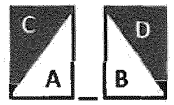


Figure 1

Figure 2

a)



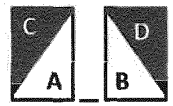
Acceptor domain A (≠ B)	Acceptor domain B (≠ A)
e.g.	e.g.
IgG1, 2, 3, 4 CH1 —	IgKappa, Lambda constant
IgA1, 2 CH1 —	IgKappa, Lambda constant
IgD CH1 —	IgKappa, Lambda constant
IgE CH1 —	IgKappa, Lambda constant
IgM CH1 —	IgKappa, Lambda constant
TCR alpha constant —	TCR beta constant
FcRn aplha3	Beta 2 microglobulin
HLA-A, B alpha3	Beta 2 microglobulin
HLA-D alpha2	HLA-D beta2

Potentially heterodimerizing elements

FcRn and inter-Ig domain interacting elements

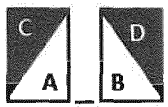
— Possible disulfide bond

b)



Donor domain C (= D)	Donor domain D (= C)
e.g.	e.g.
IgG1, 2, 3, 4 CH3 or CH1	IgG1, 2, 3, 4 CH3 or CH1
IgA1, 2 CH3 or CH1	IgA1, 2 CH3 or CH1
IgD CH3 or CH1	IgD CH3 or CH1
IgE CH4 or CH1	IgE CH4 or CH1
IgM CH4 or CH1	IgM CH4 or CH1
IgKppa constant	IgKppa constant
IgLampbda constant	IgLampbda constant

c)



Donor domain C (≠ D)	Donor domain D (≠ C)
e.g.	e.g.
IgG1, 2, 3, 4 CH1	IgKppa or Lambda constant
IgA1, 2 CH1	IgKppa or Lambda constant
IgD CH1	IgKppa or Lambda constant
IgE CH1	IgKppa or Lambda constant
IgM CH1	IgKppa or Lambda constant

Figure 3

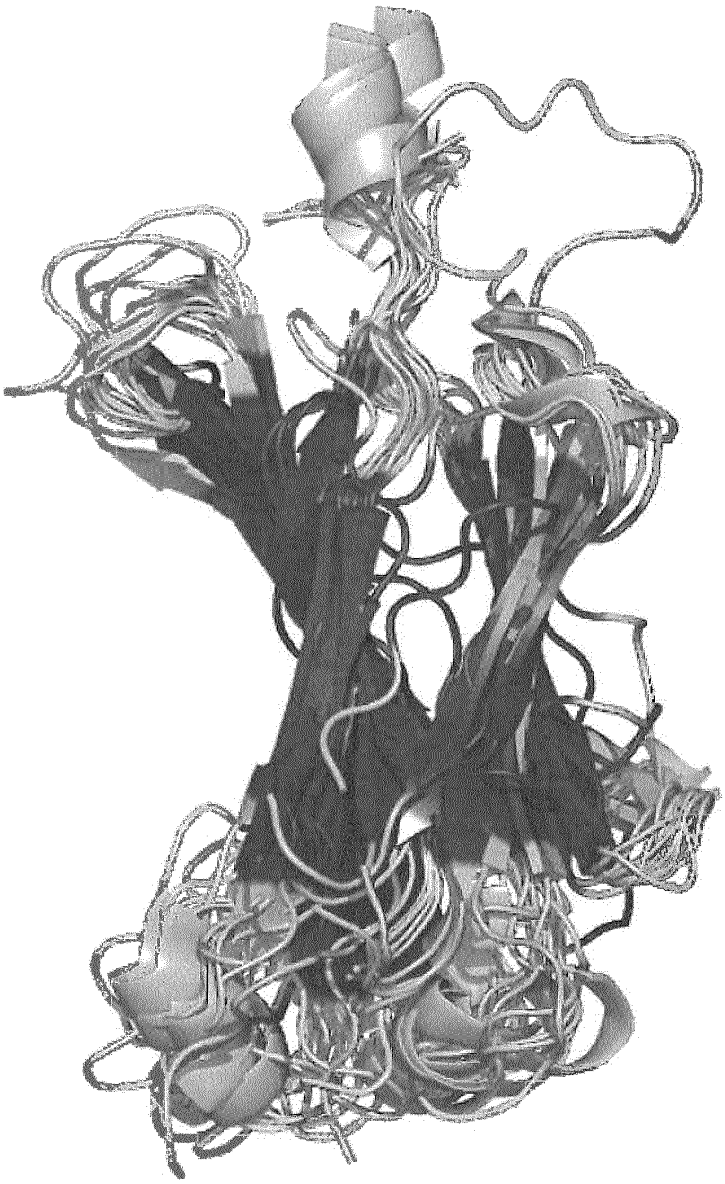


Figure 4

Beta sheet:
Beta strand predicted by PDB/JPred
Residues excluded manually from beta strands A-G according to structural alignment
Residues added to beta strands A-G according to sequence and structural alignment
* No or insufficient structural data available: alignment according to JPred prediction of secondary structures (longest cumulated prediction) and multisequence alignment
*1 Alignment was revised, taking into account the structure of the related mouse domain

Figure 5

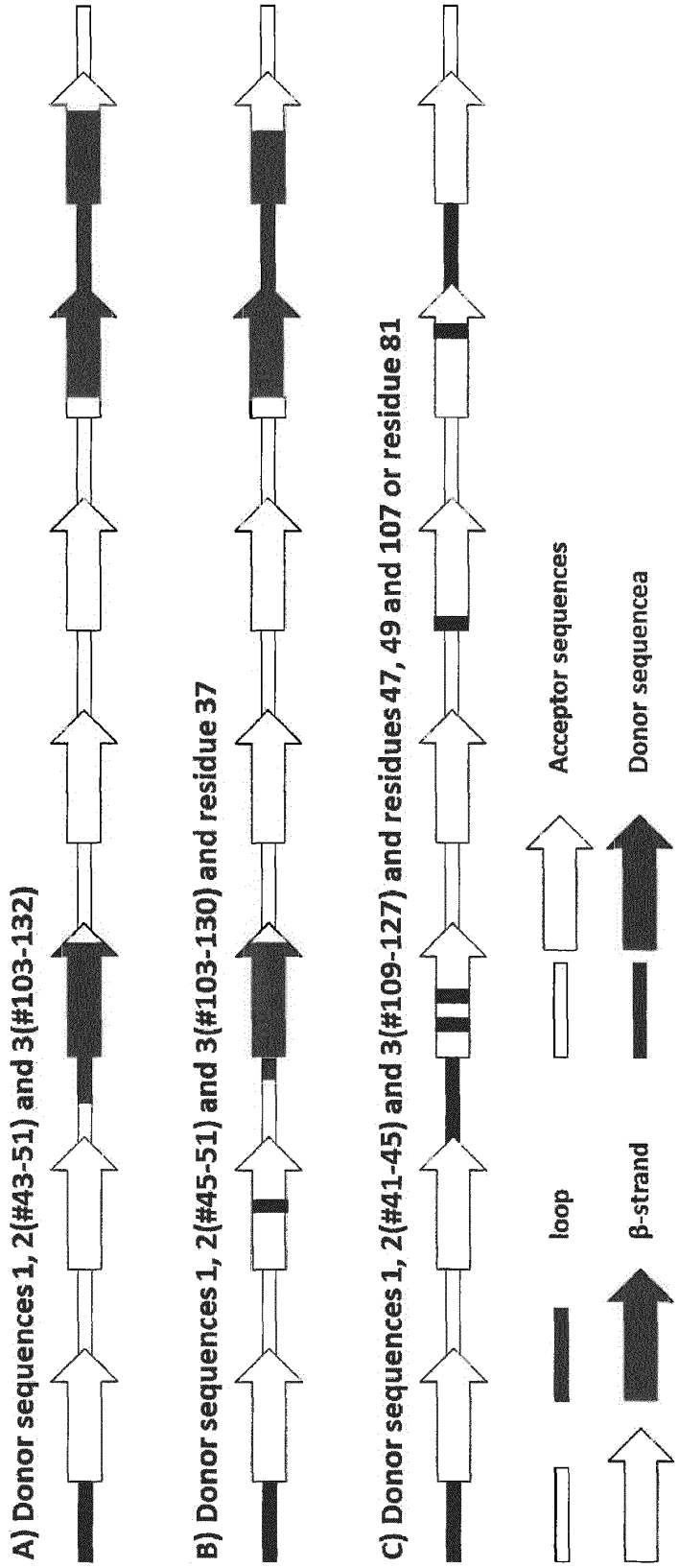

A) Donor sequences 1, 2(#43-51) and 3(#103-132)
B) Donor sequences 1, 2(#45-51) and 3(#103-130) and residue 37
C) Donor sequences 1, 2(#41-45) and 3(#109-127) and residues 47, 49 and 107 or residue 81
Acceptor sequences
Donor sequencea
loop
β-strand

Figure 6

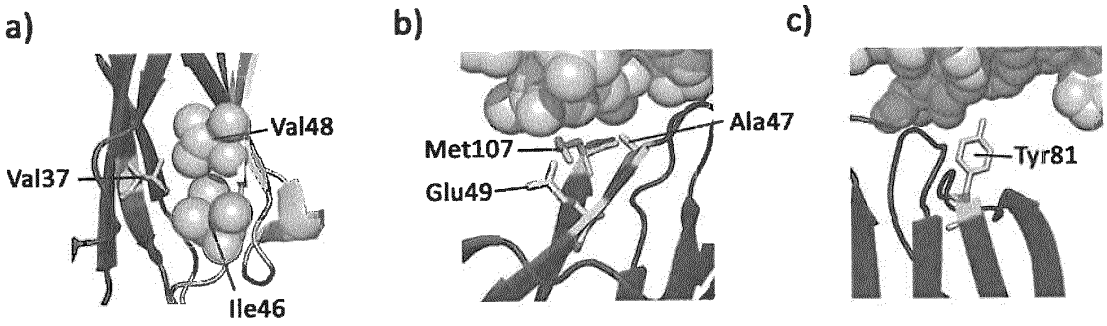


Figure 7

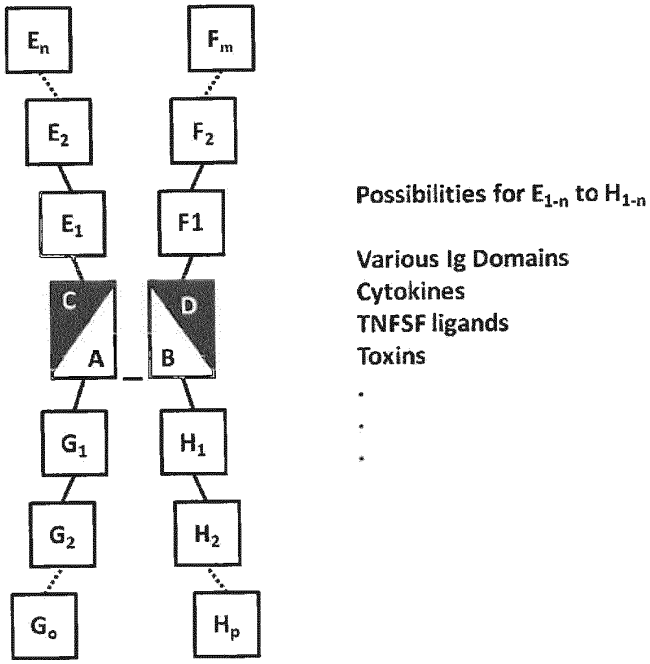


Figure 8

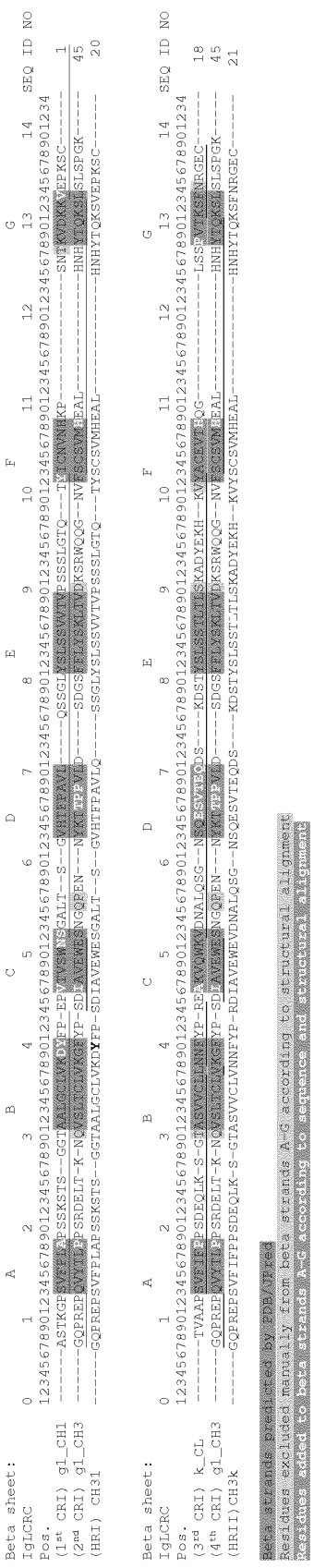

Beta sheet:
IgLCRC
Pos.
(1st CRI) gl_CH1
(2nd CRI) gl_CH3
(HRI) CH31
Beta sheet:
IgLCRC
Pos.
(3rd CRI) k_CL
(4th CRI) gl_CH3
(HRI)CH3k
Beta strands predicted by PDB/Pfed
Residues excluded manually from beta strands A-G according to structural alignment
Residues added to beta strands A-G according to sequence and structural alignment

