Electronic Supplementary Material (ESI)

Observation of liquid-liquid phase separation of ataxin-3 and quantitative evaluation of its concentration in a single droplet using Raman microscopy

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1. Figures

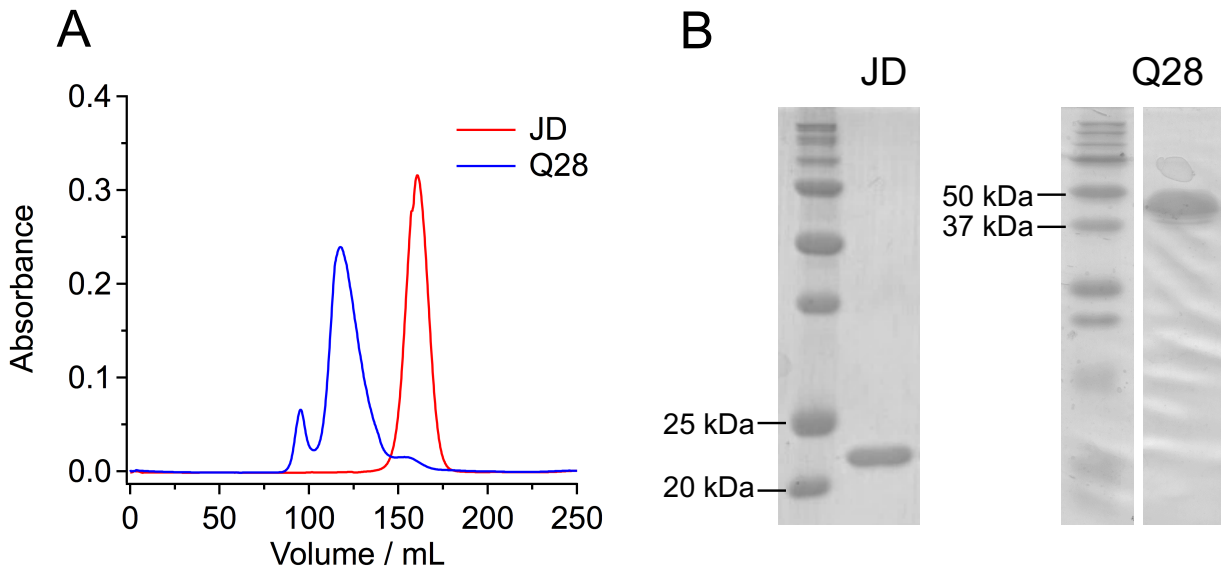

Fig. S1 Results of size-exclusion chromatography column (A) and SDS-PAGES (B) of prepared Q28 and JD solutions.

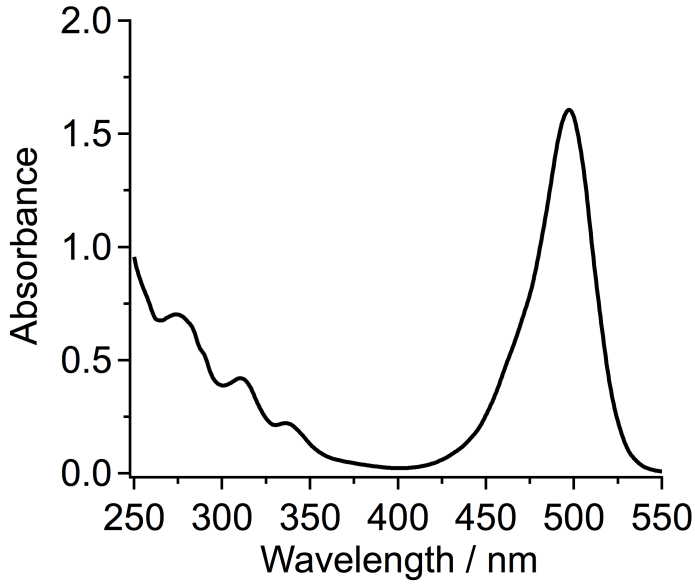

Fig. S2 Absorption spectrum of Q28 tagged with Alexa Fluor® 488 in PBS buffer.

