Posters - Membrane Proteins

P1-1

INVESTIGATIONS INTO THE BINDING SITE PROMISCUITY OF MhsT

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Neurotransmitter:sodium symporters (NSSs) are secondary transporters, placed on the presynaptic cell of the synapse. They ensure uptake of neurotransmitter molecules from the synaptic cleft, using energy released from the downward movement of Na^+ along its electrochemical gradient. Therefore, NSSs have an important role in controlling impulse signaling in neurons and a number of diseases are related to dysfunctions of neurotransmitter transporters. Insight into transporter structure and function has been obtained from structures of bacterial and eukaryotic members.

MhsT is a hydrophobic amino acid transporter [1] and a member of the NSS family. The goal of this project is to study the structural basis of the binding site promiscuity of MhsT. Crystal structures of MhsT in complex with L-Phe (2.25 Å) and a tyrosine orthologue L-4-F-Phe (2.26 Å) have been determined from crystals grown using the HiLiDe method [2]. Like the previously determined MhsT+Trp structure [3], the protein is in an occluded inward-facing conformation for the two substrates. This conformation of the protein reflects the state just before the opening of the transporter to the intracellular side, and gives insight into how sodium drives this process [3]. The binding sites, however, while similar have some important differences that mark the bound substrate. Based on these structures, binding site mutations of MhsT, to resemble that of the human neutral amino acid transporters SLC6A18 and SLC6A19, have been generated. Structural and functional investigations of these mutants will provide a better insight into the binding specificity of this transporter.

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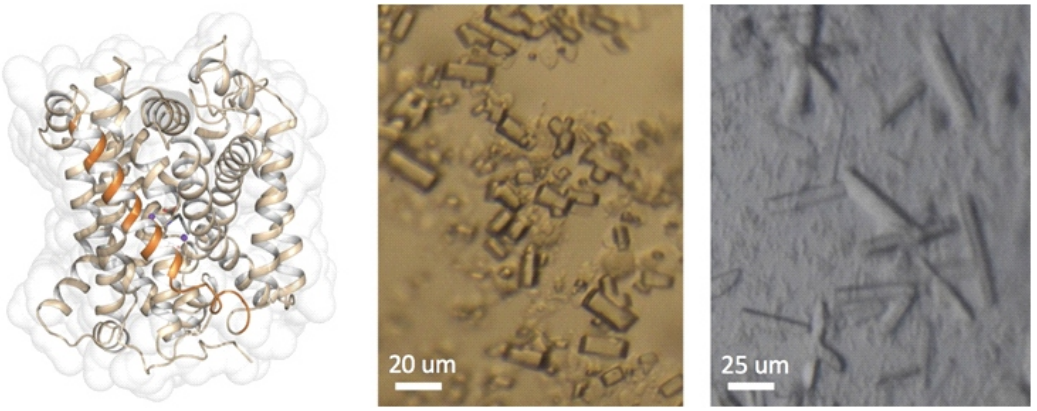

Figure 1. On left: The overall structure of MhsT in its occluded-inward facing state, what is indicated by TM5 being unwound in its intracellular part (TM5 is marked as an orange helix). On right: Various crystals of MhsT+Phe obtained using the HiLiDe method.

P1-2

RANDOM MICROSEEDING AS A NEW APPROACH APPLIED ON HALOALKANE DEHALOGENASE DbeA VARIANT CRYSTALLISATION

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A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkanii* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. The DbeA protein was crystallised using the sitting-drop vapour-diffusion method and the crystal structure of a DbeA enzyme has been solved and deposited at Worldwide Protein Data Bank under PDB ID 4k2a. The DbeA wt structure revealed the presence of two halide-binding sites. The first chloride-binding site is located in the active site in between two halide-stabilizing residues. The second chloride-binding site is unique to DbeA and has not been previously reported in any other structure of this enzyme family. To elucidate the role of the second halide-binding site, a two-point variant DbeA DCl (I44L+Q102H) lacking this site was constructed and biochemically characterized [1]. Elimination of the second halide-binding site decreased the stability and catalytic activity, and dramatically altered the substrate specificity. The two-point substitution resulted in a shift of the substrate-specificity class, which is the first time this has been demonstrated for this enzyme family. Rational design of buried halide-binding sites represents a novel strategy for engineering of enzymes with modified catalytic properties.

