Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Results

Designed Multi-stranded Heme Binding β-Sheet Peptides in Membrane

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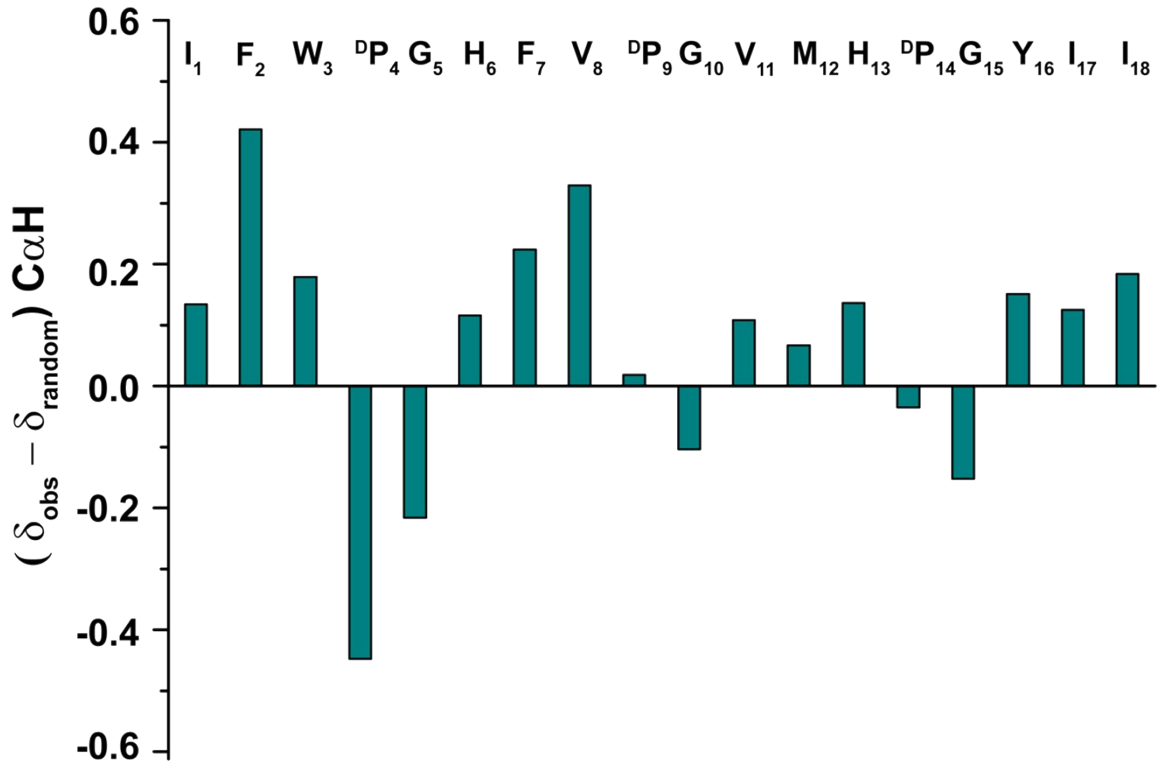
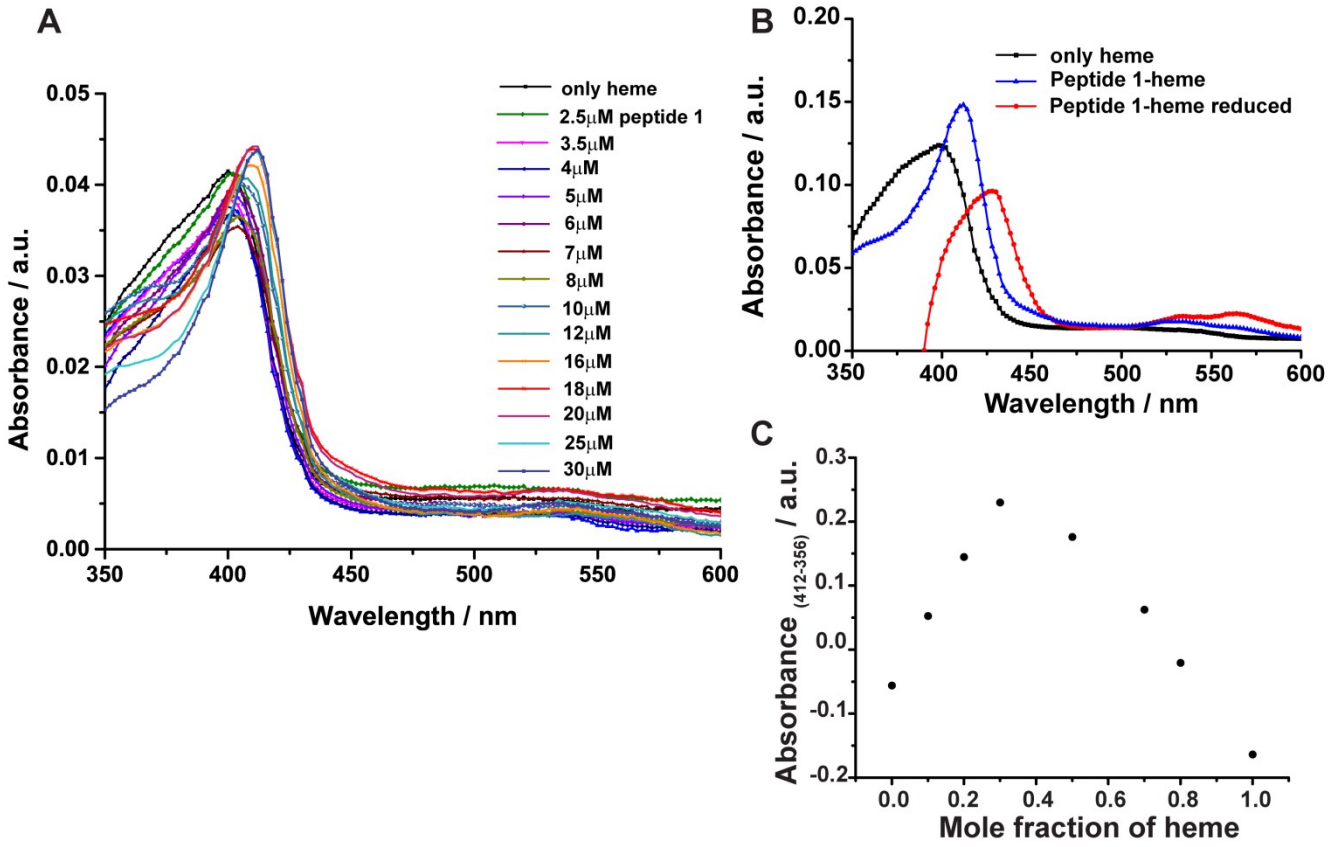
Figure S1



