Supporting information

for

Bioinspired programmable coacervate droplets and self-assembled fibers through pH regulation of monomers

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1. General Methods and Materials

Materials: All chemicals were purchased from commercial sources and were used as such without any further purification. Spectroscopic grade solvents were used for all spectroscopic measurements.

Optical Measurements: Electronic absorption spectra were recorded on JASCO V-750 UV-Visible Spectrophotometer equipped with a peltier. Fluorescence measurements were carried out on a JASCO FP-8500 spectrofluorometer equipped with a peltier. UV-Vis and emission spectra were recorded in 10 mm path-length cuvettes.

Confocal Laser Scanning Microscopy (CLSM): Confocal microscopy imaging was done at room temperature using Leica TCS SP8 laser scanning confocal microscope with a laser excitation of λ_{exc} = 405, 488 nm. Imaging was performed using Leica oil-immersion objectives: HC PL APO CS2 63x/100x with numerical aperture (NA) 1.40. Fluorescence light was specially filtered with emission filters (TD 488/561/633) and imaged with HvD detector. Confocal images were processed using LAS X (Leica) and ImageJ software.

Dynamic Light Scattering (DLS): DLS measurements were carried out using a Zetasizer ULTRA Malvern employing a 633 nm laser at a back scattering angle of 173°.

2. Synthetic Schemes and Procedures:

NDBA molecule was synthesized according to the previously reported procedure. [s1]

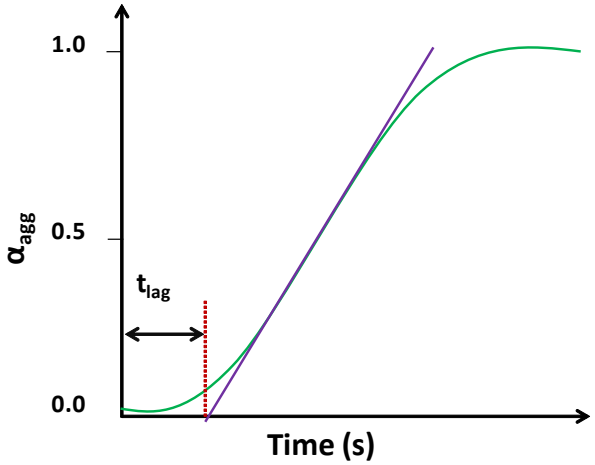
3. Experimental Procedure

Protocol I: Sample preparation protocol for self-assembly study: A stock solution of **NDBA** ($c = 5\times10^{-3}$ M) was prepared in DMSO. An appropriate volume was injected into a mixture of DMSO and aqueous buffer to adjust the final concentration and solvent composition to the required percentages (2 % DMSO in water (v/v) for self-assembled solutions).

Protocol II: Preparation of sample for transient LLPS: 5×10^{-5} M **NDBA** solution at pH 9 was kept for 60 mins to get grown coacervate droplets. On to it 15 mM citric acid, 50 mM Urea, and 25 U mL^{-1} urease were added all together.

Analysis of confocal microscopy images: Length analysis of the confocal microscopy images was done using ImageJ software package developed by US. National Institute of Health. [s2] A frequency statistics was done on the obtained diameter.

Lag phase calculation:



From the above-mentioned representative temporal change in the extent of aggregation (αagg), the data tag (lag time, after which elongation starts) was calculated.

4. Supporting Figures

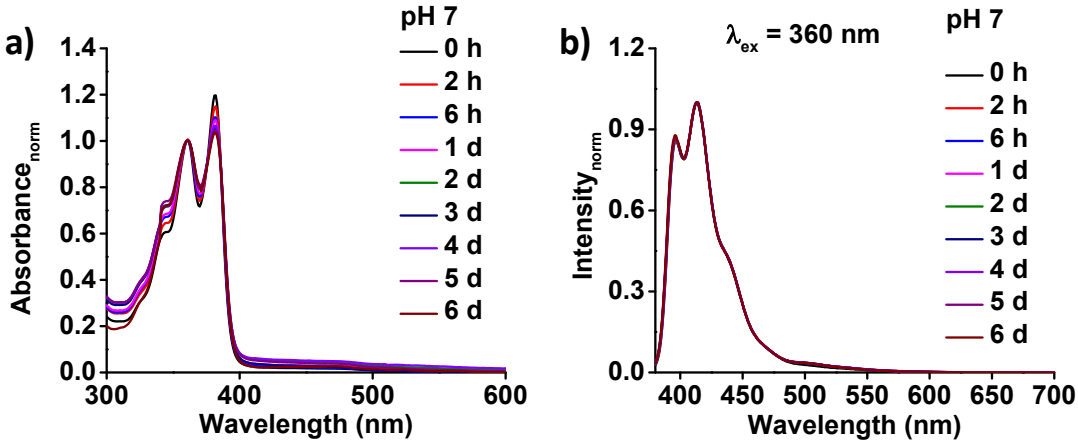


Figure S1. Time-dependent normalized a) absorption, and b) emission spectra (λ_{ex} = 360 nm) of **NDBA** monomers at pH 7. [**NDBA**] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v/v*).

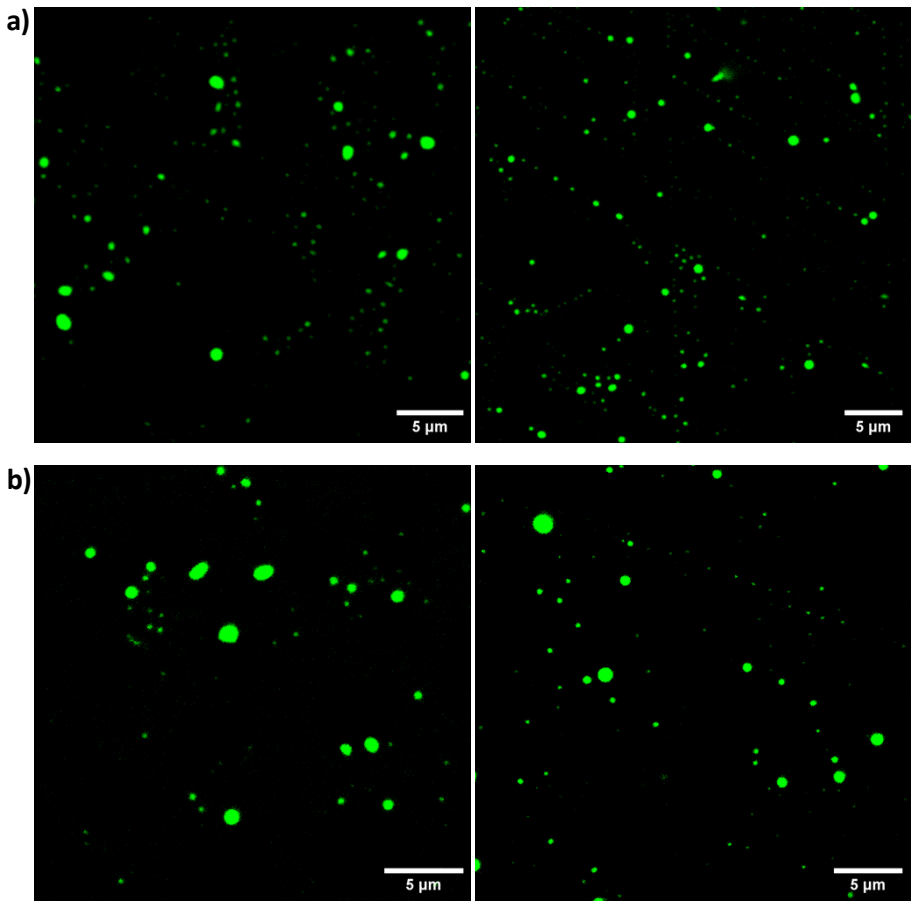


Figure S2. CLSM images of pH-triggered coacervate droplets with different urease units. a) 25 UmL^{-1}, and b) 50 UmL^{-1} urease. [**NDBA**] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v/v*), 50 mM Urea.

