Supplementary material

Acknowledgements

We thankAgResearch, Lincoln, New Zealand for partial funding of this work, and in particular to Jolon Dyer, Duane Harland and James Vernon for helpful comments and assistance with electron microscopy. We are thankful to Maite Paternostre (Institute of Biology and Technology of Saclay, France) for helping with FT-Raman spectroscopy.

S1. Experimental procedures

S1.1 Material

The four peptides were purchased from Mimotopes (Melbourne, Australia) with a purity > 95% and as acetate salts. Thioflavin T and uranyl acetate were purchased from Sigma. Fresh purified water obtained from a Millipore filtration system was used for sample preparation.

S1.2 Polarised light optical microscopy

Optical microscopy observations were performed using a Leica MZ75 microscope, at magnifications up to x100, on samples conditioned in thin layers between glass slides and coverslips. The polarizer and analyzer were in a fixed crossed position.

S1.3 Negative stain transmission electron microscopy

Carbon-coated Formvar 200-mesh copper grids (ProSciTech, Australia) were deposited successively for one minute each onto 15 μL drops of (i) peptide aqueous solutions, (ii) water (3 times) and (iii) a 2% w/w uranyl acetate solution in water. Filter paper was used to remove the excess liquid from the grids, which were then left to dry for a few hours before electron microscopy observations.

The grids were examined with a FEI Morgagni 268D transmission electron microscope operating at 80 kV, with magnifications up to x 110k. Micrographs were captured using a SIS/Olympus Megapixel III digital camera mounted above the phosphor screen.

S1.4 Circular dichroism

Far UV circular dichroism spectra were collected with a Jasco J-815 spectrometer at room temperature, using 10 mm path quartz cuvettes.

S1.5 Fourier-transform Raman spectroscopy

Fourier transform (FT)-Raman spectra were recorded at 4 cm^{-1} resolution using a Bruker IFS 66 interferometer coupled to a Bruker FRA 106 Raman module equipped with a continuous neodymium-doped yttrium aluminum garnet laser providing excitation at 1064 nm, as described in Mattioli *et al*[1]. All spectra were recorded at room temperature with backscattering geometry from concentrated samples held in standard aluminum cups. The spectra resulted from 1000 to 5000 co-added interferograms depending on the sample signal.

[1] Mattioli TA, Williams JC, Allen JP, Robert B. Changes in primary donor hydrogen-bonding interactions in mutant reaction centers from *Rhodobacter-Sphaeroides* – identification of the vibrational frequencies of all the conjugated carbonyl groups. *Biochemistry* 1994; **33**(7): 1636–1643.

S1.6 Thioflavin T fluorescence binding assays

The peptides were dissolved in a solution of Thioflavin T (ThT) in water, so that the final concentrations in each sample were 25 μM ThT and 20-30 mg/mL of peptide acetate. The other peptide concentrations resulted from dilutions with 25 μM ThT in water. The initial samples were left to equilibrate at 4°C for 24 h before recording fluorescence intensity. For the samples resulting from dilutions, fluorescence intensity was recorded just after dilution and after 24 h equilibration at 4°C.

Fluorescence intensity was measured from 50 μL samples in 96 well plates, with a Molecular Devices SpectraMax M5 fluorimeter equipped with a plate reader. The fluorescence intensity values resulted from the average of 15 recordings for each sample. Samples were excited at 440 nm, and fluorescence intensity was recorded at 482 nm, using a 25 μM ThT solution in water as a blank.

S2. Survey of the protein databank

The protein data bank was systematically searched with the word "homodimer". These results were filtered as described in the article. The 21 structures in Table S1 were considered to perform statistics on the sequences of the corresponding β-continuous interfaces (Figure S1). These statistics show that 90% of these sequences contain 11 or fewer residues (Figure S1-A), that 80% of the sequences contain at least one aromatic residue (Figure S1-B), that all the sequences include at least 30% of hydrophobic residues (Figure S1-C) and at least 15% of charged residues (Figure S1-D).

