SUPPORTING INFORMATION

ATRP under Biologically Relevant Conditions: Grafting from a Protein

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Materials. Oligo(ethylene oxide) monomethyl ether methacrylate (average molecular weight ~475, ~300, 188 g/mol, $OEOMA_{475}$, $OEOMA_{300}$, MEO_2MA respectively), BSA, mono-*tert*-butyl succinate, N-hydroxysuccinimide (NHS), trifluoroacetic acid, bromoisobutyryl bromide, 2,2'-bipyridine (bpy), , N-(n-propyl)pyridylmethanimine (PI), ascorbic acid (AA), CuCl, $CuCl_2$, CuBr, and $CuBr_2$ were purchased from Aldrich in the highest available purity. Tris(2-pyridylmethyl)amine (TPMA) was purchased from ATRP Solutions. Monomers were passed over a column of basic alumina prior to use. Poly(ethylene oxide) isobutyryl bromide (PEO-iBBr M_n = 2000) was prepared, as previously described.[1] GFP was prepared as previously outlined.[2]

Instrumentation. Molecular weight and molecular weight distribution (M_w/M_n) were determined by GPC. The GPC system used a Waters 515 HPLC Pump and Waters 2414 Refractive Index Detector using PSS columns (Styrogel 10^2, 10^3, 10^5 Å) in dimethylformamide (DMF) as an eluent at a flow rate of 1 ml/min at 50 °C and in tetrahydrofuran (THF) as an eluent at a flow rate of 1 mL/min at 35 ^oC. All samples were filtered over anhydrous magnesium sulfate and neutral alumina prior to analysis. The column system was calibrated with 12 linear polystyrene (M_n = 376 ~ 2,570,000). Monomer conversion was measured using ^1H NMR spectroscopy in D_2O, using a Bruker Avance 300 MHz spectrometer at 27 °C. Thermoresponsivity was measured by dynamic light scattering (DLS) on a Zetasizer from Malvern Instruments, Ltd. The temperature ramp used in this study was from 15 to 64 °C at 1 °C intervals. Samples were equilibrated for 2 minutes before measuring particle size. Tangential flow filtration was conducted on a Labscale TFF system from Millipore. Zebra Spin desalting columns were purchased

from Fisher and used according to the manufactures instructions. The fluorecence spectra for GFP stability testing were obtained on a Tecan Safire2 using a 384 well plate.

Stability of GFP under polymerization conditions. The following amounts of ligand were complexed with $CuCl_2$ (5.0 mg, 3.7×10^{-2} mmol) respectively: PI(12.2 mg, 8.2×10^{-2} mmol), bpy(12.8 mg, 8.2×10^{-2} mmol) and TPMA (11.9 mg, 4.1×10^{-2} mmol). The $CuCl_2$/L was precomplexed in 200 μl of water and an additional solution of $CuCl_2$ was prepared in 200 μl of water. Once clear solutions of $CuCl_2$/L and $CuCl_2$ were obtained they were added to 2 ml of GFP solution (1 mg/ml) in PBS with 10% $OEOMA_{475}$. The solutions were centrifuged at 4000 rpm to remove any particulates formed (only observed for free $CuCl_2$ and $CuCl_2$/PI). 100 μl of each solution was removed for analysis using a Tecan Safire2 plate reader with a 384 well plate.

Preparation of NHS ester initiator. Mono-*tert*-butyl succinate-EBiB **(1)**: Mono-*tert*-butyl succinate (1.0 g, 5.7×10^{-3} mol), hydroxyl-EBiB (1.3 g, 6.3×10^{-3} mol), EDC-HCl (1.4 g, 7.5×10^{-3} mol) and DMAP (0.1 g, 5.7×10^{-4} mol) were added to a 100 ml round bottom flask. The reaction mixture was dissolved in 50 ml of dichloromethane and stirred overnight. The reaction mixture was extracted once with 20 ml of water, twice with 1 N HCl, once with 1 N NaOH, once with water and brine. The organic layer was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure. ^{1}H NMR: 1.44 ppm (s, 9H), 1.93 ppm (d, 6H), 2.56 ppm (m, 4H), 4.36 ppm (s, 4H).

COOH- *tert*-butyl succinate-EBiB: Mono-*tert*-butyl succinate-EBiB (1.0 g, 2.7×10^{-3} mol) was dissolved in 50 ml of dichloromethane and trifloroacidic acid (TFA, 2.1 ml, 2.7×10^{-2} mol) was added dropwise to the reaction mixture. The reaction was stirred for 36 hours, and subsequently extracted 3 times with 30 ml of water and once with brine. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. ^{1}H NMR: 1.94 ppm (d, 6H), 2.68 ppm (m, 4H), 4.38 ppm (s, 4H).

NHS-ester initiator **(2)**: COOH-mono-*tert*-butyl succinate-EBiB (0.8 g, 2.69×10^{-3} mol), EDC-HCl (0.8 g, 4.0×10^{-3} mol) and NHS (0.5 g, 4.0×10^{-3} mol) were dissolved in 10 ml of $CHCl_3$ and stirred for 16 hours. 40 ml of ethyl acetate and 30 ml of water were then added to the reaction mixture and stirred for 10 minutes. The organic phase was separated and the aqueous phase was washed 3 times with 20 ml of ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The NHS activated initiator was directly used to modify BSA.

