The Annual Meeting of the Israeli Center of Research- Excellence (I-CORE) in Integrated Structural Cell Biology

Tuesday, May 27th, 2014

The David Lopatie Conference Centre, Weizmann Institute of Science

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Program

08:45-9:00 Gathering & light refreshments

09:00-9:15 **Prof. Gideon Schreiber**

Overview of activities of the first year, and plans for the second year

Session 1 **Chair: Dr. Ron Diskin,** Weizmann Institute of Science

09:15-9:40 **Or Szekely**, Weizmann Institute of Science

The Potential of Ultrafast 2D NMR in Kinetic Studies of Protein Folding (1)

09:40-10:05 **Dr. Doron Levin**, Weizmann Institute of Science

Multifaceted Activities of Type I Interferon Are Revealed by a Receptor Antagonist (2)

10:05-10:30 **Dr. Aya Narunskym**, Tel Aviv University

Proposing ensemble of conformations for a query protein with known structure using the ConTemplate server (3)

10:30-10:55 **Prof. Haim Wolfson**, Tel Aviv University

Computational modeling of large multimolecular complexes by integration of experimental data at various resolutions (4)

10:55-11:20 Coffee break

Session 2 **Chair: Dr. Meytal Landau,** Technion

11:20-11:45 **Dr. Sarel-Jacob Fleishman**, Weizmann Institute of Science

Design of novel protein function in antibodies and other proteins (5)

11:45-12:10 **Ori Braten,** Technion

A Systematic Approach to Identify Proteins Targeted for Proteasomal Degradation Following Mono- Rather than Polyubiquitination (6)

12:10-12:35 **Dr. Ron Diskin**, Weizmann Institute of Science

High-throughput screen for domain boundaries determination (7)

12:35-13:00 **Prof. Joel Sussman**, Weizmann Institute of Science

Proteopedia - an Outreach Tool for I-CORE (8)

13:00-14:00 Lunch

Scientific Board to meet in Presidential reception room

14:00-14:35 **Prof. Roland Dunbrack**

Session 3 **Chair: Prof. Nir Ben-Tal**, Tel Aviv University

14:35-15:00 **Dr. Meytal Landau**, Technion

Structure-based Design of Novel Antibacterial Drugs (9)

15:00-15:25 **Dr. Yael Mutsafi**, Weizmann Institute of Science

Shooting Stars with Cysteine Probes: Pulling Out Protein Components of the Mimivirus Stargate (10)

15:25-15:50 **Or Matalon**, Weizmann Institute of Science

Towards quantitative mapping of protein interactions in vivon (11)

15:50-16:15 **Prof. Arie Admon**, Technion

The nature and scale of contributions by defective ribosome products to the HLA Peptidome (12)

16:15-16:45 Coffee break

Session 4 **Chair: Dr. Emmanuel Levy**, Weizmann Institute of Science

16:45-17:10 **Prof. Nathan Nelson**, Tel Aviv University

Crystal structure of Photosystem I Complexes (13)

17:10-17:20 **Prof. Gideon Schreiber** Closing remarks

The Potential of Ultrafast 2D NMR in Kinetic Studies of Protein Folding

Or Szekely

Weizmann Institute of Science, Department of Chemical Physics

Two-dimensional Nuclear Magnetic Resonance (2D NMR) plays a uniquely important role in structural biology. Conventional acquisitions of 2D NMR spectra are generally on the time scale of minutes to hours, which is too long to observe dynamic processes of interest. Recent developments in the Frydman group [1] have made it possible to acquire 2D spectra in a single scan. The protocols developed in the Frydman group bypass the need for collecting a step-by-step incremented indirect dimension evolution time, encoding instead the same information along a spatial dimension of the sample.

This *spatio-temporal encoding* is done using a combination of frequency-swept pulses and gradients, and of an acquisition sequence where the data is decoded during free precession under gradients [2-4]. This form of *ultrafast* (UF) NMR thus accelerates the acquisition of a two-dimensional spectrum by reducing the number of required transients to just a single scan.

