Israel Crystallographic Association

Annual Meeting

Tuesday May 21th 2013, Coler-California Center

The Technion – Israel Institute of Technology

Kiryat HaTechnion, Haifa

09:30 - 10:00 ***Gathering and Coffee***

Morning Session 1, Chair: Prof. Oded Livnah

10:00 – 10:40 - Prof. Nathan Nelson (TAU): "A quest for atomic resolution of membrane supercomplexes".

10:40- 11:00 - Dr. Tali Lavy (WIS): Structural Study of the GAL Regulon in S. Cerevisiae

11:00 – 11:20 - Dr. Ailie Marx (IIT): Bridging Knowledge Gaps in Cytidine Deamination: New crystal structures of a dCMP deaminase provide details on the allosteric mechanism and functional insights into the wider zinc dependent cytidine deaminase family.

11:20 - 11:40 – Coffee Break

Morning Session 2, Chair: Prof. Israel Goldberg

11:40 – 12:00 - Dr. Ron Diskin (WIS): Targeting HIV using Structure-Based Rational Design of Antibodies

12:00 – 12:20 - Hila Nudelman (BGU): Crystallization and structure of the predicted magnetite interacting associated protein loop from magnetotactic bacteria.

12:20 – 12:40 - Dr. Hatem M. Titi (TAU): Probing halogen bonding in porphyrin assemblies

12:40 – 13:00 - Dr. Ranjit Thakuria (TAU): Stability of Caffeine-Glutaric acid polymorphic cocrystals

13:00 – 13:45: Lunch Break

13:45 – 14:00 ICA Business Meeting

Afternoon Session, Chair: Dr. Raz Zarivach

14:00 – 14:20 - Dr. Reuven Weiner (HUJI): An unusual mechanism for regulating ubiquitination by the deubiquitinating enzyme OTUB1

14:20 – 14:40 - Onit Alalouf (IIT): High resolution crystal structure of the acetylxylan esterase Axe2 from *Geobacillus stearothermophilus*

14:40 – 15:00 - Moran Shalev (IIT): Insights into the molecular mechanism of aminoglycosides action against leishmaniasis

15:00 – 15:20 - Dr. Dima Golovenko (WIS): Structural studies of DNA recognition by the restriction endonucleases Bse634I, EcoRII and BfiI

15:20 Closing Remarks

The ICA 2007 annual meeting is sponsored by Rhenium and Daniel Biotech

A quest for atomic resolution of membrane super-complexes

Yuval Mazor, Hila Toporik, Anna Borovikova, Ilanit Greenberg, Daniel Nataf, Shai Saroussi and Nathan Nelson
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The last decade yielded a wealth of information on the structure of membrane complexes. Plant Photosystem I (PSI) is supercomplex comprises two loosely bound reaction center and light-harvesting (LHC) complexes. The crystal structure at 3.1 Å resolution reported her contains 18 subunits 174 chlorophylls, 32 carotenoids 2 quinines and 3 iron-sulfur clusters. Using the new crystal structure, we examine the network of contacts among the protein subunits from the structural perspective, which provide the basis for elucidating the functional organization of the complex. In addition, much better resolution of several chlorophyll molecules was observed. We also detected 10 additional carotenoids especially in the LHC complex as well as structural lipids. The latter suggest evolutionary scenario where Lhca1 and Lhca2 coevolved with LHCII and Lhca4 coevolved with CP29. Photosystem I emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. The recently determined structure of plant PSI, which is at the top of the evolutionary tree of this kind of complexes, provided the first relatively high-resolution structural model of the supercomplex containing a reaction center and a peripheral antenna complexes. The structural information was used to generate a scenario of PSI evolution at the last 3.5 billion years.
Recently an operon encoding PSI was identified in cyanobacterial marine viruses. A PSI that mimics the most important feature was generated in *Synechocystis* 6803. This PSI is promiscuous for its electron donor and can accept electrons from respiratory cytochromes. The wild type and promiscuous PSI complexes were isolated, crystallized and their structure was solved to 4 and 3.3 Å resolution, respectively. In addition we solved the structure of PsaL minus mutant at 2.8 Å resolution. The novel structure of PSI from mesophilic cyanobacterium is different in several aspects in comparison with the previously published structure of PSI from *Thermosynechococcus elongatus*. The common properties of mesophilic cyanobacterial and plant PSI will be discussed.

