US 20110028695A1

(19) United States

(12) Patent Application Publication

(10) Pub. No.: US 2011/0028695 A1

(43) Pub. Date: Feb. 3, 2011

Revets et al.

(54) METHOD FOR OBTAINING POLYPEPTIDE CONSTRUCTS COMPRISING TWO OR MORE SINGLE DOMAIN ANTIBODIES

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(21) Appl. No.: **12/744,970**

(22) PCT Filed: **Nov. 28, 2008**

(86) PCT No.: **PCT/EP08/66369**

§ 371 (c)(1),
(2), (4) Date: **Oct. 19, 2010**

(30) **Foreign Application Priority Data**

Nov. 27, 2007 (US) 61/004,332
Dec. 4, 2007 (US) 61/005,265
Dec. 4, 2007 (US) 61/005,324
Dec. 4, 2007 (US) 61/005,331

Publication Classification

(51) **Int. Cl.**
C40B 30/00 (2006.01)
C07K 16/46 (2006.01)

(52) **U.S. Cl.** **530/387.3**; 506/7

(57) **ABSTRACT**

The present invention relates to methods for obtaining a polypeptide construct directed against one or more antigens and/or epitopes and having one or more desired characteristics, wherein the polypeptide construct comprises at least two single domain antibodies. The methods of the present invention involve producing a diversity of polypeptide constructs that are structural variants and screening the produced diversity of polypeptide constructs for a polypeptide construct having said one or more desired characteristics. The present invention further relates to polypeptide constructs directed against one or more antigens and/or epitopes having one or more desired characteristics, wherein the polypeptide construct comprises at least two single domain antibodies. The methods and polypeptide constructs according to the present invention are useful for the identification of optimal therapeutic compounds.

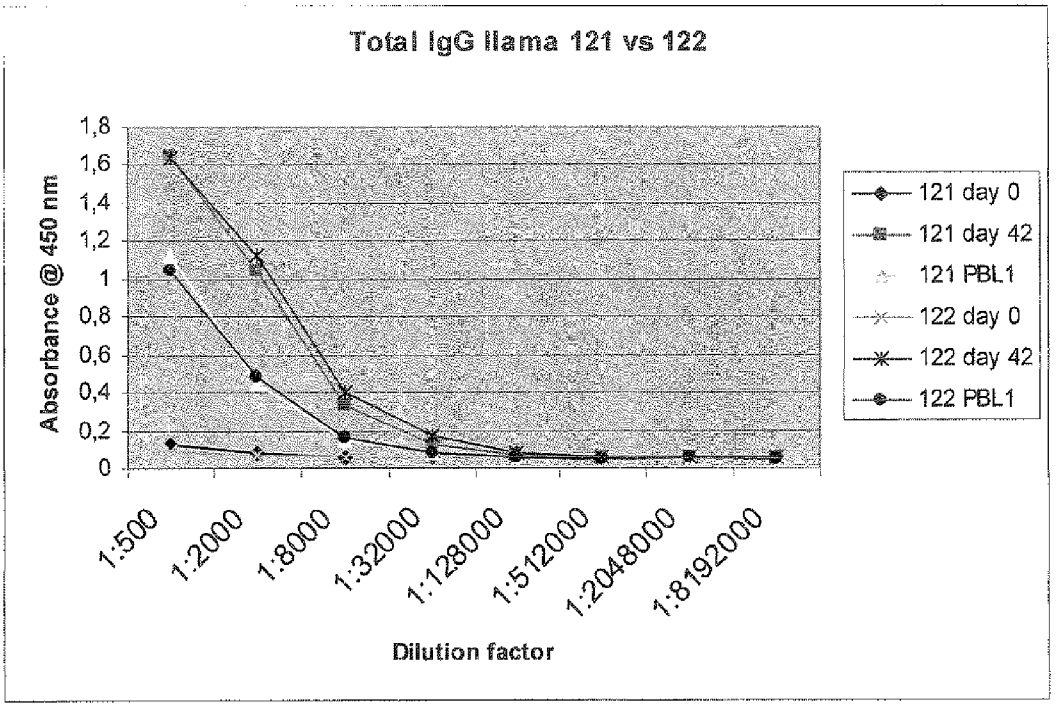

Total IgG llama 121 vs 122
Absorbance @ 450 nm
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1:500
1:2000
1:8000
1:32000
1:128000
1:512000
1:2048000
1:8192000
Dilution factor
121 day 0
121 day 42
121 PBL1
122 day 0
122 day 42
122 PBL1

Figure 1A

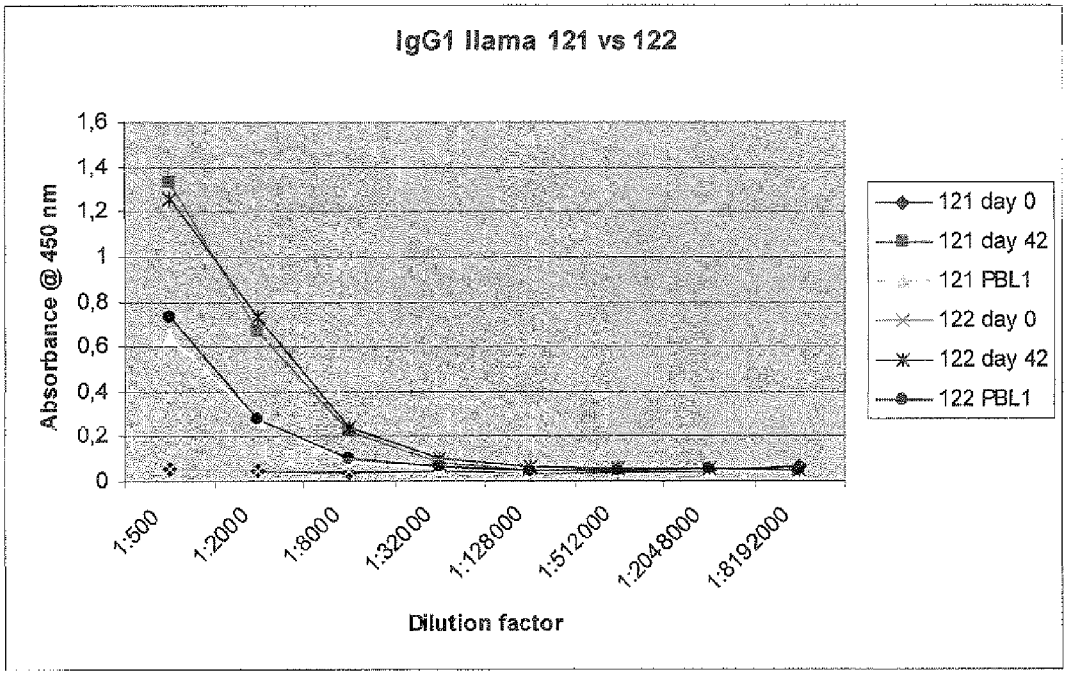

IgG1 llama 121 vs 122
Absorbance @ 450 nm
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1:500
1:2000
1:8000
1:32000
1:128000
1:512000
1:2048000
1:8192000
Dilution factor
121 day 0
121 day 42
121 PBL1
122 day 0
122 day 42
122 PBL1

Figure 1B

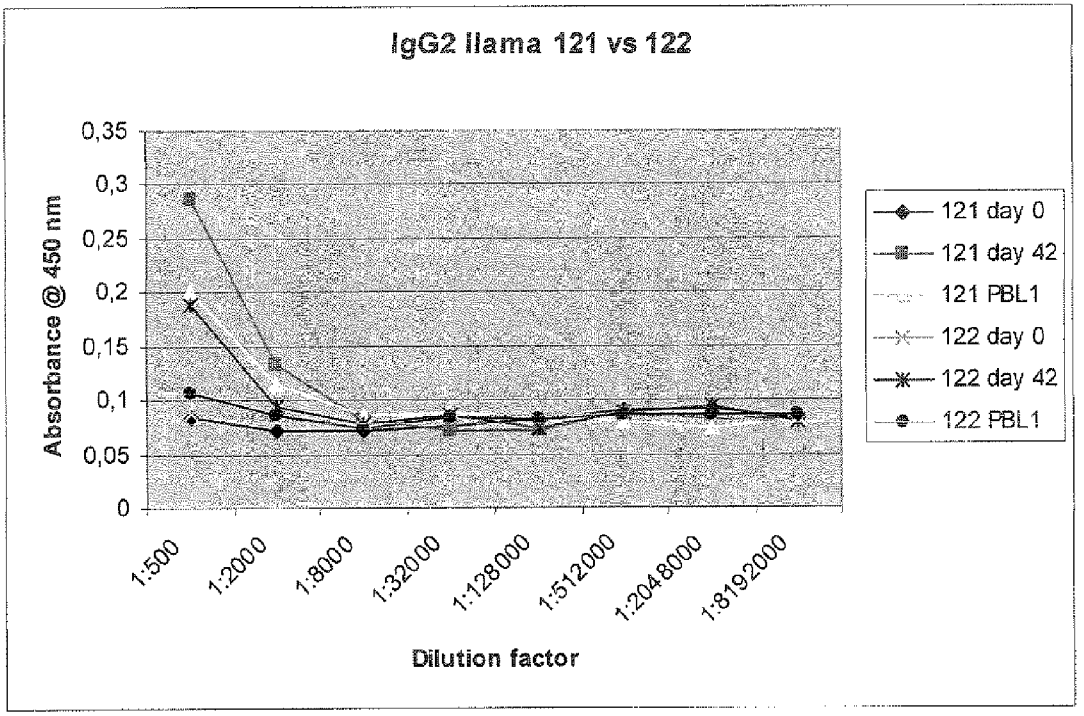

IgG2 llama 121 vs 122
Absorbance @ 450 nm
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
1:500
1:2000
1:8000
1:32000
1:128000
1:512000
1:2048000
1:8192000
Dilution factor
121 day 0
121 day 42
121 PBL1
122 day 0
122 day 42
122 PBL1

Figure 1C

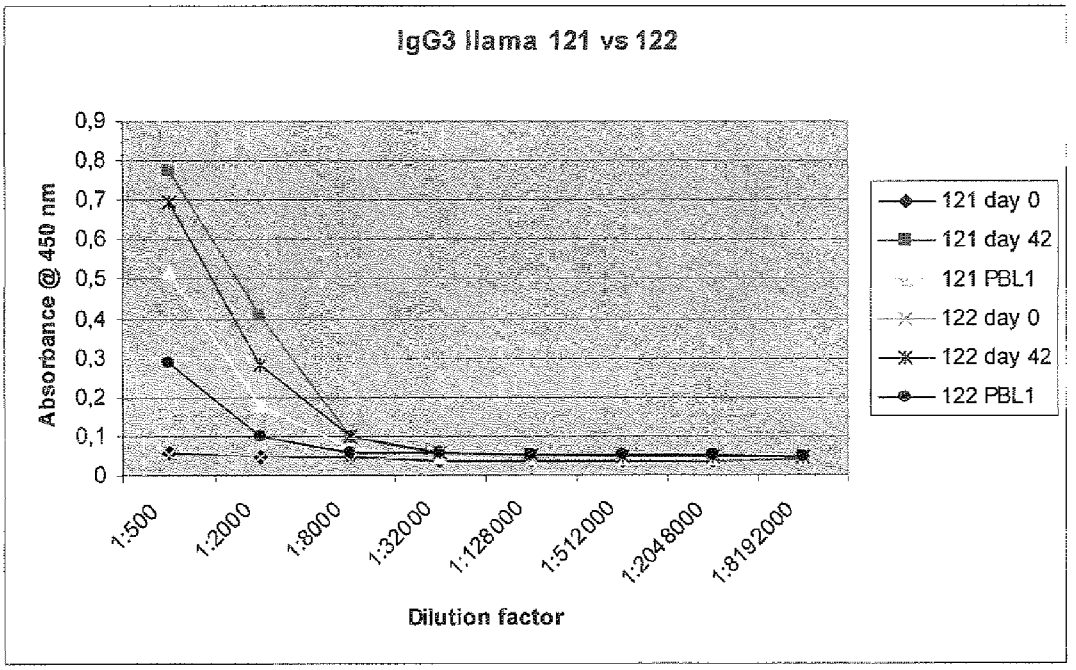

IgG3 llama 121 vs 122
Absorbance @ 450 nm
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1:500
1:2000
1:8000
1:32000
1:128000
1:512000
1:2048000
1:8192000
Dilution factor
121 day 0
121 day 42
121 PBL1
122 day 0
122 day 42
122 PBL1

Figure 1D

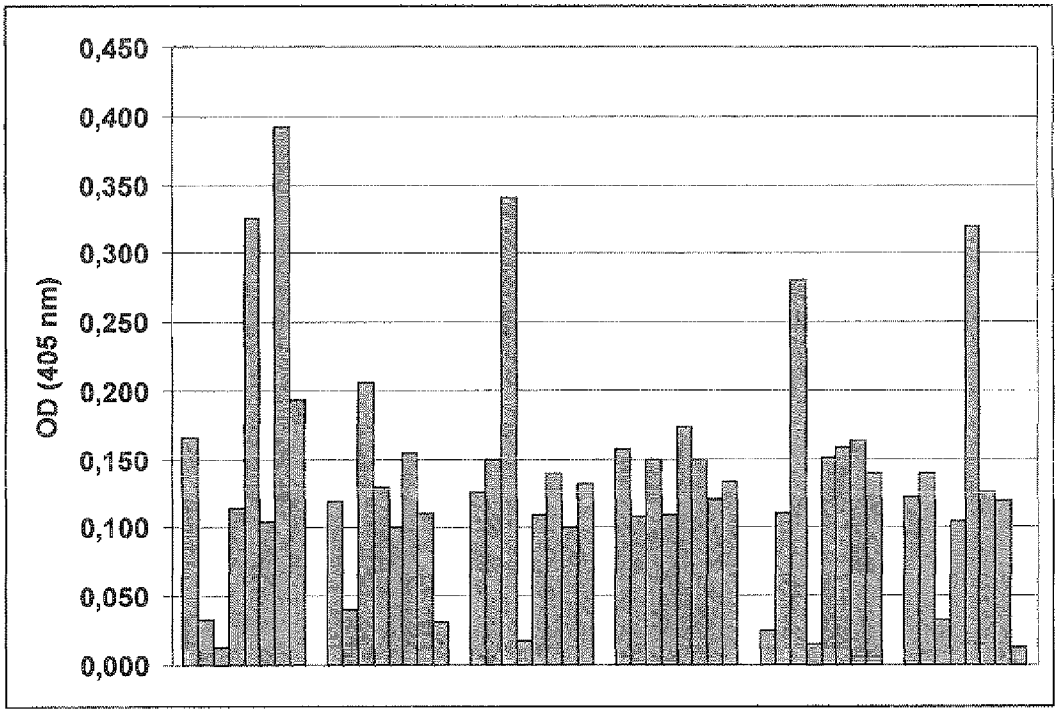


Figure 2

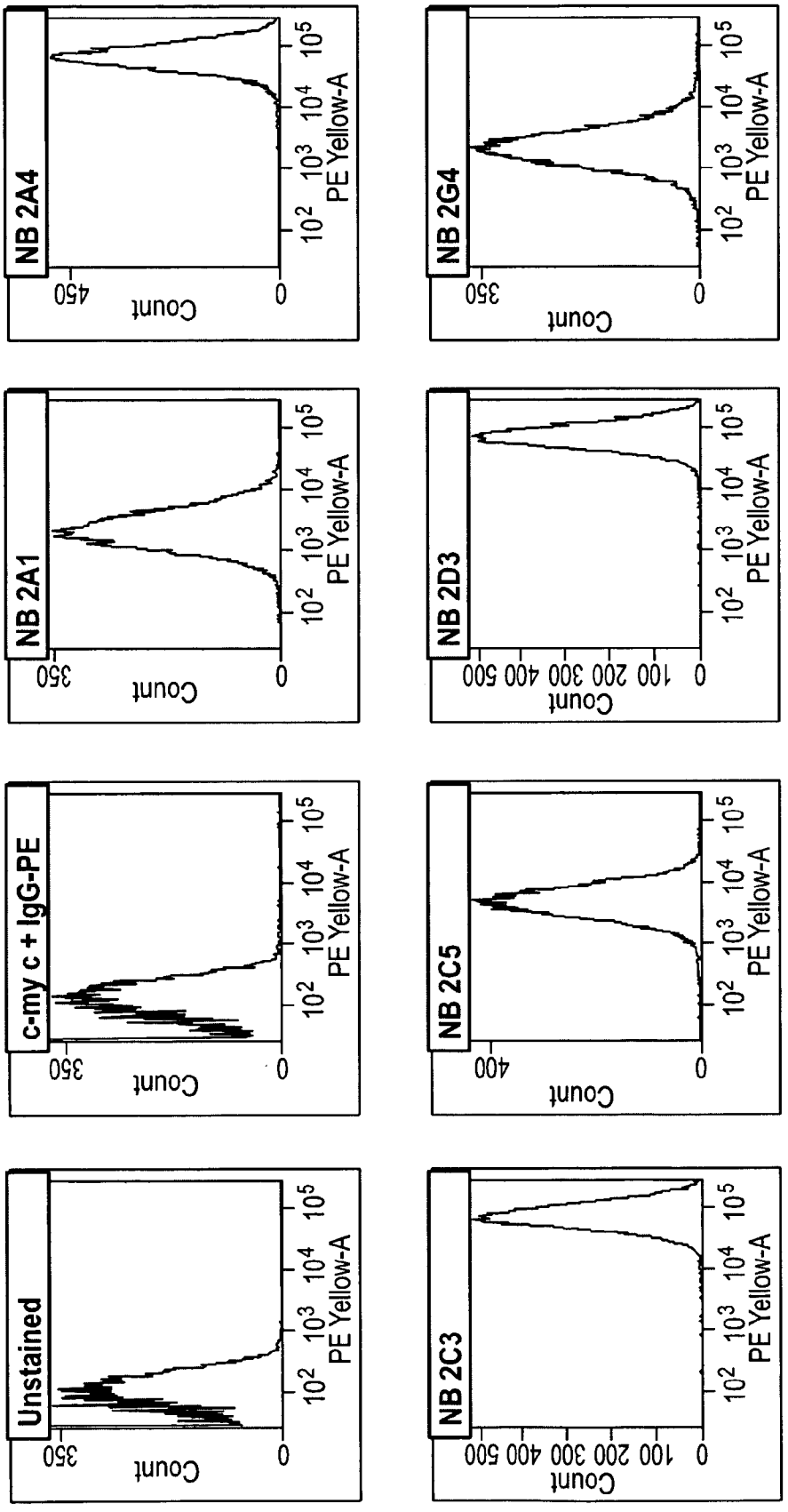

Unstained
c-myc + IgG-PE
NB 2C3
NB 2C5
NB 2D3
NB 2A1
NB 2G4
NB 2A4
Count
PE Yellow-A
0
100 200 300 400 500
350
400
450

Figure 3

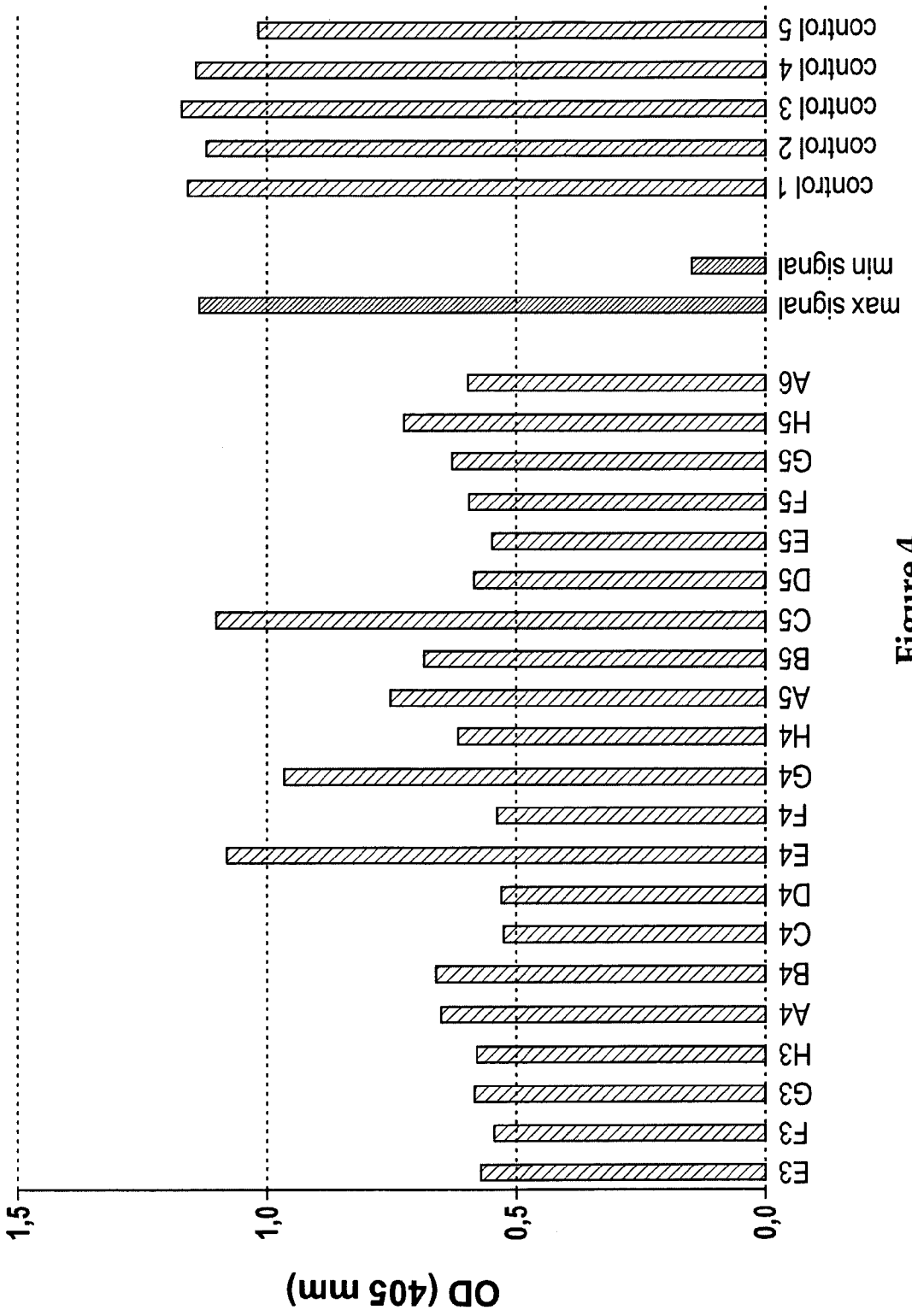
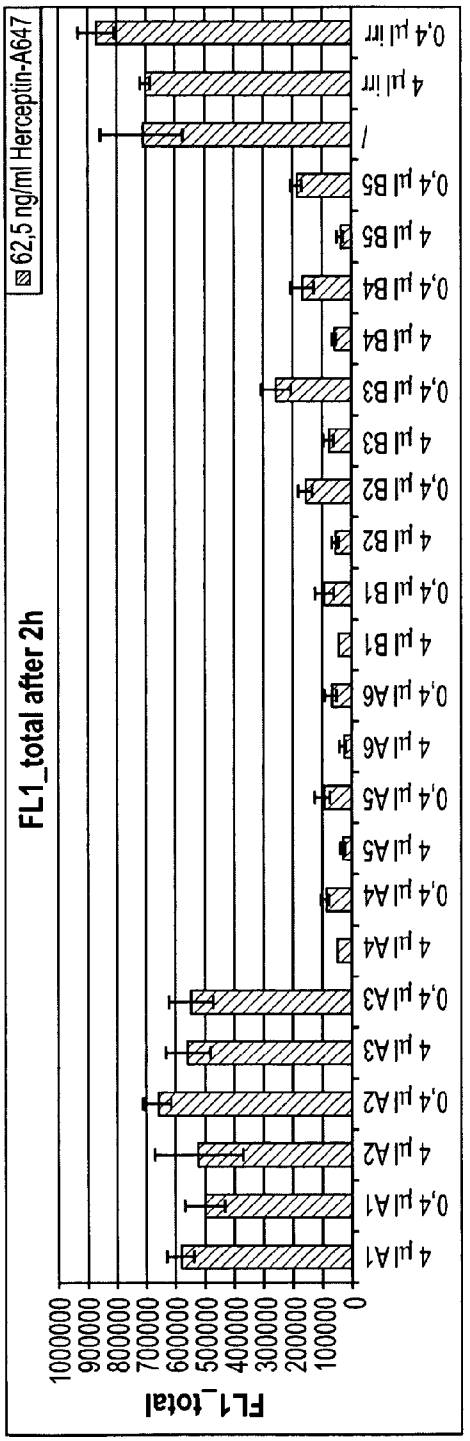

OD (405 nm)
1,5
1,0
0,5
0,0
E3
F3
G3
H3
A4
B4
C4
D4
E4
F4
G4
H4
A5
B5
C5
D5
E5
F5
G5
H5
A6
max signal
min signal
control 1
control 2
control 3
control 4
control 5

Figure 4


62,5 ng/ml Herceptin-A647
FL1_total after 2h
FL1_total
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
4 µl A1
0,4 µl A1
4 µl A2
0,4 µl A2
4 µl A3
0,4 µl A3
4 µl A4
0,4 µl A4
4 µl A5
0,4 µl A5
4 µl A6
0,4 µl A6
4 µl B1
0,4 µl B1
4 µl B2
0,4 µl B2
4 µl B3
0,4 µl B3
4 µl B4
0,4 µl B4
4 µl B5
0,4 µl B5
/
4 µl irr
0,4 µl irr

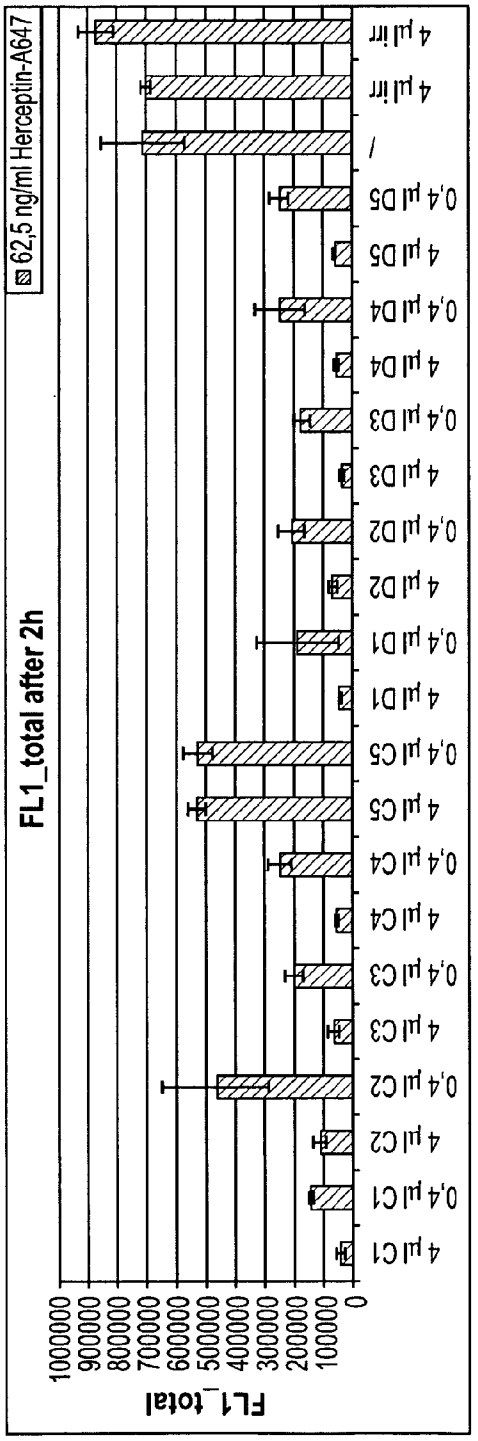

62,5 ng/ml Herceptin-A647
FL1_total after 2h
FL1_total
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
4 µl C1
0,4 µl C1
4 µl C2
0,4 µl C2
4 µl C3
0,4 µl C3
4 µl C4
0,4 µl C4
4 µl C5
0,4 µl C5
4 µl D1
0,4 µl D1
4 µl D2
0,4 µl D2
4 µl D3
0,4 µl D3
4 µl D4
0,4 µl D4
4 µl D5
0,4 µl D5
/
4 µl irr
0,4 µl irr

Figure 5

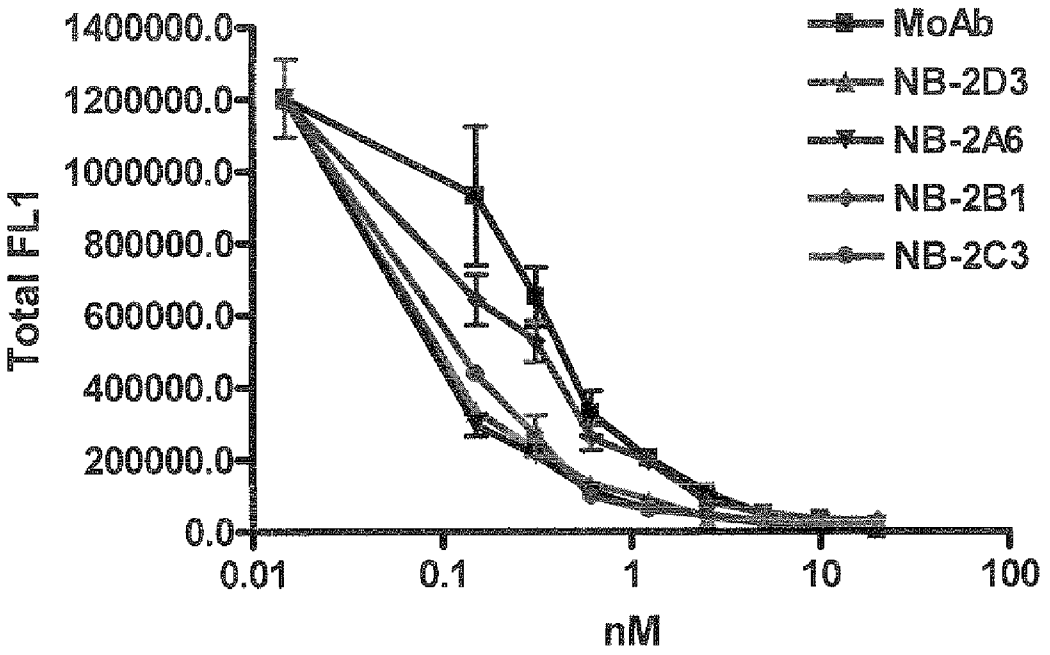

1400000.0
1200000.0
1000000.0
800000.0
600000.0
400000.0
200000.0
0.0
Total FL1
0.01
0.1
1
10
100
nM
MoAb
NB-2D3
NB-2A6
NB-2B1
NB-2C3

Figure 6

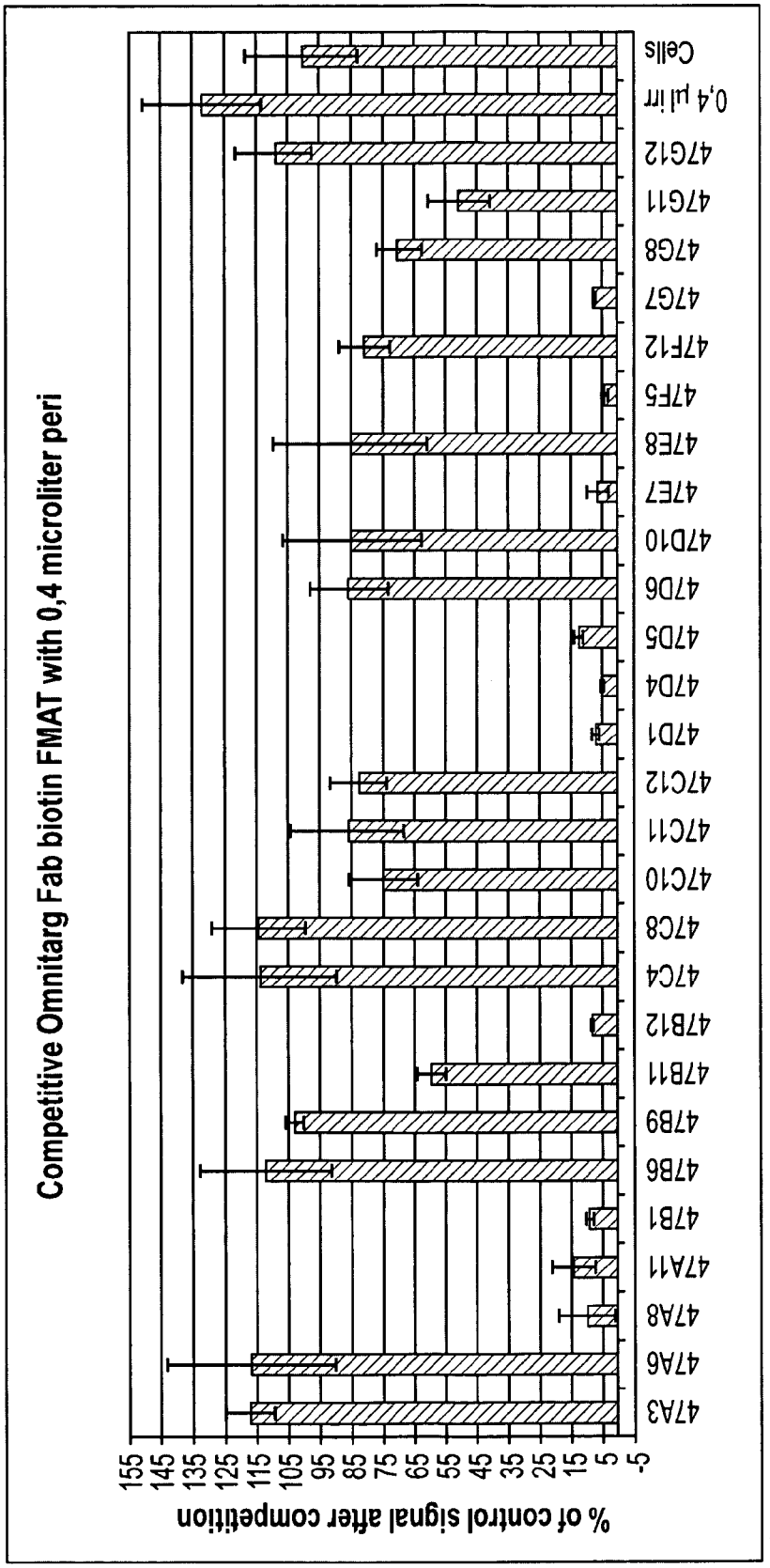

Competitive Omnitarg Fab biotin FMAT with 0,4 microliter peri
% of control signal after competition
155
145
135
125
115
105
95
85
75
65
55
45
35
25
15
5
-5
47A3
47A6
47A8
47A11
47B1
47B6
47B9
47B11
47B12
47C4
47C8
47C10
47C11
47C12
47D1
47D4
47D5
47D6
47D10
47E7
47E8
47F5
47F12
47G7
47G8
47G11
47G12
0,4 µl irr
Cells

Figure 7

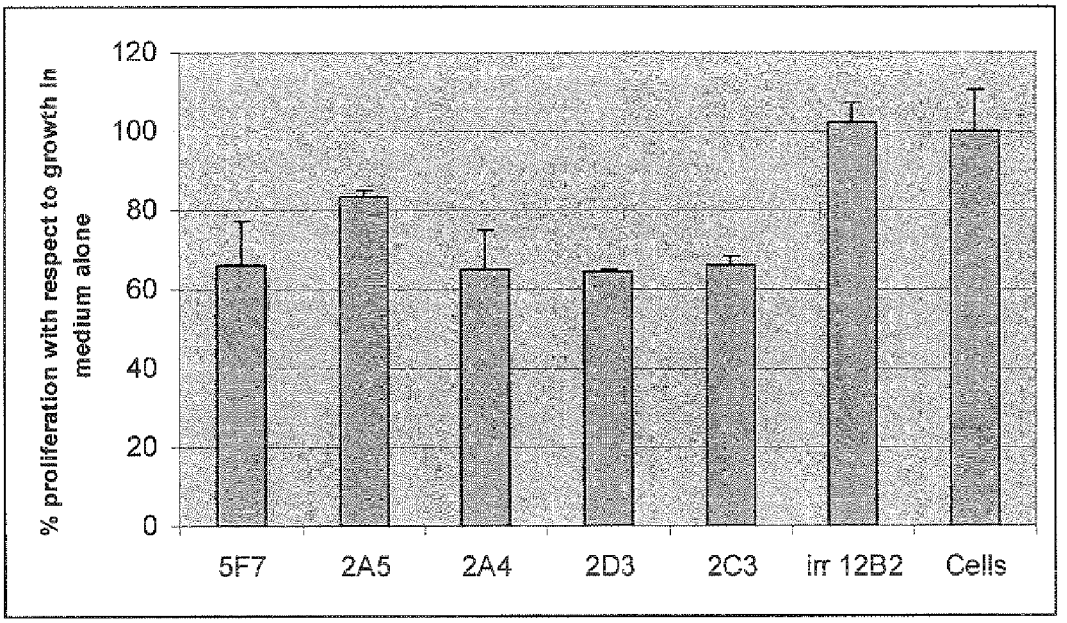

% proliferation with respect to growth in medium alone
120
100
80
60
40
20
0
5F7
2A5
2A4
2D3
2C3
irr 12B2
Cells

Figure 8

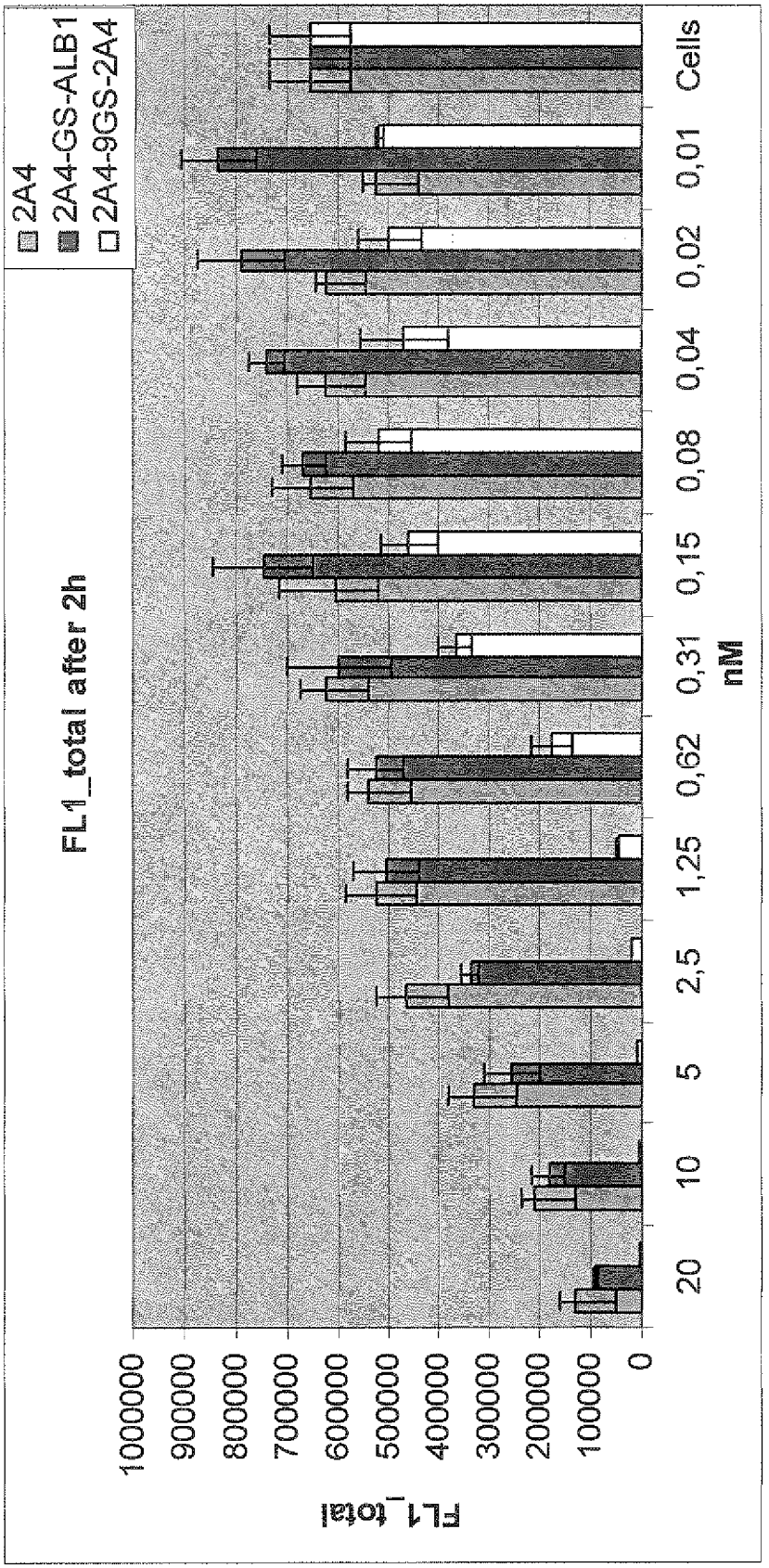

FL1_total after 2h
2A4
2A4-GS-ALB1
2A4-9GS-2A4
FL1_total
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
20
10
5
2,5
1,25
0,62
0,31
0,15
0,08
0,04
0,02
0,01
Cells
nM

Figure 9A

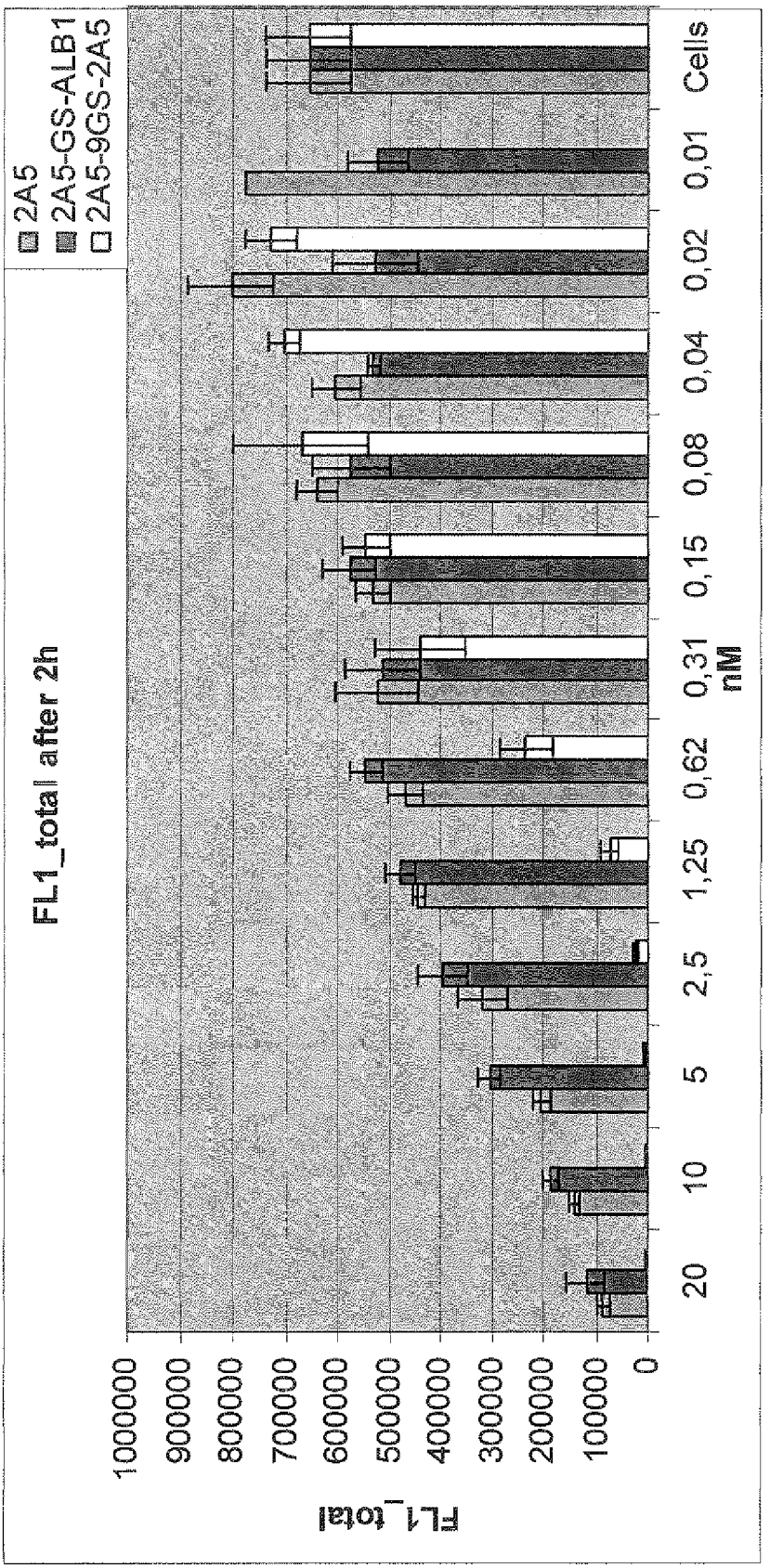

FL1_total after 2h
2A5
2A5-GS-ALB1
2A5-9GS-2A5
FL1_total
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
20
10
5
2,5
1,25
0,62
0,31
0,15
0,08
0,04
0,02
0,01
Cells
nM

Figure 9B

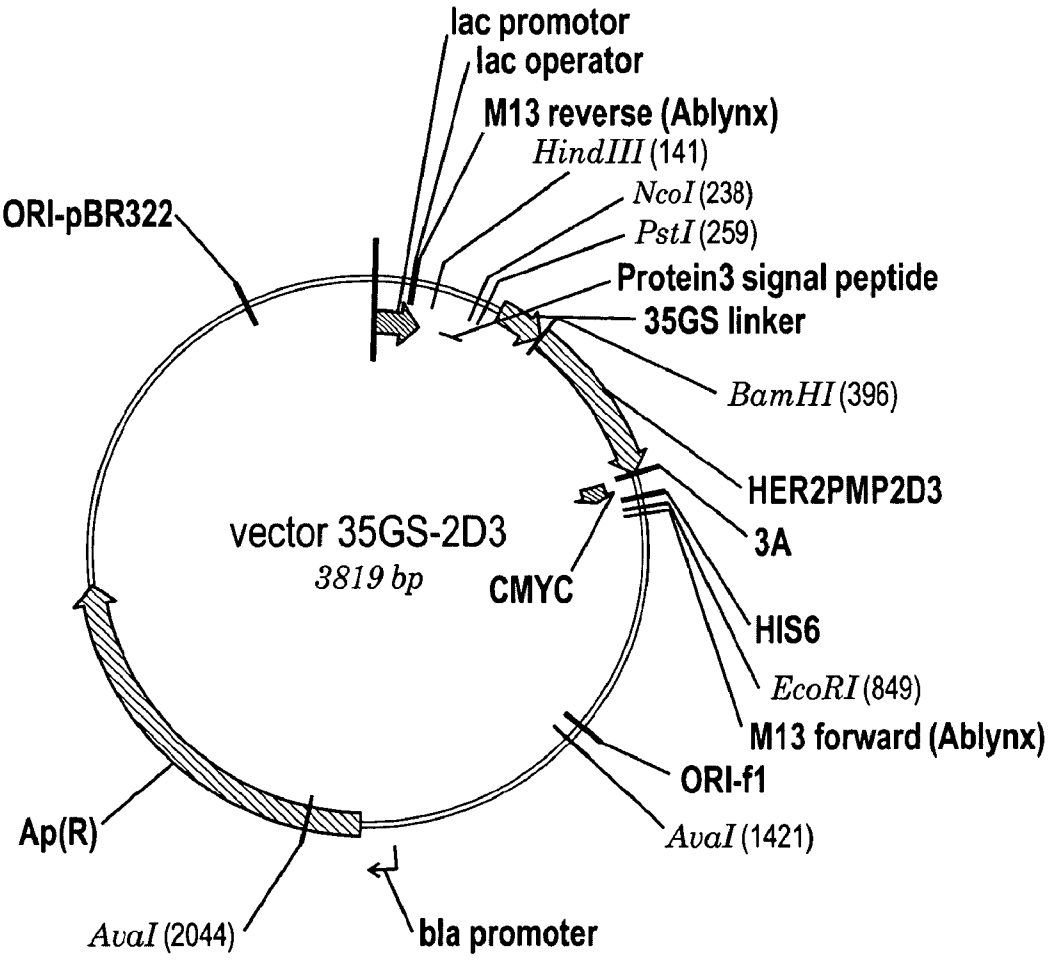

lac promotor
lac operator
M13 reverse (Ablynx)
HindIII (141)
NcoI (238)
PstI (259)
Protein3 signal peptide
35GS linker
BamHI (396)
HER2PMP2D3
3A
HIS6
EcoRI (849)
M13 forward (Ablynx)
ORI-f1
AvaI (1421)
bla promoter
AvaI (2044)
Ap(R)
ORI-pBR322
vector 35GS-2D3
3819 bp
CMYC

Figure 10

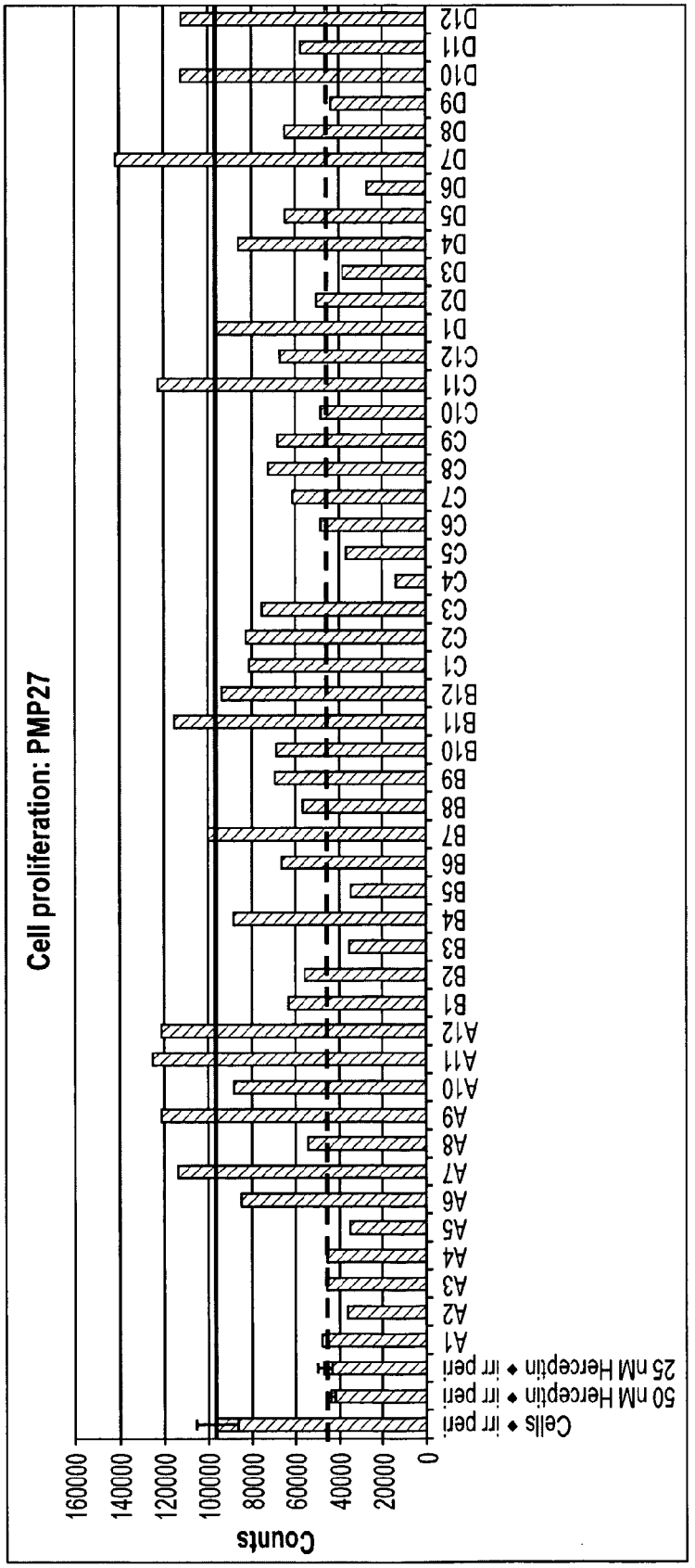

Cell proliferation: PMP27
Counts
160000
140000
120000
100000
80000
60000
40000
20000
0
Cells + irr peri
50 nM Herceptin + irr peri
25 nM Herceptin + irr peri
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
B11
B12
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
D1
D2
D3
D4
D5
D6
D7
D8
D9
D10
D11
D12

Figure 11

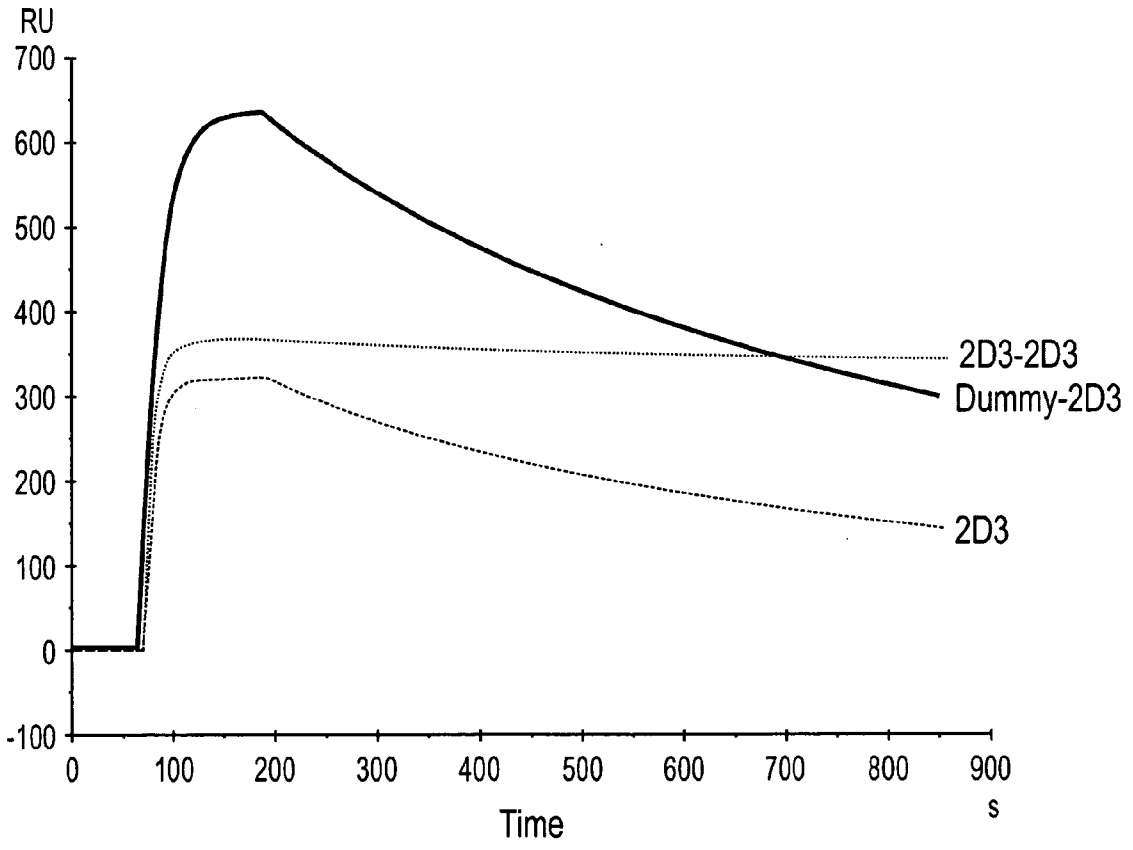

RU
700
600
500
400
300
200
100
0
-100
0
100
200
300
400
500
600
700
800
900
s
Time
2D3-2D3
Dummy-2D3
2D3

