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Supporting information

A Green and Scalable Route to Water-in-Water Emulsion Stabilizers Using Photoenzymatic Polymerization-Induced Self-Assembly

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1. Materials

N,N-Dimethylacrylamide (DMA, 99%) was purchased from Sigma-Aldrich. Diacetone acrylamide (DAAM, $\geq 99\%$), poly(ethylene glycol) (PEG, average M_n 10000), and fluorescein-5-thiosemicarbazide ($\geq 95\%$) were purchased from Aladdin. 4-((((2-Carboxyethyl)thio)carbonothioyl)thio)-4-cyanopentanoic acid (CTPA, 95%) was purchased from Laajoo. Glucose oxidase (GOx, from *Aspergillus niger*, 200 U mg⁻¹) was purchased from J&K Scientific. D-(+)-glucose ($\geq 99.5\%$) and FITC-dextran (M_w 70000) were purchased from Adamas. *N,N*-Dimethylformamide (DMF, $>99.9\%$), D₂O (99.9% D), and DMSO-*d*₆ (99.8% D) were purchased from Energy-Chemical. Dextran (Dex, M_w 70000) was purchased from Innochem.

2. Characterization

Nuclear magnetic resonance (NMR) spectroscopy: ¹H NMR spectra were acquired using a Zhongke-Niujin AS400 (400 MHz) instrument at room temperature using either D₂O or DMSO-*d*₆ as the solvent. An aliquot of the polymerizing reaction mixture was sampled and immediately quenched via dilution with either DMSO-*d*₆ or D₂O. Chemical shifts were referenced to residual solvent signals.

Gel permeation chromatography (GPC): GPC analyses were performed using an Agilent 1260 Infinity II GPC system equipped with an Optilab refractive index detector (Wyatt Technology Corporation) and an 8-angle laser light scattering detector (Wyatt DAWN8, Wyatt Technology Corporation) at 40 °C. The mobile phase consisted of HPLC-grade DMF plus LiBr (1.75 g dm⁻³) and the flow rate was 0.75 mL min⁻¹. Separation was achieved using a Shodex guard column (P8514 KD000DMF) and two Shodex analytical columns (KD-803 and KD-805, 8 mm × 300 mm each). To remove salts introduced by phosphate buffer, dried copolymer (approximately 3 mg) samples were subjected to desalting with THF and subsequently thoroughly dried. The resulting polymers were then dissolved in 1.5 mL DMF and allowed to stand overnight.

Transmission electron microscopy (TEM): TEM studies were performed using a JEM-2100 electron microscope. A 10 μ L aliquot of a freshly prepared 0.05% w/v aqueous dispersion of block copolymer nanoparticles was deposited onto a Cu grid, and after 30 min the excess liquid was carefully removed by blotting with filter paper. Imaging was performed at an accelerating voltage of 1200 kV.

Dynamic light scattering (DLS): DLS analyses were performed using a Malvern Zetasizer Pro instrument equipped with a 4 mW He-Ne 633 nm laser and an avalanche photodiode detector at 25 °C. The as-prepared aqueous dispersions of diblock copolymer nanoparticles were diluted to either 1.0% w/v or 0.2% w/v using deionized water prior to analysis.

Fluorescence microscopy: Fluorescence microscopy analysis was conducted using a Nikon Ts2-FL inverted microscope equipped with an LED illuminator and three different fluorescence units, with high-intensity LED sources employed for both diascopic and epi-fluorescence illumination. A 100 μ L pipette tip was slowly inserted into the vial. With minimal disturbance to the bulk system, a 30 μ L aliquot was gently extracted from the emulsion layer. The aliquot was then carefully deposited onto a clean glass slide and covered with a coverslip. To prevent any mechanical deformation or forced coalescence of the w/w droplets, no external pressure was applied to the coverslip. The sample was then immediately observed under fluorescence microscopy. Droplet size analysis was conducted using Cellpose and Image J software.

3. Experimental protocols

Synthesis of PDMA precursor

The synthesis of a PDMA₅₁₈ precursor is representative. CTPA (1.1769×10^{-5} mol, 3.6179 mg), DMA (7.06×10^{-3} mol, 0.70 g), GOx (7.00×10^{-9} mol, 1.05 mg), and glucose (0.175 mol, 31.528 mg) were added to a 4 mL glass vial equipped with a magnetic stirring bar. The total solution volume was adjusted to 3.5 mL by addition of desired amount of phosphate buffer (0.2 M, pH 5.8). The vial was sealed, purged with Ar for 30 min, placed in a water bath set at 30 °C, and subsequently irradiated under blue LED light (462 nm, 20.0 mW cm⁻²). After 6 h, the LED light was switched off, and the DMA polymerization was quenched by exposure to air. An aliquot of the reaction mixture was sampled for ¹H NMR and GPC analyses. The mean DP was calculated to be 518 according to the final monomer conversion determined by ¹H NMR spectroscopy. GPC analysis confirmed the synthesis of a low-dispersity PDMA homopolymer with an M_n that closely matched the theoretical value: $M_{n,th} = 51.7$ kg mol⁻¹, $M_n = 51.2$ kg mol⁻¹, $\mathcal{D} = 1.04$.