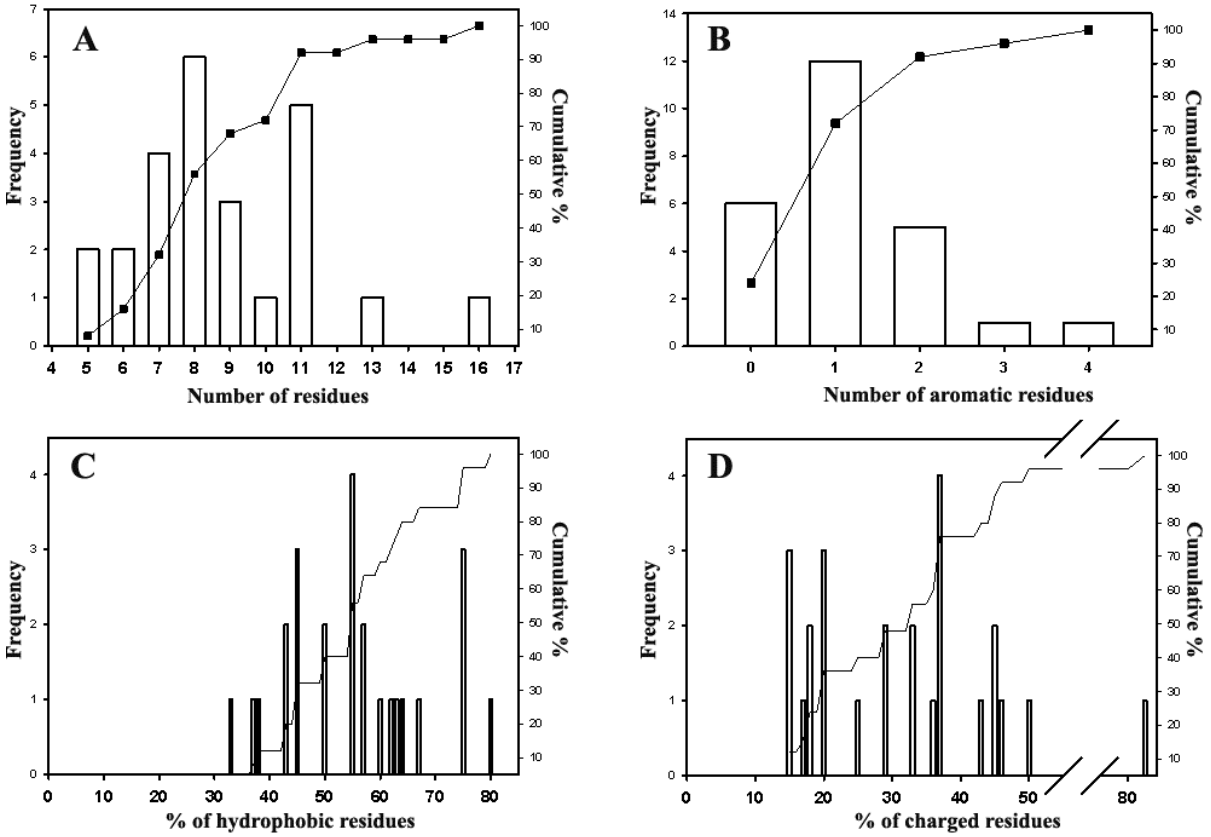

Figure S1. Statistics performed on the composition of the sequences of the β-continuous interfaces of the protein oligomers from table S1. (A) Number of residues of the sequences; (B) Number of aromatic residues; (C) percentage of hydrophobic residues in the sequences; (D) percentage of charged residues.

