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**Supporting Material**

**Protein thermostability calculations using alchemical free energy simulations**

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**Table S 1:** Experimental and calculated folding free energy differences in kJ/mol for Barnase (PDB 1bni) mutations

Mut.	$\Delta G_{exp}$	$\Delta G_{calc}$	Err.	Mut.	$\Delta G_{exp}$	$\Delta G_{calc}$	Err.	Mut.	$\Delta G_{exp}$	$\Delta G_{calc}$	Err.
A32C	-4.18	-1.57	0.34	I55V	-1.25	-0.72	0.43	T16S	-7.11	-5.26	0.57
A32F	-2.93	-2.7	0.54	I76A	-7.94	-4.39	0.64	T26A	-7.94	-7.49	0.51
A32G	-3.76	-3.0	1.83	I76V	-3.34	-0.47	0.44	T26G	-6.27	-6.47	0.63
A32H	-3.34	-2.25	0.53	I88A	-16.72	-18.44	0.5	T26N	-5.39	-9.2	1.65
A32I	-3.34	-3.9	0.59	I88G	-30.93	-38.76	0.57	T26Q	-7.19	-7.07	1.24
A32L	-1.25	3.51	0.47	I88L	-1.25	-0.31	0.4	T26S	-2.34	-2.4	0.55
A32M	-1.25	-1.21	0.5	I88V	-5.43	-7.65	0.36	T26V	-9.66	-8.11	0.5
A32N	-2.93	0.18	0.74	I96A	-13.38	-12.64	0.83	T6A	-8.78	-3.07	0.44
A32Q	-2.09	-0.3	0.65	I96G	-23.83	-30.41	0.98	T6G	-5.02	-3.34	0.5
A32S	-1.67	0.14	0.35	I96V	-3.76	-0.81	0.38	T6H	-11.08	-8.12	0.76
A32T	-3.34	-0.78	0.64	L14A	-17.97	-16.33	0.57	T6N	-5.31	-3.93	1.13
A32V	-3.76	-3.95	0.47	L33Q	-5.43	-3.03	0.68	T6Q	-7.82	-9.15	0.96
A32W	-4.18	-2.32	0.67	L89G	-29.26	-34.69	0.66	T6S	-0.92	-4.93	0.56
A32Y	-3.34	-4.45	0.57	L89T	-10.45	-9.91	0.78	T79V	1.25	0.94	0.42
F7L	-19.23	-0.21	1.19	L89V	-1.25	4.52	0.79	T99V	-11.29	-8.86	0.74
G34A	-13.04	-11.77	0.34	L95G	-19.65	-10.94	0.88	V10A	-14.21	-13.21	0.39
G34H	-11.08	-15.56	0.75	N23A	-9.2	-7.0	1.55	V10T	-10.45	-2.59	0.52
G34N	-11.49	-15.62	0.87	N58A	-11.29	-14.82	1.5	V36A	-5.43	1.55	0.39
G34S	-13.38	-10.04	0.48	N5A	-7.94	-6.71	1.01	V36T	-4.6	-1.95	0.47
G34T	-14.09	-13.85	0.58	N77A	-6.69	-0.07	0.68	V45A	-7.52	-4.88	0.43
G65S	2.09	5.3	0.46	N84A	-8.36	-6.3	1.24	V45T	-10.03	-3.14	0.45
H18A	-7.73	-6.3	0.66	Q104A	-0.84	-3.92	0.8	W94F	-4.43	3.2	1.22
H18G	-2.93	-4.37	0.81	Q15A	-0.84	-1.91	0.86	W94L	-6.65	4.92	0.77
H18N	-7.02	-13.27	1.03	Q15G	-6.69	-10.03	1.15	W94Y	-5.31	0.82	0.57
H18Q	-6.69	-2.11	1.27	Q15I	4.18	2.82	0.69	Y103F	0.0	-5.47	0.55
H18S	-9.53	-5.67	0.63	Q31A	0.42	-0.56	0.74	Y13A	-13.79	-20.79	0.85
I109A	-8.78	-11.49	0.55	Q31G	-4.1	-3.4	0.93	Y13F	-1.71	-4.54	0.38
I109V	-3.34	-1.05	0.52	Q31S	-0.84	1.42	1.07	Y13G	-26.75	-28.98	1.24
I25A	-14.63	-22.68	0.47	S28A	1.71	0.58	0.41	Y17A	-8.36	-10.91	0.8
I25V	-4.6	-8.11	0.4	S28G	-1.88	-4.42	0.49	Y17F	-1.25	-2.34	0.35
I4A	-5.85	-7.1	0.52	S85A	-0.5	-2.26	0.41	Y17G	-16.85	-21.85	1.07
I4V	-2.51	1.57	0.44	S91A	-7.94	-14.01	0.41	Y17S	-10.74	-13.98	0.6
I51A	-19.65	-19.49	0.89	S92A	-11.7	-11.13	0.5	Y24F	0.0	6.02	0.5
I51V	-7.52	-8.39	0.31	T100G	-11.7	-3.65	0.6	Y78F	-5.85	-0.52	0.6
I55A	-4.6	-9.64	0.42	T105V	-9.2	-18.21	0.44	Y97G	-27.59	-35.26	1.01
I55G	-12.96	-8.9	0.66	T16A	-1.13	-6.8	0.49				
I55T	-2.51	-1.89	0.54	T16G	-6.94	-12.35	0.54				

