Open-to-Air RAFT Polymerization in Complex Solvents —From Whisky to Fermentation Broth

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Materials and Methods

I) Materials. Unless otherwise stated, all chemicals listed below were purchased and used as received: 2-hydroxyethyl acrylate (HEA, 96%, Sigma Aldrich), N,N-dimethylacrylamide (DMA, 99%, Sigma Aldrich), N-isopropylacrylamide (NIPAm, Sigma Aldrich, >99%), 2-hydroxyethyl methacrylate (HEMA, Sigma Aldrich, ≥99%), 4-vinyl phenol (4VP, Sigma Aldrich, 10 wt% in propylene glycol), sodium 4-vinylbenzene sulfonate (4SS, Sigma Aldrich, >90%), N-(2 amino ethyl) methacrylamide hydrochloride (AEMA, Sigma Aldrich, >98%), 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, Wako), 1-propanethiol (99%, Sigma Aldrich), potassium hydroxide (>85%, Fischer Scientific), carbon disulfide (CS_2, anhydrous, ≥99%, Sigma Aldrich), bromo-2-methylpropionic acid (98%, Sigma Aldrich), glucose oxidase from *Aspergillus niger* (GO_x, Sigma Aldrich, >100,000 units/g, without added oxygen), glucose (Fisher Scientific, ACS reagent grade), SnakeSkin™ dialysis tubing (MWCO 3.5K MWCO, 22 mm, Fisher Scientific), absolute ethanol (EtOH, Fisher Scientific), LB Broth (Lennox, Sigma, EZMix™ powder microbial growth medium), methanol (MeOH, Fisher Scientific, ACS reagent grade), deuterium oxide (D_2O, 99.9 atom % D, Sigma), chloroform-*d* ($CDCl_3$, 99.96 atom % D, Sigma).

N.B. We have observed considerable bottle-to-bottle variation in the activity and appearance of the GOx enzyme as received from commercial sources. A deep yellow color may indicate some loss of activity due to exposure to oxygen or lack of refrigeration during shipping. We recommend testing the activity of the enzyme using an assay kit (ThermoFisher, 23280), prior to use. In this work, we use, *nominally*, a large excess of GOx relative to past publications[1] because positive control reactions conducted in PBS/MeOH yielded no polymerization when conducted in our test tube geometry. As mentioned in the manuscript and discussed by Chapman and coworkers,[1] the surface area to volume ratio and reactor geometry is very important as it helps determine the rate at which oxygen diffuses into the reaction mixture.

HEA, DMA, 4-vinyl phenol, sodium 4-styrene sulfonate (SS), and HEMA were passed through basic alumina to remove inhibitor prior to polymerizations. Monomers were passed through basic alumina to remove inhibitors and were stored at −20 °C for no more than a week before use. LB powder was dissolved in deionized water and autoclaved before use. A stock glucose solution (1M) was also prepared in deionized water and autoclaved before use. Fermentation broth provided by was prepared using the methods reported by Kang et al.[2] and provided by YongSoo Hong at the Korea Research Institute of BioScience and Biotechnology. The decelluarized broth was stored at −20 °C and warmed to room temperature before use. Decolorized red wine and decolorized grape juice were obtained from Black Box Cabernet Sauvignon and R.W. Knudsen Family Organic Concord Grape Juice, respectively. The wine or juice was stirred with carbon black for 10 minutes, then the carbon black was removed by filtration to yield the decolorized solvent **(Fig S13).** Attempting to decolorize with basic alumina did not fully remove the dye molecules from either solvent.

The following beers, liquors, and other drinks were donated by the coauthors and members of the Tirrell and Rowan groups: House of Stuart (40% ABV, ~$18 per 1.75 L), Rowan's Creek (40% ABV, ~$40 per 750 mL), Jameson Blended Irish Whiskey (40% ABV, ~$38 per 1.75 L), Jeppson's Malört (35% ABV, ~18 per 750 mL), Maxime Trijol Cognac Special (40% ABV, $25 per 750 mL), Death's Door Gin (47.0% ABV, $50 per 1.75 L), ZYR Vodka (40% ABV, ~$26 per 750 mL), Espolón Tequila Blanco (40% ABV, ~$20 per 750 mL), Efe Klasik Raki (45% ABV, ~$24 per 750 mL), Black Box Cabernet Sauvignon (13.5% ABV, ~$22 per 3 L), R. W. Knudsen Organic Concord Grape Juice (0% ABV, ~$6 per 32 oz bottle), Miller High Life Light (4.6% ABV, ~$0.43 per 12 oz can), Shiner Birthday Beer 108 - Cold Brew Coffee Ale (5.0% ABV, ~$0.92 per 12 oz can), Guinness Draught (4.3% ABV, ~$1.33 per 11.2 oz bottle), Angry Orchard (5.0% ABV, ~$1.67 per 12 oz bottle) and Smirnoff Ice (5.0% ABV, ~$1.33 per 11.2 oz bottle).

After opening, liquors were stored in their original bottles at −20 °C, and warmed to room temperature prior to use. Beer, wine, and juice were also warmed to room temperature and but to avoid oxidation effects these solvents were not stored and reused.

II) Instrumental Methods:

Size-Exclusion Chromatography with Multi-angle Light Scattering (SEC-MALS).

SEC-MALS experiments on poly(NIPAM) polymers were conducted using an instrument using an Agilent 1200 pump and autosampler. Separation was conducted using an eluent mobile phase of DMF with 0.05 M LiBr at a flow rate of 1.0 mL min^{-1}, and a Viscotek Model- I-MBMMW-3078 column. Signals were collected using Wyatt DAWN-HELEOS II light scattering detector (λ = 662 nm), and a Wyatt Optilab T-rEX refractometer (λ = 658 nm). The dn/dc of poly(NIPAM) in this mobile phase was estimated based on the full mass recovery assumption in batch mode.

SEC-MALS experiments on all other polymers synthesized in this work were conducted using an Agilent 1260 High Performance Liquid Chromatograph with an eluent mobile phase of 0.1 M NaH_2PO_4 with 1.0 wt % acetic acid at a flow rate of 0.4 mL min^{-1}. Separation was achieved using three columns [CATSEC1000 (7 μ, 50 × 4.6), CATSEC100 (5 μ, 250 × 4.6), CATSEC300 (5 μ, 250 × 4.6), and CATSEC1000 (7 μ, 250 × 4.6)] obtained from Eprogen Inc. (Downers Grove, IL). Signals were collected using Wyatt HELEOS II light scattering detector (λ = 662 nm), and an Optilab rEX refractometer (λ = 658 nm). SEC trace analysis was performed using Astra VI software (version 5.3.4.18) from Wyatt Technologies (Santa Barbara, CA). In this mobile phase, the dn/dc values for all polymers, excluding poly(HEA), were estimated based on the full mass recovery assumption in batch mode.

Refractometry.

The d*n*/dc value of poly(HEA) in 0.1 M NaH_2PO_4 with 1.0 wt % acetic acid was determined using an Spectronic Instruments Benchtop refractometer with a red LED light source in the SEC mobile phase at room temperature (22 °C), in which refractive index values at various polymer concentrations were collected in triplicate. The d*n*/dc value reported (0.126 mL g^{-1}) is for a linear best-fit to the mean values of measured refractive index at various polymer concentrations in triplicate **(Fig. S21).**

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H NMR experiments were performed using a Bruker AVance III HD NanoBay spectrometer operating at 400 Mhz. Spectra are reported at room temperature with a minimum of 16 transients to minimize signal-to-noise.

Thermogravimetric Analysis (TGA). TGA experiments were conducted using a TA Instruments Discovery TGA equipped with an infrared furnace, auto-sampler, and a gas delivery module. Nitrogen was used as a purge gas at a flow rate of 10 mL/min. To pre-dry adsorbed water from the poly(HEA) samples, each sample was annealed at 100 °C for 30 min and cooled to 20 °C under nitrogen prior to testing the degradation temperature by heating at a ramp rate of 10 °C min^{-1} to 600 °C.

Differential Scanning Calorimetry (DSC). DSC experiments were conducted using a TA Instruments Discovery DSC 2500. Polymer samples (~5-10 mg) were hermetically crimped in T-zero aluminum pans. Sequential heating, cooling and reheating ramps were conducted from −90 to 120 °C; at a rate of 10 °C min^{-1}. Data reported are for the second heating ramp.

pH determination. Solvent pH values were measured using a Fisher Scientific Accumet XL200 pH/Conductivity Benchtop Meter. Prior to measurement the probe was standardized with Thermo Scientific Orion 910107 buffer solutions (pH 10.01, pH 7.00, and pH 4.01). The solvents were measured in triplicate, and the probe washed thoroughly with dionized water between each measurement. The pH value reported in manuscript **Table 1** corresponds to the median value. The juice, beer and wine samples were from freshly opened bottles/cans, while the liquor, LB, and fermentation broth samples were stored for several months/weeks prior to measurement. The pH values of solvents not reported in manuscript **Table 1** are reported in **Table S1**.

III) Synthetic Methods:

RAFT CTA Synthesis. The RAFT CTA, 2-(Propylthiocarbonothioylthio)-2-methylpropionoic acid (PPA) was synthesized similarly to a literature procedure[3] and depicted in **Scheme S1.** In a round bottom flask KOH (13.72 grams, 80 mmol) was dissolved a solution of H_2O/Acetone (150 ml, 2:1 v/v). When the solution was fully mixed 1-propane thiol was added via syringe (11.2 ml, 40 mmol, 1 eq). When fully mixed, carbon disulfide (15 ml, 84 mmol, 2.1 eq) was added dropwise via syringe, and the solution changed from colorless to yellow. The reaction was stirred at room temperature for three hours, after which time a blaze orange color persisted. In a separate flask 2-bromo-2-methyl propionic acid (20.35 g, 40 mmol, 1 eq) was dispersed in a solution of H_2O/Acetone (150 ml, 2:1 v/v). This suspension was added to the reaction dropwise, and the resulting mixture stirred at room temperature for 24 hours. After this time it was acidified to pH ~2 with concentrated HCl, extracted into chloroform (600 ml), washed with water (2x 200 ml) and brine (2x200 ml), dried with $MgSO_4$, filtered, and concentrated under vacuum to yield an orange oil. This crude product was purified by sequential column chromatography (silica, mobile phase hexane gradient to 2% ethyl acetate, 1% acetic acid, two times repeated) and (silica, mobile phase hexane gradient to 2% ethyl acetate, two times repeated), recrystallized, and dried to obtain

a yellow powder in 31% yield. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.30 (t, J = 7.4, 2H), 1.78-1.71 (m, 8H), 1.03 (t, J = 7.4, 3H) (**Fig S1)**.

