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.

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

BIB DATA SHEET

CONFIRMATION NO. 1016

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/081,848	11/15/2013 RULE	435	1633	MRX5-007CN

APPLICANTS

Merus B.V., Utrecht, NETHERLANDS;

INVENTORS

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

**** CONTINUING DATA ***************************

This application is a CON of 13/866,747 04/19/2013
which claims benefit of 61/635,935 04/20/2012

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
11/29/2013

Foreign Priority claimed	☐ Yes ☑ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☐ Yes ☐ No					
Verified and Acknowledged	/MICHAEL D BURKHART/ Examiner's Signature	/MB/ Initials	NETHERLANDS	39	33	4

ADDRESS

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109
UNITED STATES

TITLE

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

FILING FEE RECEIVED	FEES: Authority has been given in Paper	☐ All Fees
2250	No._____ to charge/credit DEPOSIT ACCOUNT	☐ 1.16 Fees (Filing)
	No._____ for following:	☐ 1.17 Fees (Processing Ext. of time)
		☐ 1.18 Fees (Issue)
		☐ Other _____
		☐ Credit

BIB (Rev. 05/07).

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Tara McClain
Attorney Docket Number:	MRX5-007CN
Filed as Small Entity	

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	70	70
Utility Search Fee	2111	1	300	300
Utility Examination Fee	2311	1	360	360
Pages:				
Utility Appl Size fee per 50 sheets >100	2081	1	200	200
Claims:				
Claims in excess of 20	2202	13	40	520
Independent Claims in Excess of 3	2201	1	210	210

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1660**

Electronic Acknowledgement Receipt	
EFS ID:	17361605
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Tara McClain
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	15-NOV-2013
Filing Date:	
Time Stamp:	18:10:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1660
RAM confirmation Number	5728
Deposit Account	120080
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Oath or Declaration filed	MRX5_007_Inventor_Decs.pdf	81795 6760ce7fd7837d6f93014f1418b7cdea740cb640	no	3
Warnings:					
Information:					
2	Power of Attorney	MRX5_007CN_POA.pdf	232066 e0e70d0d883db6cfa6db395097bfb48b2c0840db	no	2
Warnings:					
Information:					
3		MRX5_007CN_Application.pdf	467267 5580d411d353bf56a2b7b72f1e7d4a31ef3fce80	yes	107

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	102
Claims	103	106
Abstract	107	107

Warnings:					
Information:					
4	Drawings-only black and white line drawings	MRX5_007CN_Figures_.pdf	4591502 daa6651af7f187ae8f4f96a663e46dc39d77800f	no	39
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
5	Application Data Sheet	MRX5_007CN_ADS.pdf	1255552 338508290b2e220e2c6f1fe89f3bc8a6bf6ad649	no	7
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	39727 afafbf1c6fea79e026171c274f8877d6bf4325c5	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	6667909	

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 a 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/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	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
Mr.	Cornelis	A.	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584CH	**Country** i	NL

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
Ms.	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584CH	**Country** i	NL

Inventor 3 Remove

Legal Name

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584 CH	**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	00959		
Email Address	IPBoston.Docketing@nelsonmullins.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES		
Attorney Docket Number	MRX5-007CN	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	39	**Suggested Figure for Publication (if any)**	

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:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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	00959		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this 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.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13866747	2013-04-19
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13866747	non provisional of	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(d). 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(h)(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

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If 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 World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

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	

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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 B.V.
Mailing Address Information:	
Address 1	Padualaan 8 (postvak 133)
Address 2	
City	Utrecht
State/Province	
Country [i] NL	Postal Code 3584CH
Phone Number	Fax Number
Email Address	

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

Non-Applicant Assignee Information:

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

Assignee 1

Complete this section only if non-applicant assignee information is desired to be included on the patent application publication in accordance with 37 CFR 1.215(b). Do not include in this section 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), as the patent application publication will include the name of the applicant(s).

Remove

If the Assignee is an Organization check here. ☐

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

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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

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

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Cynthia L. Kanik/			Date (YYYY-MM-DD)	2013-11-15
First Name	Cynthia	Last Name	Kanik	Registration Number	37320

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

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.**

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. 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 Cooperation Treaty.

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.

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

This application is a Continuation of U.S. Patent Application No. 13/866,747, filed April 19, 2013, 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 (> 1Å^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/L351D 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

Figure 1: A) schematic representation of construct vector MV1057. The stuffer region is the region into which an antibody VH region is cloned. B) schematic representation of phage display vector MV1043.

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

Figure 3: nucleotide and amino acid sequences of VH regions used for cloning into the various constructs.

Figure 4: mass spec data of transfections A, G and H.

Figure 5: mass spec data of transfections M and U.

Figure 6: mass spec data of transfection O.

Figure 7: prevention of homodimerisation by substitution of neutral amino acids for charged amino acids.

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

Figure 9: HADDOCK scores on experimentally verified mutation pairs

Figure 10: Cartoons of interactions in the CH3-CH3 interface; A) K409D:K392D/D399'K:E356'K, B) D399K:E356K/D399'K:E356'K, C) K409D:K392D/ K409'D:K392'D

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

Figure 12: Cartoons of interactions in the CH3-CH3 interface ; A) L351D/L351'D, B) L351D:S354A:R355D/ L351'D:S354'A:R355'D

Figure 13: HADDOCK scores for additional charge mutations around position L351

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

Figure 15: Cartoons of interactions in the CH3-CH3 interface

Figure 16: HADDOCK scores for variants around T366/L351

Figure 17: HADDOCK scores for additional variants around T366/L351

Figure 18: Examples of nMS spectra for bispecific IgG obtained after the co-expression of construct T366K,L351K with either construct L351D (left hand panel) or

L351D,Y349E (right hand panel), zoomed in on a single charge state of the full IgG (half bodies not shown)

Figure 19: A) Results of native MS showing relative abundances of AA, AB, BB, A and B (total of all species is 100%); B) idem but now without AB to have a better overview on the undesired species AA, BB, A and B

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

Figure 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.

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

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

Figure 24: nMS results of transfections # 1-11 from Table 20.

Figure 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.

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

Figure 27: nMS of transfections # 9 (A) and # 11 (B).

Figure 28: nMS of gel filtrated samples 1516:1516 (A), 1337:1337 (B) and 1516:1337 (C).

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

5

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 bispecifics produced	**Transfect-ion of**	**Transfection code and ratio**	**Expected species**	**Calculated mass - 2LYS**	**Experiment-al mass**	**Half molecules found (%)**	**Full IgG found (%)**	**Bispecific found (%)**	**Other molecules (%)**
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	J (1:1)	BC	144354	144382			96	4

	VII								
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**	**Transfecti on code and ratio**	**Expecte d species**	**Expected % monospecifi c 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 mAb	IV+V+VI+ II'	ZD (2:1:1:2)	BC + AB + DD	33	33 + 33
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	IX+X+II'	ZM (1:1:2)	AB + DD	50	50

mAb					
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	+-

AA substitutions in CH3	construct #	Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)
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	+-
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,	35	NT

AA substitutions in CH3	**construct #**	**Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)**
V397K		
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, Y407D	46	+
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	VdW	Electrostatic	Desolvation	Buried

	Score	**energy**	**energy**	**energy**	**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 combinations	**HADDOCK Score**	**VdW energy**	**Electrostatic energy**	**Desolvation energy**	**Buried 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/L351D 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 colour of the cells indicate the variations between T= 0 and after stress: dark grey = large change, light grey = small change and no colour = 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	2d4°C	30-50	0.003	0.9	5.1	336	7.1	507	

bispecific (AA AB BB)	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	5.1	508	57
	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	6.7	499	0
	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 greyed cells 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. Grey cells: 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 lgG 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 lgG coated in a 96-wells ELISA plate. Bound antibody was subsequently visualized using a polyclonal antihuman lgG 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 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.

2. The method of claim 1, further comprising providing said host cell with a nucleic acid molecule encoding a common light chain.

3. The method of claim 1, 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.

4. The method of claim 3, wherein said first CH3-domain-comprising polypeptide chain further comprises the amino acid substitution L351K.

5. The method of claim 3, wherein said second CH3-domain comprising polypeptide chain further comprises an amino acid substitution selected from the group consisting of Y349E; Y349D; L368E; Y349D and L388E; and Y349D and R355D.

6. The method of claim 5, wherein said second CH3-domain comprising polypeptide chain further comprises the amino acid substitution L368E.

7. The method according to claim 1, 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.

8. The method according to claim 1, wherein each of the CH3-domain comprising polypeptide chains further comprises a variable region recognizing a target epitope.

9. The method of claim 8, wherein each of the 2 variable regions of the CH3-domain comprising polypeptide chains recognizes a different target epitope.

10. The method of claim 9, wherein the different target epitopes are located on the same target molecule.

11. The method of claim 10, wherein the target molecule is a soluble molecule.

12. The method of claim 10, wherein the target molecule is a membrane-bound molecule.

13. The method of claim 9, wherein the different target epitopes are located on different target molecules.

14. The method of claim 13, wherein the different target molecules are expressed on the same cells.

15. The method of claim 13, wherein the different target molecules are expressed on different cells.

16. The method of claim 13, wherein the different target molecules are soluble molecules.

17. The method of claim 13, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

18. The method of claim 8, wherein at least one of said target epitopes is located on a tumor cell.

19. The method of claim 8, wherein at least one of said target epitopes is located on an effector cell.

20. The method of claim 19, 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.

21. The method of claim 19, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

22. The method according claim 1, wherein said heterodimeric Ig-like molecule is an antibody.

23. A heterodimeric Ig-like molecule obtainable by the methods according to the method of claim 1.

24. A heterodimeric Ig-like molecule according to claim 23, wherein said heterodimeric Ig-like molecule binds to different epitopes on the same antigen or to different epitopes on different antigens.

25. A heterodimeric Ig-like molecule according to claim 23, wherein said Ig-like molecule is an antibody.

26. 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.

27. 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.

28. A recombinant host cell according to claim 27, wherein said host cell further comprises a nucleic acid sequence encoding a common light chain.

29. A pharmaceutical composition comprising a heterodimeric Ig-like molecule according to claim 23 and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising a heterodimeric antibody according to claim 26, and a pharmaceutically acceptable carrier.

31. 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.

32. A method according to claim 31, further comprising the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.

33. The method according to claim 22 wherein antibody is human IgG.

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.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

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.

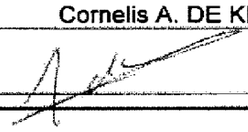
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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): 25 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)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

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
- ☒ 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: 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 (08-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

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.

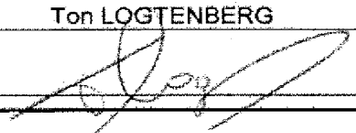
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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: 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.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B or equivalent) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5. If the Power of Attorney by Applicant form is not accompanied by this transmittal form or an equivalent, the Power of Attorney will not be recognized in the application.

Application Number	Not Yet Assigned
Filing Date	November 15, 2013
First Named Inventor	Cornelis A. DE KRUIF
Title	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	MRX5-007CN

SIGNATURE of Applicant or Patent Practitioner

Signature	/Cynthia L. Kanik/	Date	November 15, 2013
Name	Cynthia L. Kanik, Ph.D., J.D.	Telephone	(617) 202-4623
Registration Number	37,320		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications.

[X] *Total of 1 forms are submitted.

Doc Code: PA
Document Description: Power of Attorney

PTO/AIA/82B (07-12)
Approved for use through 11/30/2014 OMB 0651-0035
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
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

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.

[X] I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent): 00959

OR

[ ] I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

Name	Registration Number	Name	Registration Number

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

[X] The address associated with the above-mentioned Customer Number:

OR

[ ] The address associated with Customer Number:

OR

[ ] Firm or Individual Name	Jane E. Remillard, Esq. NELSON MULLINS RILEY & SCARBOROUGH LLP				
Address	One Post Office Square				
City	Boston	State	MA	Zip	02109-2127
Country	US				
Telephone	(800) 237-2000	Email	ipboston.docketing@nelsonmullins.com		

I am the Applicant:

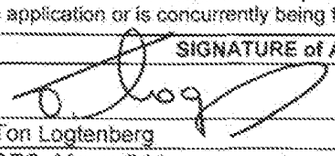
[ ] Inventor or Joint Inventor

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign

[ ] Person Who Otherwise Shows a Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

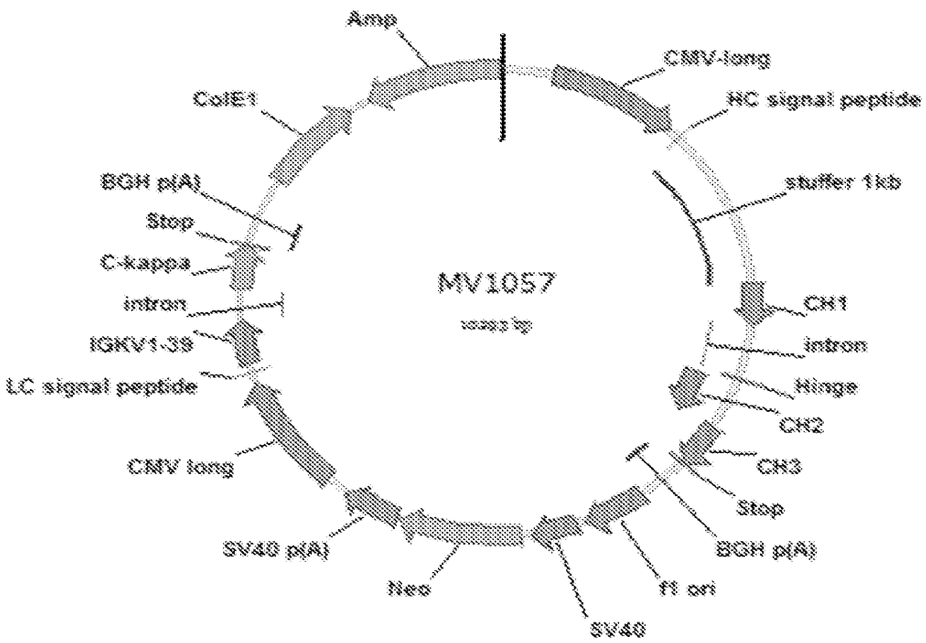
Signature		Date	May 2, 2013
Name	Ton Logtenberg	Telephone	+31302536800
Title and Company	CEO, Merus B.V.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature, see below *.

[X] *Total of 1 forms are submitted.

Figure 1:

A) schematic representation of construct vector MV1057



B) schematic representation of phage display vector MV1043

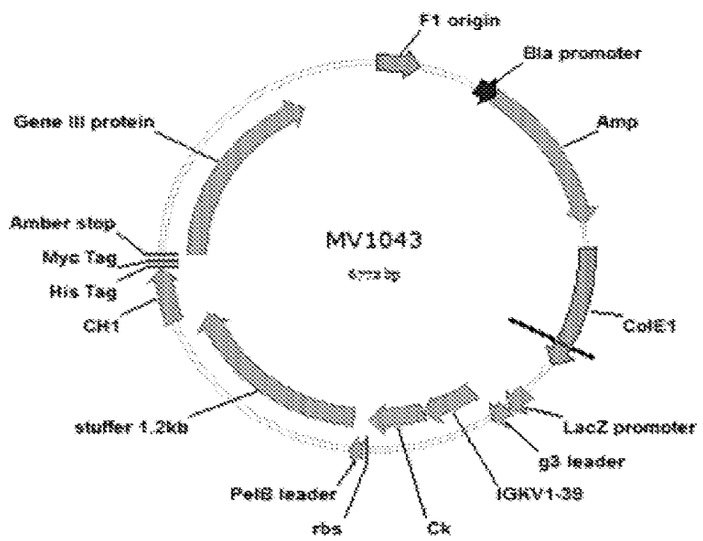


Figure 2: amino acid sequence of wildtype IgG1 Fc, as present in construct vector MV1057 (EU numbering scheme applied)

131 141 151 161 171 181

*SSK*STSGGTA 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

Figure 3: nucleotide and amino acid sequences of VH regions used

MF1025_VH
gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcag
cctctggattcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtg
ggtctcagctattagtggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtgt
attactgtgcaagggccgattggtgggcgacttttgactactggggccaaggtaccctggtcacc

MF1025_VH
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARADWWATFDYWGQGTLVT

MF1122_VH
gaggtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctgagactctcctgtgcag
cctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctggagtg
ggtggcagttatatcatatgatggaagtaataaatactatgcagactccgtgaagggccgattcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggccgtgt
attactgtgcaagagccctcttcacgaccatcgccatggactattggggccaaggtaccctggtcacc

MF1122_VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARALFTTIAMDYWGQGTLVT

MF1337 VH
gaggtgcagctggtggagactggggctgaggtgaagaagccgggggcctcagtgaaggtctcctgcaaggcttct
gactacatcttcaccaaatatgacatcaactgggtgcgccaggcccctggacaagggcttgaatggatgggatgg
atgagcgctaacactggaaacacgggctatgcacagaagttccagggcagagtcaccatgaccagggacacgtcc
ataaacacagcctacatggagctgagcagcctgacatctggtgacacggccgtttatttctgtgcgaggagtagt
cttttcaagacagagacggcgccctactatcacttcgctctggacgtctggggccaagggaccacggtcacc

MF1337
VHEVQLVETGAEVKKPGASVKVSCKASDYIFTKYDINWVRQAPGQGLEWMGWMSANTGNTGYAQKFQGRVTMTRD
TSINTAYMELSSLTSGDTAVYFCARSSLFKTETAPYYHFALDVWGQGTTVT

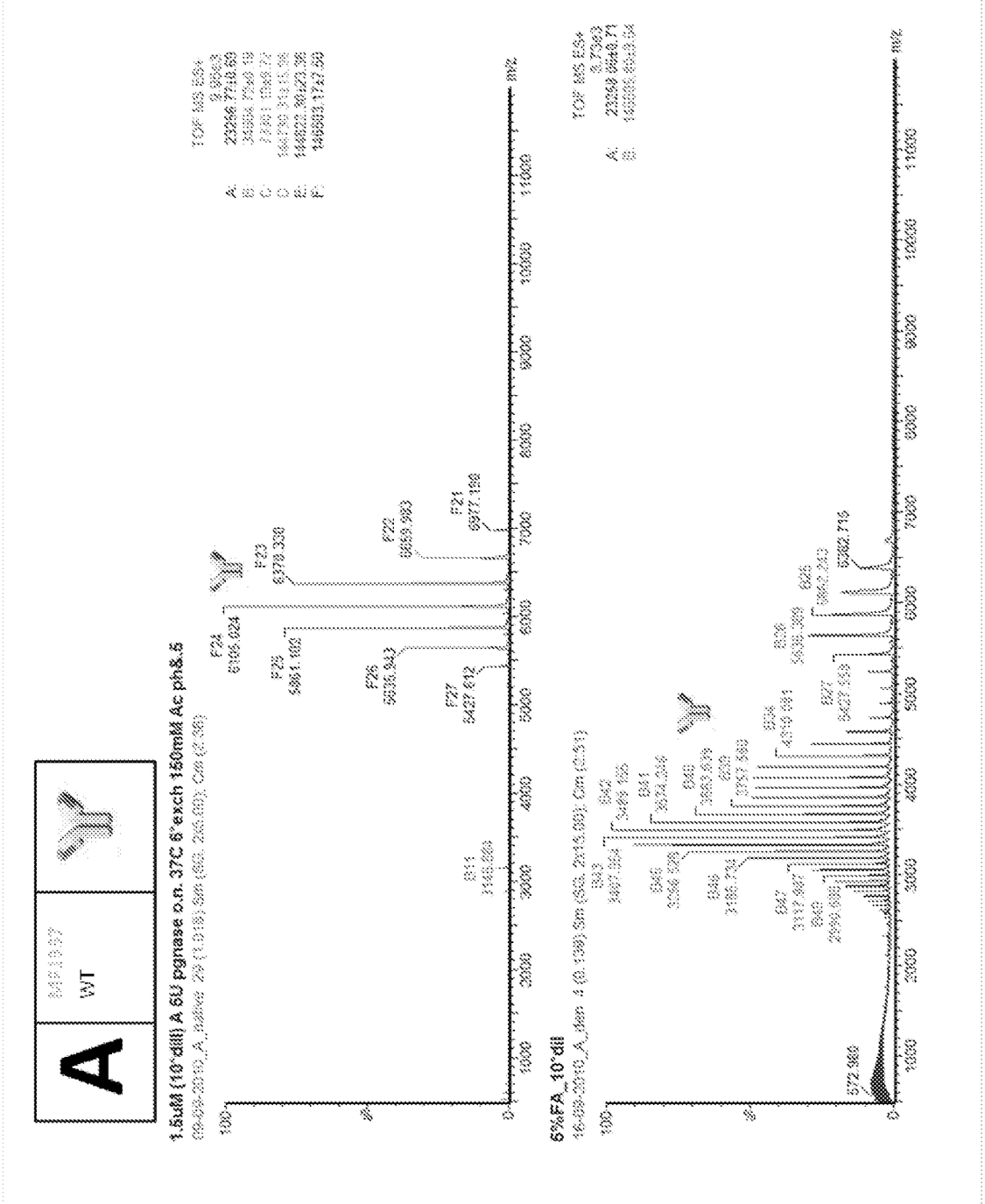

A
IMP1007
WT
1.5uM (10^diil) A 6U pgnase o.n. 37C 6'exch 150mM Ac ph6.5
16-09-2010_A_native 29 (1.019) Sm (SG, 2x6.00) Cm (2:36)
TOF MS ES+
6.69e3
A: 23256.710.69
B: 24554.230.19
C: 7761.1561.77
D: 64756.310.56
E: 144822.3642.36
F: 146883.1747.50
F22
6105.624
F23
6278.336
F25
5961.182
F22
6859.993
F21
6977.156
F26
6535.943
F27
6427.612
B11
1442.666
m/z
6%FA_10^diil
16-09-2010_A_Men 4 (0.139) Sm (SG, 2x15.00) Cm (2:31)
TOF MS ES+
8.73e3
A: 23259.664.71
B: 145205.603.34
B10
3497.554
B6
3446.765
B11
3024.204
B9
2869.979
B8
2737.569
B5
2538.709
B4
2185.714
B3
2019.881
B2
1908.399
B7
1427.559
B1
1442.243
B12
1362.263
B13
1282.716
572.966
m/z

Fig. 4-1

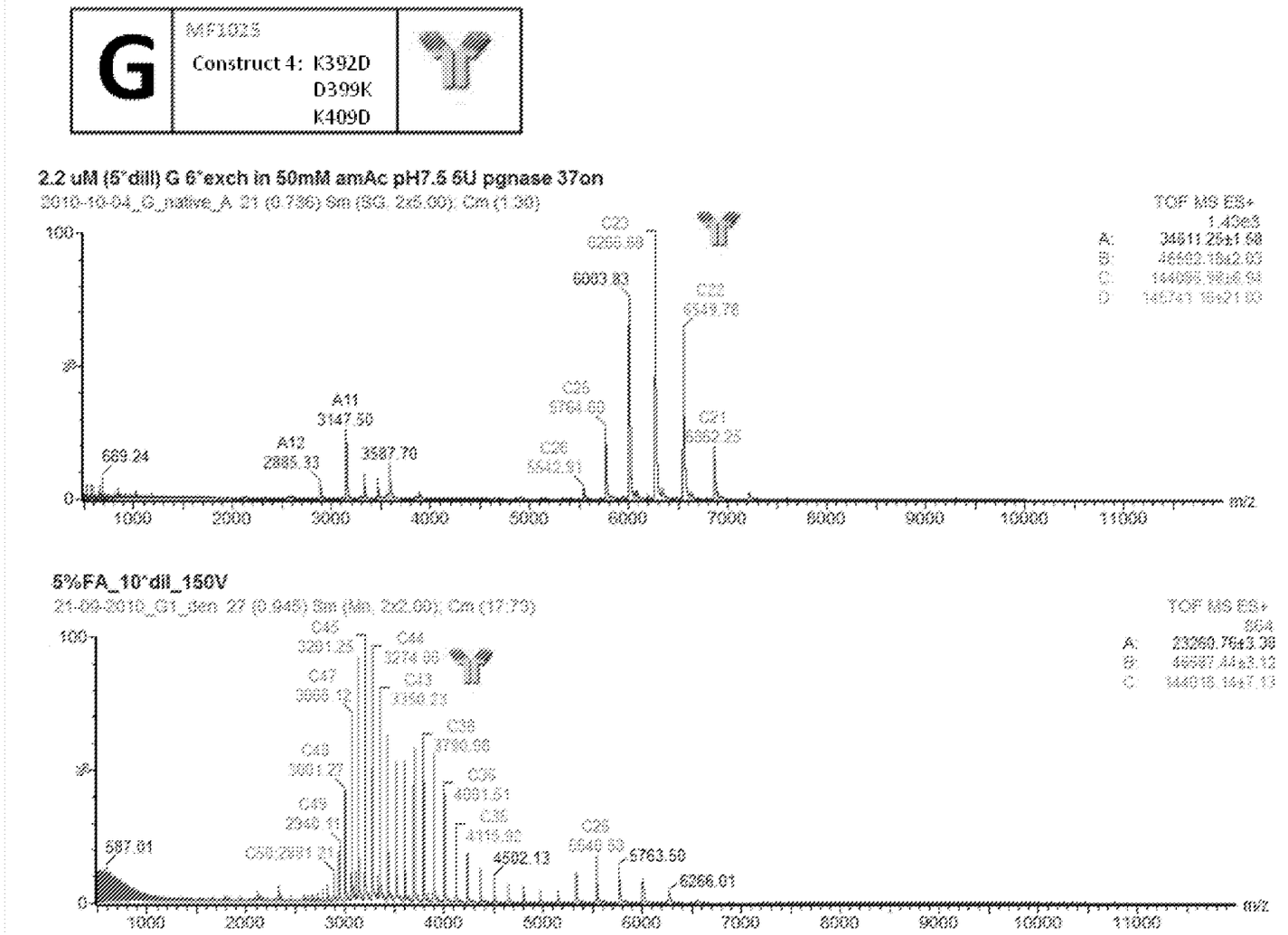

G
MF1025
Construct 4: K392D
D399K
K409D
2.2 uM (5°dill) G 6°exch in 50mM amAc pH7.5 5U pgnase 37on
TOF MS ES+
A:
B:
C:
D:
A11
3147.50
A12
2885.33
3587.70
669.24
C20
6883.83
C22
6539.76
C21
6952.25
C25
5764.69
C26
5542.59
m/z
5%FA_10°dil_150V
TOF MS ES+
A: 23260.76±3.38
B: 46687.44±8.12
C:
C45
3291.25
C44
3274.99
C47
3666.12
C43
3661.27
C49
2940.11
C50,2881.83
C48
3456.23
C38
3786.56
C36
4031.51
C35
4119.82
C28
5040.59
C37
4502.13
5763.58
6366.01
587.01
m/z

Fig. 4-2

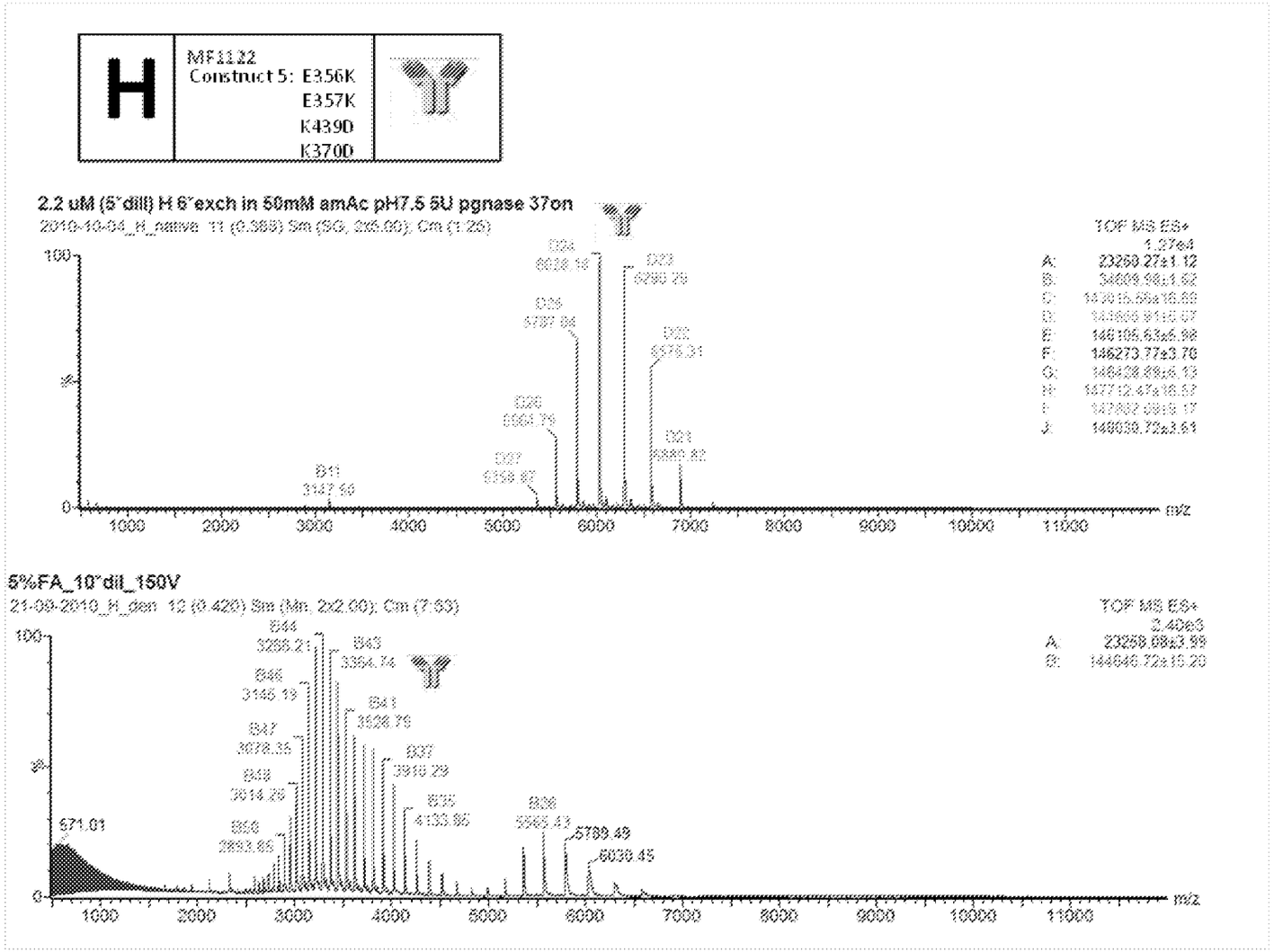

H
MF1122
Construct 5: E356K
E357K
K439D
K370D
2.2 uM (5*dill) H 6*exch in 50mM amAc pH7.5 5U pgnase 37on
2010-10-04_H_native 11 (0.389) Sm (SG, 2x5.00); Cm (1:26)
TOF MS ES+
1.27e4
A: 23260.27±1.12
B: 34609.58±1.62
C: 58815.55±16.89
D: 69569.91±0.07
E: 146105.63±6.98
F: 146273.77±3.76
G: 146428.69±6.13
H: 147712.47±16.57
I: 147857.09±9.17
J: 148039.72±3.61
%
m/z
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: 23259.68±2.99
B: 144046.72±15.20
671.01
5789.49
6039.45
m/z

