Isothermal Titration Calorimetry technique

Tatsiana Charnavets

tatsiana.charnavets@ibt.cas.cz

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Outline

Isothermal Titration Calorimetry (iTC)

- What microcalorimeter measures
- What iTC is used for
- Instrument design
- How microcalorimeter works

The raw iTC data – titration run

Evaluation of iTC data

- Thermodynamics behind binding isotherm

Advantages and disadvantages of iTC technique

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

What does microcalorimeter measure?

heat

generated or absorbed when molecules interact

1 calorie (cal) = 4.184 joules (J)

1 calorie = heat required to raise the temperature of 1 g of H_2O by 1°C

From the Latin 'calor' (heat) and the Greek 'metry' (to measure)

Heat changes detected by iTC are small, typically sub millionths of a degree Celsius

(1 µcal = 0,0000000022^{0}C)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

What iTC can be used for

Characterization of interactions of proteins, nucleic acids, lipids, antibodies, small molecules

Complete thermodynamic characterization of the binding:

- ✓ affinities (from nM to mM range)
- ✓ stoichiometry (N)
- ✓ enthalpy (ΔH)
- ✓ entropy (ΔS)

Binding to complex macromolecular targets (e.g. high order complexes, liposomes)

Material association

Competition binding

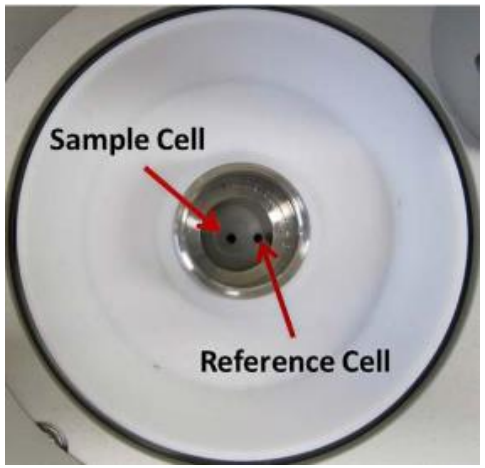
Enzyme kinetic parameters

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Malvern iTC200 microcalorimeter

Adiabatic jacket maintains temperature



Sample cell volume - 280 µl (Macromolecule)

Titration syringe volume - 40 µl (Ligand)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Malvern MicroCal PEAQ-iTC

It is sensitive to detect heats as low as 0.01 µcal (50 nJ), with noise rating of 0.00015 µcal/sec

Measures Kd ~ 10^{-3} to 10^{-9} M

The response time for the MicroCal PEAQ-ITC is 8 Seconds

A short response time = sharper and larger amplitude peaks

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

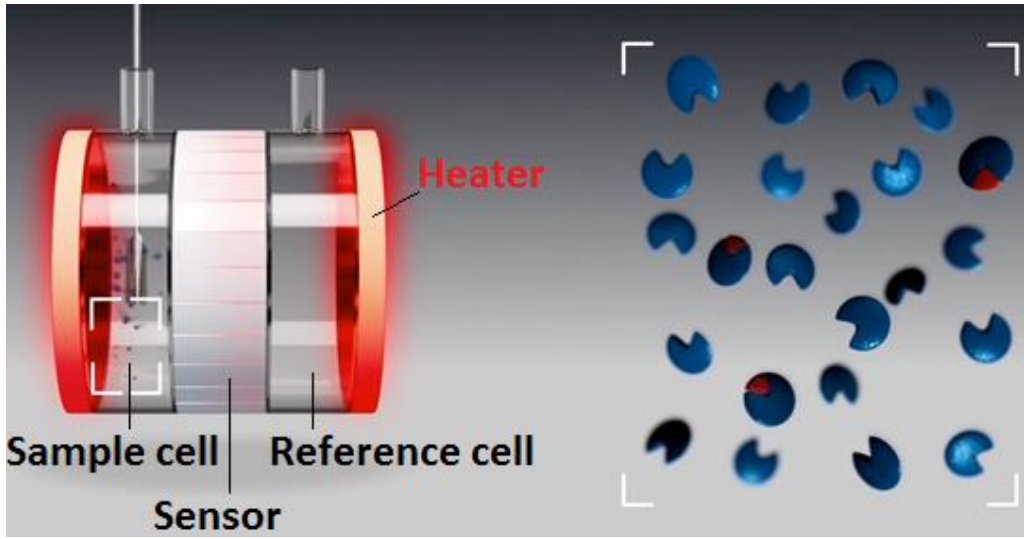
MOSBRI Molecular-Scale Biophysics Research Infrastructure