Support of the grant P207/12/0775 of Grant Agency of the Czech Republic is acknowledged.

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P1-3

INSIGHTS INTO ION SELECTIVITY AND TRANSLOCATION IN THE BACTERIAL MAGNESIUM CHANNEL CORA PROVIDED BY ANOMALOUS DIFFRACTION EXPERIMENTS

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Magnesium (Mg^{2+}) is the most abundant divalent cation in eukaryotic and prokaryotic cells, having numerous important physiological functions [1]. However, of the four main biological cations, the transport and homeostasis of magnesium remains the least understood. Members of the ubiquitous CorA family of Mg^{2+} channels contain a canonical Gly-Met-Asn ($_{312}GMN_{314}$) signature motif at the extracellular mouth of the permeation pathway, which has been proposed to form part of the Mg^{2+} selectivity filter of these channels [2-4]. In this study, we use anomalous x-ray diffraction to examine the binding at the selectivity filter of two transported substrates of CorA, cobalt (Co^{2+}) and nickel (Ni^{2+}), the non-substrate samarium (Sm^{3+}) as well as the known CorA inhibitor cobalthexammine, which is also an inert structural analogue of hexahydrated Mg^{2+}[5]. Our results indicate that while Co^{2+} and Ni^{2+} are able to bind at the $_{312}GMN_{314}$ motif coordinating with G_{312} and N_{314}, Sm^{3+} is excluded from the selectivity filter. Cobalthexammine is

also able to bind at the mouth of the CorA pore, but at a position slightly peripheral to the $_{312}GMN_{314}$ motif, suggesting that CorA allows binding of a hexahydrated Mg^{2+}, but only in a position that does not allow further penetration into the selectivity filter, explaining the inhibitory effect of cobalthexammine. Moreover, we suggest that our results strongly support a knock-on mechanism for ion transduction through the CorA pore, where a Mg^{2+} bound at G_{312} and N_{314} is pushed down the permeation pathway by an incoming Mg^{2+} bound at N_{314} (Figure 1).

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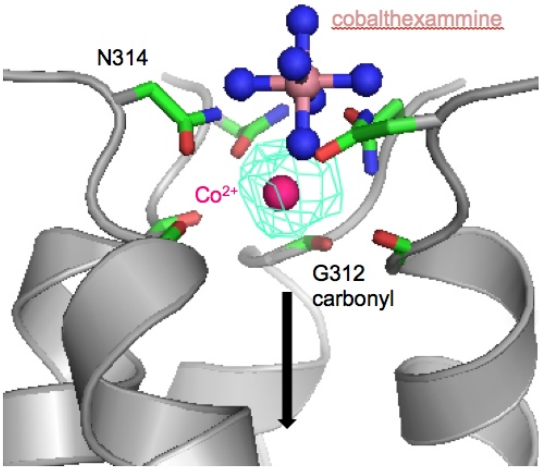

Figure 1. Co^{2+} and cobalthexammine binding at the GMN motif of CorA. The position of the cobalthexammine is superimposed onto the structure obtained with Co^{2+}. Only four protomers and the anomalous density for Co^{2+} (cyan mesh) are shown for clarity. The arrow indicates the direction of ion

P1-4

STUDYING THE OXIDATION OF WATER: THE PHOTOSYSTEM II STORY

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Photosystem II (PSII) is a key enzyme in photosynthesis that drives the synthesis of oxygen by splitting water at the Mn_4O_xCa core of the oxygen-evolving cluster (OEC) in the protein complex. Using time-resolved femtosecond X-ray crystallography (TR-SFX) [1] at an X-ray free-electron laser, conformational changes have been reported both in the OEC and its protein environment [2]. Since 2014, we have optimized the crystallization procedures for improvement of the resolution obtained from nano-crystal diffraction. Combining innovative crystallization techniques with novel techniques for imaging macromolecules from 'imperfect' crystals [3] i.e. continuous diffraction, we have been successful at collecting data beyond the highest resolution of Bragg peaks. Additionally, since this method permits iterative phasing without a need for a structural model or experimental phase information [3], it is pioneering in the field of macromolecular structural studies.