Structural Study of the GAL Regulon in *S. cerevisiae*

Tali Lavy[1], P. Rajesh Kumar[2], Hongzhen He[2], Leemor Joshua-Tor[2], [1]Weizmann Institute of Science, Rehovot, Israel; [2]Cold Spring Harbor Laboratory Cold Spring Harbor, NY 11724 USA

A wealth of genetic information and some biochemical analysis have made the *GAL* regulon of the yeast *Saccharomyces cerevisiae* a classic model system for studying transcriptional activation in eukaryotes. Galactose induces this transcriptional switch, which is regulated by three proteins: the transcriptional activator Gal4p, bound to DNA; the repressor Gal80p; and the transducer Gal3p. We showed previously that NADP appears to act as a trigger to kick the repressor off the activator. Sustained activation involves a complex of the transducer Gal3p and Gal80p mediated by galactose and ATP. We solved the crystal structure of the complex of Gal3p–Gal80p with α-D-galactose and ATP to 2.1 Å resolution. The interaction between the proteins occurs only when Gal3p is in a "closed" state induced by ligand binding. The structure of the complex provides a rationale for the phenotypes of several well-known Gal80p and Gal3p mutants as well as the lack of galactokinase activity of Gal3p

Bridging Knowledge Gaps in Cytidine Deamination: New crystal structures of a dCMP deaminase provide details on the allosteric mechanism and functional insights into the wider zinc dependent cytidine deaminase family.

Ailie Marx and Akram Alian, Technion – Israel Institute of Technology, Technion City, Haifa 32000 Israel

Zinc dependent cytidine deamination is a physiologically essential process important in diverse biological activities from the pyrimidine salvage pathway and lipid metabolism to the antibody diversification process and innate immune defense. The various family members target cytidine within different substrate contexts including DNA/RNA strands (e.g. APOBEC enzymes) and dCMP or free cytidine. dCMP deamination is distinctive due to its allosteric control and we have obtained several unique X-ray crystal structures of this enzyme including the first reported crystal structure of a dCMP deaminase in complex with the allosteric inhibitor dTTP. These new crystal structures not only provide a basis for understanding the mechanism of allosteric regulation and substrate specificity in dCMP deamination but also provide functional insights into the wider zinc dependent cytidine deaminase family. The functional importance of residues identified as critical for all family members has been supported by activity assays on dCMP and APOBEC3G mutants. Additionally, taking advantage of the structural homology between the different cytidine deaminase family members, we have constructed mutants and chimeras with the aim of defining and manipulating substrate recognition on various members of this family of enzymes.

Targeting HIV using Structure-Based Rational Design of Antibodies

Ron Diskin, Weizmann Institute of Science, Rehovot Israel

Isolating and studying broadly anti HIV neutralizing antibodies from infected individuals is a major effort in the combat against HIV. I will present crystallographic study of NIH45-46, one of the most potent anti HIV-1 antibody ever described that targets the CD4 binding site on the HIV-1 spike. The structure of NIH45-46 in complex with gp120 enabled a structure-based rational design to improve NIH45-46. Structural analysis suggested that substitution of the CDRH2 residue Gly54 might allow NIH45-46 to utilize a unique hydrophobic pocket on the surface of gp120 that is normally accommodating Phe43 of CD4. The mutated antibody exhibits a remarkable increase in breadth and potency against HIV-1, compared with NIH45-46 and is effective in controlling viremia *in-vivo* when combined with other potent neutralizing antibodies. A second-generation rationally designed antibodies with greater potency and the ability to target emerging HIV-1 escape mutants will also be presented. Altogether, this study provides potential antibody-candidates for passive immunization efforts and provides useful insights for vaccine design.

