Table S1: Selected examples of computational and/or combinatorial redesign and de novo design of protein-protein interfaces .

(Arranged by number of mutations)

Reference	Protein-Protein Interface	Design Methods	# of muts	Affinity Enhancement	Enhanced Affinity
Sammond et al. JMB 371:1392-1404	Gα--GoLoco	Computational	1	<16-fold	>6nM
Sammond et al. JMB 371:1392-1404	UbcH7--E6AP	Computational	1	<25-fold	>190nM
Reis et al. Biochem. 48(10):2180-2191	rhTRAIL--DR4	Computational	1	3-fold	8.7nM
Bajaj et al. JBC 286(6): 4329-4340	KD1--plasmin	Computational	1	6-fold	0.9nM
Li et al. JBC 284 (51): 35605-11	CRIg--C3b	Combinatorial	2	18-fold	61nM
Lippow et al. Nat. Biot. 25(10):1171-6	Cetuximab--EGFR (Ab-Ag)	Computational	3	10-fold	52pM
Reynolds et al. JMB 382:1265-1275	TEM--SHV1	Computational	3	370-fold	4.6nM
Selzer et al. Nat.Struct.Bio. 7(7):537-41	TEM--BLIP	Computational	4	250-fold	4.3pM
Sundberg et al. Structure 11:1151-61	SEC sAg--HLA-DR1 MHC	Combinatorial	4	60-fold	4.6µM
Clark et al. Prot.Sci. 15:949-960	Ab--VLA1	Computational	4	7-fold	850pM
Song et al. JBC 281(8):5042-5049	ICAM-1--LFA-1	Computational	5	20-fold	12nM
Yosef et al. JMB 385: 1470-1480	CaM--CaMKIIp	Computational	5	4-fold	350pM
Pierce et al. Biochem 49: 7050-7059	TCR--HLA-A2 MHC	Comp. (α), Comb. (β)	5	900-fold	2.3nM
Lippow et al. Nat. Biot. 25(10):1171-6	D44.1--Lysozyme (Ab-Ag)	Computational	6	140-fold	30pM
Guntas et al. PNAS 107 (45): 19296-301	E6AP--Ubc12	Comp.-dir.Combin.	7	*de novo*	34nM
Presented here	**MICA--NKG2D**	**Computational**	**7**	**50-fold**	**51nM**
Muller et al. JMB 414:545-562	Ab--PSA	Combinatorial	8	9-fold	3.7nM
Koide et al. JMB 373:481-953	Ab--Ribonuclease A	Combinatorial	9	100-fold	180pM

Reference	Protein-Protein Interface	Design Methods	# of muts	Affinity Enhancement	Enhanced Affinity
Wang et al. PNAS 108(38):15960-5	CD4--HLA-DR1 MHC	Combinatorial	11	50-fold	8µM
Jha et al. JMB 400: 257-270	Spider Roll--PAK1	Computational	12	de novo	100µM
Buonpane et al. Nat.Med. 13(6):725-9	TCR--SEB	Combinatorial	13	3 X 10^6-fold	48pM
Gilbreth et al. PNAS 108(19):7751-6	ySMB--SUMO	Combinatorial	15	100-fold	82nM
Schonfeld et al. PNAS 106 (20): 8198-3	Lcn2--CTLA-4	Combinatorial	21	*de novo*	9nM
Huang et al. Prot.Sci. 16(12): 2770-4	Protein G dimer	Computational	24	*de novo*	300µM
Dyson et al. Anal.Bioch. 417(1):25-35	Ab--SHC1	Combinatorial	chain	60-fold	7.6nM
Fleishman et al. Science 332: 816	HB36 scaffold--HA	Computational (1st)	whole helix	*de novo*	200nM
Fleishman et al. Science 332: 816	HB36 scaffold--HA	Combinatorial (2nd)	3	50-fold	4nM
Fleishman et al. Science 332: 816	HB880 scaffold--HA	Computational (1st)	whole helix	*de novo*	>5000nM
Fleishman et al. Science 332: 816	HB880 scaffold--HA	Combinatorial (2nd)	4+del.	>1000-fold	3nM
Karanicolas et al. Mol.Cell. 42:250-260	Prb--Pdar	Computational (1st)	whole int.	*de novo*	130nM
Karanicolas et al. Mol.Cell. 42:250-260	Prb--Pdar	Combinatorial (2nd)	2	720-fold	180pM

Table S2: Computational, thermodynamic and kinetic data collected for all mutants

Single Mutation Sites (Italics = First Design Strategy; Bold = Individual Rosetta run; plain type = Multiple Rosetta run)

Code	D15	R38	R64	*N69*	K71	H79	K81	D149	A150	*K152*	*K154*	T155	H156	Y157	H158	D163	Q166	GST[e]
H156A													A					
H158D															D			
H158R															R			
H79T						T												
R38E		E																
D163Y																Y		
R64K			K															
D149V								V										
K81V							V											
K71E					E													
K154D[d]											D							
D163H																H		
D149I								I										
D15N	N																	
N69W[d]				W														
R38T		T																
K71Y					Y													
A150E									E									
Q166K[f]																	K	

