Avoiding Overconcentration: An efficient way by applying *in situ* DLS

Protein purification commonly implies crucial chromatography steps. A side effect of chromatography methods is the dilution of the sample, making a downstream concentration increasing step necessary

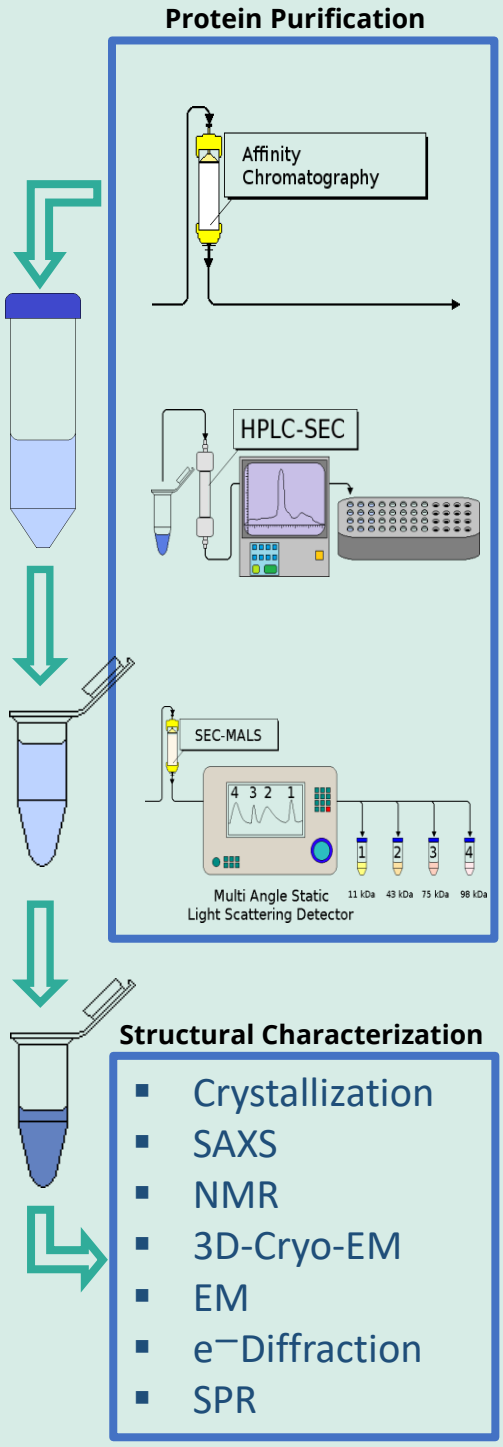
Purification in all of its variations commonly result in a pure but highly diluted sample. In order to maintain sample quality throughout the entire down stream processing, *in situ* DLS provides a highly sample efficient monitoring to prevent unwanted changes in the sample.

DLS Monitoring of the concentration steps in subsequently carried out in course of most purification protocols, due to highly diluted samples. Subsequent concentrating steps imply the risk of over concentration. Usually, loss in quality of a sample result in aggregate formation, which is mostly irreversible and thus the sample isn't well suited anymore for applying any structural characterization methods later on.

Determination of the maximum concentration in a given buffer system is therefore a key to avoid overconcentration and keeping the sample in a usable monodisperse state over the entire sample preparation procedure downstream from chromatography steps.

Sample is ready for subsequent structural characterization if the sample dispersity has not being changed in course of the down stream treatments.

On the next pages, a method will be proposed how occurrence.



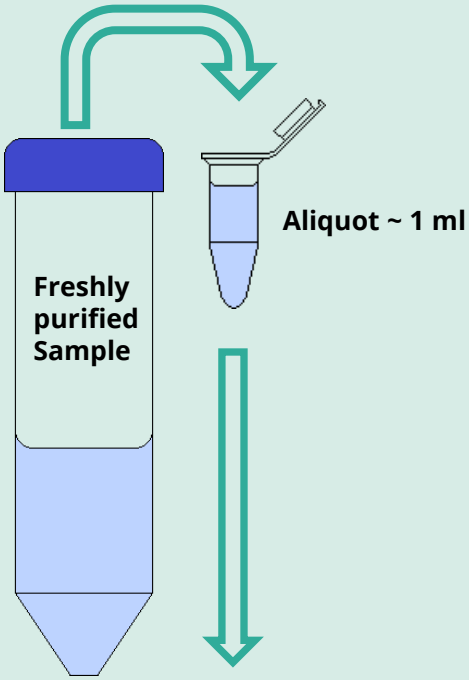


Figure 1.3

Monitoring based on Aliquots is a method to identify the maximum concentration without risking overconcentration of the entire batch. As soon as the highest concentration value has been determined via in situ DLS, the entire batch might be concentrated in the same way without reaching the previously identified maximum concentration.

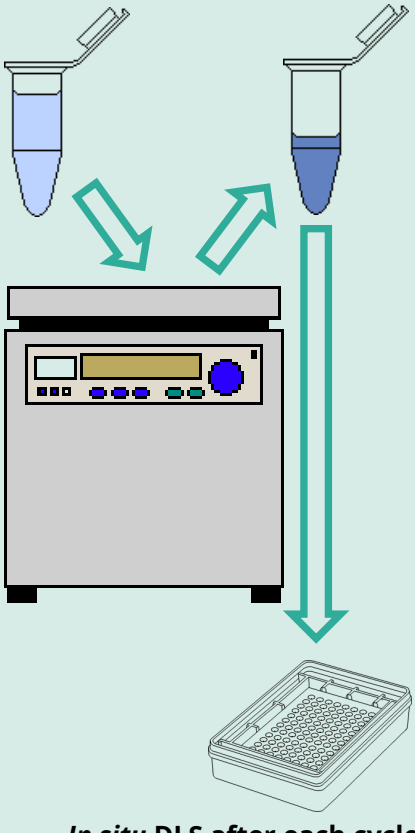
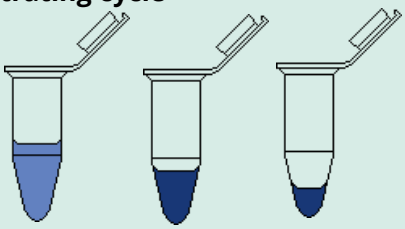


