

## **Supplemental Information: Collagen mimetic peptide discs drive supramolecular assembly of a broad range of natural protein fibers through hydrophobic interactions**

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## Materials and Methods

*Peptide synthesis and purification:* Peptides H2, H3, H4, and QH4 were synthesized and purified as previously described [1]. SH4 was synthesized, purified and verified using HPLC (Fig S8) and mass spectrometry (Fig S9) by LifeTein ([www.lifetein.com](http://www.lifetein.com)). N- and C- termini were acetylated and amidated, respectively. Peptides were dialyzed in filtered deionized water, lyophilized and kept in  $-20\text{ }^{\circ}\text{C}$ . Peptides were weighed and resuspended in 10 mM phosphate buffer, pH 7.4. Collagen peptide concentrations were confirmed by measuring the absorbance at 214 nm, using an extinction coefficient of  $2200\text{M}^{-1}\text{ cm}^{-1}$  per peptide bond on an AVIV model 14DS UV-Vis Spectrophotometer.

*Shrimp Tropomyosin (ST) extraction and purification:* Tropomyosin was extracted and purified in multiple steps from flash-frozen shrimp purchased from a local market. All steps were performed at  $4\text{ }^{\circ}\text{C}$  except where noted. 400 g of fresh, shelled, and deveined shrimp were minced in a food processor. Care was taken to ensure not to over mince shrimp so that shrimp did not fall through a cheesecloth. Minced shrimp were then rinsed with water and stirred for five minutes in a 800 mL dilute salt solution of 20 mM KCl, 1 mM  $\text{KHCO}_3$ , 0.1 mM  $\text{CaCl}_2$ , 0.1 M DTT to remove sarcoplasmic proteins. The suspension was then filtered either through a double layer of cheese cloth lightly over a funnel or centrifuged a few minutes at 5,000 rpm. This wash was repeated four times. 800 mL of 95% ethanol was then added to the residue and filtered after 10 minutes. Ethanol extraction was repeated two more times. Residue was then transferred to ice and washed three times in the chemical hood with 800 mL of chilled diethyl ether. Residue was then dried at room temperature for 2 to 3 hours or overnight. Protein powder was then stored at  $-20\text{ }^{\circ}\text{C}$ . Powder was then dissolved in an 800 mL solution of 1 M KCl, 25 mM Tris (pH 8.0), 0.1 mM  $\text{CaCl}_2$  and 1 mM DTT, filtered through a cheese cloth and placed in 400 mL of 1 M KCl. The solution was then dialyzed against 6L of 5 mM Tris (pH 7.5) containing 0.1 M  $\text{CaCl}_2$ , and 0.1 M DTT for six hours and overnight with fresh dialysis buffer. The solution was retrieved from dialysis, brought up to 133 mL by the addition of 1 M of KCl, and pH 4.6 by dropwise addition of 7.5% acetic acid. The solution was then divided into three equal aliquots to which enzyme grade powdered ammonium sulfate was gradually to two aliquots (leaving the third alone), where one aliquot was taken to 40% and the second to 53% saturation and let to sit for 30 minutes. Solutions were then centrifuged at 30,000 rpm. Ammonium sulfate was then gradually added to all three aliquots - 0 to 40%, 40 to 53%, and 53 to 70%. Each of the three precipitates was then dissolved in 66 mL of 5 mM Tris (pH 7.5) containing 0.1 M  $\text{CaCl}_2$  and 0.1 M DTT. The tropomyosin precipitate was then removed by centrifugation at 30,000 rpm and a stock solution of 6.8 mg/mL of ST in 10 mM phosphate buffer 50 mM NaCl was stored at  $-20\text{ }^{\circ}\text{C}$ . Confirmation of tropomyosin identity (a-tropomyosin from the brown shrimp *Pennaeus azteca*)

was confirmed by liquid chromatography tandem mass spectrometry. Full coverage of the shrimp sequence was confirmed.

H4 and ST solutions were combined 2:1 volume ratios and incubated at 4 °C for four days prior to imaging.

*Circular Dichroism:* CD wavelength spectra were measured (190–260 nm scans, 6 sec averaging, 4 °C) on an AVIV model 420SF spectrophotometer (Aviv Biomedical, Lakewood, NJ). Blank buffer subtraction was performed on each sample and the molar residual ellipticity (MRE) was calculated from the sample concentration, the number of residues, and a cell path length of 0.1 cm.

