Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information (ESI)

Lysozyme-Catalyzed Formation of a Conjugated Polyacetylene

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S1. General Information

All bioanalytical grade chemicals utilized were purchased from either ThermoFisher Scientific (Waltham, MA, USA) or Sigma Aldrich (St. Louis, MO, USA). NMR spectra were recorded on an Agilent DD2 750 MHz spectrometer (Agilent Technologies, Santa Clara, CA, USA) in suitable deuterated solvents. UV-Visible spectra were obtained on a Hitachi U-3010 spectrometer (Chiyoda, Tokyo, Japan). LC/MS data were collected on a 5600+ TripleTOF Mass Spectrometer (AB SCIEX, Framingham, MA, USA) with a Micro200 LC (Eksigent, Redwood, CA, USA) equipped with a hydrophilic interaction liquid chromatography column (Luna 3µ NH2 100Å, 150mm×1.0mm, Phenomenex, Torrance, CA, USA).

S2. Synthesis and Purification of the Protein-Catalyzed Polymer

Reproducible production of poly-2-EP occurs in deionized water at room temperature with concentrations of HEWL at 2 mM and 250 mM of the monomer. No other reaction components are required. Enough polymer will form overnight to turn the solution from faint brown to dark red, although allowing the reaction to proceed for five or more days will yield additional product.

The reaction is terminated by lyophilization and HEWL/poly-2-EP is harvested as a red-orange powder. The lyophilized powder is resuspended in 100% ethanol to selectively solubilize the polymer. The mixture is allowed to incubate in a 37°C shaking incubator overnight to encourage poly-2-EP release from HEWL. Insoluble material is removed via centrifugation and the supernatant is retained. This ethanol extraction is repeated several times on the insoluble material. The pooled supernatant is filtered and then evaporated; leaving behind the polymer as a shiny, black resin. This resin is resuspended in a minimum volume of dimethylformamide and once again insoluble material is pelleted and separated. To the DMF soluble polymer, sufficient diethyl ether is added cause its precipitation. The purified polymer is filtered, washed with additional ether, and allowed to dry overnight in a 60°C vacuum chamber with 30psi reduced pressure. Typical yields of purified polymer were ~10% (~70 mg from 720 mg of 2-ethynylpyridine).

It was observed that adding NaCl up to 1M could increase the rate of reactions based on UV-Vis, however purification was complicated by formation of a hydrogel-like material during ethanol extraction.

S3. Nuclear Magnetic Resonance Spectroscopy

NMR samples were suspended in 10%-D_2O/90%-H_2O solvent and all experiments were carried out at 37°C. 1D ^1H spectra were collected with WATERGATE 3919 solvent suppression and spinning at 20 Hz. Experiments were collected with 128 scans, a 90° pulse width of 7.5 µs, and an acquisition time of 340 µs. Total experiment time was 8 minutes. The kinetics experiment was observed using ^1H NMR spectrometer with 2 mM of HEWL and 250 mM of 2-EP monomer. Between measurements, the sample was kept in the NMR tube inside a 37°C incubator. 2D spectra were collected as ^1H-^{13}C coupled heteronuclear single quantum coherence (HSQC) experiments. Total experimental time was 15 hours with 32 scans, 512 increments, a 90° pulse width of 7.5 µs, and an acquisition time of 130 µs. Spectra were processed using OpenVnmrJ with sine-bell multiplication.

HEWL's folded state over the course of the polymerization reaction was also inferred using the kinetics experiment described above. Only minor shifts in 1D proton peaks for the protein are observed over the course of the two-week kinetic study. If there was significant loss of HEWL's structural integrity, most of the protein peaks would greatly shift and coalesce around 8 ppm. HEWL is an very stable protein containing four internal disulfide bridges and retention of well dispersed peaks in proton NMR over the course of the experiment indicates that it remains well folded during polymerization of 2-EP.

S4. Liquid Chromatography-Mass Spectrometry

Samples were suspended in 35%-acetonitrile/65%-H_2O (HPLC grade) at a concentration of 10 µM. An initial direct injection analysis was performed to check machine parameters. For the direct injection, 100 µL of each sample was injected at a flow rate of 10 µL/min. All analysis was performed in positive mode. The ionspray voltage was set to +5500 V, the heater gas was set to 16 psi, the ion source nebulizer gas was set at 16 psi, and the curtain gas was set to 20 psi. A time of flight (TOF) scan was performed over the mass range of 100-2,000 Da. Samples were then analyzed using the same parameters after LC separation. The mobile phases for separation consisted of a water (A) and acetonitrile (B), both supplemented with 0.1% formic acid. The samples were eluted using a 30 min gradient: 0 min 90% B, 5 min 90% B, 25 min 10% B, 29 min 10% B, 30 min 90% B pm a Phenomenex C18 5 um 150 x 2.1 mm column. Spectra were processed using Analyst 1.7®.

