The MemGold family of alpha helical membrane protein crystallization reagents: ...the science behind the screens.

In recent years significant progress has been made in determining the atomic structure of alpha helical membrane proteins using X-ray crystallography. However the identification of conditions that give crystals that are suitable for structure determination remains one of the major bottlenecks.

The most successful method for growing crystals of membrane proteins remains vapour diffusion from detergent solubilised proteins. We reasoned that important advances in membrane protein crystal screen design could be achieved by systematically mining the information present in the Protein Data Bank and associated research literature.

In 2008 we undertook an analysis of the crystallization conditions for 121 alpha helical membrane proteins to design a rationalized sparse matrix crystallization screen, MemGold (Newstead et al., 2008). Our analysis has revealed a striking success for small MW PEGs in the crystallization of channels and transporters, with larger MW PEGs being more successful for respiratory complexes and other membrane proteins that typically contain large hydrophilic domains (Figure 1).

Interestingly, when compared with similar analyses of successful crystallization data for bacterial non-membrane protein targets (Page & Stevens, 2004) from the Joint Centre for Structural Genomics in San Diego, CA and the University of Toronto, Canada, it was clear that a major difference is the success of small MW PEGs in promoting the crystallization of membrane proteins. By comparison, organic molecules such as MPD are much less successful.
Figure 2. Successful crystallization precipitants reported in our 2008 study for alpha helical membrane proteins compared with similar results reported for bacterial non-membrane proteins from the Joint Centre for Structural Genomics and University of Toronto. The reported conditions in the JCSG-67 and Toronto Core-24 were found to be the smallest subset of conditions that would have crystallized the maximum number of bacterial non-membrane protein targets in these structural genomics programs in 2004.

The rate of new membrane protein structures being determined is steadily increasing, in line with previous predictions (White, 2004), providing an ever expanding source of new crystallization information. We are continuing to mine this information to address the conundrum faced by many structural biologists in this area, which is “how many conditions are enough to adequately cover ‘crystallization space’ for a given membrane protein sample?”. This question is especially pertinent for eukaryotic MPs, when amounts are likely to be far scarcer compared to their prokaryotic counterparts. In 2012 our group undertook another detailed study of the crystallization conditions (Parker & Newstead, 2012). Many of the membrane proteins analyzed in this study crystallized in similar precipitant conditions to our 2008 study, with approximately two thirds being crystallized in a low MW PEG. However, the pH, salt and buffer components of the crystallization conditions differed substantially, suggesting successful crystallization screens should be more varied in these parameters rather than precipitant type. Based on these data a new sparse matrix style screen, MemGold 2 was developed. Our analysis of the available crystallization data suggests MemGold and MemGold 2 provide a comprehensive set of initial screening conditions for alpha helical membrane protein crystallization.

MemAdvantage: Additive screening for membrane proteins

We know from experience with non-membrane proteins that an initial crystal condition will often require optimization through the addition of small molecules, salts and specific ligands. To facilitate this task and again using information gathered from the present literature on membrane protein structures, we developed a compact, 96-reagent additive screen, MemAdvantage. Figure 3 shows the range of different small molecule and salt additives that have been reported to improve initial crystallization conditions for alpha helical membrane proteins. Multivalent ionic and polyalcohol salts appear prominently, accounting for 10 and 15 % of all reported structures respectively. A substantial increase in the number of secondary detergents and non-volatile organic molecules are also now being recorded since we first started analyzing the data in 2008, accounting for 19 and 12 % respectively.

The rationale behind the MemGold family of membrane protein crystallization products is to maximize the current information in the public databases to improve our success in crystallization and structure determination. Our goal in developing these screens is to provide useful products to assist the structural biology community in tackling the challenge of membrane protein crystallization, optimization and ultimately structure determination.

References