Electronic Supplementary Material (ESI) for Nanoscale.
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Supporting Information

Designing 1D Multiheme Peptide Amphiphile Assemblies Reminiscent of Natural Systems

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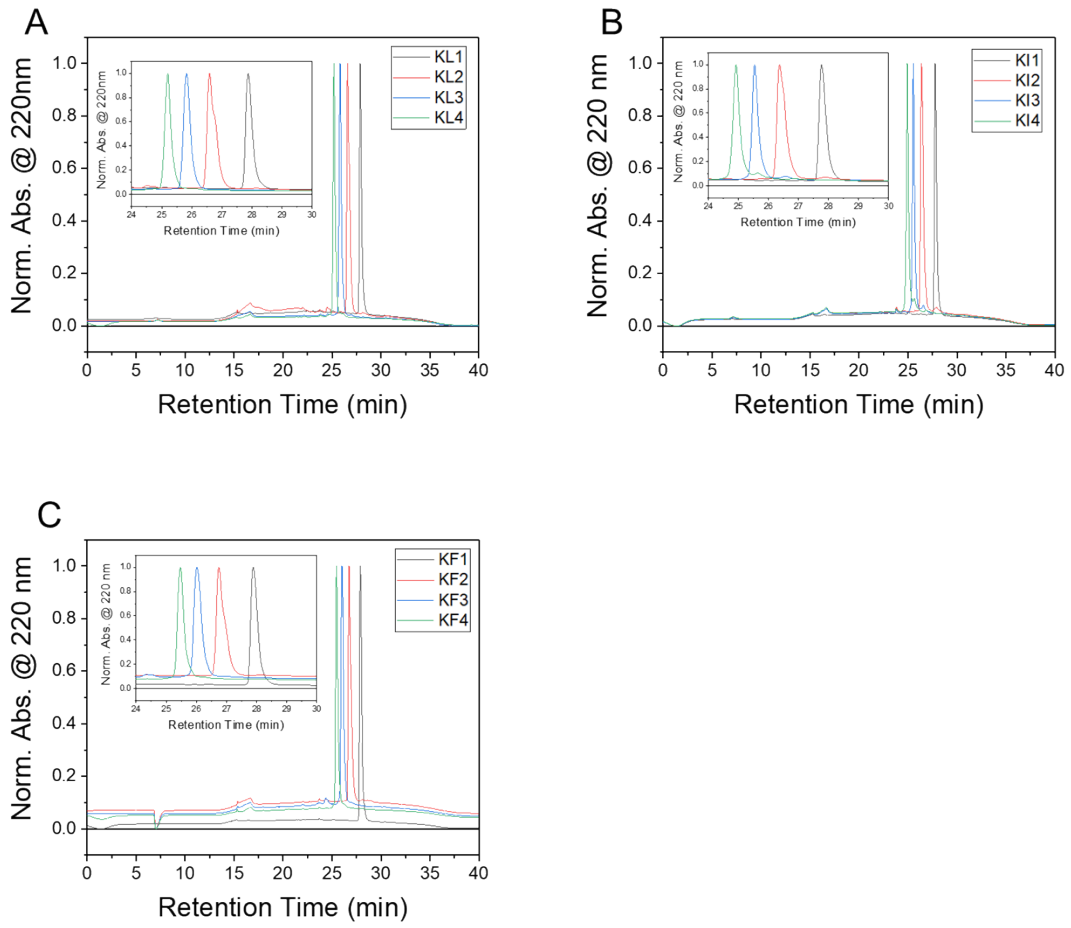

Figure S1. HPLC Results of A) PAKL*n*, B) PA-KI*n*, and C) PA-KF*n* where *n* = 1 – 4. Color code: Black, PA-Kx1; Red PA-Kx2; Blue, PA-Kx3; and Green, PA-Kx4.

