Week 8 Recitation

Exam 1

Chapter 5: Statistical Thermodynamics

Updates

- Exam 1 is graded – need to check with the other TAs before reporting final scores, details on next slide!
- Assignment 6 grades will be submitted this coming weekend
- Prevailing Doodle poll opinion is that we should cover Chapter 5 content, but will discuss a problematic exam question…
- Chapters 9 and 10 are (kinetics) all we have after Chapter 5
- In weather conditions such as this, I feel bad asking you to come to campus when all your other classes are probably online – feel free to join over Zoom, I will still be here in person out of obligation.

Exam 1, Section 20497 (pending)

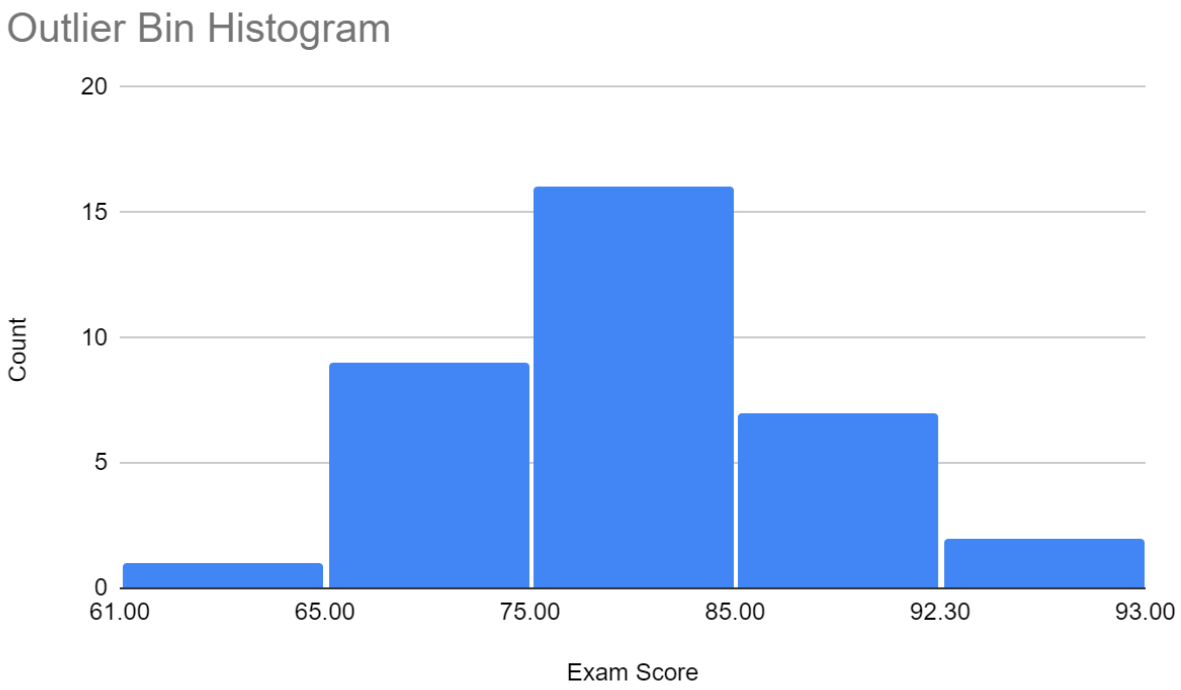
Average: 78.8

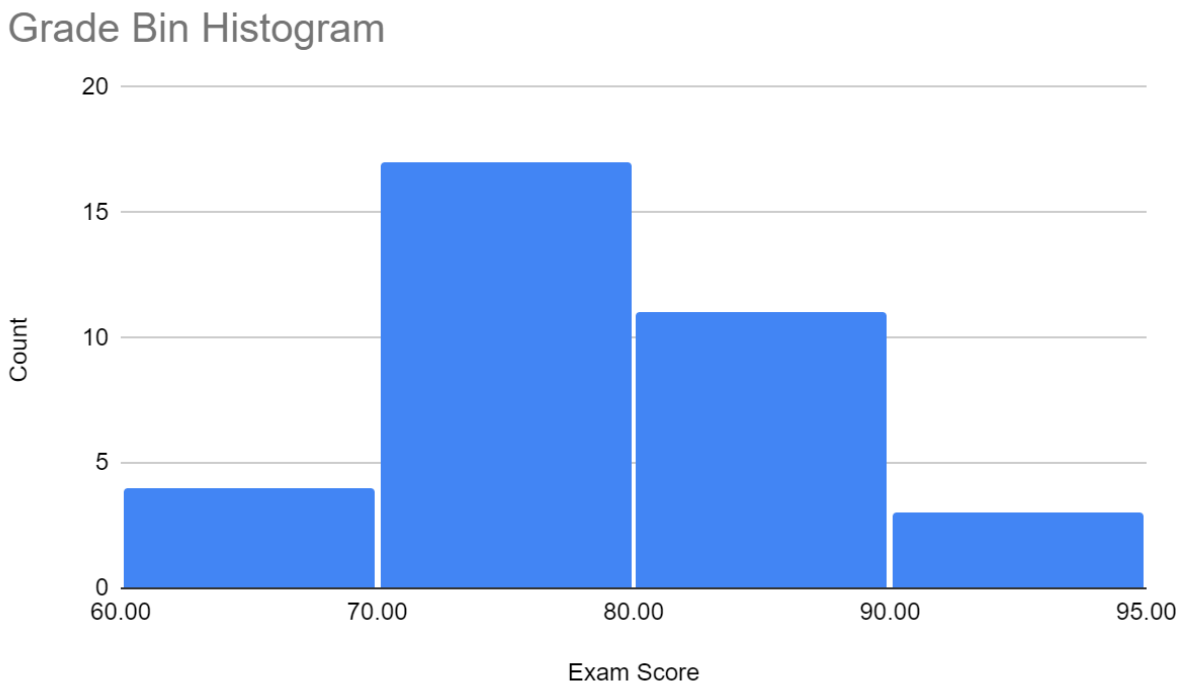
Median: 78

Standard Deviation: 7.948

High: 93 (x2)

Low: 61





Questions?

Exam 1

Section 2: Second Law, problem 4

Question: A globular protein undergoes thermal denaturation at 55˚C, what is the sign of entropy change for this process? Justify.

dS is **negative** for this process:

