Supporting Information

Membrane-less compartmentalization facilitates enzymatic cascade reactions and reduces substrate inhibition.

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Supplemental Methods

S1 Characterization of ATP-PDDA coacervates

Zeta potential measurements (ZetaSizer Nano ZSP, Malvern) were conducted on bulk solutions of HRP, GOx, dextranase, RITC-HRP, FITC-GOx, and RITC-dextranase (final concentration of 0.1 mg mL^{-1}, MES, pH 6.0), respectively. For resuspended coacervates, 0.15 µL of the ATP-PDDA coacervates alone or the enzyme-laden coacervates were resuspended in the MES buffer and the zeta potential was measured. The zeta potential values were averaged with three replicates and expressed as mean with standard deviation (Table S1).

S2 pH and ionic strength influence on ATP-PDDA coacervates in DEX-PEG ATPS

50 µL of a 10 % w/w DEX solution (DI-water, pH 7) was placed in a well within a plastic 24-well plate and 0.5 µL of the coacervate pellet was dispensed into the DEX droplet. 500 µL of a 10% w/w PEG solution (DI-water, pH 7) or PEG-NaCl solution (200 mM NaCl, DI-water, pH 7.0) was added to the well. 50 µL of 1 M HCl was added to the pure PEG solution to change pH (final pH 1).

S3 Fluorescent labelling of enzymes and determination of the partition coefficient

FITC-GOx and RITC-HRP and RITC-dextranase were prepared and characterized following a previously reported protocol (Ref 26). Briefly, 3 mL of 4 mg mL^{-1} enzyme solutions (100 mM Na_2CO_3, pH 8) were mixed with 60 µL of 2 mg mL^{-1} FITC or RITC in DMSO and incubated overnight at 4 °C. The resulting FITC-GOx, RITC-HRP, and RITC-dextranase were dialysed for 3 days (3 L of 10 mM Na_2CO_3, pH 8). The partition coefficient was determined by UV-Vis spectroscopy measurement (Ultrospec 2100 Pro, GE Healthcare). Stock solutions of RITC-HRP,

FITC-GOx, and RITC-Dextranase were prepared in a 25 mM PDDA solution (final concentration of 0.5 mg mL^{-1}) and measured by the UV-VIS spectroscopy. A 25 mM ATP-PDDA solution containing each enzyme (final concentration of 0.5 mg mL^{-1}) was centrifuged and the supernatant was measured by the UV-VIS spectroscopy. Based on the extinction coefficient (FITC: $\varepsilon^{485\ nm}$ = 50,358 M^{-1} cm^{-1} and RITC: $\varepsilon^{545\ nm}$ = 65,000 M^{-1} cm^{-1}), the mass of RITC-HRP, FITC, GOx, and RITC-Dextranase in stock solution and supernatant was calculated from the absorbance. Given the volume of the supernatant and coacervate phases, the partition coefficient of each enzyme was determined as follows.

$$K^{app} = \frac{m^{coacervate}}{m^{supernatant}}$$

where K^{app} is apparent partition coefficient and m is mass of the enzyme in the supernatant or coacervate phases. The values were averaged with three replicates and expressed as mean with standard deviation (Table S1).

S4 Bulk Dextranase Assay

The color reagent and the dextranase solutions were prepared as followed. 10 mg mL^{-1} 3,5-dinitrosalicylic acid, 2 mg mL^{-1} phenol, 0.5 mg mL^{-1} sodium sulfate, and 200 mg mL^{-1} of sodium potassium tartrate tetrahyderate were prepared in a 0.5 M NaOH solution. The total mass of free dextranase was set to be around 2 μg using a 1 mg mL^{-1} dextranase solution (DI-water). For compartmentalization, 100 μg mL^{-1} dextranase was mixed during coacervation and the dextranase-containing coacervate pellet was used (*c.a.* total 1 μg dextranase in the coacervate). The reaction was performed as followed. 1.9 mL of the dextran solution (± 150 mM NaCl) and 100 μL of the 20 μg mL^{-1} dextranase solution or DI-water as a blank condition were mixed in a tube. Alternatively, 2.0 mL of the dextran solution (± 150 mM NaCl) was directly mixed in the

dextranase-containing coacervates pellet. The mixtures were intensively pipetted and incubated at 24 °C for 30 min. 1.0 mL of the mixture were added to 1.0 mL of the color reagent solution in a tube and incubated in boiling water for 15 min. The reaction mixture was cooled down to the room temperature and absorbance at 540 nm was measured with appropriate dilution.

