Supplementary Material

Small molecule cores demonstrate non-competitive inhibition of lactate dehydrogenase

Brooke A. Andrews[a] and R. Brian Dyer[a]

[a]*Department of Chemistry, Emory University, Atlanta, Georgia, 30322*

E-mail: briandyer@emory.edu

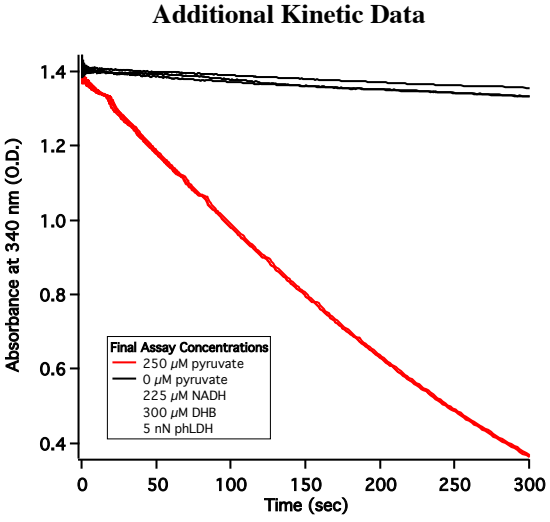

Figure S1. NADH cofactor absorbance change over time as a function of substrate concentration. Triplicate black traces demonstrate negligible change due to oxidation of cofactor in presence of inhibitor molecule DHB.

