Abstracts- Day I

High throughput protein at NYSGRC

James D. Love (Albert Einstein College of Medicine, NY)

The New York Structural Genomics Research Consortium has developed state of the art pipelines for the high throughput production of proteins in prokaryotic and eukaryotic hosts to support our structural and functional biology efforts. These highly automated pipelines can process hundreds of targets per week, and focus on the investigation of proteins to better understand enzyme mechanisms and function, human immune function and in a relatively new study, oxygen sensitive proteins from the Radical SAM superfamily.

Rapid production of IgGs, Fabs and fusion proteins thereof in *E. coli*

Itai Benhar (Tel-Aviv University)

Full-length IgG antibodies, smaller antibody fragments and antibodies that ferry a cargo to target cells are desired biopharmaceuticals and valuable research reagents. Here we describe the production of full-length IgGs, IgG-based fusion proteins and similar constructs based on smaller Fab fragments in *E. coli*. In the presented examples, the antibody heavy and light chains or fusions thereof are expressed in separate bacterial cultures, where they accumulate as insoluble inclusion bodies. Following refolding and purification, high yields (up to 50 mg /L of shake flask culture) of highly purified (>90%) full-length antibodies and antibody- fusions are obtained. The bacterially produced antibodies, named "Inclonals," equal the performance of the same IgGs that are produced using conventional mammalian cell culture in binding properties and stability.

The rapid and cost effective IgG production process and the high quality of the resultant product may make the bacterial production of full-length IgG and IgG-based fusion proteins an attractive option for antibody production and a significant contribution to recombinant antibody technology.

Protein Expression in plant cell in suspension for pharmaceutical use

Yoseph Shaaltiel (Protalix Biotherapeutics)

Gaucher Disease (GD) is probably the most prevalent sphingolipid storage disorder, caused by the inherited deficiency of the lysosomal enzyme glucocerebrosidase (GCD). The key disease features include hepatosplenomegaly, anemia and thrombocytopenia as well as bone pain and fractures.

During the past two decades, enzyme replacement therapy (ERT) with GCD has been the standard of care for symptomatic GD. All commercially available enzymes are administered as bi-weekly intravenous infusions. If one could deliver safely and efficaciously the clinically proven enzyme by the oral route - this would have the definite advantage of the well-described ERT mechanism, while improving the quality of life of patients.

Protalix developed a unique technology for expression of pharmaceutical proteins in plant cells in suspension, which will be described.

Oral administration of proteins is one of the greatest challenges of the biotherapeutic industry particularly because of early degradation of the proteins in the digestive tract. One approved intravenous ERT, taliglucerase alfa (Protalix, Carmiel Israel), is expressed as a "ready to use" enzyme within carrot cells that requires no modifications for optimal glycosylation suitable for receptor-mediated uptake in target cells. We herein present the pre-clinical and Phase 1 clinical trial with these same carrot cells expressing human recombinant GCD as vehicle for oral delivery of GCD. The composition of the plant cell wall provides protection from degradation in the stomach which in turn enables release and delivery of the plant-cell-expressed GCD in the gut.

Structure-function studies that reveal the unique binding properties of antibodies that mimic T cells specificity

Yoram Reiter (Technion- Israel Institute of Technology)

Antibody and protein engineering approaches are used in our laboratory to develop new cancer immunotherapy strategies which combine the advantage of the well-established tumor targeting capabilities of high affinity recombinant fragments of antibodies with the known efficient, specific, and potent killing ability and unique specificity of CD8 T lymphocytes directed against highly antigenic MHC/peptide complexes or other effector functions.

The molecular features of these molecules/approaches and structure-function studies that enabled us to understand at the structural level the unique properties of these molecules will be presented. The expression, purification, and structural studies of the antibody-antigen complex will be presented and discussed. The use of these novel molecules to study basic questions of tolerance will be described as well demonstrating the bridge between basic and translational immunological research.

