This file wrapper was thoroughly reviewed by our technical staff. The file history jacket cover and table of contents are missing from the original USPTO file history.

This has been brought to your attention so that you will know it has not been overlooked.

Bibliographic Data

Application No: 18/318,507

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged: /MICHAEL D BURKHART/ (Examiner's Signature) /MB/ (Initials)

Title: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
05/16/2023	424	1633	4096.0050007
RULE			

APPLICANTS

Merus N.V., Utrecht, NETHERLANDS

INVENTORS

Cornelis Adriaan DE KRUIF, Utrecht, NETHERLANDS

Linda Johanna Aleida HENDRIKS, Utrecht, NETHERLANDS

Ton LOGTENBERG, Utrecht, NETHERLANDS

CONTINUING DATA

This application is a CON of 16934925 07/21/2020

16934925 is a CON of 16417379 05/20/2019 PAT 10752929

16417379 is a CON of 15155743 05/16/2016 PAT 10329596

15155743 is a CON of 14081848 11/15/2013 PAT 9358286

14081848 is a CON of 13866747 04/19/2013 PAT 9248181

13866747 has PRO of 61635935 04/20/2012

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

07/17/2023

STATE OR COUNTRY

NETHERLANDS

ADDRESS

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005
UNITED STATES

FILING FEE RECEIVED

$6,580

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/318,507	**05/16/2023 05:34:54 PM ET**	**4096.0050007**

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	8764	FILED BY	Hayley Magann
PATENT CENTER #	62101997	FILING DATE	-
CUSTOMER #	26111	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Calvo

Documents

TOTAL DOCUMENTS: 20

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-05-16-ADS-4096-0050007.PDF	9	Application Data Sheet	1227 KB
2023-05-16-Track-One-Request-4096-0050007.PDF	1	Track One Request	114 KB
2023-05-16-EOT-Authorization-4096-0050007.PDF	1	Authorization for Extension of Time all replies	106 KB
2023-05-16-Declarations-4096-0050007.PDF	3	Oath or Declaration filed	165 KB
2023-05-16-POA-82A-4096-	1	Power of Attorney	191 KB

0050007.PDF				
2023-05-16-POA-82B-4096-0050007.PDF		1	Power of Attorney	1793 KB
4096_0050007_SequenceListing_ST26.XML		-	Sequence Listing (ST.26 XML File)	10 KB
2023-05-16-IDS-Pleading-4096-0050007.PDF		4	Transmittal Letter	124 KB
2023-05-16-IDS-SB08a-b-4096-0050007.PDF		17	Information Disclosure Statement (IDS) Form (SB08)	262 KB
Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.				
2023-05-16-Preliminary-Amendment-4096-0050007.PDF		6	-	130 KB
2023-05-16-Preliminary-Amendment-4096-0050007-A.PE.pdf	(1-1)	1	Preliminary Amendment	121 KB
2023-05-16-Preliminary-Amendment-4096-0050007-SPEC.pdf	(2-2)	1	Specification	94 KB
2023-05-16-Preliminary-Amendment-4096-0050007-CLM.pdf	(3-4)	2	Claims	122 KB
2023-05-16-Preliminary-Amendment-4096-0050007-REM.pdf	(5-6)	2	Applicant Arguments/Remarks Made in an Amendment	96 KB
2023-05-16-Specification-Claims-Abstract-4096-0050007.PDF		104	-	1285 KB

2023-05-16-Specification-Claims-Abstract-4096-0050007-SPEC.pdf	(1-99)	99	Specification	714 KB
2023-05-16-Specification-Claims-Abstract-4096-0050007-CLM.pdf	(100-103)	4	Claims	64 KB
2023-05-16-Specification-Claims-Abstract-4096-0050007-ABST.pdf	(104-104)	1	Abstract	33 KB
2023-05-16-Drawings-4096-0050007.PDF		39	Drawings-other than black and white line drawings	2721 KB
NPL_90_OA_US16934925_Apr2023.PDF		8	Non Patent Literature	476 KB
NPL_91_OA_16934925_Dec 2022.PDF		12	Non Patent Literature	748 KB
Utility-Transmittal.pdf		1	Transmittal of New Application	192 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
2023-05-16-ADS-4096-0050007.PDF	5554732BDD31E2B3F48BF48EFBD41C38D918932A26B103A27282A8B5C808AC49EECB6866718A7DD310A6AD2CE597FC395FA987CEAB54B610F328DB83348227F0
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2023-05-16-EOT-Authorization-4096-0050007.PDF	60EEE23D804DF6B9052A6C956B0D3EB4AE3155F6F0C3D211662D184E1BFC94CD1803CD1F88D27E9379C2F825CE28431A54ABD3A5CA605EB61BEB0650F613C2A6

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4096_0050007_SequenceListing_ST26.XML	8C790572741D96E94538B581C44F3C95FECEA638B3FB7BBCA680A403F2AFEFE282033510945409CAAFE9D21248065788CFE6BB085309F1324A261FFB680F8AA8
2023-05-16-IDS-Pleading-4096-0050007.PDF	E0145BD0192902168B6D73FBF5F562D47BD2BA5C0719255F4A8E259504202B4764355EA2905FA25E1656B8060A197CAEBAC9F90DFC83743EED0DC3F524F0CFF8
2023-05-16-IDS-SB08a-b-4096-0050007.PDF	58CCE48D2CD1A3C82909C67B84E047E48DEE02CF4804316F38BB21C802A2EC3287658B5A894F8931320E59EB15C6D263D17D033CE785AED1703AA1CD420F6323
2023-05-16-Preliminary-Amendment-4096-0050007.PDF	329FD74C89A834EBCD9718320144821396C08C067459B3E6BCD583D1DFAAB33670683CE5DB6F7D2FB76A02DA3578691EBEBD777F9F8E522715AA091113790673
2023-05-16-Preliminary-Amendment-4096-0050007-A.PE.pdf	BD9E3DF2C16C3AAA6ED491FB469D4A8E4F6528516B98AB0C2A7CD8259C46A9D95575D08822E144A9B925CCB1C3815641F7334BEA7B05045918D8845B09F745FC
2023-05-16-Preliminary-Amendment-4096-0050007-SPEC.pdf	02EF27CCCA9DF7F87035B3EA8377B261B41EB7A496AF8C92CAF10C433BF762B85A1C1147B1A9E15C962DF352A2BCF4C69089B2412ED061A4B367293396ACEA59
2023-05-16-Preliminary-Amendment-4096-0050007-CLM.pdf	035D3A1E5D56DD1F714478E1FFD9F64A907BBBD0C2073A10E7AE55402EA13B4DC4E84C20ECF971E94FCF05AE1F07A8B538F43989AD36E974F0AA9B5EC95A03E1

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2023-05-16-Specification-Claims-Abstract-4096-0050007.PDF	94F2D85455283EDE3F23449AF64E1B24784F22BDC6C522C2A021E8F64E41B8211497F9C84E17EE53C3AA77E789BB8B771083E2A32C251BAB45F30A783F203D63
2023-05-16-Specification-Claims-Abstract-4096-0050007-SPEC.pdf	8EF60C21345E62508316FD7389577704B3285DF7B8A4F82C49E878905739BB53A65B1623474B3DE4C2FE34B726C1254FC08FC68FECF951FD87B9D25424ECDE8F
2023-05-16-Specification-Claims-Abstract-4096-0050007-CLM.pdf	67AE7F0F50E378994F9C6B3828FB54D05EF626E6392ABED568E37D2CAC2EB279DD24A6945F487650989C56A3E4A63E1E795A980A5F0A723F93E8D45F117E085E
2023-05-16-Specification-Claims-Abstract-4096-0050007-ABST.pdf	C08B7A482DE738C325FCF260DEC64EF56108F0FBFE2E95DAE2A53F945E48516FEBCF5690C71D36ED92F956448429A59F6C62A26ED688A859CD47790E29A423E2
2023-05-16-Drawings-4096-0050007.PDF	DF4E85B779C8AAA1B4DA124C769FAD86BE6228DCBCE24E8597D2CF4A5547EDADDBF039E5F244A22A80A6D5A52AE9DF5500250D820FBE26A3056717005280E243
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NPL_91_OA_16934925_Dec2022.PDF	61176557D6BB3899CE65A5078ACF3E59E1FECAE3C7EDC85281132D478F15EFB1AD6012B57090127ADF423DFE10B0267F40D4D49DB0483943646657CF9EF50AEC
Utility-Transmittal.pdf	FDA4B29BBE6DBD8CAD85688BDE51269B64B44B9E662B2726A32E6D3BCA406BD2B2865ED47C793B3671EA377B60507A9E3D38BC118F185D9B6B6856CADF5B8E4B

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Cornelis	Adriaan	DE KRUIF	

Residence Information (Select One) US Residency ● Non US Residency Active US Military Service

City	Utrecht	Country of Residence [i]	NL

Mailing Address of Inventor:

Address 1	Uppsalalaan 17, 3rd & 4th Floor
Address 2	
City	Utrecht
State/Province	
Postal Code	3584 CT
Country [i]	NL

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Linda	Johanna Aleida	HENDRIKS	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Utrecht	Country of Residence [i]	NL

Mailing Address of Inventor:

Address 1	Uppsalalaan 17, 3rd & 4th Floor
Address 2	
City	Utrecht
State/Province	
Postal Code	3584 CT
Country [i]	NL

Inventor 3 Remove

Legal Name

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ton		LOGTENBERG	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Utrecht	Country of Residence i	NL

Mailing Address of Inventor:

Address 1	Uppsalalaan 17, 3rd & 4th Floor		
Address 2			
City	Utrecht	**State/Province**	
Postal Code	3584 CT	**Country** i	NL

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	26111		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES		
Attorney Docket Number	4096.0050007	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	39	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country i

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Publication Information:

☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	**Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	26111		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	**Continuity Type**	**Prior Application Number**	**Filing or 371(c) Date (YYYY-MM-DD)**
	Continuation of	16934925	2020-07-21

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16934925	Continuation of	16417379	2019-05-20	10752929	2020-08-25

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16417379	Continuation of	15155743	2016-05-16	10329596	2019-06-25

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15155743	Continuation of	14081848	2013-11-15	9358286	2016-06-07

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14081848	Continuation of	13866747	2013-04-19	9248181	2016-02-02

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13866747	Claims benefit of provisional	61635935	2012-04-20

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Merus N.V.		
Mailing Address Information For Applicant:			
Address 1	Uppsalalaan 17, 3rd & 4th Floor		
Address 2			
City	Utrecht	**State/Province**	
Country	NL	Postal Code	3584 CT
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

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PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Paul A. Calvo/			Date (YYYY-MM-DD)	2023-05-16
First Name	Paul A.	Last Name	Calvo	Registration Number	57,913

Additional Signature may be generated within this form by selecting the Add button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	4096.0050007
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

DocCode - SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 18318507 Document Date: 05/16/2023

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

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Form Revision Date: March 1, 2019

Doc Code - SEQ.XML

WIPO Type Code -

SLIC Placeholder Sheet for P-ELP (CMS) Content

Application Number: 18318507 Document Date: 05/16/2023

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Form Revision Date: 07 January 2022

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/318,507	05/16/2023 05:34:54 PM ET	4096.0050007

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	8764	FILED BY	Hayley Magann
PATENT CENTER #	62101997	AUTHORIZED BY	Paul Calvo
CUSTOMER #	26111	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 1005	E20235FH36327109	Hayley Magann

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	140.00	1	140.00
1817	REQUEST FOR PRIORITIZED EXAMINATION	4200.00	1	4200.00
1111	UTILITY PATENT APPL. SEARCH FEE	700.00	1	700.00
1011	BASIC FILING FEE - UTILITY (PAPER FILING ALSO REQUIRES NON-ELECTRONIC FILING FEE UNDER 1.16(T))	320.00	1	320.00
1311	PATENT APPL. EXAMINATION FEE	800.00	1	800.00

1081	UTILITY APPL. SIZE FEE > THAN 100 PAGES (FEE X PAGES >= 101 IN 50 PAGE INCREMENTS)	420.00	1	420.00

TOTAL AMOUNT: **$6,580.00**

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/15 (10-17)
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	4096.0050007
First Named Inventor	Cornelis Adriaan DE KRUIF
Title	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

1. ☐ **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. ☐ **Applicant asserts small entity status.** See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☑ **Specification** [*Total Pages* 104] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. ☑ **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 39]
6. **Inventor's Oath or Declaration** [*Total Pages* 3] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. ☐ Newly executed (original or copy)
   b. ☑ A copy from a prior application (37 CFR 1.63(d))
7. ☑ **Application Data Sheet** * *See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   ☐ Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. ☑ Computer Readable Form (CRF)
   b. ☐ Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers** (cover sheet & document(s))
    Name of Assignee _____
11. ☐ **37 CFR 3.73(c) Statement** (*when there is an assignee*) ☑ **Power of Attorney**
12. ☐ **English Translation Document** (*if applicable*)
13. ☑ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
14. ☑ **Preliminary Amendment**
15. ☐ **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. ☐ **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. ☐ **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☑ **Other:** Authorization Under 37 C.F.R. § 1.136(a)(3); Certification and Request for Prioritized Examination Under 37 C.F.R. § 1.102(e); Fees: $4,200 Track One, $140 Processing Fee; $1,820 Patent Application Fees, and $420 Application Size Fee

***Note**: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☑ The address associated with Customer Number: 26111 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Paul A. Calvo/	Date	5.16.2023
Name (Print/Type)	Paul A. Calvo	Registration No. (Attorney/Agent)	57,913

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Cornelis Adriaan DE KRUIF	Nonprovisional Application Number (if known):	To Be Assigned
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Paul A. Calvo/	Date 5.16.2023
Name (Print/Typed) Paul A. Calvo	Practitioner Registration Number 57,913

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☐ *Total of _____ forms are submitted.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: DE KRUIF *et al.*	Confirmation No.: *To Be Assigned*
Applicant: Merus N.V.	Art Unit: *To Be Assigned*
Application No.: *To Be Assigned*	Examiner: *To Be Assigned*
(*Continuation of Appl. No. 16/934,925; Filed: July 21, 2020*)	Atty. Docket: 4096.0050007
Filed: *Herewith*	

Title: **METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES**

Authorization to Treat a Reply as Incorporating an Extension of Time Under 37 C.F.R. § 1.136(a)(3)

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

The U.S. Patent and Trademark Office is hereby authorized to treat any concurrent or future reply that requires a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. The U.S. Patent and Trademark Office is hereby authorized to charge all required extension of time fees to our Deposit Account No. 19-0036, if such fees are not otherwise provided for in such reply.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Paul A. Calvo, Reg. No. 57,913/

Paul A. Calvo
Attorney for Applicant
Registration No. 57,913

Date: 5.16.2023

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

Title: Methods and means for the production of Ig-like molecules

This application is a Continuation of U.S. Patent Application No. 14/081,848, filed November 15, 2013, which is a Continuation of U.S. Patent No. 9,248,181, issued February 2, 2016, which claims priority to U.S. Provisional Patent Application No. 61/635,935, filed April 20, 2012. The entire contents of both of these patent applications, along with all documents cited therein, are hereby incorporated by reference.

FIELD

The invention relates to the fields of molecular biology, medicine and biological therapeutics. It particularly relates to the field of therapeutic antibodies for the treatment of various diseases.

BACKGROUND

Many currently used biological therapeutics are isolated recombinant, human or humanized monoclonal antibodies that enhance the ability of the body's immune system to neutralize or eliminate cells and/or molecules involved in disease processes or to eradicate invading pathogens or infectious agents. Monoclonal antibodies bind to a single specific area, or epitope, of an antigen and, for use in therapy, are often selected for a desirable functional property such as for example killing of tumor cells, blocking of receptor-ligand interactions or virus neutralization. Nowadays, there are about 30 FDA approved monoclonal antibodies, which are typically produced at large quantities and their biophysical and biochemical characteristics can be analyzed in great detail to ensure batch-to-batch consistency, which facilitates regulatory acceptability. Despite these favorable characteristics, monoclonal antibodies have several disadvantages, some of which relate to their monospecific nature and the complexity of diseases. Diseases processes are often multifactorial in nature, and involve redundant or synergistic action of disease mediators or up-regulation of different receptors, including crosstalk between their signaling networks. Consequently, blockade of multiple, different factors and pathways involved in pathology may result in improved therapeutic efficacy. By nature of their

monospecificity, monoclonal antibodies can only interfere with a single step within the complex disease processes which often does not have an optimal effect. In addition to not fully addressing multiple aspects of a disease process, it has become clear that targeting a single epitope on a single cellular or soluble protein or pathogen often will not suffice to efficiently treat disease because the target epitope may no longer be available for the monoclonal antibody to bind to and exert the desired effect. As an example, tumor cells often escape from monoclonal antibody therapy by down-regulation, mutation or shielding of the target epitope present on a growth factor receptor. By activating alternative receptors and/or their ligands, tumor cells than may exploit a different path leading to continued growth and metastasis. Similarly, viruses and other pathogens frequently mutate and lose or shield the target epitope, thereby escaping monoclonal antibody treatment. Monoclonal antibodies that bind to a single epitope often do not recruit the full spectrum of effector mechanisms evoked by polyclonal antibodies, including, amongst other things, opsonization (enhancing phagocytosis of antigens), steric hindrance (antigens coated with antibodies are prevented from attaching to host cells or mucosal surfaces), toxin neutralization, agglutination or precipitation (antibodies binding several soluble antigens cause aggregation and subsequent clearance), activation of complement and antibody-dependent cellular cytotoxicity (antibodies enable the killing of target cells by natural killer cells and neutrophils).

Polyclonal antibodies for therapeutic applications may be obtained from pooled human serum. Such serum-derived therapeutic polyclonal antibodies may for example be used to treat or prevent infections caused by viruses such as the rabies virus, cytomegalovirus and respiratory syncytial virus, to neutralize toxins such as tetanus toxin and botulinum toxin or to prevent Rhesus D allograft immunization. A more widespread use of serum-derived polyclonal antibody preparations has been prevented by the fact that source plasma is only available for a limited range of targets such as infectious diseases and toxins. Moreover, the products are highly dependent on donor blood availability, both in terms of quantity and suitability, resulting in considerable variation between batches. In addition, screening technologies fail to keep up with constantly evolving viruses, thus, immunoglobulin products carry a potential risk of infectious disease transmission. Finally, the long process of blood collection, screening

and immunoglobulin purification means plasma-derived immunoglobulins are expensive to produce.

Mixtures of monoclonal antibodies may improve the efficacy of monoclonal antibodies while avoiding the limitations associated with serum-derived polyclonal antibodies. In the art, combinations of two human or humanized monoclonal antibodies have been tested in preclinical models and in clinical trials (for example mixtures of 2 monoclonal antibodies against the HER2 receptor, mixtures of 2 antibodies against the EGFR receptor and , 2 monoclonal antibodies against the rabies virus). In the art, it has been shown that combinations of 2 monoclonal antibodies may have additive or synergistic effects and recruit effector mechanisms that are not associated with either antibody alone. For example, mixtures of 2 monoclonal antibodies against the EGFR or HER2 were shown to more potently kill tumor cells based on a combination of activities including enhanced receptor internalization, improved blockade of signalling pathways downstream of the receptors as well as enhanced immune effector-mediated cytotoxicity. For combination therapies based on 2 monoclonal antibodies, the component antibodies may be produced separately and combined at the protein level. A drawback of this approach is the staggering cost of developing the 2 antibodies individually in clinical trials and (partially) repeating that process with the combination. This would lead to unacceptable cost of treatments based on antibody combinations. Alternatively, the 2 recombinant cell lines producing the component monoclonal antibodies may be mixed in a fermentor and the resultant mixture of antibodies may be purified as a single preparation (WO 2004/061104). A drawback of this approach is the poor control over the composition and hence reproducibility of the resulting recombinant polyclonal antibody preparation, especially when considering that such compositions may change over time as the cells are being cultured.

During the past decade, bispecific antibodies have emerged as an alternative to the use of combinations of 2 antibodies. Whereas a combination of 2 antibodies represents a mixture of 2 different immunoglobulin molecules that bind to different epitopes on the same or different targets, in a bispecific antibody this is achieved through a single immunoglobulin molecule. By binding to 2 epitopes on the same or different targets, bispecific antibodies may have similar effects as compared to a

combination of 2 antibodies binding to the same epitopes. Furthermore, since bispecific antibodies of the IgG format combine 2 different monovalent binding regions in a single molecule and mixtures of 2 IgG antibodies combine 2 different bivalent binding molecules in a single preparation, different effects of these formats have been observed as well. From a technological and regulatory perspective, this makes development of a single bispecific antibody less complex because manufacturing, preclinical and clinical testing involve a single, molecule. Thus, therapies based on a single bispecific antibody are facilitated by a less complicated and cost-effective drug development process while providing more efficacious antibody therapies.

Bispecific antibodies based on the IgG format, consisting of 2 heavy and two light chains have been produced by a variety of methods. For instance, bispecific antibodies may be produced by fusing two antibody-secreting cell lines to create a new cell line or by expressing two antibodies in a single cell using recombinant DNA technology. These approaches yield multiple antibody species as the respective heavy chains from each antibody may form monospecific dimers (also called homodimers), which contain two identical paired heavy chains with the same specificity, and bispecific dimers (also called heterodimers) which contain two different paired heavy chains with different specificity. In addition, light chains and heavy chains from each antibody may randomly pair to form inappropriate, non-functional combinations. This problem, known as heavy and light chain miss-pairings, can be solved by choosing antibodies that share a common light chain for expression as bispecific. But even when a common light chain is used, expression of two heavy chains and one common light chain in a single cell will result in 3 different antibody species, i.e. two monospecific 'parental' antibodies and the bispecific antibody so that the bispecific antibody of interest needs to be purified from the resulting antibody mixture. Although technologies have been employed to further increase the percentage of bispecific antibodies in the mixtures of parental and bispecific antibodies and to decrease the percentage of miss-paired heavy and light chains, there remains a need for bispecific formats that eliminate or minimize some of the disadvantages mentioned above.

Taken together, the art provides a variety of technologies and methods for generating monoclonal antibodies, bispecific antibodies, mixtures of monoclonal antibodies, or mixtures of monospecific and bispecific antibodies that can subsequently be used for

therapeutic application in patients. However, as discussed above, each of these existing technologies and methods have their drawbacks and limitations.
There is thus a need for improved and/or alternative technologies for producing biological therapeutics in the form of mixtures or bispecific approaches for targeting multiple disease-modifying molecules

DESCRIPTION OF THE INVENTION

The invention provides methods and means for improved and/or alternative technologies for producing biological therapeutics in the form of mixtures or bispecific approaches for targeting multiple disease-modifying molecules, as well as products and uses resulting from these methods and means.
Various approaches are described in the art in order to promote the formation of a certain bispecific antibody of interest, thereby reducing the content of undesired antibodies in the resulting mixture.
For antibodies, it is well-known that the CH3-CH3 interaction is the primary driver for Fc dimerization (Ellerson JR., et al., J. Immunol 1976 (116) 510-517; Deisenhofer J. biochemistry 1981 (20) 2361-2370). It is furthermore well-known that when two CH3 domains interact with each other they meet in a protein-protein interface which comprises "contact" residues (also called contact amino acids, interface residues or interface amino acids). Contact amino acids of a first CH3 domain interact with one or more contact amino acids of a second CH3 domain. Contact amino acids are typically within 5.5 Å (preferably within 4.5 Å) of each other in the three-dimensional structure of an antibody. The interaction between contact residues from one CH3 domain and contact residues from a different CH3 domain may for instance be via Van der Waals forces, hydrogen bonds, water-mediated hydrogen bonds, salt bridges or other electrostatic forces, attractive interactions between aromatic side chains, disulfide bonds, or other forces known to one skilled in the art. It was previously shown that approximately one-third of the contact amino acid side chains at the human IgG1 CH3 domain interface can account for the majority of contributions to domain folding and association. It can further be envisaged that other (neighbouring) amino acid residues may affect the interactions in the protein-protein interface.

Approaches to interfere with the dimerization of antibody heavy chains have been employed in the art. Specific engineering in the CH3 domains was applied in order to favour heterodimerization over homodimerization. Examples of such engineering of the CH3-CH3 interface include the introduction of complementary protuberance and cavity mutations, also known as 'knob-into-hole' approaches as described for instance in WO1998/050431, Ridgeway et al., 1996 and Merchant et al. 1998.
Generally, the method involves introducing a protuberance at the interface of a first polypeptide and a corresponding cavity in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heteromultimer formation and hinder homomultimer formation. "Protuberances" or "knobs" are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" or "holes" of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by synthetic means such as altering the nucleic acid encoding the polypeptides or by peptide synthesis.

Using the knob-into-hole technology alone, the proportion of a bispecific antibody of interest is at best 87% of the mixture of the 2 parental and bispecific antibodies. Merchant et al., succeeded in raising the proportion of bispecific antibodies to 95% of the mixture by introduction of an additional disulfide bond between the two CH3 domains in the CH3-CH3 interface. Still, in order to use such bispecific antibody as a medicament, the bispecific antibody has to be purified (separated) from the homodimers and formulated into a pharmaceutically acceptable diluent or excipient. Purification of heterodimers from such mixtures poses a major challenge because of the similarity in physico-chemical properties of the homodimers and heterodimers. It is one object of the present invention to provide methods for producing a bispecific antibody in a single cell clone with a further improved proportion of the bispecific antibody in the mixture. According to the invention, knob-into-hole technology can thus be used as one of the means, alone or together with other means, to achieve said further improved bispecific proportion in a mixture.

Another example of such engineering of the CH3-CH3 interface is provided by a heterodimeric Fc technology that supports the design of bispecific and asymmetric fusion proteins by devising strand-exchange engineered domain (SEED) CH3 heterodimers. These SEED CH3 heterodimers are derivatives of human IgG and IgA CH3 domains that are composed of alternating segments of human IgA and IgG CH3 sequences which results in pairs of complementary human SEED CH3 heterodimers, the so-called SEED-bodies (Davis JH. Et al., Protein Engineering, Design & Selection 2010(23)195-202; WO2007/110205).

Yet another approach for the production of a given bispecific antibody of interest is based on electrostatic engineering of contact residues within the CH3-CH3 interface that are naturally charged, as for example described in EP01870459 or US2010/0015133, WO2007/147901, WO2010/129304, Gunasekaran et al (2010) and WO 2009/089004. These publications describe mutations in the CH3 domains of heavy chains wherein naturally occurring charged amino acid contact residues are replaced by amino acid residues of opposite charge (i.e. a charge reversal strategy). This creates an altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation.

It was described that within the CH3-CH3 interface four unique charges residue pairs are involved in the domain-domain interaction. These are D356/K439', E357/K370', K392/D399' and D399/K409' (numbering according to Kabat (1991) where residues in the first chain are separated from residues in the second chain by '/' and where the prime (') indicates the residue numbering in the second chain). As the CH3-CH3 interface displays a 2-fold symmetry, each unique charge pair is represented twice in intact IgG (i.e., also K439/D356', K370/E357', D399/K392' and K409/D399' charge interactions are present in the interface). Taking advantage of this two-fold symmetry, it was demonstrated that a single charge reversion, e.g. K409D in the first chain, or D399'K in the second chain resulted in diminished homodimer formation due to repulsion of identical charges. Combining different charge reversions further enhanced this repulsive effect. It was demonstrated that expression of different CH3

domains comprising different, complementary charge reversions, could drive heterodimerization, resulting in an increased proportion of the bispecific species in the mixture.

Using the approach described above, it is possible to produce a bispecific antibody in a single cell with proportions ranging between about 76% and about 96%. It is an object of the present invention to provide methods for producing a bispecific antibody in a single cell with a further improved percentage of desired bispecific antibodies. According to the present invention, electrostatic engineering technology can be used as one of the means, alone or together with other means, e.g knob-into-hole approaches, to achieve said further improved percentages of desired (bispecific) antibodies.

In one aspect, the present invention provides a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain,

b) a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

c) a third nucleic acid molecule encoding a 3rd CH3 domain-comprising polypeptide chain, and

d) a fourth nucleic acid molecule encoding a 4th CH3 domain-comprising polypeptide chain,

wherein at least two of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3-domain comprising polypeptides, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.

It is often desired to produce more than one (bispecific) antibody, for instance in order to more efficiently interfere with multiple biological pathways involved in a disease process or with the invasion, replication and/or spreading of a pathogen.

A mixture of more than one bispecific antibody is also particularly useful for the treatment of certain diseases. For example, tumor cells use many different strategies to develop resistance during treatment with antibodies or small molecule drugs. Resistance may involve multiple cell surface receptors and soluble molecules and it is considered beneficial to develop antibody-based treatments for cancers that address multiple such disease- and escape-associated molecules simultaneously. In case more than 2 such disease- and escape-related target molecules or epitopes are involved, a mixture of bispecific antibodies provides an innovative and attractive therapeutic format. Preferably, such mixtures of bispecific antibodies are produced by a single cell to facilitate a drug development process that is less complicated from a regulatory point of view and cost-effective and feasible from a drug manufacturing and clinical development point of view. In a single cell-based approach, it is desirable to use methods that allow controlled and efficient production of the bispecific antibodies, thus reducing or even completely abrogating the need of separating the desired mixture of bispecific IgG molecules from non-desired monospecific IgG molecules. In the prior art, mixtures of monospecific and bispecific antibodies have been produced by a single cell (WO2004/009618), but these mixtures represent complex concoctions of several different bispecific and monospecific antibody species. It is a further object of the present invention to provide means and methods for producing defined mixtures of bispecific antibodies in single cells. Preferably, methods are provided which result in mixtures of (bispecific) antibodies with a proportion of at least 95%, at least 97% or even more than 99% of dimeric IgG molecules, irrespective of the amount of monomeric by-products, see herein below. Typically, in a cell where multiple intact IgG molecules are produced, half molecules (monomeric by-products) may be present that can be simply removed by size exclusion chromatography known in the art.

In one embodiment the present invention provides methods for producing a defined mixture of at least two different Ig-like molecules in single cells, instead of a single (bispecific) antibody of interest, wherein the formation of other, undesired dimeric antibody species is diminished or even absent. The resulting mixture is well defined

and its composition is controlled by the design of CH3 domain mutants. Furthermore, regulation of expression levels and/or different transfection ratios used for expression affects the composition of the mixture. In a method according to the invention, a first nucleic acid molecule encodes a CH3 domain which preferentially pairs with a CH3 domain encoded by a second nucleic acid molecule, and a third nucleic acid molecules encodes a CH3 domain which preferentially pairs with a CH3 domain encoded by a fourth nucleic acid molecule. The present invention also provides mixtures of at least two different Ig-like molecules obtainable by the methods of the invention.

As used herein, the term "preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides" means that essentially all the resulting dimers comprising the 1st CH3 domain-comprising polypeptide and/or the 2nd CH3 domain-comprising polypeptide will be dimers consisting of one 1st CH3 domain-comprising polypeptide paired with one 2nd CH3 domain-comprising polypeptide. Likewise, the term "preferential pairing of said 3rd and 4th CH3 domain-comprising polypeptides" means that essentially all of the resulting dimers comprising the 3rd CH3 domain-comprising polypeptide and/or the 4th CH3 domain-comprising polypeptide will be dimers consisting of one 3rd CH3 domain-comprising polypeptide paired with one 4th CH3 domain-comprising polypeptide. As a result, when nucleic acid molecules encoding four different (A, B, C, D) CH3 domain-comprising polypeptides are introduced in a single cell, instead of a mixture of 10 different Ig-like dimers (AA, AB, AC, AD, BB, BC, BD, CC, CD and DD), a mixture of predominantly two specific Ig-like molecules is produced.

As explained herein below in more detail, in a preferred embodiment said first CH3-domain comprising polypeptide chain comprises the amino acid substitution T366K, and said second CH3-domain comprising polypeptide chain comprises the amino acid substitution L351D. These amino acid changes are preferred means for preferential pairing of said first and second CH3-domain comprising polypeptide chains. Said first CH3-domain comprising polypeptide chain preferably further comprises the amino acid substitution L351K. Moreover, said second CH3-domain comprising polypeptide chain preferably further comprises an amino acid substitution selected from the group consisting of Y349E, Y349D and L368E, most preferably L368E. In yet another preferred embodiment, said third CH3-domain comprising polypeptide chain comprises the amino acid substitutions E356K and D399K, and said fourth CH3-

domain comprising polypeptide chain comprises the amino acid substitutions K392D and K409D.

In a method according to the present invention, each of the CH3-domain comprising polypeptide chains preferably further comprises a variable region recognizing a target epitope. The variable regions that are part of the CH3-domain comprising polypeptide chains preferably share a common light chain. In that case only the VHs of the variable regions differ whereas the VL in all variable regions is essentially the same. Hence, in a preferred aspect a method according to the invention is provided, which further comprises providing said host cell with a nucleic acid molecule encoding a common light chain. In one particularly preferred embodiment, each of said 4 variable regions of the 4 CH3-domain comprising polypeptide chains recognizes a different target epitope. For instance, if the first nucleic acid molecule encodes a heavy chain that further contains a variable domain with specificity for antigen A, the second nucleic acid molecule encodes a heavy chain that further contains a variable domain with specificity for antigen B, the third nucleic acid molecule encodes a heavy chain that further contains a variable domain with specificity for antigen C, and the fourth nucleic acid molecule encodes a heavy chain that further contains a variable domain with specificity for antigen D, a mixture will then be produced containing bispecific Ig-like molecules that are specific for AB and bispecific Ig-like molecules that are specific for CD. The formation of monospecific antibodies (with AA, BB, CC or DD specificity) or bispecific antibodies with specificity for AC, AD, BC or BD is lowered or even absent due to the means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3 domain-comprising polypeptides. It is, of course, possible to use further nucleic acid molecules, for instance encoding a 5th and a 6th CH3 domain-comprising polypeptide, in order to produce defined mixtures comprising more than two different Ig-like molecules.

Of note, the ratio of the nucleic acids used in a method according to the invention does not need to be 1:1:1:1 and the ratio of the resulting Ig-like molecules that are expressed does not need to be 1:1. It is possible to use means known in the art to produce mixtures of antibodies with optimized ratios. For instance, expression levels of nucleic acid molecules and hence the ratios of the resulting Ig-like molecules produced may be regulated by using different genetic elements such as promoters,

enhancers and repressors or by controlling the genomic integration site of copy number of the DNA constructs encoding antibodies.

Said means for preferential pairing preferably may comprise engineered complementary knob-into-hole mutations, disulfide bridges, charge mutations including charge reversal mutations, or combinations thereof. The skilled person will appreciate that said means for preferential pairing may be chosen within a certain type of mutations, i.e. all at least 4 nucleic acid molecules encoding CH3-domain comprising polypeptide chains may for example comprise charge mutations as means for preferential pairing. Additionally, also non-engineered wildtype CH3 may in certain instances be used for preferential pairing of two wildtype CH3-domain comprising polypeptide chains. In a particularly preferred embodiment, said means for preferential pairing comprise at least one CH3 mutation selected from Table B, as explained elsewhere in this application. One preferred embodiment thus provides a method according to the present invention, wherein all 4 of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3-domain comprising polypeptides, wherein said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides are different from those means for preferential pairing of said 3rd and 4th CH3-domain comprising polypeptides.

One aspect of the present invention provides a method according to the invention, wherein said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides are different from said means for preferential pairing of said 3rd and 4th CH3-domain comprising polypeptides. By 'different' it is meant that the means for preferential pairing of said 1st and 2nd CH3 domain comprising polypeptides are designed such that preferential pairing of the 1st and 2nd chain is favoured. The design is such that essentially no interaction between the 1st and the 3rd and/or 4th CH3 domain comprising polypeptide chain will take place. In other words, dimerization between said 1st CH3 domain comprising polypeptide and said 3rd or 4th polypeptide is reduced to essentially zero and so forth. The 3rd and the 4th CH3 domain-comprising polypeptides may either be wildtype or may comprise means for preferential pairing that are different from the means for preferential pairing of the 1st and 2nd CH3 domains. Current studies have focused on the production of a single

bispecific antibody, using for instance the knob-into-hole technology or mutations (reversions) of charged contact amino acids present in CH3 domains. Production of defined mixtures of at least two (bispecific) Ig-like molecules, without significant co-production of other dimeric by-products, has, however, not been realized prior to the present invention.

The present invention provides methods for the efficient and controlled production of a well-defined mixture of Ig-like molecules, with a high proportion of bispecifics in the mixture. Even a proportion of (two) bispecifics of at least 95%, at least 97% or more is obtained in a system where two bispecifics are desired. This means that only at most 5%, at most 3% or less monospecific bivalent by-products are obtained. Of note, the amount of monomeric by-products, i.e. half molecules, is less important since these half-molecules are easily separated from dimers using their size difference.

In another preferred embodiment, the variable regions of the 1st and the 2nd CH3-domain comprising polypeptide chains recognize different target epitopes, whereas the variable regions of the 3rd and the 4th CH3-domain comprising polypeptide chains recognize the same target epitopes. This will result in the predominant production of one kind of bispecific Ig-like molecule and one kind of monospecific Ig-like molecule. For instance, if the variable regions of the 1st and the 2nd CH3-domain comprising polypeptide chains recognize different target epitopes and if the variable regions of the 3rd and the 4th CH3-domain comprising polypeptide chains both recognize the same target epitope which is different from the target epitopes recognized by the 1st and the 2nd CH3-domains, a mixture of Ig-like molecules having specificity for AB or CC will be formed. Further provided is therefore a method according to the invention, wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain comprising polypeptide chain is the same, but different from the target epitope recognized by the variable region of the 1st or the 2nd CH3-domain comprising polypeptide chain.

Alternatively, when the variable regions of the 1st and the 2nd CH3-domain comprising polypeptide chains recognize different target epitopes and when the variable regions of the 3rd and the 4th CH3-domain comprising polypeptide chains both recognize the same epitope as the 1st or the 2nd CH3-domain comprising polypeptide chains, a

mixture of Ig-like molecules having specificity for AB and AA, or AB and BB will be formed. A method according to the invention, wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain comprising polypeptide chain is the same as the target epitope recognized by the variable region of the 1st or the 2nd CH3-domain comprising polypeptide chain is therefore also herewith provided.

It is another object of the present invention to provide means and methods for producing defined mixtures of bispecific antibodies and monospecific antibodies in a single cell culture. A non-limiting example of such well-defined mixture is a mixture of bispecific antibodies with specificity AB and monospecific antibodies with specificity AA. Another example is a mixture of bispecific antibodies with specificity AB and monospecific antibodies with specificity BB. Yet another example is a mixture of bispecific antibodies with specificity AB and monospecific antibodies with specificity CC. Again, preferably means and methods are provided which yield mixtures of antibodies of interest with at least 90%, more preferably at least 95% and most preferably at least 97% or even more than 99% of desired antibodies.

In yet another embodiment, a method according to the invention is provided wherein the variable regions of the 1st and the 2nd CH3-domain comprising polypeptide chains recognize the same target epitope, whereas the variable regions of the 3rd and the 4th CH3-domain comprising polypeptide chains recognize a second target epitope which differs from the target epitope recognized by said 1st and 2nd variable regions. This will result in the predominant production of monospecific Ig-like molecules having either specificity for AA or specificity for BB. The formation of bispecific Ig-like molecules is diminished or even avoided. In several embodiments it is preferred to produce mixtures of monospecific antibodies in a single cell, rather than mixtures of bispecific antibodies. For instance when cross-linking of two identical target molecules is desired, or when two targets are located too far away from each other so that they cannot be bound by a single bispecific antibody. It can also be advantageous to produce mixtures of monospecific antibodies in a single cell as the mixture can be regarded as a single therapeutic product. In the art, the therapeutic efficacy and safety of various monospecific antibodies has already been proven and market authorisation has been obtained. Production of mixtures of monospecific antibodies in a single cell will thus facilitate the testing for efficacy and safety of several of such

mixtures and will reduce the efforts and costs for regulatory approval and manufacturing. There are, however, currently no methods available for producing specific mixtures of monospecific antibodies in a single cell wherein the formation of bispecific by-products is reduced to below 5%. It is another object of the present invention to provide means and methods for producing such well-defined homodimeric antibody mixtures in single cells wherein the formation of bispecific antibodies is reduced to below 5%.

Hence, a method according to the present invention is suitable for the production of any desired mixture of bispecific and/or monospecific Ig-like molecules. Again, it is possible to use further nucleic acid molecules, for instance encoding a 5th and a 6th (and 7th and 8th and so forth) CH3 domain-comprising polypeptide, in order to produce defined mixtures comprising more than two different Ig-like molecules.

Preferably, in a method according to the present invention at least two CH3 domains are used that comprise at least one combination of mutations provided by the present invention. Through these mutations novel specific interactions are formed between two CH3 domains. These mutations according to the present invention are discussed below in more detail.

The term 'Ig-like molecule' as used herein means a proteinaceous molecule that possesses at least one immunoglobulin (Ig) domain. Said Ig-like molecule comprises a sequence comprising the function of at least an immunoglobulin CH3 domain, preferably the sequence comprises an IgG1 CH3 domain. Proteinaceous molecules that possess at least a CH3 domain can be further equipped with specific binding moieties. The CH3 domains of the present invention, containing means for preferential pairing, can thus be used for preferential pairing of two CH3-domain comprising proteinaceous molecules to design desired heterodimeric binding molecules or mixtures of binding molecules. Binding moieties that can be engineered to the CH3-domain comprising proteinaceous molecules can be any binding agent, including, but not limited to, single chain Fvs, single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, a BiTE®, a Fab, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®,

Centyrins® or a KALBITOR®. In a preferred embodiment, the binding moieties are antibody variable regions (i.e. VH/VL combinations). Variable regions that are part of the CH3-domain comprising polypeptide chains preferably share a common light chain. In that case, only the VHs of the variable regions differ whereas the VL in all variable regions is essentially the same.

Alternatively, or in addition, other molecules can be engineered to the CH3 domains of the present invention, including cytokines, hormones, soluble ligands, receptors and/or peptides.

In a more preferred embodiment, said Ig-like molecule comprises a full length Fc backbone. In a most preferred embodiment, the Ig-like molecules are antibodies. The variable regions of these antibodies preferably share a common light chain, but they may differ in their VH regions. The term 'antibody' as used herein means a proteinaceous molecule belonging to the immunoglobulin class of proteins, containing one or more domains that bind an epitope on an antigen, where such domains are derived from or share sequence homology with the variable region of an antibody. Antibodies are known in the art and include several isotypes, such as IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, and IgM. An antibody according to the invention may be any of these isotypes, or a functional derivative and/or fragment of these. In a preferred embodiment, Ig-like molecules are produced that are antibodies of the IgG isotype because IgG antibodies e.g. have a longer half life as compared to antibodies of other isotypes.

Antibodies produced with methods according to the present invention can have sequences of any origin, including murine and human sequences. Antibodies can consist of sequences from one origin only, such as fully human antibodies, or they can have sequences of more than one origin, resulting for instance in chimeric or humanized antibodies. Antibodies for therapeutic use are preferably as close to natural antibodies of the subject to be treated as possible (for instance human antibodies for human subjects). Antibody binding can be expressed in terms of specificity and affinity. The specificity determines which antigen or epitope thereof is bound by the binding domain. The affinity is a measure for the strength of binding to a particular antigen or epitope. Specific binding is defined as binding with affinities (K_D) of at least $1x10^{-5}$ M, more preferably $1x10^{-7}$ M, more preferably higher than $1x10^{-}$

^{9}M. Typically, monoclonal antibodies for therapeutic applications have affinities of up to $1x10^{-10}$ M or even higher. The term 'antigen' as used herein means a substance or molecule that, when introduced into the body, triggers the production of an antibody by the immune system. An antigen, among others, may be derived from pathogenic organisms, tumor cells or other aberrant cells, from haptens, or even from self structures. At the molecular level, an antigen is characterized by its ability to be bound by the antigen-binding site of an antibody. Also mixtures of antigens can be regarded as 'antigen', i.e. the skilled person would appreciate that sometimes a lysate of tumor cells, or viral particles may be indicated as 'antigen' whereas such tumor cell lysate or viral particle preparation exists of many antigenic determinants. An antigen comprises at least one, but often more, epitopes. The term 'epitope' as used herein means a part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. Although epitopes are usually thought to be derived from non-self proteins, sequences derived from the host that can be recognized are also classified as epitopes.

The term 'CH3 domain' is well known in the art. The IgG structure has four chains, two light and two heavy chains; each light chain has two domains, the variable and the constant light chain (VL and CL) and each heavy chain has four domains, the variable heavy chain (VH) and three constant heavy chain domains (CH1, CH2, CH3). The CH2 and CH3 domain region of the heavy chain is called Fc (Fragment crystallizable) portion, Fc fragment, Fc backbone or simply Fc. The IgG molecule is a heterotetramer having two heavy chains that are held together by disulfide bonds (-S-S-) at the hinge region and two light chains. The heavy chains dimerize through interactions at the CH3-CH3 domain interface and through interactions at the hinge region. The number of hinge disulfide bonds varies among the immunoglobulin subclasses (Papadea and Check 1989). The Fc fragment of an immunoglobulin molecule is a dimer of the two C-terminal constant regions, i.e. CH2 and CH3 domains, of the heavy chain. Among its physiological functions are interactions with the complement system and with specific receptors on the surface of a variety of cells. Interactions between the CH3 domains of two individual heavy chains are known to play an important role in driving heavy chain dimerization. Thus, CH3 domains direct the association of antibody heavy chains, and it is known that the interface between CH3 domains contains more than 20 contact residues from each chain that play a role

in the CH3-CH3 interaction (Deisenhofer J., Biochemistry 1981(20)2361-2370; Miller S., J. Mol. Biol. 1990(216)965-973; Padlan, Advances in Protein Chemistry 1996 (49) 57-133). The CH3 variants of the present invention can thus be used in association with other antibody domains to generate full length antibodies that are either bispecific or monospecific. The specificity of the antibody as defined by the VH/VL combinations typically does not affect the heavy chain dimerization behaviour that is driven by the CH3 domains.

The terms 'contact residue', 'contact amino acid', 'interface residue' and 'interface amino acid' as used herein typically refers to any amino acid residue present in the CH3 domain that can be involved in interdomain contacts, as can be calculated by technologies known in the art, including calculating solvent accessible surface area (ASA) of the CH3 domain residues in the presence and absence of the second chain (Lee and Richards J. Mol. Biol.1971(55)379) where residues that show difference (> lÅ^2) in ASA between the two calculations are identified as contact residues. Contact residues that have been identified include residues at positions 347, 349, 350, 351, 352, 353, 354, 355, 356, 357, 360, 364, 366, 368, 370, 390, 392, 394, 395, 397, 399, 400, 405, 407, 409, 439 according to the EU numbering system (Table A).

Table A: List of CH3 domain interface residues

Interface residue in chain A	**Contacting residues in chain B**
Q347	K360
Y349	S354, D356, E357, K360
T350	S354, R355
L351	L351, P352, P353, S354, T366
S354	Y349, T350, L351
R355	T350
D356	Y349, K439
E357	Y349, K370
K360	Q347, Y349
S364	L368, K370
T366	L351, Y407
L368	S364, K409
K370	E357, S364
N390	S400
K392	L398, D399, S400, F405
T394	T394, V397, F405, Y407
P395	V397
V397	T394, P395
D399	K392, K409
S400	N390, K392
F405	K392, T394, K409
Y407	T366, T394, Y407, K409
K409	L368, D399, F405, Y407
K439	D356

Contact residues within the CH3-CH3 interface can either be amino acids that are charged, or amino acid residues that are neutral. The term 'charged amino acid residue' or 'charged residue' as used herein means amino acid residues with electrically charged side chains. These can either be positively charged side chains, such as present in arginine (Arg, R), histidine (His, H) and lysine (Lys, K) or can be negatively charged side chains, such as present in aspartic acid (Asp, D) and glutamic acid (Glu, E). The term 'neutral amino acid residue' or neutral residue as used herein refers to all other amino acids that do not carry electrically charged side chains. These neutral residues include serine (Ser, S), threonine (Thr, T), asparagine (Asn, N), glutamine (GLu, Q), Cysteine (Cys, C), glycine (Gly, G), proline (Pro, P), alanine (Ala, A), valine (Val, V), isoleucine (Ile, I), leucine (Leu, L), methionine (Met, M), phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, T).

The term 'CH3-CH3 domain interface', or 'CH3 interface', 'CH3-CH3 pairing', 'domain interface' or simply 'interface', as used herein, refers to the association between two

CH3 domains of separate CH3-domain comprising polypeptides that is a result of interacting amino acid residues, i.e. at least one interaction between an amino acid of a first CH3 domain and an amino acid of a second CH3 domain. Such interaction is for instance via Van der Waals forces, hydrogen bonds, water-mediated hydrogen bonds, salt bridges or other electrostatic forces, attractive interactions between aromatic side chains, the formation of disulfide bonds, or other forces known to one skilled in the art.

As used herein, said means for preferential pairing of the first and second CH3 domain-comprising polypeptides and said third and fourth CH3 domain-comprising polypeptide can be any means known in the art. In one embodiment, at least one nucleic acid molecule encodes a CH3 domain which contains at a contact residue position a large amino acid residue (i.e. a "knob" or "protuberance") such as for instance R, F, Y, W, I or L, whereas at least one other nucleic acid molecule encodes a CH3 domain which contains at a complementary contact residue position a small amino acid residue (i.e. a "hole" or "cavity") such as for instance G, A, S, T or V. The resulting CH3 domains will preferentially pair with each other due to the steric conformation of said contact amino acids. The knob-into-hole technology is described herein before in more detail. In a further embodiment of the present invention, at least one nucleic acid molecule encodes a CH3 domain which contains at a contact residue position that is naturally charged, i.e. a naturally occurring K, H, R, D or E, an amino acid that now carries the opposite charge as compared to wildtype, whereas at least one other nucleic acid molecule encodes a CH3 domain which contains at a complementary contact residue position that is naturally charged, an amino acid that now carries the opposite charge as compared to wildtype. The resulting engineered CH3 domains will preferentially pair with each other due to the opposite charges of said contact amino acids, whereas pairing of identical CH3 domains will be diminished due to electrostatic repulsion. In one embodiment, CH3 mutations as described in EP01870459, WO 2009/089004, Gunasekaran et al (2010), are used. In one embodiment, the means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides are "knob" and "hole" amino acid residues and the means for preferential pairing of said 3th and 4th CH3 domain-comprising polypeptides are charge-engineered amino acids. Preferably, both said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3th and 4th CH3

domain-comprising polypeptides are charge-engineered amino acids. In one embodiment, different amino acid residues are engineered for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides as compared to the amino acid residues that are engineered for preferential pairing of said 3th and 4th CH3 domain-comprising polypeptides. In a particularly preferred embodiment at least a first and a second nucleic acid molecule encode CH3 domains with novel mutations as provided by the present invention. As described herein below in more detail, the present invention provides novel CH3 mutations which enable the production of certain bispecific Ig-like molecules of interest without a significant amount of undesired (dimeric) by-products. The present invention also provides novel CH3 mutations which enable the production of certain monospecific Ig-like molecules of interest without a significant amount of undesired (dimeric) by-products. The use of at least one of these CH3 mutations according to the present invention is, therefore, preferred.

The term 'polypeptide', 'polypeptide molecule' or 'polypeptide chain' as used herein refers to a chain of amino acids that are covalently joined together through peptide bonds. Proteins are typically made up of one or more polypeptide molecules. One end of every polypeptide, called the amino terminal or N-terminal, has a free amino group. The other end, with its free carboxyl group, is called the carboxyl terminal or C-terminal. Polypeptides according to the present invention may have gone through post-translational modification processes and may e.g. be glycosylated. The CH3 domain-comprising polypeptide chains of the present invention thus refer to polypeptide chains that at least encompass an Ig CH3 domain and that may have gone through post-translational modification processes.

The term "nucleic acid molecule" as used herein is defined as a molecule comprising a chain of nucleotides, more preferably DNA and/or RNA. In one embodiment, double-stranded RNA is used. In other embodiments a nucleic acid molecule of the invention comprises other kinds of nucleic acid structures such as for instance a DNA/RNA helix, peptide nucleic acid (PNA), locked nucleic acid (LNA) and/or a ribozyme. Hence, the term "nucleic acid molecule" also encompasses a chain comprising non-natural nucleotides, modified nucleotides and/or non-nucleotide building blocks which exhibit the same function as natural nucleotides.

The present invention further provides a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain comprising polypeptide chain, wherein at least two of said nucleic acid sequences are provided with means for preferential pairing of said first and second CH3-domain comprising polypeptides and said third and fourth CH3-domain comprising polypeptides, wherein said nucleic acid sequences are introduced consecutively or concomitantly.

It is a further aspect of the present invention to provide a method for making a host cell for production of a heterodimeric Ig-like molecule, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first and a second CH3-domain comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and wherein said second CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue, wherein said nucleic acid sequences are introduced consecutively or concomitantly. Said methods for making said host cells preferably further comprise the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.

Also provided herein is a recombinant host cell comprising nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain comprising polypeptide chain, wherein at least two of said nucleic acid molecules are provided with means for preferential pairing of said first and second CH3-domain comprising polypeptides and said third and fourth CH3-domain comprising polypeptides.
The invention furthermore provides a recombinant host cell comprising nucleic acid sequences encoding at least a first and a second CH3-domain comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and wherein said second CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue.

A recombinant host cell according to the invention preferably further comprises a nucleic acid sequence encoding a common light chain.

A "host cell" according to the invention may be any host cell capable of expressing recombinant DNA molecules, including bacteria such as for instance *Escherichia* (e.g. *E. coli*), *Enterobacter, Salmonalla, Bacillus, Pseudomonas, Streptomyces*, yeasts such as *S. cerevisiae, K. lactis, P. pastoris, Candida*, or *Yarrowia*, filamentous fungi such as *Neurospora, Aspergillus oryzae, Aspergillus nidulans* and *Aspergillus niger*, insect cells such as *Spodoptera frugiperda* SF-9 or SF-21 cells, and preferably mammalian cells such as Chinese hamster ovary (CHO) cells, BHK cells, mouse cells including SP2/0 cells and NS-0 myeloma cells, primate cells such as COS and Vero cells, MDCK cells, BRL 3A cells, hybridomas, tumor-cells, immortalized primary cells, human cells such as W138, HepG2, HeLa, HEK293, HT1080 or embryonic retina cells such as PER. C6, and the like. Often, the expression system of choice will involve a mammalian cell expression vector and host so that the antibodies can be appropriately glycosylated. A human cell line, preferably PER.C6, can advantageously be used to obtain antibodies with a completely human glycosylation pattern. The conditions for growing or multiplying cells (see e. g. Tissue Culture, Academic Press, Kruse and Paterson, editors (1973)) and the conditions for expression of the recombinant product may differ somewhat, and optimization of the process is usually performed to increase the product proportions and/or growth of the cells with respect to each other, according to methods generally known to the person skilled in the art. In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach (M. Butler, ed., IRL Press, 1991). Expression of antibodies in recombinant host cells has been extensively described in the art (see e.g. EP0120694; EP0314161; EP0481790; EP0523949; US patent 4,816,567; WO 00/63403). The nucleic acid molecules encoding the light and heavy chains may be present as extrachromosomal copies and/or stably integrated into the chromosome of the host cell, the latter is preferred.

It is a further aspect of the present invention to provide a culture of recombinant host cells according to the invention, or a culture of recombinant host cells obtainable or obtained by a method according to the invention, said culture either producing at

least two different Ig-like molecules or a heterodimeric Ig-like molecule.

To obtain expression of nucleic acid sequences encoding the CH3 domain-comprising polypeptides, it is well known to those skilled in the art that sequences capable of driving such expression can be functionally linked to the nucleic acid sequences encoding the CH3 domain-comprising polypeptides. Functionally linked is meant to describe that the nucleic acid sequences encoding the CH3 domain-comprising polypeptides or precursors thereof is linked to the sequences capable of driving expression such that these sequences can drive expression of the CH3 domain-comprising polypeptides or precursors thereof. Useful expression vectors are available in the art, e.g. the pcDNA vector series of Invitrogen. Where the sequence encoding the polypeptide of interest is properly inserted with reference to sequences governing the transcription and translation of the encoded polypeptide, the resulting expression cassette is useful to produce the polypeptide of interest, referred to as expression. Sequences driving expression may include promoters, enhancers and the like, and combinations thereof. These should be capable of functioning in the host cell, thereby driving expression of the nucleic acid sequences that are functionally linked to them. Promoters can be constitutive or regulated, and can be obtained from various sources, including viruses, prokaryotic, or eukaryotic sources, or artificially designed. Expression of nucleic acids of interest may be from the natural promoter or derivative thereof or from an entirely heterologous promoter. Some well-known and much used promoters for expression in eukaryotic cells comprise promoters derived from viruses, such as adenovirus, e.g. the E1A promoter, promoters derived from cytomegalovirus (CMV), such as the CMV immediate early (IE) promoter, promoters derived from Simian Virus 40 (SV40), and the like. Suitable promoters can also be derived from eukaryotic cells, such as methallothionein (MT) promoters, elongation factor 1α (EF-1α) promoter, actin promoter, an immunoglobulin promoter, heat shock promoters, and the like. Any promoter or enhancer/promoter capable of driving expression of the sequence of interest in the host cell is suitable in the invention. In one embodiment the sequence capable of driving expression comprises a region from a CMV promoter, preferably the region comprising nucleotides –735 to +95 of the CMV immediate early gene enhancer/promoter. The skilled artisan will be aware that the expression sequences used in the invention may suitably be combined with elements that can stabilize or enhance expression, such as insulators, matrix attachment regions, STAR

elements (WO 03/004704), and the like. This may enhance the stability and/or levels of expression.

