Microcalorimetric techniques

Isothermal titration calorimetry (ITC)

Differential scanning calorimetry (DSC)

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Isothermal titration calorimetry (ITC)

- characterization of binding reactions between proteins and ligands or other macromolecules

- enzyme kinetics

Differential scanning calorimetry (DSC)

- characterization of the thermal stability of proteins and other macromolecular assemblies

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Isothermal titration calorimetry (ITC)

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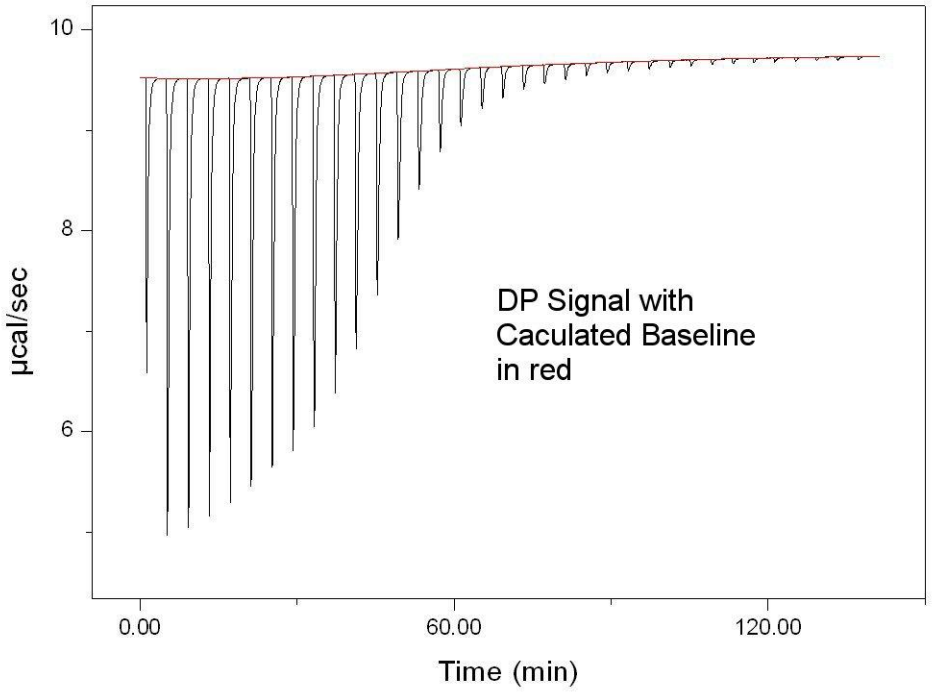
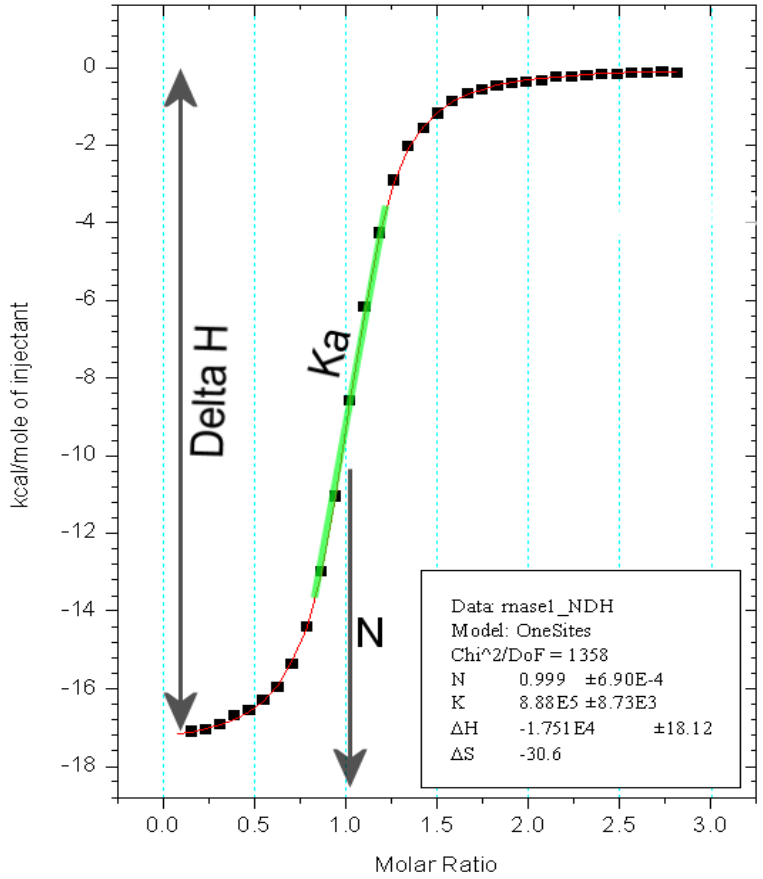
Isothermal titration calorimetry (ITC)

