Supporting information for

Coacervate microdroplets incorporating J-aggregates towards photoactive membraneless protocells

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Materials and methods

Materials: Tetrakis(4-sulfonatophenyl)porphine (TPPS, ≥98.0%, Sigma), diethylamine ethyl dextran (DEAE-dextran 20k, M_w = 20 kDa, Sigma; DEAE-dextran 70k, M_w = 70 kDa, Sigma), poly (diallyldimethylammonium chloride) (PDDA, 20 wt.% in H_2O, M_w = 100-200 kDa, Sigma), poly(allylamine) (PAH, M_w = 65 kDa, Sigma), poly-l-lysine (PLL, M_w = 4-15 kDa, Sigma), sodium iodide (NaI, ≥99.5%, Macklin), 1,3-diphenylisobenzofuran (DPBF,

>97%, Aladdin), dimethyl sulfoxide (DMSO, ≥99.5%, Concord), benzoxadiazol-phosphoethanolamine (NBD-PE, >99%, Avanti), 9-(diethylamino)-5H-benzo[a]phenoxazinone (Nile Red, >99%, Acros organics), bis[N,N−bis(carboxymethyl) aminomethyl]fluorescein (calcein, ≥96%, Sigma), rhodamine 6G (Sigma), fluorescein isothiocyanate dextran (FITC-dextran, M_w = 250 kDa, Sigma), fluorescein isothiocyanate (FITC, ≥90%, HPLC), bovine serum albumin (BSA, ≥98%, M_w = 66,400 Da) and silver nitrate ($AgNO_3$, ≥99.0%, Sigma) were all purchased from commercial suppliers and used without further purification unless otherwise stated. Rhodamine B isothiocyanate-PDDA (RITC-PDDA, labelling ratio = 10 mol%) was kindly provided by E. Wischerhoff and A. Laschewsky, Fraunhofer Institute for Applied Polymer Research, Potsdam, Germany. Single-stranded DNA oligonucleotides (99 nucleotides in length) modified with 5-carboxytetramethylrhodamine (TAMRA-ssDNA) was purchased from Integrated DNA Technologies as HPLC purified oligonucleotides and dissolved in nuclease-free buffer (50 mM Tris-HCl containing 100 mM NaCl, pH 7.4) to give a solution of 0.1 mM. FITC-BSA was prepared as reported.[18] All aqueous solutions were prepared with 18.2 MΩ•cm deionized water from a Milli-Q System (Millipore, USA).

***Preparation of TPPS/DEAE-dextran coacervate microdroplets*:** The TPPS/DEAE-dextran coacervate suspensions at total concentration of 0.3 mM and a monomer molar ratio of 1:2 were prepared by direct mixing of aqueous TPPS and DEAE-dextran solutions at pH 5.0. Typically, 200 μL TPPS solution (0.2 mM, pH 5.0) was added into 200 μL DEAE-dextran 20k solution (0.4 mM, M_w = 20 kDa, pH 5.0) at room temperature. The freshly prepared coacervate suspensions were incubated for *ca.* 15 min before use. The average molecular weight of DEAE-dextran monomer was calculated from degree of modification of DEAE group (Mw = 101.2 Da) and dextran monomer (Mw = 210.2 Da). According to the nitrogen

content of DEAE-dextran of 3.5%, calculation of degree of substitution of DEAE group was 0.7. Therefore, the average molecular weight of DEAE-dextran monomer was 280.8 Da. The TPPS/DEAE-dextran 70k coacervate suspensions were prepared with the same procedure.

***Phase diagram*:** The phase diagram was determined by mixing TPPS (pH 5.0), DEAE-dextran (pH 5.0) solutions with different molar ratio and concentrations to obtain a total solution volume of 200 μL. The samples were incubated for *ca.* 10 min and observed under optical microscope to check if they were solutions, coacervates or precipitations. For each data on the phase diagram, more than three samples had been used for the reproducibility test. Phase diagrams for TPPS/DEAE-dextran 20k and TPPS/DEAE-dextran 70k systems have been drawn separately by the above method.

***Sequestration properties of the coacervate microdroplets*:** For 50 μL of upper suspension in TPPS/DEAE-dextran coacervate (300 μL, total concentration of 2.1 mM, monomer molar ratio of 1:2, pH 5.0) in eppendorf tubes, 1 μL of NBD-PE (5 μM in methanol), Nile Red (10 μM in methanol), calcein (5 μM), rhodamine 6G (5 μM), FITC-dextran (0.5 mg/mL), FITC-BSA (0.5 mg/mL), RITC-PDDA (0.1 mg/mL), TAMRA-ssDNA (5 μM) were added, respectively. The final concentrations of fluorescent dyes were 0.1 μM. The resulting suspensions were added to the sample cell and the dye distribution was observed using a confocal laser scanning microscopy (CLSM).