N.B. a red contaminant with a similar polarity (r.f. spot 1 =0.5, r.f. spot 2 =0.46, 80:20 hexanes/ethyl acetate mobile phase, silica plate) co-elutes when acetic acid is used on the column, which may be why an earlier publication reported this molecule as a dark orange product.[1] The contaminants are best visualized in TLC by using a vanillin stain.

Polymer synthesis, general considerations:

For polymerizations, stock solutions of PPA CTA (10-30 mg ml^{-1} in 40% v/v aqueous ethanol), GOx (16 mg ml^{-1} in PBS), Va-044 initiator (30 mg ml^{-1} in water), and Glucose (1M in DI water) were prepared and added by syringe. Solvent and monomer were added directly, also added by syringe. The GOx stock solution was freshly prepared before each polymerization, CTA, glucose, and VaO44 solutions were prepared and stored at −20 °C.

Initial control reactions in methanol, ethanol, or HoSt:

RAFT polymerization experiments in closed systems (positive control) were conducted in test tubes (18x150 mm geometry) sealed with a rubber septum (sleeve stopper style with a bottom outside diameter of 15.7 mm, secured tightly on the outside with copper wire and electrical tape). Controlled RAFT polymerizations in open test tubes (negative controls) were conducted in the same geometry but were not sealed with a septum. Detailed procedure are listed below, results are summarized in **Table S2.**

Methanol, closed tube, no enzyme or glucose: To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), aq. methanol (8.9 ml, 40% *v*/*v*), and HEA monomer (1.5 ml) were

added. The tube was sealed with a septum, secured by wire and electrical tape, and sparged for 20 min by bubbling nitrogen through the solution with long needle (venting to a bubbler). After this time, the dynamic nitrogen flow was removed and the sealed tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR analysis (**Figure S2**), and the remaining sample was purified by dialysis. The purified sample was lyopholized to dry prior to SEC, DSC, TGA, and ^{1}H NMR analysis.

Ethanol, closed tube, no enzyme or glucose: To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), aq. ethanol (8.9 ml, 40% *v*/*v*), and HEA monomer (1.5 ml) were added. The tube was sealed with a septum, secured by wire and electrical tape, and sparged for 20 min by bubbling nitrogen through the solution with long needle (venting to a bubbler). After this time, the dynamic nitrogen flow was removed and the sealed tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR analysis (**Figure S2**), and the remaining sample was purified by dialysis. The purified sample was lyopholized to dry prior to SEC, DSC, TGA, and ^{1}H NMR analysis.

HoSt, closed tube, no enzyme or glucose: To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), HoSt (8.9 ml), and HEA monomer (1.5 ml) were added. The tube was sealed with a septum, secured by wire and electrical tape, and sparged for 20 min by bubbling nitrogen through the solution with long needle (venting to a bubbler). After this time, the dynamic nitrogen flow was removed and the sealed tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR

analysis (**Figure S2**), and the remaining sample was purified by dialysis. The purified sample was lyopholized to dry prior to SEC, DSC, TGA, and ^{1}H NMR analysis.

Methanol, open tube, no enzyme or glucose:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), aq. methanol (8.9 ml, 40% *v*/*v*), and HEA monomer (1.5 ml) were added. After mixing, the open tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR analysis, no evidence of polymerization was observed.

Ethanol, open tube, no enzyme or glucose:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), aq. ethanol (8.9 ml, 40% *v*/*v*), and HEA monomer (1.5 ml) were added. After mixing, the open tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR analysis, no evidence of polymerization was observed.

HoSt, open tube, no enzyme or glucose:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), HoSt (8.9 ml), and HEA monomer (1.5 ml) were added. After mixing, the open tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (3 h). A crude aliquot was removed for ^{1}H NMR analysis, no evidence of polymerization was observed.

HoSt, open tube, no enzyme, added glucose:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), HoSt (8.9 ml), aq. Glucose (0.43 ml, 1M), and HEA monomer (1.5 ml) were added. After mixing, the open tube was heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment (25 h). A crude aliquot was removed for ^{1}H NMR analysis, no evidence of polymerization was observed.

HoSt, open tube, added enzyme, no glucose:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.0 ml, 25 mg ml^{-1}), Va-044 stock solution (100 µl, 30 mg ml^{-1}), HoSt (8.9 ml), and HEA monomer (1.5 ml) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.38 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. A crude aliquot was removed for ^{1}H NMR analysis, no evidence of polymerization was observed.

Chain Extension:

Chain extension experiments were conducted in open tubes with GOx using HoSt whisky as a solvent. First, we prepared poly(HEA) targeting ~10 kg mol^{-1}. After purification, drying and characterization, this polymer was used as a macromolecular CTA for the polymerization of HEA, targeting a higher molar mass homopolymer of ~40 kg mol^{-1}. Briefly, the macromolecular CTA was fully dissolved in HoSt (4.0 ml) in a vial, then transferred to a test tube containing a magnetic stir flea. Next, Va-044 stock solution (215 µl, 30 mg ml^{-1}), aq. Glucose (1.29 ml, 1M), and HEA monomer (1.0 ml), were added to a test tube and briefly mixed by votex. To avoid

competition between re-initiation and CTA hydrolysis, the reaction was adjusted slightly through the addition of HCl (starting solution pH ~5.0), measured using Hydrion pH indicator paper .[4] [5]. To this solution, GOx stock solution (1.1 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. After incubating at room temperature for 10 min, the polymerization was immersed in a 45 °C oil bath, stirring open to the air. After 24 h, the polymer was purified by dialysis and dried, resulting in a 37 kg mol^{-1} homopolymer with modest dispersity ($Đ$ =1.3).

Kinetics Methods.

Polymerization kinetics were conducted in both open test tubes and closed round bottom flasks, outlined in further detail below:

Open experiment: To mitigate the effects of volume changes on ethanol evaporation rate and oxygen diffusion, one reaction solution was prepared at three times the typical volume (~30 ml total volume) and split into three separate test tubes (A, B, and C, each ~10 ml total volume). These three reactions were run in parallel and aliquots were removed in alternating turns from each (for example the first 0.5 ml aliquot was sampled from the tube A, and the second from tube B, and so on). Specifically, CTA stock solution (5.85 ml, 10 mg ml^{-1}), Va-044 stock solution (105 µl, 30 mg ml^{-1}), HoSt (27 ml), aq. Glucose (1.29 ml, 1M), and HEA monomer (3.0 ml), were added to a test tube and briefly mixed by votex. To this solution, GOx stock solution (1.1 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube were then split into three 18x150 mm test tubes, each equipped with a magnetic stir flea (8x3 mm) incubated at room temperature without stirring for 10 min. After this time the test tubes were transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. Aliquots (~ 0.5 ml volume) were removed at 0 m (room temp prior to reaction), 15 m, 30 m, 60 m, 90 m, 2 h, 3 h, 4 h, 5h, and 25 h. ^{1}H NMR spectroscopy experiments on the crude aliquots were used to determine the monomer conversion. Due to insufficient quantities of

polymer in the early time points (i.e., low conversion and low molar mass), only polymers from the 2 h, 3 h, 4 h, 5 h, and 25 h aliquots taken were isolated. These samples were purified by dialysis. A total of six exchanges of 4 L water were performed for each sample. The purified samples were lyophilized to dry prior to SEC and ^{1}H NMR analysis.

Closed experiments: Two different closed experiments were conducted, each at a 12 ml total volume. Both experiments were conducted without added GOx, but were otherwise similar to the open polymerizations described previously. To one 25 ml round bottom flask containing a magnetic stir bar, CTA stock solution (1.95 ml, 10 mg ml^{-1}), Va-044 stock solution (35 µl, 30 mg ml^{-1}), aqueous ethanol (8.9 ml, 40% v/v), aq. Glucose (0.43 ml, 1M), and HEA monomer (1.0 ml), were added. To a second 25 ml round bottom flask containing a magnetic stir bar, CTA stock solution (1.95 ml, 10 mg ml^{-1}), Va-044 stock solution (35 µl, 30 mg ml^{-1}), HoSt (8.9 ml), aq. Glucose (0.43 ml, 1M), and HEA monomer (1.0 ml) were added. The two flasks were sealed with a septum, secured by wire and electrical tape, and sparged for 20 min by bubbling nitrogen through the solution with long needle (venting to a bubbler). After this time, the dynamic nitrogen flow was removed and the sealed flasks were heated in an oil bath pre-equilibrated at 45 °C while stirring at ~500 rpm for the duration of the experiment. Aliquots (~ 0.5 ml volume) were removed under a nitrogen stream at 0 m (room temp, after degassing but prior to heating), 15 m, 30 m, 60 m, 90 m, 2 h, 3 h, 4 h, 5h, and 25 h. ^{1}H NMR experiments were used to determine the monomer conversion. Due to insufficient quantities of polymer in the early time points (i.e., low conversion and low molar mass), only polymers from the 2 h, 3 h, 4 h, 5 h, and 25 h aliquots taken were isolated. These samples were purified by dialysis. A total of six exchanges of 4 L water were performed for each sample. The purified samples were lyophilized to dry prior to SEC and ^{1}H NMR analysis.

Solvent screening:

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.95 ml, 10 mg ml^{-1}), Va-044 stock solution (35 µl, 30 mg ml^{-1}), *a solvent (4.3 ml), aq Glucose (0.43 ml, 1M), and HEA monomer (2.5 ml) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.38 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. A crude aliquot was removed for ^{1}H NMR analysis, and the remaining sample was purified by dialysis. The purified sample was lyophilized to dry prior to SEC and ^{1}H NMR analysis.

*N.B.: All solvent screening experiments were conducted using the same method described above, with the specific solvents being those described in Table 1 of the manuscript.

