

Supporting Information

For

Uricase -containing coacervate microdroplets as enzyme active membrane-free protocells for detoxification of uric acid in serum†

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Supplementary Experimental Section

S1. Materials and reagents

Polydiallyldimethyl ammonium chloride (PDDA) solution (20.0% in weight), Carboxymethyl-dextran (CM-dextran) sodium salt, uric acid (UA), catalase (CAT), Amplex red and Hoechst 33258 were obtained from Sigma-Aldrich. Uricase (Ur) and horseradish peroxidase (HRP) were purchased from Sangon Biotech. 3,3',5,5'-Tetramethylbenzidine (TMB), 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (Dil), and calcein were purchased from Thermo Fisher. Glacial acetic acid was purchased from Sinopharm group. All aqueous solutions of the experiments were used Milli-Q-purified water (18.2 M Ω ·cm) at 25 °C.

S2. Instrument and characterization

All fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer. Ultraviolet-visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-2600 spectrometer. Confocal laser scanning microscopic imaging (CLSM) was performed by TI-E+A1 Nikon confocal laser scanning microscope with Ar laser. Dynamic light scattering measurements and zeta potentials were performed by Nano Zetasizer analyzer (Malvern).

S3. Preparation of PDDA/CM-dextran microdroplets

PDDA/CM-dextran microdroplets were prepared by mixing of PDDA solution (10.0 mg·mL⁻¹, pH=7) and CM-dextran solution (10.0 mg·mL⁻¹, pH=7) at different weight ratios (0.10-0.32). Typically, 24 mL of 10.0 mg·mL⁻¹ PDDA were mixed with 100 mL of 10.0 mg·mL⁻¹ CM-dextran solution. After aging for 30 min, a suspension of negative charged (-1.0 \pm 0.5 mV) PDDA/ CM-Dextran coacervate microdroplets with hydrodynamic size of 6.9 \pm 0.5 μ m was obtained and used for most of the experiments. Uricase was sequestered into the coacervate droplets by adding the enzyme to the CM-dextran solution prior to addition of PDDA.

S4. Structural stability of coacervate microdroplets

Structural stability of coacervate microdroplets at different ion strengths and different pH were investigated. Typically, 10 mg·mL⁻¹ PDDA/CM-dextran microdroplets were added with different concentrations of NaCl (0-100 mg·mL⁻¹). After incubation for 15 min, the absorption at 420 nm was monitored. Similar experiments were undertaken but with different pH value (pH 1-14).

S5. Determination of dielectric constant

Preferential sequestration of the water-insoluble dye, Nile red into the PDDA/CM-dextran microdroplets was used to estimate the dielectric constant (ϵ) of the

coacervate interior. Microdroplets containing Nile red molecules were prepared by mixing of 290 mL 10 mg·mL⁻¹ PDDA/CM-dextran with 10 µL of Nile red/DMSO solution (0.2 mM). The coacervate phase was isolated by centrifugation at 4,000 rpm for 5 min and then examined using UV-Vis spectroscopy. The dielectric constant of the droplet interior was estimated from the absorbance peak position and compared with those for Nile red in dodecane (500 nm, $\epsilon = 2.0$), DMSO (552 nm, $\epsilon = 47.2$), and for micromolar concentrations of Nile red in water (591 nm, $\epsilon = 80.0$) assuming a linear relationship between dielectric constant and absorption peak position.

S6. Crosslinking of FITC and uricase

FITC was dissolved in phosphate buffer solution, (PBS, 10 mM, pH=7.4) at a concentration of 1.0 mg·mL⁻¹. Mixture of FITC and uricase solution at a proportion of 10: 1 (FITC: uricase in molar) was incubated at 4°C for 12 h in the dark. After dialysis for more than 4 times, the crosslinked products (FITC-Ur) were collected and stored in dark at 4°C.

