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Shen, A., Armes, S.P. and An, Z. (2026) A green and scalable route to water-in-water emulsion stabilizers using photoenzymatic polymerization-induced self-assembly. Science China Chemistry. ISSN: 1674-7291

<https://doi.org/10.1007/s11426-026-3499-2>

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ARTICLES

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

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Received *****; Accepted *****; Published online *****

Abstract Water-in-water (w/w) emulsions are biphasic all-aqueous systems with promising applications in bioreactors and drug delivery. However, their stabilization is challenging due to an extremely low interfacial tension. We report a green and scalable strategy based on photoenzymatic reversible addition-fragmentation chain transfer (RAFT) aqueous dispersion copolymerization to prepare well-defined diblock copolymer nanoparticles and demonstrate their efficacy as stabilizers for w/w emulsions. Using poly(*N,N*-dimethylacrylamide) as a macromolecular chain transfer agent, we synthesized a series of spherical nanoparticles with controlled molecular weights and tunable diameters via statistical copolymerization of diacetone acrylamide with *N,N*-dimethylacrylamide. These sterically-stabilized nanoparticles effectively stabilize w/w emulsions composed of immiscible aqueous solutions of poly(ethylene glycol) and dextran. Notably, nanoparticles of intermediate diameter ($D_h = 199$ nm) conferred extraordinary stability, maintaining emulsion stability for more than 50 h. This study establishes an environmentally benign and scalable approach to prepare tailorable nanoparticles for biocompatible w/w emulsions, which offer potential biomedical applications.

Keywords water-in-water emulsion, polymerization-induced self-assembly (PISA), photoenzymatic RAFT polymerization.

Introduction

Water-in-water (w/w) emulsions are all-aqueous, heterogeneous systems formed by mixing two incompatible salt or polymer solutions to produce a biphasic system whereby one aqueous phase is dispersed as microdroplets within the other.[1, 2] These systems are inherently biocompatible and have attracted significant interest as versatile platforms for biomedical applications, including cell micropatterning, biomolecule encapsulation, and serving as media for chemical reactions.[3-6] However, stabilizing w/w emulsions against droplet coalescence is challenging due to their extremely low interfacial tension and susceptibility towards Ostwald ripening.[7-9]

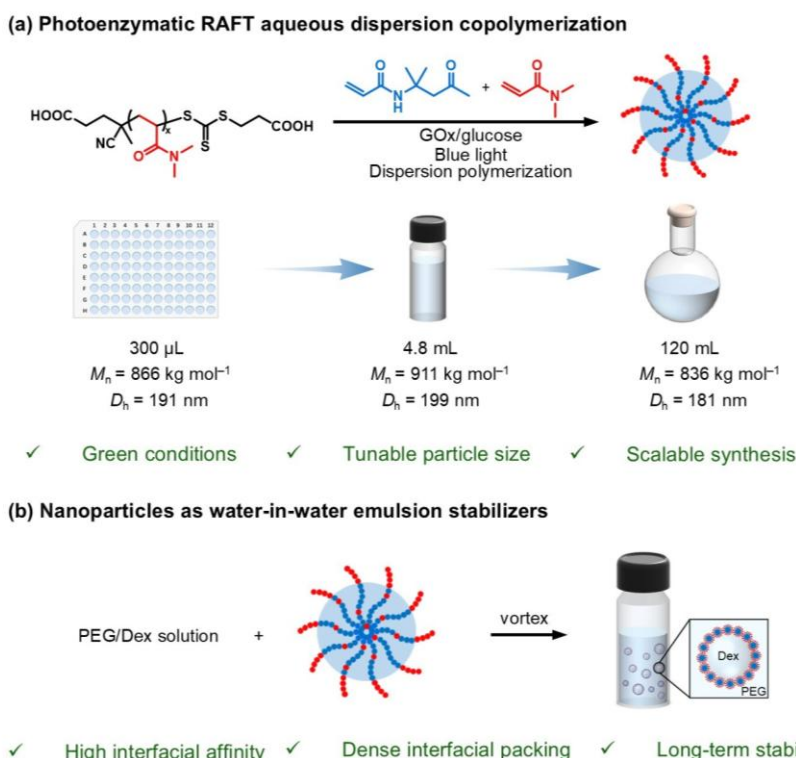
Conventional surfactants are generally ineffective in stabilizing w/w emulsions, which has led to the evaluation of various alternative stabilizers such as bespoke block copolymers and colloidal particles.[10-17] In particular, Pickering stabilization using silica particles, microgels, or 2D diblock copolymer platelets has proven to be effective.[18-20] The efficacy of such particles depends critically on their size, shape, and interfacial contact angle, which must be optimized to achieve irreversible adsorption and hence form a robust physical barrier against droplet coalescence. Despite their effectiveness, the current range of stabilizers often lacks the synthetic versatility and scalability needed for further fine-tuning of these key parameters. Therefore, developing a generic, scalable method to produce nanoparticles of

programmable size and surface properties for the preparation of w/w emulsions remains a key objective.

Polymerization-induced self-assembly (PISA) has emerged as an efficient and scalable method for preparing bespoke block copolymer nanoparticles.[21-46] This powerful technique enables the one-pot synthesis of a wide range of morphologies at $\geq 20\%$ w/w solids, making it an industrially relevant platform. Recent advances have focused on developing greener PISA protocols, such as photoinitiated and enzymatic formulations, which operate under mild, energy-efficient, and environmentally-benign conditions.[47-56] Despite the maturity of PISA and its efficiency in the generation of well-defined nanoparticles, its potential for producing next-generation w/w emulsifiers has yet to be fully explored.

Herein, we report the first application of block copolymer nanoparticles prepared by PISA for the effective stabilization of w/w emulsions. More specifically, we employed photoenzymatic reversible addition-fragmentation chain transfer (RAFT) aqueous dispersion copolymerization,[52, 57-63] which enables the efficient preparation of concentrated dispersions of well-defined nanoparticles directly in water under ambient conditions via visible light irradiation. This approach provides exceptional and straightforward control over the mean nanoparticle diameter simply by varying the degree of polymerization (DP) of the core-forming block. We demonstrate that these PISA-derived nanoparticles are highly effective Pickering stabilizers for a model poly(ethylene glycol)/dextran w/w emulsion.[8, 19, 64] Furthermore, we report an unexpected non-monotonic dependence of emulsion stability on nanoparticle size, with particles of *intermediate* size conferring exceptional long-term stability. This work establishes photoenzymatic PISA as a versatile and scalable

platform for designing next-generation stabilizers for w/w emulsions, opening up new avenues for potential biomedical applications.



Scheme 1 (a) Synthesis of block copolymer nanoparticles via photoenzymatic RAFT aqueous dispersion copolymerization and (b) nanoparticles as water-in-water emulsion stabilizers.

