Supporting Information for:

Thermodynamic Analysis of Autonomous Parallel β-Sheet Formation in Water

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Synthesis:

Synthesis of **3** began with the preparation in solution of the fragment Alloc-Glu-Val-D-Pro-DADME-Fmoc, as previously described for a linear molecule related to **1**.[1] This fragment was attached to Rink amide resin via the Glu side chain carboxyl group, and the amino acid residues corresponding to Arg-11 through Lys-17 were appended via standard Fmoc-based solid-phase peptide synthesis on an Applied Biosystems model 432A Synergy solid phase peptide synthesizer using standard Fmoc synthesis procedures. Fmoc-amino acids were activated as HOBt esters with HBTU and DIEA. All amino acids with reactive side chains were protected. The linker unit was treated as an amino acid and coupled using the same protocol. The α-amino group of Lys-17 was then coupled to the mono-2-(trimethylsilyl)ethyl ester of succinic acid (generated by using 2-(trimethylsilyl)ethanol to open succinic anhydride). The Alloc protecting group was removed with a Pd^0 catalyst, and the amino acid residues corresponding to Ile-6 through Gly-1 were introduced via standard methods. The Fmoc group was removed from Gly-1, fluoride was used to remove the 2-(trimethylsilyl)ethyl group from Succ-18, and cyclization was achieved on resin by treatment with standard coupling reagents (HBTU, HOBt and iPr_2EtN in DMF).

Figure A1: Synthetic strategy employed in the construction of cyclic peptide **3**. A) Generation of monoprotected succinic acid ester. B) Structure of linker unit used in the synthesis of peptides **1** and **3**. The linker was constructed in solution via typical amide bond forming chemistry from appropriately protected amino acids. C) Abbreviations used in figure part D D) Synthetic strategy employed in the construction of cyclic peptide **3.** One letter abbreviations are used to denote amino acids. Asterisks indicated side chain protection. Normal solid phase synthesis was performed on a Applied Biosystems model 432A Synergy solid phase peptide synthesizer using standard Fmoc synthesis procedures. Fmoc-amino acids were activated as HOBt esters with HBTU and DIEA. All amino acids with reactive side chains were protected. The linker unit was treated as an amino acid and coupled using the same protocol. All peptides were synthesized using dimethoxybenzhydrylamine resin with a Rink amide handle. Coupling reactions were carried out at room temperature in DMF. All peptides were synthesized on a 25 umol scale, using a threefold excess of amino acid in each step. The reaction progress was monitored by solution conductivity.

