

One-pot synthesis of high-concentration mixed-shell polymeric

micelles as nanochaperones for renaturation of bulk proteins

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1. Synthesis of N-(3-methacrylamidopropyl) guanidinium chloride(GUA)

The monomer of GUA was synthesized according to the reported procedure.¹

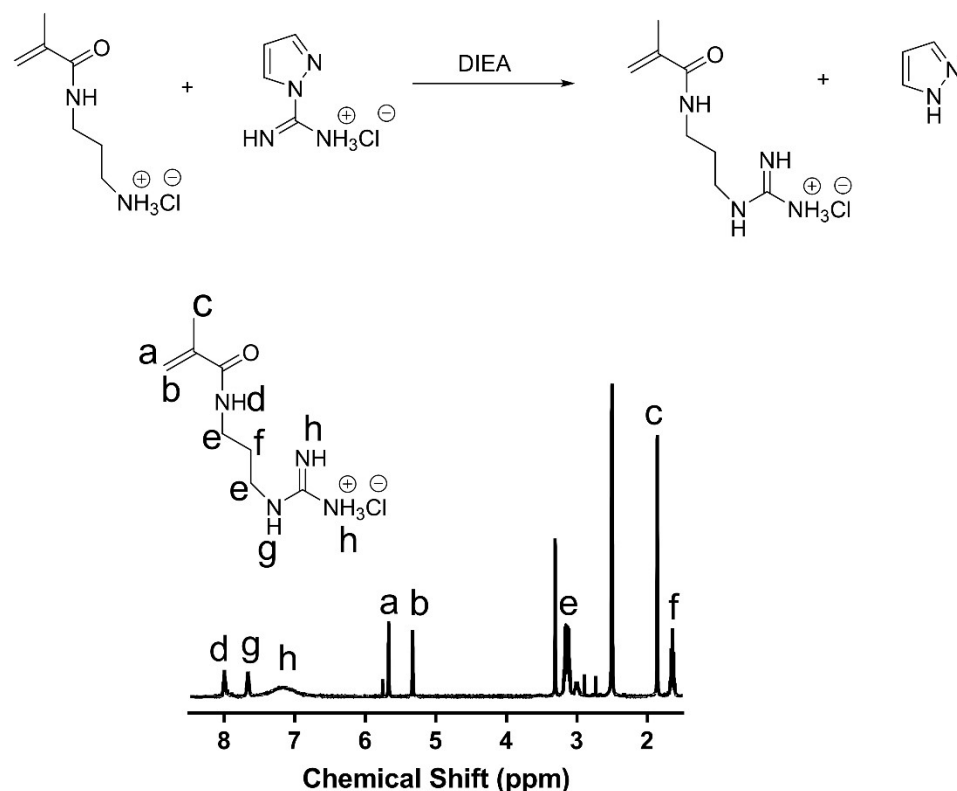
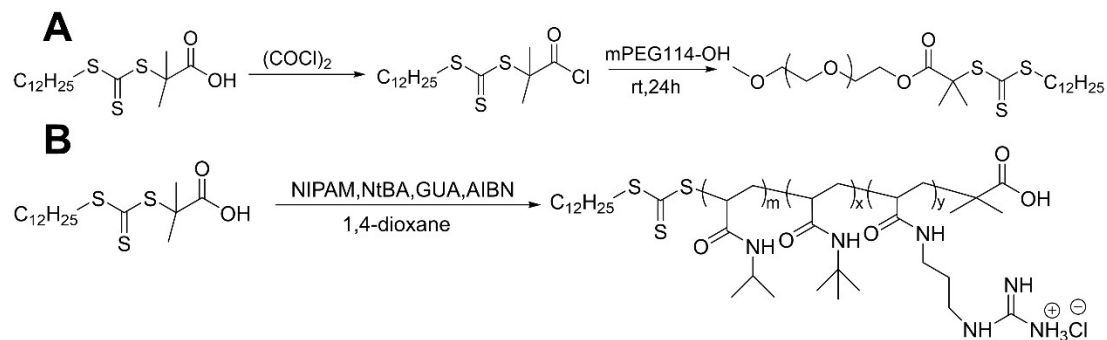