Results and discussion

Photoenzymatic RAFT polymerization using glucose oxidase (GOx) as a catalyst is an efficient, oxygen-tolerant, and versatile technique for synthesizing well-defined polymer under mild conditions.[65] When combined with high-throughput microtiter screening, this method has recently provided convenient access to ultrahigh molecular weights (UHMW; $M_n > 1000 \text{ kg mol}^{-1}$) up to 4100 kg mol^{-1} with low dispersities ($D = 1.25$).[58] Inspired by its excellent capability for molecular weight control, we employed this method for the RAFT aqueous dispersion copolymerization of diacetone acrylamide (DAAM) with *N,N*-dimethylacrylamide (DMA) using a poly(*N,N*-dimethylacrylamide) (PDMA) macromolecular chain transfer agent (macro-CTA).[66-69] This approach enables systematic tuning of the molecular weight of the core-forming block and, consequently, the size of the diblock copolymer nanoparticles, allowing us to examine the effect of nanoparticle size on the long-term stability of w/w emulsions.

The PDMA precursors were prepared via photoenzymatic RAFT solution polymerization of DMA using the water-soluble chain transfer agent 4-(((2-carboxyethyl) thio) carbonothioyl) thio)-4-cyanopentanoic acid (CTPA). This method consistently yielded low-dispersity PDMA homopolymers with predictable molecular weights (see ESI), which were subsequently used as macro-CTAs for chain extension via dispersion copolymerization of DAAM with DMA targeting 20% w/v solids. Such solution and dispersion

polymerization syntheses were both conducted at 30 °C under blue light irradiation (462 nm, 20.0 mW cm⁻²).

We first optimized the RAFT aqueous dispersion polymerization formulation by screening the molecular weight of the PDMA precursor, the core-forming comonomer composition, and the target DP of the core-forming block. The excellent deoxygenation ability conferred by GOx[70] enabled high-throughput screening to be conducted using a microtiter plate (300 μL polymerization solution in each well) without prior degassing.[71, 72] Initial attempts using a PDMA₂₃₄ macro-CTA ($M_n = 21.9 \text{ kg mol}^{-1}$; $D = 1.02$) for the RAFT aqueous dispersion polymerization of DAAM resulted in block copolymers with broad molecular weight distributions ($D = 1.47\text{--}1.98$) and significant macroscopic precipitation when targeting a PDAAM DP of 10000 (Table S1, Figure S2). Previous studies have demonstrated that incorporating solvophilic monomers such as DMA into the core-forming block can enhance hydration and improve polymerization control by increasing chain mobility.[73] Adopting this strategy, we performed the RAFT aqueous dispersion copolymerization of DAAM with 20 mol% DMA (Scheme 1). The resulting PDMA-*b*-P(DAAM-*co*-DMA) diblock copolymers exhibited lower dispersities ($D = 1.38\text{--}1.54$), confirming enhanced polymerization control. However, macroscopic precipitation still occurred at high DPs (e.g., DP = 10000), indicating that the PDMA₂₃₄ stabilizer block provided insufficient colloidal stability for larger nanoparticles. Therefore, we synthesized a higher molecular weight PDMA₅₁₈ macro-CTA ($M_n = 51.2 \text{ kg mol}^{-1}$, $D = 1.04$) to confer more effective steric stabilization.

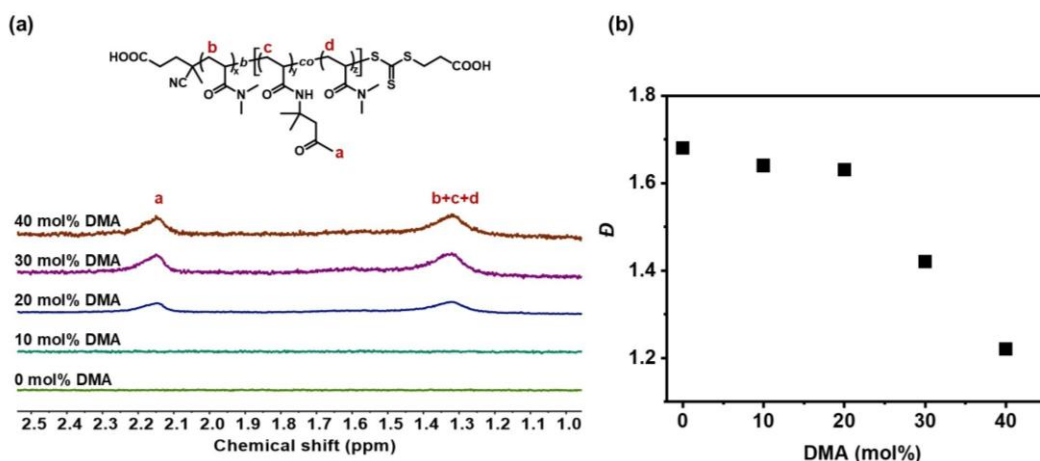


Figure 1 Photoenzymatic RAFT aqueous dispersion copolymerization using a PDMA₅₁₈ macro-CTA to target a core-forming block DP of 10000 at varying comonomer composition. (a) Evolution of dispersity ($\bar{D}$) of the resulting block copolymers vs. DMA mole fraction, and (b) ^1H NMR spectra recorded in D_2O for the corresponding diblock copolymer nanoparticles.

Gratifyingly, this PDMA₅₁₈ macro-CTA produced colloiddally stable diblock copolymer nanoparticles even at extremely high DPs, allowing us to examine the effect of comonomer composition on polymerization control for a target DP of up to 10000. Dispersion copolymerizations with various DMA mole fractions invariably reached near-quantitative conversion and yielded stable colloids within 5 h. Gel permeation chromatography coupled with multiangle laser light scattering (GPC-MALLS) analysis showed a progressive reduction in dispersity ($\bar{D} = 1.68 \rightarrow 1.22$) as the DMA mole fraction increased to 40 mol% (**Figure 1a**). However, the copolymerization became less effective above this upper limit because the diblock copolymer chains became too hydrophilic to ensure efficient self-assembly. ^1H NMR spectroscopy analysis of the nanoparticles in D_2O provided further insight

into this improved control (**Figure 1b**). At low DMA contents (< 20 mol%), the signal from the DAAM repeat units (proton *a*) was almost undetectable, indicating poorly solvated nanoparticle cores. Above 20 mol% DMA, the signals from both the DAAM repeat units and the copolymer backbone became increasingly prominent, signifying enhanced core solvation. This higher degree of solvation enables higher chain mobility, leading to more effective chain transfer and thereby greater RAFT control. Dynamic light scattering (DLS) analysis confirmed a progressive reduction in nanoparticle diameter ($D_h = 420 \text{ nm} \rightarrow 275 \text{ nm}$) with higher DMA content (**Table S2**), which is consistent with the formation of less hydrophobic core-forming blocks with reduced propensity for self-assembly and hence a lower mean aggregation number.

Table 1 Summary of characterization data obtained for diblock copolymer nanoparticles prepared by photoenzymatic RAFT aqueous dispersion copolymerization of 60 mol% DAAM with 40 mol% DMA using a PDMA₅₁₈ macro-CTA.

