IUCr webinar series

Formulation of the MORPHEUS protein crystallization screens

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Questions and Answers

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1- Did you prove that MORPHEUS screens are more efficient than other screens?

No. In fact, this would require a collaborative approach with several labs where users would proceed with a very large number of experiments following a strict protocol. However, we regularly observe MORPHEUS hits not seen in other screens. This has spared a lot of optimization work for many research groups. Testing a new formulation is particularly competitive at the crystallization facility of the LMB where many other commercial screening kits are readily available to users and challenging samples are being trialled routinely.
To see a list of initial screening kits available commercially at the LMB go to:
http://www2.mrc-lmb.cam.ac.uk/groups/JYL/WWWrobots/robot-nomenclature.html

2- You showed an example of trans-membrane protein structure solved using MORPHEUS on slide 44, a protease made of 8 transmembrane alpha-helices found in yeast (Prior Jr. *et al.*, 2013, PDB ID: 4IL3), can you have another example?

Yes, notably a Porin, made up of 14 beta-barrels found in outer membrane of the gram-negative bacteria (Van Den Berg *et al.*, 2015, PDB ID: 4RL8). It makes sense because MORPHEUS precipitant mixes are PEG-based. In addition, some additives are well-suited for membrane protein crystallization (*i.e.* divalent cations, monosaccharides and NDSBs).

3- Will false positives be observed with MORPHEUS screens?

There were no false positives when testing the final formulation of the screens with standard samples containing commercially available proteins (*e.g.* Catalase), basic salts (*e.g.* NaCl) and buffers (*e.g.* Tris). No additional false positives were observed later against samples produced at the LMB. In fact, MORPHEUS screens are optimized to avoid false positives (some reagents have been removed/replaced for this). However, considering the broad variety of buffers used to prepare protein samples trialled against the multitude of chemicals used to formulate the screens, false positives cannot be avoided entirely.

4- Where do you start when optimizing a condition?

Consider the starting mixes as your usual stock solutions made with single chemicals. The concentration of a MORPHEUS mix is the sum of the concentrations for each chemical (*e.g.* Precipitant mix 1: 10% *w*/*v* PEG 20 000 + 20% *v*/*v* PEG MME 550 = 30 %). Remember also the universal ratio of volumes for the 3 types of stock solutions (completed with water): 0.5 stock precipitants + 0.1 stock additives + 0.1 stock buffer system + 0.3 water. Typically, one would start by varying the volumes of precipitant and additive mixes. This is facilitated at the LMB with the 4-corner method for producing 2D gradient of concentrations (*A complementary webinar is available on the IUCr channel that explains optimization of MORPHEUS conditions according to the 4-corner method*). Different pH values should be tested as a third dimension (see question 5). *All the stock solutions to formulate optimized MORPHEUS conditions are available at Molecular Dimensions Ltd.*

Later, one may want to investigate other variables, such as the specificity of an additive. For this, the additive mix is replaced by a single additive (its concentration is set higher than in the original mix). The ratio precipitant:cryoprotectant can also be varied. Finally, remember that chemicals can have hidden roles. For example, some buffers are also

surfactants and hence effects of varying their concentration should be investigated when possible.

5- How do you produce a range of pH values with a MORPHEUS buffer system? Would I have a linear plot during titration?

Titration of the MORPHEUS buffer systems is not strictly linear, although they facilitate the production of stock solutions at different pH values (initial-pH +/- 0.5). Typically, one would start with an excess volume of the Acid solution and then add the corresponding Base solution until the lowest pH value required for optimization is reached (*i.e.* initial pH - 0.5). After withdrawing some of the solution (as the first buffer stock solution), titration continues to achieve the initial pH. More of the titrated solution is withdrawn (second stock) before completing the titration (*i.e.* initial pH + 0.5). The remaining titrated solution is hence the third and last buffer stock solution that will be used for producing optimization screens.

6- Are all the MORPHEUS conditions cryoprotected? How did you test them?

They are all cryoprotected. In order to avoid bias towards glycerol 7 other effective cryoprotecting agents were used across MORPHEUS conditions, namely: ethylene glycol, MPD, PEG MME 550, 1,2,4-butanetriol, 1,2,6-hexanetriol, ,5-pentanediol and 1,1,1-tris(hydroxymethyl)propane, in addition, their concentration is optimised to be minimal. During initial investigations, each cryoprotectant was mixed with different precipitants at different concentrations. Samples of these mixes and the corresponding conditions (200 μm microloops) were flash frozen into a cryostream before X-ray exposure to determine the required concentration of cryoprotectant with which no signs of ice is observed. Conditions cryoprotected with a minimum concentration of cryoprotectant (and that were also effective during crystallization assays) were integrated into the final formulation.
Ultimately, one may still observe signs of ice on diffraction patterns from crystals produced with MORPHEUS, because this also depends on the characteristics of the

protein sample, the time when the crystal is extracted from the experiment (is the experiment equilibrated?), the size of the microloop employed, *etc*.

7- You mentioned MORPHEUS III, a screen formulated with drug compounds. Can you tell us a bit more?

MORPHEUS III is in the late stages of development. The additives are not exclusively ligands well represented in the PDB, they are mainly well-known drug compounds. The underlying principle around the formulation is the same as before: introducing mixes of chemicals that may stabilize proteins, or alter positively other variables to increase the yield of useful crystals. The formulation approach is also the same as before, notably the universal ratio of volumes for the 3 types of stock solutions (see question 4). Additives well-soluble in water or ethanol solution have been selected and proceeding empirically, their final concentrations have been optimized to be relatively high. The underlying reason is to maximize the chances of observing a positive effect. Eventually, a compound can bind to an active site of the protein being investigated. MORPHEUS III combines fragment-based discovery and crystallisation approaches. I hope to publish the corresponding work later this year.