Isothermal Titration Calorimetry (ITC)

TA Instruments

TAM

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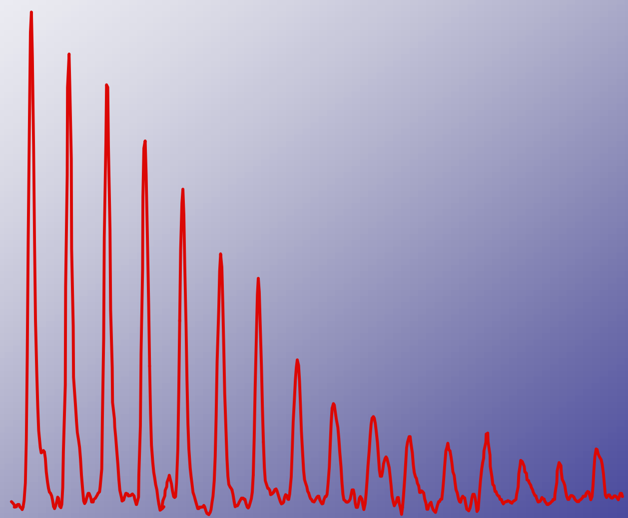
Titration Calorimetry to Measure Binding Interactions

- Universal
- No chemical modification required
- Immobilisation possible, but not necessary
- Equilibrium conditions



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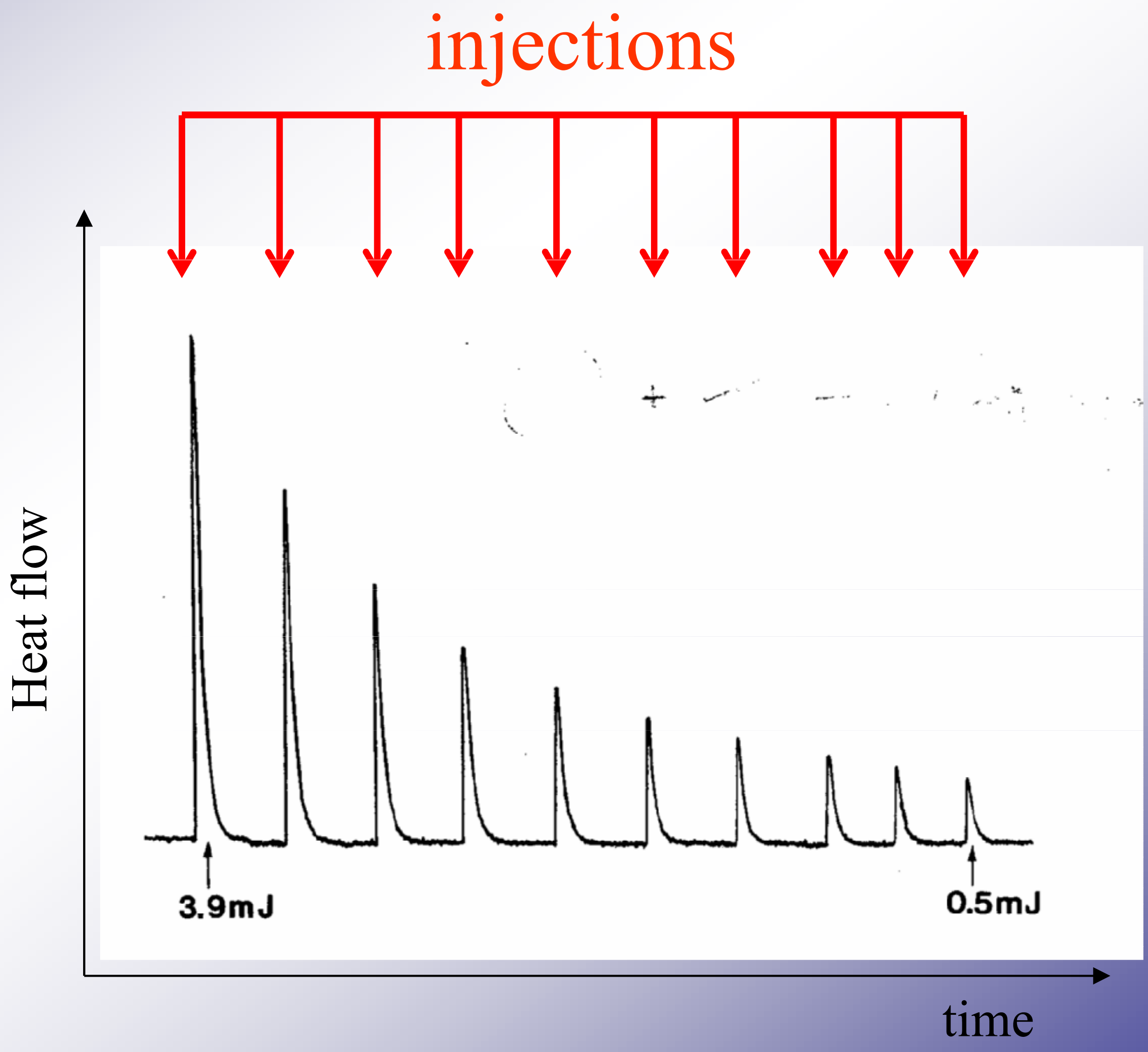
Titration calorimetry is the controlled addition (in steps or continuously) of one substance to another, whereby a response in heat flow is measured. Liquid-liquid titration is most common, but solid-liquid (dissolution) and gas-solid (sorption) can also be considered as titrations.



