Xtal Concepts

Newsletter

Quantitative and Absolute (hydrodyamic) Size Analysis

Absolute size determination is a powerful application of the SpectroLight 600. Applied at **500 nl/well** in standard plates, the accuracy reaches what is physically possible. Here, we provide an example of what this means.

The most common use of DLS is to analyse the homogeneity of a sample, which could be considered a qualitative analysis of a sample.

In contrast to this common use of DLS, the potential of this method for quantitative measurements to determine absolute sizes and size differences is often underestimated.

If three basic parameters of a sample are known, the absolute size determination of DLS can be accurate to <5 Angstroms, providing a highly efficient way to monitor protein complex assemblies.

These three Parameters are:

- The sample must be monodisperse (PD < 20%)
- The sample must be in a thermal equilibrium and the temperature must be known (relative size)
- The viscosity of the sample must be known (absolute size)

These three Parameters are easy to determine

Monodispersity can be achieved by formulation e.g. solubility buffer screens.

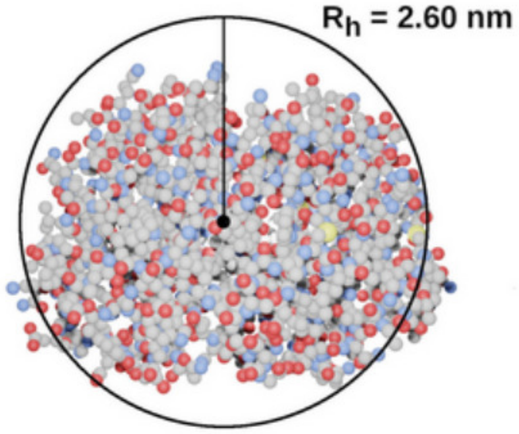
Temperature is determined automatically by our systems.

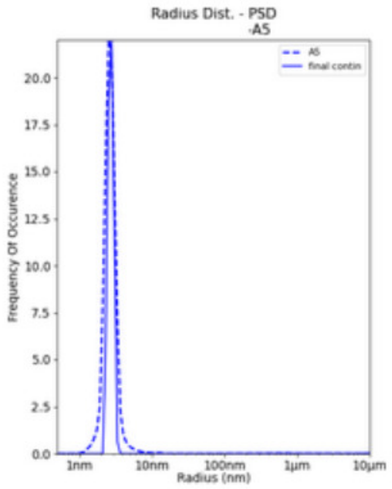
Viscosity can be determined in our systems with unrivalled efficiency using microviscosimetry (see also the scheme below).

Once these parameters are known even complexes with subunits of different sizes can be identified.

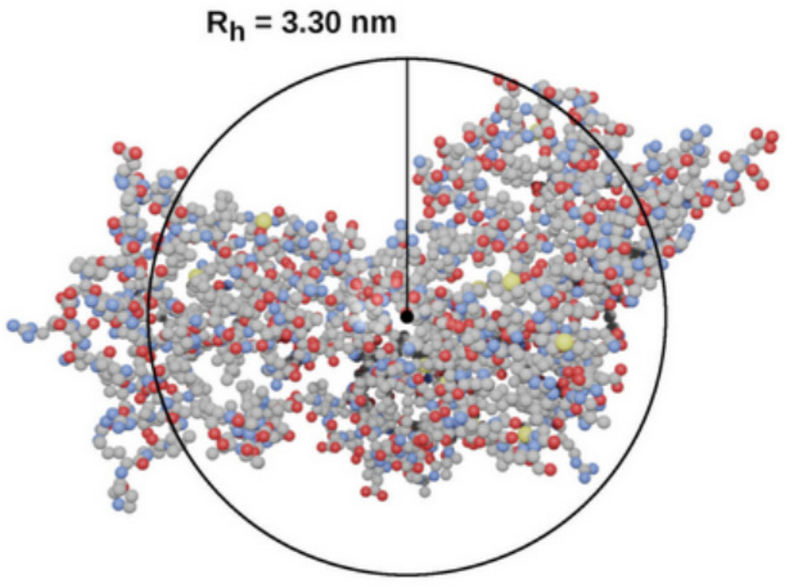
Complex Identification at the Angstrom Level of Accuracy

