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Supporting Information

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Supporting Information: Nanofibers and Lyotropic Liquid Crystals from a New Class of Self-Assembling β-Peptides

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Materials. Fmoc-(S,S)-*trans*-2-aminocyclohexanecarboxylic acid (Fmoc-S,S-ACHC) was prepared by the method outlined by Schinnerl et al[1] Fmoc-(S)-β^3-amino acids were prepared from their corresponding α-amino-acids (Novabiochem),[2] or purchaced from Peptech. Biotech grade DMF was purchased from Aldrich and stored over 50W-X8 DOWEX ion-exchange resin. Methanol, CH_2Cl_2, tetrahydrofuran, and acetonitrile were purchased from Burdick and Jackson. *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, and NovaSyn TGR® resin (0.25 mmol/g loading) were purchased from Novabiochem. iPr_2EtN was distilled from calcium hydride. All other reagents were purchased from Aldrich and used without purification.

RP-HPLC (Reverse Phase-High Pressure Liquid Chromatography)

All β-peptides were purified via RP-HPLC on a Vydac C18 semipreparative column using a flow rate of 3 mL/min. Solvent A and Solvent B for RP-HPLC were 0.1% trifluoroacetic acid (TFA) in Millipore water and 0.1% TFA in acetonitrile, respectively. β-peptide purity was assessed using a Vydac C18 analytical column using a flow rate of 1 mL/min from 10-60% B over 50 minutes monitoring at 220 and 273 nm.

MALDI-TOF-MS (matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry) data were collected on a Bruker REFLEX II spectrometer with a 337 nm laser using α-cyano-4-hydroxycinnamic acid as matrix. Measurements were calibrated using peptide standards angiotensin I ($M + H^+$) = 1296.7 and neurotensin ($M + H^+$) = 1672.9.

Optical Microscopy

β-Peptides were weighed into eppindorf tubes, diluted to the desired concentration with water, and left on an oscillatory shaker overnight to ensure dissolution. β-peptide solutions were then drawn into a 2 μL microcapillary (0.03 in. O.D., 0.011 in. I.D.; Drummond), and the ends were sealed with a high viscosity vacuum grease (Dow-Corning) Microcapillaries were placed on a glass slide and imaged on an Olympus BX-60 microscope (Tokyo, Japan) in transmission mode between crossed polarizing filters using a digital camera (Olympus C2020 Zoom). To assess the upper limit of concentration, a 1 μL droplet of a concentrated peptide solution was allowed to slowly evaporate while being monitored for birefringence.

Circular dichroism (CD) analysis

Samples were prepared by weighing lyophilized β-peptides into Eppendorf tubes and dissolving in Millipore water to yield a 2 wt % solution (11 mM) which was aliquoted and further diluted to the desired concentrations. The final concentration of each β-peptide solution was determined from the UV absorbance of a solution of known dilution. The extinction coefficient of each β-peptide at 275 nm was estimated to be 1420 cm^{-1} mol^{-1}, based on the extinction coefficient of α-tyrosine.[3] CD spectra were recorded on an Aviv 202SF spectrometer at room temperature using a 1 mm path, 0.1 mm, or 0.01 mm path length cell and 3 second averaging times. The CD signal resulting from the water alone was subtracted from the spectrum of each β-peptide solution. Data were converted to ellipticity (deg cm^2 $dmol^{-1}$) according to the equation:

$$[\Theta] = \Psi / (1000nlc),$$

where Ψ is the CD signal in degrees, n is the number of amides, l is the path length in centimeters, and c is the concentration in decimoles per cm^3.

Cryo-transmission electron microscopy (TEM)

A small quantity of the sample solution (typically 3 μL) was applied to a TEM copper grid with holey carbon film purchased from Quantifoil (400 mesh Cu grid, 1.2 μM hole diameter), and blotted with filter paper using a Vitrobot type FP 5350/60 under 100% relative humidity for two seconds to create a thin layer of sample on the surface of the grid. The grid was plunged into liquid ethane and quickly transferred to liquid nitrogen. The sample was analyzed using JEOL 2010 TEM at an accelerating voltage of 200 kV under low-dose imaging conditions.

