Isothermal Titration Calorimetry (ITC)
for the Evaluation of
Macromolecule-Ligand Interactions

[M] + [L] <=> [ML]

Some methods for obtaining thermodynamic parameters:

1) van't Hoff analysis

2) Calorimetry

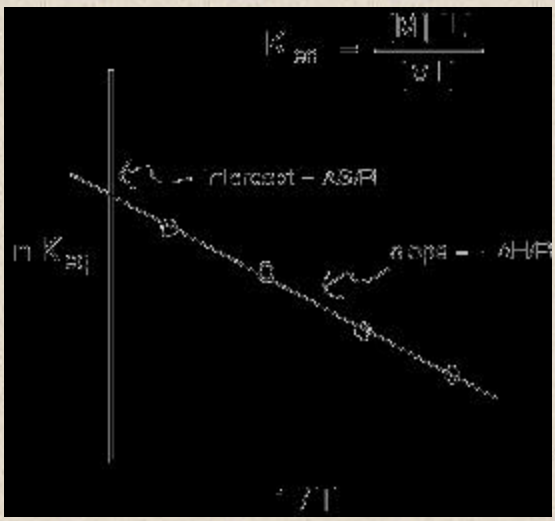
van't Hoff analysis can be performed any time you can measure equilibrium constant as a function of temperature.

$$\Delta G = - RT \ln K_{eq} \quad \text{and} \quad \Delta G = \Delta H - T\Delta S$$

Therefore: $- RT \ln K_{eq} = \Delta H - T\Delta S$

Rearrange: $\ln K_{eq} = - (\Delta H/RT) + (\Delta S/R)$

$y = m\,x + b$



$\ln K_{eq} = - (\Delta H/RT) + (\Delta S/R)$

What's nice about van't Hoff analysis?

If you can measure [M], [L], [ML] by any spectroscopic method, as a function of temperature, you can obtain thermodynamic parameters.

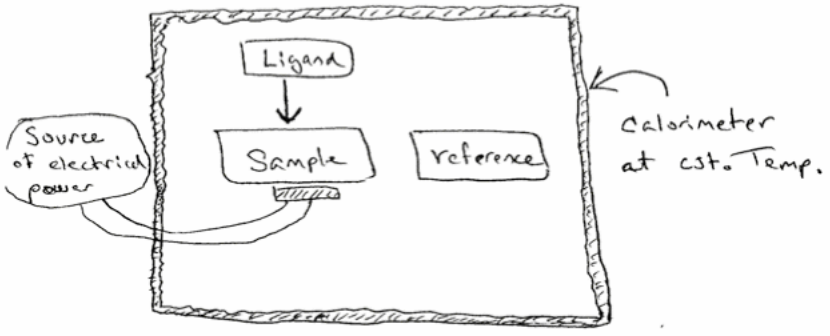
Or, any time that you can measure Keq at different temperatures, you can obtain thermodynamic parameters.

Why is van't Hoff analysis flawed?

- van't Hoff analysis assumes ΔH & ΔS & sample's heat capacity do not vary with temperature (this is not exactly correct).
- usually only a small temperature range is accessible when working with biochemical systems.

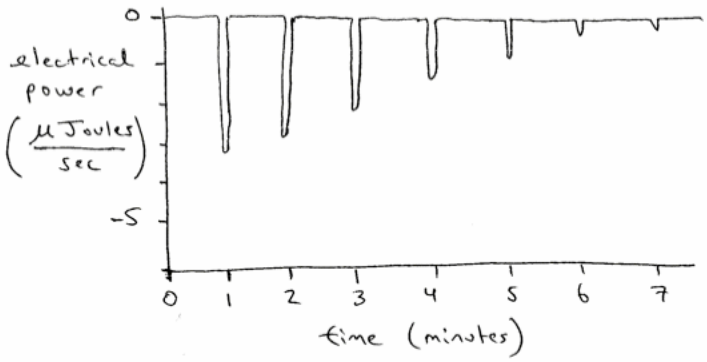
Isothermal Titration Calorimetry (ITC) provides a more rigorous alternative to van't Hoff analysis.

