SUPPORT INFORMATION

Optimization of nanobody caplacizumab via computational design

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Figures

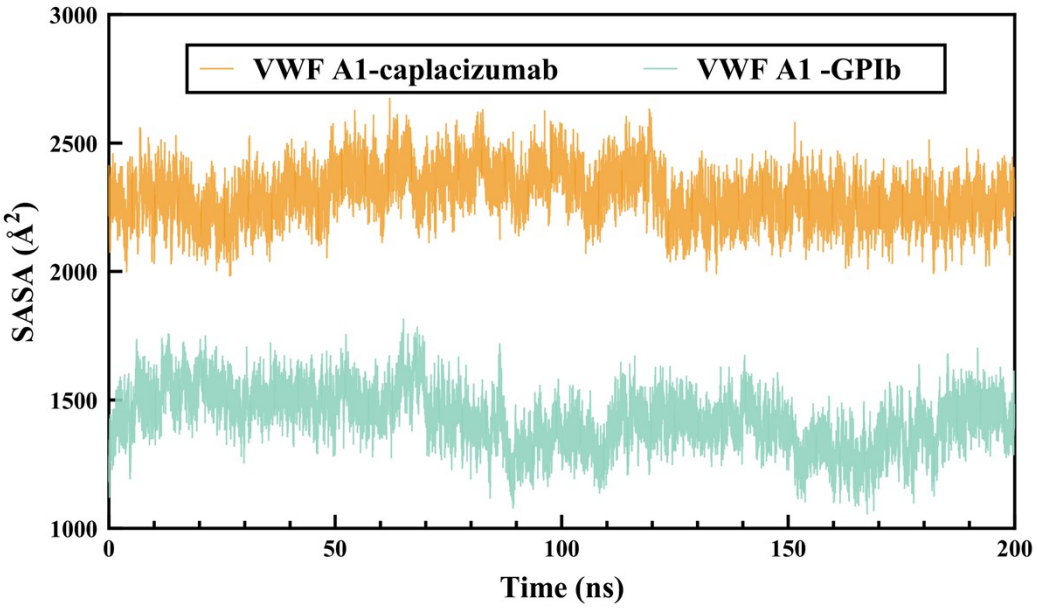

Figure S1.VWF A1bound interfacial residues in different systems SASA.

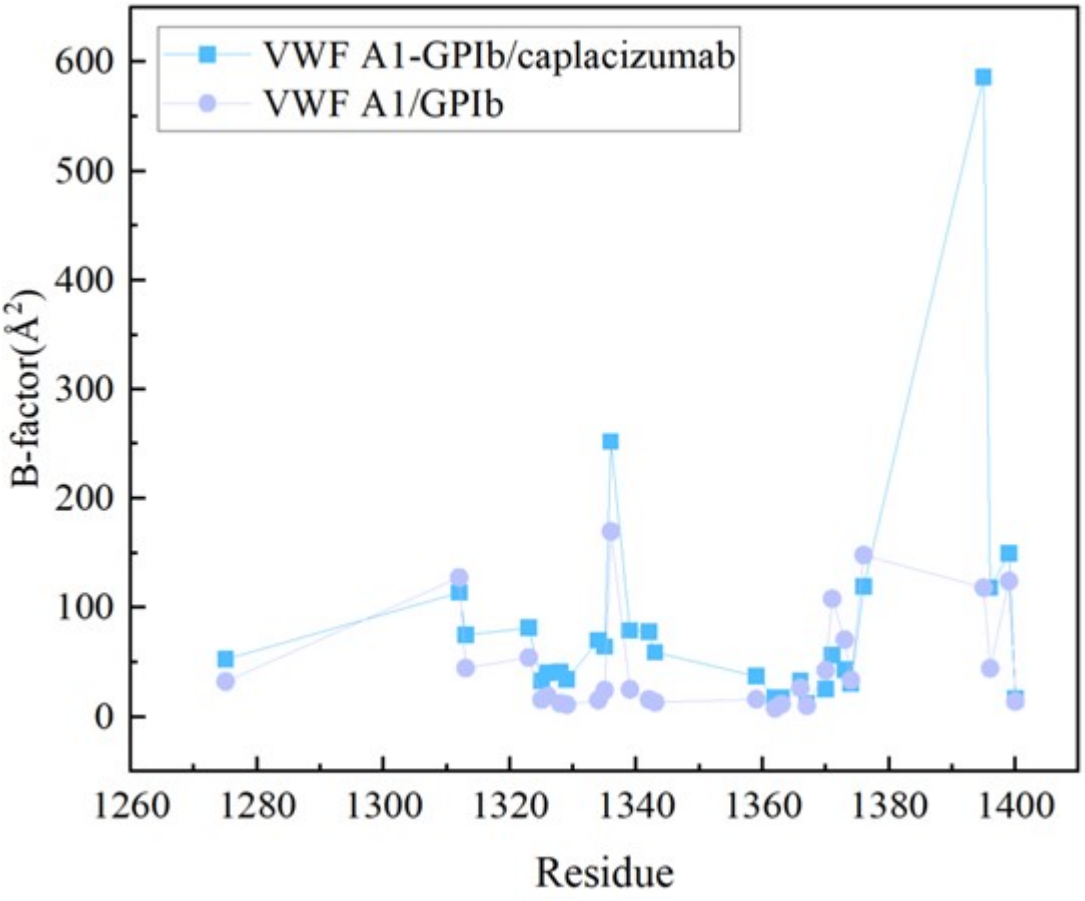

Figure S2. B-factor in VWF A1-GPIb/caplacizumab and VWF A1/GPIb systems.

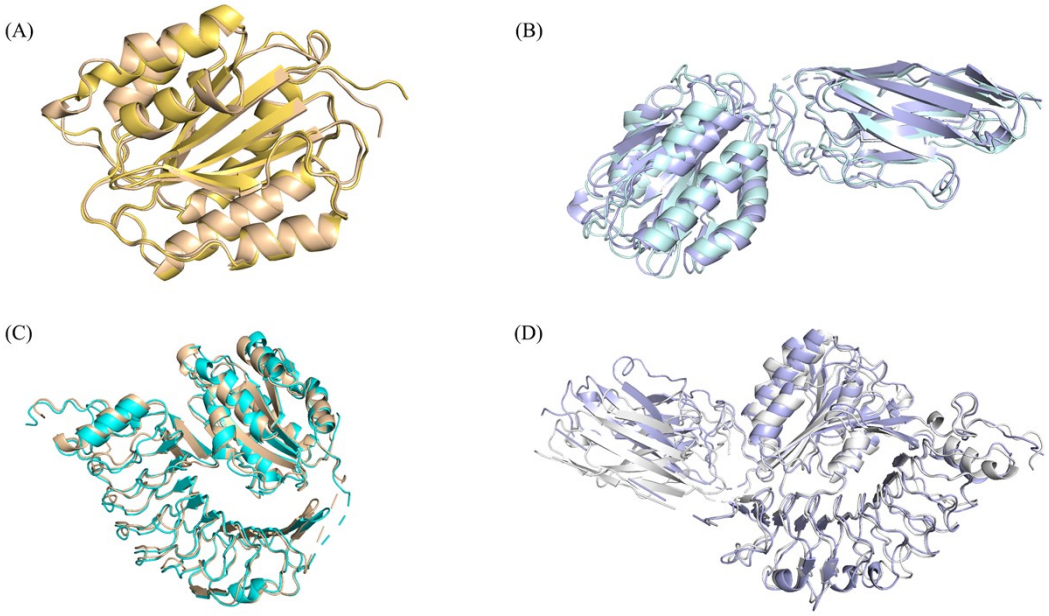

Figure S3. Structural overlay plots of different systems obtained by clustering analysis of molecular dynamics simulation trajectories. (A) VWF A1 system alone superposition plot, RMSD=0.65. (B) VWF A1/caplacizumab system superposition plot, RMSD=1.94.(C) VWF A1/GPIb system superposition plot RMSD=0.89; (D) caplacizumab/VWF A1/GPIb system superposition plot, RMSD= 1.72.

