Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2019

Supplementary Information

Thermally robust solvent-free biofluids of M13 bacteriophage engineered for high compatibility with anhydrous ionic liquids

Alex P. S. Brogan[1†], Nimrod Heldman[3,4†], Jason P. Hallett[1*], Angela Belcher[3,4,5*].

1. Department of Chemical Engineering, Imperial College London, London, SW7 2AZ, UK
2. Department of Chemistry, King's College London, Britannia House, London, SE1 1DB
3. Department of Biological Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, 76-561, Cambridge, USA
4. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Avenue, 76-561, Cambridge, USA
5. Department of Materials Science and Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, 76-561, Cambridge, USA

†Authors contributed equally

Materials and Methods

Preparation of M13 bacteriophage

The bacteriophage clone with an N-terminal fusion peptide sequence of ENKVE was constructed as reported elsewhere.[1]

Amplification of the bacteriophage clone was performed as follows. Two 500 mL inoculated one only with *E. coli* and the other with *E. coli* infected with the bacteriophage clone and grown overnight. The next day 2 L of LB media in 2 L Erlenmeyer flasks with the same concentration of tetracycline were inoculated with the *E. coli* and grown for 3 hours to an O.D. of approximately 0.4 and then infected with the bacteriophage infected *E. coli* and grown overnight. The growth media was harvested and centrifuged for 30 min at 8000 rpm in a JLA 8.1 rotor (Beckman Coultor). The bacteriophage containing supernatant was separated from the *E. coli* pellet and a solution of 20 % 8000 molecular weight polyethylene glycol and 2.5 M NaCl was added at a final ratio of 1:6 to precipitate the bacteriophage at 4 °C overnight. The precipitated bacteriophage was pelleted by centrifuging at 8000 rpm for one hour. The bacteriophage pellet was resuspended in 10 mL phosphate buffer saline pH 7 and PEG/NaCl was added again at a 1:6 final volume for a second purification step. After an overnight incubation at 4 °C the bacteriophage were pelleted at 14000 rpm in an Allegra 64R centrifuge with a F085 rotor. The pellet was resuspended in 5 mL water to a final Bacteriophage concentration of 1.33×10^{14} phage particles.mL^{-1} (3.69 mg.mL^{-1}) calculated by absorbance at 269 and 320 nm.[2]

Formation of solvent-free M13 biofluids

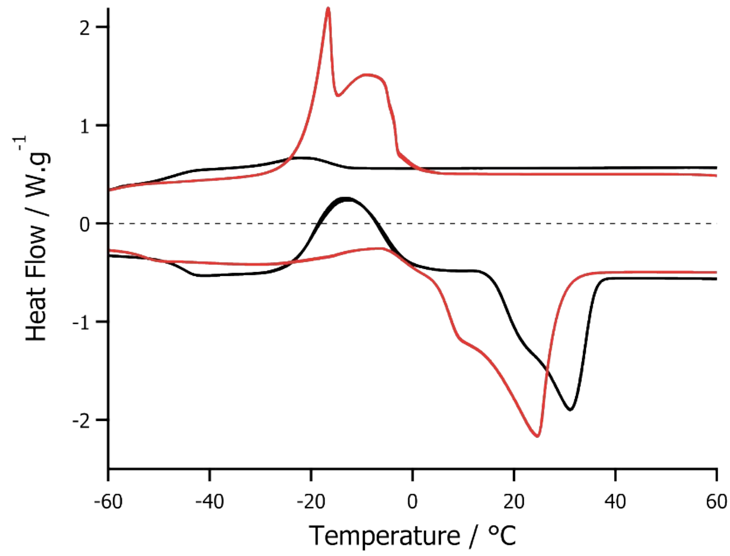
M13-polymer surfactant nanoconjugates were prepared using an adapted form of established methods[3]. Here, M13 (0.25 mg.mL^{-1}) was cationized via EDC mediated coupling of N,N′-bis(2- aminoethyl)-1,3-propanediamine to surface available acidic residues (C−M13), with pH kept above 6.5 to avoid phage aggregation. After extensive dialysis against ultrapure water for 24 h, neutralized solutions of anionic polymer-surfactants – poly(ethylene glycol) 4-nonylphenyl 3-sulfopropyl ether (S_1), and glycolic acid ethoxylate lauryl ether (S_2) - were added and stirred for 12 h. The resultant [C-M13][S] nanoconjugates were dialysed against ultrapure water for 24 h, freezedried for 48 h, and then annealed at 80 °C, to yield solvent-free biofluids of M13.