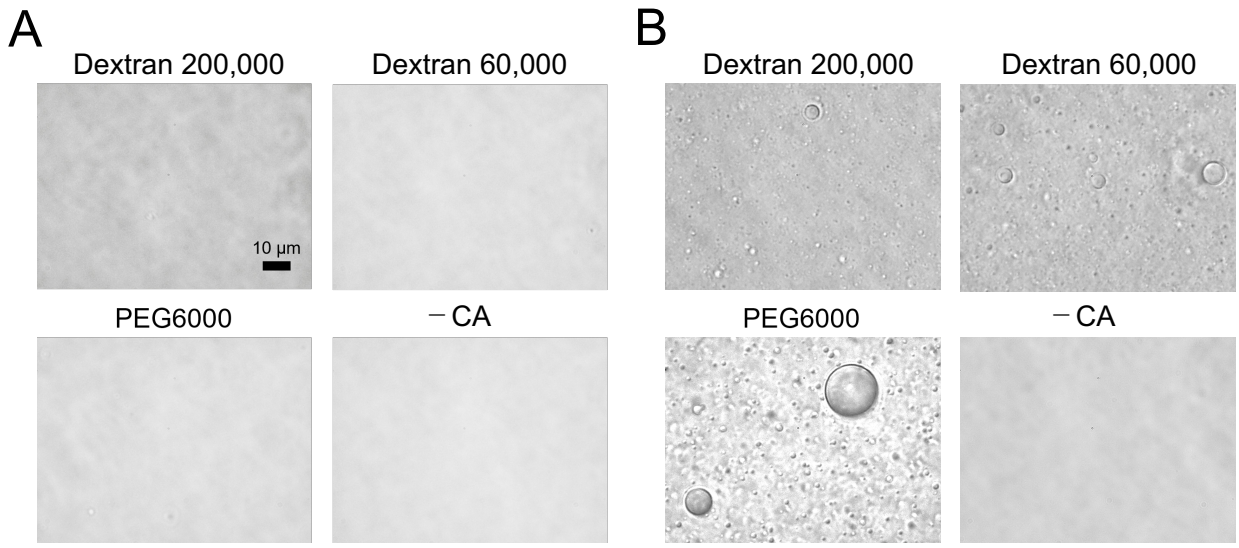

Fig. S3 Bright field images of JD (A) and Q28 (B) in Buffer A including a 20 wt% crowding agent of dextran 200,000 (molecular weight (MW): 180,000~210,000), dextran 60,000 (MW: 50,000~70,000) or PEG (MW: 7,400~10,200), together with those of the protein solution without a crowding agent (–CA). The protein concentration was 100 μM.

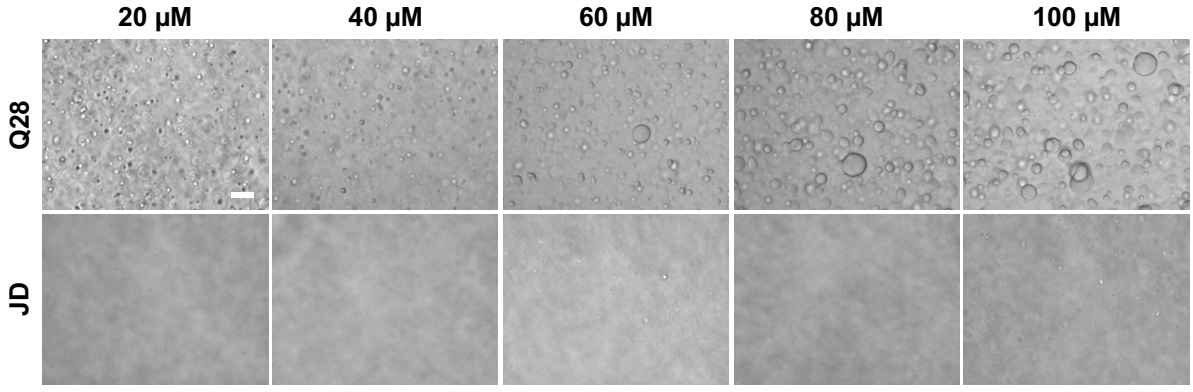

Fig. S4 Bright field images of Q28 and JD in Buffer A with 20 wt% PEG (MW: 7,400~10,200) at different protein concentrations (20, 40, 60, 80 and 100 μM).

