

1/21

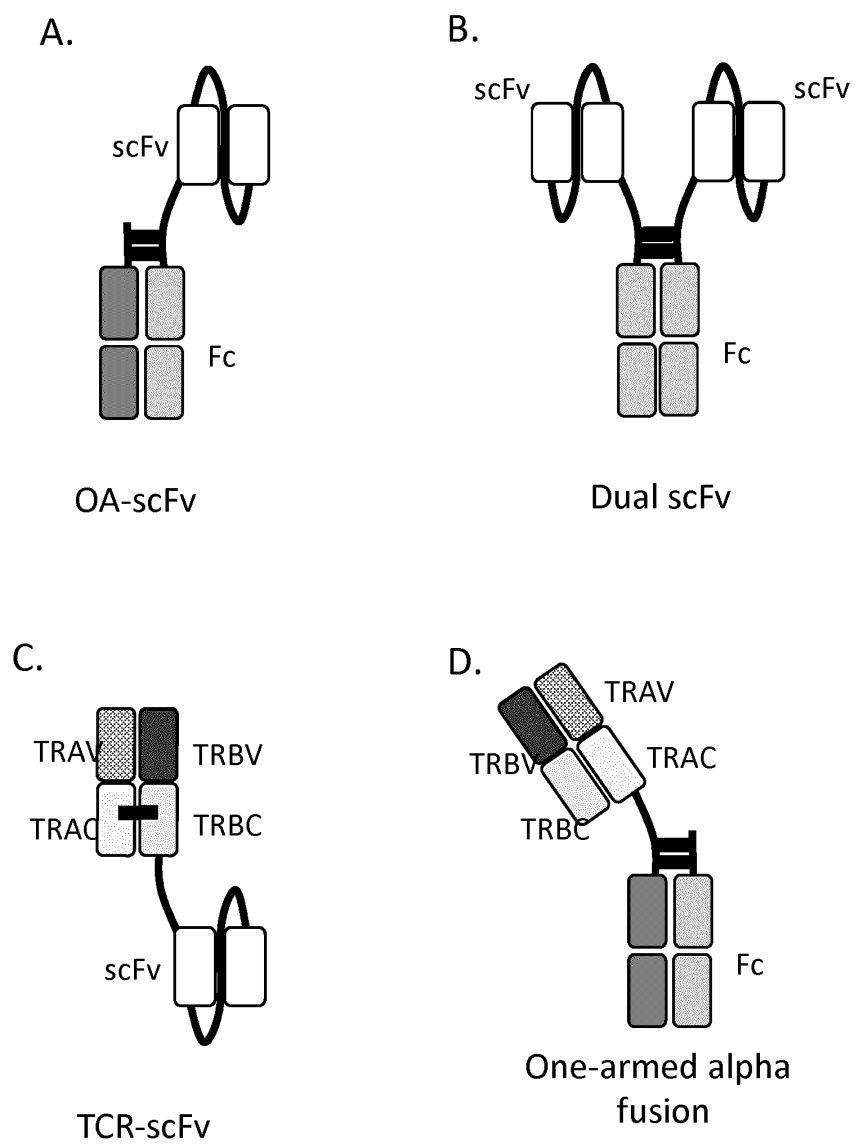


Fig. 1

2/21

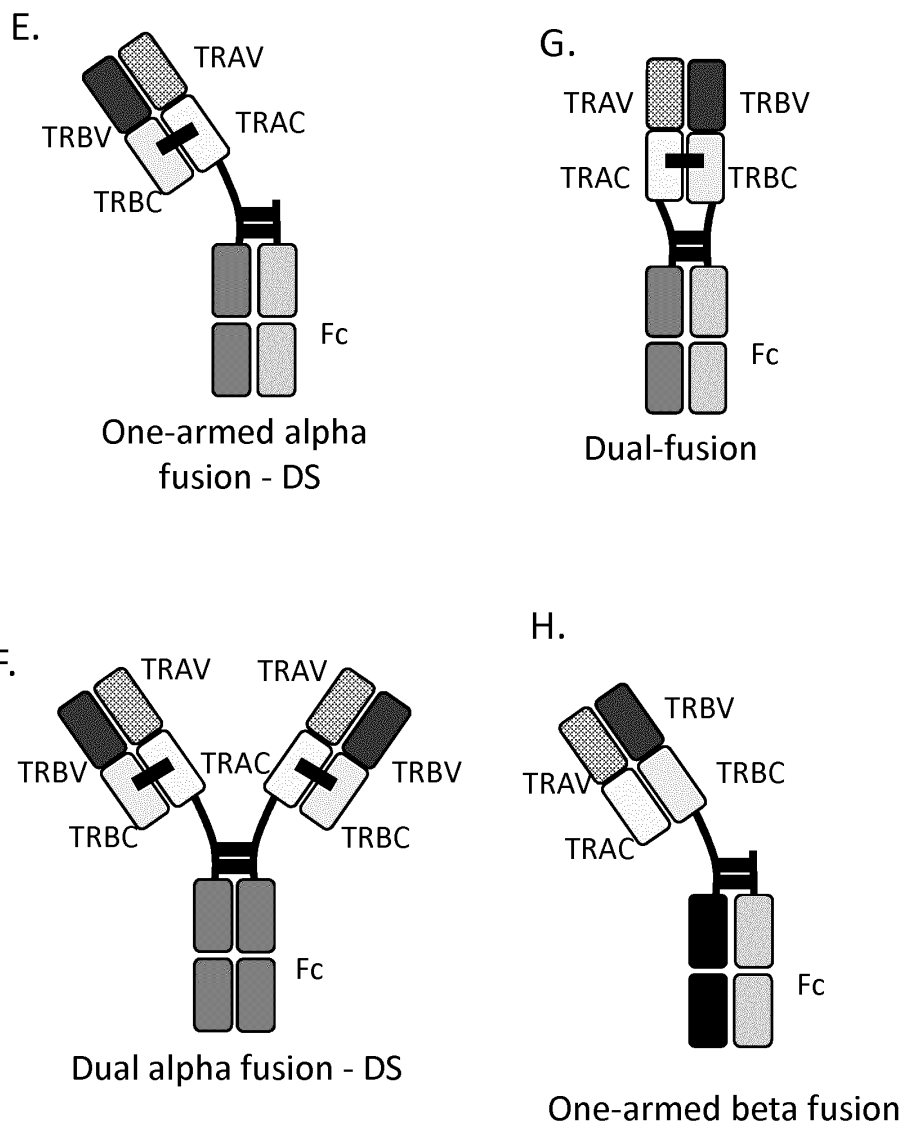


Fig. 1 (Con.)

