Introduction

ITC is a tool used for the study of biomolecular interactions, with applications in many areas of life sciences, such as characterization of protein-protein and protein-ligand binding, DNA binding, drug hit validation and enzymatic activity.

An ITC experiment involves injecting small aliquots of a solution containing one binding partner (a ligand) into a sample cell containing the other binding partner (often a macromolecule such as a protein). When the two molecules interact, heat may be released or absorbed, depending on the nature of the interaction. The ITC quantifies this interaction based on the change in cell temperature resulting from the endothermic / exothermic reaction.

Features of Isothermal Titration Calorimetry:

Label free.
By directly determining the heats of the interaction, extra labels are not necessary. Molecules can be kept in their native state in a solution in order to give a more accurate description of the interaction.

Broad dynamic range.
The measurement of molecules in solution allows for the preservation of biological conditions and the instrument's high sensitivity allows for the detection of interactions with a wide range of affinities.

Information-rich data.
One ITC experiment gives all the relevant thermodynamic properties for the interaction including the binding affinity (K_D), reaction stoichiometry (n) and changes in enthalpy (ΔH), entropy (ΔS) and Gibb's energy (ΔG).

MicroCal PEAQ-ITC Automated

The MicroCal automated PEAQ-ITC allows for full automation and unattended operation. It stores samples in four 96-well plates within a temperature controlled environment and has built-in programs, to improve assay reliability, and user friendly software to streamline the workflow and data analysis.

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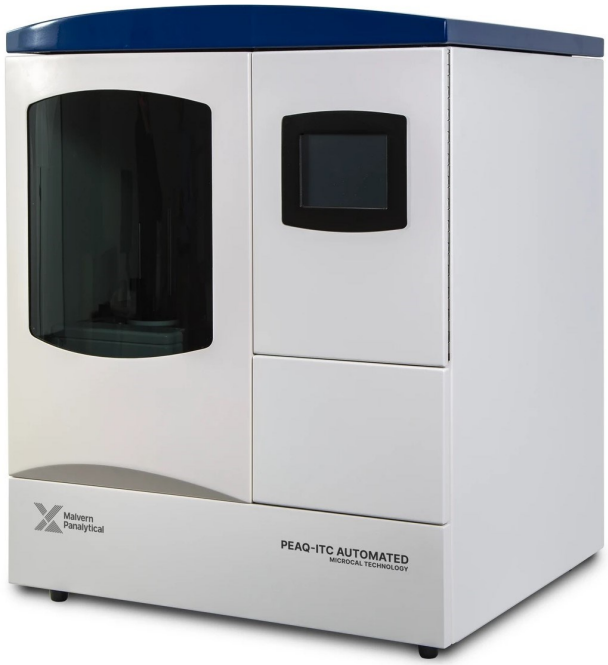


ITC—Malvern Panalytical MicroCal

MicroCal PEAQ-ITC Automated Isothermal Titration Calorimetry

- Study biomolecular interactions
- Determine thermodynamic parameters (K_d, ΔH, n, ΔG, ΔS)
- Characterize enzymatic activity and reaction mechanisms
- Fully automated operation with the integrated autosampler

Isothermal Titration Calorimetry

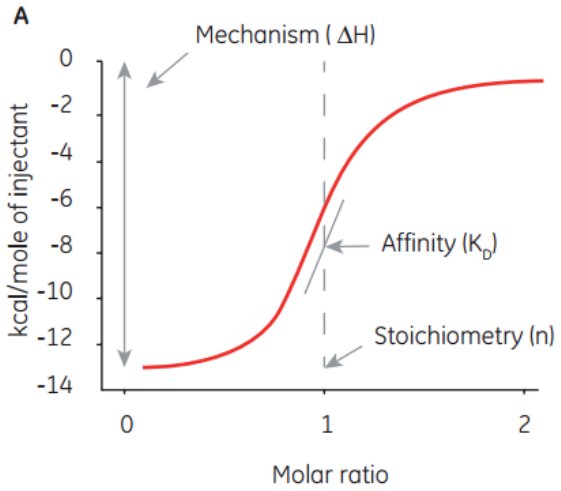
Experimental background

In an ITC experiment, a syringe injects a solution of one interacting partner (e.g. ligand) into a cell containing the other partner (e.g. protein). As the injection occurs, the two molecules interact and either release (exothermic) or absorb (endothermic) heat.

