

Supplementary Information

I. Proton signal assignments for Trp-cage in 30%HFIP/50mM phosphate buffer at pH 7

<i>Residue</i>	<i>Proton</i>	<i>TFE, 7°C</i>	<i>HFIP, 5°C</i>	<i>HFIP, 25°C</i>	<i>HFIP, 45°C</i>
ASN1	HA	4.180	3.804	3.799	3.793
ASN1	HB2	3.160	2.861	2.869	2.812
ASN1	HB3	3.160	2.932	2.910	3.018
ASN1	HD21	7.450	7.429	7.296	7.165
ASN1	HD22	7.060	6.204	6.204	6.186
LEU2	HA	4.150	4.170	4.187	4.190
LEU2	HB2	1.507	1.504	1.550	1.636
LEU2	HB3	1.737	1.631	1.636	1.563
LEU2	HG	1.757	1.782	1.768	1.759
LEU2	HN	8.478	7.819	7.777	7.736
LEU2	QD1	1.003	0.984	0.998	0.996
LEU2	QD2	0.927	0.948	0.954	0.934
TYR3	HA	4.185	4.253	4.285	4.292
TYR3	HB2	3.145	3.202	3.208	3.193
TYR3	HB3	3.032	3.063	3.208	3.193
TYR3	HN	8.600	8.164	8.131	8.064
TYR3	QD	7.020	7.080	7.084	7.093
TYR3	QE	6.805	6.835	6.852	6.838
ILE4	HA	3.832	3.872	3.899	3.908
ILE4	HB	2.062	2.024	2.024	2.019
ILE4	HG12	1.652	1.719	1.714	1.688
ILE4	HG13	1.360	1.403	1.417	1.428
ILE4	HN	7.357	8.197	7.829	7.739
ILE4	QD1	0.990	1.003	1.016	1.012
ILE4	QG2	0.990	0.962	0.968	0.980
GLN5	HA	3.905	3.881	3.902	3.924
GLN5	HE21	7.447	7.642	7.549	7.434
GLN5	HE22	6.915	6.673	6.566	6.446
GLN5	HN	7.820	7.903	8.080	7.952
GLN5	QB	2.233	2.269	2.281	2.279
GLN5	QG	2.412	2.446	2.441	2.444
TRP6	HA	4.200	4.360	4.365	4.370
TRP6	HB2	3.663	3.805	3.791	3.758
TRP6	HB3	3.168	3.336	3.327	3.312
TRP6	HD1	7.057	7.083	7.106	7.114
TRP6	HE1	9.855	9.903	9.862	9.797
TRP6	HE3	7.269	7.514	7.555	7.559
TRP6	HH2	7.149	7.126	7.138	7.147

