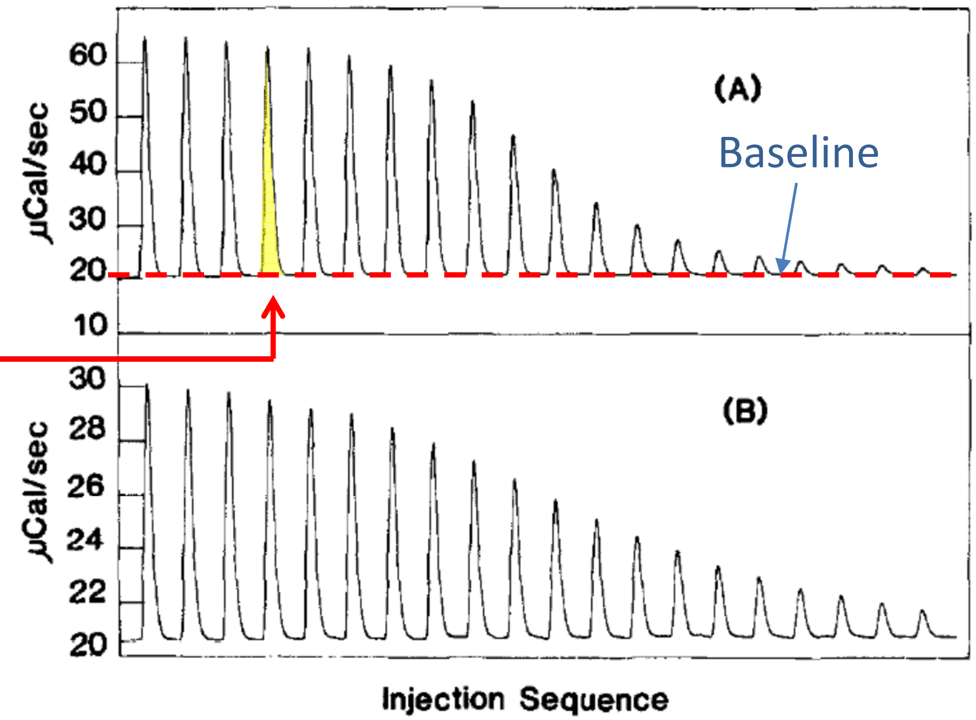
Transformation #1:
Calculating Heats from Power

- Plot of power vs. time: area under the curve is heat



$$q_i = \int_{start}^{end} P(t)dt$$

This is the heat evolved as the system re-equilibrates after an injection. Divide by known # of moles of titrant. (units = cal mol^{-1})

Wiseman, et. al.

Transformation #2:
Injection Number → Mole Ratio

- Concentration of titrant in syringe is known (L_s) as well as initial macromolecule (M_0)
- Typical sample cell is 1.5 mL (V_0), injections are 5 µL (ΔV)

- For injection n:

$$\frac{L_T}{M_T} = \frac{L_S \cdot n\Delta V}{M_0 V_0}$$

ITC Data

- Result of transformations:
  - **X-axis:** L_T/M_T
  - **Y-axis:** Heat per mole of titrant added

- How do we relate this to $\bar{\nu}$ (or anything else for that matter?)
  - Remember that $\boldsymbol{\bar{\nu} \cdot M_T = L_B}$ (concentration of titrant bound)

Thermodynamics of Binding

M + L ⇄ ML $\qquad \Delta \overline{H}^0 = x \text{ kJ mol}^{-1}$

- For every 1 mol of L that binds, x kJ of heat are evolved

- If $[L_{b,i}]$ and $[L_{b,i+1}]$ are the concentrations of bound protein for two consecutive injections, then:

$$q_{i \to i+1} = (L_{b,i+1} - L_{b,i}) \cdot V \cdot \Delta \overline{H}^0 = \underbrace{\Delta L_b \cdot V}_{\text{Concentration} \cdot \text{Volume} = \text{Moles}} \cdot \Delta \overline{H}^0$$