S5. Size Exclusion Chromatography

All samples were run on a HiLoad 16/60 Superdex 75 pre-packed column from General Electric Healthcare Life Sciences (Pittsburgh, PA, USA) with a bed volume of 120 mL. The stationary phase is highly cross-linked agarose covalently bonded to dextran. The mobile phased contained 40 mM sodium phosphate pH buffered to ~5.0. For samples containing protein 150 mM NaCl was required for efficient elution. Samples were run with a flow rate of 1.0 mL/min.

S6. Lysozyme Activity Assay

Lysozyme activity was assayed in complex with known inhibitors and poly-2-EP using the EnzChek Lysozyme Assay Kit from ThermoFisher Scientific. This kit uses cell walls from *Micrococcus lysodeikticus* which are labeled with fluorescein. Cell wall digestion by lysozyme relives quenching on the fluorescein and the resulting increase in fluorescence is proportional to lysozyme activity. Experiments were carried out using a 96-well plate with a total reaction volume of 100 μL. Each data point was run in triplicate with 250 U/mL of lysozyme per well. Inhibitor and poly-2-EP concentrations ranged from 0-200 μM. The samples were allowed to incubate at 37 °C for 1 hour before fluorescence was measured using excitation/emission wavelengths of 494 and 518 nm respectively. Error bars were calculated using standard deviation.