How microcalorimeter monitors heat change within the sample cell

Sample sell – macromolecule

Reference cell – pure water

Cells are from Hastelloy



Heaters supply a constant flow of the heart to maintain the cells temperature at set point (normally 25°C)

Sensor detects temperature difference between sample and reference cell

Binding results change of temperature within the sample cell

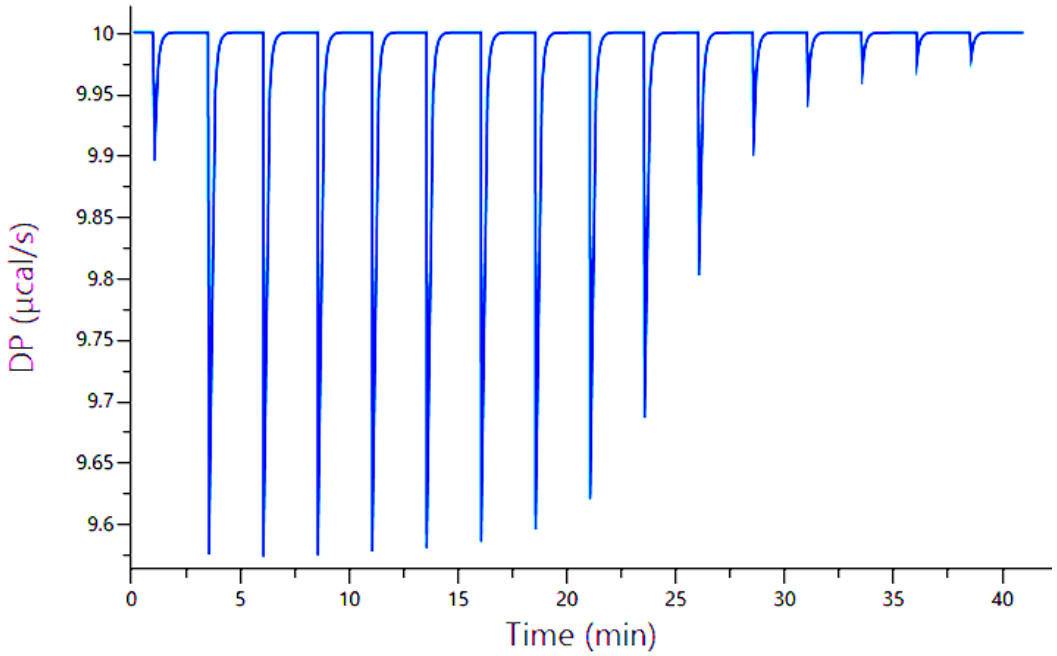
Sensor detects ΔT and gives a feedback differential power (DP) to the heater which compensate to this

