

Relaxation and Dynamics Data for B1 Domain A53 Mutant

	R1	dR1	R2	dR2	NOE	dNOE	S2	dS2
Q2	2.25	0.04	4.67	0.04	0.52	0.01	0.68	0.01
Y3	2.36	0.02	4.44	0.04	0.48	0.01	0.71	0.00
K4	2.36	0.01	4.64	0.04	0.53	0.01	0.72	0.01
L5	2.21	0.04	4.16	0.06	0.59	0.01	0.68	0.01
A6	2.31	0.04	4.57	0.04	0.59	0.01	0.74	0.01
L7	2.24	0.03	4.00	0.12	0.46	0.01	0.66	0.01
N8	2.27	0.04	5.14	0.04	0.56	0.01	0.70	0.01
G9	2.29	0.03	3.89	0.08	0.53	0.01	0.41	0.06
K10	2.06	0.02	4.27	0.06	0.41	0.01	0.61	0.01
T11	2.06	0.02	4.94	0.06	0.42	0.01	0.61	0.01
L12	1.74	0.03	3.03	0.11	0.31	0.01	0.50	0.01
K13	2.15	0.04	4.11	0.09	0.45	0.01	0.65	0.01
G14	2.04	0.02	3.54	0.04	0.50	0.01	0.49	0.03
E15	2.07	0.05	3.99	0.05	0.55	0.01	0.65	0.01
T16	2.33	0.04	4.09	0.08	0.50	0.01	0.69	0.01
T17	2.21	0.03	4.62	0.10	0.53	0.01	0.67	0.01
T18	2.33	0.05	4.37	0.06	0.48	0.01	0.70	0.01
E19	2.14	0.03	4.25	0.05	0.55	0.01	0.67	0.01
A20	2.18	0.04	4.12	0.07	0.52	0.01	0.66	0.01
V21	2.27	0.04	4.60	0.11	0.55	0.01	0.71	0.01
D22	2.37	0.04	5.67	0.05	0.55	0.01	0.73	0.01
A23	2.55	0.03	5.13	0.06	0.53	0.01	0.79	0.01
A24	2.38	0.04	5.24	0.14	0.68	0.01	0.77	0.01
T25	2.58	0.04	5.33	0.06	0.57	0.01	0.80	0.01
A26	2.40	0.03	4.88	0.05	0.58	0.01	0.76	0.01
E27	2.56	0.03	4.97	0.06	0.59	0.01	0.78	0.01
K28	2.42	0.04	5.28	0.05	0.59	0.01	0.79	0.01
V29	2.39	0.02	4.94	0.05	0.61	0.01	0.76	0.01
F30	2.51	0.03	5.26	0.06	0.63	0.01	0.78	0.01
K31	2.38	0.03	4.88	0.05	0.59	0.01	0.75	0.01
Q32	2.48	0.03	5.20	0.05	0.55	0.01	0.77	0.01
A34	2.30	0.03	5.10	0.08	0.61	0.01	0.72	0.01
N35	2.40	0.03	4.84	0.06	0.55	0.01	0.73	0.01
D36	2.30	0.03	4.83	0.04	0.59	0.01	0.72	0.01
N37	2.29	0.02	4.49	0.05	0.52	0.01	0.71	0.01
G38	2.35	0.05	4.73	0.07	0.52	0.01	0.72	0.01
V39	2.28	0.04	4.86	0.04	0.55	0.01	0.70	0.01
D40	2.05	0.04	4.27	0.07	0.35	0.01	0.62	0.01
G41	1.62	0.03	3.94	0.03	0.17	0.01	0.46	0.01
W43	2.33	0.03	4.57	0.05	0.46	0.01	0.70	0.01
A44	2.41	0.03	4.43	0.05	0.51	0.01	0.72	0.01
Y45	2.12	0.06	4.08	0.07	0.48	0.01	0.65	0.01
D46	2.41	0.04	5.09	0.05	0.55	0.01	0.74	0.01
D47	2.34	0.05	4.74	0.06	0.52	0.01	0.74	0.01
A48	2.10	0.04	4.73	0.06	0.55	0.01	0.65	0.01
T49	2.37	0.02	6.16	0.06	0.50	0.01	0.72	0.01
K50	2.50	0.04	4.56	0.07	0.49	0.01	0.74	0.01
T51	2.51	0.05	4.57	0.06	0.50	0.01	0.75	0.01