Small Angle X-ray Scattering

Small Angle X-ray Scattering (SAXS) measurements were performed at Bruker AXS (Fitchburg, WI) using a Bruker NanoStar® instrument equipped with a sealed tube source to generate copper K_α X-rays (λ =0.154nm). The X-ray beam was focused through a pair of cross-coupled Göbel mirrors and collimated by a three-pinhole system with diameters of 750μm, 400μm and 1000μm placed at distances of 1125mm, 1607mm and 1642mm from the source, respectively. The first two pinholes define the divergence and the beam profile, the third acts as an anti-scattering pinhole which helps to reduce the background scattering. The diffraction pattern was recorded on a Bruker AXS HI-STAR position sensitive area detector. A beam stop of diameter 3.0mm was placed in front of the detector. The sample to detector distance was 655mm giving access to a q (scattering vector) range from 0.008$Å^{-1}$ to 0.3$Å^{-1}$. The scattering wave-vector q is related to the wavelength of the X-rays, λ, and the scattering angle, 2θ, by the relationship $q=4\pi\sin\theta/\lambda$.

β-Peptides were weighed into microcentrifuge tubes, diluted to the desired concentration with water, mixed under vortex and left overnight to ensure dissolution. Samples were mixed under vortex immediately prior to measurement to ensure a homogeneous sample. A sample of β-peptide liquid crystal (approximately 30uL) was placed in the central section of a fixed quartz capillary (diameter 1mm) directly in the path of the X-ray beam in order to minimize the amount of material required for measurement. The capillary was cleaned thoroughly and dried under vacuum prior to use. The sample-containing fixed capillary was sealed prior to measurement in an evacuated chamber to prevent evaporation. The sample temperature was maintained at 25°C for the duration of measurement. The data were azimuthally averaged and corrected for detector efficiency, empty capillary scattering and background radiation. The transmission of each sample was determined by comparison with a previously calibrated polymeric standard. This data processing was performed using the software SAXS for Windows provided by Bruker AXS.

General procedure for the microwave-assisted solid phase synthesis of *β*-peptides. All 14-helical *β*-peptides were synthesized on solid phase in a CEM MARS microwave reactor. Microwave irradiation used a maximum power of 600 W. Reaction mixtures were agitated by magnetic stirring during irradiation. Reaction temperature was monitored using a fiberoptic temperature sensor. Coupling and deprotections used the following conditions: couplings: (600 W maximum power, 80^{o}C, ramp 2 minutes, hold 4 minutes); deprotections: (600 W maximum power, 90^{o}C, ramp 2 minutes, hold 2 minutes). For difficult couplings[4] an additional temperature ramping cycle was included: (600 W maximum power, 80^{o}C; ramp 2 minutes, 0W, 25^{o}C; 10 minutes hold, 3x).

Representative example of microwave-assisted synthesis of *β*-peptide (A). *β*-peptide **A** was synthesized on a 10 μmol scale on NovaSyn TGR® resin in a microwave reactor (CEM, MARS system). All coupling and deprotection reactions were carried out at atmospheric pressure under microwave irradiation as described above. Prior to coupling, the resin was swelled in CH_2Cl_2 in a solid phase extraction tube (Alltech). The resin was washed 3 times with DMF. In a separate vial, Fmoc-*β*-amino acid (30 μmol) was dissolved in 400 μL of DMF and activated with *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 60 μL of 0.5 M solution in DMF), 1-hydroxybenzotriazole monohydrate (HOBT, 60 μL of 0.5 M solution in DMF), and iPr_2EtN (60 μL of 1.0 M solution in DMF). The coupling solution was vortexed, added to the resin, and the mixture was irradiated at 80°C as described above. The resin was washed (3 × DMF, 3 × CH_2Cl_2, and 3 × DMF). Deprotection solution (750 μL of 20% piperidine in DMF (v/v)) was added to the resin, and the mixture was irradiated at 90°C and washed as before. All ACHC residues were double-coupled and double-deprotected. Difficult coupling reactions for ACHC-4 was subjected to the temperature ramping cycle immediately following the second coupling. The coupling/deprotection cycles were repeated until the last residue was deprtoected. The *β*-peptide was cleaved from the resin in a mixture of 95/2.5/2.5

TFA/H_2O/triisopropylsilane for 2 hr, followed by evaporation of the solvent under a nitrogen stream. The crude *β*-peptide was then purified by RP-HPLC and lyophilized to yield a white powder.