iTC measures power difference between the reference and sample cells

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

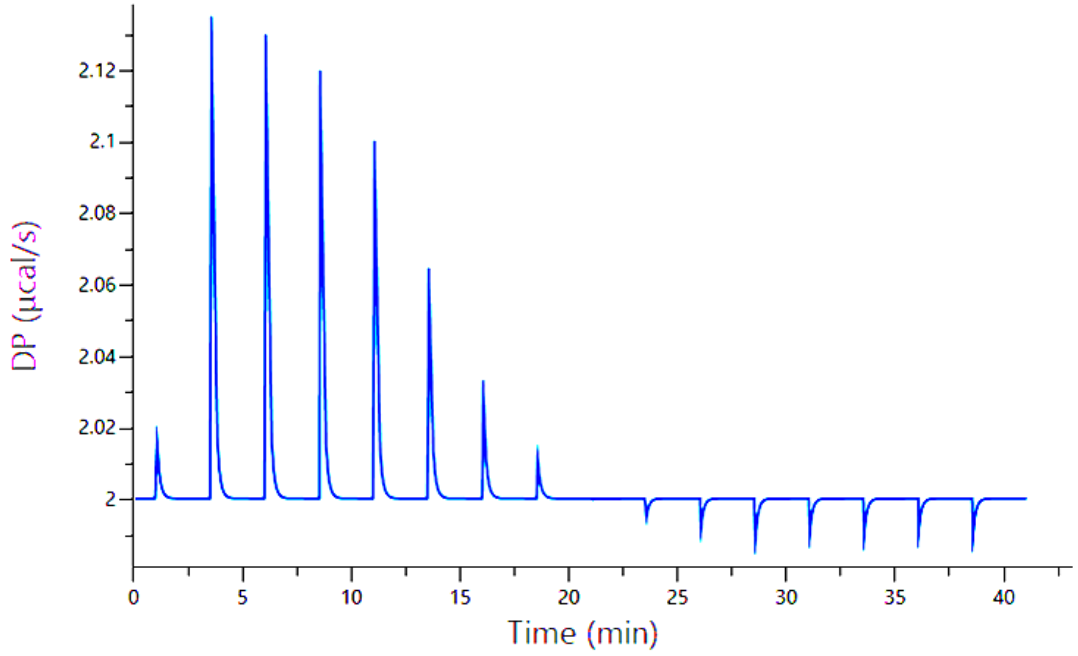
MOSBRI Molecular-Scale Biophysics Research Infrastructure

For maintaining the experiment temperature, instrument gives relevant power to the sample cell, depending on interactions



Exothermic interaction

sample cell gets warmer than reference cell
⇒ iTC supplies less power to heat the cell



Endothermic interaction

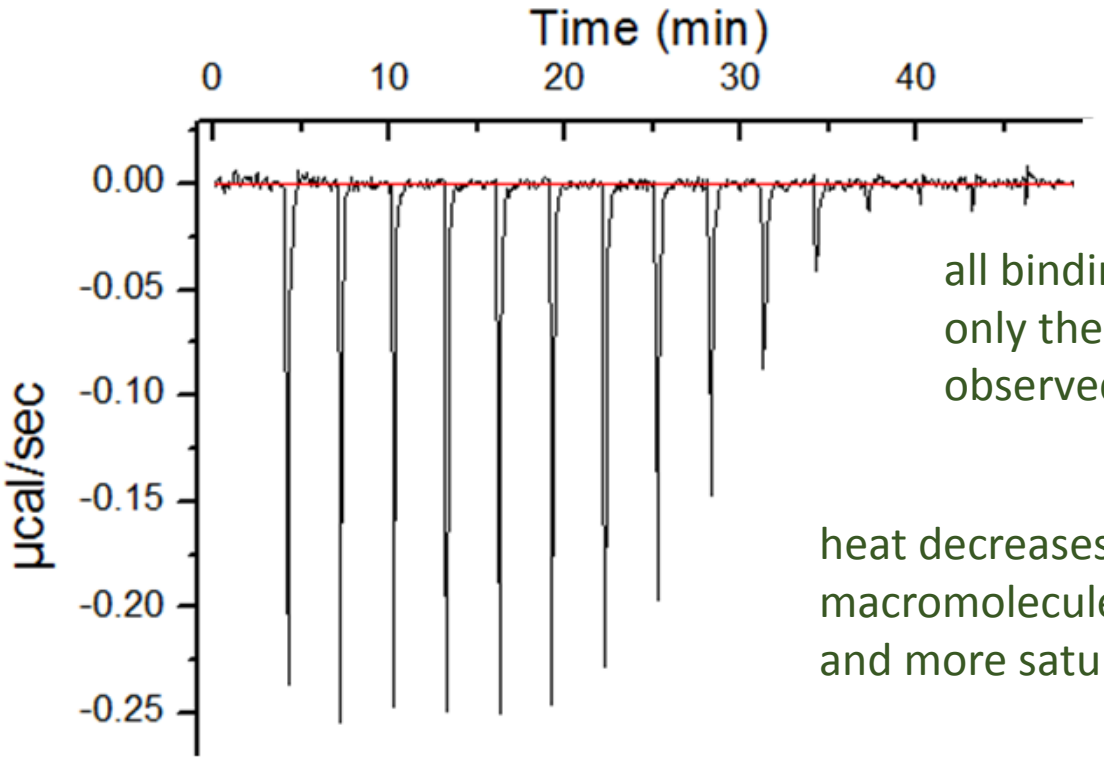
sample cell gets colder than reference cell
⇒ iTC applies the energy to heat the cell