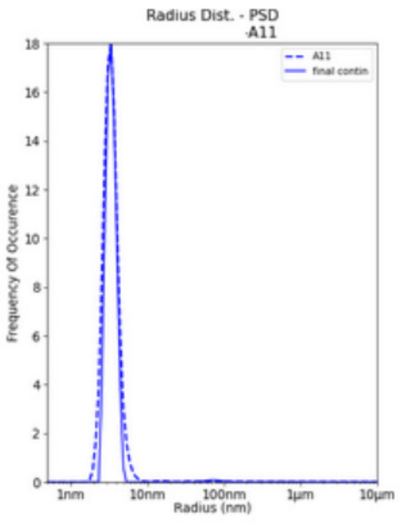
As an example, here is a **36 kDa target protein** that can be specifically bound by an ubiquitin protein ligase. The absolute hydrodynamic radius was determined to be **2.60 nm.**



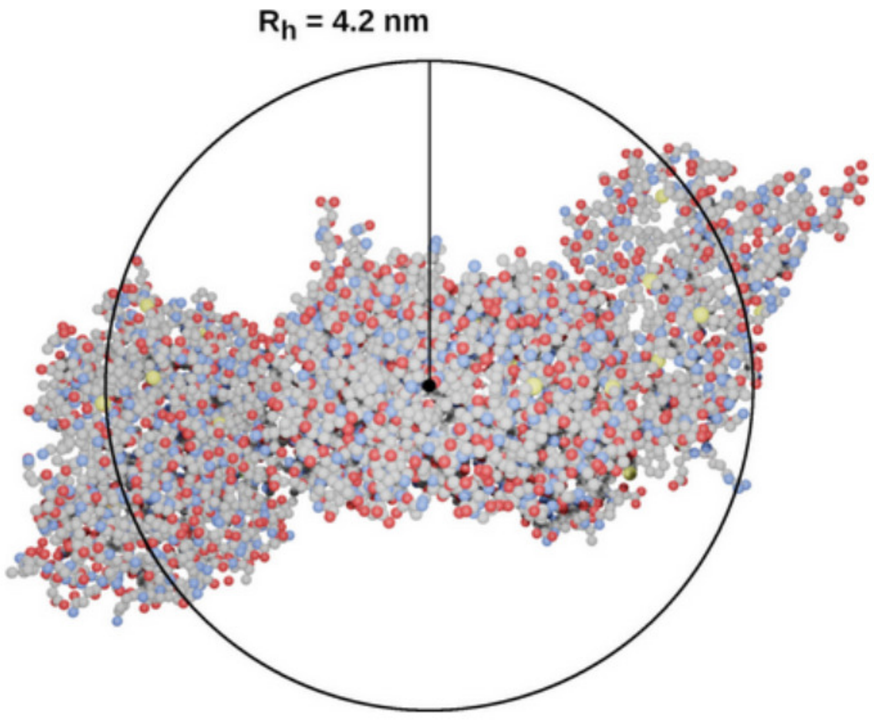


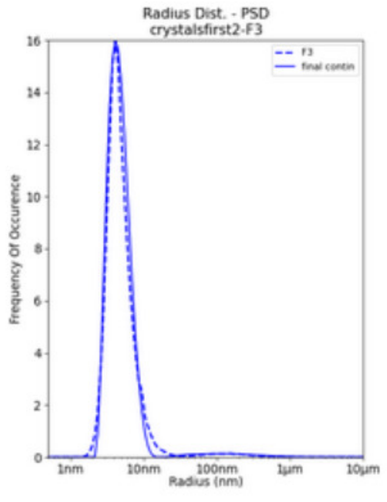
The other binding partner is a **ubiquitin protein ligase**. It has a **molecular weight of 51 kDa**. Its hydrodynamic size was determined to be **3.30 nm**.





