A Synthetic Biomolecular Condensate from Plant Proteins with Controlled Colloidal Properties

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Supplemental information

S.1. Fluorescent Dye Conjugation of the Protein Condensates for FRAP analysis. Synthetic biomolecular condensates (sBCs) composed of QZ 70, or PZ 70 were fluorescently labeled with fluorescein isothiocyanate (FITC) for FRAP analysis. A predetermined amount of sBCs (QZ70 or PZ70) were first formed and concentrated using centrifugal filters. The concentrated nanoparticle suspension was then resuspended in 500 µL of phosphate-buffered saline (PBS, pH 7.4). In a separate vial, FITC was dissolved in dimethylformamide (DMF) to prepare a stock solution at a concentration of 2 mg/mL. 500 µL pre-prepared sBC solution was added to the dye-containing vial. To this solution, a 2 µL of triethylamine (TEA) was added to facilitate the reaction. The mixture was stirred at room temperature for 3 h in the dark to prevent photobleaching. Following the reaction, the FITC-labeled sBCs were purified by dialysis against deionized water using a dialysis membrane with a molecular weight cutoff (MWCO) of 3.5 kDa for 8 h to remove unreacted FITC and organic solvents. Dialysis was performed under gentle stirring in the dark at room temperature, and the purified sBCs were stored at 4 °C until further use. Fluorescence recovery after photobleaching (FRAP) experiments were conducted to assess the dynamic properties of FITC-tagged QZ self-assembled bio-condensates (sBCs). 10 µl of the FITC-labeled sBC suspension was dispensed into each well of an 8-well glass chamber slide (ibidi), and samples were covered with a clean glass coverslip to minimize evaporation and drift during imaging. The FRAP experiment was performed using a ZEISS LSM 900 confocal microscope equipped with Airyscan 2 under controlled room temperature conditions. The imaging was carried out with a 488 nm laser line for FITC excitation. A defined square region of interest (ROI) was selected for photobleaching, and bleaching was conducted using 100% laser intensity with 10 iterations to ensure complete fluorescence loss in the selected area. Post-bleaching recovery was

monitored over 100 cycles using a time series with a 1-second interval between frames. Fluorescence

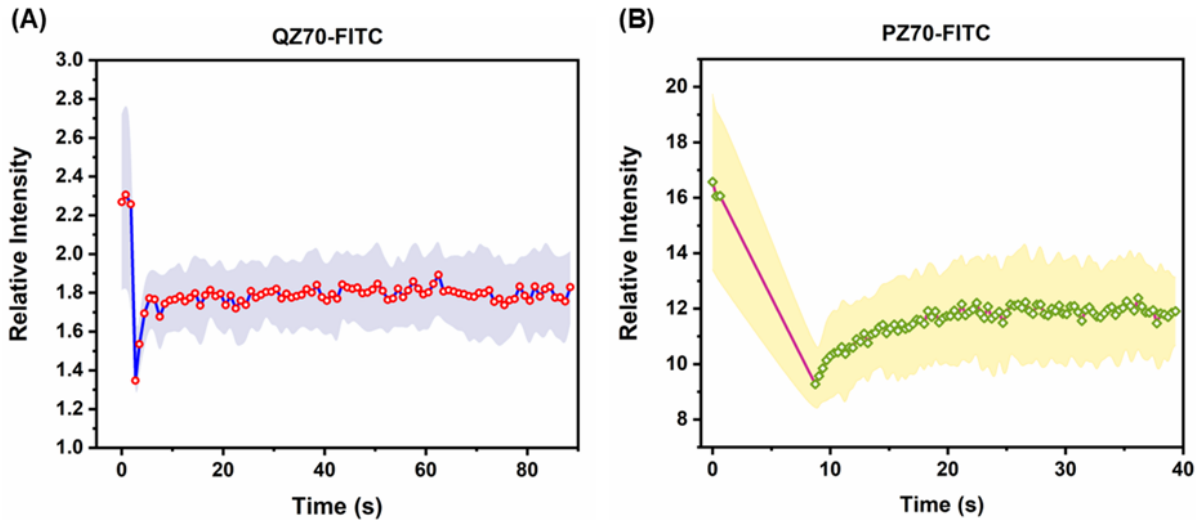

Fig. S1. Fluorescence recovery after photobleaching (FRAP) analysis of FITC-labeled sBCs. (A) FRAP recovery profile of QZ70 labelled with FITC. (B) FRAP recovery profile of QZ70 labelled with FITC.

intensity in the bleached region was measured over time.