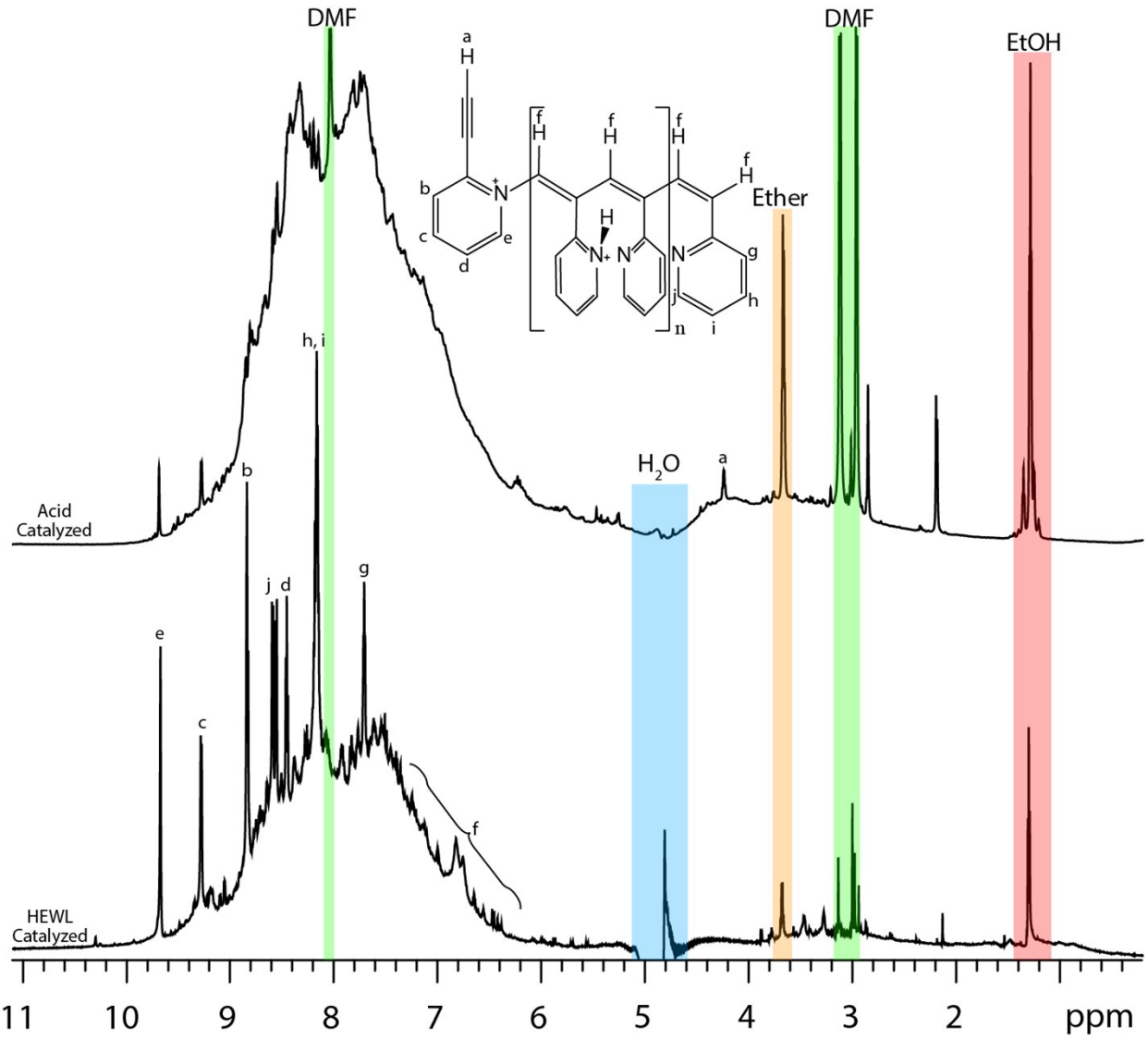

Fig. S1 ^{1}H NMR spectra with assignments for the acid- and HEWL-catalyzed poly-2-EP products. Peaks for the initiation-terminal pyridine ring can be observed at 9.75, 9.25, 8.90, and 8.49 ppm. The initiation terminus still has an intact alkyne group at 4.25 ppm, however, if left in aqueous solutions a hydration occurs and the alkyne is converted to an acetyl with a peak appearing at 2.18 ppm. Peaks for the polymeric pyridines are broadened from 9.50 to 6.50 ppm. The vinyl backbone peaks are also broadened from 7.60 to 6.30 ppm.

