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Supplemental Information

Programmable ATP-Fueled DNA Coacervates by Transient Liquid-Liquid Phase Separation

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1. Materials and methods

1.1 Instrumentation

ThermoMixer (Eppendorf), UVsolo *touch* Gel electrophoresis documentation system (Analytik Jena), gel electrophoresis chambers (biostep), power source 250 V (VWR), Image J, ScanDropR UV-VIS spectrometer (Analytik Jena), Confocal laser scanning microscopy (Leica TCS SP8, Mannheim), STX Stage Top Incubator System (Tokai Hit, Japan), and Spark® Multimode Microplate Reader (Tecan).

1.2 Reagents and materials

T4 DNA ligase (HC, 20 WU/µL, recombinant *E. coli* strain) was supplied by Promega and BsaI-HF®v2 (20 units/µL, *NEB #R3733*) was ordered from New England Biolabs (*NEB*). ATP solution (10 mM in 1 mM Tris-HCl pH 7.5) was purchased from Invitrogen. Agarose low EEO was supplied by PanReac AppliChem. Gene ruler 1k bp and 50 bp DNA ladders (ready to use), DNA gel loading dye (6×), horseradish peroxidase-conjugated streptavidin (N100), and streptavidin coated magnetic microparticles (Dynabeads™ MyOne™ Streptavidin C1) were supplied by ThermoFisher Scientific. Streptavidin-glucose oxidase was ordered from Fitzgerald (65R-S124). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, biology grade) was supplied by CALBIOCHEM. Sodium chloride (NaCl, 99%), hexadecane (99%), tris (hydroxymethyl)aminomethane hydrochloride pH 8.0 (Tris-HCl), trizma base, D-glucose (> 99.5%), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$, > 98%) were ordered from Sigma-Aldrich. Acetic acid glacial (ACS, Reag. Ph. Eur. Analytical reagents) was supplied by VWR Chemicals. RotiR-GelStain (1,1'3,3',5,5'6,6'-octamethyl-2,2'-spiro(2,3-dihydro-1H-benzimidazol) was supplied by Carl Roth. All oligonucleotides were supplied by Integrated DNA Technologies Inc.. Corning® high content imaging plates (96 wells, black wells (with 0.2 mm glass bottom), half area) were supplied by Sigma-Aldrich.

Buffer compositions

T4 DNA Ligase Storage Buffer (Promega): 10 mM Tris-HCl (pH 7.4 at 25 °C), 50 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 50% glycerol.

BsaI-HF®v2 storage buffer (NEB): 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/mL BSA, 50% glycerol.

NEB CutSmart® Buffer: 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 µg/mLBSA.

Annealing Buffer: 10 mM Tris-HCl (pH 8.0), 50 mM NaCl.

TAE Buffer: 40 mM Tris, 20 mM acetic ccid, 1 mM EDTA.

Quenching Buffer: 200 mM EDTA, 10 mM Tris-HCl (pH 8.0), 50 mM NaCl.

CSF buffer: *CutSmart® Buffer* supplemented with 0.05% Pluronic® F-127.

Milli-Q water was used throughout of this study.

Supplementary Table 1: Oligonucleotide sequences used, with their name in individual Figure, the sequence codes used for ordering at IDT, and their ID in experimental section.

Name	Oligonucleotide sequence (5'-3')	Purification	ID
M1	/5Phos/GATTAGAGACCGTACCTACATATAGCTACTG ATACTCT	HPLC	D_1
	/5ATTO488N/AGCTATATGTAGGTACGGTCTCT	HPLC	D_2
M2	/5Phos/AATCAGAGTATCTTTTTCGAATAGAGG	HPLC	D_3
M3	/5Phos/ATGAAGAGACCGTACCTACATATAGCTACTA CTTGATA	HPLC	D_4
	/5ATTO647NN/AGCTATATGTAGGTACGGTCTCT	HPLC	D_5
M4	/5Phos/TCATTATCAAGTTTT TTCCTCTATTCG	HPLC	D_6
M5	/5Phos/GATTAGAGACCGTACCTACATATAGCTACTG ATACTCT	HPLC	D_1
	AGCTATATGTAGGTACGGTCTCT	HPLC	D_7
DoS	CATAGGTAGAAGTGT/3ATTO488N/	HPLC	D_8
	/5BiosG/ACACTTCTACCTATGTTTTTTTTCACCTCTAT TCG	Desalting	D_9
$2/3M_{EC}$	/5Phos/GTCAAGATATCGTACCTACATATAGTCACTTC AATAGC	HPLC	D_{10}
	/5BiosG/GAGTATATGTAGGTACGATATCT	HPLC	D_{11}
$1/3M_{EC}$	/5Phos/TGACGCTATTGATTTTTCCTCTATTCG/3Cy3Sp/	HPLC	D_{12}
M6	/5Phos/GTCAAG AGA CCG TAC CTA CAT ATA GCT ACT CAT TCC AA	HPLC	D_{13}
	/5ATTO488N/AGC TAT ATG TAG GTA CGG TCT CT	HPLC	D_{14}
M7	/5Phos/TGACTTGGAATGTTT TTC TCA TAC T	HPLC	D_{15}
M8	/5Phos/CTTAAG AGA CCG TAC CTA CAT ATA GCT ACT CCT ATG TG	HPLC	D_{16}
	AGCTATATGTAGGTACGGTCTCT	HPLC	D_7

