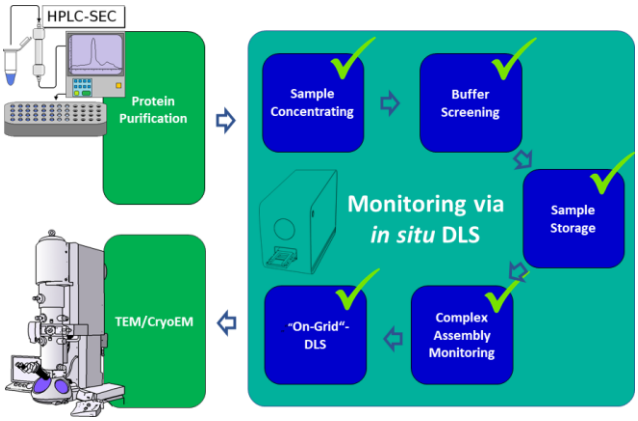
DLS for Single Particle 3D Cryo-EM

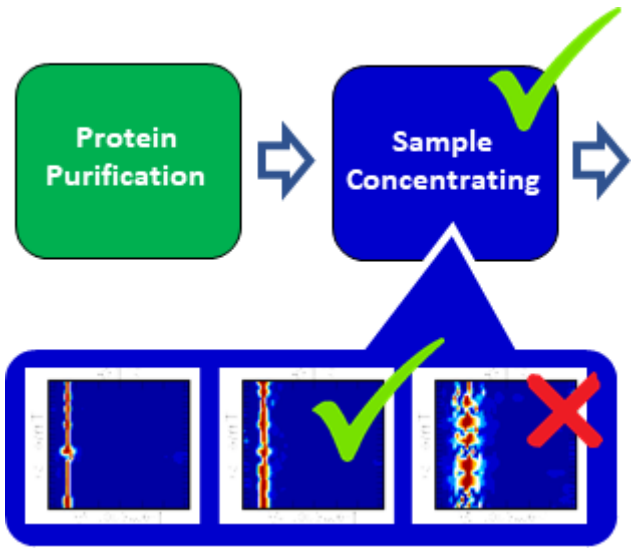
Successful data collection via single particle cryo-EM depends on suitable buffer conditions preserving integrity of proteins or high molecular weight complexes. However, identification of such specific buffer conditions is often in a repetitive trial and error process when applying electron microscopy itself. In contrast to that, *in situ* DLS provides the possibility to identify buffer conditions highly sample efficient, fast and independently from any electron microscopy equipment but highly reliable. In fact *in situ* DLS is capable to monitor almost the entire sample processing and preparation pipeline, and therefore closes the informational gap between purification and single particle 3D electron microscopy.

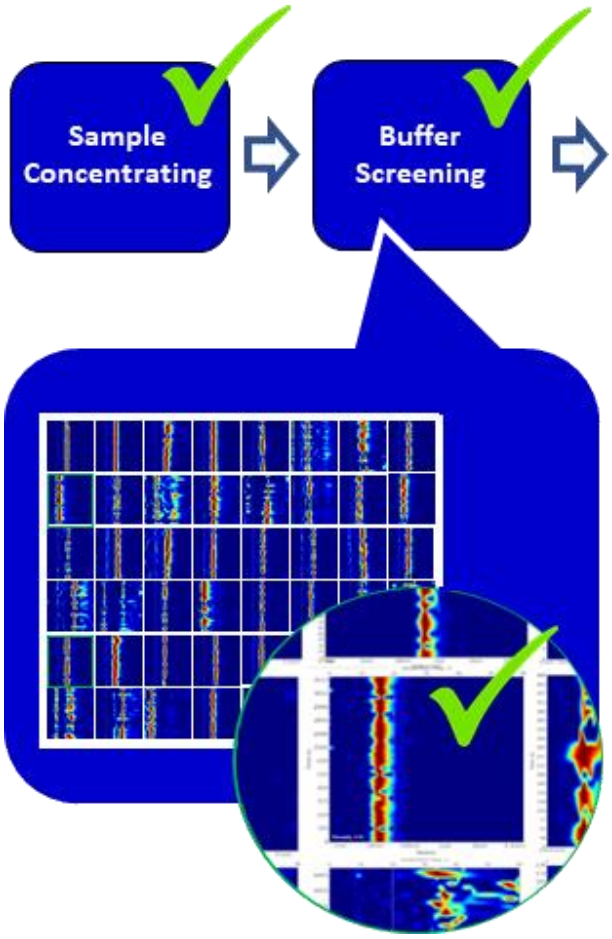
This document summarizes results from comprehensive *in situ* DLS monitoring of cryo-EM sample preparation from purification to single particle 3D cryo-EM.