Table S1. Examples of β-continuous interfaces from the protein data bank

Protein	PDB code	Type of interface	Interface sequence*	N(residues)	N(aromatic)	%hydrophobic	%charged
Peroxiredoxin 3 (bovine)	1ZYE	dimer	IKHLSVN	7	1	57	43
B-Lactoglobulin (bovine)	2AKQ, 2Q39	dimer	MHIRLSFN	8	2	62	37
Diaminopimelate decarboxylase (*M. tuberculosis*)	2O0T	tetramer	HEFISTAH	8	3	75	37
Umud' (E.Coli)	1I4V	dimer	IHVVKAMR	8	1	75	37
PlyG cell wall binding domain	2L48	dimer	ADFIL	5	1	80	20
CRO repressor (bacteriophage λ)	2CRO	dimer	AEEVKPFP	8	1	37	37
Dynein light chain km23	1Z09	dimer	TFLRIRS	7	1	57	29
GTPase-activating protein MglB (*Myxococcus Xanthus*)	3T1R	tetramer	QQEVHQ	6	1	33	83
A-Crystallin domain in αβ-Crystallin oligomers	2KLR	dimer	FISREFHRKYR	11	4	45	45
K-Bungarotoxin (*Bungarus multicinctus*)	1KBA	dimer	YRSLLCCTT	9	1	55	11
Galectin-1 (strand1) (human)	2KM2	dimer	KCVAF	5	1	75	20
Galectin-1 (strand2) (human)	2KM2	dimer	CGLVASN	7	0	43	14
Filamin A dimerisation domain (human)	3CNK	dimer	CEEILVKH	8	1	50	50
DSY0195(21-82) protein (*Desulfitobacterium hafniense*)	2KYI	dimer	QFLSLT	6	1	67	17
ZO2-PDZ2 domain-swapped dimer (strand 1) (*Zonula occludens*)	2OSG	dimer	MIGVLLMKSR	10	0	60	20
ZO2-PDZ2 domain-swapped dimer (strand 2) (*Zonula occludens*)	2OSG	dimer	EYGLRLGSQIFVKEMT	16	2	50	15
Histidine triad protein BLR8122 (*Bradyrhizobium japonicum*)	3I4S	dimer	KLNIAALGN	9	0	55	33
3-Isopropylmalate dehydrogenase with mutations at the C-terminus (*Thermus thermophilus* HB8)	1DR8	dimer	EAEAWNTERYSKP	13	2	38	46
Monocyte chemoattractant protein-1 (human)	1DON	dimer	NAPVTCCYNFT	11	2	45	18
Secretion chaperone CsaA (*Agrobacterium Tumefaciens*)	2Q2I	dimer	IVLAAVERPVP	11	0	64	18
Myomesin-1 domain 12 and 13 (human) (strand1)	2R15	dimer	LPDVVTIQ	8	0	63	25
Murine soluble epoxide hydrolase (*Mus musculus*)	1CQZ	dimer	NDVSHGYVT	9	2	55	33
Siroheme biosynthesis protein MET8 (*Saccharomyces cerevisiae*) – strand1	1KYQ	dimer	VKSLQLAHQLK	11	1	55	45
Siroheme biosynthesis protein MET8 (*Saccharomyces cerevisiae*) – strand2	1KYQ	dimer	DRLQILISTNG	11	0	45	36
Lsr2 protein dimerization domain (strand1) (*Mycobacterium tuberculosis*)	4E1R	dimer	VEFGLDG	7	1	43	29

*sequence determined with the help of the program PISA

** number of aromatic residues in the sequences if considering histidine, or not (into brackets)

S3. Birefringence of Peptide 3 samples

No optical texture could be obtained for peptide 3 samples. However, birefringence was observed under polarised light (Figure S3).

