

Crystallization of protein targets from the Midwest Center for Structural Genomics.

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ABSTRACT:

PURPOSE: Microfluidic devices are among a number of approaches used at the Midwest Center for Structural Genomics (MCSG) to increase the success rate in the structure determination pipeline. The Crystal Former from Microlytic contains 16 closed capillaries in which protein and precipitant diffuse in a countercurrent fashion, forming protein and precipitant concentration gradients that promote protein crystallization in a near convection-free environment.

METHODOLOGY: A set of proteins that had failed the vapor diffusion-based pipeline of the MCSG were selected for crystallization using the Crystal Formers and a set of 48 crystallization conditions that comprise the SmartScreen from Microlytic.

RESULTS: Of the 9 proteins selected for screening with the Crystal Formers, 3 proteins produced crystals from at least one condition from the SmartScreen crystallization screen. The crystals for one protein, 5-methyltetrahydrofolate-homocysteine methyltransferase from *Bacteriodes vulgates*, were harvested and the X-ray diffraction measured. Diffraction data for this protein were collected at 3.6 Å.

CONCLUSIONS: The Microlytic Crystal Former, coupled with the SmartScreen, constitute a simple screening technique that provides an alternative approach to the production of protein crystals when vapor diffusion methods have failed. Although still a work in progress, the MCSG plans to expand the use of the Crystal Former to a larger number of proteins that failed the standard vapor diffusion MCSHG crystallization protocol.

INTRODUCTION: The crystallization of proteins is the crucial step, and major bottleneck, in the course of protein structure determination via X-ray diffraction. Protein crystallization relies on the gradual reduction in protein solubility through modulation of the chemical environment of the target protein. Under special conditions that drive self-association, weak interactions promote the formation of a regular, periodic crystal lattice rather than protein precipitation. The process of protein crystal nucleation and growth is especially remarkable given the highly irregular nature of protein surface and intrinsic protein properties, including conformational heterogeneity, electrostatic surface potential and protein flexibility.

Crystallization conditions for a given protein cannot yet be deduced *ab initio* and, thus, must be determined through exhaustive and systematic screening of chemical space. Such screening involves sampling a wide range of buffer identities and pH, salt identities and concentration as well as concentrations of “precipitating reagents” that promote the decreased solubility of many proteins. In recent years, this screening approach has become highly automated for both vapor diffusion and microbatch screening formats. However, despite the current ability of high throughput centers to rapidly screen thousands of chemical conditions for a given protein, many well-expressed and soluble proteins fail to crystallize in a single crystallization format. Recent studies have suggested that a more comprehensive crystallization screening approach would involve the use of two or more orthogonal crystallization formats early in the process, leading to a reduction in the total number of conditions to be sampled in any given format while increasing the number of identified crystallization conditions.

To test the contribution of orthogonal methods within the high throughput pipeline of the Midwest Center for Structural Genomics (MCSG), the Microlytic Crystal Former, along with the 48 conditions comprising the SmartScreen, were selected as a salvage method for several proteins that had failed the extensive vapor diffusion protocols typically employed at this center. Of 9 bacterial proteins selected for the initial screen, crystallization conditions were identified for 3 proteins in the first crystallization pass. One of these initial conditions produced crystals suitable for X-ray data collection. The compatibility of the Crystal Former with many standard robotic systems for crystallization and crystal imaging, along with the ability to harvest crystals directly for data collection and structure determination, makes the system an attractive alternative to the standard MCSG crystallization protocol.

MATERIALS & METHODS

Crystallization

A set of 9 target proteins (Table 1) from the Midwest Center for Structural Genomics were selected for crystallization with the Crystal Former owing to their failure to crystallize in the vapor diffusion pipeline. Each protein was screened against the 48 conditions that comprise the Smart Screen (Microlytic, DK). Protein solution (0.3 - 1.0 µl) was deposited into one of two inlets bounding each capillary. Next, (0.3 - 1.0 µl) of precipitant solution was added to each opposing inlet. All inlets were sealed with foil to prevent dehydration. The Crystal Formers were then placed in snap-in holders (SH-1, Microlytic, DK) and incubated at 16°C.

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Crystals were obtained for 3 of the 9 proteins. Crystals for 5-methyltetrahydrofolate-homocysteine methyltransferase were grown in 20% (w/v) PEG 3350, 0.2 M magnesium formate pH 5.9. Further optimization of the crystals was achieved by varying the volume ratios of the protein and precipitant in the Crystal Formers.

Crystals for a conserved protein from the human gut bacterium, *Eubacterium ventriosum*, were grown in 20% PEG 6000, 0.1 M citric acid pH 5.0. Crystals for the proline dipeptidase from *Enterococcus faecalis* were grown in 28% PEG 400, 0.2 M calcium chloride, 0.1 M Hepes pH 7.5.