Fig. 4-3

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

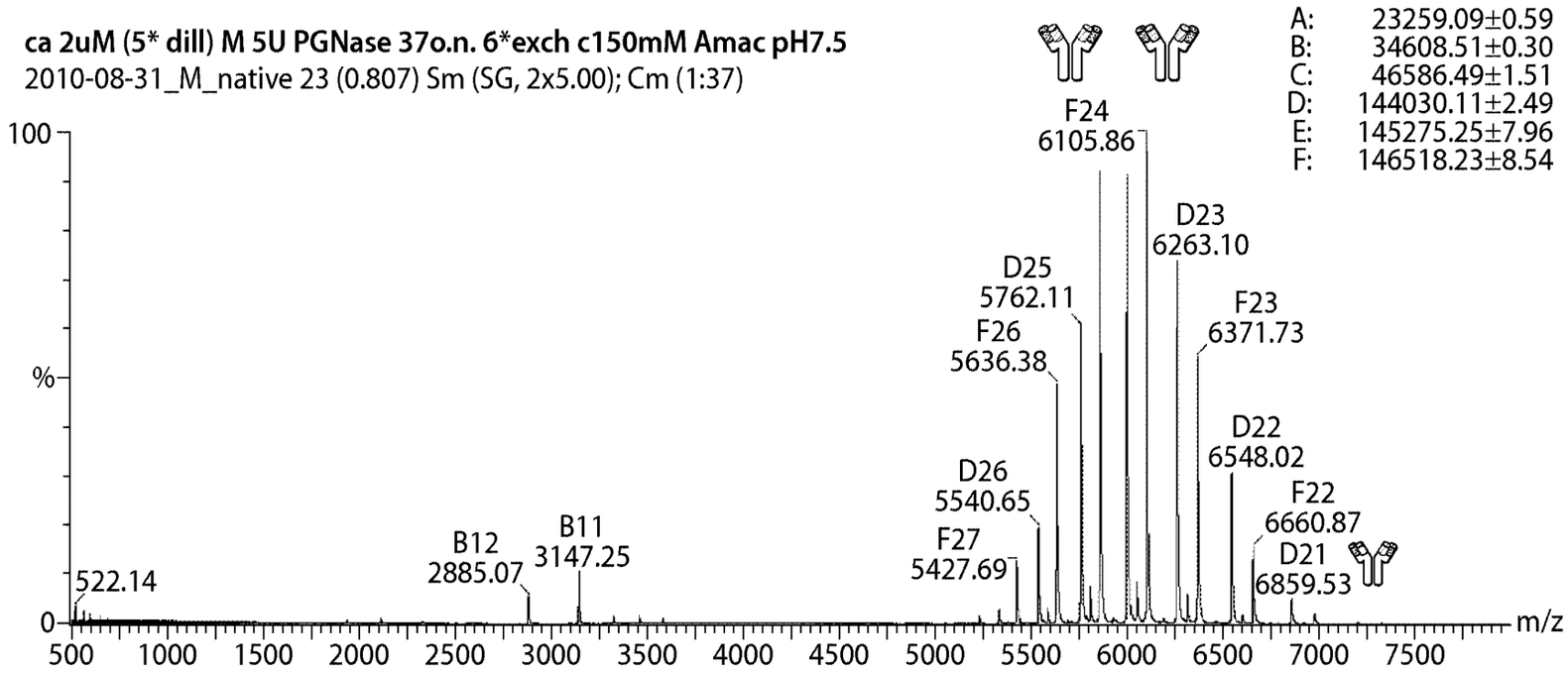


Fig. 5-1

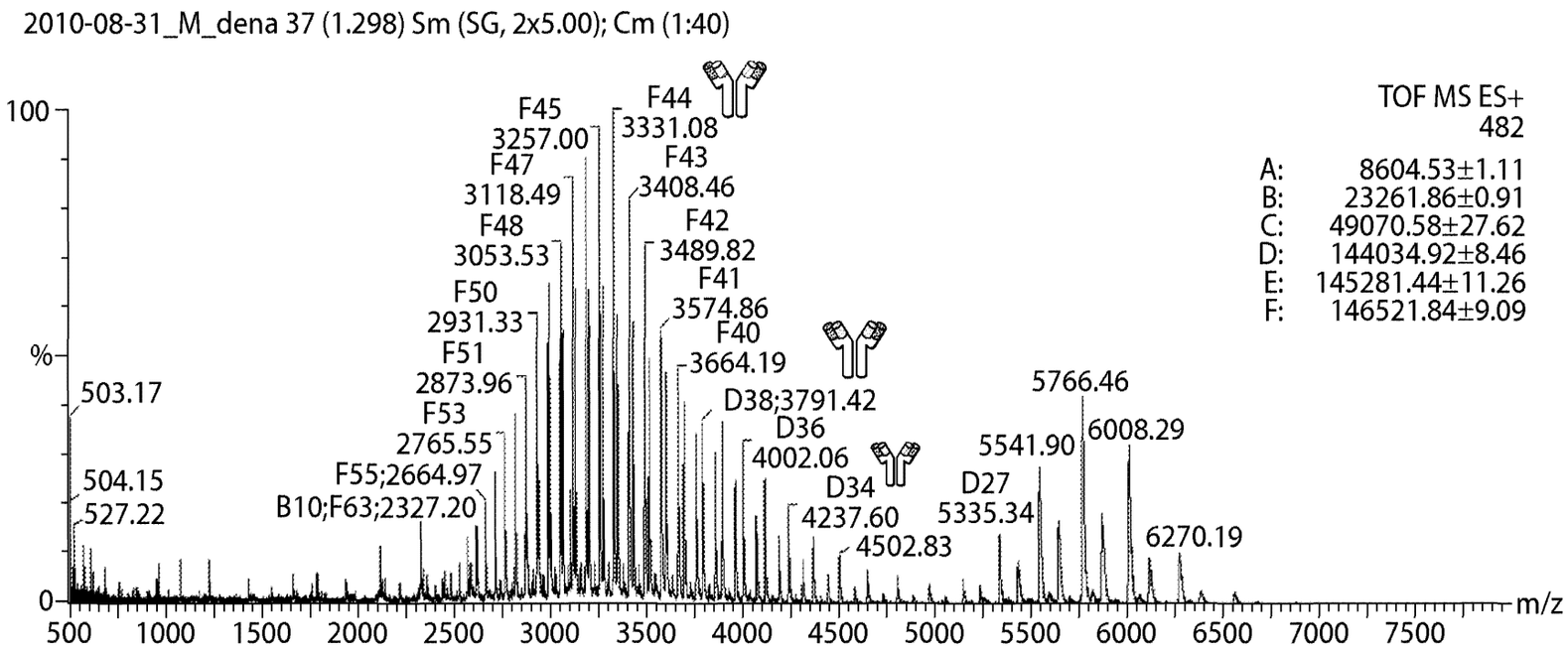

2010-08-31_M_dena 37 (1.298) Sm (SG, 2x5.00); Cm (1:40)
TOF MS ES+
482
A: 8604.53±1.11
B: 23261.86±0.91
C: 49070.58±27.62
D: 144034.92±8.46
E: 145281.44±11.26
F: 146521.84±9.09
100
%
0
m/z
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500
503.17
504.15
527.22
B10;F63;2327.20
F55;2664.97
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. 5-2

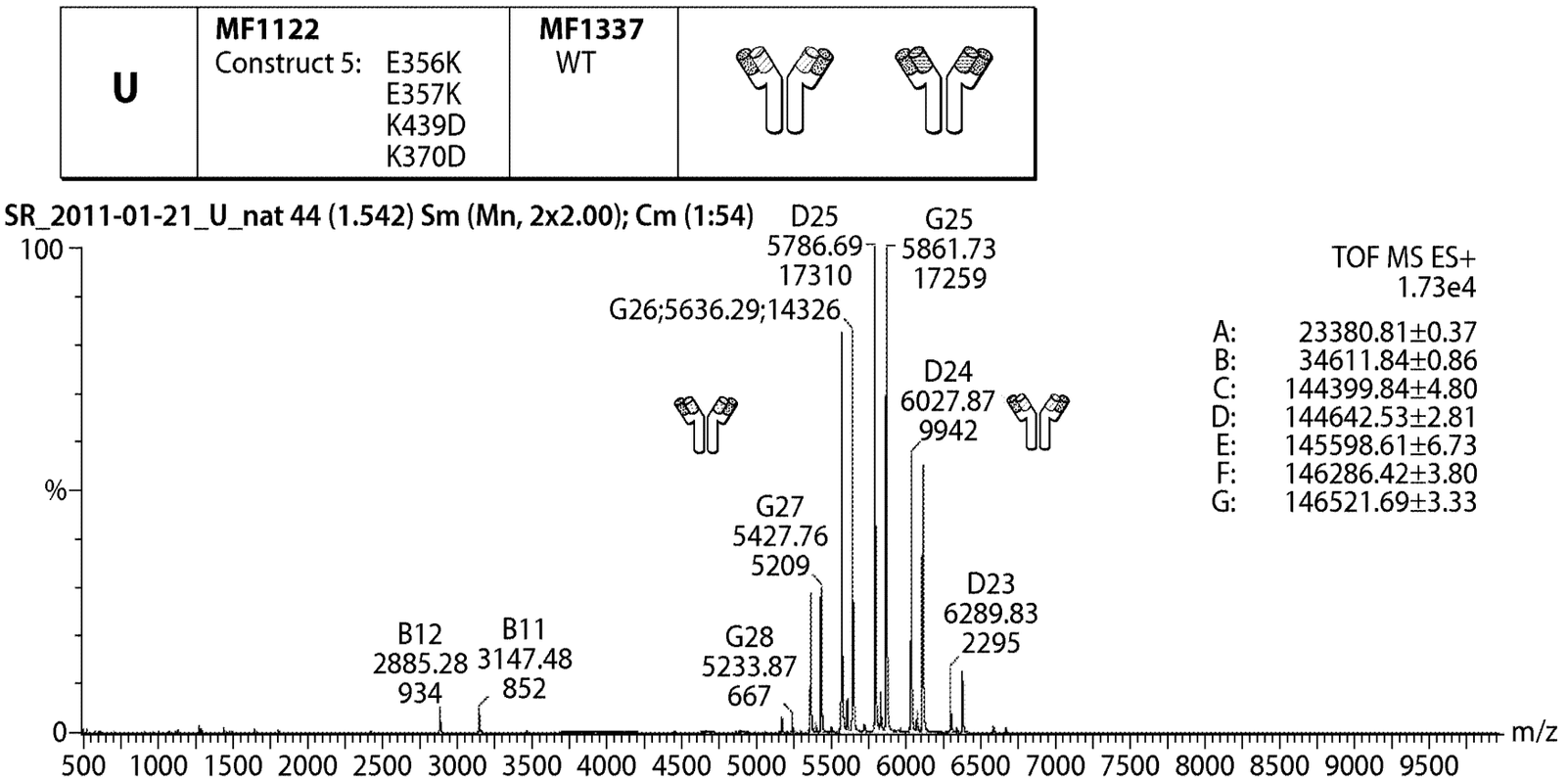

U
MF1122
Construct 5: E356K
E357K
K439D
K370D
MF1337
WT
SR_2011-01-21_U_nat 44 (1.542) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
1.73e4
A: 23380.81±0.37
B: 34611.84±0.86
C: 144399.84±4.80
D: 144642.53±2.81
E: 145598.61±6.73
F: 146286.42±3.80
G: 146521.69±3.33
D25
5786.69
17310
G25
5861.73
17259
G26;5636.29;14326
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. 5-3

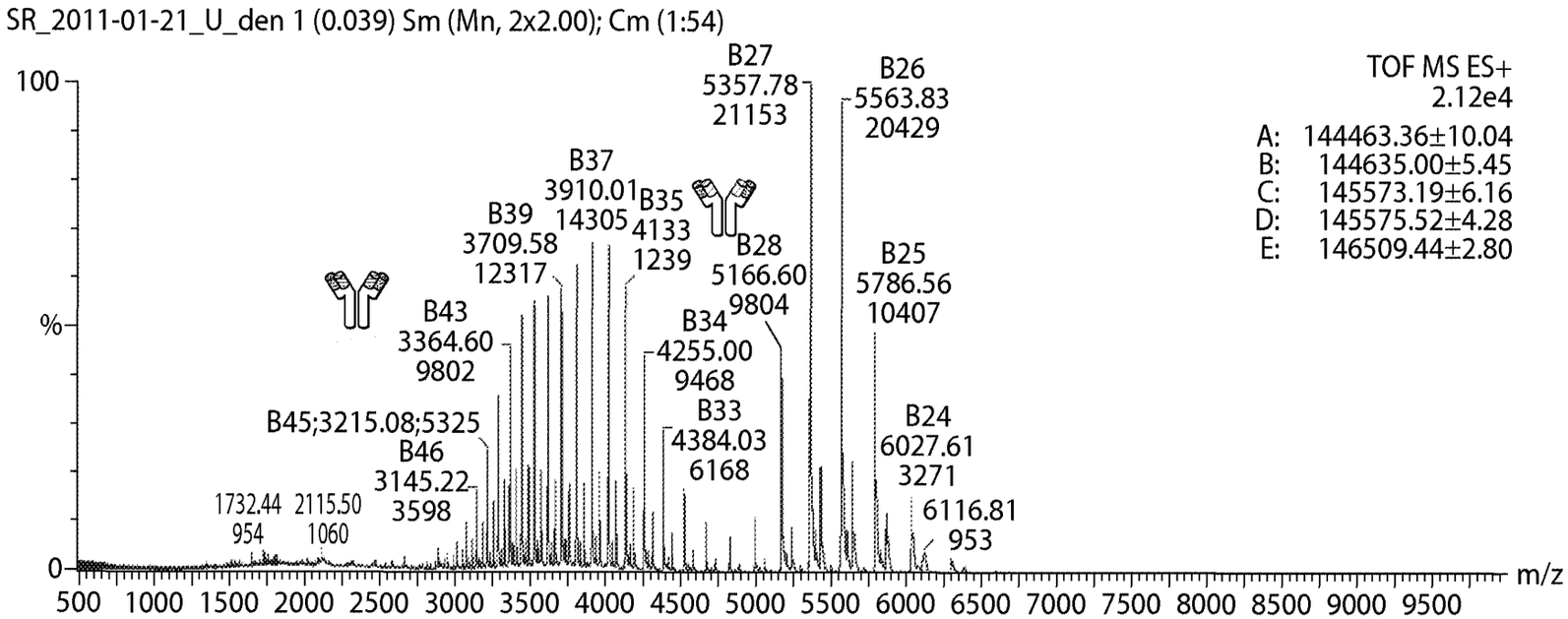

SR_2011-01-21_U_den 1 (0.039) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
2.12e4
A: 144463.36±10.04
B: 144635.00±5.45
C: 145573.19±6.16
D: 145575.52±4.28
E: 146509.44±2.80
B27
5357.78
21153
B26
5563.83
20429
B37
3910.01
14305
B39
3709.58
12317
B35
4133
1239
B28
5166.60
9804
B25
5786.56
10407
B43
3364.60
9802
B34
4255.00
9468
B33
4384.03
6168
B24
6027.61
3271
B45;3215.08;5325
B46
3145.22
3598
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. 5-4

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

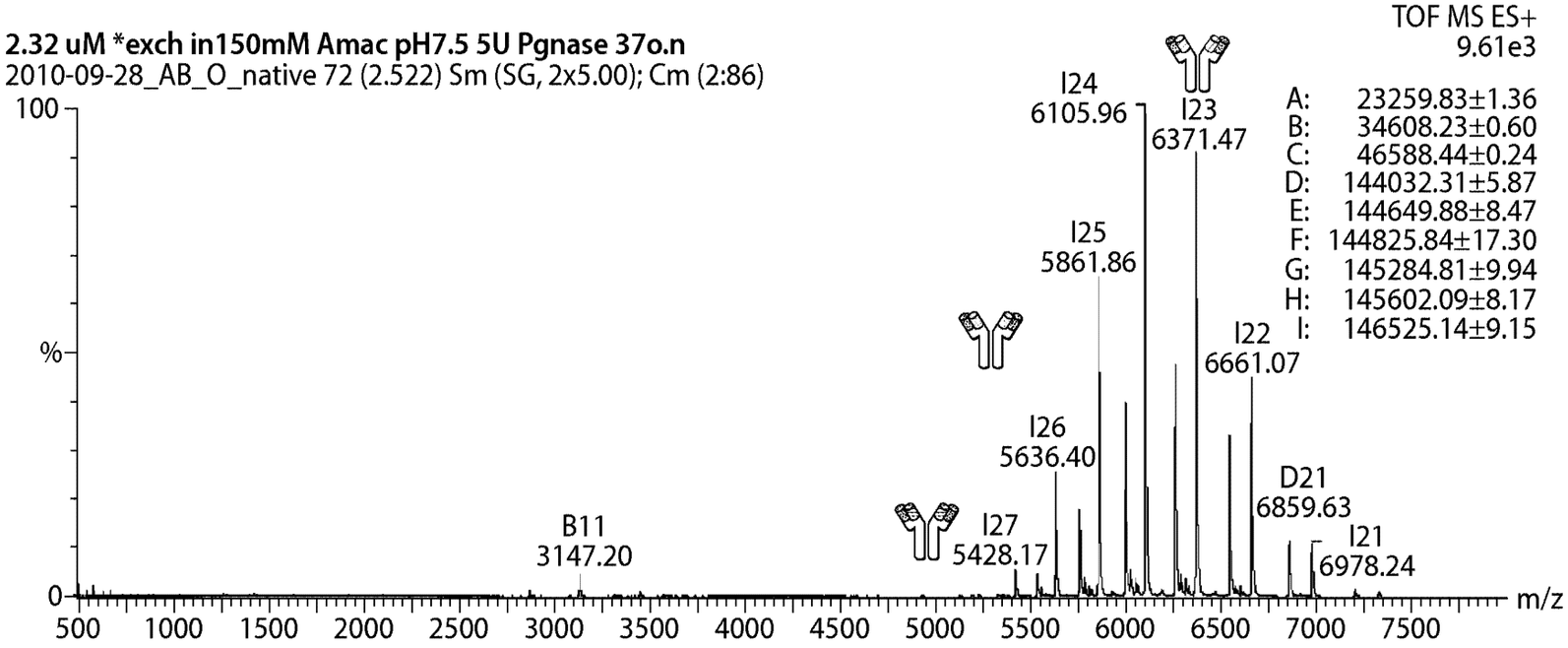


Fig. 6-1

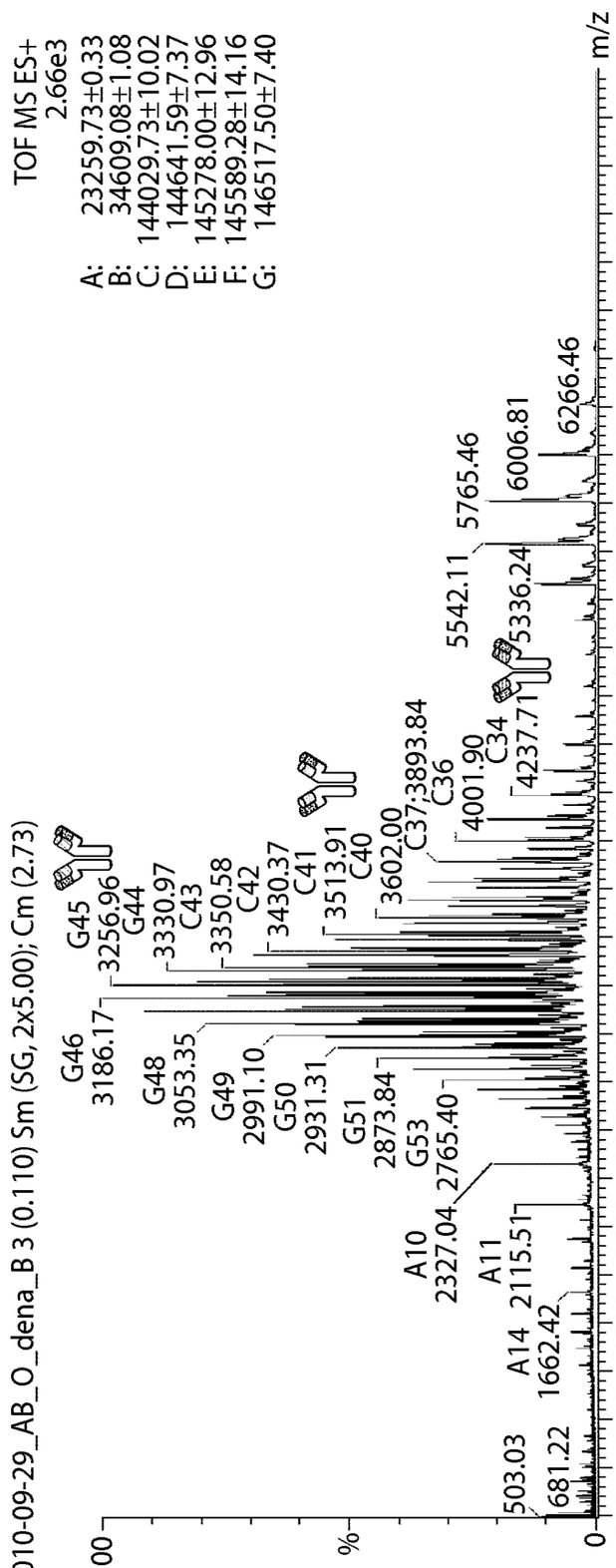

2010-09-29_AB_O_dena_B 3 (0.110) Sm (SG, 2x5.00); Cm (2.73)
TOF MS ES+
2.66e3
A: 23259.73±0.33
B: 34609.08±1.08
C: 144029.73±10.02
D: 144641.59±7.37
E: 145278.00±12.96
F: 145589.28±14.16
G: 146517.50±7.40
100
%
0
503.03
681.22
A14 1662.42
A11 2115.51
A10 2327.04
G53 2765.40
G51 2873.84
G50 2931.31
G49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

Fig. 6-2

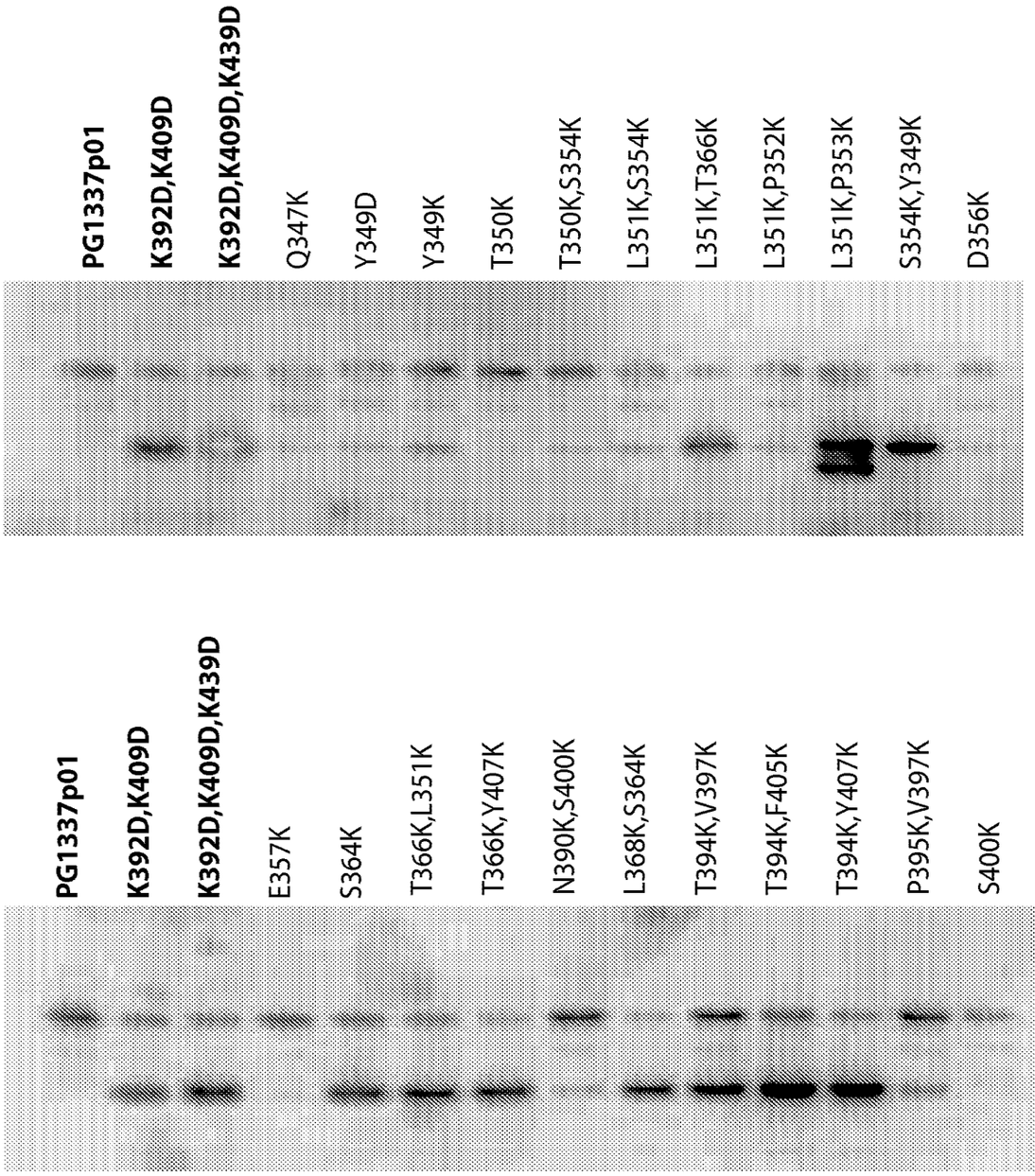

PG1337p01
K392D,K409D
K392D,K409D,K439D
E357K
S364K
T366K,L351K
T366K,Y407K
N390K,S400K
L368K,S364K
T394K,V397K
T394K,F405K
T394K,Y407K
P395K,V397K
S400K
PG1337p01
K392D,K409D
K392D,K409D,K439D
Q347K
Y349D
Y349K
T350K
T350K,S354K
L351K,S354K
L351K,T366K
L351K,P352K
L351K,P353K
S354K,Y349K
D356K

Fig. 7-1

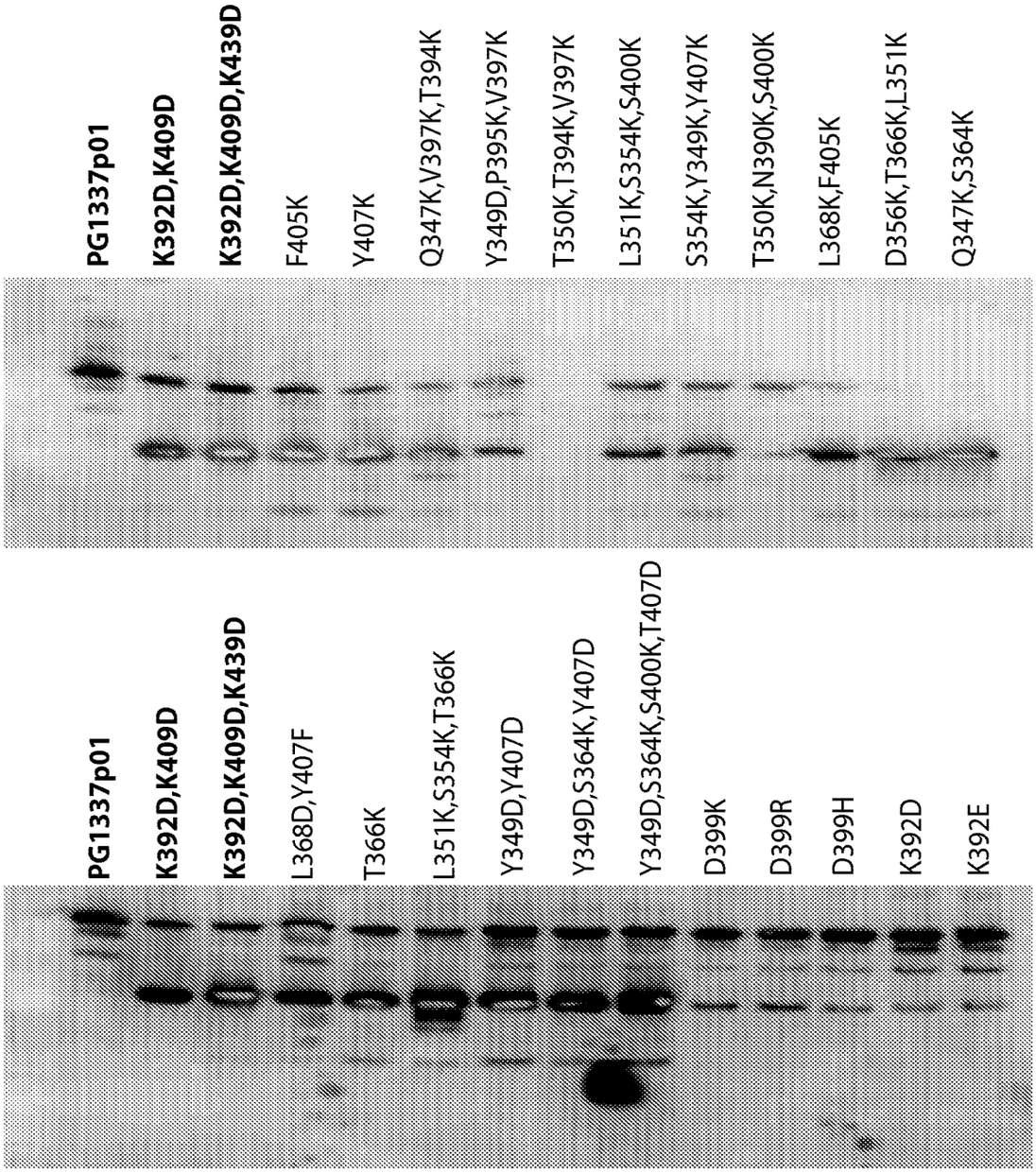

PG1337p01
K392D,K409D
K392D,K409D,K439D
L368D,Y407F
T366K
L351K,S354K,T366K
Y349D,Y407D
Y349D,S364K,Y407D
Y349D,S364K,S400K,T407D
D399K
D399R
D399H
K392D
K392E
PG1337p01
K392D,K409D
K392D,K409D,K439D
F405K
Y407K
Q347K,V397K,T394K
Y349D,P395K,V397K
T350K,T394K,V397K
L351K,S354K,S400K
S354K,Y349K,Y407K
T350K,N390K,S400K
L368K,F405K
D356K,T366K,L351K
Q347K,S364K

