

Supporting Information for

Enzyme-Deoxygenated Low Parts per Million Atom Transfer Radical

Polymerization in Miniemulsion and *Ab Initio* Emulsion

Yi Wang,^a Liye Fu,^a and Krzysztof Matyjaszewski*

Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

^aThese authors contributed equally.

Experimental Section

Materials. Copper(II) bromide (CuBr₂, 99%), sodium dodecyl sulfate (SDS, 99%), hexadecane (HD, 99%), tetrahydrofuran (THF, > 99%), ethyl α -bromoisobutyrate (EBiB, 98%), ethyl α -bromophenylacetate (EBPA, 97%), sodium pyruvate (99%), glucose (96%), glucose oxidase from *Aspergillus niger* (GOx, 100,000-250,000 units/g solid) and ascorbic acid (AsAc) were purchased from Sigma-Aldrich. Sodium bromide (NaBr, 99%) was purchased from Fisher Scientific. Tetraethylammonium hexafluorophosphate (Et₄NPF₆, 98%) was purchased from Alfa Aesar. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, 98%) and 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70, 98%) were purchased from Wako Chemical Corporation. Tris(2-pyridylmethyl)amine (TPMA) and poly(ethylene glycol) bromophenylacetate (PEO_{2k}-BPA) were prepared according to published procedures.¹ *n*-Butyl methacrylate (BMA, 99%, Aldrich), *n*-butyl acrylate (BA, 99%, Aldrich) and *tert*-butyl acrylate (*t*BA, 99%, Aldrich) were passed through a column filled with basic alumina prior to use to remove polymerization inhibitors.

Apparatus configuration and characterization.

Ultrasound treatment was carried out using Sonics & Materials VCX500 ultrasonicator.

M_n and M_w/M_n were determined by GPC equipped with Polymer Standards Services (PSS) columns (SDV, 10⁵, 10³, and 10² Å) and a differential refractive index detector (Waters, 2410), with THF as eluent at a flow rate 1.00 mL/min ($T = 35$ °C). GPC traces were processed by WinGPC 8.0 software (PSS) using a calibration based on linear polystyrene (PS) standards.

Photo-mediated polymerization was conducted in a 4.9 mW/cm² MelodySusie[®] UV lamp.

Cyclic voltammogram (CV) and electrolysis were conducted using Gamry REF 600 potentiostat.

Particle size and size distribution were determined by using a Zetasizer Nano from Malvern Instruments, Ltd.

Miniemulsion polymerization of BMA by ARGET ATRP. A stock solution of BMA (2 mL, 12.57 mmol), EBPA (7.86 μ L, 0.045 mmol, to target degree of polymerization (DP) = 280), and HD (0.25 mL, 0.853 mmol) was prepared. Cu^{II}Br₂/TPMA stock solution (0.20 mL, 0.05 M in water), NaBr (0.129 g, 1.000 mmol), SDS (0.082 g, 0.285 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The organic and aqueous solutions were mixed (total volume \approx 10 mL), placed in an ice bath, and dispersed by a probe sonicator, amplitude = 20% for 20 min (application and rest time of 1 s each). The miniemulsion and GOx (0.0032 g, 2×10^{-5} mmol) was added to a vial. The vial was immersed to 44 °C oil bath after 3 min stirring and AsAc solution was injected to start the reaction. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

Synthesis of PBA-*b*-PtBA. A PBA-Br macroinitiator (MI) ARGET ATRP polymerization was conducted following the same procedure for BMA polymerization as mentioned above using the GOx-degassing method. After 20 h, the polymerization solution was transferred to a 50 mL centrifuge tube. 20 mL of water and 20 mL of MeOH were added and then the tubes were placed in the centrifuge for 20 min at 5000 rpm. About 90% of the polymer was recovered after precipitation. The PBA-MI was re-dissolved in THF, passed through neutral alumina column and dried in vacuum.