Synthesis of diblock copolymer nanoparticles via photoenzymatic RAFT dispersion copolymerization in 96-well plates

The synthesis of PDMA₅₁₈-*b*-P(DMA₂₀₀₀-*co*-DAAM₈₀₀₀) is representative. The following components were loaded into an individual well on a 96-well plate: PDMA₅₁₈ precursor solution without further purification (1.9384×10^{-5} mol, 1.9216 mg), with the unreacted DMA incorporated into the comonomer feed for the second block, DAAM (2.9937×10^{-4} mol, 50.6593 mg), DMA (7.2193×10^{-5} mol, 7.1565 mg), GOx (6.0×10^{-10} mol, 0.09 mg), glucose (6.0×10^{-6} mol, 1.08 mg), 3 μ L DMF (serve as an internal standard), and phosphate buffer (0.2 M, pH 5.8) were added to make up a total volume of 300 μ L. 10 μ L of this reaction mixture was extracted before the wells were sealed with a plastic cover. The plate was then placed in an incubator shaker (200 rpm, 30 °C) under blue LED light irradiation (462 nm, 20.0 mW cm⁻²). The plate was removed from the LED light after 4 h and the solution was exposed to air to quench the copolymerization. An aliquot was taken from the reaction mixture for ¹H NMR, GPC, DLS and TEM analyses.

Scale-up synthesis of diblock copolymer nanoparticles via dispersion photoenzymatic RAFT polymerization

In order to prepare sufficient amounts of diblock copolymer nanoparticles for emulsion studies, we scaled up the dispersion polymerization from 300 μ L to 4.8 mL and 120 mL under otherwise identical conditions. These larger scale polymerizations produced diblock copolymer nanoparticles of comparable molecular weights and diameters, thus demonstrating the scalability of this synthetic approach.

Preparation of water-in-water emulsions using diblock copolymer nanoparticles as stabilizers

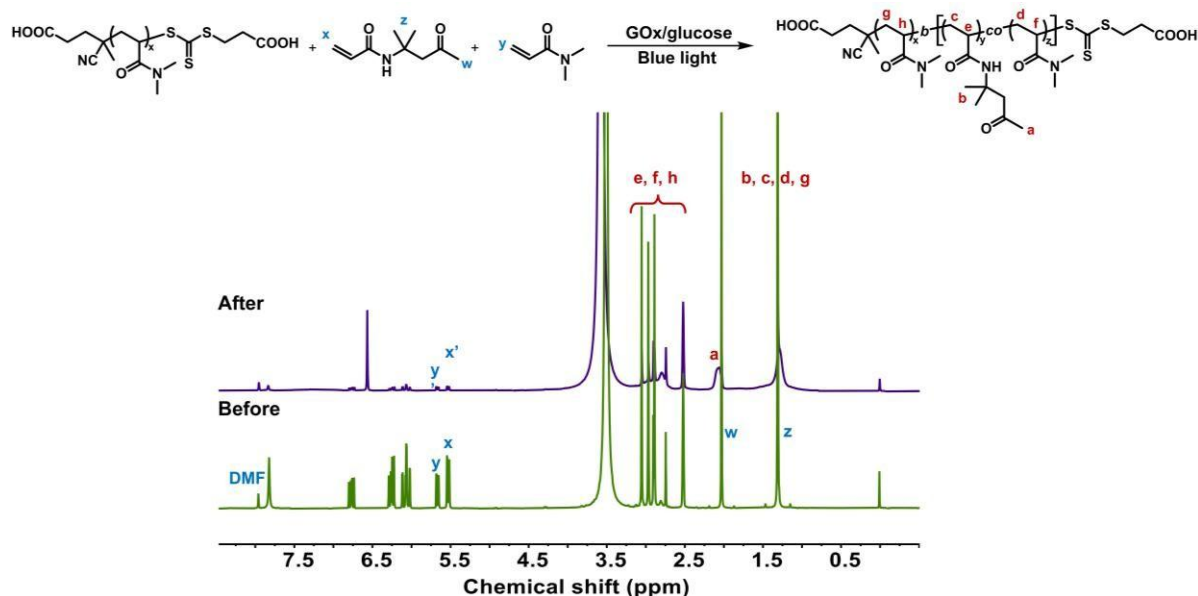
The w/w emulsions were composed of Dex_{70k} (150 mg, 7.5 wt %), PEG_{10k} (280 mg, 14.0 wt %), and diblock copolymer nanoparticles (1.0 wt %). 1.2 mL of a dextran stock solution (18.75 wt%, containing 1% w/w FITC-Dex as a fluorescent label) and 1.5 mL of a PEG stock solution (28.00 wt%) were added to a 4 mL vial, followed by the addition of diblock copolymer nanoparticles. The mixture was vortexed for 60 seconds at 3500 rpm to form an emulsion. Control samples without diblock copolymer nanoparticles were similarly prepared for comparison.