Protein production in recombinant host cells has been extensively described, e.g. in Current Protocols in Protein Science, 1995, Coligan JE, Dunn BM, Ploegh HL, Speicher DW, Wingfield PT, ISBN 0-471-11184-8; Bendig, 1988. Culturing a cell is done to enable it to metabolize, and/or grow and/or divide and/or produce recombinant proteins of interest. This can be accomplished by methods well known to persons skilled in the art, and includes but is not limited to providing nutrients for the cell. The methods comprise growth adhering to surfaces, growth in suspension, or combinations thereof. Several culturing conditions can be optimized by methods well known in the art to optimize protein production yields. Culturing can be done for instance in dishes, roller bottles or in bioreactors, using batch, fed-batch, continuous systems, hollow fiber, and the like. In order to achieve large scale (continuous) production of recombinant proteins through cell culture it is preferred in the art to have cells capable of growing in suspension, and it is preferred to have cells capable of being cultured in the absence of animal- or human-derived serum or animal- or human-derived serum components. Thus purification is easier and safety is enhanced due to the absence of additional animal or human proteins derived from the culture medium, while the system is also very reliable as synthetic media are the best in reproducibility.

Ig-like molecules are expressed in host cells and are harvested from the cells or, preferably, from the cell culture medium by methods that are generally known to the person skilled in the art. After harvesting, these Ig-like molecules may be purified by using methods known in the art. Such methods may include precipitation, centrifugation, filtration, size-exclusion chromatography, affinity chromatography, cation- and/or anion-exchange chromatography, hydrophobic interaction chromatography, and the like. For a mixture of antibodies comprising IgG molecules, protein A or protein G affinity chromatography can be suitably used (see e.g. US patents 4,801,687 and 5,151,504).

Ig-like molecules, and/or mixtures thereof, produced with methods according to the present invention preferably have a common light chain. Further provided is,

therefore, a method according to the invention, further comprising providing said host cell with a nucleic acid molecule encoding a common light chain. This is a light chain that is capable of pairing with at least two different heavy chains, thereby forming functional antigen binding domains. A functional antigen binding domain is capable of specifically binding to an antigen. Preferably, a common light chain is used that is capable of pairing with all heavy chains produced with a method according to the invention, thereby forming functional antigen binding domains, so that mispairing of unmatched heavy and light chains is avoided. In one aspect, only common light chains with one identical amino acid sequence are used. Alternatively, those of skill in the art will recognize that "common" also refers to functional equivalents of the light chain of which the amino acid sequence is not identical. Many variants of said light chain exist wherein mutations (deletions, substitutions, additions) are present that do not materially influence the formation of functional binding regions. Such variants are thus also capable of binding different heavy chains and forming functional antigen binding domains. The term 'common light chain' as used herein thus refers to light chains which may be identical or have some amino acid sequence differences while retaining the binding specificity of the resulting antibody after pairing with a heavy chain. It is for instance possible to prepare or find light chains that are not identical but still functionally equivalent, e.g. by introducing and testing conservative amino acid changes, and/or changes of amino acids in regions that do not or only partly contribute to binding specificity when paired with the heavy chain, and the like. A combination of a certain common light chain and such functionally equivalent variants is encompassed within the term "common light chain". Reference is made to WO 2004/009618 for a detailed description of the use of common light chains.

Preferably, a common light chain is used in the present invention which is a germline-like light chain, more preferably a germline light chain, preferably a rearranged germline human kappa light chain, most preferably either the rearranged germline human kappa light chain IgVκ1-39/Jκ or IGVκ3-20/Jκ.

Alternatively, the skilled person may select, as an alternative to using a common light chain and to avoid mispairing of unmatched heavy and light chains, means for forced pairing of the heavy and light chain, such as for example described in WO2009/080251, WO2009/080252 and/or WO2009/080253.

The present invention provides novel engineered CH3 domains as well as novel combinations of CH3 mutations. Before the present invention, charged contact amino acids of CH3 domains that were known to be involved in CH3-CH3 pairing were substituted by amino acids of opposite charge (charge reversal), thereby influencing the CH3-CH3 pairing. The mutations according to the present invention are an inventive alternative to this approach, because now CH3 amino acids that are non-charged or neutral in wildtype CH3 are substituted with charged residues. The present invention in this embodiment does not exchange charged contact amino acids by amino acids of opposite charge but substitutes non-charged CH3 amino acids for charged ones. The approach of the present invention provides not only a method for efficiently steering the dimerization of CH3 domains but also has the advantage that at least one additional charge-charge interaction in the CH3 interface is created. In view of this additional charge-charge interaction on top of the existing charge-pairs in the CH3-CH3 interface, the dimers according to the invention are generally more stable as compared to the wild type dimers (the wild type dimer is defined as a bispecific IgG (AB) without CH3 engineering in contrast to its parental homodimers (AA or BB)). Moreover, it has surprisingly become possible to increase the proportion of one or more Ig-like molecules of interest in a mixture even further. As described herein before, methods known in the art for preferential production of a bispecific antibody typically involves the production of some undesired dimeric side products. For instance, the proportion of a bispecific antibody of interest using the knob-into-hole technology is at best 87%, whereas the electrostatic engineering approach wherein charged contact amino acids are substituted by amino acids of opposite charge, also results in proportions of up to 96% (see for instance Example 11). Quite surprisingly, the present inventors have succeeded in introducing mutations that further enhance the proportion of an Ig-like molecule of interest in a mixture. For instance, Example 17 discloses a method using mutations according to the present invention, wherein the proportion of a bispecific antibody of interest was raised to such extent that no dimeric by-product was detectable in the resulting mixture at all. Unpaired half-molecules consisting of only a single heavy chain paired with a common light chain were present to some extent in the mixtures, but these are the result of unbalanced expression of the heavy chains and can be easily separated from the mixture by size exclusion chromatography. Hence, with such mutations according to the present invention, a bispecific Ig-like molecule can be produced in a single cell

with a high proportion with essentially no contaminating dimeric by-products being present, which is particularly suitable for the production of a pharmaceutical composition.

One preferred embodiment of the present invention therefore provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a. A first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain,

b. A second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and wherein said second CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue, said method further comprising the step of culturing said host cell and allowing for expression of said two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

Said method preferably further comprises the step of providing said host cell with a nucleic acid molecule encoding a common light chain, which has advantages as outlined herein before.

The amino acids at position 366 of one CH3 domain and position 351 of a second CH3 domain have been reported to be a pair of contact residues in the CH3-CH3 interface, meaning that they are located sufficiently close to each other in the three-dimensional conformation of the resulting Ig-like molecule in order to be capable of interacting with each other. Hence, the first CH3 domain will preferentially pair with the second CH3 domain.

In one embodiment, threonine (T) at position 366 of a first CH3 domain is replaced by a first charged amino acid and leucine (L) at position 351 of a second CH3 domain is replaced by a second charged amino acid, wherein said first and second charged amino acids are of opposite charge. If the first CH3 domain-comprising polypeptide, that

carries a charged residue at position 366, further comprises a variable domain which has specificity for antigen A, and if the second CH3 domain-comprising polypeptide, that carries an oppositely charged residue at position 351, further comprises a variable domain which has specificity for antigen B, bispecific Ig-like molecules with an AB specificity will be predominantly formed. Further provided is therefore a method according to the present invention, wherein said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides or said means for preferential pairing of said 3rd and 4th CH3 domain-comprising polypeptides are a substitution of threonine at position 366 of said 1st or 3rd CH3 domain by a first charged amino acid and substitution of leucine at position 351 of said 2nd or 4th CH3 domain by a second charged amino acid, wherein said first and second charged amino acids are of opposite charge.

One preferred combination of mutations according to the present invention is the substitution of threonine (T) by lysine (K) at position 366 of a first CH3 domain-comprising polypeptide which further comprises a variable domain (for instance with specificity A) and the substitution of leucine (L) by aspartic acid (D) at position 351 of a second CH3 domain-comprising polypeptide which further comprises a variable domain (for instance with specificity B). This is denoted as a T366K/L351'D pair mutation. As explained before, the amino acids at position 366 of one CH3 domain and position 351 of a second CH3 domain have been reported to be a pair of contact residues in the CH3-CH3 interface. The lysine that is introduced at position 366 and the aspartic acid introduced at position 351 have opposite charges, so that these amino acids will electrostatically attract each other. Hence, the first CH3 domain will preferentially attract the second CH3 domain and Ig-like molecules comprising a first CH3 domain containing lysine at position 366 paired with a second CH3 domain containing aspartic acid at position 351 will be predominantly formed. If the first CH3 domain-comprising polypeptide has specificity for antigen A, and if the second CH3 domain-comprising polypeptide has specificity for antigen B, bispecific Ig-like molecules with 'AB' specificity will be predominantly formed. Nota bene, in some embodiments the specificity of the variable domains of both said first and second CH3-domain comprising polypeptide chains may be the same, which will result in the formation of monospecific Ig-like molecules (for instance with 'AA' specificity) . As mentioned above, one of the advantages of the mutations according to the present

invention is the fact that a novel interaction between a newly introduced pair of charged amino acids is created, instead of replacing existing charged amino acid interactions. This was not previously disclosed or suggested. One aspect of the invention therefore provides a method according to the present invention for producing at least two different Ig-like molecules from a single host cell, wherein said 1st CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K, and said 2nd CH3 domain-comprising polypeptide chain comprises the amino acid substitution L351D. One embodiment provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and
- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitution L351D, said method further comprising the step of culturing said host cell and allowing for expression of said two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

Using the above mentioned amino acid substitutions according to the present invention, it has become possible to produce a heterodimeric Ig-like molecule from a single cell, whereby the presence of contaminating homodimers is less than 5%, preferably less than 2%, more preferably less than 1%, or, most preferably, whereby contaminating homodimers are essentially absent. One embodiment therefore provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein the presence of contaminating homodimers is less than 5%, preferably less than 2%, more preferably less than 1%, and most preferably contaminating homodimers are essentially absent, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and

- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitution L351D, said method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

Preferably, a method according to the present invention for producing at least two different Ig-like molecules, or a method according to the invention for producing a heterodimeric Ig-like molecule, is provided wherein said first CH3-domain comprising polypeptide chain further comprises the amino acid substitution L351K. It is further preferred that said second CH3-domain comprising polypeptide chain further comprises an amino acid substitution selected from the group consisting of Y349E, Y349D and L368E. Most preferably said second CH3-domain comprising polypeptide chain further comprises the amino acid substitution L368E.

Thus, in a preferred embodiment the above mentioned T366K/L351'D mutations according to the present invention are further combined with the substitution of leucine (L) by glutamic acid (E) at position 368 of the second CH3 domain. This is, for example, denoted as a T366K/L351'D,L368'E mutation (but alternative ways of denoting are also possible, such as T336K/L351D-L368E or T366K/L351D,L368E or T366K - L351D,L368E). As shown in Example 17, introduction of this mutation according to the invention into a first CH3 domain-comprising polypeptide with specificity for antigen A, and a second CH3 domain-comprising polypeptide with specificity for antigen B results in a particular good proportion of bispecific Ig-like molecules with dual AB specificity. With this mutational pair it has even become possible to obtain bispecific antibody without any detectable amount of homodimers formed. A particularly preferred embodiment therefore provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein the presence of contaminating homodimers is less than 5%, preferably less than 2%, more preferably less than 1%, and most preferably contaminating homodimers are essentially absent, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and
- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitutions L351D and L368E, said method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

In yet another preferred embodiment, threonine (T) is substituted by lysine (K) at position 366 of a first CH3 domain and leucine (L) is substituted by aspartic acid (D) at position 351 of a second CH3 domain and tyrosine (Y) is substituted by glutamic acid (E) at position 349 of said second CH3 domain. This is for example denoted as a T366K/L351'D,Y349'E mutation but other ways of denoting these mutations may include for example T366K – L351D:Y349E, or T366K/L351D,Y349E or simply T366K/L351DY349E. Residue Y349 is a neighboring residue of the residue at position 351 that may contribute to dimer interactions. According to in silico data, Y349E adds to the stability of the heterodimer (lower in silico scores) as well as to the destabilization of the monodimer (higher in silico scores) and glutamic acid (E) on position 349 is more favorable than aspartic acid (D). Thus, introduction of a second amino acid substitution in the second CH3 domain comprising polypeptide, comprising already the amino acid substitution at position 351, favors heterodimerization further.

A particularly preferred embodiment therefore provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein contaminating homodimers are less than 5%, more preferably less than 2%, even more preferably less than 1%, and most preferably essentially absent, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and

- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitutions L351D and Y349E, said method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

In yet another preferred embodiment, threonine (T) is substituted by lysine (K) at position 366 of a first CH3 domain and leucine (L) is substituted by aspartic acid (D) at position 351 of a second CH3 domain and tyrosine (Y) is substituted by glutamic acid (E) at position 349 of said second CH3 domain and leucine (L) is substituted by glutamic acid (E) at position 368 of said second CH3 domain. This is denoted as a T366K/L351'D,Y349'E,L368'E mutation. The two residues Y349 and L368 are residues that may contribute to dimer interactions. According to the in silico data, Y349E and L368E add to the stability of the heterodimer (lower in silico scores) as well as to the destabilization of the BB dimer (higher in silico scores) and glutamic acids (E) on positions 349 and 368 are more favorable than aspartic acids (D). Thus, introduction of a second and third amino acid substitution in the B-chain, which already comprises the amino acid substitution at position 351, favors heterodimerization further. A particularly preferred embodiment therefore provides a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein contaminating homodimers are less than 5%, more preferably less than 2%, even more preferably less than 1%, and most preferably essentially absent, said method comprising providing in said cell:
- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and
- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitution T366K and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitutions L351D and Y349E and L368E, said

method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

In yet another preferred embodiment, threonine (T) is substituted by lysine (K) at position 366 of a first CH3 domain and leucine (L) is substituted by lysine (K) at position 351 of said first CH3 domain and leucine (L) is substituted by aspartic acid (D) at position 351 of a second CH3 domain and leucine (L) is substituted by glutamic acid (E) at position 368 of said second CH3 domain. This is denoted as a T366K,L351K/L351'D,L368'E mutation. This mutation also enhances the proportion of the (bispecific) antibody of interest, as shown in the Examples. Also with this mutation it has become possible to obtain bispecific antibody without any detectable amount of homodimers formed. Further provided is therefore a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein contaminating homodimers are less than 5%, preferably less than 2%, more preferably less than 1%, and most preferably essentially absent, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and
- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitutions T366K and L351K, and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitutions L351D and L368E, said method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

In yet another preferred embodiment, threonine (T) is substituted by lysine (K) at position 366 of a first CH3 domain and leucine (L) is substituted by lysine (K) at position 351 of said first CH3 domain and leucine (L) is substituted by aspartic acid (D) at position 351 of a second CH3 domain and tyrosine (Y) is substituted by aspartic acid (D) at position 349 of said second CH3 domain and arginine (R) is substituted by

aspartic acid (D) at position 355 of said second CH3 domain. This is denoted as a T366K,L351K/L351'D,Y349'D,R355'D mutation. The T366K-L351K/L351'D-Y349'D pair may be further improved by the R355'D mutation in the B-chain, which results in a higher BB-in silico score, but also the AB in silico score is slightly higher. Further provided is therefore a method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface and wherein contaminating homodimers are less than 5%, more preferably less than 2%, even more preferably less than 1%, and most preferably essentially absent, said method comprising providing in said cell:

- a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain, and
- a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises the amino acid substitutions T366K and L351K, and wherein said second CH3 domain comprising polypeptide chain comprises the amino acid substitutions L351D and Y349D and R355D, said method further comprising the step of culturing said host cell and allowing for expression of said at two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

Table B provides an overview of mutations that can be introduced in CH3 domains as preferred means for preferential pairing to create either heterodimers or homodimers.

Table B:

AA substitutions in CH3	**Construct #**	**Preferentially pairs with**
- (wildtype)	-	Wildtype
E356K, D399K	1	Construct 2 or 3
K392D, K409D	2	Construct 1
K392D, K409D, K439D	3	Construct 1
K392D, D399K, K409D	4	Construct 4
E356K, E357K, K439D, K370D	5	Construct 5
T366W	6	Construct 7
T366S, L368A, Y407V	7	Construct 6

T366K	43	Construct 63, 69, 70, 71, 73
L351D	63	Construct 43, 68
T366K, L351K	68	Construct 63, 69, 70, 71, 72, 75
L351D, L368E	69	Construct 43, 68
L351E, Y349E	70	Construct 43, 68
L351D, Y349E	71	Construct 43, 68
L351D, R355D	72	Construct 43, 68
L351D, Y349E, L368E	73	Construct 43
L351D, Y349D, R355D	75	Construct 68

A method according to the present invention for producing at least two different Ig-like molecules, or a method according to the invention for producing a heterodimeric Ig-like molecule, wherein said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and/or said means for preferential pairing of said 3rd and 4th CH3 domain-comprising polypeptides comprise at least one combination of mutations as depicted in Table B is therefore also provided herewith. Preferably, said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said means for preferential pairing of said 3rd and 4th CH3 domain-comprising polypeptides comprise at least two combinations of mutations as depicted in Table B.

The present invention also provides novel combinations of CH3 mutations with which it has become possible to produce a mixture of at least two monospecific Ig-like molecules in a single cell, wherein contaminating bispecific Ig-like molecules are less than 5%, preferably more than 2%, even more preferably less than 1%, and most preferably even essentially absent. These mutations according to the invention are, therefore, particularly suitable for the production of a mixture of monospecific antibodies, which is for instance advantageous when a high level of crosslinking of two identical target molecules is desired, when the density of antibodies on a target cells needs to be high enough to recruit certain effector functions such as complement-mediated lysis of a tumor cell, or when two targets are located too far away from each order so that they cannot be bound by as single bispecific antibody, or in order to simplify regulatory approval procedures. In such cases, it is often desired to optimize

the production platform for such monospecific antibodies. As shown in Example 10, the present invention provides the insight that when lysine (K) at position 392 of a first CH3 domain-comprising polypeptide (for instance having specificity A) is substituted by aspartic acid (D) and when aspartic acid (D) at position 399 of said first CH3 domain-comprising polypeptide is substituted by lysine (K) and when lysine (K) at position 409 of said first CH3 domain-comprising polypeptide is substituted by aspartic acid (D), it has become possible to produce a mixture of at least two different monospecific Ig-like molecules in a single cell, including monospecific Ig-like molecules with specificity AA, wherein the formation of bispecific by-products (bispecific Ig-like molecules) is reduced to below 5%, or even to below 3%, or even essentially not detectable at all. Hence, the above mentioned combination of mutations (denoted herein as K392D, D399K, K409D) is particularly preferred for the production of a mixture of monospecific Ig-like molecules. The skilled person will appreciate that functional variants thereof, i.e., K392E, D399R, K409E, may result in similar effects. Additionally, double mutants comprising D399K and K409D substitutions, or other functional variants such as e.g. K392D and K409D, D399R and K409E and so forth, may also result in similar effects.

The same holds true for a combination of mutations wherein glutamic acid (E) at position 356 of a first CH3 domain-comprising polypeptide is substituted by lysine (K) and wherein glutamic acid (E) at position 357 of said first CH3 domain-comprising polypeptide is substituted by lysine (K) and wherein lysine (K) at position 439 of said first CH3 domain-comprising polypeptide is substituted by aspartic acid (D) and wherein lysine (K) at position 370 of said first CH3 domain-comprising polypeptide is substituted by aspartic acid (D). This combination of mutations (denoted herein as E356K, E357K, K439D, K370D) is also particularly preferred for the production of a mixture of monospecific Ig-like molecules. The skilled person will appreciate that functional variants thereof, i.e., E356R, E357R, K439E, K370E, may result in similar effects. Additionally, triple or double mutants comprising E356K and K439D, and E357K and K370D substitutions, or other functional variants may also result in similar effects. A further embodiment therefore provides a method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain having a specificity A,
b) a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain having a specificity B,
wherein said first CH3 domain-comprising polypeptide chain comprises a K392D, D399K, K409D mutation and said second CH3 domain-comprising polypeptide chain comprises either a wildtype CH3 domain or comprises a E356K, E357K, K439D, K370D mutation, said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.
An alternative embodiment provides a method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell
a) a first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain having a specificity A,
b) a second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain having a specificity B,
wherein said first CH3 domain-comprising polypeptide chain comprises either a wildtype CH3 domain or comprises a K392D, D399K, K409D mutation and said second CH3 domain-comprising polypeptide chain comprises a E356K, E357K, K439D, K370D mutation, said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.

As shown in **Example 10**, two monospecific Ig-like molecules can be produced in a single cell, wherein the formation of bispecific Ig-like molecules is essentially undetectable. The skilled person may select a 3rd nucleic acid molecule encoding a wildtype or engineered CH3 domain-comprising polypeptide chain to provide to said host cell such that a mixture of 3 monospecific antibodies is produced, and so forth.

In one aspect of the invention, a method according to the invention for producing at least two different Ig-like molecules or for producing a heterodimeric Ig-like molecule is provided wherein each of the CH3-domain comprising polypeptide chains further

comprises a variable region recognizing a different target epitope, wherein the target epitopes are located on the same molecule. This often allows for more efficient counteraction of the (biological) function of said target molecule as compared to a situation wherein only one epitope is targeted. For example, a heterodimeric Ig-like molecule may simultaneously bind to 2 epitopes present on, e.g., growth factor receptors or soluble molecules critical for tumors cells to proliferate, thereby effectively blocking several independent signalling pathways leading to uncontrolled proliferation, and any combination of at least two Ig-like molecules may simultaneously bind to 2, or even 3 or 4 epitopes present on such growth factor receptors or soluble molecules.

In a preferred embodiment, the target molecule is a soluble molecule. In another preferred embodiment, the target molecule is a membrane-bound molecule.

In another aspect of the invention, a method according to the invention for producing at least two different Ig-like molecules or for producing a heterodimeric Ig-like molecule is provided wherein each of the CH3-domain comprising polypeptide chains further comprises a variable region recognizing a target epitope, wherein the target epitopes are located on different molecules. In this case, each of the different target molecules may either be a soluble molecule or a membrane-bound molecule. In one embodiment, the different target molecules are soluble molecules. Alternatively, one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule. In yet another alternative, both target molecules are membrane bound molecules. In one embodiment the different target molecules are expressed on the same cells, whereas in other embodiments the different target molecules are expressed on different cells. As a non-limiting example, any heterodimeric Ig-like molecule or any combination of at least two Ig-like molecules may be suitable for simultaneously blocking multiple membrane-bound receptors, neutralizing multiple soluble molecules such as cytokines or growth factors for tumor cells or for neutralizing different viral serotypes or viral strains.

One preferred embodiment provides a method according to the invention for producing at least two different Ig-like molecules or for producing a heterodimeric Ig-like molecule, wherein at least one of said target epitopes is located on a tumor cell.

Alternatively, or additionally, at least one of said target epitopes is located on the surface of an effector cell. This is for instance suitable for recruitment of T cells or NK cells for tumor cell killing. For instance, at least one Ig-like molecule is produced with a method according to the invention that is capable of recruiting immune effector cells, preferably human immune effector cells, by specifically binding to a target molecule located on immune effector cells. In a further embodiment, said immune effector cell is activated upon binding of the Ig-like molecule to the target molecule. Recruitment of effector mechanisms may for instance encompass the redirection of immune modulated cytotoxicity by administering an Ig-like molecule produced by a method according to the invention that is capable of binding to a cytotoxic trigger molecule such as the T cell receptor or an Fc gamma receptor, thereby activating downstream immune effector pathways. The term 'immune effector cell' or 'effector cell' as used herein refers to a cell within the natural repertoire of cells in the mammalian immune system which can be activated to affect the viability of a target cell. Immune effector cells include cells of the lymphoid lineage such as natural killer (NK) cells, T cells including cytotoxic T cells, or B cells, but also cells of the myeloid lineage can be regarded as immune effector cells, such as monocytes or macrophages, dendritic cells and neutrophilic granulocytes. Hence, said effector cell is preferably an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

Target antigens present on immune effector cells may include CD3, CD16, CD25, CD28, CD64, CD89, NKG2D and NKp46. Further provided is therefore a method according to the invention for producing at least two different Ig-like molecules or for producing a heterodimeric Ig-like molecule, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.
The viability of a target cell may include cell survival, proliferation and/or ability to interact with other cells.

In one aspect the present invention thus provides methods according to the invention for producing a heterodimeric Ig-like molecule, wherein each of the CH3-domain comprising polypeptide chains further comprises a variable region recognizing a target epitope. In one embodiment, each of the 2 variable regions of the CH3-domain comprising polypeptide chains recognizes the same target epitope but with different

affinities. In another embodiment, each of the 2 variable regions of the CH3-domain comprising polypeptide chains recognizes a different target epitope. In another embodiment, the different target epitopes are located on the same target molecule, which can be either a membrane-bound molecule or a soluble molecule. In another embodiment, the different target epitopes are located on different target molecules, which can be either expressed on the same cells or on different cells. Alternatively, the different target molecules can be soluble molecules, or one target molecule can be a soluble molecule whereas the second target molecule is a membrane bound molecule. In a preferred embodiment, at least one of the target molecules of the heterodimeric Ig-like molecule is located on a tumor cell. In yet another preferred embodiment, at least one of the target molecules of the heterodimeric Ig-like molecule is located on an effector cell (i.e. an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte, and said target epitope may be located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule).

In a preferred embodiment, a method according to the invention for producing at least two different Ig-like molecules or for producing a heterodimeric Ig-like molecule is provided, wherein said at least two different Ig-like molecules are antibodies, most preferably antibodies of the IgG isotype, even more preferably the IgG1 isotype, as described herein above.

Further provided is an Ig-like molecule, a heterodimeric Ig-like molecule, or a mixture of at least two Ig-like molecules, obtainable by a method according to the present invention. Said (heterodimeric) Ig-like molecule or mixture of Ig-like molecules preferably comprises at least one CH3 mutation as depicted in Table B. An (heterodimeric) Ig-like molecule or a mixture of at least two Ig-like molecules, comprising at least one mutation as depicted in Table B is therefore also herewith provided, as well as a pharmaceutical composition comprising at least one Ig-like molecule, or a mixture of at least two Ig-like molecules, according to the present invention. In one embodiment said Ig-like molecule is a bispecific Ig-like molecule, such as a bispecific antibody. In another embodiment said Ig-like molecule is a monospecific Ig-like molecule, such as a monospecific antibody. One preferred embodiment provides a mixture of at least two different Ig-like molecules obtainable

by a method according to the invention, wherein said at least two different Ig-like molecules bind to different epitopes on the same antigen and/or to different epitopes on different antigens. Further provided is a heterodimeric Ig-like molecule obtainable by a method according to the invention, wherein said heterodimeric Ig-like molecule binds to different epitopes on the same antigen and/or to different epitopes on different antigens. Advantages and preferred uses of such mixtures and antibodies are described herein before. The invention also provides a mixture of at least two different Ig-like molecules obtainable by a method according to the invention, wherein said at least two different Ig-like molecules comprise at least one heterodimeric Ig-like molecule. In one embodiment, two of said at least two different Ig-like molecules are heterodimeric Ig-like molecules. Yet another preferred embodiment provides a heterodimeric antibody comprising two CH3 domains, wherein one of said two CH3 domains comprises the amino acid substitutions L351D and L368E and wherein the other of said two CH3 domains comprises the amino acid substitutions T366K and L351K. These amino acid substitutions are preferred means for preferential pairing of said two CH3 domains, as explained before. The amino acid substitutions L351D and L368E in one of said two CH3 domains and the amino acid substitutions T366K and L351K in the other of said two CH3 domains are together dubbed the 'DEKK combination of mutations', 'DEKK variant', 'DEKK pair', 'DEKK engineered CH3 domains', 'DEKK' or alternative names referring to DEKK are used. The CH3 domain that carries the amino acid substitutions L351D and L368E is also dubbed 'the DE-side' and the CH3 domain that carries the amino acid substitutions T366K and L351K is also dubbed 'the KK-side'.

Also provided is a pharmaceutical composition comprising a (heterodimeric) Ig-like molecule, or a mixture of at least two Ig-like molecules obtainable by any method according to the invention. Said (heterodimeric) Ig-like molecule, or said at least two Ig-like molecules according to the invention is/are preferably (an) antibody/antibodies. Said pharmaceutical composition may comprise said (heterodimeric) Ig-like molecule, a mixture comprising monospecific or bispecific Ig-like molecules, or a combination of monospecific and bispecific Ig-like molecules. In addition, a pharmaceutical composition according to the invention comprises a pharmaceutically acceptable carrier. As used herein, such 'pharmaceutically acceptable carrier' includes any and all solvents, salts, dispersion media, coatings, antibacterial and antifungal agents,

isotonic and absorption delaying agents, and the like that are physiologically compatible. Depending on the route of administration (e.g., intravenously, subcutaneously, intra-articularly and the like) the Ig-like molecules may be coated in a material to protect the Ig-like molecules from the action of acids and other natural conditions that may inactivate the Ig-like molecules. In one aspect, a pharmaceutical composition comprising a mixture of at least two Ig-like molecules obtainable by any method according to the invention is provided, wherein said at least two different Ig-like molecules have been produced by recombinant host cells according to the present invention. Furthermore, a pharmaceutical composition is provided comprising a heterodimeric Ig-like molecule obtainable by any method according to the invention, wherein said heterodimeric Ig-like molecule has been produced by recombinant host cells according to the present invention.

A nucleic acid molecule encoding a CH3 domain-comprising polypeptide chain that comprises at least one mutation as depicted in Table B is also provided herewith, as well as a recombinant host cell comprising at least one nucleic acid molecule encoding a CH3 domain-comprising polypeptide chain that comprises at least one mutation as depicted in Table B.

The invention is further illustrated by the following examples. These examples are not limiting the invention in any way, but merely serve to clarify the invention.

Brief description of the drawings

Fig. 1A: schematic representation of construct vector MV1057. The stuffer region is the region into which an antibody VH region is cloned.

Fig. 1B: schematic representation of phage display vector MV1043.

Fig. 2: amino acid sequence of wildtype IgG1 Fc (SEQ ID NO: 1), as present in construct vector MV1057 (EU numbering scheme applied).

Fig. 3: nucleotide and amino acid sequences (SEQ ID NOs: 2-7) of VH regions used for cloning into the various constructs.

Fig. 4A and Fig. 4B: mass spec data of transfection A.

Fig. 4C and Fig. 4D: mass spec data of transfection G.

Fig. 4E and Fig. 4F: mass spec data of transfection H.

Fig. 5A and Fig. 5B: mass spec data of transfection M.

Fig. 5C and Fig. 5D: mass spec data of transfection U.

Fig. 6A and Fig. 6B: mass spec data of transfection O.

Fig. 7A, Fig. 7B, Fig. 7C and Fig. 7D: prevention of homodimerisation by substitution of neutral amino acids for charged amino acids.

Fig. 8A: Native MS spectrum of transfection sample ZO (T366K/L351'D)

Fig. 8B: Convoluted MS spectrum of transfection sample ZO (T366K/L351'D). The second/main peak represents the bispecific molecule.

Fig. 9: HADDOCK scores on experimentally verified mutation pairs

Fig 10A: Cartoons of interactions in the CH3-CH3 interface K409D:K392D/D399'K:E356'K.

Fig. 10B: Cartoons of interactions in the CH3-CH3 interface D399K:E356K/D399'K:E356'K.

Fig. 10C: Cartoons of interactions in the CH3-CH3 interface K409D:K392D/ K409'D:K392'D.

Fig. 11: HADDOCK scores for various 366/351' charge mutants

Fig. 12A: Cartoons of interactions in the CH3-CH3 interface L351D/L351'D.

Fig. 12B: Cartoons of interactions in the CH3-CH3 interface L351D:S354A:R355D/ L351'D:S354'A:R355'D

Fig. 13: HADDOCK scores for additional charge mutations around position L351

Fig. 14: HADDOCK scores for additional charge mutations around position T366 in chain A and position L351 in chain B.

Fig. 15A and Fig. 15B: Cartoons of interactions in the CH3-CH3 interface

Fig. 16: HADDOCK scores for variants around T366/L351

Fig. 17: HADDOCK scores for additional variants around T366/L351

Fig. 18A: Example of nMS spectra for bispecific IgG obtained after the co-expression of construct T366K,L351K with construct L351D, zoomed in on a single charge state of the full IgG (half bodies not shown).

Fig. 18B: Example of nMS spectra for bispecific IgG obtained after the co-expression of construct T366K,L351K with L351D,Y349E , zoomed in on a single charge state of the full IgG (half bodies not shown).

Fig. 19A: Results of native MS showing relative abundances of AA, AB, BB, A and B (total of all species is 100%).

Fig. 19B: idem but now without AB to have a better overview on the undesired species AA, BB, A and B.

Fig. 20: Results of thermostability assay. Squares: wildtype; triangles: charge reversal pair E356K:D399K/K392D:K409D; circles: mutant CH3 combinations as indicated above each graph.

Fig. 21: Results of 10x freeze-thaw experiment. 1122= 1st parental antibody BB; 1337= 2nd parental antibody AA; wildtype=AA, AB, BB; CR= bispecific of charge reversal pair E356K:D399K/K392D:K409D; 3-6 and 9-12=bispecific molecules from combinations 3-6 and 9-12 from Table 15.

Fig. 22A: Results in serum stability, measured by ELISA using fibrinogen as coated antigen. ELISA data with IgG samples diluted to 0.5 µg/ml.

Fig. 22B: Results in serum stability, measured by ELISA using fibrinogen as coated antigen. ELISA data with IgG samples diluted to 0.05 µg/ml;. Results are normalized to the T=0 days time point (100%). 1337= 2nd parental antibody AA; wildtype=AA, AB, BB; CR= bispecific of charge reversal pair E356K:D399K/K392D:K409D; 3-6 and 9-12=bispecific molecules from combinations 3-6 and 9-12 from Table 15.

Fig, 23A: nMS results of ratio experiments with transfection ratios from 1:5 to 5:1. DEKK combination of mutations, with specificity 'A' on the DE-side and 'B' on the KK-side.

Fig. 23B: nMS results of ratio experiments with transfection ratios from 1:5 to 5:1. DEKK combination of mutations, with specificity 'C' on the DE-side and 'B' on the KK-side.

Fig. 23C: nMS results of ratio experiments with transfection ratios from 1:5 to 5:1 charge reversal combination of mutations, with specificity 'A' on the E356K:D399K-side and 'B' on the K392D:K409D-side.

Fig. 24: nMS results of transfections (tr.) # 1-11 from Table 20.

Fig. 25: HADDOCK scores for dimers with different CH3 engineered vectors. Grey bars: Desired species AB and CD; black bars: undesired species AA, BB, CC, DD, AC, BC, AD, BD.

Fig. 26: SDS-PAGE of transfections # 1-11 from Table 20. Control samples DE/KK, DE/DE and KK/KK are also included.

Fig. 27A and Fig. 27B: nMS of transfection # 9.

Fig. 27C and Fig. 27D: nMS of transfection # 11.

Fig. 28A: nMS of gel filtrated samples 1516:1516.

Fig. 28B: nMS of gel filtrated samples 1337:1337.

Fig. 28C: nMS of gel filtrated samples 1516:1337.

Fig. 29: serum levels of samples of DEKK engineered antibody and its two parental antibodies (pK study).

Examples

Example 1: amino acid substitutions to create various different CH3-domains

In order to have a wide variety of Ig-like molecules that differ in their CH3 domains such that pairing of CH3-domain comprising Ig-like molecules is preferentially promoted or inhibited, a number of amino acid substitutions that were known to promote heterodimer formation, as well as a number of alternative amino acid substitutions that were not previously reported nor tested but that were chosen to promote homodimer formation, were introduced into a construct vector (construct vector MV1057; Figure 1A). The construct vector MV1057 comprises nucleic acid sequences encoding the normal wildtype IgG1 Fc part, as depicted in figure 2. Table 1 lists the amino acid substitutions that were introduced in this wildtype Fc, resulting in a series of seven constructs. All constructs were made at Geneart. Constructs 1, 2 and 3, or alternatives thereof, have previously been described to drive heterodimerization (EP01870459, WO2009/089004) as have constructs 6 and 7 (WO98/50431). Constructs 4 and 5 are new and are designed to promote homodimerization.

Table 1

AA substitutions in CH3	**construct #**	**Will pair with**	**% bispecific product reported**
- (wildtype)	-	- (wildtype)	~50%
E356K, D399K	1	Construct 2 or 3	~100%
K392D, K409D	2	Construct 1	~100%
K392D, K409D, K439D	3	Construct 1	~100%
K392D, D399K, K409D	4	Construct 4	
E356K, E357K, K439D, K370D	5	Construct 5	
T366W	6	Construct 7	~86,7%
T366S, L368A, Y407V	7	Construct 6	~86,7%

Example 2: cloning of VH into constructs with CH3 mutations

Several antibody VH regions with known specificities and known ability to pair with the human IGKV1-39 light chain were used for cloning into these constructs.

As indicated earlier, all CH3 variants can be used in association with other antibody domains to generate full length antibodies that are either bispecific or monospecific. The specificity of the antibody as defined by the VH/VL combinations will not affect the heavy chain dimerization behaviour that is driven by the CH3 domains. Model VH/VL combinations were used throughout the studies, wherein all VLs are based on the germline human IGKV1-39 and VHs vary. Figure 3 provides full sequences and specificities of the antibody VH regions used throughout the studies. The MF coding refers to internal Merus designation for various VHs, e.g. VH MF1337 has specificity for tetanus toxoid, MF1025 for porcine thyroglobulin, MF1122 for bovine fibrinogen.

VH regions present in phage display vector MV1043 (Figure 1B) are digested with restriction enzymes SfiI and BstEII (New England Biolabs/ cat# R0123L and R0162L/ according to manufacturer's instructions) that release the VH fragment from this vector. Vector MV1057 is digested with SfiI and BstEII according to standard procedures (according to manufacturer's instructions). Fragments and vector are purified over gel (Promega/ cat# V3125/ according to manufacturer's instructions) to isolate the cut vector and VH gene inserts. Both are combined by ligation after which the ligation is transformed into E. coli DH5α (Invitrogen/ cat# 12297-016/ according to manufacturer's instructions). After overnight selection single colonies are picked and vectors with a correct insert identified by sequencing.

Example 3: transfection and expression of full IgG in HEK293T cells

Transfection of the various plasmids encoding the recloned VH variants, and further encoding the common light chain huIGKV1-39, in HEK293T cells was performed according to standard procedures such that IgG could express (de Kruif etal Biotech Bioeng. 2010). After transfection, IgG expression levels in supernatants were measured using the ForteBIO Octet-QK system, which is based on Bio-Layer Interferometry (BLI) and which enables real-time quantitation and kinetic characterization of biomolecular interactions; for details see www.fortebio.com. When expression levels exceeding 5 µg/ml were measured, the IgG was purified using Protein A affinity purification.

Example 4: purification of IgG

Culture supernatants were purified using protein A columns (GE Healthcare/ cat# 11-0034-95/ according to manufacturer's instructions) and eluted in 0,1 M citrate buffer pH 3.0 and immediately neutralized in an equal volume of 1,0 M Tris-HCL pH 8.0 or directly rebuffered to PBS using a desalting column. Alternatively one could purify IgG using protein A beads (sepharose beads CL-4B, GE healthcare cat #170780-01)

Example 5: Ag-specific ELISA's

Antigen specific ELISAs were performed to establish binding activity against the antigens and capture ELISAs were carried out to demonstrate binding activity of the bispecific antibodies. Biotinylated second antigen was used for detection of the complex. (de Kruif etal Biotech Bioeng. 2010)

Example 6: SDS-PAGE

The purified IgG mixtures were analysed by SDS-PAGE (NuPAGE® 4-12% bis-tris gel/ Invitrogen/ cat# NP0323BOX) under reduced and non-reducing conditions according to standard procedures, and staining of proteins in gel was carried out with colloidal blue (PageBlue™ protein staining solution/ Fermentas/ cat# RO571).

Example 7: Enzymatic deglycosylation of IgG1

As there is heterogeneity in the glycosylation of the IgGs, the proteins were deglycosylated in order to create a single product with a distinct mass, suitable for mass spectrometric analysis. One unit of N-glycosidase F (PNGase F; Roche Diagnostics, Mannheim, Germany) was incubated per 10 µg of IgG1, overnight at 37°C. Buffer exchange using 10 kDa MWCO centrifugal filter columns (Millipore) was performed to remove the original purification buffer (0,1 M citrate buffer pH 3.0 / 1,0 M Tris-HCL pH 8.0) and to rebuffer to PBS. Similar buffer exchange procedures were performed to remove the detached glycan chains, and to change the buffer to 150 mM ammonium acetate pH 7.5. Filters were washed with 200 µl 150 mM ammonium acetate pH 7.5, for 12 min 11,000 rpm and 4°C. After washing 50 µl deglycosylated IgG was loaded on the filter and 450 µl of 150 mM ammonium acetate pH 7.5 was added, subsequently followed by another centrifugation round of 12 min at 11,000 rpm at 4°C. In total the centrifugation was repeated 5 times, each time fresh 150 mM ammonium acetate pH 7.5 buffer was added to a total volume of 500 µl. After the last

centrifugation step the remaining buffer exchanged deglycosylated IgG1, approximately 25 µl, was collected and transferred to an eppendorf tube, ready for mass spectrometric analysis.

Example 8: Native mass spectrometric analysis

Mass Spectrometry was used to identify the different IgG species in the purified IgG mixtures and to establish in what ratios these IgG species are present. Briefly, 2-3 µl at a 1 µM concentration in 150 mM ammonium acetate pH 7.5 of IgG's were loaded into gold-plated borosilicate capillaries made in-house (using a Sutter P-97 puller [Sutter Instruments Co., Novato, CA, USA] and an Edwards Scancoat six sputter-coater [Edwards Laboratories, Milpitas, CA, USA]) for analysis on a LCT 1 mass spectrometer (Waters Corp., Milford, MA, USA), adjusted for optimal performance in high mass detection (Tahallah et al., RCM 2001). A capillary voltage of 1300 V was used and a sampling cone voltage of 200 V; however, these settings were adjusted when a higher resolution of the 'signal-to-noise' ratio was required. The source backing pressure was elevated in order to promote collisional cooling to approximately 7.5 mbar. To measure the IgG1's under denaturing conditions the proteins were sprayed at a 1 µM concentration in 5% formic acid.

Example 9: Data processing and quantification

Processing of the acquired spectra was performed using MassLynx 4.1 software (Waters Corp., Milford, MA, USA). Minimal smoothing was used, after which the spectra were centered. The mass of the species was calculated using each charge state in a series. The corresponding intensities of each charge state were assigned by MassLynx and summed. This approach allowed the relative quantification of all species in a sample. Alternatively, quantification of the peaks can be performed using area-under-the-curve (AUC) methods, known in the art. All analyses were repeated three times to calculate standard deviations of both the masses of the IgG's as well as their relative abundance.

Example 10: mixtures of 2 or 3 monospecific antibodies from a single cell

Several antibody VH regions with known specificities and known ability to pair with the human IGKV1-39 light chain (Figure 3) were used for recloning into the wildtype construct vector MV1057, or in construct 4 or construct 5 of Table 1, resulting in

vectors I-III (Table 2). The resulting vectors I, II and III, each containing nucleic acid sequences encoding for the common human light chain as well as an Ig heavy chain with different CH3 region and different VH specificity, were subsequently transfected into cells, either alone to demonstrate formation of intact monospecific antibodies only, or in combination with one or two other construct vectors to obtain mixtures of two monospecific or three monospecific antibodies. Table 3 depicts the transfection schedule and results.

Table 2: **VH specificity inserted in different constructs**

Vector	**VH gene**	**Antigen specificity**	**VH mass (Da)**	**Merus designation**	**Cloned in construct #**
I	IGHV 1.08	Tetanus (A)	13703	MF1337	wildtype
II	IGHV 3.23	Thyroglobulin (B)	12472	MF1025	4
III	IGHV 3.30	Fibrinogen (C)	12794	MF1122	5

Table 3: **transfection schedule and results**

# different mono-specifics produced	**Transfection of**	**Transfection code and ratio**	**Expected species**	**Calculated mass - 2LYS**	**Experimental mass**	**AA found (%)**	**BB found (%)**	**CC found (%)**	**Other molecules (%)**
1	Only vector I	A	AA	146521	146503	100			
1	Only vector II	G	BB	144032	144087		100		
1	Only vector III	H	CC	144647	144656			100	

2	Vector I and II	M (I:II=1:1)	AA BB	146521 144032	146518 144030	51	45		4
2	Vector I and III	N (I:III=1:1)	AA CC	146521 144647	146509 144633	88		9	3
		U (I:III= 1:5)	AA CC	146521 144647	146522 144643	47		48	5
2	Vector II and III	nd	BB CC						
3	Vector I, II and III	O (I:II:III=1: 1:1)	AA BB CC	146521 144032 144647	146525 144032 144650	66	4	30	
		V (I:II:III=1: 1:10)	AA BB CC	146521 144032 144647	146531 144043 144654	8	81	9	2

nd= not done.

It was observed that transfections A, G and H resulted in formation of homodimers only, and 100% of bivalent monospecific AA, BB or CC was retrieved from cells transfected with any one of vectors I, II or III (Fig. 4). Although this was to be expected and previously demonstrated for transfection A, it is actually now shown for the first time that homodimerisation of CH3-engineered Ig heavy chains containing either the triple amino acid substitution of construct 4 (i.e., K392D, D399K, K409D) or the quadruple amino acid substitution of construct 5 (i.e., E356K, E357K, K439D, K370D) is reported (transfections G and H).

Next, co-expression experiments of two vectors in a single cell were performed. Interestingly, transfections M and N show that wildtype and CH3 engineered Ig heavy chains can be co-expressed in a single cell together with a common light chain resulting in mixtures of two species of monospecific antibodies without the presence of undesired bispecific antibodies and with as little as 4-5% contaminating 'other molecules' present in the mixture. 'Other molecules' is defined as all molecules that do not have the mass of an intact IgG, and includes half molecules consisting of a single heavy and light chain pair. Importantly, the fraction 'other' does not include bispecific

product. In transfection M, the ratio of AA:BB was close to 1:1 upon transfection of equal ratios of vector DNA. However, transfection N resulted in an almost 10:1 ratio of AA:CC. Therefore, this transfection was repeated with adjusted ratios of DNA (transfection U). Indeed, a 1:5 ratio of vector DNA I:III equalized the ratio of AA:CC antibody product in the mixture towards an almost 1:1 ratio. Thus, transfections M and U show that it is possible to express two different, essentially pure, monospecific antibodies in a single cell, without undesired by products (i.e., no abundant presence of AC or half molecules A or C) (fig. 5). The novel CH3 modifications of constructs 4 and 5 differ substantially from wildtype CH3 such that heterodimerization between wildtype and 4, or wildtype and 5, does not occur, which is advantageous for application in large scale production of mixtures of monospecific antibodies from single cells.

Analogous to these results, also transfection of two different CH3 engineered Ig heavy chains (constructs 4 and 5) are expected to result in mixtures of two different monospecific antibodies only, without further undesired species present. It is reasoned that the CH3 modifications of construct 4 differ substantially from the CH3 modifications of constructs 5 such that heterodimerization does not occur. In that case, co-expression of CH3-engineered heavy chains of constructs 4 and 5, together with wildtype CH3 heavy chains in a single cell would results in 3 monospecific antibodies only.

Indeed, this was observed to be the case as it was found that also a mixture of three pure monospecific antibodies could be obtained by expression of three different Ig heavy chains, designed to form homodimers over heterodimers, together with a common light chain in a single cell, with no contaminations present in the mixture (transfection O) (Fig. 6). As is clear from Table 3, with equal ratios of vector DNA used during transfection O, no 1:1:1 ratio of AA:BB:CC antibodies was obtained. Transfections with altered vector DNA ratios (1:1:10, transfection V) demonstrated that ratios of AA:BB:CC in the mixtures can be steered towards desired ratios. Taken together, these experiments show that two or three essentially pure monospecific antibodies can be expressed in a single cell without undesired by products, offering advantages for large scale production of mixtures of therapeutic monospecific antibodies.

Example 11: mixtures of 2 bispecific antibodies from a single cell

Whereas use of CH3-engineered heavy chains for production of single bispecific antibodies has been reported elsewhere, this experiment was designed to investigate whether it is feasible to produce mixtures of 2 different bispecific antibodies from a single cell.

Antibody VH regions with known specificities and known ability to pair with the human IGKV1-39 light chain (fig. 3) were used for recloning into vectors containing constructs 1-3 or 6-7 of Table 1 resulting in vectors IV-X (Table 4). Vectors IV-X, each containing nucleic acid sequences encoding the common human light chain as well as an Ig heavy chain with different CH3 region and different VH specificity, were subsequently transfected into cells, either alone to demonstrate that formation of intact monospecific antibodies was hampered, or in combination with another construct vector to obtain bispecific antibodies or mixtures of two bispecific antibodies. Table 5 depicts the transfection schedule and results.

Table 4: **VH specificity inserted in different constructs**

Vector	**VH gene**	**Antigen specificity**	**VH mass (Da)**	**Cloned in construct #**
IV	IGHV 3.23	Thyroglobulin (B)	12472	1
V	IGHV 3.30	Fibrinogen (C)	12794	2
VI	IGHV 1.08	Tetanus (A)	13703	2
VII	IGHV 3.30	Fibrinogen (C)	12794	3
VIII	IGHV 1.08	Tetanus (A)	13703	3
IX	IGHV 1.08	Tetanus (A)	13703	6
X	IGHV 3.23	Thyroglobulin (B)	12472	7

Table 5:

# different bispecifi	**Transfect-ion of**	**Transfection code and**	**Expected species**	**Calculated mass - 2LYS**	**Experiment-al mass**	**Half molecules found**	**Full IgG found (%)**	**Bispecific found (%)**	**Other molecules (%)**

cs produced		**ratio**				**(%)**			
0	vector IV	B	Half B	144082	144066	40	60		
0	vector V	C	Half C	144651	144622	77	23		
0	vector VI	D	Half A	146469	146459	23	77		
0	vector VII	E	Half C	144625	144643	76	24		
0	vector VIII	F	Half A	146443	146468	64	36		
0	vector IX	P	Half A	146691	146677	82	18		
0	vector X	Q	Half B	143818	143844	58	42		
1	Vector IV and V	I (1:1)	BC	144367	144352			96	4
1	Vector IV and VII	J (1:1)	BC	144354	144382			96	4
2	Vector IV, V and VI	K(1:1:1)	BC + AB	144367 + 145276	144351+ 145260			38 + 47	15 (A + C)
		S(2:1:1)	BC + AB	144367 + 145276	144371 + 145277			42 + 55	3 (BB)
2	Vector IV, VII and VIII	L (1:1:1)	BC + AB	144354 + 145263	144346 + 145255			16 + 60	24 (A + C)
		T (2:1:1)	BC + AB	144354 + 145263	144385 + 145292			58 + 39	3 (BB)

It was previously demonstrated that CH3-engineered Ig heavy chains encoded by constructs 1 and 2 are still able to form homodimers when expressed alone in single cells (WO2009/089004). However, WO2009/089004 further reports that CH3 domains that are engineered to comprise triple charge pair mutations, such as present in construct 3, are no longer capable of forming homodimers when expressed alone.

In the present study, these findings were only partly confirmed. Indeed, the results of transfections B, C and D demonstrated the presence of full IgGs, in addition to a high proportion of unpaired half molecules, demonstrating some homodimerization of CH3 domains encoded by constructs 1 and 2. Transfections E and F also resulted in production of full IgGs in addition to unpaired half molecules, demonstrating that the triple charge mutations of construct 3 do not fully impair homodimerisation.

It was furthermore demonstrated that also the 'knob' and 'hole' CH3 variants of constructs 6 and 7 form homodimers (18% homodimers for 'knob-knob' and 42% homodimers for 'hole-hole').

CH3 variants that fully prevent homodimerisation when expressed alone are preferred, to prevent or minimize undesired byproducts (homodimers) upon co-expression with a second CH3 variant for heterodimerization.

Interestingly, the present experiments demonstrate for the first time that also mixtures of bispecific antibodies can be expressed in single cells with virtually no homodimers in the mixture. Transfections K and L clearly show that the expected bispecific species BC + AB are indeed obtained (38% + 47% in transfection K, and 16% + 60% in transfection L). In both transfections a relatively high percentage of undesired half molecules was observed (15% half molecule A + half molecule C in transfection K, and 24% half molecule A + half molecule C in transfection L). The relatively high percentage of half molecules still present was attributed to low amounts of matching heavy chains of vector IV due to unbalanced expression of heavy chains in a matched pair. Therefore, transfections were repeated with an adjusted ratio of vector DNA, 2:1:1, in transfections S and T. This resulted in equal amounts of IgG heavy chains constituting a matched pair and pure mixtures of bispecific IgG without the presence of half IgG molecules and with as little as 3% homodimeric BB present. Ideally, this low proportion of contaminating monospecific product should be reduced to essentially zero. It is therefore desired to find additional CH3-mutants that would result in mixtures of bispecific antibodies with minimal contaminating monospecific antibodies present.

The present study demonstrates for the first time that essentially pure mixtures of two bispecific antibodies recognizing 3 different target epitopes can be produced in a single cell, with minimal presence of monospecific antibodies in the mixture.

Example 12: varieties of mixtures

As it was demonstrated that production of mixtures of 2 bispecific antibodies recognizing 3 epitopes from a single cell, or production of mixtures of 2 or 3 monospecific antibodies from a single cell is technically feasible, we next explored the feasibility of controlled production of a variety of other mixtures. A fourth antibody VH region with known specificity and known ability to pair with the human IGKV1-39 light chain will be used for recloning into vectors containing constructs 1-3 or 7 of Table 1, resulting in vectors I', II', III' or X' (the ' indicating a different specificity as compared to corresponding vector numbers). The resulting vectors I'-III', X' and IV-IX, each containing nucleic acid sequences encoding for the common human light chain as well as an Ig heavy chain with different CH3 region and different VH specificity, will subsequently be transfected into cells, in combination with other construct vectors to obtain a variety of mixtures of bispecific and/or monospecific antibodies. The variety of mixtures that will be obtained include mixtures of 2 bispecific antibodies recognizing 4 epitopes, 2 bispecific antibodies and one monospecific antibody, or mixtures of 1 bispecific and one monospecific antibody from a single cell. Table 6 depicts the transfection schedule and expected results.

Table 6

Variety of mixture	**Transfect-ion of**	**Transfection code and ratio**	**Expected species**	**Expected % monospecific IgG**	**Expected % Bispecific**
2 BsAbs, 4 epitopes	IV+V+IX+ X'	ZA (1:1:1:1)	BC + AD	0	50 + 50
2 BsAbs, 4 epitopes	IV+VII+IX +X'	ZB (1:1:1:1)	BC + AD	0	50 + 50
2 bsAbs + 1 mAb	IV+V+VI+ wt'	ZC (2:1:1:2)	BC + AB + DD	33	33 + 33
2 bsAbs + 1	IV+V+VI+	ZD (2:1:1:2)	BC + AB	33	33 + 33

mAb	II'		+ DD		
2 bsAbs + 1 mAb	IV+V+VI+ III'	ZE (2:1:1:2)	BC + AB + DD	33	33 + 33
1 bsAb + 1 mAb	IV+V+wt'	ZF (1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IV+V+II'	ZG(1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IV+V+III'	ZH(1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IV+VII+wt'	ZI (1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IV+VII+II'	ZJ (1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IV+VII+III'	ZK (1:1:2)	BC + DD	50	50
1 bsAb + 1 mAb	IX+X+wt'	ZL (1:1:2)	AB + DD	50	50
1 bsAb + 1 mAb	IX+X+II'	ZM (1:1:2)	AB + DD	50	50
1 bsAb + 1 mAb	IX+X+III'	ZN (1:1:2)	AB + DD	50	50

Although, theoretically, production of all mixtures should be feasible, it is known from previous work by others that large scale production of classical knob-into-hole variants is hampered by instability issues. Mixtures resulting from transfections ZA, ZB, ZL, ZM and ZN are thus expected to become problematic when transferred to larger scale production.