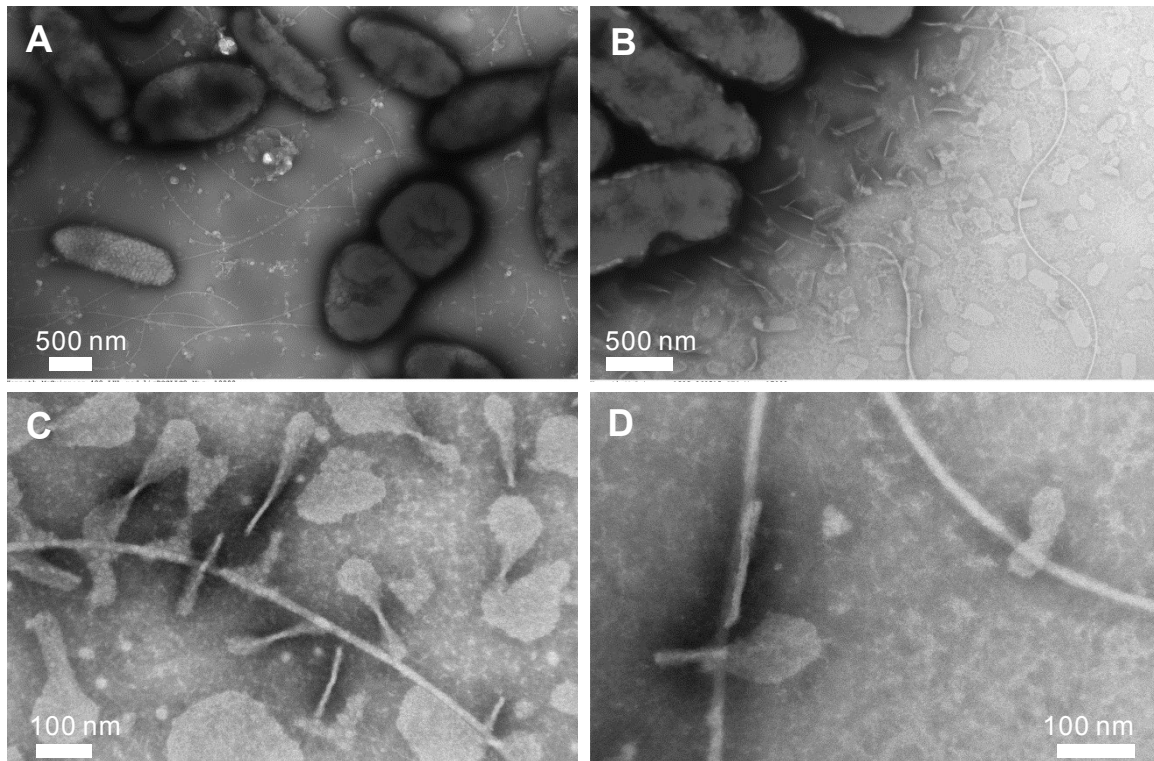
*CMP nanodisc assembly:* H-series peptide samples were prepared in 10 mM sodium phosphate buffer pH 7.3 at 3-5 mM and incubated at 4°C for 2 weeks prior to incubation with proteins.

*$\alpha$ -Synuclein assembly:* Non-acetylated alpha-synuclein ( $\alpha$ -Syn) purification and fibril formation, monitored by Thioflavin T (20  $\mu$ M) while shaking at 600 rpm, was performed according to previous protocols [2]. Lyophilized aSyn was dissolved in 10 mM sodium phosphate pH 7.3. After fibril formation, the solution was spun down at 14000 rpm to remove higher order oligomers and the supernatant filtered through a 3K filter to remove truncated species. The resultant solution was 385  $\mu$ M and diluted to 100  $\mu$ M with a 1:1 mixture of H4.