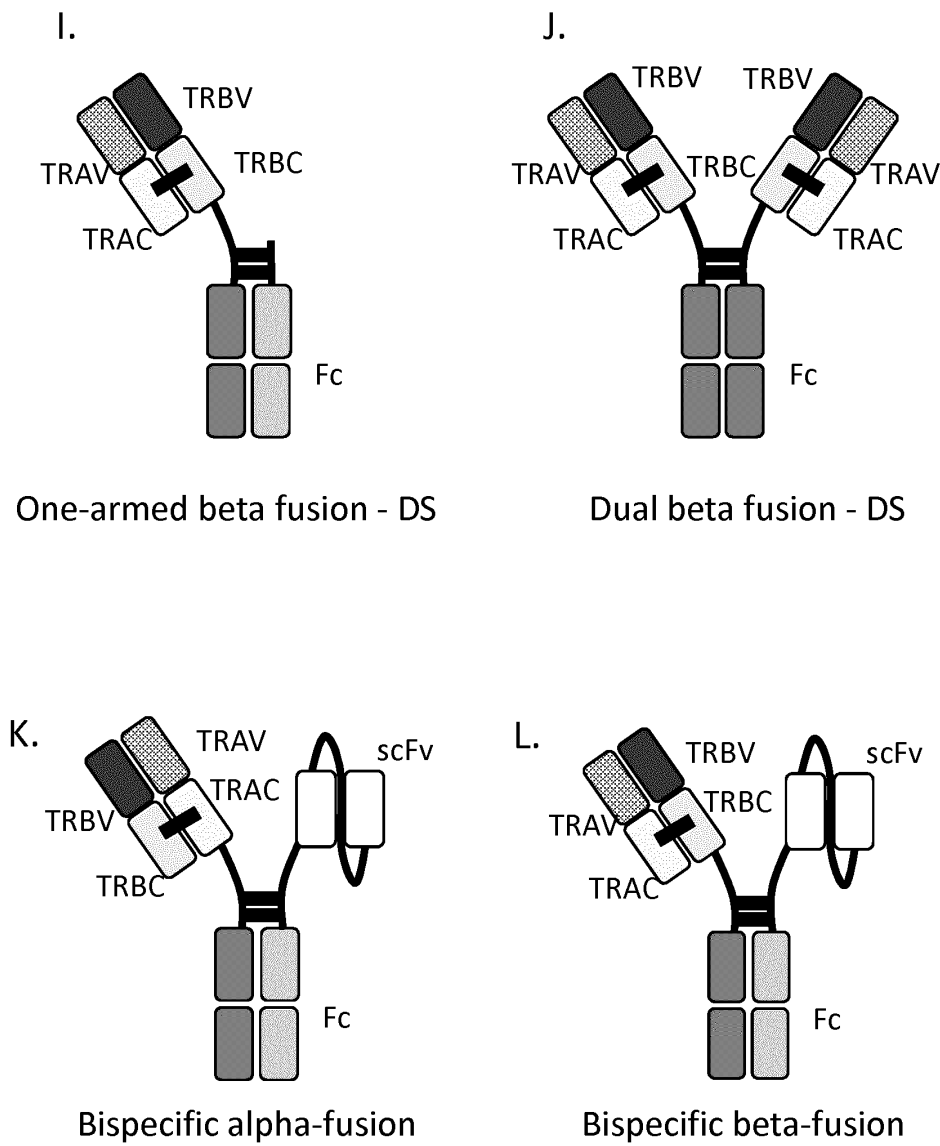


Fig. 1 (Con.)

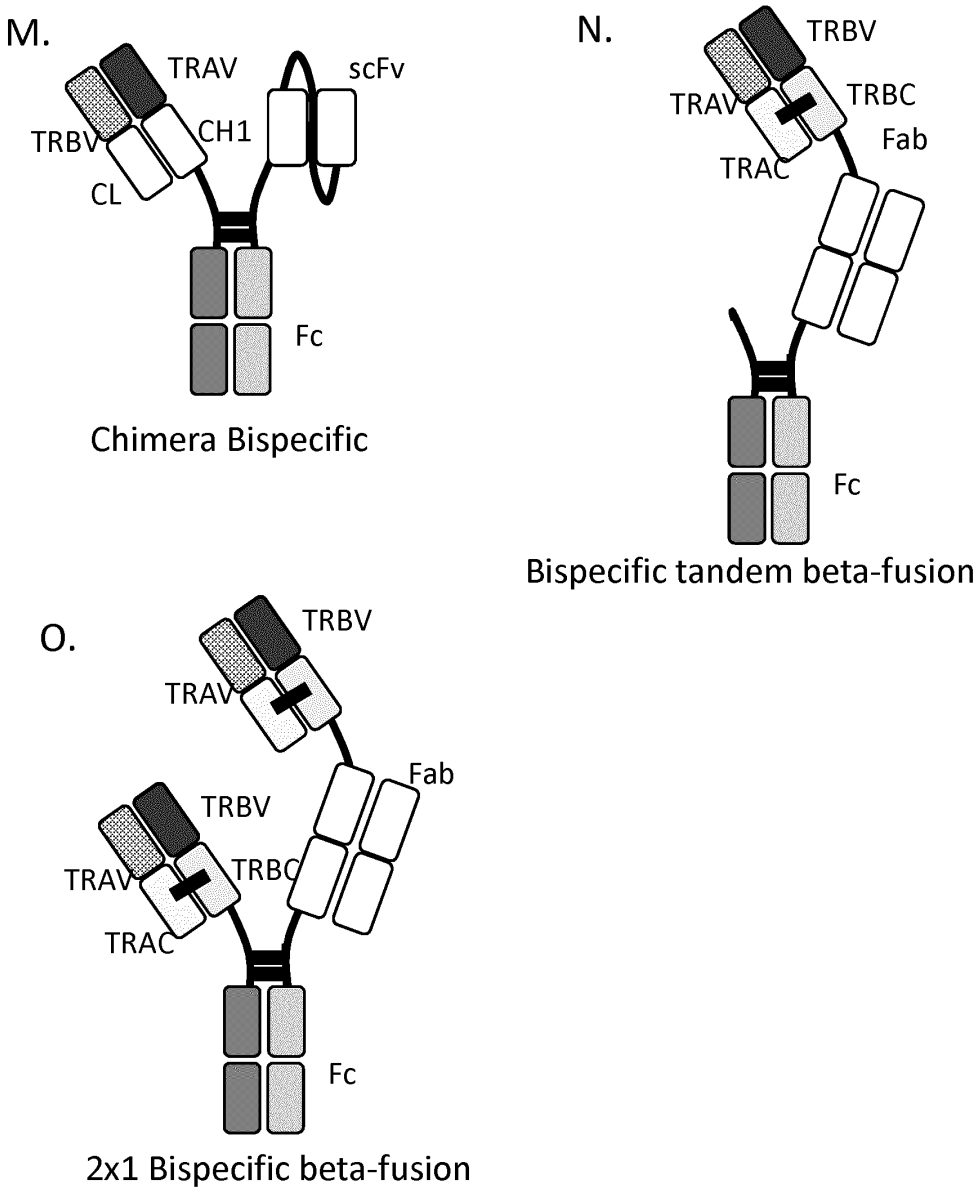


Fig. 1 (Con.)