Dynamic Light Scattering

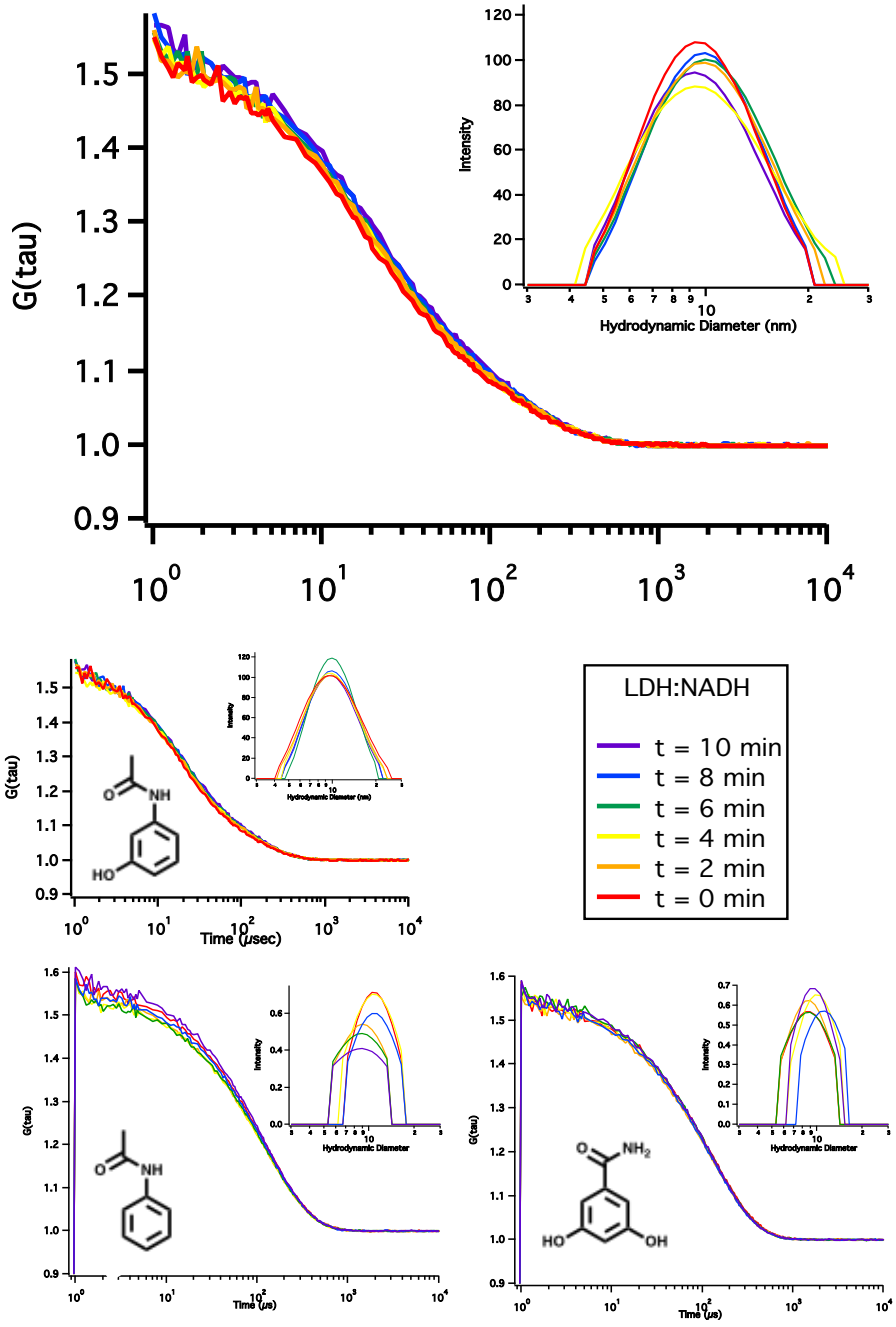

Figure S2. Dynamic light scattering autocorrelation function decay curves for LDH:NADH binary complex with and without inhibitor, at increasing incubation times. No change in the autocorrelation function decay and thus in the calculated hydrodynamic diameter is observed over ten minute incubation with or without 3-AP, DHB, or acetanilide. Corresponding inhibitor structures are overlaid on the plots.