Photo-induced oxidation of water by PSII forms the basis for the development of synthetic water splitting devices [4]. Hence, an improved understanding of the natural process would enhance our efforts towards obtaining sustainable clean energy. Our work presents multi-disciplinary scientific collaboration because it encompasses various research fields of nano-crystallization, liquid sample delivery development, laser optics, along with, crystallographic data processing and evaluation.

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P1-5

MULTIPHASE LIQUID CRYSTALLINE NANOASSEMBLIES WITH PROTEIN ORDERING

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Confinement of proteins in nanostructured particles provides a means for substantial concentration of the biomacromolecules and for optimal contacts between the neighbouring molecules towards protein ordering and crystallization. Liquid crystalline nanoparticles of self-assembled lipids and amphiphiles have been known for their advantages as templates for nucleation and growth of nanocrystals. To fulfil the need of 3D biomacromolecular ordering that precedes the protein crystallization, we followed the loading and confinement of a charged therapeutic protein brain-derived neurotrophic factor (BDNF) in PEGylated lipid nanoparticles of liquid crystalline inner organization. The latter were obtained by the method of self-assembly and hydration of lipid mixtures [1-4]. The neurotrophin BDNF plays a key functional role in the differentiation, proliferation, growth, plasticity, and survival of neurons in the central and peripheral nervous systems. This protein exerts its biological activity as a dimmer, although its dimeric form has not been crystallized yet.

Here the dynamic nanoscale organization of lipid/neurotrophic protein assemblies was studied upon progressive loading of the neurotrophin BDNF in lipid membrane particles. Synthetic lipids and human recombinant BDNF of maximal purity were employed for sample preparation. Millisecond time-resolved small-angle X-ray scattering (SAXS) experiments were *in situ* performed using a rapid-mixing stopped-flow setup coupled to synchrotron SAXS measurements. The neurotrophin BDNF (which lacks conformational flexibility) demonstrated capacity to considerably modify the curvature of the studied flexible lipid membranes. Time-resolved SAXS monitoring of protein molecules entrappment in lipid nanoparticulate containers established the formation and coexistence of double diamond cubic *Pn3m* (D), gyroid cubic *Ia3d* (G), and lamellar (L) structures within the investigated nano-objects (Figure 1). The obtained ordered arrays of uploaded BDNF biomacromolecules were able to rapidly alter the membrane curvature at the initial stage of protein loading. Subsequently, the curved packing in the membrane domains was accompanied by protein accumulation in more concentrated areas inside the nanoparticles. This was associated with the formation of cubic and well ordered lamellar domains containing nanoconfined proteins.

The performed cryogenic transmission electron microscopy (Cryo-TEM) study revealed the morphological patterns and shapes associated with the protein ordering. It

