Supporting Information

Construction of coacervate-in-coacervate multi-compartment protocells for spatial organization of enzymatic reactions

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Legends

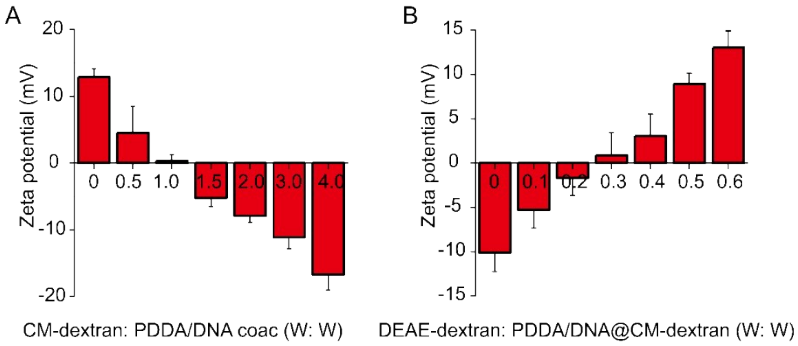

Fig. S1. (A) Zeta potential measurements of PDDA/DNA@CM-dextran coacervate microdroplets after addition of different weight ratios of CM-dextran into PDDA/DNA coacervates (0-4.0 in weight). (B) The zeta potential measurements of PDDA/DNA@CM-dextran/DEAE-dextran coacervate microdroplets by addition of different ratio of CM-dextran and DEAE-dextran based on 0.1 mL PDDA/DNA coacervate.

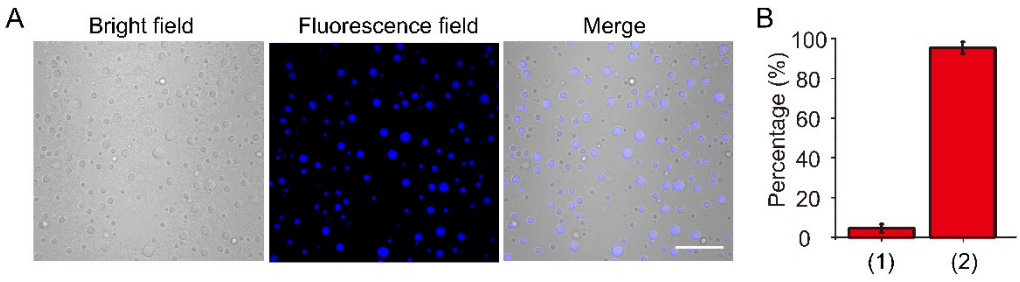

Fig. S2. Confocal images of PDDA/DNA@DEAE-dextran/CM-dextran coacervate microdroplets and the quantitative statistical analysis of the multi-compartment structures. (A) Bright field, (B) fluorescence field, (C) Merge field. (D) Statistical charts for the number percentage of the single-compartment (1), and multi-compartment (2). The microdroplets were prepared at DEAE-dextran/CM-dextran weight ratios of 0.3. Scale bar: 50 μm.

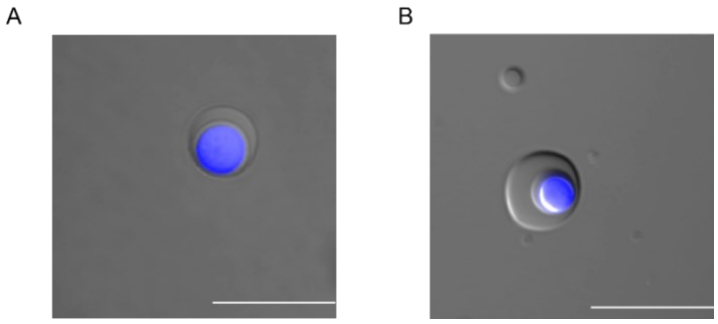

Fig. S3. PDDA/DNA coacervate encapsulated in individual CM-dextran/DEAE-dextran coacervate microdroplet determined from optical microscopy image. The two-tiered coacervate were prepared under constant conditions but at different DEAE-dextran/CM-dextran mass ratios of (**A**) 0.3, (**B**) 1.0. Scale bar: 20 μm.