S7. Determination of equilibrium partitioning constant

The partition constant (K) was determined from $K = [\text{Guest object}]_{\text{in}}/[\text{Guest object}]_{\text{out}}$, where $[\text{Guest object}]_{\text{in}}$ was equal to $([\text{Guest object}]_{\text{total}} \times V_{\text{total}} - [\text{Guest object}]_{\text{out}} \times V_{\text{out}})/V_{\text{in}}$, and $[\text{Guest object}]_{\text{out}}$ and V_{out} , and $[\text{Guest object}]_{\text{in}}$ and V_{in} , were the concentrations and volumes (V) of guest object in the continuous aqueous phase and coacervate phase, respectively. Absorption of the guest objects in the aqueous solution was monitored directly using UV-vis spectroscopy, while partitioning in the coacervate phase was ascertained after decomposition using 0.5 M NaCl.

Typically, 220 µL of PDDA (10 mg·mL⁻¹) solutions and 780 µL CM-dextran (10 mg·mL⁻¹) were mixed with a solution of the guest component to produce 1 mL of a coacervate dispersion. The coacervate phase was separated from the continuous solution by centrifugation at 2,000 rpm for 5 min. The concentration of the guest in the aqueous solution (upper layer, A1) was monitored directly using UV-vis spectroscopy, while partitioning in the coacervate phase (lower layer, A2) was determined after disassociation of the coacervate phase using NaCl solution (0.5 M NaCl) to minimize scattering artifacts. Concentrations of Hoechst, Dil, calcein, and uric acid in the bulk coacervate and aqueous phase were determined from their absorption measurements at 350 nm, 495 nm, 549 nm, and 293 nm, respectively.

S8. Determination of enzyme activity

The enzymatic activity of uricase with all samples was evaluated following H₂O₂-TMB colorimetric assay by UV-vis spectroscopy. In this experiment, phosphate buffer

solutions (10 mM, pH 7.4) containing $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ HRP and 1 mM TMB were added with uric acid (0-0.5 mM). To determinate the enzyme activity of uricase ($0.5 \text{ mg}\cdot\text{mL}^{-1}$), the time-dependent UV-vis absorption change of using TMB as substrates in coacervate was recorded to compare with that of homogeneous solution system by monitoring the absorption of TMB_{OX} at 652 nm.

S9. Detoxification of uric acid in PBS

Uric acid was dissolved in PBS (2.5 mM) to explore the initial velocity of uric acid detoxification experiment. The Ur concentration in the aggregates was adjusted from 12.5 to $50.0 \mu\text{g}\cdot\text{mL}^{-1}$. Incubated at 37°C , the method of H_2O_2 -Amplex red reaction was adopted to observe the decomposition of UA by examining the production of hydrogen peroxide in PBS buffer. In the presence of H_2O_2 and HRP ($0.1 \mu\text{g}\cdot\text{mL}^{-1}$), fluorescence products with a wavelength of 590 nm will be generated by Amplex red (0.2mM). Immediately after the addition, the fluorescence intensity of this point will be measured, and the initial velocity of the reaction process can be measured.

S10. Detoxification of uric acid in serum

Uric acid-containing new-born bovine serum (2.5 mM) was used for the experiment of the uric acid detoxification. The coacervate microdroplets loaded with uricase ranging from 12.5 to $50.0 \mu\text{g}\cdot\text{mL}^{-1}$ were added to the serum solution together with excess catalase to remove the H_2O_2 intermediate. During the incubation at 37°C , the uric acid remained in the serum was monitored at different time intervals (1 -24 h) through H_2O_2 -TMB colorimetric assay. Prior to H_2O_2 -TMB colorimetric assay, the protein macromolecules in serum samples were removed through ultrafiltration (10 kD). The uric acid removal by uricase ($25 \mu\text{g}\cdot\text{mL}^{-1}$) was also further characterized by UV-vis. The solution was diluted by 10-fold and the absorption intensity of the product at 652 nm was determined. It was observed that the absorption was gradually decreased, indicating that uric acid was effectively removed within 24 h.