S. 1.1. Observation from the FRAP assay: FRAP analysis was performed to evaluate the mobility of fluorescently labeled components within QZ70 and PZ70 nanoparticles. QZ70-FITC exhibited a rapid fluorescence recovery, reaching a plateau within ~10 seconds at approximately 70% of the initial intensity shown in **Fig. S1A**, indicating moderate molecular movement. As expected for PZ70, the recovery of FITC-labeled PZ70 were slow and these systems exhibited less complete recovery, most likely for the restricted molecular diffusion due to increased steric hindrance from the conjugated PEG moiety.

S.2. Kinetic stability of sBCs. The kinetic stability of the sBCs composed of zein mixed with QZ or PZ are evaluated by comparing the autocorrelation function obtained from the dynamic light scattering by the resulting particles. **Fig. S2A** shows the comparison between autocorrelation value with time of different compositions of sBCs composed of QZ. **Fig. S2B** shows the difference between the autocorrelation values of different compositions of PZ sBCs, indicating that conjugation of a charged species, such as GTMAC, or PEG within the sBCs can increase the stability of the particles.

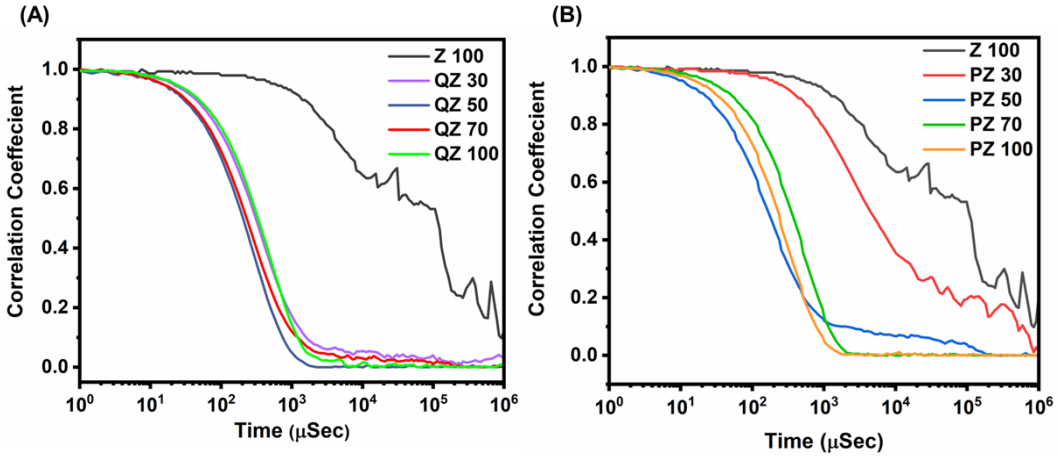

Fig. S2. (A) Autocorrelation function of different QZ sBC compositions. **(B)** Autocorrelation function of different sBCs composed of PZ.

S.3. ***Morphology of sBCs is composed of different ratios of quaternized and PEG-conjugated zein mixed with the unmodified protein.*** High resolution TEM (HR-TEM) studies of sBCs prepared via mixing different ratios of modified and unmodified zein showed the morphology of the resulting condensates. These images demonstrate that these proteins aggregate as stable, monodispersed particles.

