Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for

Liquid–Liquid Phase Separation Induced by Crowding Condition Affects Amyloid-β Aggregation Mechanism

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Prediction of intrinsically disordered regions (IDRs) of Aβ (1-42)

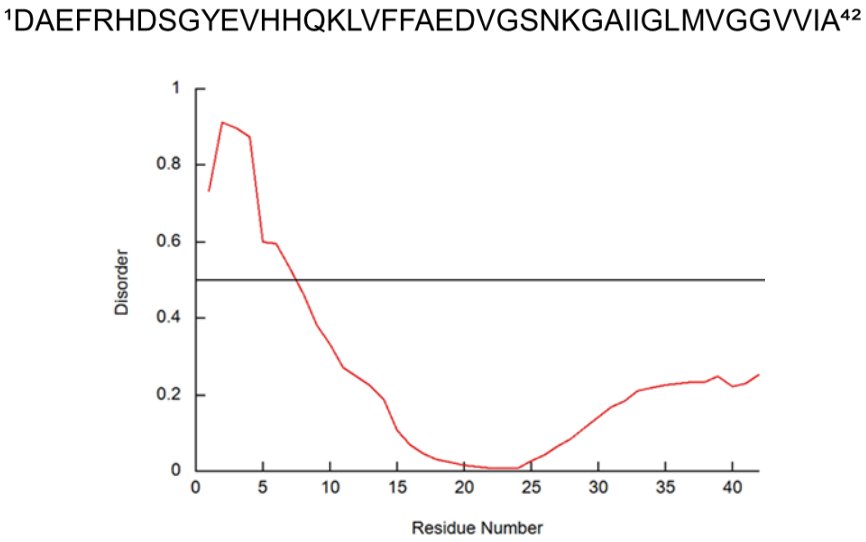

Figure S1. Disordered regions predicted based on the amino acid sequence of Aβ (1-42) are shown using PONDR (http://www.pondr.com/).

Effects of different types of electrolytes on the LLPS dynamics of Aβ

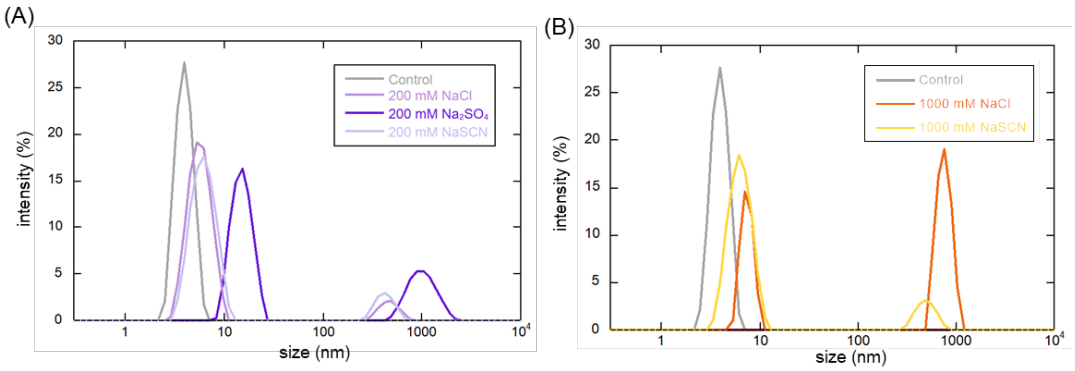

Figure S2. (A) DLS measurements of 200 mM of different types of electrolytes (NaCl, Na_2SO_4, and NaSCN) added to a solution of Aβ (5 μM) with 20% (w/v) PEG. (B) DLS measurements of 1,000 mM of different types of electrolytes (NaCl, Na_2SO_4, and NaSCN) added to a solution of Aβ (5 μM) with 20% (w/v) PEG.

As shown in Figure 2A, the sample at 1,000 mM Na_2SO_4 formed droplets larger than 10 μm, beyond the detectable range of DLS and the measurement could not be performed due to multiple scattering. Thus, the data of the sample at 1,000 mM Na_2SO_4 showed the intensity of 0% at all the measurable range. In the system containing the kosmotrope Na_2SO_4, the scattering intensity was near the microscale when 200 mM was added. In the system containing NaCl, the scattering intensities on the microscale were observed as the amount of NaCl increased. However, no microscale scattering intensity was observed in the NaSCN added system, regardless of the amount of NaSCN added.