Figure 9

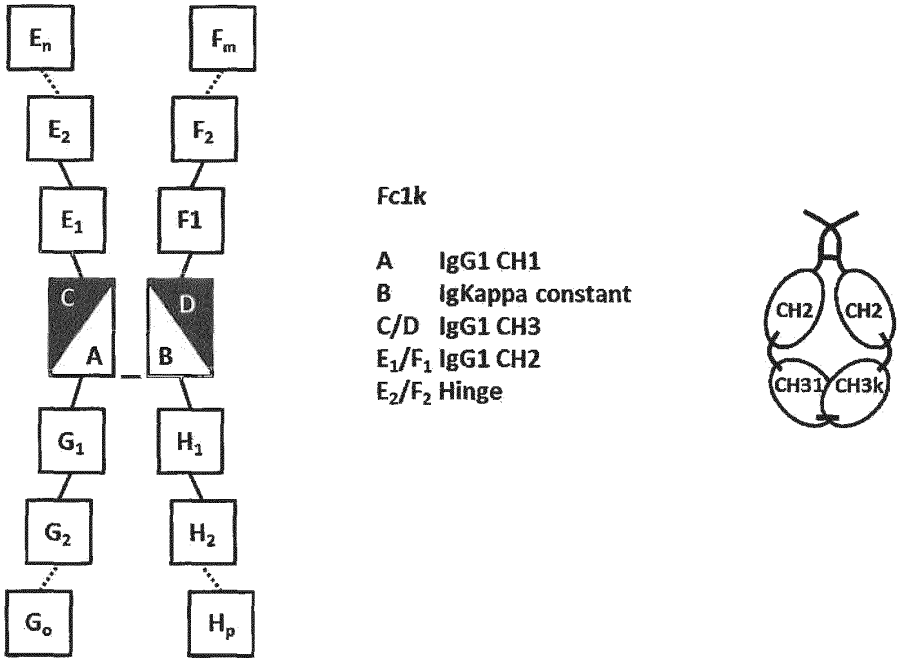


Figure 10

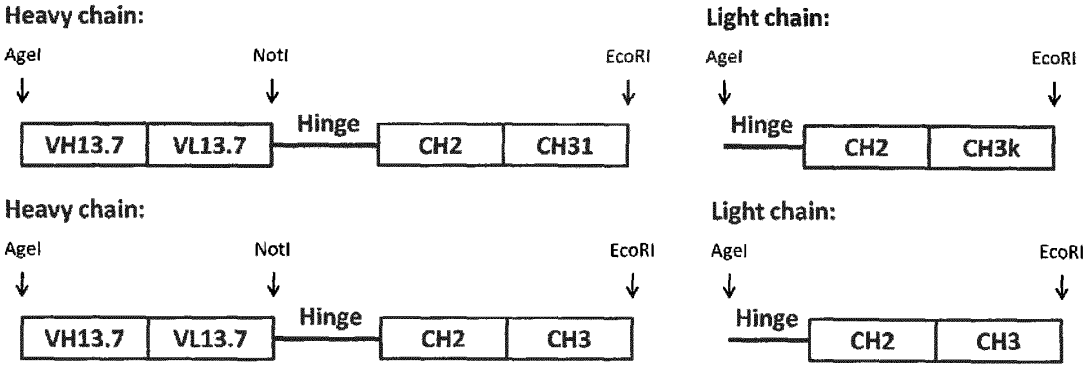


Figure 11

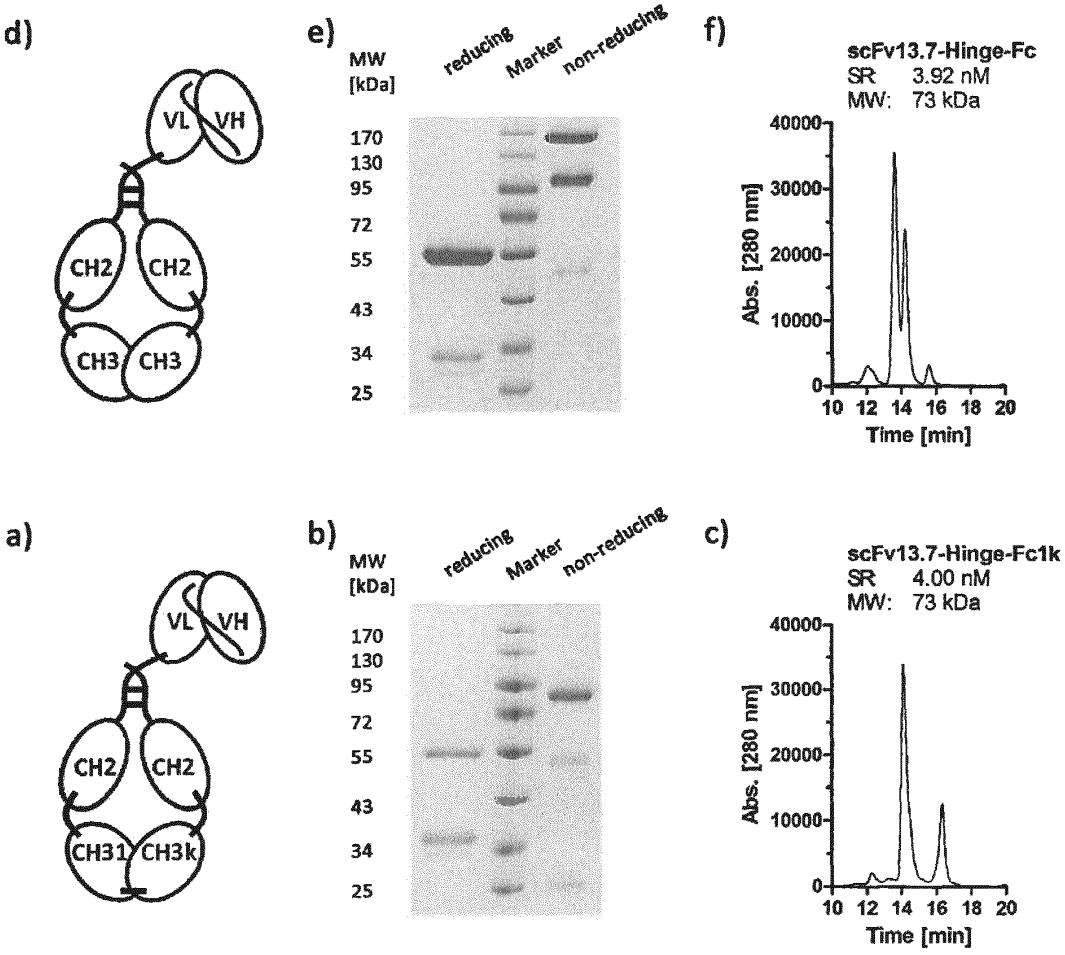
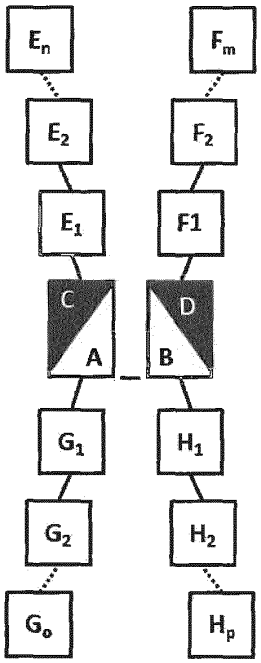
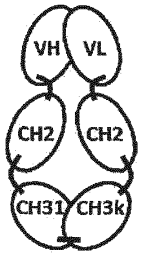

d)
VL
VH
CH2
CH2
CH3
CH3
e)
MW
[kDa]
reducing
Marker
non-reducing
170
130
95
72
55
43
34
25
f)
scFv13.7-Hinge-Fc
SR 3.92 nM
MW: 73 kDa
Abs. [280 nm]
40000
30000
20000
10000
0
10 12 14 16 18 20
Time [min]
a)
VL
VH
CH2
CH2
CH31
CH3k
b)
MW
[kDa]
reducing
Marker
non-reducing
170
130
95
72
55
43
34
25
c)
scFv13.7-Hinge-Fc1k
SR 4.00 nM
MW: 73 kDa
Abs. [280 nm]
40000
30000
20000
10000
0
10 12 14 16 18 20
Time [min]

Figure 12



Example 2
Fv13.7-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Linker sequence
E_3	VH13.7
F_3	VL13.7



Heavy chain:



Light chain:

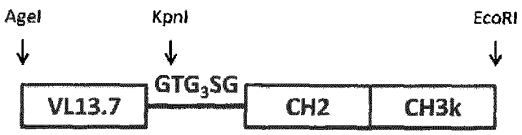


Figure 13

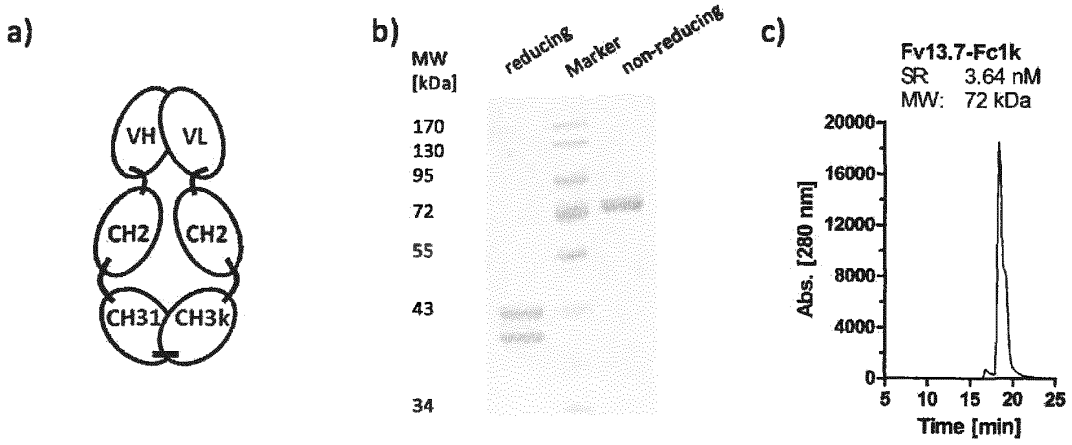


Figure 14

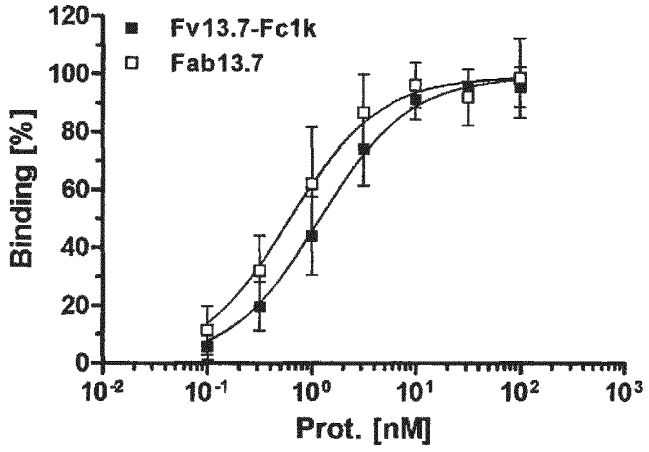


Figure 15

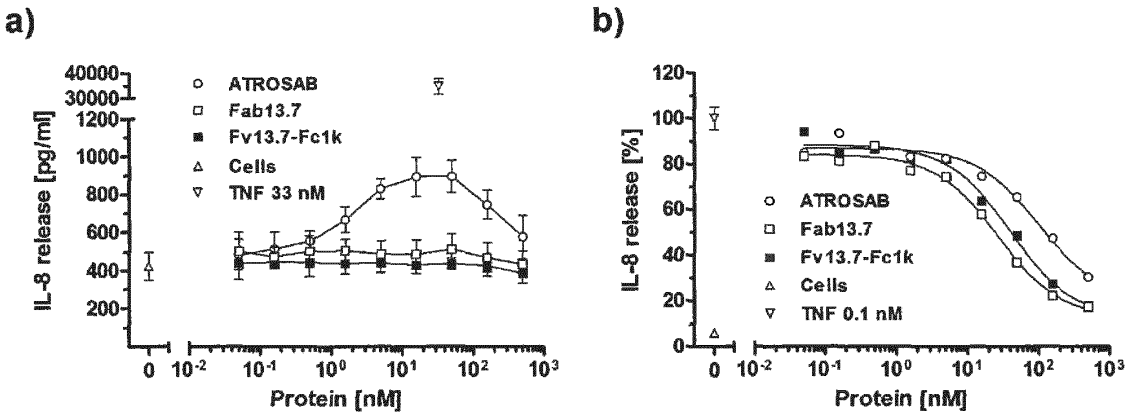


Figure 16

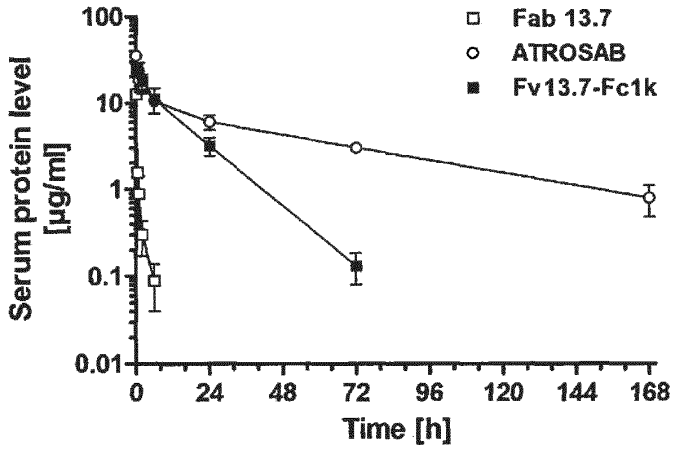
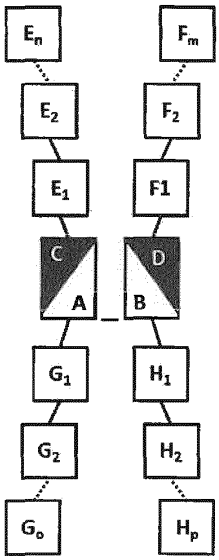
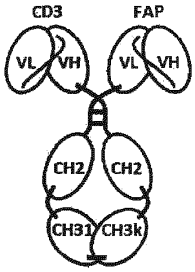


Figure 17



Example 3a
FAP_NCD3_N-hFc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
E_3	scFvhu36
F_3	scFvhuU3



Example 3b
FAP_NCD3_N-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge w/o cysteines
E_3	scFvhu36
F_3	scFvhuU3

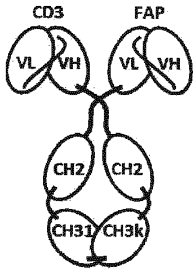
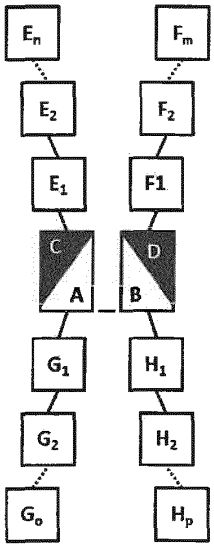
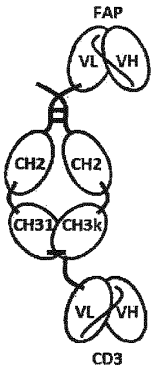


Figure 18



Example 4a
FAP_NCD3_C-hFc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
E_3	scFvhu36
H_1	Linker
H_2	scFvhuU3



Example 4b
FAP_NCD3_C-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge w/o cysteines
H_1	Linker
E_3	scFvhu36
H_2	scFvhuU3

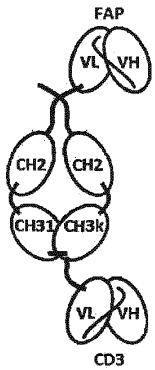
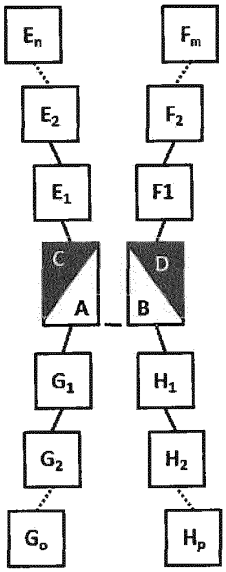
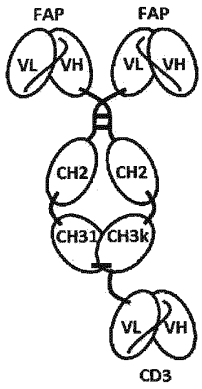


Figure 19



Example 5a
$FAP_{NN}CD3_C$-hFc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
H_1	Linker
E_3/F_3	scFvhu36
H_2	scFvhuU3



Example 5b
$FAP_{NN}CD3_C$-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge w/o cysteines
H_1	Linker
E_3/F_3	scFvhu36
H_2	scFvhuU3

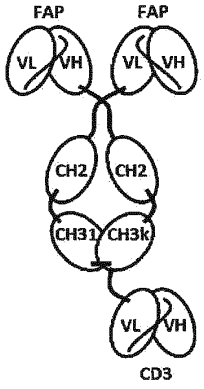
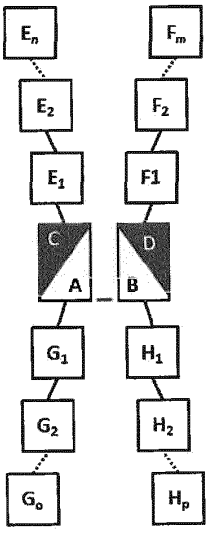
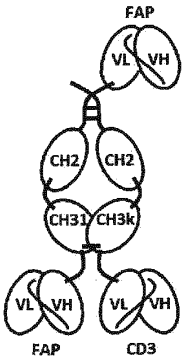


Figure 20



Example 6a
$FAP_{NC}CD3_C$-hFc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
G_1/H_1	Linker
E_3/G_2	scFvhu36
H_2	scFvhuU3



Example 6b
$FAP_{NC}CD3_C$-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge w/o cysteines
G_1/H_1	Linker
E_3/G_2	scFvhu36
H_2	scFvhuU3

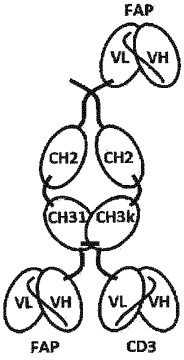


Figure 21

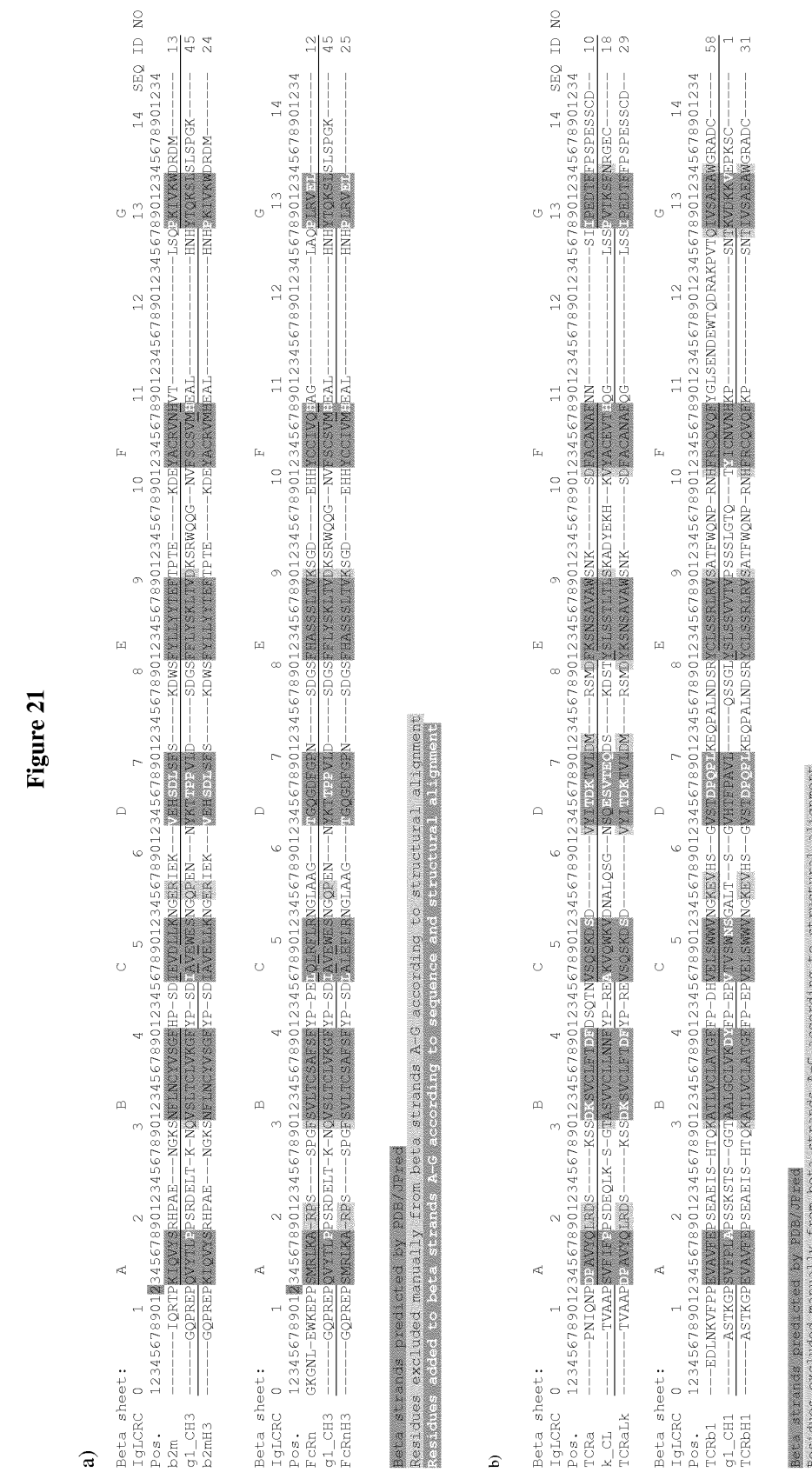
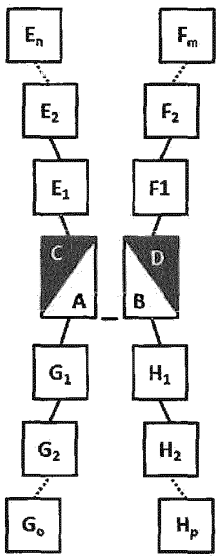


Figure 22



Example 7a
Bispecific-IgG

A	FcRn alpha 3
B	beta2 microglobulin
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
G_1	Fab fragment (Antigen X)
H_1	Fab as described in Expl. 7b

Example 7b
Alternatively dimerized Fab

A	TCR alpha constant
B	TCR beta constant
C	IgG1 CH1
D	Igkappa CL
E_1	VH (anti Y)
F_1	VL (anti Y)

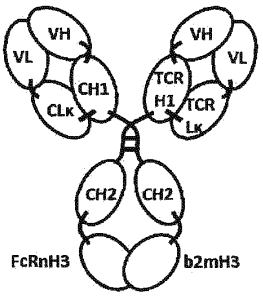
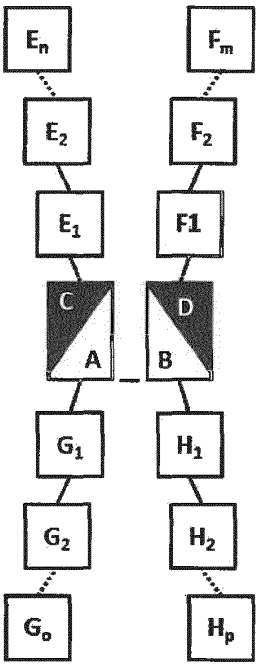
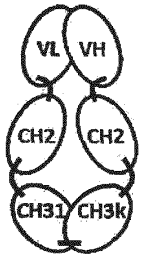


Figure 23



Example 8
Fv13.7$_X$-Fc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Linker sequence
E_3	VL13.7
F_3	VH13.7