Biophysical processes, such as protein folding, may take place on a timescale of milliseconds-to-seconds, and are therefore suitable candidates for studies by means of ultrafast 2D NMR. Thus, with the reduced acquisition time inherent to UF 2D NMR, and with additional sensitivity gained from a careful optimization of the experimental parameters, the method can be adapted to monitor folding processes of proteins at mM concentrations. This work aims to find the appropriate conditions and parameters to increase signal-to-noise ratio (SNR) and to produce a high-quality time-resolution series for the application of UF NMR in the kinetic studies of protein folding and protein dynamics.

1. Frydman, L., Scherf, T., and Lupulescu, A., Proceedings of the National Academy of Sciences, 2002. 99(25): 15858-15862.

2. Gal, M., and Frydman, L., in Encyclopedia of Magnetic Resonance. 2008, John Wiley & Sons, Ltd.

3. Mishkovsky, M., and Frydman, L., Annual Review of Physical Chemistry, 2009. 60(1): 429-448.

4. Tal, A., and Frydman, L., Progress in Nuclear Magnetic Resonance Spectroscopy, 2010. 57(3): 241-292.

Multifaceted Activities of Type I Interferon Are Revealed by a Receptor Antagonist

Doron Levin, Victoria Urin and Gideon Schreiber

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Type I interferons (IFNs) are a family of homologous multifunctional cytokines that activate different cellular responses by binding a common receptor consisting of two subunits, IFNAR1 and IFNAR2. However, the molecular basis driving differential activation is still not well understood. We utilized an IFNα2 mutant named IFN-1ant that binds tightly to IFNAR2 whereas binding to IFNAR1 is undetectable. IFN-1ant activates only the robust antiviral activity of IFN, while antagonizing its tunable immunomodulatory and antiproliferative activities. IFN-1ant antagonistic properties were also demonstrated in SIV-infected monkeys, ultimately resulting with progression to AIDS and death. Further mutating the IFNAR1 binding site of IFN-1ant resulted in an improved modulator with enhanced antiviral activity, but still no antiproliferative activity. This suggests that both IFNα2 variants can be interesting candidates for the treatment of virus infection without inducing the immunomodulatory functions of wild type IFN. Comprehensive analysis of IFN-induced gene expression revealed that the robust antiviral activity of IFN is driven by a set of IFN sensitive genes that are controlled by canonical IFN response elements, which also drive their high basal expression. Conversely, no enrichment of these elements was found in the promoters of tunable genes. In addition, while robust genes are similarly activated in five different cell lines, the antiviral activity is virus and cell-type-specific. On the contrary, tunable gene activation is cell-line-specific and is related to the antiproliferative potency of IFNs in the different cell lines.

Proposing ensemble of conformations for a query protein with known structure using the ConTemplateserver

Aya Narunsky †, Haim Ashkenazy§, Rachel Kolodny‡ and Nir Ben-Tal†

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Proteins often alternate between several conformations, e.g., active and inactive states of receptors, open and closed states of channels, etc. However, in many cases only one conformation is known. The prediction of additional (biologically-relevant) conformations of a protein can provide more insight into its function in health and disease. We suggest here a method and a web-server we developed aiming at predicting such possible conformations. Our method, ConTemplate (http://bental.tau.ac.il/contemplate), suggests an ensemble of conformations for a query protein with at least one known conformation, based on its similarity to other proteins in the protein databank (PDB), and alternative conformations of these proteins.

Briefly, ConTemplate creates an ensemble of conformations for the query using the following three steps process. First, the entire PDB is scanned, and proteins whose structural-similarity to the query is above a preset threshold are collected. Second, for each of the collected proteins, additional known conformations are indicated, and clustered. In the third step, the server calculates models of the query in various conformations using the structure-based sequence alignments found in the first step, and the centers of the clusters found in the second step as templates.

