SAROmics Biostructures

Sending Samples for Crystallization and Sample Handling at SARomics Biostructures

Preparation for shipping

In order to set up crystallization screens for crystal structure determination, we generally need 0.2-0.5 ml of around 10 mg/ml protein concentration, although it is possible to start with smaller amounts or lower protein concentration if solubility of the sample is an issue. For proteins with unknown crystallization conditions, we normally set up a large number of crystallization screens (which corresponds to many hundreds of drops) with a variation of precipitants and precipitant concentrations, buffers and pH, temperature, etc.

Shipping the sample

The protein solution can be sent in a variety of ways, most commonly frozen and shipped on dry ice (using FedEx or similar). If the sample is sensitive to freezing (which sometimes causes aggregation when the sample is thawed), glycerol may be added prior to freezing. The protein may also be sent in a diluted state, if dilution does not denature it.

Initial sample handling at SARomics Biostructures

Upon arrival of the sample, we apply an extensive characterization procedure to assess solubility, stability and polydispersity of the protein solution. We use the method of dynamic light scattering to assess the presence of aggregates in solution and differential scanning fluorimetry (Thermofluor) to identify the best conditions at which the protein is most stable. Prior to the crystallization trials we may perform a DSF buffer screen to find the optimal buffer for crystallization. The samples may be dialyzed or diluted and buffer exchanged to replace the original buffer. If required, the sample may also be concentrated.

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, longer time periods may be required.

Data collection from well-diffracting crystals and subsequent structure determination are usually relatively rapid. In cases when a close homologue (generally above 40-50% sequence identity) with known three-dimensional structure can be found in the PDB, the method of molecular replacement may be applied for structure determination. For "de novo" protein structure determination, where no structural homologues are available, we strongly recommend selenomethionine (SeMet)-labeled protein material, which we can produce if the customer is not

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able to. This enables rapid structure determination using synchrotron radiation. If SeMet labeling is impossible, we may use other methods, one of which is halide soaking of the crystals. In such cases structure determination may take longer.

If you require any additional information, or if you want to discuss your project please contact us using the contact form at the website!

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