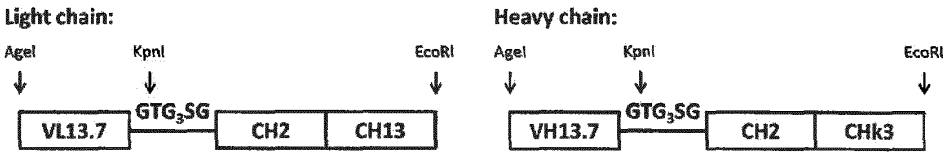


Figure 24

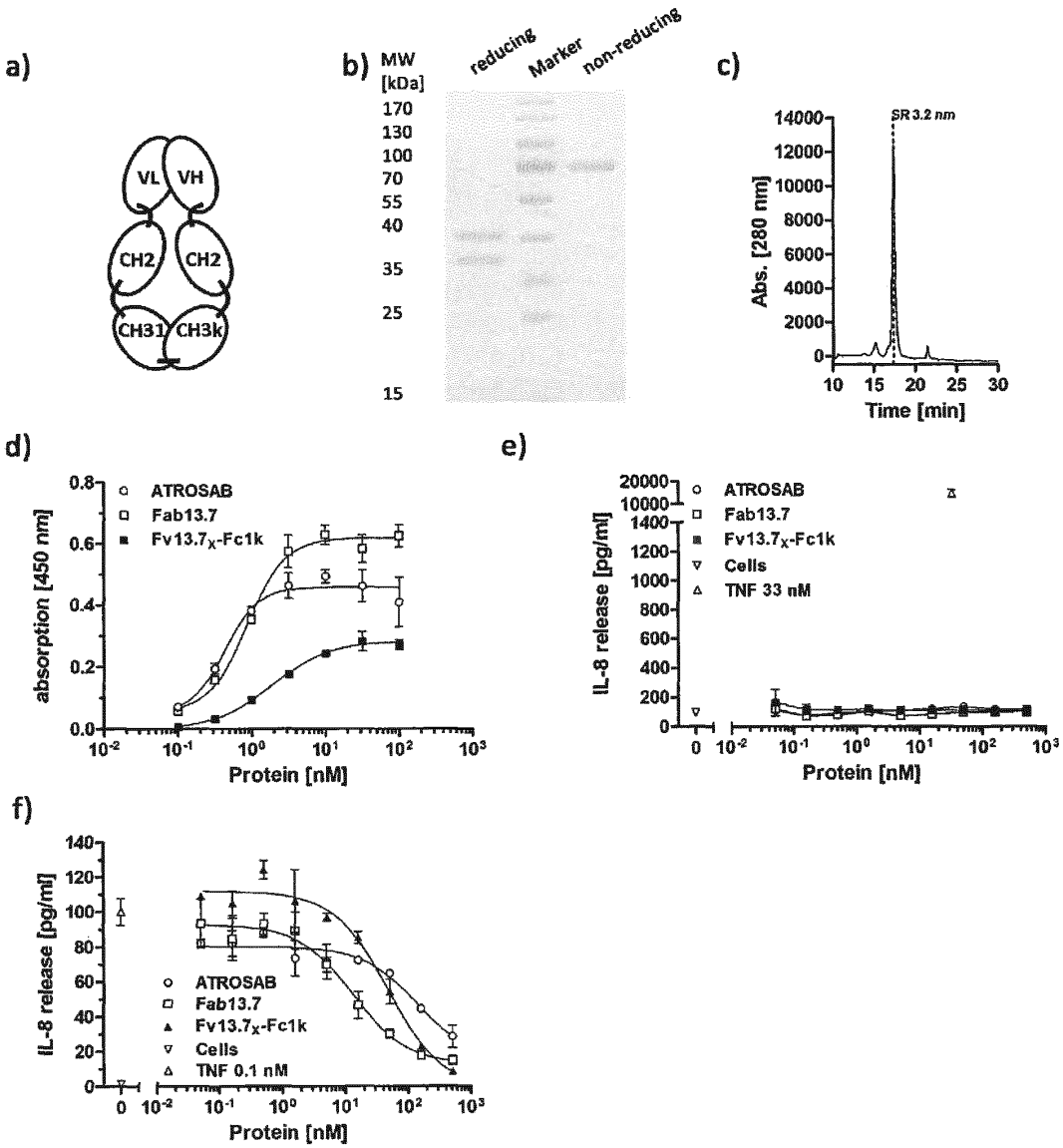
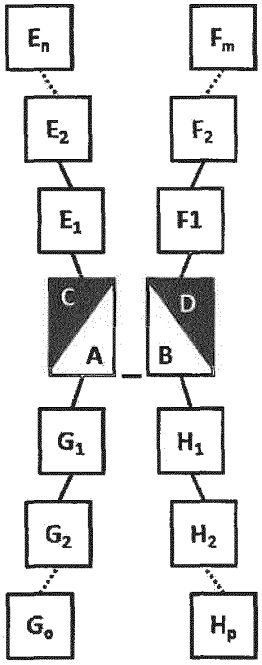
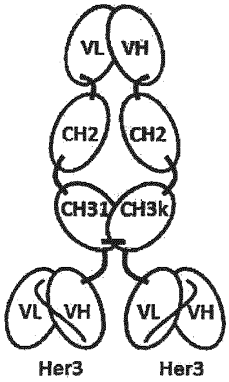

a)
VL
VH
CH2
CH2
CH31
CH3k
b)
MW [kDa]
reducing
Marker
non-reducing
170
130
100
70
55
40
35
25
15
c)
SR 3.2 nm
Abs. [280 nm]
Time [min]
d)
ATROSAB
Fab13.7
Fv13.7X-Fc1k
absorption [450 nm]
Protein [nM]
e)
ATROSAB
Fab13.7
Fv13.7X-Fc1k
Cells
TNF 33 nM
IL-8 release [pg/ml]
Protein [nM]
f)
ATROSAB
Fab13.7
Fv13.7X-Fc1k
Cells
TNF 0.1 nM
IL-8 release [pg/ml]
Protein [nM]

Figure 25



Example 9
FvCD3-Fc1k-scFvHer3$_2$

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Linker sequence
E_3	VLCD3
F_3	VHCD3
G_1/H_1	Linker
G_2/H_2	scFvHer3



Genotypic Arrangement

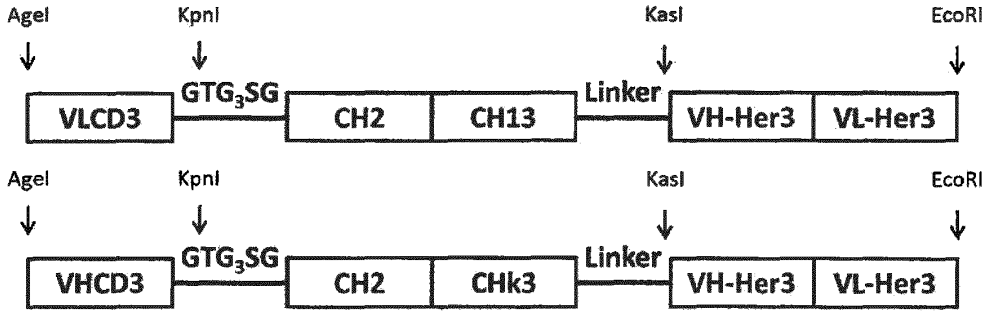


Figure 26

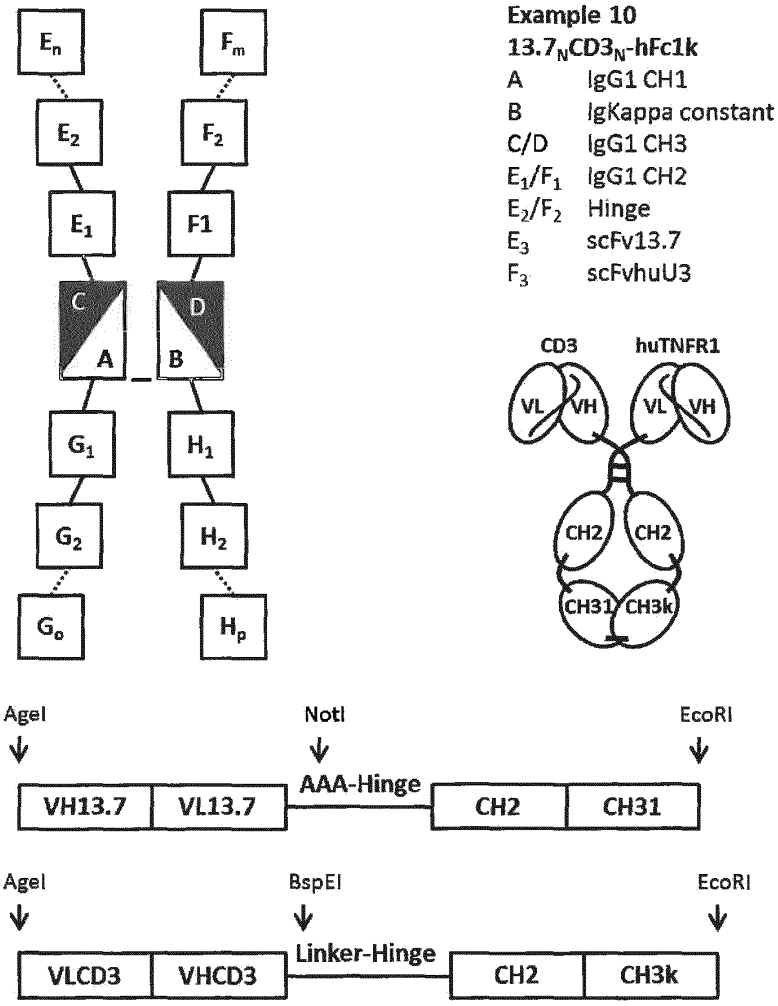

Example 10
13.7NCD3N-hFc1k
A IgG1 CH1
B IgKappa constant
C/D IgG1 CH3
E1/F1 IgG1 CH2
E2/F2 Hinge
E3 scFv13.7
F3 scFvhuU3
En
Fm
E2
F2
E1
F1
C
D
A
B
G1
H1
G2
H2
Go
Hp
CD3
huTNFR1
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
AgeI
NotI
EcoRI
VH13.7
VL13.7
AAA-Hinge
CH2
CH31
AgeI
BspEI
EcoRI
VLCD3
VHCD3
Linker-Hinge
CH2
CH3k

Figure 27

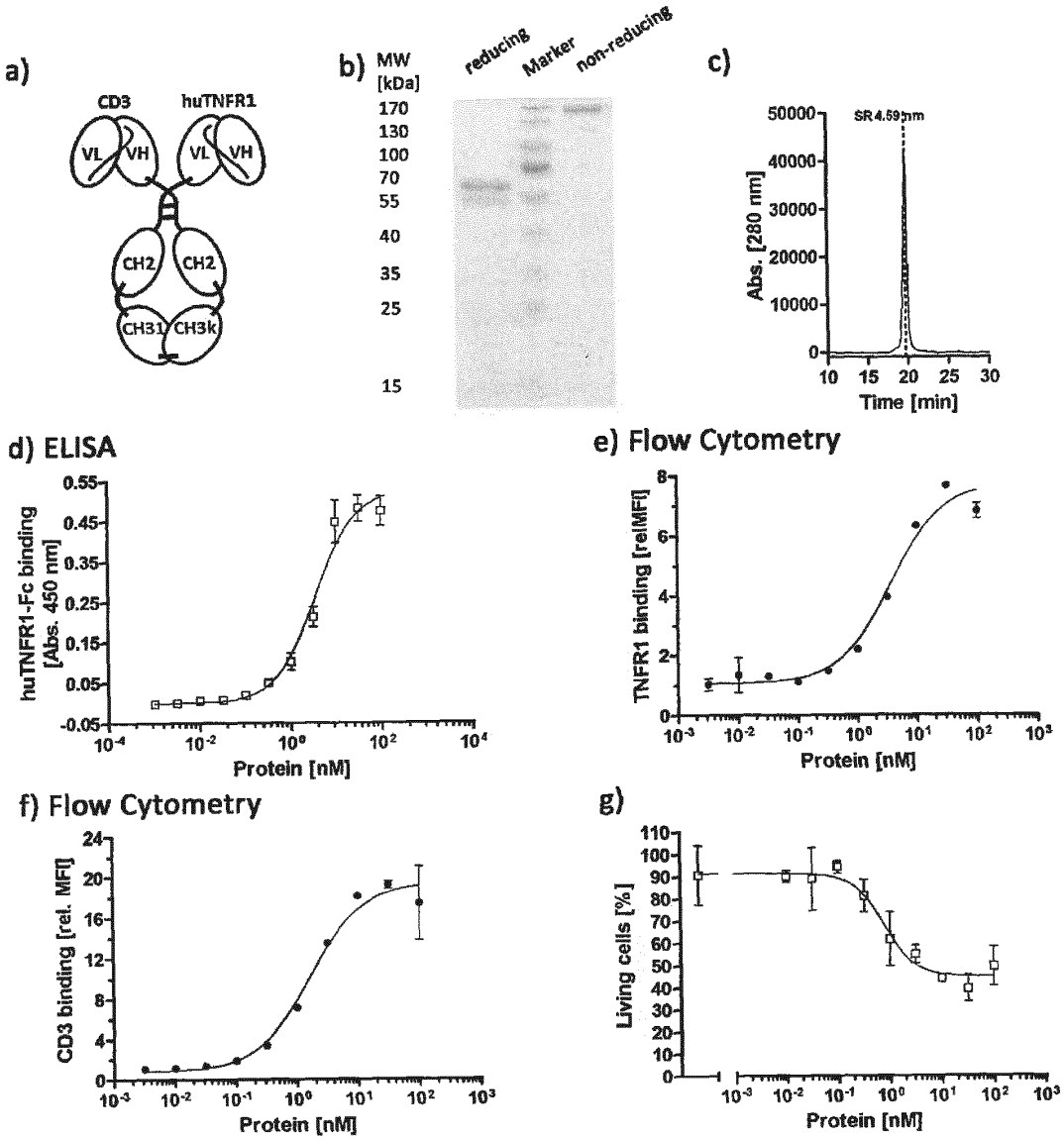

a)
CD3
huTNFR1
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
b)
MW [kDa]
reducing
Marker
non-reducing
170
130
100
70
55
40
35
25
15
c)
SR 4.59 nm
Abs. [280 nm]
Time [min]
d) ELISA
huTNFR1-Fc binding [Abs. 450 nm]
Protein [nM]
e) Flow Cytometry
TNFR1 binding [relMFI]
Protein [nM]
f) Flow Cytometry
CD3 binding [rel. MFI]
Protein [nM]
g)
Living cells [%]
Protein [nM]

