SUPPORTING INFORMATION

SYNTHESIS OF PMMA MICROPARTICLES WITH A NARROW SIZE DISTRIBUTION BY PHOTOINITIATED RAFT DISPERSION POLYMERIZATION WITH A MACROMONOMER AS THE STABILIZER

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ADDITIONAL EXPERMENTAL DETAILS

Photoinitiated dispersion polymerization of MMA using PEGMA as the reactive stabilizer in

the absence of a RAFT agent

In a typical experiment, 10 wt % monomer (MMA, 2.0 g) relative to the system, 2.5 wt % macromonomer (PEGMA2000, 0.05 g) relative to MMA and 3 wt % photoinitiator (Darocur 1173, 0.06 g) relative to MMA were dissolved in an ethanol/water mixture (40/60, w/w) under magnetic stirring to form a homogenous solution. The irradiation protocol and the product isolation were the same as that in the main text. Without the RAFT agent present, the reaction mixture turned turbid after 40 s UV irradiation. At the end of the reaction, the dispersion was diluted with distilled water and a sample was prepared on a mica film for SEM analysis. The particles were not washed prior to preparing samples for SEM.

Photoinitiated dispersion polymerization of MMA using PEGMA as the reactive stabilizer in

the presence of methyl 2-(dodecylthiocarbonothioylthio)-2-methylpropionate

In a typical experiment, 10 wt % monomer (MMA, 2.0 g) relative to the system, 2.5 wt % macromonomer (PEGMA2000, 0.05 g) relative to MMA, 0.5 wt % methyl 2-(dodecylthiocarbonothioylthio)-2-methylpropionate and 3 wt % photoinitiator (Darocur 1173, 0.06 g) relative to MMA were dissolved in an ethanol/water mixture (40/60, w/w) under magnetic stirring to form a homogenous solution. The irradiation protocol followed that described in the main text. Here, the reaction mixture turned turbid after 90 s UV irradiation. The obtained product was sedimented by centrifugation and washed three times with an ethanol/water (40/60, w/w) mixture. The dispersion was diluted with distilled water and a sample was prepared on a mica film for SEM analysis. The particles were not washed prior to preparing samples for SEM.

Surface acid titration of the carboxylated PMMA microbeads

Simultaneous potentiometric and conductometric titrations were carried out in air at room temperature using a Fisher Scientific conductivity meter and Ecomet pH probe). Samples of particles were diluted in distilled water (25 g dispersion, solids content 1%). A standardized 0.1 M NaOH solution was added by micropipette and stirred magnetically. The solution was then

back-titrated using 0.025 M HCl added in volume increments of 20 µL. A blank titration to correct for CO_2 uptake was carried out by the same way.

The mean number of carboxyl groups per particle can be calculated based on the follow equations.

$N_{MAA\text{-}titr} = C_{HCl} \times V_{HCl} \times N_{Av} = 0.1 \times (220 \times 10^{-6}) \times (6.02 \times 10^{23}) = 1.32 \times 10^{19}$

$S_{particle} = 4 \times \pi \times R^2 = 4 \times 3.14 \times (1.37/2/1000)^2 = 5.89 \times 10^6 \text{ nm}^2$

$V_{particle} = 4/3 \times \pi \times R^3 = 4/3 \times 3.14 \times (1.37/2/10000)^3 = 1.35 \times 10^{-12} \text{ cm}^3$

$m_{particle} = \rho \times V_{particle} = 1.19 \times (1.35 \times 10^{-12}) = 1.61 \times 10^{-13} \text{ g}$

$N_{particle} = m_{sample} \times$ solids content % / $m_{particle} = 25 \times 1\% / (1.61 \times 10^{-13}) = 1.56 \times 10^{12}$

$N_{MAA}/particle = (1.32 \times 10^{19}) / (1.67 \times 10^{12}) = 7.9 \times 10^6$

$N_{MAA}/nm^2 = N_{MAA}/particle / S_{particle} = (7.9 \times 10^6) / (5.89 \times 10^6) = 1.34$

In these equations, $N_{MAA\text{-}titr}$ is the number of –COOH groups titrated, R is the mean particle radius, $S_{particle}$ is the mean surface area per particle, $V_{particle}$ is the mean particle volume, ρ (= 1.19 g/cm^3) is the density of PMMA, N_{Av} is Avogadro's number, $m_{particle}$ is the mean particle mass, m_{sample} is the mass of sample to be titrated, $N_{particle}$ is the number of PMMA particles in the titrated sample, $N_{MAA}/particle$ is the number of surface –COOH groups per particle and N_{MAA}/nm^2 is the number of surface –COOH groups per nm^2.