Fluorescent labeling of nanoparticles using fluorescein-5-thiosemicarbazide

Fluorescent labeling was performed on NP 2 according to a previously published protocol.¹ The as-prepared NP 2 dispersion (300 μ L, containing 0.2367 mmol of DAAM units) was diluted with phosphate buffer (3.0 mL, 0.2 M,

pH 5.8), to which fluorescein-5-thiosemicarbazide (1.4 mg, 0.002367 mmol) was then added. The mixture was stirred at 200 rpm for 36 h at room temperature and subsequently dialyzed against water using a dialysis bag (MWCO 8–10 kDa) until no fluorescence was detected in the outside aqueous solution. The final fluorescently labeled nanoparticles were then characterized by DLS and the data compared to that of the original nanoparticles.

4. Supplementary Figures and Tables



DMF was employed as an internal standard, and its NMR signal integral was normalized to “1” both before and after polymerization. The monomer conversions were calculated as follows:

$$\text{Conv. of DMA (\%)}: (1 - y'/y) \times 100\%$$

$$\text{Conv. of DAAM (\%)}: (1 - x'/x) \times 100\%$$

1

Figure S1. ^1H NMR spectra recorded in $\text{DMSO-}d_6$ for the synthesis of a $\text{PDMA}_{518}\text{-}b\text{-P(DMA}_{3096}\text{-co-DAAM}_{5142})$ diblock copolymer before and after copolymerization.

Table S1. Photoenzymatic RAFT dispersion copolymerization of DMA with DAAM using a PDMA_{234} precursor.

Entry	DMA (mol%) ^a	Target DP ^b	DMA conversion (%) ^c	DAAM conversion (%) ^c	$M_{n,th}$ (kg mol ⁻¹) ^d	M_n (kg mol ⁻¹) ^e	$\mathcal{D}^e$
1 ^f	0	2000	—	95.1	345	519	1.98
2 ^f	0	6000	—	93.5	973	1900	1.70
3 ^f	0	10000	—	96.2	1650	1640	1.47
4 ^g	20	2000	93.2	97.2	324	278	1.38
5 ^g	20	6000	89.9	95.8	909	903	1.54
6 ^g	20	10000	73.3	92.8	1430	1650	1.40

Polymerization conditions: 20% w/v, volume = 300 μL , $[\text{GOx}] = 60 \text{ U mL}^{-1}$, $[\text{glucose}] = 20 \text{ mM}$, phosphate buffer (0.2 M, pH 5.8), blue light (462 nm, 30 mW cm^{-2}), 200 rpm, 30 $^{\circ}\text{C}$. [a] DMA mole fraction in the comonomer feed. [b] Total degree of polymerization for the core-forming block. [c] Monomer conversion determined by ^1H NMR spectroscopy studies performed in $\text{DMSO-}d_6$. [d] Theoretical molecular weight calculated from the comonomer conversion. [e] Molecular weight and dispersity determined by GPC analysis. [f] After 3 h. [g] After 4 h.

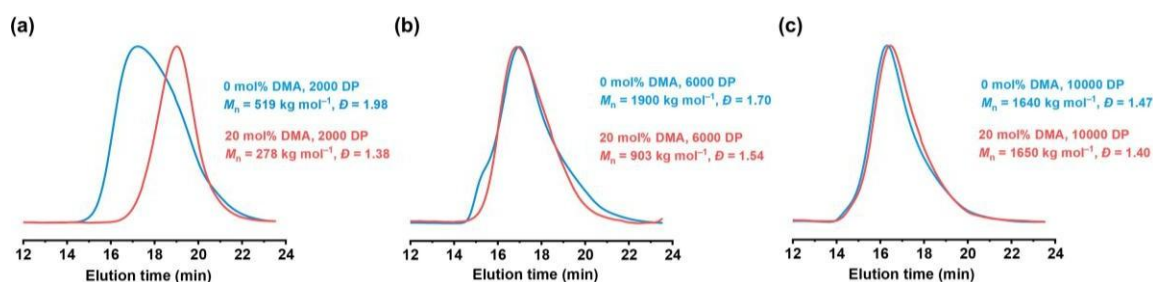


Figure S2. GPC traces recorded for photoenzymatic RAFT dispersion copolymerization targeting (a) DP = 2000, (b) DP = 5000 and (c) DP = 10000 in the presence of 0 or 20 mol% DMA comonomer with a PDMA_{234} precursor.

