FIGURE 11

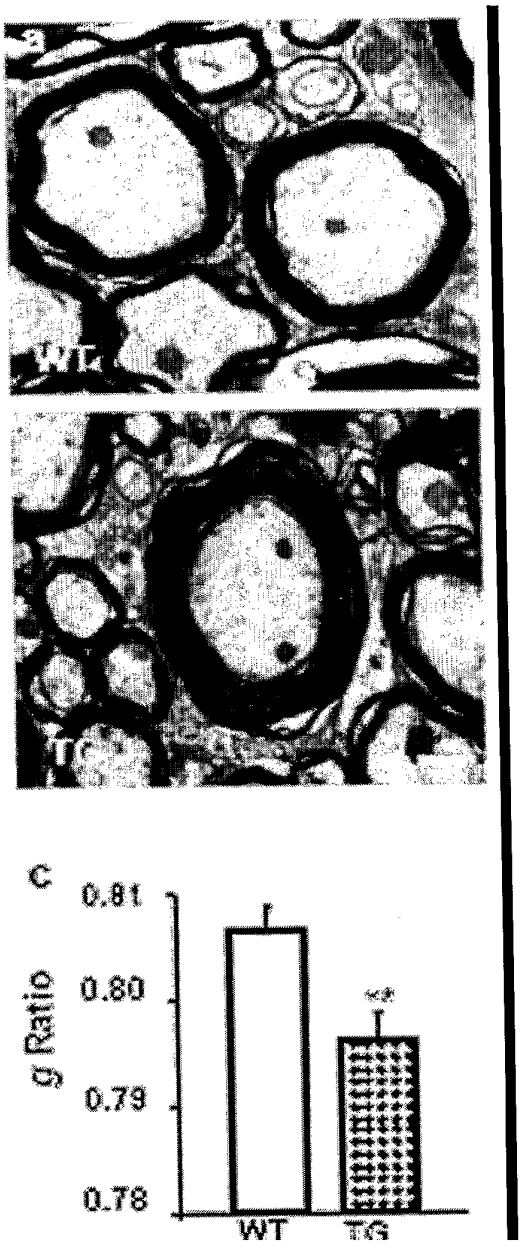


FIGURE 12

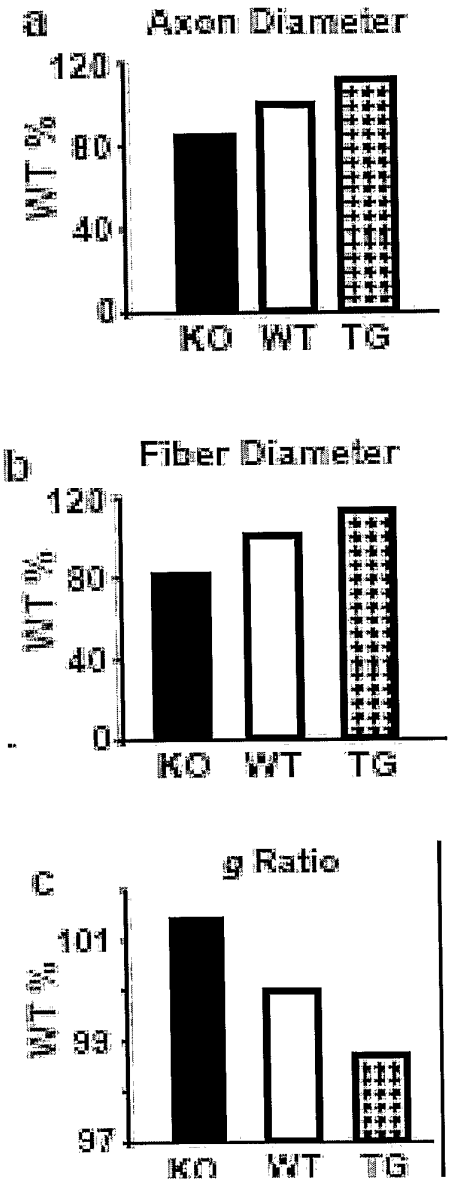

a
Axon Diameter
120
80
40
0
WT %
KO WT TG
b
Fiber Diameter
120
80
40
0
WT %
KO WT TG
c
g Ratio
101
99
97
WT %
KO WT TG

FIGURE 13

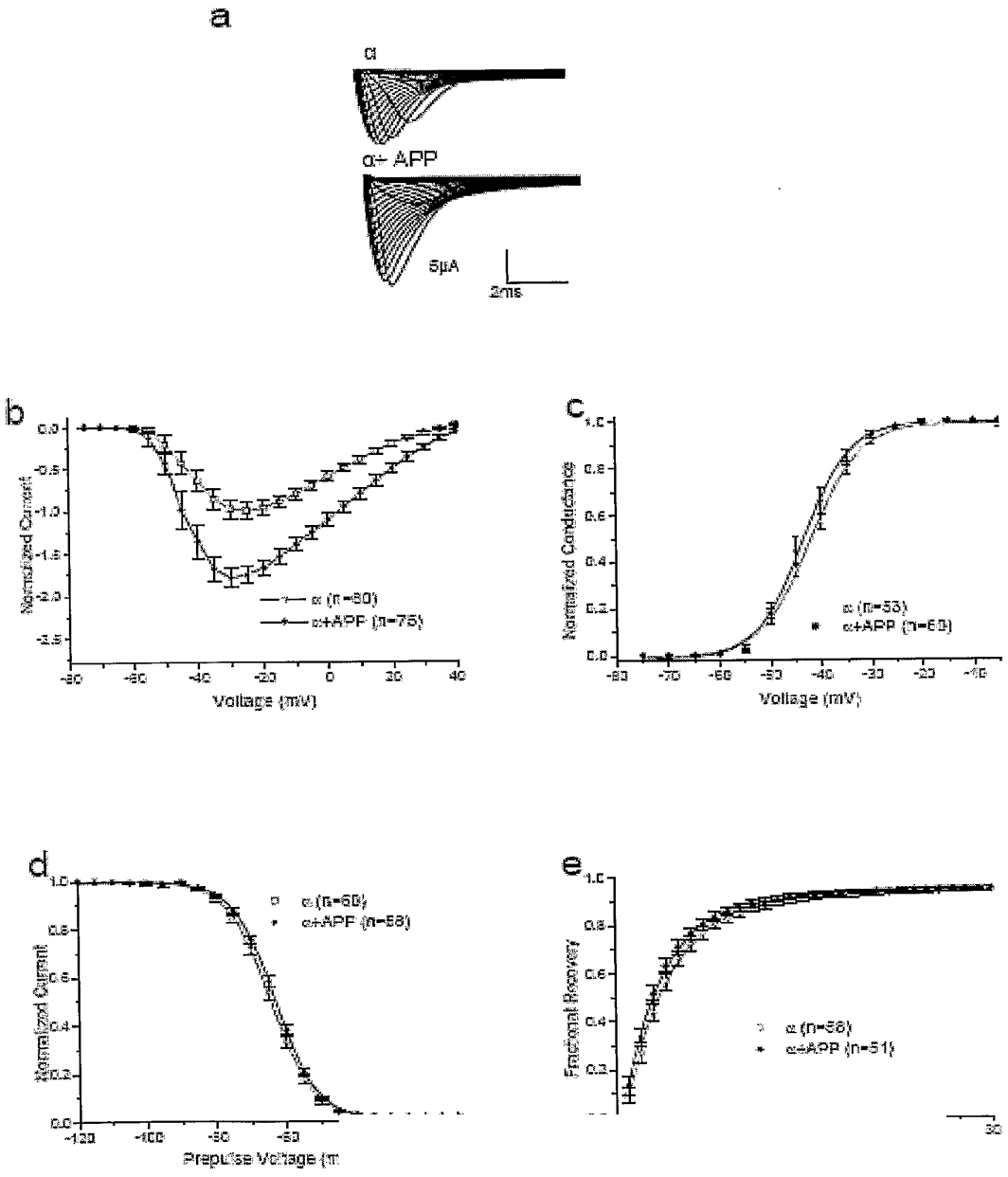
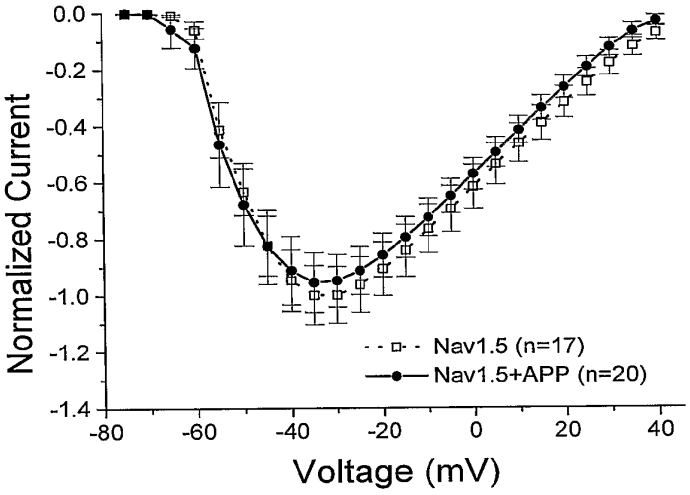
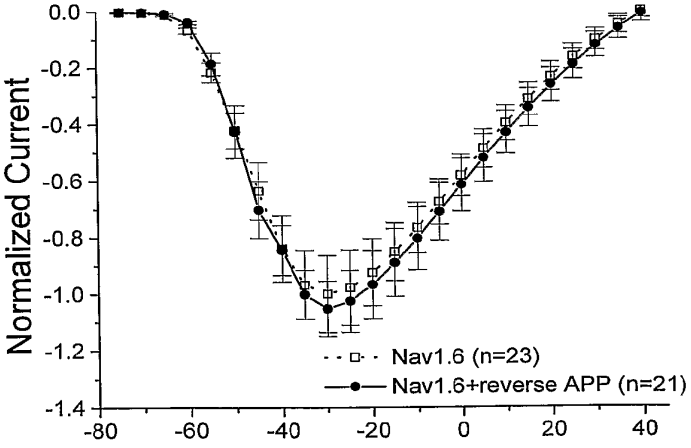

a
α
α+ APP
5μA
2ms
b
Normalized Current
Voltage (mV)
α (n=60)
α+APP (n=75)
c
Normalized Conductance
Voltage (mV)
α (n=55)
α+APP (n=63)
d
Normalized Current
α (n=69)
α+APP (n=58)
Prepulse Voltage (m
e
Fractional Recovery
α (n=56)
α+APP (n=51)

FIGURE 13

f



g



Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6004

FIGURE 14

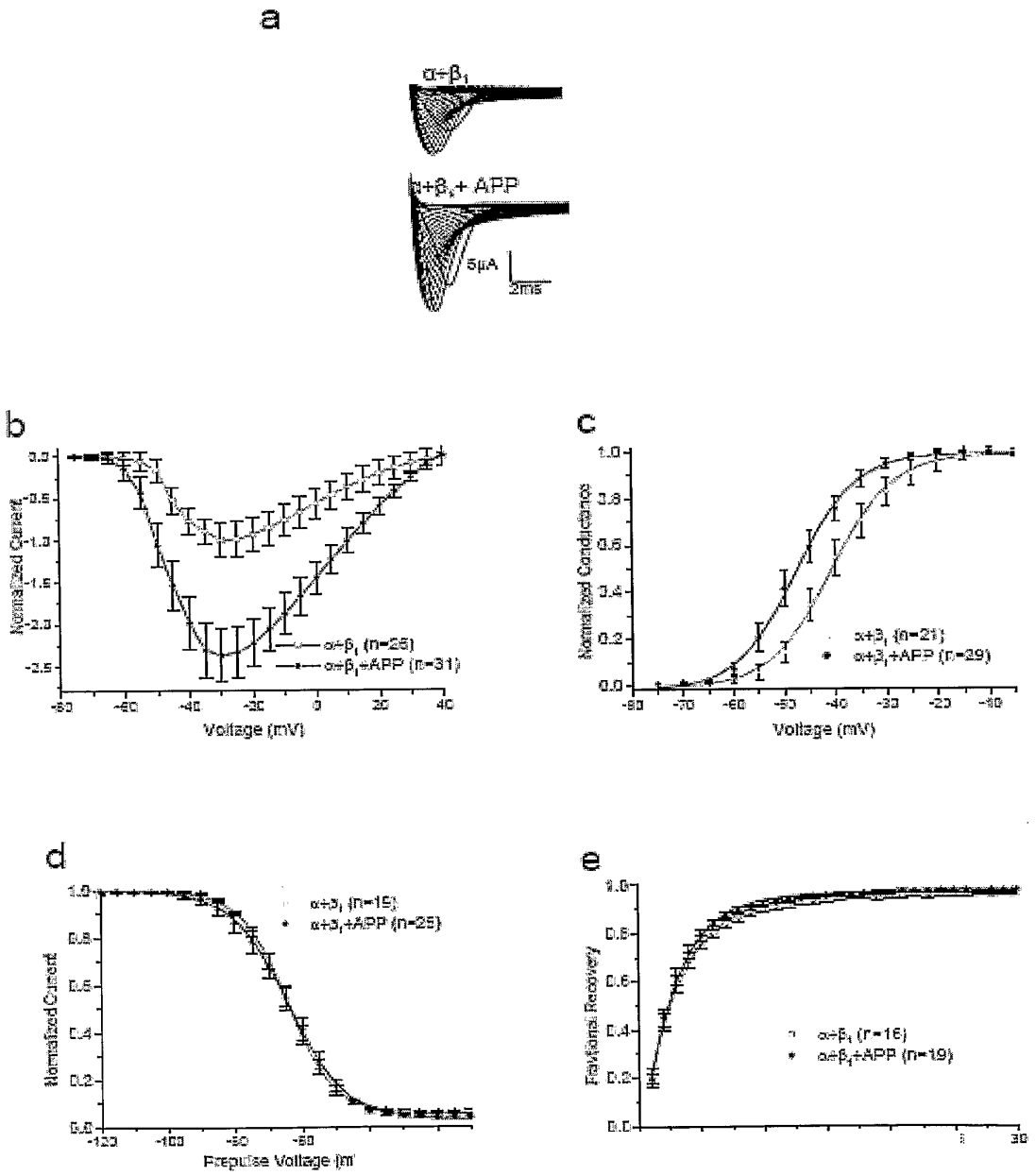

a
α+β1
α+β1+ APP
5μA
2ms
b
Normalized Current
Voltage (mV)
α+β1 (n=26)
α+β1+APP (n=31)
c
Normalized Conductance
Voltage (mV)
α+β1 (n=21)
α+β1+APP (n=29)
d
Normalized Current
Prepulse Voltage (m
α+β1 (n=15)
α+β1+APP (n=25)
e
Fractional Recovery
α+β1 (n=16)
α+β1+APP (n=19)

FIGURE 15

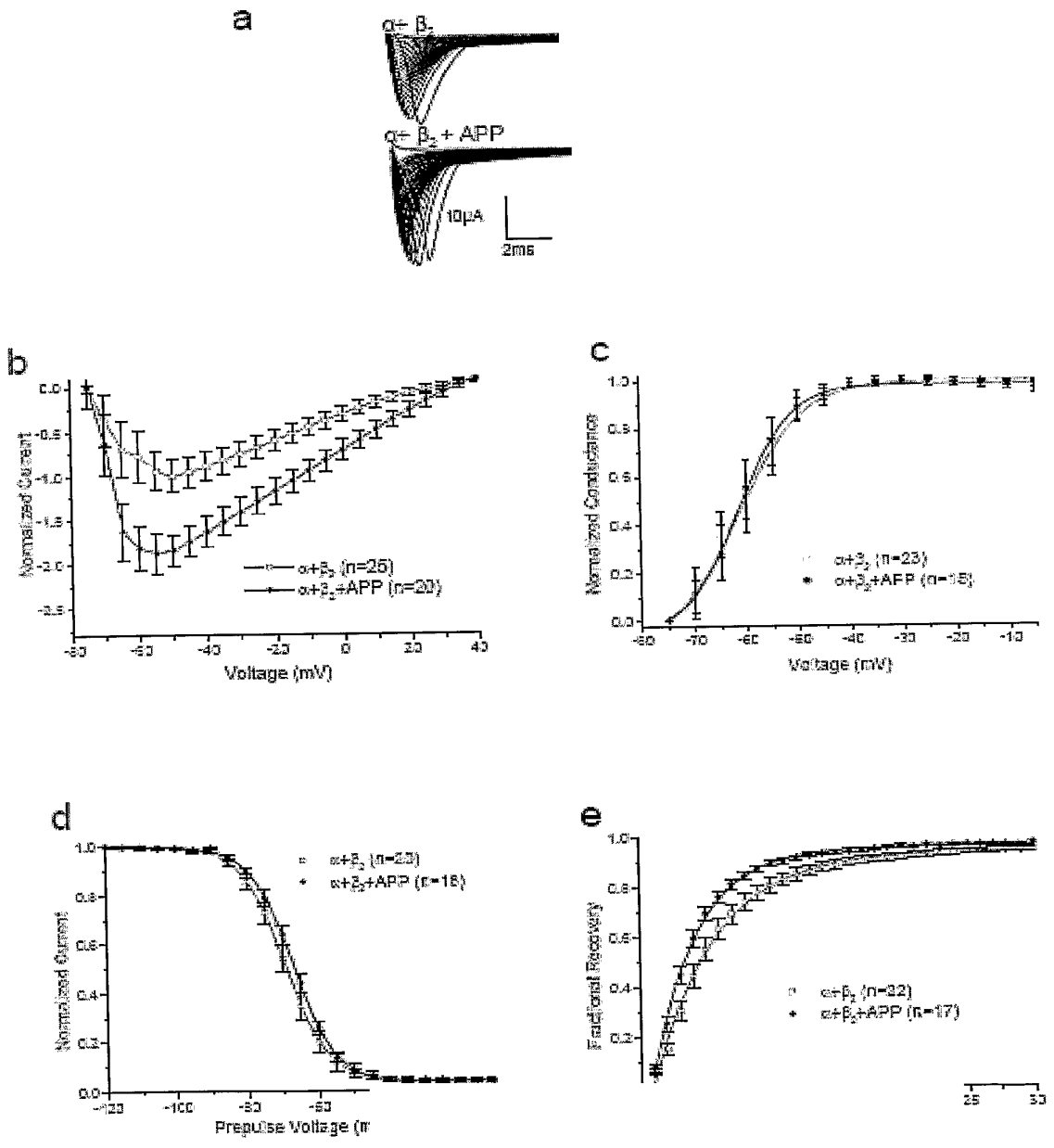

a
α+ β2
α+ β2 + APP
10μA
2ms
b
Normalized Current
Voltage (mV)
α+β2 (n=25)
α+β2+APP (n=20)
c
Normalized Conductance
Voltage (mV)
α+β2 (n=23)
α+β2+APP (n=16)
d
Normalized Current
α+β2 (n=23)
α+β2+APP (n=16)
e
Fractional Recovery
α+β2 (n=22)
α+β2+APP (n=17)

FIGURE 16

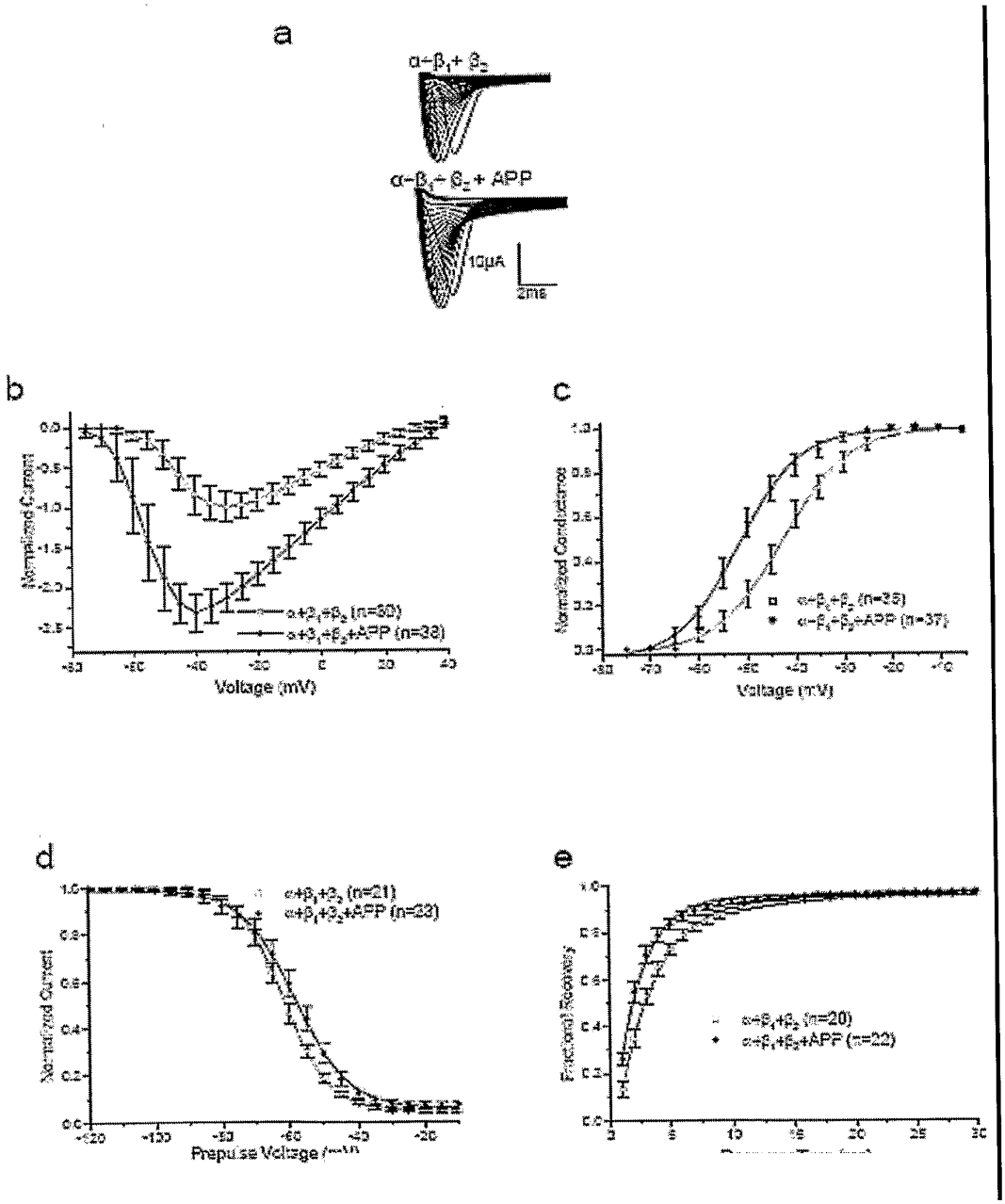

a
α+β1+ β2
α+β1+ β2+ APP
10μA
2ms
b
Normalized Current
Voltage (mV)
α+β1+β2 (n=30)
α+β1+β2+APP (n=32)
c
Normalized Conductance
Voltage (mV)
α+β1+β2 (n=38)
α+β1+β2+APP (n=37)
d
Normalized Current
α+β1+β2 (n=21)
α+β1+β2+APP (n=22)
e
Fractional Recovery
α+β1+β2 (n=20)
α+β1+β2+APP (n=22)

FIGURE 17

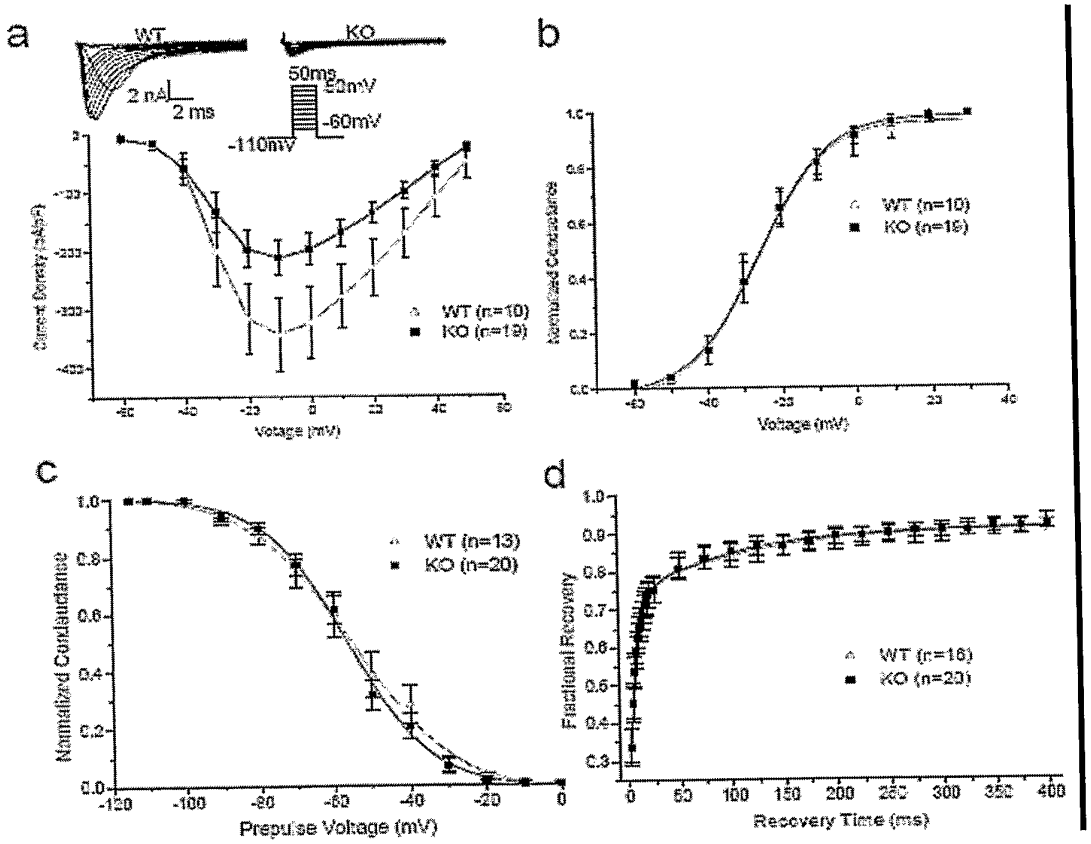

a
WT
KO
2 nA
2 ms
50ms
20mV
-60mV
-110mV
WT (n=10)
KO (n=19)
Voltage (mV)
b
WT (n=10)
KO (n=19)
Voltage (mV)
c
WT (n=13)
KO (n=20)
Normalized Conductance
Prepulse Voltage (mV)
d
WT (n=16)
KO (n=20)
Fractional Recovery
Recovery Time (ms)

FIGURE 18

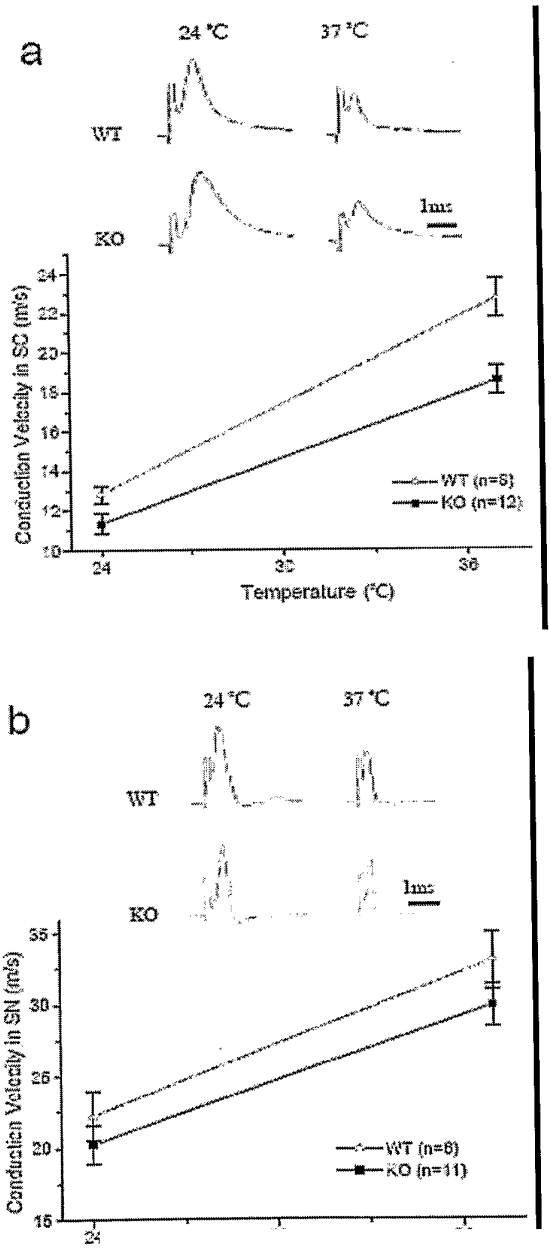

a
24 °C
37 °C
WT
KO
1ms
Conduction Velocity in SC (m/s)
WT (n=8)
KO (n=12)
Temperature (°C)
b
24 °C
37 °C
WT
KO
1ms
Conduction Velocity in SN (m/s)
WT (n=8)
KO (n=11)

FIGURE 19

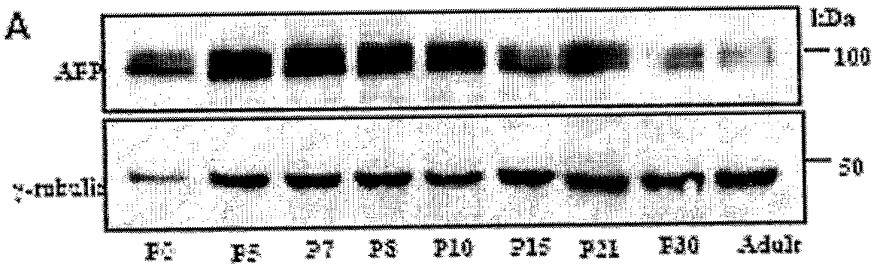




FIGURE 20

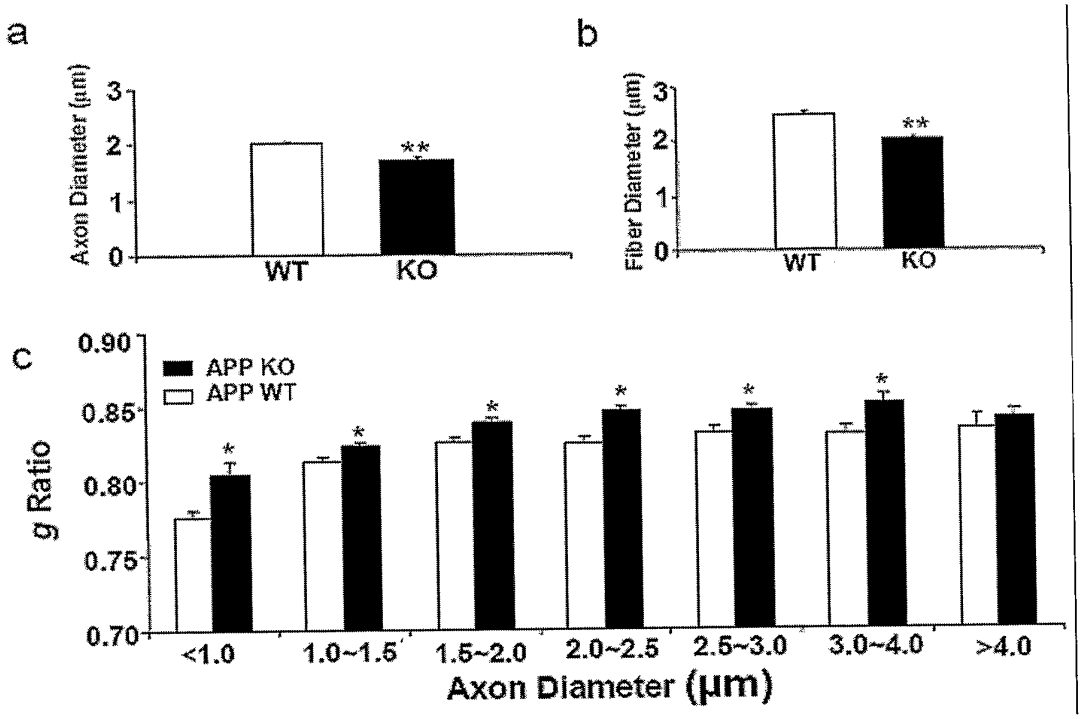

a
Axon Diameter (μm)
3
2
1
0
**
WT
KO
b
Fiber Diameter (μm)
3
2
1
0
**
WT
KO
c
g Ratio
0.90
0.85
0.80
0.75
0.70
APP KO
APP WT
*
<1.0
1.0~1.5
1.5~2.0
2.0~2.5
2.5~3.0
3.0~4.0
>4.0
Axon Diameter (μm)

FIGURE 21

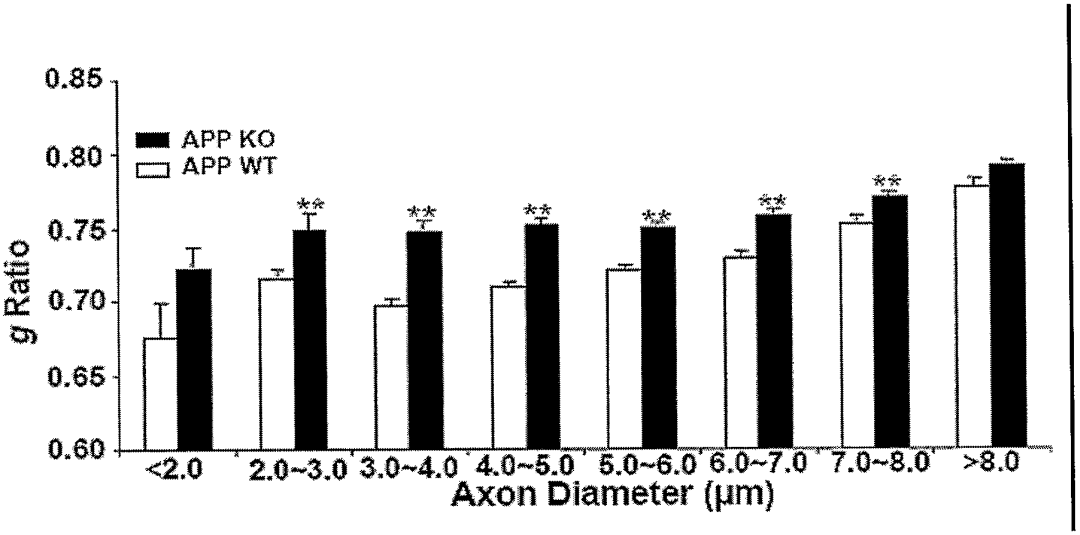


FIGURE 22

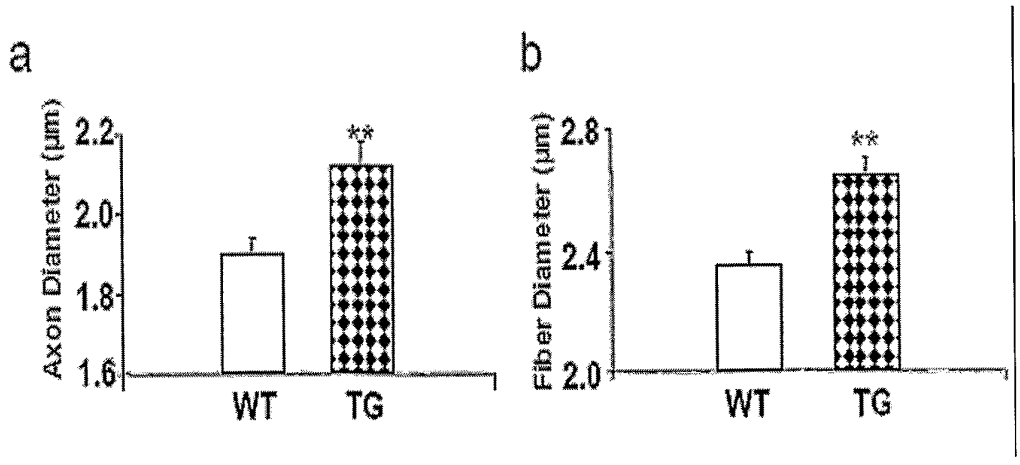


FIGURE 23

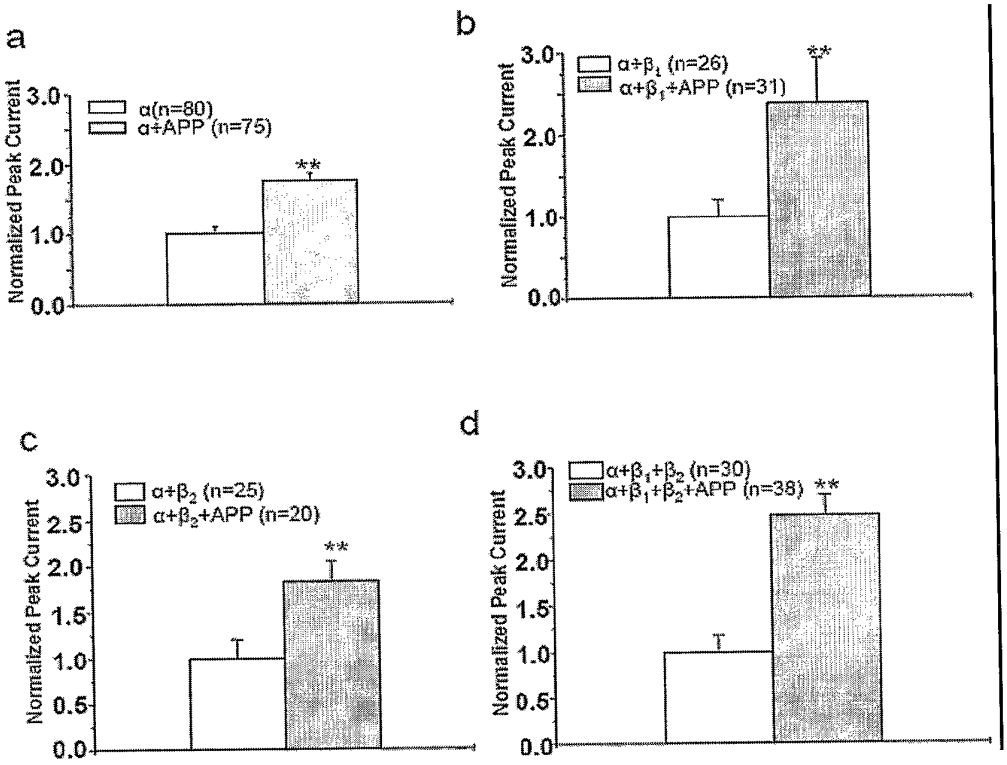

a
Normalized Peak Current
3.0
2.0
1.0
0.0
α(n=80)
α+APP (n=75)
**
b
Normalized Peak Current
3.0
2.0
1.0
0.0
α+β1 (n=26)
α+β1+APP (n=31)
**
c
Normalized Peak Current
3.0
2.5
2.0
1.5
1.0
0.5
0.0
α+β2 (n=25)
α+β2+APP (n=20)
**
d
Normalized Peak Current
3.0
2.5
2.0
1.5
1.0
0.5
0.0
α+β1+β2 (n=30)
α+β1+β2+APP (n=38)
**

FIGURE 24

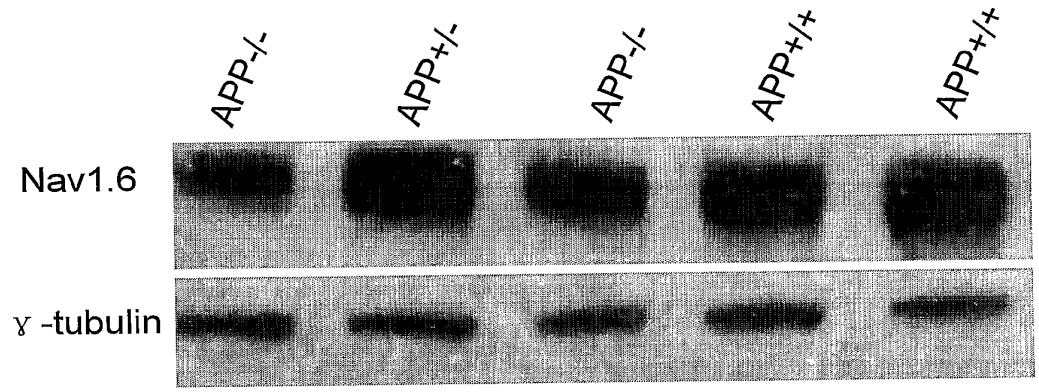


FIGURE 25

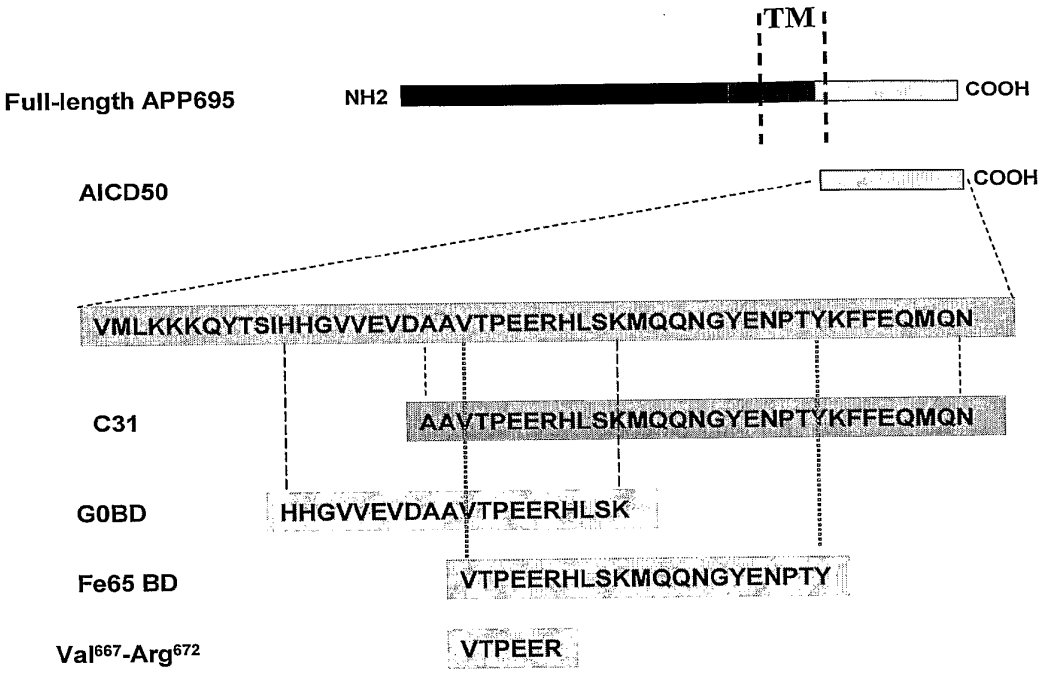

TM
Full-length APP695
NH2
COOH
AICD50
COOH
VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN
C31
AAVTPEERHLSKMQQNGYENPTYKFFEQMQN
G0BD
HHGVVEVDAAVTPEERHLSK
Fe65 BD
VTPEERHLSKMQQNGYENPTY
Val667-Arg672
VTPEER

FIGURE 26

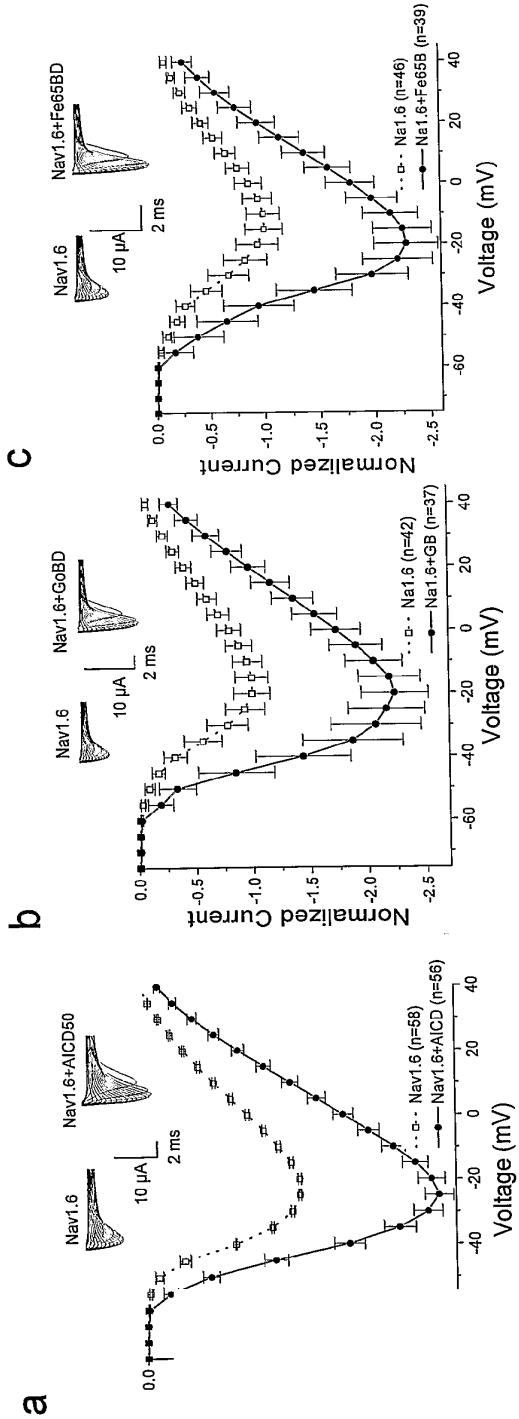

a
Nav1.6
Nav1.6+AICD50
10 µA
2 ms
0.0
Nav1.6 (n=58)
Nav1.6+AICD (n=56)
Voltage (mV)
40
20
0
-20
-40
b
Nav1.6
Nav1.6+GoBD
10 µA
2 ms
Normalized Current
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Na1.6 (n=42)
Na1.6+GB (n=37)
Voltage (mV)
40
20
0
-20
-40
-60
c
Nav1.6
Nav1.6+Fe65BD
10 µA
2 ms
Normalized Current
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Na1.6 (n=46)
Na1.6+Fe65B (n=39)
Voltage (mV)
40
20
0
-20
-40
-60

FIGURE 26

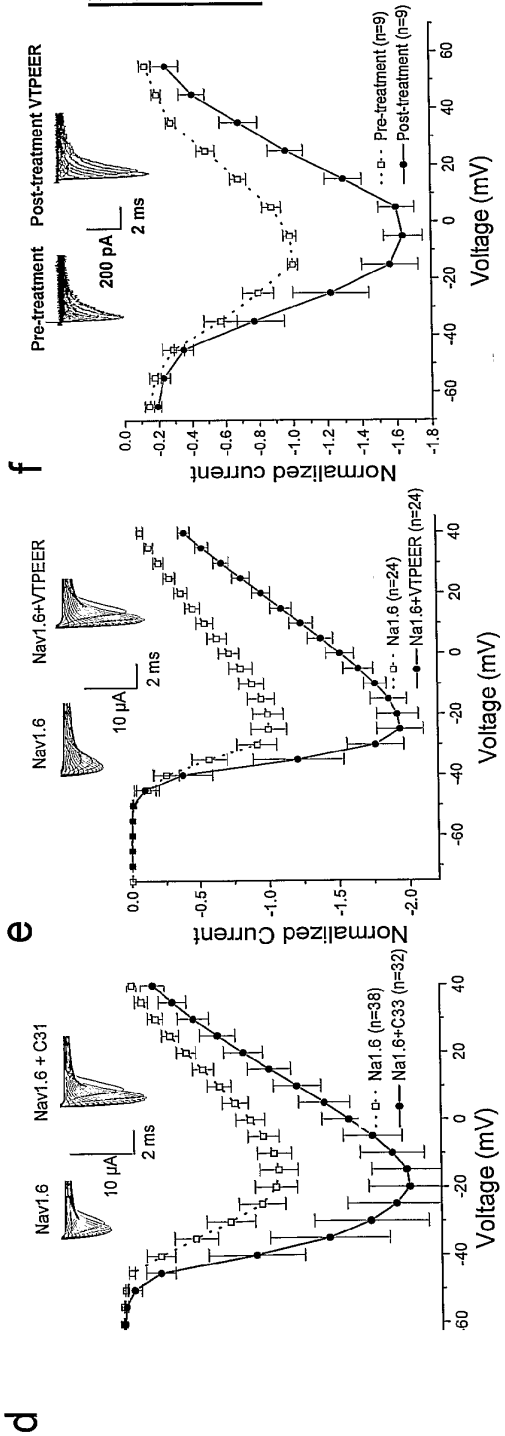

f
Pre-treatment
Post-treatment VTPEER
200 pA
2 ms
Pre-treatment (n=9)
Post-treatment (n=9)
Normalized current
Voltage (mV)
e
Nav1.6
Nav1.6+VTPEER
10 µA
2 ms
Na1.6 (n=24)
Na1.6+VTPEER (n=24)
Normalized Current
Voltage (mV)
d
Nav1.6
Nav1.6 + C31
10 µA
2 ms
Na1.6 (n=38)
Na1.6+C33 (n=32)
Voltage (mV)

FIGURE 27

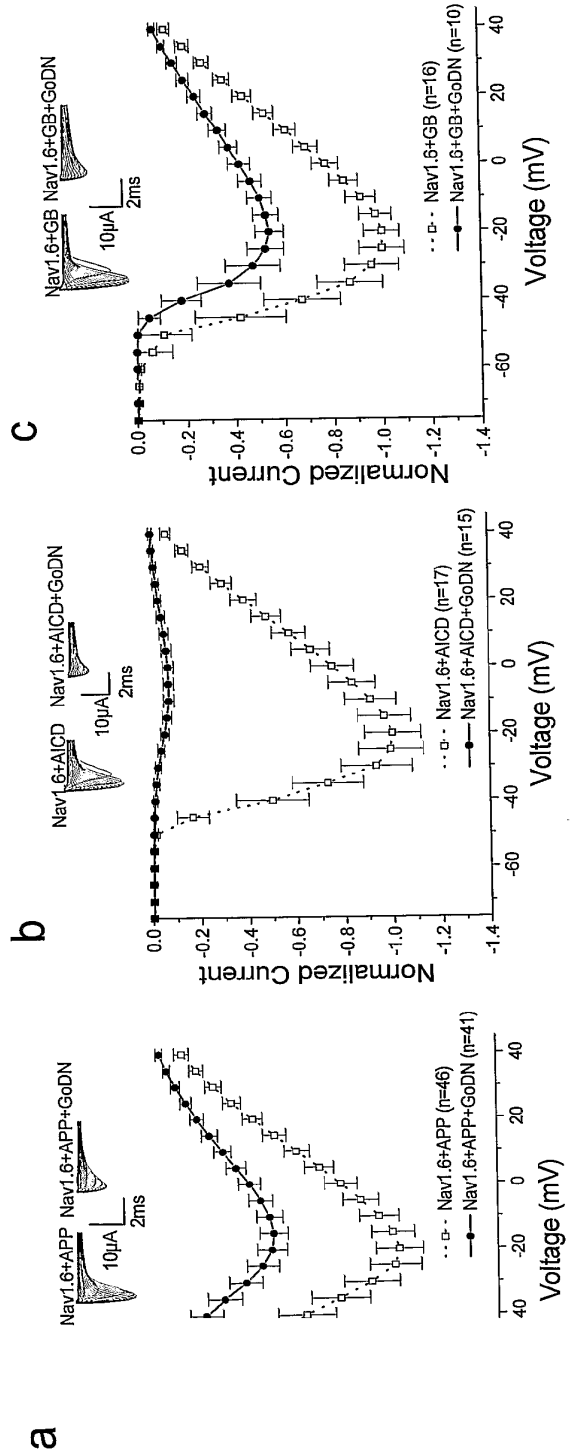

a
Nav1.6+APP
Nav1.6+APP+GoDN
10μA
2ms
Nav1.6+APP (n=46)
Nav1.6+APP+GoDN (n=41)
Voltage (mV)
b
Nav1.6+AICD
Nav1.6+AICD+GoDN
10μA
2ms
Normalized Current
Nav1.6+AICD (n=17)
Nav1.6+AICD+GoDN (n=15)
Voltage (mV)
c
Nav1.6+GB
Nav1.6+GB+GoDN
10μA
2ms
Normalized Current
Nav1.6+GB (n=16)
Nav1.6+GB+GoDN (n=10)
Voltage (mV)

FIGURE 27

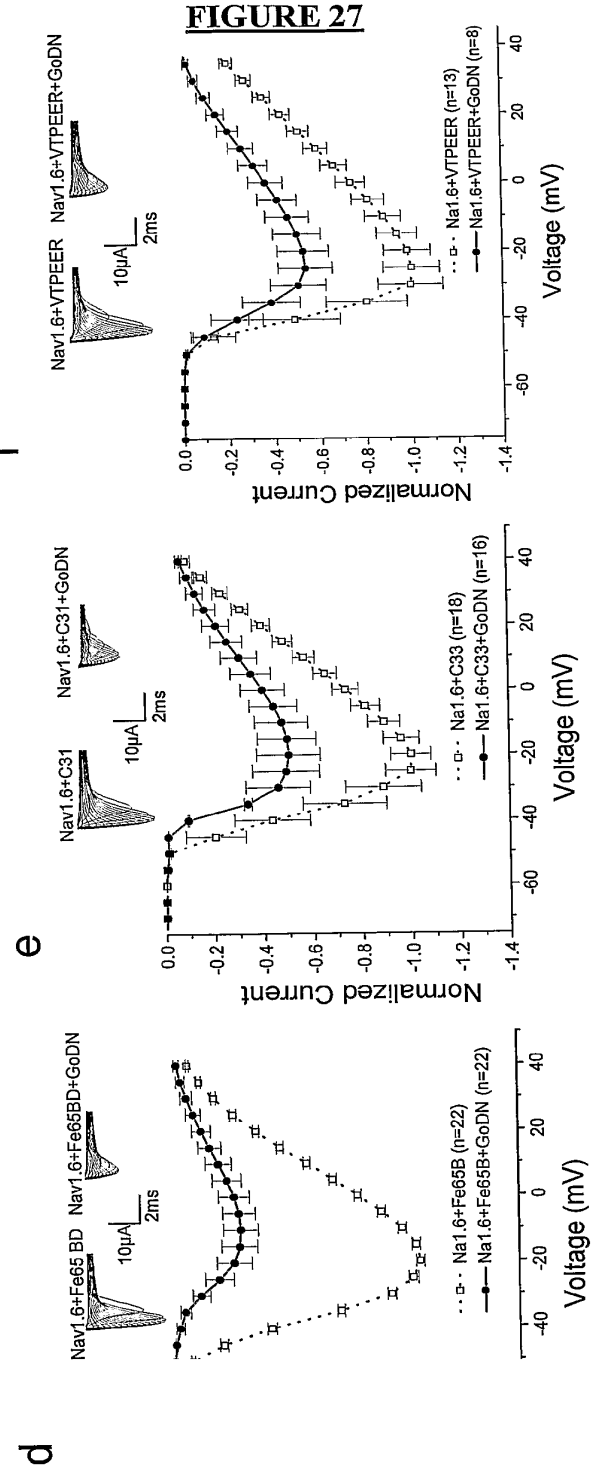

f
Nav1.6+VTPEER
Nav1.6+VTPEER+GoDN
10μA
2ms
Normalized Current
Voltage (mV)
Na1.6+VTPEER (n=13)
Na1.6+VTPEER+GoDN (n=8)
e
Nav1.6+C31
Nav1.6+C31+GoDN
Na1.6+C33 (n=18)
Na1.6+C33+GoDN (n=16)
d
Nav1.6+Fe65 BD
Nav1.6+Fe65BD+GoDN
Na1.6+Fe65B (n=22)
Na1.6+Fe65B+GoDN (n=22)

FIGURE 28

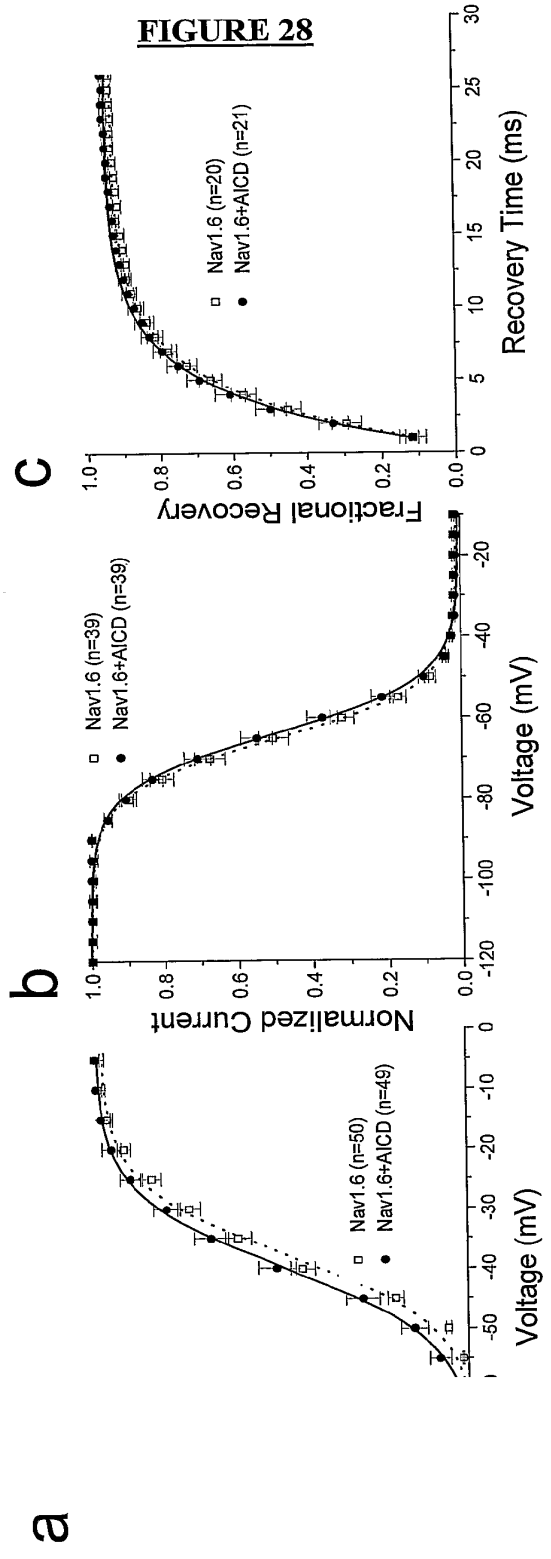
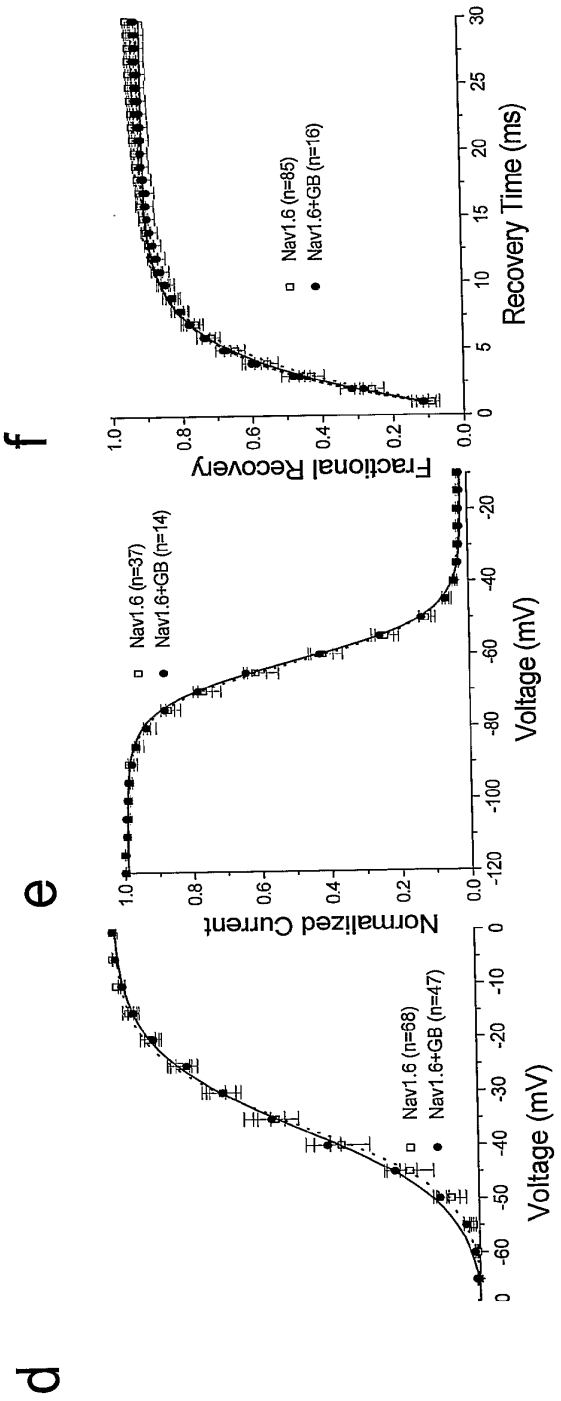

a
Nav1.6 (n=50)
Nav1.6+AICD (n=49)
Voltage (mV)
b
Nav1.6 (n=39)
Nav1.6+AICD (n=39)
Normalized Current
Voltage (mV)
c
Nav1.6 (n=20)
Nav1.6+AICD (n=21)
Fractional Recovery
Recovery Time (ms)

FIGURE 28


f
Fractional Recovery
Nav1.6 (n=85)
Nav1.6+GB (n=16)
Recovery Time (ms)
e
Normalized Current
Nav1.6 (n=37)
Nav1.6+GB (n=14)
Voltage (mV)
d
Nav1.6 (n=68)
Nav1.6+GB (n=47)
Voltage (mV)

31/42

FIGURE 28

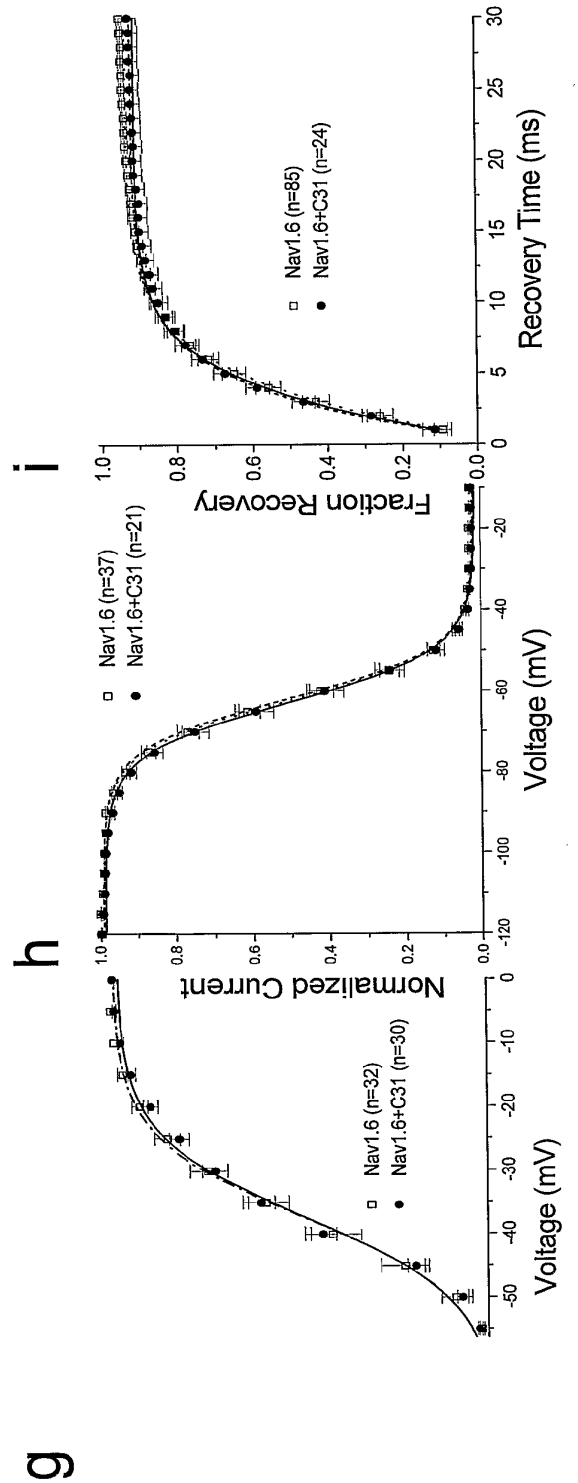


FIGURE 28

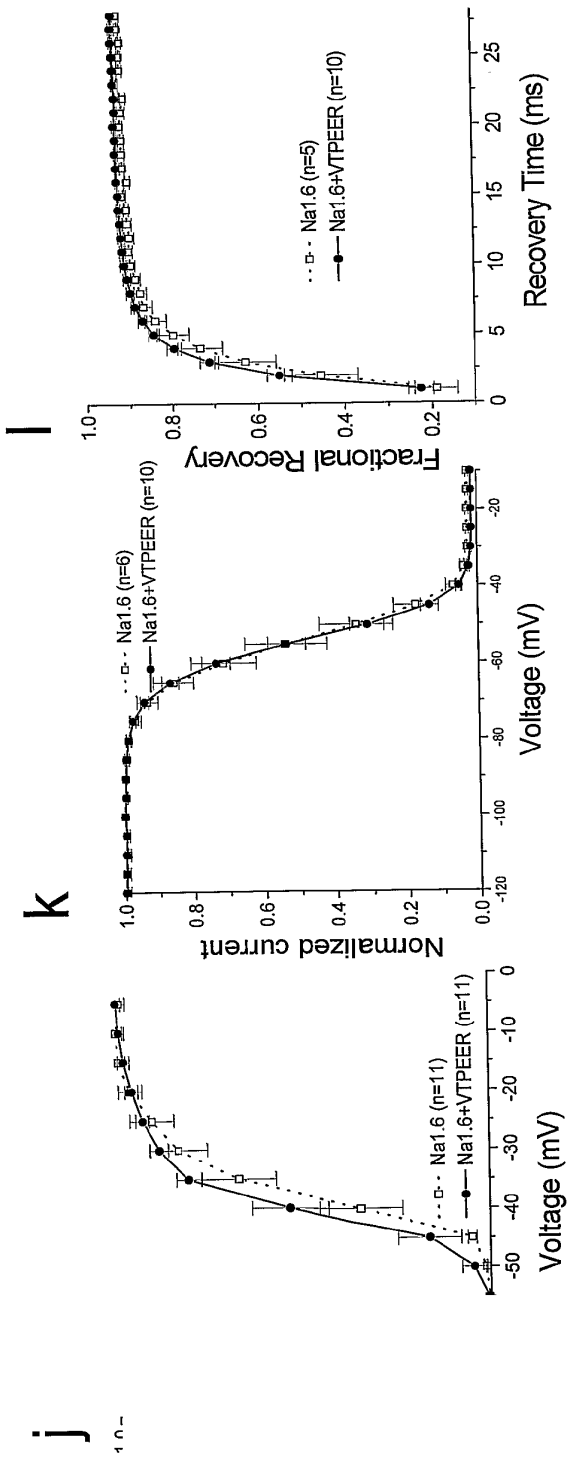

l
Fractional Recovery
Recovery Time (ms)
Na1.6 (n=5)
Na1.6+VTPEER (n=10)
k
Normalized current
Voltage (mV)
Na1.6 (n=6)
Na1.6+VTPEER (n=10)
j
Voltage (mV)
Na1.6 (n=11)
Na1.6+VTPEER (n=11)

FIGURE 29

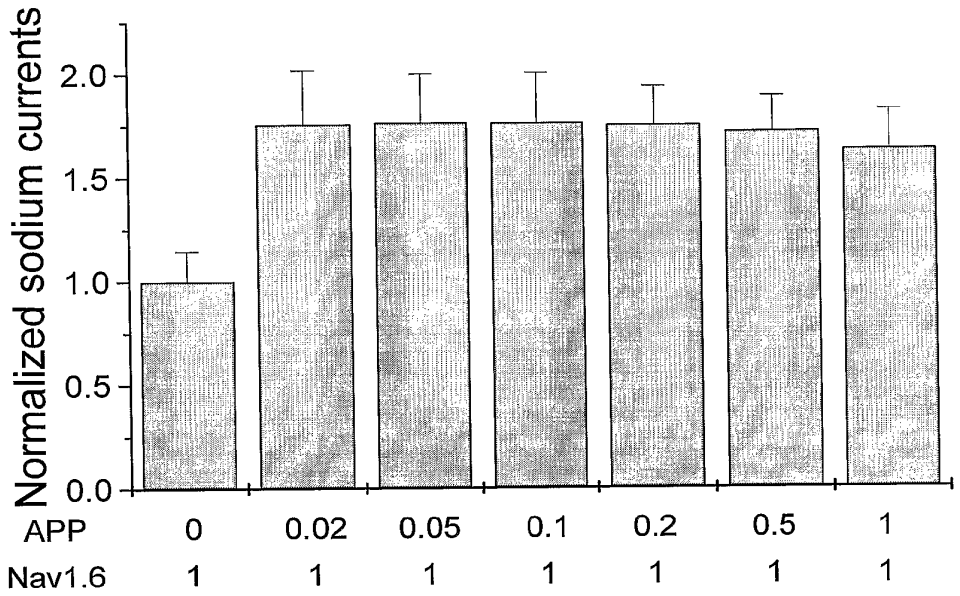

Normalized sodium currents
2.0
1.5
1.0
0.5
0.0
APP 0 0.02 0.05 0.1 0.2 0.5 1
Nav1.6 1 1 1 1 1 1 1

FIGURE 30

A

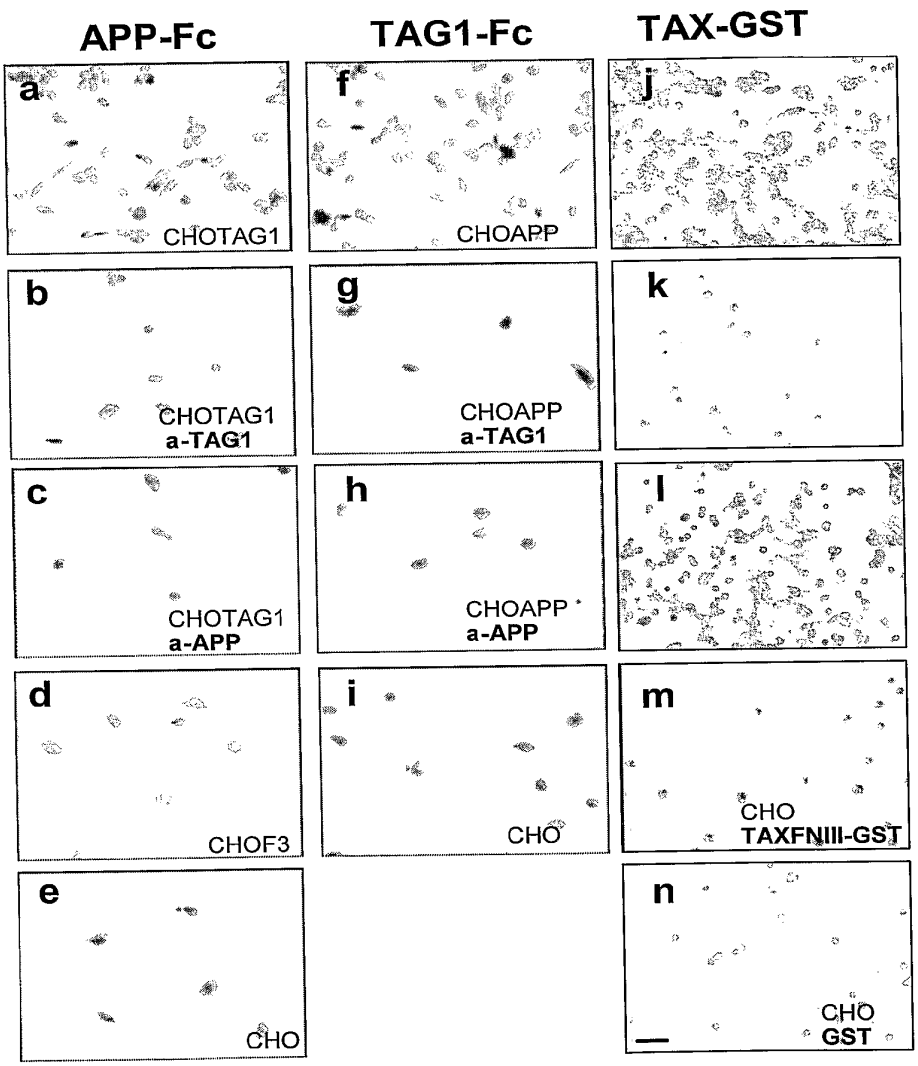

APP-Fc
TAG1-Fc
TAX-GST
a
CHOTAG1
f
CHOAPP
j
b
CHOTAG1
a-TAG1
g
CHOAPP
a-TAG1
k
c
CHOTAG1
a-APP
h
CHOAPP
a-APP
l
d
CHOF3
i
CHO
m
CHO
TAXFNIII-GST
e
CHO
n
CHO
GST

FIGURE 30

B

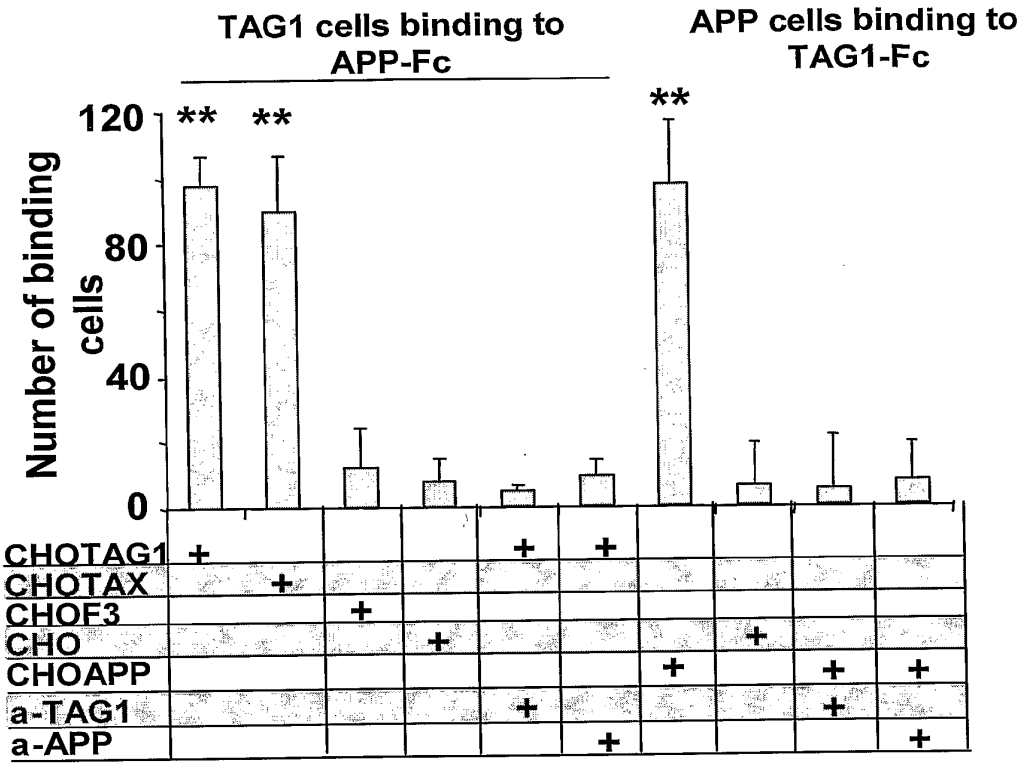

TAG1 cells binding to APP-Fc
APP cells binding to TAG1-Fc
Number of binding cells
120
80
40
0
**
**
**
CHOTAG1
CHOTAX
CHOF3
CHO
CHOAPP
a-TAG1
a-APP

FIGURE 31

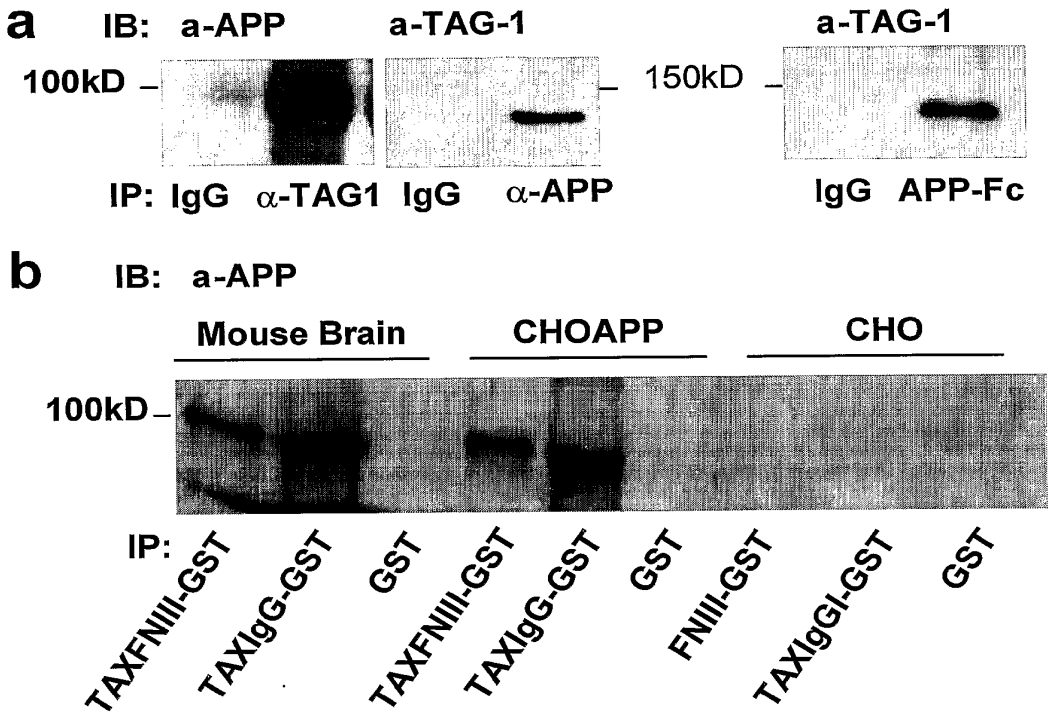

a
IB: a-APP
a-TAG-1
a-TAG-1
100kD
150kD
IP: IgG α-TAG1 IgG α-APP
IgG APP-Fc
b
IB: a-APP
Mouse Brain
CHOAPP
CHO
100kD
IP:
TAXFNIII-GST
TAXIgG-GST
GST
TAXFNIII-GST
TAXIgG-GST
GST
FNIII-GST
TAXIgGI-GST
GST

FIGURE 32

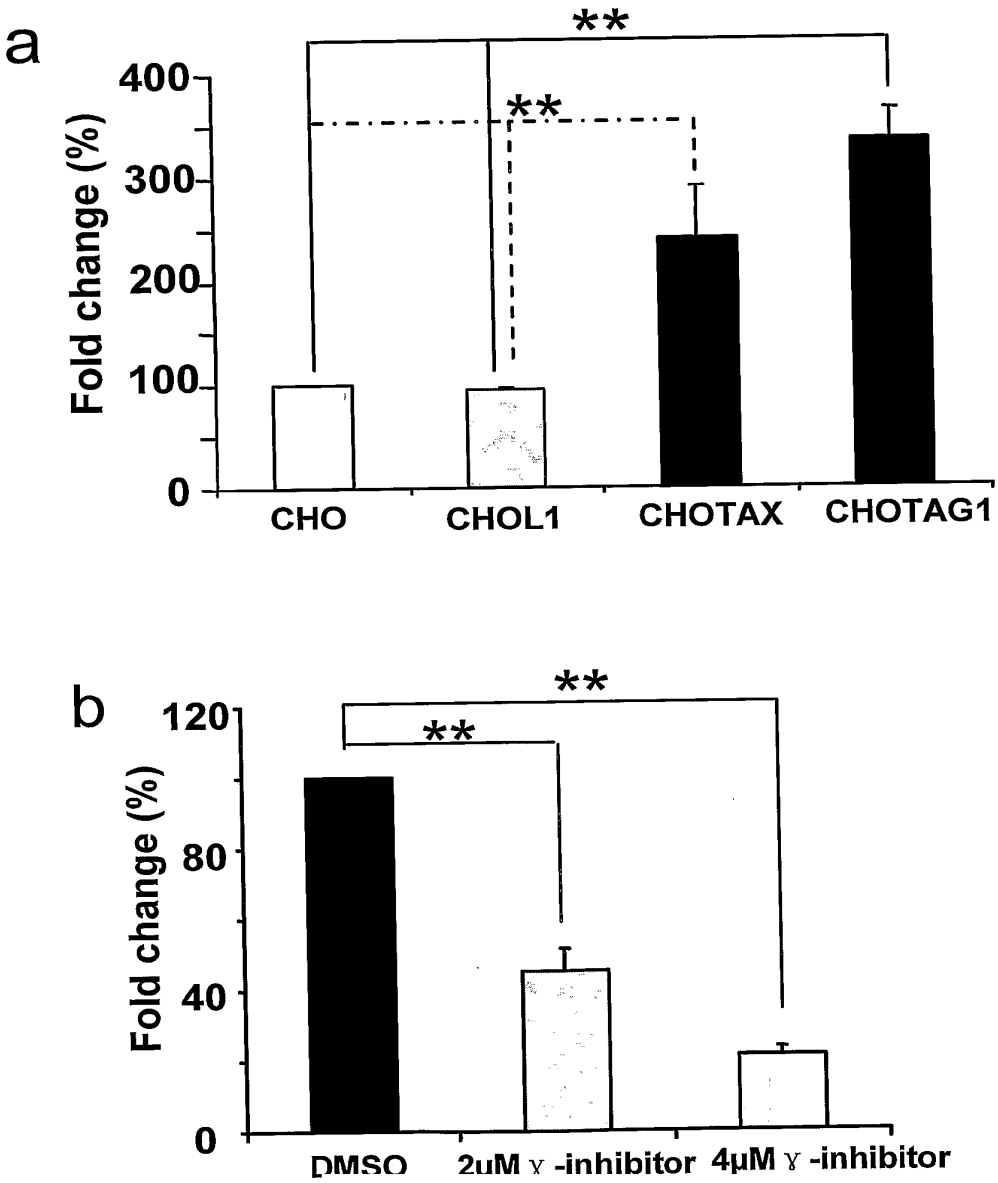

a
**
**
400
300
200
100
0
Fold change (%)
CHO
CHOL1
CHOTAX
CHOTAG1
b
**
**
120
80
40
0
Fold change (%)
DMSO
2μM γ -inhibitor
4μM γ -inhibitor

