Supplementary Material

Concerted evolution of structure and function in a miniature protein

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NMR Spectroscopy. p007 was dissolved at a concentration of approximately 1.5 mM in 90%H_20/10%D_20 containing 4 mM KCl, 205 mM NaCl, 6.5 mM Na_2HPO_4, 2.1 mM KH_2PO_4 (pH 7.4). Chemical shifts were referenced in ppm from the internal standard 3- (trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt.

All spectra were recorded on a Varian 800 MHz Inova instrument at 2 ˚C with a sweep width of 9000 Hz. NOESY experiments were performed using a waterflip-watergate pulse sequence[***1***] for water suppression with 4096t2 x 500 t1 complex points. Data was acquired at mixing times of 50, 150 and 300 ms. DQF-COSY spectra (with a 60 ms mixing time) were acquired with 2048t2 x 300t1 complex points.

Data was processing was performed on a Silicon Graphics Workstation using Felix 98 (MSI Inc.). Prior to Fourier transform of the free induction decays, a Gaussian window function[***2***] was applied to NOESY spectra, while a Kaiser window function[***2***] was applied to DQF-COSY spectra. The digital resolution of the NOESY spectra was 2.2 Hz/pt. DQF COSY data was zero filled to yield a 8192 x 8192 matrix with a digital resolution of 1.1 Hz/pt. Spectra were assigned by standard methods[***3***].

Table S1. 1H-NMR assignments for p007

Residue	NH	CαH	CβH	other
Gly 1				
Gly 2	8.61	4.09		
Ser 3	8.59	4.51	3.88, 3.91	
Arg 4	8.59	4.39	1.67,1.80,1.9[a]	CδH 3.23, NεH 7.29
Ala 5	8.52	4.34	1.41	
Thr 6	8.27	4.32	4.18	CγH 1.21
Met 7	8.62	4.82	1.97	CγH 2.56, 2.67 CεH 2.08
Pro 8		4.41	1.93	CγH 2.01, 2.07, 2.33[a] CδH 3.68, 3.83
Gly 9	8.69	3.99		
Asp 10	8.29	4.59	2.75, 2.67	
Asp 11	8.48	4.61	2.61, 2.69	
Ala 12	8.16	4.56	1.38	
Pro 13		4.48	1.93	CγH 2.03, 2.09, 2.32[a] CδH 3.66, 3.81
Val 14	8.45	4.01	2.09	CγH 0.97, 0.99
Glu 15	8.72	4.23	1.97, 2.03	CγH 2.26, 2,29

Residue	NH	CαH	CβH	other
Asp 16	8.41	4.59	2.65, 2.74	
Leu 17	8.31	4.28	1.66, 1.75[a]	CδH 0.89, 0.96
Lys 18	8.32	4.14	1.60, 1.70, 1.86[b]	CγH 1.39, 1.49 CεH 3.0
Arg 19	8.10	4.19	1.81, 1.91[a]	CδH 3.17 NεH 7.36
Phe 20	8.26	4.57	3.14, 3.22	CδH 7.29 CεH 7.37
Arg 21	8.37	4.15	1.66, 1.77, 1.88[a]	CδH 3.22 NεH 7.29
Asn 22	8.45	4.79	2.86 2.96	NH_2 7.81
Thr 23	8.02	4.48	4.3	CγH 1.31
Leu 24	8.41	4.07	1.56, 1.66[a]	CδH 0.88, 0.92
Ala 25	8.37	4.11	1.47	
Ala 26	8.03	4.17	1.46	
Arg 27	8.31	4.05	1.69, 1.85, 1.97[a]	CδH 3.17 NεH 7.19
Arg 28	8.52	4.21	1.66, 1.84, 1.92[a]	NεH 7.33
Ser 29	8.25	4.29	4.02	
Arg 30	8.25	4.15	1.65, 1.9, 1.97[a]	CδH 3.32 NεH 7.42
Ala 31	8.13	4.28	1.53	
Arg 32	8.28	4.21	1.65, 1.83, 1.95[a]	NεH 7.41

Residue	NH	CαH	CβH	other
Lys 33	8.18	4.10	1.60, 1.71, 1.85, 1.92[b]	CγH 1.45 CεH 3.01
Ala 34	8.20	4.26	1.49	
Ala 35			1.5	
Arg 36			1.66, 1.78, 1.88[a]	
Ala 37	8.12	4.26	1.48	
Ala 38	8.07	4.25	1.47	
Ala 39	7.93	4.25	1.47	

a) β or γ proton, b) β or δ proton.

Table S2. Long range (***i***, ***i+5*** and longer) NOEs observed in p007.