Multiple Mutation Sites (Italics = First Design Strategy)

Code	D15	R38	R64	*N69*	K71	H79	K81	D149	A150	*K152*	*K154*	T155	H156	Y157	H158	D163	Q166	GST[e]
A	N	T	K			T		V	E				A		R	H		
B	N	T				T		V										X
C					E	T							A		R	H		
Sept.	N	T		W				V		E	D				D			
Sext.	N	T						V		E	D				D			
D		T												F	D			
Quadr.	N	T						V							D			
E		T													D			
F	N	T													D			
Quint.	N	T						V					A		D			
G	N	T						V										X
H	N	T																
I	N	T	K					I										X
J	N	T						V					A					X
K	N	T											A					X
L	N				E								A		R			
M	N				E			V							D			
N	N														D			
O	N											S			D			
P													A		D			
Q	N			W						E	D							
R[d]				W						E	D							

Single	Total Design Score[a]			Design Tests			Kinetic Parameters[b]			
Code	Unbound	Bound	Bd-Unbd	i	ii	iii	log rel k+1	log rel k-1	log rel k+2	log rel k-2
H156A	-20.29	-19.5	0.79	X	X		0.294	0.433	-0.218	0.458
H158D	-4.81	-6.71	-1.9				0.447	0.005	0.077	0.170
H158R	-4.06	-6.71	-2.65				0.066	0.175	-0.156	0.359
H79T	-0.48	-4.86	-4.38			X	0.110	0.029	-0.365	0.428
R38E	-2.46	-2.36	0.1				0.277	-0.051	0.187	-0.066
D163Y	-1.19	-2.13	-0.94				-0.147	0.022	-0.075	0.032
R64K	-1.61	-1.88	-0.27			X	-0.149	0.046	0.203	0.024
D149V	-1.8	1.86	-0.06				0.306	0.114	0.070	-0.020
K81V	-1.53	-1.69	-0.16				-0.014	-0.139	-0.347	0.162
K71E	-1.47	-1.49	-0.02				-0.299	0.170	-0.274	0.271
K154D[d]	-1.62	-1.48	0.14				0.017	n/a	n/a	0.010
D163H	-0.19	-1.27	-1.08				0.307	0.056	-0.136	0.268
D149I	-1.32	-0.96	0.36				0.069	-0.111	0.219	-0.004
D15N	-0.77	-0.7	0.07				0.712	0.184	0.204	0.080
N69W[d]	0.86	0.79	-0.07				0.541	0.207	-0.033	0.080
R38T	9.1	5.13	-3.97				0.461	-0.078	0.397	0.030
K71Y	0.33	5.14	4.81				-0.641	0.066	-0.322	0.092
A150E	63.57	11.24	-52.33	X	X		-0.003	-0.122	0.072	0.107
Q166K[f]	-5.28	20.32	25.6	X	X		-0.724	0.101	0.114	0.125

Multi.	Total Design Score[a]			Design Tests			Kinetic Parameters[b]			
Code	Unbound	Bound	Bd-Unbd	i	ii	iii	log rel k+1	log rel k-1	log rel k+2	log rel k-2
A	43.41	-20.51	-63.92	X	X	X	-0.235	-0.372	-0.054	-0.170
B	5.99	-2.39	-8.38			X	n/a			
C	-26.51	-33.95	-7.44	X	X		n/a			
Sept.	12.98	6.66	-6.32				1.018	0.474	0.057	0.230
Sext.	12.32	6.1	-6.22				0.994	-0.225	0.148	0.056
D	17.15	11.22	-5.93				0.373	0.086	-0.005	0.216
Quadr.	1.71	-4.19	-5.9				0.821	0.311	0.087	0.137
E	4.29	-1.58	-5.87				0.378	0.085	-0.044	0.238
F	3.52	-2.33	-5.85				0.653	0.202	0.056	0.172
Quint.	-18.58	-23.69	-5.11	X			1.075	-0.334	0.167	-0.089
G	6.53	2.52	-4.01				-0.023	-0.119	0.058	0.189
H	8.33	4.38	-3.95				1.008	-0.148	0.152	-0.095
I	5.38	1.46	-3.92			X	0.154	0.491	-0.539	0.572
J	-13.77	-16.94	-3.17				-0.286	0.213	-0.061	0.234
K	-11.97	-15.12	-3.15				0.473	-0.398	0.389	-0.148
L	-26.6	-28.53	-1.93	X	X		0.264	-0.360	-0.666	0.081
M	-8.88	-10.73	-1.85				0.002	-0.156	-0.117	0.104
N	-5.58	-7.41	-1.83				0.537	0.042	0.117	0.100
O	-5.7	-7	-1.3				0.203	-0.338	-0.148	0.200
P	-25.1	-26.16	-1.06	X	X		0.354	0.224	-0.330	0.220
Q	10.55	10.24	-0.31				0.768	0.124	0.093	0.211
R[d]	11.32	11.04	-0.28				1.072	0.106	0.162	0.129

