MOSBRI Molecular-Scale Biophysics Research Infrastructure Basic Level School

# SOP for Protein Homogeneity Assessment by DLS

## Introduction - Guidelines:

### Reminder

The minimum concentration depends on the molecular weight of the protein. For example, for a 50 kDa protein of interest, the minimum concentration to use would be 0.3 $mg.mL^{-1}$.

For protein quality control, we recommend not centrifuging the sample in order to detect any aggregates present; if the DLS is used for other purposes, it is recommended to centrifuge the sample. If the protein is not detectable due to the presence of aggregates, the sample should be centrifuged using a benchtop centrifuge (15 min at 27,000 g). However, it is recommended to measure the concentration before and after centrifugation.

Always place 20 µL of buffer as a blank to check the quality of the buffer.

Avoid the formation of bubbles during loading. However, if bubbles are present, centrifuge the plate: 1 min at 3,000 rpm (1,200g).

### Precautions when loading samples into the microplate wells

Load 20 µL of the sample into the well avoiding bubbles. To use the pipette correctly, here are some general tips:

- Hold the pipette in a nearly vertical position and gently press the plunger to the first stop position; then dip the pipette tip into the liquid and allow the plunger to gently rise to the rest position. Wait one second to allow all the liquid to rise to the tip ;
- Place the pipette at an angle between 10 and 45° against the inner wall. Gently press the plunger to the first stop position and 2 seconds into the bottom of the well. Do not press to the second stop position to prevent bubbles formation. In case bubbles are still present, centrifuge 1 min at 3,000 rpm (1,200g) the plate.

## Protocol:

### Start-up

1. Turn on the DynaPro Plate Reader III and select the experimental temperature.
2. Wait for the temperature to stabilize for at least 10 min.
3. Start the Dynamics software for data collection and analysis
4. Load the microplate with 20 µL of sample per well, according to the guidelines above.
5. Cover the plate with a protective adhesive film.
6. Open the DynaPro door using the button on the instrument's LED panel or via the Dynamics software.
7. Insert the plate into the instrument with the A1 position toward the front of the instrument.