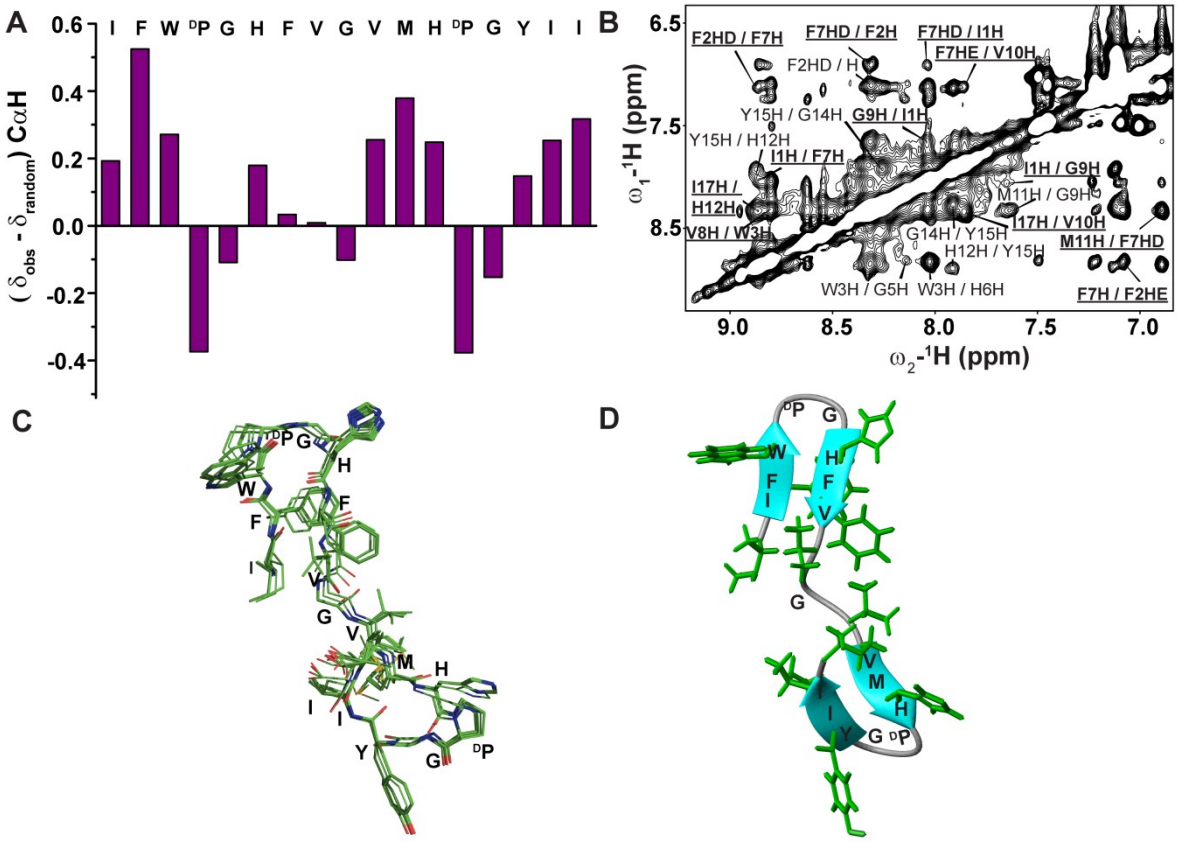
Supplementary Figure 1: αH chemical shift deviation from random coil values of peptide-1. The positive deviation of residues I1, F2, W3, H6, F7, V8, V11, M12, H13, Y16, I17 and I18 indicates four stranded β-sheet structure

