Name__________________________ Section_________

7.013 Problem Set 2 – Spring 2003
The completed problem must be turned into the wood box outside 68-120 by 4:40 pm, Thursday, February 27. Problem sets will not be accepted late.

Solutions will be put on the web after the due time.

Question 1

You have purified a thermostable DNA polymerase enzyme from bacteria that normally live in hot springs, and are now trying to replicate a short, linear piece of DNA in a test tube. However, you do not have any other enzymes that normally facilitate DNA replication at your disposal. Luckily, you are pretty smart, and you realize that you can use heat to separate the two strands of a double stranded DNA molecule (also known as DNA denaturation). Shown below are the two DNA molecules you have.

1. 5’ AATATTTTAAACGCCATAA 3’
   3’ TAATTAATAAATTGGCGGTATT 5’

2. 5’ ATTAATAGGCCCGCCATAA 3’
   3’ TAATTAATCCGGCGCCGTATT 5’

a) If you want to use the molecule that denatures at a lower temperature, would you choose number 1 or 2? Why?

You are all ready to proceed with the DNA molecule that has a lower denaturation temperature, when you remember that you are also missing the primase enzyme. Fortunately, your best friend (who’s also a smart cookie) has just figured out how to synthesize short single stranded primers. After looking at your DNA sequence, he offers you four different primers shown below. (These DNA primers will work as well as RNA primers.)

1. 5’ ATTAA 3’
2. 5’ TTATG 3’
3. 5’ CATAA 3’
4. 5’ TTATTG 3’

b) Which two primers will be useful for completely replicating the DNA molecule you have chosen? Explain your choices
Finally, you are ready to start your experiment. You add all the necessary components, including DNA, primers, DNA polymerase, and dNTPS. However, it turns out that while you were relaxing at the CBC last night, your evil lab mate has replaced your stock of dCTP with ddCTP (shown below).

\[ \text{\begin{align*}
\text{PO}_4^{2-} & \quad \text{PO}_3^- \\
\text{PO}_3^- & \quad \text{HCH} \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{Cytosine Base} & \\
\end{align*}} \]

c) How is \textit{dideoxy CTP} different from \textit{deoxy CTP}?

d) How will this difference affect your experiment?

e) Write the sequences of the two resulting double stranded DNA molecules (after 1 round of replication). Please indicate polarity (5' and 3') and designate ddCTP as "c'." Use the molecule with the lower denaturation temperature as the template.

After figuring out what went wrong, you repeat your experiment using dCTP and finally succeed in replicating your DNA molecule. Yeah!
Question 2

Although Valentine's Day has passed, you are still waiting to take the current love of your life out for a fancy dinner. One would have to be a sucker to go out on Valentine's Day: the crowds, the overpriced fixed menus...yuck! So at the end of February the two of you finally go to Giacomo's, your favorite Italian restaurant. As soon as you sit down, the waiter brings out some fresh bread with both butter and olive oil. Reminiscing about a recent 7.013 lecture, you draw structures of molecules that are likely to be present in both butter and olive oil on your napkin (see below).

1.

![Butter Molecule](image1)

2.

![Olive Oil Molecule](image2)

a) Which of the above molecules is likely to be more abundant in butter____, in olive oil____? Why?

b) Please indicate the hydrophilic as well as the hydrophobic regions of each molecule you chose in a).
You stick to olive oil, because you hear that it’s better for you. Unfortunately, you somehow manage to drip some of it onto your brand new shirt. You have the following substances at your disposal.

Choice 1. H₂O and NaCl

Choice 2. H₂O and....

Choice 3. White wine (about 10% CH₃CH₂OH)

c) Which do you chose to get out the olive oil stain? Circle one.

Choice 1  Choice 2  Choice 3

d) Explain your choice in c).

Question 3

As a last ditch effort to become competitive against Pepci and Coka-Cola, a struggling soft drink company decided to develop a new soft drink additive. They succeeded in creating a drug, named alerteine, which similar to caffeine, acts as a nervous system stimulant. Alerteine acts by competing with adenosine, a compound normally present in the body, for binding to the adenosine receptor.