Fig S1. Synthesis and ¹H NMR spectrum of N-(3-methacrylamidopropyl) guanidinium chloride

2. Synthesis process of mPEG-CTA and PNIPAM-co-NTBA-co-GUA-CTA

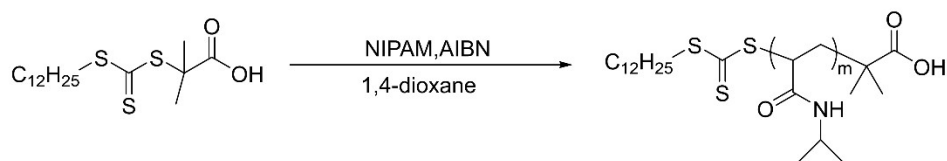


Scheme S1. (A) Synthesis of macro-RAFT agent of mPEG-CTA. (B) Synthesis of macro-RAFT agent of PNIPAM-co-NTBA-co-GUA-CTA.

3. Synthesis of PNIPAM-CTA:

The PNIPAM-CTA macro-RAFT agent was synthesized by solution RAFT polymerization of NIPAM in 1,4-dioxane at 70 °C using DDMAT as the RAFT agent and AIBN as the initiator. Briefly, DDMAT (42.63 mg, 0.117mmol), NIPAM (661.5 mg, 5.846 mmol) and AIBN(6.4 mg,0.039 mmol) were added into the reaction flask and then 4 ml of 1,4-dioxane was added. After three freeze-degas-thaw cycles, polymerization was conducted at 70 °C for 12 h. The mixture was diluted with 1,4-dioxane and then precipitated into excess amount of diethyl ether. The precipitate was

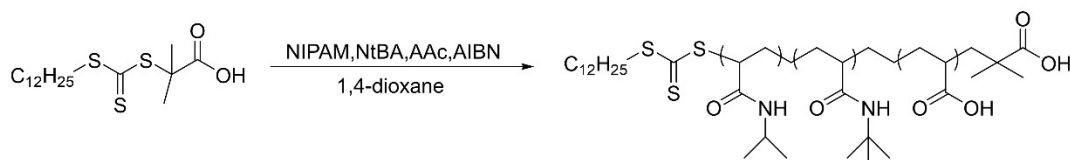
dried in a vacuum oven at room temperature to afford PNIPAM-CTA.



Scheme S2. Synthesis of macro-RAFT agent of PNIPAM-CTA.

4. Synthesis of PNIPAM-*co*-NTBA-*co*-AAc-CTA:

The PNIPAM-*co*-NTBA-*co*-AAc-CTA macro-RAFT agent was synthesized by solution RAFT polymerization of NIPAM, NTBA and AAc in 1,4-dioxane at 70°C using DDMAT as the RAFT agent and AIBN as the initiator. Briefly, DDMAT (42.63 mg, 0.117 mmol), NIPAM (661.5 mg, 5.846 mmol), NTBA(223.06 mg,1.75 mmol), AAc (126.38 mg,1.75 mmol) and AIBN(6.4 mg,0.039 mmol) were added into the reaction flask and then 4 ml of 1,4-dioxane was added. After three freeze-degas-thaw cycles, polymerization was conducted at 70 °C for 12 h. The mixture was diluted with 1,4-dioxane and then precipitated into excess amount of diethyl ether. The precipitate was dried in a vacuum oven at room temperature to afford PNIPAM-*co*-NTBA-*co*-AAc-CTA.



Scheme S3. Synthesis of macro-RAFT agent of PNIPAM-*co*-NTBA-*co*-AAc-CTA.