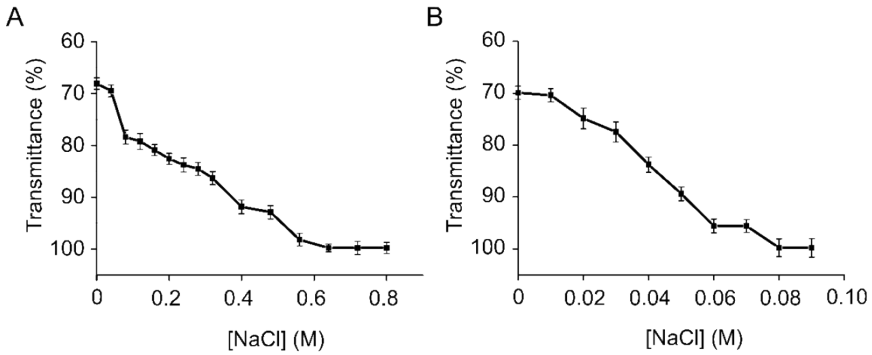

Fig. S4. (**A**) Transmittance determination of PDDA/DNA coacervates under different concentrations of NaCl (0-0.8 M). PDDA/DNA coacervate: 5 mg·ml^{-1}. pH = 8.0. (**B**) Transmittance determination of DEAE-dextran/CM-dextran coacervates under different concentrations of NaCl (0-0.09 M). DEAE-dextran/CM-dextran coacervate: 5 mg·ml^{-1}. pH = 8.0.

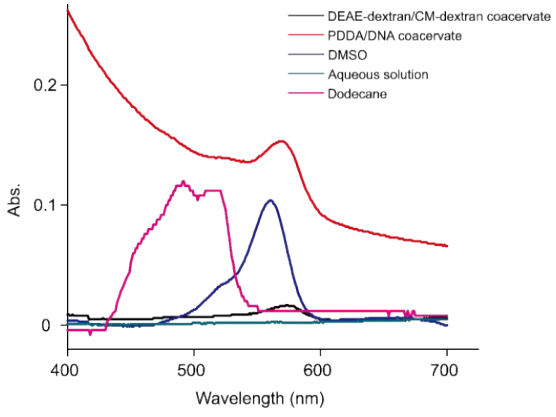

Fig. S5. UV-vis spectrum of Nile red at different solutions. Nile red-containing DEAE-dextran/ CM-dextran microdroplets (black), Nile red-containing PDDA/DNA coacervate (red), Nile red DMSO solution (blue), Nile red aqueous solution (azure), and dodecane solution (pink). Nile red in DEAE-dextran/CM-dextran and PDDA/DNA microdroplets at pH = 8 showing broad band with absorption

peak at 574, and 570 nm respectively.

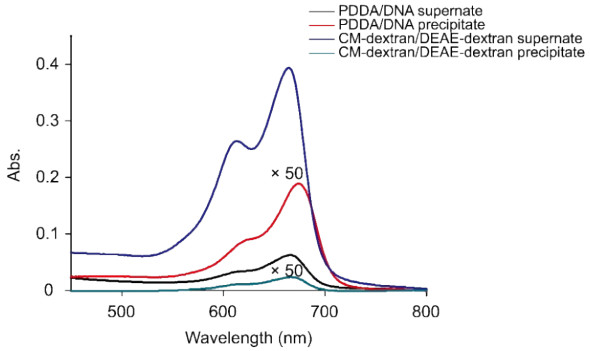

Fig. S6. UV-Vis absorption spectra of methylene blue (Peak: 674 nm) in coacervate phase and supernatant continuous phase. Coacervate microdroplet was separated from bulk aqueous phase by centrifugation at 12,000 rpm for 15 min. The PDDA/DNA and CM-dextran/DEAE-dextran coacervate phase were diluted with 50 times. The partition coefficients of methylene blue in PDDA/DNA and DEAE-dextran/CM-dextran coacervate phases were determined to be 169 and 3.8, respectively.

