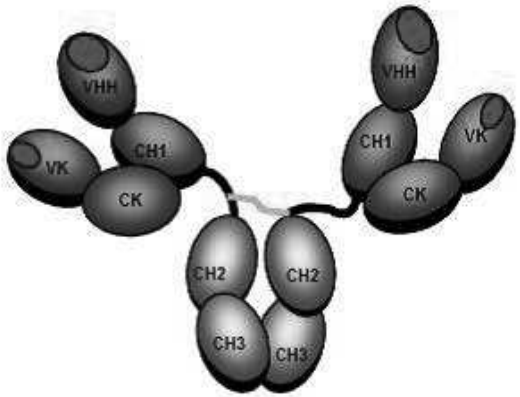
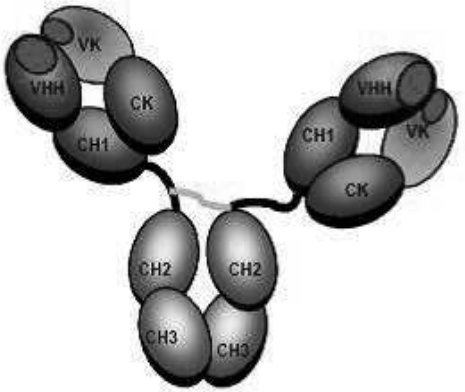
Fig. 1
1/24

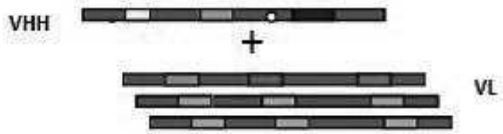
High-affinity aggregation non-resistant VHH-based antibody



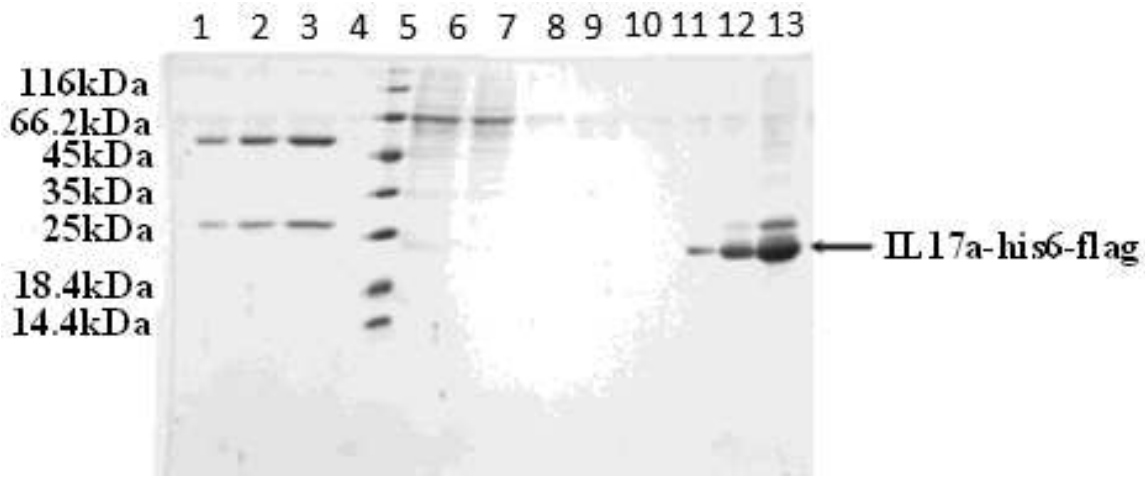
High-affinity aggregation resistant antibody on the basis of mutant VHH



Mutagenesis by VHH position 44+45 plus light chain shuffling



Display libraries and selection under tight conditions (low antigen concentrations, long-term washing, incubation with competitors, increased temperature, etc.). High-performance robotic screening including the testing of affinity and aggregation stability



A - Human recombinant IL17A-His6-FLAG product (15% PAGE+ β-ME).

1) 9E10 0.25 µg+ β-ME

2) 9E10 0.5 µg + β-ME

3) 9E10 1 µg + β-ME

4) ---

5) Enzyme marker unstained

6) Medium before application to IMACBioRad

7) Medium after application to IMACBioRad

8) Washing 1

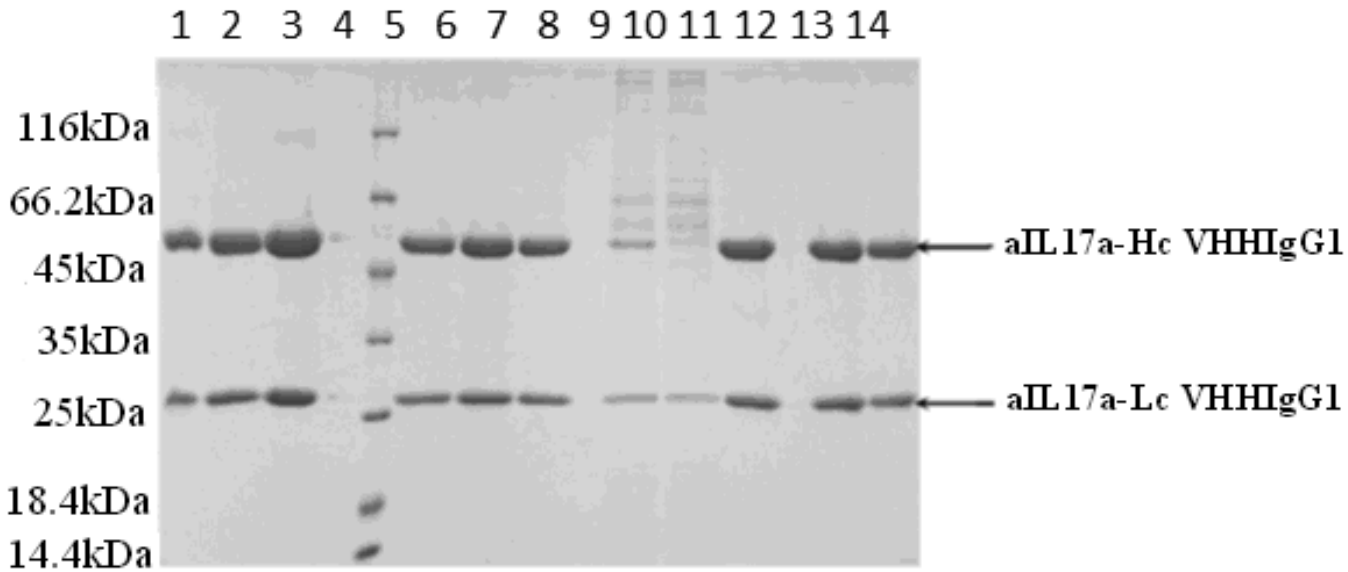
9) Washing 2

10) Washing 3

11) Elution.5 µL

12) Elution 5 µL

13) Elution 10 µL.



B - VHHIgG1 products with various amino acid substitutions at position 45 of VHH domain (12% PAGE+ β-ME).

1) Control IgG1 2.5 µg

2) Control IgG1 5 µg

3) Control IgG1 10 µg

4) -

5) Enzyme PW marker unstained

6) VHHIgG1 aIL17 A45/K8 5 µL

7) VHHIgG1 aIL17 D45/K8 5 µL

8) VHHIgG1 aIL17 F45/K8 5 µL

9) -

10) VHHIgG1 aIL17 G45/K8 cult liq before 5 µL

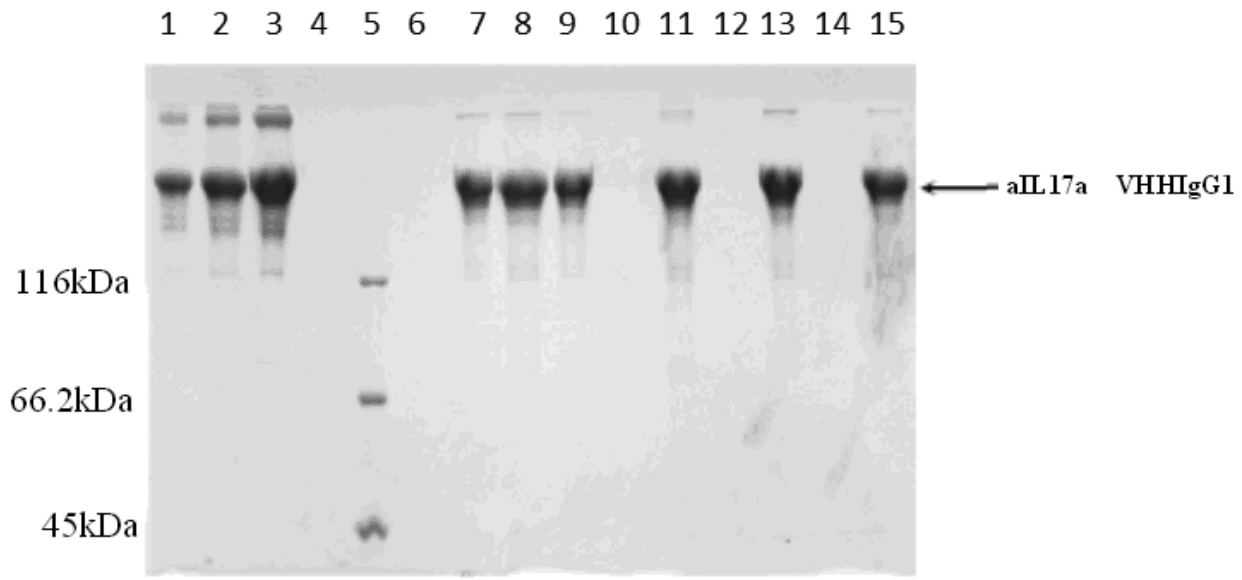
11) VHHIgG1 aIL17 G45/K8 cult liq after 5 µL

12) VHHIgG1 aIL17 G45/K8 5 µL

13) -

14) VHHIgG1 aIL17 H45/K8 5 µL.