5. GPC characterization of macro-RAFT agents

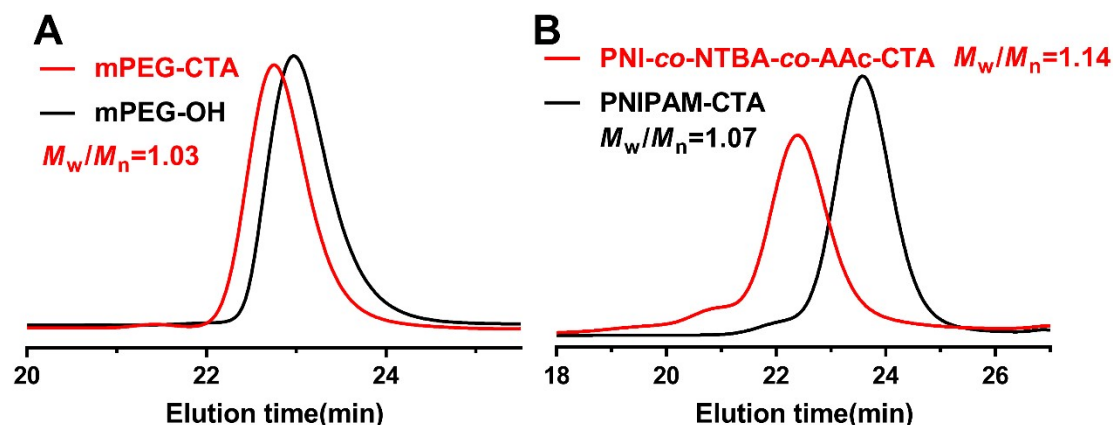


Fig S2. The GPC traces of three kinds of macro-RAFT agents. (A) mPEG-CTA, (B) PNIPAM-CTA and PNI-*co*-NTBA-*co*-AAc-CTA.

6. Table S1. Summary of the number-average molecular weight and the polydispersity index (PDI) of the synthesized four kinds of macro-RAFT agents

Polymer	Mn(H NMR)	Mn(GPC)	PDI
PEG _{5k} -CTA	5347	8245	1.03
PNIPAM ₄₂ -CTA	5110	5833	1.07
PNIPAM _{42-co-NtBA} _{13-co-AAc} ₁₁ -CTA	7553	9740	1.14
PNIPAM _{40-co-NtBA} _{12-co-GUA} ₁₄ -CTA	9488	--	--

*The PDI of the synthesized polymers were measured by GPC.