Denaturation exposes hydrophobic regions in the protein interior to the solvent. Hydrogen bonds between water (solvent) and protein are disrupted, which causes an ordering of water molecules around the protein (water cage). This increase in order is observed as a negative change in entropy for the system. (see pages 221-22)

The Partition Function

Definition: The weighted average number of states occupied at a given temperature (thermodynamic equilibrium, pg. 161).

$$Q = \sum_i g_i e^{-E_i/k_B T}$$

- i is the index of the microstate
- $\beta = 1/k_B T$
- E_i is the energy of the microstate
- g_i is the degeneracy factor – number of microstates possessing the same energy level i.e. $E_i = E_j$
- Ground (lowest) energy state has $E_i = 0 \therefore e^{-E_i/k_B T} = 1$ ($e^0 = 1$)

Ligand Binding: Independent Binding (K)

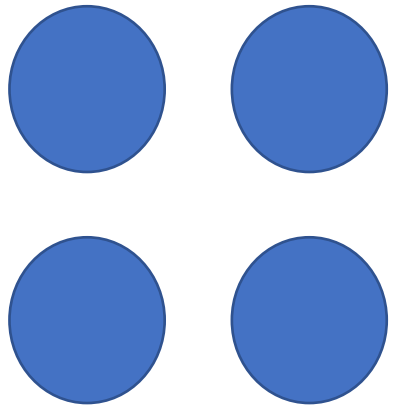
System: Evaluating protein P with **4 identical and independent** binding sites for ligand L:

$$P + L \rightleftharpoons PL \qquad K_1 = \frac{[PL]}{[P][L]}$$

$$PL + L \rightleftharpoons PL_2 \qquad K_2 = \frac{[PL_2]}{[PL][L]}$$

$$PL_2 + L \rightleftharpoons PL_3 \qquad K_3 = \frac{[PL_3]}{[PL_2][L]}$$

$$PL_3 + L \rightleftharpoons PL_4 \qquad K_4 = \frac{[PL_4]}{[PL_3][L]}$$



Note: No lines, independent.