15) VHHIgG1 aIL17 I45/K8 5 µL.



C - Products with various amino acid substitutions at position 45 of VHH domain (8% PAGE, free of β-ME).

1) Control IgG1 2.5 µg

2) Control IgG1 5 µg

3) Control IgG1 10 µg

4) –

5) Enzyme PW marker unstained

6) –

7) VHHIgG1 aIL17 A45/K8 5 µL

8) VHHIgG1 aIL17 D45/K8 5 µL

9) VHHIgG1 aIL17 F45/K8 5 µL

10) -

11) VHHIgG1 aIL17 G45/K8 cult liq before 5 µL

12) -

13) VHHIgG1 aIL17 H45/K8 cult liq after 5 µL

14) -

15) VHHIgG1 aIL17 I45/K8 5 µL

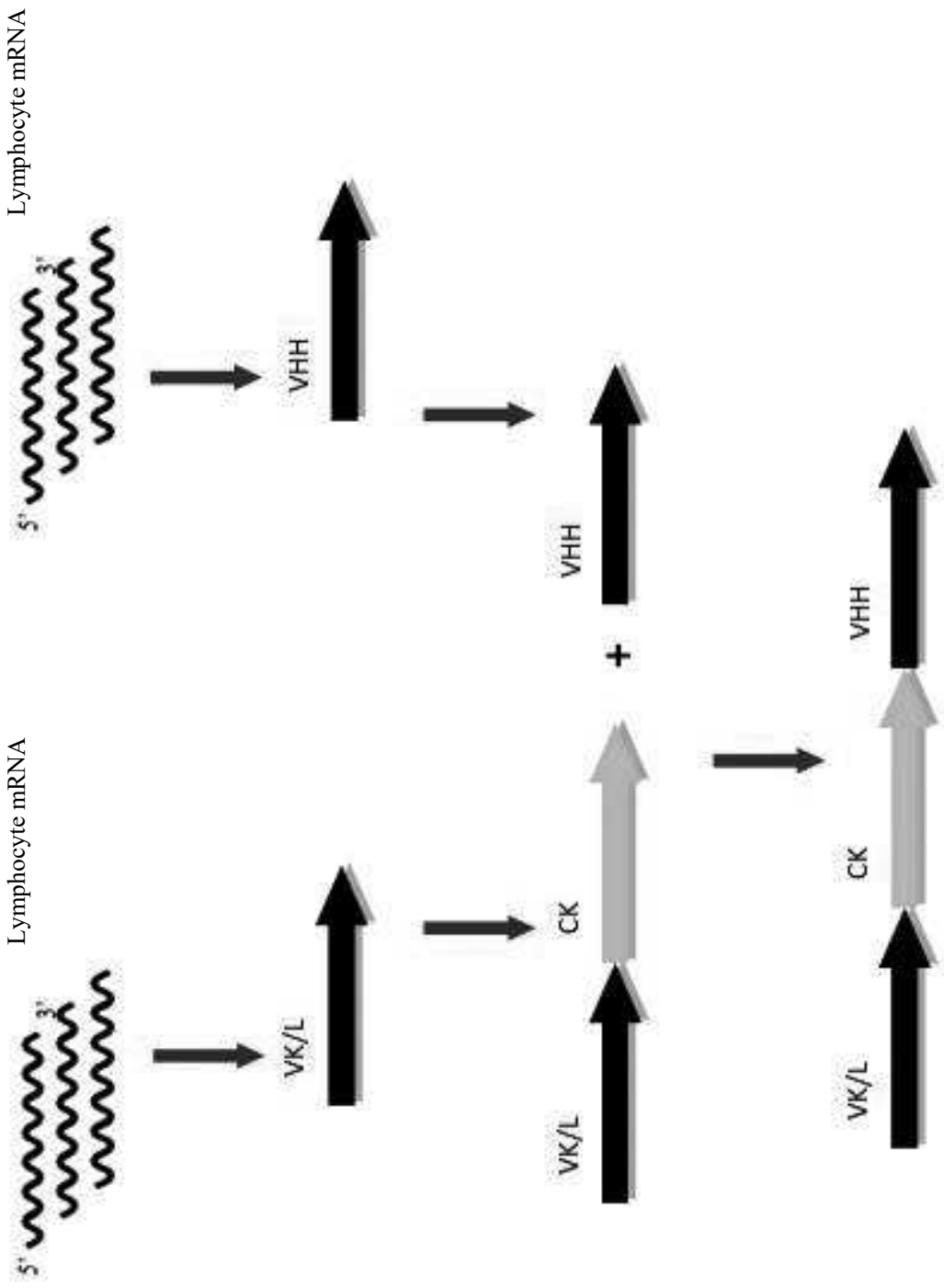
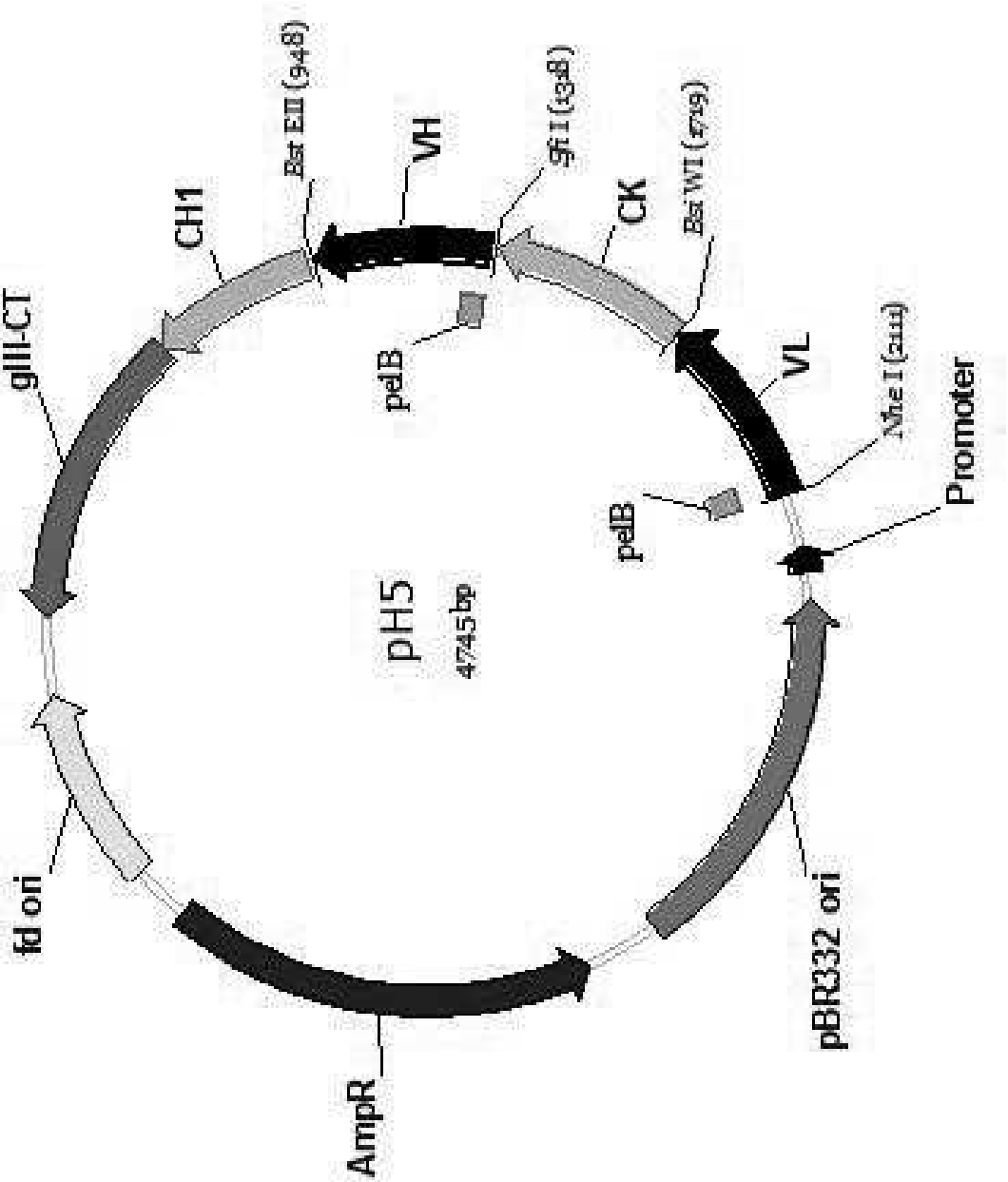


Fig. 5



gIII-CT
CH1
Bst EII (948)
VH
Sfi I (1318)
CK
Bst WI (1719)
VL
Nhe I (2111)
Promoter
pelB
pelB
pH5
4745 bp
fd ori
AmpR
pBR332 ori

Fig. 6

A

1VHH

QVQLQQSGGGSVQTGGSLTLTCAASGLTFEANSLGW**F**RQSPGK**ER**E**F**VAAVSFTKRIDYADSVK
GRFFISRDNTMNTVYLQMNSLKPEDTGIYTCAADPLLISNKRANI-----WGQGTMVTVSS

2VHH

QVQLVQSGGGLVQPGGSLRLSCAASPRVISIHDMAW**Y**RQAPGK**ER**E**L**VAGITTRGITDYGYSVK
GRFTISRDDAKNTLFLQMNDLKPEDTAVYYCNLRHYEV----------WGQGTLVTVSS