TRP6	HN	8.198	8.165	8.178	8.152
TRP6	HZ2	7.215	7.187	7.205	7.191
TRP6	HZ3	7.155	7.197	7.221	7.216
LEU7	HA	3.478	3.568	3.580	3.586
LEU7	HB2	2.136	2.233	2.206	2.155
LEU7	HB3	1.378	1.410	1.447	1.478
LEU7	HG	1.882	2.027	2.027	2.002
LEU7	HN	8.391	8.439	8.416	8.360
LEU7	QD1	0.986	1.006	1.005	0.994
LEU7	QD2	0.898	0.961	0.958	0.951
LYS8	HA	3.917	3.925	3.948	3.959
LYS8	HN	8.762	9.208	9.099	8.940
LYS8	QB	1.950	1.994	2.009	2.001
LYS8	QD	1.661	1.659	1.666	1.669
LYS8	QE	2.933	2.995	3.017	3.034
LYS8	QG	1.446	1.510	1.556	1.533
ASP9	HA	4.674	4.698	4.732	4.766
ASP9	HB2	3.074	2.989	2.974	2.955
ASP9	HB3	2.946	2.848	2.843	2.823
ASP9	HN	7.883	8.035	7.990	7.924
GLY10	HA2	3.487	3.484	3.521	3.526
GLY10	HA3	4.211	4.256	4.267	4.264
GLY10	HN	7.707	7.742	7.705	7.665
GLY11	HA2	0.948	0.821	0.942	1.119
GLY11	HA3	2.880	3.050	3.039	3.044
GLY11	HN	8.465	8.755	8.687	8.576
PRO12	HA	4.539	4.551	4.555	4.548
PRO12	HB2	2.075	2.049	2.068	2.073
PRO12	HB3	2.501	2.548	2.542	2.518
PRO12	HD2	3.671	3.828	3.822	3.806
PRO12	HD3	3.294	3.451	3.422	3.367
PRO12	HG2	2.070	2.166	2.180	2.050
PRO12	HG3	2.160	2.195	2.209	2.133
SER13	HA	4.424	4.415	4.418	4.421
SER13	HN	7.698	7.711	7.694	7.670
SER13	HB2	3.954	4.096	4.097	4.078
SER13	HB3	3.954	3.926	3.944	3.943
SER14	HA	4.221	4.237	4.261	4.285
SER14	HB2	3.654	3.616	3.655	3.687
SER14	HB3	3.954	3.949	3.962	3.968
SER14	HG	4.700	5.138	4.899	-
SER14	HN	8.162	8.238	8.197	8.133
GLY15	HA2	3.804	3.803	3.819	3.800
GLY15	HA3	4.300	4.310	4.326	4.295

GLY15	HN	7.905	7.761	7.744	7.734
ARG16	HA	5.003	5.138	5.090	5.049
ARG16	HB2	1.956	1.930	1.951	1.952
ARG16	HB3	1.874	1.887	1.854	1.837
ARG16	HD2	3.290	3.331	3.338	3.268
ARG16	HD3	3.268	3.232	3.252	3.189
ARG16	HE	7.450	7.273	7.090	7.081
ARG16	HN	8.141	8.206	8.163	8.070
ARG16	HG2	1.779	1.721	1.752	1.766
ARG16	HG3	1.741	1.721	1.752	1.766
PRO17	HA	4.671	4.732	4.727	4.717
PRO17	HB2	1.856	1.915	1.930	1.928
PRO17	HB3	2.277	2.307	2.309	2.299
PRO17	HD2	3.683	3.616	3.699	3.682
PRO17	HD3	3.884	3.800	3.820	3.798
PRO17	QG	2.014	2.028	2.047	2.045
PRO18	HA	2.655	2.655	2.760	2.907
PRO18	HB2	1.329	1.321	1.351	1.393
PRO18	HB3	0.412	0.188	0.368	0.586
PRO18	HD2	3.452	3.464	3.447	3.447
PRO18	HD3	3.379	3.438	3.447	3.447
PRO18	HG2	1.723	1.690	1.725	1.726
PRO18	HG3	1.494	1.532	1.530	1.552
PRO19	HA	4.350	4.244	4.267	4.293
PRO19	HB2	1.963	1.667	1.841	1.861
PRO19	HB3	2.170	2.042	2.049	2.051
PRO19	HD2	2.992	2.880	2.881	2.890
PRO19	HD3	3.153	3.062	3.055	3.064
PRO19	QG	1.820	1.815	1.677	1.701
SER20	HA	4.361	4.217	4.245	4.252
SER20	HB2	3.889	3.913	3.852	3.845
SER20	HB3	3.813	3.850	3.852	3.845
SER20	HN	7.852	7.298	7.279	7.256

The data in the column labeled TFE are from Neidigh, J. W., Fesinmeyer, R. M., Anderson, N. H. (2002) *Nature Struct. Biol.* **9**, 425-430 as reported in the BMRB (entry 5292). Data for HFIP were collected in this work. The TFE sample contained 30% TFE and 15 mM phosphate at pH 7. DSS was the reference compound. The HFIP sample contained 30% hexafluoroisopropanol-d₂ and 50 mM phosphate at pH 6.8, with TSP as the reference compound. Each shift results from averaging the coordinates of several dozen NOE and TOCSY cross peaks. The estimated uncertainty of the shifts for the present work is ± 0.005 ppm.

