Isothermal Titration Calorimetry

- Supplemental reading (Lewis and Murphy)
- Van Holde, Section 2.5
- Wiseman, *et al.* (1989) *Anal. Biochem.* **179**: 131-137.
- Freyer and Lewis. (2008) *Meth. Cell Biol.* **84:** 79-112.

Review: Binding

Reaction	Bound	Degeneracy	Weight Expression	Weight
	0	1	[M]/[M]	1
M + L ⇄ ML	1	N	[ML]/[M]	$KL = S$
ML + L ⇄ ML_2	2	$\frac{N!}{2!\,(N-2)!} = \binom{N}{2}$	[ML_2]/[M]	$(KL)^2 = S^2$
…	…	…	…	…
ML_{N-1} + L ⇄ ML_N	N	1	[ML_N]/[M]	S^N

- N independent, identical sites, each with association constant K
- Partition function: $Z = (1 + S)^N$

Degree of Binding

- Important relationship:

$$\bar{\nu} = \frac{moles\ L\ bound}{total\ moles\ macromolecule} = \frac{S}{Z}\left(\frac{\partial Z}{\partial S}\right)$$

$$= \frac{L_{total} - L_{free}}{total\ macomolecule} = \frac{[L_T] - [L]}{[M_T]}$$

- Note that:

$$L_{bound} = \bar{\nu} \cdot M_{tot}$$

Solution: N Independent, Identical Sites

- We know: M_T, L_T (experimental conditions)
- Fit parameters: N, K

- Equation: $\bar{\nu} = \frac{NKL}{1+KL}$

- Key insight: $L = L_T - M_T\bar{\nu}$

Solution: N Independent, Identical Sites

- Use quadratic formula to solve for $\bar{\nu}$:

$$\bar{\nu} = \frac{1}{2}\left[\left(N + \frac{L_T}{M_T} + \frac{1}{M_T K}\right) - \sqrt{\left(N + \frac{L_T}{M_T} + \frac{1}{M_T K}\right)^2 - 4N\left(\frac{L_T}{M_T}\right)}\right]$$

- Can use least squares (e.g. Gnuplot) to optimize N, K

More Complex Binding

- Examples:
  - Independent, non-identical
  - Cooperative
- No explicit solution may exist for $\bar{\nu}$ vs. M_T, L_T, and parameters

Requirements for Measuring Binding

- $M_T K \geq 1$; Preferably, this value should be ≥ 10 (see Wiseman, fig. 3)
  - Since $K = K_d^{-1}$, this is equivalent to saying $M_T \geq K_d$
  - Otherwise, nearly all of M is bound

- L_T must be soluble above K_d
  - Otherwise, cannot saturate by adding more L

ITC - Overview

- Macromolecule ("stationary" component) is held in a calorimetry cell
- Titrant is added in small volumes
- Binding will evolve heat
- When binding is saturated, no more heat is evolved

