Electronic Supplementary Material (ESI) for Soft Matter.

ELECTRONIC SUPPLEMENTARY INFORMATION

Enzymatically activated emulsions stabilised by interfacial nanofibre networks

Inês P. Moreira,[a] Ivan Ramos Sasselli,[a] Daniel A. Cannon,[a] Meghan Hughes,[a,b] Dimitrios A. Lamprou[c,d], Tell Tuttle,[a]† Rein V. Ulijn[a,b]†

[a]WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral St, Glasgow G1 1XL, U.K.

[b]Advanced Science Research Center (ASRC), City University of New York, 85 St Nicholas Terrace, New York NY10031, USA.

[c]Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, 161 Cathedral Street, G4 0RE Glasgow, U.K.

[d]EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation (CMAC), Technology and Innovation Centre, Glasgow, U.K.

Corresponding Author

† To whom the correspondence should be addressed:

Dr. Tell Tuttle, email: tell.tuttle@strath.ac.uk

Prof. Rein Ulijn, email: rein.ulijn@asrc.cuny.edu

Synthesis of Fmoc-YL

Fmoc-Tyr-OH (1 g), L-Leucine tert-butyl HCl (0.666 g) and HBTU (1.13 g) were dissolved in approximately 15 mL of dimethylformamide (DMF). 1.08 mL (0.742 $g.cm^{-3}$ density) of DIPEA was added and the solution was stirred for 24 hours. The product was precipitated out by adding sodium bicarbonate solution and extracted into ethyl acetate. The mixture was then washed in duplicate with equal volumes of brine, 1 M HCl and brine again. The resulting organic layer was then dried using magnesium sulphate, filtered and the ethyl acetate removed by vacuum evaporation. The solid was then purified by column chromatography using 2.5 - 5% methanol in dichloromethane (DCM) as eluent. Fractions were tested using TLC under UV (254 nm) light to visualize the compound spots and the ones containing the compound were combined and evaporated in vacuum. The compound was dissolved in DCM and added 10 mL of trifluoroacetic acid (TFA) to remove the tert-butyl group. The reaction mixture was stirred overnight and the DCM removed by evaporation in vacuum. Toluene was added to the resulting solid to remove the TFA. The mixture was dissolved by ultrasonication and toluene was removed by evaporation in vacuum (procedure carried out in triplicate). The resulting solid was washed with cold diethyl ether for 6 times and the product dried under vacuum to obtain a white powder (Purity 88.25%, 0.604 g). The compound was further purified by preparative HPLC using acetonitrile/water and only collecting the compound at its retention time. The collected samples were combined and freeze-dried, resulting in a final purity of 100%.

H δ (400 MHz, DMSO): 12.53 (1H, s, OH), 9.12 (1H, s, Tyr OH), 8.15 (1H, d, J = 7.9 Hz, NH), 7.88 (2H, d, J = 7.5 Hz, 2 Ar CH), 7.657 - 7.616 (2H, m, 2 Ar CH), 7.47 (1H, d, J = 8.9 Hz, NH), 7.423 - 7.383 (2H, m, 2 Ar CH), 7.330 – 7.230 (2H, m, 2 Ar CH), 7.10 (2H, d, H = 8.4 Hz, 2 Ar CH), 6.64 (2H, d, J = 8.4 Hz, 2 Ar CH), 4.23 - 4.11 (5H, m, Fmoc CH, Fmoc CH_2, 2 $C_{\alpha}H$), 3.37 - 3.32 (1H, m, Tyr CH), 2.88 - 2.69 (1H, m, Tyr CH), 2.655 - 2.505 (1H, m, Leu CH), 1.56 - 1.32

(2H, m, Leu CH_2), 0,88 (3H, d, J = 6.5 Hz, Leu CH_3), 0.84 (3H, d, J = 6.5 Hz, Leu CH_3).