A solution of *t*BA (1.67 mL, 11.40 mmol), PBA-MI (0.57 g, 0.023 mmol) to target DP = 500 was prepared. Cu^{II}Br₂/TPMA stock solution (0.20 mL, 0.01 mmol), NaBr (0.103 g, 1 mmol), SDS (0.082 g, 0.28 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The organic and aqueous solutions were mixed (total volume \approx 10 mL), placed in an ice-water bath, and dispersed by a probe sonicator, amplitude = 20% for 20 min (application and rest time of 1 s each). The miniemulsion and GOx (0.0032 g, 2×10^{-5} mmol) was

added to a vial. The vial was immersed to 44 °C oil bath after 3 min stirring to start the reaction. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

Miniemulsion polymerization of BMA by photoATRP. A stock solution of BMA (2 mL, 12.57 mmol), EBPA (11.0 μ L, 0.063 mmol, to target DP = 200), and HD (0.25 mL, 0.853 mmol) was prepared. $\text{Cu}^{\text{II}}\text{Br}_2/3\text{TPMA}$ stock solution (0.20 mL, 0.05 M in water), NaBr (0.129 g, 1.000 mmol), SDS (0.082 g, 0.285 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The organic and aqueous solutions were mixed (total volume \approx 10 mL), placed in an ice bath, and dispersed by a probe sonicator, amplitude = 20% for 20 min (application and rest time of 1 s each). The miniemulsion and GOx (0.0032 g, 2×10^{-5} mmol) was added to a vial. The UV lamp was placed on a stir plate with a stir rate = 500 rpm. The vial was inserted into the cavity of the UV lamp. The lamp was turned on to start the reaction. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

Miniemulsion polymerization of BMA by eATRP. A stock solution of BMA (2 mL, 12.57 mmol), EBPA (11.0 μ L, 0.063 mmol, to target DP = 200) and HD (0.25 mL, 0.853 mmol) was prepared. $\text{Cu}^{\text{II}}\text{Br}_2/\text{TPMA}$ stock solution (0.20 mL, 0.05 M in water), NaBr (0.129 g, 1.000 mmol), SDS (0.082 g, 0.285 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The organic and aqueous solutions were mixed (total volume \approx 10 mL), placed in an ice bath, and dispersed by a probe sonicator, amplitude = 20% for 20 min (application and rest time of 1 s each). The miniemulsion and GOx (0.0032 g, 2×10^{-5} mmol) was added to a five-neck electrochemical cell. The vial was immersed to 44 °C oil bath after 3 min stirring. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

Miniemulsion polymerization of BMA by ICAR ATRP (taking hydrophobic initiator, V-70 as example). A stock solution of BMA (2 mL, 12.57 mmol), EBPA (11.0 μ L, 0.063 mmol, to target DP = 200), V-70 (0.058 g, 0.019 mmol, [V-70]/[EBPA] = 0.3/1) and HD (0.25 mL, 0.853 mmol) was prepared. $\text{Cu}^{\text{II}}\text{Br}_2/\text{TPMA}$ stock solution (0.20 mL, 0.05 M in water), NaBr (0.129 g, 1.000 mmol), SDS (0.082 g, 0.285 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The organic and aqueous solutions were mixed

(total volume \approx 10 mL), placed in an ice bath, and dispersed by a probe sonicator, amplitude = 20% for 20 min (application and rest time of 1 s each). The miniemulsion and GOx (0.0032 g, 2×10^{-5} mmol) was added to a vial. The vial was immersed to 44 °C oil bath after 3 min stirring to start the reaction. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

Ab initio emulsion polymerization of BMA by ARGET ATRP. $\text{Cu}^{\text{II}}\text{Br}_2/\text{TPMA}$ stock solution (0.20 mL, 0.05 M in water), PEG_{2k}-BPA (0.053 g, 0.025 mmol, to target DP = 500), NaBr (0.129 g, 1.000 mmol), SDS (0.082 g, 0.285 mmol), glucose (0.36 g, 2 mmol) and Na pyruvate (0.11 g, 1 mmol) were dissolved in 8 mL of distilled water. The aqueous solution and GOx (0.0032 g, 2×10^{-5} mmol) was added to a Schlenk flask. BMA (2 mL, 12.57 mmol) was slowly injected to the flask. The vial was immersed to 44 °C oil bath after 3 min stirring and AsAc solution was injected to start the reaction. Samples were withdrawn periodically to follow the monomer conversion by gravimetric analysis, while M_n and M_w/M_n were determined by GPC.

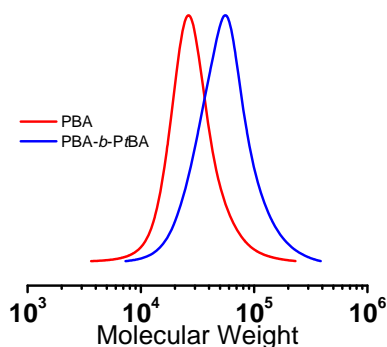


Figure S1. Chain extension of PBA-MI with *t*BA in miniemulsion. Reaction condition: *t*BA 1.67mL, [*t*BA]/[PBA MI]/[CuBr₂]/[TPMA] = 500/1/0.02/0.02, [SDS] = 4.6 wt% relative to *t*BA, [NaBr] = 0.1 M, [AsAc] = 2.5 mM. [glucose] = 0.2 M, [GOx] = 2 μ M, [sodium pyruvate] = 0.1 M.