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	Γ^g (mg m ⁻²)
NP 1	2000	93	94	317	368	1.12	134	0.04	1.85
NP 2	5000	95	96	727	911	1.26	199	0.05	1.75
NP 3	7500	98	97	1080	1320	1.24	240	0.06	1.50
NP 4	10000	97	98	1430	1900	1.22	306	0.09	1.45
NP 5	20000	95	98	2790	2930	1.26	372	0.03	0.98

Polymerization conditions: 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), 200 rpm, 30 °C, 5 h. [a] NP stands for block copolymer nanoparticles. [b] Total target DP of the core-forming block. [c] Comonomer conversions determined by ^1H NMR spectroscopy studies performed in DMSO-*d*₆. [d] Theoretical molecular weight: $M_{n,th} = M_{\text{macro-CTA}} + M_{\text{DMA}} \times \text{total target DP} \times \text{DMA mol\%} \times \text{DMA conversion (\%)} + M_{\text{DAAM}} \times \text{total target DP} \times \text{DAAM mol\%} \times \text{DAAM conversion (\%)}$. [e] Molecular weight and dispersity determined by GPC analysis. [f] Hydrodynamic diameter (D_h) and polydispersity index (PDI) determined by dynamic light scattering (DLS) studies. [g] Adsorbed amount of PDMA₅₁₈ stabilizer per unit surface area of nanoparticles (in terms of mg).

To better understand such nanoparticle syntheses, a kinetic study was conducted during the synthesis of PDMA₅₁₈-*b*-P(DMA₄₀₀₀-*co*-DAAM₆₀₀₀). DAAM consistently exhibited slightly higher conversions than DMA throughout the copolymerization, with these two comonomers attaining 88% and 83% conversion respectively within 320 min (**Figure 2a**). Moreover, this dispersion copolymerization displayed a characteristic two-stage profile^[74]: an initial homogeneous

solution polymerization with a relatively low apparent rate constant ($k_p^{\text{app}} = 0.0023 \text{ min}^{-1}$), followed by significantly faster polymerization within monomer-swollen particles ($k_p^{\text{app}} = 0.0067 \text{ min}^{-1}$) after micellar nucleation occurred at $\sim 10\%$ conversion (**Figure 2b**). DLS analysis indicated the presence of relatively large species ($D_h = 116 \text{ nm}$) after just 5 min, but transmission electron microscopy (TEM) analysis of an aliquot taken at this time point revealed no well-defined particles. We

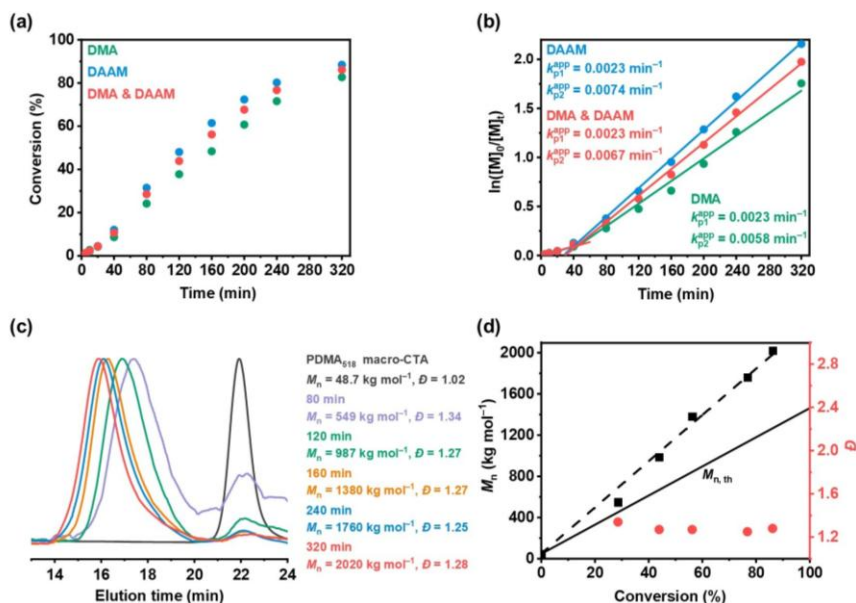


Figure 2 Kinetic data obtained for the synthesis of PDMA₅₁₈-*b*-P(DMA₄₀₀₀-*co*-DAAM₆₀₀₀) via photoenzymatic RAFT aqueous dispersion copolymerization: 20% w/v, [GOx] = 60 U mL⁻¹, [glucose] = 50 mM, phosphate buffer (0.2 M, pH 5.8), blue light (462 nm, 20 mW cm⁻²), 30 °C, 200 rpm. (a) Comonomer conversion curves versus time, (b) corresponding plots of ln([M]₀/[M]_t) versus time, (c) GPC traces for the resulting diblock copolymers recorded at varying reaction times, and (d) evolution of molecular weight and dispersity versus comonomer conversion.

postulate that loose, highly solvated diblock copolymer aggregates with relatively low light scattering intensities may be formed during this initial stage (Figure S4). Such ill-defined aggregates may not be readily discernible by TEM. In contrast, well-defined spherical nanoparticles with a mean diameter of 327 ± 25 nm were observed by TEM at the end of the copolymerization (Figure S5). GPC analysis indicated efficient chain extension of the macro-CTA, with the whole molecular weight distribution shifting to lower elution times owing to the formation of block copolymer chains (Figure 2c). M_n values increase linearly with comonomer conversion, albeit with a slight deviation from theoretical values due to gradual

macro-CTA consumption (Figure 2c, d). Notably, low-dispersity UHMW diblock copolymers were prepared in the form of colloidal stable dispersions. Thus, photoenzymatic RAFT aqueous dispersion polymerization offers a highly convenient, low-viscosity route to the preparation of well-defined sterically-stabilized nanoparticles (Figure S6). Moreover, such formulations are amenable to scale up from microtiter plates (300 μ L) to glass vials (4.8 mL) to a round-bottomed flask (120 mL), with each synthetic protocol yielding diblock copolymer nanoparticles with similar M_n values and mean diameters (Figure 1).

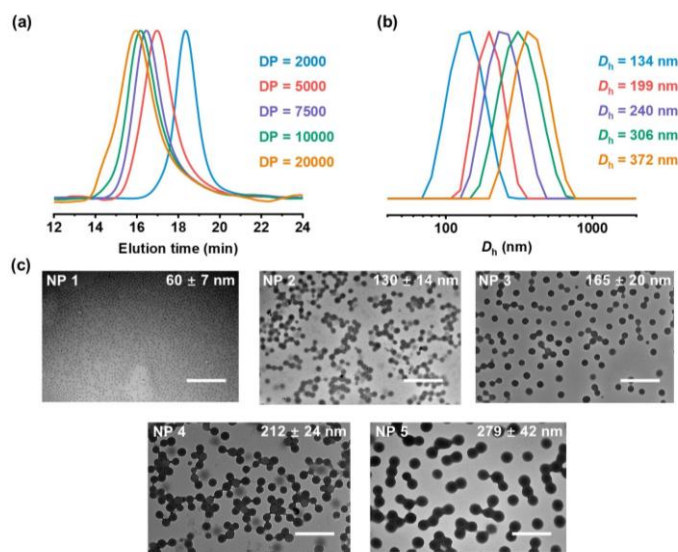


Figure 3 Characterization of the five examples of diblock copolymer nanoparticles reported in Table 1. (a) GPC traces recorded for the diblock copolymer chains, (b) DLS intensity-average size distributions for the corresponding nanoparticles, and (c) representative TEM images (Scale bar = 1 μ m).

