Supporting Information

Modular Design of Peptide Fibrillar Nano- to Microstructures

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Abbreviations

DIPEA – diisopropylethylamine, EDT – 1,2-ethanedithiol, Fmoc – 9-fluorenylmethoxycarbonyl, HBTU – *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate; EPPS – 3-[4-(2-hydroxyethyl)-1-piperazinyl] propanesulfonic acid; RP-HPLC – reversed phase high pressure liquid chromatography; MALDI-ToF – matrix-assisted laser desorption/ionization time of flight; MOPS - (3-(N-morpholino)propanesulfonic acid; TCEP – (tris(2-carboxyethyl)phosphine); TIS – triisopropyl silane; TFA – trifluoroacetic acid.

Design Notes

In Constructs IVa and IVb, D-Val residues were used instead of D-Ile residues. Valine at ***a*** positions of the heptad repeat directs the same oligomerization state as isoleucine, but avoids the complicating side-chain chirality. In Construct VI cysteine residues occupy ***b*** and ***c*** positions which form the exterior or solvent-exposed faces of individual coiled-coil fibrils. The fibrils bundle up through the association of these faces. Similar to alanines, cysteines are sufficiently small to allow close contacts between the coiled-coil exteriors. However, unlike alanines, cysteine residues can react with the formation of disulphide bonds. This leads to the cross-linking or locking of the faces thus limiting the surface area of the fibrils and subsequently their thickening. Peptide sequences of all constructs are given in Table S1.

Experimental Notes

Peptide synthesis. Peptides were synthesized using standard solid phase Fmoc-based protocols. Wang resins pre-loaded with C-terminal amino acids were used. Amino-acid couplings were performed with HBTU/DIPEA. Synthesized peptides were purified by RP-HPLC following deprotection (95% TFA, 2.5% TIS, 2.5% water for modules I-IV and V; and 95% TFA, 5% EDT for module VI) and work up. The identities of the peptides were confirmed by analytical RP-HPLC and MALDI-ToF:

MS $[M+H]^+$: module I –m/z 3068.6 (calc), 3069.3 (found); module II – m/z 3068.6 (calc), 3068.8 (observed); module III – m/z 3495 (calc), 3496 (observed); module IVa – m/z 3040.6 (calc), 3041 (observed); module IVb – m/z 3040.6 (calc), 3041.4 (observed); module V – m/z 1827.4 (calc), 1828.6 (observed); module V (without C_{18}) – m/z 1561.2 (observed); 1560.9 (calc.); module VI – m/z 3196.9 (calc), 3197.5 (observed).

Supporting Information

High Performance Liquid Chromatography. Analytical and semi-preparative gradient RP-HPLC was performed on a JASCO HPLC system using Vydac C_{18} analytical (5μm, 4.6 mm i.d. x 250 mm) and semi-preparative (5μm, 10 mm i.d. x 250 mm) columns. Both analytical and semi-prep runs used a 10-60% B gradient over 50 min at 1 mL/min and 4.5 mL/min respectively with detection at 230 and 280 nm. Buffer A – 5% and buffer B – 95% aqueous CH_3CN, 0.1% TFA.

Circular dichroism. Circular dichroism spectroscopy was performed on a JASCO J-715 and J-810 spectropolarimeters fitted with a Peltier temperature controller. All measurements were taken in ellipticities in mdeg and converted to molar ellipticities ([θ], deg cm^2 dmol res^{-1}) by normalizing for the concentration of peptide bonds. Aqueous peptide solutions (300 μL volume; 100 μM in peptide) were prepared in filtered (0.22 μm) 10 mM MOPS (morpholinepropanesulfonic acid), pH 7.4 and 10 mM EPPS, pH 7.9-9. Stock solutions of all modules were in water, except for module V which was kept in aqueous 1 mM TCEP.