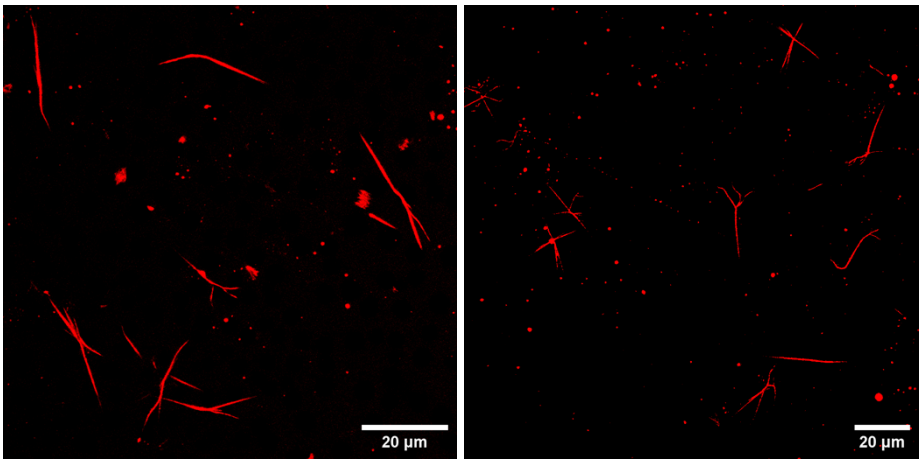


Figure S3. CLSM images after 96 h of the temporally grown thermodynamically stable fiber state with 1 µM Nile red. [**NDBA**] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v/v*).

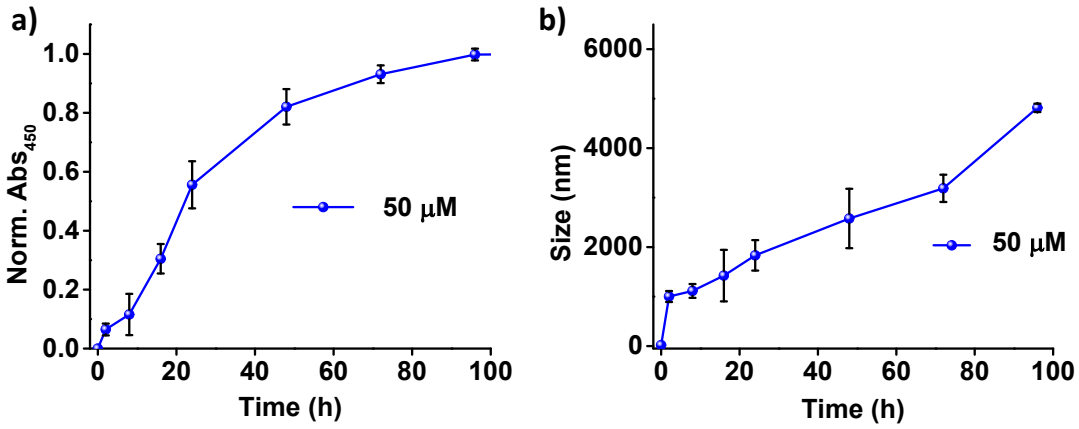


Figure S4. Supramolecular polymerization process was monitored by a) absorption kinetics (at 450 nm) and change in size in b) dynamic light scattering.

Figure S5. Time-dependent a) absorption, and b) emission spectra during the transient dissolution of coacervate droplets. 15 mM citric acid, 50 mM Urea, and 25 U mL^{-1} urease, [**NDBA**] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v*/*v*).

Figure S6. Disassembly study of kinetically grown fiber with 100 mM citric acid. Temporal change in a) pH profile, and b) absorption spectra after the addition of 100 mM citric acid to a kinetically grown fiber solution. [**NDBA**] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v/v*).