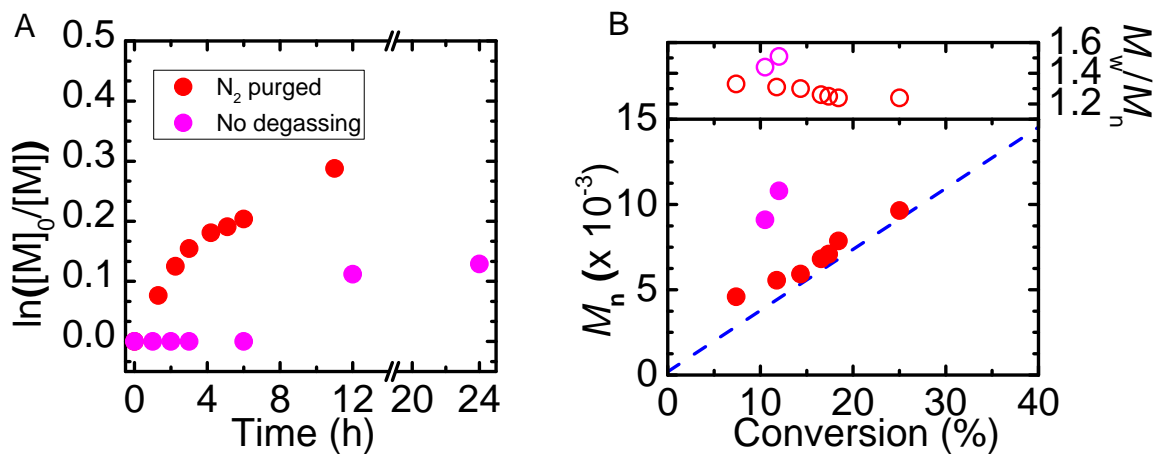


Figure S2. GOx degassed miniemulsion ARGET ATRP of BMA using (●) 30 min mild nitrogen bubbling; (●) no deoxygenation. (A) Kinetic plots; (B) Evolution of M_n and D vs conversion. Reaction conditions: BMA 2 mL (20 vol %), $[BMA]/[EBPA]/[CuBr_2/TPMA] = 280/1/0.2$, $[HD] = 10$ wt % relative to BMA, $[SDS] = 4.6$ wt % relative to BMA, $[NaBr] = 0.1$ M, $T = 44$ °C. AsAc was fed at a rate of 3.7 $\mu\text{mol/h}$.