G2 CαH R30 CδH

G2 CαH S29 CβH

R4 CδH R27 CαH

A5 CβH F20 CεH

M7 NH L17 CδH

M7 CβH F20 CδH

M7 CβH F20 CεH

M7 CεH F20 CδH

M7 CεH F20 CεH

P8 CδH 17 CδH

P8 CδH 20 CεH

Figure S1. The alpha-amide region of p007 in a 300 ms NOESY spectrum.

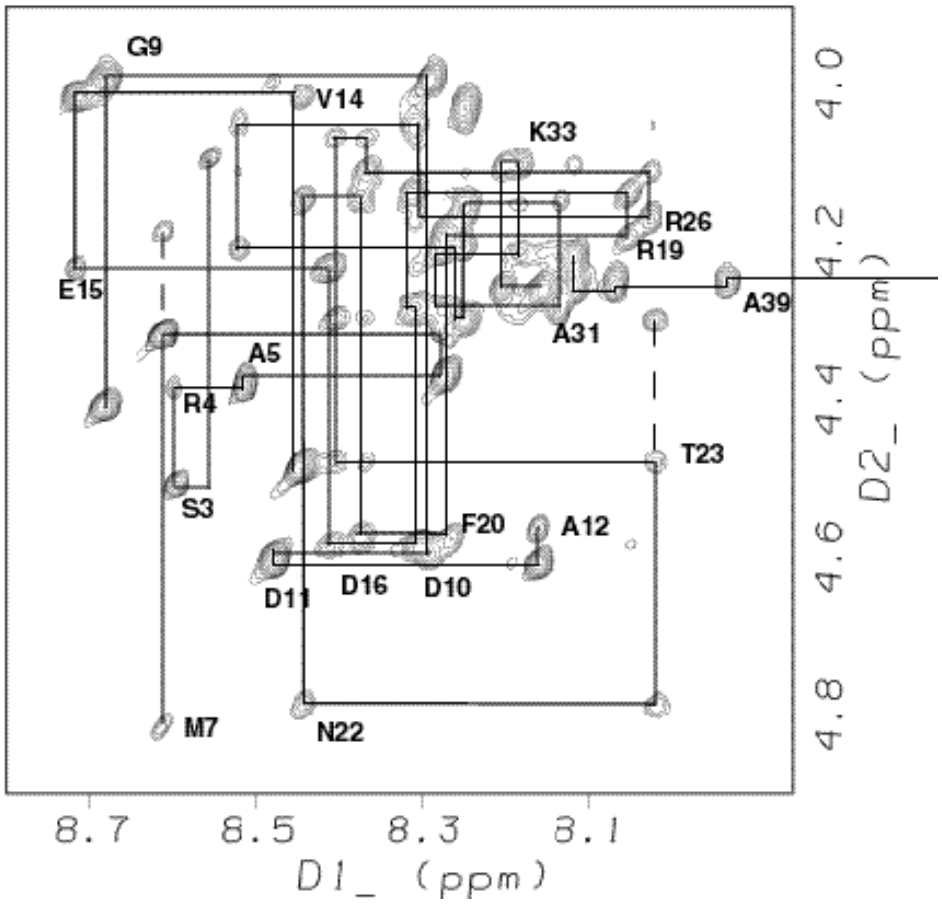


Figure S2. The amide-amide region of p007 in a 300 ms NOESY spectrum.

