Figure 1

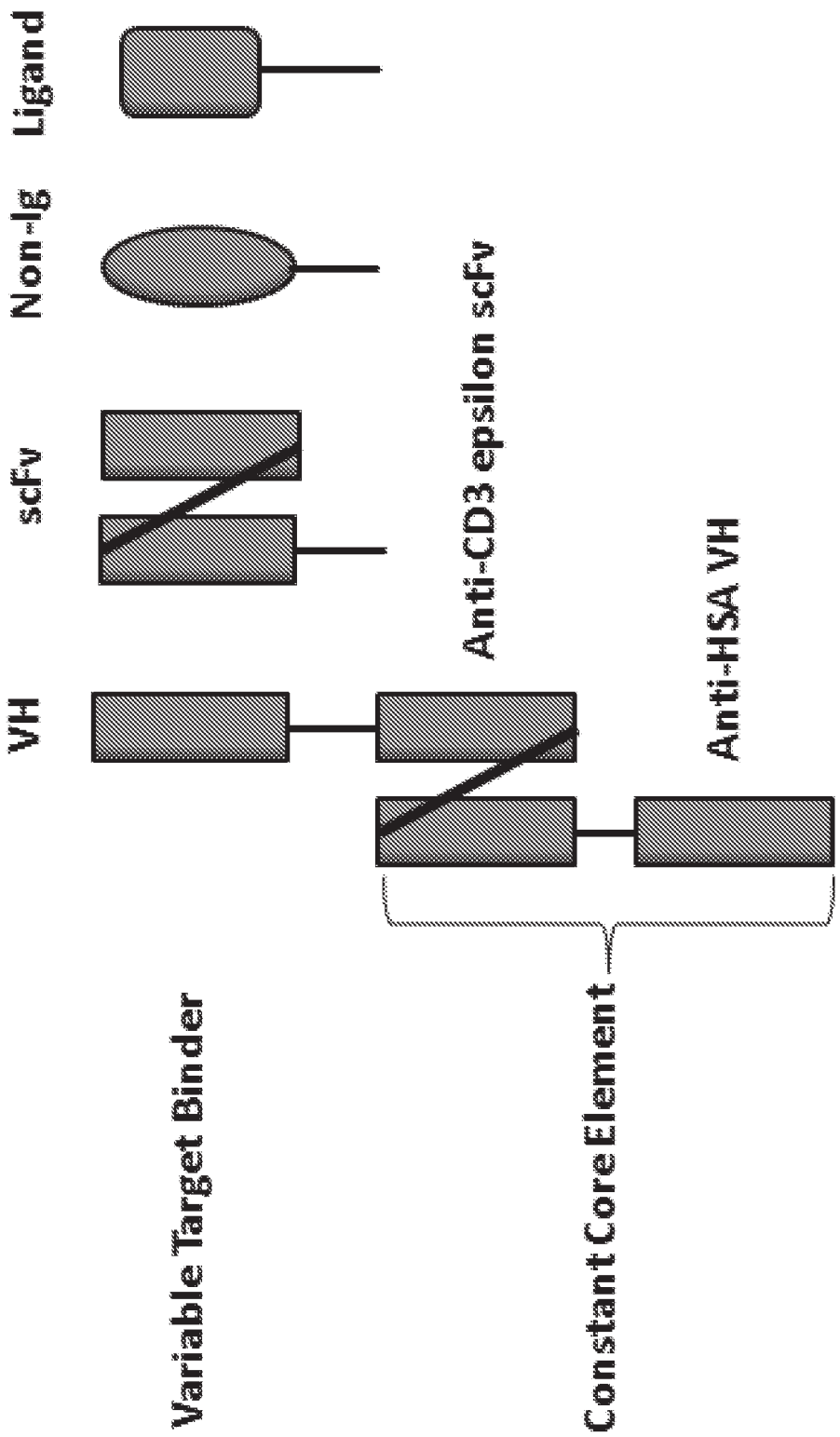

VH
scFv
Non-Ig
Ligand
Anti-CD3 epsilon scFv
Anti-HSA VH
Variable Target Binder
Constant Core Element