3VHH

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGW**F**RQAPGK**ER**E**G**VAAISPSGGDRIYDDSV
KGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSYYTGDYDSWGQGTLVTVSS

B

VK4B11DVVMTQSPSSVTASAGETVTINCKSSQSVAYKSNQKNYLAWYQQRPGQSPRLLIYYAS
TRTSGVPDRFSGSGSTTDFTLTISSFQPEDAAVYYCQQGYSAPYSFGSGTKLEIK

VLB5

AVLTQLSSMSGSPGQTVTITCTGSITNIGQYRVNWYQHLPGTAPKLLIYSNANRVSGVPDRFSG
SKSGSTASLTIAGVQAEDEADYYCSAWDGSLNGYVFGGGTKVTVLQR

VLF4QAVLTQPPSVSGSPGQTVTISCTGTSDDVGSGNYVSWYQQVPGMAPKLLIYNAGTRRAGI
TGRFSASKSGNTASLTISGLQSEDEADYYCASYRKINKYVFGGGTKLTVLQR

Fig. 7

C

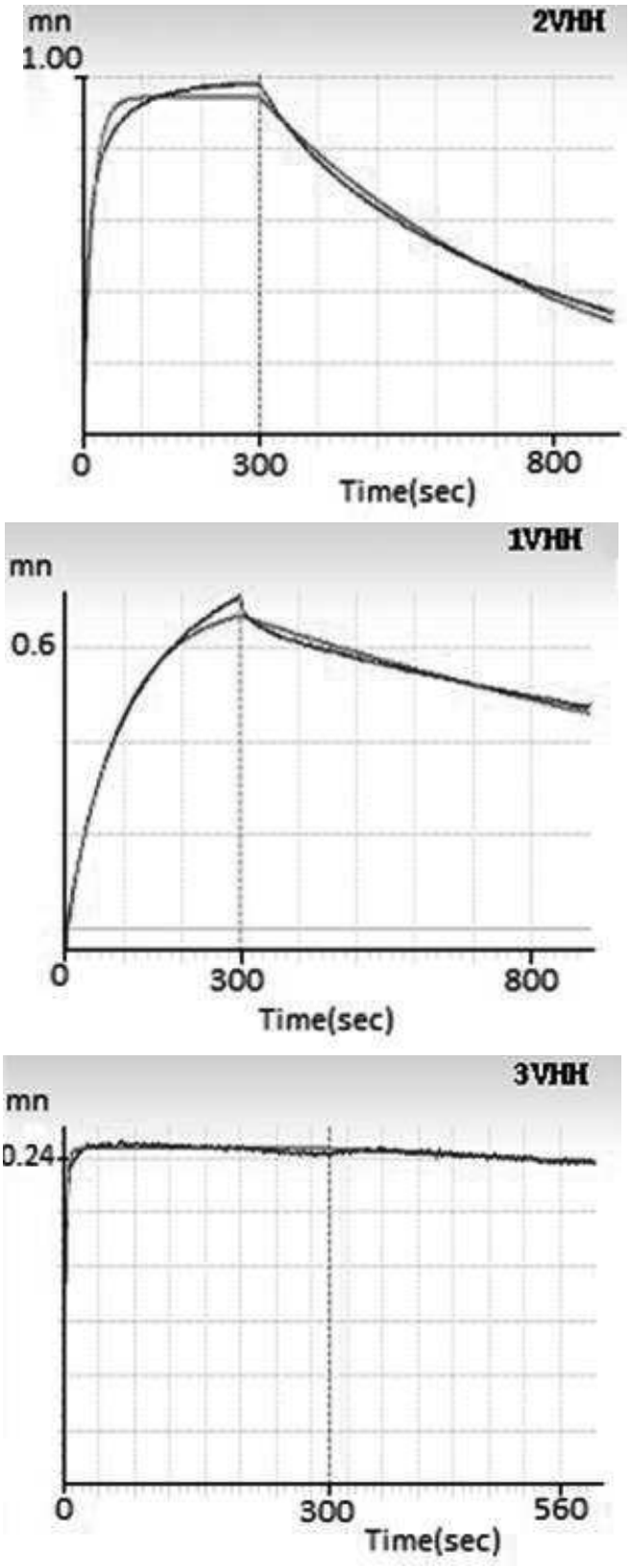

mn
1.00
2VHH
0
300
800
Time(sec)
1VHH
mn
0.6
0
300
800
Time(sec)
3VHH
mn
0.24
0
300
560
Time(sec)

Fig. 8

Wild 3VHHFab

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGK**ER**EFVAAISPS
GGDRIYDDSVKGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut1

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGK**GL**EFVAAISPS
GGDRIYADSVKGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut2

QVQLQQSGGG**L**VQAGGSLRLSCAASGGTFATSPMGWLRQAPGK**G**RE**W**VAAISP
SGGDRIYADSVKGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSY
YTGDYDSWGQGTLVTVSS

mut3

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKE**L**EFVAAISPS
GGDRIYADSVKGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut4

QVQLQQSGGG**L**VQAGGSLRLSCAASGGTFATSPMGWLRQAPGK**G**REFVAAISP
SGGDRIYADSVKGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSY
YTGDYDSWGQGTLVTVSS

Fig. 9

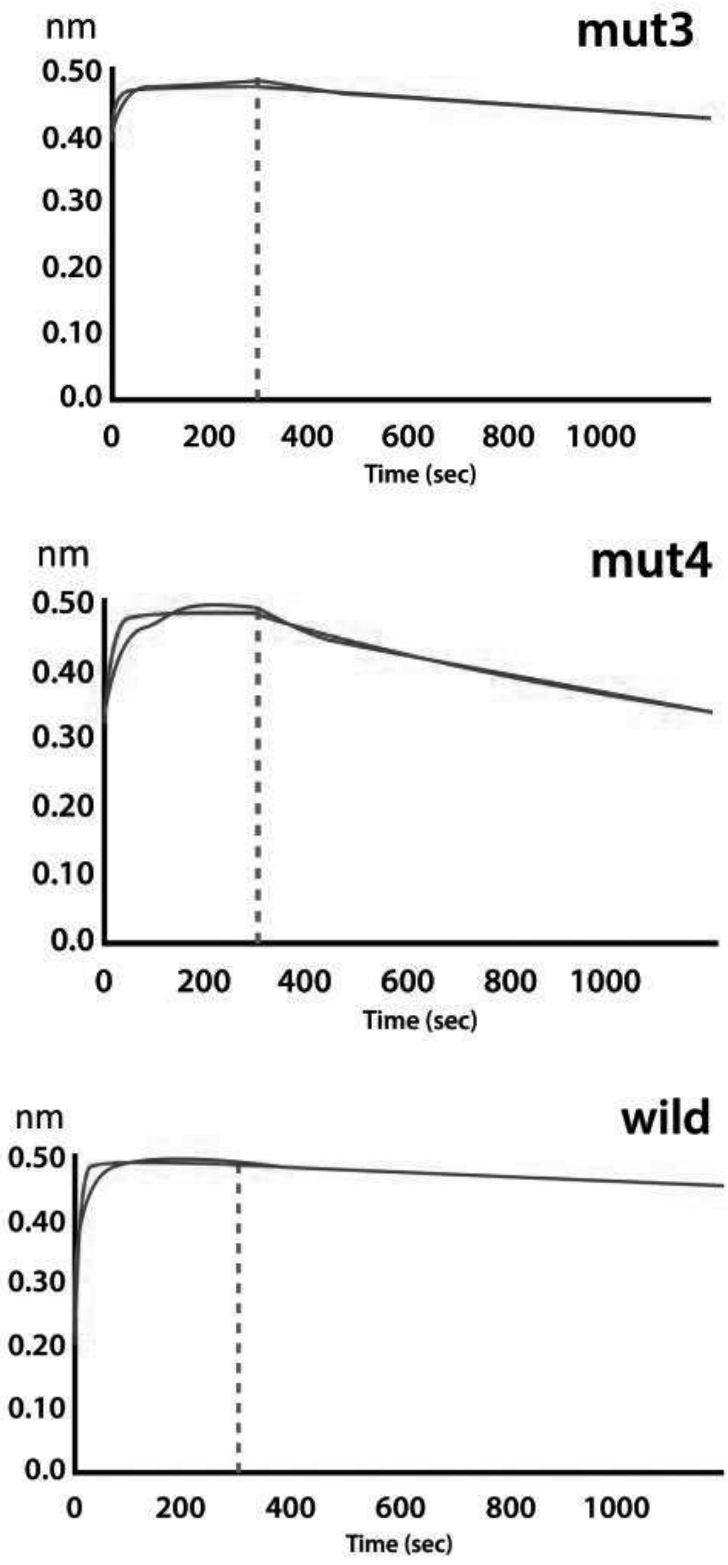

nm
mut3
0.50
0.40
0.30
0.20
0.10
0.0
0
200
400
600
800
1000
Time (sec)
nm
mut4
0.50
0.40
0.30
0.20
0.10
0.0
0
200
400
600
800
1000
Time (sec)
nm
wild
0.50
0.40
0.30
0.20
0.10
0.0
0
200
400
600
800
1000
Time (sec)