Figure 12

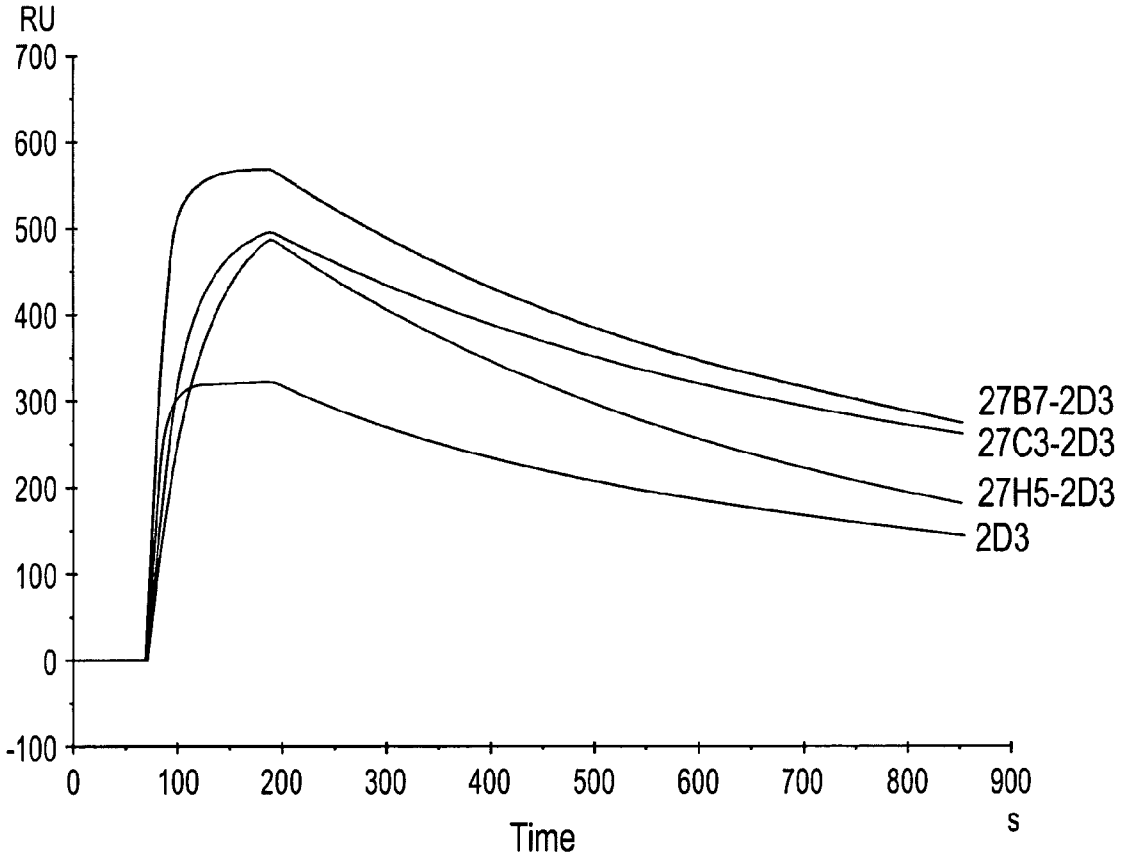

RU
700
600
500
400
300
200
100
0
-100
0
100
200
300
400
500
600
700
800
900
s
Time
27B7-2D3
27C3-2D3
27H5-2D3
2D3

Figure 13

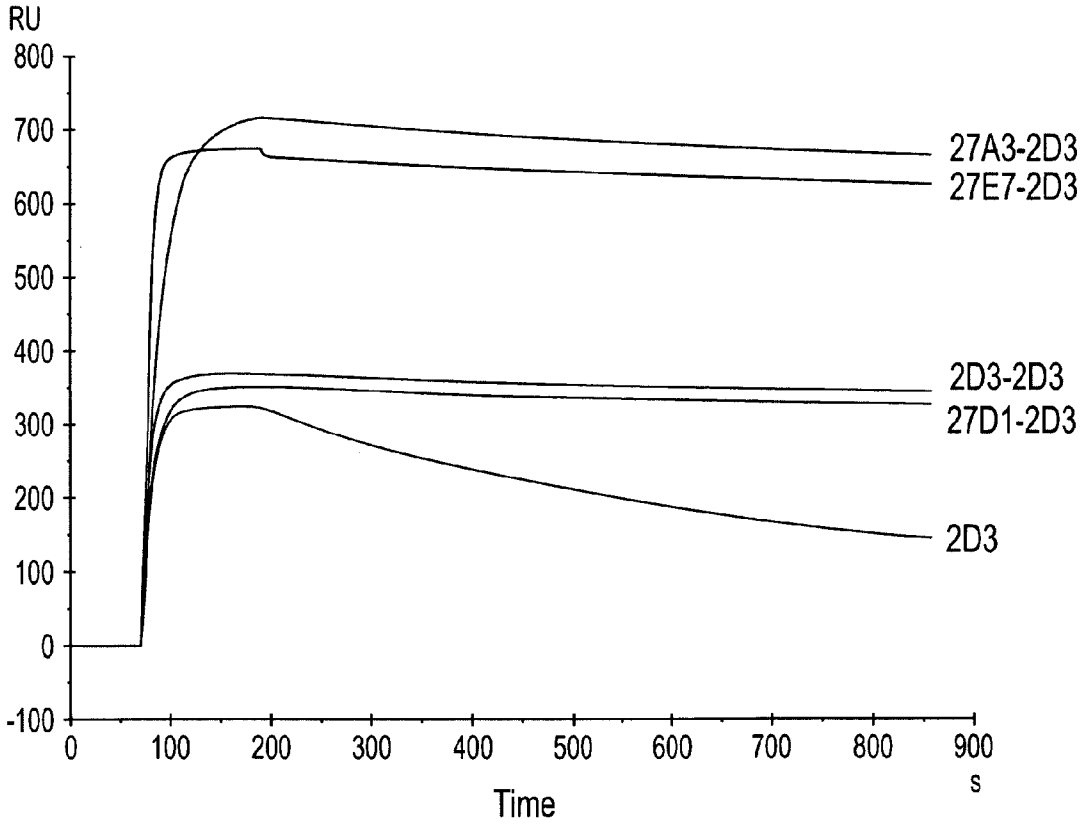

RU
800
700
600
500
400
300
200
100
0
-100
0
100
200
300
400
500
600
700
800
900
s
Time
27A3-2D3
27E7-2D3
2D3-2D3
27D1-2D3
2D3

Figure 14

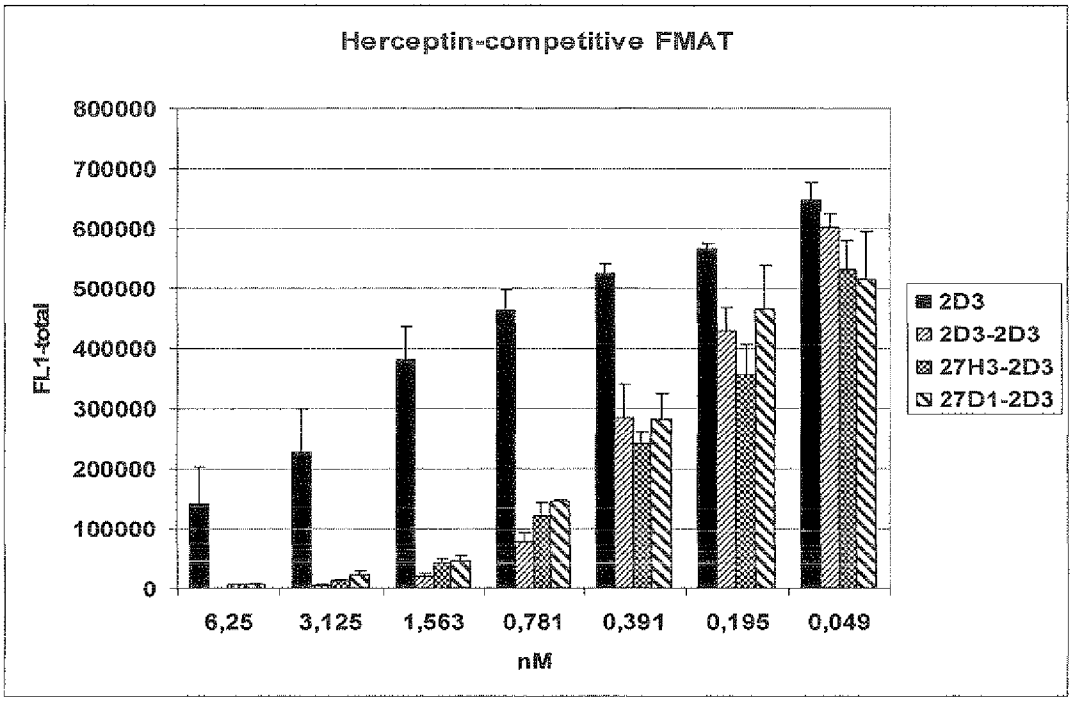

Herceptin-competitive FMAT
800000
700000
600000
500000
400000
300000
200000
100000
0
FL1-total
6,25
3,125
1,563
0,781
0,391
0,195
0,049
nM
2D3
2D3-2D3
27H3-2D3
27D1-2D3

Figure 15A

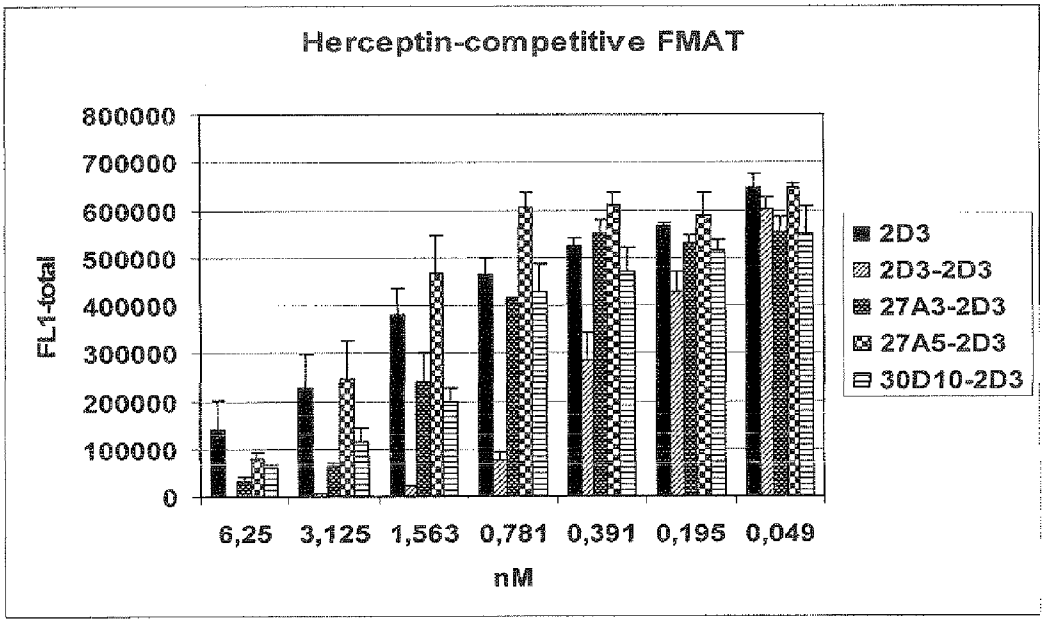

Herceptin-competitive FMAT
FL1-total
800000
700000
600000
500000
400000
300000
200000
100000
0
6,25
3,125
1,563
0,781
0,391
0,195
0,049
nM
2D3
2D3-2D3
27A3-2D3
27A5-2D3
30D10-2D3

Figure 15B

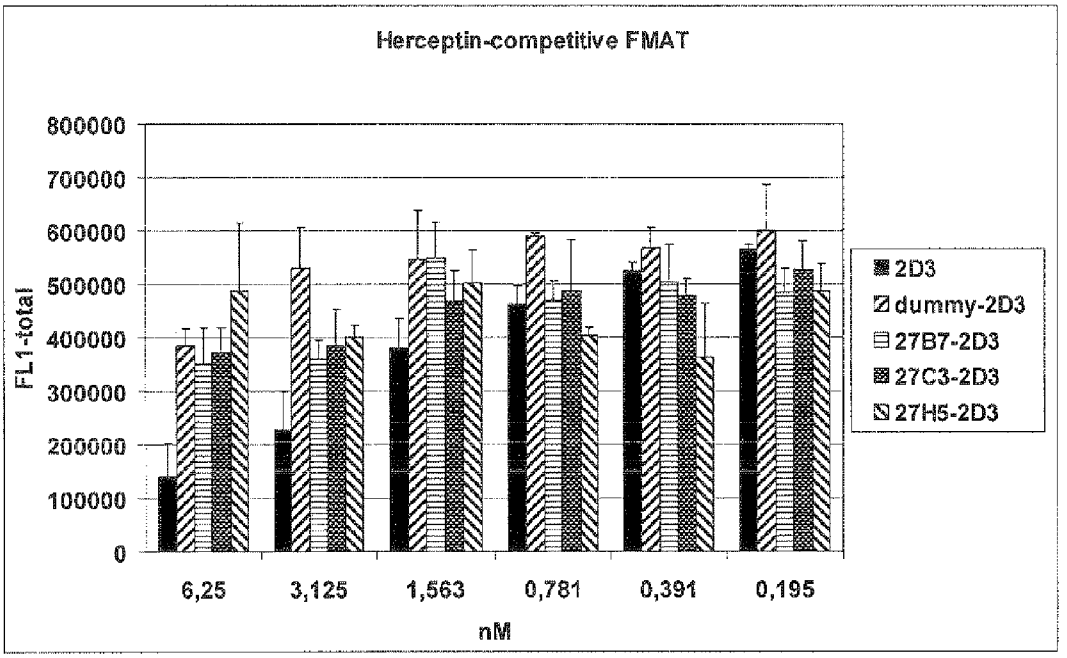

Herceptin-competitive FMAT
800000
700000
600000
500000
400000
300000
200000
100000
0
FL1-total
6,25
3,125
1,563
0,781
0,391
0,195
nM
2D3
dummy-2D3
27B7-2D3
27C3-2D3
27H5-2D3

Figure 15C

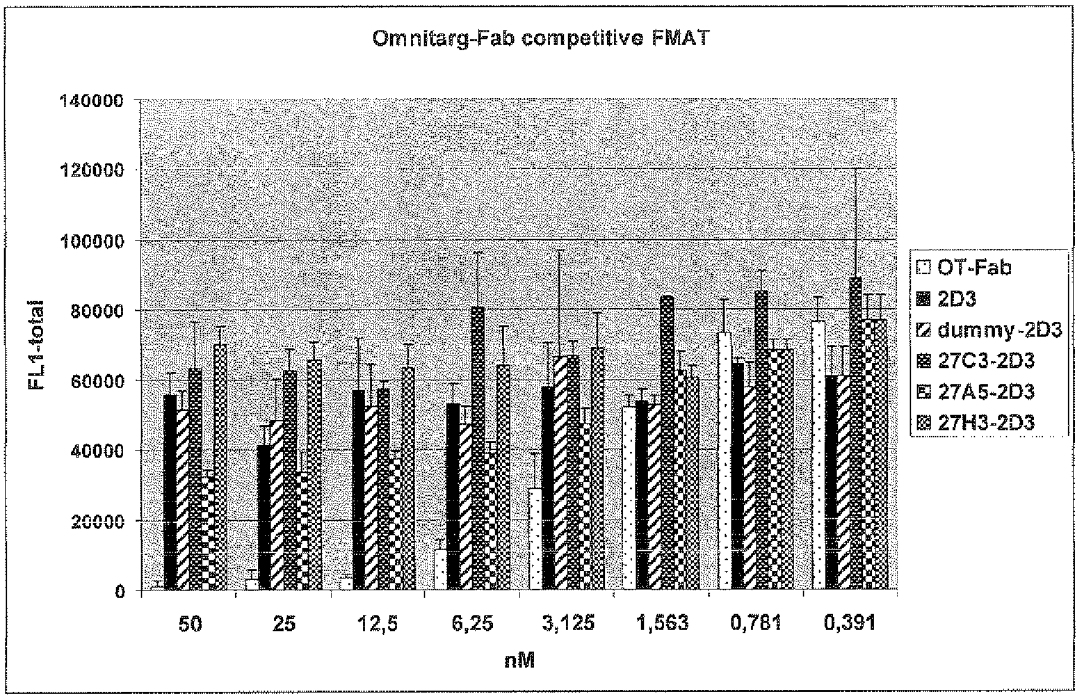

Omnitarg-Fab competitive FMAT
FL1-total
140000
120000
100000
80000
60000
40000
20000
0
50
25
12,5
6,25
3,125
1,563
0,781
0,391
nM
OT-Fab
2D3
dummy-2D3
27C3-2D3
27A5-2D3
27H3-2D3

Figure 16

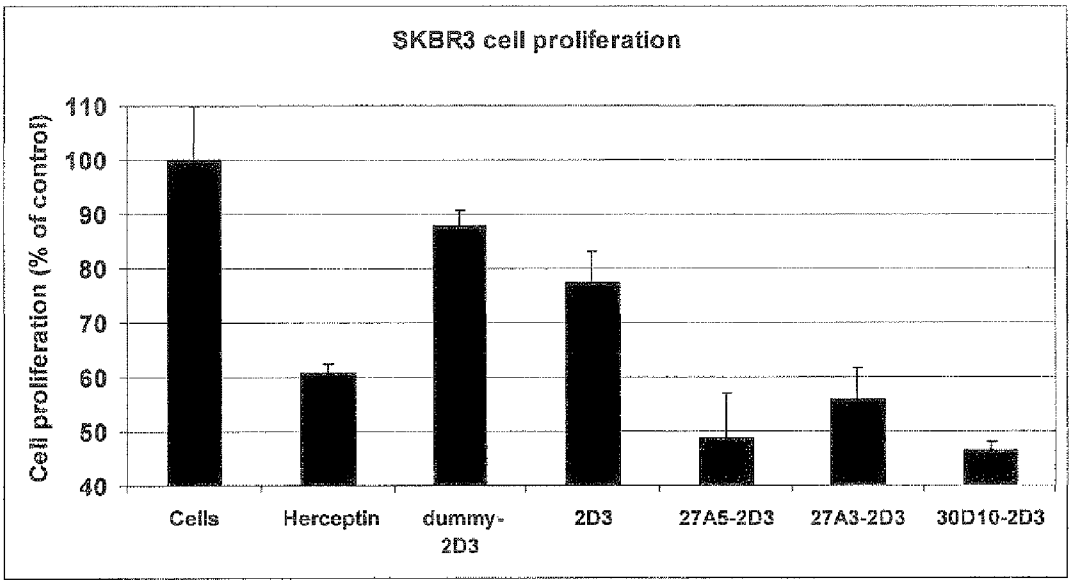

SKBR3 cell proliferation
Cell proliferation (% of control)
110
100
90
80
70
60
50
40
Cells
Herceptin
dummy-2D3
2D3
27A5-2D3
27A3-2D3
30D10-2D3

Figure 17

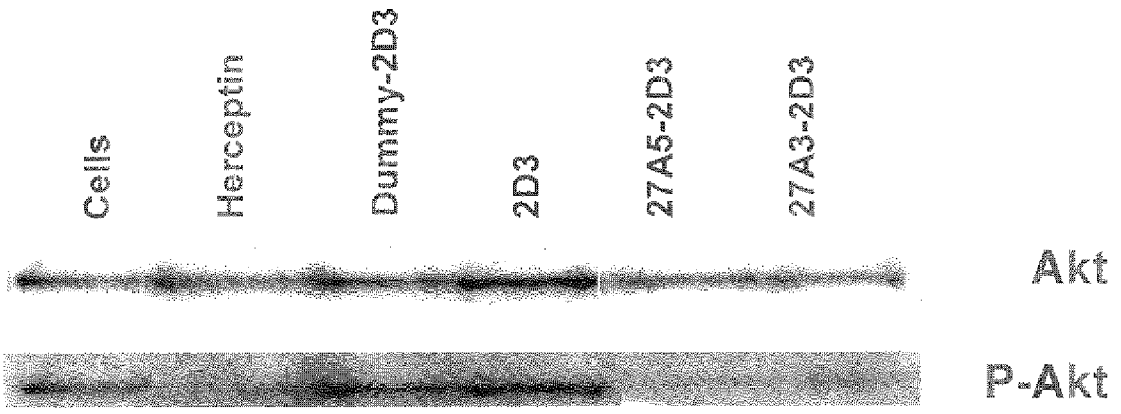


Figure 18

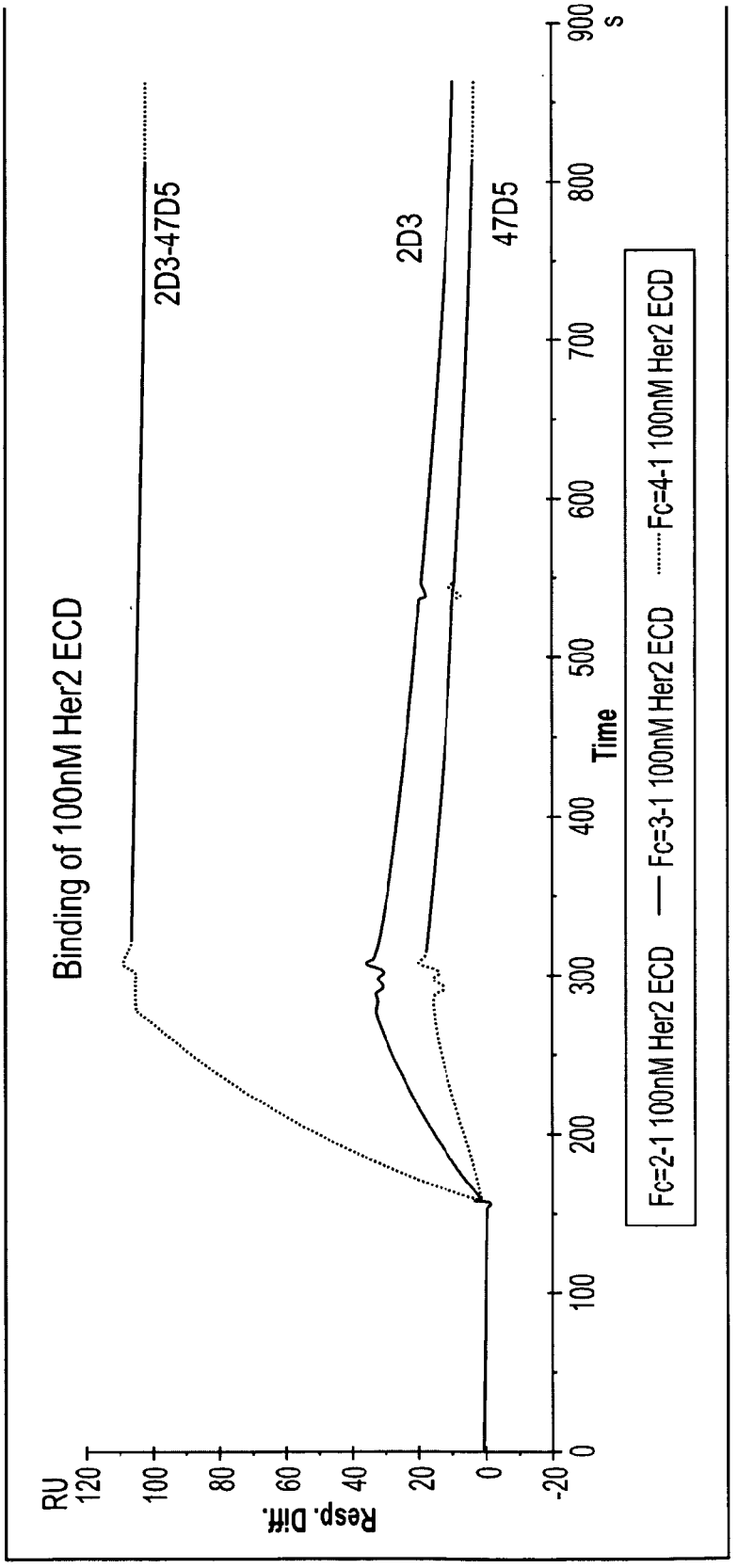

Binding of 100nM Her2 ECD
2D3-47D5
2D3
47D5
RU
120
100
80
60
40
20
0
-20
Resp. Diff.
0
100
200
300
400
500
600
700
800
900
s
Time
Fc=2-1 100nM Her2 ECD
Fc=3-1 100nM Her2 ECD
Fc=4-1 100nM Her2 ECD

Figure 19

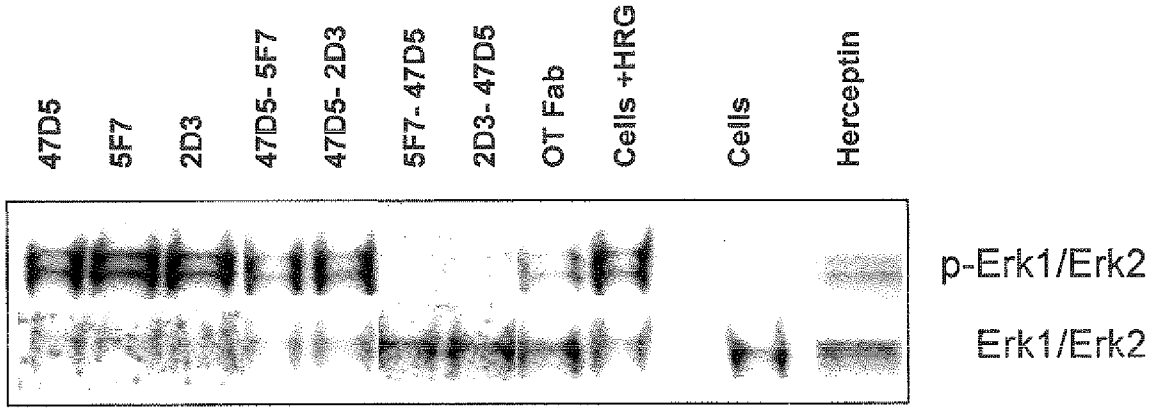

47D5
5F7
2D3
47D5- 5F7
47D5- 2D3
5F7- 47D5
2D3- 47D5
OT Fab
Cells +HRG
Cells
Herceptin
p-Erk1/Erk2
Erk1/Erk2

Figure 20

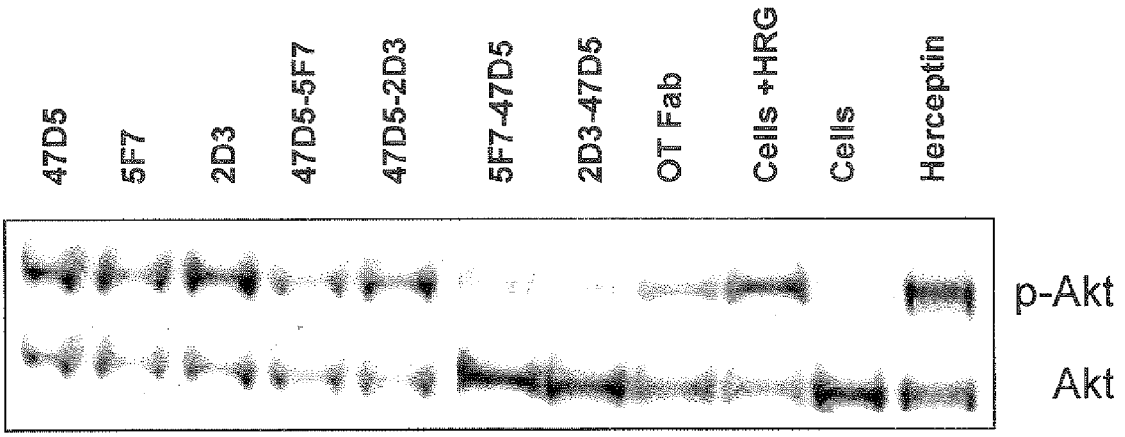

47D5
5F7
2D3
47D5-5F7
47D5-2D3
5F7-47D5
2D3-47D5
OT Fab
Cells +HRG
Cells
Herceptin
p-Akt
Akt

Figure 21

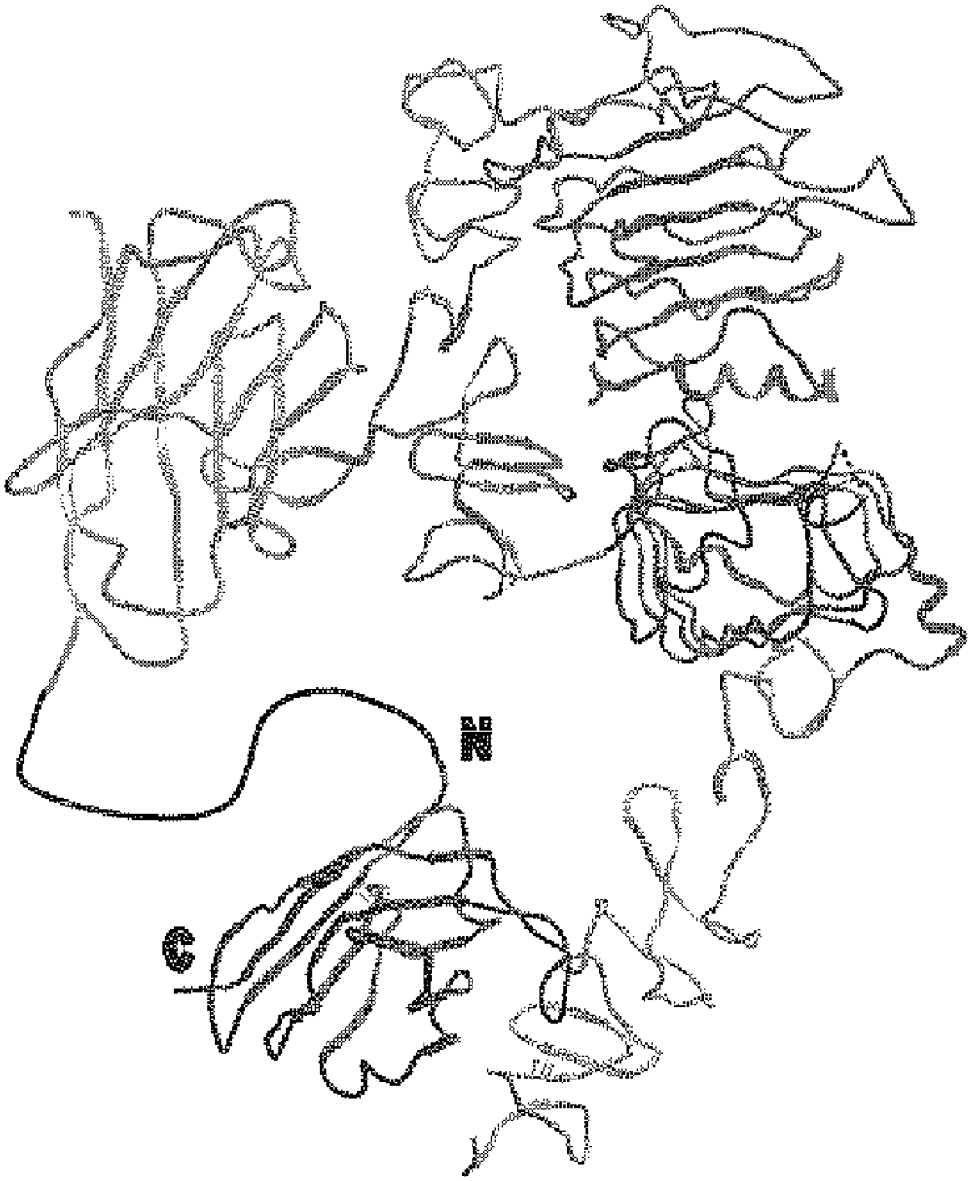


Figure 22A

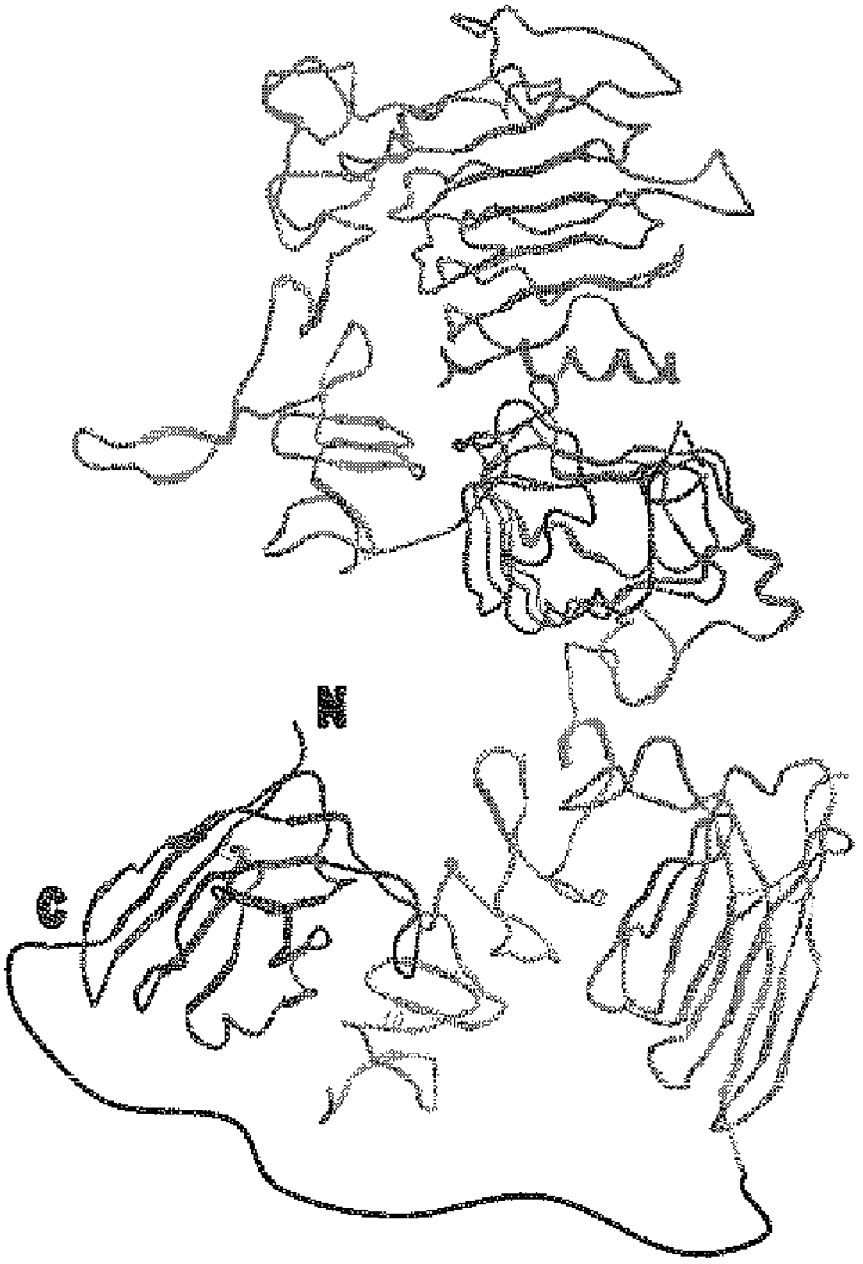


Figure 22B

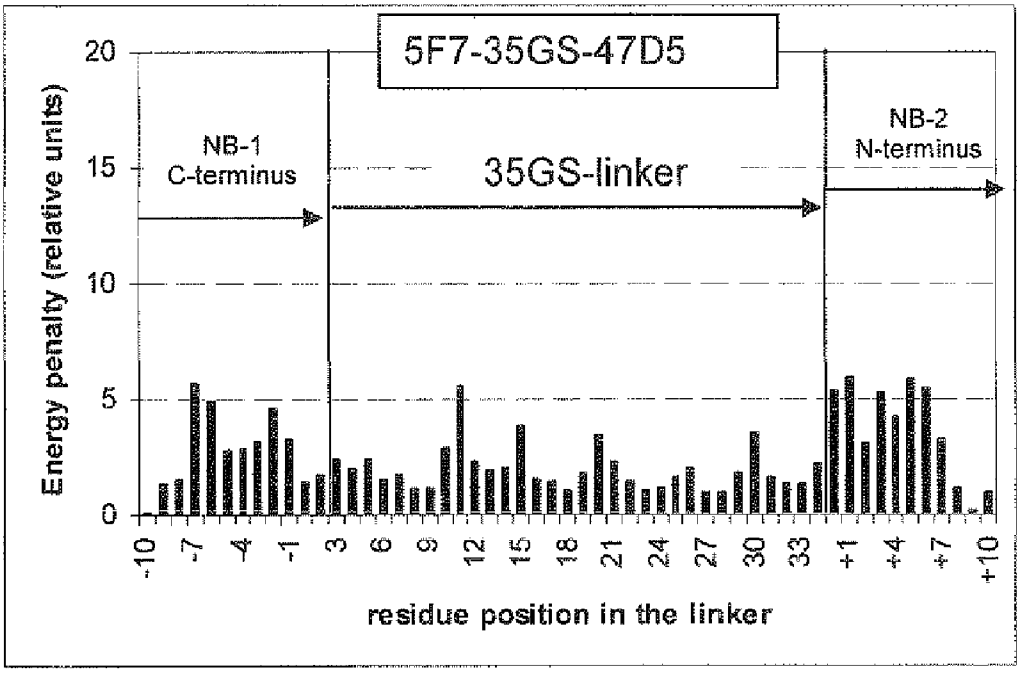

5F7-35GS-47D5
NB-1
C-terminus
35GS-linker
NB-2
N-terminus
Energy penalty (relative units)
20
15
10
5
0
-10
-7
-4
-1
3
6
9
12
15
18
21
24
27
30
33
+1
+4
+7
+10
residue position in the linker

Figure 23

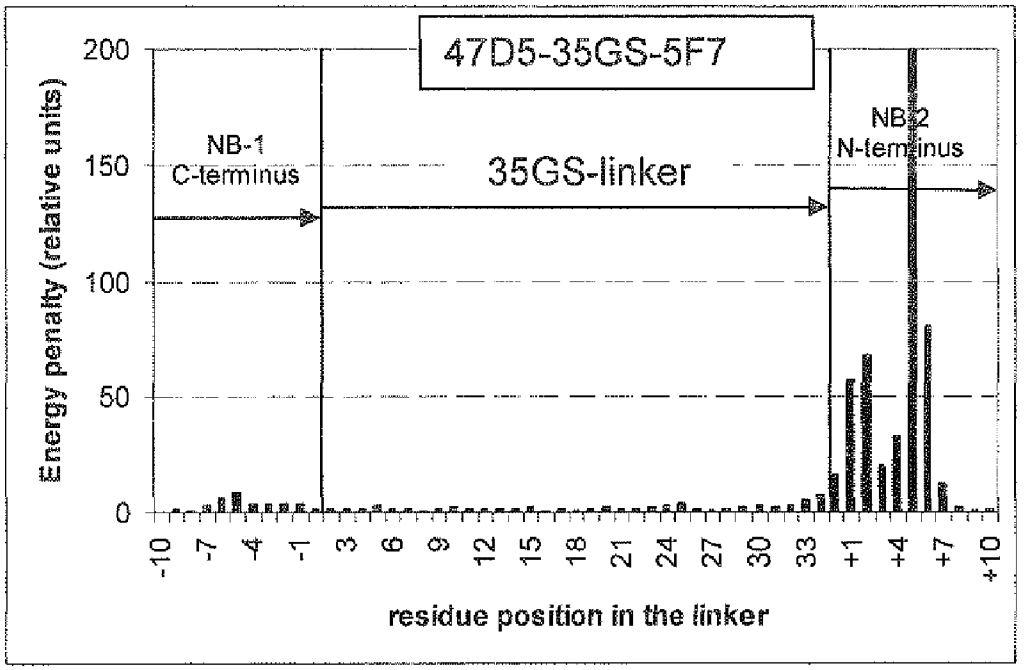

47D5-35GS-5F7
NB-1
C-terminus
35GS-linker
NB-2
N-terminus
Energy penalty (relative units)
200
150
100
50
0
-10
-7
-4
-1
3
6
9
12
15
18
21
24
27
30
33
+1
+4
+7
+10
residue position in the linker

Figure 24

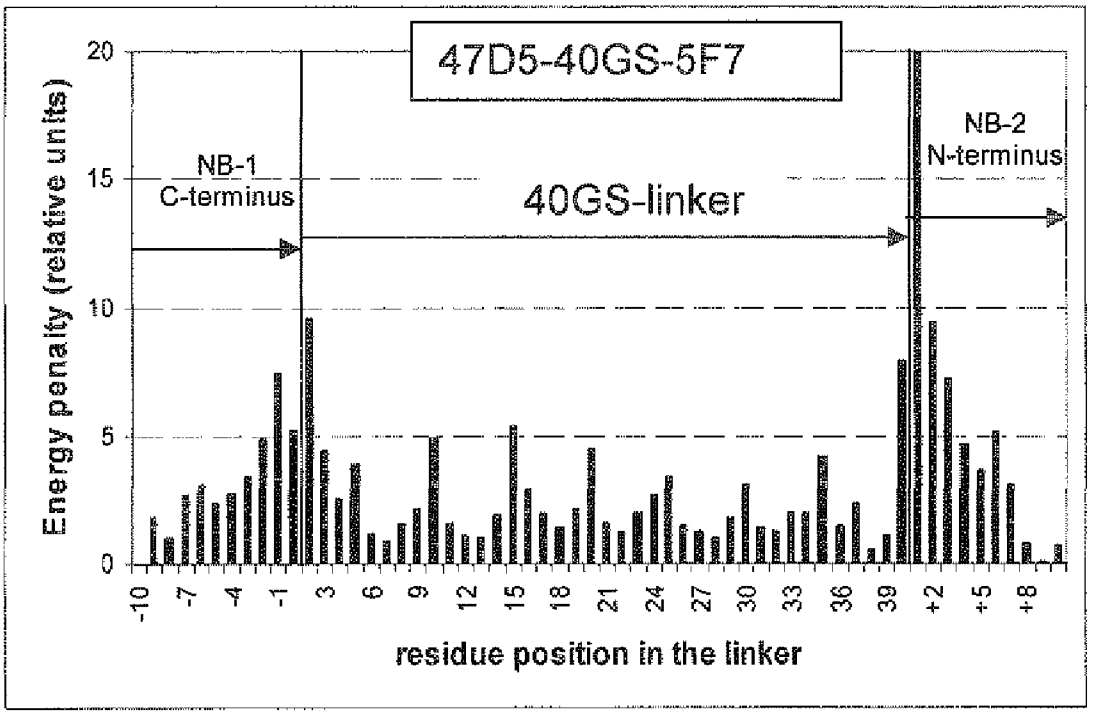

47D5-40GS-5F7
NB-1
C-terminus
40GS-linker
NB-2
N-terminus
Energy penalty (relative units)
20
15
10
5
0
-10
-7
-4
-1
3
6
9
12
15
18
21
24
27
30
33
36
39
+2
+5
+8
residue position in the linker

Figure 25

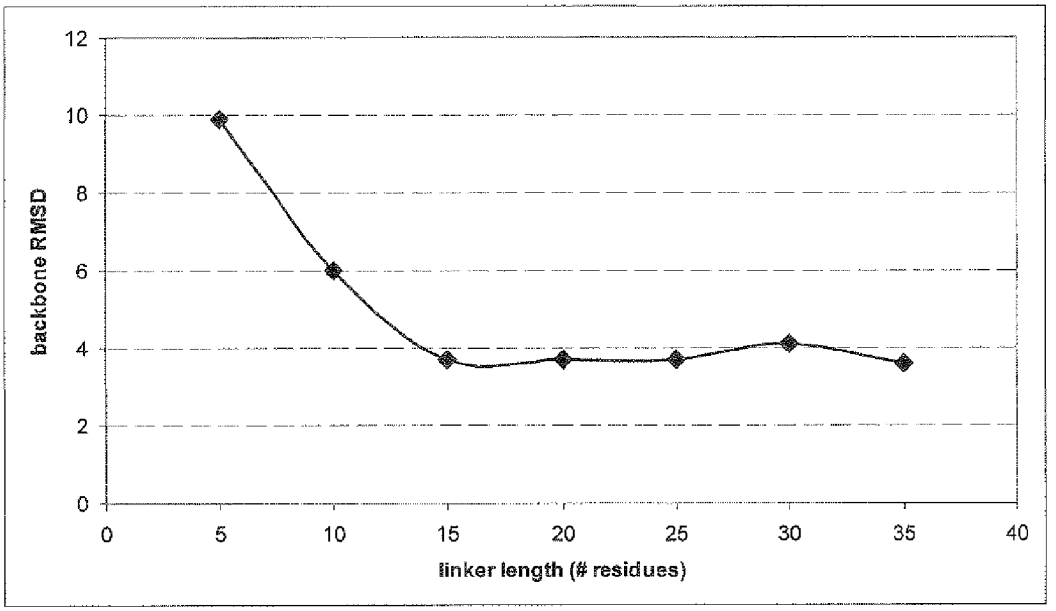

backbone RMSD
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
linker length (# residues)

Figure 26A

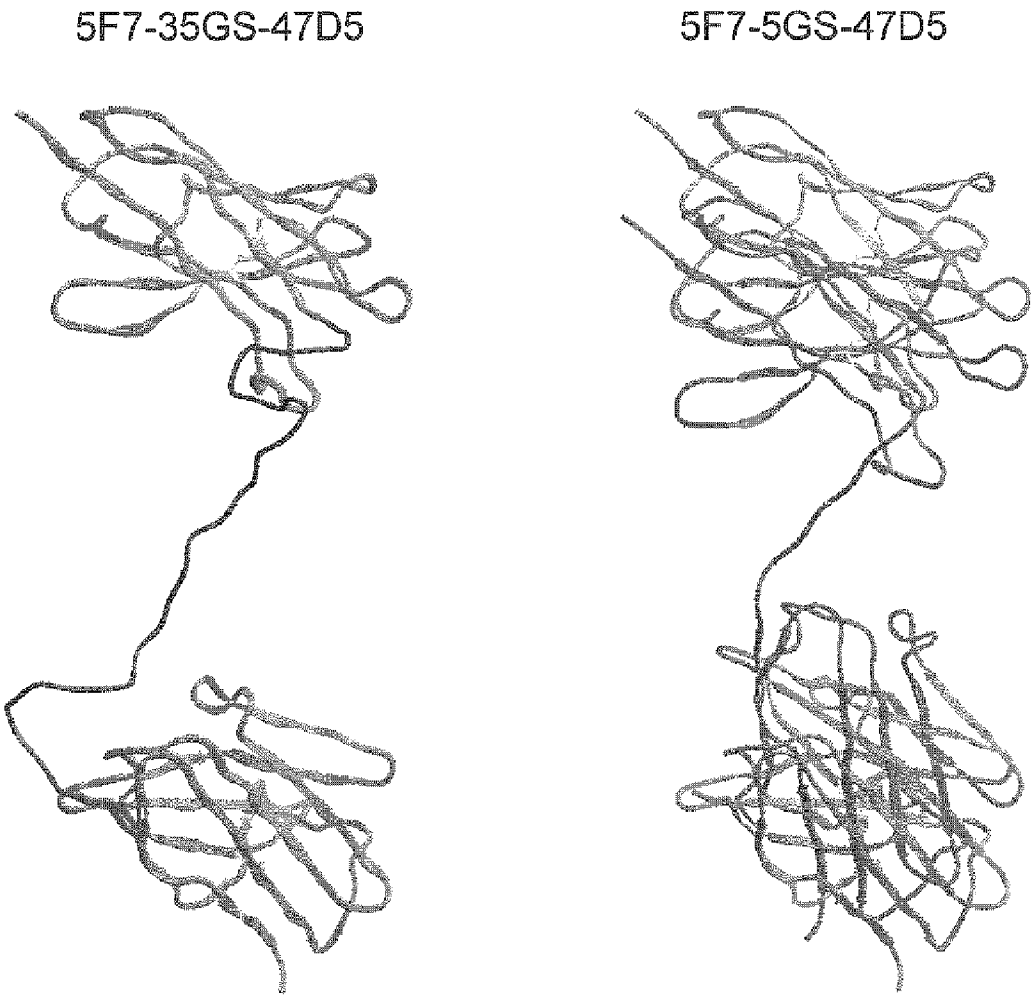


Figure 26B

FIGURE 27A

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (1)

- Approach : Combine Herceptin-competing Nanobody with library of HER2-binding Nanobodies and selecting suitable partner that antagonizes with *higher potency*

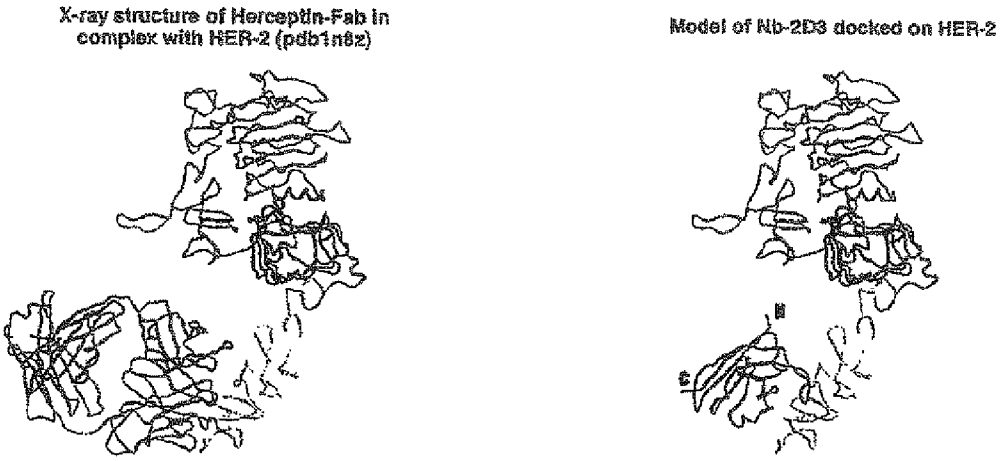


FIGURE 27B

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (1)

- Approach : Combine Herceptin-competing Nanobody with library of HER2-binding Nanobodies and selecting suitable partner that antagonizes with *higher potency*

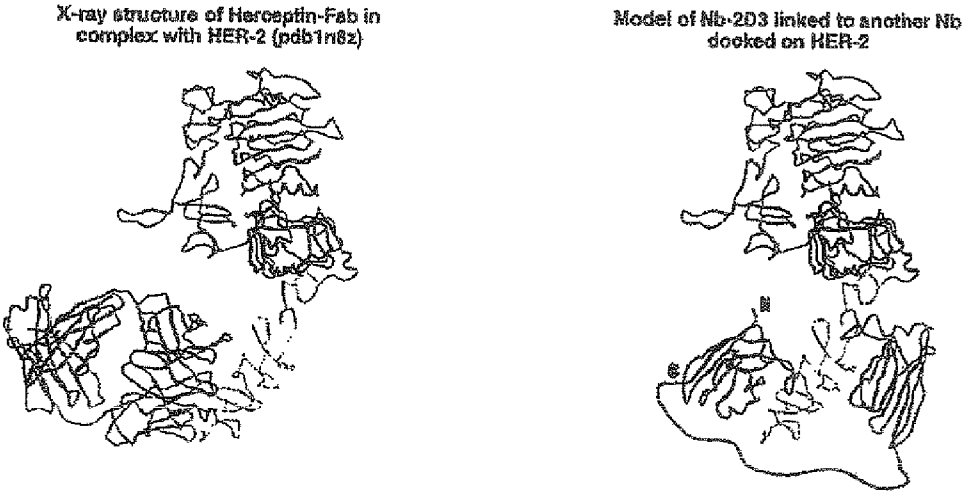


FIGURE 27C

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (1)

- Approach : Combine Herceptin-competing Nanobody with library of HER2-binding Nanobodies and selecting suitable partner that antagonizes with *higher potency*

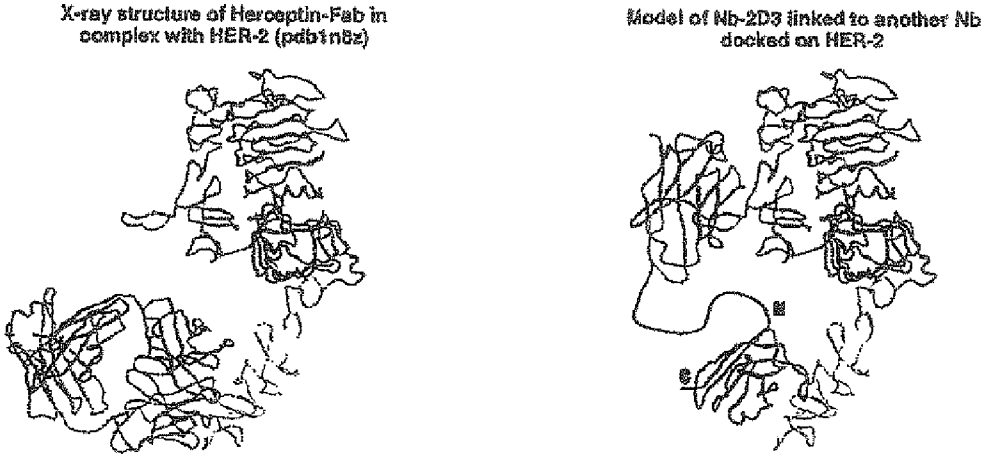


FIGURE 27D

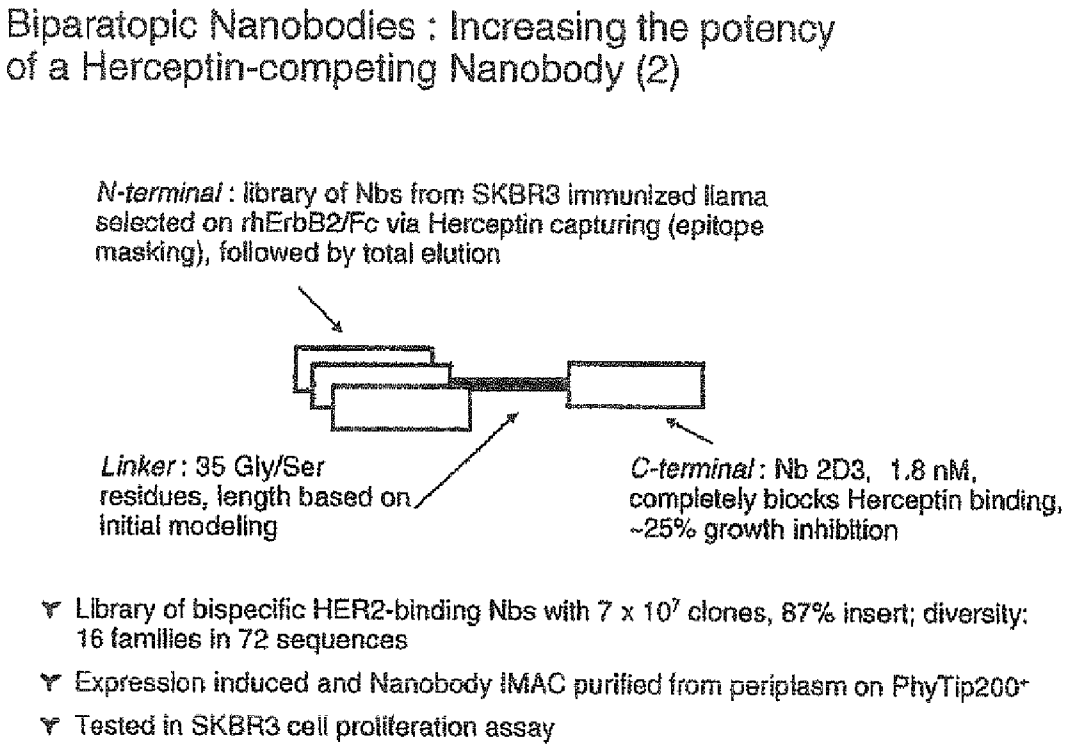
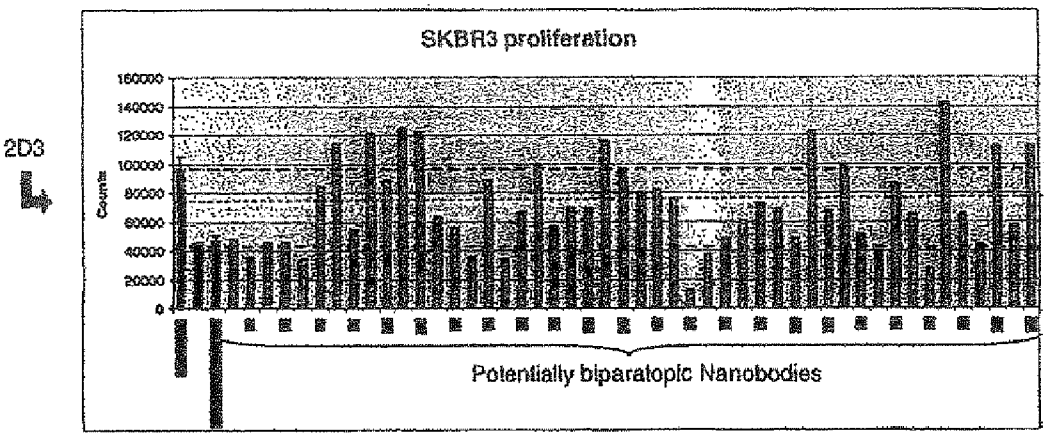