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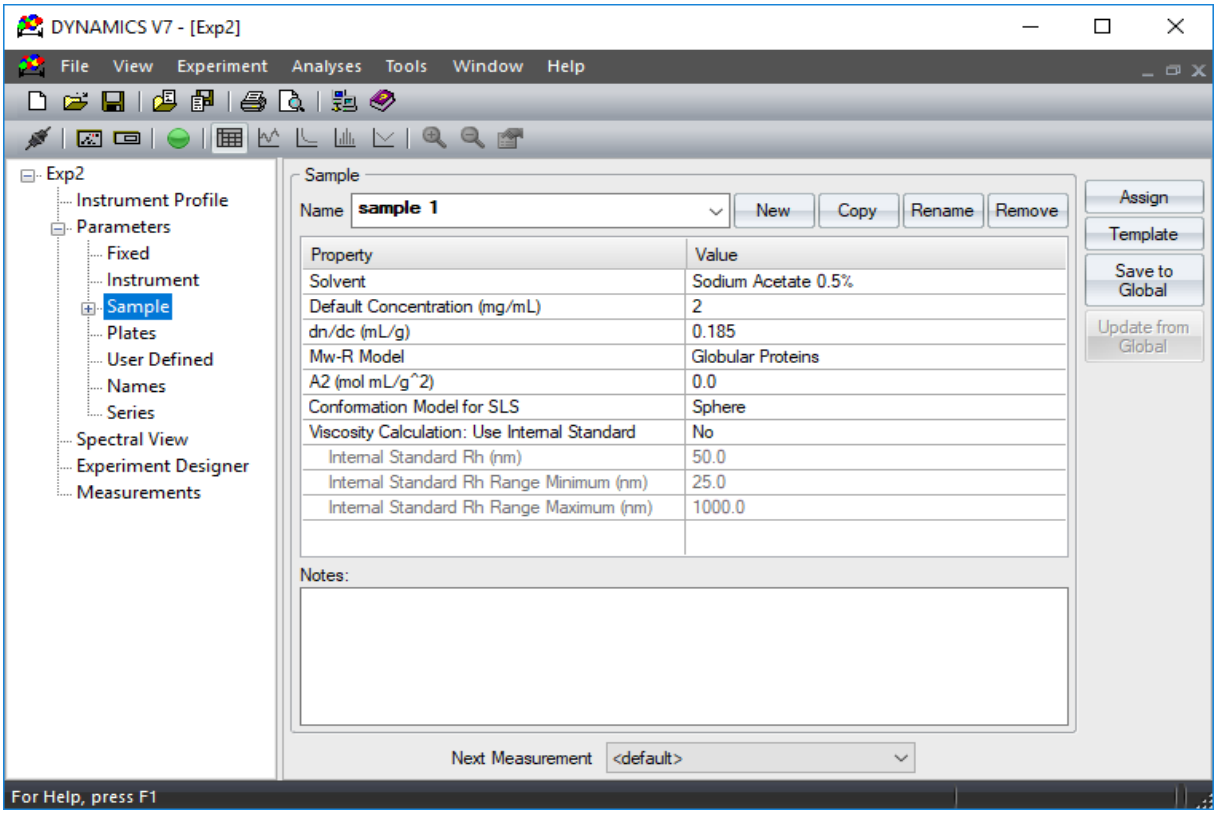
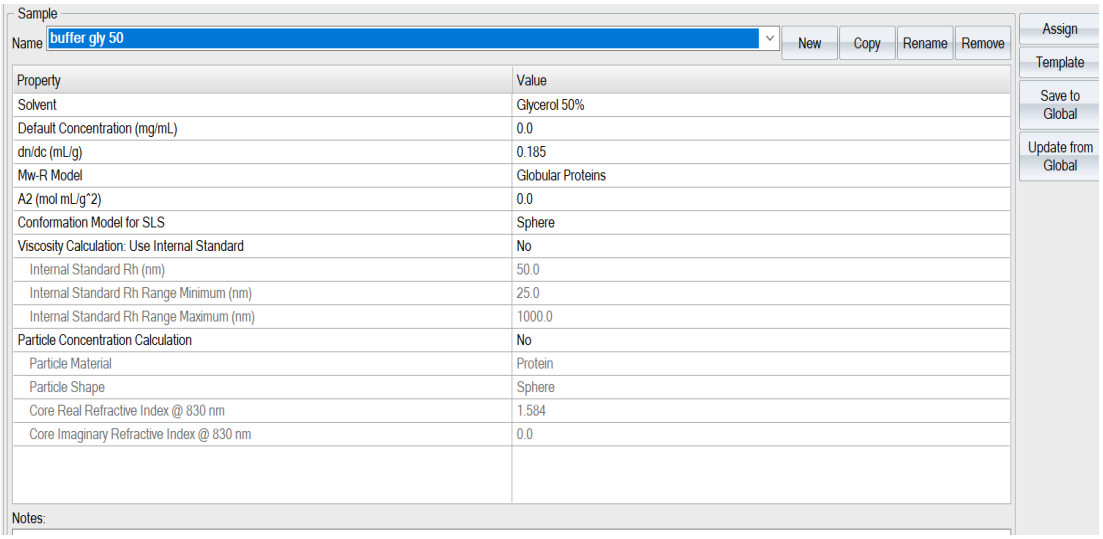
## Experiment setup via Dynamics program

1. Launch the Dynamics software for data collection and analysis.
2. Select "Parameters" then "sample", then click on "New:"
3. Assign a sample name.