Figure 2

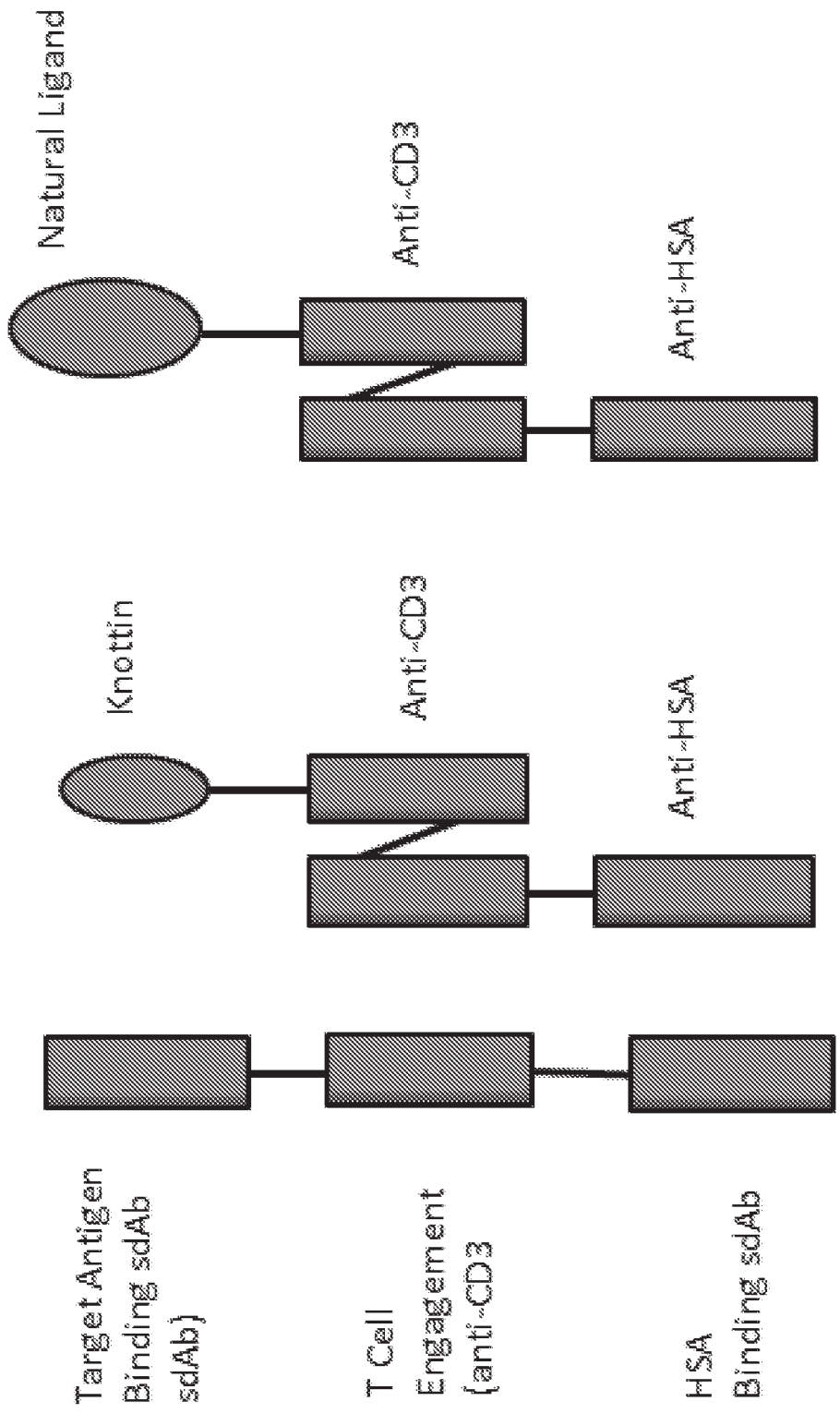

Natural Ligand
Anti-CD3
Anti-HSA
Knottin
Anti-CD3
Anti-HSA
Target Antigen Binding sdAb sdAb)
T Cell Engagement (anti-CD3
HSA Binding sdAb