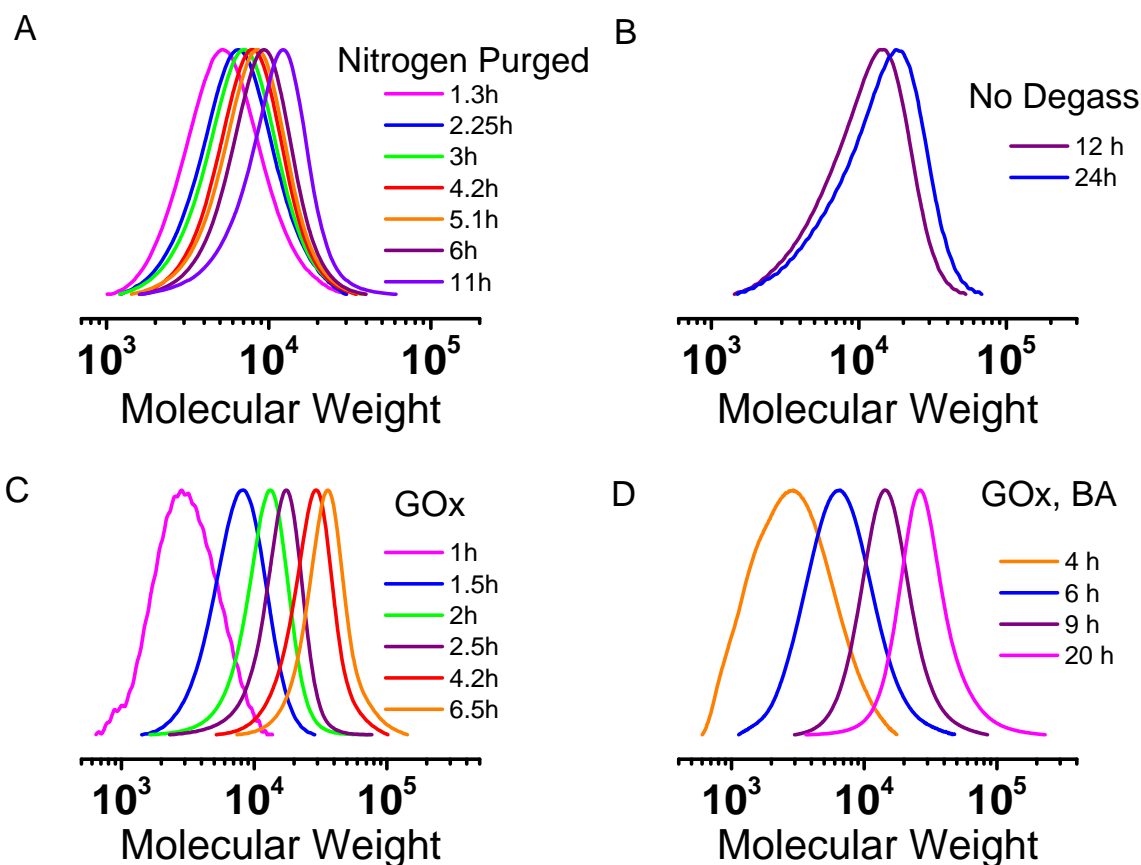


Figure S3. Evolution of GPC traces for BMA and BA miniemulsion polymerization using ARGET ATRP with different deoxygenation methods: (A) BMA, traditional nitrogen purging; (B) BMA, no degassing; (C) BMA, degassed by GOx; (D) BA, degassed by GOx. Reaction conditions: BMA or BA (monomer, M) 2 mL (20 vol %), $[M]/[I]/[CuBr_2/TPMA] = 280/1/0.2$ (I = EBPA for BMA; EBiB for BA), $[HD] = 10$ wt % relative to M, $[SDS] = 4.6$ wt % relative to M, $[NaBr] = 0.1$ M, $T = 44$ °C. AsAc was fed at a rate of $3.7 \mu\text{mol/h}$. The GOx-degassing reagents in (C) (D) are: $[\text{glucose}] = 0.2$ M, $[\text{GOx}] = 2 \mu\text{M}$, $[\text{sodium pyruvate}] = 0.1$ M.