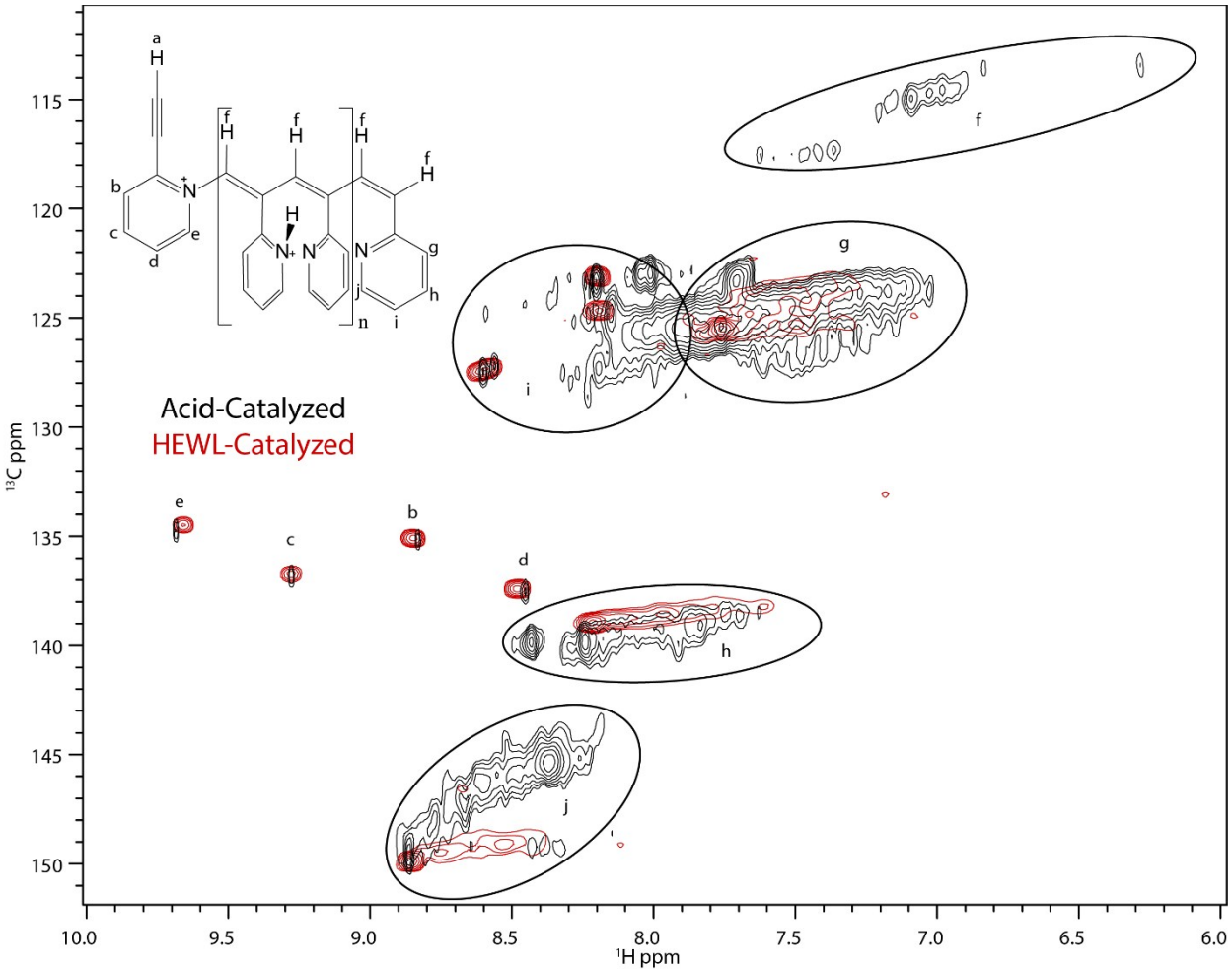

Fig. S2 ^{1}H-^{13}C correlated NMR spectra with assignments for the of acid- and HEWL-catalyzed poly-2-EP products. Four peaks for the initiation-terminal pyridine ring are still observable at 9.75, 9.25, 8.90, and 8.49 ppm in the ^{1}H spectrum and from 134 to 138 ppm in the ^{13}C spectrum. Four regions can be identified for each of the polymeric pyridine protons (black circles). These have major peaks at 8.75, 8.45, 8.10, and 7.70 ppm while spanning from 123 to 150 ppm in carbon. Finally, some peaks for the vinyl group can be observed from 7.60 to 6.30 ppm in the ^{1}H spectrum and from 113 to 118 ppm in the ^{13}C spectrum.

