Sample handling and preparation

for measurements with Prometheus Panta

1. General sample handling

The following instructions on general sample handling and loading can also be found in the instrument manual:

- Clean the capillary tray surface
    - 99.8% ethanol
    - Dust- and scratch-free tissues
- Avoid scratches on the tray
- Avoid liquid on the outside of the capillary
- Load capillaries completely and align them centered before placing the lid

2. Sample preparation

Prometheus Panta performs measurements over a large protein concentration range. If you are unsure what concentration to start with, we recommend the following:

- 1 mg/ml for nanoDSF, prepare higher concentrations for membrane proteins and DLS
- 2 mg/ml for DLS, prepare higher concentrations for small proteins (below 50 kDa)

For nanoDSF measurements alone, no specific sample preparation is required. The fluorescence reading is very robust and can even deal with proteins in complex mixtures, such as lysates.

However, for DLS measurements it is important to avoid dirt and dust by all means, since they will interfere with the measurement and create artefacts in your data. For this reason, we recommend you doing the following:

- Keep capillary vial closed when not in use
- Use fresh boxes of pipet tips and bags of sample containers (Eppis) that were not laying around open in the lab
- Keep boxes for pipet tips or Eppis closed when not in use
- Filter all buffers (see 2.1.)
- Filter samples or spin down (see 2.3.).

1.1. Filtration of water and buffers

To record high-quality DLS data, it is advisable to work with highly pure water (e.g. AnalaR NORMAPUR; VWR #102927G) and buffer components. Filter water and buffer prior to usage with filters having a cutoff of 0.2 µm (e.g. Filter devices: Nalgene 25 mm; Thermo Fisher Scientific #724-2020 or Nalgene 13 mm; Thermo Fisher Scientific #720-1320)

To get the best possible result, filter water/buffer twice through the same filter into fresh and clean 15 ml/ 50 ml tubes.

1.2. Cleaning of tubes

Similar to filtering water and buffer, keeping sample solutions in clean and dust-free sample containers can improve DLS data quality. If you are unsure whether your sample/buffer containers are free of any particulate matter or detect dust-artifacts in your data despite having filtered all solutions, you may try pre-rinsing sample containers:

1. Place sample tubes to be cleaned in a sample holder
2. Set a pipet to the matching volume
3. Rinse the pipet tip once with filtered water and discard the water
4. Use the clean tip to fill filtered water into all tubes
5. Clean tubes by shaking / inverting
6. Discard water by tipping it out (make sure no water remains in the tube, e.g. in the lid)
7. Close tubes again to avoid contamination

1.3. Filtration of protein solutions

Working with dirt- and dust-free sample solutions is key to determine the correct size of particles in your sample solution. It can therefore be advisable to filter protein solutions directly before starting an experiment.

Of course, to filter or not to filter depends on your application. If you are, for example, interested in the long-term stability of proteins, you might want to filter the sample at the beginning of the experiment, but then keep it as it is for all following measurements to assess if larger particles are forming over time.

An alternative method to remove dirt and dust is spinning down the sample directly before the experiment (e.g. 14.000g, 15 min in a tabletop centrifuge).

When filtering protein solutions, we recommend carrying out the following steps:

1. Work with clean tubes (as described under 2.2.)
2. Filter protein solution:
    a. For standard DLS application, filter protein solution through 0.22 µm pore size filters with a sufficiently small dead volume.
    e.g. Nalgene 13 mm; Thermo Fisher Scientific #720-1320 or Costar Spin-X Centrifuge Tube filter; Corning Inc. # CLS8161-100EA
    b. For optimal DLS results, filter protein solution through 0.02 µm pore size filter
    e.g. Whatman Anotop 10 Plus; GE Healthcare #6809-3002
3. Take a fresh, clean 1ml syringe
4. Fill the syringe with the protein solution and filter it into the clean tube (discard first droplet or subsequently filter the sample for a 2nd time)

Note:

For all subsequent pipetting / dilution steps, always use clean tubes and pipet tips pre-rinsed with filtered water!

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