***Photocatalytic iodide ion oxidation reaction in TPPS/DEAE-dextran coacervate microdroplets*:** Typically, 2 μL of 400 mM NaI solution was added into 300 μL of TPPS/DEAE-dextran coacervate suspension (total concentration of 0.3 mM, monomer molar ratio of 1:2, pH 5.0, incubated for 15 min before usage) in a quartz cuvette (300 μL volume, 1

mm optical path length). The resultant suspension was then exposed to visible light through a 400 nm cut-off filter and AB25 attenuator from a 300 W Xe lamp for 3 h in ice water bath. During this time, the absorption spectroscopy was recorded by a UV-vis spectrophotometer (Perkin Elmer LAMBDA950) for every 10 min to detect the concentration of triiodide. Parafilm was used to seal the quartz cuvette to avoid water evaporation during irradiation. For comparison, 300 μL of TPPS solution (0.1 mM, pH 5.0) was used for irradiation with the same condition to compare with the TPPS/DEAE-dextran coacervate suspension as control experiment. Also TPPS/DEAE-dextran coacervate suspension (total concentration of 0.3 mM, monomer molar ratio of 1:2, pH 5.0, incubated for 15 min before usage) and 300 μL of TPPS solution (0.1 mM, pH 5.0) were placed in dark with the same condition as control experiments. The calculation of triiodide concentration was referred to "*Equilibrium partitioning constant for iodide in PDDA/ATP coacervate microdroplets*".

Characterization of iodide ion distribution: After adding 2 μL of 400 mM NaI solution into 300 μL of TPPS/DEAE-dextran coacervate suspension, the dispersions were centrifuged at 7,000 rpm for 10 min. 50 μL of the supernatant was removed and added into 50 μL of 2 M silver nitrate solution to observe whether there was yellow precipitate formed. For comparison, 2 μL of 400 mM NaI solution were added into 300 μL deionized water and then processed in the same steps as above to compare with the TPPS/DEAE-dextran coacervate suspension as control experiment.

***Equilibrium partitioning constant for iodide in TPPS/DEAE-dextran coacervate microdroplets*:** The partition constant (K) was determined from $K = \frac{[iodide]_{lower\ dispersion}}{[iodide]_{supernatant}}$, where $[\text{iodide}]_{\text{lower dispersion}}$ was equal to $K = \frac{[iodide]_{total}V_{total} - [iodide]_{supematant}V_{supematant}}{V_{lower\ diapersion}}$, and

$[iodide]_{supernatant}$, $V_{supernatant}$, $[iodide]_{lower\ dispersion}$ and $V_{lower\ dispersion}$ were the concentrations and volumes of iodide in the continuous aqueous phase and coacervate phase, respectively. Typically, 900 μL of the supernatant equilibrium solution of a TPPS/DEAE-dextran/Iodide coacervate droplet dispersion was prepared by mixing 450 μL TPPS solution (0.2 мм, pH 5.0), 2 μL of 400 мм NaI solution, and 450 μL DEAE-dextran 20k solution (0.4 мм, M_w = 20 kDa, pH 5.0) at room temperature. The mixture was centrifuged to remove sediment. The coacervate phase was separated from the continuous solution by centrifugation at 7,000 rpm for 15 min, and the concentrations of iodide in the bulk coacervate and aqueous phase were determined by absorption intensity measurements at 226 nm. The concentration of iodide (c) was determined from $c = \frac{A}{\varepsilon l}$, where A was the absorbance of iodide at 226 nm measured by UV-vis spectra, and ε was the extinction coefficient of iodide ion at 226 nm. The value was determined to be 12900 $L \cdot mol^{-1} \cdot cm^{-1}$ which was in consistent with the value (13400 $L \cdot mol^{-1} \cdot cm^{-1}$) reported in literature [A. D. Awtrey and R. E. Connick, *J. Am. Chem. Soc.*, 1951, **73**, 1842-1843.]. l represents the optical path length of the quartz cuvette (0.1 cm) used to measure absorbance. 3 μL of the TPPS/DEAE-dextran coacervate was dilute 120 times by adding 0.3 м NaCl prior to the UV-vis absorption analysis to ensure that the absorption peak intensity was proportional to the iodide ion concentration and to avoid uneven distribution of iodide ions of the coacervate phase.

***Characterization of singlet oxygen*:** Typically, 3 μL of DPBF solution (10 μм, in DMSO) was added into 300 μL of TPPS/DEAE-dextran coacervate suspension (total concentration of 2.1 мм, monomer molar ratio of 1:2, pH 5.0, incubated for 15 min before usage) in a quartz cuvette (300 μL volume, 1 mm optical path length). The resultant suspension was then exposed to visible light through a 400 nm cut-off filter and AB25 attenuator from a 300 W Xe lamp for 30 s in ice/water bath. The fluorescence spectra of the suspension were

monitored on a microplate reader (CLARIOstar Plus) with the excitation wavelength of 405 nm.

***Optical and fluorescence imaging of coacervate microdroplets*:** Optical and fluorescence microscopic imaging of the coacervate microdroplets was carried out with an inverted microscope (Leica DM18) equipped with a ×100 oil immersion lens and a confocal laser scanning microscopy (CLSM, Zeiss LSM880, Germany) equipped with a ×63 oil immersion lens, respectively. Diode laser (405 nm for DPBF), Argon laser (488 nm for FITC, NBD-PE, calcein and TPPS, 514 nm for rhodamine 6G) and a HeNe543 laser (543 nm for Nile Red, TAMRA and RITC) are used for the dye excitation. The detection bands were set at 475-603 nm for DPBF, 500-550 nm for FITC, NBD-PE and calcein, 521-699 nm for rhodamine 6G, 573-675 nm for Nile Red, 548-664 nm for TAMRA, 515-653 nm RITC and 680-760 nm for TPPS, respectively. Image analysis was performed with Image J software.