Thus, the current set of constructs present in Table 1 would not allow production of all theoretical mixtures from single cells at a larger scale, as knob-into-hole variants are reported to be unstable, and it cannot be excluded that CH3 domains comprising a 'knob' or a 'hole' will dimerize with either charge variants or wildtype CH3 domains. It is thus desired to design new CH3-variants that are engineered to preferentially

form homodimers or heterodimers only and which will not homo- or heterodimerize with constructs 1-5 of Table 1 as to allow for co-expression in single cells.

Example 13: identification of novel charge pair mutants

The objective of this study was to engineer the IgG CH3 region to result in the production of only heterodimers or only homodimers upon mixed expression of different IgG heavy chains in a single cell, wherein the novel engineered CH3 domains will not homo- or heterodimerize with known engineered CH3 domains, or with wildtype CH3 domains. Therefore, as a first step in identifying novel engineered CH3 domains that would meet the criteria, many interface contact residues in the IgG CH3 domain were scanned one by one or in groups for substitutions that would result in repulsion of identical heavy chains – i.e., reduced homodimer formation - via electrostatic interactions. The objective was to obtain a list of residues that, when substituted by a charged residue, would result in repulsion of identical chains such that these mutations may be used to drive homo- and/or heterodimer formation upon mixed expression of different IgG heavy chains, whereby the obtained full length IgGs are stable and are produced with high proportions. In a follow up, the identified substitutions will be used to generate bispecific antibodies or mixtures of bispecific or monospecific antibodies by engineering matched pairs of CH3 residues in one or more IgG heavy chains - CH3 regions. Additionally, newly identified charge mutant pairs may be combined with existing pairs, such that multiple nucleic acid molecules encoding different heavy chains, all carrying different and complementing CH3 mutations, can be used for expression in cells such that mixtures of monospecific antibodies only, or bispecific antibodies only, or mixtures of defined monospecific and bispecific antibodies can preferentially be obtained. The residues to be tested in the present study are contact residues as previously identified (Deisenhofer J., 1981; Miller S., 1990; Padlan, 1996, Gunasekaran, 2010). The rationale for this approach is that repulsive charges are engineered into each available pair of contacting residues. Samples are subsequently analyzed on non-reducing SDS-PAGE to identify pairs in which dimer formation is reduced, as visualized by the presence of bands of approximately 72 kD. All available pairs will be screened as single mutations or in combination with a single other mutation as the repulsive electrostatic interaction between one non-matching pair may or may not be sufficient to result in sufficient

amounts of half-molecules for detection by this method, the mutations are also combined.

Amino acid substitutions were introduced in construct vector MV1057 by Geneart according to the table 7 and expression of constructs was performed by transfection in HEK293T cells, according to standard procedures. IgG expression levels were measured in Octet. When production failed twice, the mutation was considered to be detrimental to expression and the mutation was not pursued further.

Table 7: list of amino acid substitutions in the various constructs that were made (EU numbering)

AA substitutions in CH3	**construct #**	**Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)**
Q347K	8	-
Y349D	9	+-
Y349K	10	+-
T350K	11	-
T350K, S354K	12	+-
L351K, S354K	13	+-
L351K, T366K	14	++
L351K, P352K	15	+-
L351K, P353K	16	++
S354K, Y349K	17	++
D356K	18	-
E357K	19	-
S364K	20	++
T366K, L351K	21	++
T366K, Y407K	22	+++
L368K	23	NT
L368K, S364K	24	++
N390K, S400K	25	+-

AA substitutions in CH3	**construct #**	**Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)**
T394K, V397K	26	+
T394K, F405K	27	+++
T394K, Y407K	28	+++
P395K, V397K	29	+-
S400K	30	-
F405K	31	+++
Y407K	32	++
Q347K, V397K, T394K	33	+
Y349D, P395K, V397K	34	+
T350K, T394K, V397K	35	NT
L351K, S354K, S400K	36	+
S354K, Y349K, Y407K	37	+-
T350K, N390K, S400K	38	+-
L368K, F405K	39	++
D356K, T366K, L351K	40	+++
Q347K, S364K	41	+++
L368D, Y407F	42	+
T366K	43	+
L351K, S354K, T366K	44	+
Y349D, Y407D	45	+
Y349D, S364K,	46	+

AA substitutions in CH3	**construct #**	**Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)**
Y407D		
Y349D, S364K, S400K, T407D	47	+
D399K	48	+-
D399R	49	+-
D399H	50	+-
K392D	51	+-
K392E	52	+-
K409D	53	+

Supernatants containing ≥5 µg/ml IgG were analyzed in SDS-PAGE and IgG was purified using protein A. The proteins were stained using colloidal blue. Homodimers were visible as a band of approximately 150 kD. Smaller bands of approx 75 kD represented the presence of half molecules (see negative control: K392D, K409D). Blots are shown in Figure 7.

The results of SDS-PAGE gels were analyzed and scored as presented in table 7, right hand column. A number of residues were considered promising for further testing in combination, including residues Q347, S354, Y349, L351, K360, T366, T394, and V397. The choice was based on high scores in the inhibition of formation of homodimers combined with the availability of contacting residues that can be modified without running into issues such as other non-complementary charges. For example, it is known that residues F405 and Y407 have multiple interactions at the CH3-CH3 interface, including interactions with residues that are already charged, which may be problematic after introduction of multiple charge mutations among these interacting residues (see Table A). New constructs were made in vector MV1057 (Table 8), and antibody VH regions with known specificities and known ability to pair with the human IGKV1-39 light chain were used for recloning into vectors containing

these new constructs (see Table 9) such that combinations could further be tested. Table 10 depicts the transfection schedules and results.

Table 8:

AA substitutions in CH3	**construct #**
L351K	61
T394K	62
L351D	63
T366D	64
S354D, Y349D	65
V397D	66
K360D	67

Table 9: VH specificity inserted in different constructs

Vector	**VH gene**	**Antigen specificity**	**VH mass (Da)**	**Cloned in construct #**
XI	IGHV 1.08	Tetanus (A)	13703	8
XII	IGHV 1.08	Tetanus (A)	13703	17
XIII	IGHV 1.08	Tetanus (A)	13703	43
XIV	IGHV 1.08	Tetanus (A)	13703	61
XV	IGHV 1.08	Tetanus (A)	13703	62
XVI	IGHV 3.30	Fibrinogen (C)	12794	63
XVII	IGHV 3.30	Fibrinogen (C)	12794	64
XVIII	IGHV 3.30	Fibrinogen (C)	12794	65
XIX	IGHV 3.30	Fibrinogen (C)	12794	66
XX	IGHV 3.30	Fibrinogen (C)	12794	67

Table 10:

Transfection of	**Transfection code (ratio)**	**Expected species**	**AA found**	**AC found (%)**	**CC found (%)**	**Half A found (%)**	**Half C found (%)**	**other (%)**

			(%)					
XIII + XVI	ZO (1:1)	AC	0	69	7	24	0	0
	ZT (3:1)	AC	10	45	16	27	0	0
	ZU (1:1)	AC	5	61	10	13	0	0
	ZV (1:3)	AC	3	61	23	13	0	0
	ZW (1:1)	AC	0	88.3	2.4	7	0	2.3
XIV + XVII	ZP	AC	30	52	13	0	0	5
XII + XVIII	ZQ	AC	4	51	33	2	1	8
XV + XIX	ZR	AC	20	42	11	0	1	26
XI + XX	ZS	AC	34	41	15	0	0	10

Combinations of CH3 variants were expressed, and analyzed in SDS-PAGE (data not shown) and in native mass spectrometry (MS). Results are summarized in Table 10. The ZO transfection resulted in the highest proportion of heterodimers in the mixtures (69% AC). Interestingly, in the ZO transfection, the AA homodimer was not present whereas the CC homodimer comprised a small proportion (7%). Mass spectrometric analysis unveiled that the remaining protein in the mixture consisted of half A molecules, probably resulting from unequal expression of the A and C heavy chains. The raw MS data from transfection sample ZO are shown in Figure 8. Surprisingly, whereas transfection ZO resulted in fair amounts of bispecific product, the reverse charge pair of transfection ZP (L351K/T366'D versus T366K/L351'D of ZO) did not result in similar results, and only 52% of bispecific product was observed, with considerable amounts of the two homodimers being present (30% AA and 13% CC). An explanation for this may be that the negatively charged D structurally closely resembles T, hence the T366D may not be potent enough to repulse itself and T366D will thus still form homodimers, as was indeed observed.

It can be envisaged that subtle variants of the newly found T366K/L351'D pair (e.g. by testing all permutations including new constructs T366R and L351E) may result in similar percentages of BsAbs.

Example 14: HADDOCK for design of new CH3 mutants to drive efficient heterodimerization.

As described in example 13, the newly found charge pair T366K/L351'D increases the proportion of heterodimers in the mixture (69 %) with a small fraction of undesired CC homodimers (7%) (L351D/L351'D) and a substantial fraction of half A molecules (24%) 'contaminating' the mixture. In this example, an in silico approach was used to generate further insight in amino acid residues involved CH3 interface interactions, to test complementary substitutions in opposing CH3 regions and to find novel CH3 pairs containing complementary substitutions that further increase efficient heterodimerization while preventing efficient formation of homodimers of the two heavy chains.

HADDOCK (High Ambiguity Driven protein-protein DOCKing) is an information-driven flexible docking approach for the modeling of biomolecular complexes. HADDOCK distinguishes itself from ab-initio docking methods in the fact that it encodes information from identified or predicted protein interfaces in ambiguous interaction restraints (AIRs) to drive the docking process. (de Vries et al., 2010).

The input for the HADDOCK web server consists of a protein structure file, which can be a crystal structure, NMR structure cluster or a modeled structure. After the docking or refinement, HADDOCK returns a so-called HADDOCK score, which is a weighted average of VanderWaals energy, electrostatic energy, buried surface area and desolvation energy. The HADDOCK score can be interpreted as an indication of binding energy or affinity, even though a direct translation to experimental data is often hard to achieve. In addition to this, HADDOCK provides structure files for the 'top four' structures that resulted from the docking run. These structure files can be downloaded and visualized, enabling the detailed analysis of the interactions of the individual residues.

In this example, the interactions between the CH3-domains of the IgG1 heavy chains were studied. A high-resolution crystal structure of the Fc part of the IgG (structure 1L6X) was used as starting structure (http://www.rcsb.org/pdb/explore/explore.do?structureId=1l6x ; Idusogie, E.E. et al., J.I. 2000(164)4178-4184).

In example 13, it was found that co-transfection of vectors XIII and XVI resulted in the formation of the CC homodimeric contaminant (Table 10). HADDOCK was used to search for additional mutations to the T366K/L351'D pair that prevent homodimerization.

The HADDOCK output consists of a set of calculated energies, a HADDOCK score (which is a weighted average of the energies) and four structure files corresponding to the four lowest-energy structures found by the program. The HADDOCK-scores are used to compare different structures; the other energies are merely used to get an indication about what is happening in the structures (e.g. good electrostatic interactions, smaller buried surface, high Van der Waals energy). The lower the HADDOCK score, the better. For each mutation pair, the scores were calculated for the AA, AB and BB dimers.

Sets of mutation pairs from example 12 were run in HADDOCK to see whether the calculated energies would correlate to the experimental data. Table 11 presents all theoretical energies, which are visualized in Figure 9.

Table 11:

Construct combinations	**HADDOCK Score**	**VdW energy**	**Electrostatic energy**	**Desolvation energy**	**Buried surface area**
Wildtype-wildtype	-208.2	-62.8	-773	9.2	2505.8
1-2 (E356KD399K – K392DK409D)	-225.8	-56.4	-862	3	2458.3
2-2 (K392DK409D – K392DK409D)	-180.3	-67.9	-562.1	0.1	2312.5
1-1 (E356KD399K – E356KD399K)	-176.7	-75.5	-469.3	-7.3	2349.6
1-3 (E356KD399K – K392DK409DK439D)	-220.6	-67.9	-793.8	6.1	2499.8
3-3 (K392DK409DK439D - K392DK409DK439D)	-150.1	-76.6	-387.6	4.1	2261.2
6-7 (T366W – T366SL368AY407V)	-221.3	-65.8	-735.5	-8.3	2509.0
6-6 (T366W – T366W)	1916.9*	2072.3	-681.3	-19.2	2499.9
7-7 (T366SL368AY407V – T366SL368AY407V)	-191.9	-55.0	-683.2	-0.2	2427.2
43-63 (T366K – L351D)	-210.6	-64	-758.4	5.1	2456.5
43-43 (T366K – T366K)	-191.7	-71.2	-634.1	6.3	2533.5
63-63 (L351D – L351D)	-212.5	-60.4	-774	2.6	2445.6

*this value is unusually high due to high VanderWaals energy score, probably due to steric clash of T366W/T366'W

With 2 wildtype CH3 domains, the HADDOCK scores are the same for AA, AB and BB because the A and B CH3 regions are identical. In most other cases, the AB pair has the lowest score, which is as expected. For the T366K/L351D pair the BB score is slightly better than the AB score (-210.6 vs. -212.5), but this difference is within the error of the calculations. Using HADDOCK, the structures of the heterodimers of these pairs were visualized. For example, the construct combinations 1-2, 1-1 and 2-2 are presented in Figure 10. From these visualizations it is apparent that salt bridges are formed in the heterodimer (Figure 10A *left hand panel*) whereas electrostatic repulsion occurs between residues of identical chains (Figure 10B and C, *middle and right hand panel*). The higher HADDOCK scores for the homodimers can thus be explained by the electrostatic repulsion of the mutated interface residues. These residues have to bend away from each other and don't have interaction with residues on the other chain, causing a drop in the affinity.

Table 11 and Figure 9 confirm what was observed in example 13. The T366K/L351'D AC heterodimer and the L351D/L351'D CC homodimer form with a similar energy, explaining the presence of both the heterodimer and homodimer in the mixture. The T366K/T366'K AA homodimer, on the other hand, is barely detectable in the mixture although T366K half A molecules are present. Table 11 and Figure 9 indeed show that the HADDOCK score for the T366K/T366'K AA homodimer is higher than the score for the AC heterodimer; hence formation of this homodimer is energetically less favorable.

Example 15: 366/351 variations

In example 13, it is hypothesized that alternatives for the T366K/L351'D mutant charge pair can be designed that may have similar results in terms of percentage of bispecific antibodies in the mixture. Alternatives may include substitutions T366R, T366D, T366E, L351E, L351K and L351R. The proportion of CC homodimers of L351D/L351'D may be diminished by creating variants of the 366/351 pair. All possible mutation pairs were run in HADDOCK and the resulting scores are presented in Table 12 and visualized in Figure 11.

Table 12

Construct	HADDOCK	VdW	Electrostatic	Desolvation	Buried

combinations	Score	energy	energy	energy	surface area
T366K – L351D	-210.6	-64	-758.4	5.1	2456.5
T366K – T366K	-191.7	-71.2	-634.1	6.3	2533.5
L351D – L351D	-212.5	-60.4	-774	2.6	2445.6
T366K – L351E	-216.9	-55.7	-854.7	9.8	2532.7
L351E – L351E	-217.9	-65.5	-802.2	8	2532
T366R – L351D	-210.5	-68.8	-760.8	10.4	2514.5
T366R – T366R	-201.8	-77.4	-626.4	0.9	2608
T366R – L351E	-225.8	-56.2	-874.8	5.4	2579.2
T366D – L351R	-211.2	-71.3	-723.6	4.8	2455.6
T366D – T366D	-198.1	-58.1	-713.4	2.1	2477
L351R – L351R	-220.7	-75.5	-806.5	16.1	2552.2
T366D – L351K	-223.9	-62.1	-810.1	0.3	2487.8
L351K – L351K	-224.4	-75.6	-812.1	13.6	204.5
T366E – L351R	-222.3	-69	-783	3.4	2557.2
T366E – T366E	-201.9	-57.6	-741	4	2487.5
T366E – L351K	-215.9	-58.4	-808.9	4.3	2486

When looking at the HADDOCK scores, it was observed that some of the mutations have a similar 'pattern' when compared to T366K/L351'D. For most permutations the AA homodimer was found to have a higher HADDOCK-score than the AB heterodimer, but the BB homodimer appeared as favorable as the AB heterodimer. Even though the 351 residue is known to be a 'neighbor' to itself on the other chain, i.e. residue 351 of chain A pairs with residue 351 of chain B at the CH3-CH3 interface, there is barely a negative influence of the identical charges when the BB dimer is formed. Looking at the L351D/L351'D structure this is explained by the aspartic acids bending away from each other and the stabilizing influence of at least the naturally occurring Arginine at position 355 and also some stabilization of negative charge by the naturally occurring Serine at position 354 (see Figure 12A). Mutation of these residues (S354A and R355D) provides only little improvement. From figure 12B it is clear that the backbone-hydrogen of A354 causes stabilization of

the homodimer. From this series, the T366R/L351'E pair seems to be the most favorable, with the lowest HADDOCK score for the bispecific molecule.

Example 16: mutations around T366K/L351'D

In the series of HADDOCK analyses in this example, the T366K/L351'D or T366K/L351'E pair were taken as a starting structure. In order to identify additional mutations that would further increase the predicted percentage of bispecifics of these A and B chains, additional mutations on the B-chain were used to calculate the HADDOCK-scores and energies. When the structure of the CH3 domain is studied using a viewer for visualization of protein structures at a molecular level (YASARA, www.yasara.org), one can calculate the distances between individual residues. While doing so, it was observed that the two residues Y349 and L368 are neighboring residues that may contribute positively or negatively to dimer interactions and these have been mutated in this example –in addition to the L351D mutation– to study the result on dimer formation of the homo- and heterodimers (see figure 13). Both residues seem to add to the stability of the heterodimer (lower HADDOCK scores) as well as to the destabilization of the BB dimer (higher HADDOCK scores). Glutamic acids (E) on positions 349 and 368 seem to be more favorable than aspartic acids (D). Thus, introduction of a second amino acid substitution in the B-chain, comprising already the amino acid substitution at position 351, seems to favor heterodimerization further.

In a next set of HADDOCK analyses, the T366K/L351'D pair was again taken as starting structure. In addition to the substitutions in the B chain that further increased heterodimerization (i.e. Y349D/E and L368E), additional mutations were added to the A-chain which already comprises the T366K substitution. As shown in Figure 14, there are several mutation pairs that seem favorable towards the formation of bispecific heterodimers. In the T366K-L351K/L351'D-Y349'D pair, all four mutated residues are involved in the heterodimeric pairing, which is not de case for T366K-L351K/L351'E-L368'E in which K351 is not directly involved in the binding. However, the HADDOCK-score for this latter heterodimer is -228.9; significantly lower than the -214.2 for the T366K/ L351'E-L368'E, which can be explained by hydrogen bonding interactions of the K at position 351 (see Figure 15). The T366K-L351K/L351'D-Y349'D pair may be further improved by the R355'D mutation in the B-chain, which results in a higher BB-HADDOCK score, but also the

AB HADDOCK score is slightly higher. Overall the additional L351K results in lower AB scores and similar AA and BB scores when compared to the sole T366K mutation in the A chain. Theoretically this would result in higher amounts of bispecific heterodimers in the samples.

As is apparent from figure 11, having an R rather than a K at position 366 may be more potent in driving heterodimerization. Therefore, some of the HADDOCK analyses shown in figure 13 were repeated but now with T366R rather than T366K in the A-chain. It was demonstrated that it is not favourable to combine an R366 in chain A with double mutations in chain B (figure 16). This may be due to the large size of this residue, interfering with other interface interactions, even though all the expected salt-bridges with R366 are present in the structures. Also, the HADDOCK score for the AA homodimer is lower for R366 than for K366, which also doesn't contribute favorably to heterodimer formation. Therefore no further HADDOCK analyses were performed using R366 in the interface.

A total of 14 best performing pairs, according to HADDOCK predictions, have been selected (see Table 13 and Figure 17). In some pairs, an R355D substitution is included to remove the stabilizing influence of the naturally occurring R355 on the L351/L351'D interaction.

Table 13:

Construct combinations	**HADDOCK Score AB**	**HADDOCK Score AA**	**HADDOCK Score BB**
Wildtype-wildtype	-208.2	-208.2	-208.2
T366K – L351D	-210.6	-191.7	-212.5
T366K – L351E	-216.9	-191.7	-217.9
T366R – L351E	-225.8	-201.8	-217.9
T366E – L351R	-222.3	-201.9	-220.3
T366K – L351DY349E	-215.9	-191.7	-190
T366K – L351DL368E	-223.3	-191.7	-198.9
T366K – L351EY349E	-214.5	-191.7	-187.5
T366KL351K – L351D	-233.2	-205	-212.5
T366K – L351DY349EL368E	-207.5	-191.7	-179.5

T366KL351K – L351DY349D	-255.2	-205	-204.3
T366KL351K – L351DY349E	-227.2	-205	-190
T366KL351K – L351DL368E	-243.9	-205	-198.9
T366KL351K – L351DR355D	-233.6	-205	-211.9
T366KL351K – L351DY349DR355D	-242.8	-205	-183.5
T366D – L351KY349K	-237.9	-198.1	-228.4

Example 17: in vitro expression of bispecifics using CH3 mutants based on HADDOCK predictions

The analysis in example 16 suggested that some CH3 variants with additional mutations around the T366K/L351'D pair would yield mixtures with higher proportions of the bispecific component and potentially lower proportions of the homodimeric component. These best performing pairs were selected for production and further analysis. In addition, the constructs T366R and L351E were also generated. Table 14 lists the constructs that were made and which were used for recloning antibody VH regions with known specificities and known ability to pair with the human IGKV1-39 light chain. Expression of the IgGs that contain the individual constructs was previously reported in example 13, and was repeated for the constructs as listed in Table 14. Aim was to assess which of the constructs homodimerize in the absence of a matching heterodimerization partner. Ideally, high percentages of half bodies would be formed and low percentages of homodimers. As a control, constructs containing previously reported charge mutations and constructs containing the previously reported knob-in-hole mutations were also used for expression as whole IgG by recombinant cells. Protein A purified supernatants were analyzed in SDS-PAGE; results were analyzed and scored as presented in Table 14

Table 14:

AA substitutions in CH3	Construct #	% IgG	% half molecule
E356K, D399K	1	64.2	35.8
K392D, K409D	2	30.9	69.1
K392D, K409D, K439D	3	24.5	75.5
T366W	6	27.6	72.4
T366S, L368A, Y407V	7	58.6	41.4
T366K	43	32.9	67.1
L351D	63	89.8	10.2
T366D	64	89.6	10.4
T366K, L351K	68	34.7	65.3
L351D, L368E	69	83.7	16.3
L351E, Y349E	70	67.8	32.2
L351D, Y349E	71	79.7	20.3
L351D, R355D	72	100	-
L351D, Y349E, L368E	73	79.3	20.7
L351D, Y349D	74	88.6	11.4
L351D, Y349D, R355D	75	89.9	10.1
L351K, L368K	76	56.6	43.4
L351R	77	100	-
T366E	78	44.4	55.6
T366R	79	29.6	70.4
L351E	80	100	-

The results of co-expression of a common light chain and two different heavy chains carrying the amino acid substitutions of constructs shown in Table 14 or heavy chains carrying the amino acid substitutions of previous constructs are presented in Table 15. Expression of two different heavy chains comprising the amino acid substitutions T366K and L351'D:L368'E respectively resulted in approximately 87% of the bispecific AB heterodimer in the mixture with no AA or BB homodimers present (combination nr. 3 of Table 15). About 12% half molecules (half A) comprising the T366K substitution was observed. Furthermore, it was found that the percentage of

bispecific AB heterodimer increased when the additional amino acid substitution L351K was introduced in the first heavy chain. For example, co-expression of two different heavy chains comprising the amino acid substitutions T366K:L351K and L351'D:L368'E respectively resulted in approximately 92% of bispecific AB heterodimer whereas AA and BB homodimers are essentially absent in the mixture (combination nr. 12 of Table 15). Combinations 10 and 11 also resulted in favorable distributions of high percentages heterodimers and virtually absence of homodimers. The absence of homodimers is advantageous, because the fraction containing the intact IgG molecules is composed of AB heterodimer only. For purification and subsequent therapeutic application, the half molecules can be removed by standard approaches such as size exclusion chromatography. Hence, applying these newly identified charge mutants in the production process for generating bispecific antibodies provides advantages over known charge mutants and knobs-into-holes mutants where the presence of 'contaminating' homodimeric antibodies is not excluded. In addition, the T366K/L351'D:L368'E and T366K:L351K/ L351'D:L368'E charge pairs have an additional advantage over the previously described E356K:D399K/K392'D:K409'D and E356K:D399K/K392'D:K409'D:K439'D charge reversal pairs, in that the previously described charge variants are based on the reversal of existing charges within the CH3-CH3 interface whereas the newly identified charge variants are adding additional charge pairs (charge-charge interactions) to the CH3-CH3 interface. The introduction of additional charge pairs in the CH3-CH3 interface may further increase the stability of the interface and thereby of the intact antibody. The same holds true for the mutations used in combinations nrs. 4, 5, 6, 9, 10, and 11, which also resulted in favorable proportions of bispecific heterodimer with exceedingly low proportions of AA and BB homodimers present in the mixtures.

Table 15:

Combination of 2 different heavy chains	chain A* / mutations (construct #)	chain B**/ mutations (construct #)	% AA found	% AB found	% BB found	% half A found	% half B found
1	T366E (78)	L351R (77)	3	81	2	13	0
2	T366K (43)	L351D (63)	0	88	3	9	0
3	T366K (43)	L351D,L368E (69)	0	87	0	12	0
4	T366K (43)	L351E,Y349E (70)	2	85	0	11	0
5	T366K (43)	L351D,Y349E (71)	2	92	1	5	0
6	T366K (43)	L351D,Y349E,L368E (73)	0	96	1	4	0
7	T366K,L351K (68)	L351D (63)	0	77	12	10	1
8	T366K,L351K (68)	L351D,R355D (72)	0	79	8	10	1
9	T366K,L351K (68)	L351D,Y349D,R355D (75)	1	93	2	4	1
10	T366K,L351K (68)	L351D,Y349D (74)	1	95	1	3	0
11	T366K,L351K (68)	L351D,Y349E (71)	1	95	0	3	1
12	T366K,L351K (68)	L351D,L368E (69)	0	92	0	8	0
13	T366K (43)	L351E (80)	0	70	10	18	2
14	T366R (79)	L351E (80)	4	38	36	21	1
15	T366D (64)	L351K, L368K (76)	3	92	2,5	2,5	0
16	T366D (64)	L351R (77)	30	69	1	0	0

* chain A carries specificity of MF1337 (=tetanus toxoid); ** chain B carries specificity of MF1122 (=fibrinogen)

Native MS

Native MS was performed on all bispecific samples. The obtained graphs were analyzed to determine the relative ratio's of the present species in two ways: by peak height and by peak area. Peak area is the more scientifically correct way of analysis, but since all previous analyses for other studies were done based on peak height, both methods were included in the analysis, for comparison purposes. The differences between the methods were within the error of measurement, and therefore only the peak area values were used for future measurements. Two typical spectra are shown

in Figure 18. An overview of the results is shown graphically in Figure 19, the numerical values can be found in Table 15. In about half of the samples the total contamination of monospecific IgG is less than 5%, and only in three cases it is > 10% while for wt IgG it is expected to find about 50% of monospecific IgG in the mixture.

A panel of ten combinations of 2 different heavy chains was selected from Table 15 for further analyses. These ten combinations included combinations 1, 2, 3, 4, 5, 6, 9, 10, 11 and 12 (Table 15). Selection of these ten was based on low percentages of homodimers present in the mixtures as determined by nMS, but also based on their overall physico-chemical properties, including production yields, SDS-PAGE, as well as the number of mutations present in the CH3 domain.

Example 18: IgG stability analyses

In this study, a series of CH3 mutation pairs that resulted in high proportions of bispecific heterodimers in the intact IgG fraction and very low amounts (<5%) of parental IgGs will be further analyzed for stability of the Fc part of the IgG molecule. The mutated CH3 domains that are used to promote the heterodimerization of the heavy chains may have unexpected destabilizing effects on the Fc region of the IgG, that may result in undesirable properties such as a reduction of *in vivo* half life, reduction in effector function and/or an increase in immunogenicity. The newly identified charge pairs will be compared to wildtype bispecifics and a bispecific containing previously identified charge mutations (chain A comprising construct 1 and chain B comprising construct 2). All bispecifics in this study will contain the same heavy and light chain variable regions, ensuring that the observed effects are caused by mutations in the Fc-part of the molecule and not by variation in the variable regions.

A series of stability studies will be performed on these bispecifics. These studies include spectroscopic (UV-Vis absorbance, fluorescence and light-scatter) and microscopic (light and fluorescence microscopy with Nile Red staining) analyses that provide information on the aggregation state of the CH3 variants.
The UV-Vis absorbance spectra will be recorded with a double beam, two monochromators Cary 300 Bio spectrophotometer at 25°C. The spectra will be

monitored between 250 and 400 nm using a path length of 1 cm. The absorbance at wavelengths of 320 nm and longer provides information on the aggregation state of the IgG.

Intrinsic fluorescence spectra will be monitored at 25°C using a FluoroMax spectrofluorimeter. The fluorescence method will be optimized. The fluorescence emission will provide information on conformation and aggregation properties.

90° light-scattering spectra will be monitored at 25°C using a FluoroMax spectrofluorimeter by running a synchronous scan ($\lambda_{em} = \lambda_{ex}$) between 400 nm and 750 nm with an integration time of 0.01s. Excitation and emission slits will be optimized. For example, right angle light-scattering can distinguish between IgG samples that have no and 5% dimers.

For fluorescence microscopy with Nile Red staining, just prior to measurements, Nile Red in ethanol will be added to the sample. The samples will be filled in a microscopy slide and analyzed by fluorescence microscopy. Particles will be counted. The lower size limit of the particles that can be observed by fluorescence microscopy is approximately 0.5 μm.

Application of stress such as temperature, pH, mechanical stress or denaturants on proteins might result in a conformation change (e.g. unfolding) and/or aggregation. As it was previously reported that charge-engineered bispecific antibodies have reduced melting temperature of the modified CH3 (Gunasekaran 2010), these studies aim to discriminate between the novel charge mutants of the present invention and existing known charge mutants.

Thermo-stability studies using the Octet are explored, both with Protein A biosensors and by using FcRn binding to IgG. To examine the thermal stability of CH3-engineered IgGs, the samples will be incubated at a concentration of 100 ug/ml (in PBS) at 4, 50, 55, 60, 65, 70 and 75°C for 1 hour using a PCR machine. Following this the samples will be cooled down slowly during a period of 15 minutes to 25°C and kept at this temperature for 2 hours, after which they will be stored overnight at 4°C. Precipitated antibodies will be removed by centrifugation, after which the total IgG concentration of soluble antibodies will be determined by Octet using the protein A Biosensor (1/10 dilution in PBS). Assays that measure binding of the CH3 engineered IgG to FcRn using the Octet are being explored. Either protein L biosensors are used to bind the light chain of IgG to the sensor, followed by incubation with FcRn in solution, or anti-penta-HIS biosensors are used to bind His-tagged FcRn protein,

followed by incubation with the IgG of interest. These methods may be more sensitive than using the protein A Biosensor and can also be used for thermal stability studies. All samples will also be analyzed for serum stability. Briefly, (engineered) IgG samples will be incubated at 37°C in human serum, control samples will be kept at 4°C. After 1, 2, 3 and 4 weeks, samples are centrifuged to remove precipitated IgG. Subsequently the sample is titrated in antigen-specific ELISA to determine the relative amounts of functional IgG. Purified control antibody freshly spiked in human serum will be used as a reference.

Example 19: stability analyses

In previous experiments, high percentages of bispecific antibodies were obtained by co-expression of two different heavy chains comprising CH3 mutations, and a common light chain (example 17).

A panel of eight combinations of 2 different heavy chains was selected from Table 15 for further analyses. These eight combinations included combinations 3, 4, 5, 6, 9, 10, 11 and 12 (Table 15). In this study, these eight combinations were analyzed, with a strong focus on stability of the Fc part of the IgG. As controls, wildtype bispecifics (i.e. without CH3 mutations) and/or bispecifics based on previously reported CH3 charge mutations were included. Note that for wildtype bispecifics, 2 heavy chains and the common light chain are co-expressed without means for preferential steering towards heterodimers. These 'wildtype bispecifics' thus represent a mixture of AA, AB and BB. All bispecifics in this study were designed to carry the same VH/VL-combinations, ensuring that the observed effects are caused by mutations in the Fc-part of the molecule and not by variation(s) in the Fab parts.

It was hypothesized that the mutational pairs that were used to promote the heterodimeric pairing of the two different heavy chains could be associated with unexpected structural or otherwise destabilizing effects on the Fc region of the IgG. This could subsequently result in undesired issues that would hamper further clinical development, such as a reduction of *in vivo* half life, a reduced effector function and/or increased immunogenicity due to the presence of these mutations.

Thermo stability

Application of stress such as increases or decreases in temperature might result in a conformation change (e.g. unfolding) and/or aggregation of proteins. To examine the thermal stability of CH3-engineered IgGs, the bispecific molecules from combinations 3-6 and 9-12 (Table 15), as well as wildtype bispecifics and bispecific molecules obtained when using constructs 1 and 2 (E356K:D399K/ K392D':K409D' combination, also dubbed 'charge reversal' pair) were incubated at a concentration of 100 µg/ml (in PBS) at 4, 60, 62.5, 65, 67.5, 70 and 72.5°C for 1 hour using a PCR machine. Following this the samples were cooled down slowly during a period of 15 minutes to 25°C and kept at this temperature for 2 hours, after which they were stored overnight at 4°C. The next day, precipitated antibodies were removed by centrifugation (18,000 rpm; 4°C, 20 min), after which the total IgG concentration of soluble antibodies was determined by Octet using the protein A Biosensor (1/10 dilution in PBS). Results are shown in figure 20. It was observed that the control CH3 engineered bispecific antibody (the charge reversal E356K:D399K/ K392D':K409D' combination (triangles)) has a reduced thermal stability as compared to the wildtype bispecific (squares). The bispecific molecules from combinations 3-6 and 9-12 (diamonds) also demonstrated a reduced thermal stability as compared to wildtype. Remarkably, three combinations, however, demonstrated an improved stability as compared to the control CH3 engineered bispecific antibody. Bispecifics of combinations 9, 10 and 11 are significantly more stable than the other CH3 engineered (charge reversal) bispecifics and are as stable as wildtype bispecifics at the highest temperature measured.

Freeze-thaw stability

To examine the stability of CH3-engineered IgGs upon repetitive freezing and thawing, the bispecific molecules from combinations 3-6 and 9-12 (Table 15), as well as wildtype bispecifics and bispecific molecules obtained when using constructs 1 and 2 (E356K:D399K/ K392D':K409D' combination (charge reversal pair)) were exposed to ten subsequent freeze-thaw cycles by putting the samples at -80°C for at least 15 minutes until they were completely frozen. Thereafter, samples were thawed at room temperature. When they were completely thawed, the freeze-thaw cycle was repeated. After 10 freeze-thaw cycles, precipitated antibodies were removed by centrifugation (18,000 rpm; 4°C, 20 min), after which the total IgG concentration of soluble antibodies was determined by Octet using the protein A Biosensor (1/10 dilution in

PBS). The freeze-thaw stability test was repeated three times. Results are shown in figure 21. It was observed that the control charge reversal CH3 engineered bispecific antibody seemed to have a slightly reduced stability as compared to the wildtype bispecific. In contrast, the bispecific molecules from combinations 3, 4 and 9 seemed to have a slightly improved stability as compared to the wildtype bispecific. Overall, it can be concluded that the stringent conditions of freeze-thaw cycles do not cause major stability issues for the CH3 engineered variants.

In vitro serum stability

To examine the stability of CH3-engineered IgGs in serum kept at 37°C, the bispecific molecules from combinations 3-6 and 9-12 (Table 15), as well as wildtype bispecifics and the charge reversal bispecific molecules were incubated at 37°C in 10% human serum. Control samples were kept in human serum at 4°C. After 1, 2 or 5 days, precipitated antibodies were removed by centrifugation. Thereafter, the samples were titrated in a fibrinogen-specific ELISA, to determine the relative amounts of functional IgG. Purified control antibody freshly spiked in human serum was used as reference.

Data of the fibrinogen ELISA show that all samples were quite stable in 10% human serum at 37°C for 5 days. At the lower IgG concentration bispecific molecules from combinations 4 and 5 seem to be slightly less stable, especially at T=1 and T=2, but the difference is only minimal at the end-point of this experiment (see Figure 22).

Example 20: Further stability tests

A further series of analytical methods was used to assess the stability of the variant IgGs. Bispecific molecules from combinations 3-6 and 9-12 (Table 15), as well as wildtype bispecifics (AA, AB, BB), the individual parental antibodies (AA and BB) and bispecific molecules obtained when using constructs 1 and 2 (E356K:D399K / K392D':K409D' combination (charge reversal pair)) were used as samples in these stability assays. All IgGs were diluted to 0.2 mg/ml and several stress conditions (2 days at 50°C, 2 weeks at 40°C, 5x freeze-thawing) were applied, aiming to be able to discriminate between the different samples. Of note, these high stress levels resulted in conditions in which one of the parental antibodies (the BB parental, carrying two 1122 Fabs) as used in all bispecifics became unstable. At 2 days at 50°C, aggregation of this protein was detected by UV absorbance. This suggested that this stress

condition may not differentiate between instability of the Fab and the CH3 in the bispecific and data resulting from the 50°C incubation should be used cautiously.

The results are summarized in Table 16. Analytical methods that were used included:

- Fluorescence microscopy with Nile Red ('Nile Red particles' in Table 16); to observe the amount of particles > 0.5 µm after addition of Nile Red dye.
- UV spectrometry at 350 nm ('UV 350 nm'); a change in absorption at wavelengths > 320 nm gives information about the aggregation state of the protein.
- 90° Light scatter at 400 nm ('LS 400 nm'); a sensitive technique to observe changes in protein aggregation, e.g. the difference between monomers and dimers of IgG.
- Intrinsic fluorescence; the fluorescence wavelength maximum and intensity of the aromatic residues in a protein change upon changes in the environment (e.g. unfolding)
- 1,8-ANS fluorescence spectroscopy; 1,8-ANS binds through electrostatic interactions to cationic groups through ion pair formation and changes in protein structure and/or conformation can be detected

UV-VIS spectroscopy

UV-Vis absorbance spectra were measured at 25°C with a double beam, two monochromators Cary 300 Bio spectrophotometer from Varian in different quartz cuvettes (such as black low volume Hellma cuvettes with a pathlength of 1.0 cm and clear Hellma cuvettes of 0.2 cm x 1.0 cm). The spectra were monitored between 220 and 450 nm using a pathlength of 1.0 cm. The absorbance around 280 nm provides information on the protein concentration. The region between 320 nm and 450 nm can provide information on the aggregation state of the samples.

90° light-scattering

The 90° light-scattering spectral method was developed to study protein aggregation and was performed as described in Capelle, 2005; Demeule, 2007a. 90° light-scattering spectra were monitored at 25°C using a FluoroMax spectrofluorimeter (Spex, Instruments S.A., Inc. U.K.) by running a synchronous scan ($\lambda_{em} = \lambda_{ex}$)

between 400 nm and 750 nm with an integration time of 0.01 s. Different slits settings were tested in order to find the optimal conditions. After optimization, the same slit settings were used for all measurements.

Steady-state fluorescence emission

The fluorescence emission of tryptophan, tyrosine and phenylalanine residues gives information on the local environment of these fluorophores. Changes or differences in hydrophobicity and/or rigidity are measured. Typically, a more hydrophobic and rigid environment leads to an increase in the fluorescence intensity and a blue shift of the emission maximum. Intrinsic fluorescence spectroscopy can provide information on the current state of the protein and monitor changes in the physical and chemical properties. More information on the fluorescence of tyrosine and tryptophan can be found in the book of Lakowicz [Lakowicz, 2006].

The fluorescence emission and excitation spectra were recorded at 25°C in different quartz cuvettes. The samples were excited at different wavelengths. Integration times and slit settings were optimized. After optimization, the same integration times and slit settings were applied for all samples.

Fluorescence microscopy with Nile Red staining

The Nile Red staining method was developed to visualize protein aggregates and was performed as described in Demeule et al., 2007b.

The microscopy observations were performed on a Leica DM RXE microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a mercury lamp. The images were acquired with a Sony NEX-5 camera and its firmware. The objectives were 10x, 20x and 40x. For microscopy investigations slides with a fixed distance of 0.1 mm between the slide and the cover glass were used. The size of the 4x4 grids is 1 mm x 1 mm and corresponds to 0.1 µl.

1,8-ANS fluorescence spectroscopy

1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) is an uncharged small hydrophobic fluorescent probe (Mw 299.34 Da) used to study both membrane surfaces and proteins.

1,8-ANS is essentially non-fluorescent in water and only becomes appreciably fluorescent when bound to membranes (quantum yields ~0.25) or proteins (quantum

yields ~0.7). This property of 1,8-ANS makes it a sensitive indicator of protein folding, conformational changes and other processes that modify the exposure of the probe to water. References on 1,8-ANS can be found on the Internet home page of Molecular Probes, www.probes.com.

The fluorescence emission spectra of 1,8-ANS were recorded using a FluoroMax spectrometer. A direct comparison of the 1,8-ANS fluorescence between IgGs will not be performed. Each IgG can have different number of 1,8-ANS binding sites and can therefore not be compared. In principle, the lower the 1,8-ANS fluorescence, the less 1,8-ANS molecules are bound to the antibody. The changes in the 1,8-ANS fluorescence intensity and emission wavelength due to stress will be evaluated.

Table 16: Overview of the different forced degradation results on various IgG samples after dilution to 0.2 mg/ml. The labels of the cells indicate the variations between T= 0 and after stress: (###)= large change, (##) = small change and no label = no change (=stable).

* 'combi. #' refers to the combination of mutations as listed in Table 15; ** very small particles by fluorescence microscopy, relevance of these particles unknown; 2d4°C= 2 days at 4°C; 2d50°C= 2 days at 50°C; 2w4°C= 2 weeks at 4°C; 2w40°C= 2 weeks at 40°C; T0= start of experiment; 5FT=5 freeze thaw cycles

Protein sample	**Stress**	**Nile red particles**	**UV 350 nm**	**LS 400 nm (10^7 cps)**	**Intrinsic fluorescence**		**1,8-ANS fluorescence**		
					fluo.int. (10^6cps)	**λ Max. (nm)**	**1,8-ANS int. (10^6cps)**	**λ Max. (nm)**	**Shift (nm)**
BB	2d4°C	0-10	0.001	0.7	4.2	335			
	2d50°C	0-10	0.013 (###)	0.8	4.2	335			
AA	2d4°C	10-20	0	1.2	5.7	338			
	2d50°C	10-20	0.002	1.0	5.5	338			
Wildtype bispecific (AA AB BB)	2d4°C	30-50 (##)	0.003	0.9	5.1	336	7.1	507	
	2d50°C	>10000**	0.007	0.9	5.0	336	7.1	507	

			(##)						
	2w4°C		0	0.9	5.0	336			
	2w40°C	>2000**	0	0.8	5.0	336			
	T0		0.001	0.8	5.0	336			
	5FT	>2000**	0.009 (##)	1.2	4.8	336			
Charge reversal bispecific (E356K,D399K/ K392D,K409D)	2d4°C	10-20	0.001	1.3	5.9	336	7.0	507	
	2d50°C	10-20	0.002	1.2	5.7	336	7.0	507	
	2w4°C	>2000**	0	1.1	5.5	336			
	2w40°C	>2000**	0.002	1.1	5.5	336			
	T0		0.001	1.3	5.7	336			
	5FT	30-50 (##)	0.007 (##)	1.8 (##)	5.5	336			
Combi. # 3*	2d4°C	30-50 (##)	0	0.9	5.0	337			
	2d50°C	30-50 (##)	0.001	0.8	4.9	337			
Combi. # 4	2d4°C	20-30	0	1.0	6.2	337	7.5	505	
	2d50°C	>3000**	0.001	1.0	6.2	337	7.5	505	
	2w4°C		0.001	1.0	6.3	337			
	2w40°C	>2000**	0.003	0.9	6.3	337			
	T0		0.002	1.1	6.3	337			
	5FT	>2000**	0.003	1.2	6.0	337			
Combi. # 5	2d4°C	>2000**	0.001	1.1	4.9	337			
	2d50°C	>10000**	0.001	0.9	5.0	337			
Combi. # 6	2d4°C	10-20	0	0.7	4.3	337			
	2d50°C	20-30	0.001	0.7	4.3	337			
Combi. # 9	2d4°C	30-50 (##)	0	1.0	5.5	337	7.5	507	
	2d50°C	50-100 (###)	0	1.0	5.5	337	8.1 (###)	500 (###)	-7 (###)
	2w4°C	>2000**	0	0.9	5.1	337			
	2w40°C	>2000**	0	0.9	5.2	337			
	T0		0.002	0.8	5.1	337			
	5FT	>2000**	0.007 (##)	1.4 (##)	4.9	337			
Combi. # 10	2d4°C	30-50	0.002	1.0	5.6	337	7.0	505	

		(##)							
	2d50°C	150-200 (###)	0.001	1.1	5.9	337	8.7 (###)	499 (###)	-6 (###)
	2w4°C	>2000**	0	0.9	5.2	337			
	2w40°C	>2000**	0	0.9	5.4	337			
	T0		0.005	1.0	5.3	337			
	5FT	20-30	0.004	1.1	5.4	337			
Combi. # 11	2d4°C	20-30	0	0.9	4.9	337			
	2d50°C	30-50 (##)	0.002	0.9	5.1	337			
	2w4°C	>2000**	0	0.8	5.0	337			
	2w40°C	>2000**	0	0.8	5.1	337			
	T0		0.004	1.1	5.0	337			
	5FT	>2000**	0.002	1.2	5.0	337			
Combi. # 12	2d4°C	10-20	0.001	0.8	3.8	337	6.2	511	
	2d50°C	10-20	0.002	0.7	3.8	337	6.5 (##)	508 (##)	-3 (##)
	2w4°C	>2000**	0.003	0.6	3.6	337			
	2w40°C	>2000**	0.001	0.5	3.5	337			
	T0		0.005	0.6	3.7	337			
	5FT		0.004	0.7	3.6	337			

Taken together, these data indicate that the various IgG samples are remarkably stable. Severe stress conditions (e.g. 2 days at 50°C) were needed to generate measurable differences between the tested samples. Under these conditions, samples of combinations # 9 and # 10 seem to aggregate more than other samples.

The most discriminating factors for stability between the proteins are the freeze-thaw cycles and increased temperature. Taking into account the very stringent stress factor of incubating at 50°C, the T366K/L351E,Y349E (combi.#4) and T366K,L351K/L351D,Y349E (combi.#11) variants are the two most stable proteins within panel, closely followed by T366K,L351K/L351D,Y349D (combi.#10) and T366K,L351K/L351D,L368E (combi.#12).

Example 21: Native MS on ratio experiments; transfection ratio's from 1:5 to 5:1

To become more knowledgeable about the behavior of the CH3 mutated IgGs in skewed transfection mixtures, in particular about the T366K:L351K/L351D':L368E' combination (from now on dubbed KK/DE or DEKK), a more elaborate ratio experiment was conducted.

Previously used antibody VH regions with known ability to pair with the common light chain IGKV1-39 were used for recloning into constructs 1, 2, 68 and 69, resulting in vectors I-V of Table 17. Vectors I-V, each containing nucleic acid sequences encoding the common human light chain as well as an Ig heavy chain with different CH3 region and different antigen specificity, were subsequently transfected into cells with different transfection ratios as indicated in Table 18. Results are shown in figure 23.

Table 17:

Vector	**VH gene**	**Antigen specificity**	**VH mass (Da)**	**Merus designation**	**Cloned in construct #**
I	IGHV 3.30	Fibrinogen (A)	12794	MF1122	69 (L351D, L368E)
II	IGHV 3.23	RSV (C)	13941	MF2729	69 (L351D, L368E)
III	IGHV 1.08	Tetanus (B)	13703	MF1337	68 (T366K, L351K)
IV	IGHV 3.30	Fibrinogen (A)	12794	MF1122	1 (E356K, D399K)
V	IGHV 1.08	Tetanus (B)	13703	MF1337	2 (K392D, K409D)

Table 18:

Transfection nr	**vectors**	**ratio**
1	I and III	5:1
2	I and III	3:1

3	I and III	1:1
4	I and III	1:3
5	I and III	1:5
6	II and III	5:1
7	II and III	3:1*
8	II and III	1:1
9	II and III	1:3
10	II and III	1:5
11	IV and V	5:1
12	IV and V	3:1
13	IV and V	1:1
14	IV and V	1:3
15	IV and V	1:5

*due to a technical error, this sample has not been measured.

Figures 23A and B show that for the DEKK combination of mutations, when an excess of A or C is present (A or C are on the 'DE side' and B is on the 'KK side'), AB or BC is formed but the surplus of A or C is present as a mixture of both homodimers and half bodies in all cases. However, when an excess of B is present (B is on the 'KK side' and A or C are on the 'DE side'), there is a clear difference. AB or BC is still formed but the surplus of B is essentially absent as homodimer and only half bodies are formed. Percentages were again measured by peak height Nota bene: peaks detected in the range of 2% or lower are below the threshold of what the nMS technology as applied can accurately measure. Measurements of <2% are therefore regarded to be within the noise level of analysis and therefore ignored. It is striking that the excess of B results in high percentages of half body B only. Especially at the 1:3 and 1:5 ratios of A:B, high percentages of half body B were observed (Fig. 23A and 23B) in the absence of homodimer BB, indicating that the CH3 mutations of the KK-side disfavour homodimerization. The absence of homodimers offers a crucial advantage, as this 'KK side' of the DEKK combination can be chosen to incorporate a specificity that may have known adverse effects when present as a homodimer (for example cMET or CD3 antibodies are known to have undesired adverse side effects when present as bivalent homodimers in therapeutic compositions).

The observed findings for the different ratio's of DE:KK are in contrast to the control charge reversal CH3 mutations in vectors IV and V. Figure 23C shows that for the E356K:D399K/K392D':K409D' combination of mutations when an excess of A is present (A is on the 'K392D:K409D side'), the surplus of A is present as a mixture of both homodimers and half bodies in all cases, but also when an excess of B is present (B is on the 'E356K:D399K side'), the surplus of B is present as a mixture of both homodimers and half bodies in all cases. Even at the higher ratios 1:3 and 1:5 no half bodies B are observed although homodimers are present, indicating that the E356K:D399K side does not disfavour homodimerization as much as the KK-side of the DEKK combination.

Taken together, the DEKK combination of mutations offers a clear benefit over the charge reversal CH3 mutations, in that one of the chains of the heterodimer does not form homodimers.

Example 22: varieties of mixtures using the DEKK combination

As it was demonstrated that the DEKK combination of mutations drives the formation of bispecific IgG molecules ('AB') with high purity, we next explored the feasibility of controlled production of more complex antibody mixtures from one cell, such as 'AB and AA' or 'AB and AC' mixtures. Previously used model Fabs were incorporated in vectors that contain either the 'DE construct' or the 'KK construct' and various combinations of these vectors were co-expressed to create mixtures, to demonstrate the versatility of the technology. Model Fabs MF1337 (tetanus toxoid), MF1122 (fibrinogen) and MF1025 (thyroglobulin) were chosen based on their overall stable behaviour, good expression levels and mass differences between the IgGs containing these Fabs (see Table 19)

Table 19:

Specificity	**Fab name**	**IgG mass**	**Δ-mass MF1122**
Tetanus (A)	(MF)*1337	146747.03	+1842.05
Fibrinogen (B)	(MF)1122	144904.98	0
Thyroglobulin (C)	(MF)1025	144259.87	-645.11

* MF=Merus Fab, designations such as MF1337 and 1337 are both used interchangeably.

Table 20: Transfection schedule:

Tr. #	**Heavy chain 1**	**Heavy chain 2**	**Heavy chain 3**	**Tr. ratio**	**Expected species (%)**	**Observed species (%)**
1	1337-KK	1122-DE	1025-DE	2:1:1	AB (50%) AC (50%)	AB (43%) AC (57%)
2	1337-DE	1122-KK	1025-KK	2:1:1	AB (50%) AC (50%)	AB (40%) AC (54%) AA (6%)
3	1337-KK	1122-DE	1025-KK	1:2:1	AB (50%) BC (50%)	AB (54%) BC (46%)
4	1337-KK	1122-KK	1025-DE	1:1:2	AC (50%) BC (50%)	AC (66%) BC (33%) CC (1%)
5	1337-KK	1337-DE	1122-DE	2:1:1	AA (50%) AB (50%)	AA (57%) AB (43%)
6	1337-KK	1122-KK	1122-DE	1:1:2	AB (50%) BB (50%)	AB (75%) BB (25%)
7	1337-KK	1337-DE	1025-DE	2:1:1	AA (50%) AC (50%)	AA (46%) AC (54%)
8	1337-KK	1025-KK	1025-DE	1:1:2	AC (50%) CC (50%)	AC (60%) CC (40%)
9	1337-KK	1122-DE		1:1	AB (100%)	AB (>98%)
10	1337-KK	1025-DE		1:1	AC (100%)	AC (>98%)
11	1122-KK	1025-DE		1:1	BC (100%)	AC (>98%)

SDS-PAGE analysis demonstrated that most samples consisted of predominantly full IgGs and in some cases half bodies were present at small percentages. Furthermore, many of the samples showed two bands at ca. 150 kDa on non-reduced gels, reflecting the presence of two distinct IgG species in the sample. Also on the reduced gels, two heavy chain bands were visible in some samples (data not shown).

Native MS was performed on all samples and the percentages of observed species were calculated based on peak height (% of observed species in Table 20). Results are presented in Figure 24. In all eight samples where three heavy chains were co-expressed, two main peaks were observed which corresponded to the expected species. In two of these samples (transfections 2 and 4), and in transfection 11, a small amount of contaminating DE-DE homodimer was observed. Half bodies were detected in very small amounts in most of the samples (less than 2%), which are not

problematic as they can be easily separated from the full length IgG fraction as discussed previously. After nMS it was discovered that the observed mass of the IgG in sample 11 corresponded to a different species than expected, and it was concluded that this was due to an transfection error, i.e. in sample 11 apparently 1025-DE was co-transfected with 1337-KK instead of 1122-KK.

The IgG samples were further tested in a sandwich ELISA to confirm the functional presence of the desired specificities. Coating of ELISA plates was done with fibrinogen or thyroglobulin and detection was performed with fluorescein-labelled thyroglobulin or - tetanus toxoid. The detection antigens were labelled with fluorescein (Pierce NHS-fluorescin Antibody Labeling kit, cat. # 53029) according to the manufacturer's instructions. Fluorescein-labeled antigens could subsequently be detected by a FITC-conjugated anti-fluorescein antibody (Roche diagnostics, cat. # 11426346910).

Results of the bispecific ELISA (OD450 values) are summarized in Table 21. The cells labelled with (#) indicate the expected species for each transfection. Generally, the results meet the expected outcome with view exceptions as indicated in italic or bold. In transfections 1-3, the supposed 'negative' well for species BC (tr. #1 and 2) or AC (tr.# 3) demonstrated a significant background signal. It is known from previous studies that bispecific ELISAs may suffer from high background levels. These background levels may also be caused by the potential presence of half-bodies in the sample. Of note is that the results of bispecific ELISA indeed confirmed that an error had occurred in transfection #11, as the species AC (bold value) was detected rather than BC.

