The Israel Structural Proteomics Center (ISPC): From Gene to Structural Analysis

Department of
Structural Biology

Prof. Joel L. Sussman

Yigal Burstein, Gideon Schreiber, Israel Silman, Yoav Peleg, Tamar Unger, Orly Dym, Shira Albeck, Jaime Prilusky, Anna Branzburg, Yossi Jacobovitch, David Mualem, Anat Hodis, Ada Dantes, Reut Rotem-Bernheim, Metial Rubin-Yona, Moshe Ben-David, Eran Hodis

☎ 972 8 934 4531
FAX 972 8 934 4159
@ joel.sussman@weizmann.ac.il
www.weizmann.ac.il/ISPC

With the success of the genome project, a great deal of life science research has shifted to the functional and structural analyses of proteins. The use of high-throughput technologies to aid in 3D structure determination of proteins and protein complexes, i.e. *Structural Proteomics* (SP), can help identify functions of putative genes, and contribute to the understanding of how proteins function as well as to how they interact with protein and nucleic acid partners. These in turn, contribute to our understanding of the principles that underlie all cellular processes as well as to structure-based drug design.

The Israel Structural Proteomics Center (ISPC) has assembled a robust pipeline for SP research that is part of the European SPINE2-Complexes initiative (http://www.spine2.eu/SPINE2) to determine the 3D structures of proteins and their complexes. The ISPC provides service to the Weizmann Institute (WIS) and to the entire Israeli scientific community. Its main goal is to determine the 3D structures of proteins and protein complexes related to human health and disease, so as to aid in understanding their biochemical mode of action and its relationship to the underlying biology. To be able to handle multiple targets, the ISPC has developed methodologies for cloning, expression, purification, crystallization, structure determination and structure analysis. The center also accepts purified proteins for crystallization, as well as providing highly purified proteins for other biochemical and biophysical studies. The ISPC has developed bioinformatic tools, an interactive web resource that presents 3D structures (*Protopedia*) as well as a Proteomics Laboratory Universal Management System (PLUMS). We are continuously engaged in the development of new methodologies as well as in implementation of state-of the art technologies at all stages of the pipeline. In the last year, the ISPC has seen the fruit of the investment; we have solved and analyzed many new structures, and have contributed to several intriguing studies performed in the labs of Weizmann Institute (WIS) scientists. These activities, of which a few are described below, illustrate the interdisciplinary contribution of such a center to scientific activity at the WIS.

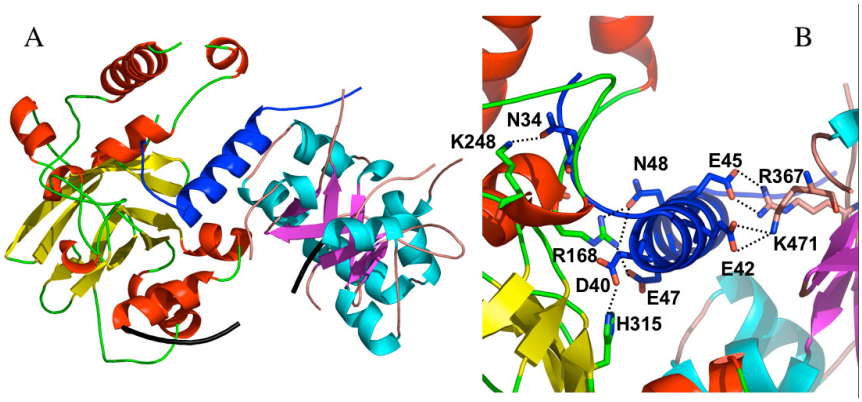

Fig. 1 *The VirE1-VirE2 complex. A) Ribbon representation of the two domains of VirE2 showing the N terminal domain depicted in red and yellow, while the C terminal domain is shown in cyan and magenta. The inter-domain flexible linker is shown in black. The helix of VirE1 is shown in blue. B) Electrostatic interactions between residues from VirE1 and VirE2.*