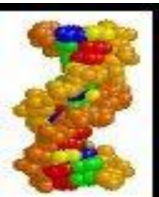
VP-ITC

PEAQ-ITC

ITC – binding







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ITC – enzyme kinetics

- based on simple Michaelis-Menten mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$v = \frac{k_{cat}[E]_{tot}[S]}{K_m + [S]}$$

- Two experimental methods:
  - single injection method (SIM)
  - multiple injection method (MIM)

k_{cat} - constant that describes the turnover rate of an enzyme-substrate complex to product and enzyme

K_M - Michaelis constant that describes the amount of substrate needed for the enzyme to obtain half of its maximum rate of reaction

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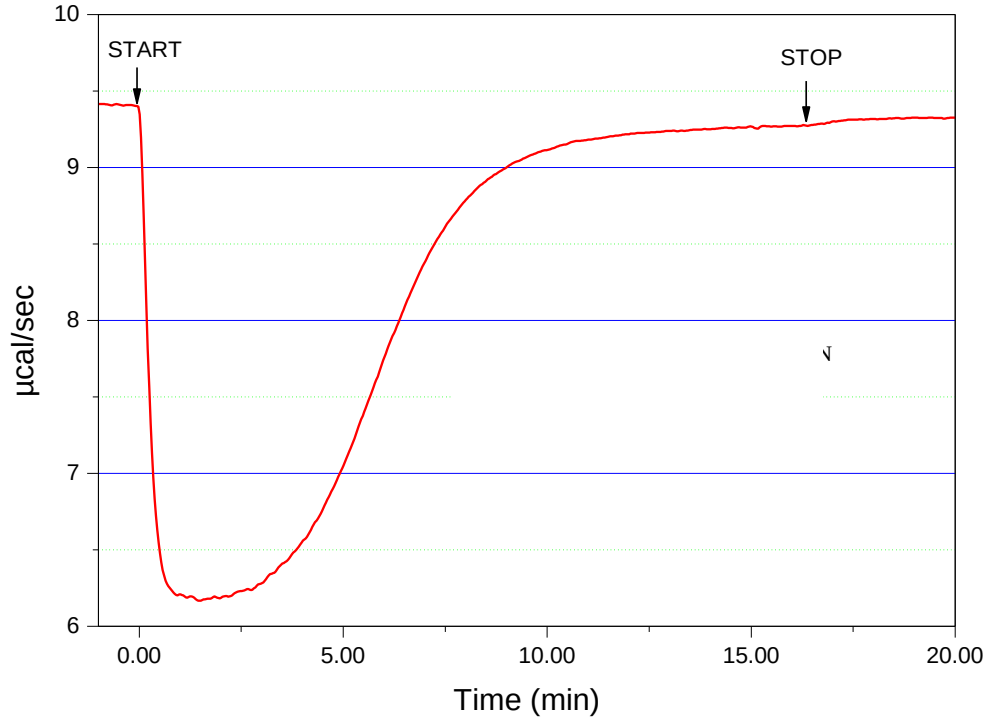
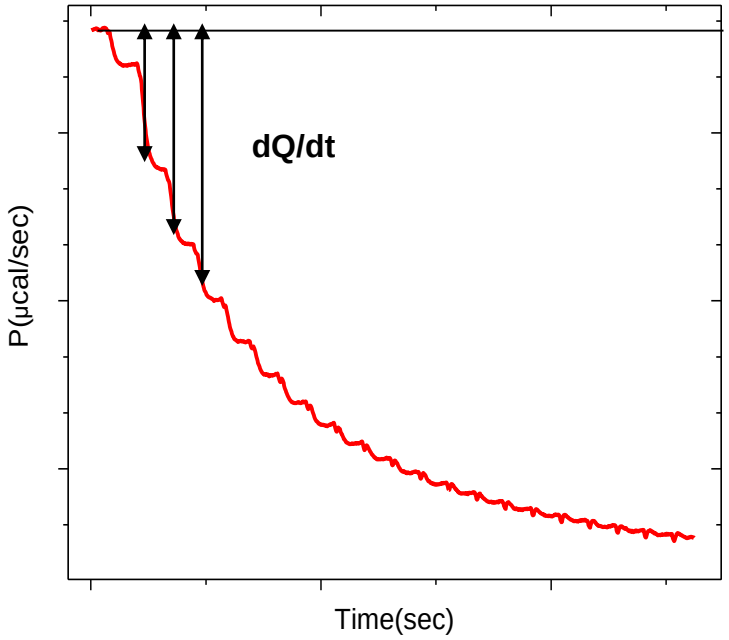
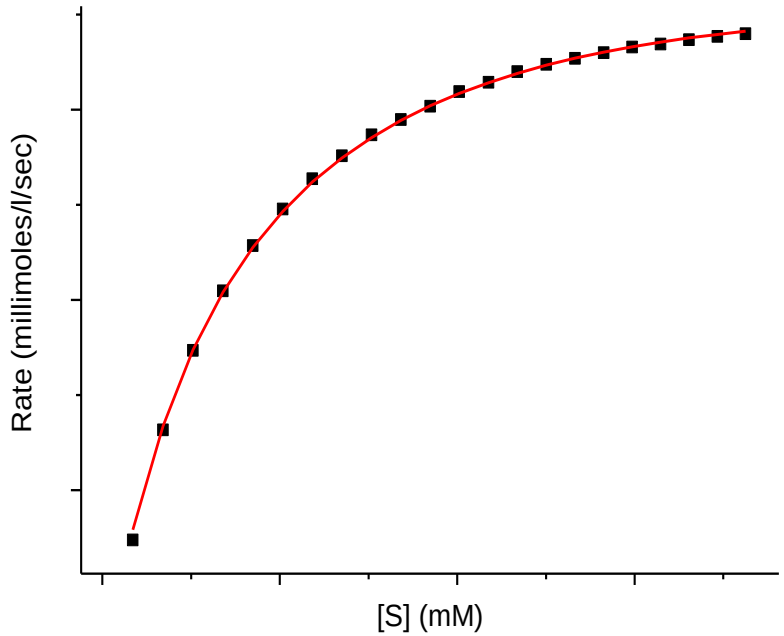
ITC – enzyme kinetics - SIM



