

## SUPPORTING INFORMATION

### BIOCATALYTICALLY ACTIVE MICROGELS BY PRECIPITATION POLYMERIZATION OF *N*-ISOPROPYL ACRYLAMIDE IN THE PRESENCE OF AN ENZYME

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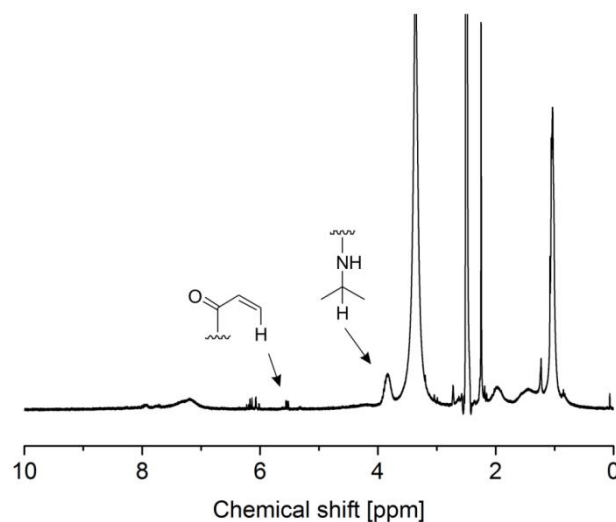
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#### **Analysis of the supernatant of the microgel dispersion**

The fraction of solid material that remains in the supernatant after removal of the crosslinked microgel material via centrifugation was investigated via <sup>1</sup>H-NMR and gel electrophoresis (SDS-PAGE) in order to gather information about residual fractions of monomer and the degree of conjugation of the present protein to the polymeric material.

<sup>1</sup>H-NMR was recorded on a Bruker 500 MHz FT-NMR spectrometer using DMSO-d<sub>6</sub> as solvent. Figure S1 shows the spectrum of the soluble fraction obtained from the supernatant of the microgel dispersion after freeze drying. The microgel synthesis was performed according to the standard protocol described in the Experimental part of the main manuscript and only BSA was used without any other protein. The relative integral values of the marked signals

are 1 for the olefin peak and ~16 for the tertiary proton of the isopropyl moiety of NIPAm. These values indicate that more than 90 % of monomer has been converted. The aromatic signals indicate the presence of the BSA.

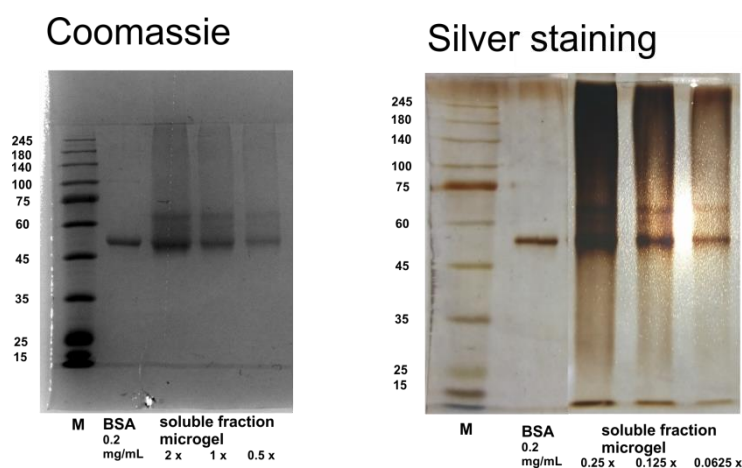


**Figure S1.**  $^1\text{H}$ -NMR spectrum recorded of the solid material dissolved in the supernatant of the microgel dispersion after removal of all crosslinked material. The microgel was synthesized according to the standard reaction protocol described in the main manuscript in the presence of BSA and no other protein. The spectrum was recorded in DMSO- $d_6$ .

