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Structure based drug design and high throughput protein crystallisation

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There is an increasing need for the determination of protein structures by X-ray crystallography. Automated systems for the monitoring of crystal growth and subsequent handling of crystals greatly improve the efficiency of the process.

Structure based drug design is the process whereby a protein is isolated and its three dimensional structure determined. The structure is then studied to find active sites where drugs can be bound to inhibit the biological reactivity of the protein. By blocking binding sites or inhibiting enzymatic activity, biological processes can be controlled.

Over the past decade, structure based drug design has begun to evolve as an essential tool in drug discovery. As the time that it takes to determine protein structure decreases and the number of known protein structures grows, structure based drug design is becoming a more and more important approach to lead generation and optimisation. This rational method for pharmaceutical development is already showing increased results over more traditional high throughput screening (HTS) and combinatorial methods. Some of the advantages over the more established HTS methods include a reduced need for handling large libraries of chemicals and better hit rates for small molecule leads when HTS fails.

The advent of high throughput characterisation of protein crystal structures is the result of rapid development in laboratory instrumentation in this field. Faster, larger detectors, improved X-ray sources and higher speed computing algorithms make protein structure elucidation quite accessible for even the smallest lab. The availability of the technique makes structure based, intelligent drug design an achievable goal.

To establish protein structures, researchers first isolate the protein through cloning, expression and chromatography-based purification. The purified protein samples then need to be crystallised. Finding the proper conditions to grow a crystal from the purified protein is often a "hit and miss" type of experiment. This task involves setting up many combinations of solvents and reagents.

Traditionally, protein crystal growth involved hands-on, labour intensive pipetting of solutions into crystallisation plates. These plates can literally contain many hundreds of experimental conditions in an attempt to find ideal crystallisation conditions for a given protein. Typically, at least four hundred conditions are tried per protein with some researchers setting up initial screens covering thousands of different conditions. As laboratory automation has developed, hand placement of protein crystallisation drops is now being replaced by liquid handling robots. More and more plates are being produced in an attempt to crys-

Figure 1. The Crystal Farm carries out automatic imaging of plates at user-defined intervals to look for the formation of crystals.

tallise a wide variety of proteins.
The next step is a "sit and wait" experiment. Protein crystals can form in as little as fifteen minutes or take as long as five months or more. Environmental conditions must be kept constant to ensure reproducibility, as researchers wait patiently for a crystal to form out of solution. Periodic optical imaging of the drops is currently the only method for identifying a crystal in solution.
Once again, optical imaging of protein drops has, until recently, been a manual operation, often involving laboratory technicians sitting in 4°C cold rooms manipulating crystallisation plates under an optical microscope. With the ever-increasing requirement for the determination of protein structures, a need for high throughput imaging and data-basing of experimental conditions has arisen. New instrumentation has evolved to fill this technological gap.

One such instrument is the Crystal Farm, developed and produced by Discovery Partners International of San Diego. The Crystal Farm [Figure 1] is able to automate a large portion of the imaging process by replacing expensive technicians with robotics. Crystallisation plates are set up and entered into the Farm. Plates are optically imaged daily, weekly or perhaps even monthly, to look for the formation of crystals. The plates are maintained at a constant temperature and imaged according to a user-defined schedule. The Crystal Farm publishes the pictures of the individual wells of the plates as a web page, viewable from any web browser on the internet.

An extra bonus feature of the system is that it also has built-in image recognition software. The Crystal Resolve software package attempts to find crystals automatically in the wells using a combination of neural-net and object finding algorithms that attempt to find solid objects in the digital image. The automated high throughput protein crystal imaging that is carried out by the Crystal Farm system is, of course, only one step in the overall process of establishing structures on which drug design can be based.

Robotic sample changers for X-ray diffractometers have, in recent years, moved from being rare development projects located at synchrotrons to being commercially available solutions. Companies, like Bruker AXS of Madison, Wisconsin, already offer commercial solutions for the handling of mounted protein crystals under cryogenic conditions. The Bruker sample handling robot, BruNO, has been designed to meet the need for improved productivity in X-ray instrumentation. The accelerating pace of research in the field of rational drug design made this more and more imperative [Figure 2].

However, despite all the impressive technological progress,

Figure 2. Sample handling robots capable of handling protein crystals under cryogenic conditions significantly improve overall efficiency. Shown here is the BruNO system which has a special gripper to handle Kryo-Vials and has access to a table-top Dewar flask through its lid.

removal of the fragile crystals from the crystallisation solutions, and their subsequent mounting and freezing still requires careful human handling. This rate-limiting manual operation has become a real "bottleneck" in the overall process and has thus far resisted all attempts at automation. Crystal harvesting requires precise complex motions that exceed the capabilities of current, standard industrial robots. Handling extremely small crystals in mother liquors that can have a wide range of viscosities presents quite difficult complications. A system that could effectively meet these challenges would be a critical enabling technology in the overall process of a fully automated, high throughput facility handling the entire pathway from protein to structure.

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