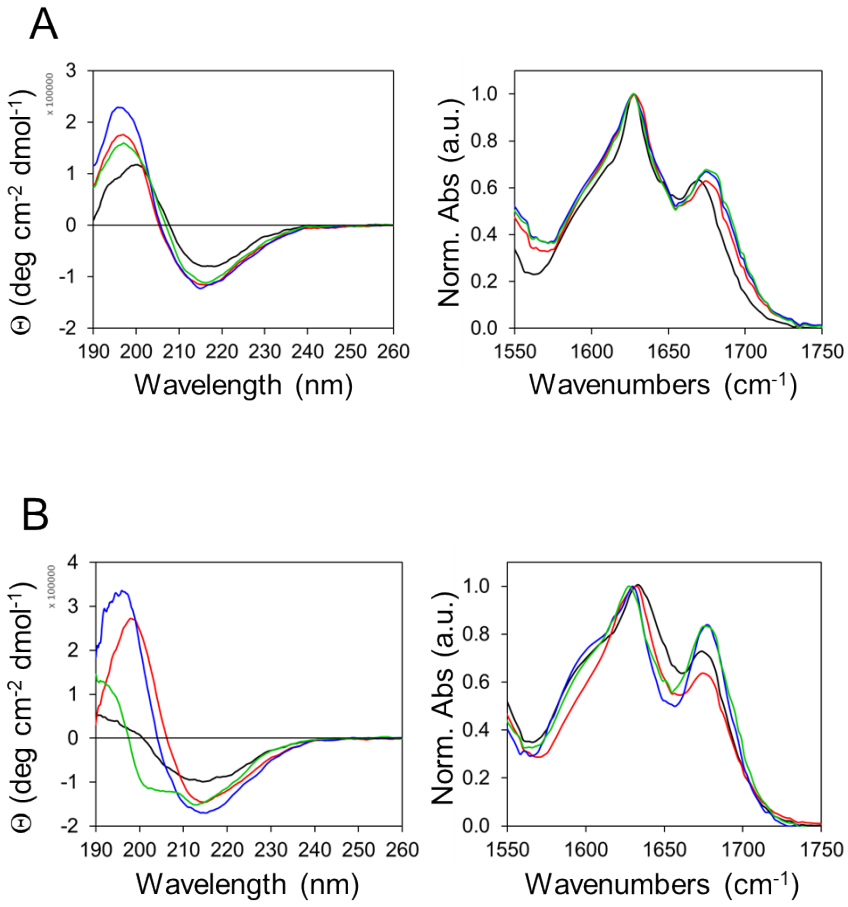

Figure S2. Secondary structure characterization of A. PA-KI*n* and B. PA-KF*n* (Left) in Tris buffer, pH 8 using CD and (Right) dried films using FTIR. Color coding: Black, PA-Kx1; Red, PA-Kx2; Blue, PA-Kx3; and Green, PA-Kx4.

Table S1. Secondary structure distribution according to the CD spectral fitting using BetStSel. It is highly unlikely that short amphipathic sequences would yield α-helices. The fitting parameters may not consider the extended β-sheet network that exist PA assemblies.

	PA-KL*n*			**PA-KI*n***			**PA-KF*n***		
n	α-helices	β-sheets	other	α-helices	β-sheets	other	α-helices	β-sheets	other
1	31.2	19.8	49	13	60.9	26.1	41.5	18.7	39.8
2	3.7	57.2	39.2	19.8	36	44.1	31.7	36.6	31.6
3	0	56.1	43.9	14.9	34.3	50.8	53.2	20.4	26.3
4	4	46.4	49.5	5.7	29.1	65.3	7.9	36.1	56

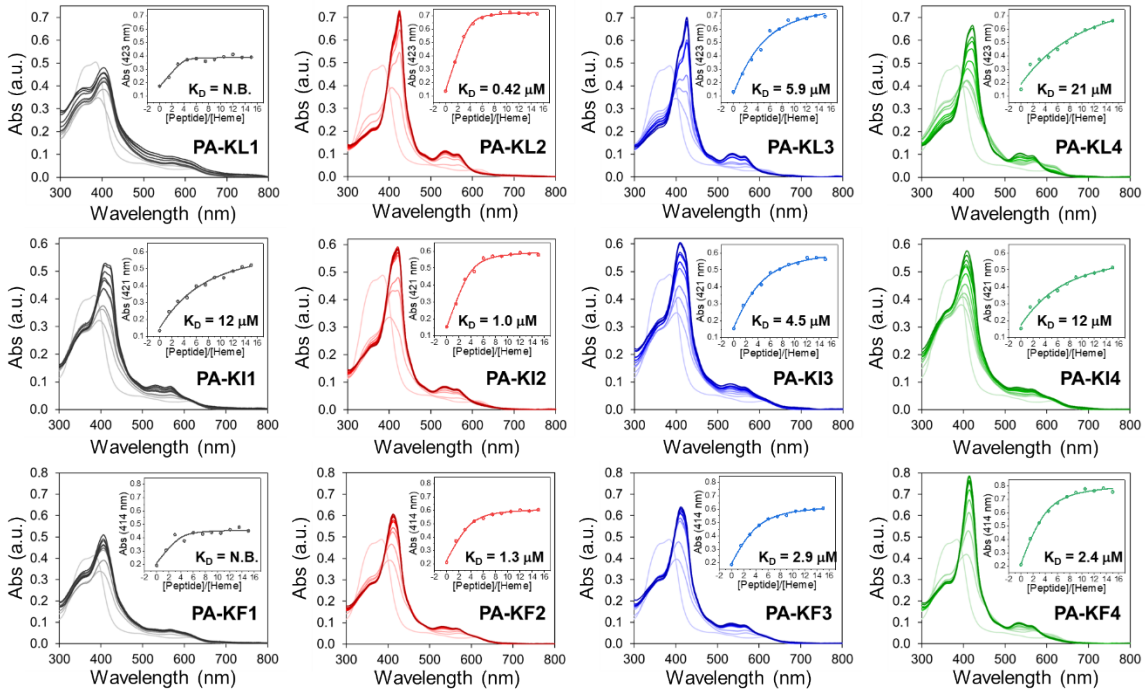

Figure S3. Binding constant analysis of PA-Kx*n* (0 – 150 μM) into heme (10 μM) in Tris buffer, pH 8 monitored by UV/visible spectroscopy. Top Row: PA-KL1-4. Middle Row: PA-KI1-4. Bottom Row: PA-KF1-4. Color coding: Black, PA-Kx1; Red, PA-Kx2; Blue, PA-Kx3; and Green, PA-Kx4.

