THE USE OF DIFFERENTIAL SCANNING CALORIMETRY IN LIFE SCIENCES

Bertrand Morel

- Technical principles

- Design and optimisation of an experiment

- Different types of applications

- Troubleshooting

PROTEIN STABILITY AND FOLDING

- A protein's function depends on its 3D-structure
- Loss of structural integrity with accompanying loss of activity is called denaturation
- Proteins could be denatured by:
  - heat or cold
  - pH extremes
  - organic solvents
  - chaotropic agents: urea and guanidinium hydrochloride

METHODS TO MEASURE PROTEIN UNFOLDING

- Temperature induced denaturation by fluorescence, Circular Dichroism, Differential Scanning Calorimetry.

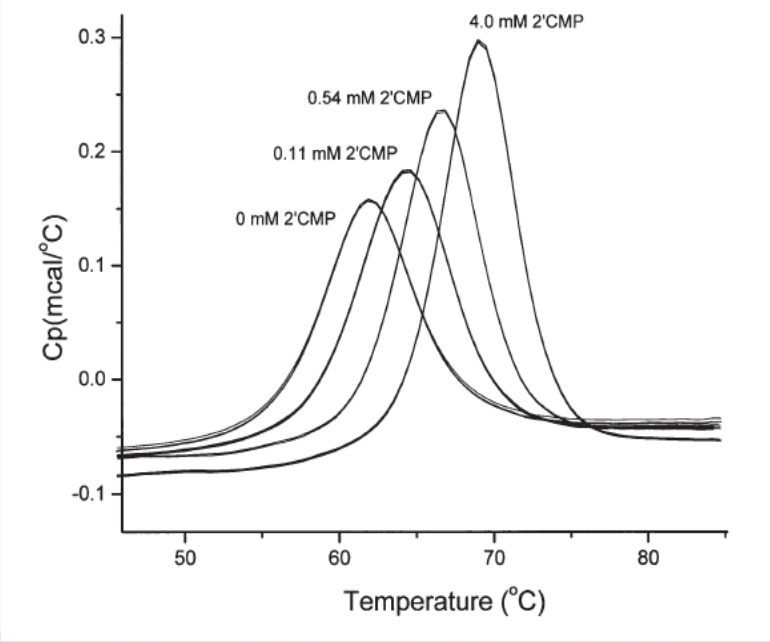
- Chemically-induced denaturation curves by fluorescence or Circular Dichroism

STABILITY AND SPECIFIC BINDING

Protein stability could be modulated by external factors.

Co-solute could increase the stability (Tm) of a protein, then probable increase in shelf-life-formulations.

If a compound at stoichiometric concentrations increases Tm then we have specific binding.



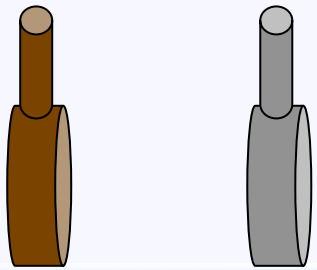
DSC : TECHNICAL PRINCIPLES

- Differential Scanning Calorimetry (DSC) measures the temperatures and heat flows associated with transitions in materials as a function of time and temperature in a controlled atmosphere.

- These measurments provide qualitative and quantitative information about physical and chemical changes that involve exothermic or endothermic processes or changes in heat capacity.

DSC : TECHNICAL PRINCIPLES

- The DSC contains two sample cells:
  - One cell contains biomolecule (e.g. protein) in buffer
  - The other cell contains only the buffer
  - DSC cells are either capillary or “lollipop” in shape, and there are always two of them:

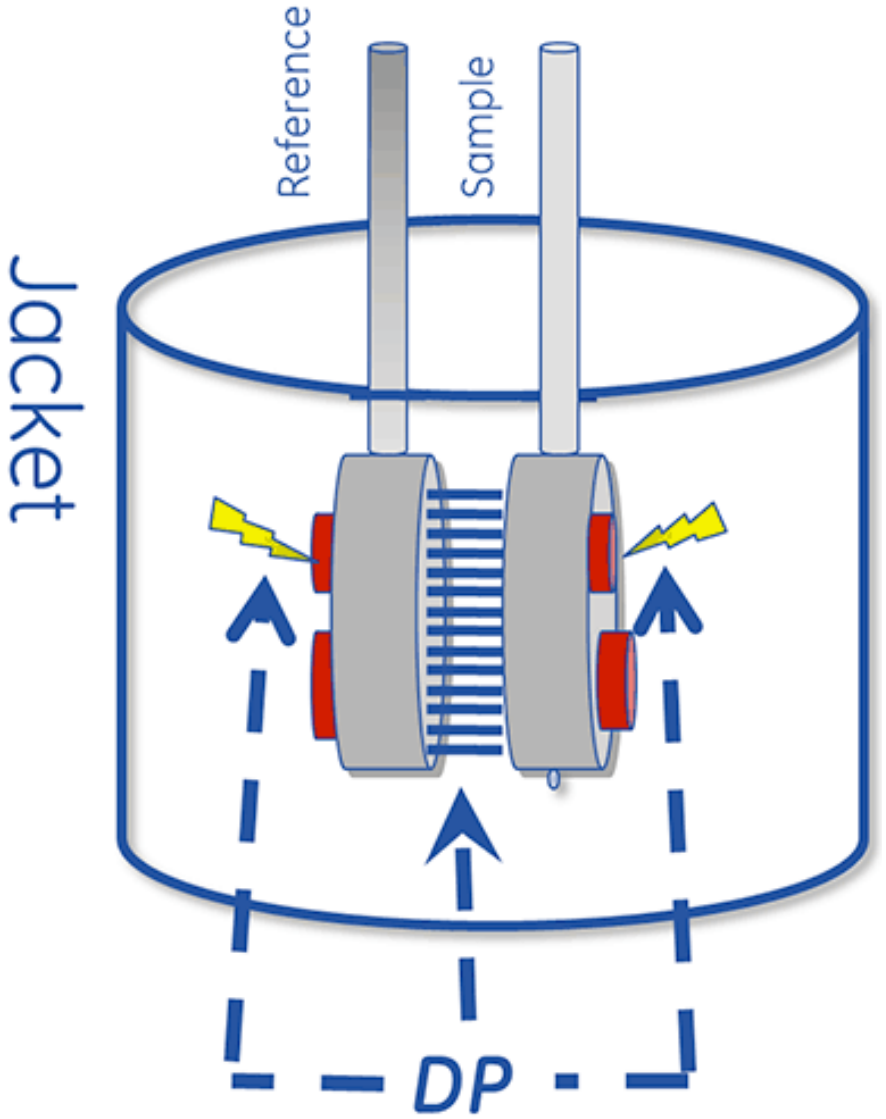
“sample” cell  “reference” cell

DSC : TECHNICAL PRINCIPLES

The DSC cells are contained in an insulated "adiabatic" chamber.

The device is designed to maintain the two cells at the same temperature, as they are heated.

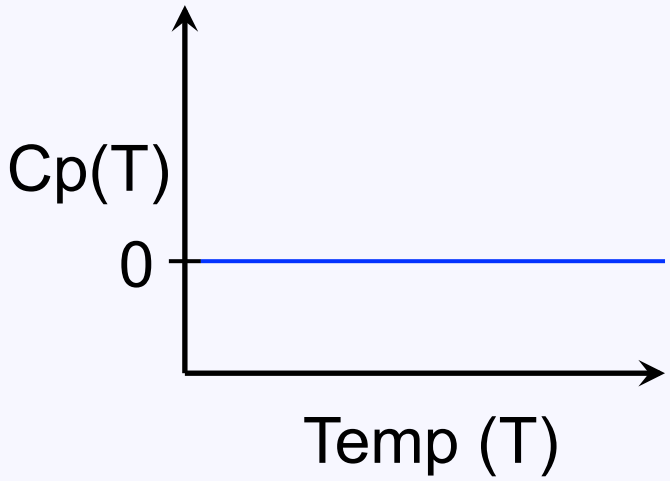
The DP is the differential power added to maintain $\Delta T \sim 0$ between the cells (data collected in an experiment).