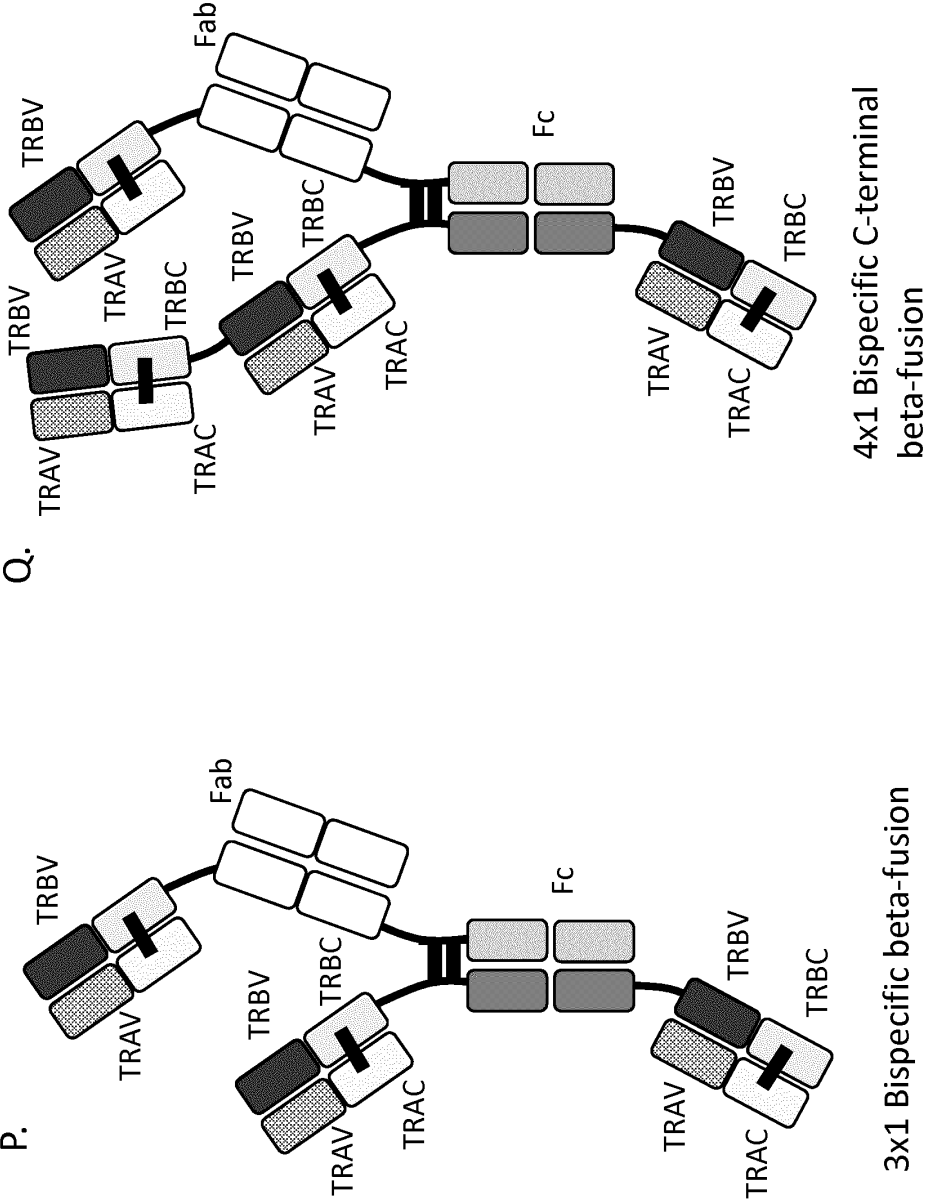


Fig. 1 (Con.)

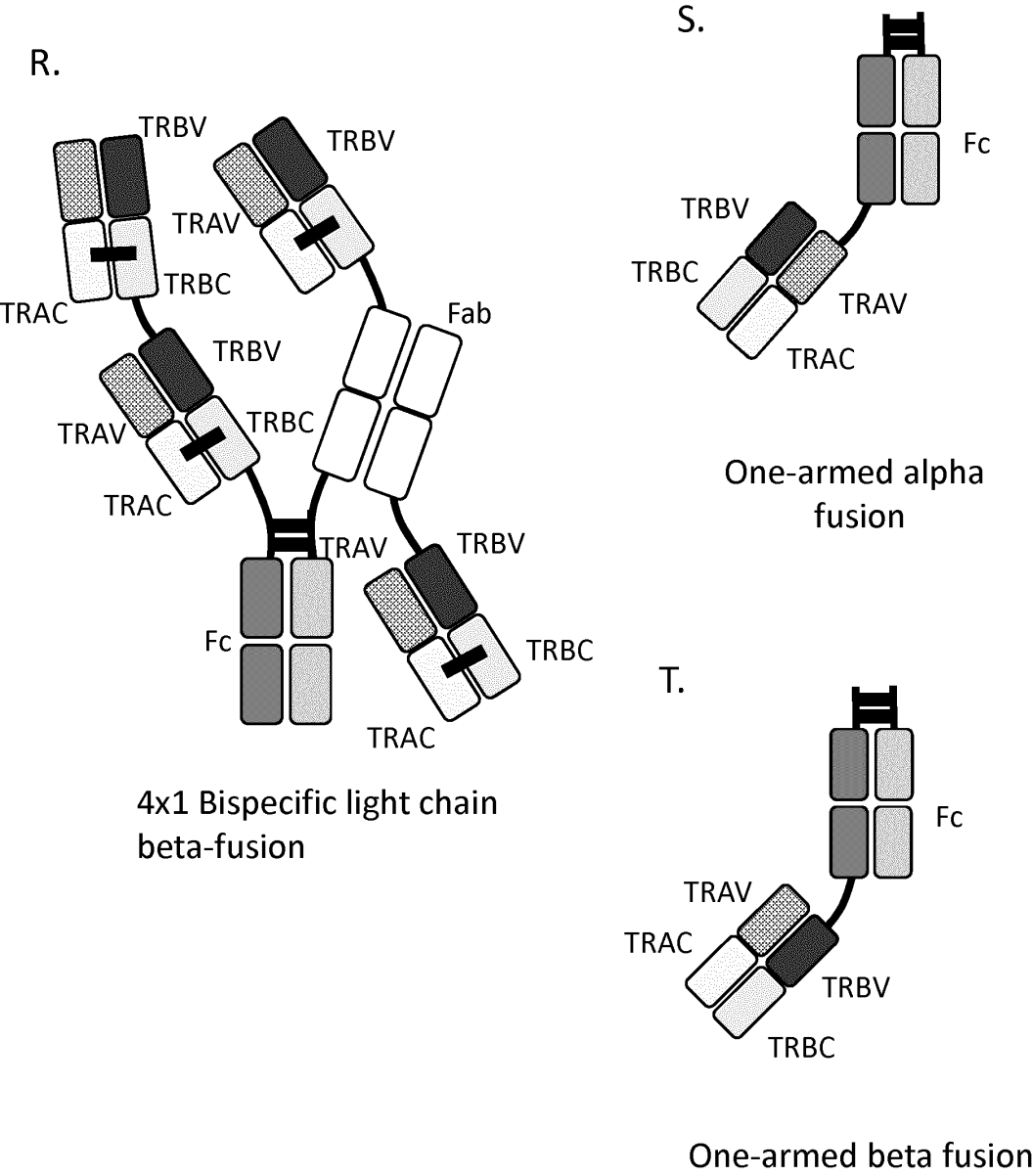


Fig. 1 (Con.)