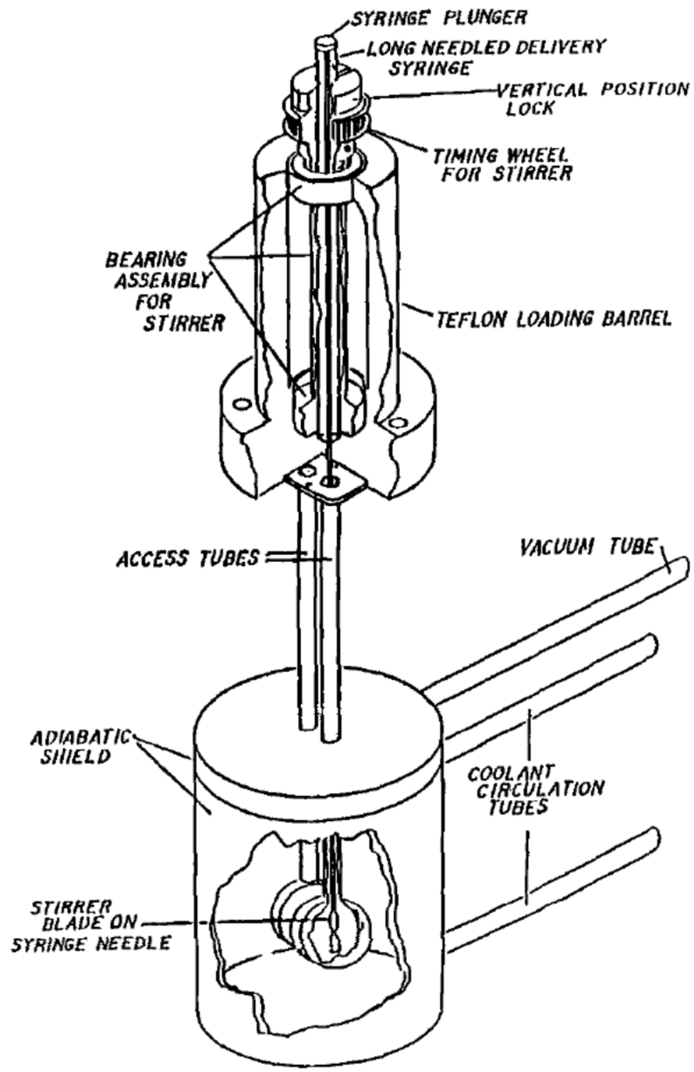
ITC - Advantages

- Can obtain several thermodynamic parameters:
  - Obtained directly: $\Delta\overline{H}^0$ (measure heat)
  - Free energy: $\Delta\bar{G}^0 = -RT\ln K$
  - Entropy: $T_{ref}\Delta\bar{S}^0 = \Delta\overline{H}^0 - \Delta\bar{S}^0$

  } **Model dependent!**

- Range: $10^4 - 10^8$ (heat evolved must be ~0.1 µcal or more)
  - Tighter binding possible using competition

ITC Apparatus



Wiseman, et. al.