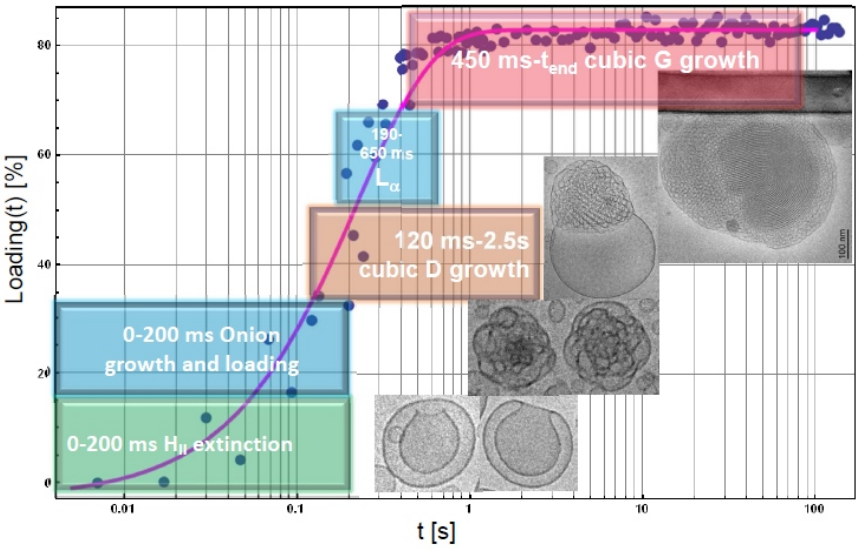

Figure 1. Percentage of protein loading that is associated with the induction of structural transformations of vesicular lipid membranes into growing cubic and mixed liquid crystalline structures with embedded proteins.

confirmed the transformation of the inner liquid crystalline lipid structures into organized lipid/protein complexes. Ordered protein patterns emerged as a result of the induction of domains of new ordering upon protein accommodation in the lipid supramolecular assemblies. The obtained structural results evidenced the stages of the protein loading and ordering in lipid nanoparticles and suggested that protein concentrations higher than 4 mg/ml would be required for biomacromolecules assembly into nuclei for 3D protein crystallization.

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P1-6

CHALLENGING CRYSTALLIZATION OBSTACLES AT THE ISPC

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I'm working as a lab technician at the crystallization unit of the Israel Structural Proteomics Center (ISPC http://www.weizmann.ac.il/ISPC)) at the Weizmann institute. In the line of my work I'm operating and maintaining our state-of-the-art crystallization and visualization robots that increase the efficiency and accuracy of the protein crystallization experiments. In the last seven years at the ISPC, I have setup thousands of crystallization screening experiments on few hundreds of proteins. This was followed by several of sophisticated optimization experiments, which I design in attempts to yield a single crystal suitable for X-ray analysis. I have also implemented a number of seeding strategies for crystallizing proteins. An interesting example was crystallizing the Conk2 and Conk3 proteins. Cones are small polypeptides with disulfide-rich conotoxins that can be classified into families according to their respective ion-channel targets and patterns of cysteine-cysteine disulfides. Conk2 and Conk3 proteins are potassium-channel pore-blocking toxin. These proteins have helix at their C-terminus, which is crucial for channel binding and they share 53% identity. Initially I managed to crystallize the Conk2 (Fig. 1), and the structure was solved 1.46 Å. However, crystallizing the Conk3 protein was very challenging and in fact yielded no crystals from experiments using different crystallization methods at different temperatures. As last resort I used the Conk2 crystals as seed to preform micro seeding and obtained Conk3 crystals (with 53% identity to Conk2) (Fig. 2) for which the structure was solved to 2.4 Å.

Another challenging crystallization experiment was on the synaptic enzyme, acetylcholinesterase (AChE), which is an important target for drug design. AChE inhibitors are employed in the treatment of myasthenia gravis and other neuromuscular diseases; the first generation of drugs for the management of Alzheimer's disease are also AChE inhibitors. Furthermore, AChE is the principal target of nerve agents and of many insecticides. It took me several years to get single crystals suitable for X-ray data collection of the human AChE (hAChE) in the presence of adenosine 3',5'-monophosphate (AMP). Initially I got crystals from the crystallization screens which did not diffract (Fig. 3).

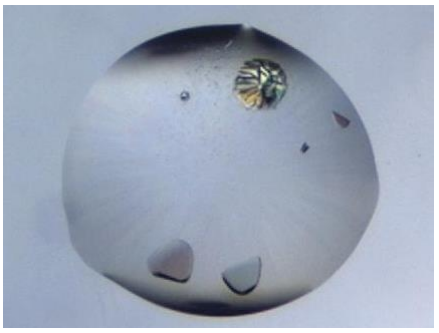

Figure 1. Conk2 crystals.

