Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Decorated Networks of Native Proteins: Nanomaterials with Tunable Mesoscopic Domain Size

Fig. S1 Protein precipitation using different concentrations of YCl_3. Both proteins retained their fluorescence upon salt addition. Upper row shows eGFP gels, whereas bottom row shows few aggregates formed with native mCherry. Scale bars are 10 μm and 25 μm for eGFP and mCherry, respectively.

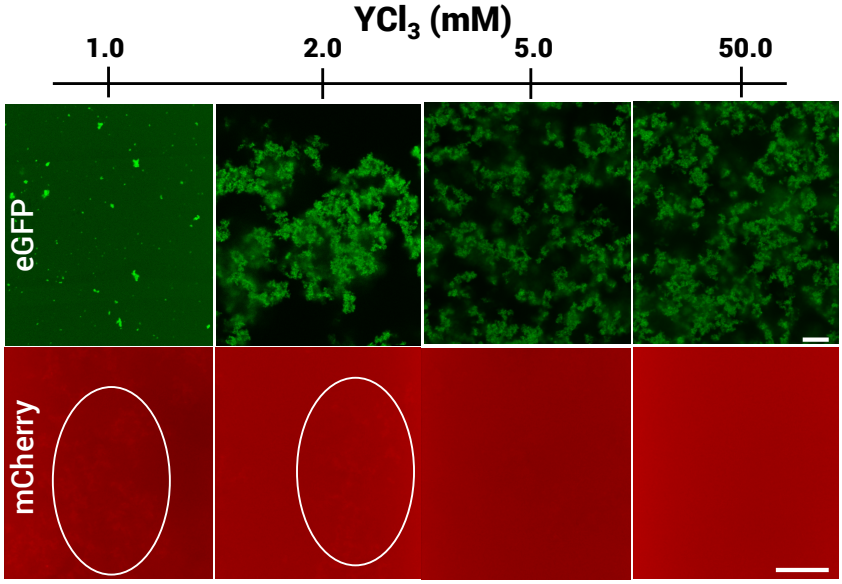

Fig. S2 Precipitation of cationised mCherry. (a) No precipitation is observed when using YCl_3. (b) The protein gels readily without loosing its fluorescence when 3 M of ammonium sulphate is added. Scale bars are 7.5 μm and 10 μm for (a) and (b), respectively.

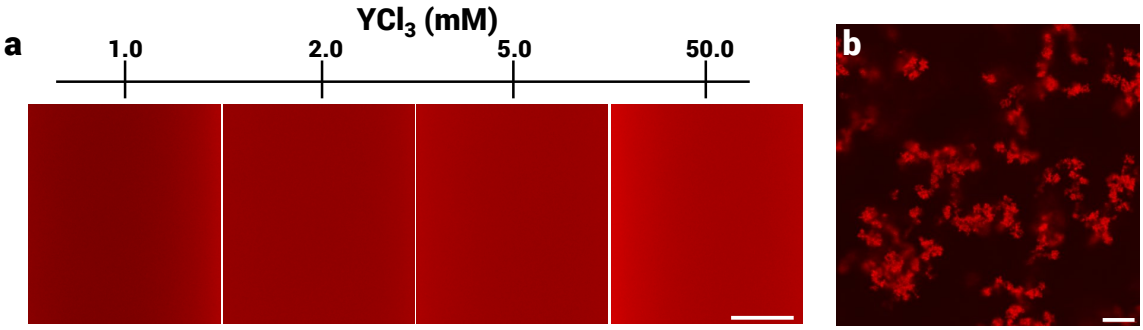

Fig. S3 Thermal denaturation curves for cationised mCherry as a function of temperature. The level 1 mL of protein folding (0.08 mg/mL) was monitored via the emission of the chromophore excited at 587 nm whilst increasing the temperature using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies) fitted with a Peltier thermoelectric bath. Before each measurement, the temperature was allowed to equilibrate for 5 min.