We demonstrate the method with the kinase domain of the Epidermal Growth Factor Receptor (EGFR). Using the inactive conformation as our query, we reproduce the active conformation with root mean square deviation (RMSD) of 1.76Å, based on the query's structural similarity to the inactive conformation of Abl tyrosine-kinase, together with the known active conformation of the latter kinase. The sequence identity between the two kinase domains is only 40%, and the fact that they share similar active and inactive conformations might not be obvious.

The idea of inferring new conformations of a protein of interest based on known conformations in related proteins is not new. However, to the best of our knowledge, ConTemplate is the first automated implementation of this approach.

Computational modeling of large multimolecular complexes by integration of experimental data at various resolutions

Dan Cohen, Naama Amir, Haim J. Wolfson

Tel Aviv University, School of Computer Science

Modeling large multi-molecular assemblies at atomic resolution is a key task in elucidation of cell function. Since, there is no single experimental method that can deliver atomic resolution structures of such large molecules, hybrid methods, which integrate data from various experimental modalities, are being developed for this task. We have developed a new integrative method, which combines atomic resolution models of individual assembly components with intermediate resolution (8-10 Å) electron microscopy map of the full assembly. Our method also naturally accommodates cross link data, if available. The method was highly successful and efficient on all the intermediate resolution EM complexes from the 2010 Cryo-EM Modeling Challenge. Remarkably, a 6.8 Å resolution 20S proteasome map, consisting of 28 (structurally homologues) units was modeled at 1.5 Å RMSD from native in about 10 minutes on a Core i7 laptop. In case of missing (or poorly modeled) individual subunits, the method can return partial solutions, thus, enabling interactive modeling.

Design of novel protein function in antibodies and other proteins

Sarel J Fleishman

Weizmann Institute of Science, Department of Biological Chemistry

Computational protein design holds great promise for deepening our understanding of the physical principles that underlie biomolecular function and for generating proteins with novel functions including enzymes, diagnostics, and therapeutics. We recently described methods and the first *de novo* designed proteins of potentially broad use, including proteins that neutralize infectivity from a wide range of influenza strains and proteins that serve as affinity reagents for human antibody purification. Despite such successes our methods fail to design large, polar, and concave binding surfaces, limiting their utility. We have now extended computational protein design to generate new antibodies not seen in nature. Antibodies are the most versatile class of binders known, but existing methods for antibody engineering often fail to isolate specific, high-affinity antibodies that target surfaces of choice. We developed a new strategy for computational antibody design, which combines backbone fragments from natural antibodies to generate a very diverse set of potential binders. Each binder's sequence and conformation are refined and the antibody is restricted to interact with a particular surface of choice on the target molecule. The designed antibodies exhibit dozens of mutations relative to mammalian germ-line sequences, suggesting that they span sequence and functional space more broadly than natural antibodies. We implemented a fast computational/experimental characterization cycle in which we compute dozens of designed antibodies using resources provided by Google, experimentally clone the antibodies, and test them using yeast cell-surface display. Results from expression and binding measurements then inform the next design cycle. Thanks to this iterative process we have made significant progress in designing stable antibodies and have isolated one antibody that binds a target protein with no known antibody binders.

Future plans for making computational antibody design robust, reliable, and a viable alternative to conventional strategies will be discussed.

A Systematic Approach to Identify Proteins Targeted for Proteasomal Degradation Following Mono- Rather than Polyubiquitination

Ori Braten, Aaron Ciechanover

Technion - Israel Institute of Technology, Faculty of Medicine

Protein ubiquitination is an important post-translational modification involved in the regulation of numerous basic cellular pathways, including cell cycle, DNA damage response and signal transduction. Typically, protein substrates are conjugated with an ubiquitin chain consists of multiple ubiquitin moieties linked to one another via an isopeptide bond between the C-terminal Gly residue of the distal moiety and the ε-NH2 group of Lys 48 in the proximal one. These polyubiquitinated substrates are the 'canonical' substrates degraded by the 26S proteasome. A chain of four ubiquitin moieties was regarded as the minimal required signal for proteasomal recognition. However, recent studies have surprisingly unraveled several substrates that are degraded following the conjugation of a single or multiple single ubiquitin moieties. This monoubiquitination-mediated proteasomal degradation is an intriguing phenomenon suggesting the existence of different ubiquitin conjugation machineries and proteasomal recognition modes. In our project, we apply an unbiased proteomic approach for systematic identification of poly- and monoubiquitinated proteasomal substrates. Substrate monoubiquitination is enforced in human and yeast cells by replacing the endogenous ubiquitin with a non-polymerizable, lysine-less ubiquitin. This is followed by ubiquitin-protein conjugate enrichment utilizing the emerging technology of immunoprecipitating with antibodies directed against Gly-Gly-Lys. Gly-Gly is the C-terminal of ubiquitin, and the Lys linked to it via an isopeptide bond represents the substrate. An antibody was developed against this tripeptide which is supposed to precipitate Gly-Gly-attached to an endogenous Lys in the substrate. The Gly-Gly is detached from the rest of the upper stream part of the chain by trypsin, which cleaves the Arg residue N-terminal to the Gly-Gly. The immunoprecipitate is resolved and the peptides with the Gly-Gly attached to them are identified and quantified using mass spectrometry. We look for proteins that diminish along time despite not being conjugated by a polymerizable wild type ubiquitin. Our work may enable identification of novel ubiquitin-proteasome substrates, elucidate novel aspects of conjugation and recognition mechanisms, and will help to identify substrates targeted by different modes of ubiquitination (e.g. mono- vs. poly).

High-••throughput screen for domain boundaries determination

Ron Diskin

Weizmann Institute of Science, Department of Structural Biology

A major goal of our lab is to understand the molecular mechanisms that govern transport of membrane proteins into primary cilia. Cilia are important sensory organelles that project from cell membrane and harbor many important receptors and sensory-related signal transduction pathways. Proper function of the cilium relies on active import of membrane imbedded proteins from the ER or the golgi apparatus. A recently described protein complex, termed the BBSome, is mediating this transport.

This is a large protein complex made of 8 different BBS proteins most of them consist of several individual domains. The BBSome was suggested to function as novel coat machinery that can recognize membrane embedded proteins, form membrane-coated vesicles, and mediate their transport to the cilium. The function of the BBSome was so far studied at the cellular level and only little is known about its function at the molecular level. We initiated in our lab a project that is aimed to characterize and investigate the molecular structure of the complete BBSome. Our current efforts focus on producing soluble fragments of the various BBS proteins for structural studies. I will describe a newly established collaborative effort for identifying domain boundaries of the various BBS proteins.

Proteopedia - an Outreach Tool for I-CORE

Joel L. Sussman1, Jaime Prilusky2

Weizmann Institute of Science, 1The Israel Structural Proteomics Center, 2Biological Services Unit

Scientists and students are now able to easily access 3D images of biomacromolecules underlying biological functions and disease. Rather than relying on text and 2D images to try to understand the function of these structures, a collaborative website called *Proteopedia*1,2 provides a new resource by linking written information and 3D structural information. The *Proteopedia* wiki web resource, **http://proteopedia.org**, displays protein structures and other biomacromolecules in an interactive format. Proteopedia works all computer platforms, including PCs, MACs or Linux as well as tablets3 such as iPads and Androids. The interactive images on each page are surrounded by descriptive text containing hyperlinks that change the appearance (such as view, representations, colors or labels) of the adjacent 3D structure to reflect the concept discussed in the text. Some examples of the more the 100,000 *Proteopedia* pages are:

- **http://proteopedia.org/w/HIV-1_protease**
- **http://proteopedia.org/w/Ribosome**
- **http://proteopedia.org/w/Proton_Channels**

Proteopedia can be a very useful tool to disseminate the results of the I-CORE project and examples of how to create your own pages in *Proteopedia*4,5, including

- Adding 3D interactive scenes via a user friendly GUI for Jmol
- Adding text to *Proteopedia* pages, with hyperlinks to the interactive scenes

Proteopedia serves as a outreach tools for students to learn about structural biology, e.g., see a page made by high school students in Wisconsin on the β2-Adrenergic Receptor:

http://proteopedia.org/w/Group:SMART:A_Physical_Model_of_the_β_2-Adrenergic_Receptor

We have established an outreach program, in Israel, to work with high school and undergraduate students to learn to use *Proteopedia* via studying particular areas in structural biology so as to be able to make pages like this the ones discussed above.

