Supporting information for: Flex ddG: Rosetta Ensemble-Based Estimation of Changes in Protein-Protein Binding Affinity Upon Mutation

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Table S1: **SHA1 Git version of Rosetta used for benchmarking**

Git SHA1	Protocol
69aa5266f0d5	flex ddG
69aa5266f0d5	no backrub control
3b2aa5cc3798	ddG monomer

Table S2: Flex ddG performance comparison, when backrub is run with a sampling temperature (kT) of 1.2 or 1.6 and 10,000 backrub steps (this differs from Table 2, which is shown at 35,000 backrub steps). N = number of cases in the dataset or subset. R = Pearson's R. MAE = Mean Absolute Error. FC = Fraction Correct. Best performance for each metric and dataset is shown in bold.

Mutation Category	Prediction Method	N	R	MAE	FC
Complete dataset	flex ddG	1240	0.63	0.98	**0.76**
	flex ddG (1.6 kT)		**0.64**	**0.93**	0.75
Small-to-large mutation(s)	flex ddG	130	0.62	0.84	**0.72**
	flex ddG (1.6 kT)		**0.64**	**0.81**	**0.72**
Single mutation to alanine	flex ddG	748	0.50	0.76	**0.75**
	flex ddG (1.6 kT)		**0.51**	**0.72**	**0.75**
Multiple mutations	flex ddG	273	**0.63**	1.63	**0.79**
	flex ddG (1.6 kT)		**0.63**	**1.52**	0.75
Multiple mutations, none to alanine	flex ddG	45	**0.65**	1.40	**0.67**
	flex ddG (1.6 kT)		0.62	**1.38**	0.58

Mutation Category	Prediction Method	N	R	MAE	FC
pdb-1A22	flex ddG	142	**0.32**	**0.61**	**0.79**
	no backrub control		0.18	0.77	0.74
	ddG monomer		0.12	0.91	0.73
	ZEMu paper		0.19	0.68	0.78
pdb-1A4Y	flex ddG	45	0.81	1.34	0.71
	no backrub control		0.79	1.47	**0.78**
	ddG monomer		0.77	1.91	0.62
	ZEMu paper		**0.87**	**1.12**	0.73
pdb-1ACB	flex ddG	6	0.28	2.89	0.83
	no backrub control		0.23	2.37	0.83
	ddG monomer		0.58	**1.57**	**1.00**
	ZEMu paper		**0.79**	2.17	0.83
pdb-1AHW	flex ddG	10	-0.83	1.31	0.4
	no backrub control		-0.42	1.42	0.4
	ddG monomer		-0.34	1.26	0.5
	ZEMu paper		**0.30**	**0.93**	**0.6**
pdb-1AK4	flex ddG	15	**0.73**	**0.53**	**0.73**
	no backrub control		0.35	1.01	0.47
	ddG monomer		0.63	1.35	0.60
	ZEMu paper		0.44	1.63	0.53
pdb-1CBW	flex ddG	15	0.01	**0.59**	**0.87**
	no backrub control		**0.05**	0.83	0.67
	ddG monomer		-0.09	0.72	0.67
	ZEMu paper		-0.26	0.71	0.67
pdb-1CSE	flex ddG	6	0.44	1.94	0.67
	no backrub control		0.37	2.03	0.67
	ddG monomer		0.46	1.88	0.67

	ZEMu paper		**0.87**	**0.81**	**1.00**
pdb-1DAN	flex ddG	118	0.65	**0.53**	**0.88**
	no backrub control		**0.69**	0.59	0.85
	ddG monomer		0.61	0.71	0.83
	ZEMu paper		0.32	0.88	0.76
pdb-1DFJ	flex ddG	20	0.70	1.35	**0.70**
	no backrub control		**0.83**	**1.04**	0.60
	ddG monomer		0.69	1.38	0.55
	ZEMu paper		0.55	1.40	0.55
pdb-1DQJ	flex ddG	34	**0.44**	**1.67**	0.79
	no backrub control		0.39	1.93	0.65
	ddG monomer		0.37	1.87	**0.82**
	ZEMu paper		0.28	2.08	0.59
pdb-1DVF	flex ddG	38	0.63	1.58	0.53
	no backrub control		**0.65**	**1.50**	0.66
	ddG monomer		0.61	1.54	**0.71**
	ZEMu paper		0.57	1.54	0.53
pdb-1E96	flex ddG	6	**0.52**	**0.84**	0.50
	no backrub control		0.51	0.91	0.50
	ddG monomer		0.45	0.96	0.50
	ZEMu paper		0.50	0.85	**0.67**
pdb-1EAW	flex ddG	27	0.03	0.58	0.85
	no backrub control		0.07	0.73	0.81
	ddG monomer		**0.13**	0.61	0.89
	ZEMu paper		0.00	**0.49**	**0.93**
pdb-1EMV	flex ddG	51	**0.89**	**0.86**	**0.86**
	no backrub control		0.84	0.98	0.84
	ddG monomer		0.84	0.96	0.80

	ZEMu paper		0.87	0.89	0.84
pdb-1F47	flex ddG	12	0.56	**0.72**	0.50
	no backrub control		0.58	0.87	**0.58**
	ddG monomer		**0.60**	0.87	**0.58**
	ZEMu paper		0.51	1.02	0.42
pdb-1FC2	flex ddG	9	-0.07	**0.84**	0.56
	no backrub control		-0.09	1.01	0.67
	ddG monomer		-0.39	1.19	0.44
	ZEMu paper		**0.28**	0.89	**0.78**
pdb-1FCC	flex ddG	8	-0.21	1.56	**0.5**
	no backrub control		-0.22	1.96	**0.5**
	ddG monomer		-0.06	1.50	**0.5**
	ZEMu paper		**0.16**	**1.35**	**0.5**
pdb-1GC1	flex ddG	56	0.12	**0.35**	**0.91**
	no backrub control		-0.15	0.43	0.86
	ddG monomer		0.28	0.38	0.86
	ZEMu paper		**0.36**	0.55	0.84
pdb-1HE8	flex ddG	10	0.39	0.67	**0.5**
	no backrub control		0.62	0.91	**0.5**
	ddG monomer		0.26	**0.66**	0.3
	ZEMu paper		**0.81**	1.23	**0.5**
pdb-1IAR	flex ddG	36	0.64	**0.72**	0.78
	no backrub control		0.35	1.32	0.67
	ddG monomer		**0.66**	0.98	**0.81**
	ZEMu paper		0.45	0.86	0.78
pdb-1JCK	flex ddG	7	0.46	1.22	0.57
	no backrub control		0.44	0.97	**0.71**
	ddG monomer		0.75	1.25	**0.71**

	ZEMu paper		**0.85**	**0.94**	**0.71**
pdb-1JRH	flex ddG	53	**0.58**	**1.09**	0.66
	no backrub control		0.50	1.25	0.60
	ddG monomer		0.52	1.29	**0.75**
	ZEMu paper		0.57	1.15	0.58
pdb-1JTG	flex ddG	118	0.44	1.87	0.84
	no backrub control		0.39	**1.77**	0.83
	ddG monomer		0.40	2.12	**0.86**
	ZEMu paper		**0.51**	**1.77**	0.75
pdb-1KTZ	flex ddG	27	**0.86**	**0.63**	0.74
	no backrub control		0.76	1.16	**0.85**
	ddG monomer		0.71	1.13	0.81
	ZEMu paper		0.80	0.83	0.70
pdb-1LFD	flex ddG	25	**0.55**	**0.68**	**0.72**
	no backrub control		0.13	1.21	0.60
	ddG monomer		0.28	0.97	**0.72**
	ZEMu paper		0.27	0.84	0.60
pdb-1MLC	flex ddG	16	-0.28	1.01	0.62
	no backrub control		0.22	0.82	0.75
	ddG monomer		0.28	0.83	0.56
	ZEMu paper		**0.79**	**0.42**	**0.88**
pdb-1NMB	flex ddG	6	0.21	0.83	**0.83**
	no backrub control		0.48	0.83	0.67
	ddG monomer		0.62	**0.60**	**0.83**
	ZEMu paper		**0.78**	1.97	0.17
pdb-1REW	flex ddG	24	**0.89**	**0.67**	**0.92**
	no backrub control		0.78	1.19	0.75
	ddG monomer		0.76	1.20	0.79

	ZEMu paper		0.65	1.03	**0.92**
pdb-1S1Q	flex ddG	6	0.17	**0.68**	**0.67**
	no backrub control		0.22	0.75	**0.67**
	ddG monomer		**0.34**	0.71	**0.67**
	ZEMu paper		-0.07	1.09	0.50
pdb-1TM1	flex ddG	21	0.33	1.71	0.43
	no backrub control		0.23	1.81	0.43
	ddG monomer		0.23	1.72	0.62
	ZEMu paper		**0.58**	**1.37**	**0.71**
pdb-1UUZ	flex ddG	5	0.79	0.62	**0.8**
	no backrub control		**0.92**	**0.52**	**0.8**
	ddG monomer		0.83	0.80	**0.8**
	ZEMu paper		0.42	1.60	0.2
pdb-1VFB	flex ddG	43	0.58	**0.95**	**0.70**
	no backrub control		0.21	1.50	0.67
	ddG monomer		**0.64**	1.40	**0.70**
	ZEMu paper		0.60	1.07	0.65
pdb-1XD3	flex ddG	18	0.46	0.95	0.72
	no backrub control		0.28	1.43	0.67
	ddG monomer		0.51	1.15	0.67
	ZEMu paper		**0.56**	**0.71**	**0.83**
pdb-2I9B	flex ddG	5	-0.81	0.53	**1.0**
	no backrub control		-0.00	**0.49**	**1.0**
	ddG monomer		-0.56	0.55	**1.0**
	ZEMu paper		**0.65**	0.62	**1.0**
pdb-2JEL	flex ddG	43	**0.67**	0.64	**0.84**
	no backrub control		0.62	**0.63**	0.81
	ddG monomer		0.64	0.70	**0.84**

	ZEMu paper		0.48	0.81	0.74
pdb-2PCB	flex ddG	6	**0.28**	**0.44**	**1.00**
	no backrub control		0.23	0.64	0.67
	ddG monomer		-0.70	0.90	**1.00**
	ZEMu paper		-0.63	0.95	0.83
pdb-2PCC	flex ddG	12	**0.10**	**1.27**	**0.5**
	no backrub control		0.04	1.49	**0.5**
	ddG monomer		-0.29	1.90	**0.5**
	ZEMu paper		-0.24	1.53	**0.5**
pdb-2VLJ	flex ddG	14	0.40	**0.84**	0.57
	no backrub control		**0.42**	1.75	0.43
	ddG monomer		0.26	0.93	0.50
	ZEMu paper		0.15	0.89	**0.64**
pdb-2WPT	flex ddG	32	0.54	1.60	0.62
	no backrub control		**0.63**	**1.39**	**0.75**
	ddG monomer		0.56	1.63	0.69
	ZEMu paper		0.45	1.63	0.56
pdb-3BK3	flex ddG	13	**0.72**	**0.51**	0.69
	no backrub control		0.70	0.98	0.62
	ddG monomer		0.65	0.55	**0.85**
	ZEMu paper		0.31	1.45	0.54
pdb-3BN9	flex ddG	25	0.48	**0.39**	**0.88**
	no backrub control		**0.53**	0.40	**0.88**
	ddG monomer		0.31	0.66	**0.88**
	ZEMu paper		-0.09	0.66	0.84
pdb-3NPS	flex ddG	27	0.18	**0.71**	0.74
	no backrub control		**0.26**	0.87	**0.78**
	ddG monomer		0.15	0.84	**0.78**
	ZEMu paper		-0.21	0.89	0.67

Table S3: Flex ddG performance by PDB structure for all complexes with 5 or more cases. Backrub steps = 35000. N = number of cases (variants) for each complex. R = Pearson's R. MAE = Mean Absolute Error. FC = Fraction Correct. Best performance for each metric and dataset is shown in bold.

Table S4: Selection of key data shown in Fig. 3

Run	Subset	Models	R	MAE
flex ddG	Complete dataset	1	0.54	1.23
flex ddG	Complete dataset	20	0.63	0.99
flex ddG	Complete dataset	30	0.63	0.98
flex ddG	Complete dataset	40	0.63	0.97
flex ddG	Complete dataset	50	0.63	0.96
no backrub control	Complete dataset	1	0.57	1.14
no backrub control	Complete dataset	20	0.57	1.12
no backrub control	Complete dataset	30	0.57	1.12
no backrub control	Complete dataset	40	0.56	1.12
no backrub control	Complete dataset	50	0.56	1.12
flex ddG	Small-to-large mutation(s)	1	0.40	1.11
flex ddG	Small-to-large mutation(s)	20	0.64	0.82
flex ddG	Small-to-large mutation(s)	30	0.64	0.80
flex ddG	Small-to-large mutation(s)	40	0.65	0.79
flex ddG	Small-to-large mutation(s)	50	0.65	0.78
no backrub control	Small-to-large mutation(s)	1	0.39	1.12
no backrub control	Small-to-large mutation(s)	20	0.40	1.12
no backrub control	Small-to-large mutation(s)	30	0.41	1.12
no backrub control	Small-to-large mutation(s)	40	0.41	1.11
no backrub control	Small-to-large mutation(s)	50	0.41	1.11
flex ddG	Multiple mutations, none to alanine	1	0.33	1.88
flex ddG	Multiple mutations, none to alanine	20	0.66	1.46
flex ddG	Multiple mutations, none to alanine	30	0.62	1.44
flex ddG	Multiple mutations, none to alanine	40	0.62	1.42
flex ddG	Multiple mutations, none to alanine	50	0.63	1.38
no backrub control	Multiple mutations, none to alanine	1	0.45	1.64
no backrub control	Multiple mutations, none to alanine	20	0.45	1.63
no backrub control	Multiple mutations, none to alanine	30	0.45	1.63
no backrub control	Multiple mutations, none to alanine	40	0.44	1.64
no backrub control	Multiple mutations, none to alanine	50	0.44	1.66
flex ddG	Single mutation to alanine	1	0.42	0.99
flex ddG	Single mutation to alanine	20	0.50	0.76
flex ddG	Single mutation to alanine	30	0.50	0.76
flex ddG	Single mutation to alanine	40	0.51	0.75
flex ddG	Single mutation to alanine	50	0.51	0.75
no backrub control	Single mutation to alanine	1	0.44	0.91
no backrub control	Single mutation to alanine	20	0.44	0.90
no backrub control	Single mutation to alanine	30	0.44	0.90
no backrub control	Single mutation to alanine	40	0.44	0.90
no backrub control	Single mutation to alanine	50	0.44	0.90

Table S5: Selection of key data shown in Fig. S2

Run	Subset	Models	R	MAE
ddG monomer	Complete dataset	1	0.41	1.87
ddG monomer	Complete dataset	20	0.59	1.25
ddG monomer	Complete dataset	30	0.60	1.22
ddG monomer	Complete dataset	40	0.61	1.19
ddG monomer	Complete dataset	50	0.62	1.16
no backrub control	Complete dataset	1	0.57	1.14
no backrub control	Complete dataset	20	0.57	1.12
no backrub control	Complete dataset	30	0.57	1.12
no backrub control	Complete dataset	40	0.56	1.12
no backrub control	Complete dataset	50	0.56	1.12
ddG monomer	Small-to-large mutation(s)	1	0.18	1.95
ddG monomer	Small-to-large mutation(s)	20	0.47	1.19
ddG monomer	Small-to-large mutation(s)	30	0.50	1.14
ddG monomer	Small-to-large mutation(s)	40	0.50	1.11
ddG monomer	Small-to-large mutation(s)	50	0.52	1.05
no backrub control	Small-to-large mutation(s)	1	0.39	1.12
no backrub control	Small-to-large mutation(s)	20	0.40	1.12
no backrub control	Small-to-large mutation(s)	30	0.41	1.12
no backrub control	Small-to-large mutation(s)	40	0.41	1.11
no backrub control	Small-to-large mutation(s)	50	0.41	1.11
ddG monomer	Multiple mutations, none to alanine	1	0.35	2.96
ddG monomer	Multiple mutations, none to alanine	20	0.54	1.73
ddG monomer	Multiple mutations, none to alanine	30	0.57	1.59
ddG monomer	Multiple mutations, none to alanine	40	0.56	1.58
ddG monomer	Multiple mutations, none to alanine	50	0.58	1.48
no backrub control	Multiple mutations, none to alanine	1	0.45	1.64
no backrub control	Multiple mutations, none to alanine	20	0.45	1.63
no backrub control	Multiple mutations, none to alanine	30	0.45	1.63
no backrub control	Multiple mutations, none to alanine	40	0.44	1.64
no backrub control	Multiple mutations, none to alanine	50	0.44	1.66
ddG monomer	Single mutation to alanine	1	0.24	1.22
ddG monomer	Single mutation to alanine	20	0.43	0.82
ddG monomer	Single mutation to alanine	30	0.46	0.79
ddG monomer	Single mutation to alanine	40	0.47	0.76
ddG monomer	Single mutation to alanine	50	0.50	0.76
no backrub control	Single mutation to alanine	1	0.44	0.91
no backrub control	Single mutation to alanine	20	0.44	0.90
no backrub control	Single mutation to alanine	30	0.44	0.90
no backrub control	Single mutation to alanine	40	0.44	0.90
no backrub control	Single mutation to alanine	50	0.44	0.90