Single	Thermodynamic Parameters[c]						
Code	ΔΔG	SE	ΔΔG1	ΔΔG2	ΔΔH	SE	-TΔΔS
H156A	-1.26	0.1	-1.00	-0.40	5.7	0.8	-6.9
H158D	-0.85	0.1	-0.65	-0.32	5.0	1.1	-5.8
H158R	-0.51	0.1	-0.34	-0.28	8.5	0.8	-9.0
H79T	-0.28	0.2	-0.16	-0.18	7.0	1.8	-7.3
R38E	-0.41	0.1	-0.32	-0.16	-1.9	2.9	1.5
D163Y	0.30	0.2	0.26	0.07	n/a		
R64K	-0.06	0.2	0.14	-0.31	n/a		
D149V	-0.63	0.1	-0.59	-0.07	1.8	0.8	-2.4
K81V	0.32	0.1	0.20	0.24	12.3	2.2	-11.9
K71E	0.09	0.2	0.13	-0.07	n/a		
K154D[d]	-0.10	0.1	7.4	1.7	-7.5[d]		
D163H	-0.58	0.1	-0.48	-0.17	4.5	2.6	-5.1
D149I	-0.13	0.2	0.04	-0.27	n/a		
D15N	-1.48	0.1	-1.24	-0.39	0.7	0.9	-2.2
N69W[d]	-1.10	0.1	-1.00	-0.06	0.6	0.7	-1.7
R38T	-0.93	0.2	-0.59	-0.52	n/a		
K71Y	0.99	0.2	0.87	0.29	n/a		
A150E	0.09	0.2	0.20	-0.16	n/a		
Q166K[f]	0.60	0.2	0.79	-0.32	n/a		

Multiple	Thermodynamic Parameters[c]						
Code	ΔΔG	SE	ΔΔG1	ΔΔG2	ΔΔH	SE	-TΔΔS
A	1.15	0.2	1.00	0.33	n/a		
B	0.16	0.1	6.8	1.4	-6.7	3	n/d
C	1.06	0.1	10.7	2.5	-9.6	3	n/d
Septuple	-2.29	0.1	-2.06	-0.37	-2.8	0.8	0.5
Sextuple	-1.27	0.1	-1.10	-0.27	-4.7	2.7	3.4
D	-0.83	0.1	-0.65	-0.3	-1.7	3.1	0.9
Quadruple	-1.74	0.1	-1.57	-0.28	-2.5	0.9	0.8
E	-0.86	0.1	-0.70	-0.3	-1.6	1.9	0.7
F	-1.41	0.1	-1.24	-0.3	-1.8	1.5	0.4
Quintuple	-1.09	0.1	-1.04	-0.08	3.1	1.4	4.2
G	-0.06	0.1	0.18	-0.4	-2.4	2.4	2.4
H	-1.24	0.2	-1.20	-0.07	-1.7	1.7	0.5
I	-0.78	0.2	-0.74	-0.07	3.7	4.2	-4.5
J	0.01	0.1	0.18	-0.28	0.8	1.2	-0.8
K	-0.24	0.1	-0.04	-0.34	3.1	2.4	-3.3
L	0.39	0.1	0.12	0.73	5.0	1.4	-4.6
M	0.15	0.1	0.17	0.0	0.4	1.3	-0.3
N	-1.00	0.1	-0.82	-0.30	-1.4	1.5	0.4
O	0.10	0.1	0.20	-0.1	-0.1	1.0	0.2
P	-0.73	0.1	-0.81	0.15	-1.4	1.8	0.7
Q	-1.58	0.1	-1.32	-0.40	-5.4	0.7	3.9
R[d]	-1.8	0.1	-1.6	-0.39	-4.4	0.9	2.6

a: Calculated by RosettaDesign with 100 runs as described in methods, subtracted from calculated values for wild-type

b: Kinetic data reported as log of ratio (mutant/wild-type) for association steps or (wild-type/mutant) for dissociation steps so that positive values indicate rate enhancement.

Values for each step were determined by the two-step fit in BIAevaluation 3.0.

Wild-type values are as reported in Lengyel et al. (2007).

For mutants without kinetic parameters, free energies of binding were determined by equilibrium methods, shown in Lengyel *et al.* (2007) to be similar for this system.

c: Thermodynamic data (kcal/mol) calculated according to methods and relative to values described in Lengyel *et al.* (2007).

SE: Standard error. ΔΔG1 and 2: Free energy of binding for steps 1 and 2 individually. -TΔΔS = ΔΔG at 25°C - van't Hoff ΔΔH

d: Thermodynamic and kinetic values for these mutants were previously collected and reported in Lengyel *et al.* (2007).

e: Checked mutants had a glutathione-S-tranferase (GST) tag attached to N-terminus of MICA.

f: This mutant was predicted to significantly destabilize the NKG2D-MICA complex and was expressed in order to test this prediction.