Hot Toddy. The Hot Toddy solvent was prepared according to the following recipe: for each serving, cinnamon (1 stick broken into thirds, McCormick), cloves (4 whole, Woodland foods brand), and nutmeg (~¼ tsp, McCormick) were combined in a T-Sac size 2 tea filter, which was folded shut and secured with a staple. In a cardboard coffee cup, water (2 oz, Culligan), 1 slice of lemon (~1/10 of one medium whole lemon), and honey (1 tbsp., Breitsamer Mountain Flower Raw Honey), were mixed and then heated using a conventional microwave for 1.33 min. After heating, the tea bag was allowed to seep for a few minutes in the hot honey solution. When the contents of the cup had cooled slightly, the tea bag was removed and whisky (2 oz, House of Stuart), was added and the contents stirred using a coffee stirrer. Hot Toddies used for polymerization experiments were then cooled to room temperature prior to the addition of monomer, CTA, and GOx enzyme. Control experiments were conducted in which the lemon was

omitted from this recipe. The pH value of the Hot Toddy at room temperature was measured to be 4.80 without lemon, and 2.01 with lemon.

For the Hot Toddy reactions targeting a theoretical molar mass of ~30 kg mol^{-1}, we examined the effects of reactor geometry, pH, and omission of added GOx. Raw honey contains native GOx; [6] however, in the sample we used, the concentration was not high enough to allow the polymerization reaction to proceed without added enzyme. With lemon, we speculate that extensive chain coupling occurred due to the loss of GOx activity at lower pH. The polymerization in the shot class did not proceed, perhaps because the surface area to volume ratio was much higher in this geometry than in the test tube.

Monomer screening:

All solvent screening experiments were conducted using the method described above, except and HEA monomer was replaced with 4SS, AEMA, 4VP, NIPAm, DMA, or HEMA.

Polymerization of DMA (target molar mass 10 kg mol^{-1})

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.95 ml, 10 mg ml^{-1}), Va-044 stock solution (33 µl, 30 mg ml^{-1}), HoSt (8.9 ml), aq. Glucose (0.43 ml, 1M), and N,N-dimethylacrylamide monomer (0.882 g) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.4 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. The sample was purified by dialysis. The purified sample was lyophilized to dry prior to SEC and ^{1}H NMR analysis.

Polymerization of NIPAm (target molar mass 12 kg mol^{-1})

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (2.0 ml, 10 mg ml^{-1}), Va-044 stock solution (35 µl, 30 mg ml^{-1}), HoSt (9 ml), aq. Glucose (0.43 ml, 1M), and N-isopropylacrylamide monomer (1.007 g) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.4 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. Due to an LCST in whisky, the polymer precipitated during the reaction. The heterogeneous sample was purified by dialysis. The purified sample was lyophilized to dry prior to SEC and ^{1}H NMR analysis.

Polymerization of HEMA (target molar mass 14kg mol^{-1})

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (1.7 ml, 10 mg ml^{-1}), Va-044 stock solution (43 µl, 30 mg ml^{-1}), HoSt (7.7 ml), aq. Glucose (0.43 ml, 1M), and 2-hydroxyethyl methacrylate monomer (1.002 g) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.40 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. A crude aliquot was removed for ^{1}H NMR analysis, and the remaining sample was purified by dialysis. N.B due to extensive overlap in the ^{1}H NMR spectrum between ethanol, residual monomer, and polymer signals; calculation of conversion may be inaccurate (**Figure S20**). The sample was insoluble in the aqueous GPC mobile phase.

Polymerization of 4VP (target molar mass 12 kg mol^{-1})

To a test tube (18 mm x 150 mm) containing a magnetic stir flea (8 mm x 3 mm), CTA stock solution (0.2 ml, 10 mg ml^{-1}), Va-044 stock solution (33 µl, 30 mg ml^{-1}), fermentation broth (8.0 ml), aq. glucose (0.43 ml, 1M), and 4-vinyl phenol monomer (0.10 g as a 10% solution in propylene glycol) were added. The solution was mixed well by vortex. To this solution, GOx stock solution (0.4 ml, 16 mg ml^{-1} in PBS) was added and the tube was flicked gently to mix. The contents of the tube incubated at room temperature without stirring for 10 min. After this time the test tube was transferred to an oil bath pre-equilibrated at 45 °C, and stirred at ~500 rpm for the duration of the experiment. The resulting polymer sample was purified by dialysis, and lyophilized to dry.

We note that with this monomer side reactions involving the phenolic hydroxyl group may occur, however further work is required to determine the level of control. Unfortunately, we have been unable to characterize these samples by GPC. The sample was soluble in the aqueous GPC mobile phase (0.1 M NaH_2PO_4 with 1.0 wt % acetic acid), however column interactions and low detector response made molar mass estimates inaccurate. Further characterization of controlled polymerization of renewable monomers in fermentation broth remains an ongoing investigation.

Table S1. pH values of Solvents not listed in Manuscript Table 1.

Solvent Sample	Brand	[a]pH
Red Wine	Black Box Cabernet Sauvignon	3.65
Decolorized Red Wine	Black Box Cabernet Sauvignon	6.50
Grape Juice	R.W. Knudsen Family Organic Concord	3.55
Decolorized Grape Juice	R.W. Knudsen Family Organic Concord	4.10
Hot Toddy, No Lemon	Made with House of Stuart whisky	4.80
Hot Toddy, with Lemon	Made with House of Stuart whisky	2.01
LB	—	7.80
Fermentation broth	—	6.09

[a]pH values are for solvents only, no monomer, CTA, glucose, or initiator.

Table S2. Characterization summary of control RAFT poly(HEA) systems.

Solvent	Vessel	GOx	Glucose	% Conv.[a] (%)	M_n [b] (kg mol^{-1})	$Đ$[b]
MeOH (40%, *v*/*v*)	Open	No	No	0	N/A	N/A
EtOH (40%, *v*/*v*)	Open	No	No	0	N/A	N/A
House of Stuart	Open	No	No	0	N/A	N/A
House of Stuart	Open	No	Yes	0	N/A	N/A
House of Stuart	Open	Yes	No	0	N/A	N/A
MeOH (40%, *v*/*v*)	Closed	No	No	91	11.8	1.02
EtOH (40%, *v*/*v*)	Closed	No	No	91	12.2	1.03
House of Stuart	Closed	No	No	90	11.2	1.03

[a] Total monomer conversion, determined by ^{1}H NMR spectroscopy of the crude aliquot in D_2O. [b] Absolute number-average molar mass and dispersity, experimentally measured by SEC-MALS using 0.1 M NaH_2PO_4 with 1.0 wt % acetic acid as the mobile phase at 35 °C with a measured dn/dc value of 0.126 mL g^{-1} by refractometry.

Table S3. [a]Summary of Hot Toddy reactions.

[b]Geometry	Lemon	GOx	[c]Conv. (%)	[d]M_n (kg mol^{-1})	[d]$Đ$
Test Tube	No	Yes	89	23.6	1.08
Test Tube	No	No	0	N/A	N/A
Test Tube	Yes	Yes	89	46.3	1.27
Shot Glass	Yes	Yes	0	N/A	N/A

[a]All Hot Toddy reactions were conducted with no added glucose, however glucose is a major component of honey (comprising 20-40% of all sugar). [b]Test tube dimensions were: 18 mm x 150 mm (38 ml capacity), the polymerization was conducted on a ~6 ml scale; surface area to volume ratio 2.5:6. Approximate shot glass dimensions were: top o.d. 50 mm, bottom o.d. 35 mm, height 60 mm (44 ml capacity), the polymerization was conducted on a ~12 ml scale; surface are to volume ratio estimated as ≥4:6, based on angle of taper. [c]Total monomer conversion, determined by ^{1}H NMR spectroscopy of the crude aliquot in D_2O. [d]Absolute number-average molar mass and dispersity, experimentally measured by SEC-MALS using 0.1 M NaH_2PO_4 with 1.0 wt % acetic acid as the mobile phase at 35 °C with a measured dn/dc value of 0.126 mL g^{-1} by refractometry.

Table S4. Comparison of polymers synthesized in kinetics experiment [manuscript figure 2].

[a]Solvent	Time (h)	Open?	[b]Conv. (%)	[c]M_n (kg mol^{-1})	[c]$Đ$
HoSt	25	No	98	16.8	1.05
EtOH (aq. 40%)	25	No	97	17.1	1.04
HoSt	25	Yes	92	15.6	1.02

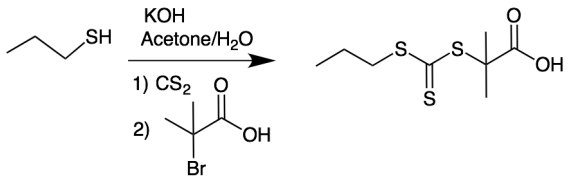
[a]HoSt = House of Stuart Whisky, 40% alcohol by volume. [b]HEA conversion, determined by ^{1}H NMR. [c]Determined using size exclusion chromatograph, aqueous mobile phase.

Table S5. Monomer Screening

[a]Monomer	[b]Conv. (%)	M_n (kg mol^{-1})	$Đ$
4SS	0%	-	-
AEMA	0%	-	-
4VP	[c]60%	-	-
HEMA	70	-	-
NIPAM	[d]-	[e]14	[e]1.1
DMA	92	[f]13	[f]1.1

All polymerizations were conducted in House of Stuart Whisky (40% alcohol by volume) and run at 45 °C for 12 h. [a]Monomer abbreviations are as follows: sodium 4-vinylbenzene sulfonate (4SS), N-(2 amino ethyl) methacrylamide hydrochloride (AEMA), 4-vinyl phenol (4VP), 2-hydroxyethyl methacrylate (HEMA), N-isopropylacrylamide (NIPAm), N,N-dimethylacrylamide (DMA).[b]Monomer conversion, determined by ^{1}H NMR. [c]Polymer was insoluable, the conversion was determined by mass recovery of filtered and dried polymer. [d]NIPAM conversion not determined. Since the PNIPAM had a LCST in the solvent, the polymer was separated by filtration and dialyzed prior to SEC. [e]Determined by size exclusion chromatograph, DMF mobile phase. [f]Determined by size exclusion chromatograph, aqueous mobile phase.