Crystallization and structure of the predicted magnetite interacting associated protein loop from magnetotactic bacteria.

Hila Nudelman, Geula Davidov and Raz Zarivach

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Magnetotactic bacteria (MTB) can navigate through the earth magnetic field. These bacteria synthesize organelles called "megnetosomes", which are contain magnetic nanoparticle (Fe_3O_4) or greigit (Fe_3S_4) and surrounded by lipid membrane[1]. It was shown that magnetosome membrane contains a unique set of proteins that are thought to direct the biomineralization of magnetite crystals. In MTB, most of the magnetosome formation involved genes that are located in genomic magnetosome island (MAI)[2]. One of the proteins that involved in biomineralization of magnetite crystals is an integral membrane protein. It is small protein (~15kDa) with two transmemebrane helices. To understand its function we attached its magnetosomal loop (located between H1 to H2) onto The C-terminal of MBP (maltose binding protein). By using X-ray crystallography we determined the MBP -loop structure from magnetotactic bacteria to 2.6A. Based on our results we identify a possible patch which may be important to biomineralization of magnetite and used this structure to predict homologues proteins from other MTB species.

[1] C. Jogler, M. Kube, S. Schübbe, S. Ullrich, H. Teeling, D. a Bazylinski, R. Reinhardt, and D. Schüler, "Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides further evidence for horizontal gene transfer.," *Environmental microbiology*, vol. 11, no. 5, pp. 1267–77, May 2009.

[2] A. Komeili, "Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria.," *FEMS microbiology reviews*, vol. 36, no. 1, pp. 232–55, Jan. 2012.

Probing halogen bonding in porphyrin assemblies

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Halogen bonding is one of the most investigated fields in crystallography in recent years[1]. Halogen bonds have been exploited as a significant tool for crystal engineering with molecular and coordination compounds. Halogen interactions are specific and directional, and they have been proven to play a crucial role in supramolecular materials. However, only a few examples of porphyrin structures based on halogen-pyridyl linkers are available[2]. The expression of halogen bonds in organic crystal structures is often affected by the presence of other non-covalent interactions. In this work, we explored a series of six-coordinate Sn-porphyrin complexes with various substitutions of the halogen and pyridyl functions on the porphyrin and the axial ligands. It was aimed to explore the mutual effect of the competing non-covalent interactions in the analyzed assemblies. Theoretical investigations have probed the electrostatic potentials of a number of halogen bonded complexes, to study the fundamental features of these interactions in the porphyrin environment. The results led to interesting observations regarding the competitive nature of the halogen and hydrogen bonding interactions.

References:

1. Parisini E, Metrangolo P, Pilati T, Resnati G and Terraneo G. *Chem. Soc. Rev.* **2011**, 40, 2267-2278.

2. Hatem M. Titi, Anirban Karmakar and Israel Goldberg, *J. Porphyrins Phthalocyanines*, **2011**; 15, 1250-1257. Sankar Muniappan, Sophia Lipstman and Israel Goldberg, *Chem. Commun.*, **2008**, 1777–1779

Stability of Caffeine-Glutaric acid polymorphic cocrystals

Ranjit Thakuria and William Jones, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW (UK)

The stability of an API to atmospheric moisture is an important factor in the pharmaceutical industry as relative humidity varies from geographical region to region and is also related to drug processing, packaging, formulation and storage. It is known that some drug formulations are stable under specific humidity conditions whereas some decompose or convert to hydrates at a high relative humidity. In such cases, solid form selection is often employed to search for a polymorph, cocrystal or salt form that exhibits greater stability.

