Supporting information for

Impact of Polymer Bioconjugation on Protein Stability and Activity Investigated with Discrete Conjugates: Alternatives to PEGylation

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Table of Contents

^{1}H NMR Spectra of NHS Ester-Functionalized Polymers

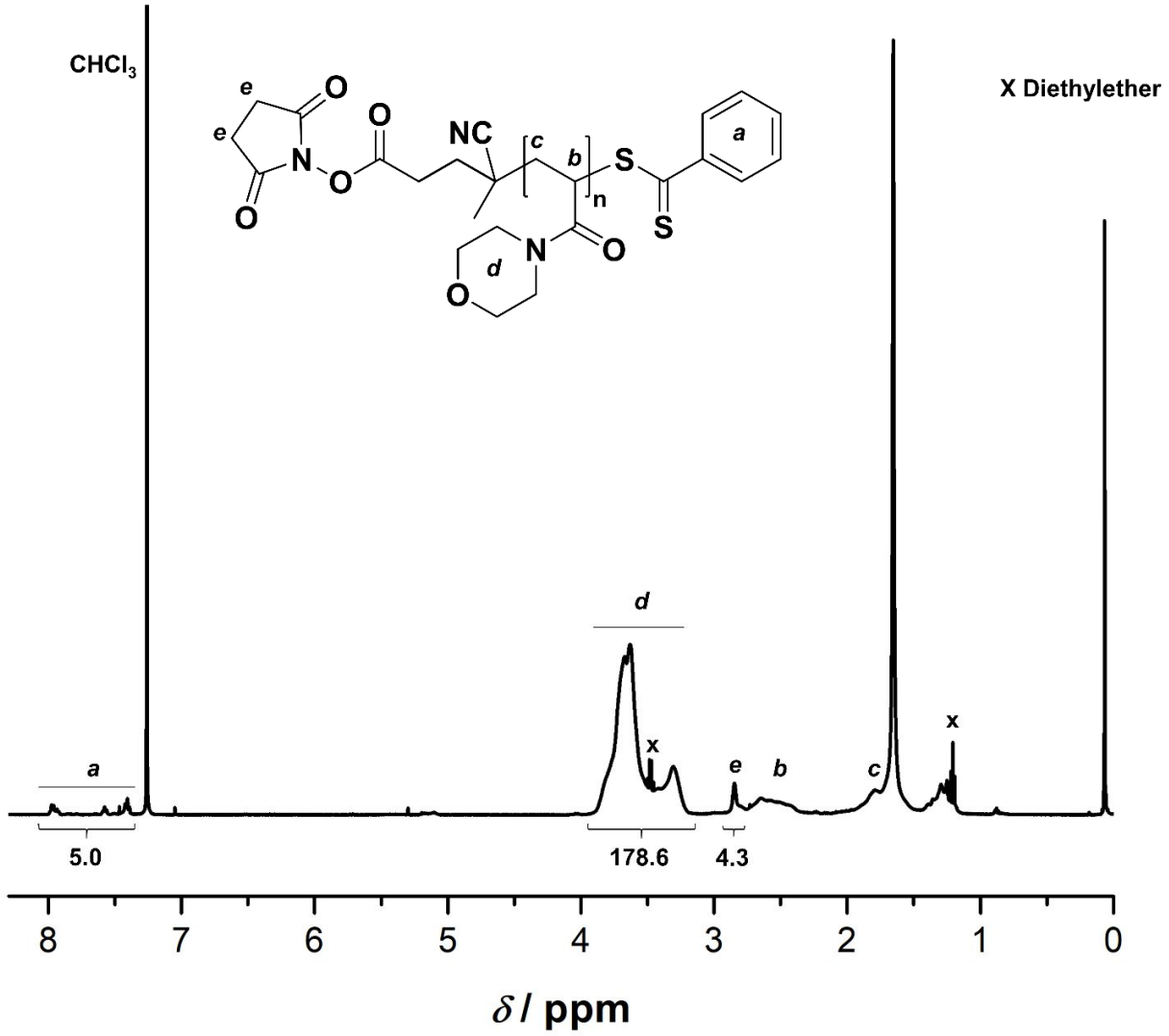

Figure S1. ^{1}H NMR spectrum of $PNAM_{3.4kDa}$.