II. Observed $^1\text{H}\{^1\text{H}\}$ NOEs

	Residue	Proton		Residue	Proton	TFE 7°C	HFIP 5°C	HFIP 25°C	HFIP 45°C
1	ASN	HA	2	LEU	HN	*	*		
1	ASN	HB2,HB3	2	LEU	HN	*	*		
1	ASN	HA	2	LEU	HG		*		
1	ASN	HA	4	ILE	HD1	*	*		
2	LEU	HN	2	LEU	HB2,HB3		*		
2	LEU	HN	2	LEU	HG		*		
2	LEU	HN	2	LEU	QD1,QD2		*		
2	LEU	HN	3	TYR	HN	*	*		
2	LEU	HN	4	ILE	HN	*			
2	LEU	HA	2	LEU	HG		*		
2	LEU	HA	2	LEU	QD1,QD2		*	*	*
2	LEU	HA	3	TYR	HN	*	*		
2	LEU	HB2,HB3	3	TYR	HN	*	*	*	
2	LEU	HA	4	ILE	HN		*		
2	LEU	HA	5	GLN	QB	*	*	*	*
2	LEU	HA	5	GLN	HN	*		*	*
2	LEU	HA	5	GLN	QG		*	*	
3	TYR	HN	3	TYR	HA			*	
3	TYR	HN	3	TYR	HB2,HB3	*	*		
3	TYR	HN	4	ILE	HN	*		*	*
3	TYR	HA	5	GLN	HN		*		
3	TYR	HA	3	TYR	HB2,HB3	*			
3	TYR	HA	3	TYR	QD		*		
3	TYR	HA	4	ILE	HN	*			
3	TYR	HA	6	TRP	HN	*			
3	TYR	HA	6	TRP	HB2,HB3	*	*	*	*
3	TYR	HA	6	TRP	HE3	*	*		
3	TYR	HA	19	PRO	HB2,HB3	*			
3	TYR	HA	19	PRO	HD2,HD3	*	*		
3	TYR	HA	19	PRO	QG				
3	TYR	HB2,HB3	5	GLN	HN		*		

3	TYR	HB2,HB3	6	TRP	HD1			*	*
3	TYR	HB2,HB3	19	PRO	HB2,HB3		*		
3	TYR	HB2,HB3	19	PRO	QG	*			
3	TYR	QD	3	TYR	HB2,HB3	*			
3	TYR	QD	6	TRP	HE3	*	*		
3	TYR	QD	7	LEU	QD1	*	*	*	
3	TYR	QD	7	LEU	QD2		*	*	
3	TYR	QD	19	PRO	HB2,HB3		*		
3	TYR	QD	19	PRO	HD2,HD3	*	*	*	
3	TYR	QE	6	TRP	HE3	*			
3	TYR	QE	6	TRP	HZ3	*			
3	TYR	QE	7	LEU	QD1,QD2	*	*	*	
3	TYR	QE	18	PRO	HB2,HB3	*	*		
3	TYR	QE	19	PRO	HB2,HB3		*		
4	ILE	HN	4	ILE	HB	*		*	*
4	ILE	HN	4	ILE	QD1		*	*	
4	ILE	HN	4	ILE	HG12		*	*	*
4	ILE	HN	4	ILE	HG13		*	*	*
4	ILE	HN	5	GLN	HN	*	*	*	*
4	ILE	HN	6	TRP	HN	*			
4	ILE	HA	4	ILE	HB	*	*	*	
4	ILE	HA	4	ILE	QD1		*	*	*
4	ILE	HA	5	GLN	HN	*			
4	ILE	HA	7	LEU	HN	*	*	*	*
4	ILE	HA	7	LEU	HB2,HB3		*	*	*
4	ILE	HA	7	LEU	QD1	*	*		
4	ILE	HA	7	LEU	QD2		*		
4	ILE	HB	4	ILE	QD1		*		
4	ILE	HB	4	ILE	HG12			*	*
4	ILE	HB	4	ILE	HG13			*	*
4	ILE	HB	5	GLN	HN	*	*	*	*
4	ILE	HB	6	TRP	HN		*		
4	ILE	QD1	5	GLN	HN				*
4	ILE	HG12	5	GLN	HN		*		
4	ILE	HG13	5	GLN	HN		*		