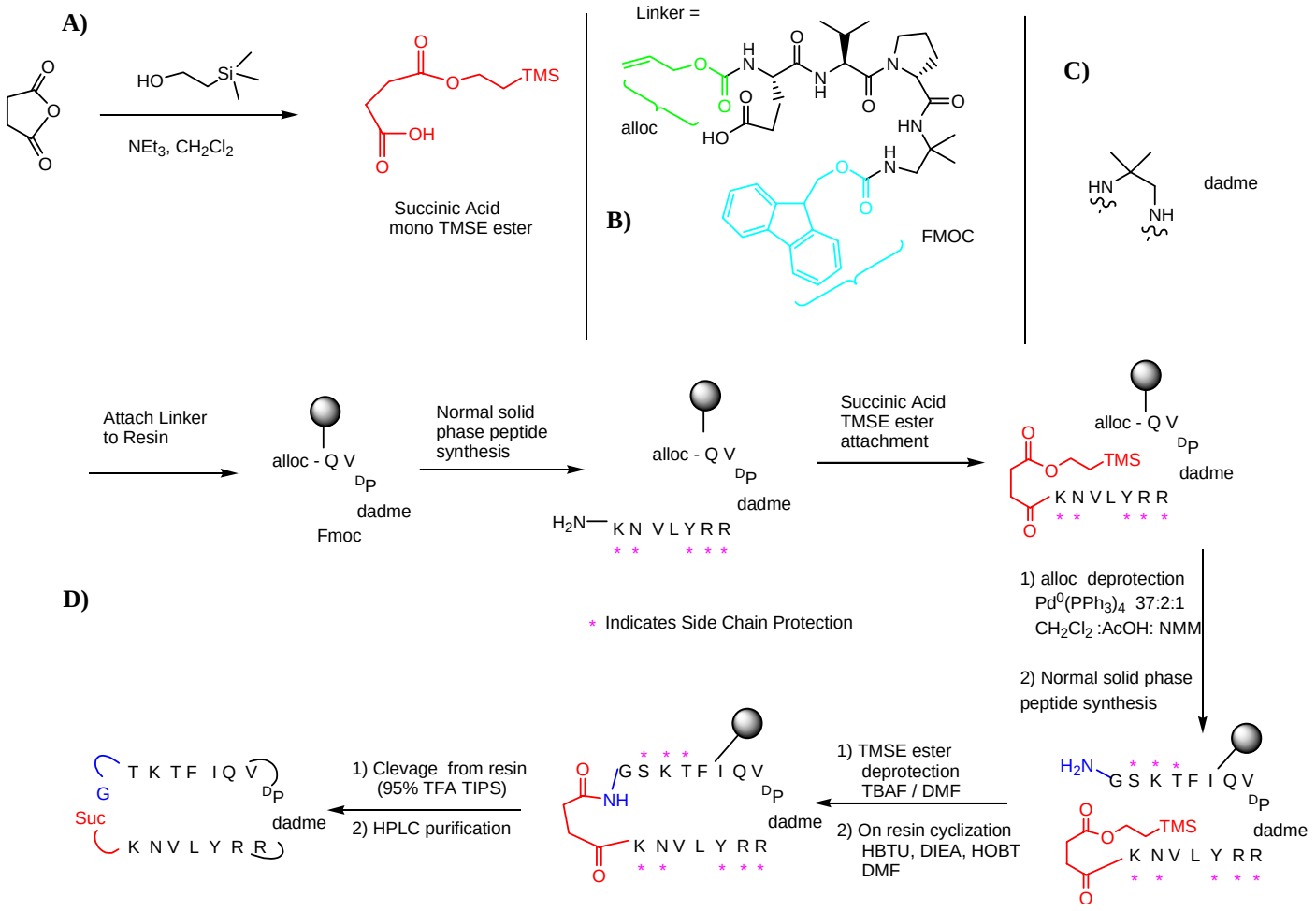

Figure A1: Synthetic strategy employed in the construction of cyclic peptide **3** (see previous page for caption).

NMR Sample Preparation and Experiments:

NMR samples were prepared by dissolving lyophilized peptides in 9:1 H_2O/D_2O, 100 mM pH 3.8 sodium deuterioacetate buffer (buffer pH was not corrected for isotope effects). The samples often contained dust or undissolved material and were filtered using PVDF low peptide binding syringe filters (Chromacol, Trumbull, CT) directly into NMR tubes. Peptide concentrations were generally 1-3 mM, as judged by mass of material dissolved, but this concentration range should be taken as an upper limit because of material loss during the filtration. Spectroscopic determinations of concentration generally yielded values 70-80% of the concentration calculated based on mass. Samples were prepared with total volumes of approximately 300 μL for 3 mm tubes and 600 μL for 5 mm NMR tubes. Trace amounts of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS; Merck, Rahway, NJ) were added to samples as an internal reference. The NMR samples were stable in solution for weeks to months showing no apparent precipitation of peptide or decrease in NMR signal strength over the entire period of study. In all cases sharp lines were observed in 1D spectra indicating that the peptides were not aggregated in solution.

NMR experiments were performed on a Varian INOVA 500 and 600 MHz spectrometers between set temperatures of 275 K and 354 K using 3 mm or 5 mm probes:

Inova 600 probes
- Varian 3 mm $^{1}H/^{13}C/^{15}N$ with 3-axis PFG
- Varian 5 mm $^{1}H/^{13}C/^{15}N$ with 3-axis PFG
- Varian 5 mm $^{1}H/^{31}P/X$ broad band with z-axis PFG

Inova 500 probes:
- Nalorac 3 mm inverse $^{1}H/^{13}C/^{15}N$ with 3-axis PFG
- Varian 5 mm inverse $^{1}H/^{13}C/X$ with z-axis PFG

The probe thermocouple temperatures were calibrated with methanol and ethylene glycol chemical shift thermometers. Chemical shift measurements were performed at 5 K intervals set via the probe thermocouple and the reported temperatures were derived from the thermocouple calibration. The reported temperatures are presumed to be accurate to ~ 1 K. Solvent suppression was achieved by 0.6-1.5 second solvent presaturation during the relaxation delay. Watergate solvent suppression as implemented in Varian's Protein Pack suite of experiments was used instead of presaturation in some NOESY and TOCSY experiments. Spectral windows of 5500 Hz (500 MHz spectrometer) or 6600 (600 MHz spectrometer) were used. Standard Varian pulse sequences were used, and data were processed using Varian VNMR 6.1 software and analyzed with XEASY[2] or Sparky[3] programs. Shifted sin bell window functions were generally applied before fourier transformation. For all samples GCOSY spectra where obtained in absolute value mode with gradient echo coherence

selection; TOCSY,[4] NOESY,[5] and ROESY[4,6] spectra were acquired in the phase-sensitive mode with hypercomplex phase cycling (States-Haberkorn method). All experiments were performed by collecting 2048 points in f2 and 300-600 points in f1. TOCSY experiments employed a standard MLEV-17 spin lock sequence with a spin lock field of 7-8 kHz and mixing time of 80 ms. ROESY experiments used spinlocking fields of 3 kHz and 200-250 ms mixing times. NOESY spectra utilized mixing times of 200-300 ms. The ^{1}H chemical shift assignment of the peptides was achieved by the sequential assignment procedure.[7] NOEs / ROEs where characterized as strong, medium or weak based on visual comparison to either the C-terminal amide cross peak or cross peaks of well resolved geminal proton pairs.

(1) Fisk, J. D.; Gellman, S. H.; *J. Am. Chem. Soc.*, **2001**; *123*, 343.

(2) Bartels, C.; Xia, T. H.; Billeter, M.; Guntert, P.; Wuthrich, K.; *J. Biomol. Nmr* **1995**, *6*, 1-10.

(3) Goddard, D. T.; Kneller, D. G. SPARKY 3, University of California, San Francisco.

(4) Bax, A.; Davis, D. G.; *J. Mag. Res.*, **1985**; *65*, 355.

(5) Jeener, J. M., B.H.; Bachmann, P.; Ernst, R. R.; *J. Chem. Phy.* **1979**, *29*, 1012-1014.

(6) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W.; *J. Am. Chem. Soc.*, **1984**; *106*, 811-813.