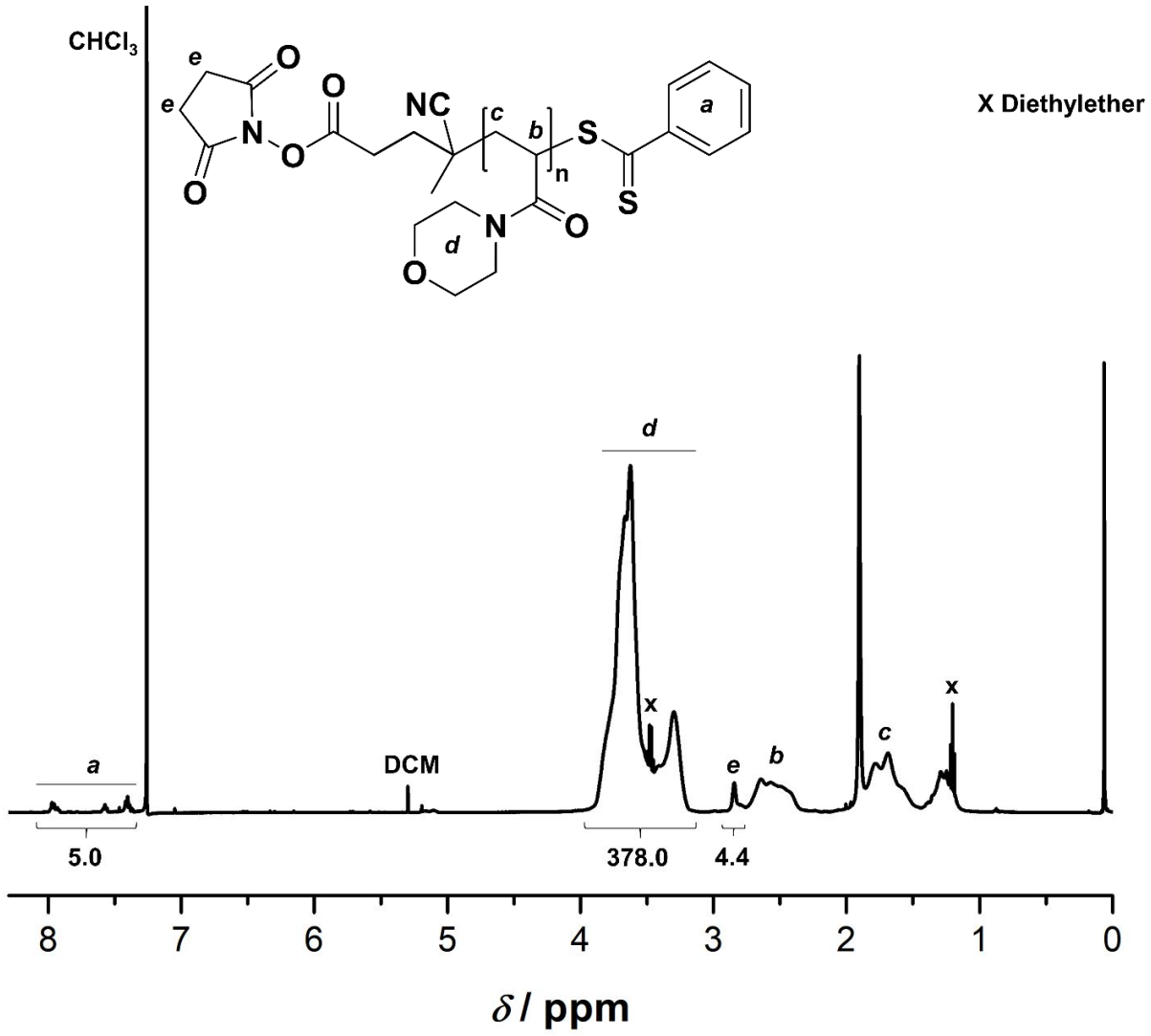

Figure S2. ^{1}H NMR spectrum of $PNAM_{6.9kDa}$.