Scheme S-1. Synthesis of 2-(propylthiocarbonothioylthio)-2-methylpropionoic acid (PPA).



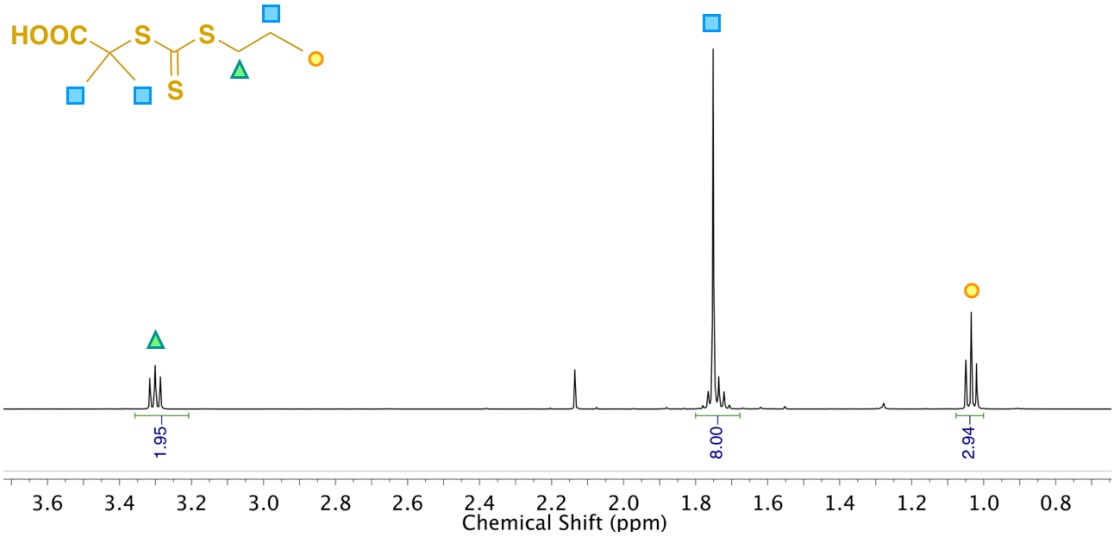


Figure S1. ^{1}H NMR spectrum of RAFT CTA PPA in $CDCl_3$.

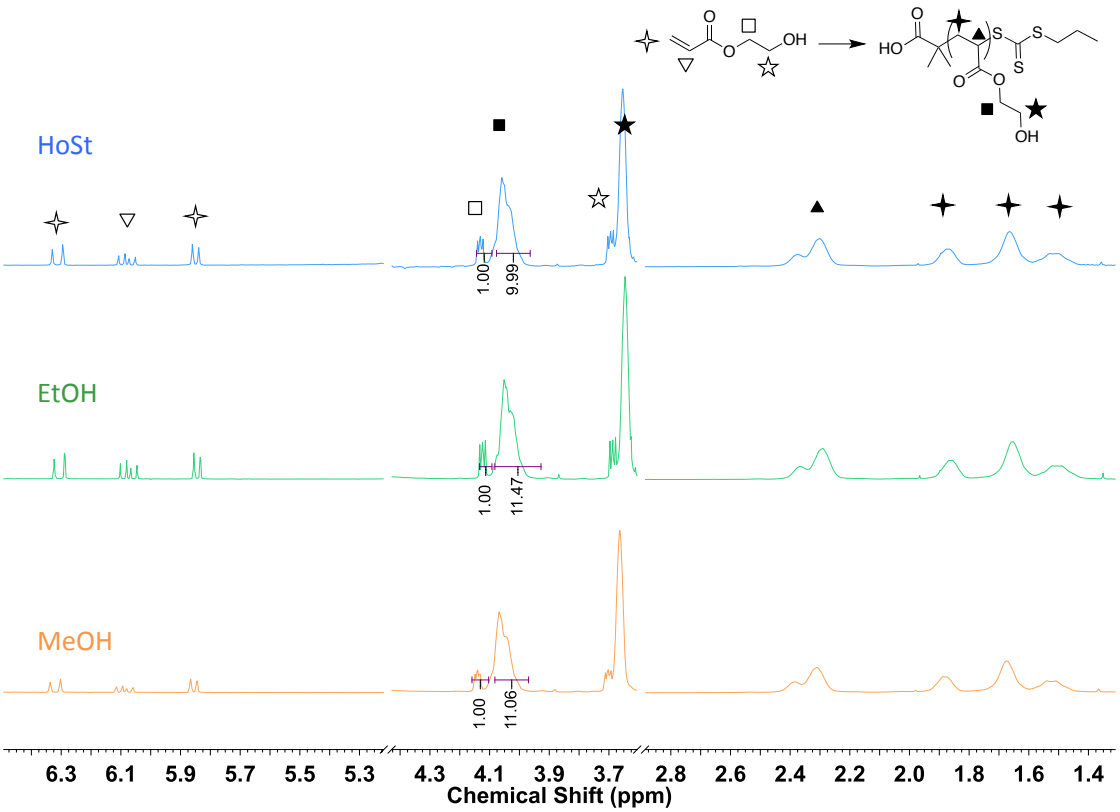


Figure S2 – ^{1}H NMR (D_2O) showing conversion calculation for closed polymerization of HEA in the indicated solvent. The integrals show the regions used to calculate conversion, the break in the axis removes the peaks characteristic of solvents.

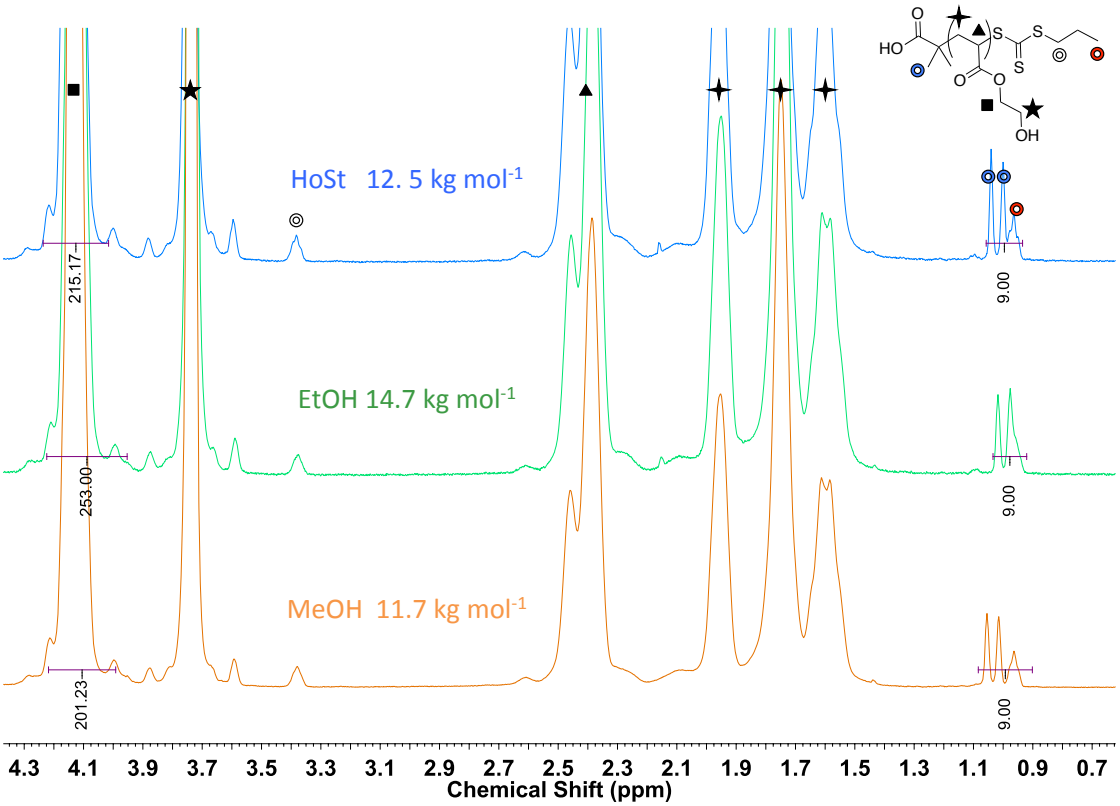


Figure S3–^{1}H NMR (D_2O) showing purified poly(HEA) polymers synthesized in the indicated solvent. The integrals show the regions used for end group analysis to calculate M_n.

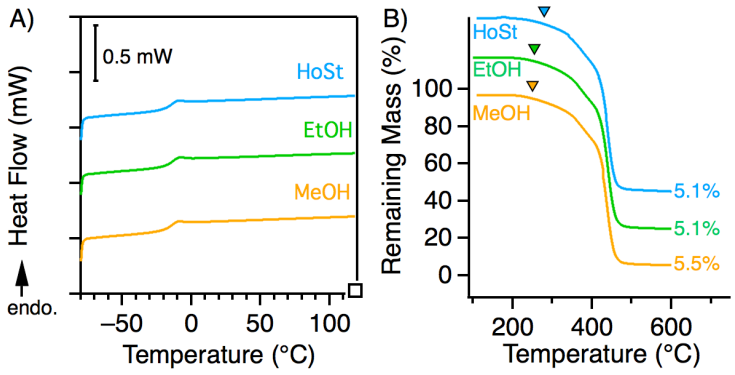


Figure S4. Expanded version of manuscript **Figure 1 B**, and **C (A)** Overlay of DSC traces for poly(HEA) synthesized in in the indicated solvent: aqueous methanol (MeOH, 40% v/v), aqueous ethanol (EtOH, 40% v/v), HoSt whisky. The data were collected on the second heating cycle at a ramp rate of 10 °C min^{-1}. **(B)** TGA for the same poly(HEA) samples shown in A, data were collected on a heating ramp rate 10 °C min^{-1}, under nitrogen and have been offset progressively (20%) for clarity. Degradation temperature, T_d (▼), is defined as the temperature at which 5% mass is lost. T_d = 279, 254, and 249 °C for the polymers synthesized in HoSt, EtOH, and MeOH, respectively.