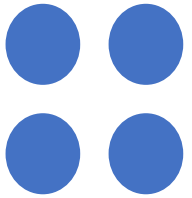
If there is some intrinsic binding constant K, then probability dictates observed binding, such that: $K_1 = 4K$, $K_2 = (3/2)K$, $K_3 = (2/3)K$, $K_4 = (1/4)K$

Ligand Binding: Independent Binding (Q)

System: Evaluating protein P with **4 identical and independent** binding sites for ligand L:

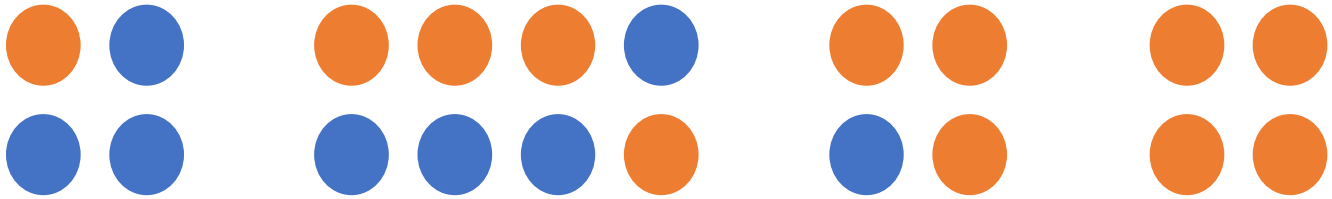
Important equations to be remember…

$Q = [P] + [PL] + [PL_2] + [PL_3] + [PL_4]$ AND $\tau^i S^j$



Unbound (ground) state, 1 poss.

Forms?



4 poss. 4 poss. 2 poss. 4 poss. 1 poss.

possibilities = degeneracy

Remember g_i?

$Q = 1 + 4S + 6S^2 + 4S^3 + 4S^4 = (1 + S)^4$, does this look familiar?

This is all well and good, but what about cooperative binding events?

Ligand Binding: Cooperative Binding (K)

System: Evaluating protein P with **4 identical and mutually cooperative** binding sites for ligand L:

How different is this from the previous case?

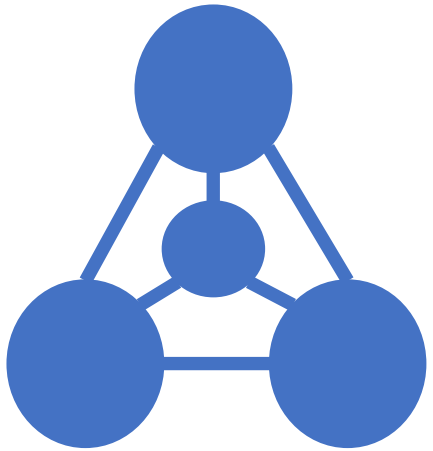
Ex. 5.12 (pg. 189): NAD^+ binding to tetramer

$K_1 = \mathbf{10^{11}}\ M^{-1}$

$K_2 = \mathbf{10^{9}}\ M^{-1}$

$K_3 = 3.3 \times \mathbf{10^{6}}\ M^{-1}$

$K_4 = 3.9 \times \mathbf{10^{4}}\ M^{-1}$



Note: Lines, dependent.

If there is some intrinsic binding constant K, then the binding constant for each event can be expressed as: $K_1 = K$, $K_2 = \tau K$, $K_3 = \tau^2 K$, $K_4 = \tau^3 K$.

Question: What is the approximate value of τ? Is this (+) or (-) cooperativity?

Ligand Binding: Cooperative Binding (Q)

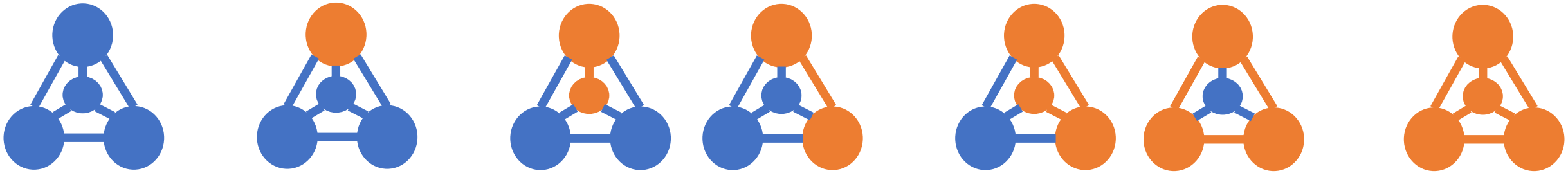
System: Evaluating protein P with **4 identical and mutually cooperative** binding sites for ligand L:

