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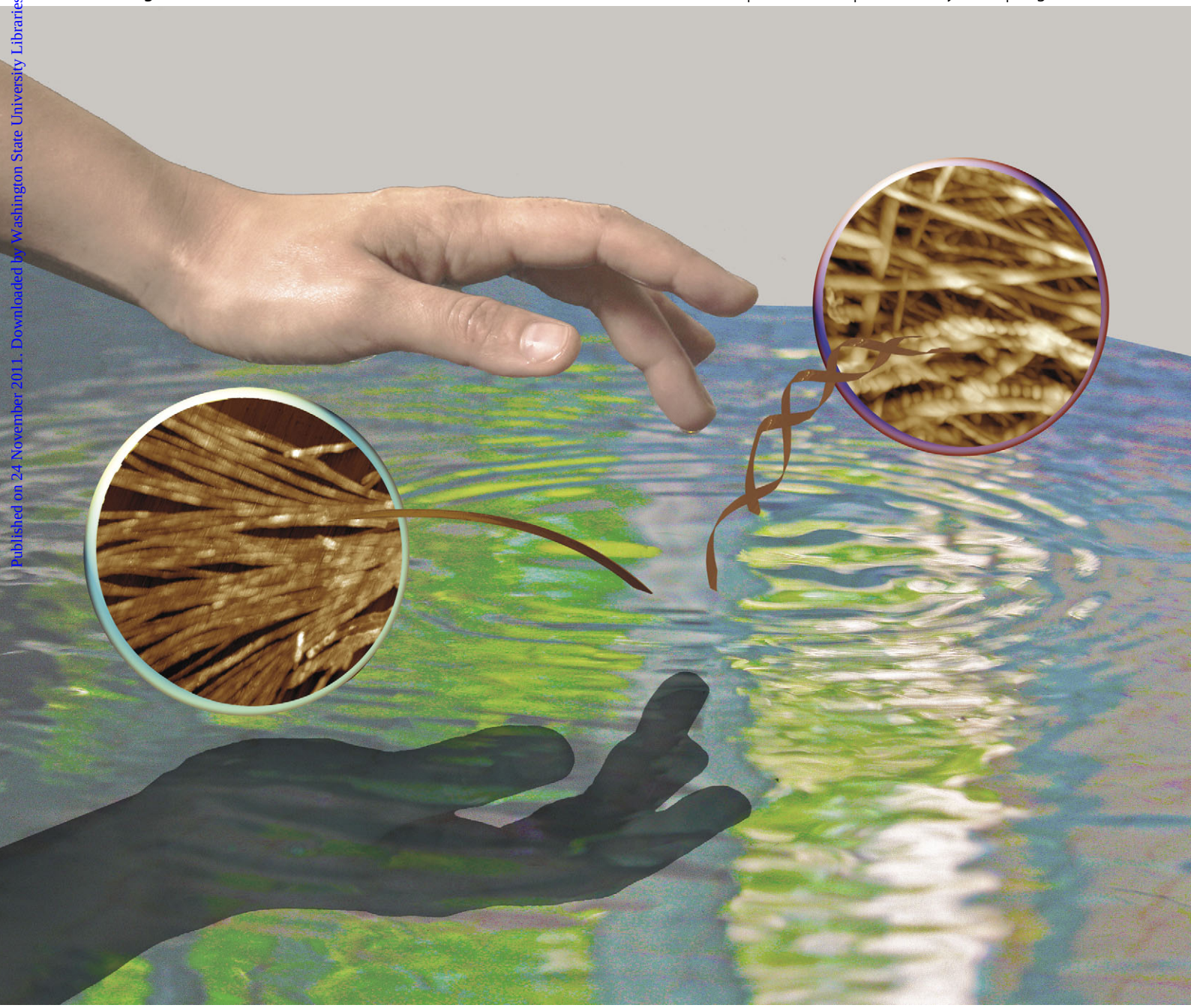

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COMMUNICATION

Tripeptide self-assembled hydrogels: unexpected twists of chirality†

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Change of chirality of the first *N*-terminal amino acid of tripeptides VFF and FFV from L to D results in self-assembled hydrogels at physiological pH from non-assembling L-analogues. Interestingly, changing the chirality of F yields very different nanostructures; nanotapes are observed for ^{D}VFF, twisted fibers for ^{D}FFV.