FIGURE 33

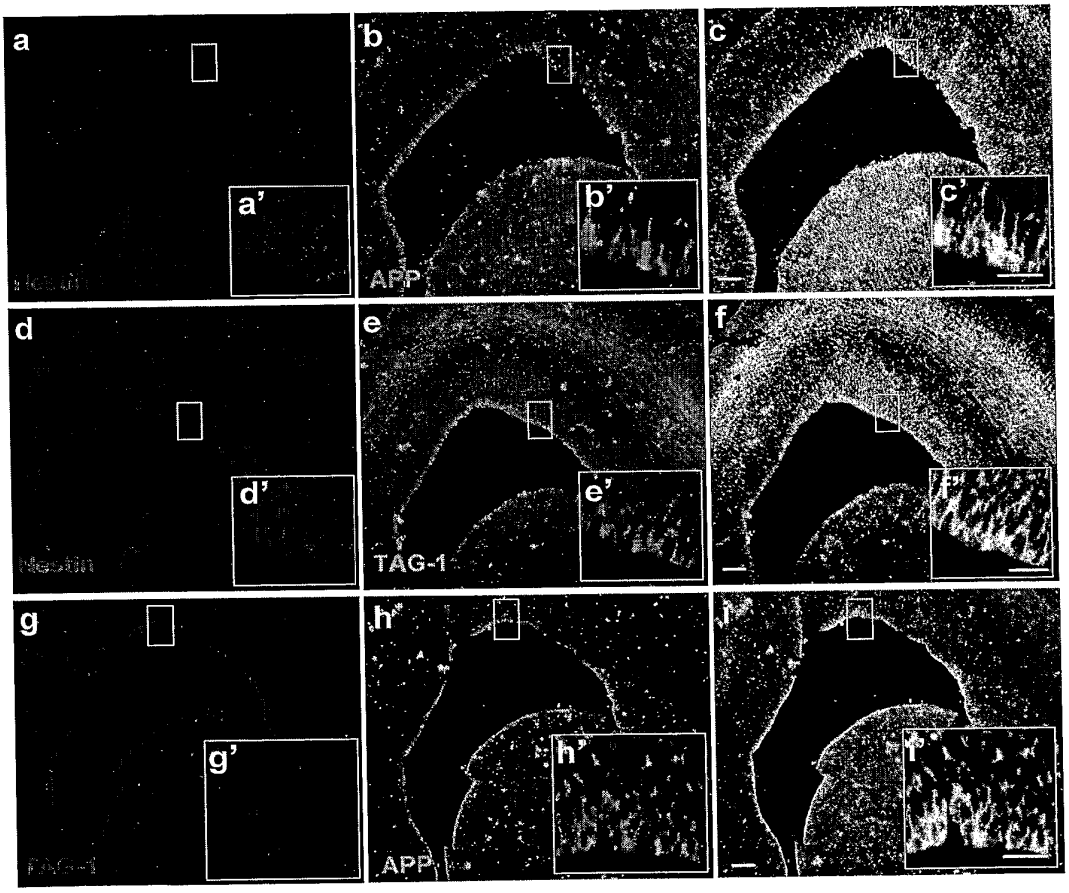


FIGURE 34

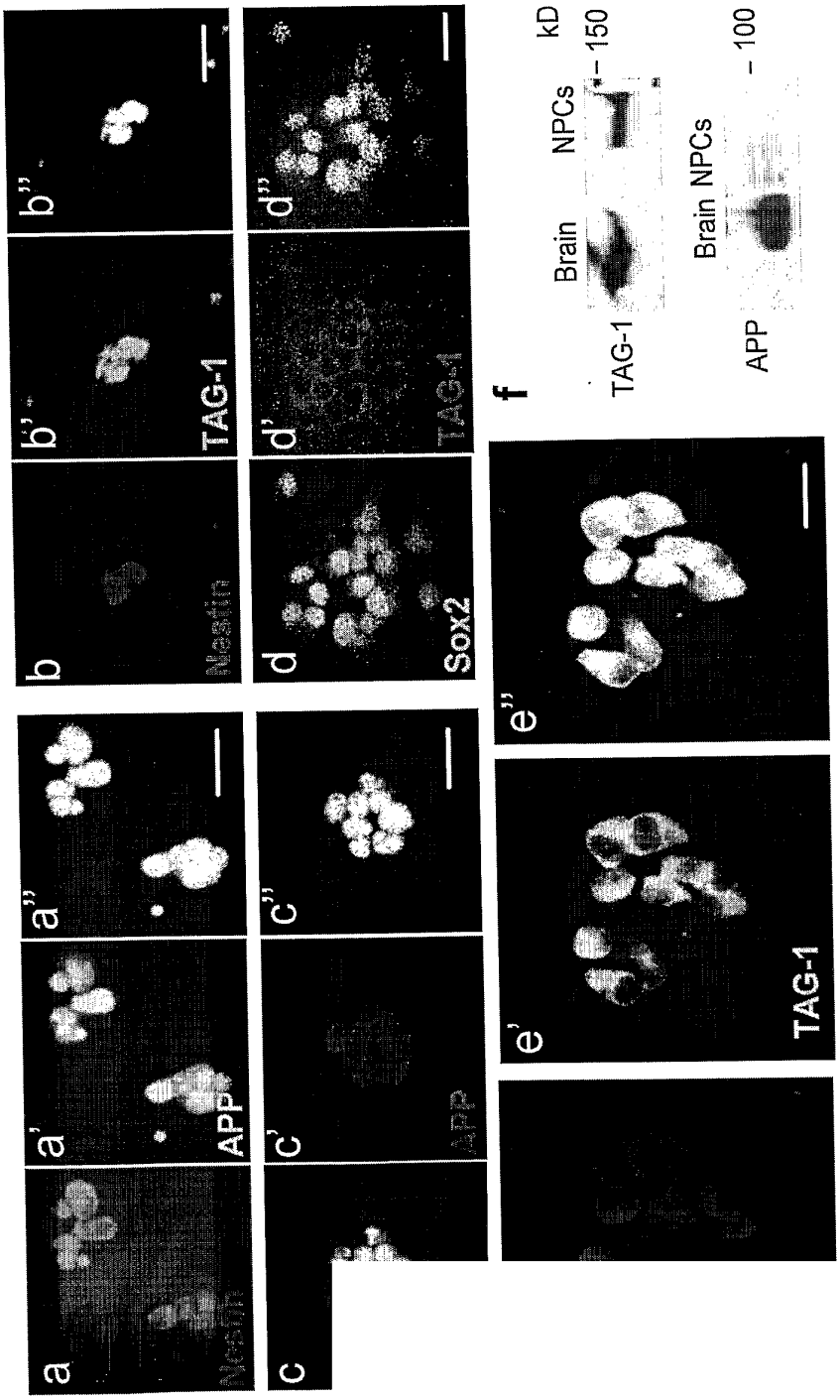

a
Nestin
a'
APP
a''
c
c'
APP
c''
b
Nestin
b'
TAG-1
b''
d
Sox2
d'
TAG-1
d''
f
kD
Brain
NPCs
150
TAG-1
Brain
NPCs
100
APP
e'
TAG-1
e''

FIGURE 35

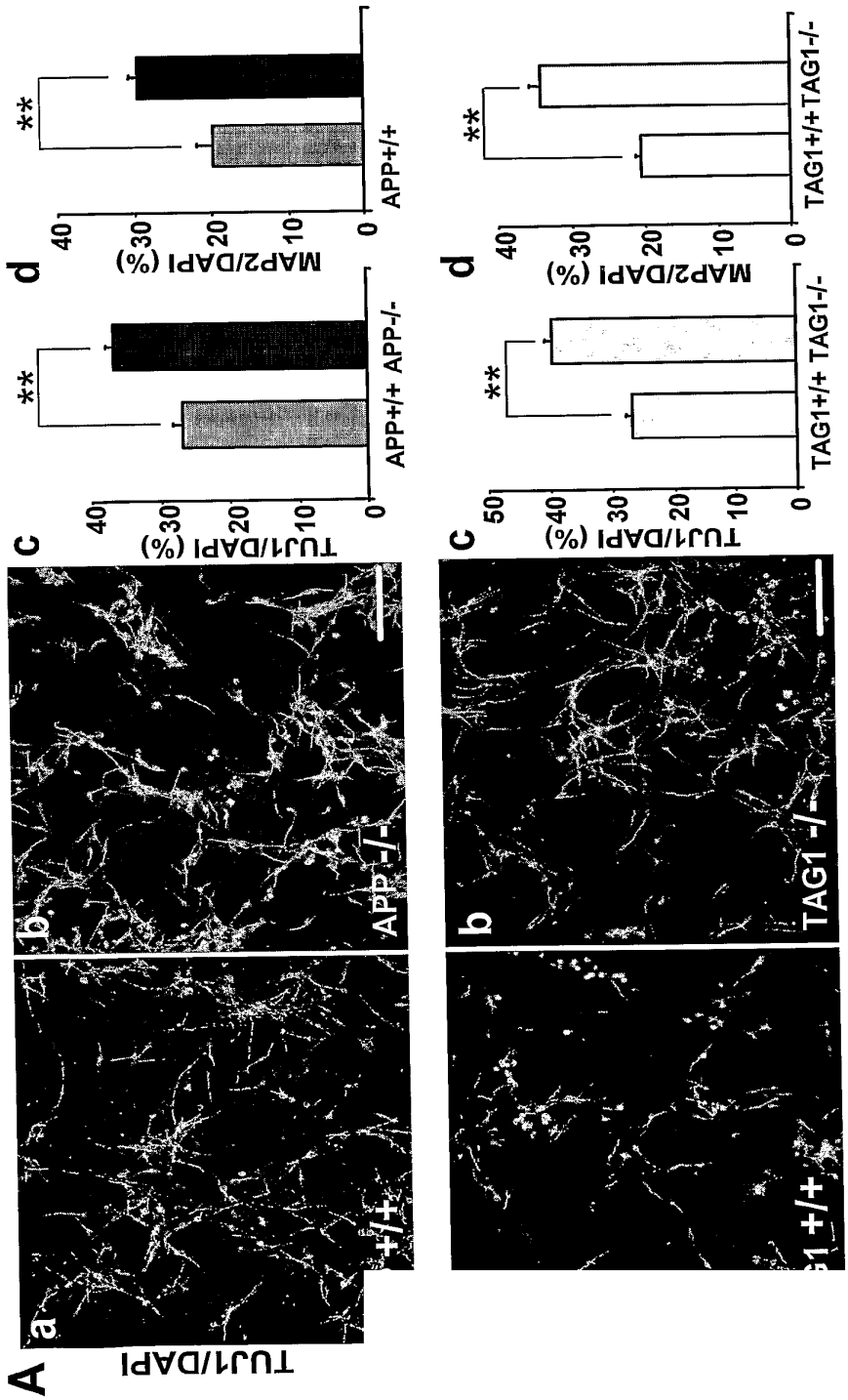

A
TUJ1/DAPI
a
+/+
b
APP -/-
c
TUJ1/DAPI (%)
APP+/+ APP-/-
**
d
MAP2/DAPI (%)
APP+/+
**
b
TAG1 -/-
c
TUJ1/DAPI (%)
TAG1+/+ TAG1-/-
**
d
MAP2/DAPI (%)
TAG1+/+TAG1-/-
**

FIGURE 36

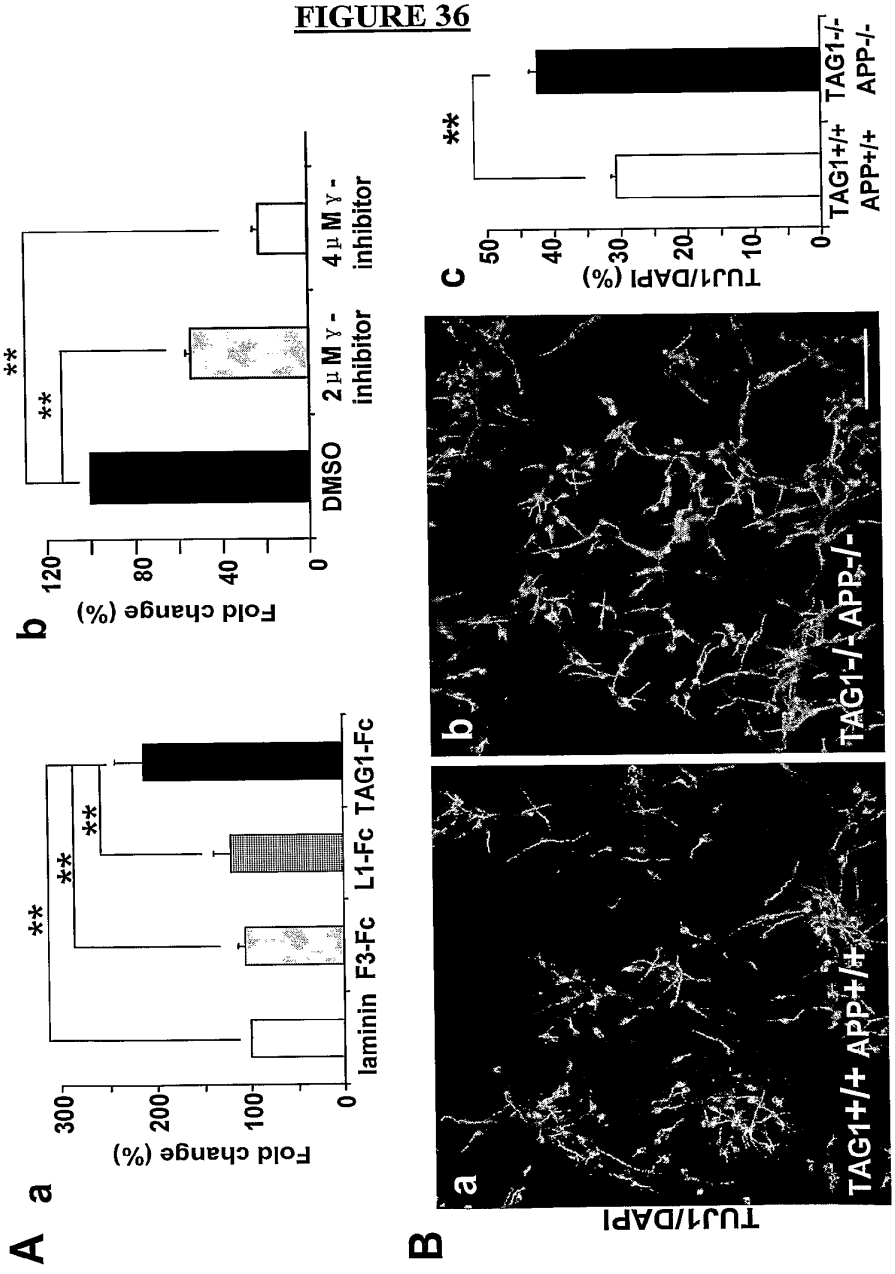
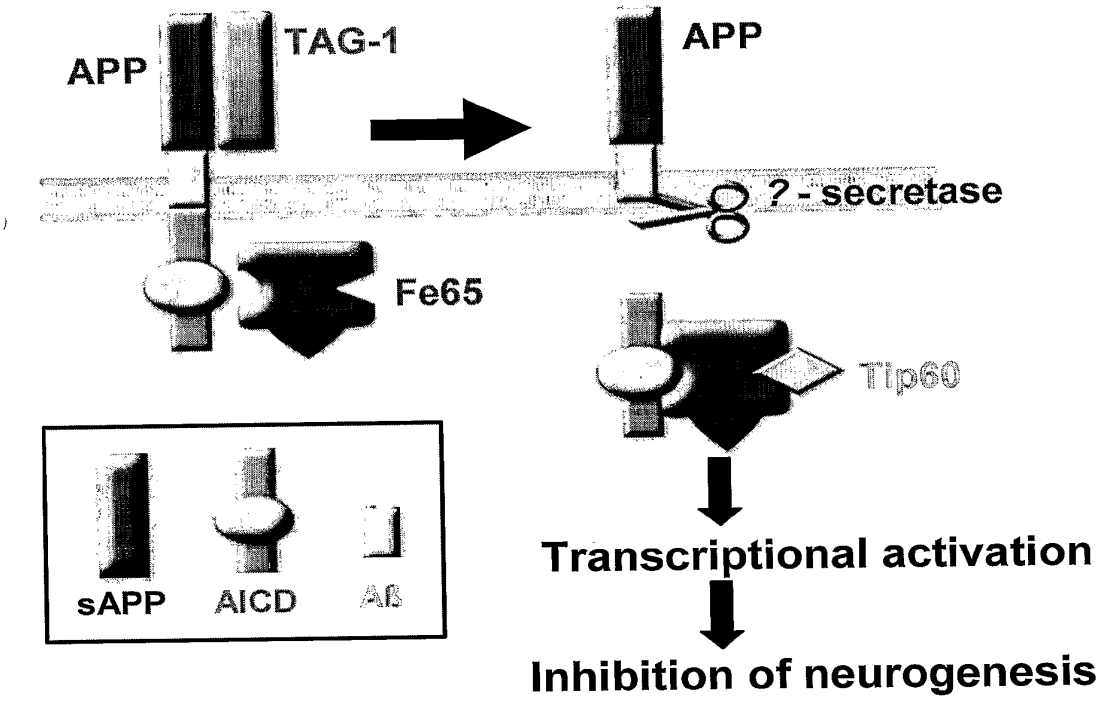

A
a
Fold change (%)
300
200
100
0
laminin
F3-Fc
L1-Fc
TAG1-Fc
**
**
**
b
Fold change (%)
120
80
40
0
DMSO
2 μM γ-inhibitor
4 μM γ-inhibitor
**
**
B
TU J1/DAPI
a
TAG1+/+ APP+/+
b
TAG1-/- APP-/-
c
TU J1/DAPI (%)
50
40
30
20
10
0
**
TAG1+/+ APP+/+
TAG1-/- APP-/-

FIGURE 37


APP
TAG-1
APP
? - secretase
Fe65
Tip60
sAPP
AICD
Aß
Transcriptional activation
Inhibition of neurogenesis

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2007/000283

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 38/02 (2006.01) ***A61P 25/00*** (2006.01) ***G01N 33/50*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, WPIDS, CAPLUS, EMBASE: amyloid precursor protein, TAG-1, Caspr2, NgR, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3 protein, contactin, OMgp, RPTPbeta, NG2, Go()protein, sodium channel, sodium channel complex protein, potassium channel, soluble APP, sAPP, APP-695, VTPEER peptide, amyloid precursor protein intracellular domain, anaesthetic, veratridine, batrachotoxin, scorpion toxin, sea anemone toxin, conus toxin, saxitoxin, tetrodotoxin, antiepileptic, antiarrhythmic, Abeta deposition, myelination, stem cell function, sodium channel function, nerve tissue repair, analgesia, anaesthesia, neural system repair, neurological condition, Alzheimer, multiple sclerosis, brain injury or spinal cord injury, axonal-injury related disorder, epilepsy, neurodegenerative, demyelination, pain, axonal conduction of action potentials and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THORNTON, Emma et al., Soluble amyloid precursor protein α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. Brain Research. 2006, Vol. 1094, No. 1, pages 38-46 Abstract, page 39 right column paragraph 3- page 40 right column paragraph 2, Figures 2 and 4	1-4, 7-10, 12-14, 17-19, 29, 31, 35-42
Y		5, 11, 16, 30, 34

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2007	26 OCT 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **TERESA SUTHERLAND** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6283 3174

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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/023858 A1 (CELLZOME AG) 17 March 2005 Page 2 paragraph 3 – page 10 paragraph 4, page 15 paragraphs 1-3, page 17 paragraphs 3 and 5, page 22 paragraphs 2-4, page 47 paragraph 6- page 51 paragraph 3, page 54 paragraph 3, page 55 paragraph 2, page 56 paragraph 4, Table 2, page 98 paragraph 4, page 110 paragraph 2, page 113 paragraph 2-page 114 paragraph 2, page 133 paragraph 2, SEQ ID Nos 28-30	1-4, 13, 14, 17-19, 29, 31, 35, 37, 38, 40, 42, 43, 45
Y		5, 11, 16, 30, 34
X	EP 564946 A1 (MILES INC.) 13 October 1993 Whole document, especially abstract, page 3 lines 16-21, page 4 lines 2-page 5 line 9, Figure 1, Example 5	17, 29, 35-37, 40-45
X	CHAUHAN, Neelima B., Anti-amyloidogenic effect of Allium sativum in Alzheimer's transgenic model Tg2576. Journal of Herbal Pharmacotherapy. 2003, Vol. 3, No. 1, pages 95-107 Whole document	1-4, 13, 14, 17-19
X	WO 2003/059276 A2 (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 24 July 2003 Abstract, paragraphs 22-25, 29, 31, 66, 141-148, Examples 12-16	13, 22, 23, 25, 26
X	NITSCH, R.M. et al., Release of amyloid (beta)-protein precursor derivatives by electrical depolarization of rat hippocampal slices. PNAS. 1993, Vol. 90, No. 11, pages 5191-5193 Abstract, page 5191 right column paragraphs 2 and 3, Figures 1 and 2	29, 32, 33
X	WO 2005/102440 A2 (INNOSPINE, INC.) 20 October 2005 Abstract, claim 26	20, 21, 24, 25
X	LE, R. et al., Plaque-induced abnormalities in neurite geometry in transgenic models of Alzheimer disease: Implications for neural system disruption. Journal of Neuropathology and Experimental Neurology. 2001, Vol. 60, No. 8, pages 753-758 Abstract, page 755 right column paragraph 2	15, 27, 28
X	RASBAND, Matthew N. et al., Dysregulation of axonal sodium channel isoforms after adult-onset chronic demyelination. Journal of Neuroscience Research 2003, Vol. 73, No. 4, pages 465-470 Abstract	15, 27, 28
X	PARK, J.H. et al., Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid. Journal of Neuroscience. 2006, Vol. 26, No. 5, pages 1386-1395 Abstract, page 1391 right column paragraph 2-page 1392 right column paragraph 1	6

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-45 (partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The claims relate to an extremely large number of possible compounds. The search has been carried out for those parts of the claims which appear to be supported and disclosed, namely the compounds listed in claims 5, 9-12, 30, 35-41, 43 and 44. Terms listed in claims 6 and 8 were also included, but the search will not cover all compounds that inherently modulate APP or these or any other cellular components that interact with it.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2005/023858	AU	2003/298171	AU	2004/315111	AU	2004/315399
		CA	2537844	CA	2554353	CA	2554362
		CA	2560869	CA	2575190	CA	2575300
		CA	2589561	CN	1925869	CN	1934251
		EP	1670903	EP	1713501	EP	1718741
		EP	1732649	EP	1778837	EP	1786442
		EP	1814991	US	2006216292	US	2007161554
		WO	2005/023833	WO	2005/074971	WO	2005/074980
		WO	2005/075632	WO	2005/092443	WO	2006/015621
		WO	2006/015622	WO	2006/056259		
EP	0564946	AU	1993/033187	CA	2086165	IL	105216
		JP	7051096	MX	9302021	NZ	247356
		US	5981208				
WO	2003/059276	AU	2003/202988	AU	2003/209259	AU	2003/216058
		CA	2473503	CA	2474141	CN	1739196
		CN	1762052	EP	1471918	EP	1471970
		EP	1584106	EP	1604397	KR	2005008835
		KR	2005011818	US	6673696	US	6962857
		US	7238588	US	2003139410	US	2003148513
		US	2003219898	US	2004137742	US	2004180509
		US	2005095807	WO	2003/060082	WO	2003/060085
		WO	2004/066368	WO	2004/084299		
WO	2005/102440	AU	2005/235099	CA	2563912	EP	1747037
		KR	2007000405	MX	PA06011821	US	2005234425

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/825,809 15 September 2006 (15.09.2006) US

(71) Applicant *(for all designated States except US)*: **FOLDRX PHARMACEUTICALS, INC.** [US/US]; 100 Acorn Park Drive, 5th Floor, Cambridge, Massachusetts 02140-2303 (US).

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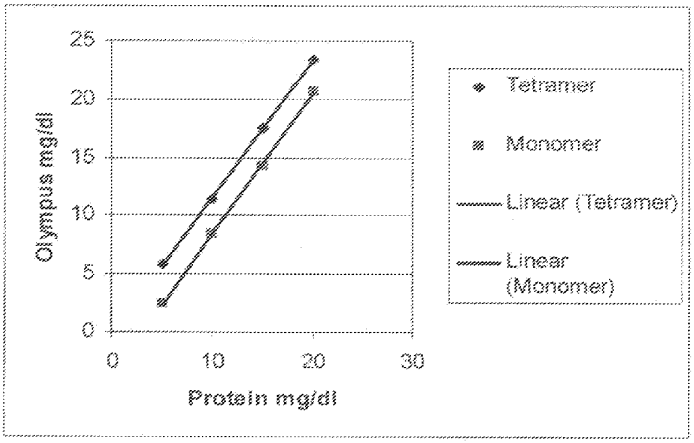
(74) Agent: **BRENNAN, Jack**; Fish & Richardson P.c., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

(54) **Title:** ASSAYS FOR DETECTING NATIVE-STATE PROTEINS AND IDENTIFYING COMPOUNDS THAT MODULATE THE STABILITY OF NATIVE-STATE PROTEINS



(57) **Abstract:** This invention relates to assays for detecting native-state proteins and to screening methods to identify compounds that modulate the stability of the native-state of proteins. Compounds identified by such screens can be used to treat diseases associated with protein misfolding, such as Alzheimer's Disease, familial amyloidotic polyneuropathies, and lysosomal storage diseases.

WO 2008/034016 A2

ASSAYS FOR DETECTING NATIVE-STATE PROTEINS AND IDENTIFYING COMPOUNDS THAT MODULATE THE STABILITY OF NATIVE-STATE PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application No. 60/825,809, filed September 15, 2006. The entire content of the prior application is incorporated herein by reference.

TECHNICAL FIELD

This invention relates to assays for detecting the native-state of a protein and screening methods to identify compounds that modulate the stability of the native-state of a protein.

BACKGROUND

Maintaining the native-state of a protein (proper assembly and folding of a protein) is a complex process and dysregulation of this process can carry significant pathological consequences. Numerous diseases are associated with a disruption(s) of the native-state of protein. For example, mutations or deletions in the homo-tetrameric p53 tumor suppressor that prevent its homotetramerization are associated with the development of certain cancers (Soussi T et al. (2001) Nat. Rev. Cancer 3:233-240; and Varley et al. (1997) Brit. J. Cancer 76:1-14). Misfolding of certain oligomer-forming polypeptides can also result in disease. Misfolding of the microtubule-associated protein Tau, for example, is a contributing factor to numerous neurodegenerative diseases including Alzheimer's disease (Johnson et al. (2004) J. Cell Sci. 117:5721-5729; and Kar et al. (2005) Alzheimer Dis. Assoc. Disord. 19(1):S29-S36). In addition, gain or loss-of-function mutations that affect protein folding in the endoplasmic reticulum can also manifest in profound effects on organismal viability. Defects in alpha-galactosidase A, beta-glucocerebrosidase (beta-glucosidase), or alpha-

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glucosidase can give rise to lysosomal storage diseases such as Fabry disease, Gaucher diseases, and Pompe disease respectively (Sawkar et al. (2002) Proc. Natl. Acad. Sci. USA 99(24):15428-15433; Ulloa-Aguirre et al. (2004) Traffic 5(11):821-837).

Transthyretin (TTR), an oligomer-forming polypeptide also known as prealbumin, is a self-associating, 55 kDa homotetrameric protein complex involved in the systemic transport of thyroxin (T4) and retinol (Damas et al. (2000) J Struct. Biol. 130(2-3):290-299 and Schussler G.C. (2000) Thyroid 10(2):141-149). Transthyretin protein is produced in liver and choroid plexus and secreted into serum and cerebrospinal fluid, respectively (Schussler G.C. (2000) Thyroid 10(2):141-149 and Schreiber G. (2002) Clin. Chem. Lab Med. 40(12):1200-1210). The transthyretin tetramer contains two conical-shaped cavities, symmetric along its structural C_2-axis, which bind two T4 molecules (Foss et al. (2005) Biochemistry 44:15525-15533; Monoco et al. (1995) Science 268(5213):1039-1041; Enegvist et al. J. Biol. Chem. (2004) 279(25):26411-26416; and Green et al. (2005) Proc. Natl. Acad. Sci. USA 102(41):14545-14550). However, less than 1% of transthyretin complexes are occupied with T4 at homeostasis.

Inherited mutations in transthyretin are causative defects for both familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy (FAC). Disease results from neurodegeneration and/or organ dysfunction associated with transthyretin amyloid fibril deposits in a variety of tissues, particularly the peripheral and central nervous system and heart. There are now over 80 point mutations in transthyretin associated with familial amyloid disease (Foss et al. (2005) Biochemistry 44:15525-15533; Lashuel et al. (1999) Biochemistry 38:13560-13573; Westermark et al. (1990) Proc. Natl. Acad. Sci. USA 87:2843-2845; and Moses et al. (1990) J. Clin. Invest. 86:2025-2033), though Senile Systemic Amylodosis (SSA), another transthyretin-related amyloid condition, results from the aggregation of normal transthyretin in ~25% of individuals over 80 years old. Treatment options for transthyretin-associated familial amyloidosis include surgical removal of fibril deposits and in some cases liver transplant. The latter is a gene therapy approach introducing a wild-type gene into the patient. The effectiveness of transplantation in treating familial amyloid disease is limited by continued production of mutant transthyretin by the choroid plexus. Transplant options are non-viable for SSA patients, since wild-type transthyretin fibrils are deposited.

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Transthyretin aggregation occurs through a multi-step process. The first, rate-limiting step, involves the dissociation of tetramer into monomers. In subsequent steps, monomeric transthyretin misfolds (Foss et al. (2005) Biochemistry 44:15525-15533), and unfolded monomers form fibrils and amyloid. The V30M point mutation, the most prevalent in FAP, has been shown by X-ray crystallographic studies to affect β-sheet folding in the transthyretin monomer by 1 angstrom, which distorts the T4-binding cavity (Foss et al. (2005) Biochemistry 44:15525-15533 and Green et al. (2005) Proc. Natl. Acad. Sci. USA 102(41):14545-14550). Since the transthyretin tetramer is stabilized by ligand binding, this provides one rationale for small molecule-based therapies in the treatment of transthyretin amyloid diseases.

SUMMARY

This invention is based, at least in part, on the discovery that treatment of a native-state protein with a denaturant (and optionally a cross-linking agent) allows for the native-state and non-native-state forms of the protein to be distinguished using an immuno-turbidity assay. This discovery led to the development of methods of detecting the presence or amount of stabilized native-state protein (e.g., stabilized tetrameric transthyretin) in a sample. The discovery also led to the development of methods to identify compounds that modulate (e.g., stabilize or destabilize) the native-state of a protein in the presence of a denaturant.

Provided herein is a method of detecting the presence or amount of a stabilized native-state protein in a sample. The method includes the steps of: contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the protein, resulting in mixture (b); contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein

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results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (c), wherein detection of insoluble aggregates indicates directly or indirectly the presence or amount of stabilized native-state protein in the sample.

Also provided is a method of detecting the presence or amount of a stabilized native-state protein in a sample that includes the steps of: contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (b), wherein detection of insoluble aggregates indicates directly or indirectly the presence or amount of stabilized native-state protein in the sample. In some embodiments of the method, mixture (a) can be contacted with a diluent prior to contacting mixture (a) with the antibody.

In some embodiments of any of the methods described above, the sample can contain a candidate compound.

In some embodiments of the methods described above, the detection of insoluble aggregates can be a direct indication of the presence or amount of stabilized native-state protein in the sample. In other embodiments of the method described above, the detection of insoluble aggregates can be an indirect indication of the presence or amount of stabilized native-state protein in the sample, the presence or amount of stabilized native-state protein in the sample being inversely proportional to the insoluble aggregates detected.

Featured herein is a method of identifying a compound that stabilizes the native-state of a protein. The method includes the steps of: contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the

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protein, resulting in mixture (b); contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (c), wherein (i) an increase in the formation of insoluble aggregates of the antibody and cross-linked native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native-state of the protein, or (ii) a decrease in the formation of insoluble aggregates of the antibody and cross-linked non-native state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native-state of the protein. In some embodiments, the protein can be contacted with the candidate compound *in vitro* or *in vivo*.

Also featured is a method of identifying a compound that stabilizes the native-state of a protein that includes the steps of: contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (b), wherein (i) an increase in the formation of insoluble aggregates of the antibody and native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native state of the protein, or (ii) a decrease in the

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formation of insoluble aggregates of the antibody and non-native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native state of the protein. In some embodiments of the method, mixture (a) can be contacted with a diluent prior to contacting mixture (a) with the antibody. In some embodiments, the protein can be contacted with the candidate compound *in vitro* or *in vivo*.

Any of the methods described above can further include the step of measuring the amount of insoluble aggregates of (i) the antibody and native-state protein or (ii) antibody and non-native-state protein.

In some embodiments of any of the methods described herein, the protein can be transthyretin. Transthyretin can be wild-type, mature (processed) transthyretin (i.e., SEQ ID NO:2) or an amyloidogenic form of transthyretin. The amyloidogenic form of transthyretin can be the V30M mutant form of transthyretin (i.e., a processed, human transthyretin mutant polypeptide having the amino acid sequence: GPTGTGESKCPLMVKVLDAVRGSPAINVAMHVFRKAADDTWEPFASGKTSESGELH GLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDSGPRRYTIAALLSPY SYSTTAVVTNPKE (SEQ ID NO: 3)), the V122I mutant form of transthyretin, the Y78F mutant form of transthyretin, the L55P mutant form of transthyretin, the L55Q mutant form of transthyretin, the A25T mutant form of transthyretin, the C10R mutant form of transthyretin, the L12P mutant form of transthyretin, the D18E mutant form of transthyretin, the D18G mutant form of transthyretin, the V20I mutant form of transthyretin, the S23N mutant form of transthyretin, the P24S mutant form of transthyretin, the V28M mutant form of transthyretin, the V30A mutant form of transthyretin, the V30L mutant form of transthyretin, the V30L mutant form of transthyretin, the V30G mutant form of transthyretin, the F33I mutant form of transthyretin, the F33L mutant form of transthyretin, the F33V mutant form of transthyretin, the R34T mutant form of transthyretin, the L35N mutant form of transthyretin, the A36P mutant form of transthyretin, the D38A mutant form of transthyretin, the E42G mutant form of transthyretin, the E42D mutant form of transthyretin, the F44S mutant form of transthyretin, the A45D mutant form of transthyretin, the A45S mutant form of transthyretin, the A45T mutant form of transthyretin, the G47R mutant form of transthyretin, the G47A mutant form of transthyretin, the G47V mutant form of

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transthyretin, the G47E mutant form of transthyretin, the T49A mutant form of transthyretin, the T49I mutant form of transthyretin, the S50R mutant form of transthyretin, the S50I mutant form of transthyretin, the E51G mutant form of transthyretin, the S52P mutant form of transthyretin, the G53E mutant form of transthyretin, the E54G mutant form of transthyretin, the E54K mutant form of transthyretin, the L55R mutant form of transthyretin, the H56R mutant form of transthyretin, the L58H mutant form of transthyretin, the L58R mutant form of transthyretin, the T59K mutant form of transthyretin, the T60A mutant form of transthyretin, the E61K mutant form of transthyretin, the F64L mutant form of transthyretin, the F64S mutant form of transthyretin, the I68L mutant form of transthyretin, the Y69H mutant form of transthyretin, the K70N mutant form of transthyretin, the V71A mutant form of transthyretin, the I73V mutant form of transthyretin, the S77F mutant form of transthyretin, the S77Y mutant form of transthyretin, the I84S mutant form of transthyretin, the I84N mutant form of transthyretin, the I84T mutant form of transthyretin, the E89Q mutant form of transthyretin, the E89K mutant form of transthyretin, the A91S mutant form of transthyretin, the A97G mutant form of transthyretin, the A97S mutant form of transthyretin, the I107V form of transthyretin, the I107M mutant form of transthyretin, the A109S mutant form of transthyretin, the L111M mutant form of transthyretin, S112I mutant form of transthyretin, the Y114C mutant form of transthyretin, the Y114H mutant form of transthyretin, the Y116S mutant form of transthyretin, the A120S mutant form of transthyretin, the Val122 deletion mutant form of transthyretin, or the V122A mutant form of transthyretin (the amino acid numbering system for the aforementioned amyloidogenic mutant forms of transthyretin is based on the amino acid numbering of the mature form of human, wild-type transthyretin (127 amino acids) as depicted in SEQ ID NO:2).

In any of the methods described herein the native-state of transthyretin can be a tetramer. In other embodiments of the methods described herein, the native-state of transthyretin can be a properly-folded monomeric form of transthyretin and the corresponding non-native-state of transthyretin can be a misfolded (or amyloid) form of monomeric transthyretin.

In any of the methods described herein, a human subject (e.g., a human patient) can have, or be at risk of developing (e.g., have a genetic predisposition to developing), a transthyretin amyloid disease. As used herein, a “transthyretin amyloid disease” is any

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pathology or disease associated with dysfunction or dysregulation of transthyretin that results in formation of transthyretin-containing amyloid fibrils. Dysregulation or dysfunction of a transthyretin polypeptide includes an enhanced or increased propensity (e.g., by virtue of mutation of one or both copies of the transthyretin gene) of tetrameric transthyretin to disassociate into monomers. Dysregulation or dysfunction of transthyretin can also include an increased propensity of transthyretin monomers to denature (misfold) or otherwise misassemble into amyloid or other abnormal quaternary structures associated with transthyretin amyloid diseases. The misfolding and/or aggregation of a transthyretin can be misfolding or aggregation of a wild-type or mutant form of transthyretin. Mutant forms of transthyretin polypeptides include, for example, any of the mutant forms or mutations described herein. Mutations in a transthyretin gene can also lead to, for example, dysregulated expression of a transthyretin molecule (e.g., overexpression of transthyretin). Overexpression or disease-causing expression of transthyretin can be overexpression of a mutant form of transthyretin or a wild-type transthyretin polypeptide. Transthyretin amyloid diseases include, but are not limited to, familial amyloid polyneuropathy, familial amyloid cardiomyopathy, senile systemic amyloidosis, cardiac amyloidosis following liver transplantation, peripheral nerve amyloidosis following liver transplantation, leptomeningeal amyloidosis, transthyretin mutant-associated carpal tunnel syndrome, vitreous deposition, or transthyretin mutant-associated skin amyloidosis.

In any of the methods described herein, the protein can be an amyloidogenic protein such as beta-amyloid, alpha-synuclein, tau, an immunoglobulin light chain, serum amyloid A protein, serum amyloid P protein, apoA-I, beta2-microglobulin, gelsolin, a lysozyme, insulin, fibrinogen, a prion protein, superoxide dismutase, calcitonin, cystatin C, and atrial natriuretic peptide. Prion proteins include, for example, scrapie prion, transmissible mink encephalopathy prion, chronic wasting disease prion, bovine spongiform encephalopathy prion, feline spongiform encephalopathy, exotic ungulate encephalopathy prion, Kuru prion, Creutzfeldt-Jakob disease prion, variant Creutzfeldt-Jakob disease prion, Gerstmann-Staussler-Scheinker syndrome prion, or fatal familial insomnia prion.

In any of the methods described herein, the protein can be a trafficking-defective protein. The trafficking defective protein can be a wild-type form of the protein (e.g., overexpression of a non-mutant form of a protein such as alpha-glucosidase) or can be a

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mutant form of a trafficking-defective protein. Trafficking-defective proteins can include, but are not limited to, gonadotropin-releasing hormone receptor, water-channel aquaporin-2, alpha1-antitrypsin, alpha1-antitrypsin variant, alpha-subunit of hNav1.5, nephrin, multi-drug resistance protein 2, the PHEX gene product, pendrin, sulfonylurea receptor 1, AE1, ceruloplasmin, palmitoyl protein thioesterase 1, cartilage oligomeric matrix protein, the ELOVL4 gene product, aspartyl-glucosaminidase, connexin 32, rhodopsin, cystic fibrosis transmembrane conductance regulator protein, HFE, tyrosinase, protein C, complement C1 inhibitor, alpha-D-galactosidase, beta-hexosaminidase, sucrase-isomaltase, UDP-glucuronosyl-transferase, insulin receptor, growth hormone receptor, myeloperoxidase (MPO), preproparathyroid hormone, Human Ether-a-go-go-Related gene (HERG), tyroxine binding globulin, lipoprotein lipase, low-density lipoprotein receptor, microsomal triglyceride transfer protein, apolipoprotein a, thyroglobulin, type I pro-collagen, fibrinogen, alpha 1-antichymotrypsin (ACT), phosphoinositide-dependent kinase 1, phosphoinositide-dependent kinase 2, vasopressin precursor protein prepro-vasopressin neurophysin II, peripheral myelin protein 22, proteolipid protein, presenilin, von Willebrand factor, ERGIC-53/p58, or sedlin. Common trafficking-defective mutations in cystic fibrosis transmembrane conductance regulator protein include, for example, the ΔF508 mutant form of the cystic fibrosis transmembrane conductance regulator protein. Mutations of beta-glucosidase can include the N370S mutant of beta-glucosidase. Further examples of trafficking-defective mutations giving rise to diseases such as Fabry disease and glycogen storage disease type II can be found, for example, in Eng et al. (1993) Am. J. Hum. Genet. 53(6):1186-1197 and Hermans et al. (2004) Hum. Mut. 23(1):47-56.

In other embodiments of any of the methods described herein, the native-state of a protein can be an oligomeric-form of a protein. Oligomer-forming polypeptides useful in any of the methods described herein include Alpha-Beta protein, superoxide dismutase, Abri/ADan, glial fibrillary acidic protein, ATP7B, hemoglobin, amyloid A, beta-2-microglobulin, custatin C, lysozyme, fibrinogen, AH and AL immunoglobulin proteins, ApoAI, ApoAII, gelsolin, lactoferrin, lactohedrin, survivin, EGF-R, Erb-B2, or IL-12.

In any of the embodiments of the methods described herein, the sample can be provided or obtained from a mammal (e.g., a human (e.g., a human patient) or a primate (e.g., a chimpanzee, a baboon, or a monkey), a mouse, a rat, a rabbit, a guinea pig, a gerbil, a

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hamster, a horse, any one of a livestock (e.g., a cow, a pig, a sheep, or a goat), a dog, a cat, or a whale.

In any of the methods described herein, a human subject can have, or be at risk of developing, Alzheimer's disease, Dutch cerebrovascular amyloidosis, Flemish cerebrovascular amyloidosis, Italian cerebrovascular amyloidosis, progressive supranuclear palsy, progressive subcortical gliosis, Pick's disease, dementia pugilisticas, Parkinson's Disease, kuru, Creutzfeldt-Jakob disease, Alexander Disease, Wilson Disease, Lou Gehrig's Disease, sickle cell anemia, cystic fibrosis, diabetes, and Huntington's disease.

In some embodiments of any of the methods described herein, a human subject can have, or be at risk of developing, a lysosomal storage disease. As used herein, a "lysosomal storage disease" is any disorder or pathology that in many cases is caused by genetic defects that affect one or more lysosomal enzymes. Lysosomal storage diseases result from a deficiency in a particular protein (e.g., enzyme) activity present in the lysosomal compartment. Deficiencies of particular protein activities can be the result of lowered or absent expression of the protein or can be the result of one or more mutations in the protein. One or more mutations of the protein can affect the activity of the protein in a number of ways. Mutations can occur in the active site of the protein (e.g., the active site of an enzyme (e.g., the kinase domain of a kinase)). Mutations can also be mutations that destabilize the native-state of a protein. In some embodiments, the one or more mutations of a protein can impair the trafficking of the protein through the endoplasmic reticulum as described above. Examples of lysosomal storage diseases include, but are not limited to, Fabry disease, Gaucher disease (type 1, type 2, or type 3), Pompe disease, Hurler/Scheie syndrome, MPS type I, GM1gangliosidosis, galactosialidosis, Morquio syndrome B, MPS type IVB, Sandhoff disease, Tay-Sachs disease, beta-mannosidosis, alpha-L-fucosidosis, Maroteaux-Lamy syndrome, MPS type VI, metachromatic leukodystrophy, Schindler disease, aspartylglycosaminuria, Hunter syndrome, MPS type II, Sanfilippo syndrome A, MPS type IIIA, Sanfilippo syndrome B, MPS type IIIB, Sanfillipo syndrome C, MPS type IIIC, Sanfilippo syndrome D, MPS type IID, Morquio syndrome A, MPS type IVA, Sly syndrome, MPS type VII, hyaluronidase deficiency, MPS type IX, multiple sulfatase deficiency, alpha-mannosidosis, sialidosis, X-linked ictiosis and multiple sulfatase deficiency, mucolipidodisis II, mucolipidosis III, Wolman disease, Farber disease, Niemann-Pick disease A, Niemann-

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Pick disease B, glycogenosis type II, neuronal ceroid lipofucsinosis infantile type, neuronal ceroid lipofucsinosis late infantile type, neuronal ceroid lipofucsinosis juvenile type, Krabbe disease, lysosomal acid phosphatase deficiency, pycnodysostosis, cystinosis, sialic acid storage disease, cobalamin deficiency type F, Niemann-Pick disease type C, galactosialidosis, metachromatic leukodystrophy variant, Gaucher disease variant, Tay-Sachs disease type AB, or glycogen storage disease.

Alternatively, the invention also features methods for detecting the non-native-state of the protein. The method includes the steps of: contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the protein, resulting in mixture (b); contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (c), wherein detection of insoluble aggregates indicates directly or indirectly the presence or amount of non-native-state protein in the sample. In another embodiment, the method includes the steps of: contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (b), wherein

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detection of insoluble aggregates indicates directly or indirectly the presence or amount of non-native-state protein in the sample. In some embodiments of the method, mixture (a) can be contacted with a diluent prior to contacting mixture (a) with the antibody. In some embodiments of any of the methods of detecting non-native-state protein, the sample can contain a candidate compound.

The invention also provides a method of identifying a compound that destabilizes the native-state of a protein that includes the steps of: contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the protein, resulting in mixture (b); contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (c), wherein (i) a decrease in the formation of insoluble aggregates of the antibody and cross-linked native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound destabilizes the native-state of the protein, or (ii) an increase in the formation of insoluble aggregates of the antibody and cross-linked non-native state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound destabilizes the native-state of the protein. In some embodiments, the protein can be contacted with the candidate compound *in vitro* or *in vivo*. In some embodiments of the method, the protein can be a protein whose native-state causes a disease, e.g., an oncogene. The oncogene can be, for example, ras, myc, HER-2/neu (erB-2), hTERT, Bcl-2, src, raf, Bruton's agammaglobulinemia tyrosine kinase (BTK), platelet-derived growth factor receptor, vascular endothelial growth factor receptor, epidermal growth factor receptor, and

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aurora kinases. The protein can also be a viral protein such as, but not limited to, any of the viral proteins described herein.

Also featured is a method of identifying a compound that destabilizes the native-state of a protein that includes the steps of: contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a); contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and detecting the presence or amount of insoluble aggregates in mixture (b), wherein (i) a decrease in the formation of insoluble aggregates of the antibody and native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound destabilizes the native state of the protein, or (ii) an increase in the formation of insoluble aggregates of the antibody and non-native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound destabilizes the native state of the protein. In some embodiments of the method, mixture (a) can be contacted with a diluent prior to contacting mixture (a) with the antibody. In some embodiments, the protein can be contacted with the candidate compound *in vitro* or *in vivo*. In some embodiments of the method, the protein can be a protein whose native-state causes a disease, e.g., an oncogene. The oncogene can be, for example, any oncogene described herein. The protein can also be a viral protein such as, but not limited to, any of the viral proteins described herein.

In some embodiments of any of the methods described herein, the sample can be, or can contain, blood, plasma, serum, urine, semen, lachrymal fluid, cerebrospinal fluid, vitreous humor, or a tissue sample.

The denaturant in any of the methods described herein can be, for example, urea, guanadinium hydrochloride, guanadinium sulfite, guanadinium thiocyanate, sodium n-dodecyl sulfate, Nonindet-40 (NP40), n-lauryl sarcosine, high or low pH (e.g., base or acid

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treatment), or heat. The cross-linking agent in any of the methods described herein can be glutaraldehyde, succinimidyl acetylthioacetate (SATA), ethylene glycol disuccinate di-(N-succinimidyl) ester (EGS), isocyanate, ultra-violet light, bis-(maleimideo)-methyl ether (BMME), or carbodiimide 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide hydrochloride (EDC).

Provided herein is a kit for detecting the presence or amount of stabilized native-state protein. The kit contains: a denaturant; a cross-linking agent; an antibody specific for the protein; instructions for use in detection of stabilized native-state protein, and optionally instructions for use in measuring the presence or amount of stabilized native-state protein.

Also featured is a kit for detecting the presence or amount of stabilized native-state protein containing: a denaturant; an antibody specific for the protein that preferentially binds to native-state protein as compared to non-native-state protein; instructions for use in detection of stabilized native-state protein, and optionally instructions for use in measuring the presence or amount of stabilized native-state protein.

Any of the kits described herein can contain any of the denaturants (e.g., urea) or cross-linking agents (e.g., glutaraldehyde) described herein.

In some embodiments of any of the kits described herein, the protein can be transthyretin and the native-state of transthyretin can be tetrameric transthyretin.

"Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. The proteins used in any of the methods of the invention can contain or be wild-type proteins or can be variants that have not more than 50 (e.g., not more than one, two, three, four, five, six, seven, eight, nine, 10, 12, 15, 20, 25, 30, 35, 40, or 50) conservative amino acid substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine. All that is required is that such variants of oligomer-forming polypeptides have at least 25% (e.g., at least: 30%; 40%; 50%; 60%; 70%; 75%; 80%; 85%; 90%; 95%; 97%; 98%; 99%; 99.5%, or 100% or even greater) of the ability of the wild-type, full-length, mature oligomer-forming polypeptide from which they were derived to assemble into oligomeric forms.

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A "polypeptide fragment," "protein fragment" as used herein, refers to a segment of the polypeptide that is shorter than a full-length, unprocessed polypeptide. A "functional fragment" of a protein is a fragment of the protein that still retains the ability of the full-length, mature, wild-type protein to assume its native-state. Fragments of a polypeptide include terminal as well internal deletion variants of a polypeptide. Deletion variants can lack one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid segments (of two or more amino acids) or non-contiguous single amino acids.

As used herein, the terms "native-state" or "native-state of a protein" refer to the properly folded or assembled form of a protein. In most cases, the native-state of a protein is the operable or functional form of the protein. The native-state of the protein includes the natural or physiologic form of the protein that occurs in a wild-type cell or whole organism, the cell or whole organism being unaffected by any exogenous factors that directly or indirectly affect the stability of the polypeptide. Where the protein is a monomeric protein or exists as a monomeric form the protein (e.g., a form of the protein that includes only one polypeptide), the "native-state" of the protein indicates a properly folded form of the monomeric protein as compared to a denatured, unfolded, or improperly folded form of the monomeric protein. Where the operable or functional form of a protein is an oligomeric form of a protein (e.g., a homodimer, a homotrimer, a homotetramer, a heterodimer, a heterotrimer, or a heterotetramer), the native-state of the protein includes the oligomeric form of the protein. For example, the operable or functional physiologic form (i.e., the native-state) of transthyretin is a homo-tetrameric form of transthyretin. In some instances, a protein (e.g., certain enzymes or other non-structural proteins) can have more than one native-state where the protein operates or undergoes regulation by conformational transitioning between these one or more native-states. In addition, in some instances a protein can have an oligomeric native-state (e.g., a transthyretin tetramer) and a properly-folded monomeric native-state (e.g., a properly-folded transthyretin monomer), where the native-state monomeric form is properly folded form that can denature or misfold into a non-native-state of the native-state monomer. As used herein, a "non-native-state" of a polypeptide, as compared to the "native-state" of the protein, is a form of the protein that is

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not operable or functional or is a form of the protein that is an unfolded, denatured, or otherwise improperly-folded (e.g., an amyloid form of transthyretin or tau).

As used herein, a "trafficking-defective" protein is a protein that fails to properly transit (e.g., fails to properly fold and/or oligomerize where the protein is an oligomer-forming polypeptide) the endoplasmic reticulum. Typically a protein that fails to properly transit the endoplasmic reticulum is a protein that has misfolded during its biosynthesis or processing in the endoplasmic reticulum. Misfolding can result from one or more mutations in a protein that destabilize the native-state of the protein. Misfolding can also result from heightened or overexpression of a wild-type form (or mutant) of the protein. Other factors that may trigger protein misfolding include aberrant temperature, oxidative stress, activation of signaling pathways associated with protein folding and quality control, or lack of chaperone constituents that are necessary to help guide proper conformation of a protein.

As used herein, an "oligomer" or "oligomeric form" of a polypeptide is a form of the polypeptide that consists of two or more (e.g., two, three, four, five, six, seven, eight, nine, ten, or up to 20) monomeric forms of the polypeptide. Where the oligomeric forms of the oligomer-forming polypeptide are composed of only one oligomer-forming polypeptide, the oligomer is a homo-oligomer. Where the oligomeric form of a polypeptide is an oligomeric form composed of one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, or up to 20) monomers of at least two (e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least ten) different polypeptides, the oligomers are hetero-oligomers (e.g., heterodimers of two proteins). Oligomeric forms can include forms where the polypeptide monomers are covalently joined together (e.g., covalently joined by disulfide linkage), or can include oligomeric forms that are joined non-covalently based on protein-protein interactions (e.g., through a protein dimerization domain (e.g., the dimerization domain of a transthyretin polypeptide).

An "oligomer-forming polypeptide" refers to any polypeptide that is capable of assembling into an oligomeric form. The oligomer-forming polypeptide can be a polypeptide which naturally self-assembles into an oligomer, for example, transthyretin that self-assembles into a homo-tetramer. The oligomer-forming polypeptide can also be a polypeptide that is artificially made to oligomerize, e.g., a polypeptide subjected to certain experimental conditions, or modified in such ways, that promote a non-physiological

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assembly of the polypeptide (e.g., a fusion polypeptide of a non-oligomer-forming polypeptide and a polypeptide or fragment of an oligomer-forming polypeptide (e.g., a glutathione-S-transferase fusion with a non-oligomer-forming polypeptide)).

Transthyretin, as used herein, contains, or is: (a) a full-length, wild-type human transthyretin molecule (SEQ ID NO:1); (b) a mature, processed form of the wild-type human transthyretin molecule (SEQ ID NO:2); (c) a disease-associated or "amyloidogenic" mutant of transthyretin; (d) a functional fragment of (a), (b), or (c); or (e) any one of (a), (b), (c), or (d) with not more than 50 (see above) conservative substitutions. Disease-associated or amyloidogenic mutant variants of transthyretin include, but are not limited to, any of the amyloidogenic mutant forms of transthyretin described herein. Transthyretin can also be from any species (e.g., bird, reptile, or mammal (e.g., a mouse, rat, dog, cat, goat, pig, cow, horse, whale, or monkey)) that expresses a homolog of a human transthyretin polypeptide (SEQ ID NO:1). "Functional fragments" of a transthyretin include fragments that (a) comprise at least 20 (e.g., at least 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, or 140) contiguous amino acids of wild-type transthyretin, but are (b) shorter than the full-length transthyretin polypeptide by at least one amino acid (e.g., at least one amino acid, at least two amino acids, at least three amino acids, at least 5 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 80 amino acids, at least 90 amino acids, at least 95 amino acids, at least 100 amino acids, at least 105 amino acids, at least 110 amino acids, at least 120 amino acids, or at least 125 amino acids) and that still retain the ability to oligomerize (e.g., form tetramers).

As used herein, the term "stabilized" (in the context of the native-state of a protein such as transthyretin) refers to the effect of a stabilizing agent (e.g., a test compound or a candidate therapeutic agent) in preventing or reducing conversion of native-state of a protein to a non-native-state of the protein in the presence of a denaturant (e.g., urea). Thus, a transthyretin tetramer, for example, is said to be "stabilized" by a stabilizing agent when one or more of the transthyretin tetramers in a sample are preserved in the presence of a denaturant. Likewise, "unstabilized" (e.g., "unstabilized transthyretin tetramer") refers to native-state protein that undergoes partial or complete conversion to non-native-state protein

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in the presence of a denaturant. Unstabilized transthyretin tetramer, for example, includes transthyretin tetramer not exposed to a stabilizing agent (e.g., a negative control in an assay) or to transthyretin tetramer exposed to a test compound that does not stabilize the tetramer. Stabilization of native-state protein by a stabilizing agent can be 100% (i.e., 100% of the native-state protein is refractory to dissociation in the presence of the denaturant) or can be less than 100% (e.g., 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%). Stability can depend on both (a) the stabilizing agent (e.g., the test compound) used and (b) the limits of detection for the particular assay method.

As used herein, "insoluble aggregate" is a protein complex formed of (i) native-state protein and one or more of an antibody specific for the protein, where the antibody preferentially binds to native-state protein as compared to non-native-state protein (e.g., a transthyretin-specific antibody complexed with tetrameric transthyretin) or (ii) non-native-state protein and one or more of an antibody specific for a protein, where the antibody preferentially binds to non-native-state protein as compared to native-state protein. The binding of one or more of the antibody to native-state or non-native-state protein results in the formation of large complexes that are no longer soluble in the solution from which they were dissolved. Such insoluble aggregates can be detected in a solution using photometric methods as described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., inhibiting survival of cancer cells, will be apparent from the following description, from the drawings and from the claims.

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DESCRIPTION OF THE DRAWINGS

FIG. 1 is a line graph depicting the concentration of purified, recombinant monomeric (diamonds) and tetrameric (squares) forms of transthyretin (TTR) in solution as determined using the Olympus OSR6175 reagent. The X-axis represents the known, input concentration of the proteins and the Y-axis is the concentration determined by the immuno-turbidimetric assay. The concentrations of the proteins are given in units of milligrams (mg)/ deciliter (dL).

FIG. 2 is a line graph showing the effect of glutaraldehyde cross-linking on the detection of purified, recombinant monomeric (squares) and tetrameric forms of transthyretin (TTR) in solution as determined using the Olympus OSR6175 reagent. Samples of purified transthyretin monomer or tetramer were treated with urea buffer (final concentrations: 4.8 M urea, 25 mM sodium phosphate, 50 mM potassium chloride, pH 7.4) followed by the immediate addition of the indicated concentration of glutaraldehyde (0-40 mM final) (X-axis). The samples were analyzed using the Olympus OSR6175 reagent, and the measured concentration of the proteins are given in units of milligrams (mg)/ deciliter (dL). The input concentration of transthyretin monomer and tetramer was 10 mg/dL final.

FIG. 3 is a flow chart depicting the steps of the immuno-turbidimetric assay in the presence and absence of the test compound 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Cmp. 1). DMSO refers to dimethyl sulfoxide and is the solvent in which the compound was dissolved.

FIG. 4A is a flow chart depicting the alternative detection steps of the protein stability assay (western blot and immuno-turbidimetric assay formats).

FIG. 4B is an immunoblot depicting the stability of transthyretin tetramers in plasma samples subjected to urea treatment in the presence and absence of the test compound 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid. Plasma samples were solubilized in Laemmli buffer, subjected to sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting using antibodies specific for transthyretin. The position of transthyretin tetramer on the immunoblot is indicated at the left of the immunoblot, and the time point at which the samples were collected for assay (0 or 3 days) are indicated above the immunoblot.

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FIG. 5 is a bar graph depicting the concentration of purified, recombinant monomeric and tetrameric forms of transthyretin as determined by immuno-turbidimetric assay using Beckman reagents in the presence and absence of 4.8 M urea. The concentration of the proteins are expressed in units of mg/dL.

FIG. 6 is a line graph depicting the effect of different concentrations of 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Compound 2 (Cmp. 2)) on transthyretin tetramer stabilization using immuno-turbidimetric assay 1. The X-axis represents time and is given in days; the Y-axis represents the percentage of tetramer remaining in solution after treatment with urea. Concentrations of Cmp. 2 were 2.2 μM (circles), 4.3 μM (cross), 8.6 μM (square), or 0 μM (DMSO control, triangles) as indicated.

FIG. 7 is a line graph depicting the effect of different concentrations of 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Compound 2 (Cmp. 2)) on transthyretin tetramer stabilization using immuno-turbidimetric assay 2. The X-axis represents time and is given in hours; the Y-axis represents the percentage of tetramer remaining in solution after treatment with urea. Concentrations of Cmp. 2 were 4.6 μM (square), 9.2 μM (triangle), or 0 μM (DMSO control, diamond) as indicated.

FIG. 8 is a line graph depicting destabilization kinetics using plasma samples containing different concentrations of tetrameric transthyretin in the presence of 7.2 μM 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Compound 2 (Cmp. 2)). The X-axis represents time and is given in days; the Y-axis represents the percentage of tetramer remaining in each sample following treatment with urea. Concentrations of tetrameric transthyretin are reported as "high" (~30.1 mg/dL; diamonds), "med" (medium, ~25.4 mg/dL; squares), or "low" (~17.7 mg/dL; triangles).

FIG. 9 is a scatter plot depicting the destabilization kinetics of tetrameric transthyretin using plasma samples from patients harboring the V30M transthyretin mutation. Pooled plasma samples were treated in triplicate with Cmp. 2 (3.6 or 7.2 μM), or DMSO as a control, for 15 minutes prior to the addition of a urea buffer. This experiment was conducted on three separate days, which is indicated by the filled circles (experiment 1), open circles (experiment 2), and open diamonds (experiment 3). The X-axis represents the dose of Cmp. 2 (in μM) and the amount of time (in hours) that the samples were subjected to the urea

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buffer. The Y-axis represents the percentage of the initial ("Fraction of initial") transthyretin tetramer concentration remaining in each sample following treatment with urea.

DETAILED DESCRIPTION

Methods of Detecting Native-State Protein

Featured herein are immuno-turbidimetric methods for detecting the presence or amount of stabilized native-state protein in a sample.

The methods can be useful as a diagnostic test (e.g., a test for a biomarker) for the presence or amount of native-state protein in samples obtained from a subject. Such a method can be useful, for example when the protein is transthyretin, in identifying a subject (e.g., a human patient) as having, or at risk of developing (e.g., having a genetic predisposition to developing), any transthyretin amyloid disease described herein. Where the protein is any one of the amyloidogenic proteins described herein, the method can also be useful in identifying subjects having, or at risk of developing, Alzheimer's disease or Dutch, Flemish, or Italian cerebrovascular amyloidoses (AB protein); progressive supranuclear palsy, progressive subcortical gliosis, Pick's disease, dementia pugilisticas, and other tauopathies (Tau protein); Parkinson's Disease (alpha-synuclein); kuru or Creutzfeldt-Jakob disease (Prion proteins); Alexander Disease (GFAP); Wilson Disease (ATP7B); Lou Gehrig's Disease (SOD-1); sickle cell anemia (hemoglobin); cystic fibrosis (CFTR); diabetes (insulin); or Huntington's disease (polyglutamate) (disease-associated amyloid protein is indicated in parentheses; Howlett D.R. (2003) Curr. Med. Chem.-Immun., Endoc., & Met. Agents 3:371-383; Aridor et al. (2000) Traffic 1:836-851; Aridor et al. (2002) Traffic 3:781-790; and U.S. Patent Publication No. US2004/0166554 A1; all of which are incorporated herein by reference in their entirety). Where the protein is a protein associated with a lysosomal storage disease (e.g., alpha1-galactosidase or alpha-glucosidase), the method can also be used in identifying subjects having, or at risk of developing a lysosomal storage disease such as any of those described herein.

Where the protein of the method is contacted with a compound *in vivo*, the method can be used to evaluate the effectiveness of a compound, administered to a subject, in stabilizing the native-state (e.g., a transthyretin tetramer) of a protein (e.g., transthyretin).

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The subject can be a patient having, or at risk of developing, familial amyloid polyneuropathy, familial amyloid cardiomyopathy, senile systemic amyloidosis, cardiac amyloidosis following liver transplantation, peripheral nerve amyloidosis following liver transplantation, leptomeningeal amyloidosis, transthyretin mutant-associated carpal tunnel syndrome, vitreous deposition, or transthyretin mutant-associated skin amyloidosis or any subject having any amyloidogenic mutation in transthyretin (e.g., a subject or patient expressing any amyloidogenic transthyretin described herein (e.g., the V30M mutant form of transthyretin)). The subject can also be a patient having, or at risk of developing, a lysosomal storage disease such as any of the lysosomal storage diseases described herein. The presence or amount of the native-state of a polypeptide (as a measure of the presence or amount of insoluble aggregates) can be assayed in samples taken from a subject before and after administering to the subject a test or candidate compound. In this case, an increase in the presence or amount of stabilized native state protein present in the sample from the subject following administration of the compound as compared to the presence or amount of native-state protein in the sample obtained (i.e., taken or provided) before administration of the compound is an indication that the compound increases the stability of the native-state protein. The subject can alternatively be an animal (e.g., a test animal (e.g., a mouse, a rat, a guinea pig, a rabbit, a dog, a cat, or a monkey used in an animal model or study)) used to evaluate the efficacy, toxicity, and/or pharmacokinetics of a given compound, for example, during clinical development of a compound. A sample obtained or provided from a subject can also be contacted with the compound *in vitro*.

Where the sample is contacted with a compound *in vitro*, the method can be used in biochemical validation studies to test or confirm the mechanism of action of a compound and/or the efficacy of a compound to modulate the stability of native-state protein (e.g., to stabilize tetrameric transthyretin). For example, a compound that was first determined to be useful in treating a familial amyloidotic polyneuropathy in an animal model could be evaluated to assess its mechanism of action. The method can generally involve contacting a sample containing a protein in a buffer with the test compound. Following the denaturation step of the method, the stability of the native-state protein can be evaluated in the presence and absence of the test compound. In this case, an increase in native-state protein present in the sample contacted with the compound as compared to the amount of native-state protein in

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the absence of the compound, is an indication that the compound increases the stability of the native-state of the protein.

Alternatively, the method can be used in biochemical validation studies to test or confirm the mechanism of action of a compound and/or the efficacy of a compound to destabilize native-state protein (e.g., to destabilize the native-state of an oncogene such as ras or myc). For example, a compound that was first determined to be useful in treating one or more cancer types in animal models could be evaluated to assess its mechanism of action. In this case, a decrease in native-state protein present in the sample contacted with the compound as compared to the amount of native-state protein in the absence of the compound, is an indication that the compound decreases the stability of the native-state of the protein.

The method can also be used generally for scientific research relating to the stability of the native-state of proteins (e.g., transthyretin) or to any other research where monitoring the stability of the native-state of a protein can be useful. The protein can be a protein associated with a disease state (e.g., a human disease such as familial amyloid polyneuropathy) or a protein not directly associated with a disease state, for example, heat shock proteins of bacteria (e.g., GroEL of *E. coli*).

Examples of immuno-turbidimetric assays are described in, e.g., Liu et al. (2006) Dement. Geriatr. Cogn. Disord. 21:155-161; Bence et al. (2005) Vet. Clin. Pathol. 34(4):335-341; Ledue et al. (2002) Clin Chem Lab Med. 40(5):520-528; Soltys et al. (1998) Blood Purif. 16(3):123-134; and Luo et al. (2004) World J Gastroenterol. 10(9):1292-1296. Such methods can be used for the qualitative or quantitative measurement of drugs or biomarkers (e.g., proteins such as native-state or non-native-state proteins) in bodily fluids such as blood, serum, or plasma. The immuno-turbidimetric assays described herein are based on the preferential binding of an antibody to a native-state or to a non-native-state of a protein. The binding of the antibody to native-state protein, if present in the sample, causes rapid agglutination (aggregation) of the native-state protein and antibody leading to the formation of insoluble aggregates of the antibody and native-state protein in the sample. By linking one or more antibodies to one or more native-state proteins, the antibodies are able to bind many antigen molecules simultaneously. This greatly increases the speed and robustness of the visible reaction (i.e., the immuno-turbidity of the sample). The presence or amount of the insoluble aggregates in the sample can be determined photometrically and is proportional to

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the difference between incident light delivered to a sample versus the transmitted light emitted from the sample (i.e., the absorbance). The higher the absorbance of a sample, the more insoluble aggregates are present in the sample, which indicates that more native-state protein is present in the sample. Machines or instruments useful in detecting insoluble aggregates are described in the Examples below, and include spectrophotometers such as the Bayer Advia 1650 Chemistry Analyzer (Diamond Diagnostics, Holliston, MA), Olympus AU400, Olympus 640, Beckman Synchron CX, Abbott Aeroset, Cobas Mira, Hitachi 704, Hitachi 902, ILAB 600, and Toshiba 80FR NEO. Where the antibody preferentially binds to the non-native-state of the protein, the binding of an antibody to non-native-state protein, if present in the sample, causes rapid agglutination of the non-native-state protein and antibody leading to the formation of insoluble aggregates of the antibody and non-native-state protein in the sample.

Detection of insoluble aggregates can be a direct indication of the presence or amount of stabilized native-state protein in the sample or, where the antibody preferentially binds to non-native-state protein as compared to native-state protein, the detection of insoluble aggregates is an indirect indication of the presence or amount of stabilized native-state protein in the sample. In the latter case, the presence or amount of stabilized native-state protein is inversely proportional to the insoluble aggregates detected (i.e., the more insoluble aggregates of antibody and non-native-state protein, the less stabilized native-state protein in the sample). Methods of assessing the immuno-turbidity of a sample can be quantitative, semi-quantitative, or qualitative. An example of a quantitative determination of the amount of native-state protein is comparing the absorbance of a test sample against the absorbance value obtained from samples of known concentration and thus generating a discreet numerical calculation of the amount of native-state protein in a sample (e.g., in units of mg/dL). A baseline or background for the immuno-turbidimetric assay can be established by detecting or measuring the immuno-turbidity of samples in the absence of the compound, samples containing only the solvent or carrier in which the compound is dissolved (e.g., DMSO), and/or samples containing no protein (native-state protein). The assay can also include a sample or samples that serve as positive controls. As used herein, "positive controls" refer to test samples containing known quantities of reagents (e.g., diluents, cross-linking agents, denaturants, or compounds) and protein (e.g., a known protein concentration

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of transthyretin) that when assayed by the methods described herein will give a known or expected value. Alternatively, the immuno-turbidity of a sample can be expressed or indicated using a variety of semi-quantitative/qualitative systems known in the art. Thus, the immunoturbidity of a sample can be expressed as, for example, (a) one or more of "excellent", "good", "satisfactory", "unsatisfactory", and/or "poor"; (b) one or more of "very high", "high", "average", "low", and /or "very low"; or (c) one or more of "+++++", "++++", "+++", "++", "+", "+/-", and/or "-".

For any of the methods described herein, the sample can be, for example, any bodily or biological fluid such as blood, plasma, serum, urine, semen, lachrymal fluid, cerebrospinal fluid, or the sample can be a tissue sample. A solid tissue sample can be readily converted to a soluble or semi-soluble form for use in the methods by techniques known to those of skill in the art, including for example, maceration techniques, or passing the tissue through a filter such as cheese-cloth. When the protein, or native-state of a protein, to be tested is in a cell, the sample can also be a lysate prepared from whole cells or cell fractions (e.g., a cell membrane fraction, cell nuclear fraction, or mitochondrial fraction). The sample can optionally be prepared in vitro and contain a purified and/or recombinant protein in an aqueous buffer (optionally with certain salts (e.g., salts of magnesium, manganese, sodium, or potassium) and/or free of other constituents. A buffer can also be used to maintain the protein (e.g., the native-state protein) in a neutral or stable pH, and can include buffer chemicals such as Tris-HCl, HEPES, MOPS, or citrate. The sample can also be free of a protein (either native-state or non-native-state protein), e.g., a negative control for use in the immuno-turbidimetric assays.

Antibodies or antibody fragments that specifically bind to a native-state protein, and useful in the methods described herein, can be generated, for example, by immunization, e.g., using an animal, or by *in vitro* methods such as phage display. A polypeptide that includes all or part of a given protein can be used to generate an antibody or antibody fragment, for example, mature, full length, wild-type human transthyretin polypeptide with the following amino acid sequence:
MASHRLLLLCLAGLVFVSEAGPTGTGESKCPLMVKVLDAVRGSPAINVAVHVFRKA
ADDTWEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVV
FTANDSGPRRYTIAALLSPYSYSTTAVVTNPKE (SEQ ID NO: 1). In some

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embodiments, a portion of the protein (e.g., portion of transthyretin, e.g., with a length of 10, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 140, 141, 142, 143, 144, 145, or 146 amino acids; a mature form of transthyretin with the following amino acid sequence:
GPTGTGESKCPLMVKVLDAVRGSPAINVAVHVFRKAADDTWEPFASGKTSESGELH
GLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDSGPRRYTIAALLSPY
SYSTTAVVTNPKE (SEQ ID NO: 2)) can be used as an immunogen to generate antibodies that can be screened for reactivity to a native-state or non-native-state protein. In some embodiments, a cell expressing all or part of a protein (e.g., all or part of transthyretin) can be used as an immunogen to generate antibodies.

Methods for testing whether an antibody is specific for a given antigen (i.e., a native-state or non-native state of a protein) such as a transthyretin polypeptide include tests, for example, such as enzyme-linked immunosorbent assays (ELISAs), or immunoblotting techniques such as SDS-PAGE/western blotting, or dot-blot procedures. Specific antibodies generated to an oligomer-forming polypeptide (e.g., an antibody specific for human transthyretin) can be tested for utility in any immuno-turbidimetric assay described herein by routine experimentation and as described in Example 1. Where a positive control antibody exists, i.e., an antibody already known to preferentially bind to a native-state or non-native-state polypeptide and to work in the immuno-turbidimetric assays, the newly generated or test antibody can be used in a parallel immuno-turbidimetric assays and compared against the positive control antibody for efficacy (e.g., the ability to cause the formation of insoluble aggregates in a sample upon preferential binding to native-state or non-native state proteins). While the methods are not limited to any particular reagent, examples of anti-transthyretin antibodies useful in the methods described herein include the anti-transthyretin antibody reagent of the Olympus OSR6175 kit (Olympus, Dublin, Ireland), the anti- transthyretin antibody reagent of the Beckman Prealbumin kit (Prealbumin PAB #475106, Beckman Coulter, Fullerton, CA), and the anti-transthyretin antibody reagent of the Bayer Advia reagents (Bayer Healthcare (Diagnostics Group), Tarrytown, NY).

An antibody specific for a protein useful in the methods described herein can be an antibody that preferentially recognizes the native-state of a protein, for example, an antibody that preferentially binds to the tetrameric form of transthyretin (e.g., the anti-transthyretin

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antibody of the Beckman Prealbumin kit (Prealbumin PAB #475106, Beckman Coulter, Fullerton, CA) or the anti-transthyretin antibody of the Bayer Advia reagents). The antibody specific for a protein useful in the methods can also be an antibody that preferentially recognizes a non-native-state of a protein. Antibodies that recognize both the native-state and non-native-state of the protein, for example, an anti-transthyretin antibody that recognizes both tetrameric and monomeric transthyretin (e.g., the anti-transthyretin antibody of the Olympus OSR6175 reagent) can also be useful in the methods described herein. Methods of testing whether an antibody can preferentially bind to the native-state of a protein polypeptide, the non-native-state of a protein, or to both native-state and non-native-state proteins are described in the examples below. Briefly, samples containing only the native-state of a protein (e.g., tetrameric form of transthyretin) or the non-native-state of a protein (e.g., a transthyretin monomer) can be incubated with a candidate antibody and assayed by, for example, immuno-turbidity assay (see Example 1 for determining monomer and tetramer specificity for an anti-transthyretin antibody). Further examples of antibodies exhibiting binding preferences for native-state or non-native-state protein (as compared to the other) are well known to those of skill in the art of molecular biology. For example, numerous antibodies are useful for immunoprecipitation methods (i.e., methods where the protein in its native-state is bound by the antibody) but not for techniques (e.g., western blotting or dot-blotting) where the protein in a non-native-state (e.g., denatured by reducing agents and detergents) is bound by the antibody. Examples of such antibodies with preferences for native-state versus non-native-state protein are described by and commercially available from companies such as BD Biosciences/Pharmingen (Franklin Lakes, NJ) and Santa Cruz Biotechnologies Inc. (Santa Cruz, CA).

The methods provided herein include the step of contacting a sample with a denaturant. As used herein, "denaturant" refers to any agent that induces the conversion of the native-state of a protein to a non-native state of a protein (e.g., causing the native-state of the protein to unfold or an oligomeric, native-state protein to dissociate). Such conversion (denaturation) can, for example, result in some or all of the original properties of the folded native-state protein, especially their biological activity, being diminished or eliminated. Examples of such agents are described herein and generally include, but are not limited to, heat, alkali, or acid. Further examples include: urea, guanadinium hydrochloride,

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guanadinium sulfite, guandinium thiocyanate, sodium n-dodecyl sulfate, Nonindet-40 (NP40), n-lauryl sarcosine, or other detergents well known to those of skill in the art. The denaturant is generally applied to the sample for a length of time sufficient for the native-state protein to denature. Exemplary concentrations and time duration for denaturation using urea as the denaturant, for example, 1-10M urea (e.g., 1 M urea, 2 M urea, 3 M urea, 4 M urea, 4.8 M urea, 5 M urea, 6 M urea, 7 M urea, 8 M urea, 9 M urea, or 10 M urea) for one or more days (e.g., one day, two days, three days, four days, or five days).

Some of the methods described herein include the step of contacting the sample with a cross-linking agent. As used herein, a "cross-linking agent" is any agent that can covalently join two or more proteins together (e.g., covalently joining two or more transthyretin polypeptides) or covalently join one or more non-adjacent regions within a single protein as predicted from the primary amino acid sequence of the protein. Examples of such cross-linking agents include, for example, UV or X-ray light, chemical cross-linking agents such as glutaraldehyde, succinimidyl acetylthioacetate (SATA), ethylene glycol disuccinate di-(N-succinimidyl) ester (EGS), isocyanate, ultra-violet light, bis-(maleimideo)-methyl ether (BMME), or carbodiimide 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide hydrochloride (EDC). The cross-linking agent is generally applied to the sample for a length of time sufficient for the oligomeric forms of an oligomer-forming polypeptide to be cross-linked, or covalently joined together. Exemplary concentrations and time intervals for cross-linking with glutaraldehyde include approximately 40 mM (e.g., approximately 1 mM, approximately 10 mM, approximately 40 mM, approximately 50 mM, approximately 100 mM, approximately 500 mM, approximately 1 M, or approximately 5 M) for approximately 4 minutes (e.g., approximately 1 minute, approximately 2 minutes, approximately 3 minutes, approximately 4 minutes, approximately 5 minutes, or approximately 6 minutes).

As an alternative to the cross-linking step, the methods described herein can include a step of contacting the sample with a diluent. As used herein, a diluent is any agent that is used to dilute the concentration of any particular compound of a sample or solution, for example, diluting the concentration of a denaturant (e.g., urea) in a sample or a solution. The diluent can be any buffer compatible with the assay, e.g., any aqueous buffer. When only a few (e.g., one, two, three, or four) components of a sample are intended to be diluted, the diluent can optionally contain sufficient concentrations of the other components of the test

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sample such that the concentration of the other components are not affected by the diluent addition. For example, a diluent containing 50 mM NaCl added 1:1 to a sample containing 50 mM NaCl and 2 M urea would only dilute the urea in the sample. An exemplary dilution ratio for the immuno-turbidimetric assays is approximately 1:5 (e.g., approximately 1:2, approximately 1:3, approximately 1:4, approximately 1:5, approximately 1:6, or approximately 1:10).

Screening Assays to Identify Compounds that Modulate the Stability of Native-State Protein

Maintaining the native-state of a protein (proper assembly and folding of a protein) is a complex process and dysregulation of this process can often result in disease. Described herein are immuno-turbidimetric screening assay methods for identifying a compound that modulates the stability of native-state protein (e.g., stabilizes the native-state of a protein). Such compounds can be used to treat a variety of diseases associated with misfolded or misassembled proteins.

The methods can be useful, for example, in identifying compounds that stabilize the native-state of a transthyretin. Such identified compounds can be useful in the treatment of subjects (e.g., humans (e.g., human patients)) having, or at risk of developing, a transthyretin amyloid disease (e.g., familial amyloid polyneuropathy, familial amyloid cardiomyopathy, senile systemic amyloidosis, cardiac amyloidosis following liver transplantation, peripheral nerve amyloidosis following liver transplantation, leptomeningeal amyloidosis, transthyretin mutant-associated carpal tunnel syndrome, vitreous deposition, or transthyretin mutant-associated skin amyloidosis). Examples of compounds useful as a positive control in the screening assay to identify compounds that stabilize the tetrameric forms of transthyretin are described below, and include, for example, genistein, thyroxin (T4), and derivatives of some non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., diflunisal, diclofenac, flufenamic acid, and derivatives thereof) (Green et al. (2005) Proc. Natl. Acad. Sci. USA 102(41):14545-14550; Alves et al. (1997) Eur. J. Biochem. 249:662-668; and Almeida et al. (2005) Curr. Drug Targets CNS Neurol Disord. 4(5):587-596). Other compounds useful in the method include 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid and 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid (see the Examples below).

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The methods can also be used to identify similar "pharmacological chaperones" (i.e., compounds that stabilize the native-state of proteins) for any other proteins described herein. Pharmacological chaperones identified for the cystic fibrosis transmembrane conductance regulator protein can be useful, for example, in the treatment of cystic fibrosis. Pharmacological chaperones identified for alpha-galactosidase A, beta-glucocerebrosidase, or alpha-glucosidase can be useful in the treatment of Fabry disease, Gaucher diseases, and Pompe disease, respectively. Examples of pharmacological chaperones can be found, e.g., in Sawkar et al. (2002) Proc. Natl. Acad. Sci. USA 99(24):15428-15433; Robert et al. (2005) J Biol. Chem. 280(51):42198-42206; and Robben et al. (2006) Mol. Biol. Cell 17:379-386.

The above-described methods can be useful in identifying compounds that inhibit or reduce amyloid fibril formation by transthyretin. These compounds can be useful in the treatment of subjects (e.g., humans (e.g., human patients)) having, or at risk of developing familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy (FAC). Examples of such compounds are known to those of skill in the art and include, for example, 4'-iodo-4'deoxydoxorubicin (I-DOX) (Palha et al. (2000) Am. J. Pathol. 156:1919-1925).

Similarly, the methods can also be useful in identifying compounds that inhibit or reduce the formation of neurofibrillary tangles or filaments associated with disease-inducing amyloid forms of Alpha-Beta (AB) protein, alpha-synuclein, or Tau. Such identified compounds can be useful in the treatment of subjects (e.g., humans (e.g., human patients)) having, or at risk of developing Parkinson's disease, Alzheimer's disease or any other tauopathy (i.e., pathologies where disease-associated oligomer-forming polypeptide is a Tau polypeptide).

The screening methods can also serve to identify compounds useful in inhibiting or reducing the amyloid aggregation of prion proteins. These compounds can be useful in treating a subject having, or at risk of developing, a prion-related disease including, e.g., scrapie, transmissible mink encephalopathy, chronic wasting disease, bovine spongiform encephalopathy, feline spongiform encephalopathy, exotic ungulate encephalopathy prion, Kuru, Creutzfeldt-Jakob disease, Variant Creutzfeldt-Jakob disease, Gerstmann-Staussler-Scheinker syndrome, or fatal familial insomnia.

In instances where the native-state of a protein causes or contributes to a disease (e.g., overexpression of oncogenes like ras or myc in certain cancers), the immuno-turbidimetric

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screening assay method can be used to identify compounds that stimulate conversion of native-state protein to non-native-state protein.

The protein whose native-state causes or contributes to disease can be, for example, an oncogene. Such oncogenes include, but are not limited to, ras, myc, HER-2/neu (erB-2), hTERT, Bcl-2, src, raf, Bruton's agammaglobulinemia tyrosine kinase (BTK), platelet-derived growth factor receptor, vascular endothelial growth factor receptor, epidermal growth factor receptor, and aurora kinases. Compounds identified that destabilize the native-state of oncogenes (i.e., stimulate the conversion of native-state protein to non-native-state protein) can be useful, for example, in the treatment of cancers.

The protein whose native-state causes or contributes to disease can also be a viral protein. In this case, the method can be useful in identifying compounds that stimulate the dissociation (e.g., conversion of native-state protein to non-native-state protein) of oligomeric proteins (oligomer-forming polypeptides) of viral coats or capsids. Examples of such oligomeric proteins include, but are not limited to: the p24 capsid polypeptide of an HIV-1 virus; the VP5, VP19c, VP21, VP23,VP24, or VP26 capsid polypeptides of a Herpes Virus (e.g., HSV-1); the L1 capsid polypeptide of a Papillomaviruses (e.g., HPV-16 or HPV-18); or the VP1 capsid polypeptides of a Hepatitis viruses (e.g., Hepatitis-A virus). Compounds identified by the method can be useful in *in vitro* or *in vivo* methods of disrupting viral particle assembly or inhibiting infection of a cell by a virus. Where the oligomer-forming polypeptides are of the HIV-1 capsid (e.g., a p24 capsid protein of an HIV-1 virus), the compounds can be useful in treating a subject (e.g., a mammal, e.g., a human patient) infected, likely to become infected, or a risk of becoming infected with an HIV-1 virus.

In any of the methods described above, the protein can be contacted with the compound *in vitro* or *in vivo*. Where the protein is contacted with the compound *in vivo*, such methods can be used, for example, for screening of compounds in animal models of diseases (e.g., any of the transthyretin amyloid diseases described herein). Alternatively, such methods can be used for evaluating the efficacy of clinical candidate compounds administered to humans.

Screening assays can also be performed in any format that allows for rapid preparation, processing, and analysis of multiple reactions. This can be, for example, in

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multi-well assay plates (e.g., 96 wells or 386 wells). Stock solutions for various agents can be generated manually or robotically, and all subsequent pipetting, diluting, mixing, distribution, washing, incubating, sample readout, data collection and analysis can be done robotically using commercially available analysis software, robotics, and detection instrumentation (e.g., a spectrophotometer) capable of detecting the immuno-turbidimetric signal generated from the assay.