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (2)
N-terminal : library of Nbs from SKBR3 immunized llama selected on rhErbB2/Fc via Herceptin capturing (epitope masking), followed by total elution
Linker : 35 Gly/Ser residues, length based on initial modeling
C-terminal : Nb 2D3, 1.8 nM, completely blocks Herceptin binding, ~25% growth inhibition
Library of bispecific HER2-binding Nbs with 7 x 10^7 clones, 87% insert; diversity: 16 families in 72 sequences
Expression induced and Nanobody IMAC purified from periplasm on PhyTip200+
Tested in SKBR3 cell proliferation assay

FIGURE 27E

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (3)



- Biparatopic Nanobodies in HTS identified showing either :
  - (1) No effect on cell proliferation
  - (2) Agonistic effect on cell proliferation (20-40% max)
  - (3) Stronger growth inhibitory effect compared to 2D3 and some compare to Herceptin

FIGURE 27F

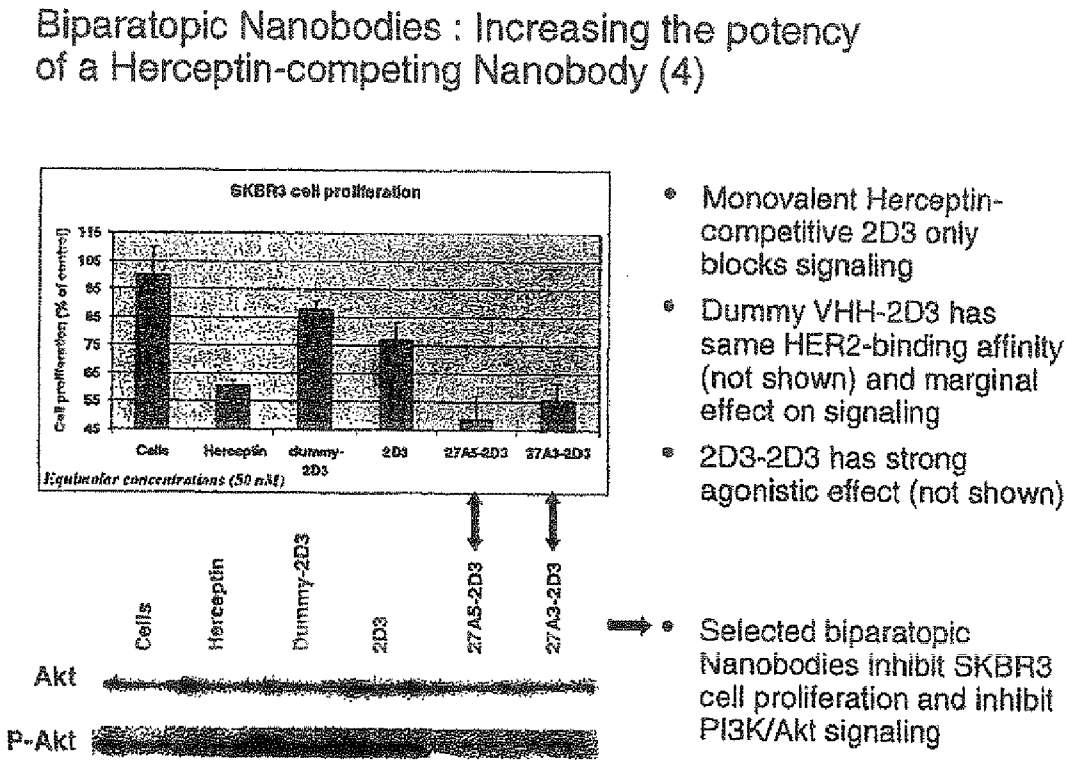

Biparatopic Nanobodies : Increasing the potency of a Herceptin-competing Nanobody (4)
SKBR3 cell proliferation
Cell proliferation (% of control)
115
105
95
85
75
65
55
45
Cells
Herceptin
dummy-2D3
2D3
27A5-2D3
27A3-2D3
Equimolar concentrations (50 nM)
Cells
Herceptin
Dummy-2D3
2D3
27A5-2D3
27A3-2D3
Akt
P-Akt
Monovalent Herceptin-competitive 2D3 only blocks signaling
Dummy VHH-2D3 has same HER2-binding affinity (not shown) and marginal effect on signaling
2D3-2D3 has strong agonistic effect (not shown)
Selected biparatopic Nanobodies inhibit SKBR3 cell proliferation and inhibit PI3K/Akt signaling

FIGURE 27G

Biparatopic Nanobodies : blocking two signaling pathways (1)

- Example : HER2, combining the mechanisms of actions of Herceptin and Omnitarg
- Concept validated by mixture of 2 mAbs : Friess et al., ESMO 2006 (animal models), Baselga et al., ASCO 2007 (Phase II combination trial)

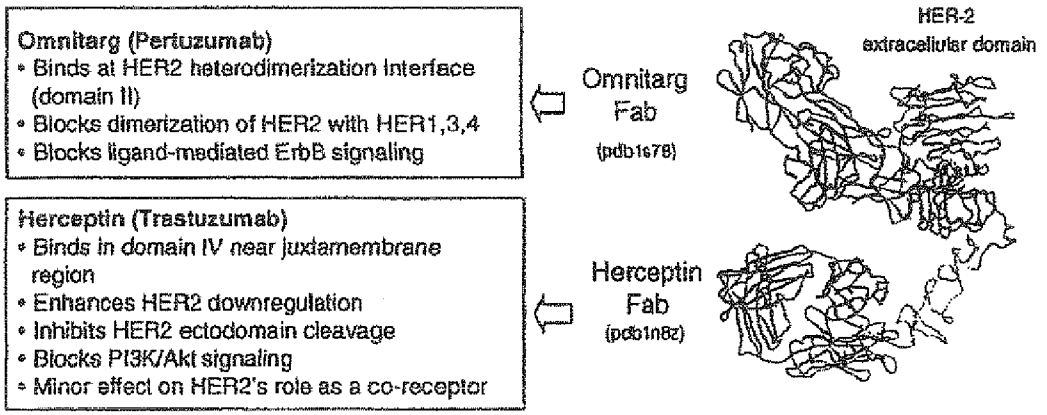
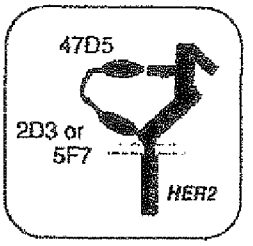


FIGURE 27H

Biparatopic Nanobodies : blocking two signaling pathways (2)

- Approach : combine the mechanisms of actions of Herceptin and Omnitarg, by genetically fusing Nanobodies that compete with the respective binding regions and have a suitable linker/format that allows *intramolecular recognition*
- Building blocks :
  - Omnitarg-competing Nanobody : 47D5
  - Herceptin-competing Nanobodies : 2D3 and 5F7
  - Affinity for HER2 of 2D3, 5F7 and 47D5 not affected if placed C-terminally
- Linker : 35 Gly/Ser residues
- Biparatopics :
  - Fusions made with N and C-terminal moieties exchanged
  - Activity measured in MCF7-cells, measuring Heregulin-mediated activation of Erk1/2 and Akt signaling



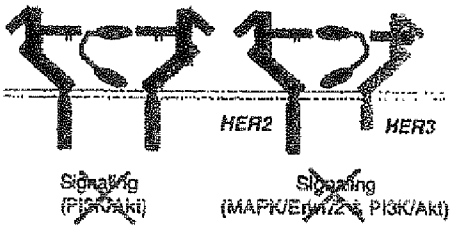
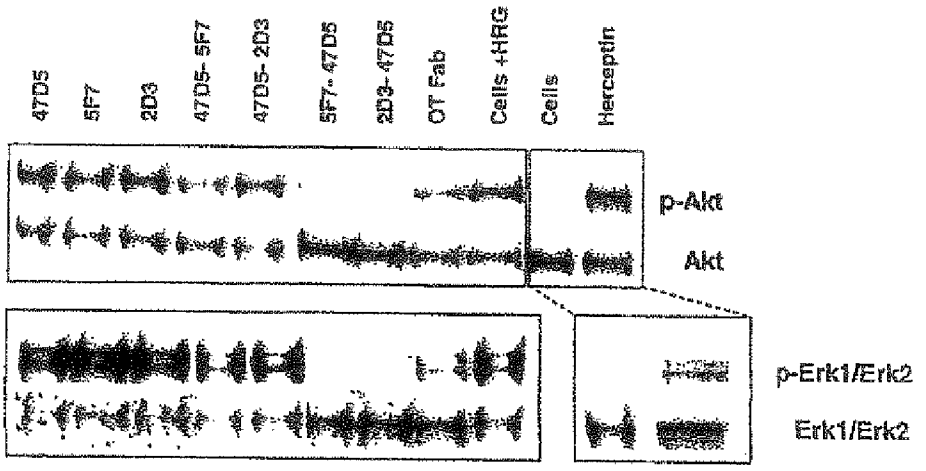


FIGURE 27I

Biparatopic Nanobodies : blocking two signaling pathways (3)



- Biparatope formats combining Herceptin and Omnitarg competitive Nanobodies block Heregulin-mediated activation of MAPK/Erk1/2 *and* PI3/Akt signaling to greater extent than Herceptin or OT-Fab
- Format of building blocks impacts on potency

FIGURE 27J

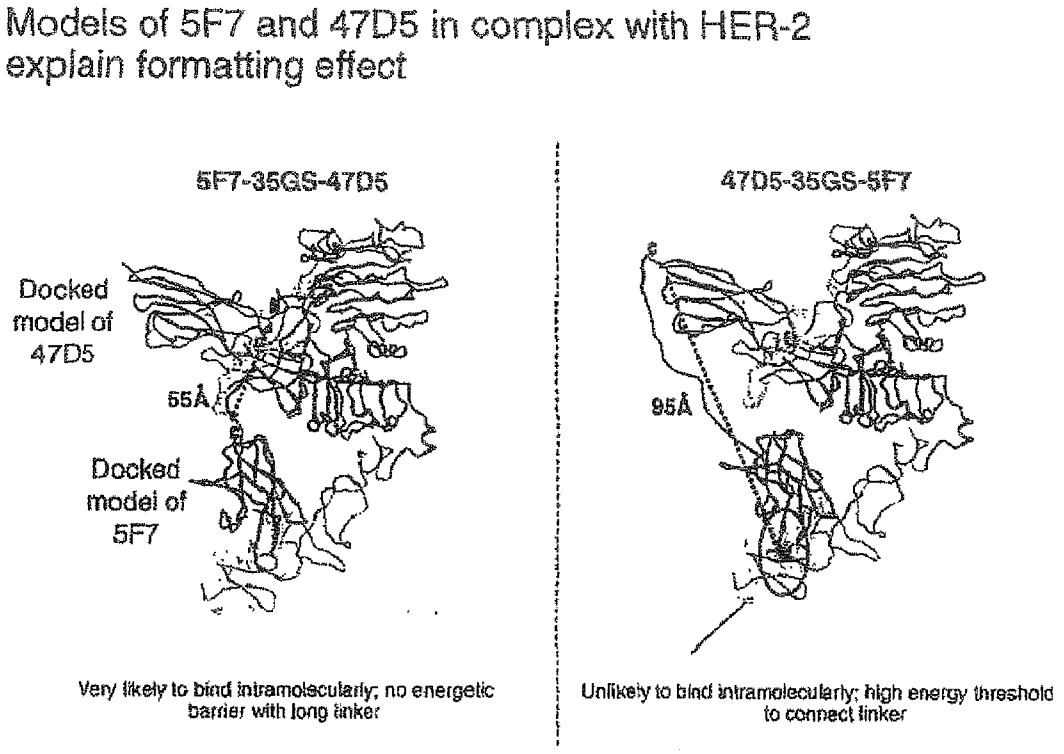
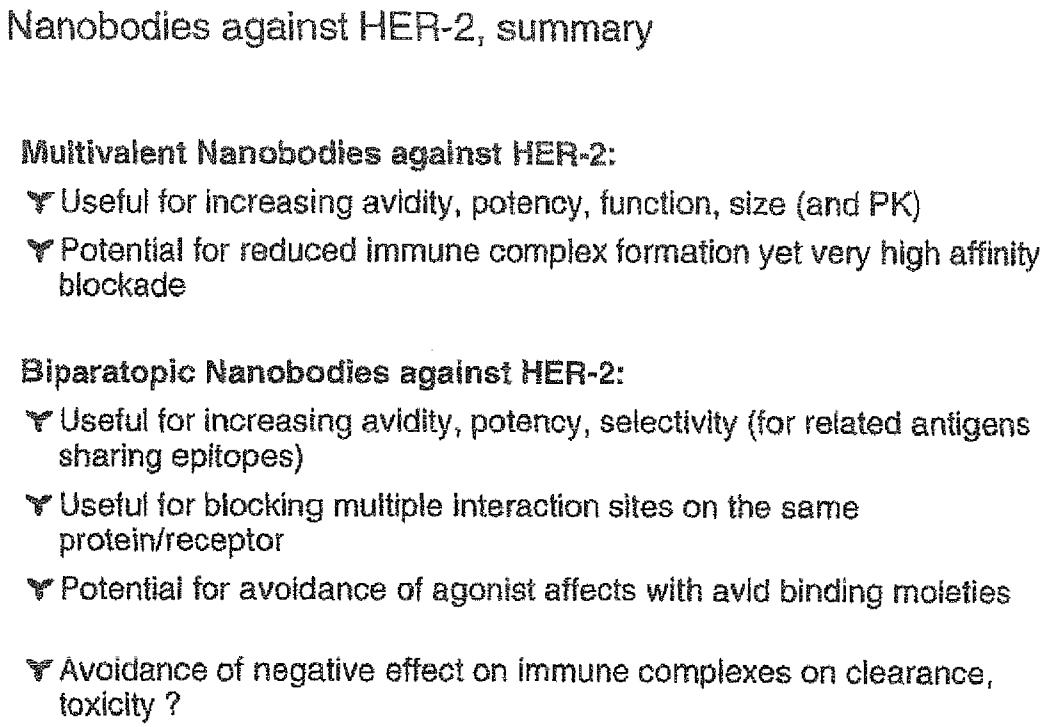

Models of 5F7 and 47D5 in complex with HER-2 explain formatting effect
5F7-35GS-47D5
Docked model of 47D5
55Å
Docked model of 5F7
Very likely to bind intramolecularly; no energetic barrier with long linker
47D5-35GS-5F7
95Å
Unlikely to bind intramolecularly; high energy threshold to connect linker

FIGURE 27K


Nanobodies against HER-2, summary
Multivalent Nanobodies against HER-2:
Useful for increasing avidity, potency, function, size (and PK)
Potential for reduced immune complex formation yet very high affinity blockade
Biparatopic Nanobodies against HER-2:
Useful for increasing avidity, potency, selectivity (for related antigens sharing epitopes)
Useful for blocking multiple interaction sites on the same protein/receptor
Potential for avoidance of agonist affects with avid binding moieties
Avoidance of negative effect on immune complexes on clearance, toxicity ?

METHOD FOR OBTAINING POLYPEPTIDE CONSTRUCTS COMPRISING TWO OR MORE SINGLE DOMAIN ANTIBODIES

FIELD OF THE INVENTION

[0001] The present invention relates to the area of antibody engineering and in particular concerns a method for obtaining polypeptide constructs directed against one or more antigens and/or epitopes, said polypeptide constructs having one or more desired characteristics.

BACKGROUND OF THE INVENTION

[0002] Antibody constructs comprising more than one binding site and/or binding unit are known to have several advantages compared to antibodies and antibody fragments comprising only one single binding site, such as an improved potency, bi- or multispecificity, multifunctionality, etc.

[0003] Typically, individual binding units are first isolated and extensively characterized, prior to composing an antibody construct combining these binding units. The method of linking the binding units by means of linker sequences, the composition of these linker sequences and the orientation and order of the binding units, as well as the choice of the binding unit combination itself all can affect the specific characteristics of the antibody construct, such as for example the affinity and/or avidity for one or more antigens, the expression levels and/or the stability of the antibody construct, etc. For instance, although some binding units may function very well individually, their binding behavior may be impaired upon linkage to another binding unit and/or component. Alternatively, some binding units may perform suboptimally when tested in their individual form but could nevertheless make suitable linkage partners in the context of a particular antibody construct, e.g. their characteristics may be complementary to those of other binding units and/or components.

[0004] McGuinness et al. (Nature Biotechnology 14: 1149-1154 (1996)) developed a method that allows the generation and screening of repertoires of bispecific antibody constructs, called "diabodies". Although these diabody libraries allow screening for the optimal bispecific molecule with regard to particularly desired properties, such as binding affinity or epitope recognition, this known production method is hampered by its complex and laborious cloning procedures.

[0005] The published international applications WO 03/002609, WO 04/003019, WO 04/058821 and WO 08/096, 158 disclose methods for producing dual-specific ligands by screening libraries of heavy and light chain variable domains derived from conventional four chain antibodies for a particular heavy chain variable domain and a particular light chain variable domain and subsequently combining these to form a dual-specific ligand. It is furthermore described in these applications that optionally libraries of structural and/or functional variants of the obtained dual-specific ligand can be produced in order to select the most optimal dual-specific ligand.

[0006] However, the potential of the methods described in the prior art is limited since only libraries composed of bivalent and/or bispecific fragments can be produced (i.e. precluding the possibility to produce libraries composed of more complex antibody constructs with multivalency and/or multispecificity). In addition, the format of antibody constructs present in the libraries of the prior art is less suitable for the production of biparatopic molecules (i.e. antibody constructs comprising at least two binding units that bind to two different epitopes on the same antigen). Finally, it is noted that the antibody construct libraries of the prior art are produced at random and therefore are characterized by the presence of quite a lot of unfavorable combinations, which hampers screening for the optimal bispecific molecule.

SUMMARY OF THE INVENTION

[0007] The present invention provides rapid and efficient methods for obtaining a polypeptide construct, which methods overcome the drawbacks and limitations of the methods described in the prior art. By using the smallest antigen binding antibody fragments (i.e. single domain antibodies) as basic building blocks for the production of polypeptide constructs, the methods of the invention allow to easily and rapidly prepare and screen large numbers of multivalent, multispecific and/or multiparatopic polypeptide constructs in order to obtain a particular polypeptide construct having specific desired characteristics. The system bypasses the need to first extensively characterize the individual binding units, by testing the performance of these binding units directly in the context of a particular polypeptide construct, potentially revealing functional features not exhibited by the individual binding units.

[0008] The invention provides methods wherein a template polypeptide construct is selected, a diversity of structural variants for the template are generated, and the diversity of constructs is screened for a polypeptide construct having one or more suitable characteristics, more particularly having two or more suitable characteristics.

[0009] Thus, according to one aspect, the present invention relates to methods for obtaining a polypeptide construct having one or more desired characteristics, wherein the polypeptide construct comprises at least two single domain antibodies and is directed against one or more antigens and/or epitopes, which methods at least comprise the steps of:

[0010] (i) selecting a template polypeptide construct

[0011] (ii) producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct of step (i), wherein said structural variants each comprise at least two single domain antibodies, and

[0012] (iii) screening the produced diversity of polypeptide constructs of step (ii) for a polypeptide construct having said one or more desired characteristics, wherein said polypeptide construct comprises at least two single domain antibodies and is directed against one or more antigens and/or epitopes.

[0013] In particular embodiments, the polypeptide constructs obtained according to the methods of the invention comprising at least two single domain antibodies comprise at least two single domain antibodies selected from domain antibodies, "dAbs", Nanobodies®, V_{HH} sequences and other single variable domains including combinations thereof. For instance, particular embodiments of the methods of the invention comprise the production of polypeptide constructs wherein the at least two single domain antibodies are selected from V_H domains and/or V_L domains (both derived from conventional four-chain antibodies) and/or V_{HH} domains (derived from heavy chain antibodies). More particular embodiments of the invention relate to the production of polypeptide constructs wherein the single domain antibodies consist essentially of only one type of domain antibody (i.e. corresponding to either heavy or light chain domains). Most par-

ticularly it is envisaged that the methods of the present invention involve the generation of polypeptide constructs wherein the single domains exclusively consist of heavy chain domain antibodies (e.g. V_H or V_{HH}). Further particular embodiments of the invention involve methods wherein the polypeptide constructs generated comprise at least two single domains, whereby the single domains of the construct consist exclusively of V_{HH} domains or exclusively consist of heavy chain variable domains derived from heavy chain antibodies.

[0014] In a particular embodiment of the invention, the diversity of polypeptide constructs can be a set, collection or library of polypeptide constructs. More particularly, the diversity of polypeptide constructs can be a library of polypeptide constructs.

[0015] Also, the diversity of polypeptide constructs may be a set, collection or library of polypeptide constructs comprising at least two single domain antibodies that are exclusively heavy chain variable domains (e.g. V_H or V_{HH}). More particularly, the diversity of polypeptide constructs may be a set, collection or library of polypeptide constructs comprising at least two single domain antibodies that are exclusively heavy chain variable domains of heavy chain antibodies (V_{HH} domains).

[0016] In further particular embodiments, step (ii) of the methods of the invention of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct of step (i), comprises producing one or more of the following:

[0017] structural variants with regard to the number and/or identity of said single domain antibodies in said polypeptide constructs, and/or,

[0018] structural variants with regard to the relative position of said single domain antibodies within the polypeptide constructs, and/or,

[0019] structural variants with regard to the amino acid residues of the CDR regions of said single domain antibodies in said polypeptide constructs, and/or,

[0020] structural variants with regard to the amino acid residues of the framework regions of said single domain antibodies in said polypeptide constructs, and/or,

[0021] structural variants with regard to the codon usage of said selected template polypeptide construct.

[0022] It is envisaged that, in the methods of the invention the selected template polypeptide construct can a theoretical polypeptide construct, for which in the methods described herein a diversity of polypeptide constructs are generated which are structural variants. This is typically the case when the starting point is only a desired feature (e.g. antigen binding). Alternatively, the selected polypeptide construct comprises a known (i.e. previously identified single domain antibody) and the diversity of polypeptide constructs comprises structural variants of the template polypeptide construct.

[0023] In particular embodiments, said step (ii) of the methods of the invention of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct of step (i) comprises producing structural variants each comprising at least two single domain antibodies that are linked via one or more peptide linkers. In particular embodiments, modifying the peptide linkers may favorably affect one or more desirable characteristics of the polypeptide construct.

[0024] Accordingly, in further particular embodiments of the methods described herein, step (ii) of the methods of the invention of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct of step (i), wherein said structural variants each comprise at least two single domain antibodies that are linked via one or more peptide linkers, comprises producing one or more of the following

[0025] structural variants with regard to the composition of said one or more peptide linkers, and/or,

[0026] structural variants with regard to the number of said one or more linkers, and/or,

[0027] structural variants with regard to the relative position of said one or more linkers in said polypeptide constructs.

[0028] Again, as described above, the template polypeptide construct may be a theoretical construct. Alternatively, the template polypeptide construct comprises a known linker.

[0029] It will be understood to the skilled person that methods are equally envisaged wherein, upon generating a diversity of polypeptide constructs, structural variants with regard to both the single variable domains and the peptide linkers are also envisaged.

[0030] The methods of the present invention further comprise step (iii) of screening the diversity of polypeptide constructs produced in step (ii) for a polypeptide construct having one or more desired characteristics. In particular embodiments, the one or more desired characteristics may be selected from characteristics such as (but not limited to) a suitable binding affinity, a suitable solubility, a suitable stability, a suitable efficacy, and/or a suitable potency. It will be understood to the skilled person that, in the selection of the template polypeptide construct, particular characteristics may be implied (such as binding to the antigen of interest) and the screening step encompasses screening for one or more additional characteristics.

[0031] The methods of the present invention, involve the identification of polypeptide constructs which comprise at least two single domain antibodies. In particular embodiments, the methods envisage the generation of polypeptide constructs directed against one antigen. In these methods, the generated polypeptide constructs may comprise two or more single domain antibodies directed against the same epitope or directed against at least two different epitopes of the same antigen. In yet further embodiments, the methods according to this embodiment may involve the generation of polypeptide constructs comprising two or more single domain antibodies directed against different antigens. In particular embodiments of the methods described herein, the diversity of polypeptide constructs and the selected polypeptide construct comprise for instance at least three single domain antibodies, wherein the at least three single domain antibodies may be directed against the same or one or more (two or all three) different epitopes, which may be present on the same antigen or which may be present on different antigens (such as e.g. two single domain antibodies directed against the same epitope and one single domain antibody directed against a different epitope on the same antigen, two single domain antibodies directed against the same epitope on a first antigen and one single domain antibody against a different antigen; two single domain antibodies directed against a different epitope on the first same antigen and one single domain antibody against a different antigen; three single domain antibodies each directed against a different epitope on the same antigen; three single domain antibodies each directed against a different antigen).

DETAILED DESCRIPTION OF THE INVENTION

[0032] Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms,

have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention. Reference is for example made to the standard handbooks, such as Sambrook et al., "Molecular Cloning: A Laboratory Manual" (2nd. Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic Engineering", 2nd edition, University of California Press, Berkeley, Calif. (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10^{th} Ed. Blackwell Publishing, UK (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as to the general background art cited herein.

[0033] As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

[0034] The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps.

[0035] The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

[0036] With the term "polypeptide construct" as used herein is meant a compound or polypeptide comprising one or more (preferably at least two) binding units that are linked to each other (optionally via linker sequences such as a peptide linker sequence) and/or optionally to other groups, residues, moieties or binding units (e.g. via disulphide bridges or via linker sequences, such as peptide linker sequences). Preferably, said one or more other groups, residues, moieties or binding units are amino acid sequences.

[0037] In the context of the present invention, the polypeptide construct (also referred to as "polypeptide construct of the invention") comprises at least two single domain antibodies (as defined herein), being directed against one or more antigens (as defined herein) and/or epitopes (as defined herein). In the method of the present invention polypeptide constructs are screened, selected and/or obtained that exhibit one or more desired characteristics. The one or more desired characteristics may be (but are not limited to) a suitable binding affinity (as described herein), a suitable solubility (as described herein), a suitable stability (as described herein), a suitable efficacy (as described herein) and/or a suitable potency (as described herein). A polypeptide construct of the invention with one or more desired characteristics is obtained using the method of the present invention by screening a diversity of polypeptide constructs for said one or more desired characteristics.

[0038] The polypeptide construct of the invention comprising two or more single domain antibodies are also be referred to herein as "multivalent" polypeptide constructs of the invention. Single domain antibodies present in such polypeptide constructs will also be referred to herein as being in a "multivalent format". For example a "bivalent" polypeptide construct of the invention comprises two single domain antibodies, optionally linked via a linker sequence, whereas a "trivalent" polypeptide of the invention comprises three single domain antibodies, optionally linked via two linker sequences; etc.;

[0039] In a multivalent polypeptide of the invention, the two or more single domain antibodies may be the same or different, and may be directed against the same antigen or antigenic determinant (for example against the same part(s) or epitope(s) or against different parts or epitopes) or may alternatively be directed against different antigens or antigenic determinants; or any suitable combination thereof. For example, a bivalent polypeptide construct of the invention may comprise (a) two identical single domain antibodies; (b) a first single domain antibody directed against a first antigenic determinant of a protein or antigen and a second single domain antibody directed against the same antigenic determinant of said protein or antigen which is different from the first single domain antibody; (c) a first single domain antibody directed against a first antigenic determinant of a protein or antigen and a second single domain antibody directed against another antigenic determinant of said protein or antigen; or (d) a first single domain antibody directed against a first protein or antigen and a second single domain antibody directed against a second protein or antigen (i.e. different from said first antigen). Similarly, a trivalent polypeptide construct of the invention may, for example and without being limited thereto. comprise (a) three identical single domain antibody; (b) two identical single domain antibody against a first antigenic determinant of an antigen and a third single domain antibody directed against a different antigenic determinant of the same antigen; (c) two identical single domain antibody against a first antigenic determinant of an antigen and a third single domain antibody directed against a second antigen different from said first antigen; (d) a first single domain antibody directed against a first antigenic determinant of an antigen, a second single domain antibody directed against a second antigenic determinant of said antigen and a third single domain antibody directed against a third antigenic determinant of the same antigen; (e) a first single domain antibody directed against a first antigenic determinant of a first antigen, a second single domain antibody directed against a second antigenic determinant of said first antigen and a third single domain antibody directed against a second antigen different from said first antigen; or (f) a first single domain antibody directed against a first antigen, a second single domain antibody directed against a second antigen different from said first antigen, and a third single domain antibody directed against a third antigen different from said first and second antigen.

[0040] Polypeptide constructs of the invention that contain at least two single domain antibody, in which at least one single domain antibody is directed against a first antigenic determinant on an antigen and at least one single domain antibody is directed against a second antigenic determinant on the same antigen will also be referred to as "multiparatopic" polypeptide constructs of the invention, and the single domain antibody present in such polypeptide construct will also be referred to herein as being in a "multiparatopic format". Thus, for example, a "biparatopic" polypeptide construct of the invention is a polypeptide construct that comprises at least one single domain antibody directed against a first antigenic determinant on an antigen and at least one further single domain antibody directed against a second antigenic determinant on the same antigen, whereas a "triparatopic" polypeptide construct of the invention is a

polypeptide construct that comprises at least one single domain antibody directed against a first antigenic determinant on an antigen, at least one further Nanobody directed against a second antigenic determinant on the same antigen and at least one further Nanobody directed against a third antigenic determinant on the same antigen; etc.

[0041] Accordingly, in its simplest form, a biparatopic polypeptide construct of the invention is a bivalent polypeptide construct of the invention (as defined herein), comprising a first single domain antibody directed against a first antigenic determinant on the antigen, and a second single domain antibody directed against a second antigenic determinant on the same antigen, in which said first and second single domain antibody may optionally be linked via a linker sequence (as defined herein); whereas a triparatopic polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first single domain antibody directed against a first antigenic determinant on the antigen, a second single domain antibody directed against a second antigenic determinant on the same antigen and a third single domain antibody directed against a third antigenic determinant on the same antigen, in which said first, second and third single domain antibody may optionally be linked via one or more, and in particular one and more, in particular two, linker sequences.

[0042] Polypeptide constructs of the invention that contain at least two single domain antibody, in which at least one single domain antibody is directed against a first antigen and at least one single domain antibody is directed against a second antigen (different from the first antigen), will also be referred to as "multispecific" polypeptides of the invention, and the single domain antibody present in such polypeptide constructs will also be referred to herein as being in a "multispecific format". Thus, for example, a "bispecific" polypeptide construct of the invention is a polypeptide construct that comprises at least one single domain antibody directed against a first antigen and at least one further single domain antibody directed against a second antigen (i.e. different from the first antigen), whereas a "trispecific" polypeptide of the invention is a polypeptide that comprises at least one single domain antibody directed against a first antigen, at least one further single domain antibody directed against a second antigen (i.e. different from the first antigen) and at least one further single domain antibody directed against a third antigen (i.e. different from both the first and the second antigen); etc.

[0043] Accordingly, in its simplest form, a bispecific polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first single domain antibody directed against a first antigen, and a second single domain antibody directed against a second antigen, in which said first and second single domain antibody may optionally be linked via a linker sequence (as defined herein); whereas a trispecific polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first single domain antibody directed against a first antigen, a second single domain antibody directed against a second antigen and a third single domain antibody directed against a third antigen, in which said first, second and third single domain antibody may optionally be linked via one or more, and in particular one and more, in particular two, linker sequences.

[0044] For multivalent and multi specific polypeptides containing one or more V_{HH} domains and their preparation, reference is also made to Conrath et al., J. Biol. Chem., Vol. 276, 10. 7346-7350, 2001; Muyldermans, Reviews in Molecular Biotechnology 74 (2001), 277-302; as well as to for example WO 96/34103 and WO 99/23221. Some other examples of some specific multispecific and/or multivalent polypeptide of the invention can be found in the applications by Ablynx N.V. referred to herein.

[0045] A "binding unit" as used herein refers to an amino acid sequence capable of binding an epitope. In the context of the present invention, a binding unit essentially consists of a single domain antibody (as defined herein).

[0046] The term "single domain antibody" as used herein refers to a binding sequence comprising an amino acid sequence that is suitable for use as a domain antibody and includes but is not limited to a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody® (as described herein, and including but not limited to a V_{HH} sequence), other single variable domains such V_H or V_L, or a suitable fragment of any one thereof.

[0047] The term "template polypeptide construct" as used herein refers to a theoretical or physically existing polypeptide construct, which is used as a starting point for producing a diversity of polypeptide constructs comprising at least two single domain antibodies that are structural variants for the template polypeptide construct.

[0048] The term "identified single domain antibody" as used herein refers to a single domain antibody, which as been generated previously (before the method of the present invention is applied). In most cases, the "identified single domain antibody" has a specified amino acid sequence and/or a specified antigen and/or epitope specificity.

[0049] The term "diversity" as used herein and particularly in the context of a diversity of polypeptide constructs refers to a set, group, collection or library of polypeptide constructs which may contain any suitable number of sequences, such as 1, 2, 3 or about 5, 10, 50, 100, 500, 1000, 5000, 10^4, 10^5, 10^6, 10^7, 10^8 or more sequences. Depending on the one or more desired characteristics of the polypeptide construct that one would like to obtaine with the method of the invention, the skilled person can assess what the preferred number of sequences in the diversity of polypeptide construct. The preferred number of sequences in the diversity of polypeptide constructs may be about 5, about 10, about 50, about 100, or more than 1000, more than 10^4, or more.

[0050] The term "antigen(s)" refers to the target molecule (s) recognized by the antigen-binding unit and more in particular by the antigen-binding site of said antigen-binding unit.

[0051] The term "epitope(s)" refers to the particular site on the antigen recognized by the antigen-binding unit (such as a binding unit essentially consisting of a (single) domain antibody or a polypeptide construct of the invention) and more in particular by the antigen-binding site of said antigen-binding unit, and can also be referred to as the "antigenic determinant (s)". Accordingly, the terms "epitope(s)" and "antigenic determinant(s)" may be used interchangeably herein.

[0052] A binding unit, such as a single domain antibody or a polypeptide construct (or a fragment thereof), that can (specifically) bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "against" or "directed against" said antigenic determinant, epitope, antigen or protein.

[0053] In respect of a target or antigen, the term "interaction site" on the target or antigen means a site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is a site for binding to a ligand, receptor or other binding partner, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in multimerisation (such as homomerization or heterodimerization) of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen. More generally, an "interaction site" can be any site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen to which a polypeptide construct of the invention can bind such that the target or antigen (and/or any pathway, interaction, signalling, biological mechanism or biological effect in which the target or antigen is involved) is modulated.

[0054] The term "specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule (such as a binding unit, e.g. a single domain antibody, or a polypeptide construct of the invention) can bind. The specificity of an antigen-binding molecule can be determined based on affinity and/or avidity.

[0055] All documents cited in the present specification are hereby incorporated by reference in their entirety.

[0056] The Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

[0057] In a first aspect, the invention provides methods for obtaining polypeptide constructs directed against one or more antigens or epitopes. Such methods are based on the observation that single domain antibodies (as defined herein) can appropriately be used as building blocks in the generation of a diversity of polypeptide constructs, from which suitable polypeptide constructs having one or more desired characteristics can be obtained.

[0058] As will be clear from the further description herein, the invention involves the use of binding units essentially consisting of single domain antibodies as "building blocks" to form polypeptide constructs (such as, but without limitation, the bi- or multiparatopic, the bi- or multivalent and/or the bi- or multispecific polypeptide constructs of the invention described herein), i.e. by suitably combining them with other groups, residues, moieties or binding units, such that the formed polypeptide constructs of the present invention may exhibit one or more desired characteristics or biological functions.

[0059] It is envisaged that in the polypeptide constructs of the invention, single domain antibodies can be linked to each other and/or optionally to other groups, residues, moieties or binding units (e.g. via disulphide bridges or via linker sequences, such as peptide linker sequences). Preferably, such one or more other groups, residues, moieties or binding units are amino acid sequences. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties may or may not provide further functionality to the polypeptide constructs of the invention and may or may not modify the properties of the polypeptide constructs of the invention. In the polypeptide constructs of the invention, the one or more amino acid sequences of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, or moieties are amino acid sequences, the linkers may also be amino acid sequences, so that the resulting polypeptide construct is a fusion (protein) or fusion (polypeptide).

[0060] In particular, the invention provides methods for obtaining polypeptide constructs having one or more, more particularly two or more desired characteristics, wherein the polypeptide constructs comprise at least two single domain antibodies and are directed against one or more antigens or epitopes, which methods comprise at least the steps of: (i) selecting a template polypeptide construct, (ii) producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct, wherein said structural variants each comprise at least two single domain antibodies, and (iii) screening the produced diversity of polypeptide constructs for polypeptide constructs having the one or more, more particularly two or more desired characteristics, wherein the polypeptide constructs comprise at least two single domain antibodies and are directed against one or more antigens and/or epitopes.

[0061] The step of selecting a template polypeptide construct may also involve determining a theoretical template, e.g. "a polypeptide construct capable of binding to a certain antigen X of interest". The selection of a theoretical template implies that there are no particular structural requirements or preferences for the polypeptide construct with the desired characteristics that one would like to obtain from the methods of the invention and/or that no template polypeptide construct is available as a starting point for generating the diversity of polypeptide constructs.

[0062] Alternatively, the step of selecting a template antibody may involve selecting a physically existing (i.e. previously generated) polypeptide construct comprising at least one single domain antibody. In particular embodiments, the physically existing polypeptide construct is directed against the antigen or epitope of interest and the methods of the invention comprise the generation of a diversity of polypeptide constructs which comprises structural variants of the template polypeptide construct (as described herein below) from which a polypeptide construct can be selected according to the methods of the invention having particular (additional) suitable and/or desired characteristics, more particularly in addition to the feature of binding to the antigen of interest. In alternative embodiments, the template polypeptide construct is a physically existing polypeptide construct directed against one or more antigens or epitopes, which are different from the antigen or epitopes of interest. In such embodiments selection can include selection based on a characteristic other than binding affinity e.g. solubility. Template polypeptide constructs combining one or more physically existing or identified single domain antibodies with one or more theoretical single domain antibodies or linkers are also envisaged to be selected in the methods of the invention.

[0063] Thus, in particular embodiments, methods for obtaining a polypeptide construct directed against antigen or epitope X may involve the selection of a particular template polypeptide construct comprising one or more single domain antibodies directed against antigen or epitope X, the generation of a diversity of structural variants of polypeptide constructs directed against antigen or epitope X (for the nature of the diversity see below), and the selection of a polypeptide

construct directed against antigen or epitope X, having one or more suitable characteristics, in addition to the binding to antigen or epitope X.

[0064] In further embodiments, methods of the invention are used for the generation of a polypeptide construct directed against more than one antigen, for instance against antigens or epitopes X and Y. In such methods, the selection of the template polypeptide construct may comprise the selection of a (physically existing or theoretical or combined existing/theoretical) template polypeptide construct directed (ore envisaged to be directed) against the two or more antigens or epitopes of interest (X and Y), e.g. comprising two (identified) single domain antibodies, each directed to one of the antigens or epitopes of interest (one single domain directed against X, one single domain antibody directed against Y). Such methods further comprise the step of generating a diversity of structural variants of polypeptide constructs directed against antigens or epitopes X and Y (this being determined by the nature of the template polypeptide construct, see below), and the selection of a polypeptide construct directed against antigens or epitopes X and Y, having one or more suitable characteristics, in addition to the binding to antigens or epitopes X and Y.

[0065] In these embodiments, the template polypeptide construct may be a theoretical construct or may comprise two or more single domain antibodies, possibly comprising one or more identified single domain antibodies known to be directed against X or Y. Accordingly, particular embodiments are envisaged where the template polypeptide construct comprises at least one identified single domain antibody known to be directed against one of the antigens (e.g. against antigen or epitope X) and at least one theoretical single domain antibody (e.g. a single domain antibody envisaged to be directed against antigen or epitope Y), the step of generating a diversity of structural variants may comprise generating a diversity of combinations (i.e. fusion proteins) of (e.g. one or more copies of) the identified single domain antibody known to be directed against X with e.g. a collection of different single domain antibodies (optionally directed against the other antigen or epitope Y, or random sequences which can be screened for binding to antigen or epitope Y). Alternatively, the template polypeptide construct may comprise two or more identified (i.e. previously generated) single domain antibodies each against one of both antigens or epitopes and the step of generating a diversity of structural variants may comprise generating polypeptide constructs comprising different combinations of the identified single domain antibodies (e.g. differing in number and/or relative position in the construct), and/or constructs comprising different modifications of (such as modifications of the CDR or FR regions of) either one or both of the identified single domain antibodies. In the selection step, a polypeptide construct having, in addition to the ability to recognize X and Y, one or more suitable characteristics such as suitable affinity (against X and/or Y), solubility etc. (as further described herein).

[0066] Accordingly, it is envisaged that the template polypeptide construct need not determine the number or the nature of the single domain antibodies in the diversity of polypeptide constructs generated nor in the polypeptide construct one would like to obtain from the methods of the invention. More particularly, it is noted that for the purpose of generating a polypeptide construct with improved affinity against an antigen, a template antibody can be selected which comprises one (physically existing or theoretical) single domain antibody against the antigen. In such embodiments, the step of producing a diversity of polypeptide constructs may comprise generating polypeptide constructs comprising different numbers of the same single domain antibody and/or different combinations of single domain antibodies directed against different epitopes of the same antigen, with the aim of obtaining a polypeptide construct with the desired suitable affinity for the antigen. The same applies when the methods of the invention are used for obtaining polypeptide constructs directed against two or more antigens.

[0067] Methods according to the present invention further comprise step (ii) of producing a diversity of polypeptide constructs that are structural variants of the selected template polypeptide construct. A diversity of structural variants of the template polypeptide construct according to the invention comprises different polypeptide constructs, each comprising at least two single domain antibodies that may be linked to each other and optionally to one or more other groups, residues, moieties or binding units.

[0068] Depending on the nature of the characteristic(s) desired for the polypeptide construct that one envisages to obtain with methods according to the invention, the produced diversity can comprise different types of structural variants for the template polypeptide construct. As detailed above, where the template polypeptide construct comprises one or more identified (i.e. previously generated) single domain antibodies, linkers or other structures or moieties, the diversity may comprise structural variants of the template polypeptide construct. Where the template polypeptide construct is a theoretical construct, the diversity needs to be generated starting from different newly generated single domain antibodies, linkers, structures or moieties.

[0069] In particular embodiments, the step of producing a diversity of structural variants of said template polypeptide construct may comprise producing a diversity of structural variants with regard to the number and/or identity of single domain antibodies in the polypeptide constructs. In particular embodiments, the template polypeptide construct comprises one single domain antibody and producing a diversity of structural variants for the template polypeptide construct may involve producing a diversity of antibody cosntructs comprising at least two single domain antibodies but more particularly three, four, five, six or more single domain antibodies.

[0070] According to particular embodiments the template antibody comprises an identified (i.e. previously generated) single domain antibody, and the one or more of the additional single domain antibodies present in the diversity of polypeptide constructs are either the same as or different from the identified single domain antibody envisaged in the template construct and may be the same or may be different from each other. For instance, where the template polypeptide construct is selected to comprise one single domain antibody of identity A, a diversity of structural variants with regard to the number of single domain antibodies may comprise polypeptide constructs comprising, in addition to single domain antibody A, one or more additional single domain antibodies of types A. Such structural variants may further be with regard to both the number and the identity of the single domain antibody A, such that the diversity of structural variants may comprise polypeptide constructs comprising, in addition to single domain antibody A, one or more single domain antibodies B, C, D, etc (such as e.g. without being limiting A-A, A-A-A, A-A-A-A, A-B, A-A-B, A-A-A-B, A-B-C, A-A-B-C, A-B-C-

D, etc.). A similar diversity may be generated starting from a template polypeptide construct comprising two or more single domain antibodies.

[0071] Accordingly, in particular embodiments, the production of a diversity of structural variants for the template polypeptide construct may comprise obtaining structural variants having fewer, an equal number of or more single domain antibodies compared to the template polypeptide construct, wherein the single domain antibodies may be the same or may be different from each other, provided that each structural variant of the diversity comprises at least two single domain antibodies.

[0072] In further particular embodiments, the methods of the invention encompass generating a diversity of structural variants with regard to the relative position of single domain antibodies within the polypeptide constructs. According to more particular embodiments, a template polypeptide construct is selected comprising one or more single domain antibodies having a certain relative position within the template polypeptide construct, and the generation of a diversity of structural variants comprises generating polypeptide constructs comprising the same single domain antibodies of the template polypeptide construct, but with different relative positions within these structural variants compared to the template polypeptide construct. For instance, when a particular template polypeptide construct is selected comprising three single domain antibodies A, B and C that are positioned relative to one another in a configuration A-B-C (wherein "-" represents the linkage, e.g. via a linker sequence such as a peptide linker, between A and B), a diversity of structural variants may comprise structural variants comprising the same three single domain antibodies A, B and C positioned relative to one another in one of the configurations A-B-C, B-A-C, or B-C-A, or A-C-B, or C-A-B, or C-B-A.

[0073] As indicated above, it is further envisaged that structural variants may involve variants with regard to additional groups which can influence the desired characteristics of the polypeptide construct. Accordingly, in particular embodiments, the diversity of polypeptide constructs comprises variants with regard to the relative position of the single domain antibodies, including variants with respect to other groups, residues, moieties present in the polypeptide constructs. For instance, when a particular template polypeptide construct is selected comprising two single domain antibodies A and B that are positioned relative to one another in a configuration A-B (wherein "-" represents the linkage, e.g. via a linker sequence such as a peptide linker, between A and B), a diversity of structural variants may comprise structural variants comprising the same two single domain antibodies A and B positioned relative to one another in one of the configurations A-B, B-A, or . . . X-A-B, X-B-A, or A-B-X, B-A-X . . . or A-X-B, B-X-A, or B-X-X- . . . -A, or A-X-X- . . . B, wherein X may be other groups, moieties present in the diversity of structural variants.

[0074] In yet further particular embodiment, the generation of a diversity of structural variants for template polypeptide construct comprises generating a diversity of structural variants with regard to the amino acid residues of the CDR regions of the single domain antibodies in the polypeptide constructs. For instance, where a template antibody is selected comprising one or more single domain antibodies that are characterized by three specific CDR regions, a diversity of structural variants for the template polypeptide construct may be generated by providing different polypeptide constructs comprising the one or more single domain antibodies of the template polypeptide construct whereby in at least one of the three CDR regions of at least one of the single domain antibodies, one or more amino acid residues are different compared to the corresponding amino acid residues in the corresponding CDR regions in the template polypeptide construct.

[0075] For instance, starting from a template polypeptide construct comprising two or more identified (i.e. previously generated) single domain antibodies, each comprising three CDR regions, a diversity of structural variants with regard to the amino acid residues of the CDR regions of the single domains antibodies in the polypeptide construct may comprise a diversity of structural variants all comprising essentially the same single domain antibodies but having in at least one of the three CDR regions (such as in one, e.g. preferably in CDR3, in two, e.g. preferably in CDR2 and CDR3, or in all three) of at least one of the single domain antibodies, at least one amino acid substitution (i.e. an amino acid residue which has been replaced by another amino acid residue).

[0076] In a further particular embodiment, where the selected template polypeptide construct comprises two or more identified (i.e. previously generated) single domain antibodies, each comprising three CDR regions, a diversity of structural variants of the template polypeptide construct with regard to the amino acid residues of the CDR regions of the single domain antibodies in the polypeptide construct may comprise different polypeptide constructs each comprising essentially the same single domain antibodies, but having in at least one (such as in one, e.g. preferably in CDR3, in two, e.g. preferably in CDR2 and CDR3, or in all three) of the three CDR regions of at least one of the single domain antibodies, at least one amino acid residue which is deleted or added, compared to the template polypeptide construct.

[0077] For example, one or more of the CDR regions in the single domain antibody may be altered in order to provide single domain antibodies and/or polypeptide construct with increased affinity compared to the wild type single domain antibody and/or polypeptide construct (also referred to as affinity maturation). Alterations of the CDR regions may include (without being limiting) the addition, deletion and/or changing of one or more of the amino acid residues in the CDR region (e.g. applying point mutations at certain specified positions); CDR grafting, veneering, the (partially or fully) randomization of the amino acid residues in the CDR); DNA shuffling, chain shuffling, look-through mutagenesis, walk-through mutagenesis and any other technique known in the art or any suitable combination of any of the foregoing. Reference is for example made to (without being limiting) the techniques described in WO 91/15581, WO 05/003345, International applications by Ablynx N.V. PCT/EP2008/058617 and PCT/EP2008/058618 as well as U.S. provisional application 61/077,924 by Albynx N.V. filed on 7 Jul. 2008 entitled "Methods for providing improved immunoglobulin sequences".

[0078] Such diversity of polypeptide constructs comprising an alteration in one or more of the CDR regions can be generated by any method known in the art (such as e.g. PCR assembly of an appropriate series or pool of oligonucleotides and similar techniques for engineering immunoglobulin sequences well known to the skilled person, followed by suitable expression).

[0079] In yet further particular embodiments of the methods described herein, the step of producing a diversity of

polypeptide constructs starting from a template polypeptide construct comprising e.g. two or more identified (i.e. previously generated) single domain antibodies comprises producing a diversity of structural variants of the polypeptide construct with regard to the amino acid residues of the framework regions of the single domain antibodies in the polypeptide constructs. For instance, a diversity of structural variants of a template polypeptide construct comprising two or more single domain antibodies that are each characterized by four specific framework regions, may be a diversity of structural variants wherein, for one or both of the single domain antibodies, in at least one of the four framework regions one or more amino acid residues are different compared to the corresponding amino acid residues in the corresponding framework regions of the template polypeptide construct.

[0080] For instance, starting from a template polypeptide construct comprising two or more identified (i.e. previously generated) single domain antibodies, each comprising four framework regions, producing a diversity of structural variants with regard to the amino acid residues of the framework regions of the single domain antibodies, may comprise producing a diversity of polypeptide constructs comprising the essentially the same single domain antibodies but wherein in at least one (in one (such as in FR2 or FR4), in two (such as in FR2 and FR4 or in FR2 and FR3 or in FR3 and FR4), in three (such as in FR2, FR3 and FR4) or in all four) of the four framework regions (of one or more, such as two, three or more of the single domain antibodies) at least one amino acid residue has been replaced by another amino acid residue.

[0081] In further envisaged embodiments, when a template polypeptide construct is selected comprising two or more single domain antibodies, each comprising four framework regions, a diversity of structural variants of said template polypeptide construct with regard to the amino acid residues of the framework regions of the single domain antibodies in the polypeptide constructs, may comprise a diversity of structural variants comprising the same single domain antibodies, wherein in at least one (in one (such as in FR2 or FR4), in two (such as in FR2 and FR4 or in FR2 and FR3 or in FR3 and FR4), in three (such as in FR2, FR3 and FR4) or in all four) of the four framework regions (in one or more, such as one, two, three or more of the single domain antibodies of the construct) at least one amino acid residue has been added or deleted.

[0082] In a specific embodiment the amino acid sequence of the framework regions may be altered by "camelization" of specific amino acid residues in the framework regions. Camelization refers to the replacing or substitution of one or more amino acid residues in the amino acid sequence of a (naturally occurring) V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description herein. Such "camelizing" substitutions are preferably inserted at amino acid positions that form and/or are present at the V_H-V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (see for example WO 94/04678, Davies and Riechmann FEBS Letters 339: 285-290, 1994; Davies and Riechmann Protein Engineering 9 (6): 531-537, 1996; Riechmann J. Mol. Biol. 259: 957-969, 1996; and Riechmann and Muyldermans J. Immunol. Meth. 231: 25-38, 1999).

[0083] In a specific embodiment the amino acid sequence of the framework regions may be altered by "humanization" of specific amino acid residues in the framework regions. In particular, humanized single domain antibodies may be single domain antibodies in which at least one amino acid residue is present (and in particular, in at least one of the framework residues) that is and/or that corresponds to a humanizing substitution. Potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring single domain antibody sequence with the corresponding framework sequence of one or more closely related human V_H sequences. The potentially useful humanizing substitutions (or combinations thereof) thus determined can be introduced into the polypeptide construct comprising said single domain antibody sequence (in any manner known per se, as further described herein). The resulting diversity of polypeptide constructs comprising at least one humanizing substitution in at least one single domain antibody can subsequently be screened for one or more desired properties.

[0084] In yet further particular embodiments of the methods of the invention, a template polypeptide construct is selected comprising two or more single domain antibodies, and the generation of a diversity of structural variants of the template polypeptide construct may also involve producing a diversity at the DNA level, i.e. a diversity of structural variants with regard to the codon usage in the selected template polypeptide construct. For instance, if a template polypeptide construct is characterized by an amino acid sequence that is obtained by expressing a particular nucleic acid sequence, wherein the nucleic acid sequence consists of a sequential number of codons, a diversity of structural variants may comprise a diversity of nucleic acid sequences encoding the same amino acid sequences wherein in each of the nucleic acid sequences at least one of the codons is different compared to the corresponding codons in the nucleic acid sequence coding for the amino acid sequence of the template polypeptide construct.

[0085] For instance, where a template polypeptide construct is selected comprising one or more single domain antibody amino acid sequences which are encoded by one or more selected nucleic acid sequences, the nucleic acid sequences consisting of a sequential number of codons, the step of producing a diversity of structural variants may involve producing structural variants wherein one or more nucleotide base pair changes have been introduced, such as by substitution, deletion or addition of one or more base pairs, compared to the one or more sequences encoding the template polypeptide construct. As a consequence, producing a diversity of structural variants of a template construct antibody with regard to codon usage may imply introducing variations at the nucleotide sequence level of the template polypeptide construct and accordingly may or may not result in variations in the amino acid sequences encoded by the structural variants.