4	ILE	QG2	5	GLN	HN	*			
5	GLN	HN	5	GLN	QB	*			
5	GLN	HN	5	GLN	QG			*	*
5	GLN	HN	6	TRP	HN		*	*	*
5	GLN	HN	7	LEU	HN	*			
5	GLN	HN	7	LEU	QD1,QD2		*	*	
5	GLN	HA	6	TRP	HN	*	*		
5	GLN	HA	8	LYS	HN	*	*	*	*
5	GLN	HA	8	LYS	QB	*	*		
5	GLN	HA	8	LYS	QD		*	*	
5	GLN	HA	8	LYS	QG		*		
5	GLN	QB	6	TRP	HN	*	*		
6	TRP	HN	6	TRP	HB2,HB3	*	*	*	*
6	TRP	HN	6	TRP	HE3	*	*	*	
6	TRP	HN	6	TRP	HD1		*	*	
6	TRP	HN	7	LEU	HN	*	*	*	*
6	TRP	HN	7	LEU	QD1,QD2		*		
6	TRP	HA	6	TRP	HB2,HB3	*			
6	TRP	HA	6	TRP	HD1	*	*	*	*
6	TRP	HA	6	TRP	HE3		*		
6	TRP	HA	7	LEU	HN	*			
6	TRP	HA	9	ASP	HN	*	*	*	*
6	TRP	HA	9	ASP	HB2,HB3	*	*	*	*
6	TRP	HB2,HB3	6	TRP	HD1	*	*		
6	TRP	HB2,HB3	6	TRP	HN	*	*		
6	TRP	HB2,HB3	7	LEU	HN	*	*	*	*
6	TRP	HD1	7	LEU	QD1,QD2			*	
6	TRP	HD1	16	ARG	HN		*		
6	TRP	HD1	16	ARG	HB2,HB3		*	*	*
6	TRP	HD1	16	ARG	HD2,HD3	*		*	
6	TRP	HD1	16	ARG	HG2,HG3	*		*	*
6	TRP	HD1	19	PRO	HA			*	
6	TRP	HD1	18	PRO	HA	*	*	*	*
6	TRP	HD1	19	PRO	HD2,HD3	*	*	*	*

6	TRP	HE3	7	LEU	HN	*	*	*	*
6	TRP	HE3	7	LEU	HA	*	*	*	*
6	TRP	HE3	7	LEU	HB2,HB3		*		
6	TRP	HE3	7	LEU	QD1,QD2	*	*	*	*
6	TRP	HE3	7	LEU	HG	*	*	*	*
6	TRP	HZ3	7	LEU	HA		*		
6	TRP	HZ3	7	LEU	HG	*	*		
6	TRP	HZ3	7	LEU	QD1,QD2	*	*	*	*
6	TRP	HZ3	12	PRO	HA			*	*
6	TRP	HZ3	12	PRO	HD2,HD3			*	*
6	TRP	HZ3	18	PRO	HD2,HD3				*
6	TRP	HH2	7	LEU	QD1,QD2		*	*	*
6	TRP	HH2	12	PRO	HA	*	*	*	*
6	TRP	HH2	12	PRO	HB2,HB3		*		
6	TRP	HH2	12	PRO	HG2,HG3	*	*	*	
6	TRP	HH2	12	PRO	HD2,HD3	*	*	*	*
6	TRP	HH2	18	PRO	HB2,HB3	*			
6	TRP	HH2	18	PRO	HG2,HG3	*	*	*	
6	TRP	HE1	16	ARG	HN	*			
6	TRP	HE1	16	ARG	HB2,HB3	*	*	*	*
6	TRP	HE1	17	PRO	HA	*	*	*	
6	TRP	HE1	18	PRO	HA	*	*	*	*
6	TRP	HZ2	7	LEU	QD1,QD2			*	*
6	TRP	HZ2	12	PRO	HA	*	*	*	*
6	TRP	HZ2	12	PRO	HB2,HB3		*		
6	TRP	HZ2	12	PRO	HG2,HG3		*	*	
6	TRP	HZ2	12	PRO	HD2,HD2		*	*	
6	TRP	HZ2	18	PRO	HA		*		
6	TRP	HZ2	17	PRO	HA	*	*	*	
6	TRP	HZ2	18	PRO	HB2,HB3	*		*	
6	TRP	HZ2	18	PRO	HG2,HG3	*		*	
6	TRP	HZ2	18	PRO	HD2,HD3	*			
6	TRP	HZ2	19	PRO	HD2,HD3		*		
7	LEU	HN	7	LEU	HB2,HB3	*	*	*	*
7	LEU	HN	7	LEU	HG	*	*	*	*