Fig. 10

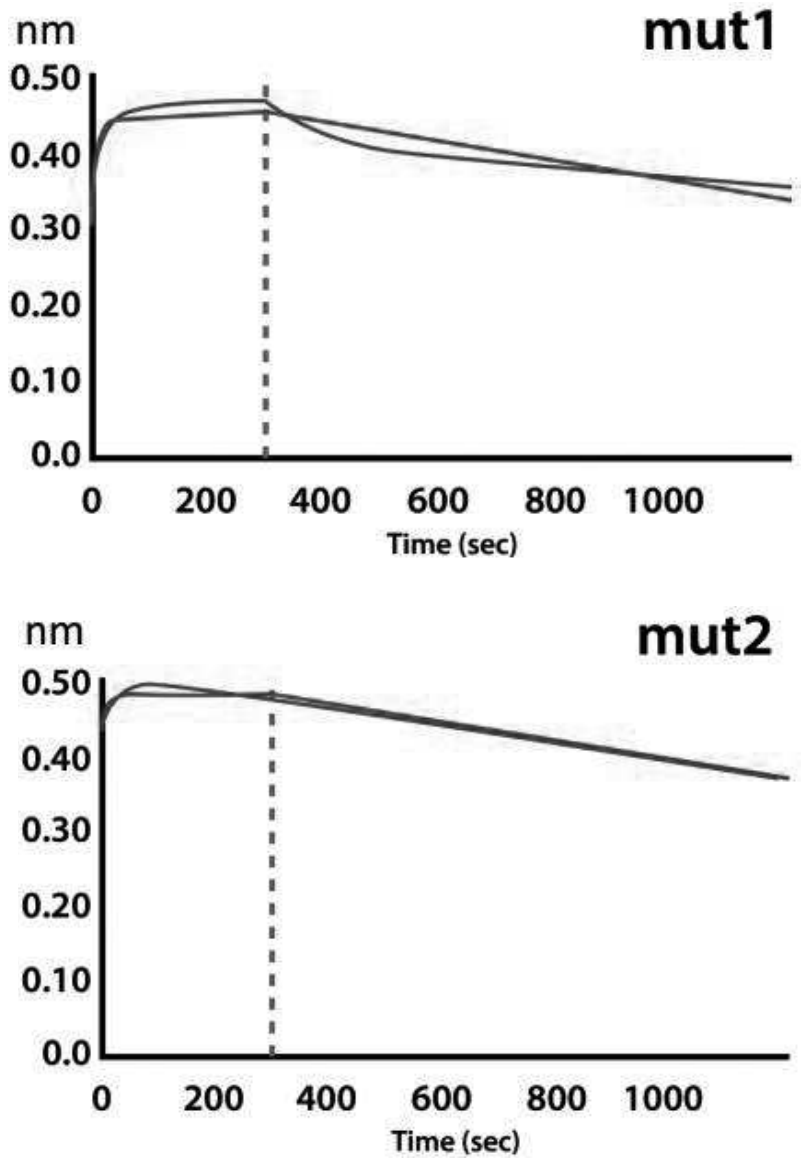

nm
mut1
0.50
0.40
0.30
0.20
0.10
0.0
0
200
400
600
800
1000
Time (sec)
nm
mut2
0.50
0.40
0.30
0.20
0.10
0.0
0
200
400
600
800
1000
Time (sec)

Fig. 11

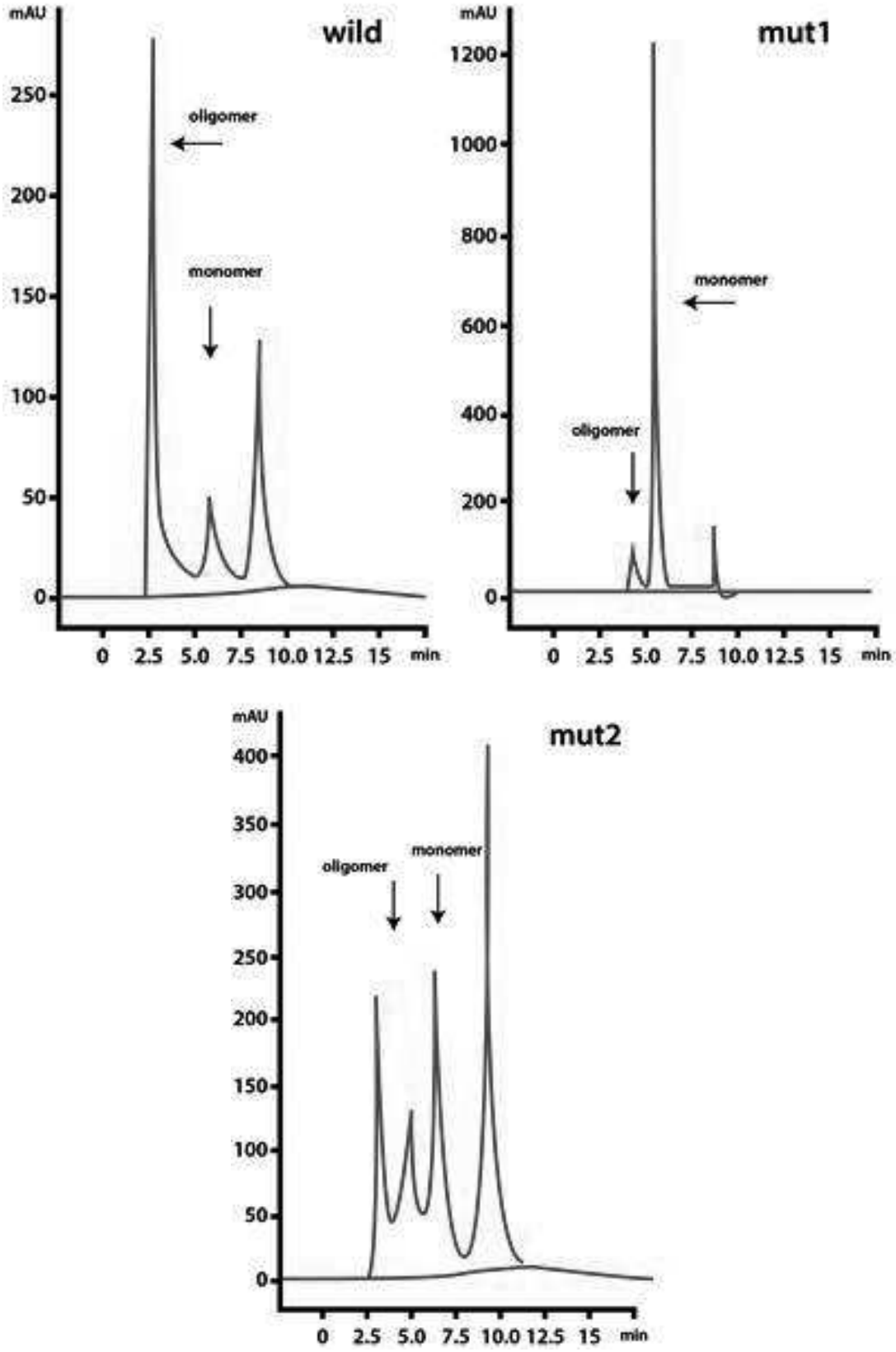

wild
mAU
250
200
150
100
50
0
oligomer
monomer
0 2.5 5.0 7.5 10.0 12.5 15 min
mut1
mAU
1200
1000
800
600
400
200
0
monomer
oligomer
0 2.5 5.0 7.5 10.0 12.5 15 min
mut2
mAU
400
350
300
250
200
150
100
50
0
oligomer
monomer
0 2.5 5.0 7.5 10.0 12.5 15 min

Fig. 12

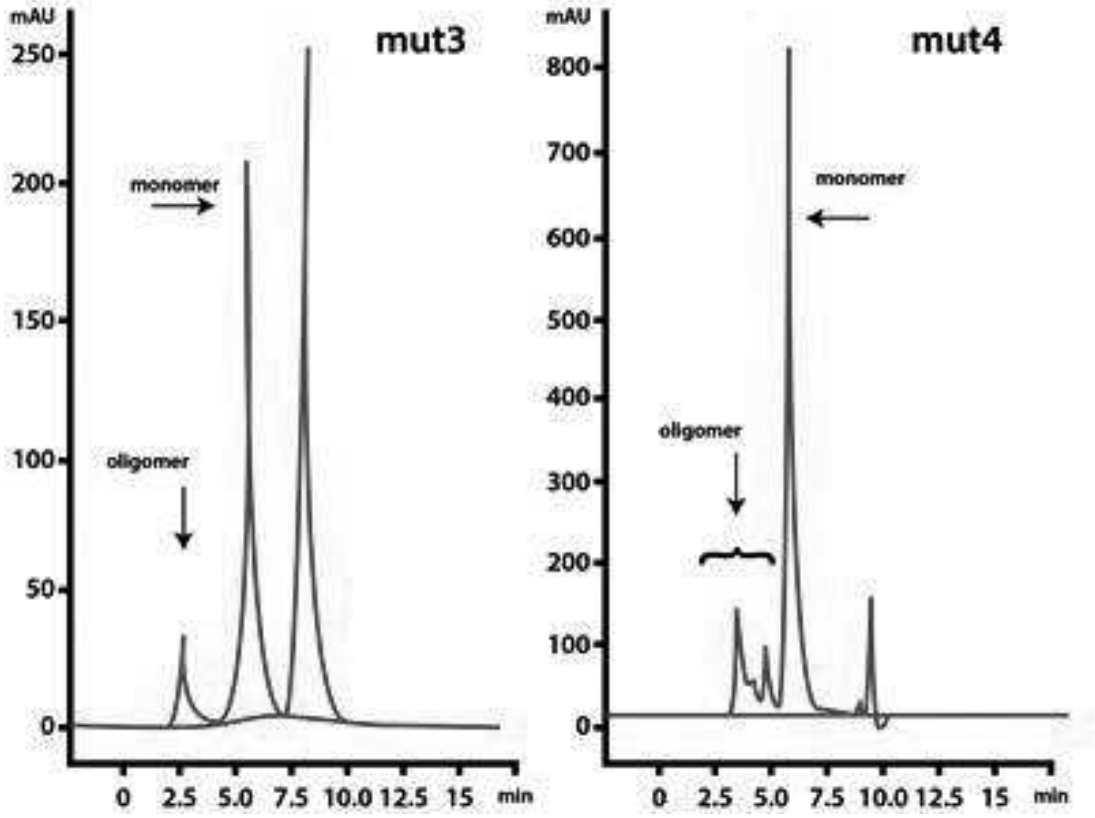

mut3
mAU
250
200
150
100
50
0
monomer
oligomer
0 2.5 5.0 7.5 10.0 12.5 15 min
mut4
mAU
800
700
600
500
400
300
200
100
0
monomer
oligomer
0 2.5 5.0 7.5 10.0 12.5 15 min