β-peptide **A**: 29.5-39.5% B over 20 min. MALDI-TOF-MS (*m/e*) calculated for ($C_{82}H_{122}N_{14}O_{11}$; M =1479.0); found: ($M+H^+$) = 1479.6; ($M+Na^+$) = 1501.7.

β-peptide ***iso*-A**: 42-52% B over 20 min. MALDI-TOF-MS (*m/e*) calculated for ($C_{82}H_{122}N_{14}O_{11}$; M = 1479.0); found: ($M+H^+$) = 1479.7; ($M+Na^+$) = 1501.7; ($M+K^+$) = 1517.7.

β-peptide **B**: 34-44% B over 20 min. MALDI-TOF-MS (*m/e*) calculated for ($C_{82}H_{140}N_{14}O_{11}$; M =1497.08); found: ($M+H^+$) = 1497.7 ($M+Na^+$) =1519.7 ($M+K^+$) = 1524.6

β-peptide **C**: 23-33% B over 20 min. MALDI-TOF-MS (*m/e*) calculated for ($C_{64}H_{110}N_{14}O_{11}$; M =1250.8); found: ($M+H^+$) = 1251.7 ($M+Na^+$) =1273.7 ($M+K^+$) = 1289.9

β-peptide **D**: 29.5-39.5% B over 20 minutes MALDI-TOF-MS (*m/e*) calculated for ($C_{81}H_{117}N_{13}O_{13}$; M = 1479.9); found: ($M+H^+$) = 1480.9; ($M+Na^+$) = 1502.9; ($M+K^+$) = 1518.9; ($M+2Na^+$) = 1524.9

β-peptide **E**: 29-39% B over 20 minutes MALDI-TOF-MS (*m/e*) calculated for ($C_{81}H_{117}N_{13}O_{13}$; M =1478.9); found: ($M+H^+$) =1480.1; ($M+Na^+$) =1502.1; ($M+K^+$) =1518.0

Structural analysis of β-peptides using circular dichroism (CD)

We undertook circular dichroism (CD) measurements to determine whether β-peptides remain 14-helical under conditions approaching those required for the nanofiber formation. To test whether our β-peptides change conformation at high concentration we looked for significant deviations from the 14-helical CD signature, which is characterized by a minimum near 214 nm.[5] β-Peptides **A** and ***iso*-A** displayed strong 14-helical signatures up to 2 wt % (11 mM, Figure S1), the concentration at which nanofibers begin to form for **A**. Interestingly, β-Peptide ***iso*-A** exhibits a slight blue shift in its global minimum to 210 nm. Similar distortions of globally amphiphilic β-peptides have been observed by both Daniels et al.[6] and Cheng and Degrado.[7] Unfortunately, these β-peptides could not be analyzed by CD at higher concentrations because of the high absorption of light by the amide chromophores, which results in loss of signal. A CD spectrum was also recorded for β-peptide **D** (0.8 wt %, 4.4 mM), which is just below the minimum LC concentration. β-Peptide **D** at this high concentration displays a broad minimum near 214 nm (Figure S1), which is similar to the CD spectrum of a dilute solution of **D** (0.05 wt %, 0.28 mM, Figure S2). These results suggest that nanofiber formation results from self-assembly of non-globally amphiphilic β-peptides in the 14-helical conformation and argue against a change in β-peptide folding upon self-assembly.