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The titration experiment

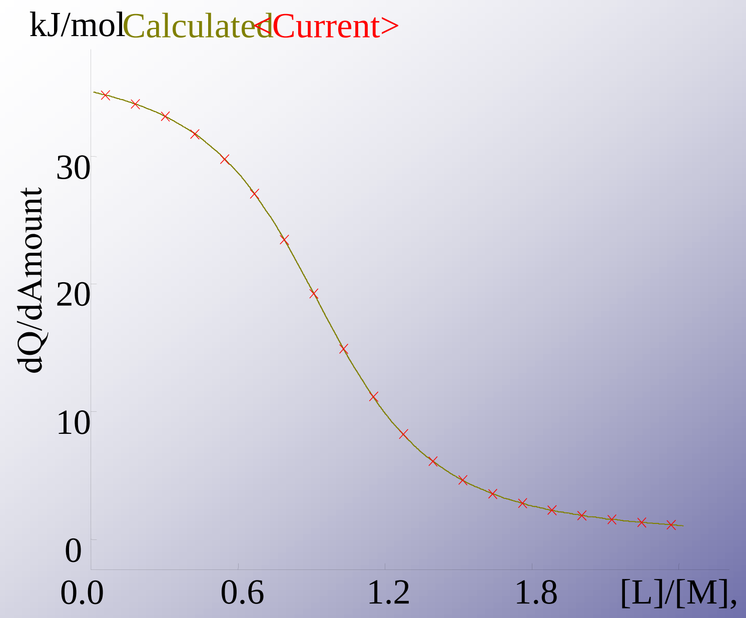
The relation between ΔH and K
(1:1 binding)

$$M + L \Leftrightarrow ML$$

$$K = [ML] / [L][M]$$

$$Q = ([ML] / [M]_{total}) \cdot \Delta H$$

$$Q = (K \cdot [L] \cdot \Delta H) / (1 + K \cdot [L])$$



The Titration ampoule

For studying interaction phenomena;

-in liquid solution

-between solid state samples and gas

Computer controlled
injection pump

Reaction
vessel

Typical Application areas

- Interactions of biological macromolecules (*e.g.* ligand binding, protein-protein interaction)
- Interaction between drugs and release modifiers (e.g. cyclodextrins, PVP)
- Adsorption/binding to particles in suspension
- Dissolution/dilution of liquids
- Gas titration