Synthesis of BSA-O-[iBBr]$_{30}$. NHS-ester initiator (1.0 g, 2.5 mmol) was dissolved in 2 ml of DMSO. BSA (1.0 g, 0.5 mmol Lys) was dissolved in 500 ml of 0.1 M PBS (pH 7.4) and **2** was added dropwise. The reaction was stirred overnight and purified using tangential flow filtration with a 30-kDa molecular weight cut off membrane. 15 dia-volumes of water were used to purify BSA-O-[iBBr]$_{30}$.

Polymer cleavage from protein. Polymers were cleaved from proteins by adding 200 μl of reaction mixture to 200 μl of 5% KOH solution and left at room temperature for 2 hours.

Synthesis of POEOMA by ATRP from PEO$_{2000}$iBBr/BSA-O-[iBBr]$_{30}$. PEO$_{2000}$iBBr (10.0 mg 5×10^{-3} mmol) or BSA-O-[iBBr]$_{30}$ (12.5 mg (protein), 5.0×10^{-3} mmol (initiator)) was dissolved in 3.5 ml of Millipore water and placed in a 10 ml Schlenk flask. OEOMA$_{475}$ (476.2 mg, 1.1 mmol) and 50 μl of DMF (internal standard for NMR) were added dropwise to the initiator solution. The flask was sealed and bubbled for 20 min, while stirring, with nitrogen to deoxygenate the reaction mixture. After the solution was deoxygenated, 1 ml of catalyst stock solution was added *via* gastight syringe to the reaction mixture to initiate polymerization. The polymerization was carried out at 30 °C. Samples were taken at allotted times throughout the reaction for GPC and NMR analysis. Stock solutions of CuX/L were prepared in 10 ml of deoxygenated ultra pure water as follows: ***X=Br, L=bpy***: CuBr (7.2 mg, 0.5×10^{-1} mmol), $CuBr_2$ (101.0 mg, 4.5×10^{-1} mmol), and bpy (164.3 mg, 1.1 mmol). ***X=Cl, L=bpy***: CuCl (5.0 mg, 0.5×10^{-1} mmol), $CuCl_2$(60.5 mg, 0.3 mmol) and bpy(164.3 mg, 1.1 mmol). ***X=Br, L=TPMA***: CuBr (7.2 mg, 0.5×10^{-1} mmol), $CuBr_2$ (101.0 mg, 0.5 mmol), and TPMA (160.0 mg, 5.5 mmol). ***X=Cl, L=TPMA***: CuCl (5.0 mg, 0.5×10^{-1} mmol), $CuCl_2$(60.5 mg, 0.3 mmol) and TPMA (160.0 mg, 5.5 mmol).

AGET ATRP from PEO-iBBr/BSA-O-[iBBr]$_{30}$. PEO$_{2000}$iBBr (40.0 mg 0.2×10^{-1} mmol) or BSA-O-[iBBr]$_{30}$ (50.0 mg (protein), 0.2×10^{-1} mmol (initiator)), OEOMA$_{475}$ (2.0 g, 4.5 mmol), $CuBr_2$ (44.6 mg, 0.2 mmol), and TPMA (63.8 mg, 0.2 mmol) were dissolved in 18.4 ml of pure water and charged into a 25 ml Schlenk flask. 0.4 ml of DMF was added as internal standard. Next, the reaction mixture was purged with N_2 for 20 minutes then placed in an oil bath at 30 °C. Then AA was added either at the beginning of the reaction, or slowly fed in *via* a syringe pump.

Synthesis of POEOMA by ATRP in DMSO/water from BSA-O-[iBBr]$_{30}$. BSA-O-[iBBr]$_{30}$ (12.5 mg (protein), 5.0×10^{-3} mmol(initiator)) was dissolved in 3 ml of Millipore water and added to a 10 ml Schlenk flask. OEOMA$_{475}$ (476.2 mg, 1.1 mmol) and 50 μl of DMF (internal standard for NMR) were dissolved in 500 μl of DMSO and added dropwise to the stirring protein solution (to avoid protein precipitation). The flask was sealed and bubbled for 20 min, while stirring, with nitrogen to deoxygenate the reaction mixture. After the solution was deoxygenated, 1 ml of catalyst stock solution was added to

the reaction mixture to initiate polymerization. The polymerization was carried out at 30 °C. Samples were taken several times throughout the reaction for GPC and NMR analysis.

Synthesis of P(MEO_2MA-*co*-$OEOMA_{475}$) from BSA-O-[iBBr]$_{30}$. BSA-O-[iBBr]$_{30}$ (12.5 mg (protein), 5.0×10^{-3} mmol (initiator)) was dissolved in 3 ml of Millipore water and added to a 10 ml Schlenk flask. $OEOMA_{475}$ (144.7 mg, 0.5 mmol), MEO_2MA (92.5 ml, 0.5 mmol) and 50 μl of DMF were dissolved in 800 μl of DMSO and added dropwise to the well stirred protein solution (to avoid protein precipitation). The flask was sealed and nitrogen was bubbled for 20 min, with stirring to deoxygenate the reaction mixture. After the solution was deoxygenated, 1 ml of catalyst stock solution (X = Cl, L = bpy) was added to the reaction mixture to initiate polymerization. Samples were taken at several times throughout the reaction for GPC and NMR analysis. Prior to DLS analysis samples were passed through a Zebra Spin desalting column to remove solvent, monomer and catalyst species.

