In addition, Fragment-Based approaches for drug discovery and even conventional SBDD protocols quite often encounter difficulties in introducing ligands either by soaking or co-crystallization of low affinity compounds. Often, this is because the active sites of the targets of interests are ocuppied by salts, additives or other chemicals that preclude the succesful crystallization/soaking of target:ligand complexes.

We have explored the use of relative humidity control of protein crystals to overcome some of these issues. We have used crystals of PurE (EC.4.1.1.21), an enzyme from the purine biosynthesis pathway of *B. anthracis* as a test case. Our findings can be summarized as follows: i) using humidity control, it is possible to improve/optimize the diffraction quality of crystals soaked with ligands/inhibitors; ii) optimization of the relative humidity can compensate for the deterioration of the diffraction pattern that is observed upon desalting crystals grown in high salt; iii) combining de-salting protocols with PEG addition it is possible to achieve very high concentrations of weak ligands (5-10 mM range) in soaking solutions; and iv) fine control of the relative humidity of the crystals soaked in these solutions can compensate for the deterioration of crystal diffraction and restore 'high resolution' diffraction for SBDD and FBDD.

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Keywords: Free-Mounting System, Relative humidity control, PurE (EC.4.1.1.21)

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Structural studies of an endotoxin biosynthesis enzyme from *neisseria meningitidis*

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Neisseria meningitidis is the causative agent of meningitis and septic shock. Septic shock results from the recognition of the neisserial endotoxin by the receptor complex, MD-2/TLR-4, that leads to high level induction of the pro-inflammatory cytokines. Endotoxin is a glycolipid that binds MD-2 via the lipid portion, lipid A, which can be modified with phosphoethanolamine (PEA).

The transfer of PEA to the lipid A is catalysed by the enzyme NmLptA. The addition of the PEA groups to lipid A appear to play important roles in moderating the ability of the bacterium to attach to host cells and increases resistance. Lastly, the potency of meningococcal lipid A to stimulate the inflammatory response via MD-2 is determined by the amount of PEA decorating the lipid A. As such, NmLptA may be suitable target for the future development of small molecule inhibitors for the treatment of meningococcal infections.

NmLptA is an integral membrane protein consisting of a transmembrane domain and a globular domain. In order to understand how NmLptA catalyses the addition of PEA to lipid A, we have solved the X-ray structure of the soluble domain of the enzyme by MAD phasing. The fold of the enzyme is strikingly similar to the alkaline phosphatase family, and the structure contains a zinc ion, which may be indicative of the location of the enzyme active site. In addition, a ligand covalently bound to Thr280 at the active site is apparent in the electron density map. These observations give important insight into NmLptA, and enable the identification of key catalytic residues essential for substrate binding and catalysis.

Keywords: protein crystallography, enzyme mechanism, structural biology

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Structure of respiratory complex I

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NADH-ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Complex I is implicated in many human neurodegenerative diseases, as well as in aging. We study bacterial complex I as a "minimal" model of human enzyme. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm. Previously, we have determined the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus*, revealing the arrangement of NADH, flavin and nine Fe-S clusters in an electron transfer chain [1,2].

The mechanism of coupling between the electron transfer and proton translocation in complex I is currently not established. Recently, we have crystallised the membrane domain of complex I from *E. coli* and determined, by X-ray crystallography, its α-helical structure [3]. We have also crystallised the entire complex I from *T. thermophilus* and determined its structure by molecular replacement with the previously determined structure of the hydrophilic domain and the α-helical structure of the *E. coli* membrane domain. The overall architecture of complex I, thus revealed, provides strong clues about the coupling mechanism. The conformational changes at the interface of the two main domains may drive the unusual long amphipathic α-helix in a piston-like motion, tilting nearby discontinuous trans-membrane helices in three similar antiporter-like subunits, resulting in proton translocation.

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Keywords: electron transport, proton pumping, membrane protein

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Dissecting Enzyme Mechanisms

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MS.93.4

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Structure of lysine oxidase with a cysteine tryptophylquinone in the active site

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