[0086] According to further embodiments of the methods of the present invention, the step of producing a diversity of structural variants may comprise producing a diversity of structural variants and/or variations which involve more than one type of variants and/or variations described above, i.e. a combination of structural variants or variations such as but not limited to the following combinations of variants and/or variations, which include combinations of:

[0087] the number of the single domain antibodies in the polypeptide constructs (as described above) and the

relative position of said single domain antibodies within said polypeptide constructs (as described above); or

[0088] the number and/or identity of the single domain antibodies in said polypeptide constructs (as described above) and the amino acid residues of the CDR regions of one or more single domain antibodies in the polypeptide constructs (as described above); or

[0089] the number of the single domain antibodies in the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0090] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the sequence encoding the selected template polypeptide construct (as described above); or

[0091] the relative position of the single domain antibodies within the polypeptide constructs (as described above) and the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0092] the relative position of the single domain antibodies within the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0093] the relative position of the single domain antibodies within the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0094] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0095] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0096] the amino acid residues of the framework region (s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0097] the number of said single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within said polypeptide constructs (as described above) and the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0098] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within said polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of the single domain antibodies in the polypeptide constructs (as described above); or

[0099] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0100] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of on or more of the single domain antibodies in the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0101] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0102] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the framework region (s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0103] the relative position of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above); or

[0104] the relative position of one or more of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in said polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0105] the relative position of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the framework region (s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0106] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0107] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the amino acid residues of the framework region(s) of one or

more of the single domain antibodies in the polypeptide constructs (as described above) or;

[0108] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0109] the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above), the relative position of the single domain antibodies within the polypeptide constructs (as described above), the amino acid residues of the framework region (s) of one or more of the single domain antibodies in the polypeptide constructs (as described above) and the codon usage in the nucleic acid sequences encoding the polypeptide constructs; or

[0110] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the codon usage in the nucleic acid sequences encoding the polypeptide constructs and the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above); or

[0111] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the codon usage in the nucleic acid sequences encoding the polypeptide constructs and the relative position of the single domain antibodies within the polypeptide constructs (as described above) or;

[0112] the amino acid residues of the CDR region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the amino acid residues of the framework region(s) of one or more of the single domain antibodies in the polypeptide constructs (as described above), the codon usage in the nucleic acid sequences encoding the polypeptide constructs, the relative position of the single domain antibodies within the polypeptide constructs (as described above) and the number and/or identity of the single domain antibodies in the polypeptide constructs (as described above).

[0113] The length, the degree of flexibility and/or other properties of the linker(s) used in polypeptide constructs may have some influence on the properties of the final polypeptide construct of the invention, including but not limited to the affinity, specificity or avidity for one or more particular antigens or epitopes.

[0114] Therefore, the methods of the present invention, also encompass in step (ii) producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct of step (i) comprises producing structural variants with regard to the peptide linkers. In particular embodiments modifying the peptide linkers may favorably affect one or more desirable characteristics of the polypeptide construct.

[0115] Accordingly, in further particular embodiments of step (ii) the methods described herein comprising producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct, comprises producing structural variants according to one or more of the following (non limiting) examples:

[0116] structural variants with regard to the composition and/or length of the one or more peptide linkers, and/or,

[0117] structural variants with regard to the number of the one or more linkers, and/or,

[0118] structural variants with regard to the relative position of the one or more linkers in the polypeptide constructs.

[0119] As described above, the template polypeptide construct selected in the methods of the present invention may be a theoretical template construct, comprising one or more single domain antibodies that are envisaged to be linked to each other and/or to other groups or moieties via suitable (peptide) linkers or spacers. Alternatively, the template polypeptide construct may be an isolated polypeptide construct, comprising one or more identified (i.e. previously generated) single domain antibodies that are linked to each other and/or to other groups or moieties via actual physical linkers and/or spacers.

[0120] Suitable spacers or linkers for use in multivalent (and optionally multispecific or multiparatopic) polypeptide constructs will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, the linker and/or spacer is suitable for use in producing polypeptide constructs that are intended for pharmaceutical use.

[0121] Some particularly suitable linkers or spacers include the linkers and/or spacers that are used in the art to link antibody fragments or antibody domains. These include the linkers generally known in the art, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, its should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide constructs of the invention, since each single domain antibody by itself forms a complete antigen-binding site).

[0122] Some preferred examples of such amino acid sequences include gly-ser linkers, for example of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077 and the GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as hinge-like regions, such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678).

[0123] Some other particularly preferred linkers are polyalanine (such as AAA), as well as the linkers GS35, GS30 (SEQ ID NO: 85 in WO 06/122825) and GS9 (SEQ ID NO: 84 in WO 06/122825).

[0124] Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in

proteins for pharmaceutical use. For instance, polyethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

[0125] According to particular embodiments of the methods of the present invention, the step of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct comprising at least two single domain antibodies that are linked via one or more peptide linkers, comprises producing structural variants with regard to the composition and/or length of the one or more peptide linkers. In this respect, the different linkers in the diversity of polypeptide constructs may have one or more different amino acid residues in their amino acid sequence or they may have less or more amino acid residues resulting in a different linker length compared to the template polypeptide construct.

[0126] For instance, the step of producing a diversity of polypeptide constructs may comprise producing structural variants with regard to the amino acid sequence of the one or more peptide linkers in the template polypeptide construct.

[0127] Accordingly, starting from a template polypeptide construct comprising two or more identified (i.e. previously generated) single domain antibodies linked via one or more peptide linkers, generating a diversity of structural variants with regard to the amino acid sequence of the one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising essentially the same linkers but wherein in at least one of the linkers at least one amino acid residue has been replaced by another amino acid residue.

[0128] For instance, producing a diversity of structural variants of polypeptide constructs with regard to the amino acid sequence of the one or more peptide linkers may involve producing structural variants comprising different types of Gly-Ser linkers, for example different types of $(Gly_xSer_y)_z$.

[0129] Alternatively, the step of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct comprising at least two single domain antibodies that are linked via one or more peptide linkers may comprise producing structural variants with regard to the length of the one or more peptide linkers.

[0130] Accordingly, starting from a template polypeptide construct comprising two or more identified (i.e. previously generated) single domain antibodies linked via one or more peptide linkers, generating a diversity of structural variants with regard to the length of said one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising essentially the same linkers but wherein in at least one of the linkers at least one amino acid residue has been added and/or deleted.

[0131] For instance, producing a diversity of structural variants of polypeptide constructs with regard to the length of the one or more peptide linkers may involve producing structural variants comprising peptide linkers having a length varying between 1 and 50, such as a length varying between 1 and 35, or such as a length varying between 1 and 10 amino acid residues (such as e.g. Gly-Ser type linkers $(Gly_4Ser)_3$, $(Gly_4S)_5$, $(Gly_4Ser)_6$, etc.).

[0132] It is also encompassed in particular embodiments of the methods of the present invention that the step of producing a diversity of polypeptide constructs that are structural variants with regard to the composition and/or length of the one or more peptide linkers in the polypeptide constructs, may comprise producing structural variants with regard to the amino acid sequence of the one or more peptide linkers as well as with regard to the length of the one or more peptide linkers (such as e.g. Gly-Ser type linkers $(Gly_4Ser)_3$, $(Gly_4S)_5$, $(Gly_4Ser)_6$, etc. and $(Gly_4Ser)_3$ or $(Gly_3Ser_2)_3$).

[0133] Thus, where the template polypeptide construct comprises two or more identified (i.e. previously produced) single domain antibodies linked via one or more peptide linkers, generating a diversity of structural variants with regard to the amino acid sequence composition as well as with regard to the length of said one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising essentially the same linkers but wherein in at least one of the linkers at least one amino acid residue has been substituted, added and/or deleted.

[0134] According to further particular embodiments of the methods of the present invention, the step of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct comprising at least two single domain antibodies that are linked via one or more peptide linkers, comprises producing structural variants with regard to the number of said one or more peptide linkers.

[0135] For instance, starting from a template polypeptide construct comprising two identified (i.e. previously produced) single domain antibodies linked via one peptide linker, generating a diversity of structural variants with regard to the number of said one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising the same two single domain antibodies that are directly linked, i.e. without using said one peptide linker.

[0136] Also, for example starting from a template polypeptide construct comprising three identified (i.e. previously generated) single domain antibodies linked via two peptide linkers, generating a diversity of structural variants with regard to the number of said one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising the same three single domain antibodies, wherein said diversity of structural variants comprises none or one peptide linker.

[0137] Alternatively, starting from a template polypeptide construct comprising two identified (i.e. previously generated) single domain antibodies one or more other groups, moieties or binding units, wherein said polypeptide construct comprises three peptide linkers, generating a diversity of structural variants with regard to the number of said one or more peptide linkers, may comprise generating a diversity of polypeptide constructs comprising the same two single domain antibodies one or more other groups, moieties or binding units, wherein said diversity of structural variants comprises variants without linker, and/or variants with one or more peptide linkers, such as none, one, two, three, four, five, six, etc., wherein the one or more peptide linkers may link (a) said single domain antibodies to each other, (b) said one or more groups, moieties or binding units to each other, or (c) may interlink said single domain antibodies to said one or more other groups, moieties or binding units.

[0138] It should be stressed that the present embodiment also encompasses the production of a diversity of polypeptide constructs wherein one or more linkers are present or absent and thus a diversity of polypeptide constructs comprising polypeptide constructs wherein the single domain antibodies are directly linked to each other (or to one or more other groups, moieties or binding units) as well as comprising polypeptide constructs wherein the single domain antibodies are linked via a (peptide) linker to each other (or to one or more other groups, moieties or binding units).

[0139] According to yet further particular embodiments of the methods of the present invention, the step of producing a diversity of polypeptide constructs that are structural variants for the selected template polypeptide construct comprising at least two single domain antibodies that are linked via one or more peptide linkers, comprises producing structural variants with regard to the relative position of said one or more peptide linkers in the polypeptide constructs.

[0140] Accordingly, a template polypeptide construct may be selected comprising one or more peptide linkers having a certain relative position within the template polypeptide construct, and the generation of a diversity of structural variants comprises generating polypeptide constructs comprising the same peptide linkers as present in the template polypeptide construct, but wherein the peptide linkers have different relative positions within these structural variants compared to the template polypeptide construct.

[0141] For instance, when a particular template polypeptide construct is selected comprising four identified single domain antibodies (e.g. A, B, C, D) that are linked via three peptide linkers (e.g. "-", "---" and "-------") that are positioned relative to one another in a particular configuration (e.g. A-B---C-------D), a diversity of structural variants may comprise structural variants comprising the same four single domain antibodies (e.g. A, B, C and D) that are linked via the same three peptide linkers (e.g. "-", "---" and "-------"), wherein said peptide linkers are positioned relative to one another in one of the following non-limiting configurations A-B---C------D, A---B-C-------D, A-B-------C---D, A---B-------C-D, A-------B-C---D, or A-------B---C-D.

[0142] As indicated above, it is further envisaged that structural variants may involve variants with regard to additional groups which can influence the desired characteristics of the polypeptide construct. Accordingly, in particular embodiments, the diversity of polypeptide constructs comprises variants with regard to the relative position of the one or more peptide linkers, including with respect to one or more other groups, moieties or binding units present in the polypeptide constructs. For instance, when a particular template polypeptide construct is selected comprising two identified single domain antibodies (e.g. A and B) and one other group, moiety or binding unit (e.g. X), linked to each other via two peptide linkers (e.g. "---" and "----------") that are positioned relative to one another in a particular configuration (e.g. A----B----------X and A----B----------X), a diversity of structural variants may comprise structural variants comprising the same two single domain antibodies (e.g. A and B) and the same one other group or moiety (e.g. X), linked to each other via the same two peptide linkers (e.g. "----" and "----------"), wherein said peptide linkers are positioned relative to one another in the configuration (e.g. A----------B-----X).

[0143] According to further embodiments of the methods of the present invention, the step of producing a diversity of structural variants may comprise generating a diversity of structural variants which involve more than one type of variation of the one or more peptide linkers, i.e. a combination of structural variations such as but not limited to the following combinations of variations, which include combinations of:

[0144] the composition and/or length of the one or more peptide linkers in the polypeptide constructs (as described above) and the number of the one or more peptide linkers in the polypeptide constructs (as described above); or

[0145] the composition and/or length of the one or more peptide linkers in the polypeptide constructs (as described above) and the relative position of the one or more peptide linkers in the polypeptide constructs (as described above); or

[0146] the number of the one or more peptide linkers in the polypeptide constructs (as described above) and the relative position of the one or more peptide linkers in the polypeptide constructs (as described above); or

[0147] the composition and/or length of the one or more peptide linkers in the polypeptide constructs (as described above), the number of the one or more peptide linkers in the polypeptide constructs (as described above) and the relative position of the one or more peptide linkers in the polypeptide constructs (as described above).

[0148] It will be understood to the skilled person that methods are equally envisaged wherein, upon generating a diversity of polypeptide constructs, said diversity of polypeptide constructs may encompass both structural variants with regard to both the single domain antibodies as well as structural variants with regard to the (peptide) linkers or spacers.

[0149] For example, in multivalent polypeptide constructs of the invention that comprise single domain antibodies directed against a multimeric antigen (such as a multimeric receptor, ligand or other protein), the length and flexibility of the linker are preferably such that it allows each single domain antibody of the invention present in the polypeptide construct to bind to the antigenic determinant on each of the subunits of the multimer. Similarly, in a multispecific polypeptide construct of the invention that comprises single domain antibodies directed against two or more different antigenic determinants on the same antigen (for example against different epitopes of an antigen and/or against different subunits of a multimeric receptor, channel or protein), the length and flexibility of the linker are preferably such that it allows each single domain antibody to bind to its intended antigenic determinant. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention.

[0150] This particular embodiment of the present invention is particularly suited for the selection of or screening for polypeptide construct comprising at least two single domain antibodies that preferentially show intramolecular binding to a certain antigen compared to intermolecular binding. By "intramolecular" binding is meant that the polypeptide construct of the invention can simultaneously bind two epitopes on the same antigen (these two epitope can be the same, e.g. if the antigen is a multimer; or these epitopes may be different, e.g. when the method of the invention is used for screening multipartopic polypeptide constructs (as is further defined herein)).

[0151] The choice of linker length in biparatopic, triparatopic or multiparatopic polypeptides of the invention can also be such that only a limited epitope space on the antigen is covered. Linker length restriction can, for example, help to avoid targeting epitopes which should not be neutralized (e.g. those essential for a function of the antigen) or to target regions relatively adjacent to a first 'guiding' single domain antibody.

[0152] The choice of the format (N- or C-terminal position of the different single domain antibodies) of the biparatopic, triparatopic or multiparatopic polypeptides of the invention and linker length can also be used to obtain molecules that

bind avidly to the target antigen (via two, or more, binding sites), yet are purposely not agonistic. By optimising the format and linker length and composition, the binding sites can be positioned in such way that simultaneous binding of two or more single domain antibodies to the same target antigen (i.e. intramolecular binding) will be highly favoured compared to binding to separate antigens in proximity of one another (intermolecular binding, such as e.g. on a cell surface). This could, for example, reduce the chance on agonism (which might not be desired in a good therapeutic compound). Screening and/or selection methods (as further described herein) will allow for the isolation of avidly binding domains positioned in relation to one another and to the antigen of interest in such way as to have an antagonistic function only.

[0153] In another aspect of the invention, biparatopic, triparatopic or multiparatopic polypeptides of the invention can also be selected to be purposely agonistic. For example, a combination of two identical or two different Nanobodies that bind to the Herceptin®-binding site on HER2 and are genetically fused to one another can be agonistic (e.g. 2D3-2D3 or 2D3 fused to other Herceptin®-competing Nanobodies). The current invention also provides a way to select for such agonistic biparatopic, triparatopic or multiparatopic polypeptides of the invention using appropriate screening and/or selection procedures (as further described herein) of members of the diversity of structural variants. Agonists could, for example, be desired and/or interesting for triggering certain receptors.

[0154] It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the Nanobodies of the invention). For example, linkers containing one or more charged amino acid residues (see Table A-2 on page 48 of the International application WO 08/020,079) can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification.

[0155] Methods for the production of a diversity of polypeptide constructs according to the embodiments described above, are known in the art and include for example the production of single domain antibodies, including dAb's, Nanobodies, V_{HH}'s and other single variable domains and subsequently linking these single domain antibodies to each other and optionally to other groups, moieties or binding units via suitable (peptide) linkers, such that different structural variants comprising combinations of the individual single domain antibodies and optionally other groups, moieties or binding units are formed.

[0156] In this respect, naturally occurring V_{HH} domains against a particular antigen or target, can be obtained from (naïve or immune) libraries of Camelid V_{HH} sequences. Such methods may or may not involve screening such a library using said antigen or target, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known per se. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from (naïve or immune) V_{HH} libraries may be used, such as V_{HH} libraries obtained from (naïve or immune) V_{HH} libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[0157] Yet another technique for obtaining V_{HH} sequences or Nanobody sequences directed against a particular antigen or target involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_{HH} sequences or Nanobody sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_{HH} sequences directed against said antigen or target, starting from said sample, using any suitable technique known per se (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens et al., Proc. Natl. Acad. Sci. USA. 2006 Oct. 10; 103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (e.g. human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

[0158] For the generation of (single) domain antibodies from conventional four-chain antibodies, reference is made to EP 0 368 684, to Ward et al. (Nature 1989 Oct. 12; 341 (6242): 544-6), to Holt et al., Trends Biotechnol., 2003, 21(11):484-490; as well as to for example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd.

[0159] It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single domain antibodies or single variable domains can be generated from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

[0160] In particular embodiments of the methods of the invention, the production of structural variants may typically involve generating structural variants with a variation with respect to the single domain antibody and/or linker (as described above), known to affect the one or more desired characteristics. For instance, where stability is a desired characteristic, the template polypeptide construct may comprise two identified (i.e. previously generated) single domain antibodies and the generation of a diversity of structural variants of the template polypeptide construct may involve introducing chemical modifications to the template polypeptide construct which are envisaged to affect the half-life thereof (for example, by means of different forms of pegylation); alternatively, such structural variants may comprise at least one binding unit consisting of a single domain antibody directed against a serum protein (such as serum albumin), or such structural variants may comprise at least one additional moiety (and in particular at least one additional amino acid sequence) that increases the half-life of the template polypeptide construct of the invention.

[0161] Examples of structural variants which can be generated in this regard include but are not limited to structural variants comprising:

[0162] structural variants comprising at least two single domain antibodies (e.g. those of the template polypeptide construct) each suitably linked to a different poly (ethylene glycol) polymer chain;

[0163] structural variants comprising at least two single domain antibodies (e.g. those of the template polypeptide construct) each suitably linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof); or

[0164] structural variants comprising at least one single domain antibody linked to one or more single domain antibodies which bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrine);

[0165] structural variants comprising at least one single domain antibodylinked to an Fc portion (such as a human Fe) or a suitable part or fragment thereof, or linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489 and to WO 08/068,280.

[0166] In particular embodiments of methods of the present invention, the generation of a diversity of polypeptide constructs comprises generating polypeptide constructs which contain two or more binding units which are single domain antibodies that are directed against different antigenic determinants, epitopes, parts, domains, subunits or conformations (where applicable) of the same antigen. Generally, such polypeptide constructs bind to the antigen with increased avidity compared to a binding unit comprising only one single domain antibody directed against the antigen. Such polypeptide constructs may for example comprise two single domain antibodies i.e. one "first" single domain antibody that is directed against a first antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of an antigen (which may or may not be an interaction site); and a "second" single domain antibody that is directed against a second antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) different from the first antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of the antigen (and which again may or may not be an interaction site). A diversity of such polypeptide constructs may comprise combinations of different "first" and "second" single domain antibodies. Additionally or alternatively, a diversity may comprise a varying number of copies of the "first" and "second" single domain antibody. Additionally a diversity can be generated comprising more than two single domain antibodies directed against a different epitope (i.e. polypeptide constructs may comprise "third", "fourth", etc. single domain antibodies). In particular embodiments, in such "biparatopic" and/or "multiparatopic" (as defined herein) polypeptide constructs generated in methods of the invention, at least one single domain antibody is directed against an interaction site (as defined herein), although the invention in its broadest sense is not limited thereto.

[0167] Accordingly, in particular embodiments, the methods of the present invention for obtaining a polypeptide construct directed against one or more antigens and/or epitopes having one or more desired characteristics, wherein the polypeptide construct comprises at least two single domain antibodies, involve the generation of biparatopic and/or multiparatopic (such as e.g. triparatopic, tetraparatopic etc.) polypeptide constructs.

[0168] Without being limited thereto, methods for generating a diversity of bi- and mulitparatopic polypeptide constructs directed against a particular antigen and comprising at least two single domain antibodies may for example comprise at least the step of providing a nucleic acid sequence encoding a first amino acid sequence binding a first antigenic determinant, epitope, part, domain, subunit or conformation and fusing it to a set, collection or library of nucleic acid sequences encoding amino acid sequences; and ensuring adequate expression thereof.

[0169] The methods for generating a polypeptide construct of the invention typically further comprise screening the diversity of expressed polypeptide constructs for constructs capable of binding to the antigen of interest with increased affinity (and/or avidity) and/or for constructs that can bind to a second antigenic determinant, epitope, part, domain, subunit or conformation of the antigen different from the antigenic determinant, epitope, part, domain, subunit or conformation recognized by the first amino acid sequence; optionally the methods may involve screening for one or more further desired characteristics.

[0170] Further embodiments of the methods of the invention may optionally comprise isolating the nucleic acid sequence encoding the polypeptide construct comprising the first amino acid sequence fused to a second nucleic acid sequence identified in the screening step, followed by expressing the encoded amino acid sequence.

[0171] For example, where the antigen is HER2, without being limited thereto, methods for generating a diversity of bi- and multiparatopic polypeptide constructs directed against HER2 comprising at least two single domain antibodies may for example comprise at least the step of providing a nucleic acid sequence encoding a HER2 binding amino acid sequence (such as a single domain antibody) (binding a first antigenic determinant of HER2) and fusing it to a set, collection or library of nucleic acid sequences encoding amino acid sequences (such as single domain antibodies); and ensuring adequate expression thereof. Accordingly, the methods of generating a polypeptide construct directed against HER2 typically further comprise screening the so obtained diversity of expressed polypeptide constructs for constructs capable of binding to HER2 with increased affinity (and/or avidity) and/or for constructs that can bind to and/or have affinity for a (second) antigenic determinant of HER2 different from the first antigenic determinant; the methods of the invention may further optionally comprise screening for one or more further desired characteristics.

[0172] Further, the methods of the invention may optionally comprise isolating the nucleic acid sequence encoding the polypeptide construct comprising the HER2 amino acid sequence fused to the nucleic acid sequence identified in the screening step, followed by expressing the encoded amino acid sequence.

[0173] It will be understood that the nucleic acid sequences encoding the biparatopic polypeptide constructs obtained according to the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences and again be screened for nucleic acid sequences that encode polypeptide constructs that can bind to and/or have affinity for an antigenic determinant on the antigen (e.g. HER2) different from the first and second antigenic determinants of the antigen (e.g. HER2), in order to obtain a triparatopic or further multiparatopic polypeptide constructs.

[0174] According to particular embodiments, methods for generating a polypeptide construct directed against HER2, which involve generating bi- and mulitparatopic polypeptide constructs may for example comprise at least the step of:

[0175] a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on HER2 that is fused (optionally via a linker sequence) to a second amino acid sequence, in which essentially each second amino acid sequence (or most of these) is a different member of a set, collection or library of different amino acid sequences;

[0176] b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on HER2 different from the first antigenic determinant, part, domain or epitope on HER-2;

[0177] and

[0178] c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on HER2 different from the first antigenic determinant, part, domain or epitope on HER-2, obtained in b), optionally followed by expressing the encoded amino acid sequence.

[0179] In these embodiments of the methods of the invention, the first amino acid sequence in the polypeptide construct (fusion protein) encoded by said set collection or library of nucleic acid sequences may be the same amino acid sequence for all members of the set, collection or library of nucleic acid sequences encoding the polypeptide construct (fusion protein); or the first amino acid sequence in the polypeptide construct (fusion protein) encoded by said set collection or library of nucleic acid sequences may also be a member of a set collection or library of different amino acid sequences.

[0180] In particular embodiments of methods of the invention wherein HER2 is the antigen of interest, in step b) as described above, the set, collection or library of nucleic acid sequences may also be screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both a first antigenic determinant, part, domain or epitope on HER2 and a second antigenic determinant, part, domain or epitope on HER2. This may for example be performed in a subsequent steps (i.e. by in a first step screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the second antigenic determinant, part, domain or epitope on HER2, and subsequently in a second step selecting or screening for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first antigenic determinant, part, domain or epitope on HER2; or visa versa) or in a single step (i.e. by simultaneously screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both the first antigenic determinant, part, domain or epitope on HER2 and the second antigenic determinant, part, domain or epitope on HER2).

[0181] In further particular embodiments of the above-described methods, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Herceptin® binding site on HER2 (and may in particular be directed against domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or (ii) competes with Herceptin for binding to HER-2; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Omnitarg® binding site on HER2 (and may in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or (ii) an amino acid sequence that can compete with Omnitarg® (or the Omnitarg Fab used in Example 9) for binding to HER-2.

[0182] Alternatively, in particular embodiments, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Omnitarg® binding site on HER2 (and may in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or (ii) competes with Omnitarg for binding to HER-2; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Herceptin® binding site on HER2 (and in particular domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or (ii) an amino acid sequence that can compete with Herceptin for binding to HER-2.

[0183] In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with Herceptin® or Omnitarg, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

[0184] It is also possible, in step b) of the methods described above, to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Omnitarg® binding site on HER2 (and in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or that can compete with Omnitarg® (or the Omnitarg Fab used in Example 9) for binding to HER-2; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the Herceptin® binding site on HER2 (and in particular domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or that can compete with Herceptin® for binding to HER-2. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of Herceptin® and/or Omnitarg, as applicable.

[0185] It will also be clear to the skilled person that the above methods may be performed by screening a set, collection or library of amino acid sequences that correspond to (e.g. are encoded by) the nucleic acid sequences used in the above method; and such methods form further aspects of the invention.

[0186] In further particular embodiments of the methods of the invention, the step of generating a diversity of polypeptide constructs involves generating a diversity of biparatopic polypeptide constructs which are structural variants with regard to the linker sequence, linking the single domain antibodies. The step of generating a diversity thus may comprise

providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a polypeptide construct (a fusion protein) that comprises a first amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on an antigen of interest (such as HER2) that is fused via a linker sequence to a second amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on the antigen of interest (which may be the same or different as the first antigenic determinant, part, domain or epitope on the antigen of interest), in which essentially each nucleic acid sequence (or most of these) encodes a fusion protein with a different linker sequence so as to provide a set, collection or library encoding different polypeptide constructs (fusion proteins);

[0187] The methods of the present invention for generating a polypeptide construct against an antigen of interest typically will further comprise the step of screening the so obtained set, collection or library of nucleic acid sequences for nucleic acid sequences that encode a polypeptide construct (fusion protein) that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on the antigen of interest (e.g. HER2). Moreover, in particular embodiments, the methods of the present invention may further comprise isolating the nucleic acid sequences that encode polypeptide construct (fusion protein) that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on the antigen of interest, optionally followed by expressing the encoded amino acid sequence.

[0188] As will be clear to the skilled person, these methods can be used to screen for suitable or even optimal linker lengths for linking the first and second amino acid sequence. For example, in this aspect, where the antigen of interest is HER2, the first amino acid sequence (such as a single domain antibody) may be an amino acid sequence (such as a single domain antibody and preferably a Nanobody) that can bind to and/or has affinity for the Omnitarg® binding site on HER2 (and may in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or that can compete with Omnitarg® (or the Omnitarg Fab used in Example 9); and the second amino acid sequence (such as a single domain antibody) may be an amino acid sequence (such as a single domain antibody and preferably a Nanobody) that can bind to and/or has affinity for the Herceptin® binding site on HER2 (and in particular domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or that can compete with Herceptin® for binding to HER-2 (or visa versa). The screening and selection may be performed as further described above.

[0189] In yet further embodiments of methods of the present invention, the methods comprise at least the steps of:

[0190] a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences (such as single domain antibodies);

[0191] b) screening said set, collection or library of nucleic acid sequences for a set, collection or library of nucleic acid sequences that encode an amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for the antigen of interest, such as for instance HER2;

[0192] c) ligating said set, collection or library of nucleic acid sequences that encode an amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for the antigen of interest to another nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for the antigen of interest (e.g. a nucleic acid sequence that encodes an amino acid sequence that competes with Herceptin® for binding HER2);

[0193] and

[0194] d) from the set, collection or library of nucleic acid sequences obtained in c), isolating the nucleic acid sequences encoding a biparatopic polypeptide construct that can bind to and/or has affinity for the antigen of interest (and e.g. further selecting for nucleic acid sequences that encode a biparatopic amino acid sequence that antagonizes with higher potency compared to the monovalent amino acid sequences), followed by expressing the encoded polypeptide construct.

[0195] The nucleic acid sequences encoding the biparatopic polypeptide construct obtained in the methods above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences (such as a single domain antibodies) that can bind to and/or have affinity for the antigen of interest in order to obtain a triparatopic or multiparatopic amino acid sequence respectively. In addition the steps described above can also be used in the generation of polypeptide constructs directed against two (or more) different antigens (e.g. HER2 and CD3, HER2 and CD16) so as to obtain polypeptide constructs which are bispecific, trispecific or multispecific.

[0196] Similarly, the steps described above can also be used in the generation of polypeptide constructs directed against two (or more) (different) epitopes on the same antigens as well as against two (or more) different antigens so as to obtain polypeptide constructs which are bispecific, trispecific and/or multispecific in addition to being biparatopic, triparatopic and/or multiparatopic.

[0197] In yet further particular embodiments of the methods of the invention for generating a polypeptide construct directed against an antigen of interest (such as HER2), the methods comprise at least the steps of:

[0198] a) providing a first set, collection or library of nucleic acid sequences encoding amino acid sequences (such as single domain antibodies);

[0199] b) screening said first set, collection or library of nucleic acid sequences for a nucleic acid sequence that encodes an amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on an antigen of interest (such as HER2);

[0200] c) ligating the nucleic acid sequence encoding said amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on the antigen of interest to another set, collection or library of nucleic acid sequences encoding amino acid sequences (such as single domain antibodies) to obtain a set, collection or library of nucleic acid sequences that encode fusion proteins;

[0201] d) screening said set, collection or library of nucleic acid sequences obtained in step c) for a nucleic acid sequence that encodes an amino acid sequence (such as a single domain antibody) that can bind a second antigenic determinant, part, domain or epitope on the antigen of

interest different from the first antigenic determinant, part, domain or epitope on the antigen of interest;

[0202] and

[0203] e) isolating the nucleic acid sequence that encodes an amino acid sequence (such as a single domain antibody) that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on the antigen of interest, optionally followed by expressing the encoded polypeptide construct.

[0204] Similar to the embodiments above, the biparatopic polypeptide construct obtained in these methods can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences (such as single domain antibodies) that can bind to and/or have affinity for the antigen of interest in order to obtain a triparatopic or multiparatopic polypeptide construct respectively.

[0205] In addition the steps described above can also be used in the generation of polypeptide constructs directed against two (or more) different antigens (e.g. HER2 and CD3, HER2 and CD16) so as to obtain polypeptide constructs which are bispecific, trispecific or multispecific.

[0206] Similarly, the steps described above can also be used in the generation of polypeptide constructs directed against two (or more) (different) epitopes on the same antigens as well as against two (or more) different antigens so as to obtain polypeptide constructs which are bispecific, trispecific and/or multispecific in addition to being biparatopic, triparatopic and/or multiparatopic.

[0207] In particular embodiments of the above method, the antigen of interest is HER2 and the first amino acid sequence (such as a single domain antibody) obtained in step b) described above is preferably such that (i) it can bind to and/or has affinity for Herceptin® binding site on HER2 (and may in particular be directed against domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or (ii) competes with Herceptin® for binding to HER-2; and in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Omnitarg binding site on HER2 (and may in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or (ii) an amino acid sequence that can compete with Omnitarg (or the Omnitarg Fab used in Example 9) for binding to HER-2.

[0208] In alternative embodiments of the methods of the present invention aimed at generating polypeptide constructs against HER2, the first amino acid sequence obtained in step b) described above is preferably such that (i) it can bind to and/or has affinity for the Omnitarg binding site on HER2 (and may in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or (ii) competes with Omnitarg for binding to HER-2; and in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Herceptin® binding site on HER2 (and in particular domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or (ii) an amino acid sequence that can compete with Herceptin® for binding to HER-2.

[0209] In the above methods wherein the antigen of interest is HER2 screening or selecting for (nucleic acid sequences that encode) amino acid sequences (such as the single domain antibodies or polypeptide constructs) that compete with Herceptin® or Omnitarg, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

[0210] It is also possible, in particular embodiments of the invention aimed at generating suitable polypeptide constructs directed against HER2, that step d) as described above, encompasses screening for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Omnitarg® binding site on HER2 (and in particular domain II of HER2, more in particular the middle of domain II of HER2) and/or that can compete with Omnitarg® (or the Omnitarg Fab used in Example 9) for binding to HER-2; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the Herceptin® binding site on HER2 (and in particular domain IV of HER2, more in particular the C-terminus of domain IV of HER2) and/or that can compete with Herceptin® for binding to HER-2. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of Herceptin® and/or Omnitarg, as applicable.

[0211] In the different embodiments of the methods of the invention described herein, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

[0212] Additionally or alternatively, in the methods described herein, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

[0213] In further particular embodiments, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with the antigen of interest such as HER2 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, the antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

[0214] The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

[0215] In the above methods, the nucleic acid sequence encoding an amino acid sequence binding the antigen/epitope of interest fused to the set, collection or library of nucleotide

sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences (such as the single domain antibodies or polypeptide constructs) will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

[0216] Further reference is made to the international application of Ablynx N.V. entitled "Amino acid sequences directed against HER2 and polypeptides comprising the same for the treatment of cancers and/or tumors", which has a filing date of Nov. 27, 2008.

[0217] Methods according to the present invention further comprise step (iii) of screening the produced diversity of polypeptide constructs of step (ii) for a polypeptide construct having one or more, more particularly two or more desired characteristics. As detailed above, in particular embodiments, the methods involve screening the produced diversity of polypeptide constructs for a polypeptide construct which, in addition to its ability to recognize the antigen/epitope of interest, has one or more additional desired characteristics. These methods may or may not involve the screening for antigen-binding.

[0218] The methods of the present invention envisage obtaining a polypeptide construct which is a polypeptide construct (as defined herein) comprising at least two single domain antibodies (as defined herein), being directed against one or more antigens (as defined herein) and/or epitopes (as defined herein) and exhibiting one or more desired characteristics. It is envisaged that the methods of the present invention can be used for obtaining a polypeptide construct with any desired characteristic that can be screened for. Most typically in the methods of the invention, screening is done for one or more desired characteristics selected from (but not limited to) a suitable binding affinity, avidity, a suitable solubility, a suitable stability, suitable efficacy, a suitable potency and/or any appropriate combinations thereof. The suitable polypeptide construct is identified within the diversity of polypeptide constructs by screening the diversity of polypeptide constructs for one or more desired characteristics. The nature of the screening step(s) in the methods described herein is thus determined, at least in part, by the envisaged one or more desired characteristics of the polypeptide construct to be obtained.

[0219] In particular embodiments of the methods described herein, the one or more desired characteristics include a suitable affinity for the antigen and/or epitope.

[0220] The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding molecule: the lesser the value of the K_D, the stronger the binding strength between an antigenic determinant and the antigen-binding molecule (alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$).

[0221] The "suitable" or desired affinity of a polypeptide construct obtained with the methods of the invention will be determined by its intended purpose. In particular embodiments it is envisaged that suitable affinity refers to the fact that the polypeptide construct binds to the one or more antigens and/or epitopes with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that the polypeptide construct:

[0222] binds to at least one of the one or more antigens or epitopes with a k_{on}-rate of between 10^2 M^{-1} s^{-1} to about 10^7 M^{-1} s^{-1}, preferably between 10^3 M^{-1} s^{-1} and 10^7 M^{-1} s^{-1}, more preferably between 10^4 M^{-1} s^{-1} and 10^7 M^{-1} s^{-1}, such as between 10^5 M^{-1} s^{-1} and 10^7 M^{-1} s^{-1};

and/or such that the polypeptide construct:

[0223] binds to at least one of the one or more antigens or epitopes with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^2 s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

[0224] In particular embodiments, where the methods of the invention envisage the generation of a polypeptide construct which is directed against two or more antigens and/or epitopes the screening for suitable affinity involves the screening of the diversity of polypeptide constructs for an affinity as described above for one, some or all of the antigens and/or epitopes against which the construct is intended to be directed.

[0225] Specific binding of an antigen-binding molecule to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or (competitive) binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich (competition) assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein.

[0226] The dissociation constant may be the actual or apparent dissociation constant, as will be clear to the skilled person. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned herein. In this respect, it will also be clear that it may not be possible to measure dissociation constants of more then 10^{-4} moles/liter or 10^{-3} moles/liter (e.g. of 10^{-2} moles/liter). Optionally, as will also be clear to the skilled person, the (actual or apparent) dissociation constant may be calculated on the basis of the (actual or apparent) association constant (K_A), by means of the relationship [K_D=1/K_A].

[0227] The affinity denotes the strength or stability of a molecular interaction. The affinity is commonly given as by the K_D, or dissociation constant, which has units of mol/liter (or M). The affinity can also be expressed as an association constant, K_A, which equals $1/K_D$ and has units of $(mol/liter)^{-1}$ (or M^{-1}). In the present specification, the stability of the interaction between two molecules (such as a binding unit, e.g. a single domain antibody or a polypeptide construct of the invention and its intended target, antigen and/or epitope) will mainly be expressed in terms of the K_D value of their interaction; it being clear to the skilled person that in view of the relation $K_A=1/K_D$, specifying the strength of molecular interaction by its K_D value can also be used to calculate the corresponding K_A value. The K_D-value characterizes the strength of a molecular interaction also in a thermodynamic sense as it is related to the free energy (DG) of binding by the well known relation $DG=RT\cdot\ln(K_D)$ (equivalently $DG=-RT\cdot\ln$

(K_A)), where R equals the gas constant, T equals the absolute temperature and in denotes the natural logarithm.

[0228] The K_D for biological interactions which are considered meaningful (e.g. specific) are typically in the range of 10^{-10} M (0.1 nM) to 10^{-5} M (10000 nM). The stronger an interaction is, the lower is its K_D.

[0229] The K_D can also be expressed as the ratio of the dissociation rate constant of a complex, denoted as k_{off}, to the rate of its association, denoted k_{on} (so that $K_D=k_{off}/k_{on}$ and $K_A=k_{on}/k_{off}$). The off-rate k_{off} has units s^{-1} (where s is the SI unit notation of second). The on-rate k_{on} has units M^{-1} s^{-1}. The on-rate may vary between 10^2 M^{-1} s^{-1} to about 10^7 M^{-1} s^{-1}, approaching the diffusion-limited association rate constant for bimolecular interactions. The off-rate is related to the half-life of a given molecular interaction by the relation $t_{1/2}=\ln(2)/k_{off}$. The off-rate may vary between 10^{-6} s^{-1} (near irreversible complex with a $t_{1/2}$ of multiple days) to 1 s^{-1} ($t_{1/2}=0.69$ s).

[0230] The affinity of a polypeptide construct of the invention against one or more antigens and/or epitopes can be determined for example using the general techniques for measuring K_D. K_A, k_{off} or k_{on}. The affinity of a molecular interaction between two molecules can be measured via different techniques known per se, such as the well known surface plasmon resonance (SPR) biosensor technique (see for example Ober et al., Intern. Immunology, 13, 1551-1559, 2001) where one molecule is immobilized on the biosensor chip and the other molecule is passed over the immobilized molecule under flow conditions yielding k_{on}, k_{off} measurements and hence K_D (or K_A) values. This can for example be performed using the well-known BIACORE instruments.

[0231] It will also be clear to the skilled person that the measured K_D may correspond to the apparent K_D if the measuring process somehow influences the intrinsic binding affinity of the implied molecules for example by artifacts related to the coating on the biosensor of one molecule. Also, an apparent K_D may be measured if one molecule contains more than one recognition sites for the other molecule. In such situation the measured affinity may be affected by the avidity of the interaction by the two molecules.

[0232] Another approach that may be used to assess affinity is the 2-step ELISA (Enzyme-Linked Immunosorbent Assay) procedure of Friguet et al. (J. Immunol. Methods, 77, 305-19, 1985). This method establishes a solution phase binding equilibrium measurement and avoids possible artefacts relating to adsorption of one of the molecules on a support such as plastic.

[0233] However, the accurate measurement of K_D may be quite labour-intensive and as consequence, often apparent K_D values are determined to assess the binding strength of two molecules. It should be noted that as long as all measurements are made in a consistent way (e.g. keeping the assay conditions unchanged) apparent K_D measurements can be used as an approximation of the true K_D and hence in the present document K_D and apparent K_D should be treated with equal importance or relevance.

[0234] Finally, it should be noted that in many situations the experienced scientist may judge it to be convenient to determine the binding affinity relative to some reference molecule. For example, to assess the binding strength between molecules A and B, one may e.g. use a reference molecule C that is known to bind to B and that is suitably labelled with a fluorophore or chromophore group or other chemical moiety, such as biotin for easy detection in an ELISA or FACS (Fluorescent activated cell sorting) or other format (the fluorophore for fluorescence detection, the chromophore for light absorption detection, the biotin for streptavidin-mediated ELBA detection). Typically, the reference molecule C is kept at a fixed concentration and the concentration of A is varied for a given concentration or amount of B. As a result an IC_{50} value is obtained corresponding to the concentration of A at which the signal measured for C in absence of A is halved. Provided $K_{D\,ref}$, the K_D of the reference molecule, is known, as well as the total concentration c_{ref} of the reference molecule, the apparent K_D for the interaction A-B can be obtained from following formula: $K_D=IC_{50}/(1+c_{ref}/K_{D\,ref})$. Note that if $c_{ref}<<K_{D\,ref}$, $K_D\approx IC_{50}$. Provided the measurement of the IC_{50} is performed in a consistent way (e.g. keeping c_{ref} fixed) for the binders that are compared, the strength or stability of a molecular interaction can be assessed by the IC_{50} and this measurement is judged as equivalent to K_D or to apparent K_D throughout this text.

[0235] In proteins, avidity is a term used to describe the combined strength of multiple bond interactions. Avidity is distinct from affinity, which is a term used to describe the strength of a single bond. As such, avidity is the combined synergistic strength of bond affinities rather than the sum of bonds. It is commonly applied to antibody interaction, where multiple, weak, non-covalent bonds form between antigen and antibody. Individually, each bond is quite readily broken, however when many are present at the same time, the overall effect results in synergistic, strong binding of antigen to antibody.

[0236] In the context of the present invention, the avidity of a polypeptide construct is referred to as the combined strength of bond affinities in the complex formed between the polypeptide construct and its antigen. This combined strength of bond affinities is obtained by the binding of each individual single domain antibody in the polypeptide construct to its respective epitope on the antigen.

[0237] In particular embodiments, the methods of the present invention are aimed at obtaining a polypeptide construct having a suitable solubility and the methods of the invention involve screening the diversity of polypeptide constructs for polypeptide constructs with a suitable solubility. Suitable solubility values of polypeptide constructs obtainable by the methods of the present invention will be determined by their intended use or purpose and will be clear to the skilled person based on the common general knowledge and the prior art as cited herein. Without being limiting, the polypeptide constructs obtained with the method of the invention may have a solubility from 5 to 500 mg per ml, more preferably from 10 to 250 mg per ml, even more preferably from 50 to 200 mg per ml, such as around 20 mg per ml, 50 mg per ml, 100 mg per ml or 150 mg per ml. With regard to measuring or determining the solubility of the polypeptide constructs obtainable according to the methods of the present invention, suitable methods are available in the art (e.g. solubility can be measured in a the dilution method, by concentration of the polypeptide construct until precipitation of the polypeptide construct occurs, e.g. via an ultrafiltration membrane (via centrifugation or via crossflow filtration); or can be measured indirectly e.g. by addition of agents such as PEG) and will be clear to the skilled person.

[0238] In particular embodiments, the methods of the present invention are aimed at obtaining a polypeptide construct having a suitable stability and the methods of the invention involve screening the diversity of polypeptide constructs

for polypeptide constructs with a suitable stability. The desired stability of a polypeptide construct will be determined by its intended purpose. More particularly with regard to stability, it is envisaged that a polypeptide construct may be considered to have a suitable stability where it has a suitable half-life for its intended purpose (e.g. for use as a human or animal therapeutic).

[0239] In particular embodiments it is envisaged that suitable stability refers to the fact that the polypeptide construct has a suitable half-life. The "half-life" of a polypeptide construct of the invention can generally be defined as the time taken for the serum concentration of the polypeptide construct to be reduced by 50%, in vivo, for example due to degradation of the polypeptide construct and/or clearance or sequestration of the polypeptide construct by natural mechanisms. Suitable half-life values may be a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the selected template polypeptide construct per se. For example, the polypeptide construct of the invention with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the selected template polypeptide construct per se. For example, a polypeptide construct having a suitable stability according to the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

[0240] Accordingly, in particular embodiments, the screening step of the method according to the present invention encompasses determining the in vivo half-life of the diversity of polypeptide constructs of the invention. The in vivo half-life of polypeptide construct of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering to a warm-blooded animal (i.e. to a human or to another suitable mammal, such as a mouse, rabbit, rat, pig, dog or a primate, for example monkeys from the genus *Macaca* (such as, and in particular, cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*) and baboon (*Papio ursinus*)) a suitable dose of the polypeptide construct of the invention; collecting blood samples or other samples from said animal; determining the level or concentration of the polypeptide construct of the invention in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence, compound or polypeptide of the invention has been reduced by 50% compared to the initial level upon dosing. Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinete analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). As will also be clear to the skilled person (see for example pages 6 and 7 of WO 04/003019 and in the further references cited therein), the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). In the present specification, an "increase in half-life" refers to an increase in any one of these parameters, such as any two of these parameters, or essentially all three these parameters. As used herein "increase in half-life" or "increased half-life" in particular refers to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

[0241] In further particular embodiments, the screening methods of the present invention may comprise determining certain characteristics (such as the conformation, binding, activity, molecular weight, amino acid sequence, etc.) of the polypeptide constructs after or during exposure to one or more specific (such as e.g. denaturing condition, presence of acids, presence of basics, presence of guanidinium chloride, presence of urea, high or low temperature, high or low pressure, shear, certain time, presence of certain human tissues (such as e.g. lung tissue, liver tissue, etc.) or fluids (such as e.g. saliva, mucus, BAL, blood, urine, gastric juice, etc.), etc.) conditions.

[0242] In further particular embodiments, the screening methods of the present invention may comprise determining the expression level of the polypeptide constructs under specified growth and/or inducing conditions. Suitable expression levels for the polypeptide constructs are defined by their intended use or purpose and will be clear to the skilled person based on the common general knowledge and the prior art as cited herein.

[0243] In particular embodiments the methods of the present invention are aimed at generating a polypeptide construct having a suitable efficacy (in a particular assay or model) and the methods of the invention involve screening the diversity of polypeptide constructs to determine for polypeptide constructs that display a suitable efficacy. A suitable efficacy value of polypeptide constructs will be determined by the intended use or purpose and will be clear to the skilled person based on the common general knowledge and the prior art as cited herein. With regard to measuring or determining the efficacy of the polypeptide constructs obtainable according to the methods of the present invention, polypeptide constructs of the invention can be tested using any suitable in vitro assay, cell-based assay, in vivo assay and/or animal model known per se, or any combination thereof, depending on the intended use or purpose of the polypeptide construct. The selection of a suitable assay for use in the screening step will be determined by e.g. the antigen(s) and/or epitope(s) that should be bound by the polypeptide construct of the invention, by the specific (therapeutic) activity (such as e.g. blocking of receptor/ligand binding; inhibition of enzymatic activity; competing with and/or blocking a reference antibody; modulating certain signalling pathways; inducing apoptosis; etc.) the polypeptide construct of the invention should have, etc. Based on the knowledge of the desired characteristics of the polypeptide construct of the invention, suitable assays and animal models will be clear to the skilled person, and for example include the assays and animal models used in the experimental part below and in the prior art cited herein.

[0244] In particular embodiments the methods of the present invention are aimed at generating a polypeptide construct having a suitable potency and the methods of the invention involve screening a diversity of polypeptide constructs obtained according to methods described herein for polypeptide constructs that display a suitable potency. More particularly, suitable potency values of polypeptide constructs will

be determined by their intended use or purpose and will be clear to the skilled person based on the common general knowledge and the prior art as cited herein. With regard to measuring or determining the potency of the polypeptide constructs of the present invention, these can generally be tested using any suitable in vitro potency assay, cell-based potency assay, in vivo potency assay and/or animal model known per se, or any combination thereof, depending on the intended use or purpose of the polypeptide construct. Suitable potency assays and animal models will be clear to the skilled person, and for example include the potency assays and animal models used in the experimental part below and in the prior art cited herein.

[0245] Methods according to the present invention allow the identification, from a diversity of polypeptide constructs, of specific (candidate) polypeptide constructs having one or more desired characteristics, which specific (candidate) polypeptide constructs are either directed against one antigen (whereby the polypeptide constructs may specifically bind to one or more epitopes thereof) or against different antigens (whereby the polypeptide construct may potentially specifically hind to one or more epitopes of each of the different antigens). Thus, the methods of the present invention are not limited to or defined by specific antigenic determinants, epitopes, parts, domains, subunits or conformations (where applicable) of the antigens against which the polypeptide constructs of the invention are directed. For example, the polypeptide constructs of the invention may or may not be directed against an "interaction site" (as defined herein). However, it is generally assumed and preferred that the polypeptide constructs of the invention are preferably directed against an interaction site (as defined herein).

[0246] Also, polypeptide constructs according to the invention contain one or more binding units consisting of single domain antibodies that are directed against one or more antigens or epitopes. Generally, such polypeptide constructs will bind to said one or more antigens or epitopes with increased avidity (as defined herein) compared to the binding unit consisting of a single domain antibody. Such a polypeptide construct may for example comprise two single domain antibodies that are directed against the same antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of an antigen (which may or may not be an interaction site); or comprise at least one "first" single domain antibody that is directed against a first antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of an antigen (which may or may not be an interaction site); and at least one "second" single domain antibody that is directed against a second antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) different from the first (and which again may or may not be an interaction site). Preferably, in such "biparatopic" polypeptide constructs of the invention, at least one single domain antibody is directed against an interaction site (as defined herein), although the invention in its broadest sense is not limited thereto.

[0247] Also, when the antigen is part of a binding pair (for example, a receptor-ligand binding pair), the polypeptide constructs of the invention may be such that they compete with the cognate binding partner (e.g. the ligand, receptor or other binding partner, as applicable) for binding to the antigen, and/or such that they (fully or partially) neutralize binding of the binding partner to the antigen. Methods and assays for polypeptide constructs that compete with a cognate binding part and/or that neutralize binding of the binding partner will be clear to the skilled person based on the common general knowledge and the available prior art.

[0248] It is also within the scope of the invention that, where applicable, a polypeptide construct of the invention can bind to two or more antigenic determinants, epitopes, parts, domains, subunits or conformations of the same antigen. In such a case, said antigenic determinants, epitopes, parts, domains or subunits may be essentially the same (for example, if said antigen contains repeated structural motifs or occurs in a multimeric form) or may be different (and in the latter case, the polypeptide constructs of the invention may bind to such different antigenic determinants, epitopes, parts, domains, subunits of said antigen with an affinity and/or specificity which may be the same or different). Also, for example, when said antigen exists in an activated conformation and in an inactive conformation, the polypeptide constructs of the invention may bind to either one of these conformations, or may bind to both these conformations (i.e. with an affinity and/or specificity which may be the same or different). Also, for example, the polypeptide constructs of the invention may bind to a conformation of an antigen in which it is bound to a pertinent ligand, may bind to a conformation of an antigen in which it not bound to a pertinent ligand, or may bind to both such conformations (again with an affinity and/or specificity which may be the same or different). (all depending on the desired characteristics of the polypeptide construct of the invention). Methods and assays for screening for polypeptide constructs that bind one or more specific conformations of an antigen will be clear to the skilled person based on the common general knowledge and the available prior art.

[0249] It is also expected that the polypeptide constructs of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of said antigen; or at least to those analogs, variants, mutants, alleles, parts and fragments of said antigen that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant(s) or epitope(s) to which the polypeptide constructs of the invention bind in the wild-type of said antigen. Again, in such a case, the polypeptide constructs of the invention may bind to such analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that are the same as, or that are different from (i.e. higher than or lower than), the affinity and specificity with which the polypeptide constructs of the invention bind to (wild-type) antigen. It is also included within the scope of the invention that the polypeptide constructs of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of said antigen, but not to others. (all depending on the desired characteristics of the polypeptide construct of the invention). Methods and assays for screening for polypeptide constructs that bind one or more analogs, variants, mutants, alleles, parts and fragments of an antigen will be clear to the skilled person based on the common general knowledge and the available prior art.

[0250] When said antigen exists in a monomeric form and in one or more multimeric forms, it is within the scope of the invention that the polypeptide constructs of the invention only bind to said antigen in monomeric form, only bind to an antigen in multimeric form, or bind to both the monomeric and the multimeric form. Again, in such a case, the polypeptide constructs of the invention may bind to the monomeric form with an affinity and/or specificity that are the same as, or

that are different from (i.e. higher than or lower than), the affinity and specificity with which the polypeptide constructs of the invention bind to the multimeric form. (all depending on the desired characteristics of the polypeptide construct of the invention). Methods and assays for screening for polypeptide constructs that bind one or more forms (monomeric or multimeric) of an antigen will be clear to the skilled person based on the common general knowledge and the available prior art.

[0251] Also, when said antigen can associate with other proteins or polypeptides to form protein complexes (e.g. with multiple subunits), it is within the scope of the invention that the polypeptide constructs of the invention bind to said antigen in its non-associated state, bind to said antigen in its associated state, or bind to both. In all these cases, the polypeptide constructs of the invention may bind to such multimers or associated protein complexes with an affinity and/or specificity that may be the same as or different from (i.e. higher than or lower than) the affinity and/or specificity with which the polypeptide constructs of the invention bind to said antigen in its monomeric and non-associated state. (all depending on the desired characteristics of the polypeptide construct of the invention). Methods and assays for screening for polypeptide constructs that bind one or more forms (monomeric or multimeric) of an antigen will be clear to the skilled person based on the common general knowledge and the available prior art.

[0252] In one aspect of the invention, the method described herein can be used to screen for and so provide a polypeptide construct that is directed against a heterodimeric protein, polypeptide, ligand or receptor.

[0253] In this aspect of the invention, the polypeptide construct that is screened for and so obtained will at least comprise at least a first (single) domain antibody and/or Nanobody that is directed against a first subunit of said heterodimeric protein, polypeptide, ligand or receptor, and at least a second (single) domain antibody and/or Nanobody that is directed against a second subunit of said heterodimeric protein, polypeptide, ligand or receptor, different from the first subunit.

[0254] Accordingly, the diversity, collection, library or set of polypeptide constructs that is used in the screening step (for example, the structural variants of the selected template) will be a collection of such polypeptide constructs in which each polypeptide construct comprises a first (single) domain antibody and/or Nanobody that is directed against a first subunit of said heterodimeric protein, polypeptide, ligand or receptor, and at least a second (single) domain antibody and/or Nanobody that is directed against a second subunit of said heterodimeric protein, polypeptide, ligand or receptor.

