**Analytical cumulative examination** **February 2, 2008**

Mass spectrometry is a very powerful analytical method. In order to demonstrate your basic understanding of this method, please answer the following questions.

1. Why are reactions important in analytical applications (not fundamental studies) of mass spectrometry? (give at least two reasons) (10 pts)

*Most ionization methods are based on chemical reactions (5 pts)*
*Chemical reactions are needed to obtain structural information for the analytes (5 pts)*

2. Most mass spectrometric reactions occur in the gas phase. Why do most mass spectrometers operate under vacuum (*i.e.*, no collisions)? (5 pts)

*High vacuum is needed to be able to manipulate the ions in the mass spectrometer (5 pts)*

3. Bimolecular gas-phase reactions often occur substantially faster than the corresponding solution reactions. Why? (give at least three reasons) (15 pts)

*Collision rates are greater. Collision complexes are long-lived. The ion/dipole and ion induced/dipole attractive forces provide energy to the collision complex. The TS energies are lower (due to better solvation of reactants than TS in solution). Solvent molecules need not be removed before reaction (5 pts each)*

4. How can you determine the rate at which a gas-phase ion and molecule collide in high vacuum? Give the relevant equations. Give a typical collision rate (15 pts)

*Calculations: ADO:* $k_{ADO} = 2\pi q(\alpha/\mu)^{1/2} + 2q\mu_D C(2\pi/\mu kT)^{1/2}$
*Trajectory:* $k = 2\pi q(\alpha/\mu)^{1/2}[(P + 0.509)^2/10.526 + 0.9754]$ *if* $0 < P \leq 2$;
$P = \mu_D/(2\alpha kT)^{1/2}$; *when* $P \geq 2$, $k = 2\pi q(\alpha/\mu)^{1/2}(0.4767P + 0.62)$ *(10 pts) ; typical* $k = 10^{-9}$ $cm^3 molecule^{-1} s^{-1}$ *(at* $10^{-7}$ *torr, about 3 collisions/second) (5 pts)*

5. Unimolecular gas-phase fragmentation reactions often satisfy the assumptions of quasi-equilibrium theory. Give these assumptions (10 pts)

*Activation is faster than dissociation. Redistribution of energy over all internal degrees of freedom is faster than dissociation. System is isolated and in an internal energy equilibrium. Reactions are competing and consecutive (2.5 pts each)*

# CUMULATIVE EXAMINATION IN BIOCHEMISTRY

Kinetics and Ligand Binding

Feb. 2, 2008

5 questions, 20 pts each

1. You measure binding to a preparation of steroid hormone receptor using radioactive progesterone. You determine a maximal specific binding of 20,000 cpm (counts per minute) in 100 ul of your protein sample. Your sample is 2 mg/ml in protein and your progesterone has a specific radioactivity of 30,000 cpm per pmol.

   a. (10 pts) What is the specific activity of your sample? (give in pmol/mg or similar units). 0.666 pmol/0.2 mg or 3.33 pmol/mg

   b. (10 pts) If your receptor has a molecular mass of 40,000, how pure is your sample (in % wt/wt)?
   1mol/40000g x 1g/1000mg x $10^{12}$ pmol/mol = 25,000 pmol/mg if pure protein
   3.33 pmol/mg / 25,000 pmol/mg x 100 = 0.0133%

2. a. (10 pts) An enzyme that follows Michaelis-Menten kinetics has a Vmax of 250 umol/min/mg and a Km of 0.2 mM. What concentration of substrate would be required to ensure that the initial velocity is at least 80% of Vmax? 0.8 mM

   b. (10 pts) If a competitive inhibitor with a Ki of 50 uM is added at a concentration of 100 uM, to the experiment described in part (a), what will be the observed rate of the reaction? Include units. 140 umol/min/mg

3. a. (10 pts) If the $K_D$ of a ligand binding to a protein is 10 uM and the association rate is diffusion limited ($10^8$ $M^{-1}s^{-1}$), what is the dissociation rate constant $k_-$? Include units.
Kd = $k_-/k_+$ $10^3$ $s^{-1}$

   b. (10 pts) The $K_D$ of biotin for streptavidin is 1 femtomolar ($10^{-15}$ M). You want to carry out an experiment that will accurately determine how fast biotin dissociates from streptavidin. What, do you anticipate, will be the half-life of the complex, ie how long should it take for 50% of the ligand to dissociate? Kd = $k_-/k_+$ ; $k_-$ = $10^{-15}$ x $10^8$ = $10^{-7}$ ; $t_{1/2}$ = .693/$k_-$ = 6.9 x $10^6$ sec or 80 days