Figure 3

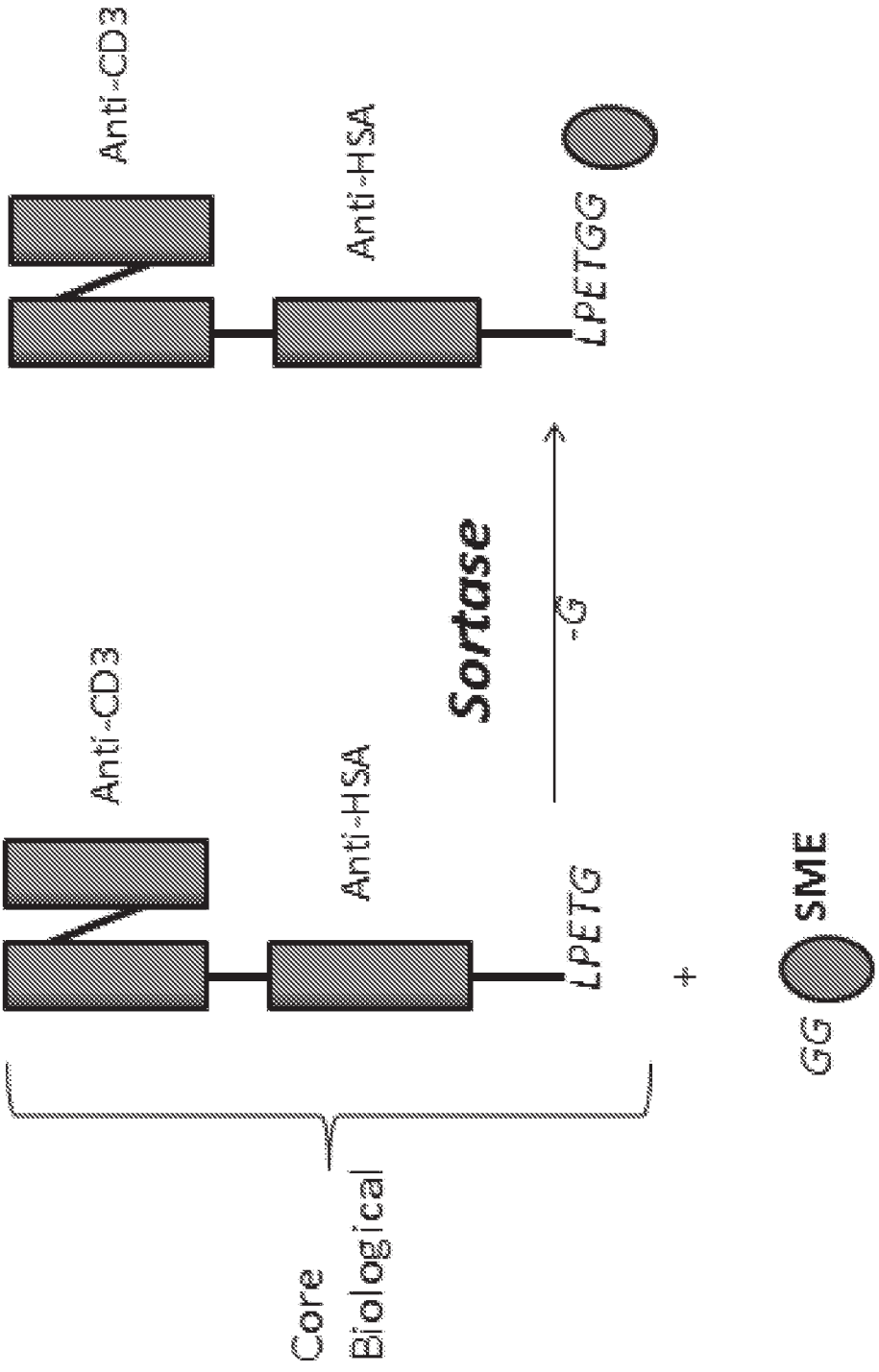

Anti-CD3
Anti-HSA
LPETGG
Sortase
-G
Anti-CD3
Anti-HSA
LPETG
+
GG
SME
Core
Biological