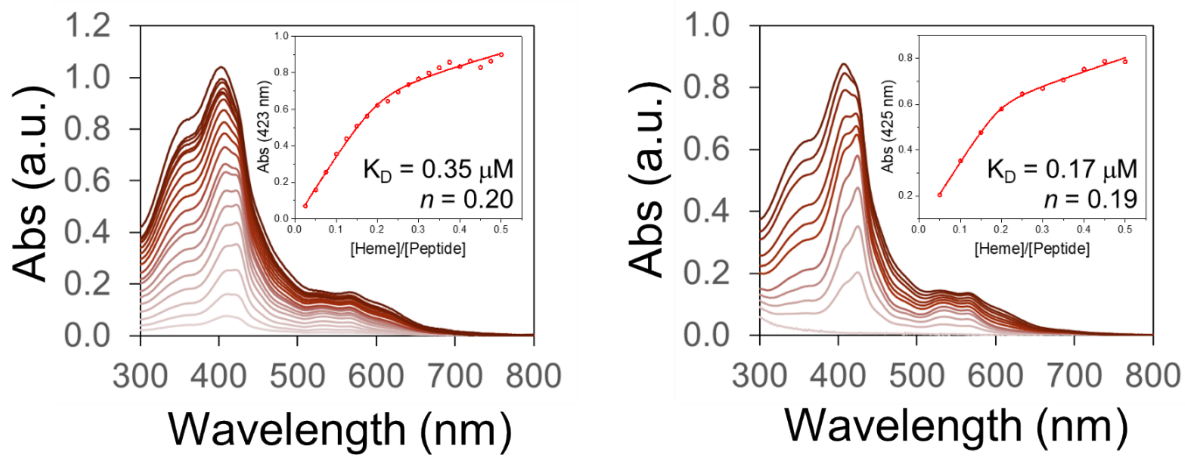

Figure S4. Heme affinity measurements. Left: Heme (2 µM aliquots) titrated into a 40 µM solution of PA-KL2 in Tris buffer, 1 mm quartz cuvette. Spectra measured immediately after addition of heme. Right: Heme (4 µM aliquots) titrated into a 40 mM solution of PA-KL2 in Tris buffer, 1 mm quartz cuvette. Spectra measured 24 hrs after addition of heme. Results: K_D are on the same order of magnitude as what is reported in the manuscript. The stoichiometry found here 5:1 (peptide:heme) is similar to what is reported in the manuscript.

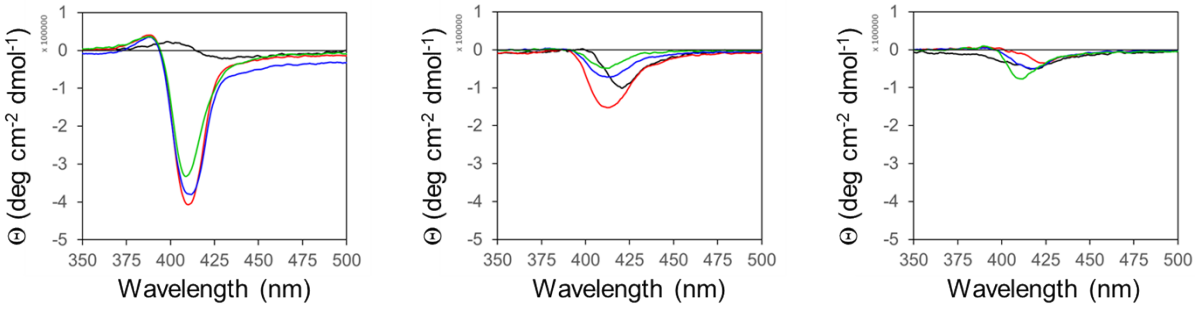

Figure S5. Circular Dichroism spectra of the visible region of heme coordinated to assemblies of A. PA-KL*n*, B. PA-KI*n* and C. PA-KF*n*. Color coding: Black, PA-Kx1; Red, PA-Kx2; Blue, PA-Kx3; and Green, PA-Kx4.

