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EXPLORING LOL PROTEINS IN GRAM-NEGATIVE BACTERIA:

Structural Analysis and Interaction with Antibiotics

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Akademisk avhandling

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Title
Exploring Lol Proteins in Gram-Negative Bacteria: Structural Analysis and Interactions with Antibiotics

Abstract
Antimicrobial resistance (AMR) poses an escalating global threat, with drug-resistant 'superbugs' undermining a century of medical progress. Misuse and overuse of antibiotics have accelerated the rise of resistance, while the development of new antibiotics has slowed due to economic, regulatory, and profitability challenges. This has created an urgent need for novel antibacterial targets.The outer membrane (OM) of Gram-negative bacteria represents a promising target for the development of novel antibiotics. As no current antibiotics specifically target OM assembly, it presents a unique opportunity to develop drugs against which bacteria have not yet developed resistance.

Lipoproteins play crucial roles in OM biogenesis and bacterial virulence, with their localization to the OM facilitated by the Lol pathway, composed of the LolCDE ABC transporter, the periplasmic chaperone LolA, and the OM-anchored receptor LolB. Despite extensive biochemical, functional, and structural studies on Lol proteins in *E. coli* (a representative of γ-proteobacteria), our understanding of Lol proteins in other bacterial phyla and classes remains limited. Studies have shown that the components of the Lol machinery vary across different bacterial classes and phyla, making it essential to explore Lol proteins beyond *E. coli* to gain a comprehensive understanding of these variations. This research aims to bridge that gap by investigating Lol proteins from a diverse array of bacterial species, potentially guiding the design of narrow- or broad-spectrum antibiotics.

In this work, LolA and LolB proteins were purified and their crystal structures determined from *Vibrio cholerae*, *Helicobacter pylori*, *Porphyromonas gingivalis*, and *Gluconobacter oxydans*. Comparative structural analysis revealed that the structural determinants critical for Lol protein functionality in *E. coli* are not universally conserved, even within the same phylum or class of bacteria. Further, we conducted ITC binding assays with the antibiotics polymyxin and A22, demonstrating that polymyxin binding correlates with the negative surface charge potential of Lol proteins, suggesting that polymyxin B could be a potential lead compound for targeting LolA proteins in drug development.

A significant discovery was the identification of a LolB-like protein in *P. gingivalis* using AlphaFold, a bacterium previously thought to lack LolB. We showed by in vitro experiments that this LolB-like protein interacts specifically with LolA from *P. gingivalis* but not from *V. cholerae* or *H.pylori*, and its structure shares features with both LolA and LolB. Protein-membrane association assays indicated a strong affinity of the LolB-like protein for phospholipids, further suggesting its functional relevance in lipoprotein transport. Additionally, we optimized the expression of LolCDE from *P. gingivalis* and the subsequent purification in SMALPs, conducting preliminary cryo-EM analyses. Functional analysis indicated that LolCDE can transport triacylated lipoproteins despite the absence of the *lnt* gene, which is typically required for triacylation in other bacteria.

Overall, this work broadens our understanding of Lol machinery across diverse bacterial species, offering new insights into its structural and functional diversity. This expanded repertoire of Lol proteins provide a foundation for the development of antibiotics targeting the Lol pathway, a critical component of Gram-negative bacterial cell envelope biogenesis. Disrupting the Lol machinery could destabilize the bacterial OM, rendering pathogens like *V. cholerae*, *H. pylori*, and *P. gingivalis* more susceptible to existing antibiotics.

Keywords
Outer-membrane, *V.cholerae*, *H. pylori*, *P.gingivalis*, and *G.oxydans*, Lol Machinery, Antibitoics, ITC, Polymyxin, Alphafold, Structural biology, X-ray crystallography, Cryo-EM

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