Table S6: Selection of key data shown in Fig. S5

Run	Subset	Models	R	MAE
flex ddG	Complete dataset	1	0.54	1.24
flex ddG	Complete dataset	20	0.63	0.98
flex ddG	Complete dataset	30	0.63	0.97
flex ddG	Complete dataset	40	0.63	0.96
flex ddG	Complete dataset	50	0.63	0.96
no backrub control	Complete dataset	1	0.56	1.12
no backrub control	Complete dataset	20	0.57	1.12
no backrub control	Complete dataset	30	0.56	1.12
no backrub control	Complete dataset	40	0.56	1.12
no backrub control	Complete dataset	50	0.56	1.12
flex ddG	Small-to-large mutation(s)	1	0.57	1.09
flex ddG	Small-to-large mutation(s)	20	0.65	0.79
flex ddG	Small-to-large mutation(s)	30	0.66	0.76
flex ddG	Small-to-large mutation(s)	40	0.66	0.77
flex ddG	Small-to-large mutation(s)	50	0.65	0.78
no backrub control	Small-to-large mutation(s)	1	0.40	1.11
no backrub control	Small-to-large mutation(s)	20	0.41	1.12
no backrub control	Small-to-large mutation(s)	30	0.41	1.11
no backrub control	Small-to-large mutation(s)	40	0.41	1.11
no backrub control	Small-to-large mutation(s)	50	0.41	1.11
flex ddG	Multiple mutations, none to alanine	1	0.44	1.88
flex ddG	Multiple mutations, none to alanine	20	0.61	1.40
flex ddG	Multiple mutations, none to alanine	30	0.63	1.37
flex ddG	Multiple mutations, none to alanine	40	0.62	1.39
flex ddG	Multiple mutations, none to alanine	50	0.63	1.38
no backrub control	Multiple mutations, none to alanine	1	0.45	1.66
no backrub control	Multiple mutations, none to alanine	20	0.44	1.66
no backrub control	Multiple mutations, none to alanine	30	0.44	1.66
no backrub control	Multiple mutations, none to alanine	40	0.44	1.66
no backrub control	Multiple mutations, none to alanine	50	0.44	1.66
flex ddG	Single mutation to alanine	1	0.45	0.98
flex ddG	Single mutation to alanine	20	0.50	0.77
flex ddG	Single mutation to alanine	30	0.51	0.76
flex ddG	Single mutation to alanine	40	0.51	0.75
flex ddG	Single mutation to alanine	50	0.51	0.75
no backrub control	Single mutation to alanine	1	0.44	0.90
no backrub control	Single mutation to alanine	20	0.44	0.90
no backrub control	Single mutation to alanine	30	0.44	0.90
no backrub control	Single mutation to alanine	40	0.44	0.90
no backrub control	Single mutation to alanine	50	0.44	0.90

Table S7: Selection of key data shown in Fig. 4

Run	Subset	Backrub Step	R	MAE
no backrub control	Complete dataset	0	0.56	1.12
flex ddG	Complete dataset	2500	0.64	0.99
flex ddG	Complete dataset	10000	0.63	0.98
flex ddG	Complete dataset	20000	0.63	0.97
flex ddG	Complete dataset	30000	0.63	0.96
flex ddG	Complete dataset	35000	0.63	0.96
flex ddG	Complete dataset	40000	0.63	0.97
flex ddG	Complete dataset	50000	0.63	0.96
no backrub control	Small-to-large mutation(s)	0	0.41	1.11
flex ddG	Small-to-large mutation(s)	2500	0.56	0.89
flex ddG	Small-to-large mutation(s)	10000	0.62	0.84
flex ddG	Small-to-large mutation(s)	20000	0.63	0.82
flex ddG	Small-to-large mutation(s)	30000	0.65	0.80
flex ddG	Small-to-large mutation(s)	35000	0.65	0.78
flex ddG	Small-to-large mutation(s)	40000	0.63	0.81
flex ddG	Small-to-large mutation(s)	50000	0.65	0.82
no backrub control	Multiple mutations, none to alanine	0	0.44	1.66
flex ddG	Multiple mutations, none to alanine	2500	0.61	1.44
flex ddG	Multiple mutations, none to alanine	10000	0.65	1.40
flex ddG	Multiple mutations, none to alanine	20000	0.62	1.40
flex ddG	Multiple mutations, none to alanine	30000	0.62	1.39
flex ddG	Multiple mutations, none to alanine	35000	0.63	1.38
flex ddG	Multiple mutations, none to alanine	40000	0.64	1.39
flex ddG	Multiple mutations, none to alanine	50000	0.61	1.36
no backrub control	Single mutation to alanine	0	0.44	0.90
flex ddG	Single mutation to alanine	2500	0.50	0.78
flex ddG	Single mutation to alanine	10000	0.50	0.76
flex ddG	Single mutation to alanine	20000	0.50	0.75
flex ddG	Single mutation to alanine	30000	0.51	0.75
flex ddG	Single mutation to alanine	35000	0.51	0.75
flex ddG	Single mutation to alanine	40000	0.51	0.75
flex ddG	Single mutation to alanine	50000	0.52	0.73

Table S8: Performance comparison of the standard flex ddG protocol (using Rosetta's Talaris energy function) with flex ddG run with the REF score function, and 35000 backrub steps. Data for the flex ddG method with the Talaris energy function are as in Table 2 in the main text. "res <= 1.5 Ang." indicates data points for which the resolution of the input wild-type crystal structure is less than or equal to 1.5 Å. N = number of cases in the dataset or subset. R = Pearson's R. MAE = Mean Absolute Error. FC = Fraction Correct. Best performance for each metric and dataset is shown in bold.

Mutation Category	Prediction Method	N	R	MAE	FC
Complete dataset	flex ddG	1240	**0.63**	**0.96**	**0.76**
	flex ddG (REF energy)		**0.63**	1.19	0.75
Small-to-large mutation(s)	flex ddG	130	**0.65**	**0.78**	0.71
	flex ddG (REF energy)		0.57	1.01	**0.72**
Single mutation to alanine	flex ddG	748	**0.51**	**0.75**	**0.76**
	flex ddG (REF energy)		0.49	0.90	0.74
Multiple mutations	flex ddG	273	**0.62**	**1.62**	0.78
	flex ddG (REF energy)		0.59	2.12	**0.80**
Res. <= 1.5 Ang.	flex ddG	52	0.47	**0.93**	0.73
	flex ddG (REF energy)		**0.65**	1.10	**0.75**
Res. >= 2.5 Ang.	flex ddG	457	**0.49**	**0.73**	**0.76**
	flex ddG (REF energy)		0.48	0.90	0.75

Table S9: Performance for GAM-fit predictions on the complete benchmark data set, with Talaris[S1–S3] and REF[S4] energy functions. Backrub steps = 35000. N = number of cases in the dataset. R = Pearson's R. MAE = Mean Absolute Error. FC = Fraction Correct.

Prediction Method	N	R	MAE	FC
Flex ddG (Talaris) GAM	1240	0.68	0.88	0.76
Flex ddG (REF) GAM		0.68	0.88	0.75
No backrub control GAM		0.62	0.93	0.75

Table S10: Selection of key data shown in Fig. S3

Run	Subset	Models	R	MAE
flex ddG	Loop	1	0.62	1.29
flex ddG	Loop	20	0.75	1.00
flex ddG	Loop	30	0.75	1.00
flex ddG	Loop	40	0.75	1.00
flex ddG	Loop	50	0.75	0.99
no backrub control	Loop	1	0.66	1.16
no backrub control	Loop	20	0.66	1.17
no backrub control	Loop	30	0.66	1.17
no backrub control	Loop	40	0.66	1.17
no backrub control	Loop	50	0.66	1.17
flex ddG	Alpha Helix	1	0.42	1.15
flex ddG	Alpha Helix	20	0.51	0.91
flex ddG	Alpha Helix	30	0.53	0.88
flex ddG	Alpha Helix	40	0.53	0.88
flex ddG	Alpha Helix	50	0.52	0.88
no backrub control	Alpha Helix	1	0.41	1.13
no backrub control	Alpha Helix	20	0.41	1.11
no backrub control	Alpha Helix	30	0.41	1.12
no backrub control	Alpha Helix	40	0.41	1.12
no backrub control	Alpha Helix	50	0.41	1.12
flex ddG	Strand	1	0.46	1.09
flex ddG	Strand	20	0.57	0.82
flex ddG	Strand	30	0.58	0.81
flex ddG	Strand	40	0.58	0.82
flex ddG	Strand	50	0.58	0.82
no backrub control	Strand	1	0.52	0.96
no backrub control	Strand	20	0.53	0.95
no backrub control	Strand	30	0.53	0.95
no backrub control	Strand	40	0.53	0.95
no backrub control	Strand	50	0.53	0.95
flex ddG	Turn	1	0.60	1.14
flex ddG	Turn	20	0.70	0.94
flex ddG	Turn	30	0.71	0.92
flex ddG	Turn	40	0.70	0.93
flex ddG	Turn	50	0.70	0.92
no backrub control	Turn	1	0.68	0.98
no backrub control	Turn	20	0.67	0.98
no backrub control	Turn	30	0.67	0.98
no backrub control	Turn	40	0.67	0.98
no backrub control	Turn	50	0.67	0.98

Table S11: Selection of key data shown in Fig. S4

Run	Subset	Models	R	MAE
flex ddG	Mean burial score < 0.10	1	0.51	1.63
flex ddG	Mean burial score < 0.10	20	0.56	1.39
flex ddG	Mean burial score < 0.10	30	0.56	1.38
flex ddG	Mean burial score < 0.10	40	0.56	1.37
flex ddG	Mean burial score < 0.10	50	0.56	1.37
no backrub control	Mean burial score < 0.10	1	0.49	1.57
no backrub control	Mean burial score < 0.10	20	0.50	1.56
no backrub control	Mean burial score < 0.10	30	0.50	1.57
no backrub control	Mean burial score < 0.10	40	0.50	1.57
no backrub control	Mean burial score < 0.10	50	0.50	1.57
flex ddG	Mean burial score >= 0.10	1	0.50	1.03
flex ddG	Mean burial score >= 0.10	20	0.64	0.75
flex ddG	Mean burial score >= 0.10	30	0.65	0.74
flex ddG	Mean burial score >= 0.10	40	0.64	0.74
flex ddG	Mean burial score >= 0.10	50	0.64	0.74
no backrub control	Mean burial score >= 0.10	1	0.57	0.88
no backrub control	Mean burial score >= 0.10	20	0.57	0.88
no backrub control	Mean burial score >= 0.10	30	0.57	0.88
no backrub control	Mean burial score >= 0.10	40	0.57	0.88
no backrub control	Mean burial score >= 0.10	50	0.57	0.88
flex ddG	Mean burial score < 0.25	1	0.52	1.48
flex ddG	Mean burial score < 0.25	20	0.59	1.21
flex ddG	Mean burial score < 0.25	30	0.60	1.20
flex ddG	Mean burial score < 0.25	40	0.60	1.19
flex ddG	Mean burial score < 0.25	50	0.60	1.19
no backrub control	Mean burial score < 0.25	1	0.54	1.37
no backrub control	Mean burial score < 0.25	20	0.54	1.36
no backrub control	Mean burial score < 0.25	30	0.54	1.36
no backrub control	Mean burial score < 0.25	40	0.54	1.36
no backrub control	Mean burial score < 0.25	50	0.54	1.36
flex ddG	Mean burial score >= 0.25	1	0.41	0.86
flex ddG	Mean burial score >= 0.25	20	0.56	0.62
flex ddG	Mean burial score >= 0.25	30	0.57	0.60
flex ddG	Mean burial score >= 0.25	40	0.56	0.61
flex ddG	Mean burial score >= 0.25	50	0.56	0.61
no backrub control	Mean burial score >= 0.25	1	0.46	0.73
no backrub control	Mean burial score >= 0.25	20	0.44	0.74
no backrub control	Mean burial score >= 0.25	30	0.44	0.74
no backrub control	Mean burial score >= 0.25	40	0.44	0.74
no backrub control	Mean burial score >= 0.25	50	0.44	0.74

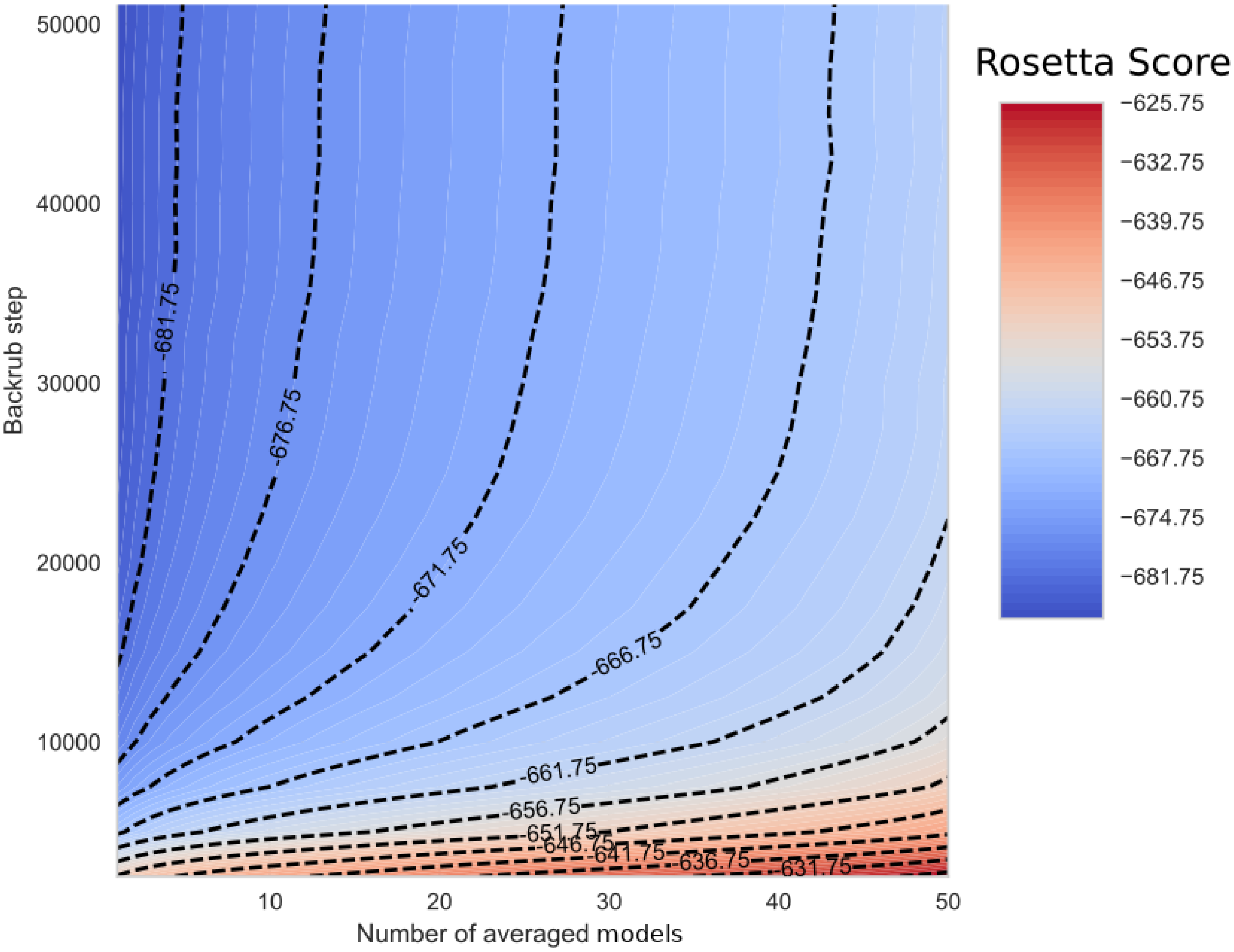

Figure S1: Contour plot showing the effect of backrub sampling on the average wild-type complex score, for increasing numbers of averaged models. As the number of averaged models is increased along the x-axis, the average total score of the ensemble of wild-type complex models (shown as colored contours in the body of the plot) also increases, as the wild-type complex models are first sorted according to their total scores and included in the averaged ensemble in order of increasing score. As the number of backrub sampling steps at which the ensemble is generated increases (along the y-axis), the total score of an ensemble of any number of models tends to decrease, indicating that flex ddG is able to find lower-energy conformations (as measured by the Rosetta energy function) as the simulation progresses. However, using only the lowest energy models does not produce higher correlations with experimental $\Delta\Delta G$ values, as shown in Fig. 3. Rosetta scores are in Rosetta Energy Units (REU) using the Rosetta Talaris energy function.[S1–S3]