4. An ion channel has four independent, identical sites for the ligand L, i.e. the ligand binds each site with the same $K_D$. The channel is only open when all four sites are occupied by ligand. Thus channel activity is proportional to the probability that all four sites are occupied simultaneously.

$$R + L \rightarrow RL + L \rightarrow RL_2 + L \rightarrow RL_3 + L \rightarrow RL_4 \rightarrow AL_4$$

The probability that any one site will be occupied by ligand is given by the fractional saturation (f) in the binding isotherm:

$$f = \frac{[RL]}{R_0} = \frac{[L]}{[L] + K_D}$$

Thus, the probability that all four sites will be occupied simultaneously is $f^4$.

a. (7 pts) What will the Hill coefficient, n, be for measuring binding to this channel?
n = 1. Binding is to identical, non-interacting sites. All have same Kd, simple binding with Hill coefficient = 1.

b. (7 pts) You measure channel activity as a function of the concentration of L and generate a Hill plot. What will the Hill coefficient be at low concentrations of L ($L<<K_D$)?
A plot of channel opening as a function of [L] will display a sigmoid shape…($f^4 = [L]^4/([L] + Kd)^4 \sim ([L]/Kd)^4$ when [L]<<Kd, so n ~ 4 at low [L].
(n = 4)

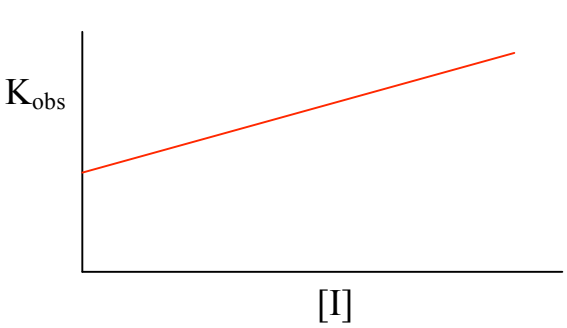
c. (6 pts) Is there cooperativity in ligand binding? No

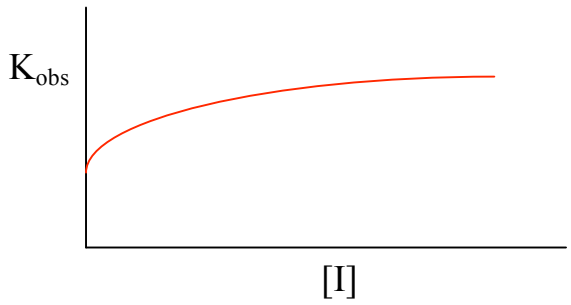
5. You have discovered a new compound, "mellowamine" [I], that appears to be a powerful sedative drug. You also identified a receptor [R] that mellowamine binds to, and found that mellowamine inhibits the normal signalling pathway by decreasing the apparent affinity of the receptor for the stimulatory ligand "highwired " [L].

You want to determine if mellowamine acts by binding to the same site as highwired (i.e. acting a competitive inhibitor of the action of highwired), or if it binds at a distinct site, allosterically modulating the activity. The best way to distinguish these possibilities is to examine the effect of different concentrations of mellowamine on the observed binding constant for highwired ($K_{obs}$).

Draw a graph of the anticipated relationship between $K_{obs}$ and [I] for these two possible scenarios (9 pts each), explaining how the experiment distinguishes these two types of inhibition (2 pts). (Note that you have already ruled out other types of inhibition, such as uncompetitive or noncompetitive, that might increase the apparent affinity, or leave it unchanged).

Competitive: Kobs = Km(1 + [I]/Ki), no saturation
Allosteric: [I] mediates shift from high affinity to low affinity conformation, R <-> T. Kobs reaches a max