Experimental arrangement:



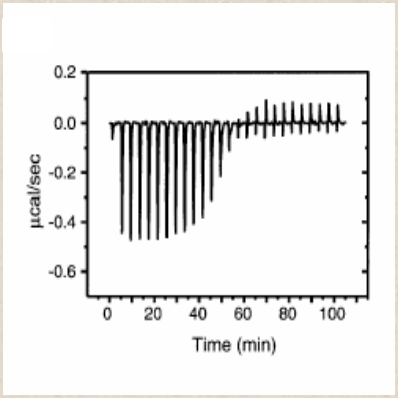
* Ligand is added to sample a few microliters at a time, at ≈ 1 minute intervals.
* Electrical energy is used to keep sample and reference at same temperature.

What is recorded:



* An aliquot of ligand solution is added to the sample once per minute.
* This releases heat.
* Electrical energy is required to re-equilibrate the sample and reference temperatures.

Some typical ITC data - mixing of two proteins that bind, with the release of heat:



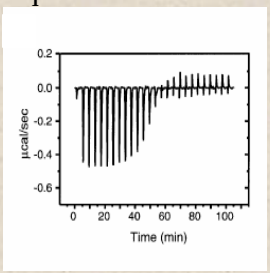
As ligand is added, binding sites on the macromolecule become saturated (this results in progressively less electrical energy being required to re-equilibrate the sample and reference cells).

Information regarding Keq for ligand binding is contained within the ITC data, in addition to information regarding ΔH.

Data analysis:

Δq(i) = enthalpy change upon each mixing event *i*.
Δq(i) is obtained by integrating each peak in ITC trace.

Δq(i) is a function of n, [M], [L], V, ΔH, K_{eq}



where:

n	=	# binding sites
[M]	=	conc. Macromolecule
[L]	=	conc. Ligand
V	=	sample volume
ΔH	=	enthalpy change upon ligand binding
K_{eq}	=	equilibrium cst for ligand binding

[M], [L], V are usually known.

n, DH, Keq are found by minimizing the difference between the observed and calculated peak areas in the ITC trace.

DG can be calculated from Keq
DS can be calculated using DH & DG

n, ΔH, Keq are found by minimizing the difference between the observed and calculated peak areas in the ITC trace.

$$\Delta q_i = n [M]_{total} V_{cell} \cdot \Delta H \cdot R$$

where R ≡ root of quadratic equation:

$$\gamma_i^2 - \gamma_i \left(1 + \frac{1}{n K_{eq} [M]_{total}} + \frac{[L_i]_{total}}{n[M]_{total}}\right) + n[L_i]_{total}[M]_{total} = 0$$

and $\gamma_i = \frac{[L_i]_{bound}}{[M]_{total}}$

or just remember:

Δq_i = function of $n, [M], [L], V, \Delta H, K_{eq}$

An experimental consideration:

[M] and [L] must be chosen so that there is a significant amount of both free and bound ligand present during the titration.

(as a consequence, K_d must be nM or greater for ITC to be useful)

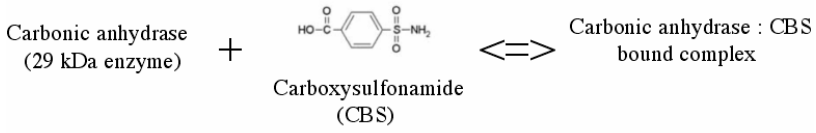
∴ for very tight binding ($K_d \simeq 10^{-9}$ M) the ITC experiment must be performed at very low ligand concentrations. This results in only very small power pulses needed to keep temperature constant, which results in significant experimental error.

Another experimental consideration:

Only a very small amount of heat is released in each ligand binding event, so a large amount of sample must be used so there is enough heat released to be detectable.

(as a consequence, typically 1 to 10 mg amounts of macromolecule are needed for an ITC titration)

Example of an application of ITC:



$$[M] + [L] \iff [ML]$$

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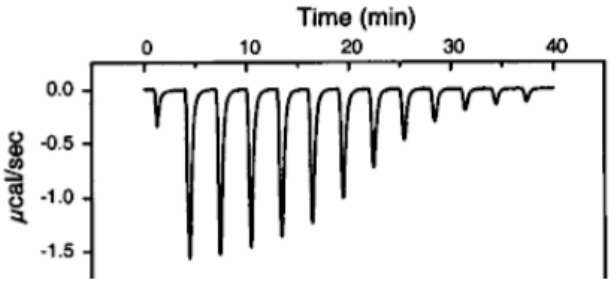
Protein Science (2002), 11:1017–1025.