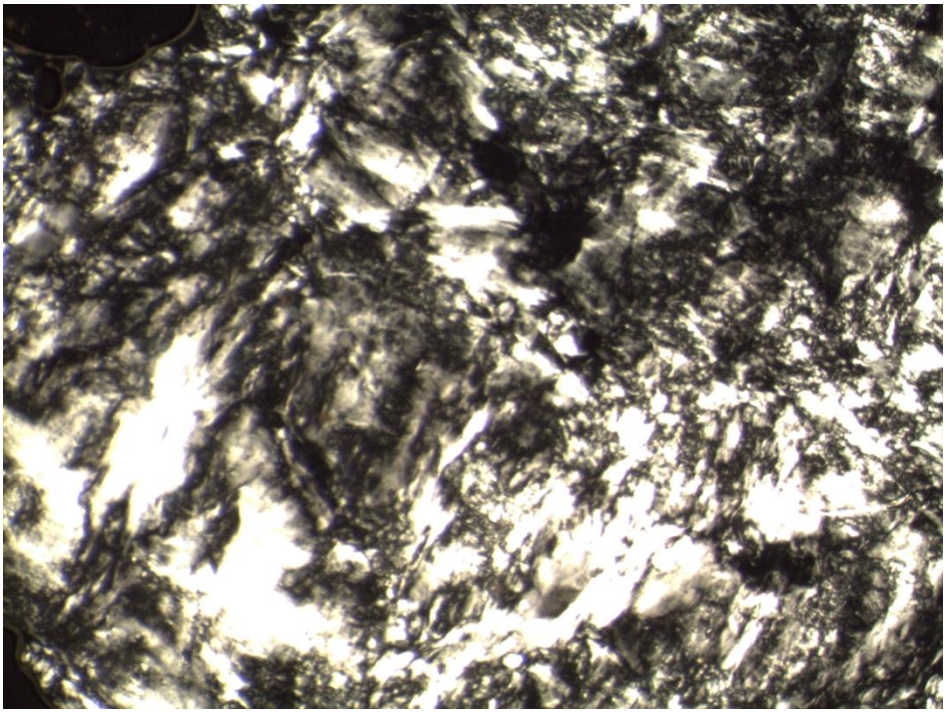

Figure S3. Birefringence (polarised light) of peptide 3 samples (peptide designed from the tetrameric β-continuous interface of diaminopimelate decarboxylase from TB). Magnification x100.

S4. Morphological and conformational analysis under high pH conditions

The assembly properties of the four peptides were investigated in 100 mM imidazole pH 8. This buffer was chosen to ensure the deprotonation of the histidine residues present in the peptide sequences. The four peptides were found to form nanostructures of similar elongated morphologies as in pure water (Figure S4-1). These structures were found to bind ThT, as shown by the enhanced fluorescence of the dye (Table S4-1), thus supporting the hypothesis that the peptides form extended β-sheet networks. Although the absorbance of imidazole was a limiting factor for circular dichroism studies[2], CD spectra consistent with β-sheet conformations were obtained for the four peptides through the weak negative shoulders recorded between 230 nm and 250 nm (Figure S4-2). Below these wavelengths, the far-UV absorbance of imidazole covered the signal, as previously reported[2].