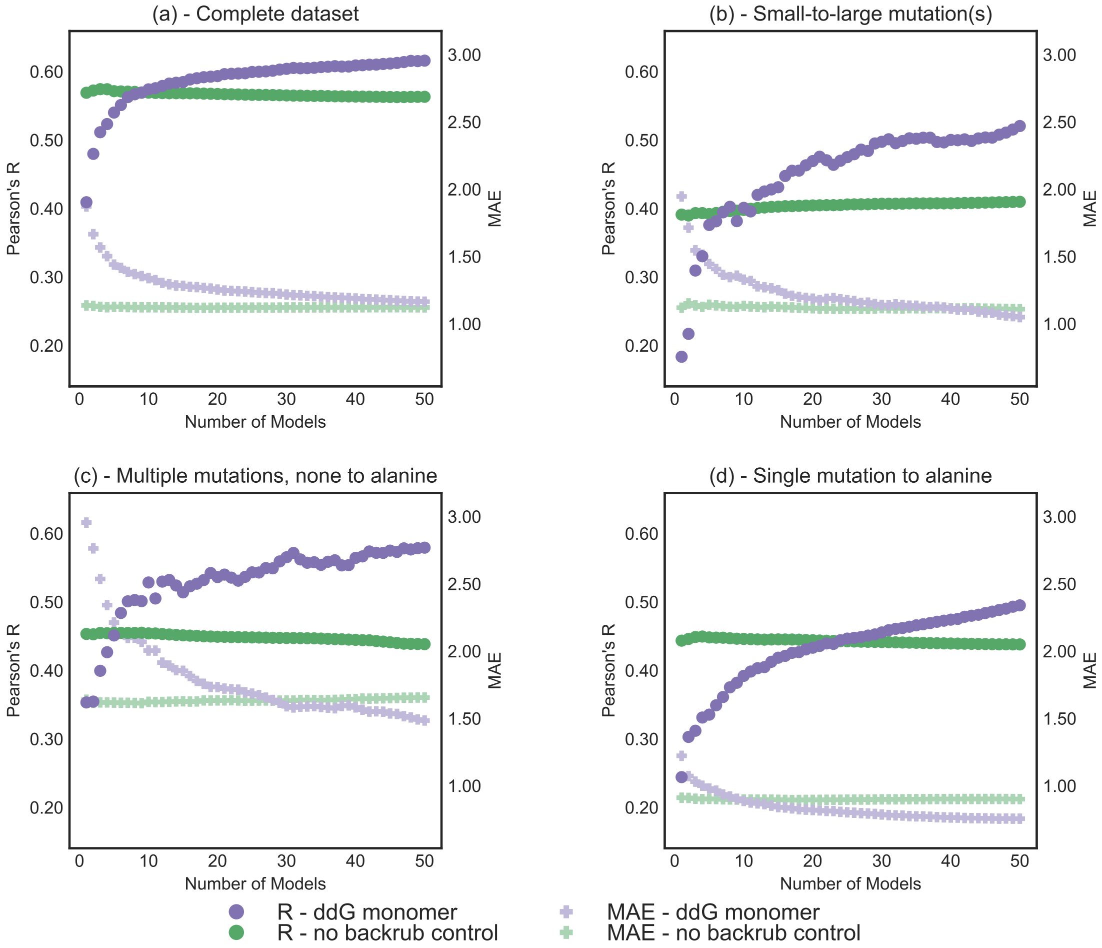

Figure S2: Correlation (Pearson's R, left y-axis) and MAE (Mean Absolute Error, right y-axis) vs. number of averaged models (x-axis), on the complete ZEMu set, and subsets. Pearson's R is shown as circles, and MAE as faded plusses. Predictions generated with the ddg_monomer protocol are shown in purple. Predictions generated with the no backrub control protocol are shown in green. A selection of key data underlying this figure can be found in Table S5. Structures are sorted by their minimized wild-type complex energy. (a) Complete dataset (n = 1240) (b) Small-to-large mutation(s) (n = 130) (c) Multiple mutations, none to alanine (n = 45) (d) Single mutation to alanine (n = 748).

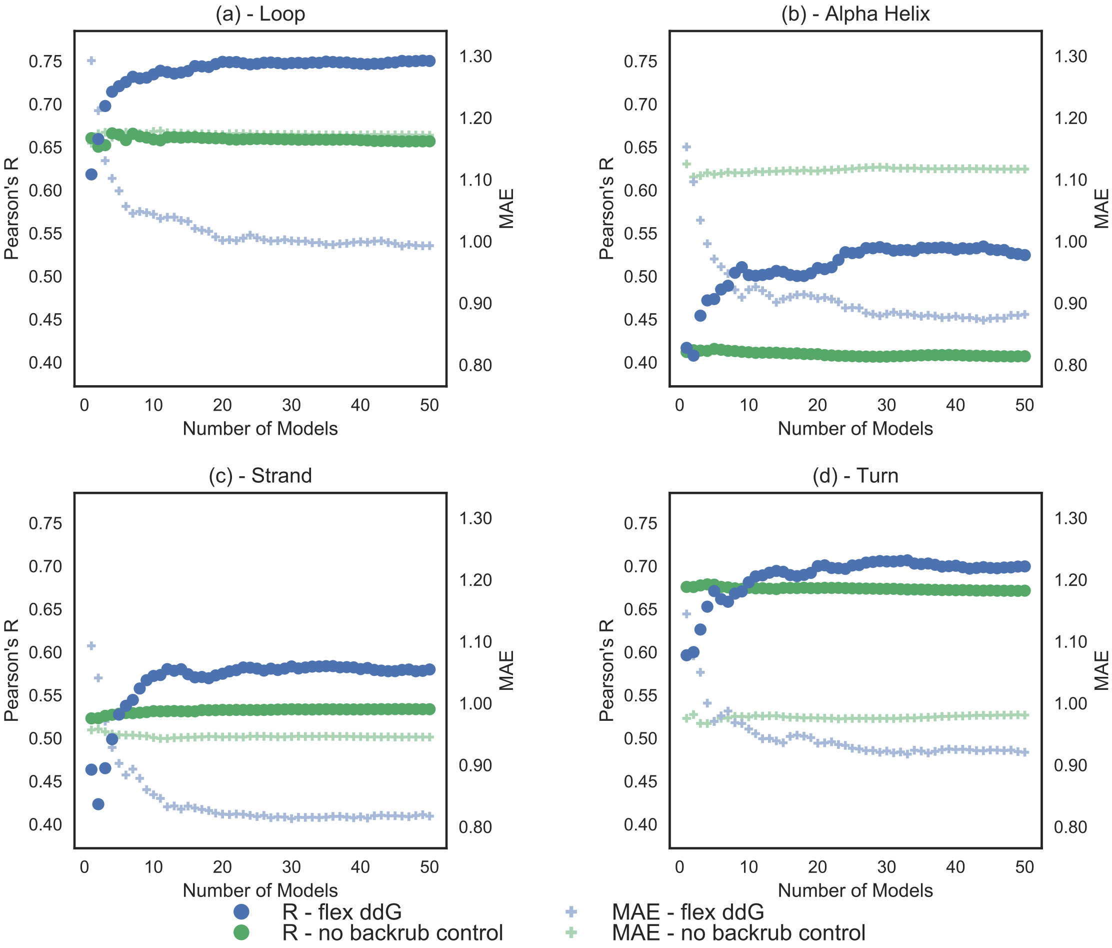

Figure S3: Correlation (Pearson's R, left y-axis) and MAE (Mean Absolute Error, right y-axis) vs. number of averaged models (x-axis), on subsets of the complete ZEMu set binned by DSSP-assigned[S5,S6] secondary structure at the mutation site. For cases with multiple mutations, all mutation sites must share the same secondary structure to be considered. Pearson's R is shown as circles, and MAE as faded plusses. Predictions generated with the Flex ddG protocol are shown in blue. Predictions generated with the no backrub control protocol are shown in green. A selection of key data underlying this figure can be found in Table S10. Flex ddG is run with 35000 backrub steps. Structures are not sorted, and are randomly added to the ensemble. (a) Loop (n = 349) (b) Alpha helix (n = 198) (c) Strand (n = 280) (d) Turn (n = 183).

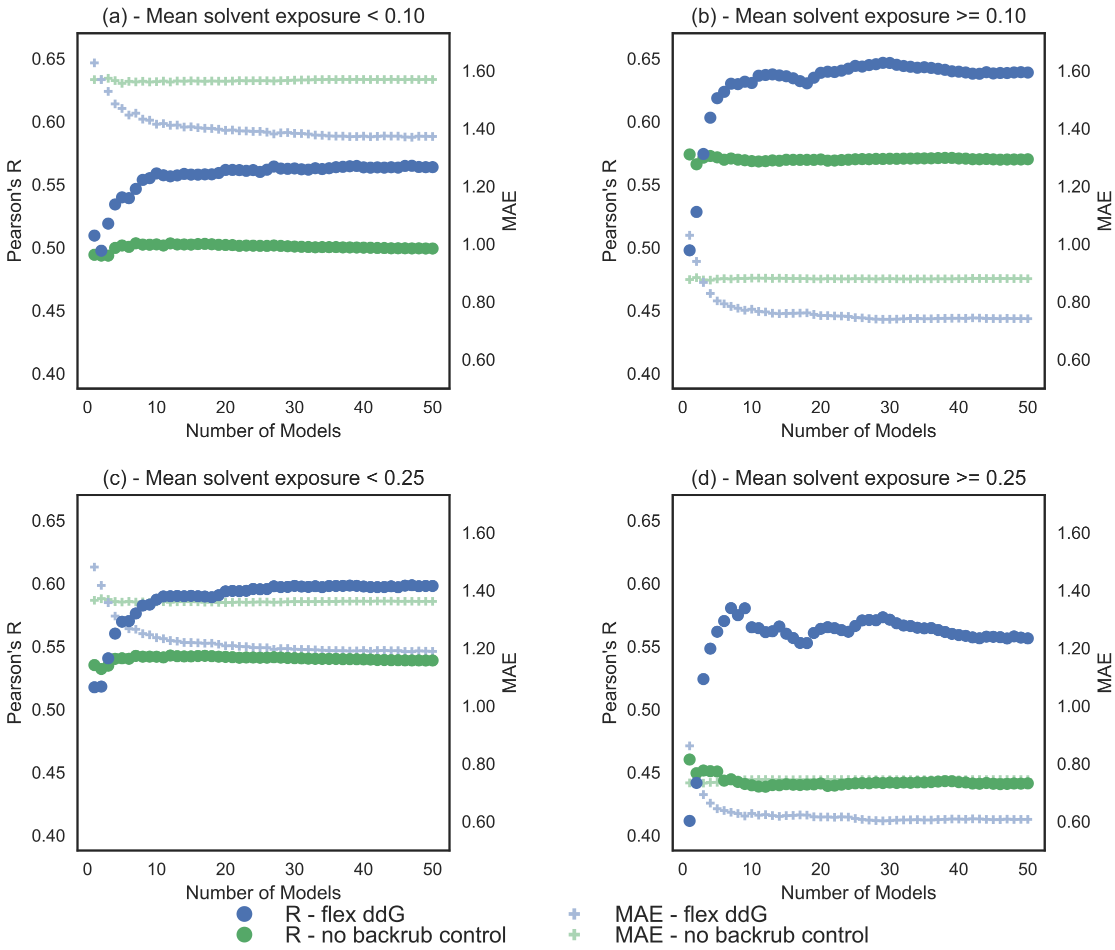

Figure S4: Correlation (Pearson's R, left y-axis) and MAE (Mean Absolute Error, right y-axis) vs. number of averaged models (x-axis), on subsets of the complete ZEMu set binned by DSSP-calculated[S5,S6] solvent exposure. Solvent exposure is calculated for all mutation position sites in the context of the input wild type crystal structure. For dataset cases with multiple mutation positions, solvent exposure is averaged. Pearson's R is shown as circles, and MAE as faded plusses. Predictions generated with the Flex ddG protocol are shown in blue. Predictions generated with the no backrub control protocol are shown in green. A selection of key data underlying this figure can be found in Table S11. Flex ddG is run with 35000 backrub steps. Structures are not sorted, and are randomly added to the ensemble. (a) Mean solvent exposure $<$ 0.10 (n = 436) (b) Mean solvent exposure $>=$ 0.10 (n = 804) (c) Mean solvent exposure $<$ 0.25 (n = 758) (d) Mean solvent exposure $>=$ 0.25 (n = 482).

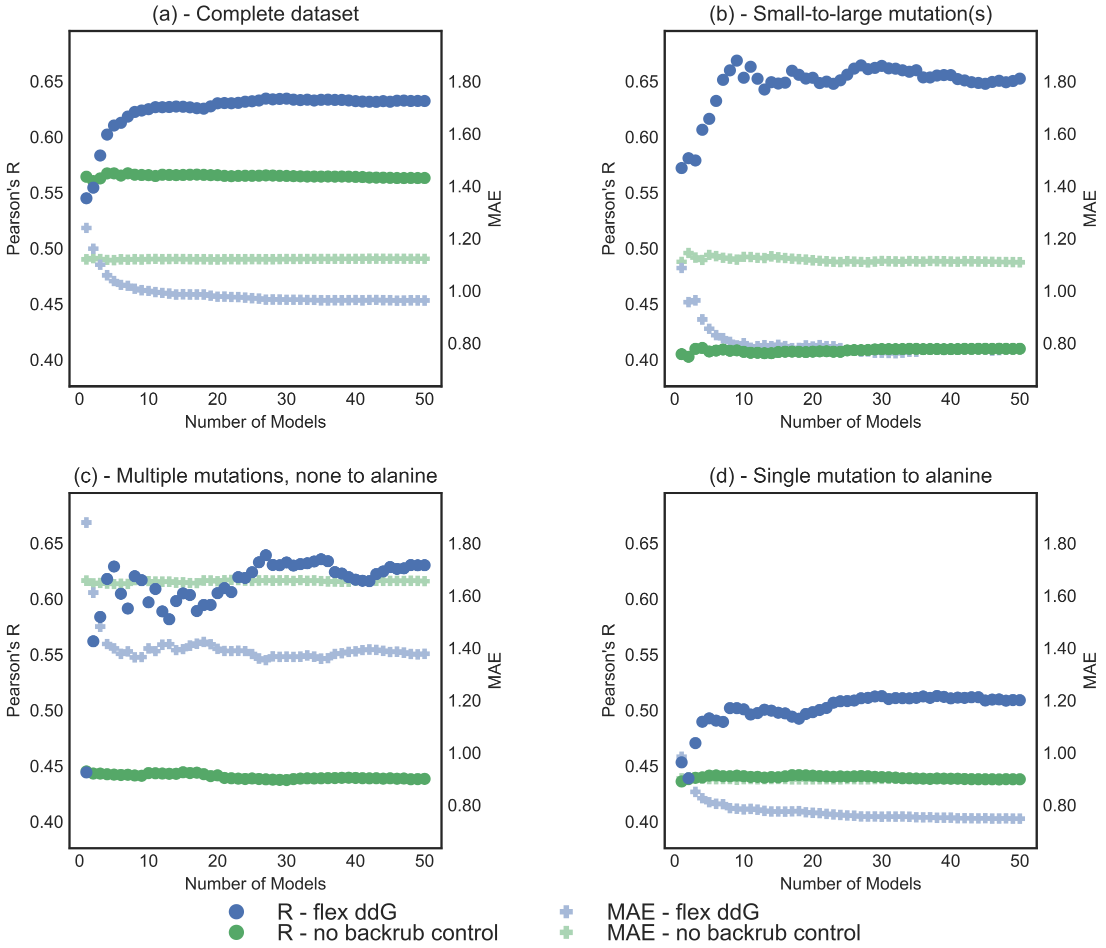

Figure S5: Correlation (Pearson's R, left y-axis) and MAE (Mean Absolute Error, right y-axis) vs. number of averaged models (x-axis), on the complete ZEMu set, and subsets. This plot is analogous to Fig. 3 in the main manuscript, except that the models used for averaging are not sorted by score. Pearson's R is shown as circles, and MAE as faded plusses. Predictions generated with the Flex ddG protocol are shown in blue. Predictions generated with the no backrub control protocol are shown in green. A selection of key data underlying this figure can be found in Table S6. Flex ddG is run with 35000 backrub steps. Structures are not sorted, and are randomly added to the ensemble. (a) Complete dataset (n = 1240) (b) Small-to-large mutation(s) (n = 130) (c) Multiple mutations, none to alanine (n = 45) (d) Single mutation to alanine (n = 748).

