NANOTEMPER

nano-Differential Scanning Fluorimetry (nanoDSF)

nano-Differential scanning fluorimetry, or nanoDSF, is a biophysical characterization technique used for assessing the conformational stability of a biological sample.

It uses the intrinsic fluorescence of a protein to monitor how it responds to stress inputs such as temperature or chaotropes. This information is used to determine conformational stability of the protein, and to rank candidates or buffer formulations based on their impact on this stability.

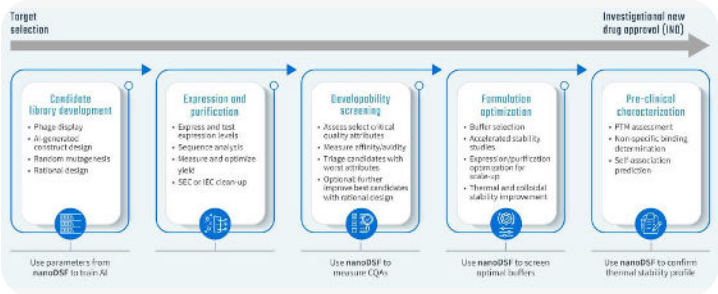
nanoDSF is a valuable tool for many researchers in therapeutic spaces

nanoDSF is a useful tool for anyone developing biologics or gene therapies. Complex biological samples such as enzymes, monoclonal antibodies, or AAVs that will be used for treatments must be highly stable for storage, transport, and clinical administration.

nanoDSF enables you to monitor the conformational stability of your sample. This information helps you optimize your sample by making changes to the sequence or buffer environment, and measuring how those changes impact stability. Stability parameters are used to rank candidates for therapies and select those with greater stability for further characterization and development.

Apply nanoDSF measurements in many aspects of therapeutic development, including:

- During expression and purification
- Pre-formulation phase
- Developability assessment
- Buffer formulation and optimization
- Comparability studies during scale-up or process changes



The affinity constant K_d is derived by plotting the ratio of the fluorescence signal detected simultaneously at 670 and 650 nm against the ligand concentration on a logarithmic scale.

nanoDSF provides insight about the stability of your samples

Conformational stability indicates how likely a protein sample is to remain folded, and how well it holds up in stressful conditions. When a thermal or chemical denaturant gradient is applied to a protein sample, it will begin to unfold under thermal or chemical duress. Researchers use the point at which unfolding occurs as an indication of the overall impact a modification or condition has on stability.

Changes to the primary sequence or buffer environment impact the stability of a protein. When screening libraries of candidates or conditions, it's important to only pass on candidates with improved stability. The parameters generated from nanoDSF data enable ranking candidates for increased stability attributes.

Melting temperature

Or T_m is the temperature along a thermal denaturation gradient at which 50% of the protein is folded, and 50% is unfolded. T_m is a commonly used parameter for ranking the most thermostable candidates – the higher the T_m, the more stable the candidate, and the more optimal it is for further development.

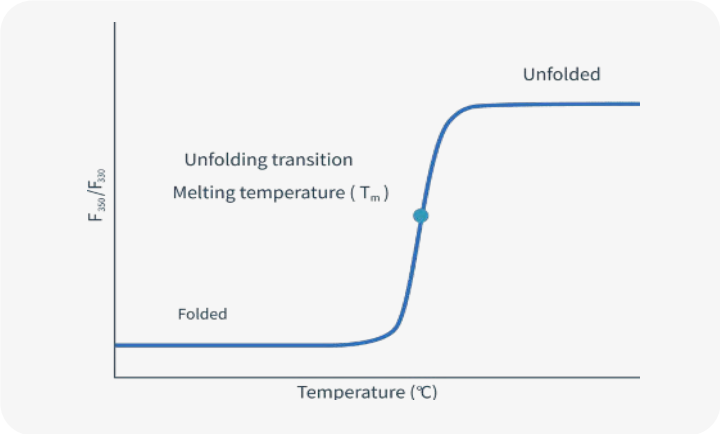
Onset of unfolding

Or T_{on}, is the temperature at which a protein begins to unfold.

Modifications to the protein sequence or buffer do not always impact the T_m. T_{on} is an additional parameter to indicate the change in thermal stability when changes are made to a candidate.

Slope at IP (inflection point)

Is a measure of how sharp the transition from folded to unfolded is. A steeper slope indicates a sharper transition, which is interpreted as a more stable candidate. Think of this by thinking of a protein that remains very well-folded until the moment the change in temperature becomes too great, and suddenly unfolds all at once. The T_{on} and T_m values will be closer together.

