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**Supplemental Information**

**Improving the Accuracy of Protein Thermostability Predictions for Single Point Mutations**

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## Supporting Material

### Improving the accuracy of protein thermostability predictions for single point mutations

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**Table S1. FEP+ predictions using different unfolded model in comparison with experiment and Residue scanning. The values in parentheses are prior to outlier analysis**

PDB	Wild type	Res Num	Mutant	$\Delta\Delta G$ Exp	Mono peptide	Tri peptide	Penta peptide	Hepta peptide	Residue Scan.
1EY0	THR	22	CYS	0.9	-0.15	-0.35	0.63	-0.35	7.29
1EY0	THR	22	VAL	0.9	0.94	1.05	1.18	1.14	2.31
1EY0	VAL	23	LEU	0.1	-0.61	-0.34	-0.02	-0.5	-3.29
1EY0	LEU	25	ILE	1.7	2.08	1.72	1.91	1.84	14.51
1EY0	THR	33	VAL	-0.4	-0.53	-0.51	-0.14	-0.4	-5.29
1EY0	THR	41	CYS	-0.6	1.59	-0.52	-0.31	-1.08	13.83
1EY0	THR	41	ILE	-0.7	2.87	-1.4	-1.27	-0.57	2.93
1EY0	THR	41	SER	1.1	2.63	1.04	1.24	1.04	9.47
1EY0	THR	41	VAL	-0.8	2.79	-1.69	-1.59	-1.45	2.54
1EY0	THR	44	VAL	-0.1	-0.57	0.04	-0.45	0.45	-0.93
1EY0	SER	59	ALA	-0.5	-0.54	-0.73	-0.43	0.26	-5.31
1EY0	THR	62	SER	2.1	0.2	1.09 (-0.34)	-0.17	0.04	6.38
1EY0	THR	62	VAL	0.2	-2.61	-2.43 (-2.61)	-2.43	-2.43	4.52
1EY0	VAL	66	ILE	1	-0.12	-0.5 (-0.3)	0.15	0.37	4.01
1EY0	VAL	66	LEU	0.3	-3.4	-2.71	-2.82	-2.52	-2.88

						(-3.12)			
1EY0	VAL	66	LYS	7.5	13.16	6.69 (16.28)	15.04	15.25	45.03
1EY0	ILE	72	LEU	0.2	0.08	0.54	0.25	0.68	2.35
1EY0	ILE	72	VAL	1.2	1.35	1.43	1.45	1.57	8.92
1EY0	THR	82	SER	0.7	0.91	0.92	1.02	0.68	3.08
1EY0	ILE	92	VAL	0.4	1.01	1.04	0.82	0.44	8.03
1EY0	LYS	116	GLY	-1	-5.85	-2.88	-1.62	-1.37	-0.11
1EY0	PRO	117	ALA	-0.8	-9.86	-1.42	-0.62	-0.65	6.15
1EY0	PRO	117	GLY	-0.9	-9.01	-2.06	-1.45	-1.13	7.63
1EY0	THR	120	CYS	1.7	1.4	1.62	1.48	0.77	2.51
1EY0	THR	120	SER	0.6	0.76	0.83	0.29	-0.26	1.64
1EY0	THR	120	VAL	1.8	3.16	4.11	3.57	3.35	1.51
1EY0	SER	128	ALA	-0.7	-2.02	-2.01	-2.22	-2.1	-2.96
1BNI	PHE	7	LEU	4.1	2.28	2.03	2.19	2.25	8.44
1BNI	LEU	14	ALA	4.5	4.13	3.48	3.1	3.32	24.63
1BNI	THR	26	ALA	1.7	1.23	0.32	0.46	1.64	2.69
1BNI	ILE	51	VAL	1.1	2.28	2.48	2.33	2.11	8.38
1BNI	ILE	76	ALA	1.7	0.76	0.23	0.59	0.07	23.6
1BNI	ILE	76	VAL	1	0.61	0.61	0.69	0.46	9.7
1BNI	TYR	78	PHE	1.1	-0.21	-1.04	-0.44	-0.16	5.17
1BNI	ILE	88	ALA	4	5.36	4.89	4.93	5.02	25.98
1BNI	ILE	88	VAL	1.6	2.32	2.16	1.84	2.19	8.64
1BNI	LEU	89	VAL	0.5	0.45	0.88	0.35	0.42	12.34
1BNI	SER	91	ALA	2.4	1.49	1.65	1.41	1.11	1.68
1BNI	ILE	96	ALA	3.2	4.61	3.88	3.53	3.86	27.03
1BNI	ILE	96	VAL	3.1	1.93	1.78	1.84	1.49	10.38
1L63	SER	38	ASN	0	-0.23	-0.34	-0.65	-0.53	-0.13
1L63	LYS	43	ALA	1	1.34	0.97	0.88	1.29	-4.72
1L63	SER	44	ALA	-0.3	-0.31	-0.11	-0.1	-0.77	1.16
1L63	LEU	46	ALA	1.9	1.88	1.82	1.3	1.08	23.65