M9	/5Phos/TAAGCACATAGGTTTTTAGTATGAG	HPLC	D_{17}

2. Experimental Protocols

2.1 Hybridization of the double-stranded DNA (dsDNA) tiles

All DNA strands were used as received, to which certain amounts of annealing buffer were added to make stock solutions and the concentrations were calculated via UV-VIS spectroscopy. The dsDNA tiles in this study were annealed from two complementary single-stranded (ssDNA) with the same stoichiometry at room temperature overnight. The annealed stock solutions (ca. 0.5 mM) were stored at -20 °C for further use.

2.2 Coacervates fusion

Typical experiments were conducted in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M1, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.0 U/μL BsaI as well as 0.2 mM ATP without shaking. The reaction solution was sealed in a well of an imaging plate by a layer of hexadecane. After reacting for 2 h, time-dependent measurements of the coacervates fusion were carried out under CLSM by time-lapse imaging in 2 min intervals over 5 h (Results in Figure S5 and Supplementary Video 1).

2.3 New DNA tile integration and dynamization in a running transient coacervates system

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.0 U/μL BsaI, and 0.2 mM of ATP, under orbital shaking at 80 rpm, followed by addition of another 0.002 mM M1 and 0.0024 mM M2 after 2 h running of the transient coacervation reaction. CLSM was implemented to characterize the coacervates at different time intervals, and the CLSM measurements were conducted consecutively with the same microscopy settings.

2.4 ATP-driven sorted LLPS for multicomponent coacervates

Typical experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.01 mM M6, 0.012 mM M7, 0.01 mM M8, 0.012 mM M9, 0.92 WU/μL T4 DNA ligase, 1.0 U/μL BsaI, and 0.12 mM ATP, under orbital shaking at 80 rpm. At different time intervals, CLSM was used to characterize the multicomponent coacervates, and the CLSM measurements were conducted consecutively with the same microscopy settings.

2.5 Immobilization of the docking strand on streptavidin-coated magnetic colloids

10 μL of streptavidin-coated magnetic particles (Dynabeads™ MyOne™ Streptavidin C1, ThermoFisher Scientific) were washed with 10 μL CSF buffer for 3 times. Afterwards, the particles were re-suspended in 20 μL CSF buffer with 0.05 mM docking strand (DoS, D_8/D_9) and the reaction was carried out at room temperature for 1 h. The particles were then washed by 10 μL CSF buffer 5 times, re-suspended in 10 μL CSF buffer, and stored in the refrigerator for further use.

2.6 ATP-driven transient colloids trapping and releasing in the DNA coacervates

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.0 U/μL BsaI, ca. 66.8 μg/mL DoS-modified colloids (ca. 4.68-6.68×10^7 beads/mL) as well as 0.06 mM ATP, under orbital shaking at 80 rpm. The reaction solution was sealed in a well of an imaging plate by a layer of hexadecane. At different time intervals, CLSM was applied to characterize transient colloid assembly and superstructure. CLSM measurements were conducted consecutively with the same microscopy settings. For another experiment, lower amount of colloids was used to control the structure of the assemblies. The experiments were performed at the same conditions as above but only 10 % of the colloids were used.

2.7 Synthesis of enzyme carrier (biotin modified BsaI-inert SfNAP)

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.05 mM 2/3M_{EC} (D_{10}/D_{11}), 0.06 mM 1/3M_{EC} (D_{12}), 0.92 WU/μL T4 DNA ligase, and 0.6 mM ATP for 12 h. Afterwards, the remaining ATP was removed by spin filtration washed by annealing buffer with 3 k molecular weight cut-off. The concentration of the products in terms of the amount of repeating units was calculated by the absorbance of Cy3 dye at 550 nm to be 0.04 mM. The obtained solution was stored in the refrigerator for further use.