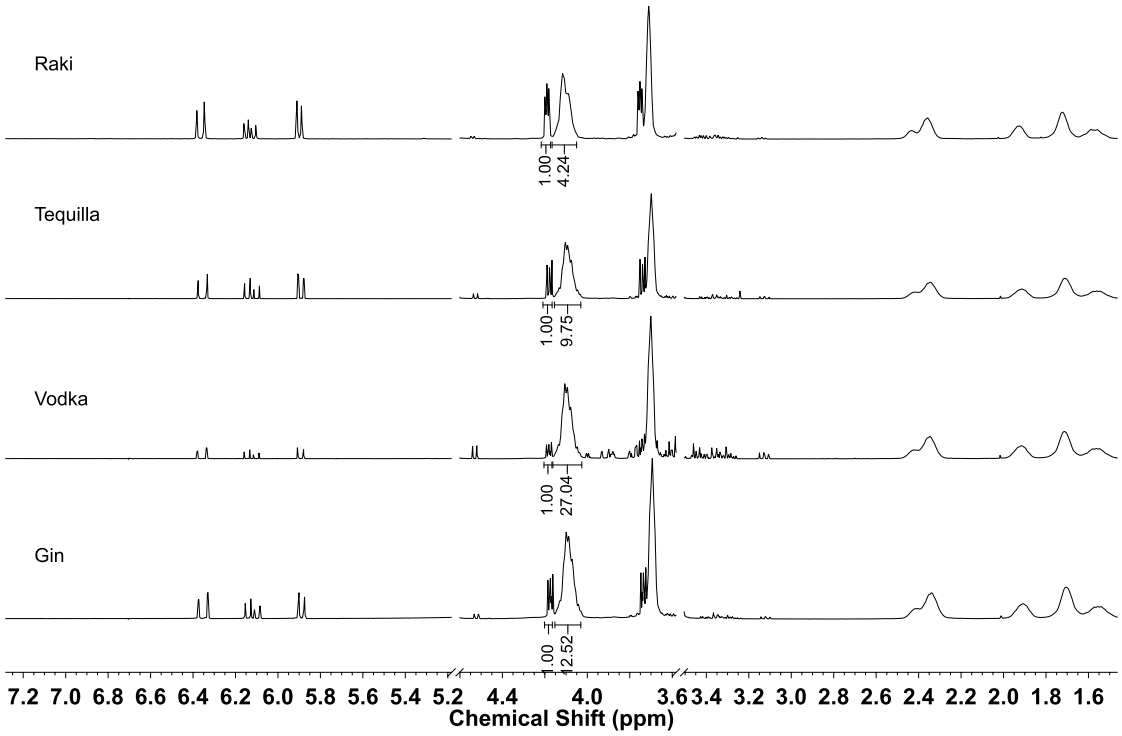


Figure S5- ^{1}H NMR (D_2O) overlay of crude poly(HEA) samples from polymerizations in clear liquors. The integrals show the regions used to calculate conversion, the break in the axis removes the peaks characteristic of solvents.

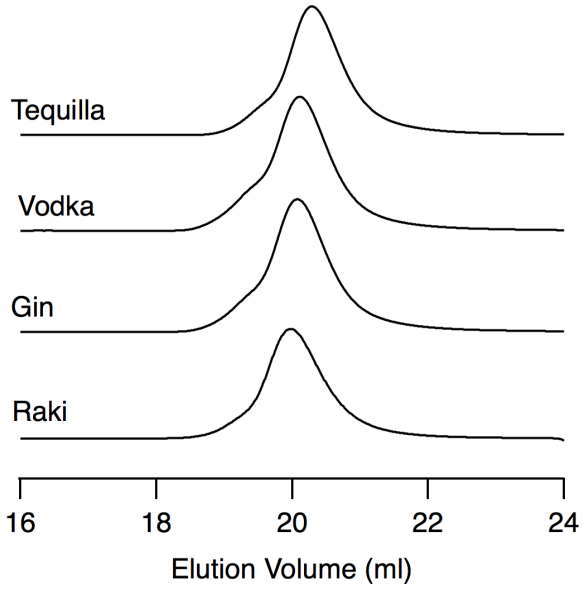


Figure S6- SEC overlay of polymers synthesized in colorless liquors, aqueous mobile phase.

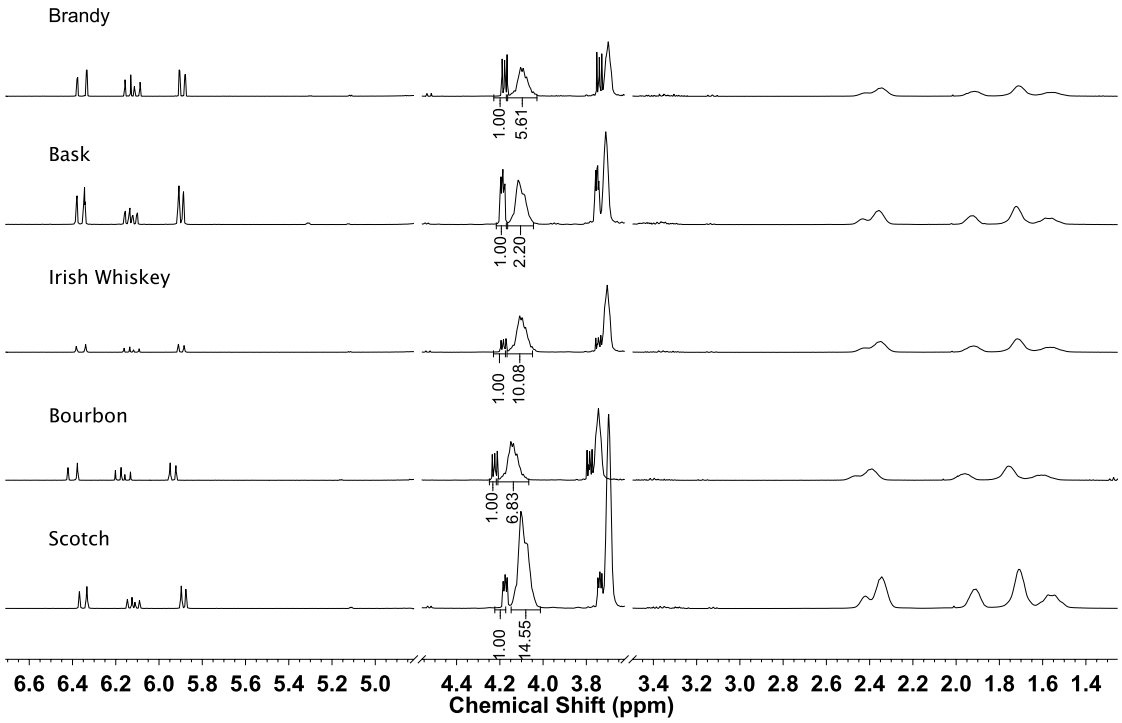


Figure S7- ^{1}H NMR overlay (D_2O) of crude poly(HEA) samples from polymerizations in brown/amber liquors. The integrals show the regions used to calculate conversion, the break in the axis removes the peaks characteristic of solvents.

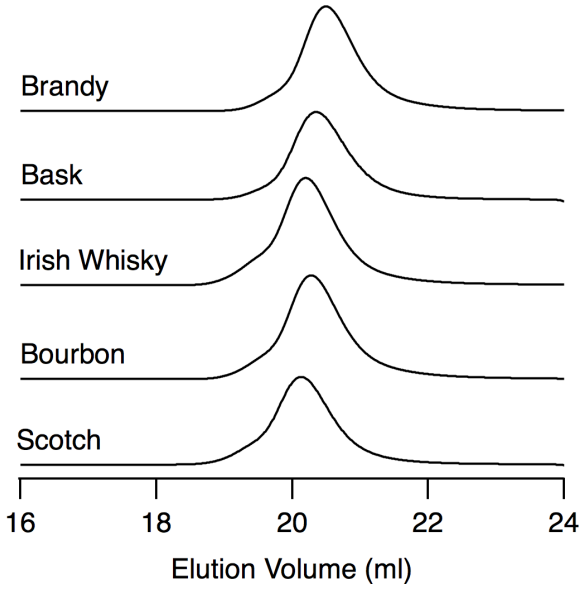


Figure S8- SEC overlay of polymers synthesized in brown/amber liquors, aqueous mobile phase.

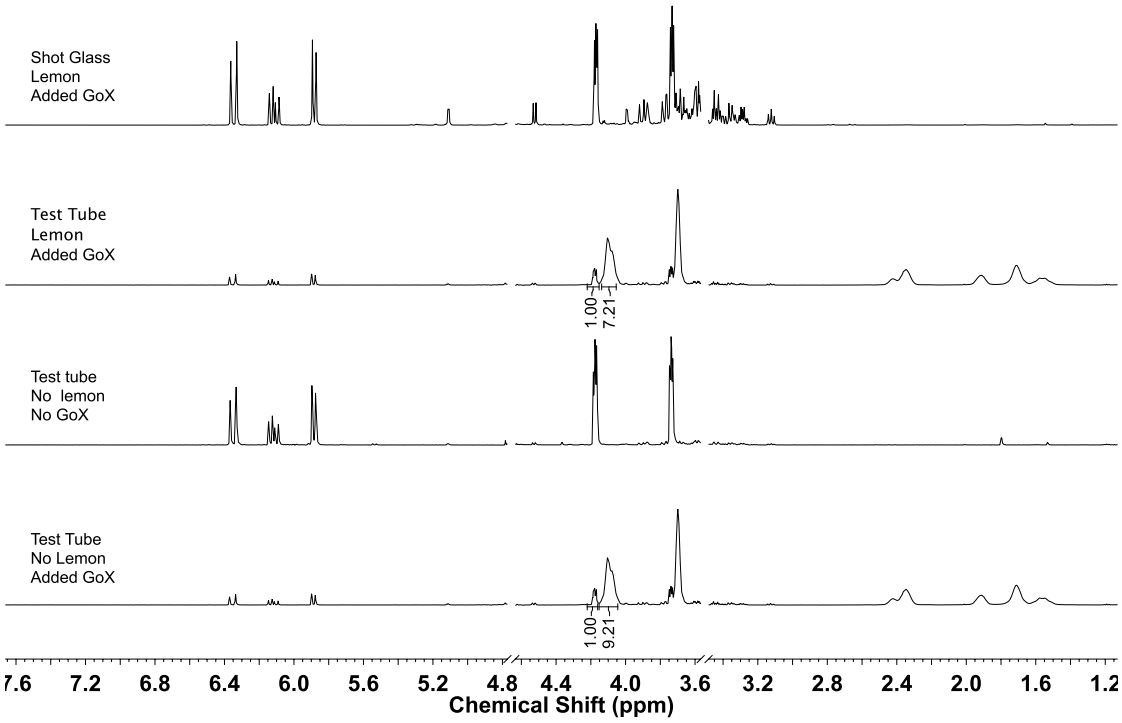


Figure S9- ^{1}H NMR (D_2O) overlay of crude poly(HEA) samples from polymerizations in Hot Toddy mixtures.. The increased concentration of sugars in the top trace is due to the increased evaporation of alcohol in the shot glass geometry.

