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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 species in the world and is estimated to infect approximately 200 million people. *S. mansoni* lacks the “de novo” purine pathway and depends on its 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, due to 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 successfully 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 \leftrightarrow AMP + ADP. Two complexes were solved up to 2.25Å: the ternary complex AK-adenosine-AMP and the complex between AK-tubercidin. Several differences were 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 complex 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 methylothioadenosine 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.

[1] L. Malinina, M. Malakhova, A. Teplov, R.E. Brown, D.J. Patel, *Nature* **2004**, *430*, 1048-1053. [2] L. Malinina et al. *PLoS Biol.* **2006**, *4*, e362.

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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.

Deleting the *nat* gene and inhibiting the NAT enzyme prevents survival of the mycobacteria in macrophages and induces cell wall alterations, rendering the mycobacterium sensitive to antibiotics to which it is normally resistant. However, TBNAT has been very difficult to generate as a soluble protein. To date, NAT from *M. marinum* (MMNAT) is considered the best available model for the TBNAT enzyme. Inhibitors have been developed by high throughput screening using homologous NAT enzymes and shown to be active against TBNAT [1]. In this work we present the first structural study on the MMNAT-inhibitor complexes. Understanding the ligand protein interaction will give better insight on the structure activity relationship of the identified inhibitors and will help to design potent inhibitors.

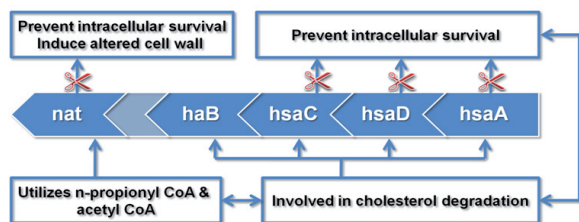


Figure 1: The *nat* operon in *M. tuberculosis*.

[1] I. Westwood, S. Bhakat, A. Russell, E. Fullam, M. Anderton, A. Kawamura, A. Mulvaney, R. Vickers, V. Bhowruth, G. Besra, A. Lalvani, S. Davies, E. Sim, *Protein Cell* **2010**, *1*, 82-95.

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ADP-Mg²⁺ bound to the ATP-grasp domain of Human ATP-citrate lyase

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ATP-citrate lyase is the cytoplasmic enzyme in humans linking energy metabolism from carbohydrates to the production of fatty acids. Acetyl-CoA, produced in mitochondria from the breakdown of carbohydrates, cannot be exported to the cytoplasm to serve as the precursor for fatty acids. Instead, citrate synthase transforms acetyl-CoA to citrate in the mitochondria, and citrate is exported to the cytoplasm where ATP-citrate lyase regenerates acetyl-CoA. Since fatty acids are required for the synthesis of both triacylglycerol and membrane lipids, ATP-citrate lyase has been considered as a possible drug target for the treatment of obesity and cancer.

Previously, a truncated form of the full-length human ATP-citrate lyase was crystallized using *in situ* proteolysis, and the structure was solved using X-ray crystallography [1]. Based on the structure, the plasmid was modified to include only the sequence encoding the 817 amino-terminal residues of the enzyme linked to a carboxy-terminal His₈-tag.

The reaction catalyzed by ATP-citrate lyase in the presence of magnesium ions is

citrate + CoA + ATP → acetyl-CoA + oxaloacetate + ADP + P_i. The amino-terminal portion of the enzyme had been shown to bind citrate or its competitive inhibitor, tartrate, and was predicted to bind ATP [1]. To investigate the nucleotide-binding site, the protein was crystallized

in the presence of tartrate, ATP and magnesium ions. The crystals diffracted to 2.3-Å resolution at the macromolecular crystallography beamline of the Canadian Light Source. They belonged to space group C2 with unit cell dimensions $a = 167.5 \text{ \AA}$, $b = 61.7 \text{ \AA}$, $c = 107.9 \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 125.5^\circ$, $\gamma = 90^\circ$. The structure shows ADP-Mg²⁺ bound to the domain that possesses the ATP-grasp fold and demonstrates that this crystal form could be used to investigate the structures of the complexes with inhibitors of ATP-citrate lyase that bind at either the citrate- or ATP-binding site.

[1] T. Sun, K. Hayakawa, K.S. Bateman, M.E. Fraser, *Journal of Biological Chemistry* **2010**, *285*, 27418-28.

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The structure of SFPQ/PSF, a multifunctional nuclear protein

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SFPQ/PSF (Polypyrimidine tract-binding protein-associated splicing factor) is a multifunctional nuclear protein that has been implicated in a variety of nuclear processes including RNA biogenesis as well as DNA repair [1]. Importantly, SFPQ is a known tumour suppressor, binding to promoters of oncogenes and repressing transcription. A recent report of interactions of SFPQ with non-coding RNAs shows that these interactions release SFPQ from a proto-oncogene and activate transcription, thus, driving transformation and tumorigenesis [2]. A tight correlation to expression level of SFPQ with the progression of prostate cancer has also been reported [3].

In addition to the functions described above, SFPQ also plays a key role in the structural integrity and functions of paraspeckles, subnuclear bodies implicated in regulation of gene expressions by nuclear retention of hyperedited mRNAs. SFPQ along with two other paraspeckle proteins, NONO/p54nrb and paraspeckle protein component 1 (PSPC1) make up the mammalian DBHS (Drosophila Behaviour/ Human Splicing) family. The conserved DBHS domains of these proteins, comprised of tandom RNA recognition motifs (RRMs) and a C-terminal coiled-coil domain, share more than 50% sequence identity. Reported interactions between DBHS proteins suggest that they exist as either homo- or heterodimers *in vivo*.

We have determined the first structure of human SFPQ homodimer and refined to 2.05 Å resolution. The structure shows the consistent arrangement of the DBHS domain with our previous structure of PSPC1/NONO heterodimer. Interestingly, the N-terminus of SFPQ reveals an additional secondary structure that was absent in the structure of PSPC1/NONO heterodimer and may be related to DNA binding activity of SFPQ. The structure of human SFPQ in comparison with PSPC1/NONO heterodimer will be discussed. In addition, DNA binding activity of SFPQ as well as possible RNA binding modes will be described in relation to the functions of SFPQ in tumorigenesis.

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Keywords: SFPQ/PSF, DBHS domain, paraspeckle