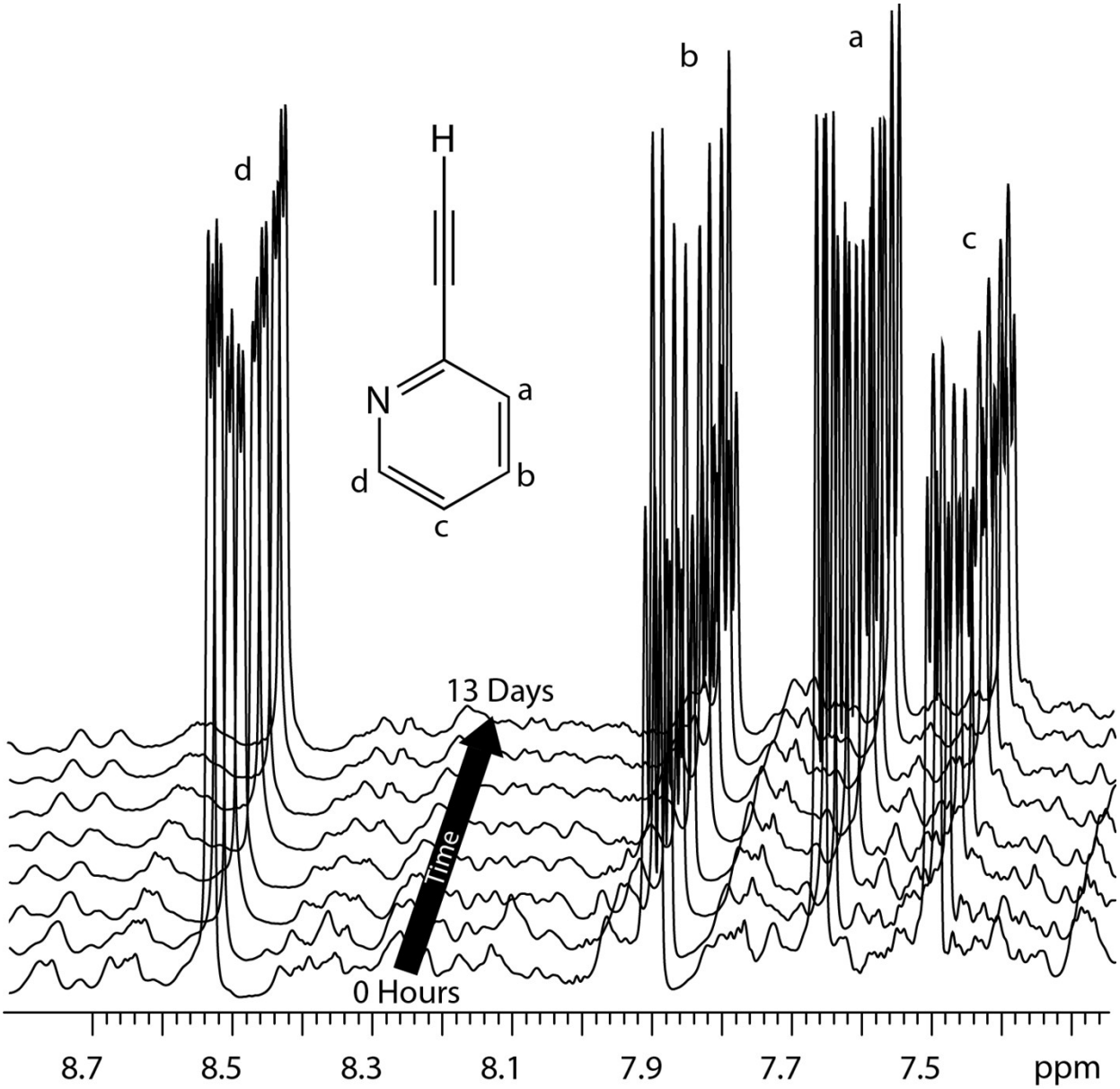

Fig. S3 Attenuation and recovery of monomer peaks in ^{1}H spectra over time during the NMR-monitored polymerization reaction. Spectra are offset with increasing experiment duration. These peaks are upfield of the same set of spectra described in Figure 2. Signal loss and recovery is a result of the monomer initially interacting with HEWL and subsequently competed away by the forming polymer.