Table S3: Design scores calculated by RosettaDesign for all mutants

Scores arranged by ΔΔ(bound-unbound)

	Total Scores				
Name/Code	**Score (MICA only)**	**Score (Complex)**	**Δunbound[a]**	**Δbound[a]**	**ΔΔ(bound-unbound)**
A	882.89	1555.63	43.41	-20.51	-63.92
A150E	903.05	1587.38	63.57	11.24	-52.33
B	845.47	1573.75	5.99	-2.39	-8.38
C	812.97	1542.19	-26.51	-33.95	-7.44
Septuple	852.46	1582.8	12.98	6.66	-6.32
Sextuple	851.8	1582.24	12.32	6.1	-6.22
D	856.63	1587.36	17.15	11.22	-5.93
Quadruple	841.19	1571.95	1.71	-4.19	-5.9
E	843.77	1574.56	4.29	-1.58	-5.87
F	843	1573.81	3.52	-2.33	-5.85
Quintuple	820.9	1552.45	-18.58	-23.69	-5.11
H79T	839	1571.28	-0.48	-4.86	-4.38
G	846.01	1578.66	6.53	2.52	-4.01
R38T	848.58	1581.27	9.1	5.13	-3.97
H	847.81	1580.52	8.33	4.38	-3.95
I	844.86	1577.6	5.38	1.46	-3.92
J	825.71	1559.2	-13.77	-16.94	-3.17
K	827.51	1561.02	-11.97	-15.12	-3.15
H158R	835.42	1569.43	-4.06	-6.71	-2.65
L	812.88	1547.61	-26.6	-28.53	-1.93
H158D	834.67	1569.43	-4.81	-6.71	-1.9
M	830.6	1565.41	-8.88	-10.73	-1.85
N	833.9	1568.73	-5.58	-7.41	-1.83
O	833.78	1569.14	-5.7	-7	-1.3
D163H	839.29	1574.87	-0.19	-1.27	-1.08
P	814.38	1549.98	-25.1	-26.16	-1.06
D163Y	838.29	1574.01	-1.19	-2.13	-0.94
K152E	851.76	1588.01	12.28	11.87	-0.41
Q	850.03	1586.38	10.55	10.24	-0.31
Rd	850.8	1587.18	11.32	11.04	-0.28
R64K	837.87	1574.26	-1.61	-1.88	-0.27
K81V	837.95	1574.45	-1.53	-1.69	-0.16
N69W	840.34	1576.93	0.86	0.79	-0.07
D149V	837.68	1574.28	-1.8	-1.86	-0.06
K71E	838.01	1574.65	-1.47	-1.49	-0.02
wild-type	**839.48**	**1576.14**	**0**	**0**	**0**
D15N	838.71	1575.44	-0.77	-0.7	0.07
R38E	837.02	1573.78	-2.46	-2.36	0.1
K154D	837.86	1574.66	-1.62	-1.48	0.14
D149I	838.16	1575.18	-1.32	-0.96	0.36
H156A	819.19	1556.64	-20.29	-19.5	0.79
K71Y	839.81	1581.28	0.33	5.14	4.81
Q166K	834.2	1596.46	-5.28	20.32	25.6

a: Relative to wild-type (=mutant value-wild-type value)

	Individual Components of Total Scores for MICA unbound			
Name/Code	fa_atr_MICA	fa_rep_MICA	fa_pair_MICA	hb_sc_MICA
A	-915.34	911.61	-16.21	-21.15
A150E	-922.42	921.3	-15.18	-20.64
B	-915.27	868.82	-15.15	-21.25
C	-914.96	838.64	-16.18	-20.77
Septuple	-918.4	880.57	-14.88	-21.72
Sextuple	-917.01	880.57	-15.44	-21.72
D	-915.76	879.56	-15.65	-21.69
Quadruple	-916.71	868	-15.66	-21.72
E	-917.23	868.09	-15.65	-21.69
F	-917.14	868.01	-15.73	-21.81
Quintuple	-912.68	848.16	-15.7	-21.59
H79T	-918.17	859.04	-15	-20.56
G	-916.98	868.58	-14.93	-21.23
R38T	-917.5	868.68	-14.92	-20.9
H	-917.41	868.59	-15	-21.32
I	-917.9	869.26	-14.98	-21.23
J	-912.95	848.74	-14.97	-21.1
K	-913.39	848.75	-15.04	-21.19
H158R	-919.01	858.22	-15.04	-20.76
L	-914.8	838.32	-15.63	-20.93
H158D	-919.68	858.22	-15.61	-21.25
M	-919.06	858.15	-16.09	-21.46
N	-919.59	858.13	-15.69	-21.55
O	-919.14	858.44	-15.67	-21.55
D163H	-921.9	866.33	-15.38	-21.22
P	-915.65	838.38	-15.65	-21.3
D163Y	-922.84	858.8	-14.69	-20.9
K152E	-920.17	871.36	-14.54	-20.94
Q	-921.56	871.28	-14.14	-21.06
R	-921.65	871.36	-14.06	-20.94
R64K	-920.66	859.51	-14.92	-20.64
K81V	-919.44	858.8	-14.87	-20.72
N69W	-921.66	858.81	-14.32	-20.94
D149V	-919.52	858.79	-14.81	-20.67
K71E	-919.85	858.83	-15.35	-20.76
wild-type	**-920.05**	**866.33**	**-14.88**	**-21.18**
D15N	-919.86	858.71	-14.96	-20.88
R38E	-917.72	857.32	-15.62	-20.23
K154D	-920.04	858.8	-14.96	-20.94
D149I	-919.73	858.76	-14.81	-20.55
H156A	-915.93	838.96	-14.92	-20.63
K71Y	-919.95	858.8	-14.88	-20.76
Q166K	-920.43	858.8	-14.69	-20.76

