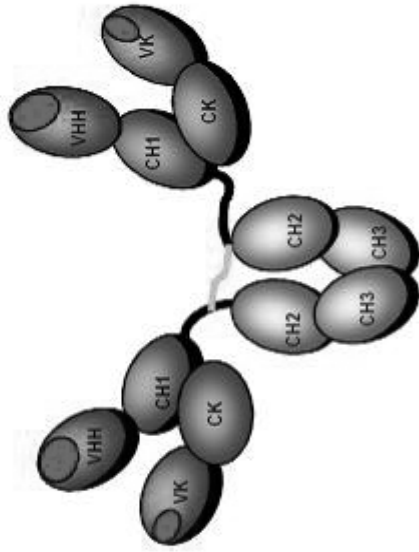
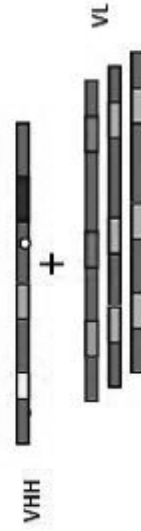


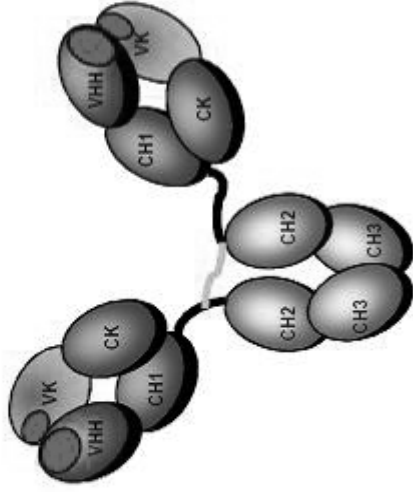
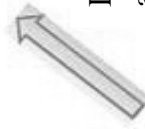
High-affinity aggregation non-resistant VHH-based antibody



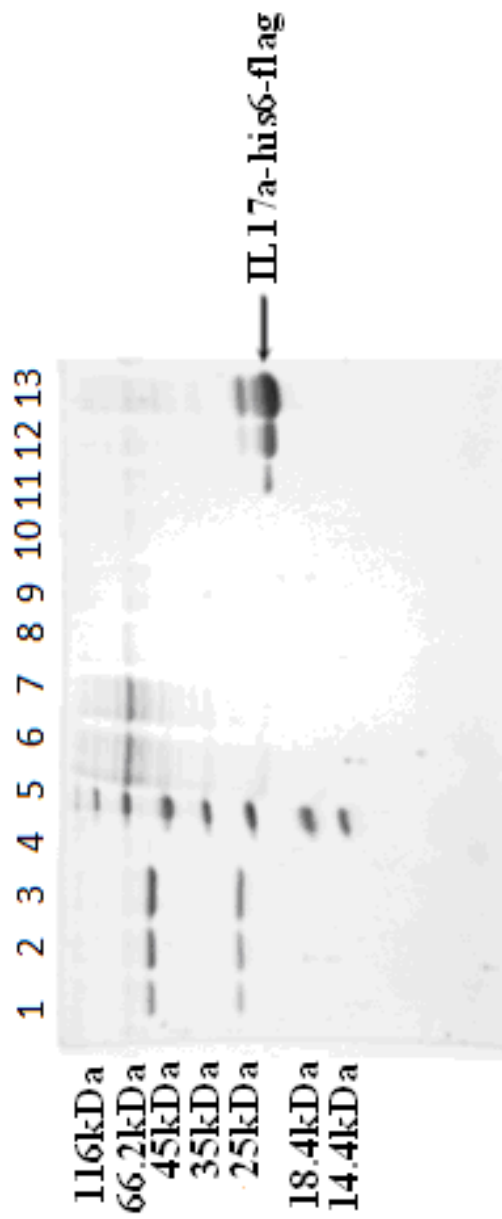
Mutagenesis by VHH position 44+45 plus light chain shuffling



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

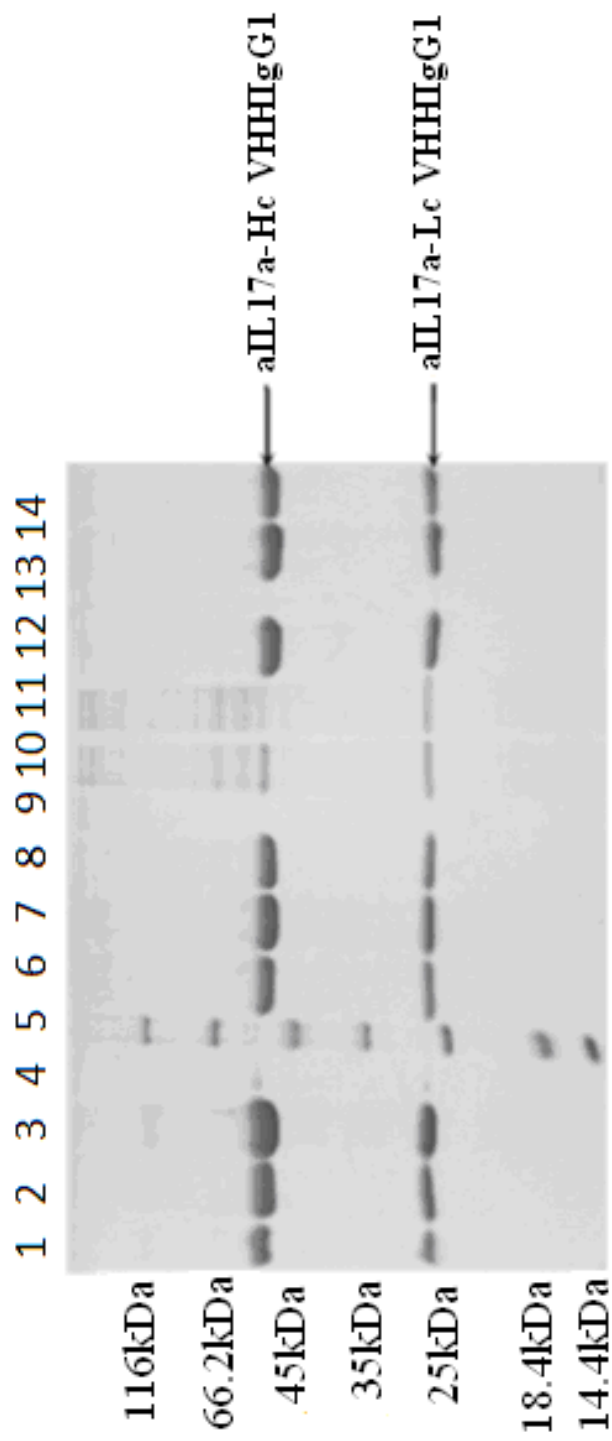


Display libraries and selection under tight conditions (low antigen concentrations, long-term washing, incubation with competitors, increased temperature, etc.). High-performance robotic screening including the testing of affinity and aggregation stability



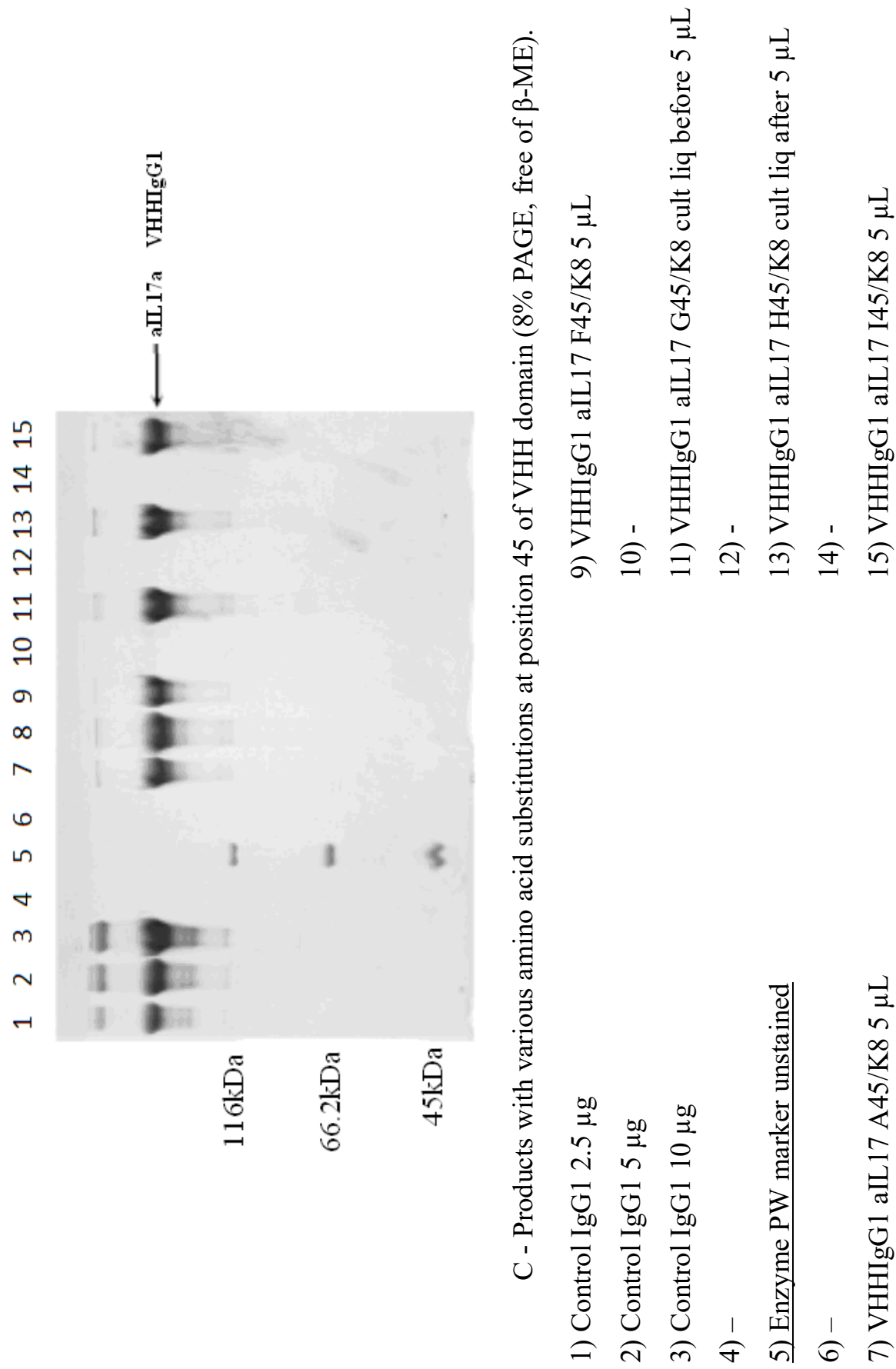
A - Human recombinant IL17A-His6-FLAG product (15% PAGE+ β -ME).