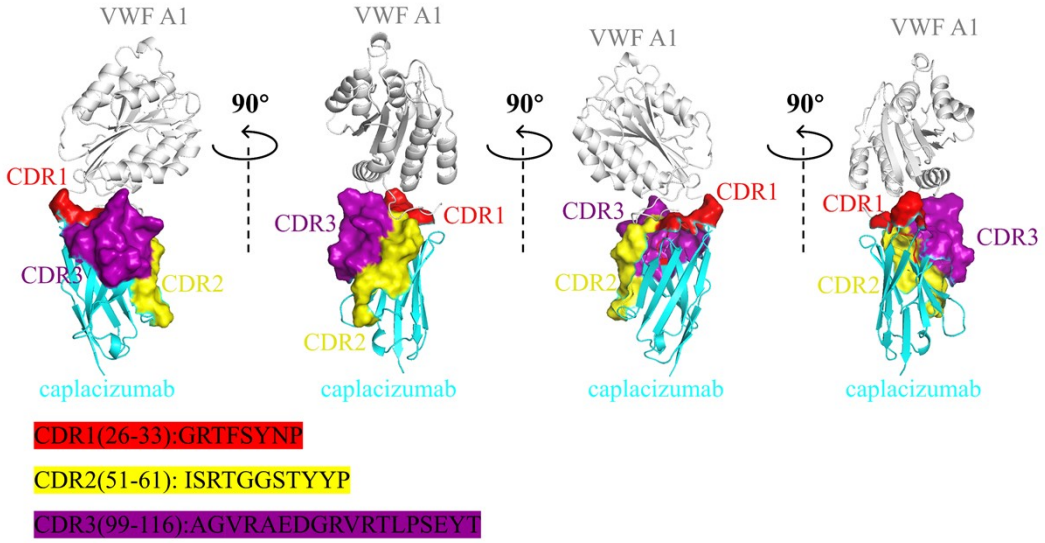

Figure S4. map of Caplacizumab and VWF A1 binding regions. The binding interface in the Caplacizumab/VWF A1 system contains three CDR regions of caplacizumab, CDR1 (red), CDR2 (yellow) and CDR3 (purple).

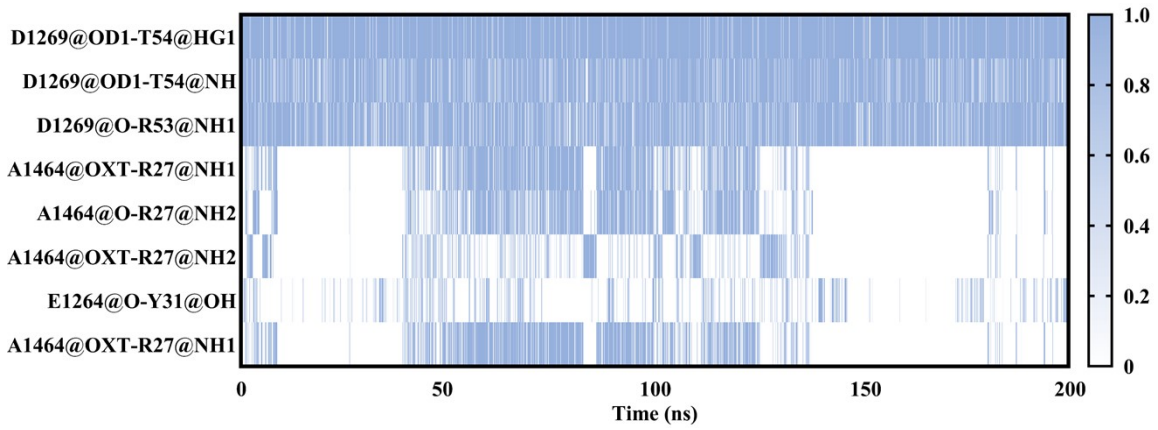

Figure S5. Stabilizing hydrogen bonds formed by caplacizumab with VWF A1.

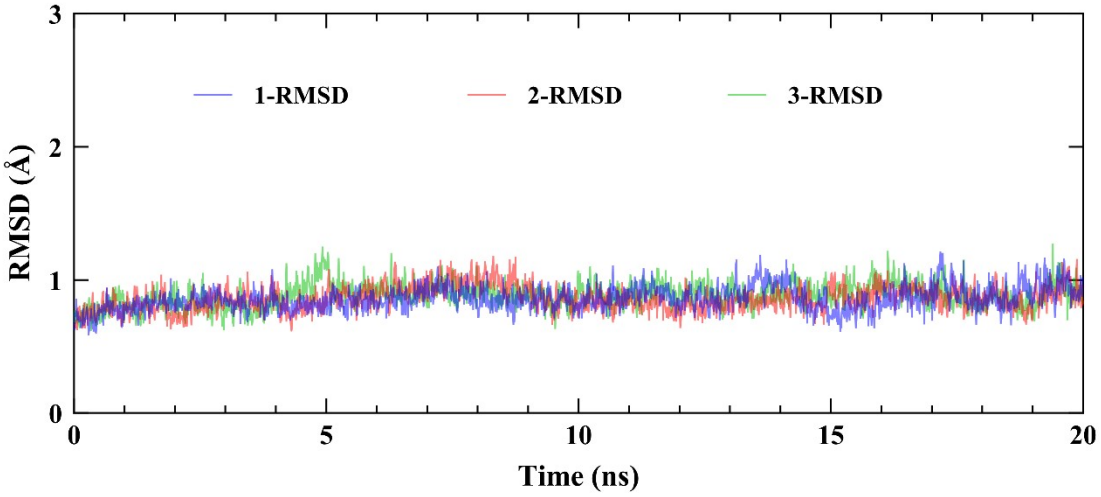

Figure S6. Four amino acids were mutated simultaneously (R27E D105W T54F T28V) and three MD simulations were performed with an RMSD of 20 ns.

