



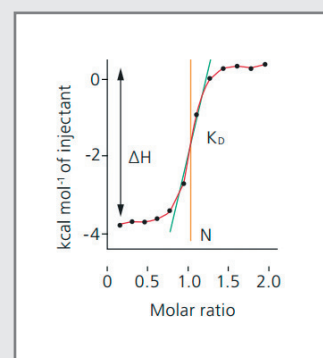
VP-ITC calorimeter

Isothermal Titration Calorimetry (ITC)

VP-ITC calorimeter is designed to measure the heat of binding. In a typical arrangement, the titrant, also referred as the ligand, is injected into the sample cell containing the macromolecule sample solution. The calorimetric measurement can be done over a range of biologically relevant conditions (temperature, salt, pH, etc.). No labelling is necessary and the complete thermodynamic profile of the interaction can be obtained in a single measurement. ITC system directly measure submillimolar to nanomolar binding constants ($10^3 - 10^9 \text{ M}^{-1}$). The interactions with nanomolar to picomolar binding constants ($10^9 - 10^{12} \text{ M}^{-1}$) can be measured using the competitive binding technique, the same principle can be used for low affinity interactions ($10^3 - 10^2 \text{ M}^{-1}$).

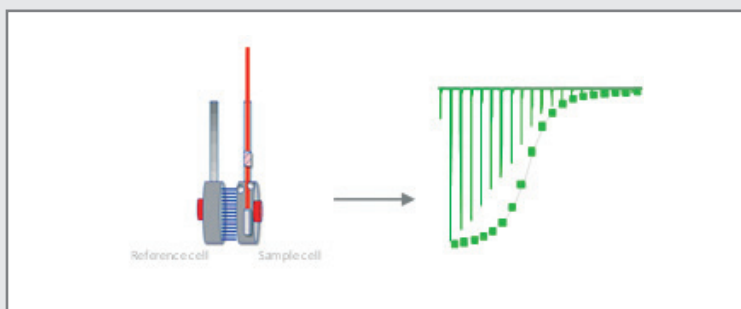
■ ITC method can be used for

- characterization of biomolecular interactions of small molecules, proteins, antibodies, nucleic acids, lipids and others
- enzyme kinetics studies, biological activity or the effect of molecular structure changes on binding mechanism determination
- determination of thermodynamic parameters K_A , ΔH and ΔS values, stoichiometry or kinetics parameters K_m and k_{cat}



■ Technical Specifications

Instruments: VP-ITC (Malvern)



Features:

- operating temperature range is of 2°C to 80°C
- calorimetric cell - volume 1400 ml, Hastelloy, coin-shaped

Assemblies:

- ThermoVac - device for thermostating and degassing samples (0-80°C)

Operational mode:

VP-ITC measurement is performed manually by the user itself after training

Data evaluation software: Origin software, NITPIC (possibility to train people in data processing)

Provided services:

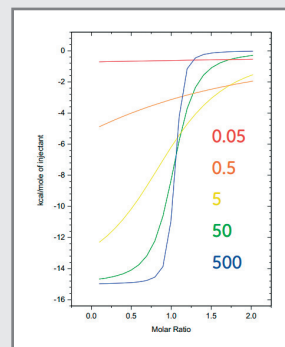
- instrument user training
- basic ITC data evaluation training
- consulting/assistance

Data collection:

- Standard titration method or Single injection method

■ Sample requirements - importance of sample preparation

- **Proper sample preparation is crucial** for the successful ITC measurement. The buffer solution, in which the macromolecule and the ligand are dissolved, **should be exactly the same** (dialysis or lyophilisation and dissolution in the buffer for ITC). The pH should be checked before the measurement.
- **The macromolecule sample** (the sample placed in the cell): 1800 μl
- **The ligand solution** (the sample placed in the injection syringe): 450 μl
- Sample concentrations must be determined precisely.
- Generally a concentration of ligand should be 10 times higher than the concentration of macromolecule otherwise the concentration should be optimized.
- High affinity interactions can be studied at low concentrations. In this case the minimum concentration of macromolecule sample which causes measurable heat is 10 μM . For low affinity interactions the macromolecule sample concentration should be 5 times of K_D or higher, but higher concentration may be limited by availability or solubility of samples.
- **Calculating the cell sample concentration** - $M = c / (n \times K_A) = c / n \times K_D$
M ... molar concentration of the cell sample; c-value ... should lie between 10-500;
n ... binding stoichiometry; K_A ... association constant; K_D ... dissociation constant
- At least 10 ml of the used buffer must be sent for each measurement (for VP-ITC).
- If it is possible, choose a pH buffer with low heat ionization in order to minimize artifactual heats of buffer ionization.
- If the presence of reducing agent is required for a protein stability, then β -mercaptoethanol (less than 5 mM) or TCEP (less than 2 mM) should be used rather than DTT.



The shape of the binding isotherms depend on a unitless constant c

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance

■ Contacts

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