Mixing the two proteins in a 1:1 molar ratio resulted in a **protein complex** with a **molecular weight of 87 kDa**. The hydrodynamic size was determined to be **4.20 nm.**





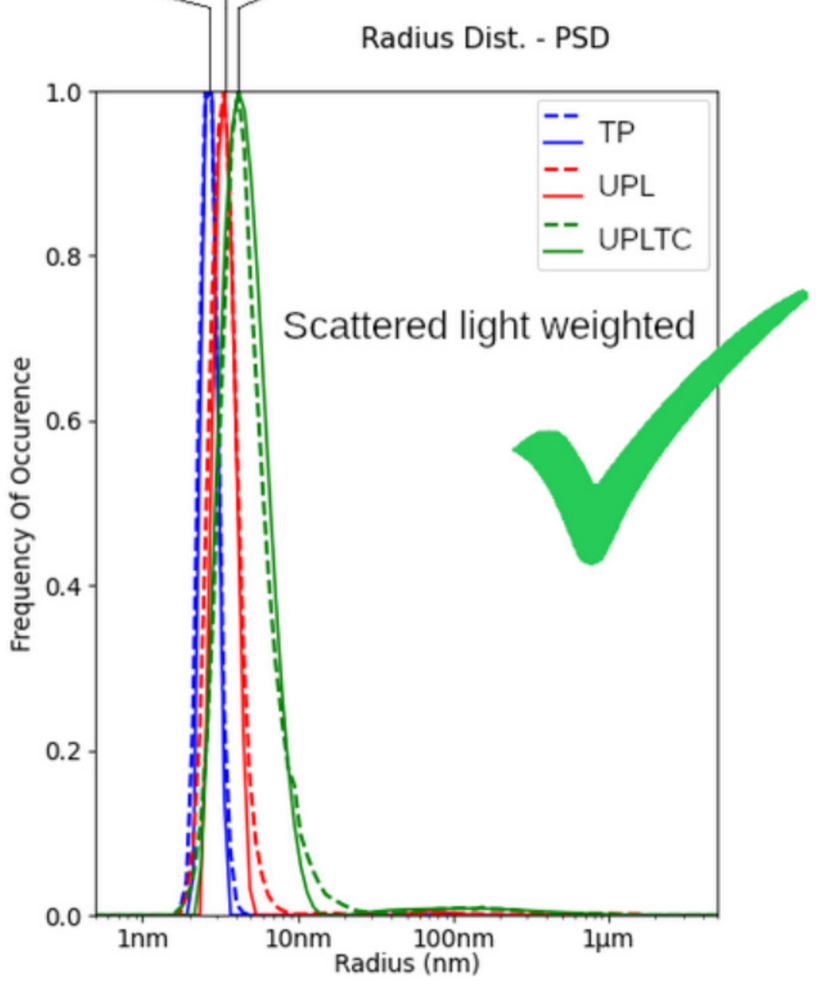
Absolute Size Comparisons reveal the successfully assembled Complex

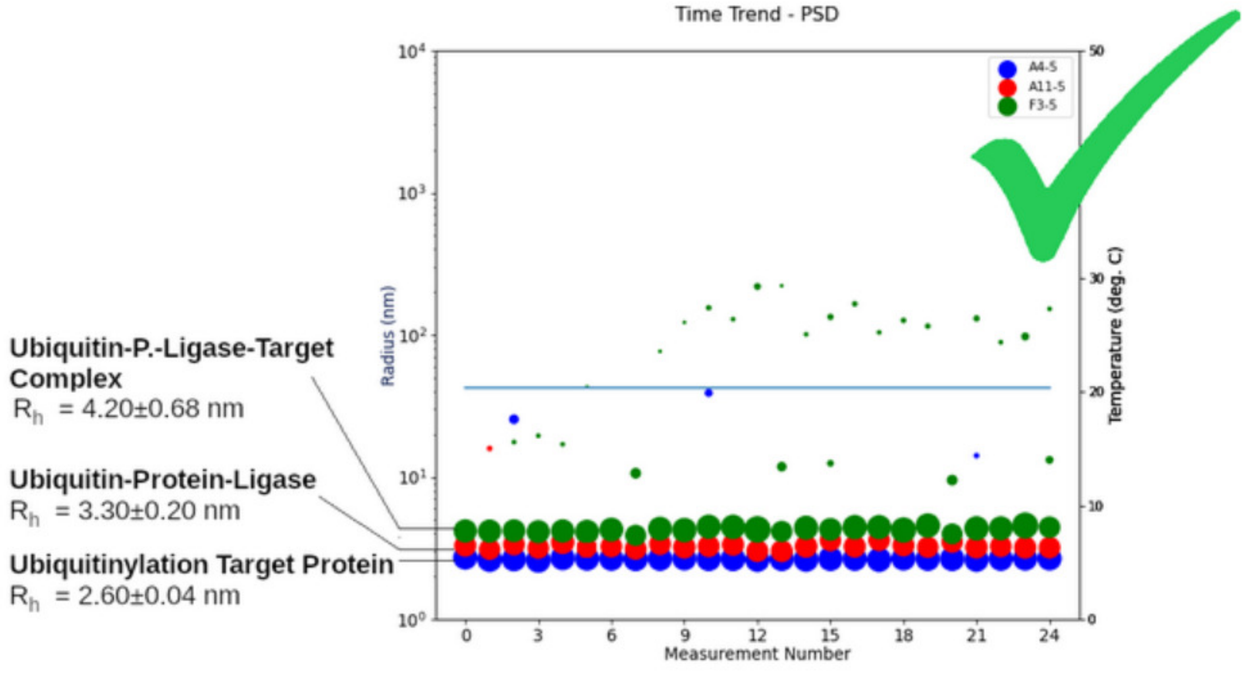
Before DLS measurements the sample has been incubated for 30 min. in order to achieve thermal equilibrium of the sample.