Additional Methods for Detecting Native-State and Non-Native-State Proteins

Additional methods for detecting (e.g., measuring) the presence or amount of either non-native-state or native-state proteins (e.g., transthyretin) are described below in the Examples. The additional methods can be used, for example, in validation studies (e.g., as secondary or confirmatory tests run parallel with the immuno-turbidimetric assays). Alternatively, the additional methods can be used to detect the native-state or non-native-state proteins, for example, from the same sample or from parallel samples used for any of the immuno-turbidimetric assays described herein. Additional methods include, for example, high-performance liquid chromatography (HPLC), western blotting techniques, and circular dichroism. The presence or amount of native-state protein or non-native state protein can be determined by analyzing the rate of its physical passage through a stationary phase matrix (e.g., HPLC or thin-layer chromatography (TLC) methodology). For the HPLC method, samples can be resuspended in an appropriate solvent (or liquid phase) and actively or passively passaged over a stationary-phase, size-exclusion matrix, which can retard (i.e., increase the retention time of), for example, the native-state of a protein on the basis of physical properties (e.g., size, hydrophobicity or charge). Larger forms of protein (e.g., native-state oligomeric forms of an oligomer-forming polypeptide) can generally pass over the matrix uninhibited, whereas smaller (e.g., non-native-state monomeric forms) of a protein can get trapped in the small pores of the matrix, effectively slowing their flow-rate over the column. The presence, amount, or relative ratio of native-state protein can be determined by measuring the retention time between the passage of the native-state protein compared to non-native-state protein over the stationary phase matrix. For more details about HPLC methodology, see, for example, Nageswara-Rao et al. J. Pharm. Biomed. Anal. 2003 Oct 15;33(3):335-77). Alternatively, following the reaction step of the procedure, the mixture

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can be resuspended in Laemmli buffer and subjected to polyacrylamide gel electrophoresis (PAGE). The PAGE can also involve a detergent, like SDS, provided that the detergent does not disrupt the stability of native-state protein. PAGE-resolved protein complexes (e.g., native-state proteins when present in a sample), separated by size can then be transferred to a filter membrane (e.g., nitrocellulose) and subjected to western blot techniques using antibodies specific for the protein (as described above). The relative amount or ratio of native-state protein, optionally in the presence or absence of a test compound, can be detected (determined) by comparison to the relative amount of the non-native-state protein. In some cases, adjustments are made to correct for reactivity differences between different forms of the protein. Examples of western-blotting variations of the methods are described in the Examples below.

Circular dichroism (CD) spectroscopy measures or detects differences in the absorption of left-handed polarized light versus right handed polarized light which arise due to structural asymmetry. The absence of regular structure results in zero CD intensity, while an ordered structure results in a spectrum which can contain both positive and negative signals. Changes in secondary structure, monitored in the far-UV CD region, can be determined with as little as 50 μg of protein, at concentrations of 0.2 mg/ml. Detection of native-state and non-native-state protein generally works best if the individual forms (i.e., native-state or non-native-state) of the protein have CD spectra which are quite different from each other, such that changes at specific wavelengths can be monitored to follow changes in the corresponding protein. In such cases, it is also possible to determine an increase in stability of native-state protein following, for example, oligomerization or denaturation of the protein (see, for example, McCann et al. (2006) J. Org. Chem. 71(16):74-98; Stephens et al. (2006) J. Nat. Prod. 69(7):1055-1064; and Oakley et al. (2006) Chirality 18(5):340-347).

Compounds

Compounds that can be screened by the methods described herein include various chemical classes and are typically small organic molecules having a molecular weight in the range of 50 to 2,500 daltons. These compounds can comprise functional groups necessary for structural interaction with proteins (e.g., hydrogen bonding), and typically include at least

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an amine, carbonyl, hydroxyl, or carboxyl group, and preferably at least two of the functional chemical groups. These compounds often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures (e.g., purine core) substituted with one or more of the above functional groups.

The compounds can also include biomolecules including, but not limited to, peptides, polypeptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives or structural analogues thereof, polynucleotides, and polynucleotide analogs.

Other examples of small molecule compounds are nucleic acid aptamers which are relatively short nucleic acid (DNA, RNA or a combination of both) sequences that bind with high avidity to a variety of proteins and inhibit the binding to such proteins of ligands, receptors, and other molecules. Aptamers are generally about 25 – 40 nucleotides in length and have molecular weights in the range of about 18 – 25 kDa. Aptamers with high specificity and affinity for targets can be obtained by an *in vitro* evolutionary process termed SELEX (systemic evolution of ligands by exponential enrichment) [see, for example, Zhang et al. (2004) Arch. Immunol. Ther. Exp. 52:307-315, the disclosure of which is incorporated herein by reference in its entirety]. For methods of enhancing the stability (by using nucleotide analogs, for example) and enhancing *in vivo* bioavailability (e.g., *in vivo* persistence in a subject's circulatory system) of nucleic acid aptamers see Zhang et al. (2004) and Brody et al. [(2000) Reviews in Molecular Biotechnology 74:5-13, the disclosure of which is incorporated herein by reference in its entirety].

Compounds can be identified from a number of potential sources, including: chemical libraries, natural product libraries, and combinatorial libraries comprised of random peptides, oligonucleotides, or organic molecules. Chemical libraries consist of a diversity of chemical structures, some of which are analogs of known compounds or analogs or compounds that have been identified as "hits" or "leads" in other drug discovery screens, while others are derived from natural products, and still others arise from non-directed synthetic organic chemistry. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms, or (2) extraction of plants or marine organisms. Natural product libraries include polypeptides, non-ribosomal peptides,

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and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed or large numbers of peptides, oligonucleotides, or organic compounds as a mixture. These libraries are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are non-peptide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997). Identification of test compounds through the use of the various libraries herein permits subsequent modification of the test compound "hit" or "lead" to optimize the capacity of the "hit" or "lead" to, for example, stabilize the native-state of a protein.

In addition, the inhibitory compounds can be antibodies, or antigen-binding antibody fragments, specific for a protein (e.g., an antibody or antigen-binding fragment specific for transthyretin). The antibody can be a purified or a recombinant antibody, or antibody fragments and chimeric antibodies and humanized antibodies made from non-human (e.g., mouse, rat, gerbil, or hamster) antibodies.

The compounds identified above can be synthesized by any chemical or biological method. The compounds identified above can also be pure, or may be in a heterologous composition (e.g., a pharmaceutical composition), and can be prepared in an assay-, physiologic-, or pharmaceutically- acceptable diluent or carrier. These compositions can also contain additional compounds or constituents which do not stabilize native-state protein, but are useful in the application of various methods described herein (e.g., a composition may contain one or more solvents, diluents, or buffers).

Exemplary compounds useful in any of the methods described herein as, for example, positive controls, are compounds known to stabilize native-state protein. Compounds that are known to stabilize tetrameric transthyretin complexes include, for example, genistein and thyroxin (T4) (Green et al. (2005) Proc. Natl. Acad. Sci. USA 102(41):14545-14550; and Alves et al. (1997) Eur. J. Biochem. 249:662-668). Other compounds useful as positive controls in methods of identifying compounds that stabilize a transthyretin tetramer include 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid and 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid, or any other transthyretin-stabilizing compound disclosed in

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U.S. Publication No. US 2004/0152140 A1, which is incorporated herein by reference in its entirety.

The following examples are intended to illustrate, not limit, the invention.

EXAMPLES

Example 1. Determining Transthyretin Tetramer Stability Using Immuno-Turbidimetric Assay 1

To assay for the ability of an anti-transthyretin antibody to detect different forms of transthyretin (monomeric or tetrameric forms of transthyretin), samples containing various concentrations [5-20 mg/dL] of either (a) tetrameric transthyretin, or (b) monomeric transthyretin (a transthyretin polypeptide containing two point mutations in the dimerization domain which prevents oligomer assembly; see Jiang et al. (2001) Biochemistry 40(38):11442-52) were incubated with a commercially available transthyretin-specific antibody, OSR6175 reagent (Olympus Life and Material Science Europa GmbH, Ireland)), and the turbidity, as a measure of the difference between incident and transmitted light, was calculated per manufacturer's instructions. OSR6175 accurately measured the known concentration of tetrameric transthyretin in each sample (Fig. 1). OSR6175 also detected the known concentration of monomeric transthyretin in each of the samples, although the correlation between known input amount and calculated amount was not 1:1 (Fig. 1).

Three day treatment of transthyretin containing samples with urea resulted in nearly complete loss of detection of transthyretin using the immuno-turbidimetric assay. In view of this finding, tetrameric transthyretin was preserved in a sample by treatment with the protein cross-linking agent glutaraldehyde. To determine the ability of OSR6175 to detect different forms of transthyretin in the presence of glutaraldehyde, samples containing 10 mg/dL of either tetrameric or monomeric transthyretin (see above) were incubated with glutaraldehyde at various final concentrations from 0 to 40 mM. The mixtures were incubated for 4 minutes at room temperature. The cross-linking reaction was stopped by the addition of 54 μL of 1.85 M freshly prepared sodium borohydride in 0.1 N sodium hydroxide, followed by a 5 minute incubation at room temperature. Tetrameric and monomeric transthyretin were

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readily detected in the samples at 0 mM glutaraldehyde (Fig. 2). However, upon treatment with glutaraldehyde, the detection of monomeric was severely attenuated, whereas tetrameric transthyretin was detectable at all concentrations of glutaraldehyde tested (Fig. 2). These results indicated that the OS6175 reagent could detect tetrameric transthyretin and distinguish tetrameric versus monomeric transthyretin, in a sample in the presence of at least up to 40 mM glutaraldehyde.

Next, the immuno-turbidity assay was evaluated in human plasma samples containing transthyretin and in the presence and absence of a test compound 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid (a transthyretin stabilizing compound, see Published U.S. Application No. 20040152140). A flow chart of the experimental method is provided in Fig. 3. A 1 mL plasma sample was incubated with 1 μL of 2-(2,6-Dichloro-phenyl)-benzoxazole-6-carboxylic acid (solubilized in dimethyl sulfoxide (DMSO), or a control solution containing only DMSO, for 15 minutes at room temperature. The final test concentration of 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Cmp. 1) was 7.2 μM. Experimental (with compound) or control (only DMSO) samples were then each combined with 1.5 mL of 8 M urea to a final urea concentration of 4.8 M. A 1 mL aliquot was removed immediately (no incubation (no-inc)) for cross-linking and the remaining 1.5 mL mixture was incubated for 3 days at room temperature (3day). Immediately for the no-inc 1 mL aliquot or following the 3 day incubation for the 3day samples, 1 mL of the samples was cross-linked by adding 16 μL of 25% (2.5 M) glutaraldehyde in water to a final concentration of 40 mM. The mixtures were incubated for 4 minutes at room temperature. The cross-linking reaction was stopped by the addition of 54 μL of 1.85 M freshly prepared sodium borohydride in 0.1 N sodium hydroxide, followed by a 5 minute incubation at room temperature. For analysis, the OSR6175 reagent was added to sample per manufacturer's instructions, and the turbidity, as a measure of the difference between incident and transmitted light, was calculated per manufacturer instruction. Three-day treatment of the samples with urea caused almost a complete loss of tetrameric transthyretin (Table 1). In contrast, pretreatment of the samples with 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid nearly completely stabilized tetrameric transthyretin. These results were compared to an alternative, western blotting method. The method was performed as described above, however, following the cross-linking step, samples were solubilized in Laemmli buffer and subjected to sodium dodecyl

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sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 4A). Size-resolved proteins were transferred from the gel to nitrocellulose and immunoblotted using antibodies specific for transthyretin. As shown in Fig. 4B, and in agreement with the OSR6175 immuno-turbidity assay, 3 day treatment of the sample with urea nearly completely abolished tetrameric transthyretin, whereas tetrameric transthyretin was almost completely stabilized in the presence of 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid.

Table 1. Effect of Cmp. 1 on Transthyretin Tetramer Stability (Immuno-turbidimetric Assay No. 1)

Glut. mM	Day	Comp. 1	Normalized (mg/dL)
40	0	-	22.7
40	3	-	1.07
40	0	+	23.5
40	3	+	21.9

Glut. is glutaraldehyde, expressed in concentration units of millimolar (mM).
Cmp. 1 is Compound 1, or 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid, and was used at a concentration of 7.2 μM. "+" indicates the presence of and "-" indicates the absence of the Compound in the Sample.
Normalized refers to a mathematical correction of the Olympus (IT) reagent determination of protein concentration, adjusting for the dilution step of the assay.

Example 2. Determining Transthyretin Tetramer Stability Using Immuno-Turbidimetric Assay 2

The first immuno-turbidity assay format (i.e., the Immuno-turbidimetric Assay 1 above) contains a cross-linking step that eliminates monomer recognition (see Fig. 2). An alternate form of the assay was developed using antibody reagents that distinguish monomeric and tetrameric forms of transthyretin. One example of such a reagent is the anti-transthyretin antibody reagent of the Beckman Prealbumin kit (Prealbumin PAB #475106, Beckman Coulter, Fullerton, CA). The selectivity of the Beckman Prealbumin kit antibody reagent was demonstrated by assaying solutions of purified recombinant tetramer or monomer as described by the manufacturer. Both preparations contained 10 mg/dL transthyretin as measured by A280. Using the non-cross-linking immuno-turbidimetric assay format, tetrameric transthyretin was detected preferentially to monomeric transthyretin; this

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selectivity was observed in the presence or absence of 4.8 M urea (Fig. 5). The selectivity of transthyretin immuno-turbidimetric reagents of the ADVIA prealbumin kit (Bayer Corporation, Diagnostics Division, Tarrytown, NY) were also tested. Solutions containing 20 mg/dL of either purified recombinant transthyretin tetramer or monomer were assayed using the protocol provided with the Bayer kit. Concentration values, determined from this kit, were 22.3 and 0.62 mg/dL, for the tetramer and monomer respectively. Thus, the Bayer reagents demonstrate strong selectively for the transthyretin tetramer, displaying a greater than 20-fold differential binding affinity for the transthyretin tetramer over monomer.

In this second assay, the cross-linking step is replaced with a step of terminating denaturation (caused by the urea treatment) by dilution of the sample. Briefly, a 1 ml sample of human plasma was combined with either 1 μl of a test compound (Cmp. 1: 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid or Cmp. 2: 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid; see Published U.S. Application No. 20040152140) solubilized in DMSO or a control solution of DMSO, and the samples were incubated for 15 minutes at room temperature prior to addition of urea. The final concentration of the test compounds was 7.2 μM in DMSO. A solution of urea was added such that the final concentration of urea in the sample was 4.8 M. A 1 mL aliquot was removed immediately (no incubation (no-inc)) for dilution (see below) and the remaining 1.5 mL mixture was incubated for 3 days at room temperature (3day) (Fig.3). Immediately (for the no-inc) or following the three day incubation (for the 3day samples), the samples were diluted 1:5 (four volumes diluent were added per one volume equivalent of sample). Potassium chloride (KCl) and sodium phosphate (NaPO4) were also added to the samples at final concentrations of 50 mM and 25 mM, respectively.

As shown in Table 2, and consistent with the previous immuno-tubidimetric assay format, incubation of the plasma samples with urea for 3 days results in near complete loss of tetrameric transthyretin (7.3 mg/dL at day 0 to ~0.8 mg/dL at day 3). However, in the presence of 7.2 μM Cmp. 1, the transthyretin tetramer was almost completely stabilized. Similarly, Cmp. 2 significantly stabilized the transthyretin tetramer in two duplicate experiments (Table 2).

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Table 2. Effect of Test Compounds on Transthyretin Stability (Immuno-turbidimetric Assay No.2)

Comp.	Comp. μM	Day	Olympus Normalized (mg/dL)	Beckman Normalized (mg/dL)
0	0	0	19.45	10.53
0	0	3	2.00	4.13
Cmp. 1	7.2	0	22.12	10.53
Cmp. 1	7.2	3	23.45	12.26
Plasma	0	0	24.7	27.3
Cmp. 2	7.2	0	22.12	
Cmp. 2	7.2	3	13.59	
Cmp. 2	7.2	0	23.72	
Cmp. 2	7.2	3	13.59	

Cmp. 1 is Compound 1, or 2-(2,6-dichloro-phenyl)-benzoxazole-6-carboxylic acid, and was used at a concentration of 7.2 μM.
Cmp. 2 is Compound 2, or 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid, and was used at a concentration of 7.2 μM.
Normalized refers to a mathematical correction of the determination of protein concentration, adjusting for the dilution step of the assay

Example 3. Dose-Dependent Stabilization of Transthyretin

To test the effect of different dosages of 2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid (Cmp. 2) on transthyretin stabilization, plasma samples (a pool from 20 individual donors) containing 4.3 μM transthyretin were treated with 2.2 μM, 4.3 μm, or 8.6 μm Cmp. 2, or DMSO as a negative control, for 15 minutes at room temperature. Following the incubation, the urea denaturant was added to the samples resulting in a final urea concentration of 4.8 M (see Fig. 6). As above, aliquots were removed immediately (day 0) and after incubation at room temperature for 1, 2, and 3 days. Cross-linking (glutaraldehyde) and detection of transthyretin using the Olympus OSR6175 reagent were performed as described under Example 1. For each concentration of Cmp. 2, the transthyretin level at days 1, 2, and 3 was divided by the initial concentration at day 0 to yield the percent of

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tetramer remaining (Fig. 6). These experiments indicated that the higher concentrations of Cmp. 2 (8.6 μM Cmp 2, squares of Fig. 6) were more effective than lower concentrations of Cmp. 2 (2.2 μM Cmp. 2, circles of Fig. 6) at stabilizing tetrameric transthyretin (Fig. 6).

Dose-dependent stabilization of transthyretin by Cmp. 2 was also monitored using immuno-turbidimetric assay 2 (Example 2). Plasma samples (a pool from 16 individual donors) containing 4.6 μM transthyretin were treated with 4.6 μm, or 9.2 μm Cmp. 2, or DMSO as a negative control, for 15 minutes at room temperature. Following the incubation, urea was added to the samples resulting in a final urea concentration of 3.2 M (see Fig. 7). Aliquots were removed immediately (day 0) and after incubation at room temperature for 8, 13, 24, 32, and 48 hours. Immediate detection of transthyretin using the Bayer ADVIA prealbumin kit was performed as described by the manufacturer. For each concentration of Cmp. 2, the assay-determined transthyretin concentration at 8, 13, 24, 32, and 48 hours was divided by the assay-determined initial concentration at day 0 to yield the percent of tetramer remaining (Fig. 7). Higher concentrations of Cmp. 2 were more effective than lower concentrations of Cmp. 2 at stabilizing tetrameric transthyretin (Fig. 7).

Example 4. Efficacy of Compound 2 Against Different Sample Concentrations of Transthyretin

Since the concentration of transthyretin fluctuates from day to day in an individual subject and can vary between different subjects (e.g., different patients), the efficacy of Cmp. 2 to stabilize tetrameric transthyretin was tested for different concentrations of transthyretin. Three plasma samples containing 17.7 mg/dL transthyretin (a pool of two individuals; designated as "low"), 25.4 mg/dL transthyretin (a pool of 16 individuals; designated as "medium"), or 30.1 mg/dL transthyretin (a pool of two individuals; designated as "high") were pre-treated with 7.2 μM Cmp. 2 or DMSO for 15 minutes at room temperature. Samples were taken either immediately (day 0) or at 1 day intervals of 1, 2, or 3 days. Samples were then subjected to denaturation and cross-linking, and stabilized tetrameric transthyretin was measured as described above in Examples 1 and 3. In the presence and absence of Cmp. 2, and at day 1, 2, and 3, the tetrameric transthyretin concentration at days 1, 2, and 3 was divided by the initial concentration at day 0 to yield the percent of tetramer remaining (Fig. 8). These experiments showed not only that 7.2 mM Cmp. 2 was

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equivalently effective on different concentrations of transthyretin, but also that the kinetics of tetrameric transthyretin stabilization in the presence of 7.2 μM Cmp. 2 were equivalent (Fig. 8).

Example 5. Efficacy of Compound 2 Against Samples from Patients with Mutant TTR (V30M)

The ability of Cmp. 2 to stabilize tetrameric transthyretin in plasma samples from familial amyloid polyneuropathy patients harboring the V30M transthyretin mutation was tested. Aliquots of pooled plasma samples (from nine individuals) containing a final concentration of 22 mg/dL of transthyretin were first treated with 3.6 μM or 7.2 μM of Cmp. 2 or with DMSO for 15 minutes at room temperature. The samples were then subjected to denaturation for 24, 31 or 36, or 48 hours and then cross-linked (as above). Stabilized tetrameric transthyretin was measured as described above in Examples 1 and 3. In the presence and absence of Cmp. 2, the tetrameric transthyretin concentration at 24, 31 or 36, or 48 hours was divided by the initial concentration present in the untreated samples to yield the percent of tetramer remaining (Fig. 9). These experiments demonstrated that tetrameric transthyretin in plasma samples from patients harboring a V30M transthyretin mutation is stabilized in a dose-dependent manner by Cmp. 2.

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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What is claimed is:

1. A method of detecting the presence or amount of a stabilized native-state protein in a sample, the method comprising:

contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a);

contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the protein, resulting in mixture (b);

contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and

detecting the presence or amount of insoluble aggregates in mixture (c), wherein detection of insoluble aggregates indicates directly or indirectly the presence or amount of stabilized native-state protein in the sample.

2. A method of detecting the presence or amount of a stabilized native-state protein in a sample, the method comprising:

contacting a sample comprising a protein with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a);

contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state

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protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and

detecting the presence or amount of insoluble aggregates in mixture (b), wherein detection of insoluble aggregates indicates directly or indirectly the presence or amount of stabilized native-state protein in the sample.

3. The method of claim 1 or 2, wherein detection of insoluble aggregates is a direct indication of the presence or amount of stabilized native-state protein in the sample.

4. The method of claim 1 or 2, wherein detection of insoluble aggregates is an indirect indication of the presence or amount of stabilized native-state protein in the sample, the presence or amount of stabilized native-state protein in the sample being inversely proportional to the insoluble aggregates detected.

5. The method of any of claims 2-4, further comprising contacting mixture (a) with a diluent prior to contacting mixture (a) with the antibody.

6. The method of any of claims 1-5, wherein the sample further comprises a candidate compound.

7. A method of identifying a compound that stabilizes the native-state of a protein, the method comprising:

contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a);

contacting mixture (a) with a cross-linking agent for a time sufficient to cross-link the protein, resulting in mixture (b);

contacting mixture (b) with an antibody that specifically binds to the protein to form mixture (c), wherein (i) the antibody preferentially binds to cross-linked native-state protein as compared to cross-linked non-native-state protein and binding of the antibody to cross-linked native-state protein results in the formation of insoluble aggregates of the antibody

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and cross-linked native-state protein, or (ii) the antibody preferentially binds to cross-linked non-native-state protein as compared to cross-linked native-state protein and binding of the antibody to cross-linked non-native-state protein results in the formation of insoluble aggregates of the antibody and cross-linked non-native-state protein; and

detecting the presence or amount of insoluble aggregates in mixture (c), wherein (i) an increase in the formation of insoluble aggregates of the antibody and cross-linked native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native-state of the protein, or (ii) a decrease in the formation of insoluble aggregates of the antibody and cross-linked non-native state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native-state of the protein.

8. A method of identifying a compound that stabilizes the native-state of a protein, the method comprising:

contacting a sample comprising a protein and a candidate compound with a denaturant for a time sufficient to induce conversion of unstabilized native-state protein to a non-native-state of the protein, resulting in mixture (a);

contacting mixture (a) with an antibody that specifically binds to the protein to form mixture (b), wherein (i) the antibody preferentially binds to native-state protein as compared to non-native-state protein and binding of the antibody to native-state protein results in the formation of insoluble aggregates of the antibody and native-state protein, or (ii) the antibody preferentially binds non-native state protein and binding of the antibody to non-native-state protein results in the formation of insoluble aggregates of the antibody and non-native-state protein; and

detecting the presence or amount of insoluble aggregates in mixture (b), wherein (i) an increase in the formation of insoluble aggregates of the antibody and native-state protein in the presence of the candidate compound as compared to in the absence of the candidate compound indicates that the candidate compound stabilizes the native state of the protein, or (ii) a decrease in the formation of insoluble aggregates of the antibody and non-native-state protein in the presence of the candidate compound as compared to in the absence of the

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candidate compound indicates that the candidate compound stabilizes the native state of the protein.

9. The method of claim 8, further comprising contacting mixture (a) with a diluent prior to contacting mixture (a) with the antibody.

10. The method of any of claims 1-9, further comprising measuring the amount of insoluble aggregates.

11. The method of any of claims 7-10, wherein the protein is contacted with the candidate compound *in vitro*.

12. The method of any of claims 7-10, wherein the protein is contacted with the candidate compound *in vivo*.

13. The method of any of claims 1-12, wherein the protein is transthyretin.

14. The method of claim 13, wherein transthyretin is wild-type, mature transthyretin.

15. The method of claim 13, wherein transthyretin is an amyloidogenic form of transthyretin.

16. The method of claim 15, wherein the amyloidogenic form of transthyretin is the V30M mutant form of transthyretin.

17. The method of claim 15, wherein amyloidogenic form of transthyretin is the V122I mutant form of transthyretin, the Y78F mutant form of transthyretin, the L55P mutant form of transthyretin, the L55Q mutant form of transthyretin, the A25T mutant form of transthyretin, the C10R mutant form of transthyretin, the L12P mutant form of transthyretin, the D18E mutant form of transthyretin, the D18G mutant form of transthyretin, the V20I mutant form of transthyretin, the S23N mutant form of transthyretin, the P24S mutant form

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of transthyretin, the V28M mutant form of transthyretin, the V30A mutant form of transthyretin, the V30L mutant form of transthyretin, the V30L mutant form of transthyretin, the V30G mutant form of transthyretin, the F33I mutant form of transthyretin, the F33L mutant form of transthyretin, the F33V mutant form of transthyretin, the R34T mutant form of transthyretin, the L35N mutant form of transthyretin, the A36P mutant form of transthyretin, the D38A mutant form of transthyretin, the E42G mutant form of transthyretin, the E42D mutant form of transthyretin, the F44S mutant form of transthyretin, the A45D mutant form of transthyretin, the A45S mutant form of transthyretin, the A45T mutant form of transthyretin, the G47R mutant form of transthyretin, the G47A mutant form of transthyretin, the G47V mutant form of transthyretin, the G47E mutant form of transthyretin, the T49A mutant form of transthyretin, the T49I mutant form of transthyretin, the S50R mutant form of transthyretin, the S50I mutant form of transthyretin, the E51G mutant form of transthyretin, the S52P mutant form of transthyretin, the G53E mutant form of transthyretin, the E54G mutant form of transthyretin, the E54K mutant form of transthyretin, the L55R mutant form of transthyretin, the H56R mutant form of transthyretin, the L58H mutant form of transthyretin, the L58R mutant form of transthyretin, the T59K mutant form of transthyretin, the T60A mutant form of transthyretin, the E61K mutant form of transthyretin, the F64L mutant form of transthyretin, the F64S mutant form of transthyretin, the I68L mutant form of transthyretin, the Y69H mutant form of transthyretin, the K70N mutant form of transthyretin, the V71A mutant form of transthyretin, the I73V mutant form of transthyretin, the S77F mutant form of transthyretin, the S77Y mutant form of transthyretin, the I84S mutant form of transthyretin, the I84N mutant form of transthyretin, the I84T mutant form of transthyretin, the E89Q mutant form of transthyretin, the E89K mutant form of transthyretin, the A91S mutant form of transthyretin, the A97G mutant form of transthyretin, the A97S mutant form of transthyretin, the I107V form of transthyretin, the I107M mutant form of transthyretin, the A109S mutant form of transthyretin, the L111M mutant form of transthyretin, S112I mutant form of transthyretin, the Y114C mutant form of transthyretin, the Y114H mutant form of transthyretin, the Y116S mutant form of transthyretin, the A120S mutant form of transthyretin, the Val122 deletion mutant form of transthyretin, or the V122A mutant form of transthyretin.

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18. The method of any of claims 13-17, wherein the native-state of transthyretin is a tetramer.

19. The method of any of claims 1-12, wherein the protein is an amyloidogenic protein.

20. The method of claim 19, wherein the amyloidogenic protein is selected from the group consisting of beta-amyloid, alpha-synuclein, tau, an immunoglobulin light chain, serum amyloid A protein, serum amyloid P protein, apoA-I, beta2-microglobulin, gelsolin, a lysozyme, insulin, fibrinogen, a prion protein, superoxide dismutase, calcitonin, cystatin C, and atrial natriuretic peptide.

21. The method of any of claims 1-12, wherein the protein is a trafficking-defective protein.

22. The method of claim 21, wherein the trafficking-defective protein is the ΔF508 mutant form of cystic fibrosis transmembrane conductance regulator protein.

23. The method of claim 21, wherein the trafficking-defective protein is alpha-galactosidase A, beta-glucocerebrosidase, or alpha-glucosidase.

24. The method of claim 21, wherein the trafficking-defective protein is gonadotropin-releasing hormone receptor, water-channel aquaporin-2, alpha1-antitrypsin, alpha1-antitrypsin variant, alpha-subunit of hNav1.5, nephrin, multi-drug resistance protein 2, the PHEX gene product, pendrin, sulfonylurea receptor 1, AE1, ceruloplasmin, palmitoyl protein thioesterase 1, cartilage oligomeric matrix protein, the ELOVL4 gene product, aspartyl-glucosaminidase, connexin 32, rhodopsin, cystic fibrosis transmembrane conductance regulator protein, HFE, tyrosinase, protein C, complement C1 inhibitor, alpha-D-galactosidase, beta-hexosaminidase, sucrase-isomaltase, UDP-glucuronosyl-transferase, insulin receptor, growth hormone receptor, myeloperoxidase, preproparathyroid hormone, Human Ether-a-go-go-Related gene, tyroxine binding globulin, lipoprotein lipase, low-

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density lipoprotein receptor, microsomal triglyceride transfer protein, apolipoprotein a, thyroglobulin, type I pro-collagen, fibrinogen, alpha 1-antichymotrypsin, phosphoinositide-dependent kinase 1, phosphoinositide-dependent kinase 2, vasopressin precursor protein prepro-vasopressin neurophysin II, peripheral myelin protein 22, proteolipid protein, presenilin, von Willebrand factor, ERGIC-53/p58, or sedlin.

25. The method of any of claims 1-12, wherein the protein is an oligomer-forming polypeptide.

26. The method of claim 25, wherein the oligomer-forming polypeptide is selected from the group consisting of Alpha-Beta protein, superoxide dismutase, Abri/ADan, glial fibrillary acidic protein, ATP7B, hemoglobin, amyloid A, beta-2-microglobulin, custatin C, lysozyme, fibrinogen, AH and AL immunoglobulin proteins, ApoAI, ApoAII, gelsolin, lactoferrin, lactohedrin, survivin, EGF-R, Erb-B2, and IL-12.

27. The method of any of claims 1-26, wherein the sample is obtained from a mammal.

28. The method of claim 27, wherein the mammal is a human.

29. The method of claim 28, wherein the human has, or is at risk of developing, a transthyretin amyloid disease.

30. The method of claim 29, wherein the transthyretin amyloid disease is familial amyloid polyneuropathy, familial amyloid cardiomyopathy, senile systemic amyloidosis, cardiac amyloidosis following liver transplantation, peripheral nerve amyloidosis following liver transplantation, leptomeningeal amyloidosis, transthyretin mutant-associated carpal tunnel syndrome, vitreous deposition, or transthyretin mutant-associated skin amyloidosis.

31. The method of claim 28, wherein the human has, or is at risk of developing, a disorder selected from the group consisting of Alzheimer's disease, Dutch cerebrovascular

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amyloidosis, Flemish cerebrovascular amyloidosis, Italian cerebrovascular amyloidosis, progressive supranuclear palsy, progressive subcortical gliosis, Pick's disease, dementia pugilisticas, Parkinson's Disease, kuru, Creutzfeldt-Jakob disease, Alexander Disease, Wilson Disease, Lou Gehrig's Disease, sickle cell anemia, cystic fibrosis, diabetes, and Huntington's disease.

32. The method of claim 28, wherein the human has, or is at risk of developing, a lysosomal storage disease.

33. The method of claim 32, wherein the lysosomal storage disease is Fabry disease, Gaucher disease, Pompe disease, Hurler-Scheie syndrome, MPS type I, GM1gangliosidosis, galactosialidosis, Morquio syndrome B, MPS type IVB, Sandhoff disease, Tay-Sachs disease, beta-mannosidosis, alpha-L-fucosidosis, Maroteaux-Lamy syndrome, MPS type VI, metachromatic leukodystrophy, Schindler disease, aspartylglycosaminuria, Hunter syndrome, MPS type II, Sanfilippo syndrome A, MPS type IIIA, Sanfilippo syndrome B, MPS type IIIB, Sanfillipo syndrome C, MPS type IIIC, Sanfilippo syndrome D, MPS type IID, Morquio syndrome A, MPS type IVA, Sly syndrome, MPS type VII, hyaluronidase deficiency, MPS type IX, multiple sulfatase deficiency, alpha-mannosidosis, sialidosis, X-linked ictiosis and multiple sulfatase deficiency, mucolipidodisis II, mucolipidosis III, Wolman disease, Farber disease, Niemann-Pick disease A, Niemann-Pick disease B, glycogenosis type II, neuronal ceroid lipofucsinosis infantile type, neuronal ceroid lipofucsinosis late infantile type, neuronal ceroid lipofucsinosis juvenile type, Krabbe disease, lysosomal acid phosphatase deficiency, pycnodysostosis, cystinosis, sialic acid storage disease, cobalamin deficiency type F, Niemann-Pick disease type C, galactosialidosis, metachromatic leukodystrophy variant, Gaucher disease variant, Tay-Sachs disease type AB, or glycogen storage disease.

34. The method of claim 33, wherein the lysosomal storage disease is Gaucher disease type 1, Gaucher disease type 2, or Gaucher disease type 3.

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35. The method of any of claims 1-34, wherein the sample comprises blood, plasma, or serum.

36. The method of any of claims 1-34, wherein the sample comprises urine, semen, lachrymal fluid, cerebrospinal fluid, vitreous humor, or a tissue sample.

37. The method of any of claims 1-36, wherein the denaturant is urea.

38. The method of any of claims 1-36, wherein the denaturant is guanadinium hydrochloride, guanadinium sulfite, guanadinium thiocyanate, sodium n-dodecyl sulfate, Nonindet-40 (NP40), or n-lauryl sarcosine.

39. The method of any of claims 1-36, wherein the denaturant is high or low pH.

40. The method of any of claims 1-36, wherein the denaturant is heat.

41. The method of any of claims 1, 3-7, or 9-40, wherein the cross-linking agent is glutaraldehyde.

42. The method of any of claims 1, 3-7, or 9-40, wherein the cross-linking agent is succinimidyl acetylthioacetate (SATA), ethylene glycol disuccinate di-(N-succinimidyl) ester (EGS), isocyanate, ultra-violet light, bis-(maleimideo)-methyl ether (BMME), or carbodiimide 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide hydrochloride (EDC).

43. A kit for detecting the presence or amount of stabilized tetrameric transthyretin, comprising:

a denaturant;

a cross-linking agent;

a transthyretin-specific antibody; and

instructions for use in detection of stabilized tetrameric transthyretin.

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44. A kit for detecting the presence or amount of stabilized tetrameric transthyretin, comprising:

a denaturant;

a transthyretin-specific antibody that preferentially binds to tetrameric transthyretin as compared to monomeric transthyretin; and

instructions for use in detection of stabilized tetrameric transthyretin.

45. The kit of claim 43 or 44, wherein the denaturant is urea.

46. The kit of claim 43 or 45, wherein the cross-linking agent is glutaraldehyde.

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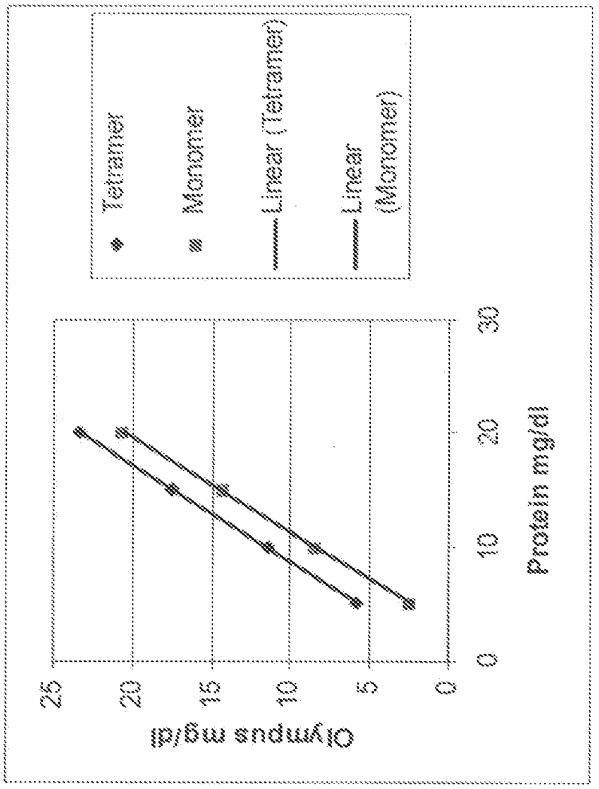

Tetramer
Monomer
Linear (Tetramer)
Linear (Monomer)
Olympus mg/dl
25
20
15
10
5
0
0
10
20
30
Protein mg/dl

FIG. 1

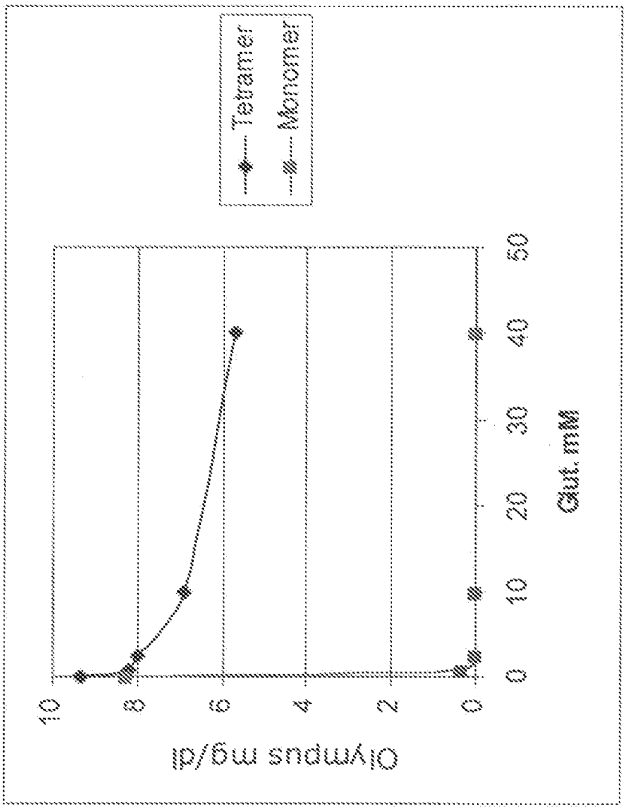


FIG. 2

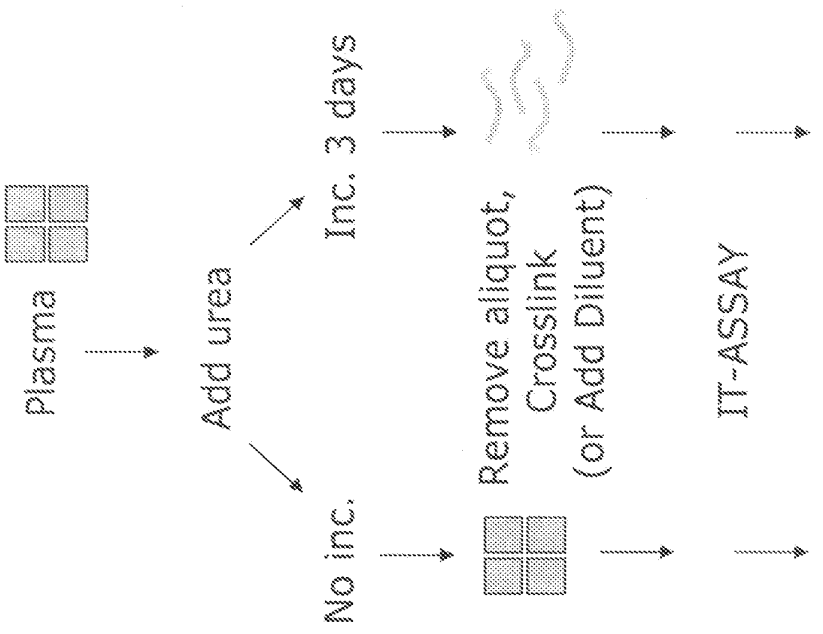

Plasma
Add urea
Inc. 3 days
No inc.
Remove aliquot,
Crosslink
(or Add Diluent)
IT-ASSAY

FIG. 3

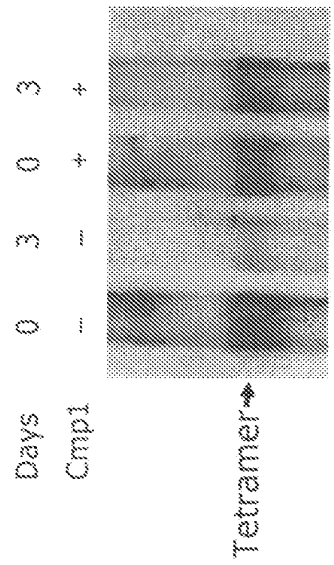

Days 0 3 0 3
Cmp1 - - + +
Tetramer

FIG. 4B

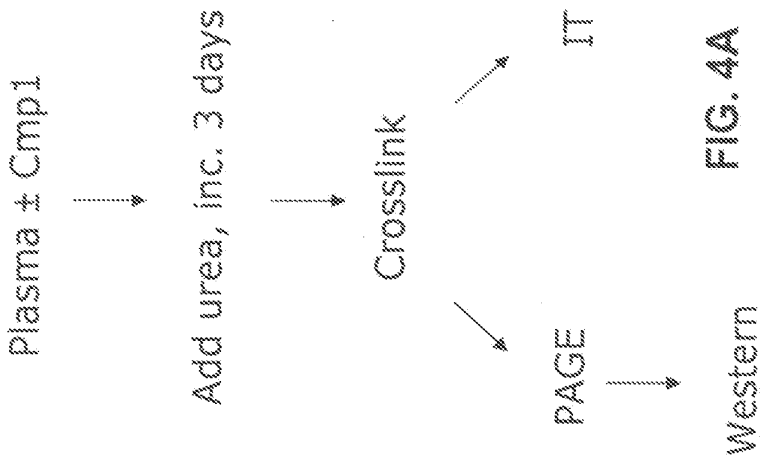

Plasma ± Cmp1
Add urea, inc. 3 days
Crosslink
IT
PAGE
Western

FIG. 4A

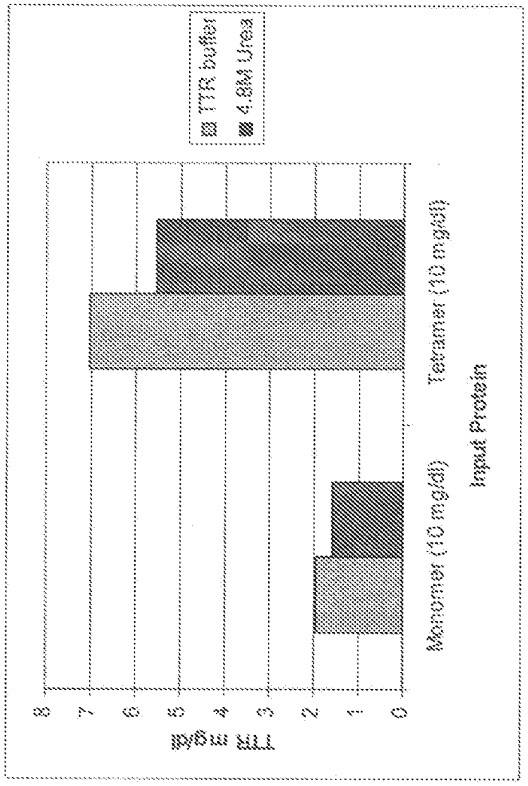

TTR buffer
4.8M Urea
TTR mg/dl
0
1
2
3
4
5
6
7
8
Monomer (10 mg/dl)
Tetramer (10 mg/dl)
Input Protein

FIG. 5

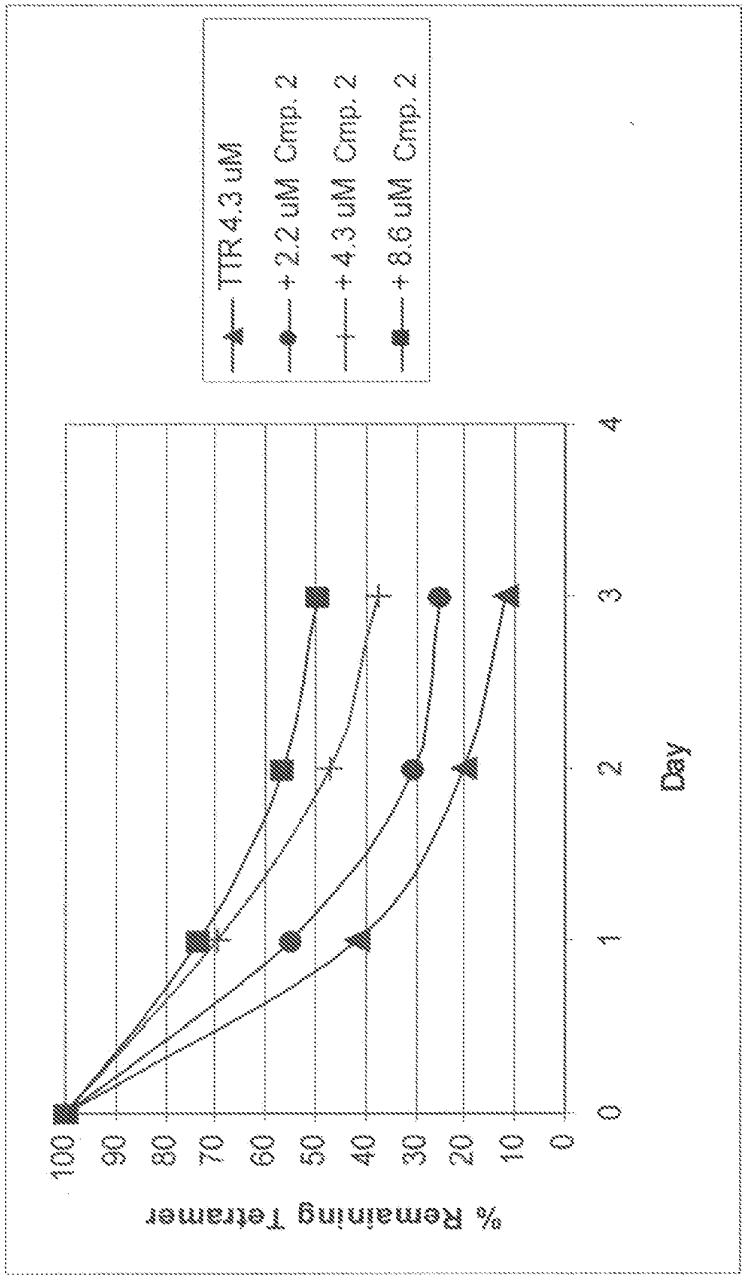

TTR 4.3 uM
+ 2.2 uM Cmp. 2
+ 4.3 uM Cmp. 2
+ 8.6 uM Cmp. 2
% Remaining Tetramer
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
Day

FIG. 6

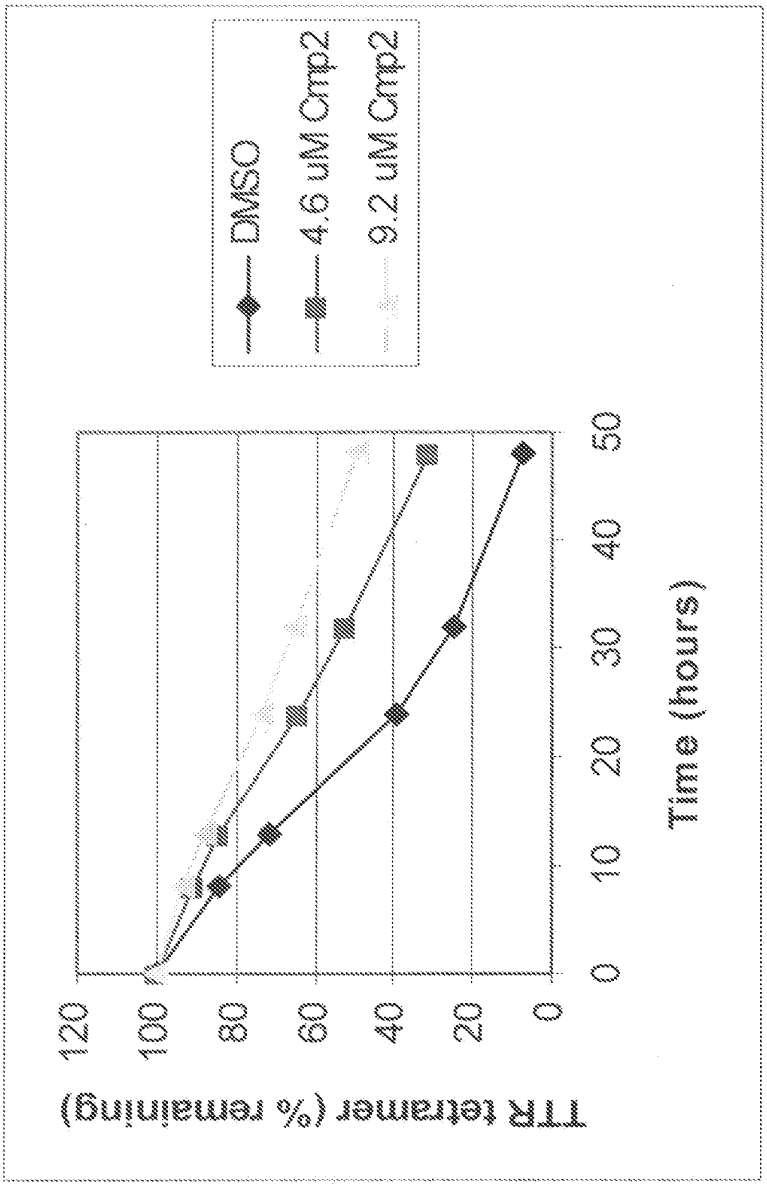

DMSO
4.6 uM Cmp2
9.2 uM Cmp2
TTR tetramer (% remaining)
120
100
80
60
40
20
0
0
10
20
30
40
50
Time (hours)

FIG. 7

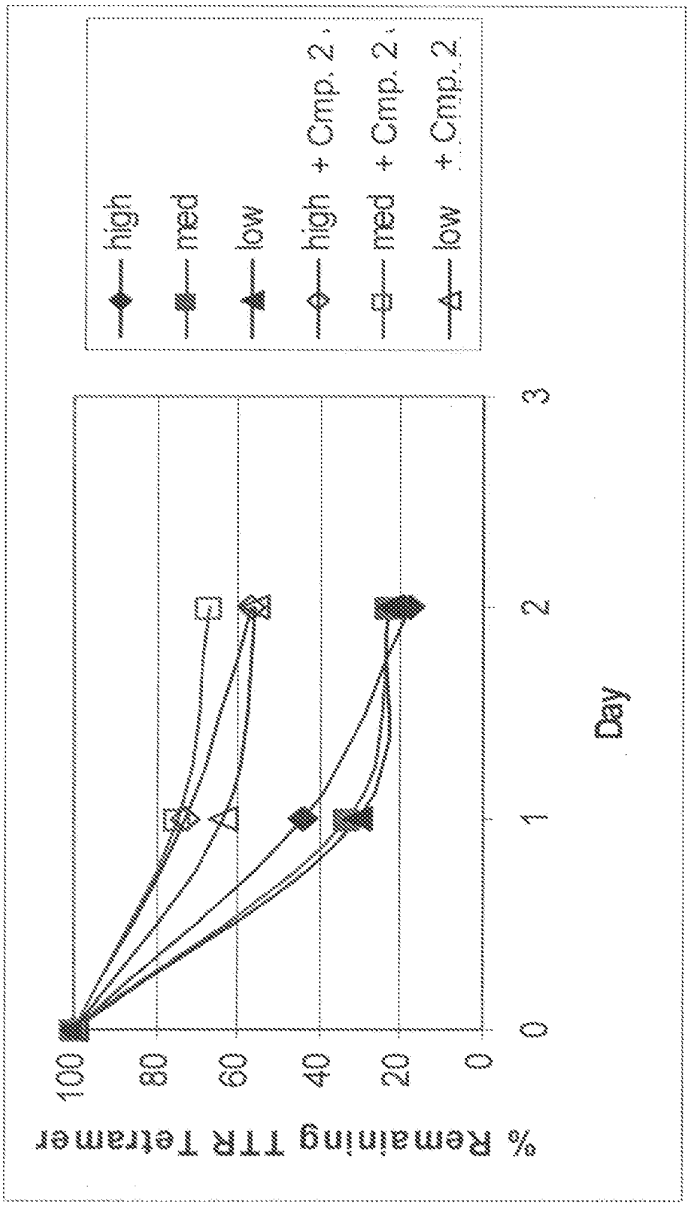

high
med
low
high + Cmp. 2
med + Cmp. 2
low + Cmp. 2
% Remaining TTR Tetramer
100
80
60
40
20
0
0
1
2
3
Day

FIG. 8

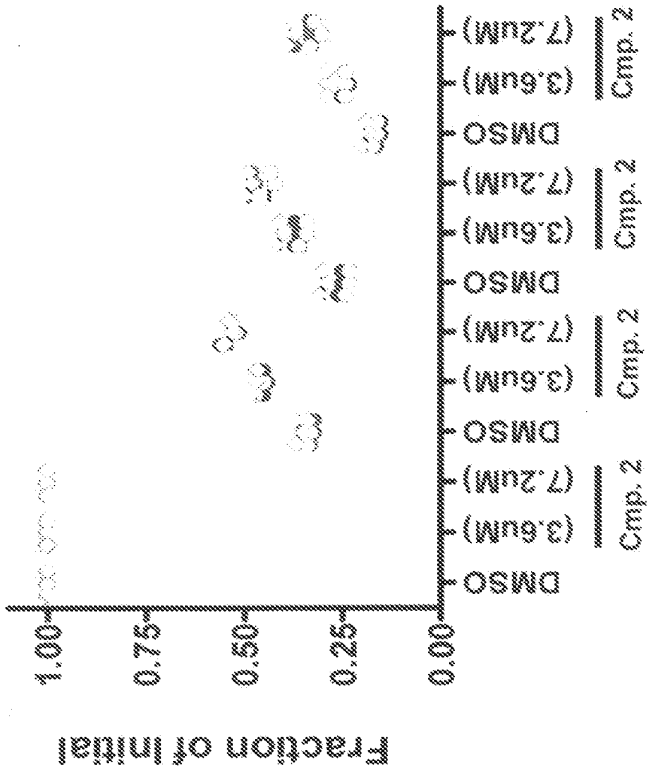

Fraction of Initial
1.00
0.75
0.50
0.25
0.00
DMSO
Cmp. 2 (3.6uM)
Cmp. 2 (7.2uM)
DMSO
Cmp. 2 (3.6uM)
Cmp. 2 (7.2uM)
DMSO
Cmp. 2 (3.6uM)
Cmp. 2 (7.2uM)
DMSO
0hr
24hr
36/31hr
48hr

FIG. 9

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(54) Title: ANTIBODIES

(57) Abstract: The present invention is related to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of disorders and abnormalities associated with amyloid protein such as Alzheimer's disease. The present invention provides novel methods and compositions comprising highly specific and highly effective antibodies having the ability to specifically recognize and bind to specific epitopes from a range of j3-amyloid proteins. The antibodies enabled by the teaching of the present invention are particularly useful for the treatment of diseases and disorders which are caused by or associated with amyloid or amyloid- like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD).

ANTIBODIES

The present invention is related to methods and compositions for diagnosis and treatment of amyloidosis, a group of disorders and abnormalities associated with amyloid protein such as Alzheimer's disease.

Amyloidosis is not a single disease entity but rather a diverse group of progressive disease processes characterized by extracellular tissue deposits of a waxy, starch-like protein called amyloid, which accumulates in one or more organs or body systems. As the amyloid deposits accumulate, they begin to interfere with the normal function of the organ or body system. There are at least 15 different types of amyloidosis. The major forms are primary amyloidosis without known antecedent, secondary amyloidosis following some other condition, and hereditary amyloidosis.

Secondary amyloidosis occurs during chronic infection or inflammatory disease, such as tuberculosis, a bacterial infection called familial Mediterranean fever, bone infections (osteomyelitis), rheumatoid arthritis, inflammation of the small intestine (granulomatous ileitis), Hodgkin's disease, and leprosy.

Amyloid deposits include amyloid P (pentagonal) component (AP), a glycoprotein related to normal serum amyloid P (SAP), and sulphated glycosaminoglycans (GAG), complex carbohydrates of connective tissue. Amyloid protein fibrils, which account for about 90% of the amyloid material, comprise one of several different types of proteins. These proteins are capable of folding into so-called "beta-pleated" sheet fibrils, a unique protein configuration which exhibits binding sites for Congo red resulting in the unique staining properties of the amyloid protein.

Many diseases of aging are based on or associated with amyloid-like proteins and are characterized, in part, by the buildup of extracellular deposits of amyloid or amyloid-like material that contribute to the pathogenesis, as well as the progression of the disease. These diseases include, but are not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity

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such as, for example, mild cognitive impairment (MCI), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex. Other diseases which are based on or associated with amyloid-like proteins are progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

Although pathogenesis of these diseases may be diverse, their characteristic deposits often contain many shared molecular constituents. To a significant degree, this may be attributable to the local activation of pro-inflammatory pathways thereby leading to the concurrent deposition of activated complement components, acute phase reactants, immune modulators, and other inflammatory mediators (McGeer et al., 1994).

Alzheimer's Disease (AD) is a neurological disorder primarily thought to be caused by amyloid plaques, an accumulation of abnormal deposit of proteins in the brain. The most frequent type of amyloid found in the brain of affected individuals is composed primarily of Aβ fibrils. Scientific evidence demonstrates that an increase in the production and accumulation of beta-amyloid protein in plaques leads to nerve cell death, which contributes to the development and progression of AD. Loss of nerve cells in strategic brain areas, in turn, causes reduction in the neurotransmitters and impairment of memory. The proteins principally responsible for the plaque build up include amyloid precursor protein (APP) and two presenilins (presenilin I and presenilin II). Sequential cleavage of the amyloid precursor protein (APP), which is constitutively expressed and catabolized in most cells, by the enzymes β and γ secretase leads to the release of a 39 to 43 amino acid Aβ peptide. The degradation of APPs likely increases their propensity to aggregate in plaques. It is especially the Aβ(1-42) fragment that has a high propensity of building aggregates due to two very hydrophobic amino acid residues at its C-terminus. The Aβ(1-42) fragment is therefore believed to be mainly involved and responsible for the initiation of neuritic plaque formation in AD and to have, therefore, a high pathological potential. There is therefore a need for agents to prevent the formation of amyloid plaques and to diffuse existing plaques in AD.

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The symptoms of AD manifest slowly and the first symptom may only be mild forgetfulness. In this stage, individuals may forget recent events, activities, the names of familiar people or things and may not be able to solve simple math problems. As the disease progresses, symptoms are more easily noticed and become serious enough to cause people with AD or their family members to seek medical help. Mid-stage symptoms of AD include forgetting how to do simple tasks such as grooming, and problems develop with speaking, understanding, reading, or writing. Later stage AD patients may become anxious or aggressive, may wander away from home and ultimately need total care.

Presently, the only definite way to diagnose AD is to identify plaques and tangles in brain tissue in an autopsy after death of the individual. Therefore, doctors can only make a diagnosis of "possible" or "probable" AD while the person is still alive. Using current methods, physicians can diagnose AD correctly up to 90 percent of the time using several tools to diagnose "probable" AD. Physicians ask questions about the person's general health, past medical problems, and the history of any difficulties the person has carrying out daily activities. Behavioral tests of memory, problem solving, attention, counting, and language provide information on cognitive degeneration and medical tests such as tests of blood, urine, or spinal fluid, and brain scans can provide some further information.

The management of AD consists of medication-based and non-medication based treatments. Treatments aimed at changing the underlying course of the disease (delaying or reversing the progression) have so far been largely unsuccessful. Medicines that restore the deficit (defect), or malfunctioning, in the chemical messengers of the nerve cells (neurotransmitters), in particular the cholinesterase inhibitors (ChEIs) such as tacrine and rivastigmine, have been shown to improve symptoms. ChEIs impede the enzymatic degradation of neurotransmitters thereby increasing the amount of chemical messengers available to transmit the nerve signals in the brain.

For some people in the early and middle stages of the disease, the drugs tacrine (COGNEX®, Morris Plains, NJ), donepezil (ARICEPT®, Tokyo, JP), rivastigmine (EXELON®, East Hanover, NJ), or galantamine (REMINYL®, New Brunswick, NJ) may help prevent some symptoms from becoming worse for a limited time. Another drug, memantine (NAMENDA®, New York, NY),

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has been approved for treatment of moderate to severe AD. Medications are also available to address the psychiatric manifestations of AD. Also, some medicines may help control behavioral symptoms of AD such as sleeplessness, agitation, wandering, anxiety, and depression. Treating these symptoms often makes patients more comfortable and makes their care easier for caregivers. Unfortunately, despite significant treatment advances showing that this class of agents is consistently better than a placebo, the disease continues to progress, and the average effect on mental functioning has only been modest. Many of the drugs used in AD medication such as, for example, ChEIs also have side effects that include gastrointestinal dysfunction, liver toxicity and weight loss.

Lewy body dementia (LBD) is a neurodegenerative disorder that can occur in persons older than 65 years of age, which typically causes symptoms of cognitive (thinking) impairment and abnormal behavioral changes. Symptoms can include cognitive impairment, neurological signs, sleep disorder, and autonomic failure. Cognitive impairment is the presenting feature of LBD in most cases. Patients have recurrent episodes of confusion that progressively worsen. The fluctuation in cognitive ability is often associated with shifting degrees of attention and alertness. Cognitive impairment and fluctuations of thinking may vary over minutes, hours, or days.
Lewy bodies are formed from phosphorylated and nonphosphorylated neurofilament proteins; they contain the synaptic protein alpha-synuclein as well as ubiquitin, which is involved in the elimination of damaged or abnormal proteins. In addition to Lewy Bodies, Lewy neurites, which are inclusion bodies in the cell processes of the nerve cells, may also be present. Amyloid plaques may form in the brains of patients afflicted with DLB, however they tend to be fewer in number than seen in patients with Alzheimer's disease. Neurofibrillary tangles, the other micropathological hallmark of AD, are not a main characteristic of DLB but are frequently present in addition to amyloid plaques.

Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons. In some ALS patients, dementia or aphasia may be present (ALS-D). The dementia is most commonly a frontotemporal dementia (FTD), and many of these cases have ubiquitin-positive, tau-negative inclusions in neurons of the dentate gyrus and superficial layers of the frontal and temporal lobes.

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Inclusion-body myositis (IBM) is a crippling disease usually found in people over age 50, in which muscle fibers develop inflammation and begin to atrophy—but in which the brain is spared and patients retain their full intellect. Two enzymes involved in the production of amyloid-ß protein were found to be increased inside the muscle cells of patients with this most common, progressive muscle disease of older people, in which amyloid-ß is also increased.

Another disease that is based on or associated with the accumulation and deposit of amyloid-like protein is macular degeneration.

Macular degeneration is a common eye disease that causes deterioration of the macula, which is the central area of the retina (the paper-thin tissue at the back of the eye where light-sensitive cells send visual signals to the brain). Sharp, clear, 'straight ahead' vision is processed by the macula. Damage to the macula results in the development of blind spots and blurred or distorted vision. Age-related macular degeneration (AMD) is a major cause of visual impairment in the United States and for people over age 65 it is the leading cause of legal blindness among Caucasians. Approximately 1.8 million Americans age 40 and older have advanced AMD, and another 7.3 million people with intermediate AMD are at substantial risk for vision loss. The government estimates that by 2020 there will be 2.9 million people with advanced AMD. Victims of AMD are often surprised and frustrated to find out how little is known about the causes and treatment of this blinding condition.

There are two forms of macular degeneration: dry macular degeneration and wet macular degeneration. The dry form, in which the cells of the macula slowly begin to break down, is diagnosed in 85 percent of macular degeneration cases. Both eyes are usually affected by dry AMD, although one eye can lose vision while the other eye remains unaffected. Drusen, which are yellow deposits under the retina, are common early signs of dry AMD. The risk of developing advanced dry AMD or wet AMD increases as the number or size of the drusen increases. It is possible for dry AMD to advance and cause loss of vision without turning into the wet form of the disease; however, it is also possible for early-stage dry AMD to suddenly change into the wet form.

The wet form, although it only accounts for 15 percent of the cases, results in 90 percent of the blindness, and is considered advanced AMD (there is no early or intermediate stage of wet

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AMD). Wet AMD is always preceded by the dry form of the disease. As the dry form worsens, some people begin to have abnormal blood vessels growing behind the macula. These vessels are very fragile and will leak fluid and blood (hence 'wet' macular degeneration), causing rapid damage to the macula.

The dry form of AMD will initially often cause slightly blurred vision. The center of vision in particular may then become blurred and this region grows larger as the disease progresses. No symptoms may be noticed if only one eye is affected. In wet AMD, straight lines may appear wavy and central vision loss can occur rapidly.

Diagnosis of macular degeneration typically involves a dilated eye exam, visual acuity test, and a viewing of the back of the eye using a procedure called fundoscopy to help diagnose AMD, and—if wet AMD is suspected—fluorescein angiography may also be performed. If dry AMD reaches the advanced stages, there is no current treatment to prevent vision loss. However, a specific high dose formula of antioxidants and zinc may delay or prevent intermediate AMD from progressing to the advanced stage. Macugen® (pegaptanib sodium injection), laser photocoagulation and photodynamic therapy can control the abnormal blood vessel growth and bleeding in the macula, which is helpful for some people who have wet AMD; however, vision that is already lost will not be restored by these techniques. If vision is already lost, low vision aids exist that can help improve the quality of life.

One of the earliest signs of age-related macular degeneration (AMD) is the accumulation of extracellular deposits known as drusen between the basal lamina of the retinal pigmented epithelium (RPE) and Bruch's membrane (BM). Recent studies conducted by Anderson et al. have confirmed that drusen contains amyloid beta. (Experimental Eye Research 78 (2004) 243-256).

Ongoing research continues with studies exploring environmental, genetic, and dietary factors that may contribute to AMD. New treatment strategies are also being explored, including retinal cell transplants, drugs that will prevent or slow down the progress of the disease, radiation therapy, gene therapies, a computer chip implanted in the retina that may help stimulate vision and agents that will prevent the growth of new blood vessels under the macula.

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An important factor to consider when developing new drugs is the ease of use for the target patients. Oral drug delivery, -specifically tablets, capsules and softgels-, account for 70% of all dosage forms consumed because of patient convenience. Drug developers agree that patients prefer oral delivery rather than subjecting themselves to injections or other, more invasive forms of medicinal administration. Formulations resulting in low dosing intervals (i.e. once a day or sustained release) are also preferable. The ease of administering antibiotics in oral dosage forms results in an increase of patient compliance during treatment.

What is needed are effective methods and compositions for preventing or addressing the complications associated with amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration. In particular what is needed are agents capable of counteracting the physiological manifestations of the disease such as the formation of plaques associated with aggregation of fibers of the amyloid or amyloid-like peptide.

Anti-amyloid antibodies elicited by the inoculation of $A\beta_{1-42}$ mixed with Freund complete or incomplete adjuvant were reported to reduce the amyloid burden in transgenic mice for human Alzheimer disease (Schenk et al., 1999). Intraperitoneal inoculation of tetrapalmitoylated $A\beta_{1-16}$ reconstituted in liposomes to NORBA transgenic mice elicited significant titers of anti-amyloid antibodies, which were reported to solubilize amyloid fibers and plaques *in vitro* and *in vivo*. (Nicolau et al., 2002).

A possible mechanism by which the dissolution of amyloid plaques and fibres occurred was first suggested by Bard et al., (2000), who concluded that the antibodies opsonized the plaques, which were subsequently destroyed by the macrophages of the microglia. De Mattos et al., (2001) indicated that a mAb directed against the central domain of β-amyloid was able to bind and completely sequester plasma amyloid. They argued that the presence of these mAbs in

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circulation shifted the equilibrium of Aβ between brain and plasma, favoring the peripheral clearing and catabolism instead of deposition within the brain.

Prolonged human therapy with rodent antibodies may result in an antiglobulin response which is detectable at about 8-12 days after administration and reaches a peak at about 20-30 days. If such an antiglobulin response is encountered, the treatment must be discontinued after not more than about 10 days and re-treatment at a latter date is usually precluded because it will lead to rapid onset of a secondary antiglobulin response. Although rodent antibodies share a considerable degree of sequence conservation with that of human antibodies, there are many sequence differences between rodents and human antibodies sufficient for the rodent antibodies to be immunogenic in humans.

This problem may be overcome by generating antibodies directly in humans or by the creation of "humanized' (a.k.a. "reshaped' antibodies). Humanized antibodies have a variable region amino acid sequence that contains the rodent-derived CDRs interspersed into human or human-like framework sequences. Since the specificity of the humanized antibody is provided by the rodent-derived CDRs, their residues are to be used essentially unchanged with only minor modifications being allowable, which do not significantly interfere with the affinity and specificity of the antibody for its target antigen. Framework residues may be derived from any primate or, particularly, from any human variable region or may be a combination thereof and the resultant designed variable region would be considered reshaped.

To maximise the likelihood that affinity will be retained in the reshaped antibody it is important to make a proper selection of the framework region. It is known that the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding. In order to maintain the affinity of the antibody for its antigen it is advantageous to select human framework sequences that are most similar to the sequences of the rodent frameworks. It then may still be necessary to replace one or more amino acids in the human framework sequence with the corresponding residue in the rodent framework to avoid losses with the affinity. This replacement may be aided by computer modelling.

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The present invention provides novel methods and compositions comprising highly specific and highly effective antibodies, particularly chimeric antibodies including fragments thereof, more particularly partially or fully humanized antibodies including fragments thereof, having the ability to specifically recognize and bind to specific epitopes from a range of β-amyloid antigens, which my be presented to the antibody in a monomeric, dimeric, trimeric, etc, a polymeric form, in form of an aggregate, fibers, filaments or in the condensed form of a plaque. The antibodies enabled by the teaching of the present invention are particularly useful for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, to name just a few.

SUMMARY OF THE INVENTION

In one embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, and more particularly to at least three distinct binding sites on the β-amyloid protein wherein said one, said at least two and said at least three binding sites each comprise at least one or two consecutive amino acid residues predominantly involved in the binding of the antibody.

In particular, the chimeric antibody or a fragment thereof, or the humanized antibody or a fragment thereof according to the invention binds to at least two, particularly to at least three distinct binding sites on the β-amyloid protein wherein at least two of the three distinct binding sites comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody and at least one of the three distinct binding sites comprise at least one amino acid residue.

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The at least two distinct binding sites comprising at least two consecutive amino acid residues predominantly involved in the binding of the antibody are located in close proximity to each other on the antigen, separated and/or flanked by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to said at least two consecutive amino acid residues, thus forming a conformational discontinuous epitope.

The at least three distinct binding sites comprising at least two consecutive amino acid residues and at least one amino acid residue, respectively, which are predominantly involved in the binding of the antibody are located in close proximity to each other on the epitope, separated and/or flanked by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues, which are predominantly involved in the binding of the antibody, thus forming a conformational discontinuous epitope.

In particular, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said at least one or said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- embedded within the following core sequence (SEQ ID NO: 9):

Xaa_3 - Phe – Phe – Xaa_4 – Xaa_5 – Xaa_6, wherein
Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;
Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;
Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,
Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_3 Xaa_4, Xaa_5 and Xaa_6 are not involved in antibody binding or to a significantly smaller extent as compared to the –Phe-Phe- binding site.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

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Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Xaa_5 is Glu or Asp, but particularly Glu;

Xaa_6 is Glu or Asp, but particularly Asp.

In particular, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- and the at least one amino acid residue is –His- embedded within the following core sequence:

$-Xaa_1-His-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Phe-Phe-Xaa_7-Xaa_8-Xaa_9-$,

wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg

Xaa_3 is an amino acid residue selected from the group consisting of Asn and Gln

Xaa_4 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg

Xaa_5 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_7 is an amino acid residue selected from the group consisting of Ala, Val, Leu and Ile

Xaa_8 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_9 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_1, Xaa_3, Xaa_6, Xaa_7, Xaa_8 and Xaa_9, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His- and the –Phe-Phe- binding site, respectively..

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

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Xaa_3 is Gln or Asn, but particularly Gln;

Xaa_4 is Lys

Xaa_5 is Leu

Xaa_6 is Val or Leu, but particularly Val;

Xaa_7 is Ala or Val, but particularly Ala;

Xaa_8 is Glu or Asp, but particularly Glu; and

Xaa_9 is Asp or Glu, but particularly Asp.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein, wherein said at least one or said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a second binding site are –Lys-Leu- embedded within the following core sequence (SEQ ID NO: 10):

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile; and wherein said amino acid residues Xaa_2, Xaa_3, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –Lys-Leu- binding site.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids, which are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues

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predominantly involved in the binding of the antibody, are -His- and –Lys-Leu-, respectively, embedded within the following core sequence:

His – Xaa_2 - Lys - Leu – Xaa_3– Xaa_4– Xaa_5–Xaa_6- – Xaa_7 — Xaa_8 – wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_5 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_7 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_8 is an amino acid residue selected from the group consisting of Glu and Asp

and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_6, Xaa_7, Xaa_8, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His- and the –Lys-Leu- binding site, respectively.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Phe

Xaa_5 is Phe

Xaa_6 is Ala or Val, but particularly Ala;

Xaa_7 is Glu or Asp, but particularly Glu; and

Xaa_8 is Asp or Glu, but particularly Asp.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acids are separated by at

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least one amino acid residue not involved in antibody binding or to a significantly smaller extent than said consecutive amino acid residues, which are –Phe-Phe- and –Lys-Leu-, respectively, representing a first and second binding site embedded within the following core sequence:

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 - Phe – Phe - $Xaa_4 – Xaa_5 – Xaa_6$, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5 and Xaa_6 are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –Lys-Leu- and –Phe-Phe- binding site, respectively.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues, which are predominantly involved in the binding of the antibody, and wherein said amino acid residues are –His- and –Phe-Phe- and –Lys-Leu-, respectively, embedded within the following core sequence:

His – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe - Xaa_4 _ Xaa_5 _ Xaa_6, wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

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Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His-, the –Lys-Leu- and the –Phe-Phe- binding site, respectively.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Xaa_5 is Glu or Asp, but particularly Glu; and

Xaa_6 is Asp or Glu, but particularly Asp.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acids are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent than said consecutive amino acid residues, which are –Phe-Phe- and –Lys-Leu-, respectively, representing a first and second binding site embedded within the following core sequence:

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 - Phe – Phe - $Xaa_4 – Xaa_5 – Xaa_6$, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Val, Ala, Leu, Met, Phe, norleucine and Ile

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

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Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the —Lys-Leu- and the –Phe- Phe binding site, respectively.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_1 is His or Arg, but particularly His;

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Xaa_5 is Glu or Asp, but particularly Glu; and

Xaa_6 is Asp or Glu, but particularly Asp.

In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, which are - Phe - Phe – Ala – Glu -, particularly - Phe - Phe – Ala –,but especially - Phe - Phe – and - Lys - Leu –, respectively, and wherein said at least two distinct binding sites exhibit amino acid sequence –Val - Phe - Phe - Ala - Glu – Asp - shown in SEQ ID NO: 7 and amino acid sequence His - Gln - Lys - Leu – Val - shown in SEQ ID NO: 8, respectively.

In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein the said at least one or said at least two distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, which are - Phe - Phe – and - Lys - Leu –, and –His-, respectively, wherein said distinct binding sites are embedded in the amino acid sequence –Val - Phe - Phe - Ala – Glu-, and amino acid sequence -His - Gln - Lys - Leu – Val -, respectively.

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In another embodiment of the invention, the chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof comprises an antigen recognition and binding site which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues within the amino acid sequence given in SEQ ID NOs: 7 and 8, respectively, wherein said consecutive amino acid residues, particularly –Phe- Phe- and –Lys-Leu-, are predominantly involved in the binding of the β-amyloid protein.

In a further specific embodiment of the invention, an antibody or a fragment thereof according to the invention is provided, which binds to 4 distinct binding sites on the β-amyloid protein wherein said 4 distinct binding sites include 2 binding sites each comprising one amino acid residue and 2 binding sites each comprising two consecutive amino acid residues, which residues are predominantly involved in the binding of the antibody, wherein said 4 distinct binding sites are located in close proximity to each other on the β-amyloid protein, and wherein said 4 binding sites are separated by at least one amino acid residue not involved in antibody binding or involved in binding but to a significantly smaller extent as compared to said one amino acid residue and said two consecutive amino acid residues of the 4 distinct binding sites thus forming a conformational discontinuous epitope.

In particular, the first of the two consecutive amino acid residues predominantly involved in the binding of the antibody is –Lys-Leu-, and the second of the at least two consecutive amino acid residues is –Phe-Phe-, the first of the single amino acid residues is -His- and the second of the single amino acid residues is -Asp- embedded within the following core sequence:

- Xaa_1– His – Xaa_2 – Lys – Leu –Xaa_3 – Phe – Phe – Xaa_4 – Xaa_5 – Asp – Xaa_6

wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg, but particularly His;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln ;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile, particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile, particularly Ala;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, particularly Glu;

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Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile, particularly Val; and wherein said amino acid residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or are involved in binding but to a significantly smaller extent as compared to the –His-, –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

In one embodiment, the invention relates to an antibody or a fragment thereof according to the invention, which binds to 4 distinct binding sites on the β-amyloid protein, wherein said 4 distinct binding sites include two binding sites each comprising one amino acid residue and two binding sites each comprising two consecutive amino acid residues, wherein the first of the two consecutive amino acid residues predominantly involved in the binding of the antibody is –Lys-Leu-, and the second of the at least two consecutive amino acid residues is –Phe-Phe-, the first of the single amino acid residues is -His- and the second of the single amino acid residues is -Asp- embedded within the following core sequence:

- Xaa_1– His – Xaa_2 – Lys – Leu –Xaa_3 – Phe – Phe – Xaa_4 – Xaa_5 – Asp. – Xaa_6

wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg, but particularly His;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln ;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile, particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile, particularly Ala;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, particularly Glu;

Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile, particularly Val; and wherein said amino acid residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or are involved in binding but to a significantly smaller extent as compared to the –His-, –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

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In a specific embodiment of the invention, the recognition and binding sites as defined herein before are forming a conformational discontinuous epitope localized in a region of the β-amyloid protein between amino acid residue 12 to 24, particularly between residues 14 to 23, more particularly between amino acid residues 14 and 20, wherein the at least two distinct recognition and binding sites each comprising at least 2 amino acid residues, are located at position 16 and 17 and at position 19 and 20, respectively, and wherein the at least one distinct recognition and binding site comprising at least 1 amino acid residue is located at position 14, which residues are predominantly involved in the binding of the β-amyloid protein and wherein said distinct recognition and binding sites are at least on one side flanked by amino acid residues, particularly residues 21 and 22, and separated by one amino acid residue located at position 15 and 18, which amino acid residues are not directly involved in the binding of the antigen or, at least, to a substantially smaller extent.

In still another embodiment of the invention the said at least three distinct recognition and binding sites are flanked on both sides by amino acid residues, particularly residues 12 and 13, and residues 21 and 22 and are separated by one amino acid residue located at position 15 and 18, which amino acid residues are not directly involved in the binding of the antigen or, at least, to a substantially smaller extent.

In a specific embodiment, said consecutive amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region:

Val–	His–	His–	Gln–	Lys–	Leu–	Val–	Phe–	Phe–	Ala–	Glu–	Asp
12	13	14	15	16	17	18	19	20	21	22	23

In another specific embodiment, said amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, and –His- at position 14, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region:

Val–	His–	His–	Gln–	Lys–	Leu–	Val–	Phe–	Phe–	Ala–	Glu–	Asp–	Val–	Gly–
12	13	14	15	16	17	18	19	20	21	22	23	24	25

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In another embodiment of the invention, a humanized antibody or a fragment thereof is provided which comprises in the light chain and heavy chain variable region, respectively, at least one CDR of non-human origin, particularly two CDRs of non-human origin, more particularly three CDR of non-human origin, embedded in one or more human- or primate-derived framework regions and, optionally, a constant region derived from a human or primate source antibody, which humanized antibody or fragment thereof is capable of specifically recognizing and binding β-amyloid protein, particularly a β-amyloid monomeric peptide, more particularly a β-amyloid polymeric peptide, even more particularly β-amyloid fibers, fibrils or filaments in isolation or as part of a β-amyloid plaque, at an epitope comprising the following amino acid sequence (SEQ ID NO: 11):

Xaa_1 – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe- Xaa_4 - Xaa_5 - Xaa_6, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, but particularly His;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln; and

Xaa_3 is an amino acid residue selected from the group consisting of Val, Leu, and Ile, but particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala and Val, but particularly Ala;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu;

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Asp.

In still another embodiment of the invention, a humanized antibody or a fragment thereof is provided which comprises in the light chain and heavy chain variable region, respectively, at least one CDR of non-human origin, particularly two CDRs of non-human origin, more particularly three CDR of non-human origin, embedded in one or more human- or primate-derived framework regions and, optionally, a constant region derived from a human or primate source antibody, which humanized antibody or fragment thereof is capable of specifically recognizing and binding β-amyloid protein, particularly a β-amyloid monomeric peptide, more particularly a β-amyloid polymeric peptide, even more particularly β-amyloid fibers, fibrils or

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filaments in isolation or as part of a β-amyloid plaque, at an epitope comprising the following amino acid sequence:

His – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe- Xaa_4 - Xaa_5 - Xaa_6, wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln; and

Xaa_3 is an amino acid residue selected from the group consisting of Val, Leu, and Ile, but particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala and Val, but particularly Ala;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu;

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu; and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller extent as compared to the –His- and the –Lys-Leu- and the -Phe-Phe- binding site.

In a specific embodiment of the invention, the CDR of non-human origin is obtained from a donor antibody, but particularly from a murine donor antibody, raised against an antigen fragment which does not contain said distinct binding site. This shift in the epitopic region may have at least partially been caused by the use of a supramolecular antigenic construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide, particularly of β-amyloid peptide $A\beta_{1-16}$, modified with a hydrophilic moiety such as, for example, polyethylene glycol (PEG), wherein said hydrophilic moiety is covalently bound to each of the termini of the antigenic peptide through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophilic moiety to the peptide fragment, as described herein below in the immunization process. When a PEG is used as the hydrophilic moiety, the free PEG termini are covalently bound to phosphatidylethanolamine or any other compound suitable to function as the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome as described herein.

In particular, the CDR of non-human origin is obtained from a murine donor antibody which exhibits the characteristic properties of ACI-01-Ab7C2 (also named "mC2" throughout the

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application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty under accession no DSM ACC2750).

In one embodiment of the invention, the CDR of non-human origin is obtained from murine donor antibody ACI-01-Ab7C2 (also named "mC2" throughout the application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty under accession no DSM ACC2750).

Also the use of lipid A as part of the immunization protocol may have contributed to a shift in the epitopic region.

In a specific embodiment, the invention relates to a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

In another embodiment, the invention relates to a humanized antibody or a fragment thereof, wherein said humanized antibody comprises integrated into human- or primate-derived heavy chain framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).
In still another embodiment, the invention relates to a humanized antibody or a fragment thereof, wherein said humanized antibody comprises integrated into human- or primate-derived light chain framework regions a peptide with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

In particular, the invention relates to a Light Chain Variable Region (LCVR) comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

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In another specific embodiment, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

The invention further relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least two peptides, which peptides are different and exhibit an amino acid sequence selected from the group of sequences consisting of SEQ ID NO:1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR) wherein the same CDR cannot be present twice in the antibody. In particular, if the at least two CDRs present are both CDRs of the Light Chain Variable Region (LCVR), at least on of said CDRs must be CDR1 represented by SEQ ID NO: 4.

