Transient Supramolecular Reconfiguration of Peptide Nanostructures using Ultrasound

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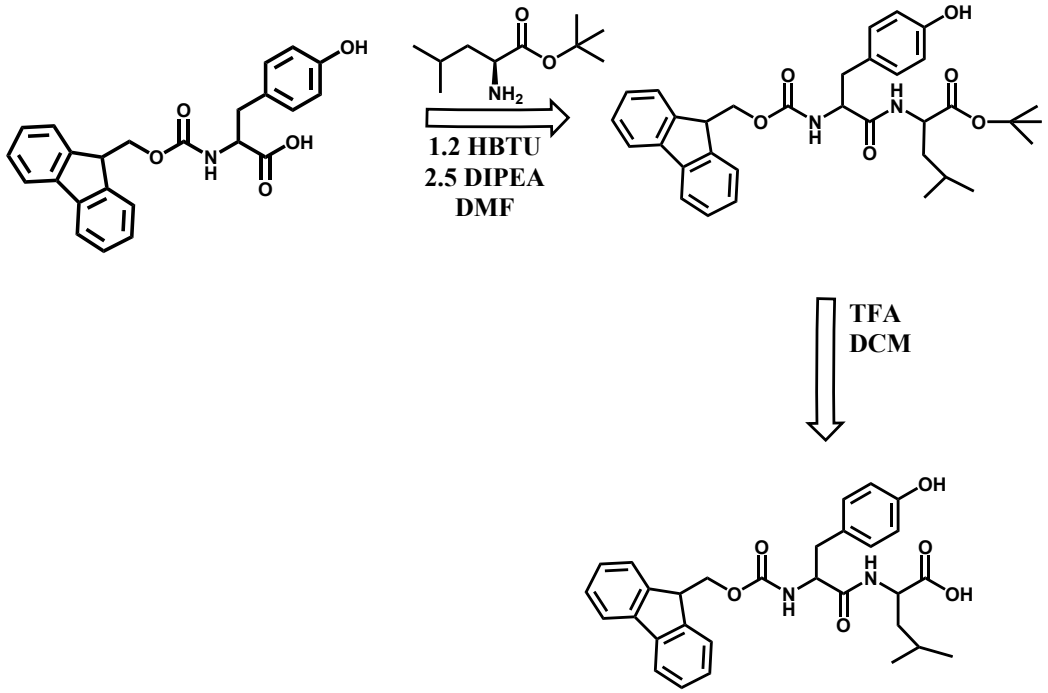
Materials: Fmoc-Tyrosine, Leu-Otbu HCl, Diisopropylethylamine (DIPEA) (>99.5%) were purchased from Sigma-Aldrich and used as received. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Novabiochem. All other reagents and materials from commercial sources were used without further purification. Silica gel used in chromatographic separations was obtained VWR (Silica Gel, ultrapure, 40-60 mm). NMR spectra were recorded in AVANCE 400 MHz (BRUKER) spectrometer.

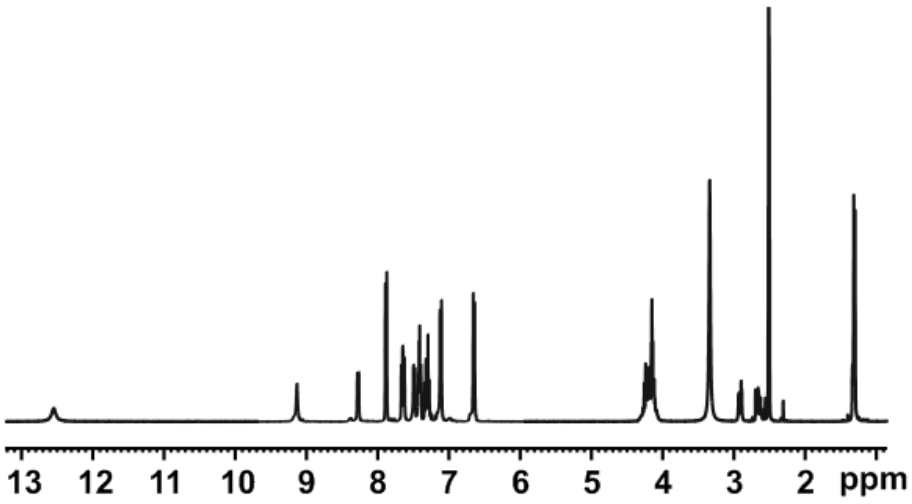
Synthesis of Fmoc-Tyr-Leu-OH (Fmoc-YL)

