Physics 4251 / Fall 2017
Problem Set 3, Sept. 15
Due: Friday, Sept. 22 before noon

Problem 1: Force of an entropic spring

A 1D freely-jointed chain composed of N segments each of length a responds to an applied force, F, by generating a restoring force, F_{restore}. In static equilibrium $F_{\text{restore}} = -F$; the restoring force contributes energy to the object/device applying the tension. The free energy of the **system** can thus be written as $G = -FL - TS$ (recall that the freely-jointed chain cannot store internal energy).

(a) What is the expected end-to-end length, L, in the case of no applied force (purely entropic)? What about at $T = 0$ (purely enthalpic)?

(b) Derive an expression for the entropy S in terms of N and n_R, the number of segments that point to the right.

(c) The relationship between force and end-to-end length shown in class was in the limit that $L << Na$, where Na is the fully extended length. Now, find the length that minimizes the free energy, i.e., $\partial G/\partial L = 0$, without taking this limit. You should find

$$L = aN \tanh(\frac{aF}{kT}) \tag{1}$$

Possible hint: Keep in mind that the end-to-end length L can be expressed in terms of n_R and n_L.

Problem 2: The cleavage mechanism of the autocatalytic transporter EspP

For this problem, we'll examine the structure of the protein EspP. EspP is an autotransporter expressed in the outer membrane of Gram-negative bacteria that secretes its own N-terminal domain to the outside of the cell. After secretion, this domain, a virulence factor, is auto-cleaved by the transporter and released.

(a) Download and examine the PDB for EspP (PDB ID 3SLT) in VMD. The site of catalysis is residue Asn1023, which can undergo "asparagine cyclization" to cleave the peptide bond between it and residue 1024. However, to crystallize the pre-cleavage state, Asn1023 had to be mutated to another amino acid. Which amino acid was used in this structure?

(b) Make a figure of the residues in the active site around residue 1023. Use a cartoon representation for the protein, but do not include the barrel. Then use licorice for all the residues within 8 Å of residue 1023. Color them by restype. Make a separate representation for 1023 such that it stands out from the others. Render and print this figure. Does anything stand out about the residues in the vicinity of 1023?

(c) Using the tkconsole of VMD, make atom selections to answer the following questions: how many negatively charged amino acids are present in the EspP structure? How many positively charged

ones? (Hint: you need to include something in your selection that identifies each residue only once.)

(d) Now we will do a pKa calculation to determine the actual charge states of ionizable residues. Go to the website `http://nbcr-222.ucsd.edu/pdb2pqr_2.0.0/`. Input the PDB ID, keep all default options, and click submit. Look at the output file "3SLT.propka". Although a lot of data are output, focus on the part near the bottom entitled "SUMMARY OF THIS PREDICTION". Pick three residues from the list, one that is typically negatively charged, one typically positively charged, and one histidine. Using the Henderson-Hasselbalch equation, calculate the probability that each is protonated at pH 7.0.

(e) Are any of the residues predicted to be in a non-standard protonation state at pH 7.0? Where are these residues located in relation to the active site? Which residue is closest? Does it make sense to alter the protonation state from its standard state? Why or why not?

Material for this problem derives from the paper "Molecular basis for activation of a catalytic asparagine residue in a self-cleaving bacterial autotransporter." T. J. Barnard, J. Gumbart, et al. *J*MB **415**:128-142, 2012.