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Solvent-tailored ordered self-assembly of oligopeptide amphiphile to create the anisotropic meso-matrix

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Experimental Section

Materials: N-9-Fluorenylmethoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH and Fmoc-Glu(Otbu)-OH), rink amide-AM resin (100-200 mesh, loading: 0.625 mmol/g, 1% DVB), coupling reagents including 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) were provided by GL Biochem. Ltd (Shanghai, China). N, N-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), methanol (MeOH), ether and Triisopropylsilane (TIPS) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Palmitic acid (AR), trifluorocaetic acid (TFA), N, N-Diisopropylethylamine (DIEA) were bought from Aladdin Reagent Co. Ltd (Shanghai, China). Estrone (98% purity) was purchased from sigma-aldrich. Dimethyl-d_6 sulphoxide + 0.05% TMS (v/v) (DMSO-d_6), Deuterium oxide (D_2O) were obtained from Cambridge Isotope Laboratories, Inc. All materials are reagent grade and used as received without further purification.

Synthesis of OPA: With reference to the reported literature, the peptide amphiphile (OPA) of $C_{15}H_{31}$-CONH-AAAAAKEEE-$CONH_2$ was synthesized based on solid-phase peptide synthesis (SPPS).[1] In detail, rink amide-AM resin was firstly washed and swelled by DMF, and 20% piperidine/DMF (V/V) solution was poured into the resin to remove the protected Fmoc group for two times. Then, the resin was washed with DMF four times to remove the residual piperidine. Fmoc-Glu(Otbu)-OH) was conjugated to the resin with the activation of HBTU (2.4 equiv), HOBt (2.4 equiv), and DIEA (6 equiv). After the condensation reaction, 20% piperidine/DMF solution was once again used to remove the Fmoc group of amino acid twice. Repetition of the deprotection and condensation was carried out to complete the amino acid conjugation. Palmitic acid was attached to the N-terminal of peptide after the activation by HBTU and DIEA in DMF/dichloromethane. Subsequently, the resin was washed with DMF, MeOH, and DCM for three times and dried under vacuum overnight. A mixed solution of trifluoroacetic acid, triisopropylsilane, and water (TFA/TIS/H_2O=95/2.5/2.5 (v/v)) was introduced to cleave peptide from resin and concurrently removed the protective group.

The filtrate was obtained by suction filtration, and then rotary evaporated at 45°C until the solution remained about 3 mL. In order to isolate the product, 3 mL of the filtrate was precipitated with cold ether. The precipitate was further dried in a vacuum dryer to obtain the crude product. The synthesized crude peptide was purified by high pressure liquid chromatography (HPLC, LC-20AR, Shimadzu, Japan) equipped with a C18 column (SinoChrom ODS-BP, 4.6×250 mm, 5 μm). The mobile phase consisting of solvent A (0.1% TFA in 100% water) and solvent B (0.1% TFA in 100% acetonitrile) was used as the eluent, and the linear gradient started with 48% A and 52% B (42:58-17: 83, 25 minutes, v/v). The components were collected at a wavelength of 220 nm and purified to a purity of more than 95%. Then the product was then freeze-dried at -45°C for 3-4 days to obtain purified OPA.

Molecular structure characterization: Electrospray ionization mass spectrometry (ESI-MS, SHIMADZU LCMS-2010EV, Japan) was used to measure the molecular weight of purified peptides. The observed peak at 1126.5 corresponding to $[M+H]^+$ was consistent with the theoretical molecular weight. The purity of OPA was measured by a high pressure liquid chromatography (HPLC, LC-20AR, Shimadzu, Japan) equipped with a C18 column (250*4.6mm, ChromCore 120-C18-5um). A purity of 96% OPA was collected for the following experiments.

