PROTEIN CRYSTALLIZATION STRATEGIES FOR STRUCTURAL GENOMICS

edited by
Naomi E. Chayen

Preface:
We are currently living in an exciting age, where for the first time ever, human diseases are being understood at a molecular level. Protein crystallography plays a major role in this understanding because proteins, being the major machinery of living things, are often the targets for drugs. The fuction of these proteins is determined by their three-dimensional structures hence a detailed understanding of protein structure is essential for rational design of therapeutic treatments. Structural genomics, or more accurately, structural proteomics, which aims to determine the structures of thousands of proteins has emerged as a direct consequence of the genome project in which the human genes and other genes have been sequenced. Structural Genomics projects worldwide are detailed in http://www.isgo.org/.

Getting from gene to structure involves many steps: cloning, expression, solubilization, purification, crystallization, and only then, the determination of the structure. Once protein is pure and soluble, the key to crystallography is the availability of high-quality crystals. Producing high-quality crystals has always been the bottleneck to structure determination and with the advent of proteomics this problem is becoming increasingly acute. As a result, crystallization is gathering a new momentum as evidenced by the increasing numbers of commercial companies selling crystallization kits and tools, the increased investment of pharmaceutical companies in crystallization equipment and expertise, a high demand for practical courses in crystal growth, the launch by the International Union of Crystallography of a new journal, *Acta Crystallographica F*, formed in 2005 for publishing the recent explosion of data-concerning crystallization, and the establishment of the International Organization for Biological Crystallizaion (http://www.iobcr.org).

The past five years have seen some of the greatest achievements in the field of protein crystallization. It is now feasible to screen thousands of potential crystallization conditions by dispensing trials consisting of nanoliter volumes in a high-throughput mode. This has cut the time of setting up experiments from weeks to minutes, a scenario that wasunimaginable a few years ago. Even more incredible, is the revelation that diffracting crystals can be produced from protein samples in volumes as small as 5–20 nanoliter. The subsequent phase of image capture and analysis of the crystallization drops is also progressing in great strides.

Surprisingly, in spite of the impressive advances accomplished, the crystallization problem has not been solved. High throughput has not yet resulted in high output and the current challenge is to design new and improved techniques (of screening and

optimization) for the production of useful crystals. Scientists worldwide have taken on the challenge by tackling the crystallization problem from a variety of different aspects.

Research advances in recent years have opened up the scope for the development of new methods and tools to overcome the bottleneck of protein crystallization. A variety of parameters that could previously not be explored are now accessible thanks to sophisticated apparatus and he development of new science-based techniques to monitor and control the process of crystallization. However, in order to become useful to the structural genomics effort, it is vital to miniaturize and automate these techniques and adapt them to cope with the vast numbers of "leads" resulting from the high-throughput screening procedures. Such efforts are those of the immediate future and the focus of this book.

This book's aim is to assemble a selection of reviews highlighting the state of the art in techniques and strategies developed over the last four years for crystallization in the context of structural genomics. It is dedicated to the steps from the time of having pure protein to the crystallization, detection, imaging, and production of a diffracting crystal. Most researchers involved in HTP consortia are engaged in solving structures and do not have the time or inclination to develop new methodology for crystallization. I have therefore chosen most of the Authors form the crystal growth community, for their expertise in development of methodology in their respective fields within crystallization.]

The **first chapter** provides a background to structural genomics describing how it was conceived, funded, and implemented in the USA. This is just one example of many excellent Structural Genomics projects
taking place worldwide (see http://www.isgo.org/) that could compile a book in their own right.

Chapter 2 provides an overview of setting up high-throughput structural proteomics projects in different environments and guidance as to how to handle the data and know-how resulting from the numerous experiments.

Chapters 3-5 describe different ways of conducting high-throughput initial screening for obtaining the first leads for crystallization.

Chapter 3 reviews the current robotic systems for screening that are available on the market for conducting high-throughput crystallization trials. Until 2002, screening for initial crystallization conditions was performed almost exclusively using vapor diffusion and microbatch. Since then, screening by the free interface and counter-diffusion methods that were least used in the past due to handling difficulties and the requirement for large quantities of sample, have been miniaturized and automated thus gaining a new lease of life.

Chapters 4 and **5** deal with miniaturization and adaptation to high throughput utilizing free interface and counter diffusion and gels, the former by microfluidics and the latter, using capillaries.

Chapter 6 describes a procedure for screening with a built-in optimization. Screening is performed using the standard screening kits but instead of looking for leads such as crystals, precipitation etc, the aim is to find the conditions that are at the metastable zone, which contains the ideal conditions for crystal growth.

Chapter 7 deals with strategies to optimize and improve the crystal quality once a lead has been obtained. These are methods that have been performed mainly manually and have recently been automated in order to adapt them to high-throughput experiments.

Chapter 8 describes the miniaturization and automation of experiments with membrane proteins in liquid cubic phase.

Dispensing trials with such amazing speed and ease has raised the issue of observation and monitoring of the vast numbers of trials. Major effort is currently being invested in designing image processing equipment for automated follow-up and analysis of the results as described in **Chapter 9**. Full automation of the visualization and monitoring of trials is expected to be the next major breakthrough in the field of crystallization.

The success rate of obtaining high-quality crystals is improving and the coming years promise to bring further advances in the more complex techniques that will play a major role in crystallization and proteomics. This will raise the rate of producing high-quality crystals, and will equip the genome project to deal with its awesome task.

Naomi Chayen
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