Transmission Electron Microscopy. 200 μL samples (100 μM in peptide) were incubated overnight at room temperature in filtered (0.22 μm) aqueous 10 mM MOPS, pH 7.4 (unless stated otherwise). Following incubations, 8 μL drops of peptide solutions were applied to carbon-coated copper specimen grids (Agar Scientific) and dried. The grids were stained with 0.5% uranyl acetate (8 μL) for 10 – 20 s and examined in a JEOL JEM 1200 EX MKI microscope at the accelerating voltage of 100 kV. Images were digitally acquired with a fitted camera (MegaViewII).

Table and Figures

Table S1. Peptide sequences

Construct name	Sequence
I	KIAALKQEIAALEQKIAALKYEIAALEQ
II	KIAALKQKIAALKQEIAALEYEIAALEQ
III	KIAALKQKIAALK(βA)$_6$EIAALEYEIAALEQ
IVa[a]	KIAALKQKIAALKQevaaleyevaaleq
IVb[a]	kvaalkqkvaalkqEIAALEYEIAALEQ
V	C_{18}-KIAALEQKIAALEY
VI	KICCLKQKICCLKQEIAALEYEIAALEQ

[a] – D-amino acids are given in lowercase

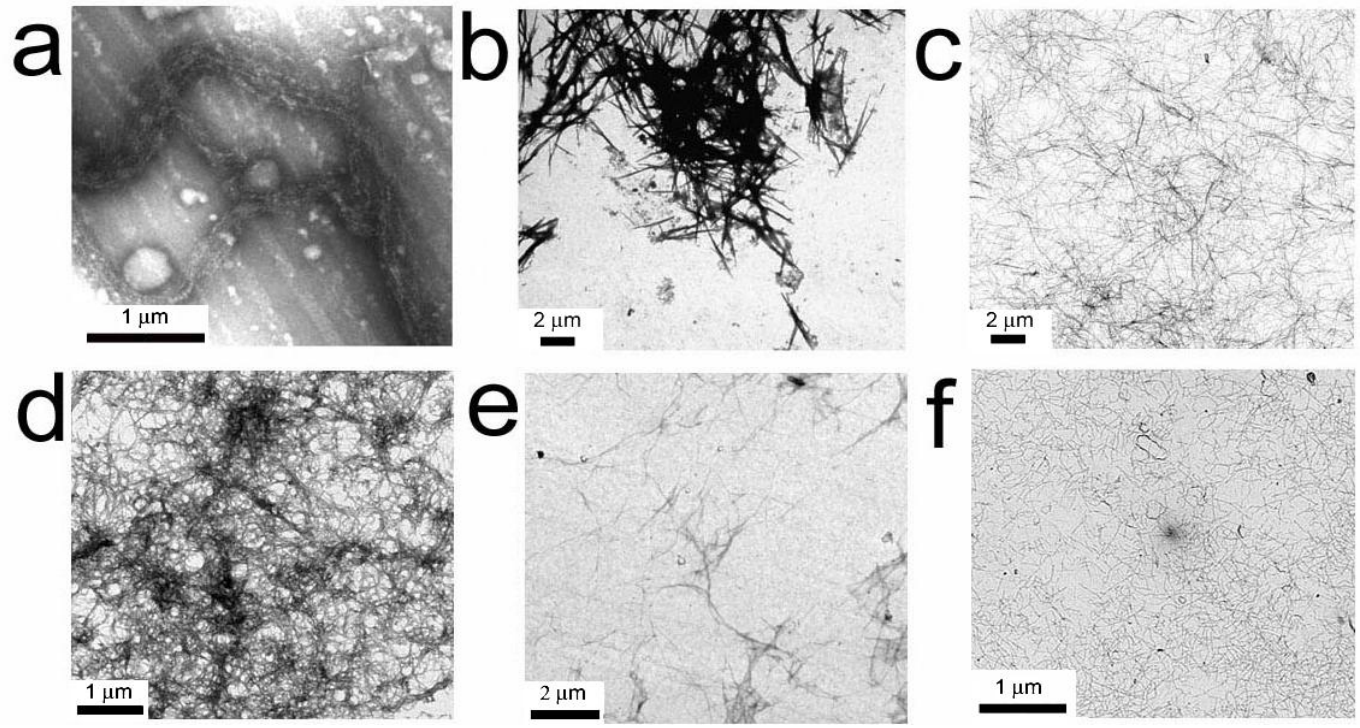

Figure S1. Low magnification electron micrographs of fibrillar nanostructures assembled from Constructs I-VI (**a-f**). Images were taken for 100 μM peptide samples in 10 mM MOPS, pH 7.4, 20 ˚C.

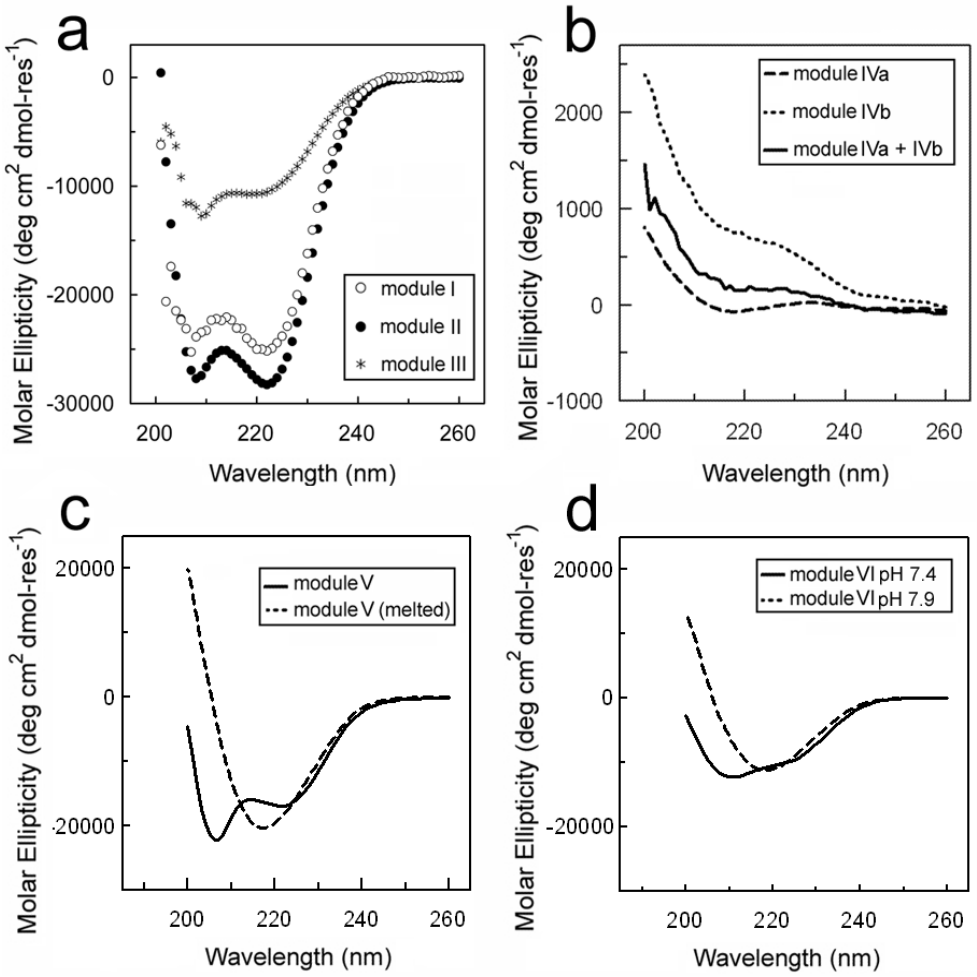

Figure S2. CD spectra of Constructs. (**a**) I-III. (**b**) IVa, IVb. (**c**) V. (**d**) VI.