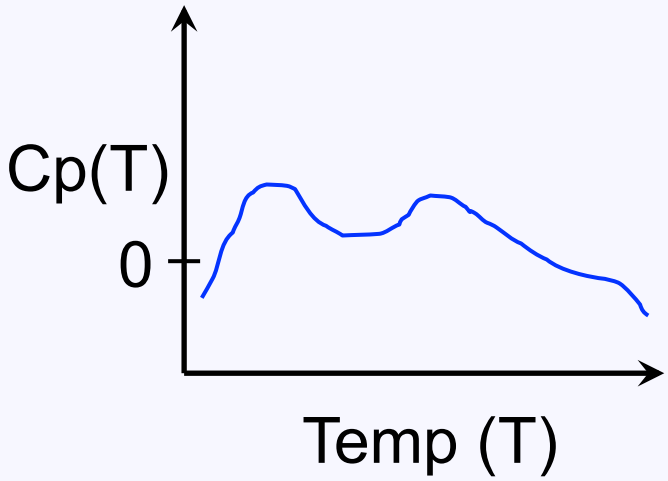
DSC: TECHNICAL PRINCIPLES

- Although the two cells in the DSC are manufactured to be as identical as possible, there will be slight differences in volume, shape, etc…

Perfectly matched cells



The typical situation



INFORMATION OBTAINED

- **Thermal transition midpoint** – Tm (melting temperature): Indication of thermal stability.
- **Enthalpy** – ΔH: Includes energy associated with changes in inter- and intramolecular interactions (hydrogen bonds, etc.).
- **Entropy** – ΔS: "Molecular disorder"
- **Heat capacity** – ΔCp: Measures ability of biomolecule to absorb heat energy without increase in temperature.
- **Gibbs free energy –** ΔG**:** $\Delta G=\Delta H-T\Delta S$. At T_m, $\Delta G_{unfolding} = 0$.

DSC PRODUCTS

VP-DSC:
Cell volume 500 µL
4 experiments/8 hours

Capillary cells
Cell Volume 160 µL
Up to 50 experiments/day
Unattempted operation
Up to 576 samples on board

PRACTICAL REQUIREMENTS

Concentration requirements:

Depends on the molecular weight of the protein

Minimum concentration 0.02 mg/ml

As starting point min 0.1-0.2 mg/ml

Maximum concentration 50 - 100 mg/ml

PRACTICAL REQUIREMENTS

Sample preparation:

Exchange material into buffer using dialysis or desalting column

Retain the exchange buffer for use as the reference solution

Centrifuge or filter sample

PRACTICAL REQUIREMENTS

Choosing a good buffer:

Compatible with many buffers

Avoid DTT (Unstable and undergoes oxidation)

Use β -mercaptoethanol or TCEP

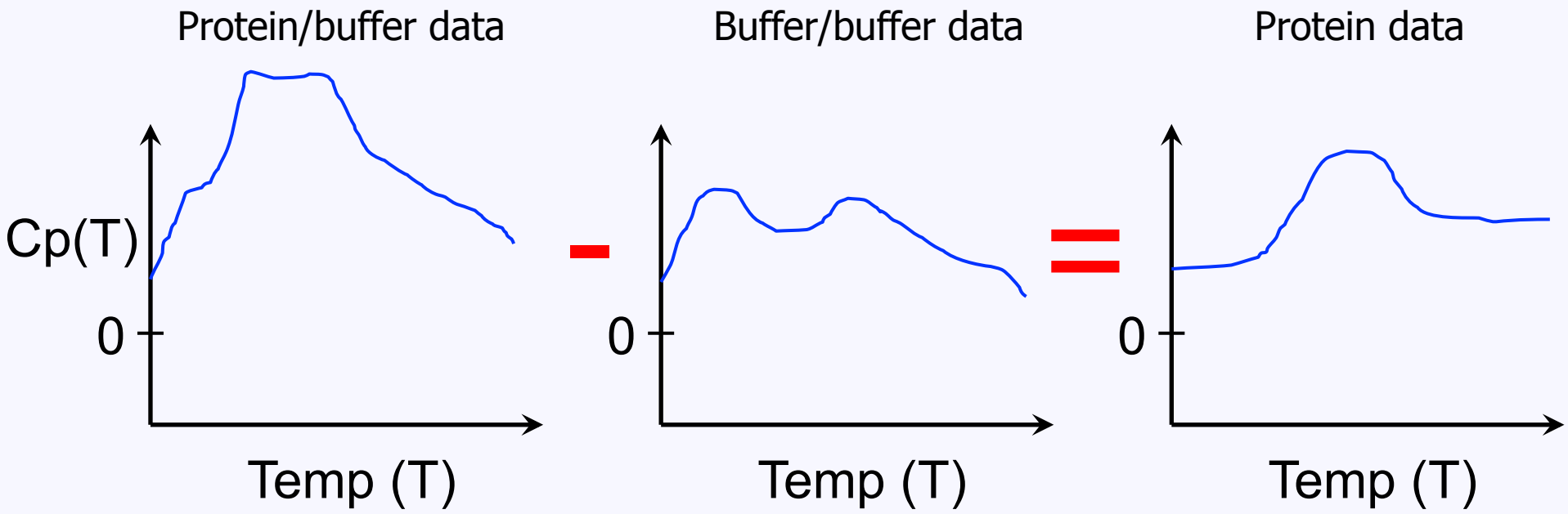
Tris buffer should not be used

First, try DSC buffer only

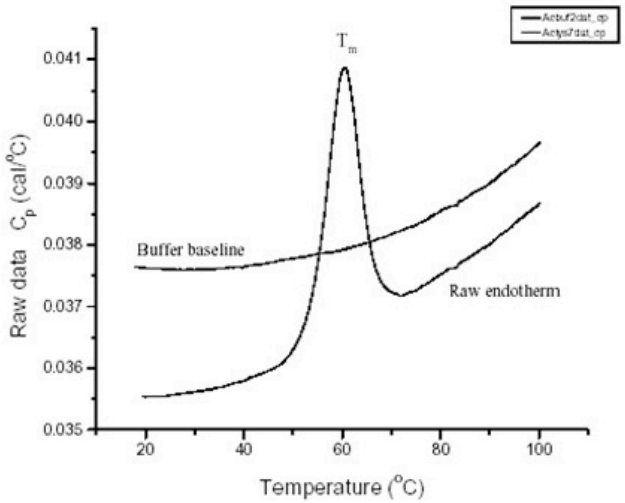
PRACTICAL EXPERIMENTAL DESIGN

- Both cells are loaded with buffer
- The instrument is setup for multiple (20) data collection runs (heating/cooling cycles)
- “Buffer/buffer” data is collected (≥3 runs)
- When the instrument is cooling down, prior to a heating cycle, the protein is introduced at 25 °C
- A “protein/buffer” data run is collected