Figure S2



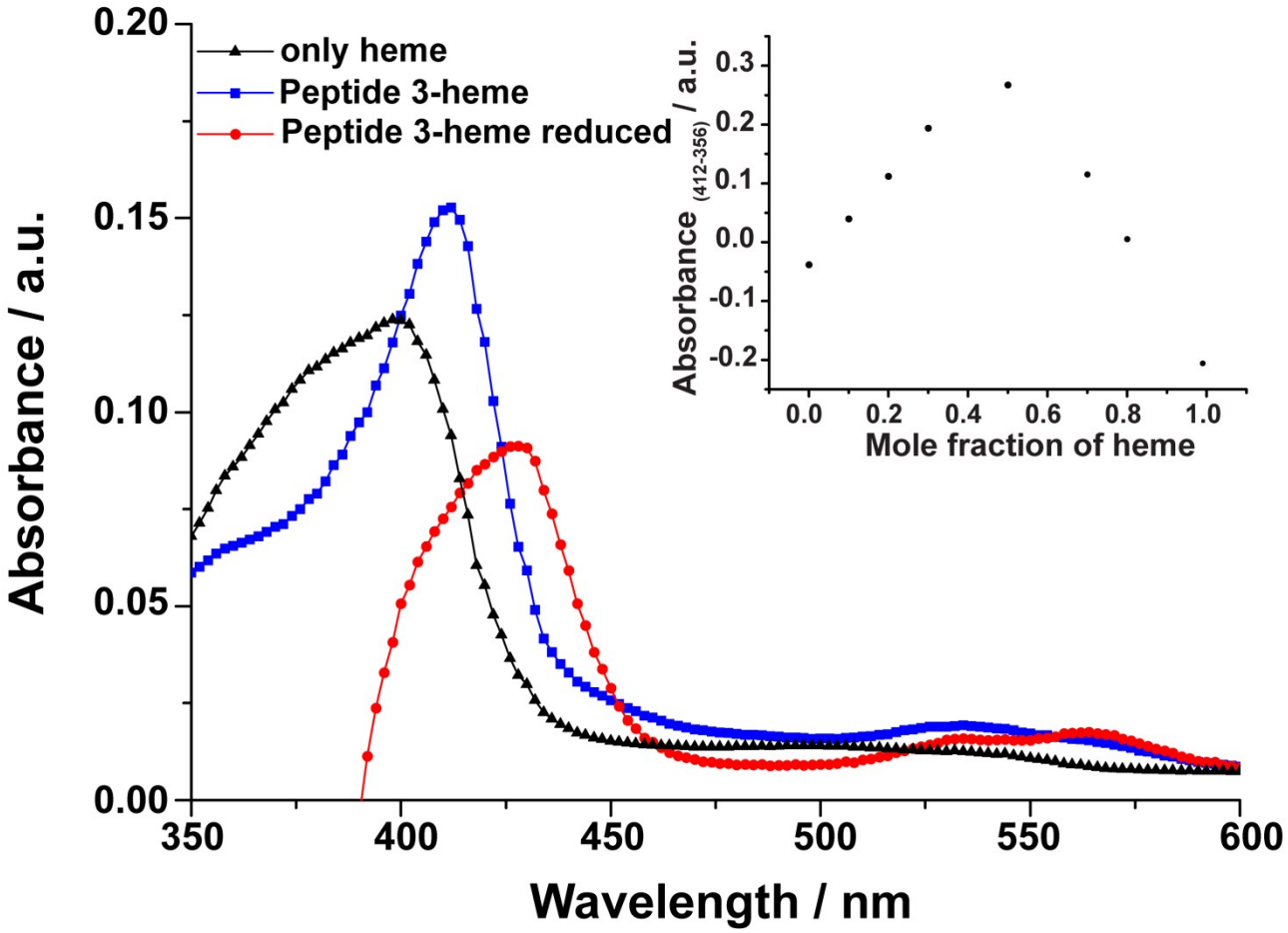
Supplementary Figure 2: (A) Absorption changes of heme (2 μM) on titrating peptide-1(0-30 μM) in 50 mM sodium phosphate buffer, pH 7.2 containing 2 mM DPC. (B) Absorption spectra of heme alone (black), peptide1-heme oxidized (blue) and peptide1-heme reduced (red). Concentration of peptide and heme was 10 μM in 2 mM DPC, 50 mM sodium phosphate buffer, pH 7.2 (C) Jobs plot of peptide-1. The shift in Soret band of heme at 412 nm upon binding to peptide-1 and further shift to 428 nm after reduction by sodium dithionite and low intense peaks at 530 and 560 nm indicated bis-histidine coordination. The job plot shows peptide:heme stoichiometry 2:1

Figure S3



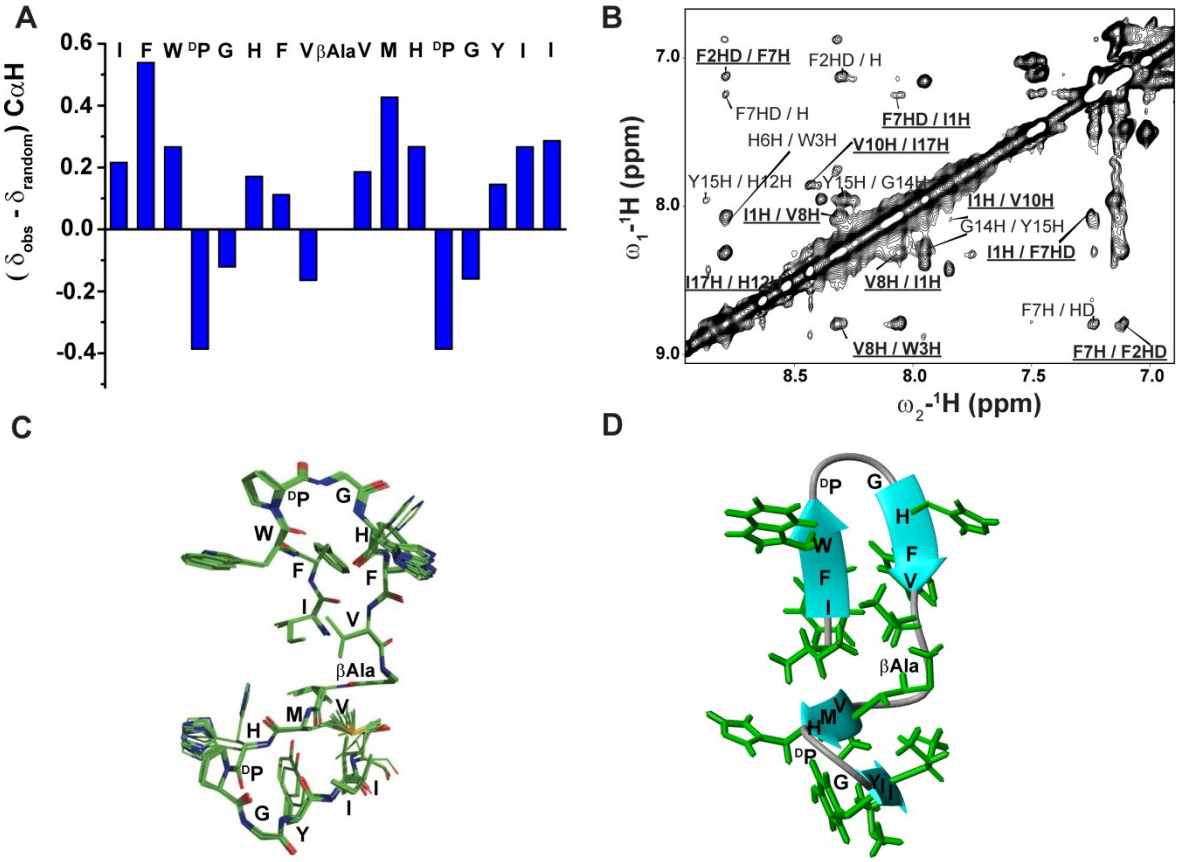
Supplementary Figure 3: (A) αH chemical shift deviation from random coil of peptide-2 (B) Section of two-dimensional ^{1}H-^{1}H NOESY spectra showing NOE connectivity between amide protons. Long range NOEs are underlined and boldfaced. (C) Superimposed twenty low energy structures of peptide-2. (D) A selected structure of peptide-2 showing side chain packing within β-sheets.

