Supporting Information

Aqueous SARA ATRP using Inorganic Sulfites

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Experimental Section

Materials

Oligo(ethylene oxide) methyl ether acrylate (OEOA\textsubscript{480}, 99%, average molecular weight 480, Sigma-Aldrich) and oligo(ethylene oxide) methyl ether methacrylate (OEOMA\textsubscript{500}, 99%, average molecular weight 500, Aldrich) were passed over a column of basic alumina (Fisher Scientific) prior to use. Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} (Sigma–Aldrich, 85 %), Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} (Sigma–Aldrich, >99 %), NaHSO\textsubscript{3} (Sigma–Aldrich, >99 %), 2-hydroxyethyl 2-bromoisobutyrate (HEBiB, 95%, Sigma-Aldrich), copper(II) bromide (99.99%, Sigma-Aldrich), sodium chloride (NaCl, 99.5%, Fisher Scientific), tetraethylammonium chloride (TEACl, 98%, Sigma-Aldrich), sodium bromide (NaBr, 99.5%, Fisher Scientific), bovine serum albumin (BSA, ≥98%, Sigma-Aldrich), water (H\textsubscript{2}O, HPLC grade, Fisher Scientific), N,N-dimethylformamide (DMF, ACS grade, Fisher Scientific), deuterium oxide (D\textsubscript{2}O, 99.9%, Cambridge Isotope Laboratories), and anhydrous magnesium sulfate (99%, Aldrich) were used as received. 1X PBS solution was prepared from 10X PBS (Thermo Fisher Scientific) and HPLC grade water. Tris(pyridin-2-ylmethyl)amine (TPMA),\textsuperscript{1} tris[2-(dimethylamino)ethyl]amine (Me\textsubscript{6}TREN)\textsuperscript{2} and BSA protein initiator (BSA-O-[iBBr]\textsubscript{30})\textsuperscript{3} were prepared as previously reported in the literature.

Techniques

A syringe pump (KDS Scientific, Legato 101) was used for the continuous feeding of the sulfites at the rate 1 μL/min (different sulfite aqueous solutions were prepared to obtained different FR\textsubscript{S}).

\textsuperscript{1}H nuclear magnetic resonance (NMR) spectroscopy measurements were performed on a Bruker Avance 500 MHz spectrometer and used to determine the monomer conversion in D\textsubscript{2}O.

The chromatographic parameters of the samples were determined using a gel permeation chromatography (GPC) system equipped with a Waters 515 HPLC pump and a Waters 2414 refractive index detector using PSS columns (Styrogel 10\textsuperscript{2}, 10\textsuperscript{3}, 10\textsuperscript{5} Å) with DMF containing 10 mM LiBr as the eluent at a flow rate of 1 mL/min at 50 °C. The system was calibrated
with low dispersity PMMA ($M_n = 800 - 1\,820\,000$) standards. Before the injection (50 μL) the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.22 μm pore.

The UV-Visible studies were performed using an Agilent 8453 UV-Vis Spectrometer. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZS at 25 °C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Bio-Rad Laboratories Mini-Protean TGX™ Precast Gels (7.5%). Staining was accomplished with Coomassie blue and washed in DI water overnight.

**General Procedure for Aqueous SARA ATRP of OEOA**

A series of aqueous SARA ATRP using OEOA as monomer were carried out with systematically different conditions to establish the optimal reactions conditions. The conditions used for polymerization of OEOA$_{480}$ generally followed this procedure: NaCl (58.4 mg, 1.0 mmol), OEOA$_{480}$ (2.40 g, 5 mmol), 100 mM stock solution HEBiB (0.2 mL, 0.02 mmol), stock solution of 25 mM CuBr$_2$ and 200 mM TPMA (40 μL, 1.0 μmol of CuBr$_2$ and 8 μmol of TPMA) were dissolved in H$_2$O (7.7 mL). DMF (0.1 mL) was added as internal standard for $^1$H NMR analysis. This mixture was added to a 25 mL Schlenk flask and purged with nitrogen for 30 min, and then the flask was placed in an oil bath at 30 °C. A Na$_2$S$_2$O$_4$ aqueous solution (64 mM) was purged with nitrogen, and the solution was continuously injected into the reaction medium using a syringe pump at the rate 1 μL/min. Samples were taken throughout the reaction for GPC and NMR analysis.