***Zeta potential measurement*:** Zeta potential measurements were performed with a Malvern Zetasizer Nano ZS90 instrument with 1 mL of TPPS/DEAE-dextran coacervate suspension (total concentration of 0.3 mM, monomer molar ratio of 1:2, pH 5.0) in disposable zeta cuvettes.

***Turbidity measurement*:** The turbidity of 100 μL TPPS/DEAE-dextran 70k coacervate suspensions with different NaCl concentration were characterized by absorbance at 600 nm and monitor by a UV-vis spectrophotometer (Perkin Elmer LAMBDA950) at room temperature.

***^{1}H NMR spectra*:** All measurements were recorded on a Bruker Avance II+ 400 spectrometer and a Bruker DMX300 spectrometer using deuterium oxide (D_2O) as internal standard.

***Flow cytometry*:** The samples were prepared with the methods mentioned above and analyzed with an ACEA Novocyte flow cytometer. The flow speed was set at 7 μL·min^{-1} for 2 min, and the particle number was counted in 30 s. All measurements were performed on a Novo Cyte 2060R flow cytometer, and data analysis was performed with FlowJo 10.4 software.

Supplementary Figures

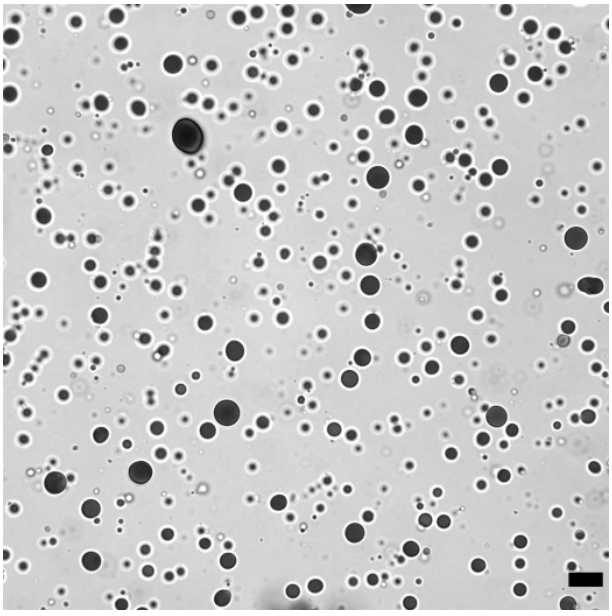

Figure S1. Large scale optical microscope image of TPPS/DEAE-dextran coacervate microdroplets for size distribution measurement. Scale bar, 10 μm.

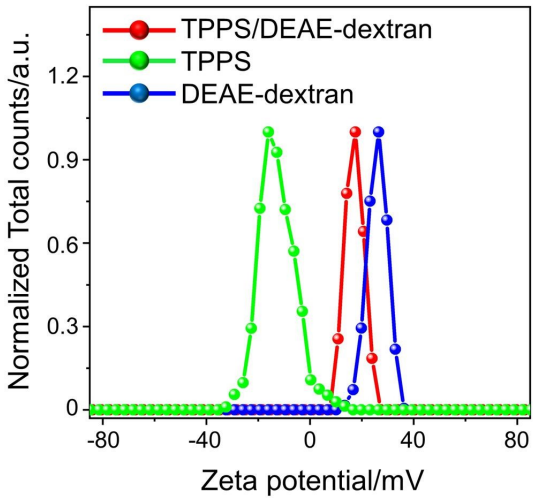

Figure S2. Zeta potential distribution of TPPS/DEAE-dextran coacervate suspension (for DEAE-dextran 20k, total concentration of 0.3 mM, monomer molar ratio of 1:2, pH 5.0, red curve), TPPS aqueous solution (0.1 mM, pH 5.0, green curve) and DEAE-dextran aqueous solution (0.2 mM, pH 5.0, blue curve) showing the positive charged microdroplets formed by oppositely charged TPPS and DEAE-dextran.

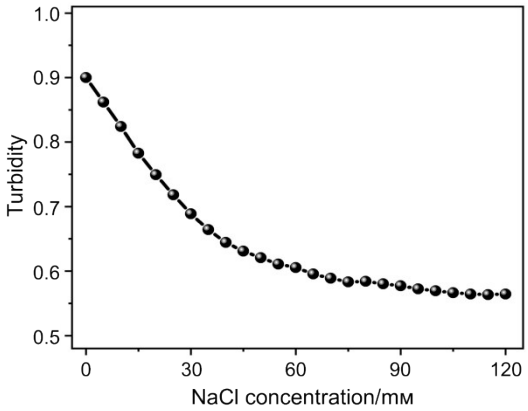

Figure S3. Change of the turbidity of TPPS/DEAE-dextran coacervate suspension (DEAE-dextran 20k, total concentration of 0.3 mM, monomer molar ratio of 1:2, pH 5.0) with the concentration of sodium chloride.