Table S2. Summary of characterization data obtained for diblock copolymers prepared via photoenzymatic RAFT dispersion copolymerization in a 96-well plate using a PDMA₅₁₈ precursor.

Entry	DMA (mol%) ^a	Target DP ^b	DMA conversion (%) ^c	DAAM conversion (%) ^c	$M_{n, th}$ (kg mol ⁻¹) ^d	M_n (kg mol ⁻¹) ^e	$\bar{D}$ ^e	D_h (nm) ^f	PDI ^f
1	0	2000	—	95.8	376	499	2.08	233	0.05
2	0	5000	—	97.0	872	1530	1.58	331	0.08
3	0	10000	—	96.1	1680	2380	1.68	420	0.16
4	10	10000	96.1	97.7	1630	2220	1.64	356	0.16
5	20	1000	90.8	97.8	202	211	1.65	181	0.02
6	20	2000	92.2	96.2	348	496	1.56	211	0.03
7	20	5000	84.6	87.9	730	1370	1.47	253	0.03
8	20	10000	90.6	93.9	1500	2340	1.63	347	0.16
9	20	15000	86.4	95.5	2250	2230	1.42	382	0.14
10	20	20000	85.5	94.3	2940	1930	1.36	421	0.12
11	30	10000	91.8	96.1	1460	2070	1.42	322	0.12
12	40	5000	95.1	97.1	733	866	1.20	191	0.03
13	40	10000	90.4	93.6	1360	1690	1.22	275	0.06

Polymerization conditions: 20% w/v, volume = 300 μ L, [GOx] = 60 U mL⁻¹, [glucose] = 20 mM, phosphate buffer (0.2 M, pH 5.8), blue light (462 nm, 20 mW cm⁻²), 200 rpm, 30 °C, 5 h. [a] Monomer conversion determined by ¹H NMR spectroscopy studies performed in DMSO-*d*₆. [b] Theoretical molecular weight calculated according to the comonomer conversion. [c] Molecular weight and dispersity determined by GPC analysis. [d] Hydrodynamic z-average diameter determined by dynamic light scattering (DLS) studies performed at 25 °C.

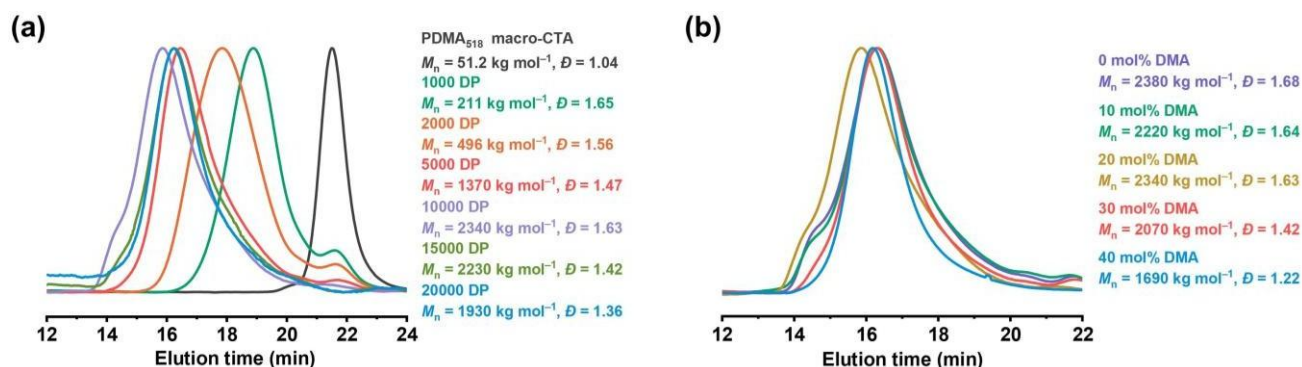
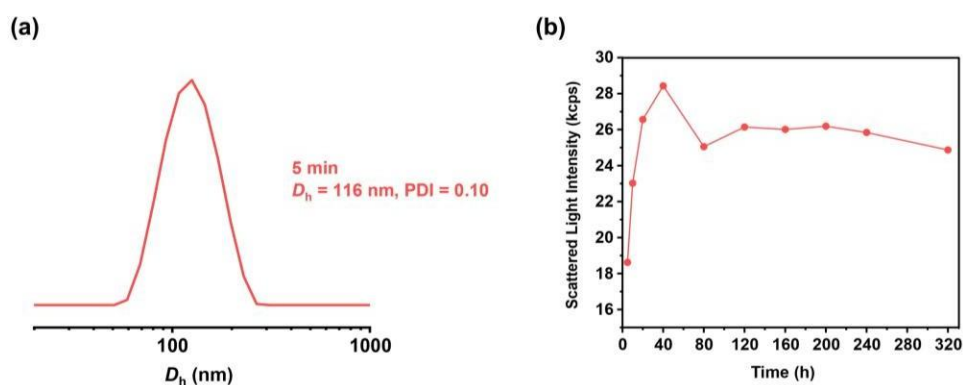


Figure S3. GPC traces recorded for the PDMA₅₁₈ precursor and the corresponding diblock copolymers prepared via RAFT dispersion copolymerization. (a) Target DPs = 1000 to 20 000 for 20 mol% DMA comonomer. (b) Target DP = 10000 for 0 to 40 mol% DMA comonomer.