Target Protein
R_h = 2.60±0.04 nm

Ubiquitin-Protein-Ligase
R_h = 3.30±0.20 nm

Ubiquitin-Ligase-Target-Complex
R_h = 4.20±0.68 nm





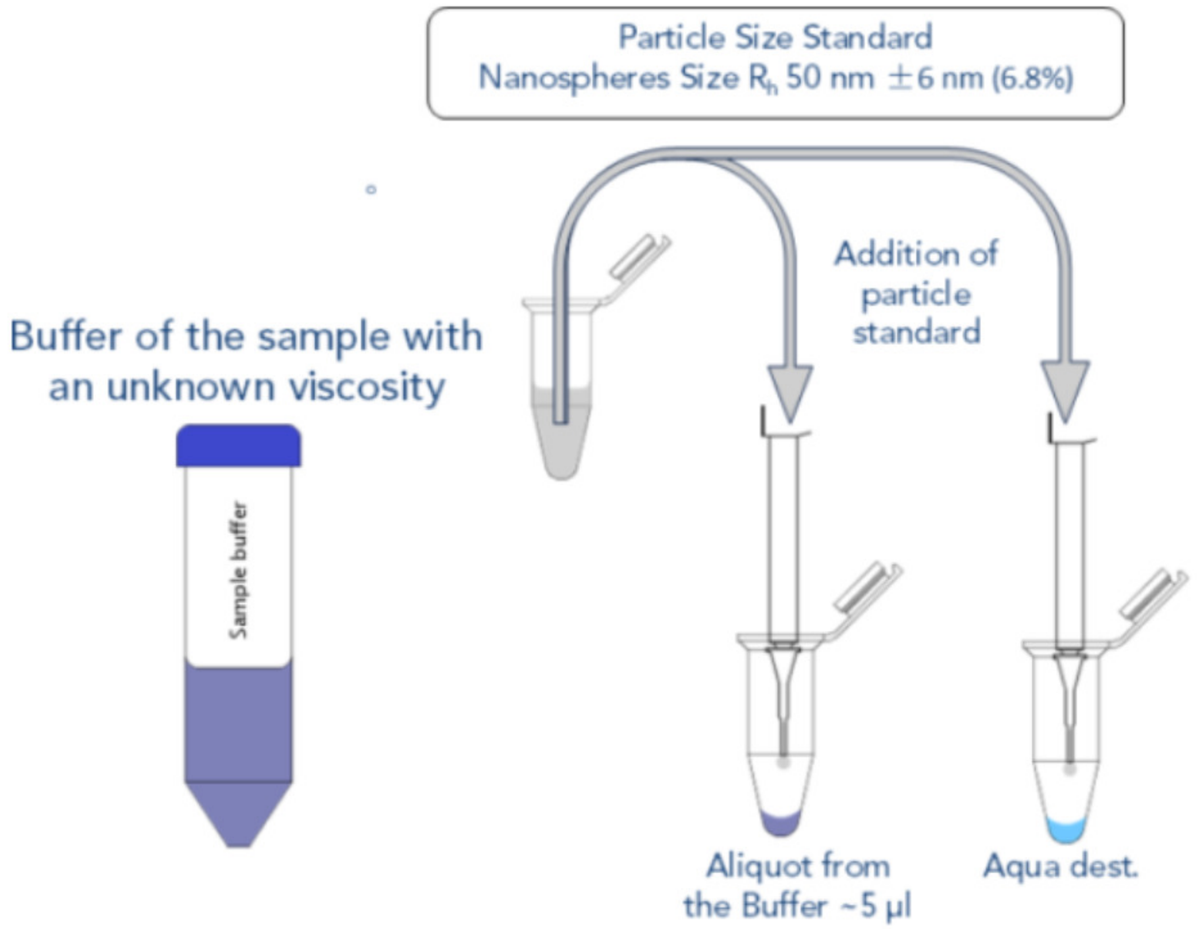
Ubiquitin-P.-Ligase-Target Complex
R_h = 4.20±0.68 nm