Caffeine is an API which exhibits instability with respect to humidity and forms a non-stoichiometric hydrate. In order to avoid hydration issues cocrystals of caffeine with dicarboxylic acids have been prepared.[1] Among them, caffeine and glutaric acid show two 1:1 cocrystal polymorphs which may be synthesised by liquid assisted grinding and solution crystallization methods. These polymorphs are further studied in this work

We studied the relative stability of single crystal and powder samples of caffeine-glutaric acid cocrystals with respect to relative humidity. The surface topography of the two cocrystal polymorphs have been studied using atomic force microscopy. Based on our experimental analysis we propose a mechanism of phase transition and dissociation of caffeine-glutaric acid polymorphic cocrystals under high humidity conditions.

1. A. V. Trask, W. D. S. Motherwell, W. Jones, *Chem. Commun.* **2004**, 890-891; *Cryst. Growth Des*. **2005**, *5*, 1013-1021.

An unusual mechanism for regulating ubiquitination by the deubiquitinating enzyme OTUB1

Reuven Weiner, The Hebrew University in Jerusalem, Hadassa Medical Center, Jerusalem, Israel: TBA

Ubiquitination of histones in response to DNA double strand breaks (DSB) promotes recruitment of repair proteins to chromatin. UBC13 is an ubiquitin conjugating enzyme (E2) that heterodimerizes with UEV1a and synthesizes K63–linked polyubiquitin (K63Ub) chains at DSB sites in concert with the ubiquitin ligase (E3), RNF168. K63Ub synthesis is regulated in a non-catalytic manner by the deubiquitinating enzyme, OTUB1, which binds preferentially to the UBC13~Ub reaction intermediate. My goal was to determine the molecular mechanism by which OTUB1 inhibits ubiquitin chain formation by UBC13.

Using crystallography and biochemistry I found that OTUB1 binding to UBC13~Ub is allosterically regulated by free ubiquitin, which binds to a second site in OTUB1 and increases its affinity for UBC13~Ub, while at the same time disrupting interactions with UEV1a. In addition, OTUB1 binds to a site on UBC13 that overlaps with the RING E3 binding site, thereby providing an additional component to inhibition of E3-catalyzed ubiquitination. My work explains how OTUB1 inhibits UBC13 as well as other E2 enzymes in non-catalytic manner.

High resolution crystal structure of the acetylxylan esterase Axe2 from *Geobacillus stearothermophilus*

Onit Alalouf[1], Shifra Lansky[2], Hodaya V. Solomon[2], , Gil Shoham[2], Yuval Shoham[1]

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The intracellular acetylxylan esterase, Axe2, from the thermophilic soil bacterium *Geobacillus stearothermophilus* T-6, removes acetyl groups from xylo-oligosaccharides. The enzyme is a GDSL hydrolase, using the catalytic triad Ser-His-Asp for hydrolysis, and presents a new undefined family of carbohydrate esterases in the CAZy database. Axe2 was biochemically characterized and its regioselectivity was determined on xylopyranoside per acetate (Alalouf *et al*. JBC 286:41993, 2011). The crystal structures of sel-Met Axe2 and its catalytic mutants, H194A, S15A, and D191A were determined at 1.7, 1.8, 1.9, and 2.12 Å resolutions (Lansky *et al*. Acta Cryst F 1;69(Pt 4):430, 2013), respectively. Conserved residues associated with the oxyanion hole in the GDSL hydrolase family are positioned at distances appropriate for hydrogen bonds stabilizing the tetrahedral intermediate in the reaction mechanism. A single phosphate anion was found in the active site, simulating the position of acetyl group in the oxyanion hole during catalysis. Although the asymmetric unit cell contained a dimer, native Axe2 appears to have a Mw of 200,000 based on gel filtration chromatography suggesting that the enzyme is an octamer. This octameric structure was predicted by the PDBePISA server and indeed transmission electron microscopy (TEM) images of the native protein revealed doughnut-like shape structures of 8-10 nm in diameter in agreement with the crystal structure. Several conserved residues putatively maintaining the octameric structure were subjected to site-directed mutagenesis. Surprisingly the replacement of Y184F resulted in a dimer structure suggesting that a single hydroxyl group is crucial for maintaining the native octameric configuration of Axe2. The salt bridge R55-E105, however, seems to be less crucial for the octamer, nonetheless, both R55A and R55E gave two structural states at different salt concentrations. The W190I replacement resulted in lower activity demonstrating the importance of the octameric configuration in stabilizing a loop in the active site. Additional residues are now being replaced to verify their exact role in the formation of the octamer and their contribution to the activity and thermal stability of the protein.