SDS-PAGE was performed using polyacrylamide gels (10 % bisacrylamide) at 90 V, 18 mA and 90 min per gel. Samples were prepared before by treating the material to be analyzed with Roti®-Load 3 protein loading buffer at 92 °C for 10 min. Then, 8 mL of each sample with varying concentration was loaded onto the gel. Silver (with Roti®-Mark BICOLOR from Carl Roth, Germany as the marker) and Coomassie (0.1 wt% Coomassie Blue R-250, 0.1 wt% in ethanol/acetic acid/water 40/10/50 v/v) staining were used to develop the gel. For the analysis a microgel was investigated that was synthesized according to the standard recipe and which only contains BSA and no other protein.

Considering the initial amount of BSA that has been used for the standard reaction protocol and the fact, that 29 % of the BSA remains in the soluble fraction after the polymerization, the maximum amount of unaffected BSA in the solution can be 0.39 mg/mL. Diluting the supernatant to the double volume before analysis matches the BSA concentration with the one that we used for the reference solution. Figure S2 indicates, that the band for the unbound BSA is significantly weaker for the diluted supernatant (column on the rightmost position in each gel) than for the reference, indicating that the majority of BSA is covalently bound to the

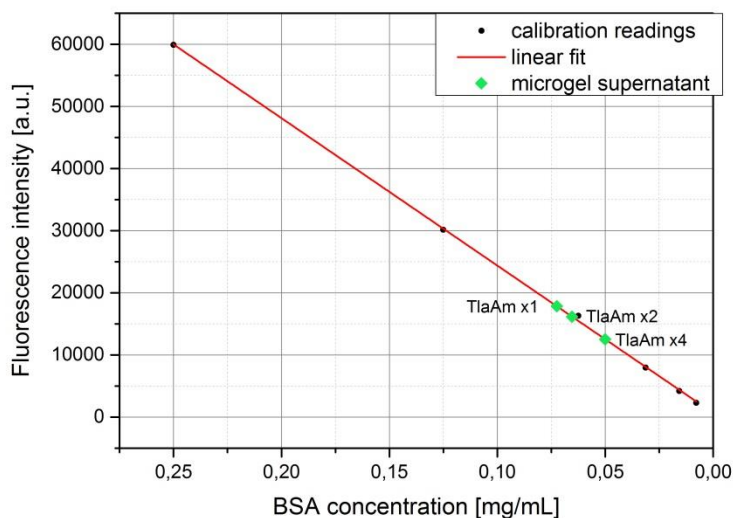
soluble polymer fraction. Note that the BSA band appears at a position below 60 kDa, which is due to the fact, that the experiment was run under non-reducing conditions. This was done to keep the extent of BSA conjugation to the polymer unchanged upon the measurement as the linking of the BSA to the polymer could also occur via disulfide bonding. The fact, that large portions of the BSA are present as protein/polymer conjugate is supported by the fact that significant staining occurs also for molecular weights higher than the one of BSA. Since the polymer should be present with a very broad molecular weight distribution ranging from short oligomers to chains with ultrahigh molecular weight and due to the fact that no (Coomassie) or only poor (silver) staining occurs at MW below the one of BSA, one can conclude, that the higher MW species carry a substantial amount of BSA.



**Figure S2.** SDS Page of the solid material dissolved in the supernatant of the microgel dispersion after removal of all crosslinked material. The microgel was synthesized according to the standard reaction protocol described in the main manuscript in the presence of BSA and no other protein.

### Protein quantification via fluorescence analysis of the supernatants

Figure S3 shows the calibration function used for the quantification of bound BSA in the microgels together with the respective data points recorded for different polymerization batches using varying amounts of protein reactive TlaAm comonomer. The highest concentration within the calibration data (0.25 mg/mL) corresponds to the initial amount of BSA in the reaction solution all data points being diluted to the same extent for the fluorescence detection. Fluorescence was recorded from the supernatants after removal of all crosslinked material via centrifugation. Except for the variation of the TlaAm content all other parameters were set according to the standard reaction protocol. This protocol, together with the detailed procedure on the fluorescence analysis is given in the Experimental part of the main manuscript.