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Results

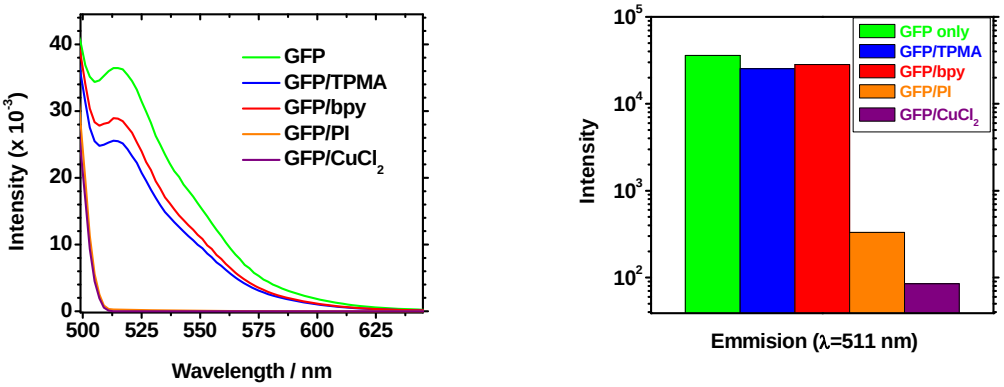
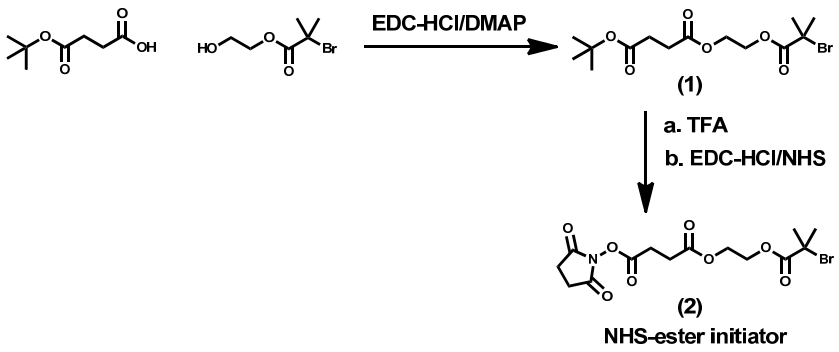


Figure S1. Effect of $CuCl_2$:L on GFP (1 mg/ml) stability. [$CuCl_2$]/[L] = 1/[bpy],[PI] and [TPMA] = 2.2 and 1.1, respectively. [$CuCl_2$]=19 mM, [$OEOMA_{475}$]$_0$ = 0.23 M



Scheme S1. Preparation of the NHS-ester initiator.

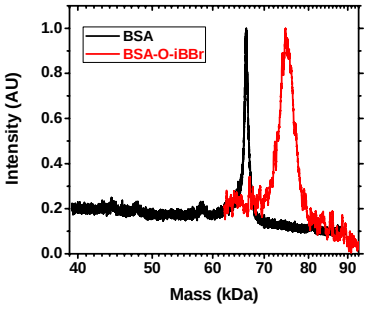
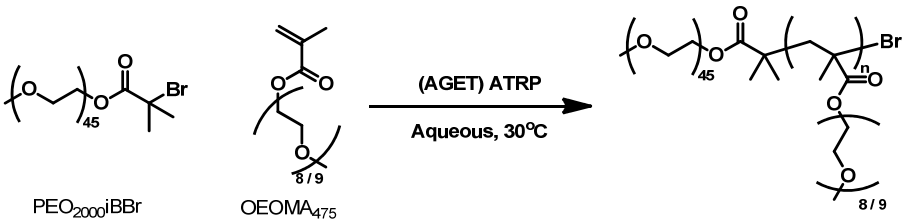
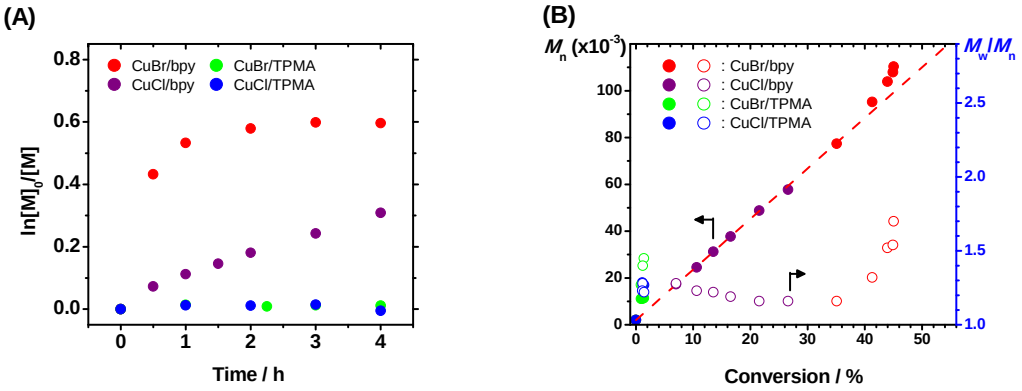


Figure S2. MALDI-TOF spectra of BSA and BSA-O-[iBBr]$_{30}$.



Scheme S2. (AGET) ATRP of OEOMA$_{475}$ from PEO$_{2000}$iBBr under biologically relevant conditions.