Fig. 13

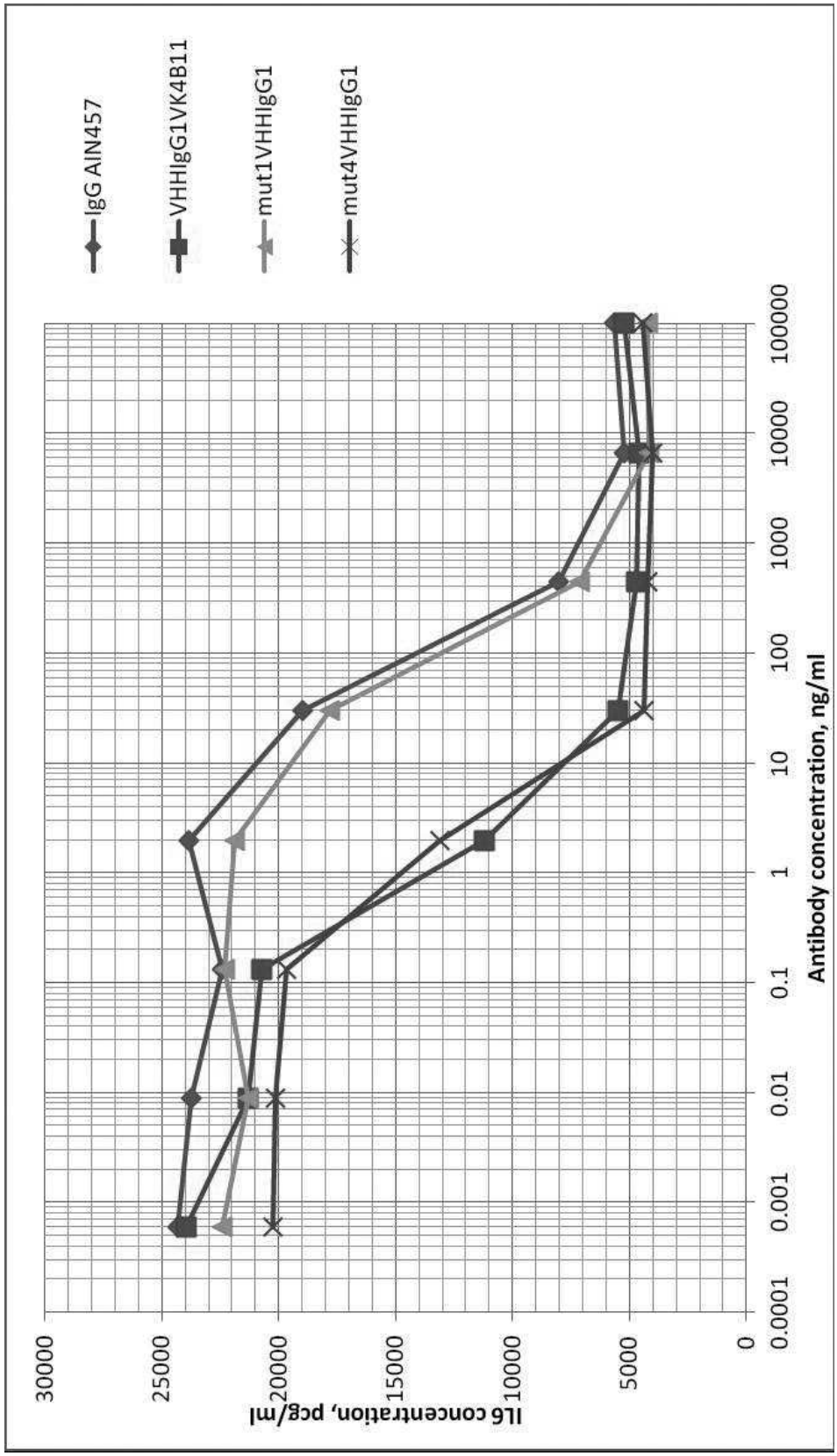

IgG AIN457
VHHIgG1VK4B11
mut1VHHIgG1
mut4VHHIgG1
IL6 concentration, pcg/ml
30000
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
100000
Antibody concentration, ng/ml

Fig. 14

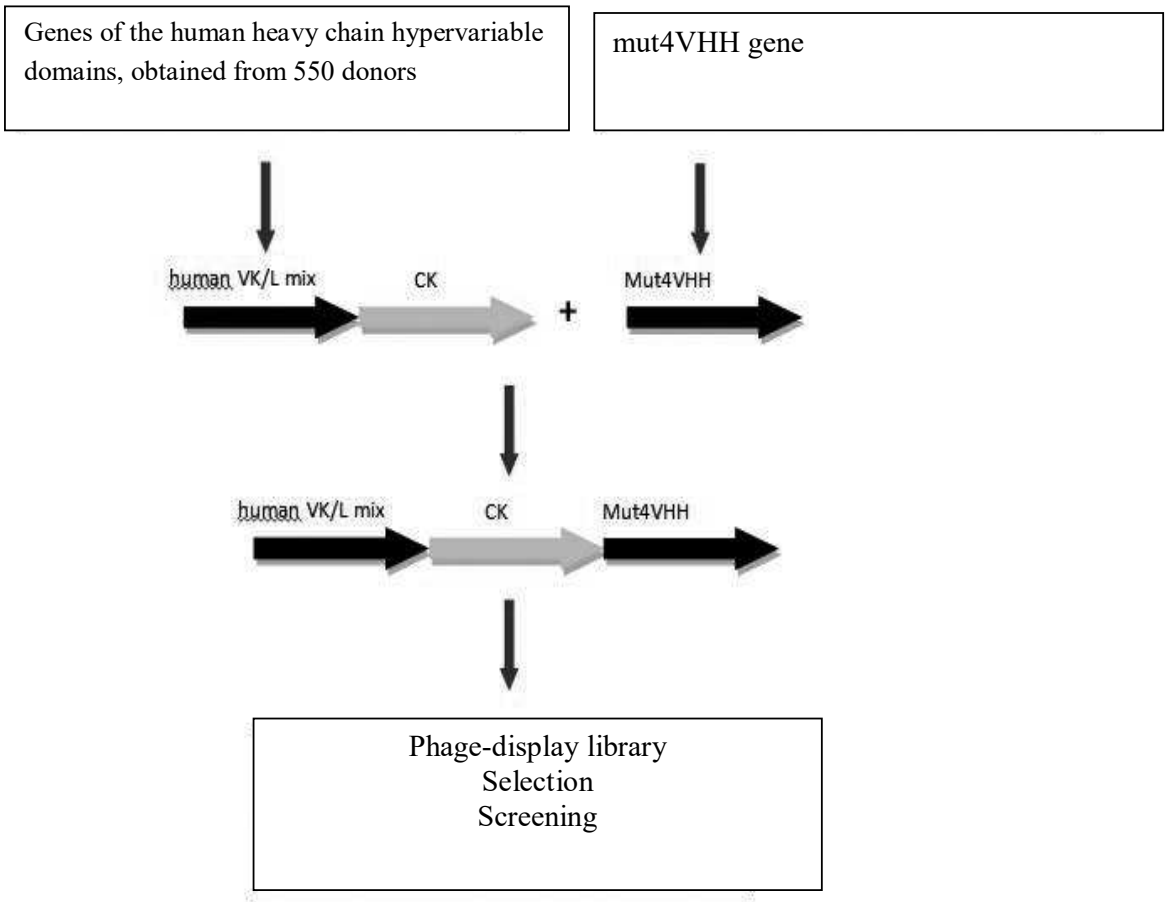

Genes of the human heavy chain hypervariable domains, obtained from 550 donors
mut4VHH gene
human VK/L mix
CK
+
Mut4VHH
human VK/L mix
CK
Mut4VHH
Phage-display library
Selection
Screening

Fig. 15

B

VK1A7
EIVLTQSPASLSASVGDRVDITCQASQSINNKIAWYQQKPGKPPKVLIYAASKLP
TGVPSRFSGSGSGTDFTLTISSLQAEDVATYYCLQDYNWPWTFGAGTKLEIK

VK3c8
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYDASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSYSPVTFGQGTKVEIK

VK3c18
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQSYYYPVTFGQGTKVEIK

VK4E12
DIQLTQSPSSLSASAGETASINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIY
WASTRESGVPDRFSGSGSGTDFTLTISSLQSEDAAVYYCQQGYSTPHTFGQGTKV
EIK