	Individual Components of Total Scores for MICA-NKG2D complex			
Name/Code	**fa_atr_complex**	**fa_rep_complex**	**fa_pair_complex**	**hb_sc_complex**
A	-1834.06	1789.79	-38.41	-62.42
A150E	-1845.21	1811.78	-37.98	-62.65
B	-1835.24	1801.84	-36.13	-61.88
C	-1836.11	1773.38	-38.8	-62.92
Septuple	-1839.21	1817.82	-36.7	-63.79
Sextuple	-1837.81	1817.82	-37.25	-63.75
D	-1836.28	1816.85	-37.83	-63.68
Quadruple	-1837.51	1805.25	-37	-63.8
E	-1837.74	1805.38	-37.83	-63.68
F	-1837.67	1805.3	-37.74	-63.8
Quintuple	-1831.32	1785.14	-37.01	-63.6
H79T	-1839.58	1796.18	-37.08	-61.53
G	-1838.33	1807.4	-36	-62.96
R38T	-1838.56	1807.54	-36.83	-62.83
H	-1838.5	1807.45	-36.74	-62.96
I	-1839.4	1807.8	-36.45	-62.38
J	-1832.14	1787.29	-36	-62.71
K	-1832.32	1787.35	-36.74	-62.76
H158R	-1844.61	1799.66	-38.2	-63.95
L	-1838.54	1779.41	-38.8	-64.11
H158D	-1841.82	1799.52	-38.19	-63.5
M	-1841.76	1799.38	-38.05	-63.68
N	-1841.76	1799.48	-38.1	-63.57
O	-1840.32	1799.79	-38.33	-63.63
D163H	-1843.21	1801.14	-37.22	-62.66
P	-1835.64	1779.46	-38.19	-63.25
D163Y	-1844.88	1801.14	-36.58	-62.78
K152E	-1842.86	1814.29	-37.3	-62.65
Q	-1844.28	1814.2	-36.7	-62.72
R	-1844.34	1814.29	-36.79	-62.55
R64K	-1842.52	1802.38	-37.64	-62
K81V	-1841.12	1801.72	-36.68	-62.48
N69W	-1844.36	1801.73	-36.63	-62.65
D149V	-1842.47	1801.68	-36.45	-62.65
K71E	-1842.81	1801.67	-37.88	-62.8
wild-type	**-1842.64**	**1801.72**	**-37.19**	**-62.65**
D15N	-1842.58	1801.63	-37.1	-62.72
R38E	-1838.81	1800.15	-37.57	-61.92
K154D	-1842.73	1801.72	-37.24	-62.6
D149I	-1843.67	1801.42	-36.45	-62.65
H156A	-1836.46	1781.62	-37.19	-62.45
K71Y	-1846.24	1809.54	-37.35	-63.68
Q166K	-1843.01	1827.36	-38.02	-62.92

Individual Components of Total Scores Relative to Wild-Type (=mutant value-wild-type value)