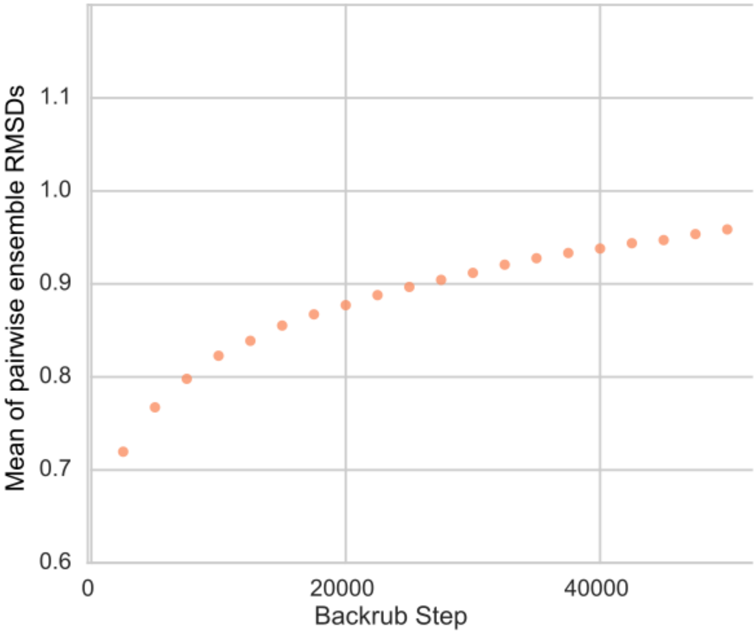

Figure S6: The average pairwise ensemble RMSD for each ensemble of 50 models is shown versus increasing backrub sampling steps. Average pairwise RMSD is computed on all atoms in residues selected as neighborhood pivot residues. To calculate pairwise RMSD, this selection of atoms is superimposed onto their positions in the last output model in the backrub trajectory before pairwise RMSDs are computed. Pairwise ensemble RMSD increases with increasing backrub sampling steps, indicating that the diversity of sampled models (as measured by RMSD) continues to increase past the point around 30k-35k sampling steps where correlation with experimental $\Delta\Delta G$ values levels out. RMSD shown is calculated for 1216 out of 1240 of the total points in the dataset.

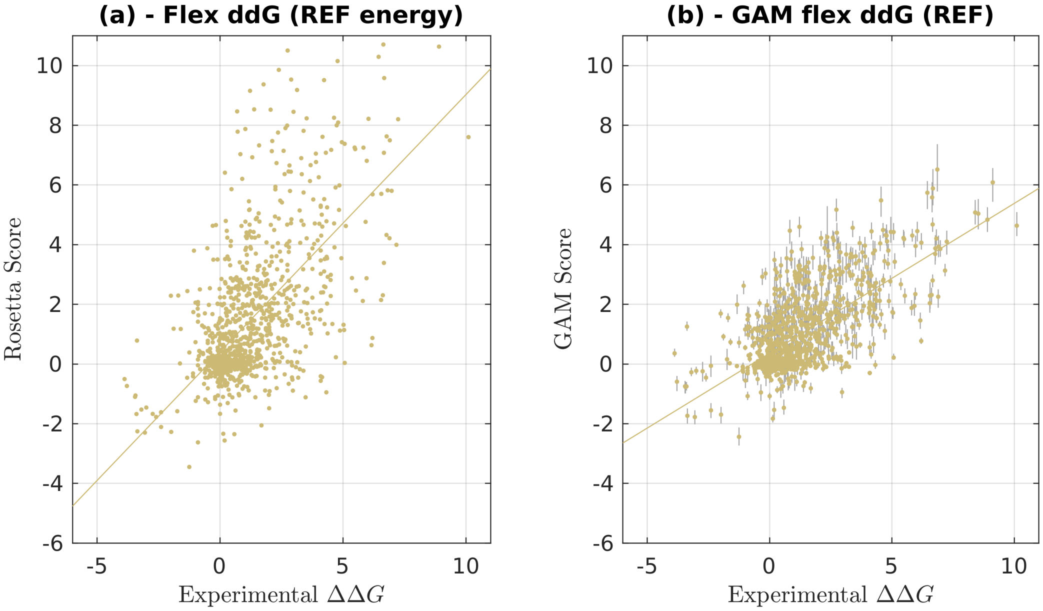

Figure S7: Experimentally determined $\Delta\Delta G$ values (x-axis) versus predictions. The error bars in gray represent the range from minimum to maximum fit predicted $\Delta\Delta G$ value for the 1000 sampled GAM (Generalized Additive Model) models. A line of best fit is shown. **(a)**: standard Rosetta output (non-fitted REF predictions) vs. experimental $\Delta\Delta G$ values. **(b)**: GAM flex ddG predictions vs. experimental data. This plot is analogous to Fig. 5b in the main text, except that GAM scores are refit from values in Rosetta Energy Units (REU) using the Rosetta REF[S4] energy function (instead of Talaris[S1–S3]).

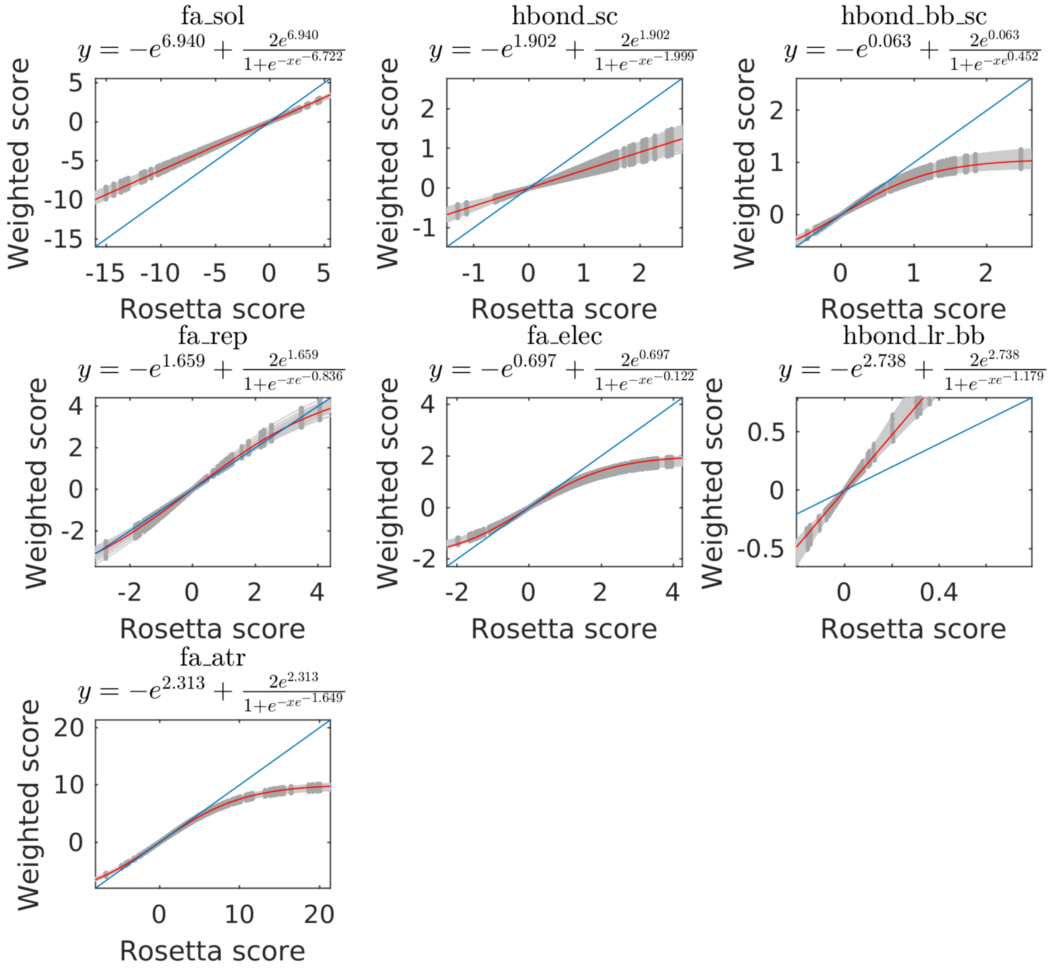

Figure S8: Sigmoid functions resulting from application of unbiased logistic scaling to individual Rosetta score terms (generated using the flex ddG protocol and the Talaris energy function[S1–S3]) in a generalized additive model. Extreme values for most score terms are downweighted, except for long-range hydrogen bond interactions between backbone atoms, which only make minor contributions (hbond_lr_bb).

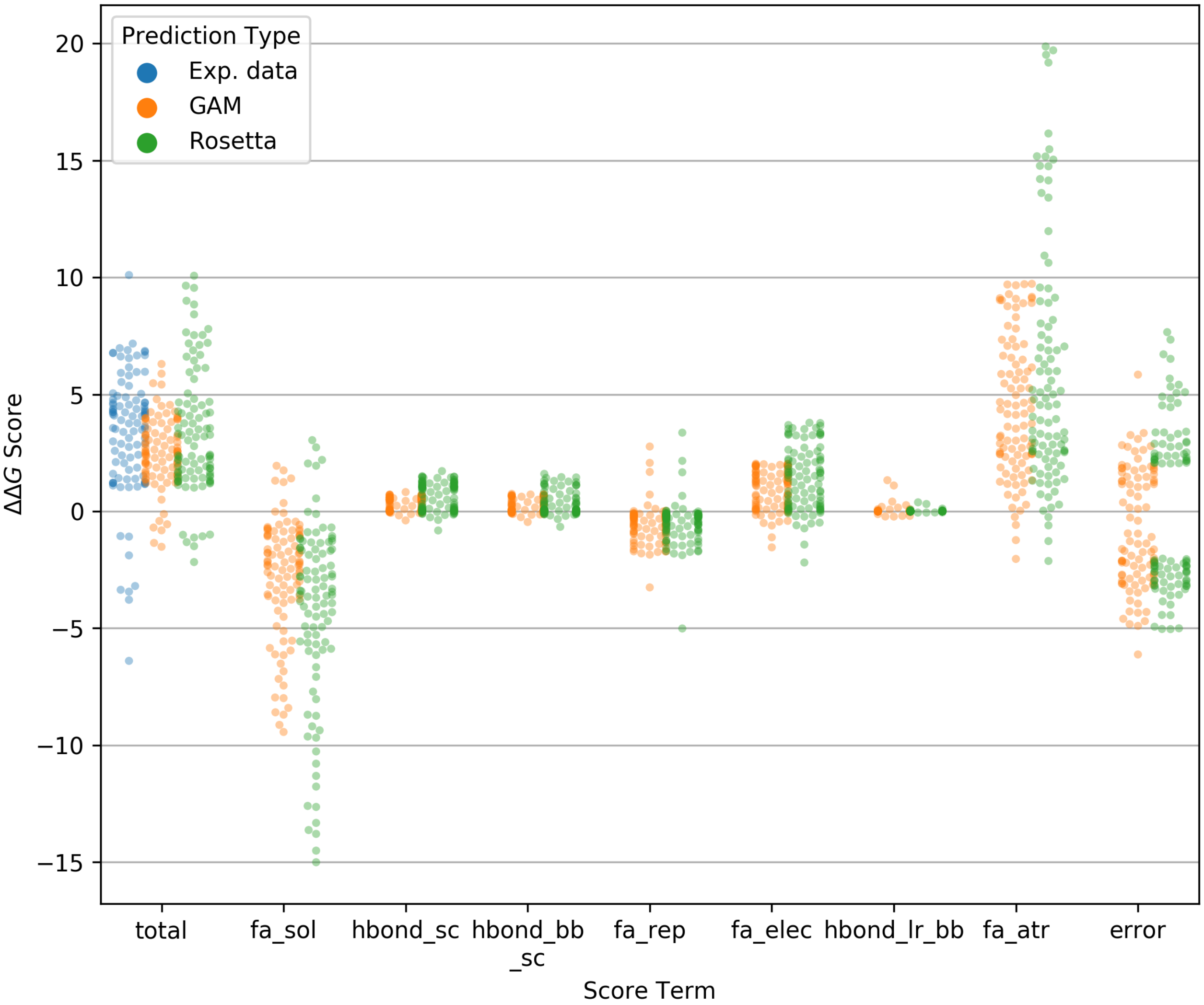

Figure S9: Total $\Delta\Delta G$ predictions and partial Rosetta score terms (Talaris energy function[S1–S3]). On the far left, total scores for the original Rosetta predictions are shown in green alongside GAM-fit predictions in orange and the experimentally determined values in blue. On the far right, the error ($\Delta\Delta G_{predicted} - \Delta\Delta G_{experimental}$) is shown. Intermediate strips show the 7 partial Rosetta score terms (fa_sol, hbond_sc, hbond_bb_sc, hbond_lr_bb, fa_rep, fa_elec, and fa_atr) which together equal the total $\Delta\Delta G$ score on the far left. Points shown are filtered from the complete prediction set according to the following criteria: neutral mutations are removed (-1.0 < experimental $\Delta\Delta G$ or Rosetta predicted $\Delta\Delta G$ score < 1.0). Also removed are points where the absolute value of the error of the original Rosetta prediction is less than 2.0

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1	1TM1	1.7	I Y61A	2.20
2	1TM1	1.7	I Y61G	4.69
3	1TM1	1.7	I R65A	3.08
4	1TM1	1.7	I R67A	2.93
5	1TM1	1.7	I R67C	3.24
6	1TM1	1.7	I R67A;I R65A	5.07
7	1CBW	2.6	I K15A	1.99
8	1A4Y	2	A Y434F	0.56
9	1A4Y	2	A Y437F	0.25
10	1A4Y	2	A Y434A;A D435A	6.81
11	1A4Y	2	A Y434A;A Y437A	6.64
12	1A4Y	2	B R5A;A Y434A	6.97
13	1A4Y	2	B R5A;A D435A	6.76
14	1A4Y	2	A D435A;B R5A;A Y434A	10.10
15	1A4Y	2	A Y434A;B R5A;A Y437A	8.53
16	1A4Y	2	B K40G	3.23
17	1A4Y	2	B K40G;A Y434F	6.66
18	1A4Y	2	A D435A;B K40G	4.11
19	1A4Y	2	B K40G;A Y437A	6.45
20	1A4Y	2	B K40G;A Y434A;A D435A	6.20
21	1A4Y	2	B K40G;A Y437A;A Y434A	9.12
22	1DFJ	2.5	I Y430F	0.12
23	1DFJ	2.5	I Y433F	2.15
24	1DFJ	2.5	I Y430A;I D431A	7.17
25	1DFJ	2.5	I Y430A;I Y433A	7.24
26	1FCC	3.2	C T25A	0.24

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
27	1FCC	3.2	C K28A	1.26
28	1FCC	3.2	C K31A	3.48
29	1FCC	3.2	C N35A	2.36
30	1FCC	3.2	C D40A	0.27
31	1FCC	3.2	C E42A	0.39
32	1FCC	3.2	C W43A	3.77
33	1FCC	3.2	C Y45A;C T44A	1.95
34	1XD3	1.45	B K6A	1.38
35	1XD3	1.45	B K27A	-0.06
36	1XD3	1.45	B R42L	-0.88
37	1XD3	1.45	B R54L	0.89
38	1XD3	1.45	B H68N	0.00
39	1XD3	1.45	B R72L	1.33
40	1XD3	1.45	B R74L	2.43
41	1XD3	1.45	B E24A	-0.25
42	1XD3	1.45	B D39A	-0.42
43	1XD3	1.45	B E51A	1.57
44	1XD3	1.45	B D52A	-0.25
45	1XD3	1.45	B D58A	-0.25
46	1XD3	1.45	B K6R	0.31
47	1XD3	1.45	B L8A	2.74
48	1XD3	1.45	B K11R	1.43
49	1XD3	1.45	B K27R	0.27
50	1XD3	1.45	B K33R	0.00
51	1XD3	1.45	B I44A	0.27
52	1JTG	1.73	A S235A	1.24

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
53	1JTG	1.73	A S130A	0.79
54	1JTG	1.73	A R243A	1.27
55	1JTG	1.73	A K234A	1.03
56	1JTG	1.73	A R243A;A S130A	1.85
57	1JTG	1.73	A S130A;A K234A	1.98
58	1JTG	1.73	A S130A;A R243A;A S235A	1.69
59	1JTG	1.73	A S130A;A K234A;A S235A	1.85
60	1JTG	1.73	A R243A;A K234A	2.73
61	1JTG	1.73	A K234A;A S235A;A R243A	2.72
62	1JTG	1.73	A S130A;A R243A;A K234A	2.51
63	1JTG	1.73	A S130A;A R243A;A S235A;A K234A	2.79
64	1DQJ	2	C Y20A	3.29
65	1DQJ	2	C R21A	1.17
66	1DQJ	2	C W63A	1.35
67	1DQJ	2	C K97A	3.52
68	1DQJ	2	C D101A	1.45
69	2I9B	2.8	E R137A	-0.29
70	2I9B	2.8	E K139A	0.67
71	2I9B	2.8	E R142A	0.36
72	2I9B	2.8	E H143A	0.66
73	2I9B	2.8	E R145A	0.42
74	1JTG	1.73	B T32K	0.20
75	1JTG	1.73	B V93K	-0.48
76	1JTG	1.73	B T140K	-0.02
77	1JTG	1.73	B N89K	-0.46
78	1JTG	1.73	B D163A	-1.34

