Supporting Information For

Protocells with hierarchical structures as regulated by liquid-liquid and liquid-solid phase separations

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Materials and Methods

Materials. Poly(L-lysine) hydrobromide, fluorescein isothiocyanate-tagged poly(L-lysine) hydrobromide (PLL / FITC-PLL, M_w = 30-70 kDa), 4-morpholineethanesulfonic acid (MES) were purchased form Sigma-Aldrich (St. Louis, MI, USA). Single-stranded oligonucleotide (oligo) with an arbitrary sequence (5′-CTTACGCTGAGTACTTCGATT-3′) and its complementary strand ($oligo_{comp}$) were obtained from Invitrogen Inc. and delivered as lyophilized samples. The melting temperature (T_m) of the dsDNA formed by oligo/$oligo_{comp}$ is about 47 ^oC. Polyvinylpyrrolidone (PVP, M_w = 30 kDa) was from Sinopharm Chemical Reagent Co. Ltd.

The stock solutions of PLL, oligo, and $oligo_{comp}$ were prepared by dissolving the samples in 20 mM MES buffer (adjusted to pH 6) to 1.0 mg/mL, 1.5 mg/mL, and 1.5 mg/mL, separately, and stored at 4 ^oC before use.

Coating of exogenous oligo$_{comp}$ on PLL/ oligo coacervate droplets. PLL/oligo coacervate droplets (± = 1.0) were prepared in a microfluidic chip by following a known procedure.[1] In brief, aqueous solutions of PLL (1.0 mg/mL) and oligo (1.5 mg/mL) were loaded into opposite wells of the microfluidic channel device (channel dimensions: 80 μm width × 25 μm depth) that was previously washed in sequence with 1.0 M NaCl, 1.0 M NaOH, 1.0 M HCl, deionized water, and 1% (w/w) polyvinylpyrrolidone to prevent adsorption of coacervate. The solutions, driven by gravity, were mixed in the central channel of the chip and produced discrete coacervate droplets. As the droplets grew to 10-15 μm in diameter, the residual oligo and PLL were replaced by MES buffer, followed by adding aqueous oligo$_{comp}$ solution with known concentrations.

Preparation of PLL/oligo/oligo$_{comp}$ complex. To prepare PLL/ oligo/oligo$_{comp}$ complex, the mixtures of oligo (1.5 mg/mL) and oligo$_{comp}$ at different concentration ratios were firstly prepared. The oligo/oligo$_{comp}$ mixture and PLL solution (1.0 mg/ml) were loaded to the opposite wells of the microfluidic channel device. The solutions were mixed in the central channel, forming complex condensates with different morphologies. After about 3 min, the residual solutions in the wells were replaced by MES buffer.

Confocal fluorescence imaging. Confocal images and fluorescent recovery after photobleaching analysis (FRAP) were conducted by using a laser scanning confocal microscope (LSCM, A1R-si, Nikon, Japan). For fluorescence imaging, the solutions of PLL containing 2.5 wt% FITC-PLL, oligo containing 0.15% cy5-oligo, and oligo$_{comp}$ containing 0.15 wt% tetramethylrhodamine(TAMRA)-oligo$_{comp}$ were used to prepare the samples. Fluorophores were excited by using the following excitation (λ_{ex}) and emission wavelength

(λ_{em}): FITC-, λ_{ex} = 488 nm and λ_{em} = 515 nm; TAMRA-, λ_{ex} = 550 nm and λ_{em} = 577 nm; cy5-, λ_{ex} = 628 nm and λ_{em} = 700 nm. All fluorescent images were processed by ImageJ software (https://imagej.nih.gov/ij/index.html).

Electric field-induced morphological change of complex. As the complex were prepared in microfluidic device, a direct current electric field at varying strength was applied along the channel by using a power supply (DYY-16D, Beijing Liuyi Instrument) and the dynamic behaviors were recorded by using LSCM.