Supplementary Table and Figures

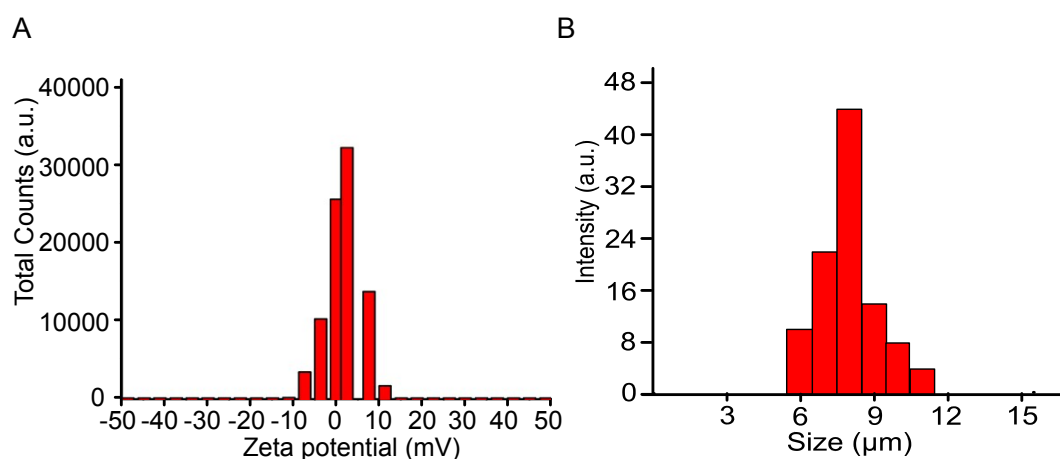


Figure S1 (A, B) Zeta potential (A) and hydrodynamic size (B) of the coacervate (PDDA: CM-dextran=0.27 in 10 mg·mL⁻¹). Zeta potential and hydrodynamic size of the coacervate microdroplets were determined to be 0.4 ± 0.5 mV, and 8.7 ± 0.4 μm, respectively.

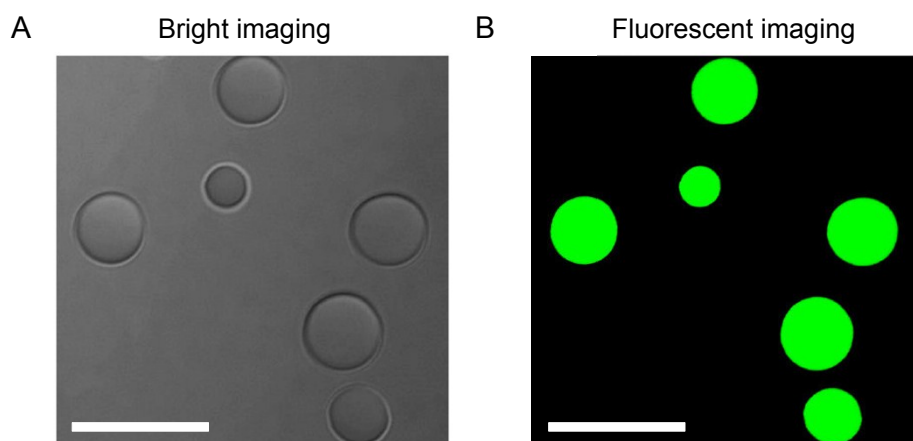


Figure S2 Confocal imaging of the coacervate microdroplets (PDDA: CM-dextran=0.27 in 10 mg·mL⁻¹). Bright imaging (A) and fluorescence imaging (B) of coacervate microdroplets. The droplet was sequestered with FITC to enhance the image contrast. Scale bar: 20 μm.