^{13}C δ (100 MHz, DMSO): 174.9 (C=O, Leu), 172.7 (C=O, Tyr), 156.6 (C=O, Fmoc), 144.6 (Ar C, Tyr), 141.5 (Ar C), 131.1 (Ar C), 129.8 (Ar C), 129.1 (Ar C), 128.5 (Ar C), 127.9 (Ar C), 126.2 (Ar C), 121,0 (Ar C), 115.7 (Ar C), 66.5 (CH_2, Fmoc), 57.1 (C_α, Tyr), 51.2 (C_α, Leu), 47.5 (CH, Fmoc), 37.5 (CH_2, Tyr), 25.2 (CH, Leu), 23.7 (CH_3, Leu), 22.3 (CH_3, Leu). 40.3 (CH2, Leu) predicted by Chemdraw, undermasked by solvent.

MM: 516.59 $g.mol^{-1}$, MS (ES+): 517.0 $[M + H]^+$, 539.2 $[M + Na]^+$.

Elemental analysis – Found: C, 69.50%; H, 6.41%; N, 5.15%; Expected for $C_{30}H_{32}N_2O_6$: C, 69.75%; H, 6.24%; N, 5.42%; O, 18.58%.

Synthesis of Fmoc-YpL

Fmoc-Tyr($PO(NMe_2)_2$-OH (1 g) was dissolved instead of Fmoc-Tyr-OH with L-Leucine tert-butyl HCl (0.499 g) and HBTU (0.847g) approximately 15 mL of dimethylformamide (DMF). 0.810 mL (0.742 $g.cm^{-3}$ density) of DIPEA was added and the solution was stirred for 24 hours. The same procedure was followed but after stirring the reaction mixture with 10 mL of trifluoroacetic acid (TFA) to remove the tert-butyl group, 1 mL of water was added and the mixture stirred for 24 hours to remove the methyl groups. The following steps were maintained as for Fmoc-YL synthesis and obtained a white powder (Purity 75.73%, 0.585 g). The compound was further purified by preparative HPLC using acetonitrile/water and only collecting the compound at its retention time. The collected samples were combined and freeze-dried, resulting in a final purity of 97.96%.

H δ (400 MHz, DMSO): 8.56 (2H, Broad s, NH&OH), 7.89 - 7.87 (2H, m, 2 Ar CH), 7.73 - 7.67 (2H, m, 2 Ar CH), 7.54 (1H, d, J = 8.16 Hz, NH), 7.44 - 7.39 (2H, m, 2 Ar CH), 7.35 - 7.29 (2H,

m, 2 Ar CH), 7.18 – 6.95 (4H, m, 4 Ar CH), 4.24 - 4.13 (5H, m, Fmoc CH, Fmoc CH_2, Tyr $C_\alpha H$, Leu $C_\alpha H$), 3.58 (2 OH, Broad s, phosphate), 2.91 - 2.68 (2H, m, Tyr CH_2), 1.58 - 1.46 (2H, m, Leu CH_2), 1.40 - 1.36 (1H, m, Leu CH), 0.86 - 0.81 (6H, m, Leu $2CH_3$).

^{13}C δ (100 MHz, DMSO): 174.21 (C=O, Leu), 171.73 (C=O, Tyr), 155.47 (C=O, Fmoc), 144.71 (Ar C), 142.61 (Ar C), 141.56 (Ar C), 130.54 (Ar C), 128.52 (Ar C), 127.98 (Ar C), 126.26 (Ar C), 121.49 (Ar C), 120.96 (Ar C), 118.97 (Ar C), 73.40 (CH_2, Fmoc), 63.98 (C_α, Tyr), 51.35 (C_α, Leu), 47.49 (CH, Fmoc), 39.12 (CH_2, Tyr), 34.78 (CH, Tyr), 25.10 (CH, Tyr), 23.59 (CH_3, Tyr), 22.55 (CH_3, Tyr).