Binding Characterization

- Binding Affinity, K
- Binding Stoichiometry
- Binding Thermodynamics: ΔG, ΔH, ΔS

$$\Delta G^o = -RT \ln K_c$$

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

$$\Delta C_p = \frac{d\Delta H^o}{dT}$$

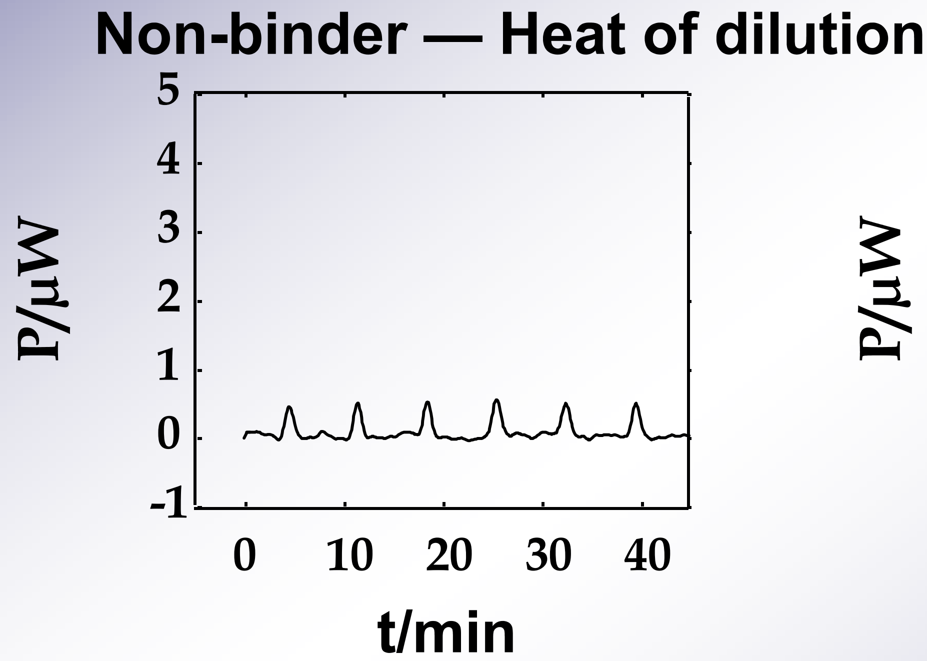
Titration Calorimetry in Biochemistry/Biophysics

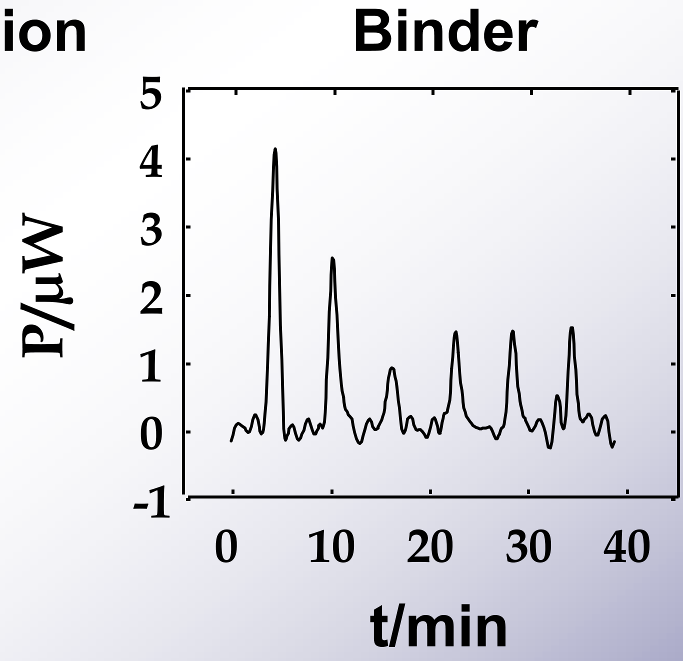
- Emerging as a premier tool for the characterization of biological macromolecules
- General applicability with high accuracy and precision
- Example:

  Antigen – Antibody
  Peptide – Protein
  Lipid – Protein
  Nucleic Acid – Protein
  Carbohydrate – Protein
  Small Molecule/Drug – Protein
  Protein – Protein
  Protein – Receptor (soluble and membrane-bound)

Rational Drug Design
-Screening of pharmaceutical hits and leads

Hallén, D., Structural Chemistry, Pharmacia, Stockholm





Non-binder: the identical peaks reflects only a dilution process

Binder: the peaks reflects dilution as well as a reaction. The peaks indicate a binding of the drug to a target molecule