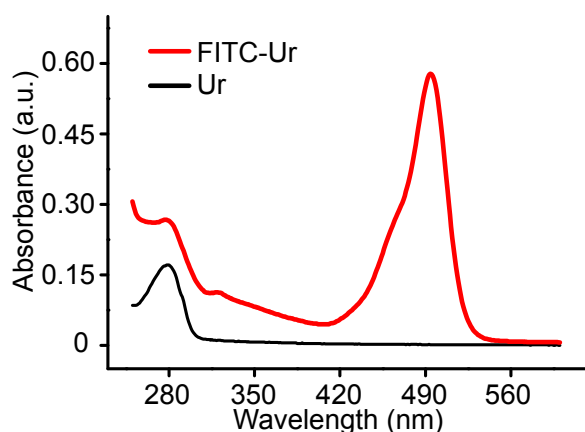


Figure S3 UV-Vis absorption spectra of FITC labelled uricase (FITC-Ur) and uricase (Ur).

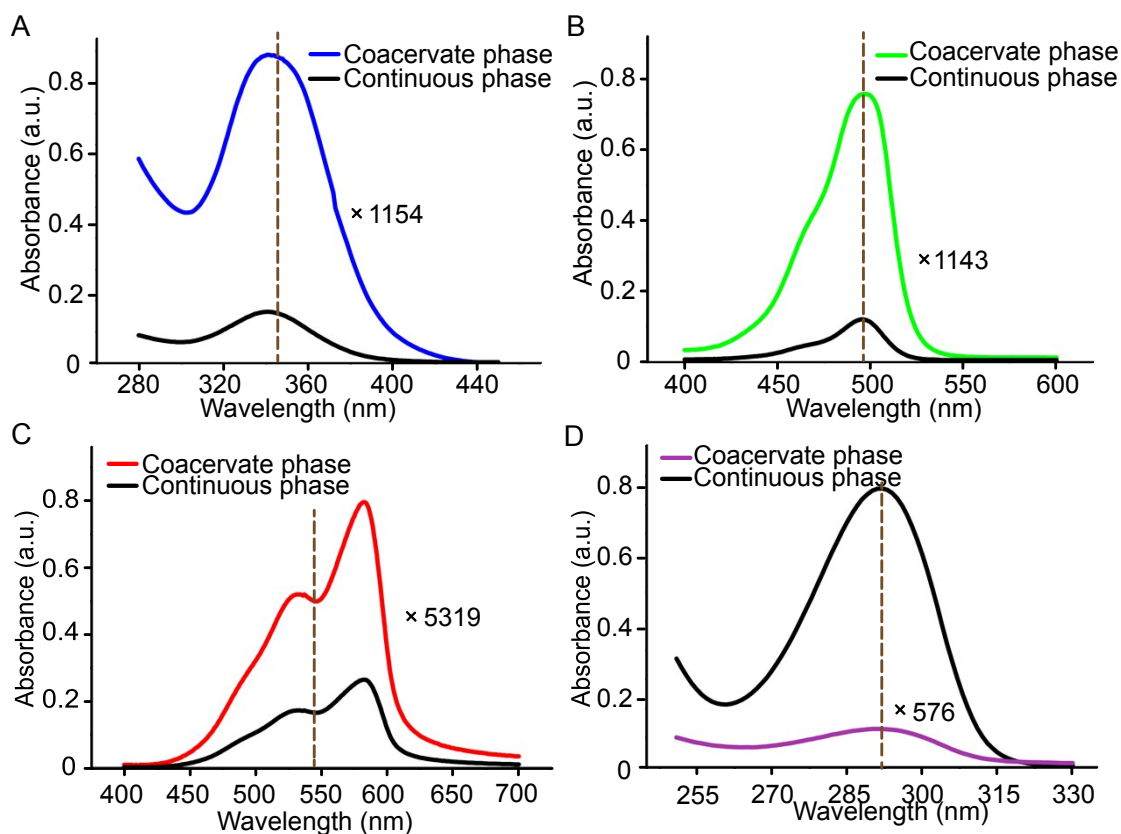


Figure S4 UV-Vis absorption spectra of different dyes and uric acid in coacervate phase and supernatant continuous phase. **(A)** Hoechst; **(B)** Calcein; **(C)** Dil; **(D)** Uric acid. The Hoechst was diluted 1154 times to measure, Calcein was diluted 1143 times to measure, Dil was diluted 5319 times to measure, and uric acid was diluted 576 times to measure. The absorption of the guest objects in the aqueous solution was monitored directly using UV-vis spectroscopy. In addition, the partition in the coacervate phase was ascertained after decomposition using 0.5 M NaCl.