S5 DEX Viscosity Measurement

The dynamic viscosity was determined based on reported values[1-4] of DEX macromolecules of a similar molecular weight (Mw: 500 kDa). The relative viscosity of DEX as a function of DEX concentration was measured by Stokes's method using a falling object. 10 mL of a DEX solution (0, 0.5, 1, 2, 3, 6, 8, 10, 15, and 20%) was added in a 10 mL mass cylinder. A spherical ball of a known density and geometry was gently dropped from a liquid surface and travel time to reach the bottom of the cylinder was recorded. The above process was repeated five times and the travel time was used to calculate the relative viscosity using 0% DEX as a standard (0.89 mPa·s). The relative viscosity of the coacervate was similarly measured using 25 mM ATP-PDDA mixture, supernatant, and pellets in a 5 mL mass cylinder. The values are expressed as mean ± standard deviation (n = 5).

Table S1. Zeta-potential and partition coefficient values

Sample	Zeta-potential (mv)	Partition coefficient (K^{app})
ATP-PDDA Coacervate	17 ± 0.32	
HRP	-7.7 ± 0.35	
GOx	-4.7 ± 0.41	
Dextranase	-6.1 ± 0.81	
RITC-HRP	-1.6 ± 0.72	$1.7 \pm 0.62 \times 10^4$
FITC-GOx	-1.8 ± 0.73	$1.8 \pm 0.14 \times 10^2$
RITC-Dextranase	-16 ± 0.81	$2.5 \pm 0.024 \times 10^2$

Table S2. A summary of the geometry of coacervate droplets used for cascade reaction experiments.

Droplet Volume / μL	1	3	9
Area[a] / mm^2	1.6 ± 0.71	5.8 ± 0.51	14 ± 1.1
Perimeter[a] / mm	4.8 ± 0.12	9.1 ± 0.39	15 ± 0.27
Height[b] / mm	0.91 ± 0.039	0.77 ± 0.067	1.0 ± 0.079
Surface Area[b] / mm^2	3.9 ± 0.081	8.5 ± 0.54	18 ± 1.1
Surface-to-Volume Ratio / mm^{-1}	3.9 ± 0.081	2.8 ± 0.18	2.0 ± 0.12

a) The values were obtained from droplet images analyzed by ImageJ

b) The values were estimated assuming a hemi-ellipsoidal droplet.

