TA Instruments
MICROCALORIMETRY

New Castle, DE USA

Lindon, UT USA

Elstree, United Kingdom

Shanghai, China

Beijing, China

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Tokyo, Japan

Seoul, South Korea

Bangalore, India

Paris, France

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Etten-Leur, Netherlands

Sollentuna, Sweden

Milano, Italy

Barcelona, Spain

Melbourne, Australia

Mexico City, Mexico

DIFFERENTIAL SCANNING **CALORIMETRY**

The Nano DSC is specifically designed to determine the thermal stability and heat capacity of proteins and other macro-molecules in dilute solution, with the versatility and precision to perform molecular stability screening, ligand binding and pressure perturbation measurements.

The TA Instruments Nano Series calorimeters represent the highest sensitivity and unmatched performance for the investigation of biological samples.

DSC **SPECIFICATIONS**

Nano DSC Specifications

Short-term Noise	0.015 µWatts
Baseline Stability	±0.028 µWatts
Response Time	7 seconds
Operating Temperature	-10 °C to 130 °C or 160 °C
Temperature Scan Rate	0.05 °C to 2°C/minute
Pressurization Perturbation	Built-in up to 6 atmospheres
Cell Volume	0.30 ml
Cell Geometry	Fixed capillary
Cell Composition	Platinum
Heat Measurement Type	Power Compensation

Automation Specifications

Sample capacity	2 standard plates x 96 wells x 1000 µL / well
Sample tray temperature control range	4 °C to Ambient
Available Wash / Rinse Buffer Ports	4 for Sample/Reference Cells; 2 for Sample Handling Syringe

NANO DSC **TECHNOLOGY**

The Nano DSC differential scanning calorimeter is designed to measure the amount of heat absorbed or released by dilute in-solution bio-molecules as they are heated or cooled. Macromolecules such as proteins respond to heating or cooling by unfolding at a characteristic temperature. The more intrinsically stable the biopolymer, the higher the midpoint temperature of the unfolding transition. As these processes often exchange microjoule levels of heat, the sensitivity of the Nano DSC is critical for successful investigation of the reaction.

The Nano DSC obtains data with less sample than competitive designs and produces unmatched short term noise (±15 nanowatts) and baseline reproducibility (±28 nanowatts). Solid-state thermoelectric elements are used to precisely control temperature and a built-in precision linear actuator maintains constant or controlled variable pressure in the cell. Increased sample throughput is realized by adding on the Nano DSC Autosampler. It provides true walk-away capability for up to 96 samples. With convenient USB connectivity, built-in pressure perturbation capability and capillary cell design, the Nano DSC provides maximum flexibility with a cell design that minimizes sample aggregation and precipitation, resulting in high quality data.

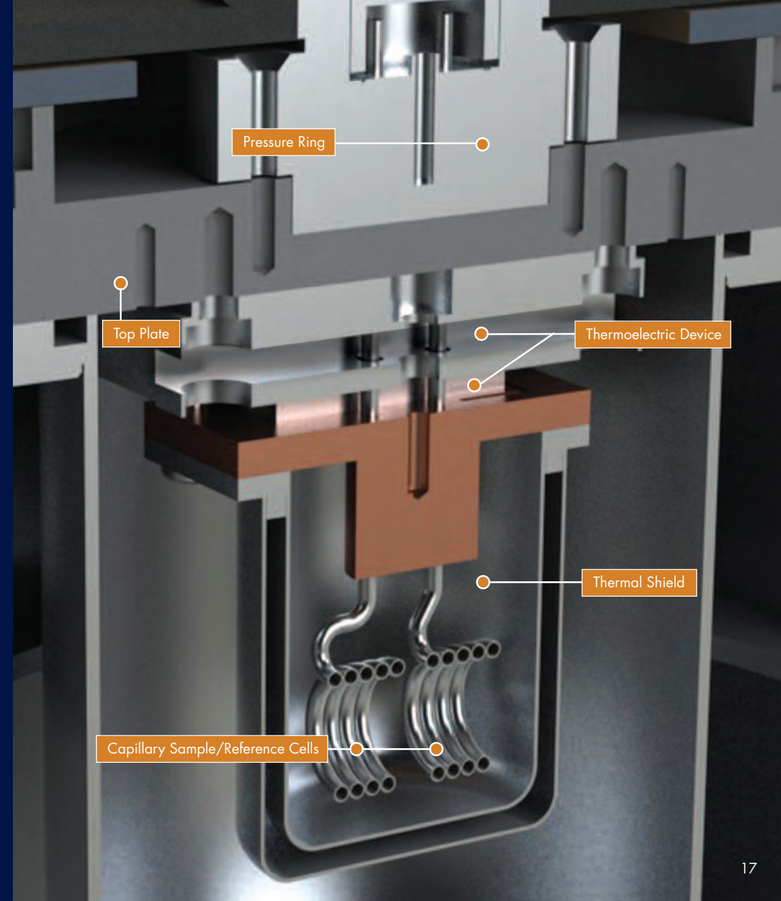
Nano DSC Capillary Cell

The capillary design of the Nano DSC provides unparalleled sensitivity, accuracy and precision. Many structurally unstable samples that show aggregation and precipitation during a scan on competitive designs can be routinely analyzed on the Nano DSC.