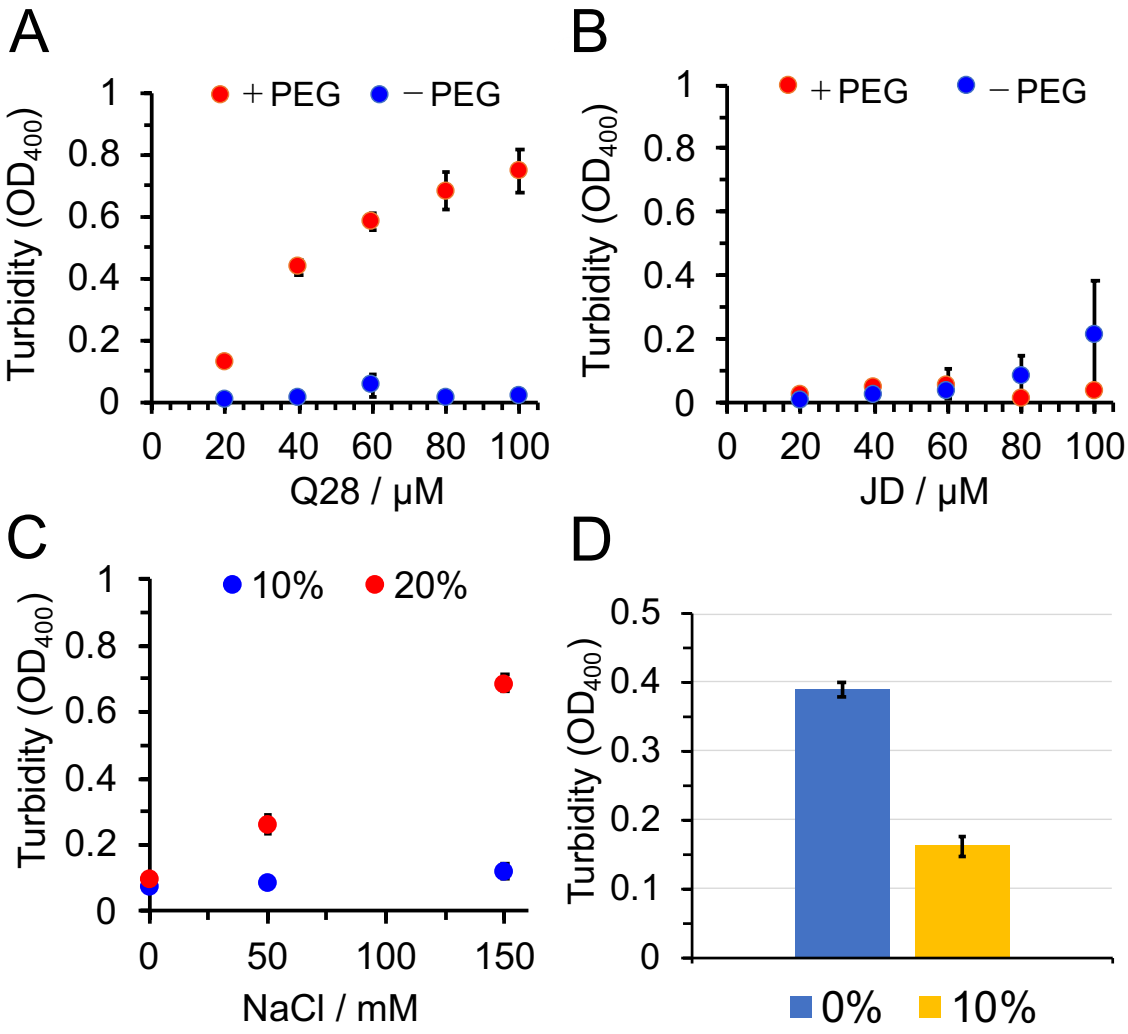

Fig. S5 (A, B) Turbidity of Q28 (A) and JD (B) in Buffer A with changing concentrations in the presence or absence of 20 wt% PEG (MW: 7,400~10,200). (C) Turbidity of 100 μM Q28 in Buffer A with 10 or 20 wt% PEG at different NaCl concentrations. (D) Turbidity of 100 μM Q28 in Buffer A with 15 wt% PEG in the presence or absence of 10 wt% 1,6-hexanediol. Error bars are SE (*n* = 3).