ITC Block Diagram

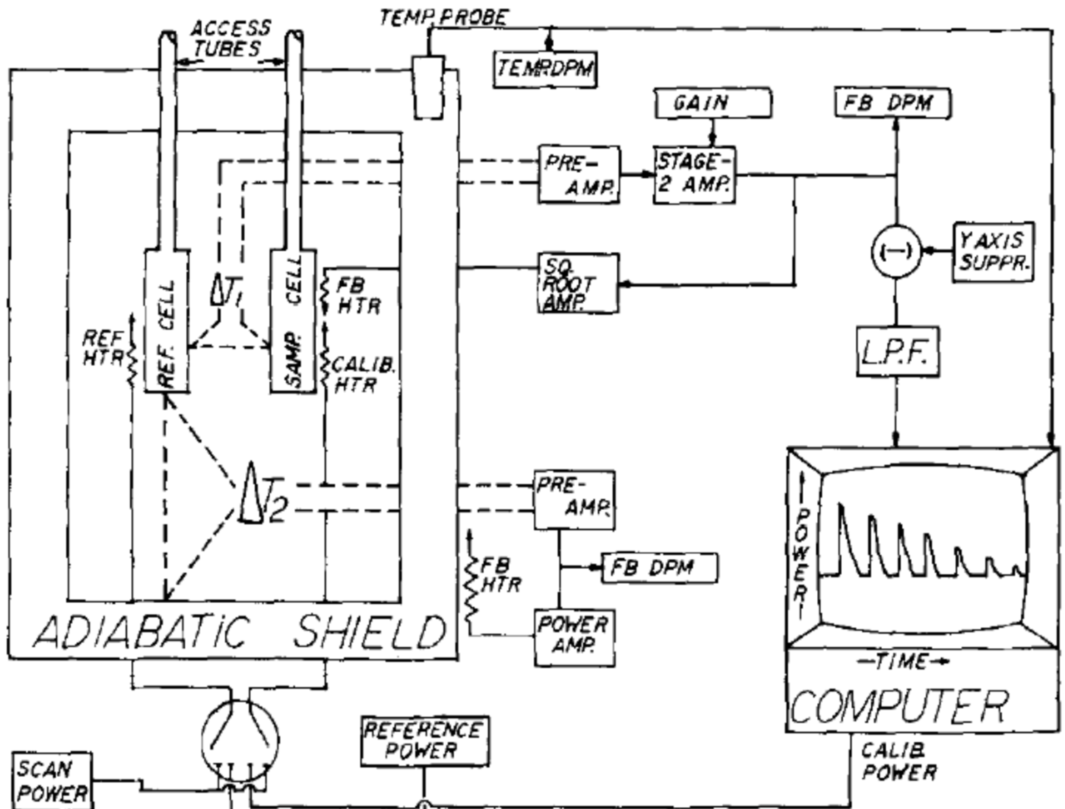


FIG. 1. Block diagram of the titration calorimeter showing the matched reference and sample cells with their access tubes, adiabatic shield, and electronic components which interface to an IBM PC/XT/AT or Series 2 computer. Although a preamplifier in the cell feedback circuit is contained within the instrument, better sensitivity results if a Keithley null detector is inserted to bypass the existing preamplifier.

Wiseman, et. al.

ITC Raw Data

- Raw Data
  - Y-axis: Power (work per time)
  - X-axis: Time
- Must integrate to get energy (heat)
- We'll discuss this in more detail next time

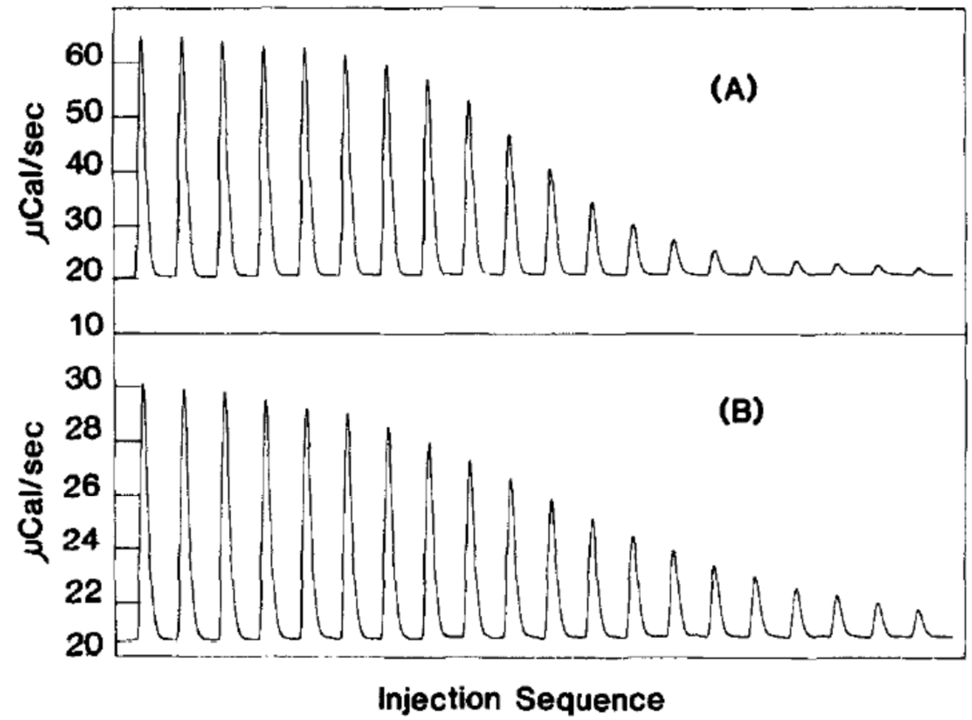


FIG. 4. Raw data obtained for 20 automatic injections, each of 4 µl, of 2′CMP solution into the sample cell containing RNase solution at a concentration of 0.651 mM (A) or 0.177 mM (B). The concentration of 2′CMP solution in the injection syringe was ca. 25 times higher than the RNase concentration in each case. Other conditions were the same (38°C, pH 5.5, 0.2 M KAc, 0.2 M KCl) for the two experiments. The total duration of each experiment was 41 min.

Wiseman, et. al.

Summary

- Any binding model is possible; use statistical methods discussed earlier
- ITC measures heat evolved as a new equilibrium is established
  - ITC is a series of ICE tables
  - Get thermodynamics using standard equations
- Apparatus measures power vs. time