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Supporting Information

Alignment of Nanostructured Tripeptide Gels by Directional Ultrasonication

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Experimental Section

Materials

Fmoc-Ile and Fmoc-Asp(otBu)-Wang resin were purchased from Novabiochem UK. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Fmoc-Phe-OH, Fmoc-^{D}Phe-OH, Piperidine, trifluoroacetic acid (TFA), diisopropyl ethyl amine (DIPEA), triisopropyl silane (TIPS) were purchased from Sigma Aldrich and used as received. Phosphate buffer solution (pH 8) was prepared by dissolving 94 mg $NaH_2PO_4 \bullet H_2O$ and 2.5 g $Na_2HPO_4 \bullet 7H_2O$ in 100 ml water.

Synthesis of Peptides

The tripeptides were synthesized by using the Fmoc-strategy.[6] Coupling reactions of the Fmoc amino acids were carried out by using a molar ratio of Fmoc-amino acid/HBTU /DIEA/resin (3:3:6:1) in anhydrous DMF. Removal of the Fmoc group was achieved by using 25% piperidine in DMF. The cleavage of the peptide from the resin was performed by using a mixture of TFA/TIPS/water in a molar ratio of 95/2.5/2.5.

^{D}FFD

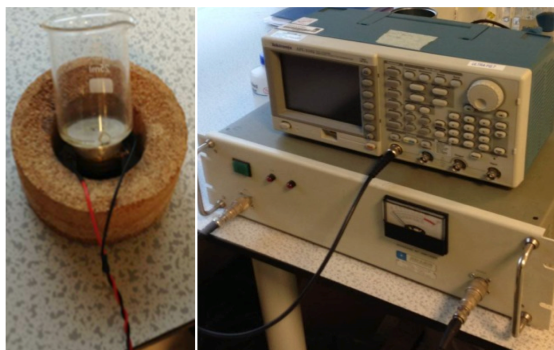
Purity by HPLC (214 nm) 97.5% ^{1}H-NMR (400 MHz, DMSO, TMS): δ 8.8 (d, 1H, NH), 8.49 (1H, NH), 7.20 (m, 10H, Ar), 4.76 (m, 1H, αCH), 4.41 (m, 1H, αCH), 3.92 (m, 1H, αCH), 3.2 (2H, CH_2 Asp), 2.9-2.618 (4H, 2xβCH_2 phenylalanine) MS (ES+): m/z 428.1 [M + H].

^{D}FFI

Purity by HPLC (214 nm) 97.7% ^{1}H-NMR (400 MHz, DMSO, TMS): δ 8.85 (d, 1H, NH), 8.42 (1H, NH), 7.23 (m, 10H, Ar), 4.86 (m, 1H, αCH), 4.20 (m, 1H, αCH), 4.01 (m, 1H, αCH), 3.094-2.692 (m, 4H, 2xβCH_2 phenylalanine), 1.47-1.22 (3H Isoleucine) and 0.9 (6H Isoleucine). MS (ES+): m/z 426.2 [M + H].

Ultrasonic Setup

For this work an ultrasonic cell was developed which was controlled by standard ultrasonic laboratory equipment to provide control of the operating parameters. An 80 kHz Tonpilz transducer (Morgan Electroceramic Ltd, UK) was bonded to the base of a 50 ml glass beaker. A Tektronix AFG 3102 signal generator was used to generate a 78 kHz continuous wave signal that was subsequently amplified through a 100W Kalmus 155CLR amplifier and used to drive the Tonpilz transducer. To improve the electrical energy transfer from the Kalmus amplifier to the transducer, the signal from the amplifier was fed into a bespoke impedance matching network that matched the amplifier output impedance to the impedance of the transducer for power efficiency. The network comprised a 1:6 transformer and reduced the electrical impedance of the transducer from ~500Ω to ~10Ω, at the operating frequency. This matching network was then connected to the transducer that generated ultrasound.



Sample preparation

Samples prepared without ultrasound: 12.5 mg of ^{D}FFI was dissolved in 1 ml sodium phosphate buffer (100 mM, pH 8). The sample was heated up to 80 °C for 4 minutes to enhance the solubility. After cooling down, a precipitate was observed. For ^{D}FFD, 10 mg was dissolved in 1 ml methanol. The sample was heated up to 50 °C for 3 minutes. After cooling down, a precipitation was observed.

