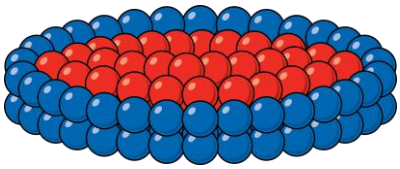
Pre-Mixed Bicelle Solutions

Bicelle Crystallization Protocol



Step 1: Prepare bicelle solutions:

Anatrace offers two solutions of pre-mixed bicelles:

- 30% 2.8:1 DMPC:CHAPSO (D606:C317 BIC MIX 0.25 ML)
- 30% 2.8:1 DMPC:DHPC (D606:D514 BIC MIX 0.25 ML)

Bicelle solutions should be stored at -20°C upon receipt. When ready to use, thaw the bicelle mixture at room temperature (the solution will become a clear gel) and place the solution on ice to transform the bicelles into a liquid phase. Ensure that the solution is heterogeneous prior to use. This may require several rounds of vortexing and cooling at 4°C. Anatrace pre-mixed bicelle solutions are stable over multiple rounds of freezing and thawing. (See Note 1)

Step 2: Reconstitute the membrane protein into bicelles:

To reconstitute membrane proteins into bicelles, bicelles are typically added to purified protein solution in a 1:4 ratio and incubated on ice. 30% bicelle solutions will allow for direct reconstitution into 6% bicelles. Pre-mixed bicelle solutions can be diluted with H_2O to screen additional bicelle concentrations or different protein:bicelle ratios can also be explored. (See Note 2)

The specific steps for membrane protein reconstitution into bicelles are:

- Prepare pure and concentrated membrane protein. Typical starting concentrations are between 8-10 mg/ml. (See Note 3)
- Add bicelle solution (kept at 4°C – liquid phase) to purified protein in a 1:4 ratio. For example, to prepare 100 µl of reconstituted protein in 6% bicelles, add 20 µl of 30% bicelle mixture to 80 µl of protein.
- Mix by pipetting up and down (do not vortex) and incubate the reconstituted protein on ice for 30 minutes.
- Prior to setup of crystallization experiments, ensure that the solution is homogeneous.
- At this stage, the reconstituted protein can alternatively be used for other biochemical assays, such as NMR, and activity assays.

Step 3: Setup of crystallization experiments:

Membrane proteins reconstituted into bicelles can be setup using standard crystallization techniques, either using vapor diffusion or the Microlytic Crystal Former. It is recommended to keep the protein:bicelle mixture cool to ensure that the solution remains liquid for easier pipetting. Crystallization experiments can be setup using any commercially available screens; however, the incidences of phase separation or false positives may be increased with some screens. For an overview of crystallization conditions that have successfully crystallized protein:bicelle mixtures see Note 4. Crystallization plates can be stored at any temperature, most successful being 20-25°C.

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Step 4: Visualization of crystallization experiments:

Some suggestions for visualization of bicelle crystallization experiments.

- ★ The use of a UV microscope is useful to separate false positives from protein crystals.
- ★ Setting up "bicelle only" experiments will allow for the identification of crystallization conditions that lead to crystalline behavior of the bicelle solutions.

Notes:

- ★ Note 1: Bicelles have unique phase properties. At lower temperatures, bicelles mixtures are liquid, and can easily be manipulated with pipettes or liquid handling robots, while at higher temperatures, the bicelles form a clear gel.
- ★ Note 2: For example, to prepare 100 µl of protein reconstituted into 5% bicelles, a 25% stock bicelle solution is needed. This can be prepared by diluting the 30% stock bicelle solution to 25% (16.7 µl 30% bicelles + 3.3 µl H2O = 20 µl 25% bicelles). Vortex the solution after dilution to ensure homogeneity. This solution can then added to 80 µl of concentrated protein solution and reconstituted as described above.
- ★ Note 3: Detergents that have worked previously with bicelle crystallization trials are DDM, OG, C8E4, LDAO, DM, Digitonin, and NG. (Ref 2).
- ★ Note 4: Table 2 in Poulos et al. (Ref 2) lists conditions that have been successful for crystallization of protein:bicelle solutions.

References:

1. Faham, S. and Bowie, J. U. (2002) J. Mol. Biol. 316(1), 1-6.
2. Poulos, S. et al. (2015) Methods in Enzymology. 557, 393-416.
3. Kimble-Hill, A. C. (2013) Front. Biol. 8(3), 261-272.
4. Agah, S. and Faham, S. (2012) Methods in Molecular Biology. 914, 3-16.

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