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Titration run - series of injections (16) of a ligand into the cell, containing macromolecules

Injections result in the exothermic heat in the sample cell



all binding sites are occupied,
only the background heat of dilution is observed

heat decreases,
macromolecules get more and more saturated

large peaks- lots of complex form
every ligand molecule becomes bound to macromolecule

At the end of the titration ligand concentration is two- to three-fold greater than the sample

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

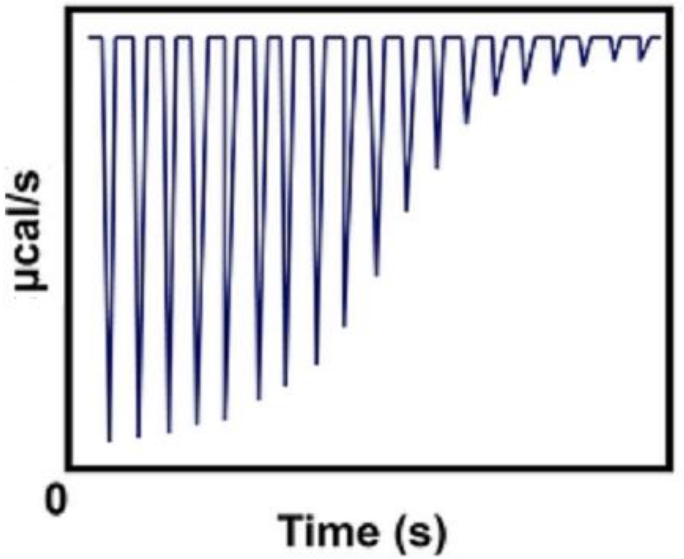
Evaluation of raw data

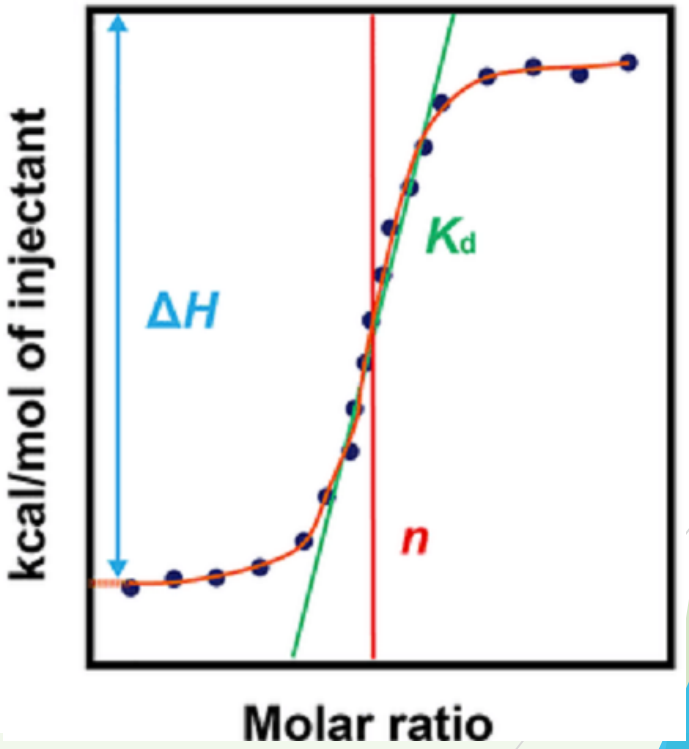
The molar ratio between the ligand and macromolecule is gradually increased through a series of ligand injections

The area of each peak is integrated and plotted versus the molar ratio of ligand to macromolecule

The resulting isotherm can be fitted to a binding model to generate the affinity, stoichiometry, enthalpy and entropy of interaction

Thereby stoichiometry, affinity and change of enthalpy, of the molecular binding can be obtained





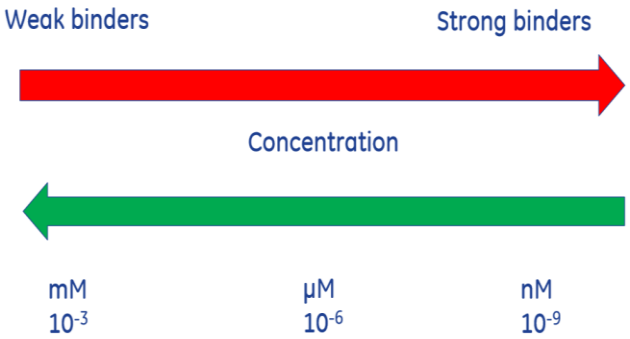
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