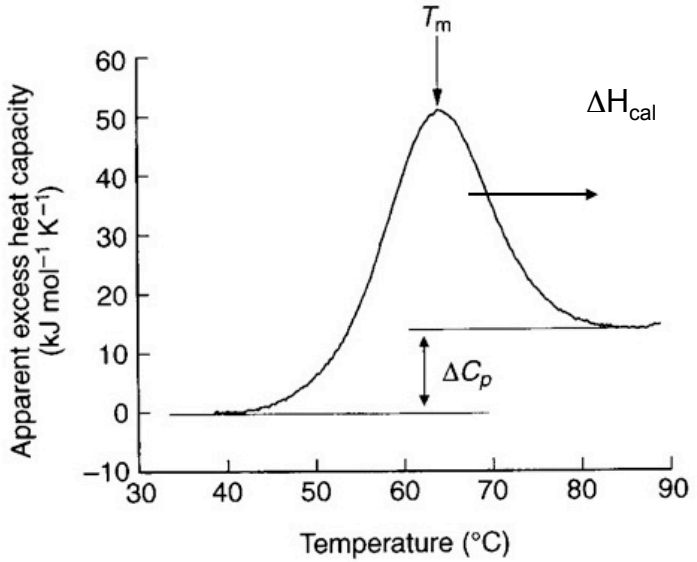
PRINCIPLES OF ANALYSIS

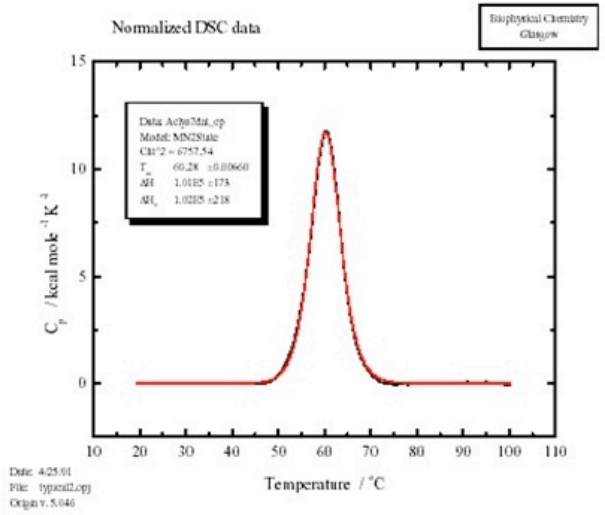


PRINCIPLES OF ANALYSIS



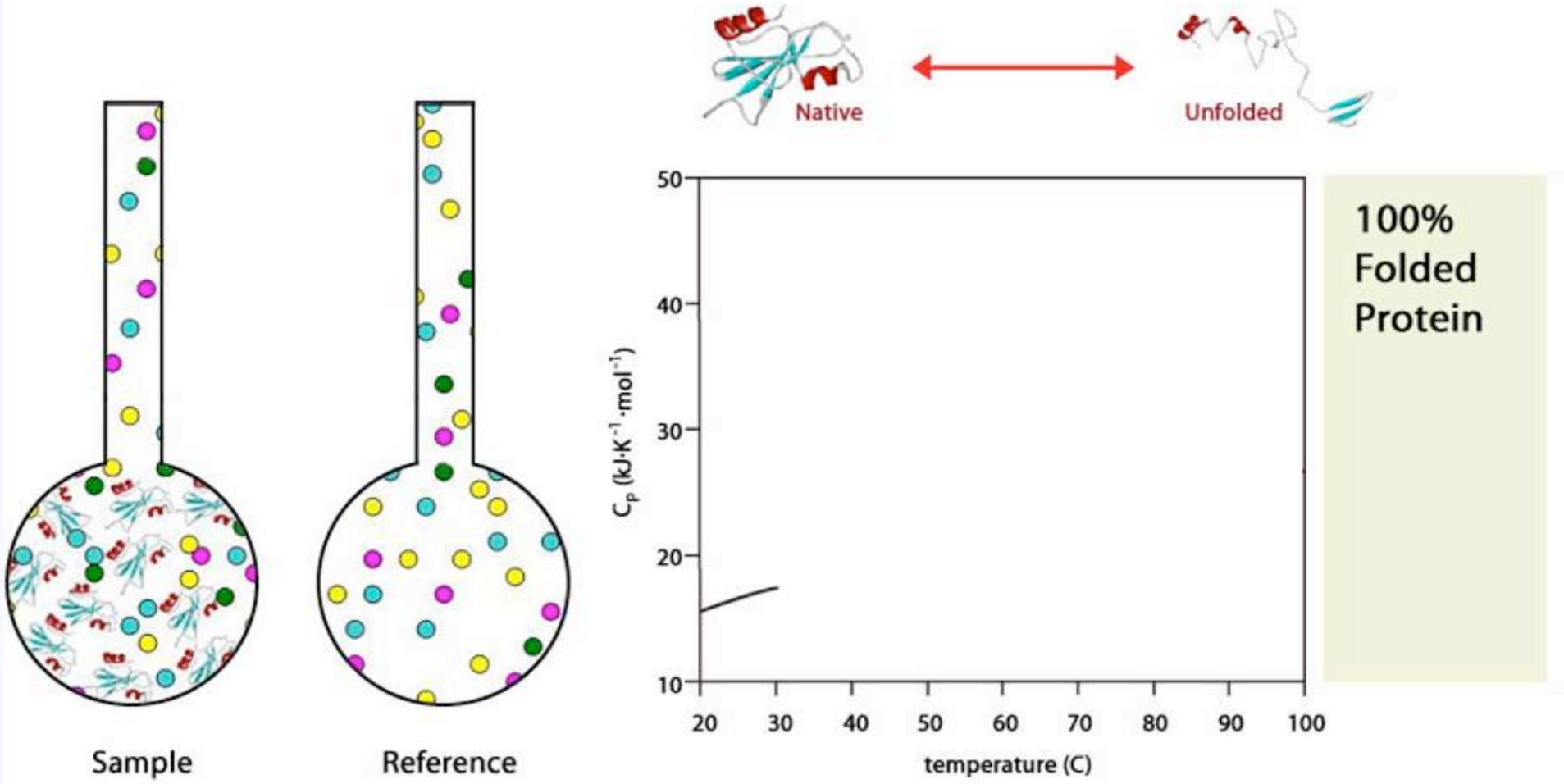
Subtract instrument baseline, normalize data to concentration





Subtract sample baseline, Fit to model

PRINCIPLES OF ANALYSIS



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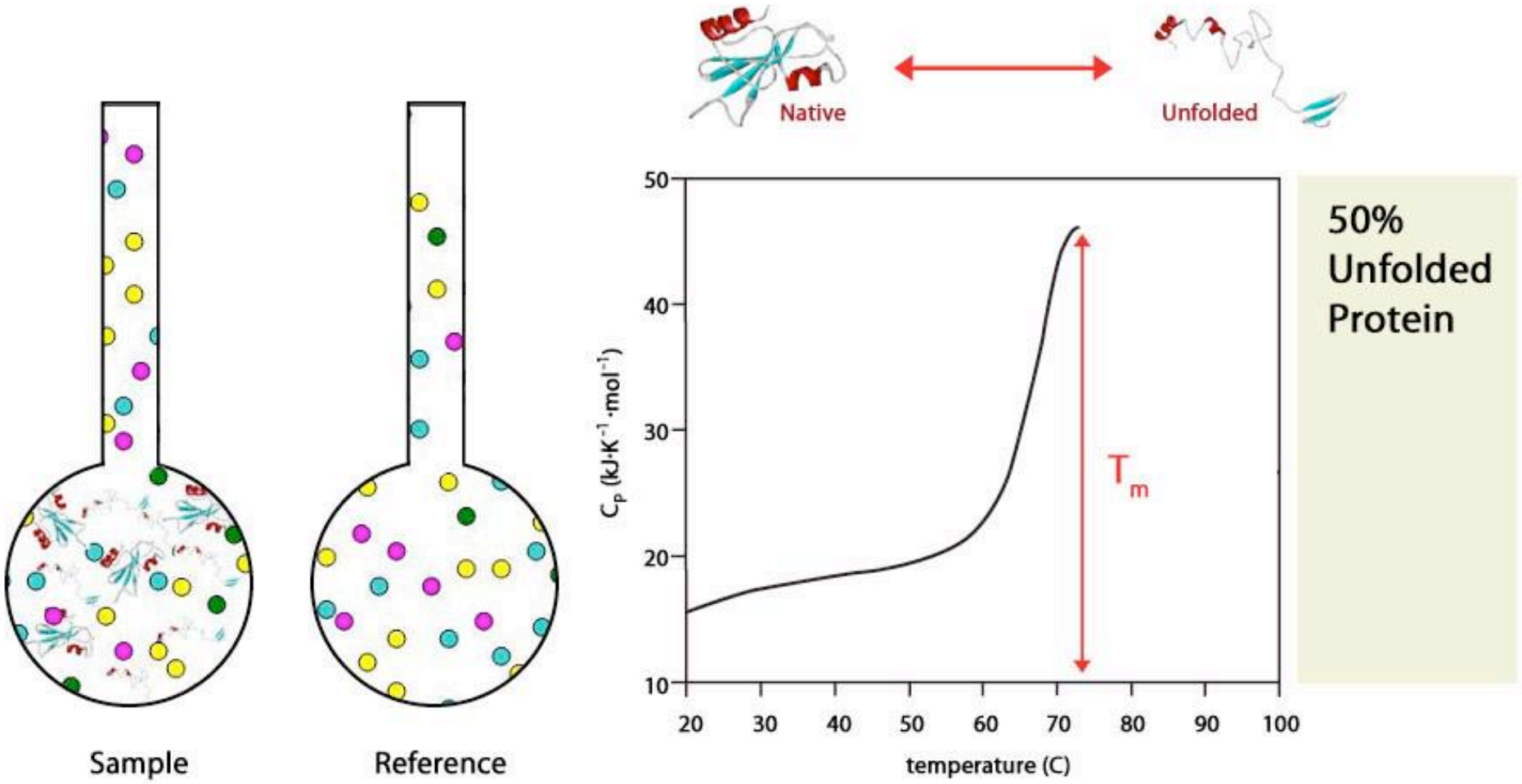