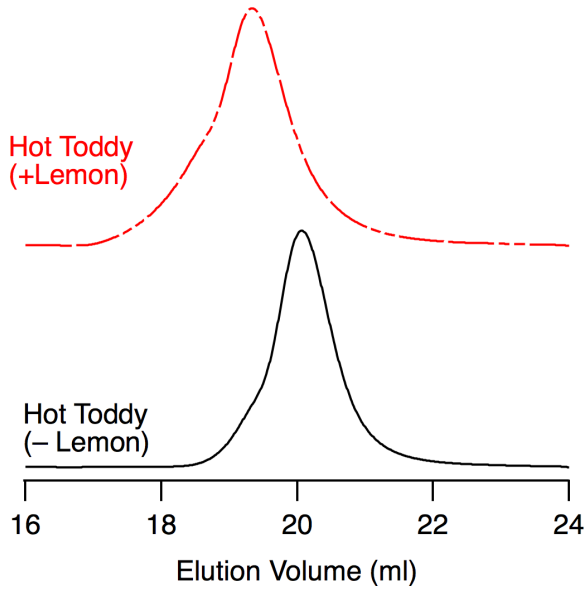


Figure S10- SEC overlay of polymers synthesized in Hot Toddy with and without lemon, aqueous mobile phase.

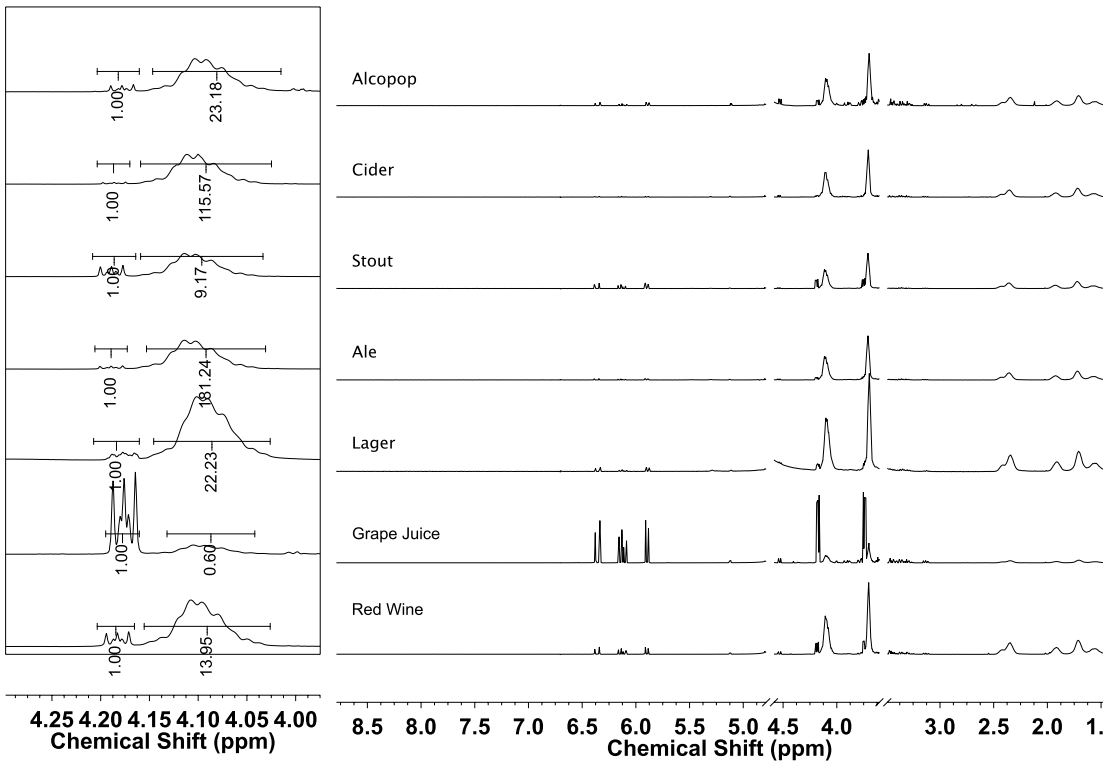


Figure S11 ^{1}H NMR (D_2O) overlay of crude poly(HEA) samples from polymerizations in low/no alcohol content solvents.

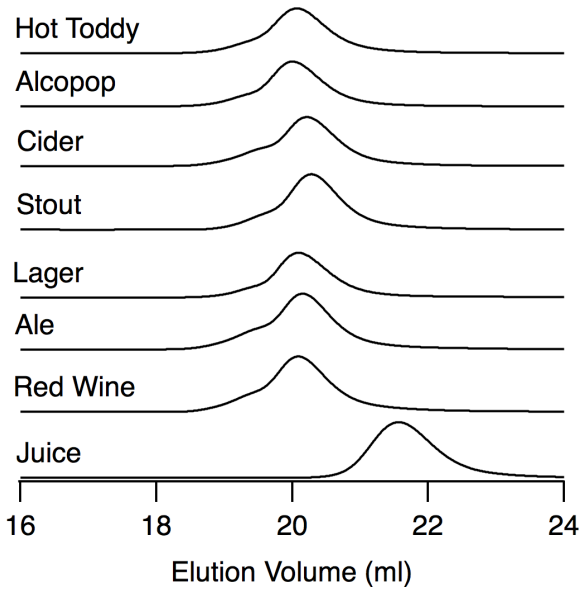


Figure S12 SEC overlay of polymers synthesized in low/no alcohol content solvents, aqueous mobile phase.

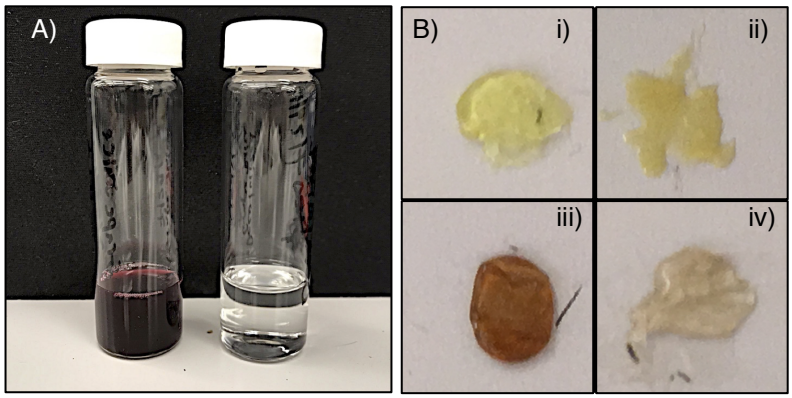


Figure S13. Physical appearance of red wine before (left) and after (right) decolorizing with carbon black B) Appearance of poly(HEA) obtained from EnzA-RAFT polymerization of HEA in respective solvents: i) Ethanol ii) HoSt, iii) red wine, iv) decolorized red wine.

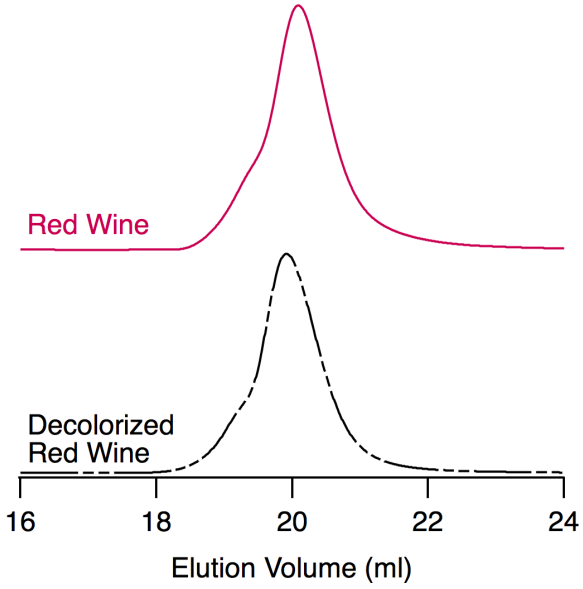


Figure S14 SEC overlay of polymers synthesized in red wine and wine decolorized by carbon black, aqueous mobile phase.