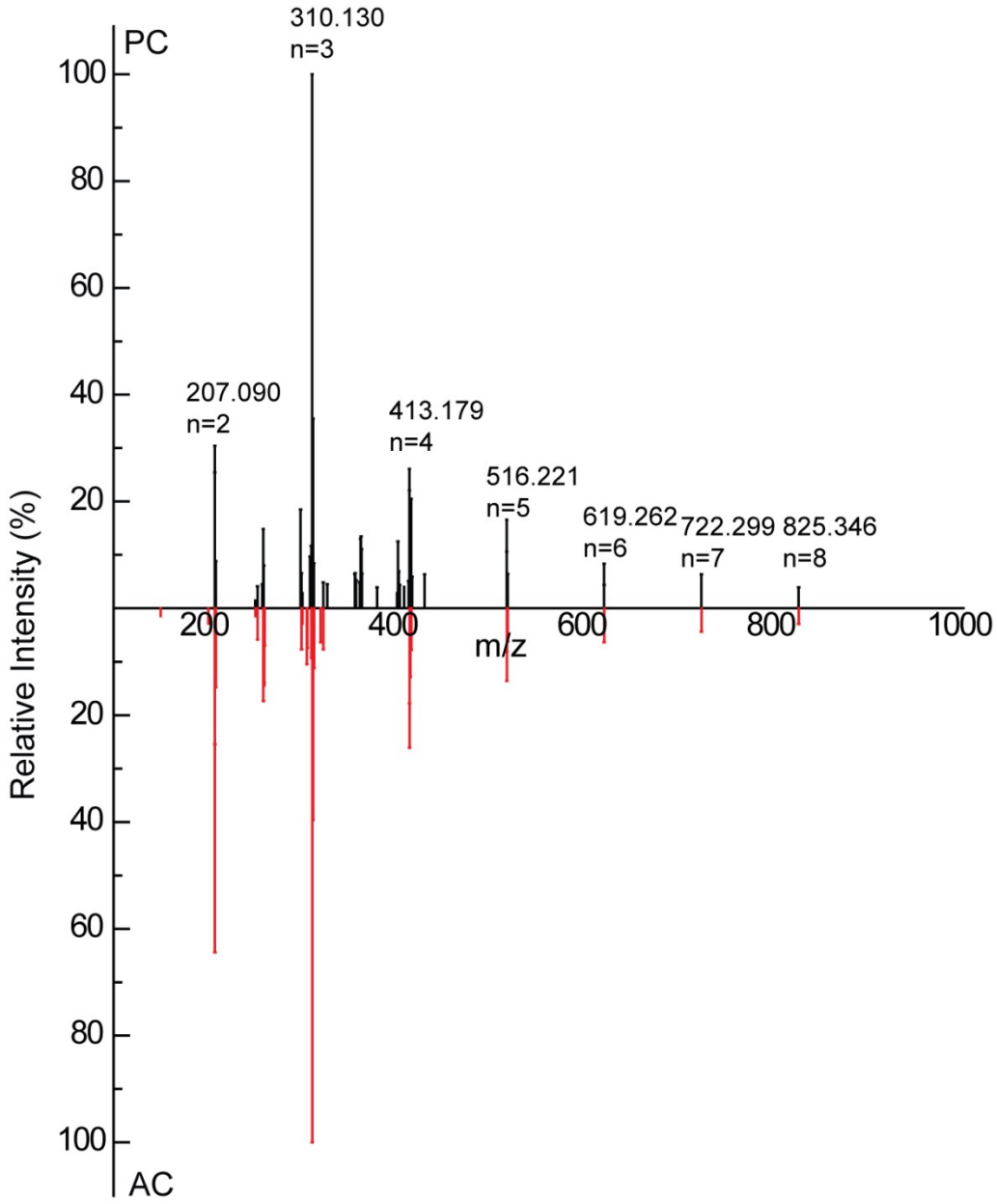

Fig. S4 Mass spectra of the protein-catalyzed (PC, top black) compared to the acid-catalyzed (AC, bottom red) poly-2-EP, after purification. Chain lengths with corresponding m/z are shown above each peak. All ions were collected in positive mode.