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
79	1JTG	1.73	B D163K	-1.99
80	1JTG	1.73	B T140K;B Q157K	-0.40
81	1JTG	1.73	B D163K;B V165K;B N89K	-2.40
82	1JTG	1.73	B D163K;B D135K;B V134K	-3.06
83	1JTG	1.73	B D163K;B V165K;B D135K;B N89K	-3.36
84	1F47	1.95	A D4A	0.69
85	1F47	1.95	A Y5A	0.87
86	1F47	1.95	A L6A	0.93
87	1F47	1.95	A D7A	1.73
88	1F47	1.95	A I8A	2.52
89	1F47	1.95	A P9A	-0.06
90	1F47	1.95	A F11A	2.44
91	1F47	1.95	A L12A	2.29
92	1F47	1.95	A K14A	-0.04
93	1F47	1.95	A Q15A	-0.05
94	1REW	1.86	A D30A;B D30A	0.67
95	1REW	1.86	B W31A;A W31A	1.09
96	1REW	1.86	B D30A;A W31A;B W31A;A D30A	2.06
97	1REW	1.86	B F49A;A F49A	1.36
98	1REW	1.86	B P50A;A P50A	1.29
99	1REW	1.86	B P50A;A F49A;B F49A;A P50A	2.88
100	1REW	1.86	B H39D;A H39D	0.20
101	1REW	1.86	A S88A;B S88A	0.20
102	1REW	1.86	B L100A;A L100A	0.23
103	1REW	1.86	A S88A;B S88A;A H39D;B H39D	0.18
104	1REW	1.86	B L100A;A H39D;B H39D;A L100A	0.12

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
105	1REW	1.86	B A34D;A A34D	0.00
106	1REW	1.86	A D53A;B D53A	-0.05
107	1REW	1.86	B E109R;A E109R	0.27
108	1REW	1.86	B A34D;A D30A;B D30A;A A34D	0.48
109	1REW	1.86	A D53A;B D53A;A A34D;B A34D	-0.18
110	1REW	1.86	B E109R;A D53A;B D53A;A E109R	0.04
111	1DFJ	2.5	I V428A;I Q426A	0.94
112	1DFJ	2.5	I E436A;I W434A;I T435A	1.98
113	1DFJ	2.5	I R453A	0.85
114	1DFJ	2.5	I I455A	0.33
115	1A4Y	2	A Q430A;A V432A	0.06
116	1A4Y	2	A S439A;A E440A;A W438A	1.83
117	1A4Y	2	A R457A	-0.22
118	1A4Y	2	A I459A	0.67
119	1DFJ	2.5	I E202A	1.02
120	1DFJ	2.5	I W257A	1.33
121	1DFJ	2.5	I W259A	2.22
122	1DFJ	2.5	I E283A	1.32
123	1DFJ	2.5	I S285A	0.80
124	1DFJ	2.5	I W314A	0.99
125	1DFJ	2.5	I K316A	1.30
126	1DFJ	2.5	I E340A	1.57
127	1DFJ	2.5	I E397A	1.31
128	1A4Y	2	B H84A	0.17
129	1A4Y	2	B W89A	0.23
130	1A4Y	2	A W261A	0.09

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
131	1A4Y	2	A W263A	1.16
132	1A4Y	2	A E287A	0.09
133	1A4Y	2	A S289A	0.03
134	1A4Y	2	A W318A	1.48
135	1A4Y	2	A K320A	-0.31
136	1A4Y	2	A E344A	0.16
137	1A4Y	2	A W375A	1.03
138	1A4Y	2	A E401A	0.86
139	1JRH	2.8	L E27A	0.54
140	1JRH	2.8	L D28A	0.43
141	1JRH	2.8	L Y30A	1.11
142	1JRH	2.8	L Y91A	0.58
143	1JRH	2.8	L W92A	2.82
144	1JRH	2.8	L S93A	-0.65
145	1JRH	2.8	L T94A	0.39
146	1JRH	2.8	L W96A	1.67
147	1JRH	2.8	H Y32A	1.43
148	1JRH	2.8	H W52A	2.69
149	1JRH	2.8	H W53A	2.42
150	1JRH	2.8	H D54A	1.89
151	1JRH	2.8	H D55A	1.67
152	1JRH	2.8	H D56A	1.85
153	1JRH	2.8	H Y58A	1.26
154	1JRH	2.8	H R95A	0.54
155	1JRH	2.8	H F98A	0.00
156	1JRH	2.8	H Y99A	1.06

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
157	1JRH	2.8	H H100bA	1.70
158	1JRH	2.8	I I57R;I E55P	-3.44
159	1JRH	2.8	I E55G;I S54G;I I57R	-3.38
160	1JRH	2.8	I I57L;I E55P	-3.46
161	1JRH	2.8	I E55P;I S54G;I I57K	-3.88
162	1JRH	2.8	I E55P	-3.79
163	1HE8	3	A K223V	0.47
164	1HE8	3	A K223V;A R226A	1.87
165	1HE8	3	A K234A;A K223V	1.50
166	1HE8	3	A K223V;A D238A	1.02
167	1HE8	3	A K223V;A K254A	1.93
168	1HE8	3	A K256A;A K223V	1.65
169	1HE8	3	A K223V;A D260A	0.93
170	1HE8	3	A E267K;A K223V	-0.10
171	1HE8	3	A K223V;A E919A	0.45
172	1HE8	3	A K223V;A E919K	-0.45
173	2PCC	2.3	A D34A	-0.91
174	2PCC	2.3	A V197A	2.09
175	2PCC	2.3	A E290A	6.19
176	2PCC	2.3	B K87A	0.89
177	2PCC	2.3	B K87A;A D34A	0.20
178	2PCC	2.3	A V197A;B K87A	1.50
179	2PCC	2.3	B K72A	0.29
180	2PCC	2.3	A V197A;B K72A	2.79
181	2PCC	2.3	A E290A;B K72A	1.09
182	2PCC	2.3	B A81G	1.90

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
183	2PCC	2.3	A D34A;B A81G	-0.10
184	2PCC	2.3	B A81G;A V197A	2.10
185	1F47	1.95	A D7S	2.06
186	1F47	1.95	A D7G	1.14
187	1S1Q	2	A V43A	0.66
188	1S1Q	2	A F44A	0.20
189	1S1Q	2	A N45A	1.21
190	1S1Q	2	A D46A	0.95
191	1S1Q	2	A W75A	0.28
192	1S1Q	2	A F88A	0.76
193	1DQJ	2	C W62A	0.76
194	1DQJ	2	C L75A	1.45
195	1DQJ	2	C T89A	0.84
196	1DQJ	2	C N93A	0.65
197	1DQJ	2	C K96A	6.16
198	1DQJ	2	C S100A	0.78
199	1DQJ	2	A N31A	2.01
200	1DQJ	2	A N32A	4.09
201	1DQJ	2	A Y50A	2.68
202	1DQJ	2	A S91A	1.43
203	1DQJ	2	A Y96A	1.14
204	1DQJ	2	B D32A	2.01
205	1DQJ	2	B Y33A	5.52
206	1DQJ	2	B Y50A	6.89
207	1DQJ	2	B Y53A	1.18
208	1DQJ	2	B W98A	4.93

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
209	1DQJ	2	C K96A;A N32A	5.80
210	1DQJ	2	C R21A;A S91A	3.17
211	1DQJ	2	A S91A;C Y20A	3.57
212	1DQJ	2	C R21A;A Y96A	3.49
213	1DQJ	2	C S100A;A Y96A	0.91
214	1DQJ	2	C K97A;B D32A	2.49
215	1DQJ	2	B Y53A;C W62A	1.29
216	1DQJ	2	C W63A;B Y53A	2.20
217	1DQJ	2	C L75A;B Y53A	1.22
218	1DQJ	2	B Y53A;C D101A	2.73
219	1DQJ	2	C S100A;B W98A	5.36
220	1DQJ	2	C K97A;B W98A	6.66
221	1DQJ	2	B W98A;C Y20A	5.11
222	1KAC	2.6	A P417S	-0.79
223	1KAC	2.6	A S489Y	-1.25
226	1A4Y	2	B R5A	2.31
227	1A4Y	2	B R32A	0.90
228	1A4Y	2	B R66A	0.20
229	1A4Y	2	B R70A	-0.25
230	1A4Y	2	B R31A	0.25
231	1A4Y	2	B R33A	0.33
232	1REW	1.86	B W28F;A W28F	0.67
233	1REW	1.86	A D53P;B D53P	2.35
234	1REW	1.86	B S69R;A S69R	1.67
235	1REW	1.86	B Y103A;A Y103A	1.21
236	1REW	1.86	C Q86A	2.66

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
237	1LFD	2.1	A R20A	1.10
238	1LFD	2.1	A S22K	-0.10
239	1LFD	2.1	A L23K	0.00
240	1LFD	2.1	A N27K	0.40
241	1LFD	2.1	A M30K	-0.90
242	1LFD	2.1	A K32A	1.30
243	1LFD	2.1	A K48A	0.20
244	1LFD	2.1	A D51A	-0.60
245	1LFD	2.1	A D51K	-1.10
246	1LFD	2.1	A K52A	1.20
247	1LFD	2.1	A N54K	-0.90
248	1LFD	2.1	A L55K	-0.60
249	1LFD	2.1	A D56A	-0.30
250	1LFD	2.1	A E57A	-0.20
251	1LFD	2.1	A E57K	-0.30
252	1LFD	2.1	A D58K	-0.90
253	1LFD	2.1	A N92K	-0.60
254	1LFD	2.1	A Y93K	0.10
255	1LFD	2.1	A D94K	-1.10
256	1LFD	2.1	A M30K;A D58K	-1.00
257	1LFD	2.1	A D94K;A M30K	-0.90
258	1LFD	2.1	A D58K;A D51K;A M30K	-2.00
259	1LFD	2.1	A D58K;A M30K;A D51K;A D94K	-1.70
260	1LFD	2.1	A D51K;A E57K;A D56K	-1.60
261	1LFD	2.1	A D58K;A D94K	-0.60
262	1TM1	1.7	I M59G	2.23

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
263	1TM1	1.7	I M59A	1.03
264	1TM1	1.7	I M59K	1.09
265	1TM1	1.7	I M59Y	0.06
266	1TM1	1.7	I M59F	1.03
267	1JTG	1.73	B K74A	3.56
268	1JTG	1.73	B F142A	2.10
269	1JTG	1.73	B Y143A	0.38
270	1JTG	1.73	B F142A;B K74A	4.85
271	1JTG	1.73	B Y143A;B K74A	3.06
272	1JTG	1.73	B Y143A;B F142A	2.84
273	1JTG	1.73	B F142A;B Y143A;B K74A	3.89
274	1JTG	1.73	A E104A	1.55
275	1JTG	1.73	B K74A;A E104A	1.58
276	1JTG	1.73	B F142A;A E104A	2.75
277	1JTG	1.73	A E104A;B Y143A	1.86
278	1JTG	1.73	B F142A;A E104A;B K74A	3.13
279	1JTG	1.73	B Y143A;B K74A;A E104A	1.05
280	1JTG	1.73	B Y143A;B F142A;A E104A	2.89
281	1JTG	1.73	B Y143A;A E104A;B K74A;B F142A	2.39
282	1JTG	1.73	A Y105A	-0.17
283	1JTG	1.73	B K74A;A Y105A	3.32
284	1JTG	1.73	B F142A;A Y105A	0.69
285	1JTG	1.73	B Y143A;A Y105A	1.07
286	1JTG	1.73	A Y105A;B K74A;B F142A	4.78
287	1JTG	1.73	B K74A;A Y105A;B Y143A	3.39
288	1JTG	1.73	B F142A;A Y105A;B Y143A	2.99

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
289	1JTG	1.73	A Y105A;B K74A;B F142A;B Y143A	4.23
290	1JTG	1.73	A E104A;A Y105A	1.03
291	1JTG	1.73	A E104A;B K74A;A Y105A	1.39
292	1JTG	1.73	B F142A;A Y105A;A E104A	1.50
293	1JTG	1.73	A Y105A;A E104A;B Y143A	2.05
294	1JTG	1.73	A Y105A;B K74A;A E104A;B F142A	3.13
295	1JTG	1.73	B K74A;A Y105A;B Y143A;A E104A	1.77
296	1JTG	1.73	B F142A;A Y105A;B Y143A;A E104A	2.84
297	1JTG	1.73	B F142A;B Y143A;A Y105A;B K74A;A E104A	2.41
298	1JTG	1.73	B K74A;A R243A	6.62
299	1JTG	1.73	A R243A;B F142A	3.39
300	1JTG	1.73	B Y143A;A R243A	2.27
301	1JTG	1.73	B K74A;B F142A;A R243A	5.49
302	1JTG	1.73	B K74A;A R243A;B Y143A	4.37
303	1JTG	1.73	B K74A;A K234A	6.55
304	1JTG	1.73	A K234A;B K74A;B F142A	5.83
305	1JTG	1.73	B Y143A;B K74A;A K234A	4.87
306	1JTG	1.73	B Y143A;A K234A;B K74A;B F142A	5.47
307	1JTG	1.73	B Y143A;A S130A;B K74A;B F142A	5.06
308	1TM1	1.7	I T58A	2.57
309	1TM1	1.7	I T58P	3.75
310	1TM1	1.7	I E60A	2.92
311	1TM1	1.7	I E60S	2.75
312	1TM1	1.7	I M59R;I E60S	3.55
313	1TM1	1.7	I R62A	1.26
314	2A9K	1.73	B G99D	2.41

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
315	2B42	2.5	A H374A	1.58
316	2B42	2.5	A H374Q	0.98
317	2B42	2.5	A H374K	2.54
318	1KTZ	2.15	B L27A	1.82
319	1KTZ	2.15	B F30A	2.98
320	1KTZ	2.15	B D32A	1.52
321	1KTZ	2.15	B D32N	2.00
322	1KTZ	2.15	B S49A	0.33
323	1KTZ	2.15	B I50A	1.90
324	1KTZ	2.15	B T51A	1.51
325	1KTZ	2.15	B S52A	0.22
326	1KTZ	2.15	B S52L	4.04
327	1KTZ	2.15	B I53A	1.37
328	1KTZ	2.15	B E55A	1.22
329	1KTZ	2.15	B V77A	0.41
330	1KTZ	2.15	B D118A	0.81
331	1KTZ	2.15	B E119A	1.49
332	1KTZ	2.15	B E119Q	1.63
333	1KTZ	2.15	B N47A	0.28
334	1KTZ	2.15	B V62A	0.65
335	1KTZ	2.15	B E75A	1.08
336	1KTZ	2.15	B H79A	0.30
337	1KTZ	2.15	B F110A	0.93
338	1KTZ	2.15	B M112A	0.87
339	1KTZ	2.15	B I125A	0.54
340	2I26	2.5	N A30V	0.07

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
341	2I26	2.5	N S61R	-0.62
342	2J12	1.5	A S299A	0.95
343	2J1K	2.3	C R515A	0.05
344	2J1K	2.3	C G370D	0.18
345	2J1K	2.3	C R384A	0.76
346	1JTG	1.73	A Q99A	0.43
347	1JTG	1.73	A N100A	-0.45
348	1JTG	1.73	A V103A	1.91
349	1JTG	1.73	A P107A	-0.38
350	1JTG	1.73	A E110A	4.06
351	1JTG	1.73	A M129A	0.74
352	1JTG	1.73	A E168A	-0.07
353	1JTG	1.73	A V216A	-0.41
354	1JTG	1.73	B F36A	3.20
355	1JTG	1.73	B H41A	3.25
356	1JTG	1.73	B Y53A	2.08
357	1JTG	1.73	B S71A	0.36
358	1JTG	1.73	B W112A	3.01
359	1JTG	1.73	B S113A	-0.17
360	1JTG	1.73	B H148A	2.75
361	1JTG	1.73	B W150A	4.25
362	1JTG	1.73	B R160A	2.22
363	1JTG	1.73	B W162A	2.34
364	1JTG	1.73	B H148A;A Q99A	3.20
365	1JTG	1.73	B W150A;A Q99A	3.82
366	1JTG	1.73	A Q99A;B R160A	3.75

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
367	1JTG	1.73	B H148A;A N100A	2.10
368	1JTG	1.73	B W150A;A N100A	4.35
369	1JTG	1.73	B R160A;A N100A	1.58
370	1JTG	1.73	A E168A;B W162A	2.08
371	1JTG	1.73	A V103A;B W162A	4.23
372	1JTG	1.73	B Y53A;A M129A	3.61
373	1JTG	1.73	B F36A;A M129A	3.63
374	1JTG	1.73	B H41A;A P107A	2.65
375	1JTG	1.73	A P107A;B Y53A	2.39
376	1JTG	1.73	B S113A;A E110A;B S71A	5.04
377	1JTG	1.73	A E110A;B S113A	4.57
378	1JTG	1.73	B S71A;A E110A	5.02
379	1JTG	1.73	A E104A;B S113A	1.86
380	1JTG	1.73	A Q99A;B W112A	3.54
381	1JTG	1.73	B W162A;A Q99A	2.89
382	1JTG	1.73	B K74A;A Q99A	4.11
383	1JTG	1.73	A Q99A;B F142A	2.82
384	1JTG	1.73	B W112A;A N100A	2.80
385	1JTG	1.73	A N100A;B W162A	2.10
386	1JTG	1.73	B K74A;A N100A	3.51
387	1JTG	1.73	A N100A;B F142A	2.29
388	1JTG	1.73	B R160A;A V103A	4.35
389	1JTG	1.73	B F142A;A V103A	4.52
390	1JTG	1.73	B W112A;A E168A	2.80
391	1JTG	1.73	B W150A;A E168A	4.11
392	1JTG	1.73	B R160A;A E168A	2.32