Global Analysis

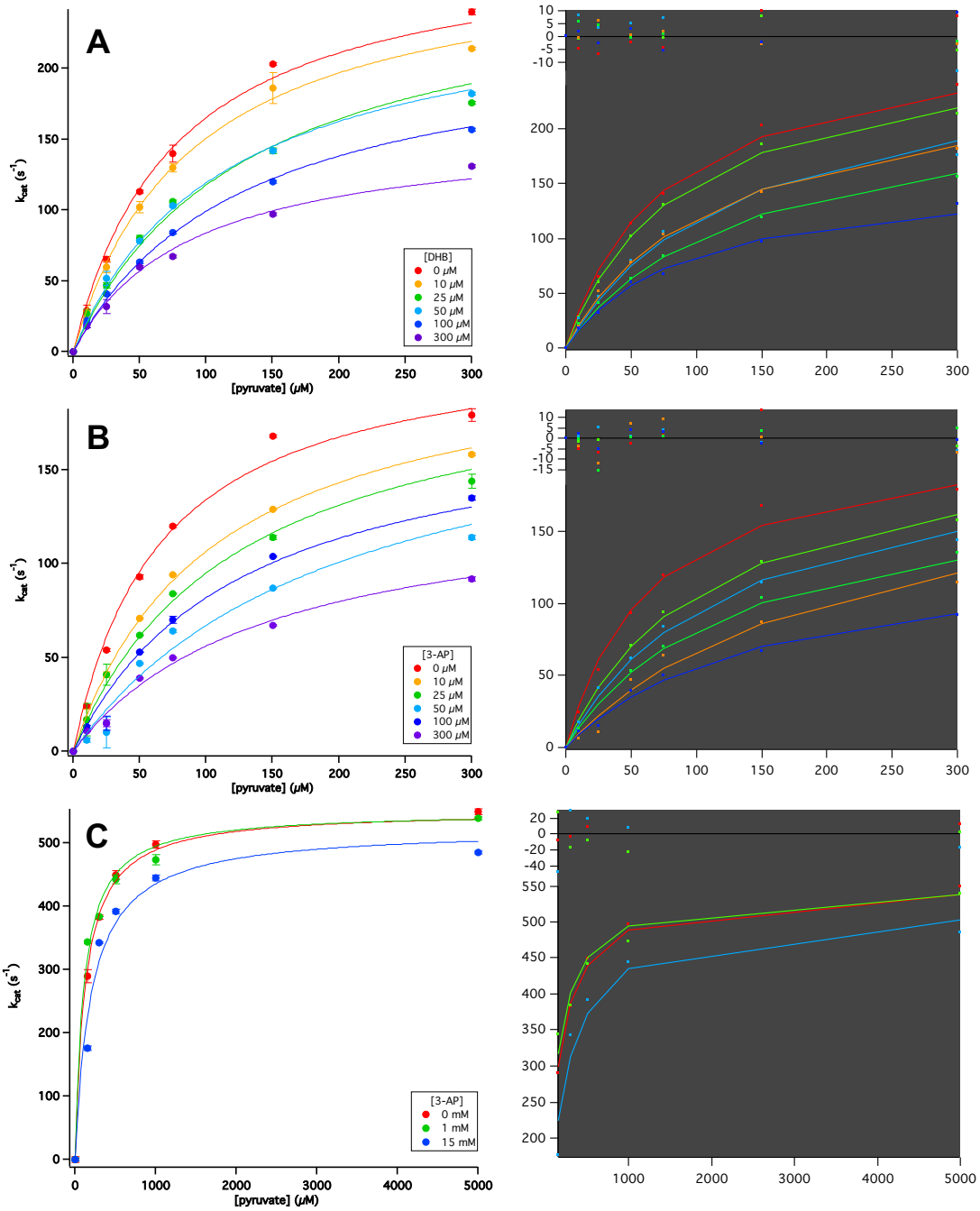

Figure S3. Global fits (left) and residuals (right) for velocity vs. substrate concentration at varying inhibitor concentrations. (A) DHB, [phLDH] = 5 nN, (B) 3-AP, [phLDH] = 5 nN, (C) 3-AP, [phLDH] = 3 μN.

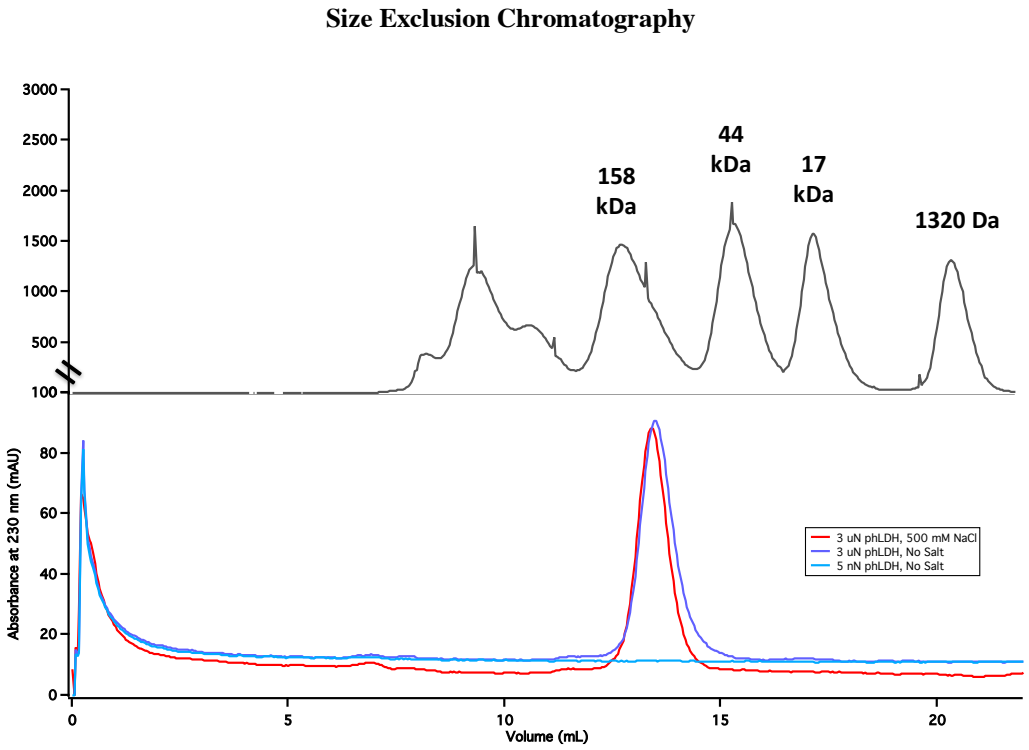

Figure S5. Size exclusion chromatography. Absorbance as a function of elution volume, MW standards shown at the top in black. 3 μN appears tetrameric, 5 nN is unable to be detected. In our hands, high salt concentration (500 mM NaCl) had no effect on the population distribution, contrary to the findings of Yamamoto (Ref. 11).