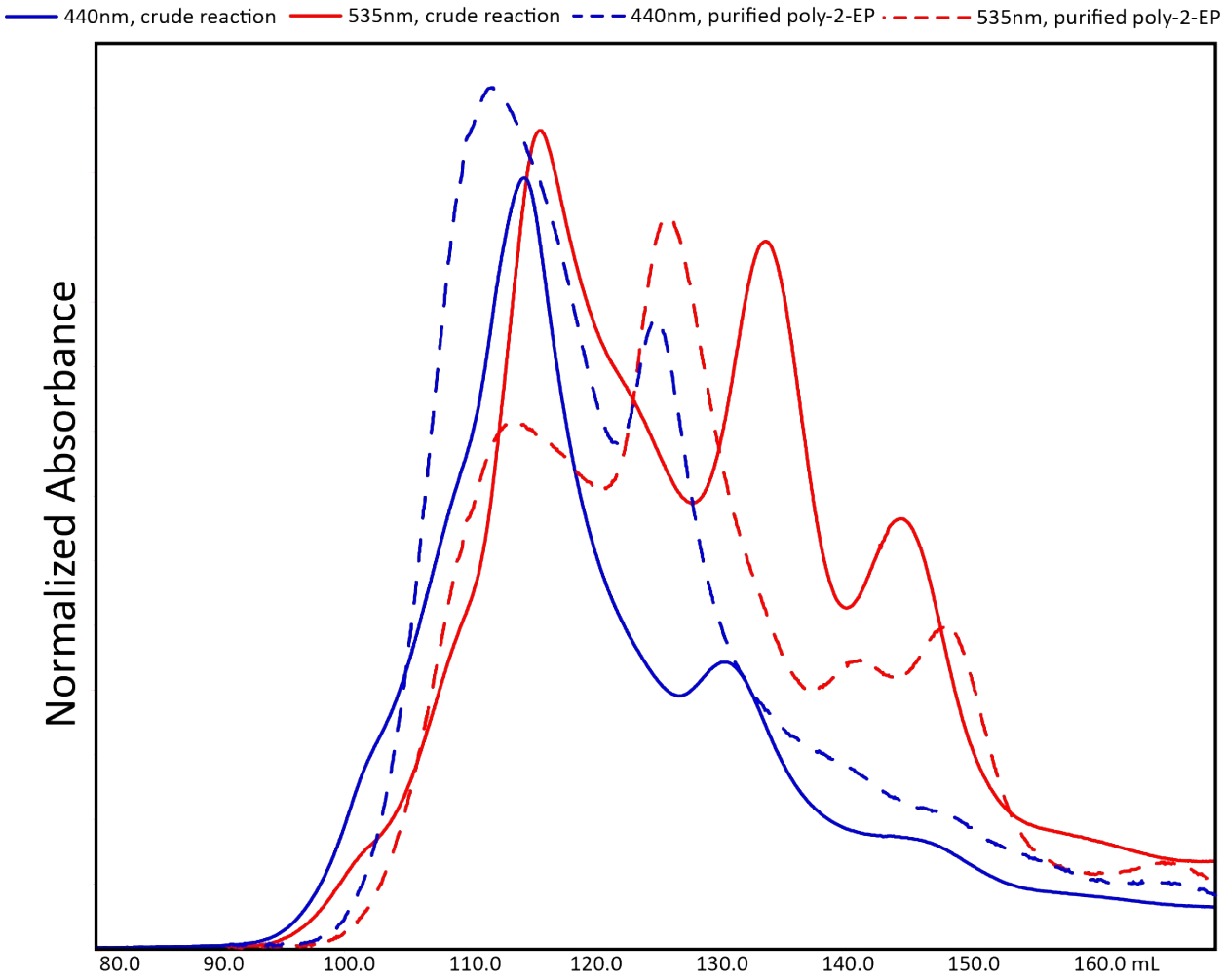

Fig. S5 Size exclusion chromatography of poly-2-EP with samples from a crude reaction mixture still containing HEWL and of the purified polymer. Samples were run with 40 mM sodium phosphate buffer as the mobile phase in both samples while the protein containing sample required 150 mM NaCl in the mobile phase for an efficient elution profile. These peaks were compared to a known protein standard, aprotinin (MW=6,500) which elutes at 90 mL. A molecular weight of 1,700 is estimated for the earliest peak shoulder that elutes at 100 mL, consistent with a polymer length of 16 units. Variances in the elution profile for peaks following can be attributed to NaCl causing disturbances in microinteractions the charged polymer has with the stationary phase and interactions between the polymer and HEWL in crude samples.