- 1) 9E10 0.25 μ g+ β -ME
- 2) 9E10 0.5 μ g + β -ME
- 3) 9E10 1 μ g + 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 μ L
- 12) Elution 5 μ L
- 13) Elution 10 μ L.



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

- 1) Control IgG1 2.5 μ g
- 2) Control IgG1 5 μ g
- 3) Control IgG1 10 μ g
- 4) -
- 5) Enzyme PW marker unstained
- 6) VHHIgG1 aIL17 A45/K8 5 μ L
- 7) VHHIgG1 aIL17 D45/K8 5 μ L
- 8) VHHIgG1 aIL17 F45/K8 5 μ L
- 9) -
- 10) VHHIgG1 aIL17 G45/K8 cult liq before 5 μ L
- 11) VHHIgG1 aIL17 G45/K8 cult liq after 5 μ L
- 12) VHHIgG1 aIL17 G45/K8 5 μ L
- 13) -
- 14) VHHIgG1 aIL17 H45/K8 5 μ L.
- 15) VHHIgG1 aIL17 I45/K8 5 μ L.



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

- 1) Control IgG1 2.5 μ g
- 2) Control IgG1 5 μ g
- 3) Control IgG1 10 μ g
- 4) -
- 5) Enzyme PW marker unstained
- 6) -
- 7) VHHIgG1 aIL17 A45/K8 5 μ L
- 8) VHHIgG1 aIL17 D45/K8 5 μ L

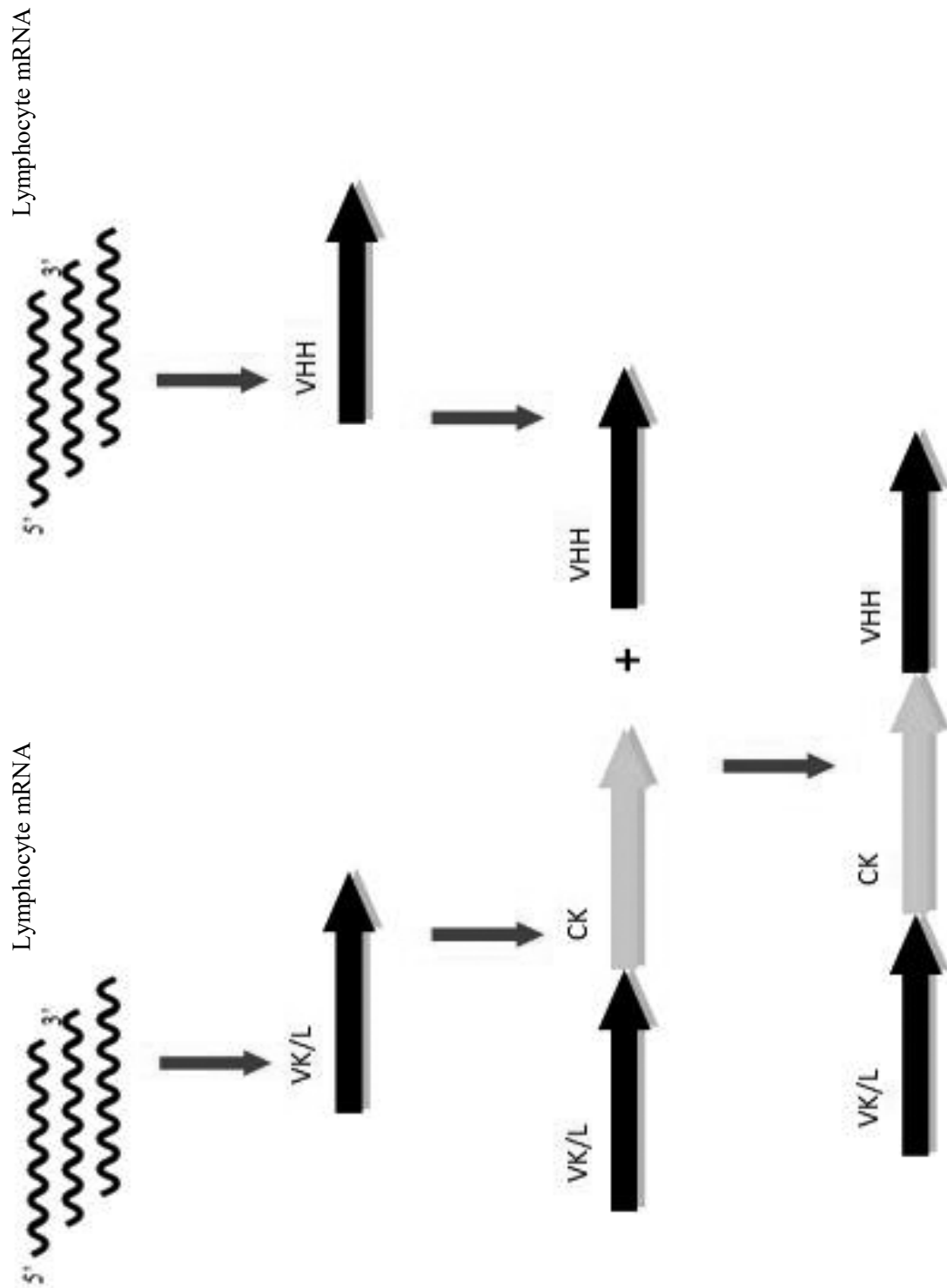


Fig. 5
5/24

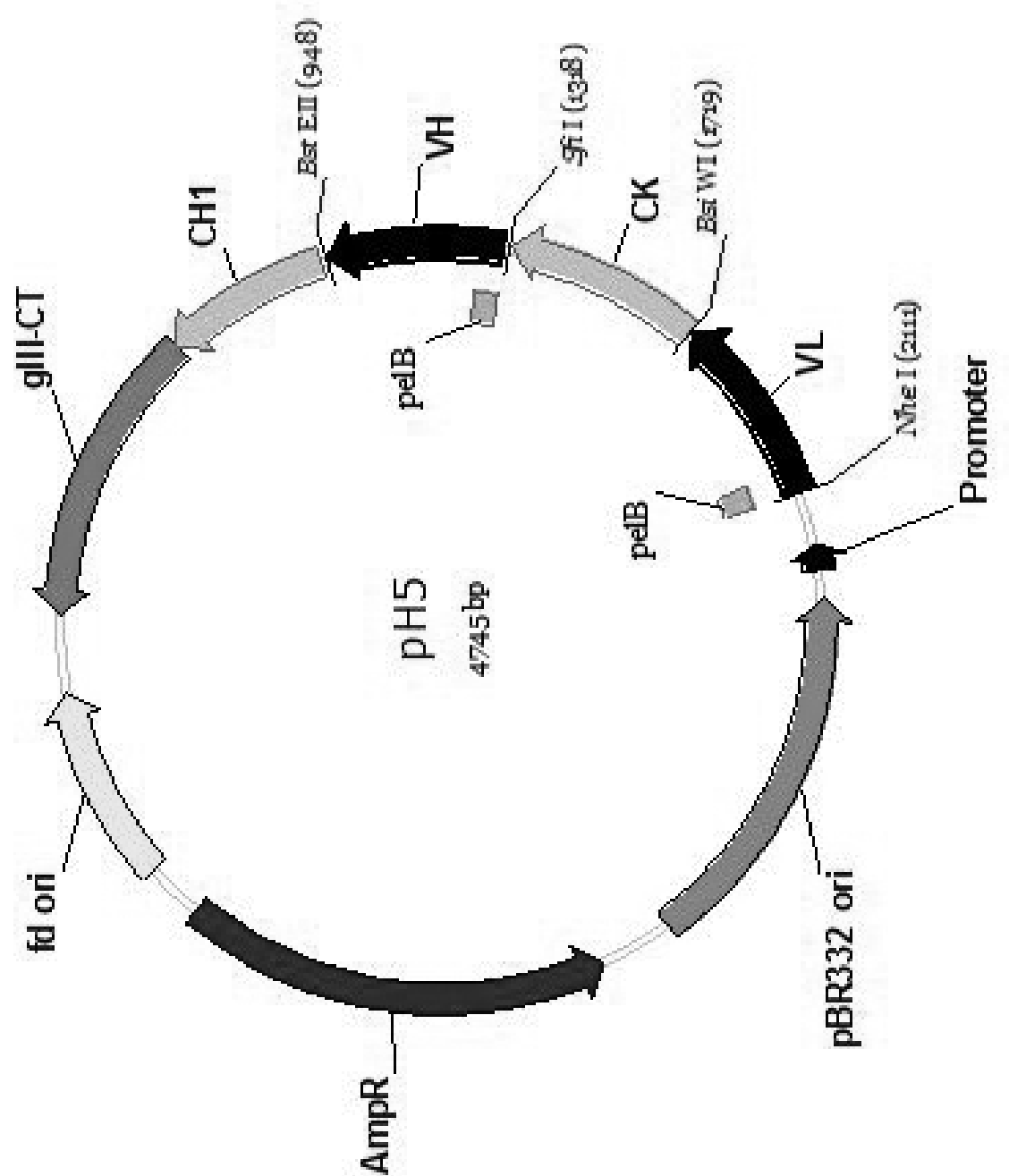


Fig. 6
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A

1VHH

QVQLQQSGGGSVQTGGSLTTLTCAASGLTFEANS LGWFRQSPGK**ERE**FVA AVSFTKRIDYADSVK
GRFFISRDNMTMNTVY LQMNSLKPEDTGIYTCAADPLLISNKRANI-----WGQGTMTVTVSS