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
393	1JTG	1.73	B K74A;A E168A	4.06
394	1JTG	1.73	A E168A;B F142A	2.58
395	1JTG	1.73	B Y50A;A E110A	3.97
396	1JTG	1.73	B S113A;A M129A;B S71A	1.67
397	2JEL	2.5	P F2Y	0.00
398	2JEL	2.5	P F2W	2.62
399	2JEL	2.5	P Q3K	4.09
400	2JEL	2.5	P Q4K	1.36
401	2JEL	2.5	P E5D	0.41
402	2JEL	2.5	P E5Q	0.71
403	2JEL	2.5	P V6F	0.00
404	2JEL	2.5	P T7N	0.41
405	2JEL	2.5	P T7S	0.00
406	2JEL	2.5	P P11E	0.00
407	2JEL	2.5	P N12D	0.00
408	2JEL	2.5	P R17G	0.00
409	2JEL	2.5	P R17K	0.00
410	2JEL	2.5	P K24E	0.00
411	2JEL	2.5	P K27E	0.00
412	2JEL	2.5	P T34Q	0.00
413	2JEL	2.5	P T36Q	0.41
414	2JEL	2.5	P N38T	0.00
415	2JEL	2.5	P S41C	1.50
416	2JEL	2.5	P S43C	0.00
417	2JEL	2.5	P S46C	0.00
418	2JEL	2.5	P Q57E	-0.41

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
419	2JEL	2.5	P T62N	0.00
420	2JEL	2.5	P T62A	0.00
421	2JEL	2.5	P S64T	4.09
422	2JEL	2.5	P E66K	4.09
423	2JEL	2.5	P E68A	0.41
424	2JEL	2.5	P D69E	0.95
425	2JEL	2.5	P E70K	4.09
426	2JEL	2.5	P E70A	2.73
427	2JEL	2.5	P Q71E	2.73
428	2JEL	2.5	P K72R	0.00
429	2JEL	2.5	P K72E	0.41
430	2JEL	2.5	P E75R	2.73
431	2JEL	2.5	P H76D	-0.65
432	2JEL	2.5	P H76A	-0.41
433	2JEL	2.5	P K79E	0.41
434	2JEL	2.5	P A82S	0.00
435	2JEL	2.5	P E83A	0.00
436	2JEL	2.5	P E85Q	0.00
437	2JEL	2.5	P E85D	0.00
438	2JEL	2.5	P E85A	0.00
439	2JEL	2.5	P E85K	0.00
440	1UUZ	1.8	A H62Q	1.52
441	1UUZ	1.8	A H62N	1.52
442	1UUZ	1.8	A H62D	3.38
443	1UUZ	1.8	A H62A	1.77
444	1UUZ	1.8	A C64A	0.65

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
445	1EAW	2.93	A Q38A	-0.52
446	1EAW	2.93	A I41A	-0.82
447	1EAW	2.93	A I60A	-0.19
448	1EAW	2.93	A D60aA	-0.17
449	1EAW	2.93	A D60bA	1.50
450	1EAW	2.93	A R60cA	0.59
451	1EAW	2.93	A F60eA	-0.43
452	1EAW	2.93	A R60fA	0.23
453	1EAW	2.93	A Y60gA	-0.08
454	1EAW	2.93	A R87A	-0.15
455	1EAW	2.93	A F94A	0.73
456	1EAW	2.93	A N95A	0.31
457	1EAW	2.93	A D96A	0.65
458	1EAW	2.93	A F97A	0.89
459	1EAW	2.93	A T98A	0.25
460	1EAW	2.93	A H143A	-0.01
461	1EAW	2.93	A Q145A	0.31
462	1EAW	2.93	A Y146A	0.50
463	1EAW	2.93	A T150A	0.09
464	1EAW	2.93	A L153A	0.50
465	1EAW	2.93	A E169A	0.70
466	1EAW	2.93	A Q174A	0.56
467	1EAW	2.93	A Q175A	-0.13
468	1EAW	2.93	A D217A	2.23
469	1EAW	2.93	A Q221aA	0.14
470	1EAW	2.93	A R222A	-0.09

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
471	1EAW	2.93	A K224A	0.48
472	3BN9	2.17	B Q38A	-0.42
473	3BN9	2.17	B I41A	0.00
474	3BN9	2.17	B I60A	0.84
475	3BN9	2.17	B D60aA	0.42
476	3BN9	2.17	B D60bA	0.31
477	3BN9	2.17	B R60cA	-0.04
478	3BN9	2.17	B F60eA	-0.04
479	3BN9	2.17	B R60fA	-0.07
480	3BN9	2.17	B Y60gA	0.02
481	3BN9	2.17	B R87A	-0.16
482	3BN9	2.17	B F94A	0.64
483	3BN9	2.17	B N95A	0.77
484	3BN9	2.17	B T98A	1.13
485	3BN9	2.17	B H143A	0.09
486	3BN9	2.17	B Q145A	0.13
487	3BN9	2.17	B Y146A	1.08
488	3BN9	2.17	B T150A	0.29
489	3BN9	2.17	B L153A	0.34
490	3BN9	2.17	B E169A	0.37
491	3BN9	2.17	B Q174A	-0.03
492	3BN9	2.17	B Q175A	2.51
493	3BN9	2.17	B D217A	0.57
494	3BN9	2.17	B Q221aA	0.71
495	3BN9	2.17	B R222A	-0.09
496	3BN9	2.17	B K224A	0.78

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
497	3NPS	1.5	A Q38A	0.03
498	3NPS	1.5	A I41A	0.64
499	3NPS	1.5	A I60A	-0.33
500	3NPS	1.5	A D60aA	0.34
501	3NPS	1.5	A D60bA	1.07
502	3NPS	1.5	A R60cA	-1.06
503	3NPS	1.5	A F60eA	0.22
504	3NPS	1.5	A R60fA	0.14
505	3NPS	1.5	A Y60gA	0.45
506	3NPS	1.5	A R87A	-0.15
507	3NPS	1.5	A F94A	1.59
508	3NPS	1.5	A N95A	0.25
509	3NPS	1.5	A D96A	1.51
510	3NPS	1.5	A F97A	0.46
511	3NPS	1.5	A T98A	0.72
512	3NPS	1.5	A H143A	1.88
513	3NPS	1.5	A Q145A	0.30
514	3NPS	1.5	A Y146A	1.77
515	3NPS	1.5	A T150A	0.17
516	3NPS	1.5	A L153A	0.30
517	3NPS	1.5	A E169A	0.62
518	3NPS	1.5	A Q174A	-0.06
519	3NPS	1.5	A Q175A	0.74
520	3NPS	1.5	A D217A	1.47
521	3NPS	1.5	A Q221aA	-0.04
522	3NPS	1.5	A R222A	-0.08

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
523	3NPS	1.5	A K224A	-0.10
524	1REW	1.86	C Q94H;C K88R;C T98D;C L95Q;C A93P;C S90T;C K92I	0.73
525	2QJ9	2.44	C S98T;C P93A;C R88K;C I92K;C T90S;C Q95L;C H94Q	-0.73
526	1REW	1.86	C T98D;C M78L;C K88R;C A74T;C S90T;C L95Q;C K79G;C Y80L;C Q94H;C K92I;C A93P	0.27
527	2QJ9	2.44	C K79G;C Y80L;C A74T;C M78L	-0.46
528	2QJA	2.6	C L78M;C T74A;C G79K;C L80Y	0.46
529	2QJA	2.6	C T74A;C Q95L;C L78M;C G79K;C S98T;C P93A;C I92K;C T90S;C R88K;C L80Y;C H94Q	-0.27
530	2QJ9	2.44	C K79G;C D46E;C Y80L;C I62M;C A74T;C M78L;C G42H;C A61T	0.36
531	2QJA	2.6	C D46E;C I62M;C A61T;C G42H	0.81
532	2QJB	2.5	C M62I;C H42G;C T61A;C E46D	-0.81
533	2QJB	2.5	C T74A;C L80Y;C G79K;C T61A;C L78M;C E46D;C M62I;C H42G	-0.36
534	2QJB	2.5	C L78M;C H42G;C T74A;C R88K;C M62I;C I92K;C G79K;C T61A;C Q95L;C L80Y;C H94Q;C E46D;C T90S;C S98T;C P93A	-1.09
535	2VLR	2.3	E A99S	0.00
536	2VLJ	2.4	E D32A	1.57
537	2VLJ	2.4	E I53V	0.20
538	2VLJ	2.4	E I53L	1.42
539	2VLJ	2.4	E N55A	1.10
540	2VLJ	2.4	E N55D	0.50

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
541	2VLJ	2.4	E D56A	0.10
542	2VLJ	2.4	E Q58A	0.50
543	2VLJ	2.4	E Q58E	0.00
544	2VLJ	2.4	E S99A	0.00
545	2VLJ	2.4	E Y101A	0.20
546	2VLJ	2.4	E Y101F	0.50
547	2VLJ	2.4	D S31A	0.63
548	2VLJ	2.4	D S32A	1.00
549	2VLJ	2.4	D Q34A	1.00
550	2WPT	1.78	B F86A;A D33A	-0.30
551	2WPT	1.78	B F86A;A N34A	1.20
552	2WPT	1.78	A V37A;B F86A	2.90
553	2WPT	1.78	B R54A	1.10
554	2WPT	1.78	B N72A	0.90
555	2WPT	1.78	B S74A	-0.90
556	2WPT	1.78	B N75A	1.20
557	2WPT	1.78	B S77A	-0.60
558	2WPT	1.78	B S78A	-0.20
559	2WPT	1.78	B S84A	-0.10
560	2WPT	1.78	B F86A	1.20
561	2WPT	1.78	B T87A	0.50
562	2WPT	1.78	B Q92A	-0.50
563	2WPT	1.78	B K97A	0.50
564	2WPT	1.78	B V98A	0.10
569	1EMV	1.7	B R54A	1.70
570	1EMV	1.7	B N72A	1.10

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
571	1EMV	1.7	B S74A	-0.20
572	1EMV	1.7	B N75A	2.30
573	1EMV	1.7	B S77A	-0.20
574	1EMV	1.7	B S78A	-0.50
575	1EMV	1.7	B S84A	-0.10
576	1EMV	1.7	B F86A	3.90
577	1EMV	1.7	B T87A	0.20
578	1EMV	1.7	B Q92A	-0.30
579	1EMV	1.7	B K97A	2.00
580	1EMV	1.7	B V98A	1.10
581	1EMV	1.7	B F86A;A L33A	6.90
582	1EMV	1.7	A V34A;B F86A	4.80
583	1EMV	1.7	B F86A;A V37A	4.30
584	1EMV	1.7	B F86A;A Y54A	8.90
585	1EMV	1.7	A Y55A;B F86A	8.40
586	3BK3	2.7	C L1A	0.04
587	3BK3	2.7	C L1R	0.08
588	3BK3	2.7	C I2A	1.04
589	3BK3	2.7	C I2R	0.65
590	3BK3	2.7	C T3P	0.54
591	3BK3	2.7	C T5P	1.79
592	3BK3	2.7	C I18A	0.54
593	3BK3	2.7	C I18R	0.47
594	3BK3	2.7	C I21A	1.31
595	3BK3	2.7	C I21R	2.12
596	3BK3	2.7	C I27A	1.26

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
597	3BK3	2.7	C I27R	1.17
598	3BK3	2.7	C A36R	1.38
599	2OOB	1.9	A K935E	-0.18
600	2OOB	1.9	A A934S;A K935E	0.16
601	2OOB	1.9	A G941S	0.24
602	2OOB	1.9	A I955E;A I966E	0.28
603	1KTZ	2.15	A V92I	0.22
604	1KTZ	2.15	A R25K	1.12
605	1KTZ	2.15	A R94K	2.20
606	1KTZ	2.15	A R25A	1.48
607	1KTZ	2.15	A R94A	2.88
608	1A22	2.6	B F296S	0.05
609	1A22	2.6	B K234A;B K237A	0.24
610	1A22	2.6	B E275A;B R271A;B R270A	0.96
611	1A22	2.6	B D319A;B K321A;B E320A	0.21
612	1A22	2.6	B D352A;B R356A;B E358A	0.31
613	1A22	2.6	B K367A;B D364A;B R361A	-0.06
614	1A22	2.6	B E383A;B K387A;B D390A;B K385A	0.12
615	1A22	2.6	B D405A;B E409A;B K406A;B E407A;B K403A	1.12
616	1A22	2.6	B R239A	0.27
617	1A22	2.6	B E242A	1.23
618	1A22	2.6	B R243A	2.12
619	1A22	2.6	B E244A	0.65
620	1A22	2.6	B R270A	0.69
621	1A22	2.6	B R271A	0.60
622	1A22	2.6	B E275A	-0.09

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
623	1A22	2.6	B E279A	-0.08
624	1A22	2.6	B K281A	0.14
625	1A22	2.6	B E282A	0.78
626	1A22	2.6	B E291A	0.18
627	1A22	2.6	B K310A	0.04
628	1A22	2.6	B D326A	0.98
629	1A22	2.6	B E327A	0.41
630	1A22	2.6	B D332A	1.09
631	1A22	2.6	B E373A	0.08
632	1A22	2.6	B E375A	-0.08
633	1A22	2.6	B K379A	0.00
634	1A22	2.6	B E380A	0.20
635	1A22	2.6	B K403A	0.24
636	1A22	2.6	B D405A	-0.05
637	1A22	2.6	B K406A	0.20
638	1A22	2.6	B E407A	-0.08
639	1A22	2.6	B E409A	-0.06
640	1A22	2.6	B R411A	0.06
641	1A22	2.6	B R413A	-0.19
642	1A22	2.6	B K415A	0.50
643	1A22	2.6	B R417A	0.20
644	1A22	2.6	B E424A	0.11
645	1A22	2.6	B W276A	0.56
646	1A22	2.6	B W280A	-0.02
647	1A22	2.6	B W304F	2.78
648	1A22	2.6	B Y295A	0.20

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
649	1A22	2.6	B P241A	0.26
650	1A22	2.6	B S247A	-0.01
651	1A22	2.6	B T251A	0.07
652	1A22	2.6	B N272A	0.27
653	1A22	2.6	B T273A	-0.44
654	1A22	2.6	B Q274A	0.00
655	1A22	2.6	B T277A	0.20
656	1A22	2.6	B Q278A	-0.41
657	1A22	2.6	B N297A	-0.26
658	1A22	2.6	B S298A	-0.33
659	1A22	2.6	B S299A	-0.51
660	1A22	2.6	B F300A	-0.01
661	1A22	2.6	B T301A	1.09
662	1A22	2.6	B S302A	-0.18
663	1A22	2.6	B I303A	0.43
664	1A22	2.6	B I305A	0.13
665	1A22	2.6	B P306A	2.63
666	1A22	2.6	B V325A	1.14
667	1A22	2.6	B I328A	0.59
668	1A22	2.6	B V329A	-0.05
669	1A22	2.6	B Q330A	-0.03
670	1GC1	2.5	C K1A	0.06
671	1GC1	2.5	C K2A	-0.02
672	1GC1	2.5	C K7A	1.00
673	1GC1	2.5	C K8A	0.11
674	1GC1	2.5	C D10A	0.00

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
675	1GC1	2.5	C T11A	0.00
676	1GC1	2.5	C T15A	0.32
677	1GC1	2.5	C T17A	-0.13
678	1GC1	2.5	C S19A	0.00
679	1GC1	2.5	C Q20A	-0.02
680	1GC1	2.5	C K21A	-0.13
681	1GC1	2.5	C K22A	0.24
682	1GC1	2.5	C S23A	0.29
683	1GC1	2.5	C Q25A	0.03
684	1GC1	2.5	C H27A	0.28
685	1GC1	2.5	C K29A	0.54
686	1GC1	2.5	C N30A	0.17
687	1GC1	2.5	C S31A	0.11
688	1GC1	2.5	C N32A	0.18
689	1GC1	2.5	C Q33A	0.11
690	1GC1	2.5	C K35A	0.32
691	1GC1	2.5	C N39A	0.47
692	1GC1	2.5	C Q40A	-0.41
693	1GC1	2.5	C S42A	0.00
694	1GC1	2.5	C L44A	1.06
695	1GC1	2.5	C T45A	-0.15
696	1GC1	2.5	C K46A	1.43
697	1GC1	2.5	C S49A	0.61
698	1GC1	2.5	C K50A	0.05
699	1GC1	2.5	C L51A	1.23
700	1GC1	2.5	C N52A	0.71