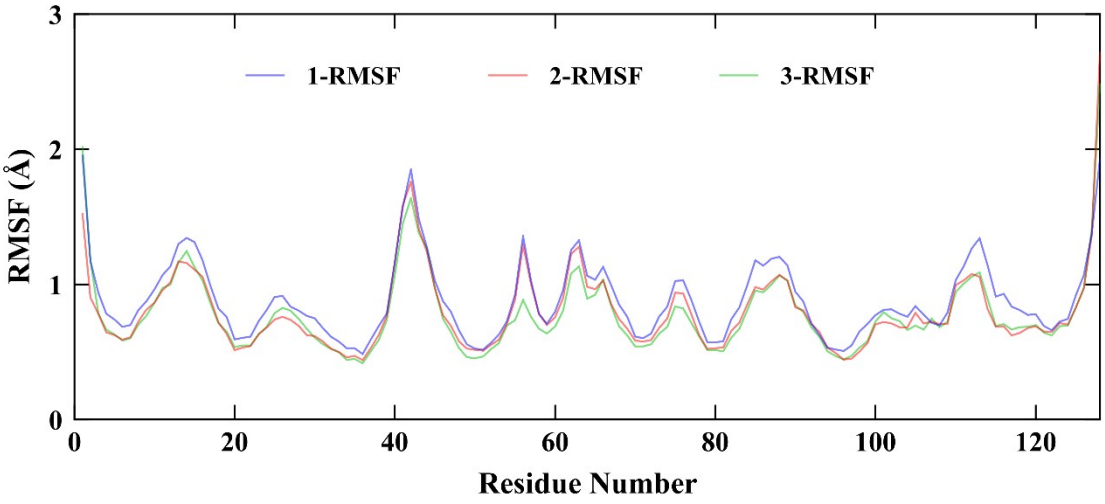

Figure S7. Four amino acids were mutated simultaneously (R27E D105W T54F T28V) and three MD simulations were performed with an RMSF of 20 ns.

Tables

Table S1. The proportion of hydrogen bonds between VWF A1 and GPIb in the VWF A1-GPIb and VWF A1-GPIb-caplacizumab systems.

Name	acceptor	donor	occupancy(VWF A1-GPIb)	occupancy(VWF A1/GPIb-caplacizumab)
H1	D18@OD2	R1334@NE	83.10%	18.30%
H2	D18@OD1	R1334@NH2	70.60%	20.70%
H3	T176@OG1	Q1367@NE2	66.10%	66.30%
H4	D235@OD1	R1342@NH1	66.00%	26.40%
H5	K237@O	A1327@N	59.20%	42.40%
H6	E1359@OE1	Y228@OH	55.10%	38.80%

Table S2. The proportion of hydrogen bonds between VWF A1 and GPIb in the VWF A1-caplacizumab and VWF A1-GPIb-caplacizumab systems.

acceptor	donor	occupancy(caplacizumab-VWF A1)	occupancy(VWF A1-GPIb-caplacizumab)
D1269@OD1	T54@OG1	95.60%	94.91%
D1269@OD1	T54@N	84.60%	85.80%
D1269@O	R53@NH1	84.30%	84.91%
A1464@OXT	R27@NH1	33.90%	9.90%
A1464@O	R27@NH2	31.30%	18.87%
A1464@OXT	R27@NH2	15.80%	13.99%
E1264@O	Y31@OH	15.70%	5.25%
A1464@O	R27@NH1	13.90%	17.35%
E104@O	Y1271@N	53.43%	62.00%

Table S3. Contribution of VWF A1-bound interfacial residue energies to the binding free energy(kcal/mol) in the VWF/GPIb system.

Residue	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta E_{GB}$	$\Delta\Delta E_{NP}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$
1275LEU	1.0±0.22	-0.2±0.01	0.0±0.04	0.1±0.03	0.9±0.22	-0.3±0.07	0.6±0.21
1312LYS	1.6±0.25	15.7±0.92	-13.8±0.80	0.4±0.05	3.9±0.40	-3.7±1.38	0.2±1.33
1313TRP	2.4±0.43	-1.4±0.30	-0.1±0.04	0.3±0.06	1.1±0.18	-0.7±0.05	0.4±0.18
1323ASP	0.7±0.34	-6.2±0.99	5.3±0.84	0.1±0.03	0.0±0.20	-0.8±0.48	-0.8±0.66
1325SER	0.4±0.16	0.1±0.32	-0.2±0.27	0.0±0.01	0.3±0.23	0.0±0.15	0.3±0.35
1326HIE	2.6±0.32	0.7±0.85	-1.1±0.61	0.1±0.04	2.4±0.45	-1.1±0.68	1.3±0.26
1328TYR	3.9±0.08	0.3±0.06	-0.4±0.07	0.2±0.01	4.0±0.08	-0.8±0.43	3.2±0.46
1329ILE	0.2±0.01	0.2±0.01	-0.2±0.00	0.0±0.00	0.1±0.01	0.0±0.04	0.1±0.03
1334ARG	6.7±0.38	23.4±0.98	-20.0±0.78	1.1±0.03	11.1±0.20	-3.3±0.47	7.8±0.34
1335LYS	0.5±0.07	12.6±0.11	-11.2±0.09	0.0±0.01	2.0±0.06	-1.0±0.23	1.0±0.28
1336ARG	0.2±0.02	10.5±0.15	-9.3±0.13	0.0±0.00	1.4±0.02	-0.4±0.06	1.0±0.07
1339GLU	0.3±0.01	-12.3±0.08	10.8±0.07	0.0±0.00	-1.2±0.02	-0.2±0.03	-1.5±0.02
1342ARG	-1.4±0.29	15.6±0.13	-12.8±0.10	0.2±0.00	1.7±0.24	-2.2±0.56	-0.4±0.79
1343ILE	1.6±0.03	0.3±0.01	-0.6±0.04	0.1±0.01	1.4±0.01	-0.2±0.09	1.1±0.08
1359GLU	0.6±0.48	-9.5±1.55	8.7±0.97	0.5±0.10	0.3±0.47	-2.5±0.97	-2.2±1.00
1362LYS	2.9±0.18	18.8±0.38	-16.0±0.38	0.6±0.01	6.4±0.18	-2.7±0.36	3.7±0.39
1363TYR	2.6±0.64	0.2±0.04	-0.5±0.03	0.2±0.04	2.4±0.66	-0.4±0.09	2.0±0.67
1366PHE	4.2±0.19	1.0±0.21	-2.2±0.18	0.4±0.02	3.3±0.17	-1.1±0.92	2.2±1.08
1367GLN	4.4±0.07	5.0±0.27	-3.2±0.19	0.6±0.02	6.7±0.10	-1.5±0.65	5.2±0.57
1370SER	0.3±0.17	0.7±0.59	-0.9±0.42	0.1±0.02	0.2±0.09	-1.1±0.44	-0.9±0.42
1371LYS	1.4±1.04	22.1±1.95	-19.3±1.53	0.5±0.05	4.6±0.62	-5.2±1.39	-0.5±2.00
1373ASP	0.2±0.04	-13.7±0.46	12.0±0.32	0.0±0.06	-1.4±0.25	-0.6±0.07	-2.0±0.25
1374ARG	0.6±0.13	10.8±0.39	-9.6±0.35	0.0±0.01	1.9±0.17	-0.5±0.17	1.3±0.13
1376GLU	0.7±0.60	-6.0±1.26	5.3±1.09	0.2±0.16	0.1±0.90	-1.4±1.06	-1.3±0.29
1395ARG	0.1±0.88	13.8±1.98	-11.9±1.45	0.2±0.08	2.3±0.28	-3.0±0.69	-0.8±0.52
1396ASN	0.2±0.02	0.9±0.84	-1.0±0.79	0.0±0.01	0.1±0.07	-1.5±1.12	-1.4±1.07
1399ARG	0.2±0.02	11.0±0.26	-9.7±0.23	0.0±0.00	1.5±0.05	-1.0±0.24	0.5±0.19
1400TYR	0.5±0.01	-0.2±0.08	0.1±0.08	0.0±0.00	0.4±0.01	-0.1±0.02	0.3±0.02
1465PRO	1.1±0.11	0.0±0.15	-0.3±0.09	0.1±0.01	0.9±0.05	-0.3±0.13	0.7±0.16
TOTAL	40.6±1.28	114.0±6.86	-102.1±5.41	6.1±0.27	58.7±1.99	-37.5±2.64	21.2±1.78

Table S4. Contribution of VWF A1 binding interface residue energies to the binding free energy(kcal/mol) in the VWF/caplacizumab system.

