Sample qualification for membrane protein crystallisation based on protein-detergent-complex identification

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Dynamic light scattering (DLS) is an appropriate method to come up one of the most commonly encountered problems in membrane protein crystallization, the identification of detergents to form protein detergent complexes (PDI). Applying DLS in situ compensates some former obstacles of this method e.g. the required sample volume, cuvette cleaning and the limit to one sample at the time. Application of DLS in situ makes the use of multi-well plates possible. In combination with the use of very low quantities of protein and an automated way to perform measurements, DLS becomes high throughput capable. These are the prerequisites for a detergent screen in which a PDI can be identified by size distribution and size comparison with the "empty" micelle. Since PDIs are a uniform population of structurally intact macromolecules it provides a clearly distinguishable distribution signature that is easy to identify among many other possible size distributions [1].

The strategy to identify the right buffer conditions to form a PDI based on in situ DLS (SpectroLight 600, XtalConcepts) and the knowledge of pure micelle sizes for comparison starts with a "blind walk". Micelles are highly uniform objects in terms of size and stoichiometry. This fact can be exploited to distinguish "empty" micelles from PDIs. The absolute size of a micelle is highly related to the structure of the detergent molecule forming it. An example for the structure-size relationship of detergents and their micelles are the n-Alkyl-β-D-maltopyranosides a class of detergents that have been used extensively to stabilize membrane proteins for biophysical and structural studies [2].

Assuming that the membrane protein is already dissolved in some kind of buffer this is the initial point. First the membrane protein in its buffer has to get in contact with the new detergent to enable a detergent exchange. The most convenient way to achieve this is by adding an excessing amount of the new detergent buffer to the sample. Since the DLS detection volume is 500 nl and the ratio of new buffer and protein sample is 4:1 only 100 nl sample are required for a single test. A huge variety of detergents of all classes are available and in the majority of our experiments, a few of them transformed the protein into a potential protein detergent complex identified by its radius distribution signature.

References

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