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
701	1GC1	2.5	C D53A	0.30
702	1GC1	2.5	C D56A	-0.07
703	1GC1	2.5	C R58A	0.13
704	1GC1	2.5	C R59A	1.18
705	1GC1	2.5	C S60A	-0.09
706	1GC1	2.5	C D63A	-0.32
707	1GC1	2.5	C Q64A	0.44
708	1GC1	2.5	C N66A	-0.03
709	1GC1	2.5	C K72A	-0.02
710	1GC1	2.5	C N73A	-0.11
711	1GC1	2.5	C K75A	0.16
712	1GC1	2.5	C E77A	0.56
713	1GC1	2.5	C Y82A	1.29
714	1GC1	2.5	C E85A	1.32
715	1GC1	2.5	C V86A	-0.07
716	1GC1	2.5	C E87A	0.22
717	1GC1	2.5	C D88A	-0.07
718	1GC1	2.5	C Q89A	0.17
719	1GC1	2.5	C K90A	0.05
720	1GC1	2.5	C E91A	-0.13
721	1GC1	2.5	C E92A	0.02
722	1GC1	2.5	C Q94A	-0.11
723	1GC1	2.5	C Q89L	-0.31
724	1GC1	2.5	C Q40A;C D63A	-0.50
725	1GC1	2.5	C Q89L;C Q40A;C D63A	-0.85
726	1A22	2.6	A T3A	-0.05

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
727	1A22	2.6	A I4A	0.41
728	1A22	2.6	A P5A	0.43
729	1A22	2.6	A L6A	0.60
730	1A22	2.6	A S7A	0.34
731	1A22	2.6	A R8A	0.20
732	1A22	2.6	A L9A	-0.04
733	1A22	2.6	A F10A	1.04
734	1A22	2.6	A N12A	0.10
735	1A22	2.6	A L15A	0.15
736	1A22	2.6	A R16A	0.24
737	1A22	2.6	A R19A	0.05
738	1A22	2.6	A F54A	0.87
739	1A22	2.6	A S55A	0.11
740	1A22	2.6	A S57A	0.20
741	1A22	2.6	A I58A	1.64
742	1A22	2.6	A P59A	0.38
743	1A22	2.6	A E66A	0.43
744	1A22	2.6	A Q69A	-0.05
745	1A22	2.6	A K70A	0.52
746	1A22	2.6	A S71A	0.41
747	1A22	2.6	A L73A	-0.20
748	1A22	2.6	A V180A	0.00
749	1A22	2.6	A Q181A	0.27
750	1A22	2.6	A C182A	1.01
751	1A22	2.6	A S184A	-0.05
752	1A22	2.6	A V185A	0.87

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
753	1A22	2.6	A G187A	0.34
754	1A22	2.6	A S188A	-0.20
755	1A22	2.6	A F191A	0.19
756	1A4Y	2	B H13A	-0.34
757	1A4Y	2	B H114A	0.62
758	1A4Y	2	B K40Q	4.25
759	1A22	2.6	A M14A	0.10
760	1A22	2.6	A H18A	-0.50
761	1A22	2.6	A H21A	0.20
762	1A22	2.6	A Q22A	-0.20
763	1A22	2.6	A F25A	-0.41
764	1A22	2.6	A D26A	-0.20
765	1A22	2.6	A Q29A	-0.60
766	1A22	2.6	A Y42A	0.20
767	1A22	2.6	A L45A	1.20
768	1A22	2.6	A Q46A	0.10
769	1A22	2.6	A P48A	0.40
770	1A22	2.6	A S51A	0.30
771	1A22	2.6	A E56A	0.40
772	1A22	2.6	A P61A	1.20
773	1A22	2.6	A S62A	0.10
774	1A22	2.6	A N63A	0.30
775	1A22	2.6	A R64A	1.60
776	1A22	2.6	A E65A	-0.50
777	1A22	2.6	A Q68A	0.60
778	1A22	2.6	A Y164A	0.30

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
779	1A22	2.6	A R167A	0.30
780	1A22	2.6	A K168A	-0.20
781	1A22	2.6	A D171A	0.80
782	1A22	2.6	A K172A	2.00
783	1A22	2.6	A E174A	-0.90
784	1A22	2.6	A T175A	2.00
785	1A22	2.6	A F176A	1.90
786	1A22	2.6	A R178A	2.40
787	1A22	2.6	A I179A	0.80
788	1A22	2.6	A R183A	0.50
789	1A22	2.6	A E186A	0.00
790	1A22	2.6	B C308A	0.00
791	1A22	2.6	B E320A	-0.18
792	1A22	2.6	B K321A	0.08
793	1A22	2.6	B C322A	0.00
794	1A22	2.6	B S324A	0.20
795	1A22	2.6	B D364A	1.48
796	1A22	2.6	B I365A	2.13
797	1A22	2.6	B Q366A	0.02
798	1A22	2.6	B K367A	0.00
799	1A22	2.6	B V371A	-0.62
800	1A22	2.6	B T394A	0.20
801	1A22	2.6	B T395A	-0.10
802	1A22	2.6	B Q416A	0.89
803	1A22	2.6	B N418A	0.30
804	1DAN	2	U E208A	0.00

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
805	1DAN	2	U V207A	1.57
806	1DAN	2	U T203A	0.14
807	1DAN	2	U K201A;U D204A	0.04
808	1DAN	2	U R200A;U N199A	0.40
809	1DAN	2	U T197A;U V198A	-0.16
810	1DAN	2	U S195A	0.00
811	1DAN	2	U R196A;U S195A	0.64
812	1DAN	2	U Y185A	-0.32
813	1DAN	2	U E183A;U N184A	-0.09
814	1DAN	2	U K181A	0.02
815	1DAN	2	U L176A	0.08
816	1DAN	2	U N173A;U E174A	-0.02
817	1DAN	2	U T172A	-0.02
818	1DAN	2	U K169A	0.12
819	1DAN	2	U T167A	0.21
820	1DAN	2	U K166A;U K165A	-0.23
821	1DAN	2	U G164R	-0.16
822	1DAN	2	U S163A	0.02
823	1DAN	2	U W158F	0.12
824	1DAN	2	U Y156L	0.16
825	1DAN	2	U I152A	0.18
826	1DAN	2	U K149A;U D150A	-0.10
827	1DAN	2	U D145A	-0.01
828	1DAN	2	U R144A	0.00
829	1DAN	2	U F140A	1.29
830	1DAN	2	U T139A	-0.01

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
831	1DAN	2	U R136A;U N138A;U N137A	0.21
832	1DAN	2	U R135A	0.52
833	1DAN	2	U L133A	-0.02
834	1DAN	2	U R131F;U E130A	-0.02
835	1DAN	2	U D129A	-0.02
836	1DAN	2	U E128A	0.09
837	1DAN	2	U K122A	-0.12
838	1DAN	2	U Q114A;U E117A	0.03
839	1DAN	2	U N107A;U Q110A	0.41
840	1DAN	2	U L104A;U E105A	-0.46
841	1DAN	2	U E99A	-0.18
842	1DAN	2	U Y94A	1.03
843	1DAN	2	T K68A	-0.07
844	1DAN	2	T Q69A;T K68A	-0.21
845	1DAN	2	T K65A;T D66A	-0.50
846	1DAN	2	T D61A;T E62A	0.31
847	1DAN	2	T D58E	1.38
848	1DAN	2	T D58A	2.00
849	1DAN	2	T E56A;T D54A	0.44
850	1DAN	2	T T52A	0.40
851	1DAN	2	T F50A	0.44
852	1DAN	2	T K48A	0.42
853	1DAN	2	T S47A	-0.13
854	1DAN	2	T K46A	0.24
855	1DAN	2	T K48A;T K46A	1.17
856	1DAN	2	T W45F	1.28

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
857	1DAN	2	T D44A	2.39
858	1DAN	2	T S42A	-0.07
859	1DAN	2	T K41A	0.33
860	1DAN	2	T D44A;T K41A	1.57
861	1DAN	2	T Q37A	0.76
862	1DAN	2	T K28A	0.12
863	1DAN	2	T E26A	0.11
864	1DAN	2	T W25F	0.62
865	1DAN	2	T E24A	0.66
866	1DAN	2	T I22A	0.65
867	1DAN	2	T T21A	-0.16
868	1DAN	2	T K20R	1.69
869	1DAN	2	T K20A	2.44
870	1DAN	2	T N18A	0.18
871	1DAN	2	T T17A	0.12
872	1DAN	2	T K15A	-0.39
873	1DAN	2	T W14F	0.69
874	1FC2	2.8	C Y133W	0.41
875	1FC2	2.8	C I135W	3.14
876	1FC2	2.8	C F149W	0.00
877	1FC2	2.8	C L163W	0.00
878	1DAN	2	T S47G;T W45R	1.54
879	1DAN	2	T W45A	1.56
880	1DAN	2	T V33A	-0.19
881	1DAN	2	T Y51A	-0.13
882	1DAN	2	T F76A	1.15

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
883	1DAN	2	T Y78A	0.65
884	1DAN	2	U P92A	-0.19
885	1DAN	2	T V36A	-0.13
886	1DAN	2	T I38A	-0.13
887	1DAN	2	T G43A	0.07
888	1DAN	2	T C57S;T C49S	-0.34
889	1DAN	2	T T60A	2.31
890	1DAN	2	U F140A;T T60A	4.50
891	1DAN	2	T K20A;T T60A	3.18
892	1DAN	2	T W45A;T D44A	1.57
893	1DAN	2	U F140A;T D44A	3.30
894	1DAN	2	T D44A;T K20A	4.16
895	1DAN	2	T D58A;T D44A	4.01
896	1DAN	2	T T60A;T D44A	4.32
897	1DAN	2	T D44A;U F140A;T K20A	5.96
898	1TM1	1.7	I T58D	2.10
899	1TM1	1.7	I E60A;I T58A	3.39
900	1TM1	1.7	I T58D;I E60A	0.75
901	1TM1	1.7	I V70A	0.02
902	1DAN	2	U Q110A	1.30
903	1DAN	2	T S16A	-0.13
904	1DAN	2	T L59A	0.00
905	1DAN	2	T D61A	0.24
906	1DAN	2	T E62A	0.00
907	1DAN	2	T I63A	0.00
908	1DAN	2	T V64A	0.00

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
909	1DAN	2	T Q69A	0.00
910	1DAN	2	T L72A	-0.06
911	1DAN	2	U E105A	-0.06
912	1DAN	2	U T106A	-0.06
913	1DAN	2	U N107A	0.00
914	1DAN	2	U R131A	0.00
915	1DAN	2	U T132A	0.00
916	1DAN	2	U R136A	-0.06
917	1DAN	2	U N138A	0.00
918	1DAN	2	U V146A	0.20
919	1DAN	2	U F147A	-0.06
920	1DAN	2	U Y157A	0.00
921	1DAN	2	U V179A	0.11
922	1DAN	2	U R196A	0.46
923	1DAN	2	U T197A	0.11
924	1DAN	2	U V198A	0.11
925	1DAN	2	U N199A	0.00
926	1FC2	2.8	C L136D	0.82
927	1FC2	2.8	C N147A	0.58
928	1FC2	2.8	C F149A	0.06
929	1FC2	2.8	C I150A	2.18
930	1FC2	2.8	C K154A	1.23
931	1DVF	1.9	A H30A	1.65
932	1DVF	1.9	A Y32A	2.03
933	1DVF	1.9	A Y49A	1.73
934	1DVF	1.9	A Y50A	0.69

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
935	1DVF	1.9	A W92A	0.34
936	1DVF	1.9	A S93A	1.16
937	1DVF	1.9	B T30A	0.91
938	1DVF	1.9	B Y32A	1.83
939	1DVF	1.9	B W52A	4.13
940	1DVF	1.9	B D54A	4.28
941	1DVF	1.9	B N56A	1.16
942	1DVF	1.9	B D58A	1.60
943	1DVF	1.9	B E98A	4.19
944	1DVF	1.9	B R99A	1.87
945	1DVF	1.9	B D100A	2.79
946	1DVF	1.9	B Y101F	2.01
947	1VFB	1.8	A H30A	0.84
948	1VFB	1.8	A Y32A	1.72
949	1VFB	1.8	A Y49A	0.80
950	1VFB	1.8	A Y50A	0.52
951	1VFB	1.8	A W92A	2.73
952	1VFB	1.8	A S93A	0.34
953	1VFB	1.8	B T30A	-0.06
954	1VFB	1.8	B Y32A;B Y32A	0.65
955	1VFB	1.8	B W52A	0.92
956	1VFB	1.8	B D54A	0.64
957	1VFB	1.8	B N56A	0.18
958	1VFB	1.8	B D58A	-0.21
959	1VFB	1.8	B E98A	1.16
960	1VFB	1.8	B R99A	-0.10

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
961	1VFB	1.8	B D100A	3.07
962	1VFB	1.8	B Y101F;B Y101F	1.75
963	1A22	2.6	A F25A;A Y42A;A Q46A	0.20
964	1A22	2.6	A Q46A;A Y42A	0.29
965	1CBW	2.6	I T11A	0.22
966	1CBW	2.6	I G12A	0.68
967	1CBW	2.6	I P13A	-0.06
968	1CBW	2.6	I R17A	0.55
969	1CBW	2.6	I I18A	1.40
970	1CBW	2.6	I I19A	0.14
971	1CBW	2.6	I R20A	0.35
972	1CBW	2.6	I F33A	0.14
973	1CBW	2.6	I V34A	0.05
974	1CBW	2.6	I Y35A	0.88
975	1CBW	2.6	I G36A	0.95
976	1CBW	2.6	I G37A	0.81
977	1CBW	2.6	I R39A	0.22
978	1CBW	2.6	I K46A	0.14
979	1VFB	1.8	A Y50S	1.42
980	1DAN	2	H R134A	0.75
981	1DAN	2	H M164A	0.74
982	1DAN	2	H K192A	-0.19
983	1DAN	2	H L144A	0.03
984	1DVF	1.9	D Y98A	4.74
985	1DVF	1.9	C Y49A	1.86
986	1DVF	1.9	D Q100A	1.63

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
987	1DVF	1.9	D N54A	1.86
988	1DVF	1.9	D R100bA	4.09
989	1DVF	1.9	D K30A	1.00
990	1DVF	1.9	D H33A	1.86
991	1DVF	1.9	D D52A	1.68
992	1DVF	1.9	D I97A	2.68
993	1DVF	1.9	B E98A;D Y98A	4.63
994	1DVF	1.9	C Y49A;B D54A	4.49
995	1DVF	1.9	D Q100A;B D58A	1.46
996	1DVF	1.9	A Y49A;D N54A	2.03
997	1DVF	1.9	A Y32A;D R100bA	4.65
998	1DVF	1.9	D Q100A;B N56A	1.49
999	1DVF	1.9	D Q100A;B W52A	4.16
1000	1DVF	1.9	B D100A;D H33A	4.15
1001	1DVF	1.9	D D52A;B D100A	4.32
1002	1DVF	1.9	D H33A;A Y49A	2.63
1003	1DVF	1.9	B D100A;D N54A	4.09
1004	1DVF	1.9	D N54A;B N56A	2.30
1005	1DVF	1.9	D H33A;B N56A	2.53
1006	1CSE	1.2	I L45G	2.25
1007	1CSE	1.2	I L45S	1.17
1008	1CSE	1.2	I L45P	6.67
1009	1CSE	1.2	I L45I	2.94
1010	1CSE	1.2	I L45D	4.35
1011	1CSE	1.2	I L45E	2.35
1012	1ACB	2	I L45G	6.03

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1013	1ACB	2	I L45S	4.95
1014	1ACB	2	I L45P	6.85
1015	1ACB	2	I L45I	4.23
1016	1ACB	2	I L45D	6.77
1017	1ACB	2	I L45E	6.55
1018	1IAR	2.3	A I5A	1.17
1019	1IAR	2.3	A I5R	0.80
1020	1IAR	2.3	A T6A	-0.10
1021	1IAR	2.3	A T6D	1.39
1022	1IAR	2.3	A Q8A	-0.02
1023	1IAR	2.3	A Q8R	0.04
1024	1IAR	2.3	A E9Q	3.11
1025	1IAR	2.3	A I11A	0.07
1026	1IAR	2.3	A K12S	-0.01
1027	1IAR	2.3	A K12E	0.14
1028	1IAR	2.3	A T13A	0.98
1029	1IAR	2.3	A T13D	-0.22
1030	1IAR	2.3	A N15A	-0.03
1031	1IAR	2.3	A N15D	-0.08
1032	1IAR	2.3	A S16A	-0.18
1033	1IAR	2.3	A S16D	-0.10
1034	1IAR	2.3	A E19A	-0.32
1035	1IAR	2.3	A E19R	-0.12
1036	1IAR	2.3	A R53Q	0.84
1037	1IAR	2.3	A K77A	0.15
1038	1IAR	2.3	A K77E	0.61

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1039	1IAR	2.3	A Q78A	0.13
1040	1IAR	2.3	A Q78E	0.25
1041	1IAR	2.3	A R81A	0.48
1042	1IAR	2.3	A R81E	1.46
1043	1IAR	2.3	A F82A	-0.09
1044	1IAR	2.3	A F82D	-0.58
1045	1IAR	2.3	A K84A	0.35
1046	1IAR	2.3	A K84D	1.88
1047	1IAR	2.3	A R85A	0.43
1048	1IAR	2.3	A R85E	1.22
1049	1IAR	2.3	A R88Q	2.83
1050	1IAR	2.3	A R88A	3.75
1051	1IAR	2.3	A N89A	1.56
1052	1IAR	2.3	A W91A	0.73
1053	1IAR	2.3	A W91D	1.31
1054	1A4Y	2	B H8A	0.90
1055	1A4Y	2	B Q12A	0.30
1056	1A4Y	2	B N68A	0.12
1057	1A4Y	2	B E108A	-0.32
1058	1A4Y	2	A Y434A	3.26
1059	1A4Y	2	A D435A	3.48
1060	1A4Y	2	A Y437A	0.82
1061	1DFJ	2.5	I Y430A	5.95
1062	1DFJ	2.5	I D431A	3.66
1063	1DFJ	2.5	I Y433A	2.62
1064	2PCB	2.8	A E291Q	0.02