MM: 596.57 $g.mol^{-1}$, MS (ES+): 597.0 $[M + H]^+$, 619.2 $[M + Na]^+$.

Elemental analysis – Found: C, 58.40%; H, 5.21%; N, 4.04%; Expected for $C_{30}H_{33}N_2O_9P$: C, 60.40%; H, 5.58%; N, 4.70%; O, 24.14%; P, 5.19;

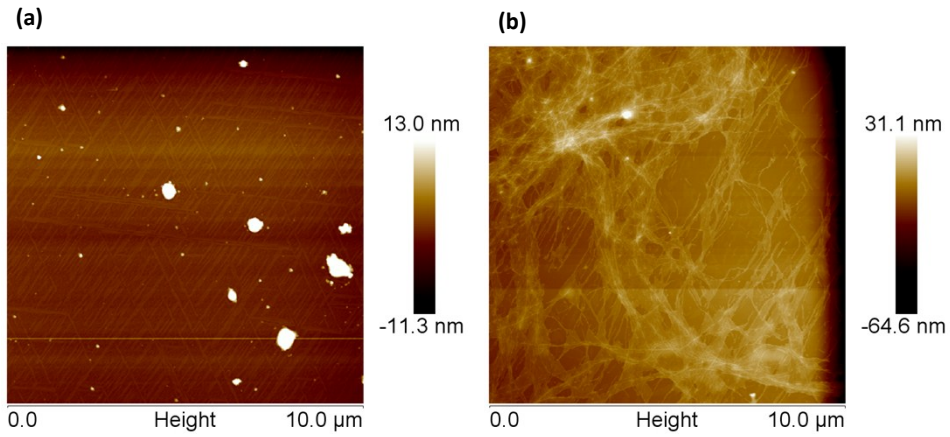

Figure S1. AFM image of Fmoc-YpL (a) and Fmoc-YL (b).

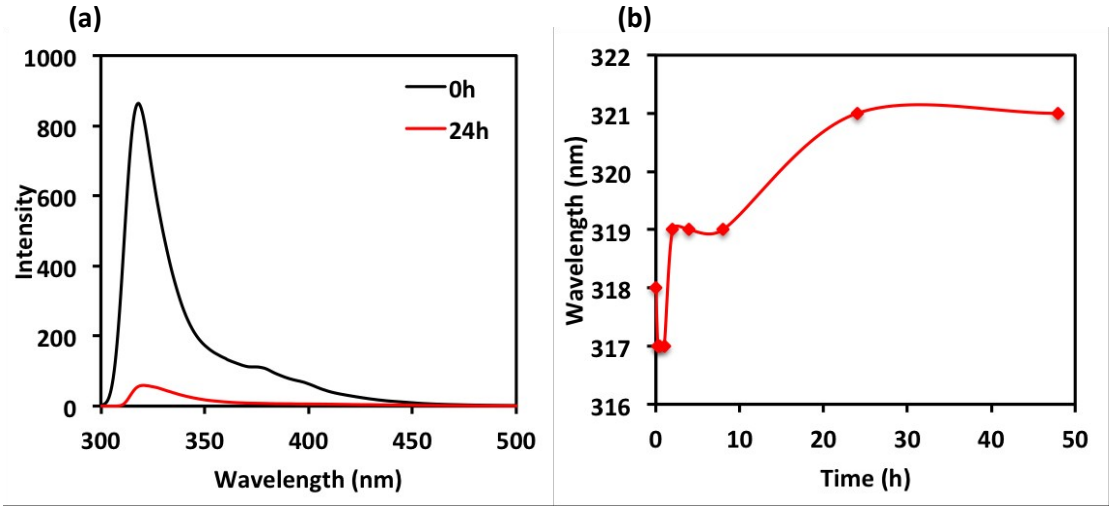

Figure S2. (a) Non-normalised fluorescence emission spectra of Fmoc-YpL (0h) and Fmoc-YL achieved 24h after enzyme addition (excitation 280 nm); (b) Representation of the lambda max wavelength at which fluorenyl peaks were observed from the time of enzyme addition, showing a gradual red-shift.