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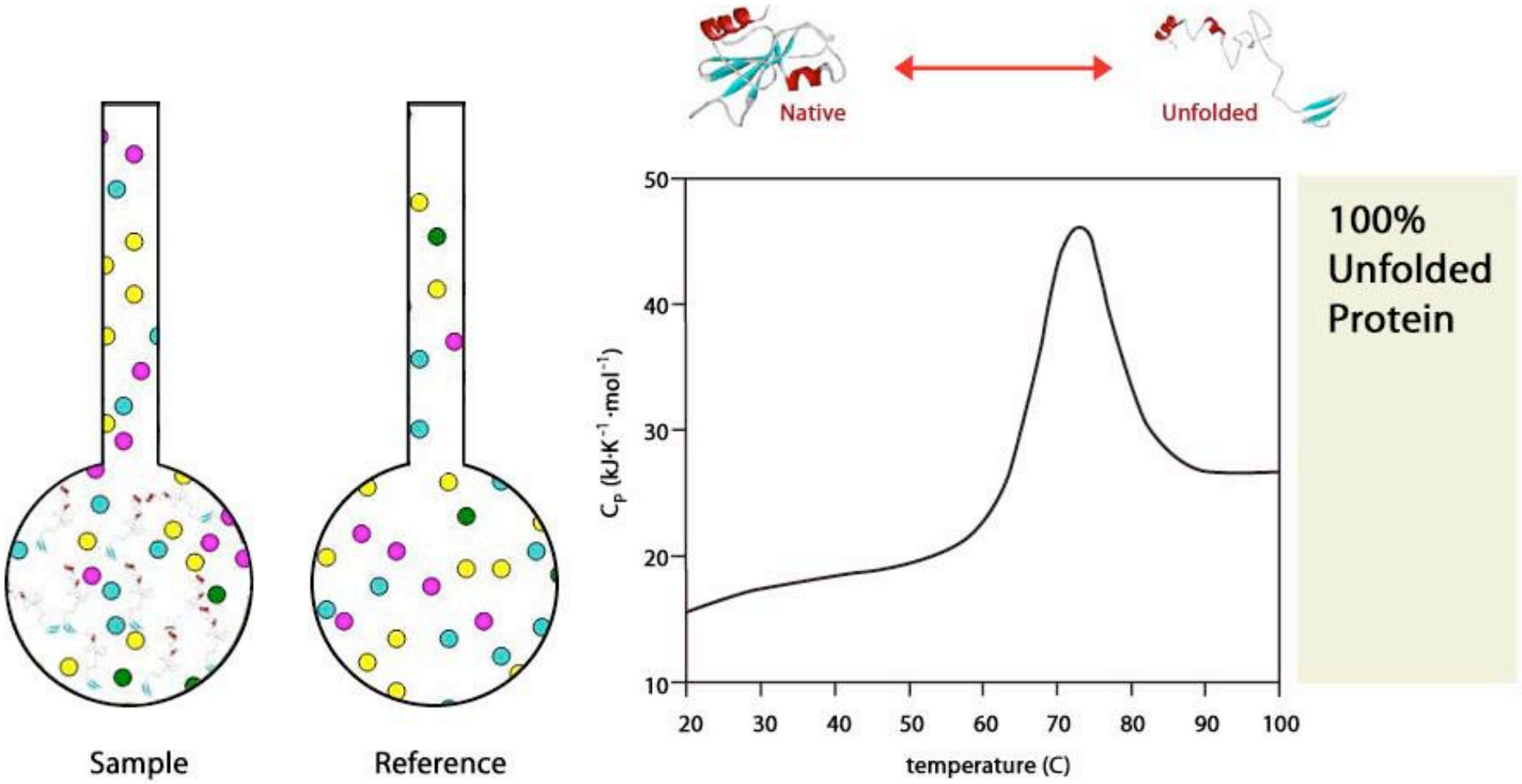
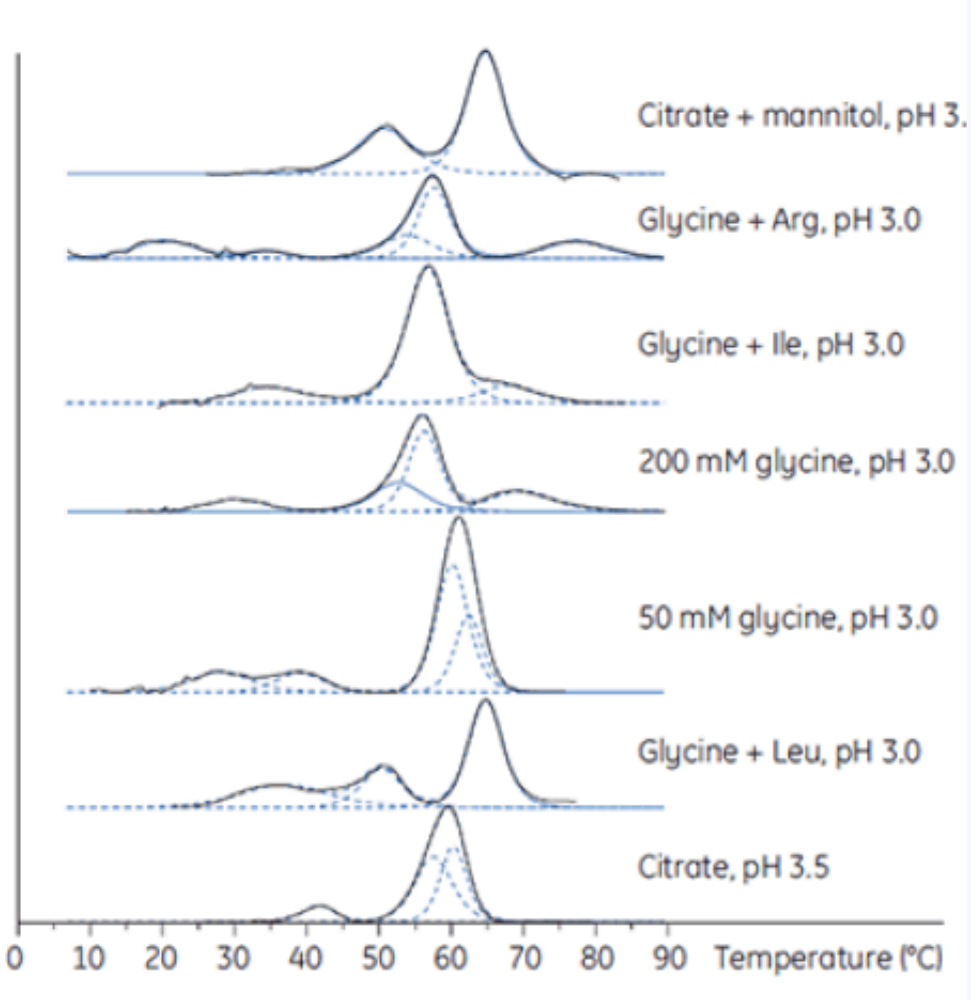