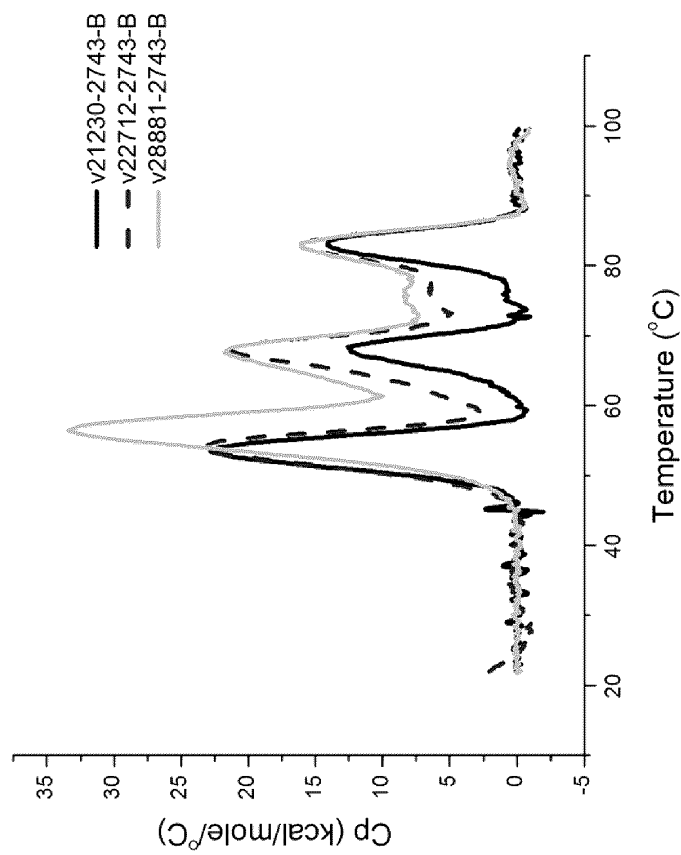


Fig. 2

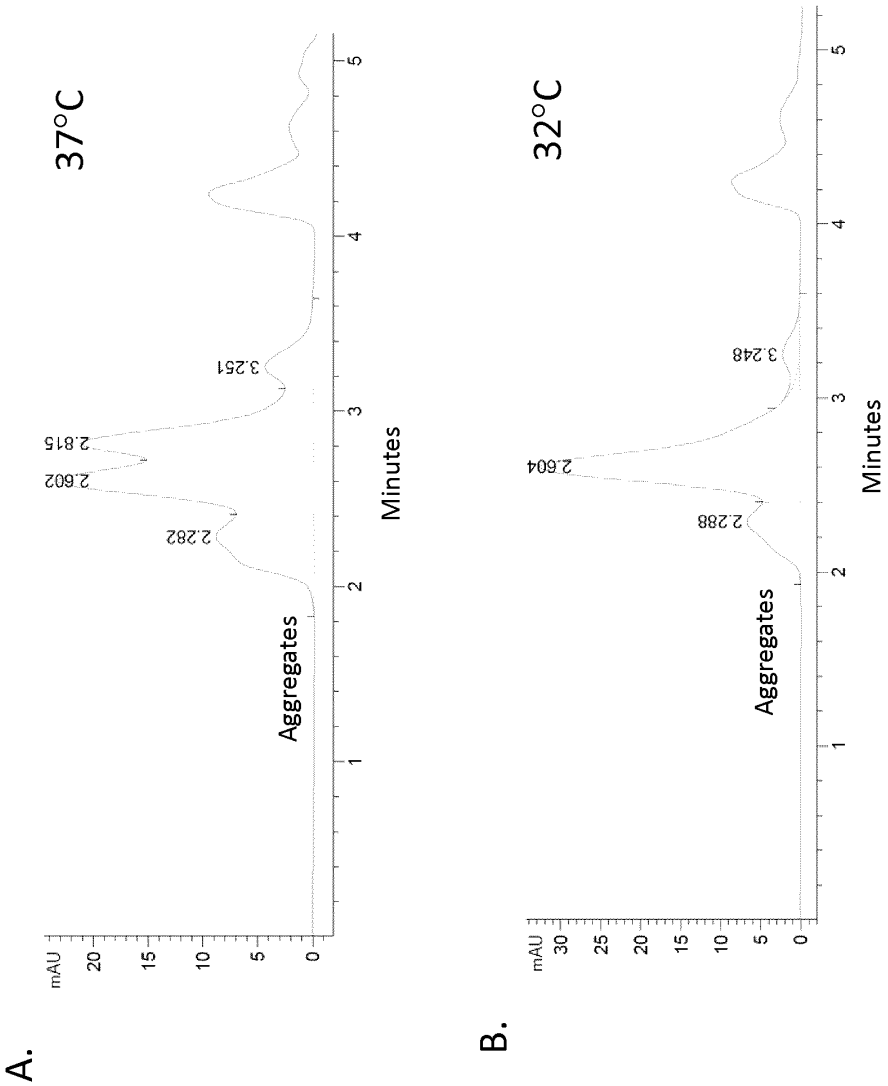


Fig. 3

9/21

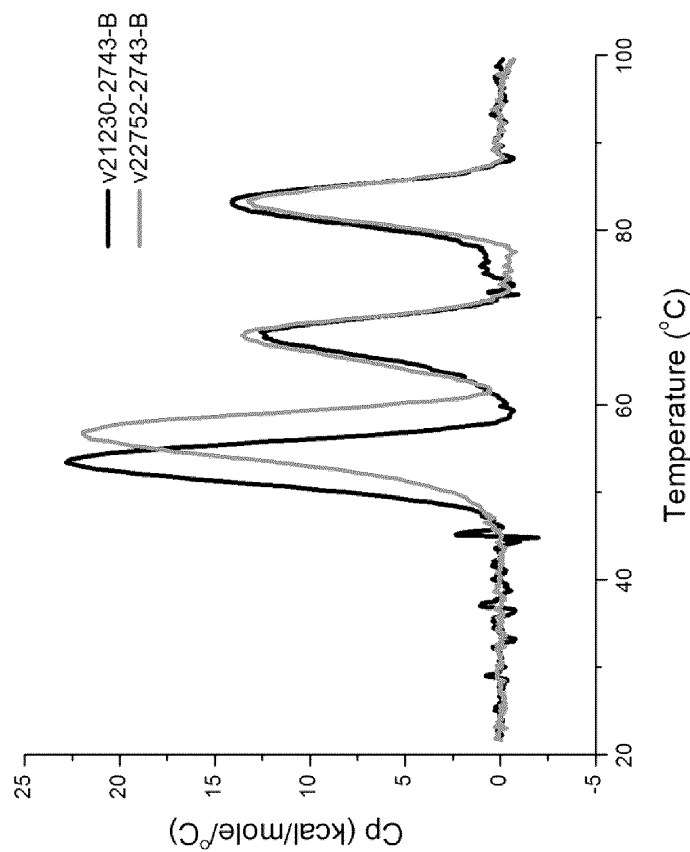