2.8 ATP-driven transient trapping of the BsaI-inert SfNAP in DNA coacervates

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.0 U/μL BsaI, inert SfNAPs of 2 nM repeating units as well as 0.06 mM ATP, under orbital shaking at 80 rpm. The reaction solution was sealed in a well of an imaging plate by a layer of hexadecane. At different time intervals, CLSM was applied to characterize transient polymer trapping and releasing.

2.9 Immobilization of glucose oxidase (GOx) and horseradish peroxidase (HRP) to enzyme carrier

100 nM streptavidin-GOx (or streptavidin-HRP) was mixed with enzyme carrier (containing 100 nM biotin) in 1× *NEB* CutSmart buffer, and the conjugation reaction was performed at a refrigerator for overnight. The obtained products were immediately used for further experiments.

2.10 ATP-driven transient enzymes encapsulation

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.33 U/μL BsaI, 0.5 nM GOx on enzyme carriers, 0.5 nM HRP on enzyme carriers, and 0.06 mM ATP, under orbital shaking at 80 rpm. The reaction solution was sealed in a well of an imaging plate by a layer of hexadecane. At different time intervals, CLSM was applied to characterize the coacervates, and the CLSM measurements were conducted consecutively with the same microscopy settings.

2.11 Multienzyme cascades in membrane-less all-DNA coacervates

The experiments were performed in 1× *NEB* CutSmart buffer at 37°C containing 0.5 nM GOx on enzyme carriers, 0.5 nM HRP on enzyme carrier, 2 mM $ABTS^{2-}$, SfNAP1 and SfNAP2 of 0.008 mM repeating units, under orbital shaking at 96 rpm (due to the lower limit of the plate

reader). After coacervating for 2 h, 1 mM glucose was added to trigger the multienzyme cascades, and the catalytic efficacy was monitored by a plate reader via absorbance at 414 nm. Control experiments were conducted by free enzymes or enzymes on the enzyme carriers but without coacervates at the same conditions as above.

3. Supplementary Figures

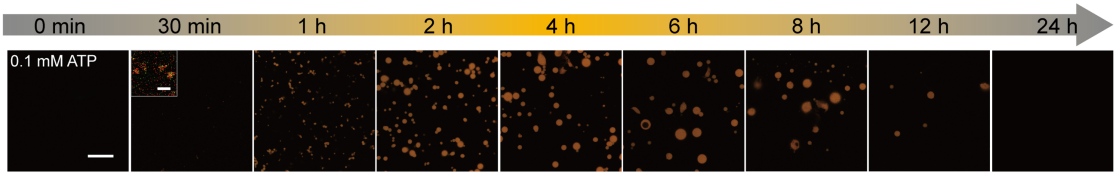

Figure S1. ATP-driven transient all-DNA coacervates. Time-dependent CLSM measurements of transient all-DNA coacervates fueled by 0.1 mM ATP. CLSM measurements were conducted consecutively with the same microscopy settings. Scale bar = 20 μm; insert = 2 μm.

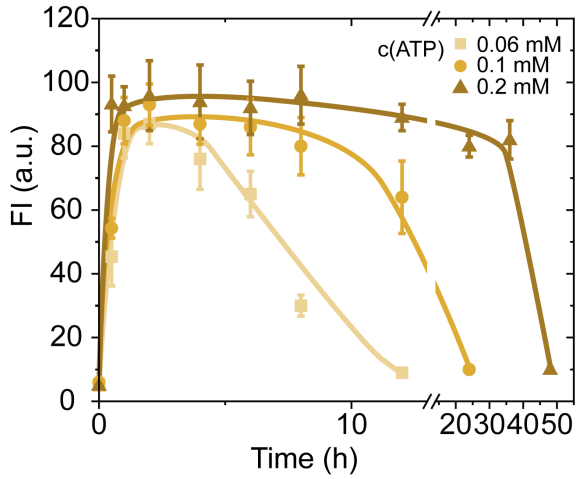

Figure S2. Time-dependent fluorescence intensity of the transient coacervates fueled with 0.06, 0.1, and 0.2 mM ATP. Error bars are standard deviations from three random fields of view of duplicate experiments. Ca. 300 coacervates were counted at the dynamic steady state, while 10-50 coacervates were counted at the degradation stage due to significantly decreased number of coacervates. Lines are guides to the eye.

