Electronic Supplementary Material (ESI) for Polymer Chemistry.

Bio-orthogonal triazolinedione (TAD) crosslinked protein nanocapsules affect protein adsorption and cell interaction

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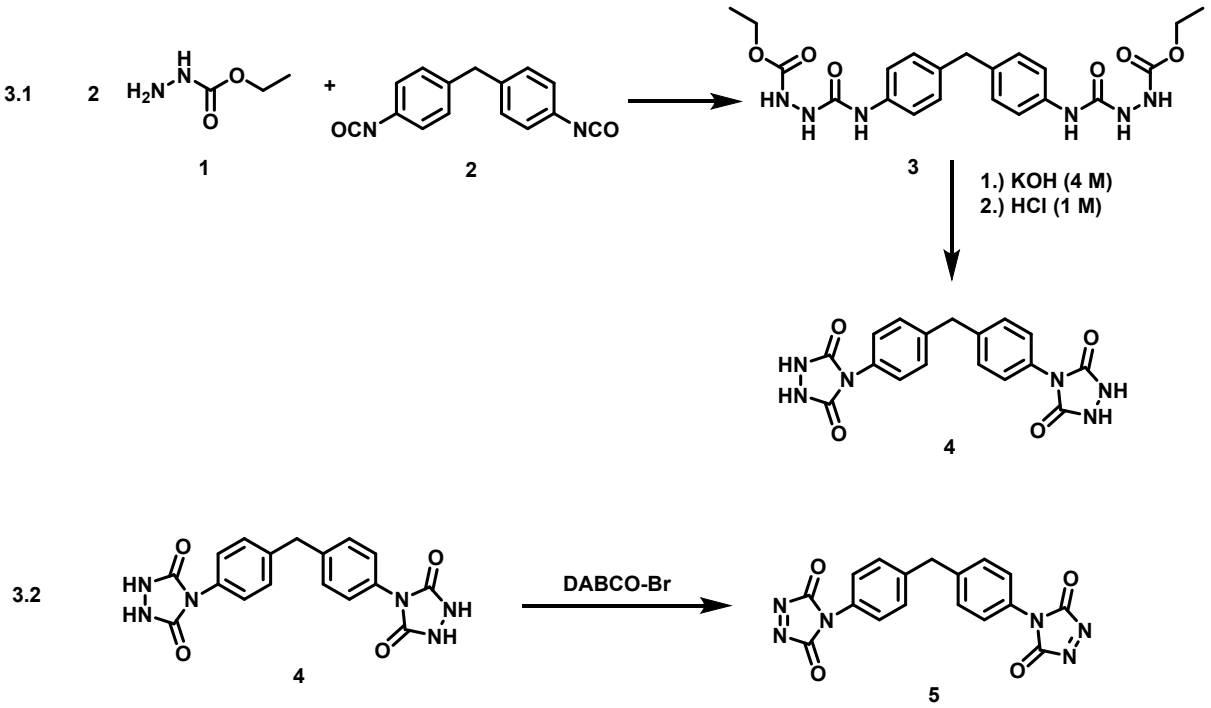
Content

1. Synthesis scheme of MDI-TAD

This procedure was adopted after a previously published procedure from Du Prez and coworkers.[1]



Scheme S1: Synthesis scheme of 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) MDI-TAD.

[1] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne and F. E. Du Prez, *Nat Chem*, 2014, **6**, 815-821.