Fig. 4

10/21

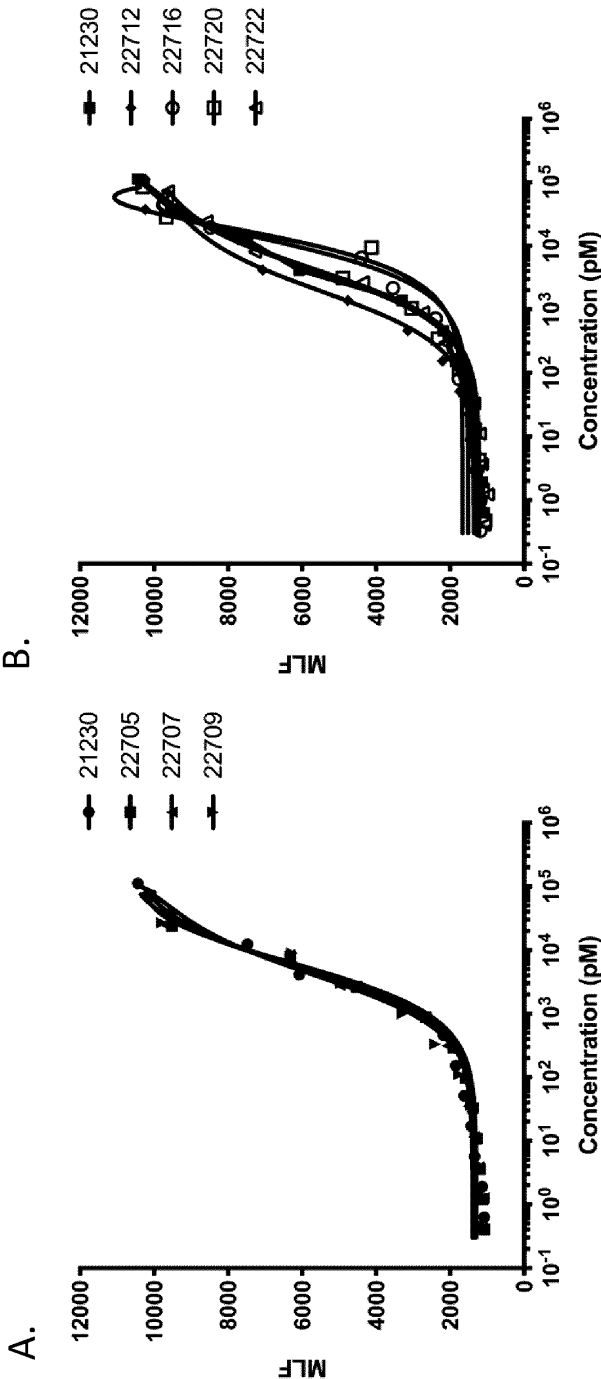


Fig. 5

11/21

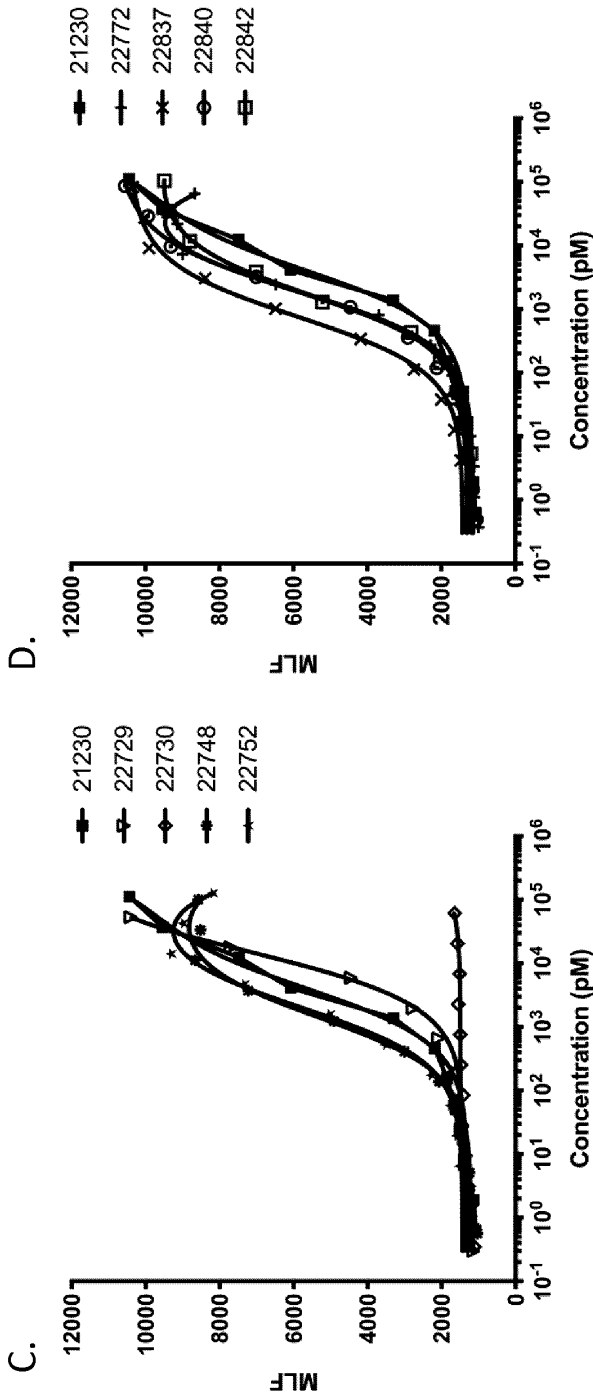


Fig. 5 (Con.)