Quantification of donkey anti-goat IgG bound to each particle

Alexa Fluor® 488 donkey anti-goat IgG (AF488-dag.IgG) has an absorption maximum at 494 nm, which can be used to quantify the concentration of AF488-dag.IgG in solution. We prepared three kinds of particle samples as described in the main text: Particles with covalently bound goat IgG via EDC-mediated coupling (PMMA-goat.IgG$_{(EDC)}$), particles with covalently bound BSA via EDC-mediated coupling (PMMA-BSA$_{(EDC)}$), and particles exposed to goat IgG without treatment with EDC (PMMA-goat.IgG$_{(no\text{-}EDC)}$).

To determine the amount of goat IgG bound to each particle sample, we incubated all three particle samples (4.0 mg/mL in PBS, 0.48 mL) with AF488-dag.IgG (2.0 mg/mL in PBS, 20 µL). After 3 h incubation, the particles were sedimented by centrifugation. A photograph showing tubes containing the pelleted particles and the supernatants under 365 nm UV light excitation is

presented in Figure S4. The concentration of unbound AF488-dag.IgG in the supernatant was measured by its absorbance at 494 nm (A(494)). A standard curve of A(494) vs the concentration of AF488-dag.IgG (c_{AF488}) is shown in Figure S5, indicating a linear relationship ranging from 0 to 80 µg/mL of the antibody in PBS. From the slope of this line, we calculate an extinction coefficient of 3.5×10^{-3} mL·µg^{-1}·cm^{-1}.

The mean number of AF488-dag.IgG bound to each particle was calculated using the equation

$$N_{AF488/particle} = \frac{N_{AF488-IgG}}{N_{particle}} = \frac{(c_{AF488} \times V_{AF488} - c_{unbound} \times V_{total})/M_{AF488}}{c_{PMMA}V_{PMMA}/\rho_{PMMA} \times \frac{4}{3}\pi(\frac{d_n}{2})^3} \qquad (S1)$$

where initially we added V_{AF488} = 20 µL of antibody solution with c_{AF488} = 2 mg/mL, and the total volume of the solution was V_{total} = 500 µL. In this experiment, the amount of PMMA microspheres was c_{PMMA} = 4.0 mg/mL in a volume V_{PMMA} = 480 µL. Values of $c_{unbound}$ were determined from the measured A(494) values and the calibration curve in Figure S5. These values are listed in Table S1. In the calculation of the mean number of AF488-dag.IgG per microbead we used 150 kDa as the molar mass of the antibody, d_n = 1.37 µm for the particle diameter and ρ_{PMMA} = 1.19 g/cm^3 for the particle density.