The self-assembly behaviour of amyloid peptides is the topic of intense research due to both pathological relevance and potential application in the production of nanostructured materials.[1] In particular, the core sequence KLVFF has been identified as the shortest key fragment responsible for the formation of β-sheet fibrils, which can yield hydrogels.[2] At present, there are no reported examples of KLVFF fragments capable of self-assembly and hydrogelation in the absence of organic solvents: uncapped di- and tripeptides are either too hydrophilic and readily dissolve in water, or too hydrophobic, and thus precipitate. In the case of FF alone, an elegant study revealed discrete nanotubes of persistent length, when a concentrated solution of the dipeptide in hexafluoroisopropanol is diluted in water.[3] Later, a few examples were reported of capped F derivatives, including di- and tripeptides, capable of self-assembly when first dissolved at high concentration in an organic solvent.[4] This is not surprising as it is known that the self-assembly process is strongly dependent on solvent.[5] Different is the case for Fmoc-FF and naphthalene-FF, that do not require the presence of organic solvents to hydrogelate, but where self-assembly is driven by the synthetic aromatic moiety.[6]

Here, we report that while the short tripeptides VFF and FFV indeed do not self-assemble into fibrils in phosphate buffer, it is sufficient to change the configuration from L to D of the *N*-terminal amino acid in both sequences to obtain self-supporting hydrogels. To the best of our knowledge, these are the first examples of uncapped FF-containing tripeptides capable of self-assembly into hydrogels at physiological pH without the aid of organic solvents. Interestingly, Cryo-TEM and AFM data show that the change of chirality in the case of ^{D}FFV results in highly twisted nanostructures, and CD, FT-IR and Thioflavin T fluorescence confirm they consist of β-sheets.

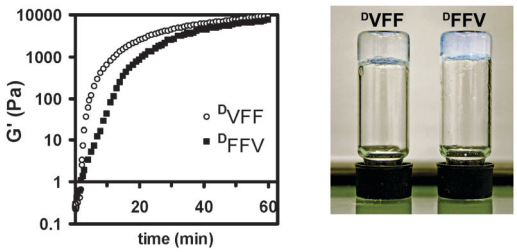

Fig. 1 Dynamic time sweep rheological data of freshly made hydrogels (left). The hydrogels formed are self-supporting (right).

In this study, we used a pH switch to promote self-assembly: peptides were first dissolved in a 0.1 M sodium phosphate solution at pH 12, while subsequent dilution to final physiological pH at ~7.4 triggered the formation of macroscopic hydrogels only for ^{D}VFF and ^{D}FFV, but not for their L-analogues, as confirmed by visual observation and rheology (Fig. 1). While both gels were initially very soft (G' of 10 kPa was reached only after 1 hour for both peptides), it is worth noting that gelation of ^{D}FFV could be markedly accelerated by sonication (see ESI†).

CryoTEM (Fig. 2) of the tripeptides containing a D-amino acid showed nanoscale fibrous morphologies, whilst no structures could be identified for their L-analogues. Interestingly, the resulting nanostructures of the two non-natural peptides were markedly different from each other. ^{D}VFF yielded long nanotapes, often running in parallel (Fig. 2A, white arrowheads) and displaying lateral stacking into wider structures (Fig. 2A, black arrowheads). ^{D}FFV self-assembled into thicker filaments, usually running in twisted pairs with a recurrent helix period of ~150–200 nm (Fig. 2B and C). These nanostructures were confirmed by AFM on dried gel samples (Fig. 3A–D). In particular, a helix period of ~150–200 nm is compatible with the AFM linescan of ^{D}FFV (line drawn in Fig. 3B and analysis in Fig. 3C) and is shown in the image in Fig. 3D. Occasionally, multiple ^{D}FFV filaments could also twist into coiled fibrils (see ESI†). In further experiments, the precursor solutions were dried onto a silicon wafer before gelation without sonication, in an attempt to gain insights into the initial phases of self-assembly. Interestingly, ^{D}VFF yielded similar structures to those observed after sonication (compare Fig. 3A and E), while ^{D}FFV formed short nanorods which suggest partial, short order alignment (Fig. 3F). It is possible

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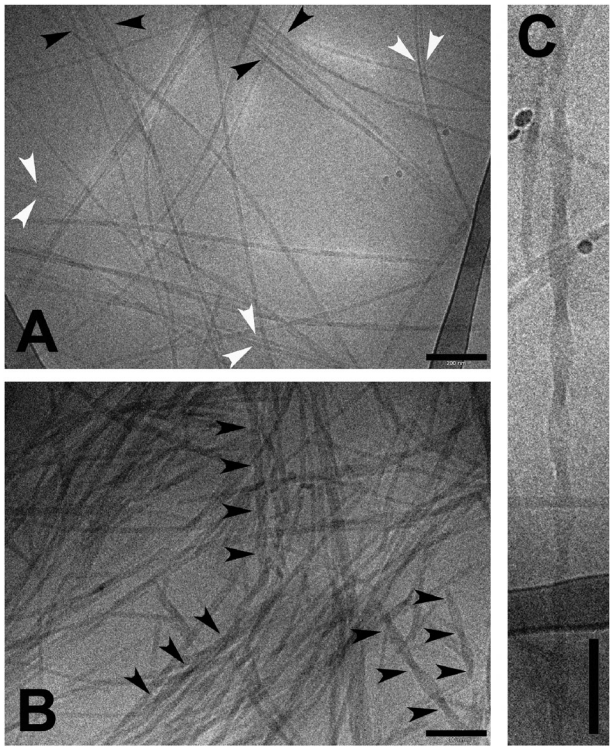

