

## Problem set 2 (Discussion on April 29)

### Problem 1

**Protein synthesis.** Bacteria divide every 20 min. Estimate the synthesis rate of ribosomes in units of amino acids per seconds assuming that there are about  $20 \cdot 10^3$  ribosomes at work. Hint: estimate the size of bacteria (see Problem Set 1 and lecture slides); estimate then the total amount of protein and the average molecular mass of an amino acid.

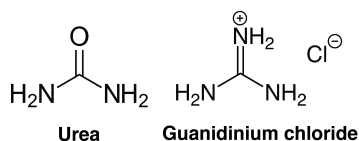
### Problem 2

**Two-state protein folding.** Consider a simple protein that undergoes *two-state folding*, i.e. there is the unfolded state  $U$  and the folded, native state  $N$ . Two-state folding is a good approximation for many small proteins. We will only consider the folding equilibrium here, i.e. we will not look at how folding or unfolding evolve over time. Under a given set of solution conditions, the concentration of folded protein is  $[N]$  and of unfolded protein  $[U]$ . We can define the chemical equilibrium for the folding reaction with the equilibrium constant  $K_{eq}$ :

$$K_{eq} = \frac{[N]}{[U]} = \frac{1 - f_U}{f_U} \quad (1)$$

where  $f_U$  is the fraction of unfolding.

- a) The fraction of unfolding is defined as  $f_U = [U]/([U] + [N])$ . From this definition, show the right hand side of Equation (1). Hint: this should be easy.
- b) The equilibrium constant is related to the free energy of the native state relative to the unfolded state:  $\Delta G_{fold} = G_N - G_U = -RT \ln K_{eq}$ , where  $R$  is the ideal gas constant and  $T$  the absolute temperature. Derive an expression for  $f_U$  as a function of  $\Delta G_{fold}$ . Note: you can derive the same expression using Boltzmann factors and partition functions.
- c) The two most frequently used denaturants for proteins are urea and guanidinium chloride (often written GdnHCl), shown below. The effect of these denaturants on the free energy of folding is often in good approximation linear:  $\Delta G_{fold}([D]) = \Delta G_0 + m \cdot [D]$ , where  $\Delta G_0$  indicates the thermodynamic stability of the protein in the absence of denaturant and  $m$  is a coefficient that describes the dependence of the free energy on the denaturant concentration  $[D]$ . Assuming values of  $\Delta G_0 = -4$  kcal/mol and  $m = 2$  kcal/(mol·M), plot  $f_U$  vs.  $[D]$  in the range 0 to 5 M.



- d) What is the fraction of unfolded protein  $f_U$  in the absence of denaturant using the data from the previous part?

### Problem 3

**Exploring the 3D structure of proteins.** The aim of this exercise is to familiarize yourself with a molecular viewer, a program that allows you to display, visualize, and manipulate the 3D structures of macromolecules.

- a) Download and install a molecular viewer of your choice on your computer - or find a computer with a viewer already installed. Popular choices are **Visual Molecular Dynamics** (VMD for short), **RasMol**, or **PyMol**. Follow the instructions on the respective websites for installation on your computer.

VMD: <http://www.ks.uiuc.edu/Research/vmd/>

RasMol: <https://www.umass.edu/microbio/rasmol/getras.htm>

PyMol: <http://www.pymol.org>

Note: The **Protein Data Bank** website at <http://www.rcsb.org> offers several in-browser visualization tools as well.

- b) Myoglobin was the very first protein for which an X-ray crystal structure was determined. It is critical for the transport of oxygen. Download a file that has the 3D structure information for the protein myoglobin. You can obtain the file by going to the website of the **Protein Data Bank** (PDB): <http://www.rcsb.org>.

To find the myoglobin structure, enter the four character structure identifier for myoglobin 1MBN into the search field. On the page with the myoglobin entry, you can find a lot of information. On the top right, you find a menu with download options (“Download Files”). Select “PDB File (Text)” and save the file to your computer.

- c) Open the PDB file with the molecular viewer. In VMD, this is accomplished by opening the “File” menu, selecting “New Molecule”, “Browse” for the PDB file that you have downloaded, and click on “Load”. Render the myoglobin structure in a way that you can inspect the  $\alpha$ -helices; for example, use the cartoon representation for the  $\alpha$ -helices (in VMD, select the “Graphics” menu, open “Representations”, and select “Cartoon” from the “Drawing Method” tab). How many  $\alpha$ -helices are there?
- d) Render the heme of myoglobin (in the “Graphical Representations” window, select “Create Rep” and type “not protein” into the “Selected Atoms” field; select for example “Drawing Method” “Licorice”) and the hydroxide ion in the oxygen binding site (create another representation for the atom selection “resid 154” and select for example “VDW” as “Drawing Method”). Is the oxygen in the plane of the heme?
- e) Select one other molecule from Jane and David Richardson’s article *Biophysical Highlights from 54 Years of Macromolecular Crystallography* (Biophysical Journal, 2014; the article is available on the course website). Include a nice rendering of your molecule of choice with the solution of your problem set.