ADDITIONAL FIGURES AND TABLES
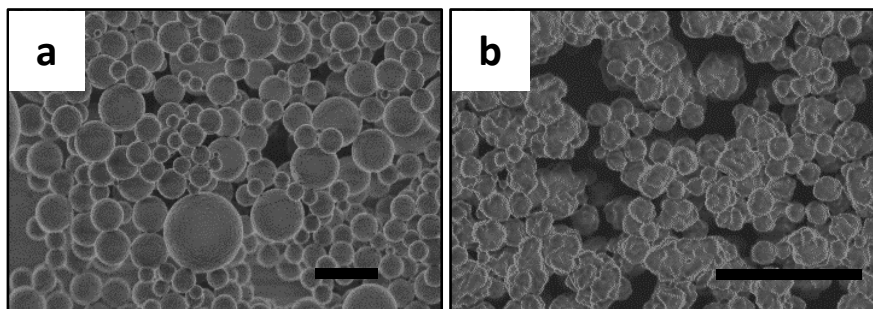
Figure S1. SEM images of PMMA microspheres prepared by photoinitiated dispersion polymerization of MMA in an ethanol/water mixture (40/60, w/w) with (a) 2.5 wt % PEGMA2000; (b) 5 wt % PEGMA2000. The concentration of photoinitiator was 3 wt % (relative to MMA) and the concentration of MMA was 10 wt % (relative to the reaction mixture). Scale bar: 2 μm.
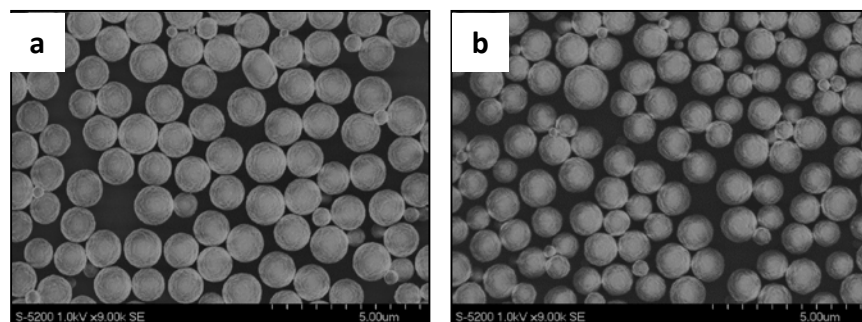