ITC data: CBS binding to carbonic anhydrase.

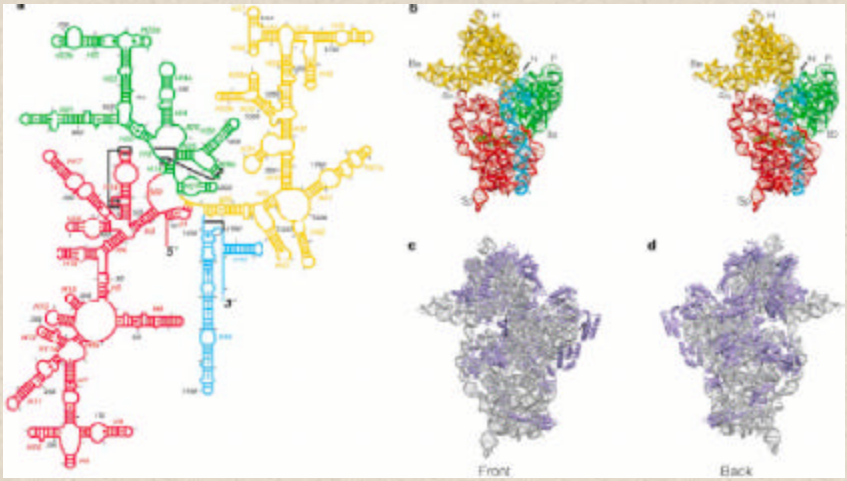


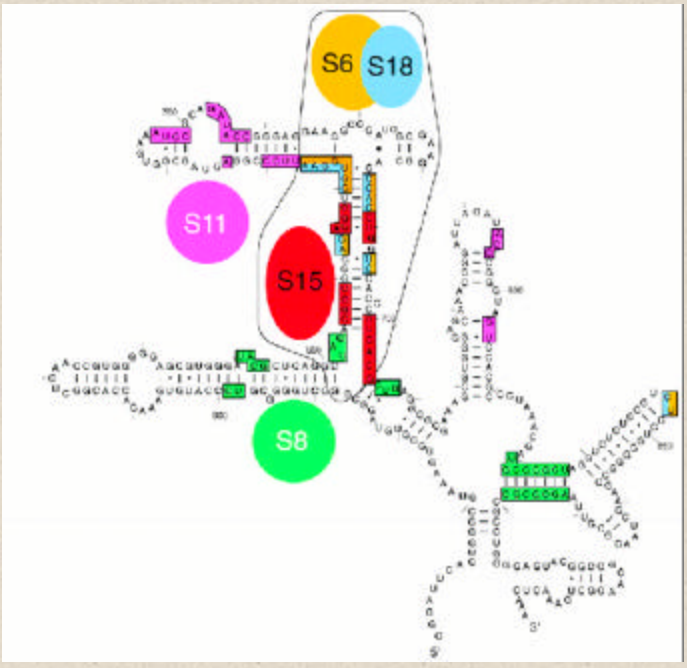
K_D (nM)	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ [cal/(mol K)]
760 ± 30	−8.3 ± 0.3	−11.6 ± 0.4	−11 ± 1

Another example of an application of ITC:

The study of ribosome assembly.

Small (16S) ribosomal subunit structure
(Ramakrishnan et al., 2000)





doi:10.1006/jmbi.2001.5018 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (2001) 313, 35–48

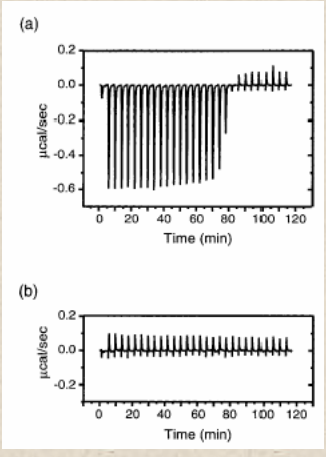
JMB

Central Domain Assembly: Thermodynamics and Kinetics of S6 and S18 Binding to an S15-RNA Complex

Michael I. Recht and James R. Williamson*

Proteins S6 and S18 form a heterodimer (without 16S RNA being present)