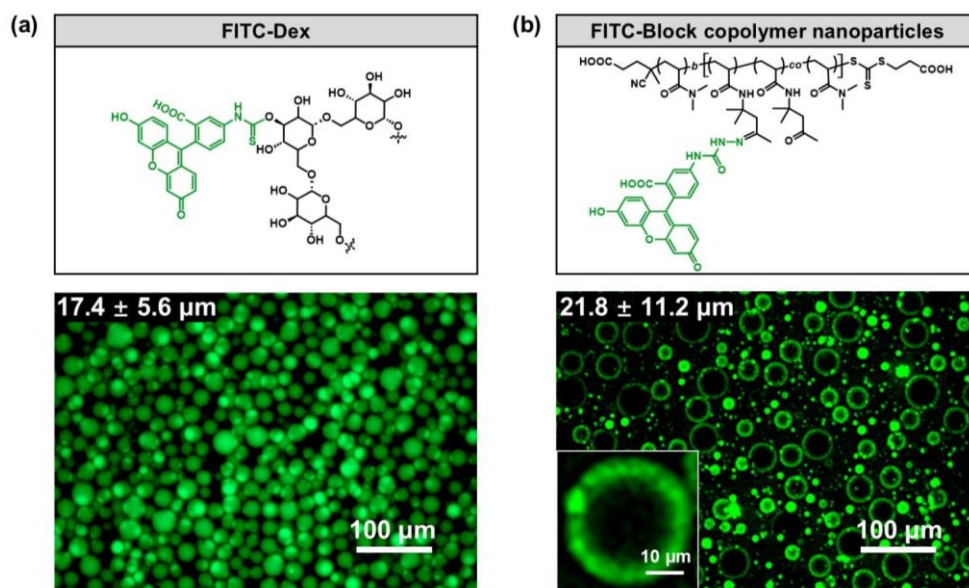


Figure 4 Fluorescence microscopy studies of w/w emulsions prepared using immiscible aqueous solutions of PEG and dextran. (a) Chemical structure of FITC-Dex and corresponding fluorescence micrograph of the dextran-in-PEG w/w emulsion droplets. (b) Chemical structure of FITC-labeled diblock copolymer nanoparticles (NP 2) and corresponding fluorescence micrograph confirming nanoparticle adsorption at the droplet surface (inset shows densely packed nanoparticles around a single aqueous dextran droplet).

Encouraged by the excellent polymerization control, efficiency, and scalability, we prepared a series of five nanoparticle dispersions with varying mean diameters by systematically increasing the target DP of the block from 2000 to 20000 (**Table 1**). All copolymerizations reached high comonomer conversions ($> 93\%$), and GPC analysis of the resulting diblock copolymers indicated a close match between theoretical and experimental molecular weights with low dispersities ($\bar{D} \leq 1.26$) (**Figure 3a**). Notably, M_n values of up to 2930 kg mol^{-1} were achieved when targeting DPs ≥ 7500 . It is important to emphasize that such UHMW diblock copolymer chains are obtained as a low-viscosity colloidal dispersion,^[75–78] in marked contrast to the extremely high viscosities that are typical of the analogous homogeneous solution polymerization syntheses,^[59, 79–91] thus highlighting the promising scalability of this route. As expected, DLS (**Figure 3b**) and TEM (**Figure 3c**) studies indicated a progressive increase in the nanoparticle diameter, with hydrodynamic diameters ($D_h = 134\text{--}372 \text{ nm}$) being consistently larger than the corresponding TEM diameters ($D_n = 60\text{--}279 \text{ nm}$) owing to solvation of the PDMA coronal chains.

Next we evaluated this series of PDMA-stabilized nanoparticles as putative stabilizers for w/w emulsions. A model system comprising poly(ethylene glycol) (PEG, 10 kg mol^{-1}) and dextran (Dex, 70 kg mol^{-1}) was used. Mixtures containing $14.0 \text{ wt\%}$ PEG, $7.5 \text{ wt\%}$ Dex, and $1.0 \text{ wt\%}$ nanoparticles were vortexed at 3500 rpm for 1 min , forming w/w emulsions with a phase volume ratio close to 2:1.

To identify the emulsion type, we incorporated fluorescein-labeled dextran (FITC-Dex). Fluorescence microscopy revealed the formation of fluorescent (green) droplets ($17.4 \pm 5.6 \mu\text{m}$) dispersed in a continuous non-fluorescent phase, confirming a Dex-in-PEG emulsion (**Figure 4a**). We also

labeled the block copolymer nanoparticles (NP 2) with fluorescein-5-thiosemicarbazide, which reacts with the pendent ketone group in the DAAM repeat units.^[66] When used to stabilize the emulsion, these fluorescein-labeled nanoparticles are strongly adsorbed at the droplet interface (**Figure 4b**), as expected for a Pickering stabilization mechanism.

Then the long-term stability of emulsions prepared using nanoparticles of varying sizes was investigated. Accordingly, five w/w emulsions prepared in turn using nanoparticles NP 1 – NP 5), were monitored over time (**Figure 5**, along with a control mixture prepared without using any nanoparticle stabilizer). In the latter case, rapid droplet coalescence led to complete macroscopic phase separation within 30 min (**Figure S8**). The w/w emulsions stabilized by NP 1 ($D_h = 134 \text{ nm}$) and NP 2 ($D_h = 199 \text{ nm}$) initially exhibited relatively uniform droplets of around $13 \mu\text{m}$ diameter. The latter emulsion exhibited remarkable stability, with no significant change in droplet size being observed over 50 h storage at ambient temperature (**Figures S10, S11**). In contrast, the emulsions prepared using NP 3 ($D_h = 240 \text{ nm}$), NP 4 ($D_h = 306 \text{ nm}$), or NP 5 ($D_h = 372 \text{ nm}$) exhibited much broader droplet size distributions and underwent macroscopic phase separation within 10 min , with the nanoparticles preferentially located at the macroscopic interface (**Figure S8**).