Fig. 16

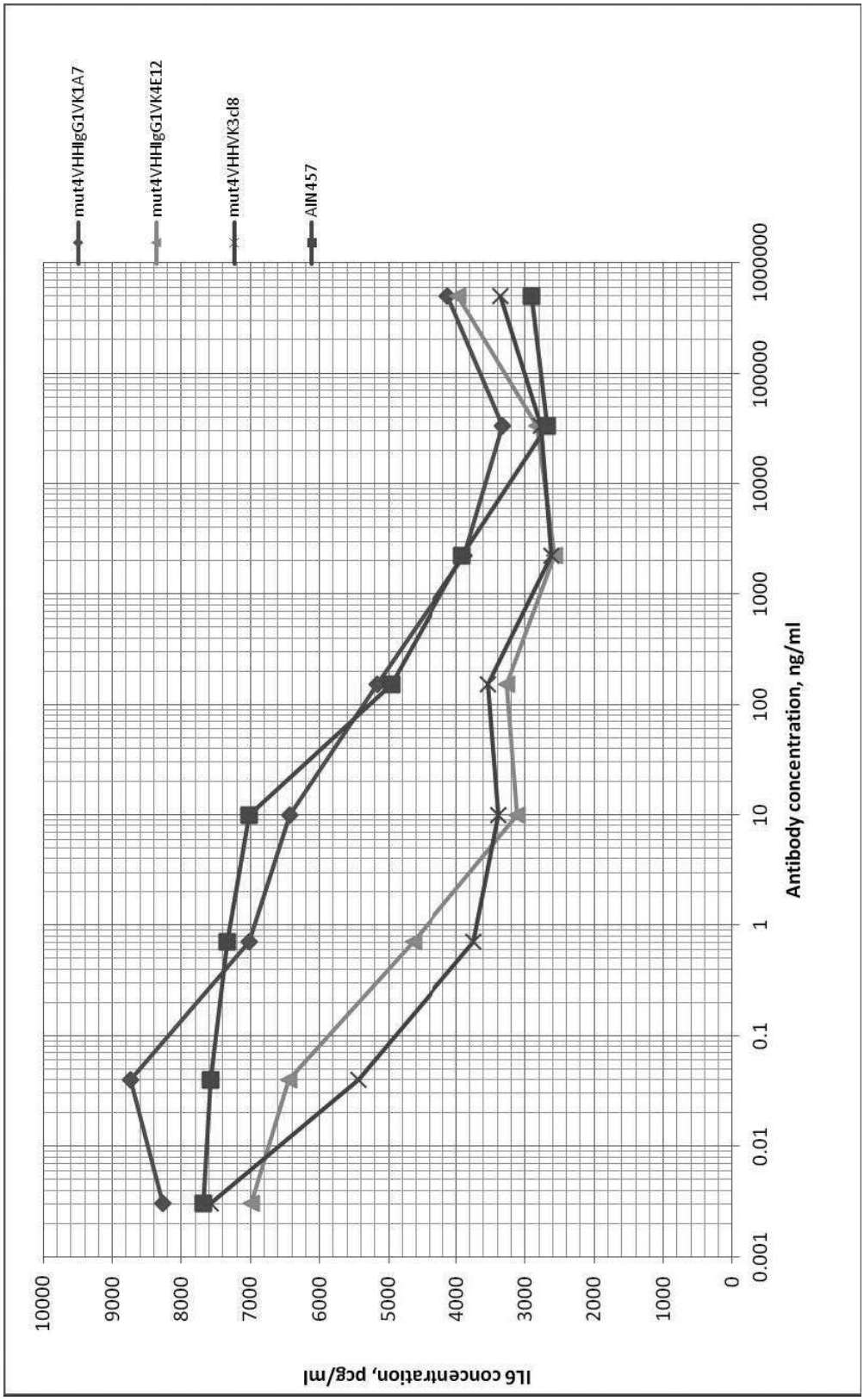
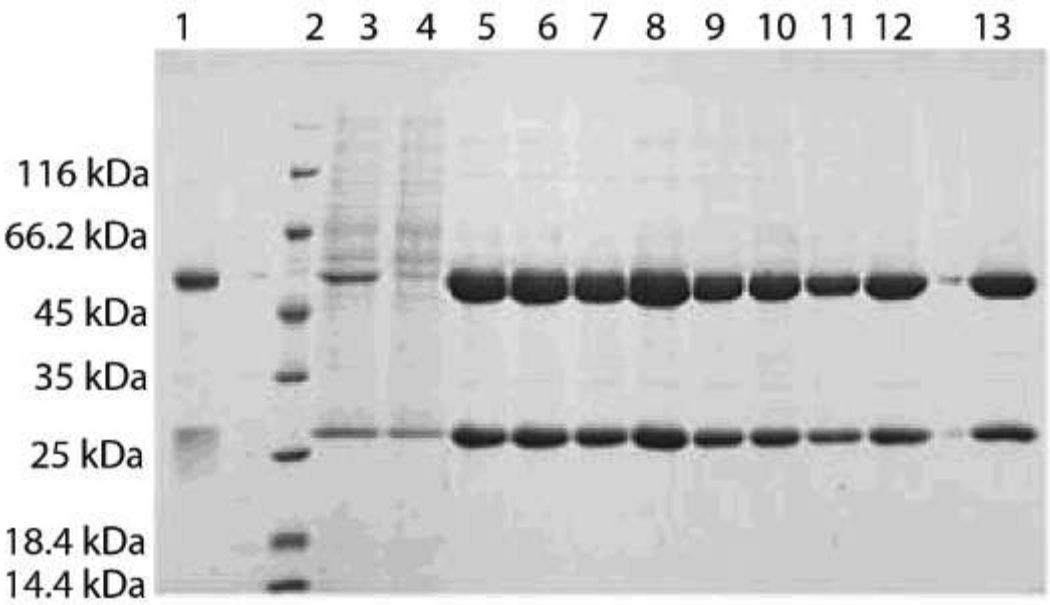

mut4VHHIgG1VK1A7
mut4VHHIgG1VK4E12
mut4VHHVK3d8
AIN457
IL6 concentration, pg/ml
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
1000
10000
100000
1000000
Antibody concentration, ng/ml

Fig. 17



Gel electrophoresis in 12% PAGE + β-ME

1) IgG1 control, 2.5 μg

2) Ferments unstained marker

3) 44V45T VHHIgG1c8 culture liquid before purification 10 μL

4) 44V45T VHHIgG1c8 culture liquid after purification 10 μL

5) 44V45T VHHIgG1c8 5 μL

6) 44D45T VHHIgG1c8 5 μL

7) 44T45T VHHIgG1c8 5 μL

8) 44A45T VHHIgG1c8 5 μL

9) 44S45T VHHIgG1c8 5 μL

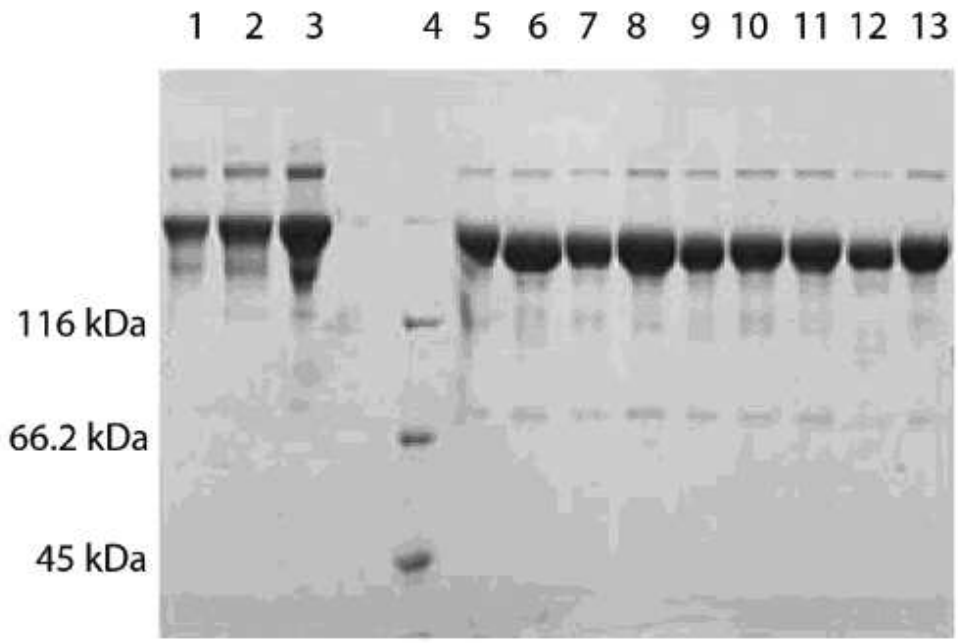
10) 44V45V VHHIgG1c8 5 μL

11) 44D45V VHHIgG1c8 5 μL

12) 44A45V VHHIgG1c8 5 μL

13) 44T45V VHHIgG1c8 5 μL.