Also comprised by the invention is a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

In particular, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

In a further embodiment, the invention relates to a humanized antibody or a fragment thereof, comprising integrated into human- or primate-derived light chain framework regions at least two

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peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

In particular, the invention relates to a Light Chain Variable Region (LCVR), which has integrated into human- or primate-derived light chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody and, in particular, at least on of said CDRs must be CDR1 represented by SEQ ID NO: 4.

The invention also relates to a humanized antibody or a fragment thereof, comprising integrated into human- or primate-derived heavy chain framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated above.

In particular, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived heavy chain framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated above.

Also comprised by the invention is a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived light chain framework regions peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated above.

In particular, the invention relates to a Light Chain Variable Region (LCVR) comprising integrated into human- or primate-derived light chain framework regions peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and

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SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated above.

The invention also relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least three peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

In another embodiment the invention relates to a humanized antibody or a fragment thereof, which antibody comprises integrated into human- or primate-derived framework regions at least four peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

In still anther embodiment, the invention relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least five peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

In still anther embodiment, the invention relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2

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and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

In a specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR), or a fragment thereof, wherein said humanized antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least a peptide with an amino acid sequence of SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, wherein said humanized antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least a peptide with an amino acid sequence of SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

In another specific embodiment, the invention relates to a humanized antibody, Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain

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framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

In another specific embodiment, the invention relates to a humanized antibody, a Light Chain Variable Region (LCVR) or a fragment thereof, which antibody, Light Chain Variable Region (LCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 5 representing CDR2 of the Light Chain Variable Region (LCVR).

In another specific embodiment, the invention relates to a humanized antibody, a Light Chain Variable Region (LCVR) or a fragment thereof, which antibody, Light Chain Variable Region (LCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

Further comprised by the invention is a humanized antibody or a fragment thereof, wherein both the Heavy Chain Variable Region (HCVR) and the Light Chain Variable Region (LCVR) of the mouse C2 antibody each contributes at least one of its CDR regions to the at least two CDR regions of the humanized antibody. The resulting humanized antibody or a fragment thereof thus may comprise

-at least an amino acid sequence of SEQ ID NO: 1 representing CDR1 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 1 representing CDR1 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR2 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR2 (LCVR);

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-at least an amino acid sequence of SEQ ID NO:2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR3 (LCVR);
-at least an amino acid sequence of SEQ ID NO:1 representing CDR1 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR3 (LCVR);
-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR2 (LCVR);
-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR3 (LCVR).

In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof as described herein before, which antibody comprises a light chain and/or a heavy chain constant region of human or primate origin.

In a further embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein at least one, particularly at least one but not more than 5, more particularly at least one but not more than 4, even more particularly at least one but not more than 3, but especially at least one but not more than 2, of the amino acids representative of the light chain and/or heavy chain CDR regions as given in SEQ ID NOs: 1 – 6 is changed through a conservative substitution such that the antibody maintains its full functionality.

In particular, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein in CDR2 of the light chain variable region (LCVR) as given in SEQ ID NO: 5, the Lys at Kabat position 50 is replaced by an amino acid residue selected from the group consisting of Arg, Gln and Glu, particularly by Arg.

In particular, the invention relates to a light chain variable region (LCVR) wherein in CDR2 as given in SEQ ID NO: 5, the Lys at Kabat position 50 is replaced by an amino acid residue selected from the group consisting of Arg, Gln and Glu, particularly by Arg.
In another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein in CDR2 of the light chain variable region (LCVR) as given in SEQ ID NO: 5, the Ser at Kabat position 53 is replaced by an amino acid residue selected from the group consisting of Asn or Thr, but particularly by Asn.

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In particular, the invention relates to a light chain variable region (LCVR) wherein in CDR2 as given in SEQ ID NO: 5, the Ser at Kabat position 53 is replaced by an amino acid residue selected from the group consisting of Asn or Thr, but particularly by Asn.

In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein the Heavy Chain Variable Region (HCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 15 and 16, respectively.

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein the Light Chain Variable Region (LCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 12 and 13, respectively.
In still another embodiment of the invention, a humanized antibody or a fragment thereof is provided, wherein at least two, but especially three, of the CDR regions of the Heavy Chain Variable Region (HCVR) have an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 1 – 3.

In a further embodiment of the invention, a humanized antibody or a fragment thereof is provided, wherein at least two, but especially three, of the CDR regions of the Light Chain Variable Region (LCVR) have an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 4 – 6.

In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before wherein the Heavy Chain Variable Region (HCVR) has an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence given in SEQ ID NO: 15 and 16, respectively.

In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before wherein the Light Chain Variable Region (LCVR) has an amino acid sequence that

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is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence given in SEQ ID NO: 12 and 13, respectively.

In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before, wherein at least one, particularly at least two, but especially three, of the CDR regions of the Heavy Chain Variable Region (HCVR) have an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the corresponding CDR region as given in SEQ ID NO: 1 – 3.

In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before, wherein at least one, particularly at least two, but especially three, of the CDR regions of the Light Chain Variable Region (LCVR) have an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the corresponding CDR region as given in SEQ ID NO: 4 – 6.

In still another embodiment, the invention relates to a humanized antibody according to the present invention and as described herein before, wherein at least one of the amino acids representative of the acceptor framework sequences obtained from human germline V_H and V_K sequences, respectively is changed through a substitution to an amino acid from the corresponding region of murine antibody ACI-01-Ab7C2 or a substitution conservative thereto.

In particular, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu such as shown in SEQ ID NO: 15.

The invention further relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT

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subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser such as shown in SEQ ID NO: 15...

In still another embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser such as shown in SEQ ID NO: 15.

The invention further relates to a Light Chain Variable Region and to a humanized antibody comprising this Light Chain Variable Region, respectively,, wherein the Gln in Kabat position 45 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region is replaced by an amino acid selected from the group consisting of Lys, Arg, Gln, and Asn, particularly by Lys and Arg, but especially by Lys.

The invention further relates to a Light Chain Variable Region and to a humanized antibody comprising this Light Chain Variable Region, respectively, wherein the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe.

The invention further relates to a Light Chain Variable Region and to a humanized antibody comprising this Light Chain Variable Region, respectively, wherein the Lys in Kabat position 50 in the CDR2 region obtained from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, such as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Arg, Gln, His, and Asn, but especially by Arg

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In still another embodiment, the invention relates to a Light Chain Variable Region and to a humanized antibody comprising this Light Chain Variable Region, respectively, wherein the Asn in Kabat position 53 in the CDR2 region obtained from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, such as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Ala, Val, Leu, Ser and Ile; but especially Ser..

In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser as shown in SEQ ID NO: 15, and the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe.

In still another embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Leu.

In still another embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by Ser such as shown in SEQ ID NO: 15.

In still another embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively,, wherein the

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Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by Ser such as shown in SEQ ID NO: 15..

In still another embodiment, the invention relates to a Light Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region is replaced by Phe.

In still another embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively,, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by Ser such as shown in SEQ ID NO: 15 and the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region is replaced by Phe..

In one embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser such as shown in SEQ ID NO: 15 and wherein the Lys in Kabat position 50 in the CDR2 region obtained from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, is replaced by an amino acid selected from the group consisting of Arg, Gln, His, and Asn, but especially by Arg.

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In one embodiment, the invention relates to a Heavy Chain Variable Region and to a humanized antibody comprising this Heavy Chain Variable Region, respectively, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser such as shown in SEQ ID NO: 15 and wherein the Asn in Kabat position 53 in the CDR2 region obtained from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, is replaced by an amino acid selected from the group consisting of Ala, Val, Leu, Ser and Ile; but especially Ser.

In a specific embodiment, the invention relates to the light chain variable region of SEQ ID NO: 12.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the light chain variable region of SEQ ID NO: 12.

In a specific embodiment, the invention relates to the light chain variable region including signal sequences as shown in SEQ ID NO: 13.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete light chain variable region including signal sequences as shown in SEQ ID NO: 13.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the light chain variable region of SEQ ID NO: 12 and the light chain constant region of SEQ ID NO: 14.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete light chain variable region of SEQ ID NO: 13 and the light chain constant region of SEQ ID NO: 14.

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In a specific embodiment, the invention relates to the heavy chain variable region of SEQ ID NO: 15.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 15.

In a specific embodiment, the invention relates to the heavy chain variable region including signal sequences as shown in SEQ ID NO: 16.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete heavy chain variable region including signal sequences as shown in SEQ ID NO: 16.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 15 and the heavy chain constant region of SEQ ID NO: 17.

In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 16 and the heavy chain constant region of SEQ ID NO: 17.

In one embodiment the humanized antibody according to the invention and as described herein, upon co-incubation with an Aβ monomeric peptide having at least 30, particularly at least 35, more particularly at least 38, even more particularly at least 40 amino acid residues and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said Aβ monomeric units, but especially with an $A\beta_{1-42}$ monomeric and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units, particularly at a molar concentration ratio of antibody to Aβ1-42 of up to 1:1000, particularly of up to 1:500, more particularly of up to 1:300, even more particularly of up to 1:200, but especially at a molar concentration ratio of between 1:10 and 1:100, inhibits the aggregation of the Aβ monomers to high molecular polymeric fibrils.

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In particular, the co-incubation of the antibody according to the invention with amyloid monomeric and/or polymeric soluble amyloid peptides is carried out for 24 hours to 60 hours, particularly for 30 hours to 50 hours, more particularly for 48 hours, but especially 24 hours, at a temperature of between 28°C and 40°C, particularly of between 32°C and 38°C, more particularly at 37°C.

In a specific embodiment of the invention, co-incubation with amyloid monomeric and/or polymeric soluble amyloid peptides is accomplished for 24 hours at a temperature of 37°C.

In particular, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof binds to $A\beta_{1-42}$ monomeric peptide and/or $A\beta$ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units and, upon co-incubation with $A\beta_{1-42}$ monomeric peptide and/or $A\beta$ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units inhibits the aggregation of the $A\beta$ monomers and/or polymers to high molecular polymeric fibrils.

In one embodiment, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the $A\beta$ monomers and/or $A\beta$ soluble polymers comprising a plurality of said $A\beta$ monomeric units to high molecular polymeric fibrils by at least 50%, particularly by at least 60%, particularly by at least 65%, more particularly by at least 75%, even more particularly by at least 80%, but especially by at least 85%-90%, or more as compared to the respective amyloid peptide monomers incubated in buffer (control), at a molar concentration ratio of antibody to $A\beta$1-42 of up to 1:1000, particularly at a molar concentration ratio of between 1:10 and 1:100, but especially at a molar concentration ratio of 1:10.

In a specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the $A\beta$ monomers and/or $A\beta$ soluble polymers comprising a

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plurality of said Aβ monomeric units to high molecular polymeric fibrils by at least 30% at a molar concentration ratio of antibody to Aβ1-42 of 1:100.

In another specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the Aβ monomers and/or Aβ soluble polymers comprising a plurality of said Aβ monomeric units to high molecular polymeric fibrils by at least 80% at a molar concentration ratio of antibody to Aβ1-42 of 1:10.

Binding of the antibodies according to the invention and as described herein to amyloidogenic monomeric and/or polymeric peptides but, particularly, to the amyloid form (1-42) leads to inhibition of the aggregation of monomeric and/or polymeric amyloidogenic peptides to high molecular fibrils or filaments. Through the inhibition of the aggregation of amyloidogenic monomeric and/or polymeric peptides the antibodies according to the present invention are capable of preventing or slowing down the formation of amyloid plaques, particularly the amyloid form (1-42), which is know to become insoluble by change of secondary conformation and to be the major part of amyloid plaques in brains of diseased animals or humans.

The aggregation inhibition potential of the antibody according to the invention may be determined by any suitable method known in the art, particularly by density-gradient ultracentrifugation followed by a SDS-PAGE sedimentation analysis on a preformed gradient and/or by a thioflavin T (Th-T) fluorescent assay.

In one embodiment, the invention relates to an antibody, particularly a humanized antibody as described herein including any functionally equivalent antibody or functional parts thereof, which antibody, upon co-incubation, particularly at a molar concentration ratio of between 1:5 and 1:1000, particularly of between 1:10 and 1:500, more particularly at a ratio of 1:10 to 1:300, even more particularly at a ratio of between 1:10 and 1:100, with preformed high molecular polymeric amyloid fibrils or filaments formed by the aggregation of Aβ monomeric peptides having at least 30, particularly at least 35, more particularly at least 38, even more particularly at least 40 amino acid residues and, but especially $A\beta_{1-42}$ monomeric peptides, is capable of disaggregating the preformed polymeric fibrils or filaments by at least 20%, particularly by at

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least 30%, more particularly by at least 35%%, even more particularly by at least 40%, but especially by at least 50% or more.

In a specific embodiment of the invention, the aggregation inhibition and the disaggregation potential of the antibody, respectively, is determined by density-gradient ultracentrifugation followed by a SDS-PAGE sedimentation analysis on a preformed gradient.

In another specific embodiment of the invention, the aggregation inhibition and the disaggregation potential of the antibody, respectively, is determined by thioflavin T (Th-T) fluorescent assay.

In another specific embodiment, the antibody according to the invention is co-incubated with amyloid preformed high molecular polymeric amyloid fibrils or filaments for 12 hours to 36 hours, particularly for 18 hours to 30 hours, more particularly for 24 hours at a temperature of between 28°C and 40°C, particularly of between 32°C and 38°C, more particularly at 37°C.

In particular, the co-incubation with preformed high molecular polymeric amyloid fibrils or filaments is done for 24 hours at a temperature of 37°C.

In a specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof is capable of disaggregating the preformed polymeric fibrils or filaments by at least 24% at a molar concentration ratio of antibody to Aβ1-42 of 1:100.

In another specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof is capable of disaggregating the preformed polymeric fibrils or filaments by at least 32% at a molar concentration ratio of antibody to Aβ1-42 of 1:10.

Through the disaggregation of amyloidogenic polymeric fibrils or filaments the antibodies according to the present invention are capable of preventing or slowing down the formation of amyloid plaques which leads to an alleviation of the symptoms associated with the disease and a delay or reversal of its progression.

Accordingly, it is a further embodiment of the invention to provide an antibody, particularly a humanized antibody, including any functionally equivalent antibody or functional parts thereof

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as described herein, which antibody is capable of decreasing the total amount of Aβ in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to increased concentration of Aβ in the brain.

In another embodiment, the invention relates to a humanized antibody according to the invention and as described herein before, which antibody is bi-effective in that it exhibits both an aggregation inhibition property as well as a disaggregation property, particularly paired with a high degree of conformational sensitivity.

In particular, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, which antibody, upon co-incubation with amyloid monomeric and/or polymeric soluble amyloid peptides, particularly with β-amyloid monomeric peptides such as, for example, Aβ monomeric peptides 1-39; 1-40, 1-41, or 1-42, and/or a polymeric soluble β-amyloid peptide comprising a plurality of said Aβ monomeric units, but especially with an $A\beta_{1-42}$ monomeric and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units,, inhibits the aggregation of the Aβ monomers into high molecular polymeric fibrils or filaments and, in addition, upon co-incubation with preformed high molecular polymeric amyloid fibrils or filaments formed by the aggregation of amyloid monomeric peptides, particularly β-amyloid monomeric peptides such as, for example, Aβ monomeric peptides 1-39; 1-40, 1-41, or 1-42, but especially $A\beta_{1-42}$ monomeric peptides, is capable of disaggregating the preformed polymeric fibrils or filaments.

In another aspect, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, which antibody is capable of inducing a transition of the β-sheet conformation towards an α-helix and/or a random coil conformation, but particularly a random coil conformation, even more particularly a random coil conformation at a given location in the molecule, especially in the environment of Tyr 10 and Val12 of the Aβ protein, which leads to an increase of the random coil conformation at the expense of the β-sheet conformation and an improved solubilization of the preformed high molecular polymeric amyloid fibrils or filaments. In particular the decrease of the β-sheet conformation amounts to at least 30%, particularly to at

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least 35%, and more particularly to at least 40% and more as compared to the respective preformed amyloid polymeric fibrils or filaments incubated in buffer (control).

The antibody's potential in inducing a transition in the secondary structure is determined by solid state 13C NMR spectroscopy but, in particular, by measuring the integral intensities of the conformations of Tyr 10 and Val 12 Cβ in the Aβ_{1-42} peptide.

In a further embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof, wherein said antibody or fragment binds to an Aβ monomer with a high binding affinity with a K_D in a range of between at least about 1 x 10^{-7} M to at least about 1 x 10^{-12} M, particularly of at least about 1 x 10^{-8} M to at least about 1 x 10^{-11} M, more particularly of at least about 1 x 10^{-9} M to at least about 1 x 10^{-10} M, even more particularly of at least about 1 x 10^{-8} M to at least about 2 x 10^{-8} M, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof, wherein said antibody or fragment binds to an Aβ fiber, fibril or filament with a high binding affinity with a K_D in a range of between at least about 1 x 10^{-7} M to at least about 1 x 10^{-12} M, particularly of at least about 1 x 10^{-8} M to at least about 1 x 10^{-11} M, more particularly of at least about 1 x 10^{-9} M to at least about 1 x 10^{-10} M, even more particularly of at least about 2 x 10^{-9} M to at least about 5 x 10^{-9} M, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

In another embodiment, the antibody according to the invention and as described herein before or a fragment thereof, exhibits an binding affinity to an Aβ fiber, fibril or filament which is at least 2 times, particularly at least 4 times, particularly at least 10 times, particularly at least 15 times, more particularly at least 20 times, but especially at least 25 times higher than the binding affinity to an Aβ monomer.

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In still another embodiment, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided as described herein before, which antibody substantially binds to aggregated Aβ, including Aβ plaques, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

In another aspect of the invention, the chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided as described herein before, which antibody substantially binds to soluble polymeric amyloid, particularly amyloid β (Aβ), including Aβ monomers, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

Further provided is a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, which antibody significantly reduces Aβ plaque burden in the mammalian, particularly the human brain. This can be achieved by either binding of the antibody to the plaque or by shifting the equilibrium between amyloid, particularly amyloid β (Aβ), in its insoluble and aggregated state towards its soluble form by disaggregating fibers to soluble poly- and monomeric forms by inducing a shift in conformation and binding and stabilizing the disaggregated and solubilized amyloid forms, particularly amyloid β (Aβ) forms, in the tissue and/or body fluids, particularly the brain. Through the activity of the antibody according to the invention the peripheral clearing and catabolism is thus favored rather than deposition within the tissue and/or body fluids, particularly the brain. The beneficial effect of the antibody according to the invention can thus be obtained without binding of the antibody to the plaque.

Through this stabilizing activity, the antibody according to the invention is able to neutralize the toxic effects of the polymeric and less aggregated soluble amyloid protein, particularly amyloid β (Aβ) protein, in the tissue and/or body fluids. In a specific embodiment of the invention the antibody according to the invention may thus achieve its beneficial effects without necessarily binding aggregated amyloid beta in the brain.

In a further aspect of the invention a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof incorporating at least one,

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particularly two and more particularly three CDR regions obtained form a mouse donor antibody, particularly from mouse antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Braunschweig, under accession no DSM ACC2750, wherein said antibody or fragment thereof has an affinity to the Aβ antigen which is at least 5 times, particularly at least 8 times, more particularly at least 10 times, but especially at least 15 times higher than that of the mouse donor antibody.

The antibody of this invention can be, in one embodiment, a whole antibody (e.g., with two full length light chains and two full length heavy chains) of any isotype and subtype (*e.g.*, IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgE, IgA1 and IgA2); but especially an antibody of the IgG4 isotype; alternatively, in another embodiment, it can be an antigen-binding fragment (e.g., Fab, $F(ab')_2$, and Fv) of a whole antibody.

The invention thus also relates to antigen-binding fragments of the antibodies described herein. In one embodiment of the invention, the fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a $F(ab)_2$fragment, and a F_v fragment, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

In another embodiment, the antibody or antigen-binding fragment of the invention is conjugated to polyethylene glycol. In yet another embodiment, the constant region of the antibody of the invention is modified to reduce at least one constant region-mediated biological effector function relative to an unmodified antibody. In still another embodiment, the antibody or antigen-binding fragment of the invention comprises a Fc region having an altered effector function.

The invention further relates to a nucleotide molecule comprising a nucleotide sequence encoding a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as disclosed herein before.

In particular, the invention relates to a nucleotide molecule comprising a nucleotide sequence encoding a stretch of contiguous amino acid molecules as given in SEQ ID NO: 2 and 3,

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respectively, or the complementary sequence, representing the Complementarity Determining Regions (CDRs) 2 and 3 of the Heavy Chain Variable Region (HCVR).

More particularly, the invention relates to a nucleotide molecule comprising a nucleotide sequence encoding a stretch of contiguous amino acid molecules as given in SEQ ID NO: 4, or the complementary sequence, representing the Complementarity Determining Regions (CDRs) 1 of the Light Chain Variable Region (LCVR).

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence as given in SEQ ID NO: 18 and SEQ ID NO: 19, or the complementary sequence, encoding the amino acid sequence of CDR 2 and CDR 3, respectively, of the Heavy Chain Variable Region (HCVR).

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence as given in SEQ ID NO: 20, or the complementary sequence, encoding the nucleotide sequence of CDR 1 of the Light Chain Variable Region (LCVR).

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 21, or the complementary sequence, encoding the light chain variable region.

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 22, or the complementary sequence, encoding the complete light chain variable region including signal sequences.

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence encoding the light chain variable region of SEQ ID NO: 22 and the light chain constant region of SEQ ID NO: 23. The invention also comprises the complementary strand of said nucleotide molecule.

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 24 encoding the heavy chain variable region. The invention also comprises the complementary strand of said nucleotide molecule.

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In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 25 encoding the complete heavy chain variable region including signal sequences. The invention also comprises the complementary strand of said nucleotide molecule.

In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence encoding the heavy chain variable region of SEQ ID NO: 25 and the heavy chain constant region of SEQ ID NO: 26 . The invention also comprises the complementary strand of said nucleotide molecule.

Also comprised by the present invention is a nucleotide sequence which hybridizes to one of the above-described antibody-encoding nucleotide sequences of the invention, particularly to the complementary strand thereof, either in isolation or as part of larger nucleotide molecule.

In particular, the invention relates to a nucleotide sequence that hybridizes under conventional hybridization conditions, particularly under stringent hybridization conditions, to any of the nucleotide sequences given in SEQ ID NOs: 18-26 and 29 - 32, particularly to the complementary strand thereof.

In another embodiment of the invention an expression vector is provided comprising the nucleic acid molecule according to the invention and as mentioned herein before.

In another embodiment of the invention a cell is provided comprising an expression vector comprising the nucleic acid according to the invention and as mentioned herein before.

In still another embodiment, the invention relates to a composition comprising the antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before including any functionally equivalent antibody or any derivative or functional parts thereof, in a therapeutically effective amount, in particular a composition which is a pharmaceutical composition optionally further comprising a pharmaceutically acceptable carrier.

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In another embodiment of the invention, said composition comprises the antibody in a therapeutically effective amount.

Further comprised by the invention is a mixture comprising an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before including any functionally equivalent antibody or any derivative or functional parts thereof, in a therapeutically effective amount and, optionally, a further biologically active substance and/or a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In particular, the invention relates to a mixture, wherein the further biologically active substance is a compound used in the medication of amyloidosis, a group of diseases and disorders associated with amyloid or amyloid-like protein such as the Aβ protein involved in Alzheimer's disease.

In another embodiment of the invention, the other biologically active substance or compound may also be a therapeutic agent that may be used in the treatment of amyloidosis caused by amyloid β or may be used in the medication of other neurological disorders.

The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the antibody according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

Generally, the other biologically active compound may include neutron-transmission enhancers, psychotherapeutic drugs, acetylcholine esterase inhibitors, calcium-channel blockers, biogenic amines, benzodiazepine tranquillizers, acetylcholine synthesis, storage or release enhancers, acetylcholine postsynaptic receptor agonists, monoamine oxidase-A or –B inhibitors, N-methyl-D-aspartate glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, antioxidants, and serotonergic receptor antagonists.

More particularly, the invention relates to a mixture comprising at least one compound selected from the group consisting of compounds effective against oxidative stress, anti-apoptotic

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compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), α-secretase activators, β- and γ-secretase inhibitors, tau proteins, neurotransmitter, β-sheet breakers, attractants for amyloid beta clearing / depleting cellular components, inhibitors of N-terminal truncated amyloid beta including pyroglutamated amyloid beta 3-42, anti-inflammatory molecules, or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine, M1 agonists and other drugs including any amyloid or tau modifying drug and nutritive supplements, and nutritive supplements, together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

The invention further relates to a mixture, wherein the compound is a cholinesterase inhibitor (ChEIs), particularly a mixture, wherein the compound is one selected from the group consisting of tacrine, rivastigmine, donepezil, galantamine, niacin and memantine.

In a further embodiment, the mixtures according to the invention may comprise niacin or memantine together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In still another embodiment of the invention mixtures are provided that comprise "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine for the treatment of positive and negative psychotic symptoms including hallucinations, delusions, thought disorders (manifested by marked incoherence, derailment, tangentiality), and bizarre or disorganized behavior, as well as anhedonia, flattened affect, apathy, and social withdrawal, together with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In a specific embodiment of the invention, the compositions and mixtures according to the invention and as described herein before comprise the antibody and the biologically active substance, respectively, in a therapeutically effective amount.

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Other compounds that can be suitably used in mixtures in combination with the antibody according to the present invention are described in WO 2004/058258 (see especially pages 16 and 17) including therapeutic drug targets (page 36-39), alkanesulfonic acids and alkanolsulfuric acids (pages 39-51), cholinesterase inhibitors (pages 51-56), NMDA receptor antagonists (pages 56-58), estrogens (pages 58-59), non-steroidal anti-inflammatory drugs (pages 60-61), antioxidants (pages 61-62), peroxisome proliferators-activated receptor (PPAR) agonists (pages 63-67), cholesterol–lowering agents (pages 68-75); amyloid inhibitors (pages 75-77), amyloid formation inhibitors (pages 77-78), metal chelators (pages 78-79), anti-psychotics and anti-depressants (pages 80-82), nutritional supplements (pages 83-89) and compounds increasing the availability of biologically active substances in the brain (see pages 89-93) and prodrugs (pages 93 and 94), which document is incorporated herein by reference.

In another embodiment, the invention relates to a mixture comprising the antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before and/or the biologically active substance in a therapeutically effective amount.

The invention further relates to the use of an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before and/or a functional part thereof and/or a pharmaceutical composition, or a mixture comprising said antibody, for the preparation of a medicament for treating or alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

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Also comprised by the present invention is a method for the preparation of an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before and/or a functional part thereof and/or a pharmaceutical composition, or a mixture comprising said antibody and/or a functional part thereof, particularly in a therapeutically effective amount, for use in a method of preventing, treating or alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration comprising formulating an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention in a pharmaceutically acceptable form.

Further comprised by the present invention is a method for preventing, treating or alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration by administering an antibody and/or a functional part thereof, but particularly a humanized antibody and/or a functional part thereof, or a composition or mixture comprising such an antibody and/or a functional part thereof, to a an animal or a human affected by such a disorder comprising administering the antibody in a therapeutically effective amount.

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It is also an object of the invention to provide a method for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), particularly a disease or condition characterized by a loss of cognitive memory capacity by administering to an animal, particularly a mammal or a human, an antibody, particularly a pharmaceutical composition according to the invention and as described herein.

In a specific embodiment the invention provides a method for retaining or increasing cognitive memory capacity but, particularly, for restoring the cognitive memory capacity of an animal, particularly a mammal or a human, suffering from memory impairment by administering to an animal, particularly a mammal or a human, an antibody, particularly a pharmaceutical composition according to the invention and as described herein before.

It is a further object of the invention to provide a therapeutic composition and a method of producing such a composition as well as a method for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), particularly a disease or condition characterized by a loss of cognitive memory capacity, using an antibody according to the invention and as described herein before.

In particular, the invention relates to the treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to the retention of cognitive memory capacity.

The invention further relates to a method of diagnosis of an amyloid-associated disease or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ* which includes the steps of

(a) bringing the sample or a specific body part or body area suspected to contain the amyloid protein into contact with an antibody, particularly a monoclonal antibody

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according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof, which antibody binds an epitope of the amyloid protein;

(b) allowing the antibody and/or a functional part thereof, to bind to the amyloid protein to form an immunological complex;

(c) detecting the formation of the immunological complex; and

(d) correlating the presence or absence of the immunological complex with the presence or absence of amyloid protein in the sample or specific body part or area.

Also comprised is a method of determining the extent of amyloidogenic plaque burden in a tissue and/or body fluids comprising

(a) obtaining a sample representative of the tissue and/or body fluids under investigation;

(b) testing said sample for the presence of amyloid protein with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof;

(c) determining the amount of antibody bound to the protein; and

(d) calculating the plaque burden in the tissue and/or body fluids.

In particular, the invention relates to a method of determining the extent of amyloidogenic plaque burden in a tissue and/or body fluids, wherein the formation of the immunological complex in step c) is determined such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

In another embodiment of the invention, a test kit for detection and diagnosis of amyloid-associated diseases and conditions is provided comprising an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof.

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In particular, the invention relates to a test kit for detection and diagnosis of amyloid-associated diseases and conditions comprising a container holding one or more antibodies according to the present invention, and/or a functional part thereof, and instructions for using the antibodies for the purpose of binding to amyloid protein to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

In another aspect, the invention provides an antibody comprising a variable region as recited in SEQ ID NO: 27, or a variant thereof. In one embodiment, a cell line expressing the antibody.

In another aspect, the invention provides an antibody gene comprising a variable region as recited in SEQ ID NO: 29, or a variant thereof. In one embodiment, a cell line expresses the antibody.

In another aspect, the invention provides a method for disaggregating preformed beta-amyloid fibers, comprising interacting an hC2 antibody with preformed beta-amyloid fibers.

In another aspect, the invention provides a humanized antibody or a fragment thereof according to any of the preceding claims, wherein said antibody or fragment thereof protects neurons from Abeta-induced degradation.

In another aspect, the invention provides a method of preventing Abeta-induced neuron degradation comprising treating neurons with an effective amount of a humanized antibody or a fragment thereof according to the disclosure herein.

In another aspect, the invention provides use of a humanized antibody or a fragment thereof according to the description herein for the preparation of a medicament for preventing degeneration of neurons upon exposure to Abeta oligomer.

In another embodiment of the invention, an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof is provided, which recognized the native conformation of amyloid in that it specifically binds to amyloid oligomers and fibers, but not to not linearized amyloid species.

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In a further embodiment of the invention, an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof according to the present invention and as described herein before, is provided which antibody or fragment binds to an Aβ monomer with a binding affinity of at least about 1 x 10^{-6} to at least about 1 x 10^{-8}, particularly of at least about 1 x 10^{-6} to at least about 1 x 10^{-7}, more particularly of at least about 1 x 10^{-7} to at least about 1 x 10^{-8}, even more particularly of at least about 1 x 10^{-7} to at least about 4 x 10^{-7}, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

In another embodiment of the invention, an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof according to the present invention and as described herein before, is provided which antibody or fragment binds to an Aβ fiber, fibril or filament with a binding affinity of at least about 1 x 10^{-7} to at least about 1 x 10^{-9}, particularly of at least about 1 x 10^{-7} to at least about 1 x 10^{-8}, more particularly of at least about 1 x 10^{-8} to at least about 1 x 10^{-9}, even more particularly of at least about 1 x 10^{-8} to at least about 5 x 10^{-8}, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

In another embodiment, the antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof according to the present invention and as described herein before exhibits an binding affinity to an Aβ fiber, fibril or filament which is at least 5 times, particularly at least 10 times, more particularly at least 15 times, higher than the binding affinity to an Aβ monomer.

Through the disaggregation of amyloidogenic polymeric fibrils or filaments the antibodies according to the present invention are capable of preventing or slowing down the formation of amyloid plaques which leads to an alleviation of the symptoms associated with the disease and a delay or reversal of its progression.

Accordingly, it is a further embodiment of the invention to provide an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof as described herein before, which antibody is capable of decreasing the total amount of Aβ in the

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brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to increased concentration of Aβ in the brain.

In another embodiment of the invention an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof as described herein before is provded, which antibody is capable of disrupting plaques thus decreasing the plaque load in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain. The antibody according to the invention including any functionally equivalent antibody or functional parts thereof decreases the plaque load in the brain by at least 20 %, particularly by at least 25%, more particularly by at least 30%, even more particularly more than 30%.

In still another embodiment of the invention an antibody, particularly a monoclonal antibody, including any functionally equivalent antibody or functional parts thereof as described herein before is provided, which antibody is capable of solubilizing plaques leading to a reduction of the amount of plaques in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain. The antibody according to the invention including any functionally equivalent antibody or functional parts thereof reduces the amount of plaques in the brain by at least 10 %, particularly by at least 15%, more particularly by at least 20%.

It is to be understood that the antibody according to the invention can exhibit one, two or more of the specific properties described herein before in various combinations.

In particular, an antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites on the β-amyloid protein wherein the said at least one or said at least two distinct binding sites comprise at least one amino acid residue and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein, in a specific embodiment of the invention, the at least one residue constituting the first distinct binding site is Leu and the at least two consecutive amino acid residues, constituting the second distinct binding site, are –Phe-Phe- embedded within the following core sequence:

$-Xaa_1-Xaa_2-Xaa_3-$ Leu $-Xaa_4-$ Phe – Phe $-Xaa_5-Xaa_6-Xaa_7-$

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wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_3 is an amino acid residue selected from the group comprising Lys, His, Asn, Gln and Arg

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp.

In another aspect, the invention relates to an antibody or a fragment thereof, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein, wherein said one or the at least two or the at least three distinct binding sites each comprise at least one, particularly at least two consecutive amino acid residues predominantly involved in the binding of the antibody.

In particular, the antibody or a fragment thereof according to the invention binds to at least two distinct binding sites on the β-amyloid protein, wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein said at least two distinct binding sites are located in close proximity to each other on the antigen, separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to said at least two consecutive amino acid residues, thus forming a conformational discontinuous epitope.

In another embodiment of the invention, an antibody or a fragment thereof according to the invention is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids, which are

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separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues predominantly involved in the binding of the antibody, are -His- and –Lys-Leu-, respectively, embedded within the following core sequence:

– His – Xaa_2 – Lys – Leu – Xaa_3– Xaa_4– Xaa_5–Xaa_6- – Xaa_7 – Xaa_8 – wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_3 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_6 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_8 is an amino acid residue selected from the group comprising Glu and Asp

and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_6, Xaa_7, Xaa_8, are not involved in antibody binding or to a significantly smaller extent as compared to the –His- and the –Lys-Leu- binding site.

In another embodiment, an antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- and the at least one amino acid residue is –His- embedded within the following core sequence:

– Xaa_1 – His – Xaa_3 – Xaa_4 – Xaa_5 – Xaa_6 – Phe – Phe – Xaa_7 – Xaa_8 – Xaa_9 –,

wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln, Lys and Arg

Xaa_3 is an amino acid residue selected from the group comprising Asn and Gln

Xaa_4 is an amino acid residue selected from the group comprising His, Asn, Gln, Lys and Arg

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Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;
Xaa_6 is an amino acid residue selected from the group comprising Ala, Val, Leu and Ile
Xaa_7 is an amino acid residue selected from the group comprising Ala, Val, Leu and Ile
Xaa_8 is an amino acid residue selected from the group comprising Glu and Asp,
Xaa_9 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_1, Xaa_3, Xaa_6, Xaa_7, Xaa_8 and Xaa_9, are not involved in antibody binding or to a significantly smaller extent as compared to the His and the –Phe-Phe- binding site.

In a specific embodiment of the invention, the first of at least two consecutive amino acid residues predominantly involved in the binding of the antibody involve –Lys- and –Leu-, and the second of the at least two consecutive amino acid residues involve –Phe-Phe- embedded within the following core sequence:

$– Xaa_1 – Xaa_2 – Lys – Leu – Xaa_4 – Phe – Phe – Xaa_5 – Xaa_6 – Xaa_7 –$

wherein
Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;
Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;
Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;
Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;
Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,
Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp, , and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –Lys-Leu and the –Phe-Phe- binding site.

In another embodiment of the invention, an antibody or a fragment thereof is provided, wherein
Xaa_1 is His or Arg, but particularly His;
Xaa_2 is Gln or Asn, but particularly Gln;
Xaa_4 is Val or Leu, but particularly Val;
Xaa_5 is Ala or Val, but particularly Ala;
Xaa_6 is Glu or Asp, but particularly Glu; and

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Xaa_7 is Asp or Glu, but particularly Asp.

In a further embodiment of the invention, the antibody or a fragment thereof according to the invention binds to at least three distinct binding sites on the β-amyloid protein wherein said at least three distinct binding sites comprise at least one amino acid residue and at least two consecutive amino acid residues, respectively, which residues are predominantly involved in the binding of the antibody, wherein said at least three distinct binding sites are located in close proximity to each other on the antigen, separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to said at least one amino acid residue and said at least two consecutive amino acid residues, respectively, thus forming a conformational discontinuous epitope.

In a specific embodiment of the invention, the first of the at least two consecutive amino acid residues predominantly involved in the binding of the antibody involve –Lys-Leu-, and the second of the at least two consecutive amino acid residues involve –Phe-Phe-, and the third at least one amino residue involves -His- embedded within the following core sequence:

–His – Xaa_2 – Lys – Leu -Xaa_4 – Phe – Phe – Xaa_5 – Xaa_6 – Xaa_7 –

wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –His-, the -Lys-Leu, and the –Phe-Phe- binding site.

In another embodiment of the invention, an antibody or a fragment thereof is provided, wherein

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_4 is Val or Leu, but particularly Val;

Xaa_5 is Ala or Val, but particularly Ala;

Xaa_6 is Glu or Asp, but particularly Glu; and

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Xaa_7 is Glu or Asp, but particularly Asp;

In a specific embodiment of the invention, the first of the at least two consecutive amino acid residues predominantly involved in the binding of the antibody involve –Lys-Leu-, and the second of the at least two consecutive amino acid residues involve –Phe-Phe-, and the third at least one amino residue involves -Asp- embedded within the following core sequence:

$-Xaa_1-Xaa_2-Lys-Leu-Xaa_4-Phe-Phe-Xaa_5-Xaa_6-Asp-$

wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

In another embodiment of the invention, an antibody or a fragment thereof is provided, wherein

Xaa_1 is His or Arg, but particularly His;

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_4 is Val or Leu, but particularly Val;

Xaa_5 is Ala or Val, but particularly Ala; and

Xaa_6 is Glu or Asp, but particularly Glu

In a further specific embodiment of the invention, an antibody or a fragment thereof according to the invention is provided, which binds to 4 distinct binding sites on the β-amyloid protein wherein said 4 distinct binding sites comprise one amino acid residue and two consecutive amino acid residues, respectively, which residues are predominantly involved in the binding of the antibody, wherein said 4 distinct binding sites are located in close proximity to each other on the antigen, separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to said one amino acid residue and said two consecutive

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amino acid residues of the 4 distinct binding sites thus forming a conformational discontinuous epitope.

In particular, the first of the two consecutive amino acid residues predominantly involved in the binding of the antibody are –Lys-Leu-, and the second of the at least two consecutive amino acid residues are –Phe-Phe-, the first of the single amino residues is -His- and the second of the single amino residues is -Asp- embedded within the following core sequence:

– His – Xaa_2 – Lys – Leu -Xaa_4 – Phe – Phe – Xaa_5 – Xaa_6 – Asp –

wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –His-, –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

In a specific embodiment of the invention, the recognition and binding sites as defined herein before are forming a conformational discontinuous epitope localized in a region of the β-amyloid protein between amino acid residue 12 to 24, particularly between residues 14 to 23, more particularly between amino acid residues 14 and 20, wherein the three distinct recognition and binding sites comprising 1 and 2 amino acid residues, respectively, are located at position 16, 17, and at position 19 and 20, and at position 14, respectively, which residues are predominantly involved in the binding of the β-amyloid protein and wherein said three distinct recognition and binding sites are separated by one amino acid residue located at position 15 and 18, respectively, which amino acids are not involved in the binding of the antigen or, at least, to a substantially smaller extent.

In a specific embodiment, said consecutive amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region:

Val– His– His– Gln– Lys– Leu– Val– Phe– Phe– Ala– Glu– Asp

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12	13	14	15	16	17	18	19	20	21	22	23

In a further specific embodiment, said consecutive amino acid residues, particularly –Lys- at position 16, -Leu- at position 17 and -Phe- Phe- at position 19 and 20, and –His- at position 14, which are predominantly involved in the binding of the β-amyloid protein are embedded into the following core region:

Val–	His–	His–	Gln–	Lys–	Leu–	Val–	Phe–	Phe–	Ala–	Glu–	Asp–
12	13	14	15	16	17	18	19	20	21	22	23

Also part of the invention is the use of a monoclonal antibody and/or a functional part thereof according to the invention and as described herein before and/or a pharmaceutical composition, or a mixture comprising said antibody, for the preparation of a medicament for treating or alleviating the effects of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD and diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

In another embodiment of the present invention a method is provided for the preparation of a pharmaceutical composition using an antibody according to the invention and/or a functional part thereof but especially a monoclonal antibody and/or a functional part thereof or a functionally equivalent antibody, for use in treating or alleviating the effects of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not

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limited to, neurological disorders such as Alzheimer's Disease (AD) and diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration comprising formulating an antibody according to the invention in a pharmaceutically acceptable form.

The antibodies and/or functional parts thereof but especially the monoclonal antibodies and/or functional parts thereof or a functionally equivalent antibody and the compositions and mixtures comprising said antibody according to the present invention may be used for the preparation of a medicament for preventing, treating or alleviating the effects of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

In a further embodiment of the invention a method is provided for reducing the plaque load in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of an antibody and/or a functional part thereof but especially of the monoclonal antibody and/or a functional part thereof or of a functionally equivalent antibody

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according to the invention and as described herein before, or a composition or a mixture comprising said antibody.

In particular, the plaque load is reduced by at least 20 %, particularly by at least 25%, more particularly by at least 30%, even more particularly by more than 30%.

In a further embodiment of the invention a method for reducing the amount of plaques in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of an antibody and/or a functional part thereof but especially of the monoclonal antibody and/or a functional part thereof or of a functionally equivalent antibody according to the invention and as described herein before, or a composition or a mixture comprising said antibody.

In particular, the the amount of plaques in the brain is reduced by at least 10 %, particularly by at least 15%, more particularly by more than 15%..

In still another embodiment of the invention a method for decreasing the total amount of soluble Aβ in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to increased concentrations of soluble Aβ in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of an antibody and/or a functional part thereof but especially of the monoclonal antibody and/or a functional part thereof or of a functionally equivalent antibody according to the invention and as described herein before, or a composition or a mixture comprising said antibody.

It is an objective of the present invention to provide a method for preventing, treating or alleviating the effects of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for

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example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, by administering an antibody, but particularly a monoclonal antibody or a composition or mixture comprising such an antibody according to the invention to an animal or a human affected by such a disorder comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of an antibody and/or a functional part thereof but especially of the monoclonal antibody and/or a functional part thereof or of a functionally equivalent antibody according to the invention and as described herein before, or a composition or a mixture comprising said antibody.

In a specific embodiment the invention provides a method for retaining or increasing cognitive memory capacity of an animal, particularly a mammal or a human suffering from memory impairment by administering to an animal, particularly a mammal or a human in need of such a treatment, an antibody, but particularly a monoclonal antibody according to the invention or a composition or mixture comprising such an antibody according to the invention and as described herein before.

In another embodiment of the present invention a method is provided for the preparation of a pharmaceutical composition using an antibody according to the invention and/ora functional part thereof but especially a monoclonal antibody and/or a functional part thereof or a functionally equivalent antibody for preventing, treating or alleviating the effects of diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins including amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as

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other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

In a specific embodiment the invention provides a method for the preparation of a pharmaceutical composition using an antibody according to the invention and/ora functional part thereof but especially a monoclonal antibody and/or a functional part thereof or a functionally equivalent antibody for retaining or increasing cognitive memory capacity of an animal, particularly a mammal or a human, suffering from memory impairment by administering to an animal, particularly a mammal or a human, an antibody, but particularly a monoclonal antibody or a composition or mixture comprising such an antibody according to the invention and as described herein before.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF FIGURES AND SEQUENCES

Figure 1 (Example 2): Expression Cassette of the mouse light chain variable region of the Chimeric Antibody;

Figure 2 (Example 2): Expression Cassette of the mouse heavy chain variable region of the Chimeric Antibody;

Figure 3 (Example 5.2): Comparison of the mouse heavy chain variable region to the closest murine germ line sequence;

Figure 4 (Example 8): Activity of purified humanized C2 antibodies;

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Figure 5 (Example 9): Binding activity of antibodies produced by transient expression of C2 modified CDRL2 constructs in conjunction with C2 chimeric heavy chain, compared to chimeric antibody C2ChVHAF/ChVK, produced by transient transfection and purified antibody;

Figure 6 (Example 11): Results of Immunohistochemical Binding Assay with chimeric antibody AF and humanized antibody H4K1;

Figure 7 (Example 12): Functionality of mC2 on Amyloid fibers;

Figure 8 (Example 12): Binding Affinity of humanized C2 in ELISA;

Figure 9 (Example 14): Conformation specific binding of mC2 to different classes of amyloid protein. Pellet preparation in the legend to this figure refers to $A\beta_{1-42}$ fibers, supernatant preparation refers to amyloid monomers;

Figure 10: Humanized C2 VK sequences compared to murine sequence and human acceptor sequences DPK15 AND J_K1;

Figure 11: Humanized C2 VH sequences compared to murine sequence and human acceptor sequences DP54 AND J_H6;

Figure 12: Complete DNA and protein sequence of light chain variable region of C2 humanized antibody, C2HuVK1;

Figure 13: Complete DNA and protein sequence of light chain constant region (human C Kappa) of humanized C2 antibody;

Figure 14: Complete DNA and protein sequence of heavy chain constant region (human IgG4 ser228-pro) of humanized C2 antibody;

Figure 15A-C (Example 15): Results of Epitope Mapping experiments;

Figure 16 (Example 13): Results of aggregation assay experiments;

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Figure 17 (Example 13): Results of disaggregation assay experiments;

Figure 18: (Example 16): Results of neuroprotection experiments with humanized antibody C2;

Figure 19: Binding of mACI-01-Ab7 C2 monoclonal antibody to amyloid species in Western blot and Dot blot;

Figure 20: Binding of mACI-01-Ab7 C2 monoclonal antibody to amyloid fibers by transmission electronic microscopy;

Figure 21: Results of a head-to-head-experiment between Th-T fluorescent assay and solid-state NMR of U-^{13}C Tyr10 and Val12-labeled β-amyloid 1-42 peptide.

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SEQ ID NO: 1 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR1)

SEQ ID NO: 2 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR2)

SEQ ID NO: 3 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR3)

SEQ ID NO: 4 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR1)

SEQ ID NO: 5 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR2)

SEQ ID NO: 6 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR3)

SEQ ID NO: 7 Amino acid sequence of Aβ epitope region 2

SEQ ID NO: 8 Amino acid sequence of Aβ epitope region 1

SEQ ID NO: 9 Amino acid sequence of Aβ epitope region 2 modified

SEQ ID NO: 10 Amino acid sequence of Aβ epitope region 1 modified

SEQ ID NO: 11 Amino acid sequence of Epitope region modified complete

SEQ ID NO: 12 Amino acid sequence of C2 HuVK 1 humanized light chain variable region

SEY ID NO: 13 Amino acid sequence of C2 humanized light chain

SEQ ID NO: 14 Amino acid sequence of humanized C2 light chain constant region

SEQ ID NO: 15 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region

SEQ ID NO: 16 Amino acid sequence of C2 humanized heavy chain

SEQ ID NO: 17: Amino acid sequence of IG GAMMA-4 CHAIN C REGION – modified

SEQ ID NO: 18: Nucleotide sequence of CDR2 of C2 HuVH AF 4 humanised heavy chain variable region

SEQ ID NO: 19: Nucleotide sequence of CDR3 of C2 HuVH AF 4 humanised heavy chain variable region

SEQ ID NO: 20: Nucleotide sequence of CDR1 of C2 HuVK 1 humanised light chain variable region

SEQ ID NO: 21: Nucleotide sequence of C2 HuVK 1 humanized light chain variable region

SEQ ID NO: 22: Nucleotide sequence of C2 humanized light chain

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SEQ ID NO: 23: Nucleotide sequence of C2 humanized light chain constant region

SEQ ID NO: 24: Nucleotide sequence of C2 HuVH AF 4 humanized heavy chain variable region

SEQ ID NO: 25: Nucleotide sequence of C2 humanized heavy chain

SEQ ID NO: 26: Nucleotide sequence of C2 humanized heavy chain constant region

SEQ ID NO: 27: Amino acid sequence of Mouse C2 Light Chain Variable Region

SEQ ID NO: 28: Amino acid sequence of Mouse C2 Heavy Chain Variable Region

SEQ ID NO: 29: Nucleotide sequence of Mouse C2 Light Chain Variable Region

SEQ ID NO: 30: Nucleotide sequence of Mouse C2 Light Chain

SEQ ID NO: 31: Nucleotide sequence of Mouse C2 Heavy Chain Variable Region

SEQ ID NO: 32: Nucleotide sequence of Mouse C2 Heavy Chain

SEQ ID NO: 33-40: Amino acid sequence variants of epitopic region on the Aβ peptide

SEQ ID NO: 41: Amino acid sequence of Mouse C2 Light Chain

SEQ ID NO: 42: Amino acid sequence of Mouse C2 Heavy Chain

SEQ ID NO: 43: Antigenic peptide $A\beta_{1-15}$

SEQ ID NO: 44: Antigenic peptide $A\beta_{1-16}$

SEQ ID NO: 45: Antigenic peptide $A\beta_{1-16(\Delta 14)}$

SEQ ID NO: 46: Antigenic peptide $A\beta_{22-35}$

SEQ ID NO: 47: Antigenic peptide $A\beta_{29-40}$

SEQ ID NO: 48: Antigenic peptide $A\beta_{1-17}$

DEFINITIONS

The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeable and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

The language "diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins" includes, but is not limited to, diseases and disorders caused by the presence or activity of amyloid-like proteins in monomeric, fibril, or polymeric state, or any

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combination of the three. Such diseases and disorders include, but are not limited to, amyloidosis, endocrine tumors, and macular degeneration.

The term "amyloidosis" refers to a group of diseases and disorders associated with amyloid plaque formation including, but not limited to, secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes, and senile cardiac amyloidosis; and various eye diseases including macular degeneration, drusen-related optic neuropathy, and cataract due to beta-amyloid deposition.

The terms "detecting" or "detected" as used herein mean using known techniques for detection of biologic molecules such as immunochemical or histological methods and refer to qualitatively or quantitatively determining the presence or concentration of the biomolecule under investigation.

"Polymeric soluble amyloid" refers to multiple aggregated monomers of amyloid peptides, or of amyloid-like peptides, or of modified or truncated amyloid peptides or of other derivates of amyloid peptides forming oligomeric or polymeric structures which are soluble in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of amyloid β (Aβ) or of modified or truncated amyloid β (Aβ) peptides or of derivatives thereof, which are soluble in the mammalian or human body more particularly in the brain.

"Amyloid β, Aβ or β-amyloid" is an art recognized term and refers to amyloid β proteins and peptides, amyloid β precursor protein (APP), as well as modifications, fragments and any functional equivalents thereof. In particular, by amyloid β as used herein is meant any fragment produced by proteolytic cleavage of APP but especially those fragments which are involved in or

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associated with the amyloid pathologies including, but not limited to, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$, $A\beta_{1-41}$ $A\beta_{1-42}$ and $A\beta_{1-43}$.

The structure and sequences of the amyloid β peptides as mentioned above are well known to those skilled in the art and methods of producing said peptides or of extracting them from brain and other tissues are described, for example, in Glenner and Wong, Biochem Biophys Res Comm129, 885-890 (1984). Moreover, amyloid β peptides are also commercially available in various forms.

By "isolated" is meant a biological molecule free from at least some of the components with which it naturally occurs.

The terms "antibody" or "antibodies" as used herein are art-recognized terms and are understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e molecules that contain a binding site that specifically binds an antigen. An immunoglobulin is a protein comprising one or more polypeptides substantially encoded by the immunoglobulin kappa and lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Also subclasses of the heavy chain are known. For example, IgG heavy chains in humans can be any of IgG1, IgG2, IgG3 and IgG4 subclass. The immunoglobulin according to the invention can be of any class (IgG, IgM, IgD, IgE, IgA and IgY) or subclass (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) of immunoglobulin molecule.

As used herein "specifically binds" in reference to an antibody means that the antibody binds to its target antigen with greater affinity that it does to a structurally different antigen(s).

A typical immunoglobulin structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition.

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The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist as full length intact antibodies or as a number of well-characterized fragments produced by digestion with various peptidases or chemicals. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab')_2$, a dimer of Fab which itself is a light chain joined to V_H-CH_1 by a disulfide bond. The $F(ab')_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $F(ab')_2$ dimer into an Fab' monomer. The Fab' monomer is essentially a Fab fragment with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that any of a variety of antibody fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo or antibodies and fragments obtained by using recombinant DNA methodologies.

"Antibodies" are intended within the scope of the present invention to include monoclonal antibodies, polyclonal antibodies, chimeric, single chain, bispecific, simianized, human and humanized antibodies as well as active fragments thereof. Examples of active fragments of molecules that bind to known antigens include separated light and heavy chains, Fab, Fab/c, Fv, Fab', and $F(ab')_2$ fragments, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, 1986.

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Recombinantly made antibodies may be conventional full length antibodies, active antibody fragments known from proteolytic digestion, unique active antibody fragments such as Fv or single chain Fv (scFv), domain deleted antibodies, and the like. An Fv antibody is about 50 Kd in size and comprises the variable regions of the light and heavy chain. A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which may be expressed from a nucleic acid including VH- and VL-encoding sequences either joined directly or joined by a peptide-encoding linker. See Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85:5879-5883. A number of structures for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778.

The combining site refers to the part of an antibody molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. The antibody variable regions comprise three highly divergent stretches referred to as "hypervariable regions" or "complementarity determining regions" (CDRs) which are interposed between more conserved flanking stretches known as "framework regions" (FRs). In an antibody molecule, the three hypervariable regions of a light chain (LCDR1, LCDR2, and LCDR3) and the three hypervariable regions of a heavy chain (HCDR1, HCDR2 and HCDR3) are disposed relative to each other in three dimensional space to form an antigen binding surface or pocket. The antibody combining site therefore represents the amino acids that make up the CDRs of an antibody and any framework residues that make up the binding site pocket.

The identity of the amino acid residues in a particular antibody that make up the combining site can be determined using methods well known in the art. For example, antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services; Johnson, G and Wu, TT (2001) Kabat Database and its applications: future directions. Nucleic Acids Research, 29: 205-206; http://immuno.bme.nwa.edu). The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others, (see Chothia and Lesk, J. Mol. Biol. 196, 901 (1987), Chothia et al., Nature 342, 877 (1989), and Tramontano et al., J. Mol. Biol. 215, 175 (1990)). Other methods include the "AbM

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definition" which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys) or the "contact definition" of CDRs by Macallum et al., ("Antibody-antigen interactions: contact analysis and binding site topography," J Mol Biol. 1996 Oct 11;262(5):732-45). The following chart identifies CDRs based upon various known definitions.

Loop	Kabat	AbM	Chothia	Contact
L1	L24 -- L34	L24 -- L34	L24 -- L34	L30 -- L36
L2	L50 -- L56	L50 -- L56	L50 -- L56	L46 -- L55
L3	L89 -- L97	L89 -- L97	L89 -- L97	L89 -- L96
H1 (Kabat Numbering)	H31 -- H35B	H26 -- H35B	H26 -- H32..34	H30 -- H35B
H1 (Chothia Numbering)	H31 -- H35	H26 -- H35	H26 -- H32	H30 -- H35
H2	H50 -- H65	H50 -- H58	H52 -- H56	H47 -- H58
H3	H95 -- H102	H95 -- H102	H95 -- H102	H93 -- H101

General guidelines by which one may identify the CDRs in an antibody from sequence alone are as follows:

LCDR1:

Start - Approximately residue 24.

Residue before is always a Cys.

Residue after is always a Trp. Typically TRP is followed with TYR-GLN, but also may be followed by LEU-GLN, PHE-GLN, or TYR-LEU.

Length is 10 to 17 residues.

LCDR2:

Start - 16 residues after the end of L1.

Sequence before is generally ILE-TYR, but also may be VAL-TYR, ILE-LYS, or ILE-PHE.

Length is generally 7 residues.

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LCDR3:

Start – generally 33 residues after end of L2.

Residue before is a Cys.

Sequence after is PHE-GLY-X-GLY.

Length is 7 to 11 residues.

HCDR1:

Start – at approximately residue 26 (four residues after a CYS) [Chothia / AbM definition] Kabat definition starts 5 residues later.

Sequence before is CYS-X-X-X.

Residues after is a TRP, typically followed by VAL, but also followed by ILE, or ALA.

Length is 10 to 12 residues under AbM definition while Chothia definition excludes the last 4 residues.

HCDR2:

Start - 15 residues after the end of Kabat /AbM definition of CDR-H1.

Sequence before typically LEU-GLU-TRP-ILE-GLY (SEQ ID NO. 1), but a number of variations are possible.

Sequence after is LYS/ARG-LEU/ILE/VAL/PHE/THR/ALA-THR/SER/ILE/ALA

Length is 16 to 19 residues under Kabat definition (AbM definition ends 7 residues earlier).

HCDR3:

Start –33 residues after end of CDR-H2 (two residues after a CYS).

Sequence before is CYS-X-X (typically CYS-ALA-ARG).

Sequence after is TRP-GLY-X-GLY.

Length is 3 to 25 residues.

The identity of the amino acid residues in a particular antibody that are outside the CDRs, but nonetheless make up part of the combining site by having a side chain that is part of the lining of the combining site (i.e., it is available to linkage through the combining site), can be determined using methods well known in the art such as molecular modeling and X-ray crystallography. See e.g., Riechmann et al., (1988) Nature, 332:323-327.

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Chimeric antibodies are those in which one or more regions of the antibody are from one species of animal and one or more regions of the antibody are from a different species of animal. A preferred chimeric antibody is one which includes regions from a primate immunoglobulin. A chimeric antibody for human clinical use is typically understood to have variable regions from a non-human animal, e.g. a rodent, with the constant regions from a human. In contrast, a humanized antibody uses CDRs from the non-human antibody with most or all of the variable framework regions from and all the constant regions from a human immunoglobulin. A human chimeric antibody is typically understood to have the variable regions from a rodent. A typical human chimeric antibody has human heavy constant regions and human light chain constant regions with the variable regions of both the heavy and light coming from a rodent antibody. A chimeric antibody may include some changes to a native amino acid sequence of the human constant regions and the native rodent variable region sequence. Chimeric and humanized antibodies may be prepared by methods well known in the art including CDR grafting approaches (see, e.g., U.S. Patent Nos. 5,843,708; 6,180,370; 5,693,762; 5,585,089; 5,530,101), chain shuffling strategies (see e.g., U.S. Patent No. 5,565,332; Rader et al., Proc. Natl. Acad. Sci. USA (1998) 95:8910-8915), molecular modeling strategies (U.S. Patent No. 5,639,641), and the like.

A "humanized antibody" as used herein in the case of a two chain antibody is one where at least one chain is humanized. A humanized antibody chain has a variable region where one or more of the framework regions are human. A humanized antibody which is a single chain is one where the chain has a variable region where one or more of the framework regions are human. The non-human portions of the variable region of the humanized antibody chain or fragment thereof is derived from a non-human source, particularly a non-human antibody, typically of rodent origin. The non-human contribution to the humanized antibody is typically provided in form at least one CDR region which is interspersed among framework regions derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity.

The humanized antibody may further comprise constant regions (*e.g.*, at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the

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case of a heavy chain). The constant regions of a humanized antibody if present generally are human.

Methods to obtain "humanized antibodies" are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured human-like polyclonal antibodies in large animals such as, for example, rabbits and mice. See, e.g. U.S. Pat No. 6,632,976.

The term constant region (CR) as used herein refers to constant regions genes of the immunoglobulin. The constant region genes encode the portion of the antibody molecule which confers effector functions. For Chimeric human antibodies and humanized antibodies, typically non-human (*e.g.*, murine), constant regions are substituted by human constant regions. The constant regions of the subject chimeric or humanized antibodies are typically derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. Further, heavy chains of various subclasses (such as the IgG subclasses of heavy chains) are responsible for different effector functions and thus, by choosing the desired heavy chain constant region, antibodies with desired effector function can be produced. Constant regions that may be used within the scope of this invention are gamma 1 (IgGl), particularly an Fc region of the gamma 1 (IgG1) isotype, gamma 3 (IgG3) and especially gamma 4 (IgG4). The light chain constant region can be of the kappa or lambda type, preferably of the kappa type. In one embodiment the light chain constant region is the human kappa constant chain (Heiter *et al.* (1980) *Cell* 22:197-207) and the heavy constant chain is the human IgG4 constant chain.

The term "monoclonal antibody" is also well recognized in the art and refers to an antibody that is the product of a single cloned antibody producing cell. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces the antibody.

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For the purpose of the present invention, "monoclonal antibody" is also to be understood to comprise antibodies that are produced by a mother clone which has not yet reached full monoclonality.

"Functionally equivalent antibody" is understood within the scope of the present invention to refer to an antibody which substantially shares at least one major functional property with an antibody mentioned above and herein described comprising: binding specificity to the β-amyloid protein, particularly to the $A\beta_{1-42}$ protein, and more particularly to the 16-21 epitope region of the $A\beta_{1-42}$ protein, immunoreactivity *in vitro*, inhibition of aggregation of the $A\beta_{1-42}$ monomers into high molecular polymeric fibrils and/or disaggregation of preformed $A\beta_{1-42}$ polymeric fibrils, and/or a β-sheet breaking property and alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, when administered prophylactically or therapeutically. The antibodies can be of any class such as IgG, IgM, or IgA, etc or any subclass such as IgG1, IgG2a, etc and other subclasses mentioned herein above or known in the art, but particularly of the IgG4 class. Further, the antibodies can be produced by any method, such as phage display, or produced in any organism or cell line, including bacteria, insect, mammal or other type of cell or cell line which produces antibodies with desired characteristics, such as humanized antibodies. The antibodies can also be formed by combining a Fab portion and an Fc region from different species.

The term "hybridize" as used refers to conventional hybridization conditions, preferably to hybridization conditions at which 5xSSPE, 1% SDS, 1xDenhardts solution is used as a solution and/or hybridization temperatures are between 35°C and 70°C, preferably 65°C. After hybridization, washing is preferably carried out first with 2xSSC, 1% SDS and subsequently with 0.2xSSC at temperatures between 35°C and 70°C, preferably at 65°C (regarding the

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definition of SSPE, SSC and Denhardts solution see Sambrook et al. loc. cit.). Stringent hybridization conditions as for instance described in Sambrook et al, supra, are particularly preferred. Particularly preferred stringent hybridization conditions are for instance present if hybridization and washing occur at 65°C as indicated above. Non-stringent hybridization conditions, for instance with hybridization and washing carried out at 45°C are less preferred and at 35°C even less.

"Homology" between two sequences is determined by sequence identity. If two sequences which are to be compared with each other differ in length, sequence identity preferably relates to the percentage of the nucleotide residues of the shorter sequence which are identical with the nucleotide residues of the longer sequence. Sequence identity can be determined conventionally with the use of computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive Madison, WI 53711). Bestfit utilizes the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2 (1981), 482-489, in order to find the segment having the highest sequence identity between two sequences. When using Bestfit or another sequence alignment program to determine whether a particular sequence has for instance 95% identity with a reference sequence of the present invention, the parameters are preferably so adjusted that the percentage of identity is calculated over the entire length of the reference sequence and that homology gaps of up to 5% of the total number of the nucleotides in the reference sequence are permitted. When using Bestfit, the so-called optional parameters are preferably left at their preset ("default") values. The deviations appearing in the comparison between a given sequence and the above-described sequences of the invention may be caused for instance by addition, deletion, substitution, insertion or recombination. Such a sequence comparison can preferably also be carried out with the program "fasta20u66" (version 2.0u66, September 1998 by William R. Pearson and the University of Virginia; see also W.R. Pearson (1990), Methods in Enzymology 183, 63-98, appended examples and http://workbench.sdsc.edu/). For this purpose, the "default" parameter settings may be used.

The antibody according to the invention may be an immunoglobulin or antibody, which is understood to have each of its binding sites identical (if multivalent) or, in the alternative, may be a "bispecific" or "bifunctional antibody".

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A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

The term "fragment" refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. Fragments can be obtained *via* chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. Exemplary fragments include Fab, Fab', F(ab')2, Fabc and/or Fv fragments. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (*i.e.*, with the intact antibody from which they were derived) for antigen binding (*i.e.*, specific binding).

Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', $F(ab')_2$, Fabc, Fv, single chains, and single-chain antibodies.

"Fragment" also refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of another polypeptide. In a specific embodiment, a fragment of a polypeptide retains at least one function of the polypeptide.

The term "antigen" refers to an entity or fragment thereof which can bind to an antibody. An immunogen refers to an antigen which can elicit an immune response in an organism,

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particularly an animal, more particularly a mammal including a human. The term antigen includes regions known as antigenic determinants or epitopes which refers to a portion of the antigen (which are contacted or which play a significant role in supporting a contact reside in the antigen responsible for antigenicity or antigenic determinants.

As used herein, the term "soluble" means partially or completely dissolved in an aqueous solution.

Also as used herein, the term "immunogenic" refers to substances which elicit the production of antibodies, T-cells and other reactive immune cells directed against an antigen of the immunogen.

An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present invention to moderate or alleviate the disorder to be treated.

The term immunogenicity as used herein refers to a measure of the ability of an antigen to elicit an immune response (humoral or cellular) when administered to a recipient. The present invention is concerned with approaches that reduce the immunogenicity of the subject human chimeric or humanized antibodies.

Humanized antibody of reduced immunogenicity refers to a humanized antibody exhibiting reduced immunogenicity relative to the parent antibody, e.g., the murine antibody.

Humanized antibody substantially retaining the binding properties of the parent antibody refers to a humanized antibody which retains the ability to specifically bind the antigen recognized by the parent antibody used to produce such humanized antibody. Preferably the humanized antibody will exhibit the same or substantially the same antigen-binding affinity and avidity as the parent antibody. Ideally, the affinity of the antibody will not be less than 10% of the parent antibody affinity, more preferably not less than about 30%, and most preferably the affinity will not be less than 50% of the parent antibody. Methods for assaying antigen-binding affinity are well known in the art and include half-maximal binding assays, competition assays, and Scatchard analysis. Suitable antigen binding assays are described in this application.

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A "back mutation" is a mutation introduced in a nucleotide sequence which encodes a humanized antibody, the mutation results in an amino acid corresponding to an amino acid in the parent antibody (*e.g.*, donor antibody, for example, a murine antibody). Certain framework residues from the parent antibody may be retained during the humanization of the antibodies of the invention in order to substantially retain the binding properties of the parent antibody, while at the same time minimizing the potential immunogenicity of the resultant antibody. In one embodiment of the invention, the parent antibody is of mouse origin. For example, the back mutation changes a human framework residue to a parent murine residue. Examples of framework residues that may be back mutated include, but are not limited to, canonical residues, interface packing residues, unusual parent residues which are close to the binding site, residues in the "Vernier Zone" (which forms a platform on which the CDRs rest) (Foote & Winter, 1992, *J. Mol. Biol.* 224, 487-499), and those close to CDR H3.

As used herein a "conservative change" refers to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants of the mutant polypeptides, respectively, as compared to the native protein. When referring to the antibodies and antibody fragments of the invention, a conservative change means an amino acid substitution that does not render the antibody incapable of binding to the subject receptor. Those of ordinary skill in the art will be able to predict which amino acid substitutions can be made while maintaining a high probability of being conformationally and antigenically neutral. Such guidance is provided, for example in Berzofsky, (1985) *Science* 229:932-940 and Bowie *et al.* (1990) *Science* 247:1306-1310. Factors to be considered that affect the probability of maintaining conformational and antigenic neutrality include, but are not limited to: (a) substitution of hydrophobic amino acids is less likely to affect antigenicity because hydrophobic residues are more likely to be located in a protein's interior; (b) substitution of physiochemically similar, amino acids is less likely to affect conformation because the substituted amino acid structurally mimics the native amino acid; and (c) alteration of evolutionarily conserved sequences is likely to adversely affect conformation as such conservation suggests that the amino acid sequences may have functional importance. One of ordinary skill in the art will be able to assess alterations in protein conformation using well-known assays, such as, but not limited to microcomplement fixation methods (Wasserman *et al.* (1961) J. Immunol. 87:290-295; Levine *et*

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al. (1967) Meth. Enzymol. 11:928-936) and through binding studies using conformation-dependent monoclonal antibodies (Lewis *et al.* (1983) Biochem. 22:948-954).

Further, the term "therapeutically effective amount" refers to the amount of antibody which, when administered to a human or animal, which is sufficient to result in a therapeutic effect in said human or animal. The effective amount is readily determined by one of skill in the art following routine procedures.

As used herein, the terms "treat," "prevent," "preventing," and "prevention" refer to the prevention of the recurrence or onset of one or more symptoms of a disorder in a subject resulting from the administration of a prophylactic or therapeutic agent.

Construction of Humanized Antibodies

The present invention may be understood more readily by reference to the following detailed description of specific embodiments included herein. Although the present invention has been described with reference to specific details of certain embodiments, thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention.

The present invention provides novel methods and compositions comprising highly specific and highly effective antibodies having the ability to specifically recognize and bind to specific epitopes from a range of β-amyloid antigens. The antibodies enabled by the teaching of the present invention are particularly useful for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, to name just a few.

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A fully humanized or reshaped variable region according to the present invention may be created within the scope of the invention by first designing a variable region amino acid sequence that contains non-human-, particularly rodent-derived CDRs, but especially CDRs derived from murine antibody ACI-01-Ab7C2 (named "mC2" throughout the application and deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty and given accession no DSM ACC2750) embedded in human-derived framework sequences. The non-human-, particularly the rodent-derived CDRs, which may be obtained from the antibody according to the present invention, provide the desired specificity. Accordingly, these residues are to be included in the design of the reshaped variable region essentially unchanged. Any modifications should thus be restricted to a minimum and closely watched for changes in the specificity and affinity of the antibody. On the other hand, framework residues in theory can be derived from any human variable region.

In order to create a reshaped antibody which shows an acceptable or an even improved affinity, a human framework sequences should be chosen, which is equally suitable for creating a reshaped variable region and for retaining antibody affinity.

In order to achieve this goal, the best-fit strategy was developed. As it is known that the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding, this strategy aims at minimizing changes that may negatively effect the three-dimensional structure of the antibody by deriving the human framework sequence used for antibody reshaping from the human variable region that is most homologous or similar to the non-human-, particularly the rodent-derived variable region. This will also maximise the likelihood that affinity will be retained in the reshaped antibody.

At its simplest level, the "best fit" strategy involves comparing the donor rodent V-region with all known human V-region amino acid sequences, and then selecting the most homologous to provide the acceptor framework regions for the humanization exercises. In reality there are several other factors which should be considered, and which may influence the final selection of acceptor framework regions. Molecular modelling predictions may be used in this regard prior to any experimental work in an attempt to maximise the affinity of the resultant reshaped

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antibody. Essentially, the goal of the modelling is to predict which key residues (if any) of the most homologous human framework should be left as in the rodent to obtain the best affinity in the reshaped antibody.

In one embodiment of the invention, the CDRs are obtainable from mouse monoclonal antibody, particularly from mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" throughout the application) described in co-pending application EP 05 02 7092.5 filed 12.12.2005, the disclosure of which is incorporated herein by reference.

Hybridoma cells FP-12H3-C2, producing mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) were deposited 01 December 2005 in co-pending application no EP05027092.5 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Braunschweig, under the provisions of the Budapest Treaty and given accession no DSM ACC2750.

The mouse antibody may be raised against a supramolecular antigenic construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide, particularly of β-amyloid peptide $A\beta_{1-15}$, $A\beta_{1-16}$ and $A\beta_{1-16(\Delta 14)}$, modified with a hydrophobic moiety such as, for example, palmitic acid or a hydrophilic moiety such as, for example, polyethylene glycol (PEG) or a combination of both, wherein the hydrophobic and hydrophilic moiety, respectively, is covalently bound to each of the termini of the antigenic peptide through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophobic and hydrophilic moiety to the peptide fragment. When a PEG is used as the hydrophilic moiety, the free PEG termini is covalently bound to phosphatidylethanolamine or any other compound suitable to function as the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome.

In particular, a mouse antibody may be raised against a supramolecular antigenic construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide $A\beta_{1-16}$ modified with a hydrophilic moiety such as, for example, polyethylene glycol (PEG) hydrophilic moiety is covalently bound to each of the termini of the antigenic peptide

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through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophobic and hydrophilic moiety to the peptide fragment. When a PEG is used as the hydrophilic moiety, the free PEG termini are covalently bound to phosphatidylethanolamine or any other compound suitable to function as the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome.

In an embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided which comprises in the variable region at least one CDR of non-human origin embedded in one or more human- or primate-derived framework regions and combined with a constant region derived from a human or primate source antibody, which chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is capable of specifically recognizing and binding β-amyloid monomeric peptide.

The CDRs contain the residues most likely to bind antigen and must be retained in the reshaped antibody. CDRs are defined by sequence according to Kabat *et al.*, Sequence of Proteins of Immunological Interest, 5th Edition, The United States Department of Health and Human Services, The United States Government Printing Office, 1991. CDRs fall into canonical classes (Chothia *et al*, 1989 *Nature*, 342, 877-883) where key residues determine to a large extent the structural conformation of the CDR loop. These residues are almost always retained in the reshaped antibody.

In the process for preparing a humanized antibody according to the invention, the amino acid sequences of the C2 heavy chain and light chain variable regions (V_H and V_K) are compared to rodent antibody V_H and V_K sequences in the NCBI and Kabat databases.

The closest match mouse germ line gene to C2 V_K is bb1, Locus MMU231201, (Schable *et al*, 1999). A comparison reveals that two amino acids differ from this germ line sequence, both located within CDRL1. Mature murine antibodies with similar, but not identical, sequence can be found. Several have an identical CDRL2 and identical CDRL3, but the CDRL1 of C2 seems to be unique. Comparison with human germ line V_K sequences shows that genes from subgroup V_KII are the best match for C2 V_K (Cox *et al*, 1994). C2 V_K can thus be assigned to Kabat subgroup MuV_KII.Sequence.

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DPK15 together with the human J region HuJ_K1 may be selected to provide the acceptor framework sequences for the humanized V_K.

The residues at the interface between the variable light and heavy chains have been defined (Chothia *et al*, 1985 *J. Mol. Biol.*, 186, 651-663). These are usually retained in the reshaped antibody. The Phe at position 87 of mouse C2 V_K is unusual at the interface, where a Tyr is more common in the V_KII subgroup, indicating that this framework residue may be important for antibody activity. Tyr 87 is present in the human germline and humanized C2VK.

The humanized V_K sequences thus may be designed such that the C2HuVK1 consists of mouse C2 V_K CDRs with frameworks from DPK 15 and human J_K1. In a specific embodiment of the invention, murine residues may be substituted in the human framework region at positions 45, and/or 87. In the CDR2 region obtainable from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, amino acid substitutions may be made at Kabat positions 50 and/or 53. Residue 45 may be involved in supporting the conformation of the CDRs. Residue 87 is located at the interface of the V_H and V_K domains. Therefore these residues may be critical for maintenance of antibody binding.

The closest match mouse germ line gene to C2 V_H AF is VH7183, Locus AF120466, (Langdon *et al*, 2000). Comparison with human germ line V_H sequences shows that genes from subgroup V_HIII are the best match for C2 V_H. C2 V_H AF can be assigned to Kabat subgroup MuV_HIIID. Sequence DP54 together with the human J region HuJ_H6 can be selected to provide the acceptor framework sequences for the humanized V_H.

The comparison shows that there are nine amino acid differences between the C2 VH sequences and the human acceptor germ line sequence DP54 and J_H6, most being located within CDRH2. Mature murine antibodies with identical or similar (one residue different) CDRH1 or with similar CDRH2 (one residue different) are found, but none with all three CDRs identical to C2 V_H AF. CDRH3 of C2 antibody is unusually short, consisting of only three residues. However, other antibodies are found in the database with CDRH3 of this length. Residue 47 of C2 V_H is Leu rather than the more common Trp, and residue 94 is Ser rather than the normal Arg, indicating that these framework residues may be important for antibody activity.

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Various humanized V_H sequences may be designed. C2HuVH1 consists of C2 V_H AF CDRs with frameworks from DP54 and HuJ_H6. In a specific embodiment of the invention, murine residues may be substituted in the human framework region at positions 47 or 94 or both. Residue 47 in framework 2 makes contact both with the CDRs and with the V_K domain. Residue 94 may be involved in supporting the conformation of the CDRs. Therefore these residues may be critical for maintenance of antibody binding.

Different HCVR and LCVR regions may be designed which comprise the non-human CDRs obtainable from the donor antibody, for example, a murine antibody, embedded into the native or modified human- or primate-derived framework regions. The modification may particularly concern an exchange of one or more amino acid residues within the framework region by non-human residues, particularly murine residues, more commonly found in this position in the respective subgroups or by residues which have similar properties to the ones more commonly found in this position in the respective subgroups.

The modification of the framework region the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding. In one embodiment of the invention measures are taken to further adapt the selected human framework sequences to make them most similar to the sequences of the rodent frameworks in order to maximise the likelihood that affinity will be retained in the reshaped antibody.

Accordingly, murine residues in the human framework region may be substituted. In particular, murine residues may be substituted in the human framework region of the Heavy Chain Variable (HCVR) region at positions 47 or 94 or both and in the human framework region of the Light Chain Variable (LCVR) region at positions 45 and/or 87. In the CDR2 region obtainable from a mouse monoclonal antibody, particularly murine antibody ACI-01-Ab7C2, amino acid substitutions may be made at Kabat positions 50 and/or 53..

The residues found in the above indicated positions in the human framework region may be exchanged by murine residues more commonly found in this position in the respective subgroups. In particular, the Trp in Kabat position 47 in the human- or primate-derived

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framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 may be replaced by an Leu or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 may further be replaced by an amino acid selected from the group consisting of norleucine, Ile, Val, Met, Ala, and Phe, particularly by Ile. Alternative conservative substitutions may be contemplated which are conformationally and antigenically neutral.

The Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 may be replaced by Ser or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 may alternatively be replaced by Thr.

In another embodiment of the invention, both residues may be replaced in the humanized antibody.

The Gln in Kabat position 45 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by Lys or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Gln in Kabat position 45 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by an amino acid selected from the group consisting of Arg, Gln, and Asn, particularly by Arg.

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The Leu in Kabat position 50 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by Lys or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Leu in Kabat position 50 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by an amino acid selected from the group consisting of Arg, Gln, and Asn, particularly by Arg.

The Asn in Kabat position 53 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by His and Gln or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Asn in Kabat position 53 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by an amino acid selected from the group consisting of Gln, His, Lys and Arg.

The Thr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by Phe or by an amino acid residue that has similar properties and the substitution of which leads to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants. In particular, the Tyr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by an amino acid selected from the group consisting of Leu, Val, Ile, and Ala, particularly by Leu.

The so obtained variable region comprising at least one CDR of non-human origin embedded in one or more human- or primate-derived framework regions may then be combined with a constant region derived from a human or primate source antibody, particularly with human IgG4

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or κ constant regions respectively. The IgG4 constant region may be modified by, for example, changing Serine at position 228 in the hinge region to Proline (HuIgG4 Ser-Pro). This mutation stabilizes the interchain disulphide bond and prevents the formation of half molecules that may occur in native human IgG4 preparations. The IgG4 constant region may be further modified by deletion of the terminal Lys in position 439 as shown in SEQ ID NO: 16.

The modified variable regions may be constructed by method known in the art such as, for example overlapping PCR recombination. The expression cassettes for the chimeric antibody, C2 ChV_H AF and C2 ChV_K, may be used as templates for mutagenesis of the framework regions to the required sequences. Sets of mutagenic primer pairs are synthesized encompassing the regions to be altered. The humanized V_H and V_K expression cassettes produced may be cloned into appropriate cloning vectors know in the art such as, for example, pUC19. After the entire DNA sequence is confirmed to be correct for each V_H and V_K, the modified heavy and light chain V-region genes can be excised from the cloning vector as expression cassettes. These can then be transferred to appropriate expression vectors such as pSV*gpt* and pSV*hyg* which include human IgG4 Ser-Pro or κ constant regions respectively.

EXPRESSION VECTORS

Expression vector *pSVgpt* is based on pSV_2gpt (Mulligan and Berg, 1980) and includes the ampicillin resistance gene for selection in bacterial cells, the *gpt* gene for selection in mammalian cells, the murine heavy chain immunoglobulin enhancer region, genomic sequence encoding the constant region gene and SV40 poly A sequences. The heavy chain variable region for expression is inserted as a *Hin*dIII to *Bam*HI fragment.

Expression vector pSVhyg includes the ampicillin resistance gene for selection in bacterial cells, the *hyg* gene for selection in mammalian cells, the murine heavy chain immunoglobulin enhancer region, genomic sequence encoding the kappa constant region gene and including the kappa enhancer and SV40 poly A sequences. The light chain variable region for expression is inserted as a *Hin*dIII to *Bam*HI fragment.

The DNA sequence is then to be confirmed to be correct for the humanized V_H and V_K in the expression vectors.

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For antibody production the humanized heavy and light chain expression vectors may be introduced into appropriate production cell lines know in the art such as, for example, NS0 cells. Introduction of the expression vectors may be accomplished by co-transfection via electroporation or any other suitable transformation technology available in the art. Antibody producing cell lines can then be selected and expanded and humanized antibodies purified. The purified antibodies can then be analyzed by standard techniques such as SDS-PAGE.

ANTIBODY WITH IMPROVED AFFINITY, SPECIFICITY, STABILITY

The CDRL2 sequence ("KVSNRFS") of the mouse C2 antibody may be modified slightly without adversely affecting antibody activity. Conservative substitutions may be made through exchange of R for K at position 50 and S for N at position 53. The two alternative CDRL2 sequences are therefore "RVSNRFS" and "KVSSRFS", respectively. These are incorporated into the murine V_K sequence with no other changes, as C2 VK-R and C2 VK-S, respectively.

The affinity, specificity and stability of an antibody according to the invention as described herein before or a fragment thereof can be modified by change of its glycosylation profile or pattern resulting in improved therapeutic values.

To achieve this change in glycosylation pattern, host cells may be engineered such that they are capable of expressing a preferred range of a glycoprotein-modifying glycosyl transferase activity which increases complex N-linked oligosaccharides carrying bisecting GlcNAc. Further, modified glycoforms of glycoproteins may be obtained, for example antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having an enhanced Fc-mediated cellular cytotoxicity.

Methods of obtaining antibodies with modified glycosylation pattern are known to those skilled in the art and described, for example, in EP1071700, US2005272128, Ferrara et al (2006) J Biol Chem 281(8), 5032-5036); Ferrara et al (2006) Biotechnology and Bioengineering 93(5), 851-861.

PHARMACEUTICAL PREPARATION AND ADMINISTRATION

The antibodies according to the invention, but particularly a monoclonal antibody according the invention, can be prepared in a physiologically acceptable formulation and may comprise a

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pharmaceutically acceptable carrier, diluent and/or excipient using known techniques. For example, the antibody according to the invention and as described herein before including any functionally equivalent antibody or functional parts thereof, in particular, the monoclonal antibody including any functionally equivalent antibody or functional parts thereof is combined with a pharmaceutically acceptable carrier, diluent and/or excipient to form a therapeutic composition. Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.

Formulation of the pharmaceutical composition according to the invention can be accomplished according to standard methodology know to those skilled in the art.

