SARomics

SARomics Biostructures AB

Sending Samples for Crystallization & Sample Handling at SARomics Biostructures

Before shipping

To set up crystallization screens, we generally use about 5 mg of protein at a concentration of roughly 10 mg/mL. However, smaller amounts or lower protein concentrations can be used if solubility is an issue. For proteins with unknown crystallization conditions, we typically prepare hundreds of crystallization drops with different precipitants, precipitant concentrations, buffers, pH levels, ionic strength, temperature, and other variables (see below).

Shipping the sample

The protein solution can be sent in various ways, but it is typically shipped frozen on dry ice (using World Courier or similar services). Make sure there is enough dry ice to keep the sample frozen until it arrives. Avoid sending samples over the weekend!

Initial handling at SARomics Biostructures

Upon receiving the sample, we typically perform a characterization process to evaluate the stability, solubility, and monodispersity of the protein in solution. For stability assessment, we use differential scanning fluorimetry (DSF) to identify the optimal conditions where the protein is most stable. To confirm monodispersity, we may perform size exclusion chromatography (SEC), dynamic light scattering (DLS), or, if required, mass spectrometry (MS).

Crystallization

- Crystallization screens and subsequent condition optimization can take as little as 3 to 6 weeks, although, depending on the protein, longer periods may be necessary. The most common crystallization protocols involve testing various conditions, with the most popular being:
- Type of buffer and its pH
- Ionic strength
- The presence of various salts in the solution
- The presence of ligands (co-factors, substrate analogs, inhibitors)
- The type of precipitant used (polyethylene glycol (PEG) and ammonium sulfate are the most common), etc.

Our services routinely use high-throughput and high-precision liquid handling and imaging robotics to set up the initial crystallization screens and optimize newly identified conditions. With robotics, we can screen 96 conditions using as little as 15 microL of protein sample, saving time and precious material. Temperature is a vital parameter for crystallization, and in the lab, we control it by storing the crystallization plates in plate hotels equipped with an imaging system (Formulatrix Rock Imager).

Data collection and structure analysis

Data collection from well-diffracting crystals and subsequent structure determination are generally quick processes. Our main X-ray source is the BioMAX beamline at the MAX IV synchrotron radiation laboratory in Lund, just a few kilometers from our facility. The close proximity of the synchrotron avoids the need to ship crystals to distant synchrotrons, ensuring that the crystals arrive safely at the beamline.

When a close homolog (typically with more than 40-50% sequence identity) that has a known three-dimensional structure is available in the PDB, molecular replacement is used for the initial data phasing. Often, an AlphaFold model of the protein can also be utilized for this purpose. If molecular replacement fails, "de novo" protein structure determination can be performed. We suggest using a selenomethionine (SeMet)-labeled protein sample, which we can produce if the customer cannot do so. This labeling allows for quick structure determination using synchrotron radiation. If SeMet labeling isn't possible, alternative methods such as halide soaking of the crystals can be employed. In such cases, structure determination may take longer.

Additional information on the experimental procedure can be found on
https://www.saromics.com/x-ray-crystallography-services/

If you require assistance or would like to discuss your project, please don't hesitate to contact us by completing the contact form on our website.

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