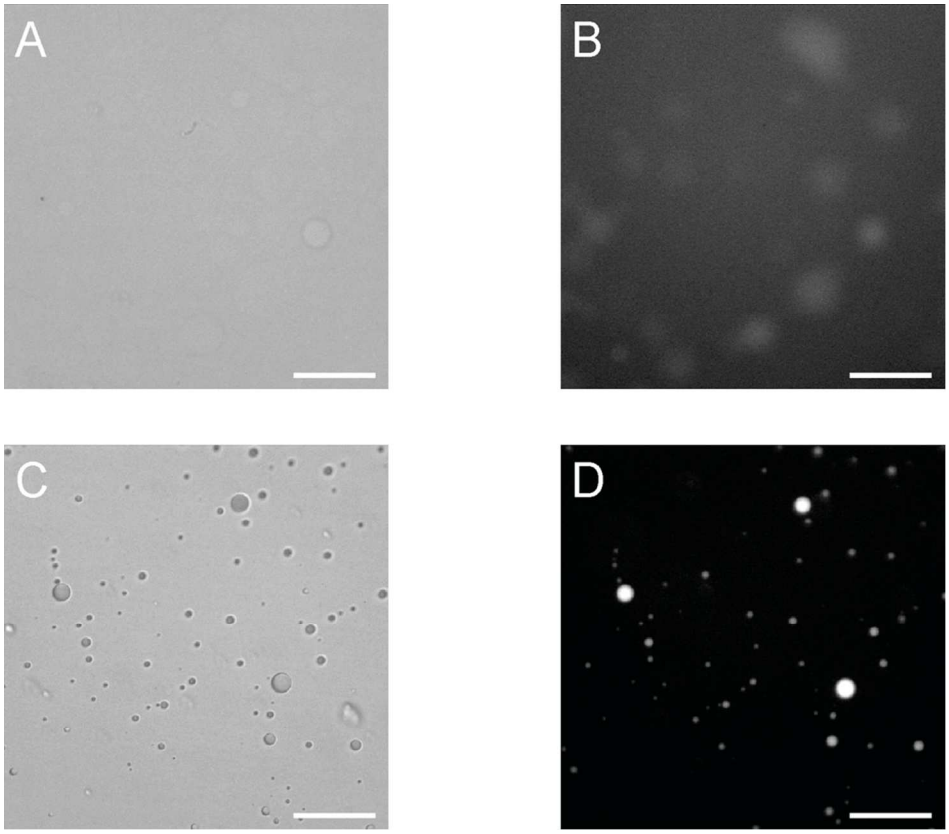

Figure S1. Wetting behavior of ATP-PDDA coacervates before and after resuspension. The ATP-PDDA coacervate suspension on a glass slide: (A) brightfield and (B) fluorescent images. The ATP-PDDA coacervate resuspension on a glass slide: (C) brightfield and (D) fluorescent images. 1 μM FITC was premixed during coacervation. Scale bar 50 μm.

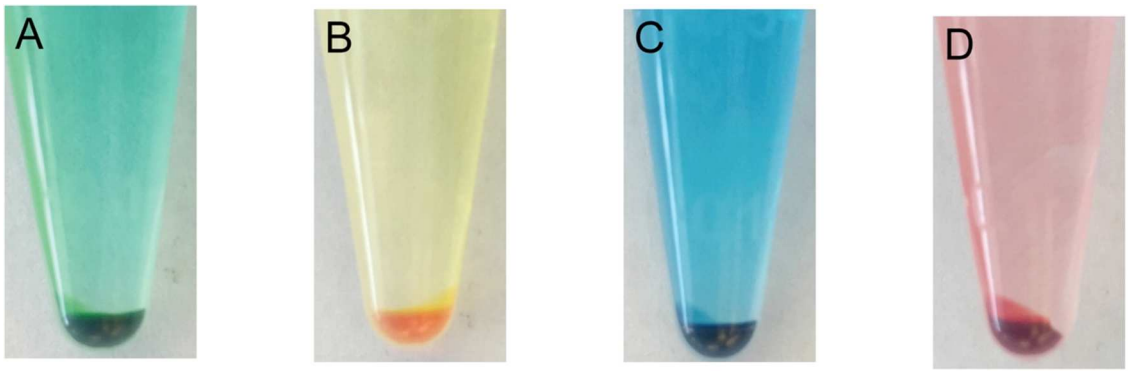

Figure S2. ATP-PDDA coacervate pellets containing food colorings. Images of the coacervate pellets after centrifugation containing (A) green, (B) yellow, (C) blue, and (D) red color dyes.

