# Supporting Information

## Feedback-induced Phase Separation of Hollow Condensates to Create Biomimetic Membraneless Compartments

Jianmian Fu,[a] Bin Wang,[a] Weiping Zhu,[a,c] Yufang Xu,[a] Xin Liang,*[a] Xuhong Qian*[a,b] and Yangyang Yang,*[a]

[a] Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai, 200237, China.
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China.
[c] Shanghai Frontiers Science Center of Optogenetic Techniques for Cell Metabolism, School of Pharmacy, East China University of Science and Technology, 130 Mei Long Road, Shanghai 200237, China

## Table of Contents

## Experimental Procedures

**Materials and reagents**

Short elastin-like polypeptides were purchased from ChinaPeptides Co., Ltd. Oligonucleotides, acryl/bis 30% solution (29:1), TBE premixed powderwere (10×), Tris-HCl solution (2 M, pH=7.8), ethylene diamine tetraacetic acid solution (EDTA, 0.5 M, pH=8.0) and bovine serum albumin (BSA, 98.0%) were purchased from Sangon Biotech (Shanghai) Co., Ltd. Sodium L-ascorbate (99.0%), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.9%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 99.0%), glucose oxidase (GOx, 95.0%) and horseradish peroxidase (HRP, 95.0%) were purchased from J&K China Chemical Co. Ltd. 1,6-hexanediol (98.0%), urea (99.0%), rhodamine 6G (95.0%), rhodamine B (95.0%), fluorescein (98.0%) and carboxyfluorescein (98.0%) were purchased from Shanghai Titan Scientific Co., Ltd. Bovine hemoglobin (Hb, 99.5%) was purchased from Shanghai yuanye Bio-Technology Co., Ltd. Sodium chloride (NaCl, 99.9%), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 99%) and nile red (98.0%) were purchased from Aladdin Industrial Corporation. Sulforhodamine B (95.0%), bodipy 493/503 (95.0%) and pyranine (90.0%) were purchased from Shanghai Canspec Scientific Instruments Co., Ltd. Calcein (95.0%), 5-fluorescein isothiocyanate (FITC, 90.0%) and rhodamine B 5-isothiocyanate (RBITC, 95.0%) were purchased from Dibai Chemical Co., Ltd. Lucifer yellow cadaverine (L. Yellow, 95.0%) was purchased from Bidepharm Co., Ltd. Porcine insulin (98.8%) was purchased from Xuzhou Wanbang Jinqiao Pharmaceutical Co., Ltd. (Jiangsu, China). FITC-Dextran (40 kDa, 95.0%) was purchased from JiQi Biotech Co., Ltd. N,N,N',N'-tetramethylethylenediamine (TEMED, 98.0%), ammonium persulphate (APS, 98.0%) and lysozyme (95.0%) were purchased from Shanghai Macklin Biochemical Co., Ltd. Porcine liver esterase (PLE, 15 U/mg) and FITC-IgG were purchased from Thermo Fisher Scientific Inc. Gelred (10000×) were purchased from Beyotime Biotechnology. All aqueous solutions were prepared with ultrapure water (18.25 MΩ·cm) supplied by Milli-Q Advantage A10 (Merck Millipore). All chemicals were used as received without further purification.

**Instrumental and characterization**

The concentration of oligonucleotides and sELP-ON conjugates were measured using a Thermo Fisher Nanodrop 2000. Denatured polyacrylamide gel electrophoresis (PAGE) was carried out in 1×TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH=8.2-8.4) at 120 V (constant voltage) for 1 h using a Bio-Rad Mini-PROTEAN® Tetra Vertical Electrophoresis Cell and visualized using DLAB GelSMART gel imaging system. UV-vis absorption and absorbance measurements were carried out with a Shimadzu UV-visible 2600 spectrometer. Dynamic light scattering (DLS) and zeta potential measurements were performed using a Malvern Zetasizer Pro instrument using a He-Ne laser (the wavelength λ = 633 nm, 4 mW) as the light source with scattering angle of 175°. The real-time fluorescence and absorbance of enzymatic reactions were measured in a BioTek Synergy NEO microplate reader. Confocal fluorescence microscope measurements were performed using a Nikon A1R inverted laser-scanning confocal microscope equipped with a 60× water objective. The microscope slide glasses (26 mm × 76 mm) and cover glasses (18 mm × 18 mm × 0.17 mm) were purchased from Beyotime Biotechnology.

**Synthesis of sELP-ON conjugates**

sELP-ON conjugates were synthesized as previously described.[1, 2] $CuSO_4$ solution (1 mM, 5 μL) and sodium L-ascorbate solution (2 mM, 5 μL) were added into a 1.5 mL Eppendorf tube and incubated for 3 min. And then, 50% (v/v) DMSO aqueous solution (200 μL), alkynyl-sELP solution (10 mg/mL, 50 μL, dissolved in DMSO), and azido-oligonucleotides solution (100 μM, 50 μL) were added into the tube. The mixed solution was stirred at 37 °C for 6 hours under nitrogen. After reaction, sELP-ON conjugates were purified using 10% denaturing polyacrylamide gel electrophoresis (PAGE) and concentrated using Amicon filtering tubes 3 kDa (Amicon bioseparations-Millipore U.S.A.). sELP-ON conjugates were stored in Tris-HCl solution (pH=7.8, 20 mM) at -40 °C.