2VHH

QVQLVQSGGGLVQPGGSLRLSCAASPRVISIHDMAWYRQAPGK**EREL**VAGITTRGITDYGYSVK
GRFTISRDDAKNTLFLQMNDLKPEDTAVYYCNLRHYEV-----WGQGT LTVTVSS

3VHH

QVQLQQSGGGSVQAGGSLRLSCAASG GTFATSPMGWFRQAPGK**EREG**VAAISP SGGDRIYDDSV
KGRFTISRDNAGYFIYLQMNSLKPEDTARYYCAVRRRFDGTSYYTGDYDSWGQGT LTVTVSS

B

VK4B11DVVMTQSPSSVTASAGETVTINC KSSQSVAYKSNQKNYLAWYQQRPGQSPRLLIYYAS
TRTSGVPDRFSGSGSTTDFTLTIS SFQPEDAAVYYCQQGYSA PYSFGSGTKLEIK

VLB5

AVLTQLSSMSGSPGQTVTITCTGSITNIGQYRVN WYQHLPGTAPKLLIY SNANRVSGVPDRFSG
SKSGSTASLTIAGVQAEDEADYYCSAWDGSLNGYV FGGGTKVTVLQR

VLF4QAVLTQPPSVSGSPGQTVTISCTGTSDDVGSGNYVS WYQQVPGMAPKLLIY NAGTRRAGI
TGRFSASKSGNTASLTISGLQSEDEADYYCAS YRKINKYVFGGGTKLTVLQR

C

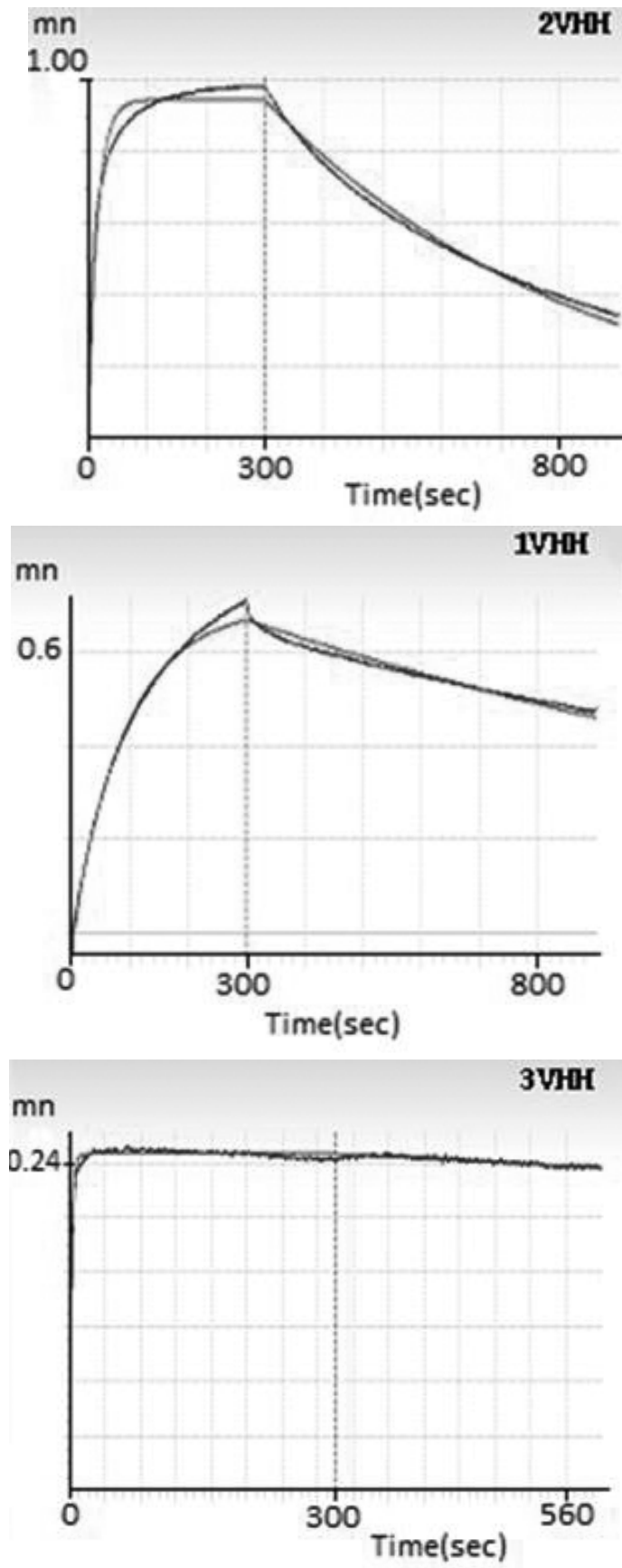


Fig. 8
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Wild 3VHHFab

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKEREFVAAISPS
GGDRIYDDSVKGRFTISRDNAGYFIYLMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut1

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKGLEFVAAISPS
GGDRIYADSVKGRFTISRDNAGYFIYLMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut2

QVQLQQSGGGLVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKGREWVAAISP
SGGDRIYADSVKGRFTISRDNAGYFIYLMNSLKPEDTARYYCAVRRRFDGTSY
YTGDYDSWGQGTLVTVSS

mut3

QVQLQQSGGGSVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKELEFVAAISPS
GGDRIYADSVKGRFTISRDNAGYFIYLMNSLKPEDTARYYCAVRRRFDGTSYY
TGDYDSWGQGTLVTVSS

mut4

QVQLQQSGGGLVQAGGSLRLSCAASGGTFATSPMGWLRQAPGKGREFVAAISP
SGGDRIYADSVKGRFTISRDNAGYFIYLMNSLKPEDTARYYCAVRRRFDGTSY
YTGDYDSWGQGTLVTVSS

