Supporting Information for

"Redesigned WW Domain Peptide Employed as an OB-fold Mimic for Selective Recognition of ssDNA"

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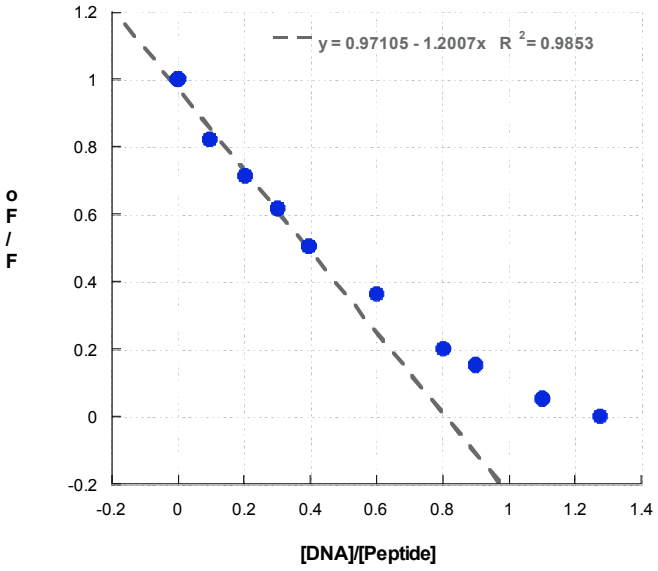


Figure S1. Molar variation plot for WW domain Mut1 (50 μM) binding to ssDNA sequence 5'-CCATCGCTACC-3' (Figure 3m; 0-60 μM). A 1:1 binding interaction was demonstrated with the average of two runs in 10 mM Na_2HPO_4, 100 mM NaCl, pH 7.0.

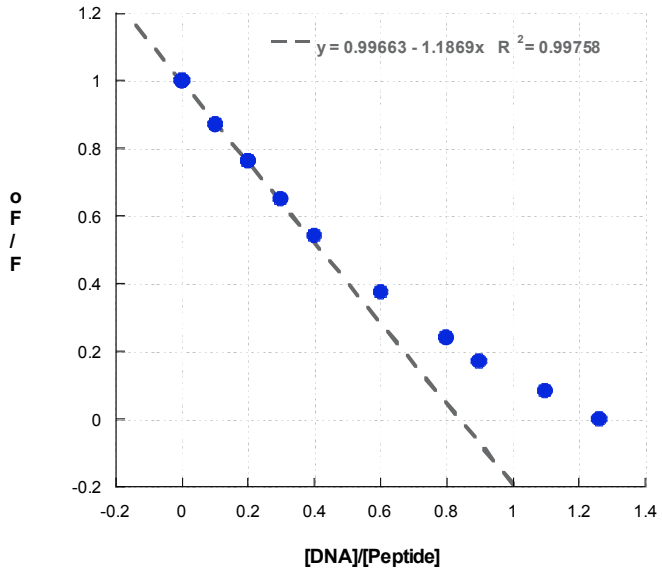
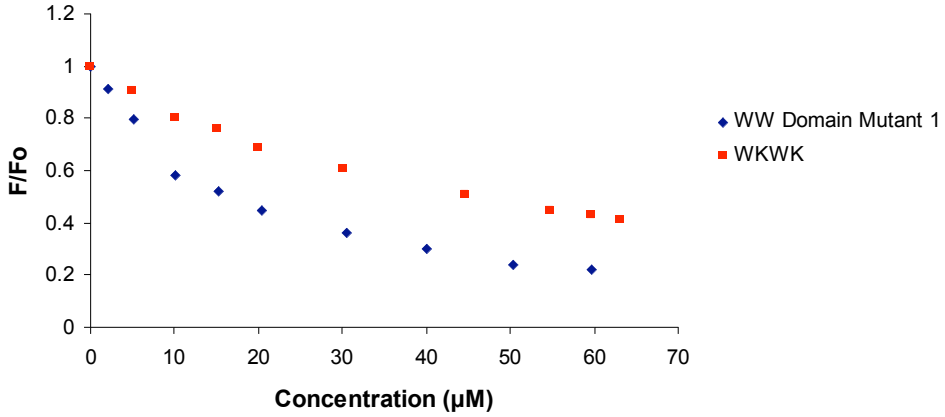