The compositions of the present invention may be administered to a subject in the form of a solid, liquid or aerosol at a suitable, pharmaceutically effective dose. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumor site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection intramuscularly, subcutaneously, intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

The compositions may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal, interdermal, intraperitoneal, or parenteral (for example, intravenous, subcutaneous, or intramuscular) routes. In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor. The method includes administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

A sustained release matrix, as used herein, is a matrix made of materials, usually polymers which are degradable by enzymatic or acid/base hydrolysis or by dissolution. Once inserted into the

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body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix desirably is chosen by biocompatible materials such as liposomes, polylactides (polylactide acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

It is well know to those skilled in the pertinent art that the dosage of the composition will depend on various factors such as, for example, the condition of being treated, the particular composition used, and other clinical factors such as weight, size, sex and general health condition of the patient, body surface area, the particular compound or composition to be administered, other drugs being administered concurrently, and the route of administration.

The composition may be administered in combination with other compositions comprising an biologically active substance or compound, particularly at least one compound selected from the group consisting of compounds against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), α-secretase activators, β- and γ –secretase inhibitors, tau proteins, neurotransmitter, β-sheet breakers, attractants for amyloid beta clearing / depleting cellular components, inhibitors of N-terminal truncated amyloid beta including pyroglutamated amyloid beta 3-42, anti-inflammatory molecules, "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine, M1 agonists and other drugs including any amyloid or tau modifying drug and nutritive supplements such as, for example, vitamin B12, cysteine, a precursor of acetylcholine, lecithin, choline, Ginkgo biloba, acyetyl-L-carnitine, idebenone, propentofylline, or a xanthine derivative, together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient and procedures for the treatment of diseases.

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Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose. Generally, the regime of administration should be in the range of between 0.1 μg and 10 mg of the antibody according to the invention, particularly in a range 1.0 μg to 1.0 mg, and more particularly in a range of between 1.0 μg and 100 μg, with all individual numbers falling within these ranges also being part of the invention. If the administration occurs through continuous infusion a more proper dosage may be in the range of between 0.01 μg and 10 mg units per kilogram of body weight per hour with all individual numbers falling within these ranges also being part of the invention.

Administration will generally be parenterally, eg intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Non-aqueous solvents include without being limited to it, propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous solvents may be chosen from the group consisting of water, alcohol/aqueous solutions, emulsions or suspensions including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and others. Preservatives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, etc.

The pharmaceutical composition may further comprise proteinaceous carriers such as, for example, serum albumin or immunoglobulin, particularly of human origin. Further biologically active agents may be present in the pharmaceutical composition of the invention dependent on its the intended use.

When the binding target is located in the brain, certain embodiments of the invention provide for the antibody or active fragment thereof to traverse the blood-brain barrier. Certain neurodegenerative diseases are associated with an increase in permeability of the blood-brain barrier, such that the antibody or active fragment thereof can be readily introduced to the brain. When the blood-brain barrier remains intact, several art-known approaches exist for transporting molecules across it, including, but not limited to, physical methods, lipid-based methods, and receptor and channel-based methods.

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Physical methods of transporting the antibody or active fragment thereof across the blood-brain barrier include, but are not limited to, circumventing the blood-brain barrier entirely, or by creating openings in the blood-brain barrier. Circumvention methods include, but are not limited to, direct injection into the brain (see, e.g., Papanastassiou et al., Gene Therapy 9: 398-406 (2002)) and implanting a delivery device in the brain (see, e.g., Gill et al., Nature Med. 9: 589-595 (2003); and Gliadel Wafers™, Guildford Pharmaceutical). Methods of creating openings in the barrier include, but are not limited to, ultrasound (see, e.g., U.S. Patent Publication No. 2002/0038086), osmotic pressure (e.g., by administration of hypertonic mannitol (Neuwelt, E. A., Implication of the Blood-Brain Barrier and its Manipulation, Vols 1 & 2, Plenum Press, N.Y. (1989))), permeabilization by, e.g., bradykinin or permeabilizer A-7 (see, e.g., U.S. Patent Nos. 5,112,596, 5,268,164, 5,506,206, and 5,686,416), and transfection of neurons that straddle the blood-brain barrier with vectors containing genes encoding the antibody or antigen-binding fragment (see, e.g., U.S. Patent Publication No. 2003/0083299).

Lipid-based methods of transporting the antibody or active fragment thereof across the blood-brain barrier include, but are not limited to, encapsulating the antibody or active fragment thereof in liposomes that are coupled to antibody binding fragments that bind to receptors on the vascular endothelium of the blood-brain barrier (see, e.g., U.S. Patent Application Publication No. 20020025313), and coating the antibody or active fragment thereof in low-density lipoprotein particles (see, e.g., U.S. Patent Application Publication No. 20040204354) or apolipoprotein E (see, e.g., U.S. Patent Application Publication No. 20040131692).

Receptor and channel-based methods of transporting the antibody or active fragment thereof across the blood-brain barrier include, but are not limited to, using glucocorticoid blockers to increase permeability of the blood-brain barrier (see, e.g., U.S. Patent Application Publication Nos. 2002/0065259, 2003/0162695, and 2005/0124533); activating potassium channels (see, e.g., U.S. Patent Application Publication No. 2005/0089473), inhibiting ABC drug transporters (see, e.g., U.S. Patent Application Publication No. 2003/0073713); coating antibodies with a transferrin and modulating activity of the one or more transferrin receptors (see, e.g., U.S. Patent Application Publication No. 2003/0129186), and cationizing the antibodies (see, e.g., U.S. Patent No. 5,004,697).

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DETECTION/DIAGNOSIS

In a further embodiment the present invention provides methods and kits for the detection and diagnosis of amyloid-associated diseases or conditions. These methods include known immunological methods commonly used for detecting or quantifying substances in biological samples or in an *in situ* condition.

Diagnosis of an amyloid-associated disease or condition in a patient may be achieved by detecting the immunospecific binding of a monoclonal antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the amyloid protein into contact with an antibody which binds an epitope of the amyloid protein, allowing the antibody to bind to the amyloid protein to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of amyloid protein in the sample or specific body part or area optionally comparing the amount of said immunological complex to a normal control value, wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an amyloid-associated disease or condition.

Monitoring minimal residual disease in a patient following treatment with an antibody or a vaccine composition according to the invention may be achieved by detecting the immunospecific binding of a monoclonal antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the amyloid antigen into contact with an antibody which binds an epitope of the amyloid protein, allowing the antibody to bind to the amyloid antigen to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of amyloid antigen in the sample or specific body part or area, optionally comparing the amount of said immunological complex to a normal control value, wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient may still suffer from a minimal residual disease.

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Predicting responsiveness of a patient to a treatment with a vaccine composition according to the invention may be achieved by detecting the immunospecific binding of a monoclonal antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ,* which includes bringing the sample or a specific body part or body area suspected to contain the amyloid antigen into contact with an antibody which binds an epitope of the amyloid protein, allowing the antibody to bind to the amyloid antigen to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of amyloid antigen in the sample or specific body part or area, optionally comparing the amount of said immunological complex before and after onset of the treatment, wherein an decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

Biological samples that may be used in the diagnosis of an amyloid-associated disease or condition are, for example, fluids such as serum, plasma, saliva, gastric secretions, mucus, cerebrospinal fluid, lymphatic fluid and the like or tissue or cell samples obtained from an organism such as neural, brain, cardiac or vascular tissue. For determining the presence or absence of the amyloid protein in a sample any immunoassay known to those of ordinary skill in the art. (See Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612) may be used such as, for example, assays which utilize indirect detection methods using secondary reagents for detection, ELISA's and immunoprecipitation and agglutination assays. A detailed description of these assays is, for example, given in WO96/13590 to Maertens and Stuyver, Zrein et al. (1998) and WO96/29605.

For *in situ* diagnosis, the antibody or any active and functional part thereof may be administered to the organism to be diagnosed by methods known in the art such as, for example, intravenous, intranasal, intraperitoneal, intracerebral, intraarterial injection such that a specific binding between the antibody according to the invention with an eptitopic region on the amyloid protein may occur. The antibody/antigen complex may be detected through a label attached to the antibody or a functional fragment thereof.

The immunoassays used in diagnostic applications typically rely on labelled antigens, antibodies, or secondary reagents for detection. These proteins or reagents can be labelled with compounds generally known to those skilled in the art including enzymes, radioisotopes, and fluorescent,

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luminescent and chromogenic substances including colored particles, such as colloidal gold and latex beads. Of these, radioactive labelling can be used for almost all types of assays and with most variations. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Antibodies useful in these assays include monoclonal antibodies, polyclonal antibodies, and affinity purified polyclonal antibodies.

Alternatively, the antibody may be labelled indirectly by reaction with labelled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibody may be conjugated with a second substance and detected with a labelled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labelled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labelled anti-hapten antibody.

Those of ordinary skill in the art will know of these and other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, J. H., et al.,1976 (Clin. Chim. Acta 70:1-31), and Schurs, A. H. W. M., et al. 1977 (Clin. Chim Acta 81:1-40). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, and others, all of which are incorporated by reference herein.

Current immunoassays utilize a double antibody method for detecting the presence of an analyte, wherein. The antibody is labeled indirectly by reactivity with a second antibody that has been labeled with a detectable label. The second antibody is preferably one that binds to antibodies of the animal from which the monoclonal antibody is derived. In other words, if the monoclonal antibody is a mouse antibody, then the labeled, second antibody is an anti-mouse antibody. For the monoclonal antibody to be used in the assay described below, this label is preferably an antibody-coated bead, particularly a magnetic bead. For the polyclonal antibody to be employed in the immunoassay described herein, the label is preferably a detectable molecule such as a radioactive, fluorescent or an electrochemiluminescent substance.

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An alternative double antibody system often referred to as fast format systems because they are adapted to rapid determinations of the presence of an analyte, may also be employed within the scope of the present invention. The system requires high affinity between the antibody and the analyte. According to one embodiment of the present invention, the presence of the amyloid protein is determined using a pair of antibodies, each specific for amyloid protein. One of said pairs of antibodies is referred to herein as a "detector antibody" and the other of said pair of antibodies is referred to herein as a "capture antibody". The monoclonal antibody of the present invention can be used as either a capture antibody or a detector antibody. The monoclonal antibody of the present invention can also be used as both capture and detector antibody, together in a single assay. One embodiment of the present invention thus uses the double antibody sandwich method for detecting amyloid protein in a sample of biological fluid. In this method, the analyte (amyloid protein) is sandwiched between the detector antibody and the capture antibody, the capture antibody being irreversibly immobilized onto a solid support. The detector antibody would contain a detectable label, in order to identify the presence of the antibody-analyte sandwich and thus the presence of the analyte.

Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes of polystyrene, magnetic, plastic or glass beads and slides which are well known in the field of radioimmunoassay and enzyme immunoassay. Methods for coupling antibodies to solid phases are also well known to those skilled in the art. More recently, a number of porous material such as nylon, nitrocellulose, cellulose acetate, glass fibers and other porous polymers have been employed as solid supports.

The present invention also relates to a diagnostic kit for detecting amyloid protein in a biological sample comprising a composition as defined above. Moreover, the present invention relates to the latter diagnostic kit which, in addition to a composition as defined above, also comprises a detection reagent as defined above. The term "diagnostic kit" refers in general to any diagnostic kit known in the art. More specifically, the latter term refers to a diagnostic kit as described in Zrein et al. (1998).

It is still another object of the present invention to provide novel immunoprobes and test kits for detection and diagnosis of amyloid-associated diseases and conditions comprising antibodies

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according to the present invention. For immunoprobes, the antibodies are directly or indirectly attached to a suitable reporter molecule, e.g., an enzyme or a radionuclide. The test kit includes a container holding one or more antibodies according to the present invention and instructions for using the antibodies for the purpose of binding to amyloid protein to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

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EXAMPLES

Materials

The development and preparation of mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) is described in co-pending application EP 05 02 7092.5 filed 12.12.2005, the disclosure of which is incorporated herein by reference.

Hybridoma cells FP-12H3-C2, producing mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) were deposited 01 December 2005 in co-pending application no EP05027092.5 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Braunschweig, under the provisions of the Budapest Treaty and given accession no DSM ACC2750.

Hybridoma cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum and antibiotics (Penicillin/Streptomycin). The isotype of the antibody produced was checked and found to be mouse IgG2b/kappa, as expected.

Assay

An ELISA for binding to Amyloid Beta provided a reliable measure of the potency of C2 antibodies. Positive control antibodies, murine FP-12H3-C2 antibody (Genovac Lot No: AK379/01), and standard Chemicon antibody 1560 (Lot no: 0508008791).

Choice of human constant regions

As immune system recruitment is not desirable for the clinical antibody candidate, the selected human constant region for the heavy chain was human IgG4, modified to change Serine at position 228 in the hinge region to Proline (HuIgG4 Ser-Pro). This mutation stabilizes the interchain disulphide bond and prevents the formation of half molecules that may occur in native human IgG4 preparations. The antibody expressed from the production cell lines will also have the terminal lysine removed. The sequences of human constant regions HuIgG4 Ser-Pro and human Kappa are given in SEQ ID NO: 17 and 14, respectively.

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Example 1 Cloning and Sequencing of Antibody Variable Regions

Total RNA was prepared from 3 x 10^6 hybridoma cells (one T175 flask) using the Qiagen RNeasy mini kit (Cat No: 74104). RNA was eluted in 50μL water and checked on a 1.2% agarose gel. The conditioned medium from the cells was retained and a sample used for testing in the antibody activity assay.

V_H and V_K cDNAs were prepared using reverse transcriptase with mouse IgG and κ constant region primers. The first strand cDNAs were amplified by PCR using a large set of signal sequence primers. The amplified DNAs were gel-purified and cloned into the vector pGem® T Easy (Promega). The V_H and V_K clones obtained were screened for inserts of the expected size by PCR and the DNA sequence of selected clones determined by automated DNA sequencing. The locations of the complementarity determining regions (CDRs) in the sequences were determined with reference to other antibody sequences (Kabat EA *et al.*, 1991). The numbering convention of Kabat for antibody variable regions is used throughout this application; hence residue numbers may differ from the strict linear number.

The DNA sequence and deduced amino acid sequence for mC2 V_K is shown in SEQ ID NO: 29 and 27, respectively. Four clones gave this identical productive sequence. A non-productive aberrant V_K sequence that arises from the hybridoma fusion partner was also found in a number of clones.

For mC2 V_H, two different productive sequences were isolated. The mC2 V_H AF sequence (see SEQ ID NO: 30) was found in a total of 29 clones, with 14 single base pair changes in individual clones. The mC2 V_H B sequence was found in a total of 8 clones. Five of these represented the majority sequence, with the other 3 clones being variations on this. It is possible that these similar V_H B sequences arose as an artifact of the PCR amplification. A non-productive aberrant V_H was also obtained from the C2 hybridoma and is attributed to defective V-D-J joining.

In order to determine which is the correct active mC2 V_H, two chimeric antibodies were prepared with the two different V_H sequences, AF and B, combined with the mC2 V_K, to be tested for the correct antibody activity.

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Example 2 Construction of Chimeric Antibody Genes

A human chimeric antibody in its most common form consists of human constant regions linked to murine (or other non-human) variable regions. A chimeric antibody provides a very useful tool, firstly for confirmation that the correct variable regions have been identified, secondly for use as a control antibody in antigen binding assays with the same effector functions and utilizing the same secondary detection reagents as a humanized or engineered antibody, and also may be used to investigate the pharmacokinetic and other properties of the human constant regions with reference to the particular target for the antibody.

Two chimeric heavy chain expression vectors were constructed consisting of mC2 V_H AF or mC2 V_H B variable regions linked to HuIgG4 (Ser-Pro) constant region in the expression vector *pSVgpt*. This is based on pSV_2*gpt* (Mulligan and Berg, 1980) and includes the ampicillin resistance gene for selection in bacterial cells, the *gpt* gene for selection in mammalian cells, the murine heavy chain immunoglobulin enhancer region, genomic sequence encoding the constant region gene and SV40 poly A sequences. The heavy chain variable region for expression is inserted as a *Hin*dIII to *Bam*HI fragment.

A chimeric light chain vector was constructed consisting of C2 VK linked to human C Kappa constant region in the expression vector pSVhyg. (Hieter PA et al, 1980) pSVhyg includes the ampicillin resistance gene for selection in bacterial cells, the *hyg* gene for selection in mammalian cells, the murine heavy chain immunoglobulin enhancer region, genomic sequence encoding the kappa constant region gene and including the kappa enhancer and SV40 poly A sequences. The light chain variable region for expression is inserted as a *Hin*dIII to *Bam*HI fragment.

Expression cassettes for the murine C2 VH and VK sequences were constructed by addition of 5' flanking sequence including the leader signal peptide, leader intron and the murine immunoglobulin promoter, and 3' flanking sequence including the splice site and intron sequence, using the vectors VH-PCR1 and VK-PCR1 as templates (Riechmann et al., 1988). The DNA sequence was confirmed to be correct for the VH and VK in the chimeric expression

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vectors. The DNA and amino acid sequences of the VH and VK genes in the expression cassettes are shown in Figures 1 and 2.

Example 3 Expression of Chimeric Antibodies

3.1 Expression in stable cell lines

The host cell line for antibody expression was NS0, a non-immunoglobulin producing mouse myeloma, obtained from the European Collection of Animal Cell Cultures, Porton UK (ECACC No 85110503). The heavy and light chain expression vectors were co-transfected into NS0 cells by electroporation. Colonies expressing the *gpt* gene were selected in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 0.8 μg/ml mycophenolic acid and 250 μg/ml xanthine. Transfected cell clones were screened for production of human antibody by ELISA for human IgG. Cell lines secreting antibody were expanded and the highest producers selected and frozen down in liquid nitrogen. The best producing cell lines for each antibody were expanded in medium as above but with only 5% FBS. Chimeric antibodies were purified using Prosep®-A (Bioprocessing Ltd). The concentration was determined by ELISA for human IgGκ antibody. The antibodies were also analyzed by SDS-PAGE.

3.2 Transient expression of chimeric antibodies

To expedite the testing of the different chimeric antibodies, transient expression was used to produce quickly small quantities of cell supernatant containing recombinant antibody for testing. The mC2 V_H and V_K expression cassettes were transferred to vectors based on pcDNA3.1 (Invitrogen) for transient expression. The heavy chain vector included a human IgG constant region. The light chain vector included a human kappa constant region. Both mC2 V_H AF and mC2 V_H B were transfected with mC2 V_K into human embryonic kidney (HEK 298) cells with Lipofectamine 2000 reagent (Invitrogen Cat No: 11668) according to the protocol supplied by the manufacturer. Conditioned medium was harvested from cells 3 days after transfection. The amount of antibody produced was determined by ELISA for human IgGκ antibody.

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Example 4 Activity of Chimeric C2 Antibodies

4.1 Activity of chimeric C2 antibodies produced by transient transfection

Samples of conditioned medium from transient transfection for the two different chimeric antibodies were tested in the ELISA for binding to Amyloid Beta. The results clearly indicate that the C2 VH AF is the correct sequence. The C2 V_H AF/C2 V_K chimeric antibody binds well in the assay, but the C2 V_H B/C2 V_K does not show any binding at all. The Chemicon 1560 murine control antibody showed good binding, but binding by the purified murine C2 antibody supplied was low. It should be noted that a different secondary antibody was employed for the murine antibodies with the mouse constant regions compared to the chimeric antibodies with human constant regions, so the results are not directly comparable. Conditioned medium from the C2 hybridoma was later found to give a good result in the assay.

4.2 Activity of purified chimeric C2 antibodies

The two different C2 chimeric antibodies were purified from stable NS0 cell lines as described and tested using the Amyloid Beta ELISA. The results obtained are in accordance with the results obtained with transiently expressed antibody. The C2 ChVH AF/ChVK antibody binds well in the ELISA and the C2 ChVH B/ChVK antibody does not bind at all.

Example 5 Design of Humanized C2 Antibody Genes

The mC2 V_H and V_K amino acid sequences were compared to rodent antibody V_H and V_K sequences in the NCBI and Kabat databases.

5.1 Light chain variable region

The closest match mouse germ line gene to mC2 V_K is bb1, Locus MMU231201, (Schable *et al*, 1999). Only two amino acids differ from this germ line sequence, both located within CDRL1. Mature murine antibodies with similar, but not identical, sequence are found. Several have an identical CDRL2 and identical CDRL3, but the CDRL1 of mC2 seems to be unique. mC2 V_K can be assigned to Kabat subgroup MuV_KII. Position 87 of mC2 V_K is F rather than the Y that is more common in the subgroup, indicating that this framework residue may be important for antibody activity. Comparison with human germ line V_K sequences shows that genes from subgroup V_KII are the best match for mC2 V_K (Cox *et al*, 1994). Sequence DPK15 together with

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the human J region HuJ_K1 were selected to provide the acceptor framework sequences for the humanized V_K.

Four humanized V_K sequences were designed. C2HuVK1 consists of mC2 V_K CDRs with frameworks from DPK 15 and human J_K1. In versions 2, 3 and 4 murine residues have been substituted in the framework at positions 45 or 87 or both. Residue 45 may be involved in supporting the conformation of the CDRs. Residue 87 is located at the interface of the V_H and V_K domains. Therefore these residues may be critical for maintenance of antibody binding.
The positions and changes that have been made in the light chain framework regions are shown in Table 6. A comparison of the humanized sequences with mC2 V_K sequence, and with DPK15 and human J_K1

5.2 Heavy chain variable region

The closest match mouse germ line gene to mC2 V_H AF is VH7183, Locus AF120466, (Langdon *et al*, 2000). The comparison is shown in Figure 3. Nine amino acids differ from this germ line sequence, most being located within CDR2. Mature murine antibodies with identical or similar (one residue different) CDR1 or with similar CDR2 (one residue different) are found, but none with all three CDRs identical to mC2 V_H AF. CDR3 of mC2 antibody is unusually short, consisting of only three residues. However, other antibodies are found in the database with CDR3 of this length. mC2 V_H AF can be assigned to Kabat subgroup MuV_HIIID. Residue 47 of mC2 V_H is L rather than the more common W, and residue 94 is S rather than the normal R, indicating that these framework residues may be important for antibody activity. Comparison with human germ line V_H sequences shows that genes from subgroup V_HIII are the best match for mC2 V_H. Sequence DP54 together with the human J region HuJ_H6 was selected to provide the acceptor framework sequences for the humanized V_H.

Four humanized V_H sequences were designed. C2HuVH1 consists of mC2 V_H AF CDRs with frameworks from DP54 and HuJ_H6. In versions 2, 3 and 4 murine residues have been substituted in the framework at positions 47 or 94 or both. Residue 47 in framework 2 makes contact both with the CDRs and with the V_K domain. Residue 94 may be involved in supporting the conformation of the CDRs. Therefore these residues may be critical for maintenance of antibody binding.

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The positions and changes that have been made in the heavy chain framework regions are shown in Table 7.

Example 6 Construction of Humanized Antibody Genes

The modified variable regions were constructed by the method of overlapping PCR recombination. The expression cassettes for the chimeric antibody, C2 ChV_H AF and C2 ChV_K, were used as templates for mutagenesis of the framework regions to the required sequences. Sets of mutagenic primer pairs were synthesized encompassing the regions to be altered. The humanized V_H and V_K expression cassettes produced were cloned into pUC19 and the entire DNA sequence was confirmed to be correct for each V_H and V_K. The modified heavy and light chain V-region genes were excised from pUC19 as *Hin*dIII to *Bam*HI expression cassettes. These were transferred to the expression vectors pSV*gpt* and pSV*hyg* which include human IgG4 Ser-pro or κ constant regions respectively, as for the chimeric antibody vectors. The DNA sequence was confirmed to be correct for the humanized V_H and V_K in the expression vectors.

Example 7 Expression of Humanized Antibodies

7.1 Expression in stable cell lines

The humanized heavy and light chain expression vectors were co-transfected into NS0 cells by electroporation, as for the expression of chimeric antibodies. Antibody producing cell lines were selected and expanded and humanized antibodies purified, exactly as for the chimeric antibody. The purified antibodies were analyzed by SDS-PAGE.

7.2 Transient expression of humanized antibodies

To expedite testing of the different humanized V_H and V_K constructs, the C2 humanized V_H and V_K expression cassettes were also transferred to the vectors for transient expression described in section 7.2. The four humanized C2 V_K constructs were co-transfected with the chimeric C2 V_H construct into HEK293 cells. Similarly, the four humanized C2 V_H constructs were co-transfected with the chimeric C2 V_K construct into HEK293 cells. Conditioned medium was

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harvested from cells three days after transfection. The amount of antibody produced was determined by ELISA for human IgGκ antibody.

Example 8 Activity of Humanized C2 Antibodies

8.1 Activity of humanized C2 antibodies produced by transient transfection

Samples of conditioned medium from the transient transfection were tested in the Amyloid Beta ELISA. The results obtained clearly indicate that the humanized VH constructs C2 HuVH AF versions 2 and 4 are functional when combined with the chimeric C2 kappa chain, and are comparable to the chimeric C2 antibody in the assay. In contrast, the antibodies containing C2 HuVH AF versions 1 and 3 combined with the chimeric C2 kappa chain show no binding at all in the assay. This indicates that the substitution of the murine residue at position 94 is essential for antibody activity. Antibodies containing the chimeric C2 heavy chain combined with the four humanized C2 kappa chains all showed good binding, comparable to the chimeric antibody, in the ELISA.

8.2 Activity of purified humanized C2 antibodies

Eight different humanized C2 antibodies comprising all combinations of two humanized heavy chains and four humanized light chains were purified from stable NS0 cell lines as described and tested using the Amyloid Beta ELISA (Figure 4).

The results obtained clearly indicate that C2 HuVH4 antibodies perform better in the assay than C2 HuVH2 antibodies. Of the C2 HuVH2 antibodies, C2 HuVH2/HuVK3 shows the best binding activity, but this is approximately 2 fold reduced compared to the chimeric control antibody C2 ChVHAF/ChVK. C2 HuVH2/HuVK2 activity is four to five fold reduced compared to the control. The activities of the antibodies comprising C2HuVH4 with the four different humanized light chains are similar. The highest activity is observed for C2HuVH4/HuVK1and all four antibodies are close to the control chimeric antibody in the assay.

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Example 9 Modifications to CDRL2

9.1 Design light chain with modified CDR 2

As noted above, many antibodies share the same CDRL2 sequence ("KVSNRFS") as the C2 antibody. It was decided to test whether CDRL2 could be modified slightly without adversely affecting antibody activity. Two conservative substitutions were selected: R for K at position 50 and S for N at position 53. The two alternative CDRL2 sequences are therefore "RVSNRFS" and "KVSSRFS". These were incorporated into the murine V_K sequence with no other changes, as mC2 VK-R and mC2 VK-S respectively.

9.2 Transient expression of modified CDRL2 antibody

The two C2 light chain constructs with modified CDRL2 described in Section 11.2.1 were cloned into the light chain vector for transient expression. Each was co-transfected with the chimeric C2 V_H vector into HEK293 cells. Conditioned medium was harvested from cells three days after transfection. The amount of antibody produced was determined by ELISA for human IgGκ antibody.

9.3 Activity of C2 antibody with modified CDRL2

Samples of conditioned medium from the transient transfection of mC2 V_Ks with modified CDRL2 combined with mC2 V_H were tested in the Amyloid Beta ELISA.(Figure 5) Both the VK-R and the VK-S antibodies are comparable to the chimeric C2 antibody, indicating that the individual modifications to CDRL2 chosen do not markedly affect the activity of the antibody in the assay.

Example 10 Affinity Determination

To assess the binding specificity and affinity of mouse (ACI-01-Ab-7-C2) chimeric (AF) and humanized antibodies (H4K1; H4K4), BIACORE.RTM. analysis was performed using amyloid beta 1-42 monomers and fibers as antigen immobilized on a CM5 chip. BIACORE.RTM. technology utilizes changes in the refractive index at the surface layer upon binding of the antibody to the antigen immobilized on the layer. Binding is detected by surface plasmon

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resonance (SPR) of laser light refracting from the surface. Analysis of the signal kinetics on rate and off rate allows the discrimination between non-specific and specific interaction. The concentration of antibody used was in the range of 0.05 μM to 1.0 μM.

Table 1: **B**inding specificity and affinity of mouse (ACI-01-Ab-7-C2) chimeric (AF) and humanized antibodies (H4K1; H4K4) for amyloid beta 1-42 monomers and fibers.

	Monomers			**Fibers**		
	k_a(1/Ms)	k_d(1/s)	KD (M)	k_a(1/Ms)	k_d(1/s)	KD (M)
Mouse ACI-01-Ab-7-C2	1.8E+04	2.7E-03	1.5E-07	2.4E+04	9.9E-04	4.1E-08
chimeric AF	4.7E+04	9.5E-04	2E-08	5.1E+04	3.3E-04	6.5E-09
humanized H4K1	5.0E+04	9.5E-04	1.9E-08	4.9E+04	2.3E-04	4.7E-09
humanized H4K4	2.5E+04	4.4E-04	1.8E-08	1.3E+05	3.0E-04	2.3E-09

Example 11 Immunhistochemical Binding Assay

11.1 Human brain sections

Brains from healthy, non-demented pre-AD and AD patients were obtained from the Universitätsklinik in Bonn after ethical approval. Brains were fixed in formaldehyde and the hippocampus region was dehydrated, embedded in paraffin and 5 μm sections were cut with a microtome. Paraffin sections were stored at RT until use. For fresh material, 5 µm cryosections were cut with a cryostat and sections stored at -80°C until use.

11.2 Immunohistochemistry

Paraffin sections were deparaffinized and rehydrated by bathing slides in xylene followed by 100% ethanol, 90% ethanol and 70% ethanol. Background was decreased by 30 minutes incubation in 10%H_2O_2, 10% methanol in water. Antigen retrieval was obtained by incubating the slides in 100% formic acid for 3 minutes. After 3 washes in Tris buffered saline (TBS, pH 7.5), non-specific labeling was blocked by a 2 hour incubation of the slides in 10 % BSA, 0.25% Triton X-100 in TBS. After washing (3 washes in TBS) blocking of endogenous antibodies was performed by adding a non-labeled anti-human IgG (Biomeda) and incubating slides in humid

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chambers overnight at RT. After another 3 washes, the primary human anti amyloid antibody was added to the slides and incubated another 24 hours at RT. Following washing, an alkaline phosphatase labeled secondary anti human IgG (Sigma) was added to the slides and incubated for 2 hours at RT. After washing, slides were developed with Liquid permanent Red (Dakocytomation) washed with water and air-dried before mounting with permanent mounting media (corbitbalsam).

Cryosection were fixed in methanol for 30 minutes at -80°C and background decreased by adding H_2O_2 to the cold methanol to a final concentration of 10% and incubating for 30 minutes at RT. After 3 washes in Tris buffered saline (TBS, pH7.5), non-specific labeling was blocked by a 2 hour incubation of the slides in 10 % BSA, 0.25% Triton X 100 in TBS as above and the same staining procedure as above was carried out.

Sections were examined with a Leica DMLB microscope and photographed using a Leica DC500 camera and Leica FireCam1.2.0 software.

Both human antibodies A and C labeled plaques of brains from AD disease patients (Figure 6). Both diffuse and cored plaques were labeled. Moreover, diffuse plaques in non -demented pre-AD patients could also be detected by the A and C antibodies. Amyloid in cerebral amyloid angiopathy (CAA) was labeled with both antibodies and some staining of neurons which may correspond to intracellular amyloid was also detected. No labeling was seen on control brains from healthy patient. Plaques could be detected on paraffin sections pretreated with formic acid but no plaques were labeled on paraffin sections without formic acid pretreatment and on cryosections fixed in methanol. The human antibody B did not detect plaques on paraffin sections and the mouse antibody did not stain either paraffin or cryosections of human brains.

Abbreviations:

A = binding chimeric antibody AF (IgG4) (mC2ChVHAF)

B = non-binding chimeric antibody B (IgG4) (mC2VHB)

C = binding humanized antibody H4K1 (IgG4) (HuVH4/HuVK1)

Mouse = ACI-01-Ab-C2 mouse antibody (IgG2b)

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Example 12 Functionality of mC2 on Amyloid Fibers

12.1 Modification of Conformation of Aâ1-42 Fibers and Initiation of Disaggregation after Binding of the mC2 antibody

In order to evaluate the mechanism by which the antibody is capable to disaggregate preformed beta-amyloid ($A\beta_{1-42}$) fibers a head-to-head comparison of Thioflavin-T (Th-T) fluorescent assay was performed measuring disaggregation and solid-state Nuclear Magnetic Resonance (NMR) of U-^{13}C Tyrosine10 and Valine12-labeled Aβ1-42 peptide analysing secondary conformation (Figure 7A). The mC2 antibody solubilised 35.4 % of the preformed Aβ1-42 fibers and simultaneously induced a shift in secondary conformation from beta sheet to random coiled. The reduction in the population of the beta sheet conformation with respect to the random coil is of the order of 35% and is therefore in close agreement with that measured using fluorescence Th-T assay (Figure 7B). These data indicate that the binding of the mC2 antibody initiates a transition of the secondary structure which potentially causes a destabilization of the parallel intermolecular arrangement of the beta sheets affecting a break of elongated fibers into smaller fragments.

12.2 Conformation-dependent Binding Affinity of mC2 antibody

Since it is well known in the scientific literature that a proportion of the antibody-antigen binding energy can be used for energy-dependent modification of the conformation of an antigen (Blond and Goldberg, 1987), a comparison experiment of the binding affinity of the C2 antibody to the whole $A\beta_{1-42}$ protein and to a smaller, nine amino acid long, peptide comprising the antibody's epitope was performed (Figure 8). For this comparison the affinities of the humanized antibody C2 were analyzed by ELISA using biotinylated peptides covering the complete amino-acid sequence of the C2's epitope (produced by Mimotopes and purchased from ANAWA Trading SA) and a biotinylated complete Aβ1-42 peptide (Bachem). The analysis was done according to the manufacturer's (Mimotopes) instructions. As demonstrated in Figure 8 andTable 2, the antibody binds with a 36.0% higher affinity to the peptide comprising its specific epitope (aminoacids 13-21 of the $A\beta_{1-42}$ sequence) than to the whole Aβ1-42 protein. It is therefore suggested that the difference in binding affinity energy was used for the energy-consuming transition of the secondary conformation of the amyloid protein to present the antigen in a more

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acceptable position for the antibody interaction. This explains why the affinity of the antibody is lower for the native (the whole amyloid protein) than for the isolated subunit.

Table 2

	O.D	
	Amyloid beta 13-21	Amyloid beta1-42
hC2	**1.225**	**0.9005**
Control IgG	**0.171**	**0.196**

Example 13 Effects of the anti-amyloid hC2 on the aggregation of amyloid beta 1-42 peptide

To evaluate the ability of the humanized anti-human amyloid beta monoclonal antibody hC2 to mediate anti-aggregating and disaggregating effects on amyloid beta (Aβ) a thioflavin T spectrofluorescence assay was accomplished.

13.1 Inhibition of Aggregation Assay

Aβ1-42 lyophilized powder was reconstituted in hexafluoroisopropanol (HFIP) to 1 mM. The peptide solution was sonicated for 15 min at room temperature, agitated overnight, and aliquots made into non-siliconized microcentrifuge tubes. The HFIP was then evaporated under a stream of argon. The resulting peptide film was vacuum dried for 10 min and stored at -80°C until used.

To assay for the antibody-mediated inhibition of Aβ1-42 aggregation the hC2 antibody was pre-diluted in PBS and an assay solution containing the following components was made in a non-siliconized incubation tube: 3.3 or 0.33 μM pre-diluted antibody, 10 μM thioflavin T, 33 μM Aβ1-42, and 8.2% DMSO. Therefore the final molar ratios of antibody to Aβ1-42 were 1:10 and 1:100. Appropriate control solutions were also prepared. The solutions were then incubated for 24 hrs at 37°C, and the spectrofluorescence (relative fluorescence units; RFU) read in six replicates in black 384-well plates (Perkin-Elmer) on a Perkin-Elmer FluoroCount spectrofluorometer. The spectrofluorescence was then measured and % disaggregation calculated as described below.

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13.2 Disaggregation Assay

To assay for antibody-mediated disaggregation of pre-aggregated Aβ1-42, a low-molecular weight Aβ1-42, prepared as described above, was made up as a 110 μM solution in 27% DMSO and 1x PBS. This solution was then allowed to aggregate at 37°C for 24 hrs after which the following were added: 3.3 or 0.33 μM pre-diluted antibody, and 10 μM thioflavin T. This resulted in a molar ratio of 1:10 and 1:100 antibody to Aβ1-42. This solution was then incubated for additional 24 hrs at 37°C. The spectrofluorescence was then measured and % disaggregation calculated as described below.

13.3 Calculation

Inhibition of aggregation or disaggregation is expressed as mean % inhibition or disaggregation, respectively, ± standard error of the mean (SEM) according to the following equation:

$$\% \text{ inhibition} = \frac{(\text{RFU of pos contrl} - \text{RFU of neg contrl}) - (\text{RFU of sample with A}\beta\text{1-42} - \text{RFU of sample without A}\beta\text{1-42})}{(\text{RFU of pos contrl} - \text{RFU of neg contrl})} \times 100\%$$

13.4 Result

13.4.1 Inhibition of Aβ1-42 aggregation

Inhibition of Aβ1-42 aggregation using the hC2 antibody is shown in Table 3 and Figure 11. At an antibody to Aβ1-42 molar ratio of 1:100 the inhibition averaged 30% (2 independent experiments), whereas at a 1:10 molar ratio the inhibition was 80% (2 independent experiments; see Table 3).

Table 3. hC2-mediated inhibition of Aβ1-42 aggregation at a 1:100 and 1:10 antibody to Aβ1-42 molar ratios.

Antibody	Molar ratio (antibody to Aβ1-42)	
	1:100	1:10
hC2	30.0 ± 4.1%	80.4 ± 6.9%

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13.4.2 Disaggregation of pre-aggregated Aβ1-42

Disaggregation of pre-aggregated Aβ1-42 using the hC2 antibody is shown in Table 4 and Figure 12. At an antibody to Aβ1-42 molar ratio of 1:100 the disaggregation averaged 24%, whereas at a 1:10 molar ratio the disaggregation was 32% (3 independent experiments; see Table 4).

Table 4. hC2-mediated disaggregation of pre-aggregated Ab1-42 at a 1:100 and 1:10 antibody to Aβ1-42 molar ratios.

Antibody	Molar ratio (antibody to Aβ1-42)	
	1:100	1:10
hC2	23.9 ± 4.4%	31.9 ± 3.5%

Using the thioflavin T assay, the bi-functional properties of the anti-Aβ humanized antibody hC2 can be demonstrated, namely to inhibit the aggregation of Aβ1-42 into pathogenic protofibrillar conformation and in addition to disaggregate preformed Aβ1-42 protofibrils. hC2 inhibited Aβ1-42 aggregation by 80% at an antibody to Aβ1-42 molar ratio of 1:10. The ability of hC2 to disaggregate pre-aggregated protofibrils of Aβ1-42 at a 1:10 molar ratio was shown to be 32%.

Example 14: Conformation-specific binding of mC2 to different classes of Amyloid Protein

In order to evaluate the specificity of mC2 to different stages of polymerized amyloid protein, monomeric, polymeric soluble and fibrillic amyloid, an ELISA coated with these different stages of polymeric beta-amyloid was performed (Figure 9). Monomers were prepared according to a modified method published by (Klein, 2002), soluble polymeric amyloid beta according to (Barghorn et al., 2005), whereas fibers were performed by incubation of amyloid (Bachem, Switzerland) with a final concentration of 1μg/μl in Tris/HCl pH 7.4 at 37°C for 5 days followed by a centrifugation step (10,000 rpm for 5 minutes). Then amyloid polymers were coated on an ELISA plates with a final concentration of 55μg/ml and binding affinity ELISA by using an anti-mouse IgG monoclonal antibody (Jackson) labelled with alkaline phosphate was performed. As demonstrated in Table 5 the mC2 antibody binds with higher affinity to soluble polymeric amyloid beta than to fibers and with the lowest to monomers. These data indicate that the antibody's binding is influenced by the amyloid epitope and by the conformation of the different amyloid aggregates.

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Table 5 Conformation-specific binding of mC2 to Amyloid Monomers, Oligomers and Fibres

mC2	O.D		
Ab Conc (ug/ml)	Oligomer	Fibers	Monomers
0.625	**2.806**	**1.620**	**1.155**
0.312	**1.724**	**0.989**	**0.649**
0.156	**1.036**	**0.631**	**0.397**
0.078	**0.652**	**0.499**	**0.333**

Example 15: Epitope mapping of AC Immune's monoclonal antibody hC2

Epitope mapping of the humanized monoclonal antibody hC2 was performed by ELISA using three different peptide libraries. One library comprised a total of 33 biotinylated peptides covering the complete amino acid (aa) sequence of Aβ1-42 (produced by Mimotopes and purchased from ANAWA Trading SA), the second library contains biotinylated peptides using peptide 12 (aa12-20 of Aβ) from the first peptide library and substituting each aa in the sequence by an alanine (see table 8 below) , and the third library contains biotinylated peptides 13, 14, or 15 (aa 13-21, 14-22 or 15-23 of Aβ) and substituting in each case the last amino acids to an alanine or to a glycine for aa 21 which is already an alanine (see table 9 below). A biotinylated complete Aβ1-42 peptide was used as positive control (Bachem). Epitope mapping was done according to the manufacturer's (Mimotopes) instructions. Briefly, Streptavidin coated plates (NUNC) were blocked with 0.1% BSA in PBS overnight at 4°C. After washing with PBS-0.05% Tween 20, plates were coated for 1 hour at RT with the different peptides from the library, diluted in 0.1% BSA, 0.1% Sodium Azide in PBS to a final concentration of 10 μM. After washing, plates were incubated for 1 hour at RT with the hC2 antibody or a non Aβ binding chimeric IgG4 antibody diluted to 200 ng/ml in 2% BSA, 0.1% Sodium Azide in PBS. Plates were washed again and incubated with alkaline phosphatase conjugated goat anti human IgG for 1h at RT. After final washing, plates were incubated with phosphatase substrate (pNPP) and read at 405 nm using an ELISA plate reader.

It was shown that the humanized monoclonal antibody hC2 bound specifically to peptides 12,13,14,15 and 16 of the first peptide library. These peptides comprise aa 12-20, 13-21, 14-22, 15-23 and 16-24 respectively of Aβ1-42, suggesting that the epitope lies in region 12-24 of Aβ. A second library with alanine substitutions was used to determine the critical aa for binding to Aβ12-20 (VHHQKLVFF). The binding of the hC2 antibody is lost completely when amino acids

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16, 17, 19 or 20 are substituted by an alanine, indicating that these aa are absolutely critical for binding of the antibody to Aβ. The binding of the hC2 antibody is partially lost when aa 15 and 18 are substituted.

The binding was also almost completely lost when aa 14 was substituted for an alanine, indicating that aa 14 is also very important for binding.
Finally, a third library was used to determine whether aa 21, 22 or 23 are critical for binding to the epitope. The binding of the antibody to aa 15-23 was reduced when aa 23 was substituted for an alanine, indicating that aa 23 is also important for binding. The binding was partially lost when aa 21 was substituted for a glycine and slightly lost when aa 22 was substituted for an alanine.

Example 16: Neuroprotection by the hC2 Antibody

The ability of antibody hC2 to protect neurons from Abeta oligomer-induced degeneration was assessed in an in vitro assay. Embryonic day 16.5-17.5 mouse cortical neurons were isolated, dissociated, and cultured in vitro in N3-F12 media. The cells were grown for nine days in total, and were fed on day 3 and on the day that Abeta oligomer, or Abeta oligomer plus anti-Abeta antibody hC2 was added. At day five ("4 days Abeta") or day six ("3 days Abeta"), certain wells of cells were treated with either 2 μM Abeta oligomer alone, or a combination of 2 μM Abeta oligomer and 50 μg/mL anti-Abeta antibody hC2.

The Abeta oligomer was prepared by dissolving Abeta 1-42 (rPeptide) in HFIP, from which Abeta peptides were aliquoted into 10μl aliquots at 1mg/ml and then evaporated in a fume hood for 30 minutes and peptide films were stored at -80C until use. Upon use, the peptide film was dissolved in 10μl of DMSO, then 78.6μl of HAMS F12, and the Abeta peptide solution was incubated at 4C for 24-48 hours (25μM final concentration of Abeta).

For control cells, DMSO-F12 alone was added at the same volume as Abeta-DMSO at day 5, and the cells were cultured for an additional 4 days without any additional treatment. On day 9, neurons from all culture conditions were fixed and stained with Tuj1 (an anti-beta-tubulin antibody), followed by staining with secondary antibodies labeled with FITC to visualize microtubules, and thus neuronal processes in general. The results are shown in Figure 13.

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Untreated mouse embryonic cortical neurons showed normal morphology after nine days of culture (Figure 13, leftmost panel). Treatment of the cells with Abeta oligomer for three days induced axon degeneration and caused a decrease in the total number of axons (Figure 13, lower center panel), and this effect was even more pronounced at four days of treatment (Figure 13, upper center panel). In contrast, the cells treated with the combination of Abeta oligomer and anti-Abeta antibody hC2 looked similar to control cells (Figure 13, upper and lower right panels). These results indicate that anti-Abeta antibody hC2 was able to protect embryonic mouse cortical neurons from Abeta oligomer-induced degeneration.

Table 6: Positions and changes made in the humanized C2 light chain framework regions

Position Light chain	45	87	50	53
Mouse $C2V_K$	K	F	K	N
Humanized $C2HuV_K1$	Q	Y	K	N
Humanized $C2HuV_K2$	Q	F	K	N
Humanized $C2HuV_K3$	K	Y	K	N
Humanized $C2HuV_K4$	K	F	K	N
Human Germline dpk15	Q	Y	L	N
Mouse $C2V_K$-R			R	
Mouse $C2V_K$-S				S

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Table 7: Positions and changes made in the humanized C2 heavy chain framework regions

Position Heavy chain	47	94
Mouse C2VHAF	L	S
Humanized C2HuVHAF1	W	R
Humanized C2HuVHAF2	W	S
Humanized C2HuVHAF3	L	R
Humanized C2HuVHAF4	L	S
Human Germline DP-54	W	R

A total of 8 different antibodies were constructed with light chains Humanized C2HuV$_K$1, C2HuV$_K$2, C2HuV$_K$3, C2HuV$_K$4 and heavy chains C2HuVHAF4 and C2HuVHAF2

Table 8. Summary of peptides used in the second library aa that are important for binding are marked in italics and underscore and aa absolutely critical for binding are marked in italics and bold.

p12-20	V	H	H	Q	K	L	V	F	F
A12	**A**	H	H	Q	K	L	V	F	F
A13	V	**A**	H	Q	K	L	V	F	F
A14	V	H	**A**	Q	K	L	V	F	F
A15	V	H	H	**A**	K	L	V	F	F
A16	V	H	H	Q	**A**	L	V	F	F
A17	V	H	H	Q	K	**A**	V	F	F
A18	V	H	H	Q	K	L	**A**	F	F
A19	V	H	H	Q	K	L	V	**A**	F
A20	V	H	H	Q	K	L	V	F	**A**
aa no.	12	13	***14***	***15***	***16***	***17***	***18***	***19***	***20***

Table 9. Summary of peptides used in the third library.
aa that are important for binding are marked in italics and underscore and aa absolutely critical for binding are marked in italics and bold

p13-21		H	H	Q	K	L	V	F	F	A		
p13-21	G21	H	H	Q	K	L	V	F	F	**G**		
p14-22			H	Q	K	L	V	F	F	A	E	
p14-22	A22		H	Q	K	L	V	F	F	A	**A**	
p15-23				Q	K	L	V	F	F	A	E	D
p15-23	A23			Q	K	L	V	F	F	A	E	**A**
aa no.		13	***14***	***15***	***16***	***17***	***18***	***19***	***20***	21	22	***23***

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Example 17 Binding of AC Immune's monoclonal Antibody mACI-01-Ab7 C2 to Amyloid Species in Western Blot and Dot Blot

To determine whether the binding of the mouse antibody mACI-01-Ab7 C2 is dependent on the native conformation of Aß a comparison of the binding to linearized amyloid by Western Blot or native amyloid on Dot Blot was performed (Figures 2a and 2b)

Amyloid monomers were generated by dissolving Aβ1-42 peptide in HFIP and the solvent evaporated under argon. Dried peptide film was stored at -80°C until use. For preparation of monomers, the peptide film was resupended in DMSO to a concentration of 2.75μg/μl and diluted in PBS to 1μg/μl. For preparation of oligomers, dried peptide film was resuspended in DMSO to 5mM, sonicated and PBS added to reach 400uM amyloid followed by the addition of SDS to a final concentration of 0.2%. After 6 hour incubation at 37 °C, the amyloid was diluted in water to a final concentration of 100μM and incubated another 18h at 37°C. The amyloid oligomers were precipitated with ice-cold 33% methanol, 4% acetic acid solution for 1h at 4°C, spun down at 16200g for 10 minutes and the pellet resuspended in 5mM Na2H2PO4, 35mM NaCl pH 7.4 to a final concentration of 1μg/μl. For preparation of fibers, peptide film was diluted in Tris-HCl 50mM buffer to get a concentration of 1mg/ml of amyloid and incubated at 37°C for 5 days. The tubes were spun at 10000g for 5 minutes and the pellet resuspended in 0.1M carbonate buffer pH 9.6 to reach 1μg/μl.
1 or 5μg of monomers, oligomers or fibers were diluted in PBS and in loading buffer and applied to a 12% SDS-PAGE and the gel transferred to nitrocellulose membranes. Alternatively, 3 or 1μg or 100 and 10ng of amyloid species were diluted in PBS and were dotted directly onto the nitrocellulose membrane and the membranes dried at RT for 1 hour. After blocking for 30 minutes with Casein solution (Vector), the membranes were incubated for 30 minutes with mACI-01-Ab7 C2 or 6E10 (Chemicon) antibodies diluted to 1μg/ml in Casein solution. After 3 washes in Casein solution, the membranes were incubated at RT for 30 minutes with HRP-labeled goat anti-mouse IgG (Dako Cytomation) diluted in Casein solution, washed 3 times and developed with DAB substrate (Dako Cytomation).

The monoclonal mouse antibody mACI-01-Ab7 C2 bound specifically to monomers, oligomers and fibers in the Dot Blot assay as did the positive control antibody 6E10. In contrast, the mACI-01-Ab7C2 antibody did not detect linearized amyloid species by Western Blot in contrast to the

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6E10 antibody which clearly recognized all linearized peptides. This result demonstrates that the binding of mACI-01-Ab7 C2 to amyloid is dependent on the native conformation of amyloid.

EXAMPLE 18: mACI-01Ab7 C2 –$A\beta_{1-42}$ interactions

The interactions between AC immune's lead antibody mACI-01-Ab7 C2 (mC2) with amyloid peptide $A\beta_{1-42}$ was studies using surface plasmon resonance. The binding of the mouse antibody mACI-01-Ab7 C2 to either monomers or fibers of $A\beta_{1-42}$ were determined.

All SPR experiments were carried out on a Biacore X instrument (Biacore AB). Reagents for immobilization (EDC, NHS and ethanolamine), sensor chips CM5 and SA as well as running and sample buffer HBS-EP were purchased from Biacore AB. Sodium acetate (10 mM, pH 5.0) was used as coupling buffer to increase coupling yield. Fibrillar $A\beta_{1-42}$ (BAchem) was prepared by adding PBS buffer to $A\beta_{1-42}$ to a final concenctration of 3 mg/ml and leaving the vials at 37 °C for 7 days. Fibrillar $A\beta_{1-42}$ was coupled to a CM5 sensor chip containing a surface-bound carboxymethyl dextran matrix. Biotinylated monomeric $A\beta_{1-42}$ (Bachem) was coupled to a Sensor chip SA consisting of carboxymethyl dextran matrix with covalently attached Streptavidin. Typically four or five concentrations of mAb were assayed by serial dilutions using running buffer. Injections were performed starting from the lowest concentration and were passed over both fc 1 and 2 at a flow rate of 30 µL/min for 3 min. Flow cell 2 was underivatised and responses were subtracted from fc 1 to correct for instrument noise and bulk refractive changes. After injection was finished, the surfaces were washed immediately with running buffer for 5 min. To remove remaining bound antibody from the $A\beta_{1-42}$ fibrils, surface regeneration was performed by injecting pulses of 10 mM NaOH. Kinetic analysis was performed using algorithms for numerical integration and global analysis using BIAevaluation 3.0. The curves obtained for injections of analyte at different concentrations were overlaid and the baselines adjusted to zero. For curve fitting, all data were fit simultaneously to a 1:1 homogeneous complex.

Binding of the mouse mACI-01-Ab7 C2 antibody to amyloid was determined to be relatively strong. As demonstrated in Table 2, the mouse antibody mACI-01-Ab7 C2 bound specifically to immobilized $A\beta_{1-42}$ fibers with an average association constant (ka) of 3.8×10^{-4} M/s, a dissociation constant (kd) of 1.1×10^{-3} s^{-1} and therefore with the resulting average KD of 3.5 x

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10^{-8} M. Association of the mACI-01-Ab7 C2 to Aβ monomers was similar or slightly faster with an average ka of 1.6 x 10^{-4} M/s but the dissociation was more rapid giving a KD of 2.5 x 10^{-7} M.

Table 2

	Monomers			Fibers		
	k_a(1/Ms)	k_d(1/s)	KD (M)	k_a(1/Ms)	k_d(1/s)	KD (M)
mACI-01-Ab7 C2 **exp. 1**	1.8E+04	2.7E-03 5.3E-03	1.5E-07	2.4E+04	9.9E-04	4.1E-08
mACI-01-Ab7 C2 **exp. 2**	1.5E+04		3.5E-07	5.60E+04	9.66E-04	1.73E-08
mACI-01-Ab7 C2 **exp. 3**				3.26E+04	1.49E-03	4.58E-08
average mACI-01-Ab7 C2	**1.6E+04** ±0.21	**4.0E-03** 1.84	**2.5E-07** 1.41	**3.8E+04** 1.66	**1.1E-03** 0.3	**3.5E-08** 1.53

EXAMPLE 19: Binding of mACI-01-Ab7 C2 monoclonal Antibody to Amyloid Fibers

To analyze the molecular binding side of the antibody on pre-formed fibers negatively contrasted transmission electronic microscopy (TEM) was performed (Figures 3a and 3b).

The antibody, mACI-01-Ab7 C2, was coupled with 8 nm colloidal gold according to [4,5]. For the co-incubation of amyloid 1-42 (Aβ1-42) fibers 6.65uM fibers were incubated for 24h at RT with the gold-labeled antibody with the molar ratio of 1:100. Subsequently 5 µl of sample were incubated on the fresh glow-discharged Cu grid (mesh 200) covered with parlodium/C film for 45 seconds, washed 3 times with water and 1 times with 2% fresh diluted and filtered uranyl acetate. Samples were stained in 2% uranyl acetate for 15-20 sec. Excess of stain on the grids was sucked and consequently air-dried. Three grids of each sample were prepared. The grids were analyzed in transmission electron microscopy Hitachi 7000.

The monoclonal antibody, mACI-01-Ab7 C2, binds directly to $A\beta_{1-42}$ fibers. Interestingly the antibody exhibits no symmetric binding to axis of single fibers but binds to particular and not all areas of side branches of the fiber network. It seemed to be the antibody targets specific regions within the side branches. The potential explanation is a specific secondary structure which occurs only in this specific side branches. This hypothesis is supported by NMR data

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demonstrating that the antibody induced transition on conformation and therefore it is likely that its binding is dependent on a conformation of the amyloid fiber comprising a β-sheet structure.

Example 20: Epitope mapping of monoclonal antibody mACI-01-Ab7 C2

Epitope mapping of the monoclonal antibody mACI-01-Ab7 C2 was performed by ELISA using three different peptide libraries. One library comprises a total of 33 biotinylated peptides covering the complete amino acid (aa) sequence of Aβ1-42 (produced by Mimotopes and purchased from ANAWA Trading SA), the second library contains biotinylated peptides using peptide 12 (aa12-20 of Aβ) from the first peptide library and substituting each amino acid in the sequence by an alanine (see table 41 below), and the third library contains biotinylated peptides 13, 14, or 15 (aa 13-21, 14-22 or 15-23 of Aβ) and substituting in each case the last amino acids to an alanine or to a glycine for aa 21 which is already an alanine (see table 5 below). A biotinylated complete Aβ1-42 peptide was used as positive control (Bachem). Epitope mapping was done according to the manufacturer's (Mimotopes) instructions. Briefly, Streptavidin coated plates (NUNC) were blocked with 0.1% BSA in PBS overnight at 4°C. After washing with PBS-0.05% Tween 20, plates were coated for 1 hour at RT with the different peptides from the library, diluted in 0.1% BSA, 0.1% Sodium Azide in PBS to a final concentration of 10 μM. After washing, plates were incubated for 1 hour at RT with the mACI-01-Ab7 C2 antibody or an isotype control mouse IgG2b antibody, diluted to 10 μg/ml in 2% BSA, 0.1% Sodium Azide in PBS. Plates were washed again and incubated with alkaline phosphatase conjugated goat anti mouse IgG for 1h at RT. After final washing, plates were incubated with phosphatase substrate (pNPP) and read at 405 nm using an ELISA plate reader.

It was shown that the monoclonal antibody mACI-01-Ab7 C2 bound specifically to peptides 12, 13, 14 and 15 of the first peptide library. These 4 peptides comprise aa 12-20 (VHHQKLVFF), 13-21 (HHQKLVFFA), 14-22 (HQKLVFFAE) and 15-23 (QKLVFFAED) of Aβ1-42 suggesting that the epitope lies in region 12-23 of Aβ. A second library with alanine substitutions was used to determine the critical aa for binding to peptide 12-20 (VHHQKLVFF). The binding of the mACI-01-Ab7 C2 antibody is lost completely when aa 16, 17, 19 or 20 are substituted by an alanine, indicating that these aa are absolutely critical for binding of the antibody to Aβ. The binding of the mACI-01-Ab7 C2 antibody is partially lost when aa 15 and 18 are substituted.

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The binding was also almost completely lost when aa 14 was substituted for an alanine, indicating that aa 14 is also very impotant for binding.

Finally, a third library was used to determine whether aa 21, 22 or 23 are critical for binding to the epitope. The binding of the antibody to aa 15-23 was reduced when aa 23 was substituted for an alanine, indicating that aa 23 is also impotant for binding. The binding was partially lost when aa 21 was substituted for a glycine and slightly lost when aa 22 was substituted for an alanine.

Table 4. Summary of peptides used in the second library.
aa that are important for binding are marked in italics and underscored and aa absolutely critical for binding are marked in italics and bold and underscored

p12-20	V	H	H	Q	K	L	V	F	F
A12	**A**	H	H	Q	K	L	V	F	F
A13	V	**A**	H	Q	K	L	V	F	F
A14	V	H	**A**	Q	K	L	V	F	F
A15	V	H	H	**A**	K	L	V	F	F
A16	V	H	H	Q	**A**	L	V	F	F
A17	V	H	H	Q	K	**A**	V	F	F
A18	V	H	H	Q	K	L	**A**	F	F
A19	V	H	H	Q	K	L	V	**A**	F
A20	V	H	H	Q	K	L	V	F	**A**
aa no.	12	13	***14***	***15***	***16***	***17***	***18***	***19***	***20***

Table 5. Summary of peptides used in the third library.
aa that are important for binding are marked in italics and underscored and aa absolutely critical for binding are marked in italics and bold and underscored

p13-21		H	H	Q	K	L	V	F	F	A		
p13-21	G21	H	H	Q	K	L	V	F	F	**G**		
p14-22			H	Q	K	L	V	F	F	A	E	
p14-22	A22		H	Q	K	L	V	F	F	A	**A**	
p15-23				Q	K	L	V	F	F	A	E	D
p15-23	A23			Q	K	L	V	F	F	A	E	**A**
aa no.		13	***14***	***15***	***16***	***17***	***18***	***19***	***20***	21	22	***23***

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Example 21: Influence of Passive Vaccination with mACI-01-Ab7 C2 on Brain Amyloid Load in single transgenic hAPP Mice

To assess the *in vivo* capacity of the mACI-01-Ab7 C2 monoclonal antibody to bind and clear soluble amyloid out of the brain, 6 month old single hAPP mice [9], gender and age matched, were used for a passive immunization study with different dose. Soluble Amyloid load was analyzed at the end of the study by harvesting the brain of the animals and by performing an Aβ 1-40 and Aβ 1-42 specific ELISA (TGC, Germany).

8-13 animals per group received two injections at an interval of one week of 100, 300 and 1000µg monoclonal antibody in 200µl PBS whereas injection of PBS alone served as control. One day after the second injection animals were sacrificed for biochemical analysis of soluble amyloid fraction. To quantify the amount of human Aβ 1-40 and human Aβ 1-42 in the soluble fraction of the brain homogenates and/or in cerebrospinal fluid (CSF), commercially available Enzyme-Linked-Immunosorbent-Assay (ELISA) kits were used (h Amyloid β 40 or β 42 ELISA high sensitive, TGC, Switzerland). The ELISA was performed according to the manufacturer's protocol. Briefly, standards (a dilution of synthetic Aβ 1-40 or Aβ 1-42) and samples were prepared in a 96-well polypropylene plate without protein binding capacity (Greiner, Germany). The standard dilutions with final concentrations of 1000, 500, 250, 125, 62.5, 31.3 and 15.6 pg/ml and the samples were prepared in the sample diluent, furnished with the ELISA kit, to a final volume of 60 µl. Since amyloid levels increase with the age of the mouse and since the actual evaluation requires that the readings of the samples are within the linear part of the standard curve, the samples for Aβ 40 analysis were diluted 2:3, the samples for Aβ 42 analysis were not diluted.

Samples, standards and blanks (50 µl) were added to the anti- Aβ -coated polystyrol plate (capture antibody selectively recognizes the C-terminal end of the antigen) in addition with a selective anti- Aβ -antibody conjugate (biotinylated detection antibody) and incubated overnight at 4°C in order to allow formation of the antibody-Amyloid-antibody-complex. The following day, a Streptavidine-Peroxidase-Conjugate was added, followed 30 minutes later by the addition of a TMB/peroxide mixture, resulting in the conversion of the substrate into a colored product and the color intensity was measured by means of photometry with an ELISA-reader with a 450

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nm filter. Quantification of the Aβ content of the samples was obtained by comparing absorbance to the standard curve made with synthetic Aβ 1-40 or Aβ 1-42. Data were expressed as individual changes to mean control value (in percent to control).

The total amount of Aβ 40 in brain homogenates could be significantly reduced and roughly non-significantly for Aβ 42 when single hAPP mice were passively immunized by two i.p. injections of monoclonal antibody ACI-01-Ab7 C2 at a dose of 300μg (Aβ 40: -27.3 ± 13.9% with $p<0.05$; Aβ 42: -8.6 ± 22.4 with $p=0.56$; unpaired Student's T test), whereas 100 and 1,000μg didn't reach significance. Immunization with 100μg lead to an increase for Aβ 40 and Aβ 42 in brain homogenates (Aβ 40: 32.3 ± 36.8%; A□42: 38.3 ± 51.4%) whereas treatment with 1,000μg elicited the right tendency of amyloid burden lowering and could be potentially effective with an increased number of animals per group (Aβ 40: -2.2 ± 26.0%; Aβ 42: -9.3 ± 15.9%). These data demonstrate that in an acute immunization protocol the antibody mACI-01-Ab7 C2 is capable to decrease the total amount of soluble Aβ in the brain of this murine AD model. Interestingly, it seems to be that the dose-relationship is transient but more studies with larger groups must be performed in order to gain significant data.

Example 22: Influence of Chronic Passive Administration of mACI-01-Ab7 C2 on Plaque Load in double transgenic hAPPxPS1 Mice

To assess the *in vivo* capacity of the mACI-01-Ab7 C2 monoclonal antibody to bind and reduce amyloid plaques in the brain, 3.5 month old double transgenic hAPPxPS1 mice [10], gender and age matched, were used for a 4 month long chronic passive immunization study. Amyloid plaques were analyzed at the end of the study by histochemistry of the brain of the animals by binding of Thioflavin S.

15 transgenic animals received 16 weekly injections of 500μg monoclonal antibody in PBS. 15 animals were injected with PBS alone, serving as controls. All injections were given intra-peritoneally. At sacrifice, mice were anaesthetized and flushed trans-cardially with physiological serum at 4°C to remove blood from the brain vessels. Subsequently, the brain was removed from the cranium and hindbrain and forebrain were separated with a cut in the coronal/frontal plane. The forebrain was divided evenly into left and right hemisphere by using a midline sagittal cut.

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One hemisphere was post-fixed overnight in 4% paraformaldehyde for histology. Sagittal vibratome sections (40 µm) were cut for free floating incubations and stored at 4°C until staining in PBS with 0.1% sodium azide. Five sections at different levels were stained for dense plaques with Thioflavin S. Sections of all animals used were randomized for staining and blind quantification. Images were acquired with a Leica DMR microscope equipped with a Sony DXC-9100P camera and analyzed with a computer using Leica Q-Win software. Light intensity and condenser settings for the microscope were kept constant throughout the image acquisition process. All acquired images were subjected to the same computer subroutines to minimize investigator bias. Density slice thresholding was applied uniformly throughout analysis. The area of the subiculum was selected for automatic quantification of the amyloid load in the Thioflavin S staining.

The total plaque load and the number of plaques in the area of subiculum could be significantly reduced when double hAPP/PS1 mice were passively immunized for 4 months as described above. In plaque load a significant decrease of 31% (mACI-01-Ab7 C2: 1.11 ± 0.21% and control: 1.61 ± 0.35%; p=0.003, Mann-Whitney U-Test) could be achieved whereas the chronic passive immunization significantly reduced the amount of plaques by 19% (mACI-01-Ab7 C2: 8.73 ± 1.36 and control: 10.78 ± 1.36; p=0.006, Mann-Whitney U-Test), indicating that plaque solubilization occurred to a slightly lesser degree than plaque disruption.

Example 23: Influence of Passive Vaccination with mACI-01-Ab7 C2 on Memory Capacity in single transgenic hAPP Mice

To analyze the *in vivo* capacity of the mACI-01-Ab7 C2 antibody to modify or increase cognitive functionality, 9 month old single hAPP mice, gender and age matched, were used for passive immunization study. Non-spatial cognition was measured at the end of the immunization period assed by new Object Recognition Task (ORT).

12 animals per group received two intra peritoneal injections of 400µg monoclonal antibody in 200µl PBS whereas injection of PBS alone served as control. One day after the second injection cognitive capability were studied in a new Object Recognition Task (ORT)[12, 13]. For ORT enrollment mice were placed for 10 minutes into a behavioral arena and faced to a new unknown object. Exploration time was recorded. Three hours later the same animals were re-placed into

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the same arena for a 2nd session but faced with the old, previously explored, and additionally with a new object. Again, exploration times for both objects were recorded and resulting cognition index was calculated as the ratio of exploration time for the new object related to total exploration time and expressed as proportional changes to the control.

Passive vaccination with mACI-01-Ab7 C2 leads to a significant increase of cognitive memory capacities in single transgenic AD mice (mACI-01-Ab7 C2: 131.6 ± 9.1% and control:100.0 ± 9.2% with $p<0.05$; unpaired Student's T test and n=12 per each group).

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Reference List

Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen H (2005) Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem 95:834-847.

Blond and Goldberg, 1987, PNAS March 1, 1987 Vol. 84 | no. 5 | 1147-1151

Cox JPL, Tomlinson IM and Winter G. Eur. J. Immunol. 1994; **24**: 827-836. A directory of human germ-line Vκ segments reveals a strong bias in their usage.

Hieter PA, Max EE, Seidman JG, Maizel JV Jr, Leder P. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments.Cell. 1980 Nov;22(1 Pt 1):197-207.

Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. Sequences of proteins of Immunological Interest, US Department of Health and Human Services, 1991.

Klein WL (2002) Abeta toxicity in Alzheimer's disease: globular soluble polymeric amyloid beta (ADDLs) as new vaccine and drug targets. Neurochem Int 41(5):345-352.

Langdon SD, Inaioki M, Kelsoe G. and Tedder TF. Immunogenetics 2000; **51**: 241-245. Germline sequences of V(H)7183 gene family members in C57BL/6 mice demonstrate natural selection of particular sequences during recent evolution

Mulligan RC and Berg P. Science 1980; 209: 1422-1427. Expression of a bacterial gene in mammalian cells.

Riechmann L, Clark M, Waldmann H, Winter G, Nature 1988; **332**: 323-327. Reshaping human antibodies for therapy.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
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Schable KF, Thiebe R, Bensch A, Brensing-Kueppers J, Heim V, Kirschbaum T, Lamm R, Ohnrich M, Pourrajabi S, Roschenthaler F, Schwendinger J, Wichelhaus D, Zocher I and Zachau HG. Eur. J. Immunol. 1999; **29**: 2082-2086. Characteristics of the immunoglobulin V kappa genes, pseudogenes, relics and orphons in the mouse genome.

Tomlinson IM, Walter G, Marks JD, Llewelyn MB and Winter G. J. Mol. Biol. 1992; **227**: 776-798. The repertoire of human germline V_H sequences reveals about 50 groups of V_H segments with different hypervariable loops

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CLAIMS:

1. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, which specifically binds to at least one epitope on the β-amyloid protein wherein the epitope comprises at least two consecutive amino acid residues predominantly involved in binding to the antibody, wherein the at least two consecutive amino acid residues are –Lys-Leu- embedded within the following core sequence (SEQ ID NO: 10):
   Xaa_1 – Xaa_2 - Lys - Leu – Xaa_3 wherein
   Xaa_1 is an amino acid selected from the group consisting of His, Asn, Gln Lys, and Arg,
   Xaa_2 is an amino acid selected from the group consisting of Asn and Gln; and
   Xaa_3 is an amino acid selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile.

2. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, which specifically binds to at least one epitope on the β-amyloid protein wherein the said epitope comprises at least two consecutive amino acid residues predominantly involved in the binding to the antibody, wherein the at least two consecutive amino acid residues are – Phe-Phe- embedded within the following core sequence (SEQ ID NO: 9):
   Xaa_3 - Phe – Phe – Xaa_4 - Xaa_5 - Xaa_6, wherein
   Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;
   Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;
   Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, and
   Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp.

3. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claims 1 or 2, which specifically binds to at least two epitopes on the β-amyloid protein wherein said at least two epitopes each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody,wherein the at least two consecutive amino acids are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent than said consecutive amino acid

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residues, which are –Phe-Phe- and –Lys-Leu-, respectively, embedded within the following core sequence:

Xaa_1 – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe - Xaa_4 - Xaa_5 - Xaa_6, wherein

Xaa_1 is an amino acid selected from the group consisting of His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid selected from the group consisting of Glu and Asp, and

Xaa_6 is an amino acid selected from the group consisting of Glu and Asp.

4. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 1 - 3, wherein

Xaa_1 is an amino acid selected from the group consisting of His and Arg;

Xaa_2 is an amino acid selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid selected from the group consisting of Val and Leu,

Xaa_4 is an amino acid selected from the group consisting of Ala and Val;

Xaa_5 is an amino acid selected from the group consisting of Glu and Asp, and

Xaa_6 is an amino acid selected from the group consisting of Glu and Asp.

5. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 4, wherein

Xaa_1 is His

Xaa_2 is Gln;

Xaa_3 is Val;

Xaa_4 is Ala;

Xaa_5 is Glu; and

Xaa_6 is Asp.

6. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 1-5, which specifically binds to at least two epitopes on the β-amyloid protein wherein said at least two epitopes each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, which are - Phe -

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Phe –and - Lys - Leu –, respectively, and wherein said at least two distinct binding sites exhibit amino acid sequence –Val - Phe - Phe - Ala - Glu – Asp - shown in SEQ ID NO: 7 and amino acid sequence His - Gln - Lys - Leu – Val - shown in SEQ ID NO: 8, respectively.

7. A humanized antibody or a fragment thereof which comprises in the variable region at least one CDR of non-human origin and one or more human- or primate-derived framework regions and, optionally, a constant region derived from a human or primate source antibody, which humanized antibody or fragment thereof is capable of specifically binding β-amyloid protein, β-amyloid monomeric peptide, polymeric soluble amyloid pepides comprising a plurality of β-amyloid monomeric units, β-amyloid fibers, fibrils or filaments, and a β-amyloid polymeric peptide in isolation or as part of a β-amyloid plaque, at an epitope comprising the following amino acid sequence (SEQ ID NO: 11):
Xaa_1 – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe- Xaa_4 _ Xaa_5 _ Xaa_6, wherein
Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, but particularly His;
Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln; and
Xaa_3 is an amino acid residue selected from the group consisting of Val, Leu, and Ile, but particularly Val;
Xaa4 is an amino acid residue selected from the group consisting of Ala and Val, but particularly Ala;
Xaa5 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu; and
Xaa6 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Asp.

8. A humanized antibody or a fragment thereof according to according to any one of claims 1-7, wherein the CDR of non-human origin is obtained from a donor antibody raised against an antigen fragment which does not contain said epitope.

9. A humanized antibody or a fragment thereof comprising variable regions with human- or primate-derived framework regions and at least one CDR with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and

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SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

10. A humanized antibody or a fragment thereof according to claim 9, wherein said humanized antibody comprises a heavy chain variable region with human- or primate-derived heavy chain framework regions and at least one CDR with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

11. A humanized antibody or a fragment thereof according to claim 9, wherein said humanized antibody comprises a light chain variable region with human- or primate-derived light chain framework regions and a CDR with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

12. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises variable regions with human- or primate-derived framework regions and at least two CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO:1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

13. A humanized antibody or a fragment thereof according to claim 12, wherein the humanized antibody comprises heavy chain variable region with human- or primate-derived heavy chain framework regions and at least two CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), wherein the same CDR cannot be present twice in the antibody.

14. A humanized antibody or a fragment thereof according to claim 12, wherein the humanized antibody comprises light chain variable regions with human- or primate-derived light chain framework regions and at least two CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5

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representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody.

15. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises heavy chain variable regions with human- or primate-derived heavy chain framework regions and CDRs with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated.

16. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises light chain variable regions with human- or primate-derived light chain framework regions and CDRs with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated.

17. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises variable regions with human- or primate-derived framework regions and at least three CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody.

18. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises variable regions with human- or primate-derived framework regions and at least four CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody.

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19. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises variable regions with human- or primate-derived framework regions and at least five CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody.

20. A humanized antibody or a fragment thereof according to claim 9, wherein the humanized antibody comprises variable regions with human- or primate-derived framework regions and CDRs with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

21. A humanized antibody or a fragment thereof according to claim 10, wherein the at least one CDR is a CDR with an amino acid sequence of SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

22. A humanized antibody or a fragment thereof according to claim 10, wherein the at least one CDR is a CDR with an amino acid sequence of SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

23. A humanized antibody or a fragment thereof according to claim 13, wherein the at least two CDRs are CDRs with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

24. A humanized antibody or a fragment thereof according to claim 13, wherein the at least two CDRs are CDRs with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

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25. A humanized antibody or a fragment thereof according to claim 13, wherein the at least two CDRs are CDRs with an amino acid sequence of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

26. A humanized antibody or a fragment thereof according to claim 14, wherein the at least two CDRs are CDRs with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 5 representing CDR2 of the Light Chain Variable Region (LCVR).

27. A humanized antibody or a fragment thereof according to claim 14, wherein the at least two CDRs are CDRs with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

28. A humanized antibody or a fragment thereof according to claim 12, wherein the at least two CDRs are selected from the group of CDRs according to any one of claims 23 to 27.

29. A humanized antibody or a fragment thereof according to any one of claims 9 to 28 comprising a light chain and/or a heavy chain constant region of human or primate origin.

30. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein at least one of the amino acids of a light chain and a heavy chain CDR region as given in SEQ ID NOs: 1 – 6 is changed through a conservative substitution such that the antibody maintains its full functionality.

31. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, according to claim 30, wherein within the amino acid sequence of CDR2 of the light chain variable region (LCVR) as given in SEQ ID NO: 5, the Lys at Kabat position 50 is replaced by Arg, Gln or Glu, but particularly by Arg, and /or the Asn at Kabat position 53 is replaced by Ala, Val, Leu, Ser and Ile; but expecially Ser.

32. A humanized antibody or a fragment thereof according to any one of claims 9 to 31, wherein the framework sequences are human germline V_K sequences of KABAT subgroup V_KII.

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33. A humanized antibody or a fragment thereof according to any one of claims 9 to 31, wherein the framework sequences are human germline V_H sequences of KABAT subgroup V_HIII.

34. A humanized antibody or a fragment thereof according to any one of claims 1 – 33, wherein at least one of the amino acids of the human- or primate-derived framework region is changed through a substitution to an amino acid from the corresponding region of murine humanized antibody ACI-01-Ab7C2 or a substitution conservative thereto.

35. A humanized antibody or a fragment thereof according to claim 34, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met , Ala, and Phe.

36. A humanized antibody or a fragment thereof according to claim 34, wherein the Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser.

37. A humanized antibody or a fragment thereof according to claim 34, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser.

38. A humanized antibody or a fragment thereof according to claim 34, wherein the Tyr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe.

39. A humanized antibody or a fragment thereof according to claim 34, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable

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Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser., and the Tyr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe.

40. A humanized antibody or a fragment thereof according to claim 35, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu.

41. A humanized antibody or a fragment thereof according to claim 36, wherein the Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser.

42. A humanized antibody or a fragment thereof according to claim 37, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu and Ile, but especially Leu.

43. A humanized antibody or a fragment thereof according to claim 38, wherein the Tyr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by Phe.

44. A humanized antibody or a fragment thereof according to claim 39, wherein the Trp in Kabat position 47 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group

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consisting of Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the human- or primate-derived framework region of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Ser and the Tyr in Kabat position 87 in the human- or primate-derived framework region of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by Phe.

45. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof antibody according to any one of claims 1 – 44, wherein the Heavy Chain Variable Region (HCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 15 and 16, respectively.

46. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 1 – 44, wherein the Light Chain Variable Region (LCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 12 and 13, respectively.

47. A humanized antibody or a fragment thereof according to any one of claims 1 – 44, wherein at least two, but especially three, of the CDR regions of the Heavy Chain Variable Region (HCVR) have an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 1 – 3.

48. A humanized antibody or a fragment thereof according to any one of claims 1 – 44, wherein at least two, but especially three, of the CDR regions of the Light Chain Variable Region (LCVR) have an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 4 – 6.

49. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof which comprises the light chain variable region of SEQ ID NO: 13.

50. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 49 which, in addition, comprises the light chain constant region of SEQ ID NO: 14.

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51. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof which comprises the light chain variable region of SEQ ID NO: 13 and the light chain constant region of SEQ ID NO: 14.

52. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof which comprises the heavy chain variable region of SEQ ID NO: 16.

53. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 52 which, in addition, comprises the heavy chain constant region of SEQ ID NO: 17.

54. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof which comprises the heavy chain variable region of SEQ ID NO: 16 and the heavy chain constant region of SEQ ID NO: 17.

55. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 50, 51, 53 and 54, wherein the N-terminal Lys of the heavy chain constant region has been removed.

56. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody is of the IgG1, IgG2, IgG3, or IgG4 isotype including a mutated IgG4, but particularly of the IgG_4 isotype.

57. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody is bi-effective in that it exhibits both an aggregation inhibition property as well as a disaggregation property, particularly paired with a high degree of conformational sensitivity.

58. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody, upon co-incubation with amyloid monomeric and/or polymeric soluble amyloid peptides, particularly with β-amyloid monomeric peptides such as, for example, Aβ monomeric peptides 1-39; 1-40, 1-41, or 1-42, and/or a polymeric soluble β-amyloid peptide comprising a plurality of said Aβ monomeric

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units, but especially with an $A\beta_{1-42}$ monomeric and/or an $A\beta$ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units, inhibits the aggregation of the $A\beta$ monomers into high molecular polymeric fibrils or filaments and, in addition, upon co-incubation with preformed high molecular polymeric amyloid fibrils or filaments formed by the aggregation of amyloid monomeric peptides, particularly β-amyloid monomeric peptides such as, for example, $A\beta$ monomeric peptides 1-39; 1-40, 1-41, or 1-42, but especially $A\beta_{1-42}$ monomeric peptides, is capable of disaggregating the preformed polymeric fibrils or filaments.

59. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds to aggregated $A\beta$ in a mammalian brain, particularly a human brain.

60. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody reduces $A\beta$ plaque burden in a mammalian brain, particularly a human brain.

61. A nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 1 – 60.

62. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody variable region comprising SEQ ID NO: 2 and 3 representing the Complementarity Determining Regions (CDRs) 2 and 3 of the Heavy Chain Variable Region (HCVR).

63. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody variable region comprising SEQ ID NO: 4 representing the Complementarity Determining Regions (CDRs) 1 of the Light Chain Variable Region (LCVR).

64. A nucleic acid molecule according to claim 62 comprising a nucleotide sequence as given in SEQ ID NO: 18 and SEQ ID NO: 19.

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65. A nucleic acid molecule according to claim 63 comprising a nucleotide sequence as given in SEQ ID NO: 20.

66. A nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of SEQ ID NO: 22.

67. A nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of SEQ ID NO: 22 and the light chain constant region of SEQ ID NO: 23.

68. A nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain variable region of SEQ ID NO: 25

69. A nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain variable region of SEQ ID NO: 25 and the heavy chain constant region of SEQ ID NO: 26.

70. An expression vector comprising the nucleotide sequence of any one of claims 60 to 69.

71. A cell comprising an expression vector comprising the nucleotide sequence of any one of claims 60 to 68 or an expression vector of claim 70.

72. A composition comprising the antibody according to any one of claims 1 to 60 including any functionally equivalent antibody or functional parts thereof in a therapeutically effective amount.

73. A composition according to claim 72 which is a pharmaceutical composition optionally further comprising a pharmaceutically acceptable carrier.

74. A composition according to any one of claims 72 to 73 comprising the antibody in a therapeutically effective amount.

75. A mixture comprising a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of claims 1 to 60 including any functionally equivalent antibody or functional parts thereof in a therapeutically effective amount and,

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optionally, a further biologically active substance and/or a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

76. A mixture according to claim 75, wherein the further biologically active substance is a compound used in the treatment of amyloidosis, a group of diseases and disorders associated with amyloid or amyloid-like protein such as the Aβ protein involved in Alzheimer's disease.

77. A mixture according to claim 76 comprising at least one compound selected from the group consisting of compounds against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), α-secretase activators, β- and γ –secretase inhibitors, tau proteins, neurotransmitter, β-sheet breakers, attractants for amyloid beta clearing / depleting cellular components, inhibitors of N-terminal truncated amyloid beta including pyroglutamated amyloid beta 3-42, anti-inflammatory molecules, or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine, M1 agonists and other drugs including any amyloid or tau modifying drug and nutritive supplements, together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

78. A mixture according to claim 77, wherein the compound is a cholinesterase inhibitor (ChEIs).

79. A mixture according to any one of claims 75 to 77, wherein the compound is one selected from the group consisting of tacrine, rivastigmine, donepezil, galantamine, niacin and memantine.

80. A mixture according to any one of claims 75 to 79 comprising the antibody and/or the biologically active substance in a therapeutically effective amount.

81. Use of a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof and/or a functional part thereof according to any one of claims 1 to 60 and/or a pharmaceutical composition according to any one of claims 72 to 74, or a mixture according to any one of claims 75 to 80 for the preparation of a medicament for treating or alleviating

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the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

82. Use according to claim 81, wherein the amyloid associated disease or condition is Alzheimer's disease.

83. Use according to claim 81, wherein the amyloid-associated condition is characterized by a loss of cognitive memory capacity in an animal, particularly a mammal or a human.

84. Use according to claim 81, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to an increase of the cognitive memory capacity.

85. Use according to claim 81, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to a complete restoration of the cognitive memory capacity.

86. A method for the preparation of a pharmaceutical composition according to any one of claims 72 to 74, or a mixture according to any one of claims 75 to 80 for use in a method of preventing, treating or alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like

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proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration comprising formulating an antibody according to the invention in a pharmaceutically acceptable form.

87. A method according to claim 86, wherein the antibody is comprised in the composition in a therapeutically effective amount.

88. A method according to claim 86, wherein the amyloid associated disease or condition is Alzheimer's Disease.

89. A method according to claim 86, wherein the amyloid-associated condition is characterized by a loss of cognitive memory capacity in an animal, particularly a mammal or a human.