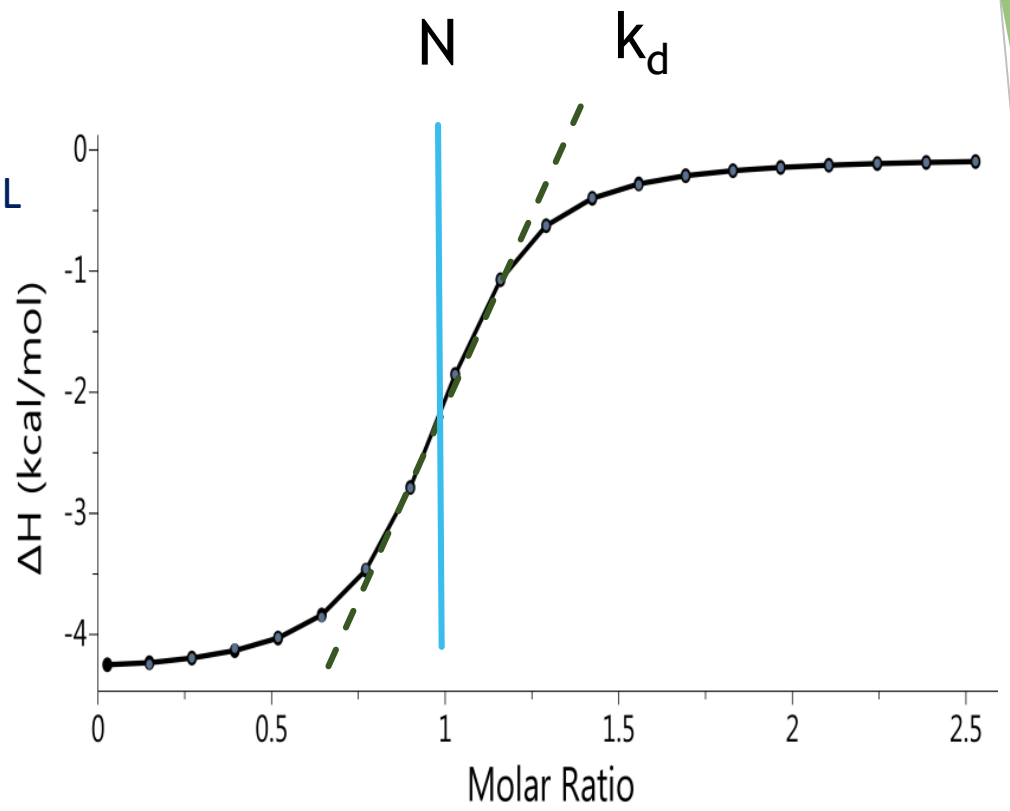
MOSBRI Molecular-Scale Biophysics Research Infrastructure

Affinity

Ligand (L) binds to the macromolecule (M) $M + L \rightleftharpoons ML$

$$K_a = \frac{[ML]}{[M][L]} = \frac{1}{K_d}$$





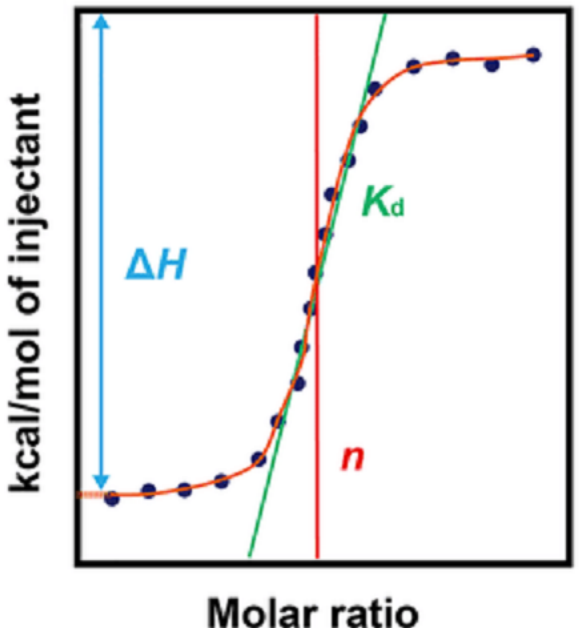
K_d - concentration of ligand
when half of the macromolecule is bound