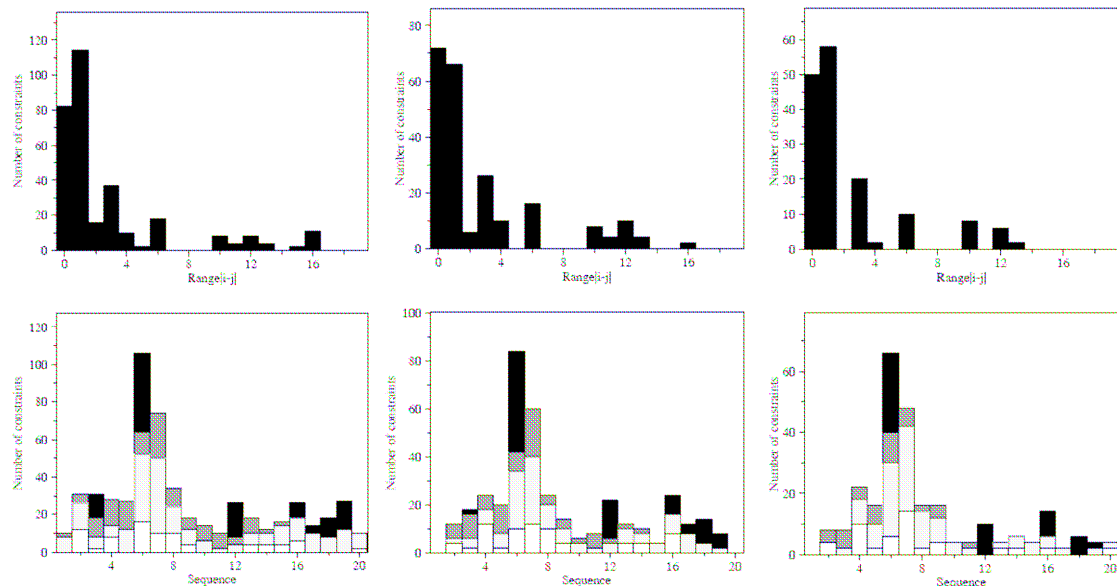
7	LEU	HN	7	LEU	QD1,QD2		*	*	*
7	LEU	HN	8	LYS	HN	*	*	*	*
7	LEU	HN	9	ASP	HN	*			
7	LEU	HA	7	LEU	HB2,HB3	*			
7	LEU	HA	7	LEU	QD1,QD2	*	*	*	*
7	LEU	HA	8	LYS	HN	*			
7	LEU	HA	9	ASP	HN	*			
7	LEU	HA	10	GLY	HN	*	*	*	
7	LEU	HA	11	GLY	HN	*	*	*	*
7	LEU	HA	11	GLY	HA2,HA3	*	*	*	
7	LEU	HB2,HB3	7	LEU	QD1	*			
7	LEU	HB2,HB3	7	LEU	HG				*
7	LEU	HB2,HB3	8	LYS	HN	*	*	*	*
7	LEU	QD1,QD2	8	LYS	QE		*		
7	LEU	QD1,QD2	11	GLY	HA2,HA3	*	*		
7	LEU	QD2	11	GLY	HN	*			
7	LEU	QD1	12	PRO	HD2,HD3				
7	LEU	QD2	12	PRO	HD2,HD3	*	*		
8	LYS	HN	8	LYS	HB2,HB3	*			
8	LYS	HN	8	LYS	QG		*	*	
8	LYS	HN	8	LYS	QD		*	*	*
8	LYS	HN	9	ASP	HN	*	*	*	*
8	LYS	HN	10	GLY	HN	*			
8	LYS	HA	8	LYS	QD		*	*	
8	LYS	HA	8	LYS	QE		*	*	
8	LYS	HA	9	ASP	HN	*			
8	LYS	HA	9	ASP	HB2,HB3				*
8	LYS	HA	10	GLY	HN		*		
8	LYS	HB2,HB3	8	LYS	QE		*	*	
8	LYS	HB2,HB3	9	ASP	HN	*	*	*	*
9	ASP	HN	9	ASP	HB2,HB3	*	*	*	*
9	ASP	HN	10	GLY	HN	*	*	*	*
9	ASP	HN	11	GLY	HN	*			
9	ASP	HA	10	GLY	HN	*			