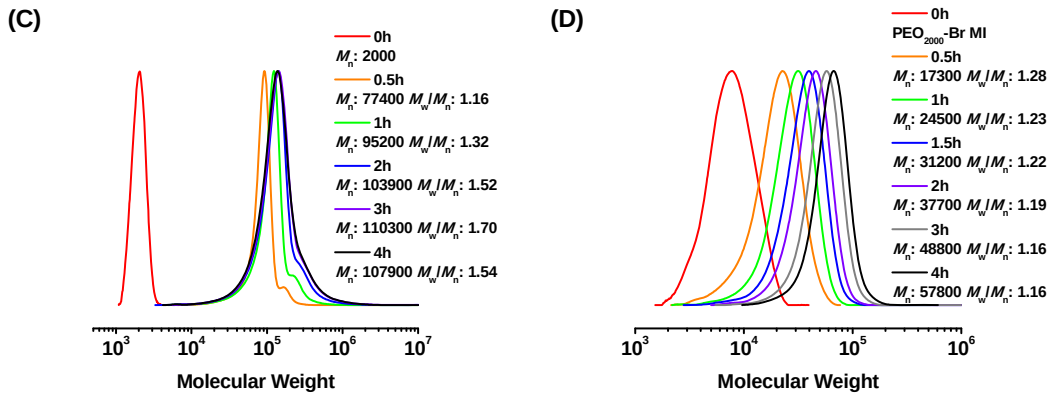


Figure S3. Effect of copper halide (X = Br or Cl) on ATRP of $OEOMA_{475}$ under aqueous conditions at 30 °C (Table 1, Entries 1-4). (A) First order kinetic plot and (B) M_n and M_w/M_n versus conversion plot; (C) GPC traces for CuBr/bpy (Table 1, Entry 1), (D) GPC traces for CuCl/bpy (Table 1, Entry 2). $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.45 M and $[OEOMA_{475}]/[I]/[L]/[CuX]/[CuX_2]$ = 455/1/11/1/9 ([L]: [TPMA] = 2[bpy]).

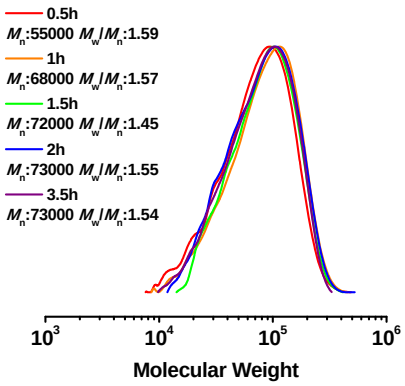


Figure S4. GPC traces for CuBr/bpy (Table 1, Entry 5). $[BSA\text{-}O\text{-}[iBBr]_{30}]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.23 M and $[OEOMA_{475}]/[I]/[bpy]/[CuBr]/[CuBr_2]$ = 227/1/22/1/9.

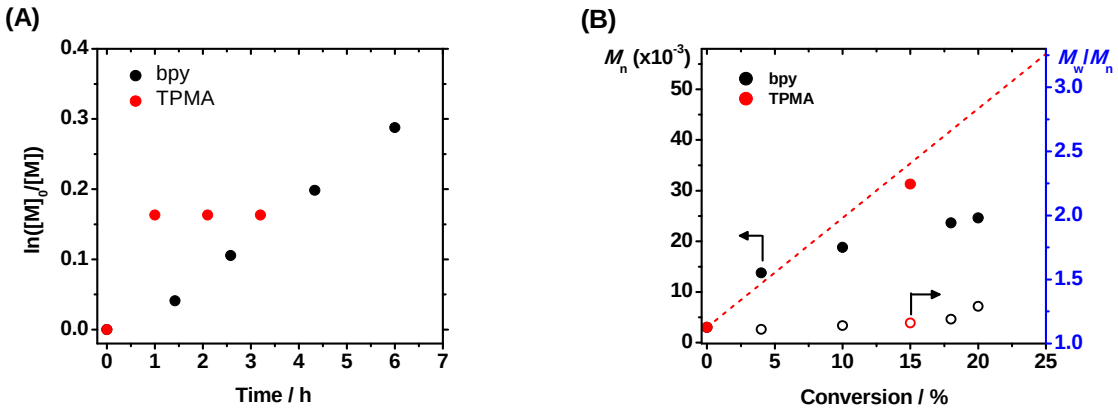


Figure S5. Effect of ligand (L = bpy or TPMA) on AGET ATRP of $OEOMA_{475}$ under aqueous conditions at 30 °C (Table 2, Entries 1-2). (A) First order kinetic plot and (B) M_n and M_w/M_n versus conversion plot. $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.45 M and $[OEOMA_{475}]/[I]/[L]/[CuBr_2]/[AA]$ = 455/1/11/10/0.1 ([L]: [TPMA] = 2[bpy]).