Following several years' efforts in collaboration with Prof. Michael Elbaum (Dept. of Materials and Interfaces), the ISPC has solved the structure of the complex of VirE1-VirE2 from *Agrobacterium tumefaciens*. This bacterium infects its plant hosts by a mechanism of horizontal gene transfer. This capability has led to its widespread use in artificial genetic transformation. In addition to DNA, the bacterium delivers an abundant single-stranded DNA (ssDNA) binding protein, VirE2, whose roles in the host include protection from cytoplasmic nucleases and adaptation for nuclear import. In *Agrobacterium*, VirE2 is bound to its acidic chaperone VirE1. When expressed *in vitro*, in the absence of VirE1, VirE2 is prone to oligomerization, and forms disordered filamentous aggregates. These filaments adopt an ordered solenoidal form in the presence of ssDNA, which was characterized previously by EM and three-dimensional image processing (Abu-Arish et al, 2004). VirE2 co-expressed *in vitro* with VirE1 forms a soluble heterodimer. VirE1 thus prevents VirE2 oligomerization and competes with its binding to ssDNA (Frenkiel et al, 2007). The ISPC has solved the crystal structure of VirE2 in complex with VirE1, showing that VirE2 is composed of two independent domains presenting a novel fold, joined by a flexible linker (Figure 1A). Electrostatic interactions with VirE1 cement the two domains of VirE2 into a locked form (Figure 1B). Comparison with the EM structure indicates that the

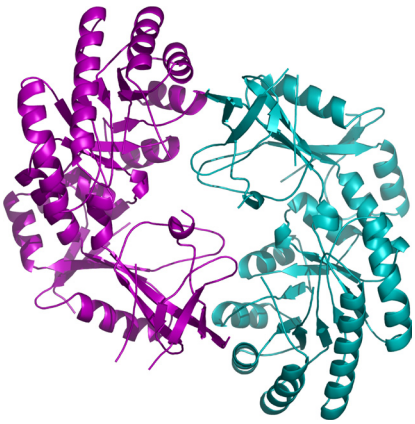

Fig.2 *Crystal structure of AzI showing its two monomers (cyan and purple). Analysis of the structure and biochemical studies have shown that this is a non-physiological dimer (see Figure 3).*

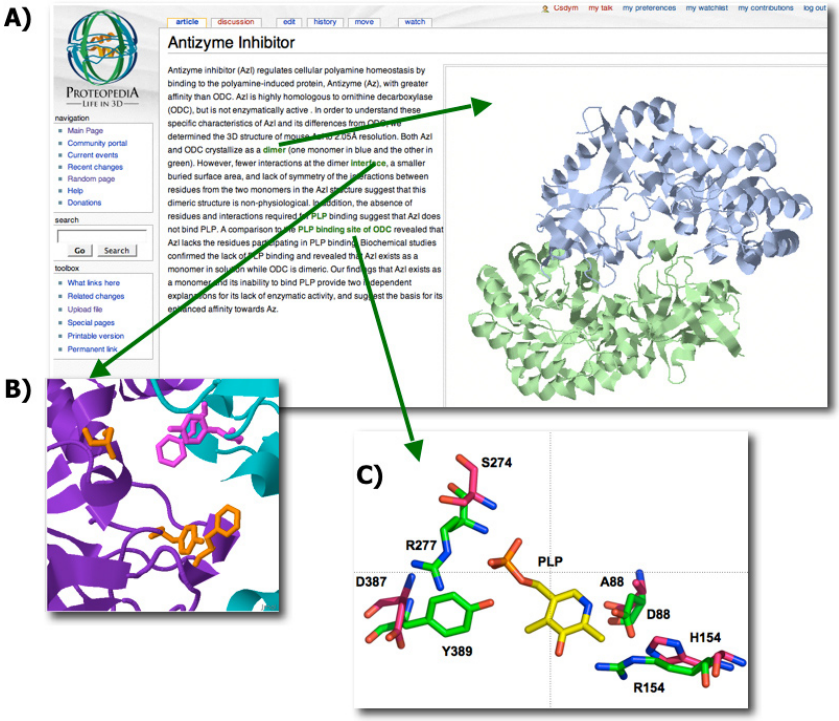

Fig. 3 *Page from Proteopedia showing the scenes that load when the user clicks on the green links; A) "dimer" showing the dimer seen in the structure of AzI (PDB 3BTN); (B) "interface" showing the few interaction in the interface between the two monomers of AZI; (C) "PLP binding site of ODC" compares the PLP binding residues in ODC and the corresponding residues in AzI.*

VirE2 domains adopt different relative orientations. Thus, the flexible linker between the domains enables VirE2 to accommodate its different binding partners. With the VirE2 structure in hand, the design of engineered analogs, which will broaden the range of susceptible hosts for gene transfer, will be possible.