Ubiquitin-Protein-Ligase
R_h = 3.30±0.20 nm

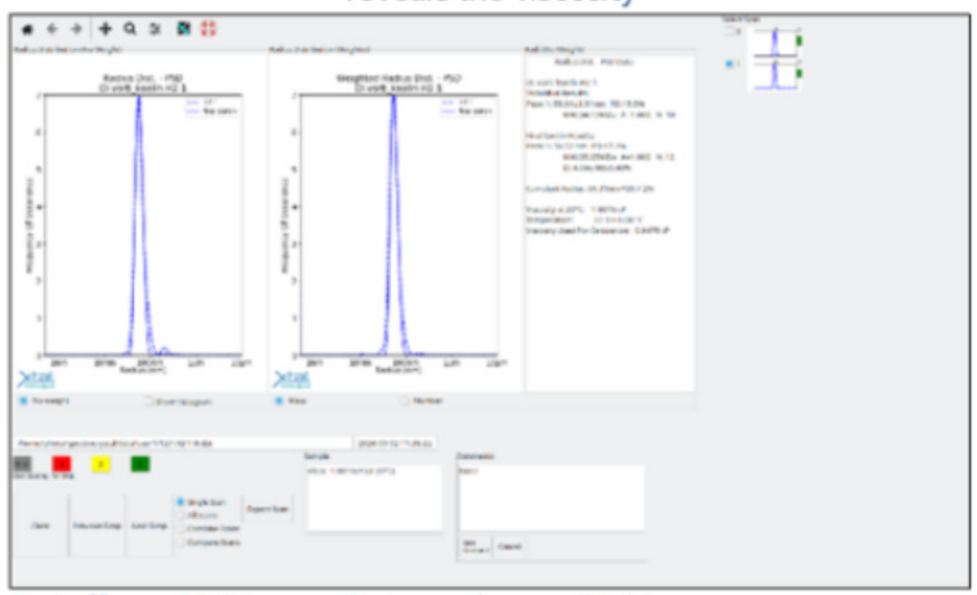
Ubiquitinylation Target Protein
R_h = 2.60±0.04 nm

Microviscosimetry, easy and efficient

A simple way to determine the viscosity of a buffer is to measure the size differences of a particle of known size in that buffer compared to the size found in water. As commercially available particle size standards are extremely highly concentrated, the amount to be added can be very small, resulting in a negligible error.



Particle size shift between Buffer and Aqua dest. reveals the viscosity



R_h Buffer = 55.91 nm R_h Aqua dest. = 50.18 nm

$$\text{Buffer Viscosity} = \frac{55.91\ nm}{50.18\ nm} * 1.0016\ \text{cp} = \underline{1.116\ \text{cp}}$$

In order to learn more about the amazing potential of in plate DLS for complex identification please get in contact to us by email post@xtal-concepts.de

More about SpectroLight 600 can be found on our web-page www.xtal-concepts.de

We hope you've enjoyed our little illustration about the capabilities of DLS for quantitative size analysis.

Your XtalConcepts Team.

March 24, 2025