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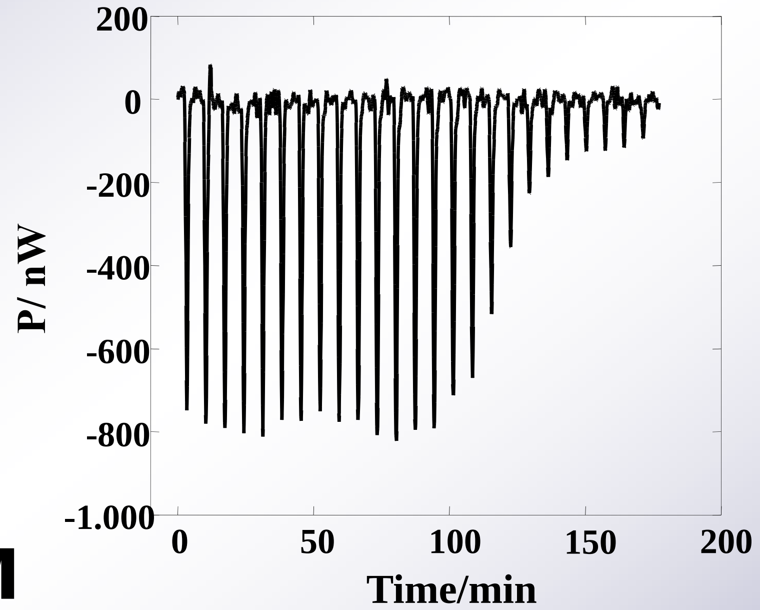
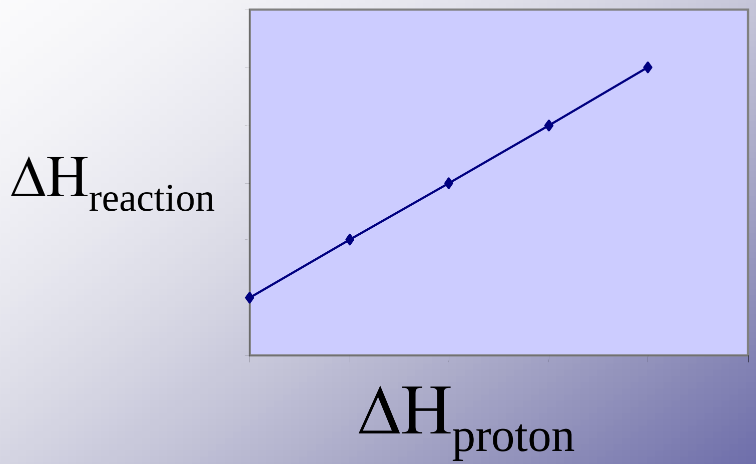
Proton Linkage

Hallén, D., Structural Chemistry, Pharmacia & Upjohn, Stockholm

- $M + L + B \Leftrightarrow ML + BH^+$; $\Delta H_{reaction}$
- $M + L \Leftrightarrow ML + H^+$; $\Delta H_{binding}$
- $B + H^+ \Leftrightarrow BH^+$; ΔH_{proton}