Figure S2. SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA in an ethanol/water mixture (40/60, w/w) with 2.5 wt % PEGMA2000 as the stabilizer in the presence of different amounts of Methyl 2-(dodecylthiocarbonothioylthio)-2-methylpropionate: (a) 0.25 wt %; (b) 0.5 wt %. The concentration of photoinitiator was 3 wt % (relative to MMA) and the concentration of MMA was 10 wt % (relative to the reaction mixture).
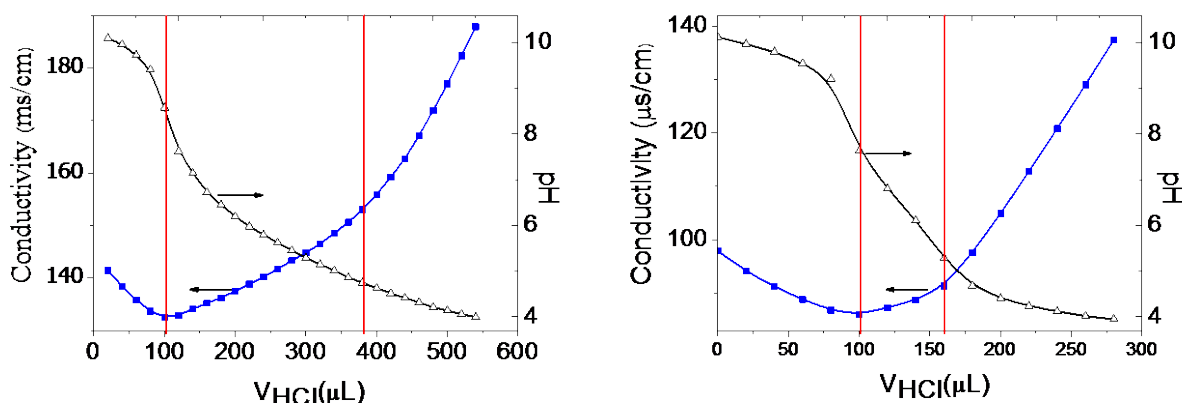
Figure S3. (left) pH and conductometric titration curves of carboxyl functional PMMA particles prepared by two-stage photoinitiated RAFT dispersion polymerization with PEGMA2000 as the stabilizer; (right) pH and conductometric titration curves of water (25 g).
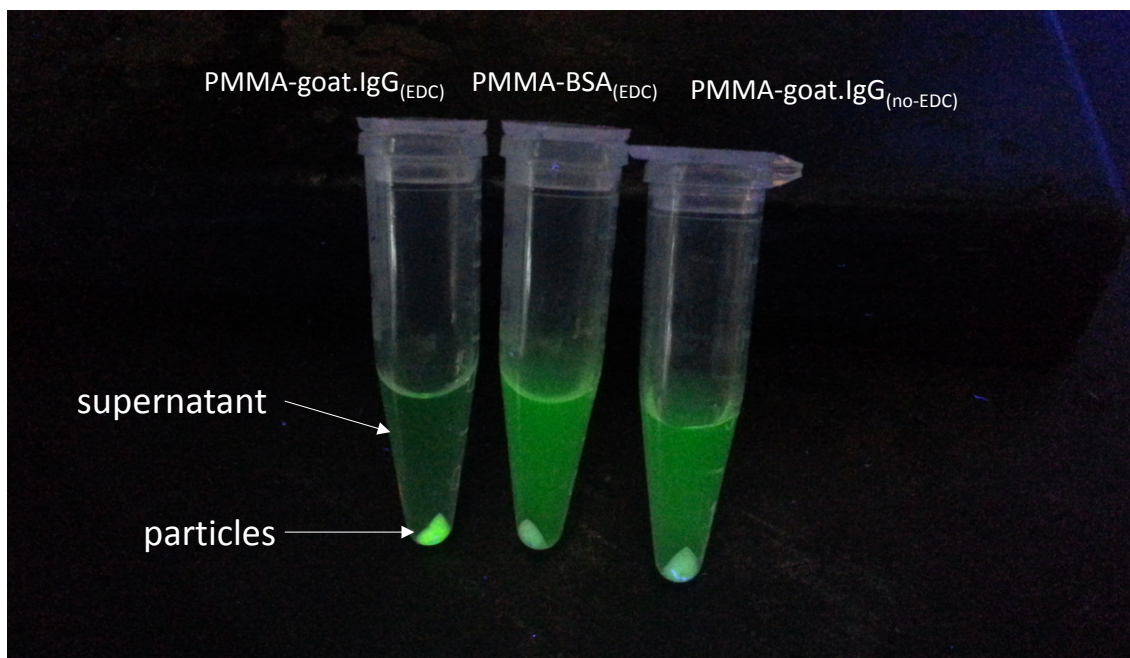
Figure S4. Optical image of the particles incubated with AF488-IgG for 3 h and pelleted by centrifugation. Unbound AF488-IgGs were left in the supernatant. Both the particles and the supernatants are excited by 365 nm UV light.
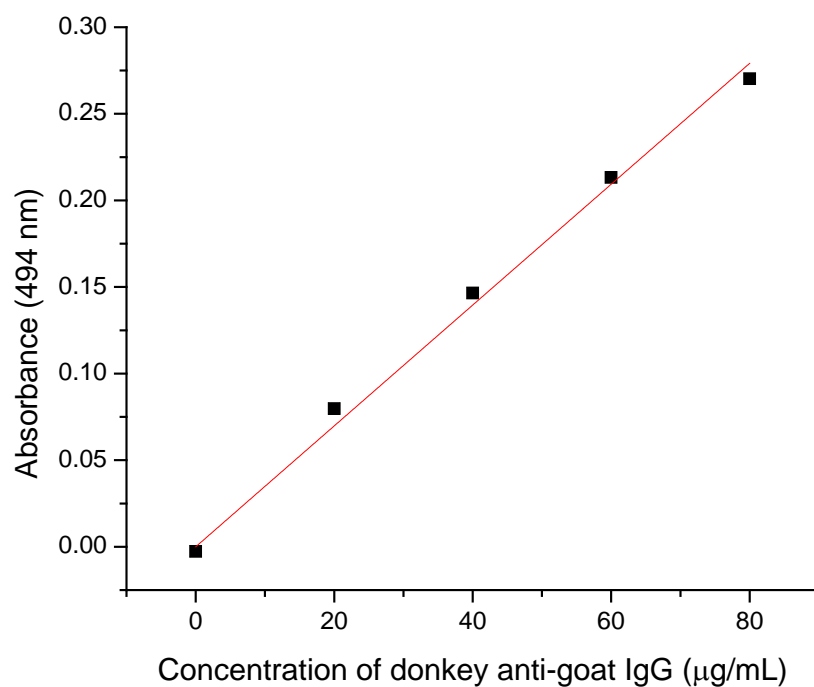
Figure S5. Standard curve of Absorbance (494 nm) versus concentration of donkey anti-goat IgG (μg/mL).