Figure 4

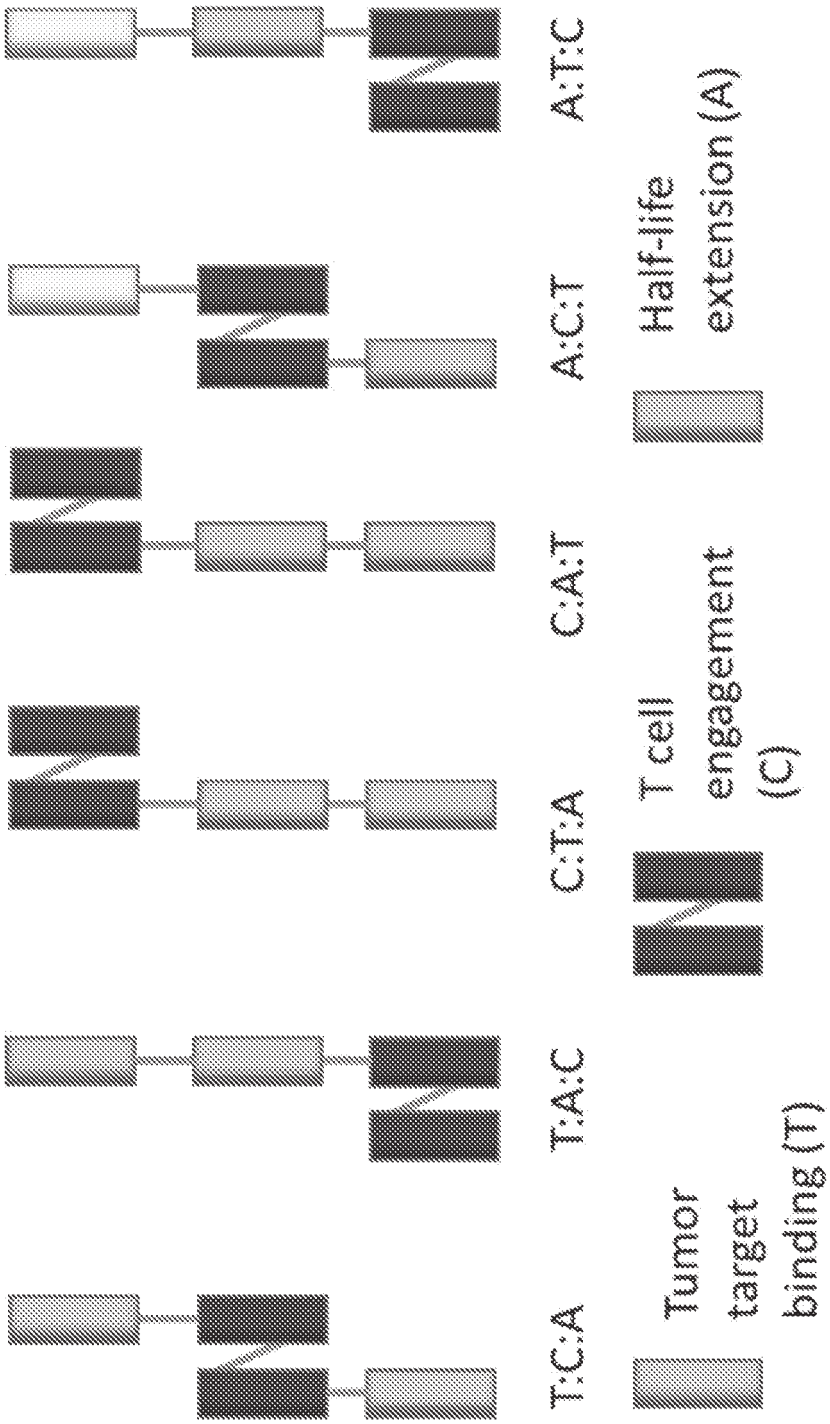
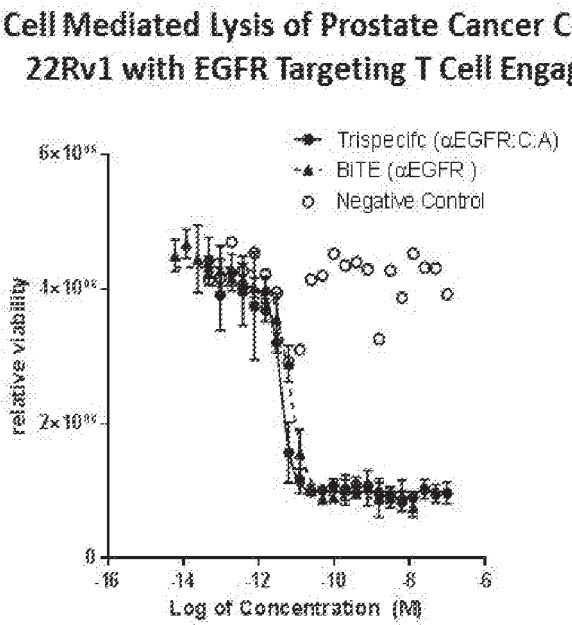
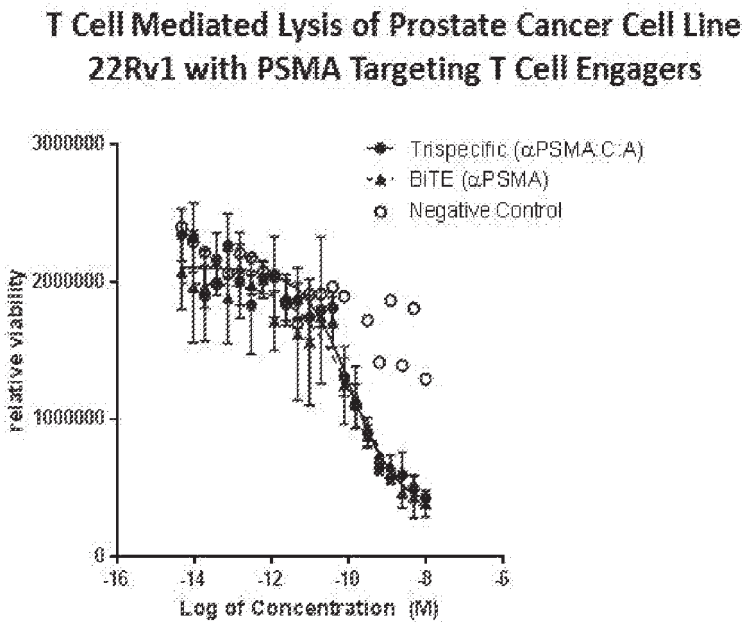