Figure S4



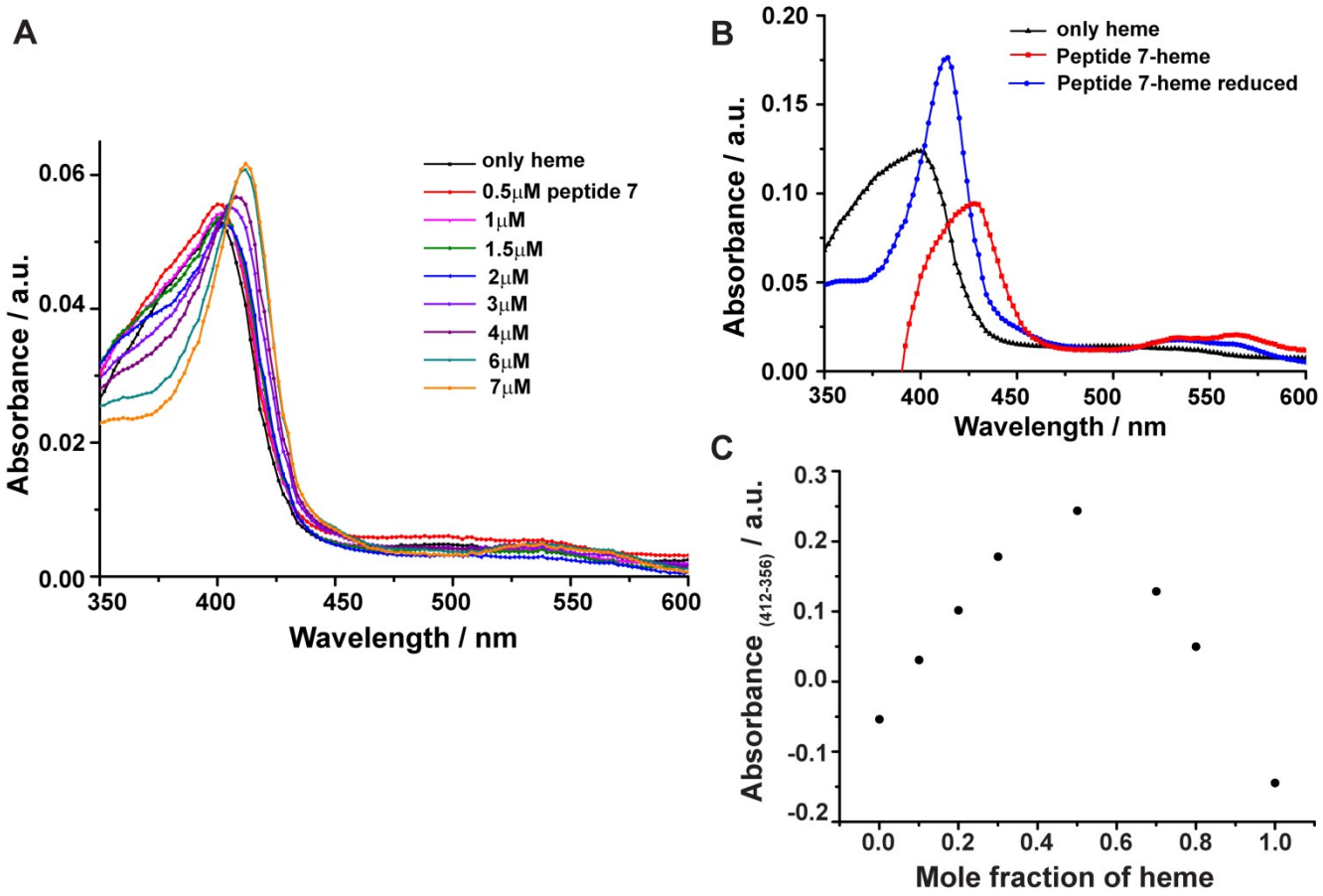
Supplementary Figure 4: Absorption spectra of heme alone (black), peptide 3-heme oxidized (blue) and peptide 3-heme reduced (red). Concentration of peptide and heme was 10 μM in 2 mM DPC, 50 mM sodium phosphate buffer, pH 7.2. Job's plot of peptide-3 (onset). The shift in Soret band of heme at 412 nm upon binding to peptide-3 and further shift to 428 nm after reduction by sodium dithionite and low intense peaks at 530 and 560 nm indicated bis-histidine coordination. The job plot shows peptide:heme stoichiometry 1:1.