Fmoc-Tyr-OH, H-Leu-OtBu.HCl and HBTU were dissolved in anhydrous dimethylformamide (~ 15 ml) and DIPEA was added. The reaction mixture was left to stir for 24 hours. Product was precipitated out by addition of saturated sodium bicarbonate solution (~ 30 mL) and extracted into ethyl acetate (~ 50 mL). The mixture was washed with equal volumes of saturated brine, 1M hydrochloric acid and brine again. The resulting organic layer was dried by anhydrous magnesium sulphate and the ethyl acetate was removed by evaporation *in vacuo*. The resulting solid was then purified by column chromatography using 2.5 % methanol in dichloromethane as eluent. Fractions were tested by TLC using UV (254 nm) light to visualise spots. Fractions containing the compound were combined and solvent removed *in vacuo*. The removal of the t-Bu group was carried out by dissolving the sample in dichloromethane and adding 10 mL of trifluoroacetic acid. The reaction mixture was stirred for four hours. The dichloromethane was removed by evaporation *in vacuo*. The TFA was

removed with the addition of toluene (~10 mL) and solvent removed by evaporation *in vacuo* (carried out in triplicate). The resulting solid was washed 6 times with cold diethyl ether and the product dried under vacuum.



Strategy for the synthesis of Fmoc-YL



Purity by HPLC (300 nm) = 97.00 % ^{1}H NMR spectrum of Fmoc-YL in DMSO-d^6 at 298K: 12.6 (1H, s, OH), 9.2 (1H, s, Tyr OH), 8.34 – 8.32 (1H, NH d, J = 8 Hz), 7.89 – 7.87 (2H, d, J = 7.5 Hz, 2 fluorenyl Ar-CH), 7.66 – 7.62 (2H, m, 2 fluorenyl Ar-CH), 7.55 – 7.53 (1H, d, J = 9 Hz, NH), 7.43 – 7.39 (2H, m, 2 fluorenyl Ar-CH), 7.34 – 7.27 (2H, m, 2 fluorenyl Ar-CH), 7.10 – 7.09 (2H, d, j = 8.3 Hz, 2 Tyr Ar-CH), 6.64 – 6.62 (2H, d, J = 8.35 Hz, 2 Tyr Ar-CH), 4.25 – 4.10 (5H, m, fluorenyl CH, fluorenyl CH_2 and $C_\alpha H$), 2.92 – 2.91 (1H, m, Tyr

CH), 2.73 – 2.71 (1H, m, Tyr CH), 1.68 -1.62 (1H, m, Leu CH) 1.55-1.52 (2H, m Leu CH_2), 0.9-0.83 (6H, m Leu $2CH_3$) MS (ES+): m/z 517.2

Fmoc-Phe-Leu (Fmoc-FL) was purchased and used without any further purification from CS Bio Co (code: CS4005). Purity (214 nm) 97%. The Fmoc-dipeptide was used as the TFA salt.

Ultrasonic set up

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 which 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 which 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 which generated ultrasound.

Ultrasonic excitation level was controlled indirectly by varying the input to the amplifier from the signal generator. A calibration curve was obtained by relating the signal generator drive voltage to the pressure measured in the reactor with a Bruel Kjaer 8103 hydrophone from which the acoustic pressure could then be calculated. This curve is shown in figure S1.

