Protocols for MOSBRI ESC3 short course: Quality control for Integral Membrane Proteins

Abstract

In this practical we will apply a quality control pipeline for integral membrane proteins. The aim is to optimise stability, aggregation behaviour and oligomerization homogeneity using three biophysical techniques.

1. Differential scanning fluorimetry using the native protein fluorescence will be used to find buffer and detergent conditions for optimal protein stability.
2. Dynamic light scattering will be used to corroborate will be used to optimize buffer conditions with regard to protein aggregation behaviour.
3. Mass photometry will be used to evaluate mass distribution, e.g. to evaluate homogeneity before structural studies such as cryo-EM or crystallography.

nDSF: detergent buffer screen

1. Check the number of Trp and Tyr residues (and their position, if known) present on your protein. This can be helpful to determine the final experimental concentration., and usually aim for a final concentration between 5 and 10 µM. This concentration can be significantly lower for large proteins or proteins with a high number of Trp and Tyr residues.
2. Prepare a set of 3 serial dilutions from your stock of protein and do an initial scan with the Prometheus to observe the signal of the different dilutions. Also add the buffer of interest as control in order to rule out signal interference caused by buffer components.
3. Screen sample preparation: Each condition was pipetted into a PCR plate and mixed with 10 µl of 2x protein buffer (without any detergent and glycerol). Protein stock (2.5 µl) was added to obtain a final protein concentration in the range of 0.1–0.5 mg/ml and thoroughly mixed by pipetting. The plate was briefly spun down in a swing-bucket centrifuge and incubated for 1 hour at room temperature prior to the thermal denaturation assay.
4. Fluorescence and scattering of detergents without proteins should be measured in 50 mM Tris, pH 7.5 and 200 mM NaCl to discard background signals.
5. Thermal denaturation assay: Each sample was used to fill two standard grade NanoDSF capillaries (Nanotemper).
6. Adjust the excitation power so that all of the samples are in the recommended regime of initial fluorescence (between 2000-15000 counts).
7. Perform a dilution of your protein in the buffers of interest using the same dilution performed for the initial scan to ensure a good initial signal for the experiment.

8. Remember to include a capillary with buffer only as a control, to discard possible fluorescence effects coming from the buffer. In case of performing a titration with a compound (e.g. ligand), a buffer control with the highest concentration of the added compound must be measured in order to discard fluorescence contribution from the compound.
9. Load the nDSF capillaries into a Prometheus NT.48 device (Nanotemper) controlled by PR.ThermControl (version 2.1.2), set a measurement from 20°C to 90°C using a heating rate of 1°C/min and start the experiment.
10. Make sure the Excitation power was pre-adjusted to get fluorescence readings above 2000 RFU for F330 and F350.
11. While running or before: Add labels for each capillary in the acquisition software to know which sample corresponds to each curve.
12. Export the processed curves from the instrument, an XLSX file with “processed data” and analyse the data using the SPC web server for the MoltenProt module (available at https://spc.embl-hamburg.de/). More details about the data analysis are listed in notes 4.2.

DLS: Check aggregation

1. Measurements should be performed at a concentration of around 0.5 mg/ml in order to obtain a good signal (starting at a concentration of around 1-2 mg/ml is usually a good starting point). The volume required per measurement depends on the instrument and cuvettes used. In our case we have used 4-5 µL of sample.
2. Before measurements, spin the samples at maximum speed for 10 minutes to avoid aggregates that could hamper measurements. Optionally, 0.22 µm mini-spin filters are recommended to be used to remove large aggregates from the sample.
3. To enhance the quality of the measurements, switch on the instrument and the laser at least 30 minutes before measuring to warm up the laser. Also, set the temperature (usually 20 or 25°C).
4. Measure the buffer to discard any signal that could come from buffer components such as detergent micelles. If the buffer contains impurities that display a particle-like auto-correlation function, it probably needs to be filtered.
5. Set the collection parameters to 30 curves with an acquisition time of 5 s and average the results. Measurements to be performed at 25 °C.
6. If the curves show a “bump” towards higher correlation-times, the sample contains large macromolecular aggregates and it is most likely not in ideal buffer conditions and therefore not suitable for structural studies. Details on the data analysis are listed in section 4.3.

Mass photometry: Check mass distribution

1. Prepare cleaned cover slides. More details about the cleaning procedure can be found in Niebling *et al.* 2022. For checking the slide cleanliness please refer to section 4.4 note 1.
2. Only use filtered buffers and check their signal prior to the protein measurement. More details can be found in section 4.4 note 2.
3. Calibrate the mass photometer with a suitable calibration standard.

   Note: More details about steps 1.-3. can be found in Niebling et al. 2022.

4. Create a pre-dilution of the protein stock with a protein concentration of 500-1000 nM using filtered buffer.

   Note: For concentrated detergent samples (those proceeding from concentrating devices) we recommend starting with a pre-dilution with protein concentration around 1.5 µM and then using only 0.5 µL of it (and 19.5 µL of non-detergent buffer on the slide) in step 3. This could highly reduce the detergent background in the cases presented here.

5. Add 18 µL of filtered detergent-free buffer into an empty well on the cover slide.
6. Add 2 µL of pre-diluted protein solution (from 1.) and mix by pipetting up and down using a 20 µL pipette.
7. Start the acquisition as soon as the mixing is finished.
8. Open the mp file with DiscoverMP and analyse data.
9. Check if the number of counts is in the recommended range for the used FOV (this depends also whether a RefeynOne or RefeynTwo is used). For a RefeynOne and the regular FOV, the recommended maximum number of counts is 3000.
10. If the number of counts is too high, repeat steps 2-6 using a smaller volume of the pre-diluted protein solution in a new well.

11. The calculated histogram of the mass photometry experiment can be used to draw conclusions regarding the different species and their distribution. Details on the data analysis can be found in notes 4.5.