2. Dynamic light scattering

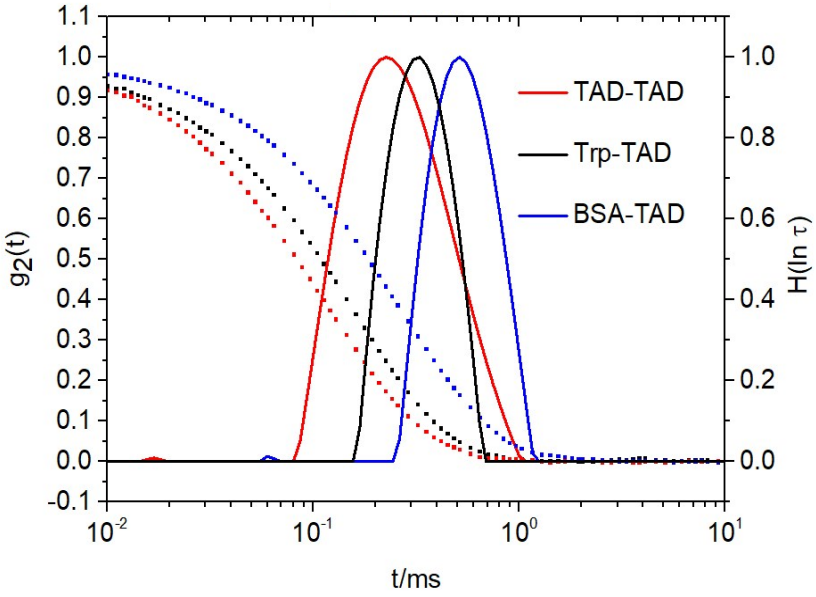


Figure S1: Size distribution of the different nanocapsules at an exemplary scattering angle of 90° in toluene represented by the distribution of relaxation times H(ln τ) (solid lines) together with the respective autocorrelation functions g2(t) (filled squares).

3. NMR spectra

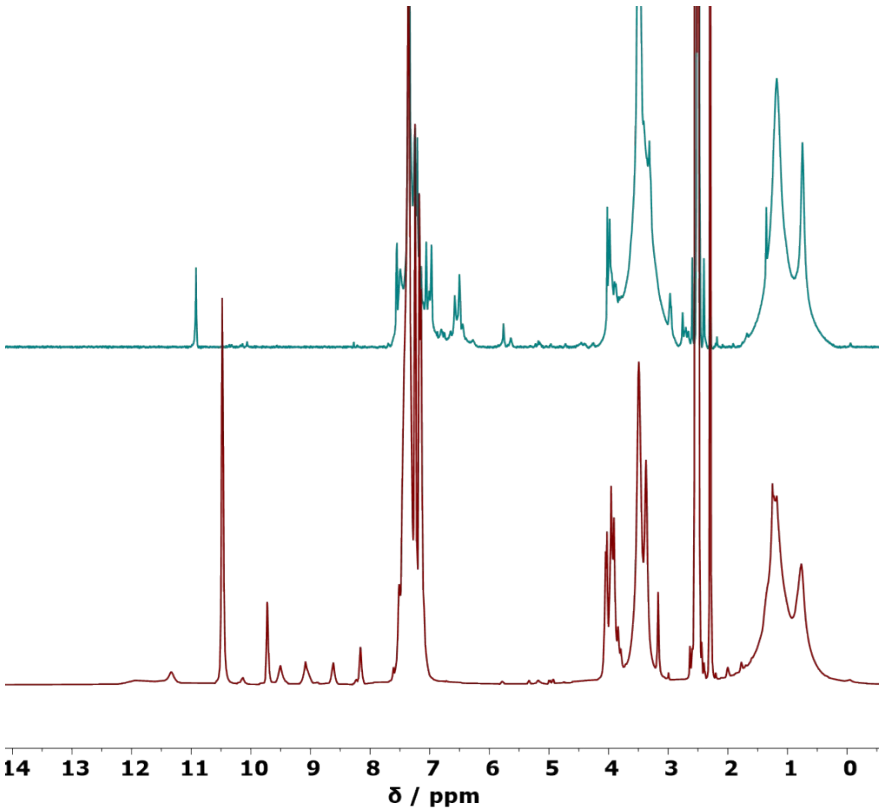


Figure S2: Full ^{1}H-NMR-Spectra of a Trp-TAD emulsion (green) in comparison to a TAD-TAD emulsion (red) in d6-DMSO.