Table S3. Polymerization kinetics data obtained during the synthesis of PDMA₅₁₈-*b*-P(DMA₄₀₀₀-*co*-DAAM₆₀₀₀).

Entry No.	Time (min)	DMA conversion (%) ^a	DAAM conversion (%) ^a	$M_{n, th}$ (kg mol ⁻¹) ^b	M_n (kg mol ⁻¹) ^c	$\bar{D}$ ^c	D_h (nm) ^d	PDI ^d
1	5	1.2	1.3	70	N.D.	N.D.	116	0.10
2	10	2.7	2.0	83	N.D.	N.D.	137	0.02
3	20	4.2	4.5	114	N.D.	N.D.	164	0.01
4	40	8.6	12.1	208	N.D.	N.D.	181	0.03
5	80	24.2	31.5	467	N.D.	N.D.	240	0.02
6	120	37.8	48.1	690	987	1.27	279	0.01
7	160	48.4	61.5	868	1380	1.27	305	0.02
8	200	60.7	72.4	1030	N.D.	N.D.	323	0.01
9	240	71.6	80.2	1150	1760	1.25	337	0.01
10	320	82.7	88.4	1280	2020	1.28	341	0.03

Polymerization conditions: 4.8 mL, 20% w/v, [GOx] = 60 U mL⁻¹, [glucose] = 50 mM, phosphate buffer (0.2 M, pH 5.8), blue light (462 nm, 20 mW cm⁻²), 30 °C, 200 rpm; 'N.D.' denotes not determined. [a] Monomer conversion determined by ¹H NMR spectroscopy studies performed in DMSO-*d*₆. [b] Theoretical molecular weight calculated according to the comonomer conversion. [c] Molecular weight and dispersity determined by GPC analysis. [d] Hydrodynamic z-average diameter determined by dynamic light scattering (DLS) studies performed at 25 °C.

**Figure S4.** DLS characterization of aliquots withdrawn during the kinetic experiments (see Table S3): (a) DLS intensity-average size distribution after 5 min; (b) light scattering intensity determined at various time points.

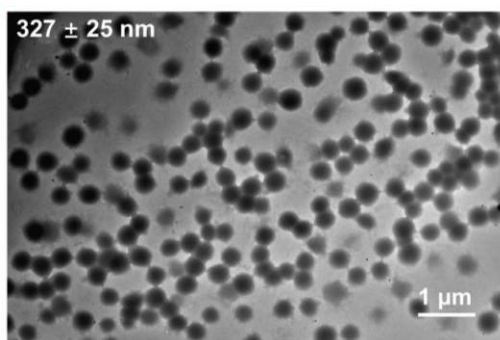
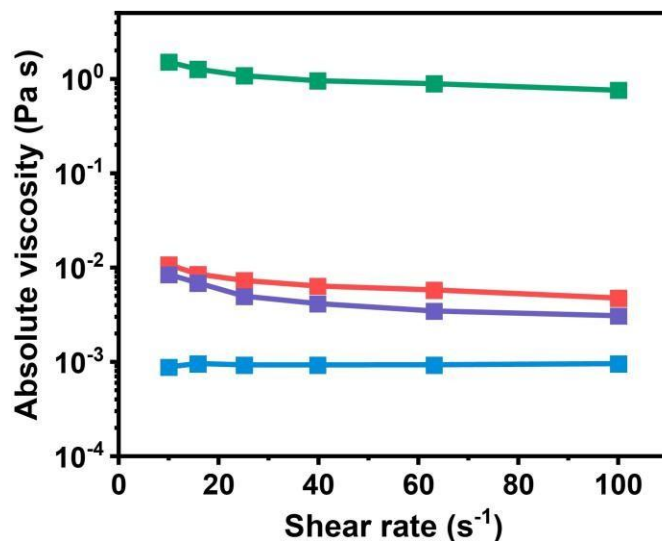


Figure S5. Representative TEM image recorded for the PDMA₅₁₈-*b*-P(DMA₄₀₀₀-*co*-DAAM₆₀₀₀) block copolymer nanoparticles (entry 10 in Table S3).



9

Figure S6. Absolute viscosity vs. shear rate plot obtained from rotational rheology studies of (i) phosphate buffer (0.2 M, pH 5.8) (blue), (ii) DMSO (purple), (iii) 10% w/v aqueous dispersion of PDMA₅₁₈-*b*-P(DMA₃₀₉₆-*co*-DAAM₅₁₄₂) diblock copolymer nanoparticles dispersed in phosphate buffer (red), and (iv) 10% w/v PDMA₅₁₈-*b*-P(DMA₃₀₉₆-*co*-DAAM₅₁₄₂) diblock copolymer chains dissolved in DMSO (green).