ITC – enzyme kinetics - MIM

$$v = \frac{d[P]}{dt} = \frac{1}{V\Delta_r H}\frac{dQ}{dt}$$





Differential scanning calorimetry (DSC)

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The Nano DSC

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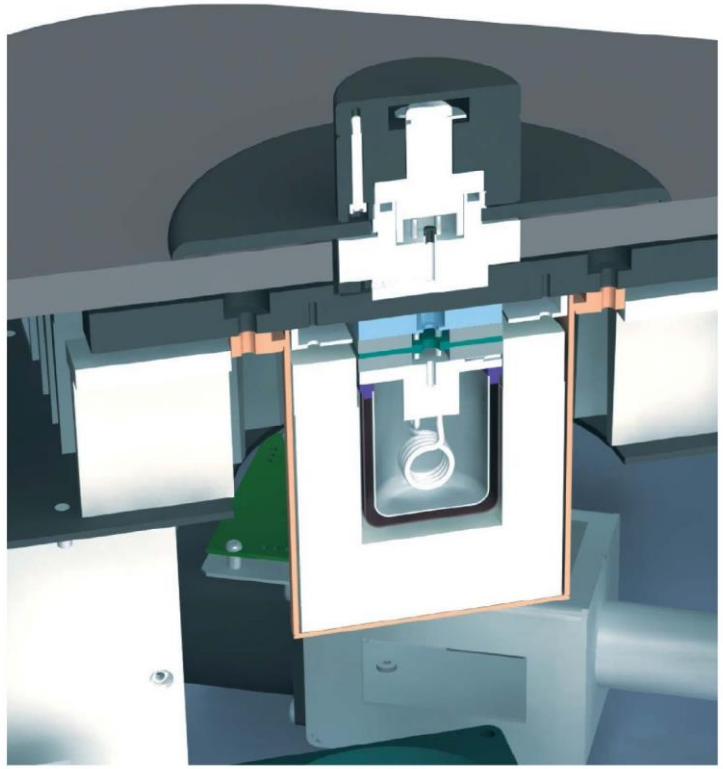
Cutaway views of the Nano-DSC

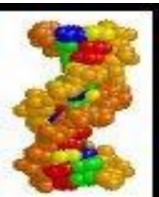


New Nano DSC

- **Platinum capillary cells**
- **New USB connection to computer**
- **Innovative sensor design**
- **Unmatched sensitivity**

The Nano DSC Cell Geometry

- Cell Construction;
  Inert to biomaterials
  99.99% Platinum
- Sample Volume 0.3 mL
- Attenuates or delays onset of aggregation
- Easy-to-fill and clean design