F52	2.35	0.02	5.05	0.04	0.50	0.01	0.71	0.01
A53	2.22	0.04	3.95	0.13	0.55	0.01	0.67	0.01
V54	2.41	0.03	4.61	0.08	0.48	0.01	0.73	0.01
T55	2.34	0.02	4.83	0.04	0.59	0.01	0.72	0.01
E56	2.18	0.03	4.92	0.05	0.41	0.01	0.64	0.01

Relaxation and Dynamics Data for B1 Domain M53 Mutant

	R1	dR1	R2	dR2	NOE	dNOE	S2	dS2
Q2	2.0179	0.0365	4.2422	0.0551	0.506	0.0105	0.61	0.011
Y3	2.2901	0.029	4.1821	0.0364	0.5277	0.0105	0.684	0.005
K4	2.3473	0.0511	4.4172	0.0412	0.5802	0.0105	0.718	0.006
L5	1.9688	0.0516	3.8332	0.0202	0.5774	0.0105	0.626	0.003
A6	2.2782	0.0481	4.354	0.04	0.5948	0.0105	0.712	0.006
L7	2.3137	0.0373	4.1372	0.0606	0.4303	0.0105	0.671	0.007
N8	2.2152	0.0297	4.9486	0.0267	0.5541	0.0105	0.678	0.009
G9	2.2922	0.0369	3.7919	0.0464	0.4979	0.0105	0.372	0.045
K10	2.1478	0.0374	4.3142	0.1119	0.5266	0.0105	0.666	0.009
T11	2.0127	0.0555	4.591	0.0552	0.3828	0.0105	0.59	0.016
L12	1.6798	0.032	3.0902	0.0741	0.272	0.0105	0.48	0.007
K13	2.0461	0.0295	3.7488	0.0285	0.4347	0.0105	0.599	0.004
G14	1.9603	0.0164	3.3754	0.0724	0.4565	0.0105	0.457	0.039
E15	2.1332	0.0446	4.0943	0.0556	0.5399	0.0105	0.661	0.007
T16	2.1807	0.0443	4.1213	0.0415	0.4853	0.0105	0.664	0.006
T17	2.2259	0.0596	4.2857	0.0774	0.5346	0.0105	0.691	0.01
T18	2.3388	0.066	4.3163	0.1404	0.5173	0.0105	0.705	0.015
E19	2.1608	0.0372	4.0525	0.0692	0.5913	0.0105	0.66	0.008
A20	2.2129	0.0423	4.2062	0.0354	0.5348	0.0105	0.677	0.005
V21	2.3544	0.0493	4.2516	0.0477	0.5658	0.0105	0.689	0.007
D22	2.1583	0.0274	5.0381	0.049	0.5572	0.0105	0.663	0.009
A23	2.3811	0.0337	4.8254	0.1243	0.5195	0.0105	0.73	0.009
A24	2.3212	0.0433	4.7472	0.054	0.6904	0.0105	0.728	0.007
T25	2.4025	0.0235	5.0716	0.0733	0.597	0.0105	0.754	0.006
A26	2.3616	0.0423	4.8218	0.0883	0.6292	0.0105	0.749	0.01
E27	2.4389	0.0522	4.8462	0.081	0.6033	0.0105	0.757	0.01
K28	2.3821	0.03	4.9396	0.0418	0.5787	0.0105	0.747	0.006
V29	2.4844	0.0409	4.9841	0.0903	0.6181	0.0105	0.775	0.009
F30	2.4954	0.0548	5.0167	0.0832	0.6289	0.0105	0.788	0.011
K31	2.2881	0.0334	4.7624	0.0393	0.6029	0.0105	0.733	0.006
Q32	2.549	0.0272	5.1352	0.1029	0.5559	0.0105	0.784	0.008
A34	2.3603	0.0423	4.9176	0.0775	0.6199	0.0105	0.75	0.009
N35	2.308	0.0191	4.5737	0.0336	0.6069	0.0105	0.705	0.004
D36	2.2859	0.0373	4.8828	0.0953	0.6386	0.0105	0.728	0.009
N37	2.3689	0.0343	4.2467	0.0339	0.5461	0.0105	0.69	0.005
G38	2.466	0.0554	4.8097	0.1576	0.544	0.0105	0.748	0.013
V39	2.1413	0.0551	4.366	0.0808	0.544	0.0105	0.671	0.01
D40	2.0668	0.0788	3.8545	0.0766	0.4387	0.0105	0.577	0.01
G41	1.6858	0.0319	4.0431	0.1486	0.1555	0.0105	0.47	0.009

