Advancing Technologies in Crystallization to Expedite Crystal to Structure Delivery

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Introduction

The role of the structural biology group at Pfizer is to provide a project with 3D-structural information to aid the drug discovery process. Highlighted are some of the technologies and approaches in use to achieve this goal rapidly and effectively, especially in the realm of protein crystallization.

RockMaker: Heart of Crystallization Process

- Software developed by Formulatrix
- Will be used by all PGRD crystallographers globally as central database for crystallization experiments
- Used to capture all crystallization experiments, to view/score images from the Robotics, and to run CrystalMation components

Crystallization Robotics: Phoenix RE

- 30 x 96 well plates
- Automated imaging, processing, and storage of crystalization plates at 4 and 13 °C (21 °C, walk-up).
- View plate images from RockMaker
- Plates created on Phoenix RE (Greiner LP 96-well), manually, and/or by CrystalMation.

Minstrel III: Automated Plate Imaging

- Automated imaging, processing, and storage of crystalization plates at 4 and 13 °C (21 °C, walk-up).
- View plate images from RockMaker
- Plates created on Phoenix RE (Greiner LP 96-well), manually, and/or by CrystalMation.

Alchemist: To Make Optimization Grids

- Liquid handling instrument used currently to make 96-well and 48-well deep well source blocks and 24- and 48-well experimental plates with crystallization screens.
- ‘Bird-feeder’ technology (no pumps, tubing, etc.)

Fluidigm: Alternate Screening Method

- Free interface diffusion technology with microchips (shown in Fig. 1)
- Can screen up to 8 protein samples with 96 conditions (8.96 chip)
- Uses ‘bird-feeder’ technology (no pumps, tubing, etc.)

From Crystal to Structure the Quickest Way!

- Pfizer La Jolla uses the synchrotron X-ray facilities of Beamlines 5.0.1 and 5.0.2 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory.
- Recent upgrade to Varimax optics (Fig. 2) has increased x-ray flux 2-3 fold.
- All detector systems are fitted with cryo streams for low temperature data collection

Synchrotron X-Rays: A Vital Adjunct

- Synchrotron X-rays are 1000-fold brighter than lab X-rays and are useful both in solving novel structures and in extending the detail of the structures beyond what is possible with lab X-ray sources.
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Summary

In order to build a strong SBDD platform and provide timely structure information for iterative compound design, the Gene to Crystal group within Structural Biology is expanding and developing new technologies to speed up the protein crystallization process to impact structure delivery. The state-of-art in house data collection and synchrotron facilities allow us to gain high resolution data from small crystals. Automated refinement protocols have been developed using advanced refinement programs for rapid and accurate structure determination.