Figure S5



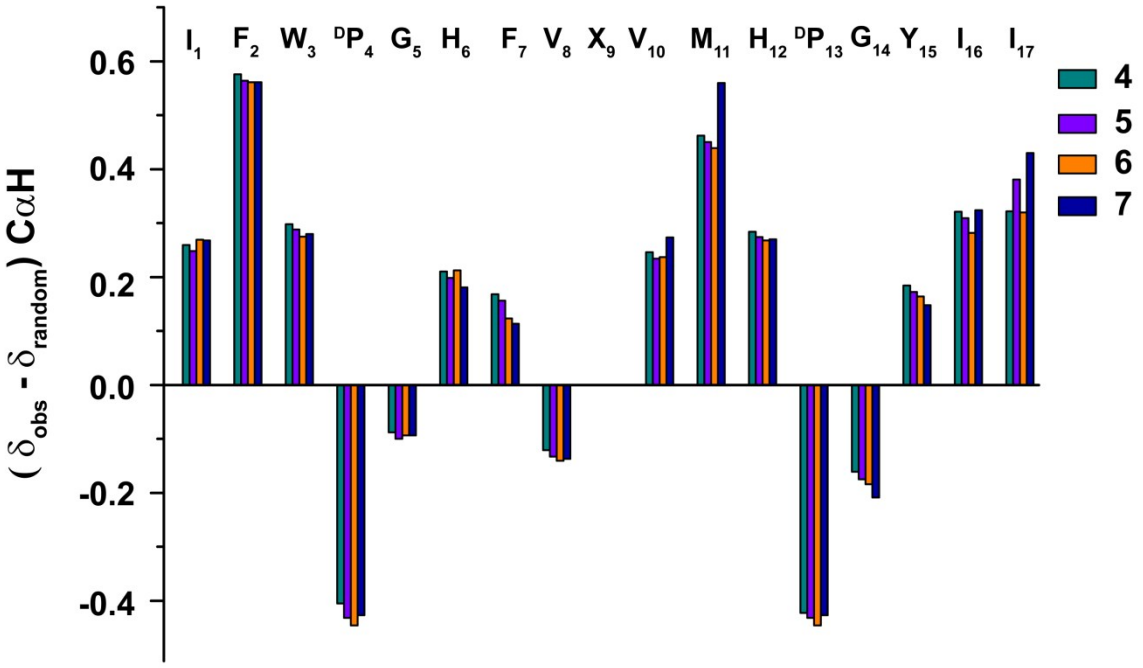
Supplementary Figure 5: (A) αH chemical shift deviation of peptide-3 (B) Section of two-dimensional ^{1}H-^{1}H NOESY spectra showing NOE connectivity between amide protons. Long range NOEs are underlined and boldfaced. (C) Superimposed twenty low energy conformers of peptide-3. (D) A selected structure of peptide-3 showing side chain packing within β-sheets.

Figure S6



Supplementary Figure 6: (A) Absorption changes of heme (2 μM) on titrating peptide-7 (0-7 μM) in 50 mM sodium phosphate buffer, pH 7.2 containing 2 mM DPC. (B) Absorption spectra of heme alone (black), peptide7-heme oxidized (blue) and peptide7-heme reduced (red). Concentration of peptide and heme was 10 μM in 2 mM DPC, 50 mM sodium phosphate buffer, pH 7.2 (C) Jobs plot of peptide-7. The shift in Soret band of heme at 412 nm upon binding to peptide-3 and further shift to 428 nm after reduction by sodium dithionite and low intense peaks at 530 and 560 nm indicated bis-histidine coordination. The job plot shows peptide:heme stoichiometry 1:1.

Figure S7



Supplementary Figure 7: Secondary chemical shift of αH peptide 4 to 7.

