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Keywords: transthyretin, amyloid, chemico-biological space.

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Structural basis for inhibition of interferon alpha signaling pathway and its therapeutic potential in SLE patients

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Increasing evidences suggest that the type I interferon (IFN) plays a critical role in the etiopathogenesis of systemic lupus erythematosus (SLE), which makes it a promising therapeutic target for the treatment of the disease. By screening a large size non-immune human antibody library, we have developed a human single-chain antibody (ScFv) AIFNα1bScFv01 and corresponding whole antibody AIFNα1bIgG01, that recognizes recombinant human interferon alpha1b (hIFNα1b) with high specificity and high affinity. The IgG antibody can down-regulate the expression of *ISG15* and *IFIT-1* induced by either recombinant hIFNα1b or naive IFN-α presented in SLE patient's sera. The crystal structure of AIFNα1bScFv01-hIFNα1b complex solved to 2.8 Å resolution reveals that both Pro26-Gln40 region in loop AB and Glu147-Arg150 region in helice E of hIFNα1b contribute to binding with AIFNα1bScFv01. Four residues of above two regions (Leu30, Asp32, Asp35 and Arg150) are critical for the formation of antigen-antibody complexes. AIFNα1bScFv01 shares partial epitopes of IFNα1b with its receptor IFNAR2. AIFNα1bIgG01 has a much higher affinity for IFNα1b than IFNAR2 (K_D = 0.747 nM versus 100 nM), making it unavailable for binding to IFNAR2 and preventing the activation of IFN-α-mediated signaling pathway. Thus, AIFNα1bIgG01 exhibits its neutralizing activity through competition with IFNRA2 to bind with IFN-α. Our results highlight the potential use of the human antibody for modulating the activity of IFN-α in SLE.

Keywords: SLE, IFNα, ScFv antibody

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Structure of peptide inhibitor of human islet amyloid polypeptide fibrillization

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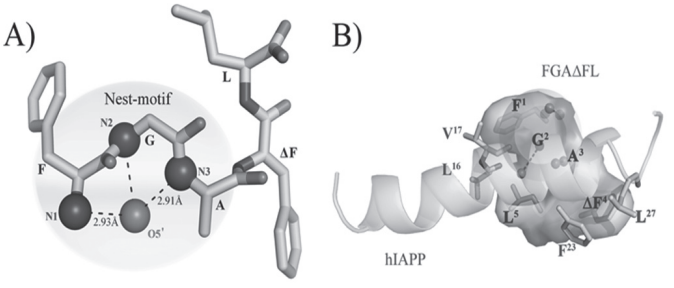
Type-2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetes worldwide. Over 100 million people worldwide have T2DM, and the prevalence is increasing dramatically in both the developed and developing countries. Amyloid deposits have been observed in a vast majority of the T2DM patients and these are primarily on account of misfolding and aggregation into fibrils of human islet amyloid polypeptide (hIAPP), a 37 residue endocrine hormone secreted by pancreatic *β*-cells. It has been suggested that intermediates produced in the process of fibrillization are cytotoxic to insulin producing *β*-cells. Hence, the inhibition of misfolding/fibrillization of hIAPP could be a possible strategy to mitigate T2DM. The misfolding of hIAPP involves structural transition from its native state (coil and/or helical and/or transient helical conformation) to *β*-sheet conformation. We have targeted hIAPP fibrillization by designing short peptides containing the helix inducing *α,β*-dehydrophenylalanine (ΔPhe or ΔF) amino acid and the fibrillization inhibition was monitored by thioflavin-T assay and electron microscopy. We find that the short peptides inhibit fibrillization without any cytotoxic effect as tested on RIN4fm pancreatic cell line. Of these, the penta-peptide, FGAΔFL is the most effective inhibitor of hIAPP fibrillization. We successfully crystallized the penta-peptide and solved its 3D structure at atomic resolution using direct methods. Molecular conformation of the peptide reveals the occurrence of a nest-motif (Fig. A) involving the stretch FGA in the penta-peptide and a type-I *β*-turn. To gain structural understanding and visualize the probable interactions of the hIAPP with FGAΔFL, molecular docking studies were performed using AutoDock4. Here, we considered the penta-peptide as receptor and $hIAPP_{6-30}$ (PDB: 2KB8) as ligand. The best ligand pose was selected from the cluster with the highest occurrence and the lowest binding energy (-6.41 kcal/mol). The interactions stabilizing FGAΔFL-hIAPP complex, are nest-motif interactions, hydrophobic interactions and aromatic interactions (Fig. B). We propose, on the basis of FGAΔFL crystal structure and molecular docking, that the penta-peptide binds to the helical conformation of hIAPP which is considered as transient in nature and/or preferred in membranous environment. Here, the penta-peptide binds at the C-terminal of helical $hIAPP_{6-30}$, stabilizes the helical conformation and makes the transition from alpha to beta structure unfavourable, thereby curtailing the fibrillization process. Thus, the crystal structure of the penta-peptide inhibitor together with computational docking studies provides an atomic level picture of the possible mechanism by which the penta-peptide manifests its fibrillization inhibition activity. Further studies are underway in our laboratories to develop even more potent inhibitors of hIAPP fibrillization and the details will be presented.



Keywords: diabetes, amyloid, inhibitor

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Studies of nucleotide metabolism from blood fluke *Schistosoma mansoni*

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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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Keywords: Schistosoma mansoni, enzymes, nucleotide metabolism.

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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.