Name/Code	Δatr_MICA	Δrep_MICA	Δpair_MICA	Δhb_sc_MICA	Δatr_comp	Δrep_comp	Δpair_comp	Δhb_sc_comp
A	4.71	45.28	-1.33	0.03	8.58	-11.93	-1.22	0.23
A150E	-2.37	54.97	-0.3	0.54	-2.57	10.06	-0.79	0
B	4.78	2.49	-0.27	-0.07	7.4	0.12	1.06	0.77
C	5.09	-27.69	-1.3	0.41	6.54	-28.34	-1.61	-0.27
Septuple	1.65	14.24	0	-0.54	3.44	16.1	0.49	-1.14
Sextuple	3.04	14.24	-0.56	-0.54	4.83	16.1	-0.06	-1.1
D	4.29	13.23	-0.77	-0.51	6.36	15.13	-0.64	-1.03
Quadruple	3.34	1.67	-0.78	-0.54	5.14	3.53	0.19	-1.15
E	2.82	1.76	-0.77	-0.51	4.9	3.66	-0.64	-1.03
F	2.91	1.68	-0.85	-0.63	4.97	3.58	-0.55	-1.15
Quintuple	7.37	-18.17	-0.82	-0.41	11.32	-16.58	0.18	-0.95
H79T	1.88	-7.29	-0.12	0.62	3.06	-5.54	0.11	1.12
G	3.07	2.25	-0.05	-0.05	4.31	5.68	1.19	-0.31
R38T	2.55	2.35	-0.04	0.28	4.08	5.82	0.36	-0.18
H	2.64	2.26	-0.12	-0.14	4.14	5.73	0.45	-0.31
I	2.15	2.93	-0.1	-0.05	3.24	6.08	0.74	0.27
J	7.1	-17.59	-0.09	0.08	10.5	-14.43	1.19	-0.06
K	6.66	-17.58	-0.16	-0.01	10.32	-14.37	0.45	-0.11
H158R	1.04	-8.11	-0.16	0.42	-1.97	-2.06	-1.01	-1.3
L	5.25	-28.01	-0.75	0.25	4.1	-22.31	-1.61	-1.46
H158D	0.37	-8.11	-0.73	-0.07	0.82	-2.2	-1	-0.85
M	0.99	-8.18	-1.21	-0.28	0.88	-2.34	-0.86	-1.03
N	0.46	-8.2	-0.81	-0.37	0.88	-2.24	-0.91	-0.92
O	0.91	-7.89	-0.79	-0.37	2.32	-1.93	-1.14	-0.98
D163H	-1.85	0	-0.5	-0.04	-0.57	-0.58	-0.03	-0.01
P	4.4	-27.95	-0.77	-0.12	7	-22.26	-1	-0.6
D163Y	-2.79	-7.53	0.19	0.28	-2.24	-0.58	0.61	-0.13
K152E	-0.12	5.03	0.34	0.24	-0.22	12.57	-0.11	0
Q	-1.51	4.95	0.74	0.12	-1.64	12.48	0.49	-0.07
R	-1.6	5.03	0.82	0.24	-1.7	12.57	0.4	0.1
R64K	-0.61	-6.82	-0.04	0.54	0.12	0.66	-0.45	0.65
K81V	0.61	-7.53	0.01	0.46	1.52	0	0.51	0.17
N69W	-1.61	-7.52	0.56	0.24	-1.72	0.01	0.56	0
D149V	0.53	-7.54	0.07	0.51	0.17	-0.04	0.74	0
K71E	0.2	-7.5	-0.47	0.42	-0.17	-0.05	-0.69	-0.15
wild-type	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
D15N	0.19	-7.62	-0.08	0.3	0.06	-0.09	0.09	-0.07
R38E	2.33	-9.01	-0.74	0.95	3.83	-1.57	-0.38	0.73
K154D	0.01	-7.53	-0.08	0.24	-0.09	0	-0.05	0.05
D149I	0.32	-7.57	0.07	0.63	-1.03	-0.3	0.74	0
H156A	4.12	-27.37	-0.04	0.55	6.18	-20.1	0	0.2
K71Y	0.1	-7.53	0	0.42	-3.6	7.82	-0.16	-1.03
Q166K	-0.38	-7.53	0.19	0.42	-0.37	25.64	-0.83	-0.27

Subtracted Columns Used in Tests ii and iii (bolded columns failed the test for the subtracted value)

Name/Code	Test ii (Δrep_comp - Δrep_MICA)	Test iii (Δhb_sc_comp - Δhb_sc_MICA)
A	**-57.21**	**0.2**
A150E	**-44.91**	-0.54
B	-2.37	**0.84**
C	-0.65	-0.68
Septuple	1.86	-0.6
Sextuple	1.86	-0.56
D	1.9	-0.52
Quadruple	1.86	-0.61
E	1.9	-0.52
F	1.9	-0.52
Quintuple	1.59	-0.54
H79T	1.75	**0.5**
G	3.43	-0.26
R38T	3.47	-0.46
H	3.47	-0.17
I	3.15	**0.32**
J	3.16	-0.14
K	3.21	-0.1
H158R	6.05	-1.72
L	5.7	-1.71
H158D	5.91	-0.78
M	5.84	-0.75
N	5.96	-0.55
O	5.96	-0.61
D163H	-0.58	0.03
P	5.69	-0.48
D163Y	6.95	-0.41
K152E	7.54	-0.24
Q	7.53	-0.19
R	7.54	-0.14
R64K	7.48	**0.11**
K81V	7.53	-0.29
N69W	7.53	-0.24
D149V	7.5	-0.51
K71E	7.45	-0.57
wild-type	0	0
D15N	7.53	-0.37
R38E	7.44	-0.22
K154D	7.53	-0.19
D149I	7.27	-0.63
H156A	7.27	-0.35
K71Y	15.35	-1.45
Q166K	**33.17**	-0.69

Table S4: Cross-validation design scores and experimental binding values

Nanomolar-affinity antibody-antigen (PDB ID: 1MLC)