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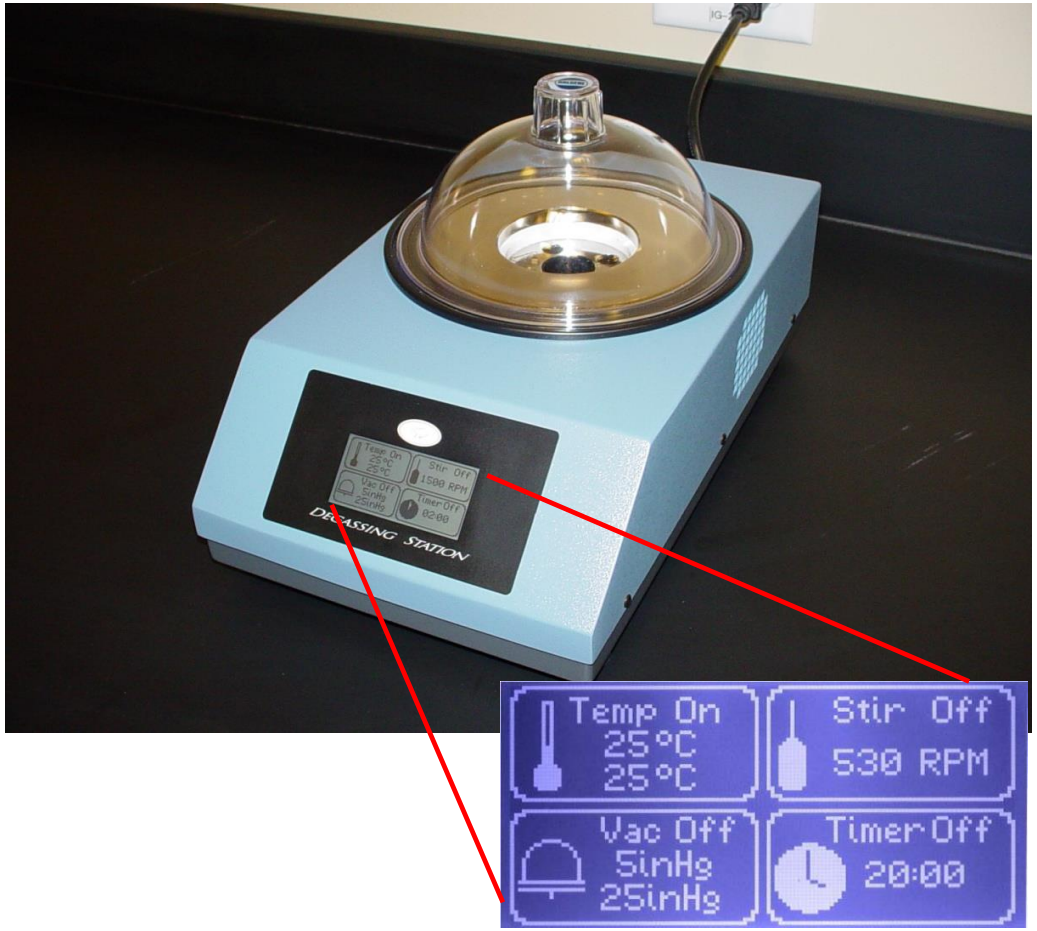
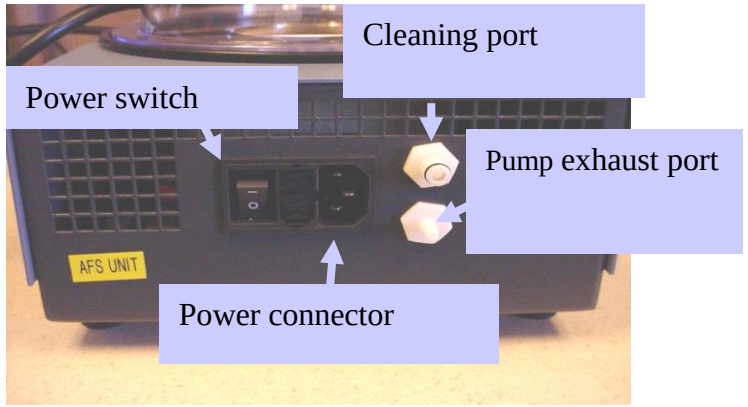
Degassing Station