4. Assign the solvent viscosity closest to the buffer of the sample used.




5. Select " Experiment Designer " then click on " Edit Experiment " :

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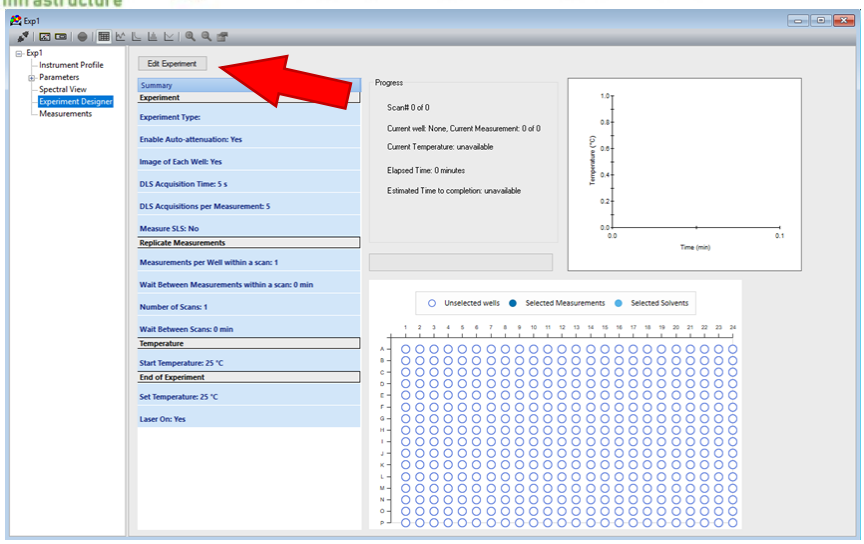
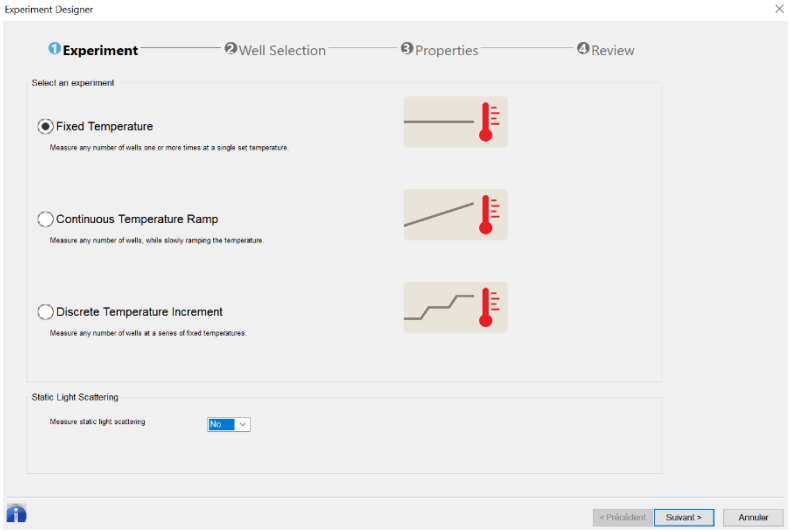
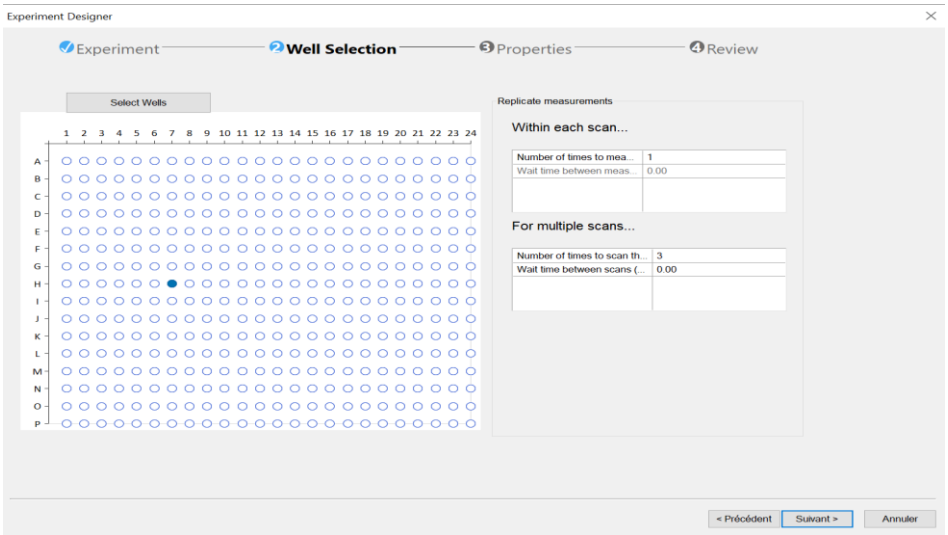