Binding of IGF-I to the soluble extracellular IGF-I receptor

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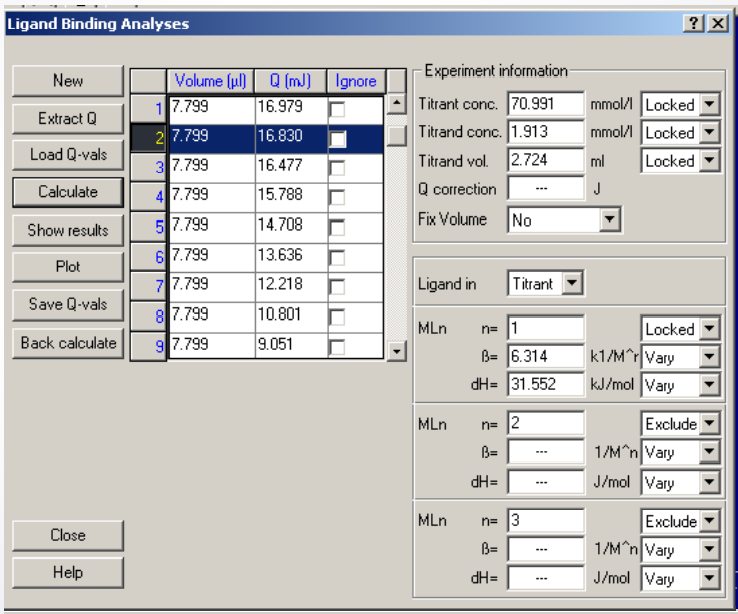
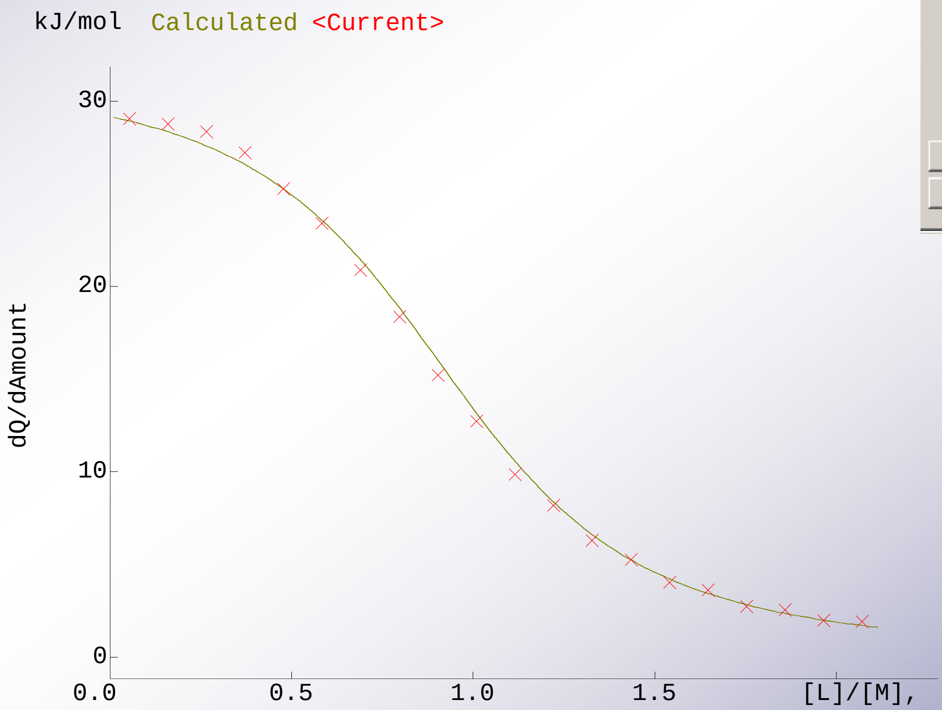
Binding of a ligand to a macrocyclic compound

Binding of $BaCl_2$ to 18-crown-6-ether

Integrated heat values plotted against ligand/host molecule





The binding process is charactersied by the equilibrium constant K_c and the reaction enthalpy ΔH.

K=5900 dm³/mol
ΔH =-31.42 kJ/mol.