Preparation of peptide liquid crystals: In order to obtain the required self-assembly medium, cosolvents with different water concentrations were prepared to dissolve the OPA. Then the sample was subjected to ultrasonic treatment (high-power NC ultrasonic cleaner, KQ-400KDE, 40KHZ, 400W) for 15 minutes to facilitate the dissolution and further self-assembly of OPA.

Polarizing photography: The birefringent pattern of self-assembled oligopeptide amphiphile media was observed by two methods, *i.e.* using two polarizers or polarized optical microscope (Nikon Eclipse Lv100npol, POM). The former was to photograph the texture of self-assembled media in NMR tube between two orthogonal polarizers. For the POM experiment, 50 microliters of OPA solution were dropped onto the slide glass and placed on the sample table for observation with a Nikon microscope.

Morphology characterization: Scanning electron microscopy (SEM) images of self-

assembled OPA nanofibers were obtained on the */SU8010 (Japan) instrument in the secondary electron imaging mode. The sample to be tested was dropped on the silicon wafer and dried in vacuum at room temperature for more than 72 hours. Before the observation, the samples were coated by gold.

Viscosity testing: The viscosity measurement was performed on a DHR rotary rheometer (TA Instruments Inc, DHR-1). The angular velocity was 1.0 rad/s, and the test temperature was 298 K. The gap between the parallel plate and the test bench was 500 μm. 1-2 mL of the prepared samples or the control solution were dispersed on the test bench for the test. The measurement of each sample was performed for 10 times to extract the average value to reduce errors.

Fluorescence experiments: Nile Red was dissolved in a cosolvent of DMSO and water to obtain a Nile Red solution with a concentration of 2×10^{-5} M. Then, the prepared OPA solution (20 mg/mL) was added to the Nile Red solution, which was further diluted to the targeted concentrations with cosolvents of different water concentrations. After the mixed solution was shaken for 24 hours in the dark, the fluorescence excitation and emission spectra were recorded by a fluorescence spectrometer (LS 55).

NMR experiments: All NMR data were performed on the Bruker Ascend IIITM 600 MHz NMR spectrometer equipped with a 5mm CPPBBO forward broadband liquid nitrogen cryogenic probe at 293 K. The 1D NMR spectra were collected using locking channel records at eight scans. For the collecting of two-dimensional [^{1}H, ^{13}C] -CLIP-HSQC spectra, the acquisition time (AQ) and the relaxation delay (RD) were 0.327 s and 2.00 s, respectively. The spectral width (SW) of each spectrum was 10.0 ppm (^{1}H) and 140 ppm (^{13}C). The chemical shift values (δ) were expressed in parts per million (ppm), and the scalar coupling constants (J) and RDCs in Hertz (Hz). The NMR data were read and processed by Bruker Topspin 3.5 pl 6 and M-spin software.