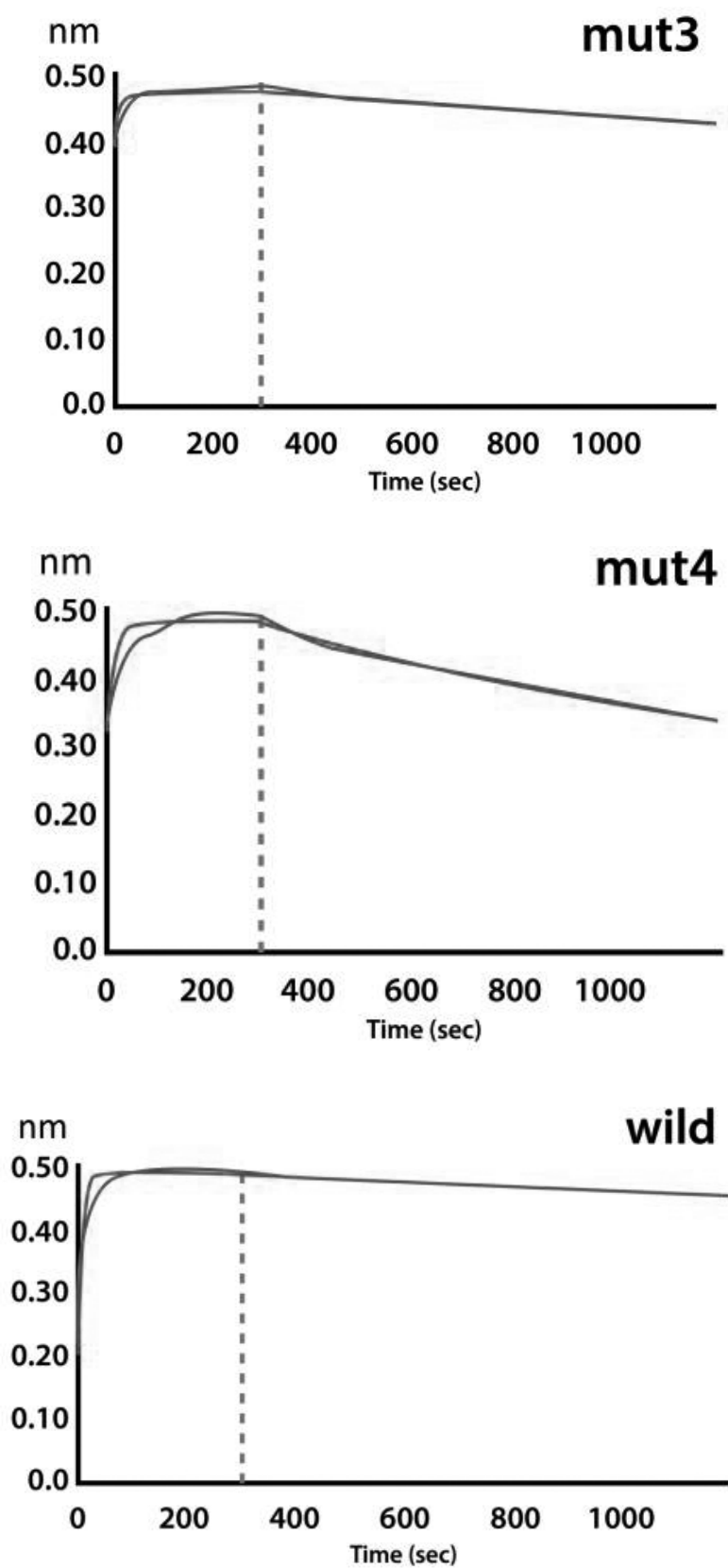


Fig. 10
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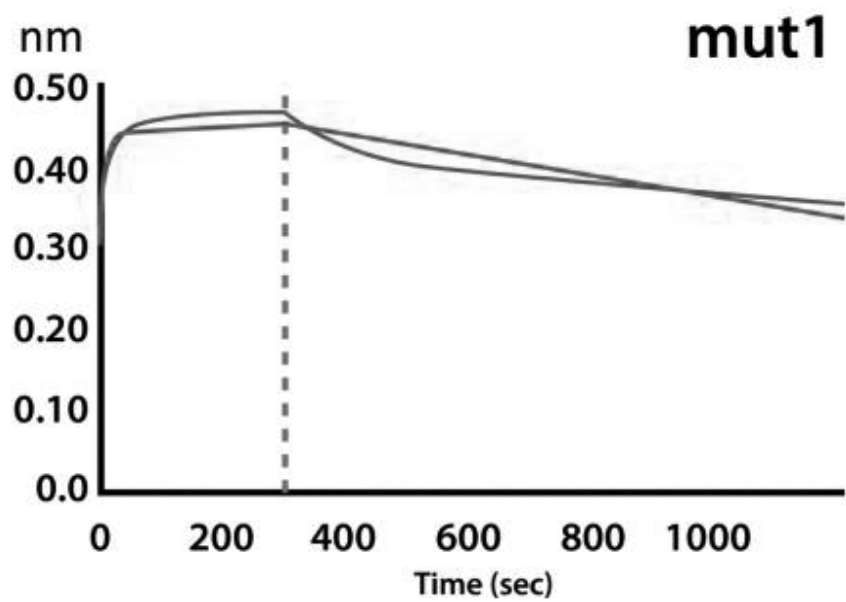
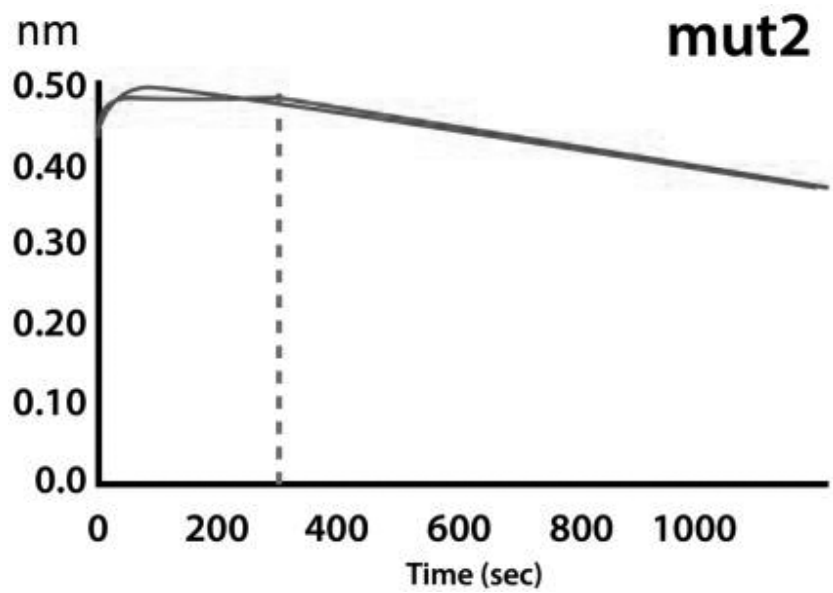