T:C:A
T:A:C
C:T:A
C:A:T
A:C:T
A:T:C
Tumor target binding (T)
T cell engagement (C)
Half-life extension (A)

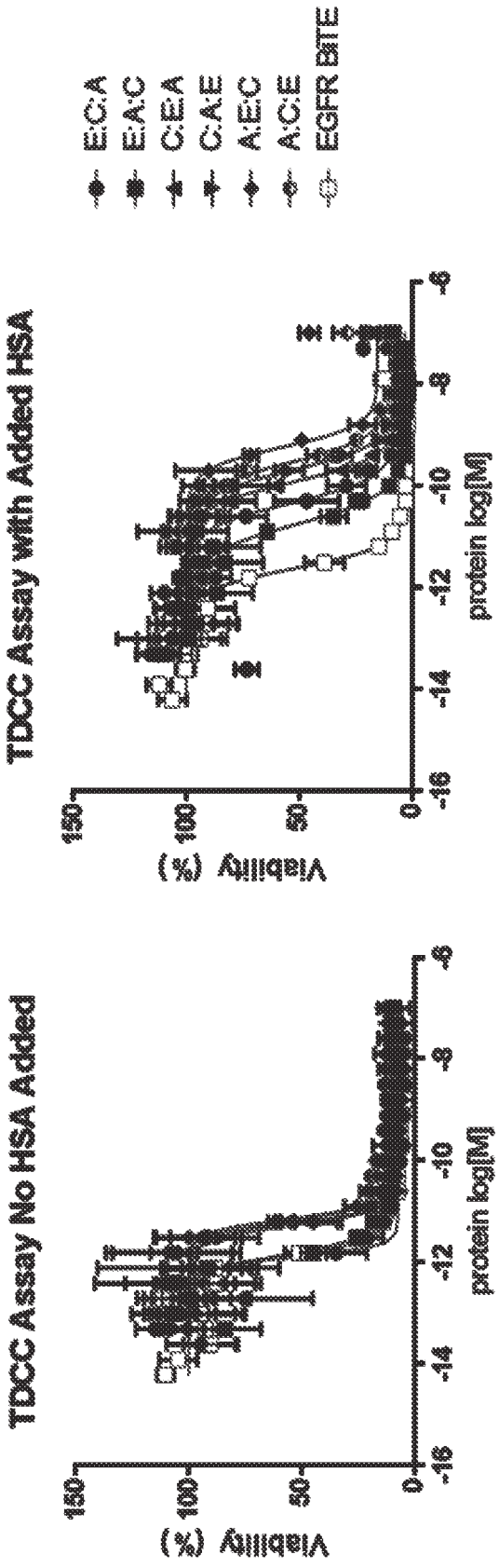
Figure 5





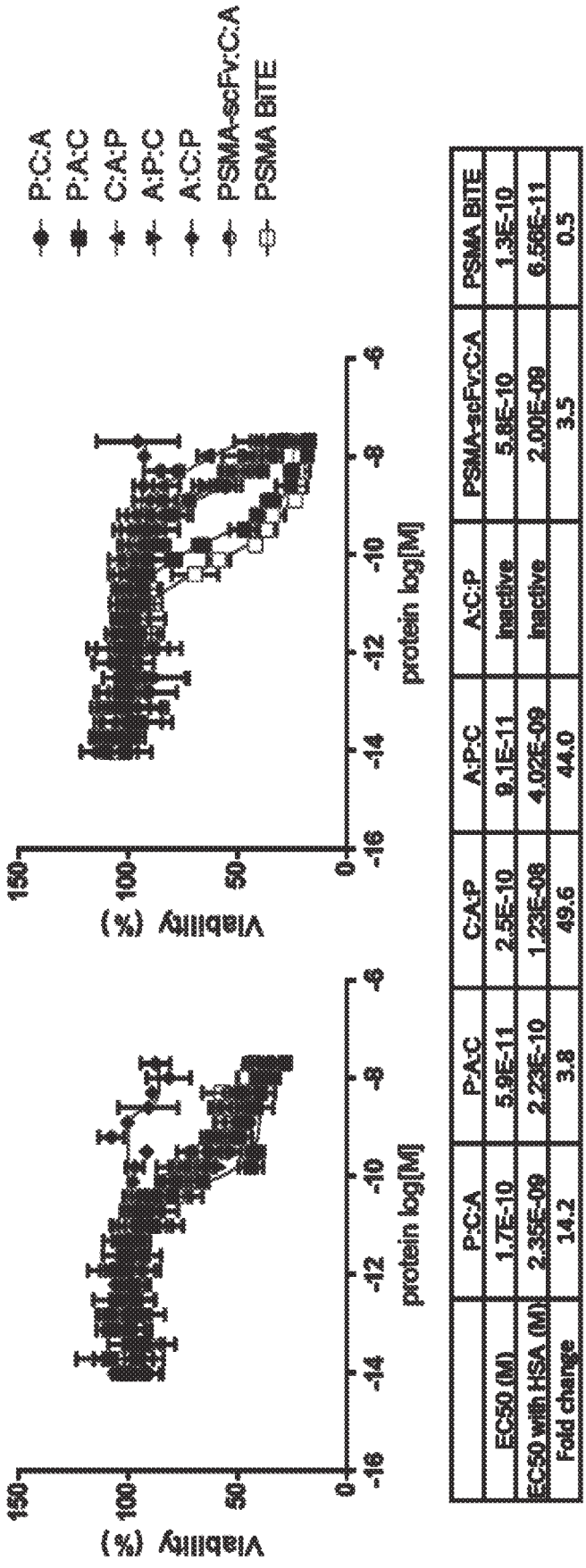
	Trispecific (EGFR)	BiTE (EGFR)	Trispecific (PSMA)	BiTE (PSMA)
EC_{50} (pM)	3.9	6.4	113	189

Figure 6



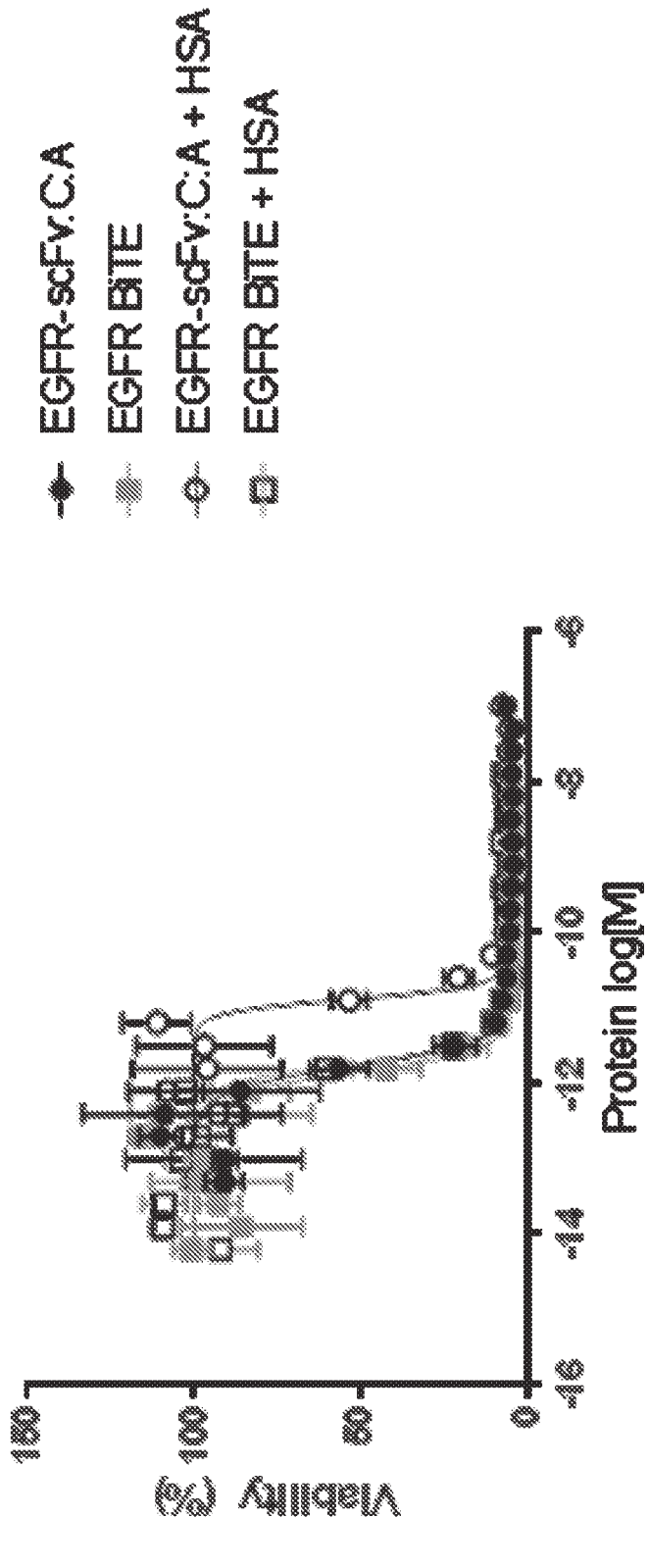
	E:C:A	E:A:C	C:E:A	C:A:E	A:E:C	A:C:E	EGFR BITE
EC50 (M)	1.3E-12	1.4E-12	5.8E-12	5.5E-12	8.9E-12	6.1E-12	1.5E-12
EC50 with HSA (M)	4.5E-11	1.7E-11	1.1E-10	2.0E-10	5.6E-10	2.8E-10	2.3E-12
Fold change	35.4	12.3	20.4	36.2	81.5	45.5	1.5