[0255] For example, it may be that the diversity, collection, library or set of polypeptide constructs comprises a set of polypeptide constructs that each share the same first (single) domain antibody and/or Nanobody (i.e. for binding to the first subunit), but in which the second (single) domain antibody (i.e. for binding to the second subunit) may differ between the different polypeptides that form the diversity, collection, library or set (or visa versa), for example in that these second (single) domain antibodies (each) have different amino acid sequences, are humanized variants of each other, are variants of each other that have been prepared and/or obtained through affinity maturation techniques (and/or as part of and/or for the purposes of affinity maturation of the original template sequence), and/or are single domain antibodies that bind to different epitopes on the second subunit; or any suitable combination thereof.

[0256] It may also be that the diversity, collection, library or set of polypeptide constructs differ in both the first and/or the second (single) domain antibody, in which both the first and/or the second (single) domain antibody, respectively, that are present in a specific polypeptide construct (i.e. that forms part of the diversity, collection, library or set that is used for screening) may again differ from the first and/or the second (single) domain antibodies, respectively, that are present in the other polypeptides from the diversity, collection, library or set in that they have different amino acid sequences, are humanized variants of each other, are variants of each other that have been prepared and/or obtained through affinity maturation techniques (and/or as part of and/or for the purposes of affinity maturation of the original template sequence), and/or are single domain antibodies that bind to different epitopes on the first and second subunit, respectively; or any suitable combination of the foregoing.

[0257] The heterodimeric protein, polypeptide, ligand or receptor may be any suitable, desired and/or intended heterodimeric protein, and may for example be a heterodimeric cytokine such as IL-12 (which consists of a p35 and a p40 subunit), IL-23 (which consists of a p19 and a p40 subunit), IL-27 (which consists of an EBI-3 and p28 subunit) or IL-35 (which consists of a p35 subunit and an EBI-3 subunit). The heterodimeric protein, polypeptide, ligand or receptor may also be a heterodimeric receptor, such as a heterodimeric receptor for a (heterodimeric) cytokine, such as the cognate receptors for IL-12, IL-23, IL-27 and/or IL-35. Further reference is made to the international application of Ablynx N.V. entitled "Amino acid sequences directed against heterodimeric cytokines and/or their receptors and polypeptides comprising the same", which has a filing date of Nov. 27, 2008. This application also contains some examples of multispecific polypeptide constructs that comprise at least one single domain antibody or Nanobody against a first subunit of a heterodimeric cytokine and at least one single domain antibody or Nanobody against a second subunit of a heterodimeric cytokine different from said first subunit (see for example FIG. **33** for p19/p40 constructs and FIG. **36** for p35/p40 constructs). It is envisaged that these and similar polypeptides may be used as templates for the methods described herein and/or that the methods described herein may be used to provide similar polypeptides (i.e. through screening of a suitable diversity, collection, library or set, as described herein).

[0258] It will also be clear that in this aspect of the invention, the diversity, collection, library or set may be screened against a group, variety, set or family of related heterodimeric proteins, polypeptides, ligands or receptors (for example, sharing a common subunit and/or belonging to the same family, such as a family that shares similar subunits), in order to screen for the polypeptides that have the optimal or desired specificity for one particular heterodimeric protein, polypeptide, ligand or receptor within said group, variety, set or family of related heterodimeric proteins, polypeptides, ligands or receptors. As a non-limiting example, a diversity, collection, library or set of polypeptides of "p19/p40 constructs", "p35/p40 constructs" or similar multispecific constructs that are directed against a heterodimeric cytokine or a cognate receptor for the same (as generally described in the international application of Ablynx N.V. entitled "Amino acid

sequences directed against heterodimeric cytokines and/or their receptors and polypeptides comprising the same") may be screened for specificity for one of IL-12, IL-23, IL-27 and/or IL-35 compared to one or more of the other heterodimeric cytokines belonging to this family (for example, p19/p40 constructs may be screened for specificity for IL-23 compared to IL-12, IL-27 and/or IL-35. Similarly, p35/p40 constructs may be screened for specificity for IL-12 compared to IL-23, IL-27 and/or IL-35). Such screening methods, and a diversity, collection, library or set that is designed for, (to be) used in and/or intended for use in such a screening method, form further aspects of this invention.

[0259] Also, as will be clear to the skilled person, polypeptide constructs that contain two or more single domain antibodies directed against said antigen may bind with higher avidity to said antigen than the corresponding monomeric amino acid sequence(s). For example, and without limitation, polypeptide constructs that contain two or more single domain antibodies directed against different epitopes of said antigen may (and usually will) bind with higher avidity than each of the individual single domain antibodies, and polypeptide constructs that contain two or more single domain antibodies directed against said antigen may (and usually will) bind also with higher avidity to a multimer of said antigen.

[0260] Generally, polypeptide constructs of the invention will at least bind to those forms of said antigen (including monomeric, multimeric and associated forms) that are the most relevant from a biological and/or therapeutic point of view, as will be clear to the skilled person.

[0261] The present invention relates to methods for obtaining polypeptide constructs comprising two or more single domain antibodies. In particular embodiments polypeptide constructs according to the invention may comprise at least two single domain antibodies which are selected from the group of domain antibodies (or an amino acid sequence that is suitable for use as a domain antibody), "dAbs" (or an amino acid sequence that is suitable for use as a dAb), Nanobodies®, V_{HH} sequences (as defined herein, and including but not limited to a V_{HH} sequence) and/or other single variable domains or combinations thereof. For instance, a polypeptide construct according to the present invention comprising at least two single domain antibodies may comprise V_H domains and/or V_L domains (both derived from conventional four-chain antibodies) and/or V_{HH} domains (derived from heavy chain antibodies). It is however preferred that in a polypeptide construct of the present invention comprising at least two single domain antibodies, said at least two single domain antibodies exclusively consist of only one type of domain antibodies (i.e. corresponding to either heavy or light chain domains). Most particularly it is envisaged that in the methods of the present invention polypeptide constructs are obtained wherein the at least two single domains exclusively consist of Nanobodies® or heavy chain domain antibodies (e.g. V_H or V_{HH}). Further particular embodiments of the invention involve methods wherein the polypeptide constructs obtained comprise at least two single domains, whereby the single domains of the construct consist exclusively of (humanized) V_{HH} domains or exclusively consist of heavy chain variable domains derived from heavy chain antibodies.

[0262] The polypeptide constructs of the invention can generally be prepared by a method which comprises at least the step of suitably linking the one or more binding units essentially consisting of single domain antibodies to each other and to the one or more other groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the polypeptide construct of the invention. Polypeptide constructs of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide construct of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide construct of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

[0263] It will also be clear to the skilled person that the method of the invention can equally be preformed at the nucleic acid level as well as at the amino acid level. Thus, the method of the invention can equally be preformed by screening a diversity, set, collection or library of nucleic acid sequences encoding a diversity, set, collection or library of single domain antibodies or encoding a diversity, set, collection or library of polypeptide construct as well as by screening a set, collection or library of single domain antibodies or a diversity, set, collection or library of polypeptide construct.

[0264] The process of selecting and/or preparing a polypeptide construct of the invention, starting from one or more binding units consisting essentially of single domain antibodies, is also referred to herein as "formatting" said single domain antibodies; and a single domain antibody that is made part of a polypeptide construct of the invention is said to be "formatted" or to be "in the format of" said polypeptide construct of the invention. Examples of ways in which a single domain antibody can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted single domain antibodies form a further aspect of the invention.

[0265] Generally, polypeptide constructs of the invention that comprise at least two single domain antibodies will be referred to herein as "multivalent" polypeptide constructs (as defined herein).

[0266] More specifically, a polypeptide constructs comprising at least two single domain antibodies that are directed against two or more different antigens are referred to herein as "multispecific" polypeptide constructs (as defined herein).

[0267] The methods of the present invention are directed at obtaining polypeptide constructs comprising two or more single domain antibodies. Such a single domain antibody may be a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody® (as defined herein, and including but not limited to a V_{HH} sequence), other single variable domains, or any suitable fragment of any one thereof.

[0268] For a general description of heavy chain antibodies and the variable domains thereof, reference is inter alia made to the prior art cited herein, to the review article by Muyldermans in Reviews in Molecular Biotechnology 74 (2001), 277-302; as well as to the following patent applications, which are mentioned as general background art: WO 94/04678, WO 95/04079 and WO 96/34103 of the Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193 of Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016

and WO 03/055527 of the Vlaams Instituut voor Biotechnologie (VIB); WO 03/050531 of Algonomics N.V. and Ablynx N.V.; WO 01/90190 by the National Research Council of Canada; WO 03/025020 (=EP 1 433 793) by the Institute of Antibodies; as well as WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858, WO 06/40153, WO 06/079372, WO 06/122786, WO 06/122787 and WO 06/122825, by Ablynx N.V. and the further published patent applications by Ablynx N.V. Reference is also made to the further prior art mentioned in these applications, and in particular to the list of references mentioned on pages 41-43 of the International application WO 06/040153, which list and references are incorporated herein by reference.

[0269] For a general description of (single) domain antibodies, reference is for example made to EP 0 368 684. For the term "dAb's", reference is for example made to Ward et al. (Nature 1989 Oct. 12; 341 (6242): 544-6), to Holt et al., Trends Biotechnol., 2003, 21(10:484-490; as well as to for example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd.

[0270] It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, (single) domain antibodies or single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

[0271] In particular, a (single) domain antibody may be a Nanobody® (as defined herein) or a suitable fragment thereof [Note: Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx N.V.]. For a general description of Nanobodies, reference is made to the further description below, as well as to the prior art cited herein (such as WO 06/040153, WO 06/122825, WO 06/122786, WO 07/042,289, WO 07/104,529, WO 08/020,079, WO 08/074,839, WO 08/071,447, WO 08/074,840, WO 08/074,867, WO 08/077,945, WO 08/101,985 by Ablynx N.V. Further reference is made to the international application of Ablynx N.V. entitled "Amino acid sequences directed against HER2 and polypeptides comprising the same for the treatment of cancers and/or tumors", which has a filing date of Nov. 27, 2008. In this respect, it should however be noted that this description and the prior art mainly described. Nanobodies of the so-called "V_H3 class" (i.e. Nanobodies with a high degree of sequence homology to human germline sequences of the V_H3 class such as DP-47, DP-51 or DP-29), which Nanobodies form a preferred aspect of this invention. It should however be noted that the invention in its broadest sense generally covers any type of Nanobody, and for example also covers the Nanobodies belonging to the so-called "V_H4 class" (i.e. Nanobodies with a high degree of sequence homology to human germline sequences of the V_H4 class such as DP-78), as for example described in WO 07/118,670.

[0272] According to particular embodiment, in the methods of the invention polypeptide constructs are obtained comprising two or more Nanobodies or V_{HH} sequences. Generally, Nanobodies (in particular V_{HH} sequences and partially humanized Nanobodies) can in particular be characterized by the presence of one or more "Hallmark residues" (as described herein) in one or more of the framework sequences (again as further described herein).

[0273] Thus, generally, a Nanobody can be defined as an amino acid sequence with the (general) structure

[0274] FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the Hallmark residues are as further defined herein.

[0275] In particular, a Nanobody can be an amino acid sequence with the (general) structure

[0276] FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which the framework sequences are as further defined herein.

[0277] The amino acid residues of a Nanobody are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun. 23; 240 (1-2): 185-195 (see for example FIG. **2** of this publication); or referred to herein. According to this numbering, FR1 of a Nanobody comprises the amino acid residues at positions 1-30, CDR1 of a Nanobody comprises the amino acid residues at positions 31-35, FR2 of a Nanobody comprises the amino acids at positions 36-49, CDR2 of a Nanobody comprises the amino acid residues at positions 50-65, FR3 of a Nanobody comprises the amino acid residues at positions 66-94, CDR3 of a Nanobody comprises the amino acid residues at positions 95-102, and FR4 of a Nanobody comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_{HH} domains—the total number of amino acid residues in each of the CDR's may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. Generally, however, it can be said that, according to the numbering of Kabat and irrespective of the number of amino acid residues in the CDR's, position 1 according to the Kabat numbering corresponds to the start of FR1 and vice versa, position 36 according to the Kabat numbering corresponds to the start of FR2 and vice versa, position 66 according to the Kabat numbering corresponds to the start of FR3 and vice versa, and position 103 according to the Kabat numbering corresponds to the start of FR4 and vice versa.

[0278] Alternative methods for numbering the amino acid residues of V_H domains, which methods can also be applied in an analogous manner to V_{HH} domains from Camelids and to Nanobodies, are the method described by Chothia et al. (Nature 342, 877-883 (1989)), the so-called "AbM definition" and the so-called "contact definition". However, in the present description, claims and figures, the numbering according to Kabat as applied to V_{HH} domains by Riechmann and Muyldermans will be followed, unless indicated otherwise.

[0279] More in particular, a Nanobody can be an amino acid sequence with the (general) structure

[0280] FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-1 below;

[0281] Nanobodies may be derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (i.e. from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences, including but not limited to "humanized" (as defined herein) Nanobodies, "camelized" (as defined herein) immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences), as well as Nanobodies that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described herein.

[0282] Also, when a Nanobody comprises a V_{HH} sequence, said Nanobody may be suitably humanized so as to provide one or more further (partially or fully) humanized Nanobodies of the invention. Similarly, when a Nanobody comprises a synthetic or semi-synthetic sequence (such as a partially humanized sequence), said Nanobody may optionally be further suitably humanized, e.g. by the methods of the present invention, again so as to provide one or more further (partially or fully) humanized Nanobodies of the invention.

[0283] Nanobodies of the invention can generally be obtained by any of the techniques (1) to (8) mentioned on pages 61 and 62 of WO 08/020,079, or any other suitable technique known per se. One preferred class of Nanobodies corresponds to the V_{HH} domains of naturally occurring heavy chain antibodies. Such V_{HH} sequences can generally be generated or obtained by suitably immunizing a species of Camelid with a particular antigen or target (i.e. so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), by obtaining a suitable biological sample from said Camelid (such as a blood sample, serum sample or sample of B-cells), and by generating V_{HH} sequences directed against said antigen or target, starting from said sample, using any suitable technique known per se. Such techniques will be clear to the skilled person and/or are described herein.

[0284] The total number of amino acid residues in a Nanobody can be in the region of 110-120., is preferably 112-115, and is most preferably 113. It should however be noted that parts, fragments, analogs or derivatives (as further described herein) of a Nanobody are not particularly limited as to their length and/or size, as long as such parts, fragments, analogs or derivatives meet the further requirements outlined herein and are also preferably suitable for the purposes described herein.

TABLE A-1

Hallmark Residues in Nanobodies

Position	Human V_H3	Hallmark Residues
11	L, V; predominantly L	L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L
37	V, I, F; usually V	$F^{(1)}$, Y, V, L, A, H, S, I, W, C, N, G, D, T, P, preferably $F^{(1)}$ or Y
44[8]	G	$E^{(3)}$, $Q^{(3)}$, $G^{(2)}$, D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably $G^{(2)}$, $E^{(3)}$ or $Q^{(3)}$; most preferably $G^{(2)}$ or $Q^{(3)}$.
45[8]	L	$L^{(2)}$, $R^{(3)}$, P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably $L^{(2)}$ or $R^{(3)}$
47[8]	W, Y	$F^{(1)}$, $L^{(1)}$ or $W^{(2)}$ G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D: preferably $W^{(2)}$, $L^{(1)}$ or $F^{(1)}$
83	R or K; usually R	R, $K^{(5)}$, T, $E^{(5)}$, Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K
84	A, T, D; predominantly A	$P^{(5)}$, S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P
103	W	$W^{(4)}$, $R^{(6)}$, G, S, K, A, M, Y, L, F, T, N, V, Q, $P^{(6)}$, E, C; preferably W
104	G	G, A, S, T, D, P, N, E, C, L; preferably G
108	L, M or T; predominantly L	Q, $L^{(7)}$, R, P, E, K, S, T, M, A, H; preferably Q or $L^{(7)}$

Notes:

[1]In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.

[2]Usually as GLEW at positions 44-47.

[3]Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF, KEREG, KQREW or KQREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), TQRE (for example TQREL), KECE (for example KECEL or KECER), KQCE (for example KQCEL), RERE (for example REREG), RQRE (for example RQREL, RQREF or RQREW), QERE (for example QEREG), QQRE, (for example QQREW, QQREL or QQREF), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.

[4]With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.

[5]Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.

[6]In particular, but not exclusively, in combination with GLEW at positions 44-47.

[7]With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.

[8]The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

[0285] A further detailed description of single domain antibodies, "dAbs", and more in particular Nanobodies®, V_{HH} sequences and/or other single variable domains or combinations thereof can be found on pages 15 to 36 and pages 59 to 105 of WO 08/020,079, which are incorporated by reference herein.

[0286] According to a further aspect, the present invention provides for a diversity of polypeptide constructs which are structural variants of a template polypeptide construct.

[0287] Also, one or more or all of the polypeptide construct sequences in the above diversity (such as set, collection or library) of structural variants may be obtained or defined by rational, or semi-empirical approaches such as computer modelling techniques or biostatics or datamining techniques.

[0288] Furthermore, such a diversity, set, collection or library of polypeptide constructs can comprise one, two or more sequences that are variants from one another (e.g. with designed point mutations or with randomized positions), compromise multiple sequences derived from a diverse set of naturally diversified sequences (e.g. an immune library), or any other source of diverse. Such a diversity, set, collection or library of sequences can be displayed on the surface of a phage particle, a ribosome, a bacterium, a yeast cell, a mammalian cell, and linked to the nucleotide sequence encoding the amino acid sequence within these carriers. This makes such a diversity amenable to selection procedures to isolate the desired polypeptide construct in methods according to the

invention. More generally, when a sequence is displayed on a suitable host or host cell, it is also possible (and customary) to first isolate from said host or host cell a nucleotide sequence that encodes the desired sequence, and then to obtain the desired sequence by suitably expressing said nucleotide sequence in a suitable host organism. Again, this can be performed in any suitable manner known per se, as will be clear to the skilled person.

[0289] In particular embodiments, the diversity of polypeptide constructs is a set, collection or library of polypeptide constructs, which may be any suitable set, collection or library of polypeptide constructs. For example, the set, collection or library of polypeptide constructs may be a set, collection or library of immunoglobulin sequences (as described herein), such as a naïve or immune set, collection or library of immunoglobulin sequences; a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

[0290] Also, diversity of polypeptide constructs may be a set, collection or library of polypeptide constructs comprising at least two single domain antibodies that are exclusively heavy chain variable domains of heavy chain antibodies (V_{HH} domains).

[0291] In a particular embodiment, the diversity, set, collection or library of polypeptide constructs may be an immune set, collection or library of polypeptide constructs, for example derived from a mammal that has been suitably immunized with a suitable antigen or antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

[0292] In the above methods, the diversity (such as set, collection or library) of polypeptide constructs may be displayed on a phage, phagemid, ribosome or suitable microorganism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) polypeptide constructs will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

[0293] According to yet a further aspect of the invention, polypeptide constructs are provided obtainable by the methods of the present invention as described herein. The present invention also relates a polypeptide construct obtained by the method of the present invention. For a detailed description of such polypeptides of the invention, reference is made to the detailed description of the method of the invention for obtaining such polypeptide constructs.

[0294] In another aspect, the invention relates to nucleic acids that encode the polypeptide constructs obtainable by the methods of the present invention and to vectors comprising such nucleic acids sequences. Such a nucleic acid sequence may for example be in the form of a genetic construct. The invention also relates to nucleic acids that encode the polypeptide constructs obtained by the methods of the present invention and to vectors comprising such nucleic acids sequences. Such a nucleic acid sequence may for example be in the form of a genetic construct.

[0295] In another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) a polypeptide construct obtainable by the methods of the present invention; and/or that contains a nucleic acid encoding said polypeptide construct. The invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) a polypeptide construct obtained by the methods of the present invention; and/or that contains a nucleic acid encoding said polypeptide construct.

[0296] The invention further relates to a product or composition containing or comprising at least one polypeptide construct of the invention (or a suitable fragment thereof) and/or at least one nucleic acid encoding said at least one polypeptide construct of the invention, and optionally one or more further components of such compositions known per se, i.e. depending on the intended use of the composition. Such a product or composition may for example be a pharmaceutical composition (as described herein), a veterinary composition or a product or composition for diagnostic use (as also described herein).

[0297] Generally, for pharmaceutical use, the polypeptide constructs of the invention may be formulated as a pharmaceutical preparation or compositions comprising at least one polypeptide construct of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc. Such suitable administration forms—which may be solid, semi-solid or liquid, depending on the manner of administration—as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein.

[0298] Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one polypeptide construct of the invention and at least one suitable carrier, diluent or excipient (i.e. suitable for pharmaceutical use), and optionally one or more further active substances.

[0299] Generally, the polypeptide constructs of the invention can be formulated and administered in any suitable manner known per se, for which reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865, WO 04/041867 and WO 08/020,079) as well as to the standard handbooks, 18th Ed., Mack Publishing Company, USA (1990), Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005); or the Handbook of Therapeutic Antibodies (S. Dubel, Ed.), Wiley, Weinheim, 2007 (see for example pages 252-255).

[0300] For example, the polypeptide constructs of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled person, and for example include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcuta-

neous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (i.e. transdermal or intradermal) administration.

[0301] Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, those mentioned on page 143 of WO 08/020,079. Usually, aqueous solutions or suspensions will be preferred.

[0302] The polypeptide constructs of the invention can also be administered using gene therapy methods of delivery (see, e.g. U.S. Pat. No. 5,399,346, which is hereby incorporated by reference in its entirety). Using a gene therapy method of delivery, primary cells transfected with the gene encoding a polypeptide construct of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

[0303] Thus, the polypeptide constructs of the invention may be systemically administered, e.g. orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the polypeptide constructs of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the polypeptide construct of the invention. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the polypeptide construct of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[0304] The tablets, troches, pills, capsules, and the like may also contain binders, excipients, disintegrating agents, lubricants and sweetening or flavouring agents, for example those mentioned on pages 143-144 of WO 08/020,079. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the polypeptide constructs of the invention, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the polypeptide constructs of the invention may be incorporated into sustained-release preparations and devices.

[0305] Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

[0306] The polypeptide constructs of the invention may also be administered intravenously or intraperitoneally by infusion or injection, as further described on pages 144 and 145 of WO 08/020,079.

[0307] For topical administration, the polypeptide constructs of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid, as further described on page 145 of WO 08/020,079.

[0308] Generally, the concentration of the polypeptide constructs of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

[0309] The amount of the polypeptide constructs of the invention required for use in treatment will vary not only with the particular polypeptide construct selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the polypeptide constructs of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

[0310] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

[0311] An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E. W., ed. 4), Mack Publishing Co. Easton, Pa. The dosage can also be adjusted by the individual physician in the event of any complication.

[0312] In another aspect, the invention relates to a method for the prevention and/or treatment of at least one disease and/or disorder, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide construct of the invention and/or of a pharmaceutical composition comprising the same.

[0313] In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or

reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

[0314] The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

[0315] The invention relates to a method for the prevention and/or treatment of at least one disease or disorder that is associated with the one or more antigens or epitopes against which a polypeptide construct according to the invention is directed, with its biological or pharmacological activity, and/or with the biological pathways or signalling in which said one or more antigens or epitopes are involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide construct of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating the one or more antigens or epitopes against which a polypeptide construct according to the invention is directed, its biological or pharmacological activity, and/or the biological pathways or signalling in which said one or more antigens or epitopes are involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide construct of the invention, and/or of a pharmaceutical composition comprising the same. In particular, said pharmaceutically effective amount may be an amount that is sufficient to modulate the one or more antigens or epitopes against which a polypeptide construct according to the invention is directed, its biological or pharmacological activity, and/or the biological pathways or signalling in which said one or more antigens or epitopes are involved; and/or an amount that provides a level of the polypeptide constructs of the invention in the circulation that is sufficient to modulate the one or more antigens or epitopes against which a polypeptide construct according to the invention is directed, their biological or pharmacological activity, and/or the biological pathways or signalling in which said one or more antigens are involved.

[0316] The invention furthermore relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering the polypeptide construct of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide construct of the invention, and/or of a pharmaceutical composition comprising the same.

[0317] More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide construct of the invention, and/or of a pharmaceutical composition comprising the same.

[0318] In another aspect, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of a polypeptide construct of the invention and/or of a pharmaceutical composition comprising the same.

[0319] In the above methods, the polypeptide constructs of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the polypeptide constructs of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

[0320] The polypeptide constructs of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific polypeptide construct of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

[0321] Generally, the treatment regimen will comprise the administration of one or more polypeptide constructs of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amounts) or doses to administered can be determined by the clinician, again based on the factors cited above.

[0322] Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the specific polypeptide construct of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the polypeptide constructs of the invention will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

[0323] Usually, in the above method, a single polypeptide construct of the invention will be used. It is however within the scope of the invention to use two or more polypeptide constructs of the invention in combination.

[0324] The polypeptide constructs of the invention may also be used in combination with one or more further pharmaceutically active compounds or principles, i.e. as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

[0325] In particular, the polypeptide constructs of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

[0326] When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

[0327] Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

[0328] The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

[0329] Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

[0330] In another aspect, the invention relates to the use of a polypeptide construct of the invention in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one disease and/or disorder; and/or for use in one or more of the methods of treatment mentioned herein.

[0331] The subject to be treated may be any warm-blooded animal, but is in particular a mammal and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

[0332] The invention also relates to the use of a polypeptide construct of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering a polypeptide construct of the invention to a patient.

[0333] More in particular, the invention relates to the use of a polypeptide construct of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of diseases and/or disorders, and in particular for the prevention and treatment of one or more of the diseases and disorders listed herein.

[0334] Again, in such a pharmaceutical composition, the one or more polypeptide constructs of the invention may also be suitably combined with one or more other active principles, such as those mentioned herein.

[0335] The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures:

FIGURE LEGENDS

[0336] FIG. **1**: Anti-HER2 humoral immune response induced after immunisation of Llama glama with HER2-overexpressing SKBR3 cells. The reactivity of pre-immune (day 0) and immune sera (day 42 and day of PBL1 take) of animals 121 and 122 immunized with whole cells was determined by ELISA using rhErbB2-Fc as antigen according to particular embodiments of the invention (e.g. see Example 3.2). A: total IgG response; B: IgG1 isotype response; C: IgG2 isotype response; D: IgG3 isotype response.

[0337] FIG. **2**: HER2-specific ELISA analysis of periplasmic preparations containing myc-tagged Nanobody protein fragments from selected clones, according to particular embodiments of the invention. Periplasmic preparations of soluble Nanobody protein fragments were added to wells of an ELISA plate, which had been coated with rhErbB2/Fc antigen and had been additionally blocked with PBS+1% casein. Detection was performed by a monoclonal anti-myc antibody followed by an alkaline phosphatase-conjugated polyclonal goat anti-mouse antibody. The ELISA was developed by a PNPP-substrate as described in Example 6. The OD-values (Y-axis) were measured at 405 nm by an ELISA-reader. Each bar represents an individual periplasmic extract.

[0338] FIG. **3**: Flow cytometric analysis of selected clones (2A1, 2A4, 2C3, 2C5, 2D3 and 2G4) according to particular embodiments of the invention. Nanobody-containing periplasmic extracts were added to ErbB2 overexpressing SKBR3 cells. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody. Nanobodies binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS+10% FBS) followed by monoclonal anti-myc antibody and PE-labeled

polyclonal anti-mouse antibody. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis.

[0339] FIG. **4**: Herceptin® competitive ELISA according to particular embodiments of the invention. An ELISA plate was coated with SKBR3 vesicles (5 μg/ml) and additionally blocked with PBS+1% casein. 2 nM Herceptin® was added to the wells, after which periplasmic preparations of soluble Nanobody protein fragments were added. Detection of Herceptin® binding to SKBR3 vesicles was performed by an alkaline phosphatase-conjugated AffiniPure Goat Anti-Human IgG, Fc Fragment Specific (Jackson ImmunoResearch Labs, Suffolk, UK). The ELISA was developed by a PNPP-substrate as described in Example 6. The OD-values (Y-axis) were measured at 405 nm by an ELISA-reader. Each bar represents an individual periplasmic extract. The OD value corresponding to the maximal signal represents the OD value measured for binding of Herceptin® without addition of periplasmic extract containing HER-binding Nanobody. The minimal signal represents the background staining of non-coated wells incubated only with alkaline phosphatase-conjugated AffiniPure Goat Anti-Human IgG, followed by detection using a PNPP-substrate. Controls 1-5 represent individual periplasmic extracts containing non-HER2 binding Nanobodies.

[0340] FIG. **5**: Herceptin®-competitive FMAT according to particular embodiments of the invention. Dilutions of periplasmic extracts containing HER2 binding Nanobodies were tested for their ability to block the binding of Herceptin® to HER2-overexpressing SKBR3 cells as described in Example 8. (−) represents the signal obtained for binding of Alexa647-labeled Herceptin® without addition of periplasmic extract. Addition of periplasmic extract containing a non-HER2 binding Nanobody (irr) had no influence on the binding of Herceptin® to SKBR3 cells. Periplasmic extracts 2A4, 2A5, 2A6, 2B1, 2B2, 2B4, 2B5, 2C1, 2C3, 2D2 and 2D3 blocked binding of Herceptin® to HER2 with more than 80%.

[0341] FIG. **6**: Herceptin®-competitive FMAT analysis according to particular embodiments of the invention. Nanobodies compete with binding of Herceptin® to SKBR3 cells in a dose-dependent manner as described in Example 8.

[0342] FIG. **7**: Omnitarg-Fab competitive FMAT according to particular embodiments of the invention. Dilutions of periplasmic extracts containing HER2 binding Nanobodies were tested for their ability to block the binding of Omnitarg-Fab (OT-Fab) to HER2-overexpressing SKBR3 cells as described in Example 9. (cells) represents the signal obtained for binding of biotinylated OT-Fab without addition of periplasmic extract. Addition of periplasmic extract containing a non-HER2 binding Nanobody (irr) had no influence on the binding of OT-Fab to SKBR3 cells. Periplasmic extracts 47A8, 47A11, 47B1, 47B12, 47D1, 47D4, 47D5, 47E7, 47F5 and 47G7 blocked binding of OT-Fab to HER2 with more than 85%.

[0343] FIG. **8**: Growth inhibitory effect of monovalent HER2 binding Nanobodies on ErbB2-overexpressing SKBR3 cells according to particular embodiments of the invention. SKBR3 cells were seeded in 96 well plates and allowed to adhere as explained in Example 11. HER2-binding Nanobodies 5F7, 2A5, 2A4, 2D3 and 2C3, non-HER2 binding irrelevant Nanobody 12B2 or medium alone were added and the cells were incubated for 3 days. During the last 24 h, cells were pulsed with 1 μCi [^{3}H]-thymidine. Incorporation of [^{3}H]-thymidine was measured as described in Example 11.

[0344] FIG. **9**: Herceptin®-competitive FMAT. Dilutions of monovalent, bivalent and bispecific Nanobodies were tested for their ability to block the binding of Herceptin® to HER2-overexpressing SKBR3 cells according to particular embodiments of the invention (e.g. as described in Example 12). Bispecific Nanobodies 2A4-9GS-ALB1 and 2A5-9GS-ALB1 blocked the binding of Herceptin® to HER2-expressing SKBR3 cells to the same extent as the monovalent 2A4 and 2A5 Nanobodies respectively. Bivalent 2A4-9GS-2A4 and 2A5-9GS-2A5 Nanobodies blocked the binding of Herceptin® to HER2-expressing SKBR3 cells to a greater extent than their monovalent format. A: 2A4 derivatives; B: 2A5 derivatives.

[0345] FIG. **10**: Design of biparatopic Nanobody expression vector according to particular embodiments of the invention (e.g. as described in Example 13.1).

[0346] FIG. **11**: SKBR3 cell proliferation assay with biparatopic Nanobodies purified from periplasmic extracts derived from plate 27 by PhyTip200$^+$ according to particular embodiments of the invention. Biparatopic Nanobodies 27A2-35GS-2D3, 27A5-35GS-2D3, 27B3-35GS-2D3, 27B5-35GS-2D3, 27C4-35GS-2D3, 27D3-35GS-2D3 and 27D6-35GS-2D3 block SKBR3 cell proliferation to a greater extent than 50 nM Herceptin®. Biparatopie Nanobodies 27A7-35GS-2D3, 27A9-35GS-2D3, 27A1'-35GS-2D3, 27A12-350S-2D3, 27B11-35GS-2D3, 27C11-350S-2D3 and 27D7-35GS-2D3 display an agonistic effect.

[0347] FIG. **12**: Sensorgram of monovalent 2D3, bivalent 2D3-35GS-2D3 and dummy-2D3 biparatopic Nanobodies according to particular embodiments of the invention.

[0348] FIG. **13**: Sensorgram of monovalent 2D3 and biparatopic Nanobodies 27B7-35GS-2D3, 27C3-35GS-2D3 and 27H5-35GS-2D3 according to particular embodiments of the invention.

[0349] FIG. **14**: Sensorgram of monovalent 2D3, bivalent 2D3-35GS-2D3 and biparatopic Nanobodies 27A3-35GS-2D3, 27E7-35GS-2D3 and 27D1-35GS-2D3 according to particular embodiments of the invention.

[0350] FIG. **15**: Herceptin®-competitive FMAT. Dilutions of monovalent 2D3, bivalent 2D3-35GS-2D3 and biparatopic Nanobodies combining the Herceptin®-competitive 2D3 and a HER2-binding or dummy Nanobody were tested for their ability to block the binding of Herceptin® to HER2-overexpressing SKBR3 cells according to particular embodiments of the invention (e.g. as described in Example 14.2). A: Bivalent 2D3-35GS-2D3 and biparatopic Nanobodies 27H3-35GS-2D3 and 27D1-35GS-2D3 block binding of Herceptin® to HER2 expressed on SKBR3 cells more efficiently than monovalent 2D3 Nanobody. B: Nanobodies 27A3, 27A5 and 30D10 have no influence on the Herceptin®-competitive behavior of Nanobody 2D3 when fused to its N-terminal end, spaced by a 35GS linker. C: Nanobodies 27B7, 27C3, 27H5 and the dummy Nanobody have an inhibitory effect on the Herceptin®-competitive potential of 2D3 when fused to its N-terminal end, spaced by a 35GS linker.

[0351] FIG. **16**: Omnitarg-Fab competitive FMAT according to particular embodiments of the invention. Dilutions of OT-Fab, monovalent 2D3 and biparatopic Nanobodies 27C3-35GS-2D3, 27A5-35GS-2D3, 27H3-35GS-2D3 and dummy-35GS-2D3 were tested for their ability to block the binding of OT-Fab to HER2-overexpressing SKBR3 cells as described in Example 14.3. None of the bipartope Nanobodies, nor monovalent 2D3 blocked the binding of OT-Fab to

HER2 expressed on SKBR3 cells. OT-Fab blocked binding of biotinylated OT-Fab in a dose-dependent manner.

[0352] FIG. **17**: Effect of biparatopic Nanobodies on SKBR3 tumor cell proliferation according to particular embodiments of the invention. Biparatopic Nanobodies 27A5-35GS-2D3, 27A3-35GS-2D3 and 30D10-35GS-2D3 significantly block proliferation of SKBR3 tumor cells and to a greater extent than the monovalent 2D3 and dummy-2D3 biparatopic Nanobody.

[0353] FIG. **18**: Effect of biparatopic Nanobodies on AKT signaling in SKBR3 cells according to particular embodiments of the invention (e.g. see Example 16). Biparatopic 27A3-35GS-2D3 and 27A5-35GS-2D3, Herceptin®, but not dummy-2D3 biparatopic or monovalent 2D3 Nanobody inhibits AKT phosphorylation in whole SKBR3 cell lysates.

[0354] FIG. **19**: Sensorgram of HER2-ECD binding to 2D3, 47D5 or the biparatopic Nanobody 2D3-35GS-47D5 according to particular embodiments of the invention.

[0355] FIG. **20**: Effect of biparatopic Nanobodies combining Herceptin®-competitive and Omnitarg competitive Nanobodies, monovalent Nanobodies 2D3, 5F7 and 47D5, Omnitarg-Fab and Herceptin® on HRG-mediated activation of mitogen-activated protein kinase (MAPK) according to particular embodiments of the invention.

[0356] FIG. **21**: Effect of biparatopic Nanobodies combining Herceptin®-competitive and Omnitarg competitive Nanobodies, monovalent Nanobodies 2D3, 5F7 and 47D5, Omnitarg-Fab and Herceptin® on HRG-mediated activation of Akt signaling according to particular embodiments of the invention.

[0357] FIGS. **22**A and **22**B: Model of NB-2D3 (blue) linked to another Nanobody (cyan) docked on HER-2 (red) according to particular embodiments of the invention. The linker is shown in black. N denotes the N-terminus of NB-2D3; C is the C-terminus of Nb-2D3.

[0358] FIG. **23**: Energy penalty values for each residue in the linker +/−10 residues of each Nanobody connected to the linker in the biparatopic construct 5F7-35GS-47D5 with appropriate linker length according to particular embodiments of the invention. None of the residues of the linker or at the connection points of the linker with the Nanobodies (NB-1 and NB-2) have a high energy penalty value.

[0359] FIG. **24**: Energy penalty values for each residue in the linker +/−10 residues of each Nanobody connected to the linker in the biparatopic construct 47D5-35GS-5F7 with unappropriate linker length according to particular embodiments of the invention. High energy penalty values are observed at the C-terminal connection of the linker with the N-terminal end of the second Nanobody (NB-2).

[0360] FIG. **25**: Energy penalty values for each residue in the linker +/−10 residues of each Nanobody connected to the linker in a biparatopic construct with the same Nanobodies as in FIG. **23** but with a longer linker length (47D5-40GS-5F7) according to particular embodiments of the invention. We see that the high energy penalty values at the connection of the C-terminal end of the linker with the N-terminal end of NB-2 are reduced suggesting a more appropriate linker length. The energy penalty values at both ends of the linker are still higher than those observed in FIG. **22**, indicating a still not optimal linker.

[0361] FIG. **26**A: Backbone RMSD ($Å^2$) between the 5F7-linker-47D5 constructs (built by homology modelling) with the individual Nanobodies 5F7 and 47D5 in their unlinked binding mode according to particular embodiments of the invention. The linker length varies from 5 to 35.

[0362] FIG. **26**B: Ribbon view of the 5F7-linker-47D5 biparatopic construct for 2 linker lengths according to particular embodiments of the invention. The binding mode of the individual Nanobodies is shown in blue; the biparatopic constructs are in red. The HER-2 target is omitted for clarity. On the left side: a 35GS linker is used between the 2 Nanobodies and a very limited deviation from the individual binding modes is observed. On the right side: a 5GS linker is used and it can clearly been observed that both Nanobodies in the biparatopic construct significantly deviate from their optimal binding mode.

[0363] FIGS. **27**A-K: Figures illustrating some of the preferred aspects and some of the advantages of the present invention, including the multiparatopic polypeptides of the invention.

EXAMPLES

Example 1

Procurement of the Extracellular Domain of HER2 for use as Selection Antigen in Phage Display

1.1 Cloning of Extracellular HER2 Domain

[0364] cDNA was isolated from SKBR3 breast cancer cells. The isolation of total RNA and cDNA synthesis was done according to standard protocols (Sambrook, Molecular cloning: Laboratory manual, 2^{nd} edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The coding sequence of the extracellular domain of the HER2 antigen was amplified by PCR using primer For-ErbB2 ECD; GCGAGCACCCAAGTGTGCACC (SEQ ID NO: 2267) and primer Rev-ErbB2 ECD: CTGCTCGGCGGGGCAGC-CCTT (SEQ ID NO: 2268). The PCR construct was then cloned into the pCR4-TOPO cloning vector (Invitrogen, Paisley, UK). Clone 4 having the correct sequence was then amplified by PCR using primers (For-pST ErbB2 ECD: GGCGCGCCGACTACAAAGACGATGACGA-CAAGAGCACCCAAGTGTGCACC (SEQ ID NO: 2269) and Rev-pST ErbB2 ECD: CGGCTCGAGCTATTAAT-GAGAATGGTGATGGTGCTCGGCGGGGCAGCCCTT (SEQ ID NO: 2270)) that were designed to introduce restriction sites at the beginning and the end of the fragment encoding the HER2-ECD. The PCR product was then cloned via AscI and XhoI into the plasmid pSecTag-HygroA (Invitrogen, Paisley, UK). As such, the coding sequence of the HER2-ECD was fused in frame with the Ig-κ chain leader sequence at its N-terminal end followed by a Flag tag and a polyhistidine tag at the C-terminus. The sequence of different clones was determined by sequencing according to standard protocols.

1.2 Expression of the Extracellular Domain of HER2 in HEK 293 Cells, Purification of the Recombinant Protein

[0365] Expression of the extracellular domain of HER2 was performed in HEK293T cells. HEK293T cells were seeded at 2×10^6 cells in 20 ml Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS in T75 tissue culture flasks and allowed to adhere overnight. The next day, culture supernatant was removed and the cells were transiently transfected with purified pSecTag-HygroA plasmid DNA using Fugene-HD (Roche, Basel, Switzerland) as transfection agent. Cells were grown for an additional 72 h in DMEM

containing 0.1% FBS, after which the culture supernatant was collected and filter-sterilized on a 0.22 μm filter (Millipore). The construct was then further purified out of the culture supernatant by immobilized metal affinity chromatography (IMAC) and size exclusion chromotagraphy (SEC).

[0366] Detection of the recombinant protein was performed by ELISA. Maxisorp 96-well plate (Num, Wiesbaden, Germany) was coated with an anti-flag monoclonal antibody (Sigma Aldrich, Bornem, Belgium). Unspecific binding was blocked with 2% milk powder in PBS for 2 hours. All prior and subsequent washes were performed with PBS. Afterward, eluate fractions were incubated for 2 hours at room temperature, followed by incubation with Herceptin®. Detection of the recombinant HER2-ECD was performed with a horseradish peroxidase conjugated anti-IgG antibody (Jackson Immunoresearch Laboratories, Suffolk, UK). Development of the ELISA was performed with TMB substrate (Pierce, Rockford, Ill.) according to the specifications of the manufacturer

Example 2

Procurement of Omnitarg-Fab for use as Competitive Agent in Phage Display and Screening Assays

2.1 Cloning of Omnitarg-Fab

[0367] Omnitarg-Fab was constructed by gene assembly. The amino acid sequence of variable light and variable heavy chain of Omnitarg was derived from patents WO 2006/044908 and WO 2004/048525. The sequence was backtranslated and codon optimized using Leto 1.0 Gene optimization software (www.entechelon.com). Oligonucleotide primers for assembly of the variable light chain (V_L), variable heavy chain (V_H), constant light chain (C_L) and constant domain 1 of the heavy chain (CH_1) of the Omnitarg-Fab were designed (Tables C-5 and C-6) and assembly PCR performed. The introduced restriction sites SfiI and BsiWI for the V_L. KpnI and BstEII for the V_H, BsiWI and AscI for the C_L, and BstEII and NotI for the CH_1 were utilized for sequential cloning into an in-house expression vector derived from pUC119 which contained the LacZ promoter, a resistance gene for ampicillin or carbenicillin, a multicloning site and the gen3 leader sequence. In frame with the Omnitarg-Fab coding sequence, the vector coded for a C-terminal c-myc tag and a (His)6 tag. Oligonucleotide sequences were designed to have a 15 nucleotide overlap with 5' and 3' overlapping oligonucleotides. Three consecutive PCR overlap extension rounds were performed using Expand High fidelity PCR system (Roche, Basel, Switzerland) to obtain V_L, V_H, C_L and CH_1 respectively. The obtained PCR fragments were cloned into the pCR4-TOPO cloning vector (Invitrogen, Paisley, UK). Plasmid DNA was prepared from clones having the correct sequence. The fragments were isolated from the pCR4-TOPO cloning vector via restriction with the appropriate enzymes and extraction of the fragments from agarose gel. The fragments were then consecutively cloned into the in-house expression vector.

2.2 Expression of the Omnitarg-Fab in *E. coli* Cells, Purification of the Recombinant Protein

[0368] The Omnitarg-Fab fragment was expressed in *E. coli* as His6-tagged protein and subsequently purified from the culture medium by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC).

[0369] Omnitarg-Fab was biotinylated using EZ-Link Sulpha-NHS-LC-Biotin labeling kit according to the manufacturer's instructions (Pierce, Rockford, Ill.). Removal of free biotin was performed on Zeba Desalt Spin columns according to the manufacturer's instructions (Pierce, Rockford, Ill.).

Example 3

Identification of HER2 Binding Nanobodies

3.1 Immunizations

[0370] After approval of the Ethical Committee of the Faculty of Veterinary Medicine (University Ghent, Belgium), 2 llamas (121, 122) were immunized, according to standard protocols, with 6 intramuscular injections at biweekly intervals of SKBR3 human tumor cells which are derived from a breast tumor and contain an amplified HER2 gene and overexpress HER2 p185 tyrosine kinase (SKBR3; ATCC HTB-30; LGC Promochem, Middlesex, UK). Each dose consisted of approximately 5×10^7 freshly harvested SKBR3 cells.

3.2 Evaluation of Induced Responses in Llama

[0371] At day 0, 42 and 81 (time of PBL collection), sera were collected to evaluate the induction of immune responses in the animals against HER2 by ELISA. In short, 2 μg/ml recombinant human ErbB2/Fc chimera (rhErb2-Fc; R&D Systems, Minneapolis, Minn.) were immobilized overnight at 4° C. in a 96 well Maxisorp plate (Num, Wiesbaden, Germany). Wells were blocked with a casein solution (1% in PBS). After addition of serum dilutions, specifically bound immunoglobulins were detected using a goat anti-llama horseradish peroxidase conjugate (Bethyl Lab. Inc., Montgomery, Tex.), showing that for all animals a significant antibody dependent immune response against HER2 was induced (FIG. **1**A). The antibody response was mounted both by the conventional and the heavy chain only antibody expressing B-cell repertoires since specifically bound immunoglobulins could be detected with antibodies specifically recognizing the conventional llama IgG1 antibodies (FIG. **1**B) or the heavy-chain only llama IgG2 (FIG. **1**C) and IgG3 (FIG. **1**D) antibodies.

3.3 Library Construction

[0372] When an appropriate immune response was induced in llama, four days after the last antigen injection, a 150 ml blood sample was collected and peripheral blood lymphocytes (PBLs) were purified by a density gradient centrifugation on Ficoll-Paque™ (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer's instructions. Next, total RNA was extracted from these cells and used as starting material for RT-PCR to amplify Nanobody encoding gene fragments. These fragments were cloned into a phagemid vector derived from pUC119 which contained the LacZ promoter, a coliphage pIII protein coding sequence, a resistance gene for ampicillin or carbenicillin, a multicloning site and the gen3 leader sequence. In frame with the Nanobody® coding sequence, the vector coded for a C-terminal c-myc tag and a (His)6 tag. Phage was prepared according to standard methods (see for example the prior art and applications filed by applicant cited herein) and stored after filter sterilization at 4° C. for further use.

3.4 Selections

[0373] Phage libraries obtained from llamas 121 and 122 were used for different selections.

[0374] In a first selection, ErbB2/Fc chimera (R&D Systems, Minneapolis, Minn., US) was coated onto Maxisorp

96-well plates (Nunc, Wiesbaden, Germany) at 20, 5 and 1 nM. Following incubation with the phage libraries and extensive washing, bound phage was specifically eluted with trypsin (1 mg/ml).

[0375] In a second selection. ErbB2/Fc chimera (R&D Systems, Minneapolis, US) was coated onto Maxisorp 96-well plates (Nunc, Wiesbaden, Germany) at 20 nM. Following incubation with the phage libraries and extensive washing, bound phage was specifically eluted with Herceptin® (Genentech, Roche).

[0376] In a third selection, soluble biotinylated ErbB2/Fc chimera was incubated with the phage libraries. After extensive washing, the biotinylated ErbB2/Fc was captured on a neutravidin coated solid phase. Bound phage was specifically eluted with trypsin (1 mg/ml).

[0377] In a fourth selection, soluble biotinylated ErbB2/Fc chimera was incubated with the phage libraries. After adding a 100-fold excess of non-labeled HER2, the biotinylated ErbB2/Fc was captured on a neutravidin coated solid phase. Bound phage was specifically eluted with trypsin (1 mg/ml).

[0378] In a fifth selection, phage libraries were incubated with Herceptin®-captured ErbB2/Fc. After extensive washing, bound phage was specifically eluted with trypsin (1 mg/ml)

[0379] In a sixth selection, soluble biotinylated ErbB2/Fc chimera was incubated with the phage libraries. After extensive washing, the biotinylated ErbB2/Fc was captured on a neutravidin coated solid phase. Bound phage was specifically eluted with Omnitarg-Fab.

[0380] In a seventh selection, phage libraries were incubated with Herceptin®-captured ErbB2/Fc. After extensive washing, bound phage was specifically eluted with Omnitarg-Fab.

[0381] In an eighth selection, phage libraries were incubated with biotinylated extracellular domain of HER2 captured on a neutravidin coated solid phase. After extensive washing, bound phage was specifically eluted with Omnitarg-Fab.

[0382] In a ninth selection, phage libraries were incubated with biotinylated extracellular domain of HER2 captured on a neutravidin coated solid phase. After extensive washing, bound phage was specifically eluted with Herceptin®.

[0383] In all selections, enrichment was observed. The output from each selection was recloned as a pool into an expression vector derived from pUC119 which contained the LacZ promoter, a resistance gene for ampicillin or carbenicillin, a multicloning site and the gen3 leader sequence. In frame with the Nanobody® coding sequence, the vector coded for a C-terminal c-myc tag and a (His)6 tag. Colonies were picked and grown in 96 deep-well plates (1 ml volume) and induced by adding IPTG for Nanobody expression. Periplasmic extracts (volume: ~80 μl) were prepared according to standard methods (see for example the prior art and applications filed by applicant cited herein).

Example 4

Detection and Isolation of HER2-Specific Heavy Chain Antibody Producing B-Cells

[0384] PBMC were isolated from peripheral blood samples from llamas immunized with HER2-Fc or SKBR3 human tumor cells using Ficoll density gradient centrifugation. These were then resuspended in cell culture medium and partially depleted from monocytes by adherence to the surface of plastic tissue culture T-flasks.

[0385] Next, non-adherent PBMC were collected from the flasks, washed with FACS buffer (PBS/10% FCS) at 4° C. and resuspended in the same ice-cold buffer. These were then stained using a combination of Alexa 488 labeled HER2-Fc (produced in-house, using Invitrogen (Paisley, UK) activated Alexa 488 and HER2-Fc recombinant protein from R&D Systems (Minneapolis, Minn.)), phycoerythrin labeled mouse-anti-llama IgG2 and -3 monoclonal antibodies (produced in-house, using purified phycoerythrin from Cyanotech, (Kailua-Kona, Hi.) crosslinked using the sulfo-SMCC heterobifunctional linker from Pierce-Endogen (Rochford, Ill.) to in-house produced and purified monoclonal antibodies originally described in Daley et al. (Clin. Diagn. Lab. Immunol. 2005, 12: 380)), Alexa 647 labeled mouse-anti-llama IgG1 monoclonal antibody (produced in-house), Alexa 647 labeled mouse-anti-llama monocyte and neutrophil antibody DH59B (purified antibody obtained from VMRD Inc. (Pullman, Wash.)) and dead cell specific dye TOPRO3 (Invitrogen, Paisley, UK). In some experiments, in-house Alexa 647 labeled recombinant human IgG1 Fc fragment (R&D Systems, Minneapolis, Minn.) was added to the stain combination as well.

[0386] Stained samples were washed thoroughly using cold FACS buffer and analyzed on a standard two-laser BD FACSAria cell sorter equipped with the ACDU microtiter plate single-cell deposition option (BD Biosciences, Franklin Lakes, N.J.). During acquisition and analysis, a gate was set on lymphocytes based on their forward/side scatter profile, which overlaps considerably with monocytes in llama. Doublet events were eliminated from acquisition and analysis by forward as well as side scatter pulse processing, eliminating all events which might be originating from more than one cell. Dead cells, monocytes and B-cells expressing conventional antibody on their cell membrane were removed from further analysis by gating out all remaining events having fluorescence over background (unstained PBMC) in the Alexa647/TOPRO3 channel. In some experiments, Alexa 647 labeled recombinant Fe fragment was used to stain the PBMC additionally. In these experiments, B-cells producing antibody binding Fc were also rejected from analysis and sorting by similar Alexa 647 channel exclusion, so as to avoid isolation of B-cells binding the Fe region of the fusion protein. In the phycoerythrin channel, B-cells displaying heavy chain antibody on their cell membrane could be clearly differentiated from any other remaining lymphocyte-type cells, and another gate was set on this population. Lastly, antigen-binding heavy chain IgG expressing B-cells cells were detected as a discrete high fluorescence intensity peak population in the Alexa 488 channel histogram distinct from the main population being no more fluorescent in this channel than when no Alexa 488 labeled antigen was added. Individual antigen binding B-cells were collected in separate wells of 96-well PCR plates in the ACDU, using DiVa software predefined stringent single-cell sorting criteria to avoid any double-cell droplet or adjacent-droplet double cell sorting. Typically, only 1-5% of heavy chain B-cells were found to bind antigen.

Example 5

Amplification and Cloning of HER2-Specific Heavy Chain Antibody Variable Regions

[0387] Individual B-cells expressing heavy chain antibodies binding HER2-Fc or the HER2 region of the fusion protein

specifically were sorted into 96-well plates containing 40 μl of RT-PCR buffer (Superscript III One-step RT-PCR kit, Invitrogen, Paisley, UK) per well, as described in Example 5, and stored at −80° C. For variable region gene sequence recovery, plates were thawed at room temperature and a mix of NP-40 (Roche Applied Sciences, Indianapolis, Ind.), gene specific 5' and 3' primers and RT-PCR enzyme mix were added to a total volume of 50 microliter per well by an automated liquid handler (Tecan, Mannedorf, Switzerland). After reverse transcription and first PCR amplification in a standard thermal cycler, a 2 microliter aliquot was removed from all wells and amplified in a nested PCR reaction using a proof-reading thermostable polymerase, or blend of polymerases containing at least one proof-reading enzyme. The 5' nested primer contains the nucleotide sequence required for directional TOPO cloning (Invitrogen, Paisley, UK). The 3' primer is designed to allow for the in-frame fusion of variable region gene framework 4 to vector encoded detection (c-myc) and purification (6His) peptide tags. Amplicons were detected from individual wells using ethidium bromide stained agarose gels and/or in microtiter plates via PicoGreen DNA binding fluorescent dye assay (Invitrogen, Paisley, UK). Typically, up to 60% of wells contained a single and sharply defined amplification product, whereas control wells in the same plate not having received any cells were completely devoid of amplification product.

[0388] The amplicons from nested PCR wells containing detectable product were then ligated into an *E. coli* expression vector in a homogenous ligation reaction, by mixing an aliquot of unpurified PCR mix with a topoisomerase-activated expression vector (in-house developed IPTG inducible *E. coli* Nanobody expression vector, adapted to allow directional TOPO cloning by Invitrogen's custom services department). The ligation mixture was then pipetted onto electrocompetent *E. coli* cells pre-aliquotted in a 96-well format electroporation chamber array (BTX Products of Harvard Apparatus, Hollisston, Mass.), and cells were transformed by electroporation using a BTX pulse generator.

[0389] Transformation mix was spread on selective agarose, multiple individual subcolonies picked and grown in 96-well deep well plates containing liquid selective medium by a QP Expression colony picker/rearrayer system (Genetix, New Milton, Hampshire, UK).

[0390] Periplasmic extracts (volume: ~80 μl) were prepared according to standard methods (see for example the prior art and applications filed by applicant cited herein).

