An Automated Platform for Miniaturized Protein Crystallization

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Abstract

The Protein Structure Factory (PSF) is the first German structural genomics initiative funded by the German government to perform high throughput protein expression combined with high throughput NMR and X-ray diffraction methods (http://www.fu-berlin.de/psf). Our crystallization strategy relies on various technologies like "sitting-drop" and "hanging-drop" vapor diffusion. We have introduced a new microplate based method which allows simultaneous "sitting-drop" and "hanging-drop" vapor diffusion.

All crystallization experiments are influenced by a number of parameters and there is not much known what is really influencing crystal formation and growth of proteins. The main hypothesis for setting up automated crystallization screening experiments is, that several hundreds of recombinant proteins might form crystals when grown under ideal conditions.

In cooperation with Greiner Bio-One, we have developed a specialized 96 well crystallization microplate which offers several advantages when used in fully automated systems. The adoption of robotic technology for plate and glass slide preparation enables "hanging drop" and "sitting drop" vapor diffusion crystallization experiment together with an increased throughput at reduced costs.

Meanwhile, we have added a huge storage system for 10.000 crystallization microplates with a pipetting devices based on solenoid ink-jet technology and an automated CCD-imaging system. Our unique crystallization procedure allows to deposit two or more proteins in a special formed triplet of crystallization wells. All crystallization wells are inspected in intervals of one to three days for the presence of crystals.

Pathway for Protein Crystallization

Development of Techniques for Pipetting and Droplet Setting

- Automated droplet setting between 0.5 and 2 μl
- Simoultanous 96-fold pipetting in microtiter-format (precipitant & reservoir)
- Optimization of crystallization techniques (sitting & hanging drop)
- Fast and reproducable performing of experimets

❶ Protein setting on glas slide

❷ Schematic use of multichannel pipetting system

❸ Precipitant setting on glas slide and reservoir setting on plate

❹ Prepared screening experiment; ready for further processing Time consumed: less than 2 min

Automated Storage System (HomeBase)

- Storage up to 10,000 plates (standard 96-well footprint)
- Automated connection to detection system
- Link to crystallization database of sub-project Tp12

Inner view

Loading

❶ Storage robot (inner side):
❷ x,y,z arm, moving profiles optimzed for hanging drops up to 10 μl
❸ 6 plates on one tray
❹ Manual tray (load/unload plates)
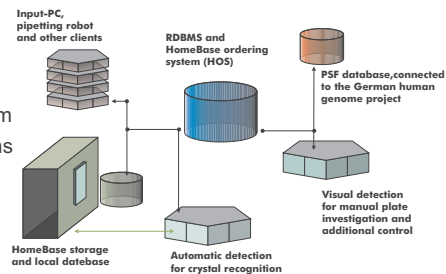❺ Automatic tray (for droplet microscopy)

Automated Crystal Detection

- Rapid screen of crystallization assays
- Reliable distinction between
  - empty droplets
  - amorphous precipitate
  - spherulites
  - crystals
- Entire scan by an automated CCD camera
- in less than 2 min per 96 well microplate

Concanavalin A (standard screen)
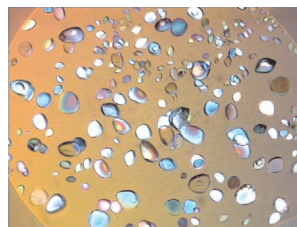
Database concept

- Plate management
- Connection to storage system
- Connection to detection system
- Automated pipetting of screens

Input-PC, pipetting robot and other clients

RDBMS and HomeBase ordering system (HOS)

PSF database,connected to the German human genome project

HomeBase storage and local database

Automatic detection for crystal recognition

Visual detection for manual plate investigation and additional control

High-Density Microplate Platform

- Reservoir solutions in 96 wells
- Sitting drop & hanging drop crystallization techniques
- Parallel screens in three microwells for sitting drops

New sitting drop microplates from Greiner Bio-One

Parallel crystallization in microwells
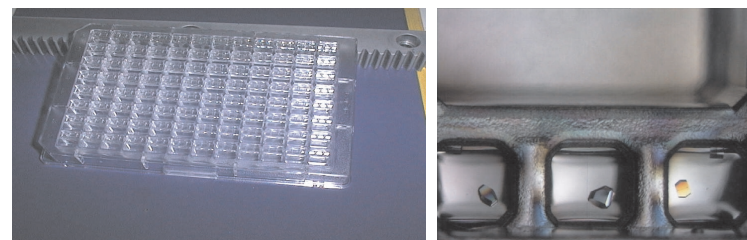
Development of Screening Strategies

- Rapid initial screening of protein samples
- Predefined optimization pathways for crystallization
- Automated pipetting of new screening patterns

- 96 different conditions for initial screening experiment
- Rapid development of start conditions
- Further optimization of startconditions on predefinied pads
- Determination of Cryo-conditions

pH →

salt conc. →