The study of Antizyme inhibitor (AzI), in collaboration with Prof. Chaim Kahanna (Dept. of Molecular Genetics), is an example of interdisciplinary work involving biochemical studies which stemmed from structural analysis performed at the ISPC (Albeck et al, 2008). AzI regulates cellular polyamine homeostasis by binding to the polyamine-induced protein, Antizyme (Az), with greater affinity than ornithine decarboxylase (ODC).

AzI is highly homologous to ODC, but is not enzymatically active. In order to understand these specific characteristics of AzI and its differences from ODC, the ISPC determined the 3D structure of mouse AzI to 2.05Å resolution (Figure 2).

Both AzI and ODC crystallize as dimers. However, fewer interactions at the dimer interface, a smaller buried surface area, and lack of symmetry of the interactions between residues from the two monomers in the AzI structure suggest that this dimeric structure is non-physiological. In addition, the absence of residues and interactions required for binding of pyridoxal 5'-phosphate (PLP) suggest that AzI does not bind PLP. Biochemical studies confirmed the lack of PLP binding, and revealed that AzI exists as a monomer in solution while ODC is dimeric. The findings that AzI exists as a monomer and its inability to bind PLP provide two independent explanations for its lack of enzymatic activity, and suggest the basis for its enhanced affinity towards Az.

Part of an ongoing project with Prof. Adi Kimchi (Dept. of Molecular Genetics) is to solve the structure of DAP5/p97. This protein was extensively studied in her laboratory due to its involvement in the cell death process. It was revealed that under apoptotic conditions DAP5/p97 undergoes proteolytic cleavage, giving rise to an 86kDa fragment (DAP5/p86) due to a single caspase cleavage event at DETD^{792} (Henis-Korenblit et al., 2000). The ISPC has solved the crystal structure of a fragment of the C-terminal region of DAP5 which includes the caspase cleavage site at position 792 (manuscript in preparation). The existence of this site is unique to DAP5, as reflected in the structural analysis. The ISPC is currently working on solving the structures of additional domains of DAP5. This will help to elucidate the unique function of DAP5, as compared to eIF4GI/II, and to clarify its role in the regulation of translation during cell death/survival.

The design of enzymes able to display non-native catalytic capabilities is one of the main challenges of protein design, and opens the door to the application of designer enzymes to industrial reactions. The groups of Prof. Dan Tawfik (Dept. of Chemical Biology) and Prof. David Baker (NIH) have designed a non-natural catalytic activity (Kemp elimination) on an existing TIM barrel scaffold, selected through a computational search. The method utilized starts with the design of an idealized active site, in which the transition state is modeled and stabilized by surrounding residues using quantum mechanics calculations. In the Tawfik lab, a first group of functional proteins was isolated with mediocre catalytic activity. These were then subjected to several rounds of directed evolution, resulting in improved versions. One, in particular, accelerates the reaction by six orders of magnitude. The ISPC solved the structures of several of the designed enzymes, thus providing a structural understanding of the enhanced enzymatic activity of the evolved mutants (Röthlisberger D. et al 2008). This work is remarkable because it represents the first *de novo* design of functional enzymes able to catalyze a non-natural reaction.

