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Electronic Supplementary Information (ESI)

Inhibition of Lysozyme's Polymerization Activity using a Polymer Structural Mimic

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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).

S2. Reaction Conditions for the HEWL Polymerization Reaction of 2-EP

Reproducible production of poly-2-EP occurs in deionized water at room temperature with concentrations of HEWL at 0.25 mM and 50 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. For bulk production of 2-EP this reaction was previously described with 2 mM of HEWL and 250 mM of the monomer versus the above concentrations.

S3. Lysozyme Activity Assay

Lysozyme activity was assayed in complex with known inhibitors, poly-2-EP, and DPP 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.

S4. 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. STD samples contained 400 μM DPP and 40 μM of HEWL while DOSY samples contained 400 μM of both reagents. All experiments were carried out at 37°C.

In the STD experiment, a 50 ms Gaussian-shaped saturation pulse with a power of 8 dB was applied to selectively saturate the methyl protons of HEWL. The mixing time was arrayed from 0 to 4 s to produce saturation build-up curves. The off-resonance experiment applied this pulse at about 18 ppm while the on-resonance experiment applied this at about 1 ppm. Varian's preinstalled BioPack dpfgse_satxfer pulse sequence was used and the on-resonance experiment was automatically subtracted from the off-resonance experiment as part of the default experiment processing. Experiments were collected with 64 scans and an acquisition time of 0.682 s with a delay of 1.5 s and 10,000 points. Spectra were processed and analyzed using VnmrJ.

DOSY experiments were recorded for each sample, DPP only and DPP/HEWL mixture, using Varian's preinstalled Dbppste sequence. A gradient pulse length of 2.0 ms and a diffusion time of 100 ms were used. Gradient strength in the analyzed data varied, over 13 DOSY slices, from 0.09 gauss/cm to 1.85 gauss/cm. VnmrJ was used for processing using default parameters from the Dbppste experiment.

S5. X-Ray Crystallography

X-Ray data were collected at 1.42 Å using a Bruker Kappa APEX II Duo equipped with an ImuS micro-focus source with QUAZAR optics (Cu Kα radiation, λ = 1.35 Å). Crystals were flash-frozen in liquid nitrogen after collection from the growth plate using a cryoloop. They were then mounted and placed under a stream of nitrogen at 100 K (Oxford Cryosystems). The detector was placed at a distance of 7.00 cm from the crystal. X-ray intensity data were collected with an exposure time of 60 s per frame. Data integration and scaling were performed using the APEX II software suite.[1] An initial solution was obtained by molecular replacement using MolRep and PDB 6LYZ as a model.[2,3] Additional model building and refinement were accomplished using Molecular Operating Environment, Coot, and Refmac5.[4–7]

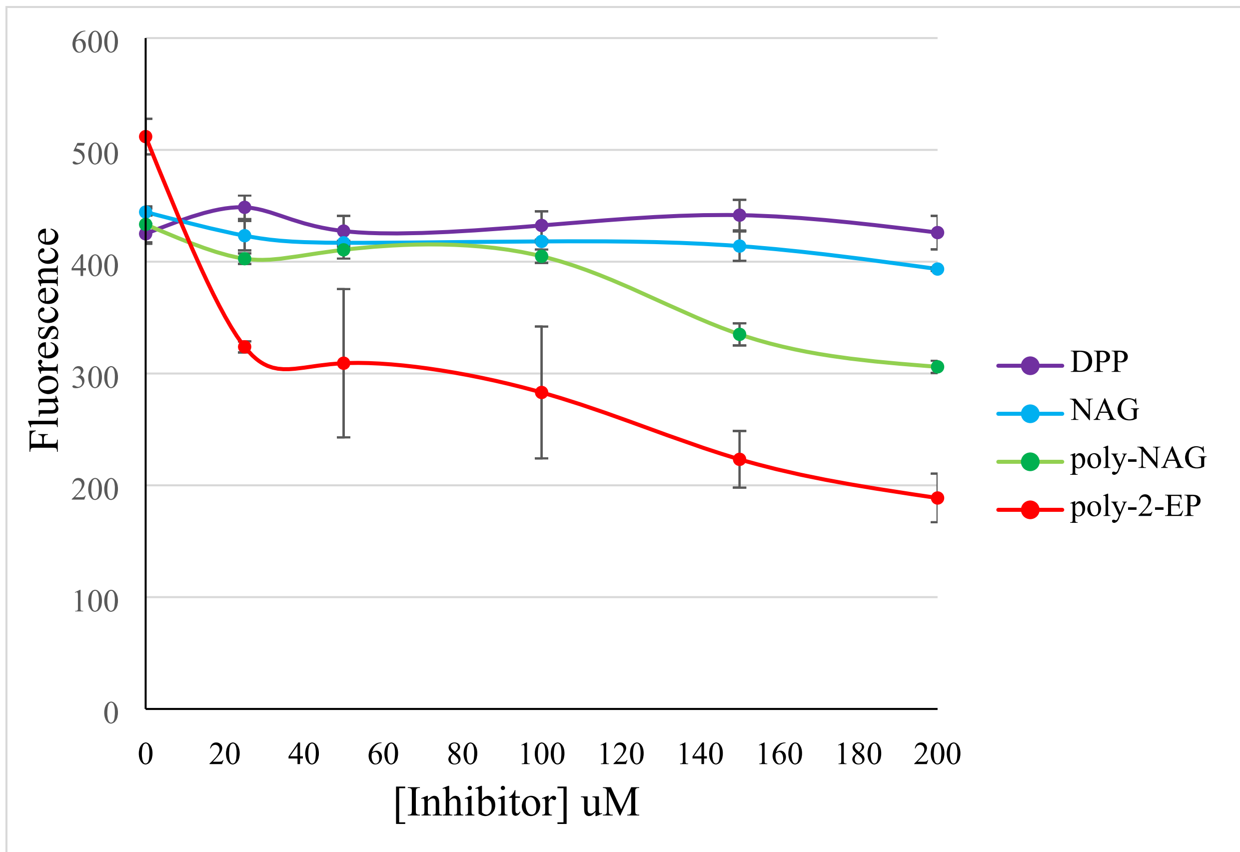

Fig. S1 Lysozyme activity assay in the presence of known inhibitors, poly-2-EP, and DPP, showing that DPP is not a strong inhibitor against HEWL's natural substrate.