Figure S2. Molar variation plot for WW domain Mutant 1 Strands 2&3 (50 µM) binding to ssDNA sequence 5'-CCATCGCTACC-3' (Figure 3m; 0-60 µM). A 1:1 binding interaction was demonstrated with the average of two runs in 10 mM Na_2HPO_4, 100 mM NaCl, pH 7.0.



FigureS3. Fluorescence titrations of WW domain Mutant 1 and WKWK with single-stranded DNA sequence 5'-CCATCGCTACC-3' (Figure 3m) corrected for the inner filter effect (see Experimental Section); 3-5 µM peptide, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25 °C.

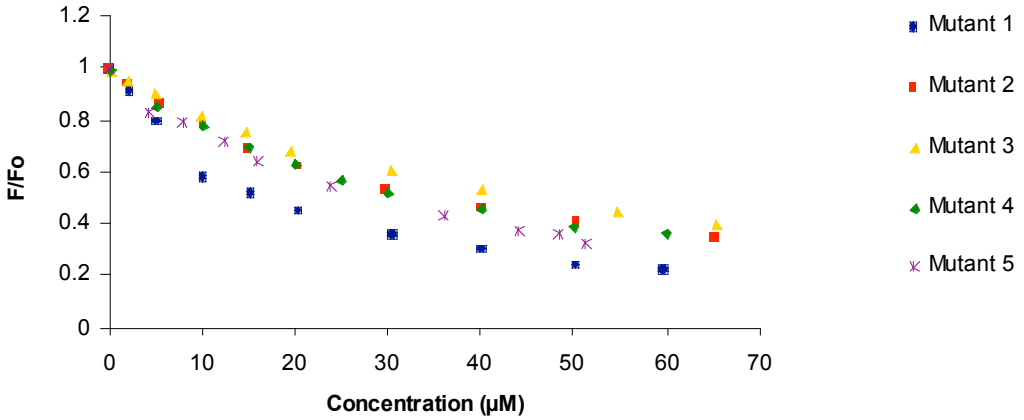


Figure S4. Fluorescence titrations of WW domain mutants with single-stranded DNA sequence 5'-CCATCGCTACC-3' (Figure 3m) corrected for the inner filter effect (see Experimental Section); 5-8 μM peptide, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25 °C.

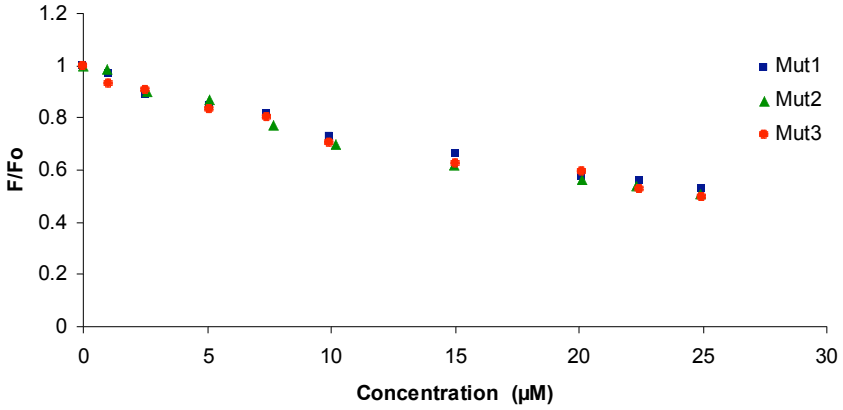


Figure S5. Fluorescence titrations of WW domain mutants with duplex DNA sequence (Figure 3n) corrected for the inner filter effect (see Experimental Section); 5-8 μM peptide, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25 °C.