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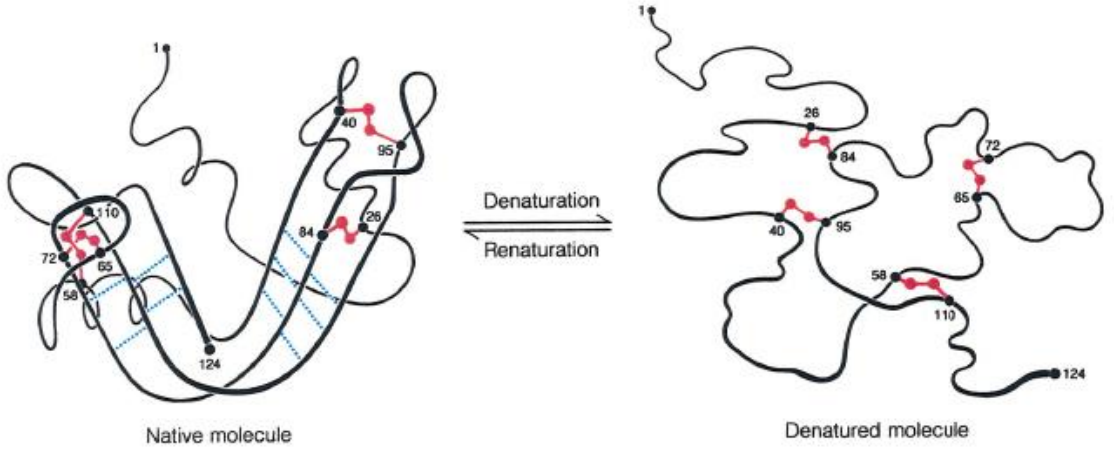
Nano DSC Cleaning Configuration

The two-state model of protein unfolding



- Heat associated with unfolding (endothermic) and folding (exothermic) is easily measured by calorimetry, allowing thermodynamic analysis of the folding/unfolding process
- Folding and unfolding of a small protein, a domain, or a subunit, is ‘cooperative’
- These small units can fold and unfold reversibly - reversibility is directly measurable by DSC.

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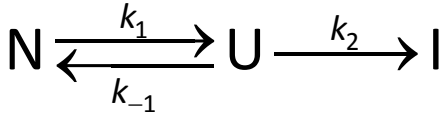
Reversibility of protein unfolding



native — reversibly unfolded — irreversibly denaturated

- k_1 – rate of unfolding
- k_{-1} – rate of folding
- k_2 – rate of denaturation

- In theory - proteins should fold and unfold reversibly
- In practice - proteins may get trapped in an irreversible denatured state
- Reversibile unfolding - equilibrium thermodynamics analysis
- Irreversibile unfolding - from first scan can be determined the enthalpy, the ‘melting’ temperature, and the change in heat capacity
- Most proteins unfold between 40 – 90 ^{o}C - temperature of unfolding of a protein is characteristic for that protein

