Kinetics of Heptosyltransferase I and Identification of Potential Inhibitors

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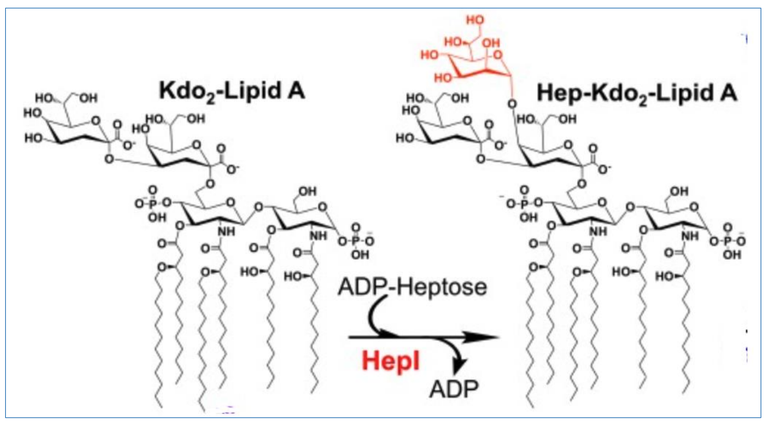
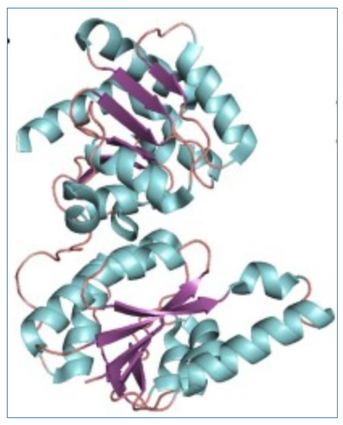
Introduction

One of the numerous resistance mechanisms that gram-negative bacteria have developed against antibiotics is the synthesis of a biofilm that protects their outer membrane from attack. *K. pneumoniae* and *V. cholerae* are few examples of such gram-negative bacteria which cause serious and life-threatening diseases. Lipopolysaccharides (LPS) are a major component of the biofilm. Heptosyltransferases are responsible for transferring heptose moieties onto growing glycolipid chains embedded in the outer membrane, which ultimately form the LPS structure.

Heptosyltransferase I catalyses the first step of the biosynthetic pathway, as outlined below, using Kdo_2-Lipid A and ADP-Heptose as substrate.