$Q = [P] + [PL] + [PL_2] + [PL_3] + [PL_4]$ AND $\tau^i S^j$

τ = cooperativity parameter

i = number of nearest-neighbor interactions

j = number of occupied sites



$Q = 1 + 4S + 6\tau S^2 + 4\tau^3 S^3 + \tau^6 S^4$

Ligand Binding: Finishing Remarks

- Familiarize with linear and square binding arrays – pay careful attention to connectivity in these cases!
- Excluded-site binding limit, cooperativity such that adjacent sites can no longer bind ligand ($\tau = 0$)
- Average number of ligands bound (v):

$v = \frac{1[PA]+\cdots n[PA_n]}{Q}$, where $[PA_n]$ = partition function term

From previous slide: $[PL_4] = \tau^6 S^4 \therefore 4\tau^6 S^4$ in numerator of v formula

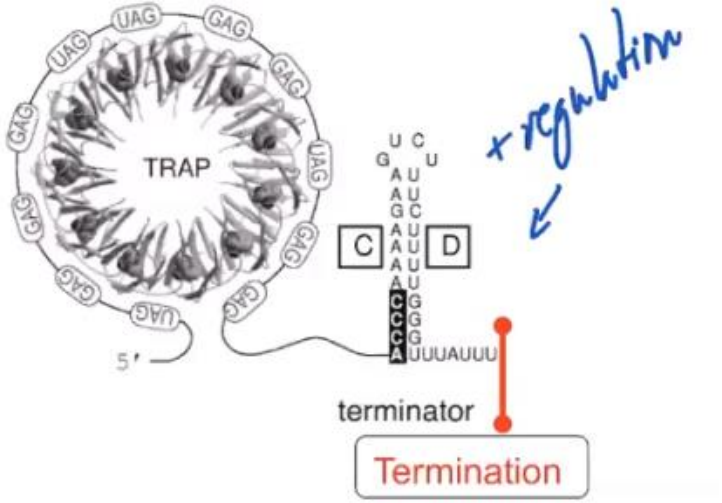
- $Y \neq v$:

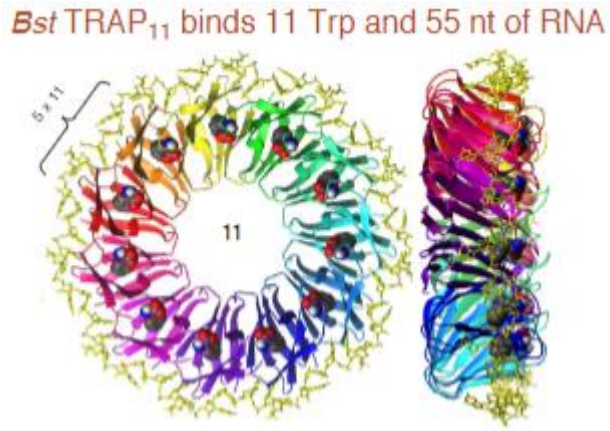
$$Y = \frac{v}{\#\ of\ binding\ sites}$$

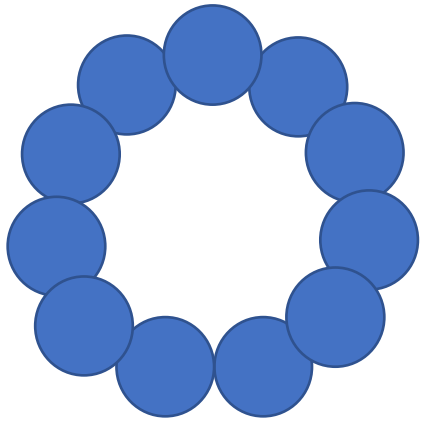
Comprehension check: Why do a random walk and identical-and-independent binding model share the same binomial coefficient?

TRAP

- Tryptophan-regulated attenuation protein (TRAP) [transcription termination]
- *trp* operon undergoes conformational switch that exposes termination hairpin







- Imagine cooperative "lines" between subunits

- The recorded lecture is a better overview of ITC – for those confused, think of the peaks as heat (calorimetry) required to keep instrument at same T (isothermal) as ligand is titrated.

Questions?

Have a good week!