**Preparation of sELP-ON condensates**

The sELP-ON conjugate was concentrated to a final concentration of 30 μM using ultrafiltration, which underwent liquid-liquid phase separation (LLPS) to the formation of sELP-ON microdroplets. To facilitate the subsequent hollow condensate formation, a gradual addition of NaOH solution (0.1 M) was employed to elevate the pH value of the solution and induce the phase transition from microdroplets to hollow condensates.

**Preparation of the observation chamber**

The observation chambers were prepared as previously described.[3] The microscope glasses were soaked in 5% (w/v) bovine serum albumin (BSA) solution for over 4 hours to prevent surface-wetting of the sELP-ON coacervates on the glass surface before being used. After the BSA coating, the glasses were washed with distilled water and dried under an airflow. The BSA-coated slide glasses and cover slips were assembled using double-sided tape. The sample solutions were immersed into the slit between the slide glass and the cover slips, and the edges were sealed with Devcon 5 Minute Epoxy to avoid evaporation of the sample solution during the observation period.

**Bright-field and confocal microscope imaging**

Samples in the observation chamber were visualized using a confocal laser scanning microscope equipped with a 60× water objective and a stage heater at 37 °C. Blue channel, excitation: 405 nm, collection: 425-475 nm; green channel, excitation: 488 nm, collection: 500-550 nm; red channel, excitation: 561 nm, collection: 570-620 nm. Three-dimensional reconstructions from Z scans were performed using NIS-Viewer software.

**Fluorescence recovery after photobleaching (FRAP)**

For FRAP measurements, the sELP-ON coacervate droplets were stained by Gelred probe. Images at attenuated laser intensity (10% intensity) were taken before photobleaching. The photobleached area was chosen in the center of a single droplet of at least 4 μm in diameter. A 2-frame pre-bleach sequence was used, followed by a 2-frame bleach at 100% 561 nm. After the area's fluorescence emission intensity was significantly decreased, the laser was then switched back to the attenuated intensity and took a frame every 10 s for at least 180 s.

**Surface-wetting**

For Surface-wetting measurements, the microscope glasses were not treated with BSA. Surface-wetting imaging was performed immediately after adding the sample solution into the observation chamber. The suspended droplets were selected for surface-wetting imaging and took a frame every 10 s for 300 s.

**Droplet fusing**

For droplet fusing measurements, the touching sELP-ON coacervate droplets were chosen for fusing imaging and took a frame every 10 s for at least 300 s.

**Dynamic light scattering (DLS) and zeta potential measurements**

sELP-ON coacervate samples (20 μM) were injected into a disposable zeta cuvette to measure the hydration dynamic diameters and zeta potentials at 37 °C. Samples were pre-incubated at 37 °C for at least 120 s before measurements and the reported data represent an average of at least three measurements.

**Transition temperature ($T_t$) measurement**

The sELP-ON conjugate samples were heated until phase separation before measurement. Immediately, the absorbance at 350 nm of sELP-ON conjugate samples was monitored using a UV-visible spectrometer equipped with a temperature sensor during cooling. The absorbance at each temperature was recorded. Origin software was used to analyze data and draw graphs. For NaCl concentration and pH effect test, sELP-ON conjugates were dissolved in buffer solution (20 mM Tris-HCl, pH=7.8), and the NaCl stock solution (5 M) was used to adjust NaCl concentration, HCl (1 M) and NaOH (1 M) were used to adjust pH.

**Fluorescent labeling of proteins**

Proteins were labeled as previously described.[4] FITC or RBITC (10 mM, 20 μL, dissolved in DMSO) was added into proteins solution (30 μM, 1000 μL, dissolved in 0.1 M $NaHCO_3$-$Na_2CO_3$, pH=9.5), the mixed solution was stirred at 37 °C for 4 hours and incubated at 4 °C for 8 hours under dark. And then, Amicon filtering tubes were used to remove excess FITC or RBITC.

**Encapsulation of molecule dyes and biomacromolecules**

Before imaging, sELP-ON coacervate droplets were incubated with molecule dyes or biomacromolecules for 2 hours at 37 °C. The concentrations of all molecule dyes were 2 μM. The concentrations of all proteins were 0.2 μM. The concentration of FITC-Dextran was 33 mg/L. The concentrations of nucleic acids were 0.5 μM. For two-component uptake experiments, the concentrations of molecule dyes were 1 μM and the concentrations of proteins were 0.1 μM.

**sELP-ON coacervate droplets for GOx-HRP cascade**

sELP-ON coacervate droplets were pre-incubated with 2.5 μM GOx/HRP for 2 hours at 37 °C. And then, 20 mM L-Arg and 40 mM glucose were added to coacervate samples. For confocal imaging, the fluorescence images were taken every 1 minute for a total of 30 minutes.