Fig. 2 (A) CryoTEM of ^{D}VFF hydrogel. Black arrowheads: wide nanotapes. White arrowheads: parallel double filaments. (B) CryoTEM of ^{D}FFV hydrogel. Black arrowheads: helix period, more clearly visible in the higher magnification image C. Scale bar = 200 nm.

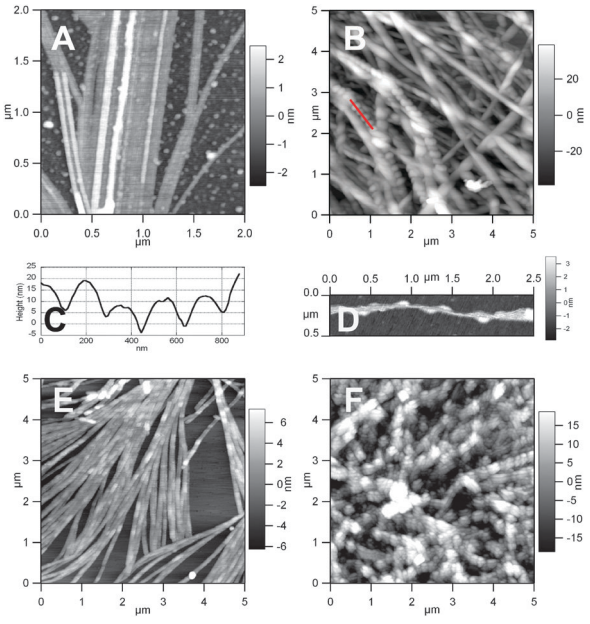

Fig. 3 AFM images of dried gels of ^{D}VFF (A) and ^{D}FFV (B) obtained after sonication; an AFM linescan (C) and a detailed view (D) of a helix period of ~150–200 nm for ^{D}FFV. Precursor solutions which were not sonicated and were dried before gelation display similar structures for ^{D}VFF (E) but only short nanorods for ^{D}FFV (F).

that these precursors eventually elongate into the twisted fibrils observed for ^{D}FFV after sonication (Fig. 3B and ESI†). In contrast, AFM did not reveal ordered nanostructures for

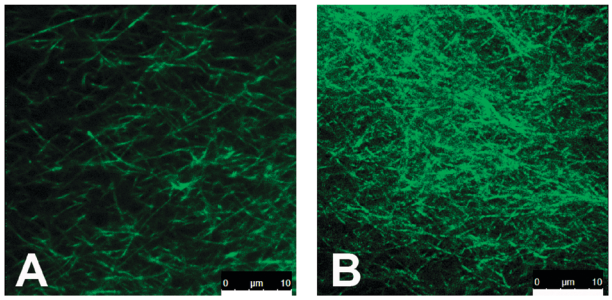

Fig. 4 Confocal images of Thioflavin T fluorescence highlight fibers of similar diameter for ^{D}VFF (A) and ^{D}FFV (B); the latter yields a denser network.

the natural peptides (see ESI†), in agreement with Cryo-TEM. It is worth noting how the different behaviour (self-assembling or not) and morphology (*i.e.* tapes *versus* twisted fibrils) results from very subtle stereochemical changes to tripeptide composition.

Thioflavin T fluorescence is a well-known method to assess β-sheet structures, although it is only recently that a binding mode has been proposed suggesting that the dye binds to an aromatic–hydrophobic groove spanning across at least four consecutive β-strands.[7] Confocal imaging (Fig. 4) of our samples treated with Thioflavin T revealed fibers of several μms in length for both ^{D}VFF and ^{D}FFV, with the latter yielding a denser network, as expected from our data. In contrast, the lack of fluorescence for the two natural analogues suggests they are devoid of extended β-sheets (not shown).

