Physics 4251/6250 – Fall 2019
Problem Set 3 Solutions
50 points in total

Problem 1: The cleavage mechanism of the autocatalytic transporter EspP

(a, 3). What amino acid was used in place of Asn1023?

Serine was used in this structure.

(b, 6). Make a figure of the active site and describe anything that may be unusual about it.

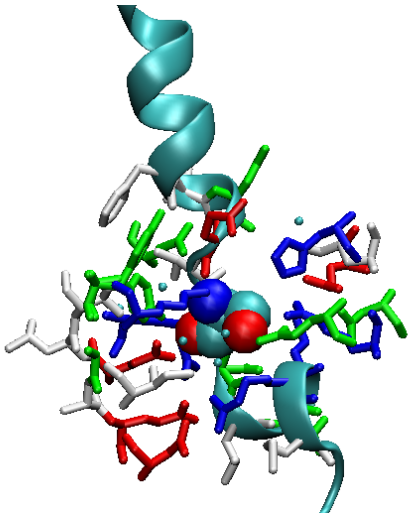

Figure 1:

See Fig. 1. For example, there are a large number of charged residues, particularly acidic (negatively charged), near the active site.

(c, 4). How many negatively/positively charged residues are in EspP?

Using the atom selection ``resname LYS ARG and name CA'', we see there are 34 positive residues (note that histidine is typically neutral). Similarly, with ``resname ASP GLU and name CA'', we find 39 negative residues.

(d, 6). Pick three residues from the pKa calculation and give their probability of being protonated.

$$\mathrm{pH} = \mathrm{pK}_a + \log(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}) \rightarrow p_{\mathrm{prot.}} = \frac{[\mathrm{HA}]}{[\mathrm{HA}]+[\mathrm{A}^-]} = \frac{1}{1+[\mathrm{A}^-]/[\mathrm{HA}]} = \frac{1}{1+10^{(\mathrm{pH}-\mathrm{pK}_a)}} \quad (1)$$

1. Negative: Asp1014, $\mathrm{pK}_a = 8.08$, $p_{\mathrm{prot.}} = 0.92$ (note that this is VERY unusual).

2. Positive: Lys1001, $\mathrm{pK}_a = 10.29$, $p_{\mathrm{prot.}} = 0.999$.

3. Histidine: His1062, $\mathrm{pK}_a = 2.63$, $p_{\mathrm{prot.}} = 4.3 \times 10^{-5}$.

(e, 6). What residues are predicted to be in a non-standard protonation state? Where are they located? Which is closest? Does it make sense to alter its state and why or why not?

An easy way to identify those residues that may be in non-standard states is to look at those basic (positive) residues with a pK_a below 7.0 and those acidic (negative) residues above 7.0 ($p_{\mathrm{prot.}} = 0.5$). Using this metric, the acidic residues Asp1014 (8.08), Glu1172 (8.23), and Glu1279 (7.38) are all predicted to be protonated. The latter two are near the active site, with Glu1172 being the closest. Given the close distance between the carbonyl O of Ser1023 and the O of Glu1172 (2.765 Å), it makes sense that it should be protonated to prevent repulsion of these two negative atoms. See Fig. 2.

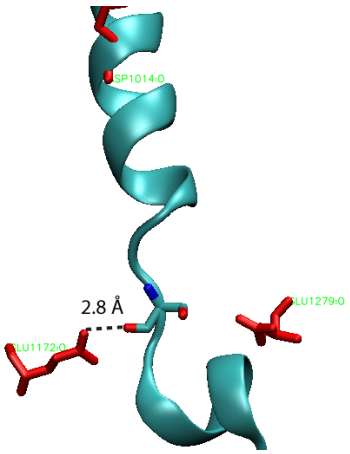

Figure 2:

Problem 2: Worm-like chain model applied to protein extension

(a, 5) What is the behavior of z in the high and low force limits?

In the high-force limit, looking at the right-hand side of Eq. 1, there are two ways to increase it, either $z \to \infty$ (clearly a physical impossibility), or for $(1 - z/L_C)^2 \to 0$. This implies that $z \to L_C$. The force dependence becomes $F \approx 1/4(1 - z/L_C)^2$.

In the low-force limit, we assume that z is small compared to L_C and approximate it as

$$\frac{z}{L_C} + \frac{1}{4(1 - z/L_C)^2} - \frac{1}{4} \approx \frac{z}{L_C} + \frac{1}{4}(1 + 2z/L_C) - \frac{1}{4} = \frac{3}{2}(\frac{z}{L_C}) \tag{2}$$

which tends toward 0.

(b, 5) What is kT at 300 K in units of pN·Å?

$$kT = 1.38 \times 10^{-23} \frac{\mathrm{J}}{\mathrm{K}} \times 300\,\mathrm{K} = 4.14 \times 10^{-21}\mathrm{J} = 41.4\ \mathrm{pN} \cdot \mathrm{\AA} \tag{3}$$

(c, 5) What is the expected value of L_C ?

The average length of each segment is 3.8 Å. Therefore, for the N terminus (residue 1) to K48, the contour length is $L_C = 47 \times 3.8\,\mathrm{\AA} = 178.6\,\mathrm{\AA}$. Using 48 residues is okay, but since pulling was done between C_α atoms, technically there are only 47 links between the two.

(d, 5) Fit Eq. 1 to the provided data and determine the persistence length.

If you look closely, you'll note that I shifted the data to start near zero force when compared to the figure in the homework. It makes the fit easier but strictly speaking is not okay to do.

Eq. 1 can be rearranged to give

$$F = \frac{1}{l_p}[kT(\frac{z}{L_C} + \frac{1}{4(1 - z/L_C)^2} - \frac{1}{4})] = \frac{1}{l_p} g(z) \tag{4}$$

with which one can carry out a least-squares linear fit between F and $g(z)$. The value obtained for l_p is 4.7 Å.

(e, 5) Plot the fitted equation and the original data and compare to the accepted value of $l_p \approx 4$ Å.

See Fig. 3. Our value of l_p is a little more than the accepted value, but reasonable.

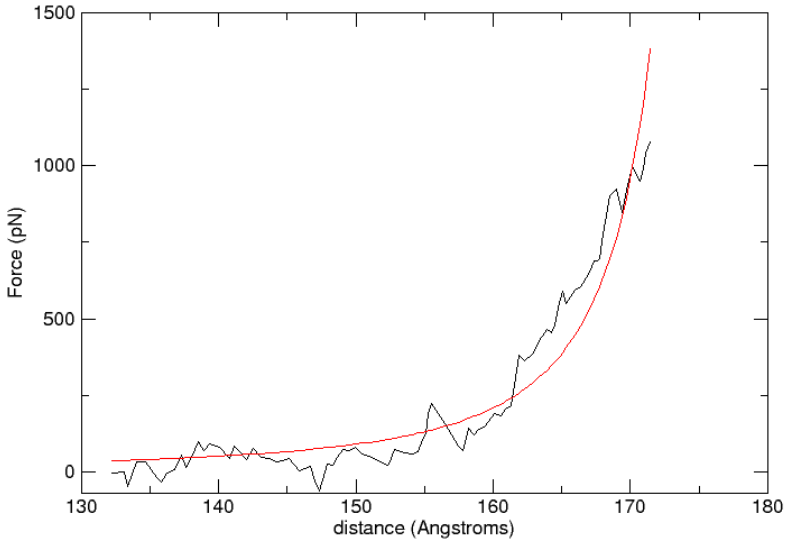

Figure 3: