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Schistosomiasis is the name of a severely debilitating disease caused by parasites of the genus *Schistosoma*, among which *Schistosoma mansoni* is the most prevalent specie in the world and is estimated to infect approximately 200 million people. *S. mansoni* lacks the "de novo" purine pathway and depends on it's host for purine requirements. In the case of pyrimidine pathways the parasite has both "de novo" and the salvage pathways. The thymidylate cycle is also functional. These pathways had been used in the past as targets for chemotherapeutic intervention. However, duo their importance for worm metabolism, little attention is devoted for this area.

The current project aims to solve crystallographic structure of all enzymes involved in the purine salvage pathway and some specifically selected from the pyrimidine salvage, "de novo" pathways and thymidylate cycle, as well as the determination of their kinetic constants.

The genes were amplified from enriched cDNA library, cloned in propagation and pET28 and/or pET28-SUMO vectors. After expression and purification the enzymes were submitted to the robotic crystallization trials. As a result we successful cloned 23 genes, performed soluble expression of 14 proteins and got crystals of 7 proteins.

Crystal structures of five proteins were solved. Adenosine kinase (AK) was the first structure solved and catalyzes the reaction: adenosine + ATP ↔ AMP +ADP. Two complexes were solved up to 2.25Å: the ternary complex AK-adenosine-AMP and the complex between AK-tubercidin. Several differences was found in the adenosine binding site that explains the preference for some adenosine analogues in contrast to the human enzyme.

Uridine phosphorylase (UP) catalyzes the phosphorolysis of uridine/thymidine to uracil/thymine plus ribose-1-phosphate, participating of pyrimidine salvage pathway. The parasite possess two isoforms for this enzyme, one with the active site totally conserved in comparison with human counterpart (UP-GQ) and other with several differences(UP-DL). We cloned the two isoforms and solved 4 structures for the UP-DL up to 2.0Å resolution. The differences in the active site between two isoforms suggests different role in the nucleotide metabolism.

Adenylate kinase (ADK) is involved in the conversion of 2 ADP into AMP +ATP, and is also has a role in energy homeostasis. The structure of ADK in apo form was obtained up to 2.05Å resolution. Despite many efforts we couldn't get complexes structures due to crystallization conditions. The kinetic assay is under way.

Dihydrofolate reductase (DHFR) is the enzyme that converts dihydrofolate into tetrahydrofolate in the presence of NADPH, and is extensively used as a drug target. We solved the structure for this enzyme up to 1.95Å resolution. The structure obtained is in apo form and the most prominent result was the conformation of the W loop that enters into folate site.

The last structure obtained for methythioadenosine phosphorylase (MTAP) was for enzyme. Two structures were obtained: in apo form and in complex with adenine, comparison between two structures shows large conformational changes in active site. All the structures were refined with acceptable values of R and R_{Free}.

These results will increase the understanding of the nucleotide metabolism for this important parasite.

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Sulfatide selective human glycolipid transfer protein

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Human glycolipid transfer protein (GLTP) represents a novel structural fold for lipid binding/transfer and reversible membrane translocation [1, 2]. GLTPs transfer glycosphingolipids (GSLs), which are key regulators of cell growth, development, surface adhesion, and neuroregeneration. Here we report structure-guided engineering of a 'designer GLTP' with enhanced transfer selectivity for sulfatide (3-O-sulfo-galactosylceramide). New crystal structures of wild-type GLTP and two mutants (D48V and A47D||D48V), each containing bound N-nervonoyl-sulfatide, reveal the molecular basis for selective anchoring of sulfated galactosylceramide by D48V-GLTP. The directed point mutation of hydrophobic pocket 'portal entrance' residues, A47 and D48, reversibly regulates 'sphingosine-in' versus 'sphingosine-out' binding modes. By serving as a homodimerization 'hot spot', the mutated portal entrance region facilitates an adaptation mechanism that controls dual chain accommodation of glycolipids with different length acyl chains. New crystal structures of *bona fide* apo-GLTP and GLTP complexed with N-oleoyl-glucosylceramide reveal 'door opening' conformational changes involving phenylalanines in the hydrophobic pocket during lipid encapsulation. The development of 'designer GLTPs' with enhanced specificity for select GSLs provides a potential new therapeutic approach for targeting GSL-mediated pathologies.

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Structural studies on novel antitubercular targets

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Latent tuberculosis remains a major health problem. The ability of *M. tuberculosis* to reside for long time inside macrophages complicates TB treatment. Targets which are essential for intracellular survival of *M. tuberculosis* are particularly attractive for TB eradication. Cholesterol is likely to be the fuel for *M. tuberculosis* inside macrophages. The arylamine N-acetyltransferase gene (*nat*) of *M. tuberculosis* and the neighbouring genes in the same operon have been shown to play role in cholesterol catabolism and intracellular survival. Four genes (*hsaA, hsaB, hsaC, hsaD*) in this operon have been shown to participate in the sterol ring catabolism while the NAT enzyme was found to be able to utilize the cholesterol metabolite n-propionyl-CoA, linking it to cholesterol metabolism.

Arylamine N-acetyltransferase from *M. tuberculosis* (TBNAT) has been proposed as a drug target for latent tuberculosis (TB) treatment.