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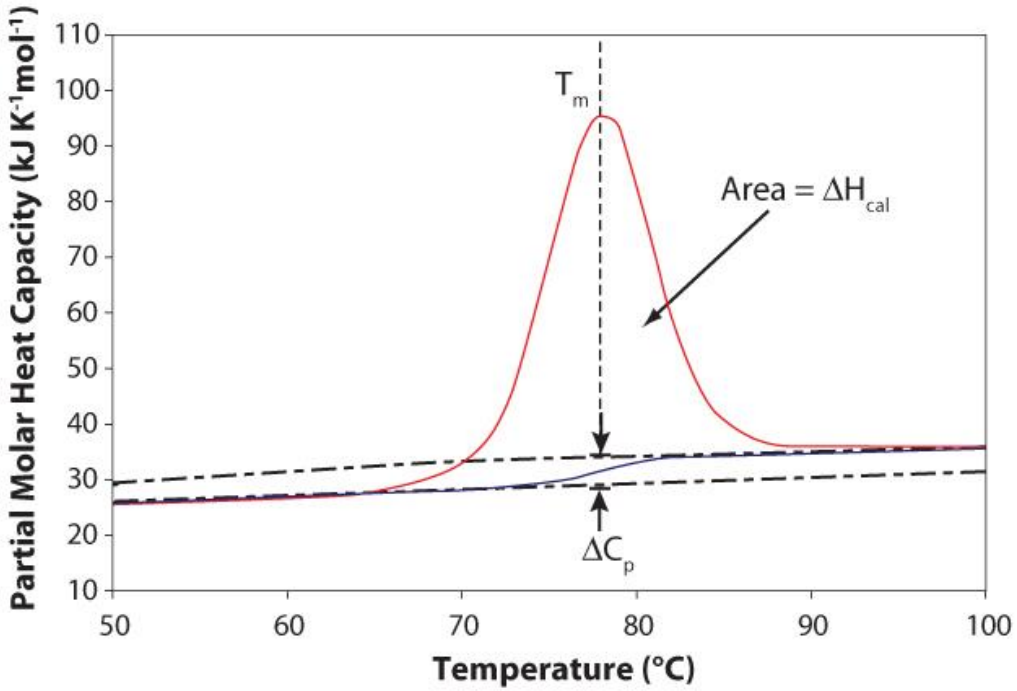
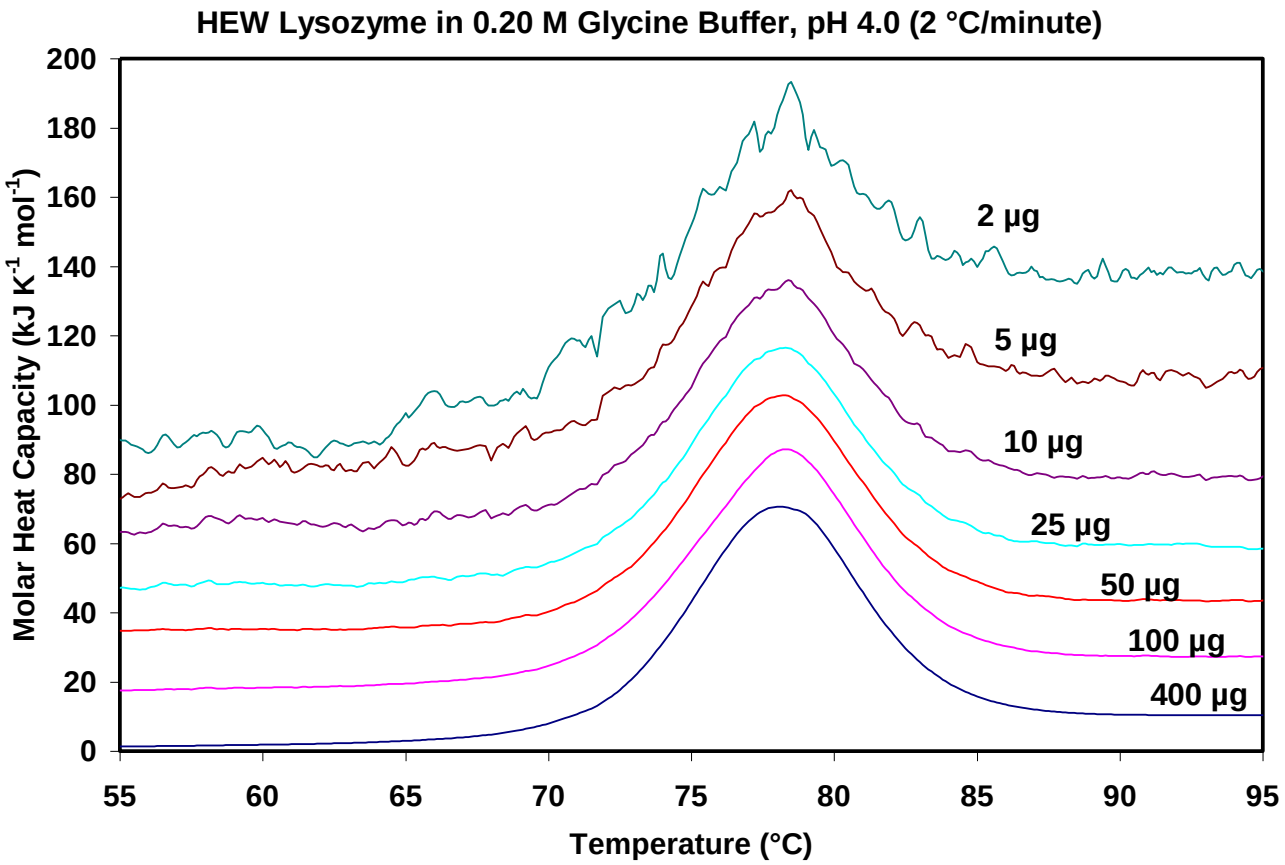
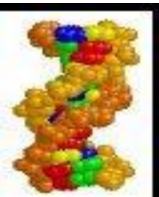
DSC scan



- Heat capacity change ($\Delta_r C_p$) - determined from baseline shift before/after unfolding
- Area under unfolding peak – calorimetric enthalpy ($\Delta_r H_{cal}$) of the unfolding reaction
- Midpoint of the thermal unfolding (T_m) - temperature at which half the molecules are unfolded - indication of the stability of the molecule
- DSC is the only technique that allows the direct measure of T_m, $\Delta_r C_p$ and $\Delta_r H$