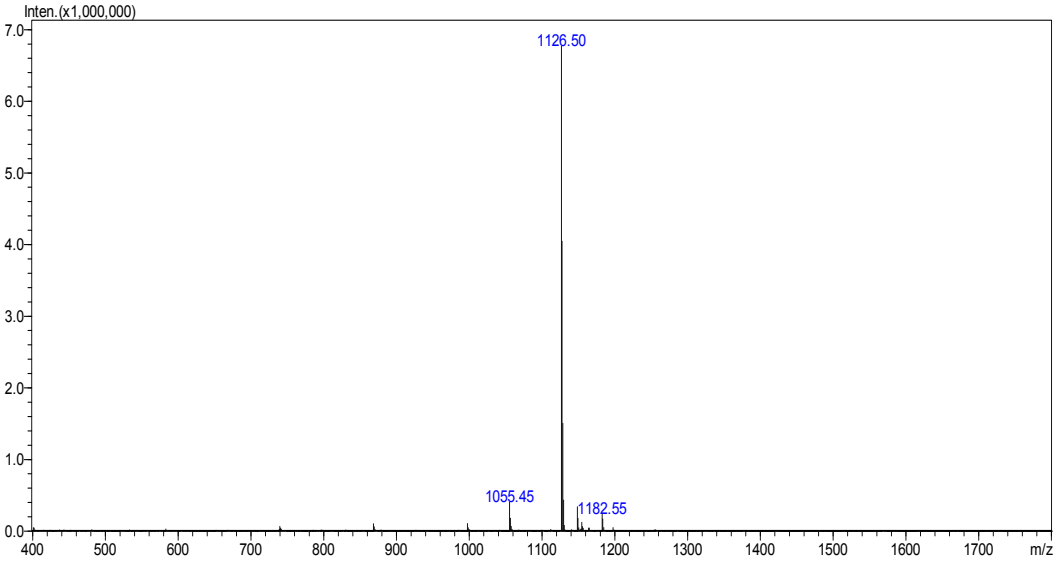

Fig. S1 ESI-MS of OPA. $[M+H]^+$ at m/z of 1126.5 was observed.

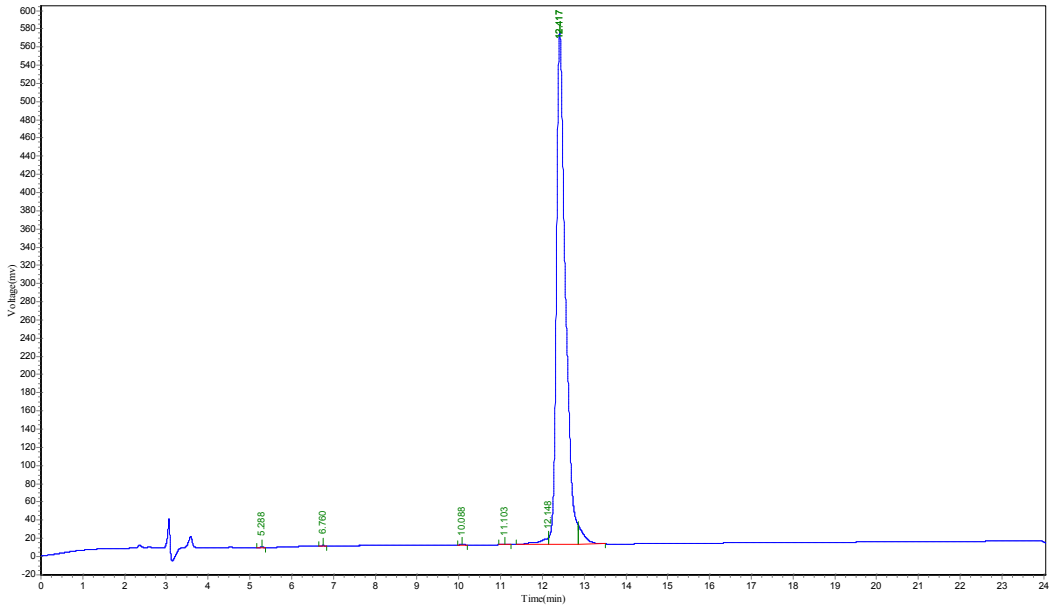

Fig. S2 HPLC profile of OPA, with a purity of 96 %.

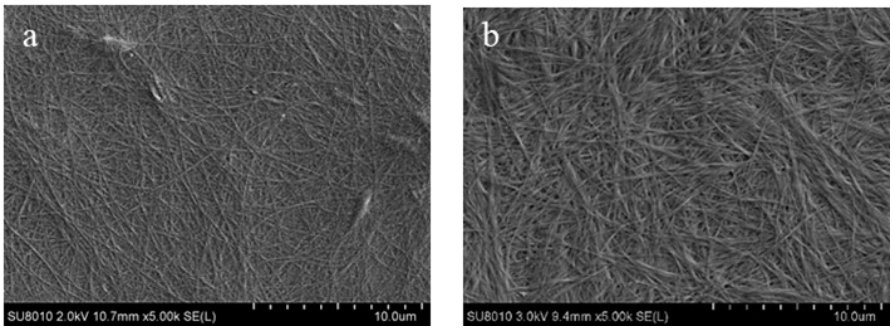

Fig. S3 SEM images of freshly prepared self-assemblies in pure DMSO (OPA/DMSO), shown long nanofibers with several microns (a: 4 wt%, b: 8 wt%).