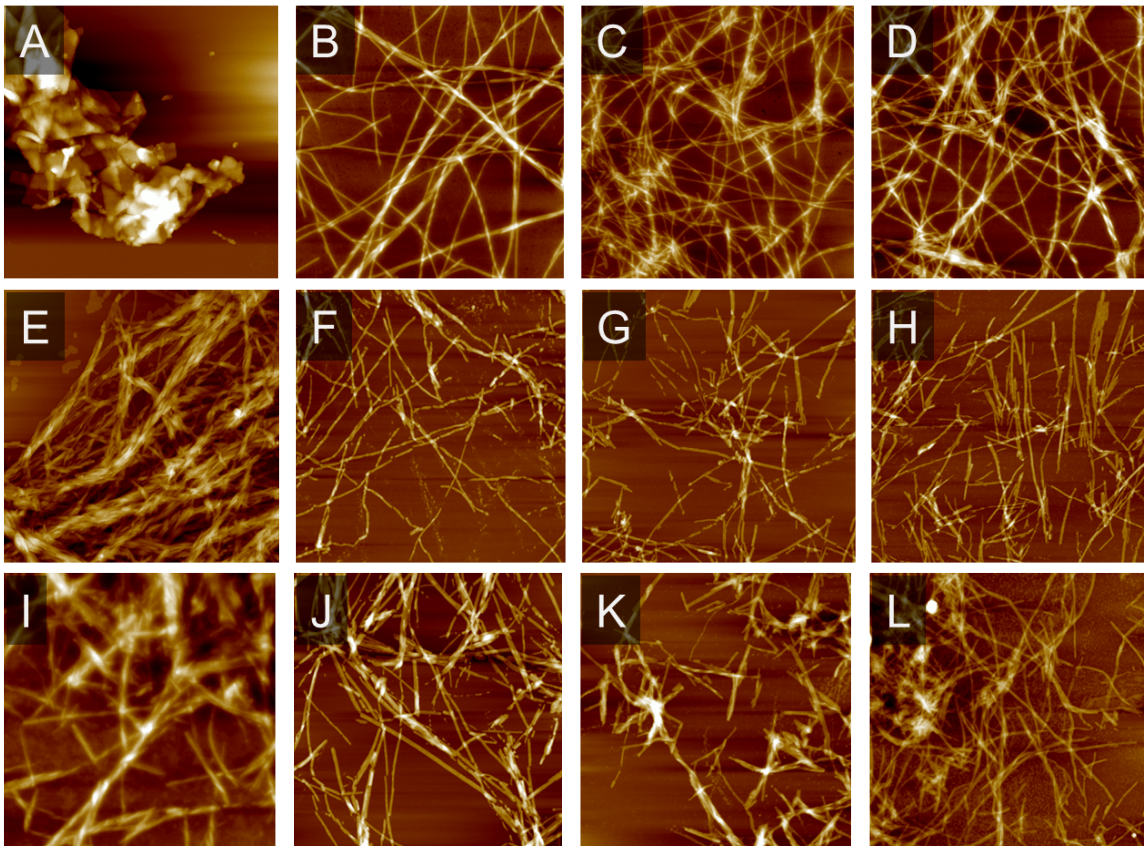

Figure S6. Atomic force micrographs of PA-KL1-4 (A-D), PA-KI1-4 (E-H), and PA-KF1-4 (I-L). All micrographs are 2 μm by 2 μm scans.

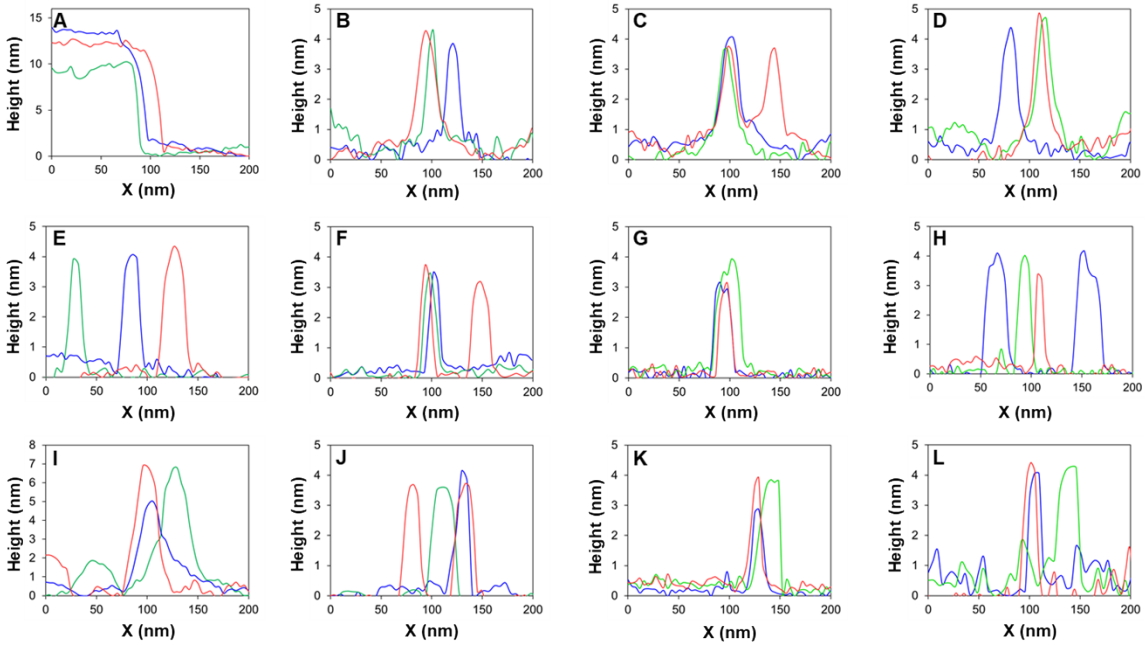

Figure S7. Height profiles of individual fibers. The panels A-L correlate with the images in Figure S6.

Table S2. Average height measurements of PA-Kx*n*.

Peptide	***n* = 1**	***n* = 2**	***n* = 3**	***n* = 4**
PA-KL*n*	12.9 ± .1	4.1 ± 0.2	3.8 ± 0.2	4.6 ± 0.2
PA-KI*n*	4.1 ± 0.2	3.6 ± 0.2	3.4 ± 0.5	3.9 ± 0.3
PA-KF*n*	6.2 ± 1.1	3.8 ± 0.3	3.5 ± 0.6	4.2 ± 0.2

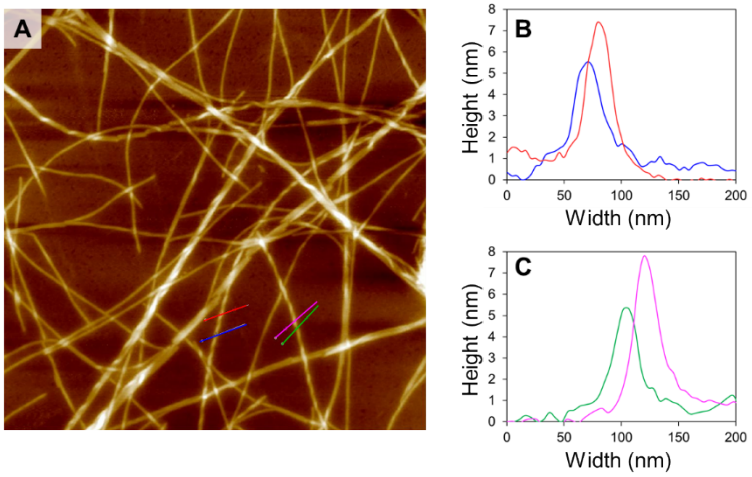

Figure S8. Height profile of twisted PA-KL2 Fiber. The height traces are color coordinated with the lines marked on the micrograph. The micrograph is identical to that found in Figure S4B and represents a 2 µm x 2 µm scan.