APPLICATIONS OF DSC

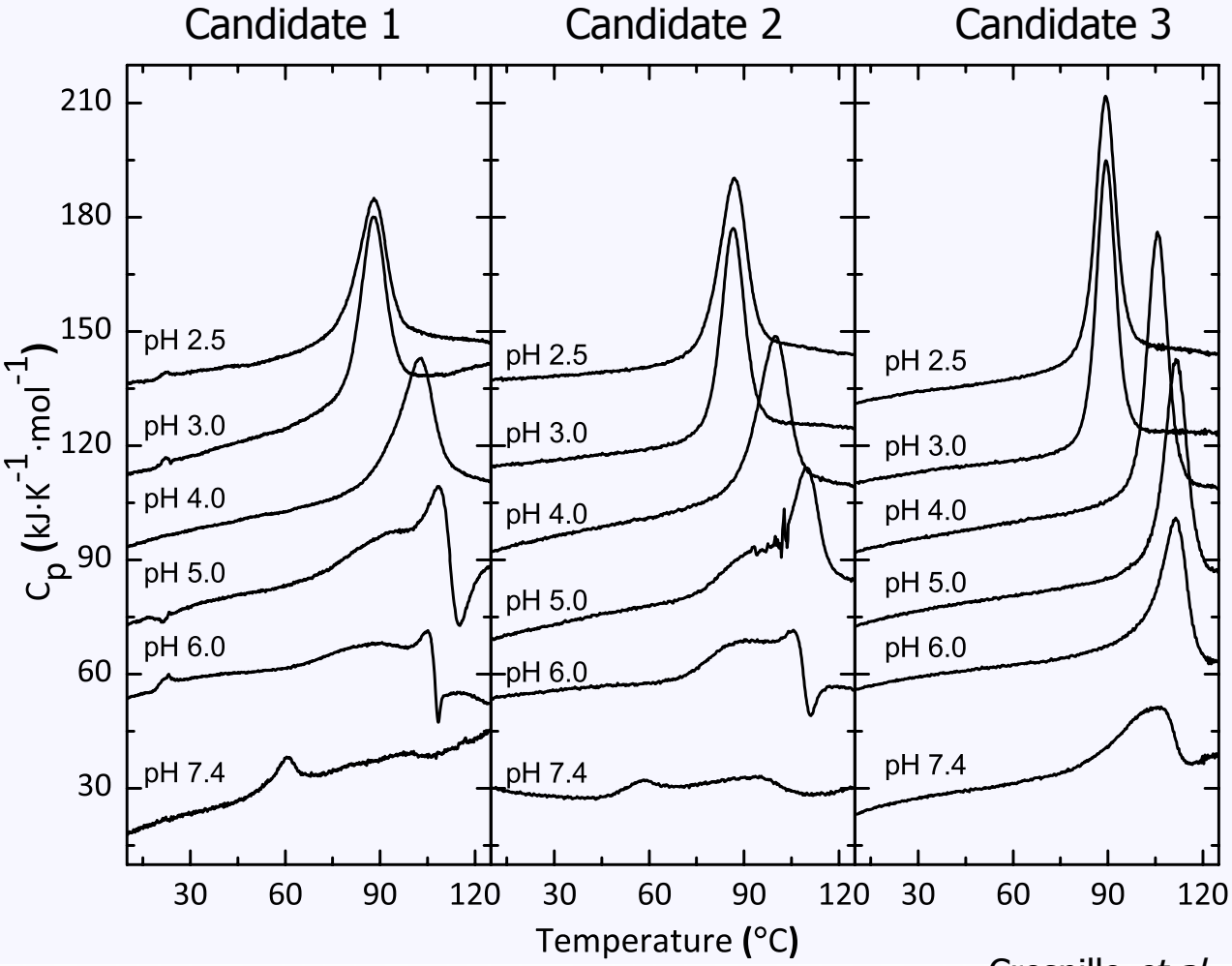
Optimization of purification conditions



Higher yield in protein purification

APPLICATIONS OF DSC

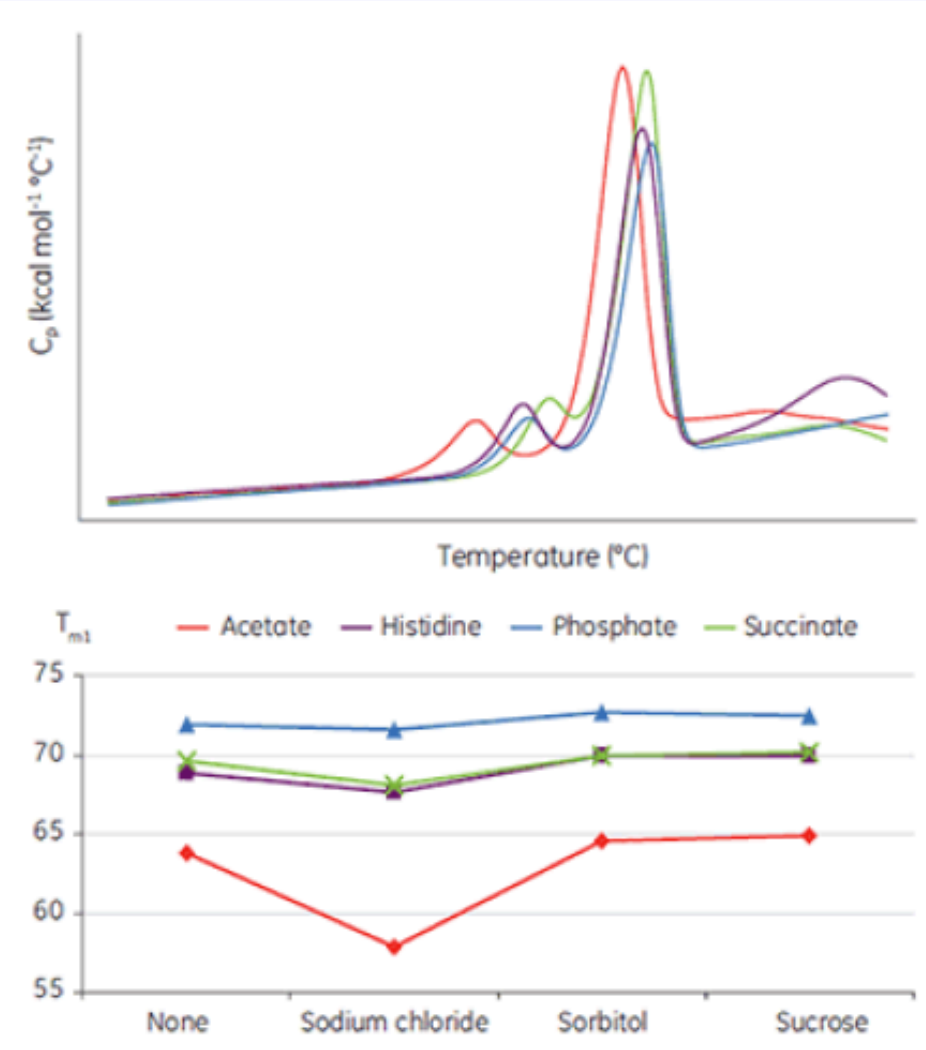
Solubility and stability study of antigens for the elaboration of a potential vaccine candidate



