

Exploring Protein Stability by nanoDSF@Prometheus NT.48

Protein unfolding and denaturation is one of the major causes for protein loss-of-function, and must therefore be carefully controlled in the drug discovery industry and in biotechnology. The Prometheus NT.48 allows experimental access to thermal unfolding profiles of proteins by detecting changes in the emission properties of the amino acid tryptophan upon unfolding. By following the shift in the fluorescence emission wavelength, the specific fluorescence ratios (F350/F330) of the folded state vs. the unfolded state is directly correlated to the respective fraction of unfolded protein at any point during the unfolding process. This information is valuable for protein quality control, either for evaluating long-term stabilities of biologicals or the effects of physical or thermal stress on protein stability. The Prometheus NT.48 uses a capillary “dip-and-read” format, meaning that the capillaries can be directly loaded by dipping into a protein solution without additional pipetting steps, measuring up to 48 capillaries at the same time, allowing to measure the effect of temperature and external agents in the unfolding process.

In this hands-on module, the participants will learn to:

- 1) Prepare protein solutions in buffer solutions with or without cosolvents
- 2) Measure a protein in buffer
- 3) Measure the influence of different concentration of cosolvents on the protein
- 4) Use the collected data from 2) and 3), evaluate the data with respect to:
 - a. Temperature of unfolding
 - b. Aggregation temperature
 - c. Unfolding kinetics
- 5) Obtain unfolding thermodynamic parameters to analyse the effect of cosolvents on the protein.