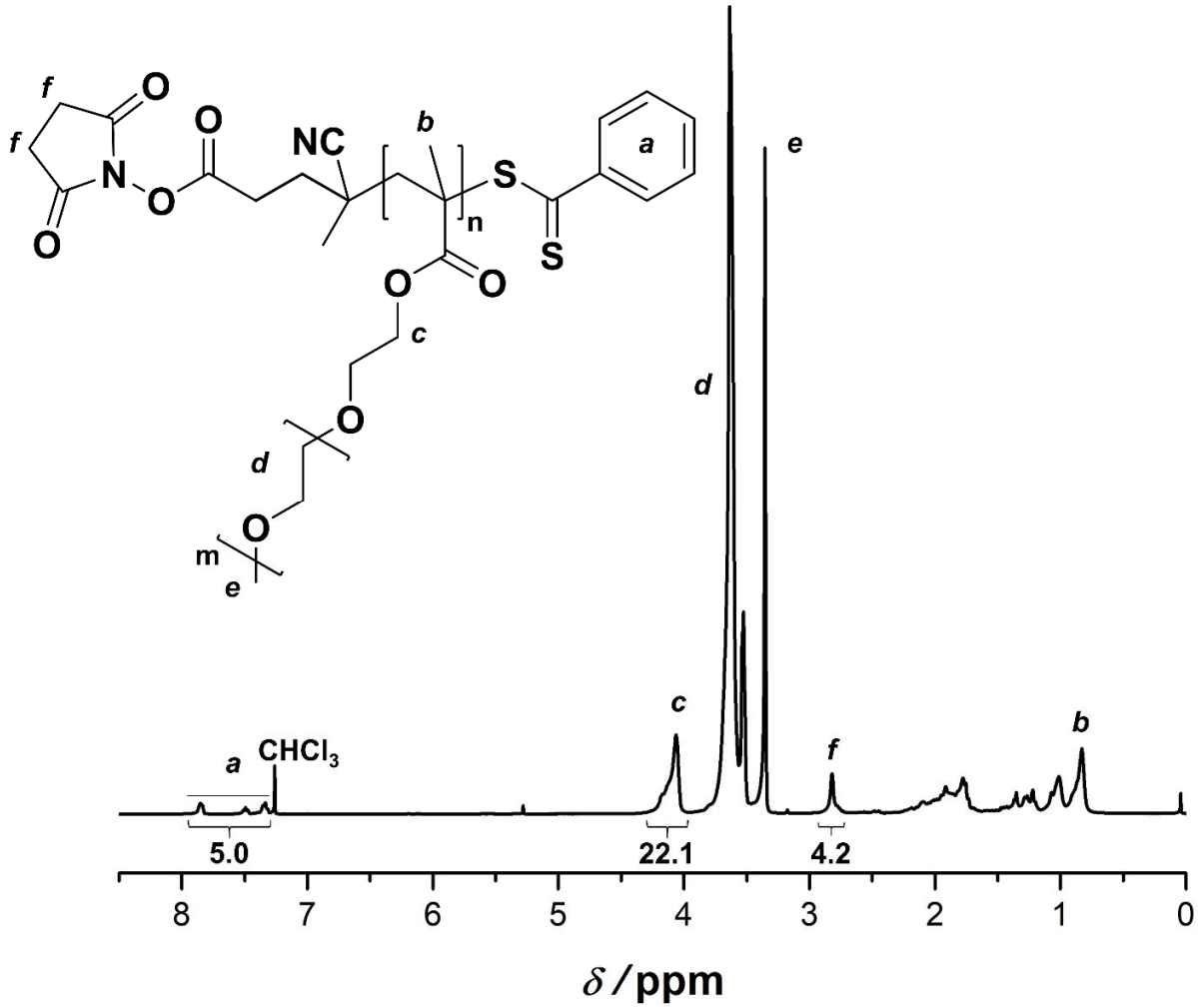

Figure S3. ^{1}H NMR spectrum of POEGMA$_{3.6kDa}$.