Fig. 11
11/24

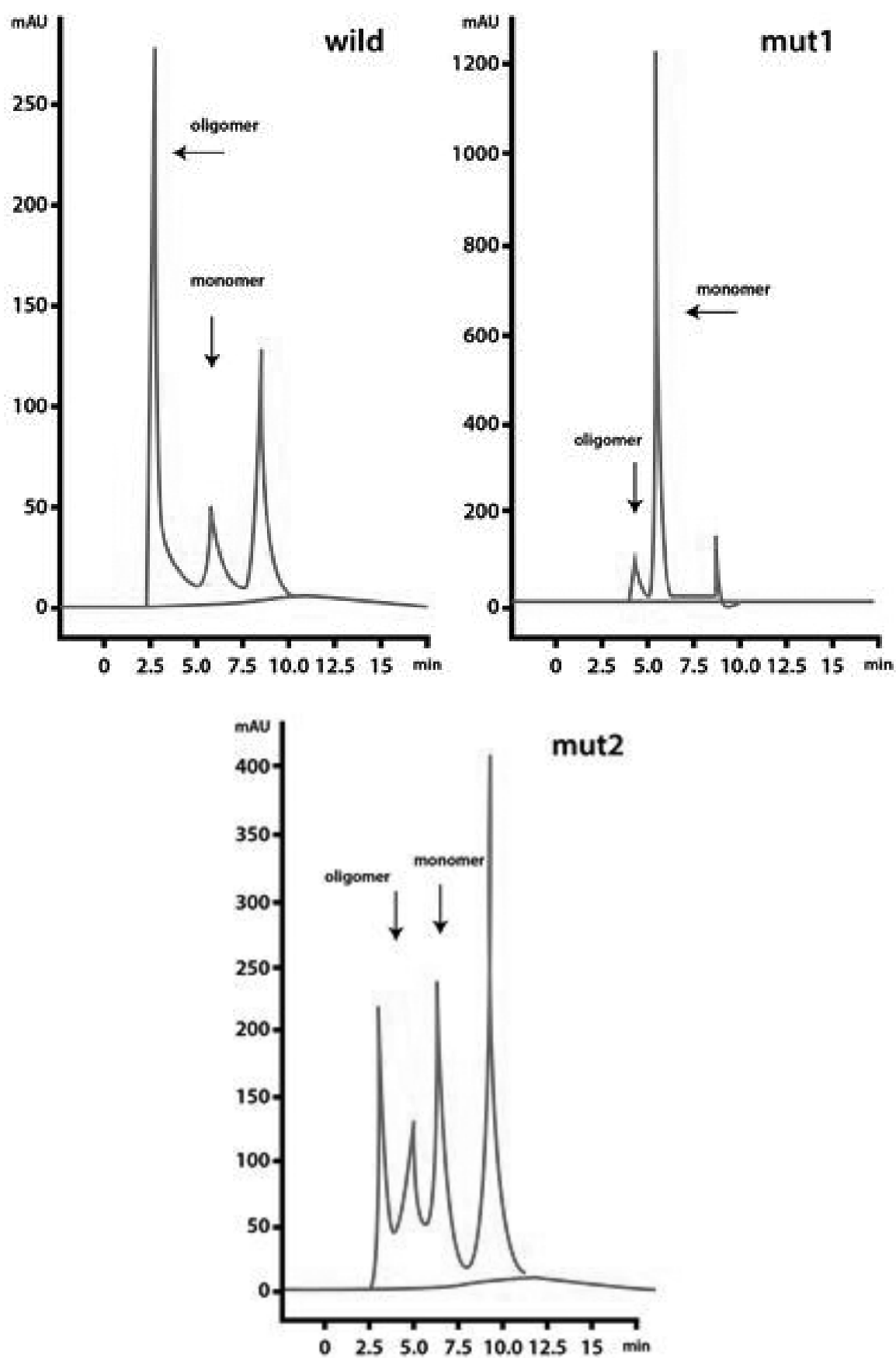


Fig. 12
12/24

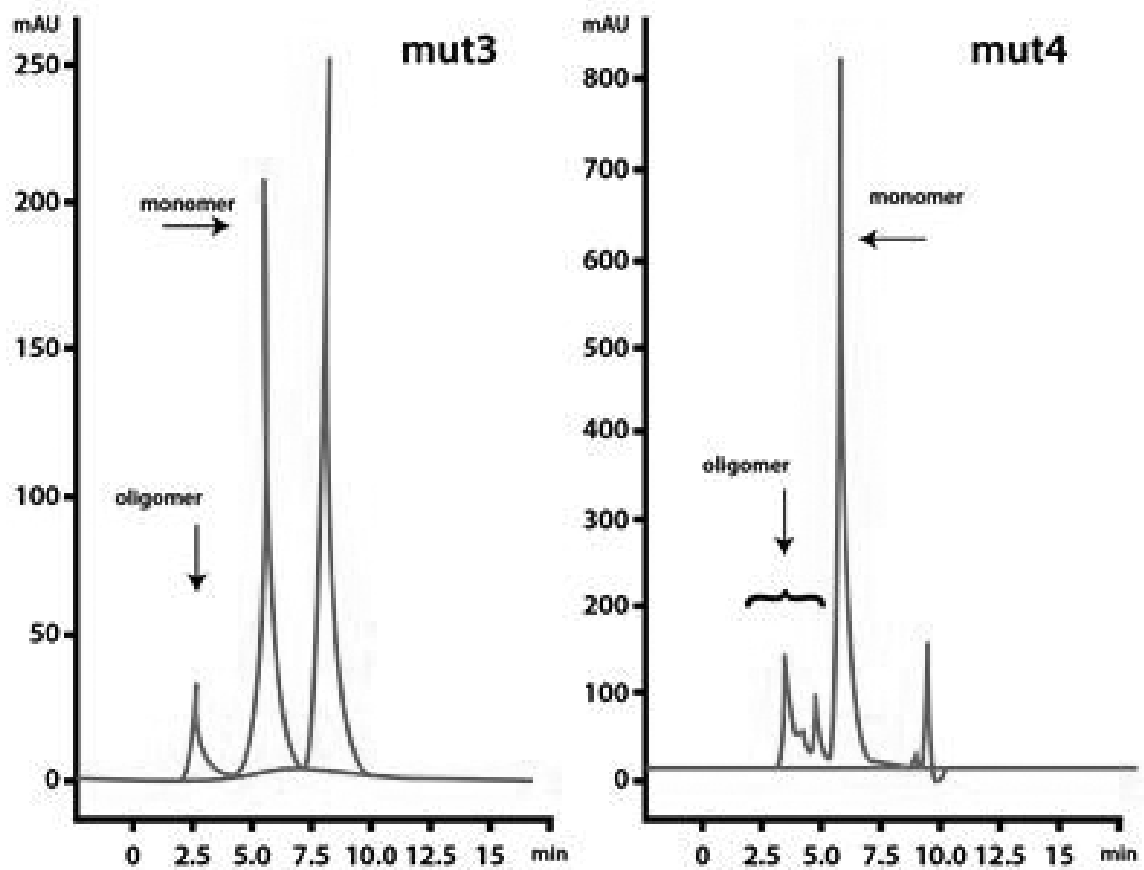


Fig. 13
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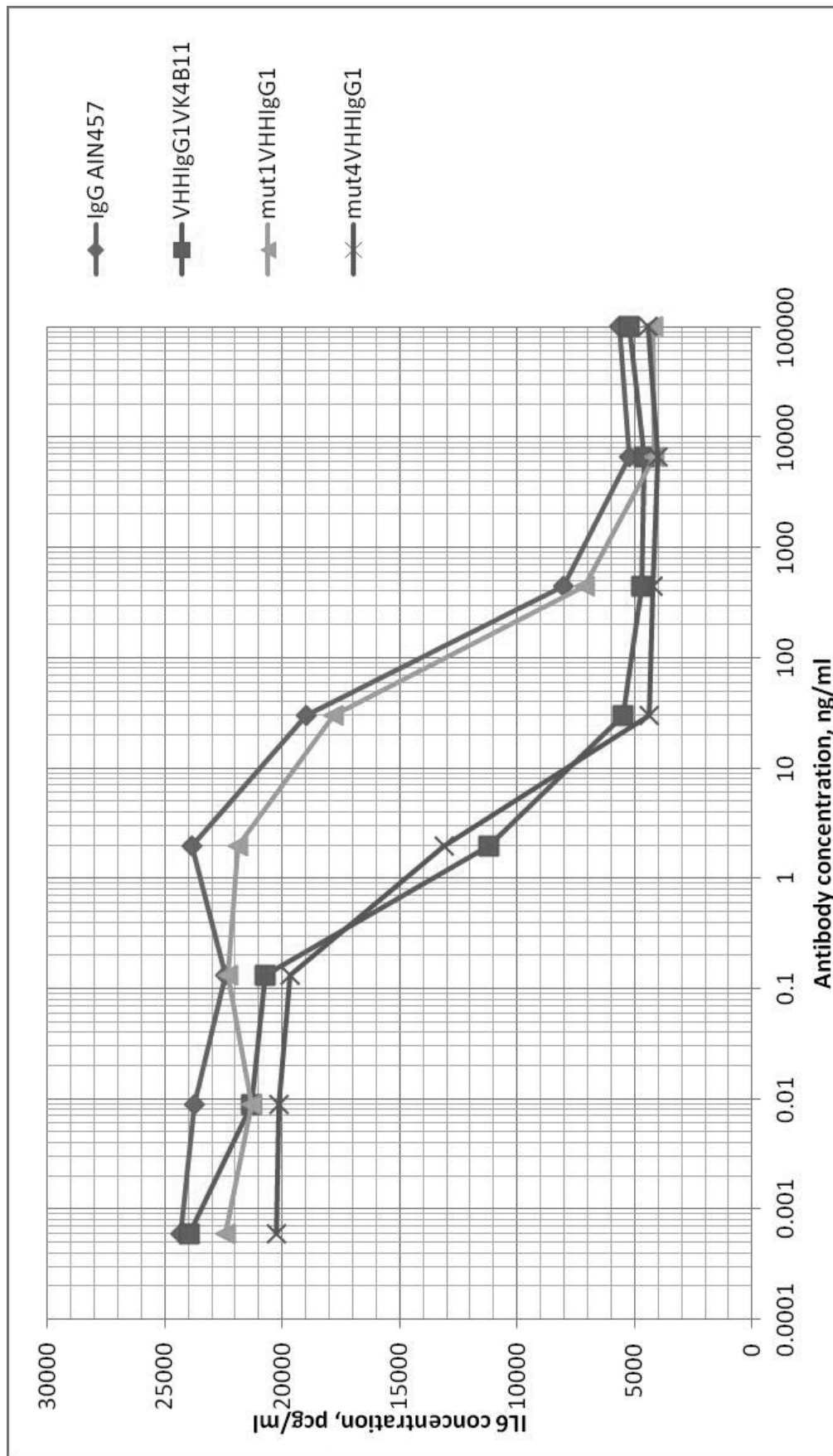


Fig. 14
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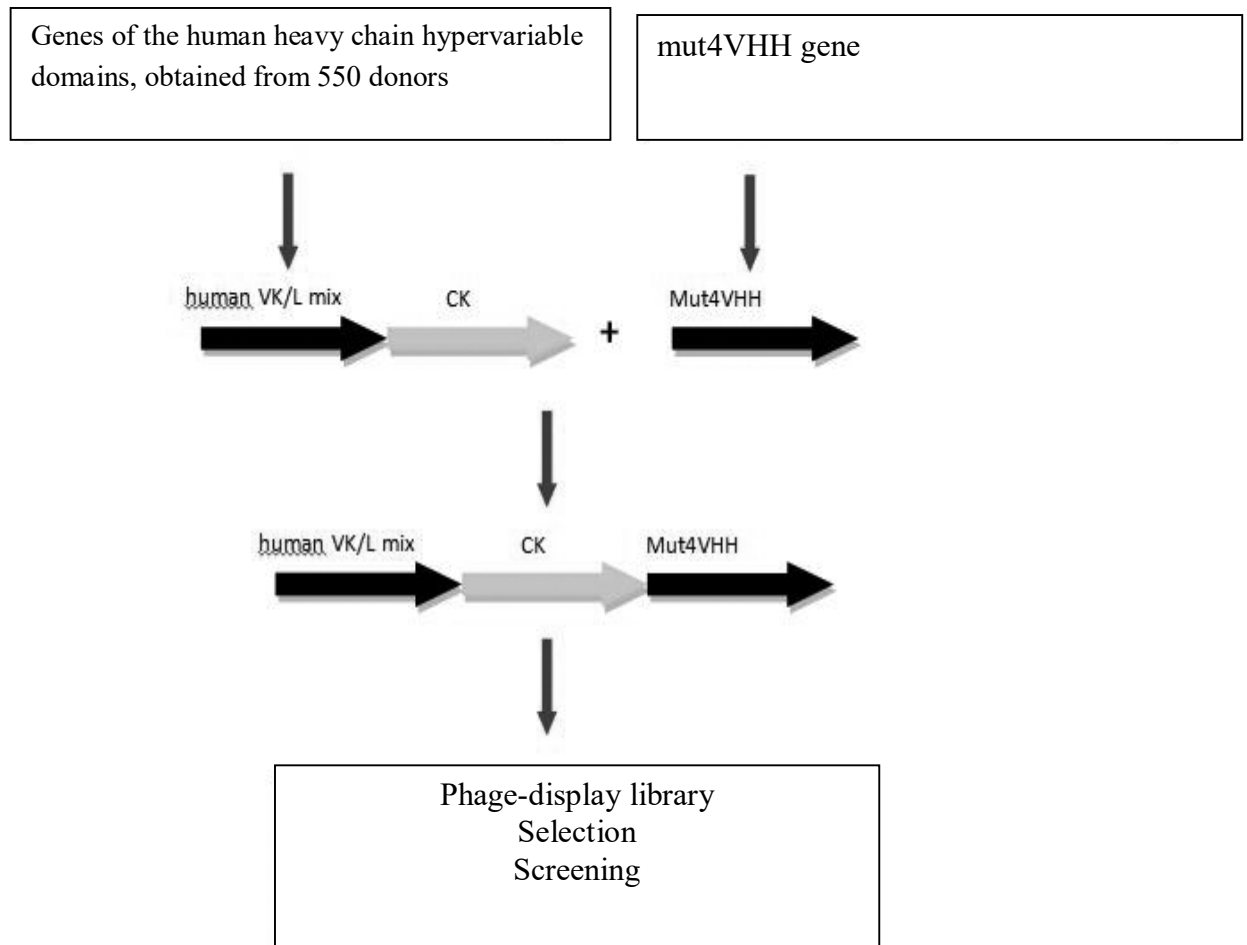


Fig. 15
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B

VK1A7

EIVLTQSPASLSASVGDRVDITCQASQSINNKIAYWYQQKPGKPPKVLIIYAASKLP
TGVPSRFSGSGSGTDFTLTISSLQAEDVATYYCLQDYNWPWTFGAGTKLEIK

VK3c18

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIIYDASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSYSPVTFGQGTKVEIK

VK3c118

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIIYGASSR
ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQSYYPVTFGQGTKVEIK

VK4E12

DIQLTQSPSSLSASAGETASINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIY
WASTRESGVPDRFSGSGSGTDFTLTISSLQSEDAAVYYCQQGYSTPHTFGQGTKV
EIK

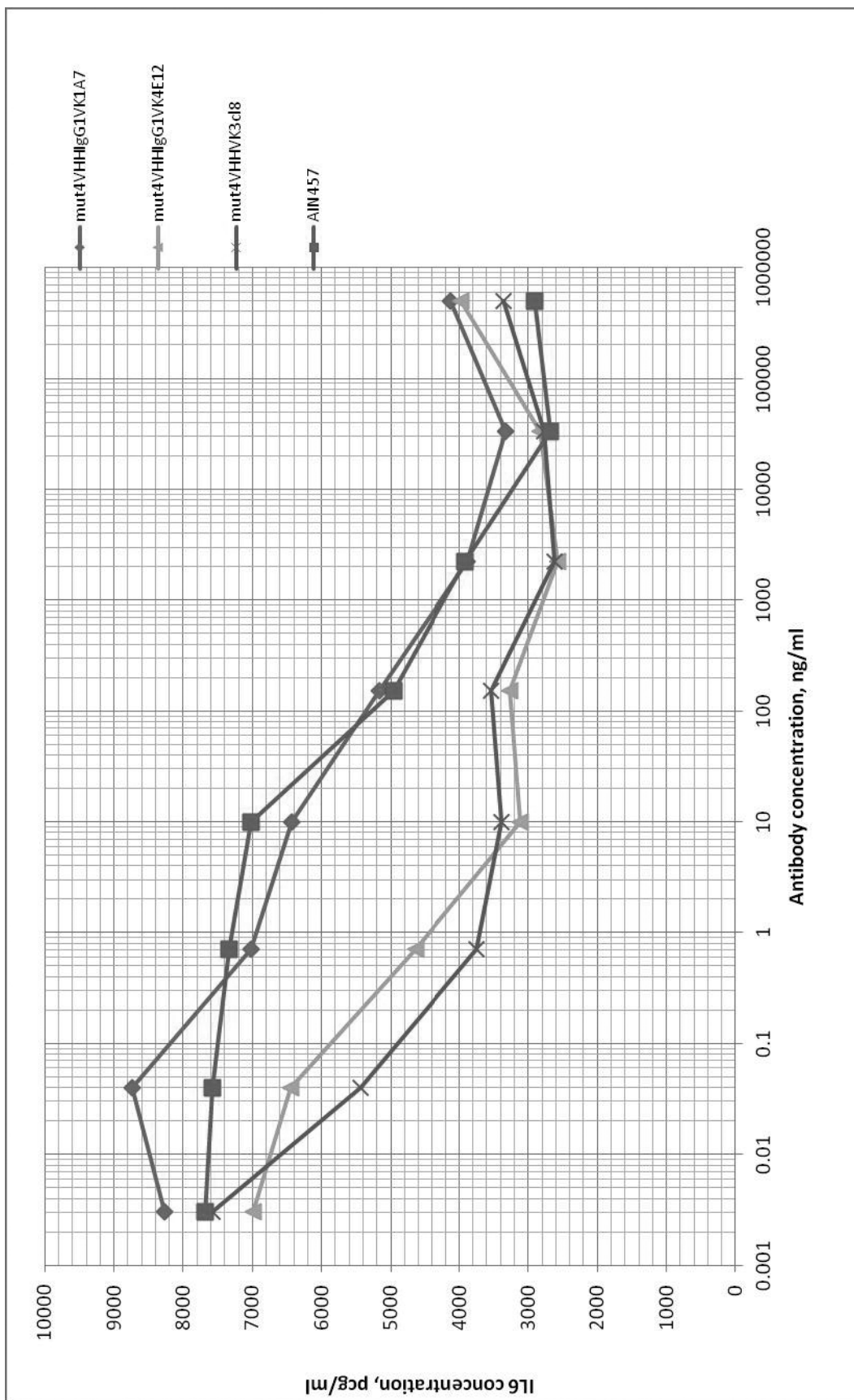
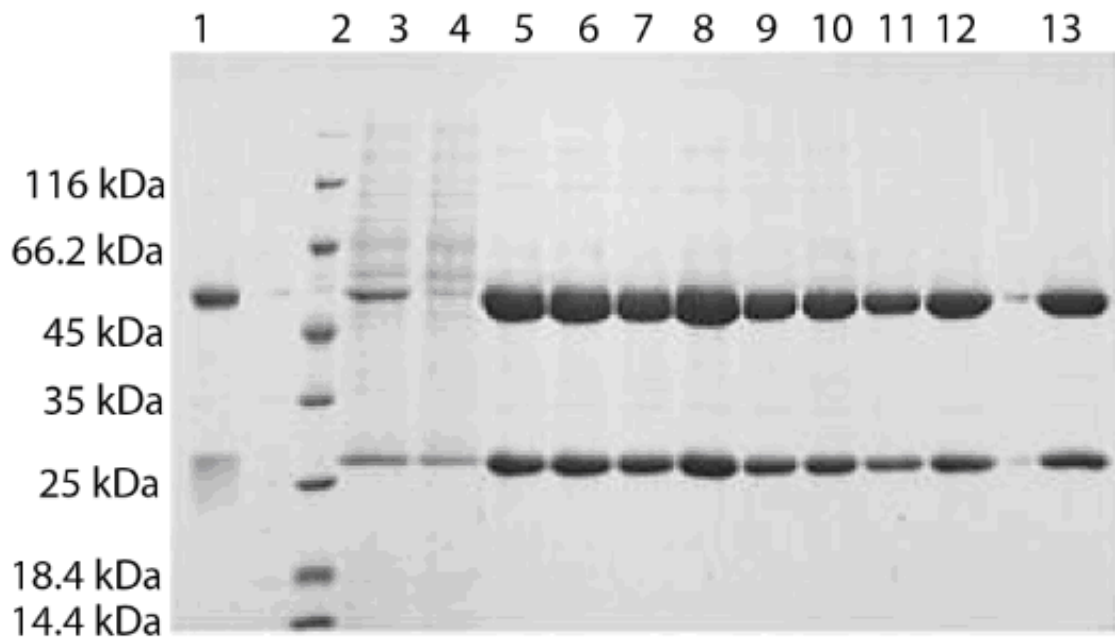
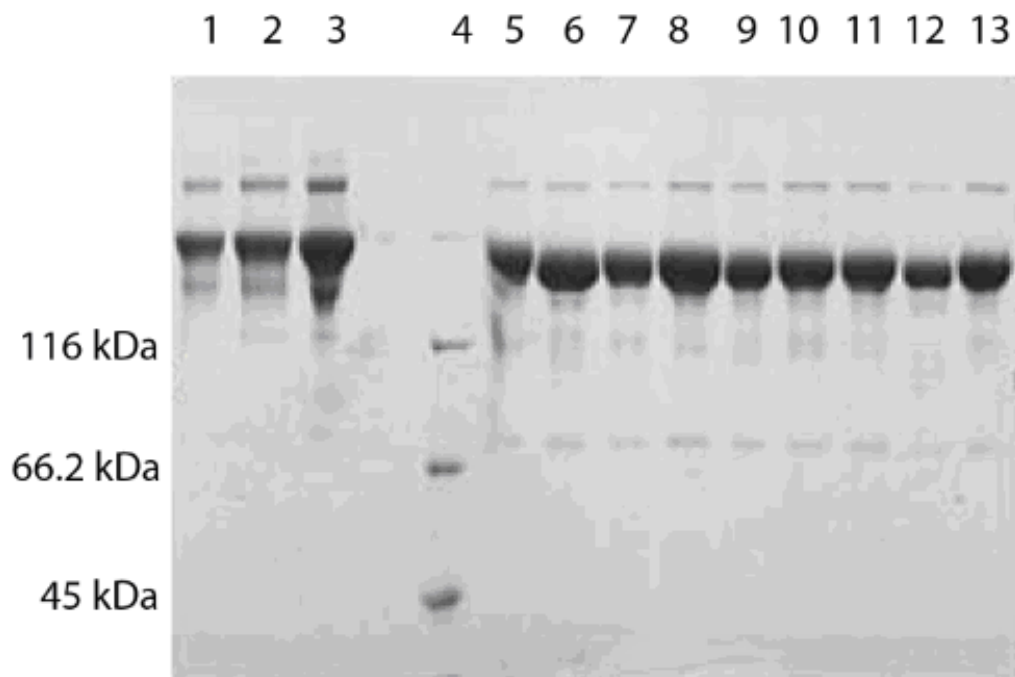


Fig. 17
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Gel electrophoresis in 12% PAGE + β -ME

- 1) IgG1 control, 2.5 μ g
- 2) Ferments unstained marker
- 3) 44V45T VHHIgG1c8 culture liquid before purification 10 μ L
- 4) 44V45T VHHIgG1c8 culture liquid after purification 10 μ L
- 5) 44V45T VHHIgG1c8 5 μ L
- 6) 44D45T VHHIgG1c8 5 μ L
- 7) 44T45T VHHIgG1c8 5 μ L
- 8) 44A45T VHHIgG1c8 5 μ L
- 9) 44S45T VHHIgG1c8 5 μ L
- 10) 44V45V VHHIgG1c8 5 μ L
- 11) 44D45V VHHIgG1c8 5 μ L
- 12) 44A45V VHHIgG1c8 5 μ L
- 13) 44T45V VHHIgG1c8 5 μ L.