Fig. 7-2

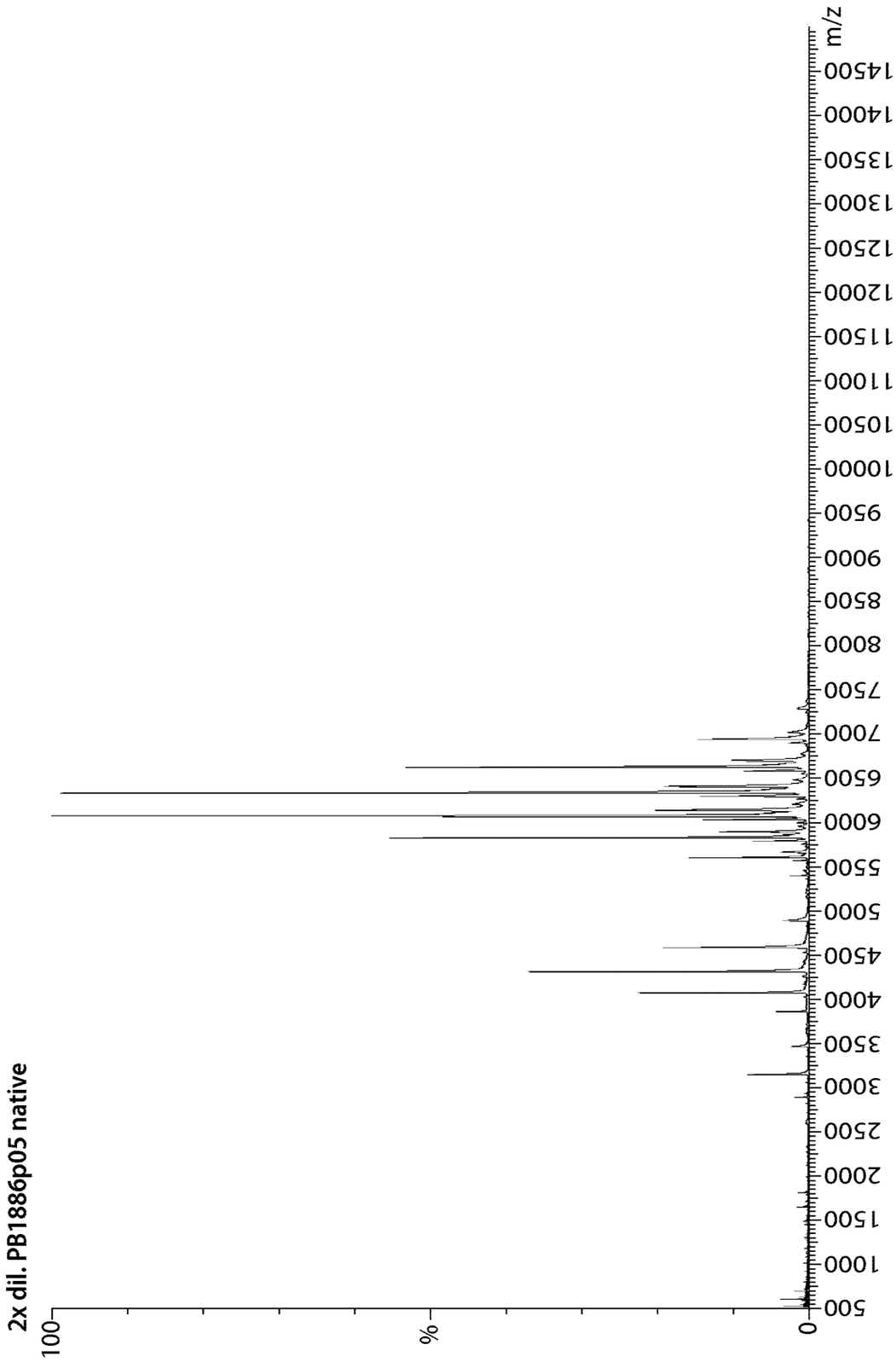
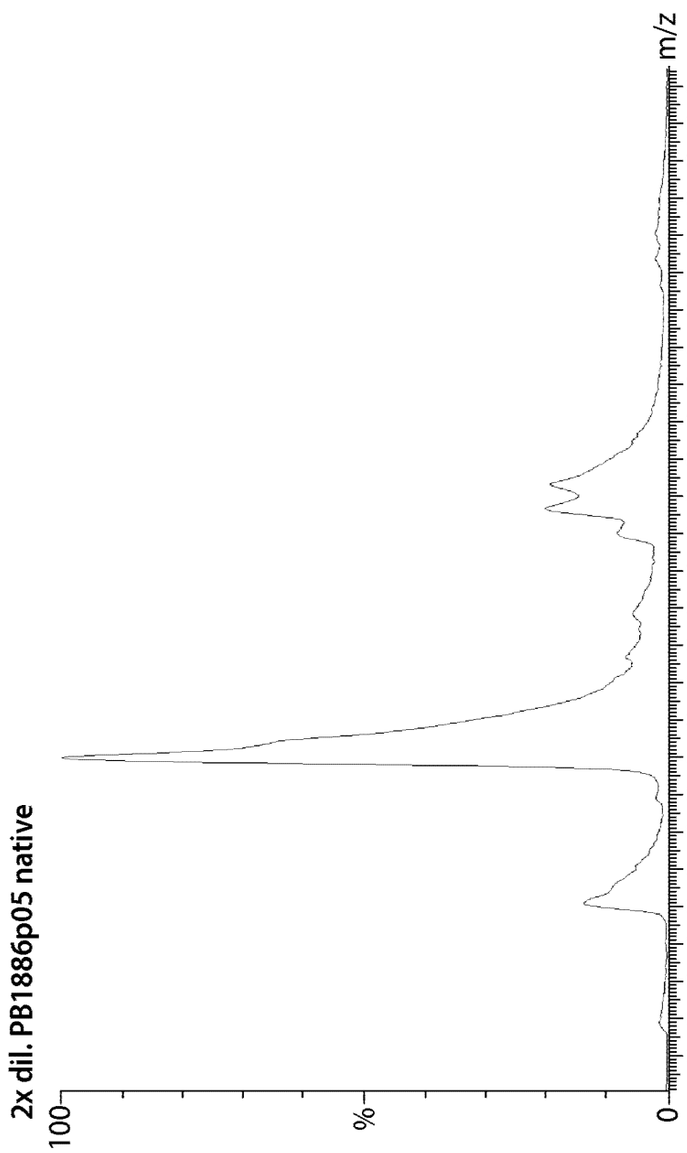

2x dil. PB1886p05 native
100
%
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000
10500
11000
11500
12000
12500
13000
13500
14000
14500
m/z
Native MS spectrum of transfection sample ZO (T366K/L351'D)

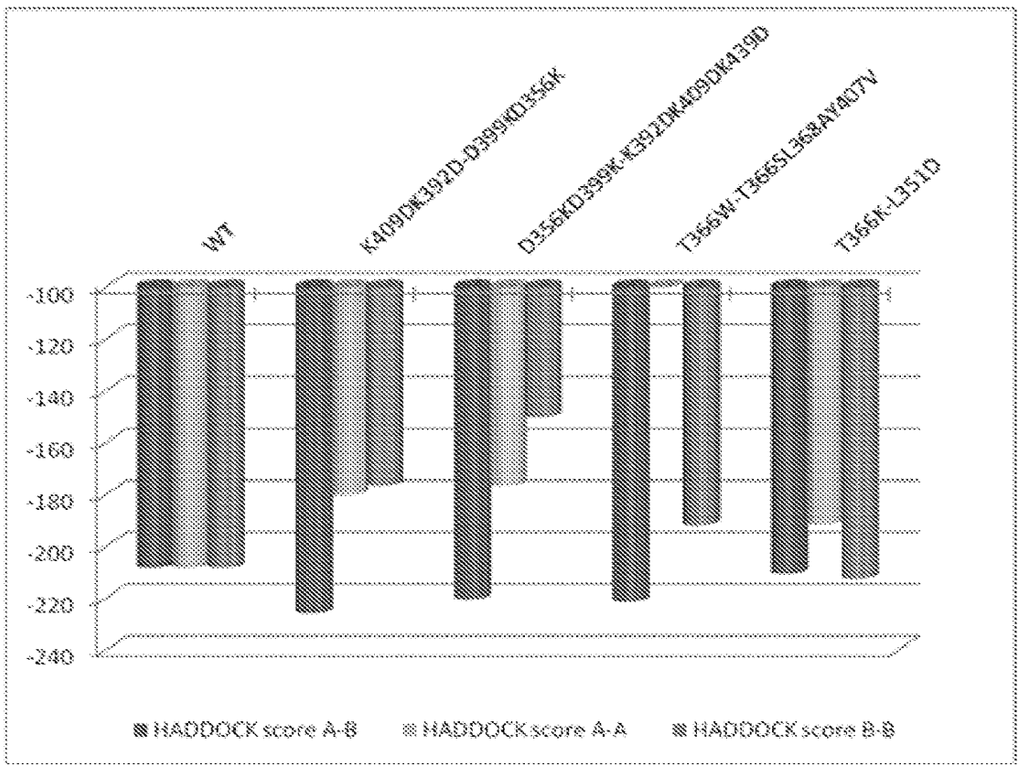
Fig. 8A

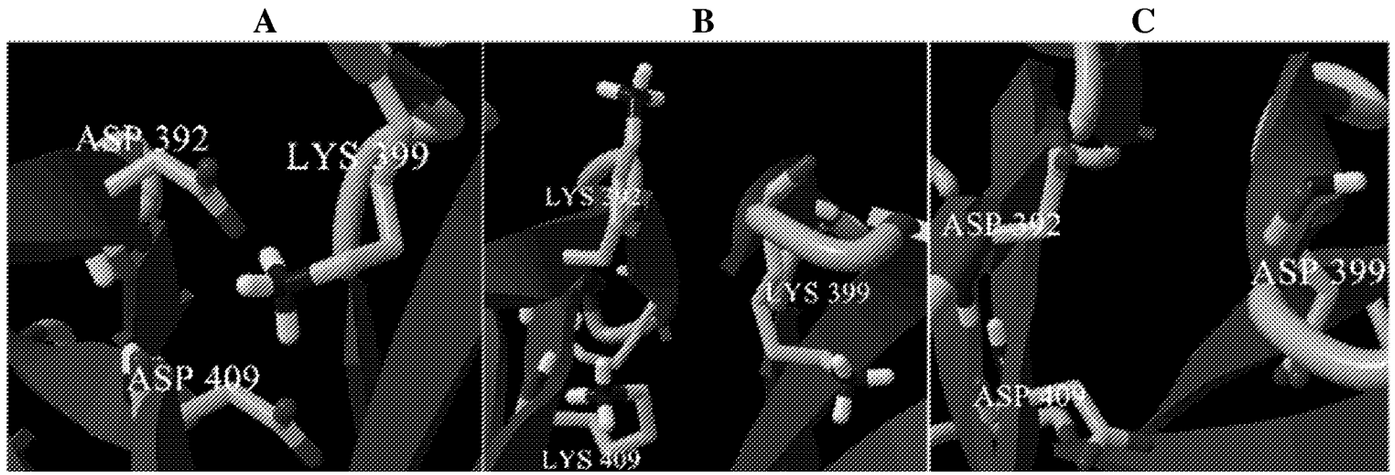


Convoluted MS spectrum of transfection sample ZO (T366K/L351'D).
The second/main peak represents the bispecific molecule.

Fig. 8B

Figure 9:


WT
K409DK392D-D399KD356K
D356KD399K-K391DK409DK439D
T366W-T366SL368AY407V
T366K-L351D
-100
-120
-140
-160
-180
-200
-220
-240
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B



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

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

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

Fig. 10

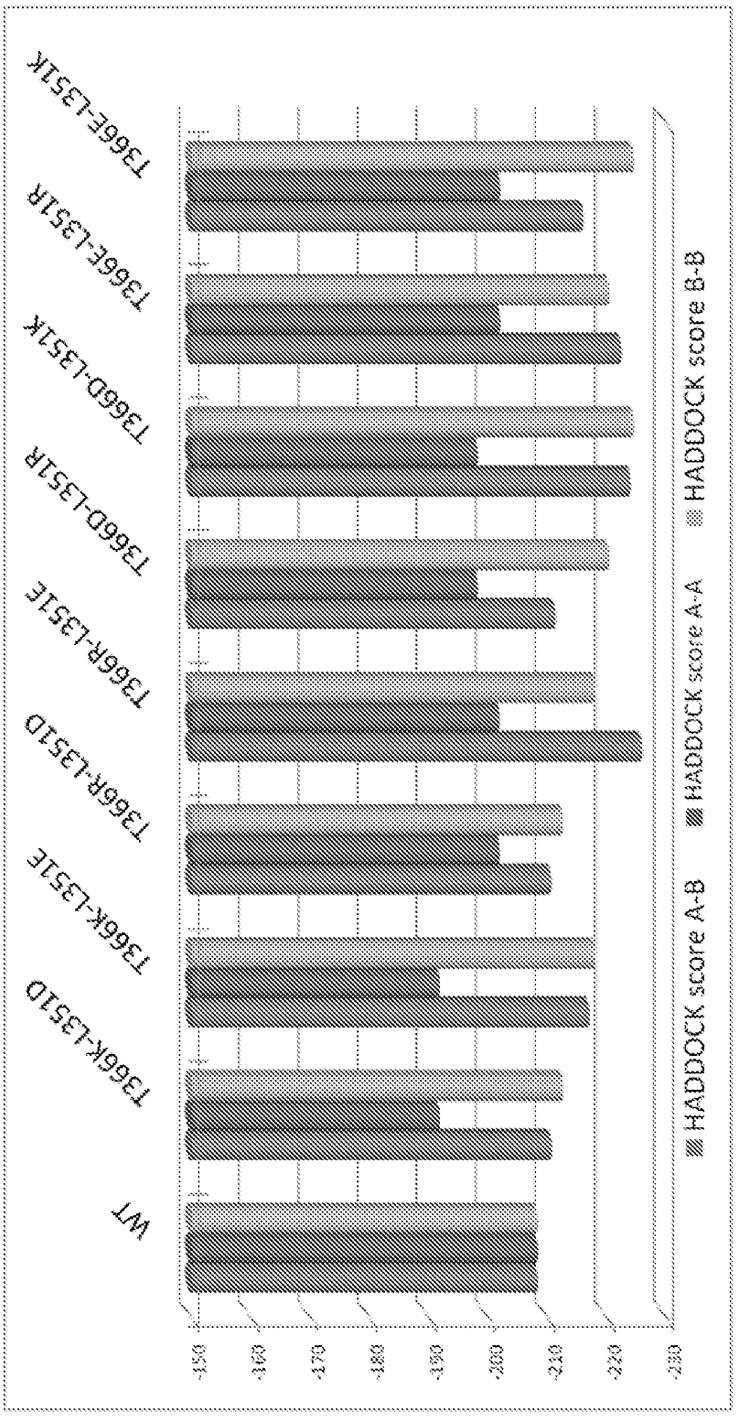

WT
T366K-L351D
T366K-L351E
T366R-L351D
T366R-L351E
T366D-L351R
T366D-L351K
T366E-L351R
T366E-L351K
-350
-360
-370
-380
-390
-400
-410
-420
-430
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig. 11

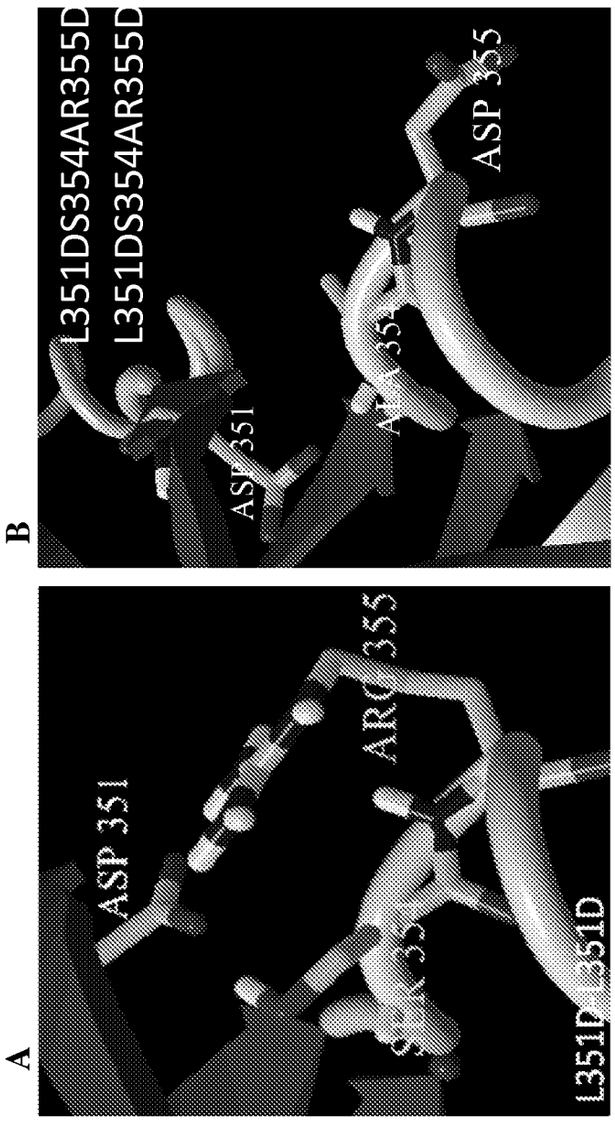

A
ASP 351
ARG 355
L351D
B
L351DS354AR355D
L351DS354AR355D
ASP 351
ASP 355

Fig. 12

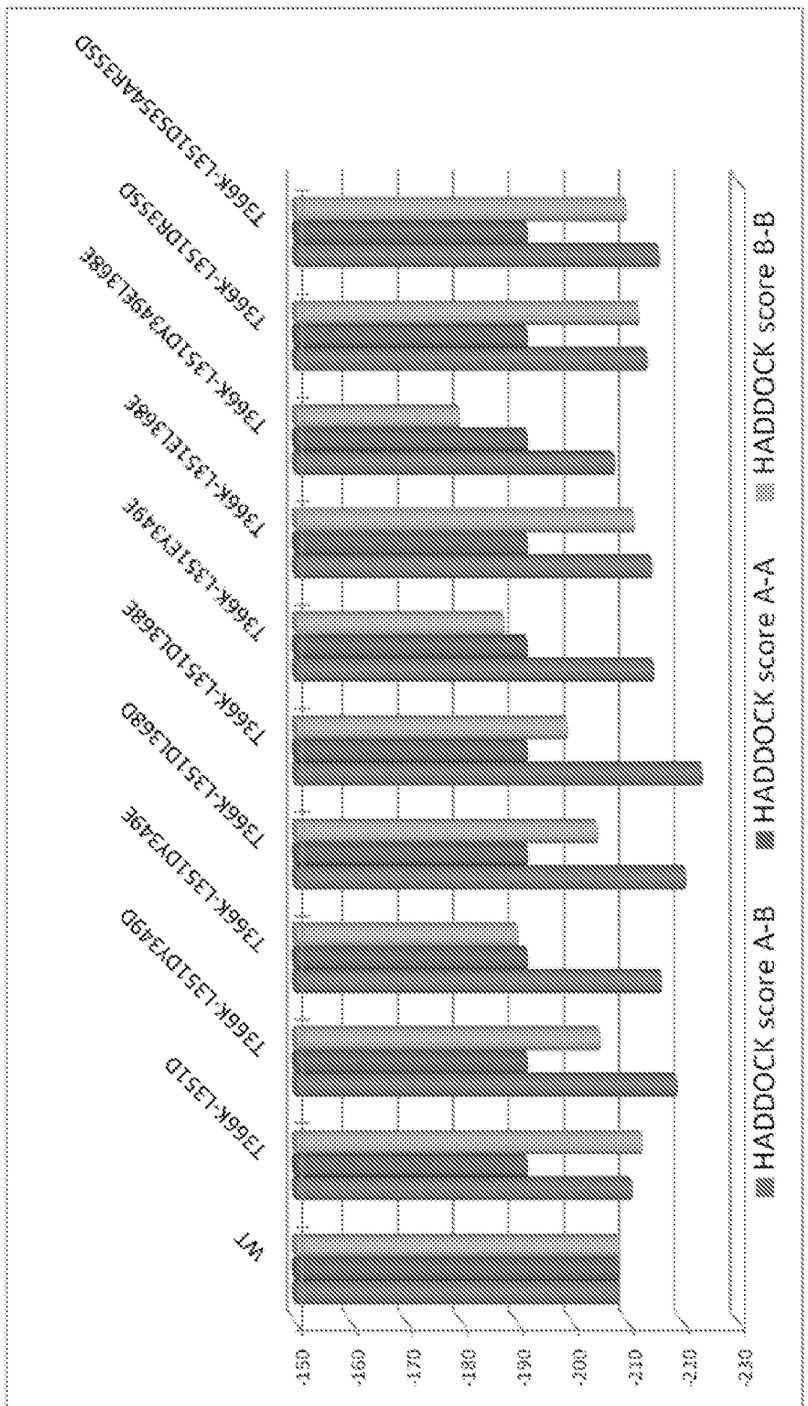

WT
T366K-L351D
T366K-L351DY349D
T366K-L351DY349E
T366K-L351DL368D
T366K-L351DL368E
T366K-L351EY349E
T366K-L351EL368E
T366K-L351DY349EL368E
T366K-L351DR355D
T366K-L351DS354AR355D
-150
-160
-170
-180
-190
-200
-210
-220
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig. 13

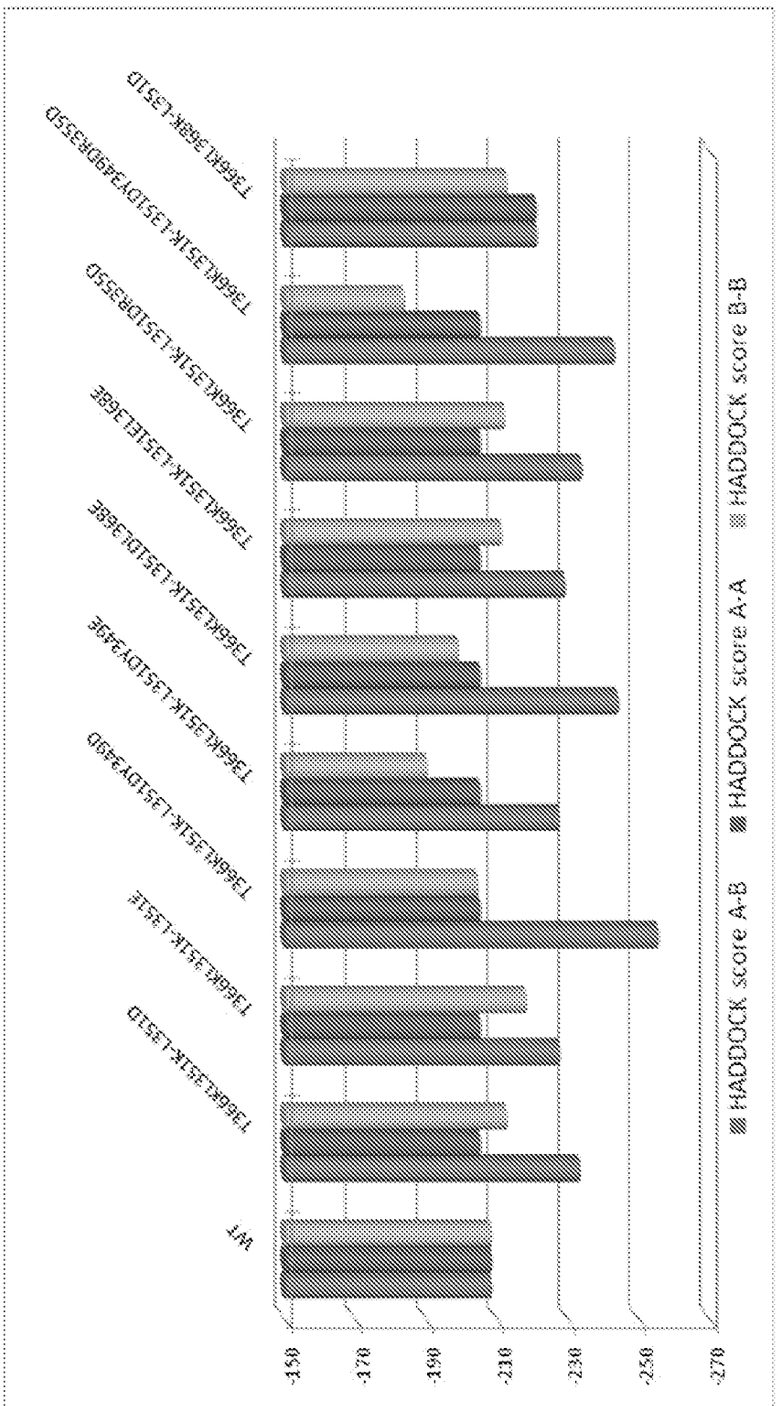

-150
-170
-190
-210
-230
-250
-270
WT
T366K,E351K-L351D
T366K,E351K-L351E
T366K,E351K-L351D,Y349D
T366K,E351K-L351D,Y349E
T366K,E351K-L351D,E368E
T366K,E351K-L351E,E368E
T366K,E351K-L351D,R355D
T366K,E351K-L351D,Y349D,R355D
T366K,E368K-L351D
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig. 14

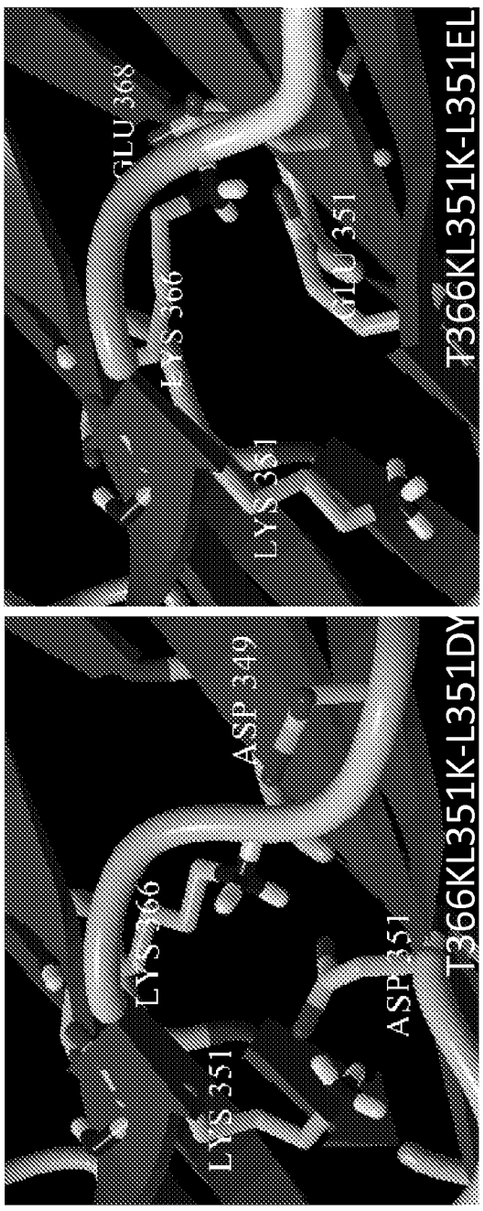

LYS 351
LYS 366
ASP 349
ASP 351
T366KL351K-L351DY
GLU 368
LYS 366
LYS 351
GLU 351
T366KL351K-L351EL

Fig. 15

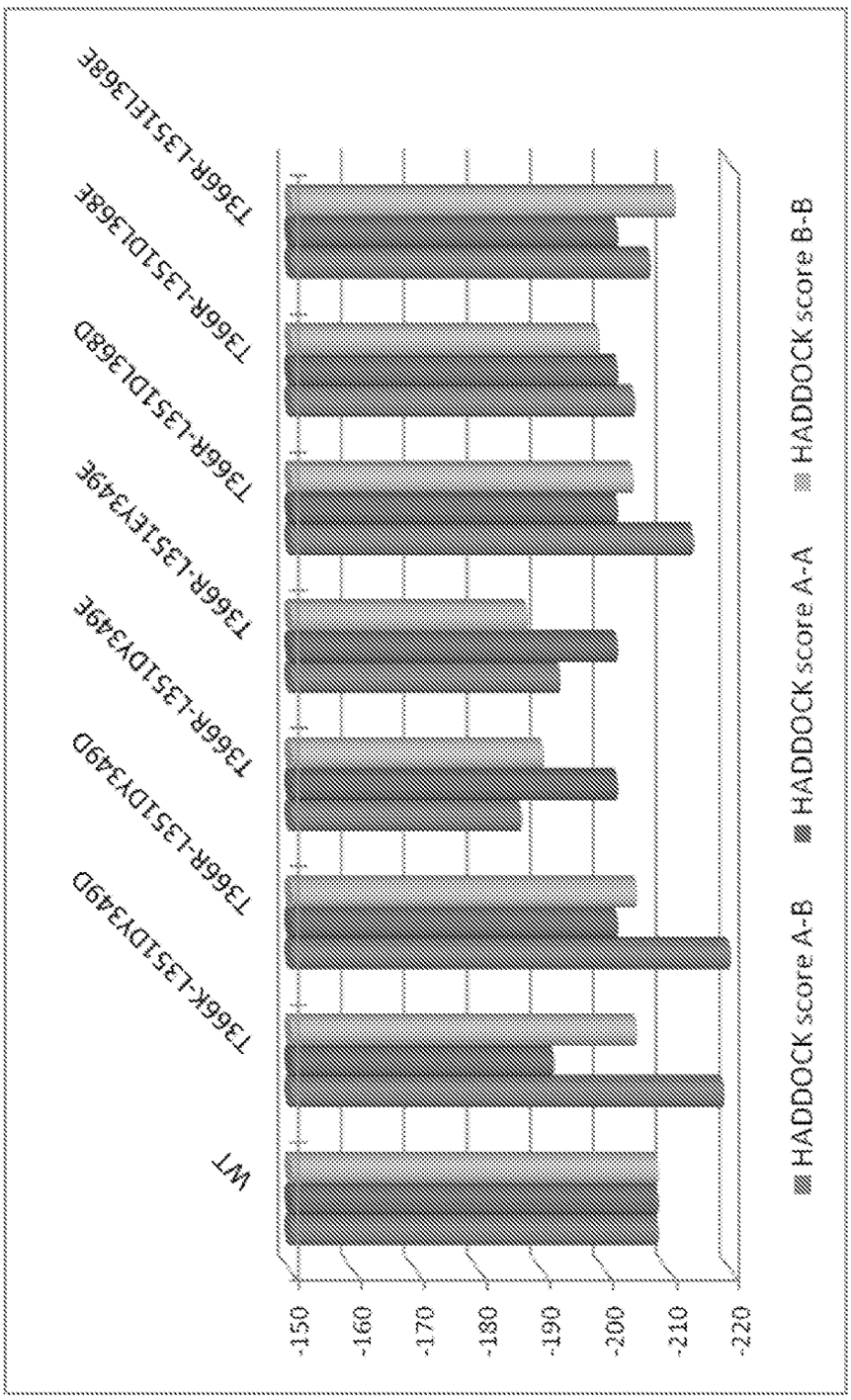

-150
-160
-170
-180
-190
-200
-210
-220
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B
WT
T366K-L351DY349D
T366R-L351DY349D
T366R-L351DY349E
T366R-L351EY349E
T366R-L351DL368D
T366R-L351DL368E
T366R-L351EL368E

Fig. 16

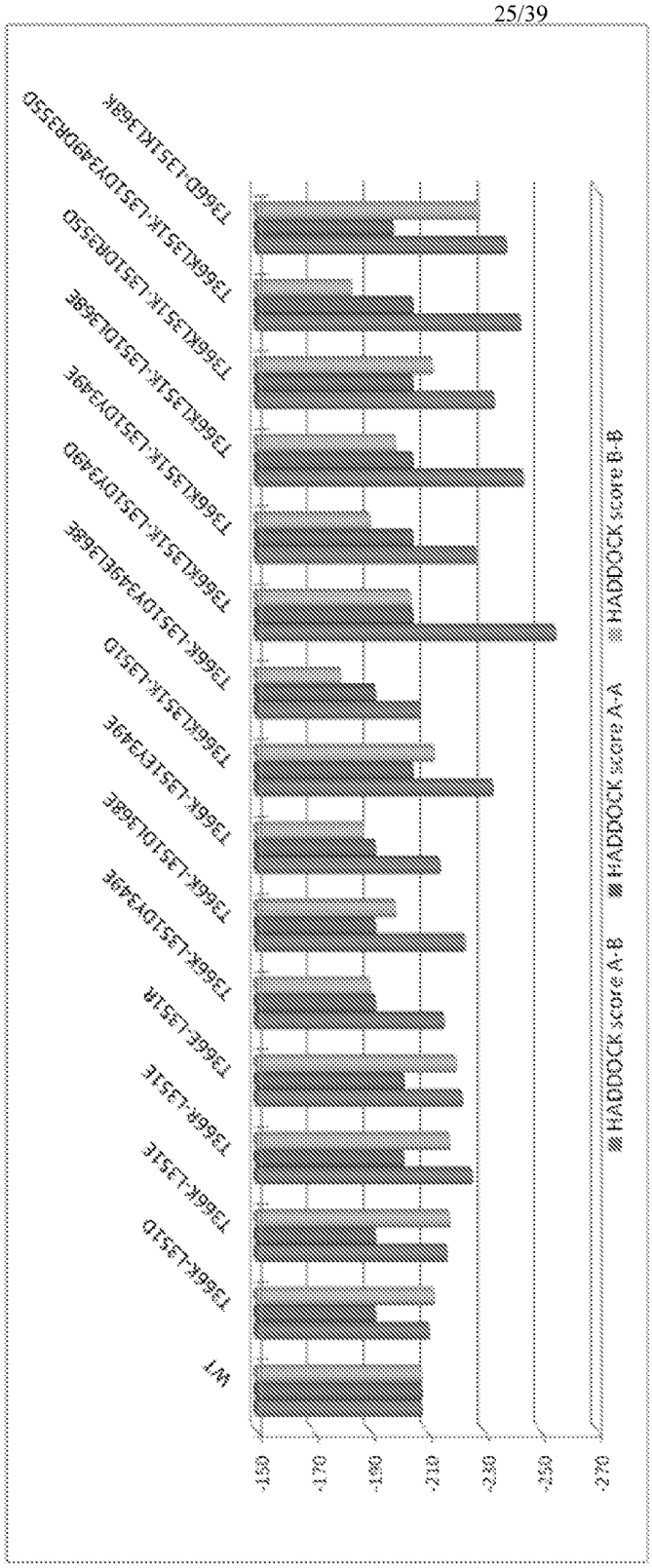

-150
-170
-190
-210
-230
-250
-270
WT
T366K-L351D
T366K-L351E
T366K-L351E
T366K-L351E
T366E-L351R
T366K-L351D-Y349E
T366K-L351D-L368E
T366K-L351E-Y349E
T366K-L351R-L351D
T366K-L351D-Y349E-L368E
T366K-L351R-L351D-Y349D
T366K-L351R-L351D-Y349E
T366K-L351R-L351D-L368E
T366K-L351R-L351D-R355D
T366D-L351K-L368K
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig. 17