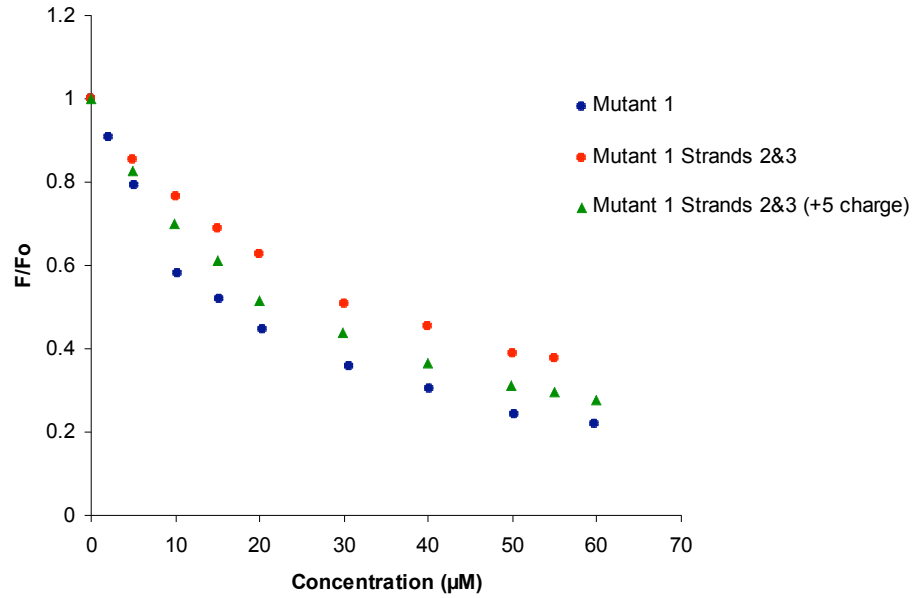


Figure S6. Fluorescence titrations of WW domain Mutant 1, Mut1-S23, and Mut1-S23 (+5 charge) with single-stranded DNA sequence 5'-CCATCGCTACC-3' (Figure 3m) corrected for the inner filter effect (see Experimental Section); 5 μM peptide, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25 °C.

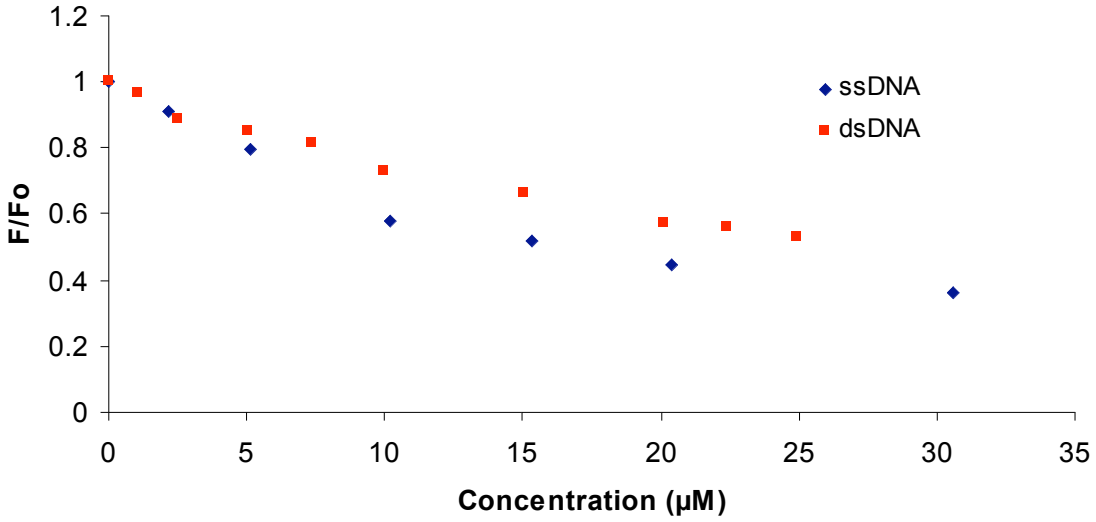


Figure S7. Fluorescence titrations of WW domain Mutant 1 with ssDNA (Figure 3m) and duplex DNA (Figure 3n) by the Trp quenching method, corrected for the inner filter effect (see Experimental Section); 5 μM peptide, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25 °C.