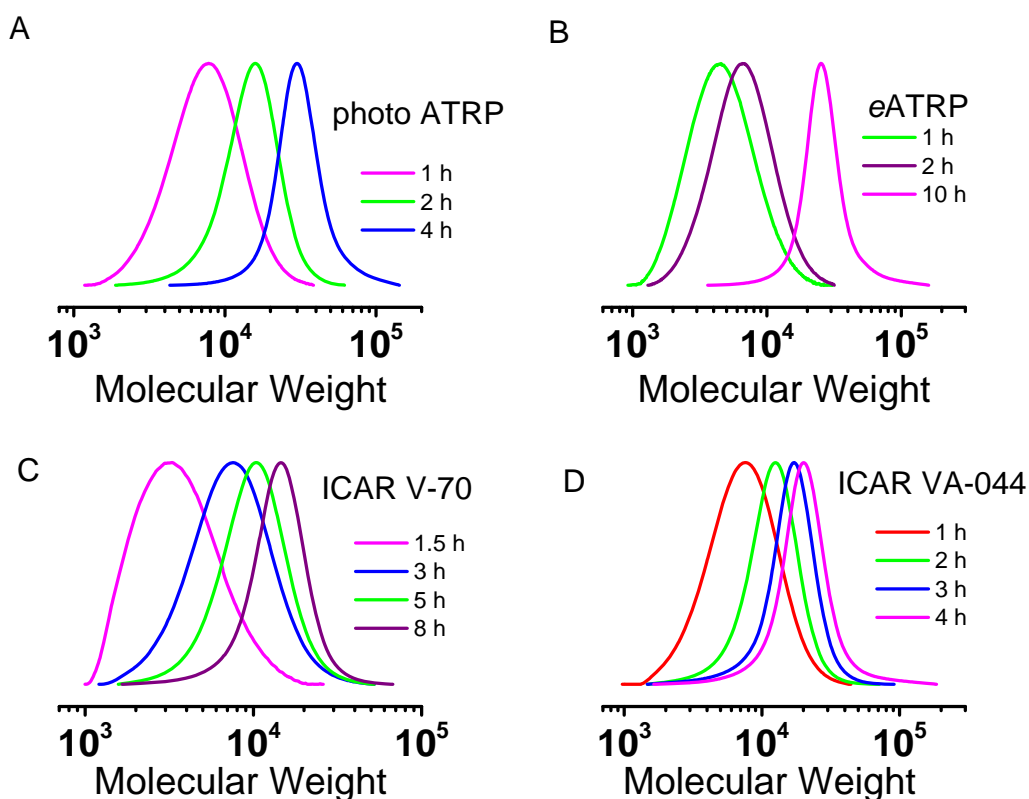


Figure S4. Evolution of GPC traces for BMA miniemulsion polymerization using different low ppm ATRP methods: (A) photo ATRP; (B) *e*ATRP; (C) ICAR ATRP mediated by V-70; (D) ICAR ATRP mediated by VA-044. Reaction conditions: BMA 2 mL (20 vol %), [BMA]/[EBPA]/[CuBr₂] = 200/1/0.16, [HD] = 10 wt % relative to BMA, [SDS] = 4.6 wt % relative to BMA, [NaBr] = 0.1 M, [glucose] = 0.2 M, [GOx] = 2 μM, [sodium pyruvate] = 0.1 M, *T* = 44 °C (30 °C when using V-70). (A) [CuBr₂]/[TPMA] = 1/3, λ_{max} = 370 nm, intensity = 5 mW/cm². (B) [CuBr₂]/[TPMA] = 1/1, *E*_{app} = *E*_{pc, CuTPMA}, working electrode = Pt mesh. (C) I = VA-044; (D) I = V-70.

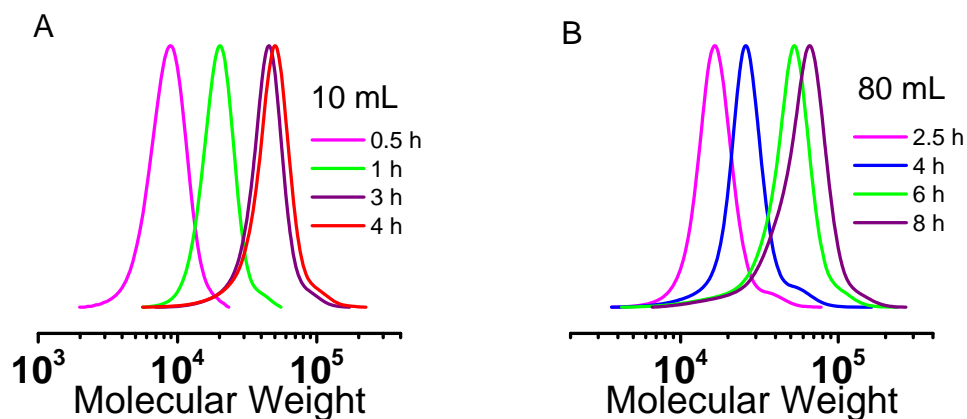


Figure S5. Evolution of GPC traces for BMA *ab initio* emulsion polymerization using ARGET ATRP. (A) $V_{\text{total}} = 10 \text{ mL}$; (B) $V_{\text{total}} = 80 \text{ mL}$. Conditions: $[\text{BMA}]/[\text{PEO}_{2k}\text{-BPA}]/[\text{NaBr}]/[\text{CuBr}_2/\text{TPMA}] = 500/1/20/0.2$, BMA 20% (v/v) in H_2O , SDS was 18.4 wt% to BMA. $[\text{glucose}] = 0.2 \text{ M}$, $[\text{GOx}] = 2 \text{ }\mu\text{M}$, $[\text{sodium pyruvate}] = 0.1 \text{ M}$, $T = 44 \text{ }^\circ\text{C}$. (A) 0.8; (B) 6.4 μmol AsAc injected at $t = 0$, then AsAc was fed with a syringe pump, feeding rate = (A) 0.8; (B) 6.4 $\mu\text{mol/h}$. Stirring rate = 250 rpm.

REFERENCES

- (a) Xia, J.; Matyjaszewski, K., Controlled/"Living" Radical Polymerization. Atom Transfer Radical Polymerization Catalyzed by Copper(I) and Picolyamine Complexes. *Macromolecules* **1999**, *32* (8), 2434-2437; (b) Fu, L. Y.; Simakova, A.; Fantin, M.; Wang, Y.; Matyjaszewski, K., Direct ATRP of Methacrylic Acid with Iron-Porphyrin Based Catalysts. *ACS Macro. Lett.* **2018**, *7* (1), 26-30.