Figure 18:

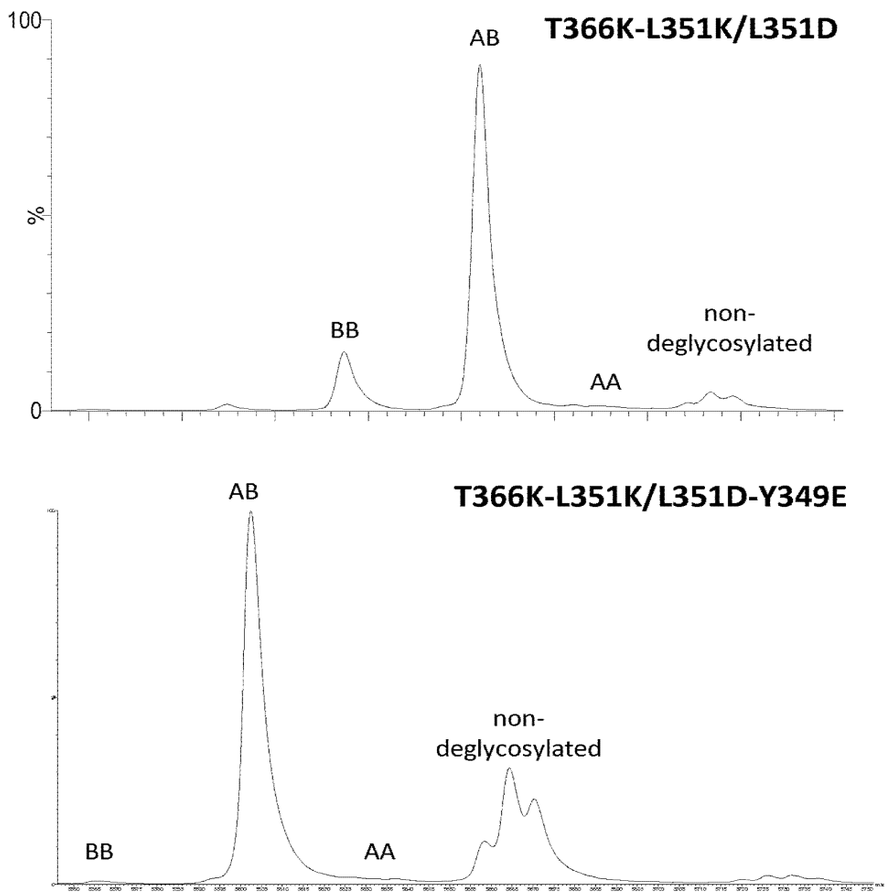

100
%
0
AB
T366K-L351K/L351D
BB
non-
deglycosylated
AA
AB
T366K-L351K/L351D-Y349E
non-
deglycosylated
BB
AA

Figure 19A:

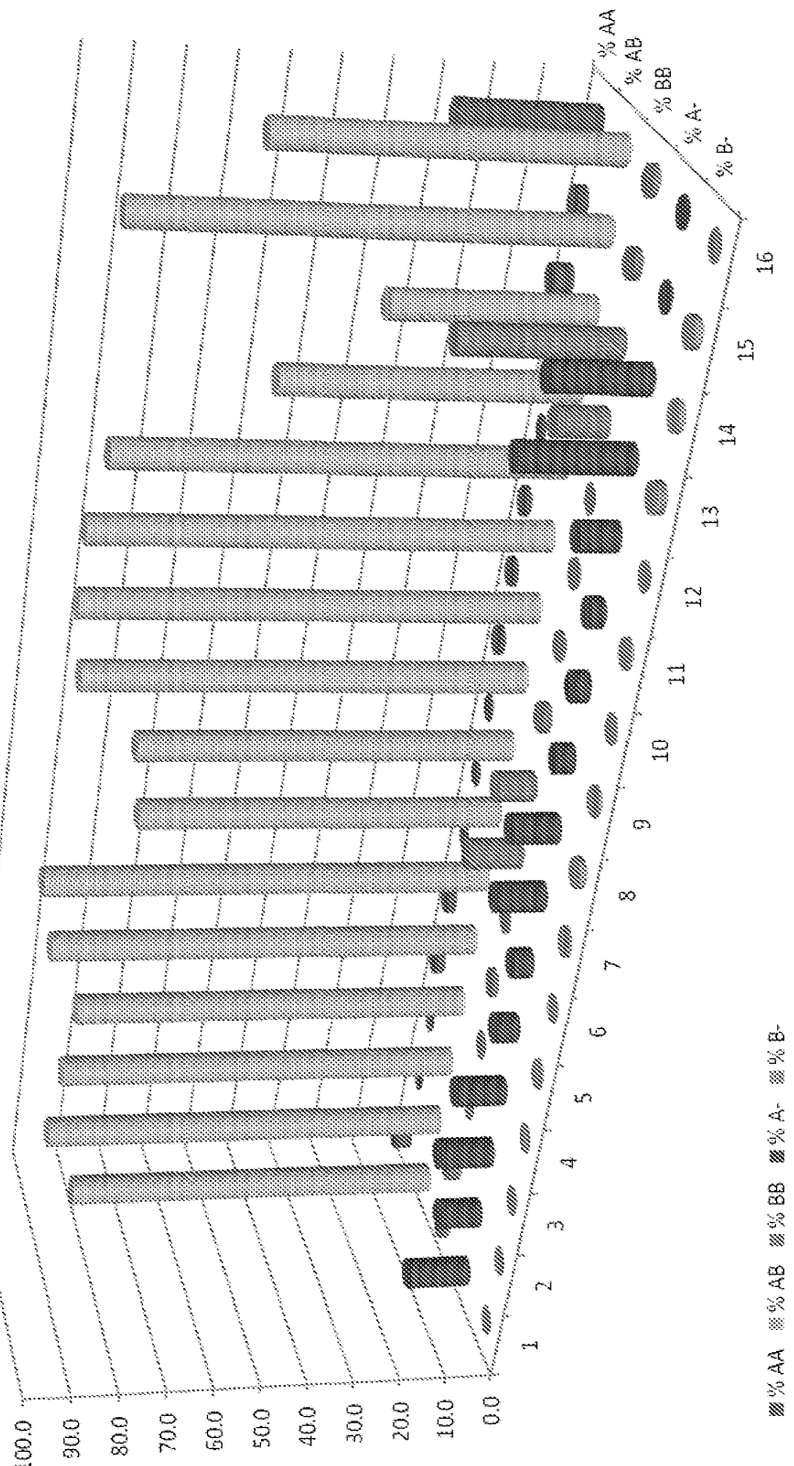

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% AA
% AB
% BB
% A-
% B-
% AA
% AB
% BB
% A-
% B-

Figure 19B:

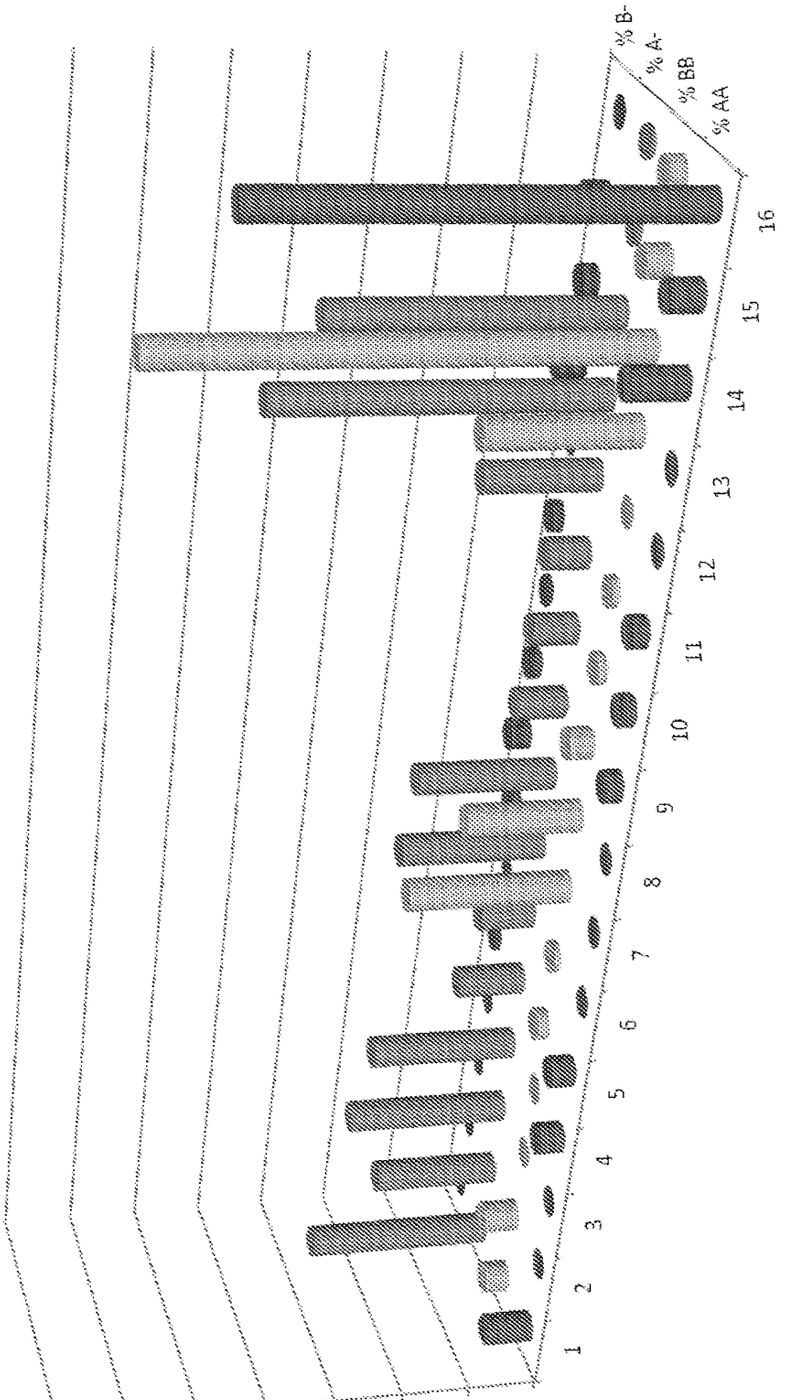

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% B-
% A-
% BB
% AA
% AA
% BB
% A-
% B-

Figure 20:

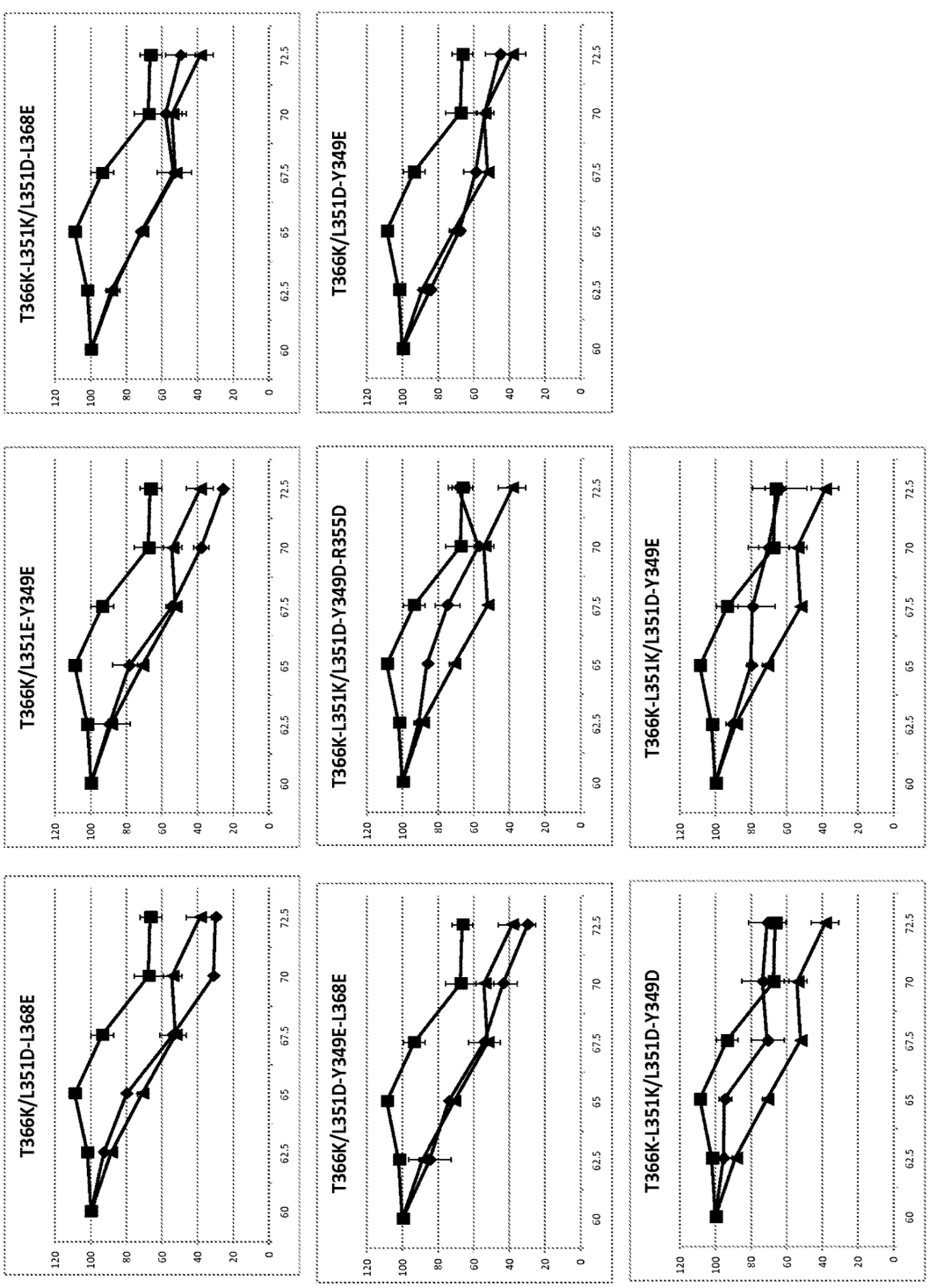

T366K/L351D-L368E
T366K/L351E-Y349E
T366K-L351K/L351D-L368E
T366K-L351K/L351D-Y349D-R355D
T366K/L351D-Y349E
T366K/L351D-Y349E-L368E
T366K-L351K/L351D-Y349E
T366K-L351K/L351D-Y349D
120
100
80
60
40
20
0
60
62.5
65
67.5
70
72.5

Figure 21:

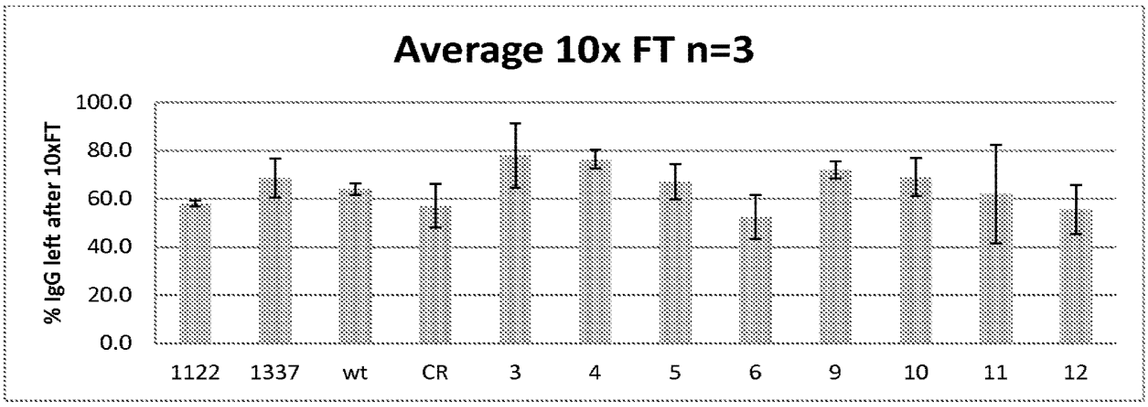
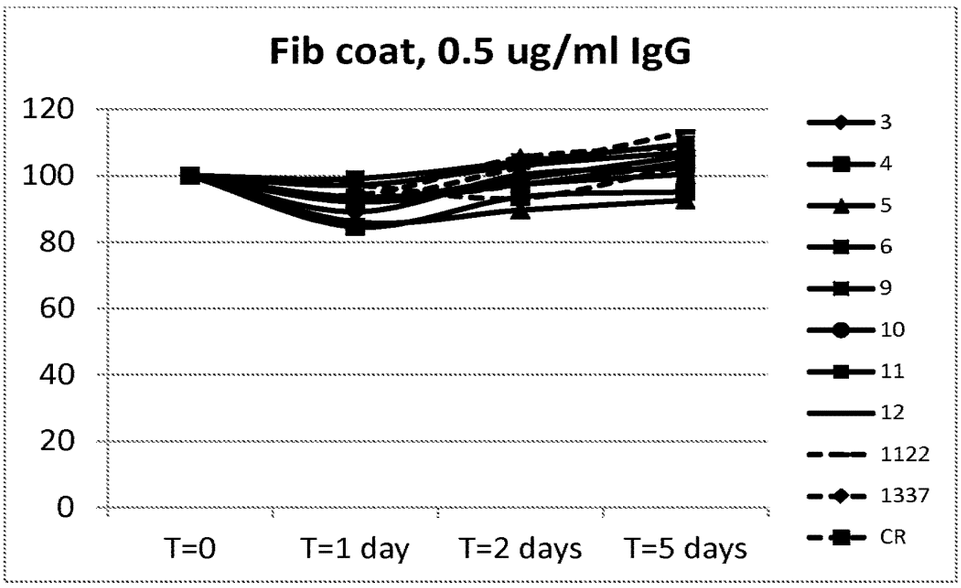

Average 10x FT n=3
% IgG left after 10xFT
100.0
80.0
60.0
40.0
20.0
0.0
1122
1337
wt
CR
3
4
5
6
9
10
11
12

Figure 22:

A


Fib coat, 0.5 ug/ml IgG
120
100
80
60
40
20
0
T=0
T=1 day
T=2 days
T=5 days
3
4
5
6
9
10
11
12
1122
1337
CR

B

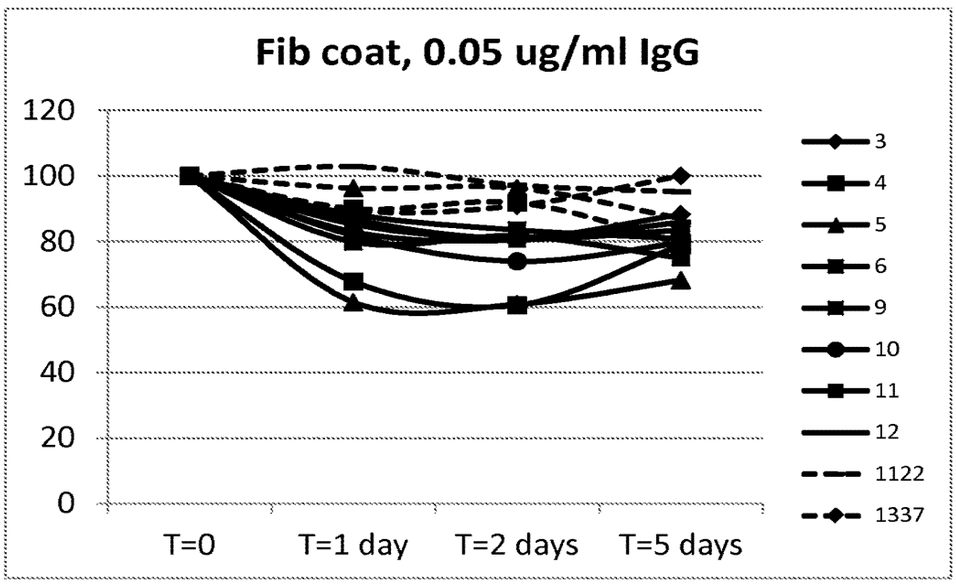
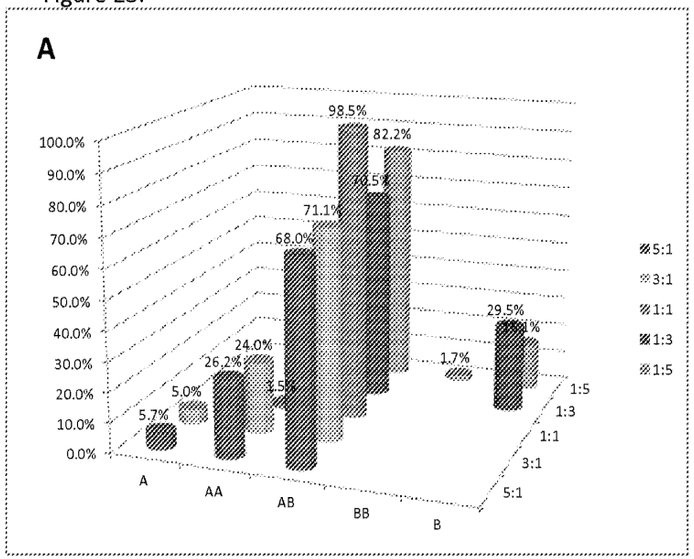
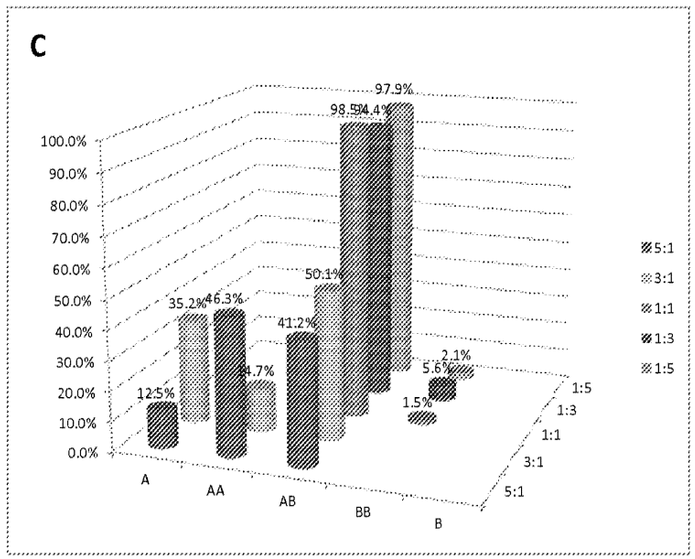
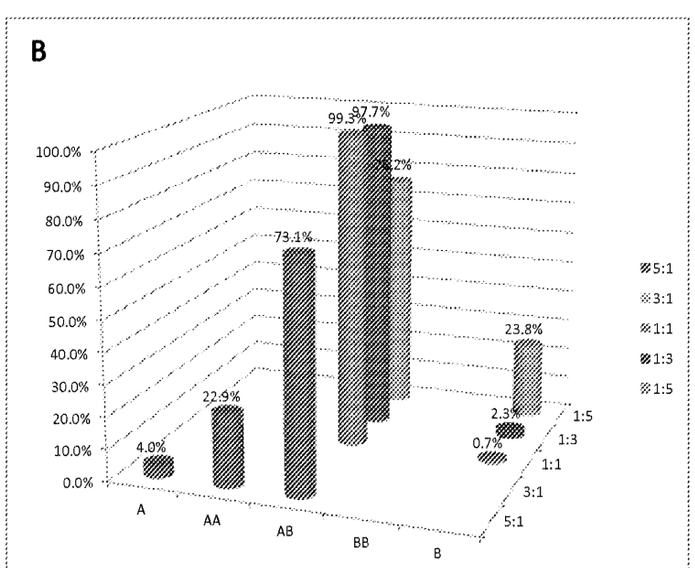

Fib coat, 0.05 ug/ml IgG
120
100
80
60
40
20
0
T=0
T=1 day
T=2 days
T=5 days
3
4
5
6
9
10
11
12
1122
1337

Figure 23:







A

	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%	82.2%
BB					1.7%
B				29.5%	16.1%

B

	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%

C

	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					

Figure 24:

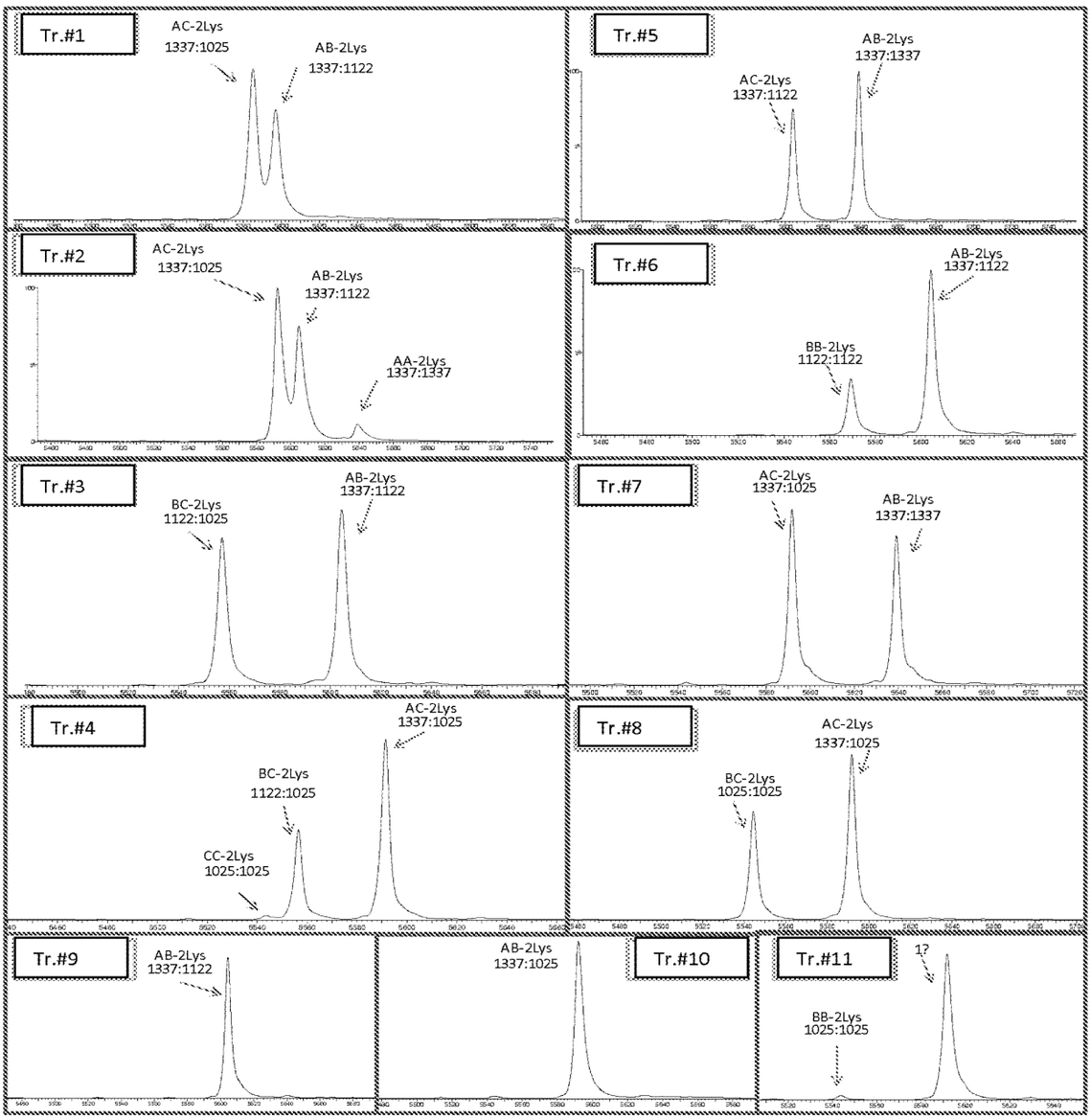

Tr.#1
AC-2Lys
1337:1025
AB-2Lys
1337:1122
Tr.#5
AB-2Lys
1337:1337
AC-2Lys
1337:1122
Tr.#2
AC-2Lys
1337:1025
AB-2Lys
1337:1122
AA-2Lys
1337:1337
Tr.#6
AB-2Lys
1337:1122
BB-2Lys
1122:1122
Tr.#3
AB-2Lys
1337:1122
BC-2Lys
1122:1025
Tr.#7
AC-2Lys
1337:1025
AB-2Lys
1337:1337
Tr.#4
AC-2Lys
1337:1025
BC-2Lys
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

Figure 25:

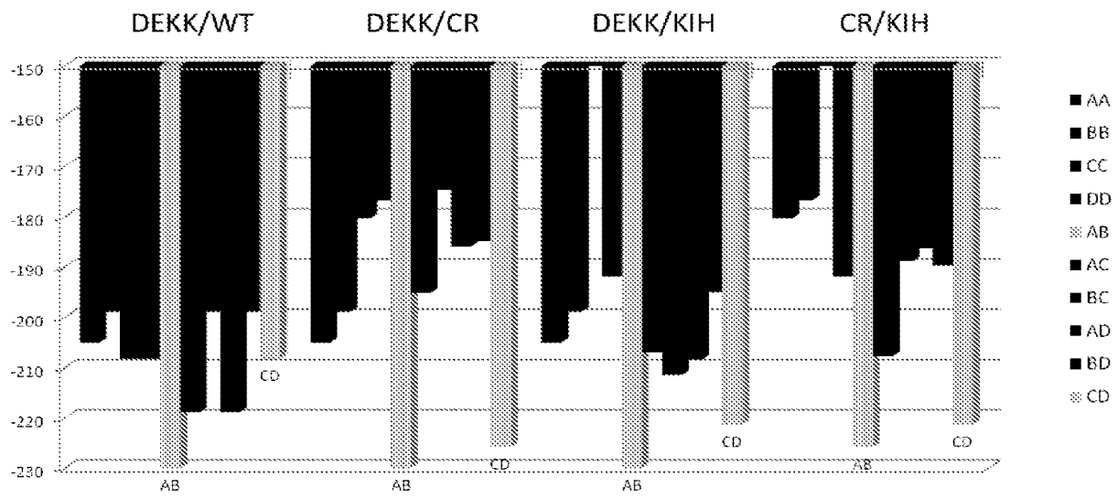

DEKK/WT
DEKK/CR
DEKK/KIH
CR/KIH
-150
-160
-170
-180
-190
-200
-210
-220
-230
AB
AB
AB
AB
CD
CD
CD
CD
CD
AA
BB
CC
DD
AB
AC
BC
AD
BD
CD

Figure 26:

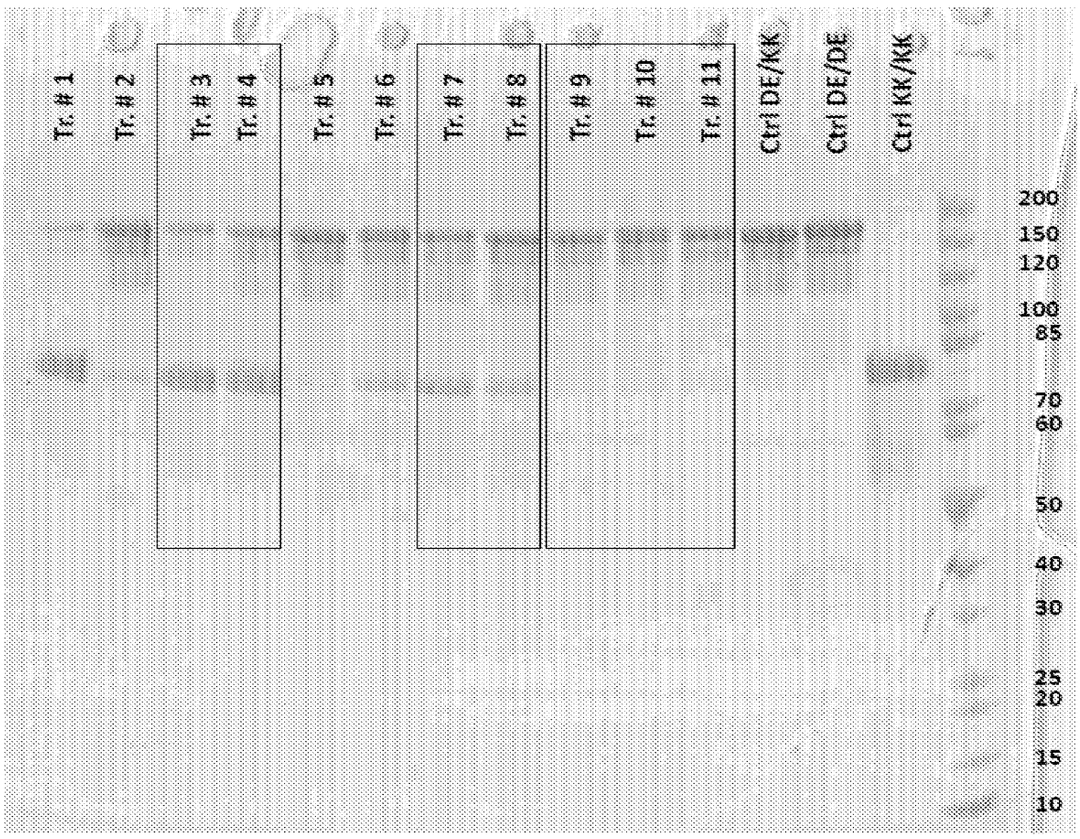

Tr. # 1
Tr. # 2
Tr. # 3
Tr. # 4
Tr. # 5
Tr. # 6
Tr. # 7
Tr. # 8
Tr. # 9
Tr. # 10
Tr. # 11
Ctrl DE/KK
Ctrl DE/DE
Ctrl KK/KK
200
150
120
100
85
70
60
50
40
30
25
20
15
10

Figure 27A:

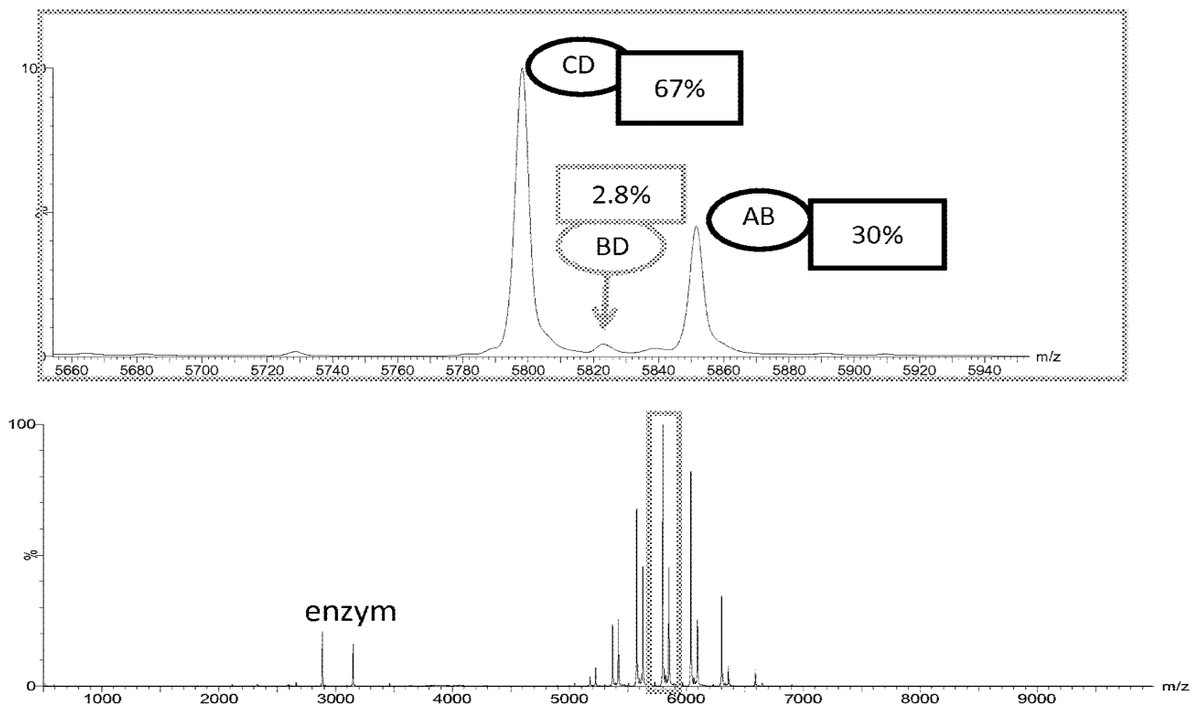
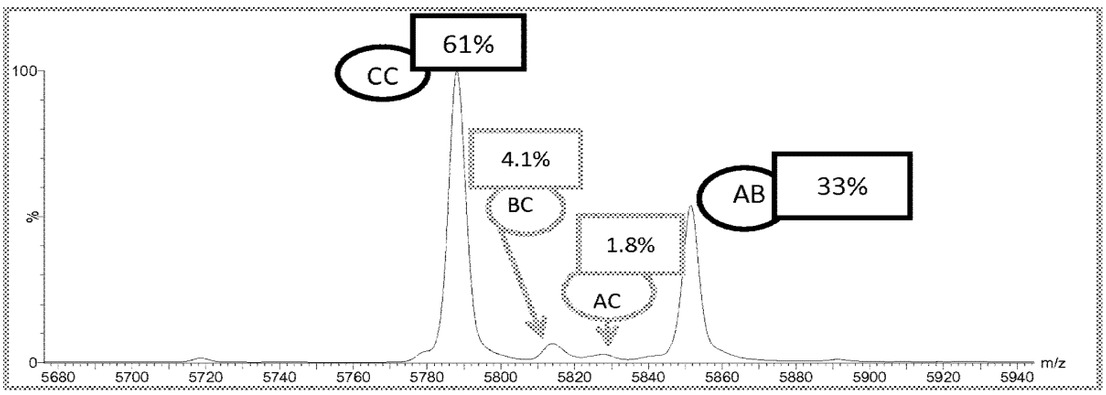

CD
67%
2.8%
BD
AB
30%
m/z
5660
5680
5700
5720
5740
5760
5780
5800
5820
5840
5860
5880
5900
5920
5940
100
%
0
enzym
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

Figure 27B:


61%
CC
4.1%
BC
1.8%
AC
AB
33%
100
%
0
5680
5700
5720
5740
5760
5780
5800
5820
5840
5860
5880
5900
5920
5940
m/z

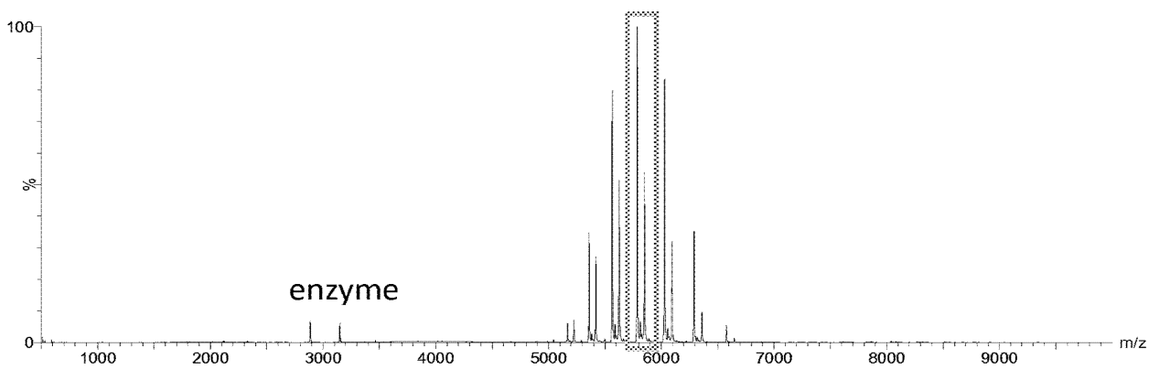

100
%
0
enzyme
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

Figure 28:

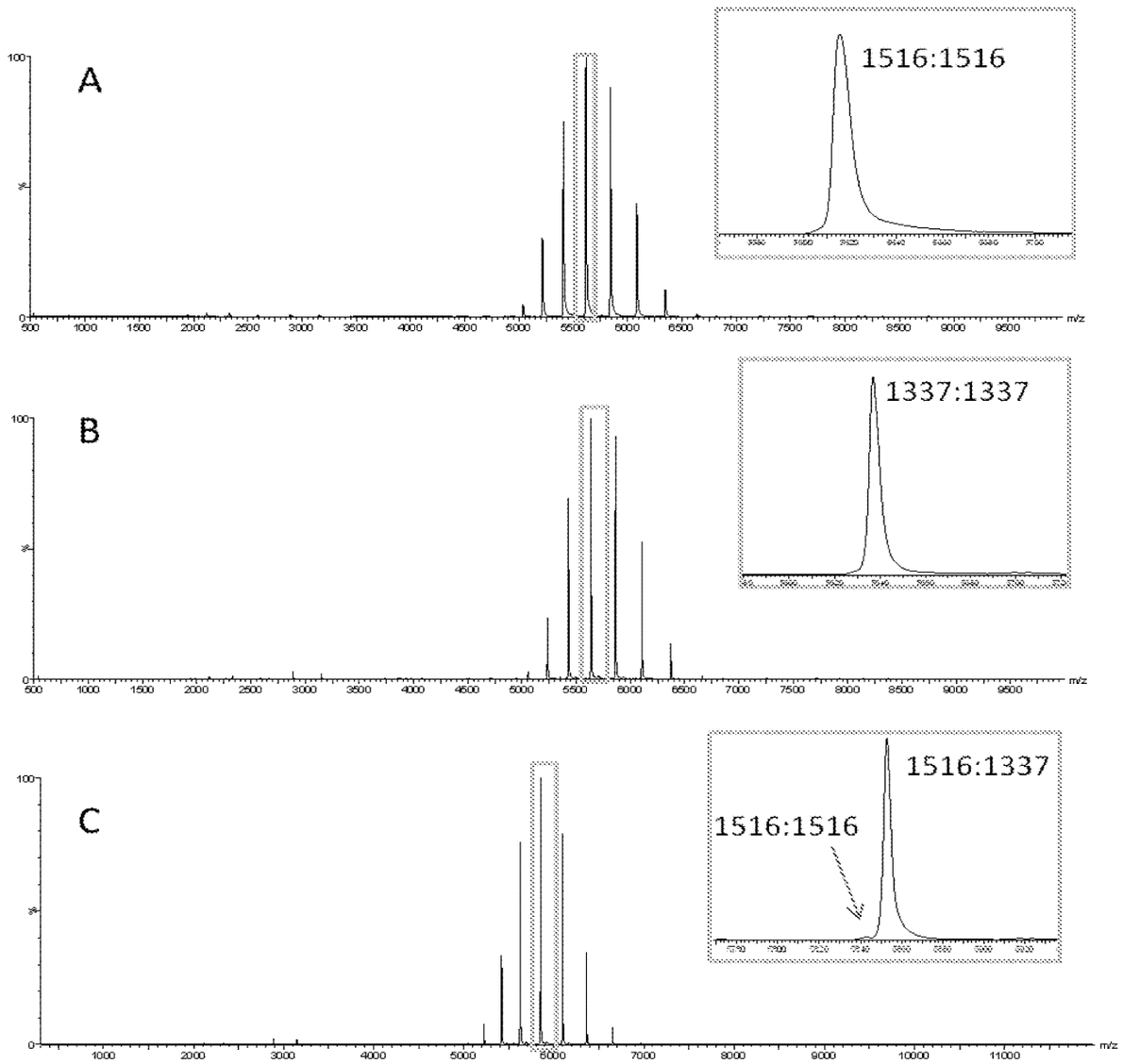
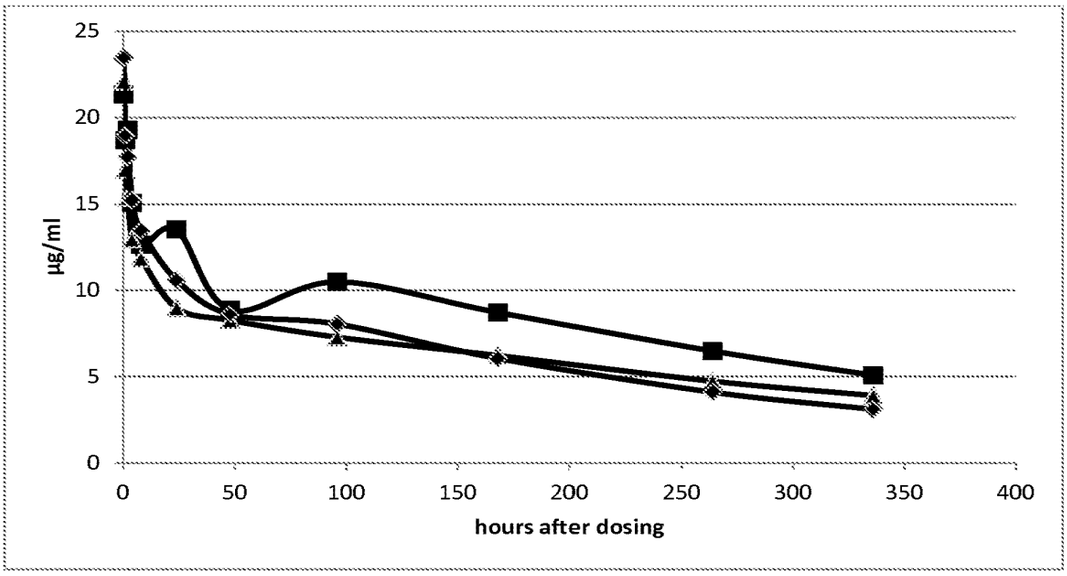


Figure 29:


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

DocCode - SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 14081848 Document Date: 11/15/2013

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.

- Drawings – Other than Black and White Line Drawings

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.

To access the documents in the SCORE database, refer to instructions below.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

FORMALITIES LETTER

OC000000065278119

Date Mailed: 12/03/2013

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Filing Date Granted

Applicant is given **TWO MONTHS FROM THE DATE OF THIS NOTICE** within which to comply with the sequence rules, 37 CFR §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR § 1.821(g). Extension of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR § 1.136.

- This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c) Applicant must provide an initial paper or compact disc copy of the "Sequence Listing", **as well as an amendment specifically directing its entry into the application** and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000).
- A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

For questions regarding compliance to these requirements, please contact:

- **For Rules interpretation, call (571) 270-5300**
- **For CRF submission help, call (571) 272-2510**
- **For PatentIn software program support technical assistance, call (866)217-9197.**

- **Send e-mail correspondence for PatentIn software program help to ebc@uspto.gov**
- **Patent software is available at http://www.uspto.gov/patents/resources/tools/checker/patentinrel.jsp .**

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/tnguyen/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

POA ACCEPTANCE LETTER

OC000000065278161

Date Mailed: 12/03/2013

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/15/2013.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/tha/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/081,848	11/15/2013	1636	1660	MRX5-007CN	33	4

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

FILING RECEIPT

OC00000065278118

Date Mailed: 12/03/2013

Receipt is acknowledged of this non-provisional 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 APPLICANT, 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 Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections 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**

Inventor(s)

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

Applicant(s)

Merus B.V., Utrecht, NETHERLANDS

Assignment For Published Patent Application

MERUS B.V., Utrecht, NETHERLANDS

Power of Attorney: The patent practitioners associated with Customer Number 00959

Domestic Priority data as claimed by applicant

This application is a CON of 13/866,747 04/19/2013
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 - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 11/29/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/081,848**

Projected Publication Date: To Be Determined - pending completion of Sequence Disclosures

Non-Publication Request: No

Early Publication Request: No
** **SMALL ENTITY** **
Title

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Preliminary Class

435

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).

SelectUSA

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 technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Electronic Acknowledgement Receipt	
EFS ID:	18079209
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Milejdi Sartori
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	31-JAN-2014
Filing Date:	15-NOV-2013
Time Stamp:	14:00:49
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	MRX5-007CN_Sequence_Listing.txt	8489	no	0
Warnings:					
Information:					

2	Sequence Listing	MRX5-007CN_Sequence_Listing.pdf	19142 6d27b11ddbe733427ca780be342b03993d242d7e	no	6
Warnings:					
Information:					
3	Response to Pre-Exam Sequence Notice	MRX5-007CN_Response_to_Notice_to_Comply.pdf	131863 4778f6d5b0406541f09c81cfb423c2ec22bd6552	no	3
Warnings:					
Information:					
4	CRF Statement Paper and CRF are the same	MRX5-007CN_Sequence_Listing_Statement.pdf	116695 920ba3b2edf6ac8c4b0a882bff08125505fcf467	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	276189		

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 a 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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 31, 2014
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Cornelis A. De Kruif *et al.*

Application No.: 14/081,848 Confirmation No.: 1016

Filed: November 15, 2013 Art Unit: 1636

For: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES Examiner: Not Yet Assigned

RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATION CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

MS Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

In response to the Notice to Comply with Requirements for a Patent Application Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, mailed December 3, 2013, please amend the above-identified U.S. Patent application as follows:

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

Remarks/Arguments begin on page 3 of this paper.

AMENDMENTS TO THE SPECIFICATION

At page 1, following the title, please insert the following new paragraph:

--SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 31, 2014, is named MRX5-007CN_Sequence_Listing.txt and is 8,489 bytes in size.--

At page 43, please replace the original paragraph starting at line 6 with the following amended paragraph:

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

At page 43, please replace the original paragraph starting at line 8 with the following amended paragraph:

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

REMARKS

Amendments to the Specification

The specification has been amended to include sequence identifiers, to correct matters of format, and to incorporate the computer readable form (CRF) of the Sequence Listing submitted concurrently herewith in compliance with 37 CFR §§ 1.821-1.825. *No new matter has been added.*

Applicant believes no fee is due with this statement. However, if a fee is due, please charge our Deposit Account No. 12-0080, under Order No. MRX5-007CN from which the undersigned is authorized to draw.

Dated: January 31, 2014 Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No.: 37,320
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 202-4623
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 31, 2014
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Cornelis A. De Kruif *et al.*

Application No.: 14/081,848 Confirmation No.: 1016

Filed: November 15, 2013 Art Unit: 1636

For: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES Examiner: Not Yet Assigned

STATEMENT PURSUANT TO 37 CFR 1.821

MS Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

Submitted herewith for filing in connection with the above-referenced patent application are paper and computer readable copies of the Sequence Listing included in the application. Applicants respectfully request entry of the Sequence Listing, which is in compliance with 37 CFR § 1.821-1.825, into the application.

I hereby state that I have reviewed the paper copy of the Sequence Listing contained on pages 1 to 6 of said Sequence Listing, as required by 37 CFR § 1.821(c), and have reviewed the computer readable form of the Sequence Listing, as required by 37 CFR § 1.821(e), and that the content of the paper and computer readable copies for the above-referenced patent application are the same as required by 37 CFR § 1.821(f). *No new matter has been added.*

Dated: January 31, 2014

Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No.: 37,320
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 202-4623 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

SEQUENCE LISTING

<110> MERUS B.V.
DE KRUIF, Cornelis A.
HENDRIKS, Linda Johanna Aleida
LOGTENBERG, Ton

<120> METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

<130> MRX5-007CN

<140> US 14/081,848
<141> 2013-11-15

<150> US 13/866,747
<151> 2013-04-19

<150> US 61/635,935
<151> 2012-04-20

<160> 7

<170> PatentIn version 3.5

<210> 1
<211> 317
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> wildtype IgG1 Fc

<400> 1

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
1 5 10 15

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
20 25 30

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
35 40 45

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
50 55 60

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
65 70 75 80

Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
85 90 95

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
100 105 110

```
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
        115                 120                 125

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
    130                 135                 140

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
145                 150                 155                 160

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
                165                 170                 175

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
            180                 185                 190

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
        195                 200                 205

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
    210                 215                 220

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
225                 230                 235                 240

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                245                 250                 255

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
            260                 265                 270

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
        275                 280                 285

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
    290                 295                 300

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
305                 310                 315


<210>  2
<211>  345
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  MF1025_VH
```

<400> 2
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt cacctttagc agctatgcca tgagctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac acggccgtgt attactgtgc aagggccgat 300

tggtgggcga cttttgacta ctggggccaa ggtaccctgg tcacc 345

<210> 3
<211> 115
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1025_VH

<400> 3

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Asp Trp Trp Ala Thr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr
115

<210> 4
<211> 348
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1122_VH

<400> 4
gaggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60

tcctgtgcag cctctggatt caccttcagt agctatggca tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtggcagtt atatcatatg atggaagtaa taaatactat 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agctgaggac acggccgtgt attactgtgc aagagccctc 300

ttcacgacca tcgccatgga ctattggggc caaggtaccc tggtcacc 348

<210> 5
<211> 116
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1122_VH

<400> 5

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Leu Phe Thr Thr Ile Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr
115

<210> 6
<211> 372
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1337 VH

<400> 6
gaggtgcagc tggtggagac tggggctgag gtgaagaagc cgggggcctc agtgaaggtc 60

tcctgcaagg cttctgacta catcttcacc aaatatgaca tcaactgggt gcgccaggcc 120

cctggacaag ggcttgaatg gatgggatgg atgagcgcta acactggaaa cacgggctat 180

gcacagaagt tccagggcag agtcaccatg accagggaca cgtccataaa cacagcctac 240

atggagctga gcagcctgac atctggtgac acggccgttt atttctgtgc gaggagtagt 300

cttttcaaga cagagacggc gccctactat cacttcgctc tggacgtctg gggccaaggg 360

accacggtca cc 372

<210> 7
<211> 126
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1337

<400> 7

Val His Glu Val Gln Leu Val Glu Thr Gly Ala Glu Val Lys Lys Pro
1 5 10 15

Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Asp Tyr Ile Phe Thr
20 25 30

Lys Tyr Asp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
35 40 45

Trp Met Gly Trp Met Ser Ala Asn Thr Gly Asn Thr Gly Tyr Ala Gln
50 55 60

Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Asn Thr
65 70 75 80

Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Gly Asp Thr Ala Val Tyr
85 90 95

Phe Cys Ala Arg Ser Ser Leu Phe Lys Thr Glu Thr Ala Pro Tyr Tyr

100 105 110

His Phe Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val Thr
115 120 125

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 14081848 Document Date: 01/31/2014

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.

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

==========
Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Douglas, Keisha (ASRC)
Timestamp: [year=2014; month=2; day=3; hr=11; min=6; sec=41; ms=398;]
==========

Validated By CRFValidator v 1.0.4

Application No: 14081848 Version No: 1.0

Input Set:

Output Set:

Started: 2014-01-31 14:00:51.174
Finished: 2014-01-31 14:00:51.212
Elapsed: 0 hr(s) 0 min(s) 0 sec(s) 38 ms
Total Warnings: 0
Total Errors: 0
No. of SeqIDs Defined: 7
Actual SeqID Count: 7

SEQUENCE LISTING

<110> MERUS B.V.
DE KRUIF, Cornelis A.
HENDRIKS, Linda Johanna Aleida
LOGTENBERG, Ton

<120> METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

<130> MRX5-007CN
<140> US 14/081,848
<141> 2013-11-15

<150> US 13/866,747
<151> 2013-04-19

<150> US 61/635,935
<151> 2012-04-20

<160> 7

<170> PatentIn version 3.5

<210> 1
<211> 317
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> wildtype IgG1 Fc

<400> 1

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
1 5 10 15

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
20 25 30

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
35 40 45

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
50 55 60

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
65 70 75 80

Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys

```
                85                  90                  95

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
            100                 105                 110

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
        115                 120                 125

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
    130                 135                 140

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
145                 150                 155                 160

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
                165                 170                 175

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
            180                 185                 190

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
        195                 200                 205

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
    210                 215                 220

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
225                 230                 235                 240

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                245                 250                 255

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
            260                 265                 270

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
        275                 280                 285

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
    290                 295                 300

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
305                 310                 315
```

<210> 2
<211> 345
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1025_VH

<400> 2
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctctggatt cacctttagc agctatgcca tgagctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac acggccgtgt attactgtgc aagggccgat 300

tggtgggcga cttttgacta ctggggccaa ggtaccctgg tcacc 345

<210> 3
<211> 115
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1025_VH

<400> 3

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

```
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ala Asp Trp Trp Ala Thr Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr
        115


<210>  4
<211>  348
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  MF1122_VH

<400>  4
gaggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc       60

tcctgtgcag cctctggatt caccttcagt agctatggca tgcactgggt ccgccaggct      120

ccaggcaagg ggctggagtg ggtggcagtt atatcatatg atggaagtaa taaatactat      180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat      240

ctgcaaatga acagcctgag agctgaggac acggccgtgt attactgtgc aagagccctc      300

ttcacgacca tcgccatgga ctattggggc caaggtaccc tggtcacc                   348


<210>  5
<211>  116
<212>  PRT
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  MF1122_VH

<400>  5

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30
```

```
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ala Leu Phe Thr Thr Ile Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr
        115
```

<210> 6
<211> 372
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> MF1337 VH

<400> 6
gaggtgcagc tggtggagac tggggctgag gtgaagaagc cgggggcctc agtgaaggtc 60

tcctgcaagg cttctgacta catcttcacc aaatatgaca tcaactgggt gcgccaggcc 120

cctggacaag ggcttgaatg gatgggatgg atgagcgcta acactggaaa cacgggctat 180

gcacagaagt tccagggcag agtcaccatg accagggaca cgtccataaa cacagcctac 240

atggagctga gcagcctgac atctggtgac acggccgttt atttctgtgc gaggagtagt 300

cttttcaaga cagagacggc gccctactat cacttcgctc tggacgtctg gggccaaggg 360

accacggtca cc 372

<210> 7
<211> 126
<212> PRT
<213> Homo sapiens

<220>

<221> misc_feature
<223> MF1337

<400> 7

Val His Glu Val Gln Leu Val Glu Thr Gly Ala Glu Val Lys Lys Pro
1 5 10 15

Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Asp Tyr Ile Phe Thr
20 25 30

Lys Tyr Asp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
35 40 45

Trp Met Gly Trp Met Ser Ala Asn Thr Gly Asn Thr Gly Tyr Ala Gln
50 55 60

Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Asn Thr
65 70 75 80

Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Gly Asp Thr Ala Val Tyr
85 90 95

Phe Cys Ala Arg Ser Ser Leu Phe Lys Thr Glu Thr Ala Pro Tyr Tyr
100 105 110

His Phe Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val Thr
115 120 125

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

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/081,848	11/15/2013	1636	1660	MRX5-007CN	33	4

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

UPDATED FILING RECEIPT

OC000000066445086

Date Mailed: 02/07/2014

Receipt is acknowledged of this non-provisional 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 APPLICANT, 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 Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections 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**

Inventor(s)

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

Applicant(s)

Merus B.V., Utrecht, NETHERLANDS

Assignment For Published Patent Application

MERUS B.V., Utrecht, NETHERLANDS

Power of Attorney: The patent practitioners associated with Customer Number 00959

Domestic Priority data as claimed by applicant

This application is a CON of 13/866,747 04/19/2013
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 - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 11/29/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/081,848**

Projected Publication Date: 05/22/2014

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Preliminary Class

435

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).

SelectUSA

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 technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

PUBLICATION NOTICE

OC00000068594726

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

Publication No.US-2014-0140999-A1
Publication Date:05/22/2014

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Cornelis Adriaan de Kruif *et al.*
Serial Number:	14/081,848
Filed:	November 15, 2013
Status:	Published as US 2014-0140999-A1 on May 22, 2014

THIRD PARTY PRE-ISSUANCE SUBMISSION UNDER 37 CFR § 1.290

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
ATTENTION: Director, Technology Center 1600 (1644)

Dear Commissioner:

The undersigned hereby submits three public documents (collectively, "Documents 1-3") of potential relevance to the examination of the above-identified patent application assigned to Merus Biopharmaceuticals B.V. ("the '848 Application"), in accordance with 37 C.F.R.§ 1.290 and 35 U.S.C. 122(e).

This submission is made before the later of six months after the date the application was first published by the Office, or the date of a first Office Action on the merits rejecting any claim, and before the date of a notice of allowance is given or mailed in the '848 Application and therefore is timely under 37 C.F.R. § 1.290(b)(2)(ii). The undersigned submits that he is not an individual who has a duty to disclose information with respect to the above-identified application under 37 C.F.R.§ 1.56.

I. List of documents being submitted

The list of documents being submitted and enclosed herewith includes the following three documents, in accordance with 37 C.F.R.§ 1.290(d)(1) and (d)(3):

U.S. Publication No. US 2010-0286374-A1 (Earliest priority date: January 7, 2008; Filed: January 06, 2009; Published: November 11, 2010 in the name of Gunasekaran Kannan, et al.; referred to herein as "the '374 Publication").

U.S. Patent No. 7,642,228 (Earliest priority date: March 01, 1995; Filed: December 7, 2001; Issued: January 5, 2010 in the name of Genentech, Inc.; referred to herein as "the '228 Patent").

International Publication No. WO 2011/143545 (Earliest priority date: May 14, 2010; Filed: May 13, 2011; Published: November 17, 2011 in the name of Rinat Neuroscience Corporation; referred to herein as "the '545 Publication").

II. Concise description of the relevance of each document

Both the '374 Publication and the '228 Patent disclose methods of generating preferentially heterodimeric Ig-like molecules by altering a pair of CH3 domains such that the ability of each CH3 domain to interact with itself is reduced while the ability of the two CH3 domains to interact with each other is increased.

In particular, the '374 Publication discloses strategies that utilize electrostatic forces to preferentially steer heterodimeric CH3-CH3 interaction. The '374 Publication discloses methods of preparing a heterodimeric Ig-like molecule that "may comprise a first CH3-containing polypeptide and a second CH3-containing polypeptide that meet together to form an interface engineered to promote heterodimer formation" (see paragraph 0008, lines 2-4). This is achieved "by replacing one or more residues that make up the CH3-CH3 interface in both CH3 domains with a charged amino acid such that homodimer formation is electrostatically unfavorable but heterodimerization is electrostatically favorable" (see paragraph 0007, lines 5-9). This document also discloses that T366, T368, L351, and Y349 are, among others, "structurally conserved and buried . . . residues" at the CH3-domain interface that are "involved in domain-domain interaction" (see paragraph 0020 and FIG. 3). Moreover, this document identifies R355 as one of the "preferred residues for mutation to enhance heterodimer formation" (see paragraph 0021, lines 3-8 and FIG. 4(a)). Preferred embodiments of this published invention include a "bispecific

antibody" that comprises two variable regions recognizing different target epitopes (see paragraph 0015, line 2).

The '228 Patent discloses methods of preparing a heteromultimeric Ig-like molecule comprising "a first polypeptide and a second polypeptide which meet at [the CH3-CH3] interface, wherein the first polypeptide has a protuberance at the interface thereof which is positionable in a cavity at the interface of the second polypeptide" (see column 5, lines 57-61). It also discloses that T366, T368, L351, and Y349 are, among others, "contact" residues at the CH3-domain interface that are involved in CH3-CH3 interaction (see column 7, lines 1-21 and FIG. 5). Preferred embodiments of this patented invention include a "bispecific antibody" that comprises two variable regions recognizing different target epitopes (e.g., see column 6, line 9).

The '545 Publication discloses heteromultimeric (e.g., heterodimeric) proteins that comprise first and second CH3 polypeptides which interact together to form a CH3 interface, wherein one or more amino acids within the CH3 interface destabilize homodimer formation and are not electrostatically unfavorable to homodimer formation (see page 4, lines 26-30). The '545 Publication also discloses a strategy to enhance the formation of a desired heteromultimeric or heterodimeric protein (e.g., a bispecific antibody) by altering or engineering an interface between a first and second immunoglobulin-like Fc region (e.g., a hinge region and/or a CH3 region) (see page 5, lines 4-7). In particular, this document discloses replacing one or more residues that make up the CH3 interface with charged residues such that the interactions within the CH3 interface further promotes heterodimer formation over homodimer formation. This document specifically discloses that heterodimer formation is enhanced by amino acid substitutions at positions 349, 368, 405, and 409 in a CH3 region (e.g., L368E, L368D and K409R) (see page 5, line 4 to page 6, line 9).

A concise explanation of the relevance of each of the three cited documents to the pending claims follows, in accordance with 37 C.F.R. § 1.290(d)(2).