- Aliquots of a solution of protein S6 are added to a solution of S18.
- Analysis of ITC data yields:
- ΔH = -16.2 kcal/mole
- K_d = 8.4 nM
- n = 1

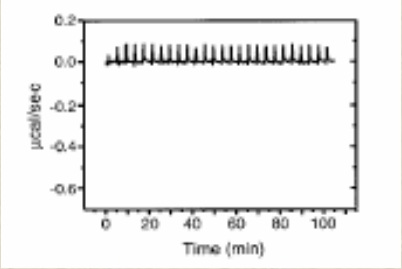


add S6 to S18

add S6 to buffer only

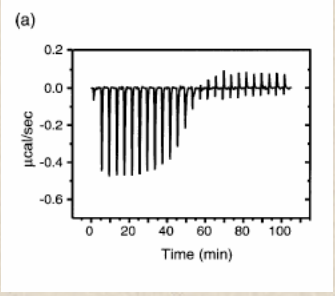
Proteins S6 & S18 do not bind to ribosomal RNA unless protein S15 is pre-bound to the RNA:

- ITC shows little energy release when protein S6 is added to ribosomal RNA
- The same result is obtained when protein S18 is added to RNA



The S6-S18 protein complex binds to ribosomal RNA when protein S15 is pre-bound:

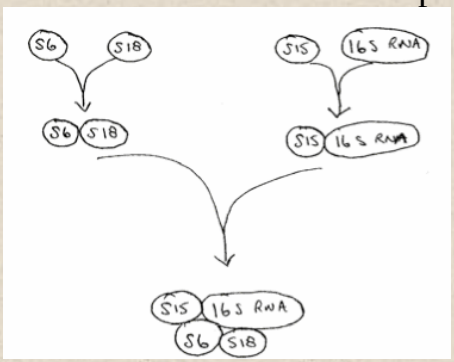
- K_d = 46 nM
- ΔH = -15.8 kcal/mole

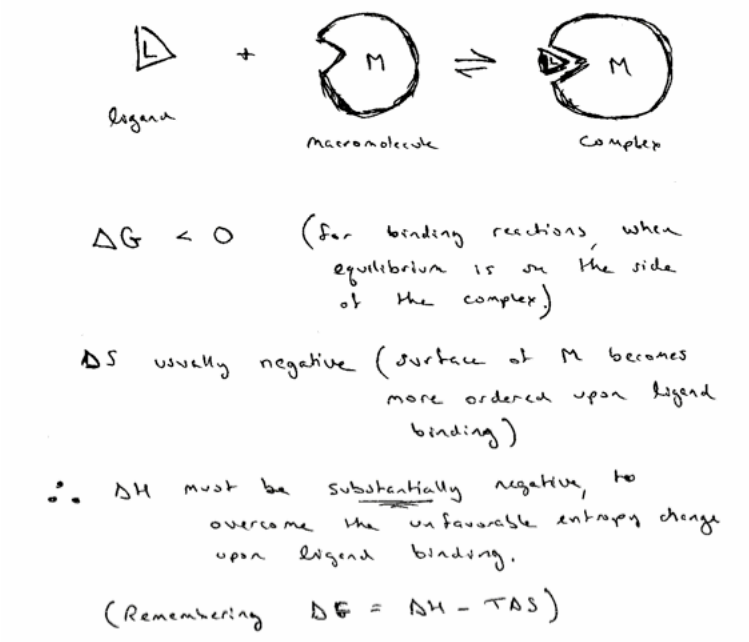


Adding S6-S18 complex to S15-16S RNA complex

Conclusions

- Proteins S6 and S18 form a tight complex, K_d = 8.4 nM
- Protein S15 and RNA form tight complex, K_d = 2 nM
- S6-S18 complex binds to S15-16S RNA complex
- S6 & S15 individually do not bind to RNA
- S18 by itself binds the S15-16S RNA complex





Summary: Isothermal Titration Calorimetry (ITC)

Good things about ITC:

Accurate determination of binding (K_d) and thermodynamic (ΔG, ΔH, ΔS) parameters for ligand-macromolecule interactions.

ITC does not make the approximations that are included in a van't Hoff type of analysis.

Not such good things about ITC:

Large (perhaps 10 mg) quantities of material are required, in order to detect the small amount of heat released upon mixing macromolecule and ligand.

In comparison, gel-mobility shift assays can be carried out with << 1 mg of material.