Example 6

Anti-HER2Nanobodies Recognize Extracellular HER2 Domain

[0391] Periplasmic extracts of individual Nanobodies were screened for HER2 specificity by ELISA on solid phase coated ErbB2/Fc chimera (R&D Systems, Minneapolis, Minn.). Detection of Nanobody fragments bound to immobilized recombinant HER2 antigen was carried out using an in house made mouse anti-myc antibody (2 mg/ml) detected with alkaline phosphatase-conjugated anti-mouse IgG (Sigma Aldrich, Bornem. Belgium). The signal was developed by adding PNPP substrate solution and detected at a wavelength of 405 nm. FIG. **2** is illustrative of typical ELISA results, showing a high hit rate of positive clones.

[0392] Sequences of different HER2 binding clones are depicted in Tables B-1, B-2 and B-3. Alignment of the different HER2 binding clones based on CDR3 similarity is depicted in Table C-1.

Example 7

Anti-HER2Nanobodies Recognize Cell Surface Exposed Receptor Epitopes

[0393] To verify whether the Nanobodies are able to recognize cell surface expressed HER2, binding to breast cancer tumor cell line SKBR3 was assessed by flow cytometry.

[0394] Cell binding assays were carried out by initially incubating 200,000 cells with Nanobody-containing periplasmic preparation obtained in Examples 3 and 5 or relevant controls. After incubation, the cells were washed with FACS buffer. Cells were subsequently incubated successively with an in-house mouse anti-myc-tag monoclonal antibody and phycoerythrin labeled goat anti-mouse F(ab')2 fragments (Jackson ImmunoResearch, Suffolk, UK). To omit signals arising from dead cells, a TOPRO-3 (Invitrogen, Paisley, UK) staining was carried out. Cells were finally analyzed on a BD FACSArray Bioanalyzer System (BD Biosciences, Franklin Lakes, N.J., US).

[0395] FIG. **3** depicts binding of several Nanobody constructs to SKBR3 cells as measured by flow cytometric analysis. It can be seen that the constructs 2A1, 2A3, 2C3, 2C5, 2D3 and 2G4 show clearly discernable shifts in fluorescence intensity as compared to the fluorescence intensity for cells incubated only with FACS buffer in the absence of any construct but with all appropriate detection agents as used for the detection of Nanobody constructs.

Example 8

Screening for Nanobodies that Compete with Herceptin® for HER2 Binding

[0396] A competition ELISA was performed to screen for Nanobodies that are able to inhibit the Herceptin® interaction with HER2. In this competition ELISA, the binding of 2 nM Herceptin® to SKBR3 vesicles was evaluated in the presence of a 1/20 dilution of Nanobody containing periplasmic extract obtained in the second selection described in Example 3. FIG. **4** shows an example of this competitive ELISA, identifying several clones that compete with binding of Herceptin® to HER2 expressed on SKBR3 vesicles.

[0397] Periplasmic extracts obtained in the second and ninth selection described in Example 3 and periplasmic extracts obtained in Example 5, were also screened in a Herceptin®-competitive homogeneous cell-based assay to evaluate the capacity of the expressed Nanobodies to block Herceptin® binding to HER2. The FMAT 8200 HTS system (Applied Biosystems, Foster City, Calif.) assay was performed as follows: SKBR3 cells expressing HER2 were grown in tissue culture flasks, collected and washed with screening buffer (PBS, 10% FCS) and resuspended in screening buffer at a concentration of 2.5×10^5 cells/ml. Alexa 647-labeled Herceptin® was diluted to 62.5 ng/ml in screening buffer. Periplasmic extracts were diluted in screening buffer to obtain final dilutions of 4, 10, 40, 100, 200 and 400. To initiate the competitive screen, 10 μl labeled Herceptin®, 10 μl periplasmic dilution and 20 μl of cells were added to each well of FMAT system 384-well plates (PE Biosystems, Foster City, Calif.) The plates were scanned after 2 hours of incuba-

tion. A well was considered positive if it had a count of over 50 events. Screening of the extracts in this Herceptin® competitive homogeneous cell-based assay identified several clones (SEQ ID NOs: 1926-1988) that can block the binding of Herceptin® to HER2 with more than 90% (FIG. **5**).

[0398] Purified Nanobodies were tested for inhibition of binding of Alexa647-labeled Herceptin® to HER2 expressed on SKBR3 cells. Serial dilutions of purified Nanobody (concentration range: 20 nM-10 pM) were added to SKBR3 cells together with 4×10^{-10} M Alexa647-labeled Herceptin® and incubated for 2 hours, after which plates were scanned. Herceptin® was included as reference (MoAb). Results are shown in FIG. **6**. Dose-response curves were observed for all Nanobodies with IC_{50}-values ranging from 40 pM to 200 pM.

Example 9

Screening for Nanobodies that Compete with Omnitarg-Fab for HER2 Binding

[0399] Periplasmic extracts obtained in the sixth and seventh selection described in Example 3, were screened in an Omnitarg-Fab (OT-Fab) competitive homogeneous cell-based assay to evaluate the capacity of the expressed Nanobodies to block OT-Fab binding to HER2. The FMAT 8200 HTS system (Applied Biosystems, Foster City, Calif.) assay was performed as follows: SKBR3 cells expressing HER2 were grown in tissue culture flasks, collected and washed with screening buffer (PBS, 10% FCS) and resuspended in screening buffer at a concentration of 2.5×10^5 cells/ml. Biotinylated OT-Fab was diluted in screening buffer to obtain a final concentration of 0.586 nM. The periplasmic extracts were diluted in screening buffer to obtain final dilutions of 100. To initiate the competitive screen, 5 μl labeled OT-Fab, 10 μl periplasmic dilution, 5 μl FMAT Blue dye-labeled streptavidin (100 ng/ml) and 20 μl of cells were added to each well of FMAT system 384-well plates (PE Biosystems, Foster City, Calif.). The plates were scanned after 2 hours of incubation. A well was considered positive if it had a count of over 50 events. Screening of the extracts in this OT-Fab competitive homogeneous cell-based assay identified clones that can block the binding of OT-Fab to HER2 with more than >90% (FIG. **7**). Sequence analysis showed that all clones that blocked binding of OT-Fab HER2 are identical and represent a single Nanobody (SEQ ID NO: 1989).

Example 10

Screening of Kinetic Off-Rate Constants Via Surface Plasmon Resonance (BIAcore)

[0400] RhErbB2-Fc was immobilized on a CM5 sensor chip surface docked in Biacore 3000. Approximately 3600RU of rhErbB2-Fc was immobilized. Experiments were performed at 25° C. Periplasmic extracts were diluted 10-fold in running buffer (HBS-EP). The samples were injected for 1 min at a flow rate of 45 μl/min over the activated and reference surfaces. Those surfaces were regenerated with a 3 s pulse of glycine-HCl pH1.5+0.1% P20. As an example, the off rate (k_{off}) of different Nanobodies is documented in Table C-2.

Example 11

Anti-HER2Nanobodies can Block SKBR3 Cell Proliferation

[0401] The growth inhibitory characteristics of isolated Nanobodies were evaluated using the breast tumor cell line SKBR3. Briefly, SKBR3 cells were detached using 0.25% (vol/vol) trypsin and suspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), glutamine, and penicillin-streptomycin at a density of 1×10^5 cells/ml. Aliquots of 200 μl (2×10^4 cells) were plated into 96-well microdilution plates and allowed to adhere. After overnight adherence, cells were washed with serum-free medium and starved for 4 hours in 100 μl serum-free medium. Then, 100 μl of 1% FCS containing medium alone or medium containing Nanobody (final concentration of 50 nM) was added. After 2 days of incubation, cells were pulsed with 1 μCi [^{3}H]-thymidine and incubated for an additional 24 h prior to freezing at −80° C. Cells were subsequently thawed and embedded on glass fiber membranes using a cell harvester (Perkin Elmer Life Sciences, Wellesley, Mass., USA). After several washings with water, filters were air-dried and counted using a γ-counter (Perkin Elmer Life Sciences). Nanobody 2A5 inhibited SKBR3 proliferation by about 18%. Up to 30% or more inhibition was achieved with Nanobodies 2C3, 2D3, 2A4 and 5F7 (FIG. **8**).

Example 12

Generation of Multivalent/Multispecific Nanobody Formats

[0402] To potentially increase the biological effect of Nanobody molecules, bivalent constructs were fused head-to-tail using a GGGGSGGGS linker.

[0403] Here we describe the construction and characterization of bivalent Nanobodies consisting of two identical anti-HER2 molecules all separated by a 9 (GS) amino acid linker peptide. DNA segments encoding Nanobodies 2A4, 2A5, 2C3, 2D3, 5F7 were head-to-tail fused resulting in constructs 2A4-9GS-2A4, 2A5-9GS-2A5, $2C_{3\text{-}9}$GS-2C3, 2D3-9GS-2D3, 5F7-9GS-5F7. Sequences of these bivalent constructs are listed in Tabel B-4. All Nanobodies were expressed in *E. coli* and purified according to standard protocols (see for example the prior art and applications filed by applicant cited herein).

[0404] The different bivalent Nanobody formats were screened in a Herceptin®-competitive homogeneous cell-based assay to evaluate their capacity to block Herceptin® binding to HER2 compared to their monovalent format. Briefly, 10 μl labeled. Herceptin® (62.5 ng/ml), 10 μl Nanobody dilution and 20 μl of cells (5×10^3 cells) were added to each well of FMAT system 384-well plates (PE Biosystems, Foster City, Calif.). The plates were scanned after 2 hours of incubation. FIG. **9** shows that the bivalent constructs are more efficient in blocking the binding of Herceptin® to HER2-expressing SKBR3 cells as compared to their monovalent formats.

[0405] To test whether selected Nanobodies have potential as anticancer agents in an animal model, a strategy to increase the serum half life is preferred (as for example described in patent application WO 04/041865), since the serum half life of a mono- or bivalent Nanobody (approximately 15 or 30 KDa, respectively) is not optimal for this therapeutic indication. Human serum albumin specific Nanobody ALB1 (SEQ ID NO: 2266), cross reactive with mouse serum albumin, was chosen. Here we describe the construction of bispecific Nanobodies consisting an anti-HER2Nanobody and ALB1, all separated by a 9 (GS) amino acid linker peptide and resulting in constructs 2A4-9GS-ALB1, 2A5-9GS-ALB1, $2C_{3\text{-}9}$GS-

ALB1, 2D3-9GS-ALB1 and 5F7-9GS-ALB1. Sequences of these bispecific constructs are given in Table B-5.

[0406] To test whether the HER2-binding Nanobodies as disclosed herein above retain their biological activity in a more complicated molecular context such as a bispecific format, Nanobody formats were screened in a Herceptin®-competitive homogeneous cell-based assay to evaluate their capacity to block Herceptin® binding to HER2 compared to their monovalent and bivalent format. Based on the results shown in FIG. **9**, it can be concluded that fusion of a Nanobody with different antigen specificity to a HER2-binding Nanobody does not affect the potency of the latter.

Example 13

Generation of Biparatopic Formats Combining a Herceptin®-Competing Nanobody with a Library of HER2 Binding Nanobodies

[0407] The structural requirement for multispecificity is to fuse two or more binding domains together, with sufficient flexibility to allow simultaneous binding to different target epitopes. The simplest bispecific is one that binds to two different and non-overlapping epitopes on the same target in such a way that simultaneous binding to the target is possible. Robert et al (Int. J. Cancer 1995, 28; 62(3): 283-90) have described the design of high avidity biparatopic antibodies directed against two different epitopes of the carcinoembryonic antigen. Binding of both arms simultaneously without a significant loss of entropy will endow 'biparatopic' antibodies with increased avidity and hence, increased binding affinity to the target. As a result, higher potency can be obtained as well as enhanced selectivity. In addition, careful selection of the epitopes targeted on the antigen by the biparatopic antibody or fragment thereof, combined with rational design of linkers to allow maximal flexibility of the two binding domains within the biparatopic antibody, may for example result in the blocking of two or more critical interaction sites of the target, leading to improved potency.

[0408] Using genetic fusion, one Herceptin®-competing Nanobody was combined with a repertoire of HER2-binding Nanobodies and this mini-repertoire was screened for biparatopics with improved binding activity and tumor cell growth inhibitory characteristics compared to the monovalent Herceptin®-competing Nanobody.

13.1 Construction of an Expression Vector for Biparatopic Design

[0409] For the construction of biparatope Nanobodies, an expression vector was adapted to contain the Herceptin®-competitive Nanobody 2D3 (which was shown to block cell proliferation between 20-30% as monovalent format (see Example 11) and which strongly competes with Herceptin® for binding to HER2-overexpressing SKBR3 cells) to which other Nanobodies with different HER2-binding specificities can be fused, spaced by a linker (FIG. **10**). For the design of this vector, a 35 GS linker was used but other linker lengths can also be used to allow flexibility between the two building blocks. The 2D3 Nanobody is placed at the C-terminal end of the construct to allow SfiI-BstEII cloning of a full selection output. Alternatively, the 2D3 Nanobody can also be placed at the N-terminal end of the construct to allow cloning of a full selection output at the C-terminal end.

13.2 Generation of a Biparatopic Library

[0410] A full selection output retrieved from a selection on Herceptin®-captured rhErbB2/Fc followed by trypsin elution (Example 3.4), was unidirectionally cloned to the 2D3 Nanobody. Sequence analysis of a selected number of individual colonies derived from the selection output showed a good diversity in the repertoire: 16 Nanobody families were identified in 72 sequences. The ligation mix was transformed into *E. coli* cells and the transformation mix spread on selective agarose. Multiple individual subcolonies were picked and grown in 96-well deep well plates containing liquid selective medium by a QP Expression colony picker/rearrayer system (Genetix, New Milton, Hampshire, UK). Fourty-eight individual colonies were sequenced and analyzed. From 32 annotated sequenced, eight different Nanobody families were identified.

[0411] Periplasmic extracts (volume: ~80 μl) were prepared according to standard methods (see for example the prior art and applications filed by applicant cited herein). The biparatopic Nanobodies were purified from the periplasmic extracts using PhyTip200$^+$ columns (Phynexus, San Jose, Calif.) by a Tecan Eva Robotic system (Promega, Madison, US) and analyzed for their effects on SKBR3 tumor cell proliferation.

13.3 Effect of biparatopic Nanobodies on SKBR3 Cell Proliferation

[0412] The growth inhibitory characteristics of Nanobodies purified from periplasmic extracts by PhyTip200$^+$ were evaluated using the breast tumor cell line SKBR3. Briefly, SKBR3 cells were detached using 0.25% (vol/vol) trypsin and suspended in DMEM supplemented with 10% fetal calf serum (FCS), glutamine, and penicillin-streptomycin at a density of 1×10^5 cells/ml. Aliquots of 200 μl (2×10^4 cells) were plated into 96-well microdilution plates and allowed to adhere. After overnight adherence, cells were washed with serum-free medium and starved for 4 hours in 100 μl serum-free medium. Then, 100 μl of 1% FCS containing medium alone or 90 μl of 1% FCS containing medium with 10 μl PhyTip200$^+$ purified periplasmic extract or 50 nM Herceptin® was added. After 2 days of incubation, cells were pulsed with 1 μCi [^{3}H]-thymidine and incubated for an additional 24 h prior to freezing at −80° C. Cells were subsequently thawed and embedded on glass fiber membranes using a cell harvester (Perkin Elmer Life Sciences, Wellesley, Mass., USA). After several washings with water, filters were air-dried and counted using a γ-counter (Perkin Elmer Life Sciences).

[0413] Herceptin® was able to inhibit cell proliferation of SKBR3 up to 50%. Different subclasses of biparatopic Nanobodies were identified: a group of biparatopic Nanobodies revealed an inhibitory effect on the ErbB2 overexpressing cell line SKBR3 to a lower extent than Herceptin®, a second group of biparatopic Nanobodies increased cell proliferation and a third group of biparatopic Nanobodies was able to inhibit cell proliferation of SKBR3 cells to an equal or greater extent than Herceptin®. FIG. **11** shows an example of this 'single hit' cell proliferation assay.

Example 14

Characterization of Biparatopic Nanobodies

[0414] The biparatopic molecules 28F6-35GS-2D3, 28G5-35GS-2D3, 29E9-35GS-2D3, 30D10-35GS-2D3, 27A5-

35GS-2D3, 31D1'-35GS-2D3, 30E10-35GS-2D3, 27A3-35GS-2D3, 27B7-35GS-2D3, 27C_{3-35}GS-2D3, 27D1-35GS-2D3, 27E4-35GS-2D3, 27E7-35GS-2D3, 27H3-35GS-2D3, 27H4-35GS-2D3, 27H5-35GS-2D3 were expressed in *E. coli* as c-myc, His6-tagged proteins and subsequently purified from the culture medium by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). A control biparatopic Nanobody consisting of a dummy (i.e. not binding to HER2) Nanobody genetically fused to the 2D3 Nanobody, spaced by a 35GS linker was used as a control.

14.1 Biparatopic Nanobodies Display Improved Binding to HER2 as Compared to the Monovalent Building Blocks

[0415] The off-rate of the biparatopic Nanobodies was determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, rhErbB2-Fc was immobilized on a CM5 sensor chip surface docked in Biacore 3000. Approximately 3600RU of rhErb B2-Fc was immobilized. Experiments were performed at 25° C. Nanobody binding was assessed at various concentrations. The samples were injected for 1 min at a flow rate of 45 μl/min over the activated and reference surfaces to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody was sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte was removed by injecting regeneration solution (Glycine/HCl pH1.5).

[0416] The monovalent 2D3 and biparatopic dummy-2D3 Nanobodies had similar off-rates in the range of 1E-3 1/s, indicating that fusion of a Nanobody to the N-terminal end of 2D3 does not interfere with binding of the latter (FIG. **12**). The off-rate of bivalent 2D3-35GS-2D3 is in the range of 1E-4 1/s, indicating simultaneous binding of the two Nanobodies.

[0417] The off-rate of the biparatopic constructs 2B7-35GS-2D3, 27C3-35GS-2D3 and 27H5-35GS-2D3 are in the range of 1E-3 1/s (FIG. **13**). These off-rates and the binding responses indicate binding by the 2D3 paratope, but lack of binding by the other paratope, either by non-specificity for rhErb2 or an extremely much lower affinity for rhErb2 compared to 2D3 or by sterical hindrance of the epitope by the Fc part or by the altered conformation after the immobilization procedure on the CM5 sensor chip.

[0418] Off-rates of the biparatopic constructs 2D3-35GS-2D3, 27D1-35GS-2D3, 27A3-35GS-2D3, 27E7-35GS-2D3 are in the range of 1E-4 1/s (FIG. **14**). These off-rates indicate simultaneous binding of the 2 paratopes.

14.2 Herceptin®-Competitive Behavior of Biparatopic Nanobodies

[0419] Biparatopic Nanobodies were screened in a Herceptin®-competitive homogeneous cell-based assay to evaluate the capacity of the expressed Nanobodies to block Herceptin® binding to HER2. The FMAT 8200 HTS system (Applied. Biosystems, Foster City, Calif.) was used as described in Example 8. Bivalent 2D3-35GS-2D3 Nanobody more efficiently blocks binding of Herceptin® to HER2 as compared to monovalent 2D3 (FIGS. **15**A and B). Likewise, biparatopic Nanobodies 27H3-35GS-2D3 and 27D1-35GS-2D3 block binding of Herceptin® to HER2 expressed on SKBR3 cells more efficiently than monovalent 2D3. Nanobodies 27A3, 27A5 and 30D 10 have no influence on the Herceptin®-competitive characteristic of Nanobody 2D3 when fused to its N-terminal end, spaced by a 35GS linker (FIG. **15**B). Finally, Nanobodies 27B7, 27C3, 27H5 and the dummy Nanobody have an inhibitory effect on the Herceptin®-competitive potential of 2D3 (FIG. **15**C).

14.3 Competitive Binding of Biparatopic Nanobodies with Omnitarg-Fab to HER2.

[0420] Biparatopic Nanobodies were screened in an Omnitarg-Fab competitive homogeneous cell-based assay to evaluate the capacity of the expressed Nanobodies to block Omnitarg-Fab binding to HER2. The FMAT 8200 HTS system (Applied Biosystems, Foster City, Calif.) was used as described in Example 9. Biparatopic Nanobodies 2D3-35GS-2D3, 27H3-35GS-2D3, 27D1-35GS-2D3, 27A3-35GS-2D3, 27A5-35GS-2D3 and 30D10-35GS-2D3 did not efficiently block the binding of biotinylated Omnitarg Fab (FIG. **16**). Non-labeled Omnitarg-Fab inhibited binding of biotinylated Omnitarg-Fab in a dose-dependent manner.

Example 15

Biparatopic Nanobodies Comprising a Herceptin-Competitive and a HER2-Binding Nanobodies Inhibit SKBR3 Cell Proliferation

[0421] The growth inhibitory characteristics of biparatopic Nanobodies were evaluated using the breast tumor cell line SKBR3. Briefly, SKBR3 cells were detached using 0.25% (vol/vol) trypsin and suspended in DMEM supplemented with 10% fetal calf serum (FCS), glutamine, and penicillin-streptomycin at a density of 1×10^5 cells/ml. Aliquots of 200 μl (2×10^4 cells) were plated into 96-well microdilution plates and allowed to adhere. After overnight adherence, cells were washed with serum-free medium and starved for 4 hours in 100 μl serum-free medium. Then, 100 μl of 1% FCS containing medium alone or 90 μl of 1% FCS containing medium with serial dilutions of IMAC/SEC purified biparatopic Nanobodies, monovalent 2D3 or 50 nM Herceptin® was added. After 2 days of incubation, cells were pulsed with 1 μCi [^{3}H]-thymidine and incubated for an additional 24 h prior to freezing at −80° C. Cells were subsequently thawed and embedded on glass fiber membranes using a cell harvester (Perkin Elmer Life Sciences, Wellesley, Mass., USA). After several washings with water, filters were air-dried and counted using a γ-counter (Perkin Elmer Life Sciences).

[0422] Biparatopie Nanobodies are able to inhibit cell proliferation of SKBR3 cells to an equal or greater extent than Herceptin®. FIG. **17** shows an example of this cell proliferation assay.

Example 16

Biparatopic Nanobodies Comprising a Herceptin®-Competitive and a HER2-Binding Nanobody Inhibit AKT Signal Transduction in SKBR3 Breast Cancer Cells

[0423] Upon overexpression, HER2 may be activated by homodimerisation. HER2 plays a major regulatory role in the signalling network involved in many cellular processes, including the p21Ras/Mitogen-Activated Protein Kinase (MAPK) and PI3K/AKT pathways. Treatment of HER2 overexpressing SKBR3 cells with Herceptin® results in reduction in HER2 phosphorylation which is linked to inhibition of AKT phosphorylation.

[0424] To assess the effect of biparatopic Nanobodies on the AKT pathway in SKBR3 cells, cells were plated in 2%

serum containing medium in 24-well culture plates. The next day, medium was refreshed and 50 nM of either biparatopic Nanobody, Herceptin®, monovalent 2D3 Nanobody or medium alone was added and incubated for 16 h. The reaction was stopped by aspirating the cell medium. Cells were lysed by addition of lysis buffer (20 mM NP40, 20 mM Tris-HCl pH8, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, complete protease inhibitor cocktail, 1% PBS). Protein concentration in the lysates was measured using BCA protein assay kit (Pierce) according to the manufacturer's indications. Equal amounts of protein were run on 10% polyacrylamide gels and electroblotted onto Invitrolon PVDF membranes (Invitrogen, Paisley, UK). The presence of poshorylated AKT was assessed by probing the blots with Phospho-AKT (Ser473) antibody (Cell Signaling, Danvers, Mass.) and total AKT was detected using AKT antibody (Cell Signaling). The blots were visualized using a chemiluminescent substrate (Perkin Elmer, Wellesley, Mass., USA).

[0425] As shown in FIG. **18**, biparatopic Nanobodies 27A5-35GS-2D3 and 27A3-35GS-2D3 significantly block AKT activation in SKBR3 cells, whereas dummy-2D3 biparatopic and monovalent 2D3 Nanobody do not have a visible effect on AKT signalling.

Example 17

Construction of Biparatopic Nanobodies Combining Herceptin®- and Omnitarg Competitive Nanobodies

[0426] For the construction of biparatopics consisting of a Herceptin®-competitive and Omnitarg-competitive Nanobody, the expression vector described in Example 13.1 was used. Herceptin®-competitive Nanobodies 2D3 and 5F7 were cloned either at the C-terminal or N-terminal end of Omnitarg-competitive Nanobody 47D5, spaced by a 35GS linker. Biparatopic Nanobodies 2D3-35GS-47D5, 47D5-35GS-2D3, 5F7-35GS-47D5 and 47D5-35GS-5F7 were expressed in *E. coli* as c-myc, His6-tagged proteins and subsequently purified from the culture medium by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). Two control biparatopic Nanobody consisting of a dummy Nanobody genetically fused to the 2D3 or 47D5 Nanobody, spaced by a 35GS linker were used as controls.

Example 18

Characterization of Biparatopic Formats Combining Herceptin®- and Omnitarg Competitive Nanobodies

18.1 Biacore Analysis

[0427] A kinetic analysis for 2D3, 5F7 and 47D5 was performed on Biacore to determine the binding affinity to HER2. In addition, the influence of a dummy Nanobody fused to the N-terminal end of 2D3 and 47D5 on the binding characteristics of the latter to HER2, was analyzed. rhErbB2-Fc was immobilized on a CM5 sensor chip surface docked in T100. Approximately 3600RU of rhErbB2-Fc was immobilized. Experiments were performed at 25° C. Different concentrations of Nanobody (100 nM-0.78 nM) were made in running buffer (HBS-EP). The samples were injected for 1 min at a flow rate of 45 μl/min over the activated and reference surfaces.

[0428] In Table 2 an overview of k_d/k_{off}, k_a, and K_d values for the Nanobodies is shown. Fusion of a Nanobody at the N-terminal end of the Nanobodies 2D3 and 47D5 does not significantly alter the binding characteristics of these Nanobodies to HER2.

[0429] The binding of the biparatopic 2D3-35GS-47D5 to HER2 was compared to the binding of the monovalent building blocks 2D3 and 47D5. Hereto, approximately 90 RU of the respective Nanobodies were immobilized and different concentrations (100-1000 nM) HER2-ECD was injected. As shown in Table C-4, the off-rate of the HER2-ECD from the 2D3-47D5 surface was 25× lower than the off-rate on each of the 2D3 and 47D5 surfaces, indicating an avidity effect caused by binding of HER2-ECD on both the 2D3 and 47D5 Nanobodies simultaneously (FIG. **19**).

18.2 Biparatopic Nanobodies Combining Herceptin®- and Omnitarg Competitive Nanobodies Inhibit Heregulin-Mediated HER2-HER3 Signaling

[0430] After ligand-binding, the HER receptors become activated by receptor dimerization between either two identical receptors (homodimerization) or different receptors of the same family (heterodimerization). After receptor dimerization, activation of the intrinsic protein kinase activity and tyrosine autophosphorylation occurs, recruiting and phosphorylating several intracellular substrates involving the Ras-Raf-MAPK, the PI3K/Akt, and other signaling pathways that regulate multiple biological processes including apoptosis and cellular proliferation. The mitogen-activated protein kinases (Erk1/Erk2) are one of the key endpoints in signal transduction pathways that ultimately trigger cancer cells to divide.

[0431] The ability of the biparatopic Nanobodies combining Herceptin® and Omnitarg-competitive Nanobodies to inhibit heregulin (HRG) activation of MAPK-Erk1/Erk2 was assessed in the following way. MCF7 cells (5×10^4/well) were plated in serum-containing media in 24-well culture plates. The next day, media were removed and fresh media containing 0.1 serum were added to each well. The next day, prior to the assay, the media were replaced with serum-free medium. Cells were then incubated for 30 min with 50 nM of biparatopic Nanobody 2D3-35GS-47D5, 47D5-35GS-2D3, 5F7-35GS-47D5 or 47D5-35GS-5F7, monovalent 2D3, 5F7 or 47D5, Omnitarg-Fab or Herceptin®. Cells were then treated with 0.2 nM HRG for 15 min. The reaction was stopped by aspirating the cell medium. Cells were lysed by addition of lysis buffer (20 mM NP40, 20 mM Tris-HCl pH8, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, complete protease inhibitor cocktail, 1% PBS). Protein concentration in the lysates was measured using BCA protein assay kit (Pierce) according to the manufacturer's indications. Equal amounts of protein were run on 10% polyacrylamide gels and electroblotted onto Invitrolon PVDF membranes (Invitrogen, Paisley, UK). The presence of poshorylated Erk1/Erk2 (p44/42 MAPK) was assessed by probing the blots with phosphor-p44/42 MAPK (Thr202/Tyr204) antibody (Cell Signaling, Danvers, Mass.) and total MAPK was detected using p44/42 MAP kinase (137F5) rabbit mAb (Cell Signaling). The blots were visualized using a chemiluminescent substrate (Perkin Elmer, Wellesley, Mass., USA).

[0432] As shown in FIG. **20**, biparatopic Nanobodies 2D3-35GS-47D5 and 5F7-35GS-47D5 significantly block HRG-mediated activation of MAPK to a greater extent than Omnitarg-Fab and Herceptin®. Surprisingly, when the Omnitarg-competing Nanobody 47D5 comprised the N-terminal Nanobody in the biparatopic constructs, i.e 47D5-35GS-2D3

and 47D5-35GS-5F7, no significant reduction in MAPK activation could be observed. Monovalent Nanobodies 2D3, 5F7 and 47D5 could not block HRG-mediated MAPK activation in MCF-7 cells.

[0433] These data suggest that the position of the Nanobodies within the biparatopic Nanobody greatly influences the potency of the molecule. In addition, the length of the linker used to genetically fuse 2 Nanobodies biparatopic may be critically important to provide maximal flexibility between the 2 Nanobodies to allow tight binding to their respective binding epitope on HER2.

[0434] Biparatopic Nanobodies 2D3-35GS-47D5 and 5F7-35GS-47D5 were also shown to inhibit heregulin (HRG)-dependent Akt activation (FIG. **21**). Activation of the PI3K signal transduction pathway is important for cell survival. Complexes formed between HER2 and either HER3 or EGFR can initiate these pathways in response to HRG. Incubation of MCF7 breast cancer cells with biparatopic Nanobodies 2D3-35GS-47D5 or 5F7-35GS-47D5 inhibited HRG-mediated Akt activation to a greater extent than Omnitarg-Fab or Herceptin®. These data suggest that the biparatopic Nanobodies 2D3-35GS-47D5 or 5F7-35GS-47D5 may inhibit HER2 ligand-activation of PI3 kinase and that this inhibition may lead to apoptosis.

Example 19

In-Silica Design of Optimal Linker Lengths in Biparatopic Nanobody Formats

[0435] In-silico design of optimal linker lengths for a biparatopic Nanobody format may for example be performed as follows. The 3-dimensional (3D) coordinates of the binding mode of each individual Nanobody to its respective epitope on the target are determined, for example from:

[0436] a. a structure of the Nanobody-target complex determined by X-ray experiments or NMR experiments.

[0437] b. a docking model of each Nanobody binding on their respective epitope on the target. Also a number of potential binding modes of each Nanobody to the target derived from docking studies can be used. Docking can be done by e.g ZDock (Chen and Weng 2002, Proteins 47(3): 281-294; Chen and Weng 2003, Proteins 51(3): 397-408; Chen et al. 2003, Proteins 52(1): 80-87) and refined by RDock (Li et al. 2003, Proteins 53(3): 693-707) or by other methods (Fernandez-Recio et al. 2003, Proteins 52(1): 113-117).

[0438] c. Binding mode of each Nanobody can be extracted from the same structure or from separate complexes. In the latter case, the binding modes of each Nanobody on a different epitope on the same target can be deduced by structural superposition of the different complexes.

[0439] A linker with a given sequence and thus of given length can be modelled between the 2 Nanobodies in different ways:

[0440] a. By homology modelling (Safi, and Blundell J. 1993, Mol. Biol. 234: 779-815)

[0441] i. The sequence of a construct Nanobody1-linker-Nanobody2 or Nanobody2-linker-Nanobody 1 is drawn and stored in a readable sequence format (e.g. Fasta)

[0442] ii. The 3-dimensional coordinates of the biparatopic construct is built by homology modelling by using the 3-dimensional coordinates (from X-ray, NMR or docking experiments) of the individual binding modes of the Nanobodies as a template.

[0443] b. By de-novo design. A linker between the 2 Nanobodies binding on a different epitope on the same target can be build by de-novo design (Hu, et al. 2007, Proc. Natl. Acad. Sci. USA 104(45): 17668-17673).

[0444] c. Several conformations of the linker are sampled and the lowest state energy conformations (1 or more) can be considered.

[0445] As a non-limiting example, the above was performed for a biparatopic construct comprising two Nanobodies. The modelling is shown in FIGS. **22**A and **22**B, which show a model of Nanobody 2D3 (blue) linked to another Nanobody (cyan) docked on HER-2 (red). Both figures show that we can dock a Nanobody to a target and predict its binding mode to the target. When doing this for several. Nanobodies binding on non-overlapping epitopes on the same target, we can design a linker between the Nanobodies to create a multivalent Nanobody construct.

[0446] The 3-dimensional coordinates of the in-silico generated linker in the biparatopic construct are evaluated on at least one of the following criteria:

[0447] a. Internal energy strain of the linker; possibly compared to a set of generated linkers of the same sequence in the free state. At least one but preferentially several energy terms are used (e.g. Van der Waals energy, electrostatics energy, dihedral angle deformation energy, etc.). To calculate the energy values an atom-based force-field (e.g. CHARMM (Brooks et al. 1983, J. Comp. Chem. 4: 187-217)) or other means of calculating potential energy (e.g. potentials of mean force (Muegge and Martin 1999, J. Med. Chem. 42: 791)) can be used.

[0448] b. Internal energy strain on at least one of the residues (amino-acids) of the linker. For three biparatopic constructs 5F7-35GS-47D5, 47D5-35GS-5F7 and 47D5-40GS-5F7 energy penalty values were calculated for each residue in the linker as well as for 10 residues of each Nanobody connected to the linker. Energy values are shown in FIGS. **22**, **23** and **24**.

[0449] c. The root-mean square deviation (RMSD) between the 3-dimensional coordinates of the 2 Nanobodies in the biparatopic construct and the 2 Nanobodies in their non-linked (monovalent) binding mode. The higher this value the less likely this linker is appropriate. FIG. **25** shows the backbone RMSD ($Å^2$) between the 5F7-linker-47D5 constructs (built by homology modelling) with the individual Nanobodies 5F7 and 47D5 in their unlinked binding mode. The linker length varies from 5 to 35. FIG. **25**A shows that the RMSD-value is at a minimum value with linker lengths larger or equal to 15 residues. When shorter linkers are used (e.g. linker length=5, 10) we see an increased RMSD indicating that the Nanobodies in the bivalent construct are deviating from their monovalent binding mode. These in-silico experiments suggest that biparatopic constructs with linker lengths lower than 15 residues will have a significant deviation from the optimal binding mode of the individual Nanobodies to the target. In FIG. **25**B a ribbon view is shown of the 5F7-linker-47D5 biparatopic construct for 2 linker lengths. The binding mode of the individual Nanobodies is shown in blue; the biparatopic constructs are in red. When a 35GS linker is used between the 2 Nanobodies and a very limited deviation from the individual binding modes is observed. How-

ever, when a 5GS linker is used, both Nanobodies in the biparatopic construct significantly deviate from their optimal binding mode.

[0450] d. Scores from scoring functions in homology modelling protocols which are derived based on a combination of experimental data and in-silico results (Sall & Overington, Protein Science 3(9):1582-1596, 1994).

[0451] As can be seen from the above results, the linker in this specific example should preferably be at least 15 amino acids in length, with linkers of between 20 and 40 amino acid residues, such as about 25, 30 or 35 amino acid residues, being particularly suited.

[0452] Also, constructs with different potentially suitable linker lengths (as determined by the above in silico analysis) may be prepared and tested for affinity/avidity, specificity, or potency using suitable binding assays or in vitro or in vivo potency assays, for example those mentioned in the present specification. In this way, optimal linker length may be determined, confirmed or verified.

Example 20

Construction of Multiparatopic Nanobodies for Broader Biological Activity

[0453] Simultaneous binding of 2 adjacent, non-overlapping epitopes by both arms of a biparatopic Nanobody without significant loss of entropy endows biparatopic Nanobodies with increased binding affinity to the target and as a result, higher potency can be obtained. For persons skilled in the art, it is evident that the engineering of Nanobody fragments to obtain an increased potency or broader activity is not limited to the construction of biparatopic Nanobody fragments. Engineering of triparatopic and even tetratopic Nanobodies with careful selection of the epitopes targeted on the antigen, combined with selction of linkers to allow maximal flexibility of the binding domains within the multiparatopic antibody, may for example result in the blocking of several critical interaction sites of the target, leading to improved potency and even an unparalleled biological activity.

Tables

[0454]

TABLE B-1

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13D11	, SEQ ID NO: 1926	; PRT; ->	EVQLVESGGGLVHPGGSLRLSCVGSGFSLDDYGM TWVRRAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLNPEDTAVYYCGQGWKI VPTNPRGHGTQVTVSS
< 2B4	, SEQ ID NO: 1927	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVGSGFSLDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI RPTIPMGHGTQVTVSS
< 2G2	, SEQ ID NO: 1928	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYTDPVKGRF TISRDNAKNTLFLQMNNLTPEDTAVYYCNRGWKI VPTDLGGHGTQVTVSS
< 13D2	, SEQ ID NO: 1929	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNNLRSEDTAVYSCNQGWKI VPTDRGGHGTQVTVSS
< 2D5	, SEQ ID NO: 1930	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKI VPTDRGGHGTQVTVSS
< 2F4	, SEQ ID NO: 1931	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKI VPTDRRGHGTQVTVSS
< 2C3	, SEQ ID NO: 1932	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKI VPTDRTGHGTQVTVSS
< 17E3	, SEQ ID NO: 1933	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTM GWYRQAPGKQRDLVASIDSSGGTNYADSVKGRFT ISRDNAKNTVYLEMNSLTPEDTAVYYCNQGWKIV PTDRTGHGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 17H3	, SEQ ID NO: 1934	; PRT; ->	EVQLMESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKI VPTDRGGHGTQVTVSS
< 17D2	, SEQ ID NO: 1935	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKI VPTDRGSHGTQVTVSS
< 2F1	, SEQ ID NO: 1936	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKELEWISSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI VPMDRRGHGTQVTVSS
< 2E2	, SEQ ID NO: 1937	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 2C2	, SEQ ID NO: 1938	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNARNTLFLQMNSLTPEDTAIYYCNQGWKI LPTDRRGHGTQVTVSS
< 2E3	, SEQ ID NO: 1939	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI LPTNRGSHGTQVTVSS
< 13B10	, SEQ ID NO: 1940	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGFEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI LPTNRGSHGTQVTVSS
< 2D1	, SEQ ID NO: 1941	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNRGWKI LPTNRGSHGTQVTVSS
< 2H3	, SEQ ID NO: 1942	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 2H1	, SEQ ID NO: 1943	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVRGRF VISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 2C1	, SEQ ID NO: 1944	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 15C5	, SEQ ID NO: 1945	; PRT; ->	EVQLVESGGGLVQPGGSLKLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWNVTHTDYAYSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 2B3	, SEQ ID NO: 1946	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDCADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 29H2	, SEQ ID NO: 1947	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNNLTPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 17E4	, SEQ ID NO: 1948	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF VISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI IPTDRRGHGTQVTVSS
< 17A2	, SEQ ID NO: 1949	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNKGWKV WPTDRGTHGTQVTVSS
< 15D1	, SEQ ID NO: 1950	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLNPEDTAVYYCNQGWKV WPTDRGTHGTQVTVSS
< 17B8	, SEQ ID NO: 1951	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI LPAERRGHGTQVTVSS
< 15C11	, SEQ ID NO: 1952	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI LPAERRGHGTPVTVSS
< 15G8	, SEQ ID NO: 1953	; PRT; ->	EVQLVESGGGLVQPGGSLKLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWNGTHTDYAYSVKGRF TISRDNAKNTLFLQMNSLTPENTAVYYCNQGWKI LPAERRGHGTQVTVSS
< 17H4	, SEQ ID NO: 1954	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLINYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLHMNNLSPEDTAVYYCGQGWKI HPADRGGHGTQVTVSS
< 27G8	, SEQ ID NO: 1955	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKI LPAERRGHGTQVTVSS
< 38C6	, SEQ ID NO: 1956	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVGSGFSLDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKI RPTIPMGHGTQVTVSS
< 2A4	, SEQ ID NO: 1957	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAM SWVRQAPGKGLEWVSAINWSGSHRNYADSVKGRF TISRDNAKKTVYLQMNSLQSEDTAVYYCGTGWQS TTKNQGYWGQGTQVTVSS
< 15G7	, SEQ ID NO: 1958	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAM SWVRQAPGKGLEWVSAINWSGTHRNYADSVKGRF TISRDNNKKTVYLQMNSLKSEDTAVYYCATGWQS TTKNQGYWGQGTQVTVSS
< 15B7	, SEQ ID NO: 1959	; PRT; ->	EVQLVESGGGLVQPGGSLKLSCAASGFIFDDYAM SWVRQAPGKGLEWVSAINWSGSHRNYADSVKGRF TISRDNAKKTVYLQMNSLQSEDTAVYYCGTGWQS TTKSQGYWGQGTQVTVSS
< 5G4	, SEQ ID NO: 1960	; PRT; ->	EVQLVESGGGLVQPGGSLTLSCAGSGFIFDDYAM SWVRQAPGKGLEWVSSINWSGSHRNYADSVKGRF TISRDNAKKTLYLQMNSLKSEDTAVYYCATGWQS TTKNQNYWGQGTQVTVSS
< 13B2	, SEQ ID NO: 1961	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWISSINWSGTHKDYADSVKGRF TISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 2E5	, SEQ ID NO: 1962	; PRT; ->	EVQLVESGGSLVQPGESLRLSCAASGFTFDDYAM SWVRQAPGKGLEWISSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 15G1	, SEQ ID NO: 1963	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 27B1	, SEQ ID NO: 1964	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWISSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 17E7	, SEQ ID NO: 1965	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM SWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 17D8	, SEQ ID NO: 1966	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAVSGFTFDDYAM SWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNMLYLQMNSLKSEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 5F8	, SEQ ID NO: 1967	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAL SWVRQAPGKGLEWISSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 2D4	, SEQ ID NO: 1968	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGD AGTTWFEKSGSAGPGTQVTVSS
< 13D8	, SEQ ID NO: 1969	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQASGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 17G8	, SEQ ID NO: 1970	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWVSSINWSGTHTGYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 2H4	, SEQ ID NO: 1971	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 2F3	, SEQ ID NO: 1972	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTGSVKGRF TISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGD AGTTWFEKSGSAGPGTQVTVSS
< 2F5	, SEQ ID NO: 1973	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 30E10	, SEQ ID NO: 1974	; PRT; ->	KVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 29H1	, SEQ ID NO: 1975	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAM SWVRQAPGKGLEWVSSINWSGTHTGYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 17E2	, SEQ ID NO: 1976	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYGM SWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 2B1	, SEQ ID NO: 1977	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGD AGTTWFEKSGSAGPGTQVTVSS
< 2A5	, SEQ ID NO: 1978	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCATSGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 13C12	, SEQ ID NO: 1979	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCATSGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 17E10	, SEQ ID NO: 1980	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDCTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 27D4	, SEQ ID NO: 1981	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQASGKGLEWVSSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 15F9	, SEQ ID NO: 1982	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTGSVKGRF TISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 30H9	, SEQ ID NO: 1983	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 39C1	, SEQ ID NO: 1984	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYGM SWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGD AGTTWFEKSGSAGQGTQVTVSS
< 27G2	, SEQ ID NO: 1985	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM TWVRQTPGKGLEWVSSINWSGTHTDYTDSVKGRF TISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGD AGTTWFEKSGSAGPGTQVTVSS
< 2D3	, SEQ ID NO: 1986	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAM SWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRF TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS
< 5F7	, SEQ ID NO: 1987	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFSINTM GWYRQAPGKQRELVALISSIGDTYYADSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAA QGTDYWGQGTQVTVSS
< 118N121_A1_4_OK/ 1-127	, SEQ ID NO: 1988	; PRT; ->	EVQLVESGGGFVQTGGSPRLSCAASGRSFSEYAA AWFRQSPGKERDLVAGIMWDGRSLFYADSVKGRF TISRDNAKNTLHLQMNSLKPEDTAVYYCAYHKTP YTTLELNRPHAFGSWGQGTQVTVSS
< 47D5	, SEQ ID NO: 1989	; PRT; ->	KVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDM AWYRQAPGKQRELVALISRVGVTSSADSVKGRFT ISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLD GSTLAYWGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 14B11	, SEQ ID NO: 1990	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGSTFSSYGM GWFRQVPGKEREFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTARYYCGVETYGS GSSLMTEYDYWGQGTQVTVSS
< 14B10	, SEQ ID NO: 1991	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVNSRTFSSYGM GWFRQAPGKEREFVATINWSGVTAYADSIKGRFT ISRDNAKETVYLQMNSLKPDDTGVYYCAAETYGS GSSLMSEYDYWGQGTQVTVSS
< 14B4	, SEQ ID NO: 1992	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSSRAFSSYGM GWFRQAPGKDREFVATINWSGVTAYADSIKGRFT ISRDNAKETVYLQMNSLKPEDTGVYYCAAETYGS GSSLMSEYDYWGQGTQVTVSS
< 14C11	, SEQ ID NO: 1993	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVNSRTFSSYGM GWFRQAPGKEREFVATINWSGATAYADSIKGRFT ISRDNAKETVYLQMNSLKPDDTGVYYCAAETYGS GSSLMSEYDYWGQGTQVTVSS
< 14B5	, SEQ ID NO: 1994	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSSRAFSSYGM GWFRQAPGKDREFVATINWSGVTAYADSIKGRFT ISRDNAKETVYLQMNSLKPDDTGVYYCAAETFGS GSSLMSEYDYWGQGTQVTVSS
< 14C6	, SEQ ID NO: 1995	; PRT; ->	EVQLVESGGGSVQAGGSLRLSCVASEGTFSSYGM GWFRQAPGKERAFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYYCATDTYGS GSSLMNEYDYWGQGTQVTVSS
< 14A4	, SEQ ID NO: 1996	; PRT; ->	EVQLVESGGGSVQAGSSLTLSCVASEGTFSSYGM GWFRQAPGKERAFVATINWSGVNAYADSVKGRFT ISRDNAKKTAYLQMNSLKPEDTAVYYCAAETYGS GSSLMNEYDYWGQGTQVTVSS
< 14B3	, SEQ ID NO: 1997	; PRT; ->	EVQLVESGGGLVQPGGSLTLSCVASEGTFSSYGM GWFRQAPGKERAFVATINWSGVNAYADSVKGRFT ISRDNAKKTAYLQMNSLKPEDTAVYYCAAETYGS GSSLMNEYDYWGQGTQVTVSS
< 14C1	, SEQ ID NO: 1998	; PRT; ->	EVQLVESGGGSVQAGGSLRLSCAASGSTFSSYGM GWFRQAPGKERAFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYYCATETYGS GSSLMNEYDYWGQGTQVTVSS
< 14A12	, SEQ ID NO: 1999	; PRT; ->	EVQLVKSGGGLVQAGGSLRLSCAASERTFSSYGM GWFRQAPGKEREFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGS GSSLISEYDYWGHGTQVTVSS
< 14A2	, SEQ ID NO: 2000	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASERTFSSYGM GWFRQAPGKEREFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGS GSSLISEYDYWGHGTQVTVSS
< 14A1	, SEQ ID NO: 2001	; PRT; ->	EVQLVESGGGSVQAGGSLRLSCAASERTFSSYGM GWFRQAPGKEREFVATINWSGVTAYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGS GSSLMSEYDYWGHGTQTVSS
< 17C3	, SEQ ID NO: 2002	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAANGLTFRRYDM GWYRQAPGQQREWVAAISGAGDINYADSVKGRFT MARDNANHTVHLQMNSLKPEDTAVYYCNANWKML LGVENDYWGQGTQVTVSS
< 46D3	, SEQ ID NO: 2003	; PRT; ->	KVQLVESGGGLVQAGGSLRLSCAASGRTFTEYSM GWFRQAPGKEREFVATISWNYGYTYYSDSVKGRF TVSRDIAENTVYLQMNTLKSEDTAVYYCAAKIGW LSIRGDEYEYWGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27H5	, SEQ ID NO: 2004	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYGI GWFRQASGKEREGVSCITSSDGSTYYADSVKGRF TISSDNAKNTVYLQMNSLKPEDTAVYYCAALPFV CPSGSYSDYGDEYDYWGQGTQVTVSS
< 17C2	, SEQ ID NO: 2005	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYAM SWVRQAPGKGLEWVSAVDSGGGRTDYAHSVKGRF TISRDNAKNTLYLQMSSLKPEDTALYYCTKHVSD SDYTEYDYWGQGTQVTVSS
< 17D11	, SEQ ID NO: 2006	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCTASGRTSSTSAM GWFRQAPGKEREFVATISRGGSATYYADSLKGRF TISRDNAKNTLYLQMNSLKPEDTAVYYCAARRSS LYTSSNVFEYDYWGQGTQVTVSS
< 15A6	, SEQ ID NO: 2007	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTRTM AWYRQAPGKQRDWVATISSHGLPVYADSVKGRFT VSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADY WGQGTQVTVSS
< 17B6	, SEQ ID NO: 2008	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASRIPFSTRTM AWYRQAPGKQRDWVATIGTSGPPRYADSVKGRFT VSRDNAKNTVYLQMNSLKAEDTAVYYCWDVNADY WGQGTQVTVSS
< 17C5	, SEQ ID NO: 2009	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTRTM AWYRQAPGKQRDWVATISSHGLPVYADSVKGRFT VSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADY WGQGTPVTVSS
< 15E11	, SEQ ID NO: 2010	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASRIPFSSRTM AWYRQAPGKQRDWVATISARGMPAYEDSVKGRFT VSRDNDKNTLYLQMNSLKPEDTAVYYCRDVNADY WGQGTQVTVSS
< 15C2	, SEQ ID NO: 2011	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTRTM AWYRQAQGKQRDWVATISSHGLPVYADSVKGRFT VSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADY WGQGTQVTVSS
< 2A3	, SEQ ID NO: 2012	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTM AWYRQAPGKPRDWVATIRNGAPVYADSVKGRFTV SRDNAKNTLYLQMNSLKPEDTATYLCRDVNGDIW GQGTQVTVSS
< 27A5	, SEQ ID NO: 2013	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTM AWYRQPPGNERDWVATIRSGAPVYADSVKGRFTV SRDNAKNTLYLQMNSLEPEDTATYYCWDVNGDIW GQGTPVTVSS
< 2C5	, SEQ ID. NO: 2014	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTM AWYRQTPGKSRDWVATIRSGTPVYADSVKGRFTV SRDNAKNTLYLRMNSLKSEDSATYTCRAVNADIW GQGTQVTVSS
< 27G5	, SEQ ID NO: 2015	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASRIPASIRTM AWYRQTPGNQRDWDATIGSSGTPAYADSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDY WGQGTQVTVSS
< 13A9	, SEQ ID NO: 2016	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASRIPASIRTM AWYRQAPGKQRDWVATIGTGGTPAYADSFKGRFT VSRDNANHTVYLQMNSLKPEDTAVYYCRDVNGDY WGQGTQVTVSS
< 29E9	, SEQ ID NO: 2017	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASRIPASIRTM AWYRQTPGNQRDWLATIGSSGTPAYADSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDY WGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 15D8	, SEQ ID NO: 2018	; PRT; ->	EVQLVESGGGLVQPGGSLKLSCVASTIPASIRTM AWYRQTPGNQRDWLATIGSSGTPAYADSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDY WGQGTQVTVSS
< 15G4	, SEQ ID NO: 2019	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGIPFRSRTM AWYRQAPGKTRDWVATIGTHGTPLYADSVKGRFT VSRDNAKNTLYLQMNSLKPEDTAVYYCWDVNGDY WGQGTQVTVSS
< 15D12	, SEQ ID NO: 2020	; PRT; ->	EVQLVESGGGLVQAGESLRLSCATSGITFKRYVM GWYRQGPGKQRELVATVNDGGTTSYADSVKGRFA ISRDNAKNTAYLQMNSLKAEDTAVYYCNAVWKLP RFVDNDYWGQGTQVTVSS
< 15E12	, SEQ ID NO: 2021	; PRT; ->	EVQLMESGGGLVQAGGSLRLSCAANGLTFRRYDM GWYRQAPGQQREWVAAISGAGDINYADSVKGRFT MARDNANHTVHLQMNSLKPEDTAVYYCNANWKML LGVENDYWGQGTQVTVSS
< 13D7	, SEQ ID NO: 2022	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAANGLTFRRYDM GWYRQAPGQQREWVAAISGAGDINYADSVKGRFT MARDNANHTVHLQMNSLKPEDTAVYYCNANWKML LGVENDYWGQGTQVTVSS
< 13A8	, SEQ ID NO: 2023	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRR TMAWYRQAPGKQRDWVATIAGDGSTVYADSMKGR FTISRDNAENTVYLQMNSLKPEDTAVYYCWDVNR DYWGQGTQVTVSS
< 15A4	, SEQ ID NO: 2024	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRR TMAWYRQAPGKQRDWVATIAGDGSTVYADSMKGR FTISRDNAKNTVYLQINSLKPEDTAVYYCWDVNR DYWGQGTQVTVSS
< 17F7	, SEQ ID NO: 2025	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGIAQSIRVM AWYRQPPGKQRDWVGTISSDGTANYADSVKGRFT ISRDNAKKTMYLQMNSLKPDDTAVYYCRDVNRDY WGQGTQVTVSS
< 15C8	, SEQ ID NO: 2026	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIAFRIRTM AWYRQAPGKQRDWVATSDSGGTTLYADSVKGRFT VSRDNAENTVYLQMNSLKPEDTAVYYCRDVNRDY WGQGTQVTVSS
< 17A10	, SEQ ID NO: 2027	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGIPSIRAIA WYRQAPGKQRDWVATSGTGYGATYDDSVKGRFTL SRDNAKNTVYLQMNSLKPEDTAVYYCRDVNRDYW GQGTQVTVSS
< 27D3	, SEQ ID NO: 2028	; PRT; ->	EVQLMESGGGLVQPGGSLRLSCAASGLGIAFSRR TMAWYRQAPGKQRDWVATIAGDGSTVYADSMKGR FTISRDNAENTVYLQMNSLKPEDTAVYYCWDVNR DYWGQGTQVTVSS
< 13B12	, SEQ ID NO: 2029	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIAFRIRTM AWYRQAPGKQRDWVATIGSDGTTIYADSVKGRFT LSRHNAENTVYLQMNSLKPEDTAVYYCRDVNRDY WGQGTQVTVSS
< 15B2	, SEQ ID NO: 2030	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGIPSSIRAM AWYRQAPGRQRDWVATIYSPSGSAVYADSVKGRF TISSDNAKSTIYLQMNSLKPDDTAVYYCRDVNRD YWGQGTQVTVSS
< 15B11	, SEQ ID NO: 2031	; PRT; ->	EVQLVESGGGSVQAGGSLRLSCVVSGIPSSIRAM AWYRQAPGRQRDWVATIYSRSGGAVYADSVKGRF TISSDNAKNTIYLQMNSLKPDDTAVYYCRDVNRD YWGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13C9	, SEQ ID NO: 2032	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGIPSIHAMA WYRQAPGKQRDWGATTYSRGGTTYNDSAKGRFTI SRDNAKKTVYLQMNSLKPEDTAVYYCRDVNRDYW GQGTQVTVSS
< 17D5	, SEQ ID NO: 2033	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGIIGTIRTM AWYRQAPGKQRDWVASIGTRGAPVYADSVNGRFT ISRDGATNTVFLQMNNLKPEDTAVYYCRDVNRDY WGQGTQVTVSS
< 27B5	, SEQ ID NO: 2034	; PRT; ->	EVQLVESGGGLVQAGGSLRLPCAASGIAFRIRTM AWYRQAPGKQRDWVATSDSGGTTLYADSVKGRFT VSRDNAENTVYLQMNSLKPEDTAVYYCRDVNRDY WGQGTQVTVSS
< 27C7	, SEQ ID NO: 2035	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIAFRIRTM AWYRQAPGKQRDWVATSDSGGTTLYADSVKGRFT VSRDNADNTVYLQMNSLKPEDTAVYYCRDVNRDY WGQGTQVTVSS
< 13D4	, SEQ ID NO: 2036	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGIPSSIRAM AWYRQAPGRQRDWVATIYSPSGSAVYADSVKGRF TISSDNAKSTIYLQMNSLEPDDTAVYYCRDVNRE YWGQGTQVTVSS
< 15G5	, SEQ ID NO: 2037	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGIPSTIRAM AWYRQAPGRQRDWVATIYSPSGSAVYADSVKGRF TISSDNAKKTIYLQMNSLKPDDTAVYYCRDVNRE YWGQGTQVTVSS
< 13C4	, SEQ ID NO: 2038	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGIPSSIRAM AWYRQAPGRQRDWVATIYSPSGSAVYADSVKGRF TISSDNAKSTIYLQMNSLKPDDTAVYYCRDVNRE YWGQGTQVTVSS
< 46G1	, SEQ ID NO: 2039	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSDDAM GWFRQAPGKERECVASLYLNGDYPYYADSVKGRF TISRDNAKNAVILQMNNLKTEDTAVYYCAAKPGW VARDPSQYNYWGQGTQVTVSS
< 46E4	, SEQ ID NO: 2040	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRAFKDDAV GWFRQAPGKERECVASMYLDGDYPYYADSVKGRF TISRDNAKNAVILQMNNLKTEDTAVYYCAAKPGW VARDPSEYNYWGQGTQVTVSS
< 17B5	, SEQ ID NO: 2041	; PRT; ->	EVQLVESGGGLVQTGGSLRLSCAASGSTFRTDMM GWYRQAPGKQREFVASITKFGSTNYADSVKGRFT ISNDNAKDTVYLQMNSLKSEDTAVYYCRNFNRDL WGQGTQVTVSS
< 15C9	, SEQ ID NO: 2042	; PRT; ->	EVQLVESGGGLVQAGGSLKLSCVNSGIPSTLRAM AWYRQAPGRQRDWVATSSNTGGTTYDDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTGVYYCRDVNRDL WGQGTQVTVSS
< 13D10	, SEQ ID NO: 2043	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASSVITLDSNA IGWFRQAPGKEREEVSCIASSDGSTYYAESVKGR FTISKDYTRNTVYLQVNSLKPEDTAVYHCATDAN PNCGLNVWNSWGQGTQVTVSS
< 17C6	, SEQ ID NO: 2044	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCAASGSTSSLDIM AWYRQAPEKQRELVASVSGGGNSDYASSVKGRFT ISGDTAKSTLYLQMNSLKPEDTAMYYCYGRDYYY MPFWGQGTQVTVSS
< 15A2	, SEQ ID NO: 2045	; PRT; ->	EVQLVESGGGLAQAGGSLSLSCAASGRFFSTRVM AWYRQTPGKQREFVASMRGSGSTNYADSARGRFA ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINEDQ WGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 17A8	, SEQ ID NO: 2046	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCAASGRFFSTRVM AWYRQTPGKQREFVASMRGSGSTNYADSVRGRFA ISRDNAKNMVYLQMNTLKPEDTAVYYCRDINEDQ WGQGTQVTVSS
< 15G10	, SEQ ID NO: 2047	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCAASGRFFSTRVM AWYRQTPGKQREFVASMRGSGSTNYADSARGRFA ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINEDQ WGQGTQVTVSS
< 27A3	, SEQ ID NO: 2048	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCVASGRFFSTRVM AWYRQTPGKQREFVASMRGSGSTNYADSVRGRFA ISRDNAKNTVYLQMNTLKPEDTAVYYCRDINEDQ WGQGTQVTVSS
< 17H10	, SEQ ID NO: 2049	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCSASGRFFSTRVM AWYRQTPGNQREFVATIHSSGSTIYADSVRGRFA ISRDNAKNTVYLQMRSLKPEDTAVYYCRDINADQ WGQGTQVTVSS
< 30D10	, SEQ ID NO: 2050	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIRTM AWYRQPPGNQREWVATIGSNGFATYPDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDI WGQGSQVTVSS
< 15H4	, SEQ ID NO: 2051	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCAPSESTVSFNTV AWYRQAPGEQREWVATISRQGMSTYPDSVKGRFT ISRDNAKNTVYLQMNNLKPEDTAVYYCRDINHDI WGRGSQVTVSS
< 17B7	, SEQ ID NO: 2052	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIISSFRTM AWYRQAPGKQRDWVATIGSDGLANYADSVKGRFT ISRDNAKKTVYLQMNSLKPEDTAVYFCRDINRDY WGQGTQVTVSS
< 15D2	, SEQ ID NO: 2053	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGVFGPIRAM AWYRQAPGKQRDWVATIGSSGHPVYTDSVKGRFT FSKDGAKNTVYLQMNSLKPEDTAVYYCRDINRDY WGQGTQVTVSS
< 17G5	, SEQ ID NO: 2054	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGIGIAFSSR TMAWYRQAPGKQRDWVATIGSGGTTNYADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINR DYWGQGTQVTVSS
< 15B6	, SEQ ID NO: 2055	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGIIGSFRTM AWYRQAPGNQRDWVATIGSAGLASYADSVRGRFT LSRDNAKKTVYLQMNSLKPEDTAIYYCRDINGDY WGQGTQVTVSS
< 27F2	, SEQ ID NO: 2056	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIISSFRTL AWYRQAPGKQRDWVATISSAGGTAYADAVKGRFT ISISRDNVEYTVDLQMDSLKPEDTAVYYCRDING DYWGQGTQVTVSS
< 17F5	, SEQ ID NO: 2057	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRR TMAWYRQAPGKQRDWVATIAGDGSTVYADSMKGR FTISRDNAKNTVYLQVNSLKPEDTAVYYCWDTNG DYWGQGTQVTVSS
< 17B2	, SEQ ID NO: 2058	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAGSGFTFSNYAM TWVRQAPGKGLEWVSGVGGDGVGSYADSVKGRFT ISRDNAKNTLYLQMNSLKPEDTALYYCTKDISTF GWGPFDYWGQGTQVTVSS
< 27H4	, SEQ ID NO: 2059	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTM GWYRQAPGKQRDLVASIDASGGTNYADSVKGRFT ISRDNAKNTVYLEMNSLKPEDTGVYYCNGRWDIV GAIWWGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13A4	, SEQ ID NO: 2060	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTM GWYRQAPGKQRDLVASIDSSGGTNYADSVKGRFT ISRDNAKNTVYLEMNSLKPEDTGVYYCNGRWDIV GAIWWGQGTQVTVSS
< 2A1	, SEQ ID NO: 2061	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASKITFRRYIM DWYRQAPGKQRELVASINSDGSTGYTDSVKGRFT ISRDNTKNTLDLQMNSLKPEDTAVYYCHGRWLEI GAEYWGQGTQVTVSS
< 15E10	, SEQ ID NO: 2062	; PRT; ->	EVQLVESGGGLVQAGGSLKLSCVASGITFFRYTM GWYRQAPGKERELVAEISSADEPSFADAVKGRFT ISRDNAKNTVVLQMNGLKPEDTAVYYCKGSWSYP GLTYWGKGTLVTVSS
< 27E7	, SEQ ID NO: 2063	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFRRYDM GWYRQFPGKERELVATILSEGDTNYVDPVKGRFT ISRDNAKNTVYLQMNDLKPEDTAVYYCNGVWRAI GRTYWGQGTQVTVSS
< 47E5	, SEQ ID NO: 2064	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASASIFGFDSM GWYRQAPGNERILVAIISNGGTTSYRDSVKGRFT IARDNAKNTVSLQMNSLKPEDTAVYYCNLDRRSY NGRQYWGQGTQVTVSS
< 2G4	, SEQ ID NO: 2065	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGNIFSHNAM GWYRQAPGKQRELVTYITINGIANYVDSVKGRFT ISRDNTKNTMYLQMVSLKPEDTAVYYCNVGGREY SGVYYYREYWGQGTQVTVSS
< 14D4	, SEQ ID NO: 2066	; PRT; ->	EVQLVESGGGLVQAGDSLRLSCAASGRALDTYVM GWFRQAPGDGREFVAHIFRSGITSYASSVKGRFT ISRDNAKNTVYLQMASLKPEDTAAYYCAARPSDT TWSESSASWGQGTQVTVSS
< 17A5	, SEQ ID NO: 2067	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTEDDYSM SWVRQATGKGLEWVSGISWNGGSTNYADSVKGRF TISRDNVKNTLYLQMNSLKSEDTAVYYCAKDLGN SGRGPYTNWGQGTQVTVSS
< 15D10	, SEQ ID NO: 2068	; PRT; ->	EVQLVESGGGLVQPGGSLKLSCAASGFTFSSYRM YWVRQAPGKGLEWVSAIKPDGSITYYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCATDCGV PGFGWTFSSWGQGTQVTVSS
< 13C2	, SEQ ID NO: 2069	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGSTFSINRM AWYRQSPGKQRELVAAVDNDDNTEYSDSVAGRFT ISRDNAKNAVHLQMNSLRLEDTAVYYCNAKQLPY LQNFWGQGTQVTVSS
< 17G11	, SEQ ID NO: 2070	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGSTFSINRW GWYRQAPGKQRELVAAIDDGGNTEYSDFVNGRFT ISRDNPETAVHLQMNSLKLEDTAVYYCNAKQLPY LQNFWGQGTQVTVSS
< 17A3	, SEQ ID NO: 2071	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCAASATLHRFDNN WYRQAPGKQRELVATIAHDGSTNYANSVKGRFTI SRDNARDTLFLQMHALQPEDTAVYMCNLHRWGLN YWGQGTQVTVSS
< 27B7	, SEQ ID NO: 2072	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSAISSGGGSITTYADSVKGR FTISRDNAKNTLYLQMSSLKPEDTALYYCAKARS SSSYYDFGSWGQGTQVTVSS
< 17A6	, SEQ ID NO: 2073	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSAISSGGGSITTYADSVKGR FTISTDNAKNTLYLQMSSLKPEDTALYYCAKARS SSSYYDFGSWGQGTQVTVSS