Enhancing Protein Secretion

Tsafi Danieli (The Hebrew University)

The ability to improve recombinant protein secretion has several biological and commercial benefits. Secreted proteins may possess enhanced biological activity, improved stability, better solubility and correct modifications; However, it is known that in many cases producing naturally secreted proteins in heterologous systems results in low productivity due to insufficient secretion rate, where a large fraction of the produced protein can get trapped in the cellular membrane in misfolded aggregates. Moreover, there are cases where secreting cytosolic proteins to the media can be of great pharmaceutical value. These cases often pose even greater challenges for protein production, since naturally non-secreted proteins are often extremely difficult to secrete. Based on high throughput screening experiments in bacterial cells, followed by

bioinformatics analysis of databases of secreted vs. cytosolic proteins, we have developed an algorithm that can predict whether a protein will be poorly or efficiently secreted.
We have tested this algorithm on several proteins experimentally known to be poorly secreted and found that they all contained specific amino acid motifs predicted to impair secretion. Disruption of these motifs by substituting a few amino acids resulted in a dramatic enhancement of secretion, often times up to several folds. We have validated our algorithm on both cytosolic proteins and secretion-impaired viral glycoproteins and were able to demonstrate secretion enhancement in both prokaryotic and eukaryotic expression systems.
We believe that by adopting our algorithm to analyze and mutate both secreted and non-secreted proteins, we will be able to dramatically increase productivity of protein secretion for pharmaceutical and research applications.

Rescuing Orphans by Engineering: A case study

Opher Gileadi (SGC, Oxford)

Protein engineering for stability has contributed spectacularly to the structural biology of GPCRs; engineering of non-membrane proteins is usually limited to selection of truncated constructs. I present a test case of an evolutionarily conserved disease-linked protein of unknown structure and function, which is marginally expressed and unstable in heterologous systems. A limited mutagenesis campaign identified a single point mutant, which can be expressed with 5-fold higher yields and can be concentrated without precipitating. The practicalities of mutant selection in the absence of a high-throughput screening assay will be discussed.

An unstable protein withholds your research? towards a fully automated protein thermostabilization algorithm for wide use

Adi Goldenzweig (Weizmann Institute of Science)

Proteins have evolved to function in the multi-constrained environment of a living cell, and their structures reflect a precise molecular compromise between various requirements. As a result, most proteins are only marginally stable, making many of them difficult and sometimes impossible to study in laboratory conditions. Library-based methods to increase stability have proven to be successful but they require high-throughput screens that are labor intensive and are not available in most research labs. We present protein thermostabilization algorithm that combines a biophysical design process with rich sequence data from homologs. We implemented this algorithm to stabilize an enzyme domain for folding research. Preliminary experimental results show that mutations offered by our algorithm successfully stabilized the domain. We are currently looking to collaborate with experimentalists seeking to stabilize specific proteins in order to fully automate and generalize the algorithm for wide use.

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Enzyme Engineering by Targeted Libraries

Moshe Goldsmith and Dan S. Tawfik (Weizmann Institute of Science)

Enzyme engineering using directed evolution and computational design is an important tool to overcome the limitations of natural enzymes as biocatalysts. It enables the improvement of critical traits of biocatalysts such as activity, selectivity, thermostability, and tolerance towards organic solvents for industrial applications. Here we outline the strategies that we applied for directed enzyme evolution using either randomly mutated or targeted libraries (i.e. libraries that diversify specific residues with pre-defined mutational compositions). We describe library construction strategies based on structure and sequence analyses, the use of ancestral libraries for low throughput screens, and the critical role of including compensatory, stabilizing mutations during library construction. Finally, we describe examples from our work with organophosphate hydrolyzing

enzymes that highlight the utility of targeted mutational libraries in the evolution of nerve-agent antidotes.

NMR Studies of Supra-molecular Machines: The Proteasome

Lewis E. Kay (University of Toronto)

The proteasome is a supra-molecular machine that is central to protein degradation. The importance of the proteasome for cellular function is underscored by its role in the removal of misfolded or otherwise damaged proteins whose accumulation in the cell is cytotoxic, by its involvement in the immune response through the production of antigenic peptides, and by its regulation of the cell cycle through controlling the lifetime of key proteins that modulate gene expression. In addition, the proteasome serves as a critical drug target for the development of pharmaceuticals against a number of cancers and neurodegenerative diseases. *Static* structural features of many stable supra-molecular complexes, such as the 20S core proteasome, can be determined by electron microscopy and x-ray crystallography. Despite the detailed pictures that have emerged, many outstanding questions remain that are related to the inherent dynamics of this machine and to its interactions with targets that cannot be solved via *static* structural studies. Nuclear magnetic resonance (NMR) spectroscopy is especially suited to study dynamics and dynamic interactions over a broad spectrum of time-scales and in the past decade my laboratory has developed new NMR experiments and labeling strategies for detailed studies of supra-molecular complexes with molecular weights as large as 1MDa. The important role of molecular dynamics in proteasome function will be illustrated

