Supporting Information

Implementing and assessing an alchemical method for calculating protein-protein binding free energy

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SI Contents

1. Tables of $\Delta\Delta G$ values of single mutations of 1BRS and 3HFM systems in SKEMPI database
2. Prediction of $\Delta\Delta G$ values of test mutations of 1BRS system as a function of transition time.
3. Prediction of $\Delta\Delta G$ values of test mutations of 3HFM system as a function of transition time.

1. Tables of $\Delta\Delta G$ values of single mutations of 1BRS and 3HFM systems in SKEMPI database

Systems	Mutations	Experimental $\Delta\Delta G$ (kcal/mol)	Average $\Delta\Delta G$ (kcal/mol)
1BRS	**D54A[1]**	**-0.17**	**-0.53**
		-0.89	
	W44F[2]	**0.06 ± 0.2**	
	W38F[2]	**1.64 ± 0.2**	
	R59K[3]	**2.49**	
	E73S[4]	**3.01 ± 0.2**	
	H102D[3]	**4.55**	
	R83Q[2]	**5.42 ± 0.2**	
	D39A[1,2,5]	**5.94**	**6.79**
		7.65 ± 0.2	
		5.41	
		4.59	
	D35A[2]	4.5 ± 0.2	4.29
		4.07	
	E60A[1]	0.51	0.09
		-0.33	
	E73A[1,4]	2.35 ± 0.2	2.35
		1.90	
		2.81	
	E73C[4]	2.53 ± 0.2	
	E73F[4]	2.24 ± 0.2	
	E73Q[4]	1.45 ± 0.2	
	E73W[4]	1.66 ± 0.2	
	E73Y[4]	2.41 ± 0.2	
	E76A[2,5]	1.36 ± 0.2	1.09
		0.82	
	E80A[2,5]	0.54 ± 0.2	0.48
		0.41	

	H102A[1–3]	6.15 ± 0.2	6.35
		6.91	
		6.25	
		6.08	
	H102G[3]	6.82	
	H102L[3]	7.67	
	H102Q[3]	4.55	
	K27A[1,2]	5.38 ± 0.2	5.13
		5.41	
		4.59	
	N58A[1]	3.07	3.09
		3.12	
	R59A[1–3]	5.25 ± 0.2	4.99
		4.64	
		5.18	
		4.89	
	R87A[1,2]	5.56 ± 0.2	5.76
		5.95	
	T42A[2]	1.86 ± 0.2	
	W35F[1]	1.41	1.26
		1.11	
	Y29A[2]	3.47 ± 0.2	
	Y29F[2]	-0.13	
3HFM	**Y20F[6,7]**	**-0.5 ± 0.06**	**-0.48**
		-0.46 ± 0.1	
	D32N[8]	**0.17 ± 0.3**	
	R21A[6–8]	**1.03 ± 0.09**	**0.90**
		0.82 ± 0.08	
		0.86 ± 0.09	
	D101K[6,9]	**1.97 ± 0.21**	**2.13**
		2.28 ± 0.15	
	W98F[8]	**3.25 ± 0.16**	
	Y50L[8]	**4.4 ± 0.12**	
	N31E[8]	**5.71 ± 0.13**	
	K97D[6]	**6.77 ± 0.14**	
	D101A[6,7,9]	1.21 ± 0.23	1.23
		1.53 ± 0.16	
		0.95 ± 0.2	

	D101E[6,9]	1.97 ± 0.21	2.13
		2.29 ± 0.14	
	D101F[6,9]	2.17 ± 0.25	2.33
		2.49 ± 0.19	
	D101G[6,9]	0.2 ± 0.31	0.36
		0.52 ± 0.27	
	D101N[6,9]	1.34 ± 0.26	1.49
		1.64 ± 0.21	
	D101Q[6,9]	1.93 ± 0.22	2.09
		2.24 ± 0.15	
	D101R[6,9]	2.12 ± 0.28	2.28
		2.44 ± 0.23	
	D101S[6,9]	1.71 ± 0.2	1.87
		2.03 ± 0.12	
	G102V[6,9]	0.24 ± 0.21	0.40
		0.55 ± 0.14	
	H15A[7]	-0.45 ± 0.18	
	K96A[8]	6.99 ± 0.27	
	K96M[6]	6.78 ± 0.12	
	K96R[7]	5.36 ± 0.12	
	K97A[6,8]	6.17 ± 0.08	5.86
		5.56 ± 0.12	
	K97E[6]	3.61 ± 0.12	
	K97G[6]	6.43 ± 0.1	
	K97M[6,8]	1.09 ± 0.08	0.95
		0.8 ± 0.12	
	K97R[6]	3.05 ± 0.32	
	L75A[7]	0.7 ± 0.1	
	N31A[8]	5.22 ± 0.15	
	N31D[8]	1.34 ± 0.28	
	N31E[8]	5.71 ± 0.13	
	N32A[8]	5.11 ± 0.22	
	N19D[9]	0.41 ± 0.37	
	N19K[9]	0.24 ± 0.25	
	N19Q[9]	-0.04 ± 0.49	
	N93A[7]	0.21 ± 0.15	
	Q53A[8]	0.95 ± 0.18	
	R21E[6,9]	2.29 ± 0.22	1.15
		2.62 ± 0.16	