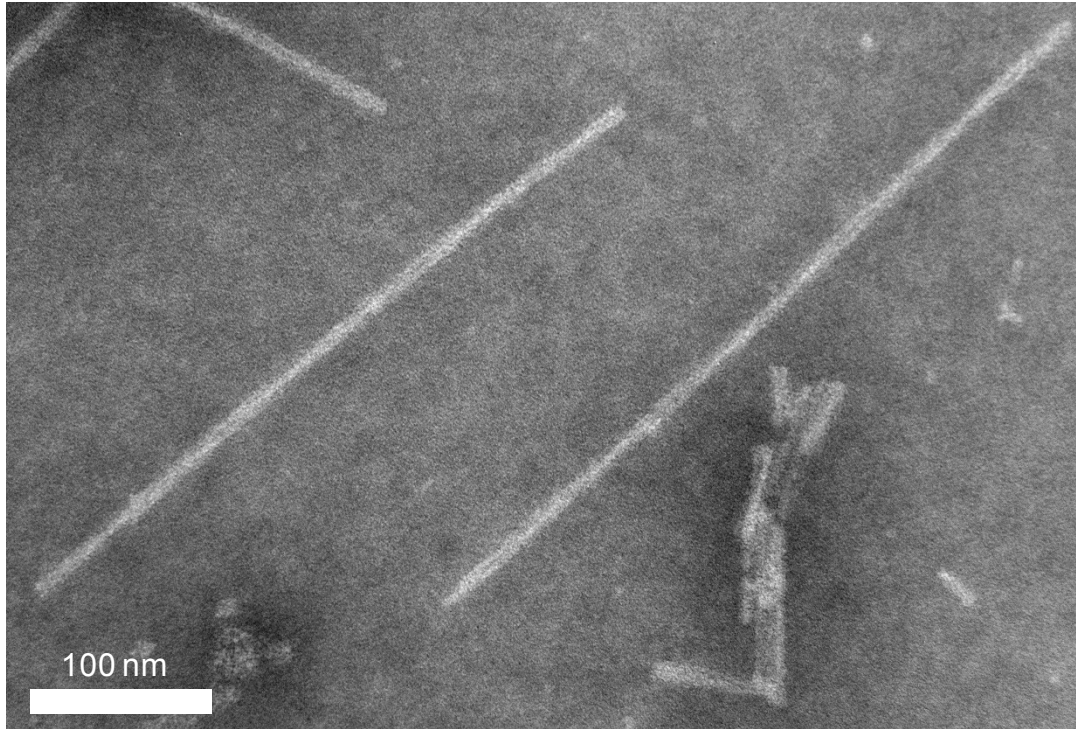
*CMP and Collagen Type-I co-assembly:* Stock solutions of 3mg/mL type-I collagen (COL1) (Elastin Products Company, Lot # 879114) were prepared in 0.02 N pH 3.3 acetic acid. COL1 fibers were formed in pH 7.3 10 mM sodium phosphate buffer as previously described [3, 4]. CMP and COL1 were combined at 1:1 volume ratios.

*Transmission Electron Microscopy:* For imaging, a volume of 5  $\mu$ L of peptide solution was deposited on a copper grid coated with carbon (Electron Microscopy Sciences CF-400-Cu), then a time of two minutes was passed to ensure deposition, and excess peptide was wicked away using filter paper. The grid was then negatively stained with 1% phosphotungstic acid (PTA) pH 7.0 for two minutes and excess stain was wicked away. Afterward, the grids were left to dry for at least two minutes in ambient conditions before imaging. Images were captured using a Philip 420 Electron Microscope at 80 kV. Structural characterizations of micrographs were performed using the ImageJ software package (<https://imagej.nih.gov/ij/>). Periodic banding patterns were selected using the line tool and the plot profiles of the lines were fit to a sin wave of period 15 and 67 nm for Tpm and COL1, respectively.