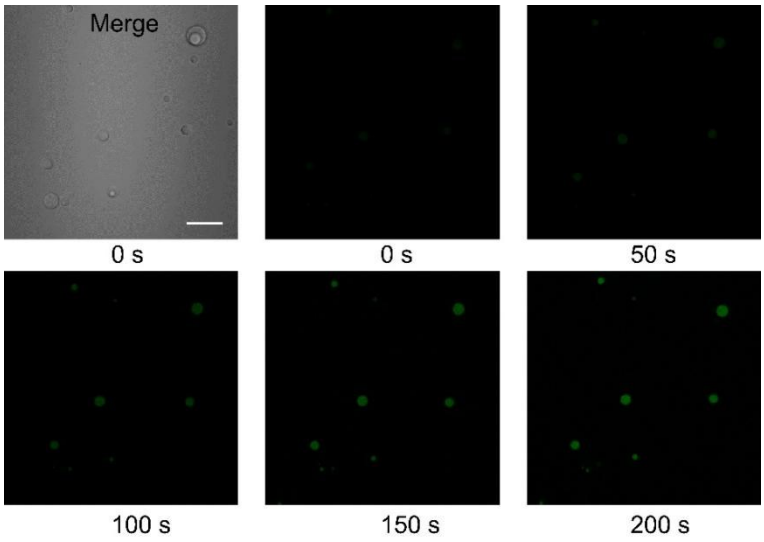

Fig. S7. The diffusion transport of small molecule (fluorescein) in PDDA/DNA @CM-dextran/ DEAE-dextran coacervate by time scan confocal imaging. PDDA/DNA@CM-dextran/DEAE-dextran coacervate was prior prepared and then added 0.02 mg/mL fluorescein dye. Scale bar: 20 μm.

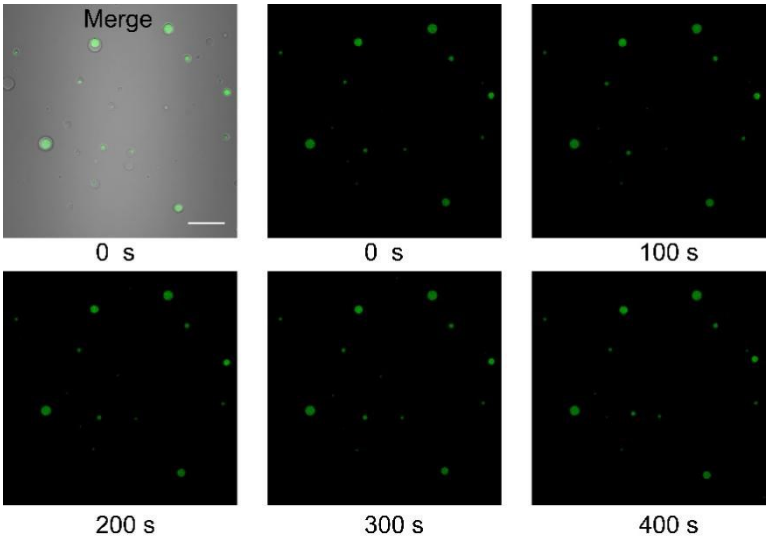

Fig. S8. The diffusion transport of macromolecule (FITC-CAT) in PDDA/DNA@CM-dextran/ DEAE-dextran coacervate by time scan confocal imaging. (FITC-CAT (0.02 mg mL^{-1})) was prior encapsulated in PDDA/DNA coacervate by layer-by-layer assembly method. Scale bar: 20 μm.