9	ASP	HB2,HB3	10	GLY	HN		*		
9	ASP	HB2,HB3	14	SER	HB2,HB3	*			
10	GLY	HN	11	GLY	HN	*	*	*	*
10	GLY	HA2,HA3	11	GLY	HN	*			
10	GLY	HA1	13	SER	HN		*		
10	GLY	HA2,HA3	13	SER	HN		*		
11	GLY	HN	13	SER	HN			*	
11	GLY	HA2	12	PRO	HD2,HD3	*			
11	GLY	HA3	12	PRO	HD2	*			
11	GLY	HA2,HA3	13	SER	HN		*		
12	PRO	HA	12	PRO	HG2,HG3	*			
12	PRO	HA	13	SER	HN	*			
12	PRO	HA	14	SER	HN	*	*	*	
12	PRO	HA	15	GLY	HN	*			
12	PRO	HB2,HB3	12	PRO	HD2,HD3	*			
12	PRO	HB2,HB3	13	SER	HN	*	*	*	
12	PRO	HD2,HD3	13	SER	HN	*	*	*	
13	SER	HN	13	SER	HB2,HB3	*	*	*	
13	SER	HN	14	SER	HN	*	*	*	*
13	SER	HA	13	SER	HB2,HB3				*
13	SER	HA	14	SER	HN	*			
13	SER	HA	15	GLY	HN	*	*		
14	SER	HN	14	SER	HB2,HB3	*	*	*	*
14	SER	HN	15	GLY	HN	*	*	*	*
14	SER	HA	14	SER	HB2,HB3			*	
14	SER	HA	15	GLY	HN	*	*		
14	SER	HB2,HB3	15	GLY	HN	*			
15	GLY	HN	15	GLY	HA2,HA3	*	*		
15	GLY	HN	16	ARG	HN	*	*	*	*
15	GLY	HA2,HA3	16	ARG	HN	*	*		

16	ARG	HN	16	ARG	HB2,HB3		*	*	
16	ARG	HN	16	ARG	HG2,HG3			*	
16	ARG	HN	16	ARG	HD2,HD3				*
16	ARG	HA	17	PRO	HD2,HD3	*	*	*	
16	ARG	HB2,HB3	16	ARG	HD2,HD3			*	
16	ARG	HB2,HB3	17	PRO	HD2,HD3	*	*	*	*
17	PRO	HB2,HB3	17	PRO	HD2,HD3	*			
17	PRO	HA	18	PRO	HD2,HD3	*	*	*	
17	PRO	HB2,HB3	18	PRO	HD2,HD3	*	*		
18	PRO	HA	19	PRO	HD2,HD3	*	*	*	
18	PRO	HB2,HB3	18	PRO	HD2,HD3	*	*		
18	PRO	HB2,HB3	19	PRO	HD2,HD3	*			
19	PRO	HA	20	SER	HN		*		*
19	PRO	HB2,HB3	20	SER	HN	*	*		
19	PRO	QG	20	SER	HN		*		
19	PRO	HD2,HD3	19	PRO	HB2,HB3	*			
20	SER	HN	20	SER	HA				*
20	SER	HN	20	SER	HB2,HB3		*		