6. Click on "Fixed Temperature": for standard dynamic light scattering measurements.




7. Then “Next”.
8. Click on “Select Wells”.



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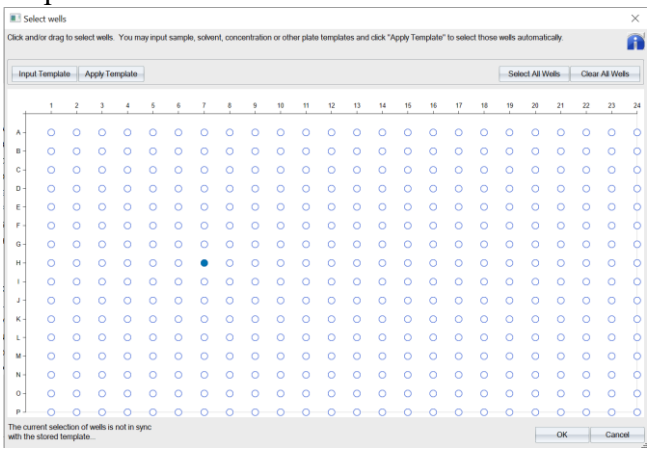
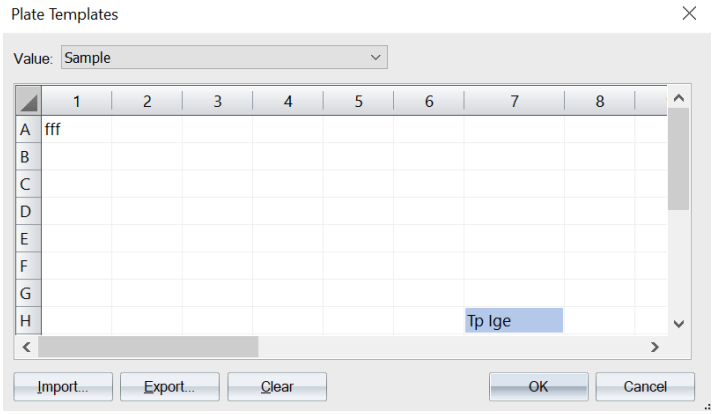
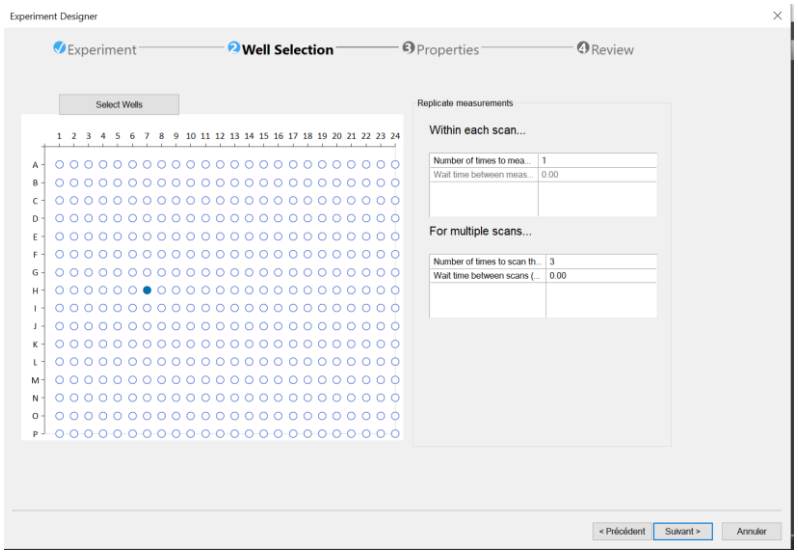
9. Click on « Input Template »