1L63	ASP	47	ALA	1	0.52	0.32	0.65	0.67	3.98
1L63	THR	59	ALA	1.5	1.52	1.46	1.66	1.74	3.2
1L63	THR	59	ASN	1.1	-0.08	0.09	-0.22	0.59	3.48
1L63	THR	59	ASP	1.2	2.29	2.77	2.45	1.76	5.66
1L63	THR	59	GLY	1.6	1.47	0.18	0.02	0.49	4.52
1L63	THR	59	SER	0.2	0.36	0.32	0.07	0.05	2.53
1L63	THR	59	VAL	1.5	2.63	2.9	2.68	2.13	-1.41
1L63	ASP	92	ASN	1.4	3.16	3.18	4.15	2.71	5.19
1L63	THR	109	ASN	-0.1	-0.54	0.11	0.16	-0.38	-0.67
1L63	THR	109	ASP	-0.6	-0.39	-0.33	0.26	-0.35	-2.91
1L63	ASN	144	GLU	-0.5	-1.18	-1.35	-1.21	-0.11	-1.75
1L63	ASP	72	PRO	2.7	12.84	3.11	0.05	1.23	40.21
2LZM	ILE	3	TYR	2.3	3.04	3.07	2.72	2.46	12.34
2LZM	ILE	3	VAL	0.4	0.71	0.8	0.29	0.46	8.95
2LZM	MET	6	ILE	1.4	3.1	2.95	3.53	3.03	31.8
2LZM	ASN	55	GLY	0.6	1.97	0.87	0.42	0.83	-3.78
2LZM	LYS	60	PRO	0	4.87	-0.21	-0.26	0.43	-7.01
2LZM	GLY	77	ALA	-0.4	-2.66	-1.5	-0.35	-0.38	1.64
2LZM	ALA	82	PRO	-0.8	8.08	-1.33	-1.43	-0.68	2.9
2LZM	GLY	113	ALA	-0.3	-2.16	-0.68	-0.42	-0.74	-3.36
2LZM	THR	115	GLU	-0.3	-1.78	-1.95	-2.1	-1.92	-4.69
2LZM	GLN	123	GLU	-0.4	-1.35	-1.09	-0.24	0	1.51
2LZM	LYS	124	GLY	0.1	3.99	2.14	1.89	1.94	-0.16
2LZM	GLY	156	ASP	2.3	3.25	3.62 (5.39)	4.49	4.92	23.98
1RGG	SER	31	PRO	-0.7	NA	-1.3	NA	NA	-0.97
1RGG	SER	42	GLY	-0.7	NA	0.44	NA	NA	1.1
1RGG	SER	48	PRO	-1.3	NA	-0.73	NA	NA	0.65
1RGG	TYR	49	PRO	0.2	NA	-0.2	NA	NA	7.31
1RGG	THR	76	PRO	-1	NA	-0.14	NA	NA	2.4
1RGG	GLN	77	GLY	-0.8	NA	-0.25	NA	NA	-1.67