Fig. 18



Gel electrophoresis in 12% PAGE + β-ME

1) IgG1 control, 2.5 µg

2) IgG1 control, 5 µg

3) IgG1 control, 10 µg

4) Fermentos unstained PW marker

5) 44V45T VHHIgG1c8 5 µL

6) 44D45T VHHIgG1c8 5 µL

7) 44T45T VHHIgG1c8 5 µL

8) 44A45T VHHIgG1c8 5 µL

9) 44S45T VHHIgG1c8 5 µL

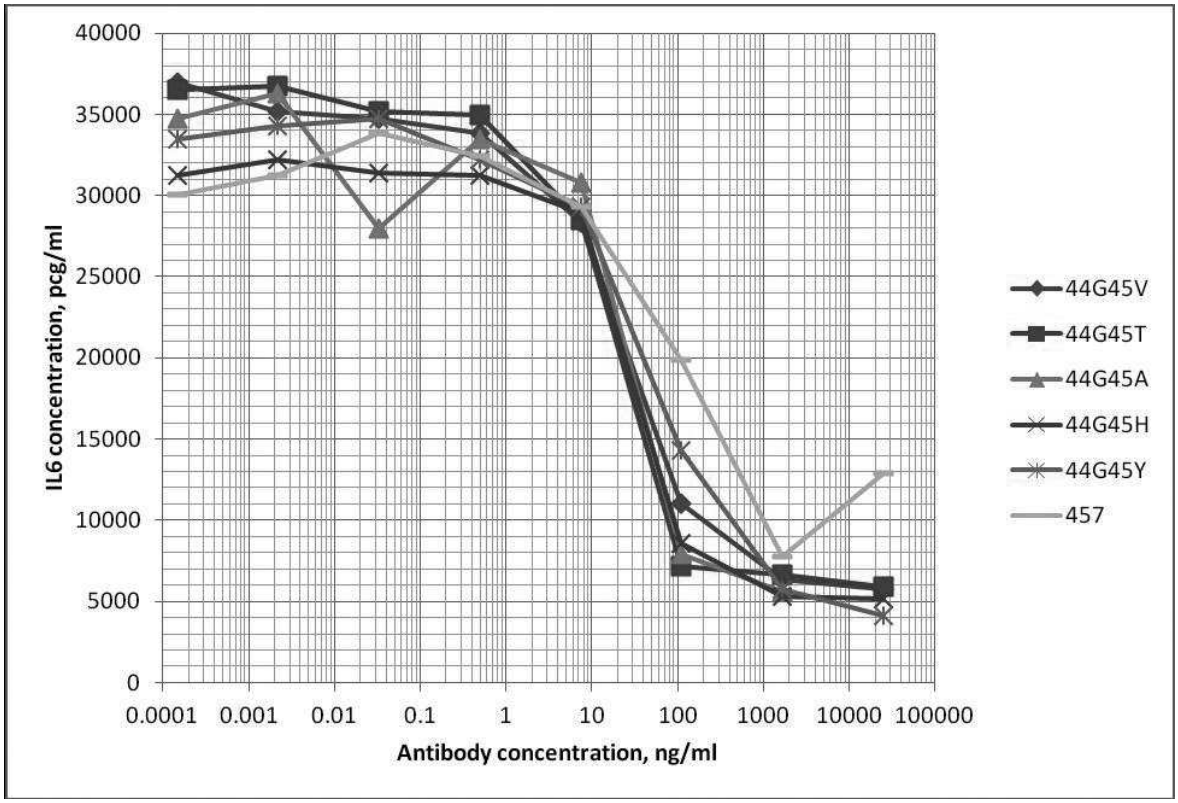
10) 44V45V VHHIgG1c8 5 µL

11) 44D45V VHHIgG1c8 5 µL

12) 44A45V VHHIgG1c8 5 µL

13) 44T45V VHHIgG1c8 5 µL.

Fig. 19


40000
35000
30000
25000
20000
15000
10000
5000
0
IL6 concentration, pcg/ml
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
100000
Antibody concentration, ng/ml
44G45V
44G45T
44G45A
44G45H
44G45Y
457

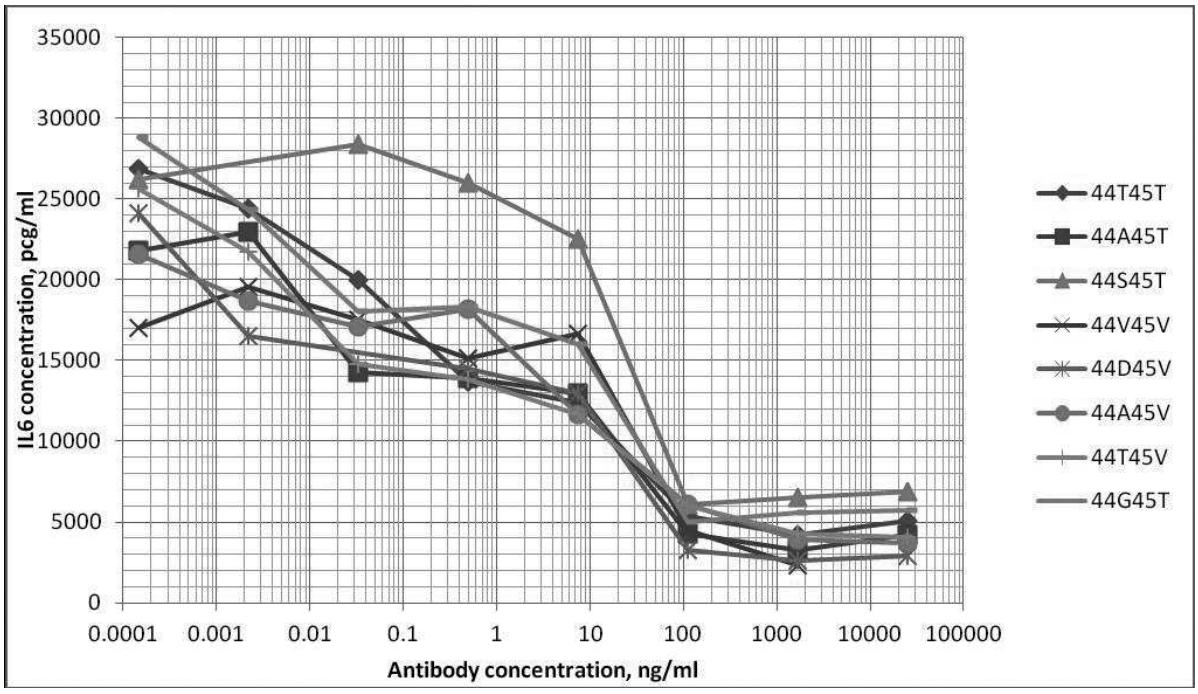
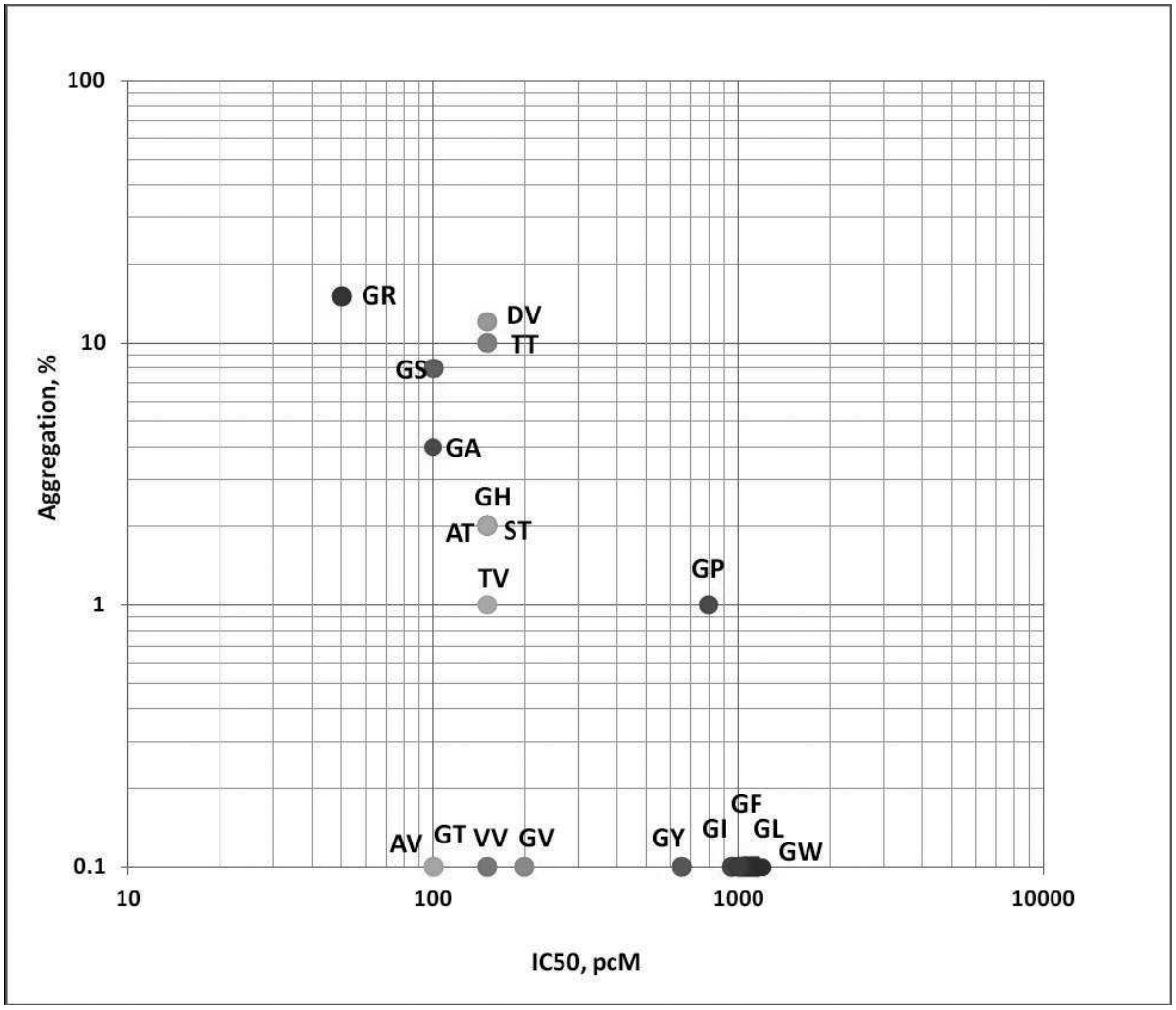

35000
30000
25000
20000
15000
10000
5000
0
IL6 concentration, pcg/ml
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
100000
Antibody concentration, ng/ml
44T45T
44A45T
44S45T
44V45V
44D45V
44A45V
44T45V
44G45T

Fig. 20



Dashed thick lines correspond to the combinations providing the maximum balanced aggregation stability and functional activity values; Dashed thin lines correspond to the suitable aggregation stability values with the high affinity remained.

