Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information

Cell-size Confinement effect on Protein Diffusion in Crowding Poly(ethylene)glycol solution

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S1. Calculation of characteristic sizes of PEG

The calculation of characteristic sizes for the polymer solutions used in current study were performed using the following equations[1, 2]:

Experimentally estimated gyration radius of PEG R_g:

$$R_g = 0.02\, M^{0.58} \quad [\text{nm}]. \tag{1}$$

M denotes the molecular weight of PEG. Overlap concentration c^* is derived from

$$c^* \cong \frac{M}{N_A}\left(\frac{4\pi}{3}R_g^{\,3}\right)^{-1}. \tag{2}$$

N_A denotes the Avogadro's number. The correlation length of the polymers ξ is calculated by

$$\xi \cong R_g\left(\frac{c}{c^*}\right)^{-3/4}. \tag{3}$$

We use equation S3 to obtain ξ for $c > c^*$. Equation S3 is correct under assumption of $\xi/R_g \cong 1$ below the overlap concentration. [3] The results are shown in Table S1.

S2. Droplets diffusion in the oil

The droplets were suspended in mineral oil. The viscosity η is calculated from the kinetic viscosity v (14–17 mm^2/s) and its density ρ (0.84 g/mL) as $\eta = \rho v$ = 12–14 mPa·s. The diffusion coefficient D of a droplet with radius R = 8~75 μm used in this study can be estimated to be $D = 3.1 \times 10^{-4} \sim 1.8 \times 10^{-3}$ μm^2/s (T = 300 K) using Einstein-Stokes equation $D = kT/6\pi\eta R$, where k is the Boltzmann coefficient and T is the temperature. Besides, the illumination area A_{FCS} of the FCS measurement is calculated from the illumination radius w_0 as $A_{FCS} = \pi w_0^2 \sim \pi(0.2\ \mu\text{m})^2 \sim 0.13\ \mu\text{m}^2$. The necessary time to move the area A_{FCS} for the droplet is calculated to $A_{FCS}/D = 5 \times 10^1 \sim 6 \times 10^2$ s. This time is much longer than the maximum correlation time of the measurement, 33 ms, which means that the droplet motion does not appear in our experimental timescale. Therefore, we conclude that the droplet motion in oil is negligible.

REFERENCES

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2. K. Devanand and J. C. Selser, *Macromolecules*, 1991, **24**, 5943-5947.
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4. B. Terry and A. Robards, *Planta*, 1987, **171**, 145-157.

TABLE

Polymer	**PEG 6k**		**PEG 20k**
R_g [nm]	3.1		6.2
c^* [mg/mL]	79		33
c [mg/mL]	50	100	100
$\varphi^\dagger$	0.6	1.3	3.0
ξ [nm]	-	2.6	2.7
	$c < c^*$		$c > c^*$

Table S1. Characteristic sizes of the polymer solutions used in the experiments.

*The hydrodynamic radius of the diffusing molecule GFP is 2.8 nm.[4]

$^\dagger\varphi$: volume fraction calculated from the gyration radius for PEG.

FIGURES

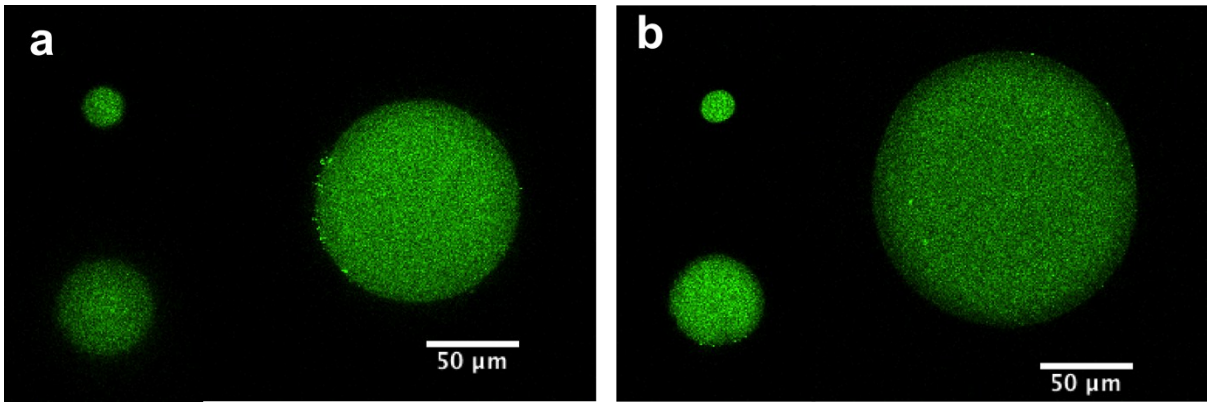

Figure S1. Distribution of GFP in water-in-oil droplets coated with a lipid layer. GFP is dissolved in (a) the buffer (pH 7.6, 50 mM HEPES, 100 mM NaCl, pH adjusted by KOH solution) and (b) 100 mg/mL PEG 20k in 50 mM sucrose.