Crespillo *et al.*, Submitted manuscript

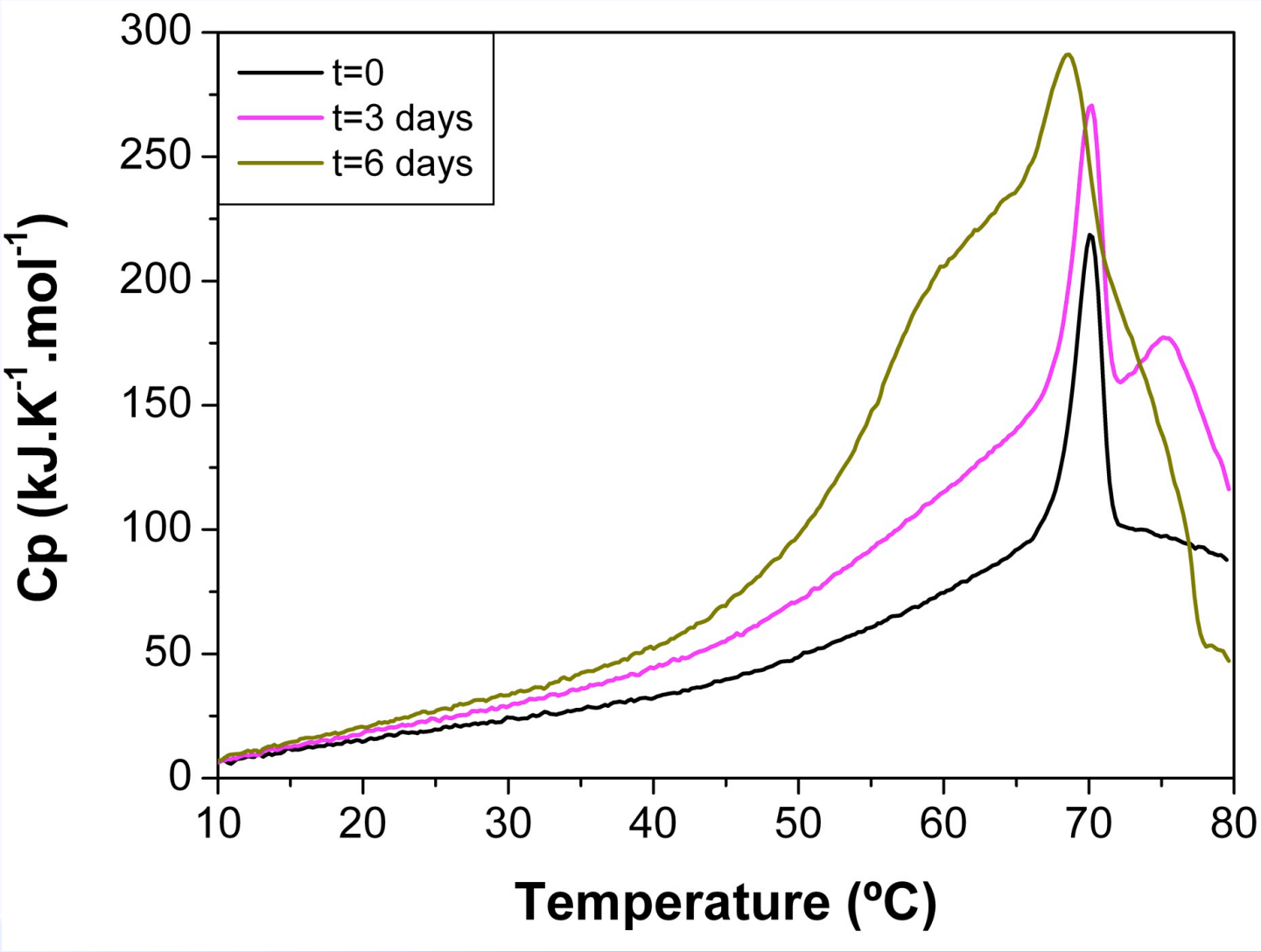
APPLICATIONS OF DSC

Stability screening for drug formulations development



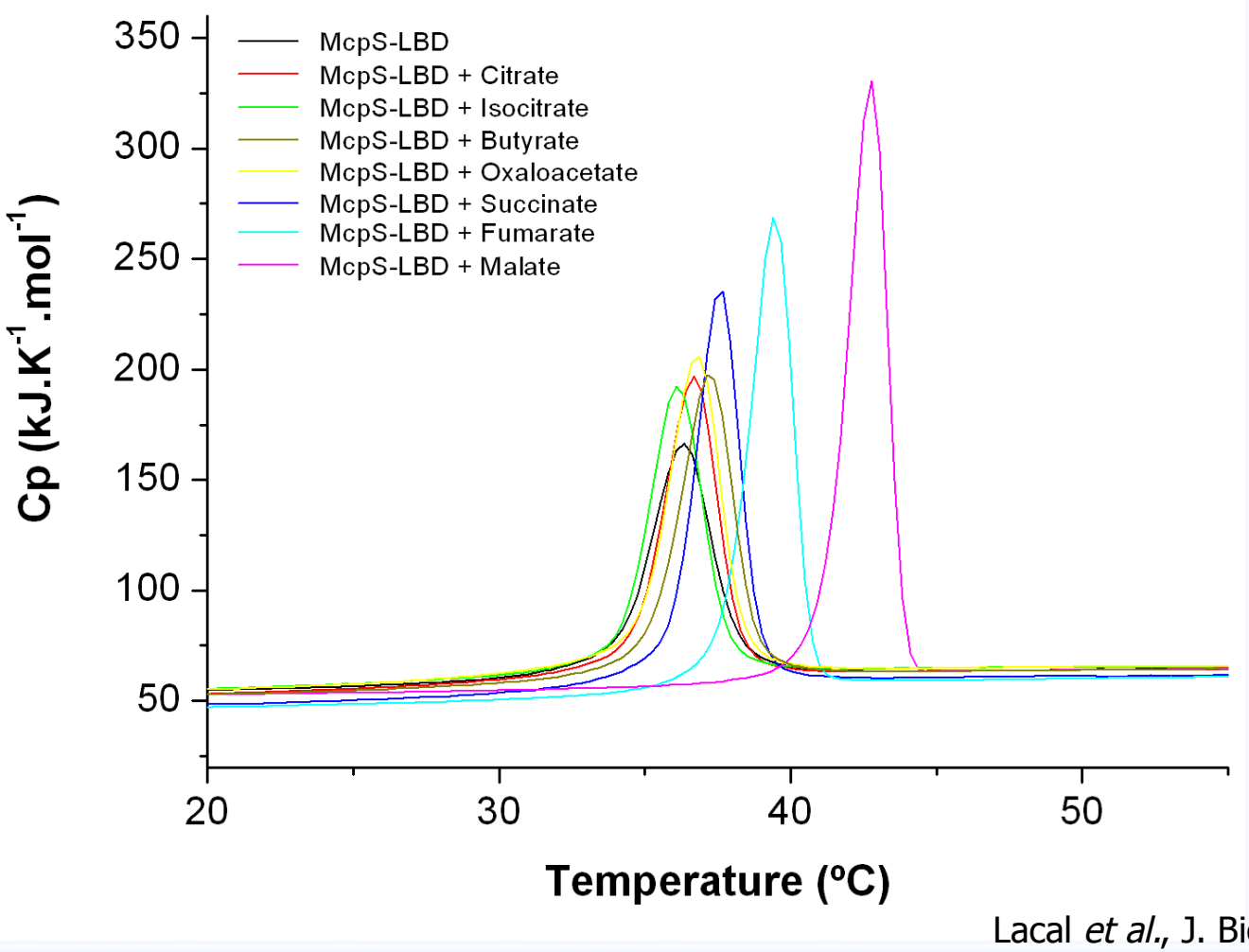
APPLICATIONS OF DSC

Protein could loose activity upon prolonged storage



Rank order binding study - High throughput technique

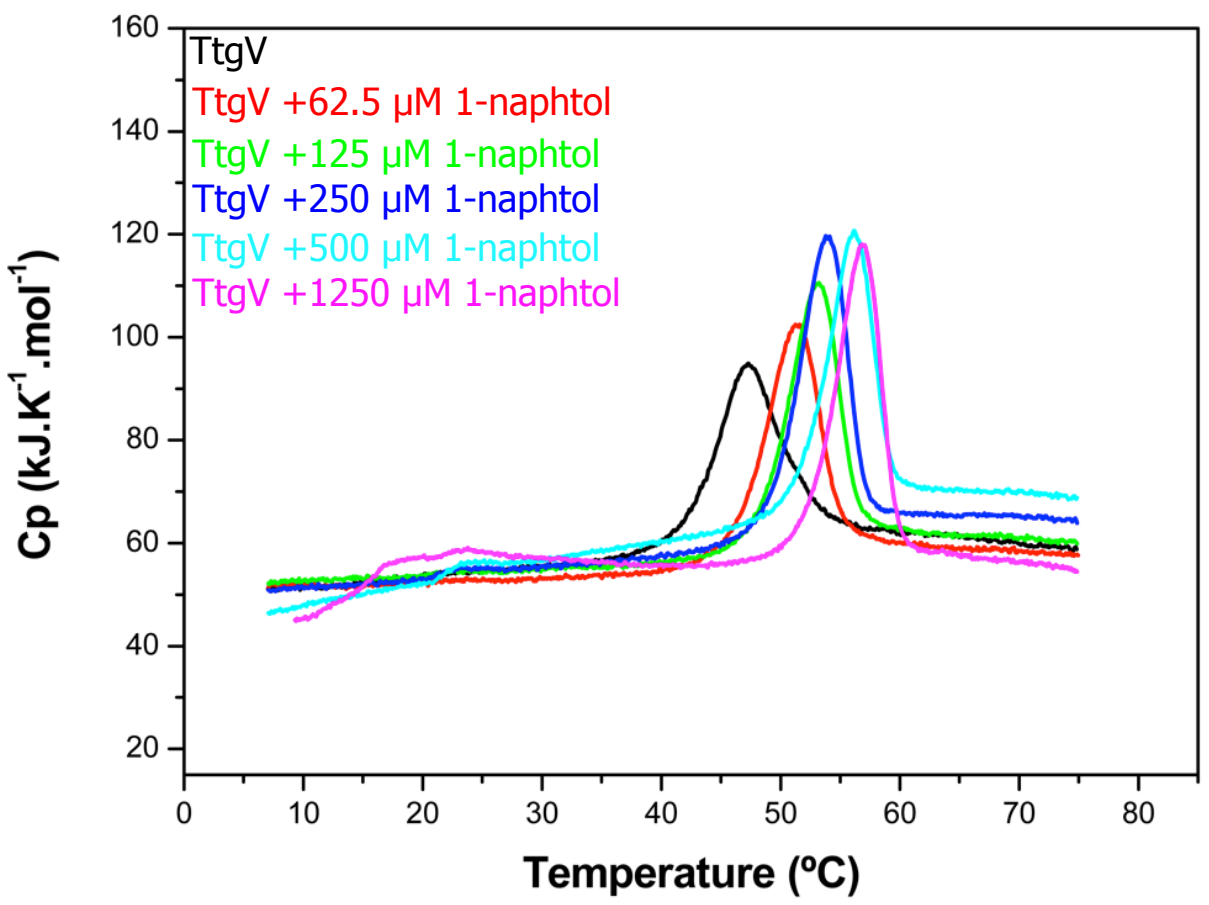
Identification of a Chemoreceptor for Tricarboxylic Acid Cycle Intermediates



