

Differential Scanning Fluorometry (nanoDSF) - Tycho

In a nutshell: Fast and comprehensive protein quality determination, including: purity, functionality, folding, similarity, and concentration.

Services: Biophysics Facility offers Tycho as an open-access instrument. This instrument is very easy to use and no formal training is required. Core staff will provide assistance for their first experiment of new users.

Location: Building 50, room 3122

Description: Differential scanning fluorometry is a term coined for the protein thermal denaturation assay, also known as protein thermal shift assay. These assays measure the change in the fluorescence of dyes such as bis-ANS or SYPRO Orange that preferentially bind to the hydrophobic regions of proteins exposed upon their thermal unfolding. An upward shift in the unfolding temperature is observed in the presence of ligand binding, and a downward shift can be induced by buffer components destabilizing the protein structure.

Unlike DSF, the Tycho instrument measures changes in the intrinsic fluorescence of proteins during thermal unfolding, and this method is called nanoDSF. When the aromatic amino acids—whose hydrophobic side chains are typically buried in the folded protein structure—are exposed to the solvent upon protein unfolding, both the intensity and the maximum wavelength of their fluorescence change. By measuring the ratio of the two emission wavelengths, Tycho can detect even a small shift of the intrinsic fluorescence.

The nanoDSF samples are loaded into capillaries. This minimizes sample consumption and prevents sample evaporation at high temperatures. A very fast temperature scanning rate minimizes the time of the experiment. Similarly to DSF, nanoDSF can be used to detect ligand binding and to study protein stability in different buffer conditions, but unlike DSF, nanoDSF does not require the addition of the reporter dye molecules.

Since not only the fluorescence intensity ratio, but also the absolute fluorescence intensity at two wavelengths is reported by Tycho, this is an ideal method to compare different protein samples, or even the same sample before and after storage; any changes in the protein condition or concentration can be easily detected during the thermal scan.

Typical applications:

- Batch-to-batch comparisons and assessment of the protein condition after storage
- Assay development, screening buffer formulation conditions, etc.
- Protein functionality validation and detection of ligand binding

Basic instrument specifications:

- Heating range: 35°C to 95°C
- Thermal ramp: 30°C per minute
- Detection wavelengths: 330 and 350 nm
- Samples per run: Up to 6
- Experimental run time: 3 minutes

Sample requirements and recommended buffers: Tycho can analyze samples in any biological buffer. The typical concentration protein range is from 0.01 to 200 mg/mL.

Minimum sample amount: Approximately 10 µL of the sample volume is loaded into the capillary.

Consumables: Tycho requires specific sample capillaries, with diameter and wall thickness different than the MST capillaries.

Consumables are provided at the manufacturer's prices.