(7) Wuthrich, K.; *NMR of Proteins and Nucleic Acids*; Wiley-Interscience: New York, 1986.

Table A.1: Proton resonances (ppm relative to DSS) for **1:**
Ac-SKTFIQV-^{D}P-dadme-RRYLVNK-ac
(~2 mM in 9:1 H2O:D2O pH 3.8, 100 mM AcOD at 275K)

Residue	N-H	Hα	Hβ	Others
Ac-1	-	-	-	CH_3 2.040
Ser-2	8.456	4.380	3.831	-
Lys-3	8.558	4.444	1.595	γ 1.326, 1.420 δ 1.660 ε 2.966 ζ 7.629
Thr-4	8.203	4.337	3.935	γ 1.117
Phe-5	8.308	4.957	2.850	δ 7.037 ε 7.267 ζ 7.221
Ile-6	8.623	4.172	1.616	γ2 0.8886 γ1 1.050, 1.345 δ 0.7557
Gln-7	8.850	4.774	2.039, 2.054	γ 2.140, 2.303 ε 7.057, 7.563
Val-8	8.854	4.599	2.040	γ 0.9135, 0.9468
^{D}Pro-9		4.296	1.941, 2.279	γ 2.006, 2.132 δ 3.753, 3.879
Dadme-10	7.969 8.036			Me 1.167, 1.311 CH_2 3.529, 3.578
Arg-11	8.755	4.708	1.820	γ 1.609 , 1.745 δ 3.234 ε 7.319 η
Arg-12	8.816	4.541	1.564, 1.701	γ 1.432 δ 2.899 ε 7.050 η
Tyr-13	8.396	5.170	2.844, 2.914	δ 7.056 ε 6.723
Leu-14	8.336	4.431	1.416	γ 1.454 δ 0.8170, 0.8182
Val-15	7.900	4.105	1.868	γ 0.6189, 0.7780
Asn-16	8.666	4.692	2.724, 2.823	δ 7.000, 7.691
Lys-17	8.475	4.333	1.718	γ 1.395, 1.444 δ 1.714 ε 2.965 ζ 7.627
Ac-18	-	-	-	CH_3 2.040

Table A.2: Proton resonances (ppm relative to DSS) for **2:**
ac-SKTFIQV-^{L}P-dadme-RRYLVNK-ac
(~2 mM in 9:1 H2O:D2O pH 3.8, 100 mM AcOD at 275 K)

Residue	N-H	Hα	Hβ	Others
Ac-1	-	-	-	CH_3 2.104
Ser -2	8.460	4.391	3.842	
Lys -3	8.645	4.396	1.728 1.826	γ 1.383, 1.437 δ 1.668 ε 2.971 ζ 7.619
Thr -4	8.180	4.266	4.091	γ 1.138
Phe-5	8.424	4.634	3.016 3.056	δ 7.202 ε 7.312 ζ 7.270
Ile-6	8.158	4.052	1.722	γ2 1.113 γ1 1.410, 1.455 δ 0.8229
Gln-7	8.536	4.273	1.918 1.964	γ 2.241, 2.307 ε 6.902 7.574
Val-8	8.466	4.394	2.095	γ 0.9747, 1.006
^{D}Pro- 9		4.283	1.889, 2.240	γ 1.941, 2.047 δ 3.668, 3.890
Dadme -10	8.168			3.471, 3.498
Arg 11	8.198	4.247	1.732	γ 1.514, 1.653 δ 3.147 ε 7.226 η
Arg-12	8.413	4.224	1.777	γ 1.599, 1.678 δ 3.215 ε 7.297 η
Tyr-13	8.262	4.540	2.968	δ 7.084 ε 6.795
Leu-14	8.347	4.311	1.546	γ 1.437 δ 0.8264 0.8976
Val-15	8.105	4.031	2.022	γ 0.8212, 0.8842
Asn-16	8.659	4.692	2.736, 2.815	δ 6.949 7.658
Lys-17	8.463	4.216	1.718 1.784	γ 1.400, 1.431 δ 1.667 ε 2.975 ζ 7.624
Ac-18	-	-	-	CH_3 2.103

Table A.3: Proton resonances (ppm relative to DSS) for **3:**
c(G-SKTFIQV-^{D}P-dadme-RRYLVNK-suc)
(~2 mM in 9:1 H2O:D2O pH 3.8, 100 mM AcOD at 275 K)

Residue	N-H	Hα	Hβ	Others
Gly-1	8.569	4.008, 4.051	-	
Ser -2	8.331	4.547	3.868	-
Lys -3	8.606	4.621	1.563	γ 1.202, 1.339 δ 1.630 ε 2.944 ζ
Thr -4	8.048	4.375	3.937	γ 1.099
Phe-5	8.592	5.272	2.717, 2.810	δ 7.029 ε 7.243 ζ 7.254
Ile-6	8.827	4.211	1.487	γ 0.9032 γ 0.975, 1.247 δ 0.716
Gln-7	8.938	4.878	2.021, 2.070	γ 2.112, 2.308 ε 7.001, 7.574
Val-8	9.002	4.628	2.060	γ 0.9267, 1.005
^{D}Pro- 9		4.304	1.936, 2.289	γ 2.009, 2.154 δ 3.735, 3.903
Dadme -10	7.907			3.499, 3.628 1.164, 1.319
Arg 11	8.871	4.798	1.828	γ 1.615, 1.769 δ 3.236 ε 7.313 η
Arg-12	8.981	4.681	1.592, 1.723	γ 1.468 δ 2.921 ε 7.048 η
Tyr-13	8.634	5.324	2.827, 2.831	δ 7.061 ε 6.728
Leu-14	8.379	4.525	1.288	γ 1.450 δ 0.728, 0.834
Val-15	8.162	4.164	1.776	γ 0.4990, 0.7362
Asn-16	8.261	4.775	2.628, 2.655	δ 7.061, 7.541
Lys-17	8.488	4.344	1.732	γ 1.283, 1.363 δ 1.634 ε 2.936 ζ 7.584
Suc-18	-	-	-	CH_2 CH_2