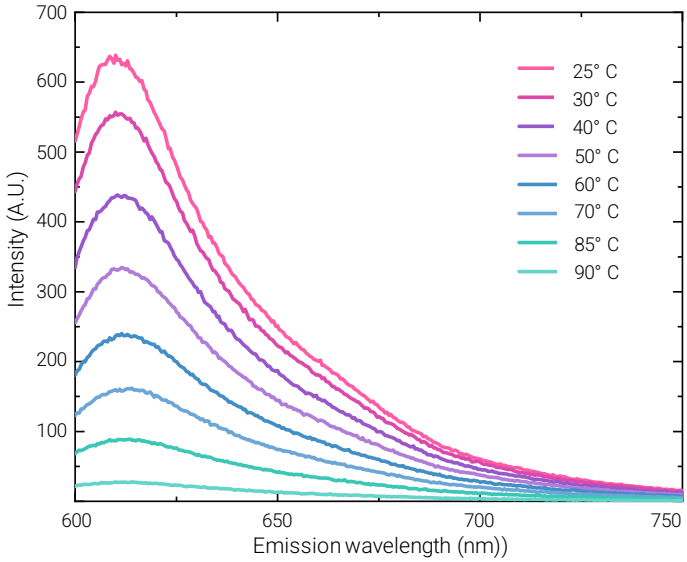

Fig. S4 Confocal image of an eGFP gel after the addition of 5 mM yttrium chloride followed by 3 M of ammonium sulphate. The gel structure shown is comparable to the correspondent one shown in Fig. 3 b in the main text and in Fig. S1. Scale bar is 10 μm.

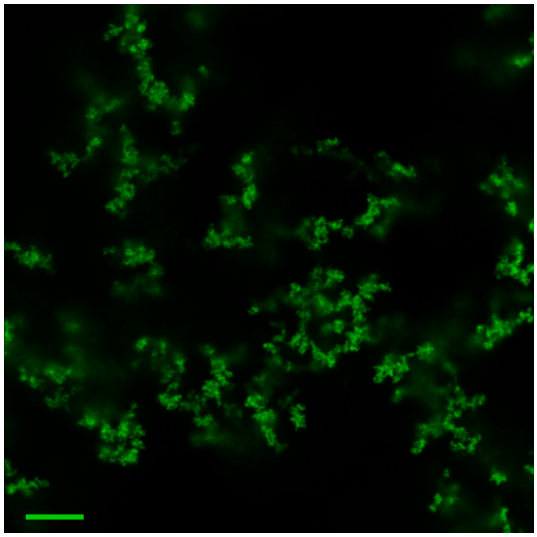

Fig. S5 One-step bicontinous gels formation through the addition of different amounts of ammonium sulphate to a 1:1 mixture of eGFP (green) and mCherry (red). The top images are an overlay of the channels. No precipitation is observed at 1 M of the salt, concentration after which small clusters of only eGFP are observed. However at 1.6 M we start seeing mCherry co-precipitation and deposition (individual protein panels). At 1.8 M all eGFP has fully precipitated (black background on eGFP individual channel) and mCherry does it by 3 M of ammonium sulphate. The binary gels are formed by a mixture of both proteins. The circled regions highlight the structures found.

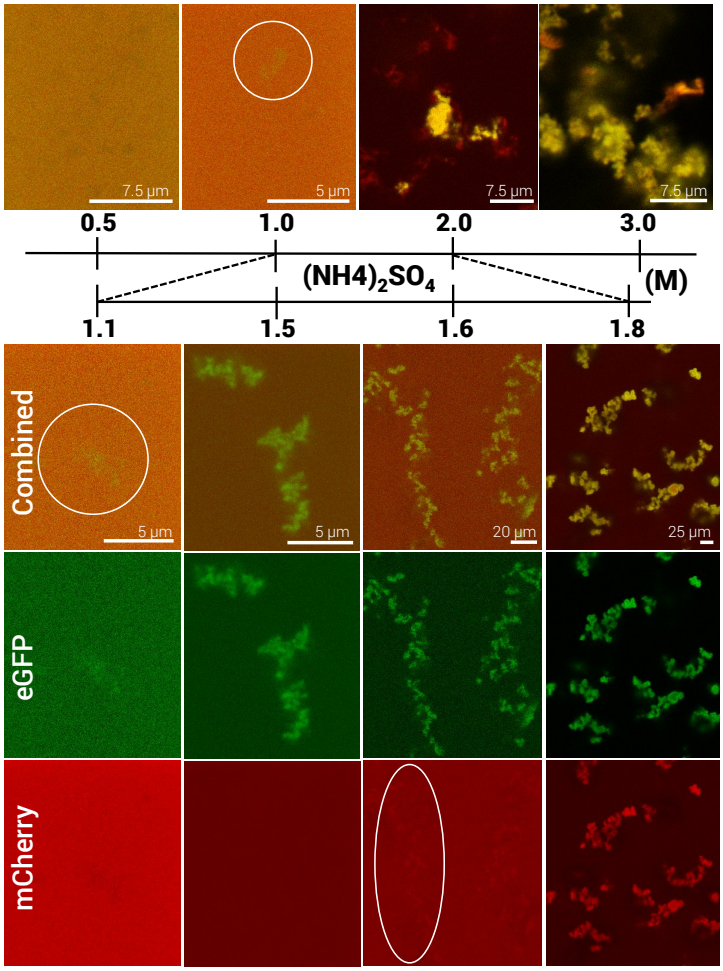

Table I. Parameters used and obtained from SASView [1] fitting using a Cylinder + Orstein-Zernicke form and structure factor, respectively

eGFP							
$[YCl_3]$ (mM)	Radius (Å)	Error Radius (Å)	Length (Å)	Error length (Å)	Correlation length, ξ (Å)	Error ξ (Å)	Fitting χ^2
No salt	20.45	0.08	82.29	0.77	23.48	0.80	1.19
0.1	29.66	0.08	74.92	0.70	18.53	0.89	1.09
0.25	19.47	0.08	74.80	0.71	189.14	96.99	1.25
0.5	18.77	0.07	132.79	1.47	246.43	31.97	1.85
0.75	18.32	0.06	221.05	3.42	245.99	27.22	1.86

eGFP Scattering Light Density = 1.894×10^{-6} A^{-2} calculated from [2]

Table II. Parameters used and obtained from SASView [1] fitting using a Cylinder Form Factor

Cationised mCherry					
$[(NH_4)_2SO_4]$ (M)	Radius (Å)	Error Radius (Å)	Length (Å)	Error length (Å)	Fitting χ^2
No salt	18.96	0.09	51.40	1.32	1.44
0.5	16.03	0.47	54.90	2.11	1.29
0.75	16.18	0.57	54.24	2.59	1.26
1	12.80	0.71	57.85	2.51	1.44
1.5	15.34	0.21	109.3	7.00	1..42

mCherry Scattering Light Density = 1.906×10^{-6} A^{-2} calculated from [2]

Table III. Comparison between the surface groups and ζ-Potential of eGFP and mCherry

Protein	Acidic Groups[a]	Cystein Residues	Other residues	native ζ-Potential[b] (mV)	ζ-Potential cationisation[b] (mV)
native eGFP [3]	32	2	2x Ca^{2+} 1X SO_4^{2-} 4x PEG	-7.02	--
native mCherry [4]	33	0	0	-7.0	+9.3

[a] Total number of glutamic and aspartic
[b] Obtained at pH = 7.4

References

[1] SasView www.sasview.org
[2] Biomolecular Scattering Length Density Calculator (2019, June) retrieved from http://psldc.isis.rl.ac.uk/Psldc/
[3] Arpino, J.A , Rizkallah, P.J., Jones, D.D., Crystal structure of enhanced green fluorescent protein to 1.35 A resolution reveals alternative conformations for Glu222, *PLOS One,* **7**, e47132 (2012).
[4] Shu, X., Shaner, N. C., Yarbrough, C. A., Tsien, R. Y., Remington, S. J., Novel chromophores and buried charges control color in mGruits,. *Biochemistry*, **45**, 9639-9647 (2006).