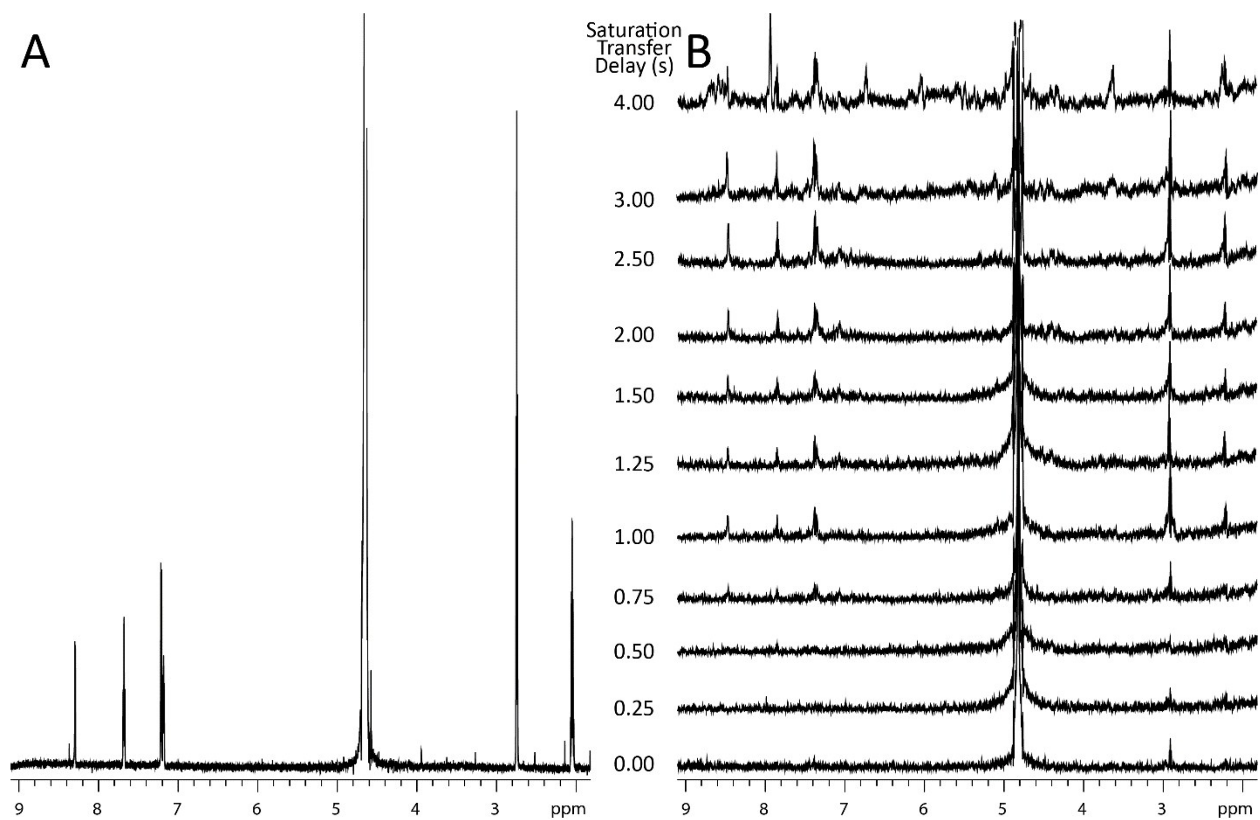

Fig. S2 A) 1D proton NMR for DPP. B) Saturation build-up experiment for a mixture containing 400 μM of DPP and 40 μM of HEWL

Table S1 Crystallographic data collection and structure parameters for PDB entry 6CIW

Property	Value
Space Group	$P4_32_12$
Cell Constants a,b,c α,β,γ	76.87Å 76.87Å 37.25Å 90.00° 90.00° 90.00°
Resolution	54.36 – 1.42 Å
% Data Completeness	98.8%
R_{merge}	0.582
$I/\sigma(I)$	1.15
Refinement Program	REFMAC
R, R_{free}	0.246, 0.0282
R_{free} test set	1064 reflections (5.29%)
Wilson B-factor (Å^2)	8.7
Anisotropy	0.014
Bulk solvent k_{sol} (e/Å^3), B_{sol} (Å^2)	0.35, 38.5
F_o,F_c correlation	0.93
Total number of atoms	1064
Average B, all atoms (Å^2)	13.0

References

1 Bruker, *APEX II*, 2013, Bruker AXS Inc., Madison, Wisconsin, USA.

2 A. Vagin and A. Teplyakov, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2010, **66**, 22–25.

3 R. Diamond, *J. Mol. Biol.*, 1974, **82**.

4 ChemicalComputingGroupInc., *Sci. Comput. Instrum.*, 2004, **22**, 32.

5 P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2010, **66**, 486–501.

6 M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2011, 67, 235–242.

7 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 1997, 53, 240–255.