The COP9-Signalosome as a model to study the structure/function relationship of large protein complexes

Ben-Nissan G., Füzesi-Levi M.G., Rozen S., Bianchi E., Zhou H., Deery M.J., Lilley K.S. , Kiss V., Levin Y., Sharon M. (Weizmann Institute of Science, Institut Pasteur and Cambridge Systems Biology Centre)

Many of the cellular processes are regulated and governed by proteins. Today, it is generally accepted that the majority of proteins act in concert within multimeric assemblies. Such protein complexes are very dynamic and often interconvert between forms in a spatial and temporal manner. Therefore, a major challenge towards understanding the function of protein complexes is to elucidate their structure and link between their structural architecture and function. Studying the structural organization of protein assemblies, however, is not a trivial task, mainly due to their large sizes, their heterogeneous composition, flexibility and asymmetric structure. In recent years, mass spectrometry has proven to be a valuable tool for analyzing such non-covalent protein complexes. Here, the COP9-Signalosome complex will be used as a model to study the structure/function relationship of a heterogenous protein complex, using various mass spectrometry methods, combined with biochemical and live imaging methods.

Lessons learned from protein-protein interaction experiments by mass spectrometry

Yishai Levin (Weizmann Institute of Science)

The combination of immunoprecipitation and mass spectrometry (IP-MS) for investigation of protein-protein interactions is a powerful and informative technique. An appropriate experimental design can result in novel discoveries and information-rich data. However a poorly designed experiment could easily produce artifactual data and erroneous results. In this workshop, the most common IP-MS methodologies will be discussed and advantages and disadvantages will be highlighted.

Use of SEC-MALS (Size Exclusion Chromatography - Multi Angle Light Scattering) for protein quality and characterization

Hadar Amartely and Mario Lebendiker (The Hebrew University)

Size exclusion chromatography (SEC) is a widely used method in protein production and characterization that separates molecules in a non-denaturative way according to their size and shape. At the analytical level, SEC is used to verify the purity of the protein and is the most accepted and reliable technique to test the oligomeric conformation of the sample. Moreover, the approximate mass of a protein sample can be extrapolated from calibration curves using globular proteins standards. One of the major drawbacks of SEC is that the molecular mass or exact oligomeric conformation cannot be obtained for non-globular proteins, such as proteins with intrinsically disordered regions. We present here the use of SEC coupled in-line with multi angle light scattering (SEC-MALS) as a methodology that can overcome some of the limitations of SEC alone.

The combination of SEC with light scattering enables calculation of an accurate molecular weight of a single protein monomer, oligomer or aggregate and the radius of the molecule. Moreover, SEC-MALS can be a valuable tool in the characterization of protein-protein or protein-ligand interactions, conformational changes and protein modifications (glycosilation, pegylation rate, etc).

Moreover, the intensity of the light scattering depends on the size of the molecule therefore very low aggregate concentrations can be easily detected. This makes SEC-MALS an extremely useful and reliable tool for protein quality, mainly in the pharmaceutical industry, and in downstream processing quality control.

Promiscuous protein-protein interactions: a burden for the cell and a tool for the biologist

Emmanuel Levy (Weizmann Institute of Science)

The interior of cells is a highly crowded environment where proteins continuously encounter each other. In this environment, functional protein-protein interactions compete with a much larger number of non-functional, or promiscuous, interactions. We will discuss how promiscuity can constrain protein evolution and be a burden for cells, as well as how we exploited them in a novel experimental strategy to measure local protein concentrations in vivo and with high accuracy.

Development of high-affinity recombinant adipokine/cytokine antagonists: blocking of leptin, IL22 and resistin

Arieh Gertler (The Hebrew University)

Most common and simple approach to block undesired activities of cytokines/adipokines is use mAbs aimed at neutralizing their action. The major disadvantage of neutralizing mAb is that their use is commonly associated with development of B and T cell allo-response, producing side effects and limiting the efficacy of treatment with time. Instead, our approach is based on development of receptor antagonists capable of binding to receptor but unable to activate it and thus competing with the wild-type cytokine/adipokine and subsequently blocking its action. Our approach has several major advantages over an antibody approach, as it manipulates the endogenous adipokine/cytokine ensuring proper binding to the receptor to induce full antagonism. As the antagonists have near complete similarity to the WT protein it also promises that none or only minor immune responses will be evoked. Increase of affinity toward their respective receptors is achieved by random mutagenesis followed by selection of high affinity mutants by yeast surface display. In the present lecture I shall demonstrate large-scale preparation and application for research and therapy of leptin, interleukin 22 and resistin antagonists.