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1065	2PCB	2.8	A E32Q	0.54
1066	2PCB	2.8	A E35Q	0.69
1067	2PCB	2.8	A A193F	0.79
1068	2PCB	2.8	A D34N	0.90
1069	2PCB	2.8	A E290N	0.91
1070	1AK4	2.36	D P485A	0.91
1071	1AK4	2.36	D V486A	0.82
1072	1AK4	2.36	D H487A	0.84
1073	1AK4	2.36	D H487Q	0.80
1074	1AK4	2.36	D H487R	1.47
1075	1AK4	2.36	D A488V	0.60
1076	1AK4	2.36	D A488G	2.48
1077	1AK4	2.36	D G489A	1.91
1078	1AK4	2.36	D G489V	2.86
1079	1AK4	2.36	D P490A	2.00
1080	1AK4	2.36	D I491A	0.07
1081	1AK4	2.36	D I491V	-0.17
1082	1AK4	2.36	D A492G	0.41
1083	1AK4	2.36	D A492V	0.19
1084	1AK4	2.36	D P493A	0.51
1085	1E+096	2.4	A N26H	1.09
1086	1E+096	2.4	A I33N	2.01
1087	1E+096	2.4	A D38N	2.16
1088	1E+096	2.4	A M45T	0.36
1089	1E+096	2.4	A K132E	-0.19
1090	1E+096	2.4	A L134R	-0.13

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1091	1JRH	2.8	I T14V	-0.03
1092	1JRH	2.8	I T19L;I E21K	-0.03
1093	1JRH	2.8	I I28V;I M25L	-0.21
1094	1JRH	2.8	I P37S;I V41I;I I35N;I V39T	0.14
1095	1JRH	2.8	I E45Q	0.10
1096	1JRH	2.8	I I61T;I H65D;I A59S	0.07
1097	1JRH	2.8	I N79V;I G75M;I S78D;I L81A;I D76Y;I S71Y;I H73Q;I D72E;I V74I	2.96
1098	1JRH	2.8	I V83A;I R88K	-0.21
1099	1JRH	2.8	I K98R;I E100K;I A95D	-0.05
1100	1JRH	2.8	I V104M;I A103L	0.63
1101	1EMV	1.7	A C23A	0.92
1102	1EMV	1.7	A N24A	0.14
1103	1EMV	1.7	A D26A	0.34
1104	1EMV	1.7	A T27A	0.73
1105	1EMV	1.7	A S28A	0.17
1106	1EMV	1.7	A S29A	0.96
1107	1EMV	1.7	A E30A	1.42
1108	1EMV	1.7	A E31A	0.31
1109	1EMV	1.7	A E32A	0.22
1110	1EMV	1.7	A L33A	3.42
1111	1EMV	1.7	A V34A	2.58
1112	1EMV	1.7	A K35A	0.19
1113	1EMV	1.7	A L36A	0.91
1114	1EMV	1.7	A V37A	1.66
1115	1EMV	1.7	A T38A	0.90

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1116	1EMV	1.7	A E41A	2.08
1117	1EMV	1.7	A E42A	0.66
1118	1EMV	1.7	A T44A	0.30
1119	1EMV	1.7	A E45A	0.21
1120	1EMV	1.7	A H46A	0.83
1121	1EMV	1.7	A P47A	0.44
1122	1EMV	1.7	A S48A	0.01
1123	1EMV	1.7	A G49A	1.49
1124	1EMV	1.7	A S50A	2.19
1125	1EMV	1.7	A D51A	5.92
1126	1EMV	1.7	A L52A	0.60
1127	1EMV	1.7	A I53A	0.85
1128	1EMV	1.7	A Y54A	4.83
1129	1EMV	1.7	A Y55A	4.64
1130	1EMV	1.7	A P56A	1.24
1131	1EMV	1.7	A D60A	0.51
1132	1EMV	1.7	A S63A	0.87
1133	1EMV	1.7	A V68A	1.86
1134	1EMV	1.7	A N69A	0.28
1137	1AHW	3	C D150A;C K149A	1.18
1138	1AHW	3	C Y157A	-1.89
1139	1AHW	3	C K165A;C K166A	-1.33
1140	1AHW	3	C T167A	-0.07
1141	1AHW	3	C T170A	1.11
1142	1AHW	3	C L176A	0.99
1143	1AHW	3	C D178A	-0.48

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1144	1AHW	3	C T197A	1.35
1145	1AHW	3	C V198A	-0.31
1146	1AHW	3	C N199A	1.08
1147	1JCK	3.5	B T20A	1.65
1148	1JCK	3.5	B Y26A	1.77
1149	1JCK	3.5	B N60A	1.64
1150	1JCK	3.5	B Y90A	2.59
1151	1JCK	3.5	B V91A	2.23
1152	1JCK	3.5	B K103A	0.68
1153	1JCK	3.5	B F176A	2.13
1154	1A22	2.6	B S419A	0.04
1155	1A22	2.6	B R243L	0.52
1156	1A22	2.6	B R243M	1.00
1157	1NMB	2.2	H D56N	2.80
1158	1NMB	2.2	H D56E	2.40
1159	1NMB	2.2	H Y99A	1.50
1160	1NMB	2.2	H Y100aF	0.50
1161	1NMB	2.2	L Y32F	1.70
1162	1NMB	2.2	L L94V	0.90
1163	1VFB	1.8	C D18A	0.34
1164	1VFB	1.8	C N19A	0.40
1165	1VFB	1.8	C Y23A	0.41
1166	1VFB	1.8	C S24A	0.85
1167	1VFB	1.8	C K116A	0.71
1168	1VFB	1.8	C T118A	0.77
1169	1VFB	1.8	C D119A	0.95

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1170	1VFB	1.8	C V120A	0.92
1171	1VFB	1.8	C Q121A	2.88
1172	1VFB	1.8	C I124A	1.23
1173	1VFB	1.8	C R125A	1.84
1174	1VFB	1.8	C L129A	0.17
1175	1VFB	1.8	C Q121A;A Y32A	1.59
1176	1VFB	1.8	A Y32A;C I124A	2.91
1177	1VFB	1.8	C D18A;A Y50A	1.27
1178	1VFB	1.8	C D119A;A Y50A	1.23
1179	1VFB	1.8	A W92A;C Q121A	3.52
1180	1VFB	1.8	C I124A;A W92A	3.85
1181	1VFB	1.8	C R125A;A W92A	3.39
1182	1VFB	1.8	A W92A;C L129A	3.31
1183	1VFB	1.8	B Y32A;C K116A	1.59
1184	1VFB	1.8	C D119A;B W52A	2.25
1185	1VFB	1.8	C T118A;B D54A	1.25
1186	1VFB	1.8	B D100A;C S24A	3.37
1187	1VFB	1.8	C D119A;B Y101F	2.74
1188	1VFB	1.8	C V120A;B Y101F	2.54
1189	1NCA	2.5	H N31Q	0.00
1190	1NCA	2.5	H E96D	0.41
1191	1NCA	2.5	H D97K	0.71
1192	1NCA	2.5	H N98Q	0.54
1193	2WPT	1.78	A D33L	-3.40
1194	2WPT	1.78	A N34V	-0.90
1195	2WPT	1.78	A R38T	-1.00

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1196	2WPT	1.78	A E39H	0.00
1197	2WPT	1.78	A R42E	-0.40
1198	2WPT	1.78	A R38T;A N34V	-2.60
1199	2WPT	1.78	A N34V;A D33L;A R38T	-6.40
1200	2WPT	1.78	A R42E;A R38T;A N34V	-3.20
1201	2WPT	1.78	A N34V;A R42E;A E39H;A R38T	-3.00
1202	2WPT	1.78	A E30A	1.60
1203	2WPT	1.78	A D33A	-0.10
1204	2WPT	1.78	A N34A	-0.40
1205	2WPT	1.78	A V37A	3.70
1206	2WPT	1.78	A E41A	4.50
1207	2WPT	1.78	A R42A	-0.30
1208	2WPT	1.78	A S50A	2.40
1209	2WPT	1.78	A P56A	2.90
1210	1JRH	2.8	I K47A	3.85
1211	1JRH	2.8	I N48A	0.63
1212	1JRH	2.8	I N48Q	0.71
1213	1JRH	2.8	I Y49A	3.66
1214	1JRH	2.8	I Y49F	1.03
1215	1JRH	2.8	I G50A	4.38
1216	1JRH	2.8	I V51A	1.48
1217	1JRH	2.8	I K52A	3.79
1218	1JRH	2.8	I N53A	4.71
1219	1JRH	2.8	I S54A	0.46
1220	1JRH	2.8	I E55A	-0.70
1221	1JRH	2.8	I W56F	0.15

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1222	1JRH	2.8	I W56Y	0.58
1223	1JRH	2.8	I N79A	0.00
1224	1JRH	2.8	I W82A	4.33
1225	1JRH	2.8	I W82F	1.10
1226	1JRH	2.8	I W82Y	1.24
1227	1JRH	2.8	I R84A	1.03
1228	1JRH	2.8	I K98A	0.67
1229	1JTG	1.73	A G238S	-1.63
1230	1JTG	1.73	A E104K	4.23
1231	1JTG	1.73	A G238S;B F142A	3.68
1232	1JTG	1.73	B F142A;A E104K	4.55
1233	1MLC	2.5	A N32G	-0.86
1234	1MLC	2.5	A N32Y	0.00
1235	1MLC	2.5	B S57V	-0.49
1236	1MLC	2.5	B S57A	-0.37
1237	1MLC	2.5	B T58D	-0.56
1238	1MLC	2.5	A N92A	-1.25
1239	1MLC	2.5	B T31V	0.53
1240	1MLC	2.5	B T31A	0.45
1241	1MLC	2.5	B T31W	0.13
1242	1MLC	2.5	B T28D	-0.15
1243	1MLC	2.5	B K65D	0.02
1244	1MLC	2.5	B T58D;B S57V	-0.99
1245	1MLC	2.5	A N32G;A N92A	-0.69
1246	1MLC	2.5	A N92A;B T58D;A N32G;B S57V	-1.74
1247	1MLC	2.5	A N92A;B T58D;B S57V	-2.40

Dataset S1: Curated ZEMu dataset with experimental $\Delta\Delta G$ values

ID	PDB	Res.	Mutations	$\Delta\Delta G$
1248	1MLC	2.5	B T58D;A N92A;B T28D;B S57V	-2.74

Listing 1: Flex ddg Rosetta Script implementation

```
<ROSETTASCRIPTS>
  <SCOREFXNS>
    <ScoreFunction name="fa_talaris2014" weights="talaris2014"/>
    <ScoreFunction name="fa_talaris2014_cst" weights="talaris2014">
      <Reweight scoretype="atom_pair_constraint" weight="1.0"/>
      <Set fa_max_dis="9.0"/>
    </ScoreFunction>
  </SCOREFXNS>

  <!-- ### Only required input - mutation resfile ### -->
  <!-- #### All residues must be set to be NATAA packable at top of resfile
↪   ### -->
  <TASKOPERATIONS>
    <ReadResfile name="res_mutate" filename="%%mutate_resfile_relpath%%"/>
  </TASKOPERATIONS>

  <RESIDUE_SELECTORS>
    <Task name="resselector" fixed="0" packable="0" designable="1"
↪   task_operations="res_mutate"/>
    <Neighborhood name="bubble" selector="resselector" distance="8.0"/>
    <PrimarySequenceNeighborhood name="bubble_adjacent" selector="bubble"
↪   lower="1" upper="1"/>
    <StoredResidueSubset name="restore_neighbor_shell"
↪   subset_name="neighbor_shell"/>
    <Not name="everythingelse" selector="restore_neighbor_shell"/>
```

```
  </RESIDUE_SELECTORS>
  <TASKOPERATIONS>
    <OperateOnResidueSubset name="repackonly"
 ↪  selector="restore_neighbor_shell">
      <RestrictToRepackingRLT/>
    </OperateOnResidueSubset>
    <OperateOnResidueSubset name="norepack" selector="everythingelse">
      <PreventRepackingRLT/>
    </OperateOnResidueSubset>
    <UseMultiCoolAnnealer name="multicool" states="6"/>
    <ExtraChiCutoff name="extrachizero" extrachi_cutoff="0"/>
    <InitializeFromCommandline name="commandline_init"/>
    <RestrictToRepacking name="restrict_to_repacking"/>
  </TASKOPERATIONS>

  <FILTERS>
  </FILTERS>

  <MOVERS>
    <StoreResidueSubset name="neighbor_shell_storer"
 ↪  subset_name="neighbor_shell" residue_selector="bubble_adjacent" />

    <AddConstraintsToCurrentConformationMover name="addcst"
 ↪  use_distance_cst="1" coord_dev="0.5" min_seq_sep="0" max_distance="9"
 ↪  CA_only="1" bound_width="0.0" cst_weight="0.0"/>
    <ClearConstraintsMover name="clearcst"/>
```

```
    <MinMover name="minimize" scorefxn="fa_talaris2014_cst" chi="1" bb="1" type="lbfgs_armijo_nonmonotone" tolerance="0.000001" max_iter="5000" abs_score_convergence_threshold="1.0"/>

    <PackRotamersMover name="repack" scorefxn="fa_talaris2014" task_operations="commandline_init,repackonly,norepack,multicool"/>
    <PackRotamersMover name="mutate" scorefxn="fa_talaris2014" task_operations="commandline_init,res_mutate,norepack,multicool"/>

    <ReportToDB name="dbreport" batch_description="interface_ddG" database_name="ddG.db3">
      <ScoreTypeFeatures/>
      <ScoreFunctionFeatures scorefxn="fa_talaris2014"/>
      <StructureScoresFeatures scorefxn="fa_talaris2014"/>
    </ReportToDB>

    <ReportToDB name="structreport" batch_description="interface_ddG_struct" database_name="struct.db3">
      <PoseConformationFeatures/>
      <PdbDataFeatures/>
      <JobDataFeatures/>
      <ResidueFeatures/>
      <PoseCommentsFeatures/>
      <ProteinResidueConformationFeatures/>
      <ResidueConformationFeatures/>
    </ReportToDB>

```

```
    <SavePoseMover name="save_wt_bound_pose" restore_pose="0"
  ↪ reference_name="wt_bound_pose"/>
    <SavePoseMover name="save_backrub_pose" restore_pose="0"
  ↪ reference_name="backrubpdb"/>
    <SavePoseMover name="restore_backrub_pose" restore_pose="1"
  ↪ reference_name="backrubpdb"/>

    <InterfaceDdGMover name="int_ddG_mover"
  ↪ wt_ref_savepose_mover="save_wt_bound_pose" chain_name="%%chainstomove%%"
  ↪ db_reporter="dbreport" scorefxn="fa_talaris2014"/>

    <ScoreMover name="apply_score" scorefxn="fa_talaris2014_cst"
  ↪ verbose="0"/>

    <ParsedProtocol name="finish_ddg_post_backrub">
      <Add mover_name="save_backrub_pose"/>
      <Add mover_name="structreport"/>

      <Add mover_name="repack"/>

      <Add mover_name="addcst"/>
      <Add mover_name="minimize"/>
      <Add mover_name="clearcst"/>

      <Add mover_name="save_wt_bound_pose"/>
      <Add mover_name="structreport"/>
      <Add mover_name="restore_backrub_pose"/>
```

```

      <Add mover_name="mutate"/>

      <Add mover_name="addcst"/>
      <Add mover_name="minimize"/>
      <Add mover_name="clearcst"/>
      <Add mover_name="structreport"/>

      <Add mover_name="int_ddG_mover"/>
    </ParsedProtocol>

    <BackrubProtocol name="backrub" mc_kt="1.2" ntrials="35000"
↪   pivot_residue_selector="restore_neighbor_shell"
↪   task_operations="restrict_to_repacking,commandline_init,extrachizero"
↪   recover_low="0" trajectory_stride="2500"
↪   trajectory_apply_mover="finish_ddg_post_backrub"/>

  </MOVERS>
  <APPLY_TO_POSE>
  </APPLY_TO_POSE>
  <PROTOCOLS>
    <Add mover_name="addcst"/>
    <Add mover_name="apply_score"/> <!-- Necessary to initialize neighbor
↪   graph -->
    <Add mover_name="neighbor_shell_storer"/>

    <Add mover_name="minimize"/>
```

```
    <Add mover_name="clearcst"/>

    <Add mover_name="backrub"/>
  </PROTOCOLS>
  <OUTPUT />
</ROSETTASCRIPTS>
```

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