The partition constant (K) was determined from $K = [\text{Guest object}]_{\text{in}}/[\text{Guest object}]_{\text{out}}$, where $[\text{Guest object}]_{\text{in}}$ are equal to $([\text{Guest object}]_{\text{total}} \times V_{\text{total}} - [\text{Guest object}]_{\text{out}} \times V_{\text{out}})/V_{\text{in}}$. Here, $[\text{Guest object}]_{\text{out}}$ and V_{out} , $[\text{Guest object}]_{\text{in}}$ and V_{in} , are the concentrations and volumes (V) of the continuous aqueous phase and coacervate phase, respectively. Thus, the partitioning constant (K) was determined to be 5,260, 5,020, 14,200 and 108 for Hoechst; Calcein; Dil; and Uric acid, respectively.

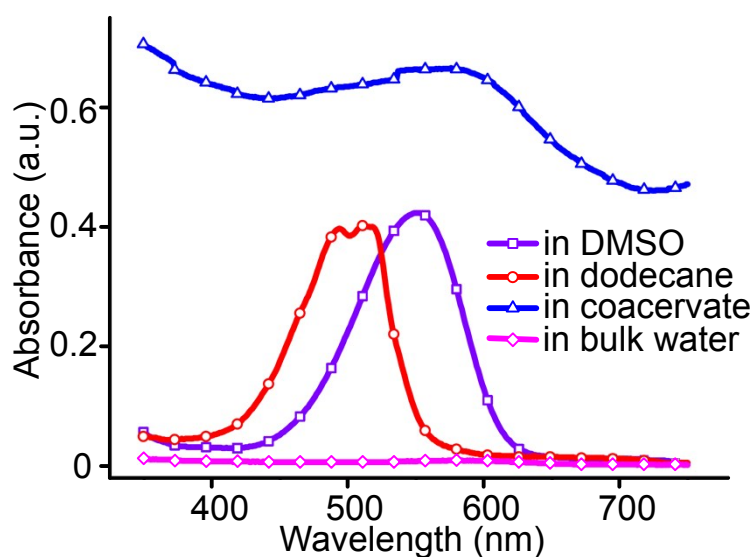


Figure S5 Dielectric constant (ϵ) was measured by adsorption spectra. UV-vis spectrum of Nile red-containing coacervate droplets at pH 7.4 showing broad band with absorption peak at 590 nm (open triangles). Corresponding solution UV-vis spectra of Nile red in DMSO (open squares) and dodecane (open circles) are also shown. The absence of Nile red in the bulk water continuous phase after centrifugation is also demonstrated (blank, open diamonds).