Abstracts- Day II

Production of human integral membrane proteins in mammalian cells

James D. Love (Albert Einstein College of Medicine, NY)

Integral membrane proteins are key targets in the understanding of health and human disease, yet producing functional material in great enough quantities for structural studies remains a formidable task. This talk will highlight the expression technologies under development that will greatly aid high-throughput efforts to produce functional human membrane proteins and complexes, specifically GPCRs, for a plethora of structural techniques.

Expression in *Pichia pastoris* of isoforms of Na,K-ATPase, purification and applications of the purified isoform complexes

Adriana Katz and Steven J.D. Karlish (Weizmann Institute of Science)

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The Na,K-ATPase, or Na,K-pump, consists of and subunits in a 1:1 complex as well as small regulatory subunits, so-called FXYD proteins. There are four isoforms of the subunit and three isoforms of the subunit, as well as seven members of the FXYD family. Combinations of FXYD complexes are expressed in a tissue-specific fashion and serve the physiological functions of the different tissues. 1 is uniquitous, while 2 is expressed mainly in muscle and brain, 3 in nervous tissue and 4 in testis. This talk will describe the *P.pastoris* system for expression of human or porcine Na,K-ATPase isoforms, purification of the expressed protein in a detergent-soluble, functional and stable state. The basic (1-3)His10-(1-3) complexes are expressed in the methanotrophic yeast and purified in the non-ionic detergent C12E8 by affinity chromatography using BD-Talon (Co) beads. In order to maintain stability of the complex it is necessary to add phosphatidyl serine together with the C12E8 and there is good evidence for specific protein-phosphatidylserine interactions. FXYD proteins are expressed separately in *E. coli*, purified in C12E8, and reconstituted in vitro to produce FXYD complexes. FXYD proteins strongly stabilize the complexes by amplifying the protein-phosphatidylserine interactions and affect kinetic properties. The purified isoform complexes are being

utilized for crystallization trials, in order to study specific lipid-protein and protein-protein interactions, and in order to develop isoform-selective cardiac glycosides (specific inhibitors of the Na,K-pump). Some of these applications will be described.

Overcoming challenges in expression, purification and NMR characterization of membrane proteins

Hadassa Shaked, Hadas Zazrin-Grynspon, Inbal Sher, Hila Shalom-Elazari, Renana Gross, Orel Hirschhorn, and Jordan H. Chill (Bar-Ilan University)

The study of membrane-associated proteins (MPs) is one of the modern frontiers of structural biology. Although MPs represent ~30% of the proteome and over 50% of pharmaceutical targets, barely 1% of currently elucidated structures come from this class of proteins. The reasons behind this are painfully clear: since MPs require a membrane-mimicking environment to survive in aqueous solution, both purification and sample-preparation present the structural biologist with significant experimental challenges. Here we describe two case studies, a pair of hydrophobic membrane-spanning peptides and a bacterial potassium channel, demonstrating the difficulties in working with MPs and methodologies for pushing such projects forward. In doing so we will focus on preparation of samples suitable for NMR characterization of these intriguing structural targets and the structural information that can be obtained, justifying the efforts invested in developing these challenging expression systems.

Towards crystallization of MdfA, a promiscuous secondary multidrug transporter

Osnat Tirosh and Eitan Bibi (Weizmann Institute of Science)

Membrane proteins represent a significant percent of the proteome and account for about 50% of drug targets. Integral membrane proteins play vital roles in all cellular life forms, performing many crucial functions. However, despite their importance, relatively few membrane protein structures have been solved by X-ray crystallography, due to the difficulties involved in their expression, purification and crystallization.

In particular, membrane proteins with conformational heterogeneity due to intrinsic structural flexibility are difficult to crystallize. One major group of membrane proteins that possess high degree of flexibility are secondary multidrug transporters, and this is reflected by their promiscuous ability to interact and translocate a large variety of dissimilar molecules across the membrane. Nevertheless, structural information is a prerequisite for deciphering the major molecular aspects of drug recognition by secondary multidrug transporters. In addition to the intellectual challenges, structural information is critical in the rational design of better drugs with improved selectivity and pharmaceutical properties.