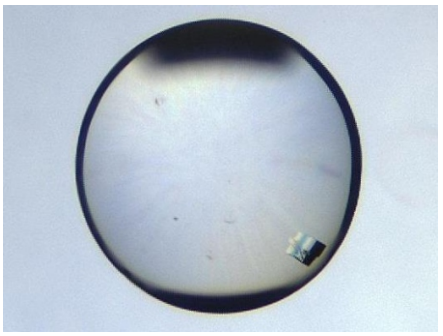

Figure 2. Conk3 crystals obtained by Micro seeding of Conk2 crystals

Additive screens yielded nice looking crystals however still did not diffract (Fig. 4). Optimization on the original crystallization conditions as well as on the additive yielded many crystals for which the structure was solved to 2.75 Å (Fig.5).

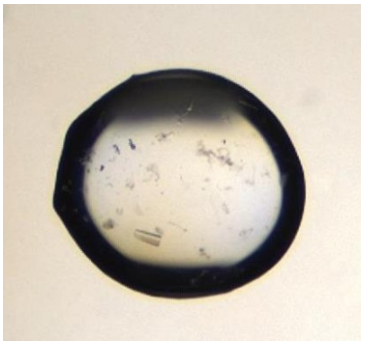

Figure 3. hAChE with AMP.

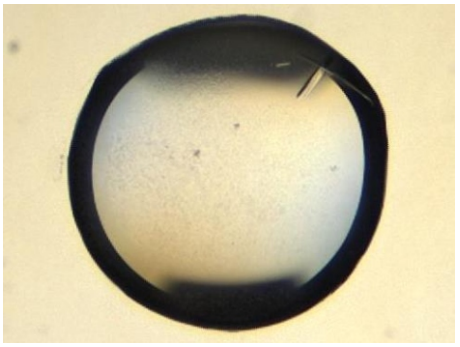

Figure 4. hAChE with AMP and Taurine as additive.

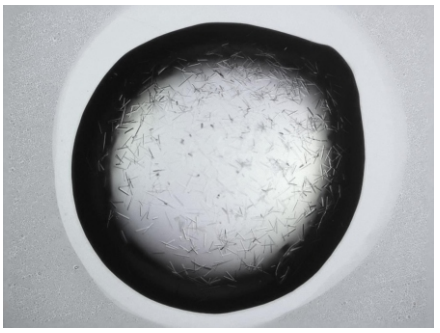

Figure 5. hAChE with AMP in the presence of Taurine and optimization of the original condition.

P1-7

TOWARDS A CRYSTAL STRUCTURE OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

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Cystic Fibrosis (CF), a fatal genetic disease affecting predominantly European descendants, has symptoms of poor growth, poor weight gain, infertility, lung disease, intestinal obstruction and untimely death due to respiratory and / or liver failure. An estimated 80,000 people suffer from CF worldwide. The gene responsible for CF codes for the cystic fibrosis transmembrane conductance regulator (CFTR), an integral membrane glycoprotein channel in the plasma membrane of epithelial mammalian cells. A high-resolution crystal structure of the full-length protein has remained elusive, leaving questions regarding CFTR's mechanism of action unanswered. Here, we explore these questions and we summarize our efforts at producing crystals of full-length CFTR using *in meso* and *in surfo* crystallization methods with a CFTR construct that is active and locked in an open conformation. We discuss small molecules known to rescue CFTR function and how they might aid high-resolution structure determination. From this work, we have gained insights regarding the direction of future crystallization trials.

P1-8

STRUCTURAL CHARACTERISATION OF AMINO ACID MEMBRANE TRANSPORTERS

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All living cells are enclosed by a selectively permeable membrane that serves as a barrier between the external environment and the contents of the cell. This membrane consists of a lipid bilayer that is not permeable to most of molecules, especially to polar substances. Ions and water-soluble molecules require the assistance of specific protein carriers to transport them across the membrane. These integral membrane proteins provide passive or active transport of their substrates when the molecules move down or against their concentration gradient respectively. Active transport can be energized from ATP hydrolysis (by ATP-binding cassette (ABC) transporters) or by utilizing the electrochemical gradient of a second substrate (co- or anti-transported by so-called secondary active transporters).