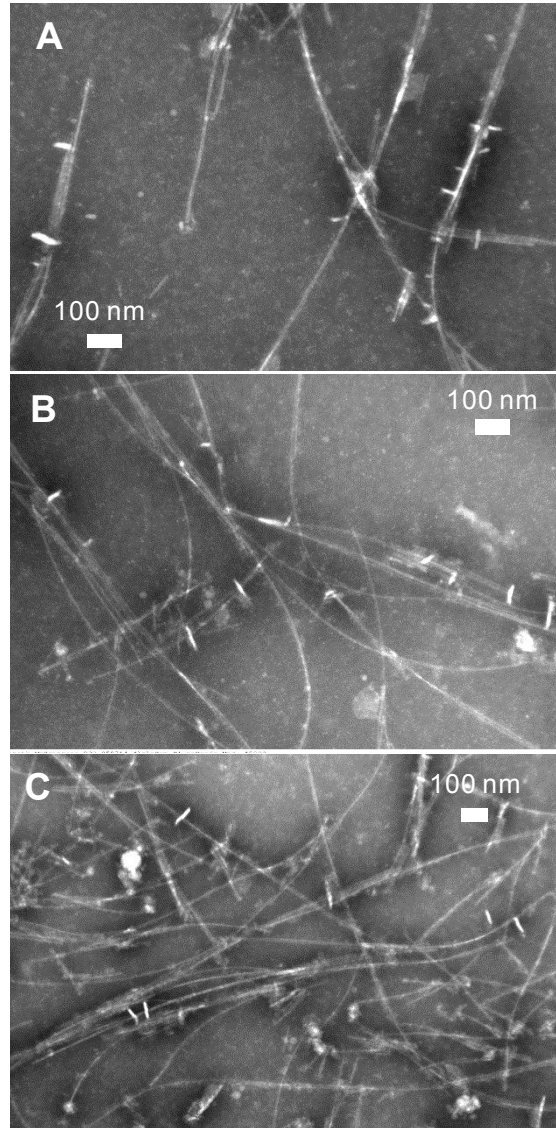
## Supplemental Figures



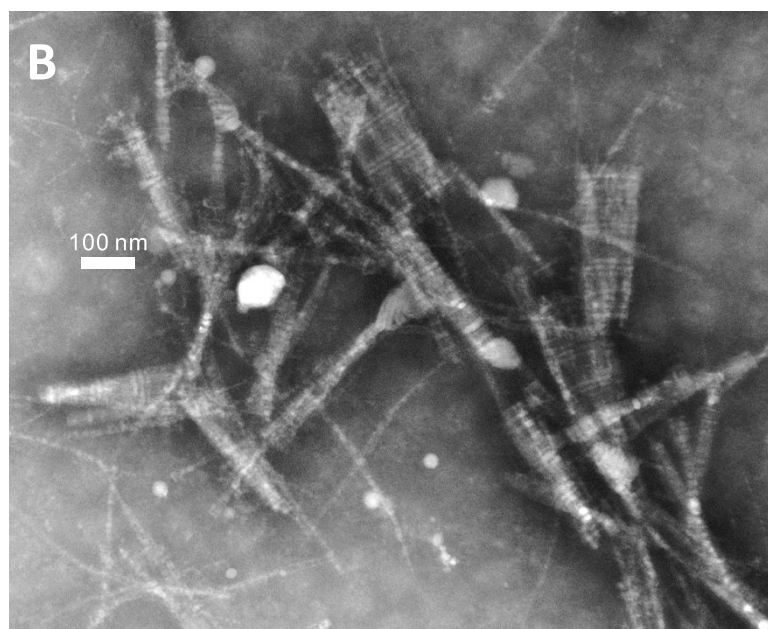
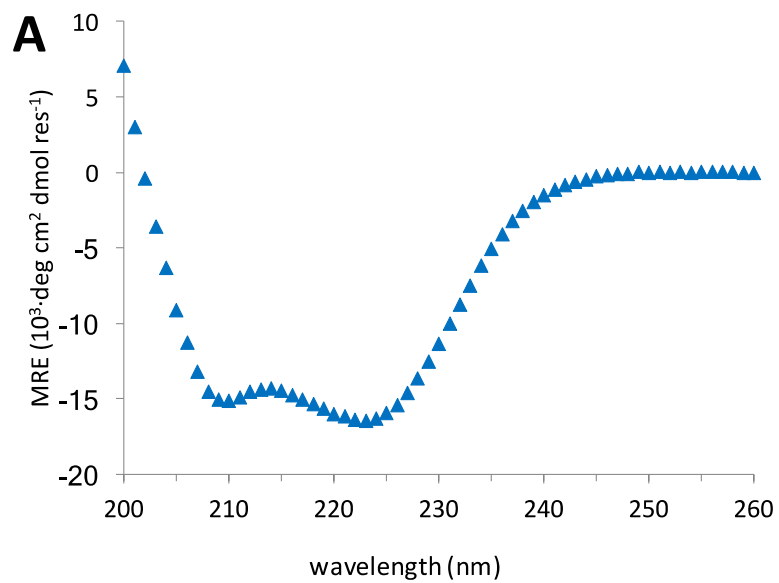
**Figure S1.** TEM micrographs of an H4 contaminated stock. (A, B) Lower magnification images of whole bacteria including flagella and H4 nanodiscs. (C, D) Close-up of disc-flagella interactions.