Thermodynamics of Binding

- If we could calculate the change in concentration of bound titrant (ΔL_b), then we could calculate heat per injection

Final, transformed heat →

$$q_{trans} = \frac{\Delta L_b \cdot V}{\Delta l_T} \cdot \Delta \overline{H}^0$$

← # of moles of titrant added; final units are kJ per mole titrant

Thermodynamics of Binding

- Alternatively, if I knew the change in ***number of moles*** Δl_b (moles, not molar) of bound titrant, then volume goes away:

$$q_{trans} = \underbrace{\frac{\Delta l_B}{\Delta l_T}}_{\text{This looks like a derivative...}} \cdot \Delta \overline{H}^0$$

Differentiation
(Here there be calculus!)

- Using N independent, identical site model:

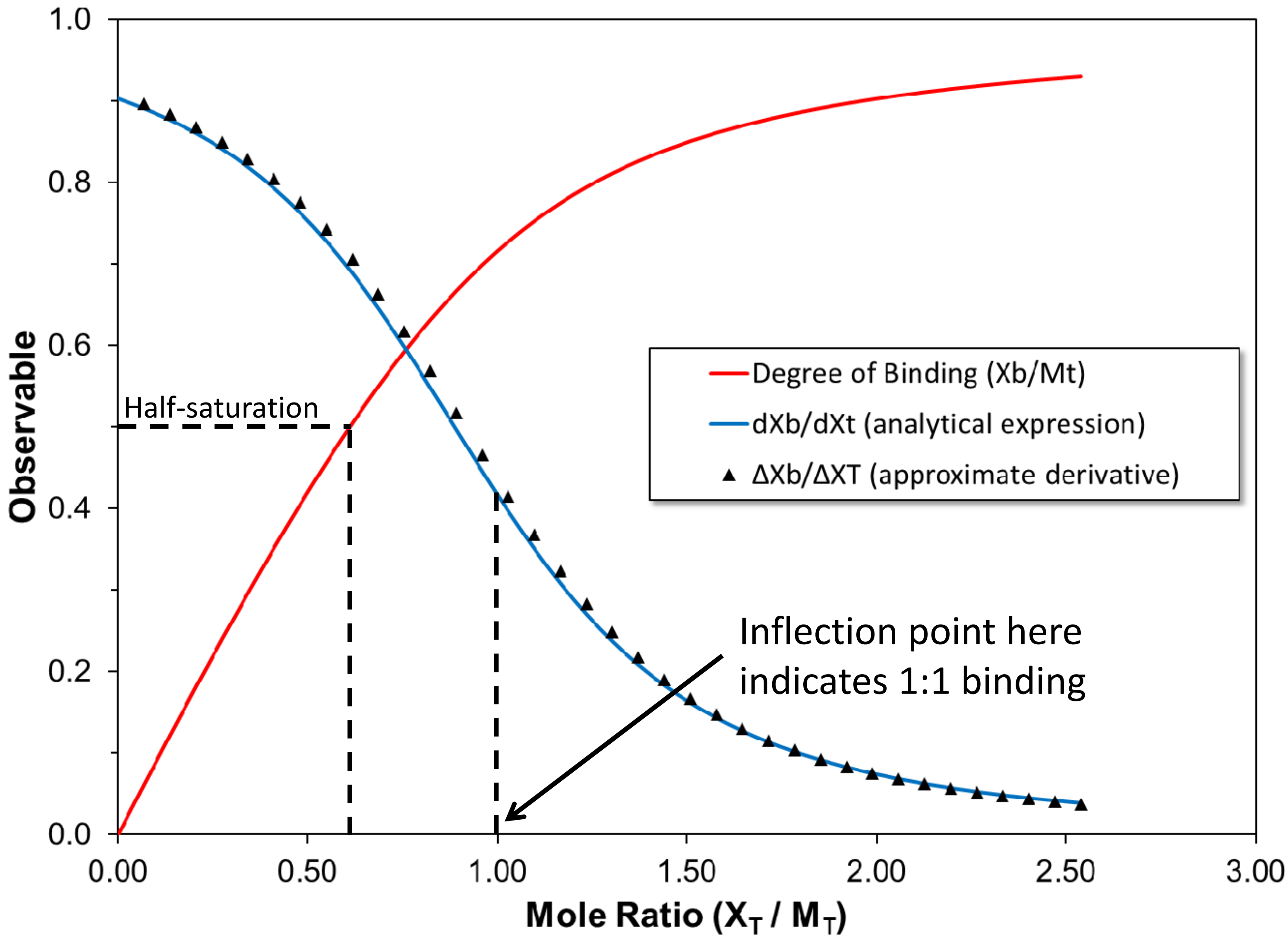
Nomenclature from Wiseman, *et al.*

$$\frac{d(MX)}{dX_{tot}} = \frac{\partial L_B}{\partial L_T} = \frac{\partial}{\partial L_T}(\bar{v} \cdot M_T)$$

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

- "How much additional binding is there when I add more ligand?"

Analytical vs. Approximate Derivative



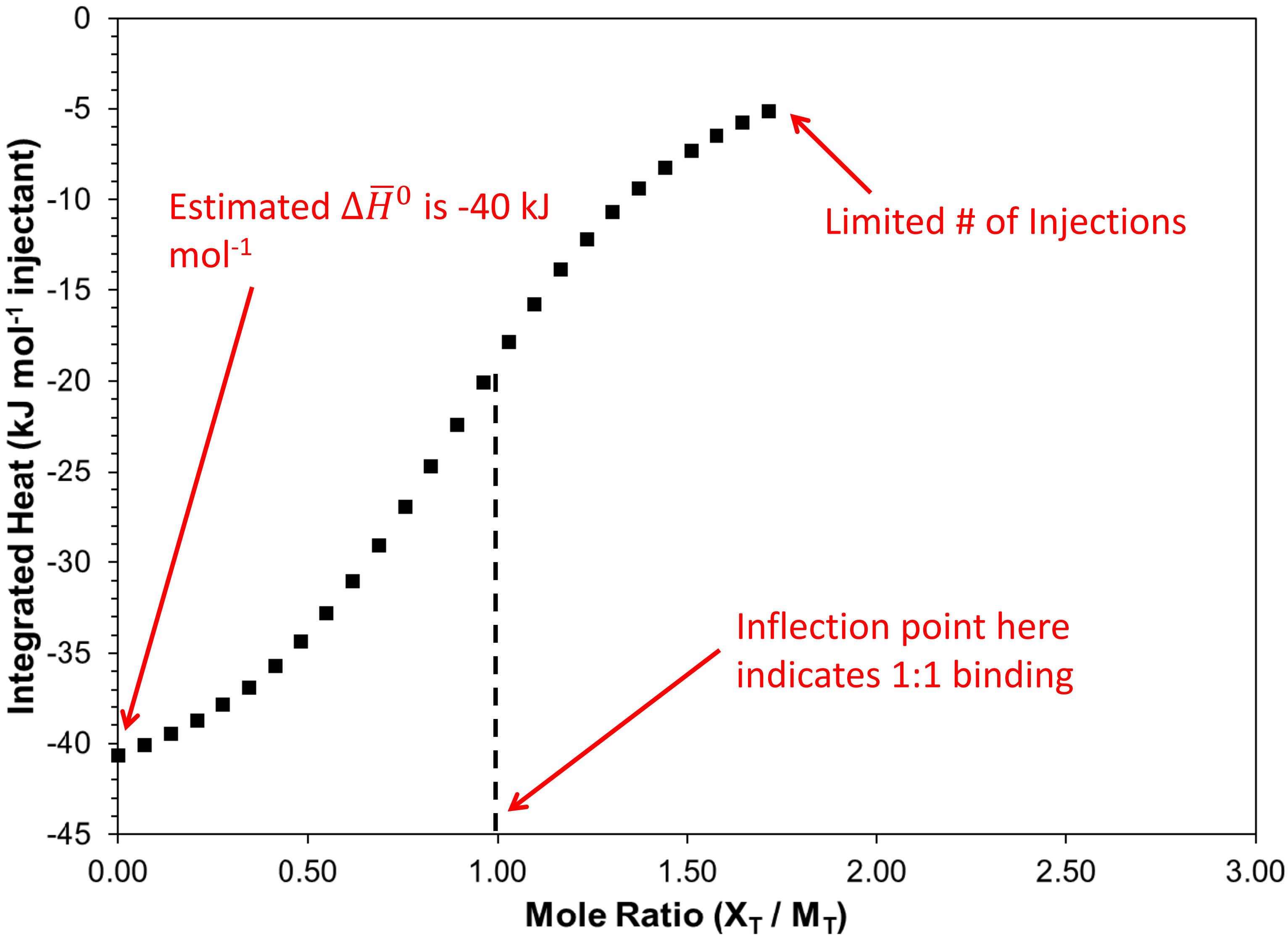
Solution for Independent, Identical Sites