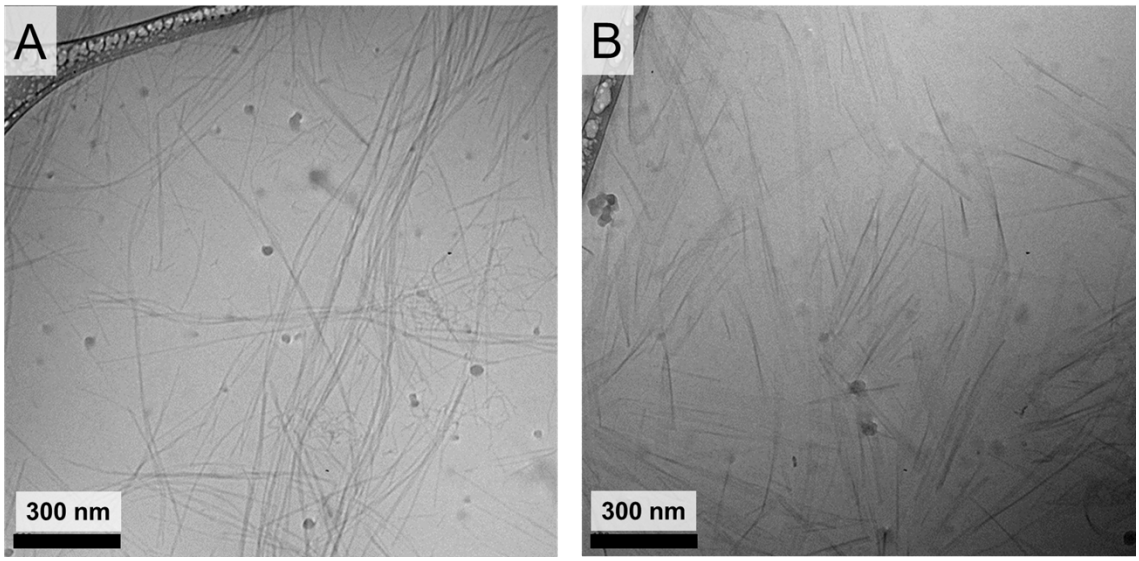

Figure S9. CryoTEM images of (A) PA-KL3 highlighting nanoscale fibers and (B) PA-KF3 highlighting nanoscale belts/tapes.

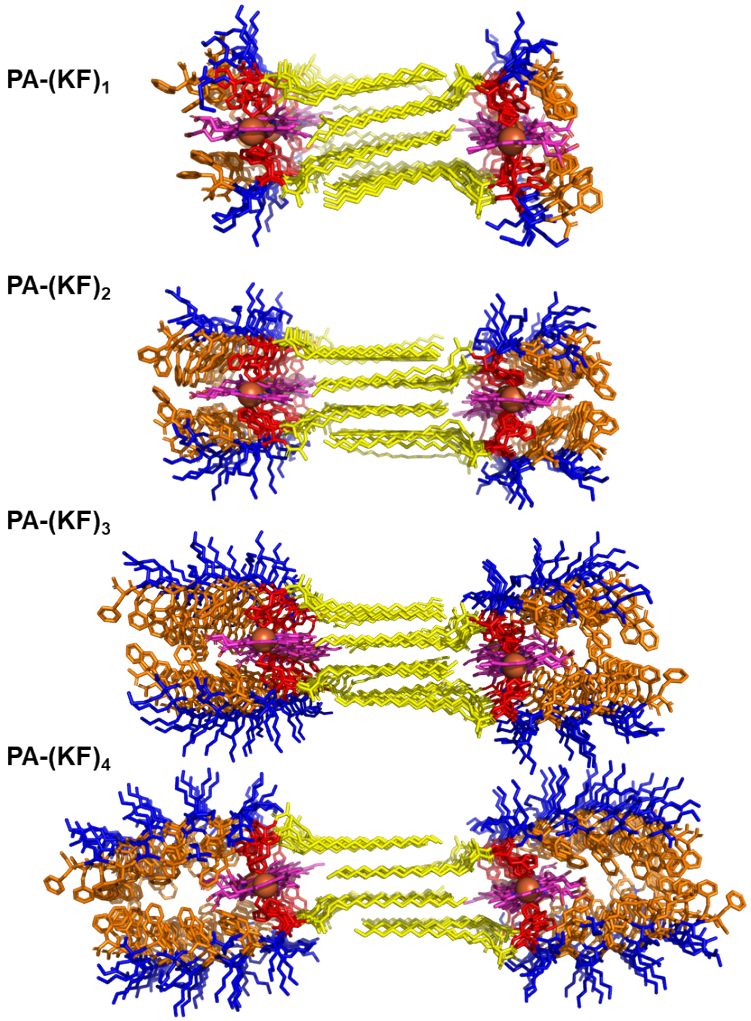

Figure S10. Energy minimized renderings of PA-KF1, PA-KF2, PA-KF3, and PA-KF4 highlighting the molecular packing around the heme molecules. The progression from PA-KF2 to PA-KF4 highlights improved van der Waals packing of the phenylalanine side chains (orange) when compared to the PA-KL*n* analogs in Figure 6.