Table 21: OD450 values from bispecific ELISA

	Detected IgG Species		
Tr. #	AB (Tet-Fib)	AC (Tet-Thyr)	BC (Fib-thyr)
1	0.989 (#)	1.792 (#)	*0.438*
2	1.085 (#)	1.852 (#)	*0.418*
3	1.419 (#)	*0.775*	1.547 (#)
4	0.205	1.795 (#)	1.22 (#)
5	1.367 (#)	0.047	0.057
6	1.359 (#)	0.043	0.06

7	0.054	1.779 (#)	0054
8	0.04	1.338 (#)	0.052
9	1.588 (#)	0.048	0.051
10	0.044	1.805 (#)	0.055
11	0.043	**1.821**	0.056 (#)

Example 23: improved mixtures of two bispecific antibodies recognizing 4 different epitopes (AB and CD) from a single cell

In example 12 it was hypothesized that mixtures resulting from transfections ZA or ZB are expected to become problematic when transferred to larger scale production, as knob-into-hole variants are reported to be unstable and it cannot be excluded that CH3 domains comprising a 'knob' or a 'hole' will dimerize with charge-engineered CH3 domains. As it was demonstrated in the above examples that novel charge pair mutants have been found that preferentially drive heterodimerization with virtually no formation of homodimers, CH3 domain-comprising polypeptide chains comprising these novel charge pair mutants can be expressed in cells together with previously known charge-engineered CH3 domain-comprising polypeptide chains or potentially with SEED bodies, and are likely to result in the preferential formation of two bispecific molecules only.

From the above examples it was clear that the DEKK combination of mutations is excellent for the production of one bispecific (AB) or two bispecifics (AB plus AC) by clonal cells where dimerization of the heavy chains is driven by the CH3 domains. However, using only one vector set of complementary CH3 mutations limits the number of possibilities of mixture-varieties that can be produced. It would be possible to produce more complex mixtures of IgGs and/or bispecifics, such as 'AB and CD' or 'AB and CC' mixtures if a second 'orthogonal' vector set could be used in combination with DEKK. When combining two vector sets, an important requirement is that the heavy chains expressed from the two different sets of CH3 engineered vectors cannot make 'crossed' dimers, which is that the heavy chains produced by one of the vector sets dimerize into full IgG with heavy chains expressed by the other vector set.

To test for such potential formation of 'crossed' dimers, an *in silico* analysis was performed using HADDOCK to obtain further insights whether possible pairing between wildtype CH3 domains and CH3 domains containing DE- or KK-mutations

would occur. Similarly, potential pairings between wildtype CH3 domains and CH3 domains containing E356K,D399K or K392D,K409D mutations were analyzed, as well as potential pairings between wildtype CH3 domains and CH3 domains containing knob-into-hole mutations and any combination of the above. Combinations of CH3-mutants that were analyzed in HADDOCK are listed in Table 22 and the resulting HADDOCK scores are summarized in Figure 25.

Table 22: CH3 variants analyzed in HADDOCK, with one letter codes for assigned for each CH3-variant carrying heavy chain. *Wildtype chains are designated 'C' and 'D' for matters of consistency; **The charge reversal variants are designated 'A and B' when combined with knob-into-hole variants, and are designated 'C and D' when combined with DE/KK variants.

CH3 combination	**Mutations**	**One letter code in HADDOCK**
DEKK	Chain 1: T366K,L351K	A
	Chain 2: L351D,L368E	B
Wildtype (WT)	Chain 1: none	C*
	Chain 2: none	D*
Charge reversal (CR)	Chain 1: K392D,K409D	A/C**
	Chain 2: E356K,D399K	B/D**
Knob-into-hole (KIH)	Chain 1: T366W	C
	Chain 2: T366S,L368A,Y407V	D

Figure 25 shows that, based on these HADDOCK predictions, combining the CH3 combinations of DEKK with charge reversal CH3 combinations is most likely to be successful in forming the desired combination of two bispecifics (AB and CD) without contaminating by-products (especially AC, AD, BC, BD) when co-transfected in a single cell. As can be seen from figure 25, these undesired bispecific species AC, AD, BC, and BD have relatively high HADDOCK scores, whereas the desired AB and CD species have the lowest HADDOCK scores. Of course, when either the CH3 combinations of DEKK or charge reversal will be put into a construct carrying the

same specificity (e.g. 'C' on the DE-side, 'C' on the KK-side, 'A' on the E356K,D399K-side and 'B' on the E356K,D399K-side, or 'A' on the DE-side, 'B' on the KK-side, 'C' on the E356K,D399K-side and 'C' on the E356K,D399K-side) this will result in the production of predominantly CC and AB upon co-expression in a cell.

In contrast, when looking at the predictions for co-expressing DEKK with wildtype, it can be seen that the HADDOCK scores for AC and AD are lower than the HADDOCK score for CD, which indicates that AC and AD are very likely contaminants when trying to produce a mixture of AB and CD by co-expression of vectors encoding for CH3 combinations of DEKK together with vectors encoding wildtype CH3. Lastly, the predictions for co-expressing either DEKK or charge reversal variants together with the knob-into-hole variants results in undesired bispecific variants with relatively low HADDOCK scores, i.e. a high likelihood that these undesired species will be produced upon co-expression.

It is thus concluded that combining the CH3 combinations of DEKK with charge reversal CH3 combinations (E356K,D399K/K392'D,K409D') is ideally suited for obtaining essentially pure 'AB and CD' and/or 'AB and CC' mixtures of antibodies.

Next, mixtures of 2 bispecifics recognizing 4 targets/epitopes (AB and CD) and mixtures of one bispecific and 1 monospecific antibody recognizing 3 targets/epitopes (AB and CC) were created by putting the above into practice. These mixtures were created using 4 different VHs that are all capable of pairing with the common light chain IGVK1-39, but the individual VH/VL combinations all have different specificities. To enable native MS analysis, the mass difference between the (expected) species has to be sufficient, i.e. > 190 Da. Four individual VHs have been selected and the masses of these were such that the expected species upon co-transfection could be identified and separated by nMS. Furthermore, the mass differences between the 4 selected VHs are also large enough to identify most of the possible contaminants in the mixtures, in addition to the two desired species. Selected VHs are listed in Table 23.

Table 23:

VH (target)	**Mass as wt IgG**
A (RTK1)	146736.78

B (Tetanus toxoid)	146106.20
C (Fibrinogen)	144904.98
D (RTK2)	145421.37

The 4 different VHs were cloned into vectors containing the 'DE' or 'KK' constructs or the charge reversal constructs, and several co-transfections were performed as indicated in Table 24. NB: as always, all vectors also contained the nucleic acid encoding the common light chain IGKV1-39. As previously indicated, when combining two vector sets, an important requirement is that the heavy chains expressed from the two different sets of CH3 engineered vectors cannot make 'crossed' dimers, which is that the heavy chains produced by one of the vector sets dimerize into full IgG with heavy chains expressed by the other vector set. To test for such potential formation of 'crossed' dimers between heavy chains containing charge reversal mutations and heavy chains containing DE or KK mutations, control transfections were performed.

Table 24:

Tr. #	**1st VH / construct #**	**2nd VH / construct #**	**Expected species**
1	D / 68	A / 68	mismatch 'KK' with 'KK'; Mostly half-bodies expected
2	D / 68	A / 69	match 'KK' with 'DE'; AD product expected
3	D / 68	A / 1	Expected mismatch 'KK' with 'E356K:D399K'
4	D / 68	A / 2	Expected mismatch 'KK' with 'K392D:K409D'
5	D / 69	A / 68	match 'DE' with 'KK'; AD product expected
6	D / 69	A / 69	mismatch 'DE' with 'DE'; mixture of half-bodies, AA, AD and DD expected
7	D / 69	A / 1	Expected mismatch 'DE' with 'E356K:D399K'
8	D / 69	A / 2	Expected mismatch 'DE' with 'K392D:K409D'

Tr. #	**1st VH / construct #**	**2nd VH / construct #**	**3rd VH / construct #**	**4th VH / construct #**	**Expected species**
9	A / 68	B / 69	C / 1	D / 2	AB and CD
10	A / 68	A / 69	C / 1	D / 2	AA and CD
11	A / 68	B / 69	C / 1	C / 2	AB and CC

Table 25 provides a further overview of masses of the expected species, and the possible contaminants, of transfections # 9-11 of Table 24.

Table 25: For each of transfections # 9-11, the species are sorted by mass, mass difference is calculated with the mass above. ***: expected (and desired) species; italics: mass difference too small to separate in nMS analysis. *Species: single letters represent half-bodies; two-letter code intact IgG.

Transfection # 9		
Species*	**Mass**	**Mass difference**
C	72464,62	
D	72684,53	219,90
B	73070,99	386,47
A	73410,46	339,47
CC	144929,2	71518,78
CD***	145149,2	219,90
DD	145369,1	219,90
BC	145535,6	*166,56*
BD	145755,5	219,90
AC	145875,1	*119,57*
AD	146095	219,90
BB	146142	*47,00*
AB***	146481,5	339,47
AA	146820,9	339,47
Transfection # 10		
Species	**Mass**	**Mass difference**
C	72464,62	
D	72684,53	219,90
A	73410,46	725,94
CC	144929,2	71518,78
CD***	145149,2	219,90
DD	145369,1	219,90
AC	145875,1	506,03
AD	146095	219,90
AA***	146820,9	725,94
Transfection # 11		
Species	**Mass**	**Mass difference**
C	72464,62	
B	73070,99	606,37
A	73410,46	339,47
CC***	144890,95	71480,49
BC	145535,61	644,66
AC	145875,08	339,47
BB	146141,98	266,90
AB***	146481,45	339,47
AA	146820,92	339,47

All purified protein samples obtained from transfections # 1-11 were analyzed on SDS-PAGE, and three control samples were included (Figure 26). In addition, nMS analysis was performed on protein samples from transfections # 9-11 to identify all species in the samples. As can be seen from Figure 26, transfections # 3 and # 4 resulted in the expected mismatch between 'KK' constructs and either 'E356K:D399K' or 'K392D:K409D' and the amount of half bodies in protein samples from these transfections exceeded the amount of full IgG molecules. Transfections # 7 and # 8 resulted in protein samples wherein both half bodies and full IgG is present in about equal amounts. However, from SDS-PAGE it cannot be deduced whether the full IgG represents a DE/DE dimer, a DE/E356K:D399K dimer or a DE/K392D:K409D dimer. Remarkably, virtually no half bodies were observed in samples from transfections # 9-11.

In Figure 27, the nMS analysis of transfections # 9 and # 11 are presented. Percentages of expected species and contaminating species were calculated by peak height. It was demonstrated that, for transfection # 9, the expected species 'AB and CD' are represented for 97% in the mixture (30% AB and 67% CD) whereas only as little of about 3% of contaminating BD is present (Figure 27A). For transfection # 11, the expected species 'AB and CC' are represented for 94% in the mixture (33% AB and 61% CC) whereas only as little of about 6% of contaminating BC (4.1%) and AC (1.8%) is present (Figure 27B). These data show that it is indeed possible to produce more complex mixtures of IgGs and/or bispecifics, such as 'AB and CD' or 'AB and CC' mixtures when a second 'orthogonal' vector set is used in combination with DEKK. Combination of the charge reversal constructs together with the DEKK constructs results in only very limited formation of 'crossed' dimers. By adjusting the transfection ratio's it is expected that the low percentages of these contaminating by-products can be even further reduced.

Example 24: single dose pharmacokinetic study in mice

To study the pharmacokinetic (pK) behavior of bispecific antibodies carrying the DEKK combination of mutations in their CH3 regions, in this study the pK parameters for three different IgG batches were determined and compared.

The three IgG batches included 1) wildtype anti-tetanus toxoid parental antibody 1337:1337 (two MF1337 Fabs on a wildtype Fc backbone); 2) wildtype anti-tetanus toxoid parental antibody 1516:1516 (two MF1516 Fabs on a wildtype Fc backbone); 3)

CH3 engineered bispecific anti-tetanus toxoid antibody 1516:1337 that carries the DEKK combination of mutations in its Fc region (MF1516 Fab on DE-side, MF1337 Fab on KK-side).

The parental antibodies 1337:1337 and 1516:1516 were chosen as specificities to be included in the DEKK-bispecific product, as it was known based on previous studies that no pre-dose serum response against these antibodies was present in several mice strains. NB: the presence of a pre-dose serum response would of course invalidate the study. In addition, there is sufficient mass difference between the parental antibodies to enable the identification of 1337:1337 (wt Fc), 1516:1337 (DEKK Fc) and 1516:1516 (wt Fc) species by nMS. The three IgG batches were prepared as previously described, but the DNA used for transfection was made using an endo-free maxiprep kit to ensure that the amount of endotoxins is as low as possible. The batches were subsequently tested for protein concentration, aggregate levels, endotoxin levels and percentage bispecific product. It was demonstrated that the acceptance criteria for subsequent use of the IgG batches in a pK study were met, i.e. the IgG concentration after gel filtration was >0.3 mg/ml, aggregate levels were <5%, endotoxin levels were <3 EU/mg protein and the DEKK batch contained > 90% bispecific IgG.
Native mass spectrometry of the gel filtrated samples showed that the expected species were present in high percentages. In sample 1516:1337 a small amount of the DE:DE homodimer is detected, which is estimated to be ca. 2% (Figure 28). It was concluded that the 3 IgG batches are qualified to be used in the pK study.

For comparison of pK parameters between the three batches, 3 groups of female C57BL/6J mice (Harlan, The Netherlands) were dosed at 1 mg/kg human IgG (5 ml/kg immunoglobulin solution/kg body weight). At dosing time, the animals were between 7-8 weeks of age and had a body weight of about 18-20 grams. Blood samples were collected pre-dose and at 15, 60 minutes, and 2, 4, 8, 24, 48, 96, 168, 268 and 336 h after dosing. Serum samples were prepared and stored at < -20 °C until analysis. Each group consisted of 3 subgroups of 4 mice, i.e. 12 mice/group. From each mice 6 time points were sampled. The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 86/609/EEC) and Dutch legislation (The Experiments on Animals Act, 1997). This study was also performed in compliance

with the Standards for Humane Care and Use of Laboratory Animals, as issued by the Office of Laboratory Animal Welfare of the U.S. National Institutes of Health under identification number 45859-01 (expiration date: 30 April 2015).

Mice of Group 1 received the full length monospecific IgG 1516:1516 antibody (triangles); Mice of Group 2 received the full length monospecific IgG 1337:1337 antibody (squares); Mice of Group 3 received the full length bispecific IgG 1516:1337 antibody (diamonds), with DEKK engineered CH3 regions (1516 on the DE-side and 1337 on the KK-side).

An ELISA assay was applied for the quantitative analysis of monoclonal human antibodies in mouse serum using a quantitative human IgG ELISA (ZeptoMetrix, NY USA; ELISA kit nr. 0801182). Briefly, the ELISA assay is based on the principle that the human monoclonal antibody binds to anti-human IgG coated in a 96-wells ELISA plate. Bound antibody was subsequently visualized using a polyclonal antihuman IgG antibody conjugated with horseradish peroxidase (HRP). The optical density (OD) of each well is directly proportional to the amount of antibody in the serum sample. Results are shown in Figure 29, and it was observed that serum levels of both the bispecific full length IgG antibody carrying the DEKK combination of mutations and its parental monospecific antibodies are strikingly similar. It is concluded that the CH3 mutations as present in the DEKK-bispecific antibody does not alter stability nor half life, and the DEKK variant is behaving as wildtype IgG.

References

Deisenhofer J., Biochemistry 1981(20)2361-2370;

Miller S., J. Mol. Biol. 1990(216)965-973;

Padlan, Advances in Protein Chemistry 1996 (49) 57-133

Ellerson JR., et al., J. Immunol 1976 (116) 510-517;

Lee and Richards J. Mol. Biol. 1971(55)379.

Gunasekaran et al J.Biol.Chem. 2010(285)19637-19646

De Vries Nature Protocols 2010(5)883

Kabat et al, (1991)

Mammalian Cell Biotechnology : a Practical Approach (M. Butler, ed., IRL Press, 1991

Merchant Nature biotechnology 1998(16)677

Ridgeway Protein Engineering 1996(9)617-621.

Davis JH. Et al., Protein Engineering, Design & Selection 2010(23)195-202

Papadea and Check. Crit Rev Clin Lab Sci. 1989;27(1):27-58.

Tissue Culture, Academic Press, Kruse and Paterson, editors (1973)

Ionescu et al., J. Pharm. Sci. 2008 (97)1414)

Current protocols in Protein Science 1995, coligan JE et al., Wingfield PT, ISBN 0-471-11184-8, Bendig 1988.

Capelle, M.A.H., Brugger, P., Arvinte, T. ***Vaccine*** 23 (2005), 1686-1694.

Demeule, B., Lawrence, M.J., Drake, A.F., Gurny, R., Arvinte, T. Biochim. Biophys. Acta 1774 (2007a), 146-153.

Demeule, B., Gurny, R., Arvinte, T., Int. J. Pharm 329 (2007b), 37-45.

Lakowicz, J.R., Principles of fluorescence spectroscopy; Second Edition Kluwer Academic/Plenum Publishers, New York, Boston, Dordrecht, London, Moscow, (2006) ISBN 0-306-46093-9.

Claims

1. A heterodimeric Ig-like molecule comprising two CH3 domains, wherein one of said two CH3 domains comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and wherein the other of said two CH3 domains comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue, wherein the at least one positively charged amino acid residue substituted in the first CH3 domain interacts with the at least one negatively charged amino acid residue substituted in the second CH3 domain.

2. A heterodimeric Ig-like molecule according to claim 1, wherein one of said two CH3 domains comprises a substitution of the amino acid residue at position 366 by a positively charged amino acid residue, and wherein the other of said two CH3 domains comprises a substitution of the amino acid residue at position 351 by a negatively charged amino acid residue.

3. A heterodimeric Ig-like molecule according to claim 1, wherein a first CH3 domain comprises a substitution of the amino acid residue at position 366 by a lysine (K) residue, and wherein a second CH3 domain comprises a substitution of the amino acid residue at position 351 by an aspartic acid (D) residue.

4. A heterodimeric Ig-like molecule according to claim 3, wherein the 1st CH3 domain further comprises a substitution of the amino acid residue at position 351 by a lysine (K) residue.

5. A heterodimeric Ig-like molecule according to claim 3 or 4, wherein the 2nd CH3 domain further comprises amino acid substitution(s) selected from the group consisting of
(i) a substitution of the amino acid residue at position 349 by a glutamic acid (E) residue;
(ii) a substitution of the amino acid residue at position 349 by an aspartic acid (D) residue;
(iii) a substitution of the amino acid residue at position 368 by a glutamic acid (E) residue;

(iv) a substitution of the amino acid residue at position 349 by an aspartic acid (D) residue, and a substitution of the amino acid residue at position 368 by a glutamic acid (E) residue; and

(v) substitution of the amino acid residues at positions 349 and 355 by aspartic acid (D) residues.

6. A heterodimeric Ig-like molecule according to claim 5, wherein the 2nd CH3 domain comprises the substitution of the amino acid residue at position 368 by a glutamic acid (E) residue.

7. A heterodimeric Ig-like molecule according to claim 1, wherein a first CH3 domain comprises an amino acid substitution at position 351 by a negatively charged amino acid residue and an amino acid substitution at position 368 by a negatively charged amino acid residue, and wherein a second CH3 domain comprises an amino acid substitution at position 366 by a positively charged amino acid residue and an amino acid substitution at position 351 by a positively charged amino acid residue.

8. A heterodimeric Ig-like molecule according to claim 7, wherein the first CH3 domain comprises an amino acid substitution at position 351 by an aspartic acid (D) and an amino acid substitution at position 368 by a glutamic acid (E), and wherein the second CH3 domain comprises an amino acid substitution at position 366 by a lysine (K) and an amino acid substitution at position 351 by a lysine (K).

9. A heterodimeric Ig-like molecule according to claim 1, that further comprises a common light chain.

10. A heterodimeric Ig-like molecule according to claim 9, wherein the common light chain is a germline light chain.

11. A heterodimeric Ig-like molecule according to claim 9, wherein the common light chain is a rearranged germline human kappa light chain.

12. A heterodimeric Ig-like molecule according to claim 9, wherein the common light chain is the rearranged germline human kappa light chain IgVκ1-39/Jκ or IgVκ3-20/Jκ.

13. A heterodimeric Ig-like molecule according to claim 1, wherein the Ig-like molecule is an antibody.

14. A heterodimeric Ig-like molecule according to claim 1, wherein the Ig-like molecule is human IgG.

15. A heterodimeric Ig-like molecule according to claim 1, wherein said heterodimeric Ig-like molecule binds to different epitopes on the same antigen or to different epitopes on different antigens.

16. A heterodimeric Ig-like molecule according to claim 15, wherein the different epitopes are located on the same target molecule.

17. A heterodimeric Ig-like molecule according to claim 16, wherein the target molecule is a soluble molecule.

18. A heterodimeric Ig-like molecule according to claim 16, wherein the target molecule is a membrane-bound molecule.

19. A heterodimeric Ig-like molecule according to claim 15, wherein the different target epitopes are located on different target molecules.

20. A heterodimeric Ig-like molecule according to claim 19, wherein the different target molecules are expressed on the same cells.

21. A heterodimeric Ig-like molecule according to claim 19, wherein the different target molecules are expressed on different cells.

22. A heterodimeric Ig-like molecule according to claim 19, wherein the different target molecules are soluble molecules.

23. A heterodimeric Ig-like molecule according to claim 19, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

24. A heterodimeric Ig-like molecule according to claim 15, wherein at least one of said epitopes is located on a tumor cell.

25. A heterodimeric Ig-like molecule according to claim 15, wherein at least one of said epitopes is located on an effector cell.

26. A heterodimeric Ig-like molecule according to claim 25, wherein said effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

27. A heterodimeric Ig-like molecule according to claim 15, wherein said epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

28. A pharmaceutical composition comprising a heterodimeric Ig-like molecule according to claim 1 and a pharmaceutically acceptable carrier.

Title: Methods and means for the production of Ig-like molecules

Abstract

The invention provides means and methods for producing one or more Ig-like molecules in a single host cell. Novel CH3 mutations enabling the production of monospecific and/or bispecific Ig-like molecules of interest are also provided.

FIG. 1A schematic representation of construct vector MV1057

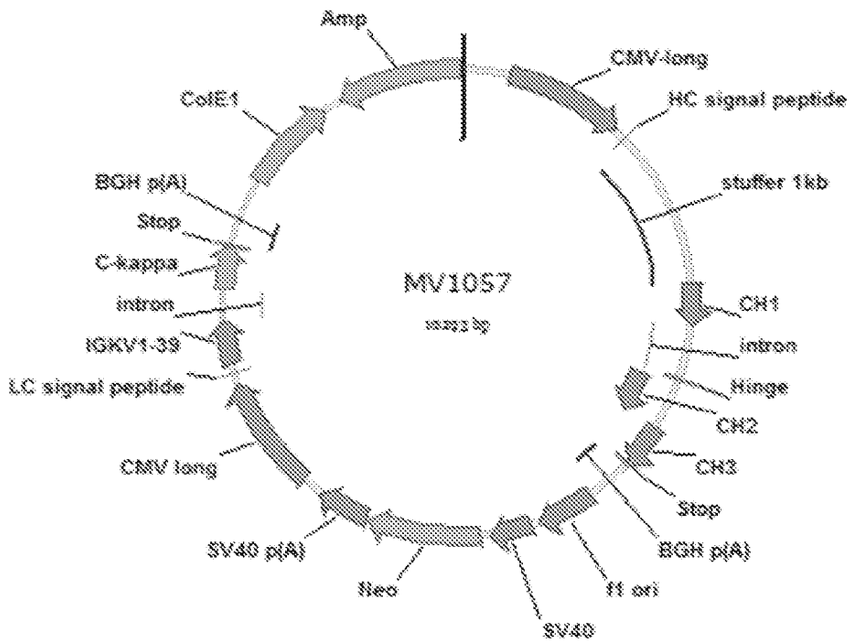


FIG. 1B schematic representation of phage display vector MV1043

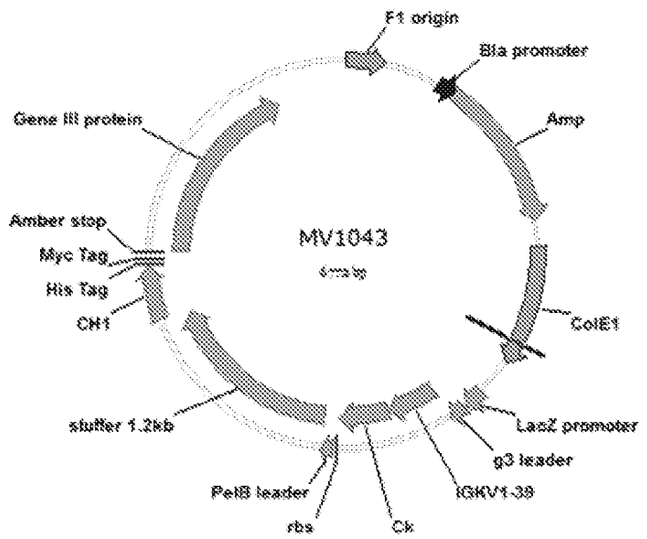


FIG. 2 amino acid sequence of wildtype IgG1 Fc, as present in construct vector MV1057 (EU numbering scheme applied)

```
131         141         151         161         171         181
SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS
191         201         211         221         231         241
SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT
251         261         271         281         291         301
LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
311         321         331         341         351         361
QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
371         381         391         401         411         421
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
431         441
ALHNHYTQKS LSLSPGK  (SEQ ID NO: 1)
```

FIG. 3 nucleotide and amino acid sequences of VH regions used

MF1025_VH (SEQ ID NO: 2)
gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcag
cctctggattcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtg
ggtctcagctattagtggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtgt
attactgtgcaagggccgattggtgggcgacttttgactactggggccaaggtaccctggtcacc

MF1025_VH (SEQ ID NO: 3)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARADWWATFDYWGQGTLVT

MF1122_VH (SEQ ID NO: 4)
gaggtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctgagactctcctgtgcag
cctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctggagtg
ggtggcagttatatcatatgatggaagtaataaatactatgcagactccgtgaagggccgattcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggccgtgt
attactgtgcaagagccctcttcacgaccatcgccatggactattggggccaaggtaccctggtcacc

MF1122_VH (SEQ ID NO: 5)
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARALFTTIAMDYWGQGTLVT

MF1337 VH (SEQ ID NO: 6)
gaggtgcagctggtggagactggggctgaggtgaagaagccgggggcctcagtgaaggtctcctgcaaggcttct
gactacatcttcaccaaatatgacatcaactgggtgcgccaggcccctggacaagggcttgaatggatgggatgg
atgagcgctaacactggaaacacgggctatgcacagaagttccagggcagagtcaccatgaccagggacacgtcc
ataaacacagcctacatggagctgagcagcctgacatctggtgacacggccgtttatttctgtgcgaggagtagt
cttttcaagacagagacggcgccctactatcacttcgctctggacgtctggggccaagggaccacggtcacc

MF1337 (SEQ ID NO: 7)
VHEVQLVETGAEVKKPGASVKVSCKASDYIFTKYDINWVRQAPGQGLEWMGWMSANTGNTGYAQKFQGRVTMTRD
TSINTAYMELSSLTSGDTAVYFCARSSLFKTETAPYYHFALDVWGQGTTVT

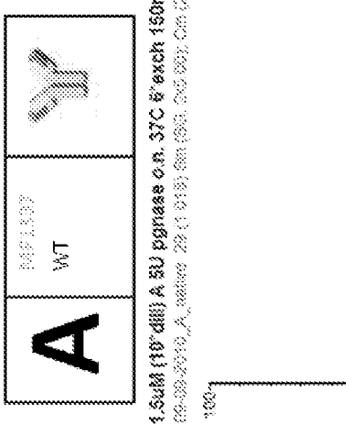
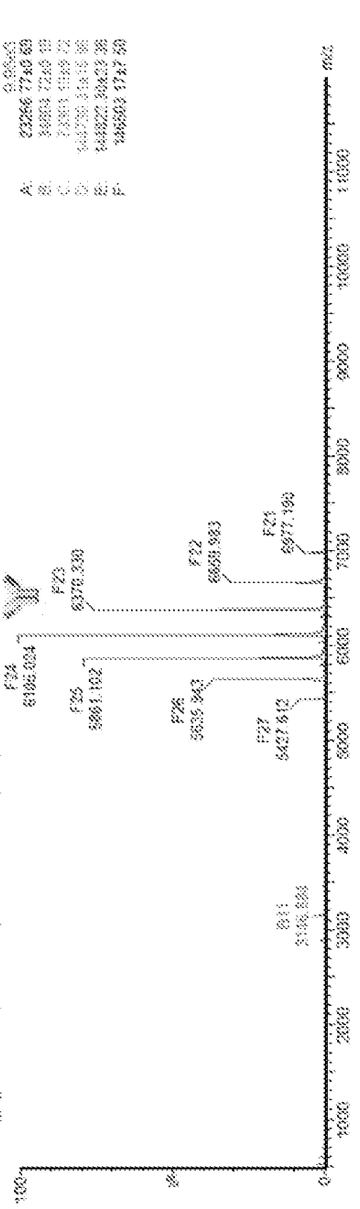

A
MP1107
WT

FIG. 4A


1.5uM (10^dil) A SU pgnase o.n. 37C 6^exch 150mM Ac ph5.5
TOF MS ES+
F24
6185.024
F23
6376.330
F25
6861.102
F22
6658.983
F26
6635.943
F21
6977.190
F27
6437.612
B11
3104.554
m/z

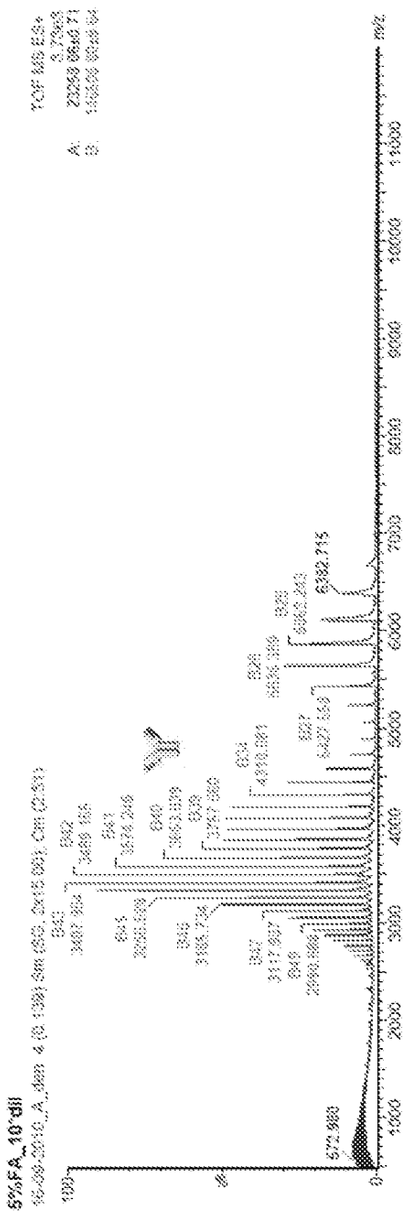

6%FA_10^dil
TOF MS ES+
3.73e5
A: 23256.6846 71
B: 14326.8528 64
B11
3087.954
B10
3488.166
B9
3841.246
B8
4220.888
B7
4600.219
B6
4979.750
B5
5359.281
B4
5738.812
B3
6118.343
B2
6497.874
B1
6877.405
572.988
m/z

FIG. 4B

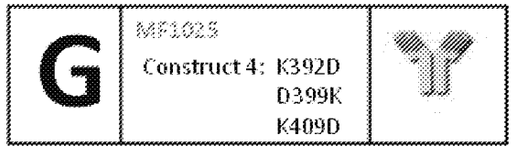

G
MF1025
Construct 4: K392D
D399K
K409D

FIG. 4C

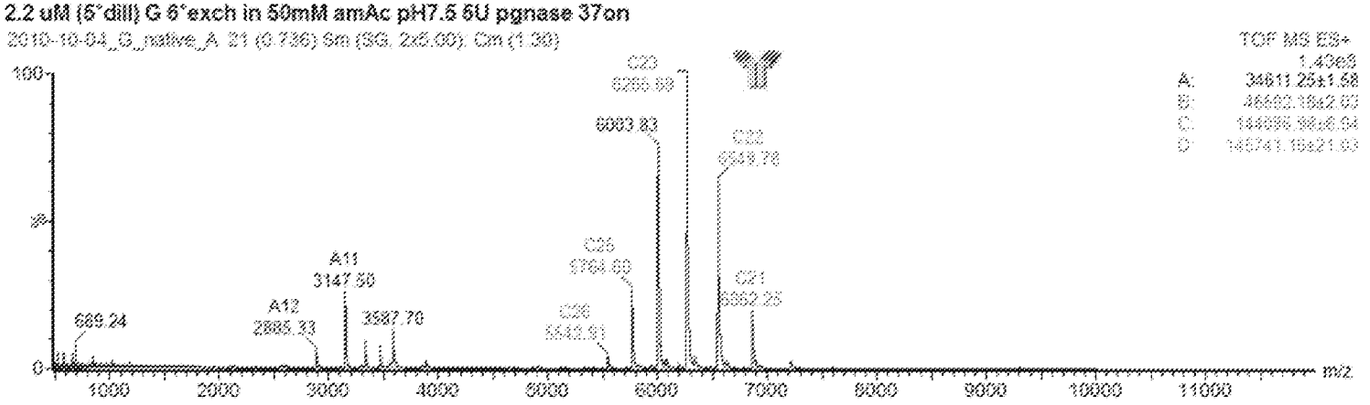
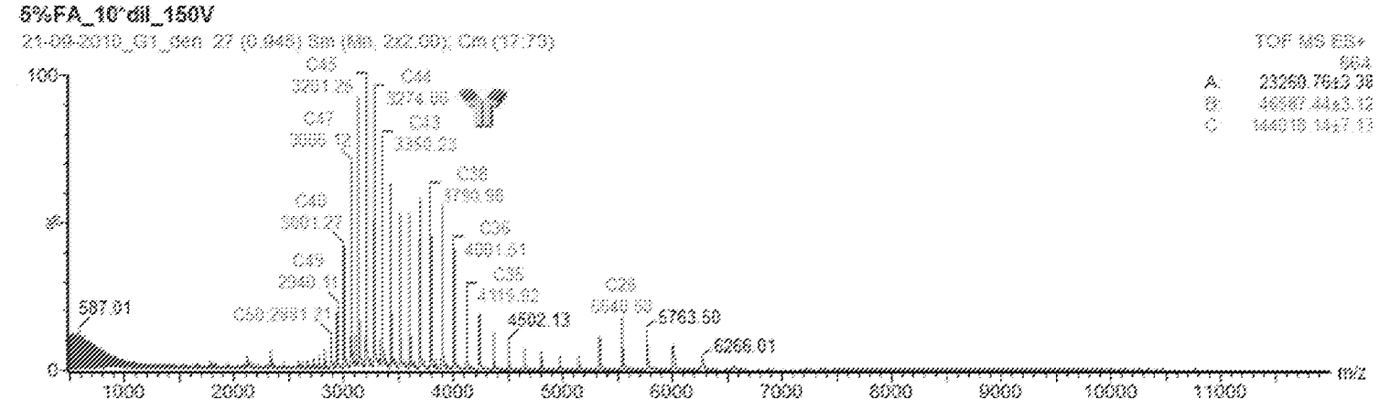

2.2 uM (5°dill) G 6°exch in 50mM amAc pH7.5 6U pgnase 37on
2010-10-04_G_native_A 21 (0.736) Sm (SG, 2x5.00); Cm (1:30)
TOF MS ES+
1.40e3
A: 34611.25±1.58
B: 46662.18±2.62
C: 144695.96±6.04
D: 145741.19±21.63
100
%
0
689.24
A12
2885.33
A11
3147.50
3587.70
C26
5443.51
C25
5764.60
C23
6265.59
6883.83
C22
6939.76
C21
7552.25
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
m/z

FIG. 4D


5%FA_10°dil_150V
21-09-2010_G1_pen 27 (0.848) Sm (Mn, 2x2.00); Cm (17:70)
TOF MS ES+
9e4
A: 23260.76±3.38
B: 46987.44±3.12
C: 144015.14±7.11
100
%
0
587.01
C50 2991.21
C45
3261.25
C47
3600.52
C40
3901.27
C49
2940.11
C44
3274.98
C43
3456.23
C38
3766.56
C36
4091.51
C35
4415.82
4502.13
C28
5040.50
5763.50
6268.01
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
m/z

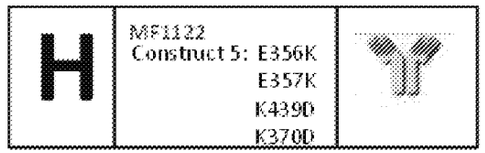

H
MF1122
Construct 5: E356K
E357K
K439D
K370D

FIG. 4E

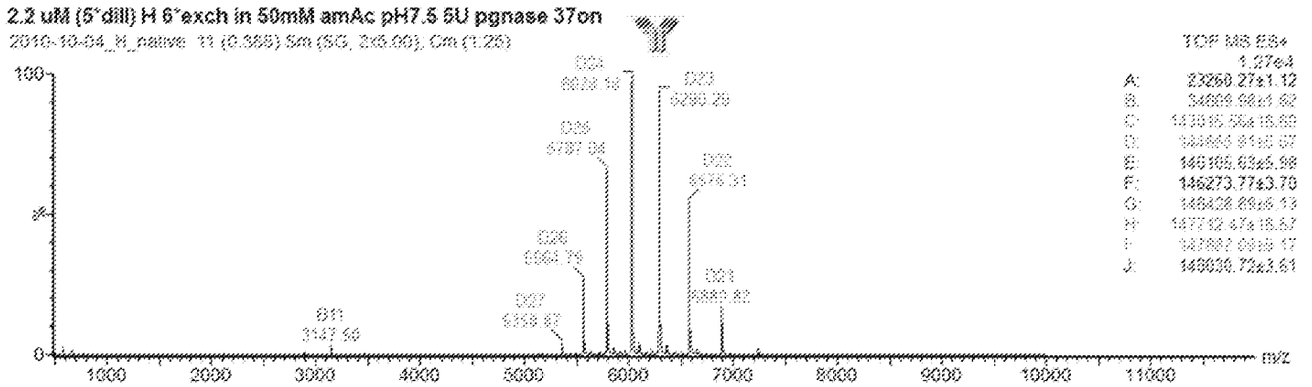
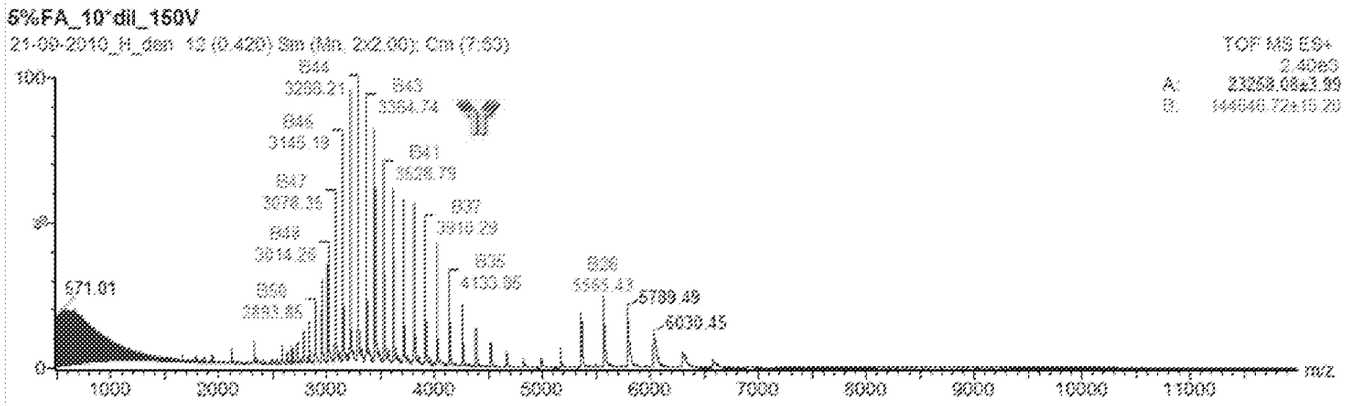

2.2 uM (5*dill) H 6*exch in 50mM amAc pH7.5 5U pgnase 37on
2010-10-04_H_native 11 (0.368) Sm (SG, 2x5.00); Cm (1:26)
TOF MS ES+
1.27e4
A: 23260.27±1.12
B: 54656.66±1.62
C: 70135.25±15.69
D: 93660.81±0.07
E: 140165.63±5.98
F: 146273.77±3.70
G: 148628.69±6.13
H: 147702.67±15.57
I: 147867.04±9.17
J: 149630.72±3.61
100
%
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
m/z

FIG. 4F


5%FA_10*dil_150V
21-09-2010_H_den 12 (0.420) Sm (Mn, 2x2.00); Cm (7:50)
TOF MS ES+
2.40e3
A: 23259.08±3.99
B: 144046.72±15.20
100
%
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
m/z

M	**MF1025** Construct 4: K392D D399K K409D	**MF1337** WT	

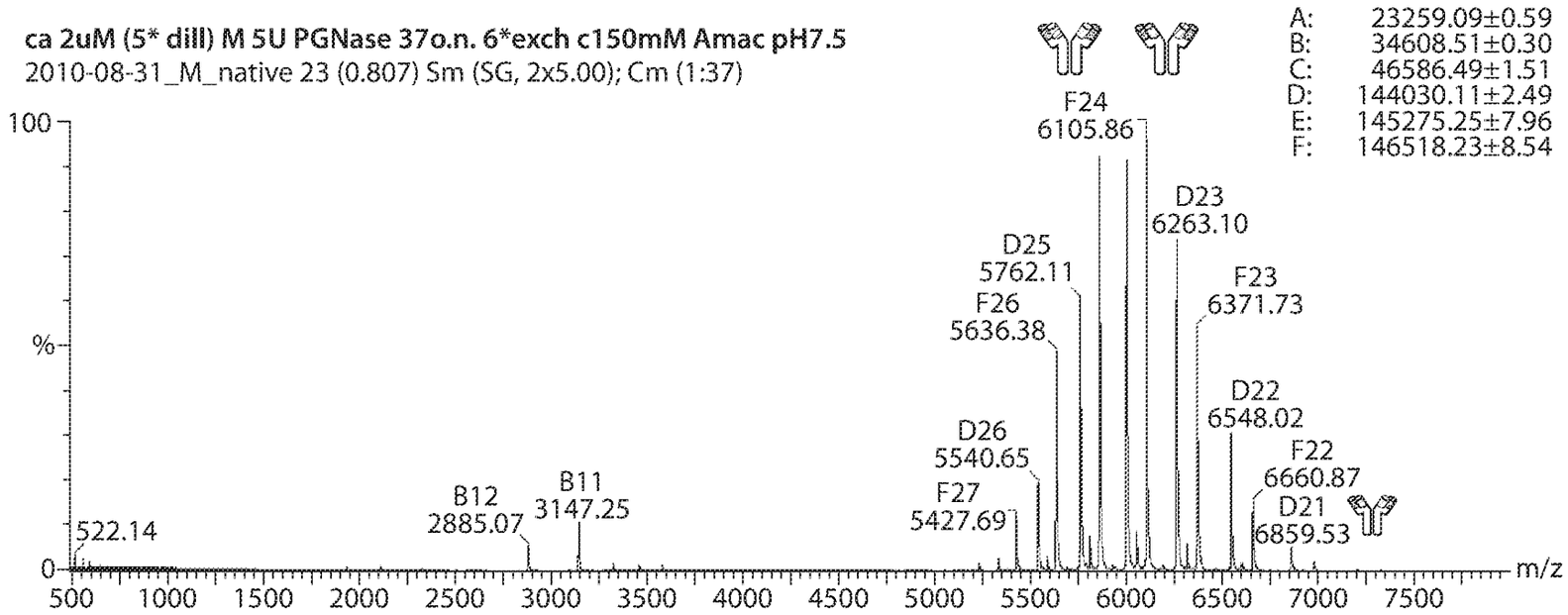


FIG. 5A

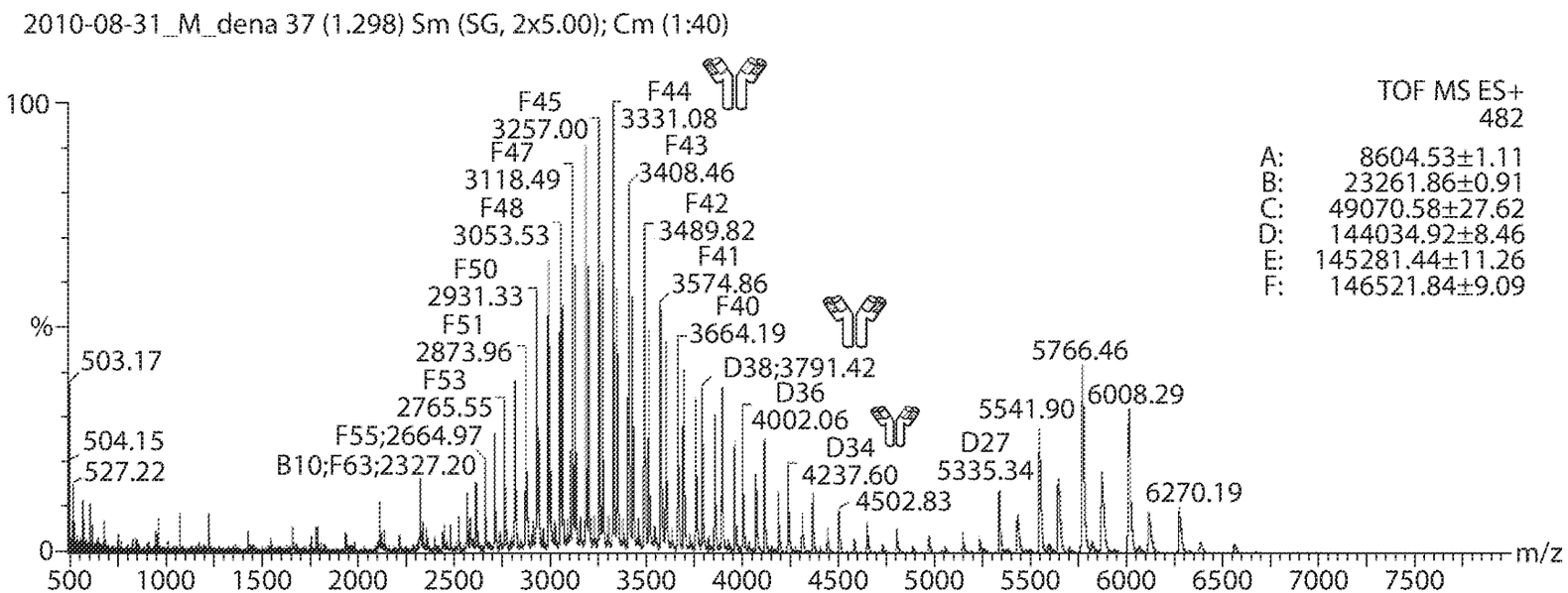

2010-08-31_M_dena 37 (1.298) Sm (SG, 2x5.00); Cm (1:40)
TOF MS ES+
482
A: 8604.53±1.11
B: 23261.86±0.91
C: 49070.58±27.62
D: 144034.92±8.46
E: 145281.44±11.26
F: 146521.84±9.09
100
%
0
m/z
500 1000 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500
503.17
504.15
527.22
B10;F63;2327.20
F55;2664.97
2765.55
F53
2873.96
F51
2931.33
F50
3053.53
F48
3118.49
F47
3257.00
F45
3331.08
F44
3408.46
F43
3489.82
F42
3574.86
F41
3664.19
F40
D38;3791.42
D36
4002.06
D34
4237.60
4502.83
D27
5335.34
5541.90
5766.46
6008.29
6270.19

FIG. 5B

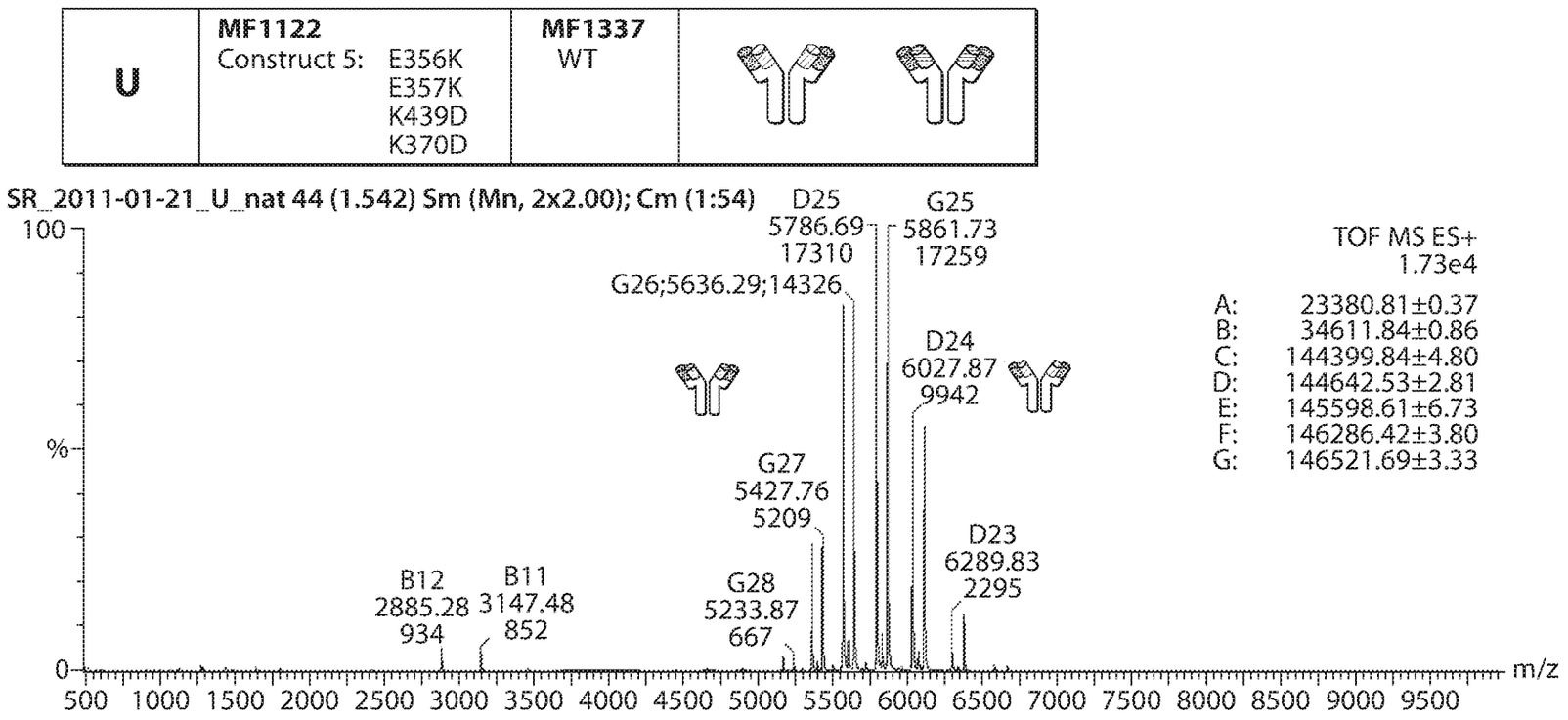

U
MF1122
Construct 5: E356K
E357K
K439D
K370D
MF1337
WT
SR_2011-01-21_U_nat 44 (1.542) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
1.73e4
A: 23380.81±0.37
B: 34611.84±0.86
C: 144399.84±4.80
D: 144642.53±2.81
E: 145598.61±6.73
F: 146286.42±3.80
G: 146521.69±3.33
D25
5786.69
17310
G25
5861.73
17259
G26;5636.29;14326
D24
6027.87
9942
G27
5427.76
5209
D23
6289.83
2295
B12
2885.28
934
B11
3147.48
852
G28
5233.87
667
100
%
0
m/z
500 1000 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500 8000 8500 9000 9500

FIG. 5C

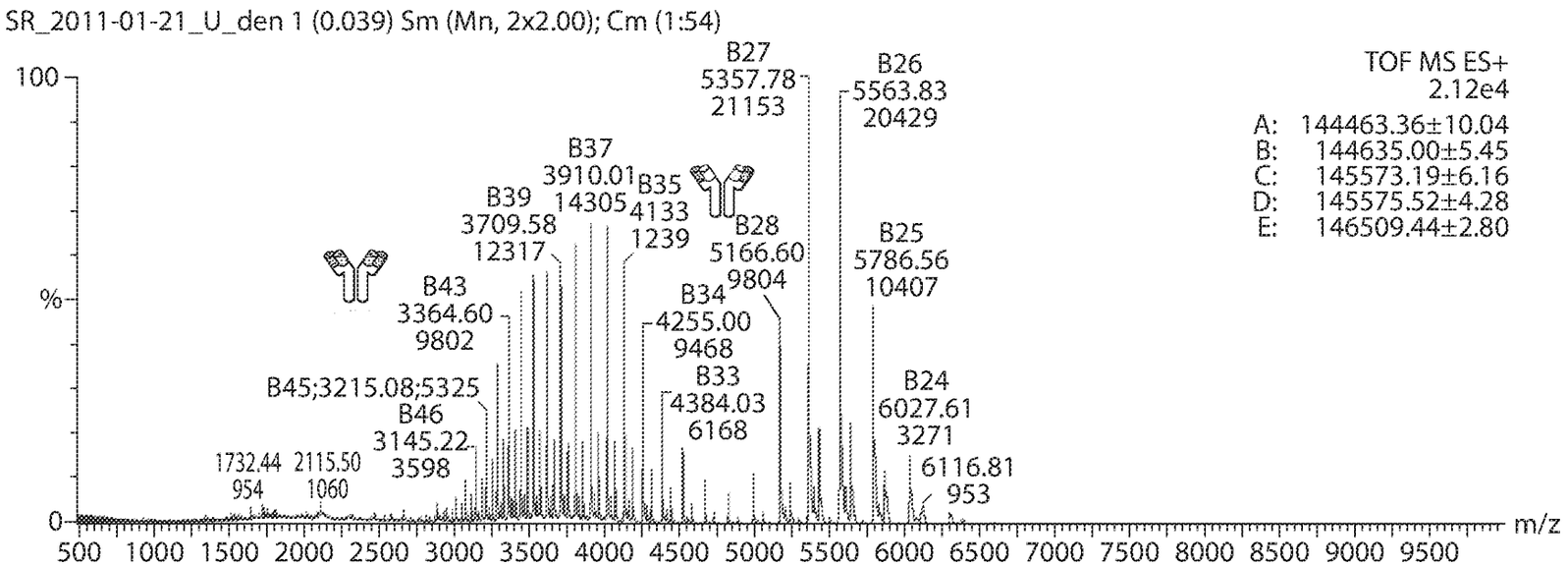

SR_2011-01-21_U_den 1 (0.039) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
2.12e4
A: 144463.36±10.04
B: 144635.00±5.45
C: 145573.19±6.16
D: 145575.52±4.28
E: 146509.44±2.80
B27 5357.78 21153
B26 5563.83 20429
B37 3910.01 14305
B35 4133 1239
B39 3709.58 12317
B28 5166.60 9804
B25 5786.56 10407
B43 3364.60 9802
B34 4255.00 9468
B33 4384.03 6168
B45;3215.08;5325
B46 3145.22 3598
B24 6027.61 3271
1732.44 954
2115.50 1060
6116.81 953
100
%
0
m/z
500 1000 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500 8000 8500 9000 9500

FIG. 5D

O	**MF1122** Construct 5: E356K E357K K439D K370D	**MF1337** WT	**MF1025** Construct 4: K392D D399K K409D	

