

Figure 1

■ = TTR negative mutant subunits
▨ = TTR positive mutant subunits

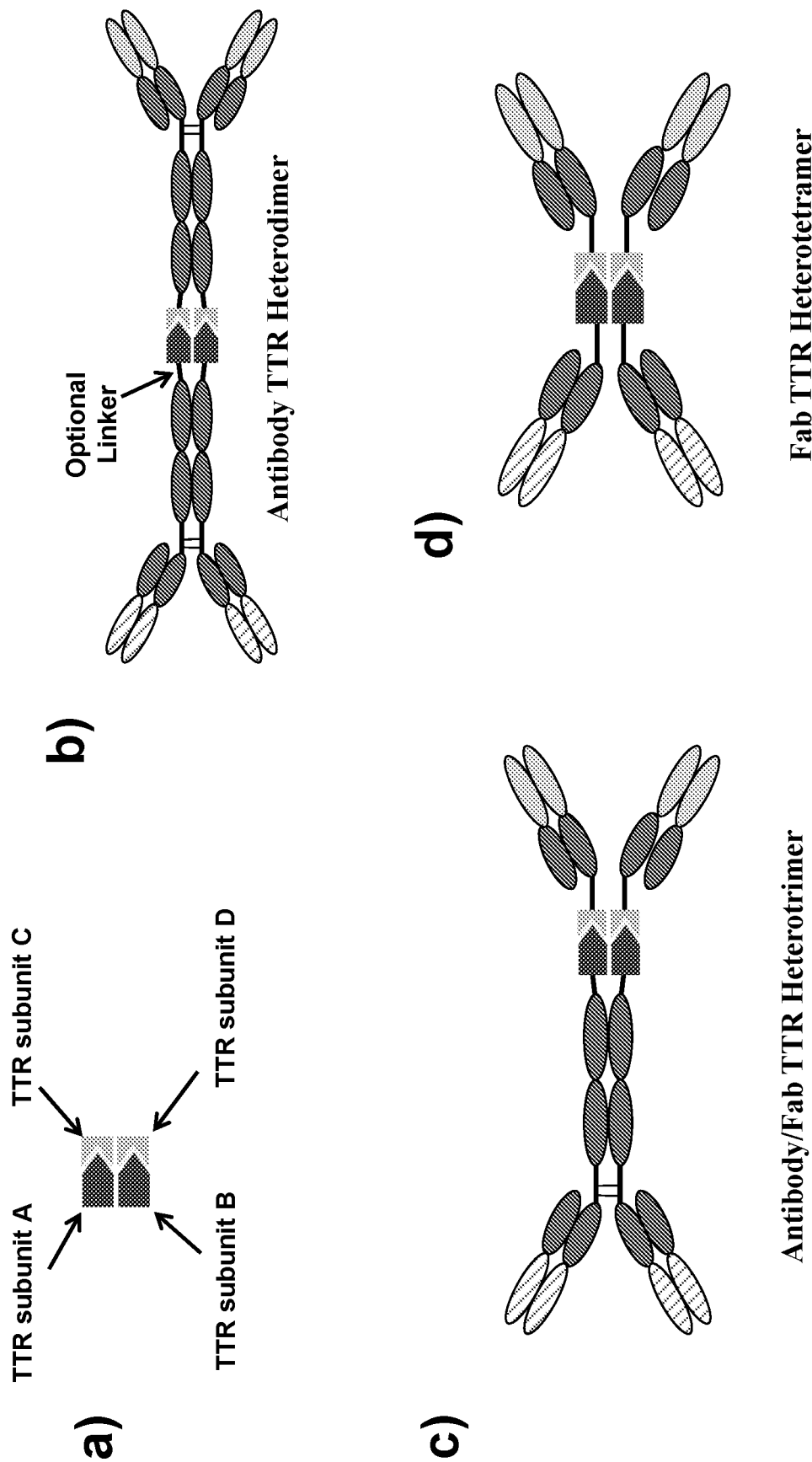


Figure 2

Exemplary heteromeric TTR - Ab/Fab bivalent bispecific constructs

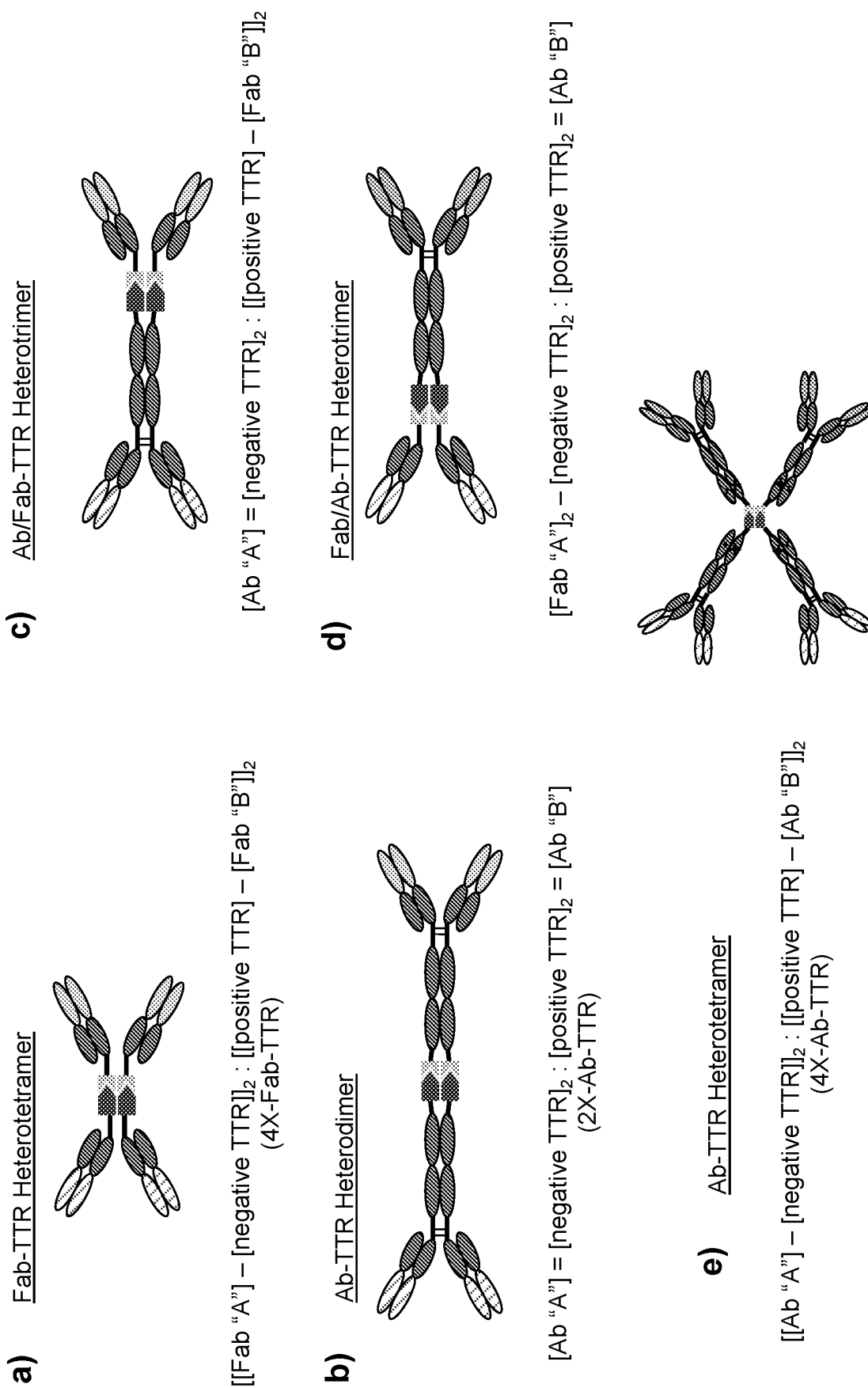


Figure 3

TTR Dimerization and Tetramerization Interfaces

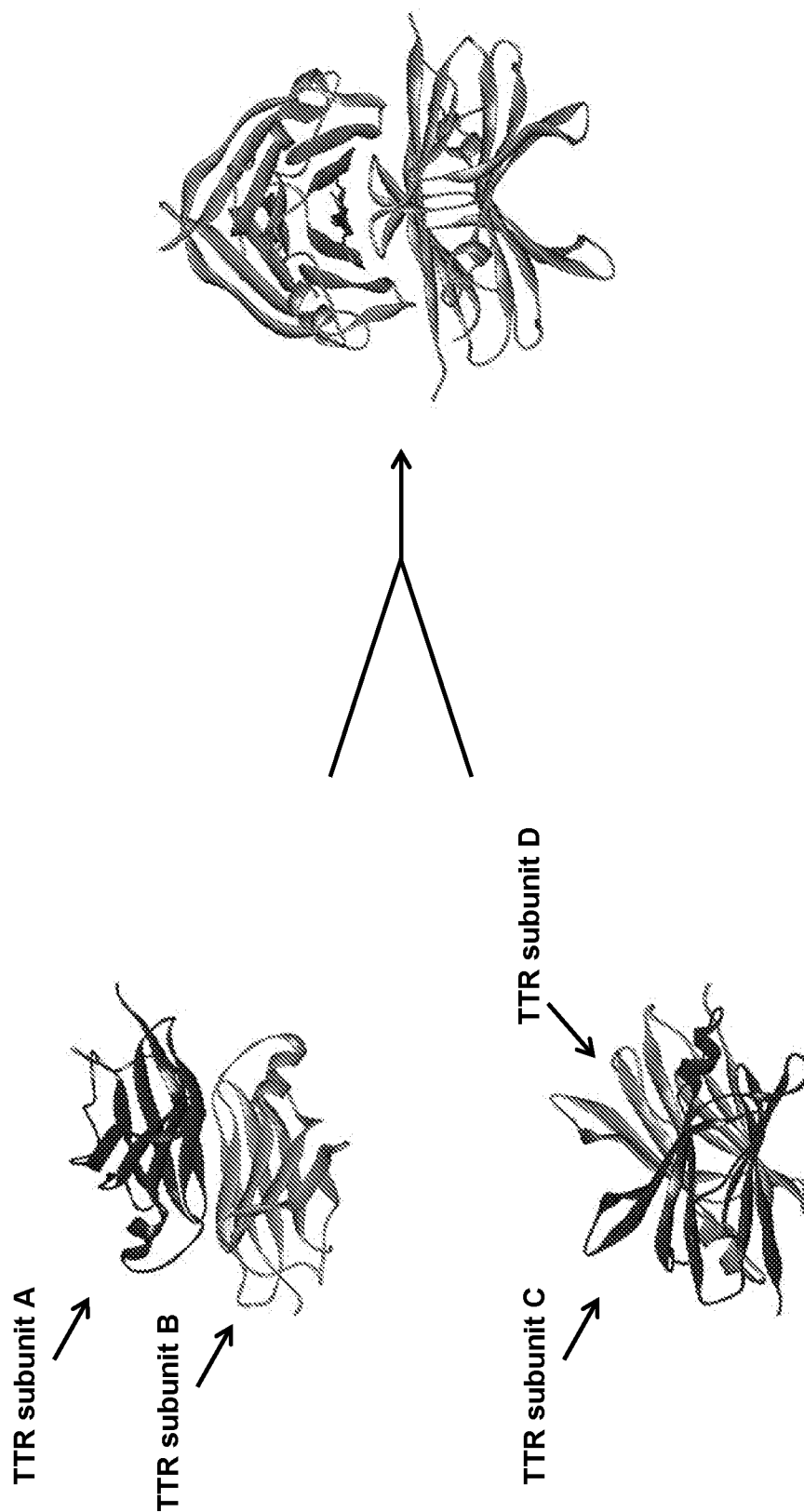
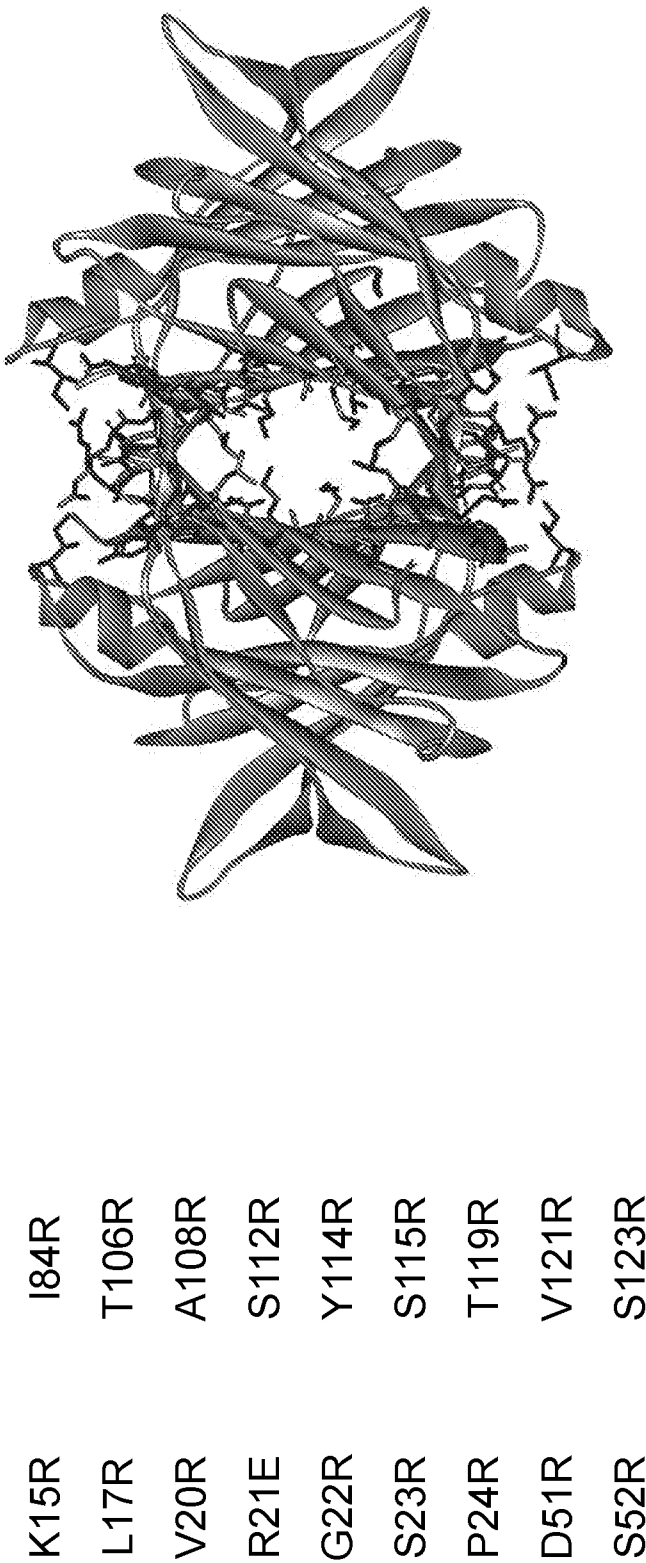


Figure 4

Tetramerization interface residues probed to identify residues that can modulate association



The TTR monomers contained the C10A/K15A mutations in addition to those listed, above in MK-6H-GG-DEVD-TTR

Figure 5
Many Variants display Disassociation of the Tetramer in the Presence of SDS

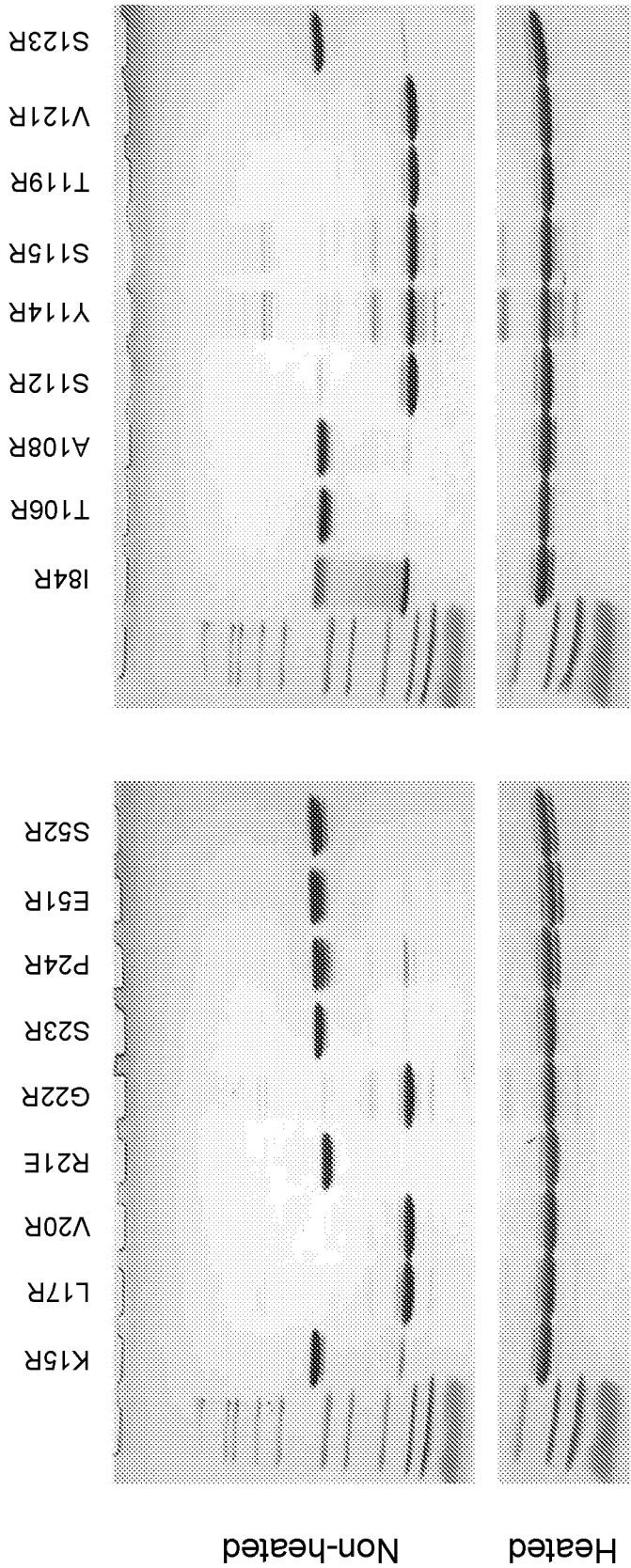


Figure 6

Effect of Non-Denaturing Conditions on TTR Variants

Variant	MolWt(kDa)	SEC	SDS PAGE	TM
K15R	132	Tetramer	Tetramer	>95
R21E	120	Tetramer	Tetramer	~5C Lower
S23R	136	Tetramer	Tetramer	>95
E51R	76	Tetramer	Tetramer	>95
S52R	83	Tetramer	Tetramer	>95
T106R	106	Tetramer	Tetramer	>95
A108R	127	Tetramer	Tetramer	>95
T123R	137	Tetramer	Tetramer	>95
P24R	127	Tetramer	Tet/Dimer	91
I84R	139	Tetramer	Tet/Dimer	87
L17R	134	Tetramer	Dimer	55
V121R	88	Tetramer	Dimer	54
V23R	155	Dimer	Dimer	ND
G22R	81	Dimer	Dimer	ND
S112R	83	Dimer	Dimer	ND
T119R	142	Dimer	Dimer	ND
Y114R	21	Dimer	Dimer	ND
S115R	27	Dimer	Dimer	ND

Figure 7
Several Combination of TTR Variants Form Heterotetramer Complexes

Variant	L17D	L17E	V20D	V20E	G22D	G22E	S112D	S112E	T119D	T119E	V121D	V121E
L17R	59	85	29	79	27	33	53	25	100	100	84	85
L17K	89	85	80	73	33	27	54	25	100	100	84	100
V20R	78	72	96	93	5	2	13	1	19	6	4	66
V20K	87	78	86	82	15	12	44	37	69	44	22	79
G22R	40	3	0	0	3	1	4	2	1	1	0	36
G22K	37	2	0	0	2	2	3	1	1	1	0	27
S112R	3	4	10	8	5	4	7	3	5	5	4	4
S112K	0	0	6	4	2	1	3	1	0	2	2	0
T119R	94	90	0	3	3	1	5	2	87	92	1	83
T119K	83	73	3	4	6	1	5	4	52	48	5	40
V121R	100	100	0	0	44	43	0	0	78	57	46	89
V121K	100	93	0	0	43	49	0	0	80	62	80	88

Variant	L17D	L17E	V20D	V20E	G22D	G22E	S112D	S112E	T119D	T119E	V121D	V121E
L17R	T/D	D	T*/D	D	D	D	D	D	T	T/D	T/D	T/D
L17K	T/D	D	T*/D	D	D	D	D	D	T	T/D	T/D	T
V20R	D	D	T	T	D	D	T*/D	D	T/D	T/D	D	D
V20K	T/D	T*/D	T*/D	D	D	D	T/D	T/D	T/D	T/D	D	T/D
G22R	T*/D	D	T*/D	D	D	D	D	D	D	D	D	D
G22K	T*/D	D	T*/D	D	D	D	D	D	D	D	D	D
S112R	D	D	T*/D	T*/D	D	D	D	D	D	D	D	D
S112K	D	D	T*/D	T*/D	D	D	D	D	D	D	D	D
T119R	T*/D	D	T*/D	D	D	D	D	D	D	D	D	D
T119K	T*/D	D	T*/D	D	D	D	D	D	D	D	D	D
V121R	T	T	T*/D	D	D	D	D	D	D	D	D	D
V121K	T	T/D	T*/D	D	D	D	D	D	D	D	D	D

“D” refers to predominantly dimer formation

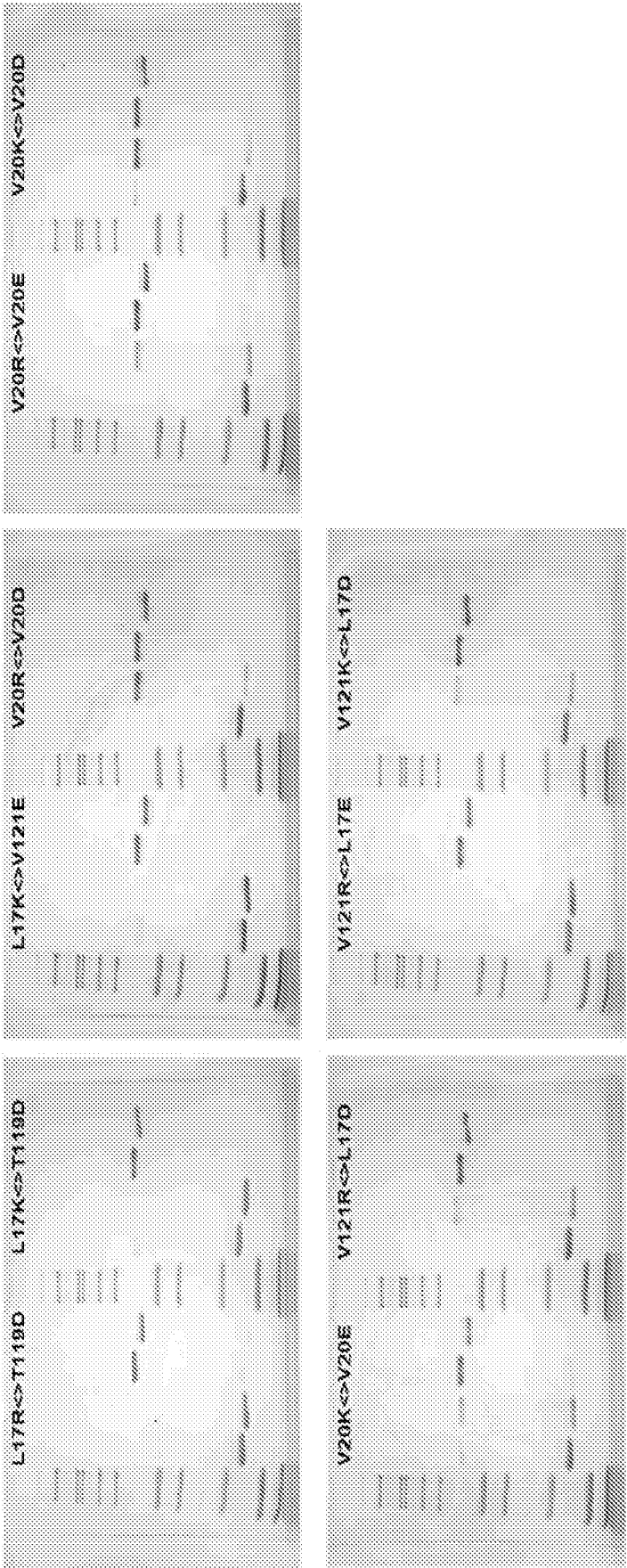
“T” refers to predominantly heterotetramer formation

T-/D, T/D, and T/D- refer to more dimer than heterotetramer, approximately equal dimer/heterotetramer, and more heterotetramer than dimer formation, respectively

T* refers to tetramers likely containing a significant amount of homotetramer (i.e., tetramers containing four negative TTR variants or four positive TTR variants)

Figure 8

Heterodimerization Confirmed by Proteolysis and SDS-PAGE



Lanes (from left to right): Markers, Basic Variant, Acidic Variant, Combination, Combination + Caspase
Basic variant = 6H-GG-DEVD-TTR
Acidic variant = 6H-GG-TTR

Figure 9
Heterodimers Demonstrate Stability at pH 5.0 by SEC Analysis

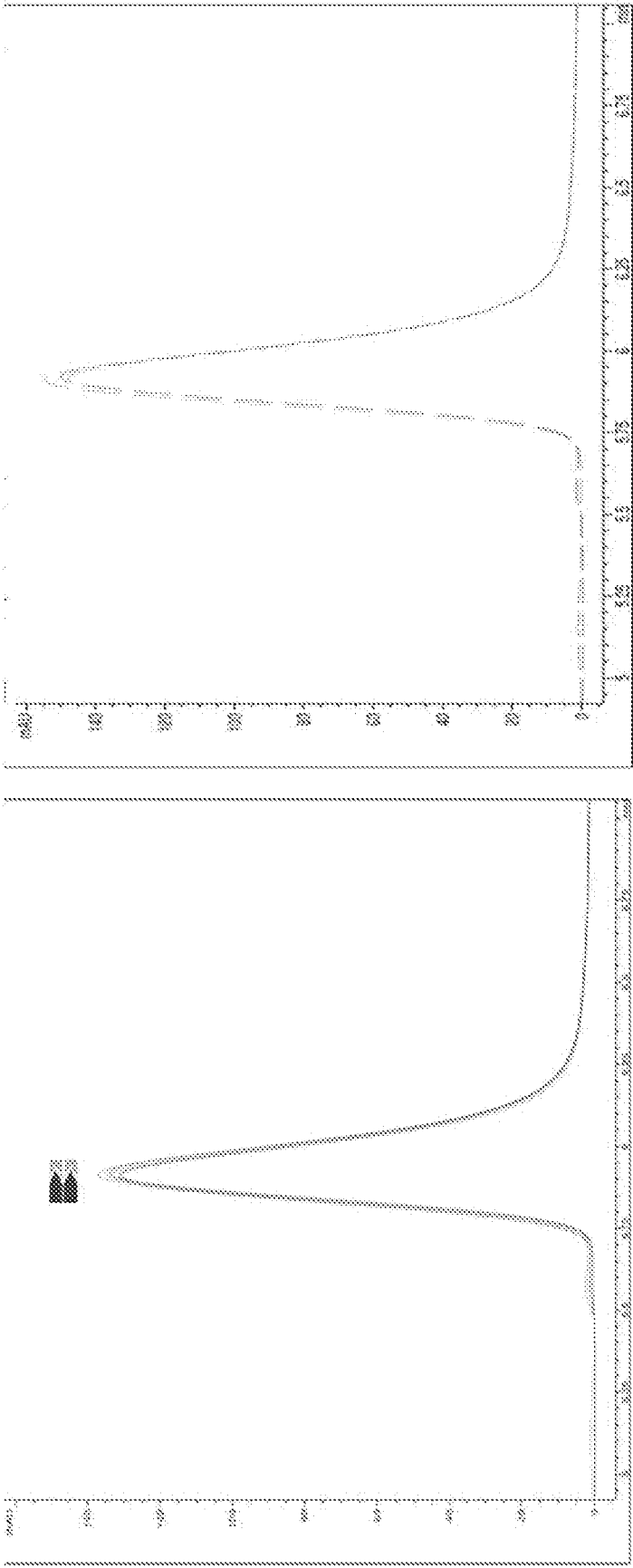
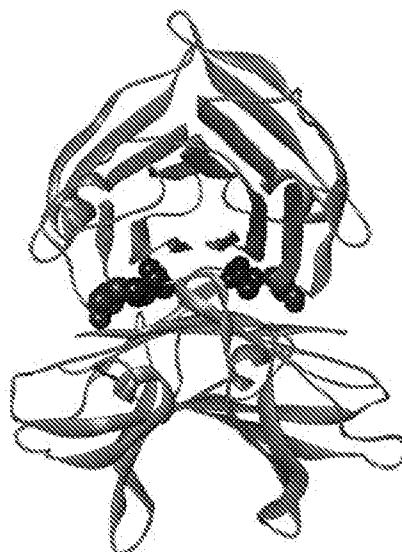


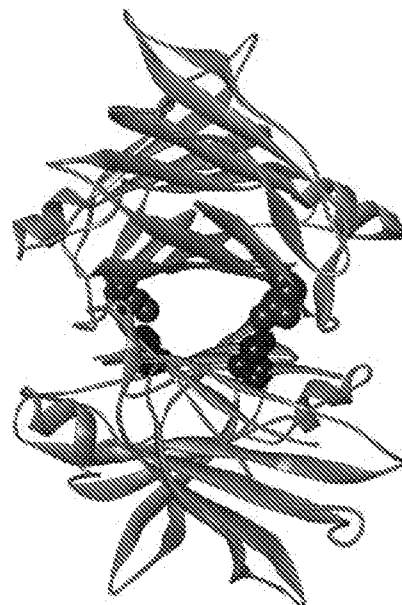
Figure 10

TTR heterotetramers successfully produced in *e. coli*

V20R/V20D
 $T_m \approx 93^\circ\text{C}$



L17R/T119D
 $T_m > 95^\circ\text{C}$



L17D/V121K
 $T_m \approx 92^\circ\text{C}$

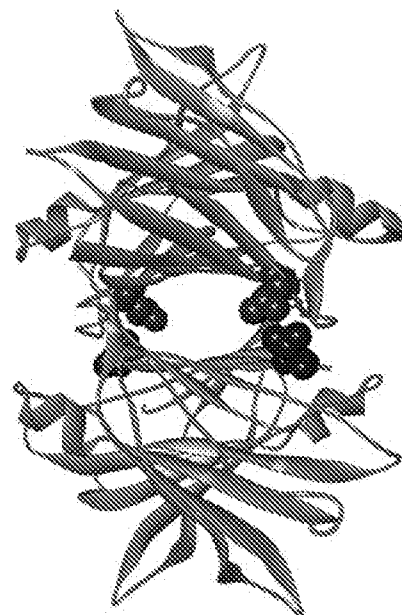


Figure 11

Ab-TTR Heterodimer Separate Expression

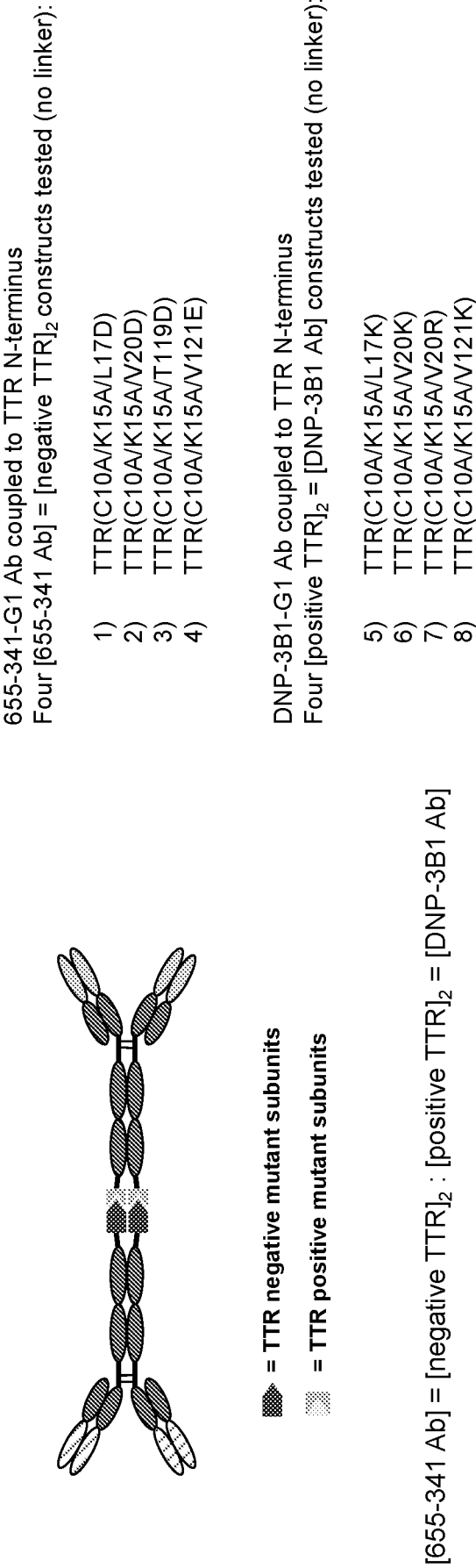


Figure 12

Ab-TTR Fusions Show Instability and Self-dimerization by SEC

Construct	Protein A > SP-HP		Protein A > G25 Buffer Exchange	
	Self-Associated (%)	Free Ab/TTR Fusion (%)	Self-Associated (%)	Free Ab/TTR Fusion (%)
DNP-3B1-L17K	38	32	68	7
DNP-3B1-V20K	39	19	62	20
DNP-3B1-V20R	47	12	75	21
DNP-3B1-V121K	32	33	64	10
655-341-L17D	56	23		
655-341-V20D	62	17		
655-341-T119D	69	11		
655-341-V121E	57	12		