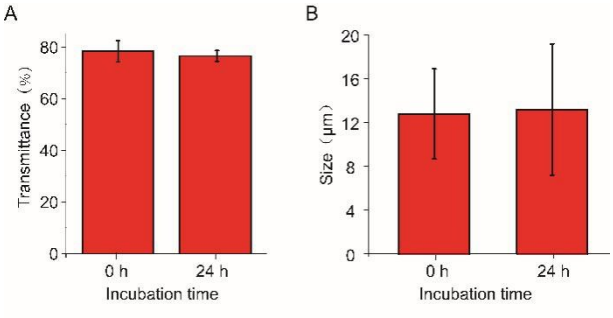

Fig. S9. (A) Transmittance determination, and (B) size determination of PDDA/DNA@CM-dextran/DEAE-dextran coacervate microdroplets at different incubation times. The transmittance of the microdroplets was determined through UV–vis absorption spectra. The size of the microdroplets was determined through dynamic light scattering. After 24 h, a shake facilitated the re-dispersion of the microdroplets.

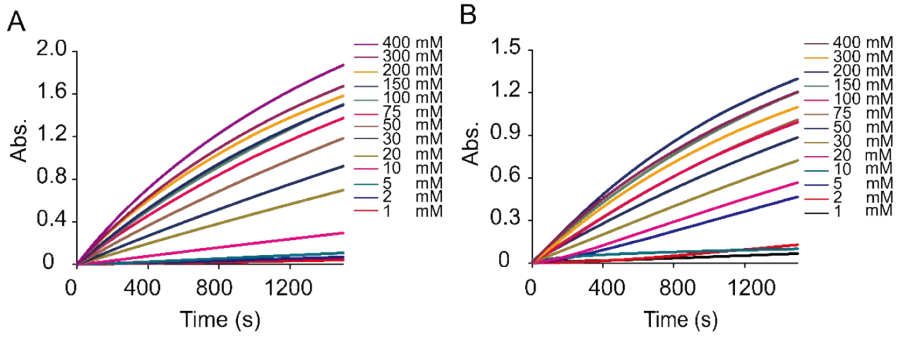

Fig. S10. Time dependent absorbance change of ABTS at 410 nm catalyzed by GOx and HRP, in the presence of glucose (1-400 mM). (A) GOx in aqueous PBS buffer solution, pH = 7.4. (B) GOx loaded in coacervate-in-coacervate structure. GOx: 0.08 μg·mL^{-1}; HRP: 0.22 μg·mL^{-1}, ABTS: 2.0 mM.

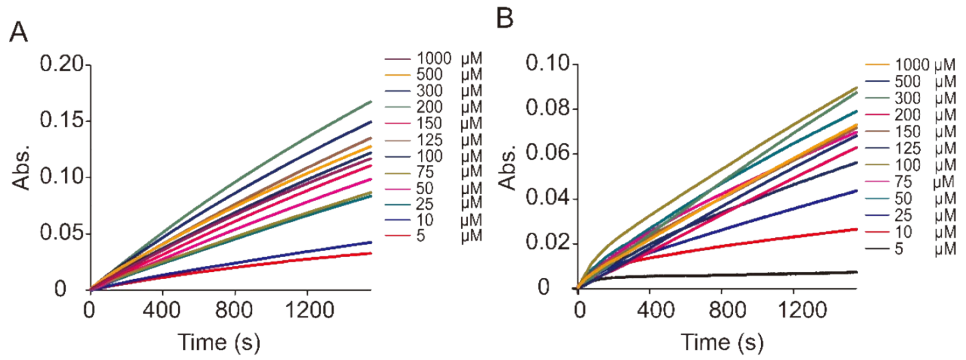

Fig. S11. Time dependent absorbance change of ABTS at 410 nm catalyzed by H_2O_2 and HRP, in the presence of different amounts of H_2O_2 (5-1000 μM). (A) HRP in aqueous PBS buffer solution, pH = 7.4. (B) HRP loaded in coacervate microdroplets. HRP: 0.022 μg·mL^{-1}, ABTS: 2.0 mM.