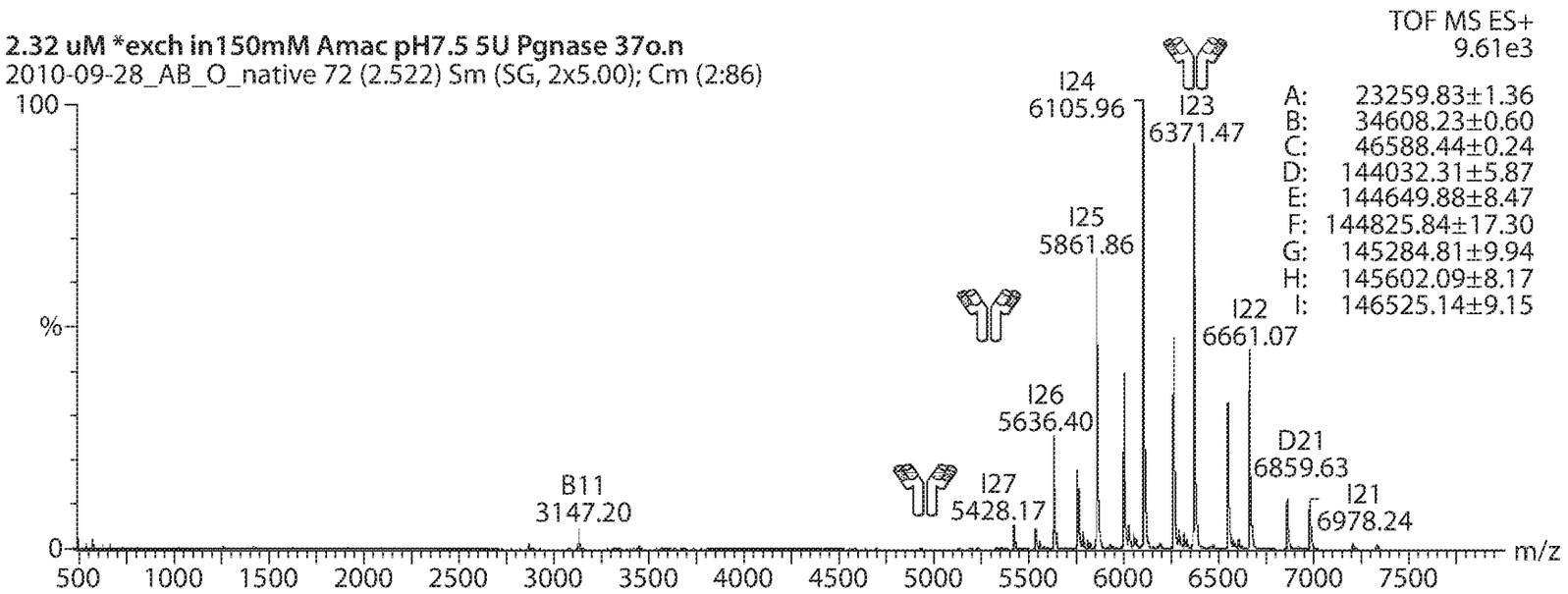


FIG. 6A

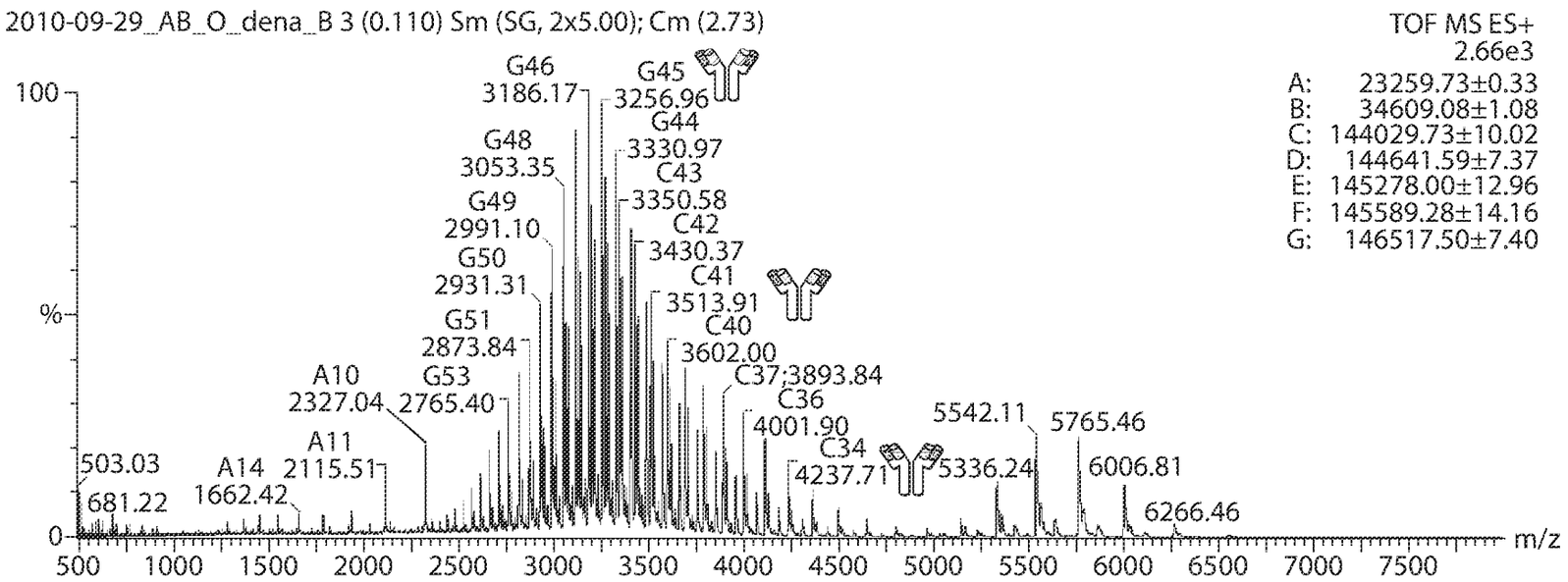

2010-09-29_AB_O_dena_B 3 (0.110) Sm (SG, 2x5.00); Cm (2.73)
TOF MS ES+
2.66e3
A: 23259.73±0.33
B: 34609.08±1.08
C: 144029.73±10.02
D: 144641.59±7.37
E: 145278.00±12.96
F: 145589.28±14.16
G: 146517.50±7.40
100
%
0
m/z
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500
G46
3186.17
G45
3256.96
G44
3330.97
G48
3053.35
C43
3350.58
G49
2991.10
C42
3430.37
G50
2931.31
C41
3513.91
G51
2873.84
C40
3602.00
A10
2327.04
G53
2765.40
C37
3893.84
C36
4001.90
A11
2115.51
C34
4237.71
A14
1662.42
503.03
681.22
5542.11
5765.46
5336.24
6006.81
6266.46

FIG. 6B

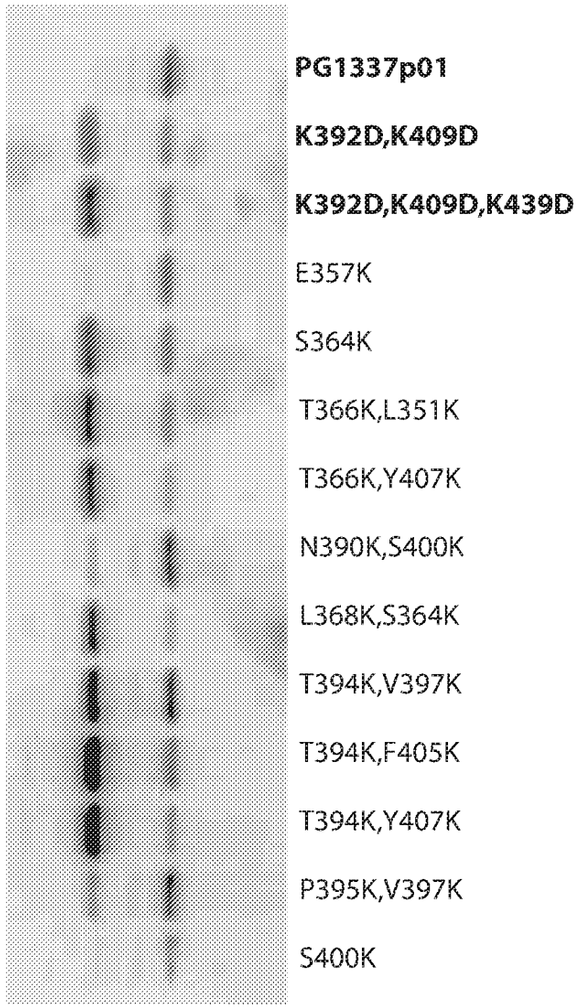

PG1337p01
K392D,K409D
K392D,K409D,K439D
E357K
S364K
T366K,L351K
T366K,Y407K
N390K,S400K
L368K,S364K
T394K,V397K
T394K,F405K
T394K,Y407K
P395K,V397K
S400K

FIG. 7A

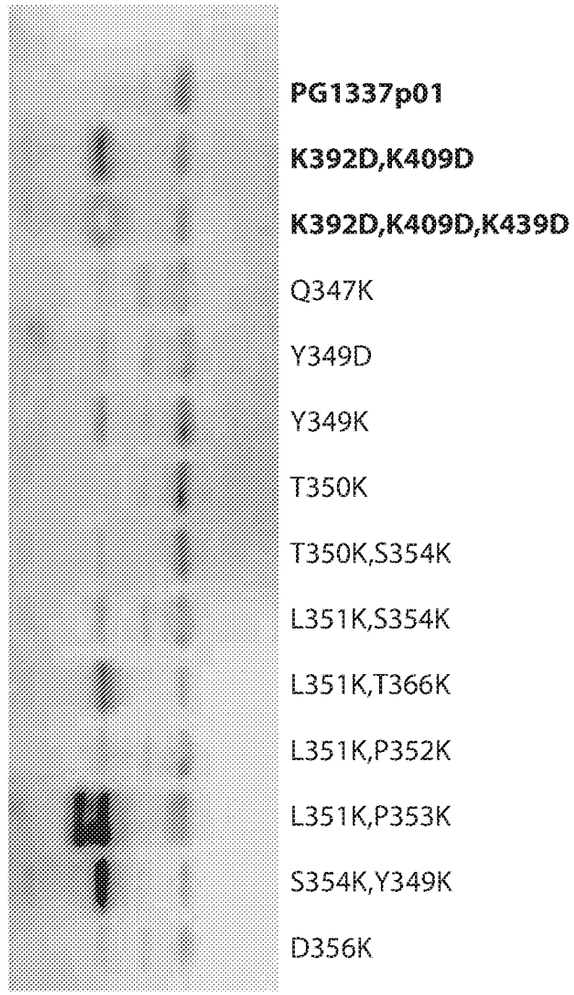

PG1337p01
K392D,K409D
K392D,K409D,K439D
Q347K
Y349D
Y349K
T350K
T350K,S354K
L351K,S354K
L351K,T366K
L351K,P352K
L351K,P353K
S354K,Y349K
D356K

FIG. 7B

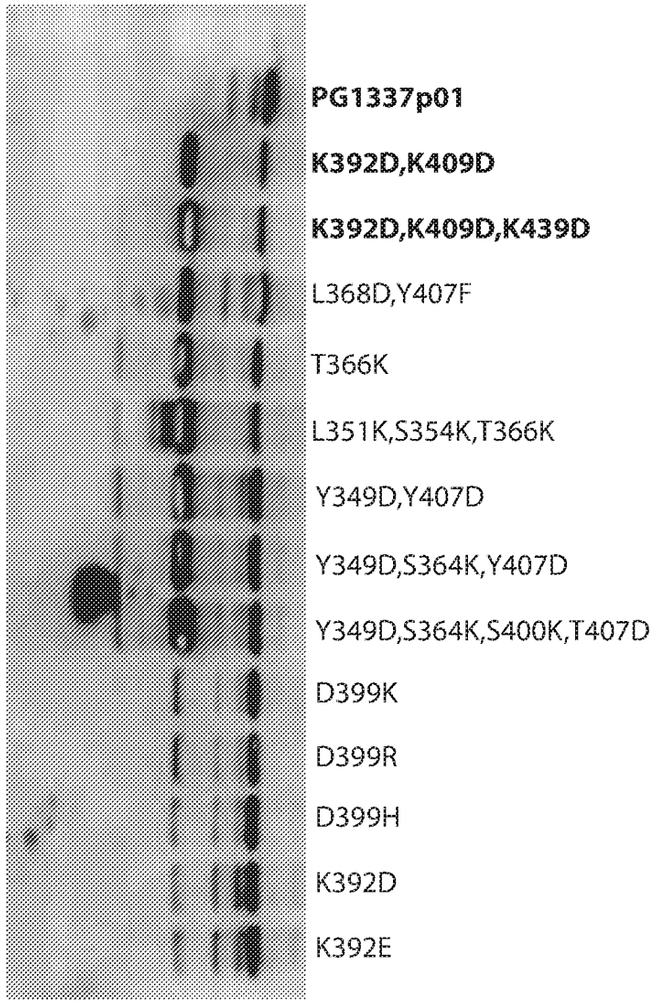

PG1337p01
K392D,K409D
K392D,K409D,K439D
L368D,Y407F
T366K
L351K,S354K,T366K
Y349D,Y407D
Y349D,S364K,Y407D
Y349D,S364K,S400K,T407D
D399K
D399R
D399H
K392D
K392E

FIG. 7C

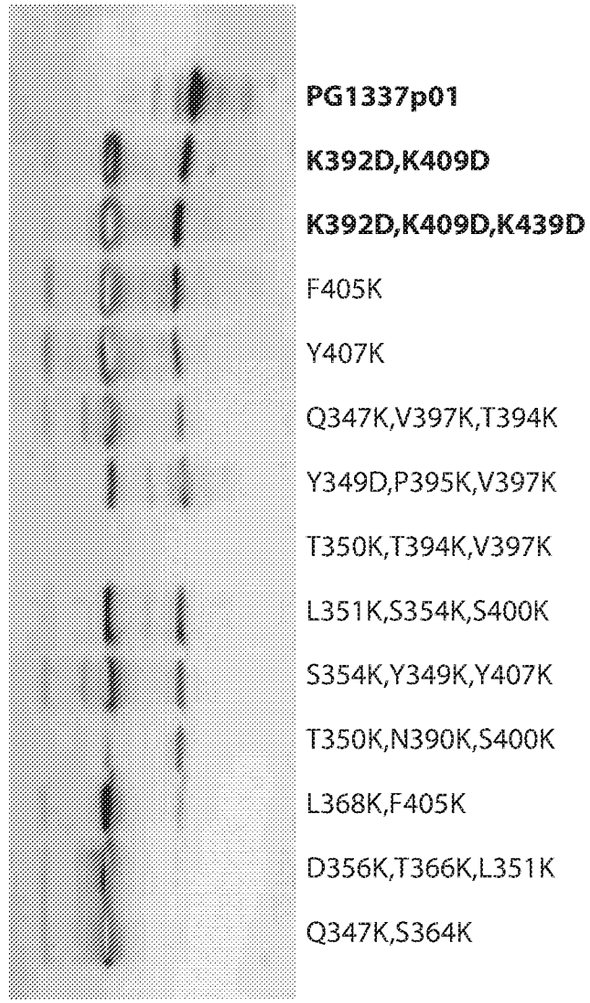

PG1337p01
K392D,K409D
K392D,K409D,K439D
F405K
Y407K
Q347K,V397K,T394K
Y349D,P395K,V397K
T350K,T394K,V397K
L351K,S354K,S400K
S354K,Y349K,Y407K
T350K,N390K,S400K
L368K,F405K
D356K,T366K,L351K
Q347K,S364K

FIG. 7D

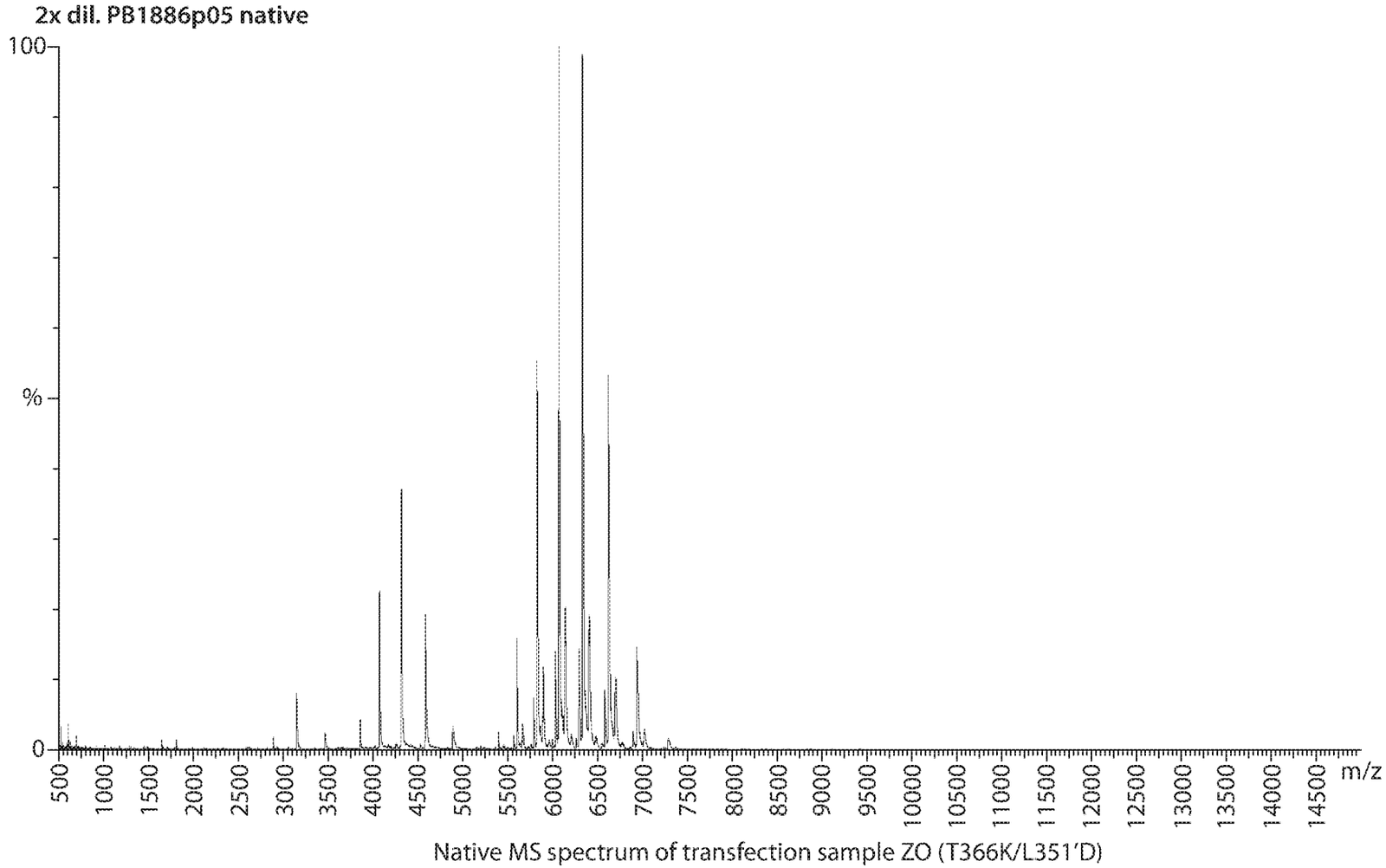
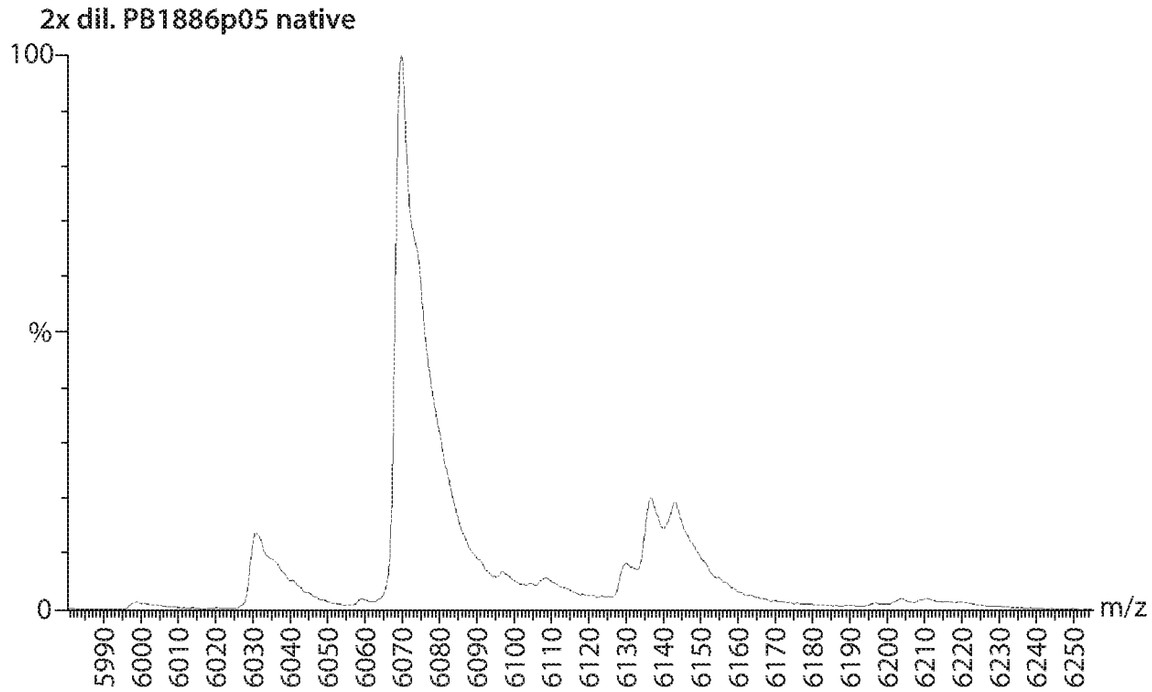

2x dil. PB1886p05 native
100
%
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000
10500
11000
11500
12000
12500
13000
13500
14000
14500
m/z
Native MS spectrum of transfection sample ZO (T366K/L351'D)

FIG. 8A



Convoluted MS spectrum of transfection sample ZO (T366K/L351'D).
The second/main peak represents the bispecific molecule.

FIG. 8B

FIG. 9

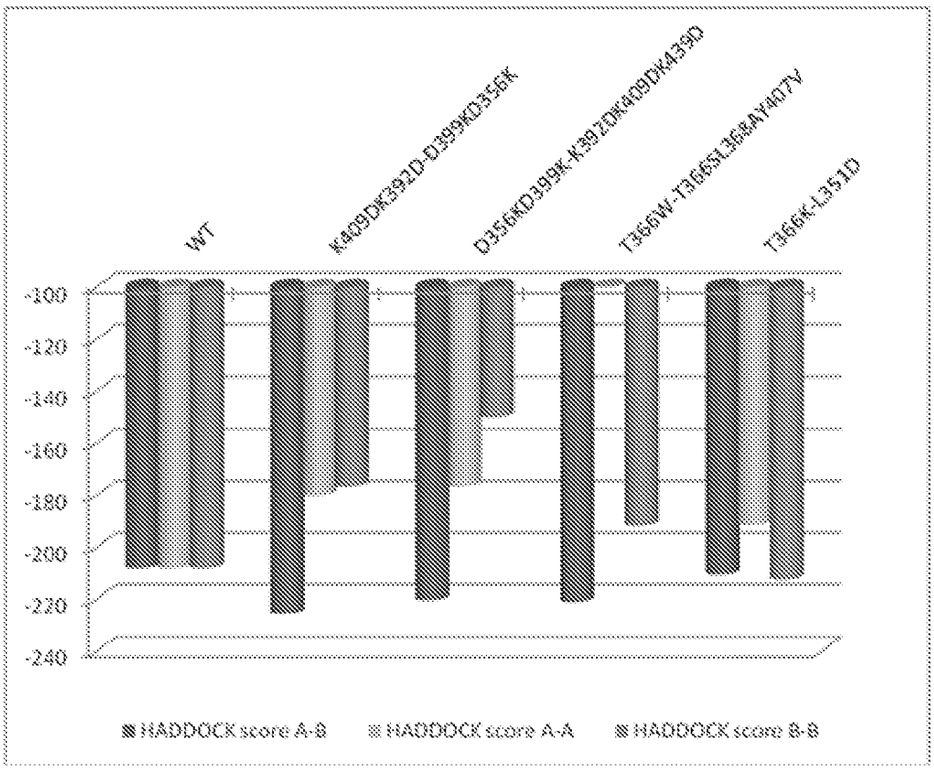
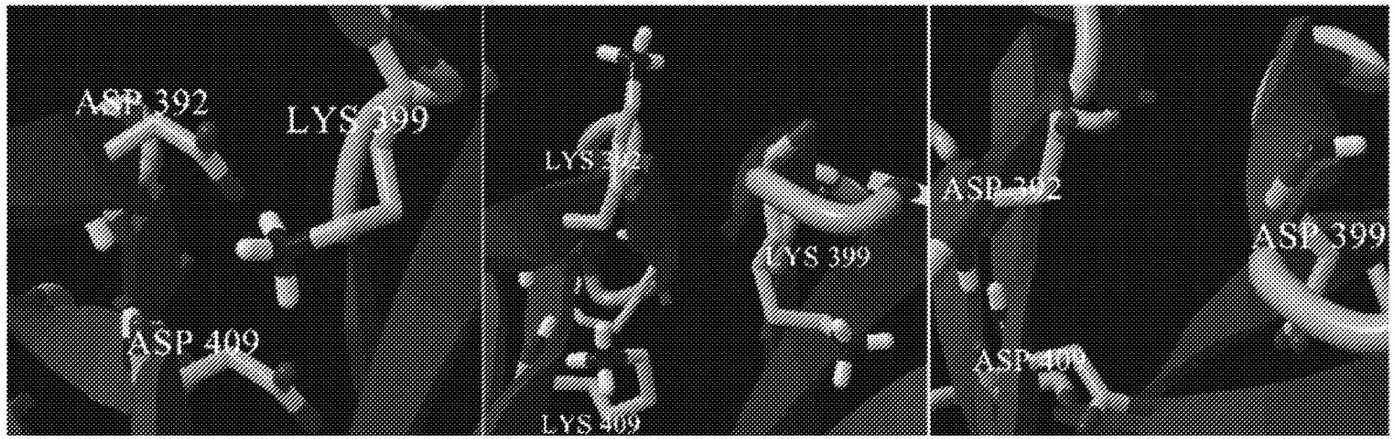

WT
K409DK392D-D399KD356K
D356KD399K-K392DK409DK439D
T366W-T366SL368AY407V
T366K-L351D
-100
-120
-140
-160
-180
-200
-220
-240
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 10A FIG. 10B FIG. 10C



A K409D:K392D/D399'K:E356'K

B D399K:E356K/D399'K:E356'K

C K409D:K392D/K409'D:K392'D

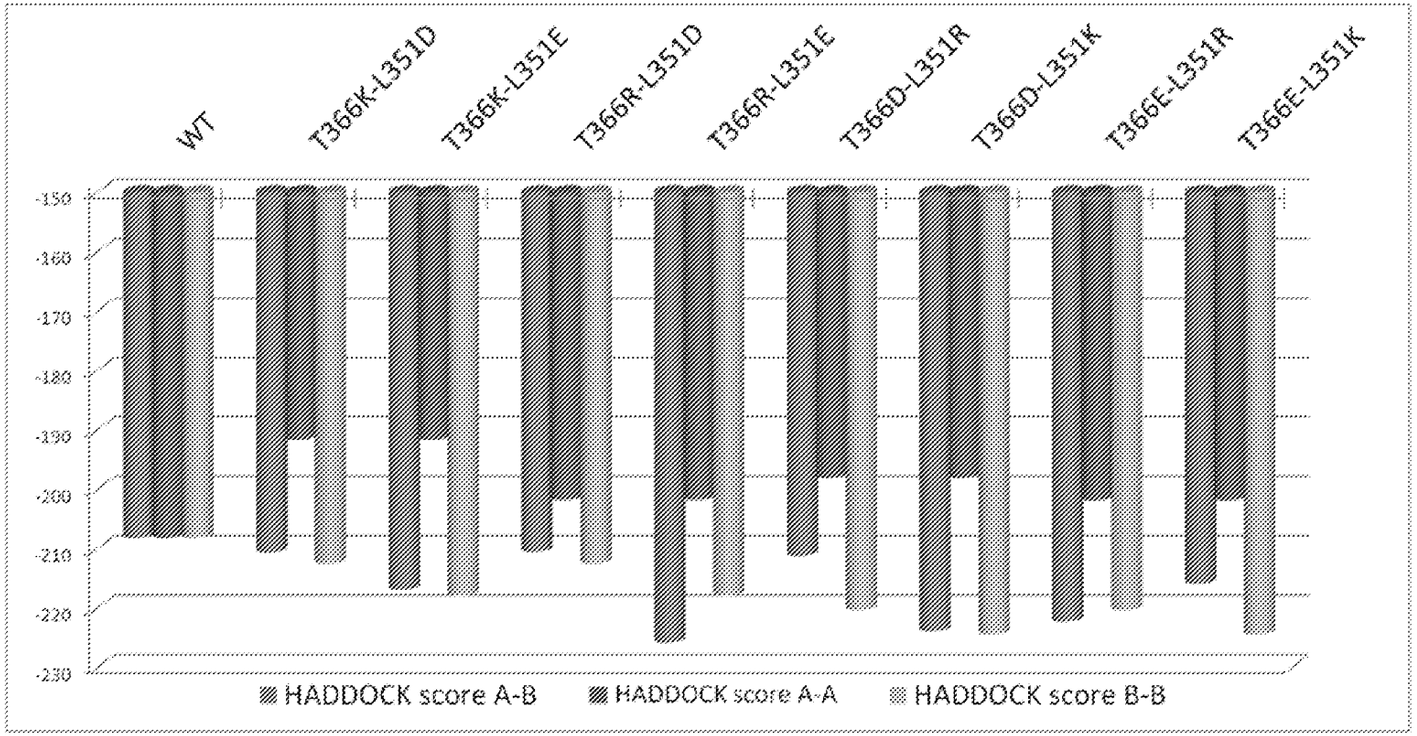

WT
T366K-L351D
T366K-L351E
T366R-L351D
T366R-L351E
T366D-L351R
T366D-L351K
T366E-L351R
T366E-L351K
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 11

FIG. 12A

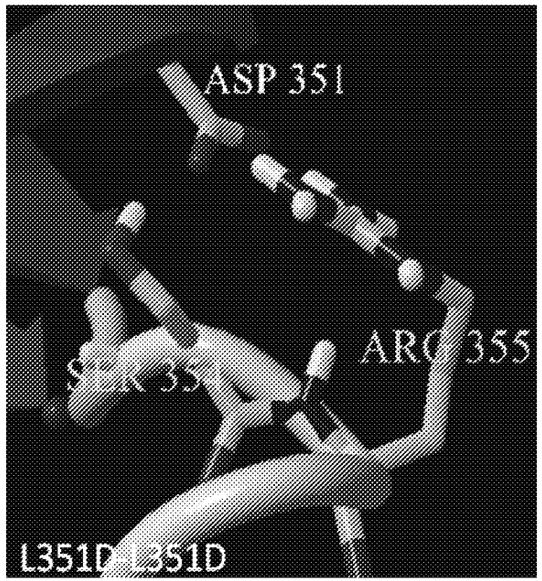
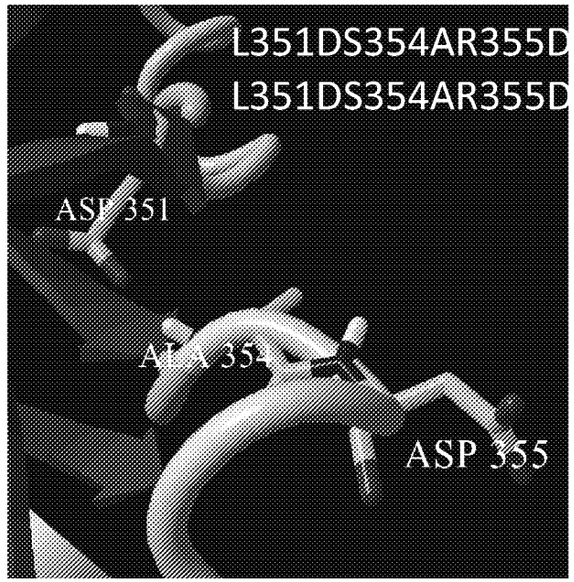


FIG. 12B



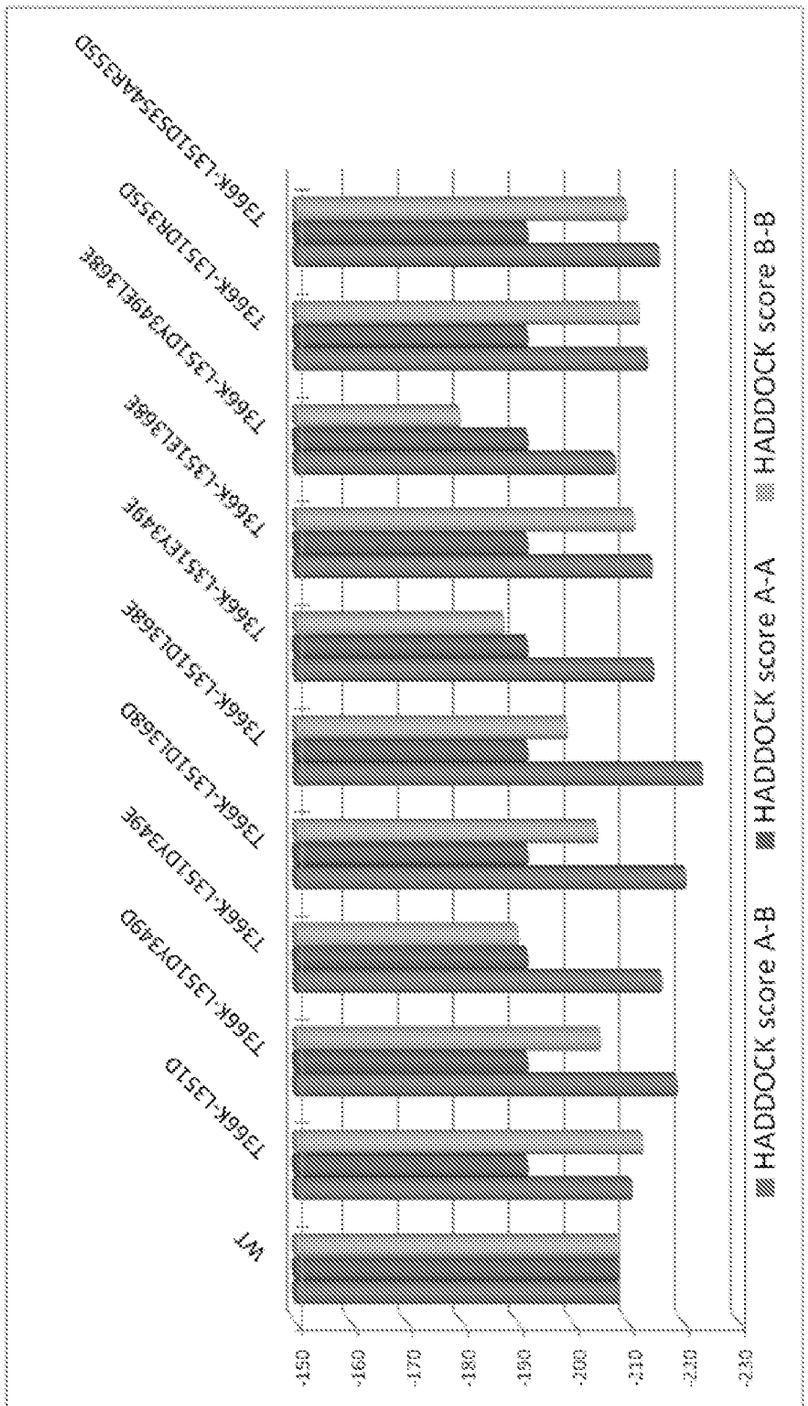

WT
T366K-L351D
T366K-L351DY349D
T366K-L351DY349E
T366K-L351DL358D
T366K-L351DL358E
T366K-L351EY349E
T366K-L351EL358E
T366K-L351DY349EL358E
T366K-L351DR355D
T366K-L351DS354AR355D
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 13

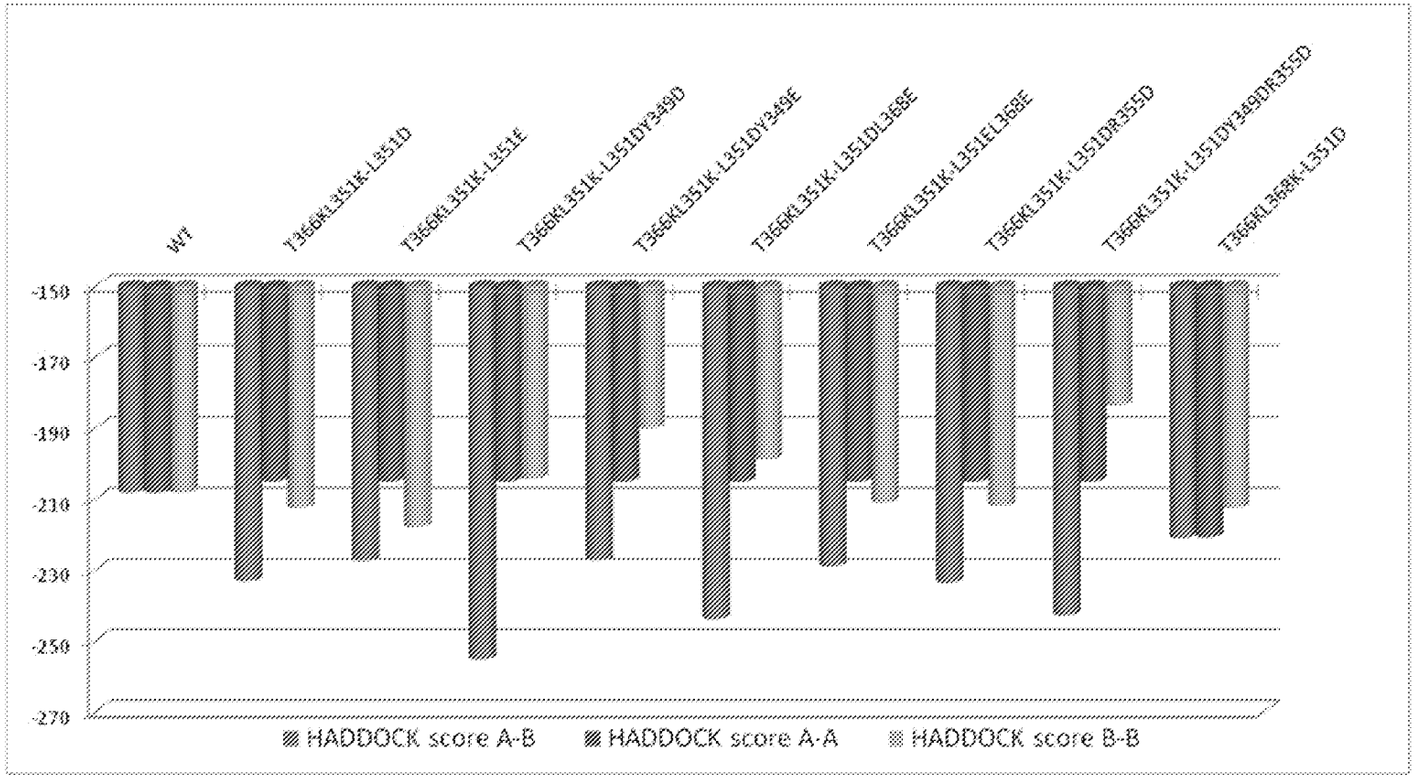

WT
T366KL351K-L351D
T366KL351K-L351E
T366KL351K-L351DY349D
T366KL351K-L351DY349E
T366KL351K-L351DL368E
T366KL351K-L351EL368E
T366KL351K-L351DR355D
T366KL351K-L351DY349DR355D
T366KL368K-L351D
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 14

FIG. 15A

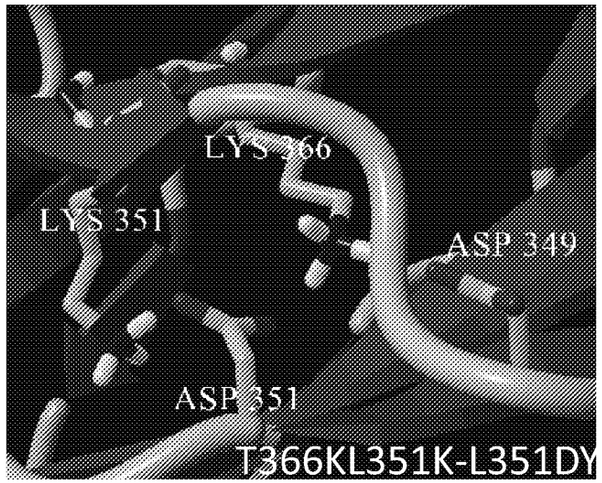
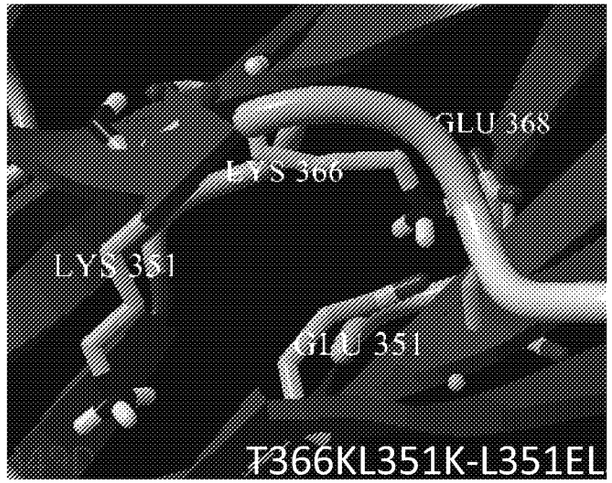


FIG. 15B



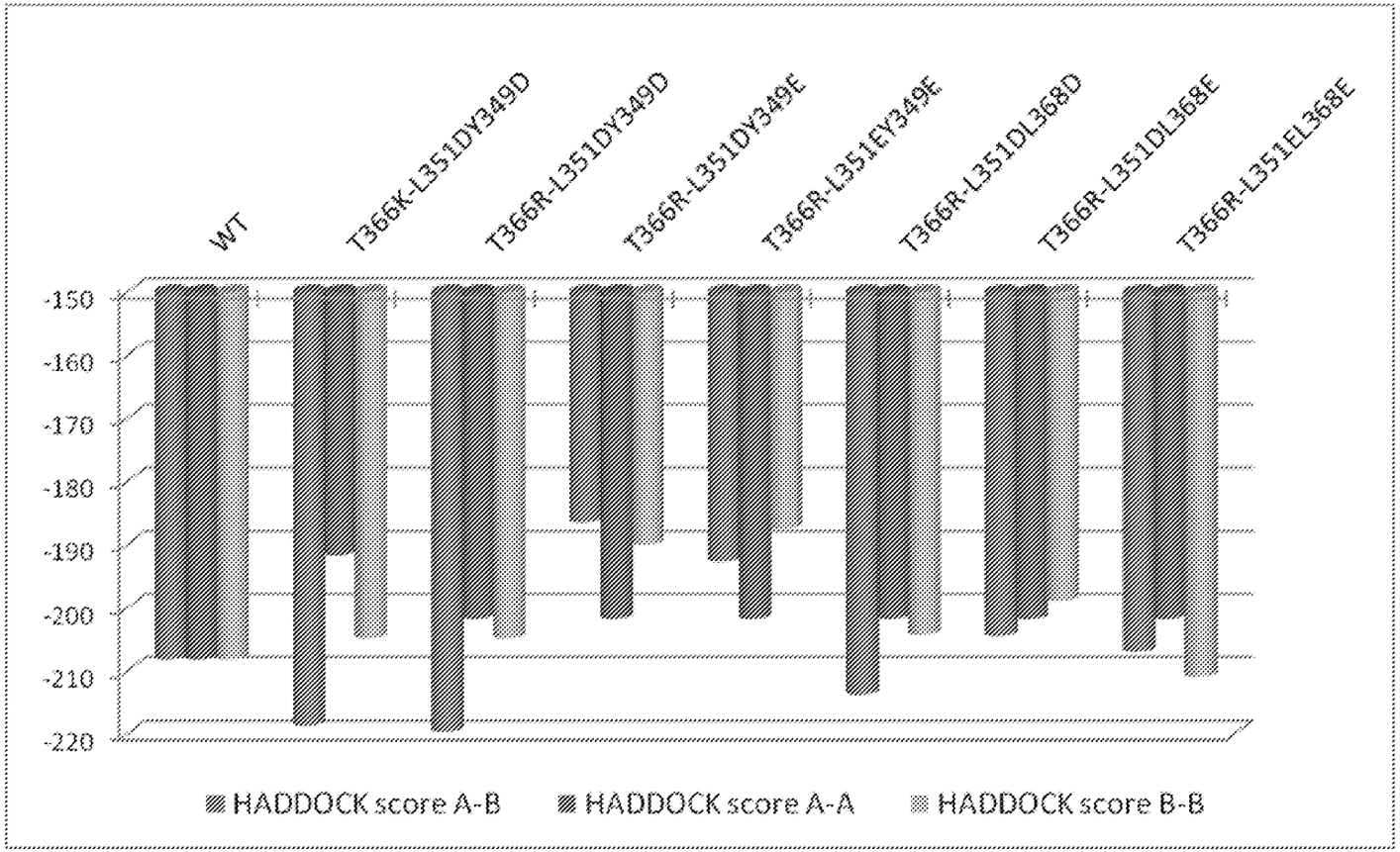

WT
T366K-L351DY349D
T366R-L351DY349D
T366R-L351DY349E
T366R-L351EY349E
T366R-L351DL368D
T366R-L351DL368E
T366R-L351EL368E
-150
-160
-170
-180
-190
-200
-210
-220
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 16

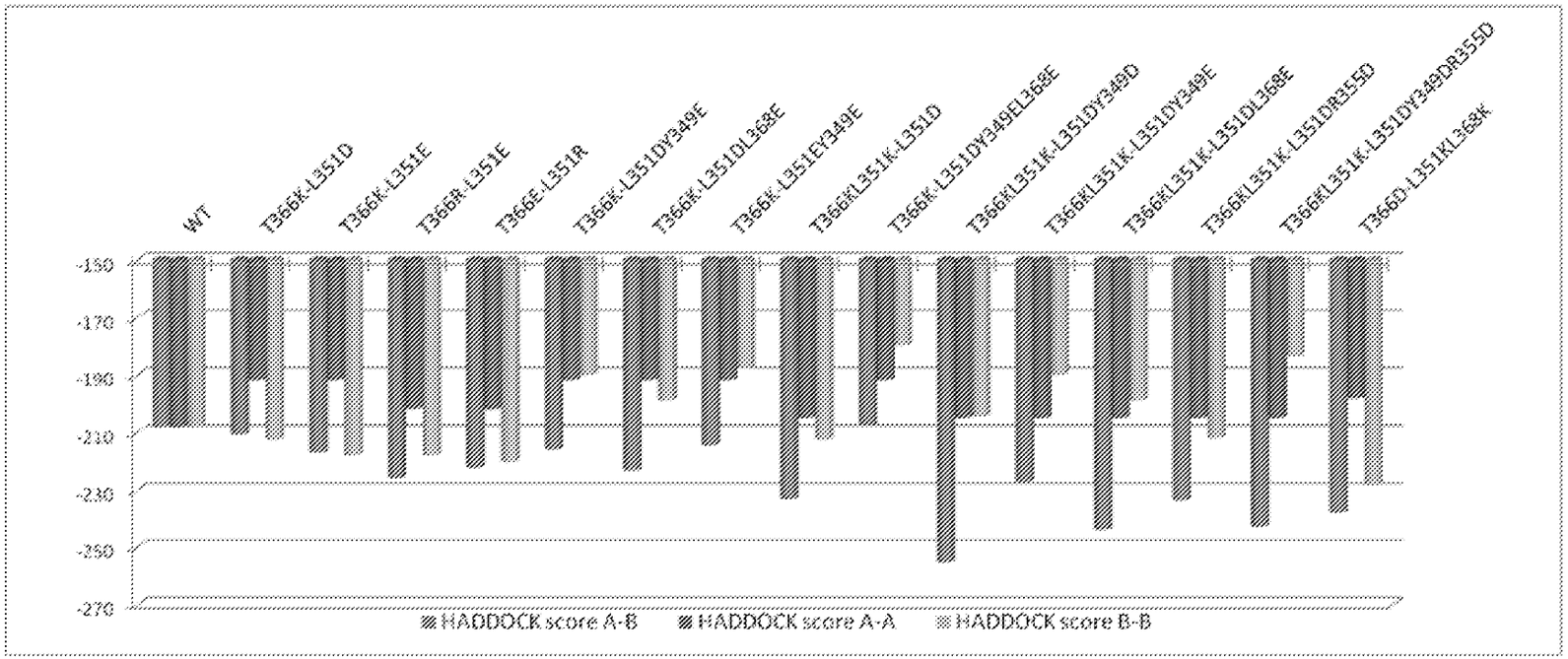

WT
T366K-L351D
T366K-L351E
T366R-L351E
T366E-L351R
T366K-L351DY349E
T366K-L351DL368E
T366K-L351EY349E
T366KL351K-L351D
T366K-L351DY349EL368E
T366KL351K-L351DY349D
T366KL351K-L351DY349E
T366KL351K-L351DL368E
T366KL351K-L351DR355D
T366KL351K-L351DY349DR355D
T366D-L351KL368K
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

FIG. 17

FIG. 18A

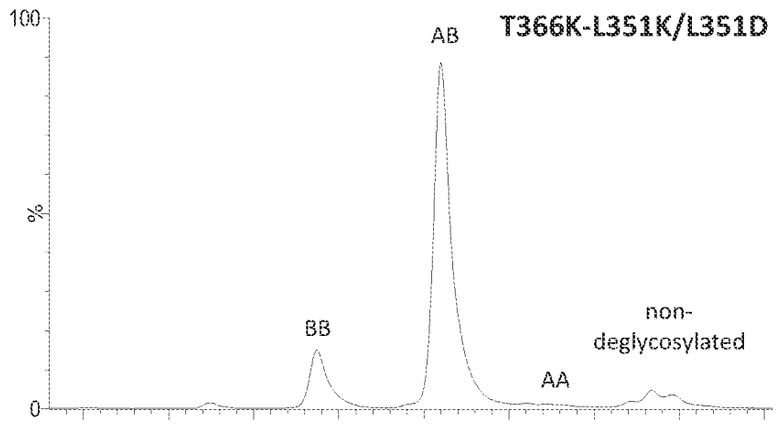
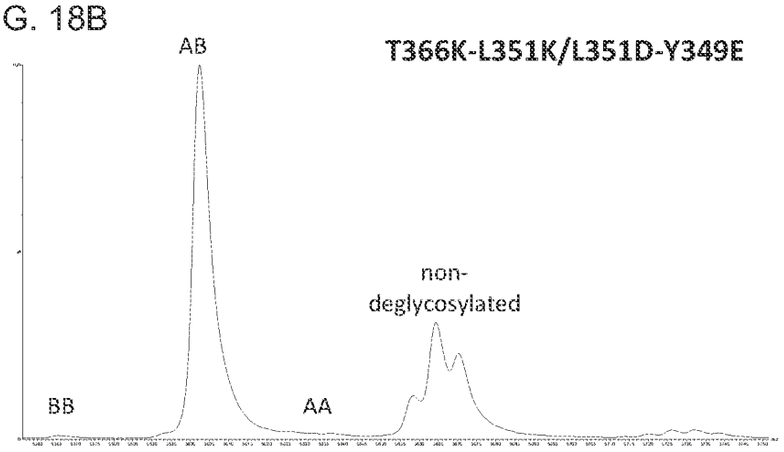


FIG. 18B



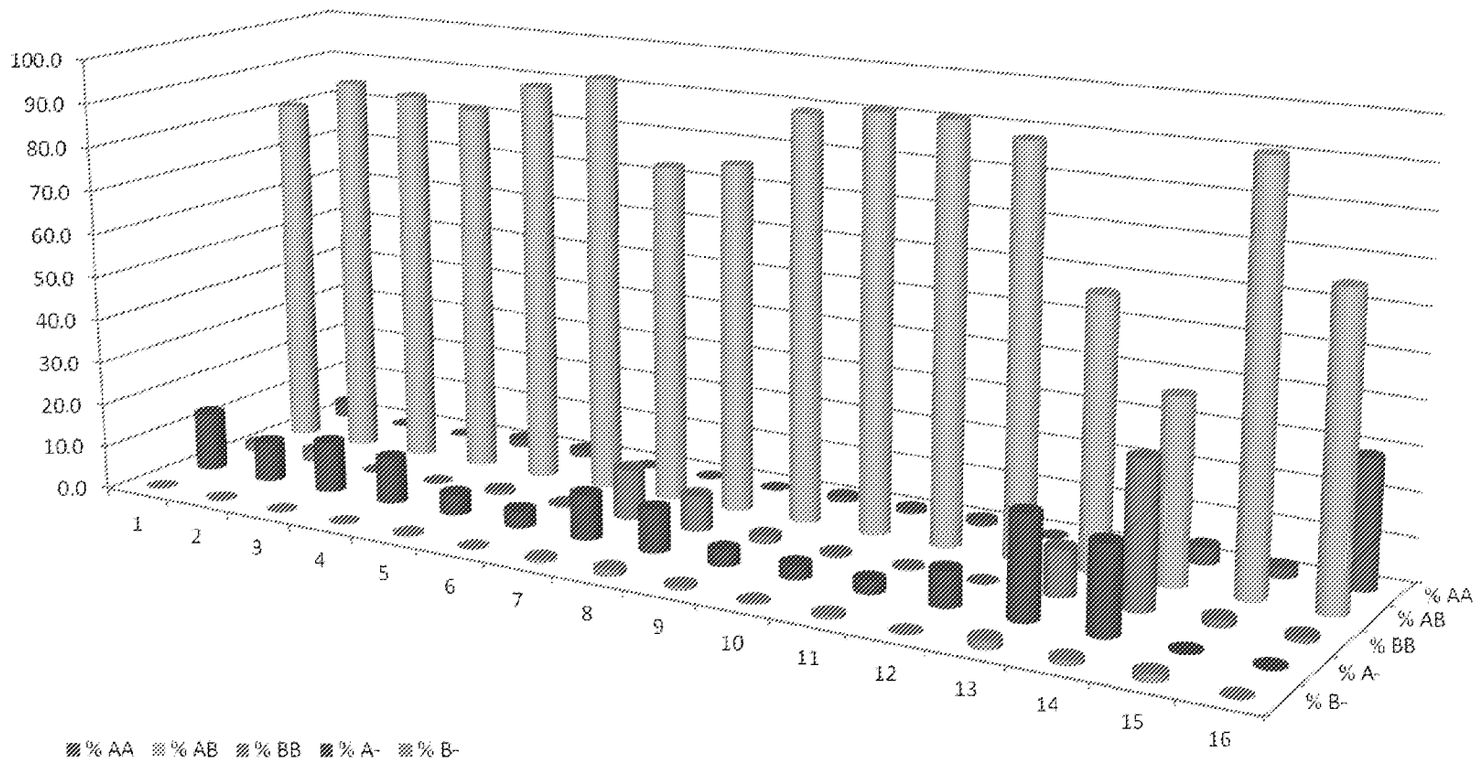

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% AA
% AB
% BB
% A-
% B-
% AA
% AB
% BB
% A-
% B-

FIG. 19A

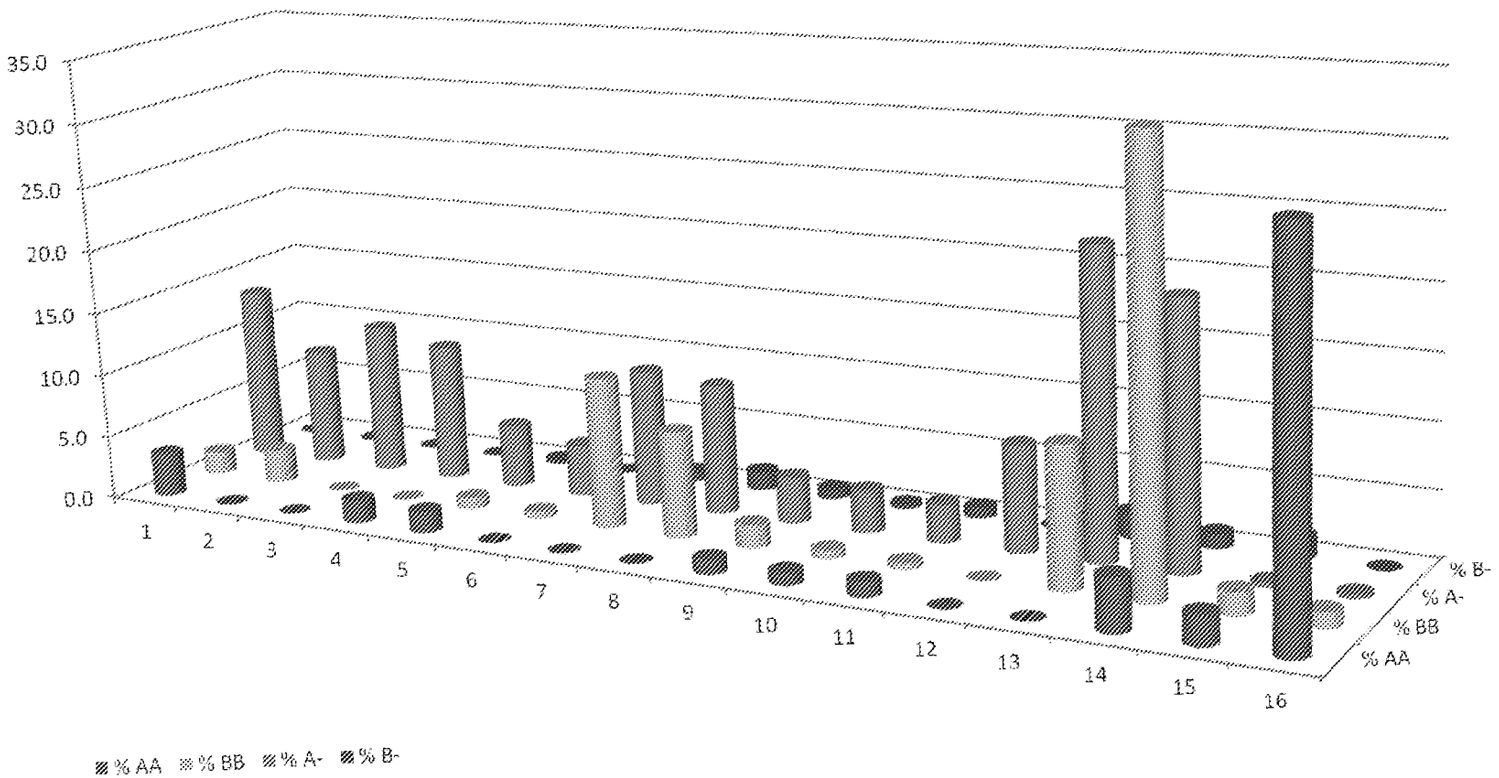

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% B-
% A-
% BB
% AA
% AA
% BB
% A-
% B-