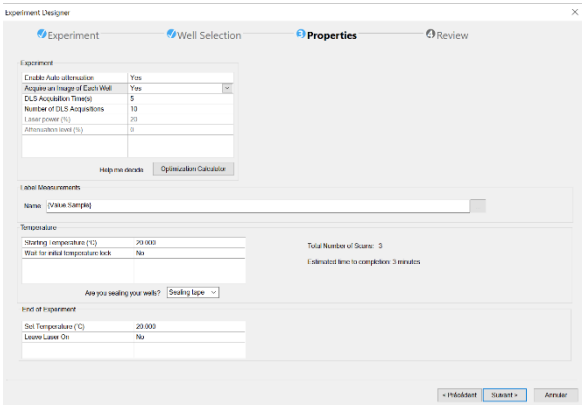

10. Choose value: sample and then click on the desired well and then search for sample name corresponding to the well.

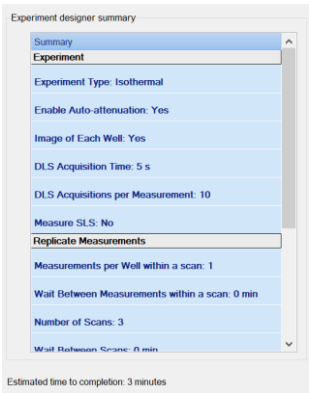


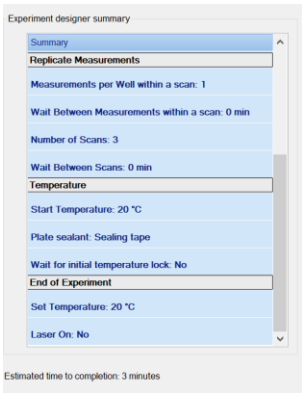

11. Click “OK” twice and then “Next”.
12. Precise 1 for " Number of times to measure " (within each scan) and 3 for " Number of times to scan… " (for multiple scans).



13. Check the Properties and click “next” (suivant)




i. Enable auto-attenuation: Yes
ii. Acquire an image of each well: Yes
iii. DLS acquisition time(s): **5** (10 for low concentrations and large sizes)
iv. Number of DLS acquisitions: **10** (20 for low concentrations and large sizes)

14. Click on « next » and then on “Finish”.

## Launching the experiment

- Save the data in your folder with the following format: QC_nameofexperiment_dateofexperiment
- Click on the Connect to Instrument icon at the top left (if not already connected).
- When connected, click on the green "Start" button to begin the acquisition.

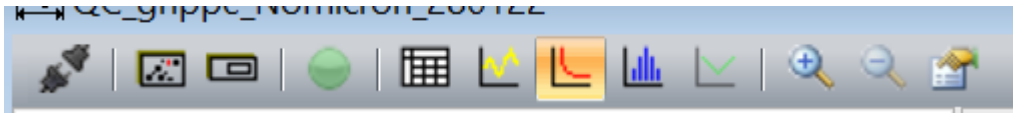
## Stop

- The experiment finishes when the green/yellow button turns red.
- Open the door using the button on the instrument's LED panel or in Dynamics.
- Remove your plate from the instrument and recover your sample.
- Close the door using the button on the instrument's LED panel or in Dynamics.
- Turn off the DynaPro III.

# Data Analysis

Data recording grid

Correlation graph



- The first thing to check is the quality of the buffer. A clean buffer has a low intensity autocorrelation curve.

- Check the shape of the autocorrelation curve for the protein.

Regularization plot-Histograms

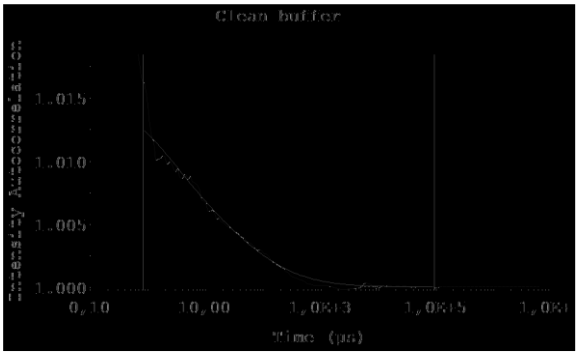
If particles with a Rh of 15-500 nm can be detected, this usually indicates the presence of aggregates.

## Notes

- Size: Refers to the radius or diameter of the particle modeled as a sphere moving or diffusing in the solution (as opposed to the molar mass of the particle). It is usually expressed as the average value of the peak of the size distribution.
- Size distribution: The way in which particle sizes are dispersed, spread out or distributed among one or more peaks; presented in a graphical form known as a histogram.
- Peak: A peak in a size distribution represents a distinct and resolvable species or population of analytes or particles. A peak is composed of several particles of different sizes, represented by boxes or bars, and is defined by a mean value and polydispersity.
- Modality: Size distribution with Refers to the number of "peaks" in the size distribution. A size distribution with only one peak is called Monomodal. A size distribution with more than one peak is called Multimodal (Bimodal, Trimodal are common terms for size distributions with 2 or 3 peaks).

