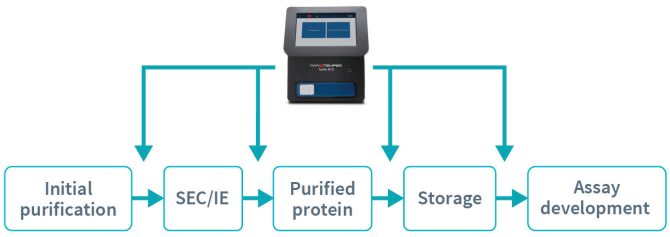
NANOTEMPER

Tycho NT.6 Application Guide

Tycho™ NT.6 is all about quick and precise protein quality checks. Test and compare the relative quality and stability of a protein sample during any step of a purification or characterization workflow. Results are generated in three minutes using Tycho NT.6 and that means better and faster decisions can be made on the next experimental steps. The influences of buffer formulation and/or storage conditions on relative stability and similarity of either freshly prepared or batch-to-batch preparations are swiftly determined. Tycho NT.6 automatically generates thermal unfolding profiles, identifies inflection temperatures (T_i), analyzes interactions effects on relative stability and monitors fluorescence sample brightness providing keen insight on sample quality and possible functionality.

Tycho NT.6 improves protein purification and characterization workflows



Generate relative stability, functionality and similarity results in 3 minutes

Initial purification Use Tycho NT.6 after the first purification steps to detect expression and also gain information on the folded state of the proteins in the preparation.

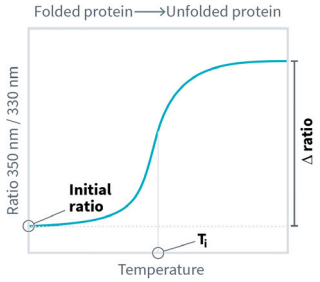
Column chromatography Directly analyze collected fractions to identify relative stability and functionality in minutes.

Purified protein Characterize sample preparations, test and optimize buffer conditions and gain a headstart on assay development.

Storage Improve formulation and storage conditions by understanding their impact on the quality of the purified preparation. Tycho NT.6 maintains reference data that is always available to access and compare.

Assay development Uncover the biological function of your target proteins faster with improved assay design using the sample quality and stability results generated by Tycho NT.6.

Interpreting Tycho NT.6 results



Monitor protein quality

T_i Inflection temperature of the unfolding transition in the 350 nm / 330 nm ratio signal.

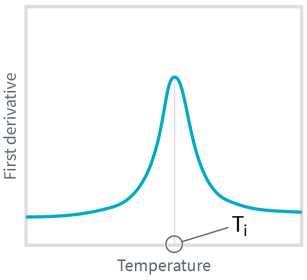
Ratio Relative units of sample brightness measured at 350 nm divided by the measurement at 330 nm. Changes in the ratio occur upon unfolding of the protein.

Initial ratio Value of the ratio at the beginning of the measurement. Serves as an indicator of the relative percentage of folded protein in a sample.

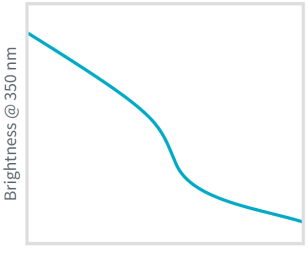
Δ Ratio Difference between the ratio at the beginning and at the end of the thermal profile.

Sample brightness Normalized fluorescence of a sample measured. Can be used for relative quantification of protein concentration.

Profile similarity An index which quantifies the similarity of the unfolding profiles of two or more samples.



A peak in the first derivative corresponds to an inflection temperature (T_i).



Some unfolding transitions only show in the single wavelength fluorescence data

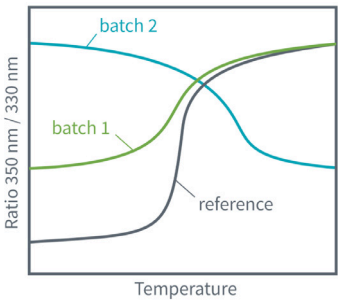
Data display options

First derivative view Tycho NT.6 automatically detects and identifies the inflection temperature (T_i) of each test sample as a function of increasing temperature. A peak in the first derivative view corresponds to the detected T_i of the test sample.

Brightness view Proteins can be complex molecules. Fluorescence measurements or brightness captured at either 350 nm or 330 nm can be displayed individually on the Tycho NT.6 to more easily identify and interpret unfolding transitions.

Tycho NT.6 Application and Data Examples

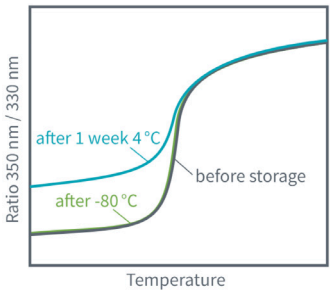
Batch-to-batch comparison



A decrease in Δ ratio compared to a reference sample indicates less folded protein (batch 1). A different unfolding profile indicates major differences between protein preparations (batch 2).

- Capture measurements during each step in protein purification preparations to ensure batch-to-batch similarity
- Identify and optimize critical steps in purification workflows
- Quickly identify major discrepancies between protein batches

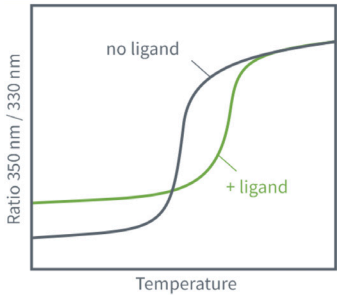
Storage + stability



A decrease in Δ ratio indicates protein denaturation during storage

- Record unfolding profiles before and after storage
- Quantify profile similarity using the comparison function to monitor relative quality and consistency of preparations
- Identical unfolding profiles before and after test conditions suggest minimal or no impact of storage effects on the protein quality.

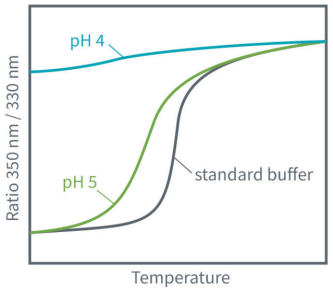
Folding and functionality



Loss of unfolding transition or loss of thermal shift during purification indicates protein denaturation

- Perform during each step of purification processes to validate protein functionality
- Rapid yes/no answer on binding of ligands, substrates, ions or small molecules
- Shift in the initial ratio and/or shift in T_i (thermal shift) in presence of ligand indicates binding and functionality of the sample

Assay development



Optimize and determine buffers or formulations that better support protein stability. Loss or shift of T_i, or decrease in Δ ratio indicate destabilization and unfolding.

- Perform better assay development and optimization
- Rapidly screen buffers for assays or immobilization conditions, for example for biosensor experiments