**Synthesis of “One-pot” POEOMA-b-POEOA Block Copolymer**

NaCl (58.4 mg, 1.0 mmol), OEOMA$_{500}$ (2.50 g, 5 mmol), 100 mM stock solution HEBiB (1.0 mL, 0.10 mmol), stock solution of 25 mM CuBr$_2$ and 200 mM TPMA (200 μL, 5.0 μmol of CuBr$_2$ and 40 μmol of TPMA) were dissolved in H$_2$O (7.7 mL). DMF (0.1 mL) was added as internal standard for $^1$H NMR analysis. This mixture was added to a 25 mL Schlenk flask, purged with nitrogen for 30 min, and the flask was placed in an oil bath at 30 °C. A Na$_2$S$_2$O$_4$ aqueous solution (64 mM) was purged with nitrogen, and the solution was continuously
injected into the reaction medium using a syringe pump at the rate 1 μL/min. Samples were taken throughout the reaction for GPC and NMR analysis. The polymerization proceeded for 18 h (98% conversion, \( M_n^{th} = 24600, \ M_n^{GPC} = 26200, \ M_w/M_n = 1.22 \)). After that, under continuous flow of nitrogen the excess of polymerization mixture was removed from Schlenk flask until reach a volume of 0.5 mL (POEOMA: 125 mg, 5 μmol; CuBr₂: 2.8 μg, 0.0125 μmol; TPMA: 29 μg, 0.10 μmol). The OEOA \(_{480}\) (2.40 g, 5 mmol) and H₂O (7.7 mL) previously bubbled with nitrogen for about 15 minutes were added. An additional Na₂S₂O₄ solution (8 mM) was purged with nitrogen, and the solution was continuously injected into the reaction medium using a syringe pump at the rate 1 μL/min allowed to copolymerize for 20 h.

**Grafting from the Protein Initiator BSA-O-[iBr]₂₅**

BSA-O-[iBr]₂₅ (25.0 mg (protein), 0.01 mmol (initiator)), OEOA\(_{480}\) (1.20 g, 2.5 mmol), stock solution of 25 mM CuBr₂ and 200 mM TPMA (20 μL, 0.50 μmol CuBr₂ and 4.0 μmol TPMA) were dissolved in 0.1 M PBS (7.7 mL). DMF (0.1 mL) was added as internal standard for \(^1\)H NMR analysis. This mixture was added to a 25 mL Schlenk flask, purged with nitrogen for 30 min, and then placed in an oil bath at 30 °C. A Na₂S₂O₄ aqueous solution (16 mM) was purged with nitrogen, and the solution was continuously injected into the reaction medium using a syringe pump at the rate 1 μL/min. The grafted polymers were cleaved from the protein by adding 200 μL of the reaction mixture to 200 μL of 5% KOH solution. The resulting solution was allowed to react for 2 h at room temperature, followed by GPC analysis, as described elsewhere.³

**Results**
Fig. S1 (a) Kinetic plots of conversion and \(\ln[M_0]/[M]\) vs. time; (b) plot of number–average molecular weights \((M_n,\text{GPC})\) and \(D (M_w/M_n)\) vs. conversion for aqueous SARA ATRP of OEOA\(_{480}\) at 30 °C; and (c) GPC traces vs. time. Conditions: \([\text{OEOA}_{480}]_0/[	ext{HEBiB}]_0/\text{Na}_2\text{S}_2\text{O}_4/\text{Cu(II)Br}_2/[\text{TPMA}]_0 = 250/1/1/0.05/0.4; [\text{NaCl}]_0 = 100 \text{ mM; } [\text{OEOA}_{480}]_0/\text{Water} = 1/3.

Fig. S2 (a and d) Kinetic plots of conversion and \(\ln[M_0]/[M]\) vs. time; (b and e) plot of \(M_n,\text{GPC}\) and \(D (M_w/M_n)\) vs. conversion for aqueous SARA ATRP of OEOA\(_{480}\) at 30 °C; and (c and f) GPC traces vs. time. Conditions: \([\text{OEOA}_{480}]_0/[	ext{HEBiB}]_0/[\text{Cu(II)Br}_2]/[\text{TPMA}]_0 = 250/1/0.05/0.4; \text{FR(Na}_2\text{S}_2\text{O}_4) = 16 \text{ (a, b and c) and 32 (d, e and f) nmol/min ; } [\text{NaCl}]_0 = 100 \text{ mM; } [\text{OEOA}_{480}]_0/\text{Water} = 1/3.