Nano DSC sensitivity





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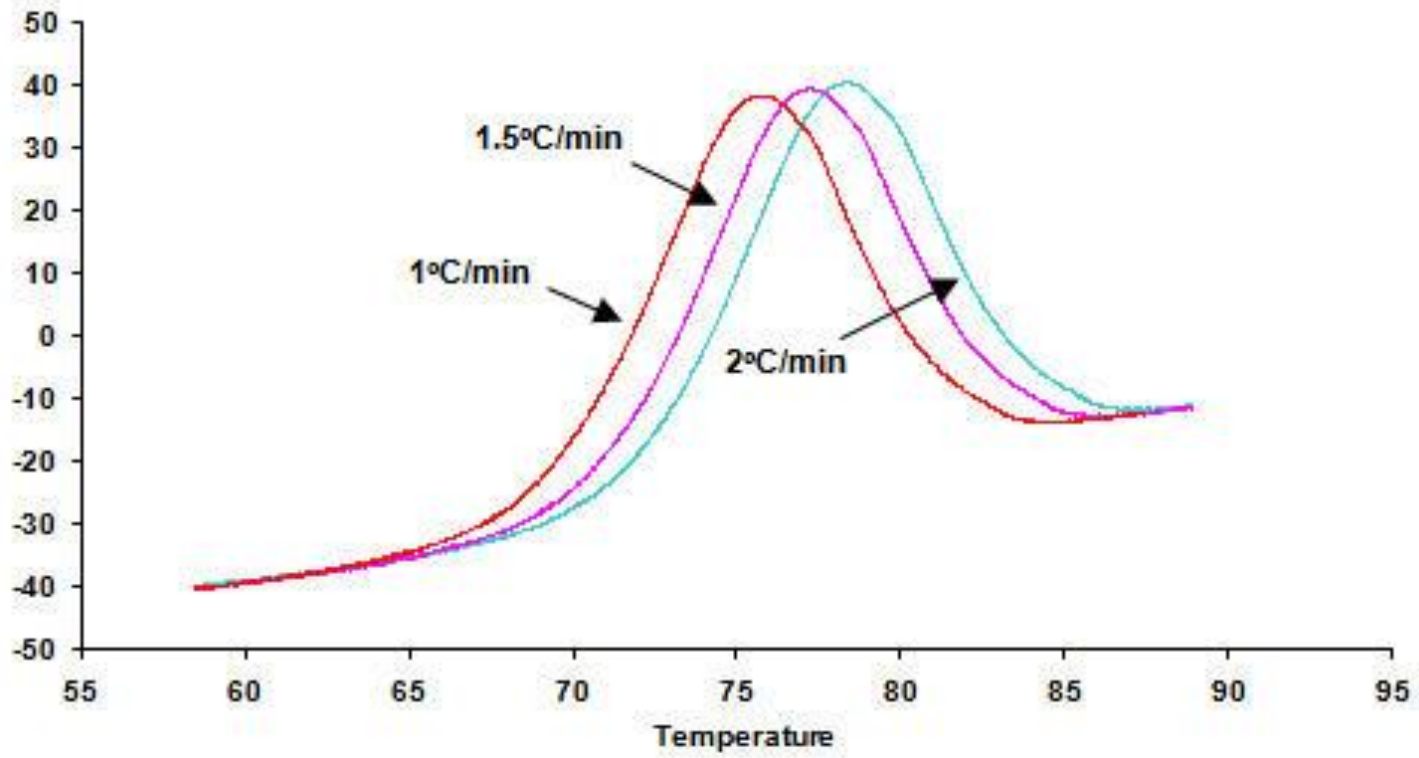
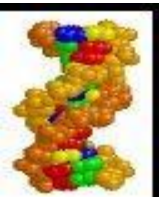
DSC scan rate



- scan rate dependence of T_m - indicates that native and unfolded protein are not in equilibrium
- kinetically controlled process