Figure 13
Expanded linker set and separate culture for Ab-TTR heterodimers

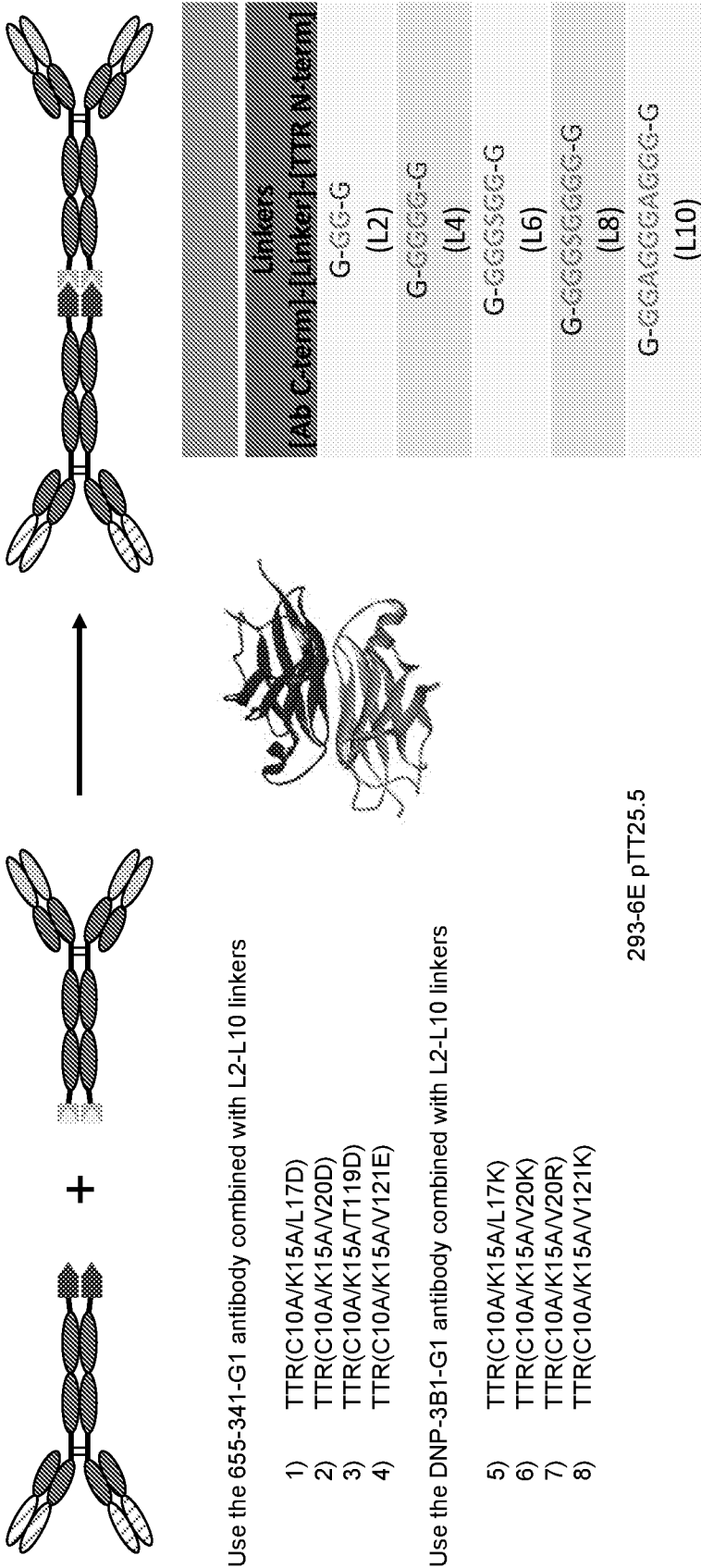


Figure 14

Ab-TTR heterodimers separate expression (no co-culture) Individual Molecules

IPS	Ab	Linker	Charge	Titer (mg/L)	Yield (mg/L)	NR-MCE % Self-Associated	NR-PAGE Free Ab/TTR Fusion (%)	SEC HMW (%)	SEC Self-Associated (%)	SEC Free Ab/TTR Fusion (%)
IPS-533076	3B1	G2	L17K	37.9	25.9	0.0	1	0.0	95.4	4.6
IPS-533077	3B1	G2	V20K	43.2	34.8	0.0	1	34.4	34.6	31.0
IPS-533078	3B1	G2	V20R	51.0	45.3	0.0	3	73.7	15.5	10.9
IPS-533079	3B1	G2	V121K	34.2	27.5	0.0	1	30.8	56.2	13.0
IPS-533080	655	G2	L17D	61.5	58.8	8.5	1	26.2	66.5	7.3
IPS-533082	655	G2	V20D	52.5	48.9	28.3	1	24.0	69.3	6.7
IPS-533083	655	G2	T119D	61.5	60.2	57.5	1	23.0	69.1	7.9
IPS-533084	655	G2	V121E	59.5	56.3	0.0	1	36.0	56.0	8.0
IPS-533085	3B1	G4	L17K	37.7	31.8	0.0	1	80.7	0.0	19.3
IPS-533086	3B1	G4	V20K	39.0	30.4	0.0	3	75.9	13.7	10.4
IPS-533087	3B1	G4	V20R	48.2	41.8	0.0	3	84.4	6.9	8.7
IPS-533088	3B1	G4	V121K	51.0	28.5	0.0	2	44.0	44.4	11.7
IPS-533089	655	G4	L17D	52.0	41.7	8.7	1	37.1	54.4	8.5
IPS-533090	655	G4	V20D	56.5	52.4	30.1	1	28.1	65.1	6.8
IPS-533091	655	G4	T119D	55.5	54.7	63.4	1	23.5	76.0	6.5
IPS-533092	655	G4	V121E	54.0	44.8	0.0	1	38.8	54.0	7.2
IPS-533093	3B1	G35-G2	L17K	34.2	27.2	0.0	1	25.6	69.6	4.2
IPS-533094	3B1	G35-G2	V20K	31.4	23.5	0.0	1	78.7	13.6	7.7
IPS-533095	3B1	G35-G2	V20R	32.8	26.1	0.0	1	63.6	7.7	8.6
IPS-533096	3B1	G35-G2	V121K	18.1	12.4	0.0	2	50.8	37.2	12.0
IPS-533097	655	G35-G2	L17D	48.4	41.9	7.4	1	46.2	46.8	7.0
IPS-533098	655	G35-G2	V20D	51.0	48.0	27.2	1	35.7	60.5	3.4
IPS-533099	655	G35-G2	T119D	55.5	50.9	64.1	1	23.9	78.3	1.8
IPS-533100	655	G35-G2	V121E	39.6	34.5	0.0	1	42.0	52.8	5.2
IPS-533101	3B1	G35-G4	L17K	24.8	18.6	0.0	1	30.3	64.1	5.6
IPS-533102	3B1	G35-G4	V20K	35.8	28.2	0.0	3	80.0	12.7	7.3
IPS-533103	3B1	G35-G4	V20R	46.9	39.6	0.0	3	84.8	6.6	8.6
IPS-533104	3B1	G35-G4	V121K	19.1	12.9	0.0	2	59.1	29.3	11.5
IPS-533105	655	G35-G4	L17D	28.9	21.4	5.0	2	56.4	35.3	8.3
IPS-533106	655	G35-G4	V20D	40.9	31.0	22.5	1	44.7	48.5	6.7
IPS-533107	655	G35-G4	T119D	45.5	40.8	69.6	1	26.9	68.5	4.6
IPS-533108	655	G35-G4	V121E	39.7	33.3	0.0	1	46.0	46.7	7.3
IPS-533109	3B1	G2AG3AG3	L17K	43.6	24.7	0.0	1	34.3	58.8	6.9
IPS-533110	3B1	G2AG3AG3	V20K	44.3	33.4	0.0	3	57.0	11.2	6.8
IPS-533111	3B1	G2AG3AG3	V20R	46.1	37.3	0.0	3	65.0	5.9	9.0
IPS-533112	3B1	G2AG3AG3	V121K	19.4	19.9	0.0	2	64.2	24.7	11.1
IPS-533113	655	G2AG3AG3	L17D	47.0	35.3	4.3	1	62.6	29.5	7.8
IPS-533114	655	G2AG3AG3	V20D	57.5	51.0	21.0	2	51.6	39.7	8.7
IPS-533115	655	G2AG3AG3	T119D	59.0	53.1	66.8	1	28.4	68.5	3.1
IPS-533116	655	G2AG3AG3	V121E	47.0	38.7	0.0	1	48.7	43.1	8.2

Figure 15
Ab-TTR heterodimers separate expression (no co-culture) Individual Molecules

IPS	Ab	Linker	Charge	Titer (mg/L)	Yield (mg/L)	NR-MCE % Self-Associated	NR-PAGE Free Ab/TTR Fusion (%)	SEC Self-Associated (%)	SEC HMW (%)	SEC Free Ab/TTR Fusion (%)
IPS:533080	655	G2	L17D	53.5	38.8	8.5	1	66.5	26.2	7.3
IPS:533089	655	G4	L17D	52.0	41.7	8.7	1	64.4	37.1	8.5
IPS:533097	655	G3S-G2	L17D	48.4	41.9	7.4	1	46.2	46.8	7.0
IPS:533105	655	G3S-G4	L17D	28.9	24.4	5.0	2	35.3	56.4	8.3
IPS:533113	655	G2AG3AG3	L17D	67.0	35.3	4.3	3	29.1	64.6	7.8
IPS:533076	3B1	G2	L17K	37.9	25.9	0.0	1	95.0	0.0	4.6
IPS:533085	3B1	G4	L17K	37.7	31.8	0.1	1	60.7	36.7	13.3
IPS:533093	3B1	G3S-G2	L17K	34.2	27.2	0.1	1	66.6	25.6	8.7
IPS:533101	3B1	G3S-G4	L17K	25.8	19.4	0.0	1	64.1	30.3	5.6
IPS:533109	3B1	G2AG3AG3	L17K	43.6	27.7	0.0	1	58.8	34.3	6.9
IPS:533083	655	G2	T119D	81.5	80.2	52.5	1	69.1	23.0	7.9
IPS:533091	655	G4	T119D	55.5	96.7	48.4	1	70.0	23.5	6.5
IPS:533099	655	G3S-G2	T119D	55.5	50.9	44.1	1	73.3	23.9	3.8
IPS:533107	655	G3S-G4	T119D	45.5	40.8	49.0	1	68.5	26.8	3.6
IPS:533115	655	G2AG3AG3	T119D	59.0	53.1	69.4	1	65.5	28.4	3.1
IPS:533084	655	G2	V121E	59.5	56.3	0.0	1	56.0	36.0	8.0
IPS:533092	655	G4	V121E	34.0	44.8	0.0	1	54.0	38.8	7.2
IPS:533100	655	G3S-G2	V121E	38.6	34.5	0.0	1	52.8	42.0	5.2
IPS:533108	655	G3S-G4	V121E	39.7	33.3	0.0	1	46.7	46.0	7.3
IPS:533116	655	G2AG3AG3	V121E	47.0	38.7	0.0	1	43.1	48.7	8.2
IPS:533079	3B1	G2	V121K	34.2	27.5	0.0	1	30.8	30.8	33.0
IPS:533088	3B1	G4	V121K	51.0	29.5	0.0	2	44.4	44.0	11.7
IPS:533096	3B1	G3S-G2	V121K	44.2	27.4	0.0	2	37.2	50.8	12.0
IPS:533104	3B1	G3S-G4	V121K	15.1	17.4	0.0	2	28.3	59.1	11.5
IPS:533112	3B1	G2AG3AG3	V121K	29.4	19.9	0.0	2	26.7	66.2	11.1
IPS:533082	655	G2	V20D	52.5	48.9	28.1	1	69.3	24.0	6.7
IPS:533090	655	G4	V20D	56.6	52.4	30.4	1	65.1	28.1	6.8
IPS:533098	655	G3S-G2	V20D	51.0	46.0	27.2	1	60.5	35.7	3.9
IPS:533106	655	G3S-G4	V20D	40.9	31.0	22.5	1	48.5	44.7	6.7
IPS:533114	655	G2AG3AG3	V20D	51.5	51.0	21.0	2	39.7	51.6	8.7
IPS:533077	3B1	G2	V20K	43.2	34.8	0.0	3	34.6	34.4	31.0
IPS:533086	3B1	G4	V20K	39.0	30.4	0.0	3	13.7	73.9	10.4
IPS:533094	3B1	G3S-G2	V20K	30.4	29.5	0.0	3	13.6	46.1	7.7
IPS:533102	3B1	G3S-G4	V20K	35.8	28.2	0.0	3	12.7	40.0	7.3
IPS:533110	3B1	G2AG3AG3	V20K	44.3	33.4	0.0	3	13.2	84.0	6.8
IPS:533078	3B1	G2	V20R	51.0	45.3	0.0	3	15.5	73.7	10.9
IPS:533087	3B1	G4	V20R	48.2	41.8	0.0	3	6.9	86.6	8.7
IPS:533095	3B1	G3S-G2	V20R	32.8	26.1	0.0	3	7.7	85.6	8.6
IPS:533103	3B1	G3S-G4	V20R	46.9	39.6	0.0	3	6.4	87.8	8.6
IPS:533111	3B1	G2AG3AG3	V20R	46.1	37.3	0.0	3	5.8	95.0	9.0

Figure 16

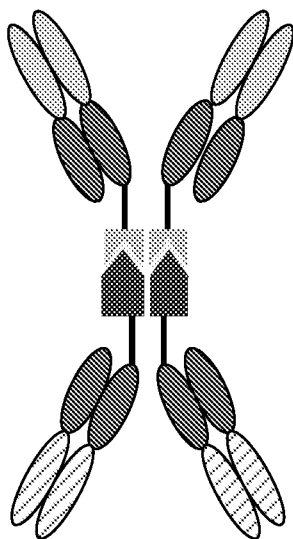
Ab-TTR heterodimers separate expression (no co-culture) Averages

