Innovative protein crystallization screens

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About the author

After a Master's degree in Biochemistry (2000, Rennes 1, France), Fabrice moved to UK where he worked for GlaxoSmithKline (2003-04) and the Structural Genomics Consortium (2005-06). As a research engineer, he participated in the development of protein crystallization technologies applied to structure determination by X-ray diffraction, including notably the TOPAZ® system (Fluidigm) and more recently the dragonfly® (TTP Labtech). Since 2007, Fabrice is responsible for the Crystallization Robotic Facility at the LMB. He enables extensive initial crystallization screening and later optimizations, always lending his support to research scientists who tackle difficult, long-term research problems. In 2012, Fabrice received an MRC Award for innovation following the development of crystallization screens commercialised by MRC Technology (MORPHEUS® and Pi screens). In 2015, he obtained a PhD for the corresponding work.

Crystallization of challenging samples

The yield of experiments containing protein crystals with sufficient quality to solve structures is often very low. In addition to sample properties, an underlying reason is the very large number of combinations of variables associated with protein crystallization. Further problems arise from a multitude of experimental biases and later reproducibility issues.

A traditional crystallization condition includes a precipitant +/- a buffer-pH +/- an additive. There are now hundreds of well-known crystallization reagents and the possible combinations to formulate initial conditions in a systematic manner (grid screen/complete factorial) grow quickly to millions. However, the demand on sample quantity, the time-consuming and costly tasks associated to crystallization prevent users to screen that many conditions. There are three traditional alternatives to formulate initial screens and reduce the number of experiments: sparse matrix, incomplete factorial and stochastic. Over the last decade, an orthogonal approach is also employed using mixes of additives ("silver bullets" approach). Additional reagents are tested individually during later optimization experiments.

Here, we present protein crystallization screens developed at the LMB with 1- The LMB sparse matrix screen 2- the Pi incomplete factorial screens 3- the MORPHEUS grid screens integrating mixes of additives and 4- the ANGSTROM additive screen that focuses on cryoprotecting polyols. Each screen integrates 96 conditions, a friendly format for automated systems. Their innovative characteristics - meant to improve the yield of quality-diffraction crystals - will be briefly discussed.

LMB sparse matrix

Because of the random nature of crystallization, the development of screens is ultimately guided by empirical results. A sparse matrix screen is a collection of conditions that are empirically selected in order to form a minimal set of conditions for crystal growth. The optimization of sparse matrices has been stimulated by the availability of data regarding successful crystallization conditions. Ultimately, formulations of sparse matrices evolve according to new findings.

An analysis was performed of published conditions for crystal growth that resulted in protein structures at the LMB between 2002-2009. In total, more than four million individual crystallization experiments were setup following standard procedures with the vapor-diffusion technique and an initial screen considered as very large (1440 conditions).

Amongst the corresponding samples (~ 2800) samples were structural proteins of the bacterial cytoskeleton and phosphoinositide signalling proteins. Amongst the crystallized proteins were 30 hetero-oligomeric complexes and the average molecular weight of the crystallized proteins was 37 kDa. Amongst 106 optimized conditions published during that period, 96 non–redundant conditions were selected to formulate the LMB sparse matrix.

Figure 1A shows the occurrence of the main precipitant classes. Polyethylene glycols (PEGs) were found to be the most successful precipitants, especially those with high molecular weight (MW ≥ 1000 Da), followed by common salts (ammonium sulfate/phosphate, sodium citrate, other) and small volatiles (ethanol, MPD, other). This trend has been observed elsewhere (21), although it may not apply to specific subsets of targets such as integral membrane proteins (22). The occurrences of pH values cluster around the pH 5.0-8.0, although a minority of samples crystallized at a wider range of pH values (3.0-9.5, fig. 1B).

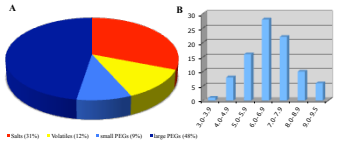

Figure 1A. Crystallization propensities of the main groups of precipitants. B. Occurrences of pH values.

Pi sampling (incomplete factorial)

Another minimal approach to formulation is the incomplete factorial where conditions are voluntarily excluded. Choices are typically made randomly, however we optimized how the formulation is balanced with a method called Pi sampling. Pi sampling uses modular arithmetic across the 96-conditon plate layout, properties of the chemicals used for the formulation and the concentrations of the corresponding stock solutions (starting from a given set of up to 36 stock solutions divided in 3 sets).

Figure 2 is a simplified representation of the Pi sampling with 3 sets of playing cards. Unlike Poker, the aim is to obtain the most diverse sets of cards (triplets). Diversity between the corresponding conditions is accentuated with varying the concentrations of reagents.

