Supplementary Information (SI) for Biomaterials Science.

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Microfluidics-Driven Templating Preparation of Polymer Vesicles with Tailorable Dimensions and Rapid Cellular Internalization

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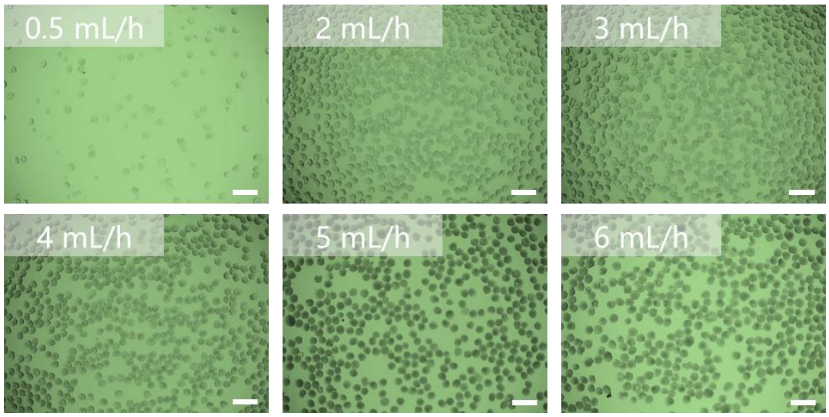

Figure S1 OM images of PEG_{113}-*b*-PLA_{167} emulsion templates with different flow rates of oil solution, by 0.5, 2, 3, 4, 5 and 6 mL/h, respectively. The flow rate of aqueous solution 35 mL/h.

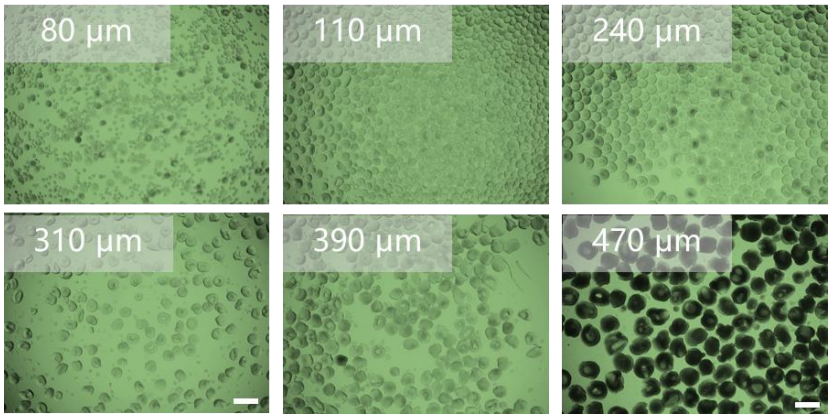

Figure S2 OM images of PEG_{113}-*b*-PLA_{167} template in PVA aqueous solution. Optical image of PEG_{113}-PLA_{125} template with 80 μm, 113 μm, 250 μm, 310 μm, 390 μm and 470 μm, respectively. The oil and PVA aqueous solution flow rates are 3 mL/h and 30 mL/h.