Linker	Titer (mg/L)	Yield (mg/L)	NR-MCE % Self- Associated	NR-PAGE Free Ab/TTR Fusion (%)	SEC HMW (%)	SEC Self- Associated (%)	SEC Free Ab/TTR Fusion (%)
L2	50.2	44.7	11.8	1.5	31.0	57.8	11.2
L4	49.2	40.9	12.8	1.6	51.6	38.6	9.9
L6	38.9	32.8	12.8	1.6	48.3	45.2	6.5
L8	35.2	28.3	12.1	1.8	53.5	39.0	7.5
L10	46.7	36.7	11.8	2.0	57.1	35.2	7.7

Mutation	Titer (mg/L)	Yield (mg/L)	NR-MCE % Self- Associated	NR-PAGE Free Ab/TTR Fusion (%)	SEC HMW (%)	SEC Self- Associated (%)	SEC Free Ab/TTR Fusion (%)
L17D	47.6	39.8	6.8	1.6	45.7	46.5	7.8
V20D	51.7	45.9	25.8	1.2	36.8	56.6	6.5
T119D	55.4	51.9	65.5	1.0	25.1	69.9	5.0
V121E	48.0	41.5	0.0	1.0	42.3	50.5	7.2
L17K	35.6	25.8	0.0	1.0	34.2	57.6	8.2
V20K	38.7	30.1	0.0	3.0	70.2	17.2	12.6
V20R	45.0	38.0	0.0	3.0	82.3	8.5	9.2
V121K	30.4	20.4	0.0	1.8	49.8	38.4	11.9

Figure 17

Fab-TTR Heterotetramer co-expression



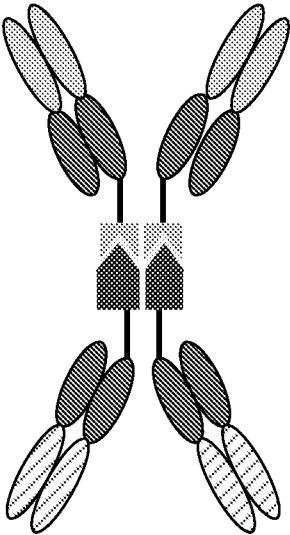
Co-production of [655-341 Fab] – [GG] – [negative TTR] and [positive TTR] – [GG] – [DNP-3B1 Fab] (each coupled to TTR N-terminus via GG linker):

- TTR(C10A/K15A/L17D) + TTR(C10A/K15A/V121R)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20K)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20R)
- TTR(C10A/K15A/T119D) + TTR(C10A/K15A/L17K)
- TTR(C10A/K15A/V121E) + TTR(C10A/K15A/L17K)

Figure 18

Fab-TTR Heterotetramer co-expression

BioReg	Linker	3B1 Mut	655 Mut	Yield (mg/L)	SEC HMW (%)	SEC Desired +/- Fab-TTR tetramer (%)	SEC LMW (%)	SEC-MS	SDS PAGE
15527-1	G2	L17K	T119D	234.0	3.3	83.6	13.12	4.0	2.0
15528-1	G2	L17K	V121E	206.3	2.3	82.3	15.34	4.0	3.0
15524-1	G2	V121R	L17D	222.3	0.1	87.7	12.16	4.0	3.0
15525-1	G2	V20K	V20D	196.9	3.4	83.1	13.43	4.0	3.0
15526-1	G2	V20R	V20D	190.8	4.6	78.1	17.33	4.0	1.0

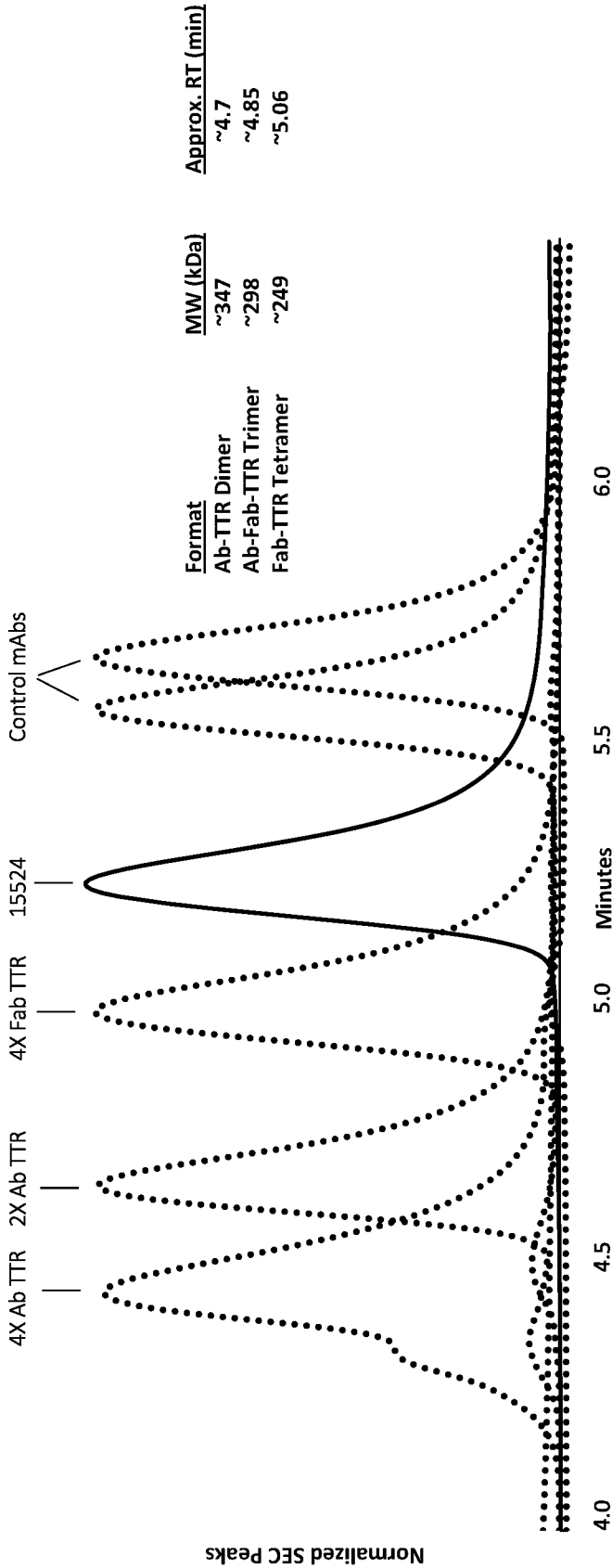


Desired +/- Fab-TTR tetramer:

SEC-MS Key	
4	Desired Product >> Other
3	Desired Product >= Other
2	Desired Product < Other
1	Desired Product << Other

Figure 19

Fab-TTR heterotetramer 15524 co-expression SEC with standards



4X Ab TTR = control [655-341 Ab]₄ – [TTR (C10A/K15A)]
2X Ab TTR = control [655-341 Ab]₂ – [TTR (C10A/K15A)]
4X Fab TTR = control [655-341 Fab]₄ – [TTR (C10A/K15A)]
15524 = [[655-341 Fab] – [GG] – [negative TTR]]₂ : [[positive TTR] – [GG] – [DNP-3B1 Fab]]₂

Figure 20

Fab-TTR heterotetramers co-expression SEC-MS and SDS-PAGE

15524

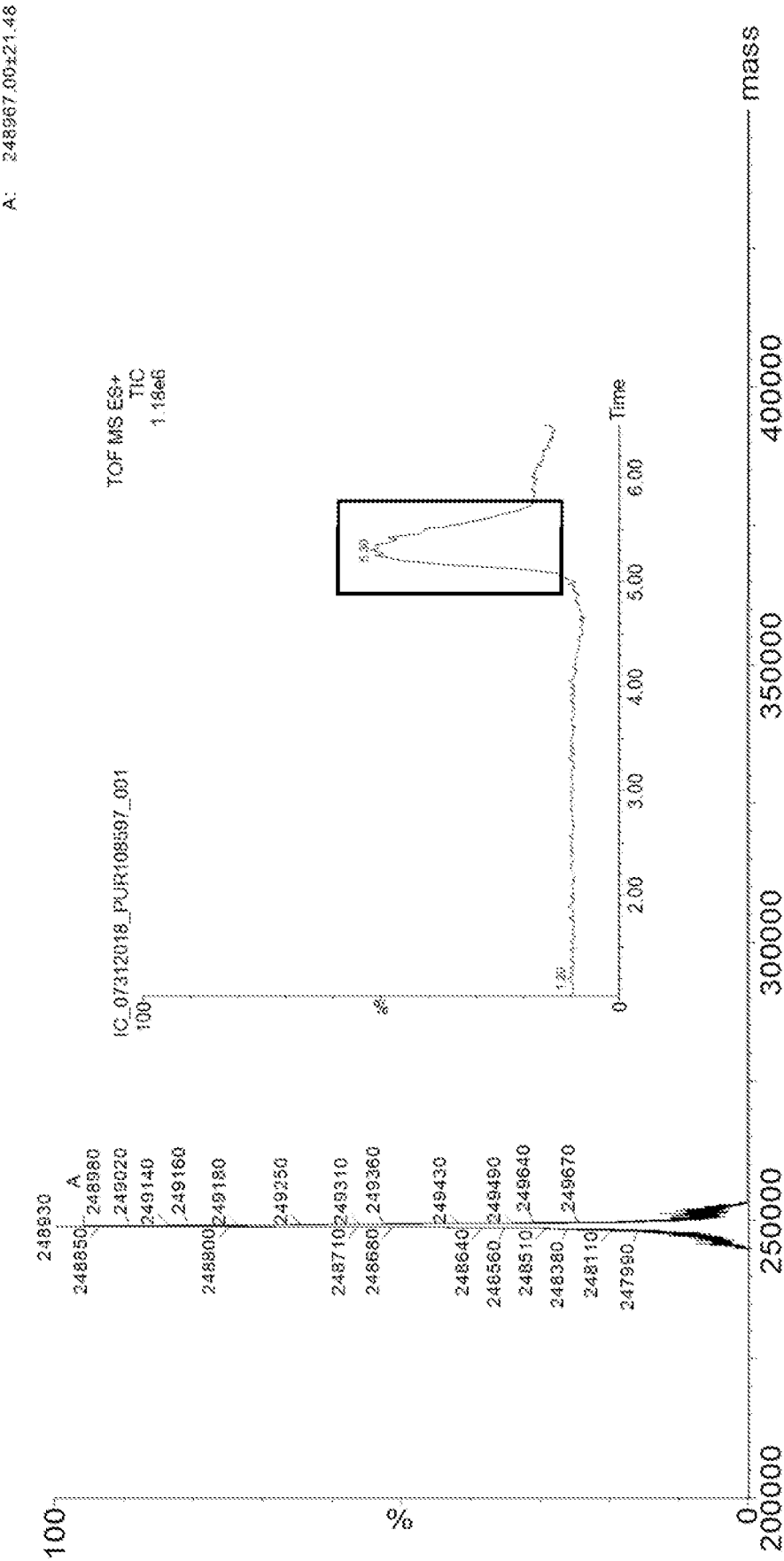
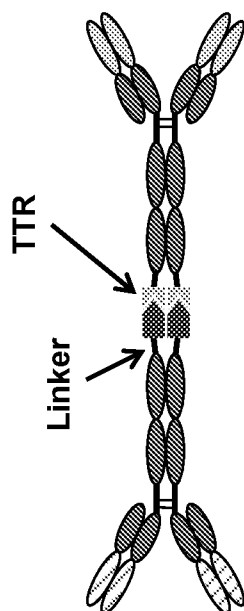


Figure 21

Ab-TTR Heterodimer co-expression



Co-expression of [655-341 Ab] = $[[\text{LX}] - [\text{negative TTR}]]_2$ and $[[\text{positive TTR}] - [\text{LX}]]_2 = [\text{DNP-3B1 Ab}]$ (each coupled to TTR N-terminus via a linker):

- TTR(C10A/K15A/L17D) + TTR(C10A/K15A/V121K); X = 0, 4, and 10
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20K); X = 0, 4, and 10
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20R); X = 0, 4, and 10
- TTR(C10A/K15A/T119D) + TTR(C10A/K15A/L17K); X = 0, 4, and 10
- TTR(C10A/K15A/V121E) + TTR(C10A/K15A/L17K); X = 0, 4, and 10

No linker (X=0)

GGGG (X=4)

GGAGGGAGGG (X=10)

Figure 22

Ab-TTR Heterodimer co-expression (Sorted by Linker)

BioReg	Linker	3B1 Mut	655 Mut	Titer (mg/L)	Yield (mg/L)	SEC HMW (%)	SEC Desired TTR Heterotetramer (X)	SEC Unassociated TTR Dimer (%)	SEC-MS
15534-1	L0	V121K	L17D	90.4	80.7	35.3	59.1	5.6	2.0
15535-1	L0	V20K	V20D	141.7	120.8	27.8	61.7	10.5	4.0
15536-1	L0	V20R	V20D	122.0	112.0	37.1	57.1	5.8	2.0
15537-1	L0	L17K	T119D	156.4	122.3	42.9	49.9	7.2	2.0
15538-1	L0	L17K	V121E	98.2	89.6	20.9	70.2	8.9	2.0
15529-1	L4	V121K	L17D	147.6	134.7	49.4	46.0	4.6	2.0
15530-1	L4	V20K	V20D	236.8	174.5	38.6	54.0	7.4	2.0
15531-1	L4	V20R	V20D	259.0	182.7	44.0	51.3	4.8	3.0
15532-1	L4	L17K	T119D	218.1	161.6	49.1	45.8	5.1	2.0
15533-1	L4	L17K	V121E	198.9	140.3	25.8	68.4	5.8	3.0
15539-1	L10	V121K	L17D	143.2	119.7	26.4	66.1	7.5	4.0
No Exp.	L10	V20K	V20D						
15540-1	L10	V20R	V20D	213.2	175.1	60.5	33.9	5.6	2.0
15541-1	L10	L17K	T119D	214.8	162.1	56.9	38.7	4.3	3.0
15542-1	L10	L17K	V121E	179.7	134.1	35.7	58.2	6.1	3.0

SEC-MS Key	
4	Desired Product >> Other
3	Desired Product >= Other
2	Desired Product < Other
1	Desired Product << Other

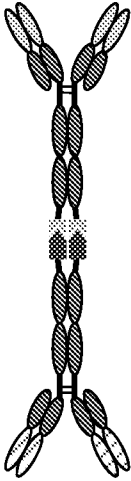
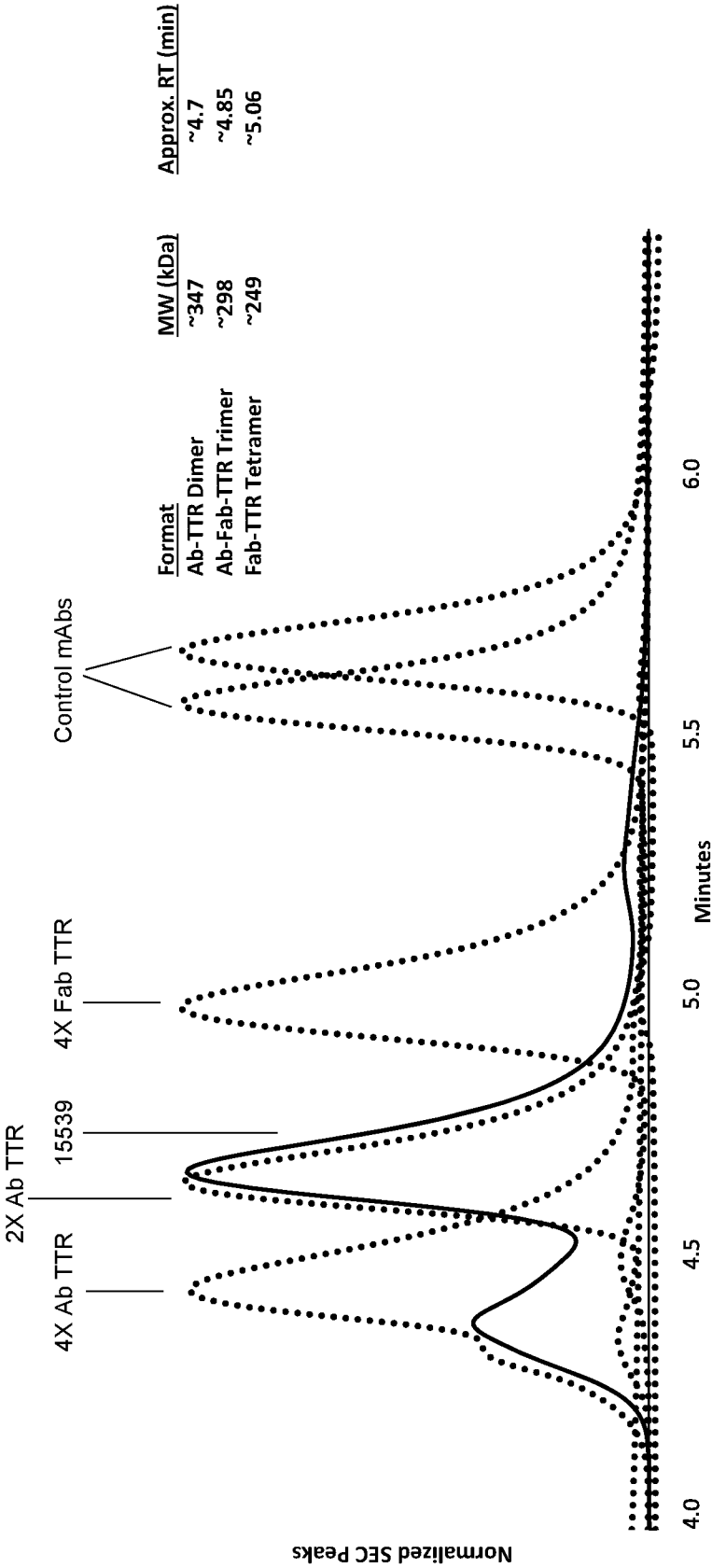