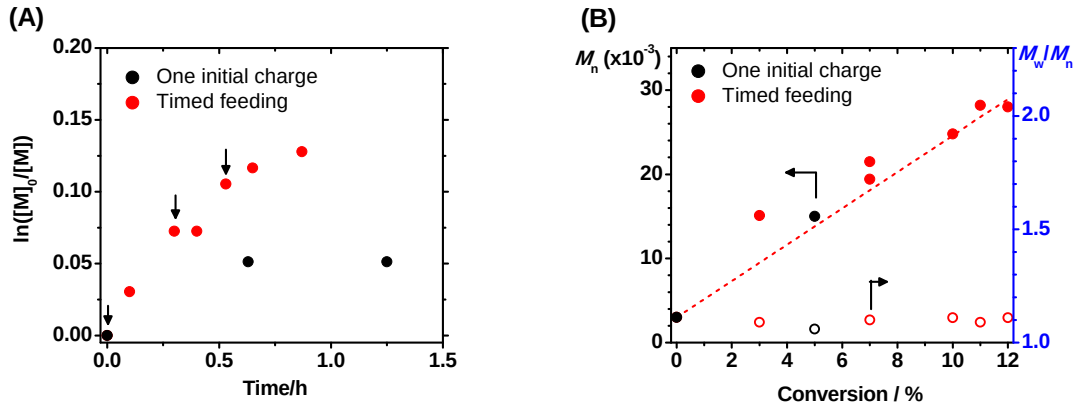


Figure S6. Effect of reducing agent addition time on ATRP of $OEOMA_{475}$ under aqueous conditions at 30 °C (Table 2, Entries 3-4,). (A) First order kinetic plot and (B) M_n and M_w/M_n versus conversion plot. $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.45 M and $[OEOMA_{475}]/[I]/[TPMA]/[CuBr_2]/[AA]$ = 455/1/11/10/(0.01 or 0.03). AA was added step-wise (times of injection indicated by black arrows).

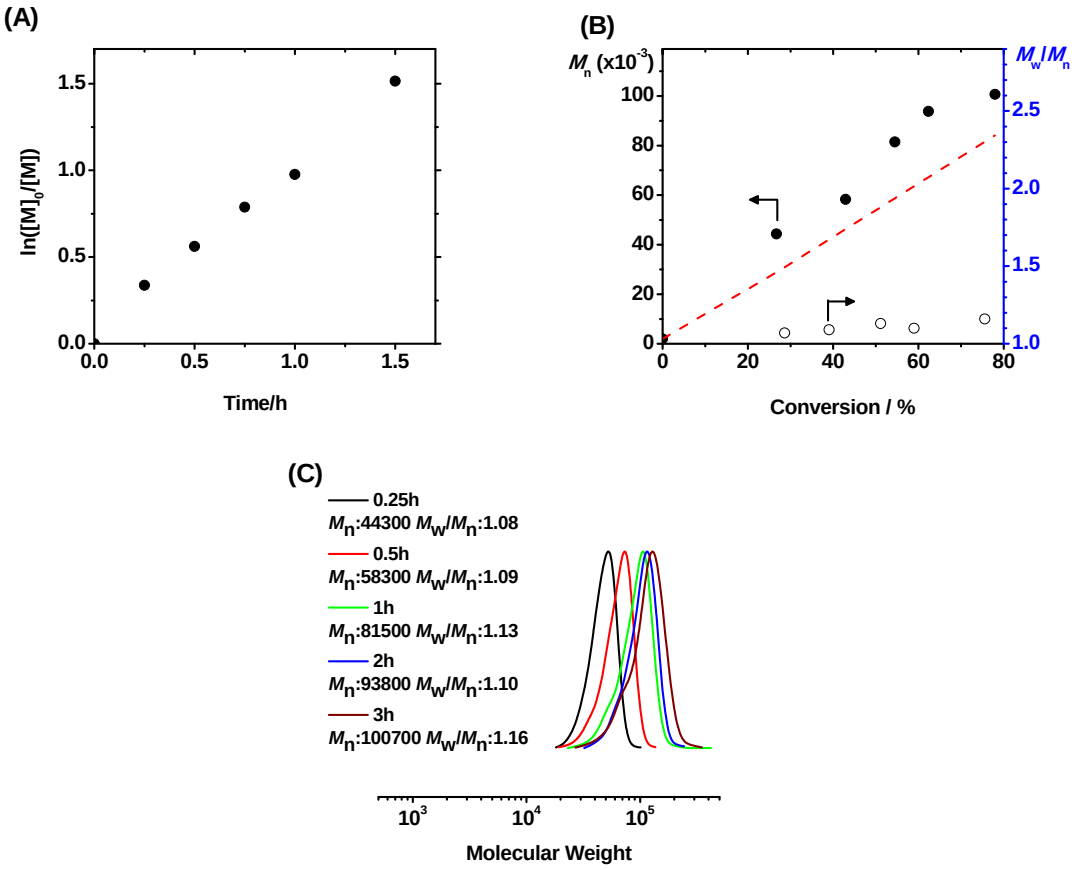


Figure S7. AGET ATRP of $OEOMA_{475}$ in water, 30 °C (Table 2, Entry 5). (A) First order kinetic plot, (B) M_n and M_w/M_n versus conversion plot, and (C) GPC traces. $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.23 M and $[OEOMA_{475}]/[I]/[TPMA]/[CuBr_2]/[AA]$ = 227/1/22/10/0.1. Rate of ascorbic acid addition was 8 nmol/min.