- Heat evolved (q_{trans}) vs. mole ratio (L_T/M_T):

$$q_{trans} = \frac{1}{2}\left[1 + \frac{N - \frac{L_T}{M_T} - \frac{1}{M_T K}}{\sqrt{\left(N + \frac{L_T}{M_T} + \frac{1}{M_T K}\right)^2 - 4N\left(\frac{L_T}{M_T}\right)}}\right]\Delta\overline{H}^0$$

- Wiseman, *et al.*: Let $\mathrm{X}_r = L_T/M_T$ and $c = M_T K = M_T/K_d$
  - Technically, c also changes, because M is being diluted, but this effect is generally small

Simulated Data for RNase A – 2'CMP



Limits of ITC

- Remember that $c = M_T K = M_T K_d^{-1}$
- Larger c → tighter binding
- When binding is too tight, can't measure K (all step functions look the same)
  - Hence the range from 10^4 - 10^8

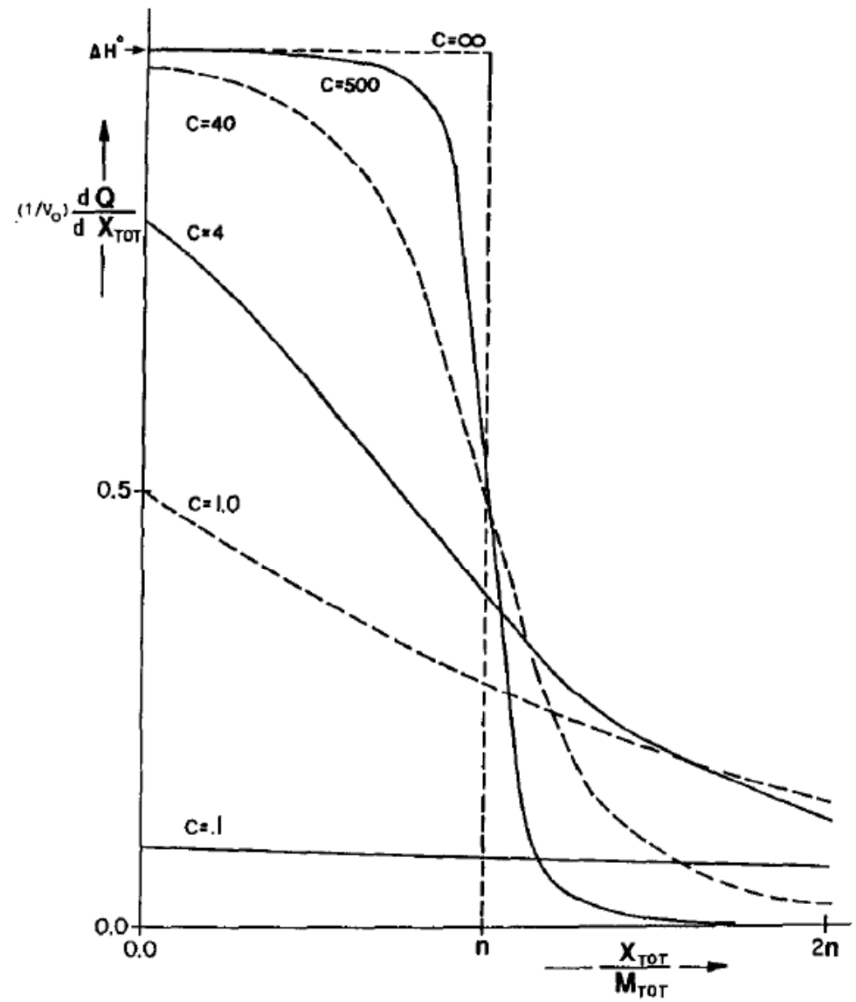
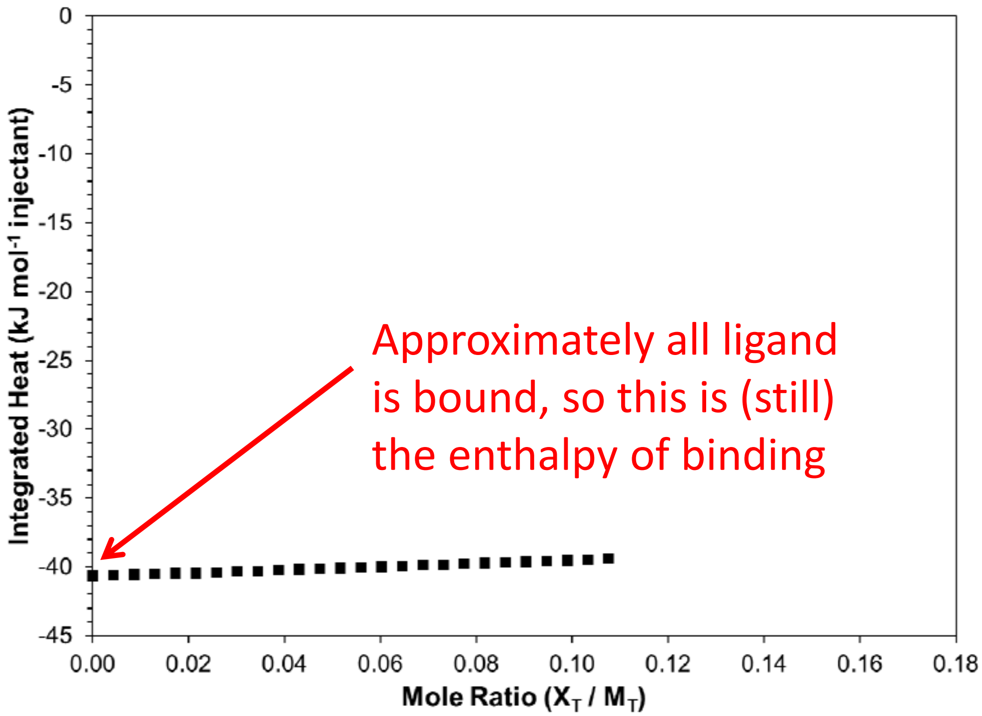


FIG. 3. Simulated binding isotherms for various values of the parameter *c* (equal to the product of the binding constant times the total macromolecule concentration), presented in derivative format. See text for details.

Wiseman, et. al.

Limits in Concentration



(This curve models a situation where the concentration titrant in syringe is low because of solubility limitations.)

- Provided that binding is tight (c > 10), one can estimate $\Delta\overline{H}^0$ even if binding can't be saturated
  - But, how do you know that c > 10? Need to test!

Your Turn: Simulate Data for Yourself!

- ITC worksheet used in this lecture has been emailed to you

- You will need to use this worksheet for your next assignment!

Summary

- Transforming raw power vs. time to useful data is not trivial
- **Key expression:** the change in bound ligand vs. the change in added ligand
  - If you know this, it is easy to calculate heat
- Number of binding sites and $\Delta \overline{H}^0$ can be estimated directly from transformed data