Insights into the molecular mechanism of aminoglycosides action against leishmaniasis

Moran Shalev, Noam Adir and Timor Baasov, Shulich Faculty of Chemistry, Technion, Haifa 32000, Israel,

Leishmaniasis, a parasitic disease caused by protozoa of the genus *Leishmania,* affects millions of people worldwide, appearing mainly in tropical and subtropical areas. The disease is transmitted by infected species of sand fly, and can be fatal if untreated. The current state-of-art in treating leishmaniasis is based on combined chemotherapy of limited array of drugs.

Aminoglycosides are mostly known as highly potent, broad-spectrum antibiotics that exert their antibacterial activity by selectively targeting the decoding A-site of the bacterial ribosome, leading to aberrant protein synthesis. Recently, aminoglycosides containing a 6'-OH group were highlighted as excellent candidates for the treatment of leishmaniasis. Nevertheless, although some aminoglycosides have already been clinically approved and are currently used worldwide for the treatment of leishmaniasis, the mechanism of which aminoglycosides induce their deleterious effect on leishmaina is rather obscure. Based on high conservation of aminoglycosides binding site in bacteria among all kingdoms, it is assumed that the putative binding site of these agents in Leishmania is the ribosomal A-site. However, while the recent X-ray crystal structures of the bacterial ribosome in complex with aminoglycosides shed light on the mechanism of aminoglycosides action as antibiotics, no such data is presently available regarding to their putative binding site in leishmania.

Herein we present the crystal structures of two aminoglycosides bound to their leishmanian binding site: G418, a potent aminoglycoside for the treatment of leishmaniasis at a 2.6Å resolution, and Apramycin, a strong binder for the leishmanian ribosome at 1.4Å resolution. The observed data provides the first demonstration of aminoglycosides binding to leishmania ribosomes; therefore illuminates the understanding of aminoglycosides mode of action in leishmania at the molecular level. The observed structural data sets ground for rational design of new derivatives as potential therapeutic agents against leishmaniasis.

Structural studies of DNA recognition by the restriction endonucleases Bse634I, EcoRII and BfiI

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Due to their unique specificity, restriction endonucleases have gained widespread application as indispensable tools for *in vitro* manipulation and cloning of DNA. The goal of this work was to explore specificity-structure relationships within the PD-(D/E)XK and phospholipase D (PLD) superfamily enzymes using X-ray crystallography. The studied proteins were Bse634I and EcoRII restriction endonucleases belonging to the Cfr10I/NgoMIV/Bse634I branch of the PD-(D/E)XK superfamily and the BfiI restriction endonuclease from the PLD superfamily. The crystal structures of the Bse634I mutant (R226A) complexed with two alternative target sites 5'-ACCGGT and 5'-GCCGGC have been solved. The analysis of the crystal structures revealed for the first time that degenerate base-pair recognition by Bse634I is achieved through the combination of direct and indirect readout mechanisms. The crystal structures of the N- and C-terminal domains of EcoRII solved in the DNA bound form revealed different structural mechanisms used for recognition of the same target sequence 5'-CCWGG. The C-terminal domain of EcoRII (EcoRII-C) flips out the central A/T nucleotides while interacting with its target site, enabling EcoRII-C to use symmetric conserved structural elements for the recognition of the CCGG core sequence. The crystal structure of the EcoRII N-terminal domain (EcoRII-N) provided the first glimpse into the B3 family domain in its DNA bound form. Finally, the crystal structure of the C-terminal DNA binding domain of BfiI (BfiI-C) bound to the target site 5'-ACTGGG allowed for the first structural comparison of two B3-family domains, EcoRII-N and BfiI-C, in their DNA-bound form.