Samples prepared with ultrasound: 12.5 mg of ^{D}FFI was dissolved in 1 ml sodium phosphate buffer (100 mM, pH 8). The sample was heated up to 80 °C for 4 minutes. Then the sample was treated by ultrasound for 30 sec and a gel-phase material was obtained (the details of setup of ultrasonic equipments, see our previous work[5g]). In the case of ^{D}FFD 10 mg was dissolved in 1 ml methanol. The sample was heated up to 50 °C for 3 minutes. Then the sample was treated by ultrasound for 60 s and a gel-phase material was obtained.

Characterization

The self-assembled structures of tripeptides were visualized by Hitachi S800 field emission scanning electron microscope (SEM) at an accelerating voltage of 10 keV and LEO 912 energy filtering transmission electron microscope (TEM) operating at 120 kV fitted with a 14 bit/2 K Proscan CCD camera.

The formation of tripeptide fibers and gels labeled by ThT were characterized by Two-photon fluorescence excitation microscopy (TPM). The TPM system consisted of a (multiphoton) scanning system (1024 MP; Bio-Rad, Hemel Hempstead, UK) coupled to the upright, fixed stage of a microscope (Eclipse E600FN; Nikon Tokyo, Japan).

Mass spectra were recorded on a Thermo Electron Exactive. 400.1 (1H) NMR spectra were recorded on Brucker Avance 400 spectrometer at room temperature using perdeuterated solvents as internal standards. Diffusion ordered spectroscopy (DOSY) spectra were acquired at 600 MHz using a Bruker Avance 600 spectrometer at 298 K. The eddy current delay (Te) was set to 5 ms. The diffusion time was adjusted to 100 ms .The duration of the pulse field gradient, δg, was optimized in order to obtain 5% residual signal with the maximum gradient strength with the resulting δ value of 3.6 ms. The pulse gradient was increased from 2 to 95% of the maximum gradient strength using a linear ramp 16k data points in the F2 dimension (20 ppm) and 16 data points in the F1 dimension were collected. Final data sizes were 16k×128.

CD and LD Spectra were measured on a Jasco J-815 spectropolarimeter with 1s integrations with a step size of 3 nm and a single acquisition with a slit width of 1 nm. Demountable cells of 0.01 cm path length were used for the measurement. The measuring wavelength was covering the region from 190-400 nm.

Infrared absorption spectra were recorded on a Bruker Vertex 70 spectrometer, averaging 25 scans per sample at a resolution of 1 cm^{-1}. Samples were sandwiched between two 2 mm CaF_2 windows separated with a 50 μm polytetrafluoroethylene (PTFE) spacer. For the samples without ultrasound treatment, a sample was taken in the form of the suspended precipitate. Spectra were corrected for background absorptions from phosphate buffer, TFA and atmospheric water where necessary.