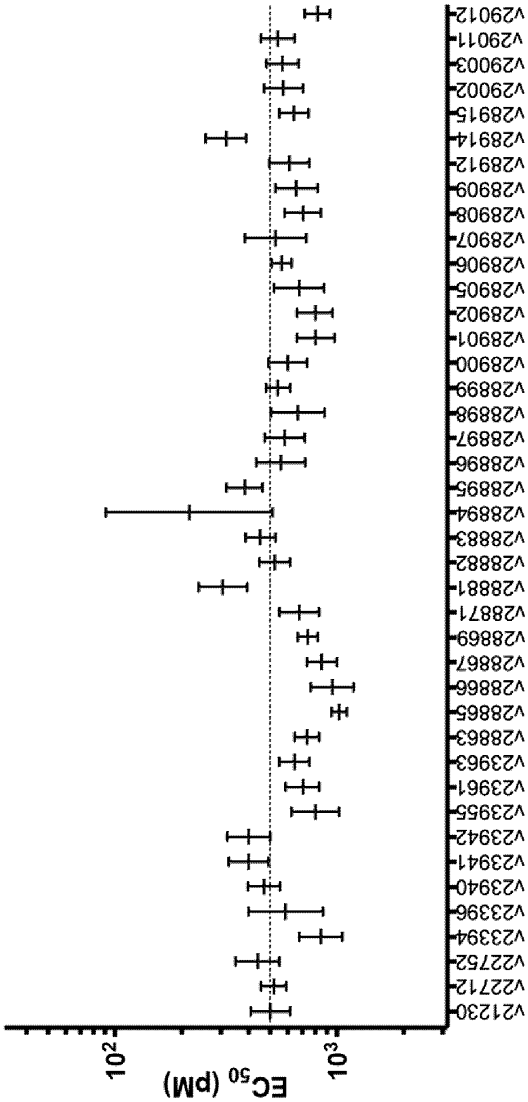


Fig. 6

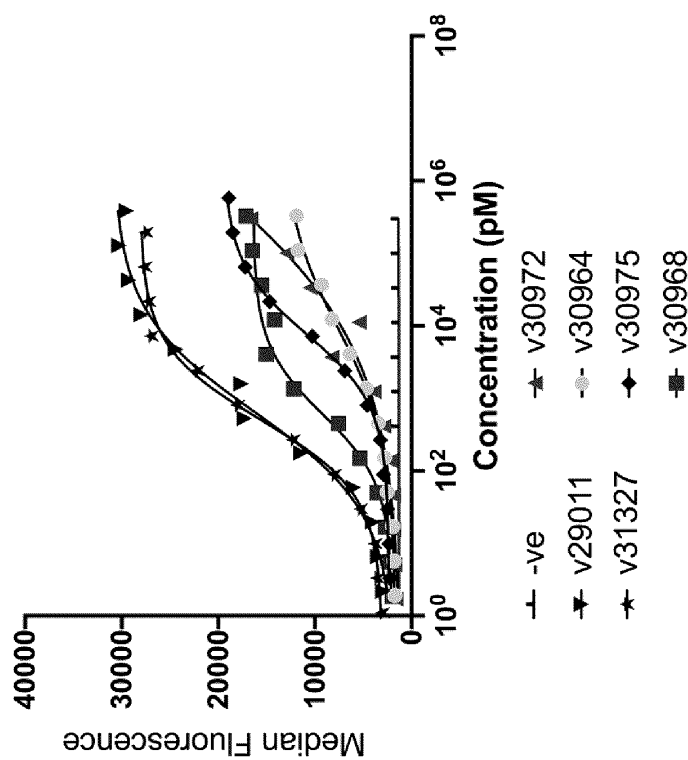


Fig. 7

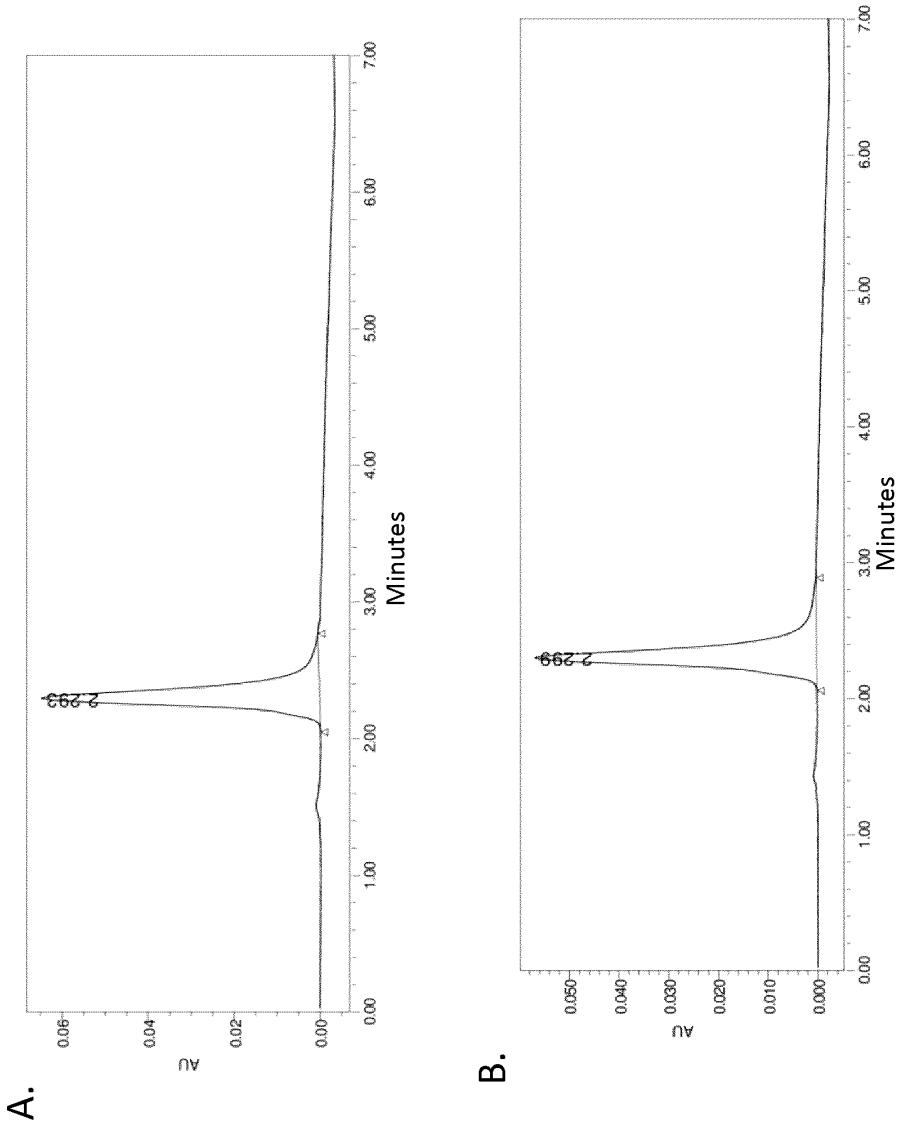


Fig. 8

IMGT	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1	2	3	4	5	6	7	8	9	10
TRAC	--	--	N	I	Q	N	P	D	P	A	V	Y	Q	L	R	D	S
TRBC1	E	D	L	N	K	V	F	P	P	E	V	A	V	F	E	P	S
TRCB2	E	D	L	K	N	V	F	P	P	E	V	A	V	F	E	P	S

