MOSBRI Molecular-Scale Biophysics Research Infrastructure Basic Level School

Differentiel scanning fluoremetry nanoDSF

Introduction - Guidelines:

Structural studies demand to reduce protein dynamics and partial unfolding under excruciating experimental conditions. Thus, high thermostability is an important feature that needs to be tested to improve the protein behaviour, e.g. to facilitate crystallization [1–3] or other structural/biophysical studies. DSF of intrinsic fluorophores monitors the intrinsic fluorescence of Trp and Tyr residues, which respond very sensitively to properties of their local microenvironment. Thermal unfolding is measured by following the intrinsic Trp and Tyr steady-state fluorescence intensity, and the position of the emission maximum [4, 5]. An empirical parameter that equals the ratio of the fluorescence intensities at two different wavelengths, 330 nm and 350 nm, is used to follow the change in the environment of Trp and Tyr residues during temperature-dependent unfolding of the protein. This ratio will show a sharp increase/decrease of the signal during thermal unfolding from which the T_m value can be calculated (see Note 1). The applicability of DSF of intrinsic fluorophores is highly dependent on the presence of Trp and Tyr in the folded core of the protein that will be exposed upon unfolding. Moreover, it is necessary to exclude that observed signal changes are caused by aggregation as this will also lead to changes in the fluorophore environment. A backscattering measurement can also be performed to test if aggregation occurs (before or after denaturation). The choice of the temperature gradient is important as it is linked to the activation energy via the Arrhenius equation [6]. Typically, a heating rate of 0.5 °C. min^{-1} is used. A comprehensive understanding of the measurement, advantages, and drawbacks can be found in [7] and an example is presented in Figure 1. Comparing the melting temperatures in different buffer compositions (ΔT_m) allows to define the optimal buffer condition as an increase in T_m corresponds to better thermal stability and reduced conformational flexibility.

Protocol:

1. Switch on the instrument, clean the reflexion mirror with ethanol and let the system warm-up for at least 15 min.
2. Prepare a stock solution of the target sample with an absorbance at 280 nm of around 1. This signal depends on the presence of tryptophans and tyrosines.
3. Dilute in PCR tubes 1 µL of protein into 10 µL of buffer to be tested. For buffer screening, if available, preparation of buffers with a robot or the use of commercial buffer screening kits are recommended to avoid mistakes and to increase reproducibility.
4. Repeat the procedure for all conditions to be tested.
5. Fill the capillaries with the diluted sample solutions and place them into the DSF instrument. Only touch the capillary extremities and not the capillary center (detection part).
6. Adjust the excitation power to obtain a fluorescence signal between 2,000 and 20,000 counts to get sharp denaturation transitions. If the signal is lower than 2,000 counts, increase

the initial concentration of the sample or use the high sensitivity capillaries with lower background fluorescence. A good practice is to run a blank buffer in parralel to rule out contamination.

7. Set the temperature range from 20 to $95°C$, select a temperature gradient and start the measurement.
8. At end of experiment, check the capillaries visually. Odd data with unexpected sharp transitions and bad replicates are sometimes caused by bubbles which may develop on heating.
9. Discard capillaries in suitable trash can, clean again the reflexion mirror with ethanol and switch off the device.

References

1. Gräslund S. & all (2008) Protein production and purification. Nature methods 5:135–46 . doi: 10.1038/nmeth.f.202

2. Medrano G. & all (2012) Quality Assessment of Recombinant Proteins Produced in Plants. In: Lorence A (ed) Recombinant Gene Expression. Humana Press, Totowa, NJ, pp 535–564

3. Dupeux F. & all (2011) A thermal stability assay can help to estimate the crystallization likelihood of biological samples. Acta Crystallographica Section D: Biological Crystallography 67:915–919. doi: 10.1107/S0907444911036225

4. Monsellier E, Bedouelle H (2005) Quantitative measurement of protein stability from unfolding equilibria monitored with the fluorescence maximum wavelength. Protein Engineering, Design and Selection 18:445–456 . doi: 10.1093/protein/gzi046

5. Moon CP, Fleming KG (2011) Using Tryptophan Fluorescence to Measure the Stability of Membrane Proteins Folded in Liposomes, 1st ed. Elsevier Inc.

6. Razinkov VI. & all (2013) Methods of high throughput biophysical characterization in biopharmaceutical development. Current drug discovery technologies 10:59–70

7. Žoldák G. & all (2017) The fluorescence intensities ratio is not a reliable parameter for evaluation of protein unfolding transitions. Protein Science 26:1236–1239 . doi: 10.1002/pro.3170

Note

Note 1: By calculating the maximum of the first derivative, the melting temperature (T_m) can be derived; it corresponds to the temperature where 50% of the proteins are unfolded.