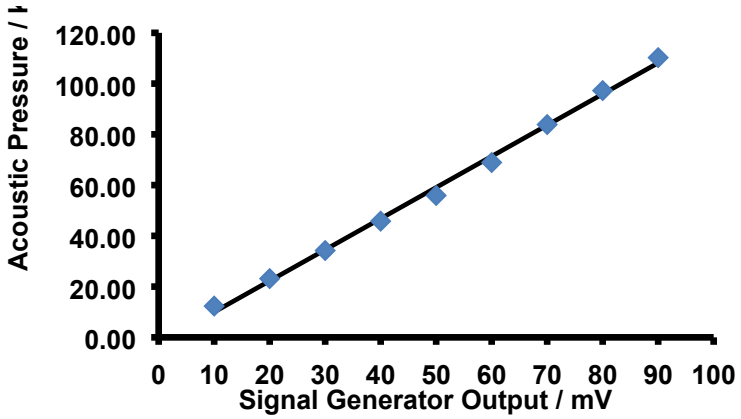


Fig. S1 Curve relating the signal generator drive voltage to the acoustic pressure in the reactor.

Hydrogel formation. 10 mg of Fmoc-YL was dissolved in 1 ml of 100 mM sodium phosphate buffer pH 8. Then the Fmoc-dipeptide was heated up to 363K for two minutes until it fully dissolved. A translucent gel like material was obtained after 20 minutes as evidenced by vial inversion. In the case of Fmoc-FL 10 mg was dissolved in 1 ml of 100 mM sodium phosphate buffer at pH 6. An opaque gel like material was obtained after 5 minutes. The ultrasound exposure for both the Fmoc-dipeptides was 5 minutes.

Fluorescence Spectroscopy. Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorometer with light measured orthogonally to the excitation light, at a scanning speed of 500 nm min^{-1}. For both the aromatic dipeptide amphiphiles the concentration was 20 mM. The excitation wavelength was 280 nm, and emission data were recorded in the range between 300 and 600 nm. The spectra were measured with a bandwidth of 3 nm with a medium response and a 3 nm data pitch.

Circular Dichroism (CD). CD Spectra were measured on a Jasco J-815 spectropolarimeter with 1 s integrations with a step size of 3 nm and a single acquisition with a slit width of 1 nm. Concentration for CD experiments was 20 mM. Demountable cells of 0.001 cm path length were used for the measurement.

FTIR spectroscopy. 20 mM of the Fmoc-dipeptides was dissolved in 1 ml of 100 mM sodium phosphate buffer pH 8 (or 6) in D_2O. FTIR spectra were acquired in a Bruker Vertex spectrometer with a spectral resolution of 2 cm^{-1}. Measurements were performed in a standard IR cuvette (Harrick Scientific), in which the sample was contained between two CaF2 windows (thickness, 2 mm) separated by a 25-mm PTFE spacer.

TEM Microscopy. Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 seconds. The support film was touched onto the gel surface or the solution (after ultrasound exposure in the case of Fmoc-YL) for 3 seconds and blotted down using filter paper. Negative stain (20mL, 1% aqueous methylamine vanadate obtained from Nanovan; Nanoprobes) was applied and the mixture blotted again using filter paper to remove the excess and dried immediately for few minutes. The dried specimens were then immediately imaged using a LEO 912 energy filtering transmission electron microscope operating at 120 kV fitted with a 14 bit/2 K Proscan CCD came.