## Supplementary Figures S1-S14 and Tables S1-S2

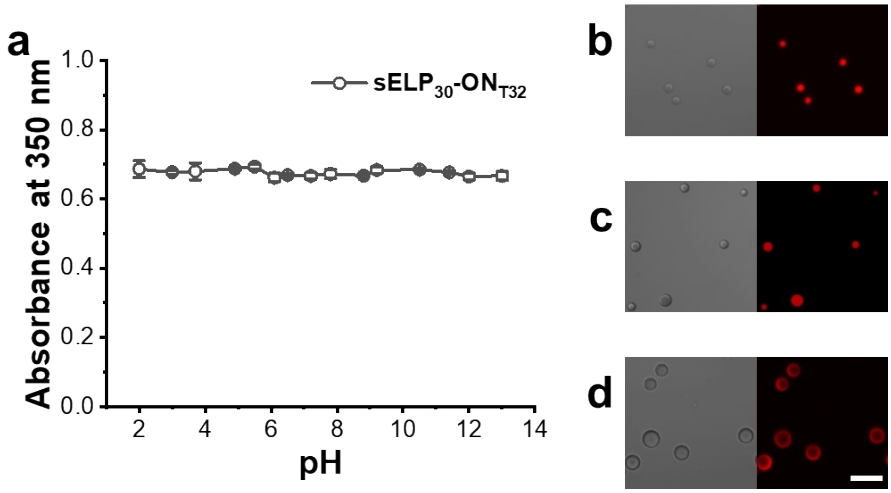


**Figure S1.** (a) Absorbance profile at 350 nm of the $sELP_{30}$-$ON_{T32}$ by varying pH from 2 to 13 under 25 °C. DIC and fluorescence micrographs of Gelred-labeled $sELP_{30}$-$ON_{T32}$ condensate structures under 25 °C at (b) pH 2, (c) pH 7.8, and (d) pH 13. Scale bar: 10 µm.

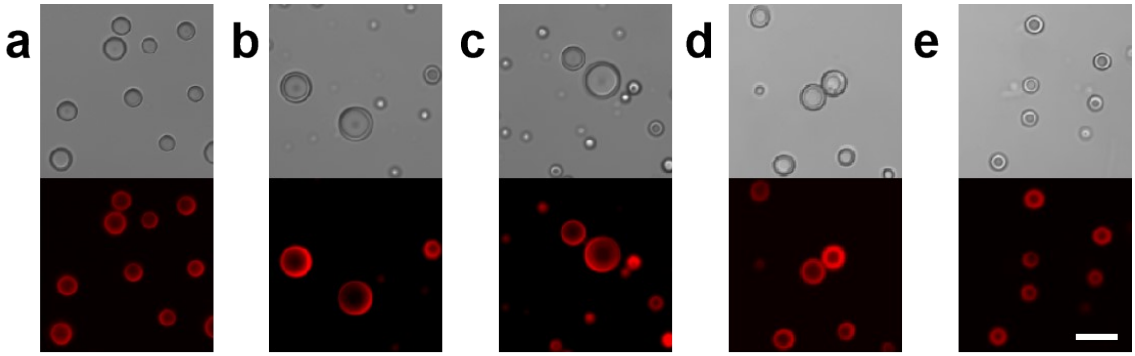


**Figure S2.** DIC and fluorescence images of Gelred-labelled sELP-ON hollow condensate under 25 °C at pH 13. (a) $sELP_{30}$-$ON_{A32}$, (b) $sELP_{30}$-$ON_{C32}$, (c) $sELP_{30}$-$ON_{32}$, (d) $sELP_{30}$-$ON_{T22}$ and (e) $sELP_{25}$-$ON_{T22}$. Scale bar: 10 µm.

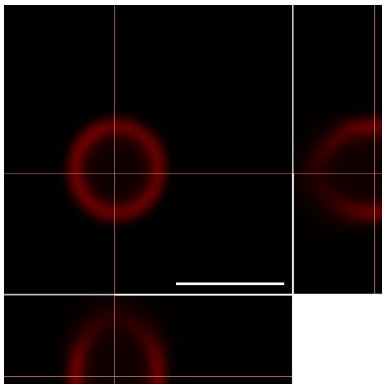

**Figure S3.** Reconstructed 3D fluoresence images of a Gelred-labelled $sELP_{30}$-$ON_{T22}$ hollow condensate. Scale bar: 10 µm.

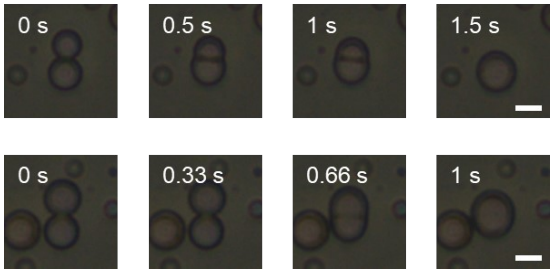


**Figure S4.** Time-lapsed bright-field images of fusion measurement of two adhering $sELP_{30}$-$ON_{T22}$ vesicle-like hollow condensates. Scale bar: 5 µm.

