## **Supporting Information**

## Capturing the Impact of Protein Unfolding on the Dynamic Assembly of Protein Networks

Matt D G Hughes<sup>1</sup>, Sophie Cussons<sup>2,3</sup>, Ahmad Borumand<sup>1</sup>, Arwen I I Tyler<sup>4</sup>, David J Brockwell<sup>2,3</sup>, and Lorna

Dougan\*1,2

1 School of Physics and Astronomy, Faculty of Engineering and Physical Sciences, University of Leeds, UK

2 Astbury Centre for Structural Molecular Biology, University of Leeds, UK

3 School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, UK

4 School of Food Science and Nutrition, Faculty of Environment, University of Leeds, UK

Corresponding Author: Lorna Dougan, L.Dougan@leeds.ac.uk



Figure S1: Dual sigmoid formation with post formation relaxation effectively fits rheology gelation curves of force labile BSA hydrogels. Example fitted gelation curve of force-labile BSA hydrogels (final concentrations: 100 mg/mL BSA, 50 mM NaPS, 100  $\mu$ M Ru(BiPy)<sub>3</sub>, 3mM DTT). Where the grey squares are the rheology data of a single BSA hydrogel repeat and the dark red line shows equation 1 fitted to the data.



**Figure S2: The addition of dithiothreitol (DTT) to BSA at 100mg/ml does not lead to a significant change in monomer shape detectable via SAXS.** SAXS curves of BSA pre-gel solutions (final concentrations: 100 mg/mL BSA, 50 mM NaPS, 100 μM Ru(BiPy)<sub>3</sub>), in the presence (force labile) and absence (mechanically robust) of 3mM DTT.



**Figure S3: Time-resolved SAXS tracks the full gelation of force-labile over 2 hours.** Additional SAXS curves of force-labile BSA (final concentrations: 100 mg/mL BSA, 50 mM NaPS, 100 μM Ru(BiPy)<sub>3</sub>, 3mM DTT) as a function of gelation time, for gelation times from 23 mins up to 121 mins, in 15 mins intervals



**Figure S4: Model independent SAXS analysis, of force labile BSA hydrogels, shows similar temporal trends observed in the extracted parameters from fractal model analysis.** The model-independent fit parameters, radius of gyration, R<sub>g</sub> (a)) and Porod exponent (b)), extracted from the SAXS curves in figure 4a as a function of gelation time for force labile BSA protein hydrogel.



Figure S5: The exposure to x-rays during the experiment are not sufficient to cause radiation damage or significant crosslinking which leads to structural changes. SAXS curves of BSA pre-gel solutions (final concentrations: 100 mg/mL BSA, 50 mM NaPS, 100  $\mu$ M Ru(BiPy)<sub>3</sub>) exposed to 1min of X-rays (light red) and 2 hours X-rays (dark red).