Lacal *et al.*, J. Biol. Chem., 2010

APPLICATIONS OF DSC

Protein interactions

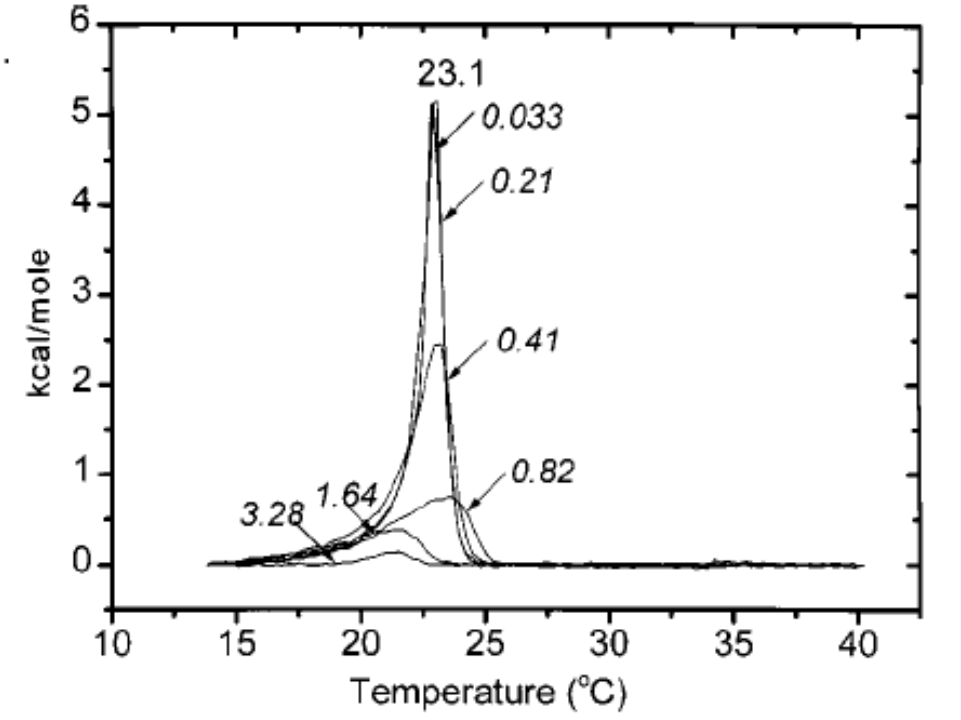


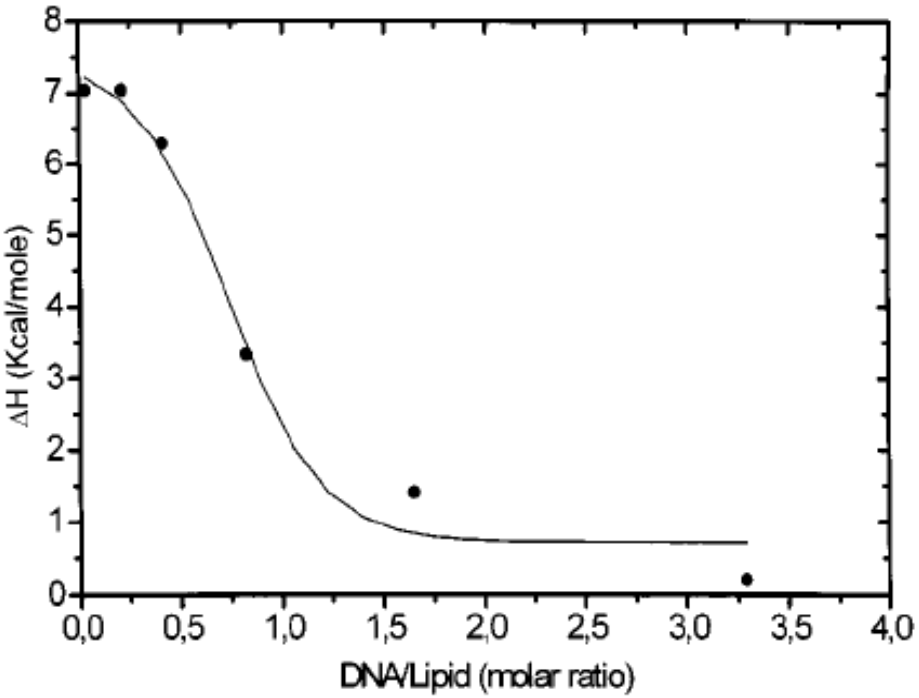
Fillet *et al.*, Proc Natl Acad Sci U S A, 2011

Using specific fitting procedure, a value of Kd could be approximately determined

APPLICATIONS OF DSC

Differential scanning calorimetry (DSC) of the DNA/lipid complex at different molar ratios.

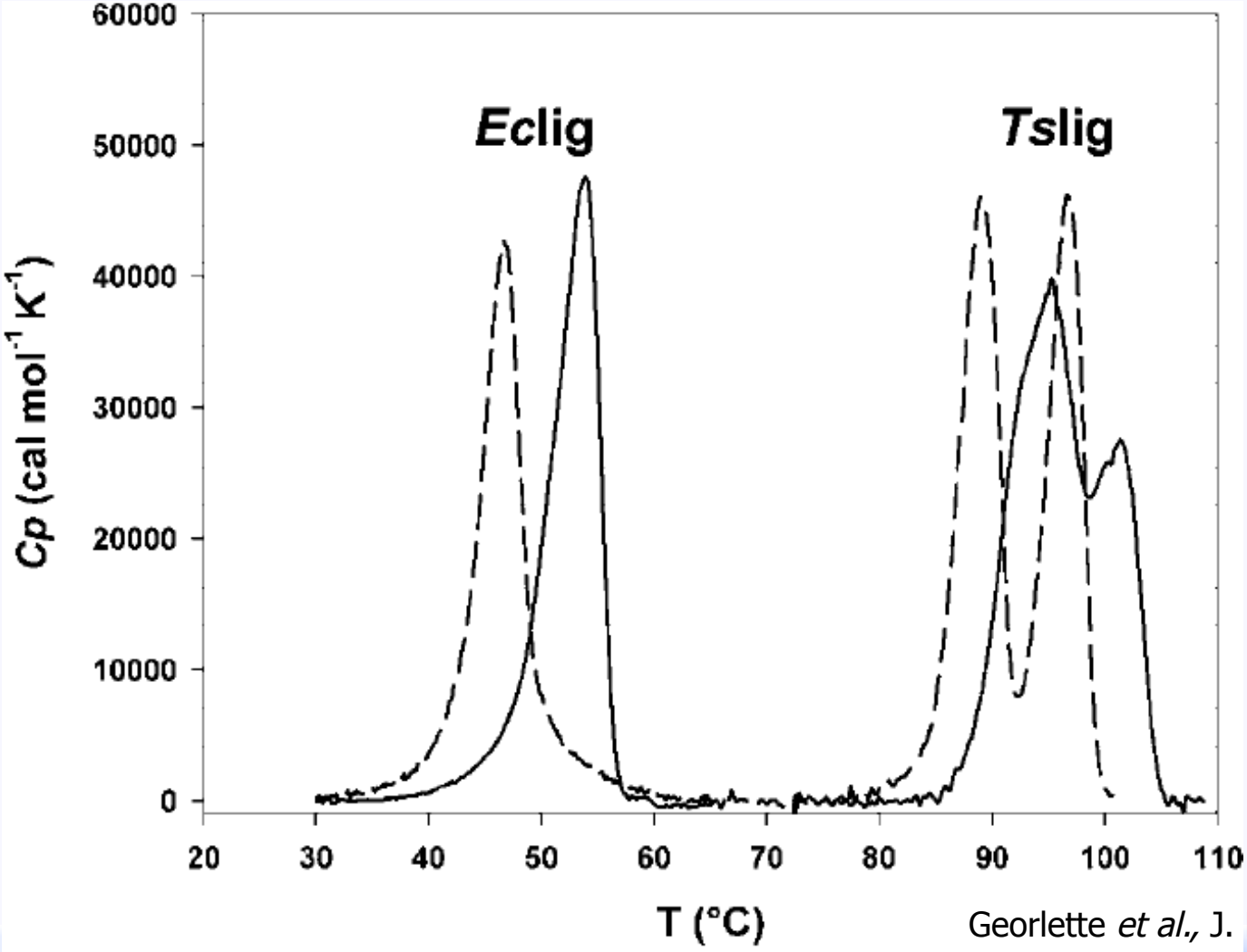




Pector *et al.,* J. Biol. Chem., 2000

APPLICATIONS OF DSC

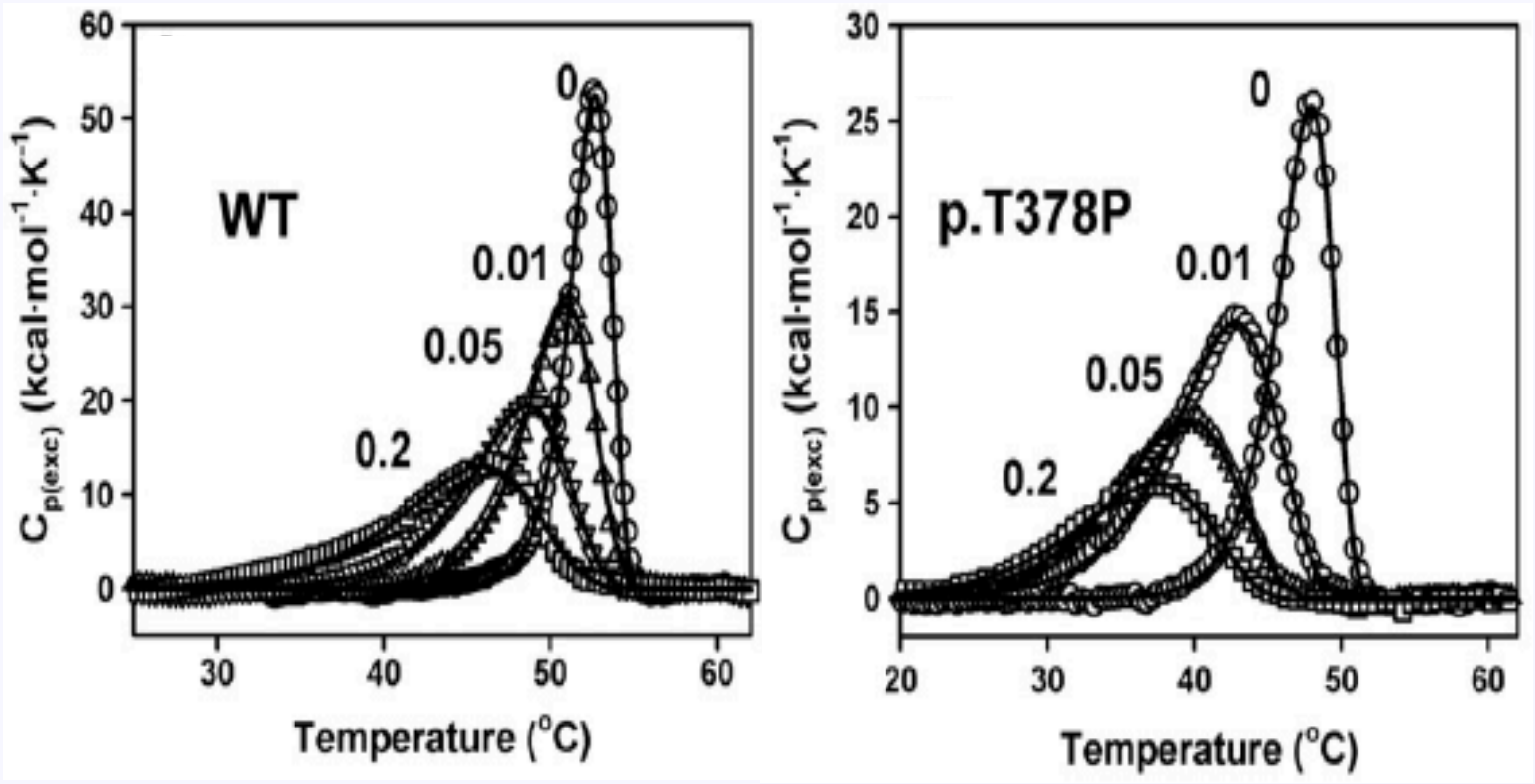
Adenylation-induced structural changes in NAD^+-DNA ligases