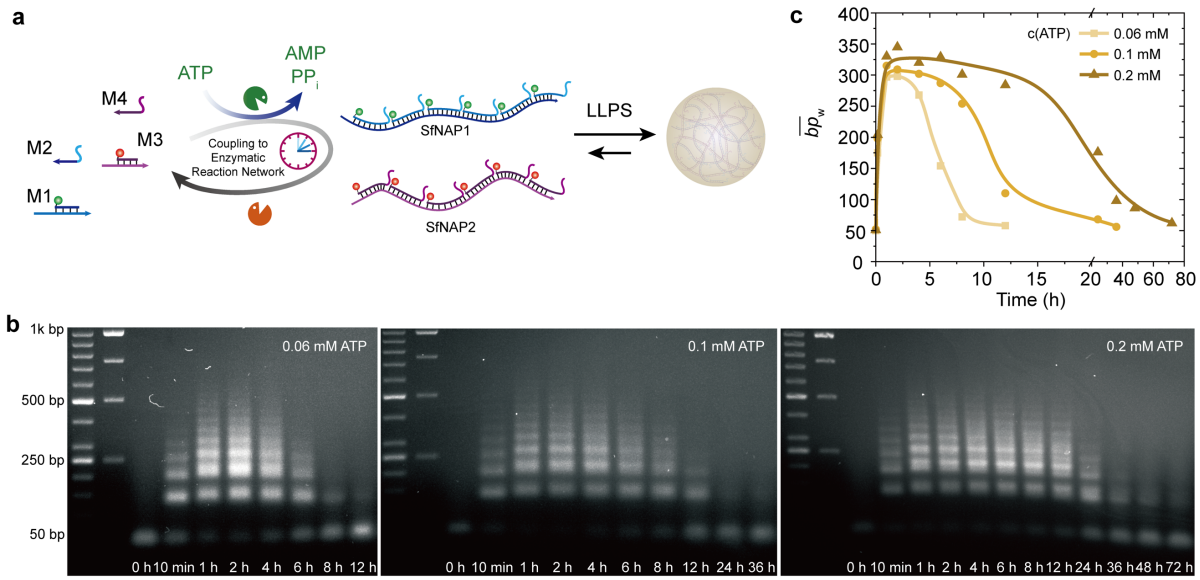

Figure S3. Analyses of the transient SfNAPs in the transient DNA coacervates. (**a**) Schematic representation of ATP-driven transient coacervates by LLPS of transient SfNAPs. (**b**) Time-dependent AGE (2 wt. %, 90 V, 2 h) for transient DNA polymerizations with programmable lifetimes by fueling with varied ATP concentration (0.06, 0.1, and 0.2 mM). (**c**) The mass-weighted average chain length ($\overline{bp_w}$) development with time by varying the ATP concentration from 0.06 to 0.2 mM. Lines are guides to the eye. Conditions: 37°C, 0.01 mM M1, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/µL T4 DNA ligase, 1.0 U/µL BsaI, and varying amounts of ATP, under orbital shaking at 80 rpm.