As a prototypic secondary multidrug transporter, we study the *E. coli* integral membrane protein MdfA. MdfA has an exceptionally wide range of dissimilar substrates. In addition to multidrug recognition, MdfA is also extremely promiscuous regarding its ability to utilize versatile modes of drug/proton exchange and thus energy coupling. Previous crystallization efforts, performed in our lab, have led to a set of conditions that yielded small, extremely anisotropic crystals of MdfA. To improve this, we have applied various methods, which were proven successful for substrate-specific transporters and GPCRs. Several of the approaches may lead to different crystal packing and improved order.

Proteins expression in inclusion bodies

Gideon Schreiber (Weizmann Institute of Science)

Despite the many advances in methods in protein production and purification, we are still faced with many proteins that refuse to be expressed in their native, soluble form. These proteins aggregate in inclusion bodies in *E. coli*. But, from our experience, this is not a reason to give up on them. Producing proteins to inclusion bodies can be very positive at times. The separating inclusion bodies from the rest of *E. coli* cells is fast and easy, leaving us with a highly enriched fraction of the protein of desire after one or two simples steps. Of course, the biggest challenge here is to refold the protein to its native structure, a challenge that is further complicated when the protein has native disulfide bridges that have to be formed. In this talk I will discuss our experience in methods of refolding of proteins form inclusion bodies, and how this disadvantage can become an advantage.

Production of prone-to-aggregate proteins

Mario Lebendiker (The Hebrew University)

Expression of recombinant proteins in Escherichia coli (*E. coli*) remains the most popular and cost effective method for producing proteins in basic research and for pharmaceutical applications.

Despite accumulating experience and methodologies developed over the years, production of recombinant proteins prone to aggregate in *E. coli*-based systems poses a major challenge in most research applications. The challenge of manufacturing these proteins for pharmaceutical applications is even greater. This review will discuss effective methods to reduce and even prevent the formation of aggregates in the course of recombinant protein production. We will focus on important steps along the production path, which include cloning, expression, purification, concentration, and storage.

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Molecular tools for facilitating DNA manipulation and protein expression

Yoav Peleg and Tamar Unger (Weizmann Institute of Science)

DNA cloning and mutagenesis are essential tools in all life-science disciplines. In recent years we have developed and implemented at the Israel Structural Proteomics Center (ISPC) Ligation Independent Cloning (LIC) strategies such as the Restriction Free (RF) and Transfer-PCR (TPCR). These methods allow integration of a gene into any plasmid and at any position by whole plasmid amplification of the insert and the target vector. Based on the above strategies we have developed new applications, which include simultaneous cloning of several DNA fragments into distinct positions, simultaneous multi-component assembly and parallel cloning of the same PCR product into series of expression vectors. We have further expanded the applications for protein engineering including multiple alterations of the target gene, simultaneous multiple-site mutagenesis and simultaneous introduction of deletions and insertions at different positions. Using

these strategies we have facilitated standard protocols for DNA manipulation and protein expression.

Spanning high-dimensional expression space using ribosome-binding site combinatorics

Niv Antonovsky (Weizmann Institute of Science)

Protein levels are a dominant factor shaping natural and synthetic biological systems. Although proper functioning of metabolic pathways relies on precise control of enzyme levels, the experimental ability to balance the levels of many genes in parallel is a major outstanding challenge.We introduce a rapid and modular method to span the expression space of several proteins in parallel. By combinatorially pairing genes with a compact set of ribosome-binding sites, we modulate protein abundance by several orders of magnitude. We demonstrate our strategy by using a synthetic operon containing fluorescent proteins to span a 3D color space. Using the same approach, we modulate a recombinant carotenoid biosynthesis pathway in Escherichia coli to reveal a diversity of phenotypes, each characterized by a distinct carotenoid accumulation profile.The methodology presented here provides an efficient tool for exploring a high-dimensional expression space to locate desirable phenotypes.