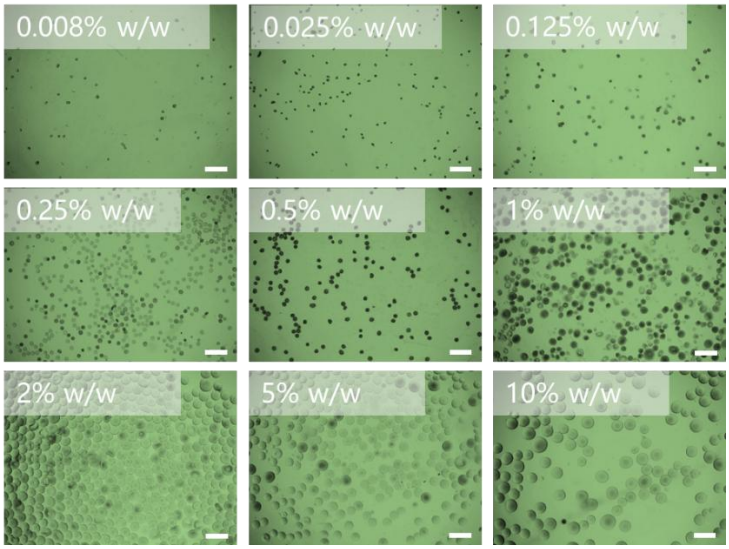

Figure S3 OM images of O/W single emulsions with different PEG$_{113}$-*b*-PLA$_{167}$ concentrations. PEG$_{113}$-*b*-PLA$_{167}$ concentrations were 0.008% w/w, 0.025% w/w, 0.125% w/w, 0.25% w/w, 0.5% w/w, 1% w/w, 2% w/w, 5% w/w, 10% w/w, respectively. The concentration of PVA aqueous solution is 0.5% w/w and the scale bar is 300 μm.

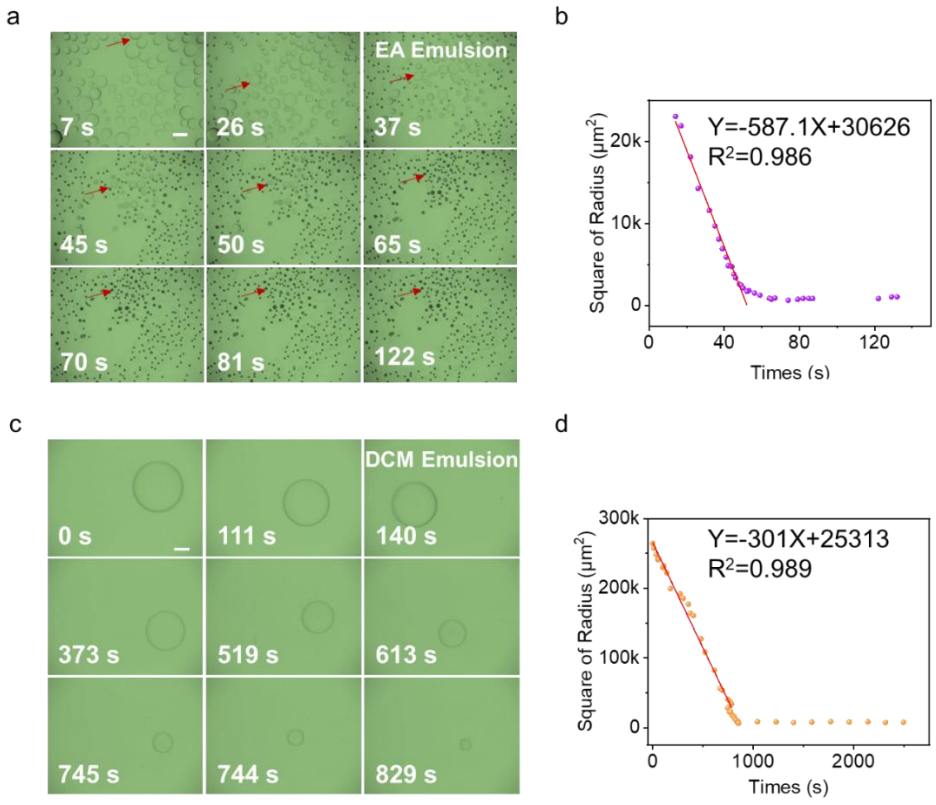

Figure S4 OM image of PEG$_{113}$-*b*-PLA$_{167}$ emulsion templates at different evaporation times. (a) OM image of PEG$_{113}$-*b*-PLA$_{167}$ EA emulsion templates transformation at different evaporation times. The arrow indicates the selected emulsion template used for measuring diameter. (b) The diameter of the EA emulsion template varies with different evaporation times. (c) OM image of PEG$_{113}$-*b*-PLA$_{167}$ DCM emulsion templates transformation at different evaporation times. (d) The diameter of the DCM emulsion template varies with different evaporation times. The scale bar is 300 μm. Both samples were 5 mg/mL (The concentration of EA sample is equal to 0.037% w/w).