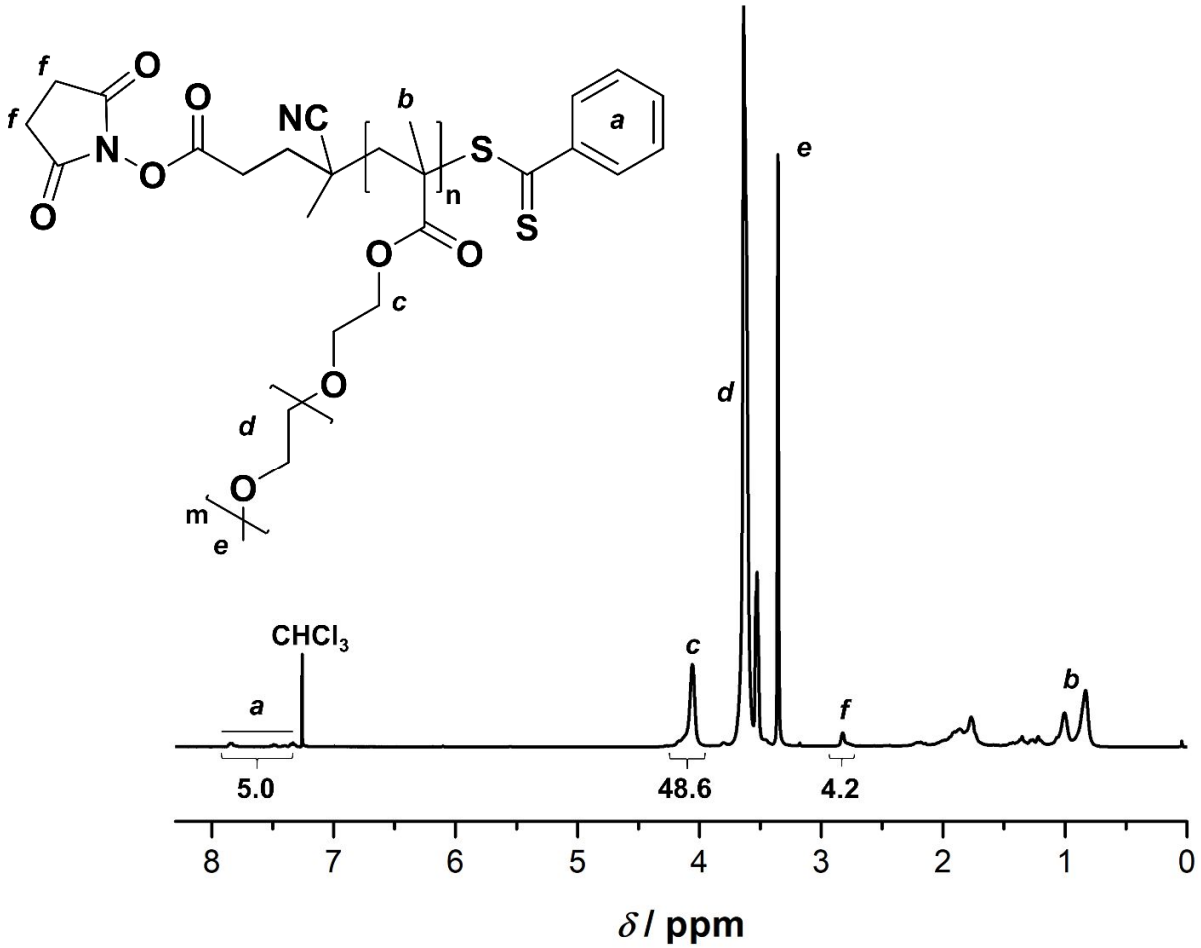

Figure S4. ^{1}H NMR spectrum of POEGMA$_{7.5kDa}$.

Table S1. Macromolecular characteristics of the NHS ester-functionalized PNAM and POEGMA used for lysozyme modification.

Polymer	$M_{n,SEC}$	$M_{n,NMR}$[a]	$Đ$
PNAM$_{3.4Da}$	1900	3500	1.08
PNAM$_{6.9kDa}$	3800	7000	1.09
POEGMA$_{3.6kDa}$	3400	3700	1.10
POEGMA$_{7.5kDa}$	6700	7700	1.10

[a]The NMR value includes the *N*-hydrosuccinimide group, which was however not taken into account to name the polymers for better comparison of the conjugates since this group is removed during coupling to the protein.

UV–Vis Spectrophotometric Data

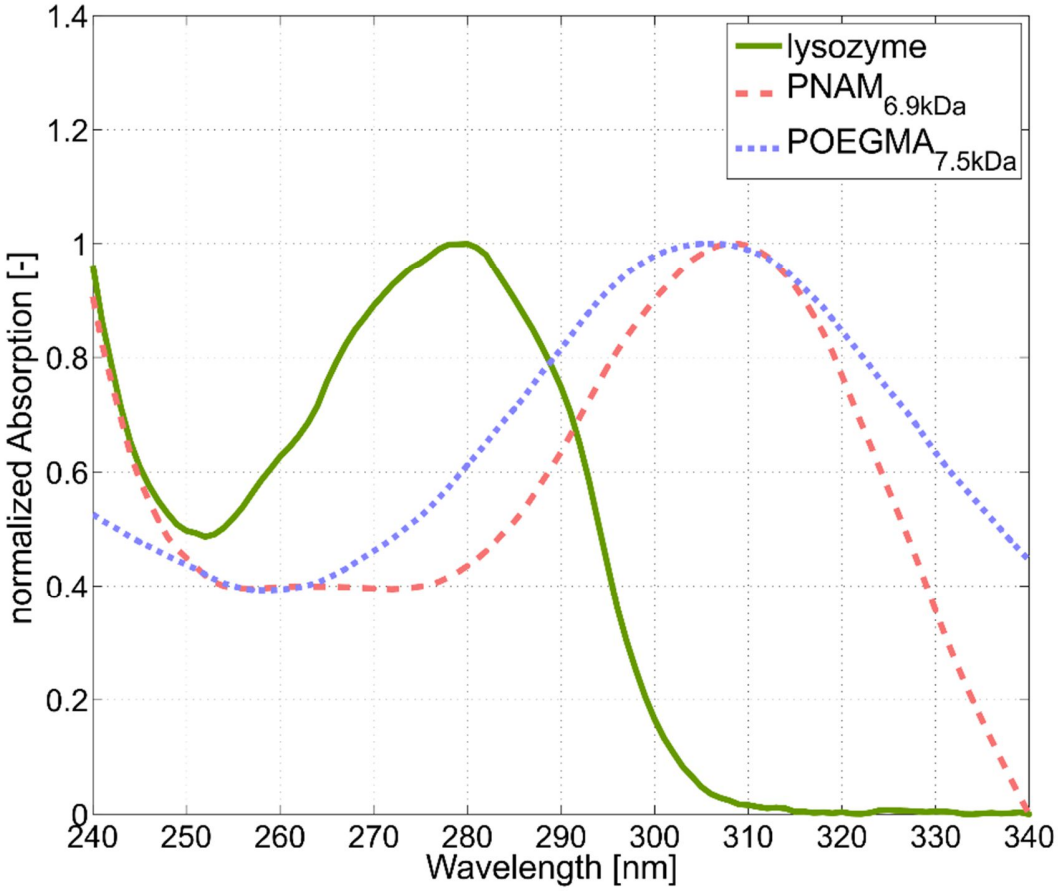

Figure S5. Absorption spectra of lysozyme, $PNAM_{6.9kDa}$, and $POEGMA_{7.5kDa}$ normalized to their absorption maximum.