IMGT	11	12	13	14	15	15.1	16	17	18	19	20	21	22	23	24	25	26
TRAC	K	--	--	--	--	--	--	S	S	D	K	S	V	C	L	F	T
TRBC1	E	A	E	I	S	H	T	Q	K	A	T	L	V	C	L	A	T
TRCB2	E	A	E	I	S	H	T	Q	K	A	T	L	V	C	L	A	T

IMGT	27	28	29	30	35	36	37	38	39	40	41	42	43	44	45	45.1	45.2
TRAC	D	F	D	S	--	Q	T	N	V	S	Q	S	K	D	S	--	--
TRBC1	G	F	F	P	D	H	V	E	L	S	W	W	V	N	G	K	E
TRCB2	G	F	Y	P	D	H	V	E	L	S	W	W	V	N	G	K	E

IMGT	45.3	45.4	45.5	77	78	79	80	81	82	83	84	84.1	84.2	84.3	84.4	84.5	84.6
TRAC	--	--	--	D	V	Y	I	T	D	K	T	V	L	D	M	R	S
TRBC1	V	H	S	G	V	S	T	D	P	Q	P	L	K	E	Q	P	A
TRCB2	V	H	S	G	V	S	T	D	P	Q	P	L	K	E	Q	P	A

Fig. 9

IMGT	84.7	85.6	85.5	85.4	85.3	85.2	85.1	85	86	87	88	89	90	91	92	93	94
TRAC	M	D	F	K	S	N	S	A	V	A	W	S	N	K	S	--	--
TRBC1	L	N	D	S	R	Y	C	L	S	S	R	L	R	V	S	A	T
TRCB2	L	N	D	S	R	Y	C	L	S	S	R	L	R	V	S	A	T

IMGT	95	96	96.1	97	98	99	100	101	102	103	104	105	106	107	108	109	110
TRAC	--	--	--	--	--	--	--	D	F	A	C	A	N	A	F	N	N
TRBC1	F	W	Q	N	P	R	N	H	F	R	C	Q	V	Q	F	Y	G
TRCB2	F	W	Q	N	P	R	N	H	F	R	C	Q	V	Q	F	Y	G

IMGT	111	111.1	111.2	111.3	111.4	111.5	111.6	112.6	112.5	112.4	112.3	112.2	112.1	112
TRAC	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TRBC1	L	S	E	N	D	E	W	T	Q	D	R	A	K	P
TRCB2	L	S	E	N	D	E	W	T	Q	D	R	A	K	P

IMGT	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127
TRAC	S	I	I	P	E	D	T	F	F	P	S	P	E	S	S
TRBC1	V	T	Q	I	V	S	A	E	A	W	G	R	A	D	--
TRCB2	V	T	Q	I	V	S	A	E	A	W	G	R	A	D	--

Fig. 9 (Con.)

Cytotoxic effects of affinity matured 1G4-33A anti-NY-ESO1 TCR in T2 T cell dependent cytotoxicity assay

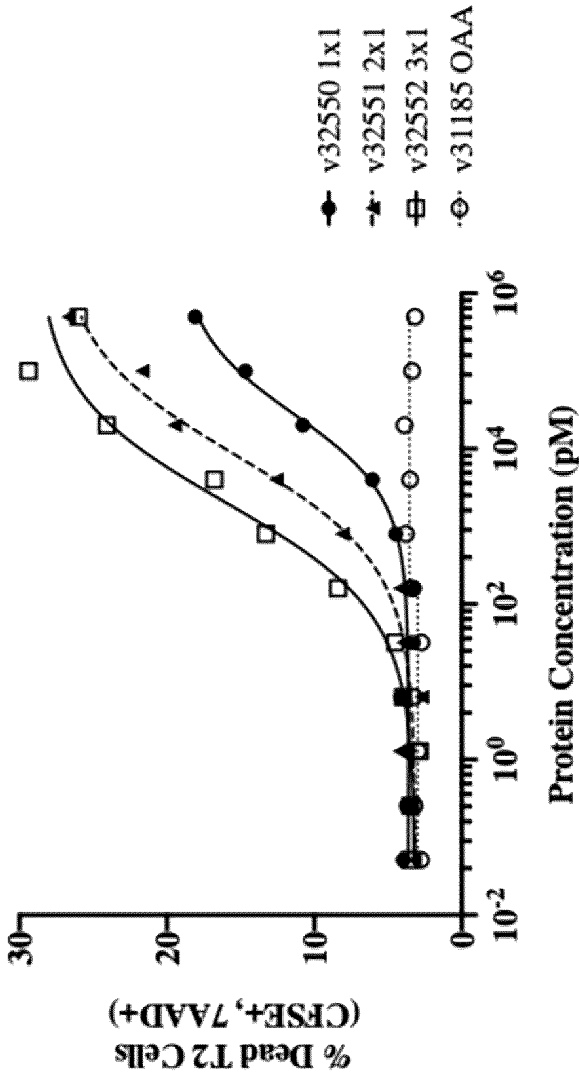


Fig. 10

Table 2

Variant Number	Point Mutations								Deletions			Interchain Disulfides										Intra-chain Disulfide		Measured Tm (°C)	HMW Species (%)	
	TRAC 4,VAL>ILE	TRAC 26,THR>ILE	TRAC 39,VAL>ILE	TRAC 85,6,ALA>VAL	TRAC 105,ALA>SER	TRBC 6,VAL>LEU	TRBC 6,VAL>ILE	TRBC 36,HIS>PHE	TRBC 86,SER>THR	TRBC 45,3,VAL>THR	Δ TRBC 84,5-85	Δ TRBC 84,4+85,3>GLY-ASN	IC Disulfide TRAC 84-TRBC 79	Disulfide TRAC 84,2-TRBC 79	TRAC-Hinge Disulfide	Disulfide TRAC 122-TRBC 12	Disulfide TRAC 124-TRBC 11	Disulfide TRAC 125-TRBC 11	Disulfide TRAC 126-TRBC 11	Disulfide TRAC 127-TRBC 11	Disulfide TRAC 128-TRBC 11	Disulfide TRAC 26-TRAC 85,5	Disulfide TRAC 39-TRAC 85			
v28906	x			x						x			x												56.1	7.5
v23940	x					x							x												54.7	12.8
v28908			x			x							x												54.9	11.8
v23941			x			x							x												55.1	13
v28894			x	x									x												56	9.4
v28895			x		x								x												55	10.3
v23961			x									x	x												54.6	11.4
v28907				x	x					x			x												56	4.5
v28909				x	x							x	x												55.3	4.8
v23942				x	x	x							x												56.1	10.3
v28896				x	x								x												55.6	5.9
v22712						x							x												54	10.7
v28898						x				x			x												54.6	7.4
v28899						x						x	x												53.6	7.4
v28912						x				x		x	x												54.1	6
v28901										x			x												54.8	10.6
v23955					x								x												53.9	9.8
v23963					x							x	x												53.6	11
v22752													x			x									56.9	11.2
v28914	x			x		x							x			x									58.6	9.6
v28915	x			x		x						x	x			x									58.1	10.3