Figure 23

Ab-TTR heterodimer 15539 co-expression SEC with standards



4X Ab TTR = control [655-341 Ab]₄ – [TTR (C10A/K15A)]

2X Ab TTR = control [655-341 Ab]₂ – [TTR (C10A/K15A)]

4X Fab TTR = control [655-341 Fab]₄ – [TTR (C10A/K15A)]

15539 = [655-341 Ab] = [[GGAGGGAGGG] – [TTR(C10A/K15A/L17D)]₂ : [[TTR(C10A/K15A/V121K)] – [GGAGGGAGGG]]₂ = [DNP-3B1 Ab]

Figure 24

Ab-TTR Heterodimer co-expression 15539-1 (L10/A) Native MS, SEC
and NR SDS-PAGE

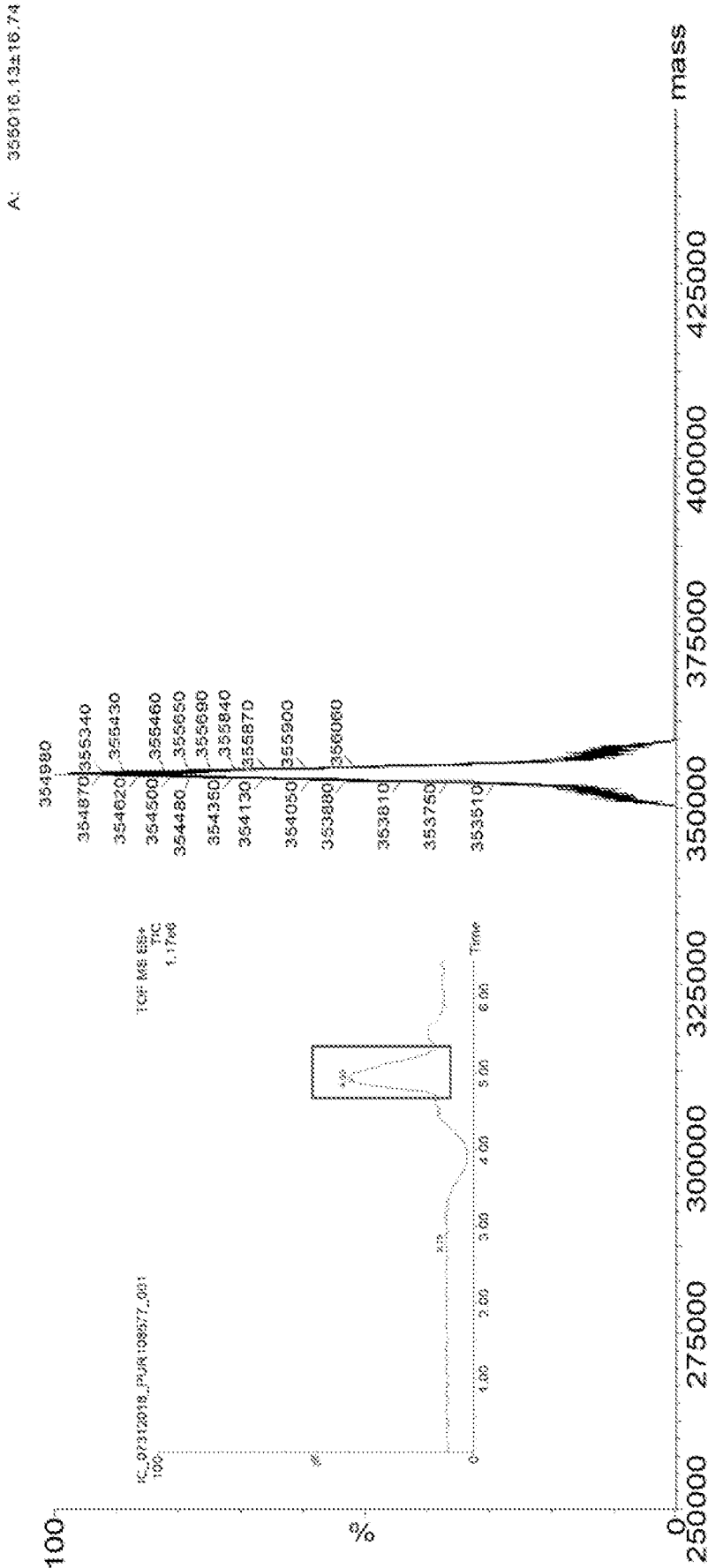


Figure 25

Ab-TTR Heterodimer co-expression Averages

Linker	Titer (mg/L)	Yield (mg/L)	SEC %HMW	SEC %2X-Ab	SEC %1X-Ab	SEC-MS
L0	121.7	105.1	32.8	59.6	7.6	2.4
L4	212.1	158.8	41.4	53.1	5.5	2.4
L10	187.7	147.8	44.9	49.2	5.9	3.0

Mutation	Titer (mg/L)	Yield (mg/L)	SEC %HMW	SEC %2X-Ab	SEC %1X-Ab	SEC-MS
L17K/T119D	196.4	148.7	49.7	44.8	5.5	2.3
L17K/V121E	158.9	121.3	27.5	65.6	6.9	2.7
V121K/L17D	127.1	111.7	37.0	57.1	5.9	2.7
V20K/V20D	189.3	147.7	33.2	57.9	8.9	3.0
V20R/V20D	198.1	156.6	47.2	47.4	5.4	2.3

SEC-MS Key	
4	Desired Product >> Other
3	Desired Product >= Other
2	Desired Product < Other
1	Desired Product << Other

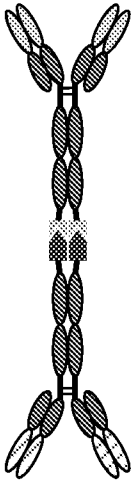
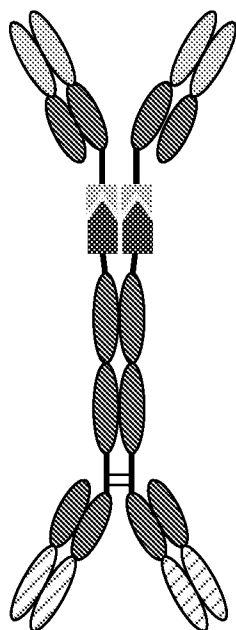


Figure 26
Ab-Fab-TTR Heterotrimer co-expression (REQ100397)



Co-expression of [655-341 Ab]=[LX] - [negative TTR]₂ and [[positive TTR] - [GG] - [DNP-3B1 Fab]]₂:

LX linkers:

No linker (X=0)

GGGG (X=4)

GGAGGGAGGG (X=10)

- TTR(C10A/K15A/L17D) + TTR(C10A/K15A/V121K)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20K)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20R)
- TTR(C10A/K15A/T119D) + TTR(C10A/K15A/L17K)
- TTR(C10A/K15A/V121E) + TTR(C10A/K15A/L17K)
- TTR(C10A/K15A/L17D) + TTR(C10A/K15A/V121K)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V121R)
- TTR(C10A/K15A/V20D) + TTR(C10A/K15A/V20R)
- TTR(C10A/K15A/T119D) + TTR(C10A/K15A/L17K)
- TTR(C10A/K15A/V121E) + TTR(C10A/K15A/L17K)

Figure 27

Ab-Fab-TTR Heterotrimer co-expression (Sorted by Linker)

BioReg	Ab Linker	Fab Linker	TTR Mutations w/DNP-3B1-Ab	TTR Mutations w/655-Ab	TTR Mutations w/DNP-3B1-Fab	TTR Mutations w/655-Fab	Titer (mg/L)	Yield (mg/L)	SEC HMW (%)	SEC Desired Ab-Fab (%)	SEC LMW (%)	SEC-MS
15548-1	L0	G2		L17D	V121R		84.4	87.3	72.4	23.3	4.2	1.9
15549-1	L0	G2		V20D	V20K		76.9	84.2	79.1	17.0	3.9	2.0
15550-1	L0	G2		V20D	V20R		87.2	91.4	56.9	39.9	3.2	2.0
15551-1	L0	G2		T119D	L17K		121.4	127.5	52.1	44.1	3.8	2.0
15552-1	L0	G2		V121E	L17K		79.5	73.0	77.5	16.2	5.4	2.0
15563-1	L0	G2	V121K			L17D	88.6	62.6	65.4	31.7	2.9	2.0
15564-1	L0	G2	V20K			V20D	127.8	113.4	74.5	20.0	5.5	2.0
15565-1	L0	G2	V20R			V20D	107.0	103.4	67.9	29.9	2.3	4.0
15566-1	L0	G2	L17K			T119D	118.5	103.1	62.4	34.2	3.4	2.0
No Exp.	L0	G2	L17K			V121E						
15543-1	L4	G2		L17D	V121R		139.5	129.3	94.5	0.0	5.5	2.0
15544-1	L4	G2		V20D	V20K		153.1	130.0	84.4	12.8	2.8	2.0
15545-1	L4	G2		V20D	V20R		149.9	129.2	66.6	29.5	3.9	2.0
15546-1	L4	G2		T119D	L17K		163.6	138.8	55.5	41.0	3.5	2.0
15547-1	L4	G2		V121E	L17K		124.8	116.3	75.5	20.5	4.0	2.0
15558-1	L4	G2	V121K			L17D	159.7	133.2	72.8	23.8	3.3	2.0
15559-1	L4	G2	V20K			V20D	210.5	158.6	81.8	15.6	2.6	2.0
15560-1	L4	G2	V20R			V20D	216.8	164.2	75.0	22.4	2.6	4.0
15561-1	L4	G2	L17K			T119D	169.4	158.1	71.9	25.6	2.5	2.0
15562-1	L4	G2	L17K			V121E	187.2	145.1	74.5	23.8	1.8	2.0
15553-1	L10	G2		L17D	V121R		173.0	140.1	66.0	28.1	5.9	2.0
15554-1	L10	G2		V20D	V20K		195.9	156.7	76.7	20.9	2.4	2.0
15555-1	L10	G2		V20D	V20R		189.2	143.4	72.8	25.0	2.2	2.0
15556-1	L10	G2		T119D	L17K		179.4	151.6	59.6	38.9	2.4	2.0
15557-1	L10	G2		V121E	L17K		158.2	129.0	55.6	40.3	4.1	2.0
15567-1	L10	G2	V121K			L17D	188.4	145.4	75.6	22.8	1.8	2.0
15568-1	L10	G2	V20K			V20D	213.9	159.7	77.2	19.8	2.9	2.0
15569-1	L10	G2	V20R			V20D	179.4	158.6	71.5	26.3	2.2	4.0
15570-1	L10	G2	L17K			T119D	206.3	155.5	72.1	25.3	2.6	2.0
15571-1	L10	G2	L17K			V121E	181.0	147.7	51.9	45.8	2.6	2.0

SEC-MS Key	
4	Desired Product >> Other
3	Desired Product >= Other
2	Desired Product < Other
1	Desired Product << Other

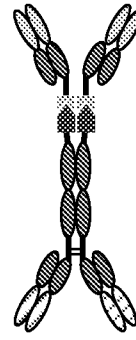
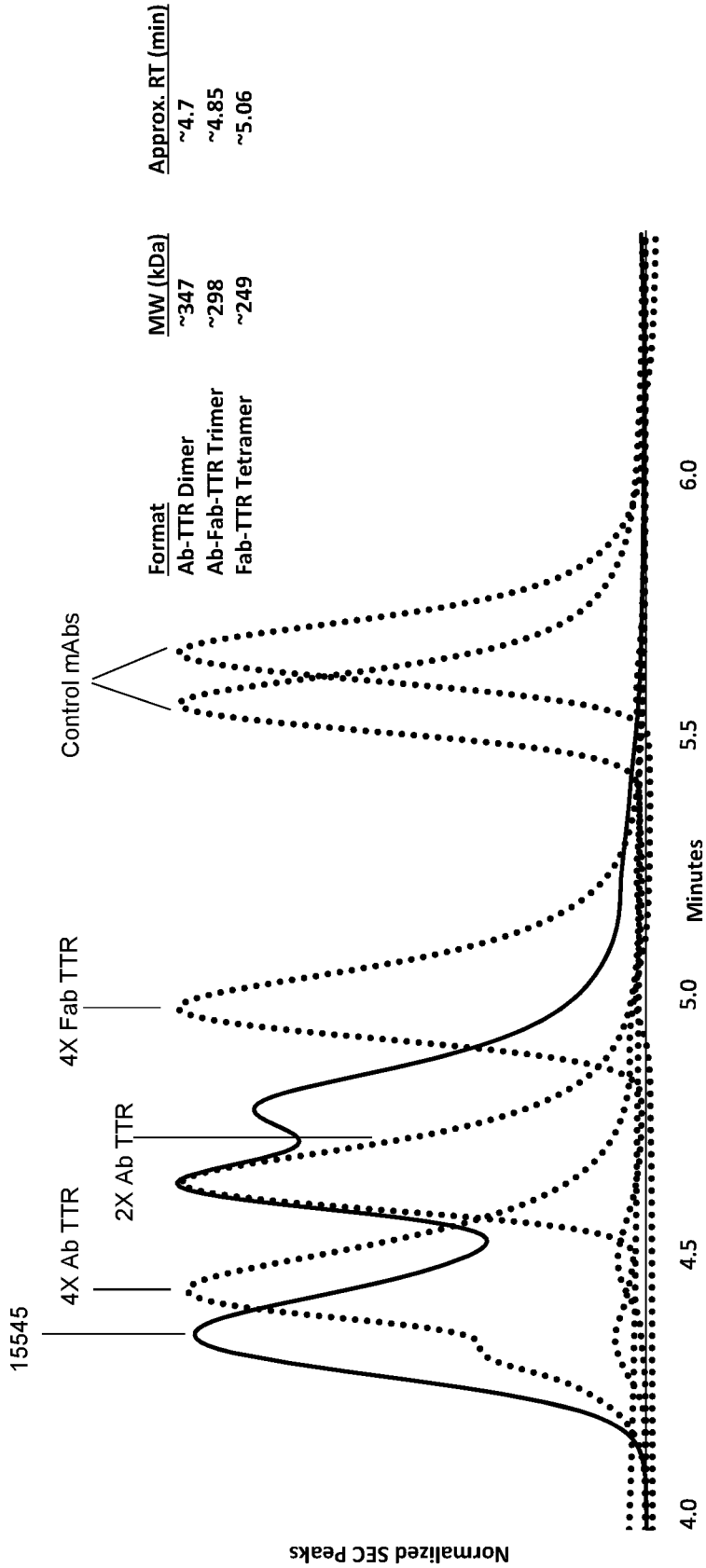