TABLE B-1-continued

Preferred Nanobodies against HER2 obtained as described in Example 3

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 17D7	, SEQ ID NO: 2074	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTLDYCAI GWFRQAPGKEREGVSCISSSDGSTYYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCATDRGS GTCYADFGSWGQGTQVTVSS
< 46D4	, SEQ ID NO: 2075	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAM SWVRQAPGKGLEWVSSINWSGTHTDYAEDMKGRF TISRDNAKKTLYLQMNSLQSEDTAVYYCAKGWGP AVTSIPVATLGTQVTVSS
< 27B3	, SEQ ID NO: 2076	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIRTM AWYRQPPGNQREWVATIGSNGFATYPDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDI WGQGSQVTVSS
< 27E5	, SEQ ID NO: 2077	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIRTM AWYRQPPGNQREWVATIGSNGFATYPDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDI WGQGSQVTVSS
< 27D6	, SEQ ID NO: 2078	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIRTM AWYRQRPGNQREWVATIGSNGFATYPDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDI WGQGSQVTVSS
< 30D10	, SEQ ID NO: 2079	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIRTM AWYRQPPGNQREWVATIGSNGFATYPDSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDI WGQGSQVTVSS
< 47G11	, SEQ ID NO: 2080	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGRIFYPMGW FRQAPGKEREFVAAIGSGDIITYYADSVKGRFTI SRDNAKNTVYLQMNSLKPEDTAVYYCASSRDYSR SRDPTSYDRWGQGTQVTVSS
< 27C3	, SEQ ID NO: 2081	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAT SWVRQAPGKGPEWVSAINSGGGSTYYADSVKGRF TISRDNAKNTLYLQMNSLKPEDTAVYYCARPRGS SLYLLEYDYWGQGTQVTVSS

TABLE B-2

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 11A101/1-120	, SEQ ID NO: 2082	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFNAMGW FRQAPGKEREFVAAISRSPGVTYYADSVKGRFTT SRDNAKNTVYLQMNDLKPEDTAVYYCAADFYLAT LAHEYDYWGQGTQVTVSS
< 11A22/1-122	, SEQ ID NO: 2083	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAM AWFRQAPGTEREFIAGIRWSDGSTYYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAADFYV STLAHEYDYWGQGTQVTVSS
< 12D44/1-122	, SEQ ID NO: 2084	; PRT; ->	KVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAM AWFRQAPGTEREFIAGIRWSDGSTYYADSVKGRF TISRANAKNTVYLQMNGLKPEDTAVYYCAADFYV STLAHEYDYWGQGTQVTVSS
< 12E11/1-122	, SEQ ID NO: 2085	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAM AWFRQAPGKEREFVGGIRWSDGSTYYADSVKGRF TISRDNAKITVYLQMNSLKPEDTAVYYCAADFYV STLAHEYDYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13G111/1-123	, SEQ ID NO: 2086	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAM GWFRQAPGKERAFVAAIRWSGGNTYYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAADTFT LSTLSHEYDYWGQGTQVTVSS
< 13E71/1-123	, SEQ ID NO: 2087	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTFSNYAL AWFRQAPGKEREFVAAINWRSGGSTYYADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLI VATLPGEYDYWGQGTQVTVSS
< 14H61/1-122	, SEQ ID NO: 2088	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSRFAM GWFRQAPGKEREFVAAVRWSDDYTYYADSVKGRF TISRDNAKNTVYLQMNSLSPEDTAVYYCAADEIL ATLPHEYDYWGQGTQVTVSS
< 22B12/1-124	, SEQ ID NO: 2089	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAM AWFRQAPGKEREFVAGINKSGGITHSADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAADAYT VIATLPHEYDYWGQGTQVTVSS
< 14H71/1-123	, SEQ ID NO: 2090	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCEASGLTISSLTM AWFRQAPGKEREFVANIKWSGDRIVYADSVKGRF TISRDSAKNAVNLQMELVESDDTAVYYCAAKHST VAGLTHEYDYWGQGTQVTVSS
< 12D51/1-120	, SEQ ID NO: 2091	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGSAFSIKSM GWYRQAPGKQRELAAVIISSGTTTYADSVKGRFT ISRDSAKNTVYLQMDSLKPEDTAVYVCNAVYVST WGNGYDYWGQGTQVTVSS
< 11A111/1-126	, SEQ ID NO: 2092	; PRT; ->	EVQLVESGGGLVQAGGSLGLSCAAAGRTFSSSLM GWFRQAPGKEREFVAAITDNGGSTYYADSVKGRF TISRDNAKNSVYLQMNSLKPEDTAIYYCAARRSG YYSLSTSPHQYAYWGQGTQVTVSS
< 13G71/1-124	, SEQ ID NO: 2093	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRAFSSYAM GWFRQAPGKERDFVAAITSSGSTNYADSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCGARVNYA AYSRLEHDYHYWGQGTQTVSS
< 13G74/1-125	, SEQ ID NO: 2094	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCATSGRTFSTYAS MGWFRQTPGKEREFVAAITSSGSTNYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCGARVNY AAYSRLEHDYHYWGQGTQVTVSS
< 11A71A/1-116	, SEQ ID NO: 2095	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGNIDGIITM GWYRQRPGKPREWVGTINSGGDTNYAGSVKGRFT IARDDAKNTMYLQMNGMKPEDTAVYYCKMNRAGI YEYWGQGTQVTVSS
< 22B101/1-123	, SEQ ID NO: 2096	; PRT; ->	EVQLVESGGGLVQTGGSLRLSCAASGPTFSDYAI GWFRQAPGKEREFVAAISSSGISTIYGDSVKGRF DISRDNAKNTVYLQMNRLKPEDTAVYYCAARLFM ATPNQGQYYYWGQGTQVTVSS
< 11B42/1-123	, SEQ ID NO: 2097	; PRT; ->	EVQLVESGGGLVQAGDSLRLSCAASGFTFSNHIM GWFRQAPGKERELIAHITWNGGSTYYADSVKGRF AISRDNALNTVYLQMNSLKPEDTAVYYCAARPSY STNNVKSYRYWGQGTQVTVSS
< 13E111/1-124	, SEQ ID NO: 2098	; PRT; ->	EVQLVESGGGLVQAGSSLRLSCALSGRTFSDYAI GWFRQAPGKEREFVAAISGWSGGTTNYADSVKGR FTISRDNGKNTVDLRMNSLKPEDTAVYYCAARPA VVHTRKESYPYWGQGTQVTVSS
< 14H12/1-125	, SEQ ID NO: 2099	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCIASERTFSSAGV GWFRQAPGKERDFVAAISWNGVTIYYADSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAARINY SVLTTTSSSYHYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13G101/1-123	, SEQ ID NO: 2100	; PRT; ->	EVQLVESGGGLVQPGDSLRLSCSASEGTLSRSRV AWFRQAPGKEREFVTVISGVGTSYADSVKGRFTI SRDDAKNTVYLQMNSLKAEDTAIYYCAADFRSTW LSSSGSSYTYWGQGTQVTVSS
< 13G41/1-121	, SEQ ID NO: 2101	; PRT; ->	EVQLVESGGGLVQPGGSLTLSCVGSGRRFSADVM GWYRQAPGKQREFVASISSGSAINYADSVKGRFT VSRDNAQNTVYLQMNSLKIEDTGVYYCNARRIVN VEGAYRDYWGQGTQVTVSS
< 22B910/1-121	, SEQ ID NO: 2102	; PRT; ->	EVQLVESGGGLVQPGGSLPLSCAASGSIFRMNDM GWYRQAPGKQRERVATLTSAGNTNYADSVKGRFT ISGDDARNTVYLQMNSLNPEDTAVYYCNAKVVVA VEGAKYDYWGQGTQVTVSS
< 21A81/1-122	, SEQ ID NO: 2103	; PRT; ->	EVQLVESGGGLAQAGGSLRLSCAVFGRSRYGMAW FRRAPGKEREFVAGIAWNGASIGSADSVRGRFTI SRDNSENTVYFEMGSLKPEDTAVYYCAICRISWC AGAESDYGYWGQGTQVTVSS
< 21A92/1-127	, SEQ ID NO: 2104	; PRT; ->	EVQLVESGGGQVQAGGSLRLSCTESGRAFNTRAM GWFRQAPEKEREFVAGITMSGFNTRYADSVKGRF TISRDNAKGTVYLQMSSLKPEDTAVYYCAADSIT DRRSVAVAHTSYYYWGQGTQVTVSS
< 22C712/1-123	, SEQ ID NO: 2105	; PRT; ->	EVQLVESGGGLVQAGGSLGLSCAASGRTFSNYAM GWFRQAPGKEREFVAGISWSGGHTFYADSVKGRF TISRDNTKNTVYLQMNSMRPEDTAVYYCAARLSS VAVASTRYDYWGQGTQVTVSS
< 11A13/1-125	, SEQ ID NO: 2106	; PRT; ->	EVQLVESGGGLVQAGDSLRLSCVASGGTFGSYAM GWFRQAPGKEREFVATIDWSGDTAFYADSVKGRF TISRDIANDVVYLQMNSLEPEDTAVYYCARNRQS GVASENLRLYTYWGQGTQVTVSS
< 13G93/1-123	, SEQ ID NO: 2107	; PRT; ->	EVQLVESGGGLAQAGDSLRLSCVDSGSSFSAYAM GWFRQAPGKEREFVAAVSWDGRNTYYADSVKGRF TISRDNAKNTLYLQTTSLRPEDTGVYYCAEDKQS GVSVNPKYAYWGQGTQVTVSS
< 12C52/1-118	, SEQ ID NO: 2108	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSGGTFESDTM AWFRQAPGKEREFVARVSWIRTTYYSDSVKGRFT ISKDNAKNTVYLQMNSLKPEDTAVYYCAAQTLGR SLYDYWGQGTQVTVSS
< 12C61/1-126	, SEQ ID NO: 2109	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSNAM AWFRQAPGNERELVSAIGWSGASTYYIDSVEGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAASRYS GGVATARRSEYHYWGQGTQVTVSS
< 21A61/1-125	, SEQ ID NO: 2110	; PRT; ->	EVQLVESGGGLVQAGDSLRLSCVASGDSFNTYTM GWFRQAPGKERFEVAAIRWSGGTTFYGDSVKGRF TISRDYAKNTWYLQMNTLKPEDTAAYYCAAVATY SRNVGSVRNYDYWGQGTQVTVSS
< 11A121/1-126	, SEQ ID NO: 2111	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSEGTFSSYSM GWFRQAPGKDREFVSAITWNGTRTYYRDSVKGRF TISRDNAKNTVQLQMNSLKPEDTAVYYCAVSQPL NYYTYYDARRYDYWGQGTQVTVSS
< 11A91/1-124	, SEQ ID NO: 2112	; PRT; ->	EAQLVESGGGLVQAGGSLRLSCTASGRTYSTTMG WFRQAPGKEREFVAAIRWSGGSAFYADSVKGRFT ISRDNAKNTVYLQMTSLMPEDTAVYYCADTPVYY QRYYDQNAYDYWGQGTQVTVSS
< 13G72/1-118	, SEQ ID NO: 2113	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRAFSSYAM GWFRQAPGKERDFVAAITSSGSTNYADSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCAAKYYSY YAYDYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 13E81/1-124	, SEQ ID NO: 2114	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFSVYHM AWFRQAPGKEREFVAAIRSSGGLFYALSVSGRFT ISRDNAKDTMYLQMNVLKPEDTAVYYCAASPVYY IDYSSQYKYGYWGQGTQVTVSS
< 11B31/1-124	, SEQ ID NO: 2115	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGAFGVYHM GWFRQAPGKEREFVAAIRSGGTTLYEDSVKGRFT ISRDNAKNTVYLRMNSLKPEDTAVYYCATQIYYR TNYYSQNAYDYWGQGTQVTVSS
< 13G81/1-124	, SEQ ID NO: 2116	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFGVYHM GWFRQAPGKEREFVAVIRSGGTTLYADSVKGRFT ISRDDAKNTVYLQMNSLKPEDTAVYLCAAQIYYR TNYYSQNNYDYWGQGTQVTVSS
< 21A53/1-124	, SEQ ID NO: 2117	; PRT; ->	EVQLVESGGGLVQAGGSLELSCAASGGAFGVYHM GWFRQAPGKEREFVAAIRSGGTTLYEDSVKGRVT ISRDDAKNTVYLRMNSLKPEDTAVYYCAAQIYYR TNYYSQNVYDYWGQGTQVTVSS
< 14H51/1-124	, SEQ ID NO: 2118	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFGVYTM AWFRQAPGKEREFVAAIRSGATTLYEDSVKGRFT ISRDDAKNTVYLRMNSLKPEDTAVYYCAAQIYYR TNYYSQNEYDYWGQGTQVTVSS
< 21A21/1-124	, SEQ ID NO: 2119	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFGVYHM GWFRQAPGTEREFVAVIRSGGTTLYEDSVKGRFT ISRDNAKNTVYLRMNSLKPEDTAVYYCAAQIYYR TNYSSQSNYDYWGQGTQVTVSS
< 21A111/1-124	, SEQ ID NO: 2120	; PRT; ->	EVQLVESGGGLVQAGGSLKLSCAVSGRTIVPYTM AWFRQAPGKEREFVAVTRSGGTTFYADSAKGRFT IARDDAKNTVYLQMNSLKPEDTAVYYCALATAYR TNYSSRDKYDYWGQGTQVTVSS
< 22B1212/1-122	, SEQ ID NO: 2121	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSAINSGGGSTSYADSVKGRF TISRDNAKNTLYLQMNSLKPEDTAVYYCAKYLSF YSDYEVYDYWGQGTQVTVSS
< 11A31/1-120	, SEQ ID NO: 2122	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFSSGVM AWFRQSPGEEREFLALITRNGETKKTADSVKGRF TISRDNAKNGVSLQMDSLKAEDTAVYYCASDPTY GSGRWTYWGQGTQVTVSS
< 13E51/1-128	, SEQ ID NO: 2123	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASRHTFSGYAM GWFRQAPGKEREFVAAIRWSGGITYYADSVKGRF TISSDNAKNTVYLQMNSLKPEDTALYYCARSVTY YSGSHAYTQEGGYARWGQGTQVTVSS
< 12D121/1-126	, SEQ ID NO: 2124	; PRT; ->	EVQLVESGGGLVQTGGSLRLSCAASGRAFSTYGM GWFRQAPGKAREFVAAISRSGTGTYYAGSMKGRF TISRDDAKNTVYLQMNSLKPEDTAVYYCAARQPY ASGSHYSSTQYTYWGQGTQVTVSS
< 13F121/1-119	, SEQ ID NO: 2125	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRSFNDYTM GWFRQTPGKEREFVARVWWNGGSAYYADSVKGRF TISIDNAKNTVYLQMNNLTPEDTAVYYCAALYRG RSVYDDWGQGTQVTVSS
< 13G121/1-127	, SEQ ID NO: 2126	; PRT; ->	EVQLVESGGGLVRAGTSLRLSCADSARTFSSAAM GWFRQAPGKEREFVSAISPIGSSKYYADSVKGRF TISRDNAKNTVYLQMDSLKPEDTAVYYCAASSYG STYYSQGRAYYYDYWGQGTQVTVSS
< 22B41/1-124	, SEQ ID NO: 2127	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCTVFGRTFSGDVI GWFRQAPGKEREFVAAISTSGGGTDSADSVKGRF TISKENAKNTVYLQMTILKPEDTAVYYCASSPYG PLYRSTHYYDYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 12D71/1-125	, SEQ ID NO: 2128	; PRT; ->	EVQLVESGGGLVQAGGSLGLSCAASGRTVSTMGW FRQAPGKEREFVTAITWSGDSTNFADSVKGRFTI SRDSAKDTVYLQMNNLKPEDTAVYYCAATTYYSG SYISTLSTSYNYWGQGTQVTVSS
< 13F42/1-111	, SEQ ID NO: 2129	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTLSTTGV GWFRQAPGKGRESVATIFVGGTTYYSDSVKGRFT ISRDNAKNAVNLQMSNLKPEDTALHYCTIGSYRG QGTQVTVSS
< 12C101/1-111	, SEQ ID NO: 2130	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTLSTTGV GWFRQAPGKERESVATIFVGGTTYYSDSVKGRFT ISRDNARNAVNPQMNNLKPEDTAVYYCTIGSYRG QGTQVTVSS
< 14H91/1-127	, SEQ ID NO: 2131	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSRDVM GWFRQAPGKEREFVAAKTWSGASTYYADSVRGRF TISRDNAKNAVYLQMNSLKPEDTAVYYCAARDSS TLDSTYYVGGSYNYWGRGTQVTVSS
< 13F41/1-111	, SEQ ID NO: 2132	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTLSTTGV GWFRQAPGKERESVATIFVGGTTYYSDSVKGRFT ISRDNAKNAVNLQMSNLKPEDTALYYCTIGSYRG QGTQVTVSS
< 14H21/1-125	, SEQ ID NO: 2133	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVRSGGYFGSYHI GWFRQAPGNEREFVAAITWNGASTSYADSVKGRF TISRSIAENTVYLQMNKVKPEDTAVYYCAARMYG SDWLPRPEDFDSWGQGTQVTVSS
< 22B610/1-120	, SEQ ID NO: 2134	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGSIFSINAM GWYRPAPGKQRELVARITSTGSTNYADSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCNADVSPS YGSRWYGWGQGTQVTVSS
< 12C32/1-127	, SEQ ID NO: 2135	; PRT; ->	EMQLVESGGGLVQAGGSLRLSCATSERTFSTYTM AWFRQAPGKEREFVVAIKSSDNSTSYRDSVKGRF TISRDNAKSTMYLQMNSLKPEDTAVYYCAARREY STIYTARYPGEYVYWGQGTQVTVSS
< 12D61/1-116	, SEQ ID NO: 2136	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASRSIFSPNVV GWYRQAPGKQRELVAAVTSGGITNYADSVKGRFT ISRDNAKNTLYLQMNSLKAEDTAVYYCNARERGI YDSWGQGTQVTVSS
< 13G31/1-125	, SEQ ID NO: 2137	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFSRYKM GWFRQAPGKEREFVAASRWSGGIKYHADSVKGRF TISRDDAKNSIYLQMNTLKPEDTAVYYCAADDYL GGDNWYLGPYDSWGQGTQVTVSS
< 22C65/1-124	, SEQ ID NO: 2138	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSGFLFDSYAM GWFRQAPGKEREFVAAIRWSGSATDYSDSVKGRF TISRDNAKNTVYLQMNSLIPEDTAVYYCAARKTY RSLTYYGEYDSWGQGTQVTVSS
< 11A71/1-125	, SEQ ID NO: 2139	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASRSIRSVSVM GWYRLAPGNQRELVATITADGITNYADSVKGRFT VSRDNGRNTVYLQMNSLKPEDTAVYYCNVDRLLY YSSGYYQTSVDVWGQGTQVTVSS
< 11B91/1-125	, SEQ ID NO: 2140	; PRT; ->	EVQLVESGGALVQPGGSLRLSCAASGSIRSINTM GWYRQAPGNQREFVAAVTEGGTTSYAASVKGRFT ISRDKAKNTVLLQMDSLKPEDTAVYYCNADRFLY YSAGRYDTGSDIWGQGTQVTVSS
< 11A81/1-125	, SEQ ID NO: 2141	; PRT; ->	EVQLVESGGALVQPGGSLRLSCAASDSIRSINIM GWYRQAPGKQREFVAAVTEDGSINYAESVKGRFT ISRDKAKNALYLQMNSLKPEDMAVYYCNADRVLY YSDSRYYTGSNYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 11B121/1-127	, SEQ ID NO: 2142	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGSSASINTM GWYRQAPGEQRELVAEITEGGIINYTDSVKGRFT ISRDNAKNTVYLEMNNLKPEDTAVYYCNADRALY RNYSDGRYYTGYDYWGQGTQVTVSS
< 12D31/1-115	, SEQ ID NO: 2143	; PRT; ->	EVQLVESGGGEVQPGGSLRLSCAASRNIFDFNDM GWYRQGPGKEREFVALINVGGVAKYEDSVKGRFT ISRDNAENTVYLQMNNLKPEDMAVYYCNARILSR NYWGQGNQVTVSS
< 11B51/1-127	, SEQ ID NO: 2144	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGTFSGRGM GWFRQAPGKEREFVAAVSWSGGNTYYADSVKGRF TISRDNAKSTVYLQMDSLKPEDTAVYYCAASRRF YSGLYYYTDDAYEYWGQGTQVTVSS
< 13G51/1-127	, SEQ ID NO: 2145	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCAASGGTFNGRAV GWFPQAPGEEREFVTGISWSGGSTDYADSVKGRF TISRDNSKNTVSLQMNSLKPEDTAVYYCAASRRF YSGLVYYSVDAYENWGQGTQVTVSS
< 13F82A/1-130	, SEQ ID NO: 2146	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAISGRTLSGRAM GWFRQAPGKEREFREFVAATSWSGGSKYVADSVT GRFTIFRDNAENTAYLQMNSLNPEDTAVYYCAVT KRYYSIKYYSTVEDYEYWGQGTQVTVSS
< 13E101/1-128	, SEQ ID NO: 2147	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSGRTFNNDHM GWFRQAPGTERELVAATGRRGGPTYYADSVKGRF TISRDNAESTVYLQMNSLKAEDTAVYYCAANRYY CSTYGCLSTPRQYDYWGQGTQVTVSS
< 22B85/1-120	, SEQ ID NO: 2148	; PRT; ->	EVQLVESGGGLVRPGGSLRLSCATSGSDIGINAM GWYRQAPGNQRELVATITGSTGTTYADSVKGRFA ISRDGAKNTVYLQMDSLKPEDTAVYYCNLRVYTG TYGGRNYWGQGTQVTVSS
< 11B12/1-118	, SEQ ID NO: 2149	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRALINYAM GWFRQAPGKEREFVSAINWSGSHTDYGDSVKGRF AISRDNAKNTVYLQMHSLKPEDTAVYHCATGYSL PAFDSWGPGTQVTVSS
< 13G61/1-118	, SEQ ID NO: 2150	; PRT; ->	EVQLVESGGGVVQAGGSLRLSCAPSGRTFSSYVM GWVRQAPGKAREFVAGITRNSGRTRYADSVKGRF TISRDNADNTVTLQMNSLKPEDTAVYYCAGGIDL YTFHYFGQGTQVTVSS
< 14H41/1-118	, SEQ ID NO: 2151	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAPSGRTFSSYVM GWVRQAPGKAREFVAGITRNSIRTRYADSVKGRF TISRDNADNTVTLQMNSLKPEDTAVYYCAGGIDL YTFDYFGQGTQVTVSS
< 11B81/1-126	, SEQ ID NO: 2152	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRPVNNYIM GWFRQALGQGREFVAAINRNGATAAYADSVKGRF TISRDNAEDLLYLQMNLLKPEDTAVYYCAANSDS GFDSYSVWAAYEYWGQGTQVTVSS
< 11C11/1-121	, SEQ ID NO: 2153	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSAYAM GWFRQAPGKERESVATIRWTGGSSSTSYADSVKG RFTISKNTAENTVYLQMNSLKPEDTAVYYCAVLL TVWDTYKYWGQGTQVTVSS
< 12D92/1-123	, SEQ ID NO: 2154	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTYNMAWF RQAPGKEREFVAAMNWSGGSTKYAESVKGRFTIS RANDNNPLYLQMNTLKPEDTAVYYCAATNRWYTG VYDLPSRYEYWGQGTQVTVSS
< 13E61/1-123	, SEQ ID NO: 2155	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCTASGQTFNMGWF RQAPGKEREFVAAISWSQYNTKYADSVKGRFTIS RDNAINSLYLQMDTLKPEDTAVYYCAATNRWFSA VYDLPSRYTYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 22B71/1-114	, SEQ ID NO: 2156	; PRT; ->	EVQLVESGGAFVQPGGSLRLSCAASGSDVWFNVM GWYRQGPGQQLELVASITYGGNINYGDPVKGRFS ISRDNALKTVYLQMNSLKPEDTAVYYCYADLPSR LWGQGTQVTVSS
< 21A121/1-123	, SEQ ID NO: 2157	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCTASGRAFNMGWF RQAPGKEREFVAGVNWGGGSTKVADSVKERFTIS RDYDNSPVYLQMNTLKPEDTAVYYGAATSRWYSA VYDLPTRYDYWGQGTQVTVSS
< 13F101/1-124	, SEQ ID NO: 2158	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCQLSGGTVSDLHM GWFRQAPGKEREFVGFTRWPSITYIAEHVKGRFT ISRDNAKNTVYLQMNSLEREDTAVYYCAADRSYS IDYRHPDSYSYWGQGTQVTVSS
< 11A43/1-123	, SEQ ID NO: 2159	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGSIFRVNHM GWYRQAPGKQREFVAAITSDHITWYADAVKGRFT ISRDNAKNTVTLQMNSLRPEDTAVYYCAADPLLF YGVGSADVDYWGQGTQVTVSS
< 12C81/1-117	, SEQ ID NO: 2160	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAGSGNIVRDNTM AWYRQAPGNQRDLVATINVGGGTYYAGPVKGRFT ISRDNAKNSVYLQMNSLKPEDTSVYYCNVISGLV QRDYWGQGTQVTVSS
< 11B21/1-124	, SEQ ID NO: 2161	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSMYLM GWFRQAPGKEREFVSTINRRGGNTYYADSVKGRF TISRDNARNTVYLQMNSLKPEDTAVYYCAAGGHL LGYDVQWEPDYWGQGTQVTVSS
< 11B71/1-126	, SEQ ID NO: 2162	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFERYAM GWFRQAPGKEREFVATISWSGGRDTVYADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHKR TYELGAHSTDFGSWGQGTQVTVSS
< 12C121/1-126	, SEQ ID NO: 2163	; PRT; ->	EVQLVESGGDLVQPGESLRLSCAVSGVTVDYSGI GWFRQAPEKEREAVSCIESGDGTTTYVDSVKGRF TISRDNAKNAVYLQMNSLKPEDTGVYYCATAVFV DSGDFSVCRGVGYWGKGTQVTVSS
< 22C51/1-121	, SEQ ID NO: 2164	; PRT; ->	EVQLVESGGGLVQAGASLRLSCAASGRTFSRYDI GWFRQAPGKGREFVAAINWSGGTTSFGDSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAALRSW PRGVDSGSWGQGTQVTVSS
< 12D11/1-123	, SEQ ID NO: 2165	; PRT; ->	EVQLVESGGGLVQTGGSLRLSCAASGRTFSGSRM GWFRQAPGKEREFVAAIRWSGGITWYAESVKSRF TISRDNTKNTIDLQINSLKPEDTAVYYCAADVIY KNIGSGSFDYWGQGTQVTVSS
< 12D14/1-123	, SEQ ID NO: 2166	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSGSRM GWLRQAPGKEREFVAAVRWSGGITWYAESVKGRF TISRDNTKNTIDLQINSLKPEDTAVYYCAADVIY KNIGSGSFDYWGQGTQVTVSS
< 12C111/1-123	, SEQ ID NO: 2167	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSGLTFSSYAM GWFRQAPGKVREFVATISRSGGRTSYADSVKGRF IVSRDNAKNTADLQMNDLKPEDTAVYYCGASKWY GGFGDTDIEYWGQGTQVTVSS
< 22B55/1-123	, SEQ ID NO: 2168	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAVSGLTFSTYAM GWFRQAPGKVREFVATISRSGGRTSYADSVKGRF IVSRDNAKNTADLQMNELKPEDTAVYYCGASKWY GGFGDTDIEYWGQGTQVTSS
< 14H121/1-113	, SEQ ID NO: 2169	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGITFRFKAM GWFRQGPGKRRELVARIAGGSTNYADSVKGRFTI SRDDAKNTVFLQMNSLKPEDTAVYYCNVDGPFGN WGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 12C71/1-125	, SEQ ID NO: 2170	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCTASGGTFGSYAL GWFRQSPGKERESVAAIDWDGSRTQYADSVKGRF TISRENVKDTMYLQMNSLQAEDTGVYYCVRSRHS GNTLSFSLKYDYWGQGTQVTVSS
< 21A31/1-125	, SEQ ID NO: 2171	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASEPTFSSVAM GWFRQGPGKEREFAATITWSGDSTYVTDSVKGRF TISRDNARNTAYLQMDSLRPEDTAVYSCAARRWS GTLSLFDNEYYYWGQGTQVTVSS
< 12C91/1-121	, SEQ ID NO: 2172	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTSSYYHM AWFRQAPGKEREFIAAINLSSGSTYYPDSVKGRF TISRGNAKNTVNLQMNSLKPEDTAVYYCAADNYR DSYLEYDYWGQGTQVTVSS
< 14H81/1-125	, SEQ ID NO: 2173	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCAASGRTFSNYRM AWFRQAPRKEREFVAAISRSGESTYFADSMKGRF TISRDNTESTGYLQMNNLKPEDTAVYYCAASWDH GDYVDGGFFYDYWGQGTQVTVSS
< 12C42/1-124	, SEQ ID NO: 2174	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSRYAM HWFRQAPGSERDFVAGISWDGGSTFYANSVKGRF TISRDNAKNMVYLQMNSLKPEDTAVYYCAAAGSA GPPSIDRQYDYWGQGTQVTVSS
< 12D102/1-118	, SEQ ID NO: 2175	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGSSLSFNAM GWSREAPGKRRELVARIISDDSTLYADSVKGRFT ISRDYAKNTAYLQMNSLKPEDTAVYYCVADVRDS IWRSYWGQGTQVTVSS
< 11A52/1-120	, SEQ ID NO: 2176	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRALSNYAM RWFRQAPGKEREFVATINWSGSHTDYRDSVKGRF TISRDNAENTVYLQMNSLTPEDTAVYYCASGWGA TQAQSGFWGQGTQVTVSS
< 14H111/1-120	, SEQ ID NO: 2177	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRALISFAM RWFRQAPGKEREFVAAINWSGTHTDYADSVKGRF TISRDNAENTVYLLMNSLIPEDTAVYYCATGWGA TQAQHGFWGQGTQVTVSS
< 11B61/1-120	, SEQ ID NO: 2178	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTSSGYGM GWFRQAPGKEREFVAAVGWYGSTYFADSVKGRFT IYRDNAQNTMYLQMNSLKPEDTAVYYCAASSSLA TISQPSSWGQGTQVTVSS
< 12E42/1-118	, SEQ ID NO: 2179	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAHSGRAFSLRTM GWYRQAPGNQRELVALISAGDSTYYPDSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCNAKAVTS RDHEYWGQGTQVTVSS
< 13F81A/1-128	, SEQ ID NO: 2180	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFSRYAM GWFRQAPGKEREFVAAISWTGGSSYYGDSVKGRS TISRENAENTVYLQMNSLKPEDTAVYYCAANSDE FYSGTLKLQSRMVEYWGQGTQVTVSS
< 11B102/1-118	, SEQ ID NO: 2181	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGGIFSSHAI SWFRQAFGKAREFVAAINWSGSHRDYADSAKGRF TISRDNAKKTAYLQMNSLRPEDTAVYYCVGGWKT DEYVKWGQGTQVTVSS
< 21A41/1-120	, SEQ ID NO: 2182	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRIFSNYAW SWFRQAPGKERGFVAAINWSGGYTDYADSVKGRF TISRDNTKNTVYLQMNSLKPEDTAVYYCRPGWVT PSYEYGNWGQGTQVTVSS
< 14H101/1-128	, SEQ ID NO: 2183	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFISSPM GWFRQAPGKEREVVAATTRSGGLPYYSDSVKGRF TISRDNAKNTVDLQMSSLKPEDTAAYYCAADQKY GMSYSRLWLVSEYEYWGQGTQVTVSS

TABLE B-2-continued

Preferred Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 12E21/1-115	, SEQ ID NO: 2184	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGSIDSIHVV GWYRKAPGKQREVVAYIGTAGATHYADSVKGRFT ISRDNAENLVYLQMNNLKPEDTAVYYCSAGWGDS AYWGQGTQVTVSS
< 13F21/1-123	, SEQ ID NO: 2185	; PRT; ->	EVQLVESGGGLVQSGGSLRLSCVASGTIVSINAT SWYRQAPGNQRELVATIIGDGRTHYADSVKDRFT ISRDAAANLVYLQMNSLKPSDTAIYSCNANGIES YGWGNRHFNYWTVGTQVTVSS
< 12E33/1-119	, SEQ ID NO: 2186	; PRT; ->	EVQLVESGGGMVQAGGSLRLSCAASGLTLSNYGM GWFRQAPGKEREFVSSINWSGTHTYDADFVKGRF IISRDNAKNTVYLQINSLKPEDTAVYYCAAGGWG TGRYNYWGQGTQVTVSS
< 13G11/1-122	, SEQ ID NO: 2187	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFISNYA MGWFRQAPGKEREFVATINWSGSHSDYADSVKGR FTISRDNAKNTVYLQMNNLKSEDTAVYYCAPGWG TAPLSTSVYWGQGTQVTVSS

TABLE B-3

Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 118N121_A1_4_OK/1-127	, SEQ ID NO: 1988	; PRT; ->	EVQLVESGGGFVQTGGSPRLSCAASGRSFSEYAA AWFRQSPGKERDLVAGIMWDGRSLFYADSVKGRF TISRDNAKNTLHLQMNSLKPEDTAVYYCAYHKTP YTTLELNRPHAFGSWGQGTQVTVSS
< 118N121_A6_2_OK/1-123	, SEQ ID NO: 2188	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGRTFSGYSV GWFRQSPGKEREFVGGINWSGRTYYVDSVKGRFT FSRDNAKNTVYLQMNSLKPEDTAIYLCAVDRFNT IANLPGEYDYWGQGTQVTVSS
< 118N121_B8_1_OK/1-135	, SEQ ID NO: 2189	; PRT; ->	EVQLVESGGGLVQDGGSLRLSCAASGQLANFASY AMGWFRQAPGKAREFVAAIRGSGGSTYIADPARS TYYADFVKGRFTISRDNAKNTVYLQMNSLKPEDT AVYYCACETFNSISNLPGEYDYWGQGTQVTVSS
< 118N121_A2_2_OK/1-124	, SEQ ID NO: 2190	; PRT; ->	KVQLVESGGGLVQAGGSLRLSCAASGRTFSNYSV GWFRQAPGKEREFVAALSKDGARTYYAASVKGRF TIYRDNAKNVVYLQMSVLNGEDTAVYYCAADHFT FMSNLPSEYDYWGQGTQVTVSS
< 118N121_A8_2_OK/1-124	, SEQ ID NO: 2191	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCVISGLTLESHAM GWFRQAPGEEREFVATIRWSGSATFYSDSVKGRF TISRDNAKNTVYLQMNSLKPEDTAVYYCAARKIY RSLSYYGDYDSWGQGTQVTVSS
< 118N121_B3_1_OK/1-123	, SEQ ID NO: 2192	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGRTFSDLAL GWFRRAPGKEREHVAAISSSGVTTIYADSVRGRF TISRDEAKNTVYLEMNSLKTDDTAVYYCAARLTM ATPNQSQYYYWGQGTQVTVSS
< 118N121_A5_2_OK/1-114	, SEQ ID NO: 2193	; PRT; ->	EVQLVESGGGSVQPGGSLRLSCVASGSISSVNAM GWHRQVSGKERELVAIVTDGFTNYADFAKGRFTI SRDNAKTTVYLQMNSLQPEDTARYYCRYSGIGTD NWGQGIEVTVSS
< 118N121_A9_2_OK/1-114	, SEQ ID NO: 2194	; PRT; ->	EVQLVESGGGSVQPGGSLRLSCVASGSISSVNAM GWHRQVPGKQRELVAIVTDGFTNYADFAKGRFTI SRDNAKTTVYLQMNSLQPEDTARYYCRYSGIGTD NWGQGIEVTVSS

TABLE B-3-continued

Nanobodies against HER2 obtained as described in Example 4

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 118N121_A7_1_OK/ 1-122	, SEQ ID NO: 2195	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGNIKSIDVM GWHRQAPGKERELVSDISFGGNTNYANSVKGRFT ISRDNAKNTVYLQMNSLKPEDTAVYYCYADILYK TDIYYRNDFWGQGTQVTVSS
< 118N121_A10_1_OK/ 1-131	, SEQ ID NO: 2196	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGFSFADYAI GWFRQAPGKEREGVSCIANSEGTKYYADSAQGRL PISSDNAKKTVYLQMDSLKPEDTAVYYCAALPYT ICPVVVKKGAVYYGVDDYWGKGTQVTVSS
< 118N121_A11_1_OK/ 1-120	, SEQ ID NO: 2197	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFPFGMYGM RWVRQAPGKGPERVSSINSDGDTTYYADSVKGRF TISRDNDENMLYLQMNSLKPEDTAVYYCATGFSD RSFAVTHKGQGTQVTVSS
< 118N121_B7_4_OK/ 1-124	, SEQ ID NO: 2198	; PRT; ->	EVQLVESGGGLEQAGGSLRLSCAASGLTFRSAAM GWFRQGPGKEREFVAAISRDGAATYYTDSVKGRF TISRDNAKNTVFLQMNSLKPEDTAIYYCAADFRL ARLRVADDYDYWGQGTQVTVSS
< 118N121_B2_1_OK/ 1-130	, SEQ ID NO: 2199	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGFSLDDRAI AWFRQAPGKAREGVSCITPHHGGIIFTRESVKGR FATSSDSAKNTVYLQMHSLKPEDTAVYYCATLRT DYSINWANCQRDSLYGYWGQGTQVTVSS
< 118N121_B7_1_OK/ 1-119	, SEQ ID NO: 2200	; PRT; ->	EMQLVESGGGLVQPGGSLRLSCAASGNIPPINAM AWYRQAPGNERELVAAVTSGGGTNYATSVKGRFI ISRDDSKNTVDLQMNSLKPEDTAVYYCNLGGWTR THPFDYWGQGTQVTVSS

TABLE B-4

Bivalent Nanobodies against HER2 as described in Example 12

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 2A4-9GS-2A4	, SEQ ID NO: 2201	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDY AMSWVRQAPGKGLEWVSAINWSGSHRNYADSV KGRFTISRDNAKKTVYLQMNSLQSEDTAVYYC GTGWQSTTKNQGYWGQGTQVTVSSGGGGSGGG SEVQLVESGGGLVQPGGSLRLSCAASGFIFDD YAMSWVRQAPGKGLEWVSAINWSGSHRNYADS VKGRFTISRDNAKKTVYLQMNSLQSEDTAVYY CGTGWQSTTKNQGYWGQGTQVTVSS
< 2A5-9GS-2A5	, SEQ ID NO: 2202	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCATSGFTFDDY AMTWVRQAPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGSEVQLVESGGGLVQPGGSLRLSCATSGF TFDDYAMTWVRQAPGKGLEWVSSINWSGTHTD YTDSVKGRFTISRNNANNTLYLQMNSLKSEDT AVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
< 2C3-9GS-2C3	, SEQ ID NO: 2203	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLRSEDTAVYYC NQGWKIVPTDRTGHGTQVTVSSGGGGSGGGSE VQLVESGGGLVQPGGSLRLSCVASGFSLDDYG MTWVRQAPGKGLEWVSSINWSGTHTDYADSVK GRFTISRDNAKNTLFLQMNSLRSEDTAVYYCN QGWKIVPTDRTGHGTQVTVSS
< 2D3-9GS-2D3	, SEQ ID NO: 2204	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMSWVRQVPGKGLEWVSSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC

TABLE B-4-continued

Bivalent Nanobodies against HER2 as described in Example 12

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
			AKNWRDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGSEVQLVESGGSLVQPGGSLRLSCAASGF TFDDYAMSWVRQVPGKGLEWVSSINWSGTHTD YADSVKGRFTISRNNANNTLYLQMNSLKSEDT AVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
< 5F7-9GS-5F7	, SEQ ID NO: 2205	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFSIN TMGWYRQAPGKQRELVALISSIGDTYYADSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCK RFRTAAQGTDYWGQGTQVTVSSGGGGSGGGSE VQLVESGGGLVQAGGSLRLSCAASGITFSINT MGWYRQAPGKQRELVALISSIGDTYYADSVKG RFTISRDNAKNTVYLQMNSLKPEDTAVYYCKR FRTAAQGTDYWGQGTQVTVSS

TABLE B-5

Bispecific Nanobodies against HER2 and against serum albumin as described in Example 12

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 2C3-9GS-ALB1	, SEQ ID NO: 2206	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLRSEDTAVYYC NQGWKIVPTDRTGHGTQVTVSSGGGGSGGGSE VQLVESGGGLVQPGNSLRLSCAASGFTFRSFG MSWVRQAPGKEPEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLKPEDTAVYYCT IGGSLSRSSQGTQVTVSS
< 2A4-9GS-ALB1	, SEQ ID NO: 2207	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDY AMSWVRQAPGKGLEWVSAINWSGSHRNYADSV KGRFTISRDNAKKTVYLQMNSLQSEDTAVYYC GTGWQSTTKNQGYWGQGTQVTVSSGGGGSGGG SEVQLVESGGGLVQPGNSLRLSCAASGFTFRS FGMSWVRQAPGKEPEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLKPEDTAVYY CTIGGSLSRSSQGTQVTVSS
< 2A5-9GS-ALB1	, SEQ ID NO: 2208	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCATSGFTFDDY AMTWVRQAPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGSEVQLVESGGGLVQPGNSLRLSCAASGF TFRSFGMSWVRQAPGKEPEWVSSISGSGSDTL YADSVKGRFTISRDNAKTTLYLQMNSLKPEDT AVYYCTIGGSLSRSSQGTQVTVSS
< 2D3-9GS-ALB1	, SEQ ID NO: 2209	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMSWVRQVPGKGLEWVSSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWRDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGSEVQLVESGGGLVQPGNSLRLSCAASGF TFRSFGMSWVRQAPGKEPEWVSSISGSGSDTL YADSVKGRFTISRDNAKTTLYLQMNSLKPEDT AVYYCTIGGSLSRSSQGTQVTVSS
< 5F7-9GS-ALB1	, SEQ ID NO: 2210	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFSIN TMGWYRQAPGKQRELVALISSIGDTYYADSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCK RFRTAAQGTDYWGQGTQVTVSSGGGGSGGGSE VQLVESGGGLVQPGNSLRLSCAASGFTFRSFG MSWVRQAPGKEPEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLKPEDTAVYYCT IGGSLSRSSQGTQVTVSS