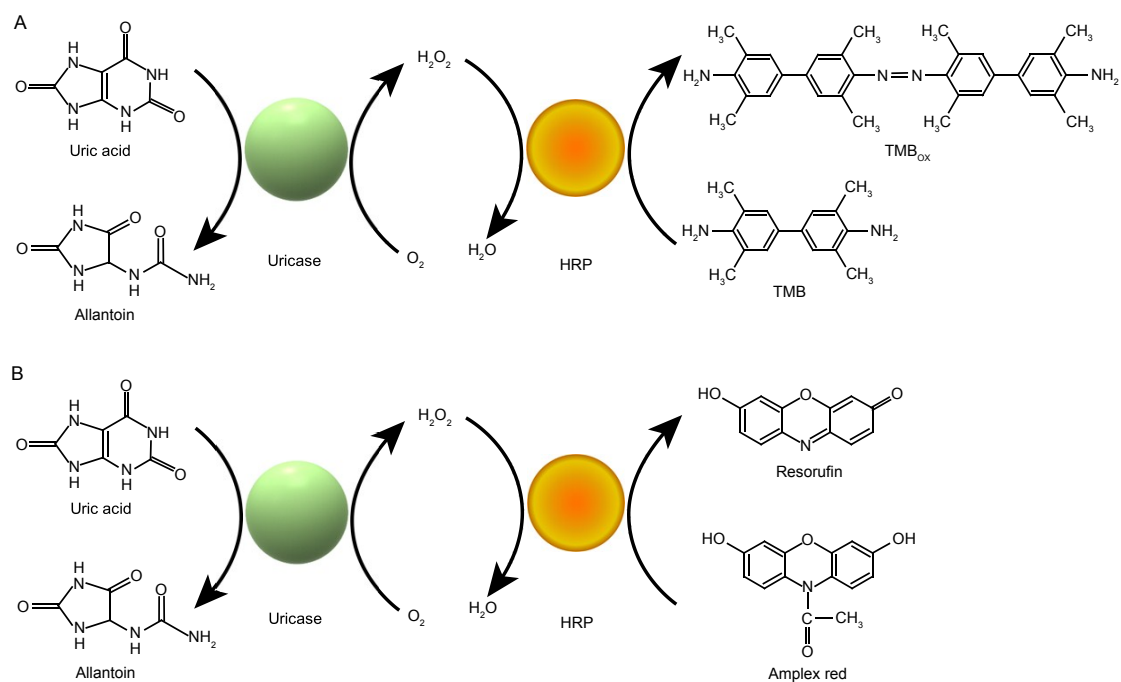


Figure S6 Illustration of TMB colorimetric assay (A) and Amplex red fluorescent assay (B).

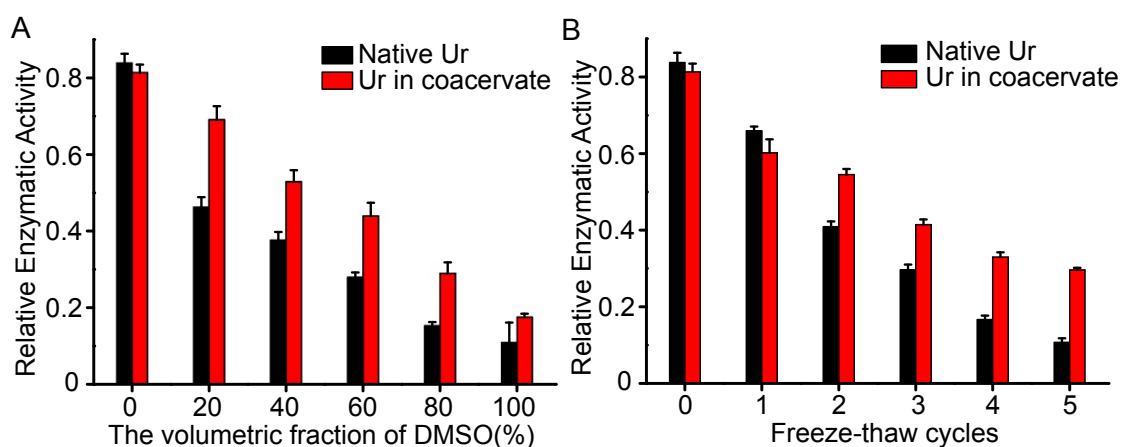


Figure S7 Immobilized uricase in coacervate microdroplets with enhanced enzymatic stability. (A) Relative activities of native uricase (Native Ur) and uricase sequestered in coacervates (Ur in coacervate) exposed to solutions containing different concentrations of DMSO. (B) Relative activities of native uricase (Native Ur) and uricase sequestered in coacervates (Ur in coacervate) subjected to five freeze-thaw cycles.