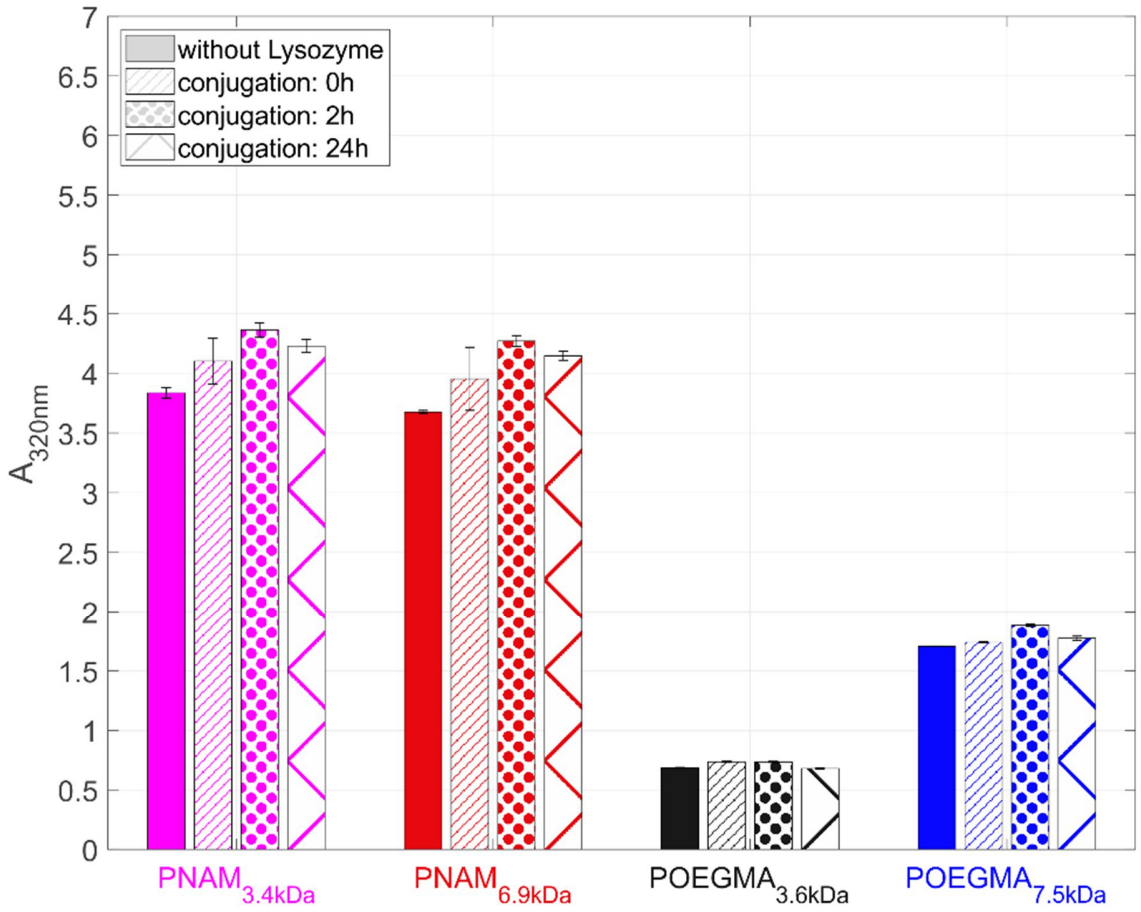

Figure S6. Absorbance at 320 nm (A_{320nm}) for all polymers during the conjugation process with only the polymer in 25 mM sodium phosphate buffer at pH 7.2. (without Lysozyme), after adding lysozyme (conjugation: 0h), after two hours conjugation time (conjugation: 2h), and after twenty-four hours conjugation time (conjugation: 24h). A_{320nm} of each sample was measured as triplicate. For none of the investigated polymers is a decrease in A_{320nm} observable over time.