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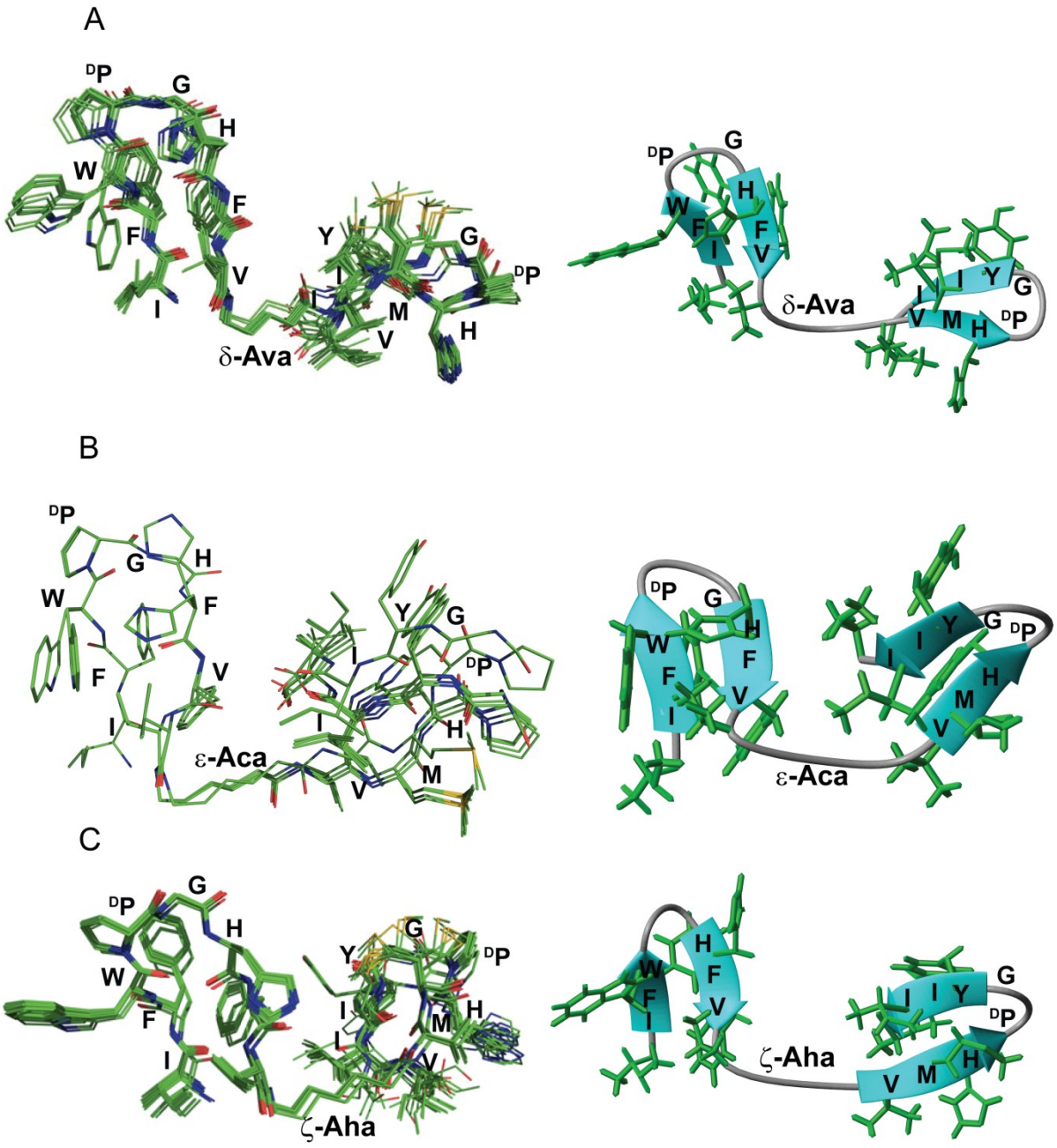
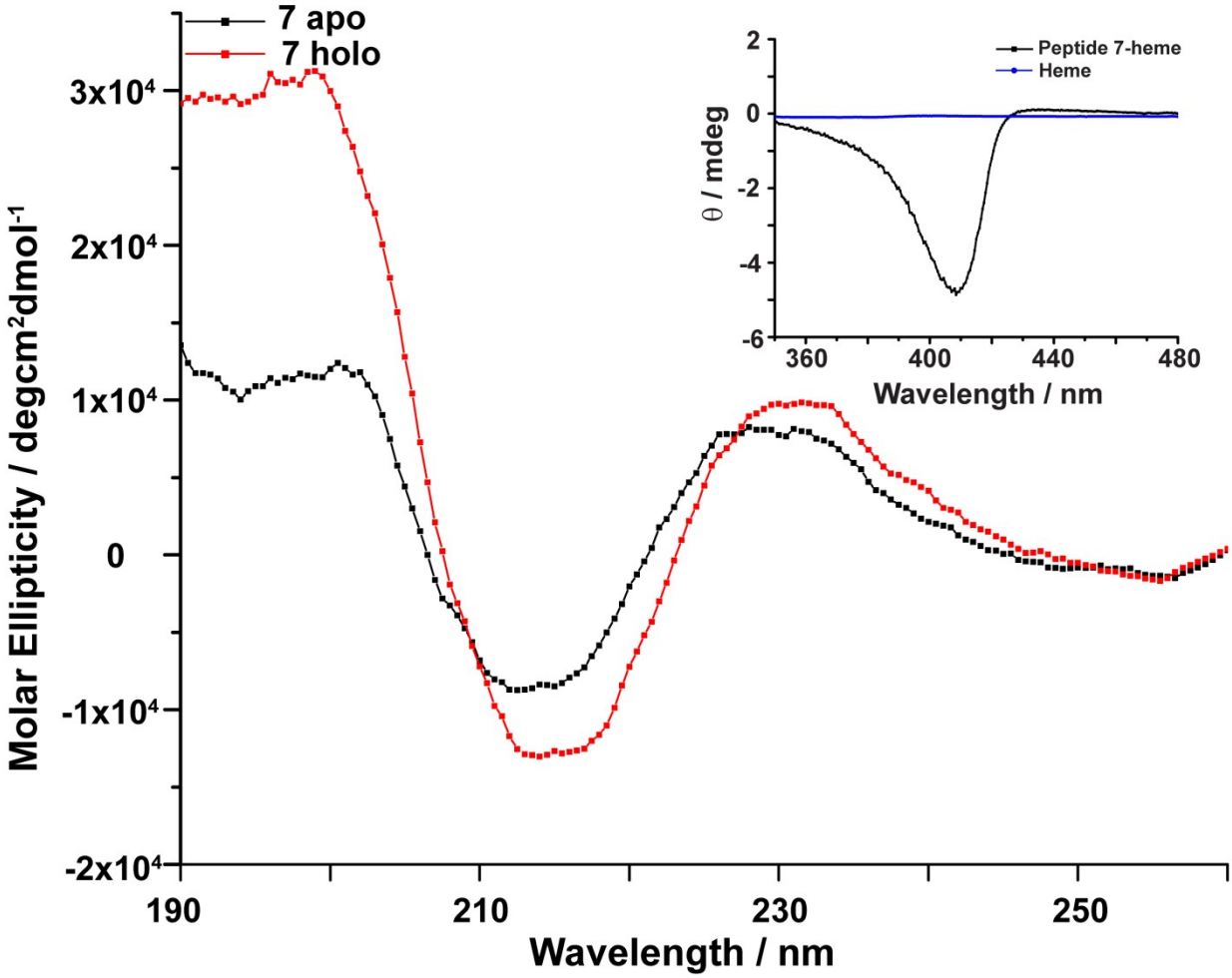
Figure S8