N - molar ratio
at the center of the binding isotherm

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Enthalpy of binding (ΔH) is a result of direct measurement by iTC



Heat gained or lost due to the binding: Q = ΔH

ΔH is the amount of heat per mole of ligand bound

Binding affinity is dictated by the Gibbs energy of binding ΔG

$$K_a = e^{-\frac{\Delta G}{RT}}$$

Once you determine K_a and ΔH,

Gibbs energy ΔG and entropy ΔS of binding can be calculated

$$\Delta G = -RT \ln K_a = RT \ln K_d$$

$$\Delta G = \Delta H - T\Delta S \Rightarrow \Delta S = \frac{\Delta H - \Delta G}{T}$$

R is the universal gas constant (1.987 cal·K^{-1}·M^{-1})

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Relationship between Gibbs free energy and affinity

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

where R= 1.98 cal mol^{-1} K^{-1}; T= 273.2 K, and RT =0.62 kcal/mol at 37°C

The larger the K_a and the more negative the Gibbs energy of binding $\Rightarrow$ stronger complex

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

$$-RT \ln K_a = \Delta H - T\Delta S$$

When is an interaction strong?

ΔG is large and negative

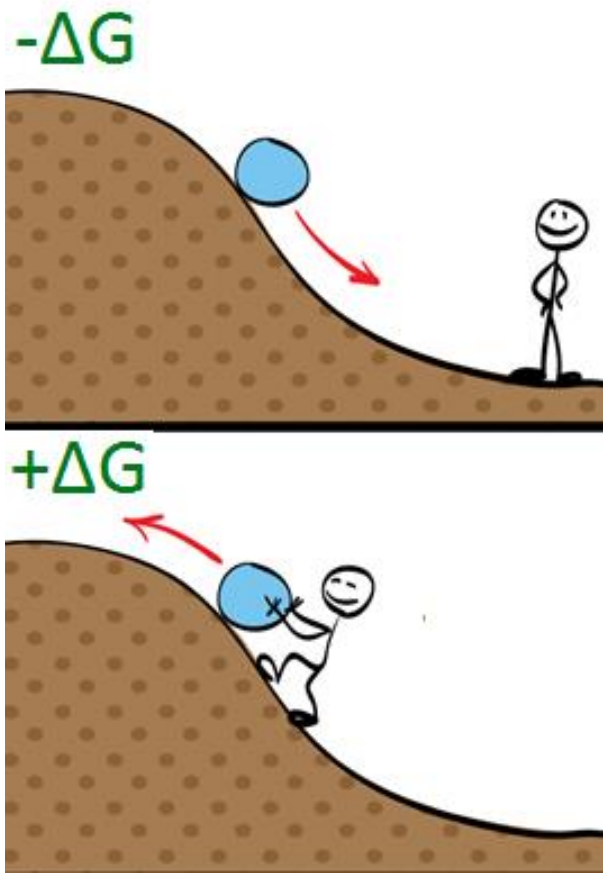
ΔH is large and negative

ΔS is large and positive

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

ΔG ≤ 0 for spontaneous binding process



If process lowers the free energy, it is "spontaneous"

- ✓ Process tends to happen
- ✓ Process occurs by itself

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Elucidation of binding mechanism

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

Enthalpic contribution

hydrogen bond formation

van der Waals interactions

electrostatic interactions

Entropic contribution

hydrophobic interaction causing release of bound solvent (favorable)

conformational changes of macromolecules and decreased conformational and rotational freedom upon complex formation (unfavorable)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Freire (2007) A new era for microcalorimetry in drug development. Eur. Pharm. Rev. 5, 73-78

MOSBRI Molecular-Scale Biophysics Research Infrastructure

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

Negative ΔH is favorable for binding

Positive $T\Delta S$ is favorable for binding

Enthalpy - changes of heat

Entropy - changes of disorder

Dominant contribution is from hydrogen bonds (energy of one hydrogen bond is 5 kcal/mol)