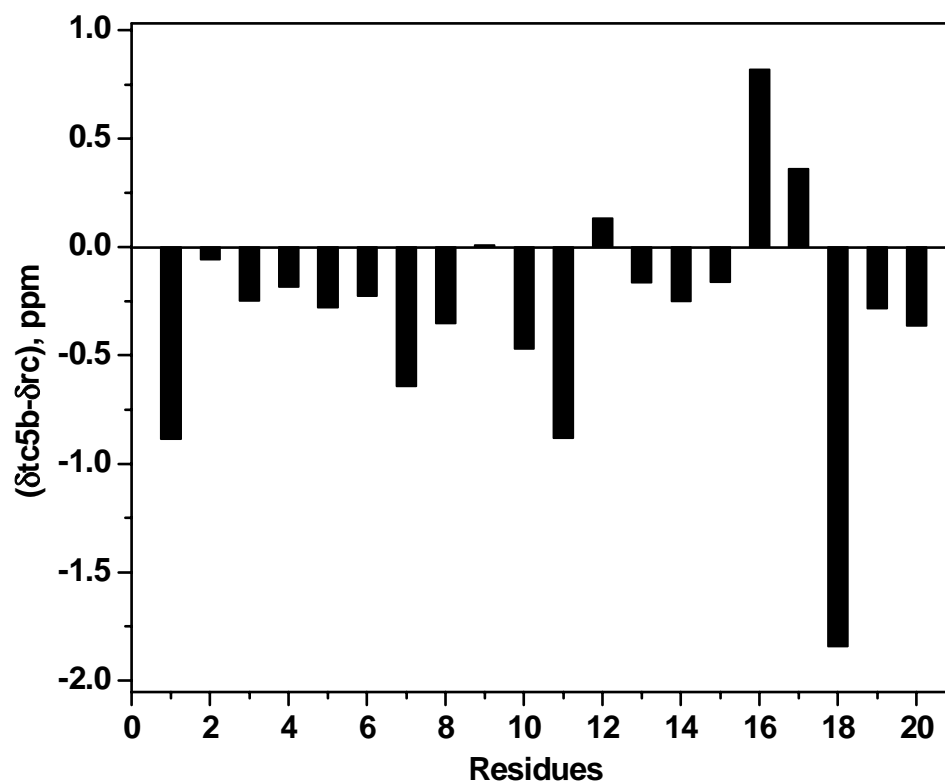
An asterisk indicates that an NOE was observed between the pair of protons indicated. When a diastereotopic pair of protons is indicated, one or more of the possible NOEs was detected. Note that some NOEs may not have been reported due to signal overlaps or shift degeneracies. The data in the column labeled TFE is from Neidigh, J. W., Fesinmeyer, R. M., Anderson, N. H. (2002) *Nature Struct. Biol.* **9**, 425-430 as reported in the BMRB (entry 5292). Data for HFIP were collected in this work.