Residue	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta E_{GB}$	$\Delta\Delta E_{NP}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$
1463GLU	2.2±0.4	10.8±1.5	-9.7±1.2	0.3±0.1	3.6±0.4	-2.0±0.8	1.6±1.2
1459ASP	-1.1±0.6	11.2±0.9	-9.1±0.6	0.2±0.0	1.1±0.2	-2.4±0.2	-1.3±0.0
1458CYS	0.5±0.2	0.0±0.3	-0.1±0.3	0.1±0.0	0.5±0.2	-0.6±0.2	0.0±0.4
1455SER	-0.1±0.8	1.9±2.3	-1.3±1.1	0.1±0.0	0.6±0.4	-0.6±0.6	0.0±0.2
1313TRP	3.0±1.1	1.6±0.9	-2.2±0.8	0.3±0.1	2.6±1.4	-2.3±0.7	0.3±1.9
1312LYS	0.1±0.0	-7.2±0.6	6.3±0.5	0.0±0.0	-0.8±0.1	-0.6±0.3	-1.4±0.2
1308ARG	2.4±0.8	-9.0±0.6	7.8±0.6	0.2±0.1	1.4±0.9	-0.9±0.4	0.5±0.5
1275LEU	0.5±0.3	-0.5±0.2	0.2±0.1	0.0±0.0	0.2±0.2	-0.1±0.1	0.1±0.1
1274ARG	0.7±0.4	-6.9±2.4	6.2±1.8	0.1±0.1	0.2±0.4	-1.5±1.2	-1.4±0.7
1273SER	0.5±0.2	-0.2±0.2	0.1±0.1	0.0±0.0	0.5±0.2	-0.1±0.1	0.4±0.3
1272CYS	0.2±0.1	-0.1±0.1	0.0±0.1	0.0±0.0	0.1±0.1	-0.1±0.0	0.1±0.1
1271TYR	5.2±0.2	0.4±0.1	-0.8±0.1	0.3±0.0	5.0±0.2	-0.2±0.1	4.8±0.2
1270PHE	4.8±0.3	0.2±0.5	-1.5±0.6	0.4±0.0	3.9±0.3	-0.7±0.3	3.2±0.6
1269ASP	2.2±0.3	16.0±0.4	-13.3±0.3	0.3±0.0	5.3±0.3	-2.0±0.7	3.3±1.0
1268HIE	0.3±0.2	1.0±0.3	-1.0±0.2	0.0±0.0	0.3±0.3	-0.1±0.1	0.3±0.2
1267LEU	0.1±0.0	0.6±0.1	-0.6±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.1±0.0
1266PRO	3.6±0.1	-0.7±0.0	0.1±0.1	0.2±0.0	3.1±0.0	-0.5±0.3	2.6±0.3
1265PRO	1.1±0.2	0.0±0.1	-0.2±0.0	0.0±0.0	0.9±0.2	-0.3±0.0	0.7±0.2
1264GLU	0.1±0.0	7.3±0.2	-6.4±0.2	0.0±0.0	1.0±0.1	-0.6±0.4	0.5±0.4
1263SER	0.3±0.3	0.1±0.1	-0.1±0.1	0.1±0.0	0.3±0.3	-0.2±0.2	0.1±0.2
TOTAL	26.7±3.1	26.5±2.8	-25.7±1.5	2.6±0.2	30.1±2.4	-15.7±0.9	14.4±3.2

Table S5. Binding free energy contributions (kcal/mol) in the caplacizumab/VWF A1 system. Values include standard deviation.

Residue	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta E_{GB}$	$\Delta\Delta E_{NP}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$
27ARG	1.9±1.4	-1.4±0.4	1.2±0.4	0.3±0.2	2.0±1.7	-4.2±3.8	-2.3±2.1
28THR	1.1±0.5	1.4±0.1	-1.4±0.1	0.1±0.0	1.3±0.5	-0.8±0.4	0.5±0.9
29PHE	0.3±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.3±0.1	-0.1±0.0	0.2±0.1
30SER	0.6±0.1	1.1±1.4	-1.5±1.5	0.1±0.1	0.3±0.1	-0.5±0.3	-0.2±0.2
31TYR	6.5±0.3	1.8±0.7	-2.3±0.5	0.6±0.0	6.6±0.1	-2.6±0.6	4.0±0.6
52SER	0.4±0.0	2.3±0.0	-2.0±0.0	0.0±0.0	0.7±0.0	-0.4±0.0	0.2±0.0
53ARG	5.9±0.5	4.7±0.6	-3.6±0.5	0.3±0.0	7.4±0.4	-1.8±0.2	5.6±0.6
54THR	0.9±0.2	4.1±0.1	-1.8±0.0	0.2±0.0	3.4±0.1	-1.4±0.1	2.0±0.1
57SER	0.0±0.0	0.6±0.0	-0.6±0.0	0.0±0.0	0.0±0.0	-0.2±0.0	-0.2±0.0
74ASN	1.1±0.1	0.9±0.3	-1.2±0.3	0.1±0.0	0.9±0.1	-0.6±0.2	0.3±0.3
77ARG	0.9±0.5	-5.8±1.0	4.9±0.9	0.1±0.1	0.1±0.6	-0.8±0.3	-0.7±0.7
102ARG	3.3±1.0	8.7±1.5	-7.2±1.0	0.6±0.1	5.3±1.6	-2.5±0.6	2.8±1.0
104GLU	2.5±0.6	-0.8±0.8	1.2±0.8	0.5±0.0	3.4±0.6	-2.4±0.3	1.0±0.8
105ASP	0.7±0.1	-6.0±1.0	5.3±0.8	0.0±0.0	0.0±0.1	-0.3±0.2	-0.3±0.2
107ARG	1.0±0.2	6.5±1.2	-5.7±1.0	0.0±0.0	1.8±0.3	-0.9±0.1	0.9±0.2
TOTAL	27.0±0.8	17.9±4.5	-14.5±3.9	2.9±0.2	33.3±1.3	-19.5±2.5	13.8±3.5

Table S6. Major hydrogen bonds in the caplacizumab/VWF A1 system with hydrogen bonding stability above 50%.

acceptor	donor	occupancy
D1269@OD1	T54@OG1	95.6%
D1269@OD1	T54@N	84.6%
D1269@O	R53@NH1	84.3%
A1464@OXT	R27@NH1	33.9%
A1464@O	R27@NH2	31.3%
A1464@OXT	R27@NH2	15.8%
E1264@O	Y31@OH	15.7%
A1464@O	R27@NH1	13.9%

Table S7. binding free energy (kcal/mol) of single point saturation mutations.