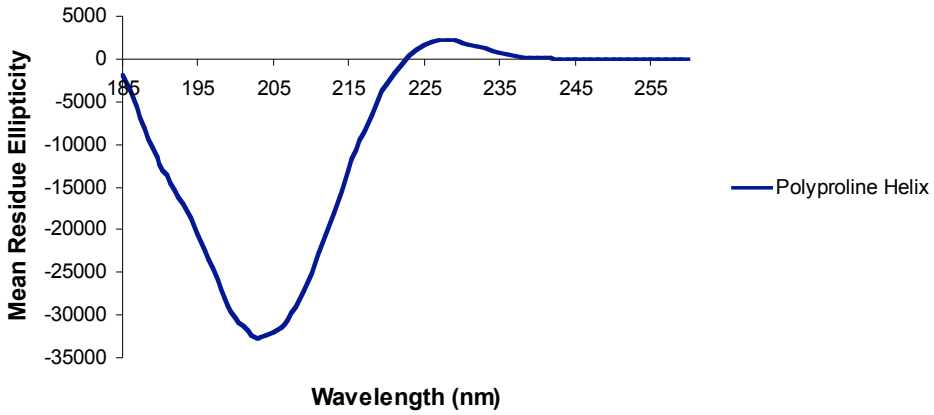


Figure S8. CD spectrum for polyproline helix. Conditions: 75 µM, pH 7, 25ºC.

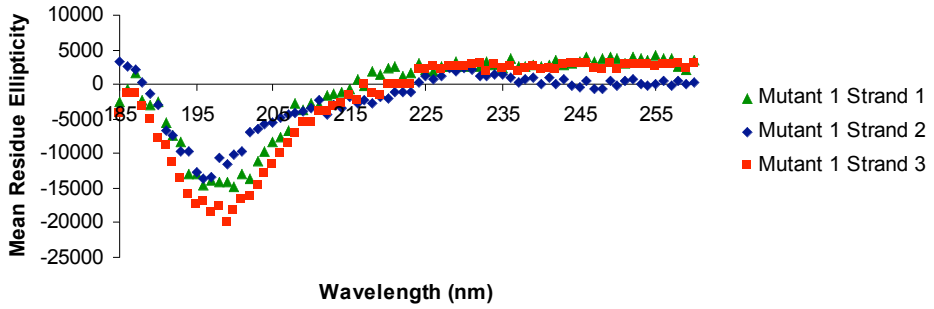


Figure S9. CD spectra for control peptides Mut1-S1, Mut1-S2, and Mut1-S3. Conditions: 30 µM, pH 7, 25ºC.

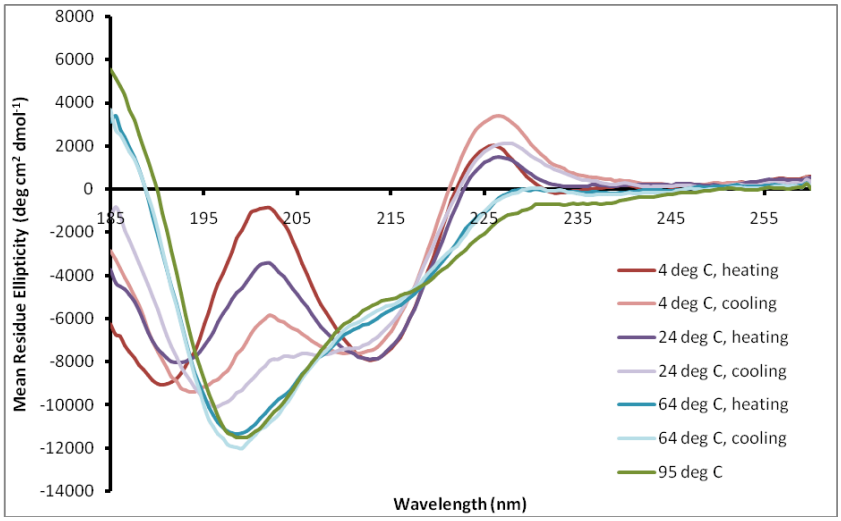


Figure S10. Thermal denaturation and renaturation CD spectra of Mut1 at 4, 24, 64, 95°C. Data were recorded at 30 µM peptide in 10 mM Na_2HPO_4, pH 7.0.