Table A4 Selected NOEs observed for **1** in aqueous solution (100 mM Acetic Acid buffer, pH 3.8, 275 K)				
Residue	**H-atom**	**Residue**	**H-atom**	**NOE Intensity**
Ser 2	Hα	Asn 16	HN	weak
Lys 3	Hδ	Phe 5	Hε	weak
Thr 4	Hα	Tyr 13	Hδ	weak
	HN	Val 15	Hα	weak
Phe 5	Hε	Arg 12	Hγ	weak
	Hα	Leu 14	HN	weak
	Hε	Leu 14	Hδ	weak
	Hζ	Leu 14	Hδ	weak
Ile 6	Hβ	Tyr 13	Hδ	medium
	Hβ	Tyr 13	Hε	weak
	Hγ	Tyr 13	Hδ	medium
	Hγ	Tyr 13	Hε	medium
	Hδ	Tyr 13	Hε	medium
	HN	Tyr 13	Hα	weak
Gln 7	Hα	Arg 12	HN	weak
	Hα	Arg 12	Hβ	weak
Val 8	HN	Arg 11	Hα	weak
	Hγ	Tyr 13	Hδ	medium
	Hγ	Tyr 13	Hε	medium
Tyr 13	Hδ	Val 15	Hγ	weak
	Hε	Val 15	Hβ	weak
	Hε	Val 15	Hγ	weak
	HN	Val 15	Hγ	weak

Table A5 Selected NOEs observed for **3** in aqueous solution (100 mM Acetic Acid buffer, pH 3.8, 297 K)

Residue	**H-atom**	**Residue**	**H-atom**	**NOE Intensity**
Ser 2	Hα	Asn 16	Hβ	weak
	Hβ	Lys 17	Hα	weak
	HN	Gly 1	HN	weak
Lys 3	Hα	Asn 16	Hβ	weak
	HN	Leu 14	Hα	weak
Thr 4	Hγ	Tyr 13	Hδ	weak
	Hγ	Tyr 13	Hε	weak
	Hβ	Val 15	Hα	weak
	HN	Val 15	Hγ	weak
		Val15	Hα	weak
	Hδ			
Phe 5	Hδ	Lys 3	Hδ	weak
	Hε	Lys 3	Hε	weak
	Hδ	Lys 3	Hδ	medium
	Hδ	Leu 14	Hβ	medium
	Hε	Leu 14	Hγ	medium
	Hε	Leu 14	Hβ	weak
	Hε	Leu 14	Hγ	medium
	Hζ	Leu 14	Hδ	medium
	Hζ	Leu 14	Hγ	weak
	Hα	Leu 14	Hδ	medium
		Leu 14	HN	weak
	Hβ			
Ile 6	Hβ	Tyr 13	Hδ	medium
	Hγ1	Tyr 13	Hε	medium
	Hγ2	Tyr 13	Hε	weak
	Hγ2	Tyr 13	Hδ	medium
	Hδ	Tyr 13	Hε	medium
	HN	Tyr 13	Hε	medium
		Tyr 13	Hα	weak
	Hα			
Gln 7	Hβ	Arg 12	HN	weak
	Hγ	Arg 11	Hα	weak
	Hβ	Pro 9	Hδ	weak
		Arg 12	HN	weak
	HN			
Val 8	Hβ	Arg 11	Hα	weak
	Hβ	Tyr 13	Hδ	weak
	Hγ	Tyr 13	Hε	weak

Val 8	Hγ	Tyr 13	Hδ	strong
		Tyr 13	Hε	medium
	Hδ			
Tyr 13	Hδ	Val 15	Hβ	weak
	Hε	Val 15	Hγ	weak
		Val 15	Hβ	weak
		Val 15	Hγ	weak

Table A6 Chemical shifts of Hα protons observed for **3** in aqueous solution with different proportions of TFE (100 mM Acetic Acid buffer / X %TFE, pH 3.8, 287 K)					
	0% TFE	15% TFE	30% TFE	40% TFE	50% TFE
Gly 1 HA1	4.027	4.04	4.115	4.13	4.162
Gly 1 HA2	3.904	3.891	3.866	3.858	3.844
Ser 2	4.558	4.601	4.661	4.675	4.678
Lys 3	4.635	4.714	4.861	4.886	4.904
Thr 4	4.367	4.373	4.421	4.431	4.449
Phe 5	5.25	5.285	5.343	5.346	5.358
Ile 6	4.213	4.219	4.257	4.27	4.27
Gln 7	4.889	4.959	4.992	5	4.992
Val 8	4.631	4.624	4.65	4.663	4.663
Pro 9					
Dadme 10	3.501	3.496	3.521	3.523	3.528
Arg 11	4.802	4.779	4.811	4.81	4.826
Arg 12	4.676	4.696	4.72	4.738	4.754
Tyr 13	5.323	5.384	5.434	5.444	5.456
Leu 14	4.518	4.537	4.57	4.577	4.581
Val 15	4.188	4.268	4.388	4.394	4.418
Asn 16	4.785	4.794	4.859	4.885	4.883
Lys 17	4.352	4.367	4.417	4.436	4.453