4. Pierce Assay

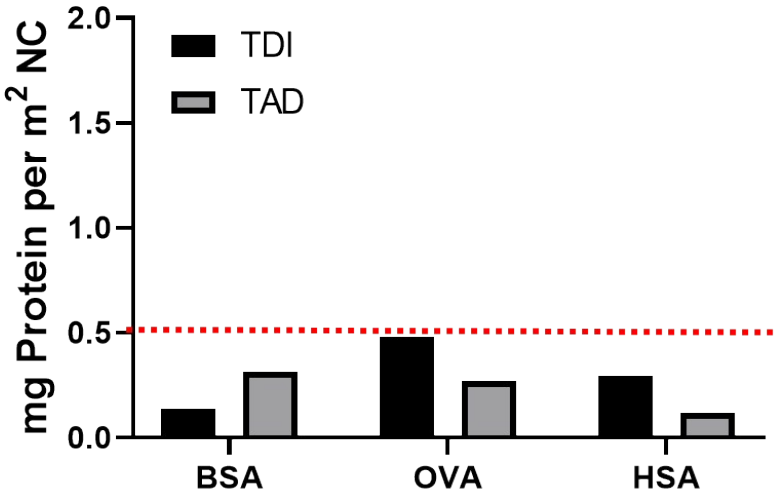


Figure S3: Quantification of the protein corona amount. Protein nanocapsules (0.05 m^2) were incubated with human serum (1 mL) for 1 h at 37 °C and the amount of all corona proteins was quantified via Pierce Assay (in mg). The red line indicates the threshold of 0.5 mg m^{-2} protein, below which quantification becomes less sensitive to differences.

5. SDS-PAGE

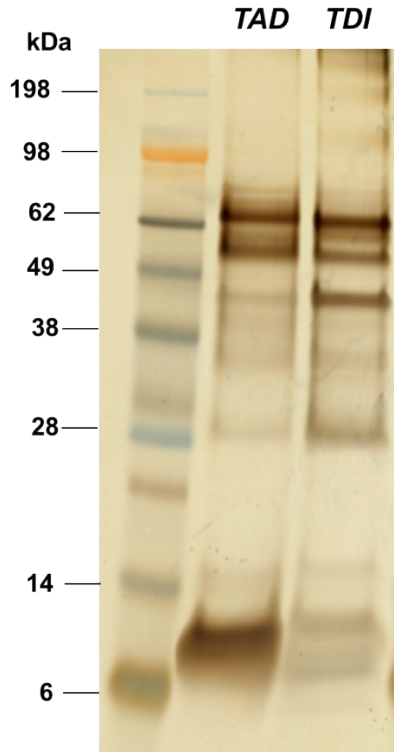


Figure S4: Protein corona profile. BSA-nanocapsules were incubated with human serum for 1 h at 37 °C and the protein corona was analyzed SDS-PAGE.

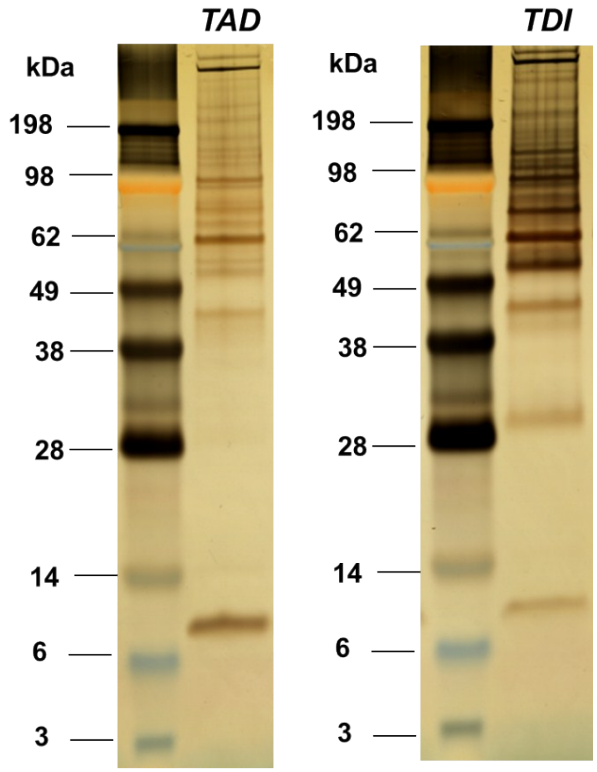


Figure S5: Protein corona profile. OVA-nanocapsules were incubated with human serum for 1 h at 37 °C and the protein corona was analyzed SDS-PAGE.