Table S4. Summary of characterization data obtained for five examples of block copolymer nanoparticles comprising with 40 mol% DMA as prepared via photoenzymatic RAFT dispersion copolymerization.

Sample ID ^a	Target DP ^b	DMA conversion (%) ^c	DAAM conversion (%) ^c	$M_{n, th}$ (kg mol ⁻¹) ^d	M_n (kg mol ⁻¹) ^e	$\bar{D}^e$	D_h (nm) ^f	PDI ^f
NP 1	2000	92.8	94.3	317	368	1.12	134	0.04
NP 2	5000	95.0	96.0	727	911	1.26	199	0.05
NP 3	7500	97.5	97.4	1080	1320	1.24	240	0.06
NP 4	10000	96.9	97.7	1430	1900	1.22	306	0.09
NP 5	20000	94.8	98.0	2790	2930	1.26	372	0.03

Polymerization conditions: 4.8 mL, 20% w/v, [GOx] = 60 U mL⁻¹, [glucose] = 50 mM, PB buffer (0.2 M, pH 5.8), blue light (462 nm, 20 mW cm⁻²), 200 rpm, 30 °C, 5 h. [a] "NP" stands for block copolymer nanoparticles. [b] Total degree of polymerization for the core-forming block. [c] Monomer conversion determined by ¹H NMR spectroscopy in DMSO-*d*₆. [d] Theoretical molecular weight calculated according to monomer conversion. [e] Molecular weight and dispersity determined by GPC. [f] Hydrodynamic diameter (D_h) and polydispersity index (PDI) determined by dynamic light scattering (DLS).

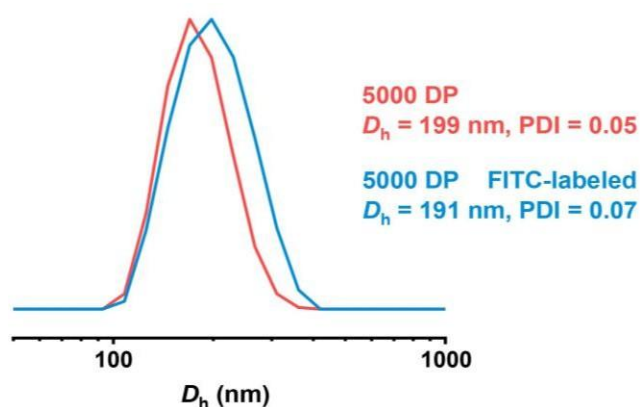


Figure S7. DLS particle size distributions recorded for the NP 2 emulsifier before and after fluorescent labeling.

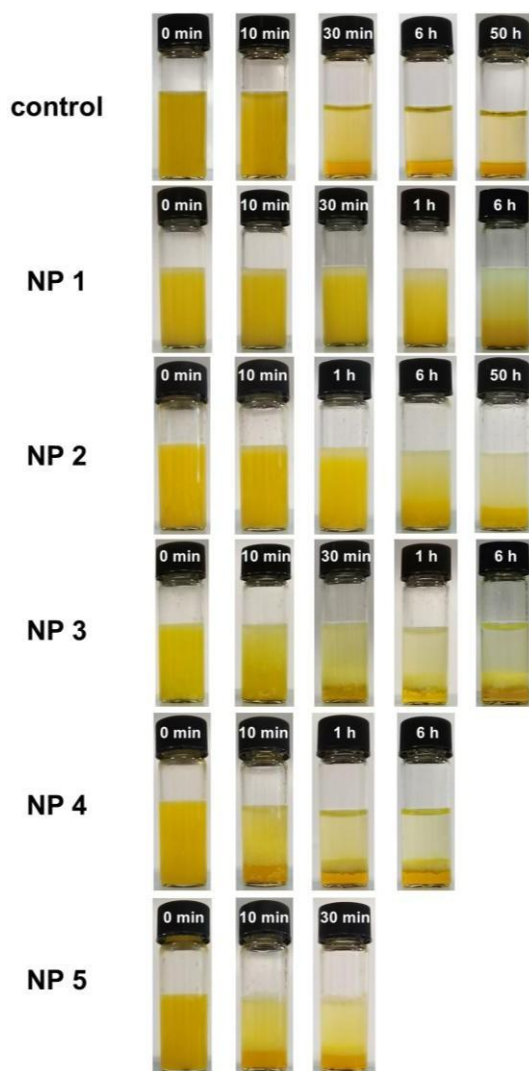


Figure S8. Digital photographs recorded for w/w emulsions at varying time points: the first row is the control group without any stabilizer, and the following five rows are the emulsions stabilized using NP 1 to NP 5, respectively.