The Nano DSC employs solid-state thermo-electric elements to accurately and precisely control the temperature of the sample. This powerful temperature control and heat sensing architecture enables active control of both heating and cooling scans.

The unique and innovative built-in high-pressure piston and pressure ring provides the highest flexibility with user-selectable functions for standard constant pressure experiments and pressure perturbation calorimetry (PPC) experiments with no extra hardware or software accessories required.

The Nano DSC's combination of a robust capillary cell design and state-of-the-art temperature control and sensor technology provides a reliable, flexible and easy-to-use calorimeter for in-solution biological samples.

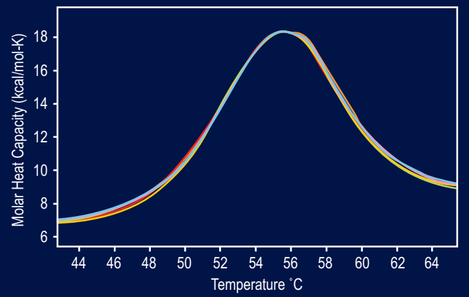


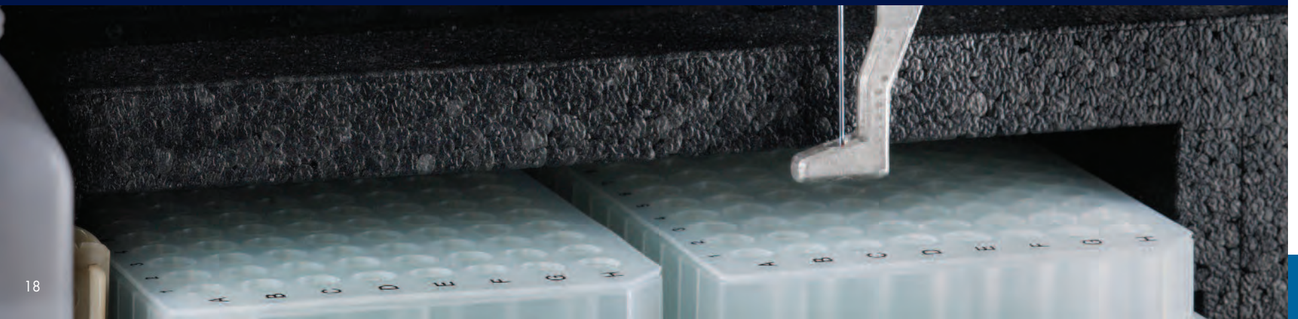
NANO DSC **AUTOMATION**

The Nano DSC Autosampler system enables true "start and walk away" capability without sacrificing either sensitivity or reliability. The autosampler stores samples and the matching buffers/solvents in a 96-well plate format at temperatures ranging from 4°C to ambient room temperature. Four (4) wash/rinse solvents are accessible through programmable ports on the autosampler interface. Two (2) exit ports enable the collection of sample and matching buffer/solvent solutions from both the sample or reference cell of the Nano DSC.

For molecular stability testing applications that require high sample throughput, the Nano DSC Autosampler system is a reliable sample handling system that increases the productivity of the most sensitive DSC on the market with true walk-away capability and proven reliability.

The figure shows overlapping plots of DSC scans of duplicate samples of five different Lysozyme sample concentrations when converted to Molar Heat Capacity. The Nano DSC Autosampler system produces superior data reproducibility and precision at low sample concentrations with no detectable sample-to-sample carry-over or sample degradation.

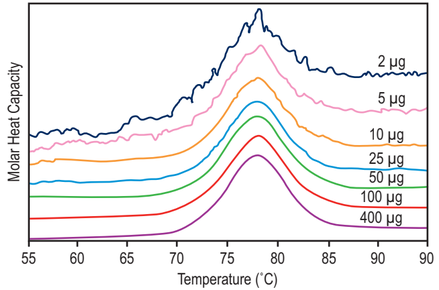




Nano DSC **Applications**

How much Protein is Required for a DSC Scan?

Determining the thermodynamic parameters of a protein by differential scanning calorimetry (DSC) using the Nano DSC requires about the same amount of protein as surface plasmon resonance or fluorescence studies. Because of the Nano DSC's extreme sensitivity and baseline reproducibility, and the sample cell's small volume (300 µL), a complete, interpretable, accurate scan can be obtained on essentially any protein of interest. The sensitivity and accuracy of the Nano DSC is demonstrated by this data. Hen egg white lysozyme (in pH 4.0 glycine buffer) was prepared at various concentrations. As little as 2 µg of lysozyme in the capillary cell is sufficient to provide quality data yielding accurate values of all four thermodynamic parameters!