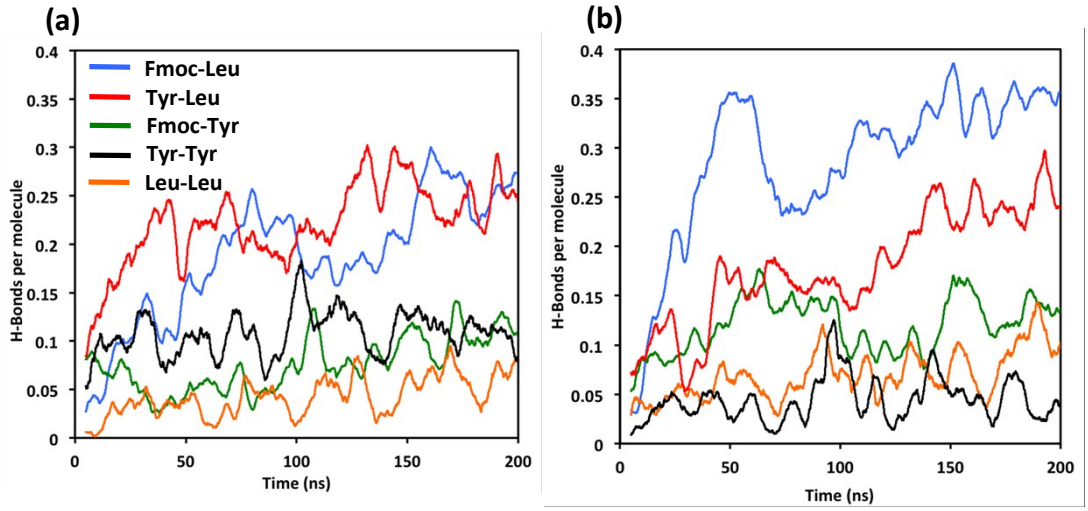

Figure S3. (a) Hydrogen bonds per molecule between Fmoc-YpL molecules throughout the aqueous system simulation; (b) Hydrogen bonds per molecule between Fmoc-YL molecules throughout the aqueous system simulation.

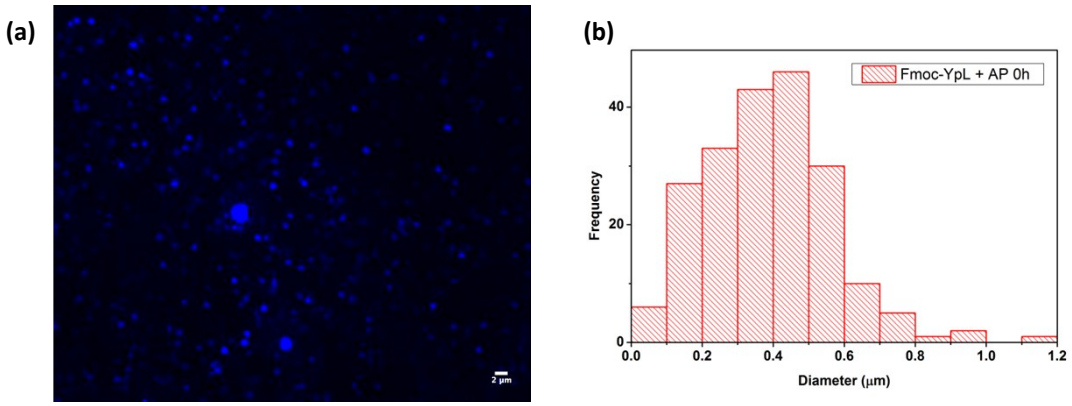

Figure S4. (a) Fluorescence microscopy and (b) Histogram of droplet size distribution of chloroform-in-water droplets stabilised by nanofibrous networks of Fmoc-YL when enzyme is added upon preparation. Bin width of 0.1 μm, defined as the 1/10 maximum drop size.