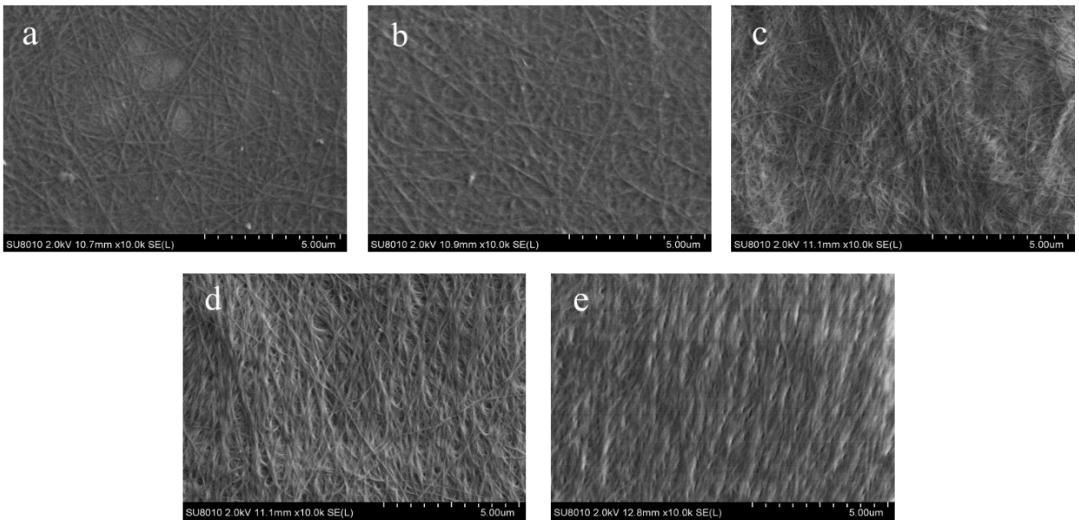

Fig. S4 SEM images of freshly prepared self-assembled OPA/DMSO media with different water concentrations. The water concentrations corresponding to the photos of a, b, c, d, e, f are 0%, 1%, 2%, 3% and 4%, respectively. The OPA concentration was 4 wt%.