Figure 28

Ab-Fab-TTR heterotrimer 15545 co-expression SEC with standards



4X Ab TTR = control [655-341 Ab]₄ - [TTR (C10A/K15A)]

2X Ab TTR = control [655-341 Ab]₂ - [TTR (C10A/K15A)]

4X Fab TTR = control [655-341 Fab]₄ - [TTR (C10A/K15A)]

15545 = [655-341 Ab] = [[GGGG] - [TTR(C10A/K15A/V20D)]]₂ : [[TTR(C10A/K15A/V20R)] - [GG] - [DNP-3B1-Fab]]₂

Figure 29

Ab-Fab-TTR Heterotrimer 15545-1 (L4C6-) Native MS, SEC and NR

SDS-PAGE

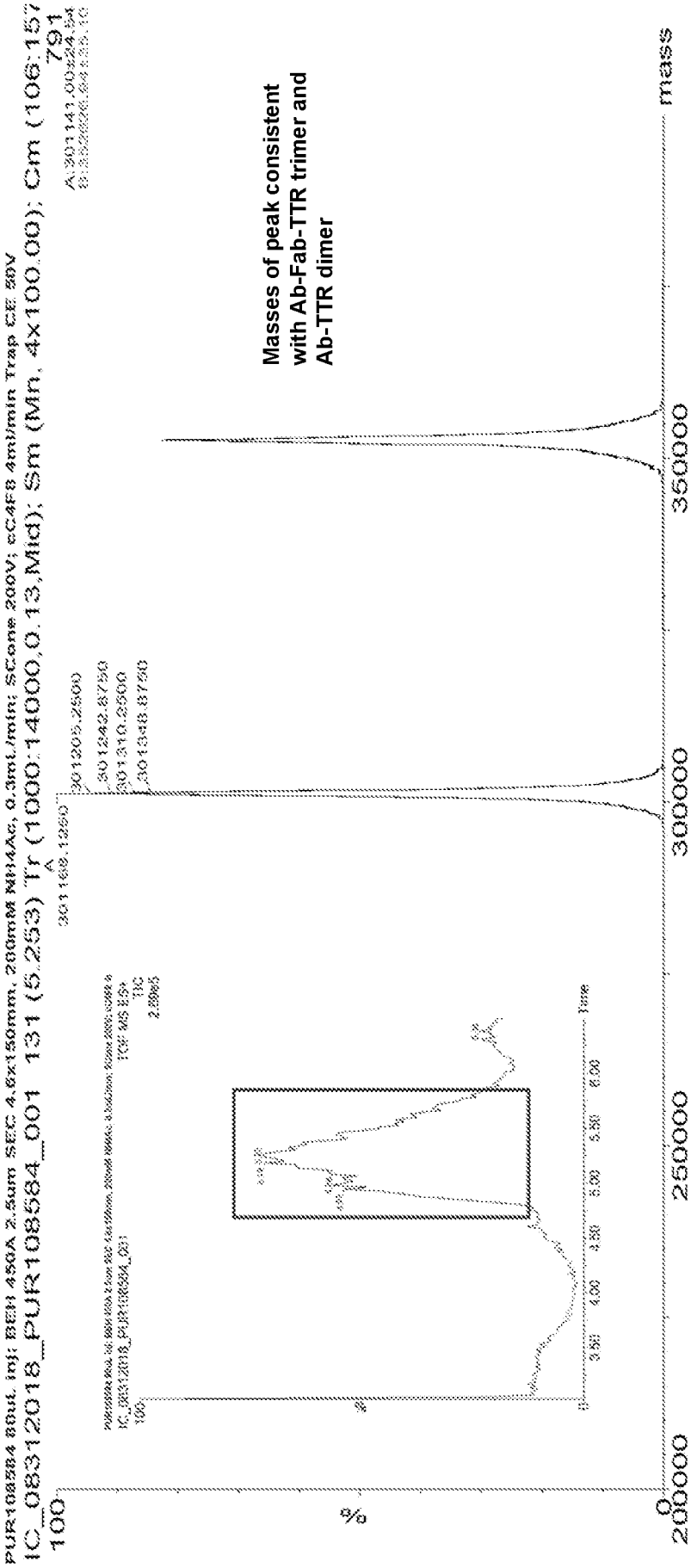


Figure 30

Ab-Fab-TTR Heterotrimer co-expression Averages

	Titer (mg/L)	Yield (mg/L)	SEC %HMW	SEC %Ab-Fab	SEC %LMW	SEC-MS
L0	95.7	94.0	67.6	28.5	3.9	2.1
L4	167.5	140.3	75.3	21.5	3.2	2.2
L10	186.5	149.7	67.8	29.3	2.9	2.2

L17K/T119D	159.8	139.1	62.1	34.9	3.0	2.0
L17K/V121E	146.1	122.2	67.0	29.3	3.7	2.0
V121K/L17D	135.6	113.7	71.3	26.1	2.6	2.0
V121R/L17D	132.3	118.9	77.6	17.1	5.2	1.7
V20K/V20D	163.0	133.6	79.0	17.7	3.4	2.0
V20R/V20D	154.9	133.4	68.4	28.8	2.7	3.0

Ab 655	138.4	121.8	69.6	26.5	3.9	1.9
Ab 3B1	166.0	137.0	71.0	26.2	2.8	2.4

SEC-MS Key	
4	Desired Product >> Other
3	Desired Product >= Other
2	Desired Product < Other
1	Desired Product << Other

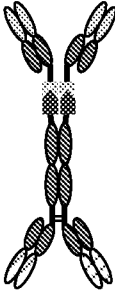


Figure 31
Double Interface Mutants Fab-TTR Heterotetramers Separate
Expression no mixing (REQ-54634A)

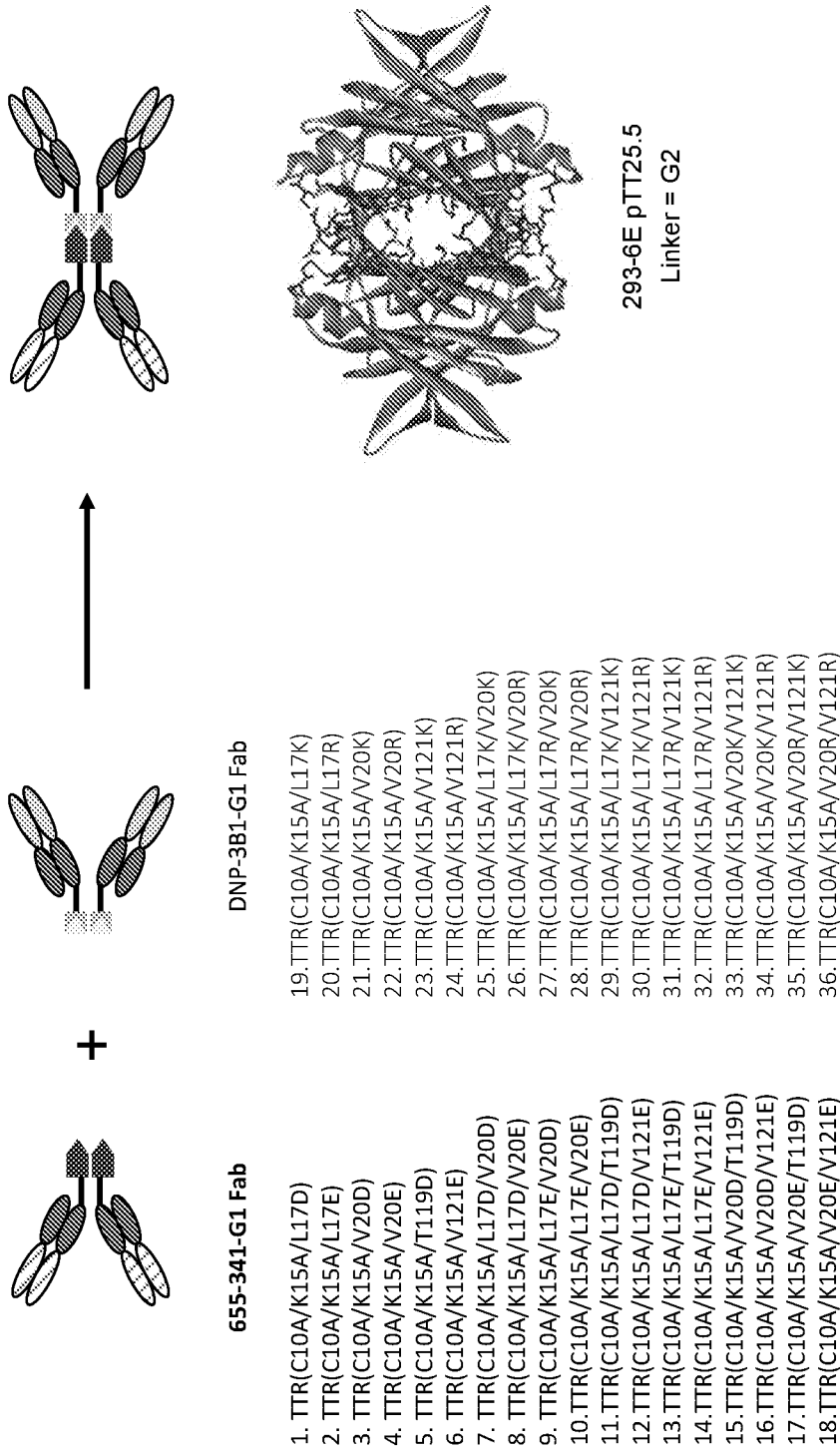
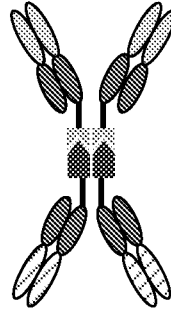


Figure 32

Fab-TTR Heterotetramer single & Double interface mutants
separate expression (SEC T=0 and ~12 months)



- Double mutations effective in preventing the formation of Fab-TTR homotetramers, particularly negative variants
- Long incubation at 4°C has only modest impact on SEC distribution indicating the molecules are stable

IPs	Target	Variant	Titer (mg/L)	Yield (mg/L)	SEC %HMW	SEC %4X-Fab	SEC %2X-Fab	NR-MCE %2X-Fab	SEC Diff HMW	SEC Diff 4X-Fab	SEC Diff 2X-Fab
IPS-533040	655	L17D	84	4.5	5.8	36.3	38.6	100.0	9.0	-23.6	-23.3
IPS-533041	655	L17E	31.6	3.99	3.3	30.7	59.0	38.7	22.2	-28.3	-5.2
IPS-533042	655	V20D	18.1	32.3	7.2	33.7	26.1	69.2	12.8	-23.1	4.7
IPS-533043	655	V20E	18.3	25.3	3.9	32.5	45.6	90.2	2.2	-13.8	6.9
IPS-533044	655	T119D	23.5	30.7	3.8	31.7	60.9	73.6	14.1	-15.3	-0.2
IPS-533045	655	V121E	16.5	28.7	3.8	38.8	59.6	100.0	0.8	-16.0	6.8
IPS-533046	655	L17D/V20D	60.5	4.3	62.5	36.0	37.5	98.0	11.9	0.0	-11.9
IPS-533047	655	L17D/V20E	66.5	3.6	40.0	30.0	58.7	96.3	15.7	0.0	-15.7
IPS-533050	655	L17D/T119D	73.1	4.8	53.8	30.0	42.3	98.3	9.5	0.0	-9.5
IPS-533051	655	L17D/V121E	72.2	6.3	21.2	10.0	76.4	100.0	5.7	0.0	-5.7
IPS-533049	655	L17E/V20E	33.6	4.7	37.8	30.0	60.8	90.0	7.2	0.0	-7.2
IPS-533052	655	L17E/T119D	72.8	4.8	50.7	30.0	42.7	100.0	13.8	0.0	-13.8
IPS-533053	655	L17E/V121E	74.6	6.5	14.4	30.0	63.3	100.0	6.0	0.0	-3.7
IPS-533054	655	V20D/T119D	82.8	4.9	13.8	12.5	68.8	98.8	11.0	-7.3	-7.7
IPS-533055	655	V20D/V121E	82.6	6.5	14.1	7.8	78.0	97.5	11.7	-5.0	-10.8
IPS-533056	655	V20E/T119D	79.4	8.0	11.0	5.8	79.2	100.0	6.0	0.0	-3.7
IPS-533057	655	V20E/V121E	13.3	13.7	10.2	38.8	82.7	97.3	5.2	0.0	-5.2
IPS-533058	381	L17K	113.6	3.9	7.8	40.1	58.3	90.0	3.7	-15.3	-10.2
IPS-533059	381	L17R	107.5	3.9	0.4	6.3	99.3	95.2	0.4	2.8	-2.6
IPS-533060	381	V20K	14.2	8.5	29.0	35.0	61.9	99.5	10.3	-1.4	-11.3
IPS-533061	381	V20R	56.7	3.9	20.0	35.0	60.4	94.0	28.7	-5.0	-27.3
IPS-533062	381	V121K	24.7	3.3	1.3	26.3	22.3	95.8	1.7	-16.0	-14.6
IPS-533063	381	V121R	19.7	12.4	1.5	39.1	59.4	100.0	0.0	-16.3	-16.2
IPS-533064	381	L17K/V20K	53.9	3.9	39.0	30.0	50.7	94.5	6.2	0.0	-6.0
IPS-533065	381	L17K/V20R	17.2	9.0	13.7	30.0	79.1	94.5	1.0	0.0	-0.3
IPS-533068	381	L17K/V121K	19.9	10.0	17.8	30.0	77.3	94.2	2.3	0.0	-6.1
IPS-533069	381	L17K/V121R	17	8.0	28.4	30.0	63.3	94.5	3.1	0.0	-3.0
IPS-533066	381	L17R/V20K	12.7	3.3	20.0	30.0	74.8	93.2	0.8	0.0	-3.7
IPS-533067	381	L17R/V20R	16.6	10.4	5.6	30.0	85.6	95.8	0.4	0.0	-4.1
IPS-533070	381	L17R/V121K	24.1	12.2	11.3	30.0	85.6	95.8	0.8	0.0	-6.4
IPS-533071	381	L17R/V121R	19	7.7	13.3	30.0	81.2	96.0	40.7	0.0	-2.0
IPS-533075	381	V20R/V121R	19.5	3.8	24.7	30.0	68.8	90.8	40.3	0.0	-5.7

Figure 33

Fab-TTR Combo Mutants Substantially Reduce Amount of 4X-Fab-TTR and Increase Amount of 2x-Fab-TTR

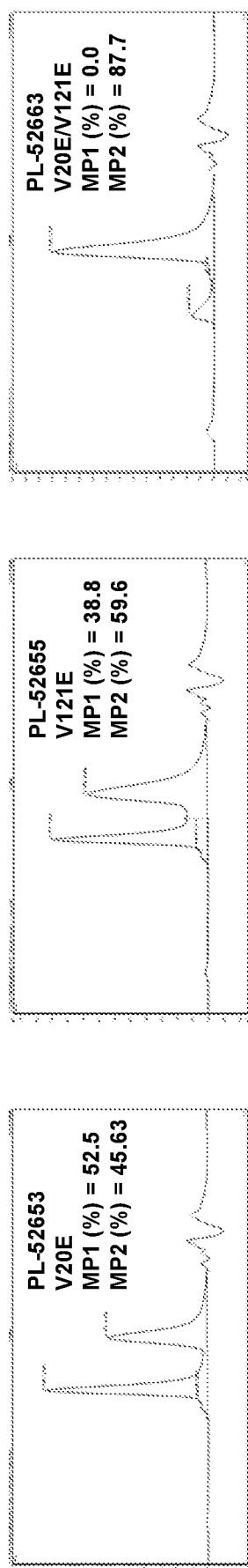


Figure 34

Double Interface Mutants Fab-TTR Heterotetramers Separate
Expression w/Post Purification Mix (REQ-54634B)