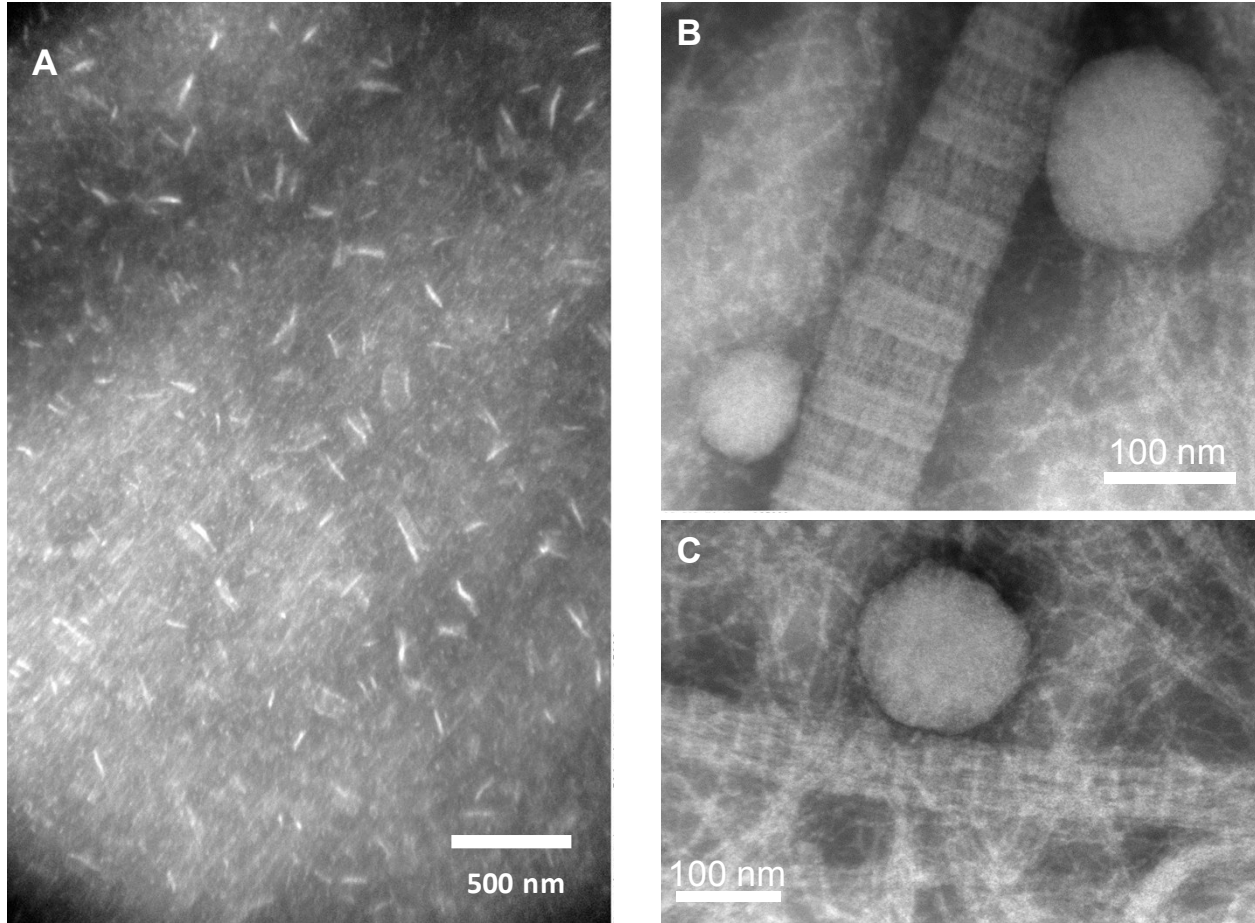
**Figure S2.**  $\alpha$ -Synuclein fibers prepared as described in the Materials and Methods.



