Crystallization of Membrane Proteins Requires Optimal Detergent Concentration, Precipitant concentration, and Use of Additives for Improved Diffraction

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I. Optimization of n-octyl-β-D-glucopyranoside (OG) concentration and glycerol concentration improved diffraction for 2 GlpF mutant proteins

- GlpF “FHC” mutant
  - Initial hit: 15-20% PEG 2000, sodium acetate pH 4.6
  - ~16 Å resolution
- GlpF “GHC” mutant
  - Initial crystal hit: ugly needle crystals
  - 4 Å Resolution

II. Magnesium chloride and isopropanol (IPA) essential for improved crystal size, quality, and resolution limit: Aquaporin Z (AqpZ)

- Without MgCl₂ & IPA
  - Initial crystal hit: small (<20 µm) hexagonal crystals
- With MgCl₂ & 4% IPA
  - Initial round of screening: PEG 2000, MES pH 7.5, 30% PEG OG
  - Lots of very small (<20 µm) bipyramidal crystals
  - No diffraction

III. Optimization of Aquifex aeolicus AmtB crystals required change in precipitant concentration, pH, and addition of polypropylene glycol (PPG)

- Initial hit: 15-20% PEG 2000, sodium acetate pH 4.6
  - Large numbers of 30-40 µm crystals
  - ~16 Å resolution
- Second round of screening: 1.2-15% PEG 2000, sodium acetate pH 5.5
  - 5% polypropylene glycol (PPG)
  - 30-45 µm crystals
  - 4 Å Resolution (Khademi et al., unpublished)

IV. Optimization of Escherichia coli AmtB crystals required change in pH, finer screen of PEG 400, and use of seleno-methionine protein

- Initial round of screening: 30% PEG 400, sodium acetate pH 6.5
  - Lots of very small (<20 µm) bipyramidal crystals
  - No diffraction
- Final round of screening: 30% PEG 400, 10% sodium acetate pH 7.5
  - 100-200 µm hexagonal crystals
  - 3 Å resolution

V. Optimization of crystals for another membrane protein required change in pH, change in buffer type, and decrease in concentration of n-octyl-β-D-glucopyranoside (OG)

- Initial hit: 15% PEG OG, 20% PEG 2000, HEPES pH 7-7.5
  - No diffraction
  - +5% Polypropylene glycol (PPG)
  - Crystals grew slowly, out of strong phase separation, 2 Å resolution

VI. Optimization of crystals for another membrane protein required a change in pH and in precipitant concentration, which resulted in improved crystal growth from phase separation and improved diffraction limit.

- Initial hit: 15-20% PEG 2000, sodium acetate pH 4.6
  - Large numbers of 30-40 µm crystals
  - ~16 Å resolution
- Second round of screening: 1.2-15% PEG 2000, sodium acetate pH 5.5
  - +5% polypropylene glycol (PPG)
  - 30-40 µm crystals
  - 4 Å Resolution (Khademi et al., unpublished)
- Final round of screening: 27-30% PEG 400, MES pH 6.5
  - 5% polypropylene glycol (PPG)
  - 20-30 µm hexagonal crystals
  - 1 Å resolution

VII. Conclusions, comments, and additional practical measures

A) Protein homogeneity and stability are of paramount importance prior to protein crystallization. For membrane proteins, in general, this depends significantly on detergent concentration and detergent type.

B) Optimal detergent concentration for membrane protein crystallization promotes nucleation of a new crystal form with better diffraction quality by ordering the protein's flexible regions.

C) Divalent cations (Mg²⁺, Ca²⁺, etc), isopropanol alcohol (plus other organics and volatiles), and all detergent types (ionic, non-ionic, zwitterionic) are additives that should be included in crystallization screens. Detergents are especially helpful for proteins prone to aggregation.

D) For optimization of membrane protein crystallization - especially the aquaporins/aquaglyceroporins - the addition of magnesium has proved very successful, perhaps in improving crystal contacts and/or promoting strong molecular interactions.

E) Isopropanol alcohol (IPA), often called a "water structure breaker," is useful when aggregation caused by hydrophobic interactions is a concern. For AQPZ, it vastly decreased the amount of non-specific aggregation, thereby improving the quality and size of the crystals.

F) In the crystallization of membrane proteins, there appears to be some level of "good," crystalline precipitation within the drops, out of which grow the protein crystals.

G) Crystal size, quantity, and morphology are dependent on precipitant type and concentration, as well buffer type and corresponding pH. Based on crystallization of membrane proteins in our lab, lower molecular weight polyethylene glycols (400, 2000 etc.) were much more successful than other types of precipitants.

H) Protein centrifuged at 100,000 ×g for 20 minutes prior to set-up of drops.

I) Protein purity assayed by SDS-PAGE (coomassie stain and western blot).

J) Protein homogeneity assessed by SEC, DLS, or TDA.

References