Capillary Gel Electrophoresis

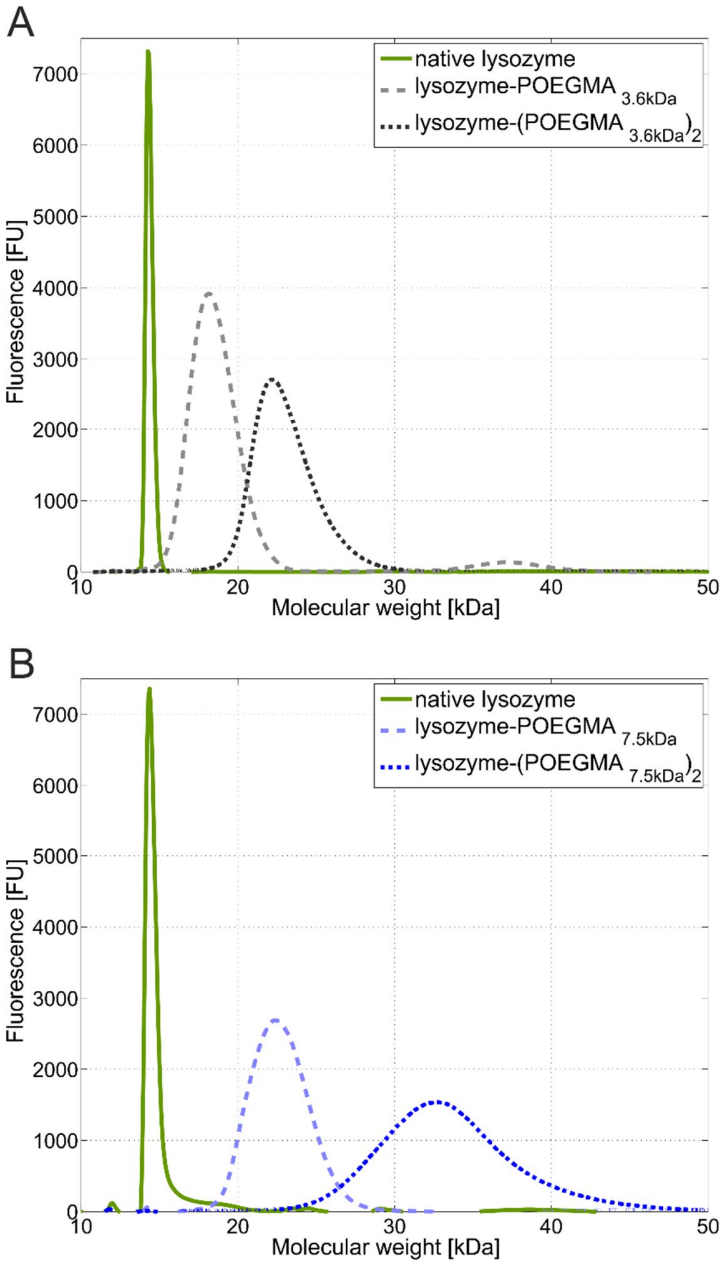

Figure S7. Analysis of the purified $POEGMA_{3.6kDa}$ (A) and $POEGMA_{7.5kDa}$ (B) conjugates using high-throughput capillary gel electrophoresis (HT-CGE), as compared to native lysozyme.[1]

Size-Exclusion Chromatography of Proteins and Conjugates

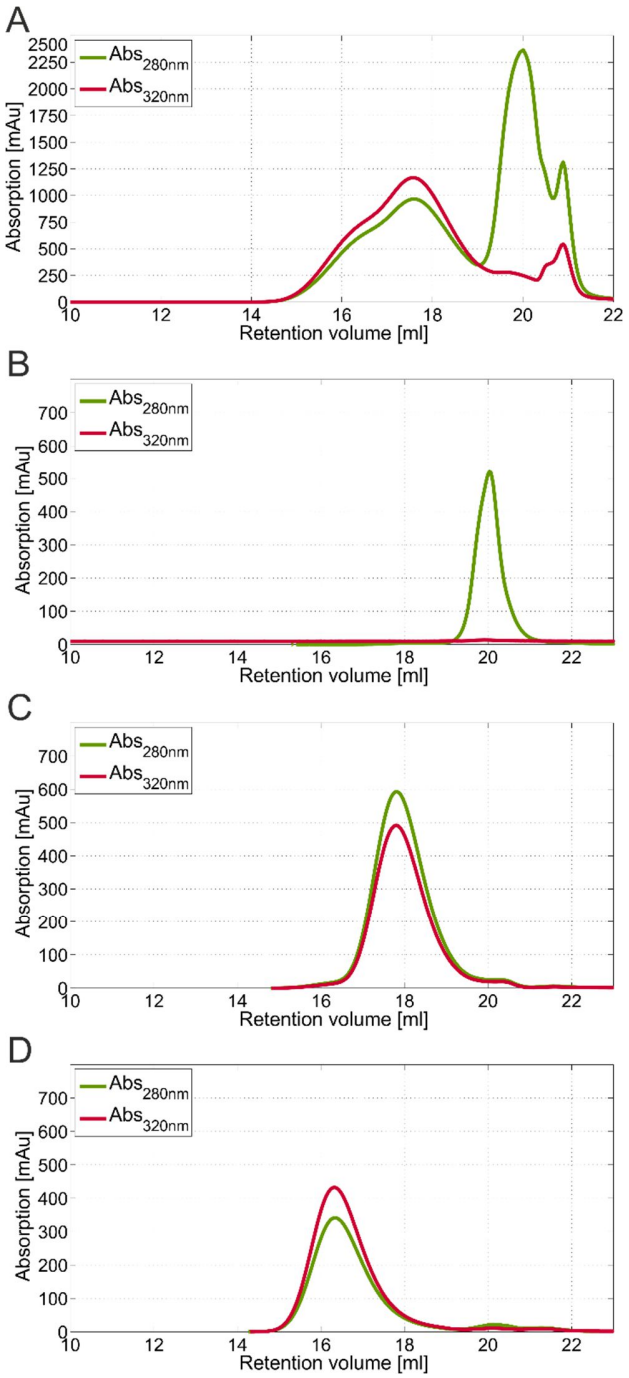

Figure S8. SEC chromatograms of the POEGMA$_{3.6kDa}$ conjugation batch (A) and the purified species of native lysozyme (B), lysozyme–POEGMA$_{3.6kDa}$ (C), and lysozyme–(POEGMA$_{3.6kDa}$)$_2$ (D). The separation was performed on a Superdex200 Increase 10/300 (GE Healthcare) using 25 mM sodium phosphate buffer with 150 mM sodium chloride at pH 7.2.

