SUPPLEMENTARY INFORMATION FOR

Arginine-specific protein modification using α-oxo-aldehyde functional polymers prepared by atom transfer radical polymerization

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Synthesis of 3-bromo-1,1-dimethoxypropan-2-one (15). 15 was prepared following a modified procedure from De Kimpe et al. by bromination of 2 (3.14 g, 15.7 mmol) in a 100 mL round-bottom flask containing 2.62 g N-bromosuccinimide (2.62 g, 14.7 mmol) in 50 mL CCl₄. The solution was deoxygenated by bubbling with nitrogen for 15 minutes then stirred under inert atmosphere for 24 h at room temperature. Solids were removed by filtration through a sand plug and 5 g strong-acid ion-exchange resin (Amberlyte IR-120) added to the filtrate, which was then agitated for 30 minutes. The resin was removed by filtration and solvent removed in vacuo. 15 was recovered as a single fraction by distillation (100–130 ºC / 10 mbar) to yield 2.20 g (71 %) of a colorless liquid, which was stored at –30 ºC until used.

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\begin{align*}
\text{(1)} & \xrightarrow{i} \text{(2) (79% isolated)} & \xrightarrow{ii} \text{(14) (>95% conversion, not isolated)} & \xrightarrow{iii} \text{(15) (71% isolated)}
\end{align*}
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Scheme S1. Synthesis of functional ATRP initiators from methylglyoxal 1,1-dimethylacetal. Reaction conditions: (i) cyclohexylamine, CaCl₂, 45 ºC; (ii) N-bromosuccinimide, CCl₄; (iii) Amberlyte IR-120, CCl₄.
Figure S1. Assigned $^1$H and $^{13}$C NMR spectra of compounds 1-5, and 15. All spectra recorded in CDCl$_3$ except for 1 in D$_2$O. Note that letter-based assignments in Figure S1 are only valid for Figure S1 and are not consistent with those of the rest of the document.
**Figure S2.** Semi-logarithmic kinetic plots for the polymerization of MMA using 15 as initiator in toluene at 90 °C ([M]:[I]:[Cu]:[L] = 100:1:1:2) using HMTETA, PMDETA, 2,2’-bipyridine or 4,4’-dimethyl 2,2’-bipyridine as ligands. Same procedure used as for polymerization of MMA using 5 (see main manuscript). Each colored line represents a different attempt at polymerization under exactly the same conditions. This Figure demonstrates substantial irreproducibility. Increasing initiator concentration by a factor of 2 or 5 did not significantly improve results.
Figure S3. Polymerization of PEGMA with $[M_0]$:[$I$] ratios of 50 (circles), and 30 (triangles) in anisole at 60 °C. (Top) Evolution of experimental $M_n$ (filled symbols) and $M_w/M_n$ versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by $^1$H NMR spectroscopy in CDCl$_3$. 
Figure S4. Polymerization of DMAEMA with [M₀]:[I] ratios of 300 (squares), 250 (circles) and 200 (triangles) in anisole at 30 °C. (Top) Evolution of experimental $M_n,SEC$ (filled symbols) and $M_w/M_n$ versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by $^1$H NMR spectroscopy in CDCl₃.
Figure S5. Polymerization of tBuMA with [M₀]:[I] ratios of 100 (squares), 50 (circles) and 33 (triangles) in toluene at 75 °C. (Top) Evolution of experimental Mₙ,SEC (filled symbols) and Mₓₙ/Mₓ versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by ¹H NMR spectroscopy in CDCl₃.
**Figure S6.** $^1$H NMR spectrum of polymerization medium (toluene, CuBr, HMTETA and 5) without monomer left to react at 75 °C for 3 h to evaluate possible routes of initiator deactivation. The principal route of deactivation of 5 appears to be proton abstraction from solvent or monomer following activation of 5 to a radical species by Cu(I). This process results in the formation of 1,1-dimetoxybutan-2-one. Assignment based on chemical shift, integration, and multiplicity considerations. Peak assignments in Scheme S2.

**Scheme S2.** 1,1-dimethoxybutan-2-one. Note that letter-based assignments in Figure S6 correspond uniquely to those found in Scheme S2 and are not consistent with those of the rest of the document.
Figure S7. Polymerization of DMEAMA with [M₀]:[I] ratios of 50 (squares), 100 (circles) and 150 (triangles) under conditions given in the main manuscript for the preparation of 7. (Top) Evolution of experimental $M_n$ and $M_w/M_n$ (filled symbols) versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Polymers with [M₀]:[I] ratios of 100 and below had relatively broad molecular weight distributions, but remained monomodal.
Figure S8. $^1$H NMR spectrum of 6 (top, $M_{n,NMR}$ 32.8 kDa) and its corresponding deprotected polymer 10 (bottom) produced by I$_2$-mediated transacetalization. Peaks assigned using letter-based assignments found in Scheme 2 in main manuscript.
Figure S9. $^1$H NMR spectra of 7 (top, $M_{n,NMR}$ 14.6 kDa) in acetone containing 3 molar eq. I$_2$ (relative to polymer end-group) and the reaction mixture obtained following incubation at 90 ºC for 2 h (bottom). The bottom spectrum shows that the acetal remains intact and peaks caused by the halogenation of the amines appear. These peaks are assigned based on chemical shift and integration considerations (i.e., peak r integrates for 18H because 3 molar eq. of amines are halogenated). Peak assignments in Scheme S3.