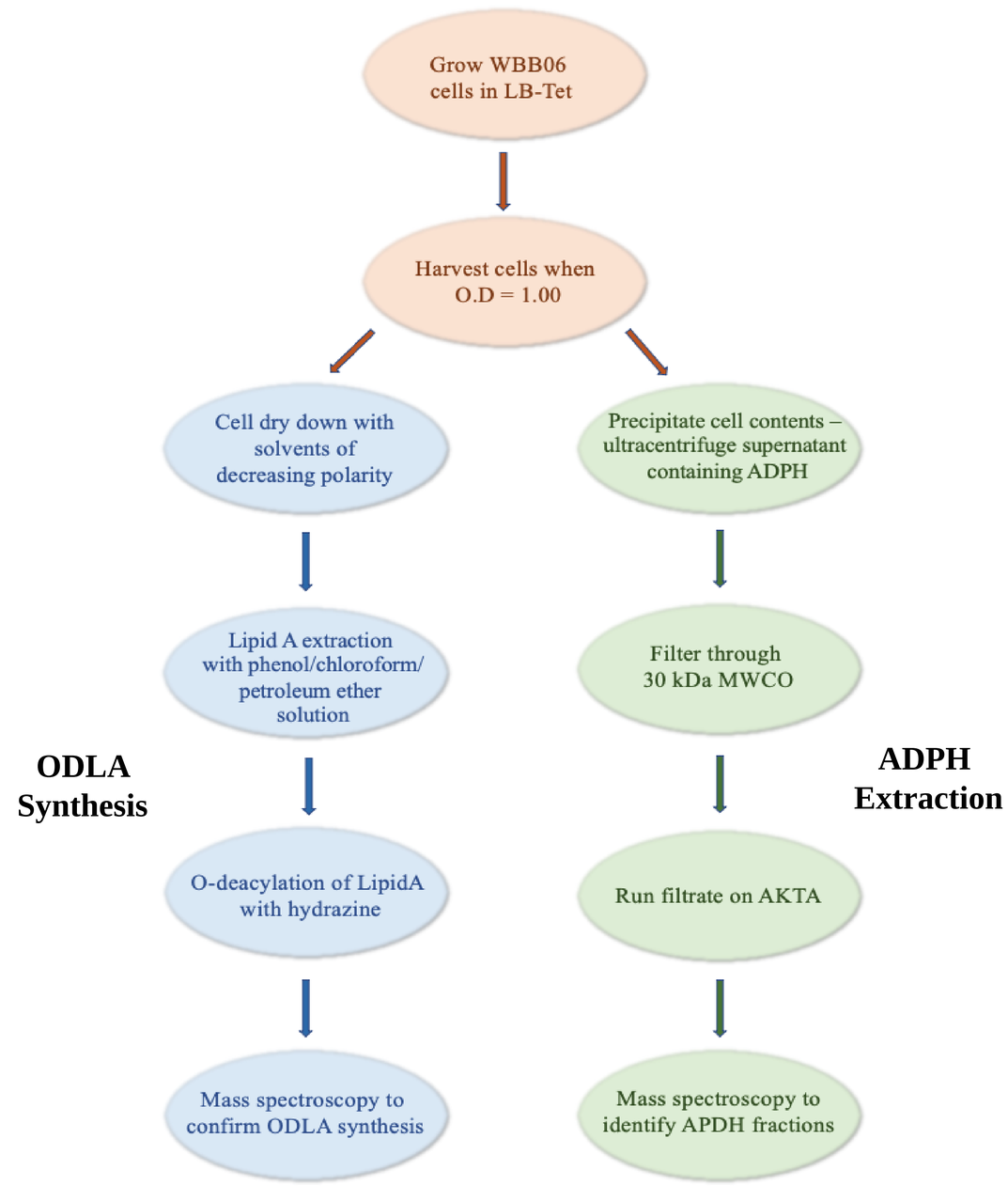


E. Coli Hep I

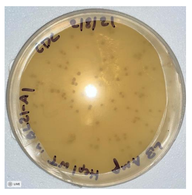
Previous research has shown that HepI knockout bacterial cell lines have truncated LPS in their membranes and are more susceptible to the effects of antibiotics. Thus, inhibiting HepI can impede LPS synthesis, disrupting biofilm formation and making antibiotic treatment much more efficient.

To identify potential HepI inhibitors, 419 compounds from an NCI library were computationally docked onto the enzyme by the Taylor Lab using AutoDock Vina. Select molecules predicted to have the best and worst binding energies were tested in kinetics assays. While multiple good inhibitors were identified, the experimental findings did not completely match the predictions. Our current goal is to analyse all of the docking data to find a possible correlation between it and the observed inhibition constants. The compounds predicted to strongly bind to HepI according to the found correlation will then also be tested in inhibition assays. The final objective is to identify adequately potent inhibitors of HepI that can be used in drug development.

Methods



HepI WT Expression and Purification



- ✓Transformation of Hep I pTOM in BL21-AI cells
- ✓Growth, induction with IPTG and 20% arabinose and propagation
- ✓Spinning down of cells and homogenizing
- ✓Purification on resin column chelated with $CoSO_4$
- ✓SDS-PAGE Gel to identify eluted protein fractions
- ✓Concentration and desalting of protein sample
- ✓Concentration and Precipitation

Kinetics

An ADP/NADH coupled kinetics assay was performed to find the k_{cat} and k_m of Hep I – parameters that have already been determined by the Taylor Lab – to validate kinetics technique.