90. A method according to claim 86, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to an increase of the cognitive memory capacity.

91. A method according to claim 86, wherein the amyloid-associated condition is characterized by an impairment of the cognitive memory capacity in an animal, particularly a mammal or a human.

92. A method according to claim 86, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to a reversal of the cognitive memory capacity and, particularly, a complete restoration of the cognitive memory capacity.

93. A method for preventing, treating or alleviating the effects of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam

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Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration by administering a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof and/or a functional part thereof according to any one of claims 1 to 60 and/or a pharmaceutical composition according to any one of claims 72 to 74, or a mixture according to any one of claims 75 to 80, to a animal or a human affected by such a disorder comprising administering the antibody in a therapeutically effective amount.

94. A method according to claim 93, wherein the amyloid associated disease or condition is Alzheimer's Disease.

95. A method according to claim 93, wherein the amyloid-associated condition is characterized by a loss of cognitive memory capacity in an animal, particularly a mammal or a human.

96. A method according to claim 93, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to an increase in the retention or cognitive memory capacity.

97. A method according to claim 93, wherein the amyloid-associated condition is characterized by an impairment of cognitive memory capacity in an animal, particularly a mammal or a human.

98. A method according to claim 93, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to a complete restoration of cognitive memory capacity.

99. Method of diagnosis of an amyloid-associated disease or condition in a patient comprising

(a) bringing the sample or a specific body part or body area suspected to contain the amyloid protein into contact with an antibody according to any one of claims 1 to 60;

(b) allowing the antibody to bind to the amyloid protein;

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(c) detecting the antibody bound to the protein; and

(d) correlating the presence or absence of antibody binding with the presence or absence of amyloid protein in the sample or specific body part or area.

100. Method of determining the extent of amyloidogenic plaque burden in a tissue and/or body fluids comprising

(a) obtaining a sample representative of the tissue and/or body fluids under investigation;

(b) testing said sample for the presence of amyloid protein with an antibody according to any one of claims 1-60;

(c) determining the amount of antibody bound to the protein; and

(d) calculating the plaque burden in the tissue and/or body fluids.

101. Test kits for detection and diagnosis of amyloid-associated diseases and conditions comprising an antibody according to any one of claims 1-60.

102. Test kit according to claim 101 comprising a container holding one or more antibodies according to the present invention and instructions for using the antibodies for the purpose of binding to amyloid protein to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

103. Light Chain Variable Region (LCVR) comprising human- or primate-derived framework regions and at least one CDR with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

104. Heavy Chain Variable Region (HCVR) comprising human- or primate-derived framework regions and at least one CDR with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

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105. Heavy Chain Variable Region (HCVR) comprising human- or primate-derived heavy chain framework regions and at least two CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

106. Light Chain Variable Region (LCVR),comprising human- or primate-derived light chain framework regions and at least two CDRs with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

107. Heavy Chain Variable Region (HCVR) comprising human- or primate-derived heavy chain framework regions and CDRs with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated above.

108. The Light Chain Variable Region (LCVR) comprising integrated into human- or primate-derived light chain framework regions and CDRs with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated above.

109. The Light Chain Variable Region (LCVR) of SEQ ID NO: 12.

110. A humanized antibody or a fragment thereof is provided, which comprises the Light Chain Variable Region (LCVR) of SEQ ID NO: 12.

111. The Light Chain Variable Region (LCVR) including signal sequences as shown in SEQ ID NO: 13.

112. A humanized antibody or a fragment thereof, which comprises the complete Light Chain Variable Region (LCVR) including signal sequences as shown in SEQ ID NO: 13.

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113. A humanized antibody or a fragment thereof, which comprises the Light Chain Variable Region (LCVR) of SEQ ID NO: 12 and the light chain constant region of SEQ ID NO: 14.

114. A humanized antibody or a fragment thereof, which comprises the complete Light Chain Variable Region (LCVR) of SEQ ID NO: 13 and the light chain constant region of SEQ ID NO: 14.

115. The Heavy Chain Variable Region (HCVR) of SEQ ID NO: 15.

116. A humanized antibody or a fragment thereof, which comprises the Heavy Chain Variable Region (HCVR) of SEQ ID NO: 15.

117. The Heavy Chain Variable Region (HCVR) including signal sequences as shown in SEQ ID NO: 16.

118. A humanized antibody or a fragment thereof, which comprises the complete Heavy Chain Variable Region (HCVR) including signal sequences as shown in SEQ ID NO: 16.

119. A humanized antibody or a fragment thereof, which comprises the Heavy Chain Variable Region (HCVR) of SEQ ID NO: 15 and the heavy chain constant region of SEQ ID NO: 17.

120. A humanized antibody or a fragment thereof, which comprises the Heavy Chain Variable Region (HCVR) of SEQ ID NO: 16 and the heavy chain constant region of SEQ ID NO: 17.

121. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds Aβ monomers but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

122. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 122, wherein said antibody or fragment binds to an Aβ monomer with a binding affinity represented by a K_D in a range of at least about 1 x 10^{-7} M to at least

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about 1 x 10^{-12} M, particularly of at least about 1 x 10^{-8} M to at least about 1 x 10^{-11} M, more particularly of at least about 1 x 10^{-8} M to at least about 1 x 10^{-10} M, even more particularly of at least about 1 x 10^{-8} M to at least about 5 x 10^{-8} M but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

123. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds Aβ fiber, fibril or filament but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

124. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 123, wherein said antibody or fragment binds to an Aβ fiber, fibril or filament with a binding affinity represented by a K_D in a range of at least about 1 x 10^{-7}M to at least about 1 x 10^{-12} M, particularly of at least about 1 x 10^{-8}M to at least about 1 x 10^{-11}M, more particularly of at least about 1 x 10^{-9}M to at least about 1 x 10^{-10}M, even more particularly of at least about 2 x 10^{-9}M to at least about 8 x 10^{-9}M, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

125. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds soluble polymeric amyloid beta but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

126. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds Aβ monomers and/or Aβ fibers, fibrils or filaments and/or soluble polymeric amyloid beta but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

127. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 126, wherein the binding affinity increases in ascending order.

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128. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 127, wherein said antibody or fragment thereof exhibits an binding affinity to an Aβ fiber, fibril or filament which is at least 10 times, particularly at least 15 times, more particularly at least 20 times, but especially at least 25 times higher than the binding affinity to an Aβ monomer.

129. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds to aggregated Aβ, including Aβ plaques, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

130. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody substantially binds to soluble fibers, including Aβ monomers, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

131. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof is capable of shifting the equilibrium between Aβ in its soluble and aggregated state towards its soluble form by disaggregating fibers to soluble poly- and monomeric forms by inducing a shift in conformation and binding and stabilizing the disaggregated and solubilized Aβ forms in the tissue and/or body fluids, particularly the brain.

132. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 131, which antibody or fragment thereof is capable of inducing a transition of the β-sheet conformation towards an α-helix and/or a random coil conformation, but particularly a random coil conformation, even more particularly a random coil conformation at a given location in the molecule, especially in the environment of Tyr 10 and Val12 of the Aβ protein, which leads to an increase of the random coil conformation at the expense of the β-sheet conformation and an improved solubilization of the preformed high molecular polymeric amyloid fibrils or filaments.

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133. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 132, wherein the decrease of the β-sheet conformation amounts to at least 30%, particularly to at least 35%, and more particularly to at least 40% and more as compared to the respective preformed amyloid polymeric fibrils or filaments incubated in buffer (control).

134. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to claim 131, which antibody or fragment thereof substantially bind to polymeric and less aggregated soluble Aβ protein species including monomeric forms thus modifying the peripheral clearing and catabolism of these Aβ protein species and reducing their toxic effects.

135. A humanized antibody or a fragment thereof according to any of the preceding claims comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof incorporating at least one, particularly two and more particularly three CDR regions obtained form a mouse donor antibody, particularly from mouse antibody ACI-01-Ab7C2, wherein said antibody or fragment thereof has an affinity to the Aβ antigen which is at least 5 times, particularly at least 8 times, more particularly at least 10 times, but especially at least 15 times higher than that of the mouse donor antibody.

136. Use according to any one of claims 83 to 85, wherein the humanized antibody or a fragment thereof does not necessarily bind aggregated amyloid beta in the brain.

137. A method according to any one of claims 89 to 91, wherein the humanized antibody or a fragment thereof does not necessarily bind aggregated amyloid beta in the brain.

138. Use according to claim 81, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to the retention of cognitive memory capacity.

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139. A method according to claim 86, wherein treatment of an animal, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity leads to the retention of the cognitive memory capacity.

140. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof is capable of shifting the equilibrium between Aβ in its soluble and aggregated state towards its soluble form.

141. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof is capable of shifting the equilibrium between Aβ in its soluble and aggregated state towards its soluble form by disaggregating fibers to soluble poly- and monomeric forms.

142. A chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof does not substantially bind Aβ in human tissue and/or body fluids, particularly the brain, but is capable of shifting the equilibrium between Aβ in its soluble and aggregated state towards its soluble form by disaggregating fibers to soluble poly- and monomeric forms by inducing a shift in conformation and binding and stabilizing the disaggregated and solubilized Aβ forms in the tissue and/or body fluids, particularly the brain.

143. A humanized antibody or a fragment thereof according to any of the preceding claims, wherein said antibody or fragment thereof exhibits an binding affinity which is at least 5 times, 10 times, particularly at least 15 times, more particularly at least 20 times, but especially at least 100 times higher than the binding affinity of the mouse antibody.

144. A humanized antibody or a fragment thereof according to any of the preceding claims, wherein the affinity, specificity and stability of said antibody or fragment thereof has been modified by a change of the glycosylation profile.

145. An antibody comprising a variable region as recited in SEQ ID NO: 27, or a variant thereof.

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146. A cell line expressing the antibody of claim 145.

147. An antibody gene comprising a variable region as recited in SEQ ID NO: 29, or a variant thereof.

148. A cell line expressing the antibody of claim 147.

149. A method for disaggregating preformed beta-amyloid fibers, comprising interacting an hC2 antibody with preformed beta-amyloid fibers.

150. A humanized antibody or a fragment thereof according to any of the preceding claims, wherein said antibody or fragment thereof protects neurons from Abeta-induced degradation.

151. A method of preventing Abeta-induced neuron degradation comprising treating neurons with an effective amount of a humanized antibody or a fragment thereof according to any of the preceding claims.

152. Use of a humanized antibody or a fragment thereof according to any of the preceding claims for the preparation of a medicament for preventing degeneration of neurons upon exposure to Abeta oligomer.

153. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites on the β-amyloid protein wherein the said at least one or said at least two distinct binding sites comprise at least one amino acid residue and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein, in a specific embodiment of the invention, the at least one residue constituting the first distinct binding site is Leu and the at least two consecutive amino acid residues, constituting the second distinct binding site, are –Phe-Phe- embedded within the following core sequence:

$$-Xaa_1-Xaa_2-Xaa_3-Leu\text{-}Xaa_4-Phe-Phe-Xaa_5-Xaa_6-Xaa_7-$$

wherein

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Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_3 is an amino acid residue selected from the group comprising Lys, His, Asn, Gln and Arg

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp.

154. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids, which are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues predominantly involved in the binding of the antibody, are -His- and –Lys-Leu-, respectively, embedded within the following core sequence:

$-His-Xaa_2-Lys-Leu-Xaa_3-Xaa_4-Xaa_5-Xaa_6--Xaa_7-Xaa_8-$ wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_3 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_6 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp,

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Xaa_8 is an amino acid residue selected from the group comprising Glu and Asp

and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_6, Xaa_7, Xaa_8, are not involved in antibody binding or to a significantly smaller extent as compared to the –His- and the –Lys-Leu- binding site.

155. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- and the at least one amino acid residue is –His- embedded within the following core sequence:

$– Xaa_1 – His – Xaa_3 – Xaa_4 – Xaa_5 – Xaa_6 – Phe – Phe – Xaa_7 – Xaa_8 – Xaa_9 –$,

wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln, Lys and Arg

Xaa_3 is an amino acid residue selected from the group comprising Asn and Gln

Xaa_4 is an amino acid residue selected from the group comprising His, Asn, Gln, Lys and Arg

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Ala, Val, Leu and Ile

Xaa_7 is an amino acid residue selected from the group comprising Ala, Val, Leu and Ile

Xaa_8 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_9 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_1, Xaa_3, Xaa_6, Xaa_7, Xaa_8 and Xaa_9, are not involved in antibody binding or to a significantly smaller extent as compared to the His and the –Phe-Phe- binding site.

156. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-

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amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the first of the at least two consecutive amino acid residues predominantly involved in the binding of the antibody are –Lys- and –Leu-, and the second of the at least two consecutive amino acid residues are –Phe-Phe- embedded within the following core sequence:

$-Xaa_1-Xaa_2-Lys-Leu-Xaa_4-Phe-Phe-Xaa_5-Xaa_6-Xaa_7-$

wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp, , and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –Lys-Leu and the –Phe-Phe- binding site.

157. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the first of the at least two consecutive amino acid residues predominantly involved in the binding of the antibody are –Lys-Leu-, and the second of the at least two consecutive amino acid residues are –Phe-Phe-, and the third at least one amino residue is -His- embedded within the following core sequence:

$-His-Xaa_2-Lys-Leu\text{-}Xaa_4-Phe-Phe-Xaa_5-Xaa_6-Xaa_7-$

wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

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Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp,

Xaa_7 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –His-, the -Lys-Leu, and the –Phe-Phe- binding site.

158. A monoclonal antibody.including any functionally equivalent antibody or functional parts thereof, which antibody recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the first of the at least two consecutive amino acid residues predominantly involved in the binding of the antibody are –Lys-Leu-, and the second of the at least two consecutive amino acid residues are –Phe-Phe-, and the third at least one amino residue is -Asp- embedded within the following core sequence:

$– Xaa_1 – Xaa_2 – Lys – Leu -Xaa_4 – Phe – Phe – Xaa_5 – Xaa_6 – Asp –$

wherein

Xaa_1 is an amino acid residue selected from the group comprising His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

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159. A monoclonal antibody including any functionally equivalent antibody or functional parts thereof, which antibody binds to 4 distinct binding sites on the β-amyloid protein wherein said 4 distinct binding sites comprise one amino acid residue and two consecutive amino acid residues, respectively, which residues are predominantly involved in the binding of the antibody, wherein said 4 distinct binding sites are located in close proximity to each other on the antigen, separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to said one amino acid residue and said two consecutive amino acid residues of the 4 distinct binding sites thus forming a conformational discontinuous epitope.

160. The monoclonal antibody according to claim 159 including any functionally equivalent antibody or functional parts thereof, wherein the first of the two consecutive amino acid residues predominantly involved in the binding of the antibody are –Lys-Leu-, and the second of the at least two consecutive amino acid residues are –Phe-Phe-, the first of the single amino residues is -His- and the second of the single amino residues is -Asp- embedded within the following core sequence:

– His – Xaa_2 – Lys – Leu -Xaa_4 – Phe – Phe – Xaa_5 – Xaa_6 – Asp –

wherein

Xaa_2 is an amino acid residue selected from the group comprising Asn and Gln;

Xaa_4 is an amino acid residue selected from the group comprising Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_5 is an amino acid residue selected from the group comprising Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group comprising Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, Xaa_7 are not involved in antibody binding or to a significantly smaller extent as compared to the –His-, –Asp-, the -Lys-Leu, and the –Phe-Phe- binding site.

161. A monoclonal antibody according to any one of claims 157-160 including any functionally equivalent antibody or functional parts thereof, wherein said consecutive amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region of the amyloid β peptide:

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Val	His	His	Gln	Lys	Leu	Val	Phe	Phe	Ala	Glu	Asp
12	13	14	15	16	17	18	19	20	21	22	23

162. A method for diagnosing a predisposition to an amyloid-associated disease or condition in a patient comprising detecting the immunospecific binding of a monoclonal antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ* which includes the steps of

(a) bringing the sample or a specific body part or body area suspected to contain the amyloid antigen into contact with an antibody according to the invention and as described herein before, which antibody binds an epitope of the amyloid protein;

(b) allowing the antibody to bind to the amyloid antigen to form an immunological complex;

(c) detecting the formation of the immunological complex; and

(d) correlating the presence or absence of the immunological complex with the presence or absence of amyloid antigen in the sample or specific body part or area,

(e) comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an amyloid-associated disease or condition.

163. A method for monitoring minimal residual disease in a patient following treatment with an antibody or a vaccine composition according to any one of the preceding claims, wherein said method comprises:

(a) bringing the sample or a specific body part or body area suspected to contain the amyloid antigen into contact with an antibody according to the invention and as described herein before, which antibody binds an epitope of the amyloid protein;

(b) allowing the antibody to bind to the amyloid antigen to form an immunological complex;

(c) detecting the formation of the immunological complex; and

(d) correlating the presence or absence of the immunological complex with the presence or absence of amyloid antigen in the sample or specific body part or area,

(e) comparing the amount of said immunological complex to a normal control value,

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wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

164. A method for predicting responsiveness of a patient being treated with an antibody or a vaccine composition according to any one of the preceding claims comprising

(a) bringing the sample or a specific body part or body area suspected to contain the amyloid antigen into contact with an antibody according to the invention and as described herein before, which antibody binds an epitope of the amyloid protein;

(b) allowing the antibody to bind to the amyloid antigen to form an immunological complex;

(c) detecting the formation of the immunological complex; and

(d) correlating the presence or absence of the immunological complex with the presence or absence of amyloid antigen in the sample or specific body part or area,

(e) comparing the amount of said immunological complex before and after onset of the treatment,

wherein an decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

165. A method for reducing the plaque load in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of a monoclonal antibody any one of claims 153 to 161.

166. A method according to claim 165, wherein the plaque load in the brain is decreased by at least 20 %, particularly by at least 25%, more particularly by at least 30%, even more particularly by more than 30%

167. A method for reducing the amount of plaques in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to an increased plaque load in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of a monoclonal antibody any one of claims 153 to 161.

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168. A method according to claim 94, wherein the amount of plaques in the brain is reduced by at least 10 %, particularly by at least 15%, more particularly by at least 20%.

169. A method for decreasing the total amount of soluble Aβ in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to increased concentrations of soluble Aβ in the brain comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of a monoclonal antibody any one of claims 153 to 161.

170. A methods for retaining or increasing cognitive memory capacity in a mammal exhibiting an amyloid-associated disease or condition comprising administering to an animal, particularly a mammal, more particularly a human in need of such a treatment, a therapeutically effective amount of a monoclonal antibody any one of claims 153 to 161.

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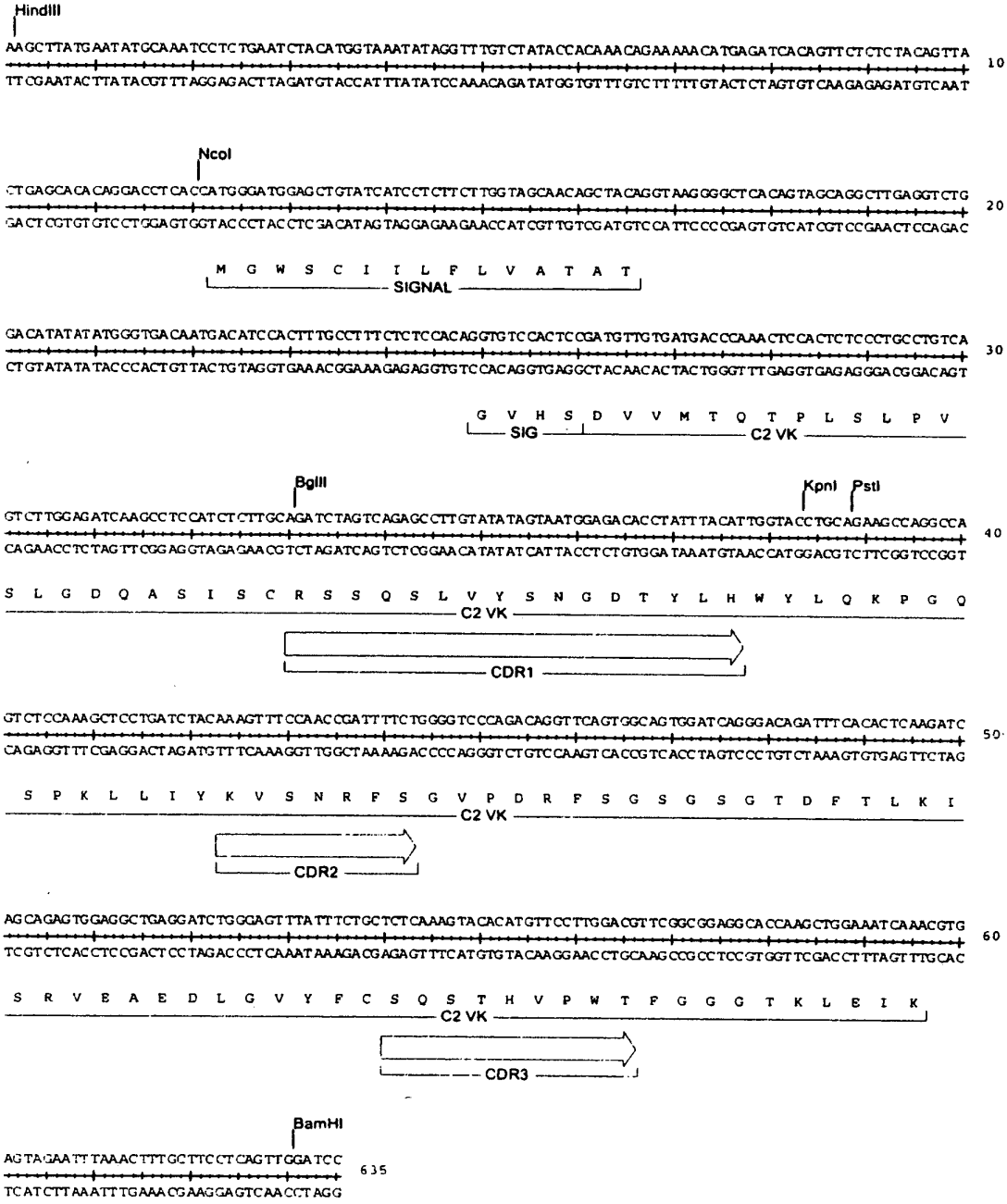


FIG. 1

(Example 2)

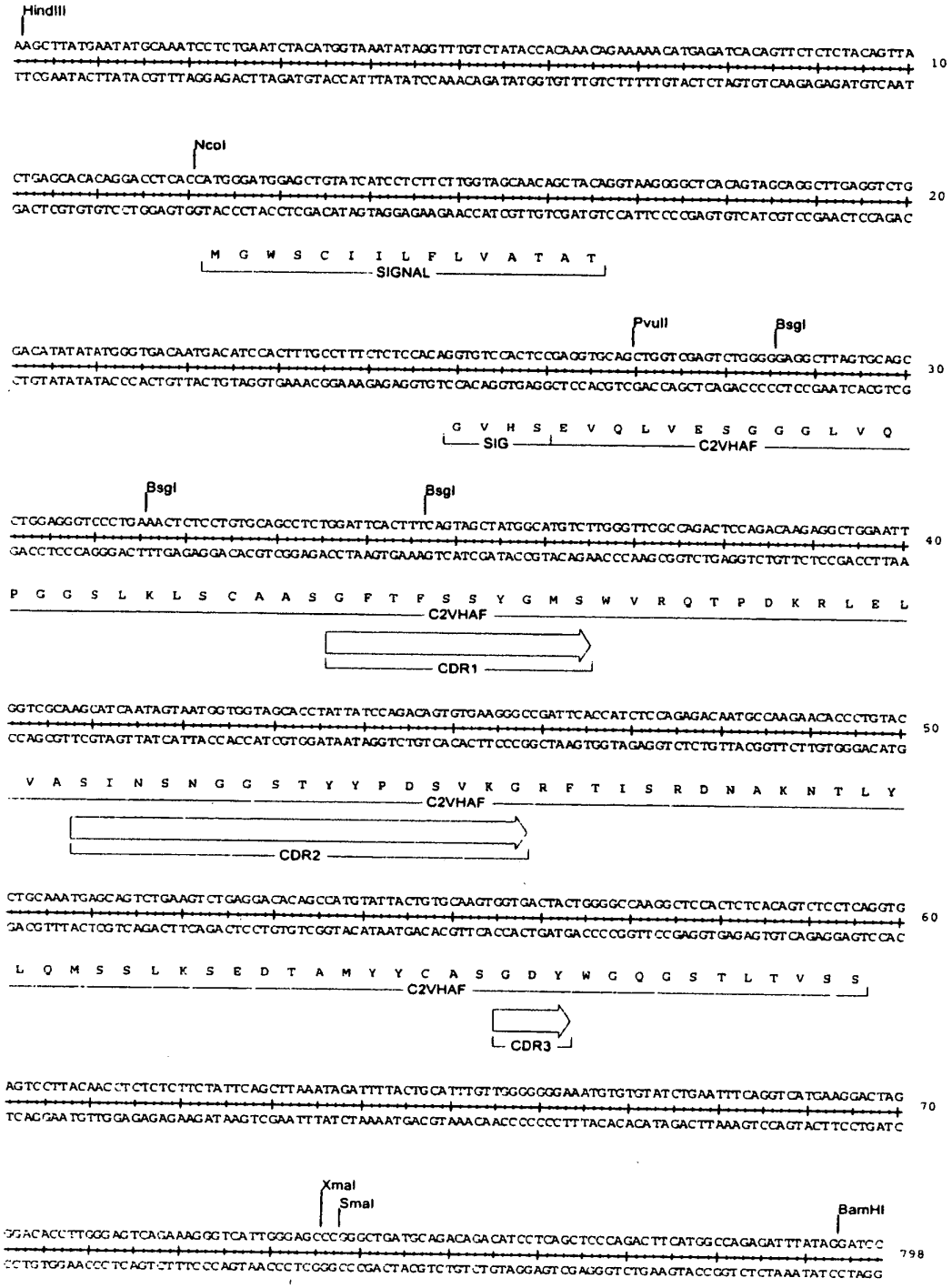


FIG. 2
(Example 2)

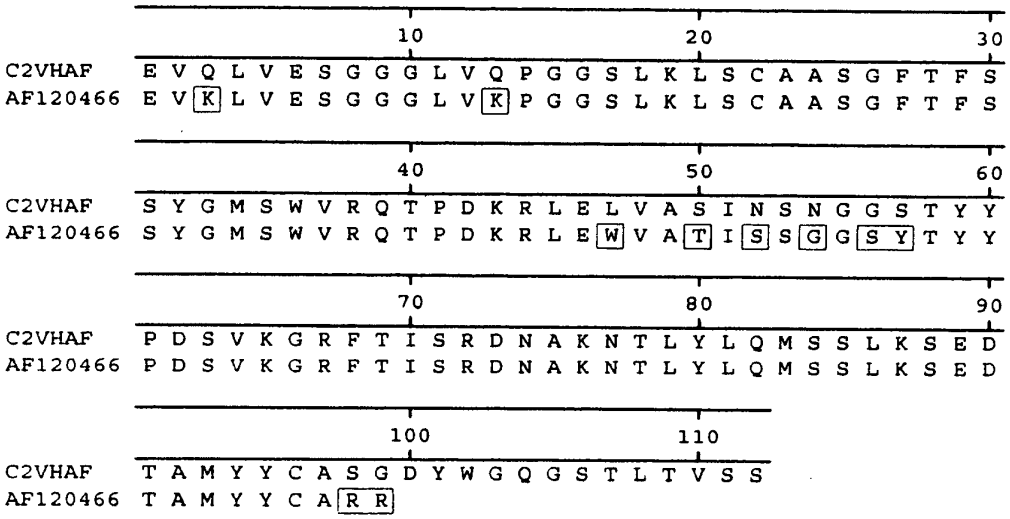


FIG. 3

(Example 5.2)

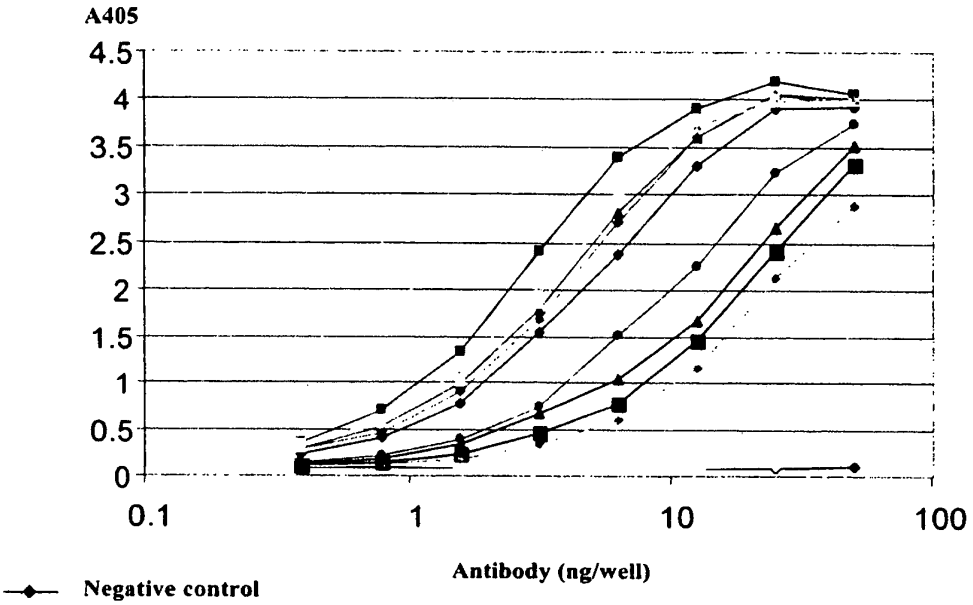


FIG. 4
(Example 8)

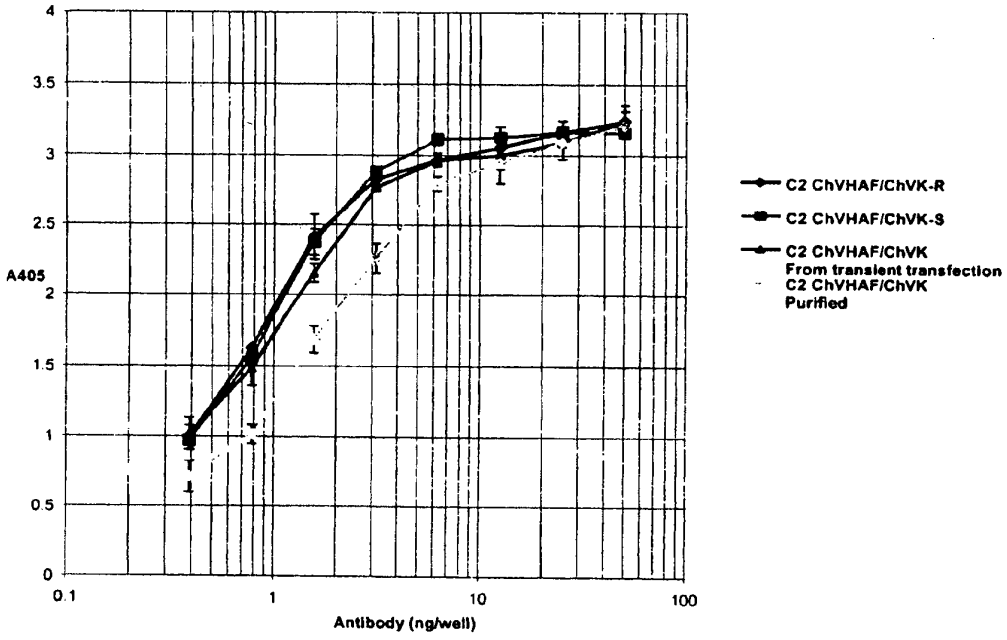


FIG. 5

(Example 9)

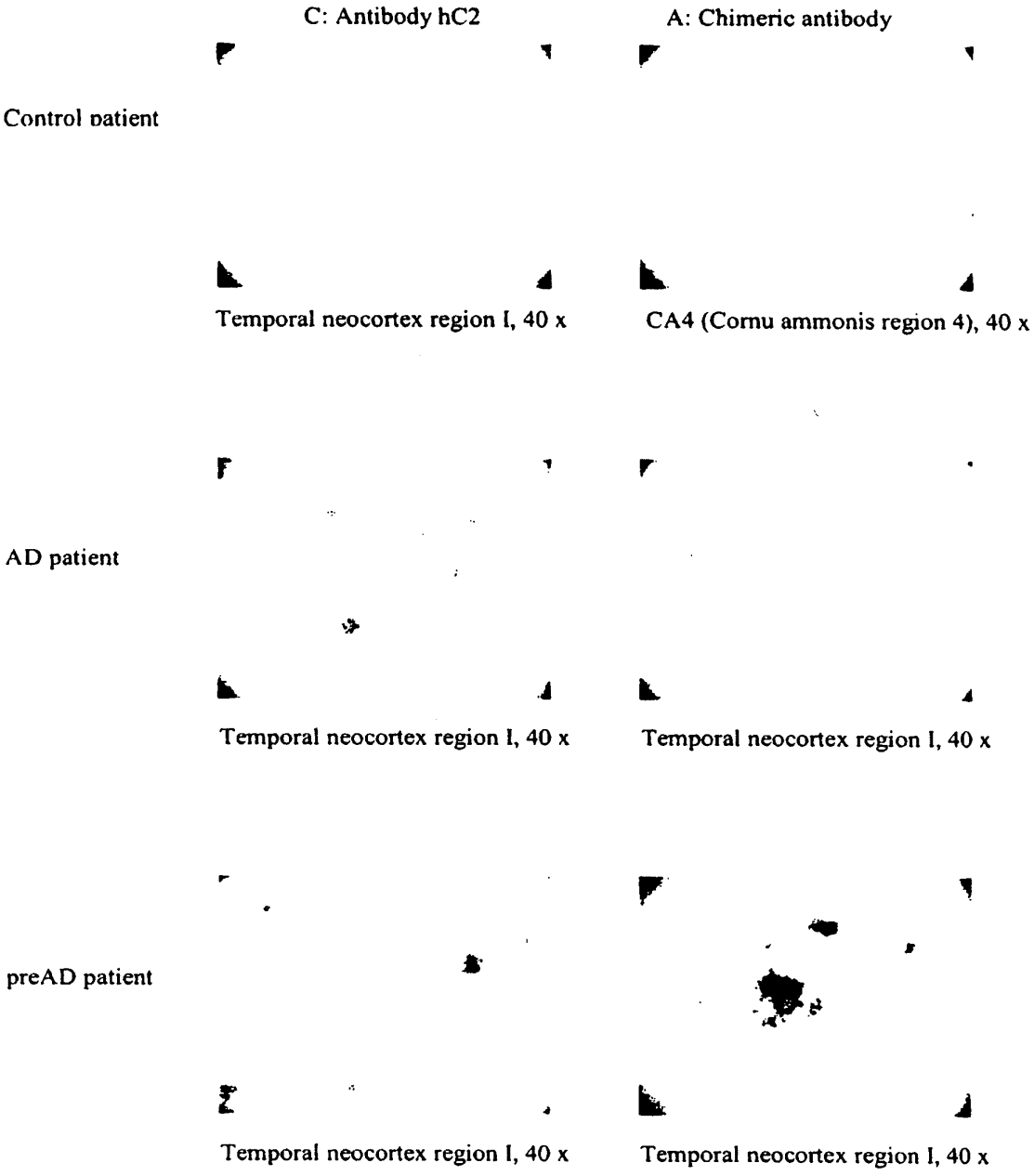
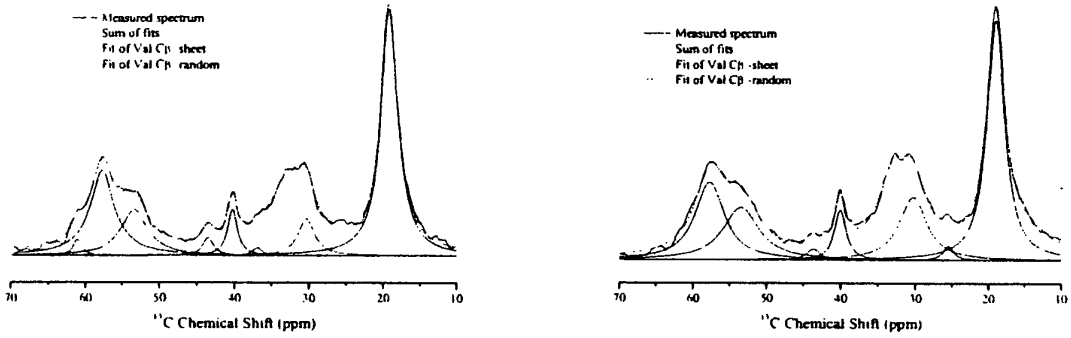


FIG. 6 (Example 11)

A)



B)

Resonance	PBS			Mouse C2		
	δ ISO (ppm)	FWHH (Hz)	% Integral Intensity	δ ISO (ppm)	FWHH (Hz)	% Integral Intensity
Val Cβ -sheet	32.60	479	81.7	33.09	366	53.5
Val Cβ -random	30.27	200	18.3	30.27	340	46.5

A) Comparison of ^{13}C CPMAS spectra and fits for U-^{13}C Tyr10 and Val12 labelled amyloid β1-42 fibres incubated with PBS (left; served as control) or ACI-7-C2 (right) for 24 hrs and then lyophilised. The fits for the two conformations of Val12 Cβ are shown in green (sheet) and blue (random coil). The peak at c33 ppm corresponds to the beta sheet conformation of the fibres whilst that at 30 ppm is a result of random coil conformation.

B): Comparison of the fitted parameters for the two conformations of Val 12 Cβ. The fitted chemical shifts for the two conformations are quite similar but the integral intensities are very different, reflecting a reduction in the original beta sheet conformation by approx 35% (1-(53.5/81.7)). This is in very close agreement with the value we obtained from the fluorescence measurement.

FIG. 7
(Example 12)

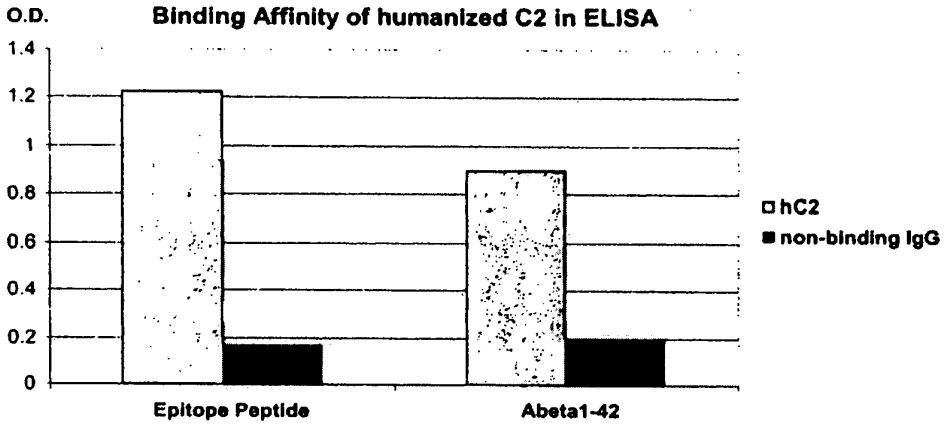


FIG. 8

(Example 12)

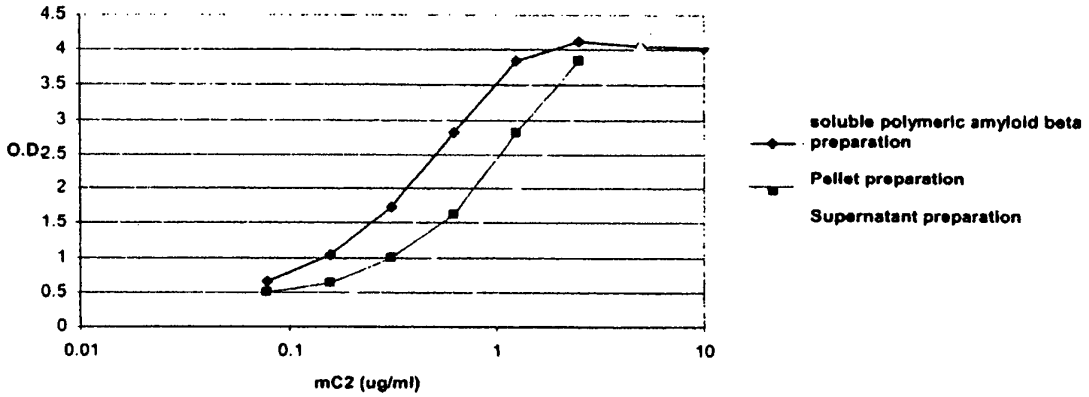


FIG. 9
(Example 13)

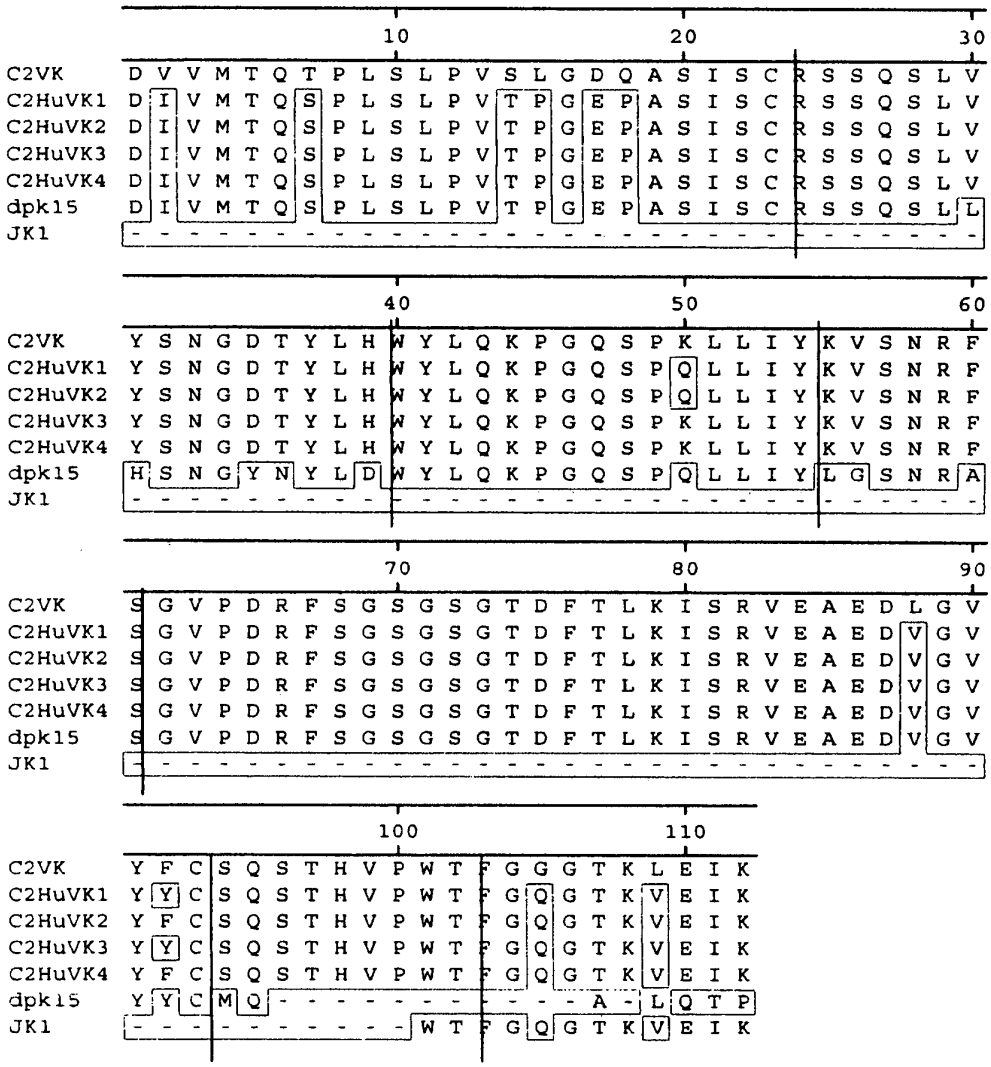

10 20 30
C2VK D V V M T Q T P L S L P V S L G D Q A S I S C R S S Q S L V
C2HuVK1 D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L V
C2HuVK2 D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L V
C2HuVK3 D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L V
C2HuVK4 D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L V
dpk15 D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L L
JK1 -
40 50 60
C2VK Y S N G D T Y L H W Y L Q K P G Q S P K L L I Y K V S N R F
C2HuVK1 Y S N G D T Y L H W Y L Q K P G Q S P Q L L I Y K V S N R F
C2HuVK2 Y S N G D T Y L H W Y L Q K P G Q S P Q L L I Y K V S N R F
C2HuVK3 Y S N G D T Y L H W Y L Q K P G Q S P K L L I Y K V S N R F
C2HuVK4 Y S N G D T Y L H W Y L Q K P G Q S P K L L I Y K V S N R F
dpk15 H S N G Y N Y L D W Y L Q K P G Q S P Q L L I Y L G S N R A
JK1 -
70 80 90
C2VK S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V
C2HuVK1 S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
C2HuVK2 S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
C2HuVK3 S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
C2HuVK4 S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
dpk15 S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
JK1 -
100 110
C2VK Y F C S Q S T H V P W T F G G G T K L E I K
C2HuVK1 Y Y C S Q S T H V P W T F G Q G T K V E I K
C2HuVK2 Y F C S Q S T H V P W T F G Q G T K V E I K
C2HuVK3 Y Y C S Q S T H V P W T F G Q G T K V E I K
C2HuVK4 Y F C S Q S T H V P W T F G Q G T K V E I K
dpk15 Y Y C M Q - - - - - - - - - - A - L Q T P
JK1 - - - - - - - - - - W T F G Q G T K V E I K

FIG. 10

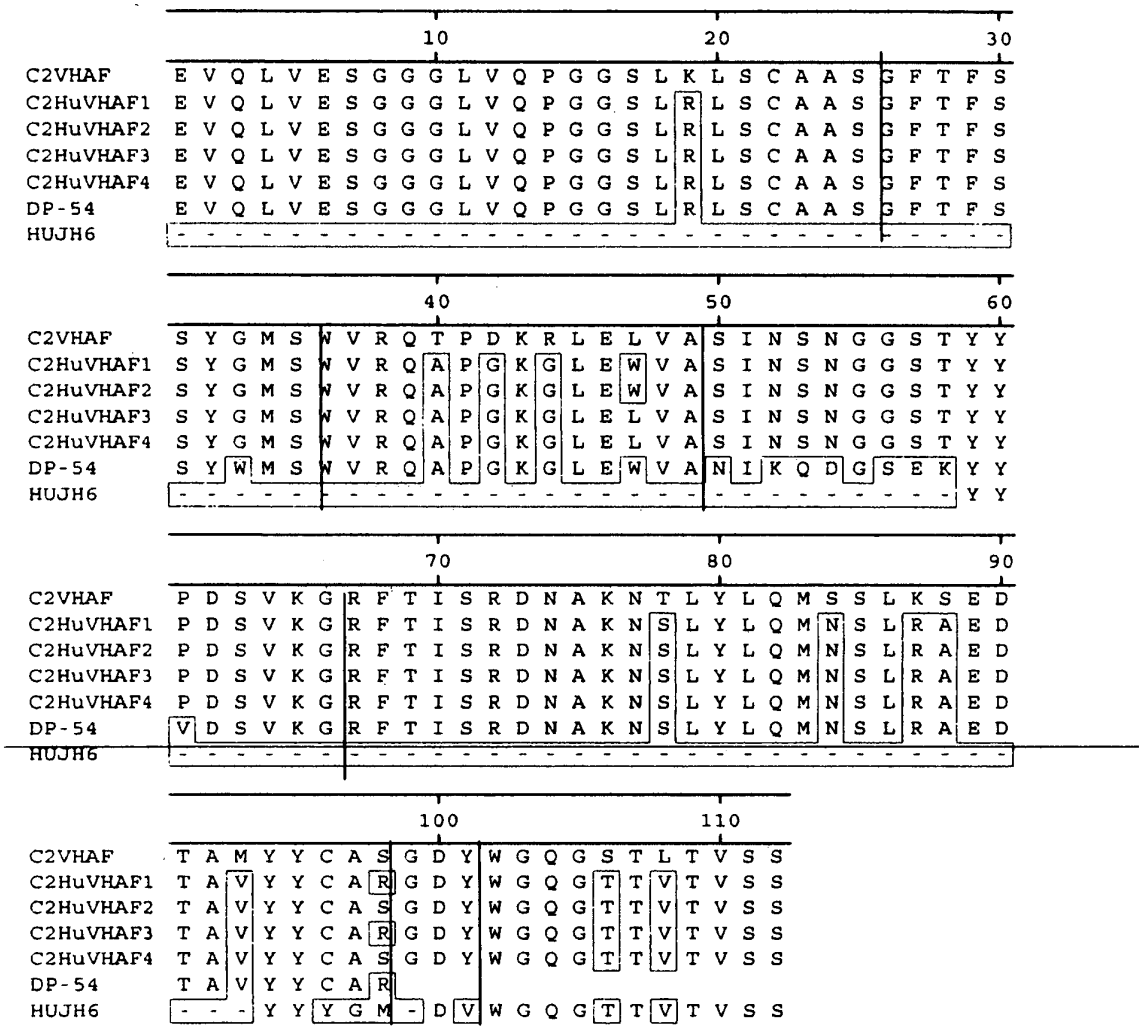

10 20 30
C2VHAF E V Q L V E S G G G L V Q P G G S L K L S C A A S G F T F S
C2HuVHAF1 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
C2HuVHAF2 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
C2HuVHAF3 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
C2HuVHAF4 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
DP-54 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
HUJH6 -
40 50 60
C2VHAF S Y G M S W V R Q T P D K R L E L V A S I N S N G G S T Y Y
C2HuVHAF1 S Y G M S W V R Q A P G K G L E W V A S I N S N G G S T Y Y
C2HuVHAF2 S Y G M S W V R Q A P G K G L E W V A S I N S N G G S T Y Y
C2HuVHAF3 S Y G M S W V R Q A P G K G L E L V A S I N S N G G S T Y Y
C2HuVHAF4 S Y G M S W V R Q A P G K G L E L V A S I N S N G G S T Y Y
DP-54 S Y W M S W V R Q A P G K G L E W V A N I K Q D G S E K Y Y
HUJH6 - Y Y
70 80 90
C2VHAF P D S V K G R F T I S R D N A K N T L Y L Q M S S L K S E D
C2HuVHAF1 P D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D
C2HuVHAF2 P D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D
C2HuVHAF3 P D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D
C2HuVHAF4 P D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D
DP-54 V D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D
HUJH6 -
100 110
C2VHAF T A M Y Y C A S G D Y W G Q G S T L T V S S
C2HuVHAF1 T A V Y Y C A R G D Y W G Q G T T V T V S S
C2HuVHAF2 T A V Y Y C A S G D Y W G Q G T T V T V S S
C2HuVHAF3 T A V Y Y C A R G D Y W G Q G T T V T V S S
C2HuVHAF4 T A V Y Y C A S G D Y W G Q G T T V T V S S
DP-54 T A V Y Y C A R
HUJH6 - - - Y Y Y G M - D V W G Q G T T V T V S S

FIG. 11

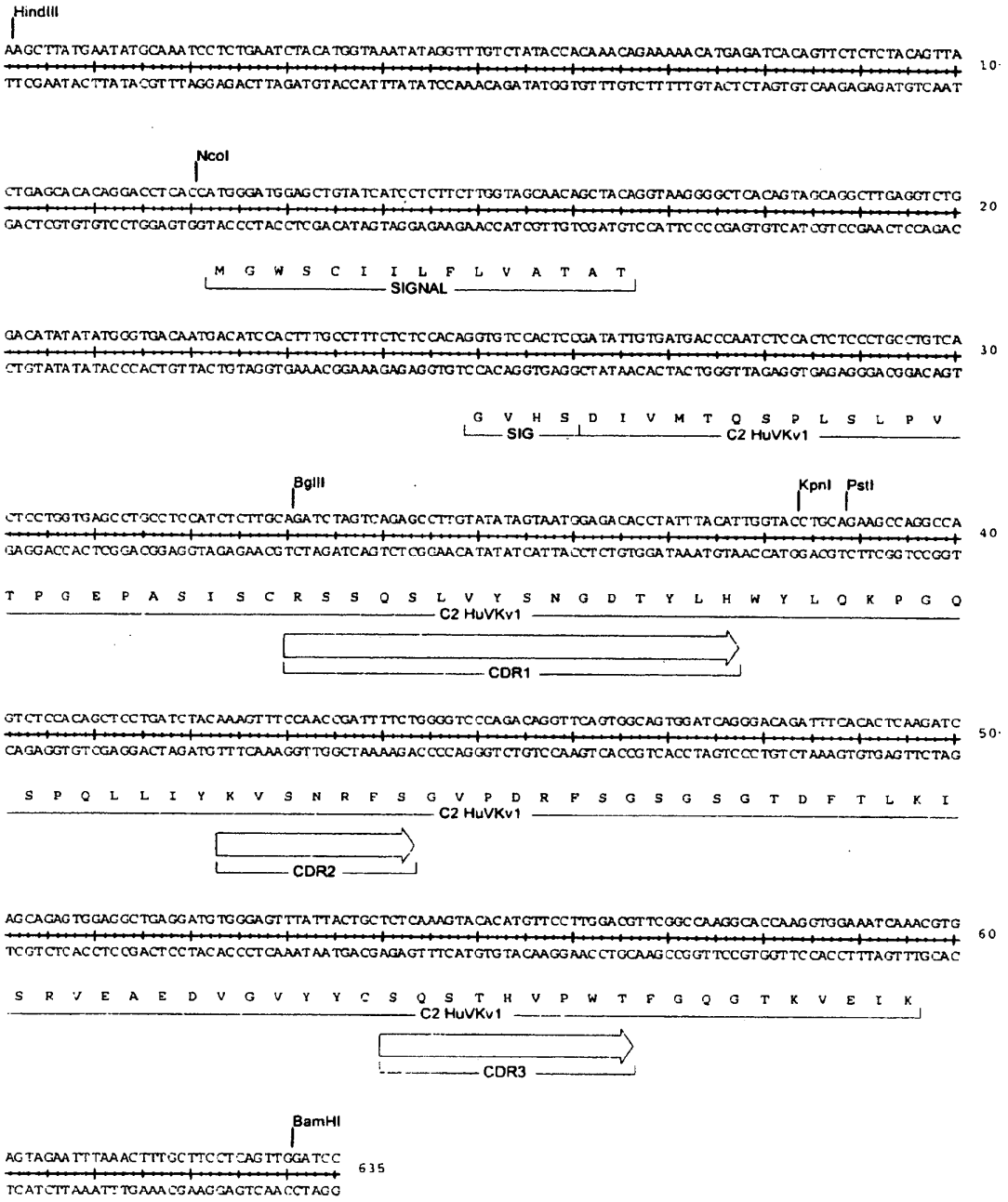

HindIII
AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACCACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTA
TTCGAATACTTATACGTTTAGGAGACTTAGATGTACCATTTATATCCAAACAGATATGGTGTTTGTCTTTTTGTACTCTAGTGTCAAGAGAGATGTCAAT
10
NcoI
CTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCACAGTAGCAGGCTTGAGGTCTG
GACTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGGAGAAGAACCATCGTTGTCGATGTCCATTCCCCGAGTGTCATCGTCCGAACTCCAGAC
20
M G W S C I I L F L V A T A T
SIGNAL
GACATATATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCGATATTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCA
CTGTATATATACCCACTGTTACTGTAGGTGAAACGGAAAGAGAGGTGTCCACAGGTGAGGCTATAACACTACTGGGTTAGAGGTGAGAGGGACGGACAGT
30
G V H S D I V M T Q S P L S L P V
SIG
C2 HuVKv1
BglII
KpnI PstI
CTCCTGGTGAGCCTGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTATATAGTAATGGAGACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCA
GAGGACCACTCGGACGGAGGTAGAGAACGTCTAGATCAGTCTCGGAACATATATCATTACCTCTGTGGATAAATGTAACCATGGACGTCTTCGGTCCGGT
40
T P G E P A S I S C R S S Q S L V Y S N G D T Y L H W Y L Q K P G Q
C2 HuVKv1
CDR1
GTCTCCACAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATC
CAGAGGTGTCGAGGACTAGATGTTTCAAAGGTTGGCTAAAAGACCCCAGGGTCTGTCCAAGTCACCGTCACCTAGTCCCTGTCTAAAGTGTGAGTTCTAG
50
S P Q L L I Y K V S N R F S G V P D R F S G S G S G T D F T L K I
C2 HuVKv1
CDR2
AGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTCTCAAAGTACACATGTTCCTTGGACGTTCGGCCAAGGCACCAAGGTGGAAATCAAACGTG
TCGTCTCACCTCCGACTCCTACACCCTCAAATAATGACGAGAGTTTCATGTGTACAAGGAACCTGCAAGCCGGTTCCGTGGTTCCACCTTTAGTTTGCAC
60
S R V E A E D V G V Y Y C S Q S T H V P W T F G Q G T K V E I K
C2 HuVKv1
CDR3
BamHI
AGTAGAATTTAAACTTTGCTTCCTCAGTTGGATCC
TCATCTTAAATTTGAAACGAAGGAGTCAACCTAGG
635

FIG. 12

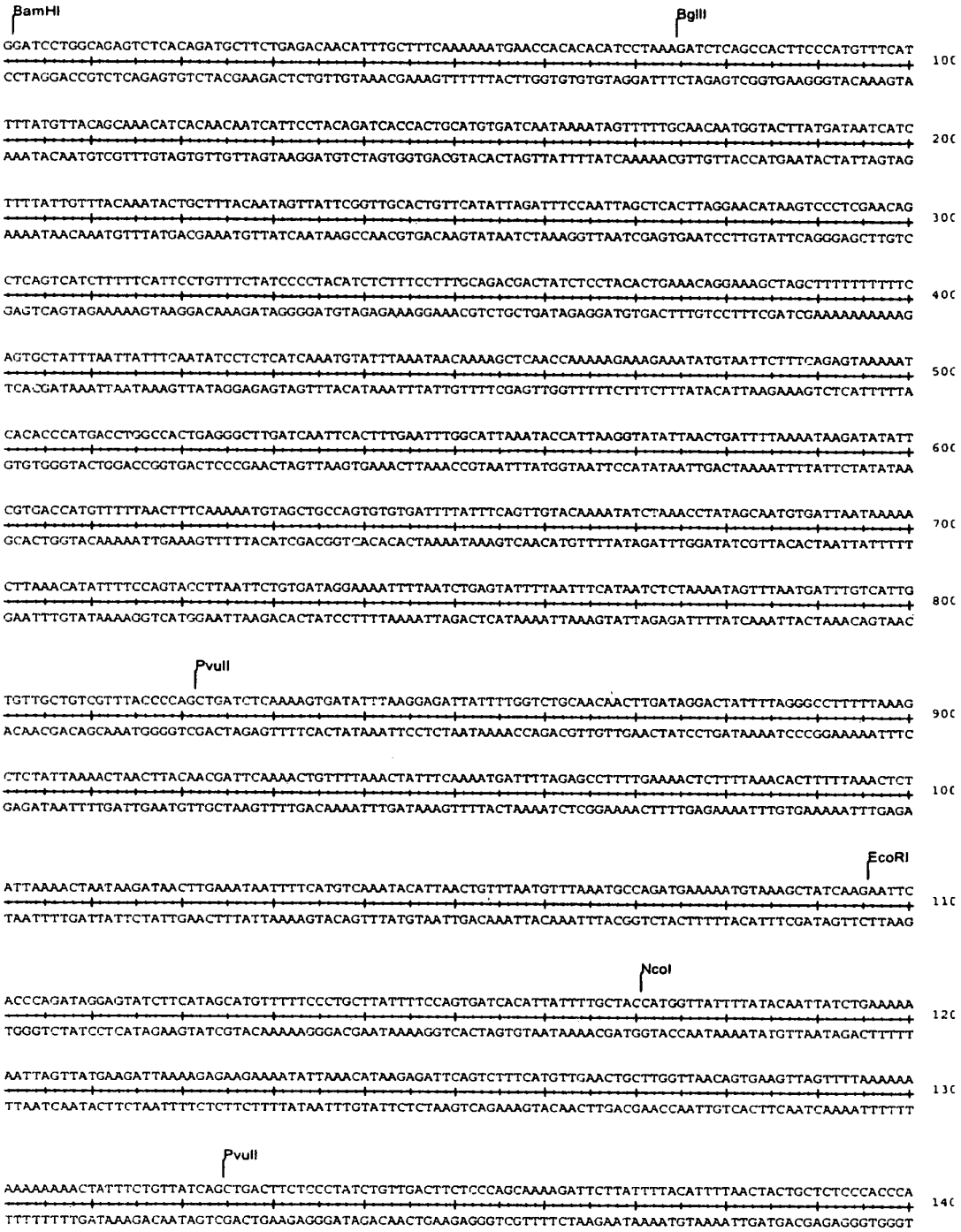

BamHI
BglII
100
200
300
400
500
600
700
800
PvuII
900
1000
EcoRI
1100
NcoI
1200
1300
PvuII
1400

FIG. 13-1

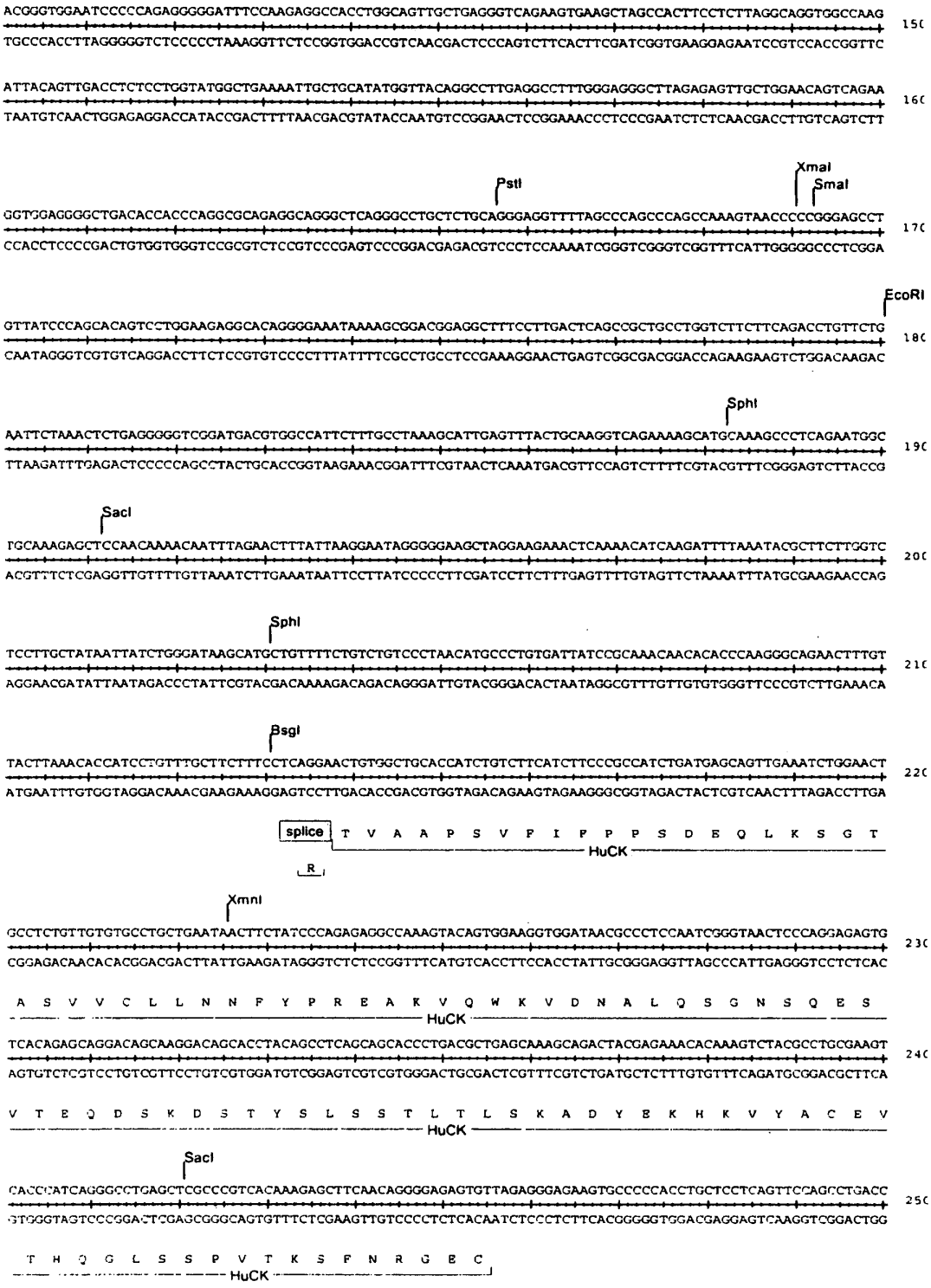

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PstI
XmaI
SmaI
GGTGGAGGGGCTGACACCACCCAGGCGCAGAGGCAGGGCTCAGGGCCTGCTCTGCAGGGAGGTTTTAGCCCAGCCCAGCCAAAGTAACCCCCGGGAGCCT
CCACCTCCCCGACTGTGGTGGGTCCGCGTCTCCGTCCCGAGTCCCGGACGAGACGTCCCTCCAAAATCGGGTCGGGTCGGTTTCATTGGGGGCCCTCGGA
EcoRI
GTTATCCCAGCACAGTCCTGGAAGAGGCACAGGGGAAATAAAAGCGGACGGAGGCTTTCCTTGACTCAGCCGCTGCCTGGTCTTCTTCAGACCTGTTCTG
CAATAGGGTCGTGTCAGGACCTTCTCCGTGTCCCCTTTATTTTCGCCTGCCTCCGAAAGGAACTGAGTCGGCGACGGACCAGAAGAAGTCTGGACAAGAC
SphI
AATTCTAAACTCTGAGGGGGTCGGATGACGTGGCCATTCTTTGCCTAAAGCATTGAGTTTACTGCAAGGTCAGAAAAGCATGCAAAGCCCTCAGAATGGC
TTAAGATTTGAGACTCCCCCAGCCTACTGCACCGGTAAGAAACGGATTTCGTAACTCAAATGACGTTCCAGTCTTTTCGTACGTTTCGGGAGTCTTACCG
SacI
TGCAAAGAGCTCCAACAAAACAATTTAGAACTTTATTAAGGAATAGGGGGAAGCTAGGAAGAAACTCAAAACATCAAGATTTTAAATACGCTTCTTGGTC
ACGTTTCTCGAGGTTGTTTTGTTAAATCTTGAAATAATTCCTTATCCCCCTTCGATCCTTCTTTGAGTTTTGTAGTTCTAAAATTTATGCGAAGAACCAG
SphI
TCCTTGCTATAATTATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCAGAACTTTGT
AGGAACGATATTAATAGACCCTATTCGTACGACAAAAGACAGACAGGGATTGTACGGGACACTAATAGGCGTTTGTTGTGTGGGTTCCCGTCTTGAAACA
BsgI
TACTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACT
ATGAATTTGTGGTAGGACAAACGAAGAAAGGAGTCCTTGACACCGACGTGGTAGACAGAAGTAGAAGGGCGGTAGACTACTCGTCAACTTTAGACCTTGA
splice
T V A A P S V F I F P P S D E Q L K S G T
HuCK
R
XmnI
GCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTG
CGGAGACAACACACGGACGACTTATTGAAGATAGGGTCTCTCCGGTTTCATGTCACCTTCCACCTATTGCGGGAGGTTAGCCCATTGAGGGTCCTCTCAC
A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S
HuCK
TCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGT
AGTGTCTCGTCCTGTCGTTCCTGTCGTGGATGTCGGAGTCGTCGTGGGACTGCGACTCGTTTCGTCTGATGCTCTTTGTGTTTCAGATGCGGACGCTTCA
V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V
HuCK
SacI
CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAGCCTGACC
GTGGGTAGTCCCGGACTCGAGCGGGCAGTGTTTCTCGAAGTTGTCCCCTCTCACAATCTCCCTCTTCACGGGGGTGGACGAGGAGTCAAGGTCGGACTGG
T H Q G L S S P V T K S F N R G E C
HuCK

FIG. 13-2

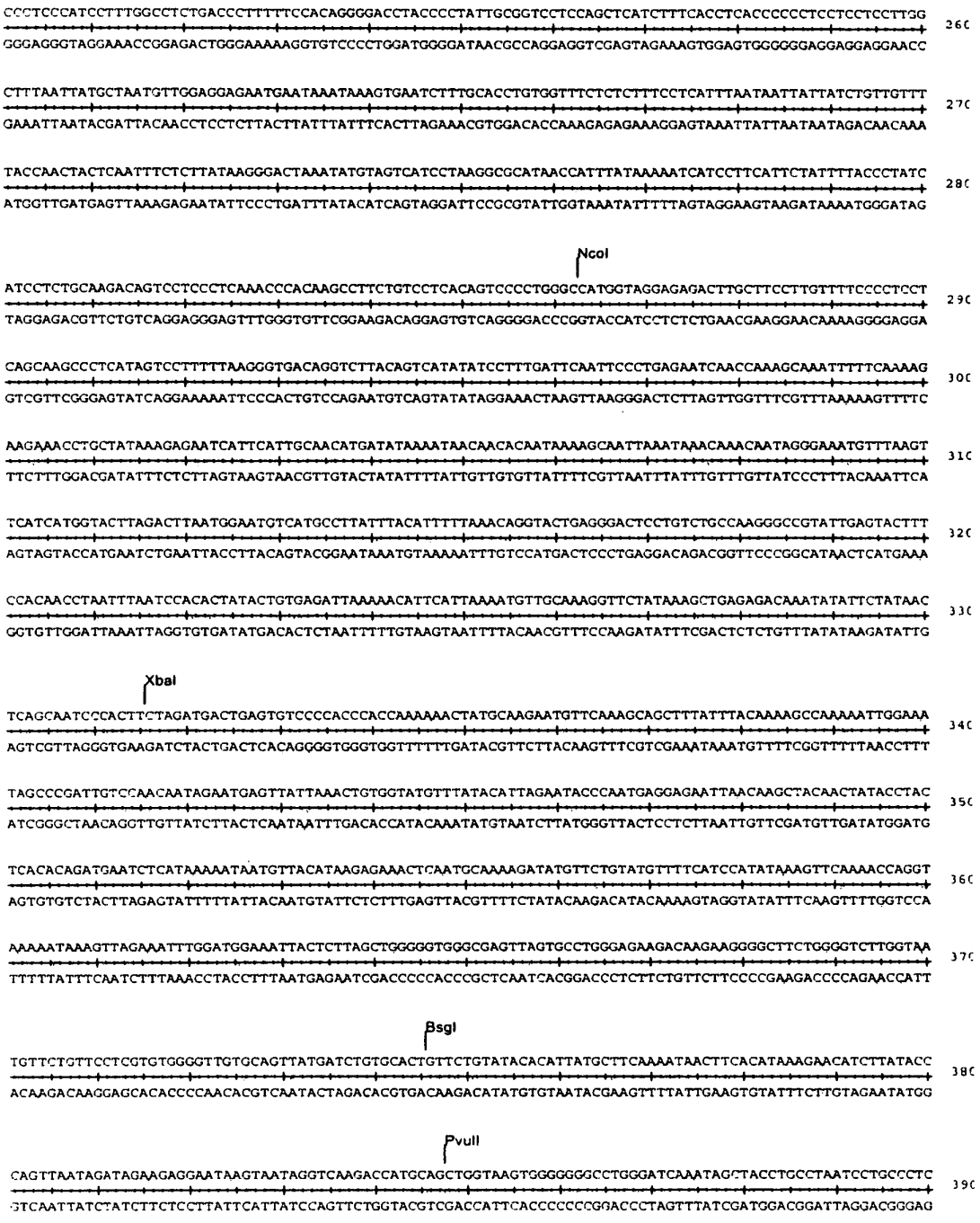


FIG. 13-3

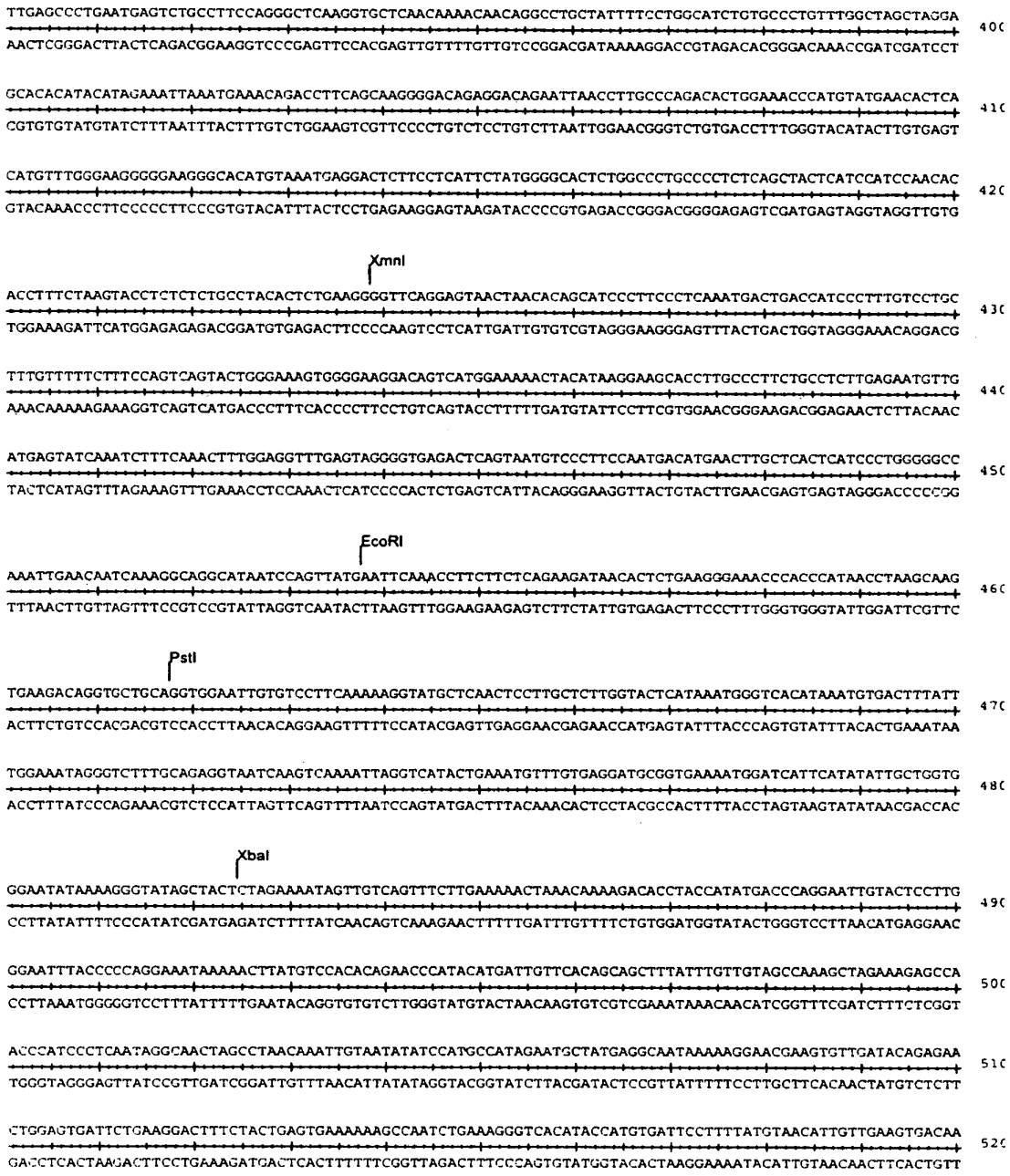

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AACTCGGGACTTACTCAGACGGAAGGTCCCGAGTTCCACGAGTTGTTTTGTTGTCCGGACGATAAAAGGACCGTAGACACGGGACAAACCGATCGATCCT
GCACACATACATAGAAATTAAATGAAACAGACCTTCAGCAAGGGGACAGAGGACAGAATTAACCTTGCCCAGACACTGGAAACCCATGTATGAACACTCA
CGTGTGTATGTATCTTTAATTTACTTTGTCTGGAAGTCGTTCCCCTGTCTCCTGTCTTAATTGGAACGGGTCTGTGACCTTTGGGTACATACTTGTGAGT
CATGTTTGGGAAGGGGGAAGGGCACATGTAAATGAGGACTCTTCCTCATTCTATGGGGCACTCTGGCCCTGCCCCTCTCAGCTACTCATCCATCCAACAC
GTACAAACCCTTCCCCCTTCCCGTGTACATTTACTCCTGAGAAGGAGTAAGATACCCCGTGAGACCGGGACGGGGAGAGTCGATGAGTAGGTAGGTTGTG
XmnI
ACCTTTCTAAGTACCTCTCTCTGCCTACACTCTGAAGGGGTTCAGGAGTAACTAACACAGCATCCCTTCCCTCAAATGACTGACCATCCCTTTGTCCTGC
TGGAAAGATTCATGGAGAGAGACGGATGTGAGACTTCCCCAAGTCCTCATTGATTGTGTCGTAGGGAAGGGAGTTTACTGACTGGTAGGGAAACAGGACG
TTTGTTTTTCTTTCCAGTCAGTACTGGGAAAGTGGGGAAGGACAGTCATGGAAAAACTACATAAGGAAGCACCTTGCCCTTCTGCCTCTTGAGAATGTTG
AAACAAAAAGAAAGGTCAGTCATGACCCTTTCACCCCTTCCTGTCAGTACCTTTTTGATGTATTCCTTCGTGGAACGGGAAGACGGAGAACTCTTACAAC
ATGAGTATCAAATCTTTCAAACTTTGGAGGTTTGAGTAGGGGTGAGACTCAGTAATGTCCCTTCCAATGACATGAACTTGCTCACTCATCCCTGGGGGCC
TACTCATAGTTTAGAAAGTTTGAAACCTCCAAACTCATCCCCACTCTGAGTCATTACAGGGAAGGTTACTGTACTTGAACGAGTGAGTAGGGACCCCCGG
EcoRI
AAATTGAACAATCAAAGGCAGGCATAATCCAGTTATGAATTCAAACCTTCTTCTCAGAAGATAACACTCTGAAGGGAAACCCACCCATAACCTAAGCAAG
TTTAACTTGTTAGTTTCCGTCCGTATTAGGTCAATACTTAAGTTTGGAAGAAGAGTCTTCTATTGTGAGACTTCCCTTTGGGTGGGTATTGGATTCGTTC
PstI
TGAAGACAGGTGCTGCAGGTGGAATTGTGTCCTTCAAAAAGGTATGCTCAACTCCTTGCTCTTGGTACTCATAAATGGGTCACATAAATGTGACTTTATT
ACTTCTGTCCACGACGTCCACCTTAACACAGGAAGTTTTTCCATACGAGTTGAGGAACGAGAACCATGAGTATTTACCCAGTGTATTTACACTGAAATAA
TGGAAATAGGGTCTTTGCAGAGGTAATCAAGTCAAAATTAGGTCATACTGAAATGTTTGTGAGGATGCGGTGAAAATGGATCATTCATATATTGCTGGTG
ACCTTTATCCCAGAAACGTCTCCATTAGTTCAGTTTTAATCCAGTATGACTTTACAAACACTCCTACGCCACTTTTACCTAGTAAGTATATAACGACCAC
XbaI
GGAATATAAAAGGGTATAGCTACTCTAGAAAATAGTTGTCAGTTTCTTGAAAAACTAAACAAAAGACACCTACCATATGACCCAGGAATTGTACTCCTTG
CCTTATATTTTCCCATATCGATGAGATCTTTTATCAACAGTCAAAGAACTTTTTGATTTGTTTTCTGTGGATGGTATACTGGGTCCTTAACATGAGGAAC
GGAATTTACCCCCAGGAAATAAAAACTTATGTCCACACAGAACCCATACATGATTGTTCACAGCAGCTTTATTTGTTGTAGCCAAAGCTAGAAAGAGCCA
CCTTAAATGGGGGTCCTTTATTTTTGAATACAGGTGTGTCTTGGGTATGTACTAACAAGTGTCGTCGAAATAAACAACATCGGTTTCGATCTTTCTCGGT
ACCCATCCCTCAATAGGCAACTAGCCTAACAAATTGTAATATATCCATGCCATAGAATGCTATGAGGCAATAAAAAGGAACGAAGTGTTGATACAGAGAA
TGGGTAGGGAGTTATCCGTTGATCGGATTGTTTAACATTATATAGGTACGGTATCTTACGATACTCCGTTATTTTTCCTTGCTTCACAACTATGTCTCTT
CTGGAGTGATTCTGAAGGACTTTCTACTGAGTGAAAAAAGCCAATCTGAAAGGGTCACATACCATGTGATTCCTTTTATGTAACATTGTTGAAGTGACAA
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FIG. 13-4

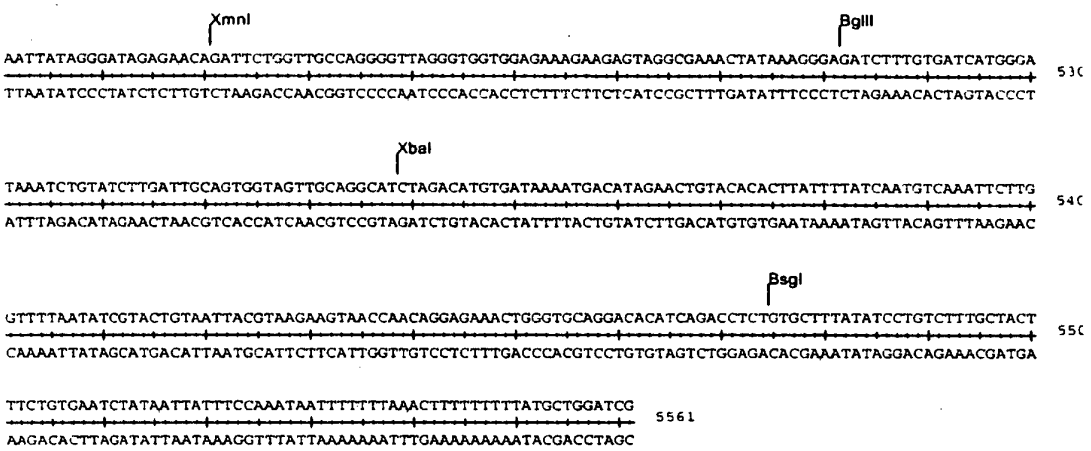

XmnI
BglII
AATTATAGGGATAGAGAACAGATTCTGGTTGCCAGGGGTTAGGGTGGTGGAGAAAGAAGAGTAGGCGAAACTATAAAGGGAGATCTTTGTGATCATGGGA
TTAATATCCCTATCTCTTGTCTAAGACCAACGGTCCCCAATCCCACCACCTCTTTCTTCTCATCCGCTTTGATATTTCCCTCTAGAAACACTAGTACCCT
530
XbaI
TAAATCTGTATCTTGATTGCAGTGGTAGTTGCAGGCATCTAGACATGTGATAAAATGACATAGAACTGTACACACTTATTTTATCAATGTCAAATTCTTG
ATTTAGACATAGAACTAACGTCACCATCAACGTCCGTAGATCTGTACACTATTTTACTGTATCTTGACATGTGTGAATAAAATAGTTACAGTTTAAGAAC
540
BsgI
GTTTTAATATCGTACTGTAATTACGTAAGAAGTAACCAACAGGAGAAACTGGGTGCAGGACACATCAGACCTCTGTGCTTTATATCCTGTCTTTGCTACT
CAAAATTATAGCATGACATTAATGCATTCTTCATTGGTTGTCCTCTTTGACCCACGTCCTGTGTAGTCTGGAGACACGAAATATAGGACAGAAACGATGA
550
TTCTGTGAATCTATAATTATTTCCAAATAATTTTTTTAAACTTTTTTTTTATGCTGGATCG
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5561

FIG. 13-5

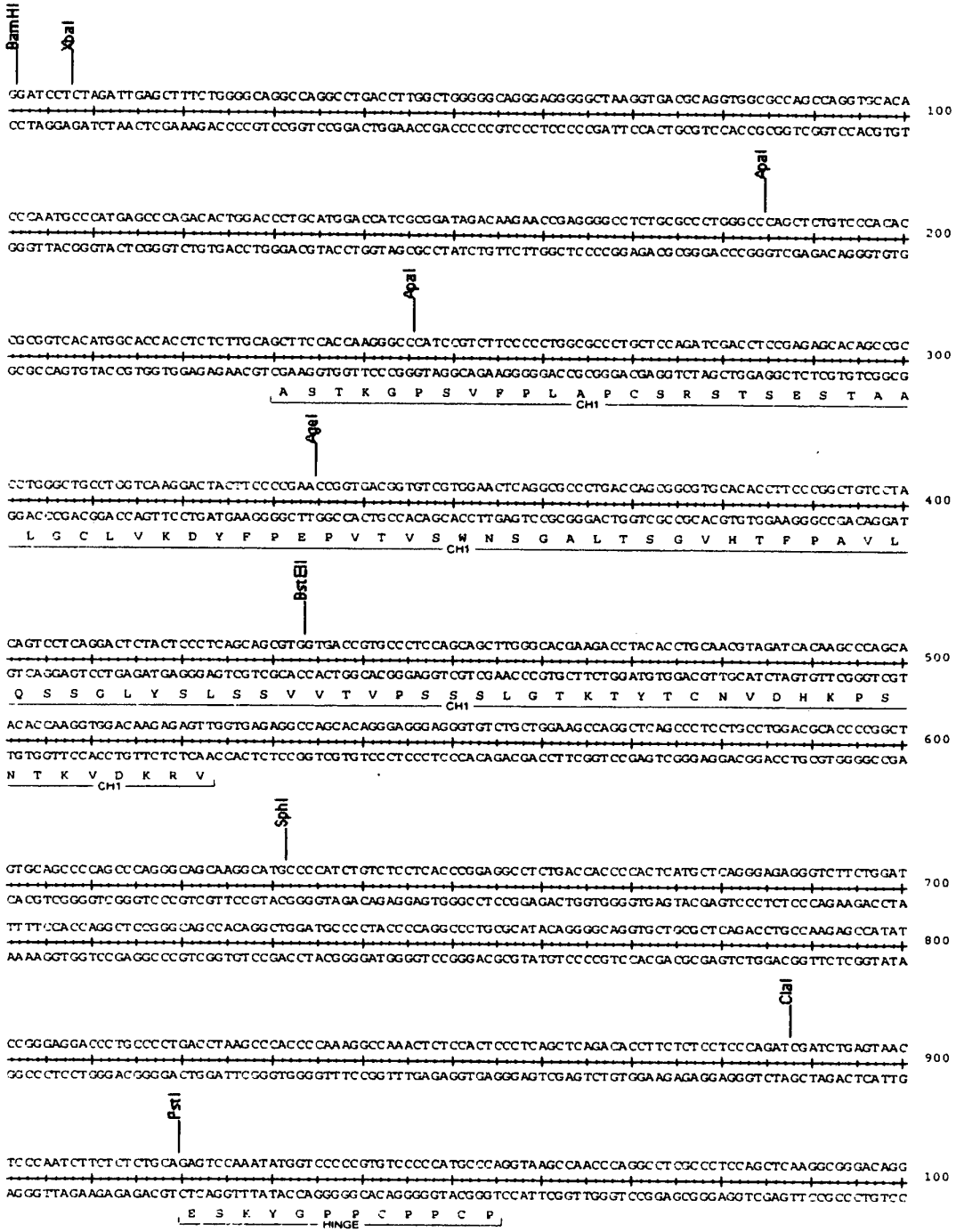

BamHI
XbaI
ApaI
ApaI
AgeI
BstEII
SphI
ClaI
PstI
CH1
CH1
CH1
HINGE
100
200
300
400
500
600
700
800
900
100

FIG. 14-1

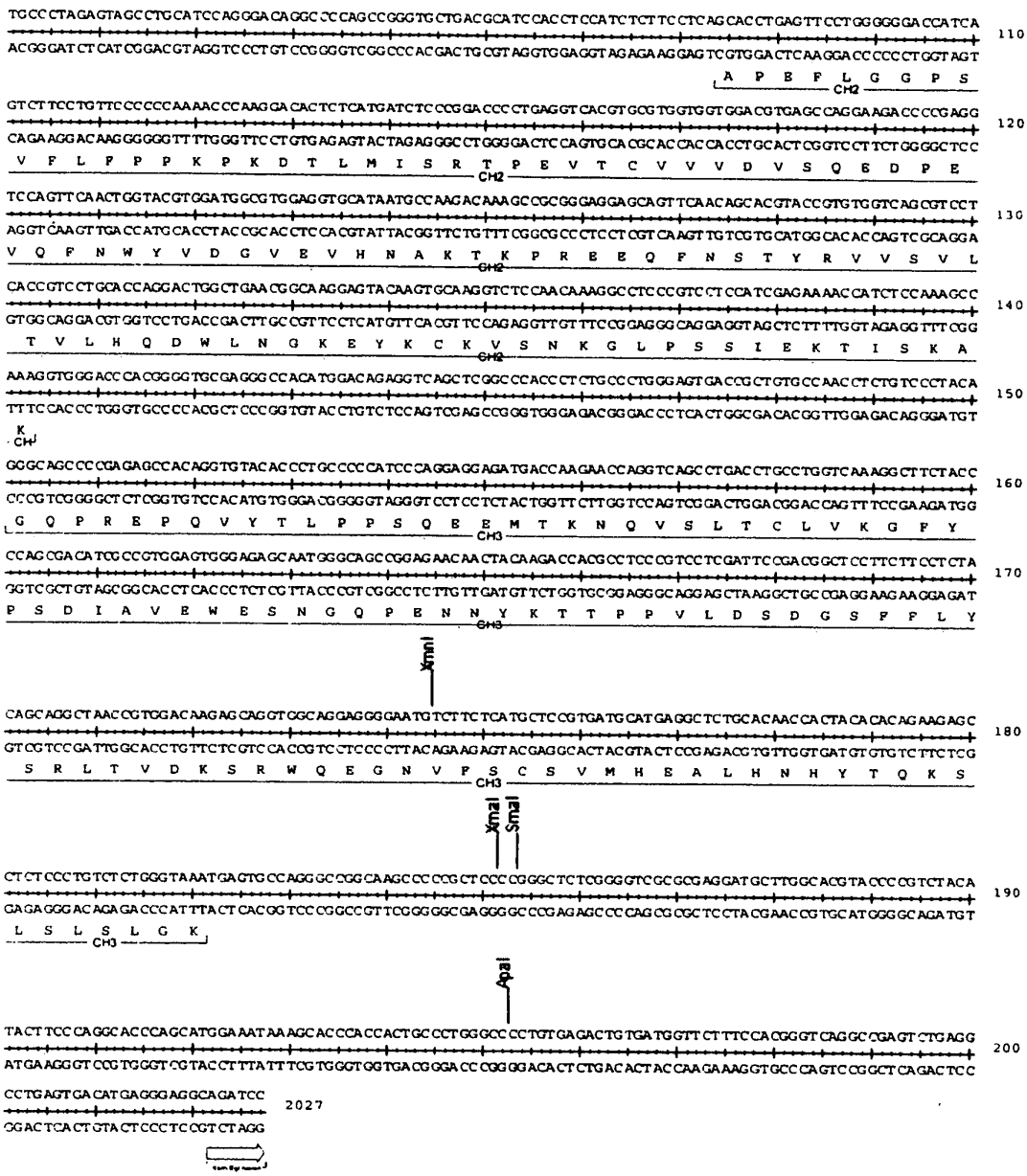


FIG. 14-2

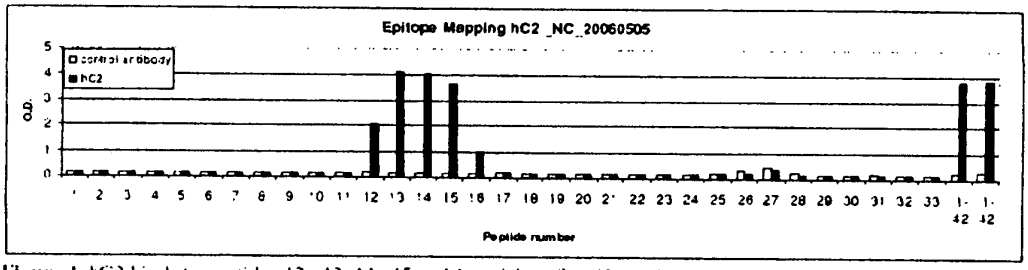


Figure 1. hC2 binds to peptides 12 , 13, 14 , 15 and 16 of the Aβ 1-42 peptide library.
Binding of hC2 to overlapping peptides of Aβ 1-42 was analyzed by ELISA. Binding to the complete Aβ 1-42 and binding of a non-binding chimeric antibody (control antibody) was used as positive and negative controls respectively. The peptide number corresponds to the amino acid in the Aβ 1-42 sequence on which the peptide starts. Results are expressed as OD.