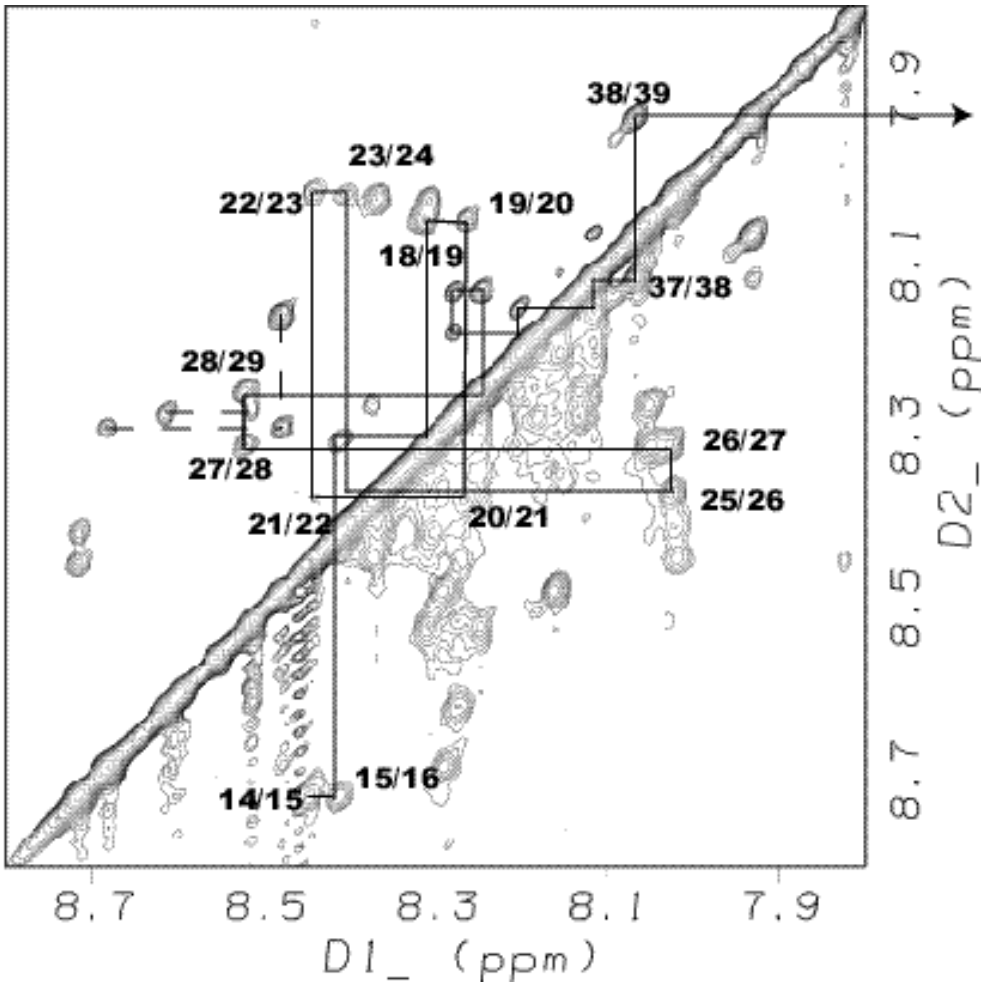


Figure S3. A summary of short and medium range NOEs for p007. A bar indicates NOE connectivity between protons on different residues. For the αN, NN or βN the height of the bar indicates the classification of the NOE as strong medium or weak.