Highly concentrated DEX dissolves Aβ fibrils

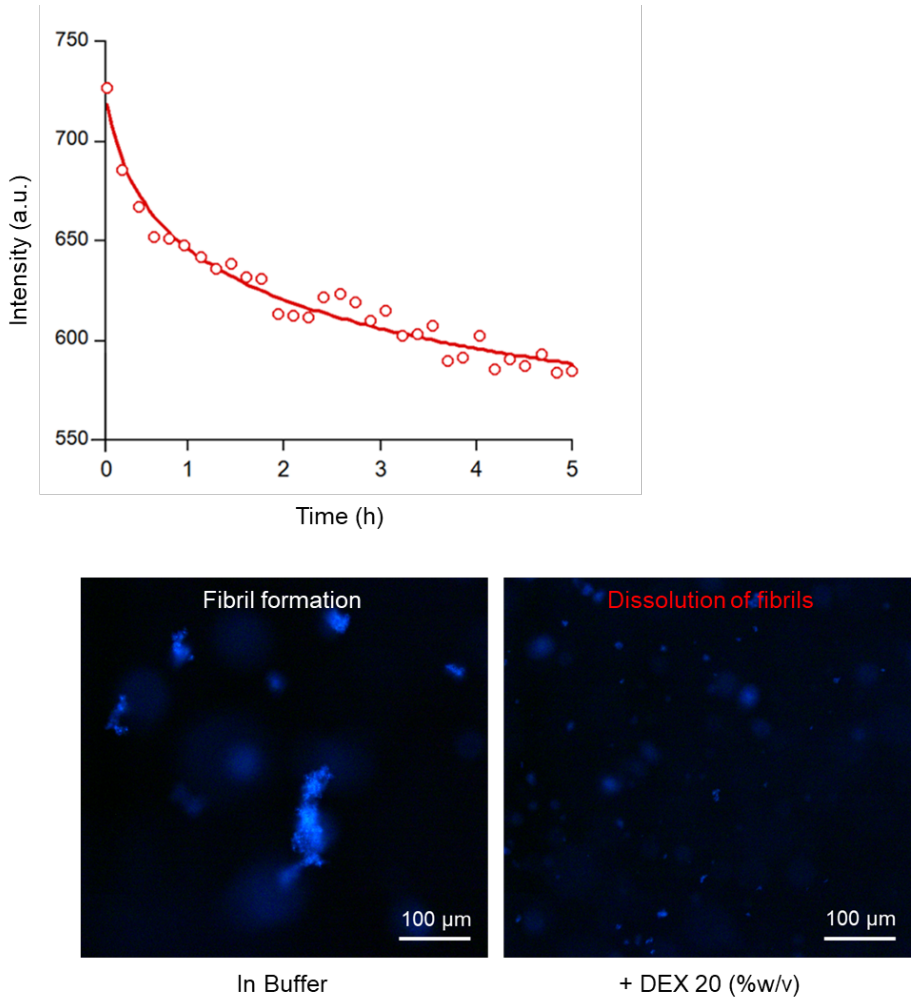

Figure S3. Measurements of ThT fluorescence intensities over time using a spectrofluorometer (Jasco FP-8300) at an excitation wavelength of 440 nm and emission wavelength of 485 nm. The temperature in the equipment was maintained at 37 °C, and the experiments were performed with excitation and emission slits at 5 nm every 10 min for 300 min. The left image shows Aβ fibrils grown in buffer at 37 °C for 24 h. The right image shows Aβ fibrils added to a 20% (w/v) DEX solution and observed in solution after measurement. Aβ fibrils grown by incubation at 37 °C for 24 h in the prepared Aβ mother solution were added to PBS and 20% (w/v) DEX solution stored at 37 °C to a final concentration of 5 μM of Aβ for measurement.

Fusion of Aβ droplets

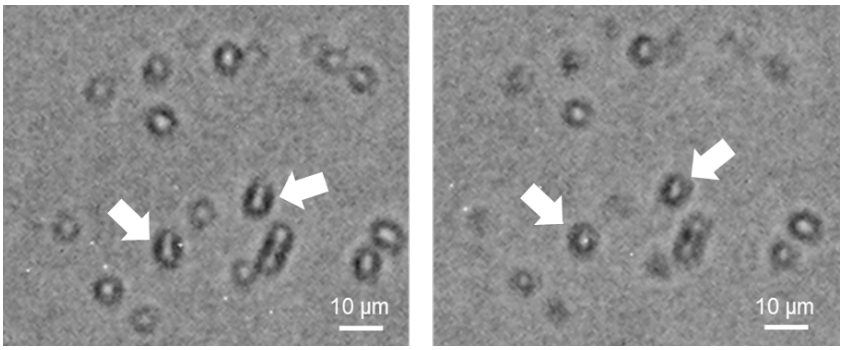

Figure S4. DIC observation image of droplet aggregates observed under DEX 20% (w/v) coexistence condition with 100 mM NaCl. In the early stage of droplet formation, droplets appeared to fuse (indicated by white arrows). Left panel shows some droplets in the prolonged state just after fusion between two droplets. After some time, these prolonged droplets underwent a structural change into more of a spherical state with lower surface area.