Analytical techniques

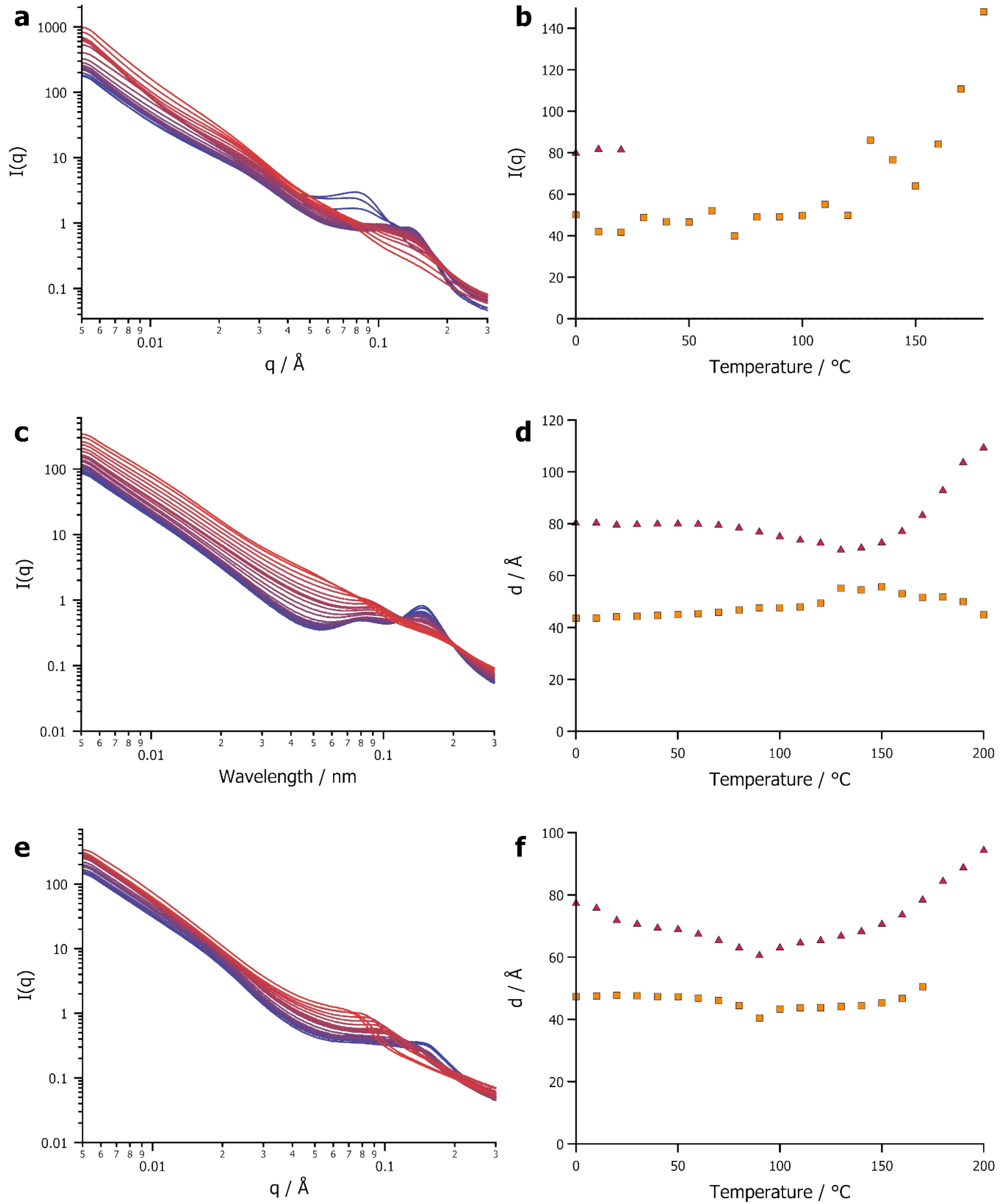
UV/Vis performed on a Shimadzu UV2600. FTIR performed on PerkinElmer Spectrum 100 with universal ATR accessory, average of 8 accumulations. SRCD performed at Diamond B23, with secondary structure estimations performed using DichroWeb with the SP175 basis set and CDSSTR algorithm.[4–6] DLS measured using Malvern Zetasizer μV. TEM performed using JEOL 2100Plus. DSC performed using TA Instruments Q2000, ramp rate of 10 °C.min^{-1}. SAXS and WAXS profiles collected at Diamond I22 (0.5 mm DSC pans, with Kapton windows).