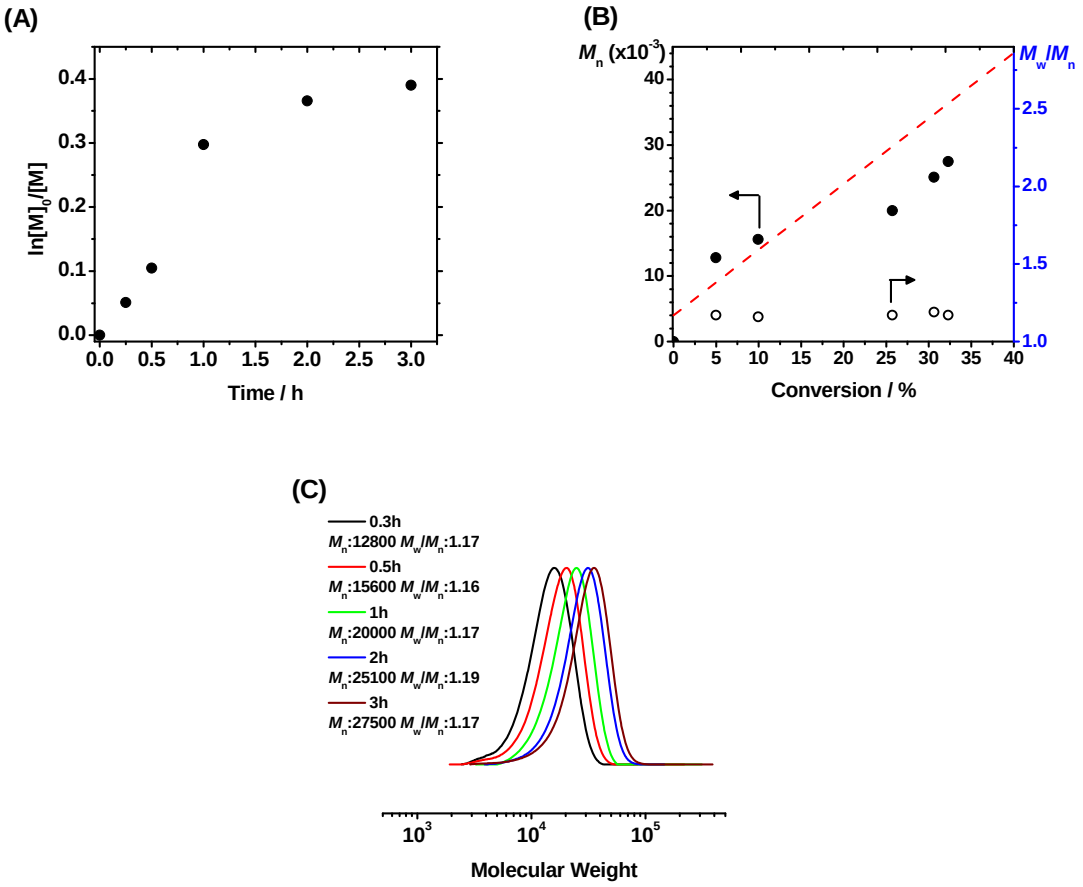


Figure S8. ATRP of $OEOMA_{475}$ in PBS, 30 °C (Table 1, Entry 12). (A) First order kinetic plot and (B) M_n and M_w/M_n versus conversion plot, and (C) GPC traces. $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.23 M and $[OEOMA_{475}]/[I]/[bpy]/[CuBr]/[CuBr_2]$ = 227/1/22/1/9.