Table S3. Summary of dimensional analysis of peptides (PA-KX*n*) and their assemblies.

Repeat Length (*n*)	Monomer Length (nm)	Assembly Width (nm)	Assembly Height (nm)	Assembly/Fiber Length
1	3.2	4.2	3	Varies
2	3.8	5.8	3	Varies
3	4.5	7.3	3	Varies
4	5.2	8.6	3	Varies

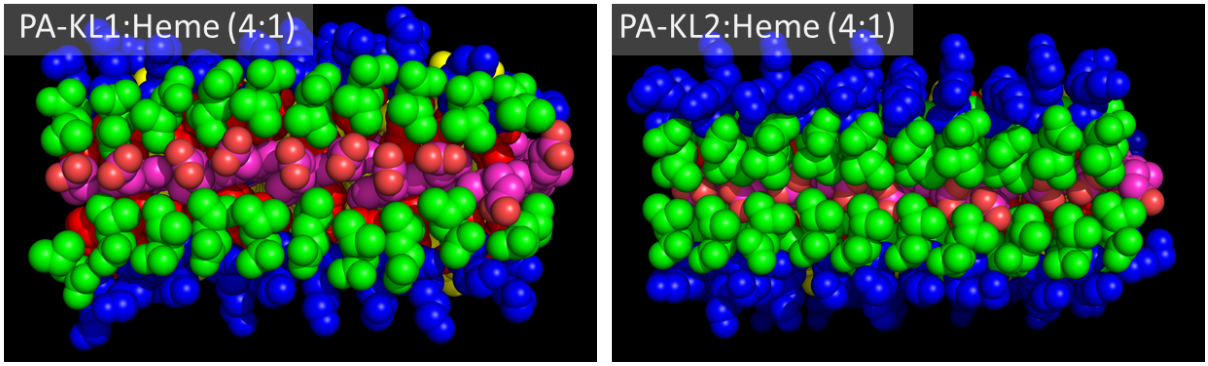

Figure S11. Side View of the heme packing in spacefill renderings of PA-KL1:Heme (4:1) and PA-KL2:Heme (4:1). The image of the PA-KL1:Heme assembly (right) indicates that the heme molecules (magenta) are partially exposed while PA-KL2:Heme assembly (left) indicates that the heme is buried and in close contact with the outermost leucine side chains (green).

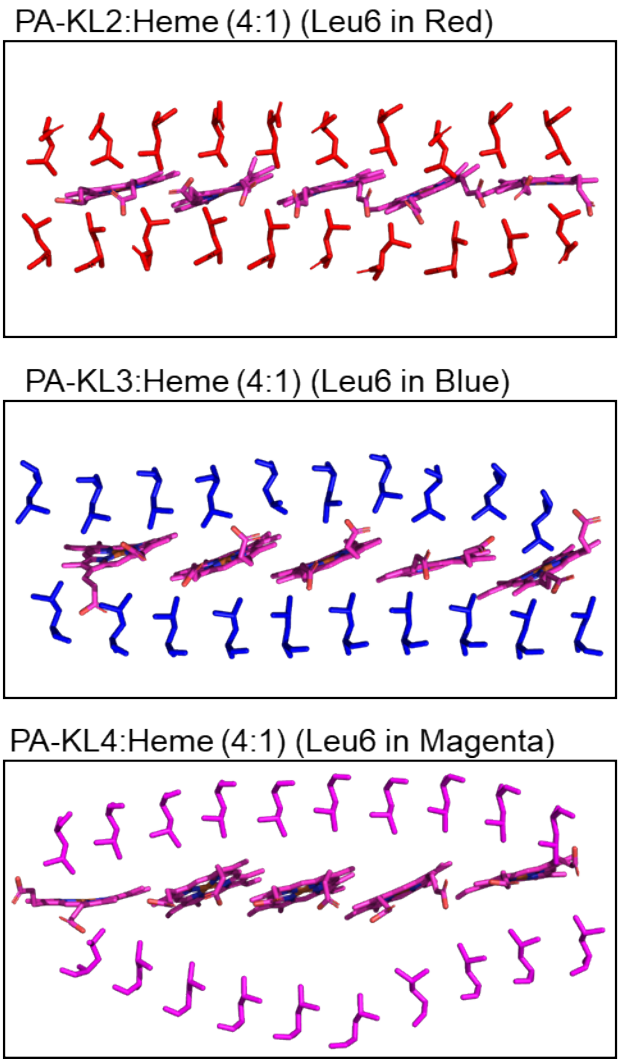

Figure S12. Mapping of the Leu6 residues in PA-KL*n* (*n* = 2-4) highlighting the impact that the increasing length of the peptide may have on the overall assembly. The Leu6-Leu6' distances are summarized in Table Sx.