Supplementary Table 1. Table showing secondary structure estimations for M13 and [C-M13][S_2], determined by deconvoluting corresponding SRCD spectra using DichroWeb.

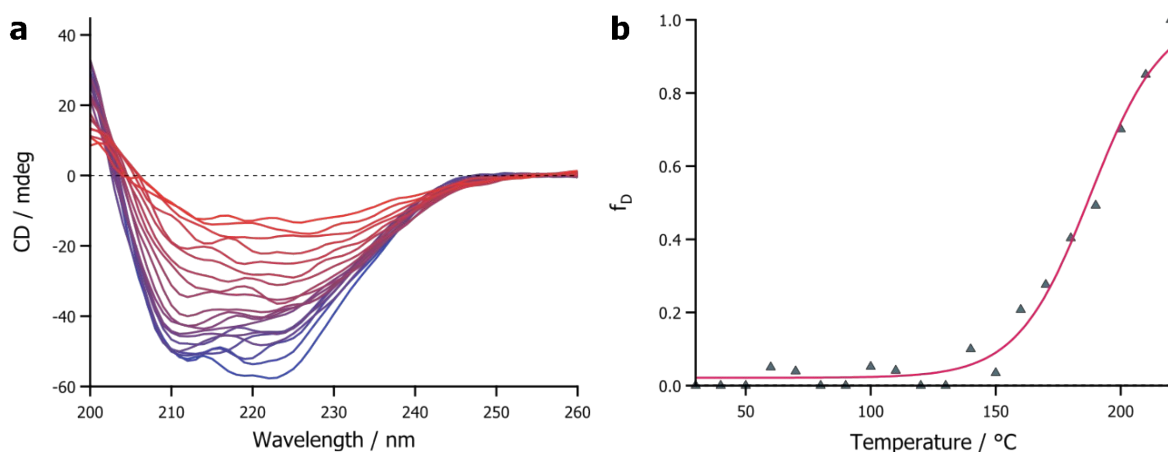
	α-helix / %	**β-sheet / %**	**Turns / %**	**Unordered / %**
M13 (aqueous)	25	24	13	39
[C-M13][S_2]				
Aqueous	49	13	12	26
Solvent-free	42	14	12	32
[bmpyrr][OTf]	39	17	13	31
[bmpyrr][$MeSO_4$]	40	13	15	32
[bmpyrr][NTf_2]	44	17	12	26