Gel electrophoresis in 12% PAGE + β -ME

- 1) IgG1 control, 2.5 μ g
- 2) IgG1 control, 5 μ g
- 3) IgG1 control, 10 μ g
- 4) Fermentos unstained PW marker
- 5) 44V45T VHHIgG1c8 5 μ L
- 6) 44D45T VHHIgG1c8 5 μ L
- 7) 44T45T VHHIgG1c8 5 μ L
- 8) 44A45T VHHIgG1c8 5 μ L
- 9) 44S45T VHHIgG1c8 5 μ L
- 10) 44V45V VHHIgG1c8 5 μ L
- 11) 44D45V VHHIgG1c8 5 μ L
- 12) 44A45V VHHIgG1c8 5 μ L
- 13) 44T45V VHHIgG1c8 5 μ L.

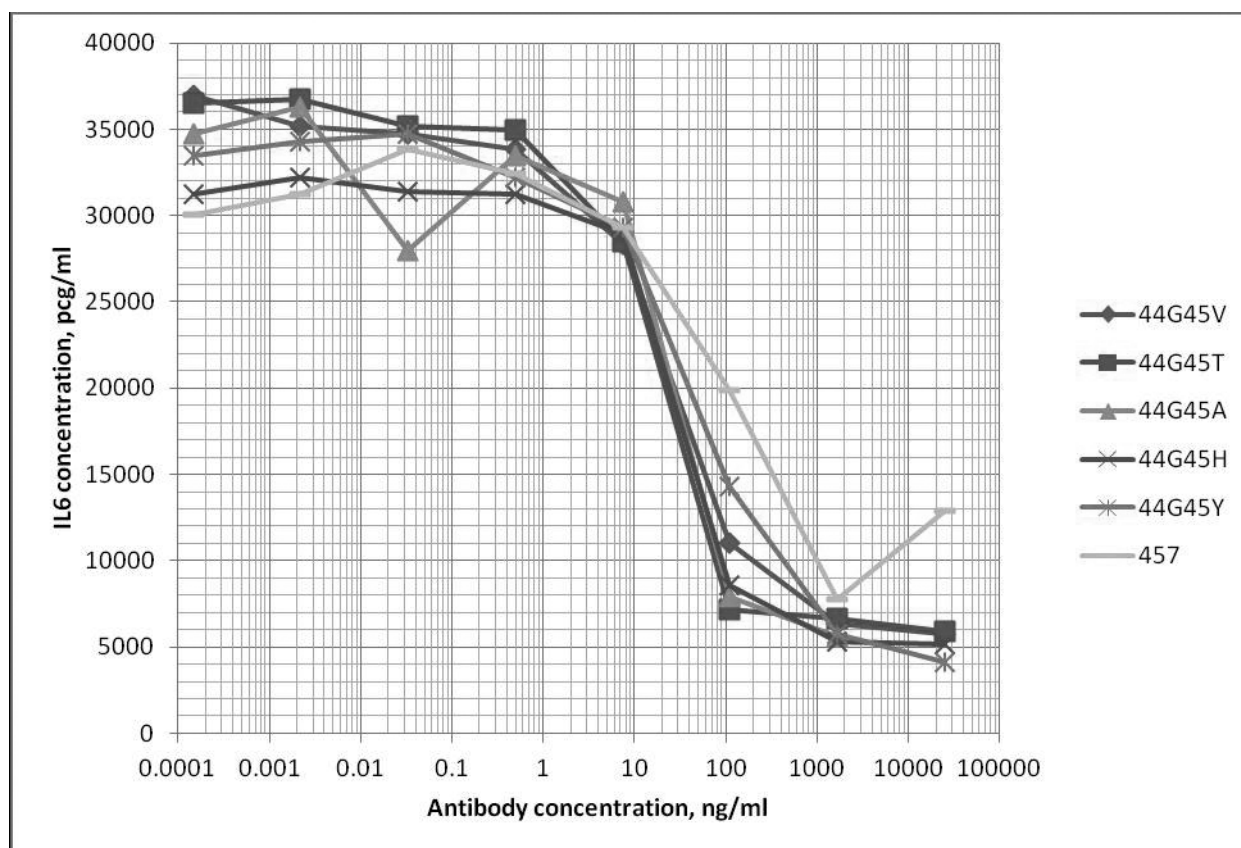
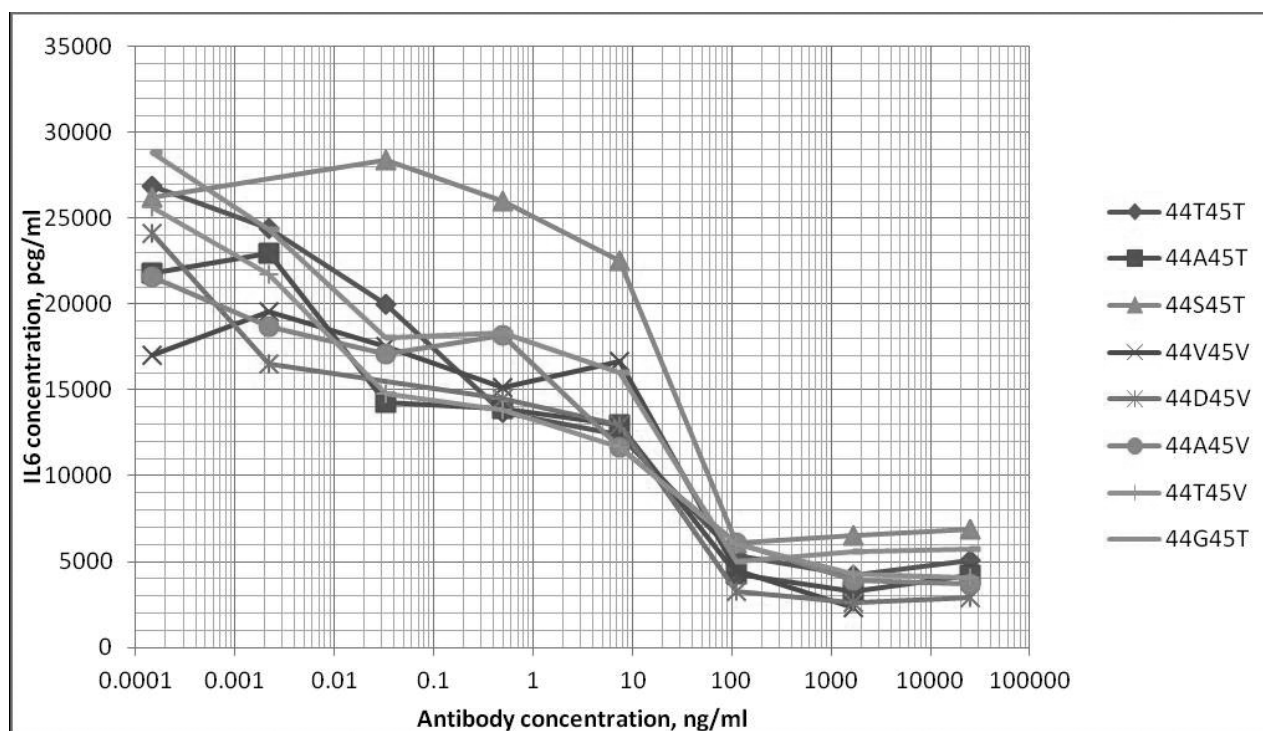
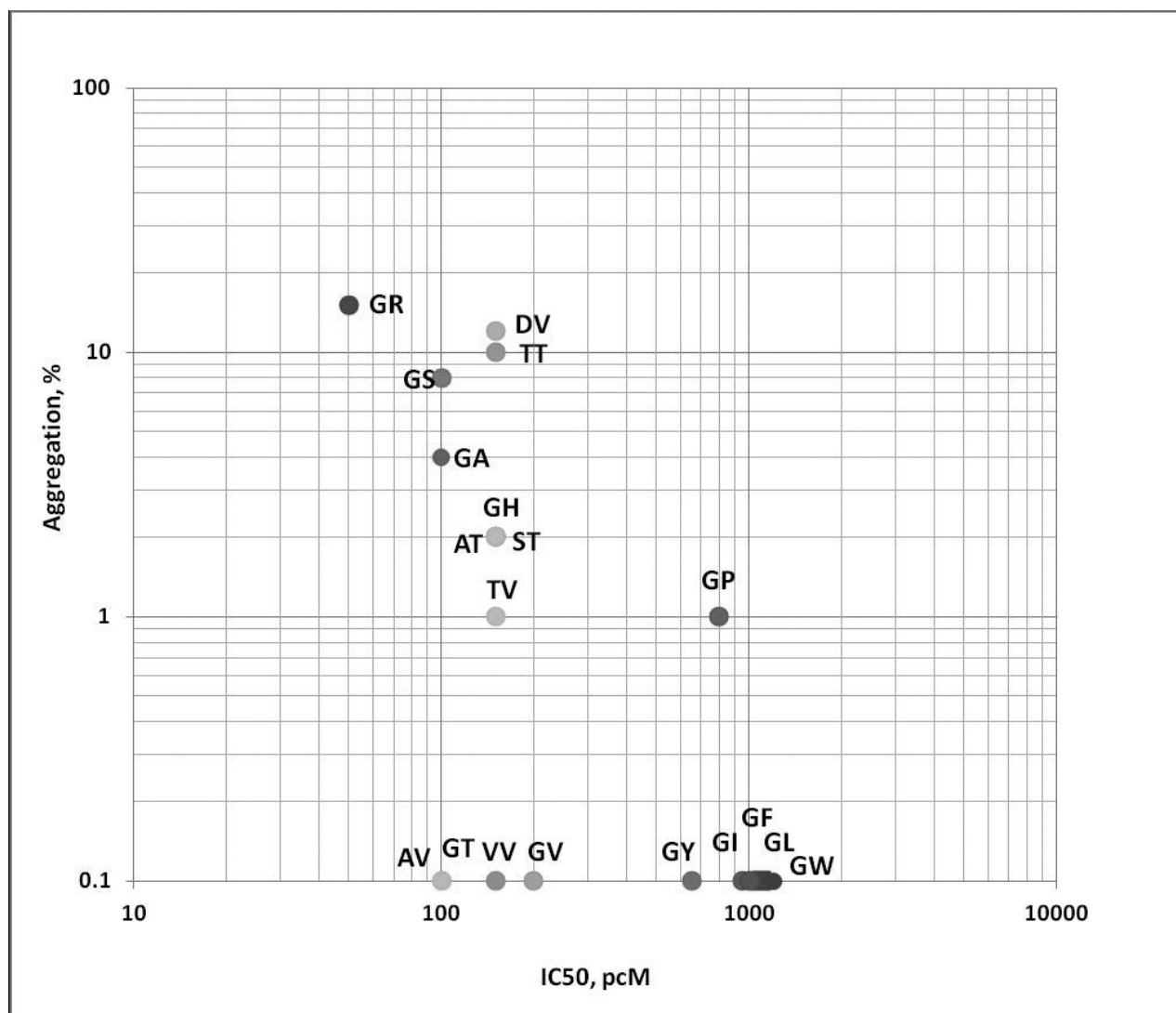