**Figure S3.** TEM micrographs showing H4 interacting with  $\alpha$ -Synuclein fibers in a discs-on-strings morphology and promoting bundling of  $\alpha$ -Synuclein fibers.

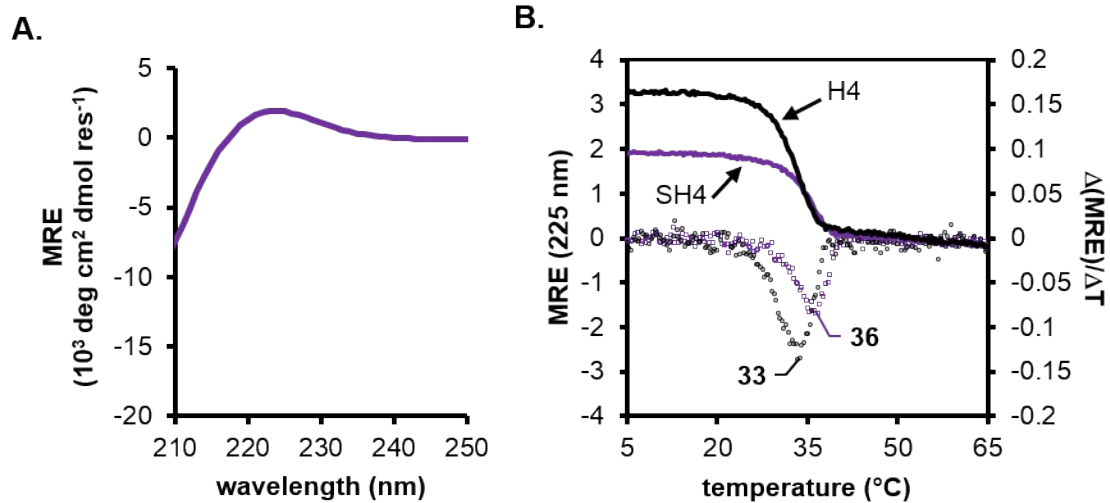


**Figure S4.** (A) CD spectrum of shrimp Tpm consistent with  $\alpha$ -helical coiled coil structure. (B) TEM micrograph of H4 interacting with Tpm.