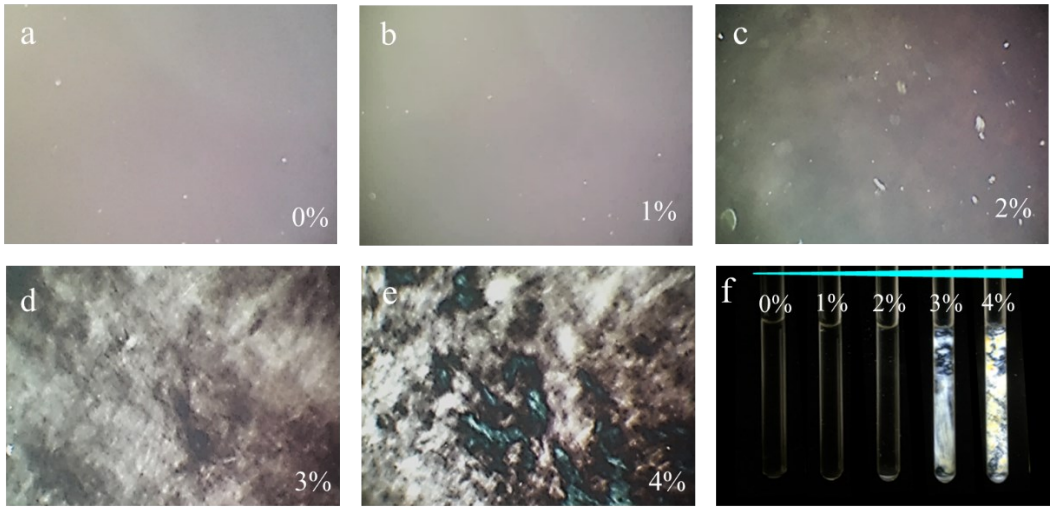

Fig. S5 The birefringence of freshly prepared self-assembled OPA/DMSO media with different water concentrations. (a-e) POM images; (f) Photo of self-assembled media in NMR tubes between two crossed polarizers.

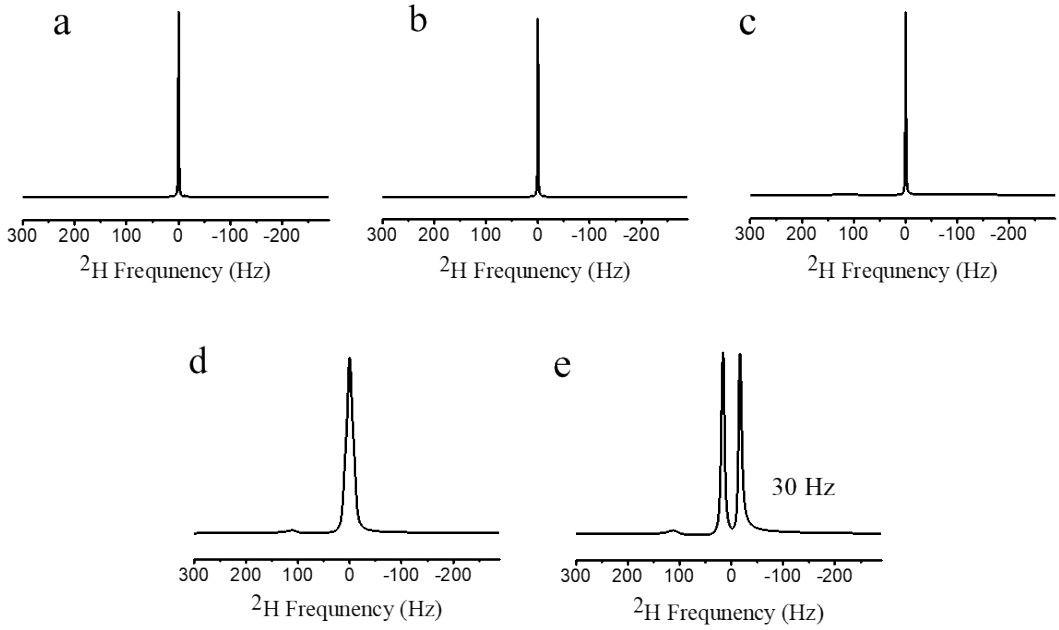

Fig. S6 1D ^{2}H NMR spectra of deuterium DMSO solvent in freshly prepared self-assembled OPA/DMSO solutions. The water concentrations corresponding to the graphs of a, b, c, d, e, f are 0%,1%, 2%, 3% and 4% respectively. The OPA concentration was 4 wt%.

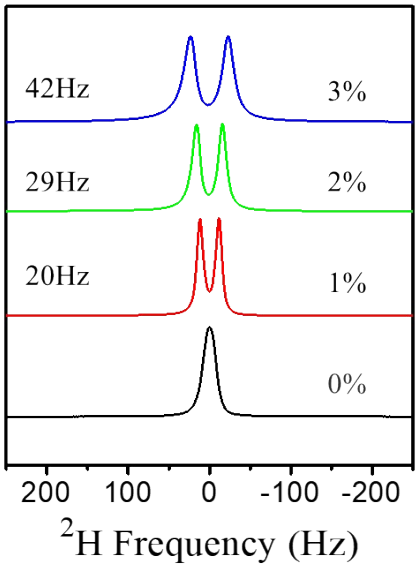

Fig. S7 1D ^{2}H NMR spectra of deuterium solvent in OPA/DMSO media with different water concentrations. The OPA concentration was 5 wt%