III. A concise description of asserted relevance for each pending claim of the '848 Application is reproduced in table format below:

Claim 1 of the '848 Application
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. **<u>The '374 Publication</u>** The '374 Publication, at paragraph 0006, lines 1-7, discloses a strategy for "altering a CH3 domain [of an antibody] to reduce the ability of the domain to interact with itself[.]" In particular, it discloses that "one or more residues that make up the CH3-CH3 interface [can be] replaced with a charged amino acid such that the interaction [between homodimers] becomes electrostatically unfavorable." At paragraph 0007, lines 1-9, the '374 Publication further discloses "a strategy for altering a pair of CH3 domains to reduce the ability of each domain to interact with itself but to increase the ability of the domains to interact with each other, i.e., form heterodimers... [O]ne or more residues that make up the CH3-CH3 interface in both CH3 domains [can be replaced] with a charged amino acid such that homodimer formation is electrostatically unfavorable but heterodimerization is electrostatically favorable." Methods for preparing such heterodimeric proteins, wherein the CH3 domains are engineered to comprise charge amino acids unfavorable to homodimer formation, are also disclosed (e.g., paragraph 0008). Thus, the '374 Publication discloses that alteration of charges at the CH3-CH3 interface of immunoglobulin chains of an antibody can be used to either promote or inhibit chain dimerization, e.g., in order to promote heterodimer formation and inhibit homodimer formation. **<u>The '228 Patent</u>** The claim 1 of the '228 Patent: "An isolated heteromultimer comprising at least a first polypeptide and a second polypeptide, wherein 1) the first polypeptide is associated with the second polypeptide via an interface, said interface having introduced therein at least one protuberance or cavity such that:

(a) the interface of the first polypeptide comprises a protuberance that is positionable in a cavity in the interface of the second polypeptide and/or

(b) the interface of the first polypeptide comprises a cavity that accommodates a protuberance of the second polypeptide, such that a greater ratio of heteromultimer forms than for a multimer having a wild-type interface, and

2) polypeptide interface via a CH3 constant domain of an antibody."

In column 5, lines 39-44 and lines 56-61, this document discloses a method (as illustrated in Figure 4) of preparing a heteromultimer preferably comprising a first CH3-domain-containing polypeptide and a second CH3-domain-containing polypeptide which meet at the CH3-CH3 interface.

Thus, the '228 Patent discloses that alteration of three-dimensional structure of the CH3-CH3 interface of immunoglobulin chains of an antibody can be used to either promote or inhibit chain dimerization, e.g., in order to promote heteromultimer formation.

The '545 Publication

On page 5, lines 4-17, this document discloses "a strategy for enhancing the formation of a desired heteromultimeric or heterodimeric protein (e.g., bispecific antibody) by altering or engineering an interface between a first and a second immunoglobulin-like Fc region (e.g., a hinge region and/or a CH3 region)… In some embodiments, one or more residues that make up the hinge interface are replaced with charged residues such that the electrostatic interactions between these charged residues electrostatically favor heterodimer formation over homodimer formation. In further embodiments, one or more residues that make up the CH3 interface are further replaced with charged residues such that the interactions within the CH3 interface further promotes heterodimer formation over homodimer formation. In some embodiments, the engineered CH3 interface destabilizes homodimer formation. In some embodiments, the engineered CH3 interface is not electrostatically unfavorable to homodimer formation. In some embodiments, the engineered CH3 interface sterically favors heterodimer formation over homodimer formation. In some embodiments, the engineered CH3 interface electrostatically favor heterodimer formation over homodimer formation."

On page 23, lines 12-21, the '545 Publication discloses a number of residues in the CH3 domain that may contain amino acid modifications. In addition, on page 6, lines 4-9, this document discloses that heterodimeric proteins as described therein comprise an "amino acid modification in the CH3 polypeptide is an amino acid substitution at a position selected from the group consisting of 349, 368, 405 and 409. In some embodiments, the amino acid modification is selected from the group consisting of K409R, L368E, and L368D..."

Additionally, claim 51 of this document recites "[a] method for producing the [heterodimeric] polypeptide... [by] introducing one or more amino acid modifications to a polypeptide comprising a wild-type immunoglobulin-like hinge polypeptide and a wild-type immunoglobulin-like CH3 polypeptide, wherein the amino acid modifications are introduced in the hinge polypeptide and the CH3 polypeptide, wherein the polypeptide has increased ability to form a heterodimeric protein with a second polypeptide, compared to the polypeptide comprising the wild-type immunoglobulin-like hinge polypeptide and the wild-type immunoglobulin-like

CH3 polypeptide."

Thus, the '545 Publication discloses that modification of the residues at the CH3 domain can affect the ability of an immunoglobulin polypeptide to heterodimerize with another immunoglobulin polypeptide. Specifically, the '545 Publication discloses substitution of a neutral amino acid to a negatively charged amino acid, e.g., acidic amino acid (e.g., L368E, and L368D).

Claims 3 and 4 of the '848 Application

3. The method of claim 1, 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.

4. The method of claim 3, wherein said first CH3-domain-comprising polypeptide chain further comprises the amino acid substitution L351K.

The '374 Publication

Figure 3 (figure legends in paragraph 0020) discloses that T366 and L351 are "structurally conserved and buried . . . residues" at "CH3 domain interface" and are "involved in the domain-domain interaction".

Figure 4(a) (figure legends in paragraph 0021, lines 1-9) discloses that T366 and L351 are residues "involved in the CH3-CH3 domain interaction" and are highly conserved among the IgGs in both human and mouse.

Table 1 discloses that both T366 and L351 are "CH3 domain interface residues". It further discloses that the T366 residue in the first CH3-domain-comprising polypeptide chain contacts the L351 residue in the second CH3-domain-comprising polypeptide chain.

Thus, the '374 Publication discloses that both T366 and L351 residues are located at the CH3 domain interface.

The '228 Patent

Figure 5 (relevant figure legends in column 7, lines 13-21) discloses that T366 and L351 are "contact" residues that are buried at the CH3-CH3 domain interface. It further discloses that T366 is one of the "optimal candidate original residues for replacement with import residues" (column 5, lines 19-21).

Claims 5 and 6 of the '848 Application

5. The method of claim 3, wherein said second CH3-domain comprising polypeptide chain further comprises an amino acid substitution selected from the group consisting of Y349E; Y349D; L368E; Y349D and L388E; and Y349D and R355D.

6. The method of claim 5, wherein said second CH3-domain comprising polypeptide chain further comprises the amino acid substitution L368E.

The '374 Publication

Figure 3 (figure legends in paragraph 0020) discloses that Y349 and L368 are "structurally conserved and buried . . . residues" at CH3 domain interface and are "involved in the domain-domain interaction".

Figure 4(a) (figure legends in paragraph 0021, lines 1-9) discloses that Y349, L368, and R355 are residues "involved in the CH3-CH3 domain interaction" and are conserved among the IgG subclasses in human. It further discloses that R355 is one of the "preferred residues for mutation to enhance heterodimer formation."

Table 1 discloses that Y349, L368, and R355 are "CH3 domain interface residues" and are involved in the CH3-CH3 domain interaction. It further discloses that R355 "could also be used for enhancing electrostatic steering effects" (Table 1, footnote b).

The '228 Patent

Figure 5 (relevant figure legends in column 7, lines 13-21) discloses that Y349 and T368 are "contact" residues that are buried at the CH3-CH3 domain interface.

Figure 7 (figure legends in column 7, lines 31-34) discloses that Y349 and T368 are "contact" residues at the interface of the CH3 domain of human IgG1.

The '545 Publication

On page 4, lines 26-30, this document discloses a heterodimeric protein that comprises "a first CH3 polypeptide and a second CH3 polypeptide which interact together to form a CH3 interface, wherein one or more amino acids within the CH3 interface destabilize homodimer formation and are not electrostatically unfavorable to homodimer formation."

On page 5, lines 18-31, this document discloses that the heterodimeric protein comprises amino acid modifications in the first and/or second CH3 regions.

On page 6, lines 4-9, this document discloses that the amino acid substitutions are at a position selected from the group consisting of 349, 368, 405 and 409. This document further discloses the specific amino acid substitutions K409R, L368E, and L368D.

Claims 8, 9,10, 13, 14, 15, and 24 of the '848 Application

8. The method according claim 1, wherein each of the CH3-domain comprising polypeptide chains further comprises a variable region recognizing a target epitope.

9. The method of claim 8, wherein each of the 2 variable regions of the CH3-domain comprising polypeptide chains recognizes a different target epitope.

10. The method of claim 9, wherein the different target epitopes are located on the same target molecule.

13. The method of claim 9, wherein the different target epitopes are located on different target molecules.

14. The method of claim 13, wherein the different target molecules are expressed on the same cells.

15. The method claim 13, wherein the different target molecules are expressed on different cells.

24. A heterodimeric Ig-like molecule according to claim 23, wherein said heterodimeric Ig-like molecule binds to different epitopes on the same antigen or to different epitopes on different antigens.

The '374 Publication

Claim 34 of the '374 Publication:

"34. The method of claim 1, wherein the heterodimeric protein is selected from the group consisting of an antibody, a *bispecific* antibody, a monospecific monovalent antibody, a *bispecific* maxibody, a monobody, a peptibody, a *bispecific* peptibody, a monovalent peptibody, and a receptor fusion protein" (emphasis added).

In paragraph 0042, lines 1-9 and FIG. 2, the '374 Publication discloses that the two CH3 domain-containing polypeptides can be engineered to have "different functionalities." For example, they can be "bispecific antibodies" that "bind to different antigens."

The '228 Patent

Claims 30 and 31 of the '228 Patent:

"30. The heteromultimer of claim 1 which is a multi-specific antibody.

31. The heteromultimer of claim 30 which is a bi-specific antibody."

In column 7, lines 65-67, the '228 Patent discloses that the patented heteromultimer comprised of the two engineered CH3 domain-containing polypeptides "[p]referably . . . has binding specificity for at least two different ligands or binding sites."

The '545 Publication

Claims 27 and 28 of the '545 Publication:

"27. The heterodimeric protein of any of claims 1-26, wherein the heterodimeric protein is selected from the group consisting of an antibody, a maxibody, a monobody, a peptibody, and an Fc fusion protein.

28. The heterodimeric protein of claim 27, wherein the heterodimeric protein is a monospecific monovalent, a *bispecific* monovalent, or a *bispecific* bivalent antibody" (emphasis added).

On page 4, in line 33, the '545 Publication discloses that "[i]n some embodiments, the heterodimeric protein is a bispecific antibody" that has two different antigen binding sites.

Claims 26, 27, and 31 of the '848 Application

26. 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.

27. 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.

31. 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.

Documents 1-3

Please see the relevant description of each Document listed for claims 1, 3, and 4 of the '848 Application.

Doc Code: 3P.RELEVANCE
Document Description: Concise Description of Relevance

U.S. Patent and Trademark Office
Department of Commerce

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	14081848

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance
1	7642228	see attached

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance
1	20100286374	see attached

FOREIGN PATENT DOCUMENTS

CiteNo	Foreign Document Number	Concise Description of Relevance
1	2011143545	see attached

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**

Doc Code:IDS.3P
Document Description: Third-Party Submission Under 37 CFR 1.290

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

U.S. PATENTS				
Cite No	Patent Number	Kind Code [1]	Issue Date (YYYY-MM-DD)	First Named Inventor
1	7642228	B2	2010-01-05	Paul Carter

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code [1]	Publication Date (YYYY-MM-DD)	First Named Inventor
1	20100286374	A1	2010-11-11	Gunasekaran Kannan

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number [3]	Country Code [2]	Kind Code [1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T [5]
1	2011143545	WO	A1	2011-11-17	RINAT NEUROSCIENCE CORPORATION	☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T [5]	E [6]
		☐	☐

EFS Web 2.1.17

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

Signature	/Charles E. Lyon/		
Name/Print	Charles E. Lyon	Registration Number (if applicable)	56630

Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

Electronic Acknowledgement Receipt	
EFS ID:	20108425
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	
First Named Inventor/Applicant Name:	
Correspondence Address:	- - - - - - - - - - -
Filer:	Charles E. Lyon
Filer Authorized By:	
Attorney Docket Number:	
Receipt Date:	12-SEP-2014
Filing Date:	
Time Stamp:	10:00:20
Application Type:	

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO_2011_143545.PDF	5963911 5394c9f0841908e87f8be90da14026d0967a36a0	no	116
Warnings:					
Information:					
2	Concise Description of Relevance	Third_Party_Pre-Issuance_Submission.pdf	112770 6814fb78cf37e271914b18c71f1687875ee76291	no	9
Warnings:					
Information:					
3	Concise Description of Relevance	Concise-description-generated.pdf	27429 85ed1a0ddc970d40f530c3afe889ff6d4d3e17a1	no	2
Warnings:					
Information:					
4	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	41598 93d6d64500d523008aa09428e2e77a9a80e722e6	no	2
Warnings:					
Information:					
5	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	19570 5cc1ef9668c3b0af867ee663cb333989d1b22fbb	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	6165278		

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 a 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.

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN	1016

959 7590 09/17/2014

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
DAHLE, CHUN WU	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
09/17/2014	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):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address : COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
14/081,848	15 November, 2013	DE KRUIF ET AL.	MRX5-007CN

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
MARIANNE SEIDEL	
ART UNIT	PAPER
1600	20140916A

DATE MAILED:

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

Commissioner for Patents

A third-party submission under 37 CFR 1.290 has been filed on 9/12/14, and is being entered in the above-identified application. Please allow a few days for the submission to be visible in the Patent Application Information Retrieval (PAIR) system.

/Marianne Seidel/
QAS, TC 1600

PTO-90C (Rev.04-03)

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN	1016

959 7590 04/10/2015

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER
BURKHART, MICHAEL D

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
04/10/2015	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):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 14/081,848	Applicant(s) DE KRUIF ET AL.	
	Examiner Michael Burkhart	Art Unit 1633	AIA (First Inventor to File) 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) *1-33* 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) *1-33* 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 *9/12/14*.

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

4) ☐ Other: _____.

DETAILED ACTION

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-33 are rejected under pre-AIA 35 USC 102(b) as being anticipated by Carter et al (US 7,642,228, of record).

The '228 patent discloses methods of preparing a heteromultimeric Ig molecule comprising "a first polypeptide and a second polypeptide which meet at the CH3-CH3 interface, wherein the first polypeptide has a protuberance at the interface thereof which is positionable in a cavity at the interface of the second polypeptide." (see column 5, lines 57-61). It also discloses that T366, T368, L351, and Y349 are, among others, "contact" residues at the CH3-domain interface that are involved in CH3-CH3 interaction (see column 5, lines 1-21 and FIG. 5). Preferred embodiments of this patented invention include a "bispecific antibody" that comprises two variable regions recognizing different target epitopes (e.g., see column 6, line 9). In column 5, lines 39-44 and lines 56-61, this document discloses a method (as illustrated in Figure 4) of preparing a heteromultimer preferably comprising a first CH3-domain-containing polypeptide and a second CH3-domain-containing polypeptide which meet at the CH3-CH3 interface.

Figure 7 (figure legends in column 7, lines 31-34) discloses that Y349 and T368 are contact residues at the interface of the CH3 domain of human IgG 1. Claims 30, 31, and 32 of the '228 patent recite a multi-specific antibody, a bi-specific antibody and a tri-specific antibody.

In column 7, lines 65-67, the '228 patent discloses that the patented heteromultimer comprised of the two engineered CH3 domain-containing polypeptides preferably has binding specificity for at least two different ligands or binding sites.

Claims 1-33 are rejected under pre-AIA 35 USC 102(b) as being anticipated by Kannan et al (US 2010/0286374, published 1/5/2010, of record).

Kannan et al, at paragraph 0006, lines 1-7, discloses a strategy for "altering a CH3 domain of an antibody to reduce the ability of the domain to interact with itself. In particular, it discloses that one or more residues that make up the CH3-CH3 interface can be replaced with a charged amino acid such that the interaction between homodimers becomes electrostatically unfavorable. At paragraph 0007, lines 1-9, Kannan et al further discloses a strategy for altering a pair of CH3 domains to reduce the ability of each domain to interact with itself but to increase the ability of the domains to interact with each other, i.e., form heterodimers. One or more residues that make up the CH3-CH3 interface in both CH3 domains can be replaced with a charged amino acid such that homodimer formation is electrostatically unfavorable but heterodimerization is electrostatically favorable. Methods for preparing such heterodimeric proteins, wherein the CH3 domains are engineered to comprise charge amino acids unfavorable to homodimer formation, are also disclosed (e.g., paragraph 0008).

Figure 3 (figure legends in paragraph 0020) discloses that T366 and L351 are "structurally conserved and buried.., residues" at the CH3 domain interface and are involved in the domain- domain interaction.

Figure 4A (figure legends in column 5, lines 6-15) discloses that T366 and L351 are residues involved in the CH3-CH3 domain interaction and are highly conserved among the IgGs in both human and mouse.

Table 1 discloses that both T366 and L351 are CH3 domain interface residues. It further discloses that the T366 residue in the first CH3-domain-comprising polypeptide chain contacts the L351 residue in the second CH3-domain-comprising polypeptide chain. In Table 1, the '374 publication discloses that, E356, E357, K370, K392, D399, K409, K439, T366, L368, Y407, L351, Y349, and R355 (all the residues listed in Table B of this application) are all CH3 domain interface residues that are involved in domain-domain interaction.

In paragraph 2, lines 13-16, the '374 Publication discloses that it is well understood that the IgG molecule can be considered as a heterotetramer having two heavy chains that are held together by disulfide bonds (-S-S-) at the hinge region and two light chains.

Figure 10 of the '374 Publication (relevant figure legends and discussions in paragraph 0055, lines 12-14) discloses that co-expression of a first CH3-domain comprising polypeptide chain comprising charge pair mutations E356K and D399K and a second CH3-domain comprising polypeptide chain comprising charge pair mutations K392D and K409D "resulted almost exclusive formation of heterodimer. Claim 34 of the '374 publication recites a method of claim 1, wherein the heterodimeric protein is selected from the group consisting of an antibody, a

bispecific antibody, a monospecific monovalent antibody, a bispecific maxibody, a monobody, a peptibody, a bispecific peptibody, a monovalent peptibody, and a receptor fusion protein.

In paragraph 0042, lines 1-9 and FIG. 2, the '374 Publication discloses that the two CH3 domain-containing polypeptides can be engineered to have "different functionalities." For example, they can be "bispecific antibodies" that "bind to different antigens."

Double Patenting

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 claims at issue 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); and *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 a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with

this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form 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 http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-33 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-36, 38 and 39 of copending Application No. 13/866,756. Although the claims at issue are not identical, they are not patentably distinct from each other because the claims of the '756 application recite methods within the scope of the instant claims, which use similar recombinant cells and produce antibodies within the scope of the instant claims.

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

Claims 1-33 of this application are patentably indistinct from claims 1-9, 22-24, 27-34 of Application No. 14/395,330. Pursuant to 37 CFR 1.78(f) or pre-AIA 37 CFR 1.78(b), when two or more applications filed by the same applicant 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.

Claims 1-33 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-13, 24, 25 30, 34, 36, 38 and 40 of copending Application No. 14/395,325. Although the claims at issue are not identical, they are not patentably distinct from each other because the claims of the '325 application recite methods within the scope of the instant claims, which use similar recombinant cells and produce antibodies within the scope of the instant claims.

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

Claims 1-4, 26-28, 31 and 32 of this application are patentably indistinct from claims 1-4, 27-29, 32-34 of Application No. 13/866747. Pursuant to 37 CFR 1.78(f) or pre-AIA 37 CFR 1.78(b), when two or more applications filed by the same applicant 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.

Conclusion

No claim is allowed.

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

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 an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Michael Burkhart/
Primary Examiner, Art Unit 1633

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MB/

Doc Code:IDS.3P
Document Description: Third-Party Submission Under 37 CFR 1.290

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

U.S. PATENTS				
Cite No	Patent Number	Kind Code [1]	Issue Date (YYYY-MM-DD)	First Named Inventor
1	7642228	B2	2010-01-05	Paul Carter

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code [1]	Publication Date (YYYY-MM-DD)	First Named Inventor
1	20100286374	A1	2010-11-11	Gunasekaran Kannan

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number [3]	Country Code [2]	Kind Code [1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T [5]
1	2011143545	WO	A1	2011-11-17	RINAT NEUROSCIENCE CORPORATION	☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T [5]	E [6]
		☐	☐

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MB/

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MB/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

Signature	/Charles E. Lyon/		
Name/Print	Charles E. Lyon	Registration Number (if applicable)	56630

Examiner Signature	/Michael Burkhart/	Date Considered	04/06/2015

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

Doc Code: DIST.E.FILE **Document Description: Electronic Terminal Disclaimer - Filed**	PTO/SB/25 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION**
Application Number	14081848
Filing Date	15-Nov-2013
First Named Inventor	Cornelis DE KRUIF
Attorney Docket Number	MRX5-007CN
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent Interest
Merus B.V.	100%

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

13866756 filed on 04/19/2013

13866747 filed on 04/19/2013

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

◉ Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.

○	I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

Applicant claims the following fee status:

◉ Small Entity

○ Micro Entity

○ Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

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 is of record in this application

Registration Number 37320

○ A sole inventor

○ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application

○ A joint inventor; all of whom are signing this request

Signature	/Cynthia L. Kanik/
Name	Cynthia L. Kanik, Ph.D., J.D.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Melissa Lebsock
Attorney Docket Number:	MRX5-007CN
Filed as Small Entity	
Filing Fees for **Utility under 35 USC 111(a)**	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Statutory or Terminal Disclaimer	1814	1	160	160
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**160**

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 14081848

Filing Date: 15-Nov-2013

Applicant/Patent under Reexamination: DE KRUIF et al.

Electronic Terminal Disclaimer filed on October 13, 2015

☒ APPROVED

This patent is subject to a terminal disclaimer

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt	
EFS ID:	23766077
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	13-OCT-2015
Filing Date:	15-NOV-2013
Time Stamp:	18:13:24
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$160
RAM confirmation Number	5698
Deposit Account	120080
Authorized User	KANIK, CYNTHIA L
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Electronic Terminal Disclaimer-Filed	eTerminal-Disclaimer.pdf	34890 7836e578de4dfa5b6b8e63ee6546be41048 6a9ad	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30439 e8b50d874860f175281037453c4c61303d5 b87e4	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	65329		

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.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a 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.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
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.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
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.

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Melissa Lebsock
Attorney Docket Number:	MRX5-007CN

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	2202	5	40	200
Multiple Dependent Claims	2203	1	390	390
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	2253	1	700	700
Miscellaneous:				
		Total in USD ($)		**1290**

Electronic Acknowledgement Receipt	
EFS ID:	23768814
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	13-OCT-2015
Filing Date:	15-NOV-2013
Time Stamp:	18:17:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1290
RAM confirmation Number	5760
Deposit Account	120080
Authorized User	BRAZELL, BIANCA
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Extension of Time	MRX5-007CN_EOT.pdf	11055 4cf53248a98a2c98084e821e8db38d91d5e6a4c7	no	1
Warnings:					
Information:					
2		MRX5-007CN_Resp_to_Nonfinal_OA.pdf	41375 45dd3e280dbab3d2affd36af7909c5dd497dc000	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	10

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	34059 94fb5ca372653e1a45f142b07c3aef27d1ee2b06	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	86489	

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 a 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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: October 13, 2015
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D. /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Cornelis A. De Kruif *et al.*

Application No.: 14/081848 Confirmation No.: 1016

Filed: November 15, 2013 Art Unit: 1633

For: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES Examiner: M.D. Burkhart

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 CFR § 1.111

In response to the non-final office action dated April 10, 2015, please amend the above-identified U.S. patent application as follows.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Enclosures: Information Disclosure Statement and SB/08 forms.

AMENDMENTS TO THE CLAIMS

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

Listing of Claims:

1. **(Currently Amended)** A method for producing a heterodimeric ~~Ig-like molecule~~ antibody from a single cell, wherein said ~~Ig-like molecule~~antibody comprises two heavy chains with CH3 domains that are capable of forming an interface, said method comprising:

a. providing ~~in said~~a host cell comprising

(i) ~~A~~a first nucleic acid molecule encoding a 1st ~~CH3 domain-comprising polypeptide~~ antibody heavy chain comprising at least one substitution of a neutral amino acid residue in the CH3 domain by a positively charged amino acid residue, and

(ii) ~~A~~a second nucleic acid molecule encoding a 2nd ~~CH3 domain-comprising polypeptide~~ antibody heavy chain comprising at least one substitution of a neutral amino acid residue in the CH3 domain by a negatively charged amino acid residue~~,~~;

~~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~~

b. culturing said host cell and allowing for expression of said two nucleic acid molecules to produce said 1st antibody heavy chain and said 2nd antibody heavy chain, wherein the at least one positively charged amino acid residue substituted in the CH3 domain of said 1st antibody heavy chain interacts with the at least one negatively charged amino acid residue substituted in the CH3 domain of said 2nd antibody heavy chain in the interface between said 1st and 2nd antibody heavy chains to produce a heterodimeric antibody; and

c. harvesting said heterodimeric ~~Ig-like molecule~~antibody from the culture.

2. **(Original)** The method of claim 1, further comprising providing said host cell with a nucleic acid molecule encoding a common light chain.

3. **(Currently Amended)** The method of claim 1, wherein said ~~first CH3 domain comprising polypeptide~~1st -antibody heavy chain comprises a substitution of the amino acid residue at position 366 in the CH3 domain by a lysine (K) residue, ~~the amino acid substitution T366K~~ and wherein said ~~second CH3 domain comprising polypeptide~~ 2nd antibody heavy chain comprises a substitution of the amino acid residue at position 351 in the CH3 domain by an aspartic acid (D) residue ~~the amino acid substitution L351D~~.

4. **(Currently Amended)** The method of any one of claims 1-3, wherein ~~said first~~the CH3-domain ~~comprising polypeptide~~ of said 1st antibody heavy chain further comprises ~~the amino acid substitution L351K~~a substitution of the amino acid residue at position 351 by a lysine (K) residue.

5. **(Currently Amended)** The method of claim ~~3~~4, wherein ~~said second CH3 domain comprising polypeptide~~the CH3 domain of said 2nd antibody heavy chain further comprises ~~an~~ amino acid substitution(s) selected from the group consisting of ~~Y349E; Y349D; L368E; Y349D and L388E; and Y349D and R355D~~
(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. **(Currently Amended)** The method of claim 5, wherein ~~said second CH3 domain comprising polypeptide~~the CH3 domain of the 2nd antibody heavy chain ~~further comprises the amino acid substitution L368E~~ comprises the substitution of the amino acid residue at position 368 by a glutamic acid (E) residue.

7. **(Original)** The method according to claim 1, 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.

8. **(Cancelled)**

9. **(Currently Amended)** The method of claim ~~8~~1, wherein each ~~of the 2 variable regions of the CH3 domain comprising polypeptide~~ variable region of the 1st and 2nd antibody heavy chains recognizes a different target epitope.

10. **(Original)** The method of claim 9, wherein the different target epitopes are located on the same target molecule.

11. **(Original)** The method of claim 10, wherein the target molecule is a soluble molecule.

12. **(Original)** The method of claim 10, wherein the target molecule is a membrane-bound molecule.

13. **(Original)** The method of claim 9, wherein the different target epitopes are located on different target molecules.

14. **(Original)** The method of claim 13, wherein the different target molecules are expressed on the same cells.

15. **(Original)** The method of claim 13, wherein the different target molecules are expressed on different cells.

16. **(Original)** The method of claim 13, wherein the different target molecules are soluble molecules.

17. **(Original)** The method of claim 13, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

18. **(Currently Amended)** The method of claim ~~8~~9, wherein at least one of said target epitopes is located on a tumor cell.

19. **(Currently Amended)** The method of claim ~~8~~9, wherein at least one of said target epitopes is located on an effector cell.

20. **(Original)** The method of claim 19, 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.

21. **(Original)** The method of claim 19, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

22. **(Currently Amended)** The method according claim 1, wherein said heterodimeric ~~Ig-like molecule is an~~ antibody is human IgG.

23. **(Currently Amended)** A heterodimeric ~~Ig-like molecule obtainable~~ antibody obtained by ~~the methods according to~~ the method of claim 1.

24. **(Currently Amended)** A heterodimeric ~~Ig-like molecule~~antibody according to claim 23, wherein said heterodimeric ~~Ig-like molecule~~antibody binds to different epitopes on the same antigen or to different epitopes on different antigens.

25. **(Currently Amended)** A heterodimeric ~~Ig-like molecule~~antibody according to claim 23, wherein said ~~Ig-like molecule is an~~ antibody is human IgG.

26. **(Currently Amended)** A heterodimeric antibody comprising two CH3 domains, wherein one of said two CH3 domains 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), ~~the amino acid substitutions L351D and L368E~~ and wherein the other of said two CH3 domains comprises an amino acid substitution at position 366 by a lysine (K) and an amino acid substitution at position 351 by a lysine (K)~~the amino acid substitutions T366K and L351K~~.

27. **(Currently Amended)** A recombinant host cell comprising nucleic acid sequences encoding at least a ~~first and a second CH3 domain comprising polypeptide~~1st and 2nd antibody heavy chain, wherein ~~said first CH3 domain comprising polypeptide~~ the CH3 domain of said 1st antibody heavy 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~~ the CH3 domain of said 2nd antibody heavy chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue.

28. **(Original)** A recombinant host cell according to claim 27, wherein said host cell further comprises a nucleic acid sequence encoding a common light chain.

29. **(Currently Amended)** A pharmaceutical composition comprising a heterodimeric ~~Ig-like molecule~~antibody according to claim 23 and a pharmaceutically acceptable carrier.

30. **(Original)** A pharmaceutical composition comprising a heterodimeric antibody according to claim 26, and a pharmaceutically acceptable carrier.

31. **(Currently Amended)** A method for making a host cell for production of a heterodimeric ~~Ig-like molecule~~antibody, the method comprising the step of introducing into said host cell nucleic ~~acid sequences~~acid molecules encoding at least a ~~first and a second CH3 domain comprising polypeptide~~ 1st and 2nd antibody heavy chain, wherein ~~said first CH3 domain comprising polypeptide~~ the CH3 domain of said 1st antibody heavy 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~~ the CH3 domain of said 2nd antibody heavy 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.

32. **(Original)** A method according to claim 31, further comprising the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.

33. **(Currently Amended)** The method according to claim 22 wherein antibody is human IgG1.

REMARKS

Claims 1-33 were pending upon issuance of the non-final Office Action dated April 10, 2014. In the present amendment, claims 1, 3-6, 9, 18-19, 22-27, 29, 31 and 33 have been amended, and claim 8 has been cancelled without prejudice or disclaimer.

Support for the claim amendments may be found throughout the specification and claims as originally filed. For example, the claims have been amended to replace the term "Ig-like molecules" with "antibodies", and to replace the term "CH3-domain comprising polypeptide chain" with "antibody heavy chain". Support for this amendment can be found throughout the specification and original claims, for example, at least in original claim 22; at page 15, lines 26-28. Accordingly, no new matter has been added.

Upon entry of the foregoing amendments, claims 1-7 and 9-33 will be pending. The amendments and claim cancellations have been made solely in the interest of expediting prosecution and allowance of the present application. Applicant reserves the right to pursue any canceled subject matter in this or one or more separate application(s).

Information Disclosure Statement

An Information Disclosure Statement and accompanying PTO/SB/08 Forms presenting publications and co-pending applications not previously submitted to the Office is submitted herewith in accordance with 37 CFR §1.97.

Claim Rejections -35 USC § 102

I. Claims 1-33 are rejected under 35 USC § 102(a) as being anticipated by Carter *et al.* (US 7,642,228, herein after "the '228 patent"), on the ground that the '228 patent allegedly discloses "a first polypeptide and a second polypeptide which meet at the CH3-CH3 interface, wherein the first polypeptide has a protuberance at the interface thereof which is positionable in a cavity at the interface of the second polypeptide," and further discloses that T366, T368, L351 and Y349 are contact residues involved in the CH3-CH3 interface (Office Action at page 2 -3). Applicant respectfully traverses this rejection.