54	ΔH	-TΔS	ΔG
H	-29.8±1.6	13.5±1.7	-16.3±1.5
D	-27.7±2.6	16.1±5.2	-11.6±3.1
R	-31.9±3.2	14.2±1.5	-17.8±4.6
F	-31.3±4.9	13.2±2.9	-18.1±7.2
A	-30.2±1.4	12.1±0.9	-18.1±0.5
C	-31.1±1.5	14.2±1.1	-16.9±1.3
G	-29.1±1.8	15.0±4.3	-14.1±4.2
Q	-29.8±2.4	12.2±0.9	-17.6±2.4
E	-27.3±0.9	12.9±3.7	-14.4±4.2
K	-30.7±3.3	14.6±2.8	-16.1±6.1
L	-29.3±2.6	13.6±2.2	-15.8±0.7
M	-30.4±2.1	14.9±2.8	-15.5±2.4
N	-29.5±4.2	13.7±1.5	-15.8±5.4
S	-28.4±2.1	13.9±7.0	-14.5±7.5
Y	-27.2±2.8	15.4±0.3	-11.8±2.6
I	-29.6±1.1	13.5±1.6	-16.1±2.0
W	-25.9±2.9	10.9±0.7	-15.1±3.2
P	-29.2±1.8	11.7±2.0	-17.5±3.2
V	-30.6±0.7	13.1±2.3	-17.6±1.9
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

57	ΔH	-TΔS	ΔG
H	-31.6±2.4	11.6±1.3	-20.0±3.0
D	-30.5±1.3	12.5±2.8	-18.0±4.1
R	-29.8±1.1	13.1±2.6	-16.7±2.5
F	-31.0±2.8	12.8±2.7	-18.2±3.1
A	-30.8±2.6	11.0±2.1	-19.8±4.7
C	-31.6±2.2	11.4±0.7	-20.2±1.6
G	-30.8±1.0	12.9±1.5	-17.9±1.6
Q	-28.7±1.6	13.6±1.4	-15.2±2.0
E	-28.3±1.3	12.7±1.4	-15.7±2.4
K	-28.5±2.1	11.9±0.5	-16.7±2.4
L	-31.9±2.0	14.6±1.7	-17.3±3.0
M	-27.5±2.7	12.0±1.9	-15.5±0.9
N	-31.2±2.4	12.6±1.5	-18.6±2.6
Y	-30.8±1.9	14.1±2.4	-16.7±1.5
T	-30.1±0.8	13.2±2.2	-17.0±1.4
I	-29.9±1.2	13.7±1.8	-16.2±2.6
W	-30.4±1.3	13.8±4.7	-16.6±5.7
P	-30.8±1.2	12.9±0.2	-18.0±1.1
V	-30.9±1.2	13.0±2.7	-17.9±1.5
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

27	ΔH	-TΔS	ΔG
H	-29.0±3.5	11.7±1.4	-17.4±4.1
D	-30.1±2.6	15.7±0.4	-14.4±2.2
F	-30.6±1.9	12.7±2.4	-17.9±2.2
A	-27.9±2.1	11.1±0.9	-16.7±2.6
C	-29.9±0.7	12.4±2.3	-17.5±2.8
G	-29.0±1.2	13.8±2.4	-15.2±3.3
Q	-29.8±3.7	12.8±0.6	-17.0±3.6
E	-32.6±0.6	15.7±1.7	-16.9±1.1
K	-29.1±0.6	12.6±0.5	-16.5±0.5
L	-27.7±0.8	11.6±1.5	-16.1±2.3
M	-29.5±1.7	14.0±3.2	-15.5±3.2
N	-30.3±2.1	14.4±3.9	-15.9±2.1
S	-28.4±1.7	12.2±1.9	-16.1±3.5
Y	-32.0±0.6	13.8±0.4	-18.1±0.2
T	-28.1±0.6	13.6±4.0	-14.5±3.5
I	-27.4±3.2	11.2±0.7	-16.2±3.8
W	-28.6±2.3	11.6±4.2	-17.0±5.9
P	-28.7±1.6	13.2±0.7	-15.6±2.0
V	-30.5±1.8	11.7±2.1	-18.9±2.6
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

52	ΔH	-TΔS	ΔG
H	-26.8±1.2	11.3±1.4	-15.5±0.4
D	-26.3±1.1	14.5±5.0	-11.9±4.8
R	-29.5±0.8	14.4±2.5	-15.1±2.2
F	-28.2±2.8	13.6±0.5	-14.6±3.2
A	-29.0±1.7	12.7±1.0	-16.3±1.6
C	-31.0±5.0	12.3±1.3	-18.7±5.4
G	-28.4±0.9	13.8±2.4	-14.5±2.0
Q	-26.4±1.7	11.3±1.4	-15.1±2.2
E	-28.4±2.1	14.3±2.4	-14.1±0.8
K	-28.6±1.9	14.4±1.8	-14.2±3.0
L	-29.0±1.0	13.0±0.8	-16.0±1.2
M	-26.8±2.9	12.0±4.6	-14.7±2.8
N	-26.4±1.1	13.9±2.3	-12.6±3.3
Y	-24.1±5.3	17.0±1.6	-7.0±6.4
T	-29.4±2.0	16.7±0.5	-12.7±2.0
I	-27.0±1.2	13.6±0.5	-13.4±0.8
W	597.1±4.5	49.5±4.2	646.7±8.7
P	-29.0±3.9	11.9±2.0	-17.2±2.6
V	-27.5±0.6	11.8±4.9	-15.7±4.5
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

74	ΔH	-TΔS	ΔG
H	-29.6±3.2	13.3±4.2	-16.3±6.3
D	-27.5±2.3	15.9±4.4	-11.7±5.3
R	-27.4±1.4	12.9±1.6	-14.4±2.2
F	-27.9±2.7	13.1±3.8	-14.7±3.6
A	-29.9±4.7	12.7±0.1	-17.2±4.5
C	-25.1±2.3	13.5±1.5	-11.6±1.4
G	-27.1±0.7	13.2±1.2	-13.9±0.6
Q	-29.9±2.0	16.5±3.7	-13.3±2.0
E	-28.3±2.3	14.7±2.0	-13.6±3.6
K	-28.4±0.4	13.4±1.2	-15.0±0.8
L	-28.1±0.9	15.5±0.9	-12.6±0.4
M	-27.0±2.9	13.8±1.0	-13.1±3.1
S	-28.6±2.5	13.5±2.9	-15.2±5.1
Y	-26.4±2.7	12.0±3.2	-14.4±2.5
T	-26.5±0.7	11.2±0.2	-15.2±0.8
I	-26.0±1.4	12.9±3.3	-13.1±2.9
W	-27.7±1.7	12.8±1.3	-14.9±0.9
P	-25.5±2.1	12.8±1.2	-12.6±1.0
V	-28.0±2.0	13.4±0.8	-14.6±2.6
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

77	ΔH	-TΔS	ΔG
H	-29.9±1.2	14.0±1.8	-15.9±1.3
D	-28.3±0.5	11.1±1.1	-17.2±1.6
F	-28.0±1.0	13.7±0.6	-14.3±1.7
A	-29.0±1.0	11.6±0.8	-17.4±1.3
C	-28.4±5.4	11.9±2.8	-16.5±6.3
G	-29.7±1.3	14.6±2.6	-15.1±1.9
Q	-28.9±0.9	12.2±1.3	-16.7±0.4
E	-27.7±0.2	11.8±1.4	-15.8±1.4
K	-28.5±1.0	14.7±1.8	-13.8±2.5
L	-26.3±2.5	10.2±1.7	-16.0±2.8
M	-29.2±3.3	12.9±2.3	-16.3±2.3
N	-28.5±1.1	12.1±2.6	-16.3±1.7
S	-29.5±1.4	10.5±2.2	-19.0±2.5
Y	-30.4±0.9	13.0±0.9	-17.4±0.1
T	-28.8±0.4	11.3±1.2	-17.6±1.4
I	-28.1±0.7	11.7±2.1	-16.3±2.9
W	-28.1±1.8	13.4±2.4	-14.7±3.0
P	-26.9±1.0	12.2±0.3	-14.8±0.6
V	-28.9±2.2	13.0±1.7	-15.9±3.9
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