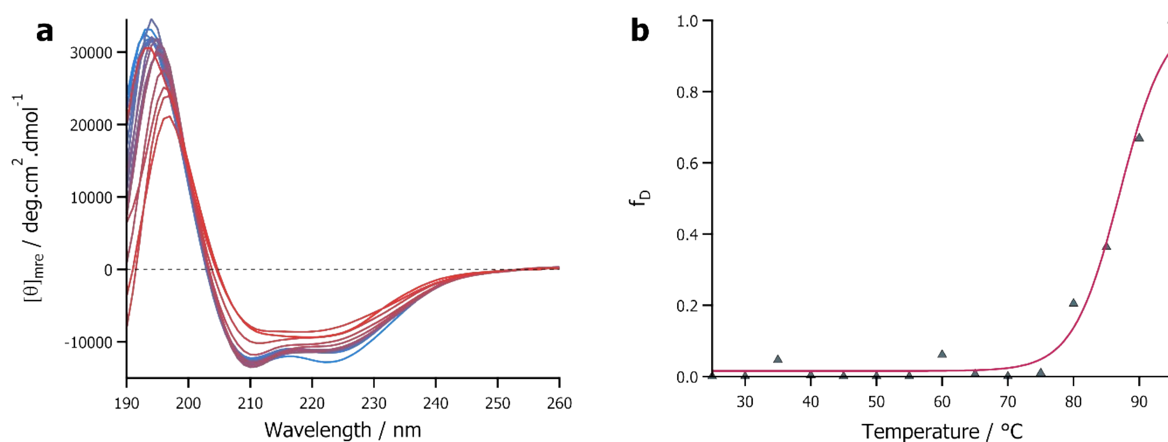
Supplementary Figure 1. DSC thermogram showing heating and cooling cycles of S_1 (black) and S_2 (red).



Supplementary Figure 2. Temperature dependent SAXS (left) plots (0 °C – blue, 200 °C – red) and corresponding plots of distance against temperature for internal diameter (yellow) and phage separation (purple) against temperature for [C-M13][S_2] in [bmpyrr][$MeSO_4$] (**a**,**b**), [bmpyrr][OTf] (**c**,**d**), and [bmpyrr][NTf_2] (**e**,**f**).



Supplementary Figure 3. (**a**) Temperature dependent SRCD spectra for solvent-free [C-M13][S_2] showing thermal denaturation from 30 °C (blue) to 220 °C (red). (**b**) Plot of fraction denatured against temperature for solvent-free [C-M13][S_2], calculated using 2-state model of denaturation from temperature dependent SRCD data (SI Fig. 3a).



Supplementary Figure 4. (**a**) Temperature dependent SRCD spectra for aqueous [C-M13][S_2] showing thermal denaturation from 25 °C (blue) to 95 °C (red). (**b**) Plot of fraction denatured against temperature for aqueous [C-M13][S_2], calculated using 2-state model of denaturation from temperature dependent SRCD data (SI Fig. 4a).

References

1 H. Park, N. Heldman, P. Rebentrost, L. Abbondanza, A. Iagatti, A. Alessi, B. Patrizi, M. Salvalaggio, L. Bussotti, M. Mohseni, F. Caruso, H. C. Johnsen, R. Fusco, P. Foggi, P. F. Scudo, S. Lloyd and A. M. Belcher, *Nat. Mater.*, 2015, **15**, 211–216.

2 C. F. I. Barbas, D. R. Burton, J. K. Scott and G. J. Silverman, *Phage display: A laboratory manual*, Cold Spring Harbor Laboratoty Press, New York, 2001.

3 A. P. S. Brogan and J. P. Hallett, *J. Am. Chem. Soc.*, 2016, **138**, 4494–4501.

4 J. G. Lees, A. J. Miles, F. Wien and B. A. Wallace, *Bioinformatics*, 2006, **22**, 1955–62.

5 L. Whitmore and B. A. Wallace, *Biopolymers*, 2008, **89**, 392–400.

6 L. Whitmore and B. A. Wallace, *Nucleic Acids Res.*, 2004, **32**, W668-73.