Fig. 11

19/21

Variant Number	Point Mutations										Deletions		Interchain Disulfides										Intra-chain Disulfide		Measured Tm (°C)	HMW Species (%)	
	TRAC 4 VAL>ILE	TRAC 26 THR>ILE	TRAC 39 VAL>ILE	TRAC 85 6ALA>VAL	TRAC 105 ALA>SER	TRBC 6 VAL>LEU	TRBC 6 VAL>ILE	TRBC 36 HIS>PHE	TRBC 86 SER>THR	TRBC 45 3 VAL>THR	Δ TRBC 84 4-85 3->GLY-ASN	Δ TRBC 84 5-85	IC Disulfide TRAC 84 - TRBC 79	Disulfide TRAC 84 2 - TRBC 79	TRAC-Hinge Disulfide	Disulfide TRAC 122 - TRBC 12	Disulfide TRAC 124 - TRBC 11	Disulfide TRAC 125 - TRBC 11	Disulfide TRAC 126 - TRBC 11	Disulfide TRAC 127 - TRBC 11	Disulfide TRAC 128 - TRBC 11	Disulfide TRAC 26 - TRAC 85 5	Disulfide TRAC 39 - TRAC 85				
v29012	X			X	X	X		X	X	X	X		X		X										58.5	10.2	
v28897				X									X		X										58.5	10.5	
v29002				X						X			X		X										58.5	6	
v29003				X		X							X		X										58.6	5.8	
v28900						X							X		X										57.1	10.3	
v28905													X		X										56.6	7.9	
v28902 ¹															X	X									52.5	6.3	
v31131															X	X									53.3	10.4	
v30934 ²															X	X									53.8	14.6	
v29011 ³	X			X	X	X		X	X	X	X			X	X										54.3	10.3	
v31095		X		X		X		X						X	X										55.9	20	
v31096		X		X		X		X						X	X										55.5	13.4	
v31098		X		X		X		X						X	X										55	10.9	
v31099		X		X		X		X						X	X										56.2	17.7	
v31100		X		X		X		X						X	X										54.6	12.9	
v31101		X		X		X		X		X				X	X										55.8	13.6	
v31102		X		X	X	X		X						X	X										55.5	14.5	
v31103		X		X		X		X				X		X	X										55.5	14.1	
v31104		X		X		X		X			X			X	X										54.8	16.2	
v31097				X		X		X						X	X										55	9.45	
v31085														X	X								X		57.8	19.9	
v31086														X	X								X		54.2	13.3	
v33047						X	X	X						X	X									X		54.8	11.2

Fig. 11 (Con.)

20/21

Variant Number	Point Mutations								Deletions		Interchain Disulfides									Intra-chain Disulfide		Measured Tm (°C)	HMW Species (%)
	TRAC 4 VAL>ILE	TRAC 26 THR>ILE	TRAC 39 VAL>ILE	TRBC 6 VAL>ILE	TRBC 36 HIS>PHE	TRBC 86 SER>THR	TRBC 45,3 VAL>THR	Δ TRBC 84,4-85,3->GLY-ASN	Δ TRBC 84,5-85	IC Disulfide TRAC 84 - TRBC 79	Disulfide TRAC 84,2 - TRBC 79	TRAC-Hinge Disulfide	Disulfide TRAC 122 - TRBC 12	Disulfide TRAC 124 - TRBC 11	Disulfide TRAC 125 - TRBC 11	Disulfide TRAC 126 - TRBC 11	Disulfide TRAC 127 - TRBC 11	Disulfide TRAC 128 - TRBC 11	Disulfide TRAC 26 - TRAC 85,5	Disulfide TRAC 39 - TRAC 85			
v33048											X	X									X	58.7	6.6
v33049										X	X							X				55.5	6.5
v33050											X							X			X	60.3	11.8
v33051											X	X									X	57.2	9.5
v33052											X	X			X						X	58.5	11.3
v33053											X	X				X					X	60.2	10.1
v33054											X	X						X			X	60.0	10.2
v33055											X	X										55.7	6.2
v33056											X	X									X	60.4	11.5
v33057		X									X	X									X	59.6	14.3

¹This variant includes the mutations TRAC/1.5.GLN->LYS and TRBC/97.GLN->ASP as described in Example 1

²This variant is based on the TRBC1 constant framework (all other variants are based on the TRBC2 constant framework)

³ Values provided are averaged from 3 different expressions of this variant

Fig. 11 (Con.)

Table 3

Variant Number	Point Mutations				Disulfide Bonds									Measured T _m (°C)	HMW Species (%)	
	TRAC 85.6,ALA->VAL	TRBC 6,VAL>ILE	TRBC 36,HIS>PHE	TRAC 26,THR>ILE	IC Disulfide TRAC 84 - TRBC 79	Disulfide TRAC 84.2 - TRBC 79	TRAC-Hinge Disulfide	Disulfide TRAC 39 - TRAC 85	Disulfide TRAC 122 - TRCB 12	Disulfide TRAC 124 - TRCB 11	Disulfide TRAC 125 - TRCB 11	Disulfide TRAC 126 - TRCB 11	Disulfide TRAC 127 - TRCB 11			Disulfide TRAC 128 TRCB 11
v28902 ¹						x	x								52.5	6.3
v31097	x	x	x			x	x								55	9.45
v33047		x	x			x		x							54.8	11.2
v33048		x	x			x	x	x							58.7	6.6
v33049						x							x		55.5	6.5
v33050		x	x			x		x					x		60.3	11.8
v33051		x	x			x		x		x					57.2	9.5
v33052		x	x			x		x			x				58.5	11.3
v33053		x	x			x		x				x			60.2	10.1
v33054		x	x			x		x						x	60.0	10.2
v33055						x			x						55.7	6.2
v33056		x	x			x		x	x						60.4	11.5
v33057		x	x	x		x		x	x						59.6	14.3

¹ This variant includes the mutations TRAC/1.5 GLN->LYS and TRBC/97.GLN->ASP as described in Example 1

Fig. 12