**Table S 2:** Experimental and calculated folding free energy differences in kJ/mol for Barnase (PDB 1bni) mutations involving charge changes

Mut.	$\Delta G_{exp}$	$\Delta G_{calc}$	Err.	Dev. from exp.	RSASA
G34D	-14.17	-21.05	0.96	6.88	0.52
D12S	-2.72	-1.65	1.2	1.07	0.6
E29G	-7.52	-6.07	0.97	1.45	0.55
D12G	-5.39	-6.11	1.01	0.72	0.6
D54N	-10.03	0.06	1.73	10.09	0.35
E29S	-5.14	-5.23	1.23	0.09	0.55
G34K	-13.04	-9.37	1.06	3.67	0.52
E29Q	0.0	1.54	1.14	1.54	0.55
G34R	-10.12	-10.7	1.67	0.58	0.52
D22M	-1.13	-4.72	1.13	3.59	1.01
S28E	1.67	4.07	0.83	2.4	0.6
R72G	-10.45	-57.67	2.07	47.22	0.4
K27G	-1.67	-8.87	1.36	7.2	0.49
D8G	-4.89	-10.73	0.94	5.84	0.73
T16R	2.09	-1.29	2.79	3.38	0.79
N41D	-10.45	-26.05	1.41	15.6	0.23
D8S	-4.05	-10.09	1.1	6.04	0.73
D93N	-17.18	-18.46	1.76	1.28	0.5
N58D	2.09	-11.39	1.55	13.48	0.4
E73G	-22.57	-15.62	1.54	6.95	0.35
D75N	-20.06	-2.06	2.49	18.0	0.26
H18D	-10.7	-13.53	1.07	2.83	0.63
H18K	-5.02	-9.57	1.69	4.55	0.63
H18R	-4.89	-7.21	2.35	2.32	0.63
R69S	-11.37	-28.43	4.76	17.06	0.26

**Table S 3:** Alchemical free energies in kJ/mol for mutations in GXG tri-peptides

	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu
Ala	-	-5.12 ± 0.25	-	-	-29.05 ± 0.34	-36.1 ± 0.18	-107.3 ± 0.29	-18.83 ± 0.41	-	-100.3 ± 0.31
Cys	4.75 ± 0.27	-	-	-	-27.12 ± 0.33	-34.54 ± 0.33	-105.1 ± 0.37	-14.26 ± 0.25	-	-99.39 ± 0.28
Asp	-	-	-	-	-	-	-	-	-	-
Glu	-	-	-	-	-	-	-	-	-	-
Phe	29.45 ± 0.31	25.53 ± 0.38	-	-	-	-6.3 ± 0.48	-78.72 ± 0.31	12.04 ± 0.31	-	-71.63 ± 0.31
Gly	37.0 ± 0.21	34.29 ± 0.37	-	-	6.63 ± 0.5	-	-70.42 ± 0.48	19.04 ± 0.46	-	-63.47 ± 0.5
His	107.6 ± 0.26	104.9 ± 0.4	-	-	78.08 ± 0.36	70.91 ± 0.42	-	92.64 ± 0.43	-	10.11 ± 0.47
Ile	18.25 ± 0.25	16.57 ± 0.32	-	-	-12.77 ± 0.3	-21.01 ± 0.44	-88.28 ± 0.37	-	-	-81.94 ± 0.34
Lys	-	-	-	-	-	-	-	-	-	-
Leu	101.4 ± 0.29	99.24 ± 0.29	-	-	71.06 ± 0.29	64.05 ± 0.46	-6.54 ± 0.35	80.64 ± 0.29	-	-
Met	32.25 ± 0.26	31.38 ± 0.28	-	-	1.55 ± 0.33	-4.97 ± 0.39	-76.36 ± 0.47	18.02 ± 0.23	-	-68.91 ± 0.28
Asn	393.9 ± 0.43	401.2 ± 0.5	-	-	365.3 ± 0.54	357.6 ± 0.57	287.5 ± 0.52	377.2 ± 0.56	-	292.4 ± 0.71
Pro	-	-	-	-	-	-	-	-	-	-
Gln	337.9 ± 0.42	336.3 ± 0.44	-	-	308.5 ± 0.71	300.9 ± 0.63	231.0 ± 0.7	319.4 ± 0.42	-	236.9 ± 0.57
Arg	-	-	-	-	-	-	-	-	-	-
Ser	53.13 ± 0.28	45.85 ± 0.27	-	-	21.89 ± 0.33	16.22 ± 0.38	-56.5 ± 0.34	32.93 ± 0.31	-	-52.09 ± 0.42
Thr	145.3 ± 0.27	136.4 ± 0.27	-	-	111.8 ± 0.38	109.0 ± 0.34	34.49 ± 0.43	120.6 ± 0.28	-	40.64 ± 0.51
Val	92.53 ± 0.23	89.1 ± 0.28	-	-	62.14 ± 0.3	52.77 ± 0.36	-14.1 ± 0.33	75.79 ± 0.2	-	-7.69 ± 0.39
Trp	37.95 ± 0.46	32.35 ± 0.39	-	-	8.03 ± 0.41	1.8 ± 0.61	17.69 ± 0.34	20.52 ± 0.42	-	-62.24 ± 0.48
Tyr	127.2 ± 0.35	125.3 ± 0.34	-	-	98.57 ± 0.24	92.02 ± 0.51	19.2 ± 0.42	111.7 ± 0.35	-	25.91 ± 0.37