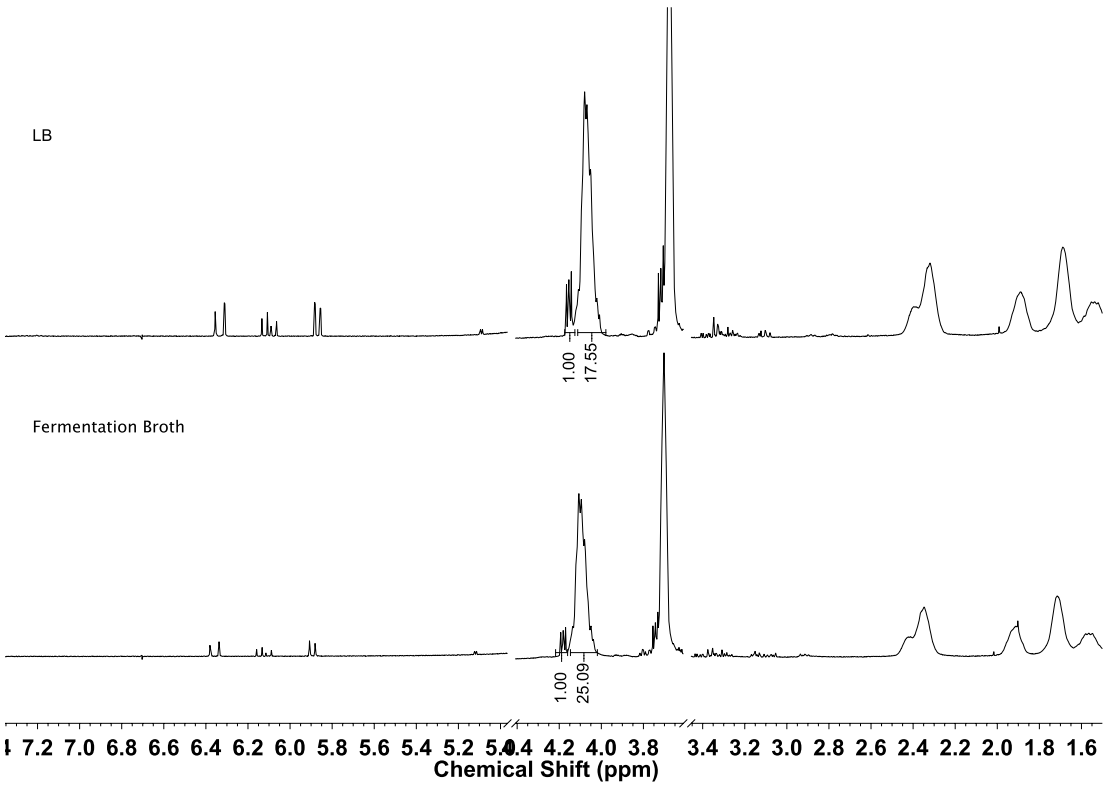


Figure S15- ^{1}H NMR (D_2O) overlay of crude poly(HEA) samples from polymerizations in LB and fermentation broth, aqueous mobile phase.

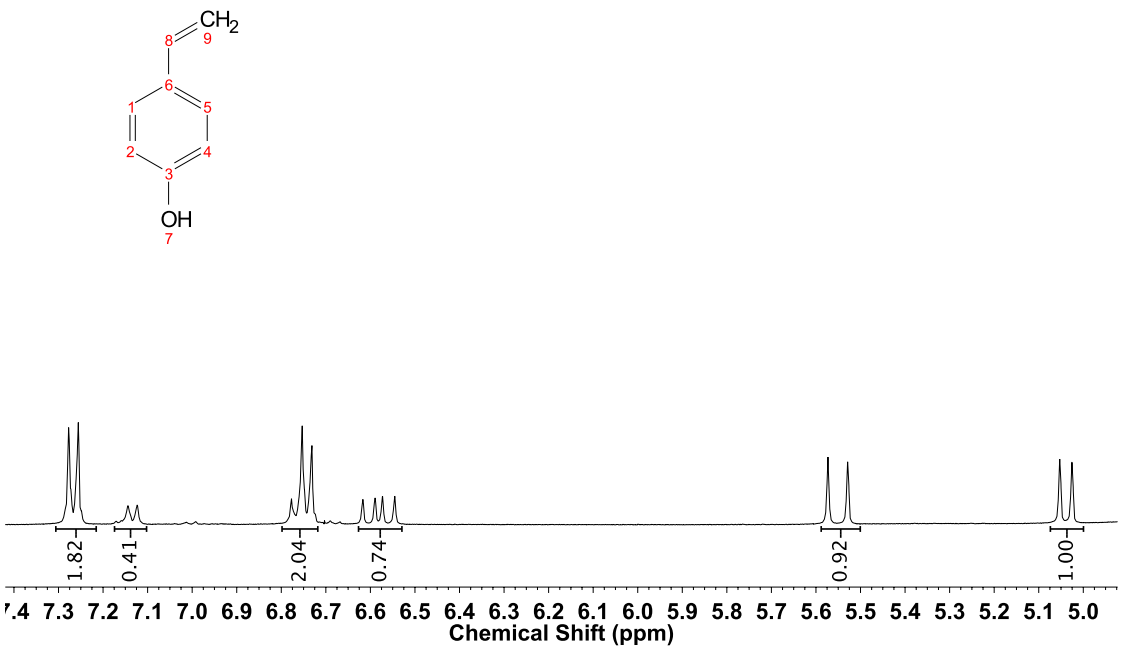


Figure S16. ^{1}H NMR (D_2O) spectrum of commercial 4-Vinylphenol monomer, 10% in propylene glycol.

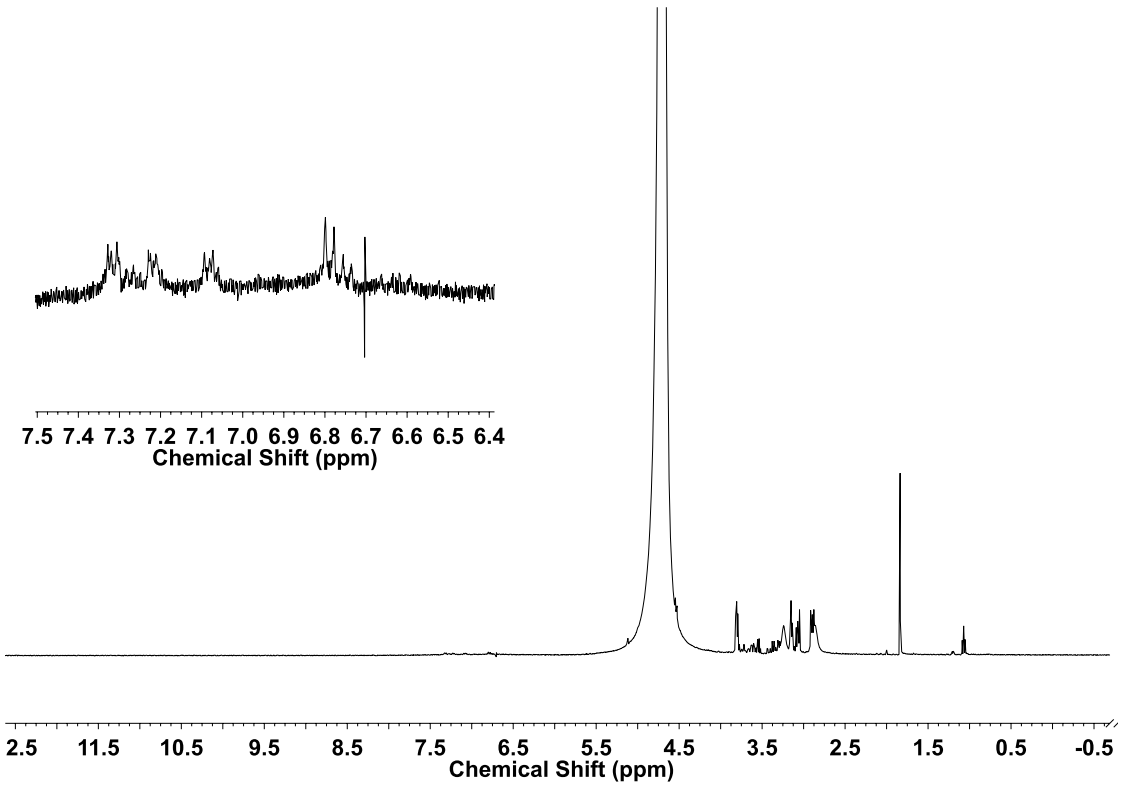


Figure S17- ^{1}H NMR (D_2O) spectrum of Fermentation broth, inset shows peaks region where vinyl phenol aromatic peaks would be expected to occur.

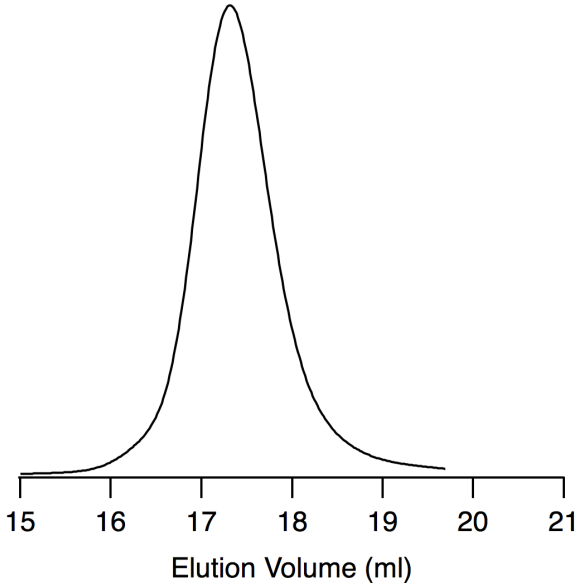


Figure S18 SEC RI trace of Enz-RAFT poly(NIPAm) synthesized in HoSt (M_n = 14 kg mol^{-1}, Đ <1.1), DMF mobile phase.

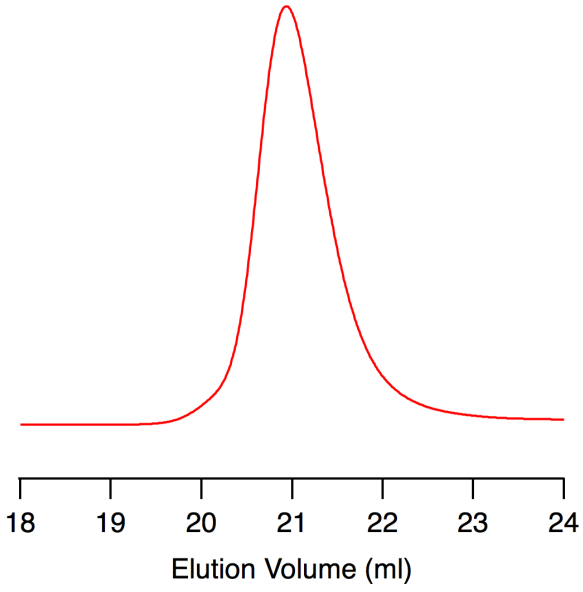


Figure S19. SEC RI trace of Enz-RAFT poly(DMA) synthesized in HoSt (M_n = 13 kg mol^{-1}, Đ = 1.1), aqueous mobile phase.