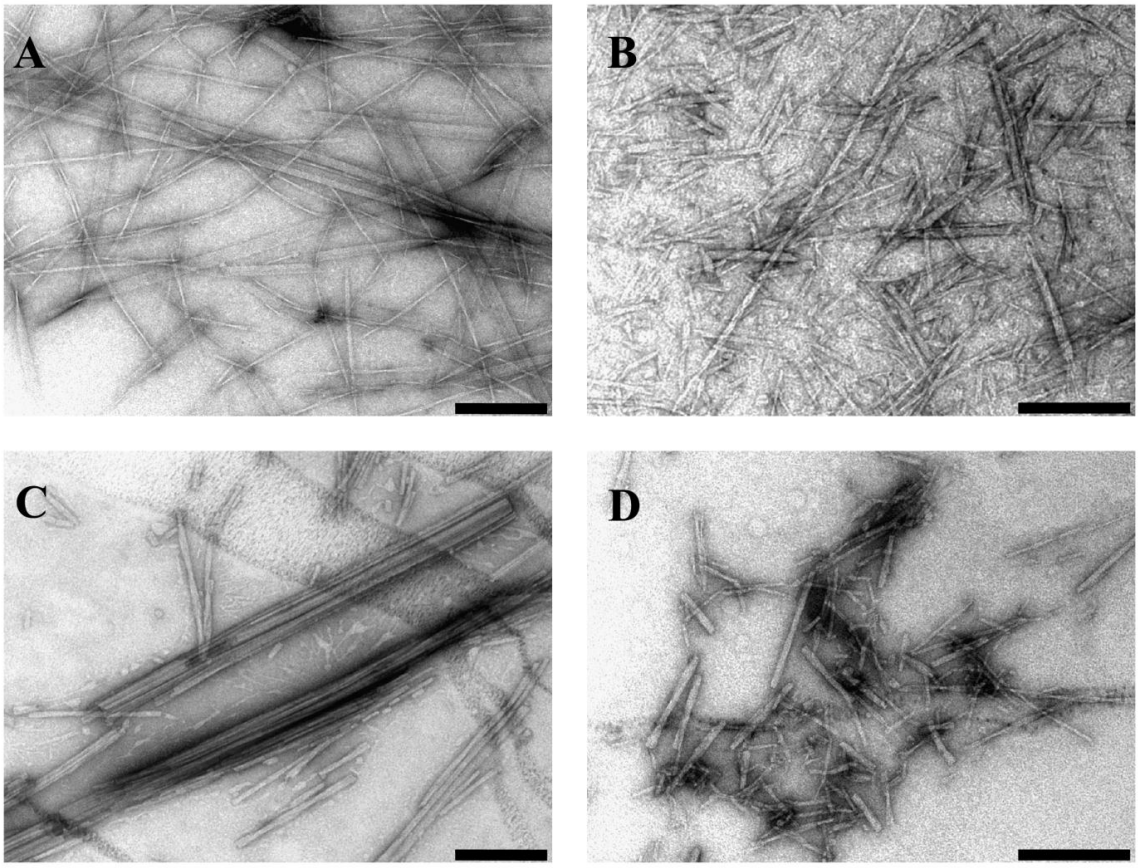

Figure S4-1. Electron micrographs of negatively stained peptide samples in 100 mM imidazole pH 8, equilibrated for 24h at 4°C. A: Prx3 peptide at 10 mg/mL; B: β-lactoglobulin peptide at 10 mg/mL; C: diaminodecarboxylase peptide at 25 mg/mL; D: umud' peptide at 50 mg/mL. Scale bars correspond to 200 nm.

Table S4-1. Relative ThT fluorescence intensity in 100mM imidazole pH 8.

Peptide	Concentration (mg/mL) in 100mM imidazole pH 8	Relative ThT fluorescence intensity (a.u.)
Peroxiredoxin peptide (peptide 1)	10	423
β-Lactoglobulin peptide (peptide 2)	10	445
DAPDC peptide (peptide 3)	25	248
Umud' peptide (peptide 4)	25	1020

[2] S. M. Kelly, T. J. Jess and N. C. Price, *BBA-Proteins Proteomics*, 2005, **1751**, 119-139.

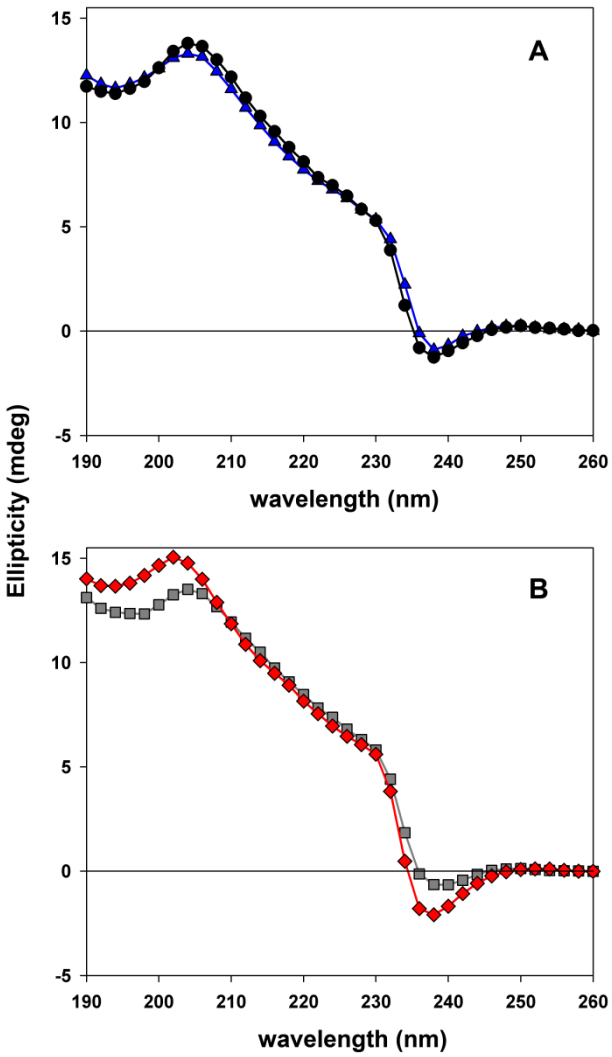

Figure S4-2. Circular dichroism of peptide samples at 0.1mg/mL in 100 imidazole pH 8, equilibrated for 24 h at 4°C. A: Prx3 peptide (black circles) and β-lactoglobulin peptide (blue triangles). B: diaminodecarboxylase peptide (red diamonds) and umud' peptide (grey squares).

S5. TEM analysis of the nanostructure reversibility

In addition to the ThT analysis shown in the article, nanostructure reversibility by dilution was further characterised by TEM analysis (Figure S5). No nanostructure could be observed for peptides 1, 3 and 4 at concentrations of 0.5 mg/mL in pure water obtained by dilution of initial 20 mg/mL samples. For peptide 2 (β-lactoglobulin peptide) in pure water, lower concentrations had to be achieved to observe the absence of nanostructures. A few small globular aggregates could be observed for peptide 2 at 0.08 mg/mL, likely corresponding to nanostructure precursors.