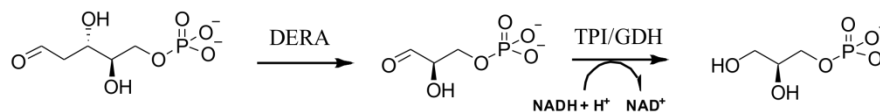


**Figure S3.** Fluorescence intensity recorded from supernatants from the microgel dispersions as a function of the BSA concentration. The black dots are reference samples used for calibration (red line). The green dots represent data gained from the respective supernatants. The microgels were synthesized under variation of the TlaAm content with all the other reaction parameters being set according to the standard reaction protocol (see Exp. part, main manuscript).

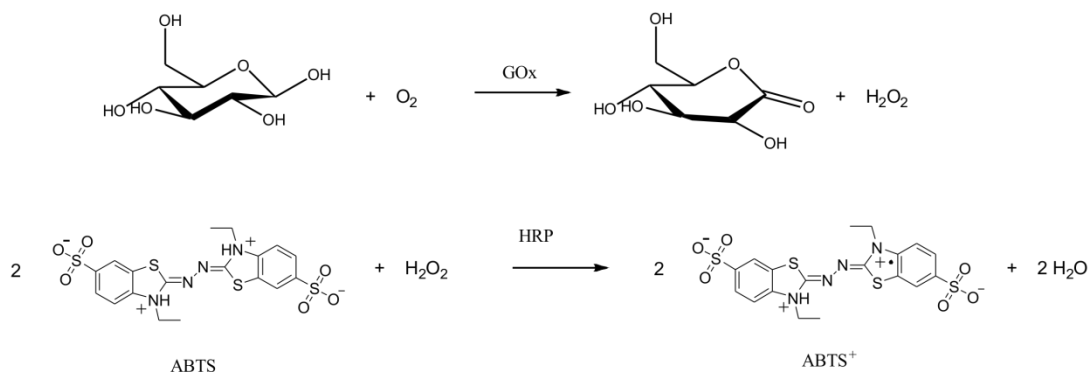
### Reaction mechanisms for the activity assays in use

Scheme S1 shows the reaction mechanisms used for the assessment of the enzymatic activity of DERA (Scheme S1A) and GOx (Scheme S1B). DERA converts its natural substrate 2-deoxy-D-ribose-5-phosphate to the corresponding retro-aldol product which is further reduced by GDH/TPI under consumption of NADH. The conversion of NADH is expressed by a drop in the detected absorption. GOx oxidizes glucose to a respective keto species under release of  $H_2O_2$ . The latter is used by HRP to oxidize ABTS yielding a cationic species that shows absorption in the range of visible light.

### A) DERA



### B) Glucose Oxidase



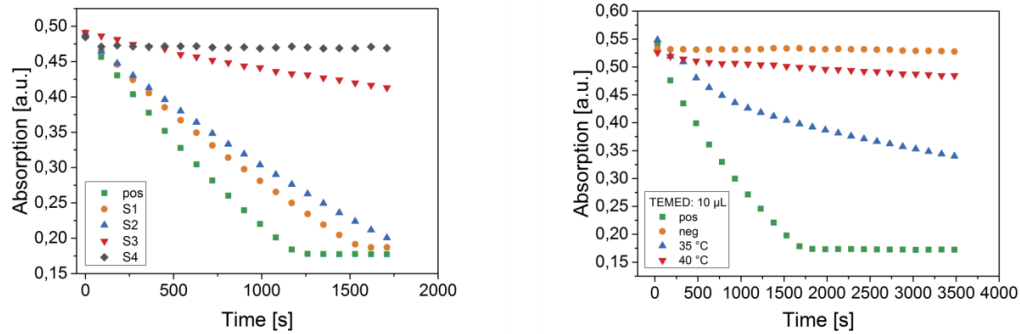
**Scheme S1.** Reaction mechanisms for the activity assay in use.