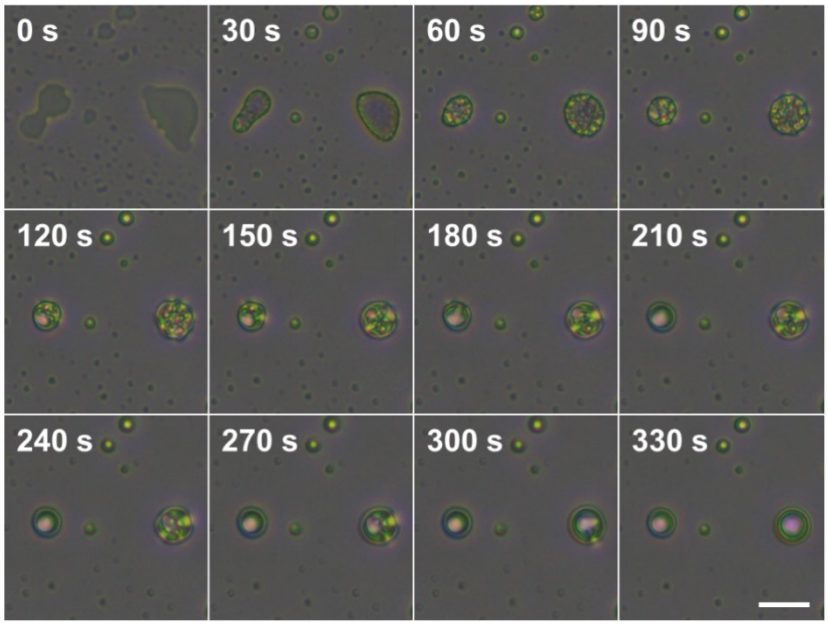


**Figure S5.** Time-lapsed bright-field images of wetted $sELP_{30}$-$ON_{T22}$ microdroplets transforming into unwetted hollow condensates after adjusting the pH to 13 using NaOH. Scale bar: 10 µm.

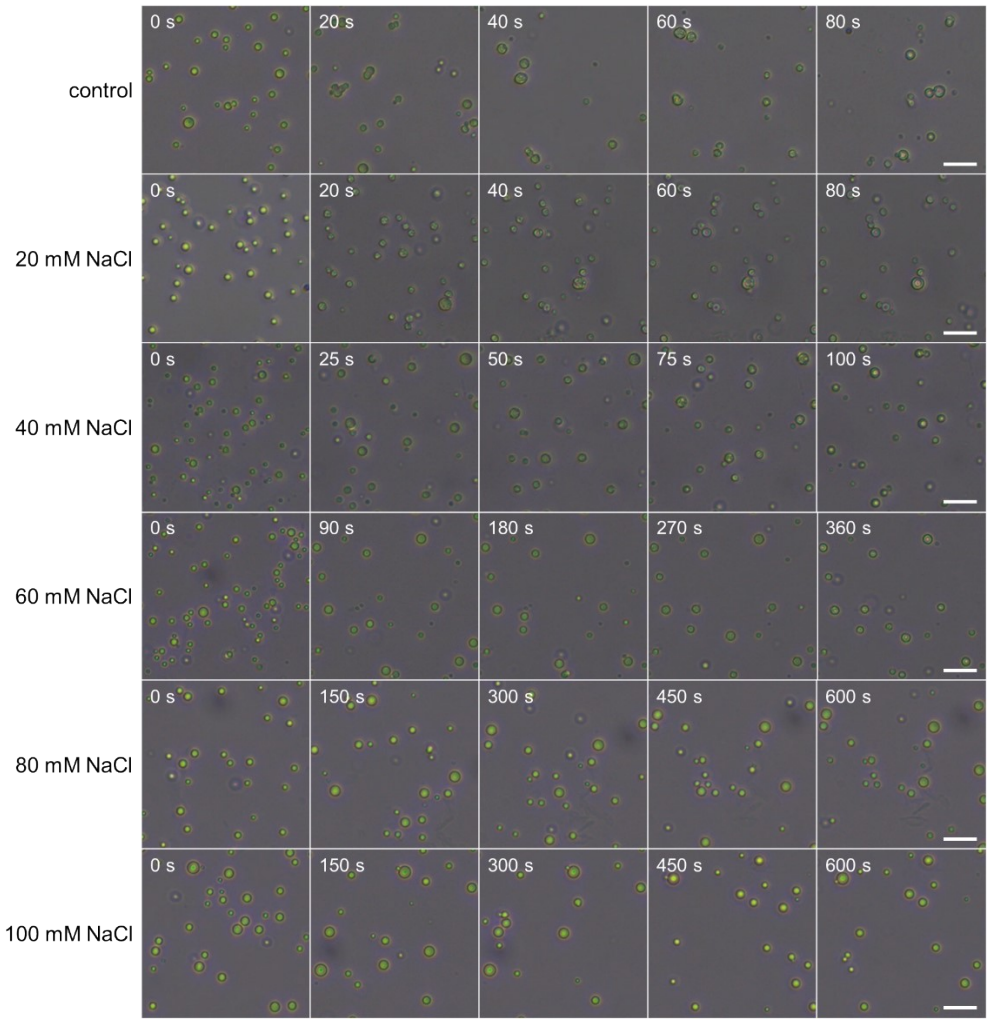


**Figure S6.** Time-lapsed bright-field images of $sELP_{30}$-$ON_{T22}$ condensates after the addition of NaOH in the presence of 0-100 mM NaCl. Scale bar: 10 µm.