A higher \(\text{FR}_S = 64 \text{ nmol/min}\) was employed at the beginning of polymerization up to 50% of monomer conversion. After that moment, the \(\text{FR}_S\) was reduced to 8 nmol/min (Fig. S3).
Fig. S3  (a) Kinetic plots of conversion and ln([M]₀/[M]) vs. time; (b) plot of number–average molecular weights (\(M_n,\text{GPC}\)) and \(D (M_w/M_n)\) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: \([\text{OEOA}_480]_0/[	ext{HEBiB}]_0/[\text{Cu(II)Br}_2]_0/[\text{TPMA}]_0 = 250/1/0.05/0.4\); FR(Na₂S₂O₄) = 64 nmol/min (until 4.5h) and FR(Na₂S₂O₄) = 8 nmol/min (until 32h); \([\text{NaCl}]_0 = 100 \text{ mM; } [\text{OEOA}_480]_0/\text{Water} = 1/3\).

Fig. S4  (a) Kinetic plots of conversion and ln([M]₀/[M]) vs. time; (b) plot of \(M_n,\text{GPC}\) and \(D (M_w/M_n)\) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: \([\text{OEOA}_480]_0/[	ext{HEBiB}]_0/[\text{Cu(II)Br}_2]_0/[\text{TPMA}]_0 = 250/1/0.05/0.1\); FR(Na₂S₂O₄) = 64 nmol/min; \([\text{NaCl}]_0 = 100 \text{ mM; } [\text{OEOA}_480]_0/\text{Water} = 1/3\).

Fig. S5  (a) Kinetic plots of conversion and ln([M]₀/[M]) vs. time; (b) plot of \(M_n,\text{GPC}\) and \(D (M_w/M_n)\) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: \([\text{OEOA}_480]_0/[	ext{HEBiB}]_0/[\text{Cu(II)Br}_2]_0/[\text{Me}_6\text{TREN}]_0 = 250/1/0.05/0.4\); FR(Na₂S₂O₄) = 64 nmol/min; \([\text{NaCl}]_0 = 100 \text{ mM; } [\text{OEOA}_480]_0/\text{Water} = 1/3\).
Fig. S6 (a, d, g and j) Kinetic plots of conversion and ln[M]/[M] vs. time; (b, e, h and k) plot of \( M_n, GPC \) and \( D (M_w/M_n) \) vs. conversion for aqueous SARA ATRP of OEOA \(_{480}\) at 30 °C; and (c, f, i and l) GPC traces vs. time. Conditions: \([\text{OEOA}_{480}]_0/[\text{HEBiB}]_0/[\text{Cu(II)Br}_2]_0/[\text{TPMA}]_0 = 250/1/0.05/0.4; \) FR\((\text{Na}_2\text{S}_2\text{O}_4) = 64 \text{ nmol/min}; [\text{OEOA}_{480}]_0/[\text{Water}] = 1/3; (a, b and c) \[\text{TEACl}]_0 = 100 \text{ mM, (d, e and f) [NaBr]}_0 = 100 \text{ mM, (g, h and i) [NaCl]}_0 = 10 \text{ mM, and (j, k and l) [Salt]}_0 = 0 \text{ mM.}
Fig. S7 (a) Kinetic plots of conversion and ln[M]₀/[M] vs. time; (b) plot of $M_n\text{GPC}$ and $D (M_w/M_n)$ vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 ºC; and (c) GPC traces vs. time. Conditions: [OEOA₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.1; FR(Na₂S₂O₄) = 64 nmol/min; [NaCl]₀ = 100 mM; [OEOA₄₈₀]₀/[Water] = 1/3.

Table S1  Aqueous SARA ATRP of OEOA₄₈₀ with Start/Stop Cycles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>[M]₀/[I]₀/[Cu(II)Br₂]₀/[TPMA]₀</th>
<th>Sulfite</th>
<th>Cu⁺ (ppm)</th>
<th>Time (h)</th>
<th>Conv. (%)</th>
<th>$M_n\text{GPC} \times 10^3$</th>
<th>$M_w\text{GPC} \times 10^3$</th>
<th>$D$</th>
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<td>Na₂S₂O₄</td>
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<td>24</td>
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<td></td>
<td></td>
<td>3 (1 h OFF)</td>
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<td></td>
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<td>90.9</td>
<td>90.9</td>
<td>1.29</td>
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</tbody>
</table>

*a All polymerizations were conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM, [NaCl]₀ = 100 mM and FR s = 64 nmol/min;

*b Calculated by the initial weight ratio of Cu to the monomer.

References