Results

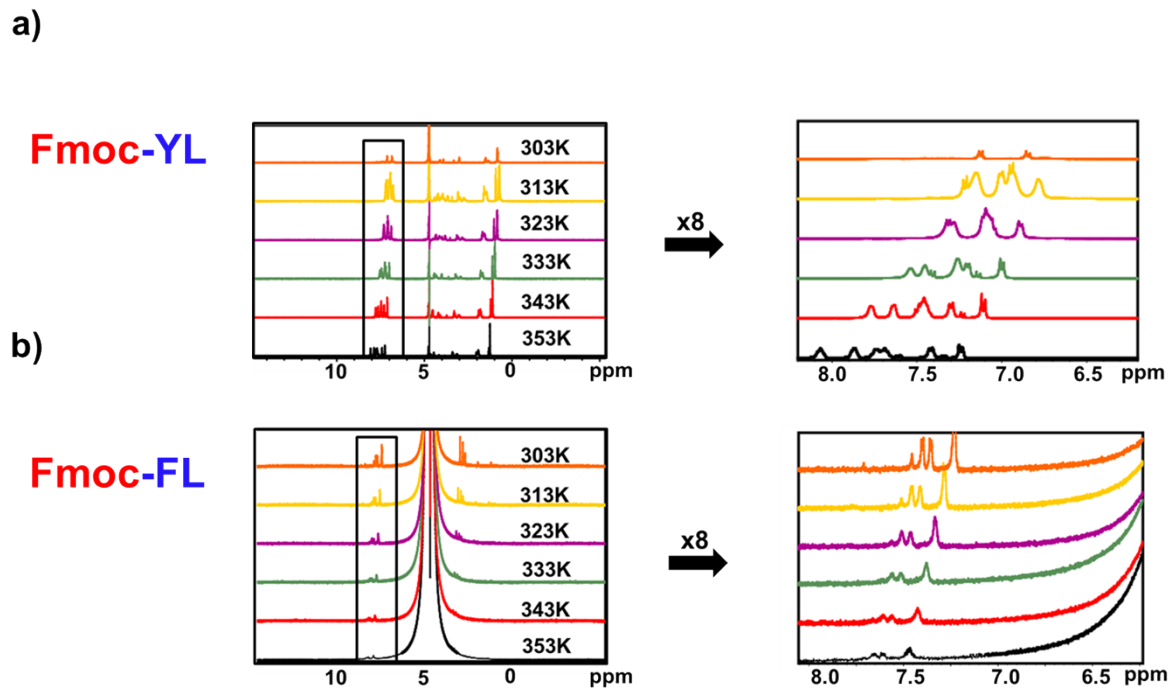


Fig. S2 **a)** Temperature dependent ^{1}H NMR spectra of 20 mM of Fmoc-YL in D_2O pH 8 from 303-353K and **b)** Fmoc-FL in D_2O pH 6 highlighting their differential aggregation behaviour upon heating. Full spectra on the left side show a water peak at 4.7 ppm just adjacent to the aromatic region. A zoomed in (8 times) image of the aromatic region is shown on the right.