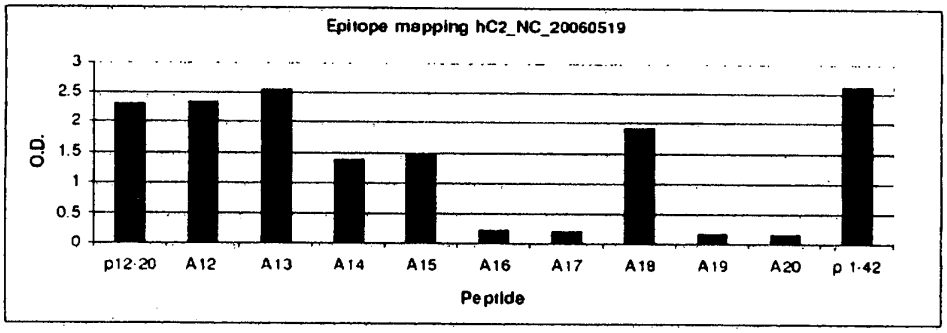


Figure 2. hC2 binding to Aβ 12-20 is completely dependent on aa 16,17,19 and 20 and partially dependent on aa 14,15 and 18.
Binding of hC2 to Aβ 12-20 and alanine substituted Aβ 12-20 was analyzed by ELISA. Binding to the complete Aβ 1-42 was used as positive control. The number corresponds to the aa that is substituted by alanine. Results are expressed as OD.

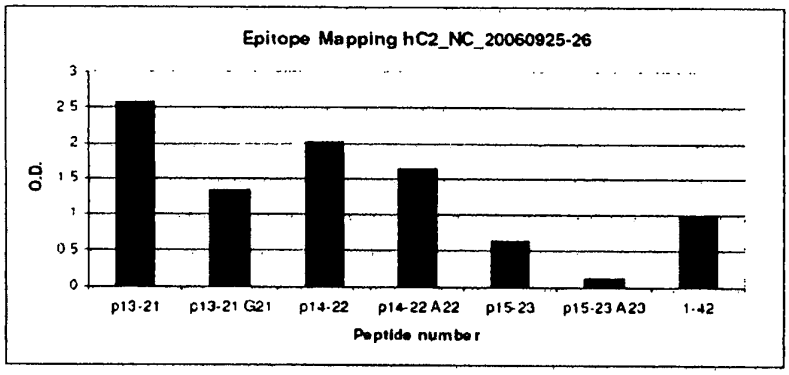


Figure 3. hC2 binding to Aβ 15-23 is dependent on aa 23 and partially on aa 21 and slightly dependent on aa 22
Binding of hC2 to Aβ 13-21, 14-22 or 15-23 and to 13-21G21, 14-22A22 or 15-23A23 was analyzed by ELISA. Binding to the complete Aβ 1-42 was used as positive control. Results are expressed as OD

FIG. 15

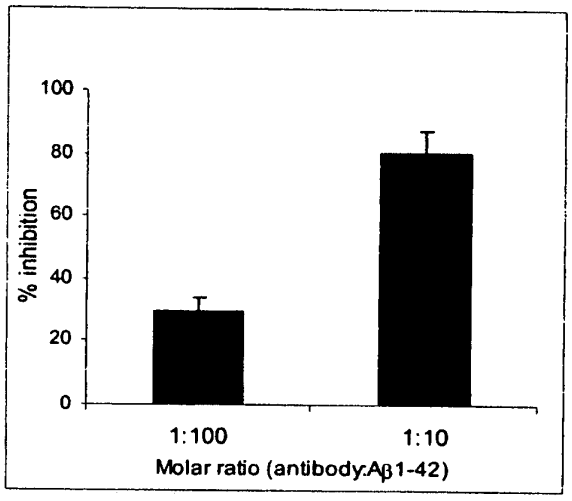

100
80
60
40
20
0
% inhibition
1:100
1:10
Molar ratio (antibody:Aβ1-42)

FIG. 16

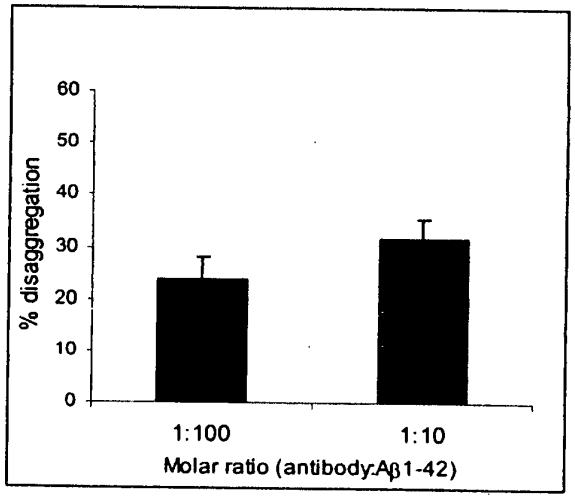

60
50
40
30
20
10
0
% disaggregation
1:100
1:10
Molar ratio (antibody:Aβ1-42)

FIG. 17

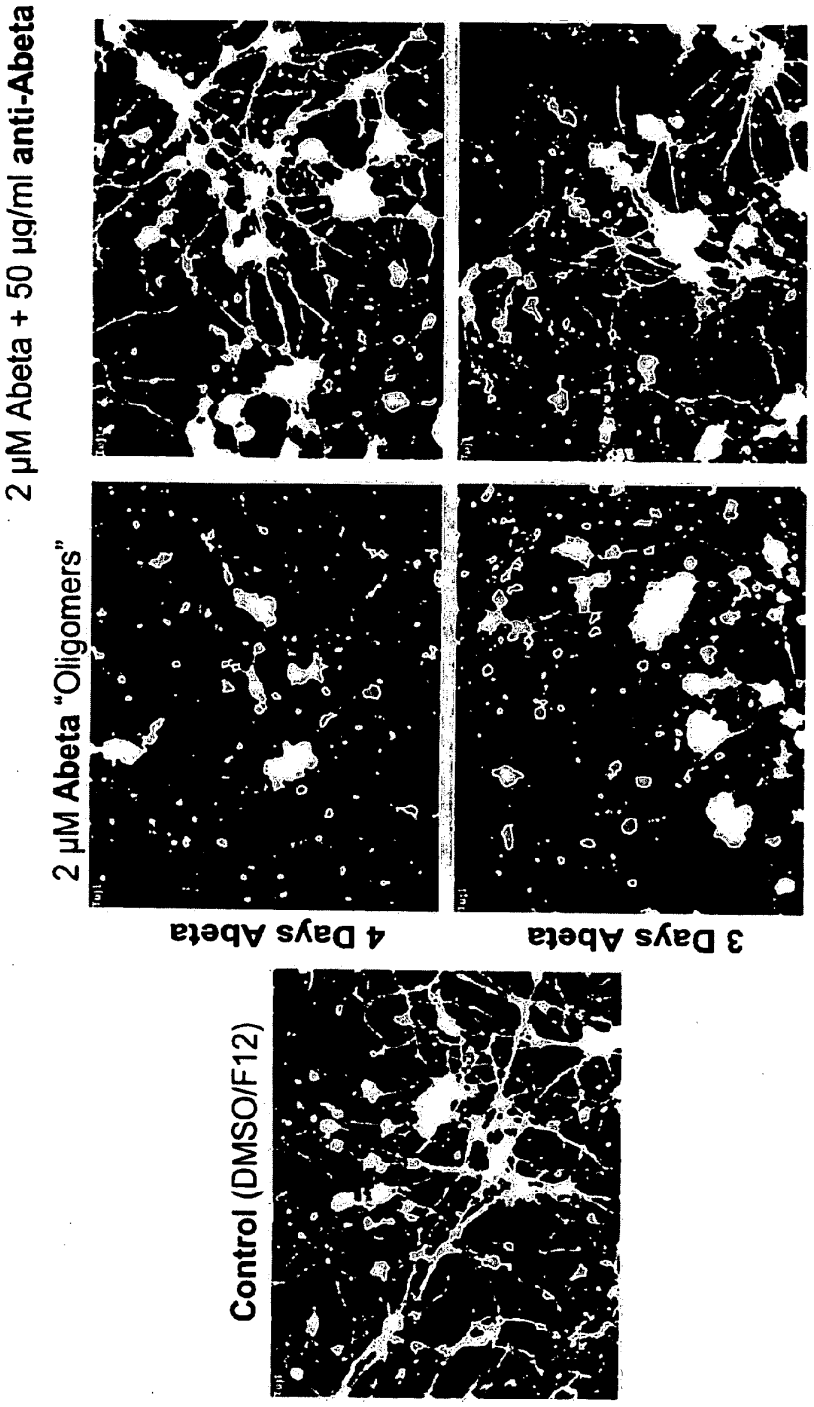

2 µM Abeta + 50 µg/ml anti-Abeta
2 µM Abeta "Oligomers"
4 Days Abeta
3 Days Abeta
Control (DMSO/F12)

FIG. 18

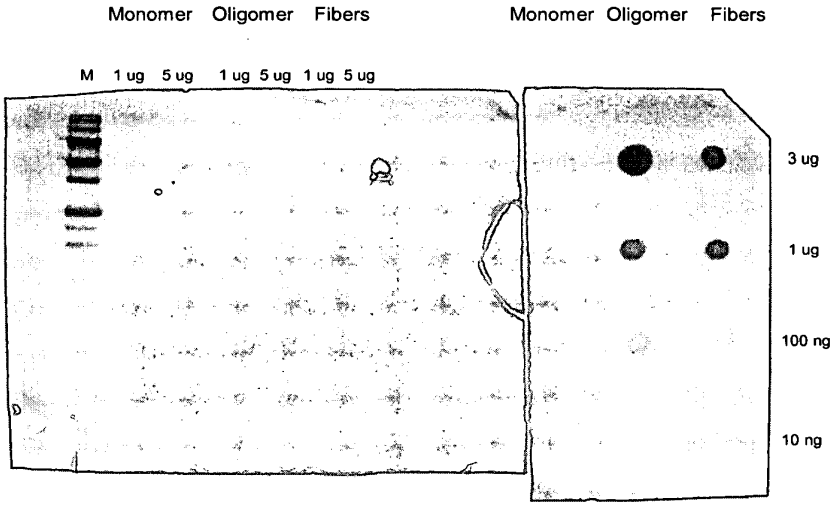


Fig 19a

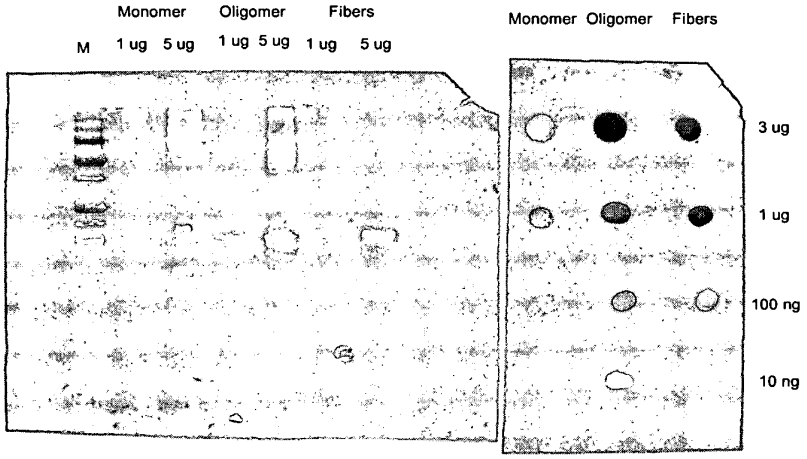


Fig 19b

FIG. 19

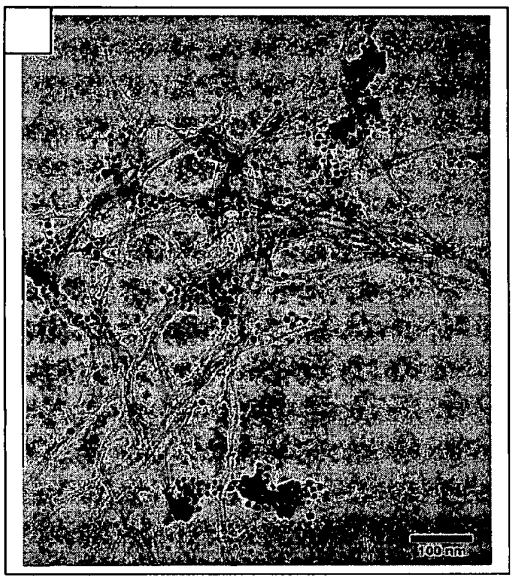


Fig 20a

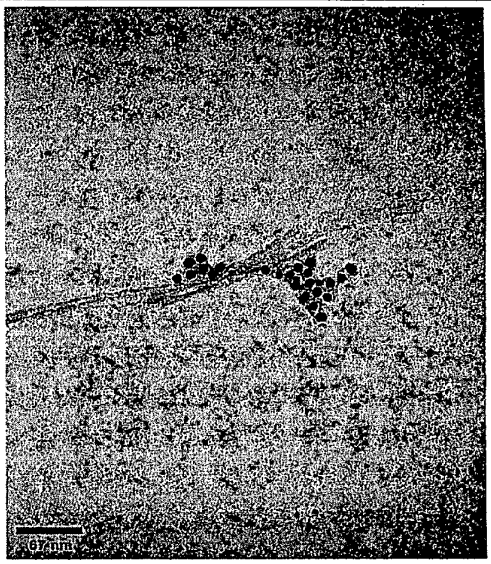
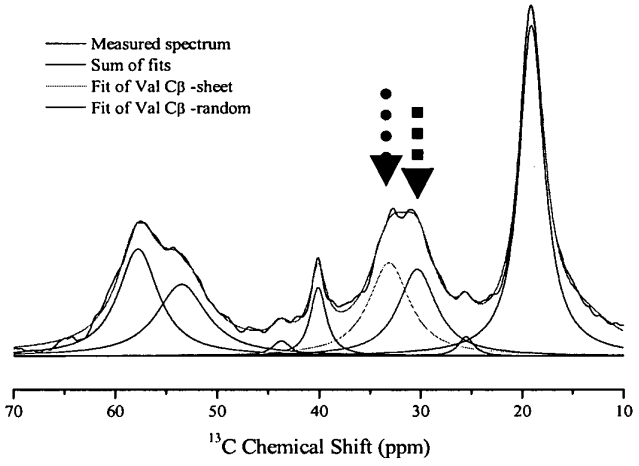
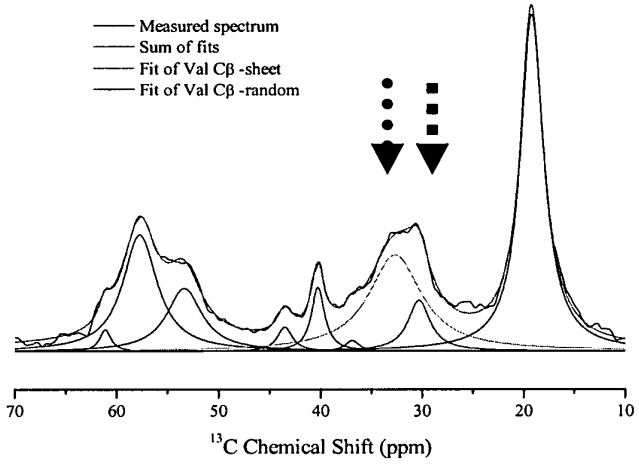


Fig 3b

Fig 20b



Control



ACI-01-Ab7

Fit for β-sheet of 13C-labeled Val12

Fit for random-coiled of 13C-labeled Val12

FIG: 21

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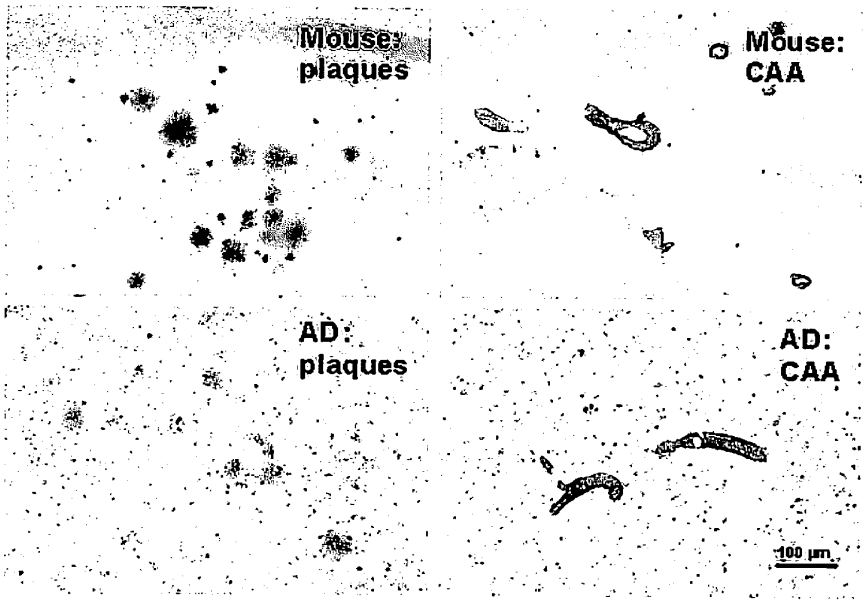
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[Continued on next page]

(54) Title: NEW Aβ CONFORMER SELECTIVE ANTI-Aβ GLOBULOMER MONOCLONAL ANTIBODIES



(57) Abstract: The subject invention relates to monoclonal antibodies that may be used in the treatment and diagnosis of Alzheimer's Disease. In particular, the present invention relates to monoclonal antibodies referred to as 10F4 and 3C5 and to other monoclonal antibodies (e.g., murine, human or humanized) having similar properties thereto.

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NEW Aβ CONFORMER SELECTIVE ANTI-Aβ GLOBULOMER MONOCLONAL ANTIBODIES

The subject application claims priority to U.S. provisional application no. 60/872,156, filed on November 30, 2006.

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to monoclonal antibodies that may be used in the treatment and diagnosis of Alzheimer's Disease. In particular, the present invention relates to monoclonal antibodies referred to as 10F4 and 3C5 and to other monoclonal antibodies (e.g., murine, human or humanized) having similar properties thereto.

Background Information

In 1907, the physician Alois Alzheimer first described the neuropathological features of a form of dementia subsequently named in his honor as Alzheimer's disease (AD). In particular, AD is the most frequent cause for dementia among the aged, with an incidence of about 10% of the population in those above 65 years of age. With increasing age, the probability of disease also rises. Globally, there are about 15 million people affected with the disease and further increases in life expectancy are expected to increase the number of people affected with the disease to about three-fold over the next decades.

From a molecular point of view, Alzheimer's disease (AD) is characterized by a deposit of abnormally aggregated proteins. In the case of extra-cellular amyloid plaques, these deposits consist mostly of amyloid-β-peptide filaments, and in the case of the intracellular neurofibrillary tangles (NFTs), mostly of the tau protein. The amyloid β (Aβ) peptide

arises from the β-amyloid precursor protein by proteolytic cleavage. This cleavage is effected by the cooperative activity of several proteases named α-, β- and γ-secretase. Cleavage leads to a number of specific fragments of differing length. The amyloid plaques consist mostly of peptides with a length of 40 or 42 amino acids (Aβ40, Aβ42). The dominant cleavage product is Aβ40; however, Aβ42 has a much stronger toxic effect. Cerebral amyloid deposits and cognitive impairments very similar to those observed in Alzheimer's disease are also hallmarks of Down's syndrome (trisomy 21), which occurs at a frequency of about 1 in 800 births.

The amyloid cascade hypothesis of Hardy and Higgins postulated that increased production of Aβ(1-42) would lead to the formation of protofibrils and fibrils (i.e., the principal components of Aβ plaques), these fibrils being responsible for the symptoms of Alzheimer's disease. Despite the poor correlation between severity of dementia and Aβ plaque burden deposited, this hypothesis was favored until recently. The discovery of soluble Aβ forms in AD brains, which correlates better with AD symptoms than plaque load does, has led to a revised amyloid-cascade-hypothesis.

Active immunization with Aβ peptides leads to a reduction in the formation as well as to partial dissolution of existing plaques. At the same time, it leads to alleviation of cognitive defects in APP transgenic mouse models. For passive immunization with antibodies directed to Aβ peptides, a reduction of an Aβ plaque burden was also found.
The results of a phase IIa trial (ELAN Corporation Plc, South San Francisco, CA, USA and Dublin, UK) of active immunization with AN-1792 (Aβ(1-42) peptide in fibrillary condition of aggregation) suggest that immunotherapy directed to Aβ peptide was successful. In a subgroup of 30 patients, the progression of disease was significantly reduced in patients with positive anti-Aβ antibody titer, measured by MMSE and DAD index.

However, this study was stopped because of serious side effects in the form of a meningoencephalitis (Bennett and Holtzman, 2005, Neurology, 64, 10-12). In particular, meningoencephalitis was characterized by neuroinflammation and infiltration of T-cells into the brain. Presumably, this was due to a T-cell immune response induced by injection of Aβ(1-42) as antigen. Such an immune response is not to be expected after passive immunization. To date, there are no clinical data with reference to this available. However, with reference to such a passive approach to immunization, concerns about the side effect profile were voiced because of preclinical studies in very old APP23 mice which received an antibody directed against an N-terminal epitope of Aβ(1-42) once a week over 5 months. In particular, these mice showed an increase in the number and severity of microhemorrhages compared to control animals treated with saline (Pfeifer et al., 2002, Science, 298, 1379). A comparable increase in microhaemorrhages was also described in very old (> 24 months) Tg2576 and PDAPP mice (Racke et al., 2005, J Neurosci, 25, 629-636; Wilcock et al. 2004, J. Neuroinflammation, 1(1):24; De Mattos et al., 2004, Neurobiol. Aging 25(S2):577). In both mouse strains, antibody injection led to a significant increase in microhemorrhages. In contrast, an antibody directed against the central region of the Aβ(1-42) peptide did not induce microhemorrhages (de Mattos et al., supra). The lack of inducing microhemorrhages was associated with an antibody treatment which did not bind to aggregated Aß peptide in the form of CAA (Racke et al., J Neurosci, 25, 629-636). Yet, the exact mechanism leading to microhemorrhages in mice transgenic for APP has not been understood. Presumably, cerebral amyloid angiopathy (CAA) induces or at least aggravates cerebral hemorrhages. CAA is present in nearly every Alzheimer's disease brain and about 20% of the cases are regarded as "severe CAA". Passive immunization should

therefore aim at avoiding microhemorrhages by selecting an antibody which recognizes the central or the carboxy terminal region of the Aβ peptide.

International Patent Application Publication No. WO2004/067561 describes stable Aβ(1-42) oligomers (Aβ(1-42) globulomers) and antibodies directed specifically against the globulomers. Digestion with unspecific proteases shows that the Aβ globulomer may be digested beginning with the hydrophilic N-terminus protruding from the globular core structure (Barghorn et al., 2005, J Neurochem, 95, 834-847). Such N-terminal truncated Aβ globulomers (Aβ(12-42) and Aβ(20-42) globulomers) represent the basic structural unit of this oligomeric Aβ and are a very potent antigen for active immunization of rabbits and mice leading to high antibody titers (WO2004/067561). The putative pathological role of N-terminally truncated Aβ forms *in vivo* has been suggested by several recent reports of their existence in AD brains (Sergeant et al., 2003, J Neurochem, 85, 1581-1591; Thal et al., 1999, J Neuropathol. Exp Neurol, 58, 210-216). During *in vivo* digestion, certain proteases found in brain, e.g. neprilysin (NEP 24.11) or insulin degrading enzyme (IDE), may be involved (Selkoe, 2001, Neuron, 32, 177-180).

In view of the above, there is a tremendous and immediate need for a treatment for Alzheimer's Disease which has few, if any, side effects (e.g., microhemmorhages). With such treatment, affected patients may be able to maintain a functional and active lifestyle for many years beyond that which is possible without such treatment. Thus, not only are there financial implications for such a treatment but "quality of life" implications as well, not only for the patients but also for their caregivers.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1

Figure 1(a) shows a dot blot analysis of the specificity of different anti-Aβ antibodies(-6E10, -3C5, 10F4). The monoclonal antibodies tested here were obtained by active immunization of mice with Aβ(12-42) globulomer (prepared as described in Example I) followed by selection of the fused hybridoma cells (except for the commercially available 6E10, Signet, Cat. No.: 9320). The individual Aβ forms were applied in serial dilutions and incubated with the respective monoclonal antibodies for immune reaction:

1. Aβ(1-42) monomer, 0.1% NH_4OH
2. Aβ(1-40) monomer, 0.1% NH_4OH
3. Aβ(1-42) monomer, 0.1% NaOH
4. Aβ(1-40) monomer, 0.1% NaOH
5. Aβ(1-42) globulomer
6. Aβ(12-42) globulomer
7. Aβ(20-42) globulomer
8. Aβ(1-42) fibril preparation
9. sAPPα (Sigma) (first dot: 1 pmol)

Figure 1(b) illustrates a quantitative evaluation which was done using a densitometric analysis of the intensity. For each Aβ form, only the dot corresponding to the lowest antigen concentration was evaluated provided that it had a relative density of greater than 20% of the relative density of the last optically unambiguously identified dot of the Aβ(1-42) globulomer (threshold). This threshold value was determined

for every dot-blot independently. The value indicates the relationship between recognition of Aβ(1-42) globulomer and the respective Aβ form for the antibody given.

Figure 2

Figure 2 illustrates the results of Aβ-peptide immunoprecipitated from Alzheimer's disease brain tissue.

Figure 2(a) represents a detailed description of the patient material that was used for analysis.

Figure 2(b) illustrates the immunoprecipitated amount of Aβ(1-40)-peptide and Aβ(1-42)-peptide as quantified by SELDI-MS analysis for the different patient and control brain samples with the antibodies 6E10, 3C5, 10F4 and the control antibody IgG2b.

Figure 2(c) illustrates the relative immunoprecipitated amount of Aβ(1-40)-peptide and Aβ(1-42)-peptide as quantified by SELDI-MS analysis for the different patient and control brain samples with the antibodies 3C5, 10F4 and the control antibody IgG2b compared to the pan-Aβ-antibody 6E10 in percent. The total amount of Aβ-peptide immunoprecipitated by antibody 6E10 was set to 100%.

Figure 2(d) illustrates the immunoprecipitated amount of Aβ-peptide as quantified by Western blot analysis for the different patient and control brain samples with the antibodies 6E10, 3C5, 10F4 and the control antibody IgG2b.

Figure 2(e) illustrates the relative immunoprecipitated amount of Aβ-peptide as quantified by Western blot analysis for the different patient and control brain samples with the

antibodies 3C5, 10F4 and the control antibody IgG2b compared to the pan-Aβ-antibody 6E10 in percent. The total amount of Aβ-peptide immunoprecipitated by antibody 6E10 was set to 100%.

Figure 3

Figure 3(a) shows a Coomassie stained SDS PAGE of:

1) standard proteins (molecular marker proteins)
2) Aβ(1-42) fibril preparation; control
3) Aβ(1-42) fibril preparation + mAb 6E10, 20h, 37°C, supernatant
4) Aβ(1-42) fibril preparation + mAb 6E10, 20h, 37°C, pellet
5) Aβ(1-42) fibril preparation + mAb 3C5, 20h, 37°C, supernatant
6) Aβ(1-42) fibril preparation + mAb 3C5, 20h 37°C, pellet
7) Aβ(1-42) fibril preparation + mAb 10F4, 20h, 37°C, supernatant
8) Aβ(1-42) fibril preparation + mAb 10F4, 20h 37°C, pellet

Figure 3(b) shows the densitometric quantitative analysis of *in vitro* antibody binding to Aβ-fibrils.

Figure 4

Figure 4 shows the binding of antibodies at different concentrations to transversal sections of the neocortices of Alzheimer's disease (AD) patients or old APP transgenic mice.

Figure 4(a) represents the verification of amyloid deposits by Congo Red staining as plaques in brain tissue and as cerebral amyloid angiopathy (CAA) in brain vessels in the APP transgenic mouse line Tg2576 and in an AD patient (RZ55).

Figure 4(b) shows the strong staining of parenchymal deposits of Aβ(amyloid plaques) in an AD patient (RZ16) occurs only with 6G1 and the commercially available antibody 6E10 while 10F4 and 3C5 show considerably weaker staining. All antibodies were tested at a concentration of 0.7 µg/mL.

Figure 4(c) shows the strong staining of parenchymal deposits of Aβ(amyloid plaques) in TG2576 mice occurs only with 6G1 and the commercially available antibody 6E10 while 10F4 and 3C5 show considerably weaker staining. All antibodies were tested at a concentration of 0.7 µg/mL.

Figures 4(d)-4(g) show the quantification of the analysis of Aβplaque staining in the histological images using image analysis. Optical density values (0% = no staining) were calculated from the greyscale values of plaques subtracted by greyscale values of background tissue. (Figure 4(d) shows the binding of 0.7 µg/mL antibody in Tg2576 mice. Figure 4(e) shows the binding of 0.07-0.7 µg/mL antibody in APP/L mice. Figure 4(f) shows the binding of 0.7 µg/mL antibody in an AD patient (RZ55), and Figure 4(g) shows the binding of 0.07-0.7 µg/mL antibody in an AD patient (RZ16).) The differences between staining of the commercially available antibodies 6E10 (starts) and 4G8 (circles) and antibodies 6G1, 10F4 and 3C5 (one asterisk/circle: $p < 0.05$, two asterisks/circles: $p < 0.01$, and three asterisks/circles: $p<0.001$ versus control; post-hoc Bonferroni's t-test after ANOVA with $p<0.001$) were statistically evaluated (Figures 4(d) and (e)). In Figures 4(e)and 4(g), the antibodies 10F4 and

3C5 showed always significantly less staining than the commercially available antibodies 6E10 and 4G8 ($p<0.05$ in post-hoc t-test after $p<0.001$ in ANOVA).

Figure 4(h) shows the strong staining of vascular deposits of Aβ(arrows) occurs only with 6G1 and the commercially available antibody 6E10 while staining with 8F5 or 8C5 was much weaker. All antibodies were tested at a concentration of 0.7 µg/mL. A qualitatively similar situation was found in Tg2576 mice (not shown here).

Figure 5

Figures 5(a), (c), (e) and (g) show the amount of Aβ(1-40) and Aβ(1-42) peptide immunoprecipitated from Alzheimer's disease patient CSF by the monoclonal antibodies 6E10, 10F4, 3C5 and 8F5. Results for 4 individual Alzheimer's disease CSF samples are shown ((a) = Alzheimer's disease patient #0504009; (c) = Alzheimer's disease patient #30027; (e) = Alzheimer's disease patient #30026; (g) = Alzheimer's disease patient #26748015).

Figure 5(b) shows the relative amount of Aβ(1-40) and Aβ(1-42) peptide immunoprecipitated from Alzheimer's disease patient CSF by the antibodies 10F4, 3C5 and 8F5 compared to the amount of Aβ-peptide immunoprecipitated by the antibody 6E10 in percent. The total amount of Aβ-peptide immunoprecipitated by mAb 6E10 antibody was set to 100%. Results for 4 individual Alzheimer's disease CSF samples are shown ((b) = Alzheimer's disease patient #0504009; (d) = Alzheimer's disease patient #30027; (f) = Alzheimer's disease patient #30026; (h) = Alzheimer's disease patient #26748015).

Figure 5(i) represents a detailed description of the Alzheimer's disease patient CSF material that was used for analysis in Figures 5(a)-5(i).

Figure 6

Figure 6(a) illustrates the DNA sequence (SEQ ID NO:1) of the variable heavy chain encoding the monoclonal antibody referred to herein as "3C5".

Figure 6(b) illustrates the DNA sequence (SEQ ID NO:2) of the variable light chain encoding the monoclonal antibody referred to herein as "3C5".

Figure 6(c) illustrates the DNA sequence (SEQ ID NO:3) of the variable heavy chain encoding the monoclonal antibody referred to herein as "10F4".

Figure 6(d) illustrates the DNA sequence (SEQ ID NO:4) of the variable light chain encoding the monoclonal antibody referred to herein as "10F4".

Figure 7

Figure 7(a) illustrates the amino acid sequence (SEQ ID NO:5) of the variable heavy chain encoding the monoclonal antibody referred to herein as "3C5".

Figure 7(b) illustrates the amino acid sequence (SEQ ID NO:6) of the variable light chain encoding the monoclonal antibody referred to herein as "3C5".

Figure 7(c) illustrates the amino acid sequence (SEQ ID NO:7) of the variable heavy chain encoding the monoclonal antibody referred to herein as "10F4".

Figure 7(d) illustrates the amino acid sequence (SEQ ID NO:8) of the variable light chain encoding the monoclonal antibody referred to herein as "10F4". (Complementarity determining regions (CDRs) are underlined in each described sequence.)

SUMMARY OF THE INVENTION

The present invention encompasses antibodies, directed against Aβ globulomers, which improve the cognitive performance of a patient in immunotherapy, while at the same time reacting only with a small portion of the entire amount of Aβ peptide in the brain. Such properties prevent a substantial disturbance of cerebral Aβ balance and lead to less side effects. (For instance, a therapeutically questionable reduction of brain volume has been observed in the study of active immunization with Aβ peptides in fibrillary condition of aggregation (ELAN Corporation Plc, South San Francisco, CA, USA and Dublin, UK) of active immunization with AN-1792 (Aβ(1-42) peptide in fibrillary condition of aggregation). Moreover, in this trial, severe side effects in form of a meningoencephalitis were observed.)

In particular, the present invention solves the above-noted side effect issues by providing Aβ globulomer antibodies possessing high affinity for Aβ globulomers. These antibodies are capable of discriminating other forms of Aβ peptides, particularly monomers, fibrils and sAPPα. Further, the antibodies of the present invention also discriminate against amyloid beta in the cerebrospinal fluid (CSF) by binding only to non-CSF amyloid beta. Additionally, the antibodies of the present invention (e.g., 10F4 and 3C5) bind less to Aβ-plaques and vascular Aβ compared to a known antibody (i.e., 6E10).

In particular, the present invention encompasses an isolated antibody having a higher affinity to Aβ(1-42) globulomer than to at least one amyloid beta protein selected from the group consisting of Aß(1-42) peptide present in cerebrospinal fluid (CSF) and b) Aß(1-40) peptide present in CSF.

The present invention also includes an isolated antibody having a binding affinity to Aβ(1-42) globulomer which is greater than the binding affinity to at least one amyloid beta protein selected from the group consisting of a) Aβ(1-42) monomer, b) Aβ(1-40) monomer, c) Aβ(1-42) fibril and d) soluble amyloid precursor protein-alpha (sAPPα). This antibody binds with greater affinity to amyloid beta protein present in non-CSF than to amyloid beta protein present in CSF.

The above-described antibodies may be, for example, murine, monoclonal, recombinant, human and/or humanized. Further, any one of more of the antibodies of the present invention may bind to at least one epitope, which is the same epitope or epitopes, to which the monoclonal antibody 10F4 (obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808) or the monoclonal antibody 3C5 (obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406) binds.

Additionally, the present invention includes an isolated antibody comprising SEQ ID NO:5, an isolated antibody comprising SEQ ID NO:6 and an isolated antibody comprising both SEQ ID NO:5 and SEQ ID NO:6.

Further, the present invention encompasses an isolated antibody comprising SEQ ID NO:7, an isolated antibody comprising SEQ ID NO:8 and an isolated antibody comprising both SEQ ID NO:7 and SEQ ID NO:8.

The above-described antibodies of the present invention may comprise at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR3 and the amino acid sequence of the light chain CDR3 of monoclonal antibody (10F4)(obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808) and b) the amino acid sequence of the heavy chain CDR3 and the amino acid sequence of the light chain CDR3 of monoclonal antibody (3C5)(obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406).

Further, the above-described antibodies of the present invention may comprise at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR2 and the amino acid sequence of the light chain CDR2 of a monoclonal antibody (10F4) (obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808) and b) the amino acid sequence of the heavy chain CDR2 and the amino acid sequence of the light chain CDR2 of a monoclonal antibody (3C5) (obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406).

Also, the antibodies of the present invention may comprise at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR1 and the amino acid sequence of the light chain CDR1 of a monoclonal antibody (10F4)(obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808) and b) the amino acid sequence of the heavy chain CDR1 and the amino acid sequence of the light chain CDR1 of a monoclonal antibody (3C5)(obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406).

Moreover, the present invention also includes an isolated antibody comprising at least one CDR selected from the group consisting of amino acid sequence: a) SHYAWN; b) YIDYSGSTRYLPSLKS; c) GSGYFYGMDY; d) HASQNINVWLS; e) KASNLHT; f) QQGQSYPYT; g) NYLIE; h) VINPGSGDTNYNENFKG; i) GVITTGFDY; j) RASGNIHNYLA; k) NAKTLAD and l) QHFWSSPRT.

Additionally, the present invention encompasses a hybridoma designated by American Type Culture Collection deposit number PTA-7808 as well as a monoclonal antibody (10F4) obtainable from or produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7808.

The invention also includes a hybridoma designated by American Type Culture Collection deposit number PTA-7406 as well as a monoclonal antibody (3C5) obtainable from or produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

Furthermore, the present invention includes an isolated nucleic acid molecule encoding the antibodies described above. The nucleotide sequence of this molecule may comprise at least one sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4. Also, the present invention includes a vector comprising the isolated nucleic acid molecule as well as a host cell comprising the vector.

Additionally, the present invention includes a method of producing an antibody, comprising culturing the host cell described above in a culture medium for a time and under conditions suitable for production of any one of the antibodies described above. The antibody produced in accordance with this method is also included within the scope of the present invention.

Also, the present invention includes a composition

comprising any one or more of the antibodies described above. This composition may further comprise a pharmaceutically acceptable carrier.

Further, the present invention encompasses a monoclonal antibody comprising an amino acid sequence encoded by at least one nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4. This antibody may be selected from the group consisting of a monoclonal antibody produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7406 and a monoclonal antibody produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7808. Also, the antibody may comprise at least one amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

The invention also includes a method for treating or preventing an amyloidosis in a patient in need of such treatment or prevention. This method comprises administering one or more of the above-described antibodies (*via* passive immunization) to the patient in an amount sufficient to effect treatment or prevention. The amyloidosis may be, for example, Alzheimer's disease or the amyloidosis of Down's sydrome.

Also, the present invention encompasses an isolated antibody which binds to at least one epitope of amyloid beta protein in the brain of a patient having amyloidosis. This antibody may be produced, for example, by a hybridoma having an ATCC deposit number selected from the group consisting of PTA-7406 and PTA-7808.

The present invention also includes a method of diagnosing Alzheimer's Disease in a patient suspected of having this disease. This method comprises the steps of isolating a biological sample (for example, a CSF sample or brain tissue sample) from the patient, contacting the biological sample with one or more of the antibodies described

above for a time and under conditions sufficient for formation of antigen/antibody complexes, and detecting presence of the antigen/antibody complexes in the sample, presence of the complexes indicating a diagnosis of Alzheimer's Disease in the patient. The antigen of the complex may be, for example, a globulomer.

Additionally, the present invention encompasses another method of diagnosing Alzheimer's Disease in a patient suspected of having this disease. This method comprises the steps of isolating a biological sample from the patient, contacting the biological sample with an antigen for a time and under conditions sufficient for the formation of antibody/antigen complexes, adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody (wherein the conjugate comprises an isolated antibody of the present invention attached to a signal generating compound capable of generating a detectable signal), and detecting the presence of an antibody which may be present in the biological sample by detecting a signal generated by the signal generating compound, the signal indicating a diagnosis of Alzheimer's Disease in the patient. The antigen used in the assay may be, for example, a globulomer.

Further, the present invention includes an additional method of diagnosing Alzheimer's Disease in a patient suspected of having Alzheimer's Disease. This method comprises the steps of isolating a biological sample from the patient, contacting the biological sample with an anti-antibody (wherein the anti-antibody is specific for one of more of the antibodies of the present invention), for a time and under conditions sufficient to allow for formation of anti-antibody/antibody complexes, the complexes containing antibody present in the biological sample, adding a conjugate

to the resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to bound antibody (wherein the conjugate comprises an antigen, which binds to a signal generating compound capable of generating a detectable signal), and detecting a signal generated by the signal generating compound, this signal indicating a diagnosis of Alzheimer's Disease in the patient.

Additionally, the present invention includes a vaccine comprising one or more of the antibodies of the present invention and a pharmaceutically acceptable adjuvant.

Further, the present invention encompasses a method of identifying compounds suitable for active immunization of a patient predicted to develop Alzheimer's Disease. This method comprises the steps of exposing one or more compounds of interest to one or more of the antibodies of the present invention, for a time and under conditions sufficient for the one or more compounds to bind to the one or more antibodies and then identifying those compounds which bind to the one or more antibodies, the identified compounds to be used in active immunization in a patient predicated to develop Alzheimer's Disease.

Also, the present invention includes a kit comprising one or more of the antibodies of the present invention and and a conjugate comprising an antibody attached to a signal-generating compound, wherein the antibody of the conjugate is different from the one or more antibodies within the kit. A package insert may also be included in the kit which describes the procedure to be utilized in carrying out the assay as well as the components of the kit.

The present invention also includes another kit comprising an anti-antibody to one or more antibodies of the present invention and a conjugate comprising an antigen attached to a signal-generating compound. The antigen may be, for example, a globulomer. Again, a package insert may be

included which describes the steps to be utilized in carrying out the assay as well as the components of the kit.

DETAILED DESCRIPTION OF THE INVENTION

The antibodies of the present invention were designed from immunization with the truncated globulomer Aβ(12-42) as described in Example 1. In particular, monoclonal antibodies 3C5 and 10F4 were generated against the truncated (12-42)-globulomer (in contrast to monoclonal antibodies 8F5 and 8C5 which have been made against the Aβ(1-42) globulomer). This Aβ (12-42)globulomer was made directly from Aβ 12-42 peptide in contrast to the procedure described in Barghorn et al. (J. Neurochem, 95, 834-847) and in Example 3, Section 6, wherein the (12-42)globulomer was made from pre-existing 1-42-globulomer by limited proteolysis. These two Aβ(12-42) globulomer variants differ in their final aggregation pattern. The one made from Aβ(12-42) peptide shows only the intermediate globulomer forms ("oligomer A" as described in WO2004/067561) and the one made from the pre-existing Aβ(1-42)-globulomer is the mature globulomer ("oligomer B" as described in WO2004/067561).

It is an object of the present invention to provide antibodies directed against Aβ globulomers which improve the cognitive performance of a patient in immunotherapy while at the same time reacting only with a small portion of the entire amount of Aβ peptide in brain. This is expected to prevent a substantial disturbance of cerebral Aβ balance and lead to less side effects. (For instance, as noted above, a therapeutically questionable reduction of brain volume has been observed in the study of active immunization with Aβ peptides in fibrillary condition of aggregation (ELAN trial with AN1792). Moreover, in this trial severe side effects in form of a meningoencephalitis were observed. The present invention solves this problem by providing globulomer-specific

antibodies possessing high affinity for Aβ globulomers. These antibodies are capable of discriminating other forms of Aβ peptides, particularly monomers and fibrils.Further, these antibodies do not bind (or bind with a lower affinity compared to commercially available antibodies (such as 6E10)(Signet Cat. no.: 9320)) to amyloid beta in cerebral spinal fluid. Consequently, the present invention relates to an antibody having a binding affinity to Aβ globulomer

The term "Aβ(X-Y)" herein refers to the amino acid sequence from amino acid position X to amino acid position Y of the human amyloid β protein including both X and Y, in particular to the amino acid sequence from amino acid position X to amino acid position Y of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IAT (corresponding to amino acid positions 1 to 43) or any of its naturally occurring variants, in particular those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβpeptide, including both position X and position Y or a sequence with up to three additional amino acid substitutions, none of which may prevent globulomer formation, preferably with no additional amino acid substitutions in the portion from amino acid 12 or X, whichever number is higher, to amino acid 42 or Y, whichever number is lower, more preferably with no additional amino acid substitutions in the portion from amino acid 20 or X, whichever number is higher, to amino acid 42 or Y, whichever number is lower, and most preferably with no additional amino acid substitutions in the portion from amino acid 20 or X, whichever number is higher, to amino acid 40 or Y, whichever number is lower, an "additional" amino acid substation herein being any deviation from the canonical sequence that is not found in nature.

The term "Aβ(1-42)" herein refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the human amyloid β protein including both 1 and 42, in particular to the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA or any of its naturally occurring variants, in particular those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβ peptide, including both 1 and 42 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation, preferably with no additional amino acid substitutions in the portion from amino acid 20 to amino acid 42. Likewise, the term "Aβ(1-40)" here refers to the amino acid sequence from amino acid position 1 to amino acid position 40 of the human amyloid β protein including both 1 and 40, in particular to the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV or any of its naturally occurring variants, in particular those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), and D23N ("Iowa") wherein the numbers are relative to the start of the Aβ peptide, including both 1 and 40 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation, preferably with no additional amino acid substitutions in the portion from amino acid 20 to amino acid 40.

The term "Aβ(12-42)" here refers to the amino acid sequence from amino acid position 12 to amino acid position 42 of the human amyloid β protein including both 12 and 42, in particular to the amino acid sequence VHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA or any of its naturally occurring variants, in particular, those with at least one mutation selected from the

group consisting of A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβ peptide, including both 12 and 42 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation, preferably with no additional amino acid substitutions in the portion from amino acid 20 to amino acid 42.

The term "Aβ(20-42)" herein refers to the amino acid sequence from amino acid position 20 to amino acid position 42 of the human amyloid β protein including both 20 and 42, in particular, to the amino acid sequence F AEDVGSNKGA IIGLMVGGVV IA or any of its naturally occurring variants, in particular those with at least one mutation selected from the group consisting of A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβ peptide, including both 20 and 42 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation, preferably without any additional amino acid substitutions.

The term "Aβ(X-Y) globulomer" (Aβ(X-Y) globular oligomer) herein refers to a soluble, globular, non-covalent association of Aβ(X-Y) peptides as defined above, possessing homogeneity and distinct physical characteristics. According to one aspect, the Aβ(X-Y) globulomers are stable, non-fibrillar, oligomeric assemblies of Aβ(X-Y) peptides which are obtainable by incubation with anionic detergents. In contrast to monomers and fibrils, these globulomers are characterized by defined assembly numbers of subunits (e.g. early assembly forms, n=4-6, "oligomers A", and late assembly forms, n=12-14, "oligomers B", as described in WO2004/067561). The globulomers have a 3-dimensional globular type structure ("molten globule", see Barghorn et al., 2005, J Neurochem, 95,

834-847). They may be further characterized by one or more of the following features:

- cleavability of N-terminal amino acids X-23 with promiscuous proteases (such as thermolysin or endoproteinase GluC) yielding truncated forms of globulomers;
- non-accessibility of C-terminal amino acids 24-Y with promiscuous proteases and antibodies;
- truncated forms of these globulomers maintain the 3-dimensional core structure of said globulomers with a better accessibility of the core epitope Aß(20-Y) in its globulomer conformation.

According to the invention and, in particular, for the purpose of assessing the binding affinities of the antibodies of the present invention, the term "Aβ(X-Y) globulomer" herein refers, in particular, to a product which is obtainable by a process as described, for example, in Example I presented below. (See also WO 04/067561.) Such a process may be used to obtain Aβ(1-42) globulomers, Aβ(12-42) globulomers, and Aβ(20-42) globulomers. Preferably, the globulomer shows affinity to neuronal cells. Preferably, the globulomer also exhibits neuromodulating effects. According to another aspect of the invention, the globulomer consists of 11 to 16, and most preferably, of 12 to 14 Aβ(X-Y) peptides.

According to another aspect of the invention, the term Aβ(X-Y) globulomer" herein refers to a globulomer consisting essentially of Aβ(X-Y) subunits, where it is preferred if on average at least 11 of 12 subunits are of the Aβ(X-Y) type, more preferred if less than 10% of the globulomers comprise any non-Aβ(X-Y) peptides, and most preferred if the content of non-Aβ(X-Y) peptides is below the detection threshold. More specifically, the term "Aβ(1-42) globulomer" herein refers to a globulomer consisting essentially of Aβ(1-42) units as defined above; the term "Aβ(12-42) globulomer" herein refers to a globulomer consisting essentially of Aβ(12-42)

units as defined above; and the term "Aβ(20-42) globulomer" herein refers to a globulomer consisting essentially of Aβ(20-42) units as defined above.

The term "cross-linked Aβ(X-Y) globulomer" as used herein refers to a molecule obtainable from an Aβ(X-Y) globulomer as described above by cross-linking, preferably chemically cross-linking, more preferably, aldehyde cross-linking, most preferably, glutardialdehyde cross-linking of the constituent units of the globulomer. In another aspect of the invention, a cross-linked globulomer is essentially a globulomer in which the units are at least partially joined by covalent bonds, rather than being held together by non-covalent interactions only. For the purposes of the present invention, a cross-linked Aβ(1-42) globulomer is, in particular, a cross-linked Aβ(1-42) oligomer.

The term "Aβ(X-Y) globulomer derivative" as used herein refers, in particular, to a globulomer that is labelled by being covalently linked to a group that facilitates detection, preferably, a fluorophore, e.g., fluorescein isothiocyanate, phycoerythrin, Aequorea victoria fluorescent protein, Dictyosoma fluorescent protein or any combination or fluorescence-active derivative thereof; a chromophore; a chemoluminophore, e.g., luciferase, preferably Photinus pyralis luciferase, Vibrio fischeri luciferase, or any combination or chemoluminescence-active derivative thereof; an enzymatically active group, e.g., peroxidase, e.g., horseradish peroxidase, or any enzymatically active derivative thereof; an electron-dense group, e.g., a heavy metal containing group, e.g., a gold containing group; a hapten, e.g., a phenol derived hapten; a strongly antigenic structure, e.g., peptide sequence predicted to be antigenic, e.g., predicted to be antigenic by the algorithm of Kolaskar and Tongaonkar; an aptamer for another molecule; a chelating group, e.g., hexahistidinyl; a natural or nature-derived

protein structure mediating further specific protein-protein interactions, e.g., a member of the fos/jun pair; a magnetic group, e.g., a ferromagnetic group; or a radioactive group, e.g., a group comprising ^{1}H, ^{14}C, ^{32}P, ^{35}S or ^{125}I or any combination thereof; or to a globulomer flagged by being covalently or by non-covalent high-affinity interaction, preferably covalently linked to a group that facilitates inactivation, sequestration, degradation and/or precipitation, preferably flagged with a group that promotes *in vivo* degradation, more preferably with ubiquitin, where is particularly preferred if this flagged oligomer is assembled *in vivo*; or to a globulomer modified by any combination of the above. Such labelling and flagging groups and methods for attaching them to proteins are known in the art. Labelling and/or flagging may be performed before, during or after globulomerization. In another aspect of the invention, a globulomer derivative is a molecule obtainable from a globulomer by a labelling and/or flagging reaction. Correspondingly, term "Aβ(X-Y) monomer derivative" herein refers, in particular, to an Aβ monomer that is labelled or flagged as described for the globulomer.

The term "greater affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer on the one hand and antibody-globulomer complex on the other is further in favor of the antibody-globulomer complex. Likewise, the term "smaller affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer on the one hand and antibody-globulomer complex on the other is further in favour of the unbound antibody and unbound globulomer. The term "greater affinity"is synonymous with the term "higher affinity" and term "smaller affinity"is synonymous with the term "lower affinity".

The term "Aβ(X-Y) monomer" herein refers to the isolated form of the Aβ(X-Y) peptide, preferably, a form of the Aβ(X-Y) peptide which is not engaged in essentially non-covalent interactions with other Aβ peptides. Practically, the Aβ(X-Y) monomer is usually provided in the form of an aqueous solution. In a particularly preferred embodiment of the invention, the aqueous monomer solution contains 0.05% to 0.2%, more preferably, about 0.1% NH_4OH. In another particularly preferred embodiment of the invention, the aqueous monomer solution contains 0.05% to 0.2%, more preferably, about 0.1% NaOH. When used (for instance, for determining the binding affinities of the antibodies of the present invention), it may be expedient to dilute said solution in an appropriate manner. Further, it is usually expedient to use said solution within 2 hours, in particular, within 1 hour, and especially within 30 minutes after its preparation.

The term "fibril" herein refers to a molecular structure that comprises assemblies of non-covalently associated, individual Aβ(X-Y) peptides, which show fibrillary structure in the electron microscope, which bind Congo red and then exhibit birefringence under polarized light and whose X-ray diffraction pattern is a cross-β structure. In another aspect of the invention, a fibril is a molecular structure obtainable by a process that comprises the self-induced polymeric aggregation of a suitable Aβ peptide in the absence of detergents, e.g., in 0.1 M HCl, leading to the formation of aggregates of more than 24, preferably more than 100 units. This process is well known in the art. Expediently, Aβ(X-Y) fibrils are used in the form of an aqueous solution. In a particularly preferred embodiment of the invention, the aqueous fibril solution is made by dissolving the Aβ peptide in 0.1% NH_4OH, diluting it 1:4 with 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, followed by readjusting the pH to 7.4, incubating the

solution at 37 °C for 20 h, followed by centrifugation at 10000 g for 10 min and resuspension in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4.

The term "Aβ(X-Y) fibril" herein refers to a fibril consisting essentially of Aβ(X-Y) subunits, where it is preferred if on average at least 90% of the subunits are of the Aβ(X-Y) type, more preferred, if at least 98% of the subunits are of the Aβ(X-Y) type and, most preferred, if the content of non-Aβ(X-Y) peptides is below the detection threshold.

The present invention also relates to antibodies having a similar binding profile to that of any one of said monoclonal antibodies, 10F4 and 3C5. Antibodies having a binding profile similar to that of any one of said monoclonal antibodies include antibodies which bind to the same epitope as monoclonal antibody 10F4 and 3C5.

The present invention also relates to antibodies which are capable of competing with at least one, preferably all, antibodies selected from the group consisting of 10F4 and 3C5. The term "competing antibodies" herein refers to any number of antibodies targeting the same molecular or stably but non-covalently linked supermolecular entity, preferably, the same molecule, wherein at least one is capable of specifically reducing the measurable binding of another, preferably, by sterically hampering the other's access to its target epitope or by inducing and/or stabilizing a conformation in the target entity that reduces the target's affinity for the other antibody, more preferably, by directly blocking access to the other's target epitope by binding to an epitope in sufficiently close vicinity of the former, overlapping with the former or identical to the former, most preferably, overlapping or identical, in particular identical. Two epitopes are said to be "overlapping" if they share part of their chemical structures, preferably their amino acid

sequences, and to be "identical" if their chemical structures, preferably their amino acid sequences, are identical. Thus, the present invention also relates to antibodies whose target epitopes are overlapping with, preferably identical to, the target epitope of at least one of the antibodies selected from the group consisting of 10F4 and 3C5. Antibodies having a similar binding profile to that of any one of said monoclonal antibodies 10F4 and 3C5 thus further include antibodies which comprise at least a portion of the antigen-binding moiety of any one of said monoclonal antibodies. Preferably, said portion comprises at least one complementary determining region (CDR) of any one of said monoclonal antibodies. Thus, according to a further particular embodiment, the present invention relates to antibodies comprising the amino acid sequence of the heavy chain CDR3 and/or the amino acid sequence of the light chain CDR3 of monoclonal antibody 10F4 or 3C5, respectively. Specific examples of such antibodies include those which also comprise the amino acid sequence of the heavy chain CDR2 and/or the amino acid sequence of the light chain CDR2 of monoclonal antibody 10F4 or 3C5, respectively. Even more specifically, such antibodies include those which also comprise the amino acid sequence of the heavy chain CDR1 and/or the amino acid sequence of the light chain CDR1 of monoclonal antibody 10F4 or 3C5, respectively.

In one aspect, the present invention thus relates to antibodies comprising a heavy chain wherein the CDR3, CDR2 and/or CDR1 domain comprises the amino acid sequence of the heavy chain CDR3, CDR2 and/or CDR1 of monoclonal antibody 10F4 or 3C5. In a further aspect, the present invention thus relates to antibodies comprising a light chain wherein the CDR3, CDR2 and/or CDR1 domain comprises the amino acid sequence of the light chain CDR3, CDR2 and/or CDR1, respectively, of monoclonal antibody 10F4 or 3C5.

In one embodiment the antibody of the invention comprises at least two variable domain CDR sets. More preferably, the two variable domain CDR sets are selected from the group consisting of: VH 10F4 CDR Set & VL 10F4 CDR Set; VH 3C5 CDR Set & VL 3C5 CDR Set (see Figures 7a – 7d).

In another embodiment the antibody disclosed above further comprises a human acceptor framework. In a preferred embodiment, the antibody is a CDR grafted antibody. Preferably, the CDR grafted antibody comprises one or more of the CDRs disclosed above. Preferably the CDR grafted antibody comprises a human acceptor framework.

In a preferred embodiment the antibody is a humanized antibody. Preferably, the humanized antibody comprises one or more of the CDRs disclosed above. More preferably, the humanized antibody comprises three or more of the CDRs disclosed above. Most preferably, the humanized antibody comprises six CDRs disclosed above. In a particular embodiment, the CDRs are incorporated into a human antibody variable domain of a human acceptor framework. Preferably, the human antibody variable domain is a consensus human variable domain. More preferably, the human acceptor framework comprises at least one framework region amino acid substitution at a key residue, wherein the key residue is selected from the group consisting of a residue adjacent to a CDR; a glycosylation site residue; a rare residue;; a residue capable of interacting with a CDR; a canonical residue; a contact residue between heavy chain variable region and light chain variable region; a residue within a Vernier zone; and a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework. Preferably, the human acceptor framework human acceptor framework comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the

sequence of said human acceptor framework and comprises at least 70 amino acid residues identical to said human acceptor framework. In yet a further aspect, the present invention relates to antibodies comprising both the heavy and light chain as defined above. Preferably, the antibody comprises at least one variable domain as described above. More preferably, the antibody comprises two variable domains as described above, wherein said two variable domains have amino acid sequences as noted in Figure 7.

In another aspect, the antibodies of the present invention comprise a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgD, IgE and human IgG1 Ala234 Ala235 mutant constant regions. In particular, the antibodies comprise a human constant region. Antibodies comprising an IgG1 heavy chain constant region are preferred.

In another embodiment the antibody is glycosylated. Preferably the glycosylation pattern is a human glycosylation pattern or a glycosylation pattern produced by any one of the eukaryotic cells disclosed herein, in particular CHO cells.

The present invention also relates to an antigen-binding moiety of an antibody of the present invention. Such antigen-binding moieties include, but are not limited to, Fab fragments, $F(ab')_2$ fragments and single chain Fv fragments of the antibody. Further antigen-binding moieties are Fab' fragments, Fv fragments, and disulfide linked Fv fragments.

The invention also provides an isolated nucleic acid encoding any one of the antibodies disclosed herein. A further embodiment provides a vector comprising the isolated nucleic acid disclosed herein. The vector may in particular be selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No.2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima, S.

and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; and pBJ.

In another aspect, a host cell is transformed with the vector disclosed herein. Preferably, the host cell is a prokaryotic cell. More preferably, the host cell is *E. coli*. In a related embodiment, the host cell is an eukaryotic cell. Preferably, the eukaryotic cell is selected from the group consisting of a protist cell, an animal cell, a plant cell and a fungal cell. More preferably, the host cell is a mammalian cell including, but not limited to, CHO and COS; or a fungal cell such as Saccharomyces cerevisiae; or an insect cell such as Sf9.

Another aspect of the invention provides a method of producing an antibody of the invention, comprising culturing any one of the host cells or a hybridoma disclosed herein in a culture medium under conditions suitable to produce the antibody. Another embodiment provides an antibody that is obtainable by the method disclosed herein. Antibodies of the present invention can be obtained in a manner known per se. B lymphocytes which, in totality, contain an antibody repertoire composed of hundreds of billions of different antibody specificities are a part of the mammalian immune system. A normal immune response to a particular antigen means selection of one or more antibodies of said repertoire which specifically bind to said antigen, and the success of an immune response is based at least partially on the ability of said antibodies to specifically recognize (and ultimately to eliminate) the stimulating antigen and to ignore other molecules in the environment of said antibodies. The usefulness of antibodies which specifically recognize one particular target antigen has led to the development of monoclonal antibody technology. Standardized hybridoma technology now allows the production of antibodies with a single specificity for an antigen of interest. More recently,

recombinant antibody techniques such as *in-vitro* screening of antibody libraries have been developed. These techniques likewise allow antibodies having a single specificity for an antigen of interest to be produced.

In the method of the invention, the antigen of interest may be allowed to act on the antibody repertoire either *in vivo* or *in vitro*. According to one embodiment, the antigen is allowed to act on the repertoire by immunizing an animal *in vivo* with said antigen. This *in-vivo* approach may furthermore comprise establishing from the lymphocytes of an animal a number of hybridomas and selecting a particular hybridoma which secretes an antibody specifically binding to said antigen. The animal to be immunized may be, for example, a mouse, rat, rabbit, chicken, camelid or sheep or may be a transgenic version of any of the animals mentioned above, for example, a transgenic mouse with human immunoglobulin genes, which produces human antibodies after an antigenic stimulus. Other types of animals which may be immunized include mice with severe combined immunodeficiency (SCID) which have been reconstituted with human peripheral mononuclear blood cells (chimeric hu-PBMC SCID mice) or with lymphoid cells or precursors thereof, as well as mice which have been treated with a lethal total body irradiation, then protected against radiation with bone marrow cells from a mouse with severe combined immunodeficiency (SCID) and subsequently transplanted with functional human lymphocytes (the "Trimera" system). Another type of an animal to be immunized is an animal (e.g., a mouse) in whose genome an endogenous gene encoding the antigen of interest has been switched off (knocked out), for example, by homologous recombination, so that after immunization with the antigen, said animal recognizes said antigen as foreign. The polyclonal or monoclonal antibodies produced by this method are characterized and selected by

using known screening methods which include, but are not limited to, ELISA and dot blot techniques.

According to another embodiment, the antigen is allowed to act on the antibody repertoire *in vitro* by screening a recombinant antibody library with said antigen. The recombinant antibody library may be expressed, for example, on the surface of bacteriophages or on the surface of yeast cells or on the surface of bacterial cells. In a variety of embodiments, the recombinant antibody library is an scFv library or an Fab library, for example. According to another embodiment, antibody libraries are expressed as RNA-protein fusions.

Another approach to producing antibodies of the invention comprises a combination of *in vivo* and *in vitro* approaches. For example, the antigen may be allowed to act on the antibody repertoire by immunizing an animal *in vivo* with said antigen and then screening *in vitro* with said antigen a recombinant antibody library prepared from lymphoid cells of said animal or a single domain antibody library (e.g., containing heavy and/or light chains). According to another approach, the antigen is allowed to act on the antibody repertoire by immunizing an animal *in vivo* with said antigen and then subjecting a recombinant antibody library or single domain library produced from lymphoid cells of said animal to affinity maturation. According to another approach, the antigen is allowed to act on the antibody repertoire by immunizing an animal *in vivo* with said antigen, then selecting individual antibody-producing cells secreting an antibody of interest and obtaining from said selected cells cDNAs for the variable region of the heavy and light chains (e.g., by means of PCR) and expressing said variable regions of the heavy and light chains in mammalian host cells *in vitro* (this being referred to as selected lymphocyte antibody method or SLAM), thereby being able to further select and manipulate the

selected antibody gene sequences. Moreover, monoclonal antibodies may be selected by expression cloning by expressing the antibody genes for the heavy and light chains in mammalian cells and selecting those mammalian cells which secrete an antibody having the desired binding affinity.

The methods of the invention for producing antibodies can be used to produce various types of antibodies. These include monoclonal, in particular recombinant antibodies, especially essentially human antibodies, chimeric antibodies, humanized antibodies and CDR graft antibodies, and also antigen-binding moieties thereof.

The present invention further relates to a hybridoma that is capable of producing (secreting) a monoclonal antibody of the present invention. Hybridomas of the present invention include those designated by an American Type Culture Collection deposit number selected from the group consisting of PTA-7808 and PTA-7406 and those producing monoclonal antibodies 10F4 and 3C5.

It is noted that the antibodies of the present invention may also be reactive with, i.e., bind to, Aβ forms other than the Aβ globulomers described herein. These antigens may or may not be oligomeric or globulomeric. Thus, the antigens to which the antibodies of the present invention bind include any Aβ form that comprises the globulomer epitope with which the antibodies of the present invention are reactive. Such Aβ forms include truncated and non-truncated Aβ(X-Y) forms (with X and Y being defined as above), such as Aβ(20-42), Aβ(20-40), Aβ(12-42), Aβ(12-40), Aβ(1-42), and Aβ(1-40) forms, provided that said forms comprise the globulomer epitope.

The present invention also relates to a composition comprising an antibody of the invention or an antigen-binding moiety thereof, as defined above. According to a particular embodiment, said composition is a pharmaceutical composition

which comprises the antibody of the invention or the antigen-binding moiety and a pharmaceutical acceptable carrier. The antibody of the invention or the antigen-binding moiety, as defined above, is preferably capable of neutralizing, both *in vitro* and *in vivo*, the activity of Aβ globulomer or a derivative thereof to which it binds. Said antibody or antigen-binding moiety may therefore be used for inhibiting the activity of said globulomer or derivative thereof, for example, in a preparation containing said globulomer or derivative thereof or in human individuals or other mammals in which said globulomer or derivative thereof is present.

According to one embodiment, the invention relates to a method of inhibiting the activity of said globulomer or derivative thereof which method comprises allowing an antibody of the invention or an antigen-binding moiety thereof to act on a globulomer or derivative thereof so as to inhibit the activity of said globulomer or derivative thereof. Said activity may be inhibited *in vitro*, for example. For instance, the antibody of the invention or the antigen-binding moiety may be added to a preparation such as a sample derived from a subject or a cell culture which contains or is suspected to contain said globulomer or derivative thereof, in order to inhibit the activity of said globulomer or derivative thereof in said sample. Alternatively, the activity of the globulomer or derivative thereof may be inhibited in an individual *in vivo*. Thus, the present invention further relates to the use of an antibody or an antigen-binding moiety as defined above for preparing a pharmaceutical composition for treating or preventing an amyloidosis, in particular, an amyloidosis selected from the group consisting of Alzheimer's disease and the amyloidosis of Down's syndrome. One aspect of said use of the invention is therefore a method of treating or preventing an amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, in a subject in need

thereof, which comprises administering an antibody or an antigen-binding moiety as defined above to the subject. Using said antibody or antigen-binding moiety for treating and especially preventing the amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, is in particular for passive immunization. Accordingly, in the method of treating or preventing an amyloidosis, in particular Alzheimer's disease or the amyloidosis of Down's syndrome, in a subject in need thereof one purpose of administering the antibody or antigen-binding moiety to the subject is passively immunizing the subject against the amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome.

The antibody of the invention or the antigen-binding moiety as defined above is preferably capable of detecting, both *in vitro* and *in vivo*, an Aβ globulomer or derivative thereof to which it binds. Said antibody or the antigen-binding moiety may therefore be used for detecting said globulomer or derivative thereof, for example, in a preparation containing said globulomer or derivative thereof or in human individuals or other mammals in which said globulomer or derivatives thereof is present.

According to one embodiment, the invention relates to a method of detecting said globulomer or derivative thereof, which method comprises allowing an antibody of the invention or an antigen-binding moiety thereof to act on a globulomer or derivative thereof so as to bind to said globulomer or derivative thereof (and thereby preferably forming a complex comprising the antibody or antigen-binding moiety thereof and the globulomer or derivative thereof). The globulomer may be detected *in vitro*, for example. For instance, the antibody of the invention or the antigen-binding moiety may be added to a preparation, for instance, a sample derived from a subject or a cell culture which contains or is suspected to contain said globulomer or derivative thereof, in order to detect said

globulomer or derivative thereof in said preparation. Alternatively, the globulomer or derivative thereof may be detected in an individual *in vivo*. Thus, the present invention further relates to the use of an antibody or an antigen-binding moiety as defined above for preparing a composition for diagnosing an amyloidosis, in particular Alzheimer's disease or the amyloidosis of Down's syndrom. One aspect of said use of the invention is a method of diagnosing an amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, in a subject suspected of having the amyloidosis, in particular Alzheimer's disease or the amyloidosis of Down's syndrom, which comprises administering to the subject an antibody or an antigen-binding moiety as defined above and detecting the formation of a complex comprising the antibody or the antigen-binding moiety with the antigen, the presence of the complex indicating the amyloidosis, in particular Alzheimer's disease or the amyloidosis of Down's syndrom, in the subject. A second aspect of said use of the invention is a method of diagnosing an amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, in a subject suspect of having the amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, which comprises providing a sample from the subject, contacting the sample with an antibody or an antigen-binding moiety (as defined) above and detecting the formation of a complex comprising the antibody or the antigen-binding moiety with the antigen, the presence of the complex indicating the amyloidosis, in particular, Alzheimer's disease or the amyloidosis of Down's syndrome, in the subject.

The binding affinities of the antibodies of the invention may be evaluated by using standardized in-vitro immunoassays such as ELISA, dot blot or BIAcore analyses (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further

descriptions, see Jönsson, U., et al. (1993) Ann. Biol. Clin. 51:19-26; Jönsson, U., et al. (1991) Biotechniques 11:620-627; Johnsson, B., et al. (1995) J. Mol. Recognit. 8:125-131; and Johnnson, B., et al. (1991) Anal. Biochem. 198:268-277.

According to a particular embodiment, the affinities defined herein refer to the values obtained by performing a dot blot and evaluating it by densitometry. According to a particular embodiment of the invention, determining the binding affinity by dot blot comprises the following: a certain amount of the antigen (e.g. the Aβ(X-Y) globulomer, Aβ(X-Y) monomer or Aβ(X-Y) fibrils, as defined above) or, expediently, an appropriate dilution thereof, for instance in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, 0.2 mg/mL BSA to an antigen concentration of, for example, 100 pmol/µL, 10 pmol/µL, 1 pmol/µL, 0.1 pmol/µL and 0.01 pmol/µL, is dotted onto a nitrocellulose membrane, the membrane is then blocked with milk to prevent unspecific binding and washed, then contacted with the antibody of interest followed by detection of the latter by means of an enzyme-conjugated secondary antibody and a colorimetric reaction; at defined antibody concentrations, the amount of antibody bound allows affinity determination. Thus the relative affinity of two different antibodies to one target, or of one antibody to two different targets, is here defined as the relation of the respective amounts of target-bound antibody observed with the two antibody-target combinations under otherwise identical dot blot conditions. Unlike a similar approach based on Western blotting, the dot blot approach will determine an antibody's affinity to a given target in the latter's natural conformation; unlike the ELISA approach, the dot blot approach does not suffer from differences in the affinities between different targets and the matrix, thereby allowing for more precise comparisons between different targets.

The term "K_d", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction as is known in the art.

The antibodies of the present invention are preferably isolated antibodies. An isolated antibody" means an antibody having the binding affinities as described above and which is essentially free of other antibodies having different binding affinities. The term "essentially free" here refers to an antibody preparation in which at least 95% of the antibodies, preferably at least 98% of the antibodies and more preferably at least 99% of the antibodies have the desired binding affinity. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The isolated antibodies of the present invention include monoclonal antibodies. A "monoclonal antibody" as used herein is intended to refer to a preparation of antibody molecules, antibodies which share a common heavy chain and common light chain amino acid sequence, in contrast with "polyclonal" antibody preparations which contain a mixture of antibodies of different amino acid sequence. Monoclonal antibodies can be generated by several novel technologies like phage, bacteria, yeast or ribosomal display, as well as by classical methods exemplified by hybridoma-derived antibodies (e.g., an antibody secreted by a hybridoma prepared by hybridoma technology, such as the standard Kohler and Milstein hybridoma methodology ((1975) Nature 256:495-497). Thus, a non-hybridoma-derived antibody with uniform sequence is still referred to as a monoclonal antibody herein although it may have been obtained by non-classical methodologies, and the term "monoclonal" is not restricted to hybridoma-derived antibodies but used to refer to all antibodies derived from one nucleic acid clone. Thus, the monoclonal antibodies of the present invention include recombinant antibodies. The term "recombinant" as used herein refers to any artificial combination of two

otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. In particular, the term "recombinant antibody" refers to antibodies which are produced, expressed, generated or isolated by recombinant means, such as antibodies which are expressed using a recombinant expression vector transfected into a host cell; antibodies isolated from a recombinant combinatorial antibody library; antibodies isolated from an animal (e.g. a mouse) which is transgenic due to human immunoglobulin genes (see, for example, Taylor, L.D., *et al.* (1992) *Nucl. Acids Res.* 20:6287-6295); or antibodies which are produced, expressed, generated or isolated in any other way in which particular immunoglobulin gene sequences (such as human immunoglobulin gene sequences) are assembled with other DNA sequences. Recombinant antibodies include, for example, chimeric, CDR graft and humanized antibodies. The person skilled in the art will be aware that expression of a conventional hybridoma-derived monoclonal antibody in a heterologous system will require the generation of a recombinant antibody even if the amino acid sequence of the resulting antibody protein is not changed or intended to be changed.

In a particular embodiment of the invention, the antibody is a humanized antibody. According to a multiplicity of embodiments, the antibody may comprise an amino acid sequence derived entirely from a single species, such as a human antibody or a mouse antibody. According to other embodiments, the antibody may be a chimeric antibody or a CDR graft antibody or another form of a humanized antibody.

The term "antibody" is intended to refer to immunoglobulin molecules consisting of 4 polypeptide chains, two heavy (H) chains and two light (L) chains. The chains are usually linked to one another via disulfide bonds. Each heavy

chain is composed of a variable region of said heavy chain (abbreviated here as HCVR or VH) and a constant region of said heavy chain. The heavy chain constant region consists of three domains CH1, CH2 and CH3. Each light chain is composed of a variable region of said light chain (abbreviated here as LCVR or VL) and a constant region of said light chain. The light chain constant region consists of a CL domain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs which are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. This structure is well known to those skilled in the art.

The term "antigen-binding moiety" of an antibody (or simply "antibody moiety") refers to one or more fragments of an antibody of the invention, said fragment(s) still having the binding affinities as defined above. Fragments of a complete antibody have been shown to be able to carry out the antigen-binding function of an antibody. In accordance with the term "antigen-binding moiety" of an antibody, examples of binding fragments include (i) an Fab fragment, i.e. a monovalent fragment composed of the VL, VH, CL and CH1 domains; (ii) an $F(ab')_2$ fragment, i.e. a bivalent fragment comprising two Fab fragments linked to one another in the hinge region via a disulfide bridge; (iii) an Fd fragment composed of the VH and CH1 domains; (iv) an Fv fragment composed of the FL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546) consisting of a VH domain or of VH, CH1, CH2, DH3, or VH, CH2, CH3; and (vi) an isolated complementarity-determining region (CDR). Although the two domains of the Fv fragment, namely VL and VH, are encoded by separate genes,

they may further be linked to one another using a synthetic linker, e.g. a poly-G_4S amino acid sequence, and recombinant methods, making it possible to prepare them as a single protein chain in which the VL and VH regions combine in order to form monovalent molecules (known as single chain Fv (ScFv); see, for example, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). The term "antigen-binding moiety" of an antibody is also intended to comprise such single chain antibodies. Other forms of single chain antibodies such as "diabodies" are likewise included here. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker which is too short for the two domains being able to combine on the same chain, thereby forcing said domains to pair with complementary domains of a different chain and to form two antigen-binding sites (see, for example, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

Furthermore, an antibody of the present invention or antigen-binding moiety thereof may be part of a larger immunoadhesion molecule formed by covalent or noncovalent association of said antibody or antibody moiety with one or more further proteins or peptides. Relevant to such immunoadhesion molecules are the use of the streptavidin core region in order to prepare a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and the use of a cystein residue, a marker peptide and a C-terminal polyhistidinyl, e. g. hexahistidinyl, tag in order to produce bivalent and

biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058).