The Pi sampling is intended to help laboratories on a day-to-day basis to formulate crystallization screen based on the properties of their macromolecules and the techniques employed for crystallization. In this perspective, Pi screens can be formulated with a freely available web-based applet (see link below). For example, a Pi screen called Pi-PEG was formulated taking into consideration general observations made about crystallization of integral membrane protein samples that increased the yield of quality diffraction crystals of the adenosine A2a receptor (a G-Protein Coupled Receptor).

Figure 2. Pi sampling represented with play-cards. Kings were excluded to show sets of only 12 cards (*i.e.* 3 sets of 12 stock solutions).

http://pisampler.mrc-lmb.cam.ac.uk/

MORPHEUS (3-D grid including mixes of additives)

The formulation of a MORPHEUS screen follows a 3D grid approach, where eight mixes of additives are combined with four precipitant mixes and three buffer-systems. The use of mixes allows one to screen more extensively for components with a positive contribution to crystallization. Also, by selecting additives on their high occurrence in the Protein Data Bank (the universal archive for protein crystal data, http://www.rcsb.org), the chances of incorporating one that stabilizes or cross-links the protein (see example fig. 3), or promotes crystallization in some other way should be increased. Finally, more than one (type of) additive may be required for crystal growth, as proteins may be crystallized with multiple additives bound.

MORPHEUS screens integrate both widespread reagents and others not seen in commercial screens. Notably, heavy-atoms were are used (e.g. rare and alkali earth metals). Heavy-atoms are poorly soluble and will usually destabilize a protein. Nevertheless, heavy-atoms can opportunately enable to solve a crystal structure when they become part of the crystals initially produced without them. The anomalous signal produced by the heavy-atoms is used to deduce information about the phase and hence structure solution is enabled (i.e. the isomorphous replacement method). In addition to additive mixes, the MORPHEUS screens integrate mixes of precipitants and buffers. Precipitants can be mixed to have a synergetic effect and to provide cryoprotection. An advantage of buffer-systems is that no concentrated acid/base is required to alter the pH and hence formulation becomes fully automated friendly.

The original MORPHEUS screen increased the yield of quality diffraction crystals during projects related to mechanisms of Phosphoinositide 3-kinase and Ubiquitin.

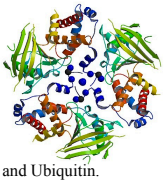

Figure 3.Crystal structure of the Human Protein Tyrosine Phosphatase Receptor Type J (PTPNJ, PDB ID: 2CFV). The protein crystallized only as a non-physiological trimer. The trimer is formed with interactions between nickel ions (blue spheres) and the engineered Histidine tags used in purification (blue ribbons). With the permission of Alastair J. Barr (University of Westminster, London).

ANGSTROM additive screen (polyols)

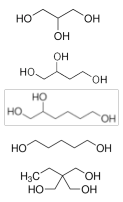

Figure 4. Glycerol-like molecules (glycerol on top).

After initial hits have been obtained, one will investigate how to reproduce the crystals and, ultimately, optimize their diffraction. In order to do this, the physico-chemical properties of the sample and the parameters of the crystallization experiments can be altered with an additive screen. We developed an additive screen, called ANGSTROM that integrates cryoprotecting polyols. The polyols used in the preparation of the screen are derivatives of glycols (fig. 4), carbohydrates (sugars/saccharides) and polymers.

Polyols are stable and soluble in most conditions, and in these respects lend themselves to be investigated as crystallization additives. Also, they have a capacity for water sorption and hence will alter crystallization mechanisms and kinetic of equilibration during crystallization experiments. They can enhance the stability of proteins too. Finally, to bypass the formation of ice crystals during flash-cooling (from 277-297 to 0 K using liquid nitrogen), crystals are previously soaked in a solution containing a cryoprotectant (most commonly glycerol). Many crystals are damaged/lost owing to the extensive handling and because of considerations such as the cooling rate and differential expansion between the crystal and the mother liquor. Since polyols alter the parameters related to the cryoprotection of crystals, they should also be thoroughly investigated at that stage.

The ANGSTROM screen has already been successfully employed for crystal optimization at the LMB. An example is shown on Figure 5 (complex ESCORT1, 30 KDa, 10 mg/ml, with the permission of Nicolas Soler).

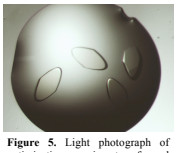

Figure 5. Light photograph of optimization experiment performed with the ANGSTROM screen.

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http://www2.mrc-lmb.cam.ac.uk/

Further reading

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