Problem set 2
(Hand in by May 11)

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

DNA thermodynamics and kinetics. Using experimental values combined with theoretical models makes it possible to estimate thermodynamic properties and calculate kinetics of interacting DNA molecules. Here, you will do some calculations to develop a feeling for time scales and conditions for DNA hybridization reactions.

a) Based on information from the lecture and the article *The Thermodynamics of DNA Structural Motifs* (Annu. Rev. Biophys. Biomol. Struct. 2004, available on the course website), calculate the free energy for the following DNA duplex using the nearest neighbor model with standard conditions (1 M NaCl, 37 °C, 1 μM concentration of strands):

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5'-CTAGATGTAT-3'
3'-GATCTACATA-5'
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b) Perform the same calculation with the web service `NUPACK` (www.nupack.org) and compare the obtained values for the free energy of the duplex. (Hint: Pay attention to enter both strands in 5' to 3' notation! Would you expect the same or different results compared to your 'manual' calculation? Why yes? Why no?)

c) Using `NUPACK` with the same conditions as in b), perform a melting curve analysis from 5 to 60 °C in 2 °C steps, export the data and try to fit it with an appropriate mathematical expression to determine the melting temperature of the duplex. (Hint: The melting temperature is defined as the temperature where 50 percent of the bases are unpaired).

d) Based on the obtained free energy value from `NUPACK` for the duplex above, estimate the dissociation constant k_{off} (also named k_- in the lecture) at 37 °C, assuming an association rate k_{on} (also called k_+ in the lecture) of $10^6(\mathrm{Ms})^{-1}$.

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) Myglobin 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 α-helices; for example, use the cartoon representation for the α-helices (in VMD, select the "Graphics" menu, open "Representations", and select "Cartoon" from the "Drawing Method" tab). How many α-helices are there?

d) Render the heme of myglobin (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.