Normally, when adenosine binds to its receptor, signals are sent that have a calming effect on the body. When caffeine or alerteine are present, they can bind to the adenosine receptor instead of adenosine. And though they bind the receptor, calming signals are not induced nor sent through the body.

Interestingly, alerteine differs from caffeine in that it only binds to adenosine receptors in the brain, and thus has fewer annoying side effects of caffeine such as frequent urination and intestinal discomfort.
a) Circle the **strongest** type of interaction between the amino acid side chains of the receptor and the drug.

i) Between leu 253 and alertiene.

   covalent hydrogen ionic van der Waals

ii) Between ser 204 and alertiene.

   covalent hydrogen ionic van der Waals

ii) Between val 58 and the drug:

   covalent hydrogen ionic van der Waals

iii) Between asp 110 and alertiene.

   covalent hydrogen ionic van der Waals

iv) Between thr 280 and alertiene.

   covalent hydrogen ionic van der Waals
Without much testing, the company produced a small quantity of soda, called “Thunderpop”, containing alerteine. To help this new drink gain popularity, they gave it away for free at several college campuses around the country (luckily, MIT wasn’t one of them).

Within a few days of Thunderpop’s distribution, many alert, yet miserable college students began to complain of inability to fall asleep ever since drinking the free soda. It turns out that alerteine is degraded much more slowly than caffeine and circulates in the body for weeks, even months. To avoid lawsuits, the soda company began frantically looking for an antidote to alerteine. Luckily for them, Starbucks had recently developed a compound shown below.

This drug, "chillpillin", appears to mimic the calming effects of adenosine. Starbucks was planning to use it in their newest beverage, the Chillout Latte.

b) Judging by its structure, could chillpillin bind in the same binding pocket as alerteine? Circle one.

YES  NO

c) If yes, what new interaction(s) could account for chillpillin’s ability to cause the adenosine receptor to elicit calming signals?

. .

d) With respect to alerteine, would chillpillin be considered a competitive or noncompetitive inhibitor or neither?

competitive   noncompetitive   neither

Explain your choice...
Question 4

Consider the following reaction:

\[
\text{A} + \text{B} \xrightleftharpoons{\text{Enzyme Z}} \text{C} + \text{D}
\]

\[\Delta G^0 = -9.5 \text{ kcal/mole}\]

a) Draw and label an energy diagram for this reaction both in the presence and in the absence of enzyme Z. Make sure to include the following information.

Include:
- the labels of both axes
- the energy levels of the products and substrates
- the activation energy
- the \(\Delta G^0\) for the reaction.
b) Addition of enzyme to the above reaction leads to the following ...

(Circle all the statements below that are correct.)

i) An increase in $K_{eq}$ of the reaction

ii) A decrease in the amount of products at equilibrium

iii) An increase in the amount of products at equilibrium

iv) Stabilization of the reaction intermediate

v) A decrease in the free energy of the reaction

vi) Increase in the rate of the reaction

vii) Equilibrium being reached quicker than without the enzyme

You decide to test whether increasing the temperature will have an effect on the rate of the reaction. You combine reactants A and B with enzyme Z (which is a human enzyme) in a test tube and determine the reaction rate, this time at 98°C, instead of the normal 37°C (human body temperature).

c) How do you expect this higher temperature to affect the rate of the reaction? Circle one.

Faster rate than with enzyme at 37°C

Slower rate than with enzyme at 37°C

Same rate as with enzyme at 37°C

Please explain why.
STRUCTURES OF AMINO ACIDS at pH 7.0

ALANINE (ala)

ARGinine (arg)

ASPAragine (asn)

ASPArtic Acid (asp)

CYSTEINE (cys)

GLUTAMIC ACID (glu)

GLUTAMINE (gln)

GLYCINE (gly)

HISTIDINE (his)

ISOLEUCINE (ile)

LEUCINE (leu)

LYSINE (lys)

METHIONINE (met)

PHENYLALanine (phe)

PROLINE (pro)

SERINE (ser)

THREONINE (thr)

TRYPTOPHAN (trp)

TYROSINE (tyr)

VALINE (val)