7. LCST of thermo-responsive macro-RAFT agents

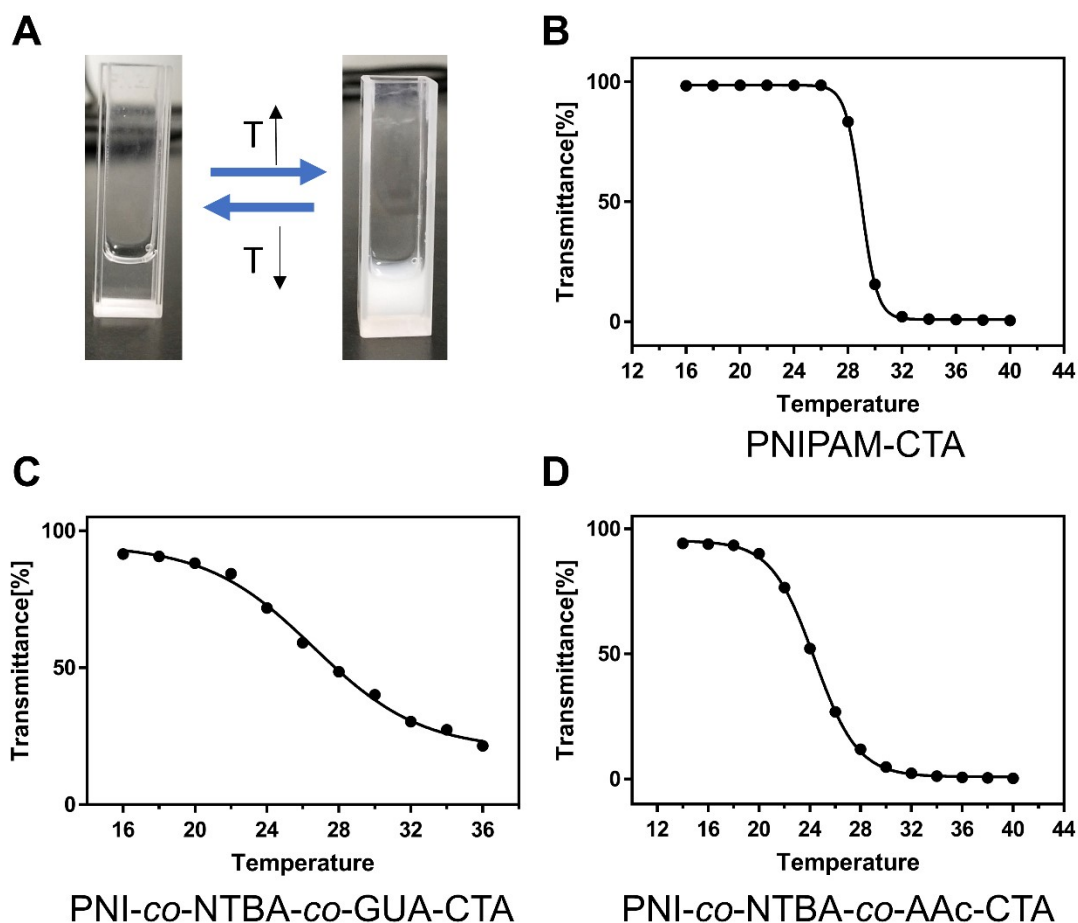


Fig S3. Temperature-dependent transmittance of three kinds of macro-RAFT agents.

8. Solubility of macro-RAFT agents

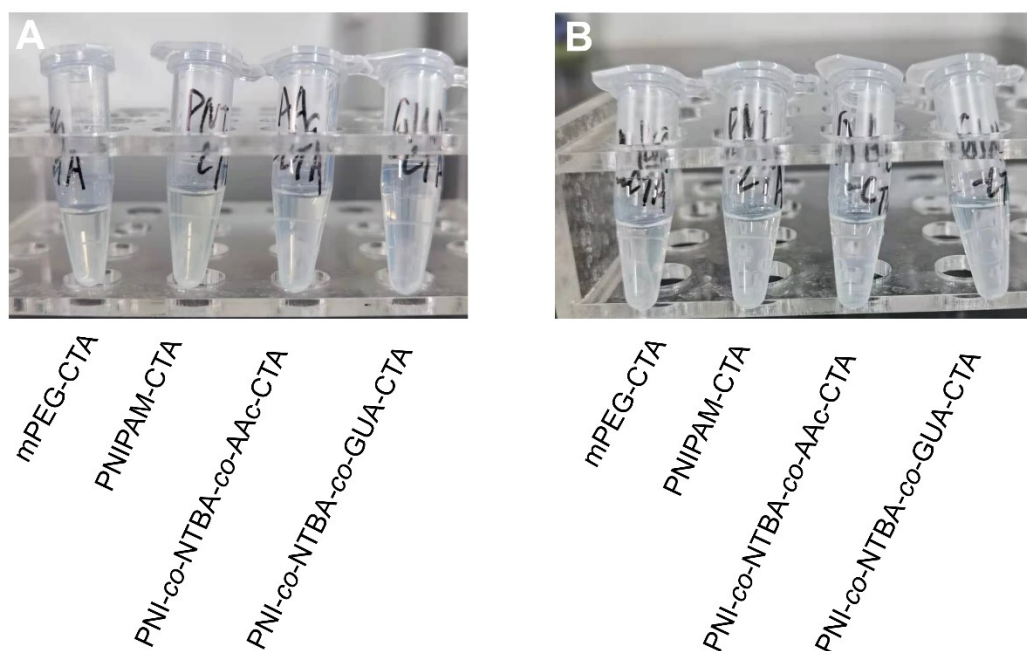


Fig S4. Photograph of four kinds of macro-RAFT agents solution. (A), (B) represents four macro-RAFT agents dissolved in 1,4-dioxane, ethanol respectively. The concentration is 10 mg ml⁻¹.