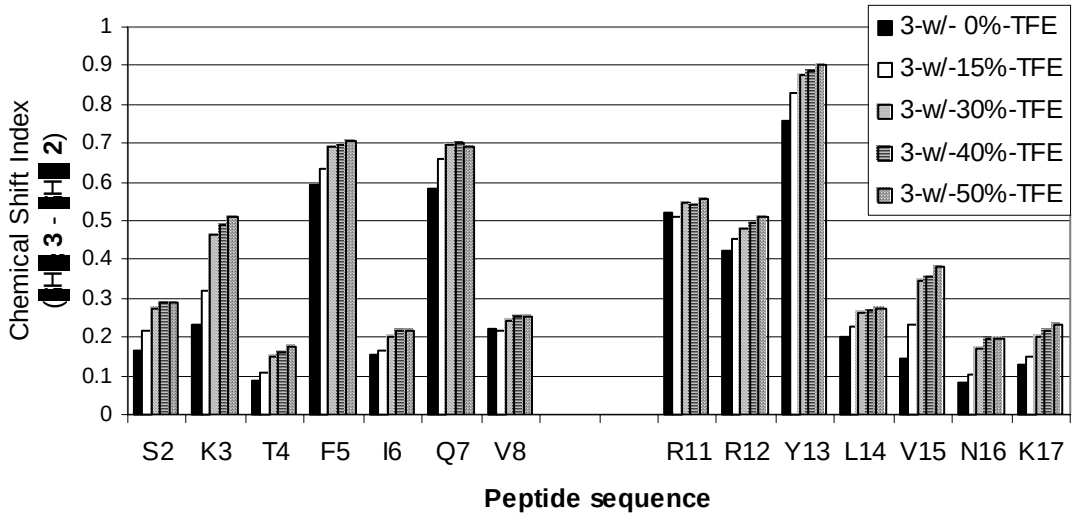

Figure A2: Effect of TFE cosolvent addition on chemical shifts in cyclic reference peptide 3 (100 mM Acetic Acid buffer / X %TFE, pH 3.8, 287 K).

Table A7: α proton chemical shifts for **1** in aqueous solution from 275 to 354 K
(100 mM Acetic Acid buffer, pH 3.8, 275-354 K)

Temp ^{o}K	275.4	280.2	286.6	291.5	297.2	302.8	308.5	313.9	319.8	325.3	331.6	343	354
Ser 2	4.376	4.379	4.382	4.385	4.387	4.396	4.398	4.399	4.403	4.405	4.407	4.417	4.376
Lys 3	4.440	4.442	4.438	4.438	4.434	4.437	4.432	4.428	4.428	4.424	4.417	4.410	4.440
Thr 4	4.294	4.295	4.292	4.291	4.291	4.292	4.291	4.288	4.290	4.288	4.285	4.275	4.294
Phe 5	4.977	4.984	4.988	4.989	4.986	4.987	4.979	4.971	4.965	4.949	4.914	4.887	4.977
Ile 6	4.165	4.170	4.174	4.176	4.176	4.183	4.182	4.182	4.184	4.185	4.188	4.187	4.165
Gln 7	4.767	4.782	4.796	4.802	4.809	4.814	4.815	4.810	4.809	4.793	4.767	4.734	4.767
Val 8	4.596	4.599	4.599	4.597	4.601	4.602	4.599	4.596	4.592	4.589	4.576	4.563	4.596
	3.529	3.526	3.518	3.515	3.507	3.511	3.507	3.509	3.497	3.499	3.497	3.482	3.529
Arg 11	4.707	4.712	4.712	4.714	4.715	4.712	4.705	4.697	4.693	4.681	4.647	4.619	4.707
Arg 12	4.538	4.542	4.545	4.547	4.540	4.538	4.532	4.523	4.518	4.506	4.482	4.459	4.538
Tyr 13	5.170	5.172	5.170	5.163	5.158	5.147	5.131	5.110	5.091	5.064	4.991	4.940	5.170
Leu 14	4.432	4.434	4.433	4.428	4.425	4.424	4.420	4.416	4.408	4.397	4.381	4.370	4.432
Val 15	4.100	4.108	4.119	4.125	4.131	4.137	4.140	4.141	4.145	4.141	4.141	4.141	4.100
Asn 16	4.690	4.692	4.691	4.693	4.692	4.695	4.692	4.693	4.695	4.699	4.693	4.694	4.690
Lys 17	4.225	4.230	4.230	4.236	4.240	4.244	4.246	4.248	4.252	4.256	4.262	4.258	4.225

Table A8 : α proton chemical shifts for **2** in aqueous solution from 275 to 354 K
(100 mM Acetic Acid buffer, pH 3.8, 275-354 K)