## Examples

Here are some general results for good and bad samples. The figure below shows the common signal for a pure buffer. The autocorrelation curve should decrease dramatically as an asymptote.




Below shows the typical curves and signals for a small protein and a large protein complex. The smooth autocorrelation curve starts with a plateau. For a large protein, the plateau is longer than for the small one. Then the curve goes down exponentially to the baseline.

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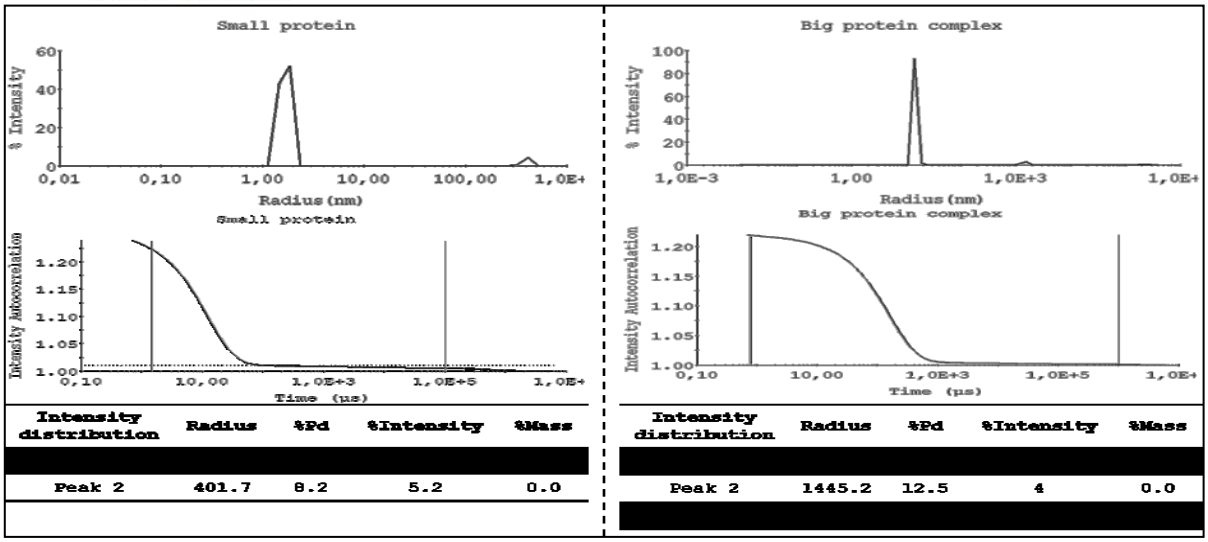
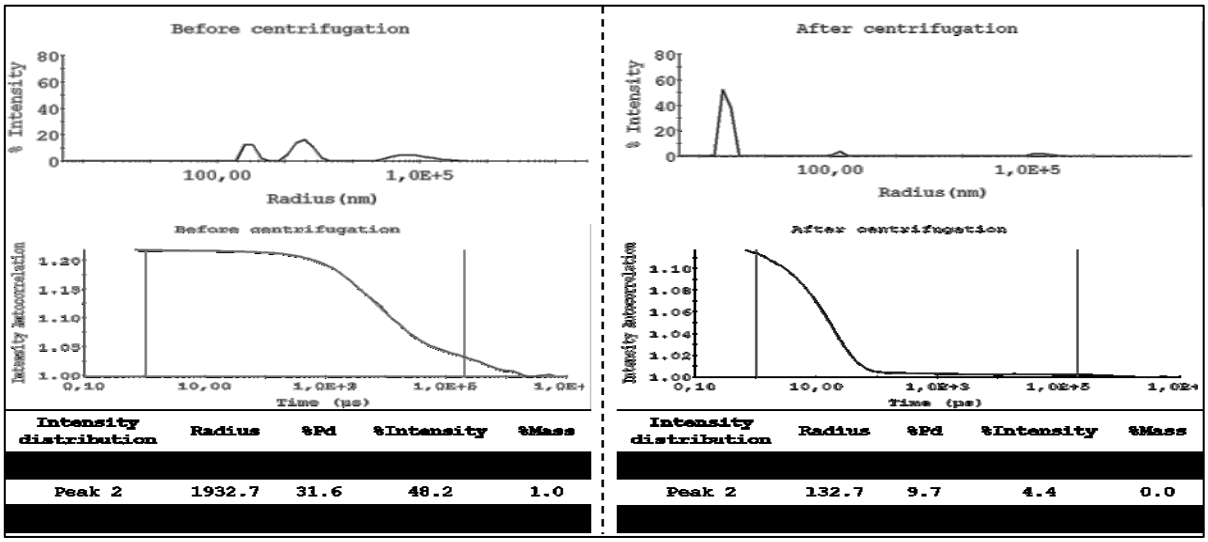



| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 401.7 | 8.2 | 5.2 | 0.0 |

| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 1445.2 | 12.5 | 4 | 0.0 |

When the autocorrelation curve signal is bad, one solution is to centrifuge. Below, after centrifugation, the signal is much better.




| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 1932.7 | 31.6 | 48.2 | 1.0 |

| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 132.7 | 9.7 | 4.4 | 0.0 |

Another remark concerns the case where the protein is not concentrated enough. The rule of thumb is:

$$c_{\mathrm{mg/mL}} = \frac{15}{MW}$$

In the figure below, a low concentration and an optimal concentration are shown. For both conditions, the hydrodynamic radius is quite similar. However, for the low concentration, the signal of the autocorrelation curve is weak but especially the percentage of polydispersity is 55 % while for the optimal concentration it is only 11 %.

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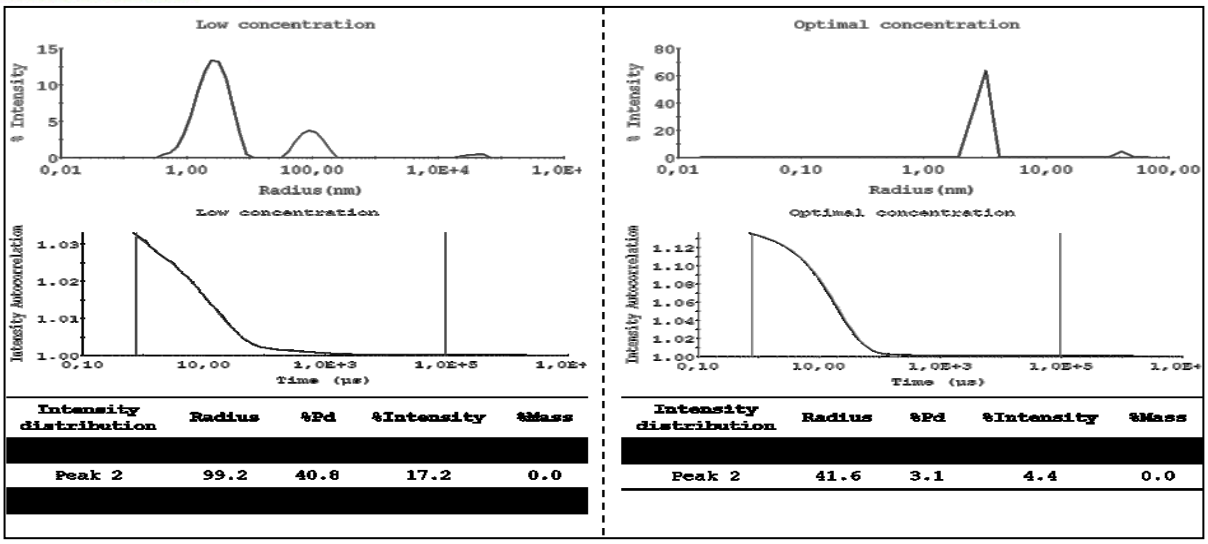


| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 99.2 | 40.8 | 17.2 | 0.0 |

| Intensity distribution | Radius | %Pd | %Intensity | %Mass |
|---|---|---|---|---|
| Peak 2 | 41.6 | 3.1 | 4.4 | 0.0 |