28	ΔH	-TΔS	ΔG
H	-30.1±1.6	13.6±0.9	-16.5±1.5
D	-28.2±1.6	15.4±3.0	-12.8±2.8
R	-28.8±3.4	13.8±5.7	-15.0±8.1
F	-27.1±1.8	14.9±1.8	-12.1±0.8
A	-29.2±1.7	13.7±3.2	-15.5±2.0
C	-28.8±1.0	16.1±2.4	-12.6±3.3
G	-28.7±2.8	12.5±0.4	-16.2±3.2
Q	-26.9±1.5	12.5±1.7	-14.4±3.2
E	-29.3±2.9	12.8±1.0	-16.5±3.9
K	-30.7±4.3	14.0±2.0	-16.6±5.5
L	-31.3±1.7	12.5±2.4	-18.8±3.7
M	-29.0±1.2	12.6±0.9	-16.4±2.1
N	-29.2±1.1	14.0±4.8	-15.3±5.3
S	-27.9±1.9	13.1±1.7	-14.9±2.0
Y	-29.4±0.5	15.5±2.6	-13.9±2.7
I	-29.0±1.3	12.5±1.6	-16.5±1.6
W	-30.4±2.6	14.1±1.6	-16.3±2.2
P	-29.4±1.2	14.0±1.3	-15.4±2.5
V	-31.3±2.2	13.9±1.6	-17.3±2.0
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

30	ΔH	-TΔS	ΔG
H	-28.5±0.6	13.1±0.9	-15.4±1.4
D	-23.6±0.6	14.9±1.4	-8.7±2.0
R	-28.5±2.4	14.7±0.8	-13.8±1.6
F	-29.9±1.1	14.3±3.4	-15.6±2.9
A	-25.7±2.8	13.0±1.5	-12.7±3.6
C	-26.1±0.9	13.2±0.4	-12.9±1.0
G	-27.9±1.8	15.3±1.3	-12.6±0.5
Q	-30.4±0.2	14.8±0.5	-15.6±0.4
E	-31.3±2.8	14.5±0.5	-16.8±3.3
K	-29.8±3.2	14.1±2.5	-15.7±1.9
L	-26.7±3.3	10.4±1.4	-16.2±2.8
M	-31.6±2.3	13.0±2.0	-18.5±2.9
N	-26.5±1.5	12.7±1.6	-13.8±1.5
Y	-30.8±0.6	14.2±1.5	-16.6±1.5
T	-26.1±1.1	10.5±0.6	-15.6±1.1
I	-26.9±2.1	12.5±3.3	-14.4±3.1
W	-26.6±0.6	12.0±3.4	-14.5±2.9
P	-27.9±2.2	13.9±3.4	-13.9±3.1
V	-26.4±1.8	12.6±3.6	-13.8±2.4
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

29	ΔH	-TΔS	ΔG
H	-28.1±2.7	14.0±2.9	-14.1±1.9
D	-28.1±0.9	12.7±1.1	-15.4±0.7
R	-28.8±0.5	13.9±2.6	-14.8±3.1
A	-27.4±0.7	12.8±2.4	-14.7±2.2
C	-27.9±2.0	13.9±1.4	-14.0±2.3
G	-27.7±0.3	15.5±1.7	-12.2±2.0
Q	-29.5±2.9	14.0±2.3	-15.5±2.5
E	-29.8±0.4	10.1±1.7	-19.7±1.5
K	-25.7±1.1	11.2±1.0	-14.5±2.0
L	-26.8±1.9	12.9±0.7	-13.9±2.3
M	-28.5±4.4	12.6±1.4	-15.8±5.8
N	-27.2±1.2	14.9±3.0	-12.3±3.5
S	-27.6±1.0	13.6±2.2	-14.0±1.8
Y	-27.3±2.1	12.7±2.5	-14.7±3.7
T	-27.4±2.1	12.8±2.2	-14.6±4.3
I	-26.7±3.0	12.6±0.4	-14.1±2.7
W	-30.3±4.3	13.8±3.2	-16.5±1.9
P	-29.4±0.7	11.9±1.4	-17.4±2.1
V	-28.4±1.4	13.4±3.5	-15.1±2.2
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

102	ΔH	-TΔS	ΔG
H	-27.6±2.7	10.9±0.4	-16.7±2.8
D	-28.3±2.1	14.4±4.9	-13.9±3.3
F	-26.3±0.2	13.8±4.2	-12.6±4.0
A	-25.7±2.2	9.0±1.4	-16.6±0.9
C	-26.4±1.8	11.6±2.7	-14.8±4.5
G	-26.3±1.1	12.8±1.1	-13.5±0.5
Q	-27.0±2.0	12.2±2.8	-14.8±0.9
E	-27.8±1.0	18.6±1.8	-9.2±2.7
K	-26.4±0.6	13.9±1.2	-12.5±0.9
L	-27.1±0.9	10.9±1.9	-16.2±2.4
M	-27.4±0.8	12.1±2.1	-15.3±1.7
N	-29.3±2.9	11.9±1.1	-17.4±1.9
S	-26.8±1.2	12.5±1.1	-14.2±1.4
Y	-27.1±1.3	11.2±1.1	-15.9±0.3
Y	-28.0±2.4	10.2±0.4	-17.8±2.7
I	-27.0±0.8	10.0±0.9	-17.0±1.6
W	-26.5±0.5	14.8±1.5	-11.7±1.9
P	-25.8±4.2	14.1±1.9	-11.7±2.6
V	-27.3±2.0	11.8±3.2	-15.5±1.3
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

107	ΔH	-TΔS	ΔG
H	-26.8±1.5	14.8±1.3	-11.9±1.9
D	-27.5±2.0	13.5±2.7	-14.0±4.0
F	-26.4±1.1	11.7±1.3	-14.7±1.9
A	-28.3±3.3	14.0±0.5	-14.3±3.7
C	-28.0±1.7	14.2±1.9	-13.8±1.0
G	-26.0±1.9	12.3±2.3	-13.7±1.6
Q	-27.7±1.5	15.2±1.3	-12.5±2.6
E	-26.4±2.0	12.8±0.9	-13.5±2.5
K	-29.5±1.8	15.3±1.9	-14.2±2.5
L	-27.5±1.7	14.3±2.2	-13.2±2.9
M	-26.4±0.3	14.9±1.8	-11.5±1.5
N	-26.9±2.7	12.9±1.0	-14.0±3.4
S	-24.4±2.4	12.5±1.8	-11.9±2.9
Y	-26.2±2.5	12.8±2.3	-13.4±0.8
T	-27.9±4.1	13.1±1.9	-14.8±2.3
I	-27.9±1.1	12.0±2.3	-15.9±1.4
W	-24.9±3.0	12.4±0.1	-12.5±2.9
P	-27.7±4.5	15.4±1.1	-12.3±3.6
V	-26.6±1.2	10.3±2.3	-16.4±1.2
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