Georlette *et al.,* J. Biol. Chem., 2003

APPLICATIONS OF DSC

Differential Scanning Proteolysis

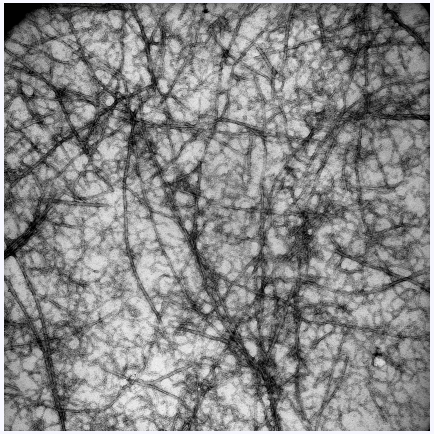


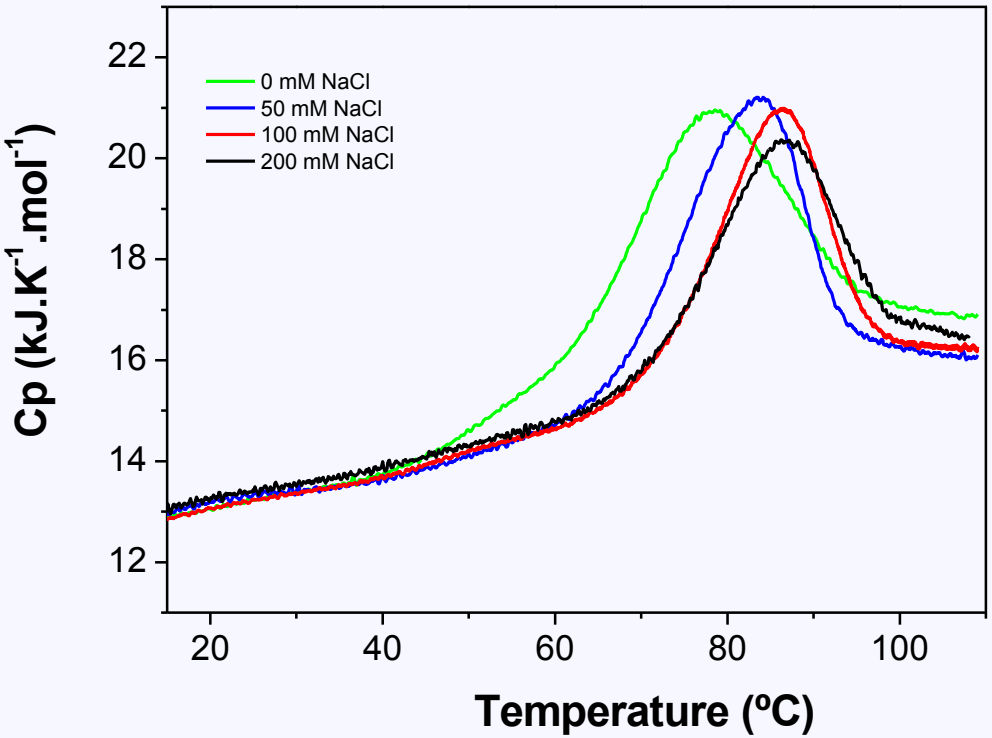
Pey Biochim. et Biophys. acta, 2013

APPLICATIONS OF DSC

Thermodynamic parameters of aggregates dissolution

Amyloid fibrils are involved in neurodegenerative diseases





Morel *et al.*, J. Phys Chem B, 2010

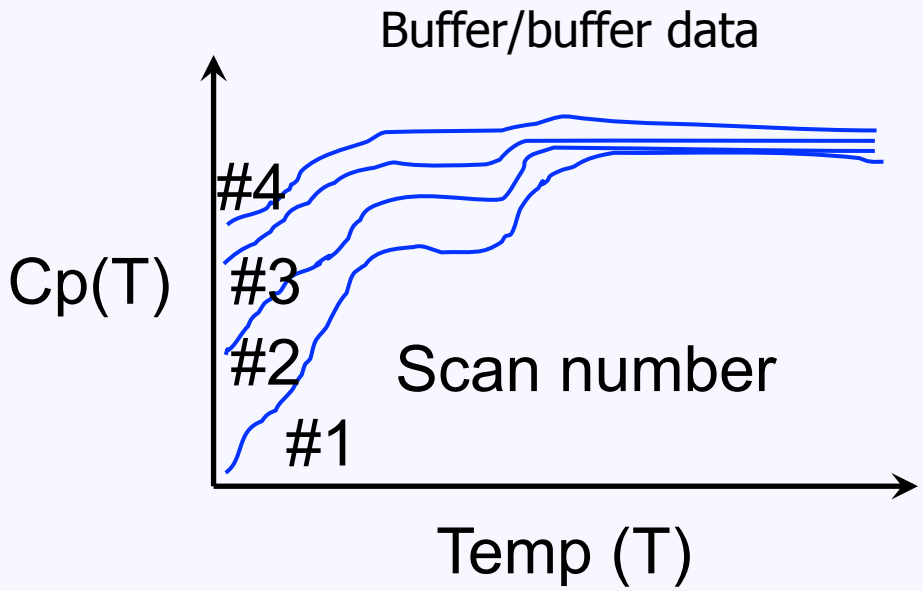
TROUBLESHOOTING

Why do DSC experiments not work as expected?

- Incorrect sample preparation
- Protein already denatured prior to DSC
- Incorrect concentrations used
- Buffer mismatch between reference and sample cells
- Incorrect filling technique
- “Thermal history” not established

PROBLEMS IN DSC DATA COLLECTION

- Air bubbles displace liquid and therefore reduce the heat capacity (yielding erroneous results).

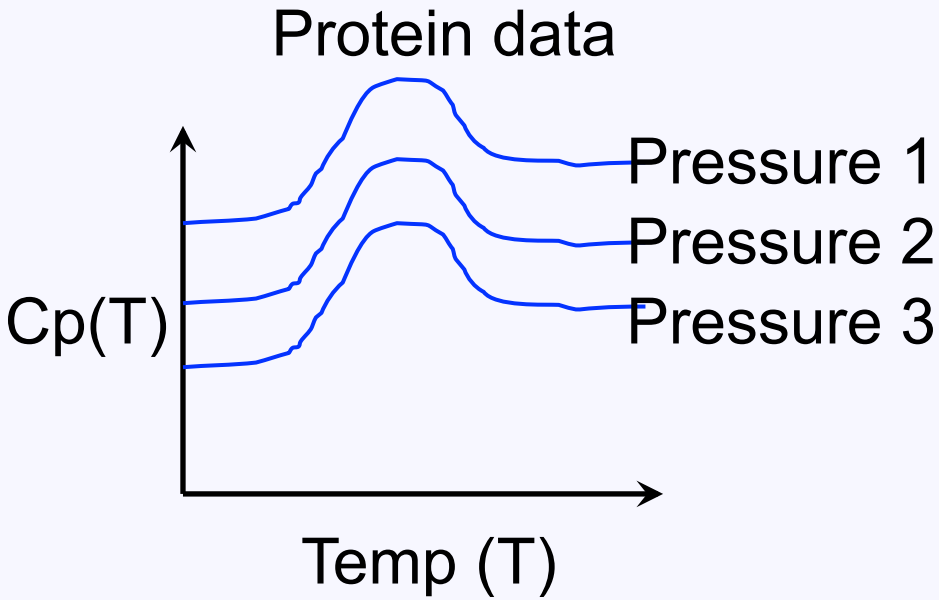


To address this issue:

- Samples & buffer are degassed (10 min)
- DSC cell is kept under pressure (~35 psi)
- A certain technique is used in filling the cells

PROBLEMS IN DSC DATA COLLECTION

Pressure changes affect the apparent heat capacity



This is not a critical issue to derive thermodynamic parameters

CONCLUSIONS

- Ideal for stability and folding studies
- Identify conditions that guarantee long term stability
- Ideal to identify ligands of unknown proteins
- Monitor reversibility of thermal processes
- Study molecules in their native state without labeling
- Can be use with solutions that interfere with optical methods including turbid or colored solutions or particulate suspensions
- Monitor conformational energetics of proteins and biopolymers