A time-efficient identification of the best conditions for cryo-EM, NMR, SAXS and crystallization applying *in situ* DLS

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After purification, a sample might not be in the desired aggregation state or a biologically-relevant complex of molecules might not have formed. Some buffer conditions might support complex formation apart from its natural environment but often such conditions are unknown. With the newly developed *in situ* Dynamic light scattering (DLS) [1,2] smallest sample volumes and a huge number of samples become accessible. It is a non-invasive technique that can provide detailed information about the state of aggregation and the absolute size of a target protein. Application of this advanced DLS method provides a time, material and manpower efficient way to qualify samples for subsequent structure determination methods e.g. single particle cryo-EM, SAXS, NMR and crystallization.

The promoted approach ensures an appropriate particle size and size distribution on a sample carrier e.g. cryo-EM grid or NMR-tube, based on radius distribution measurements in 500 nl aliquots shortly before loading the sample on a sample carrier. The droplet is stored in a standard “microbatch” plate under paraffin oil to prevent evaporation. Particle size and aggregation state determination is carried out directly in wells. The determined radius distribution in this aliquot represents what you'll find on the grid or in your NMR tube if the sample transfer and freezing procedure worked as intended. If the particles determined by DLS show sizes of oligomers or aggregation, this is what later will be find on the grid, when applying e.g. cryo-EM.

For identification of conditions supporting complex formation and preventing aggregation, a screening of various buffer conditions might be applied. This is achieved by mixing a sample with a specially designed set of buffers and additives systematically on sample aliquots as small as 100 nl. This approach results in many conditions (usually 96) far exceeding the capacities of standard DLS and most sample carriers. The aim is to identify the (few) conditions that stabilize the desired macromolecular complex. Most of the applied conditions have a negative effect on the sample, leading to a very inefficient usage of cryo-EM, NMR, SAXS and crystallization when all samples would be investigated. Here *in situ* DLS is a key technology to select conditions as well.

Manual pipetting however is a tedious work which increases the chance of errors. A significant improvement of the workflow is to apply an automated dispensing system enabling a time, material and manpower-saving approach when setting up such buffer matrices. The few good conditions can be selected for subsequent investigation, resulting in a significant increase of positive hits on the sample carriers with an easy to use and time efficient technique.

References

[1] - SpectroLight 600 an *in situ* DLS system, see www.xtal-concepts.de.
[2] - J. Birch, D. Axford, J. Foadi, A. Meyer, A. Eckhardt, Y. Thielmann, I. Moraes, in press (2018)