Figure 28

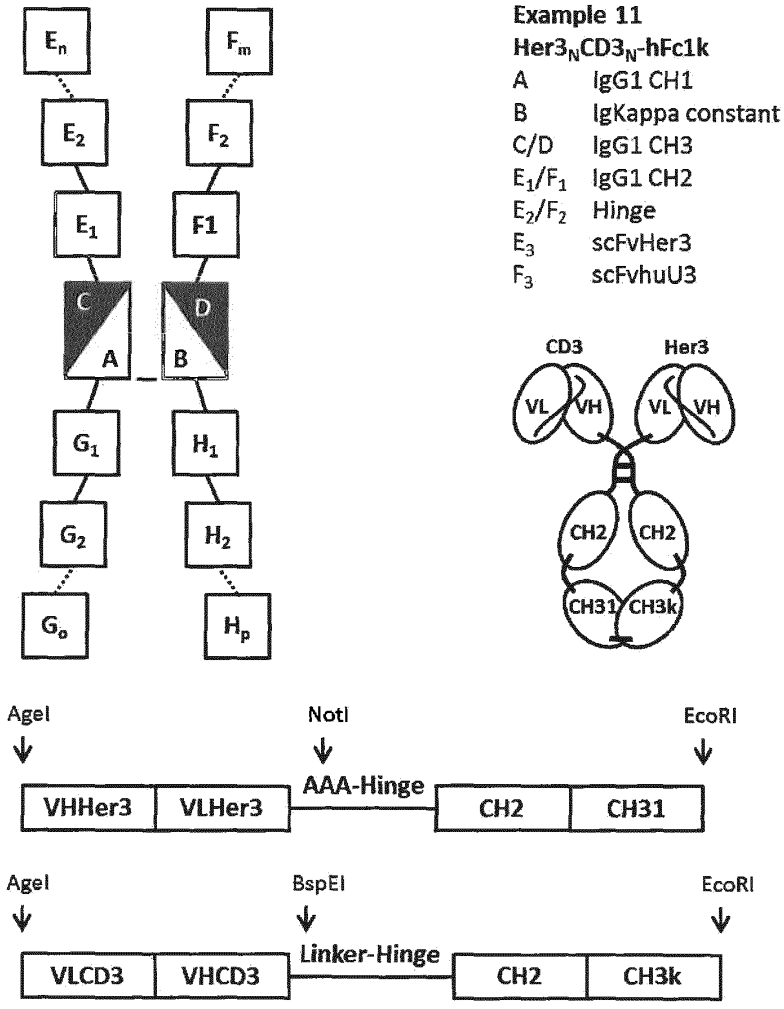

Example 11
Her3NCD3N-hFc1k
A IgG1 CH1
B IgKappa constant
C/D IgG1 CH3
E1/F1 IgG1 CH2
E2/F2 Hinge
E3 scFvHer3
F3 scFvhuU3
En
Fm
E2
F2
E1
F1
C
D
A
B
G1
H1
G2
H2
Go
Hp
CD3
Her3
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
AgeI
NotI
EcoRI
AAA-Hinge
VHHer3
VLHer3
CH2
CH31
AgeI
BspEI
EcoRI
Linker-Hinge
VLCD3
VHCD3
CH2
CH3k

Figure 29

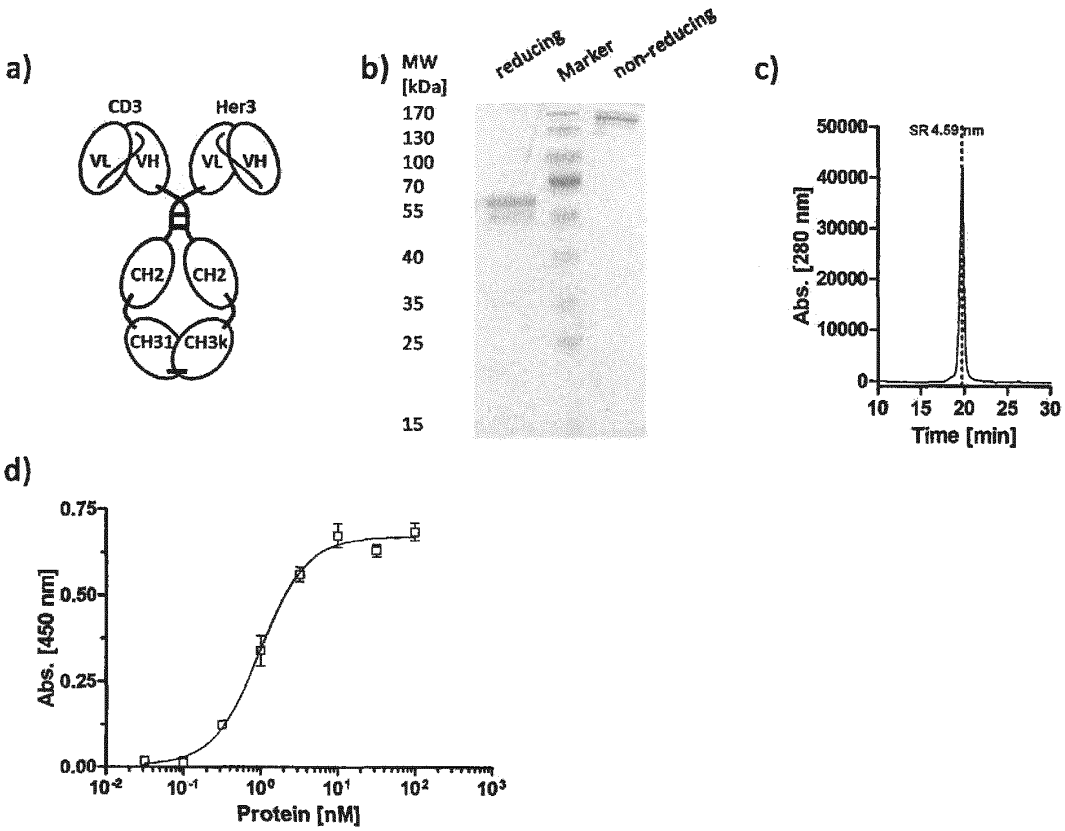

a)
CD3
Her3
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
b)
MW [kDa]
reducing
Marker
non-reducing
170
130
100
70
55
40
35
25
15
c)
SR 4.59 min
Abs. [280 nm]
50000
40000
30000
20000
10000
0
10
15
20
25
30
Time [min]
d)
Abs. [450 nm]
0.75
0.50
0.25
0.00
10-2
10-1
100
101
102
103
Protein [nM]

Figure 30

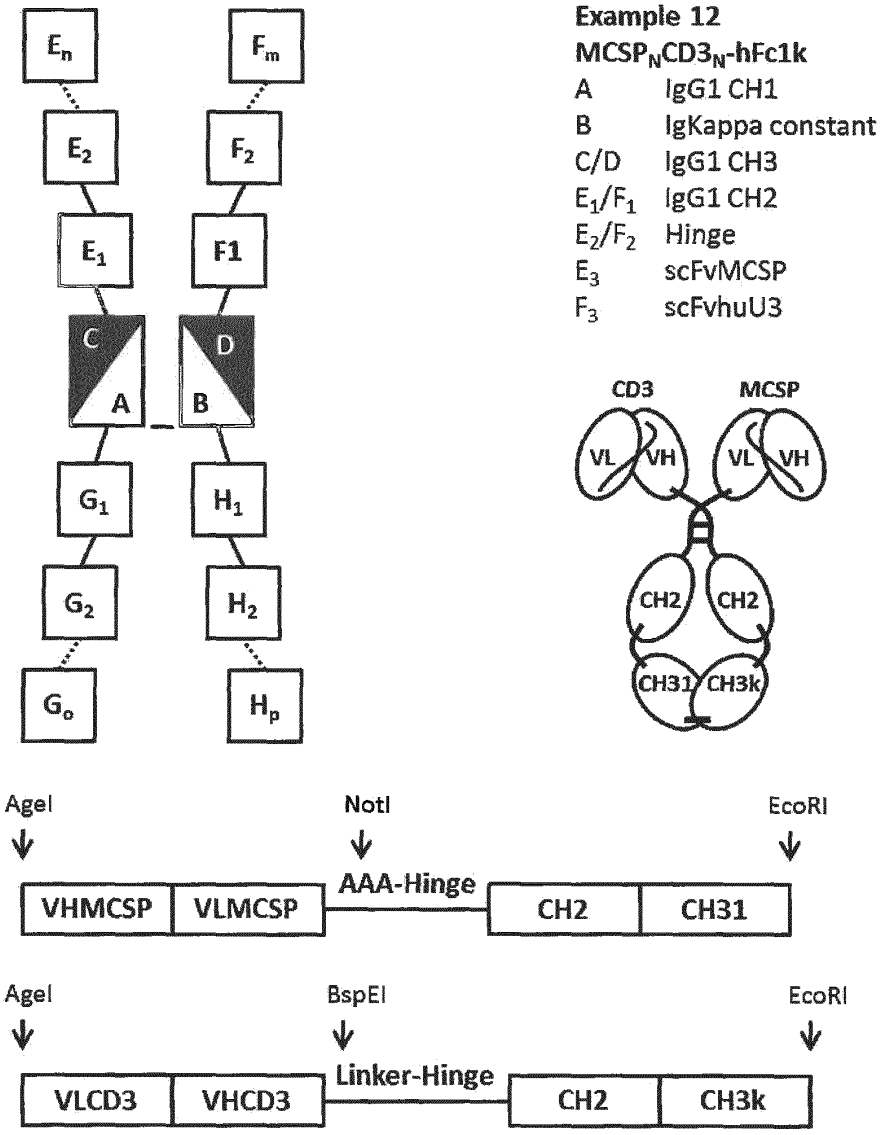

Example 12
MCSP$_N$CD3$_N$-hFc1k
A IgG1 CH1
B IgKappa constant
C/D IgG1 CH3
E_1/F_1 IgG1 CH2
E_2/F_2 Hinge
E_3 scFvMCSP
F_3 scFvhuU3
E_n
F_m
E_2
F_2
E_1
F1
C
D
A
B
G_1
H_1
G_2
H_2
G_o
H_p
CD3
MCSP
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
AgeI
NotI
EcoRI
VHMCSP
VLMCSP
AAA-Hinge
CH2
CH31
AgeI
BspEI
EcoRI
VLCD3
VHCD3
Linker-Hinge
CH2
CH3k