Temp °K	275.4	280.2	286.6	291.5	297.2	302.8	308.5	313.9	319.8	325.3	331.6	343	354
Ser 2	4.391	4.4	4.387	4.392	4.394	4.398	4.399	4.403	4.403	4.409	4.413	4.414	4.415
Lys 3	4.396	4.395	4.394	4.389	4.389	4.389	4.384	4.383	4.385	4.383	4.384	4.377	4.375
Thr 4	4.266	4.266	4.272	4.267	4.271	4.271	4.268	4.268	4.27	4.271	4.269	4.269	4.26
Phe 5	4.637	4.637	4.652	4.653	4.663	4.67	4.672	4.678	4.679	4.684	4.688	4.69	4.69
Ile 6	4.046	4.043	4.056	4.074	4.08	4.093	4.094	4.107	4.107	4.12	4.128	4.14	4.145
Gln 7	4.273	4.271	4.3	4.307	4.32	4.335	4.335	4.352	4.352	4.365	4.372	4.375	4.38
Val 8	4.394	4.392	4.408	4.411	4.416	4.422	4.423	4.428	4.43	4.436	4.439	4.443	4.445
Arg 11	4.251	4.251	4.267	4.267	4.277	4.285	4.286	4.297	4.297	4.301	4.306	4.303	4.309
Arg 12	4.223	4.223	4.243	4.248	4.259	4.274	4.272	4.286	4.286	4.296	4.303	4.307	4.313
Tyr 13	4.54	4.539	4.556	4.558	4.566	4.573	4.57	4.579	4.579	4.579	4.58	4.581	4.579
Leu 14	4.312	4.31	4.308	4.305	4.303	4.301	4.301	4.298	4.3	4.296	4.292	4.295	4.29
Val 15	4.031	4.031	4.036	4.037	4.044	4.049	4.048	4.056	4.055	4.06	4.062	4.07	4.071
Asn 16	4.692	4.69	4.689	4.69	4.69	4.692	4.692	4.691	4.693	4.689	4.691	4.684	4.687
Lys 17	4.215	4.214	4.22	4.22	4.226	4.23	4.227	4.236	4.236	4.238	4.242	4.249	4.247

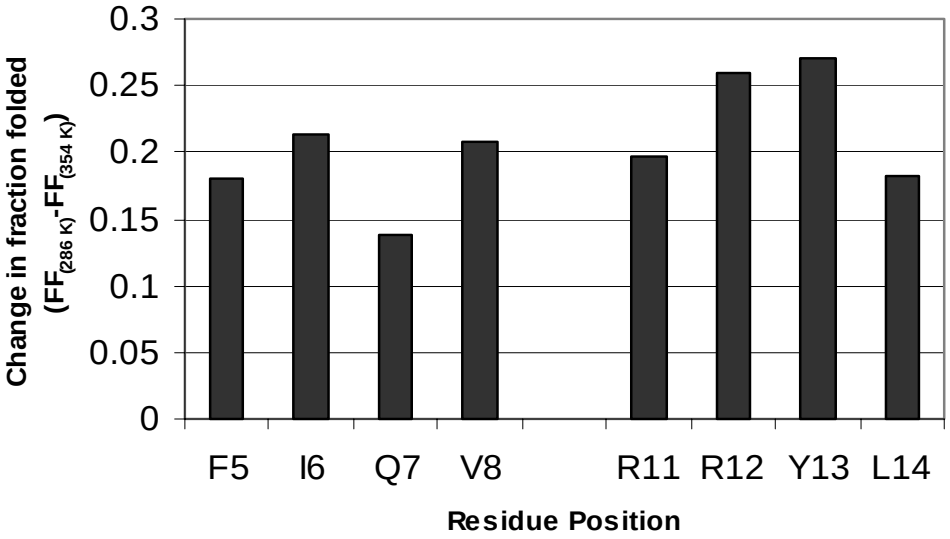

Figure A3: β-Sheet population change between 287 K and 354 K for **1** measured at core residues

Van't Hoff Analysis:

Non-linear regression fitting of the temperature dependence of the folded population of 1 to an expression of free energy in terms of entropy, enthalpy and heat capacity.

Thermodynamic analysis of folding was performed on **1** (using **2** and **3** as references for the fully folded and fully unfolded state) as described in Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**; *120*, 1996. The sum of the α proton chemical shifts from the folded "core" region of **1** (residues $F_5I_6Q_7V_8$ $R_{11}R_{12}Y_{13}L_{14}$) and the sums of the α proton chemical shifts of the same residues in **2** and **3** were used to calculate a folded populations for **1** at temperatures from 275 to 354 K according to top equation in figure A4. The chemical shifts of **1** and **2** were measured at each temperature; for the 100% reference peptide **3**, data obtained at 287 K in 50% (v/v) trifluoroethanol were used. The estimated error in the determined populations due to the estimated error in chemical shift determinations of ± 0.01 ppm is ±0.01 (i.e., populations are reported as fractions folded, from 0 to 1.00, with an error of ±0.01). The estimated error in the determination of temperatures is approximately ± 1 K. The error in the population due to this error in temperature is expected to be less than the error due to chemical shift measurement because changes in temperature of 1 K generally introduced chemical shift changes that were less than 0.01 ppm. The temperature dependence of the folded population was fit to the bottom equation in Figure A4 using non-linear regression with the program Sigma Plot. The error in the thermodynamic parameters reported below is derived from the fitting.