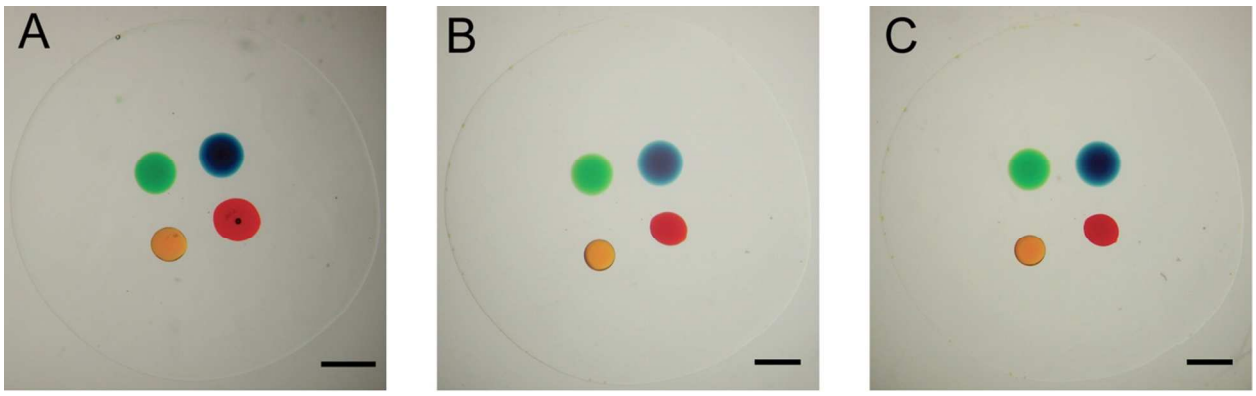

Figure S3. Patterning of multiple ATP-PDDA coacervates containing food colorings in a 10% DEX - 10% PEG system. Time-lapse images of the ATP-PDDA coacervates at (A) t = 0, (B) 24 hrs, and (C) 48 hrs. Scale bar 1 mm.

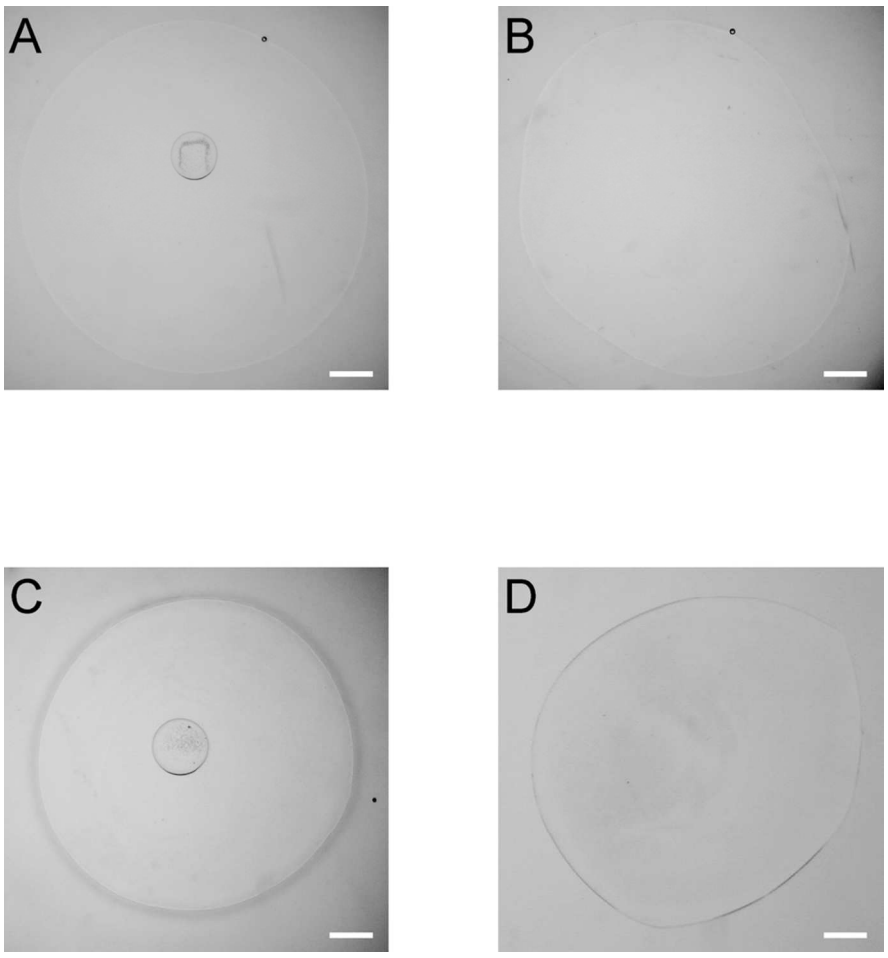

Figure S4. Sensitivity of the coacervate phase to pH and ionic strength change in the DEX-PEG system. Single ATP-PDDA coacervate the DEX-PEG system at (A) pH 7 and (B) pH 1. The coacervate in the presence of 250 mM NaCl at (C) t = 0 and (D) t = 10 min. Scale bar 1 mm.