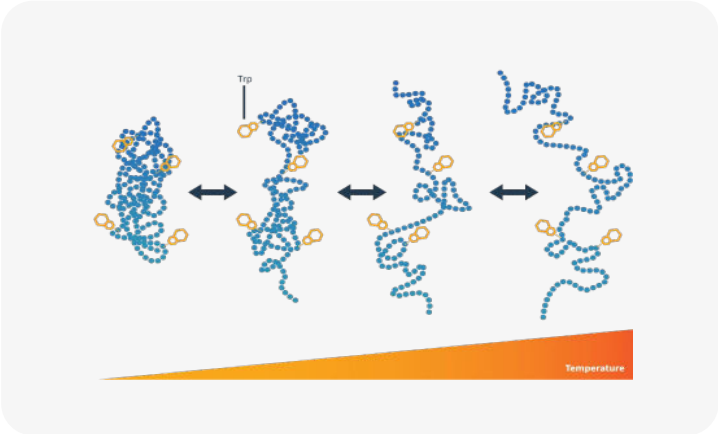


C_{50} or C_m

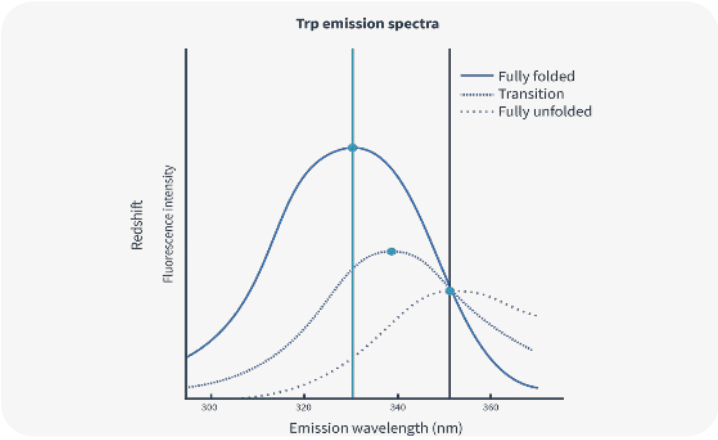
Are values reflect the concentration of a chemical denaturant or chaotrope, at which 50% of the protein is unfolded, and 50% is folded. The more denaturant is required to unfold a protein, the higher this value is. Greater C_{50}/C_m values indicate greater stability.

Energy of unfolding, ΔG

Is an energetic value parameter that tells you how much energy input is required to trigger unfolding of your sample at a given temperature. Protein unfolding for most proteins used in labs is not energetically favorable, so energy is required to disrupt the folding; the more positive ΔG, the more energy is required to trigger unfolding. Change in ΔG, ΔΔG is used to compare the change in ΔG between two different conditions.



Tryptophan residues become exposed to solution in response to a temperature (or concentration) gradient



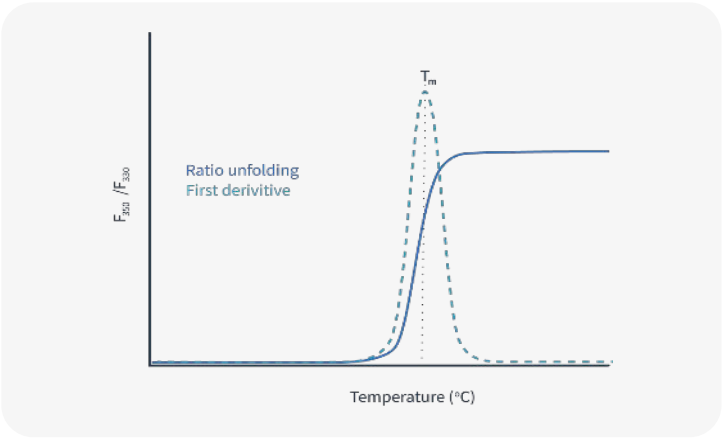
The emission maximum for Trp changes in response to a change in its chemical environment; when folded it fluorescence most highly at 330 nm, while when exposed to aqueous solution it fluoresces more highly at 350 nm

Proteins contain tryptophan residues, which absorb light at 280 nm. Their emission properties change depending on their chemical environment. When protected from aqueous solution, as when folded, they have a fluorescence emission peak at 330 nm.

When exposed to solution, the hydrophobic side chains have different emission properties, and have a fluorescence maximum at 350 nm. There is also an overall shift in tyrosine emission, which is useful in rare cases when a protein does not contain tryptophan residues.

Plotting the F_{350}/F_{330} ratio against the thermal or chemical denaturant concentration gradient results in a graph that has an inflection point at the T_m or C_{50}/C_m. The first derivative view of this plot is sometimes used to more easily visualize where the inflection takes place; the inflection point corresponds to a peak in the first derivative plot. Highly sensitive optics enable you to

monitor the shift in fluorescence attributed to unfolding due to either thermal or chemical denaturation.



First derivative overlaid onto the ratio vs gradient plot. The inflection point is easier to identify precisely by looking for the peak value.