FIG. 19B

FIG. 20

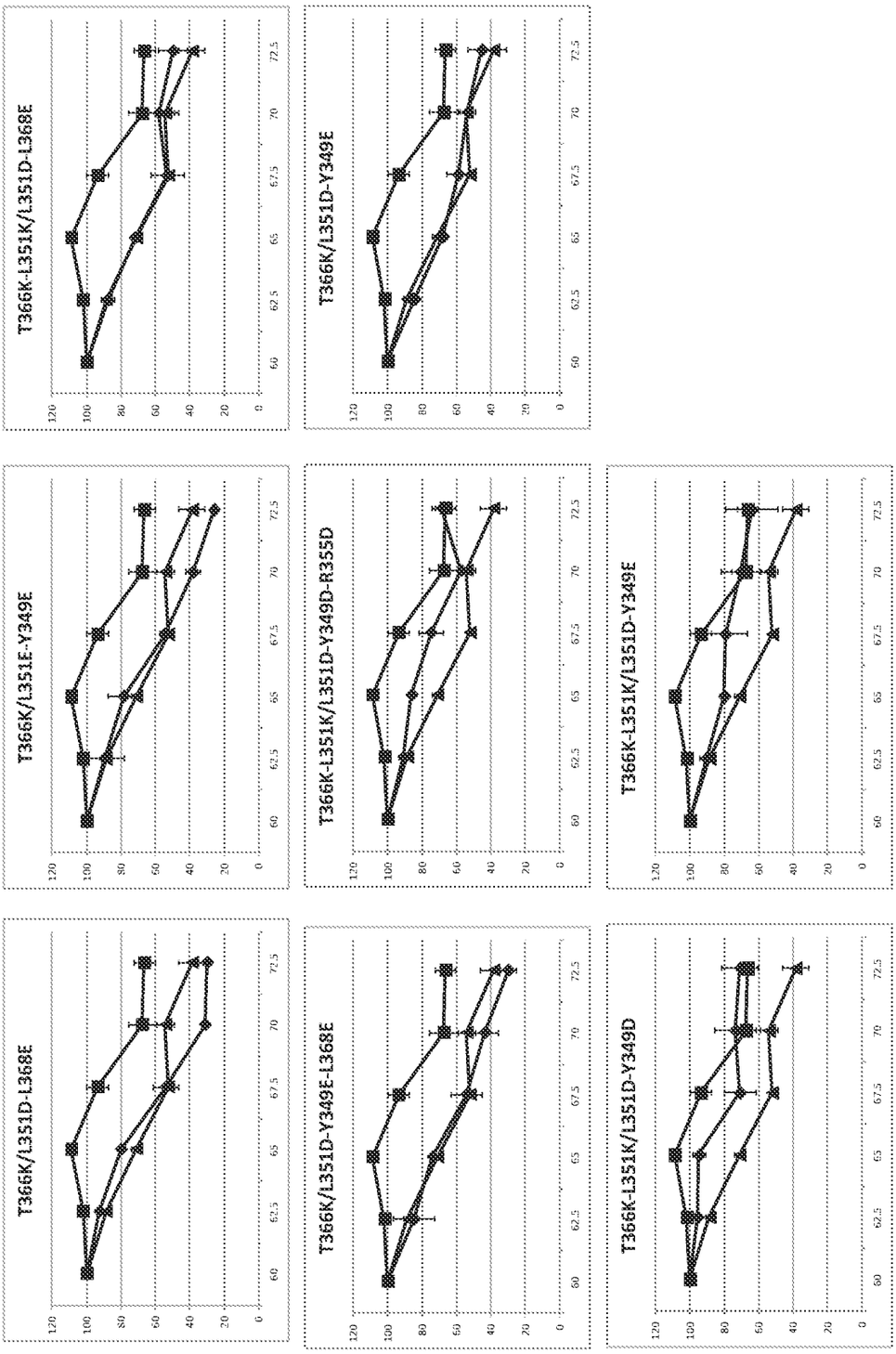

T366K/L351D-L368E
T366K/L351E-Y349E
T366K-L351K/L351D-L368E
T366K-L351K/L351D-Y349D-R355D
T366K/L351D-Y349E
T366K/L351D-Y349E-L368E
T366K-L351K/L351D-Y349E
T366K-L351K/L351D-Y349D

FIG. 21

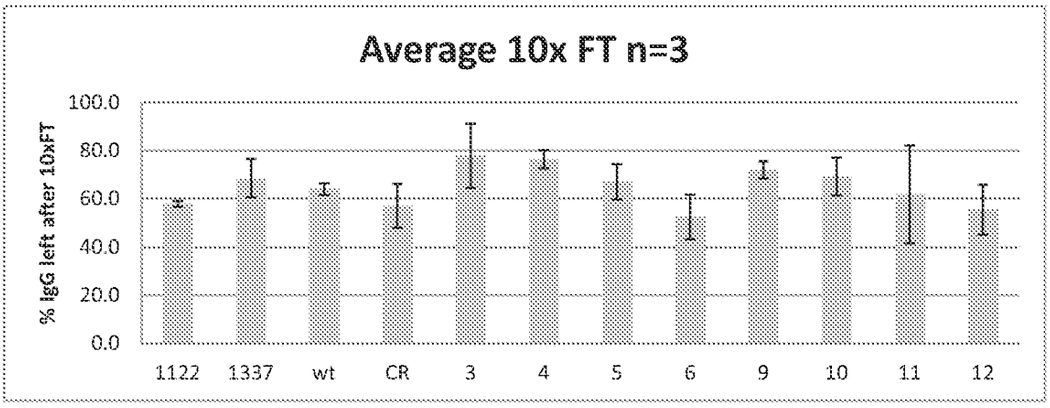

Average 10x FT n=3
% IgG left after 10xFT
100.0
80.0
60.0
40.0
20.0
0.0
1122
1337
wt
CR
3
4
5
6
9
10
11
12

FIG. 22A

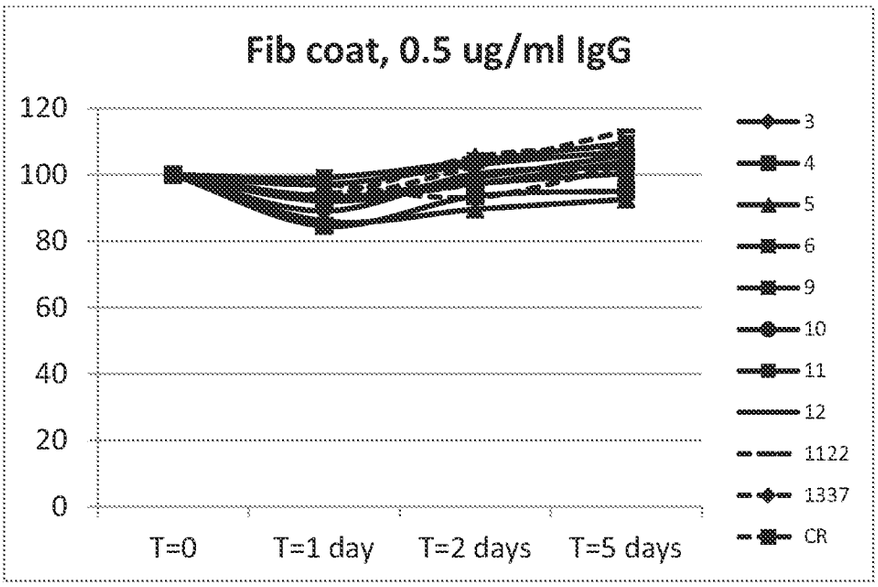
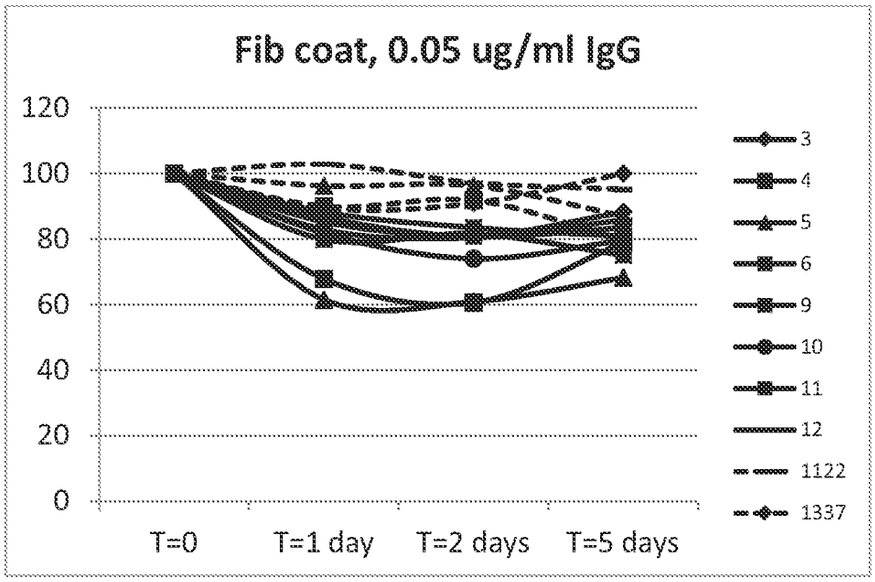


FIG. 22B



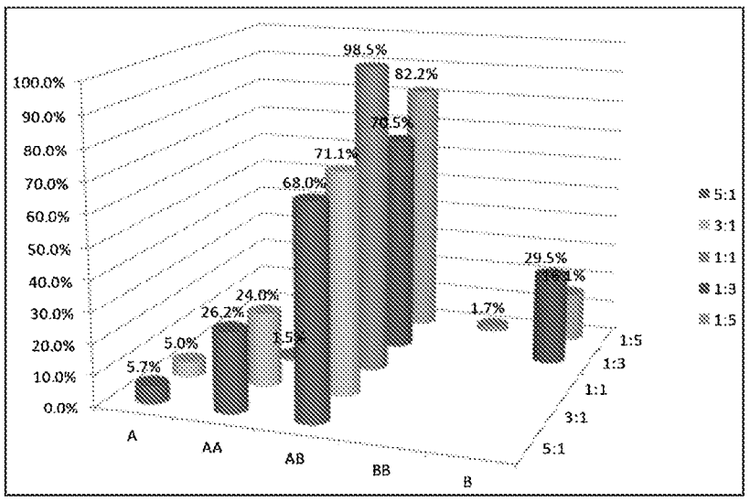


FIG.23A

	5:1	3:1	1:1	1:3	1:5
A	5.7%	5.0%			
AA	26.2%	24.0%	1.5%		
AB	68.0%	71.1%	98.5%	70.5%	67.2%
BB					1.7%
B				29.5%	16.1%

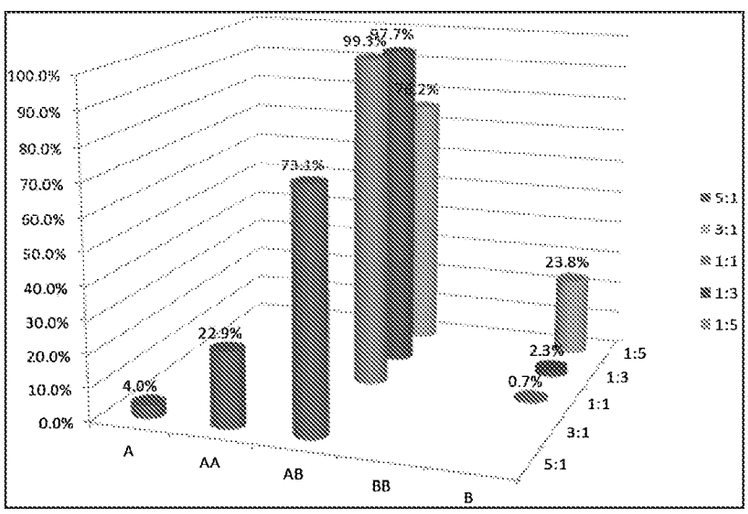


FIG.23B

	5:1	3:1	1:1	1:3	1:5
A	4.0%				
AA	22.9%				
AB	73.1%		99.3%	97.7%	76.2%
BB					
B			0.7%	2.3%	23.8%

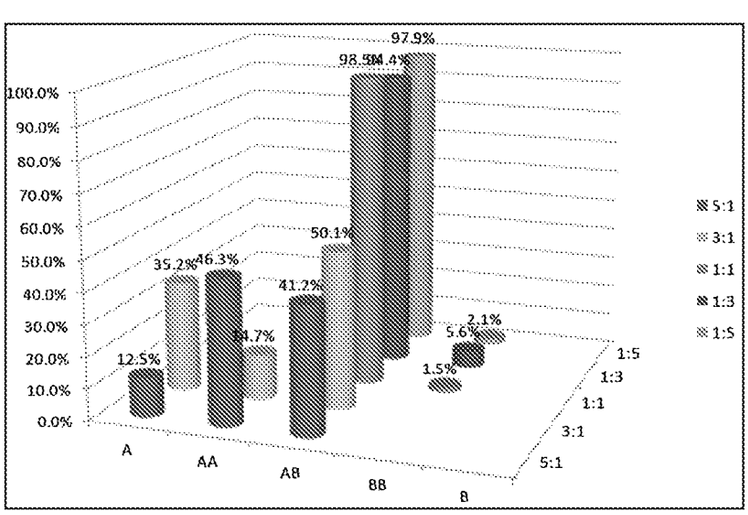


FIG.23C

	5:1	3:1	1:1	1:3	1:5
A	12.5%	35.2%			
AA	46.3%	14.7%			
AB	41.2%	50.1%	98.5%	94.4%	97.9%
BB			1.5%	5.6%	2.1%
B					

FIG. 24

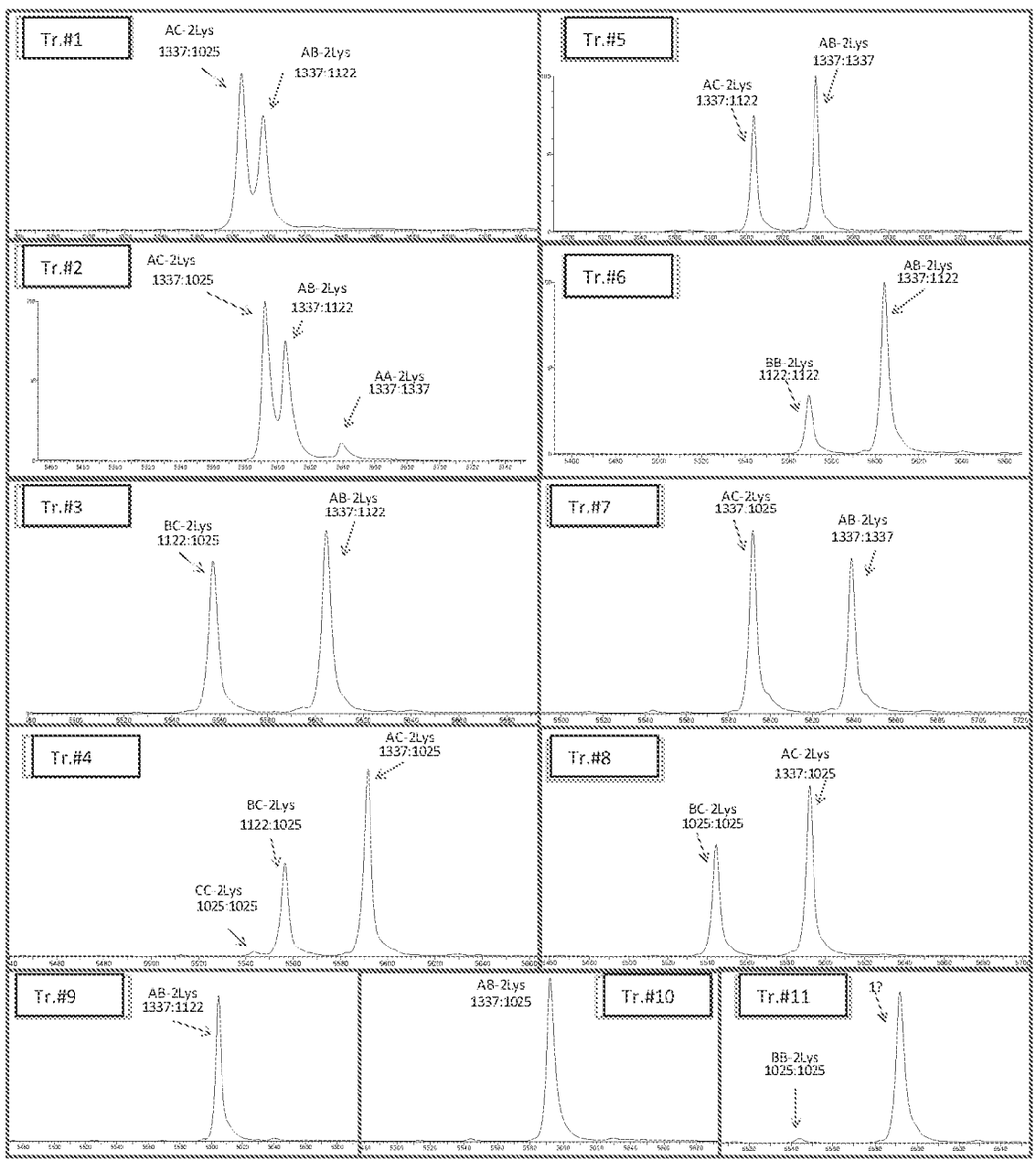

Tr.#1
AC-2Lys 1337:1025
AB-2Lys 1337:1122
Tr.#5
AB-2Lys 1337:1337
AC-2Lys 1337:1122
Tr.#2
AC-2Lys 1337:1025
AB-2Lys 1337:1122
AA-2Lys 1337:1337
Tr.#6
AB-2Lys 1337:1122
BB-2Lys 1122:1122
Tr.#3
BC-2Lys 1122:1025
AB-2Lys 1337:1122
Tr.#7
AC-2Lys 1337:1025
AB-2Lys 1337:1337
Tr.#4
AC-2Lys 1337:1025
BC-2Lys 1122:1025
CC-2Lys 1025:1025
Tr.#8
AC-2Lys 1337:1025
BC-2Lys 1025:1025
Tr.#9
AB-2Lys 1337:1122
AB-2Lys 1337:1025
Tr.#10
Tr.#11
1?
BB-2Lys 1025:1025

FIG. 25

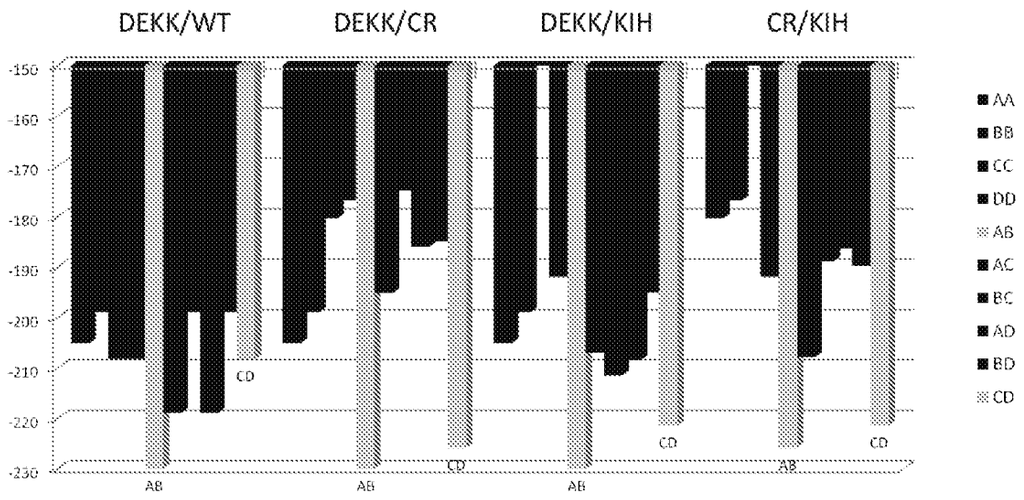

DEKK/WT
DEKK/CR
DEKK/KIH
CR/KIH
-150
-160
-170
-180
-190
-200
-210
-220
-230
AB
CD
AA
BB
CC
DD
AB
AC
BC
AD
BD
CD

FIG. 26

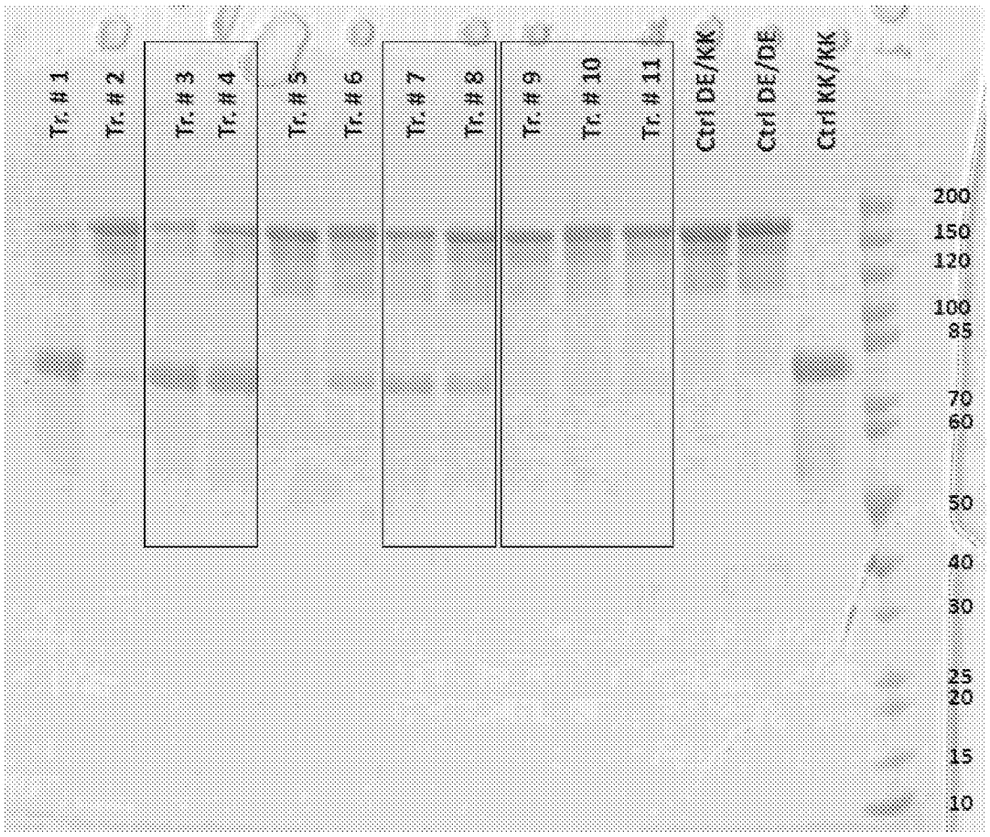

Tr. # 1
Tr. # 2
Tr. # 3
Tr. # 4
Tr. # 5
Tr. # 6
Tr. # 7
Tr. # 8
Tr. # 9
Tr. # 10
Tr. # 11
Ctrl DE/KK
Ctrl DE/DE
Ctrl KK/KK
200
150
120
100
85
70
60
50
40
30
25
20
15
10

FIG. 27A

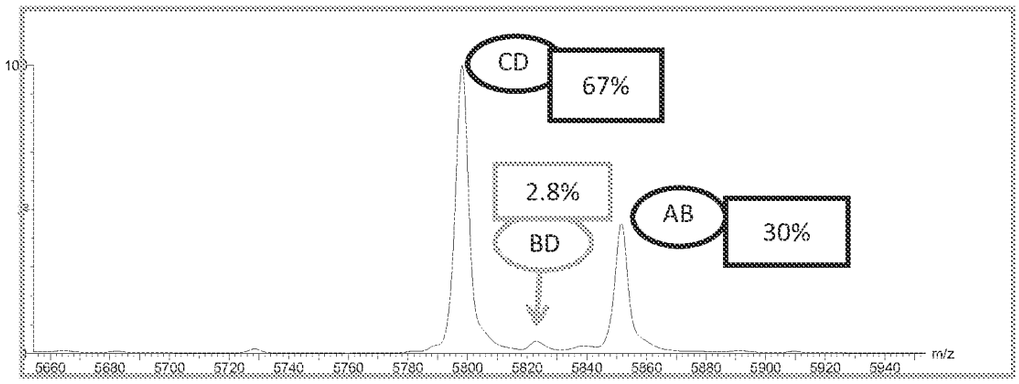


FIG. 27B

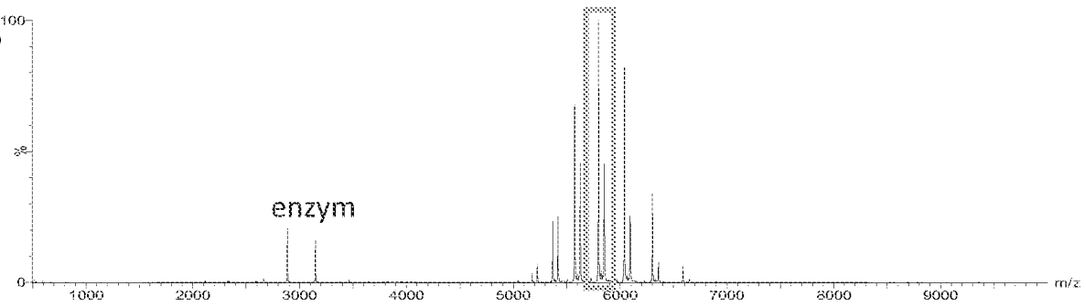


FIG. 27C

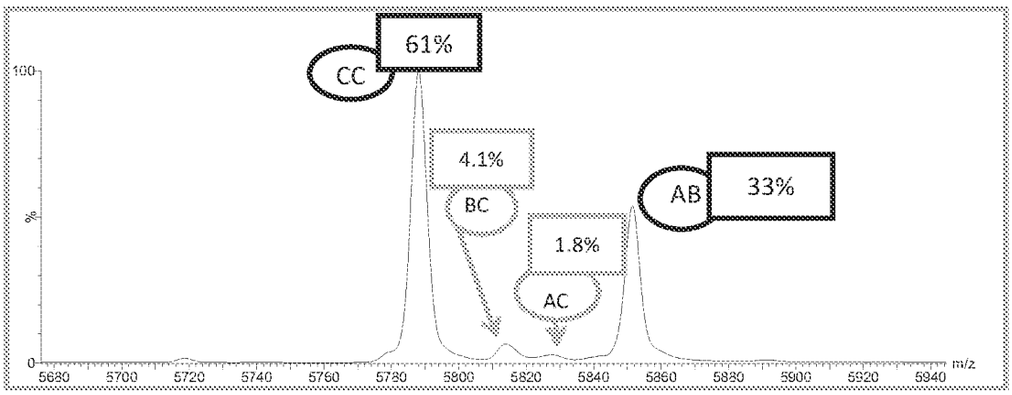


FIG. 27D

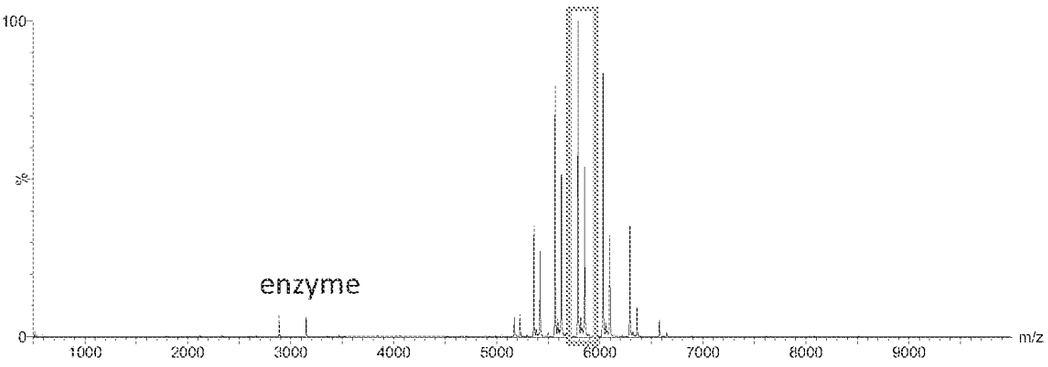


FIG. 28A

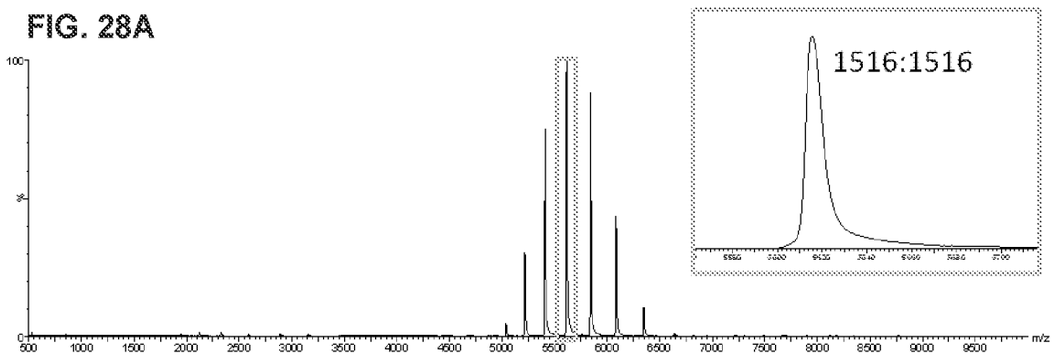


FIG. 28B

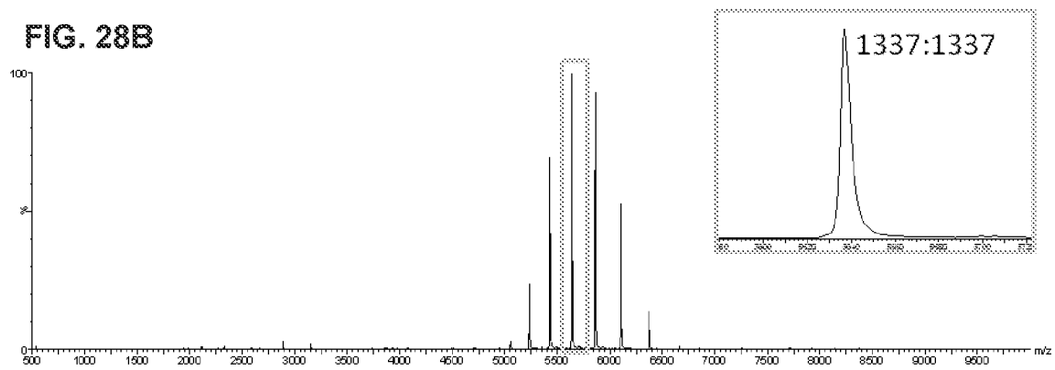


FIG. 28C

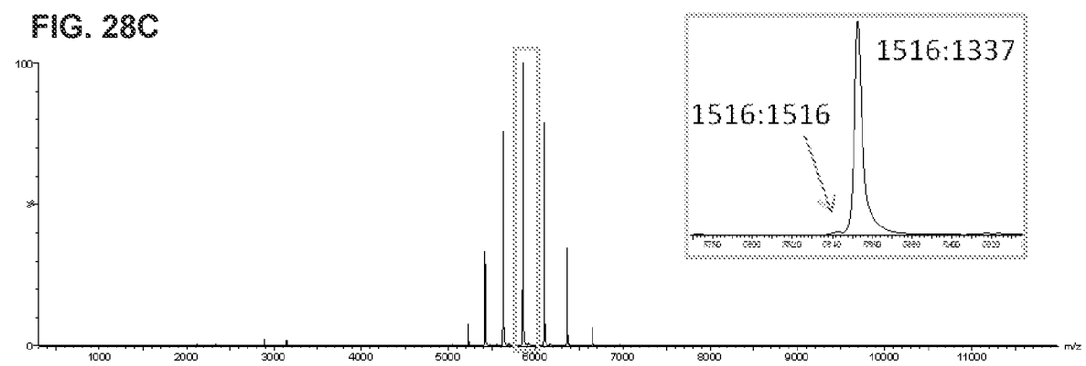
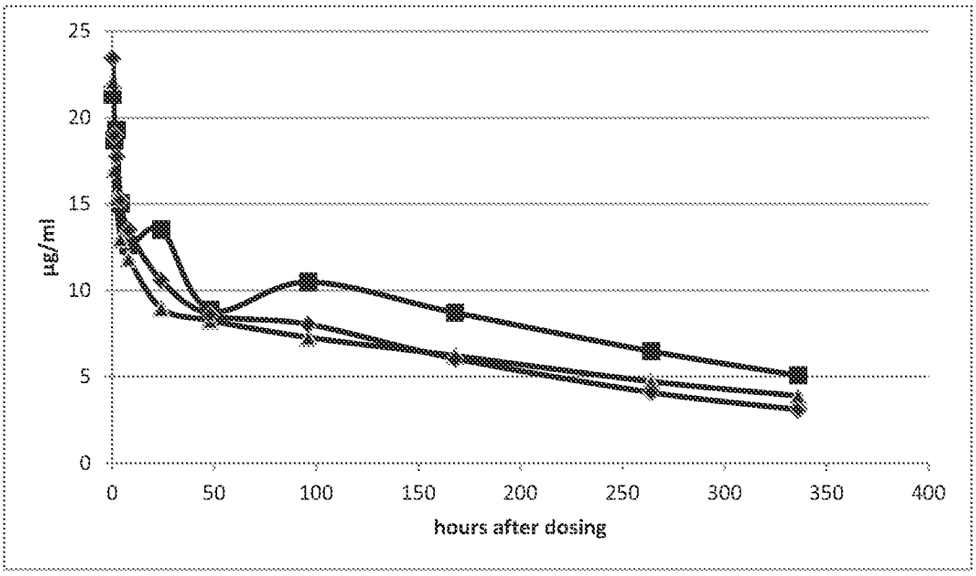


FIG. 29


25
20
15
10
5
0
µg/ml
0
50
100
150
200
250
300
350
400
hours after dosing

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

As the below named inventor, I hereby declare that:

This declaration is directed to:

- [ ] The attached application, or
- [x] United States application or PCT international application number 13/866,747 filed on 04/19/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

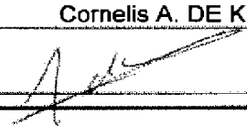
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Cornelis A. DE KRUIF Date (Optional): 24 May 13

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
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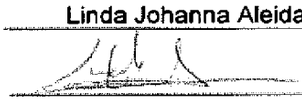
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LEGAL NAME OF INVENTOR

Inventor: Linda Johanna Aleida HENDRIKS Date (Optional): 24May13

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
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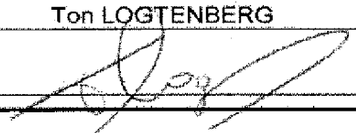
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LEGAL NAME OF INVENTOR

Inventor: Ton LOGTENBERG Date (Optional): May 24, 2013

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Cornelis Adriaan DE KRUIF	Confirmation No.: *To Be Assigned*
Applicant: MERUS N.V.	Art Unit: *To Be Assigned*
Application No.: *To Be Assigned*	Examiner: *To Be Assigned*
Filed: *Herewith*	Atty. Docket: 4096.0050007
(*Continuation of Appl. No. 16/934,925; Filed: July 21, 2020*)	

Title: **METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES**

Preliminary Amendment Under 37 C.F.R. § 1.115

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

In advance of prosecution, Applicant submits the following amendments and remarks.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 5 of this paper.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any additional fees required to continue prosecution or appeal of this application (including issue fee, fees for net addition of claims or forwarding to appeal) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments to the Specification

Please amend the Specification after the title by replacing the first paragraph with the following headers and paragraphs:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of U.S. Patent Application No. 16/934,925, filed July 21, 2020, which is a Continuation of U.S. Patent Application No. 16/417,379, filed May 20, 2019, now U.S. Patent No. 10,752,929, which is a Continuation of U.S. Patent Application No. 15/155,743, filed May 16, 2016, now U.S. Patent No. 10,329,596, which is a Continuation of U.S. Patent Application No. 14/081,848, filed November 15, 2013, now U.S. Patent No. 9,358,286, which is a Continuation of U.S. Patent Application No. 13/866,747, filed April 19, 2013, now U.S. Patent No. 9,248,181, which claims priority to U.S. Provisional Patent Application No. 61/635,935, filed April 20, 2012; the entire contents of which are hereby incorporated by reference.

REFERENCE TO SEQUENCE LISTING SUBMITTED

ELECTRONICALLY VIA EFS-WEB

The content of the electronically submitted sequence listing (Name 4096_0050007_SequenceListing_ST26.XML; Size: 10,004 bytes; and Date of Creation: April 25, 2023) filed with the application is incorporated herein by reference in its entirety.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1-28. (Canceled)

29. (New) A heterodimeric antibody comprising a first human CH3 domain comprising a positively charged amino acid residue at position 364 according to the EU numbering system, and a second human CH3 domain comprising a negatively charged amino acid residue at position 368 according to the EU numbering system.

30. (New) The heterodimeric antibody of claim 29, wherein said positively charged amino acid residue at position 364 comprises a lysine (K) or an arginine (R) residue, and wherein said negatively charged residue at position 368 comprises an aspartic acid (D) or glutamic acid (E) residue.

31. (New) The heterodimeric antibody of claim 30, wherein said positively charged amino acid residue at position 364 comprises a lysine (K) residue, and wherein said negatively charged amino acid residue at position 368 comprises an aspartic acid (D).

32. (New) The heterodimeric antibody of claim 29, wherein said positively charged amino acid residue at position 364 interacts with said negatively charged amino acid residue at position 368 in the interface between said CH3 domains.

33. (New) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody comprises two heavy chains that comprise said first or second human CH3 domains.

34. (New) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is a bispecific antibody.

35. (New) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is human IgG.

36. (New) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is human IgG1.

37. (New) A pharmaceutical composition comprising the heterodimeric antibody according to claim 29, and a pharmaceutically acceptable carrier.

Remarks

Upon entry of the foregoing amendment, claims 29-37 are pending in the application, with claim 29 being the independent claim. Claims 1-28 are sought to be canceled herein without prejudice to or disclaimer of the subject matter herein. New claims 29-37 are sought to be added. Support for the new claims can be found throughout the specification, *inter alia*, at Example 13 and Table 7, and in the claims as originally filed. These changes are believed to introduce no new matter, and their entry is respectfully requested.

The current specification has been amended to update the Reference to Earlier Filed Applications section and the Reference to Sequence Listing Submitted Electronically Via EFS-Web section. Further, the Sequence Listing file submitted herewith is identical to the Sequence Listing originally-submitted in U.S. Application No. 16/934,925, with changes made only to meet compliance of Sequence Listing standards.

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicant believes the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Paul A. Calvo, Reg. No. 57913/

Paul A. Calvo
Attorney for Applicant
Registration No. 57,913

Date: 5.16.2023

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Cornelis Adriaan DE KRUIF	Confirmation No.: *To Be Assigned*
Applicant: Merus N.V.	Art Unit: *To Be Assigned*
Application No.: *To Be Assigned*	Examiner: *To Be Assigned*
Filing Date: *To Be Assigned*	Atty. Docket: 4096.0050007

Title: **METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES**

Information Disclosure Statement

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Listed on accompanying IDS Form PTO/SB/08a and PTO/SB/08b equivalent are documents that may be considered material to the patentability of this application as defined in 37 C.F.R. §1.56, and in compliance with the duty of disclosure requirements of 37 C.F.R. §§ 1.97 and 1.98.

Applicant has listed publication dates on the attached IDS Form based on information presently available to the undersigned. However, the listed publication dates should not be construed as an admission that the information was actually published on the date indicated.

Applicant reserves the right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered.

This statement should not be construed as a representation that a search has been made, or that information more material to the examination of the present patent application does not exist. The Examiner is specifically requested not to rely solely on the material submitted herewith.

This is a filing under 37 C.F.R. § 1.97(b). This Information Disclosure Statement is being filed before the mailing date of a first Office Action on the merits. No statement or fee is required.

A concise explanation of the relevance of the non-English language documents appears below in accordance with 37 C.F.R. § 1.98(a)(3).

Document **FP11**, EP-0445625-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP11**.

Document **FP24**, JP-2006109711-A, is in a foreign language. An English language abstract was previously appended to the face page of document **FP24**.

Document **FP25**, JP-H08116978-A, is in a foreign language. An English language abstract was previously appended to the face page of document **FP25**.

Document **FP44**, WO-0246233-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP44**.

Document **FP55**, WO-03107218-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP55**.

Document **FP64**, WO-2006106905-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP64**.

Document **FP110**, WO-9301288-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP110**.

Document **FP131**, WO-9926569-A1, is in a foreign language. An English language abstract was previously appended to the face page of document **FP131**.

Copies of documents **NPL90-NPL91** are submitted. However, in accordance with 37 C.F.R. § 1.98(a)(2)(ii), no copies of U.S. Patents and Patent application publications cited as documents **US1-US150** on the attached IDS Form are submitted.

Copies of documents **FP1-FP135** and **NPL1-NPL89** were cited by or submitted to the Office in an IDS that complies with 37 C.F.R. § 1.98(a)-(c) in Application No. 16/934,925, filed July 21, 2020, which is

relied upon for an earlier filing date under 35 U.S.C. § 120. Thus, copies of these documents are not attached. 37 C.F.R. § 1.98(d).

It is expected that the examiner will review the prosecution and cited art in the parent Application No. 16/934,925, filed July 21, 2020, in accordance with MPEP 2001.06(b), and indicate in the next communication from the office that the art cited in the earlier prosecution history has been reviewed in connection with the present application.

In accordance with the Federal Circuit decision in *Dayco Prods., Inc. v. Total Containment, Inc.* 329 F.3d 1358 (Fed. Cir. 2003), Applicant submits herewith Office Actions from the co-pending U.S. Patent Application No. 16/934,925, filed July 21, 2020, as documents **NPL90** to **NPL91**. The identification of these Office Actions is not to be construed as a waiver of secrecy as to those applications now or upon issuance of the present application as a patent. The Examiner is respectfully requested to consider the cited applications and the art cited therein during examination.

It is respectfully requested that the Examiner initial and return a copy of the enclosed IDS Form, and indicate in the official file wrapper of this patent application that the documents have been considered.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Paul A. Calvo, Reg. No. 57,913/

Paul A. Calvo
Attorney for Applicant
Registration No. 57,913

Date: 5.16.2023

Atty. Dkt. No. 4096.0050007

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Washington, D.C. 20005-3934
(202) 371-2600

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT *(Use as many sheets as necessary)*				Application Number	*To Be Assigned*
				Filing Date	*Herewith*
				First Named Inventor	Cornelis Adriaan DE KRUIF
				Art Unit	*To Be Assigned*
				Examiner Name	*To Be Assigned*
Sheet	1	of	17	Attorney Docket Number	4096.0050007

U.S. PATENT DOCUMENTS

Examiner initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US1	US-4399216-A	08-16-1983	AXEL; Richard et al.	
	US2	US-4599311-A	07-08-1986	KAWASAKI; Glenn H.	
	US3	US-4634665-A	01-06-1987	AXEL; Richard et al.	
	US4	US-4801687-A	01-31-1989	NGO; That T.	
	US5	US-4816567-A	03-28-1989	CABILLY et al.	
	US6	US-4868103-A	09-19-1989	STAVRIANOPOULOS; Jannis et al.	
	US7	US-4937190-A	06-26-1990	PALMENBERG; Ann C. et al.	
	US8	US-5030002-A	07-09-1991	NORTH Jr. HOWARD L;	
	US9	US-5137809-A	08-11-1992	LOKEN; Michael R. et al.	
	US10	US-5151504-A	09-29-1992	CROZE; Edward M.	
	US11	US-5179017-A	01-12-1993	AXEL; Richard et al.	
	US12	US-5223409-A	06-29-1993	LADNER; Robert C. et al.	
	US13	US-5385839-A	01-31-1995	STINSKI; Mark F.	
	US14	US-5627037-A	05-06-1997	WARD; Anthony J. et al.	
	US15	US-5631169-A	05-20-1997	LAKOWICZ; Joseph R. et al.	
	US16	US-5641640-A	06-24-1997	HANNING; Anders	
	US17	US-5667988-A	09-16-1997	BARBAS; Carlos F. et al.	
	US18	US-5731168-A	03-24-1998	CARTER; Paul J. et al.	
	US19	US-5733779-A	03-31-1998	REFF; Mitchell E.	
	US20	US-5770429-A	06-23-1998	LONBERG et al.	
	US21	US-5772997-A	06-30-1998	HUDZIAK; Robert M. et al.	
	US22	US-5783186-A	07-21-1998	ARAKAWA; Tsutomu et al.	
	US23	US-5789208-A	08-04-1998	SHARON; Jacqueline	
	US24	US-5834237-A	11-10-1998	JACOBS; Eric et al.	
	US25	US-5885827-A	03-23-1999	WABL; Matthias et al.	
	US26	US-5888789-A	03-30-1999	RODRIGUEZ; Raymond L.	
	US27	US-5965371-A	10-12-1999	MARASCO; Wayne A. et al.	
	US28	US-6004940-A	12-21-1999	MARASCO; Wayne A. et al.	
	US29	US-6054297-A	04-25-2000	CARTER; Paul J. et al.	
	US30	US-6080560-A	06-27-2000	RUSSELL; David R. et al.	
	US31	US-6114598-A	09-05-2000	KUCHERLAPATI; Raju et al.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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				Art Unit	*To Be Assigned*
				Examiner Name	*To Be Assigned*
Sheet	2	of	17	Attorney Docket Number	4096.0050007

U.S. PATENT DOCUMENTS

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	US32	US-6180357-B1	01-30-2001	YOUNG; David S. F. et al.	
	US33	US-6207446-B1	03-27-2001	SZOSTAK; Jack W. et al.	
	US34	US-6265150-B1	07-24-2001	TERSTAPPEN; Leon WMM et al.	
	US35	US-6291740-B1	09-18-2001	BREMEL; Robert D. et al.	
	US36	US-6303341-B1	10-16-2001	HIATT; Andrew C. et al.	
	US37	US-6335163-B1	01-01-2002	SHARON; Jacqueline	
	US38	US-6596541-B2	07-22-2003	MURPHY; Andrew J. et al.	
	US39	US-7067284-B1	06-27-2006	BARBAS; Carlos F. et al.	
	US40	US-7105348-B2	09-12-2006	MURPHY; Andrew J. et al.	
	US41	US-7183076-B2	02-27-2007	ARATHOON; W. Robert et al.	
	US42	US-7262028-B2	08-28-2007	VAN BERKEL; Patrick Hendrikus Cornelis et al.	
	US43	US-7329530-B2	02-12-2008	HOUTZAGER; Erwin et al.	
	US44	US-7429486-B2	09-30-2008	VAN BERKEL; Patrick H. C. et al.	
	US45	US-7491516-B2	02-17-2009	COLLINSON; Albert et al.	
	US46	US-7579446-B2	08-25-2009	BAKKER; Alexander B. H. et al.	
	US47	US-7642228-B2	01-05-2010	CARTER; Paul J. et al.	
	US48	US-7696330-B2	04-13-2010	MEULEN; Jan H. Ter et al.	
	US49	US-7740852-B2	06-22-2010	BAKKER; Alexander Berthold Hendrik et al.	
	US50	US-7858086-B2	12-28-2010	GEUIJEN; Cecilia Anna Wilhelmina et al.	
	US51	US-7901919-B2	03-08-2011	HOUTZAGER; Erwin et al.	
	US52	US-7919257-B2	04-05-2011	MATTHEUS; Hoogenboom Hendricus R.J et al.	
	US53	US-7927834-B2	04-19-2011	VAN BERKEL; Patrick H. C. et al.	
	US54	US-7932360-B2	04-26-2011	VAN BERKEL; Patrick H. C. et al.	
	US55	US-7960518-B2	06-14-2011	THROSBY; Mark et al.	
	US56	US-7968092-B2	06-28-2011	THROSBY; Mark et al.	
	US57	US-8052974-B2	11-08-2011	THROSBY; Mark et al.	
	US58	US-8106170-B2	01-31-2012	TER; Meulen JAN H. et al.	
	US59	US-8148497-B2	04-03-2012	BAKKER; Alexander Berthold Hendrik et al.	

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U.S. PATENT DOCUMENTS

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	US60	US-8192927-B2	06-05-2012	VAN; DEN Brink Edward Norbert et al.	
	US61	US-8211431-B2	07-03-2012	THROSBY; Mark et al.	
	US62	US-8241631-B2	08-14-2012	THROSBY; Mark et al.	
	US63	US-8268756-B2	09-18-2012	LOGTENBERG; TON et al.	
	US64	US-8470327-B2	06-25-2013	THROSBY; Mark et al.	
	US65	US-8592562-B2	11-26-2013	KANNAN; Gunasekaran et al.	
	US66	US-8911738-B2	12-16-2014	THROSBY; Mark et al.	
	US67	US-9012371-B2	04-21-2015	LOGTENBERG; Ton et al.	
	US68	US-9145588-B2	09-29-2015	THROSBY; Mark et al.	
	US69	US-9248181-B2	02-02-2016	DE KRUIF; Cornelis A. et al.	
	US70	US-9248182-B2	02-02-2016	DE KRUIF; Cornelis A. et al.	
	US71	US-9358286-B2	06-07-2016	DE KRUIF; Cornelis A. et al.	
	US72	US-9758805-B2	09-12-2017	DE KRUIF; Cornelis Adriaan et al.	
	US73	US-10752929-B2	08-25-2020	DE KRUIF; Cornelis Adriaan et al.	
	US74	US-20020088016-A1	07-04-2002	BRUGGEMANN; Marianne	
	US75	US-20020138857-A1	09-26-2002	GHAYUR; Tariq	
	US76	US-20030039958-A1	02-27-2003	HOLT; Lucy Jessica et al.	
	US77	US-20030077739-A1	04-24-2003	SIMMONS; Laura et al.	
	US78	US-20030078385-A1	04-24-2003	ARATHOON; W. Robert et al.	
	US79	US-20030091561-A1	05-15-2003	VAN DE WINKEL; Jan G.J. et al.	
	US80	US-20030093820-A1	05-15-2003	GREEN; Larry L. et al.	
	US81	US-20030096225-A1	05-22-2003	LOGTENBERG; Ton	
	US82	US-20030194403-A1	10-16-2003	VAN DE WINKEL; Jan et al.	
	US83	US-20030207346-A1	11-06-2003	ARATHOON; William R. et al.	
	US84	US-20030219829-A1	11-27-2003	LOGTENBERG; TON et al.	
	US85	US-20030224408-A1	12-04-2003	HOOGENBOOM; Henricus Renerus Jacobus Mattheus et al.	
	US86	US-20050014261-A1	01-20-2005	HOUTZAGER; Erwin et al.	
	US87	US-20050037001-A1	02-17-2005	GERMERAAD; Wilfred Thomas Vincent et al.	
	US88	US-20050170398-A1	08-04-2005	VAN BERKEL; Patrick Hendrikus Cornelis et al.	

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	US89	US-20060015949-A1	01-19-2006	LONBERG; Nils et al.	
	US90	US-20060015957-A1	01-19-2006	LONBERG; Nils et al.	
	US91	US-20060088520-A1	04-27-2006	GERMERAAD; Wilfred T.V et al.	
	US92	US-20060117699-A1	06-08-2006	DI; Trapani Agostino	
	US93	US-20060160184-A1	07-20-2006	MATTHEUS HOOGENBOOM; Hendricus Renerus Jacobus et al.	
	US94	US-20060205077-A1	09-14-2006	SCHWENK; Frieder et al.	
	US95	US-20060257397-A1	11-16-2006	THROSBY; Mark et al.	
	US96	US-20070054362-A1	03-08-2007	VAN BERKEL; Patrick H. C. et al.	
	US97	US-20070059766-A1	03-15-2007	LOGTENBERG; Ton et al.	
	US98	US-20070280945-A1	12-06-2007	STEVENS; Sean et al.	
	US99	US-20080070799-A1	03-20-2008	BAKKER; Alexander Berthold Hendrik et al.	
	US100	US-20080241166-A1	10-02-2008	TOMLINSON; Ian M. et al.	
	US101	US-20090017521-A1	01-15-2009	HOUTZAGER; Erwin et al.	
	US102	US-20090054254-A1	02-26-2009	THROSBY; Mark et al.	
	US103	US-20090074765-A1	03-19-2009	CARMELIET; Peter et al.	
	US104	US-20090092582-A1	04-09-2009	BOGIN; Oren et al.	
	US105	US-20090130652-A1	05-21-2009	THROSBY; Mark et al.	
	US106	US-20090181855-A1	07-16-2009	VASQUEZ; Maximiliano et al.	
	US107	US-20090182127-A1	07-16-2009	KJAERGAARD; Kristian et al.	
	US108	US-20090263864-A1	10-22-2009	VAN BERKEL; Patrick H. C. et al.	
	US109	US-20100015133-A1	01-21-2010	IGAWA; Tomoyuki et al.	
	US110	US-20100069614-A1	03-18-2010	HOUTZAGER; Erwin et al.	
	US111	US-20100146647-A1	06-10-2010	LOGTENBERG; Ton et al.	
	US112	US-20100172917-A1	07-08-2010	TER; Meulen JAN H. et al.	
	US113	US-20100286374-A1	11-11-2010	KANNAN; Gunasekaran et al.	
	US114	US-20100297153-A1	11-25-2010	GEUIJEN; Cecilia A.W et al.	
	US115	US-20100310572-A1	12-09-2010	BAKKER; Alexander Berthold Hendrik et al.	
	US116	US-20100310586-A1	12-09-2010	DOLCETTI; Riccardo et al.	
	US117	US-20100331527-A1	12-30-2010	DAVIS; Samuel et al.	
	US118	US-20110054141-A1	03-03-2011	BEST; Kurt E. et al.	

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	US119	US-20110177073-A1	07-21-2011	VAN; Berkel Patrick H. C. et al.	
	US120	US-20110195454-A1	08-11-2011	MCWHIRTER; John et al.	
	US121	US-20110268739-A1	11-03-2011	THROSBY; Mark et al.	
	US122	US-20120021409-A1	01-26-2012	MCWHIRTER; John et al.	
	US123	US-20120039898-A1	02-16-2012	THROSBY; Mark et al.	
	US124	US-20120058907-A1	03-08-2012	LOGTENBERG; Ton et al.	
	US125	US-20120076794-A1	03-29-2012	THROSBY; Mark et al.	
	US126	US-20120093823-A1	04-19-2012	VAN DEN BRINK; Edward Norbert et al.	
	US127	US-20120141493-A1	06-07-2012	THROSBY; Mark et al.	
	US128	US-20120177637-A1	07-12-2012	HOOGENBOOM; Hendricus Renerus Jacobus Mattheus et al.	
	US129	US-20120192300-A1	07-26-2012	BABB; Robert et al.	
	US130	US-20120276115-A1	11-01-2012	VAN DEN BRINK; Edward Norbert et al.	
	US131	US-20120315278-A1	12-13-2012	THROSBY; Mark et al.	
	US132	US-20120321950-A1	12-20-2012	OKUMURA; Takefumi et al.	
	US133	US-20130096020-A1	04-18-2013	THROSBY; Mark et al.	
	US134	US-20130115208-A1	05-09-2013	HO; Wei-Hsien et al.	
	US135	US-20130145484-A1	06-06-2013	LOGTENBERG; Ton et al.	
	US136	US-20130336981-A1	12-19-2013	DE KRUIF; Cornelis A. et al.	
	US137	US-20140072579-A1	03-13-2014	DE KRUIF; Cornelis Adriaan et al.	
	US138	US-20140120096-A1	05-01-2014	BAKKER; Alexander Berthold Hendrik et al.	
	US139	US-20140140999-A1	05-22-2014	DE KRUIF; Cornelis A. et al.	
	US140	US-20140314755-A1	10-23-2014	LOGTENBERG; Ton et al.	
	US141	US-20140317766-A1	10-23-2014	LOGTENBERG; Ton et al.	
	US142	US-20150139996-A1	05-21-2015	DE KRUIF; Cornelis Adriaan et al.	
	US143	US-20150196637-A1	07-16-2015	DE KRUIF; Cornelis Adriaan et al.	
	US144	US-20160130367-A1	05-12-2016	THROSBY; Mark et al.	
	US145	US-20160177364-A1	06-23-2016	DE KRUIF; Cornelis Adriaan et al.	
	US146	US-20160238600-A1	08-18-2016	HOOGENBOOM; Hendricus Renerus et al.	