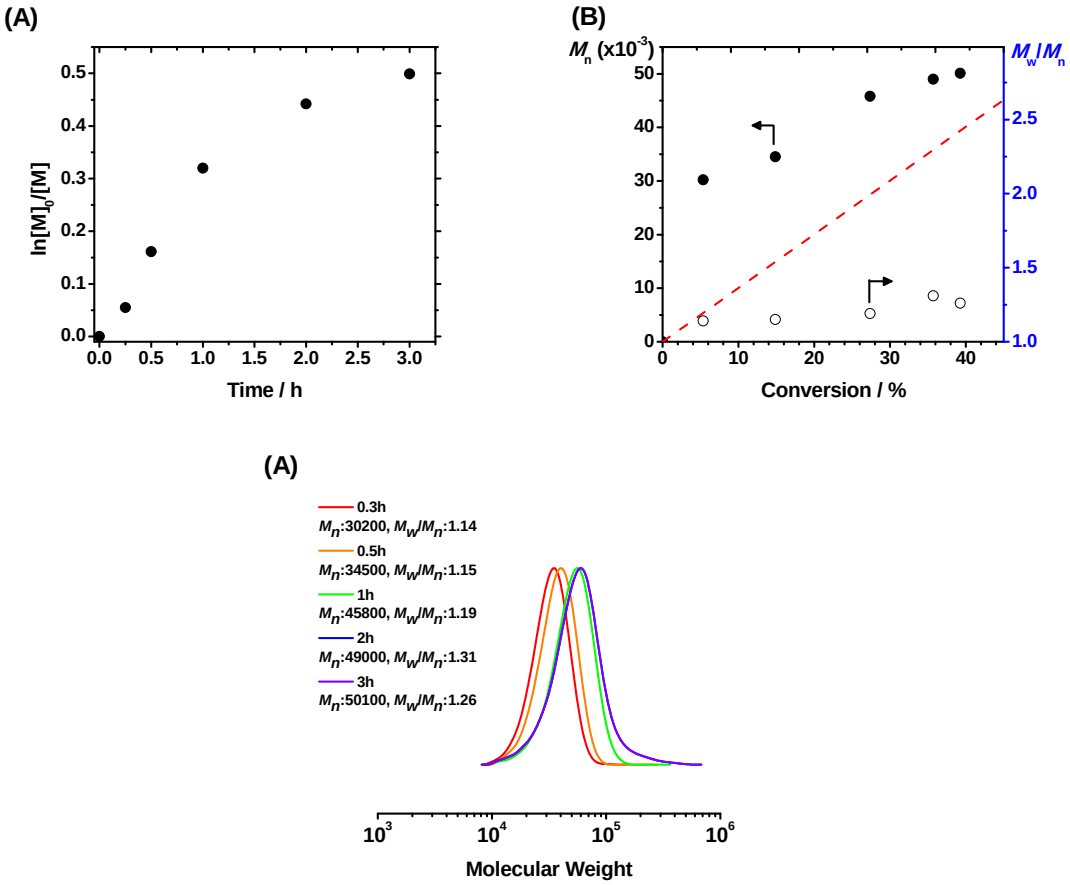


Figure S9. ATRP of $OEOMA_{475}$ g-f BSA-O-$[iBBr]_{30}$ at 30 °C in PBS (Table 1, entry 13). (A) First order kinetic plot and (B) M_n and M_w/M_n versus conversion plot. $[BSA\text{-}O\text{-}[iBBr]_{30}]_0$ = 1mM, $[OEOMA_{475}]_0$ = 0.21 M and $[OEOMA_{475}]/[I]/[CuBr]/[CuBr_2]/[L]$ = 227/1/1/9/ 22.

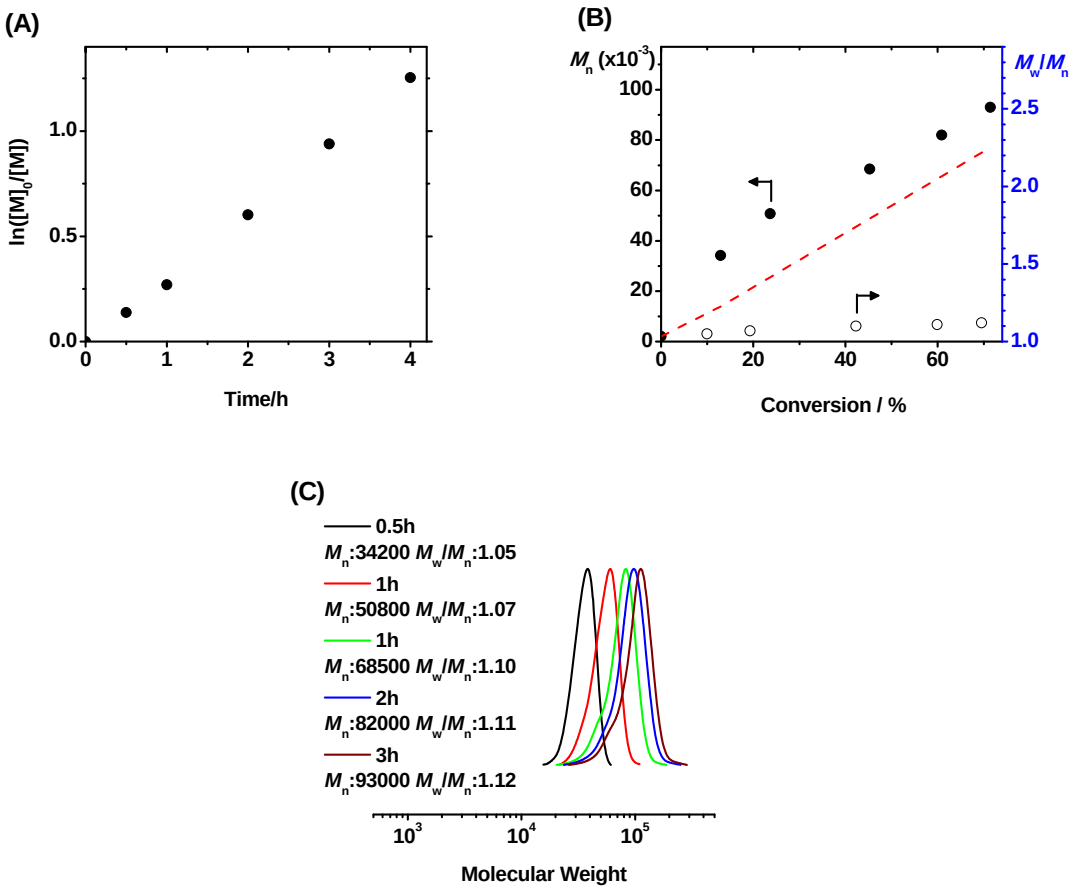


Figure S10. AGET ATRP of $OEOMA_{475}$ in PBS, 30 °C (Table 2, Entry 8). (A) First order kinetic plot, (B) M_n and M_w/M_n versus conversion plot, and (C) GPC traces. $[PEO_{2000}iBBr]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.23 M and $[OEOMA_{475}]/[I]/[TPMA]/[CuBr_2]/[AA]$ = 227/1/22/10/0.2. Rate of ascorbic acid addition was 8 nmol/min.