## Assessment of enzyme stability upon exposure to the polymerization conditions for the synthesis of the microgels

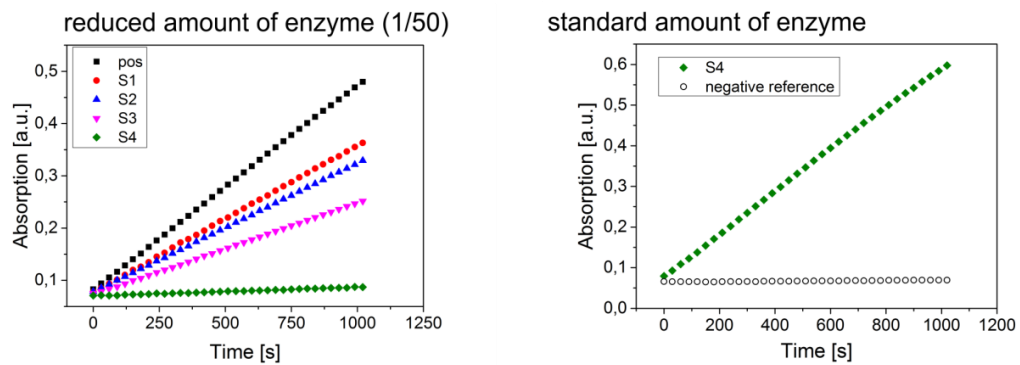
Prior to the actual incorporation of the respective enzymes into the microgel structure it was tested whether the biocatalysts are able to withstand the different manipulation steps that constitute the microgel synthesis protocol. The different steps are listed at the bottom of Figure S4. After each manipulation step, the enzyme containing solution was purified and rebuffered to an appropriate buffer by ultrafiltration followed by a run of the respective activity assay. Enzyme concentrations were matched within one measurement series, so that the raw absorption over time data could be directly compared without the need of further data processing. A detailed description for each activity assay is given in the Experimental part of the main manuscript. Deviating activities within the reference samples for DERA displayed within Figure S4 compared to the respective data in Figure 4 and 5 (main manuscript) are due to the usage of enzyme formulations with varying U/mg specifications, respectively. For HRP, the assay composition is the same as for GOx with the difference that no GOx and Glucose are added but a fixed amount of H<sub>2</sub>O<sub>2</sub> instead (8.82 mM). The results are shown in Figure S4. Note that for DERA additional experiments have been carried out where the temperature in step 4 was lowered to 40 and 35 °C in the presence of a decreased amount of TEMED (10 instead of 20 μL). For GOx, the experiments have been carried out with a

reduced amount of enzyme (1/50 of the value that is used for the microgels) due to the high activity of the enzyme. Only for the last step, the measurement has been repeated with the standard amount of enzyme and compared to a negative reference (right graph in the respective line).

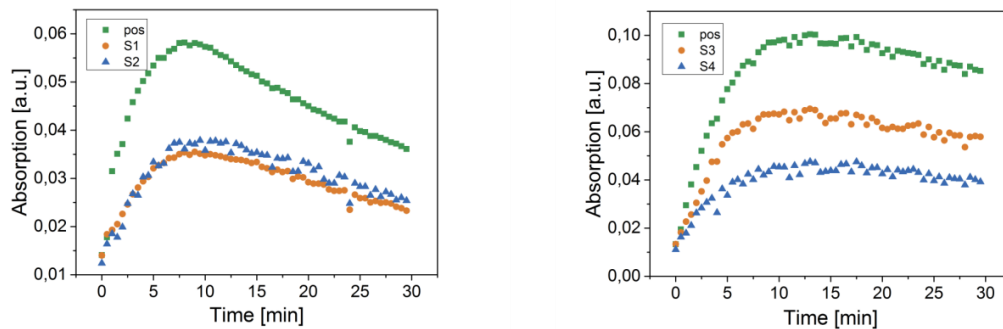
### DERA:



### Glucose Oxidase:



### Horseradish Peroxidase:



- S1: incubation with TlaAm over night at pH = 9
- S2: step 2 + exchange of medium to salt-free Milli-Q water
- S3: step 1 and 2 + addition of reaction components
- S4: step 1 to 3 + heating to 50 °C for 10 min

**Figure S4.** Raw data from the activity assays performed on DERA, GOx and HRP after exposure of the respective biocatalyst to the different manipulation steps of the microgel polymerization protocol. For each measurement series, positive reference samples were measured (“pos”) and enzyme concentrations were matched, allowing for a direct comparison.