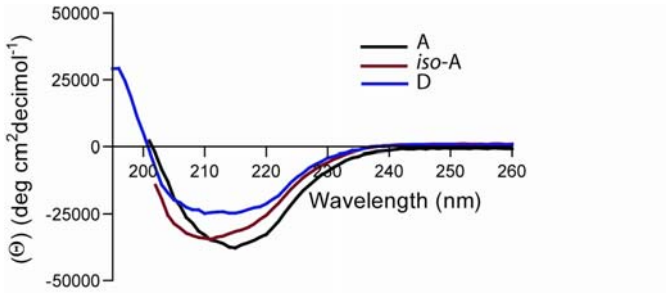

Figure S1) Circular dichroism spectra of β-peptides **A**, (2 wt %, 11 mM), ***iso*-A**, (2 wt %, 11 mM) and **D**, (0.8 wt %, 4.4 mM)

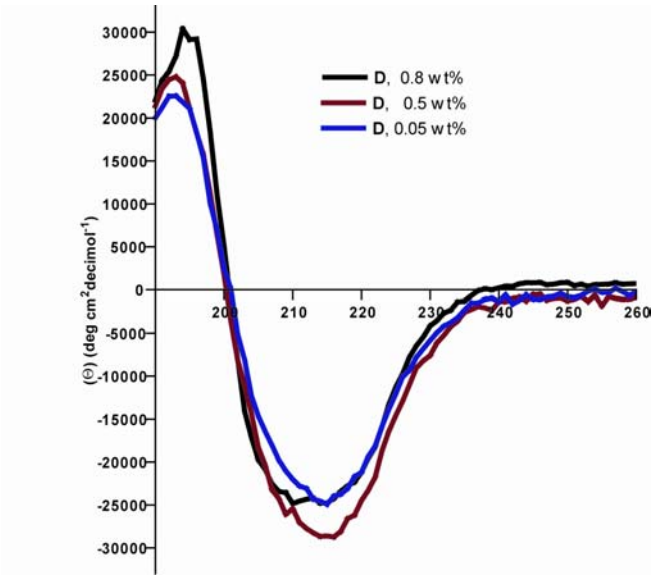

Figure S2) Circular dichroism spectra of β-peptide **D** at various concentrations, black line, **(**0.8 wt %, 4.4 mM), red line, (0.5 wt %, 2.8 mM), and blue line, (0.05 wt %, 0.28 mM).