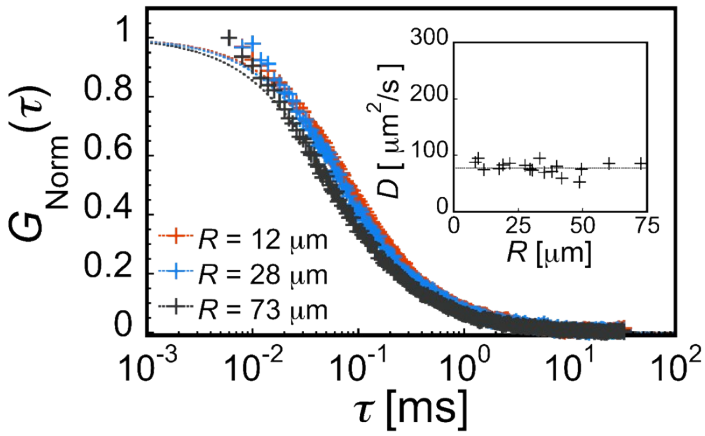

Figure S2. Normalized ACFs measured inside of microdroplets with different radius *R* for buffer solution (pH 7.6, 50 mM HEPES, 100 mM NaCl, pH adjusted by KOH). The inset shows the diffusion coefficient *D* plotted as a function of *R*.

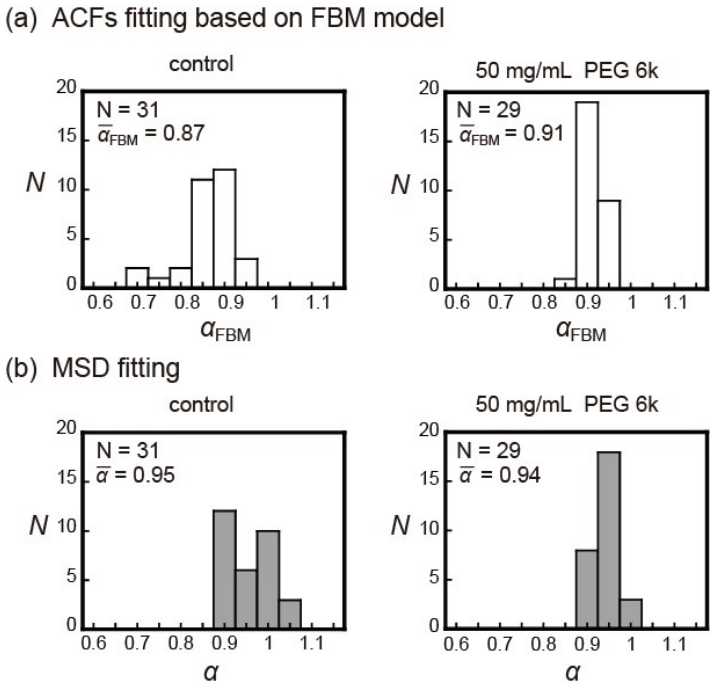

Figure S3. Histogram of the anomalous exponent derived from (a) FBM fitting α_{FBM} and (b) fitting of short-time MSD α for the control and the 50 mg/mL PEG 6k ($c < c^*$) solution.

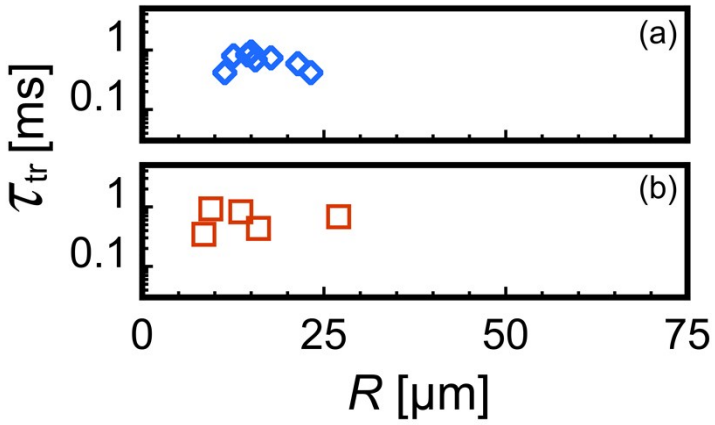

Figure S4. Transition time τ_{tr} plotted as a function of droplet radius R for (a) 100 mg/mL PEG 6k, and (b) 100 mg/mL PEG 20k. The existence of the angle is judged by the variation values in 0.1 ms < τ < 2 ms.