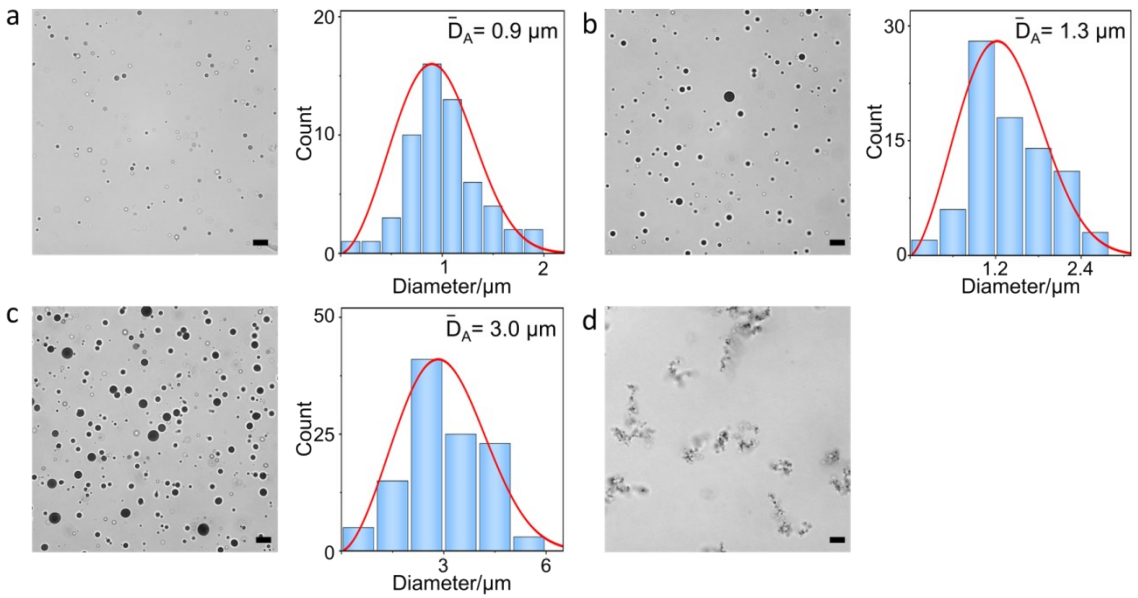

Figure S4. Optical microscope images and size distributions of TPPS/DEAE-dextran system after 10 mins' incubation at varied TPPS concentrations of (**a**) 0.05 mM, (**b**) 0.25 mM, (**c**) 0.5 mM and (**d**) 1 mM. TPPP/DEAE-dextran coacervate microdroplets fixed at the molar ratio of 1:2 displaying an average diameter of 0.9 μm, 1.3 μm and 3.0 μm as the concentration of TPPS increases. Scale bars, 5 μm.