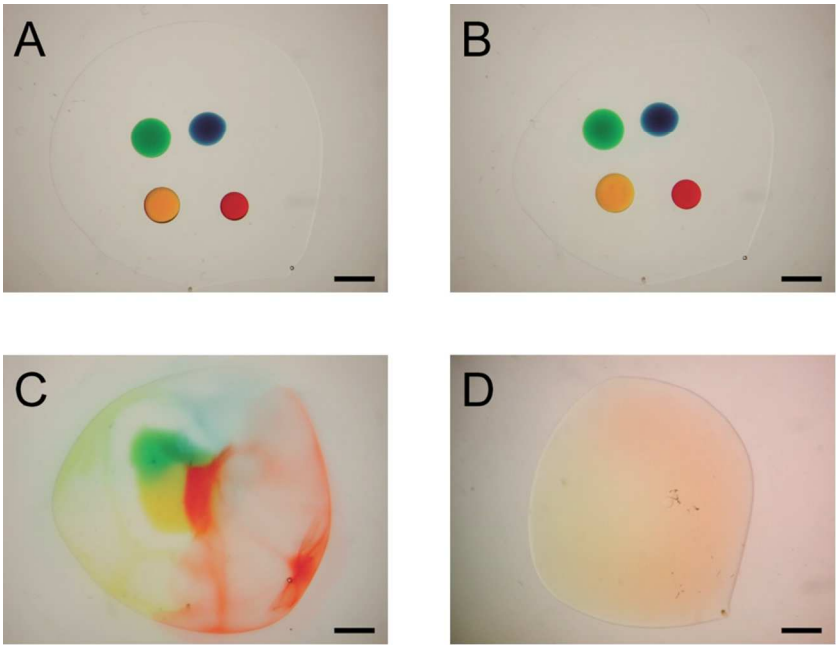

Figure S5. Selective dissociation of ATP-PDDA coacervates and subsequent release of food colorings in the DEX-PEG system. Time-lapse images of the ATP-PDDA coacervates in the presence of 150 mM NaCl at (A) t = 0 min, (B) 5 min, (C) 15 min, and (D) 1200 min. Scale bar 1 mm.

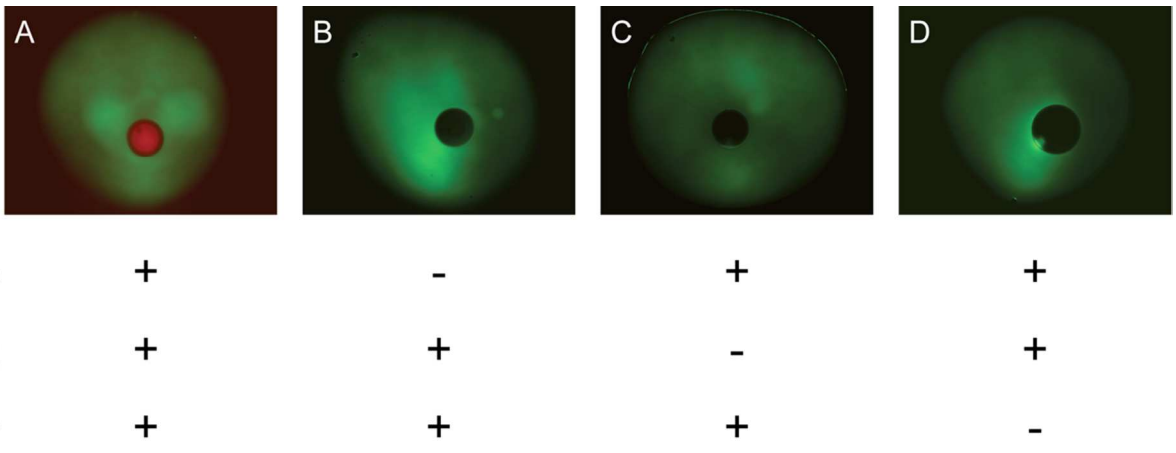

Figure S6. GOx-HRP-mediated cascade reactions across ATP-PDDA coacervate, DEX, and PEG phases. (A) β-glucose, GOx, and HRP present, (B) GOx and HRP present, (C) β-glucose and HRP present, and (D) β-glucose and GOx present. FITC-DEX (Green) and catalyzed Amplex Red™ (Red). Scale bar 1 mm.