III. Distribution of NOEs



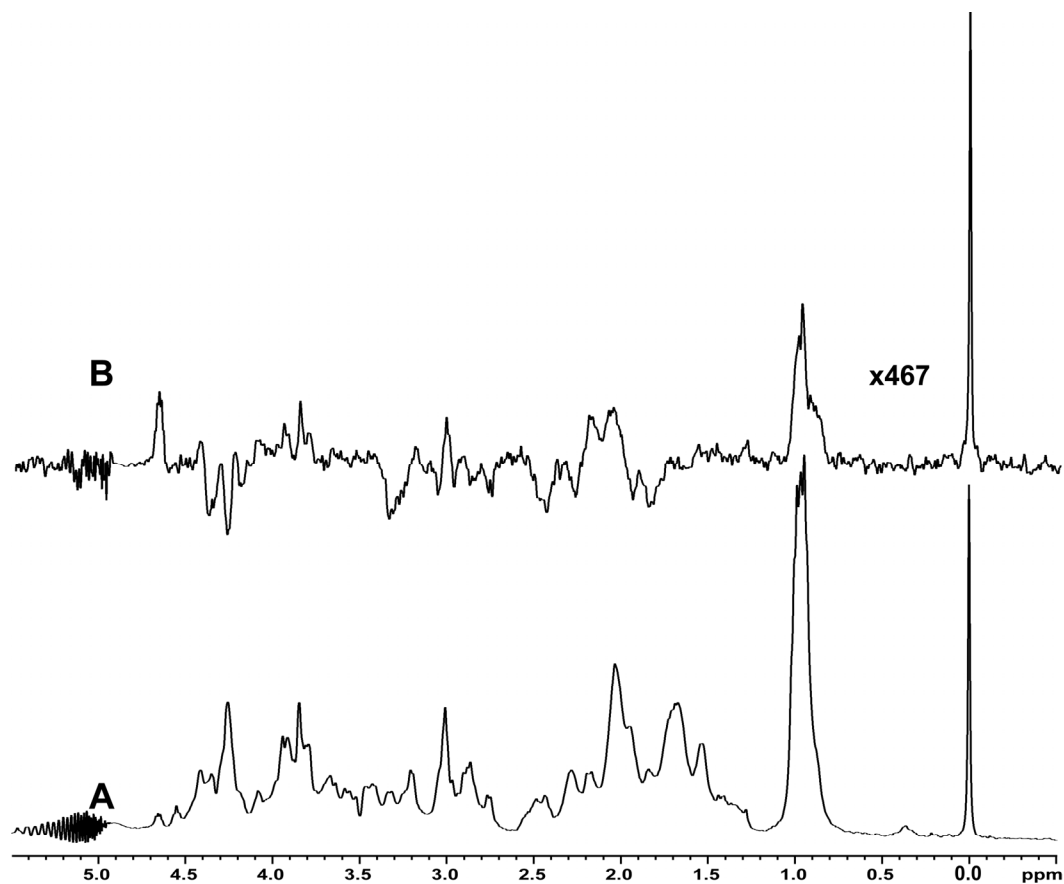
The distribution of observed NOEs at the temperatures examined in this work (5°C, 25°C, 45°C, left to right) are shown in graphs above. The plots were produced by the program DYANA (Güntert, P. *Meth. Mol. Biol.* 278, 353-378 (2004)). The top plot shows the number of NOE/distance constraints observed plotted against the residue index difference. The bottom plot shows the number of intra-residue (white), short range (light gray), medium-range (dark gray) and long-range constraints (black) to each residue.

IV. HA Chemical Shift Deviations from Random Coil Values at 5°C



Deviations of the proton chemical shifts from random coil values observed for 5 mM Trp-cage in 30% HFIP/50mM phosphate buffer 5°C. Random coil shifts are from Wishart and Sykes (1994) and were corrected for sequence dependence using the method of Schwarzingner *et al.* (2001). The generally negative deviations are consistent with the presence of α -helices.

V. Intermolecular Proton-Fluorine NOEs at 25°C



Upfield portion of proton-fluorine intermolecular NOE spectra at 500 MHz of Trp-cage in 30% HFIP at 25°C. Conditions for each experiment were the same as those used to collect the data shown in Figures 2 and 3 of the paper. Spectrum A is the observed 1D proton spectrum; spectrum B is the proton-fluorine NOE spectrum at a mixing time of 600 ms. Horizontal and vertical scales for the two sets of spectra are slightly different.