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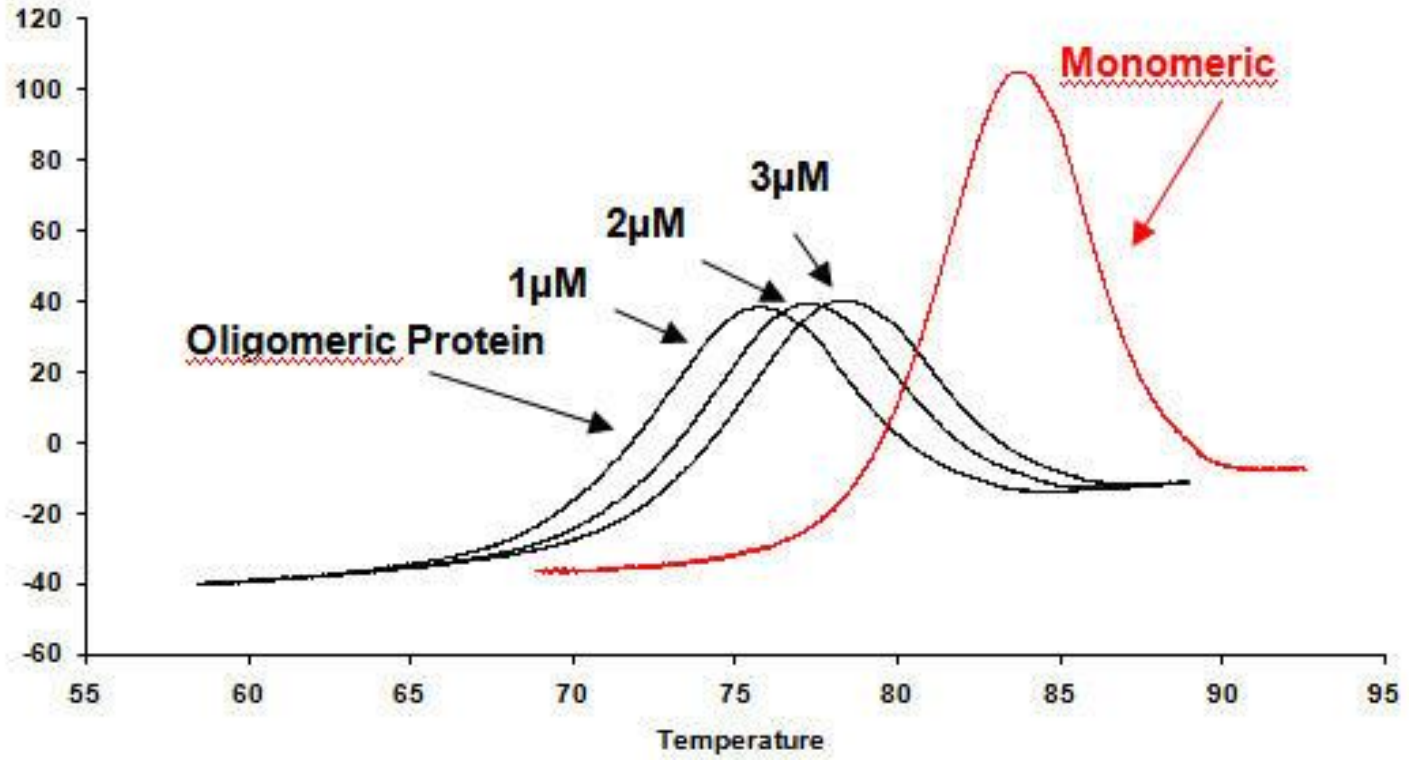
Importance of concentration determination



- Effect of sample concentration dependence of T_m - test for oligomerization

The van't Hoff enthalpy vs. the calorimetric enthalpy

- Calorimetric enthalpy ($\Delta_r H_{cal}$) - the area under the transition peak, energy required to unfold the protein
- The van't Hoff enthalpy ($\Delta_r H_{vH}$) – calculated enthalpy from two-state model
- If $\Delta_r H_{cal} = \Delta_r H_{vH}$ two-state model is valid:

$$N \rightleftarrows U$$

- If $\Delta_r H_{cal} > \Delta_r H_{vH}$ intermediate unfolded states are likely present and two-state model is invalid:

$$N \rightleftarrows Int \rightleftarrows U$$

- If $\Delta_r H_{cal} < \Delta_r H_{vH}$ protein forms oligomers and two-state model is valid:

$$N_n \rightleftarrows nU$$

P. L. Privalov, S. A. Potekhin, "Scanning Microcalorimetry in Studying Temperature-Induced Changes in Proteins", *Methods in Enzymology* **141** (1986) 4-51.

Applications

- Stability of proteins and protein structural components
- Stability of polynucleotides and oligonucleotides
- Cooperativity and reversibility of unfolding/folding reactions
- Stability of molecular assemblies (e.g. liposomes)
- Effect of ligand binding on protein-ligand complex stability

Experimental approaches are applicable to all biological macromolecules, not just proteins

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Summary

- DSC - only technique for directly determining the enthalpy of the unfolding of a biological polymer

- Comparison of $\Delta_r H_{cal}$ to $\Delta_r H_{vH}$ - provides unique information about the unfolding pathway (oligomerization, intermediates, aggregation)

- Sample concentration dependence of T_m - sensitive test of higher-order association

- Scan rate dependence of T_m - key test for equilibrium unfolding

- Interpretable experimental results - highly dependent on sample purity and concentration

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