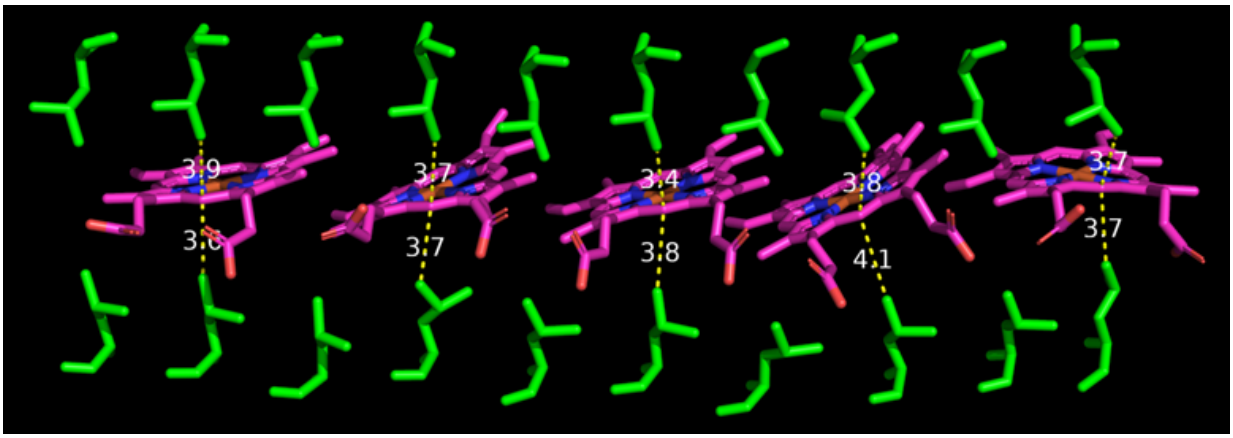

Figure S13. Example of measurements made for side chains interacting with heme, i.e Leu4-mesoCarbon. The distances for all peptides are summarized in Table Sx.

Table S4. Summary of measurements impacting the heme binding site determined from the assembly models.

	PA-KL*n*		**PA-KF*n***	
n	**Leu4-mesoC (Å)**	**Leu6-Leu6'(Å)**	**Phe4-mesoC (Å)**	**Phe6-Phe6' (Å)**
1	4.9 ± 1.1	NA	5.5 ±1.2	NA
2	3.7 ± 0.2	4.6 ± 0.4	4.7 ± 0.6	4.0 ± 0.5
3	4.9 ± 0.7	7.4 ± 0.5	4.7 ± 0.5	7.4 ± 2.3
4	5.3 ± 1.7	10.1 ± 3.2	5.3 ± 0.9	8.4 ± 2.3

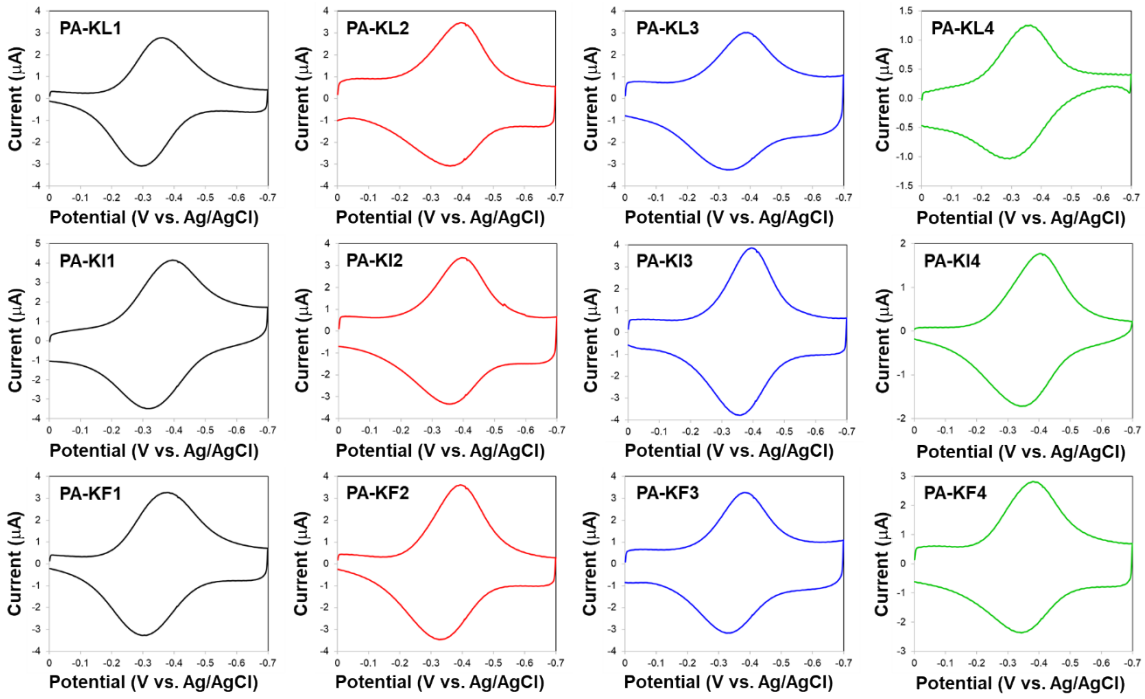

Figure S14. Thin layer cyclic voltammograms for all PA-Kx-n/Heme assemblies deposited from 1.5 mM peptide:100 μM heme solutions in Tris buffer.

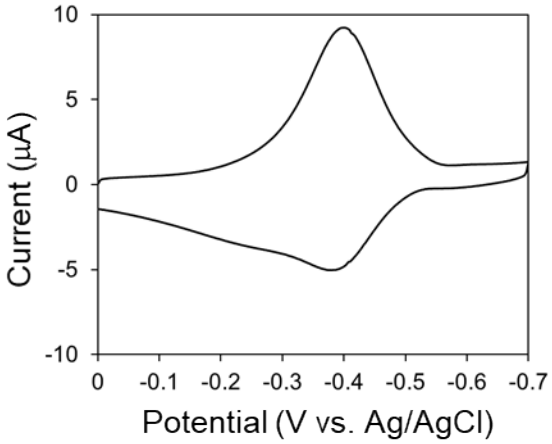

Figure S15. Thin layer cyclic voltammograms for Heme deposited from a 100 μM heme solution in Tris buffer.