The ISPC is currently involved in a collaboration with Dr. Oren Zimhony from the Kaplan Hospital that involves a series of proteins from *Mycobacterium*

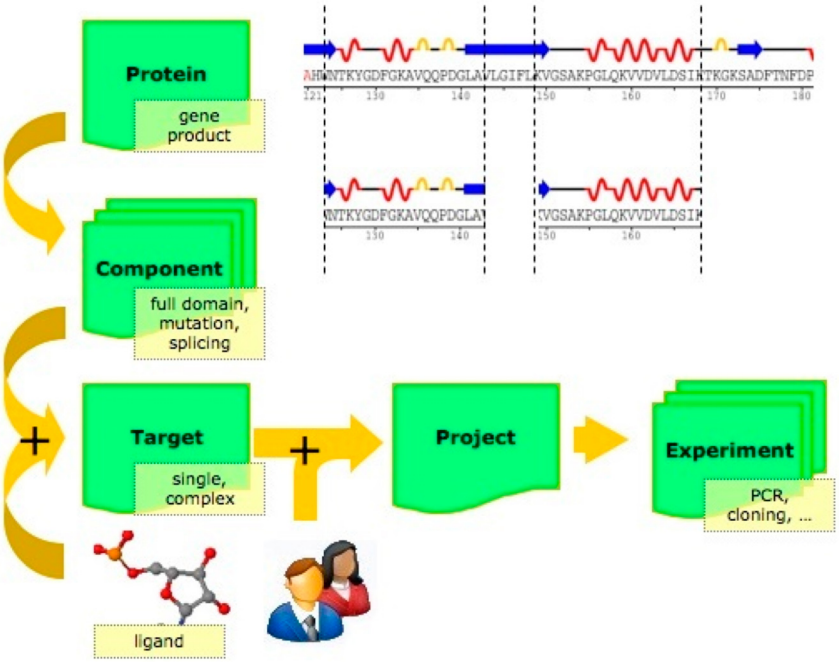

Fig. 4 *Outline of PLUMS (Proteomics Laboratory Universal Management System)*

tuberculosis. Recently, the ISPC has solved the structure of the *M. tuberculosis* AcpS (Acetyl carrier protein synthase) and we are currently analyzing this structure in order to understand how it different from its homologues in other organisms. The ISPC has also isolated and purified two mega-proteins from *M. tuberculosis* which are involved in fatty acid synthesis: Fas1 and PKS13. Both proteins were subjected to crystallization screens. Recently, we obtained crystals of FAS1 that diffract to 4Å resolution. We have shown by biochemical studies that this protein forms hexamers. We hope to be able to solve its structure, which is believed to be the putative target of the principal drug currently used for treatment of tuberculosis. With the structure in hand, we hope to design more effective drugs for combating this lethal disease.

The ISPC has also solved structures of proteins for groups outside the WIS. One example is the structure of the active form of Cyt2Ba carried out in collaboration with Prof. Zaritsky from Ben-Gurion University. This toxin, which is a member of the family of δ-endotoxins expressed by *Bacillus thuringiensis israelensis* (Bti), is of interest due to its potential use as a vehicle for targeted membrane-destruction (manuscript submitted for publication).

Proteopedia

(http://www.proteopedia.org), created at the ISPC by Jaime Prilusky and Eran Hodis, is an interactive web resource that presents 3D structures in an intuitive way by linking descriptive text to 3D images (Figure 3). It provides a graphical narrative accessible to users from all levels of expertise and knowledge. In addition, Proteopedia, which is based on the 'open source' tools of MediaWiki and Jmol, allows for real-time community annotation, via easy-to-use authoring tools, additionally fostering an open atmosphere of information sharing. A web browser is all that is needed to access the site and the 3D information, i.e. there are no downloadable viewers required to install and run.

PLUMS

A Proteomics Laboratory Universal Management System is a Laboratory Information Management System (LIMS) designed and developed for the proteomics area. It covers every step from DNA to protein, from target selection to publication of the protein structure, both on a single protein and on protein complexes (Figure 4). PLUMS is the result of a new concept, design based on the experience gained at the ISPC in development, improvement and utilization of ORALIMS, the ORACLE-based LIMS. ORALIMS is being used at the ISPC on a daily basis for recording all stages from gene to 3D protein structure. Technically, PLUMS is a distributed client-server application that allows concurrent access from multiple users, equipments and laboratories, and can be installed on multiple platforms and operating systems.

The ISPC has expressed and purified proteins for numerous scientific groups at the WIS for biochemical and biophysical analysis. The ISPC has handled individual proteins (ranging from several kDa to over a million kDa), as well as protein complexes. To date, approximately 40 structures have been solved, and over 300 proteins have been purified. Within the next few months, the ISPC will relocate to its new facility in the Meyer Building, and will be able to expand its capacity.