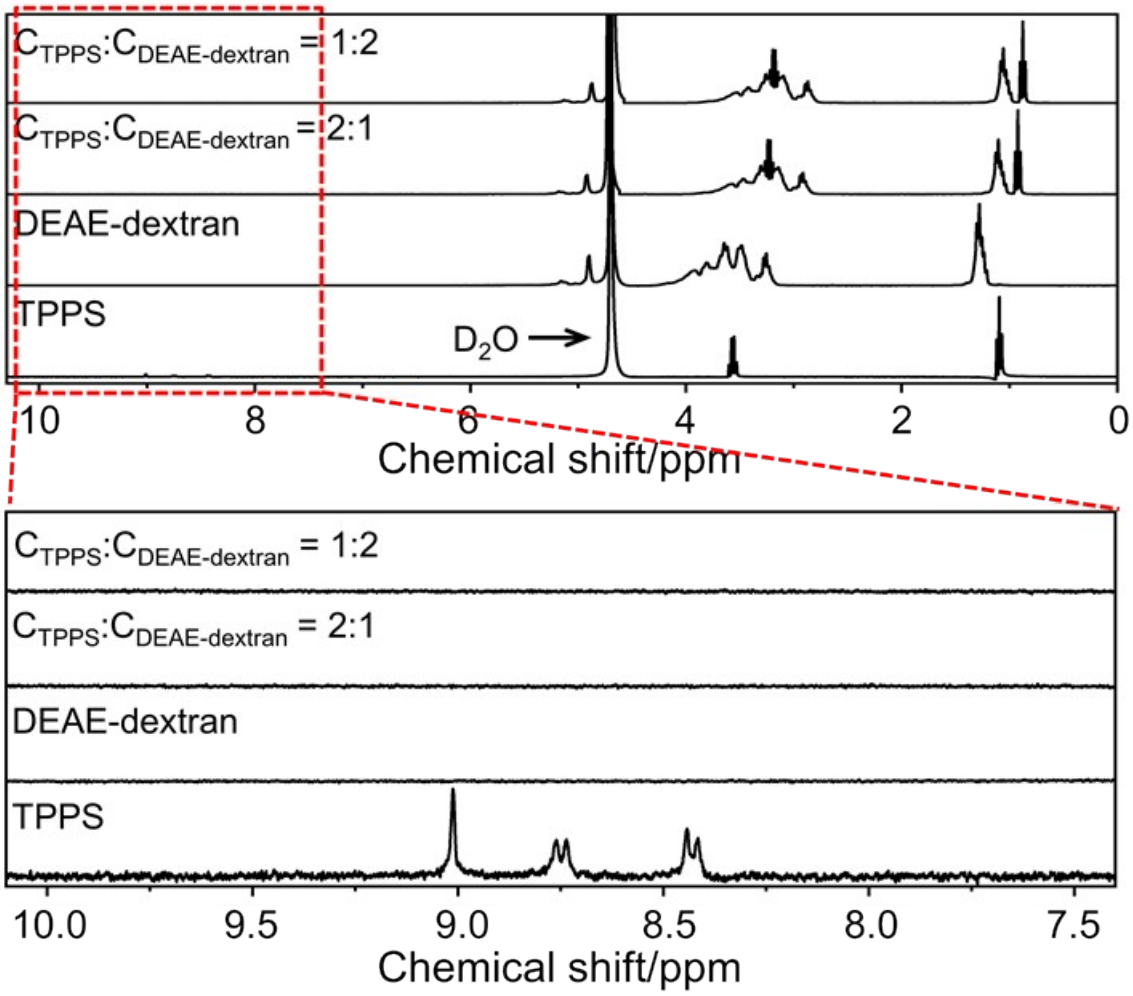

Figure S5. ^{1}H NMR spectra of TPPS (0.1 mM), DEAE-dextran (0.2 mM) solutions and TPPS/DEAE-dextran coacervate dispersion (C_{TPPS} = 0.1 mM, monomer molar ratio of 2:1 and 1:2) in D_2O. The peaks for aromatic protons of TPPS disappear after mixing with DEAE-dextran.

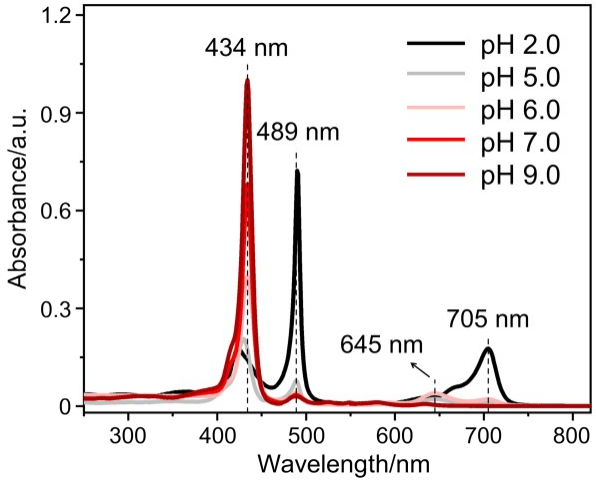

Figure S6. UV-vis absorption spectra of TPPS solution (0.1 mM) at different pH conditions showing the formation of J-aggregates in acid conditions (pH 2.0 to pH 6.0) compared with those in neutral and alkaline conditions (pH 7.0 to pH 9.0).

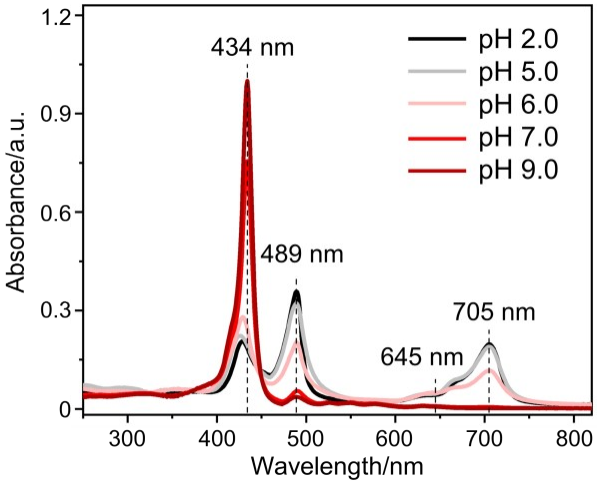

Figure S7. UV-vis absorption spectra of TPPS/DEAE-dextran suspensions (total concentration of 0.3 mM, monomer molar ratio of 1:2) at different pH conditions.