Figure 1.4

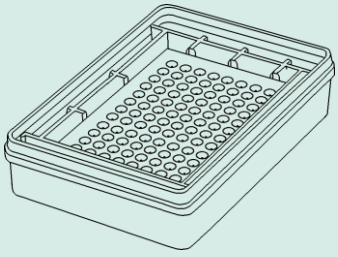
Each concentration cycle can be analyzed via *in situ* DLS, by taking above mentioned aliquot after each concentrating cycle. Since such an aliquot can be as smalls as 80 – 800 nl the procedure can be repeated many times, even when the sample volume decreases significantly due to the concentrating process itself.

Transfer of sub-µl Aliquots after each concentrating cycle to a 96 well plate, sealed with paraffin oil is usually carried out by manual pipetting in about 0.8 to 0.5 µl volumes.

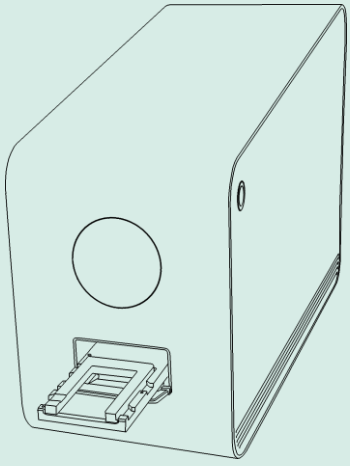
Aliquot taking after each concentrating cycle



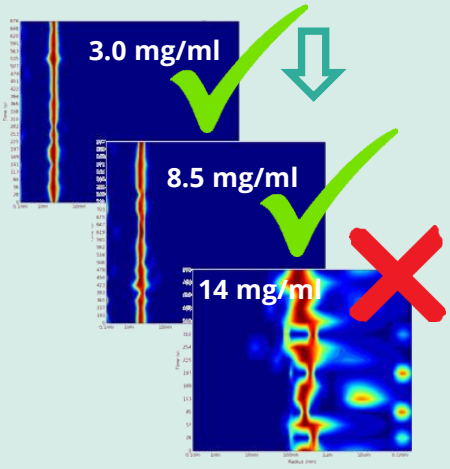
Standard 96-well batch crystallization plates are used as carrier for multiple samples. The paraffin oil seal ensures that the sample is protected from evaporation or from being negatively influenced by air mediated impurities.

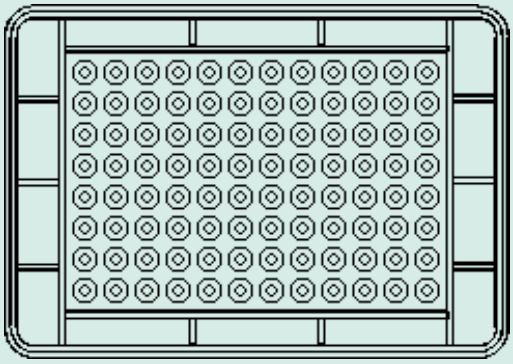


SpectroLight 600 provides a technical solution to measure particle sizes in very small aliquots (80 – 800 nl). Therefore the identification of maximum concentration can be achieved in a semi-automated way and highly sample efficient.

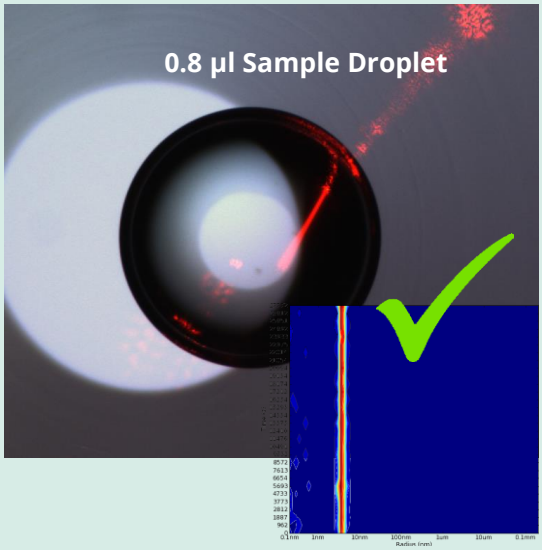


Maximum concentration: In situ DLS radius distribution plots of a sample concentration approach. A tremendous change in particle size can be detected, when the sample was concentrated to 14 mg/ml, indicating aggregate formation as a result of overconcentration





A 96 well plate is ideal for determination of the maximal possible concentration maintaining a monodisperse state. Since this format provides a sufficient number of wells, precise determination of the maximum concentration is enabled, applying small changes in terms of concentration between the taken aliquots. In combination with the ease of use and the unmatched small sample volumes/well in situ DLS is the ideal tool for this quality checks.



Clear droplets occur even when overconcentrated, but not revealed when investigated only by optical microscopy. However, the trespassing laser beam indicates a significant difference in the scattered light intensity and hence particle sizes. A more detailed analysis of the scattering light by means of dynamic light scattering accomplishes the impression of a highly aggregated sample. Evaluation based on DLS in small aliquots provides a reliable statement about solubility while optical microscopy alone is insufficient to provide this information.