References:

1. Hodis, E., Prilusky, J., Martz, E., Silman, I., Moult, J. & Sussman, J. L. (2008). *Genome Biol* **9**, R121.
2. Prilusky, J., Hodis, E., Canner, D., Decatur, W. A., Oberholser, K., Martz, E., Berchanski, A., Harel, M. & Sussman, J. L. (2011). *J Struct Biol* **175**, 244-252.
3. Hanson, R.M., Prilusky, J., Renjian, Z., Nakane, T. & Sussman, J.L. (2013). *Israel J Chem* **53**, 207-216.
4. *Proteopedia* help pages: **http://proteopedia.org/w/Help:Contents**
5. *Proteopedia* Video Guide: Several narrated videos to guide you through using *Proteopedia*: **http://proteopedia.org/w/Proteopedia:Video_Guide**

Structure-based Design of Novel Antibacterial Drugs

Orly Tabachnikov, Mickal Abd Alhadi, Asher Moshe, Hay Dvir, Meytal Landau

Technion - Israel Institute of Technology, Faculty of Medicine

The alarming rapid increase in the occurrence of antibiotic resistance to many common bacterial pathogens calls for an immediate demand for new classes of antibacterial agents. Bacterial tyrosine kinases (BTKs) have recently emerged as potential attractive drug targets, being vital for core bacterial processes while having no homology to mammalian proteins. Accordingly, developing inhibitors to BTKs might offer new safe and effective drugs. We determined the crystal structure of BTK from Burkholderia cepacia (Pseudomonas cepacia), an important human pathogen, which causes pneumonia in immunocompromised individuals, especially those having lung disease such as cystic fibrosis. In collaboration with Nir Ben-Tal, we now aim to identify and characterize novel inhibitors of the B. Cepacia BTK via structure-based drug design.
Another important drug target that makes the bacteria most resilient and resistant to modern antibiotics is the formation of biofilms, which act as a barrier to drug delivery. The major proteinaceous component in many biofilms is amyloid fibers, which are mostly notoriously known because of their involvement in fatal diseases such as Alzheimer's and prion diseases. The knowledge of the molecular structures of amyloids is necessary to control their formation, yet their polymorphic and partially disordered nature hinders structural analysis. Still, atomic structures of disease-associated fibril-forming amyloid peptides were determined by Eisenberg and co-workers (UCLA). Our working hypothesis, based on information accumulated about the biophysical and biochemical characteristics of microbial and disease-associated amyloids, is that they share their β-spine structures. Accordingly, our goals are to identify and characterize fiber-forming segments of microbial functional amyloids, and to determine their atomic structures. The structures will be used to design inhibitors of biofilm formation. We already identified four novel fiber-forming microbial segments.

Shooting Stars with Cysteine Probes: Pulling Out Protein Components of the Mimivirus Stargate

Yael Mutsafi, Yael Fridmann-Sirkis, Deborah Fass, Avi Minsky

Weizmann Institute of Science, Department of Structural Biology

Mimivirus is one of the largest viruses ever identified, so large that it is visible by light microscopy. The mimivirus genome is 1.2 Mbp, larger than the genomes of some bacteria. Mimivirus particles contain at least 114 proteins, most of them not annotated. A remarkable and unique physical feature of the mimivirus virion is the “stargate” on its surface. The five-slitted stargate distinguishes one of the vertices of the icosahedral virion from its quasi-symmetry-related corners. Functionally, the stargate is the portal for DNA release. The stargate opens when the virion is ingested by a host amoeba cell, apparently fusing with the phagosome membrane. In a new interdisciplinary collaboration facilitated by the I-CORE, we are using fractionation of virus-infected cells, electron microscopy, thiol chemistry, and mass spectrometry to identify proteins that make up the mimivirus stargate. These experiments are among the first to dissect the molecular identity and structural positions of individual proteins in the mimivirus virion. Furthermore, a molecular characterization of the stargate paves the way toward understanding its dynamic role in mimivirus infection.