Chain	Mutant	Expt. ΔΔG[a]	Failed test	Bd-Unbnd score:	fa_atr:	fa_rep:	fa_pair:	hb_sc:
A	N32G	-0.86		-0.27	1.02	0.15	-0.02	0.01
A	N32Y	0	i, ii, iii	61.55	-2.55	61.26	-0.02	0.24
A	N92A	-1.25	iii	0.1	1.28	0.18	0.14	0.11
A	N32G +N92A	-0.69	iii	-0.09	1.9	0.18	0.12	0.11
B	T28D	-0.15		-1.2	0.14	-2.45	0.21	-0.04
B	T31A	0.45		0.32	0.59	0.17	0.13	-0.15
B	T31V	0.53		0.03	0.18	0.18	0.13	-0.17
B	T31W	0.13	i, ii	238.29	-3.38	237.93	0.13	-0.09
B	S57A	-0.37		-0.48	0.63	0.15	0.03	-0.11
B	S57V	-0.49	i, ii	34.57	-1.11	36.09	0.03	-0.19
B	T58D	-0.56		-0.02	-0.02	0.18	-0.02	-0.21
B	K65D	0.02		0.07	0	0.18	0	-0.1
B	S57V +T58D	-0.99	i, ii	34.54	-1.11	36.1	0.02	-0.24

Picomolar-affinity antibody-antigen (PDB ID: 1YY9)

Chain	Mutant	Expt. ΔΔG[a]	Failed test	Bd-Unbnd score:	fa_atr:	fa_rep:	fa_pair:	hb_sc:
C	S26D	-0.2		-0.11	0	-0.01	-0.11	0
C	T31E	-0.53		0	0	0	0	0
C	N93A	-0.7	iii	0.1	0.58	0	-0.1	0.35
D	N56A	-0.04		-0.18	0.86	0.06	0.31	-0.04
D	T61E	-0.07		0.22	0.01	0.07	0.25	-0.11

Micromolar-affinity TCR-MHC (PDB ID: 1AO7)

Chain	Mutant	Expt. ΔΔG[b]	Failed test	Bd-Unbnd score:	fa_atr:	fa_rep:	fa_pair:	hb_sc:
D	D26M	0.19	iii	0.5	-0.84	1.15	-0.19	0.3
D	D26V	1.45	i, ii, iii	-128.11	0.31	-127.89	-0.19	0.29
D	D26W	-1.08	i, ii, iii	466.05	-2.9	467.18	-0.19	0.29
D	R27F	-0.22		2.41	-0.37	2.81	0.27	0.01
D	G28A	0.58		-0.8	-0.52	0.14	-0.47	0
D	G28I	-0.27	i, ii	-10.18	-1.74	-8.71	-0.47	-0.01
D	G28L	-0.46	i, ii	55.94	-3.64	57.4	-0.47	0
D	G28M	-0.51		6.2	-3.88	7.84	-0.47	0
D	G28R	2.43	i, ii	34.61	-7.86	36.27	-2.06	-0.15
D	G28T	-0.76		-2	-1.08	-1.08	-0.3	-0.01
D	G28V	0.56	i, ii	-16.05	-1.08	-14.87	-0.47	0
D	S29A	0.33		0.11	-0.03	0	0.11	0.01
D	Q30N	0.73		-1.03	2.29	0	-0.04	-1.27
D	Q30E	0.43	iii	0.3	1.29	0.02	0.27	0.64
D	S51M	-0.37		-1.43	-1.22	0.07	0.02	0.01
D	K68H	1.09	iii	0.04	0.25	0.01	-0.31	1.12
D	S100A	-0.01		-0.37	0.5	-0.09	0.07	0.01
D	S100N	1.92		2.68	-2.93	1.36	0.15	-0.09
D	S100T	-0.49		1.3	-0.35	0.07	0.03	0.02
D	S100Y	2.34	i, ii, iii	672.59	-5.83	675.71	0.07	0.22
E	I54R	1.28		-0.83	-1.69	0	-0.34	-0.86
E	A99M	-0.31		-4.63	-0.36	-6	0	0.01
E	A99K	0.1		-3.66	-0.54	-3.85	-0.14	0.03
E	G100S	-0.32	i, ii	-67.35	-0.12	-67.32	-0.2	-0.02
E	G101A	-1.09	i	0.51	-1.59	1.84	0	0.05
E	R102Q	0.45	i, ii	30.97	2.16	30.41	0.02	-0.23

a: kcal/mol reported in Lippow *et al.* (2007).

b: kcal/mol reported in Haidar *et al.* (2009).