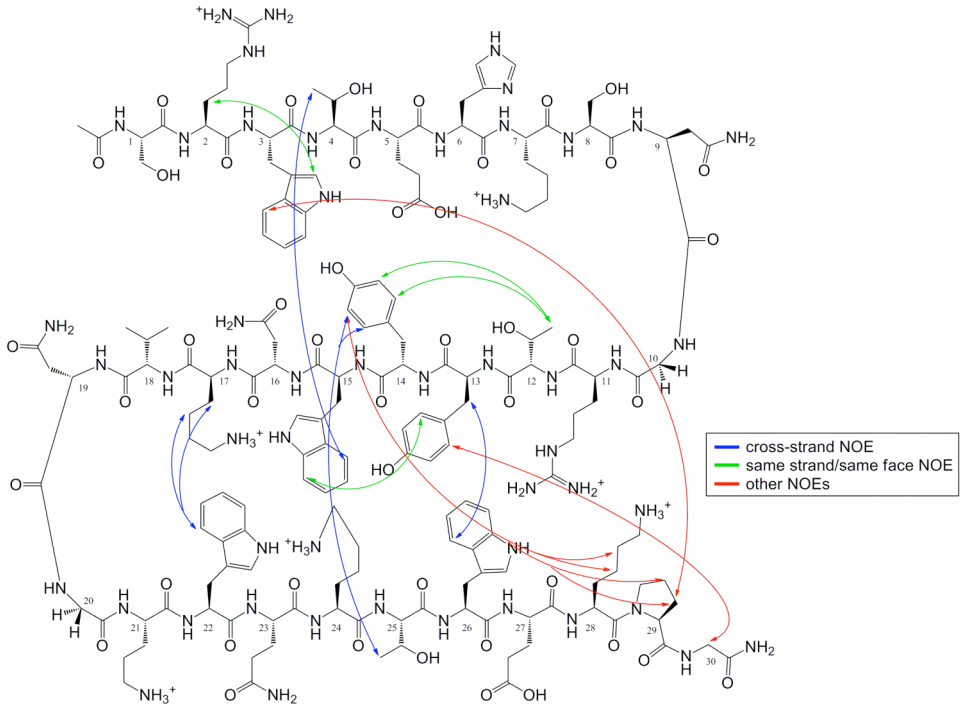
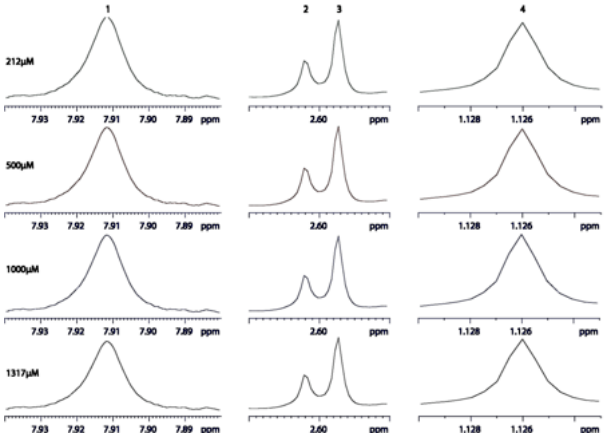


Figure S11. Long-range NOEs for Mut 1.



	Peak Width, Hz			
[Mut1], μM	**Peak 1 (7.912 ppm)**	**Peak 2 (2.602 ppm)**	**Peak 3 (2.597 ppm)**	**Peak 4 (1.126 ppm)**
212	6.30	1.14	0.96	0.93
500	6.24	1.14	0.96	0.93
1000	6.24	1.14	0.96	0.93
1317	6.24	1.14	0.96	0.93

Figure S12. Peak widths for representative peaks at 212 μM, 500 μM, 1000 μM, and 1317 μM.