W43	2.0363	0.0411	4.1239	0.044	0.5349	0.0105	0.62	0.012
A44	2.2885	0.0425	4.1765	0.0915	0.4797	0.0105	0.68	0.01
Y45	2.1327	0.0313	4.0697	0.0471	0.4734	0.0105	0.65	0.006
D46	2.429	0.0642	4.5202	0.1013	0.4981	0.0105	0.732	0.013
D47	2.1078	0.0374	4.2549	0.0996	0.5451	0.0105	0.657	0.009
A48	2.1106	0.0339	4.4395	0.0588	0.5534	0.0105	0.659	0.007
T49	2.4036	0.0434	5.5882	0.1598	0.4999	0.0105	0.728	0.014
K50	2.5105	0.0641	4.5798	0.1162	0.4909	0.0105	0.747	0.013
T51	2.5357	0.0625	4.3006	0.1386	0.4968	0.0105	0.736	0.014
F52	2.3702	0.0249	4.6081	0.0347	0.557	0.0105	0.74	0.005
A53	2.2297	0.0295	4.3222	0.0268	0.5207	0.0105	0.695	0.004
V54	2.4243	0.0643	4.3405	0.0394	0.4985	0.0105	0.705	0.006
T55	2.3339	0.0683	4.3813	0.0674	0.5715	0.0105	0.715	0.01
E56	2.3079	0.0459	5.076	0.1184	0.4168	0.0105	0.682	0.014

Relaxation and Dynamics Data for B1 Domain T53 Mutant

	R1	dR1	R2	dR2	NOE	dNOE	S2	dS2
Q2	2.0971	0.0471	3.9721	0.1259	0.5678	0.0108	0.641	0.012
Y3	2.3967	0.0633	4.2411	0.0525	0.5342	0.0108	0.695	0.008
K4	2.3484	0.064	4.12	0.087	0.6117	0.0108	0.691	0.012
L5	2.262	0.0657	4.1007	0.0458	0.5218	0.0108	0.668	0.007
A6	2.4	0.0456	4.1544	0.064	0.5819	0.0108	0.528	0.083
L7	2.1624	0.0423	3.9523	0.0822	0.5024	0.0108	0.645	0.009
N8	2.2045	0.0308	4.1002	0.1016	0.5467	0.0108	0.67	0.008
G9	2.1847	0.0268	3.9578	0.0411	0.5374	0.0108	0.535	0.029
K10	2.0998	0.0429	4.0521	0.1124	0.5339	0.0108	0.646	0.01
T11	2.0371	0.0368	3.8576	0.1053	0.4129	0.0108	0.606	0.009
L12	1.6345	0.0385	2.9585	0.0674	0.2799	0.0108	0.465	0.007
K13	1.8495	0.0536	3.4014	0.0941	0.4227	0.0108	0.544	0.011
G14	1.9381	0.0233	3.3252	0.0468	0.5478	0.0108	0.42	0.04
E15	1.9603	0.0321	3.647	0.1222	0.5541	0.0108	0.598	0.008
T16	2.1993	0.033	3.9973	0.0572	0.4663	0.0108	0.651	0.007
T17	2.0247	0.0315	4.1095	0.0389	0.5596	0.0108	0.62	0.01
T18	2.3238	0.053	4.2044	0.0599	0.516	0.0108	0.688	0.008
E19	2.0091	0.0444	3.9589	0.0627	0.5951	0.0108	0.632	0.008
A20	2.0533	0.0723	4.2964	0.1326	0.5344	0.0108	0.659	0.016
V21	2.1807	0.0384	4.1091	0.0814	0.5822	0.0108	0.666	0.009
D22	2.2182	0.0772	4.4973	0.0699	0.5736	0.0108	0.697	0.01
A23	2.3107	0.04	4.6477	0.0712	0.5472	0.0108	0.717	0.008
A24	2.1712	0.0738	4.6646	0.1385	0.7046	0.0108	0.706	0.016
T25	2.2808	0.0253	4.8051	0.0996	0.604	0.0108	0.716	0.007
A26	2.3003	0.0614	4.8083	0.1284	0.6254	0.0108	0.737	0.014
E27	2.3006	0.0695	4.6987	0.097	0.6374	0.0108	0.731	0.013
K28	2.3211	0.0563	4.6577	0.0638	0.6342	0.0108	0.717	0.009
V29	2.2542	0.0326	4.6832	0.0744	0.6407	0.0108	0.717	0.008
F30	2.3376	0.0368	4.8501	0.0689	0.636	0.0108	0.751	0.008
K31	2.3318	0.0308	4.7724	0.0957	0.6332	0.0108	0.734	0.008