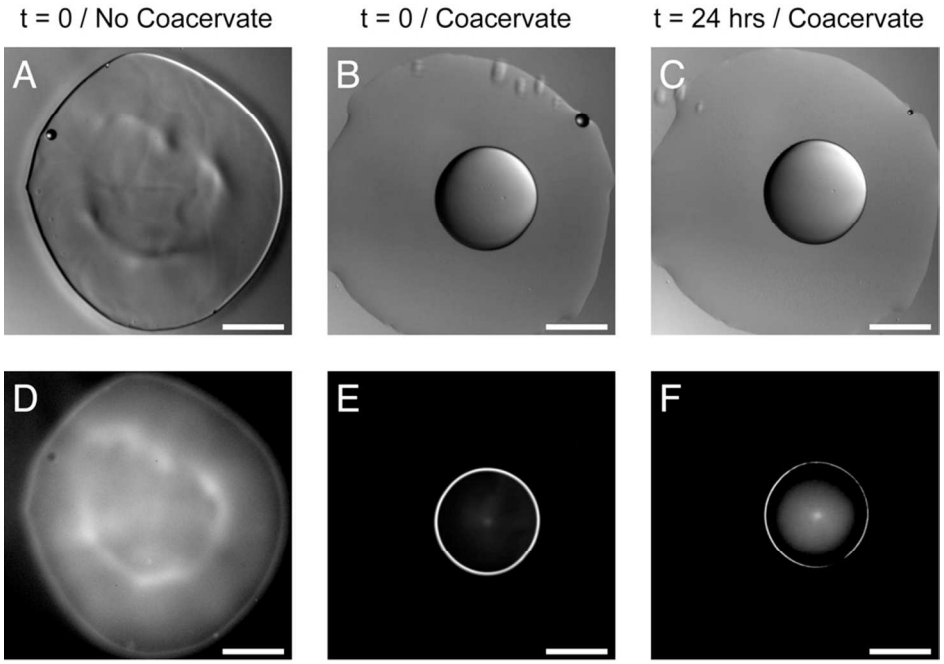

Figure S7. Time-lapse GOx partitioning to the ATP-PDDA coacervate phase in the DEX-PEG system. FITC-GOx dispensed in a DEX droplet (A and D) in the absence of the coacervate at t = 0 and in the presence of the coacervate at (B and E) t = 0 and (C and F) t = 24 hrs. (A-C) Brightfield and (D-F) fluorescent images. Scale bar 500 μm.

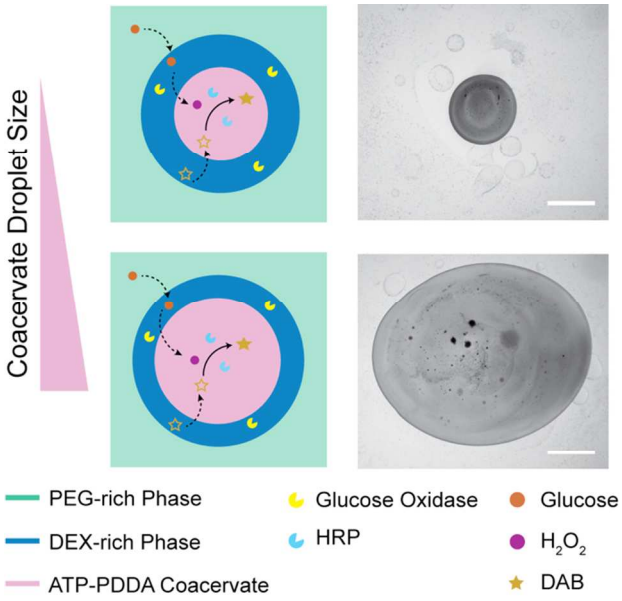

Figure S8. The reaction kinetics of the DAB-catalyzing GOx-HRP reaction. The coacervate droplets after the cascade reaction in a 10% DEX – 10% PEG system. The brightfield images show 1 µL (top) and 9 µL (bottom) coacervate droplets. Scale bar 1 mm.