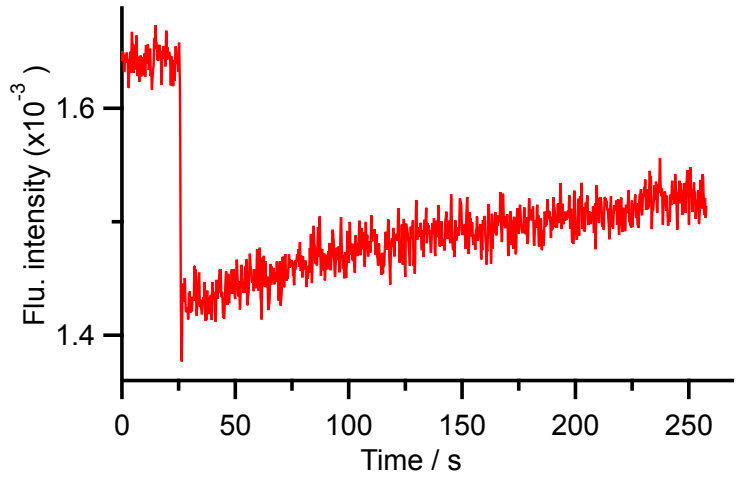

Fig. S6 The average FRAP of a droplet of Q28 labelled with Alexa Fluor® 488 in 20 wt% PEG (MW: 7,400~10,200) solution (*n* =17).

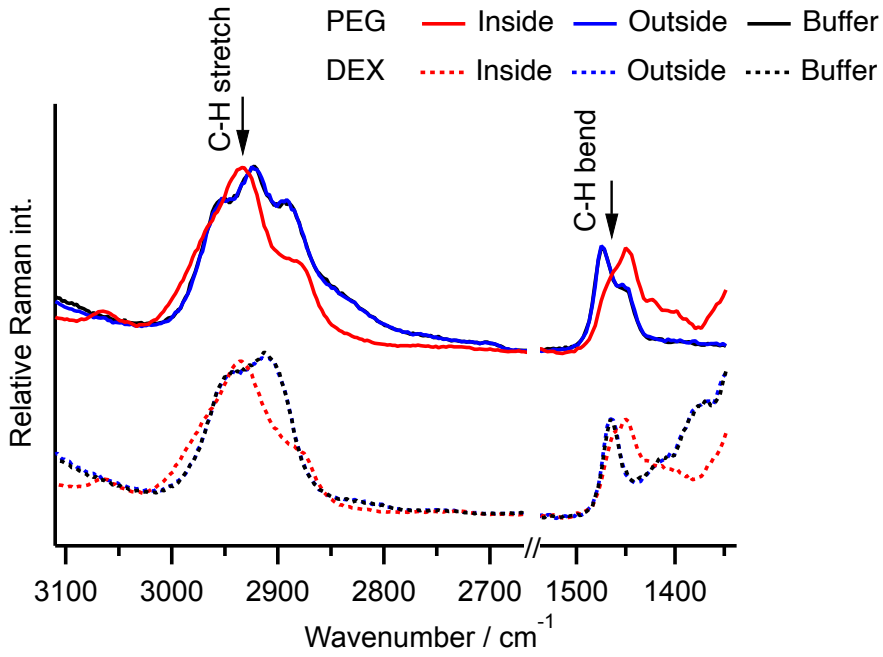

Fig. S7 Raman spectra obtained from inside and outside of a droplet of Q28 in 20 wt% PEG (MW: 7,400~10,200) solution or in 20 wt% DEX (MW: 50,000~70,000) solution in the C-H bending and stretching band regions. The concentration of Q28 was 100 μM. Red, inside a droplet; blue, outside droplets; black, only buffer solution including PEG or DEX. The Raman intensity was normalised to that of the C-H bands.