SuggesES (Suggest an Expression System)

Jaime Prilusky, Yoav Peleg and Tamar Unger (Weizmann Institute of Science)

The process of selecting the expression system for expressing a given sequence is a process that may lead us to a path of multiple decisions: effective vs inexpensive, yield vs speed, known-by-us technique vs lengthily setup of a new expression platform. Given a protein, SuggestES helps in the process of choosing the expression system that potentially will yield the best result on the simplest conditions.
SuggestES takes the protein sequence you provide and scans a large database with protein sequences with known results, both positive and negative, for different expression systems. At the time of generating a suggestion, suggestES takes into consideration

several parameters: Similarity: how similar is your sequence to the existing data in the database? The expression systems used on sequences similar to yours are preferred when creating the list of suggestions. Recentness: how recently was used a given expression system?. The older the record of the usage of a given expression system, the less this system will influence the final result. This will provide visibility to recently appearing system. Frequency: how frequently a given expression system has been used? SuggestES is freely available at http://expsys.weizmann.ac.il

Analysis of the full proteome and HLA peptidome by mass spectrometry

Arie Admon (Technion- Israel Institute of Technology)

The analysis of the full proteome and HLA peptidome by mass spectrometry and determining the dynamics of synthesis of the proteins and their derived degradation products, the HLA peptides. I will describe the use of quantitative proteomics based on label-free, chemical isotope labeling and SILAC labeling.

Novel bacterial expression systems to genetically select and purify ubiquitylated-proteins

Olga Levin-Kravets, Tal keren-Kaplan, Ilan Attali and Gali Prag (Tel-Aviv University)

Novel bacterial expression systems to genetically select and purify ubiquitylated-proteins Ubiquitylation regulates essentially all the eukaryotic cellular pathways. However, deubiquitylation imposes challenges on genetic, biochemical and biophysical characterization of ubiquitylated proteins. To circumvent this limitation we developed novel expression systems of the ubiquitylation apparatus in *E. coli,* which lacks deubiquitylation activity. In these systems the entire ubiquitylation cascade including Ub, E1, E2, E3 and substrate are co-expressed from two compatible plasmids in a polycistronic manner. The systems performances were evaluated using a large variety of known substrates, mutants and conditions. Two systems will be presented:

1. Genetic selection system to identify the components along the ubiquitylation cascades

In the system fragments of split antibiotic resistance gene are tethered onto Ub and the ubiquitylation target. Assembly of the selection marker functions in a ubiquitylation dependent manner, gives rise for bacterial growth in non-permissive conditions. The system provides a robust high-throughput synthetic biology approach for genetic studies of ubiquitylation events. Some of the discoveries derived from the system will be presented.

2. Purification system for ubiquitylated proteins:

We co-express the ubiquitylation apparatus, His_6-Ub and MBP-substrate from two compatible plasmids. Affinity chromatography using both tags produces milligrams of highly pure ubiquitylated substrates. The system allowed us to determine the first crystal structure of ubiquitylated-protein.

Process development challenges in purification and formulation of a recombinant polypeptide with limited solubility

Ram Uritski (BiondVax Pharmaceuticals)

The process of purification and formulation development of the recombinant polypeptide will be presented. Polypeptide's challenging characteristics, such as high pI, limited solubility, and tendency to aggregation will be discussed. A novel formulation process was developed, turning these challenges into advantages.

The development of INS-004 - a biosimilar therapeutic monoclonal antibody

Joel M. Van Gelder (InSight Biopharmaceuticals)

Half of the top ten best-selling drugs in 2013 were therapeutic monoclonal antibodies which go off-patent in the coming years. Due to high costs to the patient, there exists a big incentive to develop biosimilar versions of these drugs. InSight is involved in the field of development of biosimilar proteins for almost two decades.

A case study will be presented, illustrating the different stages required for the development of a biosimilar monoclonal antibody. Reverse engineering of the reference listed drug, by determining its quality target product profile, using multiple highly

sophisticated state-of-the-art analytical methods and biochemical assays, is the first step in this process which further dictates the clone selection, upstream and downstream process development. Based on physico-chemical features and biological functionality a high similarity profile of INS-004 will be presented, in comparison to the reference listed drug. Development pitfalls and regulatory prospects will be discussed.

Moving protein expression from the laboratory scale to large scale high density fermentation

Ghil Jona (Weizmann Institute of Science)

Although protein expression seems to be a simple process, many factors can directly influence both the quantity and the quality of the target protein. At the bench most of the efforts are spent in the optimization of the purification phase, and little if any time is spent to develop a robust and good expression platform, to be integrated with the downstream purification phase. The superficial upstream process development becomes even more critical when aiming to move the laboratory scale process to large-scale protein expression and purification in fermentors for example for clinical trials.

Here I will discuss the importance of planning in advance a combined upstream fermentation, side by side with the downstream purification process. I will further present an example of the benefits of developing scalable high density fermentations and protein purification processes, and how different factors can directly influence the properties of the final target protein.