The effect that errors in the determination of the extent of folding in the "100%" reference peptide have on the thermodynamic parameters calculated is described below. Qualitatively, if the fully folded reference peptide is less than 100% folded, but is assumed to be 100% folded for the calculations, then the thermodynamic parameters obtained would be larger than the true values. This is due to an underestimation of the true limiting value, which leads to an apparent increase in the calculated folded populations and an increase in the extent of unfolding observed over the temperature range. Figure A5 and Table A9 show fits and derived thermodynamic parameters for the van't Hoff analysis of **1** using **2** (in aq solution) and **3** (in 50% TFE at 287 K) assuming that peptide **3** is either 80%, 90%, or 100% folded. The curve in blue shows the data and fit if **3** is really 100% folded. The curve in red shows the corrected data and fit if the fully folded reference is really only 90% folded (made by multiplying the limiting value by 10/9). The curve in black shows the corrected data and fit if the fully folded reference is really only 80% folded (made by multiplying the limiting value by 10/8). The values obtained from these fits are all similar.

The effect of using different subsets of residues to determine folded population and thermodynamic parameters is shown in Figure A6 and Table A10. The determined thermodynamic values and trends are qualitatively similar in every case.

$$\text{Population} = \frac{\sum \Delta\delta \mathrm{H}_{\alpha}(\mathbf{1}) - \sum \Delta\delta \mathrm{H}_{\alpha}(\mathbf{2})}{\sum \Delta\delta \mathrm{H}_{\alpha}(\mathbf{3}) - \sum \Delta\delta \mathrm{H}_{\alpha}(\mathbf{2})}$$

$$= \frac{e^{((T(\Delta S^{o}_{298} + \Delta C^{o}_{p} ln(T/298)) - (\Delta H^{o}_{298} + \Delta C^{o}_{p} ln(T - 298))/RT)}}{\left(1 + e^{((T(\Delta S^{o}_{298} + \Delta C^{o}_{p} ln(T/298)) - (\Delta H^{o}_{298} + \Delta C^{o}_{p} ln(T - 298))/RT)}\right.}$$

Figure A4: Van't Hoff equation used to determine thermodynamic parameters of folding based upon population change with temperature. Adapted from Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* 1998, *120*, 1996-2007. Non-linear regression fitting was performed using the program sigma plot.

Figure A5:

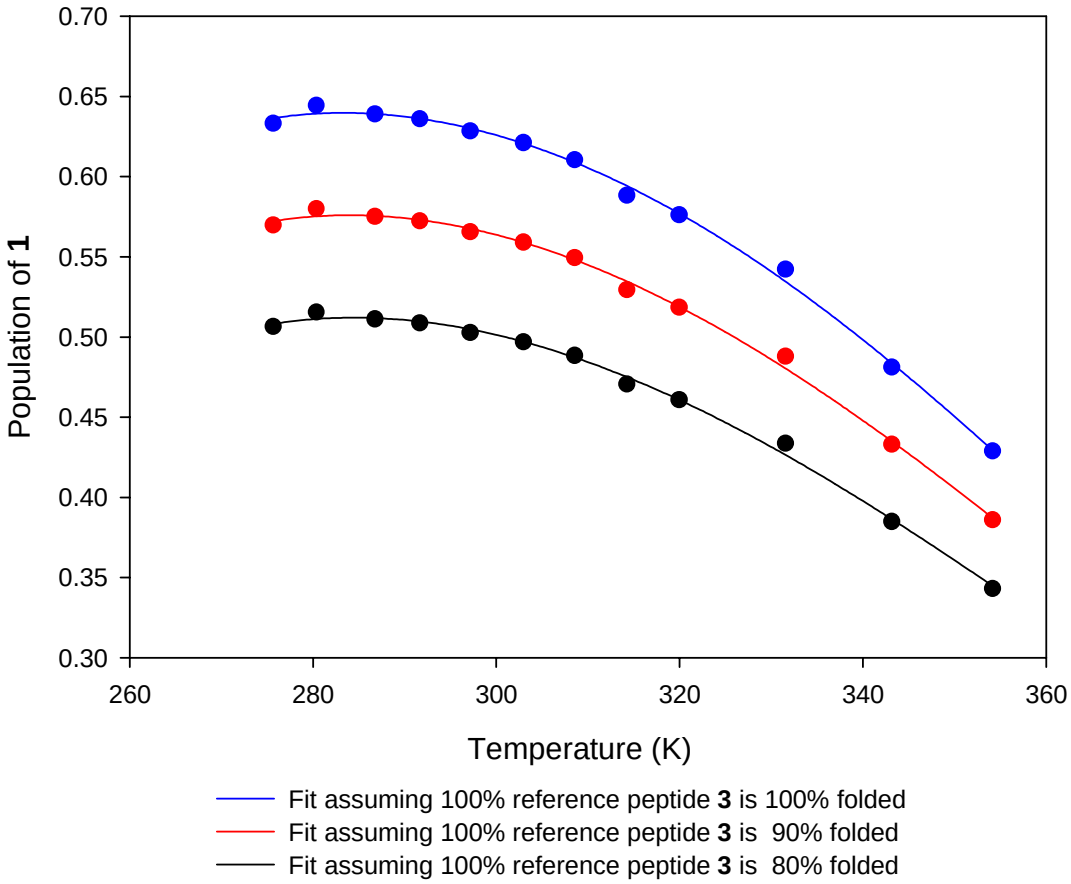

Table A9: Thermodynamic parameters obtained from the fits in figure A5

	Calculated thermodynamic parameters assuming reference peptide 3 is 100% folded (Fit reported in text)	Calculated thermodynamic parameters assuming reference peptide **3** is 90% folded	Calculated thermodynamic parameters assuming reference peptide **3** is 80% folded
ΔH (kcal mol^{-1})	- 1.1	- 0.9	- 0.8
ΔS (cal mol^{-1} ^{o}K^{-1})	- 2.6	- 2.6	- 2.7
ΔC_p(cal mol^{-1})	- 73	- 67	- 61
Initial fraction folded	0.63	0.57	0.51
Δ fraction folded from 276 to 354 ^{o}K	- 0.20	-0.18	-0.16