Figure 31

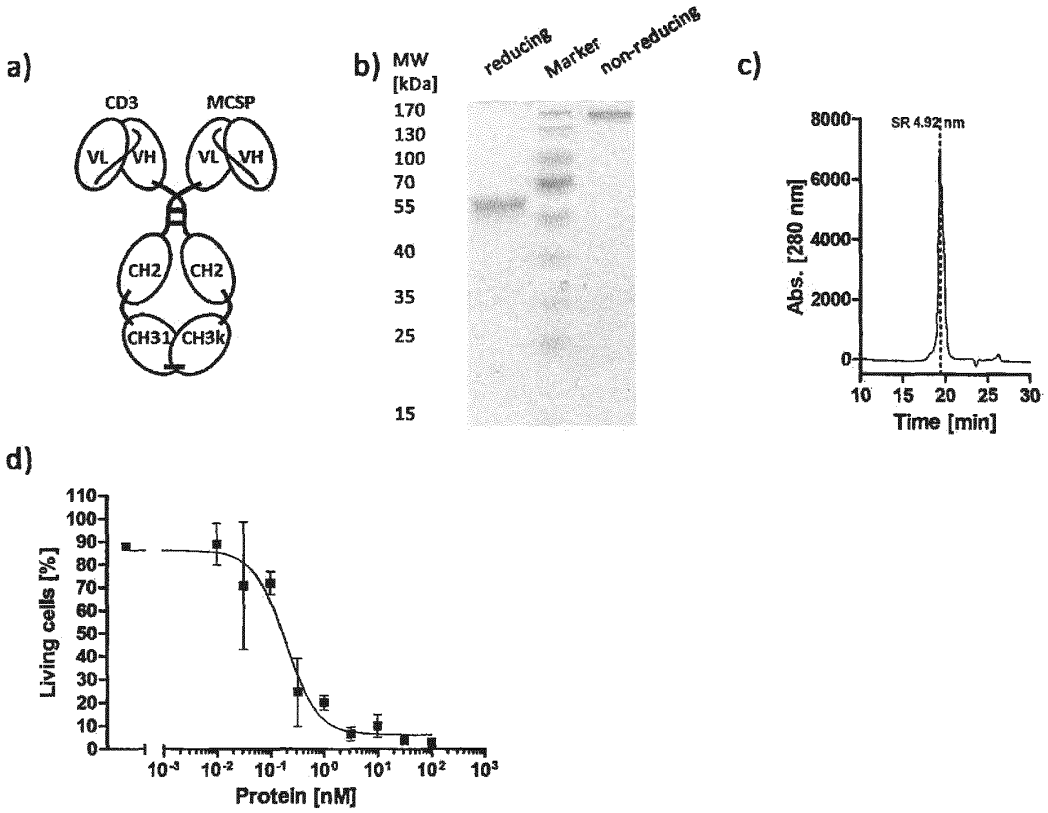
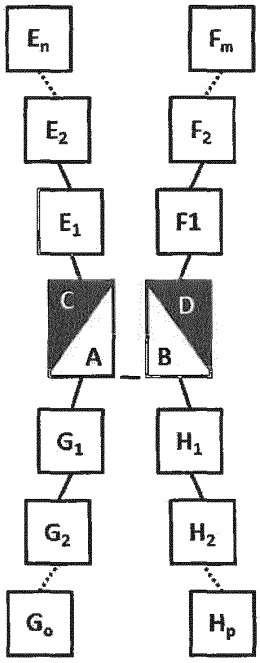

a)
CD3
MCSP
VL
VH
VL
VH
CH2
CH2
CH31
CH3k
b)
MW [kDa]
reducing
Marker
non-reducing
170
130
100
70
55
40
35
25
15
c)
SR 4.92 nm
Abs. [280 nm]
8000
6000
4000
2000
0
10
15
20
25
30
Time [min]
d)
Living cells [%]
110
100
90
80
70
60
50
40
30
20
10
0
10-3
10-2
10-1
100
101
102
103
Protein [nM]

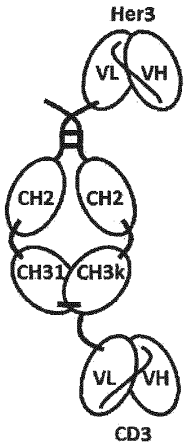
Figure 32



Example 13

$Her3_NCD3_N$-hFc1k

A	IgG1 CH1
B	IgKappa constant
C/D	IgG1 CH3
E_1/F_1	IgG1 CH2
E_2/F_2	Hinge
E_3	scFvHer3
H_1	Linker
H_2	scFvhuU3



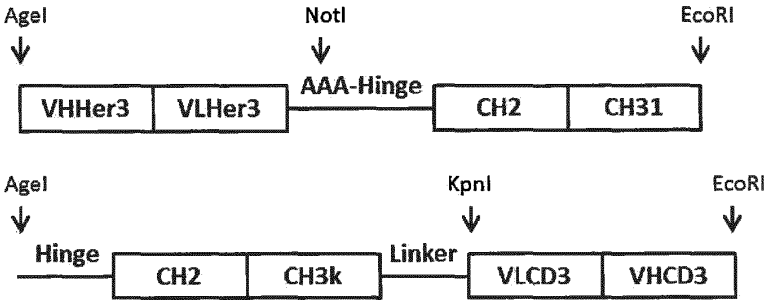


Figure 33

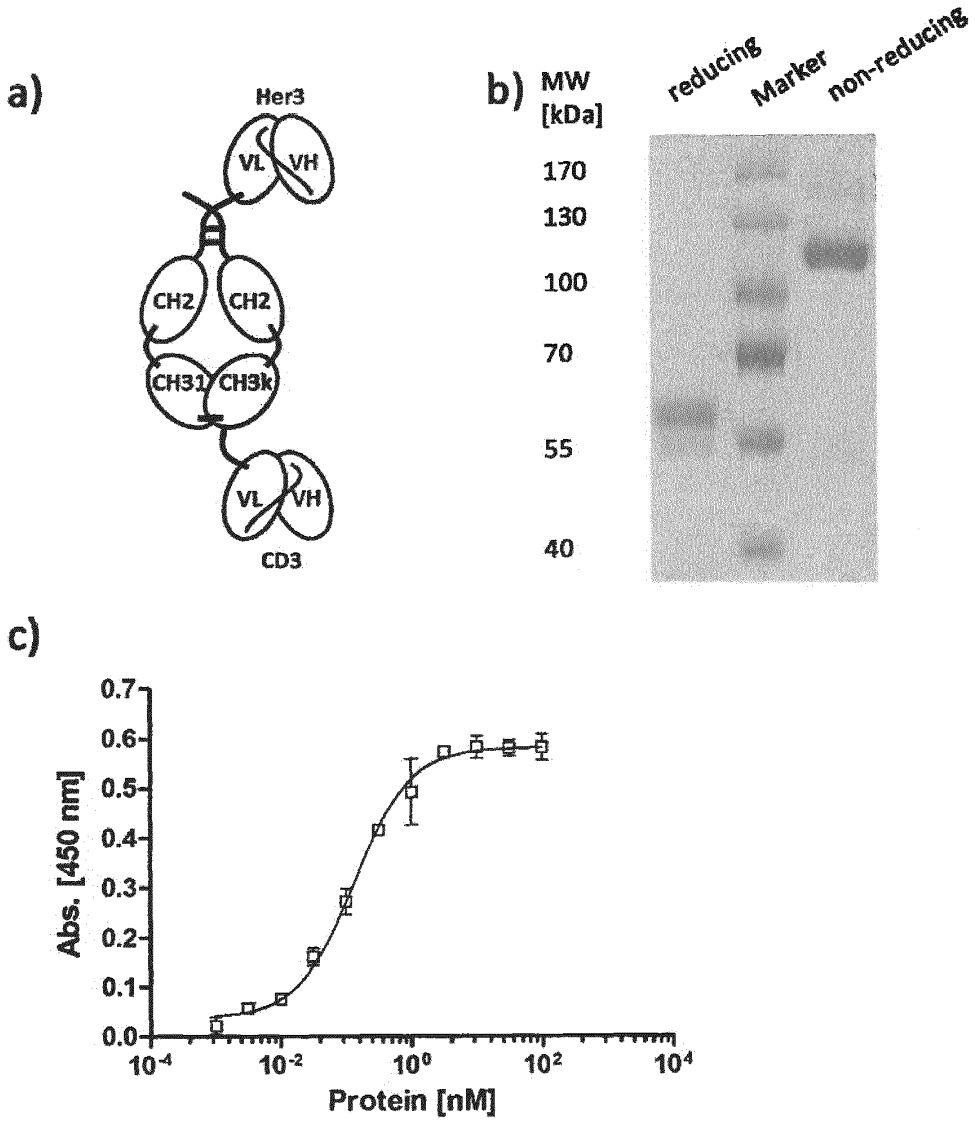

a)
Her3
VL
VH
CH2
CH2
CH31
CH3k
VL
VH
CD3
b)
MW
[kDa]
reducing
Marker
non-reducing
170
130
100
70
55
40
c)
Abs. [450 nm]
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10-4
10-2
100
102
104
Protein [nM]

Figure 34

SEQ ID NO: 1, CH1 of IgG1
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSC

SEQ ID NO: 2, CH1 of IgG2
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVD
HKPSNTKVDKTVERK

SEQ ID NO: 3, CH1 of IgG3
ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVN
HKPSNTKVDKRVELKTP

SEQ ID NO: 4, CH1 of IgG4
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD
HKPSNTKVDKRVESKYG

SEQ ID NO: 5, CH1 of IgA1
ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQDASGDLYTTSSQLTLPATQCLAGKSVTCH
VKHYTNPSQDVTVPC

SEQ ID NO: 6, CH1 of IgA2
ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVTARNFPPSQDASGDLYTTSSQLTLPATQCPDGKSVTCH
VKHYTNPSQDVTVPC

SEQ ID NO: 7, CH1 of IgD
APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTQSQPQRTFPEIQRRDSYYMTSSQLSTPLQQWRQGEYKCVV
QHTASKSKKEIFRWPESP

SEQ ID NO: 8, CH1 of IgE
ASTQSPSVFPLTRCCKNIPSNATSVTLGCLATGYFPEPVMVTWDTGSLNGTTMTLPATTLTLSGHYATISLLTVSGAWAKQMFTCR
VAHTPSSTDWVDNKTFSVC

SEQ ID NO: 9, CH1 of IgM
GSASAPTLFPLVSCENSSPSSTVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVV
CKVQHPNGNKEKDVPLPVVI

SEQ ID NO: 10, TCR α
PNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPED
TFFPSPESSCD

SEQ ID NO: 11, TCR β2
DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFW
QNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC

Figure 34 (continued)

SEQ ID NO: 12, FcRn alpha 3
GKGNLEWKEPPSMRLKARPSSPGFSVLTCSAFSFYPPELQLRFLRNGLAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQ
HAGLAQPLRVEL

SEQ ID NO: 13, β 2 micro globulin
IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVT
LSQPKIVKWDRDM

SEQ ID NO: 14, HLA-A
GKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCH
VQHEGLPKPLTLRWE

SEQ ID NO: 15, HLA-B α3
DIETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCH
VQHEGLPKPLTLRWEP

SEQ ID NO: 16, HLA-D α2
GRIPVSRGFPIAEVFTLKPLEFGKPNTLVCFVSNLFPPMLTVNWHDHSVPVEGFGPTFVSAVDGLSFQAFSYLNFTPEPSDIFSCI
VTHEPDRYTAIAYWVPRNAL

SEQ ID NO: 17, HLA-D β2
WGSLTNRTRPPSVQVAKTTPFNTREPVMLACYVWGFYPAEVTITWRKNGKLVMHSSAHKTAQPNGDWTYQTLSHLALTPSYGDTYT
CVVEHIGAPEPILRDWTPGLSP

SEQ ID NO: 18, Igκ constant region
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 19, Igλ constant region
GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS

SEQ ID NO: 20: CH31, 1st CRI: CH1(IgG1), 2nd CRI: CH3(IgG1)
GQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSDIAVEWESGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYSCSVM
HEALHNHYTQKSVEPKSC

SEQ ID NO: 21: CH3κ, 3rd CRI: CLκ, 2nd CRI: CH3(IgG1)
GQPREPSVFIFPPSDEQLKSGTASVVCLVNNFYPRDIAVEWEVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYS
CSVMHEALHNHYTQKSFNRGEC

SEQ ID NO: 22: CH1H3, 1st CRI: CH1 (IgG1), 2nd CRI: CH3 (IgG1)
GQPREPSVFPLAPSSKSTSGGTAALGCLVKDYYPSDVAVEWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVM
HEALHNHKVDKKVEPKSC

Figure 34 (continued)

SEQ ID NO: 23: CLkH3, 3rd CRI: CLκ, 4th CRI: CH3 (IgG1)
GQPREPSVFIFPPSDEQLKSGTASVVCLLNNFYPSDAAVEWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVMHEALHNHPVTKSFNRGEC

SEQ ID NO: 24: b2mH3, 1st CRI: beta 2 microglobulin, 2nd CRI: CH3 (IgG1)
GQPREPKIQVYSRHPAENGKSNFLNCYVSGFYPSDIAVELLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVMHE
ALHNHPKIVKWDRDM

SEQ ID NO: 25: FcRnH3, 3rd CRI: FcRn alpha 3 domain, 4th CRI: CH3 (IgG1)
GQPREPSMRLKARPSSPGFSVLTCSAFSFYPSDLALEFLRNGLAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVMHEALH
NHPLRVEL

SEQ ID NO: 26: TCRaH3, 1st CRI: TCR alpha chain constant domain, 2nd CRI: CH3 (IgG1)
GQPREPDPAVYQLRDSKSSDKSVCLFTDFYPSDVAQEKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANMFEALHNHIPE
DTFFPSPESSC