Figure 7



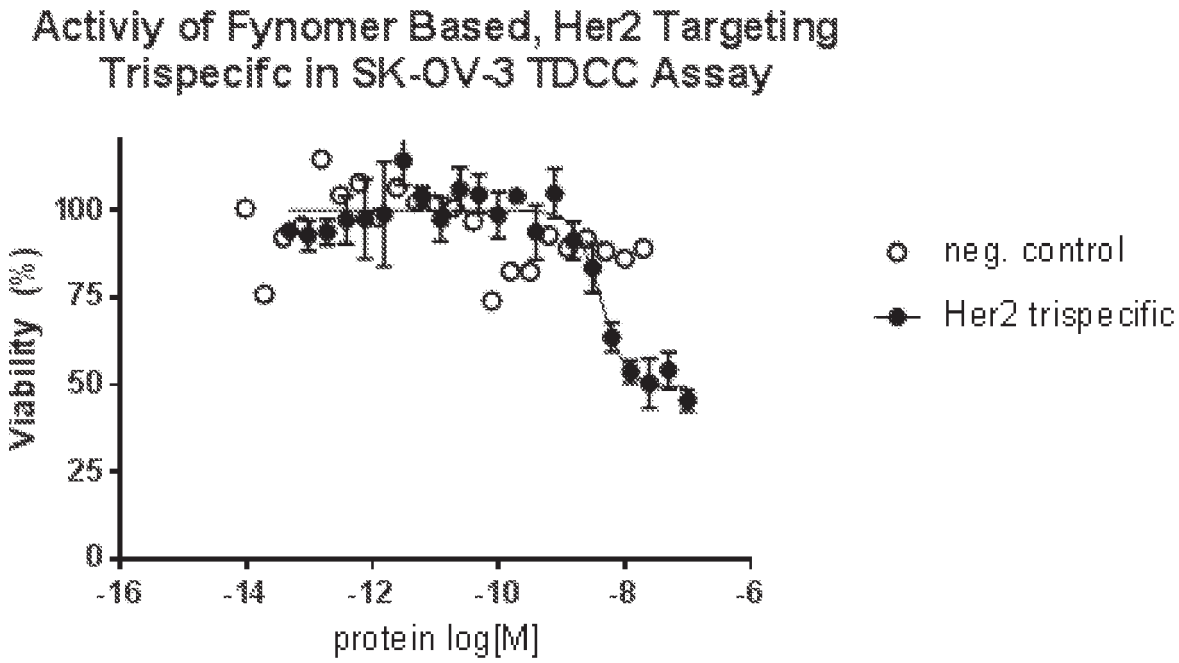
	P:C:A	P:A:C	C:A:P	A:P:C	A:C:P	PSMA-scFv:C:A	PSMA BITE
EC50 (M)	1.7E-10	5.9E-11	2.5E-10	9.1E-11	Inactive	5.8E-10	1.3E-10
EC50 with HSA (M)	2.35E-09	2.23E-10	1.23E-08	4.02E-09	Inactive	2.00E-09	6.58E-11
Fold change	14.2	3.8	49.6	44.0		3.5	0.5

Figure 8



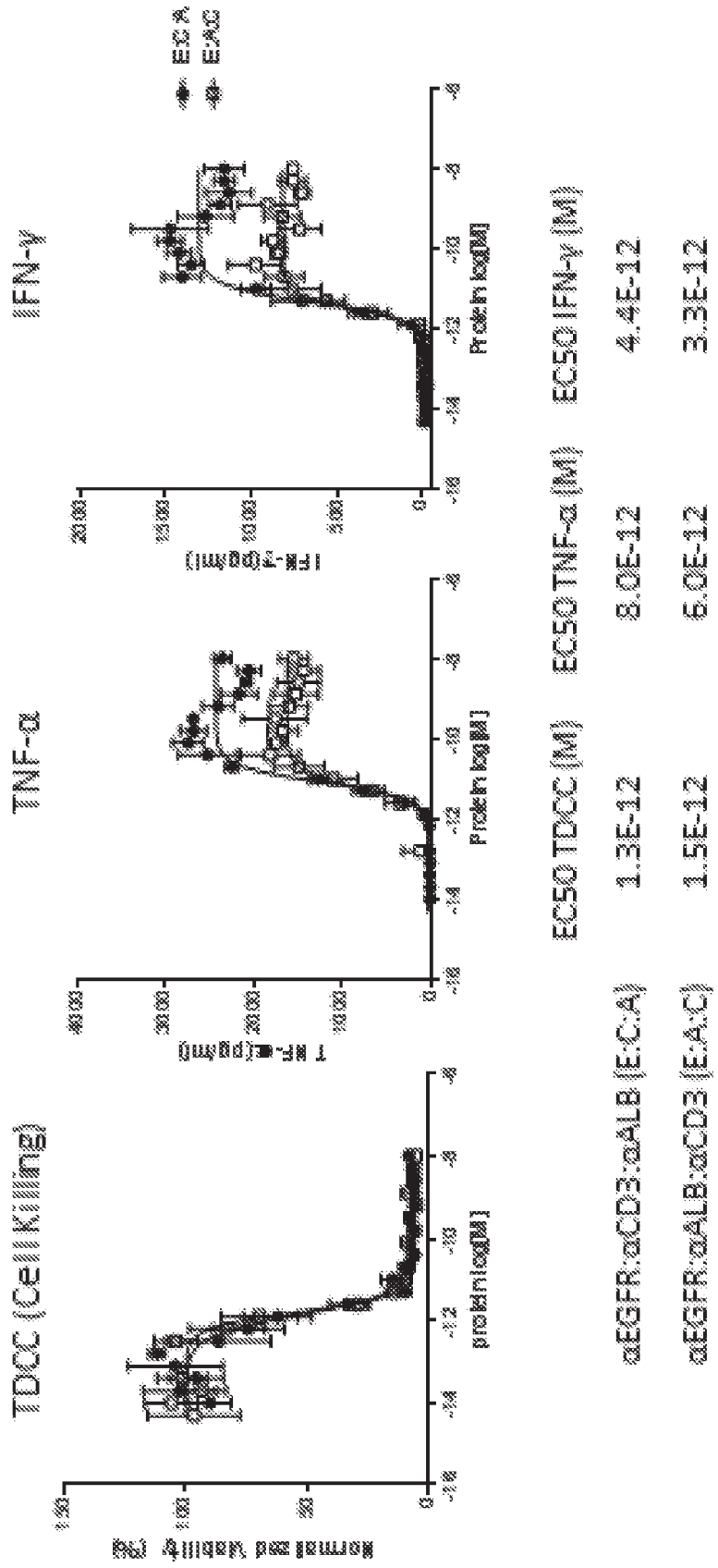
	EGFR-scFv:C:A	EGFR BiTE
EC50 (M)	1.6E-12	1.3E-12
EC50 with HSA (M)	1.3E-11	1.7E-12
Fold change	7.8	1.3