9. Preparation of uncharged nanoparticles

The uncharged nanoparticles were performed by the two macro-RAFT agents of mPEG₁₁₄-CTA and PNIPAM-CTA co-mediated crosslinking polymerization. The two macro-RAFT agents co-mediated solution crosslinking polymerization of NTBA was performed in 1,4-dioxane at 70 °C under [NTBA]:[mPEG₁₁₄-CTA]:[PNIPAM-CTA]:[Bis]:[AIBN]=200:1/7:6/7:5:0.4. Briefly, mPEG₁₁₄-CTA(7.3 mg, 0.00136 mmol), PNIPAM-CTA(41.87 mg, 0.00819 mol), NTBA(242.8 mg, 1.91 mmol), Bis(7.36 mg, 0.0478 mmol), AIBN(0.63 mg, 0.0038 mmol) and 1,3,5-trioxane(17.1 mg, 0.19 mmol, internal standard) were dissolved in 5 ml 1,4-dioxane. After three freeze-degas-thaw cycles, polymerization was conducted at 70 °C for 24 h. After the reaction was completed, the mixture was dialyzed in 1,4-dioxane firstly, then dialyzed in ethanol, and finally transferred to cold ultrapure water for dialysis for two days to obtain high-concentration nanoparticles. The conversion of monomer was calculated by comparing the vinyl signals of NTBA at 6.2,6.0, and 5.57 ppm with the signal of 1,3,5-trioxane at 5.15 ppm.

10. Preparation of negatively charged nanoparticles

The negatively charged nanoparticles were performed by the two macro-RAFT agents of mPEG₁₁₄-CTA and PNIPAM-co-NTBA-co-AAc-CTA co-mediated crosslinking polymerization. The two macro-RAFT agents co-mediated solution crosslinking polymerization of NTBA was performed in 1,4-dioxane at 70 °C under [NTBA]:[mPEG₁₁₄-CTA]:[PNIPAM-co-NTBA-co-AAc-CTA]:[Bis]:[AIBN]=200:1/7:6/7:5:0.4. Briefly, mPEG₁₁₄-CTA(6.85 mg, 0.00128 mmol), PNIPAM-co-NTBA-co-AAc-CTA (58.02 mg, 0.00768 mol), NTBA(227.6 mg, 1.792 mmol), Bis(6.9 mg, 0.0448 mmol), AIBN(0.59 mg, 0.0036 mmol) and 1,3,5-trioxane(16.2 mg, 0.18 mmol, internal standard) were dissolved in 5 ml 1,4-dioxane.

After three freeze-degas-thaw cycles, polymerization was conducted at 70 °C for 24 h. After the reaction was completed, the mixture was dialyzed in 1,4-dioxane firstly, then dialyzed in ethanol, and finally transferred to cold ultrapure water for dialysis for two days to obtain high-concentration nanoparticles. The conversion of monomer was calculated by comparing the vinyl signals of NTBA at 6.2,6.0, and 5.57 ppm with the signal of 1,3,5-trioxane at 5.15 ppm.

11. TEM of synthesized nanogel

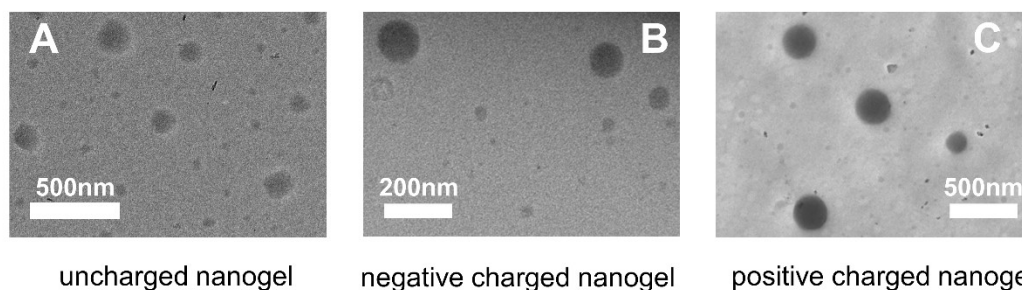


Fig S5. TEM of different charged mixed-shell nanogels. (A), (B), (C) represents the uncharged nanogels, the negatively charged nanogels and the positively charged nanogels respectively. TEM samples were prepared by dispersing the synthesized particles in 1,4-dioxane into ethanol at a volume ratio of 1:9 (1,4-dioxane : ethanol).

1.Y. Yonamine, K. Yoshimatsu, S. H. Lee, Y. Hoshino, Y. Okahata and K. J. Shea, *ACS Appl. Mater. Interfaces*, 2013, **5**, 374-379.