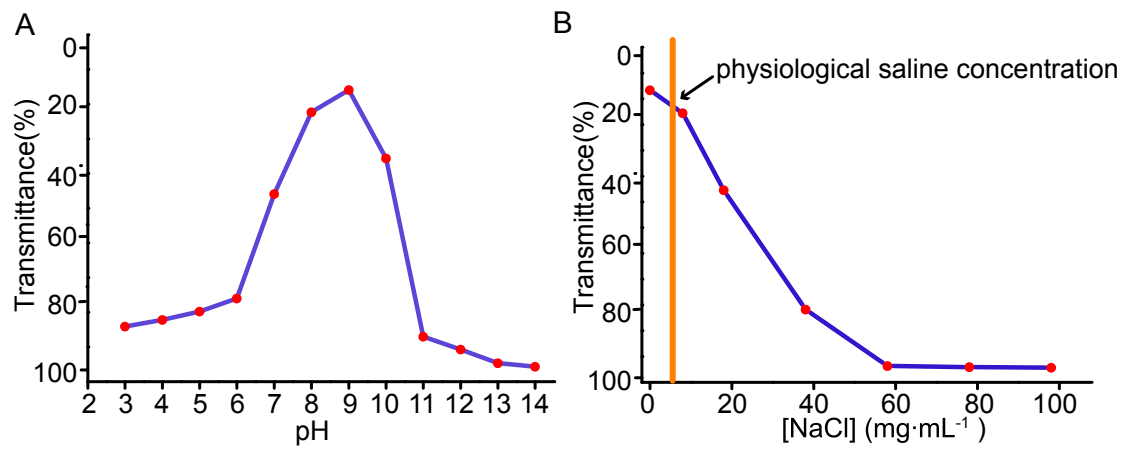


Figure S8 Stability of the coacervate against different pH values (**A**) and different concentrations of NaCl (**B**), characterized by transmittance determination.

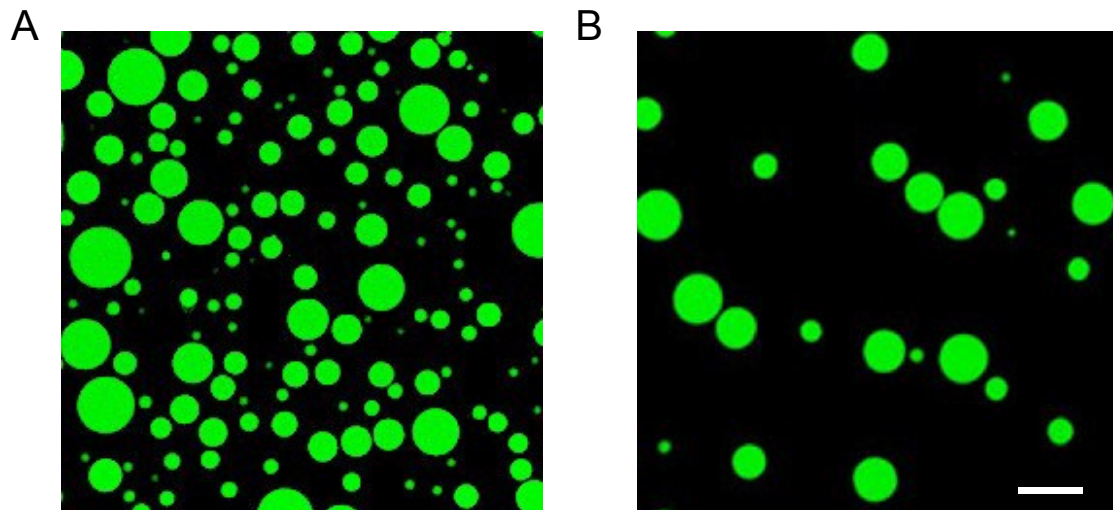


Figure S9 Fluorescent imaging of FITC-Ur containing-coacervate microdroplets before (**A**) and after dilution 10 times (**B**). Scale bar: 10 μm .