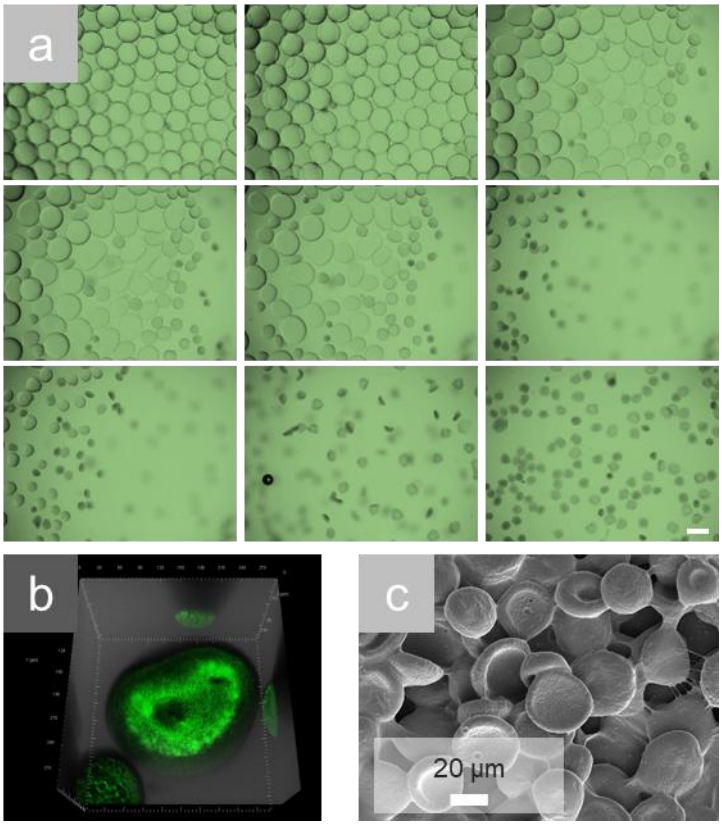

Figure S5 SEM and OM image of PEG_{113}-*b*-PLA_{167} microsphere templates at 0.15% w/w. (a) OM image of PEG_{113}-*b*-PLA_{167} O/W microsphere droplets at different evaporation times. (b) CLSM image of PEG_{113}-*b*-PLA_{167} O/W microsphere droplets. (c) SEM image of PEG_{113}-*b*-PLA_{167} O/W microsphere templates**.**

