The Efficient use of a 1536 High-Throughput Crystallization Screen to Guide Subsequent Optimization

Understanding the crystallization landscape

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Introduction

The Center for High Throughput Structural Biology (CHTSB) offers a High-Throughput crystallization screening service available to the outside community. The service performs 1536 different crystallization experiments using a microbatch under oil technique (Chayen *et. al.* 1992) with about 400 µl of macromolecule solution (10 mg/ml). Images are recorded from each experiment over time. The 1536 conditions are comprised of a incomplete factorial design (984 conditions) coupled with 552 conditions comprising commercial cocktails that use both the factorial and grid sampling approaches. Previously, results had been analyzed on the basis of crystallization hits. This required significant analysis of the results to obtain a comprehensive picture of the crystallization space, and did not serve to illustrate potentially lucrative conditions to be exploited. By presenting the results graphically as a function of the chemical landscape, chemical trends in crystallization conditions are rapidly apparent and can be readily exploited .

The 1536 cocktail screen

The 1536 cocktail makeup and how it is used is described in detail elsewhere (Luft et al., 2003). The 984 in-house conditions comprise a incomplete factorial sampling of 36 salts, eight buffers, and 5 different PEGs (table 1).

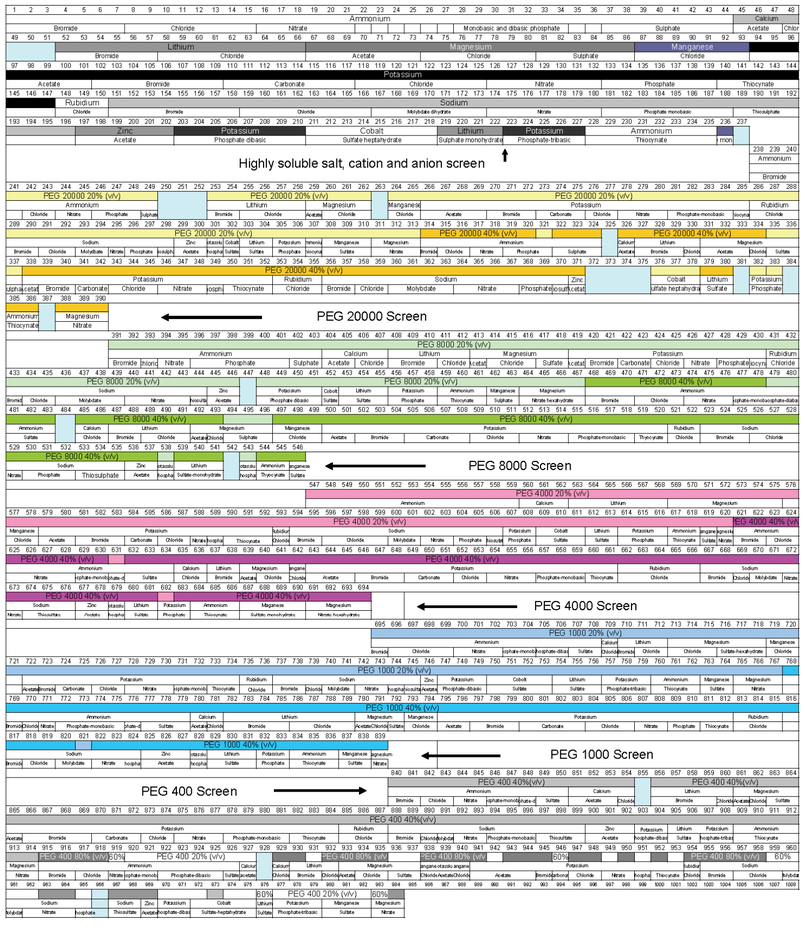


Table 1. Formulation of in-house components of 1536 screen

Within this screen are a selection of Hampton Cryo screens, shown as the pale blue blocks. These serve as control points for a related study to be described elsewhere. The remainder of 1536 cocktails are comprised of commercial screens available from Hampton Research. Specifically, in order of use; the Natrix Screen, Quick Screen, Nucleic Acid Screen, Sodium Malonate Grid, PEG/Ion, PEG 6000 Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, HT Screen, Index and the SaltRx screen.

The incorporation of a mix of incomplete factorial and grid screen designs is deliberate. The grid screens complement the incomplete factorial sampling of crystallization space providing a control to see the effect of fine sampling on a narrow region of that space

The macromolecule and cocktail solutions (200nl of each) are setup under oil and the resulting experiments imaged over time (Luft et al., 2003). The results are presented using software called Macroscope developed in-house. In the current form it displays 96 experiments at a time in the format shown in figure 1. It is possible to zoom in on individual images and display the chemical conditions underneath.

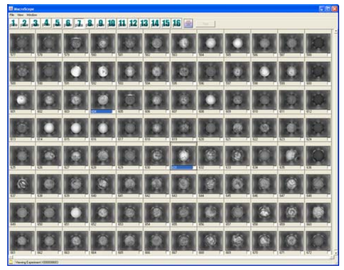
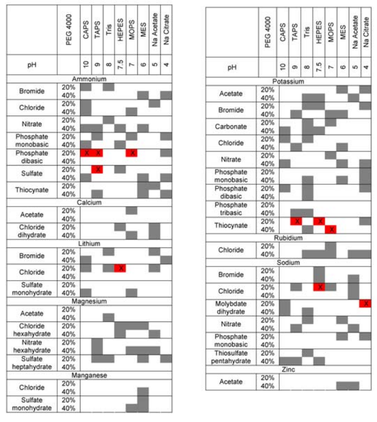


Figure 1. An example of 96 conditions for apoferritin comprising part of the PEG 8000 and PEG 4000 screens (577-672) as displayed in Macroscope. Macroscope presents the images in the order of experiment number (table 1). Highlighted images show crystals. Each crystal can be scored as a function of multiple definitions with the resulting hits exported as a webpage containing images and full chemical information.

