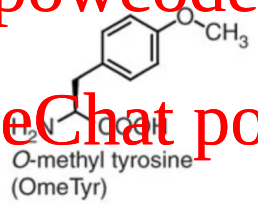
Midterm Practice Problem Set 1

Question 1: Central dogma

A synthetic mRNA has the following sequence:

5'- CAAUGGGCCUGGACUACAUACGACUCACUAUAGGGUAGGAAUAA -3'

a) What is the sequence of peptide translated from this mRNA?
b) If this mRNA naturally exists in E. Coli, what is the DNA sequence that this mRNA is transcribed from?
c) If this mRNA naturally exists in human, would the DNA have the same sequence as the answer for part (b)? Why or why not?
d) In an experiment researchers constructed DNA oligonucleotides with the same sequence as the answer for part (b), and found its melting temperature to be 66.7 °C. If you constructed a duplex oligonucleotide with the given mRNA and an RNA with complementary sequence, will its melting temperature be higher or lower than the DNA oligo? Why?
e) Researchers have constructed a tRNA carrying unnatural amino acid O-methyl tyrosine with anticodon CUA (Wang et al. *Nat Neurosci*. 2007). If this tRNA is introduced to the translation system for our synthetic mRNA, will the overall charge of translated peptide change? Why or why not?



O-methyl tyrosine
(OmeTyr)

Wang et al., Nat Neurosci (2007)

Question 2: Energy transfer

Draw the chemical structure of ATP and circle the three phosphate groups. How many high energy bonds are there in ATP? The energy release from hydrolysis of one ATP molecule (30 kJ/mol) is sufficient to form a glycosidic bond in sucrose (27 kJ/mol). Is one ATP molecule enough to fuel the formation of one hydrogen bond? Why or why not?

Question 3: Bonds and molecular interactions

Glutenin, a wheat protein rich in disulfide bonds, is responsible for the cohesive and elastic character of dough made from wheat flour. Similarly, the hard, tough nature of tortoise shell is due to the extensive disulfide bonding in its keratin. What is the molecular basis for the correlation between disulfide-bond content and mechanical properties of the protein?

Question 4: PCR

Describe the overall process of PCR. Use schematic diagrams to illustrate each step involved in the process. What is reverse transcriptase PCR and how does it differ from regular PCR? What is qPCR and what can you use it to calculate?

Question 5: Hemoglobin kinetics

Hemoglobin is an essential protein responsible for shuttling oxygen through tissues in the body. Hemoglobin is comprised of several large peptide structures, each containing a heme molecule - an organic porphyrin ring structure chelated with a central iron atom that binds oxygen in its ferrous (Fe^{2+}) state.

a) Describe the tertiary and quaternary structure of hemoglobin, as well as the type of force responsible for self-assembly into the final multi-subunit structure.
b) Oxygen binding produces conformational changes in hemoglobin from a tense state (T) to a relaxed state (R), which in turn alter oxygen binding properties. 1) Describe the transition from the unbound state (T) to the bound state (R), and the effect this has on oxygen binding. 2) Why does this make physiologic sense?
c) Steric interactions between bound oxygen and amino acids in the hemoglobin peptide chain are critical to enzyme function, in particular the His residue on the E7 chain. What would happen to oxygen binding if the His(E7) was replaced by a Glycine?
d) Draw the binding curve for hemoglobin for part c). How does this affect K_d?

Question 6: Inhibition Kinetics

You have just received your flu shot for this year. After getting the shot, you begin to notice some inflammation around the point of injection. Imagine now that you have the special ability to view the complex reactions occurring under your skin that's causing the inflammation. You observe that a certain fatty acid (let's call this FA) is being oxidized into a derivative (let's call this FAD) by an enzyme (let's call this FADase) that is causing the inflammation and some pain.

$$FA + O_2 \xrightarrow{\text{FADase}} FAD$$

You observe this reaction and note down the values for the reaction kinetics (Table 1, columns 1 and 2). In an effort to ameliorate this inflammation and associated pain, you decide to take a certain amount of an anti-inflammatory drug (let's call this antiFAD). Using your special skills to view the enzyme reactions, you take another observation of the reaction kinetics after taking the drug and record them in column 3 of Table 1.

Table 1:

Concentration of FA (mM)	**Rate of Formation of FAD (mM/ min)**	**Rate of Formation of FAD in the presence of antiFAD (mM/min)**

0.49	23.45	16.67
1.1	32.32	25.25
1.48	37.00	30.52
2.48	42.10	36.90
3.53	43.89	39.00

(a) From column 1 and 2, determine the maximum rate of formation of FAD from FA when no drug is administered. Also determine the Michaelis-Menten constant of this reaction.

(b) From columns 1 and 3, determine the maximum rate of formation of FAD from FA when the drug antiFAD is administered. Also determine the Michaelis-Menten constant of this reaction.

(c) From your calculations and plots, provide an explanation for how anti-FAD helps reduce your inflammatory response to the injection. What type of inhibition is this? Explain your reasoning.

For (a) and (b), include plots used for your calculations.

Question 7: Enzyme function

Biochemical reactions rely on enzymes, proteins that catalyze otherwise unfavorable reactions, to drive the biologic processes necessary for life. Describe how enzymes make these reactions more favorable, as well as describing three thermodynamic factors contributing to this process. Include a diagram illustrating the energy differences between normal and enzyme-catalyzed processes.

