SAROmics

SAROmics Biostructures AB

Sending Samples for Crystallization and Sample Handling at SAROmics Biostructures

Preparation for shipping

To set up screens for crystallization and crystal structure determination, we generally use around 5 mg of protein at a concentration of approximately 10 mg/ml. However, it is possible to start with smaller amounts or lower protein concentrations if the solubility of the sample is an issue. For proteins with unknown crystallization conditions, we usually set up many crystallization screens (which correspond to hundreds of drops) with varying precipitants and precipitant concentrations, buffers, pH, temperature, etc.

Shipping the sample

The protein solution can be sent in various ways, often frozen and shipped on dry ice (using World Courier or similar).

Initial sample handling at SAROmics Biostructures

Upon the sample's arrival, we can apply a characterization procedure to assess the stability of the protein in the solution. We can use the method of differential scanning fluorimetry (DSF) to identify the best conditions at which the protein is most stable. We can also run Size Exclusion chromatography (SEC), dynamic light scattering (DLS), or mass spectrometry (MS). Before the crystallization trials, we may perform a DSF buffer screen to find the most optimal buffer for crystallization.

Crystallization and structure determination

Crystallization screens and the following crystallization condition optimization can take as little as 3-6 weeks, although depending on the protein, more extended periods may be required.

Data collection from well-diffracting crystals and subsequent structure determination are usually relatively rapid. When a close homolog (generally above 40-50% sequence identity) with a known three-dimensional structure can be found in the PDB, the molecular replacement method may be applied for structure determination. In many cases, an AlphaFold model of the protein may also be used for initial phasing. In case of failure of molecular replacement, "de novo" protein structure determination can be applied. We recommend a selenomethionine (SeMet)-labeled protein sample, which we can produce if the customer cannot. This labeling enables rapid structure determination using synchrotron radiation. If SeMet labeling is impossible, we may use other

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methods, including halide soaking of the crystals. In such cases, structure determination may take longer.

Additional information on the experimental procedure can be found on https://www.saromics.com/technologies/crystallization/

If you require any assistance or want to discuss your project, please contact us by filling out the contact form on the website!

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