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New trends in protein expression

In order to benefit from the wealth of genomics data and to make new discoveries in the protein world, genes need to be converted to proteins that, in turn, must be characterized at the molecular level. This has galvanized researchers from across a broad range of biological and biomedical disciplines to study the form and function of thousands of proteins. This task can be achieved by production of stable and pure proteins for their biophysical and biochemical analysis and/or for their 3D structure determination. The broad hierarchy of structures in biological systems necessitates integration of a wide range of high and low-resolution structural determination techniques. These include X-ray and neutron crystallography, NMR, electron microscopy and tomography, small angle X-ray and neutron scattering, mass spectroscopy and advanced light microscopy techniques. Common to all these techniques is the requirement for highly pure protein samples. Consequently, high throughput protein expression and purification, as well as more versatile expression systems and techniques, are now in even greater demand to support the pressing requirements of the research pipeline. However, protein expression is not only a technology, but also an art. Each protein is unique and thus requires special and often tedious treatment. Therefore, the expression of high levels of stable and functional proteins remains a bottleneck in many scientific endeavors, including the determination of structures in a high-throughput fashion or the screening for novel active compounds in modern drug discovery. Recently, numerous developments have substantially improved the procedures for production of soluble and active proteins in heterologous expression systems. These include parallel construct design, new and more robust expression systems, modifications to the expression constructs, introduction of new and/or improved prokaryotic and eukaryotic expression systems, and the development of improved cell-free protein synthesis systems. This has driven technological developments striving for miniaturization and automation, and methods to characterize proteins prior to structural characterization, in order to achieve a better understanding of how to design constructs generating proteins more amenable to structural characterization. While many of these developments have gained momentum from the various "structural genomics/proteomics initiatives", they are now being applied to study the form and function of proteins by scientists from a broad range of biological and biomedical disciplines.

We present here a collection of papers in a special issue entitled "New Trends in Protein Expression". Our goal was to gather papers concentrating on current developments of the methods and tools that are being used in the production of proteins for structural analysis. In assembling the articles appearing in this volume of the *Journal of Structural Biology*, an attempt has been made to focus on those principles, strategies, and technologies that currently shape our thinking regarding the challenges posed by producing suitable protein samples for both structural and biomedical analysis. These articles may be classified into five broad sections, though clearly their common goal results in substantial overlap. The issue begins with three papers that provide comprehensive and insightful overviews of the "structural genomics experience". These articles describe the pipeline from gene to structure, emphasizing the approach used by three leading facilities to overcome the protein expression and purification bottlenecks. The second section deals with "Cloning Techniques". Efficient cloning methods are required for maintaining robust pipelines, and are a prerequisite to any heterologous expression endeavor. Many laboratories have largely adopted various Ligation-Independent Cloning (LIC) methods to cope with their individual cloning requirements. This section presents a single paper describing novel applications to the Restriction Free (RF) cloning technique. The third section handles methods for overcoming difficulties in the expression of troublesome proteins. The aim at the expression and purification stage is to obtain a sample that is relatively pure and homogenous. The customary route to achieve this objective is through heterologous overexpression in various strains of *Escherichia coli*. Many eukaryotic and prokaryotic proteins can be expressed in bacteria, as can human integral membrane proteins, such as G-protein-coupled receptors (GPCRs). Since bacterial expression is fast and inexpensive, in many cases it is worth trying it first, even for eukaryotic proteins. However, for challenging proteins, such as many eukaryotic targets and large protein complexes, other expression systems may be required, such as baculovirus, mammalian cell lines, yeast or the wheat germ cell-free expression system. Two papers in this section describe some of the latest developments in the use of eukaryotic expression systems, while two other papers tackle difficult proteins by altering the cloned constructs. Although membrane proteins fall into the category of "challenging proteins", we decided to devote a separate section, the fourth one, to this important group of proteins. Finally, appropriate to the dominant theme of structural biology, the issue also includes a section describing various biophysical techniques for the characterization of proteins. Biophysical characterization of target proteins serves as a useful metric to evaluate the success of the protein production pipeline.

Understanding how biological systems operate, from the level of single enzymes and other proteins, through the level of protein–protein interactions, and finally at the level of intact organelles and cellular physiological pathways, a goal of systems biology, will require detailed, quantitative characterization of cellular proteins and their interactions. These structural and functional analyses require adequate protein samples. Overcoming the obstacles encountered in their production promises to allow

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for more comprehensive molecular insights into the roles and functions of proteins in a wide range of biological processes. It is our fervent desire that these papers will offer novel ideas, strategies, and engineering applications that may pave the way to assured success. It is our hope that this special issue of the *Journal of Structural Biology* will provide a point of reference both for those researchers actively engaging "the protein world" and for those wishing to acquaint themselves with the current state of the art in this rapidly growing field.

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