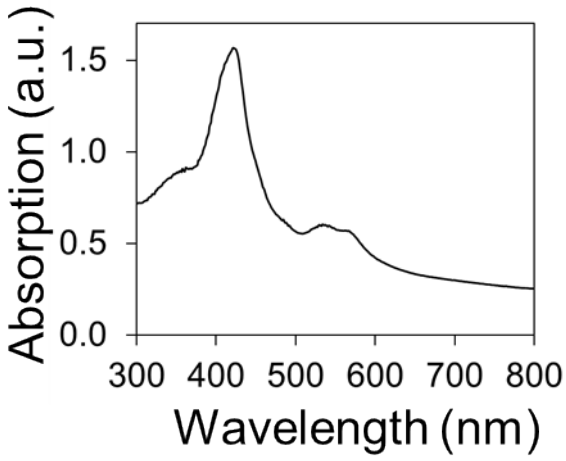

Figure S16. UV/visible spectrum of a drop cast film of PA-KL2:Heme (4:1) on a glass slide is similar to that found in solution. Soret = 424 nm and Q-band = 534, 560 nm.

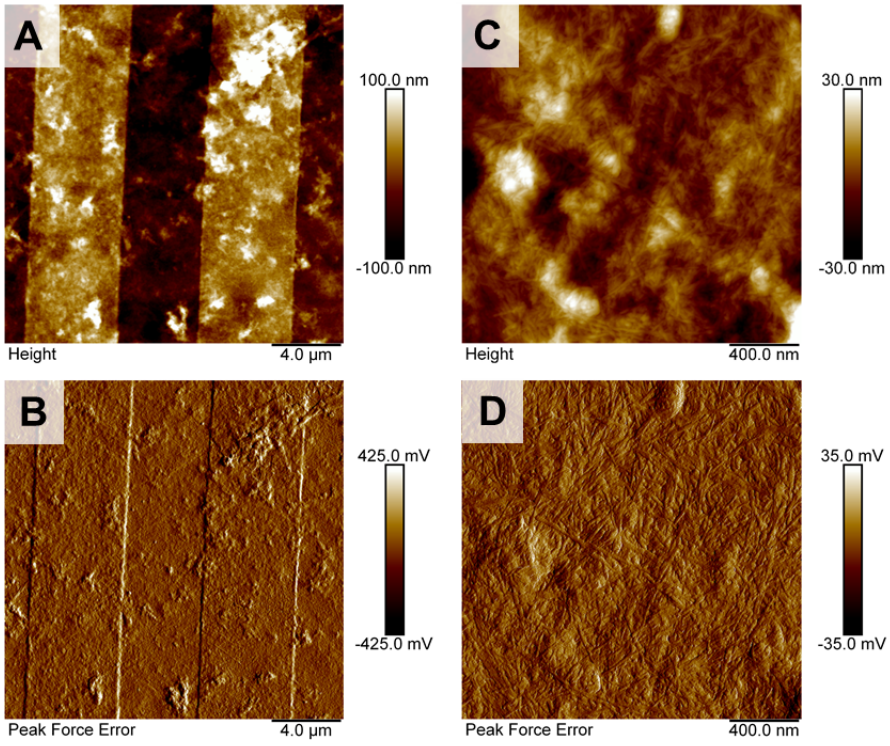

Figure S17. Atomic force micrographs of PA-KL2:Heme (4:1) deposited on interdigitated gold electrodes with 5 µm gaps. A (height profile) and B (peak force error): the gold electrodes can be seen with a

heterogeneous film spanning the intraband gap. C (height profile) and D (peak force error): the space in the intraband gap is comprised of randomly oriented fibers.

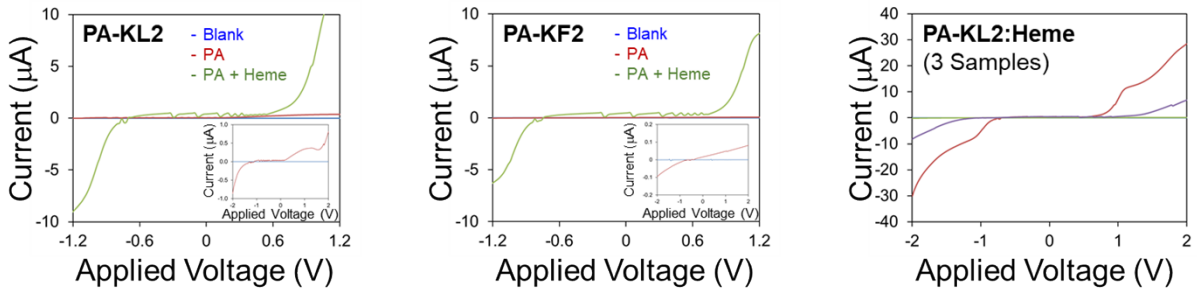

Figure S18. IV plots for samples deposited on the interdigitated electrode. A. Blank (blue), PA-KL2 (Red), and PA-KL2:Heme (4:1) (Green), PA-KF2:Heme (4:1). B. Blank (blue), PA-KF2 (Red), and PA-KF2:Heme (4:1) (Green), PA-KF2:Heme (4:1) (Inset: magnified view highlighting the blank and the peptide only plots). C. 3 different samples prepared from the same solution of PA-KL2:Heme (4:1) highlighting the difficulty in preparing reproducible samples.