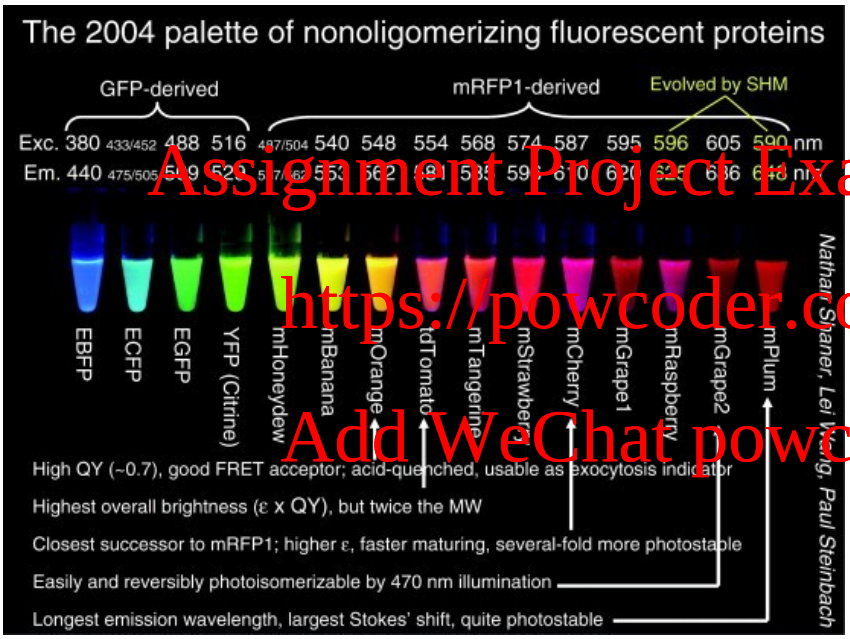
Question 8: Paper/technique question

Directed evolution has emerged as a powerful means for rapidly developing and deploying biological molecules with altered/enhanced properties.

a) How does a directed evolution-based approach differ from a rational design-based approach?
b) Researchers can take a few different approaches to directed evolution, in particular sequential random mutagenesis, random mutagenesis, and homologous recombination. For each method describe one advantage and one disadvantage.
c) What steps would you take to create a random mutagenesis library for a particular protein?

Question 9: Paper/technique question

Green Fluorescent Protein (GFP) has various applications today in *in vivo* imaging, allowing biologists to be able to track the activity of various proteins in cells through fusion protein constructs that can be transfected into cells. The original wtGFP protein was isolated successfully from the underbelly organs of the jellyfish species in 1962 by Osamu Shimamura. In 1996, its crystal structure was solved and the underlying cyclic tripeptide chromaphore responsible for the protein's fluorescence was discovered. Since then, through various site-directed mutagenesis efforts over the next 40-50 years, the cyclic tripeptide portion and other domains of the protein were modified which changed the physical properties and the excitation and emission spectra of the protein, producing a palette of variants collectively called "enhanced proteins" or EPs.



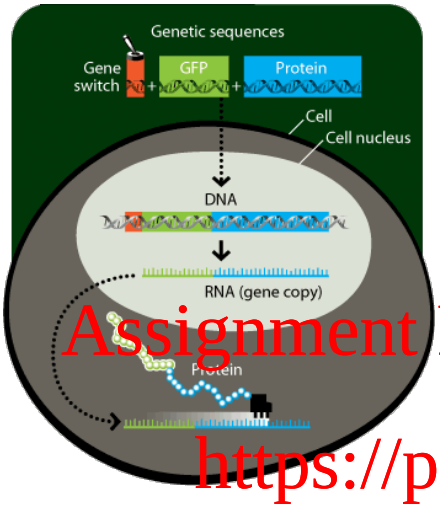
Tsien et al., FEBS Letters (2005)

1. One of the reasons why the use of EPs for live cell imaging was such a "revolutionary" in cell biology is because this protein could be used under a variety of environmental factors and can resist changes in pH, temperature, fixation in formaldehyde and even resist the action of several denaturants like urea and guanidinium hydrochloride. **Characterize the crystal structure of wtGFP and explain why this structure greatly enhances the stability of this protein**.

2. The enhanced GFP (EGFP) variant was made by a S65T modification using site-directed mutagenesis. Just this one modification made the entire protein have a completely different excitation-emission spectra. This EGFP is considered the standard for live cell imaging and in fact, wtGFP by itself is almost never used for imaging applications today. **Draw the excitation-emission spectra of wtGFP and EGFP.**

Explain why EGFP was so much better to use for live cell applications.

3. The ability to track a protein of interest in a live cell using GFP was possible by constructing fusion proteins- two separate genes connected with a linker such that they are always transcribed and translated as a single unit producing a single polypeptide. The construct was made such that the final translated product after transfection into the cell is the protein of interest connected to a GFP by a linker (see below). Thus, whenever the protein of interest was expressed in the cell, GFP would also be expressed.



(right)The touch-receptor proteins fused with GFP lights up the nervous system of C. elegans; C halfie et al., Science 1994

Besides the issue with the excitation and emission spectra of wtGFP that you answered in question found to be promoting this dimerization were A206, F223 (or Phe223), and L221.

(i) Why do these 3 sites specifically promote dimerization in wtGFP (ie what forces are promoting this?)? Why does this dimerization make wtGFP unsuitable for live cell imaging?

(ii) In EGFP and other variants, modifications through site-directed mutagenesis have been made for these three locations to remove this dimerization issue. Mention one of these modifications and explain why this modification prevents GFP dimerization.