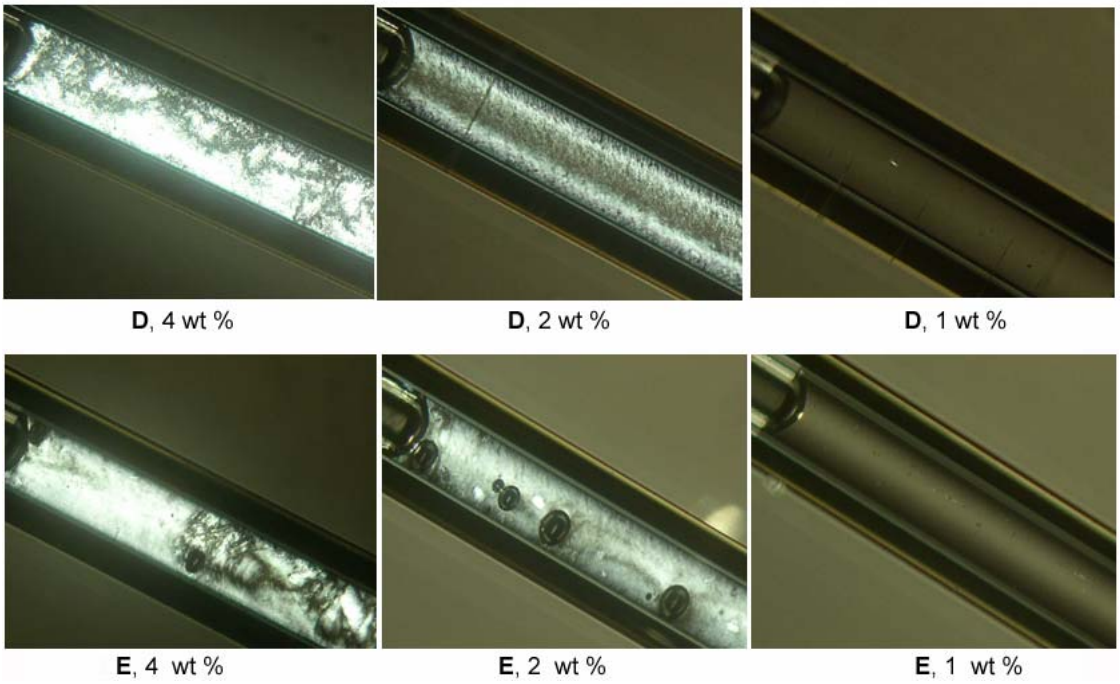

Figure S3) Optical micrographs of solutions of β-peptide **D** and **E** between crossed polarizing filters at several concentrations. The lowest concentration where birefringence was observed was determined to be the minimum LC concentration, (2 wt %, 11 mM).