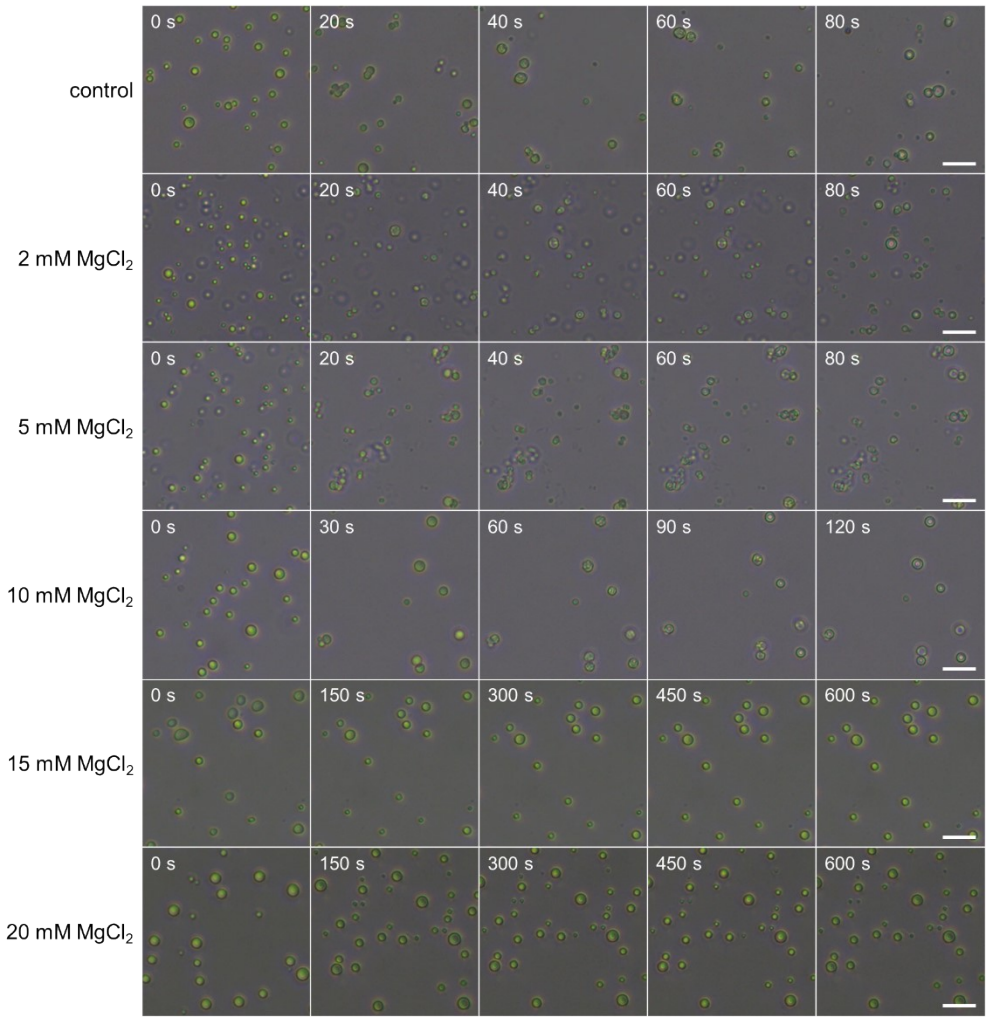


**Figure S7.** Time-lapsed bright-field images of $sELP_{30}$-$ON_{T22}$ condensates after the addition of NaOH in the presence of 0-20 mM $MgCl_2$. Scale bar: 10 μm.

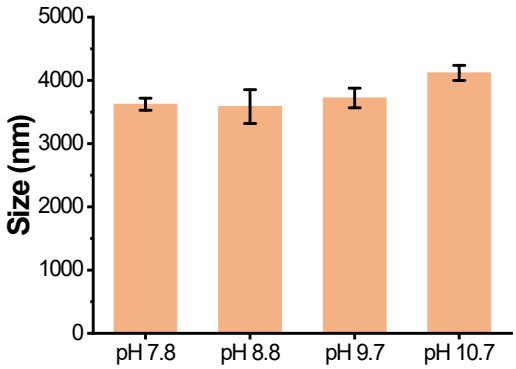


**Figure S8.** Hydrodynamic diameters of $sELP_{30}$-$ON_{T22}$ condensates in the presence of 20 mM NaCl under 37 °C at pH 7.8, 8.8, 9.7, and 10.7 respectively.