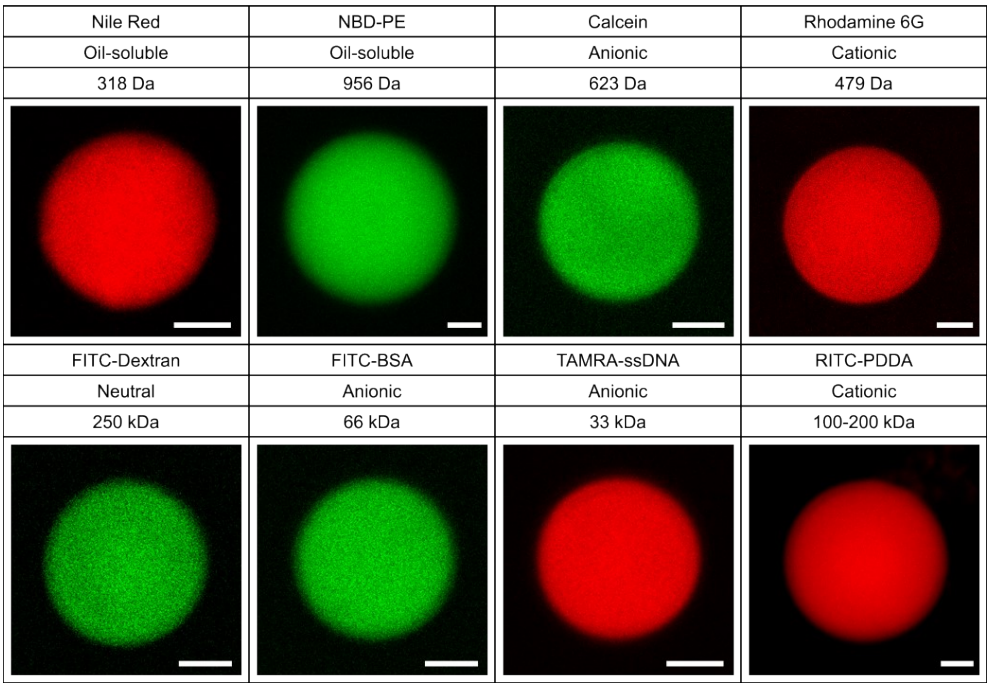

Figure S8. Sequestration of guest compounds from surroundings by TPPS/DEAE-dextran coacervate microdroplets. Small molecules, such as oil-soluble (Nile Red and NBD-PE) and water-soluble molecules (calcein and rhodamine 6G) and macromolecules, such as FITC-dextran, FITC-BSA, TAMRA-ssDNA and RITC-PDDA can all be recruited by TPPS/DEAE-dextran coacervate droplets. Scale bars, 2 μm.

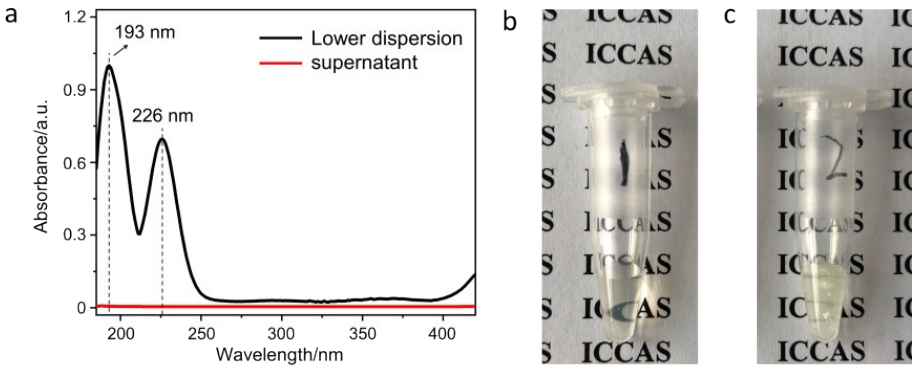

Figure S9. (**a**) UV-vis absorption spectra of supernatant (red curve) and lower dispersion (black curve) of TPPS/DEAE-dextran droplet systems after centrifugation with the addition of iodide ion. The strong absorption peaks of the lower dispersion (TPPS/DEAE-dextran coacervate microdroplets) at 193 nm and 226 nm indicate that the concentration of iodide ions dispersed is 155 times that of the supernatant. (**b,c**) Photos of silver iodide precipitation experiment for (**b**) supernatant of TPPS/DEAE-dextran coacervate suspension, and (**c**) deionized water with addition of iodide. The photos show that the iodide ion is uploaded by TPPS/DEAE-dextran coacervate and no iodide ion in tube 1.

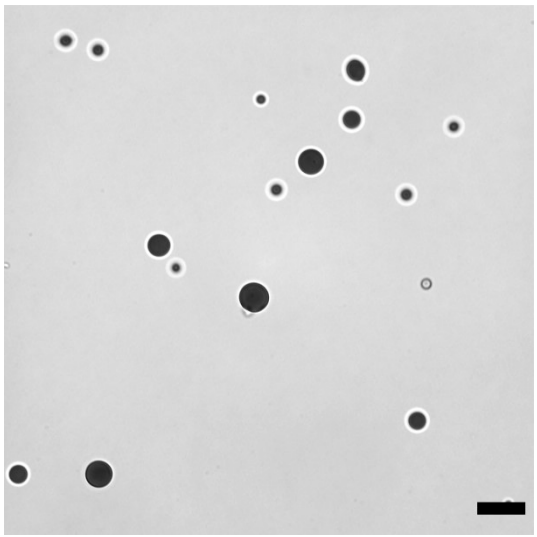

Figure S10. Optical microscope image of TPPS/DEAE-dextran coacervate microdroplets with the addition of NaI solution before irradiation. Scale bar, 5 μm.

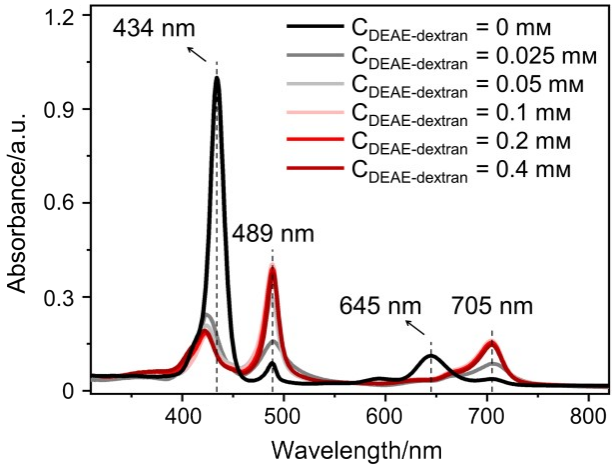

Figure S11. UV-vis absorption spectra of TPPS/DEAE-dextran mixtures (TPPS concentration of 0.05 мм, pH 5.0) with the increase of DEAE-dextran concentration (DEAE-dextran 70k, from 0 мм to 0.4 мм, pH 5.0). The characteristic absorption peak of J-aggregates at 489 nm shows a significant enhancement when increase the concentration of DEAE-dextran from 0 мм to 0.1 мм because of the coacervation between TPPS and DEAE-dextran.