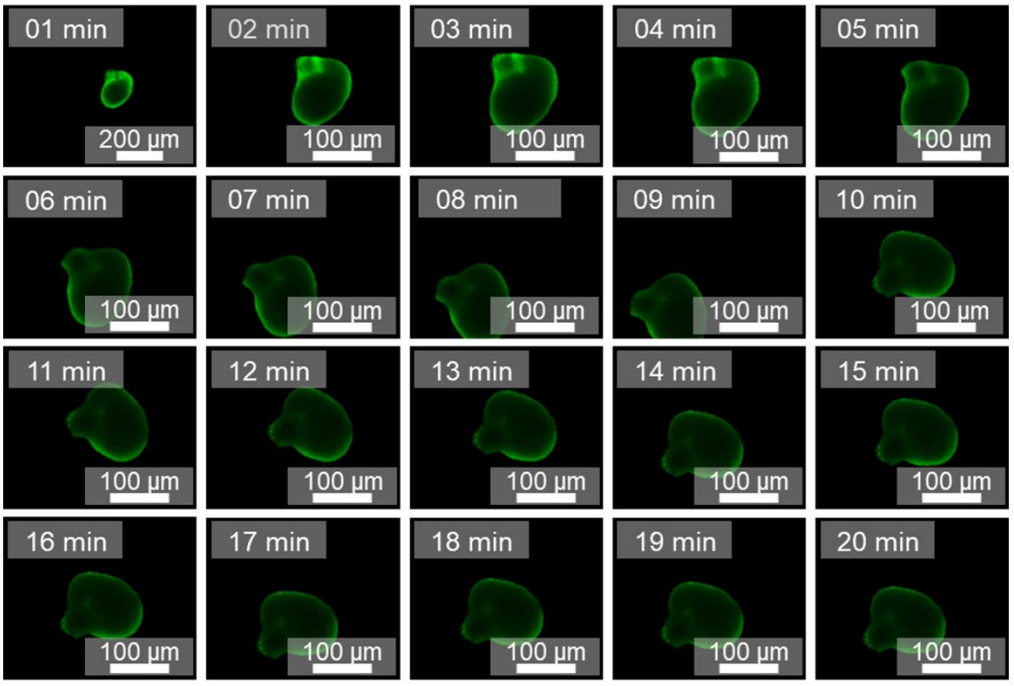

Figure S6 CLSM image of PEG_{113}-*b*-PLA_{167} emulsion (0.075% w/w) templates during evaporation at different times.

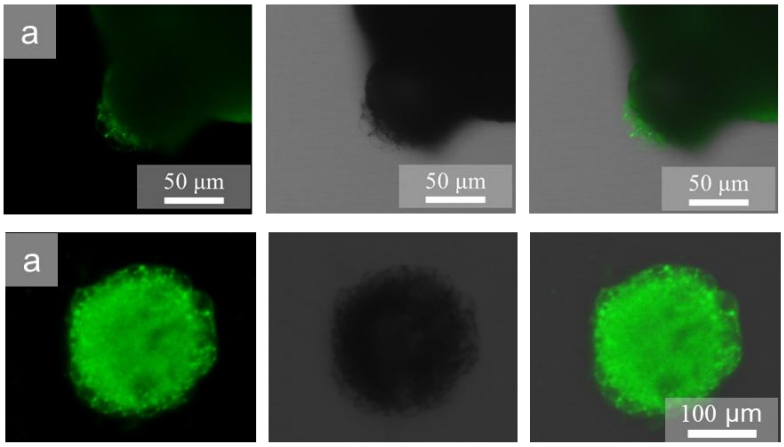

Figure S7 CLSM image of PEG_{113}-*b*-PLA_{167} O/W microsphere templates. (a) CLSM of PEG_{113}-*b*-PLA_{167} O/W emulsion at 10 min in Figure S7 (0.075% w/w, 12 h). (b) CLSM of PEG_{113}-*b*-PLA_{167} O/W emulsion (0.037% w/w, 12 h).

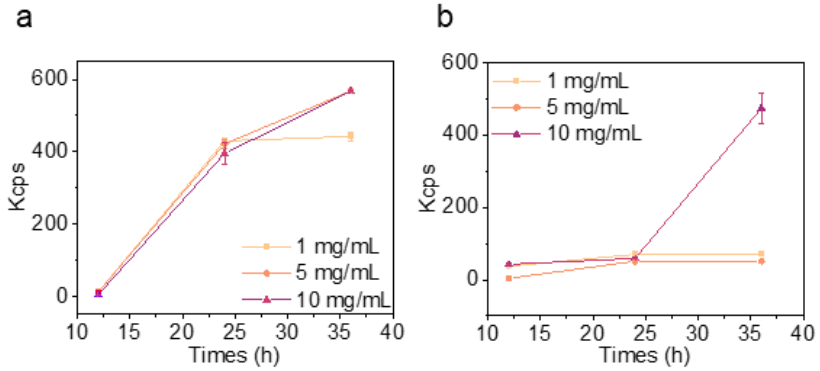

Figure S8 Kcps of PEG_{113}-*b*-PLA_{167} polymer vesicles in the solution hydrated from the microsphere templates method with different concentrations at room temperature. (a) Samples are stirred at 500 rpm. (b) Samples are stirred with 100 rpm.

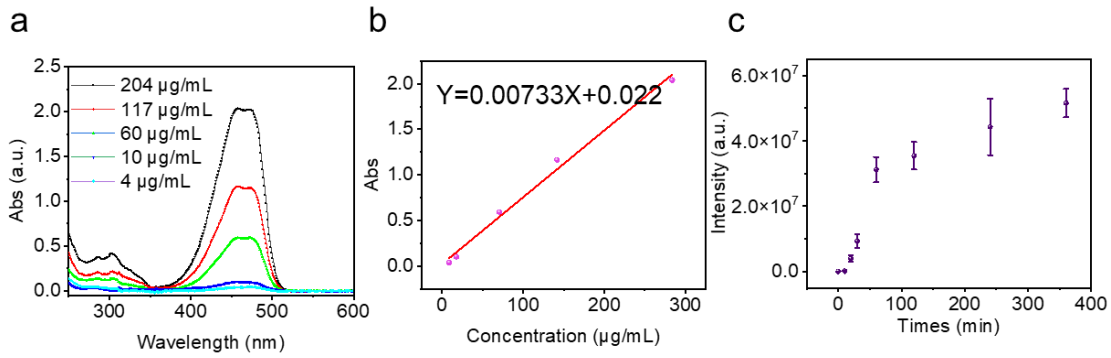

Figure S9 UV-vis absorption and fluorescence emission intensity of vesicles. (a) UV absorption of Cour-6 in DCM solution. (b) Standard curve of Cour-6 in DCM solution. (c) Fluorescence emission intensity of vesicles at 0, 10, 20, 30, 60, 120, 240, 360 min after rehydration.

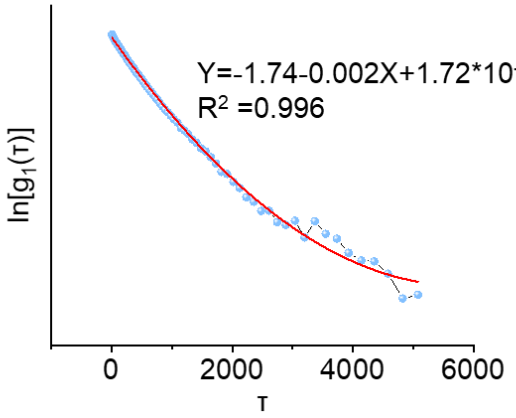

Figure S10 The curve of ln[$g_1(\tau)$] verse τ and polynomial fitting of polymer vesicle (store 5 days, 4 °C).