Figure 9



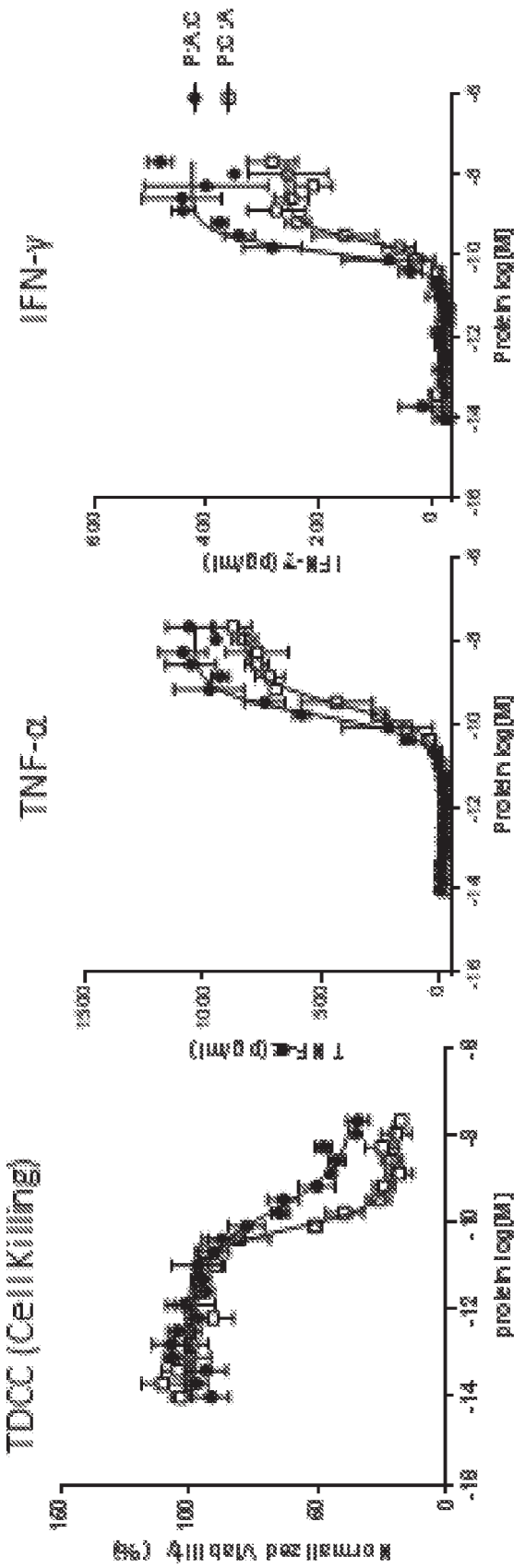
	Trispecific (Her2)
EC_{50} (nM)	4.1

Figure 10



	EC50 TDCC (M)	EC50 TNF-α (M)	EC50 IFN-γ (M)
αEGFR:αCD3:αALB (E:C:A)	1.3E-12	8.0E-12	4.4E-12
αEGFR:αALB:αCD3 (E:A:C)	1.5E-12	6.0E-12	3.3E-12

Figure 11



	EC50 TDCC (M)	EC50 TNF-α (M)	EC50 IFN-γ (M)
αPSMA:αCD3:αALB (P:C:A)	1.7E-10	2.6E-10	2.3E-10
αPSMA:αALB:αCD3 (P:A:C)	6.9E-11	1.5E-10	1.3E-10