SEQ ID NO: 27: TCRbH3, 3rd CRI: TCR beta chain constant domain, 4th CRI: CH3 (IgG1)
GQPREPEVAVFEPSEAEISHTQKATLVCLATGFYPSDVALEWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQN
PRNHFRCQVMFEALHNHIVSAEAWGRADC

SEQ ID NO: 28: TCRaH1, 1st CRI: TCR alpha chain constant domain, 2nd CRI: CH1 (IgG1)
ASTKGPDPAVYQLRDSKSSDKSVCLFTDFFPEPVSQSKDSDVYITDKTVLDMRSMDYKSNSAVAWSNKSDFACANAFKPSNTIPED
TFFPSPESSCD

SEQ ID NO: 29: TCRaLk, 3rd CRI: TCR alpha chain constant domain, 4th CRI: CLκ
TVAAPDPAVYQLRDSKSSDKSVCLFTDFYPREVSQSKDSDVYITDKTVLDMRSMDYKSNSAVAWSNKSDFACANAFQGLSSIPEDT
FFPSPESSCD

SEQ ID NO: 30: TCRaLL, 3rd CRI: TCR alpha chain constant domain, 4th CRI: CLλ
GQPKANPDPAVYQLRDSKSSDKSVCLFTDFYPGAVSQSKDSDVYITDKTVLDMRSMDYKSNSAVAWSNKSDFACANAFEGSIPEDT
FFPSPESSCD

SEQ ID NO: 31: TCRbH1, 1st CRI: TCR beta chain constant domain, 2nd CRI: CH1 (IgG1)
ASTKGPEVAVFEPSEAEISHTQKATLVCLATGFFPEPVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQN
PRNHFRCQVQFKPSNTIVSAEAWGRADC

SEQ ID NO: 32: TCRb1Lk, 3rd CRI: TCR beta chain constant domain, 4th CRI: CLk
TVAAPEVAVFEPSEAEISHTQKATLVCLATGFYPREVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNP
RNHFRCQVQFQGLSSIVSAEAWGRADC

SEQ ID NO: 33: TCRbLL, 3rd CRI: TCR beta chain constant domain, 4th CRI: CLλ
GQPKANPEVAVFEPSEAEISHTQKATLVCLATGFYPGAVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQ
NPRNHFRCQVQFEGSIVSAEAWGRADC

Figure 34 (continued)

SEQ ID NO: 34: VH13.7-CH2-CH31
HVQLVQSGAEVKKPGSSVKVSCKASGYTFTDFYINWVRQAPGQGLEWIGEIVPSQGEAKYNDKFKARVTITADKSTSTAYMELSSL
RSEDTAVYYCARWDFLDYWGQGTTVTVSSGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYF
PSDIAVEWESGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSC

SEQ ID NO: 35: VL13.7-CH2-CH3κ
DVQMTQSPSSLSASVGDRVTITCRSSQSLLHSNGNTYLHWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCSQSTHVPYTFGGGTKVEIKGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFIFPPSDEQLKSGTASVVCLVNNFYPRD
IAVEWEVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYSCSVMHEALHNHYTQKSFNRGEC

SEQ ID NO: 36: scFv13.7-Hinge-CH2-CH31
HVQLVQSGAEVKKPGSSVKVSCKASGYTFTDFYINWVRQAPGQGLEWIGEIVPSQGEAKYNDKFKARVTITADKSTSTAYMELSSL
RSEDTAVYYCARWDFLDYWGQGTTVTVSSGGGGSGGGGSGGGGSDVQMTQSPSSLSASVGDRVTITCRSSQSLLHSNGNTYLHWYQ
QKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPYTFGGGTKVEIKAAADKTHTCPPCPAPP
VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSDIAVEWESGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSC

SEQ ID NO: 37: Hinge-CH2-CH3κ
DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFIFPPSDEQLKSGTASVVCLVNNFYPRDIAVEWEVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYSCSVMHEALHNHYTQKSFNRGEC

SEQ ID NO: 38: scFvhuU3-Hinge-CH2-CH3κ
DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTISSLQPEDIATY
YCQQGNTLPWTFGQGTKLEIKRGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSGYTMNWVRQAPGQGLEWMG
LINPYKGVSTYNGKFKDRVTITADKSTSTAYMELSSLRSEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSSGGGSGGGGSDKT
HTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFIFPPSDEQLKSGTASVVCLVNNFYPRDIAVEWEVDNALQSGNSQESV
TEQDSKDSTYSLSSTLTLSKADYEKHKVYSCSVMHEALHNHYTQKSFNRGECGGGGSGGGGSGGGGSGTGGSGG

SEQ ID NO: 39: scFv3-43-Hinge-CH2-CH31
QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAQSLKSRITINPDTPKNQFSLQL
NSVTPEDTAVYYCARDGQLGLDALDIWGQGTMVTVSSGGGGSGGGGSGGGGSQAGLTQPPAVSVAPGQTASITCGRDNIGSRSVHW
YQQKPGQAPVLVVYDDSDRPAGIPERFSGSNYENTATLTISRVEAGDEADYYCQVWGITSDHVVFGGGTKLTVLAAADKTHTCPPC
PAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSDIAVEWESGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSC

Figure 34 (continued)

SEQ ID NO: 40: scFhuMCSP-Hinge-CH2-CH31
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRSWMNWVRQAPGQGLEWMGRIYPGDGDTNYNGKFKGRVTMTRDTSTSTVYMELSSL
RSEDTAVYYCARGNTVVVPYTMDYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDSYGNSF
MHWYQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQNNEDPLTFGGGTKVEIKRAAADKTHTCP
PCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSDIAVEWESGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSC

SEQ ID NO: 41: Amino acid sequence of VH13.7-CH2-CH3k
HVQLVQSGAEVKKPGSSVKVSCKASGYTFTDFYINWVRQAPGQGLEWIGEIVPSQGEAKYNDKFKARVTITADKSTSTAYMELSSL
RSEDTAVYYCARWDFLDYWGQGTTVTVSSGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFIFPPSDEQLKSGTASVVCLVNNFY
PRDIAVEWEVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYSCSVMHEALHNHYTQKSFNRGEC

SEQ ID NO: 42: Amino acid sequence of VL13.7-CH2-CH31
DVQMTQSPSSLSASVGDRVTITCRSSQSLLHSNGNTYLHWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCSQSTHVPYTFGGGTKVEIKGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSD
IAVEWESGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSC

SEQ ID NO: 43: Amino acid sequence of VHCD3-CH2-CH3k-scFvHer3
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSGYTMNWVRQAPGQGLEWMGLINPYKGVSTYNGKFKDRVTITADKSTSTAYMELSSL
RSEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFIFPPSDEQLKSGTASVV
CLVNNFYPRDIAVEWEVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYSCSVMHEALHNHYTQKSFNRGECDKTH
TAPAPPVAGQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAQSLKSRITINPDT
PKNQFSLQLNSVTPEDTAVYYCARDGQLGLDALDIWGQGTMVTVSSGGGGSGGGGSGGGGSQAGLTQPPAVSVAPGQTASITCGRD
NIGSRSVHWYQQKPGQAPVLVVYDDSDRPAGIPERFSGSNYENTATLTISRVEAGDEADYYCQVWGITSDHVVFGGGTKLTVL

SEQ ID NO: 44:Amino acid sequence of VLCD3-CH2-CH31-scFvHer3
DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTISSLQPEDIATY
YCQQGNTLPWTFGQGTKLEIKGTGGGSGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPSVFPLAPSSKSTSGGTAALGCLVKDYFPSDIAVEW
ESGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYSCSVMHEALHNHYTQKSVEPKSCDKTHTAPAPPVAGQVQLQQSGPG
LVKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAQSLKSRITINPDTPKNQFSLQLNSVTPEDTAV
YYCARDGQLGLDALDIWGQGTMVTVSSGGGGSGGGGSGGGGSQAGLTQPPAVSVAPGQTASITCGRDNIGSRSVHWYQQKPGQAPV
LVVYDDSDRPAGIPERFSGSNYENTATLTISRVEAGDEADYYCQVWGITSDHVVFGGGTKLTVL

SEQ ID NO: 45; Amino acid sequence of IgG1 CH3
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 46; Amino acid sequence of IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

Figure 34 (continued)

SEQ ID NO: 47; Amino acid sequence of IgG3 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCS
VMHEALHNRFTQKSLSLSPGK

SEQ ID NO: 48; Amino acid sequence of IgG4 CH3
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 49; Amino acid sequence of IgM CH4
VALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGE
TYTCVVAHEALPNRVTERTVDKSTGK

SEQ ID NO: 50; Amino acid sequence of IgA1 CH3
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKG
DTFSCMVGHEALPLAFTQKTIDRLAGK

SEQ ID NO: 51; Amino acid sequence of IgA2 CH3
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKG
DTFSCMVGHEALPLAFTQKTIDRLAGK

SEQ ID NO: 52; Amino acid sequence of IgD CH3
VPAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPAT
YTCVVSHEDSRTLLNASRSLEVSYVT

SEQ ID NO: 53; Amino acid sequence of IgE CH4
GPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFI
CRAVHEAASPSQTVQRAVSVNPGK

SEQ ID NO: 54, Igλ constant region
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS

SEQ ID NO: 55, Igλ constant region
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPAKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYS
CQVTHEGSTVEKTVAPTECS

SEQ ID NO: 56, Igλ constant region
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVKVAWKADGSPVNTGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPAECS

SEQ ID NO: 57, Igλ constant region
GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CRVTHEGSTVEKTVAPAECS

Figure 34 (continued)

SEQ ID NO: 58, TCR β1
EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATF
WQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC

SEQ ID NO: 59, HLA-D β2
WGSLTNRTRPPSVQVAKTTPFNTREPVMLACYVWGFYPAEVTITWRKNGKLVMHSSAHKTAQPNGDWTYQTLSHLALTPSYGDTYT
CVVEHIGAPEPILRDWTPGLSPMQTLK

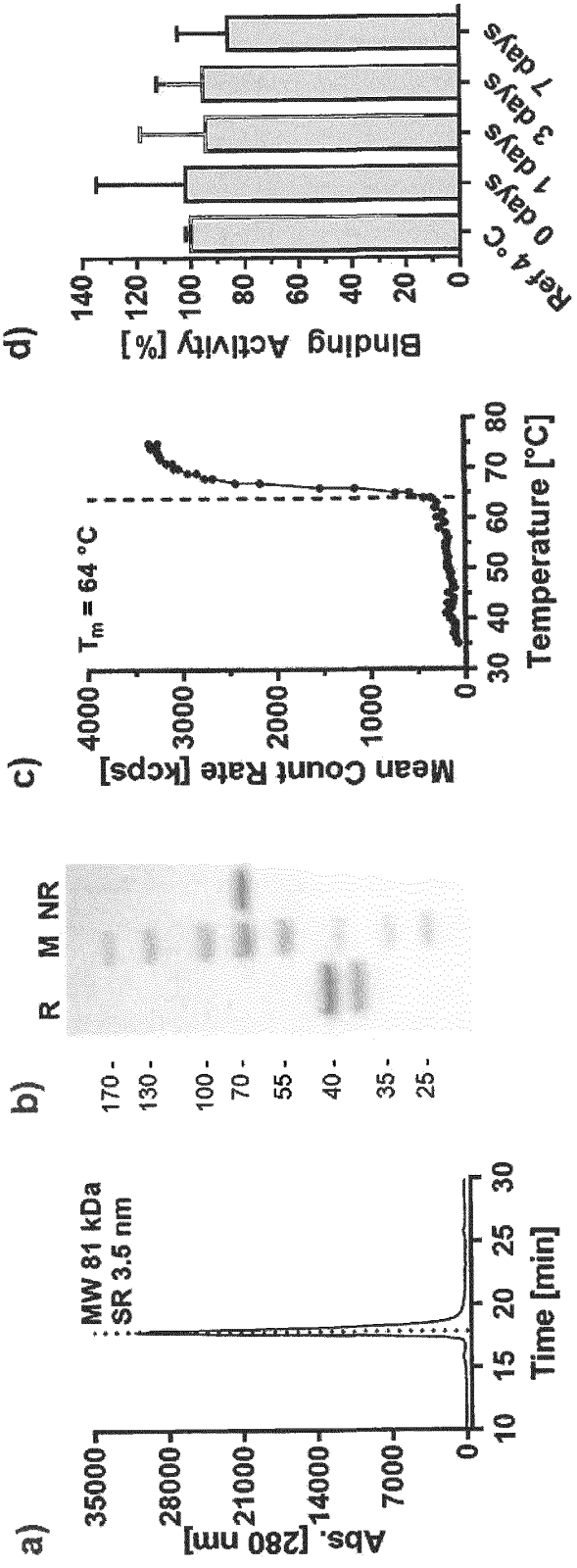

a)
Abs. [280 nm]
35000
28000
21000
14000
7000
0
10 15 20 25 30
Time [min]
MW 81 kDa
SR 3.5 nm
b)
R M NR
170 -
130 -
100 -
70 -
55 -
40 -
35 -
25 -
c)
Mean Count Rate [kcps]
4000
3000
2000
1000
0
T_m = 64 °C
30 40 50 60 70 80
Temperature [°C]
d)
Binding Activity [%]
140
120
100
80
60
40
20
0
Ref 4 °C
0 days
1 days
3 days
7 days