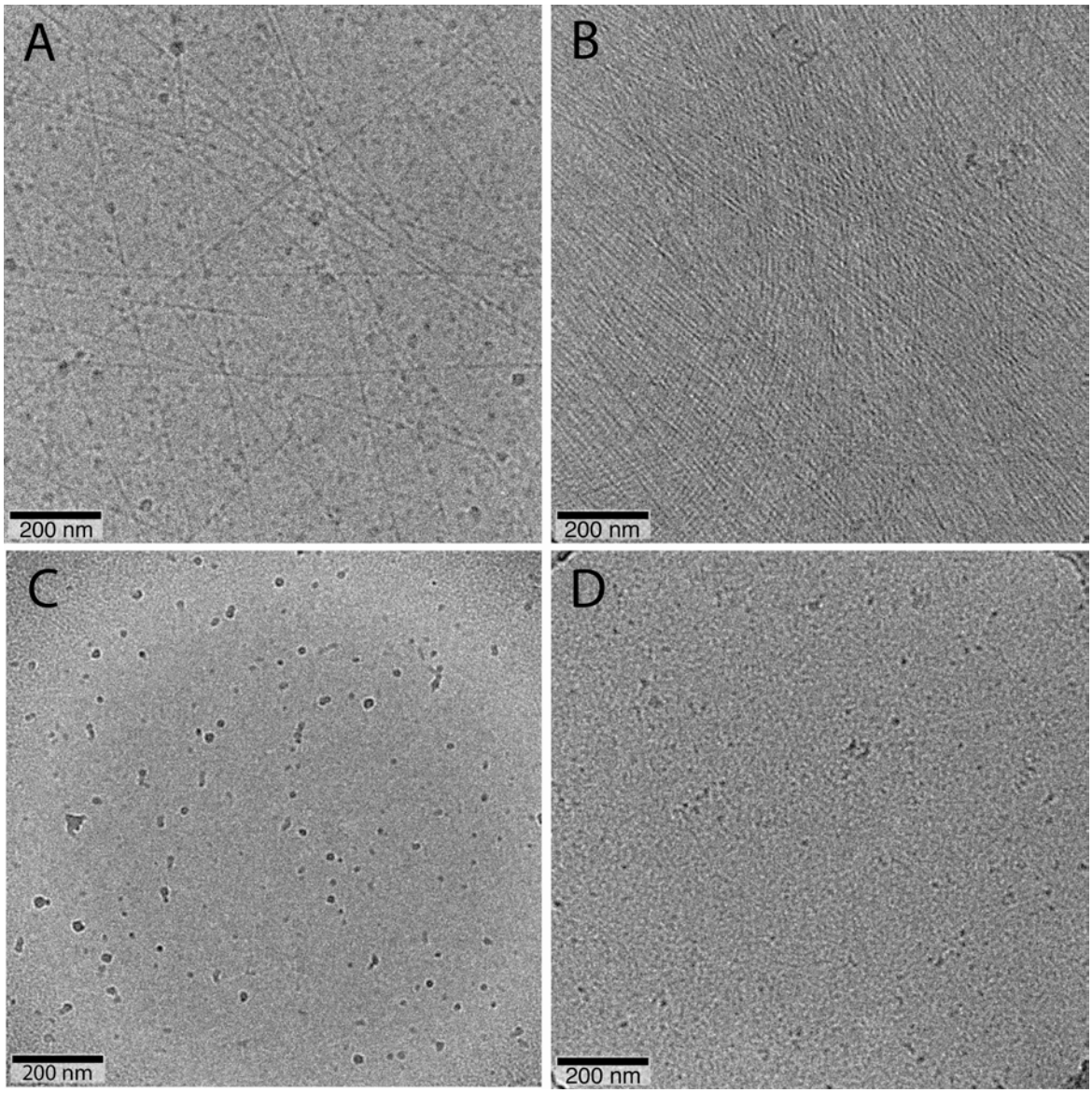

Figure S4) Enlarged Cryo-TEM micrographs of **A** and ***iso*-A** A) Cryo-TEM micrograph of **A, (**2 wt %, 11 mM B) **A, (**8 wt %, 45 mM) C) ***iso*-A, (**2 wt %, 11 mM). and D) ***iso*-A, (**8 wt %, 45 mM).