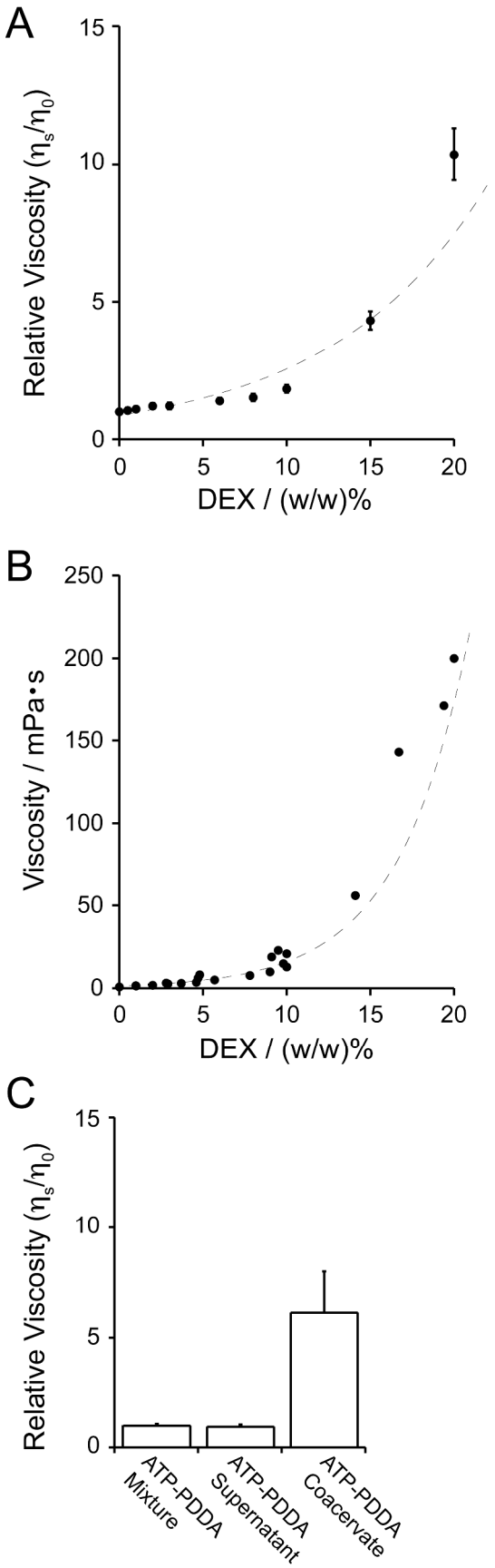
A
Relative Viscosity (η_s/η_0)
DEX / (w/w)%
B
Viscosity / mPa•s
DEX / (w/w)%
C
Relative Viscosity (η_s/η_0)
ATP-PDDA Mixture
ATP-PDDA Supernatant
ATP-PDDA Coacervate

Figure S9. Viscosity change as a function of DEX concentration. (A) The relative viscosity was obtained by Stokes's method and normalized by the viscosity of water. The fitting line (dotted) was used to estimate the viscosity of the coacervate. (B) The dynamic viscosity was estimated from reported values (Ref 1-4 below) using DEX macromolecules of a similar molecular weight (Mw: 500 kDa). The fitting line (dotted) was used to estimate the viscosity of the coacervate. (C) The relative viscosity of 25 mM ATP-PDDA coacervate solutions. (A and C) Five replicates were measured and the values are expressed as mean ± standard deviation.

References for the DEX viscosity plot

1. Carrasco, F.; Chornet, E.; Overend, R. and Costa, J., A Generalized Correlation for the Viscosity of Dextrans in Aqueous Solutions as a Function of Temperature, Concentration, and Molecular Weight at Low Shear Rates. *J. Appl. Polym. Sci.* **1989**, *37*, 2087-2098.

2. Chu, K. K.; Mojahed, D.; Fernandez, C. M.; Li, Y.; Liu, L.; Wilsterman, E. J.; Diephuis, B.; Birket, S. E.; Bowers, H. and Solomon, G. M., Particle-Tracking Microrheology Using Micro-Optical Coherence Tomography. *Biophys. J.* **2016**, *111*, 1053-1063.

3. Cote, G. and Willet, J., Thermomechanical Depolymerization of Dextran. *Carbohydr. Polym.* **1999**, *39*, 119-126.

4. Goins, A. B.; Sanabria, H. and Waxham, M. N., Macromolecular Crowding and Size Effects on Probe Microviscosity. *Biophys. J.* **2008**, *95*, 5362-5373.