105	ΔH	-TΔS	ΔG
H	-30.7±3.4	14.0±1.3	-16.6±2.1
R	-29.3±1.2	14.5±4.0	-14.8±2.9
F	-29.7±2.7	13.5±0.2	-16.2±2.9
A	-27.7±0.4	12.9±1.2	-14.7±1.4
C	-28.5±2.6	17.2±2.2	-11.3±0.8
G	-28.3±1.2	14.1±0.8	-14.3±0.5
Q	-29.2±0.6	14.6±1.0	-14.6±1.5
E	-28.7±2.4	16.3±0.9	-12.4±2.6
K	-29.3±1.3	15.1±1.6	-14.1±1.2
L	-28.4±1.9	13.5±1.7	-14.8±3.6
M	-29.3±1.4	14.8±1.4	-14.5±1.9
N	-27.6±1.9	14.3±1.8	-13.2±2.9
S	-28.2±0.9	14.7±1.9	-13.5±1.3
Y	-31.0±2.3	13.9±2.7	-17.2±4.1
T	-26.7±1.0	13.4±0.9	-13.3±0.9
I	-28.6±0.9	13.9±1.3	-14.7±1.8
W	-32.9±2.9	17.0±2.7	-15.8±1.3
P	-28.6±1.0	12.6±2.9	-16.0±2.3
V	-27.3±0.8	13.1±1.3	-14.2±0.7
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

104	ΔH	-TΔS	ΔG
H	-29.1±1.4	12.0±1.9	-17.2±0.9
D	-27.6±1.1	14.2±4.2	-13.4±3.1
R	-27.4±2.2	12.5±0.8	-15.0±2.4
F	-27.9±1.6	13.2±2.6	-14.7±4.2
A	-30.4±0.8	15.0±2.8	-15.4±3.4
C	-28.2±2.3	11.1±4.4	-17.1±6.2
G	-30.8±0.1	13.8±1.1	-17.1±1.2
Q	-31.2±2.3	13.1±2.2	-18.2±2.6
K	-31.3±0.7	14.2±2.0	-17.1±1.7
L	-27.3±0.6	9.6±1.2	-17.7±1.4
M	-29.8±3.1	15.3±1.4	-14.6±1.9
N	-30.6±2.2	14.5±0.9	-16.1±2.3
S	-30.5±0.2	12.3±0.5	-18.2±0.6
Y	-29.1±1.3	10.9±2.9	-18.2±1.6
T	-29.5±1.4	14.5±1.4	-15.0±0.7
I	-28.8±2.3	13.4±3.1	-15.5±2.9
W	-29.4±3.0	11.5±4.1	-17.8±6.9
P	-29.0±1.5	10.0±0.9	-19.0±2.1
V	-29.0±1.8	10.5±2.0	-18.5±1.5
WT	-30.1±2.4	15.7±0.9	-14.4±3.2

Table S8. Contribution of wild-type caplacizumab binding interface residues to binding free energy.

Residue	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta E_{GB}$	$\Delta\Delta E_{NP}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$
53ARG	6.48	3.98	-2.97	0.36	7.86	-1.53	6.32
31TYR	6.78	0.99	-1.76	0.61	6.62	-1.92	4.70
54THR	1.07	3.96	-1.78	0.18	3.43	-1.36	2.08
102ARG	2.13	6.91	-6.04	0.39	3.40	-1.80	1.60
107ARG	0.93	5.14	-4.55	0.05	1.57	-0.89	0.68
74ASN	1.17	1.23	-1.50	0.06	0.97	-0.31	0.66
29PHE	0.31	-0.01	0.07	0.00	0.38	-0.11	0.27
52SER	0.37	2.26	-1.93	0.00	0.69	-0.45	0.24
30SER	0.45	-0.52	0.31	0.01	0.24	-0.18	0.06
104GLU	1.85	0.09	0.26	0.50	2.70	-2.67	0.03
105ASP	0.67	-4.93	4.34	0.02	0.11	-0.11	0.01
57SER	0.03	0.52	-0.52	0.00	0.04	-0.20	-0.16
28THR	0.52	1.47	-1.40	0.08	0.66	-1.25	-0.59
77ARG	0.47	-6.81	5.86	0.02	-0.45	-0.98	-1.43
27ARG	3.44	-1.67	1.51	0.55	3.83	-8.49	-4.66
Total	26.67	12.62	-10.10	2.85	32.04	-22.24	9.80

Table S9. Contribution of binding interface residues to the binding free energy of the T54R mutant of caplacizumab.

Residue	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta E_{GB}$	$\Delta\Delta E_{NP}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$
53ARG	6.44	4.83	-3.71	0.33	7.89	-1.62	6.27
54ARG	6.46	8.38	-7.25	0.59	8.19	-5.01	3.17
31TYR	5.76	1.19	-1.74	0.50	5.71	-2.64	3.07
107ARG	1.92	7.12	-6.31	0.14	2.87	-1.01	1.86
102ARG	1.49	10.24	-8.20	0.47	4.00	-2.58	1.42
104GLU	3.30	-4.59	4.57	0.32	3.60	-2.44	1.16
30SER	0.62	-0.99	0.67	0.02	0.32	0.06	0.38
29PHE	0.28	-0.08	0.18	0.00	0.39	-0.02	0.36
105ASP	1.29	-6.66	5.83	0.06	0.52	-0.20	0.32
52SER	0.36	2.45	-2.13	0.01	0.69	-0.48	0.21
74ASN	1.09	0.87	-1.13	0.05	0.89	-0.85	0.03
57SER	0.02	0.41	-0.42	0.00	0.01	-0.29	-0.28
28THR	-0.68	3.21	-2.22	0.09	0.40	-1.80	-1.40
27ARG	1.71	-1.89	1.70	0.26	1.77	-3.25	-1.48
77ARG	0.48	-6.11	5.25	0.04	-0.34	-1.32	-1.66
Total	30.55	18.37	-14.90	2.88	36.90	-23.45	13.45

Table S10. binding free energy (kcal/mol) of double point saturation mutations. Values include standard deviation.

Mut		ΔH	-TΔS	ΔG
R27E	D105W	-35.4±0.5	22.8±2.9	-12.5±2.8
R27E	E104K	-33.7±1.1	17.3±2.9	-16.4±1.9
D105W	E104K	-33.1±1.5	16.5±1.5	-16.6±2.6
S57L	E104K	-32.6±3.7	13.1±2.4	-19.6±4.3
T54R	T28L	-32.6±0.6	15.5±1.2	-17.1±0.7
R27E	S57L	-32.4±1.7	17.4±1.7	-15.0±3.3
R27E	S52C	-32.2±0.3	15.9±2.4	-16.3±2.1
T54F	T28V	-30.9±1.8	13.1±1.3	-17.8±1.1
T54F	T28W	-30.9±4.0	11.6±1.6	-19.3±2.5
T54R	T28W	-30.8±1.1	16.4±2.2	-14.5±2.4
S30M	T54F	-30.8±4.3	11.5±1.4	-19.3±4.0
T54R	T28V	-30.6±1.0	18.4±1.5	-12.1±0.5
S30M	T28V	-30.5±1.2	13.2±1.6	-17.3±1.2
S30M	T54R	-30.3±2.1	16.7±5.2	-13.6±3.2
S57L	D105W	-30.3±1.5	13.2±1.8	-17.1±2.6
S30Y	T28L	-30.2±1.6	12.0±2.3	-18.2±1.8
S30E	T54R	-30.1±1.2	17.2±3.5	-12.9±3.7
E104K	S52C	-30.1±0.5	12.0±1.4	-18.2±1.4
T54R	T28K	-30.1±1.3	17.5±5.6	-12.6±6.5
WT		-30.1±2.4	15.7±0.9	-14.4±3.2
S30E	T28W	-30.0±1.6	12.2±3.5	-17.8±5.0
S30E	T28K	-30.0±4.4	14.3±1.5	-15.7±3.0
S30E	T54F	-29.9±4.4	12.5±1.7	-17.4±3.9
T54F	T28L	-29.8±2.6	13.3±4.1	-16.5±5.0
D105W	S52C	-29.8±2.9	14.6±3.9	-15.2±1.5
T54F	T28K	-29.3±4.4	14.6±2.1	-14.7±4.1
S30Y	T54F	-29.2±0.6	11.5±1.2	-17.7±1.8
S30Y	T28K	-29.1±1.1	12.5±0.5	-16.7±1.5
S57L	S52C	-28.9±2.7	10.9±3.1	-17.9±1.9
S30E	T28L	-28.7±3.8	15.4±1.6	-13.3±4.4
S30Y	T28V	-28.7±1.9	13.8±0.9	-14.8±1.2
S30M	T28L	-28.6±4.3	11.5±2.1	-17.1±3.3
S30Y	T54R	-28.5±0.9	13.2±2.1	-15.3±1.3
S30E	T28V	-28.3±1.7	15.1±1.2	-13.2±2.2
S30Y	T28W	-27.6±2.2	13.8±1.8	-13.8±0.6
S30M	T28K	-26.9±1.4	14.2±1.9	-12.7±1.5
S30M	T28W	-26.8±1.3	12.9±3.6	-13.9±2.5