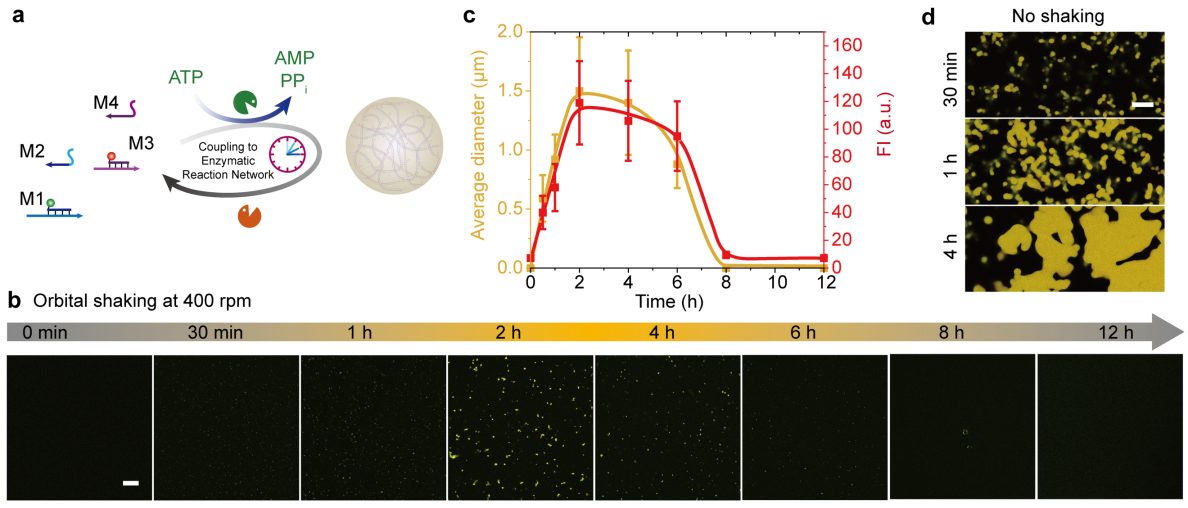

Figure S4. Mechanical responsiveness of the transient DNA coacervates. (**a**) Schematic illustration of ATP-driven transient DNA coacervates. (**b**) Time-dependent CLSM measurements of the coacervates under orbital shaking at 400 rpm. Scale bar = 20 µm. (**c**) Time-dependent average diameter and fluorescence intensity ($ATTO_{647}$) for the coacervates. Error bars are standard deviations from three random fields of view of duplicate experiments. Lines are guides to the eye. (**d**) CLSM measurements of the coacervates with time for the experiment

without shaking. Scale bar = 20 µm. Conditions: 37°C, 0.01 mM M1, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/µL T4 DNA ligase, 1.0 U/µL BsaI, and 0.06 mM ATP, under orbital shaking at (**b**) 400 and (**d**) 0 rpm.

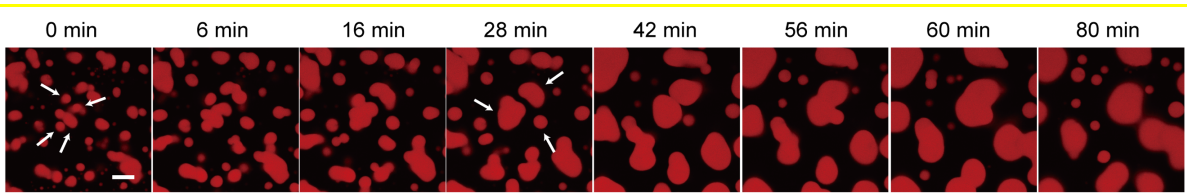

Figure S5. Coacervate fusion. Time-dependent CLSM measurements during fusion of the ATP-fueled DNA coacervates by removing shaking for maturation. Measurements were carried out 2 h after adding ATP. Scale bar = 10 µm. Conditions: 37°C, 0.01 mM M1, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/µL T4 DNA ligase, 1.0 U/µL BsaI, and 0.2 mM ATP, without shaking.