Fig. 20
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Dashed thick lines correspond to the combinations providing the maximum balanced aggregation stability and functional activity values; Dashed thin lines correspond to the suitable aggregation stability values with the high affinity remained.

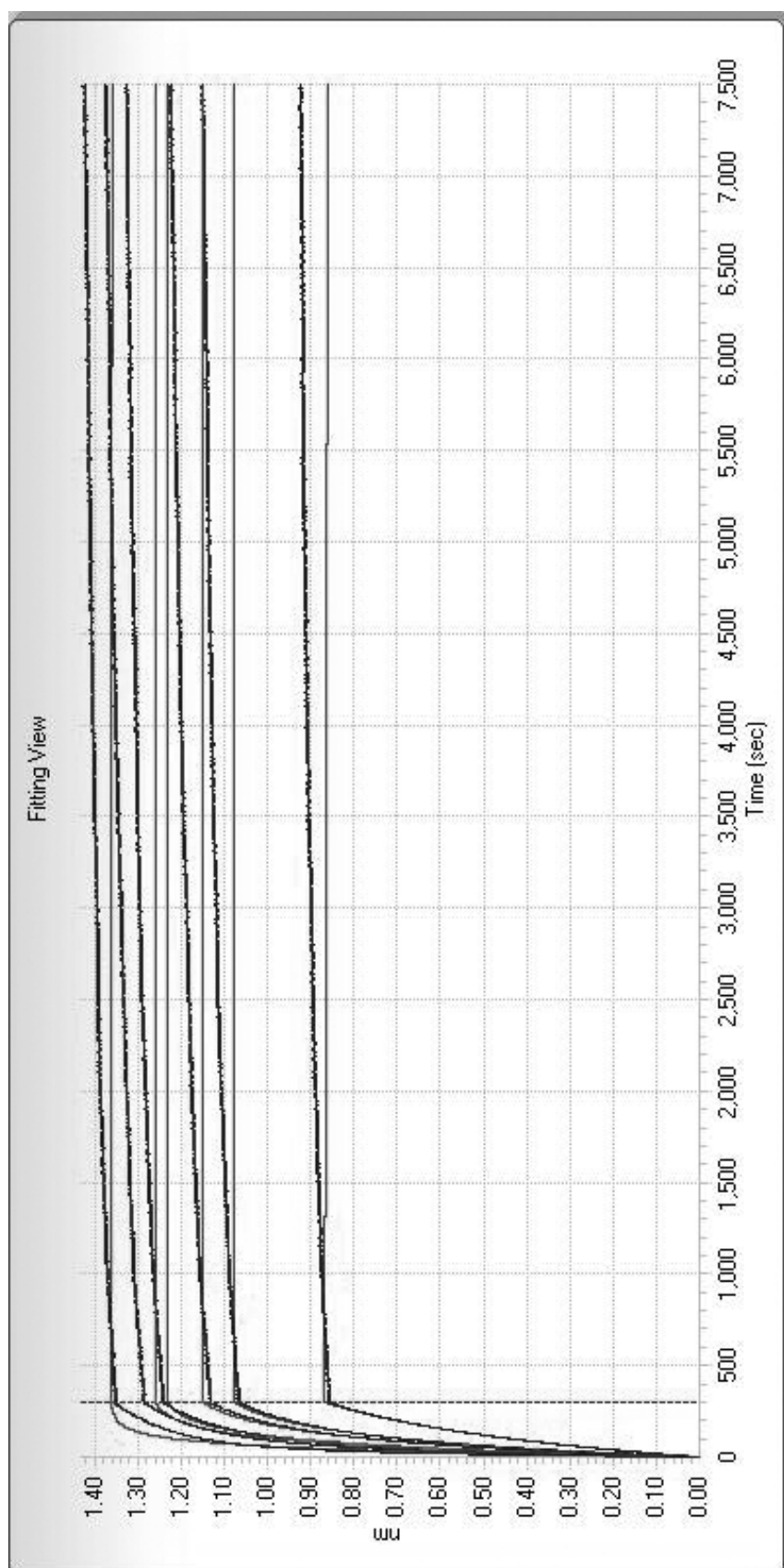


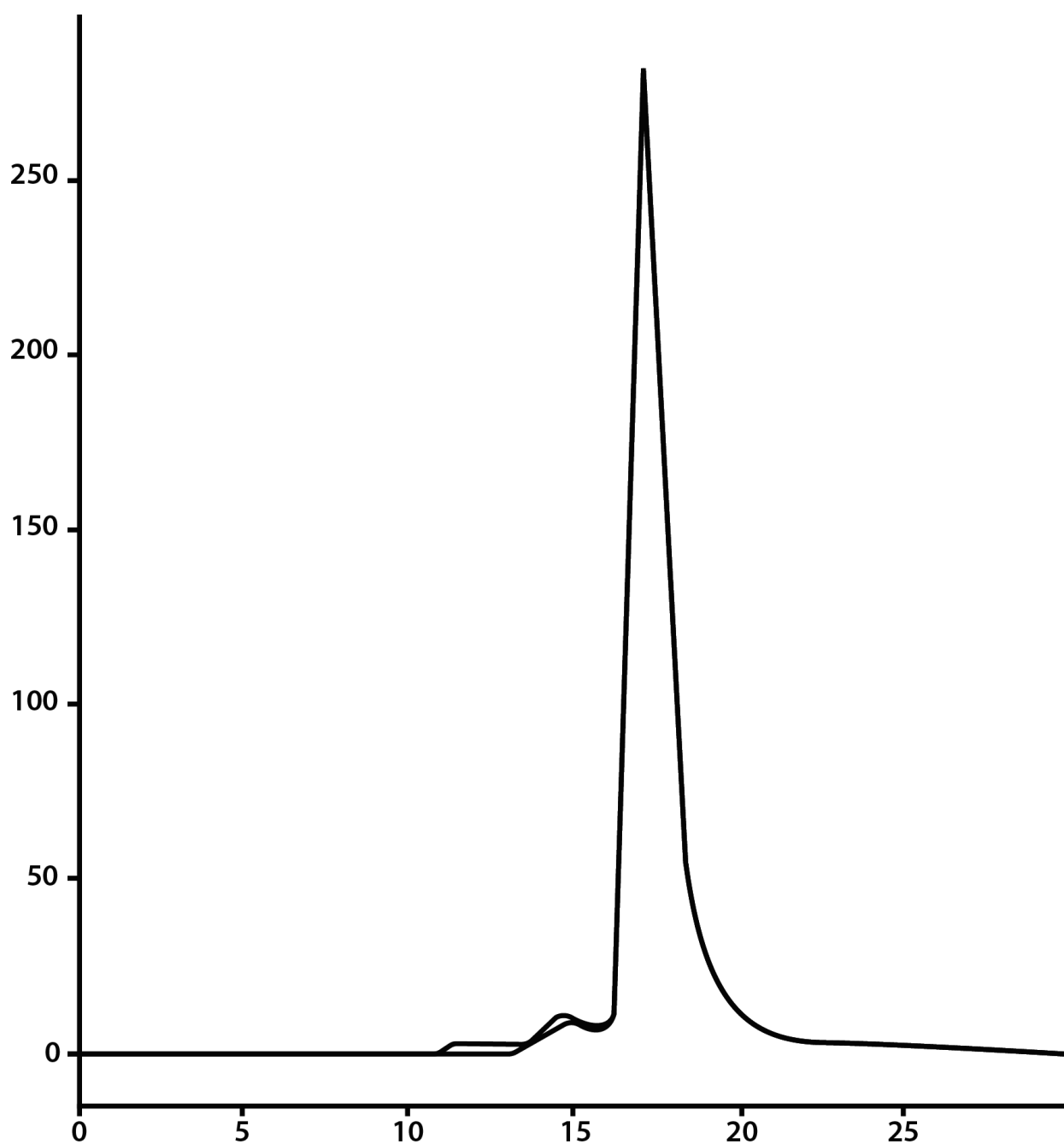
Fig. 22
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Human anti-IL17ABCD-109 cIL17 Fc

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



----- 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