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From Gene to 3D Structure at the ISPC

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The 3D structure of a protein is a key to understanding many of its features, such as identification of its active site, of its domains, its amino acids important for activity and for binding to other proteins. This ultimately contributes to understanding biological processes involving the protein and to the discovery of new lead drugs. Until now, <5% of the unique, non-redundant structures of human proteins have been determined. There is, therefore, a need to speed up all steps on the route from the gene to the protein structure. The Israel Structural Proteomics Center (ISPC) was established by scientists at the Weizmann Institute of Science to increase the efficiency of all the stages on this route, and is supported by the Israel Ministry of Science and Technology. The theme of the center deals with targets related to health and disease.

The ISPC receives DNA coding for protein targets from any species for cloning, expression, purification, crystallization and structure determination. Alternatively, the center will accept purified proteins for crystallization and structure determination. The center has a unique combination of scientific expertise and 'state-of-the-art' instrumentation for high-throughput (HTP) production and crystallization of proteins.

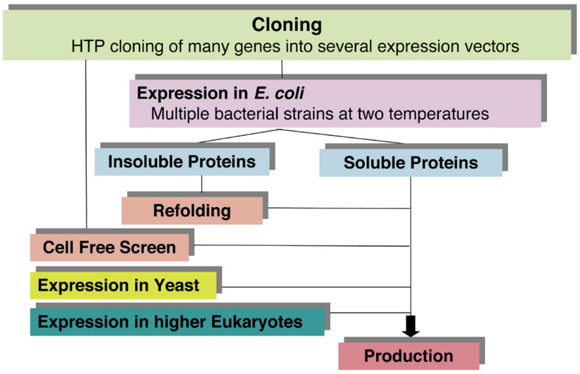

Fig. 1: *Strategy for protein production at the ISPC*

Each target is cloned into multiple vectors, using ligation-independent cloning (Gateway). Expression is extensively screened in several bacterial strains with different fusion proteins. Proteins which are expressed in insoluble form are screened for expression either in bacterial cell-free extracts or in the yeast, *Pichia pastoris*. A baculovirus expression system is being introduced, and will become operative within a year. HTP cloning and expression is assisted by using a liquid-handling workstation robot (Tecan). Parallel purification of up to 6 proteins is performed using an AKTA3D. Purified proteins are screened for crystallization using a Douglas Instruments ORYX6 robot, which employs the batch method under oil. This has yielded a high percentage of high-quality diffracting crystals. The entire process from "gene to 3D structure" is documented in our laboratory information management system (LIMS). Bioinformatics tools incorporated into the LIMS facilitate target analysis such as putative domain determination and identification of potential non-structured regions in the target protein.

The ISPC is now receiving targets from scientists both in academia and industry. We believe that making structural information accessible to the entire scientific community will stimulate novel studies and developments related to health and disease.

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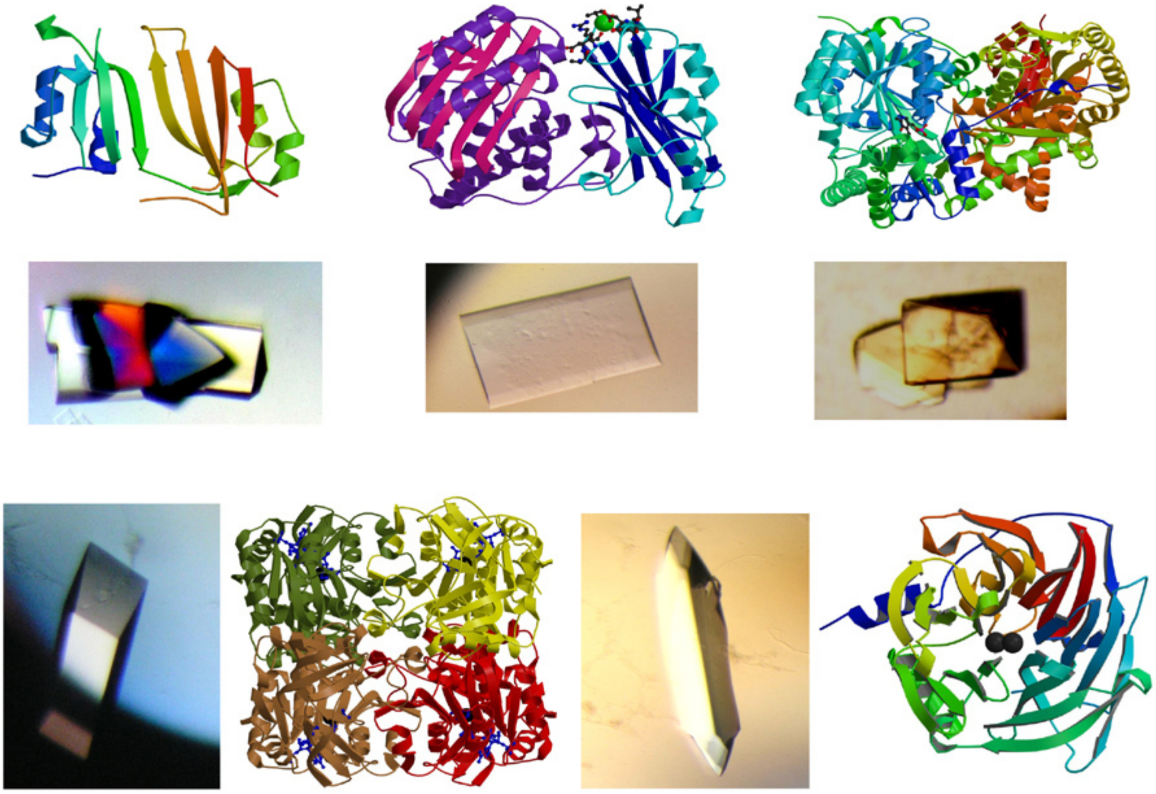

Fig. 2: *Crystallization & Structure Determination*