Cryo-EM workflow with completion of sample purification via chromatography, subsequent steps are of high significance for sample integrity. Each step outlined here can be addressed by *in situ* DLS in order to ensure that a sample maintained its integrity. On top of this, the sample can be even monitored after loading on a cryo-EM grid as the very last step before the sample will be used for plunge freezing.

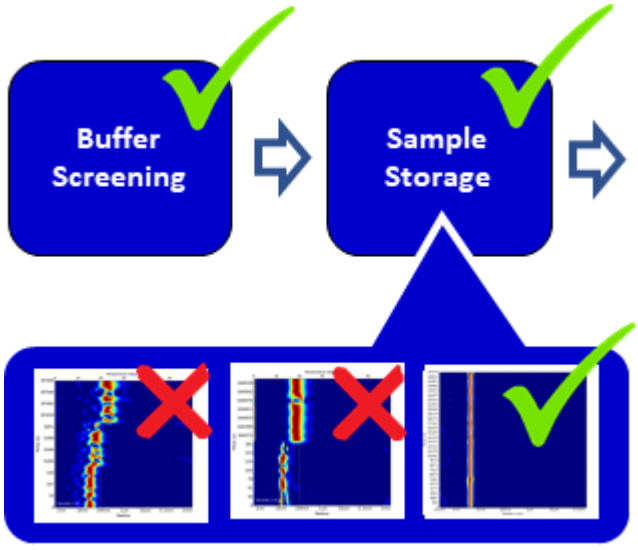


Sample concentrating is most commonly, the first processing step after purification is sample concentrating. Depending on the hydrophobicity of a protein or a complex, the solubility can be rather low.
In order to avoid to force a protein into assemblies of no biological significance or even aggregates, monitoring of the concentrating process itself is highly recommended and can be done highly sample efficient via *in situ* DLS.



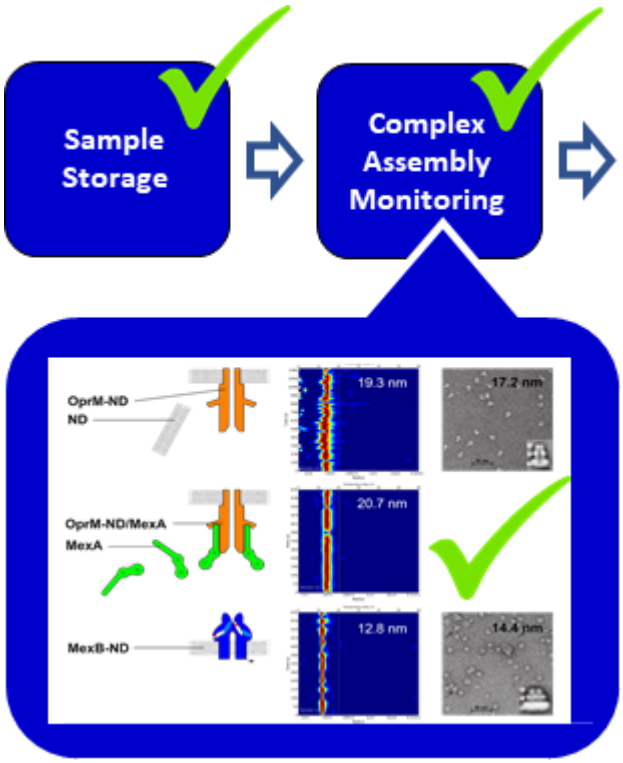


Buffer screening is a quite frequently applied step to improve stability and solubility of a sample. However, scoring and classification of buffer conditions is often misleading when indirect methods were applied. In contrast to that, *in situ* DLS is capable for rapid identification of the handful buffers that provides monodispersity of the target protein. The search for suitable buffers literally resembles the search for the needle in a haystack. However identification of the few conditions providing monodispersity among all thinkable aggregation states became handy since *in situ* DLS is available.



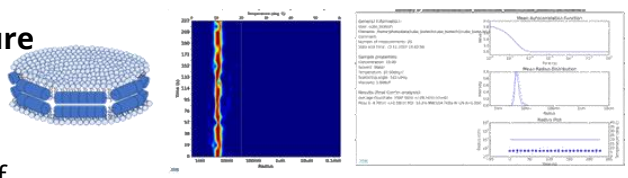
Sample Storage is often inevitable since immediate structural characterization is not always possible. Storage of a sample is commonly believed to be best for short times at 4 °C and for long term storage at 80 °C. However, freezing and melting can be harmful to a sample, but this highly depends on the sample itself. Also here, *in situ* DLS provides a reliable and rapid way to ensure sample integrity by before storage and after storage DLS-data size comparison.

Complex Assembly Monitoring
Besides qualitative analysis revealing aggregation and stability, DLS is also capable to deliver quantitative data of absolute particle sizes. This feature can be exploited to monitor high molecular complex assemblies. Incomplete assemblies or complex decaying can be detected quickly and reliable in the same way, making *in situ* DLS the ideal pre-check method for cryo-EM samples.

