Electronic Supplementary Material (ESI) for Polymer Chemistry


Electronic Supporting Information

# Block Copolymer Conjugates Prepared by Sequentially Grafting from Proteins via RAFT

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**Determination of the extinction coefficient for the *N*-hydroxy succinimide functionalized trithiocarbonate chain transfer agent (NHS-CTA).** UV-Vis spectroscopy was employed to determine the concentration of trithiocarbonate moieties present in the protein macro-chain transfer agent (macroCTA) and polymer-protein conjugates. To enable these determinations, a calibration plot (Fig. S1) was constructed according to the description in the main text.

**Fig. S1.** UV-Vis extinction coefficient determination for NHS-CTA in aqueous solution.

**RAFT polymerization of *N*-isopropylacrylamide (NIPAM) with the lysozyme (LYS)-macroCTA.** NIPAM was polymerized in the presence of a lysozyme LYS-macroCTA in phosphate buffer (pH 6.0) at 25 °C ([NIPAM]/[LYS-macroCTA]/[initiator] = 219/1/1.3). The pseudo-first-order kinetic plot is shown in Fig. S2.

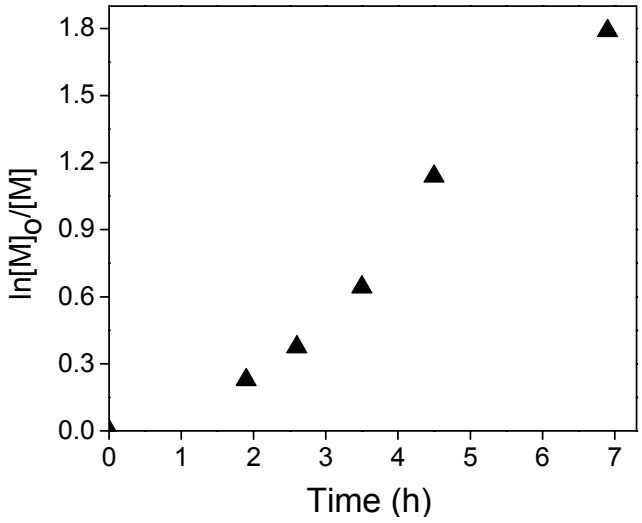


**Fig. S2.** Pseudo-first-order kinetic plot for the grafting-from reversible addition-fragmentation chain transfer (RAFT) polymerization of *N*-isopropylacrylamide (NIPAM) with the lysozyme (LYS)-macro-chain transfer agent (CTA) in phosphate buffer (pH 6.0) at 25 °C in the presence of the LYS-macroCTA ([NIPAM]/[LYS-macroCTA]/[initiator] = 219/1/1.3).

**Polymer cleavage from protein by treatment with Tergazyme.** Tergazyme was used to decompose lysozyme from the polymer-lysozyme conjugates and to isolate the remaining polymer. The decomposition procedure was as reported in the main text. After the process, no protein band was observed in the subsequent SDS-PAGE, indicating the successful decomposition of LYS (Fig. S3). As a control experiment, PNIPAM homopolymer and PNIPAM-*b*-poly(*N*,*N*-dimethylacrylamide (PDMA) block copolymer were treated with Tergazyme under the identical conditions as described above. No noticeable difference was observed in the size exclusion chromatography (SEC) traces of the polymers before and after incubation with Tergazyme (Fig. S3).

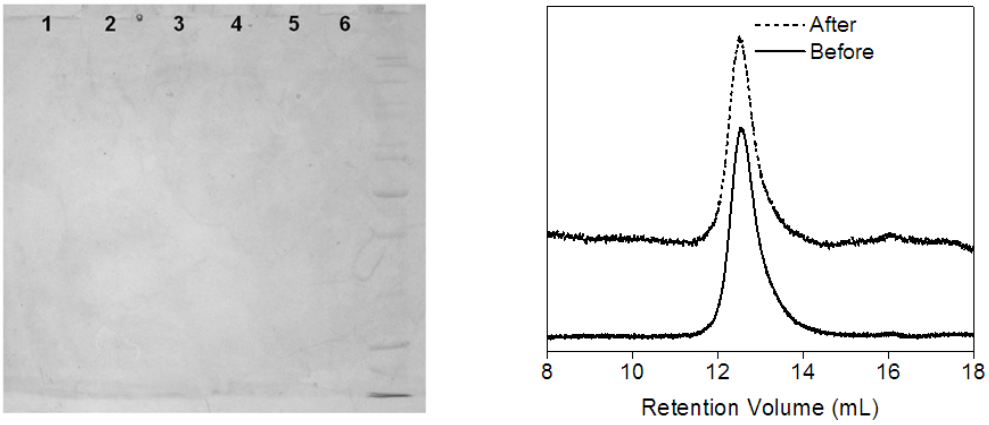


**Fig. S3.** (Left) SDS-PAGE results of cleaved PNIPAM from aliquots taken during the polymerization of NIPAM with the LYS-macroCTA (Lanes 1-6: t = 0, 1.9, 2.6, 3.5, 4.5, and 6.9 h, respectively). The absence of bands indicates successful protein decomposition. Lane numbers correspond to SEC curves in Fig. 1C. (Right) SEC traces from a control experiment in which PNIPAM-*b*-PDMA was exposed to Tergazyme for 2 days at room temperature.

**Block copolymerization of DMA from the LYS-PNIPAM macroCTA.** Chain extension of the LYS-PNIPAM macroCTA with DMA was conducted as described in the main text. A plot of $M_n$ and $M_n$/ $M_w$ versus conversion is given in Fig. S4.

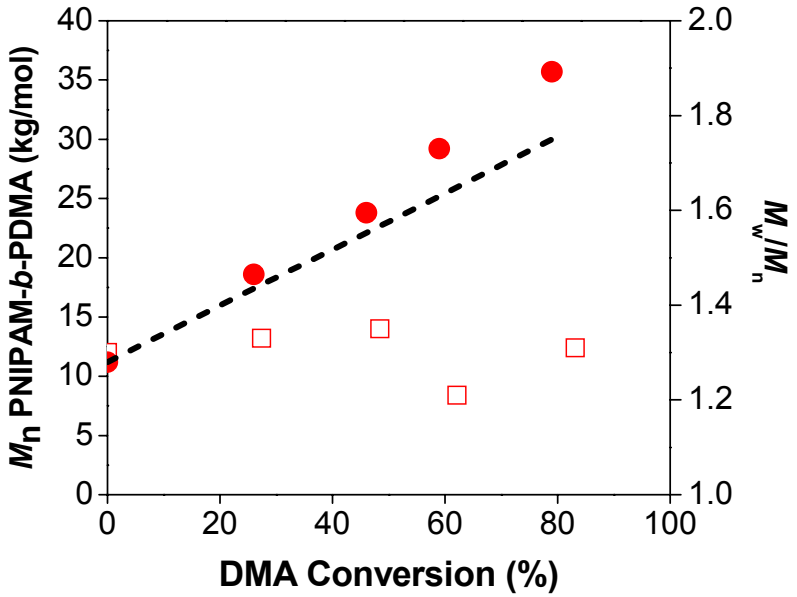


**Fig. S4.** $M_n$ and $M_w/M_n$ vs. monomer conversion for cleaved PNIPAM-*b*-PDMA after grafting from a LYS-PNIPAM macroCTA. The dotted line represents the theoretical $M_n$.