In this work we study mechanisms of active uptake of amino acids by the bacterial transporter GlnPQ and the archaeal transporter GltT using X-ray crystallography. GlnPQ is an ABC-importer with soluble substrate-binding domains that have different affinities for asparagine, glutamine and glutamate [1-3]. Archaeal GltT is a secondary transporter that catalyses the uptake of aspartate together with sodium ions [4-6]. Several structures of these transporters are already known, however some details of

their functioning remain elusive. Crystallization and structural characterization of these transporters in different states with high resolution is needed to clarify the existing structural models and results of biochemical experiments.

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Posters - Macromolecular Complexes

P2-1

CLOSED CLEFT OF O-ACETYL SERINE SULFHYDRALASE (OASS) ACTIVE SITE FROM *BRUCELLA ABORTUS* IS RESPONSIBLE FOR LOSS CSC FORMATION

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Brucellosis also known as 'undulant fever', 'Mediterranean fever' or 'malta fever' is zoonosis, an infectious disease caused by bacteria called *brucella.* It can spread from animals to humans, when person comes into contact with an animal or animal products of infected with brucella. Brucellosis is considered a significant health treat in many parts of the world especially in Mediterranean countries of Europe, north and east Africa, the Middle East, South and Central Asia and Central and South America. Activation of NADPH oxidase and low ROS production needed for the bacterium to induce premature cell death of neutrophils without inducing pro inflammatory phenotypic changes. This event majorly achieved by important antioxidative property of Cystein, involved in survival and protection of pathogenic microbes from oxidative stress and is the precursors for biosynthesis of many metabolites like glutathione, tripanthione and taurine etc., involved in maintaining cellular homeostasis, as cysteine biosynthesis pathway is crucial for inhibitor designing. In bacteria, plants and most of the pathogenic protozoans, cysteine biosynthesis is a two-step pathway. Serine acetyl transferase (SAT) catalyzes the first step of the pathway in which it transfers the acetyl group from acetyl Coenzyme A to serine to form O-acetyl serine (OAS) and in second step O-acetyl serine sulfydralase (OASS) catalyzes the condensation of O-acetyl serine with sulfide to produce cysteine.

Cysteine biosynthetic pathway is regulated by formation of a decameric complex called cysteine synthase complex (CSC), plays an important role in maintaining intracellular cysteine level. The SAT C-terminal tail binds at the active site of OASS to form CSC, which was reported earlier in E.coli, Salmonella, Hemophilus, leishmania, and Arabidopsis. And there are few structural models for CSC, although no structural evidence has been given so far.

Here in *Brucella abortus* OASS, does not interacts with its cognate SAT C-terminal tail. We recently determined the crystal structure of native BaOASS at 2.2Å resolution. The detailed comparison of BaOASS crystal structure with OASS complexes with SAT C-terminal peptides from other organisms showed the two residues 96Q and 125Y of BaOASS present instead of M and G from all other OASS respectively, occupying the active site pocket and interfering the entry of SAT C-terminal tail into the active site pocket of OASS. Thus blacking the active site pocket for entry of SAT C-terminal peptide. Point mutation of these residues (Q96 and Y125) was done to generate three mutations (Q96A, Y125A and Q96A Y125A). The binding affinity of SAT C-terminal peptides were measured by fluorescence spectrometer for all three mutants, it was found that the binding affinity (Kd) of double mutant BaOASS (Q96A Y125A) is much stronger (1000 fold) and single mutants of BaOASS (Q96A), BaOASS (Y125A) about (10 fold stronger) up on titration with SAT C-terminal mimicking peptides compared to Native BaOASS.

The Surface Plasmon Resonance (SPR) has been done for both native BAOASS and mutant BaOASS (Q96A Y125A) with BaSAT protein, where double mutant can bind to BaSAT with about 7uM binding affinity while native protein can't bind to BaSAT. These observations clearly conforms the reason for not forming CSC in BaOASS and we have validated these observations by mutational studies.