

## How to start to crystallise proteins

The sparse matrix design is probably the most common search strategy in use today for initial screening of crystallization conditions. Its popularity is due to its success and its ease of use, now that the formulations are available as commercial kits.

Recent data mining experiments<sup>1,2,3,4</sup> have revealed that there is a lot of redundancy in the screening kits in common usage. This has resulted in a number of minimal screens being proposed.

For the beginner, then, it may be prudent and worth trying such a minimal screen in the hope of a lucky result, especially as this can involve as few as 24 trial conditions. At the JCSG in Toronto it was found that out of 755 proteins the full 48 conditions of the classic screen of Jancarik & Kim<sup>5</sup> was able to crystallise 338 proteins or 45%; however, only 24 of those conditions crystallised 318 proteins or 42%.

There are typically two schools of thought about how one should proceed with a crystal growth trial. One approach would be to utilise a modern sparse matrix screen alongside a good systematic screen. JCSG *plus* and PACT *premier* would be a good choice, if there is plenty of protein, as these are both 96 condition screens: or quite often the sparse matrix is tried first.

This leads us to the second school of thought. What happens when you don't get any "hits" from a sparse matrix design? Do you proceed with another version of the sparse matrix or try a different kind of design altogether? The answer to this will depend of course on how much protein is available and if the screening is done in reiterative rounds; i.e. the results are awaited from the first screen before a second screen is attempted. Many crystallographers feel that the advantage of employing a systematic screen at an early stage, the second school of thought, is that you learn more about the solubility of your protein and its behavior in different reagents.

A very powerful approach is therefore to set up a small (24 conditions) sparse matrix screen and a solubility profiling screen simultaneously. The experimental designs are quite different. Or more often, start with the systematic profiling or a simple solubility test with ammonium sulphate. An initial test, if stability vs pH is unknown, is worth doing.

The Solubility Tool Kit™ is such a solubility profiling screen that tests the solubility of different protein salts by utilizing the effects of the net charge of the protein to evoke crystallization. This is easier to do than it might sound; all you really need to know is the pI of your protein and then select from the kit the ready-made solutions appropriate for that pI. Thus, if your protein is basic you use one set of salts but if it is neutral or acidic, you use different sets.

Other systematic approaches, such as grid screens of PEG or ammonium sulphate can, of course, be used for solubility profiling. However the advantage of The Solubility Tool Kit is that it allows you to determine the crystallization potential of proteins in the presence of different ions (i.e. protein salts), to rapidly test and identify the crystallizing agent, the limit of nucleation/precipitation and locate the concentration range on which to focus. This search is based on the net charge of the protein and the chemical nature of the crystallizing agent (Hofmeister series). In addition it is more efficient by screening ranges of salts rather than one particular condition of a phase diagram.

### Results and refining the crystallization conditions

The first aim is to detect the limit of nucleation/precipitation for one crystallizing agent (or several is even better). Different situations may occur:

**The range is found** i.e. clear drops, followed by precipitates along a salt gradient.

First transfer the coverslips with clear drops onto reservoirs of higher concentration, and those having precipitates to lower concentrations. Then, prepare a new tray with the precipitant concentrations ranging from the last concentration where the drop remained clear to the first concentration where precipitation was observed. To do this, prepare a series of reservoirs using tubes containing the two solutions from both sides of the limit, and prepare a new gradient. The first reservoir will contain 1 ml of tube N, and the last will contain 1 ml of tube N+1. In between prepare 1ml with mixtures, for example 4 conditions with 0.2, 0.4, 0.6, and 0.8 ml of tube N completed to 1 ml with 0.8, 0.4, 0.6 and 0.8 ml of tube N+1, respectively.

Wait one week before drawing any conclusions.

**All have precipitated** First verify if it is precipitation or protein denaturation. If it is precipitation, transfer the drops onto reservoirs of lower concentration.

**All drops are clear** Transfer the drops onto reservoirs of higher concentration.

### References

1. Page *et al* 2003 Acta Cryst. **D59**, 1028
  2. Newman *et al* 2005 Acta Cryst. **D61**, 1426,
  3. Kimber *et al* 2003 Proteins **51**, 562,
  4. Page & Stevens 2004 Methods **34**, 373
  5. Jancarik, J. & Kim, S.H. J. Appl. Cryst. **24**, 409-411, (1991)
  6. Riès-Kautt M, Ducruix A: Inferences from physico-chemical studies of crystallogenes and the precrystalline stage. Methods Enzymol 1997, **276**:23-59.
  7. Riès-Kautt M, Ducruix A: From solution to crystals with physico-chemical aspect. In Crystallization of Nucleic acids and proteins: A practical approach. Ed Ducruix A and Giegé R. IRL/Oxford Press
  8. Riès-Kautt M Strategy 2: An Alternative to Sparse Matrix Screens. In Protein Crystallization Techniques, Strategies, and Tips Ed: Bergfors T M IUL Biotechnology Series International University Line, La Jolla, California.
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