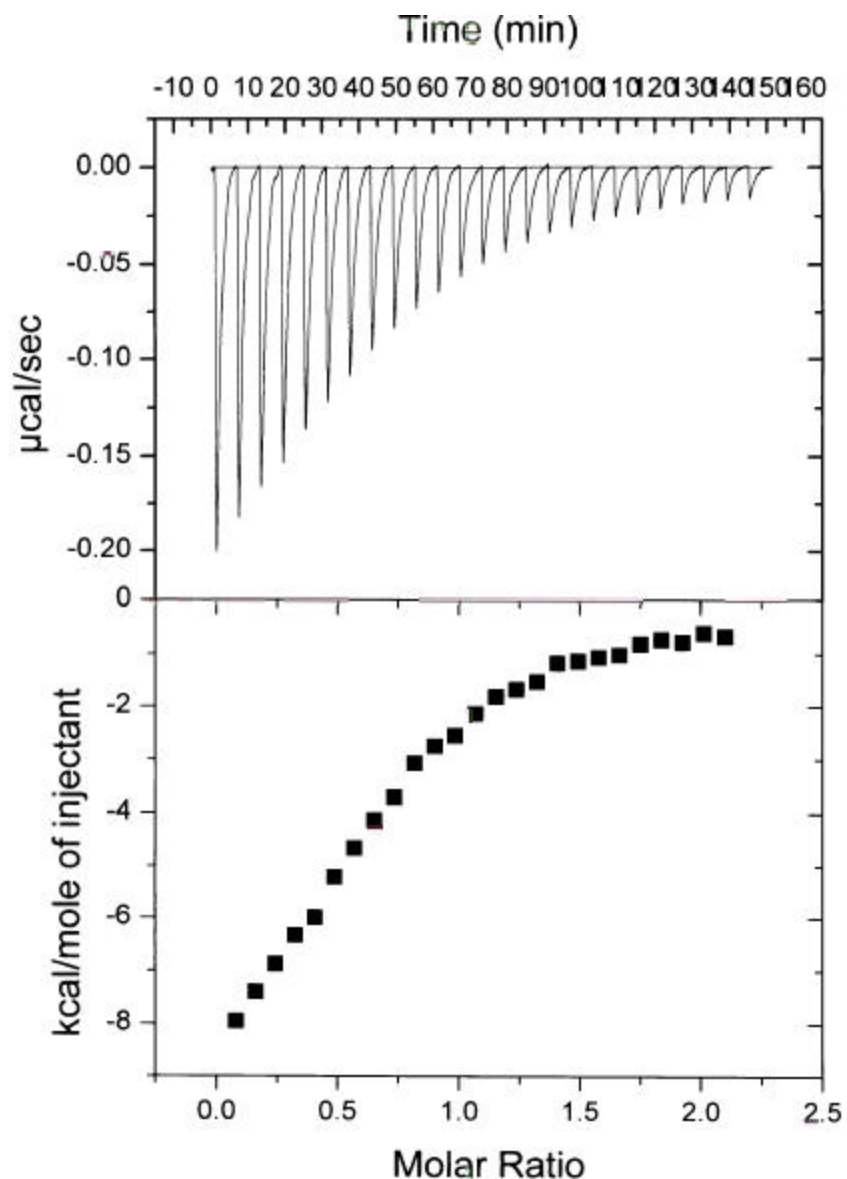


1. A given protein-ligand interaction has a  $\Delta G$  of  $-8.18$  kcal/mole and a  $\Delta S$  of  $10$  cal/mole $\cdot$ K. Calculate  $\Delta H$  (at  $25^\circ\text{C}$ ) and use this value (assume it is  $\Delta H_{\text{app}}$ ) to calculate the heat released by the 2<sup>nd</sup> injection of ligand ( $10\ \mu\text{L}$  of a  $300$  micromolar solution) into a  $1.5$  mL sample cell containing protein at a concentration of  $20 \times 10^{-6}$  M.
2. You have recently generated a small molecule (ATP-analog) kinase inhibitor for a given target protein in the lab. Your initial indications give a binding affinity (dissociation constant) of  $20$  nM. To completely characterize the inhibitor and provide some indication of how it may be improved in second- and third-stage iterations, you want to determine all of the thermodynamic parameters describing this protein-ligand interaction. For an ITC experiment (sample cell volume  $1.5$  mL), how much protein (assume  $50$  kDa for molecular weight) will be needed for a successful experiment? How much of your small molecule ligand (M.W. =  $342.3$ ) is necessary, and what concentration should you use to generate a final mole ratio of  $2:1$ ?
3. This same small molecule inhibitor is toxic to all cells (not the desired effect!) at concentrations above  $1$  micromolar. What is the maximum inhibition possible given a Kinase-ATP dissociation constant of  $75\ \mu\text{M}$  and  $[\text{ATP}] = 3$  mM?
4. As discussed in class, the pseudo-first order approximation can be used in calculating fractional saturation. What is the error associated with using this approximation if  $K_d = 10$  nM and  $[\text{L}]_o = 5[\text{P}]_o$ , and  $[\text{P}]_o = 10$  nM. How does this change if  $[\text{L}]_o = 50[\text{P}]_o$ ? Does the error change if  $K_d$  improves to  $100$  pM?
5. The following ITC data was collected in Forest White's laboratory for a certain protein-ligand binding reaction.



You will find a file on Assignments section associated with this pset called “pset2\_data”. Please download this file and load it into MATLAB using the command:

```
load 'pset2_data';
```

This will create a variable in MATLAB (a matrix) called “pset2\_data”. The first column of this matrix is the molar ratio and is unitless. This is the ratio  $[L]_T/[P]_T$  discussed in class regarding ITC data and is also the x-axis data from the lower plot above. The second column of this matrix is the Q term (in units of cal/mol) as discussed in class regarding ITC data, which is the integration of the area within each peak of the upper plot above. This corresponds to the y-axis of the plot above except for the units (the data is in cal/mol and the plot is in kcal/mol).

(a) Plot the concentration of injectant (in kcal/mol) at each molar ratio in MATLAB, creating a plot that looks like the lower plot above.

- (b) Use the given data to calculate the change in enthalpy ( $\Delta H$ ) for this reaction, in kcal/mol. The data you have been given spans the entire range for Q.
- (c) Assume that the concentration of protein stays constant in the sample cell throughout the experiment at 0.019 mM. (Note, this is not exactly true. The *amount* of protein in the sample cell does not change throughout the experiment. But a small amount of buffer is added with each ligand injection, so the concentration of protein actually decreases during the experiment from 0.02 to 0.01832). Using this assumption, estimate  $K_d$  and  $\Delta G$  for this binding reaction. This ITC experiment was performed at a temperature of 300K.
- (d) Determine  $\Delta S$  for this binding reaction.