Q32	2.3168	0.0472	4.9052	0.0883	0.5567	0.0108	0.728	0.01
A34	2.4371	0.0594	4.7553	0.0607	0.6558	0.0108	0.744	0.009
N35	2.2838	0.0742	4.6995	0.0916	0.6123	0.0108	0.714	0.012
D36	2.2224	0.0329	4.7057	0.0906	0.6494	0.0108	0.706	0.008
N37	2.2587	0.0574	4.19	0.0549	0.5528	0.0108	0.677	0.008
G38	2.1735	0.0457	4.6573	0.1095	0.5314	0.0108	0.682	0.01
V39	2.0923	0.0345	4.3331	0.0312	0.5906	0.0108	0.674	0.004
D40	1.8487	0.0398	3.7576	0.0847	0.4184	0.0108	0.552	0.008
G41	1.6164	0.0155	3.5622	0.0514	0.137	0.0108	0.448	0.004
W43	2.229	0.0517	3.7712	0.1101	0.5847	0.0108	0.656	0.012
A44	2.0762	0.1388	3.8356	0.0865	0.5664	0.0108	0.625	0.014
Y45	1.9695	0.0347	3.6441	0.1569	0.5137	0.0108	0.596	0.011
D46	2.1823	0.0863	4.1064	0.0571	0.5697	0.0108	0.671	0.009
D47	2.0285	0.0253	4.1245	0.06	0.4812	0.0108	0.628	0.006
A48	1.8571	0.0241	3.9868	0.0546	0.5914	0.0108	0.588	0.006
T49	2.2996	0.0343	4.9804	0.1245	0.523	0.0108	0.716	0.01
K50	2.294	0.0345	4.3504	0.0658	0.5121	0.0108	0.699	0.008
T51	2.2996	0.0294	4.2502	0.0607	0.518	0.0108	0.694	0.007
F52	2.4894	0.0257	4.4531	0.0849	0.5481	0.0108	0.751	0.007
A53	2.3064	0.1009	4.2916	0.0472	0.5518	0.0108	0.698	0.008
V54	2.2204	0.0372	4.0907	0.0856	0.5309	0.0108	0.671	0.009
T55	2.3012	0.0572	4.5033	0.0734	0.6059	0.0108	0.73	0.01
E56	2.0905	0.1258	4.9658	0.0563	0.4452	0.0108	0.622	0.043

Definition of Symbols

R1	^{15}N longitudinal relaxation rate (s^{-1})
R2	^{15}N transverse relaxation rate (s^{-1})
NOE	NOE
S2	Generalized order parameter
d	Prefix indicates uncertainty in the parameter

Summary of Results Obtained by Alternative Data Analysis Methods.

To ensure that the total ΔG_{conf} values were not biased by the choice of data analysis methods, several alternative methods were also investigated (in addition to the method reported in the text of the article). Fixing τ_m to the same value (3.51 ns) for all three mutants increases the magnitudes of $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{M})$ and $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{T})$ by 67% and 20%, respectively. Using different (independently selected) dynamics models for the same residue in the different mutants does not change the ΔG_{conf} values within error. Finally, limiting all residues to the original Lipari-Szabo model (optimization of only S^2 and the internal correlation time τ_e) increases the magnitudes of $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{M})$ and $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{T})$ by 63% and 46%, respectively. Irrespective of the analysis method chosen, the $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{M})$ and $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{T})$ values are always negative and at least as large in magnitude (within error) as the corresponding $\Delta \Delta G_{\text{folding}}$ values. In addition, $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{T})$ is always at least twice the magnitude of $\Delta G_{\text{conf}}(\text{A} \rightarrow \text{M})$. Thus, the conclusion that backbone conformational entropy makes a significant contribution to the stability differences between these three mutants is supported by the calculated conformational free energies.