Unfavorable entropy is caused by decreased disorder upon binding - complex is less disordered and flexible, than two molecules

Provides insight into how hydrogen bonding and electrostatic interactions mediate binding

Release of ordered water molecules and ions to the bulk solvent when hydrophobic surfaces of the ligand and target interact is the main driving force for hydrophobic interactions

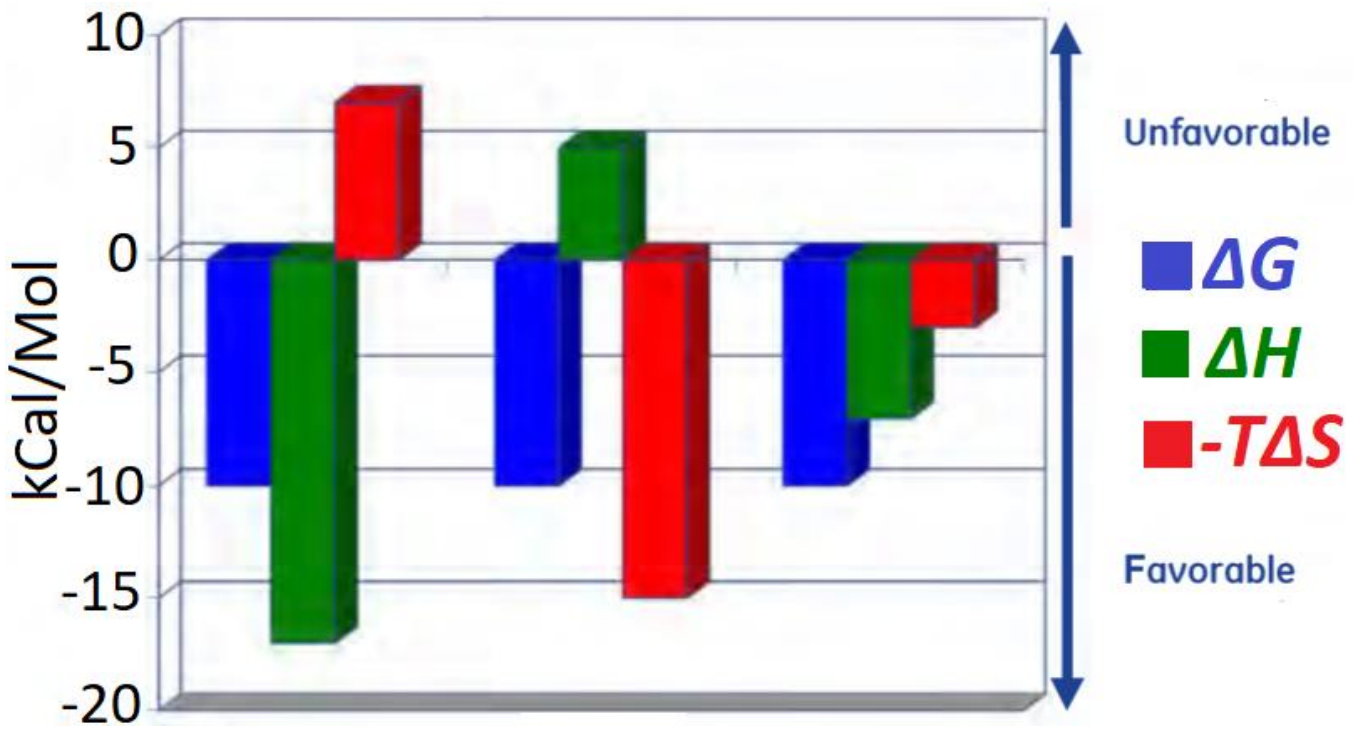
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