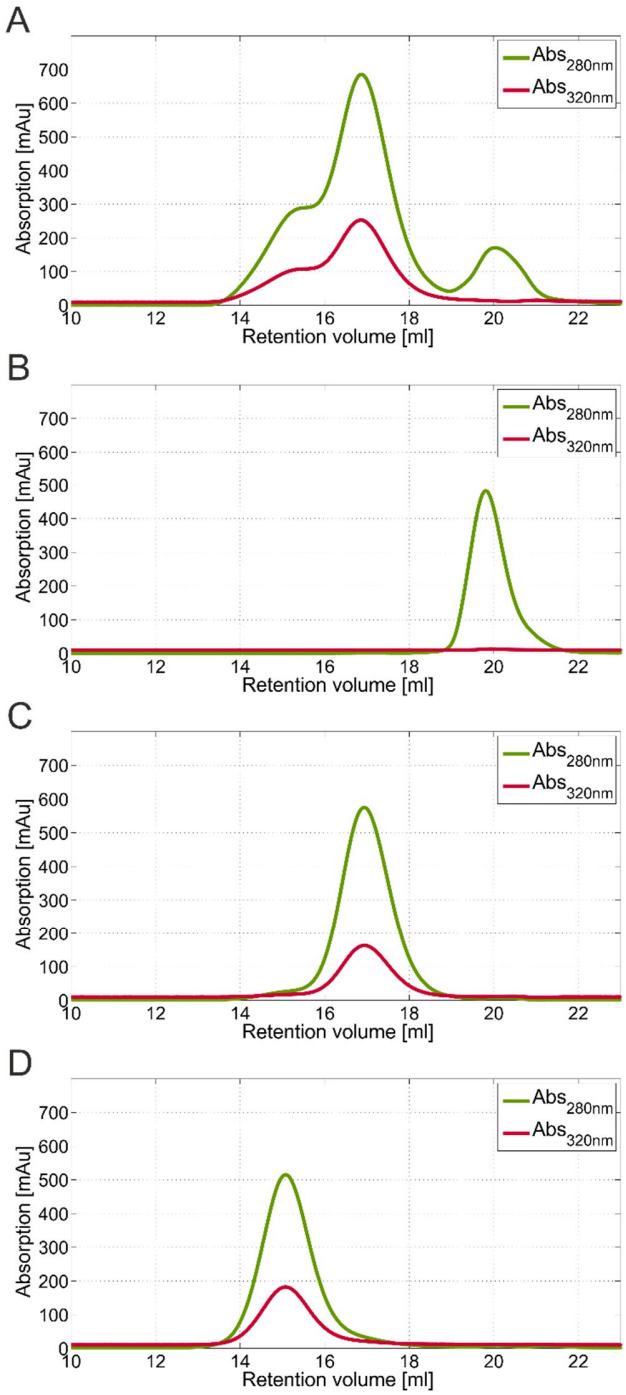

Figure S9. SEC chromatograms of the POEGMA$_{7.5kDa}$ conjugation batch (A) and the purified species of native lysozyme (B), lysozyme–POEGMA$_{7.5kDa}$ (C), and lysozyme–(POEGMA$_{7.5kDa}$)$_2$ (D). The separation was performed on a Superdex200 Increase 10/300 (GE Healthcare) using 25 mM sodium phosphate buffer with 150 mM sodium chloride at pH 7.2.

Description of the High-Throughput Solubility Study of Conjugates

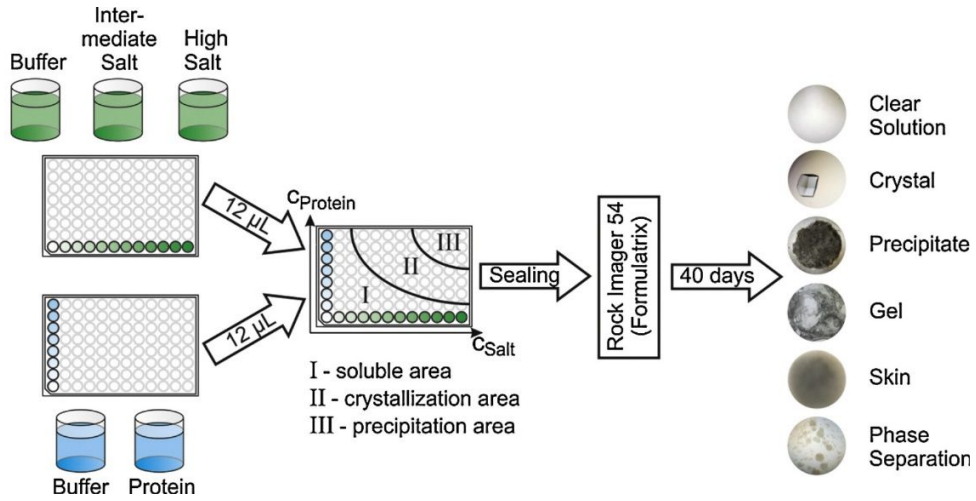

Figure S10. Schematic illustration of the experimental setup and evaluation of protein phase states for the generation of protein phase diagrams. The soluble (I), crystallization (II), and precipitation (III) areas are depicted in the phase diagram. Reproduced with permission from Elsevier.[2]