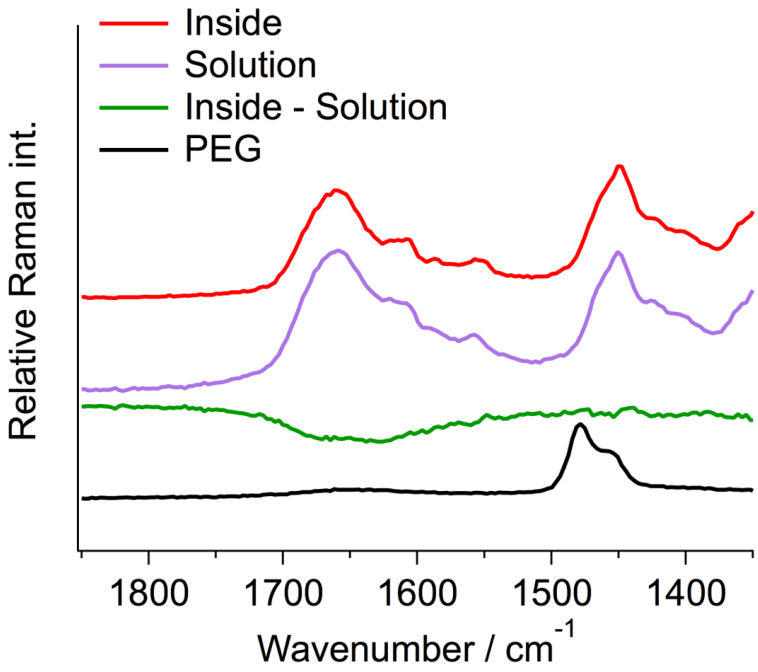

Fig. S8 Raman spectra of Q28 in Buffer A inside a droplet with 20 wt% PEG (MW: 7,400~10,200) (red), in Buffer A without PEG (purple), and its difference spectrum (green; red minus purple). The Raman spectrum of Buffer A with 20 wt% PEG is shown as a black line, in which the band at around 1480 cm^{-1} is attributed to the C–H bending band of PEG. The concentration of Q28 was 100 μM in red and 5 mM in purple.

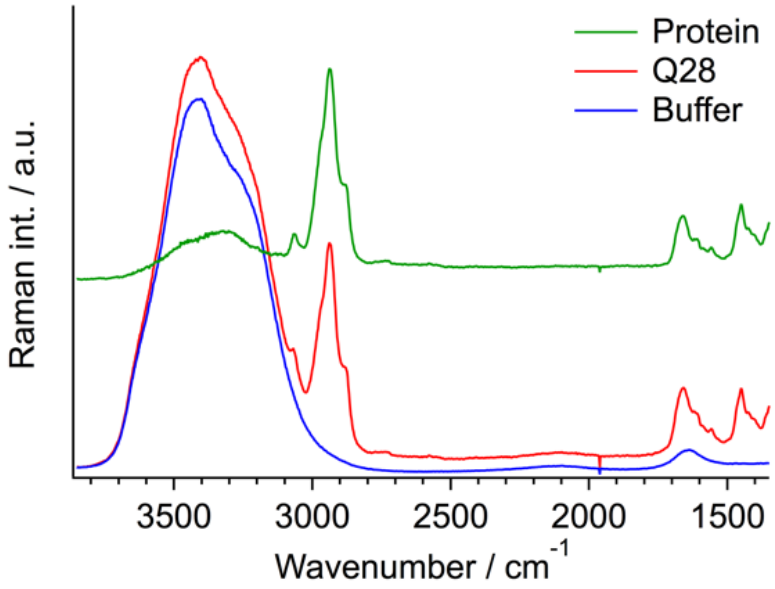

Fig. S9 Difference spectrum (green) obtained by the subtraction of the Raman spectrum of Buffer A only (blue) from that of Q28 in Buffer A (red). The concentration of Q28 was 5 mM. Crowding agent was not used in these measurements. The baseline was corrected in the difference spectrum to be flat at all wavenumbers.