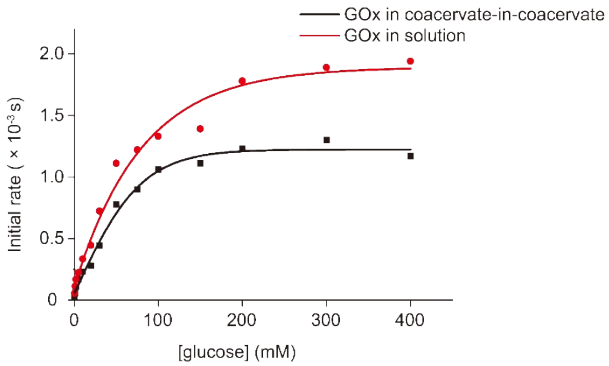

Fig. S12. Michaelis–Menten kinetics fitting of GOx via the ABTS colorimetric method, determined in 10 mM PBS buffer (pH 7.4, red line) and in a coacervate-in-coacervate structure (black line). GOx: 0.08 μg·mL^{-1}; HRP: 0.22 μg·mL^{-1}, ABTS: 2.0 mM.

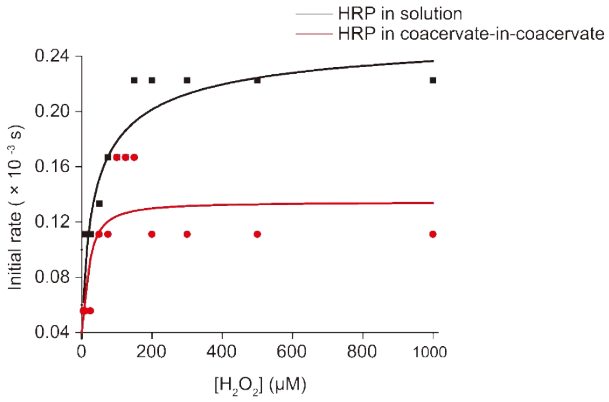

Fig. S13. Michaelis–Menten kinetics fitting of HRP via the ABTS colorimetric method, determined in 10 mM PBS buffer (pH 7.4, black line) and in a coacervate-in-coacervate structure (red line). HRP: 0.022 μg·mL^{-1}, ABTS: 2.0 mM.

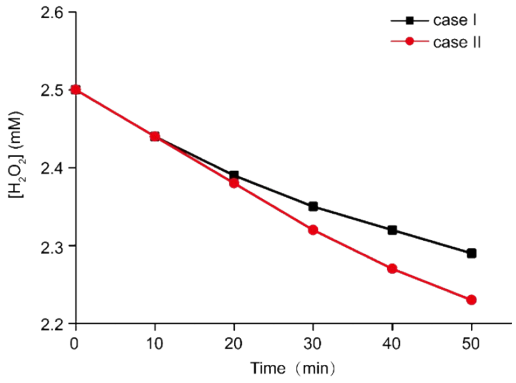

Fig. S14. The decomposition of H_2O_2 via UV-Vis absorption spectra, determined in 10 mM PBS buffer by CAT-mediated enzymatic reaction in the case I, case II, respectively.

Table S1. physical and chemical parameters of different coacervate phases.

Coacervate phases	partition coefficient	dielectric constant	Density
PDDA/DNA	169	60.3	2.1
DEAE-dextran/CM-dextran	3.8	66.3	1.5

[a] The partition coefficient was determined by using methylene blue probe;

[b] The dielectric constant was determined by using Nile red probe.

Table S2. The encapsulation efficiency of GOx,HRP and catalase in cases I and II.

encapsulation efficiency	GOx	HRP	CAT
inner coac-in-coac	97.6%	97.6%	98.6%
Outer coac-in-coac	97.5%	97.5%	98.2%

Table S3. Michaelis-Menten constant (Km) of GOx, and HRP in coacervate or solution.

	GOx /mM	HRP/ μM
Buffer solution	44.2	12.6
Coacervate matrix	96.6	30.5