Lysozyme in cell (µg)	Calorimetric	van't Hoff		
	ΔH (kJ mol^{-1})	ΔS (kJ K^{-1} mol^{-1})	T_m (°C)	ΔH (kJ mol^{-1})
400	512	1.46	78.0	515
100	512	1.46	78.0	509
50	517	1.47	77.9	513
25	513	1.46	77.8	513
10	515	1.47	78.0	515
5	490	1.40	78.0	510
2	503	1.43	77.8	499

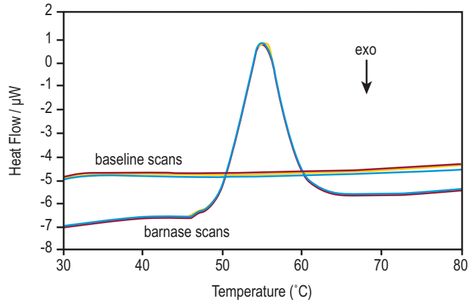
NANO DSC **APPLICATIONS**

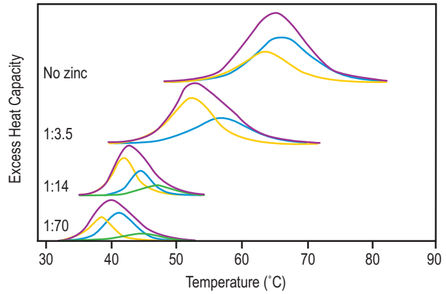
Characterization of Protein Stability

Analyzing the stability of a protein in dilute solution involves determining changes in the partial molar heat capacity of the protein at constant pressure (ΔCp). The contribution of the protein to the calorimetrically measured heat capacity (its partial Cp) is determined by subtracting a scan of a buffer blank from the sample data prior to analysis. Heating the protein sample initially produces a slightly increasing baseline but as heating progresses, heat is absorbed by the protein and causes it to thermally unfold over a temperature range characteristic for that protein, giving rise to an endothermic peak. Once unfolding is complete, heat absorption decreases and a new baseline is established. After blank subtraction, the data can be analyzed to provide a complete thermodynamic characterization of the unfolding process.

Characterization of Protein Structure

DSC can be used to characterize both the specific binding of a ligand (for example, a drug to a receptor binding site), or nonspecific binding (for example, detergents binding to hydrophobic patches on a protein surface). In some instances ligand binding, even if to a specific receptor site, results in long-range protein structural rearrangements that destabilize the entire complex. The figure shows DSC scans of Ca^{2+} saturated bovine a-lactalbumin at various protein:Zn^{2+} ratios scanned at 1 °C/min. The midpoint of the thermal unfolding of the protein decreases from 65 °C in the absence of Zn^{2+} to 35 °C at a protein:Zn^{2+} ratio of 1:70. The enthalpy of unfolding is also decreased substantially by high Zn^{2+} concentrations.



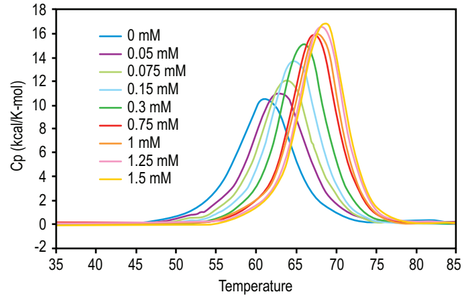


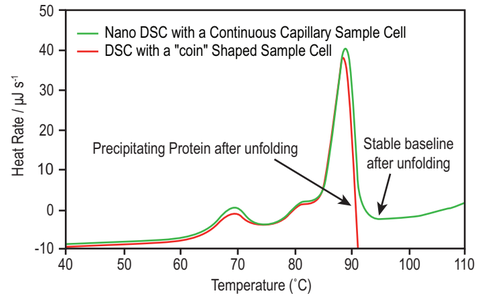
Investigation of Protein-Ligand Binding

DSC is a valuable tool for studying binding between a biological macromolecule and a ligand such as another biopolymer or a drug. Unlike ITC, DSC allows the thermo-dynamics that drive binding to be correlated with conformational changes in the macromolecule caused by the binding reaction. DSC is particularly useful for characterizing very tight or slow binding interactions. DSC also allows characterization of binding reactions that are incompatible with the organic solvent requirements of some ITC experiments (i.e., where ligand solubility for an ITC experiment requires concentrations of organic solvent not tolerated by the protein). The data shows DSC scans of RNase A bound with increasing concentrations of 2′-CMP, showing that the protein is stabilized by higher concentrations of the inhibitor. Essentially identical data were obtained in the presence of 5% DMSO, verifying that organic solvents are compatible with the DSC technique.

Nano DSC Capillary Cell Advantages

This figure shows two DSC scans of matched samples of human IgG_1 at 0.5 mg/ml in physiological buffer. The data from the DSC with a "coin" shaped sample cell shows the easily recognizable exothermic aggregation/precipitation event at approx 89-90 °C, while the data collected on the Nano DSC with a capillary sample cell shows a stable post-transition baseline that will enable complete and accurate determinations of transition temperatures (Tm) and enthalpy (ΔH).





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