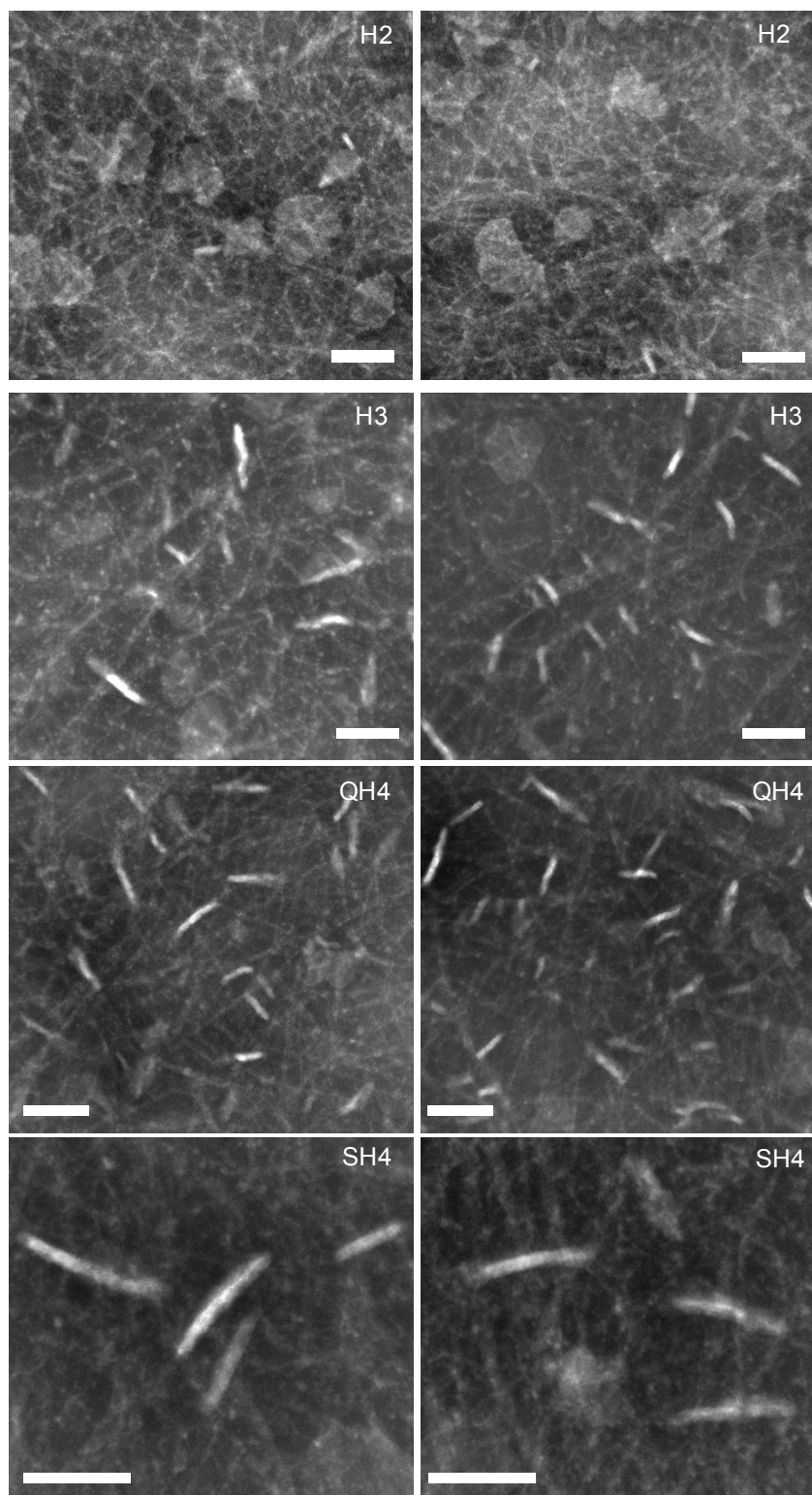


**Figure S5.** (A) H4 + COL1 maintained in a non-fiber state. (B, C) fibrillar collagen forms D-periodic bands. Spherical aggregates are proposed to contain H4. These aggregates are not observed in TEM micrographs for COL1 alone [4].