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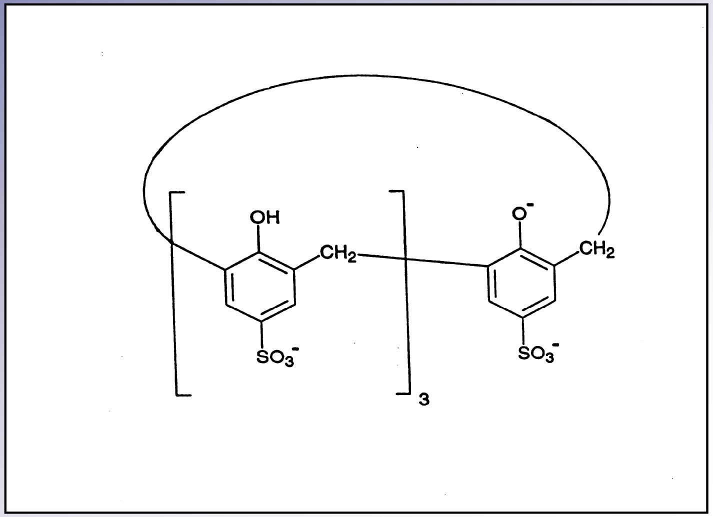
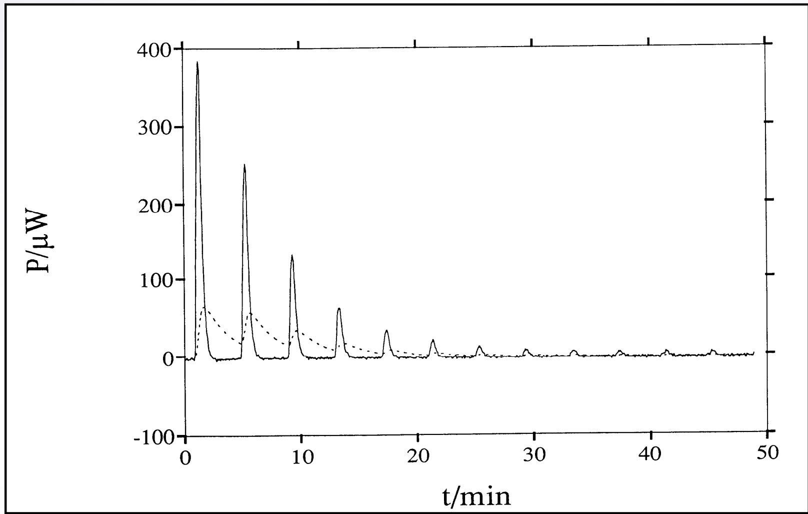
Binding of a calixarene derivative CATS and alkyl ammonium ions





Thermo-dynamics

$$\Delta G^o = -RT \ln K_c$$

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

$$\Delta C_p = \frac{d\Delta H^o}{dT}$$

n	K_c (M^{-1})	ΔH (kJ/mol)
3	13090	-16.89
4	10200	-17.94
5	6400	-20.24
6	4000	-20.42
7	2460	-20.86

Conclusion

The affinity decreases and the reaction becomes more exothermic with increasing chain length

TAM Courses

Dr. M. Stödeman, Univ. Lund

IUPAC Test Reaction for Isothermal Titration Calorimetry

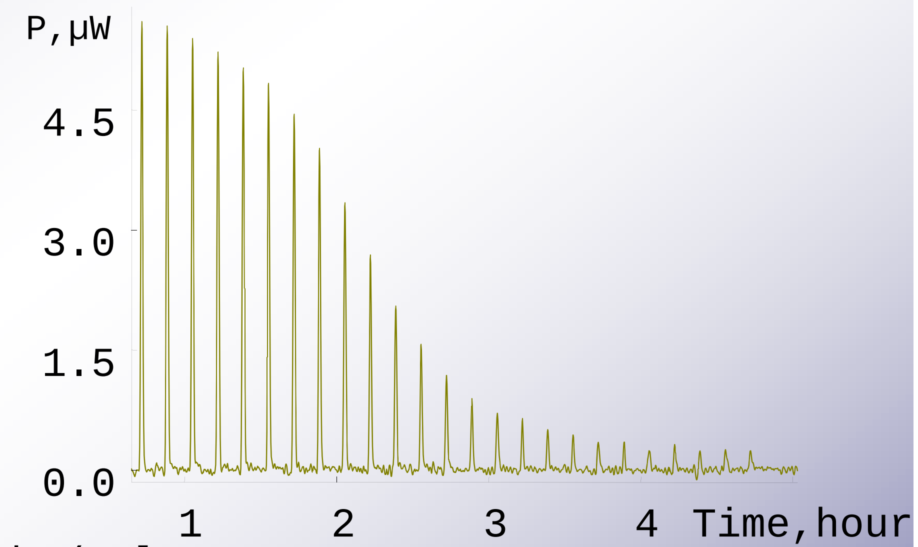
- The reaction between 2’CMP and RNaseA has been suggested by IUPAC as a test reaction in titration calorimetry
  - 2'CMP: 2-cytindine monophosphate
  - RNaseA: bovine pancreatic ribonuclease
- 1:1 binding reaction