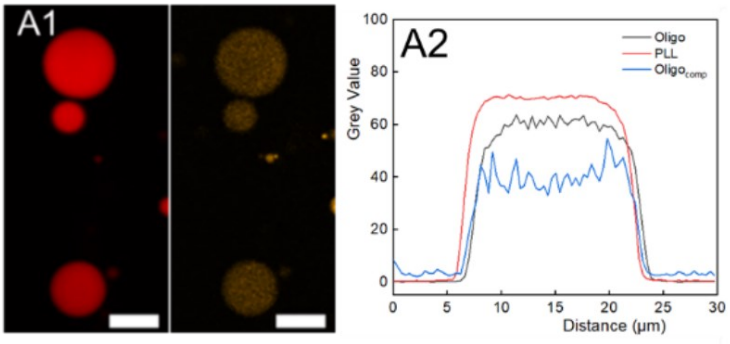

Figure S1. Controlled experiment showing that a trace amount of TAMRA-oligo$_{comp}$ (1.5 × 10^{-3} mg/mL) can permeate into the coacervate droplet. Scale bar, 10 μm.

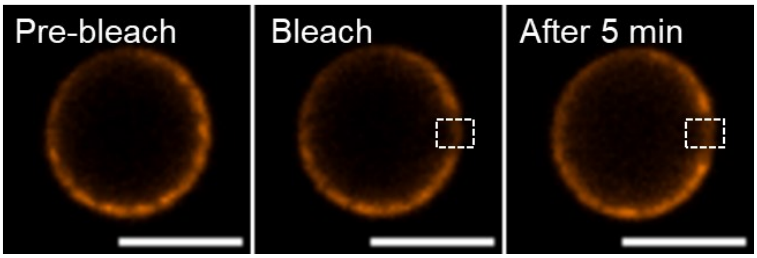

Figure S2. FRAP of TAMRA-oligo$_{comp}$ within complex membrane. Scale bar, 10 μm.

Supplementary Movies Legends

Movie S1. Droplet of PLL/oligo containing 5.0% $oligo_{comp}$ at E = 10 V cm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 3.3 minutes. Scale bar, 10 μm.

Movie S2. Droplet of PLL/oligo containing 5.0% $oligo_{comp}$ at E = 30 V cm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 3.3 minutes in real time. Scale bar, 10 μm.

Movie S3. Droplet of PLL/oligo containing 5.0% $oligo_{comp}$ at E = 50 Vcm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 3.2 minutes in real time, respectively. Scale bar, 10 μm.

Movie S4. Droplet of PLL/oligo containing 12.5% $oligo_{comp}$ at E = 10 Vcm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 3.3 minutes in real time. Scale bar, 10 μm.

Movie S5. Droplet of PLL/oligo containing 12.5% $oligo_{comp}$ at E = 30 Vcm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 3.3 minutes in real time. Scale bar, 10 μm.

Movie S6. Droplet of PLL/oligo containing 12.5% $oligo_{comp}$ at E = 50 Vcm^{-1} as shown in TAMRA channel. Movie is shown at ×30 of real-time speed at 10 frames per second. Total duration of recording was 2.7 minutes in real time. Scale bar, 10 μm.

Movie S7. PLL/oligo droplet with PLL/oligo/$oligo_{comp}$ membrane at E = 10 V cm^{-1} as shown in TAMRA channel. Movie is shown at ×6 of real-time speed at 2 frames per second. Total duration of recording was 1.0 min in real time, Scale bar, 10 μm.

Movie S8. PLL/oligo droplet with PLL/oligo/oligo$_{comp}$ membrane at E = 30 V cm^{-1} as shown in TAMRA channel. Movie is shown at ×15 of real-time speed at 5 frames per second. Total duration of recording was 1.9 min in real time. Scale bar, 10 μm.

Movie S9. PLL/oligo droplet with PLL/oligo/oligo$_{comp}$ membrane at E = 30 V cm^{-1} as shown in bright field. Movie is shown at ×15 of real-time speed at 5 frames per second. Total duration of recording was 1.9 min in real time. Scale bar, 10 μm.

Reference

1 Y. Yin, L. Niu, X. Zhu, M. Zhao, Z. Zhang, S. Mann and D. Liang, *Nat. Commun.*, 2016, **7**, 10658.