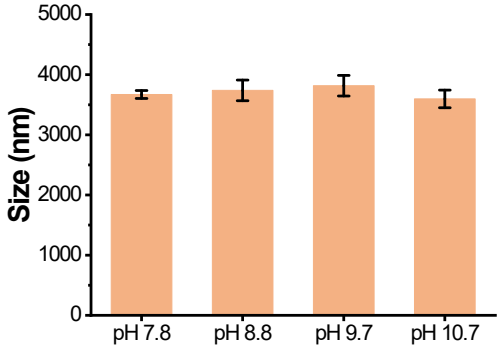


**Figure S9.** Hydrodynamic diameters of $sELP_{30}$-$ON_{T22}$ condensates in the presence of 20 mM $MgCl_2$ under 37 °C at pH 7.8, 8.8, 9.7, and 10.7 respectively.

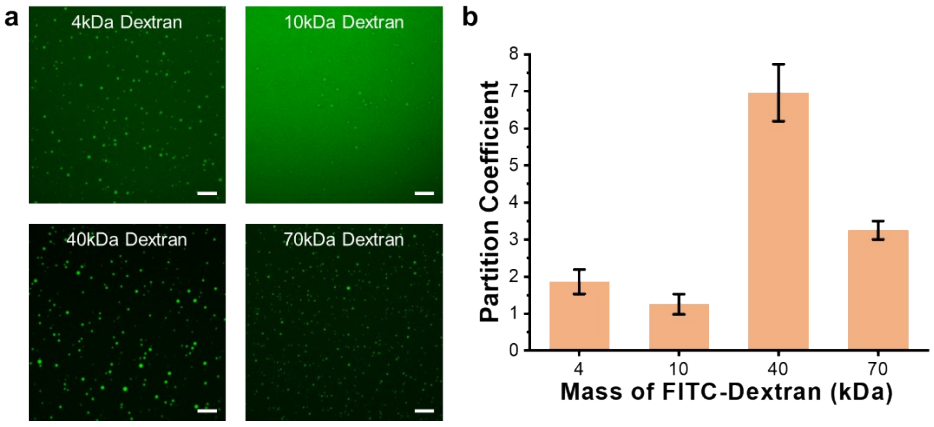


**Figure S10.** (a) CLSM images of $sELP_{30}$-$ON_{T22}$ microdroplets incubated with 4 kDa, 10 kDa, 40 kDa, and 70 kDa FITC-dextran. Scale bar: 20 μm. (b) Partition coefficient of 4 kDa, 10 kDa, 40 kDa, and 70 kDa FITC-dextran in $sELP_{30}$-$ON_{T22}$ microdroplets. At least 300 microdroplets were calculated by imageJ software.

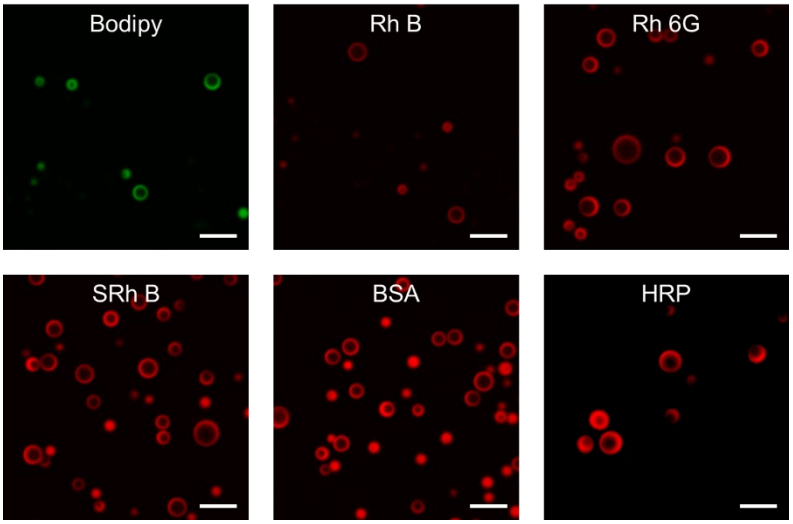


**Figure S11.** CLSM images of $sELP_{30}$-$ONT_{22}$ hollow condensates transformed by $sELP_{30}$-$ONT_{22}$ microdroplets preloaded with Bodipy, RhB, Rh 6G, SRh B, BSA, and HRP after the addition of NaOH. Scale bar: 10 μm.