	R21G[6,9]	2.27 ± 0.3	2.42
		2.58 ± 0.26	
	R21H[6,9]	2.02 ± 0.31	2.17
		2.33 ± 0.26	
	R21K[6,9]	1.62 ± 0.26	1.77
		1.91 ± 0.2	
	R21M[6]	2.05 ± 0.07	
	R21N[6,9]	2.17 ± 0.2	2.33
		2.49 ± 0.11	
	R21Q[6,9]	2.24 ± 0.23	2.40
		2.56 ± 0.16	
	R21W[6,9]	1.97 ± 0.28	2.13
		2.29 ± 0.23	
	R73A[7]	-0.33 ± 0.2	
	S31A[8]	0.17 ± 0.34	
	S100A[7]	0.27 ± 0.2	
	W98A[8]	5.51 ± 0.17	
	W63A[7]	0.32 ± 0.26	
	Y33A[8]	6.04 ± 0.15	
	Y33F[10]	1.05	
	Y33L[10]	1.97	
	Y33W[10]	1.72	
	Y50A[8]	7.32 ± 0.39	
	Y50F[8]	1.58	1.97
		2.36 ± 0.18	
	Y50L[10]	2.80	
	Y53A[10]	3.20	
	Y53F[10]	0.61	
	Y53L[10]	0.79	
	Y53W[10]	0.69	
	Y58A[10]	1.65	
	Y58F[10]	0.36	
	Y58L[10]	1.49	
	Y96A[10]	2.71 ± 0.07	
	Y96F[10]	1.4 ± 0.18	
	Y20A[7,10]	4.88 ± 0.11	4.58
		4.27 ± 0.2	
	Y20L[7]	2.19 ± 0.14	

Table S1. Experimental ΔΔ*G* values and associated experimental errors reported for single mutations for the 1BRS and 3HFM systems in the SKEMPI database. Bold font denotes the eight mutations selected for each system. The average ΔΔ*G* value was used in this study when multiple ΔΔ*G* values for a single mutation were in the database.