Figure 35

Figure 36

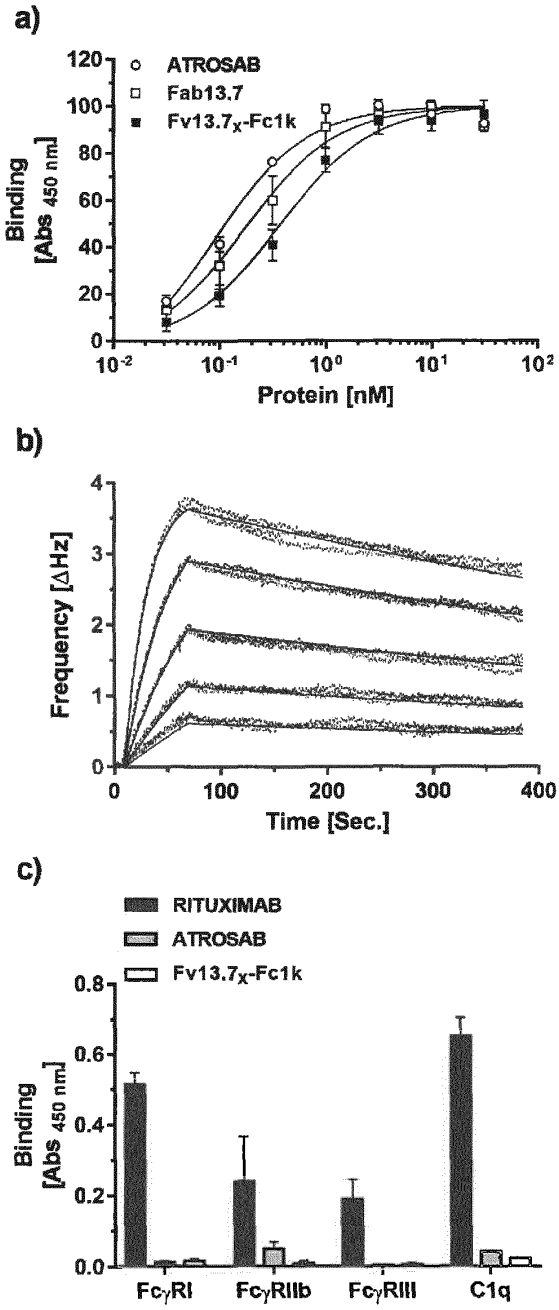

a)
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Binding [Abs 450 nm]
Protein [nM]
b)
Frequency [ΔHz]
Time [Sec.]
c)
RITUXIMAB
ATROSAB
Fv13.7x-Fc1k
Binding [Abs 450 nm]
FcγRI
FcγRIIb
FcγRIII
C1q

Figure 37

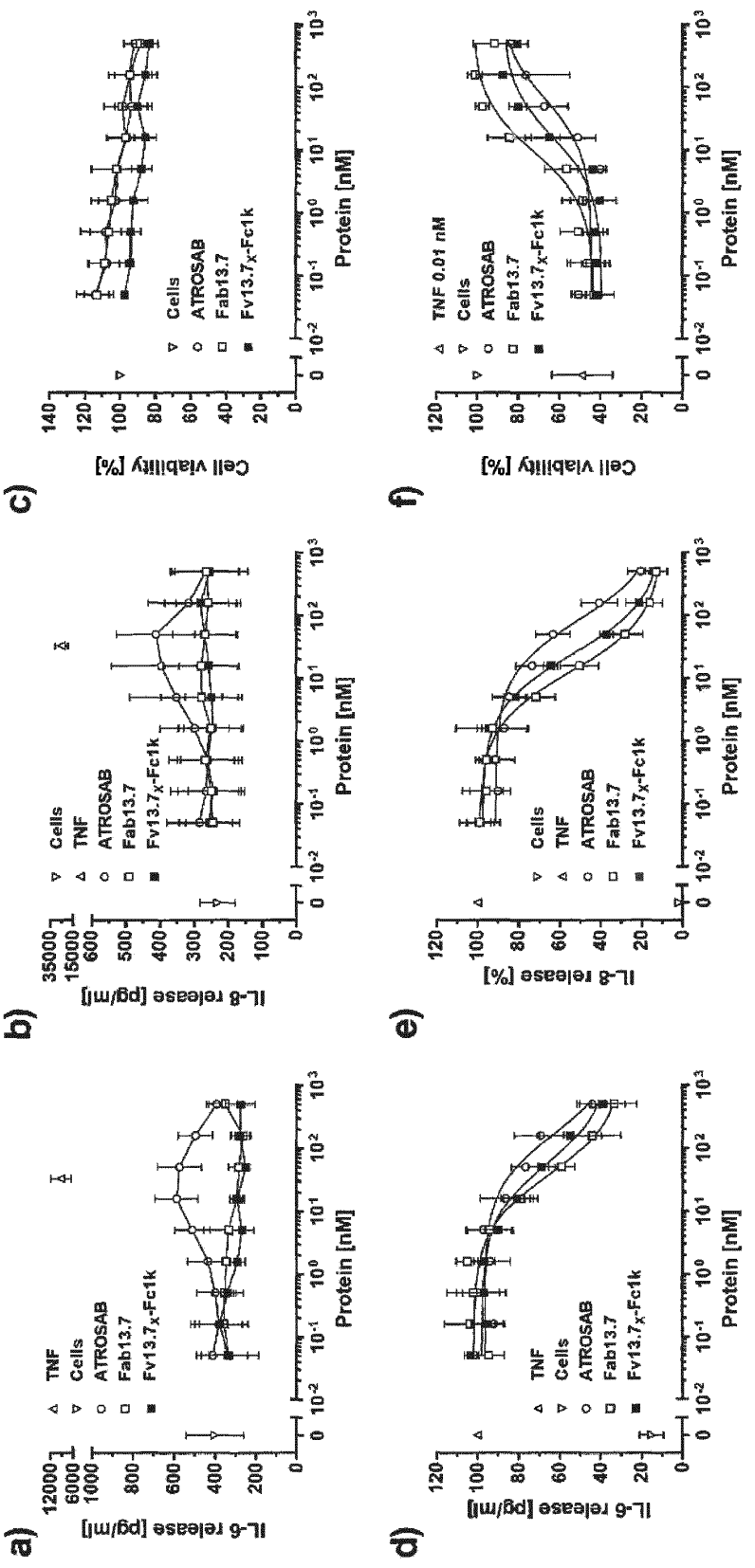

a)
IL-6 release [pg/ml]
TNF
Cells
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]
b)
IL-8 release [pg/ml]
Cells
TNF
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]
c)
Cell viability [%]
Cells
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]
d)
IL-6 release [pg/ml]
TNF
Cells
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]
e)
IL-8 release [%]
Cells
TNF
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]
f)
Cell viability [%]
TNF 0.01 nM
Cells
ATROSAB
Fab13.7
Fv13.7x-Fc1k
Protein [nM]

Figure 38

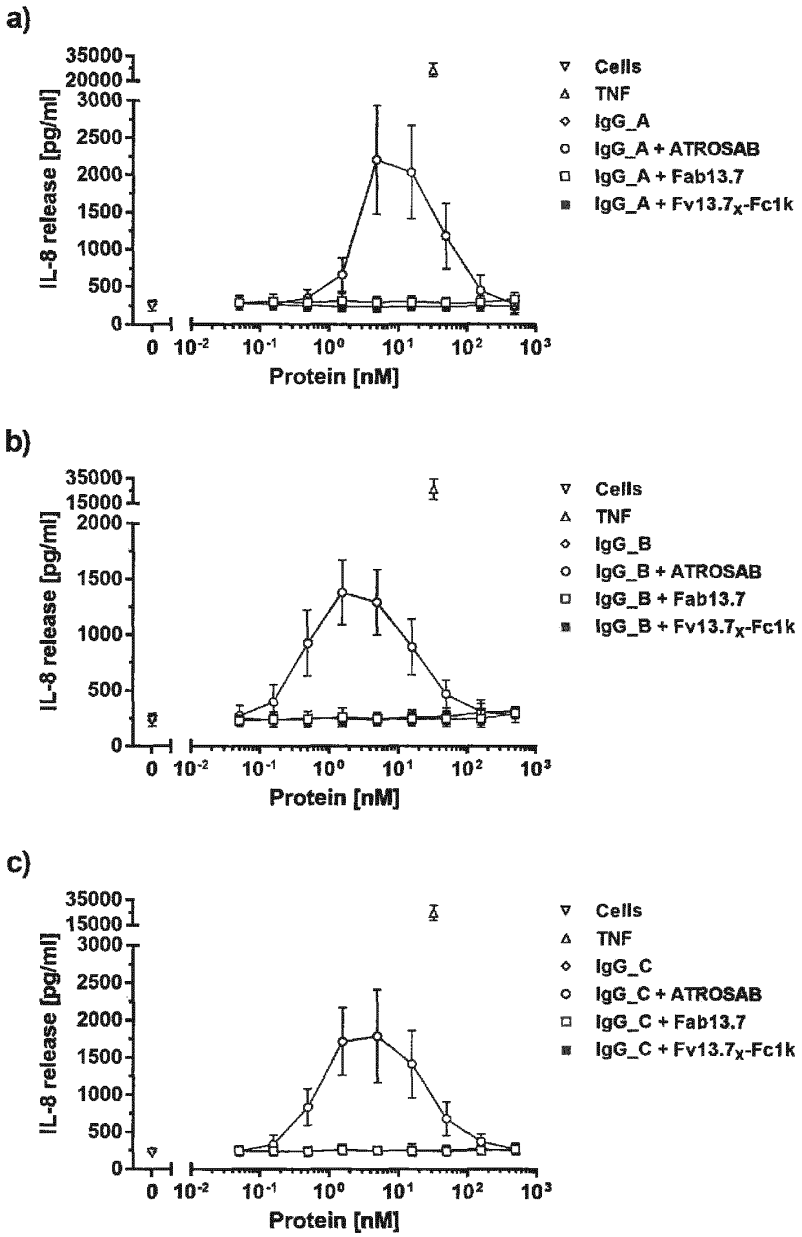
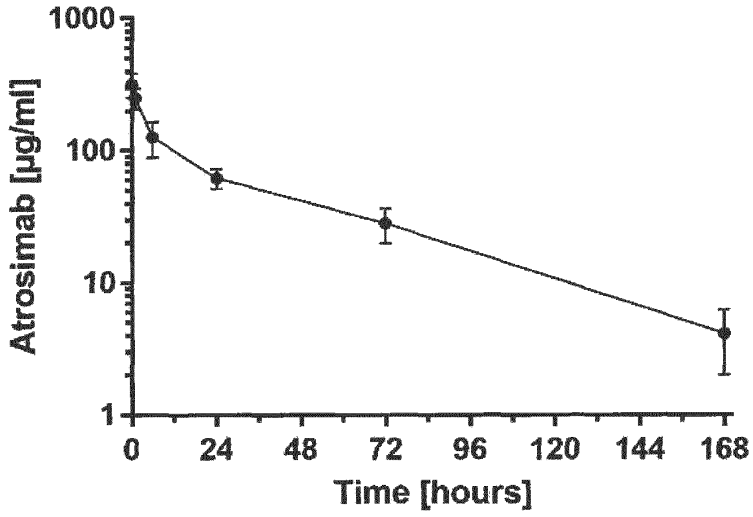

a)
IL-8 release [pg/ml]
Protein [nM]
Cells
TNF
IgG_A
IgG_A + ATROSAB
IgG_A + Fab13.7
IgG_A + Fv13.7χ-Fc1k
b)
IL-8 release [pg/ml]
Protein [nM]
Cells
TNF
IgG_B
IgG_B + ATROSAB
IgG_B + Fab13.7
IgG_B + Fv13.7χ-Fc1k
c)
IL-8 release [pg/ml]
Protein [nM]
Cells
TNF
IgG_C
IgG_C + ATROSAB
IgG_C + Fab13.7
IgG_C + Fv13.7χ-Fc1k

Figure 39


1000
100
10
1
Atrosimab [µg/ml]
0
24
48
72
96
120
144
168
Time [hours]