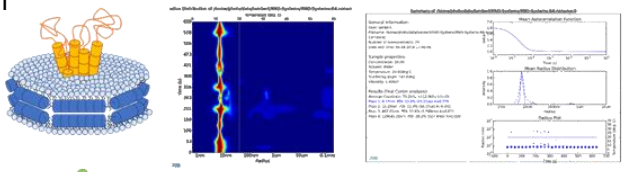


Nanodisc self Assembly Monitoring

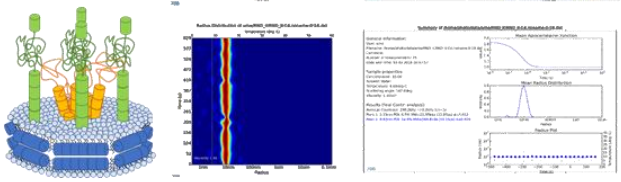
Assembled "empty" nanodisc signature
(POPC + Apoprotein (MSP1D1))
D_h = 9.4 nm, PDI 14.2%



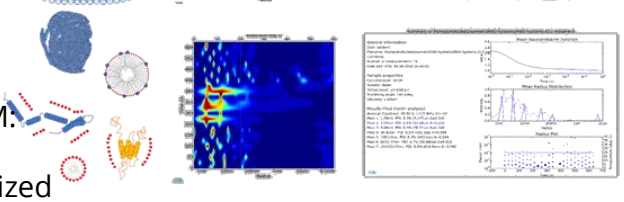
Signature of successful integration of protein A into a nanodisc (POPC + MSP1D1 + Protein A)
D_h = 12.32 nm, PDI 12%



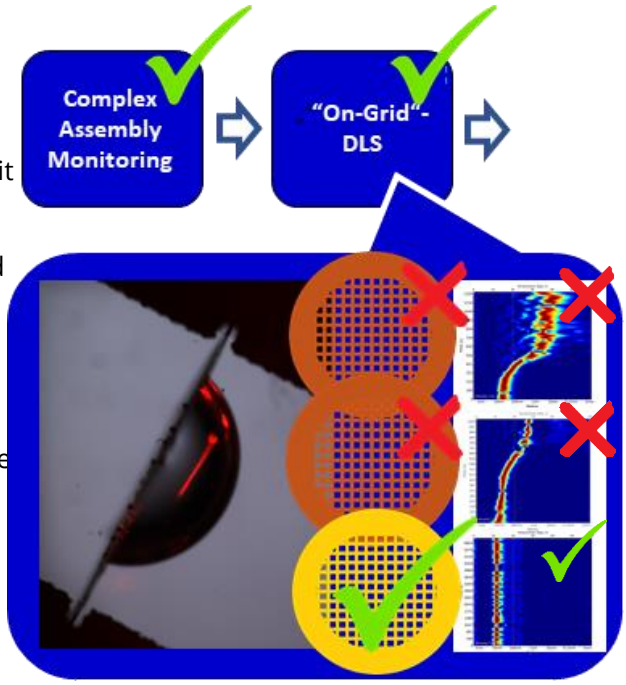
Signature of complex integration
of Protein A and Protein B (green) co-integration into a nanodisc (POPC + Apoprotein (MSP1D1))
D_h = 19.26 nm, PDI 25%



Signature of a failure:
Inhibition of the nanodisc-assembly by slightly excessive concentration of DDM: POPC + apoprotein (MSP1D1) + Proteins A, B are separated and solubilized as detergent-complexes.



"On-grid-DLS" is a unique feature of *in situ* DLS. This application allows to determine protein responses in terms of induced aggregation directly *in situ* when it got in contact with the grid surface. Proteins behave quite differently after touching a grid surface. Also different grid materials and even different grid preparation protocols possess a strong influence on the sample state. Therefore, identification of suitable grid parameters via *In situ* DLS prevents miss-attempts in advance. It is conceivable that those observed protein aggregation responses are responsible for some well known difficulties when it comes to high resolution single particle 3D cryo-EM. A stable dispersity on a grid however indicates sample integrity and increases the overall success rate in cryo-EM structure determination.



Protein Responses to Grid Surfaces:
Formation of aggregates can frequently be observed after pipetting a sample droplet on a grid. If a sample does not change its state when being attached on a grid surface, the grid material and parameters a suitable for subsequential cryo-EM imaging. As a control an aliquot on a standard plate and will be later used as a reference. Several different classes of responses can be distinguished. Spontaneous formation of such aggregation can be observed in some cases quite rapidly. In other cases aggregation happens only slightly even after hours. How fast the aggregation takes place is therefore an important evaluation criteria for selecting grid-types and their preparation parameters.