$m1 = k_{cat}$

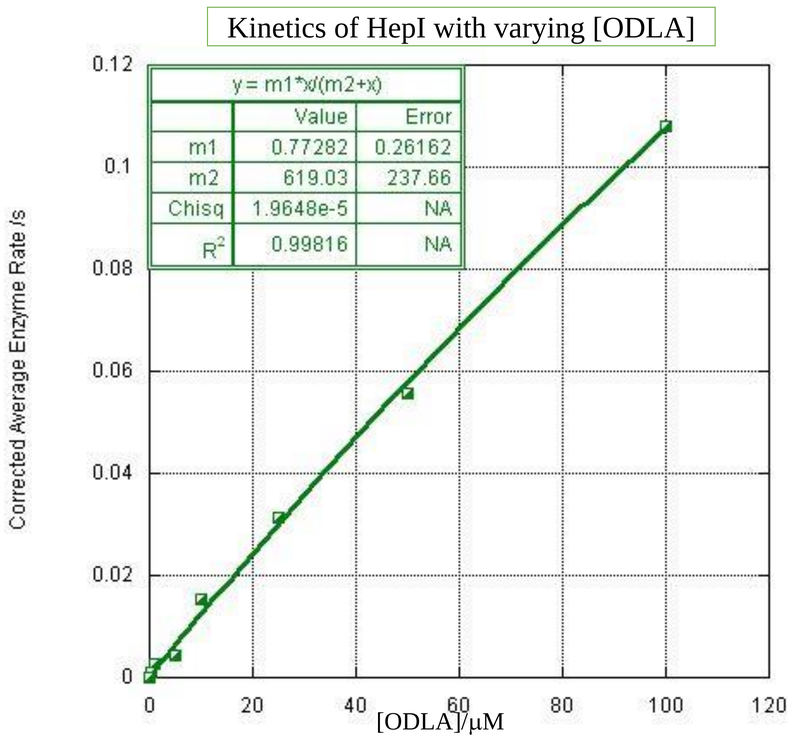
$m2 = k_m$

k_{cat} = 0.78 ± 0.26 s^{-1}

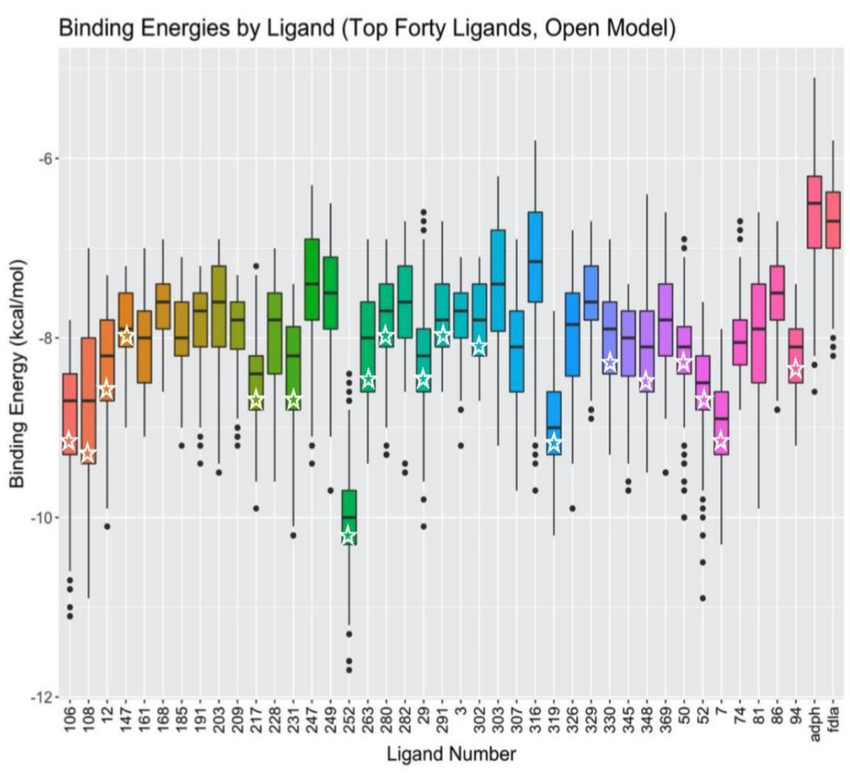
k_m = 619 ± 238 µM

The k_{cat} obtained is close to the known range of values. The k_m however is nowhere near the acceptable value (~ 3µM).

Kinetics of HepI with varying [ODLA]



Molecular Docking



➢ *The predicted binding energies for the top 40 ligands docked onto the open conformation of HepI.*

Inhibition assays were done on the top 19 compounds, and bottom 10 out of the 419 ligands. Some of the bottom 10 had low Ki while many of the top 19 had high Ki. Thus, binding energy alone is not a good indicator of potency and various other parameters have to be considered.

References

Cote, J.; Taylor, E. The Glycosyltransferases OF LPS Core: A Review of Four Heptosyltransferase Enzymes in Context. *International Journal of Molecular Sciences* **2017**, *18* (11), 2256.

Czyzyk, D. J.; Sawant, S. S.; Ramirez-Mondragon, C. A.; Hingorani, M. M.; Taylor, E. A. *Escherichia coli* Heptosyltransferase I: Investigation of Protein Dynamics of a GT-B Structural Enzyme. *Biochemistry* **2013**, *52* (31), 5158–5160.

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Future Directions

- ❑Build a statistical model to identify which parameter or combination of parameters from the docking data correctly predicts inhibition trends
- ❑Perform kinetics on HepI with only substrates to improve technique
- ❑Perform inhibition assays to test new hypotheses following statistical analysis