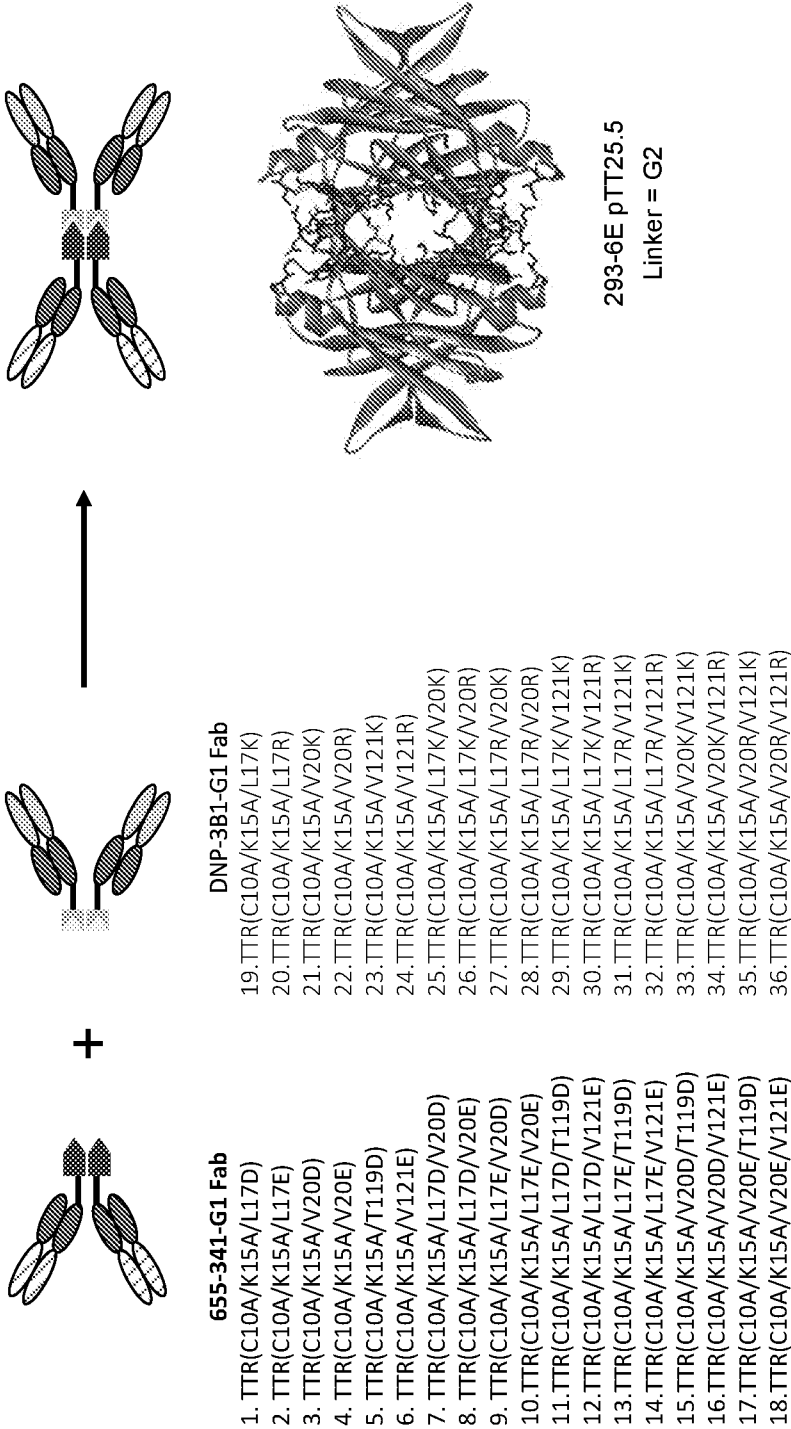


Figure 35
Fab-TTR Heterotetramer Single & Double Interface Mutants Post-Purification Mixture

3B1 Pos Mut	655 Neg Mut	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab	Increase %4X-Fab
L17K/V121R	L17D/V20E	46.2	47.8	6.0	0.0
L17K/V20K	L17D/V20E	50.6	39.4	6.0	0.0
L17K/V20R	L17D/V20E	53.5	34.3	6.0	0.0
L17R/V121K	L17D/V20E	35.4	60.2	6.0	0.0
L17R/V121R	L17D/V20E	37.1	58.3	6.0	0.0
L17R/V20K	L17D/V20E	40.6	59.0	6.0	0.0
L17R/V20R	L17D/V20E	37.1	59.9	6.0	0.0
V121K	L17D/V20E	28.8	32.8	33.4	-4.3
V121R	L17D/V20E	40.4	39.7	6.0	-19.6
V20K	L17D/V20E	43.6	34.4	1.9	-0.6
V20R	L17D/V20E	47.7	40.3	2.8	0.3
V20R/V121R	L17D/V20E	40.2	51.4	3.2	3.2
L17K/V121K	L17E	35.4	48.1	11.0	-8.4
L17K/V121R	L17E	31.3	50.5	11.4	-8.0
L17R/V121K	L17E	23.4	67.4	4.6	-34.8
L17R/V121R	L17E	23.4	45.9	23.4	4.1
V121K	L17E	18.8	49.8	61.5	4.5
V121R	L17E	17.7	11.3	68.1	20.7
V20R/V121R	L17E	28.0	59.7	6.5	-32.9
L17K	L17E/T119D	24.4	39.1	37.5	12.5
L17K/V121K	L17E/T119D	53.1	27.9	15.6	15.6
L17K/V121R	L17E/T119D	55.1	31.2	9.1	9.1
L17K/V20K	L17E/T119D	61.3	28.6	6.0	0.0
L17K/V20R	L17E/T119D	64.6	30.7	6.0	0.0
L17R	L17E/T119D	25.1	50.9	24.0	20.9
L17R/V121K	L17E/T119D	51.9	28.2	15.8	15.8
L17R/V121R	L17E/T119D	51.6	24.2	19.8	19.8
L17R/V20K	L17E/T119D	55.5	36.9	2.4	2.4
L17R/V20R	L17E/T119D	56.4	39.6	6.0	0.0
V121K	L17E/T119D	42.0	37.9	6.5	-31.1
V121R	L17E/T119D	45.2	30.9	6.0	-19.6
V20R/V121R	L17E/T119D	58.2	37.0	6.0	0.0
L17K	L17E/V121E	5.5	71.1	19.2	-0.9
L17K/V121K	L17E/V121E	20.0	73.4	6.0	0.0
L17K/V121R	L17E/V121E	24.9	58.1	8.1	8.1
L17K/V20K	L17E/V121E	39.9	60.1	6.0	0.0
L17K/V20R	L17E/V121E	17.0	80.6	6.0	0.0
L17R	L17E/V121E	4.6	82.3	7.7	4.6
L17R/V121K	L17E/V121E	16.6	76.9	6.0	0.0
L17R/V121R	L17E/V121E	15.0	71.4	4.7	4.7
L17R/V20K	L17E/V121E	19.5	70.9	6.0	0.0
L17R/V20R	L17E/V121E	15.9	80.2	6.0	0.0
V121K	L17E/V121E	8.2	60.8	25.6	-12.0
V121R	L17E/V121E	22.4	73.8	6.0	-19.6
V20R/V121R	L17E/V121E	21.9	69.5	6.0	0.0
L17K/V121K	L17E/V20E	40.6	54.0	6.0	0.0
L17K/V121R	L17E/V20E	43.0	53.3	6.0	0.0
L17K/V20K	L17E/V20E	43.7	54.5	6.0	0.0

3B1 Pos Mut	655 Neg Mut	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab	Increase %4X-Fab
L17K/V121K	L17D	15.4	29.3	54.5	16.3
L17K/V121R	L17D	16.6	34.7	44.4	6.3
L17R/V121K	L17D	14.6	28.1	54.4	16.3
L17R/V121R	L17D	14.5	29.4	51.5	15.4
V121K	L17D	16.7	35.4	67.9	-7.9
V121R	L17D	13.2	22.2	62.8	5.1
V20R/V121R	L17D	15.7	34.2	46.6	8.4
L17K	L17D/T119D	19.4	41.1	35.6	15.5
L17K/V121K	L17D/T119D	47.3	26.6	20.5	20.5
L17K/V121R	L17D/T119D	52.1	34.3	9.1	9.1
L17K/V20K	L17D/T119D	66.2	30.8	6.0	0.0
L17K/V20R	L17D/T119D	63.4	32.6	6.0	0.0
L17R	L17D/T119D	22.2	49.3	27.7	24.6
L17R/V121K	L17D/T119D	47.2	25.6	23.3	23.3
L17R/V121R	L17D/T119D	48.6	26.6	20.6	20.6
L17R/V20K	L17D/T119D	52.4	38.9	4.1	4.1
L17R/V20R	L17D/T119D	55.1	43.1	6.0	0.0
V121K	L17D/T119D	68.3	31.7	6.0	-6.7
V121R	L17D/T119D	44.3	41.4	10.5	-9.0
V20R/V121R	L17D/T119D	56.0	39.4	6.0	0.0
L17K	L17D/V121E	20.4	66.5	3.0	3.0
L17K/V121K	L17D/V121E	21.4	47.7	21.4	21.4
L17K/V121R	L17D/V121E	27.7	57.4	6.4	6.4
L17K/V20K	L17D/V121E	29.2	52.0	6.2	6.2
L17K/V20R	L17D/V121E	29.5	65.7	6.0	0.0
L17R	L17D/V121E	34.6	71.1	8.1	5.0
L17R/V121K	L17D/V121E	18.0	61.3	13.6	13.6
L17R/V121R	L17D/V121E	19.4	63.8	9.0	9.0
L17R/V20K	L17D/V121E	24.8	66.4	6.0	0.0
L17R/V20R	L17D/V121E	21.3	74.5	6.0	0.0
V121K	L17D/V121E	34.6	42.7	52.7	15.1
V121R	L17D/V121E	26.8	53.0	3.0	-16.6
V20R/V121R	L17D/V121E	26.4	65.3	6.0	0.0
L17K/V121K	L17D/V20D	62.3	25.7	6.0	0.0
L17K/V121R	L17D/V20D	55.4	37.6	6.0	0.0
L17K/V20K	L17D/V20D	72.4	21.1	6.0	0.0
L17K/V20R	L17D/V20D	83.2	18.3	6.0	0.0
L17R/V121K	L17D/V20D	40.2	54.7	6.0	0.0
L17R/V121R	L17D/V20D	43.7	50.8	6.0	0.0
L17R/V20K	L17D/V20D	49.8	42.8	6.0	0.0
L17R/V20R	L17D/V20D	45.5	50.4	6.0	0.0
V121K	L17D/V20D	23.3	30.5	17.6	26.0
V121R	L17D/V20D	80.4	19.6	6.0	19.6
V20K	L17D/V20D	54.1	37.3	2.6	0.1
V20R	L17D/V20D	52.8	37.4	3.2	-0.3
V20R/V121R	L17D/V20D	48.2	43.2	2.3	2.3
L17K/V121K	L17D/V20E	39.6	60.4	6.0	0.0

Figure 36

Fab-TTR Heterotetramer Single & Double Interface Mutants Post-Purification Mixture

3B1 Pos Mut	655 Neg Mut	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab	Increase %4X-Fab
V20R	V20D/T119D	28.0	54.9	9.8	1.0
V20R/V121R	V20D/V121E	25.6	60.5	4.7	-1.5
L17K	V20D/V121E	33.2	62.4	20.0	-2.5
L17K/V121K	V20D/V121E	24.6	68.8	3.2	-0.2
L17K/V121K	V20D/V121E	25.1	65.4	3.1	0.7
L17K/V20K	V20D/V121E	24.7	68.7	3.2	-0.2
L17K/V20R	V20D/V121E	24.2	67.9	3.9	1.5
L17R	V20D/V121E	31.5	81.9	3.2	-3.4
L17R/V121K	V20D/V121E	25.7	69.9	6.4	-2.4
L17R/V121R	V20D/V121E	22.5	69.7	3.5	0.1
L17R/V20K	V20D/V121E	24.7	66.3	4.1	1.7
L17R/V20R	V20D/V121E	22.7	70.3	2.7	0.3
V20K	V20D/V121E	25.2	68.3	6.0	-4.9
V20R	V20D/V121E	26.0	71.9	3.1	-2.8
V20R/V121R	V20D/V121E	24.2	67.6	2.6	0.2
L17K/V20K	V20E	34.6	56.3	35.3	7.1
L17K/V20R	V20E	6.7	59.5	34.2	7.9
L17R/V20K	V20E	11.9	70.0	10.2	14.0
L17R/V20R	V20E	6.7	77.2	10.9	15.3
V20K	V20E	18.1	37.1	30.8	2.1
V20R	V20E	26.2	37.1	36.7	7.9
V20R/V121R	V20E	11.3	69.1	11.8	-10.5
L17K	V20E/T119D				33.8
L17K/V121K	V20E/T119D	19.0	71.8	1.5	-1.4
L17K/V121R	V20E/T119D	21.8	63.3	3.7	0.8
L17K/V20K	V20E/T119D	31.2	52.9	7.3	4.4
L17K/V20R	V20E/T119D	32.1	33.2	7.5	4.6
L17R	V20E/T119D				-6.1
L17R/V121K	V20E/T119D	14.3	35.6	2.5	-0.4
L17R/V121R	V20E/T119D	12.6	75.3	3.1	-0.9
L17R/V20K	V20E/T119D	14.0	58.4	16.1	13.2
L17R/V20R	V20E/T119D	12.6	72.8	7.8	4.9
V20K	V20E/T119D				-5.4
V20R	V20E/T119D				-5.4
V20R/V121R	V20E/T119D	18.4	68.2	2.8	-0.1
L17K	V20E/V121E	35.5	78.3	20.0	0.0
L17K/V121K	V20E/V121E	18.3	75.6	6.9	0.0
L17K/V121R	V20E/V121E	22.6	64.8	2.5	2.5
L17K/V20K	V20E/V121E	16.3	81.5	6.9	0.0
L17K/V20R	V20E/V121E	16.5	94.9	6.0	0.0
L17R	V20E/V121E	2.2	87.6	9.3	6.1
L17R/V121K	V20E/V121E	15.1	78.3	6.0	0.0
L17R/V121R	V20E/V121E	15.2	76.8	6.9	0.0
L17R/V20K	V20E/V121E	18.2	73.7	6.1	0.0
L17R/V20R	V20E/V121E	14.4	78.0	6.9	0.0
V20K	V20E/V121E	16.7	85.3	6.1	-2.5
V20R	V20E/V121E	22.8	65.3	1.9	-1.5
V20R/V121R	V20E/V121E	20.1	70.6	6.9	0.0

3B1 Pos Mut	655 Neg Mut	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab	Increase %4X-Fab
L17K/V20R	L17E/V20E	44.4	53.8	6.0	0.0
L17K/V121K	L17E/V20E	39.7	57.9	6.0	0.0
L17R/V121R	L17E/V20E	40.2	56.7	6.0	0.0
L17R/V20K	L17E/V20E	41.2	55.6	6.0	0.0
L17R/V20R	L17E/V20E	39.6	58.0	6.0	0.0
V121K	L17E/V20E	45.7	43.2	2.6	15.0
V121R	L17E/V20E	44.8	55.2	6.0	19.8
V20K	L17E/V20E	42.6	57.4	6.0	-2.5
V20R	L17E/V20E	42.6	47.9	6.0	-2.5
V20R/V121R	L17E/V20E	41.9	54.9	6.0	0.0
L17K	T119D	32.2	47.9	45.2	9.3
L17K/V121K	T119D	22.7	62.5	11.5	-4.4
L17K/V121R	T119D	23.3	50.5	18.1	2.2
L17K/V20K	T119D	22.2	61.0	11.7	-4.2
L17K/V20R	T119D	21.7	61.5	12.2	-3.6
L17R	T119D	17.8	61.3	11.4	-7.6
L17R/V121K	T119D	15.8	26.7	52.4	16.5
L17R/V121R	T119D	15.6	28.9	49.4	33.9
L17R/V20K	T119D	18.3	18.8	45.9	16.1
L17R/V20R	T119D	15.1	38.7	41.5	25.6
L17K	V121E	7.0	65.6	22.1	17.3
L17K/V121K	V121E	16.1	60.6	23.7	4.3
L17K/V121R	V121E	14.0	68.8	10.6	-8.8
L17K/V20K	V121E	20.6	56.1	13.0	-6.4
L17K/V20R	V121E	13.5	56.4	26.6	7.2
L17R	V121E	1.6	64.2	33.4	10.9
L17R/V121K	V121E	8.8	42.1	6.6	-12.8
L17R/V121R	V121E	6.4	83.0	6.9	12.5
L17R/V20K	V121E	9.0	16.0	9.9	-9.5
L17R/V20R	V121E	5.9	80.2	8.0	-11.5
L17K/V20K	V20D	29.6	34.8	37.8	-9.0
L17K/V20R	V20D	21.9	23.3	53.8	16.9
L17R/V20K	V20D	18.1	40.9	32.1	-4.7
L17R/V20R	V20D	13.7	52.6	26.9	-9.9
V20K	V20D	28.3	19.0	36.0	-3.4
V20R	V20D	26.8	26.5	39.5	0.2
V20R/V121R	V20D	19.1	46.2	27.6	-9.3
L17K	V20D/T119D	11.6	64.3	19.1	-7.3
L17K/V121K	V20D/T119D	24.3	58.4	10.1	3.8
L17K/V121R	V20D/T119D	25.4	58.2	6.2	0.0
L17K/V20K	V20D/T119D	24.5	57.7	6.5	0.2
L17K/V20R	V20D/T119D	25.3	59.3	5.8	-0.5
L17R	V20D/T119D	12.7	51.3	30.3	20.9
L17R/V121K	V20D/T119D	22.8	60.5	9.9	3.7
L17R/V121R	V20D/T119D	23.3	62.5	8.0	1.8
L17R/V20K	V20D/T119D	23.9	46.5	23.1	16.8
L17R/V20R	V20D/T119D	26.5	60.2	5.3	-1.0
V20K	V20D/T119D	25.9	53.5	13.5	4.8