Table S11. Binding free energies (kcal/mol) of three-point saturation mutations. Values include standard deviations.

Mut			ΔH	-TΔS	ΔG
R27E	D105W	T54F	-38.4±1.0	18.1±2.3	-20.4±3.1
R27E	D105W	T28W	-37.8±1.2	18.4±3.3	-19.4±2.5
D105Y	T28K	R27E	-37.0±2.1	21.9±3.0	-15.1±3.3
R27E	D105W	T28L	-36.4±2.0	16.8±3.6	-19.7±1.9
D105W	T28W	R27F	-35.0±2.5	14.5±1.9	-20.4±2.3
R27E	D105W	T54R	-37.6±2.0	22.8±2.4	-14.7±3.5
R27E	D105W	S57L	-35.6±1.0	19.7±4.9	-15.9±4.0
R27E	D105W	T28V	-35.6±2.0	16.5±0.6	-19.1±2.2
D105W	T28W	R27Y	-33.5±3.7	12.3±1.4	-21.2±3.8
D105Y	T28W	R27F	-36.2±1.2	12.6±2.4	-23.6±1.5
D105W	T28K	R27Y	-34.6±2.5	17.9±2.1	-16.7±4.5
D105Y	T28W	R27Y	-34.6±0.5	15.3±1.5	-19.3±1.5
D105Y	T28W	R27E	-35.6±1.0	18.8±2.4	-16.8±2.1
R27E	D105W	S52C	-33.0±1.2	17.4±5.5	-15.6±4.4
D105Y	T28K	R27Y	-33.1±0.6	14.9±2.3	-18.2±3.0
R27E	D105W	30M	-33.9±1.9	19.2±5.3	-14.7±6.1
R27E	D105W	T28K	-37.1±4.0	20.0±4.1	-17.2±2.1
D105Y	T28K	R27F	-33.7±1.8	15.0±1.2	-18.7±1.3
R27E	D105W	S30Y	-33.3±3.1	16.5±5.2	-16.9±2.2
D105W	T28K	R27F	-34.4±3.1	14.8±3.6	-19.6±6.7
	WT		-30.1±2.4	15.7±0.9	-14.4±3.2
R27E	D105W	30E	-29.8±2.6	18.6±3.5	-11.2±5.6

Table S12. Binding free energies (kcal/mol) of four-point saturation mutations. Values include standard deviations.

Mut				ΔH	-TΔS	ΔG
R27E	D105W	T54F	T28K	-41.2±1.5	20.7±2.6	-20.4±3.7
R27E	D105W	T54F	104Q	-40.0±2.1	18.2±3.4	-21.8±5.4
R27E	D105W	T54F	T28V	-39.6±0.7	20.5±1.8	-19.1±2.3
R27E	D105W	T54F	T28L	-39.4±1.2	20.2±2.0	-19.2±2.0
T28K	R27E	D105W	S52C	-38.4±2.5	23.0±2.4	-15.4±4.9
R27E	D105W	T28W	T54R	-38.3±2.1	19.0±4.1	-19.3±4.1
T28K	R27E	D105W	S30E	-38.0±2.5	20.7±0.4	-17.3±2.1
R27E	D105W	T54F	T28W	-37.6±3.2	18.3±1.0	-19.4±2.2
R27E	D105W	T54F	S30M	-37.6±3.9	18.5±5.8	-19.1±1.9
R27E	D105W	T28W	S57L	-37.5±0.3	19.3±2.4	-18.2±2.2
D105Y	T28W	R27E	S30M	-36.8±2.2	21.4±4.3	-15.4±6.0
R27E	D105W	T54F	S57L	-36.8±0.4	19.0±2.1	-17.8±1.9
R27E	D105W	T28W	S52C	-36.5±1.6	16.5±3.3	-20.0±4.0
R27E	D105W	T28W	T54F	-36.5±3.0	16.9±3.5	-19.6±5.4
T28K	R27E	D105W	S57L	-36.5±1.8	19.4±7.7	-17.1±5.9
R27E	D105W	T28W	S30M	-36.4±1.9	18.4±2.5	-18.0±4.2
T28K	R27E	D105W	S30M	-36.0±5.6	22.2±3.2	-13.9±2.5
R27E	D105W	T28W	E104K	-36.0±1.8	19.2±4.2	-16.8±2.7
R27E	D105W	T28W	E104Q	-35.5±2.5	17.2±2.3	-18.3±0.4
D105Y	T28W	R27E	S52C	-35.4±1.2	23.1±3.9	-12.3±5.1
R27E	D105W	T54F	E104K	-35.3±1.5	16.8±1.8	-18.5±1.5
D105Y	T28W	R27F	S52C	-35.3±1.1	15.2±0.3	-20.1±1.2
D105Y	T28W	R27E	S57L	-35.0±2.2	19.1±2.8	-15.9±3.2
D105Y	T28W	R27F	S57L	-34.8±1.3	13.5±1.0	-21.3±1.9
R27E	D105W	T28W	S30E	-33.3±1.9	20.3±5.7	-13.0±6.4
R27E	D105W	T54F	S30E	-33.2±1.0	18.4±4.4	-14.8±4.0
R27E	D105W	T54F	S30Y	-32.4±0.4	13.4±0.9	-19.0±1.0
D105Y	T28W	R27F	S30M	-32.1±1.3	13.7±2.3	-18.4±2.3
D105Y	T28W	R27E	S30Y	-30.9±1.0	14.5±4.1	-16.4±3.4
D105Y	T28W	R27E	S30E	-30.8±3.8	17.9±3.2	-12.9±3.2
R27E	D105W	T28W	S30Y	-30.7±2.5	15.6±2.1	-15.1±1.2
T28K	R27E	D105W	S30Y	-30.5±0.2	15.6±2.5	-14.9±2.6
		WT		-30.1±2.4	15.7±0.9	-14.4±3.2
D105Y	T28W	R27F	S30E	-30.0±2.2	15.4±2.3	-14.6±4.4
D105Y	T28W	R27F	S30Y	-27.8±1.0	11.6±2.0	-16.2±1.7