For a prior art reference to anticipate under 35 U.S.C. § 102, the prior art must teach each and every element of the claimed invention. *Lewmar Marine v. Barient*, 827 F.2d 744, 3 USPQ2d 1766 (Fed. Cir. 1987). The '228 patent does *not* disclose a heterodimeric antibody in which the CH3 domain of the first heavy chain contains a substitution of a neutral amino acid with a positively charged amino acid, and the CH3 domain of the second heavy chain contains a substitution of a neutral amino acid with a negatively charged amino acid, as required by independent claims 1, 27 and 31. Nor does the '228 patent disclose a heterodimeric antibody in which the CH3 domain of the first heavy chain contains amino acid substitutions at positions 351 and 368 with aspartic acid and glutamic acid, respectively, and the CH3 domain of the second heavy chain contains amino acid substitutions at positions 366 and 351 with lysine, as required by independent claim 26.

Accordingly, the '228 patent does not anticipate the presently claimed invention and reconsideration and withdrawal of this rejection is respectfully requested.

II. Claims 1-4, 27-29 and 32-34 are rejected under 35 USC § 102(b) as being anticipated by Kannan *et al.* (US 2010/0286374 "the '374 application"), on the ground that the '374 application allegedly discloses that "one or more residues that make up the CH3-CH3 interface can be replaced with a charged amino acid such that the interaction between homodimers becomes electrostatically unfavorable" (Office Action at page 3). Applicant respectfully traverses this rejection.

The teachings of the '374 application are limited to the replacement of a charged amino acid residue in each CH3 domain of an antibody with an amino acid having an opposite charge. The '374 application does *not* teach replacement of a neutral amino acid with a positively charged amino acid in the CH3 domain of one antibody heavy chain and the replacement of a neutral amino acid with a negatively charged amino acid in a second antibody heavy chain to produce a heterodimeric antibody in which the two substituted amino acids interact within the CH3 interface (*e.g.,* form an electrostatically favorable interaction), as required by independent claim 1. Nor does the '374 application disclose a heterodimeric antibody in which the CH3 domain of the first heavy chain contains amino acid substitutions at positions 351 and 368 with aspartic acid and glutamic acid, respectively, and the CH3 domain of the second heavy chain

contains amino acid substitutions at positions 366 and 351 with lysine, as required by independent claim 26.

Accordingly, the '374 application does not anticipate the presently claimed invention and reconsideration and withdrawal of this rejection is respectfully requested.

Provisional Double Patenting Rejections

Claims 1-33 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-36, 38 and 39 of co-pending Application No. 13/866,756; and claims 1-4, 27-29 and 32-34 of co-pending Application No. 13/866,747. While in no way admitting that pending claims of the present application are obvious over the cited claims of these co-pending applications, Applicant respectfully submits herewith a terminal disclaimer complying with 37 C.F.R. §1.321, thus obviating these rejections.

Claims 1-33 also are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 30, 34 and 40 of co-pending Application No. 14/395,325. Applicant respectfully submits that Application No. 14/395,325 is abandoned, thus rendering this rejection moot.

Claims 1-33 also are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 27-29 and 32-34 of co-pending application No. 14/395,330. Applicant respectfully requests that this rejection be held in abeyance until the pending claims of the present application are indicated otherwise allowable, at which time Applicants will consider submitting terminal disclaimers complying with 37 C.F.R. §1.321 (b) and (c) if appropriate.

CONCLUSION

In view of the above amendments and remarks, Applicant respectfully submits that the above-identified application is in condition for allowance. However, if there are any remaining issues, the Examiner is urged to contact the undersigned at (617) 217-4623 in order to expedite prosecution of the application.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. MRX-007CN.

Dated: October 13, 2015

Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No. 37,320
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(800) 237-2000
(617) 742-4214 (Fax)
Attorney/Agent for Applicant

PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) MRX5-007CN
Application Number 14/081,848-Conf. #1016	Filed November 15, 2013
For METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	
Art Unit 1633	Examiner M. D. Burkhart

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☒ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 700.00
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☒ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 12-0080 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 37,320 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Cynthia L. Kanik/	October 13, 2015
Signature	Date
Cynthia L. Kanik, Ph.D., J.D.	(617) 217-4623
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Anita Costa
Attorney Docket Number:	MRX5-007CN
Filed as Small Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	2806	1	90	90
		Total in USD ($)		**90**

Electronic Acknowledgement Receipt	
EFS ID:	23768388
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	13-OCT-2015
Filing Date:	15-NOV-2013
Time Stamp:	18:14:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$90
RAM confirmation Number	5718
Deposit Account	120080
Authorized User	KANIK, CYNTHIA L
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	MRX5-007USCN_IDSTRANS.pdf	31089 7a8688debbac8a9dff9d0fc90e36e909a6f36318	no	3
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
2	Non Patent Literature	Atwell_-_Stable_heterodimers_from_remodeling_-_4822-7613-9272_v_1.PDF	352611 75a2c808ba72cc4379c7f1d7190290fe54e56980	no	10
Warnings:					
Information:					
3	Non Patent Literature	Beauerle.pdf	479202 30c00b7b9b480b56cbfa86c2f4e8523b5cd046a8	no	4
Warnings:					
Information:					
4	Non Patent Literature	BOGAN.pdf	931630 335fefd18117b81e3191bfe933c48dbbeb3fe83a	no	9
Warnings:					
Information:					
5	Non Patent Literature	Carter_-_Bispecific_human_IgG_by_design_03.pdf	644165 8463a653f061b8c930a171ef08079ff546cfcb22	no	9
Warnings:					
Information:					
6	Non Patent Literature	Carter_-_Toward_the_production_of_bispecific_-_4828-6366-9512_v_1.PDF	1678941 66b17b3a451680eca72c5e4bbc4bf24b15e704e2	no	9
Warnings:					
Information:					
7	Non Patent Literature	Davies_Antibody.pdf	1581563 8f9f00acf9ce88623252492571bb406ee70746f8	no	5
Warnings:					
Information:					

8	Non Patent Literature	DAVIS.pdf	909400 87fca32d9d7dac076317a0c87bee4e4398c989a5	no	8
Warnings:					
Information:					
9	Non Patent Literature	Diesenhofer_1981_-_4812-5325-3384_v_1.PDF	1564306 f4ebb6a54bdf58483a8d567d06663d39a8efeb42	no	10
Warnings:					
Information:					
10	Non Patent Literature	ELLERSON.pdf	882584 fdea6e9b99b3147d30f4e833504bb9522b299067	no	9
Warnings:					
Information:					
11	Non Patent Literature	Gunasekaran_-_Enhancing_antibody_Fc_Heterdimer.pdf	907385 2d969b58605ded4dc06a869ee584bb578294afae	no	10
Warnings:					
Information:					
12	Non Patent Literature	Hendsch_-_Preferential_Heterdimer_formation.pdf	124578 19f64afe04b1df666a7e03090f8ada23af159994	no	2
Warnings:					
Information:					
13	Non Patent Literature	Kumar_-_The_Second_PDZ_domain_of_INAD_-_4822-2567-6552_v_1.PDF	370041 e83c6d7db758b218d73d06ee7676903e723750fc	no	7
Warnings:					
Information:					
14	Non Patent Literature	Marvin_-_Redesigning_an_antibody_fragment.pdf	342217 41fdfa9061b422c1b9dabbb42d9cb511157a8baf	no	7
Warnings:					
Information:					
15	Non Patent Literature	McPhee_-_Engineering_human_immunodeficiency_-_4822-9357-1848_v_1.PDF	2039012 3b949a67a03442f7ee0cd06ba1d6f19809c6f43c	no	5
Warnings:					
Information:					
16	Non Patent Literature	Merchant_-_An_efficient_route_to_human.pdf	662275 3a8f6f1281f80ca175615f3f6369c1e5644528b1	no	5
Warnings:					
Information:					

17	Non Patent Literature	PR_2013.pdf	158511 349808cddd4d8c7271db7b87f840d55697d27b60	no	3
Warnings:					
Information:					
18	Non Patent Literature	PR_JAN.pdf	149492 1687e948fd02ec8812453ce18fa1e3575aa3c9ea	no	2
Warnings:					
Information:					
19	Non Patent Literature	Miller_-_Protein-protein_recognition_-_4839-3701-8120_v_1.PDF	1140756 1a96e61cb53eeb7f776aa293bb689187047b8d30	no	9
Warnings:					
Information:					
20	Non Patent Literature	Nieba.pdf	1287363 9bc7d2b449473d9f4a4a7abbd49881808a84d423	no	10
Warnings:					
Information:					
21	Non Patent Literature	Nohaile_2001.pdf	139568 1406f005be865d984ff94f747311ec142ebace24	no	6
Warnings:					
Information:					
22	Non Patent Literature	Padlan_-_X-Ray_Crystallography_of_antibodies.pdf	4133528 64621007e118bc69378d02808486effb2714dfc5	no	77
Warnings:					
Information:					
23	Non Patent Literature	PAPADEA.pdf	2065562 08efbb18d31f3a114f54d3d80add245fffab3a38	no	34
Warnings:					
Information:					
24	Non Patent Literature	Raffen.pdf	1119420 40a93a228464d2b24097e29fe737095715ffa372	no	7
Warnings:					
Information:					
25	Non Patent Literature	Ridgway_1996_-_4817-3959-6040_v_1.PDF	4323546 2723047452f156136b566d8787ae4b015198ceef	no	5
Warnings:					
Information:					

26	Non Patent Literature	Sal-Man_-_Arginine_mutations_within_a_-_4811-1890-4584_v_1.PDF	381884 31476b5fe8c5a5c9a730c8c3b140f313daaffa96	no	8
Warnings:					
Information:					
27	Non Patent Literature	Schiffer_-_Analysis_of_immunoglobulin_-_4833-8323-8920_v_1.PDF	559600 01d5222c8eefb03c46b10c3201c1163deea67503	no	4
Warnings:					
Information:					
28	Non Patent Literature	Selzer_-_Rational_design_of_faster_associating.pdf	611930 f56dc83e544d5236b1a994cc7f544834326d319d	no	5
Warnings:					
Information:					
29	Non Patent Literature	Sinha-2002.pdf	2855342 452bbeb6e088c93b1477df2b09809aa65eda911a	no	23
Warnings:					
Information:					
30	Non Patent Literature	Sinha_-_Electrostatics_in_protein_binding-current.pdf	884492 3cc9702214a03fbfe940f8c5bf86c031a0ce86a8	no	14
Warnings:					
Information:					
31	Non Patent Literature	STRELKAUSKAS.pdf	621213 c9a031497c21ab933d177eb98d7fe8d57e4a5c16	no	9
Warnings:					
Information:					
32	Non Patent Literature	VANRHENEN.pdf	795239 a74dafcd8a6dc652d8e6dbc2b8937c424e15fe1b	no	8
Warnings:					
Information:					
33	Non Patent Literature	ZHAO.pdf	1061187 4ec6c02f354602a94b079429f9d6ca500bc25fa2	no	8
Warnings:					
Information:					
34	Non Patent Literature	9098887.pdf	5774571 ef260285424d25582f91c0c30beb4aaf64de0c1b	no	8
Warnings:					
Information:					

35	Foreign Reference	EP1870459A1.pdf	5078316 1be00d7d6edbfc6c07d03013e84a095ea3a9bd66	no	86
Warnings:					
Information:					
36	Foreign Reference	WO96027011A1.pdf	3837050 6f781491075249 82f7c6a5e3adb554b4a996ba23	no	74
Warnings:					
Information:					
37	Foreign Reference	WO98050431A2.pdf	4322106 f0aab70bc58f0117dd788b44026774e38fdd6ced	no	70
Warnings:					
Information:					
38	Foreign Reference	WO2004009618A2.pdf	7606900 6c57ff4357e56e5aacc5250d8e1a1cba8d1ea7bd	no	186
Warnings:					
Information:					
39	Foreign Reference	WO2005118635A2.pdf	4288301 2fd0ccbc31f8122abc2d43f0861e451d3a0dadb7	no	84
Warnings:					
Information:					
40	Foreign Reference	WO2006028936A2.pdf	5670239 427a81cd593ec416597870cbf4c0d6687af9cf25	no	99
Warnings:					
Information:					
41	Foreign Reference	WO2006106905A1.pdf	6212060 303d43903d16aa97051e9aaff368ab492ba3b812	no	123
Warnings:					
Information:					
42	Foreign Reference	WO2007110205A2.pdf	3574230 4405f0d82ef6073ef4c4a08d69fa729ad2c1dd85	no	73
Warnings:					
Information:					
43	Foreign Reference	WO2007147901A1.pdf	4028563 c59256763edf4f41bbf046f860fce17d033fa496	no	71
Warnings:					
Information:					

44	Foreign Reference	WO2008119353A1.pdf	5233768 d51f301b3163eb52a6aea99b0d8ba23289249c9a	no	125
Warnings:					
Information:					
45	Foreign Reference	WO2009080251A1.pdf	4000420 ecf6761478f1b0d401b88abc17bbe2457c3c258f	no	102
Warnings:					
Information:					
46	Foreign Reference	WO2009080252A1.pdf	2559459 60a45473c0a5d6ad28aefe21637bfcd6386692f7	no	61
Warnings:					
Information:					
47	Foreign Reference	WO2009080253A1.pdf	2518992 139b08a02a9d632fff5afec5b8ae47702c84fd20	no	60
Warnings:					
Information:					
48	Foreign Reference	WO2009089004A1.pdf	2073845 ccdf20ce7611db218b67e1de98f54f71a178154a	no	47
Warnings:					
Information:					
49	Foreign Reference	WO2009098596A2.pdf	2975480 946a8db30448959587b36768b24fdb905a40bcad	no	61
Warnings:					
Information:					
50	Foreign Reference	WO2009051974A1.pdf	6851972 b08cfc7a8c7d1a035b47b11f34a4d4110c92f169	no	129
Warnings:					
Information:					
51	Foreign Reference	WO2010084197A1.pdf	2518324 5c7c3ecc4c58dae9a70743c2d2a8f240866a11c4	no	59
Warnings:					
Information:					
52	Foreign Reference	WO2010129304A2.pdf	6948726 07cb2ddc9134adefadf73d9fb7cdb0a29d832a4c	no	92
Warnings:					
Information:					

53	Foreign Reference	WO2011028952A1.pdf	7124014 7bcc0b7d6e20d34a4e00aeac173a583d4c16a9e2	no	130
Warnings:					
Information:					
54	Foreign Reference	WO2011028953A1.pdf	1779885 3b60a732bc1d7b7be367d53321579cdd2c6e9c8a	no	40
Warnings:					
Information:					
55	Foreign Reference	WO2012131555A2pt1.pdf	11175942 977d0a82ca5833d43b8b5a0e78516cabc009d58a	no	198
Warnings:					
Information:					
56	Foreign Reference	WO2012131555A2pt2.pdf	15120694 854b301b068fafdcec2a65635370aace12048d92	no	198
Warnings:					
Information:					
57	Foreign Reference	WO2012023053A2.pdf	4024736 ead63bbe7e47d28798c54588d87f3c8a89cbe82a	no	75
Warnings:					
Information:					
58	Non Patent Literature	Sheinerman.pdf	107529 012d9c677c904da9551311840fe8e882311b2893	no	7
Warnings:					
Information:					
59	Information Disclosure Statement (IDS) Form (SB08)	MRX5-007USCN__SB08.pdf	1039263 9e4339a2c45be9593ed694784c9575cd5e9935c6	no	10
Warnings:					
Information:					
60	Fee Worksheet (SB06)	fee-info.pdf	30622 a502cc5eca73abae1c7ea485a22a7c7167a70373	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	162265639		

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 a 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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: October 13, 2015
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Cornelis Adriaan de Kruif *et al.*

Application No.: 14/081,848 Confirmation No.: 1016

Filed: November 15, 2013 Art Unit: 1633

For: METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES Examiner: M. D. Burkhart

INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed after the mailing date of the first Office Action on the merits, but before the mailing date of any of a Final Office Action, a Notice of Allowance (37 C.F.R. § 1.97(c)) or an action that otherwise closes prosecution in the application.

For the Examiner's convenience in reviewing this continuation application, Applicants submit a consolidated PTO Form SB/08, listing all references cited during the prosecution of the parent application. The present application is a continuation of U.S. Application No. 13/866,747, filed April 19, 2013 (Atty. Docket No. MRX5-007). Applicants provide herewith copies of the

references listed on the enclosed PTO Form SB/08 which have been previously cited by or submitted to the Office in the parent-application, in accordance with 37 CFR §1.98(d).

In addition, this Information Disclosure Statement presents references not previously submitted to the Office. However, Applicants have not submitted copies of each cited U.S. patent and U.S. patent application in accordance with 37 CFR 1.98(a)(2)(ii).

Applicants call to the attention of the Examiner the following Applications:

Applications			
Application No.	*Filing Date*	*First Named Inventor*	*Docket No.*
13/866,747	04-19-2013	Cornelis A. DE KRUIF	MRX5-007
13/866,756	04-19-2013	Cornelis A. DE KRUIF	MRX5-008
14/040,023	09-27-2013	Cornelis A. DE KRUIF	MRX5-010
14/395,330	10-17-2014	Cornelis A. DE KRUIF	MRX5-011
14/395,325	10-17-2014	Cornelis A. DE KRUIF	MRX5-012

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicants' Attorney if the Examiner would like the Applicants to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicants' Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicants request that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement.

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

Please charge our Deposit Account No. 12-0080 in the amount of $90.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. MRX5-007CN.

Dated: October 13, 2015

Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No.: 37,320
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4623
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

CLK/amc

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8592562		2013-11-26	Kannan et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20090182127	A1	2009-07-16	Kjaergaard et al.	
	2	20100015133	A1	2010-01-21	Igawa et al.	
	3	20110195454	A1	2011-08-11	MCWHIRTER et al.	
	4	20130336981	A1	2013-12-19	de Kruif et al.	
	5	20140072579	A1	2014-03-13	De Kruif et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	6	20140120096	A1	2014-05-01	BAKKER et al.	
	7	20140140999	A1	2014-05-22	DE KRUIF et al.	
	8	20150139996	A1	2015-05-21	De Kruif et al.	
	9	20150196637	A1	2015-07-16	De Kruif et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	1870459	EP	A1	2007-12-26	Chugai Pharmaceutical Co LTD.		☐
	2	1996027011	WO	A1	1996-09-06	Genentech, Inc.		☐
	3	1998050431	WO	A2	1998-11-12	Genentech, Inc.		☐
	4	2004009618	WO	A2	2004-01-29	Crucell Holland BV		☐
	5	2005118635	WO	A2	2005-12-15	Novimmune SA		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	6	2006/028936	WO	A2	2006-03-16	Genentech, Inc.		☐
	7	2006106905	WO	A1	2006-10-12	Chugai Pharmaceutical Co LTD.		☒
	8	2007110205	WO	A2	2007-10-04	Merck Patent Gmbh		☐
	9	2007147901	WO	A1	2007-12-27	Novo Nordisk AS		☐
	10	2008/119353	WO	A1	2008-10-09	Genmab AS		☐
	11	2009/080251	WO	A1	2009-07-02	Hoffmann La Roche		☐
	12	2009/080252	WO	A1	2009-07-02	Hoffmann La Roche		☐
	13	2009/080253	WO	A1	2009-07-02	Hoffmann La Roche		☐
	14	2009/089004	WO	A1	2009-07-16	Amgen, Inc.		☐
	15	2009/098596	WO	A2	2009-08-13	Zymeworks, Inc.		☐
	16	2009051974	WO	A1	2009-04-23	Nuvelo, Inc.		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	17	2010/084197	WO	A1	2010-07-29	Genmab AS		☐
	18	2010/129304	WO	A2	2010-11-11	Oncomed Pharmaceuticals, Inc.		☐
	19	2011/028952	WO	A1	2011-03-10	Xencor, Inc.		☐
	20	2011/028953	WO	A1	2011-03-10	Amfit, Inc.		☐
	21	2012/131555	WO	A2	2012-10-04	Glenmark Pharmaceuticals SA		☐
	22	2012023053	WO	A2	2012-02-23	Novimmune SA		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	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, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	ATWELL, S. et al., "Stable Heterodimers from Remodeling the Domain Interface of a Homodimer using a Phage Display Library," JMB, vol. 270, pages 26-35 (1997)	☐
	2	BAEUERLE, P., et al., "Bispecific T-Cell Engaging Antibodies for Cancer Therapy," Cancer Research,vol.69(12), pages 4941-4944 (2009)	☐
	3	BOGAN, A., et. al., "Anatomy of Hot Spots in Protein Interfaces," JMB, vol. 280, pages 1-9 (1998)	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	4	CARTER, P., et al., "Bispecific human IgG by design," Journal of Immunological Methods, vol. 248, pages 7-15, (2001)	☐
	5	CARTER, P., et al., "Toward the Production of Bispecific Antibody Fragments for Clinical Applications," Journal of Hematotherapy, vol. 4, pages 463-470 (1995)	☐
	6	DAVIES, J. et al., "Antibody VH Domains as Small Recognition Units," BioTechnology, vol. 13, pages 475-479 (1995)	☐
	7	DAVIS, J., 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, vol. 23 (4) pages 195-202 (2010)	☐
	8	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, vol. 20 (9), pages 2361-2370, (1981)	☐
	9	ELLERSON, J.R., et al., "Structure and Function of Immunoglobulin Domains: III. Isolation and Characterization of a Fragment Corresponding to the C y2 Homology Region of Human Immunoglobin G," Journal of Immunology, vol. 116, pages 510-517 (1976)	☐
	10	GUNASEKARAN, K. et al., "Enhancing Antibody Fc Heterodimer Formation through Electrostatic Steering Effects, APPLICATIONS TO BISPECIFIC MOLECULES AND MONOVALENT IgG," Journal of Biological Chemistry, vol. 285 (25), pages 19637-19646 (2010)	☐
	11	HENDSCH, Z., et al., "Preferential Heterodimer Formation via Undercompensated Electrostatic Interactions," J. Am. Chem. Soc., vol. 123 (6), pages 1264-1265 (2001)	☐
	12	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," The Journal of Biological Chemistry, vol. 276 (27), pages 24971-24977 (2001)	☐
	13	MARVIN, J. et al., "Redesigning an Antibody Fragment for Faster Association with Its Antigen," Biochemistry, vol. 42 (23), pages 7077-7083, (2003)	☐
	14	MCPHEE , F. et al., "Engineering human immunodeficiency virus 1 protease heterodimers as macromolecular inhibitors of viral maturation," PNAS, vol. 93, pages 11477-11481 (1996)	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	15	MERCHANT, A., et al., "An efficient route to human bispecific IgG," Nature Biotechnology, vol. 16, pages 677-681, (1998)	☐
	16	MERUS, "Merus Presents Preclinical Data on its Novel Bispecific Antibody MCLA-117 at EHA 2013,- Clinical Candidate Designed for the Treatment of Acute Myeloid Leukemia(AML)," Press Release, www.merus.nl, 3 pages, dated June 17, 2013	☐
	17	MERUS, "Merus Selects Clinical Candidate for the Treatment of Acute Myeloid Leukemia (AML)," Press Release, www.merus.nl, 2 pages, dated January 7,2013	☐
	18	MILLER, S., "Protein-Protein Recognition and the Association of Immunoglobulin Constant Domains," J. Mol. Biol. Vol. 216, pages 965-973 (1990)	☐
	19	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, vol. 10, pages 435-444 (1997)	☐
	20	NOHAILE, M.J., et al., "Altering dimerization specificity by changes in surface electrostatics," PNAS, vol. 98(6), pages 3109-3114 (2001)	☐
	21	PADLAN, E. et al, "X-Ray Crystallography of Antibodies," Advances in Protein Chemistry, vol. 49, pages 57-133 (1996)	☐
	22	PAPADEA, C., et al., "Human Immunoglobulin G and Immunoglobulin G Subclasses: Biochemical, Genetic, and Clinical Aspects," Critical Reviews in Clinical Laboratory Sciences, vol. 27(1), pages 27-58 (1989)	☐
	23	RAFFEN, R. et al., "Reengineering Immunoglobulin Domain Interactions by Introduction of Charged Residues," Protein Engineering, vol. 11(4), pages 303-309 (1998)	☐
	24	RIDGWAY, J. et al., "Knobs-into-holes' Engineering of Antibody CH3 Domains for Heavy Chain Heterodimerization," Protein Engineering, vol. 9 (7) pages 617-621 (1996).	☐
	25	SAL-MAN, N. et al., "Arginine Mutations within A Transmembrane Domain of Tar, an Escherichia coli Aspartate Receptor, Can Drive Homodimer Dissociation and Heterodimer Association in Vivo," Journal of Biochemistry, vol. 385, pages 29-36 (2005).	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	26	SCHIFFER, M. et al., "Analysis of Immunoglobulin Domain Interactions Evidence for a Dominant Role of Salt Bridges," JMB, vol. 203, pages 799-802 (1988)	☐
	27	SELZER, T. et al., "Rational design of faster associating and tighter binding protein complexes," Nature Structural Biology, vol. 7, page 537-541 (2000)	☐
	28	SHEINERMAN, F., et al., " Electrostatic aspects of protein-protein interactions," Current Opinion in Structural Biology, vol. 10, pages 153-159 (2000)	☐
	29	SINHA, N. et al., "Differences in Electrostatic Properties at Antibody-Antigen Binding Sites: Implications for Specificity and Cross-Reactivity," Biophysical Journal, vol. 83, pages 2946-2968 (2002)	☐
	30	SINHA, N., et al. "Electrostatics in Protein Binding and Function," Current Protein and Peptide Science, vol 3, pages 601-614 (2002)	☐
	31	STRELKAUSKAS, A. et al., "Human Monoclonal Antibody: 2. Simultaneous Expression of IgG and IgM with Similar Binding Specificities by a Human Hybrid Clone," Hybridoma, vol. 6 (5), pages 479-487 (1987)	☐
	32	VAN RHENEN, A., et al., "The novel AML stem cell-associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells," Blood, vol. 110 (7), pages 2659-2666 (2007)	☐
	33	ZHAO, X., et al., "Targeting C-type lectin-like molecule-1 for antibody-mediated immunotherapy in acute myeloid leukemia," Haematologica, vol. 95(1), pages 71-78 (2010)	☐
	34	ZHU, Z. et al., "Remodeling Domain Interfaces to Enhance Heterodimer Formation," Protein Science, vol. 6, pages 781-788 (1997)	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

EFS Web 2.1.17

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Cynthia L. Kanik/	Date (YYYY-MM-DD)	2015-10-13
Name/Print	Cynthia L. Kanik, Ph.D., J.D.	Registration Number	37320

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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.**

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. 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 record s.

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 Cooperation Treaty.

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.

Notice of Allowability	Application No. 14/081,848	Applicant(s) DE KRUIF ET AL.	
	Examiner Michael Burkhart	Art Unit 1633	AIA (First Inventor to File) 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 amendment dated 10/13/2015*.
   ☐ 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 *1-7 and 9-33*. 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 10/19/2015
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 Burkhart/
Primary Examiner, Art Unit 1633

EXAMINER'S COMMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Terminal Disclaimer

The terminal disclaimer filed on 10/13/2015 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of applications 13/866,756 and 13/866,747 has been reviewed and is accepted. The terminal disclaimer has been recorded.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: the prior art does not teach or suggest replacing a neutral amino acid (considered to be any amino acid other than R, K, H, E or D) with a positively charged amino acid in the CH3 domain. The closest prior art is represented by Ho et al (US 2013/0115208 or WO 2011143545 A1), which teaches replacement of a neutral amino acid (L368) with negatively charged residues such as D or E. There is no teaching or suggestion to combine this substitution with another wherein a neutral residue is replaced with a positively charged residue (e.g. R, K or H). Rather, the prior art teaches changing positively charged residues, such as K409, with other charged residues.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

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

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 an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Michael 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

959 7590 02/12/2016

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
BURKHART, MICHAEL D	
ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 02/12/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN	1016

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	SMALL	$480	$0	$0	$480	05/12/2016

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 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity 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 and an extra copy of the form should be submitted. 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: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax **(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 including the Patent, advance orders and notification of maintenance fees 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.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

959 7590 02/12/2016

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

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 facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN	1016

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	SMALL	$480	$0	$0	$480	05/12/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
BURKHART, MICHAEL D	1633	435-455000

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/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form 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 has been filed for recordation as set forth in 37 CFR 3.11. 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. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): (**Please first reapply any previously paid issue fee shown above**)

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

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. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN	1016

959 7590 02/12/2016

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
BURKHART, MICHAEL D	
ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 02/12/2016

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 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, 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. 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 disclosure of these records is required by the Freedom of Information Act.
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 Cooperation Treaty.
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 inspection 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.

