

Improved protein crystallization output using the Crystal Former™

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ABSTRACT:

PURPOSE: Simple, effective and robust methods are needed for identification of protein crystallization conditions. The Crystal Former™ (Microlytic ApS, Denmark) is a device that is designed to facilitate crystallization by combining unique surface chemistry with highly efficient fluid mixing in a simple to use format. The Crystal Former™ can be loaded by hand within a few minutes or using automated liquid handling robots and can be incubated under standard laboratory conditions allowing crystal nucleation and subsequent growth to occur. In this study we compare the crystallization hit rate of the Crystal Former™ to conventional vapor diffusion plates for the model proteins Thaumatin, Catalase and Myoglobin.

METHODOLOGY: The proteins Thaumatin, Catalase and Myoglobin (Sigma, USA) were solubilized in appropriate buffers. For each protein 96 sparse matrix crystallization experiments were set up using sitting drop plates (Corning CrystalEX, Hampton Research, USA) and the Crystal Former™. Crystallization experiments were incubated at room temperature for 7 days and crystallization hit rate was evaluated by manual inspection of crystallization experiments as well as X-ray diffraction data collection of a representative subset of the crystals.

RESULTS: The Crystal Former™ identified 5, 28 and 8 crystallization conditions for Thaumatin, Catalase and Myoglobin, respectively. Using sitting drop vapor diffusion 1, 7 and 1 crystallization conditions were identified for Thaumatin, Catalase and Myoglobin, respectively. The diffraction properties of a subset of the crystals obtained using the Crystal Former™ were determined at the X6A beamline at NSLS at Brookhaven National Labs and all crystals tested showed protein diffraction patterns to a resolution between 1.1-3.1 Å.

CONCLUSIONS: For the proteins Thaumatin, Catalase and Myoglobin the unique mixing kinetics of the Crystal Former™ offer between 4-fold to 8-fold improved crystallization hit rate compared to conventional sitting drop vapor diffusion techniques. Additionally,

the crystals grown within the Crystal Former™ were extracted from the devices and showed good diffraction properties. This demonstrates that the Crystal Former™ offers significantly improved output of protein crystallization efforts when used as a front end protein crystallization screening device compared to vapor diffusion techniques.

INTRODUCTION: X-ray crystallography allows researchers to gain a detailed understanding of the workings of biological macromolecules at atomic resolution, that benefits our basic scientific understanding of biology as well as enables rational design of novel drugs to treat human disease. A major bottleneck in the protein crystallography pipeline is the formation of protein crystals that are suitable for X-ray diffraction studies. Crystallization of a biological macromolecule is achieved by perturbing the solubility of a concentrated pure protein solution by the addition of precipitants such as salt, polymers and other additives to promote crystal nucleation followed by crystal growth. In addition to using the appropriate precipitants for a particular protein, the mixing of the protein solution with the precipitant is known to have a significant impact on crystal formation.

Currently the most widely used technique for crystallization of proteins is vapor diffusion in which a protein solution is mixed with a precipitant solution followed by a controlled dehydration of the drop. An alternative mixing kinetics is achieved in the Crystal Former™ in which a microfluidic channel with a cross section of 100 µm by 150 µm is filled with a pure protein solution, which is put in liquid communication with a precipitant solution. The microfluidic geometry results in a diffusive mixing of the two solutions throughout the channel enabling the sampling of a continuum of protein and precipitant concentrations.

In this study we evaluate the crystallization hit rate of sitting drop vapor diffusion plates and the Crystal Former™ for the model proteins Thaumatin, Catalase and Myoglobin.

Table 1 | Preparation of model protein solutions.

Protein	Thaumatin	Catalase	Myoglobin
Concentration	50 mg/mL	30 mg/mL	62 mg/mL
Buffer	dH2O	dH2O	dH2O

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CONCLUSIONS: Using the three commercially available model protein proteins Thaumatin, Catalase and Myoglobin we determined the crystallization hit rates of the Crystal Former™ and sitting drop plates for two widely used sparse matrix screens Crystal Screen I and JCSG plus I. The results showed that the crystallization hit rate is improved 4-fold to 8-fold by using the Crystal Former™ instead of the sitting drop plates for the initial screening experiments. Furthermore, we verified by X-ray diffraction at X6A beamline at NSLS at Brookhaven National Labs, that a representative subset of the crystals obtained using the Crystal Former™ were indeed protein crystals.

In conclusion these results demonstrate that the unique mixing kinetics of the Crystal Former™ offer substantially improved crystallization hit rates compared to vapor diffusion plates and thus should be considered for primary screening of protein crystallization conditions.