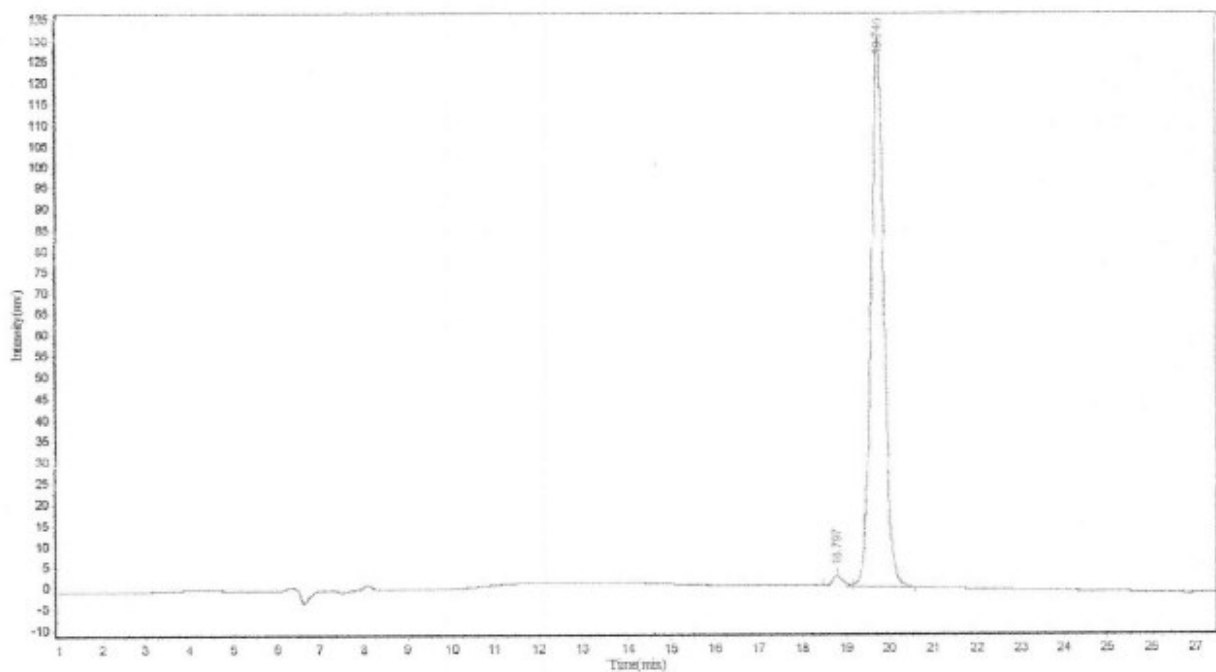




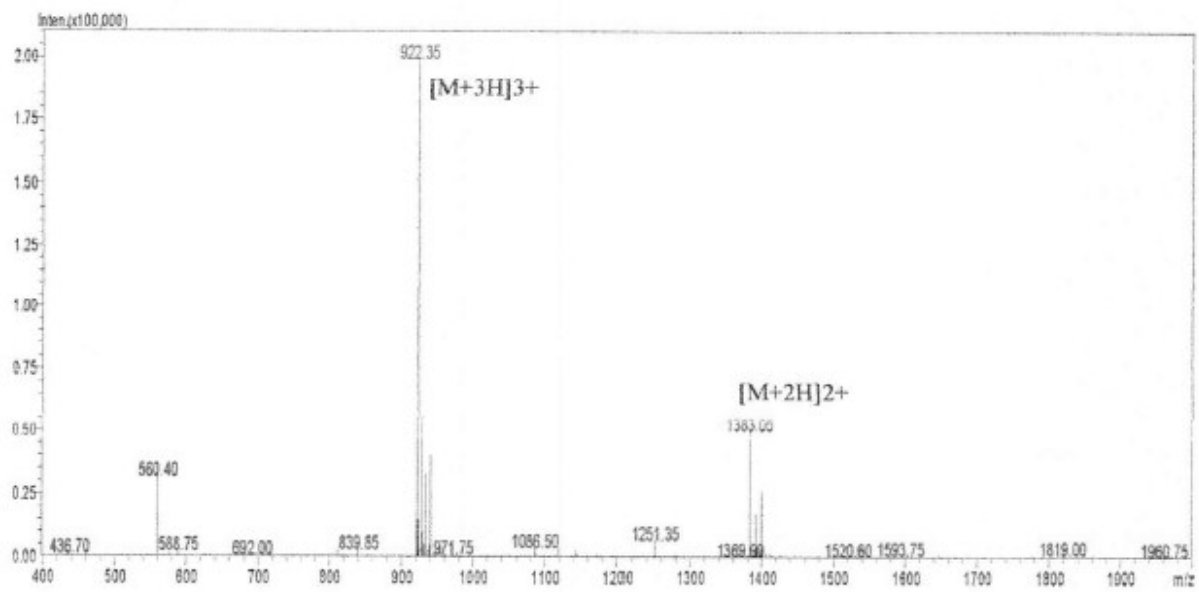
**Figure S6.** Characterization of the split-H4 peptide SH4. (A) SH4 CD spectrum with positive ellipticity at 223 nm indicative of triple-helical fold. (B) Thermal denaturation monitored by CD at 225 nm indicates a melting temperature of 36 $^{\circ}\text{C}$ , indicating peptide is folded under experimental conditions in this study. (C) TEM micrograph shows nanodisc morphology for SH4.



**Figure S7.** TEM micrographs of H-series CMPs combined with COL1. All scale bars are 100 nm.



**Figure S8.** Analytical HPLC chromatogram of SH4 purification.



**Figure S9.** Mass spectrometry characterization of SH4 (theoretical MW 2764.12 Da)

## Supplementary References

1. McGuinness, K., I.J. Khan, and V. Nanda, *Morphological diversity and polymorphism of self-assembling collagen peptides controlled by length of hydrophobic domains*. ACS Nano, 2014. **8**(12): p. 12514-23.
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3. Drzewiecki, K.E., D.R. Grisham, A.S. Parmar, V. Nanda, and D.I. Shreiber, *Circular Dichroism Spectroscopy of Collagen Fibrillogenesis: A New Use for an Old Technique*. Biophys J, 2016. **111**(11): p. 2377-2386.
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