ATPS Properties

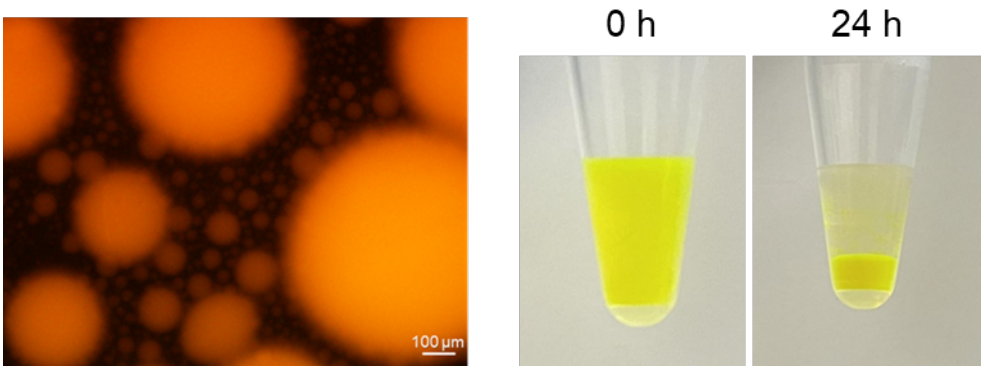

Figure S5. Observed images in the ATPS, where DEX was mixed with 10% fluorescently labeled dextran (FITC-dextran 150, TdB Labs AB, Uppsala, Sweden). Left panel shows LLPS of PEG and DEX. The droplet is DEX-rich and its outside is PEG-rich. The photograph on the right shows that the solution is in a single phase immediately after stirring, but separates into two phases when left to stand. The upper phase of the solution is PEG-rich, and the lower phase is DEX-rich.

Aβ monomer forming droplets and fibril formation

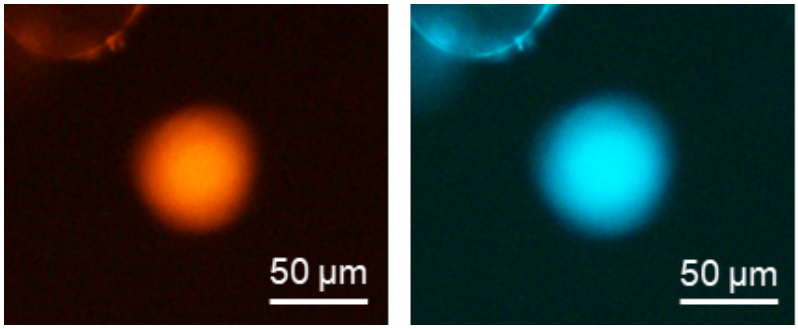

Figure S6. Fluorescence microscopy image of a droplet formed in ATPS. HiLyte Fluor 555 Aβ monomer was mixed at 1% (0.05 μM). In ATPS, a mixture of HiLyte Fluor 555 and ThT was also created. Aβ monomers were involved in droplet formation and formed β-sheet structures during fibril formation, which were fluorescently labeled with ThT.

Diffusion of Aβ fibrils incorporated into a DEX droplet

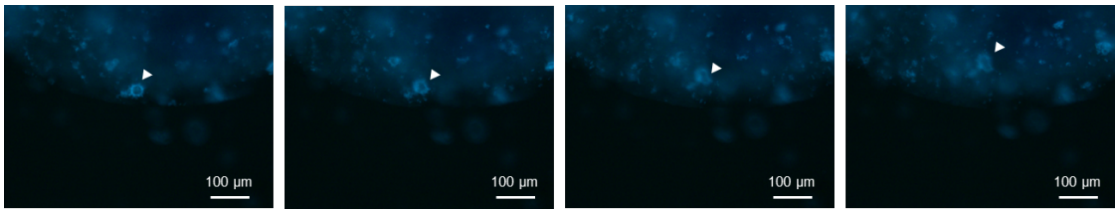

Figure S7. In ATPS, Aβ fibrils incorporated into DEX droplets diffused inside the droplets. This fluorescence microscopic image shows the liquid-like properties of the system.