Enthalpy and entropy contributions to affinity

All three interactions have the same binding energy ΔG

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

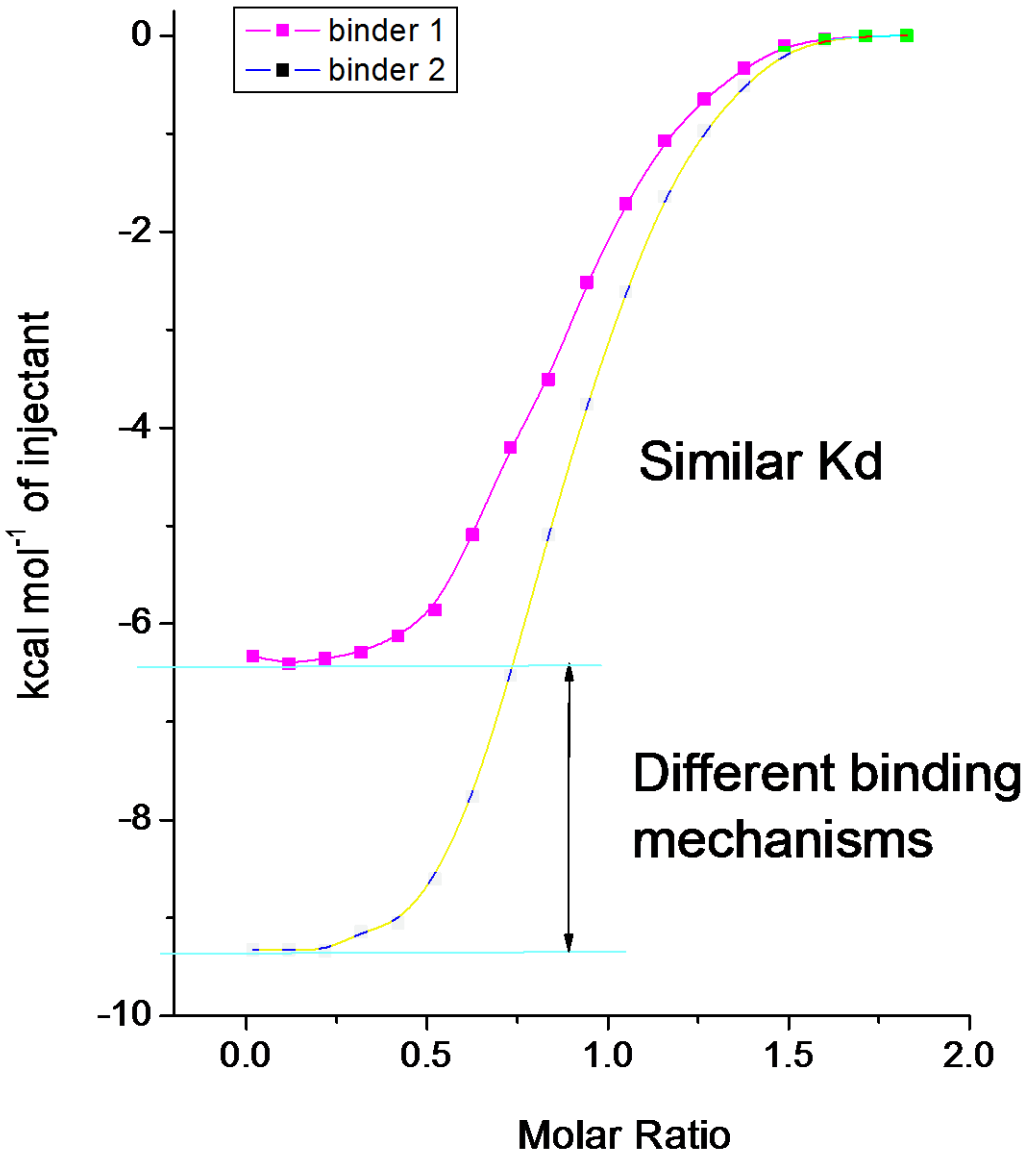


iTC gives insights to the mechanism of binding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

iTC characterizes binding forces



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

MOSBRI Molecular-Scale Biophysics Research Infrastructure

iTC technique

Advantages	Disadvantages
Complete thermodynamic characterization in a single experiment	Slow technique with a relatively low throughput (1-2 h/assay in the conventional titration experiment)
Direct determination of the binding enthalpy	Large amount of sample is needed
Universal technique - almost all reactions involve a heat exchange	Non-specific
Label-free, in solution	Signal proportional to the binding enthalpy ($\Delta H \neq 0$ required)
No need for immobilization	Kinetically slow interactions may be overlooked
No molecular weight limitations	Maintenance is required

Isothermal titration calorimetry for the biomolecular interaction analysis

tatsiana.charnavets@ibt.cas.cz

http://www.ibt.cas.cz IBT web page

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806