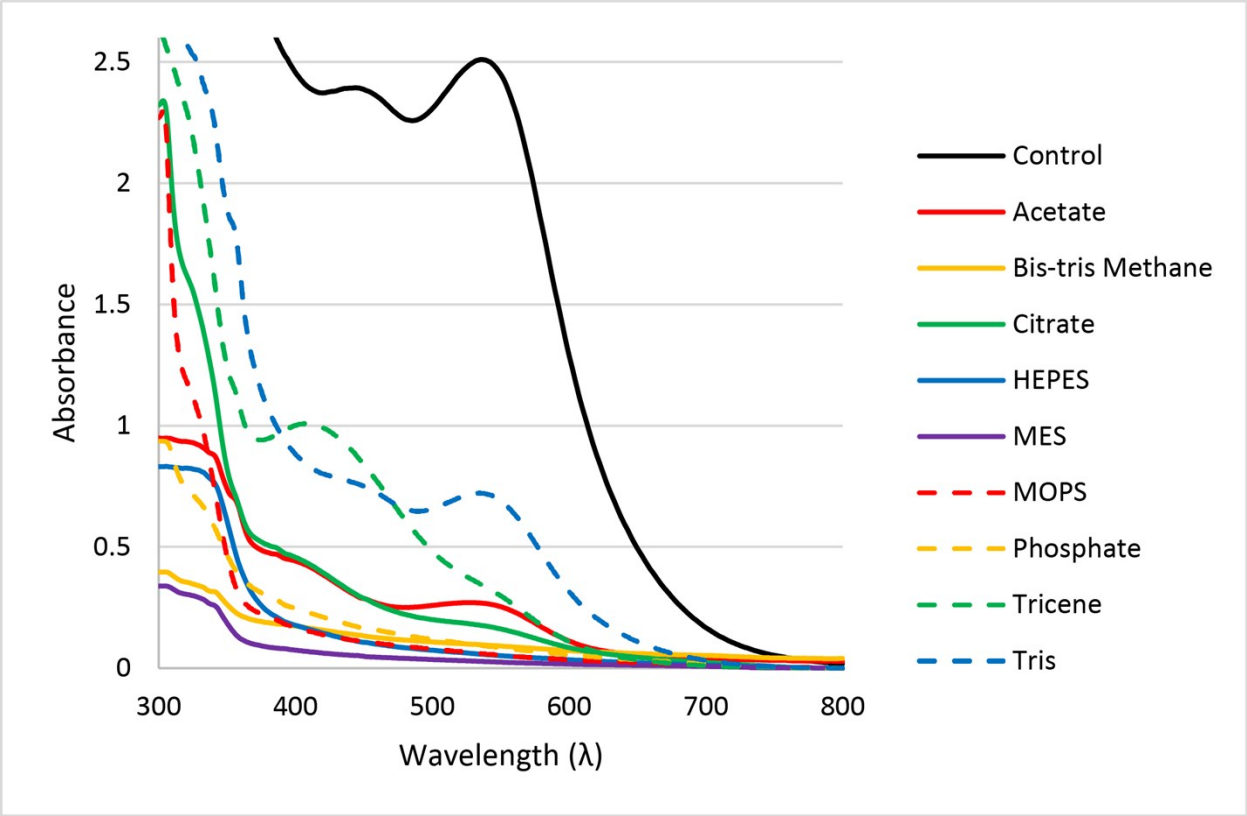

Fig. S6 UV-Visible spectra of HEWL-catalyzed polymerizations carried out in 100 mM of biologically relevant buffers after 48-hours. All solutions were buffered to pH = 7.0 using HCl or NaOH. All buffers investigated inhibit polymerization. Only acetate, citrate, tricene, and tris allow polymerization to proceed to a limited extent.

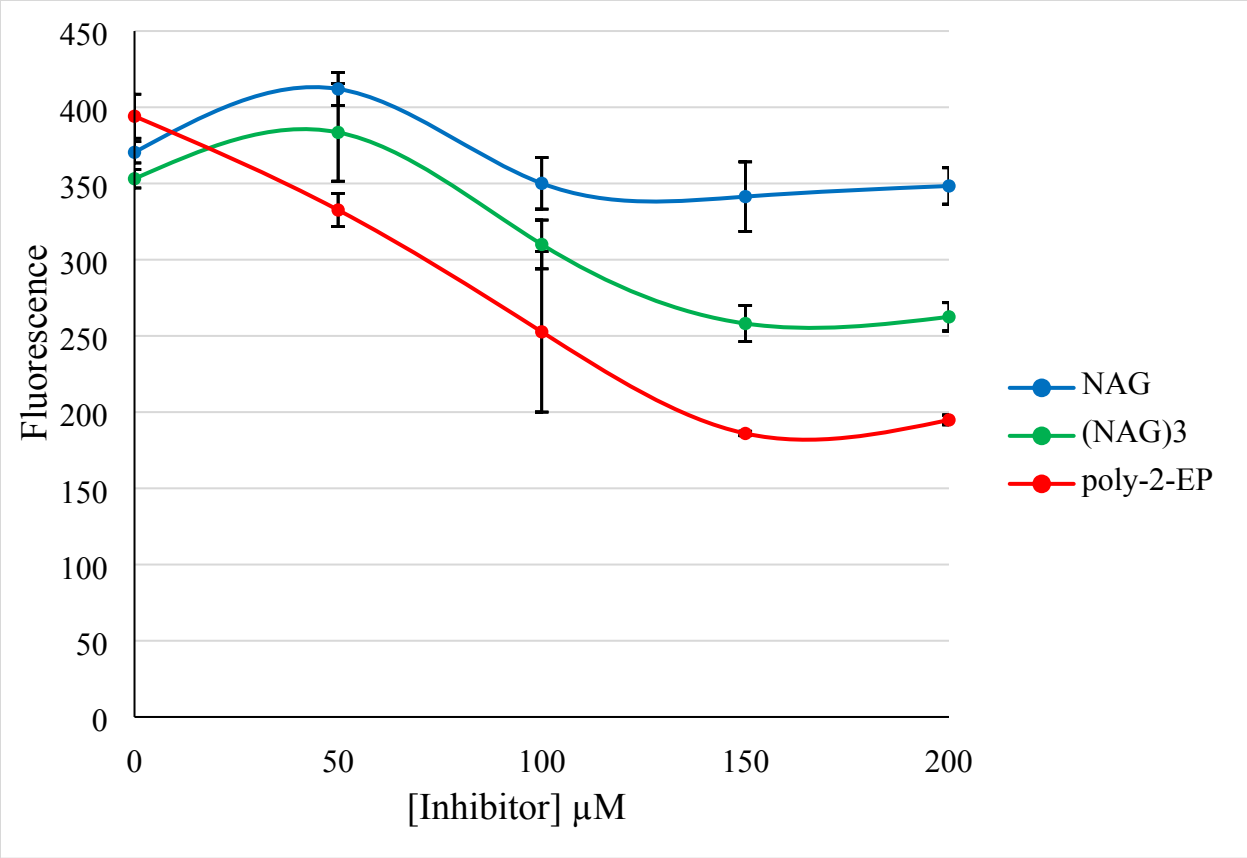

Fig. S7 Lysozyme activity assay in the presence of known inhibitors and purified poly-2-EP. This qualitatively exhibits poly-2-EP inhibiting HEWL activity similarly to $(NAG)_3$.