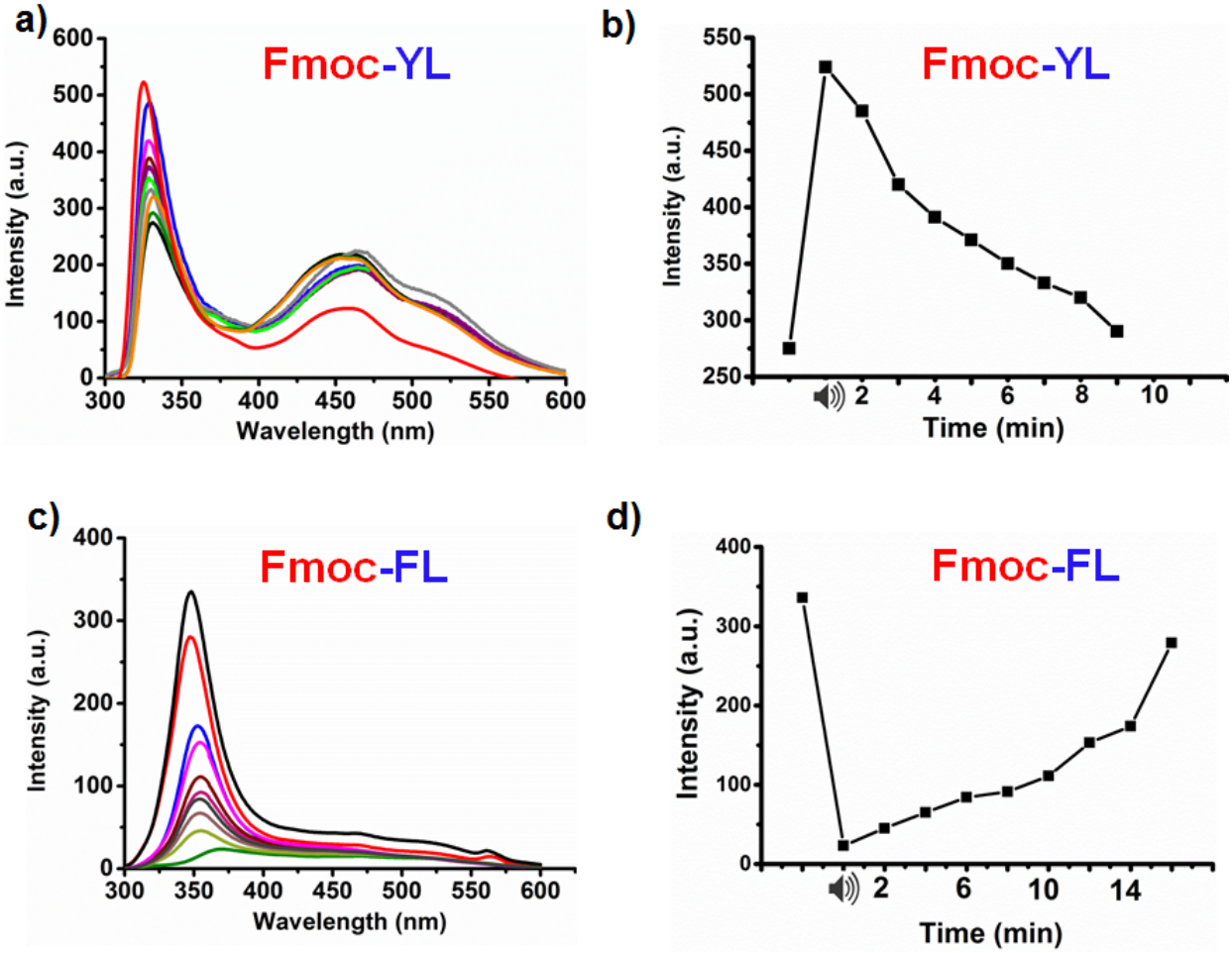


Fig. S3 **a)** Fluorescence emission spectrum of 20 mM of Fmoc-YL pH 8 before ultrasound exposure (black line), after 5 minutes ultrasound exposure (red line) and when the ultrasound is off, **b)** plot of the fluorescence intensity at 331 nm before (275 a.u.) after ultrasound exposure (524 a.u.) and when the sound is off (290 a.u.) after 10 minutes, **c)** Fluorescence emission spectra of 20 mM of Fmoc-FL pH 6 before ultrasound exposure (black line), after 5 minutes ultrasound exposure (green line) and when the ultrasound is off after 16 minutes and **d)** plot of the fluorescence intensity before (348 a.u.) after ultrasound exposure (25 a.u.) and when the sound is off (336 a.u.) for 16 minutes.