Table S1. Results of quantification of donkey anti-goat IgG bound to each particle

Sample	$A(494)$	$c_{unbound}$ (µg/mL)	$N_{AF488/particle}$
PMMA-goat.IgG$_{(EDC)}$	0.0606	16.2	1.1×10^4
PMMA-BSA$_{(EDC)}$	0.2723	78.5	2.5×10^2
PMMA-goat.IgG$_{(no\text{-}EDC)}$	0.2485	71.5	1.4×10^3

DERIVING THE RELATIONSHIP BETWEEN CV_d and PDI.

In this section, we derive the relationship between the standard deviation in a Gaussian distribution and PDI as applied to dynamic light scattering and electron microscopy measurements. We examine the parameters d_w / d_n for particles of diameter d and M_w / M_n for polymers of molar mass M as a function of their relative standard deviations σ_d and σ_M. We use the term d to describe the diameter of an individual particle in a continuous distribution and M to describe the molar mass of an individual polymer molecule in a continuous distribution. Here d_w, d_n are the weight average diameter and number average diameters, and M_w and M_n are the weight average and number average molar mass of the particle/polymer. The absolute value of the CV_d is known as the relative standard deviation (σ_d) of the diameter.

1. Derivation of the relationship between σ_d, σ_M:

Assuming that the distribution function of the mass of a particle/polymer chain obeys a normal Gaussian distribution, we can write

$$ f(M) = \left(2\pi M^{-2}\sigma_M\right)^{-\frac{1}{2}} \cdot \exp\left(-\frac{M - \overline{M}}{2\overline{M}^2 \sigma_M^2}\right), \tag{S2} $$

where σ_M is the relative standard deviation of the mass of a particle/polymer chain. For particles of diameter d, the relative standard deviation is σ_d.

The relationship between the particle diameter of a particle/polymer d and a molecular weight M follows the scaling relationship.

$$\frac{d}{\overline{d}} = \left(\frac{M}{\overline{M}}\right)^{\nu} \quad , \tag{S3}$$

The exponent ν is related to the size and shape of the particles or the conformation of the polymer in solution. For spherical particles of uniform density, $\nu = 1/3$. For a polymer chain in a good solvent, $\nu = 0.6$, whereas for a polymer in theta solvent $\nu = 0.5$.

Since $d = \overline{d} + \Delta d$ and $M = \overline{M} + \Delta M$, we can assume $\Delta M / \overline{M} \ll 1$ and write the expression

$$d = \left(\frac{M}{\overline{M}}\right)^{\nu} \cdot \overline{d} = \overline{d}\left(1 + \frac{\Delta M}{\overline{M}}\right)^{\nu} \cong \overline{d}\left(1 + \nu\frac{\Delta M}{\overline{M}}\right) \Rightarrow \Delta d \cong \nu\overline{d}\frac{\Delta M}{\overline{M}} \tag{S4}$$

Thus $\quad \langle d \rangle = \int d \cdot f(M)\mathrm{d}M = \int \overline{d} \cdot f(M)\mathrm{d}M + \int \Delta d \cdot f(M)\mathrm{d}M \quad$ and $\quad \int \overline{d} \cdot f(\mathrm{M})\mathrm{d}M = \overline{d}$

$\int \Delta d \cdot f(M)\mathrm{d}M = 0, \ \langle d \rangle = \overline{d}$

$$\langle d^2 \rangle = \int \Delta d^2 \cdot f(M) \cdot \mathrm{d}M = \int \overline{d}^2 \cdot \frac{(\Delta M)^2}{\overline{M}^2} \cdot \nu^2 \cdot f(M) \cdot \mathrm{d}M$$

$$= \int \overline{d}^2 \cdot \frac{(\Delta M)^2}{\overline{M}^2} \cdot \nu^2 \cdot f(M) \cdot \mathrm{d}M$$

$$= \frac{\overline{d}^2 \cdot \nu^2}{\overline{M}^2} \int (M^2 - 2M\overline{M} + \overline{M}^2) \cdot f(M) \cdot \mathrm{d}M$$