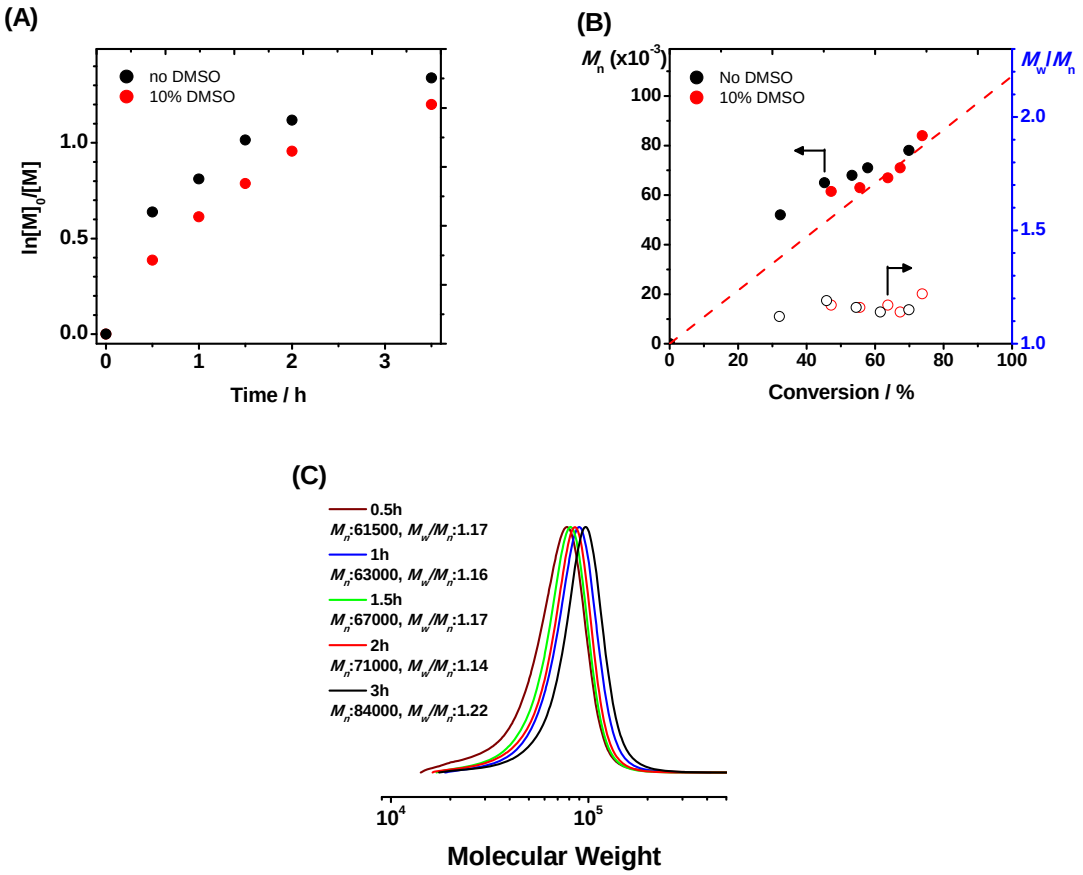


Figure S11. Effect of 10% DMSO (v/v) on ATRP of $OEOMA_{475}$ g-f BSA-O-$[iBBr]_{30}$ at 30 °C (Table 1, Entries 6 and 9). (A) First order kinetic plot, (B) M_n and M_w/M_n versus conversion plot, and (C) GPC traces (Table 1, Entry 9). $[BSA\text{-}O\text{-}[iBBr]_{30}]_0$ = 1 mM, $[OEOMA_{475}]_0$ = 0.21 M and $[OEOMA_{475}]/[I]/[CuCl]/[CuCl_2]/[bpy]$ = 227/1/1/9/22.

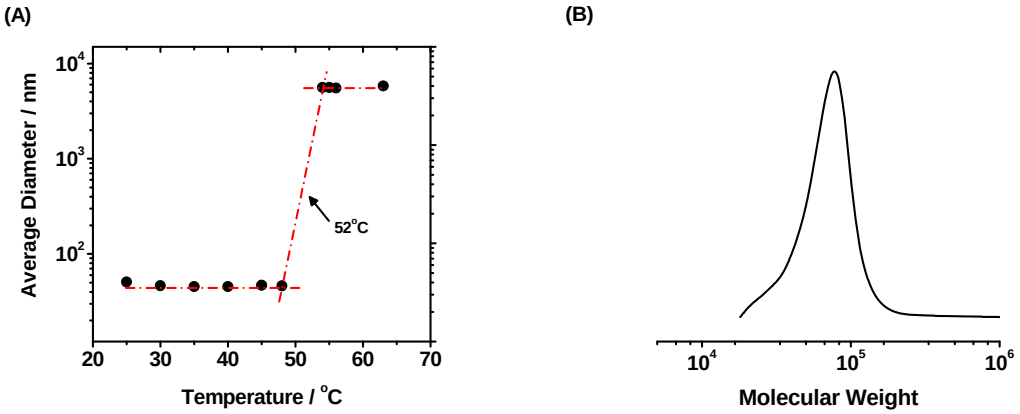


Figure S12. Thermo-responsive copolymer g-f BSA-O-$[iBBr]_{30}$ at 30 °C. (A) Increase of diameter of PPH with temperature (B) GPC of copolymer cleaved from PPH. $[OEOMA_{300}]_0$ = 0.1 M, $[MEO_2MA]_0$ = 0.1 M and $[MEO_2MA]/[OEOMA_{300}]/[I]/[CuCl]/[CuCl_2]/[bpy]$ = 100/100/1/1/10/21.

References

1. Bencherif, S. A.; Siegwart, D. J.; Srinivasan, A.; Horkay, F.; Hollinger, J. O.; Washburn, N. R.; Matyjaszewski, K. *Biomaterials* **2009,** 30, 5270-5278.

2. Peeler, J. C.; Woodman, B. F.; Averick, S.; Miyake-Stoner, S. J.; Stokes, A. L.; Hess, K. R.; Matyjaszewski, K.; Mehl, R. A. *J. Am. Chem. Soc.* **2010,** 132, 13575-13577.