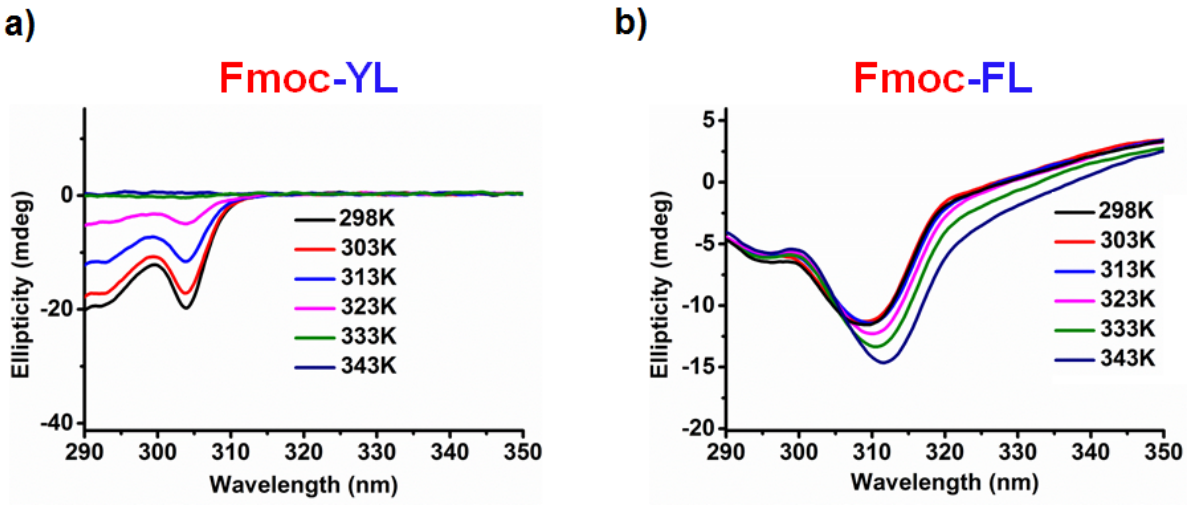


Fig. S4 Temperature dependent CD spectrum of 20 mM of **a)** Fmoc-YL pH 8 from 298-343K and **b)** 20 mM of Fmoc-FL pH 6 from 298-343K.

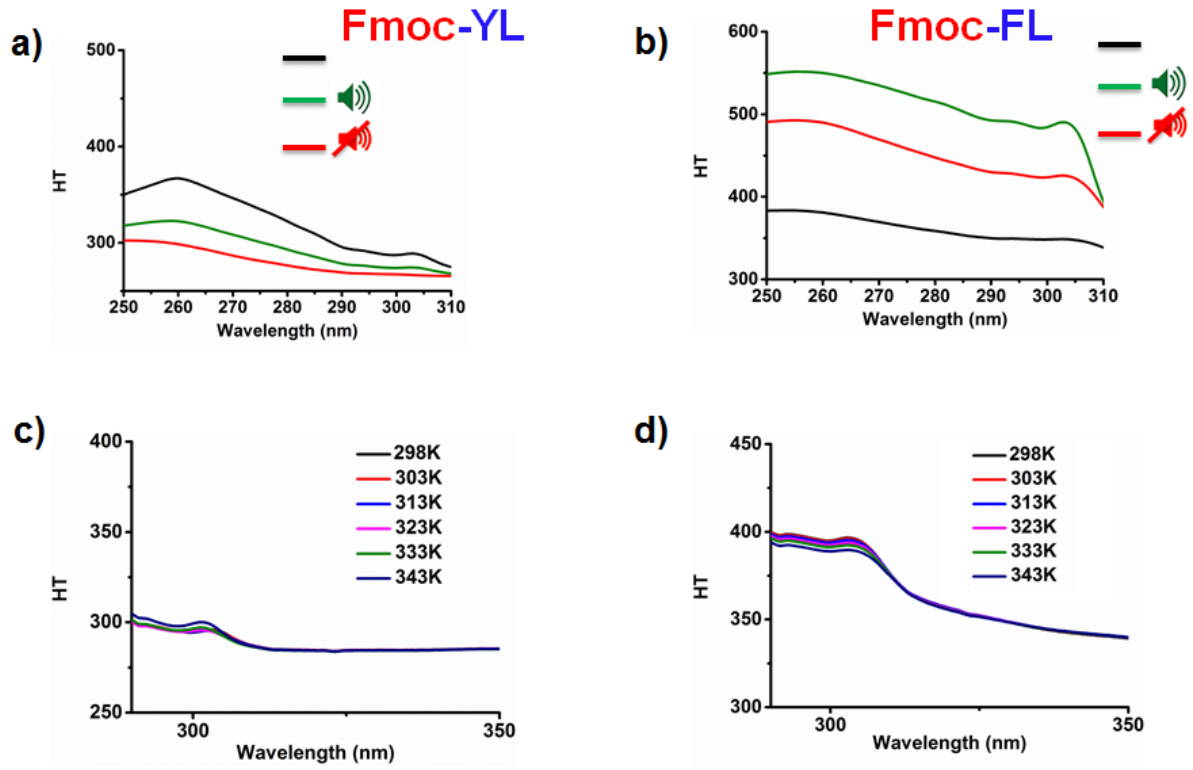


Fig. S5 HT values of the CD experiments for **a)** Fmoc-YL, **b)** Fmoc-FL before after and when the ultrasound is off and **c), d)** temperature dependent HT values for Fmoc-YL and Fmoc-FL respectively.