$$= \frac{\overline{d}^2 \cdot \nu^2}{\overline{M}^2} \left[\int M^2 \cdot f(M) \cdot \mathrm{d}M - 2\overline{M} \cdot \int M \cdot f(\mathrm{M}) \cdot \mathrm{d}M + \overline{M}^2 \cdot \int f(M) \cdot \mathrm{d}M \right]$$

$$= \frac{\overline{d}^2 \cdot \nu^2}{\overline{M}^2} \left[\overline{M}^2 + \sigma_M^2 \overline{M}^2 - 2\overline{M} \cdot \overline{M} + \overline{M}^2 \right]$$

$$= \overline{d}^2 \cdot \nu^2 \cdot \sigma_M^2 \tag{S5}$$

which implies that

$$\Rightarrow \frac{\left\langle \Delta d^2 \right\rangle}{\left\langle d \right\rangle^2} = v^2 \cdot \sigma_M^2, \tag{S6}$$

$$\text{Since } \frac{\left\langle \Delta d^2 \right\rangle}{\left\langle d^2 \right\rangle} = \sigma_d^2 \tag{S7}$$

$$\Rightarrow \sigma_d^2 = v^2 \cdot \sigma_M^2 \tag{S8}$$

Here we derived the relationship between the relative standard deviation of d and M (σ_d, σ_M) that applies both to dense particles and to polymer chains in solution. For a particle with uniform density, $v = 1/3$. For a polymer chain in a good solvent, $v = 0.6$, whereas for a polymer in theta solvent $v = 0.5$.

2. Derivation of the relationship between d_w/d_n and M_w/M_n:

Here we assume that the distribution of the particle diameter also follows a Gaussian distribution $f(d)$ and the relative standard deviation is σ_d. For particles of uniform density,

$$d_n = \frac{\sum n_i d_i}{\sum n_i} = \frac{\int d \cdot f(d) \mathrm{d}(d)}{\int f(d) \mathrm{d}(d)} = \overline{d}, \tag{S9}$$

$$d_w = \frac{\sum n_i d_i M_i}{\sum n_i M_i} = \frac{\sum n_i d_i \cdot d_i^3}{\sum n_i \cdot d_i^3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} = \frac{\int d^4 f(d) \mathrm{d}(d)}{\int d^3 f(d) \mathrm{d}(d)} \tag{S10}$$

Note that $\int d^3 f(d) \mathrm{d}(d)$ is the third moment of Gaussian function which is $\overline{d}^3 + 3\overline{d}^3 \cdot \sigma_d^2$

The fourth moment of Gaussian function $\int d^4 f(d) \mathrm{d}(d)$ is $\overline{d}^4 + 6\overline{d}^4 \cdot \sigma_d^2 + 3\sigma_d^4 \cdot \overline{d}^4$

$$\Rightarrow d_w = \frac{\overline{d}^4 (1 + 6\sigma_d^2 + 3\sigma_d^4)}{\overline{d}^3 (1 + 3\sigma_d^2)} = \overline{d}\left(\frac{1 + 6\sigma_d^2 + 3\sigma_d^4}{1 + 3\sigma_d^2}\right) \tag{S11}$$

$$\Rightarrow \frac{d_w}{d_n} = \frac{1 + 6\sigma_d^2 + 3\sigma_d^4}{1 + 3\sigma_d^2} = 1 + 3\frac{\sigma_d^2(1 + \sigma_d^2)}{1 + 3\sigma_d^2} \cong 1 + 3\sigma_d^2 \tag{S12}$$

Here we assume $(\sigma_d^2 \ll 1)$.