Figure 12

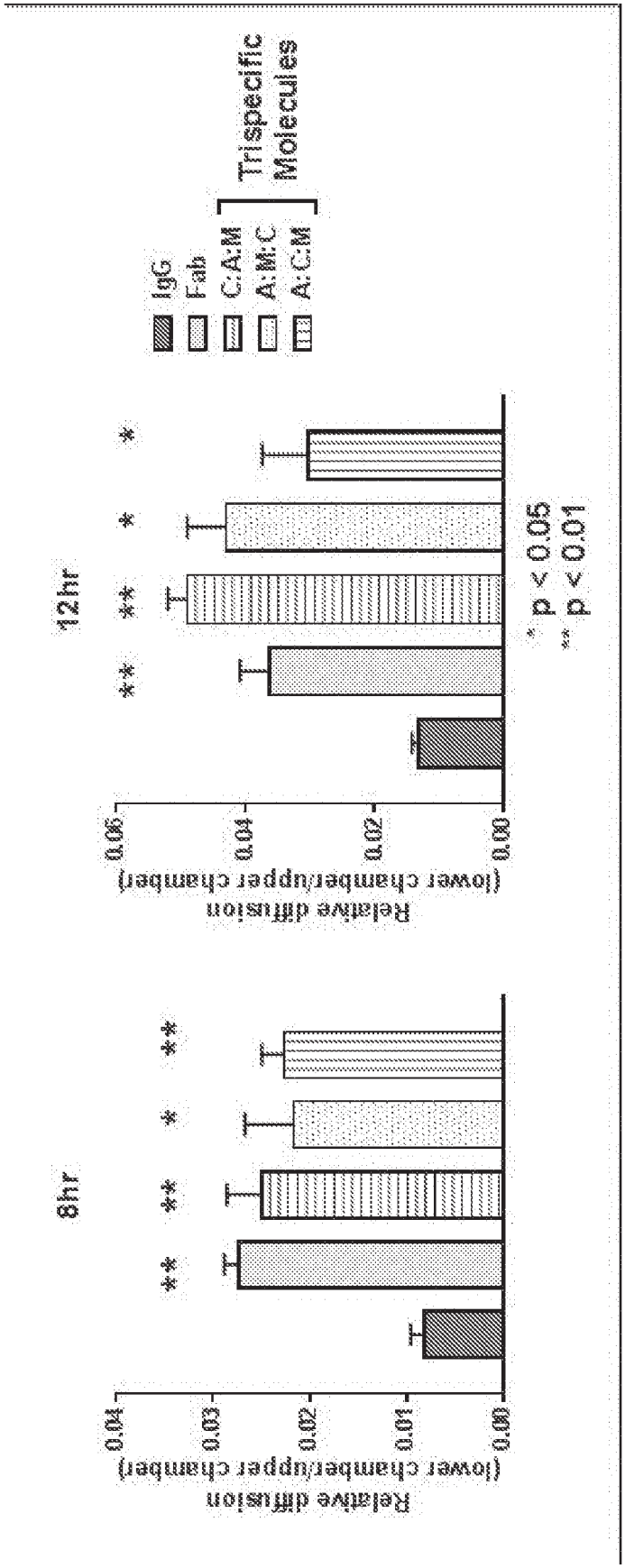
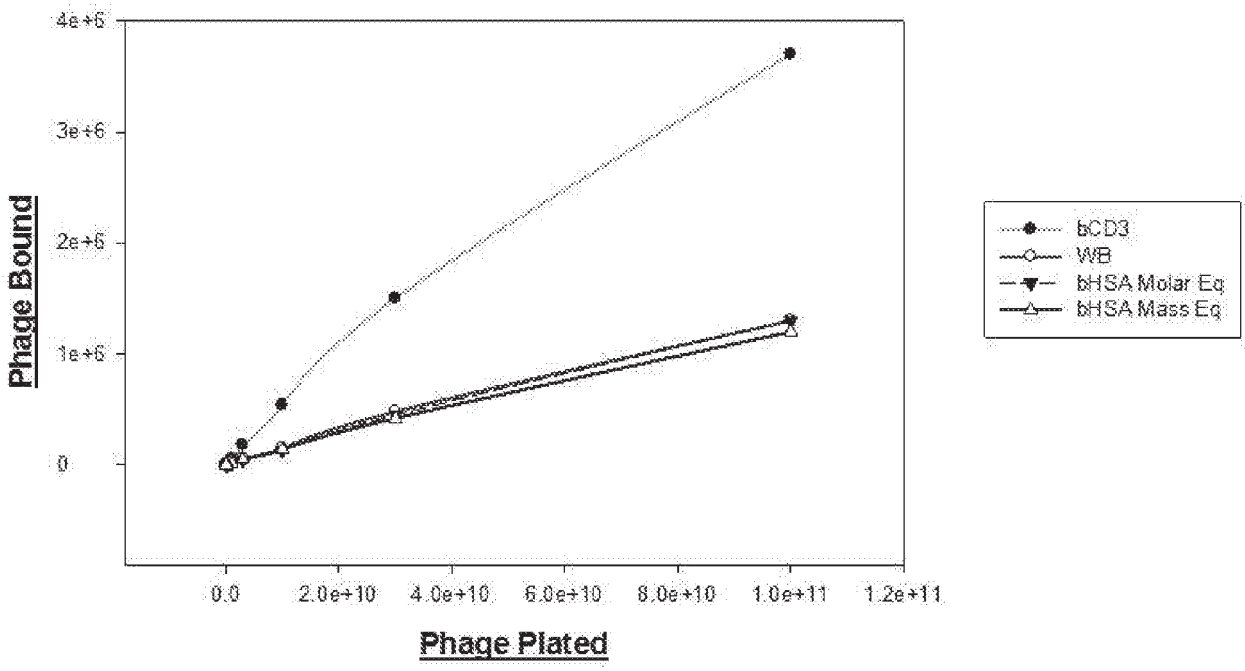

IgG
Fab
C:A:M
A:M:C
A:C:M
Trispecific Molecules
12hr
8hr
Relative diffusion (lower chamber/upper chamber)
0.06
0.04
0.02
0.00
0.04
0.03
0.02
0.01
0.00
**
*
p < 0.05
p < 0.01

Figure 13


4e+6
3e+6
2e+6
1e+6
0
Phage Bound
0.0
2.0e+10
4.0e+10
6.0e+10
8.0e+10
1.0e+11
1.2e+11
Phage Plated
bCD3
WB
bHSA Molar Eq
bHSA Mass Eq