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	US147	US-20160368988-A1	12-22-2016	BAKKER; Alexander Berthold Hendrik et al.	
	US148	US-20170369923-A1	12-28-2017	DE KRUIF; Cornelis Adriaan et al.	
	US149	US-20200017897-A1	01-16-2020	DE KRUIF; Cornelis Adriaan et al.	
	US150	US-10329596-B2	06-25-2019	DE KRUIF; Cornelis Adriaan et al	

FOREIGN PATENT DOCUMENTS

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	FP1	CA-1341364-C	06-04-2002	GENEX CORP [US]		
	FP2	CA-2114353-A1	08-02-1994	SQUIBB BRISTOL MYERS CO [US]		
	FP3	CA-2137558-A1	02-03-1994	DANA FARBER CANCER INST INC [US]		
	FP4	CA-2405961-A1	11-01-2001	ELUSYS THERAPEUTICS INC [US]		
	FP5	CA-2445255-A1	10-31-2002	UNIV ERASMUS [NL]		
	FP6	EP-0120694-A2	10-03-1984	CELLTECH LTD [GB]		
	FP7	EP-0171142-A1	02-12-1986	ZYMOGENETICS INC [US]		
	FP8	EP-0307667-A2	03-22-1989	WACOM CO LTD [JP]		
	FP9	EP-0314161-A1	05-03-1989	BRISTOL MYERS CO [US]		
	FP10	EP-0402029-A2	12-12-1990	WISTAR INST [US]		
	FP11	EP-0445625-A1	09-11-1991	BEHRINGWERKE AG [DE]		X
	FP12	EP-0469025-A1	02-05-1992	MEDICAL RES COUNCIL [GB]		
	FP13	EP-0469897-A2	02-05-1992	WISTAR INST [US]		
	FP14	EP-0481790-A2	04-22-1992	WELLCOME FOUND [GB]		
	FP15	EP-0523949-A1	01-20-1993	WELLCOME FOUND [GB]		
	FP16	EP-0666868-A1	08-16-1995	GENENTECH INC [US]		
	FP17	EP-0724639-A1	08-07-1996	GENENTECH INC [US]		
	FP18	EP-0814159-A2	12-29-1997	GENPHARM INT [US]		
	FP19	EP-1325932-A2	07-09-2003	GENENTECH INC [US]		
	FP20	EP-1349234-A2	10-01-2003	THALES PLC [GB]		
	FP21	EP-1439234-A1	07-21-2004	ARTEMIS PHARMACEUTICALS GMBH [DE]		

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	FP22	EP-1870459-A1	12-26-2007	CHUGAI PHARMACEUTICAL CO LTD [JP]		
	FP23	EP-2147594-A1	01-27-2010	MERUS B V [NL]		
	FP24	JP-2006109711-A	04-27-2006	UNIV OKAYAMA [JP]		X
	FP25	JP-H08116978-A	05-14-1996	NISSHIN SPINNING [JP]		X
	FP26	WO-0044777-A1	08-03-2000	IMCLONE SYSTEMS INC [US]		
	FP27	WO-0063403-A2	10-26-2000	INTROGENE BV [NL]		
	FP28	WO-0070023-A1	11-23-2000	DYAX CORP [US]		
	FP29	WO-0071694-A1	11-30-2000	SCRIPPS RESEARCH INST [US]		
	FP30	WO-0076310-A1	12-21-2000	ABGENIX INC [US]		
	FP31	WO-0100245-A2	01-04-2001	GENENTECH INC [US]		
	FP32	WO-0119394-A2	03-22-2001	THERAPEUTIC HUMAN POLYCLONALS [US]		
	FP33	WO-0127279-A1	04-19-2001	CAMBRIDGE ANTIBODY TECH [GB]		
	FP34	WO-0132901-A1	05-10-2001	CHIRON CORP [US]		
	FP35	WO-0148485-A2	07-05-2001	CRUCELL HOLLAND BV [NL]		
	FP36	WO-0164929-A1	09-07-2001	UNIV AUBURN [US]		
	FP37	WO-0188132-A2	11-22-2001	UNIV JEFFERSON [US]		
	FP38	WO-02059297-A2	08-01-2002	EVOLVA BIOTECH AS [DK]		
	FP39	WO-02066630-A1	08-29-2002	REGENERON PHARMA [US]		
	FP40	WO-02074969-A2	09-26-2002	UNIV LAUSANNE [CH]		
	FP41	WO-02096948-A2	12-05-2002	IDEC PHARMA CORP [US]		
	FP42	WO-0218948-A2	03-07-2002	CRUCELL HOLLAND BV [NL]		
	FP43	WO-0243478-A2	06-06-2002	MEDAREX INC [US]		
	FP44	WO-0246233-A1	06-13-2002	TECHNOPHARM [FR]		X
	FP45	WO-03002609-A2	01-09-2003	MEDICAL RES COUNCIL [GB]		
	FP46	WO-03004704-A2	01-16-2003	CHROMAGENICS BV [NL]		
	FP47	WO-03016501-A2	02-27-2003	UNIV JEFFERSON [US]		
	FP48	WO-03033670-A2	04-24-2003	SLOAN KETTERING INST CANCER [US]		
	FP49	WO-03046560-A2	06-05-2003	CA NAT RESEARCH COUNCIL [CA]		
	FP50	WO-03048306-A2	06-12-2003	IDEC PHARMA CORP [US]		
	FP51	WO-03052416-A2	06-26-2003	NEUTEC PHARMA PLC [GB]		

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	FP52	WO-03102157-A2	12-11-2003	GENENTECH INC [US]		
	FP53	WO-03106674-A2	12-24-2003	CHROMAGENICS BV [NL]		
	FP54	WO-03106684-A2	12-24-2003	CHROMAGENICS BV [NL]		
	FP55	WO-03107218-A1	12-24-2003	CELESTAR LEXICO SCIENCES INC [JP]		X
	FP56	WO-2004003211-A1	01-08-2004	IMCLONE SYSTEMS INC [US]		
	FP57	WO-2004009618-A2	01-29-2004	CRUCELL HOLLAND BV [NL]		
	FP58	WO-2004061104-A2	07-22-2004	SYMPHOGEN AS [DK]		
	FP59	WO-2004106375-A1	12-09-2004	MERUS BIOPHARMACEUTICALS B V I [NL]		
	FP60	WO-2005000894-A2	01-06-2005	CRUCELL HOLLAND BV [NL]		
	FP61	WO-2005068622-A2	07-28-2005	MERUS B V [NL]		
	FP62	WO-2005118635-A2	12-15-2005	NOVIMMUNE SA [CH]		
	FP63	WO-2006028936-A2	03-16-2006	GENENTECH INC [US]		
	FP64	WO-2006106905-A1	10-12-2006	CHUGAI PHARMACEUTICAL CO LTD [JP]		X
	FP65	WO-2006117699-A2	11-09-2006	INNATE PHARMA SA [FR]		
	FP66	WO-2007059782-A1	05-31-2007	GENMAB AS [DK]		
	FP67	WO-2007110205-A2	10-04-2007	MERCK PATENT GMBH [DE]		
	FP68	WO-2007117410-A2	10-18-2007	MEDAREX INC [US]		
	FP69	WO-2007147901-A1	12-27-2007	NOVO NORDISK AS [DK]		
	FP70	WO-2008027236-A2	03-06-2008	GENENTECH INC [US]		
	FP71	WO-2008054606-A2	05-08-2008	REGENERON PHARMA [US]		
	FP72	WO-2008076379-A2	06-26-2008	REGENERON PHARMA [US]		
	FP73	WO-2008119353-A1	10-09-2008	GENMAB AS [DK]		
	FP74	WO-2009051974-A1	04-23-2009	NUVELO INC [US]		
	FP75	WO-2009080251-A1	07-02-2009	HOFFMANN LA ROCHE [CH]		
	FP76	WO-2009080253-A1	07-02-2009	HOFFMANN LA ROCHE [CH]		
	FP77	WO-2009089004-A1	07-16-2009	AMGEN INC [US]		
	FP78	WO-2009098596-A2	08-13-2009	ZYMEWORKS INC. [CA]		
	FP79	WO-2009157771-A2	12-30-2009	MERUS B V [NL]		
	FP80	WO-2010084197-A1	07-29-2010	GENMAB AS [DK		
	FP81	WO-2010108127-A1	09-23-2010	GENENTECH INC [US]		

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	FP82	WO-2010129304-A2	11-11-2010	ONCOMED PHARM INC [US]		
	FP83	WO-2011028952-A1	03-10-2011	XENCOR INC [US]		
	FP84	WO-2011028953-A1	03-10-2011	AMFIT INC [US]		
	FP85	WO-2011063348-A1	05-26-2011	AMGEN INC [US]		
	FP86	WO-2011097603-A1	08-11-2011	REGENERON PHARMA [US]		
	FP87	WO-2011143545-A1	11-17-2011	RINAT NEUROSCIENCE CORP [US]		
	FP88	WO-2012020096-A1	02-16-2012	MEDIMMUNE LTD [GB]		
	FP89	WO-2012023053-A2	02-23-2012	NOVIMMUNE SA [CH		
	FP90	WO-2012058768-A1	05-10-2012	ZYMEWORKS INC [CA]		
	FP91	WO-2012131555-A2	10-04-2012	GLENMARK PHARMACEUTICALS SA [CH		
	FP92	WO-2012141798-A1	10-18-2012	REGENERON PHARMA [US]		
	FP93	WO-2012148873-A2	11-01-2012	REGENERON PHARMA [US]		
	FP94	WO-2013134263-A1	09-12-2013	REGENERON PHARMA [US]		
	FP95	WO-2013157953-A1	10-24-2013	MERUS B V [NL]		
	FP96	WO-2013157954-A1	10-24-2013	MERUS B V [NL]		
	FP97	WO-2013184761-A1	12-12-2013	REGENERON PHARMA [US]		
	FP98	WO-2014151433-A1	09-25-2014	ALTRIA CLIENT SERVICES INC [US]		
	FP99	WO-9002809-A1	03-22-1990	PROTEIN ENG CORP [US]		
	FP100	WO-9012878-A1	11-01-1990	MEDICAL RES COUNCIL [GB]		
	FP101	WO-9100906-A1	01-24-1991	GENETICS INST [US]		
	FP102	WO-9108216-A1	06-13-1991	GENPHARM INT [US]		
	FP103	WO-9117271-A1	11-14-1991	AFFYMAX TECH NV [NL]		
	FP104	WO-9201047-A1	01-23-1992	CAMBRIDGE ANTIBODY TECH [GB]		
	FP105	WO-9203918-A1	03-19-1992	GENPHARM INT [US]		
	FP106	WO-9209690-A2	06-11-1992	GENENTECH INC [US]		
	FP107	WO-9215679-A1	09-17-1992	PROTEIN ENG CORP [US]		
	FP108	WO-9218619-A1	10-29-1992	SCRIPPS RESEARCH INST [US]		
	FP109	WO-9220791-A1	11-26-1992	CAMBRIDGE ANTIBODY TECH [GB]		
	FP110	WO-9301288-A1	01-21-1993	DEUTSCHES KREBSFORSCH [DE]		X
	FP111	WO-9312227-A1	06-24-1993	GENPHARM INT [US]		

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	FP112	WO-9402602-A1	02-03-1994	CELL GENESYS INC [US]		
	FP113	WO-9402610-A1	02-03-1994	DANA FARBER CANCER INST INC [US]		
	FP114	WO-9423046-A1	10-13-1994	US HEALTH [US]		
	FP115	WO-9425591-A1	11-10-1994	UNILEVER PLC [GB]		
	FP116	WO-9517085-A1	06-29-1995	GENZYME TRANSGENICS CORP [US]		
	FP117	WO-9520401-A1	08-03-1995	UNIV BOSTON [US]		
	FP118	WO-9627011-A1	09-06-1996	GENENTECH INC [US]		
	FP119	WO-9742313-A1	11-13-1997	SCRIPPS RESEARCH INST [US]		
	FP120	WO-9747739-A1	12-18-1997	UNIV MICHIGAN STATE [US]		
	FP121	WO-9815627-A1	04-16-1998	UNIV SOUTHAMPTON [GB]		
	FP122	WO-9815833-A1	04-16-1998	UNIV UTRECHT [NL]		
	FP123	WO-9824923-A1	06-11-1998	UNIV WAKE FOREST [US]		
	FP124	WO-9839416-A1	09-11-1998	INFIGEN INC [US]		
	FP125	WO-9841645-A1	09-24-1998	IDEC PHARMA CORP [US]		
	FP126	WO-9850431-A2	11-12-1998	GENENTECH INC [US]		
	FP127	WO-9852976-A1	11-26-1998	BIOVATION LTD [GB]		
	FP128	WO-9915684-A2	04-01-1999	OXFORD BIOMEDICA LTD [GB]		
	FP129	WO-9920749-A1	04-29-1999	MEDICAL RES COUNCIL [GB]		
	FP130	WO-9923221-A2	05-14-1999	UNILEVER PLC [GB]		
	FP131	WO-9926569-A1	06-03-1999	MITSUBISHI PRECISION CO LTD [JP]		X
	FP132	WO-9936569-A1	07-22-1999	UNIV ILLINOIS [US]		
	FP133	WO-9945962-A1	09-16-1999	GENPHARM INT [US]		
	FP134	WO-9950657-A1	10-07-1999	RES DEV FOUNDATION [US]		
	FP135	WO-9964582-A2	12-16-1999	INTROGENE BV [NL]		

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	NPL1	ALLEN, T.M., "Ligand-Targeted Therapeutics in Anticancer Therapy," Nature Reviews Cancer 2(10):750-763, Nature Publishing Group, United Kingdom (October 2002).	
	NPL2	ATWELL, S., et al., "Stable Heterodimers from Remodeling the Domain Interface of a Homodimer Using a Phage Display Library," Journal of Molecular Biology 270(1):26-35, Elsevier, United Kingdom (1997).	
	NPL3	BAEUERLE, P., et al., "Bispecific T-Cell Engaging Antibodies for Cancer Therapy," Cancer Research 69(12):4941-4944, American Association for Cancer Research, United States (2009).	
	NPL4	BOGAN, A., et. al., "Anatomy of Hot Spots in Protein Interfaces," Journal of Molecular Biology 280(1):1-9, Elsevier, Netherlands (July 1998).	
	NPL5	CARTER, P., "Bispecific Human IgG by Design," Journal of Immunological Methods 248(1-2):7-15, Elsevier, Netherlands (2001).	
	NPL6	CARTER, P., et al., "Toward the Production of Bispecific Antibody Fragments for Clinical Applications," Journal of Hematotherapy 4:463-470, Mary Ann Liebert, Inc., United States (1995).	
	NPL7	CARTER, P., "Improving the efficacy of antibody-based cancer therapies," Nature Reviews Cancer 1(2):118-129, Nature Publishing Group, United States (2001).	
	NPL8	CHAN, A., et al., "Genomic Organization of the T Cell Receptor," Cancer Detection and Prevention 14(2):261-267, Elsevier, United Kingdom (1989).	
	NPL9	CHEONG, H.S., et al., "Affinity Enhancement of Bispecific Antibody Against Two Different Epitopes in the Same Antigen," Biochemical and Biophysical Research Communications 173(3):795-800, Elsevier, Netherlands (December 1990).	
	NPL10	Communication pursuant to Article 94(3) EPC, European Application No. 13720614.0, dated Mar. 24, 2017, 5 pages.	
	NPL11	DAVIES, J. and RIECHMANN, L., "Antibody VH Domains as Small Recognition Units," Biotechnology 13(5):475-479, Nature Publishing Group, United States (1995).	
	NPL12	DAVIS, J.H., et al., "SEEDbodies: Fusion Proteins Based on Strand-exchange Engineered Domain (SEED) CH3 Heterodimers in an Fc Analogue Platform for Asymmetric Binders or Immunofusions and Bispecific Antibodies," Protein Engineering, Design & Selection 23(4):195-202, Oxford University Press, United Kingdom (2010).	
	NPL13	DE KRUIF, J., et al., "Generation of Stable Cell Clones Expressing Mixtures of Human Antibodies," Biotechnology and Bioengineering 106(5):741-750, Wiley, United States (August 2010).	
	NPL14	DE WILDT, et al., Analysis of Heavy and Light Chain Pairings Indicates that Receptor Editing Shapes fhe Human Antibody Repertoire, Journal of Molecular Biology, 285(3):895-901, Elsevier, United Kingdom (1999).	
	NPL15	DEISENHOFER, J., "Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-A resolution," Biochemistry 20(9):2361-2370, American Chemical Society, United States (1981).	

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	NPL16	DEMEULE, B., et al., "Characterization of Protein Aggregation: the Case of a Therapeutic Immunoglobulin," Biochimica et Biophysica Acta 1774(1):146-153, Elsevier Publisher, Netherlands (January 2007).	
	NPL17	DEMEULE, B., et al., "Detection and Characterization of Protein Aggregates by Fluorescence Microscopy," International Journal of Pharmaceutics 329(1-2):37-45, Elsevier/North-Holland Biomedical Press, Netherlands (February 2007).	
	NPL18	Description of relevance of Third Party Submission in U.S. Appl. No. 15/140,321 dated Feb. 10, 2017.	
	NPL19	ELLERSON, J.R., et al., "Structure and Function of Immunoglobulin Domains. III. Isolation and Characterization of a Fragment Corresponding to the Cgamma2 Homology Region of Human Immunoglobin G1," Journal of Immunology 116(2):510-517, American Association of Immunologists, United States (February 1976).	
	NPL20	EZZELL, C., et al., "Magic Bullets Fly Again," Scientific American 285(4):34-41, Scientific American, United States (October 2001).	
	NPL21	FARNAN, D. and MORENO, G.T., "Multiproduct High-resolution Monoclonal Antibody Charge Variant Separations by pH Gradient Ion-exchange Chromatography," Analytical Chemistry 81(21):8846-8857, American Chemical Society, United States (2009).	
	NPL22	GARBER, K., "Biotech Industry Faces New Bottleneck," Nature Biotechnology 19(3):184-185, Nature America Publishing, United States (March 2001).	
	NPL23	GenBank, "H.sapiens gene for Ig kappa light chain variable region "012"," Acc. No. X59315, accessed at https://www.ncbi.nlm.nih.gov/nuccore/X59315, 2 pages.	
	NPL24	GHETIE, M.A., et al., "Homodimerization of Tumor-Reactive Monoclonal Antibodies Markedly Increases their Ability to Induce Growth Arrest or Apoptosis of Tumor Cells," Proceedings of the National Academy of Sciences USA 94(14):7509-7514, National Academy of Sciences, United States (1997).	
	NPL25	GUNASEKARAN, K., et al., "Enhancing Antibody Fc Heterodimer Formation Through Electrostatic Steering Effects: Applications to Bispecific Molecules and Monovalent IgG," Journal of Biological Chemistry 285(25):19637-19646, American Society for Biochemistry and Molecular Biology, United States (June 2010).	
	NPL26	HARJUNPAA, A., et al., "Rituximab (Anti-CD20) Therapy of B-Cell Lymphomas: Direct Complement Killing is Superior to Cellular Effector Mechanisms," Scandinavian Journal of Immunology 51(6):634-641, Blackwell Scientific Publications, United Kingdom (June 2000).	
	NPL27	HENDSCH, Z.S., et al., "Preferential Heterodimer Formation via Undercompensated Electrostatic Interactions," Journal of the American Chemical Society 123(6):1264-1265, American Chemical Society, United States (February 2001).	
	NPL28	HOLLIGER, P., et al., "Diabodies: Small Bivalent and Bispecific Antibody Fragments," Proc Natl Acad Sci USA 90(14):6444-6448, National Academy of Sciences, United States (July 1993).	

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	NPL29	HOLT, L.J., et al., "Domain Antibodies: Proteins for Therapy," Trends in Biotechnology 21(11):484-490, Elsevier Science Publishers, United Kingdom (2003).	
	NPL30	HOOGENBOOM, H.R., "Selecting and Screening Recombinant Antibody Libraries," Nature Biotechnology 23(9):1105-1116, Nature America Publishing, United States (2005).	
	NPL31	HWANG, W.Y., et al., "Immunogenicity of Engineered Antibodies," Methods 36(1):3-10, Academic Press, United States (May 2005).	
	NPL32	JAIN, M., et al., "Engineering Antibodies for Clinical Applications," Trends in Biotechnology 25(7):307-316, Elsevier Science Publishers, United Kingdom (July 2007).	
	NPL33	JAKOBOVITS, A., et al., "Analysis of Homozygous Mutant Chimeric Mice: Deletion of the Immunoglobulin Heavy-chain Joining Region Blocks B-cell Development and Antibody Production," Proceedings of the National Academy of Sciences USA 90(6):2551-2555, National Academy of Sciences, United States (March 1993).	
	NPL34	KLOHN, P.C., et al., "Ibc's 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics International Conferences and the 2012 Annual Meeting of the Antibody Society: December 3-6, 2012, San Diego, CA," Monoclonal antibody 5(2):178-201, Taylor & Francis, United States (March-April 2013).	
	NPL35	KONTERMANN, R.E., "Dual Targeting Strategies with Bispecific Antibodies," MAbs 4(2):182-197, Taylor and Francis, United States (2012).	
	NPL36	KORTT, A.A., et al., "Dimeric and Trimeric Antibodies: High Avidity scFvs for Cancer Targeting," Biomolecular Engineering 18 (3):95-108, Elsevier, Netherlands (October 2001).	
	NPL37	KROESEN, B.J., et al., "Bispecific antibodies for treatment of cancer in experimental animal models and man," Adv. Drug Deliv. Rev. 31(1-2):105-129, Elsevier, Netherlands (1998).	
	NPL38	KUMAR, R., et al., "The Second Pdz Domain of Inad Is a Type I Domain Involved in Binding to Eye Protein Kinase C. Mutational Analysis and Naturally Occurring Variants," Journal of Biological Chemistry 276(27):24971-24977, American Society for Biochemistry and Molecular Biology, United States (July 2001).	
	NPL39	LAZAR, G.A., et al., "A Molecular Immunology Approach to Antibody Humanization and Functional Optimization," Molecular Immunology 44(8):1986-1998, Pergamon Press, United Kingdom (March 2007).	
	NPL40	LEE, C.M., et al., "Selection of human antibody fragments by phage display," Nat. Protoc. 2(11):3001-3008, Nature Publishing Group, United Kingdom (2007). (Cited by applicant).	
	NPL41	LEFRANC, M.P, "Nomenclature of the Human Immunoglobulin Kappa (Igk) Genes," Experimental and Clinical Immunogenetics 18(3):161-174, Karger, Switzerland (2001).	
	NPL42	LENZ, H., et al., "Expression of Heterobispecific Antibodies by Genes Transfected Into Producer Hybridoma Cells," Gene 87(2):213-218, Elsevier/North-Holland, Netherlands (March 1990).	
	NPL43	LINDHOFER, H., et al., "Preferential Species-Restricted Heavy/Light Chain Pairing In Rat/Mouse Quadromas. Implications for a Single-Step Purification of Bispecific Antibodies," Immunology 155(1):219-225, American Association of Immunologists, United States (July 1995).	

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				Filing Date	*Herewith*
				First Named Inventor	Cornelis Adriaan DE KRUIF
				Art Unit	*To Be Assigned*
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	NPL44	LOBATO, M.N., et al., "Intracellular Antibodies and Challenges Facing Their Use as Therapeutic Agents," Trends in Molecular Medicine 9(9):390-396, Elsevier Science Ltd., United Kingdom (September 2003).	
	NPL45	LOGTENBERG, T, "Antibody Cocktails: Next-generation Biopharmaceuticals With Improved Potency," Trends in Biotechnology 25(9):390-394, Elsevier Science Publishers, Netherlands (September 2007).	
	NPL46	LONBERG, N., "Fully human antibodies from transgenic mouse and phage display platforms, Curr. Opin. Immunol. 20(4):450-459, Elsevier, Netherlands (2008).	
	NPL47	LONBERG, N., "Human Antibodies From Transgenic Animals," Nature Biotechnology, 23(9):1117-1125, Nature America Publishing, United States (September 2005).	
	NPL48	LU, D., et al., "Acquired Antagonistic Activity of a Bispecific Diabody Directed Against Two Different Epitopes on Vascular Endothelial Growth Factor Receptor 2," Journal of Immunological Methods 230(1-2):159-171, Elsevier, Netherlands (November 1999).	
	NPL49	MARVIN, J. S., et al., "Redesigning an Antibody Fragment for Faster Association With Its Antigen," Biochemistry 42(23):7077-7083, American Chemical Society, United States (June 2003).	
	NPL50	MARVIN, J.S. and ZHU, Z., "Recombinant Approaches to IgG-like Bispecific Antibodies," Acta Pharmacologica Sinica 26(6):649-658, Nature Publishing Group, United States (2005).	
	NPL51	MCGINNES, K.," B-lineage colonies from normal, human bone marrow are initiated by B cells and their progenitors," Blood 77(5):961-970, American Society of Hematology, United States (1991).	
	NPL52	MCPHEE, F., et al., "Engineering Human Immunodeficiency Virus 1 Protease Heterodimers as Macromolecular Inhibitors of Viral Maturation," Proceedings of the National Academy of Sciences of the United States of America 93(21):11477-11481, National Academy of Sciences, United States (October 1996).	
	NPL53	MERCHANT, A.M, et al., "An efficient route to human bispecific IgG," Nature Biotechnology, 16(7):677-681, Nature Publishing Group, United States (1998).	
	NPL54	Merus, "Merus presents preclinical data on its novel bispecific antibody MCLA-117 at EHA 2013," www.merus.nl, press release, 3 pages, dated Jun. 17, 2013.	
	NPL55	Merus, "Merus selects clinical candidate for the treatment of acute myeloid leukemia (AML)," www.merus.nl, press release, 2 pages, dated Jan. 7, 2013.	
	NPL56	MILLER, S, "Protein-protein Recognition and the Association of Immunoglobulin Constant Domains," Journal of Molecular Biology 216(4):965-973, Elsevier Ltd., Netherlands (December 1990).	
	NPL57	MIRICK, G.R., et al., "A Review of Human Anti-Globulin Antibody (HAGA, HAMA, HACA, HAHA) Responses To Monoclonal antibodies. Not four letter words," The Quarterly Journal of Nuclear Medicine and Molecular Imaging 48(4):251-257, Minerva Medic, Italy (December 2004).	

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	NPL58	MOHAPATRA, S., et al., "Designer monoclonal antibodies as drugs: the state of the art," Expert Rev. Clin. Immunol. 4(3):305-307, Taylor & Francis, United Kingdom (2008). (Cited by applicant)	
	NPL59	NELSON, A.L., et al., "Development trends for human monoclonal antibody therapeutics," Nat. Rev. Drug Discov. 9(10):767-774, Nature Publishing Group, United Kingdom (2010).	
	NPL60	NEMAZEE, D., "Receptor Editing in B Cells," Advances in immunology 74:89-126, Academic Press, United States (2000).	
	NPL61	NIEBA, L., et al., "Disrupting the Hydrophobic Patches at the Antibody Variable/constant Domain Interface: Improved in Vivo Folding and Physical Characterization of an Engineered Scfv Fragment," Protein Engineering 10(4):435-444, Oxford University Press, United Kingdom (April 1997).	
	NPL62	NOHAILE, M.J., et al., "Altering dimerization specificity by changes in surface electrostatics," Proceedings of the National Academy of Sciences 98(6):3109-3114, National Academy of Sciences, United States (2001).	
	NPL63	Office Action mailed April 6, 2020, in United States Patent Application No. 16/417,379, DE KRUIF; et al., filed May 20, 2019, 5 pages.	
	NPL64	PADLAN, E.A, "X-Ray Crystallography of Antibodies," Advances in Protein Chemistry 49:57-133, Academic Press, United States (1996).	
	NPL65	PAPADEA, C., and CHECK, I.J., "Human Immunoglobulin G and Immunoglobulin G Subclasses: Biochemical, Genetic, and Clinical Aspects," Critical Reviews in Clinical Laboratory Sciences 27(1):27-58, Informa Healthcare, United Kingdom (1989).	
	NPL66	PHELPS, J.L., et al., "Expression and Characterization of a Chimeric Bifunctional Antibody with Therapeutic Applications," The Journal of Immunology 145(4):1200-1204, American Association of Immunologists, United States (1990).	
	NPL67	PRESTA, L.G., "Engineering of Therapeutic Antibodies to Minimize Immunogenicity and Optimize Function," Advanced Drug Delivery Reviews 58(5-6):640-656, Elsevier Science, Netherlands (2006).	
	NPL68	RAFFEN, R., et al., "Reengineering Immunoglobulin Domain Interactions by Introduction of Charged Residues," Protein Engineering 11(4):303-309, Oxford University Press, United Kingdom (April 1998).	
	NPL69	REPP, R., et al., "Phase I Clinical Trial of the Bispecific Antibody MDX-H210 (Anti-FcgammaRI x Anti-HER-2/neu) in Combination With Filgrastim (G-CSF) for Treatment of Advanced Breast Cancer," British Journal of Cancer 89(12):2234-2243, Nature Publishing Group on behalf of Cancer Research UK, United Kingdom (December 2003).	
	NPL70	RETTER, M.W., et al., "Receptor Editing Occurs Frequently during Normal B Cell Development," J. Exp. Med. 188(7):1231-1238, Rockefeller University Press, United States (1998).	

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	NPL72	SAL-MAN, N. and SHAI, Y., "Arginine mutations within a transmembrane domain of Tar, an Escherichia coli aspartate receptor, can drive homodimer dissociation and heterodimer association in vivo," Biochemical Journal 385(Pt1):29-36, Portland Press, United Kingdom (2005).	
	NPL73	SCHAEFER, G., et al., "A Two-in-one Antibody Against HER3 and EGFR Has Superior Inhibitory Activity Compared With Monospecific Antibodies," Cancer Cell 20(4):472-486, Cell Press, United States (October 2011).	
	NPL74	SCHIFFER, M., et al., "Analysis of Immunoglobulin Domain Interactions. Evidence for a Dominant Role of Salt Bridges," Journal of Molecular Biology 203(3):799-802, Elsevier, United Kingdom (October 1988).	
	NPL75	SEGAL, D.M., et al., "Introduction: Bispecific Antibodies," Journal of Immunological Methods 248(1-2):1-6, Elsevier, Netherlands (2001).	
	NPL76	SELZER, T., et al., "Rational Design of Faster Associating and Tighter Binding Protein Complexes," Nature Structural & Molecular Biology 7(7):537-541, Nature Publishing Group, United States (July 2000).	
	NPL77	SHEINERMAN, F.B., et al., "Electrostatic Aspects of Protein-protein Interactions," Current Opinion in Structural Biology 10(2):153-159, Elsevier Science, United Kingdom (April 2000).	
	NPL78	SINHA, N., et al., "Differences in Electrostatic Properties at Antibody-antigen Binding Sites: Implications for Specificity and Cross-reactivity," Biophysical Journal 83(6):2946-2968, Cambridge, United States (December 2002).	
	NPL79	SINHA, N., et al., "Electrostatics in Protein Binding and Function," Current Protein and Peptide Science 3(6):601-614, Bentham Science Publishers, Netherlands (December 2002).	
	NPL80	SMITH, E.J., et al., "A Novel, Native-Format Bispecific Antibody Triggering T-Cell Killing of B-cells is Robustly Active in Mouse Tumor Models and Cynomolgus Monkeys," Scientific Reports 5:17943, Nature Publishing Group, United Kingdom (December 2015).	
	NPL81	STRELKAUSKAS, A.J., et al., "Human Monoclonal Antibody: 2. Simultaneous Expression of IgG and IgM with Similar Binding Specificities by a Human Hybrid Clone," Hybridoma 6(5):479-487, Mary Ann Liebert Inc., United States (1987).	
	NPL82	TADA, H., et al., "Expressionand Characterization of a Chimeric Bispecific Antibody Against Fibrin andAgainst Urokinase-Type Plasminogen Activator," Journal of Biotechnology 33:157-174, Elsevier B.V (March1994).	
	NPL83	VAN RHENEN, A., et al., "The Novel AML Stem Cell Associated Antigen Cll-1 Aids in Discrimination Between Normal and Leukemic Stem Cells," Blood 110(7):2659-2666, American Society of Hematology, United States (October 2007).	

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	NPL84	WEINER, L.M., et al., Abstract, "Fully human therapeutic monoclonal antibodies," Journal of Immunotherapy, 29(1):1-9, Lippincott Williams & Wilkins, Hagerstown, Maryland, United States (January 1, 2006).	
	NPL85	WINTER, G., et al., "Making Antibodies by Phage Display Technology," Annual Review of Immunology 12:433-455, Annual Reviews Inc., United States (1994).	
	NPL86	WU, C., et al., "Simultaneous Targeting of Multiple Disease Mediators by a Dual-variable-domain Immunoglobulin," Nature Biotechnology 25(11):1290-1297, Nature Publishing Co., United States (2007).	
	NPL87	YOO, E.M., et al., Structural requirements for polymeric immunoglobulin assembly and association with J chain," J. Biol. Chem. 274(47):33771-33777, American Society for Biochemistry and Molecular Biology, United States (1999).	
	NPL88	ZHAO, X., et al., "Targeting C-type Lectin-like Molecule-1 for Antibody-mediated Immunotherapy in Acute Myeloid Leukemia," Haematologica 95(1):71-78, Ferrata Storti Foundation, Italy (January 2010).	
	NPL89	ZHU, Z., et al., "Remodeling Domain Interfaces to Enhance Heterodimer Formation," Protein Science 6(4):781-788, Cold Spring Harbor Laboratory Press, United States (April 1997).	
	NPL90	Office Action mailed April 13, 2023, in U.S. Application No. 16/934,925, inventor: De Kruif, C.A., et al., filed July 21, 2020, 8 pages.	
	NPL91	Office Action mailed December 23, 2022, in U.S. Application No. 16/934,925, inventor: De Kruif, C.A., et al., filed July 21, 2020, 12 pages.	

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First Named Inventor	Corelis Adriaan DE KRUIF
Title	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
Art Unit	To Be Assigned
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Attorney Docket Number	4096.0050007

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Signature	/Paul A. Calvo/	Date (Optional)	
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Title (if Applicant is a juristic entity)	Attorney for Applicant		
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Elapsed: 0 hr(s) 0 min(s) 21 sec(s) 486 ms
Total Warnings: 1
Total Errors: 0
No. of SeqIDs Defined: 7
Actual SeqID Count: 7

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
18/318,507	05/16/2023		2240	4096.0050007	9	1

CONFIRMATION NO. 8764

FILING RECEIPT

26111
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 NEW YORK AVENUE, N.W.
WASHINGTON, DC 20005

OC00000005950174

Date Mailed: 07/19/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Cornelis Adriaan DE KRUIF, Utrecht, NETHERLANDS;
Linda Johanna Aleida HENDRIKS, Utrecht, NETHERLANDS;
Ton LOGTENBERG, Utrecht, NETHERLANDS;

Applicant(s)

Merus N.V., Utrecht, NETHERLANDS;

Power of Attorney: The patent practitioners associated with Customer Number 26111

Domestic Priority data as claimed by applicant

This application is a CON of 16/934,925 07/21/2020
which is a CON of 16/417,379 05/20/2019 PAT 10,752,929
which is a CON of 15/155,743 05/16/2016 PAT 10,329,596
which is a CON of 14/081,848 11/15/2013 PAT 9,358,286
which is a CON of 13/866,747 04/19/2013 PAT 9,248,181
which claims benefit of 61/635,935 04/20/2012

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/17/2023

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/318,507**

Projected Publication Date: 10/26/2023

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

26111 7590 08/07/2023

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

EXAMINER
CENTRAL, DOCKET

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
08/07/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

e-office@sternekessler.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 18/318,507	**Applicant(s)** DE KRUIF et al.	
	Examiner FIKIRTE A GEREMEW	**Art Unit** OMBL	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 16 May 2023 IS **GRANTED** .

   The above-identified application has met the requirements for prioritized examination
   - A. ☑ for an original nonprovisional application (Track I).
   - B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:
   - A. filing a **petition for extension of time** to extend the time period for filing a reply;
   - B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;
   - C. filing a **request for continued examination** ;
   - D. filing a notice of appeal;
   - E. filing a request for suspension of action;
   - F. mailing of a notice of allowance;
   - G. mailing of a final Office action;
   - H. completion of examination as defined in 37 CFR 41.102; or
   - I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to FIKIRTE GEREMEW at (703) 756-1930. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/FIKIRTE A GEREMEW/ PROGRAM SUPPORT ASSISTANT, OMBL	

U.S. Patent and Trademark Office
PTO-2298 (Rev. 02-2012)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

26111 7590 08/31/2023
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

EXAMINER
BURKHART, MICHAEL D

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
08/31/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

e-office@sternekessler.com

Office Action Summary	**Application No.** 18/318,507	**Applicant(s)** DE KRUIF et al.	
	Examiner MICHAEL D BURKHART	**Art Unit** 1633	**AIA (FITF) Status** Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 29-37 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 29-37 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 5/16/23.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Double Patenting

Claim 29 of this application is patentably indistinct from claim 53 of Application No. 16/934,925. Pursuant to 37 CFR 1.78(f), when two or more applications filed by the same applicant or assignee contain patentably indistinct claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the patentably indistinct claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822. Claim 53 of the '925 application recites heterodimeric, human antibodies (in a product-by-process-format) with CH3 domains having the same amino acid residue changes at the same positions as the instant claims. Thus, the claims at issue do not differ in scope.

Claim 34 of this application is patentably indistinct from claim 54 of Application No. 16/934,925. Pursuant to 37 CFR 1.78(f), when two or more applications filed by the same applicant or assignee contain patentably indistinct claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the

patentably indistinct claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822. Claim 54 of the '925 application is dependent upon claim 53, which recites the same antibodies as instant claim 29 for reasons above. Further, claim 54 recites that the antibodies bind two different epitopes or antigens, which is no different than the "bispecific" antibodies of instant claim 34 because this is the meaning of the term "bispecific" in the antibody art, i.e. an antibody that binds two distinct epitopes or antigens.

Claim 35 of this application is patentably indistinct from claim 55 of Application No. 16/934,925. Pursuant to 37 CFR 1.78(f), when two or more applications filed by the same applicant or assignee contain patentably indistinct claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the patentably indistinct claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822. Claim 55 of the '925 application is dependent upon claim 53, which recites the same antibodies as instant claim 29 for reasons above. Further, claim 55 recites the antibodies are human IgG, the same as instant claim 35.

Claim 37 of this application is patentably indistinct from claim 56 of Application No. 16/934,925. Pursuant to 37 CFR 1.78(f), when two or more applications filed by the same applicant or assignee contain patentably indistinct claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the patentably indistinct claims from all but one application or maintain a clear line of demarcation

between the applications. See MPEP § 822. Claim 56 of the '925 application is dependent upon claim 53, which recites the same antibodies as instant claim 29 for reasons above. Further, claim 56 and instant claim 37 are copies of each other.

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process... may obtain a patent therefor..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the claims that are directed to the same invention so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Applicant is advised that should claim 29 be found allowable, claim 32 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 608.01(m). Claim 32 merely recites an inherent property (interaction of the residues) of the CH3 domains of claim 29. Such inherency is set forth, e.g., in Table A of the specification. Thus, the claims do not differ in scope in any meaningful way.

Applicant is advised that should claim 29 be found allowable, claim 33 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 608.01(m). Claim 33 merely recites a property (two heavy chains) of the antibodies of claim 29. The antibodies or antibody molecules of the specification and relevant art that comprise CH3 domains all have two distinct heavy chains. This is illustrated in, e.g., Fig. 5A of the instant specification. Typical single-chain scFv molecules does not comprise CH3 domains, rather, they comprise two variable regions linked via a peptide linker. So-called "scFv-CH3", "scFv-Fc", "minibodies", natural IgG and the molecules of the specification that do comprise CH3 domains all comprise two heavy chains not linked by a peptide linker.

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van*

Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The filing of a terminal disclaimer by itself is not a complete reply to a nonstatutory double patenting (NSDP) rejection. A complete reply requires that the terminal disclaimer be accompanied by a reply requesting reconsideration of the prior Office action. Even where the NSDP rejection is provisional the reply must be complete. See MPEP § 804, subsection I.B.1. For a reply to a non-final Office action, see 37 CFR 1.111(a). For a reply to final Office action, see 37 CFR 1.113(c). A request for reconsideration while not provided for in 37 CFR 1.113(c) may be filed after final for consideration. See MPEP §§ 706.07(e) and 714.13.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The actual filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-

processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/apply/applying-online/eterminal-disclaimer.

Claims 29-37 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 5, 6, 8-12, 21-25, 28, 29, 32 of U.S. Patent No.9,358,286. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant compositions are produced by, or are found within the compositions of, the '286 claims. The '286 claims recite methods of producing antibodies, and antibodies produced, wherein the CH3 domains comprise substitutions of neutral amino acids with a positively charged amino acid and a negatively charged amino acid, e.g. claim 1. One such neutral to negative change is 368 to D or E (claim 5, position 368 is inherently a leucine). Looking to the specification for a definition of the claimed neutral amino acid to positive amino acid change, the '286 patent teaches S364K as one such specific substitution (Table A, Table 7).

Claims 29-37 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 7, 10-12, 17-20, 32-34 of U.S. Patent No.10,329,596. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant compositions are produced by, or are found within the compositions of, the '596 claims. The '596 claims recite antibodies wherein the CH3 domains comprise substitutions of neutral amino acids with a positively charged amino acid and a negatively charged amino acid, e.g. claim 1. One such neutral to negative change is 368 to D or E (claim 7, position 368 is inherently a leucine). Looking to the specification for a definition of the claimed neutral amino

acid to positive amino acid change, the '596 patent teaches S364K as one such specific substitution (Table A, Table 7).

Claims 29-37 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 5, 6, 9-15, 28-31, 35, 40-43 of U.S. Patent No. 9,758,805. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant compositions are produced by, or are found within the compositions of, the '805 claims. The '805 claims recite antibodies wherein the CH3 domains comprise substitutions of neutral amino acids with a positively charged amino acid and a negatively charged amino acid, e.g. claim 1. One such neutral to negative change is 368 to D or E (claim 5, position 368 is inherently a leucine). Looking to the specification for a definition of the claimed neutral amino acid to positive amino acid change, the '805 patent teaches S364K as one such specific substitution (Table A, Table 7).

Claims 29-37 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4, 5, 11-18, 53-56 of U.S. Patent No.10,337,045. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant compositions are produced by, or are found within the compositions of, the '045 claims. The '045 claims recite antibodies wherein the CH3 domains comprise substitutions of neutral amino acids with a positively charged amino acid and a negatively charged amino acid, e.g. claim 1. One such neutral to negative change is 368 to D or E (claim 4, position 368 is inherently a leucine). Looking to the specification for a definition of the claimed neutral amino

acid to positive amino acid change, the '045 patent teaches S364K as one such specific substitution (Table A, Table 7).

Claims 29-37 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 29, 31-34, 51-58 of copending Application No. 16/934,925 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the '925 claims recite methods and antibodies comprising the same substitutions (positively or negatively charged residues) at the same CH3 positions (364, 368) as the instant claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MICHAEL D BURKHART whose telephone number is (571)272-2915. The examiner can normally be reached M-F 8-5.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on 571 272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more

information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MICHAEL D BURKHART/
Primary Examiner, Art Unit 1633

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

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Substitute for form 1449/PTO				***Complete if Known***	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT *(Use as many sheets as necessary)*				Application Number	*To Be Assigned*
				Filing Date	*Herewith*
				First Named Inventor	Cornelis Adriaan DE KRUIF
				Art Unit	*To Be Assigned*
				Examiner Name	*To Be Assigned*
Sheet	1	of	17	Attorney Docket Number	4096.0050007

U.S. PATENT DOCUMENTS

Examiner initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	US1	US-4399216-A	08-16-1983	AXEL; Richard et al.	
	US2	US-4599311-A	07-08-1986	KAWASAKI; Glenn H.	
	US3	US-4634665-A	01-06-1987	AXEL; Richard et al.	
	US4	US-4801687-A	01-31-1989	NGO; That T.	
	US5	US-4816567-A	03-28-1989	CABILLY et al.	
	US6	US-4868103-A	09-19-1989	STAVRIANOPOULOS; Jannis et al.	
	US7	US-4937190-A	06-26-1990	PALMENBERG; Ann C. et al.	
	US8	US-5030002-A	07-09-1991	NORTH Jr. HOWARD L;	
	US9	US-5137809-A	08-11-1992	LOKEN; Michael R. et al.	
	US10	US-5151504-A	09-29-1992	CROZE; Edward M.	
	US11	US-5179017-A	01-12-1993	AXEL; Richard et al.	
	US12	US-5223409-A	06-29-1993	LADNER; Robert C. et al.	
	US13	US-5385839-A	01-31-1995	STINSKI; Mark F.	
	US14	US-5627037-A	05-06-1997	WARD; Anthony J. et al.	
	US15	US-5631169-A	05-20-1997	LAKOWICZ; Joseph R. et al.	
	US16	US-5641640-A	06-24-1997	HANNING; Anders	
	US17	US-5667988-A	09-16-1997	BARBAS; Carlos F. et al.	
	US18	US-5731168-A	03-24-1998	CARTER; Paul J. et al.	
	US19	US-5733779-A	03-31-1998	REFF; Mitchell E.	
	US20	US-5770429-A	06-23-1998	LONBERG et al.	
	US21	US-5772997-A	06-30-1998	HUDZIAK; Robert M. et al.	
	US22	US-5783186-A	07-21-1998	ARAKAWA; Tsutomu et al.	
	US23	US-5789208-A	08-04-1998	SHARON; Jacqueline	
	US24	US-5834237-A	11-10-1998	JACOBS; Eric et al.	
	US25	US-5885827-A	03-23-1999	WABL; Matthias et al.	
	US26	US-5888789-A	03-30-1999	RODRIGUEZ; Raymond L.	
	US27	US-5965371-A	10-12-1999	MARASCO; Wayne A. et al.	
	US28	US-6004940-A	12-21-1999	MARASCO; Wayne A. et al.	
	US29	US-6054297-A	04-25-2000	CARTER; Paul J. et al.	
	US30	US-6080560-A	06-27-2000	RUSSELL; David R. et al.	
	US31	US-6114598-A	09-05-2000	KUCHERLAPATI; Raju et al.	

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	08/26/2023

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				Art Unit	*To Be Assigned*
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Sheet	2	of	17	Attorney Docket Number	4096.0050007

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	US32	US-6180357-B1	01-30-2001	YOUNG; David S. F. et al.	
	US33	US-6207446-B1	03-27-2001	SZOSTAK; Jack W. et al.	
	US34	US-6265150-B1	07-24-2001	TERSTAPPEN; Leon WMM et al.	
	US35	US-6291740-B1	09-18-2001	BREMEL; Robert D. et al.	
	US36	US-6303341-B1	10-16-2001	HIATT; Andrew C. et al.	
	US37	US-6335163-B1	01-01-2002	SHARON; Jacqueline	
	US38	US-6596541-B2	07-22-2003	MURPHY; Andrew J. et al.	
	US39	US-7067284-B1	06-27-2006	BARBAS; Carlos F. et al.	
	US40	US-7105348-B2	09-12-2006	MURPHY; Andrew J. et al.	
	US41	US-7183076-B2	02-27-2007	ARATHOON; W. Robert et al.	
	US42	US-7262028-B2	08-28-2007	VAN BERKEL; Patrick Hendrikus Cornelis et al.	
	US43	US-7329530-B2	02-12-2008	HOUTZAGER; Erwin et al.	
	US44	US-7429486-B2	09-30-2008	VAN BERKEL; Patrick H. C. et al.	
	US45	US-7491516-B2	02-17-2009	COLLINSON; Albert et al.	
	US46	US-7579446-B2	08-25-2009	BAKKER; Alexander B. H. et al.	
	US47	US-7642228-B2	01-05-2010	CARTER; Paul J. et al.	
	US48	US-7696330-B2	04-13-2010	MEULEN; Jan H. Ter et al.	
	US49	US-7740852-B2	06-22-2010	BAKKER; Alexander Berthold Hendrik et al.	
	US50	US-7858086-B2	12-28-2010	GEUIJEN; Cecilia Anna Wilhelmina et al.	
	US51	US-7901919-B2	03-08-2011	HOUTZAGER; Erwin et al.	
	US52	US-7919257-B2	04-05-2011	MATTHEUS; Hoogenboom Hendricus R.J et al.	
	US53	US-7927834-B2	04-19-2011	VAN BERKEL; Patrick H. C. et al.	
	US54	US-7932360-B2	04-26-2011	VAN BERKEL; Patrick H. C. et al.	
	US55	US-7960518-B2	06-14-2011	THROSBY; Mark et al.	
	US56	US-7968092-B2	06-28-2011	THROSBY; Mark et al.	
	US57	US-8052974-B2	11-08-2011	THROSBY; Mark et al.	
	US58	US-8106170-B2	01-31-2012	TER; Meulen JAN H. et al.	
	US59	US-8148497-B2	04-03-2012	BAKKER; Alexander Berthold Hendrik et al.	

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	08/26/2023

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Sheet 3 of 17

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Examiner Name	*To Be Assigned*
Attorney Docket Number	4096.0050007

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	US60	US-8192927-B2	06-05-2012	VAN; DEN Brink Edward Norbert et al.	
	US61	US-8211431-B2	07-03-2012	THROSBY; Mark et al.	
	US62	US-8241631-B2	08-14-2012	THROSBY; Mark et al.	
	US63	US-8268756-B2	09-18-2012	LOGTENBERG; TON et al.	
	US64	US-8470327-B2	06-25-2013	THROSBY; Mark et al.	
	US65	US-8592562-B2	11-26-2013	KANNAN; Gunasekaran et al.	
	US66	US-8911738-B2	12-16-2014	THROSBY; Mark et al.	
	US67	US-9012371-B2	04-21-2015	LOGTENBERG; Ton et al.	
	US68	US-9145588-B2	09-29-2015	THROSBY; Mark et al.	
	US69	US-9248181-B2	02-02-2016	DE KRUIF; Cornelis A. et al.	
	US70	US-9248182-B2	02-02-2016	DE KRUIF; Cornelis A. et al.	
	US71	US-9358286-B2	06-07-2016	DE KRUIF; Cornelis A. et al.	
	US72	US-9758805-B2	09-12-2017	DE KRUIF; Cornelis Adriaan et al.	
	US73	US-10752929-B2	08-25-2020	DE KRUIF; Cornelis Adriaan et al.	
	US74	US-20020088016-A1	07-04-2002	BRUGGEMANN; Marianne	
	US75	US-20020138857-A1	09-26-2002	GHAYUR; Tariq	
	US76	US-20030039958-A1	02-27-2003	HOLT; Lucy Jessica et al.	
	US77	US-20030077739-A1	04-24-2003	SIMMONS; Laura et al.	
	US78	US-20030078385-A1	04-24-2003	ARATHOON; W. Robert et al.	
	US79	US-20030091561-A1	05-15-2003	VAN DE WINKEL; Jan G.J. et al.	
	US80	US-20030093820-A1	05-15-2003	GREEN; Larry L. et al.	
	US81	US-20030096225-A1	05-22-2003	LOGTENBERG; Ton	
	US82	US-20030194403-A1	10-16-2003	VAN DE WINKEL; Jan et al.	
	US83	US-20030207346-A1	11-06-2003	ARATHOON; William R. et al.	
	US84	US-20030219829-A1	11-27-2003	LOGTENBERG; TON et al.	
	US85	US-20030224408-A1	12-04-2003	HOOGENBOOM; Henricus Renerus Jacobus Mattheus et al.	
	US86	US-20050014261-A1	01-20-2005	HOUTZAGER; Erwin et al.	
	US87	US-20050037001-A1	02-17-2005	GERMERAAD; Wilfred Thomas Vincent et al.	
	US88	US-20050170398-A1	08-04-2005	VAN BERKEL; Patrick Hendrikus Cornelis et al.	

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	08/26/2023

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				Art Unit	*To Be Assigned*
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U.S. PATENT DOCUMENTS

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	US89	US-20060015949-A1	01-19-2006	LONBERG; Nils et al.	
	US90	US-20060015957-A1	01-19-2006	LONBERG; Nils et al.	
	US91	US-20060088520-A1	04-27-2006	GERMERAAD; Wilfred T.V et al.	
	US92	US-20060117699-A1	06-08-2006	DI; Trapani Agostino	
	US93	US-20060160184-A1	07-20-2006	MATTHEUS HOOGENBOOM; Hendricus Renerus Jacobus et al.	
	US94	US-20060205077-A1	09-14-2006	SCHWENK; Frieder et al.	
	US95	US-20060257397-A1	11-16-2006	THROSBY; Mark et al.	
	US96	US-20070054362-A1	03-08-2007	VAN BERKEL; Patrick H. C. et al.	
	US97	US-20070059766-A1	03-15-2007	LOGTENBERG; Ton et al.	
	US98	US-20070280945-A1	12-06-2007	STEVENS; Sean et al.	
	US99	US-20080070799-A1	03-20-2008	BAKKER; Alexander Berthold Hendrik et al.	
	US100	US-20080241166-A1	10-02-2008	TOMLINSON; Ian M. et al.	
	US101	US-20090017521-A1	01-15-2009	HOUTZAGER; Erwin et al.	
	US102	US-20090054254-A1	02-26-2009	THROSBY; Mark et al.	
	US103	US-20090074765-A1	03-19-2009	CARMELIET; Peter et al.	
	US104	US-20090092582-A1	04-09-2009	BOGIN; Oren et al.	
	US105	US-20090130652-A1	05-21-2009	THROSBY; Mark et al.	
	US106	US-20090181855-A1	07-16-2009	VASQUEZ; Maximiliano et al.	
	US107	US-20090182127-A1	07-16-2009	KJAERGAARD; Kristian et al.	
	US108	US-20090263864-A1	10-22-2009	VAN BERKEL; Patrick H. C. et al.	
	US109	US-20100015133-A1	01-21-2010	IGAWA; Tomoyuki et al.	
	US110	US-20100069614-A1	03-18-2010	HOUTZAGER; Erwin et al.	
	US111	US-20100146647-A1	06-10-2010	LOGTENBERG; Ton et al.	
	US112	US-20100172917-A1	07-08-2010	TER; Meulen JAN H. et al.	
	US113	US-20100286374-A1	11-11-2010	KANNAN; Gunasekaran et al.	
	US114	US-20100297153-A1	11-25-2010	GEUIJEN; Cecilia A.W et al.	
	US115	US-20100310572-A1	12-09-2010	BAKKER; Alexander Berthold Hendrik et al.	
	US116	US-20100310586-A1	12-09-2010	DOLCETTI; Riccardo et al.	
	US117	US-20100331527-A1	12-30-2010	DAVIS; Samuel et al.	
	US118	US-20110054141-A1	03-03-2011	BEST; Kurt E. et al.	