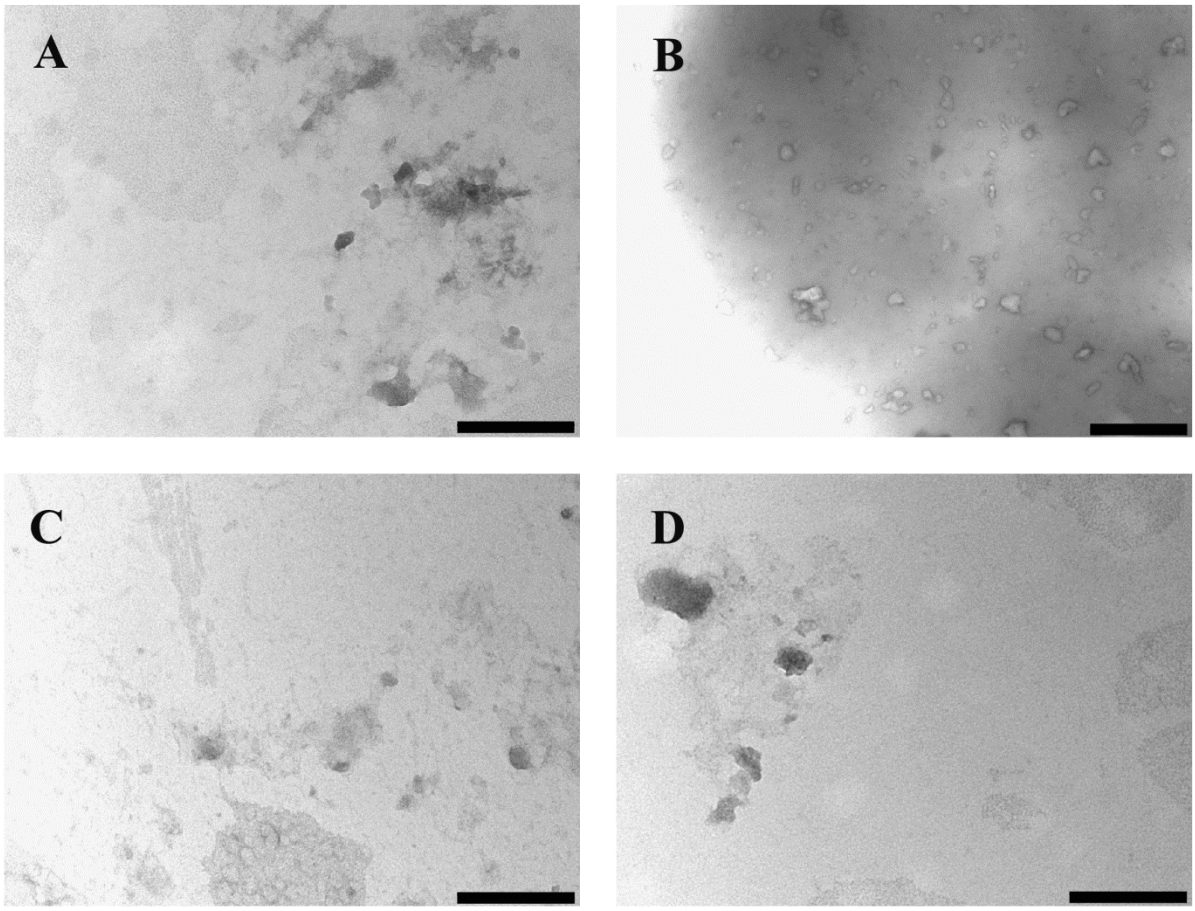

Figure S5. Electron micrographs of negatively stained samples prepared by dilution of 20 mg/mL in water initial peptide samples, both equilibrated for 24 h at 4°C. A: Prx3 peptide at 0.5 mg/mL in water showing no nanostructure; B: β-lactoglobulin peptide at 0.008 mg/mL showing no nanostructure but small aggregates; C: diaminodecarboxylase peptide at 0.5 mg/mL showing no nanostructure; D: Umud' peptide at 0.5 mg/mL showing no nanostructure. Scale bars correspond to 200 nm.