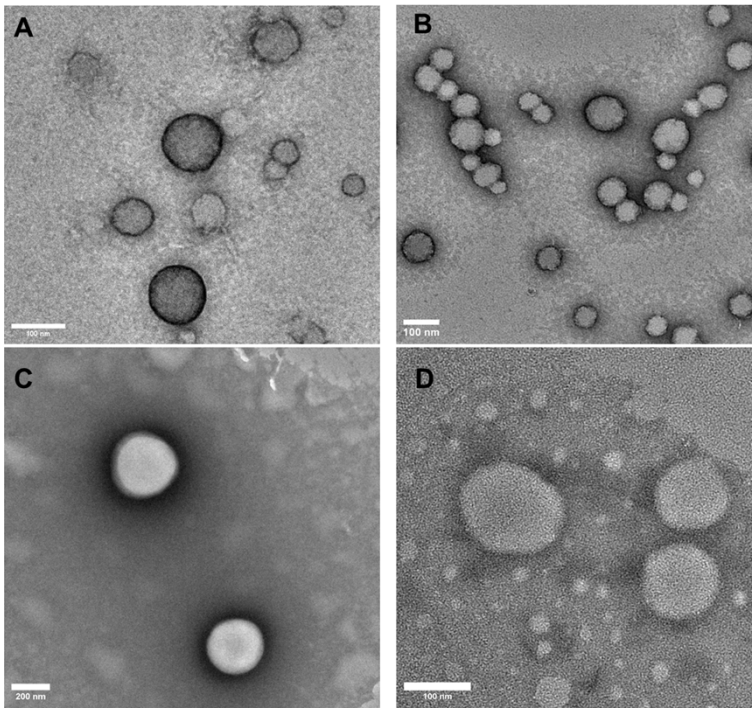

Fig. S3. TEM images of (**A**) QZ 50; **(B)** QZ 30; **(C)** PZ 50, and **(D)** PZ 30 sBCs.

S.4. Critical Aggregation Concentration of PZ 70 sBCs. Fluorescence Intensity vs. wavelength graph of different concentrations (1000 to 0.001 mg/ml of PZ70 used for the formation of corresponding sBCs presented in Fig. S4.

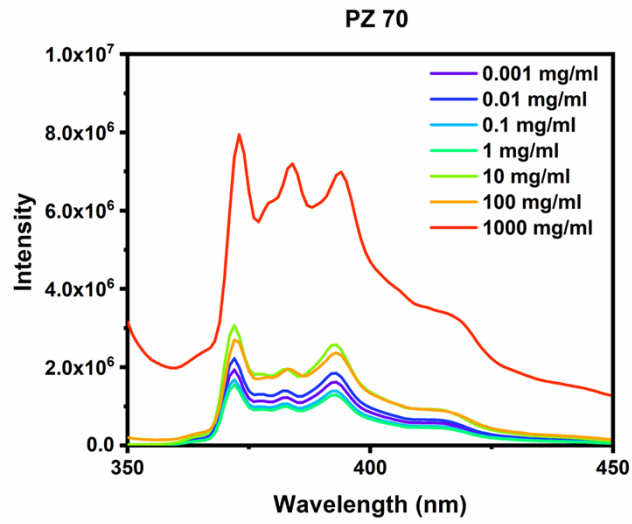

Fig. S4. Intensity Vs wavelength graph of different concentrations of PZ sBCs to determine their critical aggregation concentration.

S.4. Cumulative release profile of DOX from sBCs. Cumulative DOX dissociation from PZ 70 sBCs with compared to unbound DOX over 30 h.

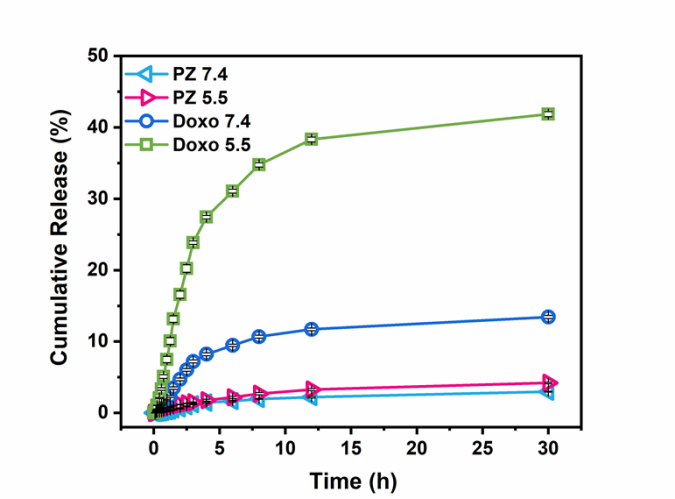

Fig. S5. Cumulative dissociation of DOX from the PZ 70 sBCs in two different pH 7.4 and 5.5.