High-Throughput Approaches for Protein Crystallization in Standardized Microplates

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Introduction

The post-genomic era has now entered a phase of producing thousands of proteins per year for structural analysis. One of the driving forces is rational drug design for individualized pharmacogenomics based on a deciphered high-resolution target protein structure (Fig. 1). A major bottleneck is still finding the right crystallization condition to obtain high-quality crystals for X-ray diffraction. This can be overcome by empirical trial and error approaches with hundreds of conditions for each protein. Often the total number of experiments is further increased by investigation of several protein concentrations and different crystallization methods in parallel. The three most common methods are shown in Fig. 2. Beside the classic hanging drop method, microbatch and sitting drop better fulfill the special needs of fully automated high-throughput screening.

Miniaturized approaches are necessary to save costs, reagents and the limited amount of protein for crystallization. The consumption of 10 mg/ml stock solution can be minimized through downsizing single experiments to the microliter range or below. This is of importance because 200 - 1500 experiments are commonly done to decipher high-resolution target protein structure (Fig. 1). The planar surface and rims around each well enable perfect sealing of the plate with either pre-greased glass sheets or ViewSeal™ adhesive. Crystallography plates are designed in a way to enable top and bottom inspection of the crystal growth with CCD-based or microscopic systems (Fig. 6). To make screening in CrystalQuick™ most efficient, each of the 96 reagent wells is associated with three crystallization wells. Checkerboard screening, with up to 288 crystallization options per plate, can utilize for investigation of optimal crystal growth conditions. Squared crystallization wells can take up 2 - 4 µl while plates with round bottom crystallization wells are dedicated for 1 µl or less protein solution (Fig. 7). Screening systems in use at PSF and GNF contain CCD-imaging systems and software for semi-automated crystal growth detection.

To use CrystalQuick™ even more efficient, the MPI for Molecular Genetics and PSF, Berlin have developed a specialized lid to enable hanging and sitting drop vapor diffusion screens at the same time. The new CrystalDrop™ lid with 192 indentations (Fig. 7) is well suited for automated hanging drop vapor diffusion. Printed light-tight masks allow improved detection of crystal growth (Fig. 8), while grooves around each of the 96 center locations are useful for air-tight sealing, either with silicon grease or any other adhesive.

Outlook

Greiner Bio-One will further expand the CrystalStar™ product family of crystallization plates (Fig. 9) with advanced plates for high-throughput, small volume microbatch crystallography.

Microbridges, necessary for sitting drop crystallography fit neatly into the flat bottom wells with a diameter of 16.2 mm. Wide rims ensure tight sealing of the plate with the transparent adhesive ViewSeal™. IMP@CT™ (Improved Microbatch Protein Crystallization Technique) offers a robot-friendly SBS footprint of a 96 well plate for 4 – 10 µl of protein solution. Conical wells provide a total volume of 19 µl.

A unique protein crystallization microplate for sitting-drop vapor diffusion protocols is CrystalQuick™. The 96 well format with standard microplate footprint (Fig. 4) is optimized for automated high-throughput protein crystal growth (Fig. 5). The planar surface and rims around each well enable perfect sealing of the plate with either pre-greased glass sheets or ViewSeal™ adhesive. Crystallography plates are designed in a way to enable top and bottom inspection of the crystal growth with CCD-based or microscopic systems (Fig. 6). To make screening in CrystalQuick™ most efficient, each of the 96 reagent wells is associated with three crystallization wells. Checkerboard screening, with up to 288 crystallization options per plate, can utilize for investigation of optimal crystal growth conditions. Squared crystallization wells can take up 2 - 4 µl while plates with round bottom crystallization wells are dedicated for 1 µl or less protein solution (Fig. 7). Screening systems in use at PSF and GNF contain CCD-imaging systems and software for semi-automated crystal growth detection.

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