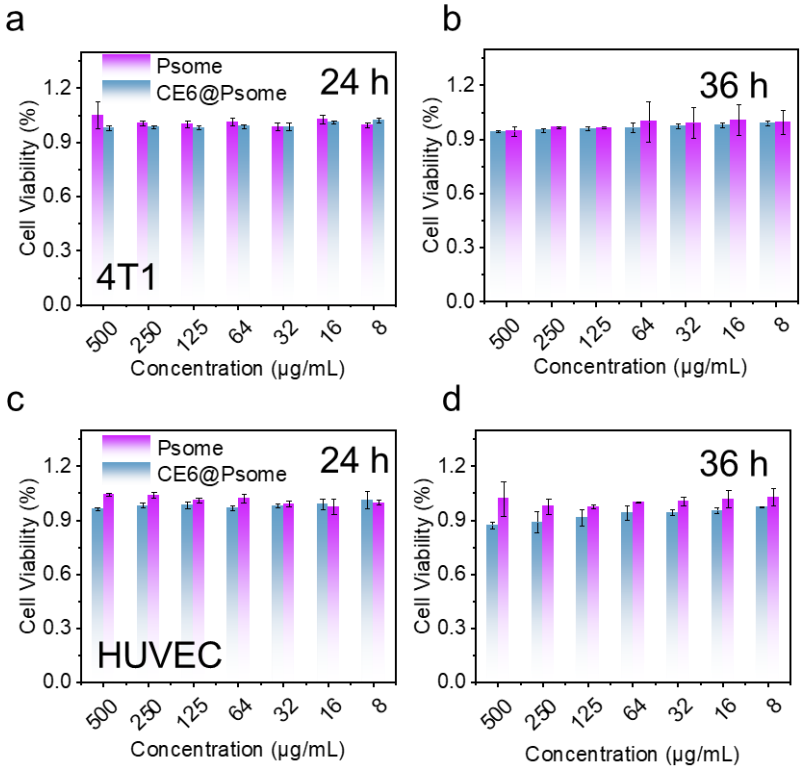

Figure S11 Cell viability of polymer vesicle and Cour-6@Polymer vesicles after incubation with 4T1 and HUVEC. (a-b) Cell viability of 4T1 cell line after incubation in polymer vesicle and Cour-6@Polymer vesicles 24 h and 36 h. (c-d) Cell viability of HUVEC cell line after incubation in polymer vesicle and Cour-6@Polymer vesicles 24 h and 36 h.