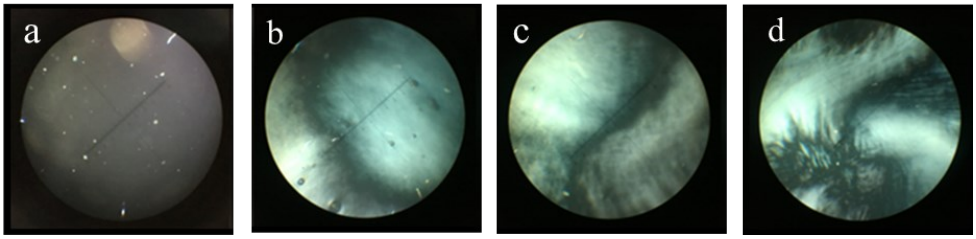

Fig. S8 POM images of self-assembled OPA/DMSO media with different water concentrations. The water concentrations corresponding to the graphs of a, b, c, d are 0%, 1%, 2% and 3%, respectively. The OPA concentration was 5 wt%.

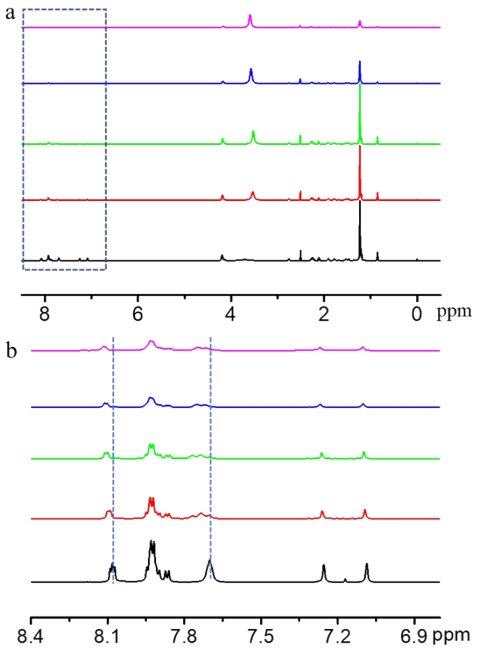

Fig. S9 (a) ^{1}H NMR spectra of the freshly prepared OPA in DMSO with different water concentrations. (b) The partial enlargement of chemical shifts from 6.9 to 8.4 ppm.

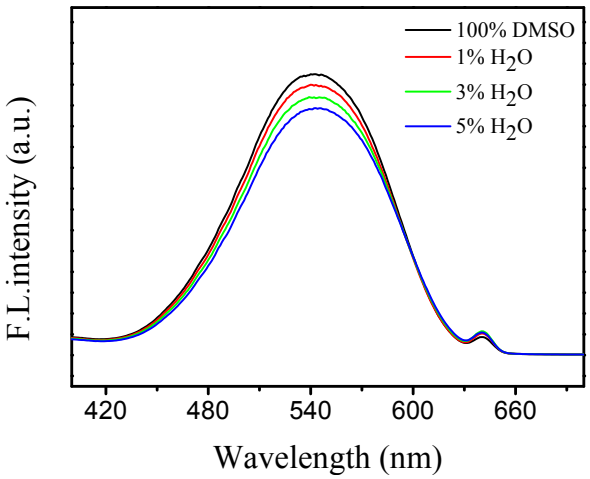

Fig. S10 Fluorescence excitation spectra of Nile Red in OPA/DMSO solution with different water concentrations. The OPA concentration was 4 wt%.