AB-TTR heterodimers separate expression (no co-culture)
Individual Molecules SEC

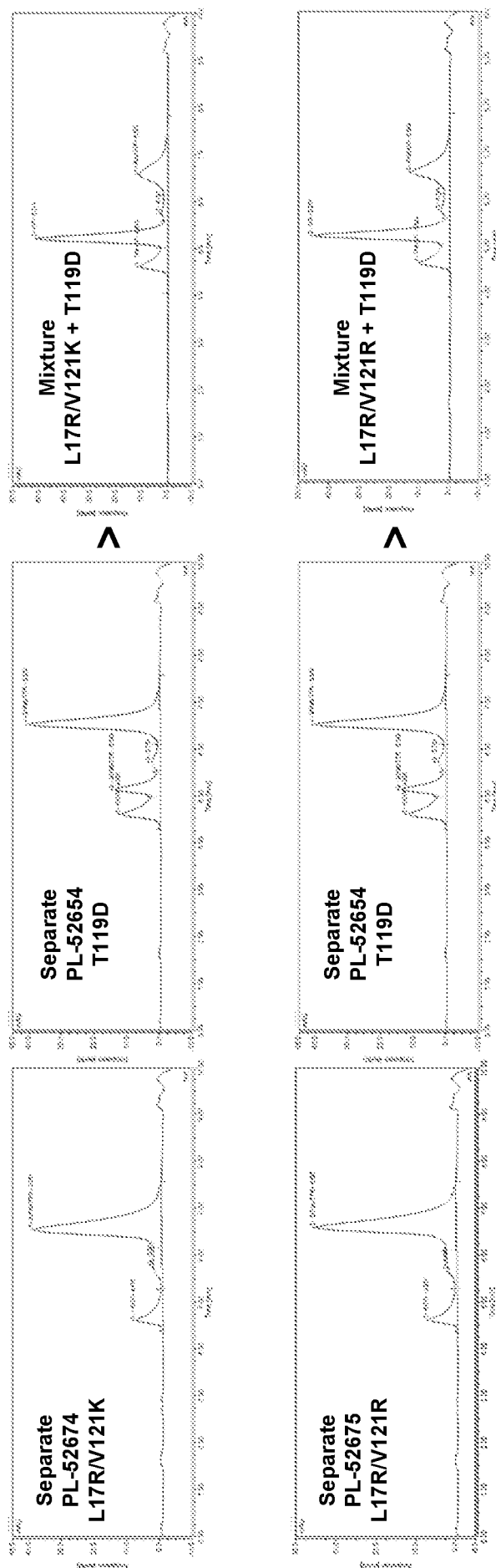


Figure 38
Fab-TTR Heterotetramers Co-culture Individual Molecule Pairs
(REQ-55327)

381 Pos Mut	655 Neg Mut	Yield (mg/L)	SEC %MW	SEC %2Fab	SEC %4Fab
V20K	L17D/V20E	35.5	75.2	70.7	4.1
V20K/V121K	L17D/V20E	39.7	73.1	78.2	4.6
V20K/V121R	L17D/V20E	39.9	75.1	70.5	3.6
V20R	L17D/V20E	36.4	78.2	70.6	1.2
V20R/V121K	L17D/V20E	35.6	77.4	68.6	1.5
V20R/V121R	L17D/V20E	34.4	73.0	61.0	1.5
L17K/V121K	L17E	42.5	74.0	81.5	4.4
L17K/V121R	L17E	35.7	71.3	82.8	6.1
V121K	L17E	108.3	8.8	66.9	23.3
V121R	L17E	38.1	8.6	88.4	7.0
V20K/V121K	L17E	96.7	18.1	77.3	4.6
V20K/V121R	L17E	107.9	19.4	77.1	3.5
V20R/V121K	L17E	32.5	20.2	75.8	4.0
L17K	L17E/T119D	44.8	8.2	62.3	25.3
L17K/V121K	L17E/T119D	7.4	5.8	37.4	13.0
L17K/V121R	L17E/T119D	7.4	5.3	40.6	13.9
L17K/V20K	L17E/T119D	5.3	11.3	73.5	3.9
L17K/V20R	L17E/T119D	5.2	5.2	58.2	1.5
L17R	L17E/T119D	33.2	8.3	69.3	32.8
L17R/V121K	L17E/T119D	35.9	6.6	70.5	17.4
L17R/V121R	L17E/T119D	35.0	8.0	58.9	29.9
L17R/V20K	L17E/T119D	7.4	10.6	77.2	4.4
L17R/V20R	L17E/T119D	5.3	11.7	75.7	1.5
V20K	L17E/T119D	2.9	11.8	75.7	5.4
V20K/V121K	L17E/T119D	37.3	21.1	56.0	8.9
V20K/V121R	L17E/T119D	37.0	17.5	58.6	2.5
V20R	L17E/T119D	22.1	32.5	59.3	3.4
V20R/V121K	L17E/T119D	34.9	22.4	77.6	1.5
V20R/V121R	L17E/T119D	32.1	21.3	76.3	2.4
L17K	L17E/V121E	73.0	15.1	64.0	13.6
L17K/V121K	L17E/V121E	8.2	7.5	30.8	4.6
L17K/V121R	L17E/V121E	19.0	6.4	76.1	13.0
L17K/V20K	L17E/V121E	7.4	10.8	78.2	3.6
L17K/V20R	L17E/V121E	5.1	7.1	84.5	1.5
L17R	L17E/V121E	8.4	7.2	89.9	4.8
L17R/V121K	L17E/V121E	38.0	12.6	76.1	5.9
L17R/V121R	L17E/V121E	37.5	13.5	64.3	13.3
L17R/V20K	L17E/V121E	87.3	13.4	74.5	4.2
L17R/V20R	L17E/V121E	19.3	10.8	78.2	4.5
V20K	L17E/V121E	22.2	12.9	75.1	5.1
V20K/V121K	L17E/V121E	40.0	17.6	65.2	7.7
V20K/V121R	L17E/V121E	39.9	32.9	58.1	7.0
V20R	L17E/V121E	5.4	9.3	82.3	3.4
V20R/V121K	L17E/V121E	34.4	8.9	94.1	1.5
L17K/V121R	L17E/V20D	36.1	34.7	64.0	13.3
L17K/V20K	L17E/V20D	95.1	27.5	70.8	1.7
L17K/V20R	L17E/V20D	41.7	28.2	74.5	1.8
L17R/V121K	L17E/V20D	46.5	32.3	64.6	1.5
L17R/V121R	L17E/V20D	41.5	7.1	82.6	1.5
L17R/V20K	L17E/V20D	46.6	32.4	80.6	1.8
L17R/V20R	L17E/V20D	31.4	25.2	73.8	1.5

Figure 39
Fab-TTR Heterotetramers Co-culture Individual Molecule Pairs
(REQ-55327)

381 Pos Mut	655 Neg Mut	Yield (mg/L)	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab
L17K/V20K	V20D/V121E	3.9	9.0	79.4	5.9
L17K/V20R	V20D/V121E	1.5	6.8	83.1	4.6
L17R	V20D/V121E	2.5	6.5	85.1	4.1
L17R/V121K	V20D/V121E	26.4	32.1	72.9	6.9
L17R/V121R	V20D/V121E	33.8	14.2	69.4	6.9
L17R/V20K	V20D/V121E	6.3	33.7	73.3	6.4
L17R/V20R	V20D/V121E	26.9	9.0	79.5	6.4
V20K	V20D/V121E	2.2	24.4	62.0	7.7
V20K/V121K	V20D/V121E	44.2	18.0	63.6	8.6
V20R	V20D/V121E	51.5	20.6	81.2	4.9
V20R/V121R	V20D/V121E	32.2	19.0	78.6	2.4
L17K/V121K	V20E	333.0	9.9	57.8	32.4
L17K/V20R	V20E	129.6	9.2	59.1	31.8
L17R/V121K	V20E	125.6	10.3	55.2	34.6
L17R/V121R	V20E	111.0	10.5	55.6	33.9
L17R/V20K	V20E	77.6	10.3	63.2	36.5
L17R/V20R	V20E	253.8	9.4	59.6	31.0
V20K/V121K	V20E	45.8	10.6	63.2	36.2
V20K/V121R	V20E	13.2	11.7	80.0	8.3
V20R	V20E	125.6	6.3	60.0	33.7
V20R/V121K	V20E	323.8	10.3	59.2	30.5
V20R/V121R	V20E	348.3	11.9	58.2	28.0
L17K	V20E/T119D	7.3	8.7	84.2	13.7
L17K/V121K	V20E/T119D	2.9	5.0	85.9	10.0
L17K/V121R	V20E/T119D	2.8	5.6	81.1	11.0
L17K/V20K	V20E/T119D	3.4	6.1	78.9	7.1
L17K/V20R	V20E/T119D	2.3	4.8	80.8	4.4
L17R/V121K	V20E/T119D	35.1	11.4	72.2	8.4
L17R/V121R	V20E/T119D	26.7	12.7	69.8	9.0
L17R/V20K	V20E/T119D	26.4	7.9	75.7	10.2
L17R/V20R	V20E/T119D	36.2	8.1	77.8	7.4
V20K	V20E/T119D	2.7	29.0	58.9	6.9
V20K/V121K	V20E/T119D	25.5	15.6	64.8	9.3
V20K/V121R	V20E/T119D	29.0	19.7	76.7	3.6
V20R	V20E/T119D	31.4	27.5	56.0	10.4
V20R/V121K	V20E/T119D	20.8	19.4	71.0	3.6
V20R/V121R	V20E/T119D	20.8	20.9	73.6	3.4
L17K	V20E/V121E	3.3	11.0	74.2	10.5
L17K/V121K	V20E/V121E	2.4	4.8	85.0	6.0
L17K/V121R	V20E/V121E	2.5	6.2	83.7	6.0
L17K/V20K	V20E/V121E	3.9	8.0	80.5	4.4
L17K/V20R	V20E/V121E	2.4	17.0	68.7	6.0
L17R	V20E/V121E	2.8	6.1	87.2	3.5
L17R/V121R	V20E/V121E	55.0	14.7	69.9	7.0
L17R/V20K	V20E/V121E	33.3	9.8	75.6	10.0
V20K/V121K	V20E/V121E	53.6	16.8	66.1	8.1
V20K/V121R	V20E/V121E	52.3	17.0	80.7	3.7
V20R/V121K	V20E/V121E	55.4	16.8	80.4	2.7
V20R/V121R	V20E/V121E	50.7	20.0	75.8	2.8

381 Pos Mut	655 Neg Mut	Yield (mg/L)	SEC %HMW	SEC %2X-Fab	SEC %4X-Fab
V121K	L17E/V20D	53.5	25.7	70.4	4.4
V121R	L17E/V20D	59.5	28.5	76.1	2.5
V20K	L17E/V20D	59.3	26.5	79.4	4.3
V20K/V121R	L17E/V20D	38.8	17.0	80.4	3.5
V20R	L17E/V20D	48.7	26.7	72.2	2.5
V20R/V121K	L17E/V20D	49.2	21.1	84.5	2.4
V20R/V121R	L17E/V20D	37.5	22.4	85.5	2.4
L17K/V121K	L17E/V20E	65.3	28.7	82.2	2.4
L17K/V121R	L17E/V20E	67.3	28.4	70.2	3.4
L17K/V20K	L17E/V20E	74.5	28.5	75.1	3.4
L17K/V20R	L17E/V20E	69.4	28.7	80.2	2.4
L17R/V121K	L17E/V20E	65.5	28.5	86.8	2.4
L17R/V121R	L17E/V20E	68.8	20.5	75.8	3.4
V121K	L17E/V20E	68.1	28.5	85.4	2.4
V121R	L17E/V20E	59.4	21.2	85.4	2.4
V20K	L17E/V20E	72.8	28.5	79.1	2.4
V20K/V121K	L17E/V20E	93.5	36.6	89.7	2.4
V20K/V121R	L17E/V20E	53.8	30.2	87.5	2.4
L17K/V121K	L17E/V20R	69.9	28.5	84.4	2.4
V20R/V121K	L17E/V20R	66.4	20.6	86.2	2.4
L17K/V121R	L17E/V20R	59.3	22.8	84.1	2.4
L17R/V121K	T119D	60.0	28.0	78.9	1.0
L17R/V121R	T119D	57.4	31.5	79.5	1.0
L17R/V20K	T119D	57.4	31.6	80.4	4.3
L17K	V121E	57.0	28.0	82.5	1.0
L17K/V121K	V121E	54.5	28.0	86.7	3.2
L17K/V121R	V121E	52.0	30.7	86.1	8.7
L17R	V121E	68.1	28.0	86.0	8.4
L17R/V20K	V121E	54.5	28.0	86.4	2.4
L17K/V121K	V20D	68.4	28.3	82.8	8.0
L17K/V121R	V20D	68.1	36.1	86.5	8.4
L17R/V20K	V20D	57.4	29.3	82.2	8.4
L17R/V121K	V20D	58.5	28.5	86.6	9.5
L17R/V121R	V20D	58.5	31.2	82.6	9.2
V20K	V20D	58.4	28.0	86.0	8.4
V20K/V121K	V20D	57.7	29.3	82.6	8.4
L17K	V20D/T119D	40.2	23.1	82.1	20.4
L17K/V121K	V20D/T119D	39.0	24.0	71.0	8.2
L17K/V121R	V20D/T119D	39.0	23.7	58.6	8.0
L17R/V121K	V20D/T119D	39.0	27.7	76.7	9.8
L17R/V121R	V20D/T119D	39.0	28.6	86.4	12.5
L17R/V20K	V20D/T119D	39.0	28.5	86.4	12.5
L17R/V20R	V20D/T119D	39.0	28.5	86.4	12.5
V20K	V20D/T119D	39.0	28.5	86.4	12.5
V20K/V121K	V20D/T119D	39.0	28.5	86.4	12.5
V20K/V121R	V20D/T119D	39.0	28.5	86.4	12.5
V20R	V20D/T119D	39.0	28.5	86.4	12.5
V20R/V121K	V20D/T119D	39.0	28.5	86.4	12.5
V20R/V121R	V20D/T119D	39.0	28.5	86.4	12.5
V20R/V121K	V20D/V121E	44.7	38.3	86.6	10.6
L17K	V20D/V121E	44.7	38.3	86.6	4.5
L17R/V121R	V20D/V121E	44.7	38.3	86.6	4.1

Figure 40

Ab-TTR Heterodimers co-culture SEC