TABLE B-6

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27B3-35GS-2D3	, SEQ ID NO: 2211	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIR TMAWYRQPPGNQREWVATIGSNGFATYPDSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCR DINRDIWGQGSQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27C3-35GS-2D3	, SEQ ID NO: 2212	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDY ATSWVRQAPGKGPEWVSAINSGGGSTYYADSV KGRFTISRDNAKNTLYLQMNSLKPEDTAVYYC ARPRGSSLYLLEYDYWGQGTQVTVSSGGGGSG GGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ LVESGGSLVQPGGSLRLSCAASGFTFDDYAMS WVRQVPGKGLEWVSSINWSGTHTDYADSVKGR FTISRNNANNTLYLQMNSLKSEDTAVYYCAKN WRDAGTTWFEKSGSAGQGTQVTVSS
< 27E5-35GS-2D3	, SEQ ID NO: 2213	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIR TMAWYRQPPGNQREWVATIGSNGFATYPDSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCR DINRDIWGQGSQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27F2-35GS-2D3	, SEQ ID NO: 2214	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIISSFR TLAWYRQAPGKQRDWVATISSAGGTAYADAVK GRFTISISRDNVEYTVDLQMDSLKPEDTAVYY CRDINGDYWGQGTQVTVSSGGGGSGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSEVQLVESGGS LVQGGSLRLSCAASGFTFDDYAMSWVRQVPG KGLEWVSSINWSGTHTDYADSVKGRFTISRNN ANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTT WFEKSGSAGQGTQVTVSS
< 27D6-35GS-2D3	, SEQ ID NO: 2215	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIR TMAWYRQPPGNQREWVATIGSNGFATYPDSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCR DINRDIWGQGSQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 30D10-35GS-2D3	, SEQ ID NO: 2216	; PRT; ->	EVQLVESGGGLVQAGGSLTLSCTASETTVRIR TMAWYRQPPGNQREWVATIGSNGFATYPDSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCR DINRDIWGQGSQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 47D5-35GS-2D3	, SEQ ID NO: 2217	; PRT; ->	KVQLVESGGGLVQGGSLRLSCAASGSIFGFN DMAWYRQAPGKQRELVALISRVGVTSSADSVK GRFTISRVNAKDTVYLQMNSLKPEDTAVYYCY MDQRLDGSTLAYWGQGTQVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVE SGGSLVQPGGSLRLSCAASGFTFDDYAMSWVR QVPGKGLEWVSSINWSGTHTDYADSVKGRFTI SRNNANNTLYLQMNSLKSEDTAVYYCAKNWRD AGTTWFEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< DUMMY-35GS-2D3	, SEQ ID NO: 2218	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFRSY PMGWFRQAPGKEREFVASITGSGGSTYYADSV KGRFTISRDNAKNTVYLQMNSLRPEDTAVYSC AAYIRPDTYLSRDYRKYDYWGQGTQVTVSSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG SEVQLVESGGSLVQPGGSLRLSCAASGFTFDD YAMSWVRQVPGKGLEWVSSINWSGTHTDYADS VKGRFTISRNNANNTLYLQMNSLKSEDTAVYY CAKNWRDAGTTWFEKSGSAGQGTQVTVSS
< 2D3-35GS-2D3	, SEQ ID NO: 2219	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMSWVRQVPGKGLEWVSSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWRDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< DUMMY-35GS-47D5	, SEQ ID NO: 2220	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGRTFRSY PMGWERQAPGKEREFVASITGSGGSTYYADSV KGRFTISRDNAKNTVYLQMNSLRPEDTAVYSC AAYIRPDTYLSRDYRKYDYWGQGTQVTVSSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG SEVQLVESGGGLVQPGGSLRLSCAASGSIFGF NDMAWYRQAPGKQRELVALISRVGVTSSADSV KGRFTISRVNAKDTVYLQMNSLKPEDTAVYYC YMDQRLDGSTLAYWGQGTQVTVSS
< 5F7-35GS-47D5	, SEQ ID NO: 2221	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFSIN TMGWYRQAPGKQRELVALISSIGDTYYADSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCK RFRTAAQGTDYWGQGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQ APGKQRELVALISRVGVTSSADSVKGRFTISR VNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDG STLAYWGQGTQVTVSS
< 47D5-35GS-5F7	, SEQ ID NO: 2222	; PRT; ->	KVQLVESGGGLVQPGGSLRLSCAASGSIFGFN DMAWYRQAPGKQRELVALISRVGVTSSADSVK GRFTISRVNAKDTVYLQMNSLKPEDTAVYYCY MDQRLDGSTLAYWGQGTQVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVE SGGGLVQAGGSLRLSCAASGITFSINTMGWYR QAPGKQRELVALISSIGDTYYADSVKGRFTIS RDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAA QGTDYWGQGTQVTVSS
< 2D3-35GS-47D5	, SEQ ID NO: 2223	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMSWVRQVPGKGLEWVSSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWRDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGSE VQLVESGGGLVQPGGSLRLSCAASGSIFGFND MAWYRQAPGKQRELVALISRVGVTSSADSVKG RFTISRVNAKDTVYLQMNSLKPEDTAVYYCYM DQRLDGSTLAYWGQGTQVTVSS
< 27F7-35GS-2D3	, SEQ ID NO: 2224	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVVSGIPSTIR AMAWYRQAPGRQRDWVATIYSPSGSAVYADSV KGRFTISSDNAKKTIYLQMNSLKPDDTAVYYC RDVNREYWGQGTQVTVSSGGGGSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSEVQLVESGGSL VQPGGSLRLSCAASGFTEDDYAMSWVRQVPGK GLEWVSSINWSGTHTDYADSVKGRFTISRNNA NNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTW FEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 28F6-35GS-2D3	, SEQ ID NO: 2225	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTR TMAWYRQPPGNERDWVATIRSGAPVYADSVKG RFTVSRDNAKNTLYLQMNSLEPEDTATYYCWD VNGDIWGQGTPVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTFDDYAMSWVRQVRGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS
< 28G3-35GS-2D3	, SEQ ID NO: 2226	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTR TMAWYRQPPGNERDWVATIRSGAPVYADSVKG RFTVSRDNAKNTLYLQMNSLEPEDTATYYCWD VNGDIWGQGTPVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTFDDYAMSWVRQVPGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS
< 28G5-35GS-2D3	, SEQ ID NO: 2227	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EYSGSAGQGTQVTVSS
< 29D9-35GS-2D3	, SEQ ID NO: 2228	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASRIPFSTR TMAWYRQAPGKQRDWVATIGTSGPPRYADSVK GRFTVSRDNAKNTVYLQMNSLKAEDTAVYYCW DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 29E9-35GS-2D3	, SEQ ID NO: 2229	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASRIPASIR TMAWYRQTPGNQRDWLATIGSSGTPAYADSVK GRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCR DVNGDYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTEDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 30E10-35GS-2D3	, SEQ ID NO: 2230	; PRT; ->	KVQLVESGGSLVPPGGSLRLSCAASGFTFDDY AMTWVRQAPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 31D11-35GS-2D3	, SEQ ID NO: 2231	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTR TMAWYRQPPGNERDWVATIRSGAPVYADSVKG RFTVSRDNAKNTLYLQMNSLEPEDTATYYCWD VNGDIWGQGTPVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTFDDYAMSWVRQVPGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27G2-35GS-2D3	, SEQ ID NO: 2232	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMTWVRQTPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSDDTAVYYC AKNWGDAGTTWFEKSGSAGPGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGSE VQLVESGGSLVQPGSSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< P27G4-35GS-2D3	, SEQ ID NO: 2233	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27G5-35GS-2D3	, SEQ ID NO: 2234	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASRIPASIR TMAWYRQTPGNQRDWLATIGSSGTPAYADSVK GRFTVSRDNAKFTVYLQMNSLKPEDTAVYYCR DVNGDYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27G7-35GS-2D3	, SEQ ID NO: 2235	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27H1-35GS-2D3	, SEQ ID NO: 2236	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27H2-35GS-2D3	, SEQ ID NO: 2237	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27H3-35GS-2D3	, SEQ ID NO: 2238	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMTWVRQASGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27H4-35GS-2D3	, SEQ ID NO: 2239	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRY TMGWYRQAPGKQRDLVASIDASGGTNYADSVK GRFTISRDNAKNTVYLEMNSLKPEDTGVYYCN GRWDIVGAIWWGQGTQVTVSSQGGGSGGGGSG GGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GSLVQPGGSLRLSCAASGFTFDDYAMSWVRQV PGKGLEWVSSINWSGTHTDYADSVKGRFTISR NNANNTLYLQMNSLKSEDTAVYYCAKNWRDAG TTWFEKSGSAGQGTQVTVSS
< 27H5-35GS-2D3	, SEQ ID NO: 2240	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDY GIGWFRQASGKEREGVSCITSSDGSTYYADSV KGRFTISSDNAKNTVYLQMNSLKPEDTAVYYC AALPFVCPSGSYSDYGDEYDYWGQGTQVTVSS GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGSLVQPGGSLRLSCAASGFTF DDYAMSWVRQVPGKGLEWVSSINWSGTHTDYA DSVKGRFTISRNNANNTLYLQMNSLKSEDTAV YYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
< 27H7-35GS-2D3	, SEQ ID NO: 2241	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIAFRIR TMAWYRQAPGKQRDWVATSDSGGTTLYADSVK GRFTVSRDNAENTVYLQMNSLKPEDTAVYYGR DVNRDYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27A3-35GS-2D3	, SEQ ID NO: 2242	; PRT; ->	EVQLVESGGGLVQAGGSLSLSCVASGRFFSTR VMAWYRQTPGKQREFVASMRGSGSTNYADSVR GRFAISRDNAKNTVYLQMNTLKPEDTAVYYCR DINEDQWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27A4-35GS-2D3	, SEQ ID NO: 2243	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTR TMAWYRQAPGKQRDWVATISSHGLPVYADSVK GRFTVSRDNANNTVYLQMNTLKPEDTAVYYCR DVNADYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27A5-35GS-2D3	, SEQ ID NO: 2244	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTR TMAWYRQPPGNERDWVATIRSGAPVYADSVKG RFTVSRDNAKNTLYLQMNSLEPEDTATYYCWD VNGDIWGQGTPVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTEDDYAMSWVRQVPGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS
< 27B1-35GS-2D3	, SEQ ID NO: 2245	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMSWVRQAPGKGLEWISSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNNLKFEDTAVYYC AKNWRDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27B2-35GS-2D3	, SEQ ID NO: 2246	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCVASGIPSIRA IAWYRQAPGKQRDWVATSGTGYGATYDDSVKG RFTLSRDNAKNTVYLQMNSLKPEDTAVYYCRD VNRDYWGQGTQVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTFDDYAMSWVRQVPGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS
< 27B5-35GS-2D3	, SEQ ID NO: 2247	; PRT; ->	EVQLVESGGGLVQAGGSLRLPCAASGIAFRIR TMAWYRQAPGKQRDWVATSDSGGTTLYADSVK GRFTVSRDNAENTVYLQMNSLKPEDTAVYYCR DVNRDYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27B7-35GS-2D3	, SEQ ID NO: 2248	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISSGGGSITTYADS VKGRFTISRDNAKNTLYLQMSSLKPEDTALYY CAKARSSSSYYDFGSWGQGTQVTVSSGGGGSG GGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ LVESGGSLVQPGGSLRLSCAASGFTFDDYAMS WVRQVPGKGLEWVSSINWSGTHTDYADSVKGR FTISRNNANNTLYLQMNSLKSEDTAVYYCAKN WRDAGTTWFEKSGSAGQGTQVTVSS
< 27C2-35GS-2D3	, SEQ ID NO: 2249	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMTWVRQASGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 27C5-35GS-2D3	, SEQ ID NO: 2250	; PRT; ->	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTR TMAWYRQPPGNERDWVATIRSGAPVYADSVKG RFTVSRDNAKNTLYLQMNSLEPEDTATYYCWD VNGDIWGQGTPVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGSLVQ PGGSLRLSCAASGFTFDDYAMSWVRQVPGKGL EWVSSINWSGTHTDYADSVKGRFTISRNNANN TLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFE KSGSAGQGTQVTVSS
< 27C7-35GS-2D3	, SEQ ID NO: 2251	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGIAFRIR TMAWYRQAPGKQRDWVATSDSGGTTLYADSVK GRFTVSRDNADNTVYLQMNSLKPEDTAVYYCR DVNRDYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKG LEWVSSINWSGTHTDYADSVKGRFTISRNNAN NTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWF EKSGSAGQGTQVTVSS
< 27D1-35GS-2D3	, SEQ ID NO: 2252	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLTPEDTAVYYC NQGWKILPAERRGHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 27D2-35GS-2D3	, SEQ ID NO: 2253	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFS RRTMAWYRQAPGKQRDWVATIAGDGSTVYADS MKGRFTISRDNAKNTVYLQVNSLKPEDTAVYY CWDTNGDYWGQGTQVTVSSGGGGSGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSEVQLVESGGS LVQPGGSLRLSCAASGFTFDDYAMSWVRQVPG KGLEWVSSINWSGTHTDYADSVKGRFTISRNN ANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTT WFEKSGSAGQGTQVTVSS
< 27D3-35GS-2D3	, SEQ ID NO: 2254	; PRT; ->	EVQLMESGGGLVQPGGSLRLSCAASGLGIAFS RRTMAWYRQAPGKQRDWVATIAGDGSTVYADS MKGRFTISRDNAENTVYLQMNSLKPEDTAVYY CWDVNRDYWGQGTQVTVSSGGGGSGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSEVQLVESGGS LVQPGGSLRLSCAASGFTFDDYAMSWVRQVPG KGLEWVSSINWSGTHTDYADSVKGRFTISRNN ANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTT WFEKSGSAGQGTQVTVSS
< 27D4-35GS-2D3	, SEQ ID NO: 2255	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMTWVRQASGKGLEWVSSINWSGTHTDYADSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 27D7-35GS-2D3	, SEQ ID NO: 2256	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLSPEDTAVYYC NQGWKILPTNRGSHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS
< 27E2-35GS-2D3	, SEQ ID NO: 2257	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLTPEDTAVYYC NQGWKIIPTDRRGHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS
< 27E4-35GS-2D3	, SEQ ID NO: 2258	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGSTFSIN RMAWYRQSPGKQRELVAAVDNDDNTEYSDSVA GRFTISRDNAKNAVHLQMNSLRLEDTAVYYCN AKQLPYLQNFWGQGTQVTVSSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GSLVQPGGSLRLSCAASGFTFDDYAMSWVRQV PGKGLEWVSSINWSGTHTDYADSVKGRFTISR NNANNTLYLQMNSLKSEDTAVYYCAKNWRDAG TTWFEKSGSAGQGTQVTVSS
< 27E7-35GS-2D3	, SEQ ID NO: 2259	; PRT; ->	EVQLVESGGGLVQAGGSLRLSCAASGITFRRY DMGWYRQFPGKERELVATILSEGDTNYVDPVK GRFTISRDNAKNTVYLQMNDLKPEDTAVYYCN GVWRAIGRTYWGQGTQVTVSSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GSLVQPGGSLRLSCAASGFTFDDYAMSWVRQV PGKGLEWVSSINWSGTHTDYADSVKGRFTISR NNANNTLYLQMNSLKSEDTAVYYCAKNWRDAG TTWFEKSGSAGQGTQVTVSS

TABLE B-6-continued

Biparatopic Nanobodies against HER2

< Name	, SEQ ID NO: #	; PRT (protein)	Amino acid sequence
< 29H1-35GS-2D3	, SEQ ID NO: 2260	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDY AMSWVRQAPGKGLEWVSSINWSGTHTGYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 30H9-35GS-2D3	, SEQ ID NO: 2261	; PRT; ->	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDY AMTWVRQAPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRNNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 39C1-35GS-2D3	, SEQ ID NO: 2262	; PRT; ->	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDY GMSWVRQAPGKGLEWVSSINWSGTHTDYTDSV KGRFTISRMNANNTLYLQMNSLKSEDTAVYYC AKNWGDAGTTWFEKSGSAGQGTQVTVSSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGSLVQPGGSLRLSCAASGFTFDDYA MSWVRQVPGKGLEWVSSINWSGTHTDYADSVK GRFTISRNNANNTLYLQMNSLKSEDTAVYYCA KNWRDAGTTWFEKSGSAGQGTQVTVSS
< 27G8-35GS-2D3	, SEQ ID NO: 2263	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAYNTLFLQMNSLTPEDTAVYYC NQGWKILPAERRGHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS
< 29H2-35GS-2D3	, SEQ ID NO: 2264	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDY GMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNNLTPEDTAVYYC NQGWKIIPTDRRGHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS
< 38C6-35GS-2D3	, SEQ ID NO: 2265	; PRT; ->	EVQLVESGGGLVQPGGSLRLSCVGSGFSLDDY AMTWVRQAPGKGLEWVSSINWSGTHTDYADSV KGRFTISRDNAKNTLFLQMNSLSPEDTAVYYQ NQGWKIRPTIPMGHGTQVTVSSGGGGSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQ VPGKGLEWVSSINWSGTHTDYADSVKGRFTIS RNNANNTLYLQMNSLKSEDTAVYYCAKNWRDA GTTWFEKSGSAGQGTQVTVSS

TABLE C-1

Sequence of HER2 binding Nanobodies aligned by family

HERCEPTIN ® COMPETING

13D11	EVQLVESGGGLVHPGGSLRLSCVGSGFSLDDYGMTWVRRAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLNPEDTAVYYCGQGWKIVPTNPRGHGTQVTVSS
2B4	EVQLVESGGGLVQPGGSLRLSCVGSGFSLDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKIRPTIPMGHGTQVTVSS
2G2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYTDPVKGRFTISRDNAKNTLFLQMNNLTPEDTAVYYCNRGWKIVPTDLGGHGTQVTVSS
13D2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNNLRSEDTAVYSCNQGWKIVPTDRGGHGTQVTVSS
2D5	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKIVPTDRGGHGTQVTVSS
2F4	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKIVPTDRRGHGTQVTVSS
2C3	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKIVPTDRTGHGTQVTVSS
17E3	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTMGWYRQAPGKQRDLVA SIDSSGGTNYADSVKGRFTISRDNAKNTVYLEMNSLTPEDTAVYYCNQGWKIVPTDRTGHGTQVTVSS
17H3	EVQLMESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKIVPTDRGGHGTQVTVSS
17D2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLRSEDTAVYYCNQGWKIVPTDRGSHGTQVTVSS
2F1	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKELEWISSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIVPMDRRGHGTQVTVSS
2E2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
2C2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNARNTLFLQMNSLTPEDTAIYYCNQGWKILPTDRRGHGTQVTVSS
2E3	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKILPTNRGSHGTQVTVSS
13B10	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGFEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKILPTNRGSHGTQVTVSS
2D1	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNRGWKILPTNRGSHGTQVTVSS
2H3	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
2H1	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVRGFVISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
2C1	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
15C5	EVQLVESGGGLVQPGGSLKLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWNVTHTDYAYSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
2B3	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDCADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
29H2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNNLTPEDTAVYYCHQGWKIIPTDRRGHGTQVTVSS
17E4	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFVISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKIIPTDRRGHGTQVTVSS
17A2	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNKGWKVWPTDRGTHGTQVTVSS

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

15D1	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLNPEDTAVYYCNQGWKVWPTDRGTHGTQVTVSS
17B8	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMSLTPEDTAVYYCNQGWKILPAERRGHGTQVTVSS
15C11	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKILPAERRGHGTPVTVSS
15G8	EVQLVESGGGLVQPGGSLKLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINMNGTHTDYAYSVKGRFTISRDNAKNTLFLQMNSLTPENTAVYYCNQGWKILPAERRGHGTQVTVSS
17H4	EVQLVESGGGLVQPGGSLRLSCVASGFSLINYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLHMNNLSPEDTAVYYCGQGWKIHPADRGGHGTQVTVSS
27G8	EVQLVESGGGLVQPGGSLRLSCVASGFSLDDYGMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVYYCNQGWKILPAERRGHGTQVTVSS
38C6	EVQLVESGGGLVQPGGSLRLSCVGSGFSLDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRDNAKNTLFLQMNSLSPEDTAVYYCNQGWKIRPTIPMGHGTQVTVSS
2A4	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAMSWVRQAPGKGLEWVSAINWSGSHRNYADSVKGRFTISRDNAKKTVYLQMNSLQSEDTAVYYCGTGWQSTTKNQGYWGQGTQVTVSS
15G7	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAMSWVRQAPGKGLEWVSAINWSGTHRNYADSVKGRFTISRDNNKKTVYLQMNSLKSEDTAVYYCATGWQSTTKNQGYWGQGTQVTVSS
15B7	EVQLVESGGGLVQPGGSLKLSCAASGFIFDDYAMSWVRQAPGKGLEWVSAINWSGSHRNYADSVKGRFTISRDNAKKTVYLQMNSLQSEDTAVYYCGTGWQSTTKSQGYWGQGTQVTVSS
5G4	EVQLVESGGGLVQPGGSLTLSCAGSGFIFDDYAMSWVRQAPGKGLEWVSSINWSGSHRNYADSVKGRFTISRDNAKKTLYLQMNSLKSEDTAVYYCATGWQSTTKNQNYWGQGTQVTVSS
13B2	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWISSINWSGTHKDYADSVKGRFTISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
2E5	EVQLVESGGSLVQPGESLRLSCAASGFTFDDYAMSWVRQAPGKGLEWISSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
15G1	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
27B1	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWISSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
17E7	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
17D8	EVQLVESGGSLVPPGGSLRLSCAVSGFTFDDYAMSWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNMLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
5F8	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYALSWVRQAPGKGLEWISSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNNLKFEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
2D4	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGDAGTTWFEKSGSAGPGTQVTVSS
13D8	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQASGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
17G8	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSSINWSGTHTGYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
2H4	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
2F3	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTGSVKGRFTISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGDAGTTWFEKSGSAGPGTQVTVSS
2F5	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
30E10	KVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

29H1	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSSINWSGTHTGYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
17E2	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
2B1	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGDAGTTWFEKSGSAGPGTQVTVSS
2A5	EVQLVESGGGLVQPGGSLRLSCATSGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
13C12	EVQLVESGGSLVQPGGSLRLSCATSGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
17E10	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDCTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
27D4	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQASGKGLEWVSSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
15F9	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTGSVKGRFTISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
30H9	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
39C1	EVQLVESGGSLVPPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
27G2	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMTWVRQTPGKGLEWVSSINWSGTHTDYTDSVKGRFTISRNNANNTLYLQMNSLKSDDTAVYYCAKNWGDAGTTWFEKSGSAGQGTQVTVSS
2D3	EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVSS
5F7	EVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTQVTVSS
PBMP118N121_A1_4_OK/ 1-127	EVQLVESGGGFVQTGGSPRLSCAASGRSFSEYAAAWFRQSPGKERDLVAGIMWDGRSLFYADSVKGRFTISRDNAKNTLHLQMNSLKPEDTAVYYCAYHKTPYTTLELNRPHAFGSWGQGTQVTVSS
	OT-FAB COMPETING
47D5	KVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVGVTSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAYWGQGTQVTVSS
	HER2 BINDING
14B11	EVQLVESGGGLVQAGGSLRLSCAASGSTFSSYGMGWFRQVPGKEREFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTARYYCGVETYGSGSSLMTEYDYWGQGTQVTVSS
14B10	EVQLVESGGGLVQAGGSLRLSCAVNSRTFSSYGMGWFRQAPGKEREFVATINWSGVTAYADSIKGRFTISRDNAKETVYLQMNSLKPDDTGVYYCAAETYGSGSSLMSEYDYWGQGTVTVSS
14B4	EVQLVESGGGLVQAGGSLRLSCAVSSRAFSSYGMGWFRQAPGKDREFVATINWSGVTAYADSIKGRFTISRDNAKETVYLQMNSLKPEDTGVYYCAAETYGSGSSLMSEYDYWGQGTQVTVSS
14C11	EVQLVESGGGLVQAGGSLRLSCAVNSRTFSSYGMGWFRQAPGKEREFVATINWSGATAYADSIKGRFTISRDNAKETVYLQMNSLKPDDTGVYYCAAETYGSGSSLMSEYDYWGQGTQVTVSS
14B5	EVQLVESGGGLVQAGGSLRLSCAVSSRAFSSYGMGWFRQAPGKDREFVATINWSGVTAYADSIKGRFTISRDNAKETVYLQMNSLKPDDTGVYYCAAETFGSGSSLMSEYDYWGQGTQVTVSS
14C6	EVQLVESGGGSVQAGGSLRLSCVASEGTFSSYGMGWFRQAPGKERAFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCATDTYGSGSSLMNEYDYWGQGTQVTVSS
14A4	EVQLVESGGGSVQAGSSLTLSCVASEGTFSSYGMGWFRQAPGKERAFVATINWSGVNAYADSVKGRFTISRDNAKKTAYLQMNSLKPEDTAVYYCAAETYGSGSSLMNEYDYWGQGTQVTVSS

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

14B3	EVQLVESGGGLVQPGGSLTLSCVASEGTFSSYGMGWFRQAPGKERAFVATINWSGVNAYADSVKGRFTISRDNAKKTAYLQMNSLKPEDTAVYYCAAETYGSGSSLMNEYDYWGQGTQVTVSS
14C1	EVQLVESGGGSVQAGGSLRLSCAASGSTFSSYGMGWFRQAPGKERAFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCATETYGSGSSLMNEYDYWGQGTQVTVSS
14A12	EVQLVKSGGGLVQAGGSLRLSCAASERTFSSYGMGWFRQAPGKEREFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGSGSSLISEYDYWGHGTQVTVSS
14A2	EVQLVESGGGLVQAGGSLRLSCAASERTFSSYGMGWFRQAPGKEREFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGSGSSLISEYDYWGHGTQVTVSS
14A1	EVQLVESGGGSVQAGGSLRLSCAASERTFSSYGMGWFRQAPGKEREFVATINWSGVTAYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCAAEPYGSGSSLMSEYDYWGHGTQVTVSS
17C3	EVQLVESGGGLVQAGGSLRLSCAANGLTFRRYDMGWYRQAPGQQREWVAAISGAGDINYADSVKGRFTMARDNANHTVHLQMNSLKPEDTAVYYCNANWKMLLGVENDYWGQGTQVTVSS
46D3	KVQLVESGGGLVQAGGSLRLSCAASGRTFTEYSMGWFRQAPGKEREFVATISWNYGYTYYSDSVKGRFTVGRDIAENTVYLQMNTLKSEDTAVYYCAAKIGWLSIRGDEYEYWGQGTQVTVSS
27H5	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYGIGWFRQASGKEREGVSCITSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPED TAVYYCAALPFVCPSGSYSDYGDEYDYWGQGTQVTVSS
17C2	EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYAMSWVRQAPGKGLEWVSAVDSGGGRTDYAHSVKGRFTISRDNAKNTLYLQMSSLKPEDTALYYCTKHVSDSDYTEYDYWGQTQVTVSS
17D11	EVQLVESGGGLVQAGGSLRLSCTASGRTSSTSAMGWFRQAPGKEREFVATISRGGSATYYADSLKGRFTISRDNAKNTLYLQMNSLKP EDTAVYYCAARRSSLYTSSNVFEYDYWGQGTQVTVSS
15A6	EVQLVESGGGLVQAGGSLRLSCVTTSRRPASTRTMAWYRQAPGKQRDWVATISSHGLPVYADSVKGRFTVSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADYWGQGTQVTVSS
17B6	EVQLVESGGGLVQPGGSLRLSCAASRIPFSTRTMAWYRQAPGKQRDWVATIGTSGPPRYADSVKGRFTVSRDNAKNTVYLQMNSLKAEDTAVYYCWDVNADYWGQGTQVTVSS
17C5	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTRTMAWYRQAPGKQRDWVATISSHGLPVYADSVKGRFTVSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADYWGQGTPVTVSS
15E11	EVQLVESGGGLVQAGGSLRLSCVASRIPFSSRTMAWYRQAPGKQRDWVATISARGMPAYEDSVKGRFTVSRDNDKNTLYLQMNSLKPEDTAVYYCRDVNADYWGQGTQVTVSS
15C2	EVQLVESGGGLVQAGGSLRLSCVTSRRPASTRTMAWYRQAQGKQRDWVATISSHGLPVYADSVKGRFTVSRDNANNTVYLQMNTLKPEDTAVYYCRDVNADYWGQGTQVTVSS
2A3	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTMAWYRQAPGKPRDWVA TIRNGAPVYADSVKGRFTVSRDNAKNTLYLQMNSLKPEDTATYLCRDVNGDIWGQGTQVTVSS
27A5	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTMAWYRQPPGNERDWVA TIRSGAPVYADSVKGRFTVSRDNAKNTLYLQMNSLEPEDTATYYCWDVNGDIWGQGTPVTVSS
2C5	EVQLVESGGGLVQAGGSLNLSCVASGIPFSTRTMAWYRQTPGKSRDWVA TIRSGTPVYADSVKGRFTVSRDNAKNTLYLRMNSLKSEDSATYTCRAVNADIWGQGTQVTVSS
27G5	EvQLVESGGGLVQPGGSLRLSCVASRIPASIRTMAWYRQTPGNQRDWLATIGSSGTPAYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDYWGQGTQVTVSS
13A9	EVQLVESGGGLVQAGGSLRLSCVASRIPASIRTMAWYRQAPGKQRDWVATIGTGGTPAYADSFKGRFTVSRDNANHTVYLQMNSLKPEDTAVYYCRDVNGDYWGQGTQVTVSS
29E9	EVQLVESGGGLVQPGGSLRLSCVASRIPASIRTMAWYRQTPGNQRDWLATIGSSGTPAYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDYWGQGTQVTVSS
15D8	EVQLVESGGGLVQPGGSLKLSCVASTIPASIRTMAWYRQTPGNQRDWLATIGSSGTPAYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNGDYWGQGTQVTVSS
15G4	EVQLVESGGGLVQAGGSLRLSCVASGIPFRSRTMAWYRQAPGKTRDWVATIGTHGTPLYADSVKGRFTVSRDNAKNTLYLQMNSLKPEDTAVYYCWDVNGDYWGQGTQVTVSS
15D12	EVQLVESGGGLVQAGESLRLSCATSGITFKRYVMGWYRQGPGKQRELVATVNDGGTTSYADSVKGRFAISRDNAKNTAYLQMNSLKAEDTAVYYCNAVWKLPRFVDNDYWGQGTQVTVSS

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

15E12	EVQLMESGGGLVQAGGSLRLSCAANGLTFRRYDMGWYRQAPGQQREWVAAISGAGDINYADSVKGRFTMARDNANHTVHLQMNSLKPEDTAVYYCNANWKMLLGVENDYWGQGTQVTVSS
13D7	EVQLAVESGGGLVQAGGSLRLSCAANGLTFRRYDMGWYRQAPGQQREWVAAISGAGDINYADSVKGRFTMARDNANHTVHLQMNSLKPEDTAVYYCNANWKMLLGVENDYWGQGTQVTVSS
13A8	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRRTMAWYRQAPGKQRDWVATIAGDGSTV YADSMKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCWDVNRDYWGQGTQVTVSS
15A4	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRRTMAWYRQAPGKQRDWVATIAGDGSTV YADSMKGRFTISRDNAKNTVYLQINSLKPEDTAVYYCWDVNRDYWGQGTQVTVSS
17F7	EVQLVESGGGLVQAGGSLRLSCVASGIAQS IRVMAWYRQPPGKQRDWVGTISSDGTAN YADSVKGRFTISRDNAKKTMYLQMNSLKPDDTAVYYCRDVNRDYWGQGTQVTVSS
15C8	EVQLVESGGGLVQAGGSLRLSCAASGIAFR IRTMAWYRQAPGKQRDWVATSDSGGTTL YADSVKGRFTVSRDNAENTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
17A10	EVQLVESGGGLVQAGGSLRLSCVASGIPSI RAIAWYRQAPGKQRDWVATSGTGYGAT YDDSVKGRFTLSRDNAKNTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
27D3	EVQLMESGGGLVQPGGSLRLSCAASGLGIAFSRRTMAWYRQAPGKQRDWVATIAGDGSTV YADSMKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCWDVNRDYWGQGTQVTVSS
13B12	EVQLVESGGGLVQAGGSLRLSCAASGIAFR IRTMAWYRQAPGKQRDWVATIGSDGTTI YADSVKGRFTLSRHNAENTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
15B2	EVQLVESGGGLVQAGGSLRLSCVVSGIPSS IRAMAWYRQAPGRQRDWVATIYSPSGSAVYADSVKGRFTISSDNAKSTIYLQMNSLKPDDTAVYYCRDVNRDYWGQGTQVTVSS
15B11	EVQLVESGGGSVQAGGSLRLSCVVSGIPSS IRAMAWYRQAPGRQRDWVATIYSRSGGAVYADSVKGRFTISSDNAKNTIYLQMNSLKPDDTAVYYCRDVNRDYWGQGTQVTVSS
13C9	EVQLVESGGGLVQAGGSLRLSCVASGIPSI HAMAWYRQAPGKQRDWGATTYSRGG TTYNDSAKGRFTISRDNAKKTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
17D5	EVQLVESGGGLVQPGGSLRLSCAASGIIGT IRTMAWYRQAPGKQRDWVA SIGTRGAPVYADSVNGRFTISRDGATNTVFLQMNNLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
27B5	EVQLVESGGGLVQAGGSLRLPCAASGIAFR IRTMAWYRQAPGKQRDWVA TSDSGGTTLYADSVKGRFTVSRDNAENTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
27C7	EVQLVESGGGLVQAGGSLRLSCAASGIAFR IRTMAWYRQAPGKQRDWVA TSDSGGTTLYADSVKGRFTVSRDNADNTVYLQMNSLKPEDTAVYYCRDVNRDYWGQGTQVTVSS
13D4	EVQLVESGGGLVQAGCSLRLSCVVSGIPSS IRAMAWYRQAPGRQRDWVA TIYSPSGSAVYADSVKGRFTISSDNAKSTIYLQMNSLEPDDTAVYYCRDVNREYWGQGTQVTVSS
15G5	EVQLVESGGGLVQAGGSLRLSCVVSGIPST IRAMAWYRQAPGRQRDWVA TIYSPSGSAVYADSVKGRFTISSDNAKKTIYLQMNSLKPDDTAVYYCRDVNREYWGQGTQVTVSS
13C4	EVQLVESGGGLVQAGGSLRLSCVVSGIPSS IRAMAWYRQAPGRQRDWVA TIYSPSGSAVYADSVKGRFTISSDNAKSTIYLQMNSLKPDDTAVYYCRDVNREYWGQGTQVTVSS
46G1	EVQLVESGGGLVQAGGSLRLSCAASGRTFSDDAMGWFRQAPGKERECVASLYLNGDYPYYADSVKGRFTISRDNAKNAVILQMNNLKTEDTAVYYCAAKPGWVARDPSQYNYWGQGTQVTVSS
46E4	EVQLVESGGGLVQAGGSLRLSCAASGRAFKDDAVGWERQAPGKERECVASMYLDGDYPYYADSVKGRFTISRDNAKNAVILQMNNLKTEDTAVYYCAAKPGWVARDPSEYNYWGQGTQVTVSS
17B5	EVQLVESGGGLVQTGGSLRLSCAASGSTFRTDMMGWYRQAPGKQREFVASITKFGSTNYADSVKGRFTISNDNAKDTVYLQMNSLKSEDTAVYYCRNFNRDLWGQGTQVTVSS
15C9	EVQLVESGGGLVQAGGSLKLSCVNSGIPSTLRAMAWYRQAPGRQRDWVATSSNTGGTTYDDSVKGRFTISRDNAKNTVYLQMNSLKPEDTGVYYCRDVNRDLWGQGTQVTVSS
13D10	EVQLVESGGGLQPGGSLRLSCAASSVITLDSNAIGWERQAPGKEREEVSCIASSDGSTYYAESVKGRFTISKDYTRNTVYLQVNSLKPEDTAVYHCATDANPNCGLNVWNSWGQGTQVTVSS
17C6	EVQLVESGGGLVQAGGSLTLSCAASGSTSSLDIMAWYRQAPEKQRELVASVSGGGNSDYASSVKGRFTISGDTAKSTLYLQMNSLKPEDTAMYYCYGRDYYYMPFWGQGTQVTVSS
15A2	EVQLVESGGGLAQAGGSLSLSCAASGRFFS TRVMAWYRQTRGKQREFVASMRGSGSTNYADSARGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCRDINEDQWGQGTQVTVSS

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

17A8	EVQLVESGGGLVQAGGSLSLSCAASGRFFS	TRVMAWYRQTPGKQREFVASMRGSGSTNYADSVRGRFAISRDNAKNMVYLQMNTLKPEDTAVYYCRDINEDQWGQGTQVTVSS	
15G10	EVQLVESGGGLVQAGGSLSLSCAASGRFFS	TRVMAWYRQTPGKQREFVASMRGSGSTNYADSARGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCRDINEDQWGQGTQVTVSS	
27A3	EVQLVESGGGLVQAGGSLSLSCVASGRFFS	TRVMAWYRQTPGKQREFVASMRGSGSTNYADSVRGRFAISRDNAKNTVYLQMNTLKPEDTAVYYCRDINEDQWGQGTQVTVSS	
17H10	EVQLVESGGGLVQAGGSLSLSCSASGRFFS	TRVMAWYRQTPGNQREFVATIHSSGSTIYADSVRGRFAISRDNAKNTVYLQMRSLKPEDTAVYYCRDINADQWGQGTQVTVSS	
30D10	EVQLVESGGGLVQAGGSLTLSCTASETTVR	IRTMAWYRQPPGNQREWVATIGSNGFATYPDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDIWGQGSQVTVSS	
15H4	EVQLVESGGGLVQAGGSLTLSCAPSESTVS	FNTVAWYRQAPGEQREWVATISRQGMSTYPDSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCRDINHDIWGRGSQVTVSS	
17B7	EVQLVESGGGLVQAGGSLRLSCAASGIISS	FRTMAWYRQAPGKQRDWVATIGSDGLANYADSVKGRFTISRDNAKKTVYLQMNSLKPEDTAVYFCRDINRDYWGQGTQVTVSS	
15D2	EVQLVESGGGLVQAGGSLRLSCVVSGVFGP	IRAMAWYRQAPGKQRDWVATIGSSGHPVYTDSVKGRFTFSKDGAKNTVYLQMNSLKPEDTAVYYCRDINRDYWGQGTQVTVSS	
17G5	EVQLVESGGGLVQPGGSLRLSCAASGIGIAFSS	RTMAWYRQAPGKQRDWVATIGSGGTTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDYWGQGTQVTVSS	
15B6	EVQLVESGGGLVQPGGSLRLSCAASGIIGS	FRTMAWYRQAPGNQRDWVATIGSAGLASYADSVRGRFTLSRDNAKKTVYLQMNSLKPEDTAIYYCRDINGDYWGQGTQVTVSS	
27F2	EVQLVESGGGLVQAGGSLRLSCAASGIISSFRTLAWYRQAPGKQRDWVATISSAGGTAYADAVKGRFTISISRDNVEYTVDLQMDSLKPEDTAVYYCRDINGDYWGQGTQVTVSS		
17F5	EVQLVESGGGLVQPGGSLRLSCAASGLGIAFSRRTMAWYRQAPGKQRDWVATIAGDGSTVYADSMKGRFTISRDNAKNTVYLQVNSLKPEDTAVYYCWDTNGDYWGQGTQVTVSS		
17B2	EVQLVESGGGLVQPGGSLRLSCAGSGFTFSNYAMTWVRQAPGKGLEWVSGVGGDGVGSYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCTKDISTFGWGPFDYWGQGTQVTVSS		
27H4	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTMGWYRQAPGKQRDLVASIDASGGTNYADSVKGRFTISRDNAKNTVYLEMNSLKPEDTGVYYCNGRWDIVGAIWWGQGTQVTVSS		
13A4	EVQLVESGGGLVQAGGSLRLSCVASKMTFMRYTMGWYRQAPGKQRDLVASIDSSGGTNYADSVKGRFTISRDNAKNTVYLEMNSLKPEDTGVYYCNGRWDIVGAIWWGQGTQVTVSS		
2A1	EVQLVESGGGLVQAGGSLRLSCVASKITFRRYIMDWYRQAPGKQRELVASINSDGSTGYTDSVKGRFTISRDNTKNTLDLQMNSLKPEDTAVYYCHGRWLEIGAEYWGQGTQVTVSS		
15E10	EVQLVESGGGLVQAGGSLKLSCVASGITFFRYTMGWYRQAPGKERELVAEISSADEPSFADAVKGRFTISRDNAKNTVVLQMNGLKPEDTAVYYCKGSWSYPGLTYWGKGTLVTVSS		
27E7	EVQLVESGGGLVQAGGSLRLSCAASGITFRRYDMGWYRQFPGKERELVATILESEGDTNYVDPVKGRFTISRDNAKNTVYLQMNDLKPEDTAVYYCNGVWRAIGRTYWGQGTQVTVSS		

TABLE C-1-continued

Sequence of HER2 binding Nanobodies aligned by family

47E5	EVQLVESGGGLVQAGGSLRLSCAASASIFGFDSMGWYRQAPGNERILVAIISNGGTTSYRDSVKGRFTIARDNAKNTVSLQMNSLKPEDTAVYYCNLDRRSYNGRQYWGQGTQVTVSS
2G4	EVQLVESGGGLVQAGGSLRLSCAASGNIFSHNAMGWYRQAPGKQRELVTYITINGIANYVDSVKGRFTISRDNTKNTMYLQMVSLKPEDTAVYYCNVGGREYSGVYYYREYWGQGTQVTVSS
14D4	EVQLVESGGGLVQAGDSLRLSCAASGRALDTYVMGWFRQAPGDGREFVAHIFRSGITSYASSVKGRFTISRDNAKNTVYLQMASLKPEDTAAYYCAARPSDTTWSESSASWGQGTQVTVSS
17A5	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYSMSWVRQATGKGLEWVSGISWNGGSTNYADSVKGRFTISRDNVKNTLYLQMNSLKSEDTAVYYCAKDLGNSGRGPYTNWGQGTQVTVSS
15D10	EVQLVESGGGLVQPGGSLKLSCAASGFTFSSYRMYWVRQAPGKGLEWVSAIKPDGSITYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATDCGVPGFGWTFSSWGQGTQVTVSS
13C2	EVQLVESGGGLVQAGGSLRLSCAASGSTFSINRMAWYRQSPGKQRELVAAVDNDDNTEYSDSVAGRFTISRDNAKNAVHLQMNSLRLEDTAVYYCNAKQLPYLQNFWGQGTQVTVSS
17G11	EVQLVESGGGLVQAGGSLRLSCAASGSTFSINRWGWYRQAPGKQRELVAAIDDGGNTEYSDFVNGRFTISRDNPETAVHLQMNSLKLEDTAVYYCNAKQLPYLQNFWGQGTQVTVSS
17A3	EVQLVESGGGLVQAGGSLSLSCAASATLHRFDNNWYRQAPGKQRELVATIAHDGSTNYANSVKGRFTISRDNARDTLFLQMHALQPEDTAVYMCNLHRWGLNYWGQGTQVTVSS
27B7	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISSGGGSITTYADSVKGRFTISRDNAKNTLYLQMSSLKPEDTALYYCAKARSSSSYYDFGSWGQGTQVTVSS
17A6	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISSGGGSITTYADSVKGRFTISTDNAKNTLYLQMSSLKPEDTALYYCAKARSSSSYYDFGSWGQGTQVTVSS
17D7	EVQLVESGGGLVQPGGSLRLSCAASGFTLDYCAIGWFRQAPGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATDRGSGTCYADFGSWGQGTQVTVSS
46D4	EVQLVESGGGLVQPGGSLRLSCAASGFIFDDYAMSWVRQAPGKGLEWVSSINWSGTHTDYAEDMKGRFTISRDNAKKTLYLQMNSLQSEDTAVYYCAKGWGPAVTSIPVATLGTQVTVSS
27B3	EVQLVESGGGLVQAGGSLTLSCTASETTV RIRTMAWYRQPPGNQREWVATIGSNGFATYPDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDIWGQGSQVTVSS
27E5	EVQLVESGGGLVQAGGSLTLSCTASETTV RIRTMAWYRQPPGNQREWVATIGSNGFATYPDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDIWGQGSQVTVSS
27D6	EVQLVEGGGGLVQAGGSLTLSCTASETTV RIRTMAWYRQPPGNQREWVATIGSNGFATYPDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDIWGQGSQVTVSS
30D10	EVQLVESGGGLVQAGGSLTLSCTASETTV RIRTMAWYRQPPGNQREWVATIGSNGFATYPDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRDINRDIWGQGSQVTVSS
47G11	EVQLVESGGGLVQPGGSLRLSCAASGRIFYPMGWFRQAPGKEREFVAAIGSGDIITYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCASSRDYSRSRDPTSYDRWGQGTQVTVSS
27C3	EVQLVESGGGLVQGGSLRLSCAASGFTFDDYATSWVRQAPGKGPEWVSAINSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCARPRGSSLYLLEYDYWGQGTQVTVSS

TABLE C-2

k_{off} rate of different Nanobodies as measured in Biacore

ID	k_{off} (s^{-1})
2A4	2.05E−03
2A5	1.42E−03
2A6	1.65E−03
2B1	1.55E−03
2C4	1.26E−03
2D2	1.61E−03
2D4	1.65E−03
2F2	1.65E−03
2F3	1.53E−03
2F5	1.57E−03
2G5	1.56E−03
2H4	1.61E−03
2B2	1.19E−03
2B3	1.25E−03
2B4	2.77E−03
2B5	1.15E−03
2C1	1.18E−03
2C2	4.12E−03
2C3	1.11E−03
2D1	1.27E−03
2D5	1.20E−03
2F1	1.77E−03
2F4	1.07E−03
2G1	1.23E−03
2G2	1.30E−03
2G3	1.20E−03
2H3	1.09E−03
2H2	1.18E−03
2H3	1.15E−03
2H5	1.21E−03

TABLE C-3

Overview of k_d/k_{off}-, k_a-, and K_d-values for binding of Nanobodies 2D3, 5F7 and 47D5 to HER2.

Nanobody ID	k_{off} (s^{-1})	K_{on} (1/Ms)	K_D (nM)
2D3	1.48E−03	1.36E+06	1.09
Dummy-2D3	1.13E−03	1.16E+06	1.77
5F7	3.02E−04	1.02E+06	0.29
47D5	8.62E−04	3.86E+05	2.23
Dummy-47D5	8.69E−04	2.71E+05	3.21

Fusion of a dummy Nanobody at the N-terminal end of the Nanobodies 2D3 and 47D5 does not significantly impact on the binding characteristics of 2D3 or 47D5 respectively.

TABLE C-4

Off-rate analysis of HER2-ECD on 2D3, 47D5 and 2D3-35GS-47D5 coated sensor chips

Analyte	Protein on sensor chip	k_{off} (1/s)
100 nM Her2 BCD	2D3-47D5	8.07E−5
100 nM Her2 BCD	2D3	2.10E−3
100 nM Her2 ECD	5F7	2.56E−3
1000 nM Her2 ECD	2D3-47D5	5.45E−5
1000 nM Her2 BCD	2D3	1.51E−3
1000 nM Her2 ECD	5F7	1.31E−3

TABLE C-5

Oligonucleotide primers used for generation of Omnitarg light chain V_L + C_L by overlap extension

For-sequences	SEQ ID NO	Rev-sequences	SEQ ID NO
>For_LCrescuepAX51 Tgattacgccaagct	2271	>Rev_LC1pAX51 TAATAACAATCCAGCGGCTGCCGTAG GCAATAGGTATTTCATGTTGAAAATCT	2283
>For_LC1pAX51 Tgattacgccaagcttgcatgca aattctatttcaaggagattttc aacatga	2272	>Rev_LC2_OT ATCGCCGACGGACGCGCTCAGGCTAC TCGGAGATTGCGTCATCTGGATGTCG GC	2284
>For_LC2pAX51_OT gctggattgttattactcgcggc ccagccggccatggccGACATCC AGATGACG	2273	>Rev_LC3_OT CCGGCTTCTGTTGATACCAAGCAACC CCGATAGATACGTCCTGACTTGCT	2285
>For_LC3_OT GCGTCCGTCGGCGATCGCGTTAC CATCACATGCAAAGCAACTCAGG ACGT	2274	>Rev_LC4_OT CCGCTGAAACGGGAAGGCACACCGGT GTAACGATATGATGCGGAGTAAAT	2286
>For_LC4_OT ATCAACAGAAGCCGGGCAAGGCT CCGAAATTGCTCATTTACTCCGC ATCA	2275	>Rev_LC5_OT ATAGTAGGTGGCGAAGTCCTCTGGCT GCAGGCTAGAGATAGTCAGGGTAA	2287
>For_LC5_OT TTCCCGTTTCAGCGGAAGCGGCT CGGGTACTGATTTTACCCTGACT ATCT	2276	>Rev_LC6_OT TACCGTACGTTTAATTTCCACTTTCG TACCCTGGCCAAAGGTATACGGGT	2288

TABLE C-5-continued

Oligonucleotide primers used for generation of Omnitarg light chain V_L + C_L by overlap extension

For-sequences	SEQ ID NO	Rev-sequences	SEQ ID NO
<For_LC6_OT TTCGCCACCTACTATTGTCAGCA ATACTATATTTACCCGTATACCT TTGG	2277	>Rev_LCrescue_VL_OT TCGGAAGGCGGAAAG	2289
>For_LC7_OT ATTAAACGTACGGTAGCTGCCCC TAGCGTGTTTATCTTTCCGCCTT CCGA	2278	>Rev_LC7 ATACGACGCTGGCCGTACCACTTTTC AGCTGCTCGTCGGAAGGCGGAAAG	2290
>For_LC8 CGGCCAGCGTCGTATGTTTACTG AATAACTTCTATCCGCGCGAAGC TAAA	2279	>Rev_LC8 CCGGACTGCAGTGCATTATCCACTTT CCATTGGACTTTAGCTTCGCGCGG	2291
>For_LC9 TGCACTGCAGTCCGGCAATTCTC AAGAATCCGTGACGGAACAAGAT AGCA	2280	>Rev_LC9 GGTCAGGGTAGAGCTCAGTGAGTAAG TGCTATCTTTGCTATCTTGTTCCG	2292
>For_LC10 AGCTCTACCCTGACCTTGTCAAA GGCAGATTATGAAAAACACAAAG TTTA	2281	>Rev_LC10 GAAAGTCCCTGATGGGTCACTTCACA GGCGTAAACTTTGTGTTTT	2293
>For_LC11 CCATCAGGGACTTTCGAGTCCGG TTACAAAGTCTTTTAACCGCGG	2282	>Rev_LC11 aaatagaattggcgcgccttattaGC ACTCACCGCGGTTAAAAGAC >Rev_LCrescue aaatagaattggcgc	2294 2295

TABLE C-6

Oligonucleotide primers used for generation of Omnitarg heavy chain V_H + CH_1 by overlap extension

For	SEQ ID NO	Rev	SEQ ID NO
>For_HCrescue gtgctaataaggcgc	2296	>Rev_HC1 AAAGGTACCACTAAAGGRATTGCGAA TAATAATTTTTTCACTATGACTGT	2309
>For_HC1 gtgctaataaggcgcgccaattctat ttcaaggagacagtcatagtgaaa	2297	>Rev_HC2_OT ACGCAGAGAACCGCCTGGCTGCACCA GCCCACCTCCGCTTTCCACCAGCT	2310
>For_HC2_OT tttagtggtacctttctattctcact ccGAGGTTCAGCTGGTGGAAAGCG	2298	>Rev_HC3_OT TTTCACGTTCACTGATTATACCATGG ATTGGGTTCGCCAGGCGCCGGGTA	2311
>For_HC3_OT GGCGGTTCTCTGCGTCTGAGCTGCGC TGCCTCCGGTTTCACGTTCACTGA	2299	>Rev_HC4_OT CCCTTAAAACGTTGGTTGTAAATTGA GCCACCAGAGTTAGGGTTTACGTC	2312
>For_HC4_OT GCCAGGCGCCGGGTAAAGGCCTGAA TGGGTGGCCGACGTAAACCCTAAC	2300	>Rev_HC5_OT TTCTGCACCCAGCGAATTCATCTGTA AATAGAGTGTGTTTTTAGAGCGAT	2313
>For_HC5_OT CCAACGTTTTAAGGGTCGTTTCACCC TGAGCGTAGATCGCTCTAAAAACA	2301	>Rev_HC6_OT TGCCTTGGCCCCAATAGTCAAAGTAA AAGGACGGGCCCAGATTGCGTGCA	2314
>For_HC6_OT TCGCTGCGTGCAGAAGACACCGCTGT TTATTACTGTGCACGCAATCTGGG	2302	>Rev_HC7 GATTTCGAGCTTGGGGCCAGCGGAAA CACTGACGGACCTTTAGTGCTTGC	2315

TABLE C-6-continued

Oligonucleotide primers used for generation of Omnitarg heavy chain V_H + CH_1 by overlap extension

For	SEQ ID NO	Rev	SEQ ID NO
>For_HC7_OT ATTGGGGCCAAGGCACGTTGGTCACC GTGAGTAGCGCAAGCACTAAAGGT	2303	>Rev_HCrescue_VH_OT GATTTCGAGCTTGGG	2316
>Rev_HC7_OT_PCR ACCTTTAGTGCTTGCGCTACTCACGG TGACCAACGTGCCTTGGCCCCAAT	2304	>Rev_HC8 GGAGACAGTGACCGGTTCCGGGAAGT AATCTTTCACCAGACAGCCCAGCG	2317
>For_HC8 CCCAAGCTCGAAATCCACGTCCGGTG GCACCGCCGCGCTGGGCTGTCTGG	2305	>Rev_HC9 TATACAAGCCGCTAGACTGCAAAACC GCAGGGAAAGTATGTACACCCGAG	2318
>For_HC9 CCGGTCACTGTCTCCTGGAACTCGGG TGCACTTACCTCGGGTGTACATAC	2306	>Rev_HC10 TGGTTCACATTGCAAATATACGTCTG GGTGCCCAGAGAGCTTGAAGGCAC	2319
>For_HC10 CTAGCGGCTTGTATAGCCTGTCAAGC GTTGTGACCGTGCCTTCAAGCTCT	2307	>Rev_HC11 TTTTTGTTCTGCGGCCGCACAGCTCT TCGGTTCCACTTTCTTATCCA	2320
>For_HC11 TTGCAATGTGAACCACAAACCGAGTA ACACCAAAGTGGATAAGAAAGTGG	2308	>Rev_HCrescue TTTTTGTTCTGCGGC	2321

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20110028695A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. A method for obtaining a polypeptide construct directed against to one or more antigens and/or epitopes and having one or more desired characteristics, wherein said polypeptide construct essentially consists or comprises at least two single domain antibodies, said method at least comprising the steps of:

(i) selecting a template polypeptide construct directed against one or more antigens or epitopes,

(ii) producing a diversity of polypeptide constructs that are structural variants of the selected template polypeptide construct of step (i), wherein said structural variants each comprise at least two single domain antibodies, and

(iii) screening the produced diversity of polypeptide constructs of step (ii) for a polypeptide construct having said one or more desired characteristics, wherein said polypeptide construct comprises at least two single domain antibodies and is directed against one or more antigens and/or epitopes.

2. The method according to claim **1**, wherein said diversity of polypeptide constructs is a library of polypeptide constructs.

3. The method according to claim **1**, wherein said structural variants of step (ii) are one or more of the following:

structural variants with regard to the number and/or identity of said single domain antibodies in said polypeptide constructs, and/or,

structural variants with regard to the relative position of said single domain antibodies within the polypeptide constructs, and/or,

structural variants with regard to the amino acid residues of the CDR region(s) of one or more of said single domain antibodies in said polypeptide constructs, and/or,

structural variants with regard to the amino acid residues of the framework region(s) of one or more of said single domain antibodies in said polypeptide constructs, and/or,

structural variants with regard to the codon usage in the nucleic acid sequences encoding the polypeptide constructs.

4. The method according to claim **1**, wherein said structural variants of step (ii) comprise at least two single domain antibodies that are linked via one or more peptide linkers.

5. The method according to claim **4**, wherein said structural variants of step ii) are one or more of the following

structural variants with regard to the composition of said one or more peptide linkers, and/or,

structural variants with regard to the number of said one or more linkers, and/or,

structural variants with regard to the relative position of said one or more linkers in said polypeptide constructs.

6. The method according to claim **1**, wherein the single domain antibodies present in said diversity of polypeptide constructs and in said polypeptide construct are all heavy chain variable domains or are all light chain variable domains.

7. The method according to claim **6**, wherein the single domain antibodies present in said diversity of polypeptide constructs and in said polypeptide construct are all heavy chain variable domains.

8. The method according to claim **7**, wherein the heavy chain variable domains are variable domains obtainable from heavy chain antibodies (VHH).

9. The method according to claim **1**, wherein in step (iii) the produced diversity of polypeptide constructs is screened for a polypeptide construct having a suitable binding affinity or avidity, for a polypeptide construct having a suitable solubility, for a polypeptide construct having a suitable stability, for a polypeptide construct having a suitable efficacy, and/or for a polypeptide construct having a suitable potency.

10.-**13**. (canceled)

14. The method according to claim **1**, wherein said diversity of polypeptide construct comprises at least two single domain antibodies that are directed against the same epitopes.

15. The method according to claim **1**, wherein said diversity of polypeptide construct comprises at least two single domain antibodies that are directed against at least two different epitopes that are present on the same antigen.

16. The method according to claim **1**, wherein said diversity of polypeptide construct comprises at least two single domain antibodies that are directed against at least two different epitopes that are present on different antigens.

17. The method according to claim **1**, wherein said polypeptide construct comprises at least three single domain antibodies.

18. The method according to claim **17**, wherein said three single domain antibodies are directed against the same epitope.

19. The method according to claim **17**, wherein said three single domain antibodies are directed against at least three different epitopes.

20. The method according to claim **19**, wherein said at least three different epitopes are present on the same antigen.

21. The method according to claim **19**, wherein said at least three different epitopes are present on at least two different antigens.

22. Polypeptide construct obtainable by the method of claim **1**.

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