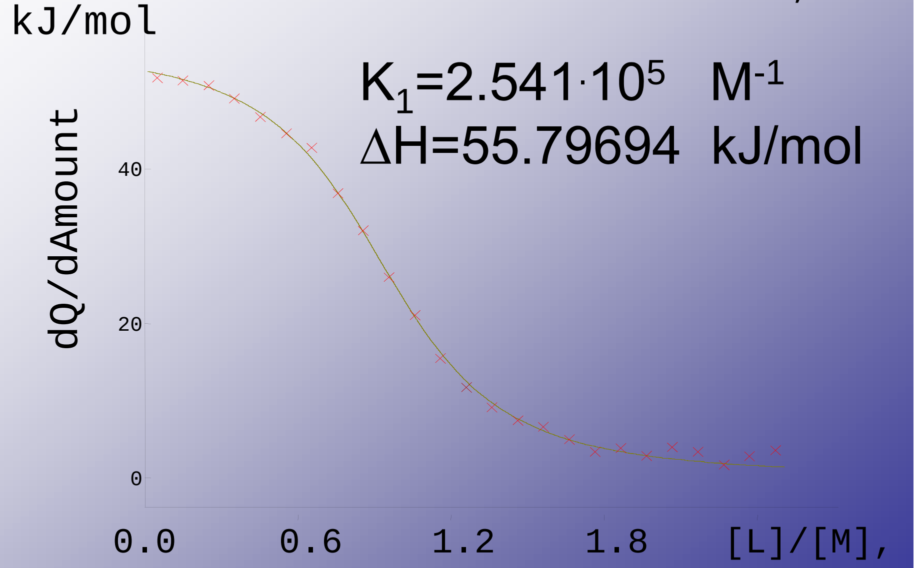
Dr. Dan Hallén and Dr. Natalia Markova at Biovitrum AB, Stockholm, Sweden.

Experimental procedure and results

- Experimental conditions
  - Conc of RNaseA in vessel: 66.670 µmol/l
  - Conc of 2'CMP in syringe: 764.702 µmol/l
  - Volume in vessel: 900.000 µl
  - Stirrer rate: 60 rpm
  - 20 mM Potassium Acetate buffer at pH5.5.

- TAM System configuration
  - 1 ml titration ampoule
  - gold stirrer
  - titration reference ampoule
  - 3 µW measuring range
  - Dynamically corrected data





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Results

- The derived thermodynamic properties are highly dependent on pH, ionic strength, temperature and RNaseA concentration.

  Therefore it is necessary to get all these parameters correct during the sample preparation.

- It was proposed that the standardised test should be performed at high salt concentrations because the enthalpy change is less sensitive to protein concentrations under these conditions.

Ligand binding

$$L + M \Leftrightarrow ML \quad \mathrm{K}, \Delta\mathrm{H}$$

$$\frac{ML}{L \cdot M} = K$$

$$L_t = L + ML \quad \Rightarrow \quad \mathrm{ML} = \mathrm{L}_t - L$$

$$M_t = M + ML \quad \Rightarrow \quad \mathrm{M} = \mathrm{M}_t - ML = M_t - L_t + L$$

$$q = V \cdot \Delta H \cdot ML$$

$$\frac{1}{V \cdot \Delta H} \frac{dq}{dL_t} = \frac{d}{dL_t}(L_t - L) = 1 - \frac{dL}{dL_t}$$