Notice of References Cited	Application/Control No. 14/081,848	Applicant(s)/Patent Under Reexamination DE KRUIF ET AL.	
	Examiner Michael Burkhart	Art Unit 1633	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-2013/0115208 A1	05-2013	Ho; Wei-Hsien	C07K16/2809	424/133.1
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

Doc Code:IDS.3P
Document Description: Third-Party Submission Under 37 CFR 1.290

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

U.S. PATENTS

Cite No	Patent Number	Kind Code [1]	Issue Date (YYYY-MM-DD)	First Named Inventor
1	7642228	B2	2010-01-05	Paul Carter

U.S. PATENT APPLICATION PUBLICATIONS

Cite No	Publication Number	Kind Code [1]	Publication Date (YYYY-MM-DD)	First Named Inventor
1	20100286374	A1	2010-11-11	Gunasekaran Kannan

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS

Cite No	Foreign Document Number [3]	Country Code [2]	Kind Code [1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T [5]
1	2011143545	WO	A1	2011-11-17	RINAT NEUROSCIENCE CORPORATION	☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)

Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T [5]	E [6]
		☐	☐

EFS Web 2.1.17

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	14081848

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

Signature	/Charles E. Lyon/		
Name/Print	Charles E. Lyon	Registration Number (if applicable)	56630

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	12/22/2015

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14081848
Filing Date	2013-11-15
First Named Inventor	Cornelis Adriaan DE KRUIF
Art Unit	1633
Examiner Name	M. D. Burkhart
Attorney Docket Number	MRX5-007CN

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8592562		2013-11-26	Kannan et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20090182127	A1	2009-07-16	Kjaergaard et al.	
	2	20100015133	A1	2010-01-21	Igawa et al.	
	3	20110195454	A1	2011-08-11	MCWHIRTER et al.	
	4	20130336981	A1	2013-12-19	de Kruif et al.	
	5	20140072579	A1	2014-03-13	De Kruif et al.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	6	20140120096	A1	2014-05-01	BAKKER et al.	
	7	20140140999	A1	2014-05-22	DE KRUIF et al.	
	8	20150139996	A1	2015-05-21	De Kruif et al.	
	9	20150196637	A1	2015-07-16	De Kruif et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	1870459	EP	A1	2007-12-26	Chugai Pharmaceutical Co LTD.		☐
	2	1996027011	WO	A1	1996-09-06	Genentech, Inc.		☐
	3	1998050431	WO	A2	1998-11-12	Genentech, Inc.		☐
	4	2004009618	WO	A2	2004-01-29	Crucell Holland BV		☐
	5	2005118635	WO	A2	2005-12-15	Novimmune SA		☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

EFS Web 2.1.17

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	6	2006/028936	WO	A2	2006-03-16	Genentech, Inc.		☐
	7	2006106905	WO	A1	2006-10-12	Chugai Pharmaceutical Co LTD.		☒
	8	2007110205	WO	A2	2007-10-04	Merck Patent Gmbh		☐
	9	2007147901	WO	A1	2007-12-27	Novo Nordisk AS		☐
	10	2008/119353	WO	A1	2008-10-09	Genmab AS		☐
	11	2009/080251	WO	A1	2009-07-02	Hoffmann La Roche		☐
	12	2009/080252	WO	A1	2009-07-02	Hoffmann La Roche		☐
	13	2009/080253	WO	A1	2009-07-02	Hoffmann La Roche		☐
	14	2009/089004	WO	A1	2009-07-16	Amgen, Inc.		☐
	15	2009/098596	WO	A2	2009-08-13	Zymeworks, Inc.		☐
	16	2009051974	WO	A1	2009-04-23	Nuvelo, Inc.		☐

EFS Web 2.1.17 ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	17	2010/084197	WO	A1	2010-07-29	Genmab AS		☐
	18	2010/129304	WO	A2	2010-11-11	Oncomed Pharmaceuticals, Inc.		☐
	19	2011/028952	WO	A1	2011-03-10	Xencor, Inc.		☐
	20	2011/028953	WO	A1	2011-03-10	Amfit, Inc.		☐
	21	2012/131555	WO	A2	2012-10-04	Glenmark Pharmaceuticals SA		☐
	22	2012023053	WO	A2	2012-02-23	Novimmune SA		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	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, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	ATWELL, S. et al., "Stable Heterodimers from Remodeling the Domain Interface of a Homodimer using a Phage Display Library," JMB, vol. 270, pages 26-35 (1997)	☐
	2	BAEUERLE, P., et al., "Bispecific T-Cell Engaging Antibodies for Cancer Therapy," Cancer Research,vol.69(12), pages 4941-4944 (2009)	☐
	3	BOGAN, A., et. al., "Anatomy of Hot Spots in Protein Interfaces," JMB, vol. 280, pages 1-9 (1998)	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

EFS Web 2.1.17

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	4	CARTER, P., et al., "Bispecific human IgG by design," Journal of Immunological Methods, vol. 248, pages 7-15, (2001)	☐
	5	CARTER, P., et al., "Toward the Production of Bispecific Antibody Fragments for Clinical Applications," Journal of Hematotherapy, vol. 4, pages 463-470 (1995)	☐
	6	DAVIES, J. et al., "Antibody VH Domains as Small Recognition Units," BioTechnology, vol. 13, pages 475-479 (1995)	☐
	7	DAVIS, J., 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, vol. 23 (4) pages 195-202 (2010)	☐
	8	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, vol. 20 (9), pages 2361-2370, (1981)	☐
	9	ELLERSON, J.R., et al., "Structure and Function of Immunoglobulin Domains: III. Isolation and Characterization of a Fragment Corresponding to the C y2 Homology Region of Human Immunoglobin G," Journal of Immunology, vol. 116, pages 510-517 (1976)	☐
	10	GUNASEKARAN, K. et al., "Enhancing Antibody Fc Heterodimer Formation through Electrostatic Steering Effects, APPLICATIONS TO BISPECIFIC MOLECULES AND MONOVALENT IgG," Journal of Biological Chemistry, vol. 285 (25), pages 19637-19646 (2010)	☐
	11	HENDSCH, Z., et al., "Preferential Heterodimer Formation via Undercompensated Electrostatic Interactions," J. Am. Chem. Soc., vol. 123 (6), pages 1264-1265 (2001)	☐
	12	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," The Journal of Biological Chemistry, vol. 276 (27), pages 24971-24977 (2001)	☐
	13	MARVIN, J. et al., "Redesigning an Antibody Fragment for Faster Association with Its Antigen," Biochemistry, vol. 42 (23), pages 7077-7083, (2003)	☐
	14	MCPHEE , F. et al., "Engineering human immunodeficiency virus 1 protease heterodimers as macromolecular inhibitors of viral maturation," PNAS, vol. 93, pages 11477-11481 (1996)	☐

EFS Web 2.1.17 ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	15	MERCHANT, A., et al., "An efficient route to human bispecific IgG," Nature Biotechnology, vol. 16, pages 677-681, (1998)	☐
	16	MERUS, "Merus Presents Preclinical Data on its Novel Bispecific Antibody MCLA-117 at EHA 2013,- Clinical Candidate Designed for the Treatment of Acute Myeloid Leukemia(AML)," Press Release, www.merus.nl, 3 pages, dated June 17, 2013	☐
	17	MERUS, "Merus Selects Clinical Candidate for the Treatment of Acute Myeloid Leukemia (AML)," Press Release, www.merus.nl, 2 pages, dated January 7,2013	☐
	18	MILLER, S., "Protein-Protein Recognition and the Association of Immunoglobulin Constant Domains," J. Mol. Biol. Vol. 216, pages 965-973 (1990)	☐
	19	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, vol. 10, pages 435-444 (1997)	☐
	20	NOHAILE, M.J., et al., "Altering dimerization specificity by changes in surface electrostatics," PNAS, vol. 98(6), pages 3109-3114 (2001)	☐
	21	PADLAN, E. et al, "X-Ray Crystallography of Antibodies," Advances in Protein Chemistry, vol. 49, pages 57-133 (1996)	☐
	22	PAPADEA, C., et al., "Human Immunoglobulin G and Immunoglobulin G Subclasses: Biochemical, Genetic, and Clinical Aspects," Critical Reviews in Clinical Laboratory Sciences, vol. 27(1), pages 27-58 (1989)	☐
	23	RAFFEN, R. et al., "Reengineering Immunoglobulin Domain Interactions by Introduction of Charged Residues," Protein Engineering, vol. 11(4), pages 303-309 (1998)	☐
	24	RIDGWAY, J. et al., "Knobs-into-holes' Engineering of Antibody CH3 Domains for Heavy Chain Heterodimerization," Protein Engineering, vol. 9 (7) pages 617-621 (1996).	☐
	25	SAL-MAN, N. et al., "Arginine Mutations within A Transmembrane Domain of Tar, an Escherichia coli Aspartate Receptor, Can Drive Homodimer Dissociation and Heterodimer Association in Vivo," Journal of Biochemistry, vol. 385, pages 29-36 (2005).	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

	26	SCHIFFER, M. et al., "Analysis of Immunoglobulin Domain Interactions Evidence for a Dominant Role of Salt Bridges," JMB, vol. 203, pages 799-802 (1988)	☐
	27	SELZER, T. et al., "Rational design of faster associating and tighter binding protein complexes," Nature Structural Biology, vol. 7, page 537-541 (2000)	☐
	28	SHEINERMAN, F., et al., " Electrostatic aspects of protein-protein interactions," Current Opinion in Structural Biology, vol. 10, pages 153-159 (2000)	☐
	29	SINHA, N. et al., "Differences in Electrostatic Properties at Antibody-Antigen Binding Sites: Implications for Specificity and Cross-Reactivity," Biophysical Journal, vol. 83, pages 2946-2968 (2002)	☐
	30	SINHA, N., et al. "Electrostatics in Protein Binding and Function," Current Protein and Peptide Science, vol 3, pages 601-614 (2002)	☐
	31	STRELKAUSKAS, A. et al., "Human Monoclonal Antibody: 2. Simultaneous Expression of IgG and IgM with Similar Binding Specificities by a Human Hybrid Clone," Hybridoma, vol. 6 (5), pages 479-487 (1987)	☐
	32	VAN RHENEN, A., et al., "The novel AML stem cell-associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells," Blood, vol. 110 (7), pages 2659-2666 (2007)	☐
	33	ZHAO, X., et al., "Targeting C-type lectin-like molecule-1 for antibody-mediated immunotherapy in acute myeloid leukemia," Haematologica, vol. 95(1), pages 71-78 (2010)	☐
	34	ZHU, Z. et al., "Remodeling Domain Interfaces to Enhance Heterodimer Formation," Protein Science, vol. 6, pages 781-788 (1997)	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/MICHAEL D BURKHART/	Date Considered	12/22/2015

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.D.B/

EFS Web 2.1.17

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14081848
	Filing Date	2013-11-15
	First Named Inventor	Cornelis Adriaan DE KRUIF
	Art Unit	1633
	Examiner Name	M. D. Burkhart
	Attorney Docket Number	MRX5-007CN

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Cynthia L. Kanik/	Date (YYYY-MM-DD)	2015-10-13
Name/Print	Cynthia L. Kanik, Ph.D., J.D.	Registration Number	37320

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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.**

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. 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 record s.

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 Cooperation Treaty.

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.

references listed on the enclosed PTO Form SB/08 which have been previously cited by or submitted to the Office in the parent-application, in accordance with 37 CFR §1.98(d).

In addition, this Information Disclosure Statement presents references not previously submitted to the Office. However, Applicants have not submitted copies of each cited U.S. patent and U.S. patent application in accordance with 37 CFR 1.98(a)(2)(ii).

Applicants call to the attention of the Examiner the following Applications:

Applications			
Application No.	*Filing Date*	*First Named Inventor*	*Docket No.*
13/866,747	04-19-2013	Cornelis A. DE KRUIF	MRX5-007
13/866,756	04-19-2013	Cornelis A. DE KRUIF	MRX5-008
14/040,023	09-27-2013	Cornelis A. DE KRUIF	MRX5-010
14/395,330	10-17-2014	Cornelis A. DE KRUIF	MRX5-011
14/395,325	10-17-2014	Cornelis A. DE KRUIF	MRX5-012

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicants' Attorney if the Examiner would like the Applicants to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicants' Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicants request that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement.

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MB/

/Michael Burkhart/ 12/22/2015

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Melissa Lebsock
Attorney Docket Number:	MRX5-007CN

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
Printed Copy of Patent - No Color	8001	2	3	6
		Total in USD ($)		**486**

Electronic Acknowledgement Receipt	
EFS ID:	25678304
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	04-MAY-2016
Filing Date:	15-NOV-2013
Time Stamp:	15:02:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$486
RAM confirmation Number	1397
Deposit Account	120080
Authorized User	KANIK, CYNTHIA L
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	MRX5-007CN_Issue_Fee_Payment.pdf	16765 7275d77274f1303f8ec79b2605262c9903bc0e07	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32329 14b85a7143a7c9aa34e06fb1bb13cdbd481e673a	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	49094		

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 a 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.

AMENDMENTS TO THE SPECIFICATION

At page 1, following the title, please insert the following new paragraph:

--SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 31, 2014, is named MRX5-007CN_Sequence_Listing.txt and is 8,489 bytes in size.--

At page 43, please replace the original paragraph starting at line 6 with the following amended paragraph:

Change(s) applied to document, /J.B./ 2/22/2016

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

At page 45, please replace the original paragraph starting at line 8 with the following amended paragraph:

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

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

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	06/07/2016	9358286	MRX5-007CN	1016

959 7590 05/18/2016

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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 Application Information Retrieval (PAIR) WEB site (http://pair.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 Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Merus B.V., Utrecht, NETHERLANDS;
Cornelis A. DE KRUIF, Utrecht, NETHERLANDS;
Linda Johanna Aleida HENDRIKS, Utrecht, NETHERLANDS;
Ton LOGTENBERG, Utrecht, NETHERLANDS;

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 USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

IR103 (Rev. 10/09)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: July 1, 2016
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Cornelis Adriaan de Kruif *et al.*

Patent No.: 9,358,286 B2

Issued: June 7, 2016

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

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.323

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted an error which should be corrected as set forth below.

On the Title Page

In field (73), "Assignee", delete "Merus B.V." and insert -- Merus N.V. --

The error now sought to be corrected is due to the name change of the Assignee. The name change assignment was recorded by the Assignment Recordation Branch on June 1, 2016. Accordingly, this correction does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Please charge our Deposit Account No. 12-0080 in the amount of $100.00 covering the fee set forth in 37 C.F.R. § 1.20(a). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. MRX5-007CN.

Dated: July 1, 2016

Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No.: 37,320
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4623 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Melissa Lebsock
Attorney Docket Number:	MRX5-007CN
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of Correction	1811	1	100	100

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**100**

Electronic Acknowledgement Receipt	
EFS ID:	26237361
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	01-JUL-2016
Filing Date:	15-NOV-2013
Time Stamp:	15:17:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$100
RAM confirmation Number	2455
Deposit Account	120080
Authorized User	Kanik, Cynthia
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	MRX5-007CN_Certificate_of_Correction.pdf	69941 8b25e7216dff162499810f289b80923d9ccb9cf7	no	1
Warnings:					
Information:					
2	Request for Certificate of Correction	MRX5-007CN_Request_for_Certificate_of_Correction.pdf	108591 ee72b9802b4a8bd2b7f9a93802026b2bb1d6c68f	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30227 c809532d4a68160754e22382e55b9005e2d182fe	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	208759		

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.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a 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.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
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.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
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/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9358286 B2
APPLICATION NO.	:	14/081,848
ISSUE DATE	:	June 7, 2016
INVENTOR(S)	:	Cornelis Adriaan DE KRUIF *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

(73) Assignee: Merus N.V., Utrecht (NL)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: July 1, 2016 Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Cynthia L. Kanik, Ph.D., J.D.
NELSON MULLINS RILEY & SCARBOROUGH LLP 1
One Post Office Square
Boston, Massachusetts 02109-2127

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

959 e 2016-08-22

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

Paper No.

Application No.:	**14/081,848**	**Date Mailed:**	**2016-08-22** **ELECTRONIC**
First Named Inventor:	Cornelis A. DE KRUIF	Examiner:	BURKHART, MICHAEL D
Attorney Docket No.:	MRX5-007CN	Art Unit:	1633
Confirmation No.:	1016	Filing Date:	11/15/2013

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

Commissioner for Patents

PTO-90c (Rev.08-06)

Response to the Request for Certificate of Correction	Patent No. 9358286	Applicant(s) DE KRUIF ET AL.
	Issue Date 07 June, 2016	Docket No. MRX5-007CN

This is in response to the request for a Certificate of Correction filed 01 July, 2016

☒ **Request Denied** – Consideration has been given to your request for the issuance of a Certificate of Correction under the provisions of 37 CFR 1.322 and/or 37 CFR 1.323. The Request is improper and denied for the reason(s) below:

1. ☒ Assignees' names and addresses (assignment data) printed in a patent, are based solely on information supplied in the appropriate space for identifying the assignment data on the Issue Fee Transmittal Form (PTOL-85b). Any request for a patent to be corrected to state the name of the assignee, must state that the assignment was submitted for recordation as set forth in in 37 CFR 3.11 before issuance of the patent. Petition under 3.81 is to be filed for consideration of correction to assignee. The petition fee set forth in 37 CFR 1.17(i)(1) (currently $140, $70, $35 for large, small and micro entities, respectively.

2. ☐ The alleged error in , is in fact an Amendment and/or Change made by the examiner and considered to be in accordance with the permissible amendments enumerated in the Manual of Patent Examining Procedure (MPEP) Section 1302.04. Applicant did not file objection or amendment under 37 CFR 1.312 prior to payment of the issue fee.

3. ☐ A petition under CFR 1.182 is required to correct the alleged errors in spelling or order of inventor's names, since inventor's names are printed solely in accordance with the type-written names, and in the order of the type-written names on the Application Data Sheet (ADS). The required fee currently under rule 1.17(f) (small entity $200, large entity $400, micro entity fee $100).

4. ☐ With respect to the alleged error in changing the inventor name on the patent due to clerical error in ADS/OATH of related patents. The inventor's name is printed in accordance with the OATH/ADS submitted at the time of filing the application. However, your attention is directed to C.F.R. 1.324, wherein a request is being made to change, add or delete inventor(s), after issuance of the patent.

5. ☐ With respect to the alleged error in , comparison of the printed patent with the corresponding location in the application file reveals that there is no discrepancy.

6. ☐ With respect to 37 CFR 1.72, the title should be brief but technically accurate and descriptive and should contain fewer than 500 characters. Inasmuch as the words "new," "improved," "improvement of," and "improvement in" are not considered as part of the title of an invention, these words should not be included at the beginning of the title of the invention and will be deleted when the Office enters the title into the Office's computer records, and when any patent issues.

7. ☐ The fee for correction under 37 CFR 1.323 is set forth in 37 CFR 1.20(a). ☐ Partial fee ☐ No fee was received with your request. Full fee payment is required before further action is taken on this request.

8. ☐ With respect to the request for corrected Letters Patent (Grant), corrections to the original Letters Patent are made under the provisions of Rule 1.322(b), not Rule1.323, unless a petition is granted.

9. ☒ Other Comments: A petition under 3.81 must also be filed.

Further correspondence concerning this matter should be filed and directed to the Certificates of Correction Branch.

Legal Instrument Examiner: **VIRGINIA TOLBERT** Phone: (571)272-0460

Certificates of Correction Branch email: CustomerServiceCoC@uspto.gov CoC Central Phone Number: (703) 756-1814

If applicable, information regarding a petition under 37 CFR 1.183 should be directed to the attention of the Commissioner for Patents using the FAX number (571) 273-8300

U.S. Patent and Trademark Office
PTO-998 (Rev. 10/2014)

Part of Paper No 20160818-1

PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9358286 B2
APPLICATION NO.	:	14/081,848
ISSUE DATE	:	June 7, 2016
INVENTOR(S)	:	Cornelis Adriaan DE KRUIF *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page

In field (73), "Assignee", delete "Merus B.V." and insert -- Merus N.V. --

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: August 23, 2016 Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Cynthia L. Kanik, Ph.D., J.D.
NELSON MULLINS RILEY & SCARBOROUGH LLP 1
One Post Office Square
Boston, Massachusetts 02109-2127

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: August 23, 2016
Electronic Signature for Cynthia L. Kanik, Ph.D., J.D.: /Cynthia L. Kanik/

Docket No.: MRX5-007CN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Cornelis Adriaan de Kruif *et al.*

Patent No.: 9,358,286 B2

Issued: June 7, 2016

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

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.323

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted an error which should be corrected as set forth below.

On the Title Page

In field (73), "Assignee", delete "Merus B.V." and insert -- Merus N.V. --

The error now sought to be corrected is due to the name change of the Assignee. The name change assignment was recorded by the Assignment Recordation Branch on June 1, 2016. Accordingly, this correction does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment, along with a Petition to Correct Assignee after Payment of Issue Fee under 37 C.F.R. 3.81(b). Patentee respectfully solicits the granting of the requested Certificate of Correction.

Please charge our Deposit Account No. 12-0080 in the amount of $240.00 covering the fee set forth in 37 C.F.R. § 1.20(a) and the associated processing fee. The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. MRX5-007CN.

Dated: August 23, 2016

Respectfully submitted,

Electronic signature: /Cynthia L. Kanik/
Cynthia L. Kanik, Ph.D., J.D.
Registration No.: 37,320
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4623 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

Doc Code: PET.AUTO **Document Description: Petition automatically granted by EFS-Web**	PTO/SB/141 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**Petition to Correct Assignee After Payment of Issue Fee (37 CFR 3.81(b))**
Application Number	14081848
Filing Date	15-Nov-2013
First Named Inventor	Cornelis DE KRUIF
Attorney Docket Number	MRX5-007CN
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES

Pursuant to 37 CFR 3.81(b), applicant hereby request that the listed assignee with respect to U.S. Patent Number 9358286 be corrected to accurately reflect the name of the assignee on the front page of the above-identified patent by way of a Certificate of Correction.

☒ I certify, in accordance with 37 CFR 1.4(d)(4), that the assignment was submitted for recordation as set forth in 37 CFR 3.11 before issuance of the patent.

Correction of Assignee

Current Assignment Listed (240 char limit)
The assignment information is currently listed as:

Merus B.V.

Update Assignment Listing to (240 char limit)

Change assignment information to the following:

Merus N.V.

☒ As required by 37 CFR 3.81, a Request for a Certificate of Correction is being filed herewith, along with the fee set forth in 37 CFR 1.20(a).

Petition Fee
◯ Small Entity
◯ Micro Entity
◉ Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

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 been given power of attorney in this application.

◯ An attorney or agent registered to practice before the Patent and Trademark Office, acting in a representative capacity.

◯ A sole inventor

◯ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application

◯ A joint inventor; all of whom are signing this request

Signature	/Cynthia L. Kanik/
Name	Cynthia L. Kanik, Ph.D., J.D.
Registration Number, if applicable	37320

Electronic Patent Application Fee Transmittal	
Application Number:	14081848
Filing Date:	15-Nov-2013
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Filer:	Cynthia L. Kanik/Melissa Lebsock
Attorney Docket Number:	MRX5-007CN

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Certificate of Correction	1811	1	100	100
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**240**

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Decision Date:	August 23, 2016
In re Patent No.	9358286
Issue Date:	07-Jun-2016
Application No	14081848
Filed date	15-Nov-2013
Attorney Docket No	MRX5-007CN

DECISION ON REQUEST
UNDER 37 CFR 3.81(b)

This is an electronic decision on the request filed August 23, 2016 under 37 CFR 3.81(b) to correct the name of the assignee of the above-identified patent by way of a Certificate of Correction.

Petitioner request that the listed assignment information be replace with updated assignment information.
Assignment Information Currently Listed As:

Merus B.V.

Change Assignment Information to the Following:

Merus N.V.

The request is GRANTED.

Telephone inquiries concerning this decision may be directed to the the Patent Electronic Business Center (EBC) at 866-217-9197.

Inquiries regarding the issuance of a Certificate of Correction should be directed to the Certificate of Correction Branch at (571) 272-4200.

The Certificate of Correction Branch will be notified of this decision granting the request under 37 CFR 3.81(b) and directing issuance of the requested Certificate of Correction.

Office of Petitions

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (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 including the Patent, advance orders and notification of maintenance fees 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.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

959 7590 02/12/2016
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127

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 facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/081,848	11/15/2013	Cornelis Adriaan DE KRUIF	MRX5-007CN	1016

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	SMALL	$480.00	$0	$0	$486.00	05/12/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
M. D. Burkhart	1633	435-455000

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/SB/122) attached.
- [ ] "Fee Address" indication (or "Fee Address" Indication form 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 Nelson Mullins Riley & Scarborough LLP
2 Jane E. Remillard, Esq.
3 Cynthia L. Kanik, Ph.D., J.D.

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 has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Merus B.V.
(B) RESIDENCE: (CITY and STATE OR COUNTRY): Utrecht, Netherlands

Please check the appropriate assignee category or categories (will not be printed on the patent) : [ ] Individual [X] Corporation or other private group entity [ ] Government

4a. The following fee(s) are submitted:
- [X] Issue Fee
- [ ] Publication Fee (No small entity discount permitted)
- [X] Advance Order - # of Copies 2

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- [ ] A check is enclosed.
- [ ] Payment by credit card. Form PTO-2038 is attached.
- [X] The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number 12-0080 (enclose an extra copy of this form).

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 /Cynthia L. Kanik/ Date May 4, 2016
Typed or printed name Cynthia L. Kanik, Ph.D., J.D. Registration No. 37,320

Electronic Acknowledgement Receipt	
EFS ID:	26702970
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Cynthia L. Kanik/Melissa Lebsock
Filer Authorized By:	Cynthia L. Kanik
Attorney Docket Number:	MRX5-007CN
Receipt Date:	23-AUG-2016
Filing Date:	15-NOV-2013
Time Stamp:	11:24:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$240
RAM confirmation Number	9645
Deposit Account	120080
Authorized User	Kanik, Cynthia
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	MRX5-007CN_Draft_Cert_of_Correction.pdf	69891 b1367240aae8c02f04ea3c0974ae8735d7ab52e7	no	1
Warnings:					
Information:					
2	Request for Certificate of Correction	MRX5-007CN_Request_for_Cert_of_Correction.pdf	109060 0b4027ed0a7194f4a09db995de855786c0eecb70	no	2
Warnings:					
Information:					
3	Petition automatically granted by EFS	petition-request.pdf	31615 64ba95768183a94aeb1b6f1be0a0893e1694dadc	no	2
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	31942 53ef708e08e17b1c2b8aea055b40bdc4d23cbe2b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	242508		

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 a 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.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.	: 9,358,286 B2
APPLICATION NO.	: 14/081848
DATED	: June 7, 2016
INVENTOR(S)	: Cornelis Adriaan De Kruif et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page

Item (73), "Assignee", delete "Merus B.V." and insert -- Merus N.V. --

Signed and Sealed this
Eleventh Day of October, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
Approved for use through 01/31/2018. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	14/081,848
Filing Date	November 15, 2013
First Named Inventor	Cornelis Adriaan DE KRUIF
Title	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
Art Unit	1633
Examiner Name	Burkhart, Michael D.
Attorney Docket Number	4096.0050003/DAS/PAC/E-H

SIGNATURE of Applicant or Patent Practitioner

Signature	/Paul A. Calvo/	Date (Optional)	2.7.2018
Name	Paul A. Calvo	Registration Number	57,913
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)	Merus N.V.		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

[X] I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: **26111**

OR

[ ] I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

[X] The address associated with the above-mentioned Customer Number

OR

[ ] The address associated with Customer Number: **26111**

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Merus N.V.

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/T. Logtenberg/	Date (Optional)	16 November 2017
Name	T. Logtenberg		
Title	CEO		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[ ] Total of forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 3 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.**

8447755 *If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Merus N.V.

Application No./Patent No.: 14/081,848 Filed/Issue Date: November 15, 2013

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

Merus N.V., a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

B. ☑ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: Inventors To: Merus B.V.

      The document was recorded in the United States Patent and Trademark Office at

      Reel 031637, Frame 0906, or for which a copy thereof is attached.

   2. From: Merus B.V. To: Merus N.V.

      The document was recorded in the United States Patent and Trademark Office at

      Reel 039038, Frame 0866, or for which a copy thereof is attached.

This collection of information is required by 37 CFR 3.73(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.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☑ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Paul A. Calvo/	2.7.2018
Signature	Date
Paul A. Calvo	57,913
Printed or Typed Name	Title or Registration 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. 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 disclosure of these records is required by the Freedom of Information Act.
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 Cooperation Treaty.
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 inspection 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.

Electronic Acknowledgement Receipt	
EFS ID:	31733851
Application Number:	14081848
International Application Number:	
Confirmation Number:	1016
Title of Invention:	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES
First Named Inventor/Applicant Name:	Cornelis A. DE KRUIF
Customer Number:	959
Filer:	Paul Anthony Calvo/Debbie Colonna
Filer Authorized By:	Paul Anthony Calvo
Attorney Docket Number:	MRX5-007CN
Receipt Date:	08-FEB-2018
Filing Date:	15-NOV-2013
Time Stamp:	10:52:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		4096_0050003_Filing_ReqCorrectApplicantName_ADS_POA_373c.pdf	1568932 0e238d8c04383db3fb48838c9e38916d04aa386b	yes	13

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Miscellaneous Incoming Letter	1	1
	Application Data Sheet	2	8
	Power of Attorney	9	10
	Assignee showing of ownership per 37 CFR 3.73	11	13
Warnings:			
Information:			
	Total Files Size (in bytes):	1568932	

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 a 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

Inventors: DE KRUIF *et al.*	Confirmation No.: 1016
Applicant: Merus N.V.	Art Unit: 1633
Application No.: 14/081,848	Examiner: Burkhart, Michael D.
Filing Date: November 15, 2013	Atty. Docket: 4096.0050003/DAS/PAC/E-H

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

Request to Correct or Update Name of the Applicant Under 37 CFR 1.46(c)(1) After an Applicant has been Specified

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

Commissioner:

Applicant hereby requests that the United States Patent and Trademark Office correct or update the name of the applicant under 37 C.F.R. § 1.46(c)(1) after an applicant has been specified in the above-captioned patent application. Specifically, Applicant requests that the Office correct or update the name of the applicant from **"Merus B.V."** to **--Merus N.V.--** This request is accompanied by a signed Application Data Sheet marked-up to show the correct name of the applicant, Merus N.V., in the Applicant Information section, an executed Power of Attorney, and a Statement Under 37 C.F.R. 3.73(c) showing chain of title to Applicant.

Prompt and favorable consideration of this request is respectfully requested. Applicant hereby authorizes the U.S. Patent and Trademark Office 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/

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

Date: 2.7.2018

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

8964587_1.docx

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	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
Mr.	Cornelis	A.	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584CH	**Country** i	NL

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
Ms.	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584CH	**Country** i	NL

Inventor 3 Remove

Legal Name

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	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	c/o Merus B.V.		
Address 2	Padualaan 8 (postvak 133)		
City	Utrecht	**State/Province**	
Postal Code	3584 CH	**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	00959		
Email Address	IPBoston.Docketing@nelsonmullins.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES		
Attorney Docket Number	~~MRX5-007CN~~ 4096.0050003/DAS/PAC/E-H	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	39	**Suggested Figure for Publication (if any)**	

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:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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	00959		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this 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.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13866747	2013-04-19
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13866747	non provisional of	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(d). 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(h)(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 [j] (if applicable)

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

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If 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 World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

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	

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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 B.V.~~ Merus N.V.

Mailing Address Information:

Address 1	~~Padualaan 8 (postvak 133)~~ Yalelaan 62		
Address 2			
City	Utrecht	**State/Province**	
Country [i]	NL	Postal Code	~~3584CH~~ 3584 CM
Phone Number		Fax Number	
Email Address			

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

Non-Applicant Assignee Information:

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

Assignee 1

Complete this section only if non-applicant assignee information is desired to be included on the patent application publication in accordance with 37 CFR 1.215(b). Do not include in this section 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), as the patent application publication will include the name of the applicant(s).

Remove

If the Assignee is an Organization check here. ☐

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

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MRX5-007CN
	Application Number	
Title of Invention	METHODS AND MEANS FOR THE PRODUCTION OF IG-LIKE MOLECULES	

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

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

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Paul A. Calvo/			Date (YYYY-MM-DD)	2018-02-07
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

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.**

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. 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 Cooperation Treaty.

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.

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/081,848	11/15/2013	Cornelis A. DE KRUIF	MRX5-007CN

CONFIRMATION NO. 1016

959
NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

POWER OF ATTORNEY NOTICE

OC000000097337222

Date Mailed: 02/12/2018

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/08/2018.

- The Power of Attorney to you in this application has been revoked by the applicant. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/qtran/

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/081,848	11/15/2013	Cornelis A. DE KRUIF	4096.0050003/DAS/PAC/E-H

CONFIRMATION NO. 1016

POA ACCEPTANCE LETTER

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

OC000000097337223

Date Mailed: 02/12/2018

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/08/2018.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/qtran/

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/081,848	Filing Date 11/15/2013	☐ 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 $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ 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

AMENDMENT 10/13/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 38	Minus	** 33	= 5	X $40 =	200
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☒ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						**390**
					TOTAL ADD'L FEE	**590**

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ 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.
** 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 number found in the appropriate box in column 1.

LIE
/Ruby Johnson/

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/081,848

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	33 minus 20 =	* 13	x 40 =	520	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 210 =	210			
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).			200			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1660		TOTAL	

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.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/081,848

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	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	33 minus 20 =	* 13	x 40 =	520	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 210 =	210			
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).			200			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1660		TOTAL	

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
	14081848	DE KRUIF ET AL.
	Examiner	Art Unit
	MICHAEL BURKHART	1633

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search (Medline, EAST), STN Keyword search (Medline, CAPlus, Embase, Biosis), USPat, USPgPub, EPO, JPO, Derwent keyword search (EAST)	4/6/2015	MB

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/M.B./ Primary Examiner.Art Unit 1633

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14081848	DE KRUIF ET AL.
	Examiner	Art Unit
	MICHAEL BURKHART	1633

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search (Medline, EAST), STN Keyword search (Medline, CAPlus, Embase, Biosis), USPat, USPgPub, EPO, JPO, Derwent keyword search (EAST)	4/6/2015	MB
Updated	12/22/2015	MB
Parent cases reviewed and references checked	12/22/2015	MB

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
none	none	12/22/2015	MB

	/M.B./ Primary Examiner.Art Unit 1633

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14081848	DE KRUIF ET AL.
	Examiner	**Art Unit**
	MICHAEL BURKHART	1633

CPC						
Symbol					**Type**	**Version**
A61K		39	/	3955	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		2317	/	14	A	2013-01-01
C07K		2317	/	526	A	2013-01-01
C07K		2317	/	94	A	2013-01-01
C07K		16	/	18	I	2013-01-01
C07K		16	/	1278	I	2013-01-01
C12P		21	/	005	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
C07K		2317	/	31	A	2013-01-01
A61K		39	/	395	I	2013-01-01
A61K		39	/	40	I	2013-01-01
A61K		2039	/	507	A	2013-01-01
C07K		16	/	1282	I	2013-01-01
C07K		2317	/	56	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 32	
/MICHAEL BURKHART/ Primary Examiner.Art Unit 1633 (Primary Examiner)	12/22/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14081848	DE KRUIF ET AL.
	Examiner	**Art Unit**
	MICHAEL BURKHART	1633

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
435	455	C	1	2	N	15 / 00 (2006.01.01)						
CROSS REFERENCE(S)		C	1	2	N	5 / 07 (2010.01.01)						
		C	1	2	P	21 / 04 (2006.01.01)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)											
435	69.6	326										

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 32	
/MICHAEL BURKHART/ Primary Examiner.Art Unit 1633 (Primary Examiner)	12/22/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office — Part of Paper No. 20151222

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14081848	DE KRUIF ET AL.
	Examiner	**Art Unit**
	MICHAEL BURKHART	1633

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☐ **T.D.** ☐ **R.1.47**

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 32	
/MICHAEL BURKHART/ Primary Examiner.Art Unit 1633 (Primary Examiner)	12/22/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none