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	US119	US-20110177073-A1	07-21-2011	VAN; Berkel Patrick H. C. et al.	
	US120	US-20110195454-A1	08-11-2011	MCWHIRTER; John et al.	
	US121	US-20110268739-A1	11-03-2011	THROSBY; Mark et al.	
	US122	US-20120021409-A1	01-26-2012	MCWHIRTER; John et al.	
	US123	US-20120039898-A1	02-16-2012	THROSBY; Mark et al.	
	US124	US-20120058907-A1	03-08-2012	LOGTENBERG; Ton et al.	
	US125	US-20120076794-A1	03-29-2012	THROSBY; Mark et al.	
	US126	US-20120093823-A1	04-19-2012	VAN DEN BRINK; Edward Norbert et al.	
	US127	US-20120141493-A1	06-07-2012	THROSBY; Mark et al.	
	US128	US-20120177637-A1	07-12-2012	HOOGENBOOM; Hendricus Renerus Jacobus Mattheus et al.	
	US129	US-20120192300-A1	07-26-2012	BABB; Robert et al.	
	US130	US-20120276115-A1	11-01-2012	VAN DEN BRINK; Edward Norbert et al.	
	US131	US-20120315278-A1	12-13-2012	THROSBY; Mark et al.	
	US132	US-20120321950-A1	12-20-2012	OKUMURA; Takefumi et al.	
	US133	US-20130096020-A1	04-18-2013	THROSBY; Mark et al.	
	US134	US-20130115208-A1	05-09-2013	HO; Wei-Hsien et al.	
	US135	US-20130145484-A1	06-06-2013	LOGTENBERG; Ton et al.	
	US136	US-20130336981-A1	12-19-2013	DE KRUIF; Cornelis A. et al.	
	US137	US-20140072579-A1	03-13-2014	DE KRUIF; Cornelis Adriaan et al.	
	US138	US-20140120096-A1	05-01-2014	BAKKER; Alexander Berthold Hendrik et al.	
	US139	US-20140140999-A1	05-22-2014	DE KRUIF; Cornelis A. et al.	
	US140	US-20140314755-A1	10-23-2014	LOGTENBERG; Ton et al.	
	US141	US-20140317766-A1	10-23-2014	LOGTENBERG; Ton et al.	
	US142	US-20150139996-A1	05-21-2015	DE KRUIF; Cornelis Adriaan et al.	
	US143	US-20150196637-A1	07-16-2015	DE KRUIF; Cornelis Adriaan et al.	
	US144	US-20160130367-A1	05-12-2016	THROSBY; Mark et al.	
	US145	US-20160177364-A1	06-23-2016	DE KRUIF; Cornelis Adriaan et al.	
	US146	US-20160238600-A1	08-18-2016	HOOGENBOOM; Hendricus Renerus et al.	

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	US147	US-20160368988-A1	12-22-2016	BAKKER; Alexander Berthold Hendrik et al.	
	US148	US-20170369923-A1	12-28-2017	DE KRUIF; Cornelis Adriaan et al.	
	US149	US-20200017897-A1	01-16-2020	DE KRUIF; Cornelis Adriaan et al.	
	US150	US-10329596-B2	06-25-2019	DE KRUIF; Cornelis Adriaan et al	

FOREIGN PATENT DOCUMENTS

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	FP1	CA-1341364-C	06-04-2002	GENEX CORP [US]		
	FP2	CA-2114353-A1	08-02-1994	SQUIBB BRISTOL MYERS CO [US]		
	FP3	CA-2137558-A1	02-03-1994	DANA FARBER CANCER INST INC [US]		
	FP4	CA-2405961-A1	11-01-2001	ELUSYS THERAPEUTICS INC [US]		
	FP5	CA-2445255-A1	10-31-2002	UNIV ERASMUS [NL]		
	FP6	EP-0120694-A2	10-03-1984	CELLTECH LTD [GB]		
	FP7	EP-0171142-A1	02-12-1986	ZYMOGENETICS INC [US]		
	FP8	EP-0307667-A2	03-22-1989	WACOM CO LTD [JP]		
	FP9	EP-0314161-A1	05-03-1989	BRISTOL MYERS CO [US]		
	FP10	EP-0402029-A2	12-12-1990	WISTAR INST [US]		
	FP11	EP-0445625-A1	09-11-1991	BEHRINGWERKE AG [DE]		X
	FP12	EP-0469025-A1	02-05-1992	MEDICAL RES COUNCIL [GB]		
	FP13	EP-0469897-A2	02-05-1992	WISTAR INST [US]		
	FP14	EP-0481790-A2	04-22-1992	WELLCOME FOUND [GB]		
	FP15	EP-0523949-A1	01-20-1993	WELLCOME FOUND [GB]		
	FP16	EP-0666868-A1	08-16-1995	GENENTECH INC [US]		
	FP17	EP-0724639-A1	08-07-1996	GENENTECH INC [US]		
	FP18	EP-0814159-A2	12-29-1997	GENPHARM INT [US]		
	FP19	EP-1325932-A2	07-09-2003	GENENTECH INC [US]		
	FP20	EP-1349234-A2	10-01-2003	THALES PLC [GB]		
	FP21	EP-1439234-A1	07-21-2004	ARTEMIS PHARMACEUTICALS GMBH [DE]		

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	FP22	EP-1870459-A1	12-26-2007	CHUGAI PHARMACEUTICAL CO LTD [JP]		
	FP23	EP-2147594-A1	01-27-2010	MERUS B V [NL]		
	FP24	JP-2006109711-A	04-27-2006	UNIV OKAYAMA [JP]		X
	FP25	JP-H08116978-A	05-14-1996	NISSHIN SPINNING [JP]		X
	FP26	WO-0044777-A1	08-03-2000	IMCLONE SYSTEMS INC [US]		
	FP27	WO-0063403-A2	10-26-2000	INTROGENE BV [NL]		
	FP28	WO-0070023-A1	11-23-2000	DYAX CORP [US]		
	FP29	WO-0071694-A1	11-30-2000	SCRIPPS RESEARCH INST [US]		
	FP30	WO-0076310-A1	12-21-2000	ABGENIX INC [US]		
	FP31	WO-0100245-A2	01-04-2001	GENENTECH INC [US]		
	FP32	WO-0119394-A2	03-22-2001	THERAPEUTIC HUMAN POLYCLONALS [US]		
	FP33	WO-0127279-A1	04-19-2001	CAMBRIDGE ANTIBODY TECH [GB]		
	FP34	WO-0132901-A1	05-10-2001	CHIRON CORP [US]		
	FP35	WO-0148485-A2	07-05-2001	CRUCELL HOLLAND BV [NL]		
	FP36	WO-0164929-A1	09-07-2001	UNIV AUBURN [US]		
	FP37	WO-0188132-A2	11-22-2001	UNIV JEFFERSON [US]		
	FP38	WO-02059297-A2	08-01-2002	EVOLVA BIOTECH AS [DK]		
	FP39	WO-02066630-A1	08-29-2002	REGENERON PHARMA [US]		
	FP40	WO-02074969-A2	09-26-2002	UNIV LAUSANNE [CH]		
	FP41	WO-02096948-A2	12-05-2002	IDEC PHARMA CORP [US]		
	FP42	WO-0218948-A2	03-07-2002	CRUCELL HOLLAND BV [NL]		
	FP43	WO-0243478-A2	06-06-2002	MEDAREX INC [US]		
	FP44	WO-0246233-A1	06-13-2002	TECHNOPHARM [FR]		X
	FP45	WO-03002609-A2	01-09-2003	MEDICAL RES COUNCIL [GB]		
	FP46	WO-03004704-A2	01-16-2003	CHROMAGENICS BV [NL]		
	FP47	WO-03016501-A2	02-27-2003	UNIV JEFFERSON [US]		
	FP48	WO-03033670-A2	04-24-2003	SLOAN KETTERING INST CANCER [US]		
	FP49	WO-03046560-A2	06-05-2003	CA NAT RESEARCH COUNCIL [CA]		
	FP50	WO-03048306-A2	06-12-2003	IDEC PHARMA CORP [US]		
	FP51	WO-03052416-A2	06-26-2003	NEUTEC PHARMA PLC [GB]		

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	FP52	WO-03102157-A2	12-11-2003	GENENTECH INC [US]		
	FP53	WO-03106674-A2	12-24-2003	CHROMAGENICS BV [NL]		
	FP54	WO-03106684-A2	12-24-2003	CHROMAGENICS BV [NL]		
	FP55	WO-03107218-A1	12-24-2003	CELESTAR LEXICO SCIENCES INC [JP]		X
	FP56	WO-2004003211-A1	01-08-2004	IMCLONE SYSTEMS INC [US]		
	FP57	WO-2004009618-A2	01-29-2004	CRUCELL HOLLAND BV [NL]		
	FP58	WO-2004061104-A2	07-22-2004	SYMPHOGEN AS [DK]		
	FP59	WO-2004106375-A1	12-09-2004	MERUS BIOPHARMACEUTICALS B V I [NL]		
	FP60	WO-2005000894-A2	01-06-2005	CRUCELL HOLLAND BV [NL]		
	FP61	WO-2005068622-A2	07-28-2005	MERUS B V [NL]		
	FP62	WO-2005118635-A2	12-15-2005	NOVIMMUNE SA [CH]		
	FP63	WO-2006028936-A2	03-16-2006	GENENTECH INC [US]		
	FP64	WO-2006106905-A1	10-12-2006	CHUGAI PHARMACEUTICAL CO LTD [JP]		X
	FP65	WO-2006117699-A2	11-09-2006	INNATE PHARMA SA [FR]		
	FP66	WO-2007059782-A1	05-31-2007	GENMAB AS [DK]		
	FP67	WO-2007110205-A2	10-04-2007	MERCK PATENT GMBH [DE]		
	FP68	WO-2007117410-A2	10-18-2007	MEDAREX INC [US]		
	FP69	WO-2007147901-A1	12-27-2007	NOVO NORDISK AS [DK]		
	FP70	WO-2008027236-A2	03-06-2008	GENENTECH INC [US]		
	FP71	WO-2008054606-A2	05-08-2008	REGENERON PHARMA [US]		
	FP72	WO-2008076379-A2	06-26-2008	REGENERON PHARMA [US]		
	FP73	WO-2008119353-A1	10-09-2008	GENMAB AS [DK]		
	FP74	WO-2009051974-A1	04-23-2009	NUVELO INC [US]		
	FP75	WO-2009080251-A1	07-02-2009	HOFFMANN LA ROCHE [CH]		
	FP76	WO-2009080253-A1	07-02-2009	HOFFMANN LA ROCHE [CH]		
	FP77	WO-2009089004-A1	07-16-2009	AMGEN INC [US]		
	FP78	WO-2009098596-A2	08-13-2009	ZYMEWORKS INC. [CA]		
	FP79	WO-2009157771-A2	12-30-2009	MERUS B V [NL]		
	FP80	WO-2010084197-A1	07-29-2010	GENMAB AS [DK		
	FP81	WO-2010108127-A1	09-23-2010	GENENTECH INC [US]		

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				First Named Inventor	Cornelis Adriaan DE KRUIF
				Art Unit	*To Be Assigned*
				Examiner Name	*To Be Assigned*
Sheet	9	of	17	Attorney Docket Number	4096.0050007

FOREIGN PATENT DOCUMENTS

Examiner initials*	Cite No.[1]	Foreign Patent Document: Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	FP82	WO-2010129304-A2	11-11-2010	ONCOMED PHARM INC [US]		
	FP83	WO-2011028952-A1	03-10-2011	XENCOR INC [US]		
	FP84	WO-2011028953-A1	03-10-2011	AMFIT INC [US]		
	FP85	WO-2011063348-A1	05-26-2011	AMGEN INC [US]		
	FP86	WO-2011097603-A1	08-11-2011	REGENERON PHARMA [US]		
	FP87	WO-2011143545-A1	11-17-2011	RINAT NEUROSCIENCE CORP [US]		
	FP88	WO-2012020096-A1	02-16-2012	MEDIMMUNE LTD [GB]		
	FP89	WO-2012023053-A2	02-23-2012	NOVIMMUNE SA [CH		
	FP90	WO-2012058768-A1	05-10-2012	ZYMEWORKS INC [CA]		
	FP91	WO-2012131555-A2	10-04-2012	GLENMARK PHARMACEUTICALS SA [CH		
	FP92	WO-2012141798-A1	10-18-2012	REGENERON PHARMA [US]		
	FP93	WO-2012148873-A2	11-01-2012	REGENERON PHARMA [US]		
	FP94	WO-2013134263-A1	09-12-2013	REGENERON PHARMA [US]		
	FP95	WO-2013157953-A1	10-24-2013	MERUS B V [NL]		
	FP96	WO-2013157954-A1	10-24-2013	MERUS B V [NL]		
	FP97	WO-2013184761-A1	12-12-2013	REGENERON PHARMA [US]		
	FP98	WO-2014151433-A1	09-25-2014	ALTRIA CLIENT SERVICES INC [US]		
	FP99	WO-9002809-A1	03-22-1990	PROTEIN ENG CORP [US]		
	FP100	WO-9012878-A1	11-01-1990	MEDICAL RES COUNCIL [GB]		
	FP101	WO-9100906-A1	01-24-1991	GENETICS INST [US]		
	FP102	WO-9108216-A1	06-13-1991	GENPHARM INT [US]		
	FP103	WO-9117271-A1	11-14-1991	AFFYMAX TECH NV [NL]		
	FP104	WO-9201047-A1	01-23-1992	CAMBRIDGE ANTIBODY TECH [GB]		
	FP105	WO-9203918-A1	03-19-1992	GENPHARM INT [US]		
	FP106	WO-9209690-A2	06-11-1992	GENENTECH INC [US]		
	FP107	WO-9215679-A1	09-17-1992	PROTEIN ENG CORP [US]		
	FP108	WO-9218619-A1	10-29-1992	SCRIPPS RESEARCH INST [US]		
	FP109	WO-9220791-A1	11-26-1992	CAMBRIDGE ANTIBODY TECH [GB]		
	FP110	WO-9301288-A1	01-21-1993	DEUTSCHES KREBSFORSCH [DE]		X
	FP111	WO-9312227-A1	06-24-1993	GENPHARM INT [US]		

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	FP112	WO-9402602-A1	02-03-1994	CELL GENESYS INC [US]		
	FP113	WO-9402610-A1	02-03-1994	DANA FARBER CANCER INST INC [US]		
	FP114	WO-9423046-A1	10-13-1994	US HEALTH [US]		
	FP115	WO-9425591-A1	11-10-1994	UNILEVER PLC [GB]		
	FP116	WO-9517085-A1	06-29-1995	GENZYME TRANSGENICS CORP [US]		
	FP117	WO-9520401-A1	08-03-1995	UNIV BOSTON [US]		
	FP118	WO-9627011-A1	09-06-1996	GENENTECH INC [US]		
	FP119	WO-9742313-A1	11-13-1997	SCRIPPS RESEARCH INST [US]		
	FP120	WO-9747739-A1	12-18-1997	UNIV MICHIGAN STATE [US]		
	FP121	WO-9815627-A1	04-16-1998	UNIV SOUTHAMPTON [GB]		
	FP122	WO-9815833-A1	04-16-1998	UNIV UTRECHT [NL]		
	FP123	WO-9824923-A1	06-11-1998	UNIV WAKE FOREST [US]		
	FP124	WO-9839416-A1	09-11-1998	INFIGEN INC [US]		
	FP125	WO-9841645-A1	09-24-1998	IDEC PHARMA CORP [US]		
	FP126	WO-9850431-A2	11-12-1998	GENENTECH INC [US]		
	FP127	WO-9852976-A1	11-26-1998	BIOVATION LTD [GB]		
	FP128	WO-9915684-A2	04-01-1999	OXFORD BIOMEDICA LTD [GB]		
	FP129	WO-9920749-A1	04-29-1999	MEDICAL RES COUNCIL [GB]		
	FP130	WO-9923221-A2	05-14-1999	UNILEVER PLC [GB]		
	FP131	WO-9926569-A1	06-03-1999	MITSUBISHI PRECISION CO LTD [JP]		X
	FP132	WO-9936569-A1	07-22-1999	UNIV ILLINOIS [US]		
	FP133	WO-9945962-A1	09-16-1999	GENPHARM INT [US]		
	FP134	WO-9950657-A1	10-07-1999	RES DEV FOUNDATION [US]		
	FP135	WO-9964582-A2	12-16-1999	INTROGENE BV [NL]		

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	NPL1	ALLEN, T.M., "Ligand-Targeted Therapeutics in Anticancer Therapy," Nature Reviews Cancer 2(10):750-763, Nature Publishing Group, United Kingdom (October 2002).	
	NPL2	ATWELL, S., et al., "Stable Heterodimers from Remodeling the Domain Interface of a Homodimer Using a Phage Display Library," Journal of Molecular Biology 270(1):26-35, Elsevier, United Kingdom (1997).	
	NPL3	BAEUERLE, P., et al., "Bispecific T-Cell Engaging Antibodies for Cancer Therapy," Cancer Research 69(12):4941-4944, American Association for Cancer Research, United States (2009).	
	NPL4	BOGAN, A., et. al., "Anatomy of Hot Spots in Protein Interfaces," Journal of Molecular Biology 280(1):1-9, Elsevier, Netherlands (July 1998).	
	NPL5	CARTER, P., "Bispecific Human IgG by Design," Journal of Immunological Methods 248(1-2):7-15, Elsevier, Netherlands (2001).	
	NPL6	CARTER, P., et al., "Toward the Production of Bispecific Antibody Fragments for Clinical Applications," Journal of Hematotherapy 4:463-470, Mary Ann Liebert, Inc., United States (1995).	
	NPL7	CARTER, P., "Improving the efficacy of antibody-based cancer therapies," Nature Reviews Cancer 1(2):118-129, Nature Publishing Group, United States (2001).	
	NPL8	CHAN, A., et al., "Genomic Organization of the T Cell Receptor," Cancer Detection and Prevention 14(2):261-267, Elsevier, United Kingdom (1989).	
	NPL9	CHEONG, H.S., et al., "Affinity Enhancement of Bispecific Antibody Against Two Different Epitopes in the Same Antigen," Biochemical and Biophysical Research Communications 173(3):795-800, Elsevier, Netherlands (December 1990).	
	NPL10	Communication pursuant to Article 94(3) EPC, European Application No. 13720614.0, dated Mar. 24, 2017, 5 pages.	
	NPL11	DAVIES, J. and RIECHMANN, L., "Antibody VH Domains as Small Recognition Units," Biotechnology 13(5):475-479, Nature Publishing Group, United States (1995).	
	NPL12	DAVIS, J.H., et al., "SEEDbodies: Fusion Proteins Based on Strand-exchange Engineered Domain (SEED) CH3 Heterodimers in an Fc Analogue Platform for Asymmetric Binders or Immunofusions and Bispecific Antibodies," Protein Engineering, Design & Selection 23(4):195-202, Oxford University Press, United Kingdom (2010).	
	NPL13	DE KRUIF, J., et al., "Generation of Stable Cell Clones Expressing Mixtures of Human Antibodies," Biotechnology and Bioengineering 106(5):741-750, Wiley, United States (August 2010).	
	NPL14	DE WILDT, et al., Analysis of Heavy and Light Chain Pairings Indicates that Receptor Editing Shapes fhe Human Antibody Repertoire, Journal of Molecular Biology, 285(3):895-901, Elsevier, United Kingdom (1999).	
	NPL15	DEISENHOFER, J., "Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-A resolution," Biochemistry 20(9):2361-2370, American Chemical Society, United States (1981).	

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	NPL16	DEMEULE, B., et al., "Characterization of Protein Aggregation: the Case of a Therapeutic Immunoglobulin," Biochimica et Biophysica Acta 1774(1):146-153, Elsevier Publisher, Netherlands (January 2007).	
	NPL17	DEMEULE, B., et al., "Detection and Characterization of Protein Aggregates by Fluorescence Microscopy," International Journal of Pharmaceutics 329(1-2):37-45, Elsevier/North-Holland Biomedical Press, Netherlands (February 2007).	
	NPL18	Description of relevance of Third Party Submission in U.S. Appl. No. 15/140,321 dated Feb. 10, 2017.	
	NPL19	ELLERSON, J.R., et al., "Structure and Function of Immunoglobulin Domains. III. Isolation and Characterization of a Fragment Corresponding to the Cgamma2 Homology Region of Human Immunoglobin G1," Journal of Immunology 116(2):510-517, American Association of Immunologists, United States (February 1976).	
	NPL20	EZZELL, C., et al., "Magic Bullets Fly Again," Scientific American 285(4):34-41, Scientific American, United States (October 2001).	
	NPL21	FARNAN, D. and MORENO, G.T., "Multiproduct High-resolution Monoclonal Antibody Charge Variant Separations by pH Gradient Ion-exchange Chromatography," Analytical Chemistry 81(21):8846-8857, American Chemical Society, United States (2009).	
	NPL22	GARBER, K., "Biotech Industry Faces New Bottleneck," Nature Biotechnology 19(3):184-185, Nature America Publishing, United States (March 2001).	
	NPL23	GenBank, "H.sapiens gene for Ig kappa light chain variable region "012"," Acc. No. X59315, accessed at https://www.ncbi.nlm.nih.gov/nuccore/X59315, 2 pages.	
	NPL24	GHETIE, M.A., et al., "Homodimerization of Tumor-Reactive Monoclonal Antibodies Markedly Increases their Ability to Induce Growth Arrest or Apoptosis of Tumor Cells," Proceedings of the National Academy of Sciences USA 94(14):7509-7514, National Academy of Sciences, United States (1997).	
	NPL25	GUNASEKARAN, K., et al., "Enhancing Antibody Fc Heterodimer Formation Through Electrostatic Steering Effects: Applications to Bispecific Molecules and Monovalent IgG," Journal of Biological Chemistry 285(25):19637-19646, American Society for Biochemistry and Molecular Biology, United States (June 2010).	
	NPL26	HARJUNPAA, A., et al., "Rituximab (Anti-CD20) Therapy of B-Cell Lymphomas: Direct Complement Killing is Superior to Cellular Effector Mechanisms," Scandinavian Journal of Immunology 51(6):634-641, Blackwell Scientific Publications, United Kingdom (June 2000).	
	NPL27	HENDSCH, Z.S., et al., "Preferential Heterodimer Formation via Undercompensated Electrostatic Interactions," Journal of the American Chemical Society 123(6):1264-1265, American Chemical Society, United States (February 2001).	
	NPL28	HOLLIGER, P., et al., "Diabodies: Small Bivalent and Bispecific Antibody Fragments," Proc Natl Acad Sci USA 90(14):6444-6448, National Academy of Sciences, United States (July 1993).	

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	NPL29	HOLT, L.J., et al., "Domain Antibodies: Proteins for Therapy," Trends in Biotechnology 21(11):484-490, Elsevier Science Publishers, United Kingdom (2003).	
	NPL30	HOOGENBOOM, H.R., "Selecting and Screening Recombinant Antibody Libraries," Nature Biotechnology 23(9):1105-1116, Nature America Publishing, United States (2005).	
	NPL31	HWANG, W.Y., et al., "Immunogenicity of Engineered Antibodies," Methods 36(1):3-10, Academic Press, United States (May 2005).	
	NPL32	JAIN, M., et al., "Engineering Antibodies for Clinical Applications," Trends in Biotechnology 25(7):307-316, Elsevier Science Publishers, United Kingdom (July 2007).	
	NPL33	JAKOBOVITS, A., et al., "Analysis of Homozygous Mutant Chimeric Mice: Deletion of the Immunoglobulin Heavy-chain Joining Region Blocks B-cell Development and Antibody Production," Proceedings of the National Academy of Sciences USA 90(6):2551-2555, National Academy of Sciences, United States (March 1993).	
	NPL34	KLOHN, P.C., et al., "Ibc's 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics International Conferences and the 2012 Annual Meeting of the Antibody Society: December 3-6, 2012, San Diego, CA," Monoclonal antibody 5(2):178-201, Taylor & Francis, United States (March-April 2013).	
	NPL35	KONTERMANN, R.E., "Dual Targeting Strategies with Bispecific Antibodies," MAbs 4(2):182-197, Taylor and Francis, United States (2012).	
	NPL36	KORTT, A.A., et al., "Dimeric and Trimeric Antibodies: High Avidity scFvs for Cancer Targeting," Biomolecular Engineering 18 (3):95-108, Elsevier, Netherlands (October 2001).	
	NPL37	KROESEN, B.J., et al., "Bispecific antibodies for treatment of cancer in experimental animal models and man," Adv. Drug Deliv. Rev. 31(1-2):105-129, Elsevier, Netherlands (1998).	
	NPL38	KUMAR, R., et al., "The Second Pdz Domain of Inad Is a Type I Domain Involved in Binding to Eye Protein Kinase C. Mutational Analysis and Naturally Occurring Variants," Journal of Biological Chemistry 276(27):24971-24977, American Society for Biochemistry and Molecular Biology, United States (July 2001).	
	NPL39	LAZAR, G.A., et al., "A Molecular Immunology Approach to Antibody Humanization and Functional Optimization," Molecular Immunology 44(8):1986-1998, Pergamon Press, United Kingdom (March 2007).	
	NPL40	LEE, C.M., et al., "Selection of human antibody fragments by phage display," Nat. Protoc. 2(11):3001-3008, Nature Publishing Group, United Kingdom (2007). (Cited by applicant).	
	NPL41	LEFRANC, M.P, "Nomenclature of the Human Immunoglobulin Kappa (Igk) Genes," Experimental and Clinical Immunogenetics 18(3):161-174, Karger, Switzerland (2001).	
	NPL42	LENZ, H., et al., "Expression of Heterobispecific Antibodies by Genes Transfected Into Producer Hybridoma Cells," Gene 87(2):213-218, Elsevier/North-Holland, Netherlands (March 1990).	
	NPL43	LINDHOFER, H., et al., "Preferential Species-Restricted Heavy/Light Chain Pairing In Rat/Mouse Quadromas. Implications for a Single-Step Purification of Bispecific Antibodies," Immunology 155(1):219-225, American Association of Immunologists, United States (July 1995).	

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	NPL44	LOBATO, M.N., et al., "Intracellular Antibodies and Challenges Facing Their Use as Therapeutic Agents," Trends in Molecular Medicine 9(9):390-396, Elsevier Science Ltd., United Kingdom (September 2003).	
	NPL45	LOGTENBERG, T, "Antibody Cocktails: Next-generation Biopharmaceuticals With Improved Potency," Trends in Biotechnology 25(9):390-394, Elsevier Science Publishers, Netherlands (September 2007).	
	NPL46	LONBERG, N., "Fully human antibodies from transgenic mouse and phage display platforms, Curr. Opin. Immunol. 20(4):450-459, Elsevier, Netherlands (2008).	
	NPL47	LONBERG, N., "Human Antibodies From Transgenic Animals," Nature Biotechnology, 23(9):1117-1125, Nature America Publishing, United States (September 2005).	
	NPL48	LU, D., et al., "Acquired Antagonistic Activity of a Bispecific Diabody Directed Against Two Different Epitopes on Vascular Endothelial Growth Factor Receptor 2," Journal of Immunological Methods 230(1-2):159-171, Elsevier, Netherlands (November 1999).	
	NPL49	MARVIN, J. S., et al., "Redesigning an Antibody Fragment for Faster Association With Its Antigen," Biochemistry 42(23):7077-7083, American Chemical Society, United States (June 2003).	
	NPL50	MARVIN, J.S. and ZHU, Z., "Recombinant Approaches to IgG-like Bispecific Antibodies," Acta Pharmacologica Sinica 26(6):649-658, Nature Publishing Group, United States (2005).	
	NPL51	MCGINNES, K.," B-lineage colonies from normal, human bone marrow are initiated by B cells and their progenitors," Blood 77(5):961-970, American Society of Hematology, United States (1991).	
	NPL52	MCPHEE, F., et al., "Engineering Human Immunodeficiency Virus 1 Protease Heterodimers as Macromolecular Inhibitors of Viral Maturation," Proceedings of the National Academy of Sciences of the United States of America 93(21):11477-11481, National Academy of Sciences, United States (October 1996).	
	NPL53	MERCHANT, A.M, et al., "An efficient route to human bispecific IgG," Nature Biotechnology, 16(7):677-681, Nature Publishing Group, United States (1998).	
	NPL54	Merus, "Merus presents preclinical data on its novel bispecific antibody MCLA-117 at EHA 2013," www.merus.nl, press release, 3 pages, dated Jun. 17, 2013.	
	NPL55	Merus, "Merus selects clinical candidate for the treatment of acute myeloid leukemia (AML)," www.merus.nl, press release, 2 pages, dated Jan. 7, 2013.	
	NPL56	MILLER, S, "Protein-protein Recognition and the Association of Immunoglobulin Constant Domains," Journal of Molecular Biology 216(4):965-973, Elsevier Ltd., Netherlands (December 1990).	
	NPL57	MIRICK, G.R., et al., "A Review of Human Anti-Globulin Antibody (HAGA, HAMA, HACA, HAHA) Responses To Monoclonal antibodies. Not four letter words," The Quarterly Journal of Nuclear Medicine and Molecular Imaging 48(4):251-257, Minerva Medic, Italy (December 2004).	

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	08/26/2023

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT *(Use as many sheets as necessary)*				Application Number	*To Be Assigned*
				Filing Date	*Herewith*
				First Named Inventor	Cornelis Adriaan DE KRUIF
				Art Unit	*To Be Assigned*
				Examiner Name	*To Be Assigned*
Sheet	15	of	17	Attorney Docket Number	4096.0050007

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published	T[2]
	NPL58	MOHAPATRA, S., et al., "Designer monoclonal antibodies as drugs: the state of the art," Expert Rev. Clin. Immunol. 4(3):305-307, Taylor & Francis, United Kingdom (2008). (Cited by applicant)	
	NPL59	NELSON, A.L., et al., "Development trends for human monoclonal antibody therapeutics," Nat. Rev. Drug Discov. 9(10):767-774, Nature Publishing Group, United Kingdom (2010).	
	NPL60	NEMAZEE, D., "Receptor Editing in B Cells," Advances in immunology 74:89-126, Academic Press, United States (2000).	
	NPL61	NIEBA, L., et al., "Disrupting the Hydrophobic Patches at the Antibody Variable/constant Domain Interface: Improved in Vivo Folding and Physical Characterization of an Engineered Scfv Fragment," Protein Engineering 10(4):435-444, Oxford University Press, United Kingdom (April 1997).	
	NPL62	NOHAILE, M.J., et al., "Altering dimerization specificity by changes in surface electrostatics," Proceedings of the National Academy of Sciences 98(6):3109-3114, National Academy of Sciences, United States (2001).	
	NPL63	Office Action mailed April 6, 2020, in United States Patent Application No. 16/417,379, DE KRUIF; et al., filed May 20, 2019, 5 pages.	
	NPL64	PADLAN, E.A, "X-Ray Crystallography of Antibodies," Advances in Protein Chemistry 49:57-133, Academic Press, United States (1996).	
	NPL65	PAPADEA, C., and CHECK, I.J., "Human Immunoglobulin G and Immunoglobulin G Subclasses: Biochemical, Genetic, and Clinical Aspects," Critical Reviews in Clinical Laboratory Sciences 27(1):27-58, Informa Healthcare, United Kingdom (1989).	
	NPL66	PHELPS, J.L., et al., "Expression and Characterization of a Chimeric Bifunctional Antibody with Therapeutic Applications," The Journal of Immunology 145(4):1200-1204, American Association of Immunologists, United States (1990).	
	NPL67	PRESTA, L.G., "Engineering of Therapeutic Antibodies to Minimize Immunogenicity and Optimize Function," Advanced Drug Delivery Reviews 58(5-6):640-656, Elsevier Science, Netherlands (2006).	
	NPL68	RAFFEN, R., et al., "Reengineering Immunoglobulin Domain Interactions by Introduction of Charged Residues," Protein Engineering 11(4):303-309, Oxford University Press, United Kingdom (April 1998).	
	NPL69	REPP, R., et al., "Phase I Clinical Trial of the Bispecific Antibody MDX-H210 (Anti-FcgammaRI x Anti-HER-2/neu) in Combination With Filgrastim (G-CSF) for Treatment of Advanced Breast Cancer," British Journal of Cancer 89(12):2234-2243, Nature Publishing Group on behalf of Cancer Research UK, United Kingdom (December 2003).	
	NPL70	RETTER, M.W., et al., "Receptor Editing Occurs Frequently during Normal B Cell Development," J. Exp. Med. 188(7):1231-1238, Rockefeller University Press, United States (1998).	

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Sheet	16	of	17	Attorney Docket Number	4096.0050007

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	NPL71	RIDGWAY, J.B., et al., "Knobs-Into-Holes' Engineering of Antibody CH3 Domains for Heavy Chain Heterodimerization," Protein Engineering 9(7):617-621, Oxford University Press, United Kingdom (1996).	
	NPL72	SAL-MAN, N. and SHAI, Y., "Arginine mutations within a transmembrane domain of Tar, an Escherichia coli aspartate receptor, can drive homodimer dissociation and heterodimer association in vivo," Biochemical Journal 385(Pt1):29-36, Portland Press, United Kingdom (2005).	
	NPL73	SCHAEFER, G., et al., "A Two-in-one Antibody Against HER3 and EGFR Has Superior Inhibitory Activity Compared With Monospecific Antibodies," Cancer Cell 20(4):472-486, Cell Press, United States (October 2011).	
	NPL74	SCHIFFER, M., et al., "Analysis of Immunoglobulin Domain Interactions. Evidence for a Dominant Role of Salt Bridges," Journal of Molecular Biology 203(3):799-802, Elsevier, United Kingdom (October 1988).	
	NPL75	SEGAL, D.M., et al., "Introduction: Bispecific Antibodies," Journal of Immunological Methods 248(1-2):1-6, Elsevier, Netherlands (2001).	
	NPL76	SELZER, T., et al., "Rational Design of Faster Associating and Tighter Binding Protein Complexes," Nature Structural & Molecular Biology 7(7):537-541, Nature Publishing Group, United States (July 2000).	
	NPL77	SHEINERMAN, F.B., et al., "Electrostatic Aspects of Protein-protein Interactions," Current Opinion in Structural Biology 10(2):153-159, Elsevier Science, United Kingdom (April 2000).	
	NPL78	SINHA, N., et al., "Differences in Electrostatic Properties at Antibody-antigen Binding Sites: Implications for Specificity and Cross-reactivity," Biophysical Journal 83(6):2946-2968, Cambridge, United States (December 2002).	
	NPL79	SINHA, N., et al., "Electrostatics in Protein Binding and Function," Current Protein and Peptide Science 3(6):601-614, Bentham Science Publishers, Netherlands (December 2002).	
	NPL80	SMITH, E.J., et al., "A Novel, Native-Format Bispecific Antibody Triggering T-Cell Killing of B-cells is Robustly Active in Mouse Tumor Models and Cynomolgus Monkeys," Scientific Reports 5:17943, Nature Publishing Group, United Kingdom (December 2015).	
	NPL81	STRELKAUSKAS, A.J., et al., "Human Monoclonal Antibody: 2. Simultaneous Expression of IgG and IgM with Similar Binding Specificities by a Human Hybrid Clone," Hybridoma 6(5):479-487, Mary Ann Liebert Inc., United States (1987).	
	NPL82	TADA, H., et al., "Expressionand Characterization of a Chimeric Bispecific Antibody Against Fibrin andAgainst Urokinase-Type Plasminogen Activator," Journal of Biotechnology 33:157-174, Elsevier B.V (March1994).	
	NPL83	VAN RHENEN, A., et al., "The Novel AML Stem Cell Associated Antigen Cll-1 Aids in Discrimination Between Normal and Leukemic Stem Cells," Blood 110(7):2659-2666, American Society of Hematology, United States (October 2007).	

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	NPL84	WEINER, L.M., et al., Abstract, "Fully human therapeutic monoclonal antibodies," Journal of Immunotherapy, 29(1):1-9, Lippincott Williams & Wilkins, Hagerstown, Maryland, United States (January 1, 2006).	
	NPL85	WINTER, G., et al., "Making Antibodies by Phage Display Technology," Annual Review of Immunology 12:433-455, Annual Reviews Inc., United States (1994).	
	NPL86	WU, C., et al., "Simultaneous Targeting of Multiple Disease Mediators by a Dual-variable-domain Immunoglobulin," Nature Biotechnology 25(11):1290-1297, Nature Publishing Co., United States (2007).	
	NPL87	YOO, E.M., et al., Structural requirements for polymeric immunoglobulin assembly and association with J chain," J. Biol. Chem. 274(47):33771-33777, American Society for Biochemistry and Molecular Biology, United States (1999).	
	NPL88	ZHAO, X., et al., "Targeting C-type Lectin-like Molecule-1 for Antibody-mediated Immunotherapy in Acute Myeloid Leukemia," Haematologica 95(1):71-78, Ferrata Storti Foundation, Italy (January 2010).	
	NPL89	ZHU, Z., et al., "Remodeling Domain Interfaces to Enhance Heterodimer Formation," Protein Science 6(4):781-788, Cold Spring Harbor Laboratory Press, United States (April 1997).	
	NPL90	Office Action mailed April 13, 2023, in U.S. Application No. 16/934,925, inventor: De Kruif, C.A., et al., filed July 21, 2020, 8 pages.	
	NPL91	Office Action mailed December 23, 2022, in U.S. Application No. 16/934,925, inventor: De Kruif, C.A., et al., filed July 21, 2020, 12 pages.	

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007

CONFIRMATION NO. 8764

26111
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

PUBLICATION NOTICE



Date Mailed: 10/26/2023

Title:METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Publication No.US-2023-0340556-A1
Publication Date:10/26/2023

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APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/318,507	11/29/2023 05:52:32 PM Z ET	4096.0050007

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	8764	FILED BY	Hayley Magann
PATENT CENTER #	63413513	AUTHORIZED BY	Paul Calvo
CUSTOMER #	26111	FILING DATE	05/16/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF

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APPROVAL LETTER

APPLICATION #	FILING DATE	APPLICANT/PATENT UNDER REEXAMINATION
18/318,507	05/16/2023	Cornelis DE KRUIF

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

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PATENT CENTER #	63413513	FILING DATE	05/16/2023
CUSTOMER #	26111	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Calvo

Documents

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grantLetter.pdf	1	Terminal Disclaimer-Electronic-Approved	19 KB

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	DD883CBDCA2345D6E9CB6B8C65D16793
grantLetter.pdf	384E45D514A6CCA0C1BD60F274189F50FE3CE999DC25931BA5BD0DC0F5D655B1F6CC375FD7432BC48DDD6FABD73B05D040CDDE8D74FD1E47D77A2EE08492D9D4

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APPLICATION #	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET #
18318507	05/16/2023	Cornelis DE KRUIF	4096.0050007

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

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Merus N.V.	100%
Total	100%

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Signature	Name	Registration #
/Paul A. Calvo/	Paul Calvo	57913

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CONFIRMATION #	8764	FILED BY	Hayley Magann
PATENT CENTER #	63426238	FILING DATE	05/16/2023
CUSTOMER #	26111	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Calvo

Documents

TOTAL DOCUMENTS: 3

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
2023-11-29-Amendment-Reply-111-4096-0050007.pdf		6	-	132 KB
2023-11-29-Amendment-Reply-111-4096-0050007-A....pdf	(1-1)	1	Amendment/Request for Reconsideration-After Non-Final Rejection	121 KB
2023-11-29-Amendment-Reply-111-4096-0050007-CLM.pdf	(2-3)	2	Claims	72 KB
2023-11-29-Amendment-Reply-111-4096-0050007-REM.pdf	(4-6)	3	Applicant Arguments/Remarks Made in an Amendment	75 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
2023-11-29-Amendment-Reply-111-4096-0050007.pdf	4ECEB5FEF6FE59DA11D90B22892EFE4C8F09CE9CDB246BD5F8CD517716C6560182A82969A5BA7D86A62FE397627774185FD31BD57024486304C081037368BA1C
2023-11-29-Amendment-Reply-111-4096-0050007-A....pdf	881FF53227E3CA25C0FF36210A3C4D95DE333C4398E3D81C2539535C191883D76CDE58C004B9036D0F07F78F1F1370866F970010E1172A633C2F86AE962F4533
2023-11-29-Amendment-Reply-111-4096-0050007-CLM.pdf	C5ABE37FF2731023AA07976FEB7B3F894014C76E7381D6646C80318C4637891E4164C9B903C84466B216FE728702352251669DD174FD61E1514A766BAA8532B7
2023-11-29-Amendment-Reply-111-4096-0050007-REM.pdf	02F932AF12CB33CA0C495597EE528B27769D07C7AB90250C3D0A44B10AB228FF6A9244ACC9E237202D0D0797F390B463B3AF9FBA8F92C2D042902087299CCB3E

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Cornelis Adriaan DE KRUIF	Confirmation No.: 8764
Applicant: MERUS N.V.	Art Unit: 1633
Application No.: 18/318,507	Examiner: MICHAEL D. BURKHART
Filed: May 16, 2023 (*Continuation of Appl. No. 16/934,925; Filed: July 21, 2020*)	Atty. Docket: 4096.0050007

Title: **METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES**

Amendment and Reply Under 37 C.F.R. § 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Mail Stop Amendment

Commissioner:

In reply to the Office Action dated **August 31, 2023**, Applicant submits the following Amendment and Remarks.

Amendments to the Claims begin on page 2 of this paper.

Remarks and Arguments begin on page 4 of this paper.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any additional fees required to continue prosecution or appeal of this application (including issue fee, fees for net addition of claims or forwarding to appeal) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1-28. (Canceled)

29. (Previously Presented) A heterodimeric antibody comprising a first human CH3 domain comprising a positively charged amino acid residue at position 364 according to the EU numbering system, and a second human CH3 domain comprising a negatively charged amino acid residue at position 368 according to the EU numbering system.

30. (Previously Presented) The heterodimeric antibody of claim 29, wherein said positively charged amino acid residue at position 364 comprises a lysine (K) or an arginine (R) residue, and wherein said negatively charged residue at position 368 comprises an aspartic acid (D) or glutamic acid (E) residue.

31. (Previously Presented) The heterodimeric antibody of claim 30, wherein said positively charged amino acid residue at position 364 comprises a lysine (K) residue, and wherein said negatively charged amino acid residue at position 368 comprises an aspartic acid (D).

32-33. (Canceled)

34. (Previously Presented) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is a bispecific antibody.

35. (Previously Presented) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is human IgG.

36. (Previously Presented) The heterodimeric antibody of claim 29, wherein said heterodimeric antibody is human IgG1.

37. (Previously Presented) A pharmaceutical composition comprising the heterodimeric antibody according to claim 29, and a pharmaceutically acceptable carrier.

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 29-31 and 34-37 are pending in the application, with claim 29 being the independent claim. Claims 32 and 33 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Double Patenting Rejections

The Office alleges that claim 29 of the present application is patentably indistinct from claim 53 of U.S. Appl. No. 16/934,925 ("the '925 application"). The Office also alleges that claim 34 of the present application is patentably indistinct from claim 54 of the '925 application. The Office also alleges that claim 35 of the present application is patentably indistinct from claim 55 of the '925 application. The Office also alleges that claim 37 of the present application is patentably indistinct from claim 56 of the '925 application. Applicant respectfully traverses the rejections.

Not in acquiescence to the propriety of the rejections, but rather solely to advance prosecution, Applicant canceled claims 53-56 in the '925 application in an amendment filed September 8, 2023. Therefore, the Office's allegations regarding claims 29, 34, 35, and 37 being patentably indistinct from claims 53-56, respectively, of the '925 application have been rendered moot.

The Office also alleges that should claim 29 be found allowable, claims 32 and 33 will be objected to under 37 C.F.R. § 1.75 as being substantial duplicates thereof. Applicant respectfully traverses the rejections. Not in acquiescence to the propriety of the rejections, but rather solely to advance prosecution, Applicant has canceled claims 32 and 33 rendering moot the Office's allegation regarding claim 29 being patentably indistinct from claims 32 and 33.

Claims 29-37 are rejected on the grounds of non-statutory obviousness type double patenting as allegedly being unpatentable over claims 1, 5-6, 8-12, 21-25, 28-29, and 32 of U.S. Patent No. 9,358,286. Claims 29-37 are also rejected on the grounds of non-statutory obviousness type double patenting as allegedly being unpatentable over claims 1, 7, 10-12, 17-20, and 32-34 of U.S. Patent No. 10,329,596. Claims 29-37 are also rejected on the grounds of non-statutory obviousness type double patenting as allegedly being unpatentable over claims 1, 5-6, 9-15, 28-31, 35, and 40-43 of U.S. Patent No. 9,758,805. Claims 29-37 are also rejected on the grounds of non-statutory obviousness type double patenting as allegedly being unpatentable over claims 1, 4-5, 11-18, and 53-56 of U.S. Patent No. 10,337,045. Claims 29-37 are also rejected on the grounds of non-statutory obviousness type double patenting as allegedly being unpatentable over claims 29, 31-34, and 51-58 of U.S. Patent Application No. 16/934,925. As an initial matter, claims 32 and 33 have been canceled rendering moot the rejection as applied to those claims. Applicant respectfully traverses the rejections as they apply to the amended claims presented herein. Not in acquiescence to the propriety of the rejections, but rather solely to advance prosecution, Applicant submits herewith a terminal disclaimer to obviate the double patenting rejection over U.S. Pat. Nos. 9,358,286; 10,329,596; 9,758,805; 10,337,045; and U.S. Pat. Appl. No. 16/934,925.

In view of the above remarks and the terminal disclaimer filed herewith, Applicant respectfully asserts that the rejections have been rendered moot. Accordingly, Applicant requests that the rejections be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Paul A. Calvo, Reg. No. 57,913/

Paul A. Calvo
Attorney for Applicant
Registration No. 57,913

Date: November 29, 2023

1101 K Street NW, 10th Floor
Washington, D.C. 20005
Main: (202) 371-2600
Direct: (202) 772-8846

Notice of Allowability	Application No. 18/318,507	Applicant(s) DE KRUIF et al.	
	Examiner MICHAEL D BURKHART	Art Unit 1633	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the response dated 11/29/2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 29-31 and 34-37 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/MICHAEL D BURKHART/
Primary Examiner, Art Unit 1633

EXAMINER'S COMMENT

Double Patenting

The rejection of claims 29, 34, 35 and 37 as patentably indistinct from claims in the 16/934,925 application is withdrawn in light of cancelation of the relevant claims in the '925 application.

Terminal Disclaimer

The terminal disclaimer filed on 11/29/2023 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of 16/934,925; 9,358,286; 10,329,596; 9,758,805 and 10,337,045 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MICHAEL D BURKHART whose telephone number is (571)272-2915. The examiner can normally be reached M-F 8-5.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on 571 272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MICHAEL D BURKHART/
Primary Examiner, Art Unit 1633

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

26111 7590 01/08/2024

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

EXAMINER
BURKHART, MICHAEL D

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 01/08/2024

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

TITLE OF INVENTION: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	04/08/2024

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

26111 7590 01/08/2024

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

TITLE OF INVENTION: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	04/08/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
BURKHART, MICHAEL D	1633	424-136100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via the USPTO patent electronic filing system ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

26111 7590 01/08/2024

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

EXAMINER	
BURKHART, MICHAEL D	

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 01/08/2024

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).
https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to:

1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
2) a federal, state, local, or international agency, in response to its request;
3) a contractor of the USPTO having need for the information in order to perform a contract;
4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
9) the Office of Personnel Management (OPM) for personnel research purposes; and
10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
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ISSUE FEE TRANSMITTAL FORM

APPLICATION #	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET #
18318507	**05/16/2023**	**Cornelis DE KRUIF**	**4096.0050007**

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Nonprovisional Application under 35 USC 111(a)	**DATE DUE**	04/08/2024
CONFIRMATION #	8764	**ISSUE FEE DUE**	$ 1200
EXAMINER	MICHAEL BURKHART	**PUBLICATION DUE**	$ 0
GROUP ART UNIT	1638	**TOTAL FEES DUE**	$1200
CLASS - SUBCLASS	424/136100	**PREV. PAID FEE**	$ 0
ENTITY STATUS	Regular Undiscounted		

1. CHANGE OF CORRESPONDENCE ADDRESS AND/OR INDICATION OF FEE ADDRESS (37 CFR 1.33 & 1.363)

Correspondence Address

CURRENT ADDRESS

Maintenance Fee Address

CURRENT ADDRESS

☐ Change of correspondence address requested,system generated AIA/122-PC form attached

☐ Fee address indication requested, system generatedSB/47-PC form attached

2. ENTITY STATUS

Change in Entity Status

NEW STATUS

3. THE FOLLOWING FEES ARE SUBMITTED

☑ Issue Fee

☑ Publication Fee

4. FEE AUTHORIZATION

☐ I authorize USPTO to apply my previously paid issue fee to the current fees due

☐ The Director is herby authorized to apply my previously paid issue fee to the current fee due and to charge deficient fees to Deposit Account Number:

☑ If in addition to the payment of the issue fee amount submitted with this form, there are any discrepencies in any amount(s) due, the Director is authorized to charge any deficiency, or credit any overpayment, to Deposit Account Number: 190036

5. FIRM AND/OR ATTORNEY NAMES TO BE PRINTED

NOTE: If no name is listed, no name will be printed
For printing on the patent front page, list to be displayed as entered

Sterne, Kessler, Goldstein & Fox P.L.L.C.

6. ASSIGNEE NAME(S) AND RESIDENCE DATA TO BE PRINTED

NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a susbstitute for filing an assignment.

Merus N.V.	Utrecht, NETHERLANDS	Corporation

Signature

I certify, in accordance with 37 CFR 1.4(d)(4) that I am an attorney or agent registered to practice before the Patent and Trademark Office who has filed and has been granted power of attorney in this application. I also certify that this Fee(s) Transmittal form is being transmitted to the USPTO via Patent Center on the date indicated below.

Signature	Name	Registration #	Date
/Paul A. Calvo/	Paul Calvo	57913	02/01/2024

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/318,507	**02/01/2024 02:57:05 PM Z ET**	**4096.0050007**

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	8764	FILED BY	Elizabeth Holemans
PATENT CENTER #	64153375	AUTHORIZED BY	Paul Calvo
CUSTOMER #	26111	FILING DATE	05/16/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 3007	**E202421E58319402**	**Paul Calvo**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1501	UTILITY ISSUE FEE	1200.00	1	1200.00
			TOTAL AMOUNT:	**$1,200.00**

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/318,507	**02/01/2024 02:57:05 PM Z ET**	**4096.0050007**

Title of Invention

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	8764	FILED BY	Elizabeth Holemans
PATENT CENTER #	64153375	FILING DATE	05/16/2023
CUSTOMER #	26111	FIRST NAMED INVENTOR	Cornelis Adriaan DE KRUIF
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Calvo

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
web85b.pdf	3	Issue Fee Payment (PTO-85B)	58 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
web85b.pdf	791F258FB6312820E1880CE10AE18AF8AC85D7471C1EB0880 26A1A96E5B2E2AC97E23BF8EB36D96321275840FA67B6C1AD 0C515ACA3662B5466A7D25920D95DC

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	03/12/2024	11926859	4096.0050007	8764

26111 7590 02/21/2024

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at https://patentcenter.uspto.gov/. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at https://certifiedcopycenter.uspto.gov/index.html. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (https://patentcenter.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional inventors):

Cornelis Adriaan DE KRUIF, Utrecht, NETHERLANDS;
Linda Johanna Aleida HENDRIKS, Utrecht, NETHERLANDS;
Ton LOGTENBERG, Utrecht, NETHERLANDS;

APPLICANT(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional applicants):

Merus N.V., Utrecht, NETHERLANDS;

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/318,507	05/16/2023	Cornelis Adriaan DE KRUIF	4096.0050007	8764

26111 7590 03/12/2024

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
10th Floor
WASHINGTON, DC 20005

EXAMINER
BURKHART, MICHAEL D

ART UNIT	PAPER NUMBER
1638	

NOTIFICATION DATE	DELIVERY MODE
03/12/2024	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

e-office@sternekessler.com

APPLICATION NO.	ISSUE DATE	PATENT NO.
18/318,507	12-Mar-2024	11926859

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1101 K Street, NW
WASHINGTON, DC 20005

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at https://patentcenter.uspto.gov

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit https://www.uspto.gov/electronicgrants

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 18/318,507	Filing Date 05/16/2023	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $480 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	11/29/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/LISA THOMAS/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/318,507

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	320
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	700
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	800
TOTAL CLAIMS (37 CFR 1.16(i))	9 minus 20 =	* 0			OR	x =	0
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x =	0
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						420
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2240

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/318,507	DE KRUIF et al.
	Examiner	Art Unit
	MICHAEL D BURKHART	1633

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Inventor name search (OC search, USPat, USPgPub, EPO, JPO, Derwent) keyword search (OC search, Google)	08/26/2023	MB
Parent cases reviewed	08/26/2023	MB

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

	/MICHAEL D BURKHART/ Primary Examiner, Art Unit 1633

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
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	Examiner	Art Unit
	MICHAEL D BURKHART	1633

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Inventor name search (OC search, USPat, USPgPub, EPO, JPO, Derwent) keyword search (OC search, Google)	08/26/2023	MB
Parent cases reviewed	08/26/2023	MB
updated	12/15/2023	MB

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
none	none	12/15/2023	MB

	/MICHAEL D BURKHART/ Primary Examiner, Art Unit 1633

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/318,507	DE KRUIF et al.
	Examiner	**Art Unit**
	MICHAEL D BURKHART	1633

CPC						
Symbol					**Type**	**Version**
C12P	/	21	/	005	F	2013-01-01
C07K	/	16	/	12	I	2013-01-01
C07K	/	16	/	26	I	2013-01-01
C07K	/	16	/	36	I	2013-01-01
C07K	/	16	/	468	I	2013-01-01
C07K	/	16	/	18	I	2013-01-01
C07K	/	16	/	1278	I	2013-01-01
C07K	/	16	/	28	I	2013-01-01
C07K	/	16	/	2809	I	2013-01-01
C07K	/	16	/	283	I	2013-01-01
C07K	/	16	/	2866	I	2013-01-01
C07K	/	16	/	2896	I	2013-01-01
A61K	/	39	/	395	I	2013-01-01
A61K	/	39	/	3955	I	2013-01-01
A61K	/	39	/	40	I	2013-01-01
C07K	/	16	/	1282	I	2013-01-01
C07K	/	2317	/	14	A	2013-01-01
C07K	/	2317	/	526	A	2013-01-01
C07K	/	2317	/	94	A	2013-01-01
C07K	/	2317	/	31	A	2013-01-01
A61K	/	2039	/	507	A	2013-01-01
C07K	/	2317	/	56	A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	7	
/MICHAEL D BURKHART/ Primary Examiner, Art Unit 1633	15 December 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20231215

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/318,507	DE KRUIF et al.
	Examiner	**Art Unit**
	MICHAEL D BURKHART	1633

INTERNATIONAL CLASSIFICATION					
CLAIMED					
C12N	/	15	/	00	

NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 7	
/MICHAEL D BURKHART/ Primary Examiner, Art Unit 1633 (Primary Examiner)	15 December 2023 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office — Part of Paper No.: 20231215

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/318,507	DE KRUIF et al.
	Examiner	**Art Unit**
	MICHAEL D BURKHART	1633

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	7	
/MICHAEL D BURKHART/ Primary Examiner, Art Unit 1633	15 December 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20231215

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L193	14	cornelius near2 (de adj kruif.in.)	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:34 PM
L194	549	ton near2 logtenberg.in.	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:34 PM
L195	78	linda near2 hendriks.in.	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L196	35402	ch3 with substitut$3	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L197	27	L153 SAME ("364" AND "368")	(US-PGPUB; USPAT; USOCR; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L198	2	L153 SAME ("s364" AND "I368")	(US-PGPUB; USPAT; USOCR; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L199	609	I368$.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/15 03:35 PM
L200	99	L144 AND s364K.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/15 03:35 PM
L201	116	(I368E.clm. OR L368D.clm.) AND s364K.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L202	0	(I368E.clm. OR L368D.clm.) AND s364R.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L203	17	(I368E.clm. OR L368D.clm.) AND s364H.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L204	18	L180 AND "L368"	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM
L205	18	L181 AND "L368"	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/12/15 03:35 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L179	14	cornelius near2 (de adj kruif.in.)	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:46 PM
L180	542	ton near2 logtenberg.in.	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:46 PM
L181	77	linda near2 hendriks.in.	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:46 PM
L182	36013	ch3 with substitut$3	(US-PGPUB; USPAT; FPRS; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:48 PM
L183	27	L153 SAME ("364" AND "368")	(US-PGPUB; USPAT; USOCR; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:48 PM
L184	2	L153 SAME ("s364" AND "I368")	(US-PGPUB; USPAT; USOCR; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:48 PM
L185	567	I368$.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/26 05:49 PM
L186	134	L144 AND s364$.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/26 05:49 PM
L187	92	L144 AND s364K.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/26 05:49 PM
L188	108	(I368E.clm. OR L368D.clm.) AND s364K.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:50 PM
L189	0	(I368E.clm. OR L368D.clm.) AND s364R.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:50 PM
L190	13	(I368E.clm. OR L368D.clm.) AND s364H.clm.	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:50 PM
L191	17	L180 AND "L368"	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:55 PM
L192	17	L181 AND "L368"	(US-PGPUB; USPAT; EPO; DERWENT)	OR	ON	ON	2023/08/26 05:56 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.