Inorganic Chem Feb 2, 2008

1. 6S, 5d + 6p

A. $z^2/s$ B. $z^2/s$ C. $xz$

D. $yz$ E. $x^2-y^2$ F. $xy$

See C&E News Jan 29, 2007

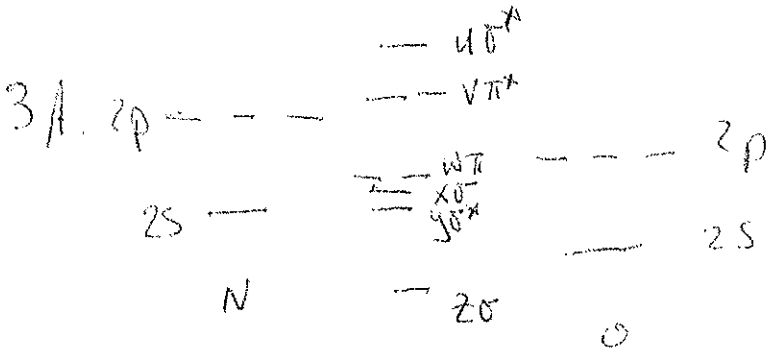
2. A. $x^2-y^2$

B. $\pi$, $yz$

C. $z^2$

See J Phys Chem A 2007, 111, 12864

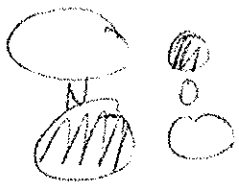
A.




B. $z\sigma^2 y\sigma^2 w\pi^4 x\sigma^2 v\pi^{*2}$ + $f v\pi^*$

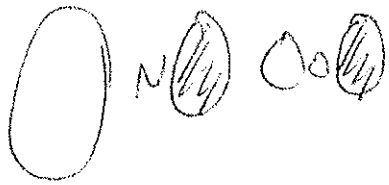
2 unpaired $e^-$

C.




$v\pi^*$

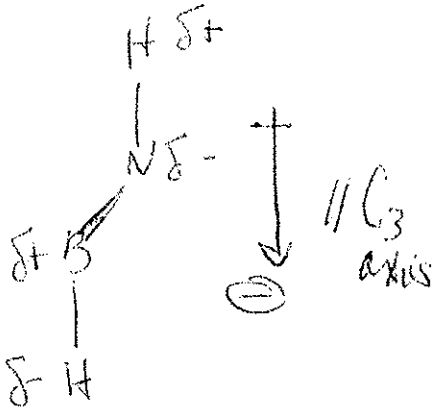
D



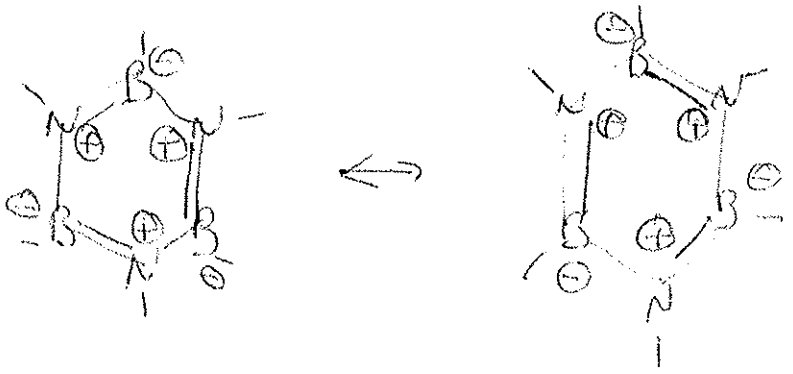

$u\sigma^*$

4.

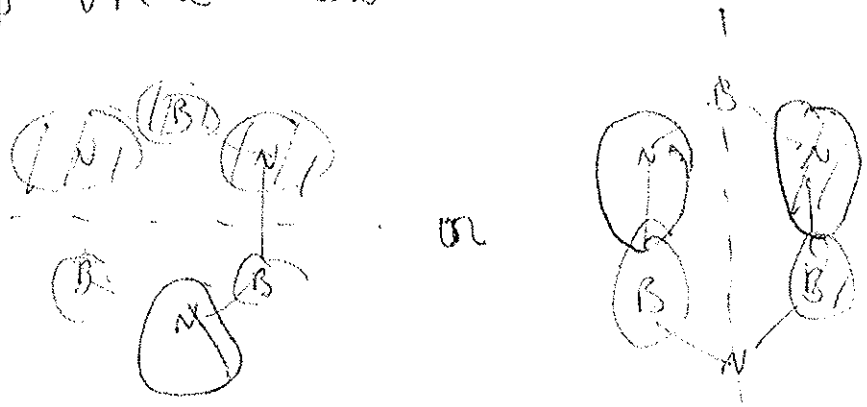
A 




Electrons B < H < N

B. 

Top view based on $C_6H_6$




For a discussion of formal charge etc. see Parkin J. Chem. Educ 2006, 83, 791.

No Organic crib available yet

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No Physical crib available yet

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