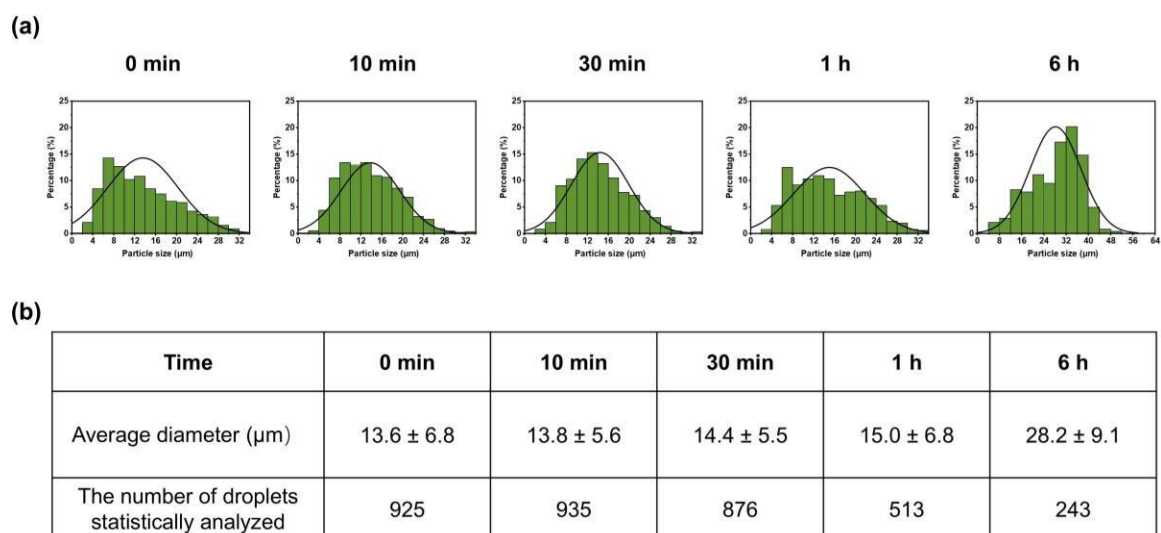
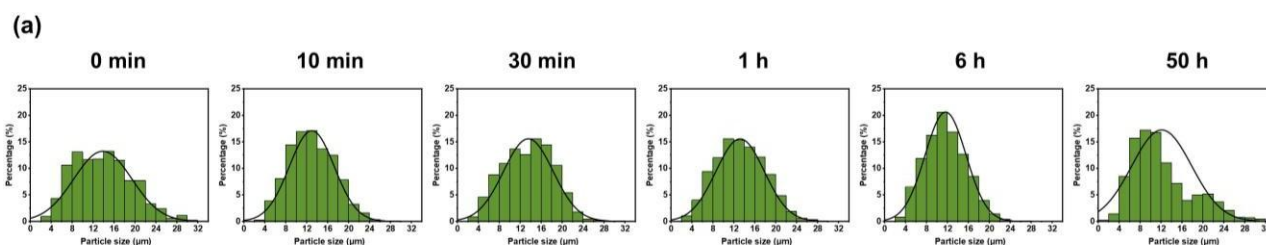


Figure S9. Droplet size data obtained for w/w emulsions stabilized using NP 1. (a) Temporal evolution of the droplet size distribution. (b) The mean droplet diameter calculated from statistical analysis, plus the number of droplets analyzed in each case.



(b)

Time	0 min	10 min	30 min	1 h	6 h	50 h
Average diameter (μm)	13.8 ± 5.5	13.0 ± 4.3	13.5 ± 4.7	13.2 ± 4.6	11.7 ± 3.9	12.1 ± 5.7
The number of droplets statistically analyzed	885	1428	1054	1177	1257	905

Figure S10. Droplet size data obtained for w/w emulsions stabilized using NP 2. (a) Temporal evolution of the droplet size distribution. (b) The mean droplet diameter calculated from statistical analysis, plus the number of droplets analyzed in each case.

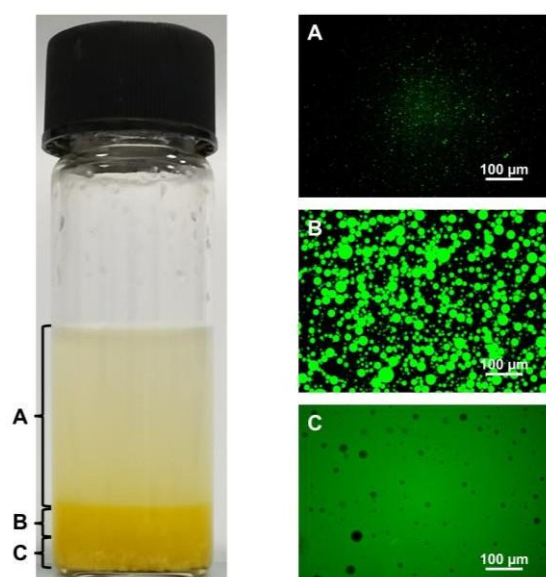


Figure S11. (a) Digital photograph recorded for a w/w emulsion (14.0 wt % PEG, 7.5 wt % Dex) stabilized by 1 wt % NP 2, recorded after 50 h. (b) Corresponding fluorescence micrographs obtained for different regions within the emulsion.