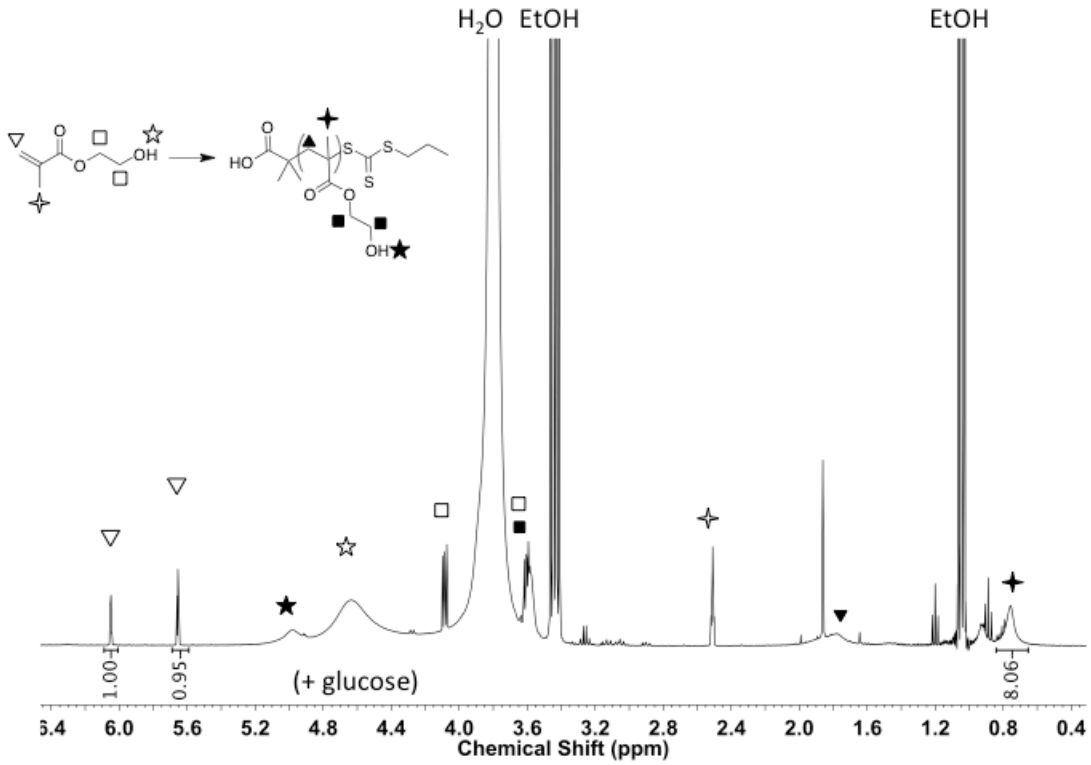


Figure S20: Crude ^{1}H NMR (D_2O) of Poly(HEMA) Synthesized in HoSt. Extensive overlap between glucose, Ethanol, residual monomer, and polymer signals makes calculation of conversion inaccurate, however based on the integrals shown we estimate a conversion of ~70%.

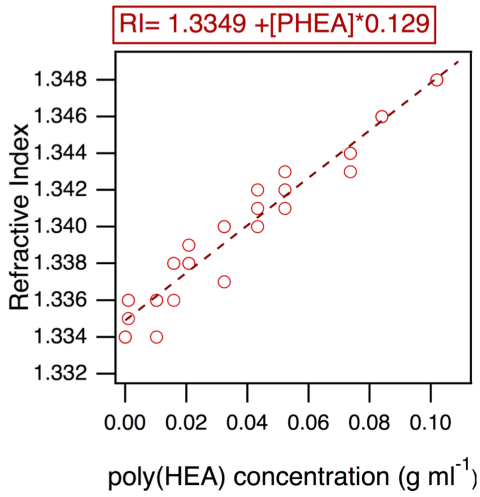


Figure S21. Refractometry experiment for poly(HEA), showing the collected triplicate measurements of refractive index. Measurements were collected using polymers dissolved in 0.1 M NaH_2PO_4 with 1.0 wt % acetic acid at 22 °C.

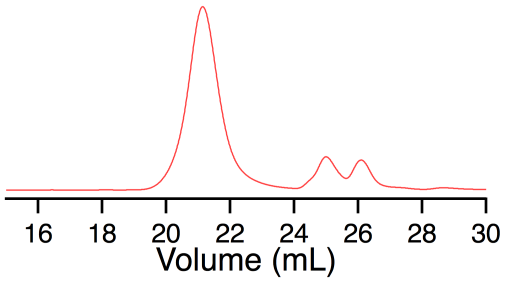


Figure S22: SEC RI trace of Enz-RAFT poly(HEA) synthesized in HoSt, 180 minute time point from manuscript Figure 2. We believe the two smaller peaks are residual ethanol and the GPC injection peak.

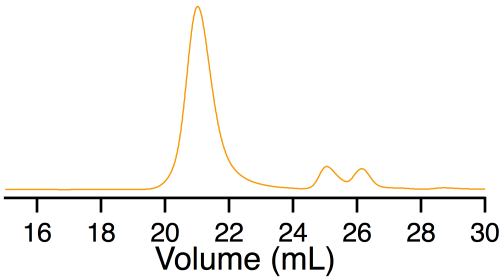


Figure S23: SEC RI trace of Enz-RAFT poly(HEA) synthesized in HoSt, 240 minute time point from manuscript Figure 2. We believe the two smaller peaks are residual ethanol and the GPC injection peak.

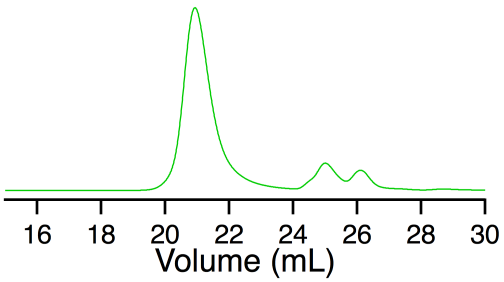


Figure S24: SEC RI trace of Enz-RAFT poly(HEA) synthesized in HoSt, 300 minute time point from manuscript Figure 2. We believe the two smaller peaks are residual ethanol and the GPC injection peak.

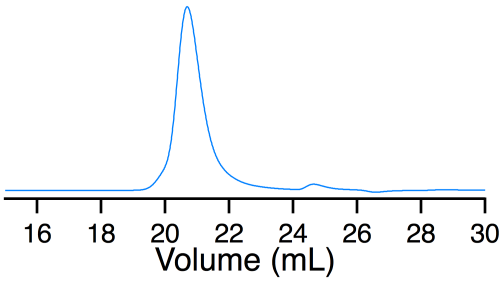


Figure S25: SEC RI trace of Enz-RAFT poly(HEA) synthesized in HoSt, 25 h time point from manuscript Figure 2. The smaller peak is characteristic of the GPC injection peak.

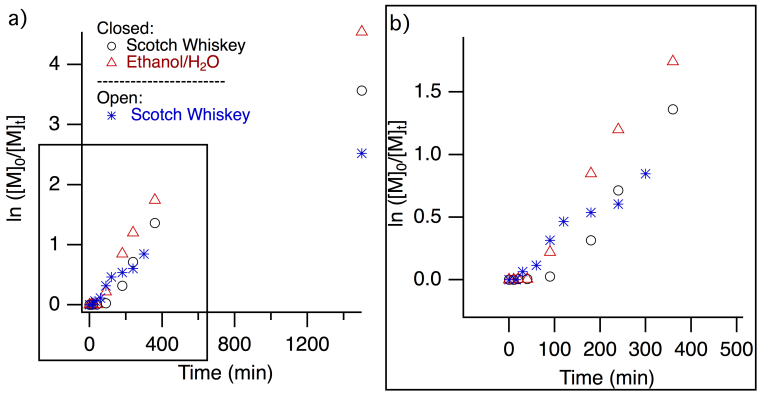


Figure S26) Pseudo First order plot of kinetics shown in manuscript figure 2A. a) Full range of data. b) Magnified plot showing low conversion to clarify induction times for the three different conditions.

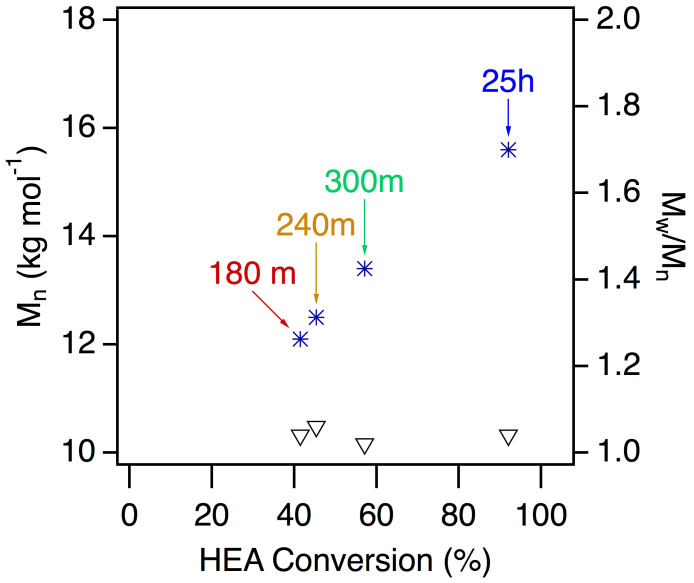


Figure S27) Relationship between monomer conversion (determined by ^{1}H NMR) and molar mass of purified polymer (determined by SEC, aqueous mobile phase) for the open to air polymerization of HEA in HoSt whiskey.

IV Author Roles and Responsibilities

All authors have read and approved the completed manuscript. D.K.S. and J.M.T. designed of experiments, conducted general synthesis and characterization experiments, drafted the preliminary manuscript, and directed the revision process. A.A.P. performed the size-exclusion chromatography experiments on all investigated samples. R.M., Jr. assisted with the purification and characterization of polymers and with experiments conducted in fermentation broth. S.J.R. provided contributions to data analyses and interpretation of the experiments. Commercial beverages were donated by the coauthors or other affiliates of the Rowan and Tirrell groups, as noted. All of the reactions that utilized alcoholic beverages as solvents were conducted by coauthors of legal drinking age.

V References

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