The superior emulsifier performance observed for NP 2 is attributed to its optimal balance of interfacial adsorption energy and three-phase contact angle.^[2] Its cross-sectional area provides strong adsorption, while its intermediate size most likely results in a contact angle that favors localization at the interface rather than preferential partitioning into either bulk phase, as evidenced by the dense surface coverage in

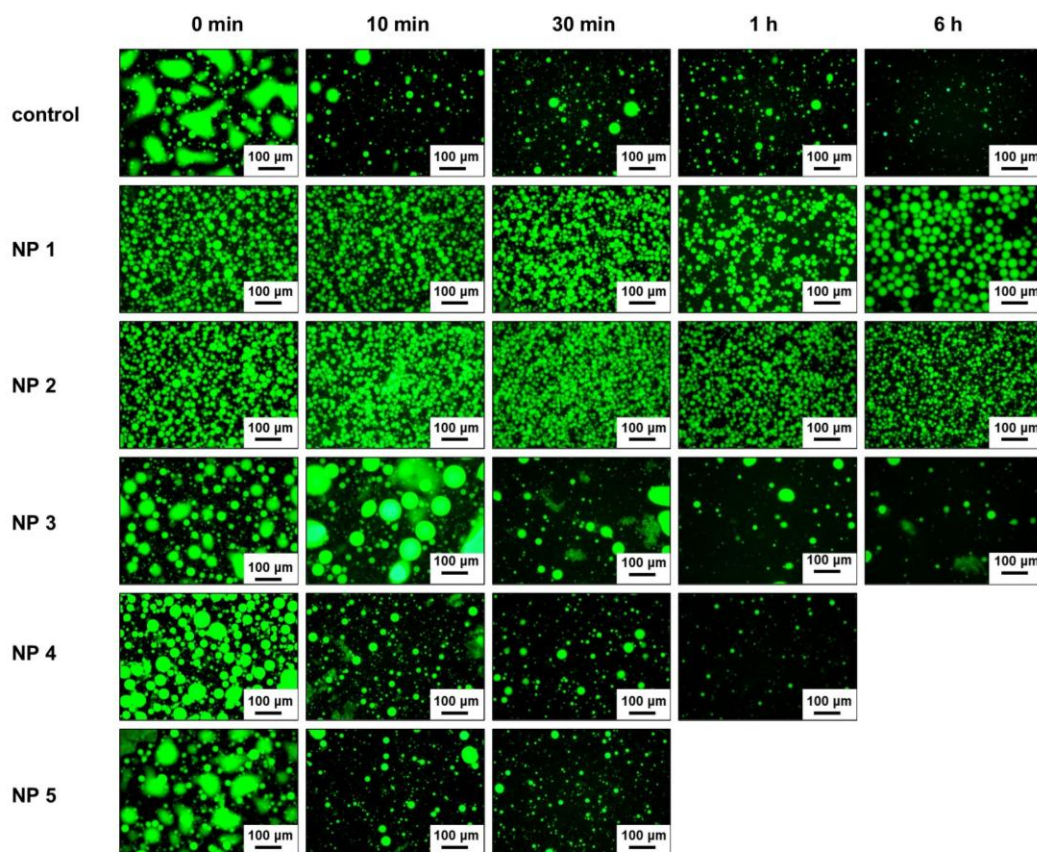


Figure 5 Fluorescence microscopy images recorded for w/w emulsions prepared using 14.0 wt% PEG ($M_n = 10 \text{ kg mol}^{-1}$) and 7.5 wt% dextran ($M_n = 70 \text{ kg mol}^{-1}$) acquired at various time points. The control mixture contained no nanoparticles, while 1.0 wt% copolymer was used in the emulsions stabilized using nanoparticles NP 1 – NP 5.

Figure 4b(inset). While larger particles have a greater cross-sectional area and can theoretically reduce interfacial free energy more effectively, nanoparticles NP 3, NP 4 and NP 5 all performed poorly. To rule out insufficient concentration, we increased the concentration of NP 4 up to 2.0 wt%, but it still failed to prevent rapid coalescence (Figure S12). We speculate that the failure of these larger nanoparticles as effective stabilizers stems from three factors. First, their adsorption increases the effective density of the aqueous dextran droplets, promoting rapid sedimentation and facilitating coalescence through close-packing. Second, their slower diffusion hinders their ability to rapidly adapt to interfacial changes and maintain dense coverage on the droplets during and after emulsification. Consequently, rapid phase separation of the emulsions was observed. Third, the surface density of the steric stabilizer chains at the surface of nanoparticles is known to influence their Pickering emulsifier performance.[92–94] For the current PISA formulation, the surface density of the PDMA steric stabilizer chains at the nanoparticle surface is necessarily reduced when targeting a higher DP for the core-forming block, since this leads to larger nanoparticle cores. Assuming that all the PDMA chains are located at the surface of the nanoparticles, the calculated adsorbed amount, Γ , is reduced from 1.85 to 0.98 mg m^{-2} for the five examples of nanoparticles (NP 1 to NP 5) listed in Table 1. For NP 3, NP 4, and NP 5, the lower surface density of the PDMA chains promotes nanoparticle flocculation, which in turn hinders the formation of an

effective steric barrier at the liquid-liquid interface. Thus this explains why such nanoparticles are less effective Pickering emulsifiers.

The above calculated surface chain densities represent a theoretical upper limit for the PDMA chains, which may not be all located at the surface of each nanoparticle. However, there is no doubt that surface chain density must be reduced when targeting longer core-forming blocks.

When elucidating the stabilization mechanism of the nanoparticles, the potential contribution of electrostatic repulsion arising from surface charges must be taken into account. [95, 96] To investigate whether surface charge density dictates the size-dependent stability, we systematically measured the zeta potentials of the entire nanoparticle series (NP 1 to NP 5) (Table S5). The results revealed that all nanoparticles exhibited near-zero zeta potentials (ranging from -1.8 to $+0.1 \text{ mV}$) measured at the emulsion's native pH. To further ascertain that this near-zero potential is independent of environmental pH, the zeta potential of NP 2 (as a representative sample) was evaluated under highly acidic conditions (pH 2.8). The zeta potential remained consistently close to 0 mV ($+0.5 \text{ mV}$). These findings confirm that the terminal carboxylic groups are completely shielded by the highly solvated PDMA corona, regardless of their protonation state. Therefore, we conclude that surface charge does not play an important role in stabilizing the system.

Conclusions

In summary, we have developed an environmentally benign and scalable approach for preparing well-defined diblock copolymer nanoparticles by photoenzymatic RAFT aqueous dispersion copolymerization. By systematically adjusting the chemical composition of the core-forming block, we can achieve excellent polymerization control and tune the molecular weight over a broad range, producing colloidally stable block copolymer nanoparticles with programmable mean diameters. A series of such nanoparticles was evaluated as Pickering stabilizers for the formation of w/w emulsions, which revealed an unexpected size-dependent effect. Nanoparticles of intermediate size (NP 2) exhibited outstanding stabilization, whereas larger nanoparticles (NP 3 – NP 5) proved to be inferior, perhaps owing to their reduced interfacial adaptability, lowered steric stabilizer density on nanoparticle surface, and enhanced tendency to undergo sedimentation. Overall, the mild, green, and scalable preparation of tailorable nanoparticles, combined with their efficacy as stabilizers for biocompatible w/w emulsions, represents a promising strategy for potential biomedical applications, such as the encapsulation and sustained release of protein therapeutics in drug delivery.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

This work was supported by the Natural Science Foundation of China (22371089, 22193020, 22193024).