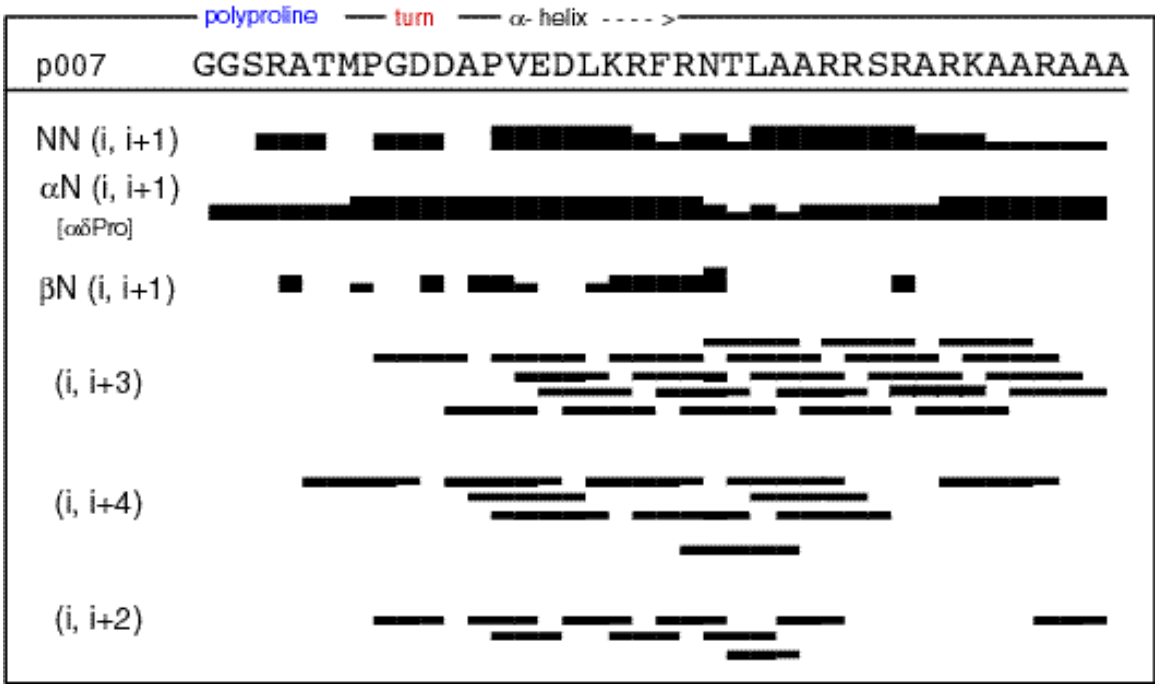
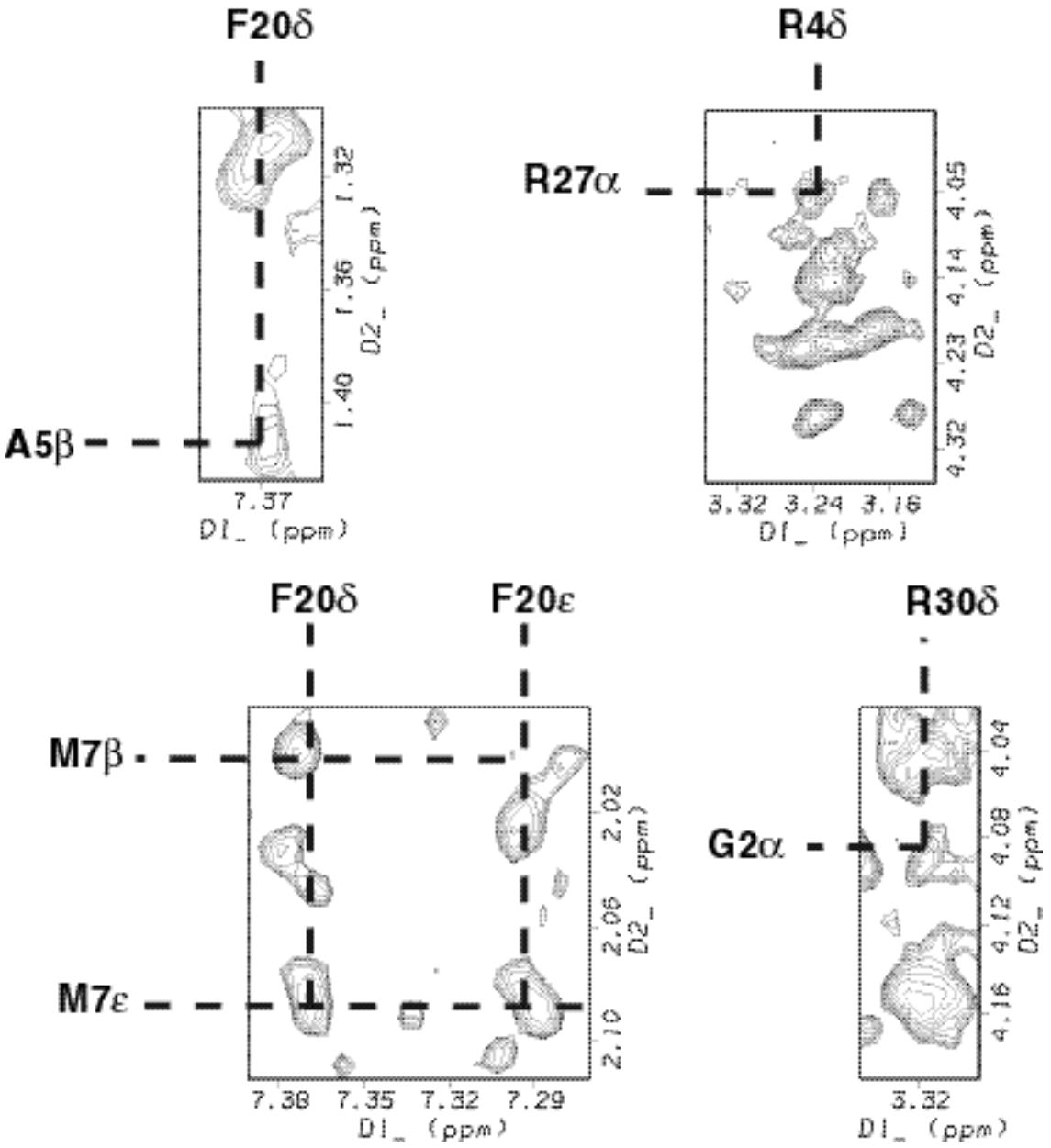


Figure S4. Long range NOEs observed for p007 in a 300 ms NOESY.



References:

1. Piotto, M., Saudek, V., and Sklenar, V. (1992) ***Journal of Biomolecular NMR 2***, 661-665.
2. Canvanagh, J., Fairbrother, W. J., Palmer, A. G., and Skelton, N. J. (1996) ***Protein NMR spectroscopy***, Academic Press, New York.
3. Wüthrich, K. (1986) ***NMR of proteins and nucleic acids***, Wiley, New York.