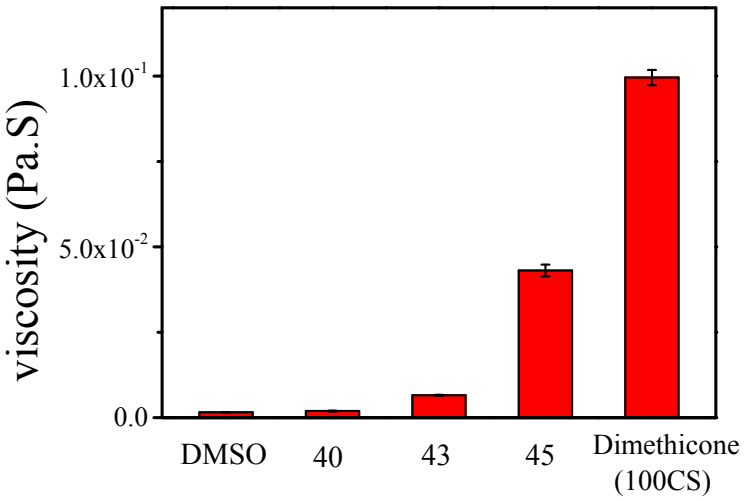

Fig. S11 Viscosity values of the self-assembled OPA/DMSO media with different water concentrations. DMSO, 40, 43, and 45 corresponding to the media with a water content of 0%, 3%, and 5%. respectively. The OPA concentration was 4 wt%.

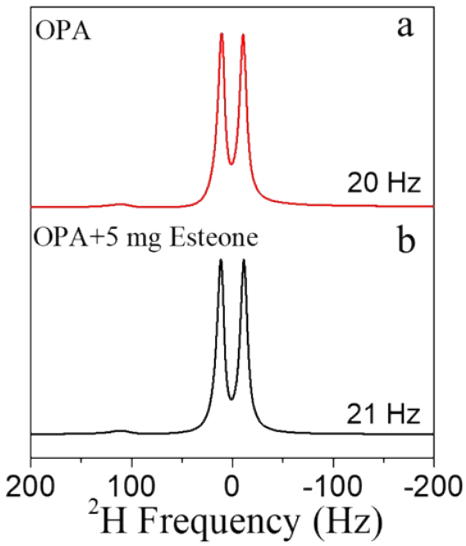

Fig. S12 ^{2}H NMR spectra of DMSO-d_6 in self-assembled media without (a) and with (b) estrone.