Scheme S3. Deprotection of PDMAEMA (7) via I$_2$-mediated transacetalization leading to the halogenated polymer, for which a simplified structure is given. The actual structure of the modified polymer involves coordination of two dimethylamino groups to one iodide anion (to give a net positive charge), with I$_3^-$ as negative counterion.$^2$ Note that letter-based assignments in Figure S9 correspond uniquely to those found in Scheme S3 and are not consistent with those of the rest of the document.
Figure S10. $^1$H NMR spectra of 8 (top, $M_{n,NMR}$ 8.8 kDa) in acetone containing 3 molar eq. I$_2$ (relative to polymer end-group) and the reaction mixture obtained following incubation at room temperature for 24 h (middle). The latter spectra shows the formation of tert-butanol and 2-methyl 1,2-propene in the supernatant above the polymer. After the 24 h incubation period, precipitation was observed. The water-soluble fraction of precipitated polymer was dissolved in H$_2$O, purified by size-exclusion chromatography, isolated by freeze-drying and a $^1$H NMR spectrum taken in D$_2$O (bottom). This spectrum shows residual tert-butyl ester groups (peak g, 1.48 ppm) as well as an intact acetal end-group (a, 3.49 ppm). The star denotes Et$_2$O contaminant. Peak assignments are given in Scheme S4.

Scheme S4. Deprotection of PrBuMA (8) via I$_2$-mediated transacetalization. Note that letter-based assignments in Figure S10 correspond uniquely to those found in Scheme S4 and are not consistent with those of the rest of the document.
Figure S11. SEC chromatograms of polymers 6-13 showing no significant perturbation of molecular weight distribution following deprotection. (A) SEC chromatograms of 6 (Mn 18,400; Mw/Mn 1.20), 10 (via TFA deprotection, Mn 16,900; Mw/Mn 1.27), and 10 (via I2 deprotection, Mn 18,900; Mw/Mn 1.22) recorded in DMF; (B) SEC chromatograms of 7 (Mn 15,800; Mw/Mn 1.16) and 11 (via TFA deprotection, Mn 14,200; Mw/Mn 1.23) recorded in THF + 5% Et3N; (C) SEC chromatogram of 8 (Mn 15,300; Mw/Mn 1.28) recorded in THF and 12 (via TFA deprotection, Mn 17,600, Mw/Mn 1.24) recorded in 10 mM NaHPO4 (pH 7.4). The red star marks the solvent elution peak; (D) SEC chromatograms of 9 (Mn 6,400; Mw/Mn 1.27) and 13 (via I2 deprotection, Mn 6,300; Mw/Mn 1.19) recorded in THF. Molecular weights for 6-11 and 13 are given relative to PMMA. Molecular weight of 12 is given relative to PEG.
**Figure S12.** MALDI-TOF mass spectra of (A) 9 and (B) 13. The quasi-single distribution seen in the top figure demonstrates that 5 is the sole initiating species during ATRP and that all polymers therefore bear a protected α-oxo-aldehyde group. Deprotection of 9 to yield 13 was accomplished by I₂-mediated transacetalization in acetone (90 °C, 15 min). The conditions used were milder than those typically used to achieve deprotection in order to visualize the hemiacetal (peak C), which is an intermediate of the deprotection reaction and confirms the deprotection mechanism. The fully deprotected polymer (peak D) corresponds to the di-hydrate of 13.

**Scheme S5.** Summary of compounds observed in the MALDI-TOF mass spectra seen in Figure S9.
Figure S13. Raw HPLC chromatograms for the modification of HEWL with 10-12 at pH 9 (left) and pH 7.4 (right).
Figure S14. (a) SDS-PAGE of reaction mixtures containing HEWL and 10-12 performed at pH 9. Reactions were quenched with NH₂OH to cleave any polymer conjugated to HEWL at lysine residues. This image shows quasi-total transformation of HEWL to a conjugate. (b) Control SDS-PAGE containing 10, 11, 12, and HEWL. This image illustrates that the polymers themselves are revealed by the silver staining used to reveal the gel.

References for Supplementary Information