Representing the data in chemical space

In figure 1, the chemical space is not represented. The current form makes it easy to visualize many images and score them, but scrambles the chemical space. When we take potential crystallization hits and plot them as a function of the chemical space, patterns that are not seen in the standard representation rapidly become apparent, table 2. In this case, for the protein apoferritin, a number of observations can be made from this table alone: a significant portion of space, ~60%, is un-sampled and, there are clusters of crystallization hits around ammonium phosphate dibasic, potassium thiocynate and sodium chloride with a single hit in lithium chloride.



When the same representation is applied to other components of the incomplete factorial screen, the crystallization landscape is revealed and predictions can be made. This highlights conditions that have not been sampled but are chemically likely to produce hits.

In figure 2 the sampled space is shown (shaded boxes) as a function of PEG type and pH. Going from PEG 1K to PEG 20K takes the experiment's outcomes from clear to precipitate. Fortunately this passes through a region of crystals, and some intelligent guesses can be made about the trajectory for crystallization.

Table 2. Representation of PEG 4000 screen in chemical space with hits for apoferritin. Grey boxes show the sampled space with hits from that space marked in red. Un-shaded boxes are not sampled by the screen.

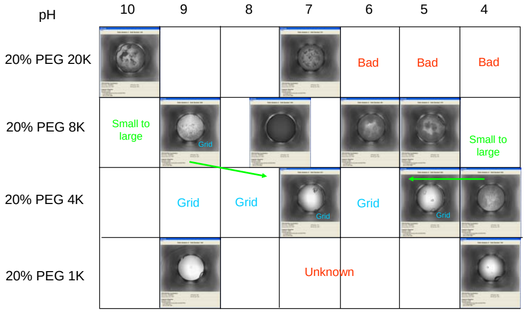


Figure 2. Crystallization results plotted on a grid showing the conditions sampled and more importantly those not. In this case PEG and pH play a major role in optimization. The "Grid" shows conditions that should be covered by a grid screen for optimization.

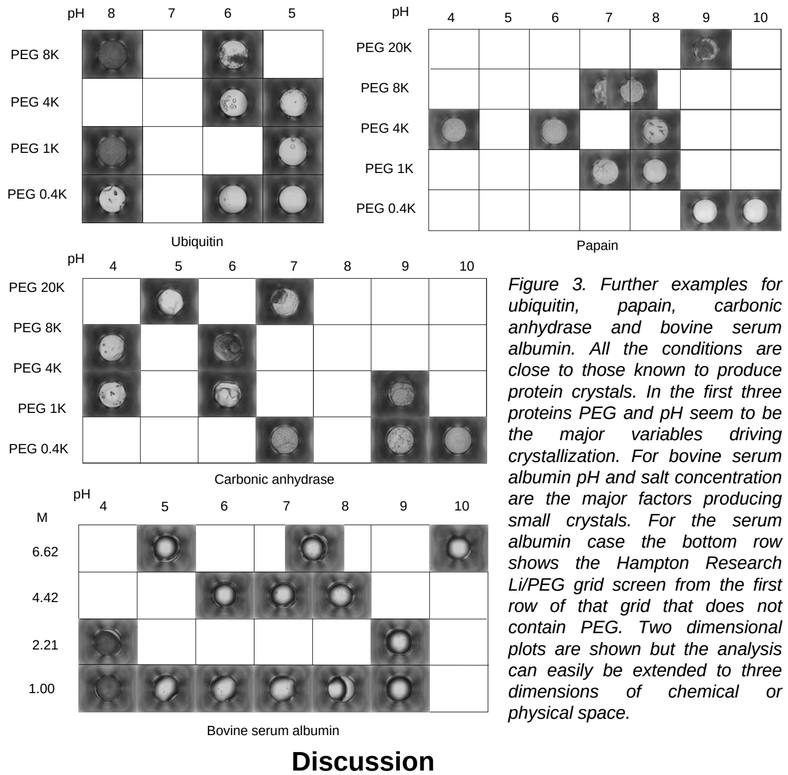


Figure 3. Further examples for ubiquitin, papain, carbonic anhydrase and bovine serum albumin. All the conditions are close to those known to produce protein crystals. In the first three proteins PEG and pH seem to be the major variables driving crystallization. For bovine serum albumin pH and salt concentration are the major factors producing small crystals. For the serum albumin case the bottom row shows the Hampton Research Li/PEG grid screen from the first row of that grid that does not contain PEG. Two dimensional plots are shown but the analysis can easily be extended to three dimensions of chemical or physical space.

Discussion

The incomplete factorial design of the screen simplifies the analysis of crystallization results as a function of pH, anion, cation and PEG. The large sampling area provides us with a crystallization map rather than a single hit to optimize around. It identifies trends and directions of approach for optimization. A software package, Architect, is in development to reform the image display automatically as a function of user defined chemical space maps. This will be combined with CHTSB developments in automated image analysis. The method can be applied to past, current and future images (all archived) so that the crystallization space can be explored as a function of the protein. The analysis complements the DVR/T optimization method (Luft *et al.* 2007) but requires an extra grid screen step before DVR/T if the sampling of space is too sparse in a region of interest. In summary, *you should know what crystallization conditions you examined but more importantly how those relate to those you did not*.

Further Information and References

Details on using the 1536 screening service are available at:
http://www.chtsb.org/About/HTS_Services/hts_services.html

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