**Table S 4:** Alchemical free energies in kJ/mol for mutations in GXG tri-peptides

	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
Ala	-31.6 ± 0.28	-393.8 ± 0.47	-	-337.2 ± 0.41	-	-53.69 ± 0.22	-144.4 ± 0.51	-91.79 ± 0.32	-36.84 ± 0.4	-127.7 ± 0.36
Cys	-30.97 ± 0.37	-403.1 ± 0.47	-	-336.4 ± 0.44	-	-45.92 ± 0.25	-136.4 ± 0.28	-87.95 ± 0.21	-33.53 ± 0.39	-125.0 ± 0.37
Asp	-	-	-	-	-	-	-	-	-	-
Glu	-	-	-	-	-	-	-	-	-	-
Phe	-2.38 ± 0.36	-362.4 ± 0.57	-	-308.9 ± 0.69	-	-22.22 ± 0.36	-115.3 ± 0.49	-59.13 ± 0.47	-8.1 ± 0.26	-99.33 ± 0.19
Gly	6.48 ± 0.44	-358.5 ± 0.57	-	-299.3 ± 0.62	-	-16.99 ± 0.34	-108.6 ± 0.33	-52.65 ± 0.4	-1.7 ± 0.64	-90.14 ± 0.53
His	76.61 ± 0.41	-288.5 ± 0.46	-	-229.9 ± 0.59	-	53.75 ± 0.33	-37.73 ± 0.37	18.0 ± 0.32	-18.6 ± 0.28	-19.05 ± 0.35
Ile	-17.59 ± 0.36	-379.2 ± 0.54	-	-318.0 ± 0.49	-	-32.69 ± 0.34	-118.3 ± 0.36	-74.4 ± 0.23	-18.0 ± 0.49	-111.4 ± 0.32
Lys	-	-	-	-	-	-	-	-	-	-
Leu	68.37 ± 0.28	-293.8 ± 0.74	-	-236.8 ± 0.44	-	51.87 ± 0.39	-39.26 ± 0.46	8.13 ± 0.7	65.35 ± 0.41	-26.42 ± 0.35
Met	-	-360.6 ± 0.73	-	-313.4 ± 0.41	-	-16.55 ± 0.4	-110.4 ± 0.42	-57.51 ± 0.34	-3.87 ± 0.5	-96.51 ± 0.35
Asn	361.3 ± 0.74	-	-	53.86 ± 0.92	-	345.3 ± 0.59	256.3 ± 0.83	303.9 ± 0.54	359.1 ± 0.68	265.5 ± 0.67
Pro	-	-	-	-	-	-	-	-	-	-
Gln	312.8 ± 0.41	-56.45 ± 0.97	-	-	-	288.4 ± 0.5	199.9 ± 0.54	248.4 ± 0.5	301.0 ± 0.91	208.5 ± 0.79
Arg	-	-	-	-	-	-	-	-	-	-
Ser	15.33 ± 0.65	-344.6 ± 0.54	-	-289.6 ± 0.49	-	-	-91.45 ± 0.28	-41.39 ± 0.32	15.62 ± 0.45	-73.5 ± 0.43
Thr	112.5 ± 0.39	-258.3 ± 0.8	-	-199.5 ± 0.55	-	88.97 ± 0.33	-	45.88 ± 0.23	108.0 ± 0.48	17.41 ± 0.4
Val	57.5 ± 0.31	-301.6 ± 0.5	-	-244.0 ± 0.47	-	38.87 ± 0.3	-47.05 ± 0.36	-	54.97 ± 0.39	-36.67 ± 0.38
Trp	3.92 ± 0.48	-357.7 ± 0.68	-	-301.5 ± 0.87	-	-16.11 ± 0.4	-106.2 ± 0.55	-51.72 ± 0.36	-	-90.68 ± 0.35
Tyr	94.93 ± 0.33	-267.3 ± 0.59	-	-210.3 ± 0.85	-	76.17 ± 0.43	-18.14 ± 0.39	33.32 ± 0.33	89.85 ± 0.36	-