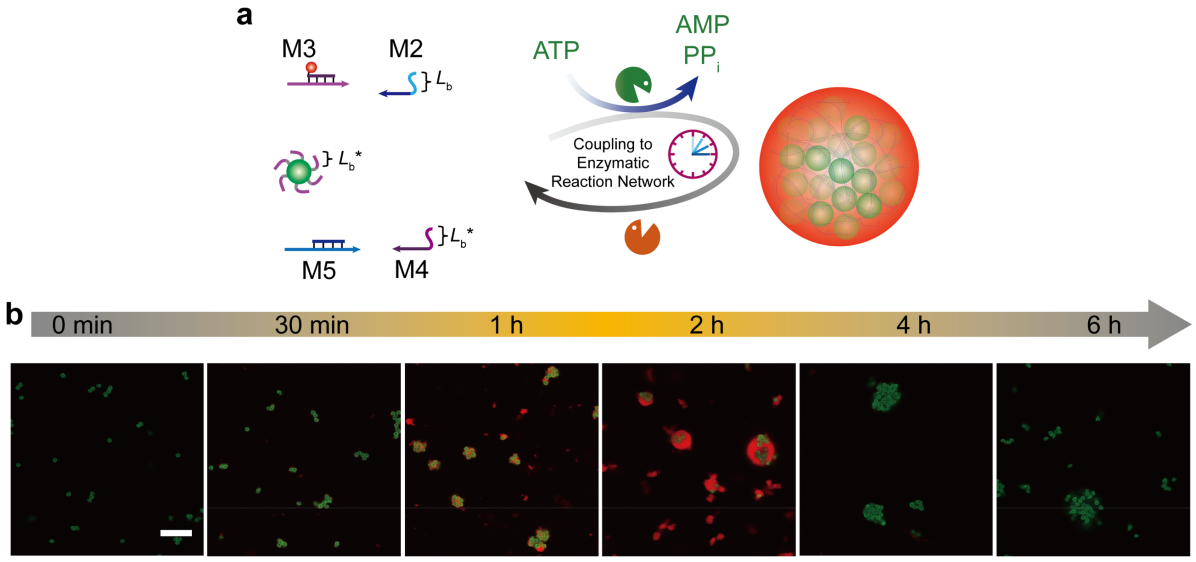

Figure S6. Transient trapping and releasing of colloids using 10 % colloids as in Figure 5c in main manuscript. (**a**) Schematic illustration of ATP-driven transient coacervates for temporal trapping micron-sized colloids. (**b**) Time-dependent CLSM measurements of the transient superstructures. Scale bar = 10 µm. Conditions: 37°C, 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/µL T4 DNA ligase, 1.0 U/µL BsaI, ca. 6.68 µg/mL DoS-modified colloids (ca. 4.68-6.68×10^6 beads/mL) as well as 0.06 mM ATP, under orbital shaking at 80 rpm.

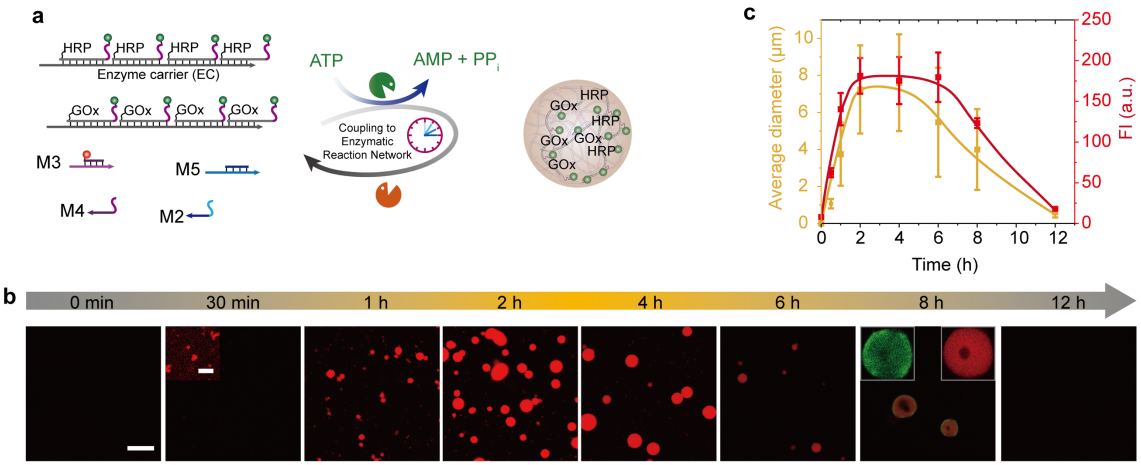

Figure S7. ATP-driven transient trapping and releasing of multienzyme complex. (**a**) Schematic illustration of ATP-driven transient encapsulation of GOx and HRP. (**b**) Time-dependent CLSM measurements of enzymes loaded coacervates. Scale bar = 20 μm; insert = 4 μm. (**c**) Average diameters and fluorescence intensities for the coacervates over time. Error bars are standard deviations from three random fields of view of duplicate experiments. Lines are guides to the eye. Conditions: 37°C, 0.01 mM M5, 0.012 mM M2, 0.01 mM M3, 0.012 mM M4, 0.92 WU/μL T4 DNA ligase, 1.33 U/μL BsaI, 0.5 nM GOx on enzyme carriers, 0.5 nM HRP on enzyme carriers, and 0.06 mM ATP, under orbital shaking at 80 rpm.

Supplementary Note 1. ATP-driven transient trapping of multienzyme complex

For proof-of-concept, we conjugated GOx and HRP to the biotin functionalized DNA polymer, enzyme carrier (EC), for transient biomolecules encapsulation in the transient coacervates. Upon running the GOx and HRP immobilized functional DNA polymers with the transient coacervate system, transient encapsulation of multienzyme complex is achieved (Figure S7b). However, the multienzyme complex shows significant inhibition on BsaI. Thus, compared to the system above without multienzyme complex, the degradation of the coacervates with multienzyme complex is more time-consuming and more restriction enzyme is needed for successful coacervates degradation. In principle, the biomolecules could be any other streptavidin, neutravidin or avidin conjugated biomolecules, and the conjugation interaction between the DNA and the biomolecules can also be changed to some other interactions, such as His-tag or click chemistry. Hence, our strategy provides a universal method to encapsulate biomolecules inside the coacervates for versatile functions.