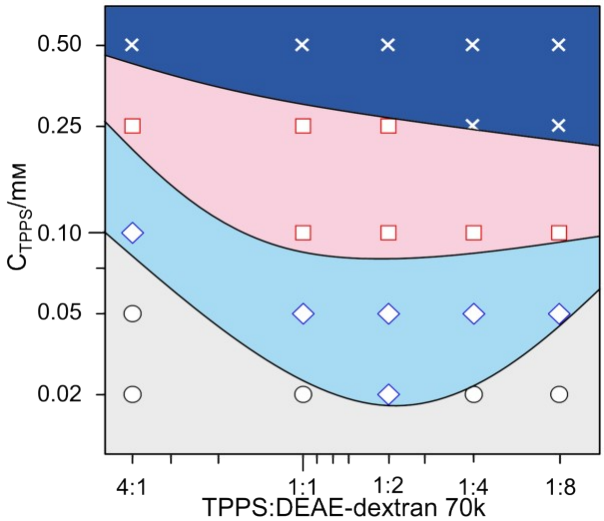

Figure S12. Phase diagram of TPPS/DEAE-dextran 70k system at different concentrations and ratios. The grey, blue, pink and dark blue domains represent the phases of aqueous solution, coacervate, co-existence of coacervate and precipitation, and precipitation respectively.

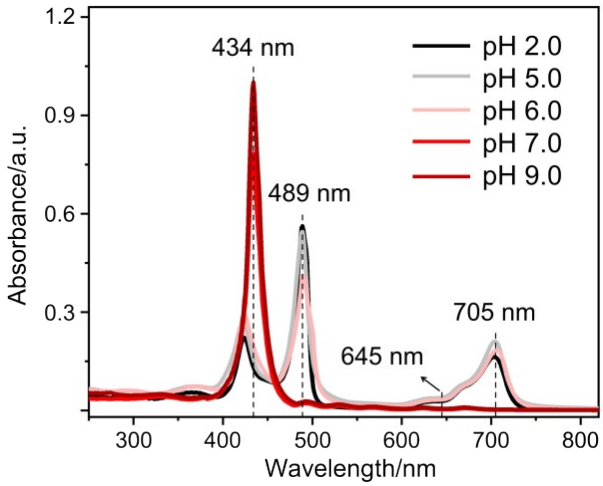

Figure S13. UV-vis absorption spectra of TPPS/DEAE-dextran suspensions (DEAE-dextran 70k, total concentration of 0.15 mM, monomer molar ratio of 1:2) at different pH.

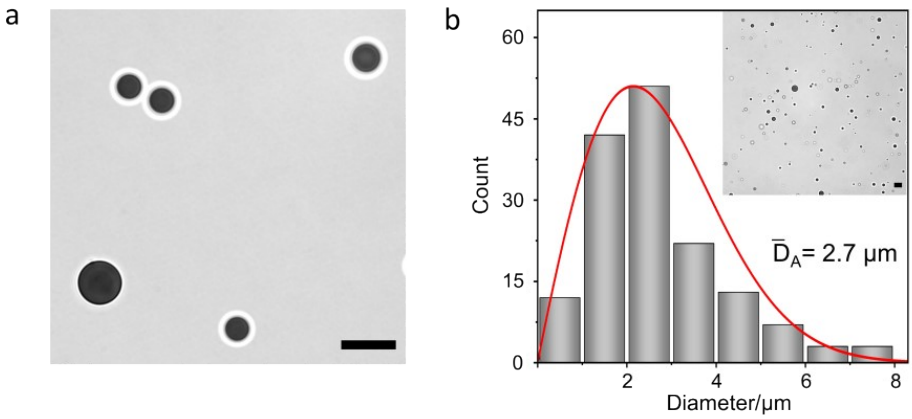

Figure S14. (**a**) Bright field image and (**b**) size distribution of TPPS/DEAE-dextran 70k coacervates microdroplets (total concentration of 0.15 mM, monomer molar ratio of 1:2, pH = 5.0) displaying an average diameter of 2.7 μm. Scale bars, 5 μm.