Figure A6:

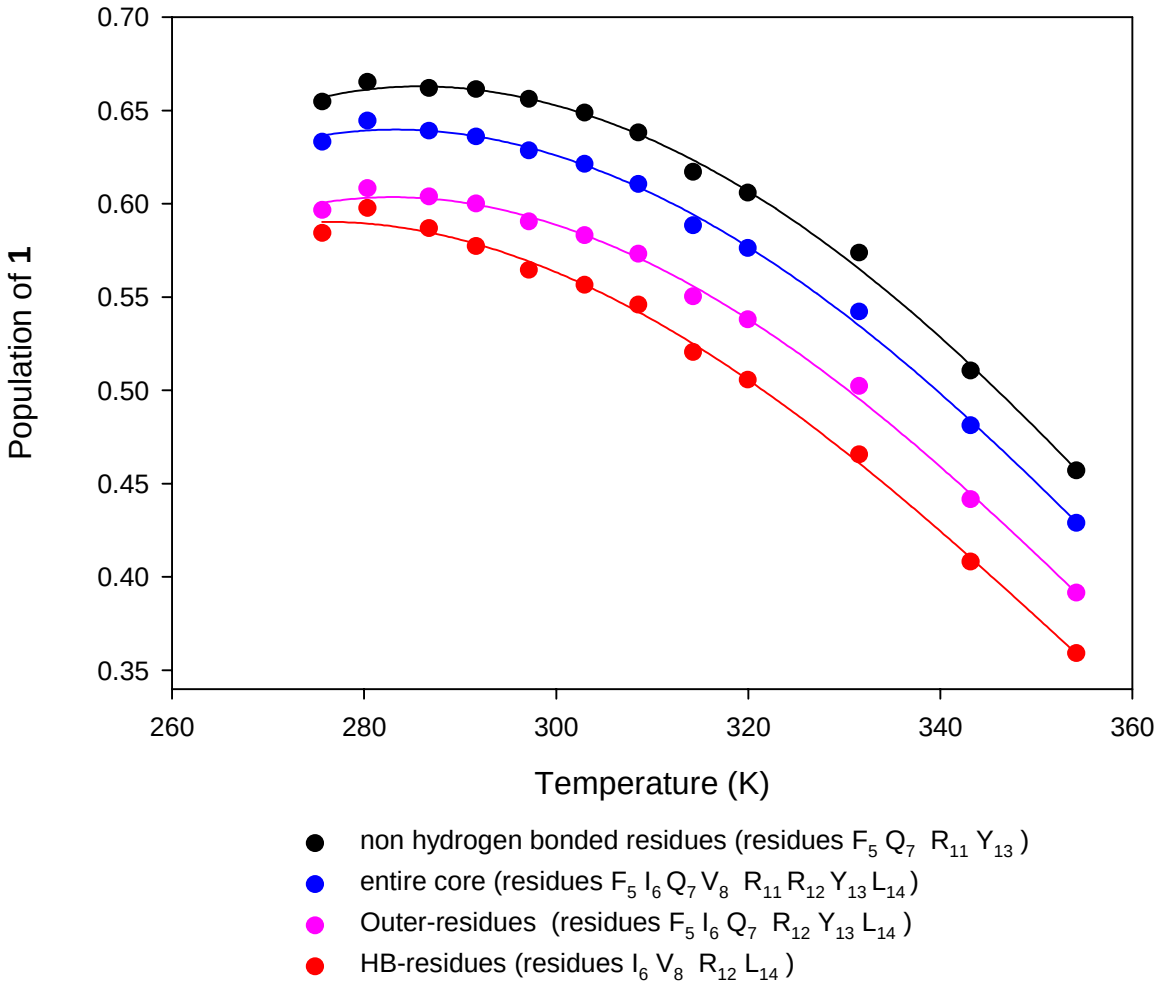

Table A10: Derived thermodynamic parameters for peptide **1** using subsets of residues in the calculations .

Subset of residue used in fitting procedure	ΔH^{o}_{298} kcal /mol	ΔS^{o}_{298} cal /mol K	$C_p{}^{o}_{298}$ cal /mol K
Entire Core (residues $F_5 I_6 Q_7 V_8$ $R_{11} R_{12} Y_{13} L_{14}$)	**-1.1 ± 0.1**	**-2.5 ± 0.2**	**-73 ± 2**
Non hydrogen bonded residues (residues $F_5 Q_7$ $R_{11} Y_{13}$)	**-1.0 ± 0.1**	**-1.9 ± 0.2**	**-78 ± 2**
Hydrogen bonded residues (residues $I_6 V_8$ $R_{12} L_{14}$)	**-1.4 ± 0.1**	**-4.3 ± 0.2**	**-66 ± 2**
Omitting residues adjacent to ^{D}Pro-dadme turn (residues $F_5 I_6 Q_7$ $R_{12} Y_{13} L_{14}$; entire core omitting residues V_8, R_{11})	**-1.1 ± 0.1**	**-2.9 ± 0.2**	**-73 ± 2**