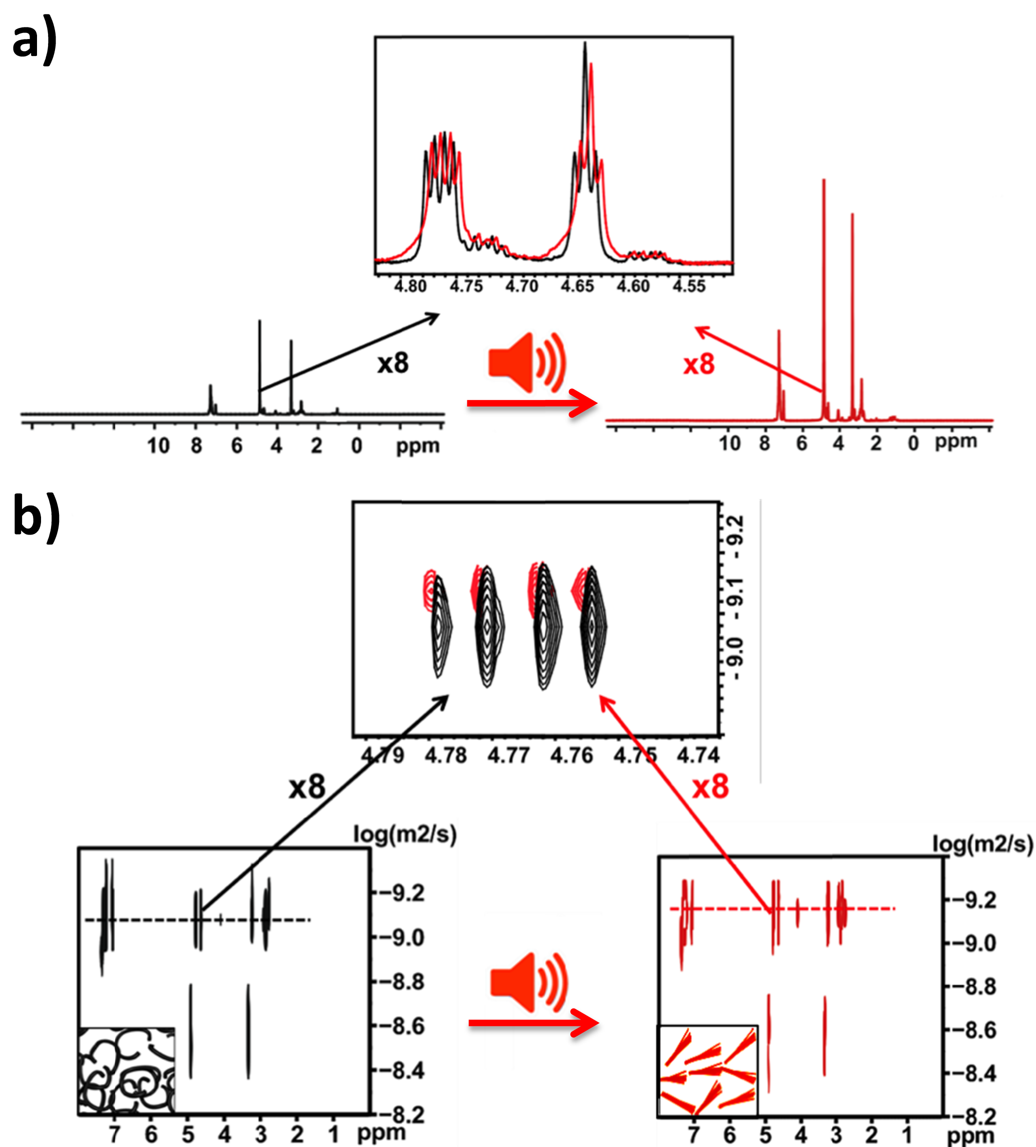


Figure S1. (a) ^{1}H NMR spectra of ^{D}FFD in CD_3OH before and after ultrasound irradiation, picture in the middle highlights a snapshot of the chiral protons (zoomed x8). (b) DOSY NMR spectra of ^{D}FFD in CD_3OH before and after ultrasound irradiation with a snapshot of the diffusion spectra for the chiral protons (zoomed x8).

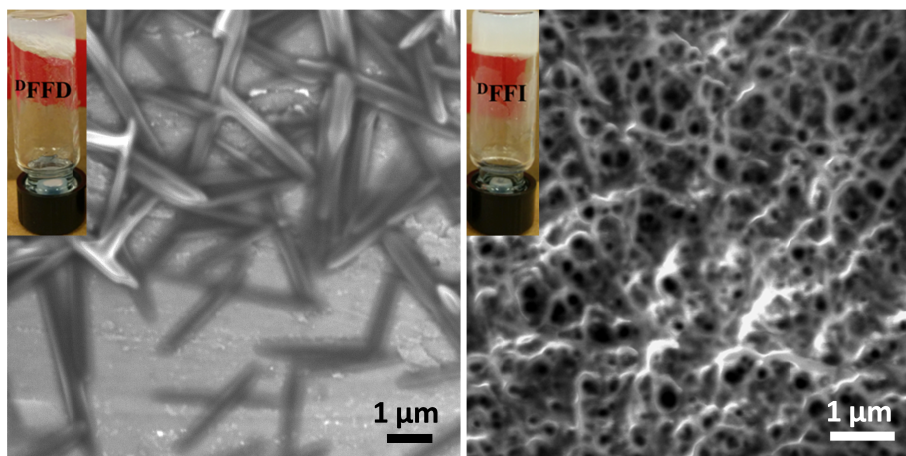


Figure S2. SEM images of ^{D}FFD in methanol (left) and ^{D}FFI in phosphate buffer (pH 8) (right) prepared by non-directional sonication bath. Inset photographs are ^{D}FFD and ^{D}FFI forming gels in methanol and buffer, respectively. The scale bars are 1 μm. The concentrations are 25 and 30 mM, respectively.