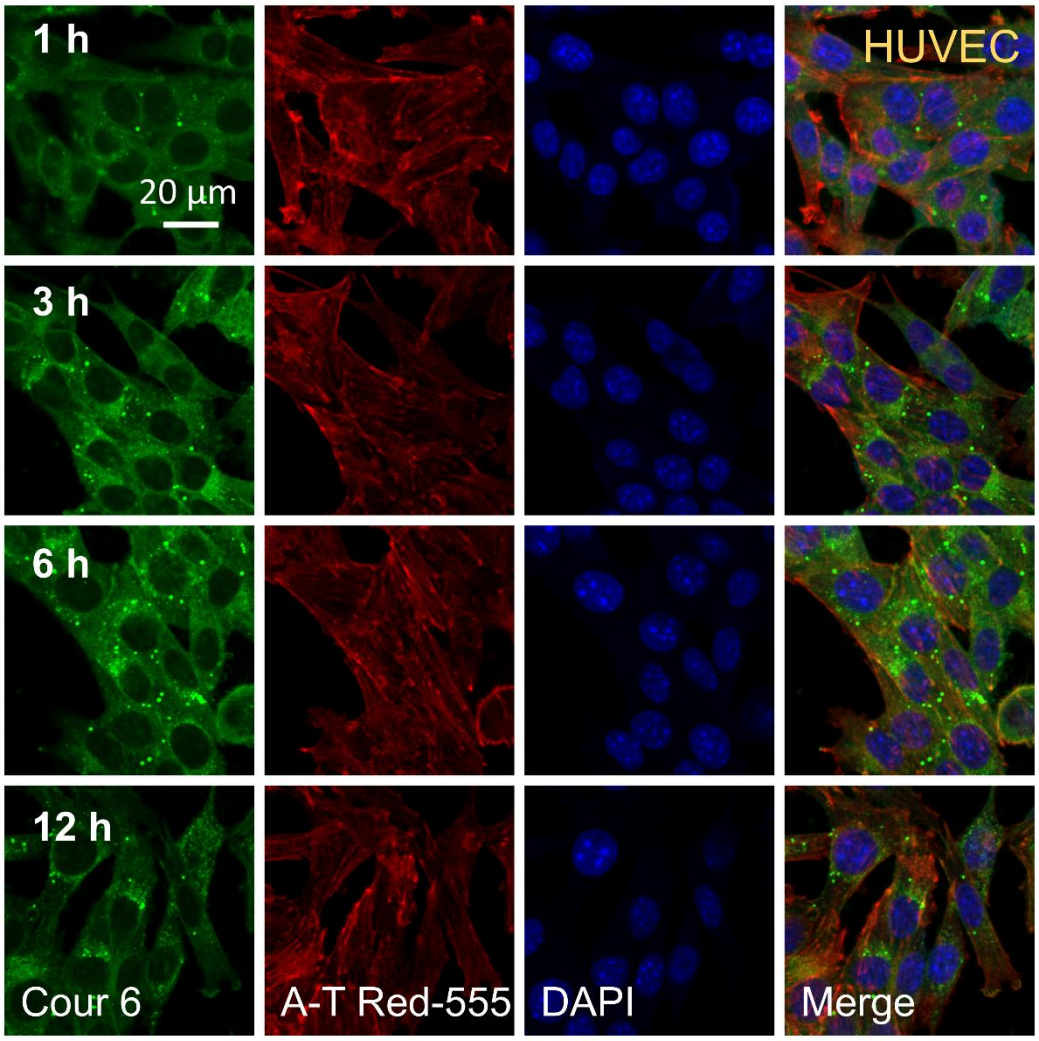

Figure S12 CLSM image of 4T1 cell lines after incubation with Cour-6@Polymer vesicles at 1 h, 3 h, 6 h, 12 h.

EquationS1-S6 for the rehydration of polymer vesicle from DLS, calculate as follow

the scattered intensity, $g_2(\tau)$ is related to the electric field $g_1(\tau)$. According to Siegert relation, the normalized autocorrelation function of the scattered intensity, $g_2(\tau)$ is related to the electric field $g_1(\tau)$:[1, 2]

$$g_2(\tau)=B+\beta\left|g_1(\tau)\right|^2 \quad \text{Equation S1}$$

$$g_1(\tau)=\sum_0^i A_i exp(-\Gamma_i t) \quad \text{Equation S2}$$

$$\Gamma=Dq^2 \quad \text{Equation S3}$$

$$q=\frac{4\pi n_0}{\lambda}\sin\left(\frac{\theta}{2}\right) \quad \text{Equation S4}$$

Where β is the coherence factor and B is the baseline. θ is the angle of the detector's position (90°) and q is the scattering vector with wavelength λ (633 nm) . Γ is associated with diffusion coefficient of the particle. D is diffusion coefficient and n_0 is the refractive index of the aqueous solution (1.331). The weighting factor A_i is proportional to the portion of the scattered intensity that this subset of particles contributes. After get the calculated $g_1(\tau)$, cumulant expansions were used through polynomial fitting **(Figure S10)**

$$ln[g_1(t)]=-K_1 t+\left(\frac{1}{2}\right)K_2^2 t^2+\cdots. \quad \text{Equation S5}$$

The coefficients Kn represent the cumulants. The first cumulant K_1 is equal to the average of the reciprocal relaxation time. The second cumulant K_2 indicates the dispersion of the reciprocal relaxation time around this average value $(\Gamma-\bar{\Gamma})^2$. If K_2 (and any higher-order cumulants) are equal to zero, the first-order correlation function simplifies to a single exponential.[3] For $g_2(\tau)$ of polymer vesicles is $g_2(\tau)=0.18*e^{(-x/481)}$. According to equation S1and Figure 8b, polymer vesicle's $g_1(\tau)$ is the Equation S6.

$$g_1(\tau)=0.424\times\exp\left(-\frac{x}{962}\right) \quad \text{Equation S6}$$

Therefore, Γ of the polymer vesical is 1/962=0.001. through the equation S4 and Equation 3, the rehydration diameter of polymer vesicle is calculated as 81 nm (store 5 days, 4 °C). Γ of the polymer vesical is 1/962=0.001 S^{-1}.

Reference

1. P. A. Hassan, S. Rana and G. Verma, Making Sense of Brownian Motion: Colloid Characterization by Dynamic Light Scattering, *Langmuir*, 2015, **31**, 3-12.
2. J. Rodriguez-Loya, M. Lerma and J. L. Gardea-Torresdey, Dynamic Light Scattering and Its Application to Control Nanoparticle Aggregation in Colloidal Systems: A Review, *Micromachines*, 2024, **15**. 24.
3. R. Pecora, Dynamic light scattering measurement of nanometer particles in liquids, *J. Nanopart. Res.*, 2000, **2**, 123-131.