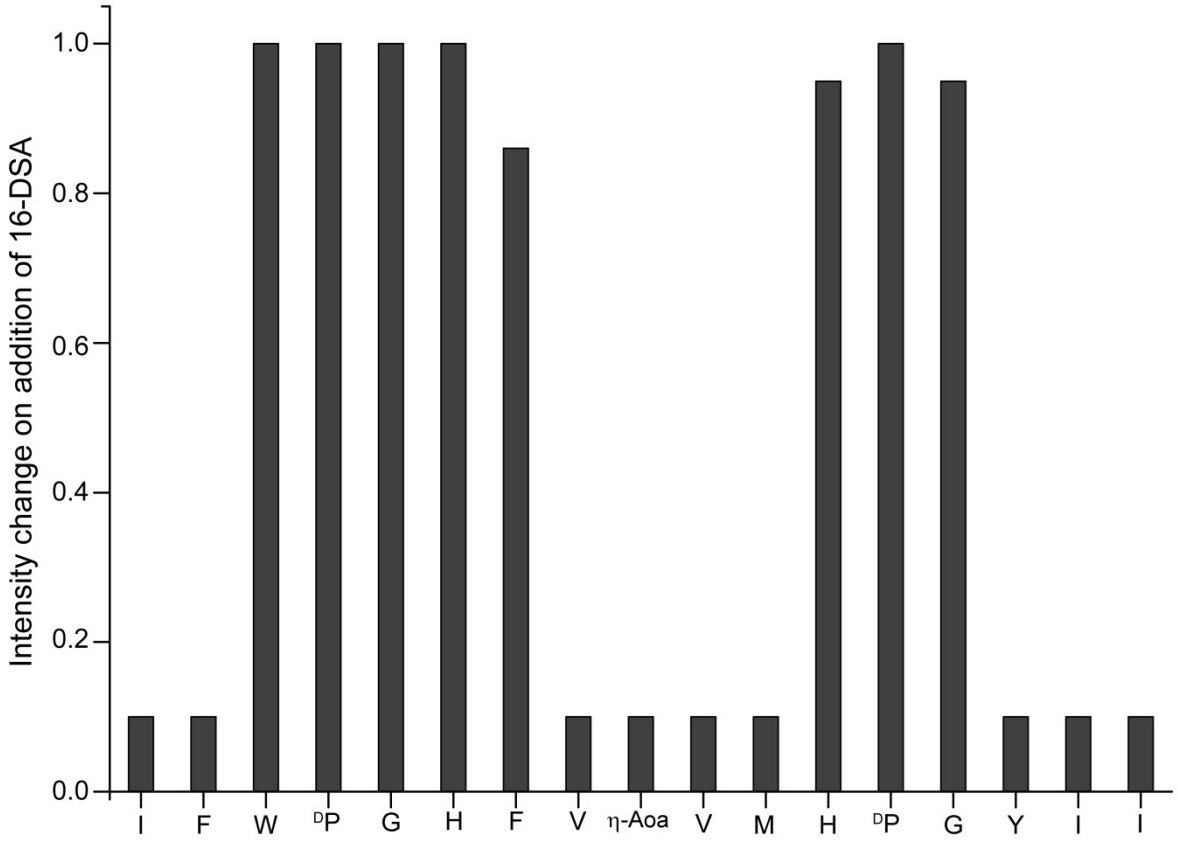
Supplementary Figure 8: Superimposed twenty low energy structures and one selected structure of each peptide showing side chain packing within β-sheets. (A) Peptide-4, (B) Peptide-5 and (C) Peptide-6

Figure S9



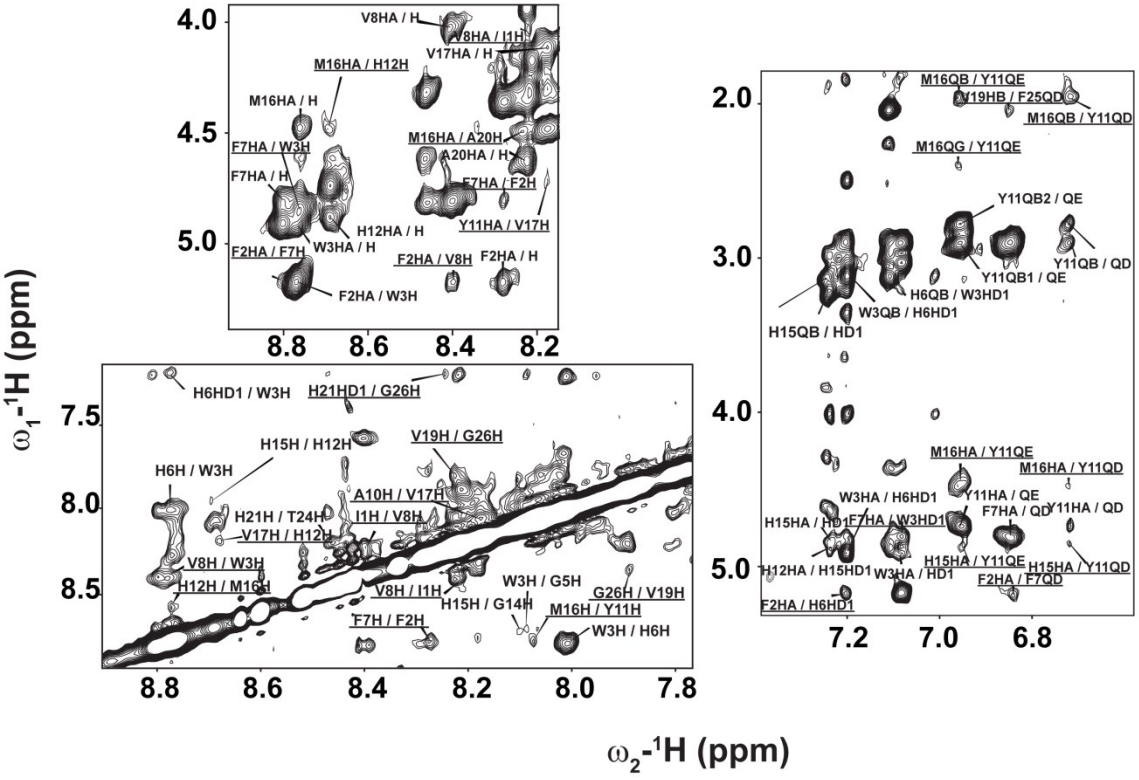
Supplementary Figure 9: Far-UV CD spectra of peptide-7 alone (black) and heme-bound (red). Concentration of peptide and heme was 100 μM in 2 mM DPC, 50 mM sodium phosphate buffer, pH 7.2. CD studies were performed using a 0.01cm path length cuvette with a 1nm bandwidth and a step size of 0.5 nm at 0.5 seconds per data set. Near-UV CD spectra of peptide-7-heme and heme alone (in blue) (onset) were acquired in a 1 cm path length cuvette to monitor the changes in heme environment. 8 μM of peptide along with 8 μM heme dissolved in 2 mM DPC, sodium phosphate buffer pH 7.2 was used for the experiment.