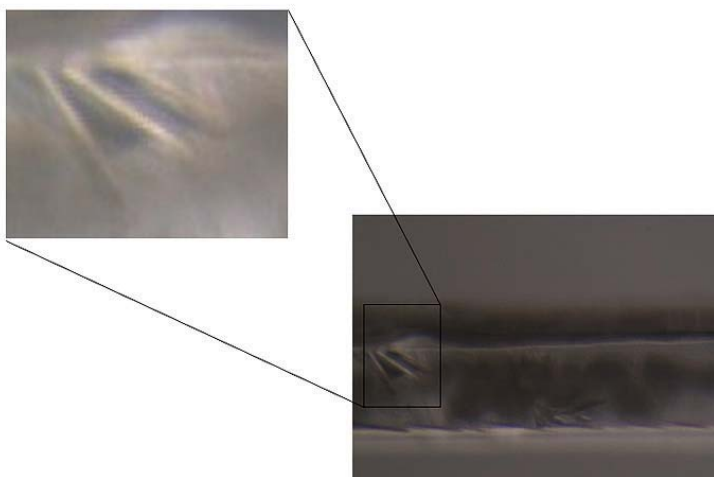
Data Collection

X-ray diffraction data for the 5-methyltetrahydrofolate-homocysteine methyltransferase crystal was collected at the 19-ID beamline of the Structural Biology Center at Argonne national Laboratory using the program SBCcollect. Diffraction data were collected at a wavelength of 0.9794 Å and recorded on an ADSC Q315r CCD detector located at 450 mm away from the crystal.

RESULTS

Initial Crystallization Results

Nine proteins from various bacterial sources were selected for crystallization trials with the Crystal Formers (Table 1). All of the selected protein targets had been subjected to extensive screening within the MCSG pipeline without success. For the Crystal Former trials, proteins were screened only against the 48 conditions of the Smart Screen (Microlytic, DK). From these initial trials, crystals were iden-



tified for 3 of the 9 proteins: (1) 5-methyltetrahydrofolate-homocysteine methyltransferase from *B. vulgates*; (2) proline dipeptidase from *E. faecalis*; (3) a conserved hypothetical protein from *E. ventriosum*. Crystals for the proline dipeptidase and the hypothetical protein were too small for diffraction analysis.

Crystal optimization and diffraction analysis of 5-methyltetrahydrofolate-homocysteine methyltransferase

Crystals for 5-methyltetrahydrofolate-homocysteine methyltransferase were optimized using the Crystal Former by varying the ratio of protein and precipitant volumes (Figure 1). Crystals were harvested directly from the microchannels of the Crystal Former with nylon cryoloops, cryoprotected and flash frozen in liquid nitrogen. Data were collected to a maximum resolution of 3.6 Å.

CONCLUSIONS

Microfluidic devices, such as the Microlytic Crystal Former, are among a number of approaches used by the MCSG to increase the success rate in the structure determination pipelines. Within days of the initial set-up, crystals were obtained for one third of the tested protein when the Crystal Formers were employed. None of these proteins had been previously crystallized. Furthermore, the Crystal Former design permitted the harvesting of crystals for data collection without optimization in another crystallization format. The data reported here underscores the advantages of employing orthogonal methods for crystallization where vapor diffusion approaches have failed in maximizing the crystallization success in a high throughput environment.

ACKNOWLEDGEMENTS

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Figure 1 | Crystals of the 5-methyltetrahydrofolate-homocysteine methyltransferase.

| Organism | Protein | Molecular Weight | Expression Vector |
|------------------------------------|---------------------------------------------------------|------------------|-------------------|
| <i>Geobacter sulfurreducens</i> | Transcriptional regulator, GntR family | 27635 | pMCSG7 |
| <i>Eubacterium ventriosum</i> | Conserved protein from human gut bacteria | 21376 | pMCSG19 |
| <i>Bacteriodes vulgates</i> | 5-methyltetrahydrofolate-homocysteine methyltransferase | 31680 | pMCSG19 |
| <i>Vibrio parahaemolyticus</i> | Putative metal-dependent phosphoesterase | 32229 | pMCSG19 |
| <i>Clorobium tepidum</i> | MoaA/nifB/pqqE family protein | 34979 | pMCSG19 |
| <i>Chlorobium tepidum</i> | Conserved hypothetical protein | 14342 | pMCSG19 |
| <i>Enterococcus faecalis</i> | Proline dipeptidase | 15008 | pMCSG7 |
| <i>Acinetobacter sp. ADP1</i> | Conserved hypothetical protein; putative ABC1 protein | 50405 | pMCSG19b |
| <i>Bacillus stearothermophilus</i> | DivIVA protein | 21090 | pMCSG7 |

Table 1 | MCSG protein targets selected for crystallization with the Crystal Formers.