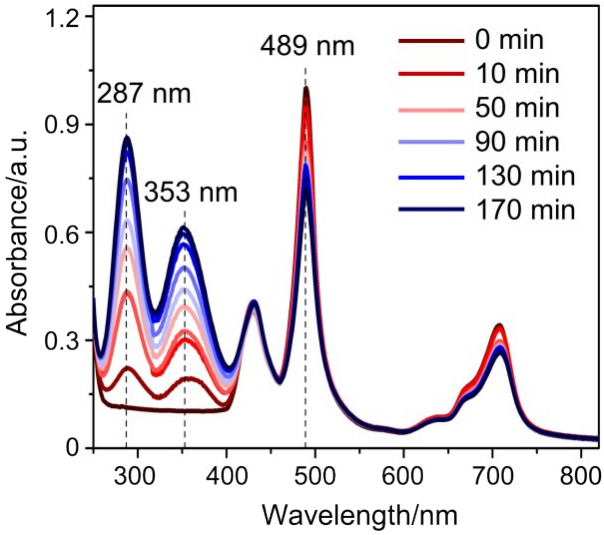

Figure S15. Kinetic UV-vis absorption spectra of the coacervate microdroplets containing TPPS/DEAE-dextran 70k with the addition of iodide, suggesting the superior photocatalytic activity of TPPS/DEAE-dextran 70k coacervate microdroplets.

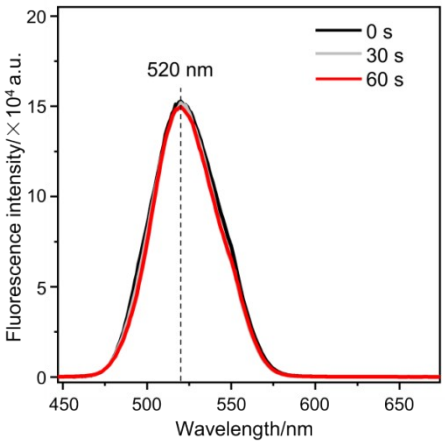

Figure S16. Fluorescence spectra of DPBF aqueous solution (0.1 μM) at different irradiation time (E_x = 405 nm). The fluorescence intensity of DPBF shows almost no change upon photo irradiation in the system.

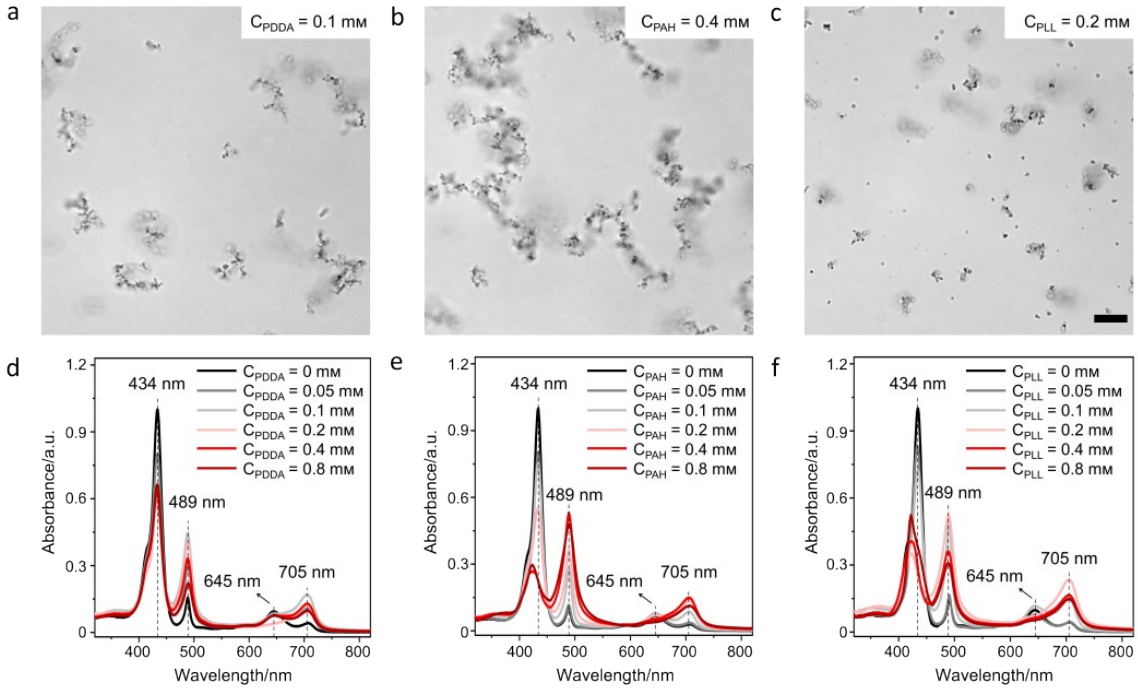

Figure S17. J-aggregation of TPPS with polycation electrolyte (PDDA, PAH, PLL). (**a-c**) Optical microscope images of TPPS (0.1 мм, pH 5.0) mixed with (**a**) PDDA (0.1 мм, pH 5.0), (**b**) PAH (0.4 мм, pH 5.0), (**c**) PLL (0.2 мм, pH 5.0) achieving the maximum J-aggregation degree. Scale bar, 10 μm. (**d-f**) UV-vis absorption spectra of TPPS/polycation electrolyte mixtures (TPPS concentration of 0.1 mM, pH 5.0) with the increase of polyelectrolyte concentration (from 0 мм to 0.8 мм, pH 5.0). The absorption peak intensity at 489 nm reaches the highest when (**d**) PDDA, (**e**) PAH, and (**f**) PLL at a concentration of 0.1 мм, 0.4 мм, 0.2 мм, respectively.