Figure S10



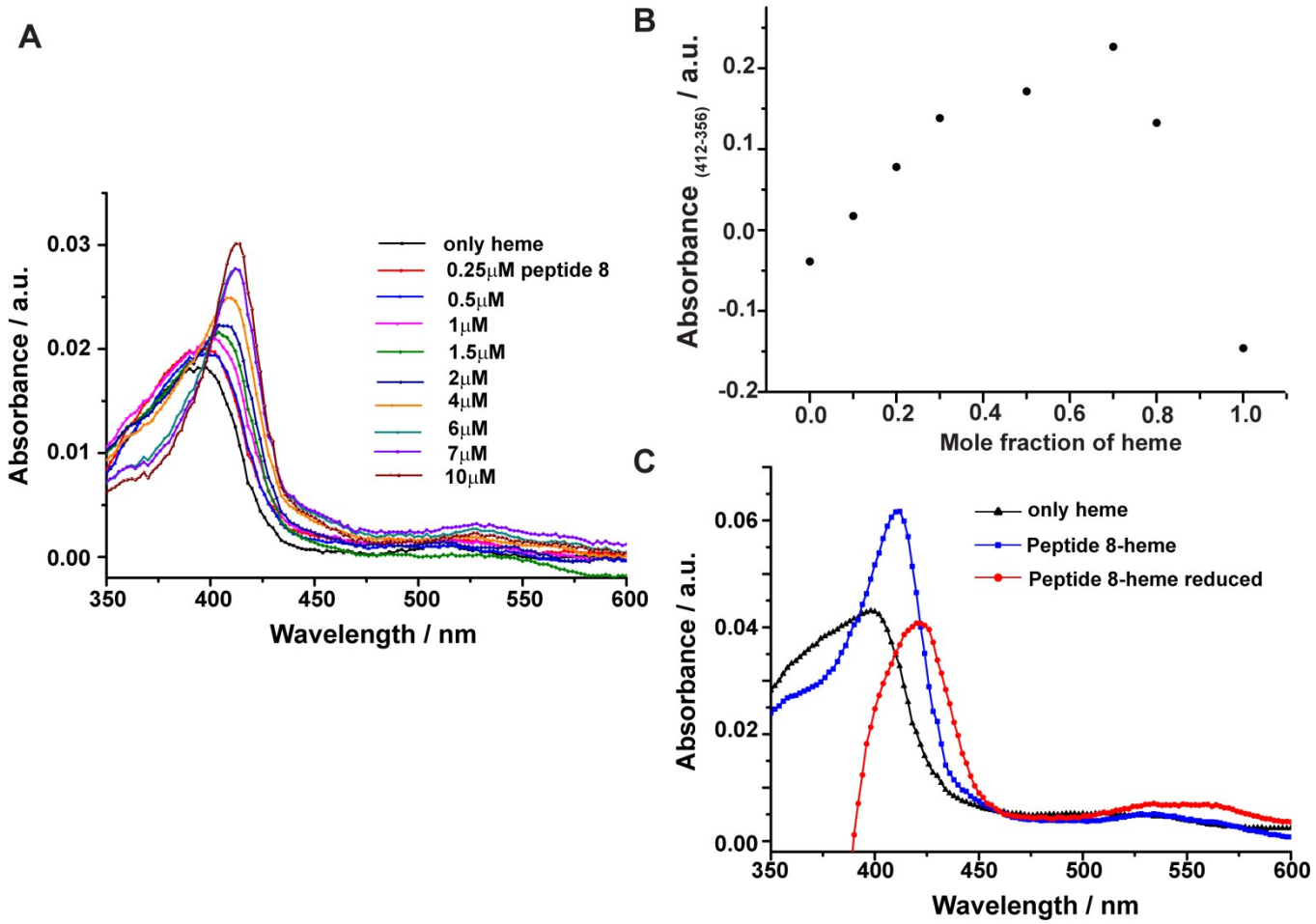
Supplementary Figure 10: The bar diagram showing ratio of intensity of CαH/NH cross-peaks obtained from two-dimensional TOCSY spectra of peptide-7 before addition of 16-DSA and in the presence of 16-DSA (2 mM). Residues experiencing perturbation by paramagnetic 16-DSA showed intensity ratio <1.

Figure S11



Supplementary Figure 11: Sections of two-dimensional ^{1}H-^{1}H NOESY spectra of peptide-8 showing NOE connectivity in the αH-NH region (top left), NH-NH region (bottom left) and aromatic region (right). Long range NOEs are underlined and boldfaced.

Figure S12



Supplementary Figure 12: (A) Absorption changes of heme (2 μM) on titrating peptide-8 (0-10 μM) in 50 mM sodium phosphate buffer, pH 7.2 containing 2 mM DPC. (B) Jobs plot of peptide-8. (C) Absorption spectra of heme alone (black), peptide 8-heme oxidized (blue) and peptide 8-heme reduced (red) Concentration of peptide and heme was 10 μM and 2 μM respectively in 2 mM DPC, 50 mM sodium phosphate buffer, pH 7.2. The shift in Soret band of heme at 412 nm upon binding to peptide-8 and further shift to 428 nm after reduction by sodium dithionite and low intense peaks at 530 and 560 nm indicated bis-histidine coordination. The job plot shows peptide:heme stoichiometry 1:2.

Supplementary Table 1: Summary of structural statistics of the designed peptides

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Distance constraints:								
Sequential [| i-j | = 1]	46	38	49	61	65	62	58	84
Medium range [1< | i-j | < 4]	12	6	12	13	19	10	16	26
Long range [| i-j | ≥ 4]	14	19	20	28	21	25	32	38
PRE driven distance restraints							18	
Total	156	131	159	200	183	185	206	278
Dihedral-angle constraints	22	22	22	22	22	22	22	36
Deviation from mean structure (Å)								
All backbone atoms	0.30	0.37	0.20	0.45	0.35	0.44	0.14	1.32
All heavy atoms	0.57	0.65	0.42	0.84	0.81	0.93	0.50	1.49
Ramachandran plot for the mean structure (%residues)								
Most favoured region	92.3	75.0	92.3	92.3	92.3	92.3	92.3	72.6
Additionally allowed region	7.7	25.0	7.7	7.7	7.7	7.7	7.7	27.3
Generously allowed region	0	0	0	0	0	0	0	0
Disallowed region	0	0	0	0	0	0	0	0