2. Prediction of ΔΔ*G* values of test mutations of 1BRS system as a function of transition time

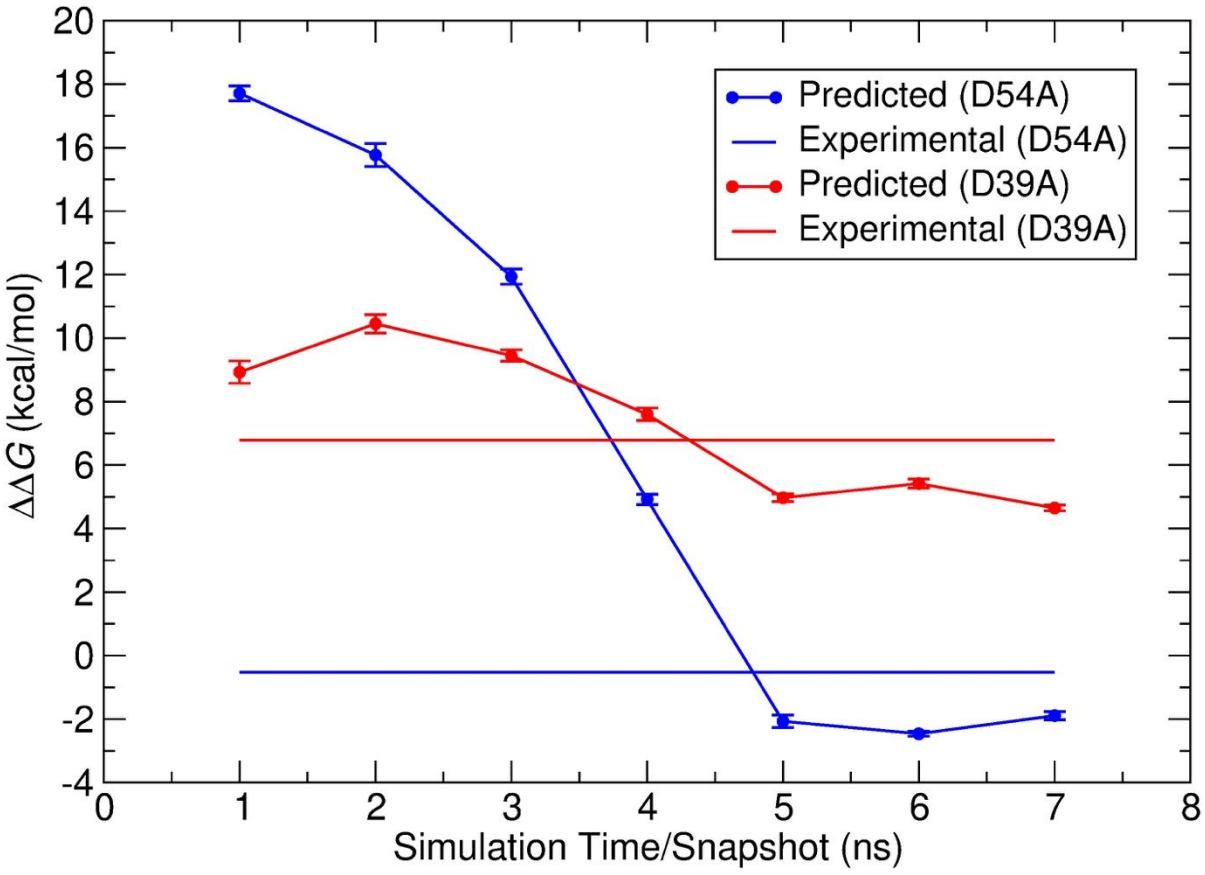

Figure S1. Predicted ΔΔ*G* values of D54A (blue) and D39A (red) mutations as a function of transition time averaged over 100 independent transitions. ΔΔ*G* values of two test mutations D54A and D39A from the 1BRS system estimated for different transition time ranging from 1 ns to 7 ns. The data points show the mean ΔΔ*G* estimates and error bars show the standard deviations from the 100 trials. The predicted ΔΔ*G* values for D54A and D39A can be compared to experiment (horizontal lines).

3. **Prediction of ΔΔ*G* values of test mutations of 3HFM system as a function of transition time**

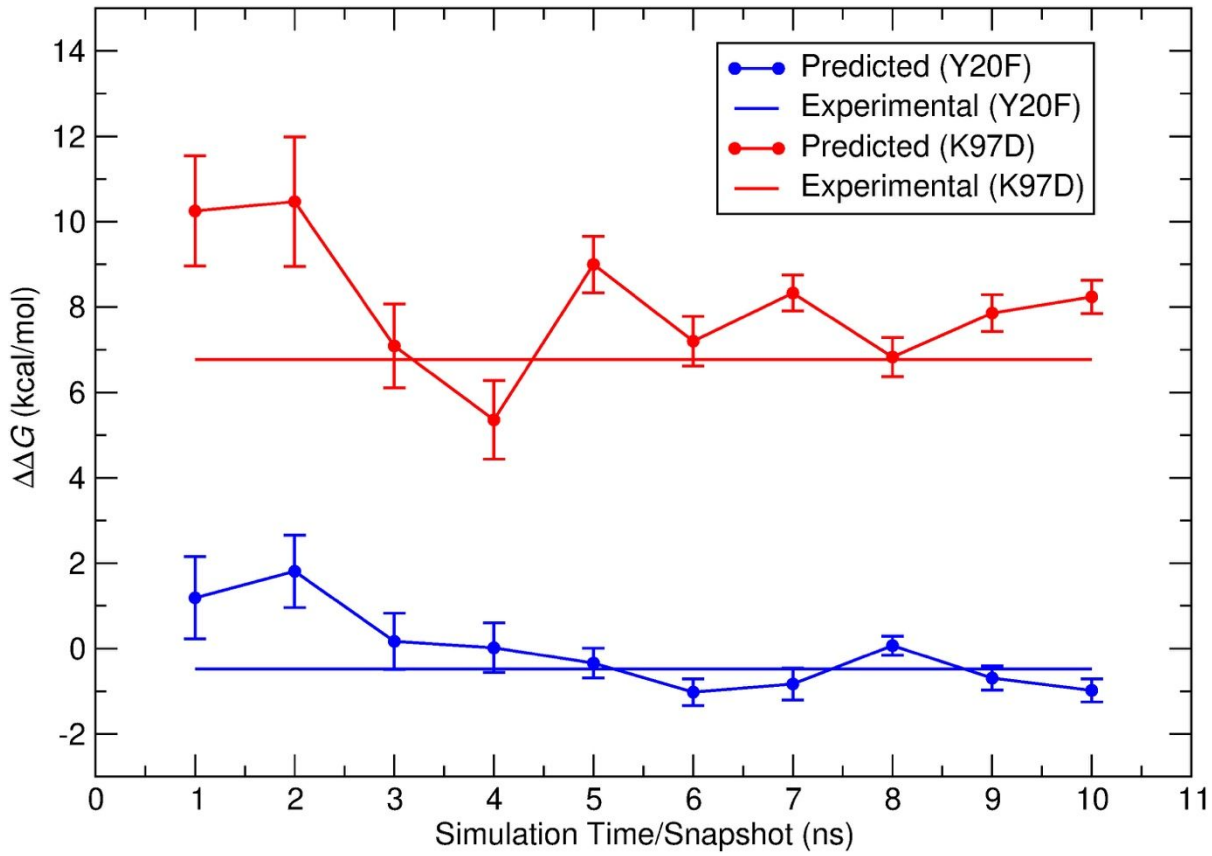

Figure S2. Predicted ΔΔ*G* values of Y20F (blue) and K97D (red) mutations as a function of transition time averaged over 100 independent transitions. ΔΔ*G* values of two test mutations Y20F and K97D from the 3HFM system estimated for different transition time ranging from 1 ns to 10 ns. The data points show the mean ΔΔ*G* estimates and error bars show the standard deviations from the 100 trials. The predicted ΔΔ*G* values for Y20F and K97D can be compared to experiment (horizontal lines).

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