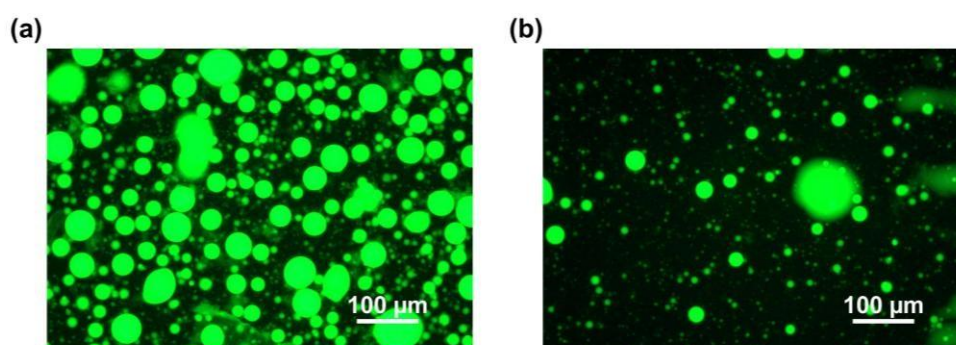


Figure S12. Fluorescence micrographs for a w/w emulsion (14.0 wt % PEG, 7.5 wt % Dex) stabilized by 2.0 wt % NP 2, recorded after (a) 0 min and (b) 10 min.

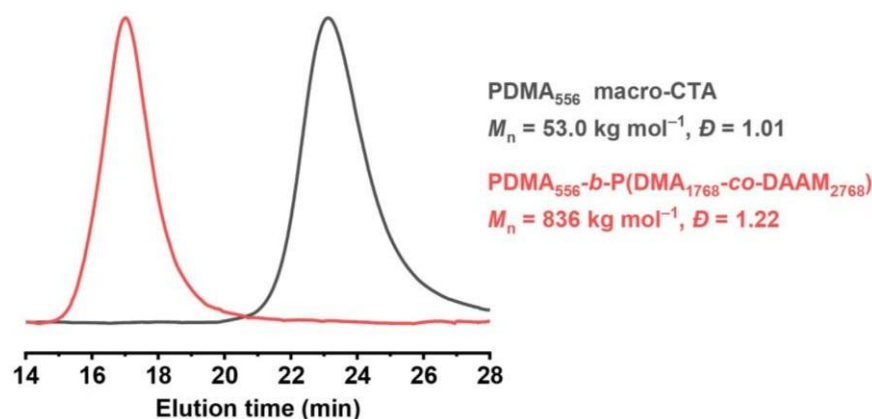


Figure S13. GPC traces recorded for the diblock copolymer synthesized in 120 mL solution and the corresponding PDMA₅₅₆ precursor.

Table S5. Zeta potential measurements of the block copolymer nanoparticles.

Sample	Zeta Potential (mV)
NP 1	-0.3
NP 2	-1.8
NP 3	-0.2
NP 4	+0.1
NP 5	-1.7

Measurement condition: To eliminate any potential viscosity-induced artifacts caused by the PEG/dextran polymer solutions, a standard 1 mM NaCl aqueous solution was employed as the background electrolyte. The pH of this background solvent was pre-adjusted to 7.7 (matching the native pH of the emulsion), and zeta potential measurements were then performed at a nanoparticle concentration of 0.1 wt%.

Calculation of the theoretical adsorbed amount of PDMA₅₁₈ chains on the nanoparticle surface:

The calculation of Γ was performed under two assumptions: (i) all PDMA chains are located at the nanoparticle surface, and (ii) the nanoparticles are ideal solid spheres. Γ was then obtained by dividing the total mass of PDMA₅₁₈ chains (with mass fraction determined by ¹H NMR) by the total nanoparticle surface area.

The calculation formula used are as follows:

$$\Gamma = \frac{\rho \times \frac{4}{3}\pi R^3 \times M_{\text{PDMA}_{518}}}{4\pi R^2 \times M}$$

M: Molecular weight of polymer chain.

ρ : Density of the nanoparticle.

R: Particle radius determined by TEM.

Reference

1. W. Zhou, Q. Qu, Y. Xu and Z. An, Aqueous Polymerization-Induced Self-Assembly for the Synthesis of Ketone-Functionalized Nano-Objects with Low Polydispersity, *ACS Macro Letters*, 2015, **4**, 495-499.