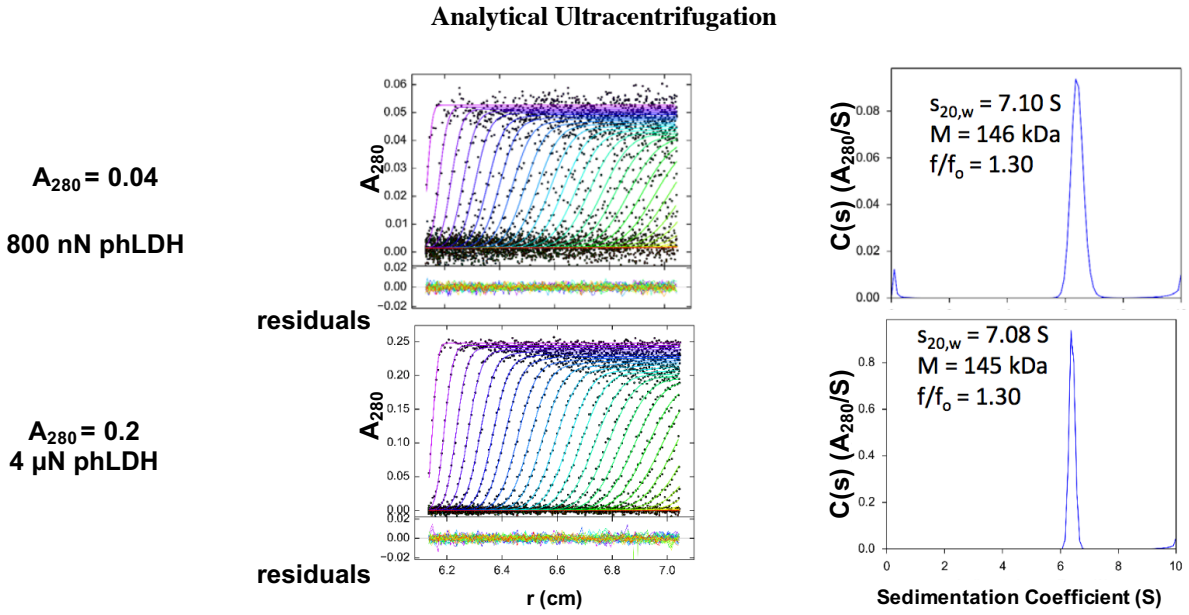

Figure S6. AUC of phLDH samples, Shown are every second scan, every third data point. Both samples are in 100 mM sodium phosphate buffer, pH 7.2 and referenced to the same. **Top**: A_{280} = 0.04 for 800 nN, **bottom**: A_{280} = 0.20 for 4 µN. Both samples appear tetrameric, A_{280} = 0.04 is the lower limit of detection for the method.

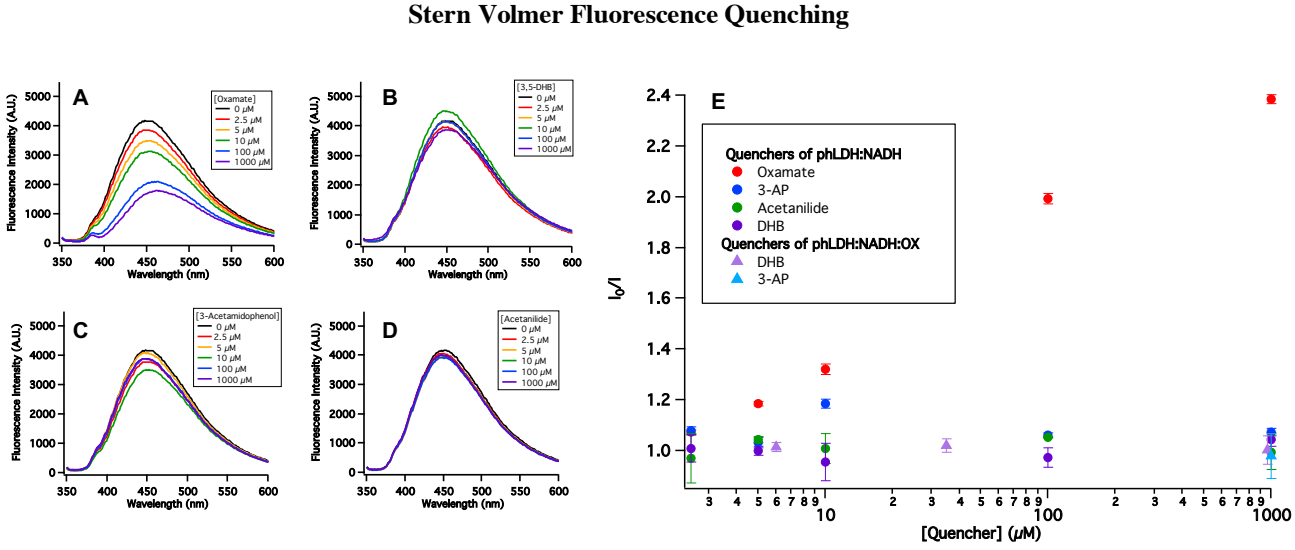

Figure S7. Flourescence spctra for titrations of (A) oxmate, (B) DHB, (C) 3-AP, and (D) acetanilide against phLDH:NADH. Stern-Volmer plot (E) demonstrates negligible quenching of the 3 μN phLDH: 6 μM NADH complex, even at 1 mM concentrations, and no change in quenched state for the ternary complex when inhibitors ae introduced. Note the x-axis is on a logarithmic scale and the presence of excess NADH precludes the determination of a SV constant for each molecule against a single species.