Selected publications

Henis-Korenblit, S., Shani, G., Sines, T., Marash, L., Shohat, G., and Kimchi, A. (2002) The caspase-cleaved DAP5 protein supports internal ribosome entry site-mediated translation of death proteins. Proc Natl Acad Sci U S A, 99, 5400-5405.

Abu-Arish, A., Frenkiel-Krispin, D., Fricke, T., Tzfira, T., Citovsky, V., Wolf, S.G., and Elbaum, M. (2004) Three-dimensional reconstruction of Agrobacterium VirE2 protein with single-stranded DNA. J. Biol. Chem., 279, 25359-25363.

Frenkiel-Krispin, D., Grayer-Wolf, S., Albeck, S., Unger, T., Peleg, Y., Jacobovitch, Y., Michael, Y., Daube, S., Sharon, M., Robinson, C.V., Svergun, D.I., Fass, D., Tzfira, T. and Elbaum, M. (2007) Plant Transformation by Agrobacterium tumefaciens, Modulation of single-stranded DNA-VirE2 complex assembly by VirE1. J. Biol. Chem.,

282, 6, 3458-3464.

Banci, L., Baumeister, W., Heinemann, U., Schneider, G., Silman, I., Stuart, D.I., and Sussman, J.L.

An idea whose time has come. Genome Biology 8, 408 (2007).

Hodis, E., Schreiber, G., Rother, K. and Sussman, J.L. (2007) eMovie: a storyboard-based tool for making molecular movies. Trends Biochem Sci. 32, 199-204.

Tsvetkov, P., Asher, G., Paz, A., Reuven, N., Sussman, J. L., Silman, I., and Shaul, Y. (2008) Operational definition of intrinsically unstructured protein sequences based on susceptibility to the 20S proteasome. Proteins 70, 1357-1366.

Paz, A., Zeev-Ben-Mordehai, T., Lundqvist, M., Sherman, E., Mylonas, E., Weiner, L., Haran, G., Svergun, D. I., Mulder, F. A. A., Sussman, J. L., and Silman, I. (2008) Biophysical Characterization of The Unstructured Cytoplasmic Domain of The Human Neuronal Adhesion Protein Neuroligin 3. Biophysical Journal (in press).

Manicka, S., Peleg, Y., Unger, T., Albeck, S., Dym, O., Greenblatt, H., Bourenkov, G., Lamzin, V., Krishnaswamy, S. & Sussman, J.L. (2008) Crystal structure of YagE, a putative DHDPS like protein from Escherichia coli K12. Proteins (in press).

Albeck, S., Dym, O., Unger, T., Snapir, Z., Bercovich, Z., and Kahana, C. (2008) Crystallographic and biochemical studies revealing the structural basis for Antizyme inhibitor function. Protein Science (in press).

Röthlisberger D, Khersonsky O, Wollacott AM, Jiang L, DeChancie J, Betker J, Gallaher JL, Althoff EA, Zanghellini A, Dym O, Albeck S, Houk KN, Tawfik DS, Baker D "Novel Kemp elimination catalysts by computational enzyme design" Nature (2008) In press

Acknowledgements

JLS is The Morton and Gladys Pickman Professor of Structural Biology

Granting agencies: European Commission Sixth Framework Research and Technological Development Programme 'SPINE2-COMPLEXES' Project, under contract No. 031220, The Israel Ministry of Science, Culture and Sport, Autism Speaks, Israel Science Foundation, Israel Academy of Sciences and Humanities, and Israel Ministry of Trade & Industry

INTERNAL support

Divadol Foundation, The Neuman Foundation, Jean and Jula Goldwurm Memorial Foundation, Helen & Milton A. Kimmelman Center for Biomolecular Structure and Assembly, Benoziyo Center for Neuroscience, Minerva Foundation, Bruce and Rosalie N. Rosen Family Foundation, Kalman and Ida Wolens Foundation, Nalvyco Foundation, M.D. Moross Institute for Cancer Research, a Research Grant from Mr. Erwin Pearl, Yeda