Fig. 21

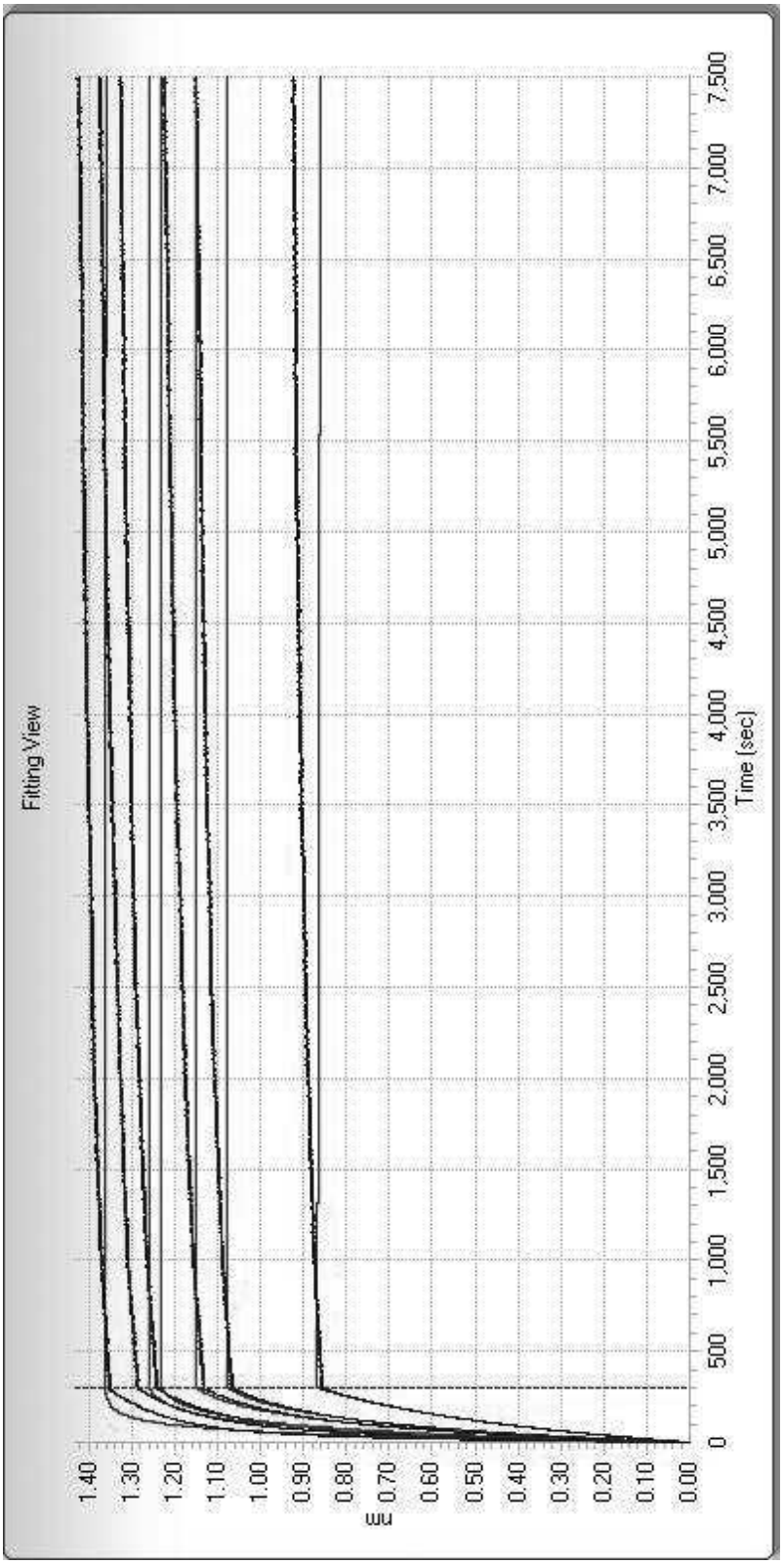

Fitting View
Time (sec)
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,500
7,000
7,500
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
1.10
1.20
1.30
1.40

Fig. 22

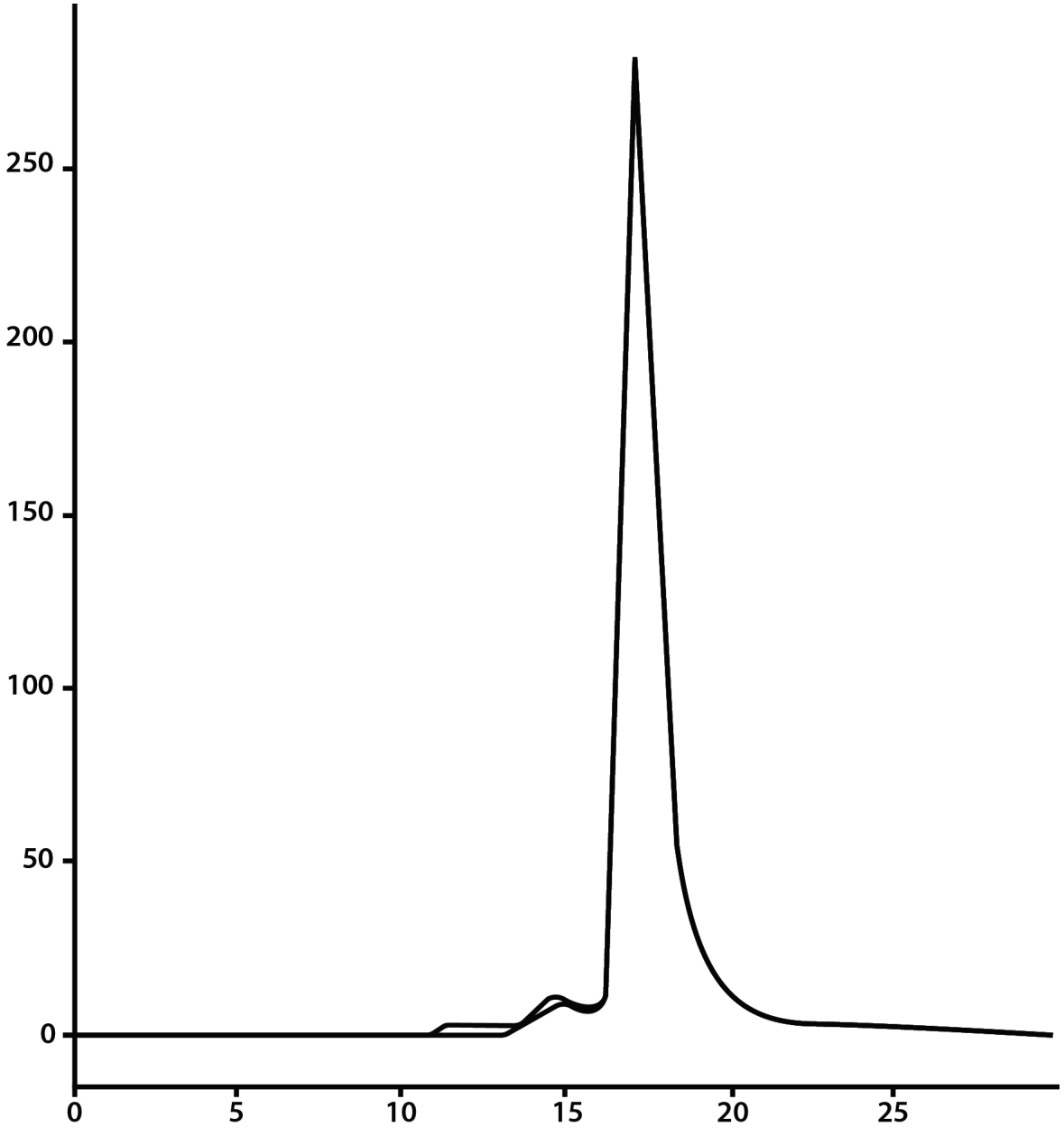
Human anti-IL17ABCD-109 cIL17 Fc

Conc. (nM)	Response	KD (M)	kon(1/Mc)	konError	kdis(1/c)	kdisError	Full R^2
68,6	1,2363	<1.0E-12	2,00E+05	3,28E+03	<1.0E-07		0,383582
34,3	1,06	<1.0E-12	2,61E+05	3,72E+03	<1.0E-07		0,744563
17,1	0,845	4,40E-12	2,82E+05	6,65E+03	1,24E-06	2,61E-07	0,775434

Macaque anti-IL17A BCD-109 c IL17 Fc

Conc. (nM)	Response	KD (M)	kon(1/Mc)	konError	kdis(1/c)	kdisError	Full R^2
68,7	1,3497	<1.0E-12	3,24E+05	3,22E+03	2,36E-07	1,71E-07	0,672159
34,4	1,2814	<1.0E-12	4,26E+05	8,35E+03	<1.0E-07		0,061979
17,2	1,127	<1.0E-12	5,71E+05	7,48E+03	<1.0E-07		0,753773

Fig. 23



------- Chromatographic profile obtained with the antibody before heating

-------- Chromatographic profile obtained with the antibody after heating

Protein concentrations were aligned and equaled 10 mg/mL in PBS

Fig. 24