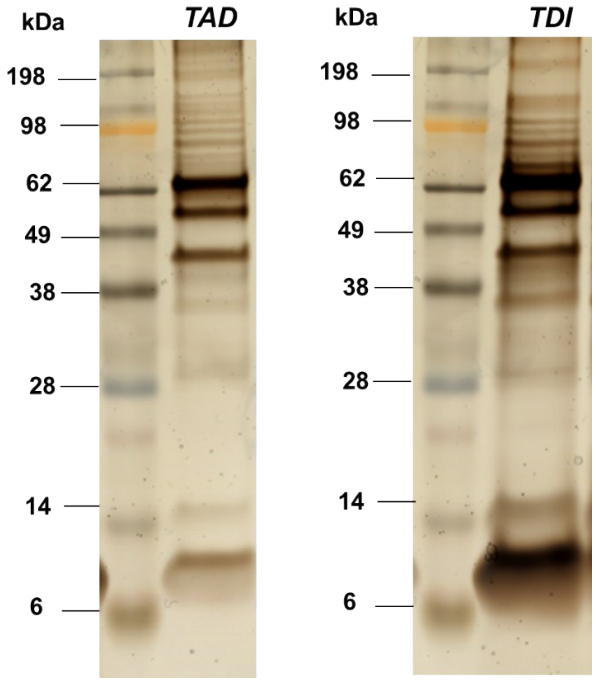


Figure S6: Protein corona profile. HSA-nanocapsules were incubated with human serum for 1 h at 37 °C and the protein corona was analyzed SDS-PAGE.