The term "human antibody" refers to antibodies whose variable and constant regions correspond to or are derived from immunoglobulin sequences of the human germ line, as described, for example, by Kabat *et al.* (see Kabat, *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). However, the human antibodies of the invention may contain amino acid residues not encoded by human germ line immunoglobulin sequences (for example mutations which have been introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs, and in particular in CDR3. Recombinant human antibodies of the invention have variable regions and may also contain constant regions derived from immunoglobulin sequences of the human germ line (see Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). According to particular embodiments, however, such recombinant human antibodies are subjected to in-vitro mutagenesis (or to a somatic in-vivo mutagenesis, if an animal is used which is transgenic due to human Ig sequences) so that the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences which although related to or derived from VH and VL sequences of the human germ line, do not naturally exist in vivo within the human antibody germ line repertoire. According to particular embodiments, recombinant antibodies of this kind are the result of selective mutagenesis or back mutation or of both. Preferably, mutagenesis leads to an affinity to the target which is greater, and/or an affinity to non-target structures which is smaller than that of the parent antibody.

The term "chimeric antibody" refers to antibodies which contain sequences for the variable region of the heavy and light chains from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (*e.g.*, CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which contain sequences of the variable region of heavy and light chains from a nonhuman species (e.g. mouse, rat, rabbit, chicken, camelid, sheep or goat) but in which at least one part of the VH and/or VL sequence has been altered in order to be more "human-like", i.e. to be more similar to variable sequences of the human germ line. One type of a humanized antibody is a CDR graft antibody in which human CDR sequences have been inserted into nonhuman VH and VL sequences to replace the corresponding nonhuman CDR sequences.

The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad, Sci.* 190:382-391 and Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S.

Department of Health and Human Services, NIH Publication No. 91-3242). .

As used herein, the terms "acceptor" and "acceptor antibody" refer to the antibody or nucleic acid sequence providing or encoding at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% of the amino acid sequences of one or more of the framework regions. In some embodiments, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding the constant region(s). In yet another embodiment, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding one or more of the framework regions and the constant region(s). In a specific embodiment, the term "acceptor" refers to a human antibody amino acid or nucleic acid sequence that provides or encodes at least 80%, preferably, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In accordance with this embodiment, an acceptor may contain at least 1, at least 2, at least 3, least 4, at least 5, or at least 10 amino acid residues not occuring at one or more specific positions of a human antibody. An acceptor framework region and/or acceptor constant region(s) may be, e.g., derived or obtained from a germline antibody gene, a mature antibody gene, a functional antibody (e.g., antibodies well-known in the art, antibodies in development, or antibodies commercially available).

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and of the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term “CDR set” as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs

have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD(1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, in spite of great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "canonical" residue refers to a residue in a CDR or framework that defines a particular canonical CDR structure as defined by Chothia et al. (J. Mol. Biol. 196:901-907 (1987); Chothia et al., J. Mol. Biol. 227:799 (1992)). According to Chothia et al., critical portions of the CDRs of many antibodies have nearly identical

peptide backbone confirmations despite great diversity at the level of amino acid sequence. Each canonical structure specifies primarily a set of peptide backbone torsion angles for a contiguous segment of amino acid residues forming a loop.

As used herein, the terms "donor" and "donor antibody" refer to an antibody providing one or more CDRs. In a preferred embodiment, the donor antibody is an antibody from a species different from the antibody from which the framework regions are obtained or derived. In the context of a humanized antibody, the term "donor antibody" refers to a non-human antibody providing one or more CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined using different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub- regions constituting a framework region. Human heavy chain and light chain acceptor sequences are known in the art.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non-lymphoid cells that have not undergone the maturation

process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., Crit. Rev. Immunol. 22(3): 183-200 (2002); Marchalonis et al., Adv Exp Med Biol. 484:13-30 (2001)). One of the advantages provided by various embodiments of the present invention stems from the finding that germline antibody genes are more likely than mature antibody genes are to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as non-self when used in that species.

As used herein, the term "key" residues refers to certain residues within the variable region that have more impact on the binding specificity and/or affinity of an antibody, in particular a humanized antibody. A key residue includes, but is not limited to, one or more of the following: a residue that is adjacent to a CDR, a potential glycosylation site (which can be either N- or O-glycosylation site), a rare residue, a residue capable of interacting with the antigen, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable region and light chain variable region, a residue within the Vernier zone, and a residue in the region that overlaps between the Chothia definition of a variable heavy chain CDR1 and the Kabat definition of the first heavy chain framework.

As used herein, the term "humanized antibody" specifically refers to an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at

least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any subclass, including without limitation IgG 1, IgG2, IgG3 and IgG4. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond exactly to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term

"consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, From Genes to Clones, Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. Where two amino acids occur equally frequently, either can be included in the consensus sequence.

As used herein, "Vernier" zone refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote and Winter (1992, J. Mol. Biol. 224:487-499). Vernier zone residues form a layer underlying the CDRs and may impact on the structure of CDRs and the affinity of the antibody.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

The term "polynucleotide" as referred to herein means a polymeric form of two or more nucleotides, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and

double stranded forms of DNA but preferably is double-stranded DNA.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or any combination thereof) that, by virtue of its origin, the "isolated polynucleotide" is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors

(e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is connected in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation", as defined herein, refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably host cells include but are not limited to the prokaryotic cell line E.coli; mammalian cell lines CHO, HEK 293 and COS; the insect cell line Sf9; and the fungal cell Saccharomyces cerevisiae.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be

performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)).

"Transgenic organism", as known in the art and as used herein, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

Methods of producing antibodies of the invention are described below. A distinction is made here between in-vivo approaches, in-vitro approaches or a combination of both.

In-vivo approaches:

Depending on the type of the desired antibody, various host animals may be used for in-vivo immunization. A host expressing itself an endogenous version of the antigen of interest may be used. Alternatively, it is possible to use a host which has been made deficient in an endogenous version of the antigen of interest. For example, mice which had been made deficient in a particular endogenous protein via homologous recombination at the corresponding endogenous gene (i.e., knockout mice) have been shown to generate a humoral response to the protein with which they have been immunized and therefore to be able to be used for production of high-

affinity monoclonal antibodies to the protein (see, for example, Roes, J. et al. (1995) *J. Immunol. Methods* 183:231-237; Lunn, M.P. et al. (2000) *J. Neurochem.* 75:404-412).

A multiplicity of nonhuman mammals are suitable hosts for antibody production in order to produce nonhuman antibodies of the invention. They include, for example, mice, rats, chickens, camelids, rabbits, sheep and goats (and knockout versions thereof), although preference is given to mice for the production of hybridoma. Furthermore, a nonhuman host animal expressing a human antibody repertoire may be used for producing essentially human antibodies to a human antigen with dual specificity. Nonhuman animals of this kind include transgenic animals (e.g., mice) bearing human immunoglobulin transgenes (chimeric hu-PBMC SCID mice) and human/mouse irradiation chimeras which are described in more detail below.

According to one embodiment, the animal immunized is a nonhuman mammal, preferably a mouse, which is transgenic due to human immunoglobulin genes so that said nonhuman mammal makes human antibodies upon antigenic stimulation. Typically, immunoglobulin transgenes for heavy and light chains with human germ line configuration are introduced into such animals which have been altered such that their endogenous heavy and light chain loci are inactive. If such animals are stimulated with antigen (e.g., with a human antigen), antibodies derived from the human immunoglobulin sequences (human antibodies) are produced. It is possible to make from the lymphocytes of such animals human monoclonal antibodies by means of standardized hybridoma technology. For a further description of transgenic mice with human immunoglobulins and their use in the production of human antibodies, see, for example, U.S. Patent No. 5,939,598, WO 96/33735, WO 96/34096, WO 98/24893 and WO 99/53049 (Abgenix Inc.), and U.S. Patent No. 5,545,806, U.S. Patent No. 5,569,825, U.S. Patent No. 5,625,126, U.S. Patent No. 5,633,425, U.S. Patent No. 5,661,016, U.S. Patent No.

5,770,429, U.S. Patent No. 5,814,318, U.S. Patent No. 5,877,397 and WO 99/45962 (Genpharm Inc.); see also MacQuitty, J.J. and Kay, R.M. (1992) *Science* 257:1188; Taylor, L.D. *et al.* (1992) *Nucleic Acids Res.* 20:6287-6295; Lonberg, N. *et al.* (1994) *Nature* 368:856-859; Lonberg, N. and Huszar, D. (1995) *Int. Rev. Immunol.* 13:65-93; Harding, F.A. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546; Fishwild, D. M. *et al.* (1996) *Nature Biotechnology* 14:845-851; Mendez, M. J. *et al.* (1997) *Nature Genetics* 15:146-156; Green, L.L. and Jakobovits, A. (1998) *J. Exp. Med.* 188:483-495; Green, L.L. (1999) *J. Immunol. Methods* 231:11-23; Yang, X.D. *et al.* (1999) *J. Leukoc. Biol.* 66:401-410; Gallo, M.L. *et al.* (2000) *Eur. J. Immunol.* 30:534-540.

According to another embodiment, the animal which is immunized may be a mouse with severe combined immunodeficiency (SCID), which has been reconstituted with human peripheral mononuclear blood cells or lymphoid cells or precursors thereof. Such mice which are referred to as chimeric hu-PBMC SCID mice produce human immunoglobulin responses upon antigenic stimulation, as has been proved. For a further description of these mice and of their use for generating antibodies, see, for example, Leader, K.A. *et al.* (1992) *Immunology* 76:229-234; Bombil, F. *et al.* (1996) *Immunobiol.* 195:360-375; Murphy, W.J. *et al.* (1996) *Semin. Immunol.* 8:233-241; Herz, U. *et al.* (1997) *Int. Arch. Allergy Immunol.* 113:150-152; Albert, S.E. *et al.* (1997) *J. Immunol.* 159:1393-1403; Nguyen, H. *et al.* (1997) *Microbiol. Immunol.* 41:901-907; Arai, K. *et al.* (1998) *J. Immunol. Methods* 217:79-85; Yoshinari, K. and Arai, K. (1998) *Hybridoma* 17:41-45; Hutchins, W.A. *et al.* (1999) *Hybridoma* 18:121-129; Murphy, W.J. *et al.* (1999) *Clin. Immunol.* 90:22-27; Smithson, S.L. *et al.* (1999) *Mol. Immunol.* 36:113-124; Chamat, S. *et al.* (1999) *J. Infect. Diseases* 180:268-277; and Heard, C. *et al.* (1999) *Molec. Med.* 5:35-45.

According to another embodiment, the animal which is immunized is a mouse which has been treated with a lethal does of total body irradiation, then protected from radiation with bone marrow cells from mice with severe combined immunodeficiency (SCID) and subsequently transplanted with functional human lymphocytes. This type of chimera, referred to as the Trimera system, is used in order to produce human monoclonal antibodies by immunizing said mice with the antigen of interest and then producing monoclonal antibodies by using standardized hybridoma technology. For a further description of these mice and of their use for generating antibodies, see, for example, Eren, R. *et al.* (1998) *Immunology* 93:154-161; Reisner, Y and Dagan, S. (1998) *Trends Biotechnol.* 16:242-246; Ilan, E. *et al.* (1999) *Hepatology* 29:553-562; and Bocher, W.O. *et al.* (1999) *Immunology* 96:634-641.

Starting from the in-vivo generated antibody-producing cells, monoclonal antibodies may be produced by means of standardized techniques such as the hybridoma technique originally described by Kohler and Milstein (1975, *Nature* 256:495-497) (see also Brown *et al.* (1981) *J. Immunol* 127:539-46; Brown *et al.* (1980) *J Biol Chem* 255:4980-83; Yeh *et al.* (1976) *PNAS* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75). The technology of producing monoclonal antibody hybridomas is sufficiently known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.*, 3:231-36). Briefly, an immortalized cell line (typically a myeloma) is fused with lymphocytes (typically splenocytes or lymph node cells or peripheral blood lymphocytes) of a mammal immunized with the Aβ globulomer of the invention or derivative thereof, and the culture supernatants of the resulting hybridoma cells are screened in order to identify a hybridoma which produces a

monoclonal antibody of the present invention. Any of the many well known protocols for fusing lymphocytes and immortalized cell lines can be applied for this purpose (see also G. Galfre *et al.* (1977) *Nature* 266:550-52; Gefter *et al. Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the skilled worker will appreciate that there are diverse variations of such methods, which are likewise useful. Typically, the immortalized cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas may be established by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the invention with an immortalized mouse cell line. Preferred immortalized cell lines are mouse myeloma cell lines which are sensitive to culture medium containing hypoxanthine, aminopterine and thymidine (HAT medium). Any of a number of myeloma cell lines may be used by default as fusion partner, for example the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma cell lines are available from the American Type Culture Collection (ATCC), Manassas, Virginia. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol (PEG). Hybridoma cells resulting from the fusion are then selected using HAT medium, thereby killing unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing monoclonal antibodies of the invention are identified by screening the hybridoma culture supernatants for such antibodies, for example, by using a dot blot assay in order to select those antibodies which have the binding affinities as defined above. The monoclonal antibodies 10F4 and 3C5 all have been generated using the above-described in-vivo approach and thereof are obtainable from a hybridoma as defined herein.

Likewise, said hybridoma can be used as a source of nucleic acid encoding light and/or heavy chains in order to recombinantly produce antibodies of the present invention, as is described below in further detail.

In-vitro approaches:

As an alternative to producing antibodies of the invention by immunization and selection, antibodies of the invention may be identified and isolated by screening recombinant combinatorial immunoglobulin libraries to thereby isolate immunoglobulin library members which have the required binding affinity. Kits for generating and screening display libraries are commercially available (e.g. the Pharmacia Recombinant Phage Antibody System, catalog No. 27-9400-01; and the Stratagene SurfZAP® Phage Display Kit, catalog No. 240612). In many embodiments, the display library is an scFv library or an Fab library. The phage display technique for screening recombinant antibody libraries has been adequately described. Examples of methods and compounds which can be used particularly advantageously for generating and screening antibody display libraries can be found, for example, in McCafferty *et al*. WO 92/01047, US 5,969,108 and EP 589 877 (describes in particular scFv display), Ladner *et al*. U.S. Patent No. 5,223,409, U.S. Patent No. 5,403,484, U.S. Patent No. 5,571,698, U.S. Patent No. 5,837,500 and EP 436 597 (describes pIII fusion, for example); Dower *et al*. WO 91/17271, U.S. Patent No. 5,427,908, U.S. Patent No. 5,580,717 and EP 527 839 (describes in particular Fab display); Winter *et al*. International Publication WO 92/20791 and EP 368,684 (describes in particular the cloning of sequences for variable immunoglobulin domains); Griffiths *et al*., U.S. Patent No. 5,885,793 and EP 589 877 (describes in particular isolation of human antibodies to human antigens by using recombinant libraries); Garrard *et al*. WO 92/09690 (describes in

particular phage expression techniques); Knappik *et al.* WO 97/08320 (describes the human recombinant antibody library HuCal); Salfeld *et al.* WO 97/29131, (describes production of a recombinant human antibody to a human antigen (human tumor necrosis factor alpha) and also in-vitro affinity maturation of the recombinant antibody) and Salfeld *et al.*, U.S. Provisional Patent Application No. 60/126,603 and the patent applications based hereupon (likewise describes production of recombinant human antibodies to human antigen (human interleukin-12), and also *in-vitro* affinity maturation of the recombinant antibody).

Further descriptions of screenings of recombinant antibody libraries can be found in scientific publications such as Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; Barbas *et al.* (1991) *PNAS* 88:7978-7982; McCafferty *et al.* *Nature* (1990) 348:552-554; and Knappik *et al.* (2000) *J. Mol. Biol.* 296:57-86.

As an alternative to using bacteriophage display systems, recombinant antibody libraries may be expressed on the surface of yeast cells or of bacterial cells. WO 99/36569 describes methods of preparing and screening libraries expressed on the surface of yeast cells. WO 98/49286 describes in more detail methods of preparing and screening libraries expressed on the surface of bacterial cells. In all *in vitro* approaches, a selection process for enriching recombinant antibodies with the desired properties form an integral part of the process, which is generally referred to as "panning" and often takes the form of affinity chromatography over columns to whose

matrix the target structure has been attached. Promising candidate molecules are then subjected to individual determination of their absolute and/or relative affinities, preferably by means of a standardized dot blot assay.

Once an antibody of interest of a combinatorial library has been identified and sufficiently characterized, the DNA sequences encoding the light and heavy chains of said antibody are isolated by means of standardized molecular-biological techniques, for example, by means of PCR amplification of DNA from the display package (e.g., the phage) which has been isolated during library screening. Nucleotide sequences of genes for light and heavy antibody chains, which may be used for preparing PCR primers, are known to one of ordinary skill in the art. A multiplicity of such sequences are described, for example, in Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 and in the database of sequences of the human germ line VBASE.

An antibody or antibody moiety of the invention may be produced by recombinantly expressing the genes for light and heavy immunoglobulin chains in a host cell. In order to recombinantly express an antibody, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the light and heavy immunoglobulin chains of said antibody, thereby expressing the light and heavy chains in the host cell and secreting them preferably into the medium in which said host cells are cultured. The antibodies can be isolated from this medium. Standardized recombinant DNA methods are used in order to obtain genes for heavy and light antibody chains, to insert said genes into recombinant expression vectors and to introduce said vectors into host cells. Methods of this kind are described, for example, in Sambrook, Fritsch and Maniatis (eds.), *Molecular Cloning; A*

Laboratory Manual, *Second Edition*, Cold Spring Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in US 4,816,397 by Boss *et al.*.

Once DNA fragments encoding VH and VL segments of the antibody of interest have been obtained, said DNA fragments may be further manipulated using standardized recombinant DNA techniques, for example, in order to convert the genes for variable regions to genes for full length antibody chains, to genes for Fab fragments or to an scFv gene. These manipulations comprise linking a VL- or VH-encoding DNA fragment operatively to another DNA fragment encoding another protein, for example a constant antibody region or a flexible linker. The term "operatively linked" is to be understood here as meaning that the two DNA fragments are linked in such a way that the amino acid sequences encoded by said two DNA fragments remain in frame. The isolated DNA encoding the VH region may be converted to a gene for a full length heavy chain by operatively linking the VH-region encoding DNA with another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are well known (see, for example, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), and DNA fragments spanning said regions may be obtained by means of standardized PCR amplification. The heavy chain constant region may be a constant region from IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgE or IgD, with preference being given to a constant region from IgG, in particular IgG1 or IgG4. To obtain a gene for a heavy chain Fab fragment, the VH-encoding DNA may be operatively linked to another DNA molecule encoding merely the heavy chain constant region CH1. The isolated DNA encoding the VL region may be converted to a gene for a full length light chain (and

a gene for an Fab light chain) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region CL. The sequences of genes of the constant region of human light chain are well known (see Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), and DNA fragments spanning said regions may be obtained by means of standardized PCR amplification. The light chain constant region may be a constant kappa or lambda region, a constant kappa region being preferred.

In order to generate an scFv gene, the VH- and VL-encoding DNA fragments may be operatively linked to another fragment encoding a flexible linker, for example the amino acid sequence $(Gly_4\text{-}Ser)_3$ so that the VH and VL sequences are expressed as a continuous single-chain protein, with the VL and VH regions being linked to one another via said flexible linker (see Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

Single domain VH and VL having the binding affinities as described above may be isolated from single domain libraries by the above-described methods. Two VH single-domain chains (with or without CH1) or two VL chains or a pair of one VH chain and one VL chain with the desired binding affinity may be useful as described herein for the antibodies of the invention.

In order to express the recombinant antibodies or antibody moieties of the invention, the DNAs encoding partial or full length light and heavy chains may be inserted into expression vectors so as to operatively link the genes to appropriate transcriptional and translational control sequences. In this context, the term "operatively linked" is to be understood to mean that an antibody gene is ligated in a

vector in such a way that transcriptional and translational control sequences within the vector fulfill their intended function of regulating transcription and translation of said antibody gene. Expediently, the expression vector and the expression control sequences are chosen so as to be compatible with the expression host cell used. The gene for the antibody light chain and the gene for the antibody heavy chain may be inserted into separate vectors or both genes are inserted into the same expression vector, this being the usual case. The antibody genes are inserted into the expression vector by means of standardized methods (for example by ligation of complementary restriction cleavage sites on the antibody gene fragment and the vector, or by ligation of blunt ends, if no restriction cleavage sites are present). The expression vector may already carry sequences for antibody constant regions prior to insertion of the sequences for the light and heavy chains. For example, one approach is to convert the VH and VL sequences to full length antibody genes by inserting them into expression vectors already encoding the heavy and, respectively, light chain constant regions, thereby operatively linking the VH segment to the CH segment(s) within the vector and also operatively linking the VL segment to the CL segment within the vector.

Additionally or alternatively, the recombinant expression vector may encode a signal peptide which facilitates secretion of the antibody chain from the host cell. The gene for said antibody chain may be cloned into the vector, thereby linking the signal peptide in frame to the N terminus of the gene for the antibody chain. The signal peptide may be an immuno-globulin signal peptide or a heterologous signal peptide (i.e. a signal peptide from a non-immunoglobulin protein). In addition to the genes for the antibody chain, the expression vectors of the invention may have regulatory sequences

controlling expression of the genes for the antibody chain in a host cell.

The term "regulatory sequence" is intended to include promoters, enhancers and further expression control elements (e.g. polyadenylation signals) which control transcription or translation of the genes for the antibody chain. Regulatory sequences of this kind are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). The skilled worker will appreciate that the expression vector design which includes selection of regulatory sequences may depend on factors such as the choice of the host cell to be transformed, the desired strength of expression of the protein, etc. Preferred regulatory sequences for expression in mammalian host cells include viral elements resulting in strong and constitutive protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), simian virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. For a further description of viral regulatory elements and sequences thereof, see, for example, U.S. Patent No. 5,168,062 to Stinski, U.S. Patent No. 4,510,245 to Bell *et al.* and U.S. Patent No. 4,968,615 to Schaffner *et al.*

Apart from the genes for the antibody chain and the regulatory sequences, the recombinant expression vectors of the invention may have additional sequences such as those which regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker genes facilitate the selection of host cells into which the vector has been introduced (see, for example, U.S. Patent Nos 4,399,216, 4,634,665 and 5,179,017, all to Axel *et al.*). For example, it is common for the selectable marker gene to render a host cell into which the vector has

been inserted resistant to cytotoxic drugs such as G418, hygromycin or methotrexate. Preferred selectable marker genes include the gene for dihydrofolate reductase (DHFR)(for use in dhfr$^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding said heavy and light chains is(are) transfected into a host cell by means of standardized techniques. The various forms of the term "transfection" are intended to comprise a multiplicity of techniques customarily used for introducing exogenous DNA into a prokaryotic or eukaryotic host cell, for example electroporation, calcium phosphate precipitation, DEAE-dextran transfection, and the like. Although it is theoretically possible to express the antibodies of the invention either in prokaryotic or eukaryotic host cells, preference is given to expressing the antibodies in eukaryotic cells and, in particular, in mammalian host cells, since the probability of a correctly folded and immunologically active antibody being assembled and secreted is higher in such eukaryotic cells and in particular mammalian cells than in prokaryotic cells. Prokaryotic expression of antibody genes has been reported as being ineffective for production of high yields of active antibody (Boss, M.A. and Wood, C.R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing recombinant antibodies of the invention include CHO cells (including dhfr$^-$ CHO cells described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, which are used together with a DHFR-selectable marker, as described, for example, in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When introducing recombinant expression vectors encoding the antibody genes into mammalian host cells, the antibodies are produced by culturing the host cells until the antibody is expressed in

said host cells or, preferably, the antibody is secreted into the culture medium in which the host cells grow. The antibodies may then be isolated from the culture medium by using standardized protein purification methods. It is likewise possible to use host cells in order to produce moieties of intact antibodies, such as Fab fragments or scFv molecules. Variations of the above-described procedure are of course included in the invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of the invention. If either light or heavy chains are present which are not required for binding of the antigen of interest, then the DNA encoding either such a light or such a heavy chain or both may be removed partially or completely by means of recombinant DNA technology. Molecules expressed by such truncated DNA molecules are likewise included in the antibodies of the invention. In addition, it is possible to produce bifunctional antibodies in which a heavy chain and a light chain are an antibody of the invention and the other heavy chain and the other light chain have specificity for an antigen different from the antigen of interest, by crosslinking an antibody of the invention to a second antibody by means of standardized chemical methods.

In a preferred system for recombinant expression of an antibody of the invention or an antigen-binding moiety thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr$^-$ CHO cells by means of calcium phosphate-mediated transfection. Within the recombinant expression vector, the genes for the heavy and light antibody chains are in each case operatively linked to regulatory CMV enhancer/AdMLP-promoter elements in order to effect strong transcription of said genes. The recombinant expression vector also carries a DHFR gene which can be used for

selecting dhfr$^-$ CHO cells transfected with the vector by using methotrexate selection/amplification. The selected transformed host cells are cultured so that the heavy and light antibody chains are expressed, and intact antibody is isolated from the culture medium. Standardized molecular-biological techniques are used in order to prepare the recombinant expression vector, to transfect the host cells, to select the transformants, to culture said host cells, and to obtain the antibody from the culture medium. Thus, the invention relates to a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention has been synthesized. The method may further comprise isolating said recombinant antibody from said culture medium.

As an alternative to screening recombinant antibody libraries by phage display, other methods known to the skilled worker may be used for screening large combinatorial libraries to identify the antibodies of the invention. Basically, any expression system in which a close physical linkage between a nucleic acid and the antibody encoded thereby is established and may be used to select a suitable nucleic acid sequence by virtue of the properties of the antibody it encodes may be employed. In one type of an alternative expression system, the recombinant antibody library is expressed in the form of RNA-protein fusions, as described in WO 98/31700 to Szostak and Roberts, and in Roberts, R.W. and Szostak, J.W. (1997) *Proc. Natl. Acad. Sci. USA* 94:12297-12302. In this system, in-vitro translation of synthetic mRNAs carrying on their 3' end puromycin, a peptidyl acceptor antibiotic, generates a covalent fusion of an mRNA and the peptide or protein encoded by it. Thus, a specific mRNA of a complex mixture of mRNAs (e.g. a combinatorial library) may be concentrated on the basis of the properties of the encoded peptide or protein

(e.g. of the antibody or a moiety thereof), such as binding of said antibody or said moiety thereof to Aβ(12-42) globulomer or a derivative thereof. Nucleic acid sequences which encode antibodies or moieties thereof and which are obtained by screening of such libraries may be expressed by recombinant means in the above-described manner (e.g. in mammalian host cells) and may, in addition, be subjected to further affinity maturation by either screening in further rounds mRNA-peptide fusions, introducing mutations into the originally selected sequence(s), or using other methods of *in-vitro* affinity maturation of recombinant antibodies in the above-described manner.

Combinations of in-vivo and in-vitro approaches

The antibodies of the invention may likewise be produced by using a combination of in-vivo and in-vitro approaches such as methods in which Aβ(12-42) globulomer or a derivative thereof is first allowed to act on an antibody repertoire in a host animal in vivo to stimulate production of Aβ(12-42) globulomer or derivative-binding antibodies and then further antibody selection and/or antibody maturation (i.e., optimization) are accomplished with the aid of one or more in-vitro techniques. According to one embodiment, a combined method of this kind may comprise firstly immunizing a nonhuman animal (e.g., a mouse, rat, rabbit, chicken, camelid, sheep or goat or a transgenic version thereof or a chimeric mouse) with said Aβ (12-42) globulomer or derivative thereof to stimulate an antibody response to the antigen and then preparing and screening a phage display antibody library by using immunoglobulin sequences of lymphocytes which have been stimulated in vivo by the action of said Aβ (12-42) globulomer or derivative. The first step of this combined procedure may be carried out in the manner described above in connection with the in-vivo approaches, while the second step of this

procedure may be carried out in the manner described above in connection with the in-vitro approaches. Preferred methods of hyperimmunizing nonhuman animals with subsequent in-vitro screening of phage display libraries prepared from said stimulated lymphocytes include those described by BioSite Inc., see, for example, WO 98/47343, WO 91/17271, US 5,427,908 and U.S. Patent No. 5,580,717.

According to another embodiment, a combined method comprises firstly immunizing a nonhuman animal (e.g., a mouse, rat, rabbit, chicken, camelid, sheep, goat or a knockout and/or transgenic version thereof, or a chimeric mouse) with an Aβ (12-42) globulomer of the invention or derivative thereof to stimulate an antibody response to said Aβ (12-42) globulomer or derivative thereof and selecting the lymphocytes which produce the antibodies having the desired specificity by screening hybridomas (prepared, for example, from the immunized animals). The genes for the antibodies or single domain antibodies are isolated from the selected clones (by means of standardized cloning methods such as reverse transcriptase polymerase chain reaction) and subjected to in-vitro affinity maturation in order to improve thereby the binding properties of the selected antibody or the selected antibodies. The first step of this procedure may be conducted in the manner described above in connection with the in-vivo approaches, while the second step of this procedure may be conducted in the manner described above in connection with the in-vitro approaches, in particular by using methods of in-vitro affinity maturation, such as those described in WO 97/29131 and WO 00/56772.

In a further combined method, the recombinant antibodies are generated from individual isolated lymphocytes by using a procedure which is known to the skilled worker as selected lymphocyte antibody methods (SLAM) and which is described in US 5,627,052, WO 92/02551 and Babcock, J.S. *et al.* (1996)

Proc. Natl. Acad. Sci. USA 93:7843-7848. In this method, a nonhuman animal (e.g., a mouse, rat, rabbit, chicken, camelid, sheep, goat, or a transgenic version thereof, or a chimeric mouse) is firstly immunized in vivo with Aβ (12-42) globulomer or a derivative thereof to stimulate an immune response to said oligomer or derivative, and then individual cells secreting antibodies of interest are selected by using an antigen-specific haemolytic plaque assay. To this end, the globulomer or derivative thereof or structurally related molecules of interest may be coupled to sheep erythrocytes, using a linker such as biotin, thereby making it possible to identify individual cells secreting antibodies with suitable specificity by using the haemolytic plaque assay. Following the identification of cells secreting antibodies of interest, cDNAs for the variable regions of the light and heavy chains are obtained from the cells by reverse transcriptase PCR, and said variable regions may then be expressed in association with suitable immunoglobulin constant regions (e.g., human constant regions) in mammalian host cells such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences derived from in vivo-selected lymphocytes may then be subjected to further in-vitro analysis and in-vitro selection by spreading out the transfected cells, for example, in order to isolate cells expressing antibodies with the binding affinity. The amplified immunoglobulin sequences may furthermore be manipulated *in vitro*.

Antibodies having the required affinities defined herein can be selected by performing a dot blot essentially as described above. Briefly, the antigen is attached to a solid matrix, preferably dotted onto a nitrocellulose membrane, in serial dilutions. The immobilized antigen is then contacted with the antibody of interest followed by detection of the latter by means of an enzyme-conjugated secondary antibody and a colorimetric reaction; at defined antibody and antigen

concentrations, the amount of antibody bound allows affinity determination. Thus the relative affinity of two different antibodies to one target, or of one antibody to two different targets, is here defined as the relation of the respective amounts of target-bound antibody observed with the two antibody-target combinations under otherwise identical dot blot conditions. Antibodies which bind to the same epitope as monoclonal antibody 10F4 or 3C5 can be obtained in a manner known per se.

In the same way as antibodies may be competing, described above, different target structures are herein said to be "competing" for a particular antibody if at least one of these structures is capable of specifically reducing the measurable binding of another, preferably by offering an overlapping or identical epitope, more preferably an identical epitope. Competing target entities are useful for directly selecting antibodies by virtue of their relative affinity to such target structures. Relative affinities may thus be determined directly by using a competition assay in which distinguishable forms of the competing entities, e.g., differently labelled competing structures, are contacted with the antibody of interest, and the relative affinity of the antibody to each of these entities is deduced from the relative amounts of these entities which are bound by the antibody. Such competition may be used to directly enrich for antibodies possessing a desired relative affinity to the target entity, by attaching the entity towards which greater affinity is desired to a solid matrix support and adding a suitable amount, preferably a molar excess, of the competing entity towards which smaller affinity is desired to the medium. Thus, the antibodies displaying the desired relative affinities will tend to bind to the matrix more strongly than others and may be obtained after washing out the less desirable forms, e.g., by washing out at low salt concentrations and then harvesting the bound

antibody by reversibly detaching it from its target by using high salt concentrations. If desired, several rounds of enrichment may be performed. In a particular embodiment of the invention, where the genotype underlying an antibody is physically linked to this antibody, e.g., in a pool of hybridomas or antigen-displaying phages or yeast cells, the corresponding phenotype may be rescued.

In another embodiment of the invention, a modified dot blot is used where the immobilized antigen competes with a solved entity for antibody binding, so that the relative affinity of the antibody can be deduced from the percentage bound to the immobilized antigen. Antibody moieties such as Fab and $F(ab')_2$ fragments may be produced from whole antibodies by using conventional techniques such as digestion with papain or pepsin. In addition, antibodies, antibody moieties and immunoadhesion molecules may be obtained by using standardized recombinant DNA techniques.

The present invention also relates to pharmaceutical agents (compositions) comprising an antibody of the invention and, optionally, a pharmaceutically suitable carrier. Pharmaceutical compositions of the invention may furthermore contain at least one additional therapeutic agent, for example one or more additional therapeutic agents for the treatment of a disease for whose relief the antibodies of the invention are useful. If, for example, the antibody of the invention binds to a globulomer of the invention, the pharmaceutical composition may furthermore contain one or more additional therapeutic agents useful for the treatment of disorders in which the activity of said globulomer is important. Pharmaceutically suitable carriers include any solvents, dispersing media, coatings, antibacterial and antifungal agents, isotonic and absorption-delaying agents, and the like, as long as they are physiologically compatible. Pharmaceutically acceptable carriers include, for example,

water, saline, phosphate-buffered saline, dextrose, glycerol, ethanol and the like, and combinations thereof. In many cases, preference is given to using isotonic agents, for example sugars, polyalcohols such as mannitol or sorbitol, or sodium chloride in addition. Pharmaceutically suitable carriers may furthermore contain relatively small amounts of auxiliary substances such as wetting agents or emulsifiers, preservatives or buffers, which increase the half life or efficacy of the antibodies. The pharmaceutical compositions may be suitable for parenteral administration, for example. Here, the antibodies are prepared preferably as injectable solutions with an antibody content of 0.1 - 250 mg/mL. The injectable solutions may be prepared in liquid or lyophilized form, the dosage form being a flint glass or vial, an ampoule or a filled syringe. The buffer may contain L-histidine (1 - 50 mM, preferably 5-10 mM) and have a pH of 5.0-7.0, preferably of 6.0. Further suitable buffers include, without being limited thereto, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate buffers. Sodium chloride may be used in order to adjust the tonicity of the solution to a concentration of 0-300 mM (preferably 150 mM for a liquid dosage form). Cryoprotectants, for example sucrose (e.g., 0-10%, preferably 0.5-1.0%) may also be included for a lyophilized dosage form. Other suitable cryoprotectants are trehalose and lactose. Fillers, for example mannitol (e.g., 1-10%, preferably 2-4%) may also be included for a lyophilized dosage form. Stabilizers, for example L-methionine (e.g., 51 - 50 mM, preferably 5-10 mM) may be used both in liquid and lyophilized dosage forms. Further suitable fillers are glycine and arginine. Surfactants, for example, polysorbate 80 (e.g., 0 - 0.05%, preferably 0.005 - 0.01%), may also be used. Further surfactants are polysorbate 20 and BRIJ surfactants.

The compositions of the invention may have a multiplicity of forms. These include liquid, semisolid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended type of administration and on the therapeutic application. Typically, preference is given to compositions in the form of injectable or infusible solutions, for example compositions which are similar to other antibodies for passive immunization of humans. The preferred route of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal or intramuscular). According to a preferred embodiment, the antibody is administered by intravenous infusion or injection. According to another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection. Therapeutic compositions must typically be sterile and stable under preparation and storage conditions. The compositions may be formulated as solutions, microemulsions, dispersions, liposomes or other ordered structures suitable for high concentrations of active substance. Sterile injectable solutions may be prepared by introducing the active compound (i.e., the antibody) in the required amount into a suitable solvent, where appropriate with one or a combination of the abovementioned ingredients, as required, and then sterile-filtering said solution. Dispersions are usually prepared by introducing the active compound into a sterile vehicle containing a basic dispersion medium and, where appropriate, other required ingredients. In the case of a sterile lyophilized powder for preparing sterile injectable solutions, vacuum drying and spray drying are preferred methods of preparation, which produces a powder of the active ingredient and, where appropriate, of further desired ingredients from a previously sterile-filtered solution. The correct flowability of a solution may be

maintained by using, for example, a coating such as lecithin, by maintaining, in the case of dispersions the required particle size or by using surfactants. A prolonged absorption of injectable compositions may be achieved by additionally introducing into the composition an agent which delays absorption, for example monostearate salts and gelatine.

The antibodies of the invention may be administered by a multiplicity of methods known to the skilled worker, although the preferred type of administration for many therapeutic applications is subcutaneous injection, intravenous injection or infusion. The skilled worker will appreciate that the route and/or type of administration depend on the result desired. According to particular embodiments, the active compound may be prepared with a carrier which protects the compound against rapid release, such as, for example, a formulation with sustained or controlled release, which includes implants, transdermal plasters and microencapsulated release systems. Biologically degradable biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid may be used. The methods of preparing such formulations are well known to the skilled worker; see, for example, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

According to particular embodiments, an antibody of the invention may be administered orally, for example, in an inert diluent or a metabolizable edible carrier. The antibody (and further ingredients, if desired) may also be enclosed in a hard or soft gelatine capsule, compressed to tablets or added directly to food. For oral therapeutic administration, the antibodies may be mixed with excipients and used in the form of oral tablets, buccal tablets, capsules, elixirs, suspensions, syrups and the like. If it is intended to administer an antibody of the invention via a route other than

the parenteral one, it may be necessary to choose a coating from a material which prevents its inactivation.

The present invention also relates to a method of inhibiting the activity of globulomers of the invention in an individual which suffers from a disorder in which the amyloid β protein is involved and in which in particular the activity of said globulomers of the invention is important. Said method comprises the administration of at least one antibody of the invention to the individual with the aim of inhibiting the activity of the globulomer to which the antibody binds. Said individual is preferably a human being. An antibody of the invention may be administered for therapeutic purposes to a human individual. In addition, an antibody of the invention may be administered to a nonhuman mammal for veterinary purposes or within the framework of an animal model for a particular disorder. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (for example for testing dosages and time courses of administration).

Disorders in which the globulomers of the invention play a part include, in particular, disorders in whose development and/or progression a globulomer of the invention is involved. These are in particular those disorders in which globulomers of the invention are evidently or presumably responsible for the pathophysiology of said disorder or are a factor which contributes to the development and/or progression of said disorder. Accordingly, those disorders are included here in which inhibition of the activity of globulomers of the invention can relieve symptoms and/or progression of the disorder. Such disorders can be verified, for example, by an increased concentration of globulomers of the invention in a biological fluid of an individual suffering from a particular disorder (e.g., increased concentration in serum, plasma, CSF, urine, etc.). This may be detected, for example, by using an

antibody of the invention. The globulomers of the invention play an important part in the pathology associated with a multiplicity of disorders in which neurodegenerative elements, cognitive deficiencies, neurotoxic elements and inflammatory elements are involved.

In another aspect of the invention, disorders that can be treated or prevented include those associated with amyloidoses. The term "amyloidoses" herein denotes a number of disorders characterized by abnormal folding, clumping, aggregation and/or accumulation of particular proteins (amyloids, fibrous proteins and their precursors) in various tissues of the body. In Alzheimer's disease and Down's syndrome, nerve tissue is affected, and in cerebral amyloid angiopathy (CAA) blood vessels are affected.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody moiety of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody moiety may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody moiety to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically

effective amount will be less than the therapeutically effective amount.

Moreover, the present invention includes a further method of preventing or treating Alzheimer's disease in a patient in need of such prevention or treatment. This method comprises the step of administering the vaccine noted above to the patient in an amount sufficient to effect the prevention or treatment.

Further, the present invention encompasses a method of identifying compounds suitable for active immunization of a patient predicted to develop an amyloidosis, e.g. Alzheimer's disease. This method comprises: 1) exposing one or more compounds of interest to one or more of the antibodies described above for a time and under conditions sufficient for the one or more compounds to bind to the antibody or antibodies; 2) identifying those compounds which bind to the antibody or antibodies, the identified compounds to be used in active immunization in a patient predicated to develop an amyloidosis, e.g., Alzheimer's disease.

Within the framework of diagnostic usage of the antibodies, qualitative or quantitative specific globulomer determination serves in particular to diagnose disease-relevant amyloid β forms. In this context, specificity means the possibility of being able to detect a particular globulomer or a derivative thereof, or a mixture thereof with sufficient sensitivity. The antibodies of the invention advantageously have detection threshold concentrations of less than 10 ng/mL of sample, preferably of less than 1 ng/mL of sample and particularly preferably of less than 100 pg/mL of sample, meaning that at least the concentration of globulomer per mL of sample, indicated in each case, advantageously also lower concentrations, can be detected by the antibodies of the invention. The detection is carried out immunologically. This may be carried out, in principle, by using any analytical

or diagnostic assay method in which antibodies are used, including agglutination and precipitation techniques, immunoassays, immunohistochemical methods and immunoblot techniques, for example Western blotting or, preferably, dot blot methods. *In vivo* methods, for example imaging methods, are also included here.

The use in immunoassays is advantageous. Competitive immunoassays, i.e., assays where antigen and labelled antigen (tracer) compete for antibody binding, and sandwich immunoassays, i.e., assays where binding of specific antibodies to the antigen is detected by a second, usually labelled antibody, are both suitable. These assays may be either homogeneous, i.e., without separation into solid and liquid phases, or heterogeneous, i.e., bound labels are separated from unbound ones, for example, via solid phase-bound antibodies. Depending on labelling and method of measurement, the various heterogeneous and homogeneous immunoassay formats can be classified into particular classes, for example RIAs (radioimmunoassays), ELISA (enzyme-linked immunosorbent assay), FIA (fluorescence immunoassay), LIA (luminescence immunoassay), TRFIA (time-resolved FIA), IMAC (immunoactivation), EMIT (enzyme-multiplied immune test), TIA (turbidometric immunoassay), I-PCR (immuno-PCR).

For the globulomer quantification of the invention, preference is given to competitive immunoassays in which a defined amount of labelled globulomer derivative serving as tracer competes with the globulomer of the sample (containing an unknown amount of unlabelled globulomers) to be quantified for binding to the antibody used. The amount of antigen, i.e., the amount of globulomer, in the sample can be determined from the amount of the displaced tracer with the aid of a standard curve.

Of the labels available for these purposes, enzymes have proved advantageous. Systems based on peroxidases, in

particular, horseradish peroxidase, alkaline phosphatase and β-D-galactosidase, may be used, for example. Specific substrates whose conversion can be monitored photometrically, for example, are available for these enzymes. Suitable substrate systems are based on p-nitrophenyl phosphate (p-NPP), 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NPT), Fast-Red/naphthol-AS-TS phosphate for alkaline phosphatase; 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPT), 3,3‘,5,5‘-tetramethylbenzidine (TMB), o-dianisidine, 5-aminosalicylic acid, 3-dimethylaminobenzoic acid (DMAB) and 3-methyl-2-benzothiazolinehydrazone (MBTH) for peroxidases; o-nitrophenyl-β-D-galactoside (o-NPG), p-nitrophenyl-β-D-galactoside and 4-methylumbelliphenyl-β-D-galactoside (MUG) for β-D-galactosidase. In many cases, these substrate systems are commercially available in a ready-to-use form, for example in the form of tablets which may also contain further reagents such as appropriate buffers and the like. The tracers used may be labelled globulomers. In this sense, a particular globulomer can be determined by labelling the globulomer to be determined and using it as tracer. The coupling of labels to globulomers for preparing tracers may be carried out in a manner known per se. The comments above on derivatization of globulomers of the invention are referred to by analogy. In addition, a number of labels appropriately modified for conjugation to proteins are available, for example biotin-, avidin-, extravidin- or streptavidin-conjugated enzymes, maleimide-activated enzymes and the like. These labels may be reacted directly with the oligomer or, if required, with the appropriately derivatized globulomer to give the tracer. If, for example, a streptavidin-peroxidase conjugate is used, then this firstly requires biotinylation of the globulomer. This applies correspondingly to the reverse order. Suitable methods to this end are also known to the skilled worker.

If a heterogeneous immunoassay format is chosen, the antigen-antibody complex may be separated by binding it to the support, for example via an anti-idiotypical antibody coupled to said support, e.g. an antibody directed against rabbit IgG. Appropriate supports, in particular microtiter plates coated with appropriate antibodies, are known and partly commercially available.

The present invention further relates to immunoassay sets having at least one antibody as described above and further components. Said sets are, usually in the form of a packaging unit, a combination of means for carrying out a globulomer determination of the invention. For the purpose of as easy handling as possible, said means are preferably provided in an essentially ready-to-use form. An advantageous arrangement offers the immunoassay in the form of a kit. A kit usually comprises multiple containers for separate arrangement of components. All components may be provided in a ready-to-use dilution, as a concentrate for diluting or as a dry substance or lyophilisate for dissolving or suspending; individual or all components may be frozen or stored at room temperature until use. Sera are preferably shock-frozen, for example at -20°C so that in these cases an immunoassay has to be kept preferably at temperatures below freezing prior to use. Further components supplied with the immunoassay depend on the type of said immunoassay. Usually, standard protein, tracer which may or may not be required and control serum are supplied together with the antiserum. Furthermore, microtiter plates, preferably antibody-coated, buffers, for example, for testing, for washing or for conversion of the substrate, and the enzyme substrate itself may also be included.

General principles of immunoassays and generation and use of antibodies as auxiliaries in laboratory and hospital can be found, for example, in Antibodies, A Laboratory Manual

(Harlow, E., and Lane, D., Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988).

The present invention also includes a method of diagnosing an amyloidosis, e.g., Alzheimer's disease, in a patient suspected of having this disease. This method comprises the steps of: 1) isolating a biological sample from the patient; 2) contacting the biological sample with at least one of the antibodies described above for a time and under conditions sufficient for formation of antigen/antibody complexes; and 3) detecting presence of the antigen/antibody complexes in said sample, presence of the complexes indicating a diagnosis of an amyloidosis, e.g., Alzheimer's disease, in the patient. The antigen may be, for example, an globulomer or a portion or fragment thereof which has the same functional properties as the full globulomer (e.g., binding activity).

Further, the present invention includes another method of diagnosing an amyloidosis, e.g., Alzheimer's disease in a patient suspected of having this disease. This method comprising the steps of: 1) isolating a biological sample from the patient; 2) contacting the biological sample with an antigen for a time and under conditions sufficient for the formation of antibody/antigen complexes; 3) adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein the conjugate comprises one of the antibodies described above, attached to a signal generating compound capable of generating a detectable signal; and 4) detecting the presence of an antibody which may be present in the biological sample, by detecting a signal generated by the signal generating compound, the signal indicating a diagnosis of an amyloidosis, e.g., Alzheimer's disease in the patient. The antigen may be a globulomer or a portion or fragment thereof having the same functional properties as the full globulomer (e.g., binding activity).

The present invention includes an additional method of diagnosing an amyloidosis, e.g., Alzheimer's disease, in a patient suspected of having an amyloidosis, e.g., Alzheimer's disease. This method comprises the steps of: 1) isolating a biological sample from said patient; 2) contacting the biological sample with anti-antibody, wherein the anti-antibody is specific for one of the antibodies described above, for a time and under conditions sufficient to allow for formation of anti-antibody/antibody complexes, the complexes containing antibody present in the biological sample; 2) adding a conjugate to resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to bound antibody, wherein the conjugate comprises an antigen, which binds to a signal generating compound capable of generating a detectable signal; and 3) detecting a signal generated by the signal generating compound, the signal indicating a diagnosis of an amyloidosis, e.g., Alzheimer's disease, in the patient.

Also, the present invention includes a kit comprising: a) at least one of the antibodies described above and b) a conjugate comprising an antibody attached to a signal-generating compound, wherein the antibody of the conjugate is different from the isolated antibody.

The present invention also encompasses a kit comprising: a) an anti-antibody to one of the antibodies described above and b) a conjugate comprising an antigen attached to a signal-generating compound. The antigen may be a globulomer or a fragment or portion thereof having the same functional characteristics as the globulomer (e.g., binding activity).

In one diagnostic embodiment of the present invention, an antibody of the present invention, or a portion thereof, is coated on a solid phase (or is present in a liquid phase). The test or biological sample (e.g., whole blood, cerebrospinal fluid, serum, etc.) is then contacted with the

solid phase. If antigen (e.g., globulomer) is present in the sample, such antigens bind to the antibodies on the solid phase and are then detected by either a direct or indirect method. The direct method comprises simply detecting presence of the complex itself and thus presence of the antigens. In the indirect method, a conjugate is added to the bound antigen. The conjugate comprises a second antibody, which binds to the bound antigen, attached to a signal-generating compound or label. Should the second antibody bind to the bound antigen, the signal-generating compound generates a measurable signal. Such signal then indicates presence of the antigen in the test sample. Examples of solid phases used in diagnostic immunoassays are porous and non-porous materials, latex particles, magnetic particles, microparticles (see U.S. Patent No. 5,705,330), beads, membranes, microtiter wells and plastic tubes. The choice of solid phase material and method of labeling the antigen or antibody present in the conjugate, if desired, are determined based upon desired assay format performance characteristics.

As noted above, the conjugate (or indicator reagent) will comprise an antibody (or perhaps anti-antibody, depending upon the assay), attached to a signal-generating compound or label. This signal-generating compound or "label" is itself detectable or may be reacted with one or more additional compounds to generate a detectable product. Examples of signal-generating compounds include chromogens, radioisotopes (e.g., 125I, 131I, 32P, 3H, 35S and 14C), chemiluminescent compounds (e.g., acridinium), particles (visible or fluorescent), nucleic acids, complexing agents, or catalysts such as enzymes (e.g., alkaline phosphatase, acid phosphatase, horseradish peroxidase, beta-galactosidase and ribonuclease). In the case of enzyme use (e.g., alkaline phosphatase or horseradish peroxidase), addition of a chromo-, fluro-, or lumo-genic substrate results in generation of a detectable

signal. Other detection systems such as time-resolved fluorescence, internal-reflection fluorescence, amplification (e.g., polymerase chain reaction) and Raman spectroscopy are also useful. Examples of biological fluids which may be tested by the above immunoassays include plasma, whole blood, dried whole blood, serum, cerebrospinal fluid or aqueous or organo-aqueous extracts of tissues and cells.

The present invention also encompasses a method for detecting the presence of antibodies in a test sample. This method comprises the steps of: (a) contacting the test sample suspected of containing antibodies with anti-antibody specific for the antibodies in the patient sample under time and conditions sufficient to allow the formation of anti-antibody/antibody complexes, wherein the anti-antibody is an antibody of the present invention which binds to an antibody in the patient sample; (b) adding a conjugate to the resulting anti-antibody/antibody complexes, the conjugate comprising an antigen (which binds to the anti-antibody) attached to a signal generating compound capable of detecting a detectable signal; and (d) detecting the presence of the antibodies which may be present in the test sample by detecting the signal generated by the signal generating compound. A control or calibrator may be used which comprises antibody to the anti-antibody.

Kits are also included within the scope of the present invention. More specifically, the present invention includes kits for determining the presence of antigens (e.g., globulomers) in a patient suspected of having Alzheimer's disease or another condition characterized by cognitive impairment. In particular, a kit for determining the presence of antigens in a test sample comprises a) an antibody as defined herein or moiety thereof; and b) a conjugate comprising a second antibody (having specificity for the antigen) attached to a signal generating compound capable of

generating a detectable signal. The kit may also contain a control or calibrator which comprises a reagent which binds to the antigen as well as a package insert describing the procedure to be used when conducting the assay.

The present invention also includes a kit for detecting antibodies in a test sample. The kit may comprise a) an anti-antibody specific (for example, one of the subject invention) for the antibody of interest, and b) an antigen or portion thereof as defined above. A control or calibrator comprising a reagent which binds to the antigen may also be included. More specifically, the kit may comprise a) an anti-antibody (such as the one of the present invention) specific for the antibody and b) a conjugate comprising an antigen (e.g., globulomer) attached to a signal generating compound capable of generating a detectable signal. Again, the kit may also comprise a control of calibrator comprising a reagent which binds to the antigen as well as a package insert describing the components of the kits and how they are to be utilized. The kit may also comprise one container such as vial, bottles or strip, with each container with a pre-set solid phase, and other containers containing the respective conjugates. These kits may also contain vials or containers of other reagents needed for performing the assay, such as washing, processing and indicator reagents.

It should also be noted that the subject invention not only includes the full length antibodies described above but also moities or fragments thereof, for example, the Fab portion thereof. Additionally, the subject invention encompasses any antibody having the same properties of the present antibodies in terms of, for example, binding specificity, structure, etc.

Advantages of the invention:

By immunization with Aβ(12-42) globulomer (as described in Example I), different monoclonal antibodies may be obtained which differ in their tolerance or recognition of different Aβ(1-42) oligomers and Aβ(X-42) oligomers, as determined by comparative dot blotting as described above. This allows development of an antibody directed to Aβ oligomers which possesses an optimal relation between cognition enhancing effect, desired specificity over other Aβ forms and minimal side effect profile. The same holds true for monoclonal antibodies for use in passive immunization. The advantage of such a specific strategy for immunization (active and passive) is that it will not induce an immune response against Aβ monomers, Aβ peptides in fibrillary states of aggregation or sAPPα. This is advantageous in several ways:

1) In the form of insoluble Aβ plaques, Aβ peptides in fibrillary states of aggregation amount to the major part of the entire Aβ peptide pool in AD brains. A massive release of Aβ by dissolution of Aβ plaques induced by reaction of anti-Aβ antibodies with these plaques is to be regarded as detrimental. This massive release of Aβ would then cross the blood-brain barrier, enter the bloodstream and potentially increase the risk of microhaemorrhages. In addition, in the ELAN trial mentioned above, this very strategy of immunization with fibrillary Aβ peptide forms required cancellation of the trial due to 6% of cases with an onset of meningoencephalitis.

2) Immune responses directed to monomeric Aβ peptide forms are undesirable, as it could be shown that the latter may exert cognition-enhancing effects. 3)Immune responses directed t reaction. Moreover, sAPPα was also shown to exert cognition-enhancing effects.

4) A response directed to vascular Aβ peptide in the form of CAA is to be avoided in order to eschew the undesirable side effect of microhaemorrhages (i.e., antibodies against the central portion of Aβ and which in addition do not bind to Aβ-peptides aggregated in the form of CAA induce fewer microhaemorrhages when compared to such against the N-terminus, see above).

5) Antibodies which specifically react with Aβ oligomers will have higher bioavailability with regard to the pathophysiologically relevant Aβ species, as they will not be bound to, e.g., fibrillary or monomeric Aβ and thus made unavailable for therapeutic effect.

Again, it should be noted that the antibodies of the present invention and, in particular, 10F4 and 3C5, do not (or with a lower binding affinity compared to commercially available antibodies like 6E10 (Signet Cat. no.: 9320)) detect amyloid beta in the cerebrospinal fluid. Thus, due to the high turnover rates of amyloid beta in the CSF, this lack of binding by the antibodies to the amyloid beta in the CSF prevents the waste of antibodies, as well as creates a more efficacious and selective sytem in comparison to those antibodies which bind to all amyloid beta found in the body (e.g., brain and CSF). Further, this property of the antibodies of the present invention allows one to reduce the amount of antibody to be administered (in connection with passive immunization), reduces the risk of side effects since the dose is lower thereby restricting antibodies to the target, increases efficacy, and also increases the therapeutic index. Furthermore, the risk of microhemmorhages is also reduced. Additionally, since the antibodies do not detect

fibrillar forms of amyloid beta, the risks associated with such complex formation are also reduced.

Deposit Information:

The hybridoma (ML45-3C5.5C10) which produces monoclonal antibody 3C5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on February 28, 2006 under the terms of the Budapest Treaty and was assigned ATCC No. PTA-7406. Hybridoma (ML43-10F4.3H8) which produces monoclonal antibody 10F4 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on August 16, 2006 under the terms of the Budapest Treaty and was assigned ATCC No. PTA-7808.

The present invention may be illustrated by use of the following non-limiting examples:

EXAMPLE I

PREPARATION OF Aβ(12-42) GLOBULOMER FOR IMMUNIZATION

The Aβ(12-42) synthetic peptide (AnaSpec Inc.; Lot # 40443) was suspended in 100% (v/v) 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at 40 mg/mL (5mg in 125 µL HFIP) and incubated for complete solubilization under shaking at 37 °C for 1 h. The HFIP acts as a hydrogen-bond breaker and is used to eliminate pre-existing structural inhomogeneities in the Aβ peptide. After centrifugation at 10000 g for 10 min the supernatant of the HFIP-dissolved Aβ(12-42) was diluted with 6.1 mL phosphate-buffered saline(PBS)(20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4) and 625 µL 2% (w/v) sodium dodecyl sulfate (SDS) (in H_2O) were added (final concentration of 0.2% (w/v) SDS) and incubated for 3 h at 37 °C. Once more, 625 µL 2% (w/v) sodium dodecyl sulfate (SDS) (in H_2O) were added (final concentration of 0.4% (w/v) SDS) and further incubated for 3 h at 37 °C. The

solution was diluted with 7 mL H_2O and incubated for 16 h at 37 °C. After centrifugation at 3000 g for 10 min the supernatant was further diluted with 15mL PBS (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4) and concentrated by ultrafiltration (5 kDa cut-off) to 0.65 mL, dialysed against 20 mM NaH_2PO_4, 140 mM NaCl, 0.05% (w/v) SDS, pH 7.4 for 16 h at room temperature, centrifuged at 10000 g for 10 min and the supernatant comprising the Aβ(12-42) globulomer withdrawn. The samples were aliquoted and stored at -80°C until further use.

EXAMPLE II

PRODUCTION OF MONOCLONAL ANTIBODIES 3C5 AND 10F4

Balb/c mice were immunized sub-cutaneous with 50 µg of Aβ (12-42) globulomer as described in Example I in CFA (Sigma) and boosted twice at one month intervals. Spleens were collected and spleen cells fused with mouse myeloma SP2/0 cells at 5:1 ratio by a PEG procedure. Fusion cells were plated in 96-well dishes in Azaserine/Hypoxanthine selection media at 2x105 cells/mL, 200 mL per well. Cells were allowed to grow to form visible colonies and supernatants assayed for Aβ oligomer reactivity by a direct ELISA assay. Hybridomas secreting antibodies to Aβ oligomers were subcloned by limiting dilution, until antibody expression appeared stable.

EXAMPLE III

DOT-BLOT PROFILE OF THE SELECTIVITY OF THE ANTI-Aβ GLOBULOMER ANTIBODIES

In order to characterize the selectivity of the monoclonal anti-Aβ globulomer antibodies, they were probed for recognition with different Aβ-forms. To this end, serial dilutions of the individual Aβ forms ranging from 100 pmol/µL to 0.01 pmol/µL in PBS supplemented with 0.2 mg/mL BSA were made. 1 µL of each sample was blotted onto a nitrocellulose membrane. For detection, the corresponding antibody was used (0.2 µg/mL). Immunostaining was done using peroxidase conjugated anti-mouse-IgG and the staining reagent BM Blue POD Substrate (Roche).

Aβ-standards for dot-blot:

1. Aβ(1-42) monomer, 0.1% NH_4OH

1 mg Aβ(1-42) (Bachem Inc., Cat. no.: H-1368) were dissolved in 0.5 mL 0.1% NH_4OH in H_2O (freshly prepared) (= 2 mg/mL) and immediately shaken for 30 sec at room temperature to obtain a clear solution. The sample was stored at −20 °C for further use.

2. Aβ(1-40) monomer, 0.1% NH_4OH

1 mg Aβ(1-40) (Bachem Inc., cat. no. H-1368) were dissolved in 0.5 mL 0.1% NH_4OH in H_2O (freshly prepared) (= 2 mg/mL) and immediately shaken for 30 sec. at room temperature to obtain a clear solution. The sample was stored at −20°C for further use.

3. Aβ(1-42) monomer, 0.1% NaOH

2.5 mg Aβ(1-42) (Bachem Inc., cat. no. H-1368) were dissolved in 0.5 mL 0.1% NaOH in H_2O (freshly prepared) (= 5 mg/mL) and immediately shaken for 30 sec. at room

temperature to obtain a clear solution. The sample was stored at –20°C for further use.

4. Aβ(1-40) monomer, 0.1% NaOH

2.5 mg Aβ(1-40) (Bachem Inc., cat. no. H-1368) were dissolved in 0.5 mL 0.1% NaOH in H_2O (freshly prepared) (= 5 mg/mL) and immediately shaken for 30 sec. at room temperature to obtain a clear solution. The sample was stored at –20°C for further use.

5. Aβ(1-42) globulomer

The Aβ(1-42) synthetic peptide (H-1368, Bachem, Bubendorf, Switzerland) was suspended in 100% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at 6 mg/mL and incubated for complete solubilization under shaking at 37 °C for 1.5 h. The HFIP acts as a hydrogen-bond breaker and is used to eliminate pre-existing structural inhomogeneities in the Aβ peptide. HFIP was removed by evaporation in a SpeedVac and Aβ(1-42) resuspended at a concentration of 5 mM in dimethylsulfoxide and sonicated for 20 s. The HFIP-pre-treated Aβ(1-42) was diluted in phosphate-buffered saline (PBS) (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4) to 400 µM and 1/10 volume 2% sodium dodecyl sulfate (SDS) (in H_2O) added (final concentration of 0.2% SDS). An incubation for 6 h at 37 °C resulted in the 16/20-kDa Aβ(1-42) globulomer (short form for globular oligomer) intermediate. The 38/48-kDa Aβ(1-42) globulomer was generated by a further dilution with three volumes of H_2O and incubation for 18 h at 37 °C. After centrifugation at 3000 g for 20 min the sample was concentrated by ultrafiltration (30-kDa cut-off), dialysed against 5 mM NaH_2PO_4, 35 mM NaCl, pH 7.4, centrifuged at 10000 g for 10 min and the supernatant comprsing the 38/48-kDa Aβ(1-42) globulomer withdrawn. As

an alternative to dialysis the 38/48-kDa Aβ(1-42) globulomer can also be precipitated by a ninefold excess (v/v) of ice-cold methanol/acetic acid solution (33% methanol, 4% acetic acid) for 1 h at 4°C. The 38/48-kDa Aβ(1-42) globulomer is then pelleted (10 min at 16200 g), resuspended in 5 mM NaH_2PO_4, 35 mM NaCl, pH 7.4, and the pH adjusted to 7.4.

6. Aβ(12-42) globulomer

2 mL of an Aβ(1-42) globulomer preparation prepared according to Example 3.5 (see above) are admixed with 38 mL buffer (5 mM sodium phosphate, 35 mM sodium chloride, pH 7.4) and 150 µl of a 1 mg/mL GluC endoproteinase (Roche) in H_2O. The reaction mixture is stirred for 6 h at RT, and a further 150 µL of a 1 mg/mL GluC endoproteinase (Roche) in H_2O are subsequently added. The reaction mixture is stirred at RT for another 16 h, followed by addition of 8 µL of a 5 M DIFP (Diisopropylfluorphosphate) solution. The reaction mixture is concentrated to approx. 1 mL via a 15 mL 30 kDa Centriprep tube. The concentrate is admixed with 9 mL of buffer (5 mM sodium phosphate, 35 mM sodium chloride, pH 7.4) and again concentrated to 1 mL. The concentrate is dialyzed at 6°C against 1 L of buffer (5 mM sodium phosphate, 35 mM NaCl) in a dialysis tube for 16 h. The dialysate is adjusted to an SDS content of 0.1% with a 1% strength SDS solution in H_2O. The sample is removed by centrifugation at 10000 g for 10 min and the supernatant is removed.

7. Aβ(20-42) globulomer

1.59 mL of Aβ(1-42) globulomer preparation prepared according to Example 2.5 (see above) are admixed with 38 mL of buffer (50 mM MES/NaOH, pH 7.4) and 200 µL of a 1 mg/mL thermolysin solution (Roche) in H_2O. The reaction

mixture is stirred at RT for 20 h. Then 80 µl of a 100 mM EDTA solution, pH 7.4, in H_2O are added and the mixture is furthermore adjusted to an SDS content of 0.01% with 400 µl of a 1% strength SDS solution. The reaction mixture is concentrated to approx. 1 mL via a 15 mL 30 kDa Centriprep tube. The concentrate is admixed with 9 mL of buffer (50 mM MES/NaOH, 0.02 % SDS, pH 7.4) and again concentrated to 1 mL. The concentrate is dialyzed at 6°C against 1 L of buffer (5 mM sodium phosphate, 35 mM NaCl) in a dialysis tube for 16 h. The dialysate is adjusted to an SDS content of 0.1% with a 2% strength SDS solution in H_2O. The sample is removed by centrifugation at 10000 g for 10 min and the supernatant is removed.

8. Aβ(1-42) fibrils

1 mg Aβ(1-42) (Bachem Inc. Cat. no.: H-1368) were solved in 500 µL aqueous 0.1% NH_4OH (Eppendorff tube) and the sample was stirred for 1min at room temperature. 100 µL of this freshly prepared Aβ(1-42) solution were neutralized with 300 µL 20 mM NaH_2PO_4; 140 mM NaCl, pH7.4. The pH was adjusted to pH 7.4 with 1% HCl. The sample was incubated for 24 h at 37°C and centrifuged (10 min at 10000 g). The supernatant was discarded and the fibril pellet resuspended with 400 µL 20 mM NaH_2PO_4; 140 mM NaCl, pH 7.4 by vortexing for 1 min.

9. sAPPα

Supplied by Sigma (cat.no. S9564; 25 µg in 20 mM NaH_2PO_4; 140 mM NaCl ; pH 7.4). The sAPPα was diluted to 0.1 mg/mL (= 1 pmol/µL) with 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, 0.2 mg/mL BSA.

Materials for dot blot:

Aβ-standards:

Serial dilution of Aβ antigens in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4 + 0.2 mg/mL BSA

1) 100 pmol/µL
2) 10 pmol/µL
3) 1 pmol/µL
4) 0,1 pmol/µL
5) 0,01 pmol/µL

Nitrocellulose:

Trans-Blot Transfer medium, Pure Nitrocellulose Membrane (0.45 µm); BIO-RAD

Anti-Mouse-POD:

Cat. No: 715-035-150 (Jackson Immuno Research)

Detection reagent:

BM Blue POD Substrate, precipitating (Roche)

Bovine Serum Albumin, (BSA):

Cat. No.: A-7888 (SIGMA)

Blocking reagent:

5 % low fat milk in TBS

Buffer solutions:

TBS

25 mM Tris / HCl buffer pH 7.5 + 150 mM NaCl

TTBS

25 mM Tris / HCl - buffer pH 7.5
+ 150 mM NaCl
+ 0.05 % Tween 20

PBS + 0.2 mg/mL BSA
20 mM NaH_2PO_4 buffer pH 7.4
+ 140 mM NaCl
+ 0.2 mg/mL BSA

Antibody solution I:

0.2 µg/mL antibody diluted in 20 mL 1 % low fat milk in TBS

Antibody solution II:

1:5000 dilution
Anti-Mouse-POD in 1 % low fat milk in TBS

Dot blot procedure:

1) 1 µL each of the different Aβ-standards (in their 5 serial dilutions) were dotted onto the nitrocellulose membrane in a distance of approximately 1 cm from each other.

2) The Aβ-standards dots were allowed to dry on the nitrocellulose membrane on air for at least 10 min at room temperature (RT) (= dot blot).

3) Blocking:
The dot blot was incubated with 30 mL 5% low fat milk in TBS for 1.5 h at RT.

4) Washing:
The blocking solution was discarded and the dot blot incubated under shaking with 20 mL TTBS for 10 min at RT.

5) Antibody solution I:
The washing buffer was discarded and the dot blot incubated with antibody solution I for 2 h at RT.

6) Washing:
The antibody solution I was discarded and the dot blot incubated under shaking with 20 mL TTBS for 10 min at RT. The washing solution was discarded and the dot blot incubated under shaking with 20 mL TTBS for 10 min at RT. The washing solution was discarded and the dot blot incubated under shaking with 20 mL TBS for 10 min at RT.

7) Antibody solution II:
The washing buffer was discarded and the dot blot incubated with antibody solution II overnight at RT.

8) Washing:
The antibody solution II was discarded and the dot blot incubated under shaking with 20 mL TTBS for 10 min at RT. The washing solution was discarded and the dot blot incubated under shaking with 20 mL TTBS for 10 min at RT. The washing solution was discarded and the dot blot incubated under shaking with 20 mL TBS for 10 min at RT.

9) Development:
The washing solution was discarded. The dot blot was developed with 10 mL BM Blue POD Substrate for 10 min. The development was stopped by intense washing of the dot blot with H_2O. Quantitative evaluation was done using a

densitometric analysis (GS800 densitometer (BioRad) and software package Quantity one, Version 4.5.0 (BioRad)) of the dot-intensity. Only dots were evaluted that had a relative density of greater than 20% of the relative density of the last optically unambiguously identified dot of the Aβ(20-42) globulomer. This threshold value was determined for every dot-blot independently. The calculated value indicates the relation between recognition of Aβ(1-42) globulomer and the respective Aβ form for the antibody given.

The monoclonal antibodies tested were obtained (except for 6E10) by active immunization of mice with Aβ(12-42) globulomer (prepared as described in Example I), followed by selection of the fused hybridoma cells. The individual Aβ forms were applied in serial dilusions and incubated with the respective antibodies for immune reaction.

1. Aβ(1-42) monomer, 0.1% NH_4OH
2. Aβ(1-40) monomer, 0.1% NH_4OH
3. Aβ(1-42) monomer, 0.1%NaOH
4. Aβ(1-40) monomer, 0.1% NaOH
5. Aβ(1-42) globulomer
6. Aβ(12-42) globulomer
7. Aβ(20-42) globulomer
8. Aβ(1-42) fibril preparation
9. sAPPα (Sigma); (first dot: 1 pmol)

Results are shown in Figure 1.

Based upon an analysis of the dot blot results, the anti-Aβ globulomer mAbs 10F4 and 3C5 have a high affinity for Aβ-

globulomer forms such as the Aβ(1-42) globulomer, Aβ(12-42) globulomer and Aβ (20-42) globulomer). They discriminate other Aβ forms such as Aβ-monomers to a certain extent and do not significantly recognize Aβ(1-42) fibrils or sAPPα. The antibodies 10F4 and 3C5 can therefore be coined 'anti-Aβ globulomer antibodies'.

EXAMPLE IV

DETECTION OF Aβ-GLOBULOMER EPITOPES IN ALZHEIMER'S DISEASE BRAIN BY 10F4 AND 3C5

A: Extraction Procedure

Reagent List :

3% SDS-buffer :

- 50 mM Tris/HCl, 150 mM NaCl, 0.5% Triton X100, 1 mM EGTA, 3% SDS, 1% Na-desoxycholate, pH7.4

Complete Protease Inhibitor Cocktail:

- dissolve 1 tablet complete inhibitor cocktail (Roche Diagnostics GmbH; Cat. no.: 1697498) in 1 mL H_2O; freshly prepared

PMSF-solution:

- 500 mM PMSF in methanol

3% SDS extraction-buffer:

- add 1/100 complete inhibitor cocktail solution to the 3% SDS-buffer
- add 1/500 PMSF solution to the 3% SDS-buffer
- prepare extraction buffer immediately before use at room temperature

Antibodies:

- mAb 10F4
- mAb 3C5
- mAb 6E10 (Signet; Cat. no.:9320)
- mAb IgG2b (control antibody, generated against a synthetical hapten, Dianova, clone NCG2B.01, Cat.No: DLN-05812)

Procedure:

0.2 g of -80°C frozen post mortem human AD and aged match control brain tissue samples were added to 1.8 mL freshly prepared 3% SDS-extraction buffer at room temperature. The sample was immediately homogenized on ice by a glass potter. The homogenized sample was transfered to a reaction vial and centrifuged at 10000 g for 5 min. The supernatant (= 3% SDS-brain extract) was collected carefully and stored in a reaction vial at -80°C for further use.

B: Activation of Dynabeads with monoclonal mouse antibodies

- the stock-suspension of dynabeads (Dynabeads M-280 Sheep anti-Mouse IgG, Invitrogen; Cat. no.: 112.02) was shaken carefully to prevent foaming
- 1 mL was aseptically removed and transfered to a 1.5 mL reaction vial
- the dynabeads were washed 3 times 5 min with 1 mL immunoprecipitation (IP)-wash buffer (IP-wash-buffer: PBS (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1%BSA). During the washing procedure the supernatant was carefully removed while the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS)
- the washed dynabeads were incubated with 40 µg Aβ-antibody in 1 mL PBS, 0.1% BSA

- the activation was carried out by overnight incubation under shaking at 4°C
- the activated dynabeads were washed 4 times 30 min (again using the MSS) with 1mL IP-wash buffer (PBS (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1%BSA)
- the activated dynabeads were resuspended with 1 mL PBS, 0.1% BSA, 0.02% Na-Azide; vortexed and centrifuged briefly
- the antibody activated dynabeads were stored at 4°C until further use

C: Immunoprecipitation (IP)

- 25 µL 3% SDS-brain extract were diluted with 975 µL 20 mM NaH_2PO_4, 140 mM NaCl; 0.05% Tween 20, pH 7.5(= 1:40 dilution).
- 25 µL of each antibody activated dynabeads of the following list were incubated with 1 mL of the 1:40 diluted 3% SDS-brain extract:
    - 6E10-Dynabeads
    - 3C5-Dynabeads
    - 10F4-Dynabeads
    - IgG2b-Dynabeads

- the immunoprecipitation was carried out by overnight incubation (~20 h) under shaking at 6°C
- the dynabeads were immobilized with the MPS
- the supernatant was carefully removed and discarded
- the dynabeads were washed as follows:
    - 2 times 5 minutes with 500 µL 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.5 + 0.1% BSA

- 1 time 3 minutes with 500 µL 2 mM NaH_2PO_4, 14 mM NaCl, pH 7.5
- important: after the last removal of the washing buffer the reaction vials were centrifuged, placed back in the MSS and the remaining drops of fluid carefully removed
- 10 µL 50% CH_3CN, 0.5% TFA in H_2O were added to the reaction vial and vortexed
- the reaction vials were incubated 10 minutes at RT under shaking
- the dynabeads were immobilized with the MSS
- the supernatant comprising the immunoprecipitated eluted Aβ species was carefully withdrawn (= IP-eluate)

D: Surface-enhanced laser desorption ionization-mass spectrometry (SELDI-MS):

- 1 µL IP-eluate was spotted onto a H4 Protein Chip Array (Ciphergen; Cat.no. C573-0028).
- the spots were allowed to dry on a warm incubator plate
- CHCA-solution:
  - 5 mg CHCA were dissolved in 150 µL acetonitrile + 150 µL 1% TFA = stock solution; stored at -20°C
  - of the stock solution 10 µL were diluted with 20 µL acetonitrile and 20 µL 1% TFA = working CHCA-solution
  - 2 µL of the working CHCA-solution was applied onto the spots
- the spots were allowed to dry on a warm incubator plate and analysed by SELDI-MS (Surface-Enhanced Laser Desorption Ionization-Mass Spectrometry)

- conditions: laser intensity 200; sensitivity 6; mass range 800Da – 10000Da; position 20-80; collect 5
- analysis: the MZ area of the respective Aβ-mass peaks was quantified

E. Western Blot analysis of imuunoprecipitated AD-brain extract:

SDS-PAGE:

SDS-sample buffer:

- 0.3 g SDS
- 4 mL 1 M Tris/HCl pH 6.8
- 8 mL glycerol
- 70 µL 1% bromphenolblue in ethanol
- add H_2O to 50 mL

Running buffer :

- 7.5 g Tris
- 36 g Glycine
- 2.5 g SDS
- add H_2O to 2.5 L

SDS-PAGE gel system:

- 18% Tris/Glycine Gel: (Invitrogen Inc., Cat. no.: EC65055BOX)

5 µL IP-eluate were added to 13 µL sample buffer (300 µL SDS-sample buffer + 10 µL 1 M Tris-solution in H_2O + 20 µL 85% gycerol). The resulting 18 µL sample are loaded onto a 18% Tris/Glycin Gel (Invitrogen Inc., Cat. no.: EC65055BOX). The SDS-PAGE is conducted at a constant current of 20 mA.

Western Blot procedure:

Subsequent to electrophoresis, the gel was blotted for 45 minutes at 75 mA onto a nitrocellulose membrane (7.5 cm x 9 cm, 0.2 µm, BioRad) using a semi-dry blotting chamber (BioRad).

Blot-buffer:

- 6 g Tris
- 28.1 g glycine
- 500 mL methanole
- add H_2O to 2.5 L

Western Blot immunostaining:

Materials:

- Anti-Aβ antibody 6E10 (Signet; Cat.No.9320)
- Anti-Mouse-POD (Jackson ImmunoResearch, Cat. no.: 715-035-150)
- Detection reagent:
  - Super Signal West Pico Substrat (Pierce, Cat. no.: 34077)
- Bovine Serum Albumin (BSA, Serva, Cat. no.: 11926)
- low fat milk powder (Lasana)
- Blocking reagent:
  - 2 % BSA in PBST
- TBS:
  - 25 mM Tris / HCl

- 150 mM NaCl Puffer, pH 7.5

- TTBS:
  - 25 mM Tris / HCl
  - 150 mM NaCl Puffer
  - 0.05% Tween 20, pH 7.5

- PBS:
  - 20 mM NaH_2PO_4 buffer
  - 140 mM NaCl buffer, pH 7.5

- PBST:
  - 20 mM NaH_2PO_4 buffer
  - 140 mM NaCl buffer
  - 0.05% Tween 20, pH 7.5

- Antibody solution I:
  - 1 µg/mL 6E10 = 1:1000 in 20 mL 3 % low fat milk in TBS

- Antibody solution II:
  - 1:10000 diluted anti-mouse -POD in 20 mL 3 % low fat milk in TBS

Procedure:

1) The Western blot was boiled for 10 minutes in PBS.

2) Blocking:
   - The Western blot was incubated for 16 h at 6°C with 50 mL blocking reagent.

3) Washing:

- The blocking solution was discarded and the Western blot washed with 50 mL TTBS for 10 minutes at room temperature.
- The blocking solution was discarded and the Western blot washed with 50 mL TBS for 10 minutes at room temperature.

4) Antibody solution I:
- The washing solution was discarded and the Western blot incubated with antibody solution I for 4 h at room temperature.

5) Washing:
- The blocking solution was discarded and the Western blot washed with 50 mL TTBS for 10 minutes at room temperature.
- The blocking solution was discarded and the Western blot washed with 50 mL TTBS for 10 minutes at room temperature.
- The blocking solution was discarded and the Western blot washed with 50 mL TBS for 10 minutes at room temperature.

6) Antibody solution II:
- The washing solution was discarded and the Western blot incubated with antibody solution II for 1 h at room temperature.

7) Washing:
- The blocking solution was discarded and the Western blot washed with 50 mL TTBS for 10 minutes at room temperature.

- The blocking solution was discarded and the Western blot washed with 50 mL TTBS for 10 minutes at room temperature.
- The blocking solution was discarded and the Western blot washed with 50 mL TBS for 10 minutes at room temperature.

8) Development and quantitative analysis:

- The washing solution was discarded.
- Two mL Super Signal West Pico Substrate Enhancer and 2 mL Peroxide Solution were mixed.
- The resulting 4 mL solution were added to the Western blot and the blot was incubated for 5 minutes in the dark.
- The blot was analyzed using a chemoluminescence imaging system (VersaDoc, BioRad). Five pictures at were taken at 30, 97.5, 165, 232.5and 300 seconds acquisition time.
- The picture at which no saturation of the trace (intensity x mm) of the Aβ-protein bands occured was quantitatively analyzed using the software package Quantity one, Version 4.5.0 (BioRad).

The results are shown in Figure 2. The extraction procedure with 3% (w/v) used herein is thought to extract soluble forms of the total Aβ-peptide pool in the brain because the buffer composition is not suffucient to solubilize Aβ-peptide in the aggregated fibrillar form. The Aβ-peptide that is bound in the Alzheimer's disease brain extract by the monoclonal antibodies 3C5 and 10F4 is therefore soluble Aβ-peptide. These soluble Aβ-species are thought to be the Alzheimer's disease relevant species, as they correlate better with the severity of the disease than fibrillar Aβ in the form of Aβ-

plaques found in AD brain (Kuo et al. 1996, J. Biol. Chem. 271, 4077-4081,; Lue et al., 1999, Am. J. Pathol. 155, 853-862). Therefore, the antibodies 10F4 and 3C5 target the disease relevant Aβ-species. Moreover, in comparison to the pan-Aβ-antibody 6E10, the monoclonal antibodies 3C5 and 10F4 bind only a to subfraction of the total soluble Aβ-pool in the Alzheimer's disease brain extract. The remaining Aβ-forms obviously do not possess the Aβ-globulomer epitope recognized by 3C5 and 10F4. Due to the fact that these Aβ-forms are not thought to be neuropathogenic, it is advantegeous not to attack them by the treatment antibody to reduce side effects and not to reduce the effective concentration of antibodies circulating in the CNS. Therefore, the dosing of the treatment antibody can be reduced resulting in a better therapeutic index.

EXAMPLE V
SEMI-QUANTITATIVE ANALYSIS VISUALIZED BY SDS-PAGE OF THE DISCRIMINATION OF ANTI-Aβ GLOBULOMER ANTIBODIES FOR Aβ(1-42) FIBRILS

Aβ(1-42) fibril preparation:

1 mg of Aβ(1-42) (Bachem, Cat. No.: H-1368) was dissolved in 500 µL 0.1% NH_4OH in H_2O and agitated for 1 min at ambient temperature. The sample was centrifuged for 5 min at 10000 g. The supernatant was collected. Aβ(1-42) concentration in the supernatant was determined according to Bradford's method (BIO-RAD Inc. assay procedure).

100 µL of Aβ(1-42) in 0.1% NH_4OH were mixed with 300 µL of 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4 and adjusted to pH 7.4 with 2% HCl. The sample was then incubated at 37 °C for 20 hours. Following which, the sample was centrifuged for 10 min at

10000 g. The supernatant was discarded, and the residue was mixed with 400 µL of 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, resuspended by vigorous agitation ("vortexing") for 1 min and centrifuged for 10 min at 10000 g. The supernatant was discarded, and the residue was mixed with 400 µL of 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, resuspended by vigorous agitation ("vortexing") for 1 min and centrifuged for 10 min at 10000 g once more. The supernatant was discarded. The residue was resuspended in 380 µL of 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4 and prompted by vigorous agitation ("vortexing").

Binding of anti-Aβ antibodies to Aβ(1-42) fibrils:
40 µL of Aβ(1-42) fibril preparation were diluted with 160 µL of 20 mM NaH_2PO_4, 140 mM NaCl, 0.05% Tween 20, pH 7.4 and agitated 5 min at ambient temperature, and then the sample was centrifuged for 10 min at 10000 g. The supernatant was discarded, and the residue was resuspended in 95 µL of 20 mM NaH_2PO_4, 140 mM NaCl, 0.05% Tween 20, pH 7.4. Resuspension was prompted by vigorous agitation ("vortexing"). Aliquots of 10 µL of the fibril preparation were each mixed with:

a) 10 µL 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4
b) 10 µL 0.5 µg/µL of 3C5 in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4
c) 10 µL 0.5 µg/µL of 10F4 in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4
d) 10 µL 0.5 µg/µL of 6E10 (Signet Cat. Nr.: 9320) in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4

The samples were incubated at 37 °C for 20 hours, and then centrifuged for 10 min at 10000 g. The supernatants were collected and mixed with 20 µL of SDS-PAGE sample buffer. The residues were mixed with 50 µL of 20 mM NaH_2PO_4, 140 mM NaCl, 0.025% Tween 20, pH 7.4 and resuspended by "vortexing". Then,