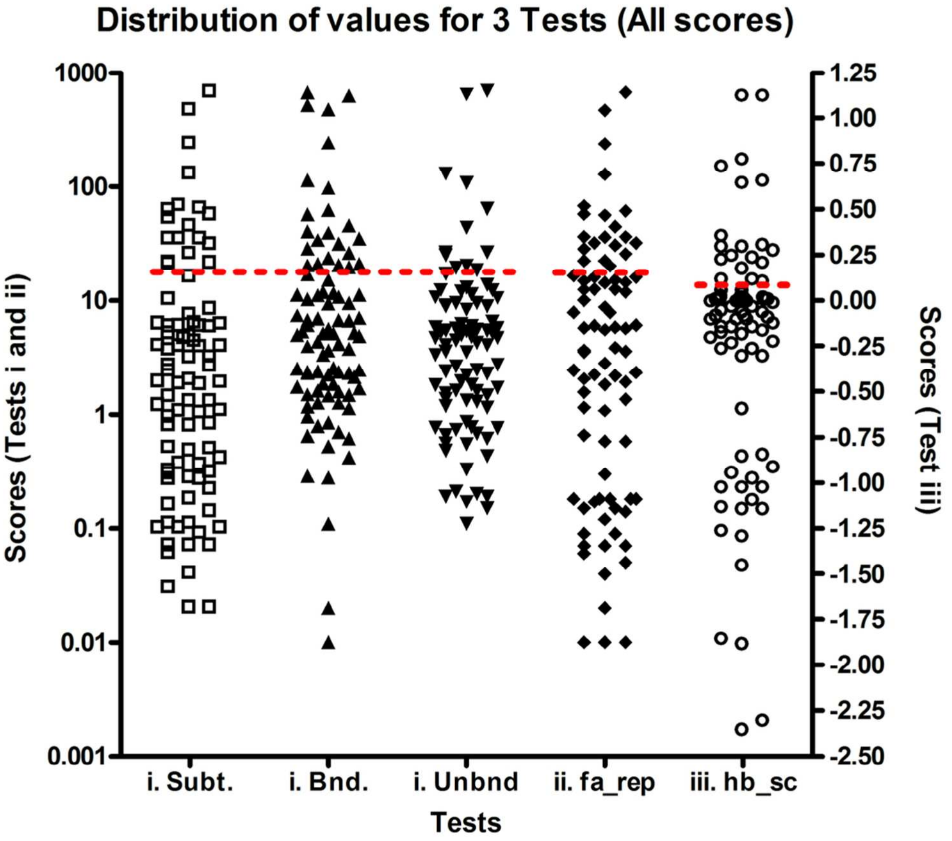

Figure S1: All scores for the five score categories plotted to show the approximate cutoff for the 80th percentile
94x84mm (300 x 300 DPI)

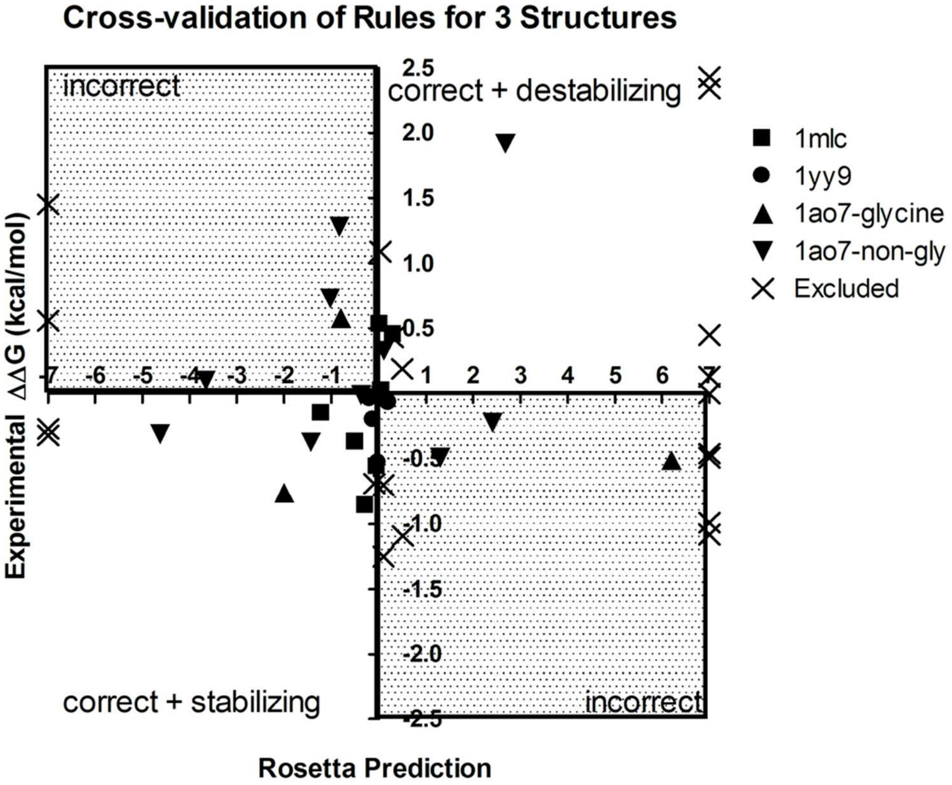

Figure S2: Experimental free energy of binding vs. subtracted total Rosetta score (cut off at +/- 7) for mutations at three protein-protein interfaces for cross-validation, listed by PDB ID
89x74mm (300 x 300 DPI)