Phase Behavior of the Polymers

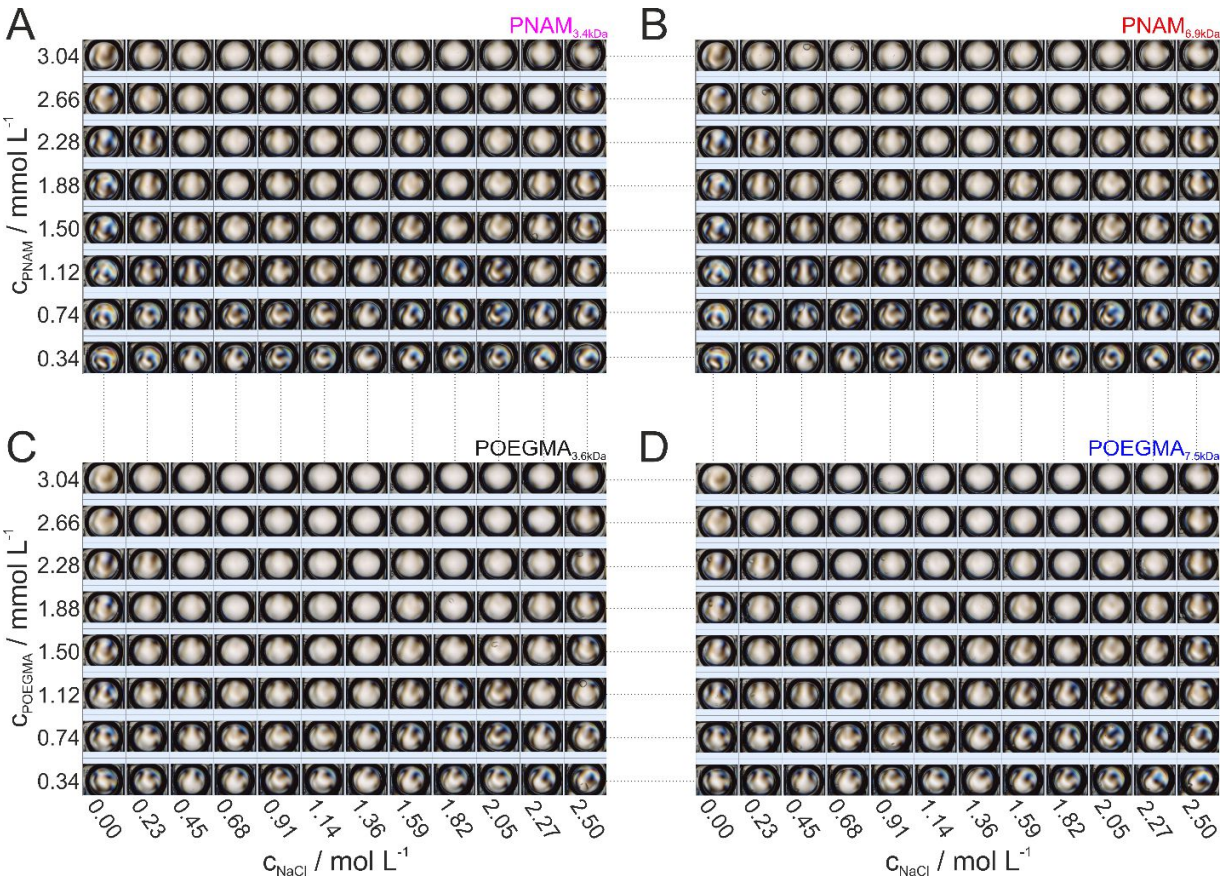

Figure S11. Phase behavior of (A) $PNAM_{3.4kDa}$, (B) $PNAM_{6.9kDa}$, (C) $POEGMA_{3.6kDa}$, and (D) $POEGMA_{7.5kDa}$ under the influence of sodium chloride (NaCl) as precipitant at pH 3. Under the studied experimental conditions none of the investigated polymers undergoes phase transition.

References

(1) Morgenstern, J.; Busch, M.; Baumann, P.; Hubbuch, J. *J. Chromatogr. A* **2016**, *1462*, 153–164.

(2) Baumgartner, K.; Galm, L.; Nötzold, J.; Sigloch, H.; Morgenstern, J.; Schleining, K.; Suhm, S.; Oelmeier, S. A.; Hubbuch, J. *Int. J. Pharm.* **2015**, *479* (1), 28–40.