1RGG	TYR	86	GLY	-0.4	NA	-0.67	NA	NA	17.98
1RGG	GLN	94	PRO	0.8	NA	0.54	NA	NA	15.03
1PGA	THR	2	PRO	2.7	NA	0.66	NA	NA	6.96
1PGA	GLY	9	PRO	2.4	NA	2.63	NA	NA	35.34
1PGA	LYS	10	PRO	0.2	NA	2.28	NA	NA	53.73
1PGA	VAL	21	PRO	-0.5	NA	-0.02	NA	NA	4.86
1PGA	ALA	23	PRO	0.3	NA	0.43	NA	NA	0.31
1PGA	ALA	24	PRO	0.5	NA	2.21	NA	NA	6.74
1PGA	THR	25	PRO	2.8	NA	5.3	NA	NA	51.79
1PGA	VAL	29	PRO	3.5	NA	4.4	NA	NA	48.62
1PGA	ASP	36	PRO	3.1	NA	3.31	NA	NA	38.38
1PGA	ALA	48	PRO	0.7	NA	1.51	NA	NA	23.34

**Table S2. MUE and RMSE for the Pucci set, with or without proline mutations. Data for Figure 2A-B**

All mutations	Monopeptide	Tripeptide	Pentapeptide	Heptapeptide
MUE	1.68	0.85	0.76	0.68
RMSE	2.79	1.11	1.05	0.90
Non-proline mutations	Monopeptide	Tripeptide	Pentapeptide	Heptapeptide
MUE	1.16	0.87	0.75	0.70
RMSE	1.67	1.14	1.03	0.92

**Table S3. Error distribution for the Pucci set, with or without proline mutations. Data for Figure 2C-D**

All mutations					
Error(log[U]/[F])	Monopeptide	Tripeptide	Post outlier analysis	Pentapeptide	Heptapeptide
< 1	66.67	75.36	80.88	76.81	79.71
1 - 2	14.49	18.84	17.65	20.29	17.39
2 - 3	8.70	4.35	1.47	1.45	1.45
> 3	10.14	1.45	0.00	1.45	1.45
Non-proline mutations					
Error(log[U]/[F])	Monopeptide	Tripeptide	Post outlier analysis	Pentapeptide	Heptapeptide
< 1	71.88	73.44	79.37	76.56	79.69
1 - 2	15.63	20.31	19.05	20.31	17.19
2 - 3	9.38	4.69	1.59	1.56	1.56
> 3	3.13	1.56	0.00	1.56	1.56

**Table S4. Comparison to common protein thermostability predictors for both forward and reverse mutations.**

	FEP+	MMGBSA	PopMuSiC <sup>sym</sup>		MUPRO		FoldX		Rosetta	
			Forw	Rev	Forw	Rev	Forw	Rev	Forw	Rev
<b>MUE</b>	0.85	NA	1.08	1.06	0.70	2.01	1.20	1.49	1.65	1.63
<b>RMSE</b>	1.11	NA	1.46	1.46	1.21	2.45	1.79	2.03	2.19	2.34
<b>R<sup>2</sup></b>	0.68	0.39	0.27	0.42	0.36	0.10	0.22	0.23	0.39	0.21
<b>Accuracy</b>	0.85	0.74	0.65	0.67	0.77	0.30	0.70	0.65	0.64	0.64
<b>Sensitivity</b>	0.89	0.46	0.68	0.79	0.32	1.00	0.68	0.79	0.53	0.76
<b>Specificity</b>	0.84	0.86	0.64	0.62	0.94	0.04	0.70	0.60	0.68	0.60