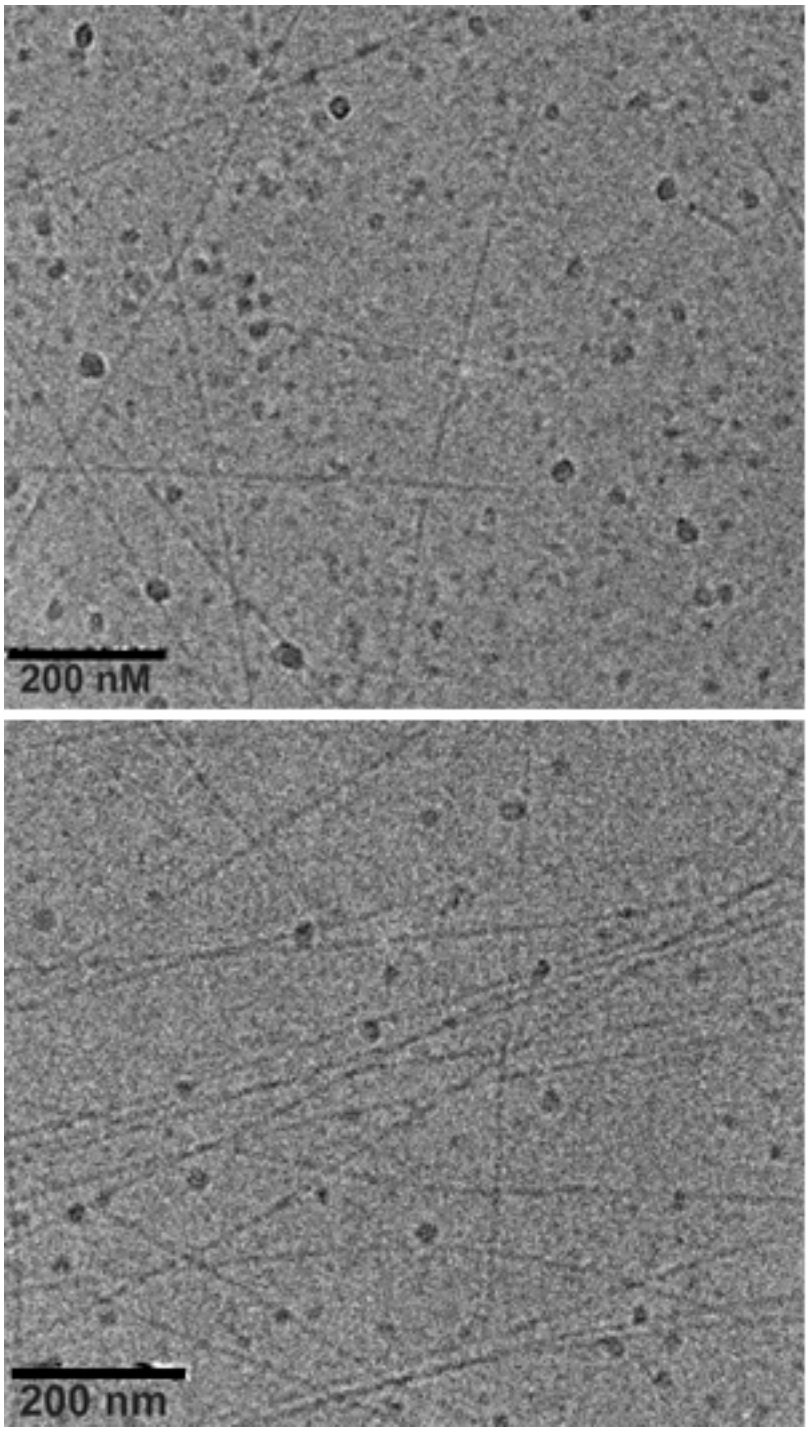

Figure S5) Enlarged Cryo-TEM micrographs of additional images of **A,** (2 wt %, 11 mM). Micrographs indicate a consistent co-existence of nanofibers and globular aggregates.

Aggregate Size Determination

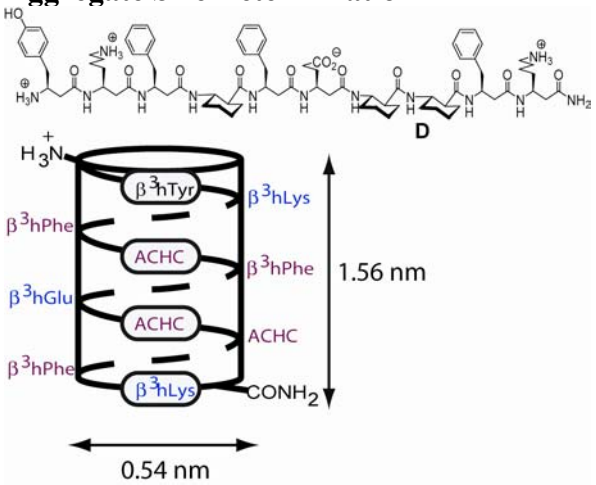

Figure S6) β-peptide **D**, idealized as a cylindrical solid

Aggregate size was determined using the calculations put forth by Onsager for hard rods.[8] This aggregate size is only a conservative estimate as it is based on a fiber one β-peptide wide stacked end-to-end. Published values of helical diameter (0.54 nm) and length 10 * (0.156 nm/residue) were used to determine the molecular volume where the side chains were ignored.[5] The minimum aggregate number was assessed by assuming a high-aspect ratio fiber with diameter equal to the width of one β -peptide, 0.54 nm. A derivation of the minimum aggregate number for a 2 wt % solution of **D** is shown below.

$C_{iso} > 3.34$ = minimum LC concentration
$C_{iso} = L/D * Phi$
L = length of the molecular aggregate
D = diameter of the molecular aggregate
Phi = molecules/volume * volume of cylinder
L = 3.34 * D/Phi
L/1.56 nm per β-peptide = minimum aggregate number.

$3.34 * 0.54/(1.12E^{-}5 \text{ molecules/cm}^3) * 0.357 \text{ nm}^3 * 1\text{cm}^3/1E21 \text{ nm}^3 = L$
L = 748

Minimum aggregate number = 748/1.56 nm per β-peptide
= 480 β-peptides

References:

[1] M., Schinnerl, J. K. Murray, J. M.; Langehan; S. H. Gellman, *Eur. J. Org. Chem.* **2003**, 721-6.

[2] D. Seebach, D.; M. Overhand; F. Kühnle, N. M. Florina; B. Martinoni; L. Oberer; U. Hommel; H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913-41.

[3] Creighton, T. E. *Proteins: Structures and Molecular Principles, 2nd ed.*; W. H. Freeman and Company: New York, 1993; p 14.

[4] J. K. Murray; S. H. Gellman, *Org. Lett.* **2005**, *7*, 1517-20.

[5] R. P. Cheng; S. H. Gellman; W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219-32.

[6] D. S. Daniels; E. J. Petersson; J. X. Qiu, *J. Am. Chem. Soc.* **2007**, *129*, 1532-3.

[7] R. P. Cheng; S. H. Gellman; W. F. DeGrado, *J. Am. Chem. Soc.* **2002**, *124*, 11564-65.

[8] L. Onsager, *Ann. NY Acad. Sci.* **1949**, *51*, 627-59