For the molecular weight of a polymer chain or the mass of a particle

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\int M^2 \cdot f(M)\mathrm{d}M}{\int M f(M)\mathrm{d}M} = \frac{\overline{M}^2 (1 + \sigma_M^2)}{\overline{M}} = \overline{M}(1 + \sigma_M^2) \tag{S13}$$

$$M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\int M f(M)\mathrm{d}M}{\int f(M)\mathrm{d}M} = \overline{M} \tag{S14}$$

$$\Rightarrow \frac{M_w}{M_n} = 1 + \sigma_M^2 \tag{S15}$$

Thus the relationship between the standard deviations σ_d, σ_M, and the ratios $\dfrac{M_w}{M_n}, \dfrac{d_w}{d_n}$ are given by the expression $\sigma_d^2 = v^2 \sigma_M^2$. For particles, $v = 1/3$, and $\sigma_d = \sigma_M / 3$. Thus

$$\frac{d_w}{d_n} \cong 1 + 3\sigma_d^2 \tag{S16}$$

$$\frac{M_w}{M_n} = 1 + \sigma_M^2 \tag{S17}$$

Expression S16 corresponds to eq 3 in the main text for the case where CV_d is equal to the relative standard deviation σ_d.

3. Proof of the relationship $\dfrac{\langle d_h{}^2 \rangle}{\langle d_h \rangle^2} = \sigma_{d_h}^2$ **for PDI from DLS measurements**

For a polydispersed system, the electric-field autocorrelation function is

$$|g_1(\tau)| = \int G(\Gamma)\exp(-\Gamma\tau)\mathrm{d}\Gamma \tag{S18}$$

$$= \int \Gamma G(\Gamma)\exp(-\Gamma\tau)\mathrm{d}\ln\Gamma \tag{S19}$$

Γ is the decay rate of the electric-field autocorrelation function and $G(\Gamma)$ represents the contribution of each Γ and is normalized.

Then

$$\frac{\partial \ln|g_1(\tau)|}{\partial \tau} = -|g_1(\tau)|^{-1}\cdot\int \Gamma G(\Gamma)\exp(-\Gamma\tau)\mathrm{d}\Gamma \tag{S20}$$

$$\left.\frac{\partial \ln|g_1(\tau)|}{\partial \tau}\right|_{\tau=0} = -\langle\Gamma\rangle \tag{S21}$$

$$\frac{\partial^2 \ln|g_1(\tau)|}{\partial \tau^2} = |g_1(\tau)|^{-1}\cdot\int \Gamma^2 G(\Gamma)\exp(-\Gamma\tau)\mathrm{d}\Gamma \tag{S22}$$

$$\left.\frac{\partial^2 \ln|g_1(\tau)|}{\partial \tau^2}\right|_{\tau=0} = \langle\Gamma^2\rangle - \langle\Gamma\rangle^2 = \langle\Delta\Gamma^2\rangle \tag{S23}$$

The Taylor expansion of $\ln|g_1(\tau)|$ at $\tau = 0$ is expressed as

$$\ln|g_1(\tau)| = -\langle\Gamma\rangle\tau + \frac{1}{2}\langle\Delta\Gamma^2\rangle\tau^2 \dots\dots \tag{S24}$$

which is an expression of the cumulant analysis employed in light scattering.

Here the PDI obtained in light scattering is defined as

$$\mathrm{PDI} = \langle\Delta\Gamma^2\rangle / \langle\Gamma\rangle^2 \tag{S25}$$

Since $\Gamma \propto D_{\mathrm{diff}} \propto d_h^{-1}$, where D_{diff} is the diffusion coefficient of a particle or a polymer chain

$$\frac{\left\langle \Delta \Gamma^2 \right\rangle}{\left\langle \Gamma \right\rangle^2} = \frac{\left\langle \Delta D_{diff}{}^2 \right\rangle}{\left\langle D_{diff} \right\rangle^2} \tag{S26}$$

Then we can use the expression $\sigma_D^2 = v^2 \cdot \sigma_M^2$ (eq S8), with $v = -1$ for the relationship between D_{diff} and d_{h}. Thus

$$\text{PDI} = \frac{\left\langle \Delta \Gamma^2 \right\rangle}{\left\langle \Gamma \right\rangle^2} = \frac{\left\langle \Delta D_{diff}{}^2 \right\rangle}{\left\langle D_{diff} \right\rangle^2} \cong \frac{\left\langle \Delta d_h{}^2 \right\rangle}{\left\langle d_h \right\rangle^2} \cong \sigma_{d_h}^2 \tag{S27}$$

Here σ_{d_h} is the relative standard deviation of the hydrodynamic diameter.