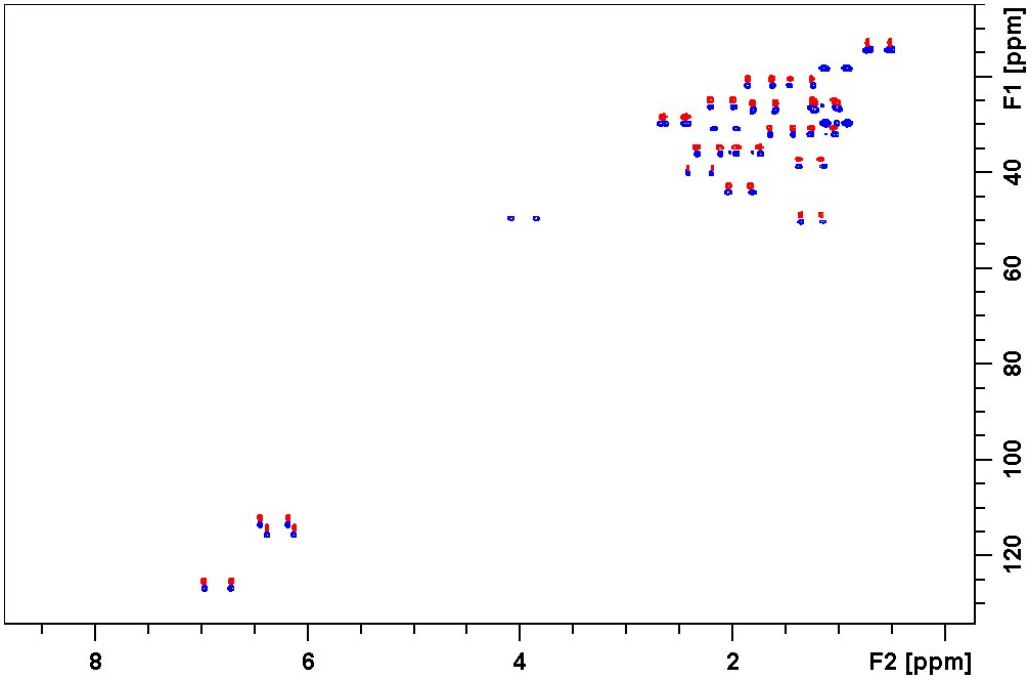

Fig. S13 Overlaid [^{1}H, ^{13}C]-CLIP-HSQC spectra of estrone in the isotropic phase (red contours) and in aligned medium (anisotropic, blue contours, down-shifted 1.5 ppm in the ^{13}C dimension).

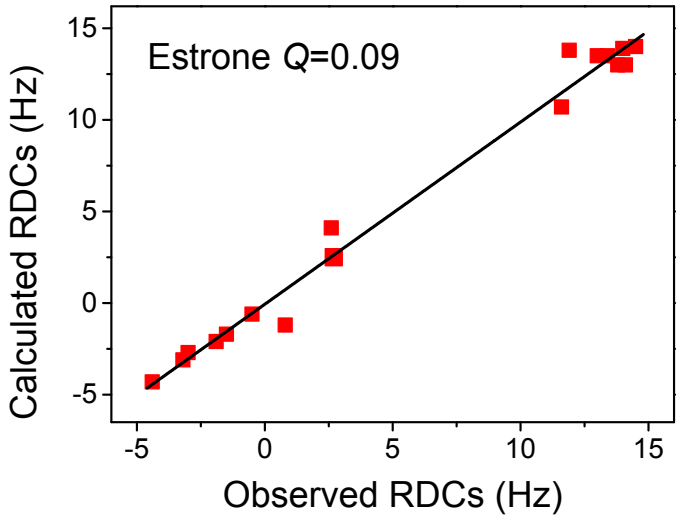

Fig. S14 Correlation between the experimental RDCs and calculated ones for estrone in the aligned medium.

Table S1 The one bond scalar ($^1J_{CH}$), total couplings ($^1T_{CH}$), residual dipolar couplings ($^1D_{CH}$) values and calculated ones of estrone.

Atom number	$^1J_{CH}$	$^1T_{CH}$	$^1D_{CH}$	$^1D_{CH}$ Calculation
C1H1	154.1	151.1	-3.0	-2.7
C2H2	158.1	156.2	-1.9	-2.1
C4H4	154.2	151.0	-3.2	-3.1
C7H7a	128.2	127.7	-0.5	-0.6
C7H7b	124.5	139.0	14.5	14.0
C8H8	124.6	138.6	14.0	13.9
C9H9	122.8	135.8	13.0	13.5
C11H11a	127.6	130.3	2.7	2.5
C11H11b	126.0	139.8	13.8	13.0
C12H12a	128.7	129.5	0.8	-1.2
C12H12b	126.5	139.9	13.4	13.5
C14H14	115.0	126.9	11.9	13.8
C15H15a	138.9	141.5	2.6	4.1
C15H15b	117.3	131.4	14.1	13.0
C16H16a	134.4	132.9	-1.5	-1.7
C16H16b	125.6	137.2	11.6	10.7
C18H18(Me)	127.8	123.4	-4.4	-4.3

Reference

1 S.-Y. Qin, S.-S. Xu, R.-X. Zhuo and X.-Z. Zhang, *Langmuir*, 2012, **28**, 2083–2090.