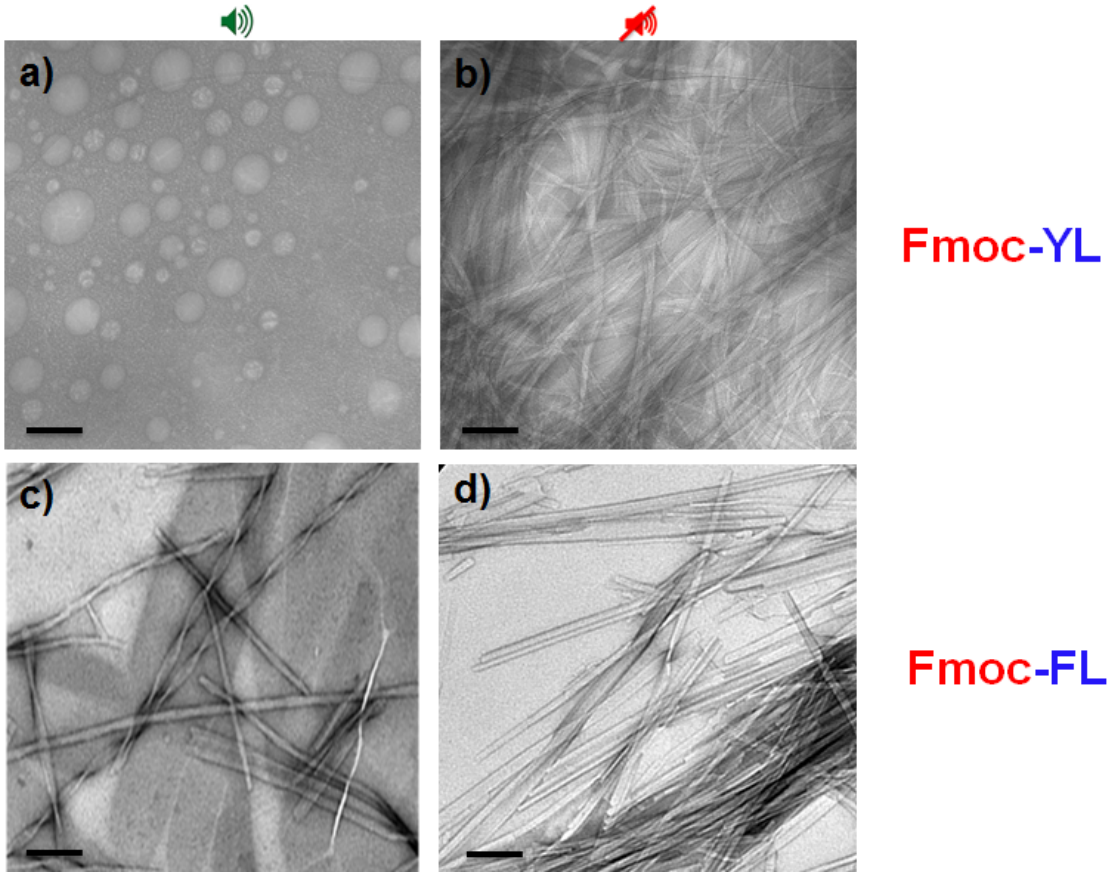


Fig. S6 TEM images of a second sonication cycle after 5 minutes ultrasound exposure and when the sound is off for Fmoc-YL **(a-b)** and for Fmoc-FL **(c-d)** respectively.