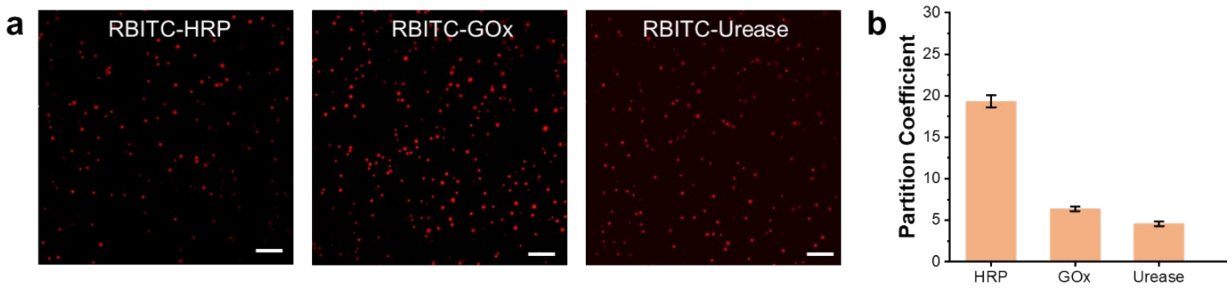


**Figure S12**. (a) CLSM images of $sELP_{30}$-$ON_{T22}$ microdroplets incubated with RBITC-HRP, RBITC-GOx, and RBITC-Urease, respectively. Scale bar: 20 μm. (b) Partition coefficient of RBITC-HRP, RBITC-GOx, and RBITC-Urease in $sELP_{30}$-$ON_{T22}$ microdroplets. At least 300 microdroplets were calculated by imageJ software.

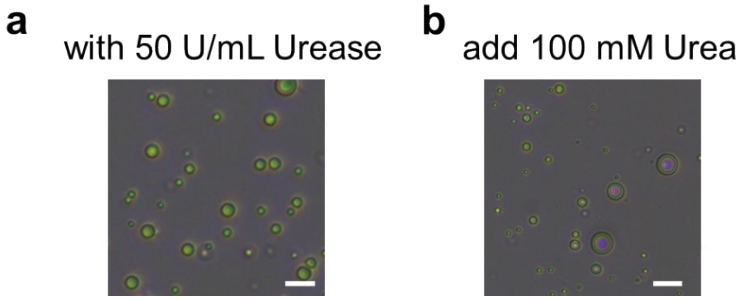


**Figure S13.** Bright-field images of $sELP_{30}$-$ONT_{22}$ condensates after (a) the addition of 50 U/mL urease and (b) the following addition of 100 mM urea. Scale bar: 10 μm.

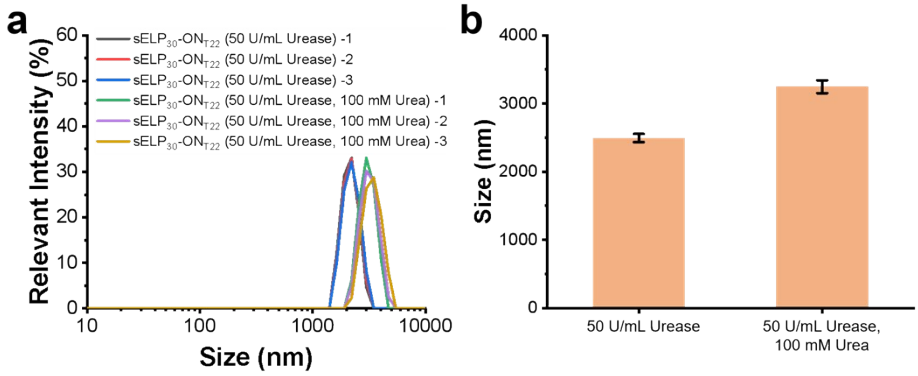


**Figure S14**. (a) Three repeated results of dynamic laser scattering analysis of $sELP_{30}$-$ON_{T22}$ microdroplets containing 100 U/mL urease before and after the addition of 100 mM urea. (b) The corresponding hydrodynamic diameters of $sELP_{30}$-$ON_{T22}$ microdroplets containing 50 U/mL urease before and after addition of 100 mM urea. The error bars indicated three repeated experiments.

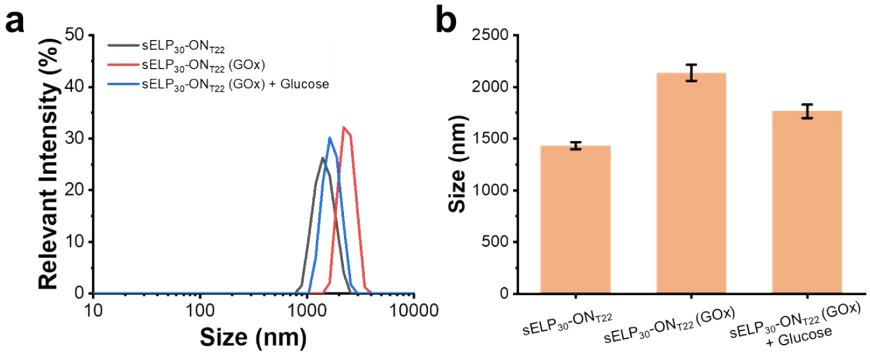


**Figure S15**. (a) Dynamic laser scattering analysis of $sELP_{30}$-$ON_{T22}$ microdroplets containing 2.5 μM GOx before and after the addition of 40 mM glucose. (b) The corresponding hydrodynamic diameters of $sELP_{30}$-$ON_{T22}$ microdroplets containing 2.5 μM GOx before and after addition of 40 mM glucose. The error bars indicated three repeated experiments.