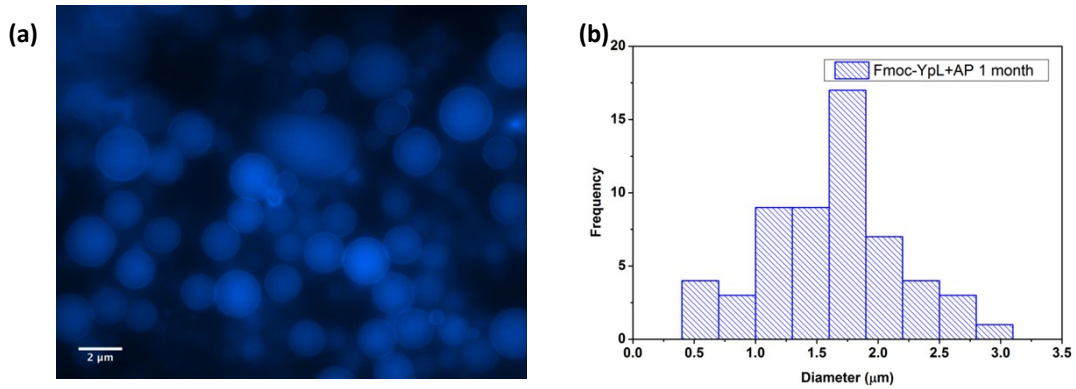

Figure S5. (a) Fluorescence microscopy and (b) Histogram of droplet size distribution of chloroform-in-water droplets stabilised by nanofibrous networks of Fmoc-YL when enzyme is added to Fmoc-YpL two-phases mixture after being stored for 1 month. Bin width of 0.3 µm, defined as the 1/10 maximum drop size.

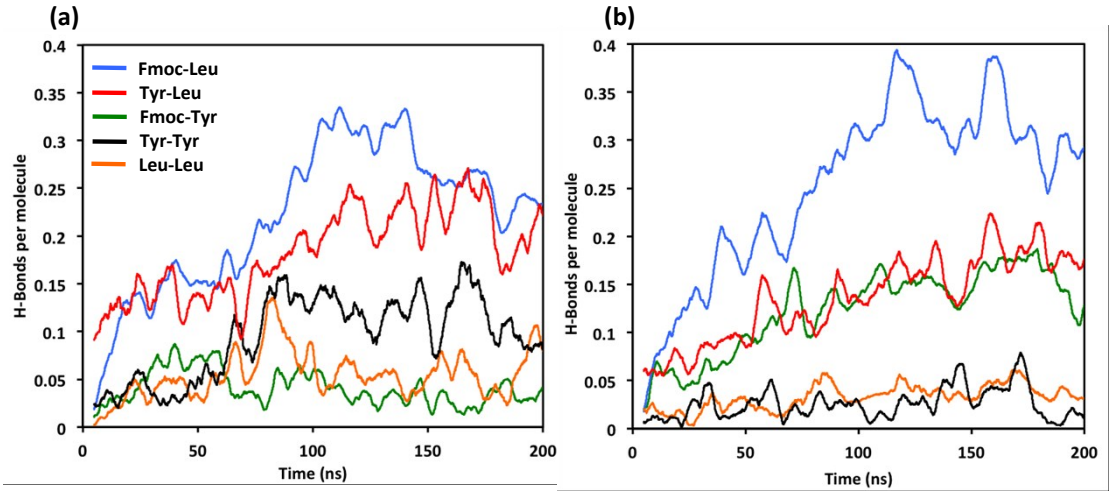

Figure S6. (a) Hydrogen bonds per molecule between Fmoc-YpL molecules throughout the biphasic system simulation; (b) Hydrogen bonds per molecule between Fmoc-YL molecules throughout the biphasic system simulation.