Supporting Information

The data supporting this article have been included as part of the supplementary information (SI), supplementary information: materials, characterization, experimental protocols, ¹H NMR spectra, GPC traces, TEM micrograph, solution viscosity plots, DLS traces, emulsion photographs, and emulsion droplet statistics. See DOI:

References

- [1] Esquena J. Water-in-water (w/w) emulsions. *Current Opinion in Colloid & Interface Science*, 2016, 25: 109-119
- [2] Chao Y, Shum HC. Emerging aqueous two-phase systems: From fundamentals of interfaces to biomedical applications. *Chemical Society Reviews*, 2020, 49(1): 114-142
- [3] Yang H, Yang Y, Li B-Z, et al. Production of protein-loaded starch microspheres using water-in-water emulsion method. *Carbohydrate Polymers*, 2020, 231: 115692
- [4] Dewey DC, Strulson CA, Cacace DN, et al. Bioreactor droplets from liposome-stabilized all-aqueous emulsions. *Nature Communications*, 2014, 5(1): 4670
- [5] Dickinson E. Particle-based stabilization of water-in-water emulsions containing mixed biopolymers. *Trends in Food Science & Technology*, 2019, 83:31-40
- [6] Zhou Y, Zhu M, Sun Y, et al. Fabrication of macroporous polymers via water-in-water emulsion-templating technique. *ACS Macro Letters*, 2023, 12(3): 302-307
- [7] Fick C, Khan Z, Srivastava S. Interfacial stabilization of aqueous two-phase systems: A review. *Materials Advances*, 2023, 4(20): 4665-4678
- [8] Nicolai T, Murray B. Particle stabilized water in water emulsions. *Food Hydrocolloids*, 2017, 68: 157-163
- [9] Cui W, Xia C, Xu S, et al. Water-in-water emulsions stabilized by self-assembled chitosan colloidal particles. *Carbohydrate Polymers*, 2023, 303: 120466
- [10] Buzza DMA, Fletcher PDI, Georgiou TK, et al. Water-in-water emulsions based on incompatible polymers and stabilized by triblock copolymers-templated polymericomes. *Langmuir*, 2013, 29(48): 14804-14814
- [11] Xue L-H, Xie C-Y, Meng S-X, et al. Polymer-protein conjugate particles with biocatalytic activity for stabilization of water-in-water emulsions. *ACS Macro Letters*, 2017, 6(7): 679-683
- [12] Vis M, Opdam J, van 't Oor ISJ, et al. Water-in-water emulsions stabilized by nanoplates. *ACS Macro Letters*, 2015, 4(9): 965-968
- [13] Nguyen BT, Nicolai T, Benyahia L. Stabilization of water-in-water emulsions by addition of protein particles. *Langmuir*, 2013, 29(34): 10658-10664
- [14] Nguyen BT, Wang W, Saunders BR, et al. Ph-responsive water-in-water pickering emulsions. *Langmuir*, 2015, 31(12): 3605-3611
- [15] Peddireddy KR, Nicolai T, Benyahia L, et al. Stabilization of water-in-water emulsions by nanorods. *ACS Macro Letters*, 2016, 5(3): 283-286
- [16] Ganley WJ, Ryan PT, van Duijneveldt JS. Stabilisation of water-in-water emulsions by montmorillonite platelets. *Journal of Colloid and Interface Science*, 2017, 505: 139-147
- [17] Binks BP, Shi H. Phase inversion of silica particle-stabilized water-in-water emulsions. *Langmuir*, 2019, 35(11): 4046-4057
- [18] Inam M, Jones JR, Pérez-Madriral MM, et al. Controlling the size of two-dimensional polymer platelets for water-in-water emulsifiers. *ACS Central Science*, 2018, 4(1): 63-70
- [19] Zhang J, Hwang J, Antonietti M, et al. Water-in-water pickering emulsion stabilized by polydopamine particles and cross-linking. *Biomacromolecules*, 2019, 20(1): 204-211
- [20] Zhang J, Kumru B, Schmidt BVKJ. Supramolecular compartmentalized hydrogels via polydopamine particle-stabilized water-in-water emulsions. *Langmuir*, 2019, 35(34): 11141-11149
- [21] Warren NJ, Armes SP. Polymerization-induced self-assembly of block copolymer nano-objects via raft aqueous dispersion polymerization. *Journal of the American Chemical Society*, 2014, 136(29): 10174-10185
- [22] Canning SL, Smith GN, Armes SP. A critical appraisal of raft-mediated polymerization-induced self-assembly. *Macromolecules*, 2016, 49(6): 1985-2001
- [23] D'Agosto F, Rieger J, Lansalot M. Raft-mediated polymerization-induced self-assembly. *Angewandte Chemie International Edition*, 2020, 59(22): 8368-8392
- [24] Liu C, Hong CY, Pan CY. Polymerization techniques in polymerization-induced self-assembly (pisa). *Polymer Chemistry*, 2020, 11(22): 3673-3689
- [25] Rieger J. Guidelines for the synthesis of block copolymer particles of various morphologies by raft dispersion polymerization. *Macromolecular Rapid Communications*, 2015, 36(16): 1458-1471
- [26] Derry MJ, Fielding LA, Armes SP. Polymerization-induced self-assembly of block copolymer nanoparticles via raft non-aqueous dispersion polymerization. *Progress in Polymer Science*, 2016, 52: 1-18
- [27] Cheng G, Pérez-Mercader J. Polymerization-induced self-assembly for artificial biology: Opportunities and challenges. *Macromolecular Rapid Communications*, 2019, 40(2): 1800513
- [28] Khor SY, Quinn JF, Whittaker MR, et al. Controlling nanomaterial size and shape for biomedical applications via polymerization-induced self-assembly. *Macromolecular Rapid Communications*, 2019, 40(2): 1800438
- [29] Le D, Keller D, Delaitre G. Reactive and functional nanoobjects by polymerization-induced self-assembly. *Macromolecular Rapid Communications*, 2019, 40(2): 1800551
- [30] Wang X, An Z. New insights into raft dispersion polymerization-induced self-assembly: From monomer library, morphological control, and stability to driving forces. *Macromolecular Rapid Communications*, 2019, 40(2): 1800325
- [31] Zhang WJ, Hong CY, Pan CY. Polymerization-induced self-assembly of functionalized block copolymer nanoparticles and their application in drug delivery. *Macromolecular Rapid Communications*, 2019, 40(2): 1800279
- [32] An NK, Chen X, Yuan JY. Non-thermally initiated raft polymerization-induced self-assembly. *Polymer Chemistry*, 2021, 12(22): 3220-3232
- [33] Cao JP, Tan YX, Chen Y, et al. Expanding the scope of polymerization-induced self-assembly: Recent advances and new horizons. *Macromolecular Rapid Communications*, 2021, 42(23): 2100498
- [34] Cornel EJ, Jiang JH, Chen S, et al. Principles and characteristics of polymerization-induced self-assembly with various polymerization techniques. *CCS Chemistry*, 2021, 3(4): 2104-2125
- [35] Shi BY, Wang GW. Application of polymerization-induced self-assembly (pisa) technology. *Acta Polymerica Sinica*, 2022, 53(1): 15-29
- [36] Wan J, Fan B, Thang SH. Raft-mediated polymerization-induced self-assembly (raft-pisa): Current status and future directions. *Chemical Science*, 2022, 13(15): 4192-4224
- [37] György C, Armes SP. Recent advances in polymerization-induced self-assembly (pisa) syntheses in non-polar media. *Angewandte Chemie-International Edition*, 2023, 62(42): e202308372
- [38] Zhang HW, Han WJ, Liu YH, et al. Stimuli responsive nano-objects via polymerization induced self-assembly. *Acta Polymerica Sinica*, 2023, 54(11): 1663-1680
- [39] Zhao XP, Sun CR, Xiong F, et al. Polymerization-induced self-assembly for efficient fabrication of biomedical nanoplateforms. *Research*, 2023, 6: 0113
- [40] Zhang S, Li R, An Z. Degradable block copolymer nanoparticles synthesized by polymerization-induced self-assembly. *Angewandte Chemie-International Edition*, 2024, 63(12): e202315849
- [41] Tan J, Liu D, Bai Y, et al. Enzyme-assisted photoinitiated polymerization-induced self-assembly: An oxygen-tolerant method for preparing block copolymer nano-objects in open vessels and multiwell plates. *Macromolecules*, 2017, 50(15): 5798-5806
- [42] Qin X, An Z. Reversible addition-fragmentation chain transfer dispersity regulation by chain transfer agent micelles. *Acta Polymerica Sinica*, 2024, 55(1): 67-78