Towards quantitative mapping of protein interactions in vivo

Or Matalon

Weizmann Institute of Science, Department of Structural Biology

Proteins interact with each other to execute most of the processes in living cells. Several methods are routinely used to map the physical organization of proteins in cells, and include tandem affinity purification coupled to mass-spectrometry (TAP-MS) or Yeast Two Hybrid (Y2H). These methods, however, only provide binary information and cannot be used to measure the affinity of an interaction between two proteins. Yet, knowing the affinity of an interaction is fundamental for the understanding of cellular processes involving, e.g., protein dosage sensitivity or dynamics of complex assembly. We thus aim to develop an assay that (i) can be applied at high-throughput, (ii) can be used in vivo, and (iii) that is quantitative. We will show preliminary results indicating that the DHFR-PCA, a protein interaction assay based on the reconstitution of a split enzyme, can meet all three requirements. Proteome-wide interactions measurements using this method will ultimately enable us to build the first quantitative protein interaction network.

Defining the MHC peptidome production pipeline by mass spectrometry based immunopeptidomics

Arie Admon

Technion - Israel Institute of Technology, Faculty of Biology

We have attempted to shed new light on the MHC peptidome production pipeline of human cancer cells. The immunopeptidomes analyses were based on large-scale dynamic-SILAC labeling of cultured cells, followed by immunoaffinity purification of the HLA molecules and capillary chromatography combined with Orbitrap mass spectrometry. Using this methodology, with and without proteasome inhibitors, we were able to follow the processing pathways that lead to the production of the immunopeptidome. The more interesting effects were observed with untreated cells and by comparing their dynamics to cells inhibited with proteasome inhibitors, such as epoxomicin and bortezomib (Velcade). The inhibitors affected in a complex manner the rate of synthesis of the cellular proteins as well as their degradation, cellular transport and formation of MHC peptidome. While (as expected) the proteasome inhibitors reduced the rates of degradation of many cellular proteins, they increased the degradation (and synthesis) rates of others. Correlating between the rates of synthesis of the source proteins of the different MHC peptides and the rates by which these newly synthesized proteins are processed into MHC peptides suggests that the contribution of the proteasomal proteolysis to the production of the HLA immunopeptidome is lower than previously thought. Furthermore, the analysis suggest that the production of HLA peptides is derived significantly from newly synthesized proteins, many of which are defective ribosome products (DRiPs) and short lived proteins (SLiPs), which were defined in this study. This is in contrast to contribution from old proteins, which finished their functional life times in the cells (retirees).

Crystal structure of Photosystem I Complexes

Yuval Mazor, Anna Borovikova, Ilanit Greenberg, Hila Toporik and Nathan Nelson

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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 3.8 Å resolution, respectively. In addition we solved the virus-like structure of PsaL and PsaI minus PSI 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.
Plant Photosystem I (PSI) is one of the most intricate membrane complexes in Nature. It comprises two loosely bound reaction center and light-harvesting (LHC) complexes containing 18 subunits 173 chlorophylls, 30 carotenoids 2 quinines and 3 ironsulfur clusters. This assembly operates with a quantum yield of close to 1 and keeps its efficiency at a large scale of light intensities. The crystal structure at 3.1 Å resolution reported her add several new features that were not detected in the previous structures.
An improved electron density map yielded identification and tracing of subunit PsaK, PsaN and PsaG. The location of an additional ten b-carotenes as well as five chlorophylls and several loop regions, are now modeled. This represents the most complete plant Photosystem I structure obtained thus far, revealing the locations of and interactions among 18 protein subunits and 211 non-covalently bound photochemical cofactors.
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 of much better resolution of several chlorophyll molecules we detected 12 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.