The heat changes are measured by the calorimeter as the amount of power needed to maintain an isothermal temperature between the sample cell and a reference cell. The raw data output is a unit of power (µcal/sec) versus time (min). An example of raw ITC data is shown on the top right.

As the experiment progresses, the binding site of the protein in the cell becomes saturated by the ligand and the peaks become smaller until the binding reaction is complete, leaving only the signal caused by the heat of dilution.

The peak areas are then integrated and plotted against the molar ratio of molecules in the syringe versus the cell in a Wiseman plot. An appropriate fitting model is applied to determine the dissociation constant (K_D), enthalpy change (ΔH) and reaction stoichiometry (n).



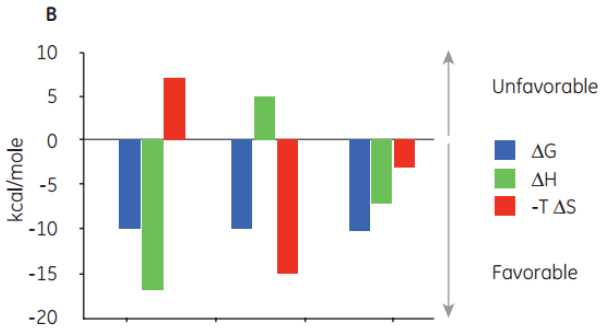
From one experiment, there is enough information to complete the thermodynamic profile of the interaction by calculating the changes in Gibb's energy (ΔG) and entropy (ΔS).

1. $K_a = \frac{1}{K_d}$

2. $\Delta G = -RT \ln K_a$

3. $\Delta G = \Delta H - T\Delta S$

Shown below are thermodynamic values for the interaction of three different pairs of molecules. Although they all appear to have similar binding affinities, they have different binding enthalpies and entropies, suggesting a different binding mechanisms for each.

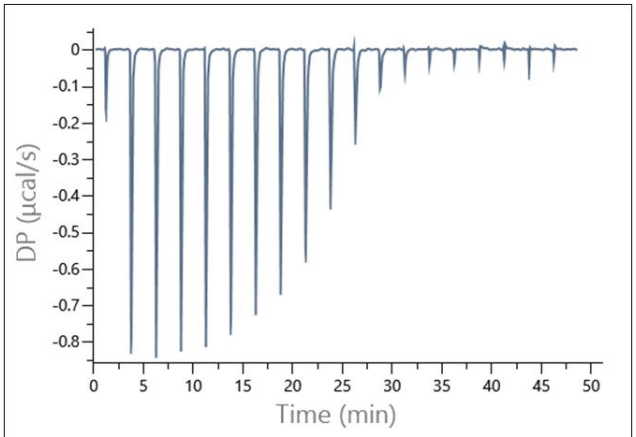


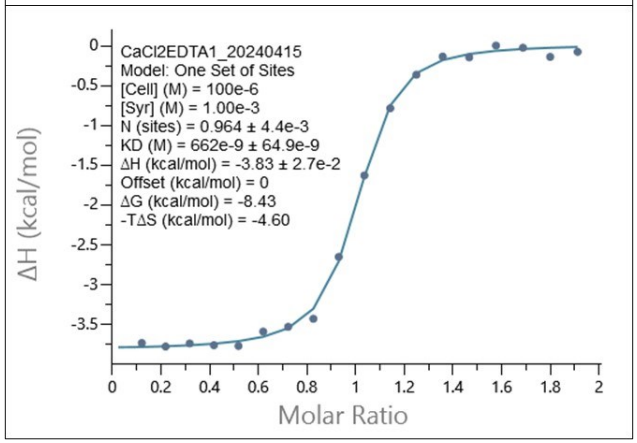
The enthalpy and entropy contribute to the strength of the interaction in different ways:

Enthalpy ΔH—Changes in hydrogen bond, van der Waals interactions.

Entropy ΔS—Conformational changes, desolvation (hydrophobic force).

This provides a deeper understanding of the molecular interaction and can help with determining the mechanism of a novel interaction or confirming a known interaction event.





Typical ITC experiment parameters	
Sample volume (cell)	200 µL (400 µL required)
Syringe sample volume	40 µL (120 µL required)
Injection volume	2 µL (adjustable)
Injection duration	4 sec.
Spacing	180 sec. between injections
Experimental temp.	25 °C (adjustable)

Optimization of an ITC experiment can include changing which molecule is in the syringe and cell, the protein and ligand concentrations, the number and volume of injections, the time between injections and the temperature of the experiment.