- [43] Han S, Gu Y, Ma M, et al. Light-intensity switch enabled nonsynchronous growth of fluorinated raspberry-like nanoparticles. *Chemical Science*, 2020, 11(38): 10431-10436
- [44] Quan Q, Wen H, Han S, et al. Fluorous-core nanoparticle-embedded hydrogel synthesized via tandem photo-controlled radical polymerization: Facilitating the separation of perfluorinated alkyl substances from water. *ACS Applied Materials & Interfaces*, 2020, 12(21): 24319-24327
- [45] Zhong F, Zhou Y, Chen M. The influence of mixing on chain extension by photo-controlled/living radical polymerization under continuous-flow conditions. *Polymer Chemistry*, 2019, 10(35): 4879-4886
- [46] Quan Q, Zhao Y, Chen K, et al. Organocatalyzed controlled copolymerization of perfluorinated vinyl ethers and unconjugated monomers driven by light. *ACS Catalysis*, 2022, 12(12): 7269-7277
- [47] Li R, Kong W, An Z. Enzyme catalysis for reversible deactivation radical polymerization. *Angewandte Chemie International Edition*, 2022, 61(26): e202202033
- [48] Wang X, Shen L, An Z. Dispersion polymerization in environmentally benign solvents via reversible deactivation radical polymerization. *Progress in Polymer Science*, 2018, 83: 1-27
- [49] Tan J, Sun H, Yu M, et al. Photo-pisa: Shedding light on polymerization-induced self-assembly. *ACS Macro Letters*, 2015, 4(11): 1249-1253
- [50] Meng L, Tang X, An Z. Visible light photoiniferter polymerization. *Acta Polymerica Sinica*, 2026, doi: 10.1177/j.issn1000-3304.2026.25326
- [51] Gong H, Gu Y, Zhao Y, et al. Precise synthesis of ultra-high-molecular-weight fluoropolymers enabled by chain-transfer-agent differentiation under visible-light irradiation. *Angewandte Chemie International Edition*, 2020, 59(2): 919-927
- [52] Chen K, Zhou Y, Han S, et al. Main-chain fluoropolymers with alternating sequence control via light-driven reversible-deactivation copolymerization in batch and flow. *Angewandte Chemie International Edition*, 2022, 61(14): e202116135
- [53] Zeng Y, Quan Q, Wen P, et al. Organocatalyzed controlled radical copolymerization toward hybrid functional fluoropolymers driven by light. *Angewandte Chemie International Edition*, 2022, 61(52): e202215628
- [54] Zhang W, He J, Lv C, et al. Atom transfer radical polymerization driven by near-infrared light with recyclable upconversion nanoparticles. *Macromolecules*, 2020, 53(12): 4678-4684
- [55] He J, Zhang W, Lv C, et al. Thermo- and ph-responsive star-like polymers synthesized by photolat. *Polymer*, 2021, 215: 123345
- [56] Chen M, Zhong M, Johnson JA. Light-controlled radical polymerization: Mechanisms, methods, and applications. *Chemical Reviews*, 2016, 116(17): 10167-10211
- [57] Li R, An Z. Achieving ultrahigh molecular weights with diverse architectures for unconjugated monomers through oxygen-tolerant photoenzymatic raft polymerization. *Angewandte Chemie International Edition*, 2020, 59(49): 22258-22264
- [58] Kong W, An Z. High-throughput photoenzymatic reversible addition-fragmentation chain-transfer polymerization: Molecular weight and dispersity control for entangled network polymers. *CCS Chemistry*, 2026, 8: 1467-1479
- [59] Wang Q, Bai F-Y, Wang Y, et al. Photoinduced ion-pair inner-sphere electron transfer-reversible addition-fragmentation chain transfer polymerization. *Journal of the American Chemical Society*, 2022, 144(43): 19942-19952
- [60] Quan Q, Gong H, Chen M. Preparation of semifluorinated poly(meth)acrylates by improved photo-controlled radical polymerization without the use of a fluorinated raft agent: Facilitating surface fabrication with fluorinated materials. *Polymer Chemistry*, 2018, 9(30): 4161-4171
- [61] Zhou Y, Han S, Gu Y, et al. Facile synthesis of gradient copolymers enabled by droplet-flow photo-controlled reversible deactivation radical polymerization. *Science China Chemistry*, 2021, 64(5): 844-851
- [62] Chen Y, Han S, Chen K, et al. Controlled radical copolymerization toward tailored f/n hybrid polymers by using light-driven organocatalysis. *Angewandte Chemie International Edition*, 2024, 63(37): e202408611
- [63] Chen K, Zhang Z, Ye Q, et al. Reversible-deactivation radical copolymerization of tetrafluoroethylene via the formation of divergent termini in dormant chains. *Chem*, 2025, 11(6): 102434
- [64] Balakrishnan G, Nicolai T, Benyahia L, et al. Particles trapped at the droplet interface in water-in-water emulsions. *Langmuir*, 2012, 28(14): 5921-5926
- [65] Zhou F, Li R, Wang X, et al. Non-natural photoenzymatic controlled radical polymerization inspired by DNA photolyase. *Angewandte Chemie International Edition*, 2019, 58(28): 9479-9484
- [66] Zhou W, Qu Q, Xu Y, et al. Aqueous polymerization-induced self-assembly for the synthesis of ketone-functionalized nano-objects with low polydispersity. *ACS Macro Letters*, 2015, 4(5): 495-499
- [67] Jiang Y, Xu N, Han J, et al. The direct synthesis of interface-decorated reactive block copolymer nanoparticles via polymerisation-induced self-assembly. *Polymer Chemistry*, 2015, 6(27): 4955-4965
- [68] Zhang L, Lu Q, Lv X, et al. In situ cross-linking as a platform for the synthesis of triblock copolymer vesicles with diverse surface chemistry and enhanced stability via raft dispersion polymerization. *Macromolecules*, 2017, 50(5): 2165-2174
- [69] Figg CA, Carmean RN, Bentz KC, et al. Tuning hydrophobicity to program block copolymer assemblies from the inside out. *Macromolecules*, 2017, 50(3): 935-943