Table S1. Proton chemical shifts (in ppm) of WW Domain Mut 1 in 50 mM KD_2PO_4 buffer, pH 7.0

Residue	α	β	γ	δ	ε
Ser	4.4315	3.821			
Arg	4.3218	1.9763,1.8866	1.5974	2.1359	
Trp	4.6857	3.3025, 3.237			
Thr	4.4265	2.7541			
Glu	4.3660	1.9265	2.2655		
His	4.6010	3.2726			
Lys	4.3000	1.5500	1.1500	1.2900	2.9100
Ser	4.4813	3.8908			
Asn	4.6209	2.9335, 2.5945			
Gly	0.26*				
Arg	4.6807	1.9265	1.721	2.9136	
Thr	4.6059	4.0303	0.9992		
Tyr	4.6558	2.9535, 2.8637			
Tyr	4.9400	3.3424, 2.7940			
Trp	4.9300	3.3424, 3.2327			
Asn	5.2192	2.7541, 2.5646			
Lys	4.5500	1.8200		1.5900	
Val	4.0825	1.8866	0.8795		
Asn	4.4215	3.0233, 2.7451			
Gly	0.68*				
Orn	4.6458	1.757, 1.6174	1.3482	2.8737	
Trp	4.9450	3.3922, 3.1330			
Gln	4.9101	2.2057, 1.9963	2.3851		
Lys	4.1500	1.660	0.735	0.475	
Thr	4.5361	3.9705	1.1886		
Trp	4.8602	3.1728, 3.0632			
Glu	4.2919	1.7869	2.1758		
Lys	4.2700	1.7830	0.4500	0.1600	2.182
Pro	4.3966	2.1857	1.9664, 1.8567		
Gly	3.8900				

*Glycine splitting values, not chemical shifts

Table S2. Alpha proton chemical shift assignments (in ppm) for random coil peptide Strand 1 Ac-Ser-Arg-Trp-Thr-Glu-His-Lys-Ser-Asn-Gly-NH_2

Residue	α
Ser	4.38
Arg	4.28
Trp	4.72
Thr	4.19
Glu	4.12
His	4.62
Lys	4.32
Ser	4.42
Asn	4.72
Gly	3.89

Table S3. Alpha proton chemical shift assignments (in ppm) for random coil peptide Strand 2 Ac-Gly-Arg-Thr-Tyr-Tyr-Trp-Asn-Lys-Val-Asn-Gly-NH_2[a]

Residue	α
Gly	3.85
Arg	4.33
Thr	4.20
Tyr	4.40
Tyr	4.51
Trp	4.51
Asn	4.66
Lys	4.15
Val	4.02
Asn	4.40
Gly	3.91

[a]Tyrosine residues could not be definitively distinguished.

Table S4. Alpha proton chemical shift assignments (in ppm) for random coil peptide Strand 3 Ac-Asn-Gly-Orn-Trp-Gln-Lys-Thr-Trp-Glu-Lys-Pro-Gly-NH_2

Residue	α
Asn	4.65
Gly	3.91
Orn	4.37
Trp	4.65
Gln	4.20
Lys	4.27
Thr	4.20
Trp	4.60
Glu	4.14
Lys	4.08
Pro	4.37
Gly	3.86

Table S5. Alpha proton chemical shift assignments (in ppm) for Mut1-S12 Ac-Ser-Arg-Trp-Thr-Glu-His-Lys-Ser-Asn-Gly-Arg-Thr-Tyr-Tyr-Trp-Asn-Lys-Val-NH_2