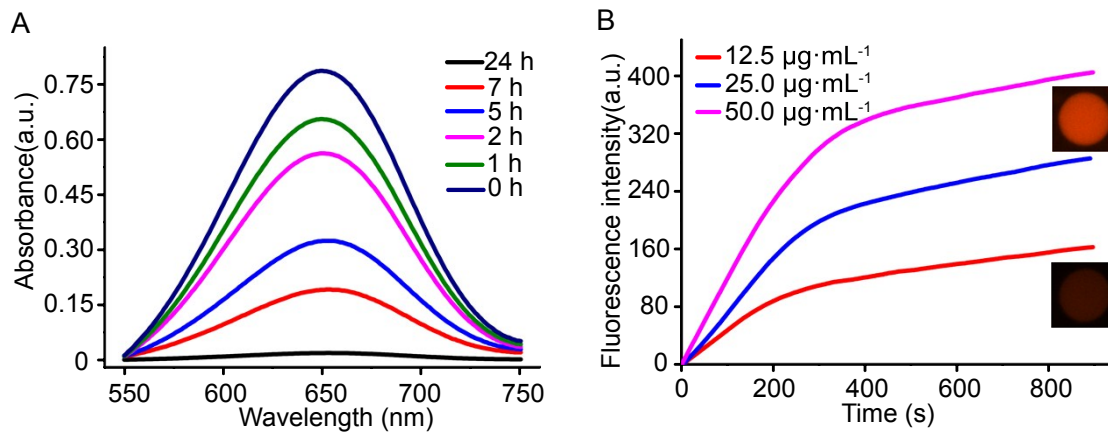


Figure S10 (A) The UV-vis adsorption of TMB_{ox} oxidized by the H₂O₂. Ur-Coac: 0.05 mg·mL⁻¹. (B) Time-dependent fluorescent kinetic of coacervate microdroplets loaded with different contents of uricase (12.5-50.0 µg·mL⁻¹), characterized by Amplex red fluorescent assay.

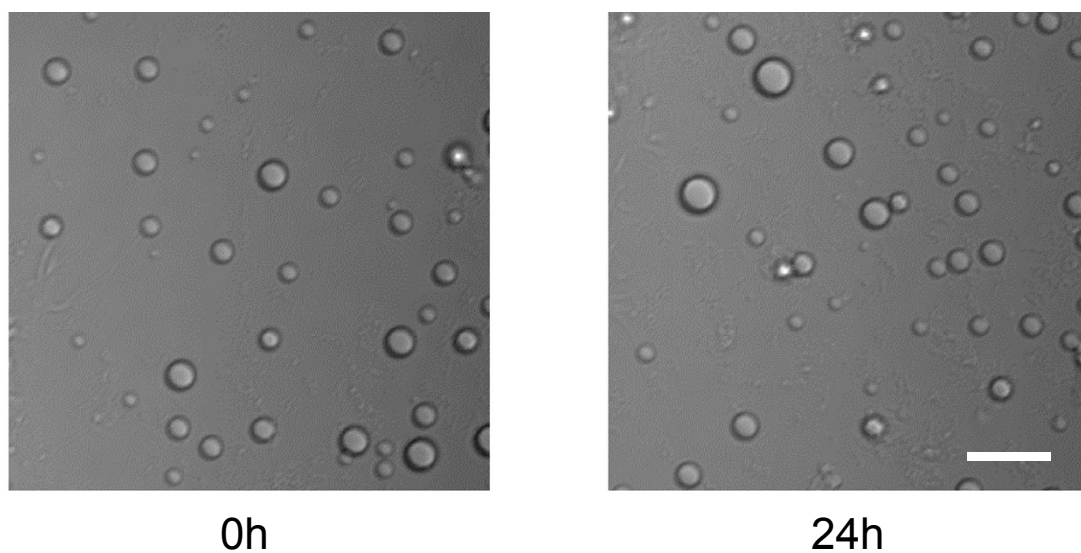


Figure S11 Confocal bright images of coacervate microdroplet in serum at 0 h and after 24 h. scale bar: 10 µm.