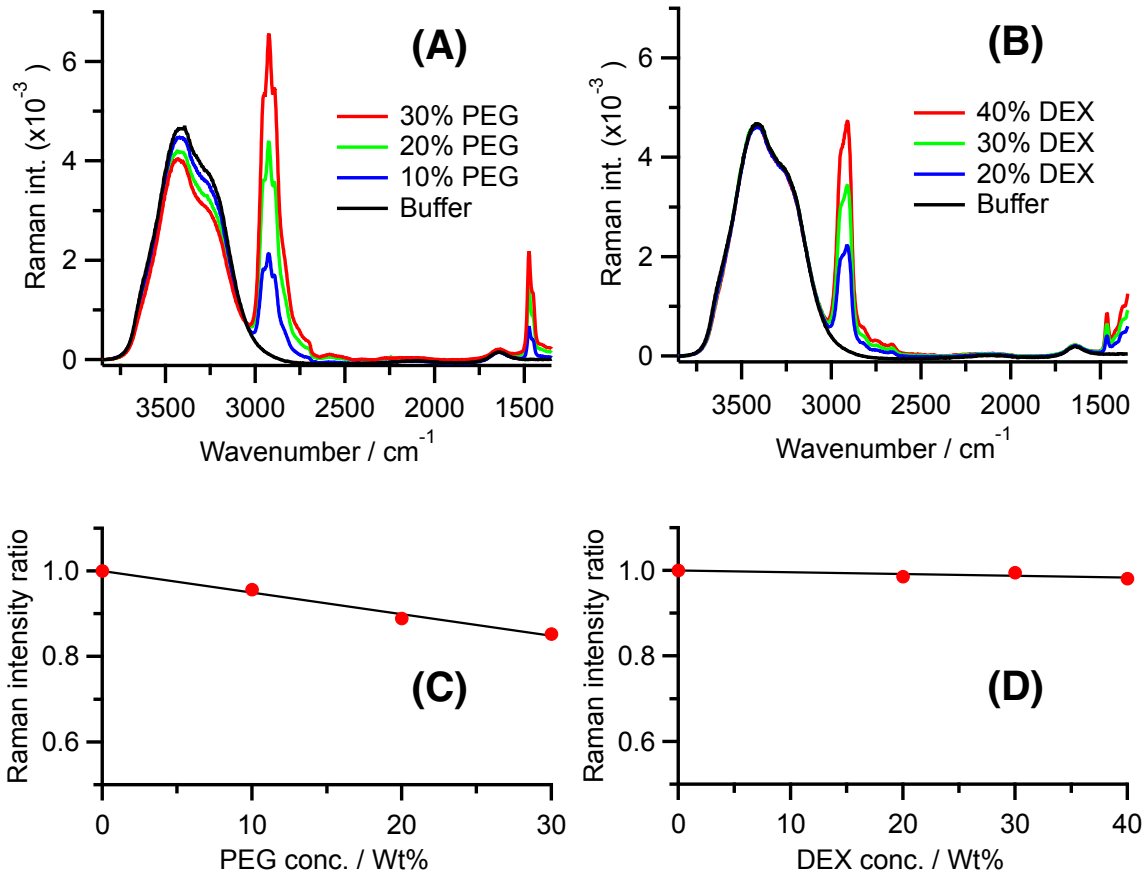

Fig. S10 (A, B) Raman spectra of PEG (A) and DEX (B) in buffer with different concentrations of PEG or DEX. (C, D) Ratio of the integrated Raman intensity of the buffer solution containing PEG (C) or DEX (D) to that of the buffer solution in the 3500-3600 cm^{-1} region. The intensity ratio decreased linearly with increasing PEG concentration (Wt%), and to compensate for the decrease in the integrated intensity due to the presence of PEG, the protein concentrations estimated by the calibration line at 20 and 30 wt% PEG were multiplied by coefficients of 0.90 and 0.85, respectively. The intensity ratio did not change with DEX concentration (Wt%), so that no correction was necessary for the DEX solution.

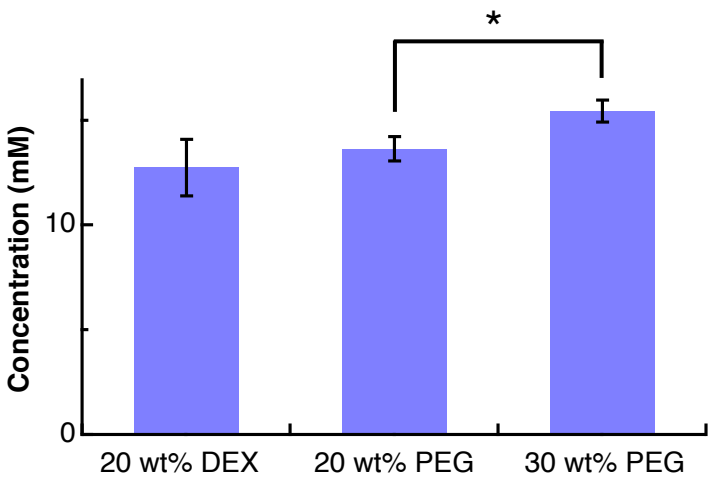

Fig. S11 Evaluated concentration of Q28 within droplets formed in 20 wt% DEX (MW: 50,000~70,000), 20 wt% PEG (MW: 7,400~10,200) and 30 wt% PEG (MW: 7,400~10,200). The Q28 solution was prepared to be 100 μM. Error bars are SE (n = 4 (20 wt% DEX), n = 8 (20 wt% PEG), n = 6 (30 wt% PEG)). *p < 0.05.