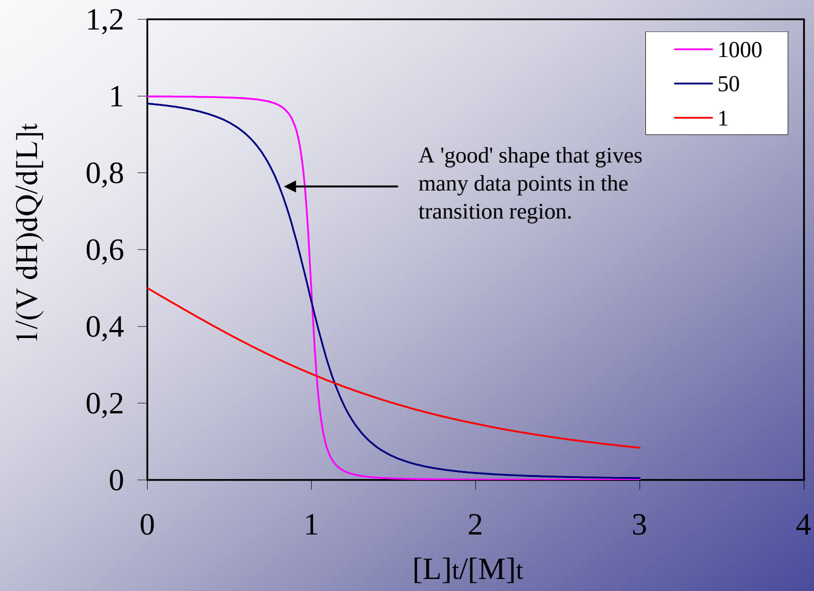
$$r = K \cdot M_t \quad \Rightarrow \quad \mathrm{M}_t = \frac{r}{K}$$

$$X = \frac{L_t}{M_t} \quad \Rightarrow \quad \mathrm{L}_t = \frac{X \cdot r}{K}$$

$$\frac{dq}{dL_t} = V \cdot \Delta H \frac{1}{2}\left(1 - \frac{1 + r \cdot (X - 1)}{\sqrt{(r - X \cdot r + 1)^2 + 4X \cdot r}}\right)$$

The left side can be plotted versus X= Lt/Mt for different values of r = K*M_t.

Note: the shape of the 'binding curve' depends on the product K*M_t. Thus, for a certain K value the concentration of M must be optimized for highest accuracy in the curve fitting procedure.



L – ligand (syringe)
M – host molecule (vessel)
K – equilibrium constant
ΔH – reaction enthalpy

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Overall / Step-wise Reaction Path

General reaction stoichiometry when L binds to M

$$M + nL \leftrightarrow ML_n$$

Overall reaction path

$M + L \leftrightarrow ML_1$ — β_1, ΔH_1

$M + 2L \leftrightarrow ML_2$ — β_2, ΔH_2 where $\beta_1 = \frac{[ML_1]}{[M][L]}$ $\beta_2 = \frac{[ML_2]}{[M][L]^2}$ $\beta_3 = \frac{[ML_3]}{[M][L]^3}$

$M + 3L \leftrightarrow ML_3$ — β_3, ΔH_3

Step-wise reaction path

$M + L \leftrightarrow ML_1$ — K_1, ΔH_{1s}

$ML_1 + L \leftrightarrow ML_2$ — K_2, ΔH_{2s} where $K_1 = \frac{[ML_1]}{[M][L]}$ $K_2 = \frac{[ML_2]}{[ML_1][L]}$ $K_3 = \frac{[ML_3]}{[ML_2][L]}$

$ML_2 + L \leftrightarrow ML_3$ — K_3, ΔH_{3s}

NOTE: In the Ligand binding analysis program of TAM Assistant the equilibrium constants (**βn**) and the reaction enthalpies for the overall reactions (**ΔH_i**) are determined.

$\beta_1 = K_1$ — ΔH1s=ΔH1

$\beta_2 = K_1 K_2$ — ΔH2s=ΔH2 - ΔH1

$\beta_{31} = K_1 K_2 K_3$ — ΔH3s=ΔH3 - ΔH2

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Conclusion

- Isothermal Titration Calorimetry (ITC) is a versatile technique that can be used to study a variety of applications
- Some characteristic of the TAM system are;
  - The calorimeter is of heat conduction type (temperature remains constant during the reaction)
  - High dynamic measuring range, i.e up to 3 μW
  - Easy to clean the reaction vessel
  - Different sizes of reaction vessels
  - The reaction vessel can be observed
  - The stirring rate can be varied
  - TAM is flexible allowing the user to developed his own technical solutions

END