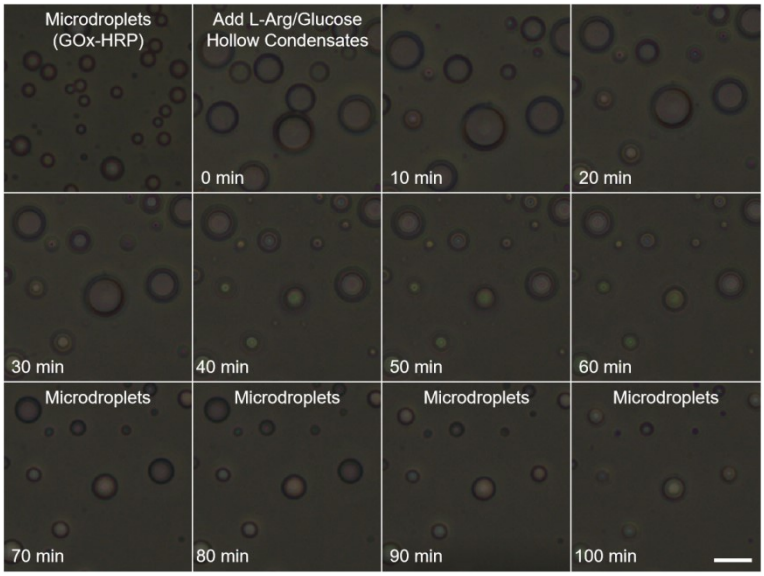


**Figure S16.** Time-lapsed bright-field images of $sELP_{30}$-$ON_{T22}$ microdroplets preloaded with 2.5 μM GOx-HRP rapidly transforming into hollow condensates and gradually returning to microdroplets after the addition of 20 mM L-Arg and 40 mM glucose. Scale bar: 10 μm.

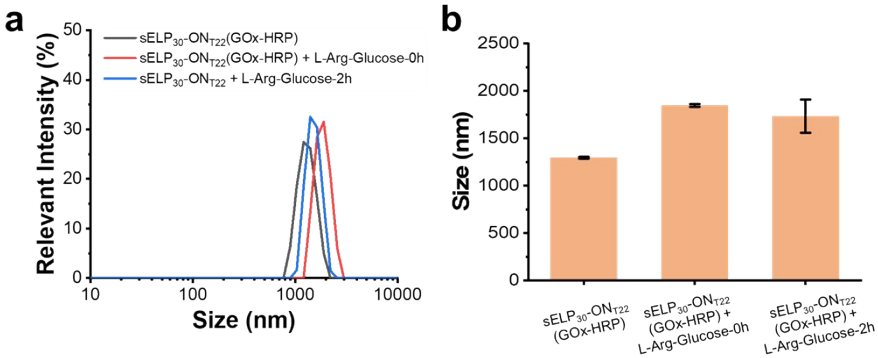


**Figure S17**. (a) Dynamic laser scattering analysis of $sELP_{30}$-$ON_{T22}$ microdroplets containing 2.5 μM GOx/HRP before and after the addition of 20 mM L-Arg and 40 mM glucose. (b) The corresponding hydrodynamic diameters of $sELP_{30}$-$ON_{T22}$ microdroplets containing 2.5 μM GOx/HRP before and after addition of 20 mM L-Arg and 40 mM glucose. The error bars indicated three repeated experiments.

**Table S1.** Elastin-like polypeptides sequences used in this study.

| sELP | Sequence (N'-C') |
|---|---|
| $sELP_{30}$ | VPGFGVPGFGVPGFGVPGFGVPGFGVPGFG-(L-Pra[a])-$NH_2$ |
| $sELP_{25}$ | VPGFGVPGFGVPGFGVPGFGVPGFG-(L-Pra)-$NH_2$ |

[a] L-Propargylglycine.

**Table S2.** Oligonucleotides polypeptides sequences used in this study.

| Oligonucleotide | Sequence (5'-3') | Base number |
|---|---|---|
| Azido-$ON_{T32}$ | Azido-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | 32 |
| Azido-$ON_{A32}$ | Azido-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | 32 |
| Azido-$ON_{C32}$ | Azido-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC | 32 |
| Azido-$ON_{32}$ | Azido-TCCCCTATAGTGAGTCGTATTAATTTCGCGGG | 32 |
| Azido-$ON_{T22}$ | Azido-TTTTTTTTTTTTTTTTTTTTTT | 22 |

## References


1 B. Wang, R. Pan, W. Zhu, Y. Xu, Y. Tian, M. Endo, H. Sugiyama, Y. Yang and X. Qian, *Soft Matter* 2021, **17**, 1184-1188.
2 S. Yao, Y. Liao, R. Pan, W. Zhu, Y. Xu, Y. Yang and X. Qian, *Chinese Chem. Lett.* 2021. DOI: 10.1016/j.cclet.2021.08.116.
3 Y. Sato, T. Sakamoto and M. Takinoue, *Sci. Adv.* 2020, **6**, eaba3471.
4 W. Mu, Z. Ji, M. Zhou, J. Wu, Y. Lin and Y. Qiao, *Sci. Adv.* 2021, **7**, eabf9000.