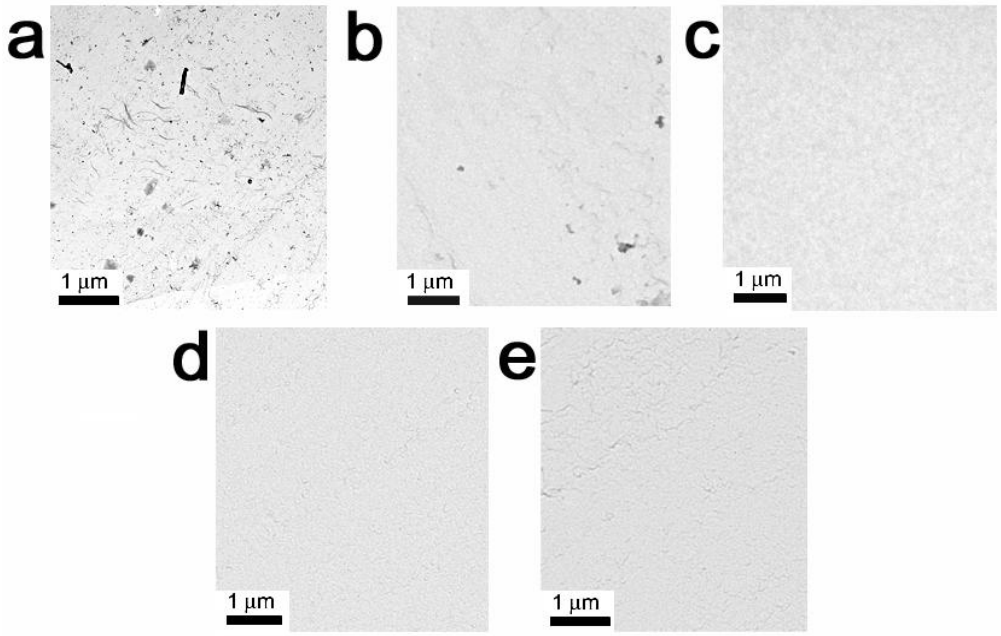

Figure S3. Low magnification electron micrographs of Constructs IVa (**a**), IVb (**b**) and V at 20°C (**c**), V without the C_{18} tail before (20°C, **d**) and after (up to 100°C, **e**) melting. Images were taken for 100 μM peptide in 10 mM MOPS, pH 7.4.

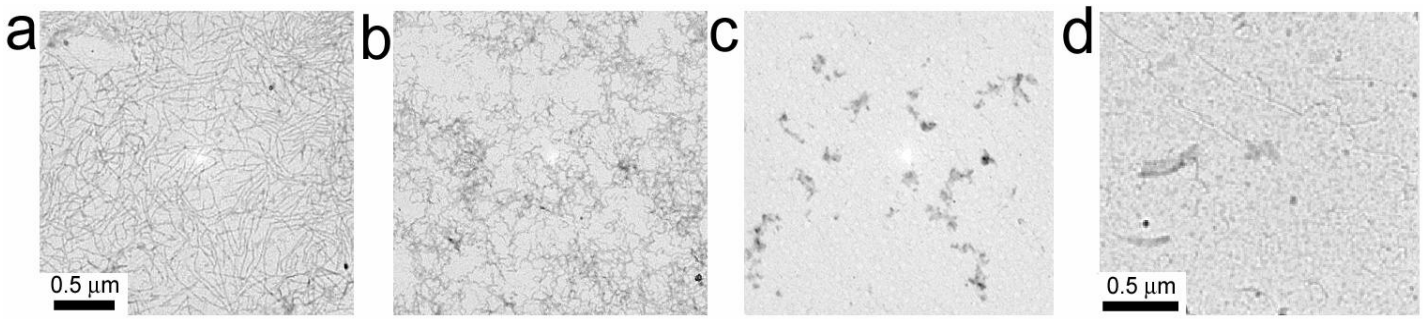

Figure S4. Low magnification electron micrographs of Construct VI at pH 7.9 (**a**), 8.5 (**b**), 9 (**c**), and Construct II at pH 8 (**d**). Images were taken for 100 μM peptide samples in 10 mM EPPS, incubated at 20 ˚C overnight.