CD was used to better assess the nature of the secondary structure of the peptide self-assembled materials (Fig. 5). Surprisingly, the CD spectra of all four peptides showed an evident maximum centered at 225–230 nm, indicative of π–π stacking of the aromatic phenylalanine residues. It is plausible that this is due to the formation of aggregates for the two L-peptides, as their limited solubility led to slow precipitation. It is worth noting that in the case of the self-assembling peptide ^{D}FFV, the intensity of the maximum was significantly reduced, due to the presence of the D-aromatic amino acid. More importantly, as expected only the two self-assembling structures containing non-natural amino acids displayed the β-sheet minimum in the region 210–220 nm (Fig. 5, bottom). The ^{D}FFV self-assembled peptide was the only one showing a broad positive signal in the near-UV region (250–270 nm), which can be attributed to phenylalanine residues with tertiary structure. The β-sheet nature of the self-assembling peptides

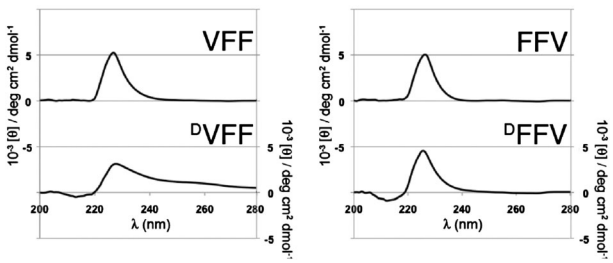

Fig. 5 CD spectra of the four tripeptides: each sample displays π–π stacking (maximum at 225–230 nm); only the two self-assembling hydrogels (bottom) display the β-sheet minimum in the region 210–220 nm.

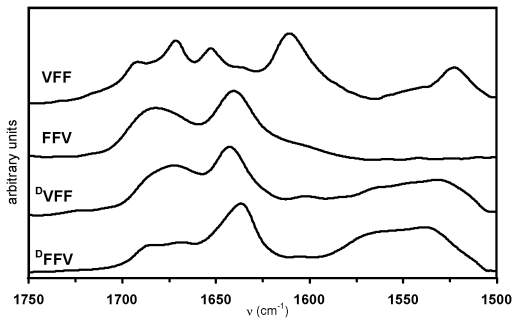

Fig. 6 FT-IR of the amide I–II regions for the four tripeptides.

was also confirmed by FT-IR spectral analysis of the amide I–II regions (Fig. 6). The natural peptide VFF revealed a mixture of secondary structures, including a predominant maximum at 1610–1620 cm^{-1} that can be attributed to antiparallel aggregates, and which is absent or barely visible in all the other samples. In comparison, the spectrum of FFV is very different, with a broad maximum centered at 1640 cm^{-1} ascribed mainly to random coils, and a dominant TFA peak in the region 1670–1680 cm^{-1} that makes it difficult to assess the presence of β-structures. Nevertheless, both VFF and FFV are likely devoid of extended β-sheets with supramolecular order, as suggested by CD analysis and lack of binding and fluorescence of Thioflavin T. TFA presence was apparent also in the spectra of the two non-natural self-assembling peptides, however, in this case the antiparallel β-sheet signature was evident at least for ^{D}FFV (1637 and 1680–1690 cm^{-1}).

Moreover, ^{D}VFF and ^{D}FFV both displayed a broad signal in the N–H amide II region, shifted to lower frequencies due to extended hydrogen bonding, indicative once again of β-sheets. More specifically, the signal at 1570 cm^{-1} suggests COO^{-}/NH_3^{+} head-to-tail interactions in the self-assembling tripeptides, as previously observed for the gel-forming Ile-Phe but not for the non-self-assembling Val-Phe.[4d] It is interesting to note that a maximum centered at 1523 cm^{-1} was present in the spectrum of VFF, but not FFV. It appears that the overall lack of supramolecular order for the two natural peptides can be ascribed to the co-existence of a number of secondary structures for VFF, and their absence for FFV, which appears to be predominantly random coil.

In conclusion, this study reports two examples where chirality acts as a key tool for the self-assembly of short peptides. This effect is not limited to isolated nanostructures, but extends to the formation of macroscopic self-supporting hydrogels, as confirmed by rheology and a variety of microscopic (Cryo-TEM, AFM and confocal) and spectroscopic (CD, FT-IR) techniques. In addition, these gels are promising candidates for the development of biomaterials as they form at physiological pH without the aid of organic solvents, or of synthetic capping end-groups, and the display of a D-amino acid at their *N*-terminus may be a convenient feature to extend their resistance to protease degradation.[3] Investigation of the structural properties of a series of analogues is currently underway in our laboratories to further elucidate how the chiral features described in this study influence intermolecular interactions and translate into the observed dramatic effects on the supramolecular structures. It can be envisaged that chirality will be a key tool to control self-assembly behavior and also to induce designed morphologies in nanostructured materials.

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