Residue	α	β	γ	δ	ε
Ser	4.37	3.76			
Arg	4.30	1.57	1.37	3.00	
Trp	4.60	3.18			
Thr	4.21	4.00	1.03		
Glu	4.19	1.89	2.14		
His	4.63	3.02			
Lys	4.39	1.72	1.33	1.63	2.90
Ser	4.43	3.84			
Asn	4.51	2.84			
Gly	3.91				
Arg	4.37	1.70, 1.55	1.33	3.06	
Thr	4.26	4.01	1.00		
Tyr	4.47	2.66			
Tyr	4.57	2.51			
Trp	4.76	3.21			
Asn	4.69	2.84			
Lys	4.16	2.13	1.29	1.59	2.89
Val	4.05	2.02	0.92		

Table S6. Alpha proton chemical shift assignments (in ppm) for Mut1 S23: Ac-Gly-Arg-Thr-Tyr-Tyr-Trp-Asn-Lys-Val-Asn-Gly-Orn-Trp-Gln-Lys-Thr-Trp-Glu-Lys-Pro-Gly-NH_2

Residue	α	β	γ	δ	ε
Gly	3.91				
Arg	4.43	2.21	1.92	3.01	
Thr	4.40	4.12	1.08		
Tyr	4.72	3.08			
Tyr	4.80	2.85			
Trp	4.97	3.38			
Asn	5.18	2.82, 2.61			
Lys	4.45	1.97	1.43	1.68	2.99
Val	4.10	1.91	0.90		
Asn	4.50	2.96, 2.76			
Gly	4.12				
Orn	4.71	1.93	1.75	3.08	
Trp	4.95	3.14			
Gln	4.95	2.19, 2.00	2.35		
Lys	4.43	1.98	1.70	1.89	2.76
Thr	4.52	3.90	1.16		
Trp	4.98	3.14			
Glu	4.28	1.89, 1.84	2.15		
Lys	4.10	1.68	1.33	1.70	3.10
Pro	4.39	2.27, 2.07	1.93	3.69, 3.50	
Gly	3.87				

Table S7. Alpha proton chemical shift assignments (in ppm) for random coil peptide Strands 1&2 Cyclic Ac-Cys-Arg-Trp-Thr-Glu-His-Lys-Ser-Asn-Gly-Arg-Thr-Tyr-Tyr-Trp-Asn-Lys-Cys-NH_2

Residue	α	β	γ	δ	ε
Cys	4.53	3.22, 2.56			
Arg	4.49	1.77	1.59, 1.48	3.14	
Trp	4.64	3.22, 2.94			
Thr	4.56	3.85	0.86		
Glu	4.41	1.91, 1.79	2.06		
His	4.75	2.96			
Lys	4.20	1.75	1.28, 1.24	1.54, 1.44	2.82
Ser	4.44	3.81			
Asn	4.42	2.86, 2.70			
Gly	4.14				
Arg	4.60	1.72	1.55	3.13	
Thr	4.57	3.96	1.08		
Tyr	4.97	2.74			
Tyr	4.67	2.43			
Trp	4.90	3.26, 3.17			
Asn	4.70	2.84, 2.72			
Lys	4.12	1.80	1.33	1.66, 1.62	2.91
Cys	4.57	2.76, 2.59			

Table S8. Alpha proton chemical shift assignments (in ppm) for random coil peptide Strands 2&3 Cyclic Ac-Cys-Thr-Tyr-Tyr-Trp-Asn-Lys-Val-Asn-Gly-Orn-Trp-Gln-Lys-Thr-Trp-Glu-Cys-NH_2

Residue	α	β	γ	δ	ε
Cys	5.30	3.31, 2.91			
Thr	4.54	4.25	1.14		
Tyr	4.71	2.90			
Tyr	4.95	3.11			
Trp	5.05	3.23			
Asn	5.31	2.91, 2.75			
Lys	4.41	0.91		0.40	2.31
Val	4.25	1.98	1.02		
Asn	4.54	3.15, 2.89			
Gly	4.28				
Orn	5.07	2.45, 2.30	2.11	3.02	
Trp	5.09	3.03			
Gln	5.46	2.07	2.45		
Lys	4.33	1.30		0.5	
Thr	4.71	3.86	1.32		
Trp	4.97	3.33			
Glu	4.69	1.80	2.00		
Cys	5.25	3.12, 2.58			