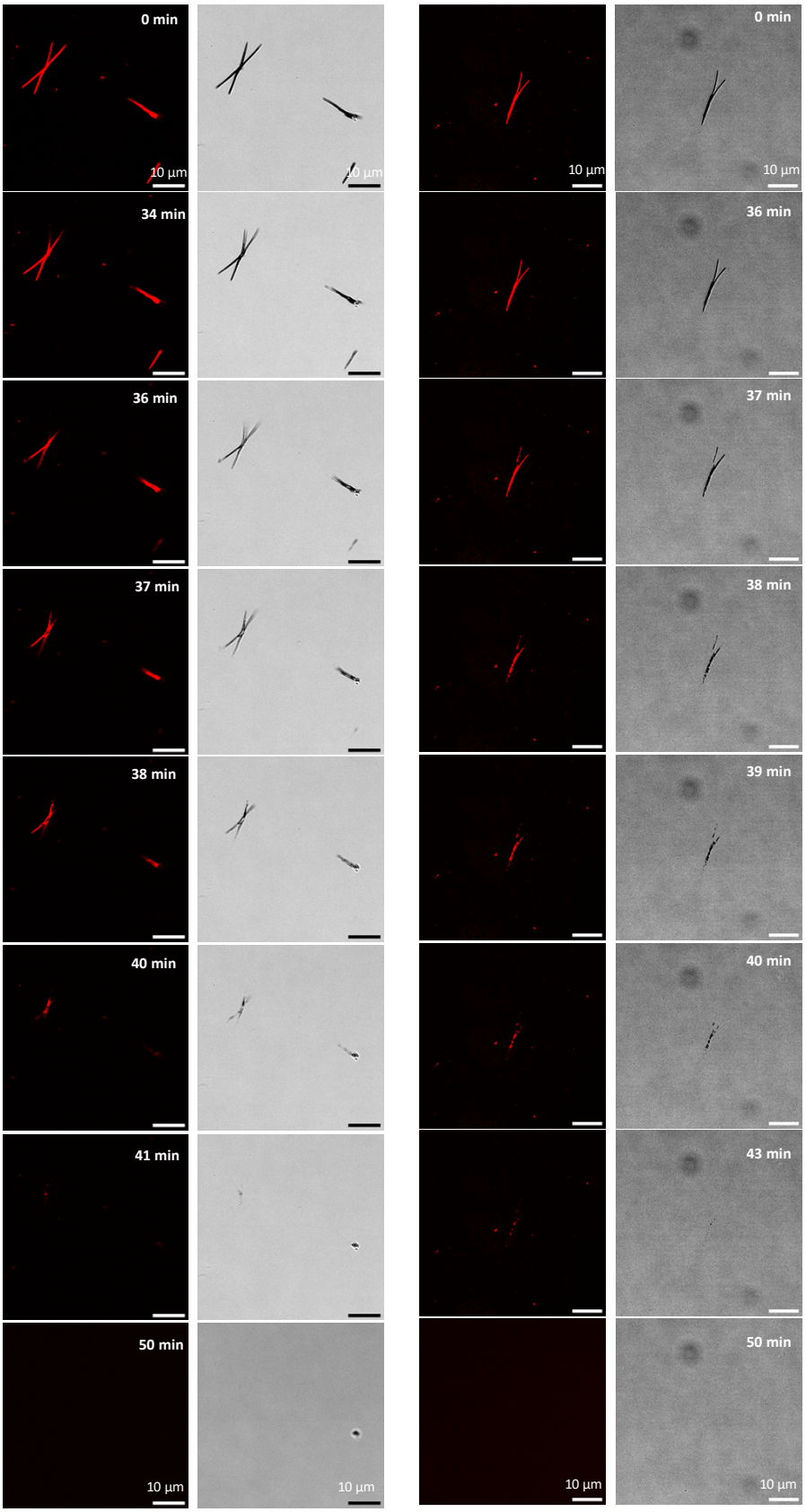


Figure S7. Time dependent CLSM (left) and corresponding bright-field (right) images of *in-situ* visualization of fiber disassembly upon the addition of 100 mM citric acid for two different sets of experiments. Scale bar 10 µm. [NDBA] = 5×10^{-5} M, H_2O/DMSO, 98/2 (*v/v*), 1 µM Nile Red.

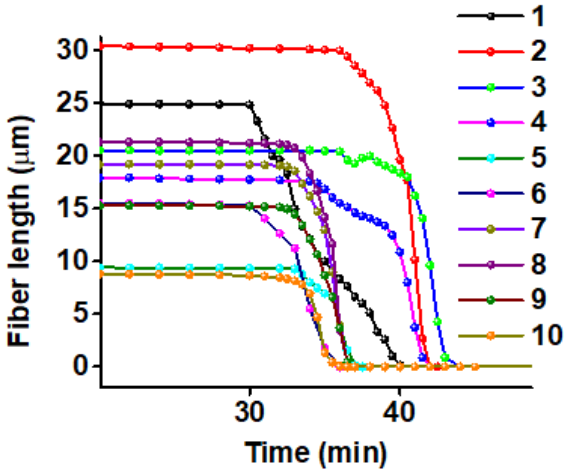


Figure S8. The Disassembly profile was calculated from time-dependent confocal images by tracing 10 individual fibers.

Movie S1 and S2 denotes the disassembly of fibers in Fig 3 main text.

5. References:

1. Patra, S.; Chandrabhas, S.; Dhiman, S.; George, S. J. Controlled Supramolecular Polymerization via Bioinspired, Liquid–Liquid Phase Separation of Monomers. *J. Am. Chem. Soc.* **2024**, *146,* 12577–12586.
2. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671-675. DOI: 10.1038/nmeth.2089.