- [70] Chapman R, Gormley AJ, Stenzel MH, et al. Combinatorial low-volume synthesis of well-defined polymers by enzyme degassing. *Angewandte Chemie International Edition*, 2016, 55(14): 4500-4503
- [71] Li R, Zhang S, Li Q, et al. An atom-economic enzymatic cascade catalysis for high-throughput raft synthesis of ultrahigh molecular weight polymers. *Angewandte Chemie International Edition*, 2022, 61(46): e202213396
- [72] Li R, Zhang S, Tang X, et al. Near-infrared photoenzymatic catalysis at ppb levels enables ultrahigh-molecular-weight polymers. *Journal of the American Chemical Society*, 2025, 147(36): 33153-33161
- [73] Tang X, Han J, Zhu Z, et al. Facile synthesis, sequence-tuned thermoresponsive behaviours and reaction-induced reorganization of water-soluble keto-polymers. *Polymer Chemistry*, 2014, 5(13): 4115-4123
- [74] Blanz A, Madsen J, Battaglia G, et al. Mechanistic insights for block copolymer morphologies: How do worms form vesicles? *Journal of the American Chemical Society*, 2011, 133(41): 16581-16587
- [75] McBride RJ, Miller JF, Blanz A, et al. Synthesis of high molecular weight water-soluble polymers as low-viscosity latex particles by raft aqueous dispersion polymerization in highly salty media. *Macromolecules*, 2022, 55(17): 7380-7391
- [76] McBride RJ, Geneste E, Xie A, et al. Low-viscosity route to high-molecular-weight water-soluble polymers: Exploiting the salt sensitivity of poly(n-acryloylmorpholine). *Macromolecules*, 2024, 57(5): 2432-2445
- [77] Eades CB, Stevens KC, Cabrera DE, et al. Ultra-high molecular weight polymer synthesis via aqueous dispersion polymerization. *Chemical Science*, 2025, 16(13): 5573-5578
- [78] Lian S, Li R, Chen Y, et al. Reversible deactivation radical polymerization in heterogeneous systems: Enhancing access to high molecular weight polymers. *Macromolecules*, 2025, 58(11): 5385-5394
- [79] Carmean RN, Becker TE, Sims MB, et al. Ultra-high molecular weights via aqueous reversible-deactivation radical polymerization. *Chem*, 2017, 2(1): 93-101
- [80] Allison-Logan S, Karimi F, Sun Y, et al. Highly living stars via core-first photo-raft polymerization: Exploitation for ultra-high molecular weight star synthesis. *ACS Macro Letters*, 2019, 8(10): 1291-1295
- [81] Read E, Guinaudeau A, James Wilson D, et al. Low temperature raft/madix gel polymerisation: Access to controlled ultra-high molar mass polyacrylamides. *Polymer Chemistry*, 2014, 5(7): 2202-2207
- [82] Xu J, Jung K, Atme A, et al. A robust and versatile photoinduced living polymerization of conjugated and unconjugated monomers and its oxygen tolerance. *Journal of the American Chemical Society*, 2014, 136(14): 5508-5519
- [83] Zhou C, Zhang Z, Li W, et al. Organocatalyzed photo-controlled synthesis of ultrahigh-molecular-weight fluorinated alternating copolymers. *Angewandte Chemie International Edition*, 2024, 63(2): e202314483
- [84] Ma Q, Qiao GG, An Z. Visible light photoiniferter polymerization for dispersity control in high molecular weight polymers. *Angewandte Chemie International Edition*, 2023, 62(48): e202314729
- [85] Liu Z, Lv Y, An Z. Enzymatic cascade catalysis for the synthesis of multiblock and ultrahigh-molecular-weight polymers with oxygen tolerance. *Angewandte Chemie International Edition*, 2017, 56(44): 13852-13856
- [86] Zhang M, Armes SP, An Z. Synthesis of star polymers with ultrahigh molecular weights and tunable dispersities via photoiniferter polymerization. *ACS Macro Letters*, 2025, 14(3): 306-312
- [87] Zhao Y, Zhang S, An Z. Amplified cascade catalysis for raft polymerization. *Angewandte Chemie International Edition*, 2025, 64(11): e202421851
- [88] Trachsel L, Levkovsky IO, Hu X, et al. Red light-driven, oxygen-tolerant raft polymerization enabled by methylene blue. *Journal of the American Chemical Society*, 2025, 147(35): 32096-32109
- [89] Zhu S, Kong W, Lian S, et al. Photocontrolled radical polymerization for the synthesis of ultrahigh molecular weight polymers. *Nature Synthesis*, 2025, 4(1): 15-30
- [90] Tang X, Meng L-Q, Li R, et al. Triplet-enhanced photoiniferter polymerization: Ultrafast, oxygen-tolerant, and biocompatible synthesis of well-defined polymers under visible light. *Angewandte Chemie International Edition*, 2026, 65(2): e22611
- [91] Lian S, Armes Steven P, An Z. Universal visible-light photoiniferter polymerization. *CCS Chemistry*, 2024, 7(8): 2304-2314
- [92] Dupin D, Armes SP, Connan C, et al. How does the nature of the steric stabilizer affect the pickering emulsifier performance of lightly cross-linked, acid-swellaible poly(2-vinylpyridine) latexes? *Langmuir*, 2007, 23(13): 6903-6910
- [93] Cunningham VJ, Alswieleh AM, Thompson KL, et al. Poly(glycerol monomethacrylate)-poly(benzyl methacrylate) diblock copolymer nanoparticles via raft emulsion polymerization: Synthesis, characterization, and interfacial activity. *Macromolecules*, 2014, 47(16): 5613-5623
- [94] Thompson KL, Armes SP, Howse JR, et al. Covalently cross-linked colloidosomes. *Macromolecules*, 2010, 43(24): 10466-10474
- [95] Hunter SJ, Penfold NJW, Chan DH, et al. How do charged end-groups on the steric stabilizer block influence the formation and long-term stability of pickering nanoemulsions prepared using sterically stabilized diblock copolymer nanoparticles? *Langmuir*, 2020, 36(3): 769-780

[96] Chan DHH, Hunter SJ, Neal TJ, et al. Adsorption of sterically-stabilized diblock copolymer nanoparticles at the oil–water interface: Effect of charged end-groups on interfacial rheology. *Soft Matter*, 2022, 18(35): 6757-6770

Table of Contents graphic

