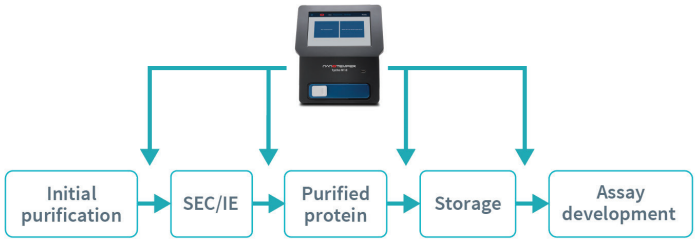
NANOTEMPER

Tycho NT.6 Application Guide

Tycho™ NT.6 is all about quick protein quality checks.
It can tell you about the presence, purity, concentration, functionality and similarity of your protein sample all within a single experiment. It does this by determining if your protein is structurally intact or properly folded. Use only 10 µL of sample to find out the quality of any protein in 3 minutes and make your assay development and purification workflows more efficient. Tycho automatically generates thermal unfolding profiles, identifies inflection temperatures (T_i), analyzes interaction effects on structural integrity and monitors fluorescence sample brightness, providing keen insight on sample quality and possible functionality.

Tycho NT.6 improves protein purification and characterization workflows



Generate functionality, purity, concentration 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 structural integrity or folded state of your protein of interest in the preparation.

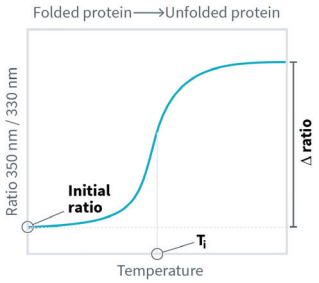
Column chromatography Directly analyze collected fractions to identify the presence, purity and functionality in minutes.

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 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.
The higher T_i, the more stable the protein in the conditions measured.

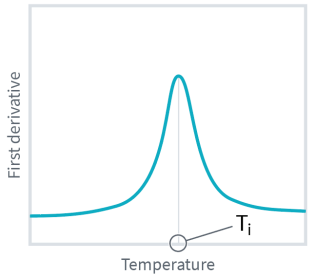
Ratio Used to follow the protein unfolding process. Relative units of sample brightness measured at 350 nm divided by the measurement at 330 nm.

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.

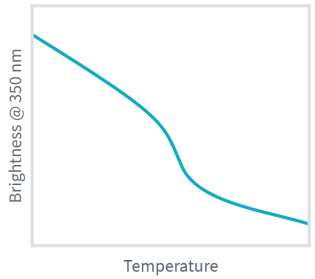
Δ ratio Difference between the ratio at the beginning and at the end of the unfolding profile. Δ ratio can be used to assess the fraction of unfolded protein contained in the sample.

Sample brightness Normalized fluorescence of a sample. Can be used for 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

Tycho NT.6 automatically detects and identifies the inflection temperature (T_i) of each tested sample as a function of increasing temperature and by default shows the ratio view (see above).

First derivative view A peak in the first derivative view corresponds to the detected T_i of the test sample. T_is are sometimes easier to spot visually in this view.

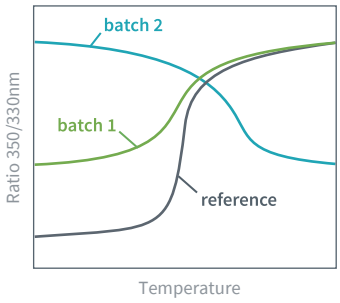
Brightness or single wavelength view Proteins can be complex molecules. Fluorescence measurements or brightness captured at either 350 nm or 330 nm can be displayed individually to provide additional information for identifying and interpreting unfolding transitions.

Tycho NT.6 Application and Data Examples

Batch-to-batch comparison

Quickly identify discrepancies between protein batches

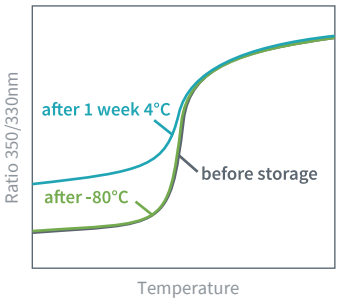
- A decrease in Δ ratio compared to a reference sample indicates less folded protein (batch 1).
- A different unfolding profile indicates major differences between protein preparations (batch 2).



Storage and stability

Understand what storage does to your protein

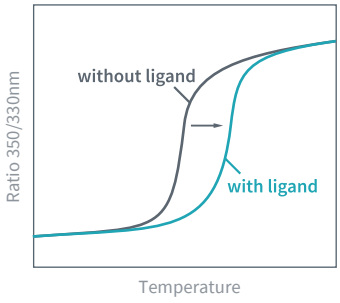
- Identical unfolding profiles before and after test conditions suggest minimal or no impact of storage effects on protein quality.
- A decrease in Δ ratio indicates protein denaturation during storage.



Folding and functionality

Validate your protein's functionality

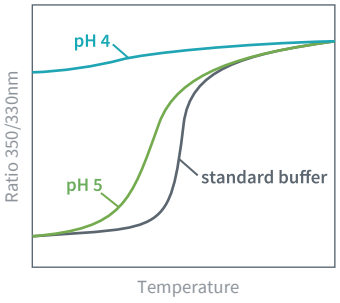
- A shift in T_i (thermal shift) and/or in the initial ratio in the presence of a ligand indicates binding and functionality of the sample.
- Loss of thermal shift (or complete loss of unfolding transition) indicates that the protein is not functional and likely denatured.



Assay development

Determine optimal buffers or formulations for your protein

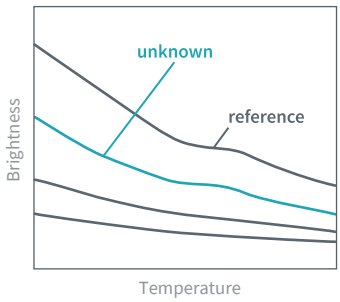
- Destabilization and unfolding is indicated by a loss or shift of T_i, or by a decrease in Δ ratio.
- Rapidly screen buffers for assays or immobilization conditions, for example for biosensor experiments.



Concentration

Check the presence and concentration of your protein of interest

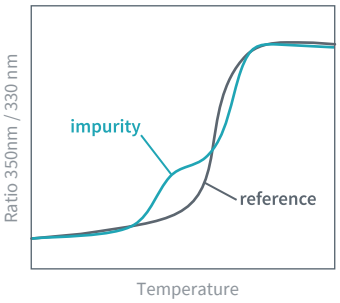
- Sample brightness correlates with concentration.
- Compare relative concentration between different batches, or get absolute numbers if you have a reference with known concentration.



Purity

Detect impurities in your protein sample

- If the unfolding profile looks different than your reference, something's off.
- Additional unfolding transitions can indicate impurities in the sample.



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