6. Confocal laser scanning microscopy (cLSM)

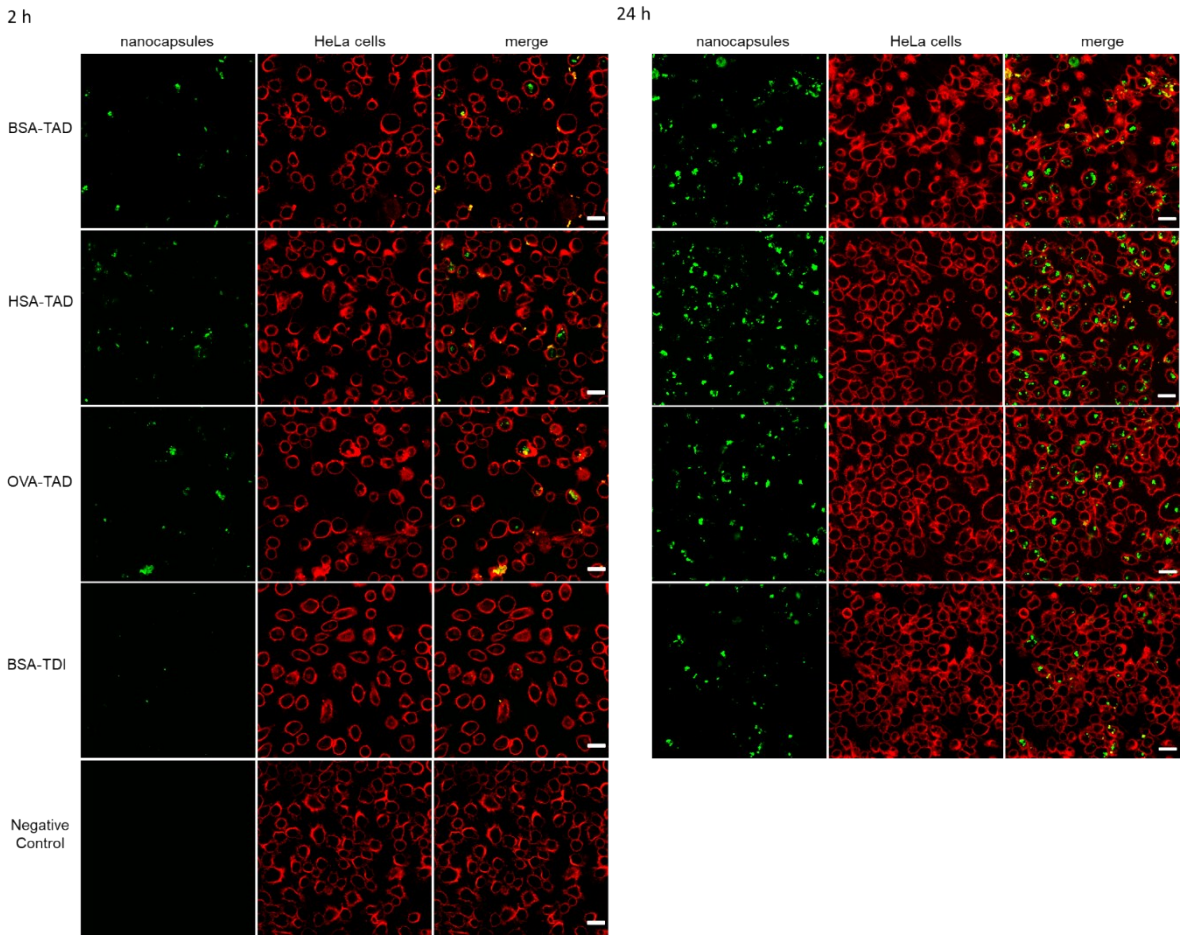


Figure S7: Confocal laser scanning microscopy (cLSM) of HeLa cells incubated with differently crosslinked protein nanocapsules. On the left panel, cells incubated with nanocapsules with a concentration of 75 µg mL^{-1} for 2 h are shown, while on the right panel, cells incubated with the same concentration (75 µg mL^{-1}) for 24 h are shown. All experiments were performed in cell culture medium containing 10% FBS. As a negative control, HeLa cells without nanocapsules treatment were stained with CellMask Deep Red only. The cell membrane is pseudo-coloured in red and the nanocapsules are pseudo-coloured in green. All scale bars represent 25 µm.

7. Cell viability

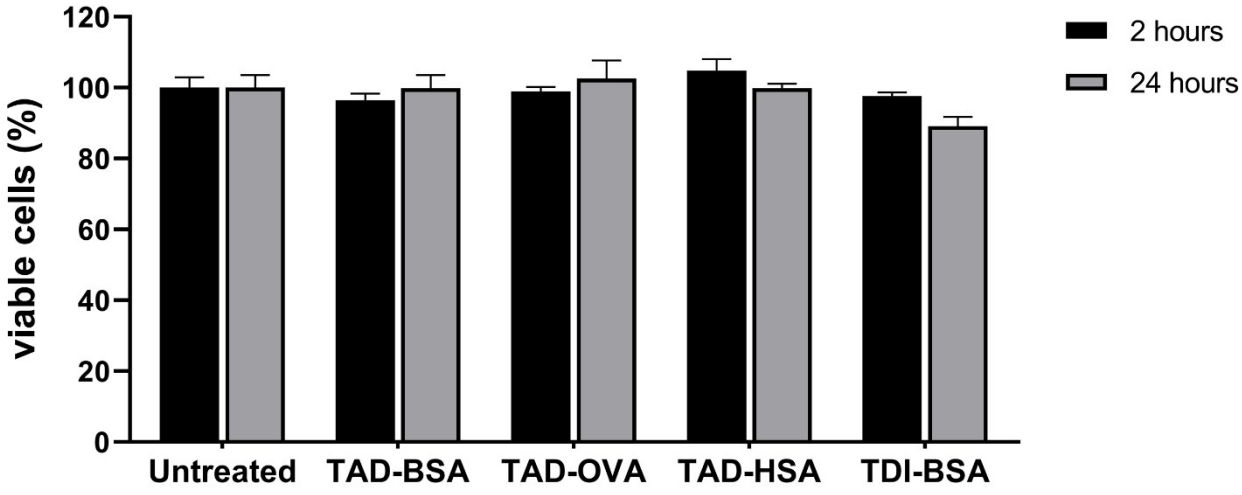


Figure S8: Cell viability of HeLa cells treated with differently crosslinked protein nanocapsules at concentrations of 75 µg mL^{-1} for 2 h and 24 h of incubation. Untreated cells were incubated with the same volume of 10% FBS supplemented DMEM. The percentage of viable cells is proportional to the measured luminescence signal.