Exam #4 Chem 471 Fall 2001 Name:_____

Work independently. Do not look at others' exams.
Do not allow your exam responses to be shared.

1. (15 points) For each scenario below, indicate whether a Michaelis-Menten kinetic analysis is likely to be appropriate (Yes) or not (No). Circle your choice.

a)	You react 0.1 µM enzyme with 0.2 µM substrate and follow the reaction until it is 1% complete.	Yes	**No**
b)	You react 0.1 µM enzyme with 0.2 mM substrate and follow the reaction until it is 1% complete.	**Yes**	No
c)	You react 10.0 µM enzyme with 2 mM substrate and follow the reaction until it is 50% complete.	Yes	**No**
d)	You react 100.0 µM enzyme with 2 mM substrate and follow the reaction until it is 98% complete.	Yes	**No**
e)	You react 10.0 µM enzyme with 2 mM substrate and follow the reaction until it is 1% complete.	**Yes**	No

The key to the above questions is remembering that Michaelis-Menten assumes that the substrate concentration does not change over the course of the measurement. Reactions that proceed to 50 or 98% have clearly consumed a large fraction of the substrate – it's concentration changes substantially. Answer (a) is also "No" because substrate is not present at large excess over enzyme. A large fraction of the substrate can be expected to be consumed in forming the ES complex.

2. (20 points) Consider a fluorescent molecule with the following measured decay parameters:

$k_f = 1.0 \times 10^8\ s^{-1}$ $k_p = 1.0 \times 10^6\ s^{-1}$
$k_t = 1.0 \times 10^9\ s^{-1}$ $k_Q = 0$

Calculate the following 3 parameters:

$$\tau = \frac{1}{k_d} = \frac{1}{k_f + k_t + k_p + k_Q Q} = \frac{1}{1.101 x 10^9 s^{-1}} = 9.1 x 10^{-10} s = 0.91 n \sec$$

$$\tau_o = \frac{1}{k_f} = \frac{1}{1.0 x 10^8 s^{-1}} = 1.0 x 10^{-8} s = 10 n \sec$$

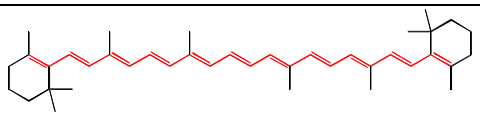
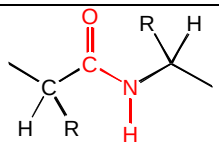
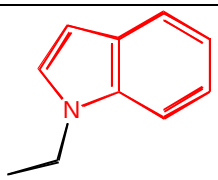
$$\phi_f = \frac{\tau}{\tau_o} = 0.091$$

3. (20 points) Consider the following biological chromophores:

a) The absorbance maxima for the above chromophores are 210 nm, 278 nm, and 440 nm (not necessarily in that order). Match these absorbances with the chromophore by writing the corresponding absorption wavelength below each structure.

b) On top of each structure below, circle the π system which represents the electronic "big box." Enclose only those atoms which are direct participants.

c) In the bottom box of each column, indicate if the substructure is active in CD and explain why it is or is not. If it is, indicate briefly how it's CD is routinely used by physical biochemists. What can it tell us?

			
λ_{max}	440 nm	210 nm	278 nm
CD useful? Why or why not? Describe typical uses, if any.	This part was not graded, since we did not talk about it in class.	Yes Chiral (asymmetric) electron distribution. Determine secondary structure in proteins	Yes Chiral (asymmetric) electron distribution. Follow changes in environment within proteins. (this was supposed to be Trp – an error in the exam may have confused you. Nucleic acid answers were accepted as well.

4. (25 points) Consider a one dimensional particle in a box of length 25.0 Å, containing one electron.

a. (10 points) Calculate the energy of the ground state of the system.

$$E_1 = \frac{h^2 1^2}{8ma^2} = \frac{\left(6.6262\ \text{x}10^{-34}\ \text{J s}\right)^2 1^2}{8\left(9.11\text{x}10^{-31}\ \text{kg}\right)\left(2.5x10^{-10} m\right)^2} = 9.64x10^{-21}\frac{J^2 s^2}{kg\ m^2}\left(\frac{kg\ m^2}{s^2}\frac{1}{J}\right) = 9.64x10^{-21} J$$

b. (10 points) Calculate the energy of the transition from the ground state to the first excited state .

$$\Delta E = E_2 - E_1 = \frac{h^2\left(2^2 - 1^2\right)}{8ma^2} = \frac{\left(6.6262\ \text{x}10^{-34}\ \text{J s}\right)^2 3}{8\left(9.11\text{x}10^{-31}\ \text{kg}\right)\left(2.5x10^{-10} m\right)^2} = 2.89x10^{-20} J$$

c. (5 points) Calculate the wavelength of the transition in nanometers.

$$E = \frac{hc}{\lambda} \qquad \lambda = \frac{hc}{E} = \frac{\left(6.6262\ \text{x}10^{-34}\ \text{J s}\right)\left(3.0\ \text{x}10^{-8}\ \text{m s}^{-1}\right)}{2.89x10^{-20} J} = 6.87x10^{-6} m = 6870 nm$$

5. (20 points) Consider the simple Michaelis-Menten mechanism for an enzyme-catalyzed reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The following data were obtained at 280 K:

$k_1 = 10^8\ M^{-1}\ s^{-1}$ $k_2 = 100\ s^{-1}$

k_{-1} = not measured $K_m = 1.0\text{x}10^{-4}$ M

a) (10 points) For [S] = 0.10 M and $[E]_o = 1.0 \text{ x } 10^{-5}$ M, calculate the rate of formation of product at 280 K.

$$\upsilon_o = \frac{k_2[E]_o}{1 + \frac{K_m}{[S]}} = \frac{\left(100 s^{-1}\right)\left(1.0x10^{-5} M\right)}{1 + \frac{1.0x10^{-4} M}{0.1 M}} = 9.99\text{x}10^{-4} M\, s^{-1} = 1.0\text{x}10^{3} M\, s^{-1}$$

b) (10 points) What is the value of the equilibrium constant at 280 K for the formation of the enzyme substrate complex ES from E and S?

$$K_m = \frac{k_{-1} + k_2}{k_1} \qquad K_m k_1 = k_{-1} + k_2$$

$$k_{-1} = K_m k_1 - k_2 = \left(1.0x10^{-4} M\right)\left(10^8 M^{-1} s^{-1}\right) - 100 s^{-1} = 9900 s^{-1}$$

$$K = \frac{k_1}{k_{-1}} = \frac{10^8 M^{-1} s^{-1}}{9900 s^{-1}} = 1.01x10^4 M^{-1}$$