Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B. This journal is © The Royal Society of Chemistry 2020

**Supporting Information for** 

## Exploring the inner environment of protein hydrogels with fluorescence spectroscopy toward understanding their drug delivery capabilities

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Figure S1: Images of different samples after gelation.



**Figure S2:** Wavelength dependent fluorescent transient decay of BSA solution before a) 0.25%, b) 8% and after gelation c) 0.25%, d) 8%.



**Figure S3:** TRANES measurements for the first 2 ns of the emission decay of Trp for different weight percentage of BSA before and after thermal treatment.



**Figure S4:** Two exponent fitted time resolved emission spectra of HPTS inside BSA (0.25 to 0.8% concentration) before and after thermal. Samples were excited at 390 nm and emission was monitored at 435 nm.



**Figure S5:** Change in ROH<sup>\*</sup> intensity with time a) before and b) after gelation, was evaluated from TRANES of each sample.



Figure S6: Steady state emission spectra of HPTS inside BSA before and after gelation normalized at RO<sup>-</sup> peak position.



**Figure S7**. Images of the loading of Dox within BSA hydrogels at different weight concentrations and at different loading pH values, together with a picture of 0.1 mM Dox solution. The volume of the gels is 2 ml. The pictures were taken following 3 days of loading.



**Figure S8**. (a) Potential field and (b) non-polar/polar ratio (green is more hydrophobic and purple is more polar) of BSA (PDB ID: 4F5S). The maps were generated using Protein-Sol (M. Hebditch and J. Warwicker, *Sci. Rep.*, 2019, **9**, 1969).