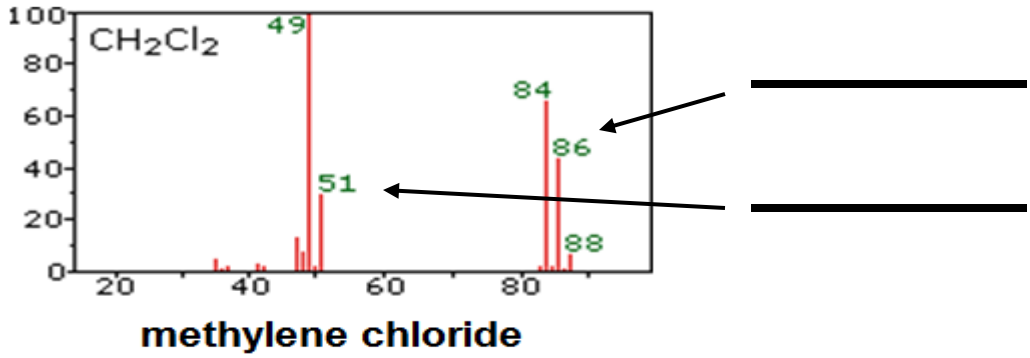
Fall '10 **CH370** Name _____
Hackert **Exam III** UTeID _____

$R = 8.314 \times 10^7$ g-cm^2 /(sec^2-mol-K) = 8.314 J/mol-K ; $\eta = 0.01$ g/(cm-sec)
$n\lambda = 2d\sin\theta$; $k = 1.38 \times 10^{-23}$ J/K; $h = 6.63 \times 10^{-34}$ J-sec; $E = (\gamma\, h\, m\, H)/2\pi$;
γ for $^1H = 26.7 \times 10^7$ rad/sec-T ; $\theta = [S]/(Kd + [S])$; $\nu = n - \nu Kd/[S]$;
(Note: You must show work including equations used for full credit to numerical problems.)

1. Mass Spec:

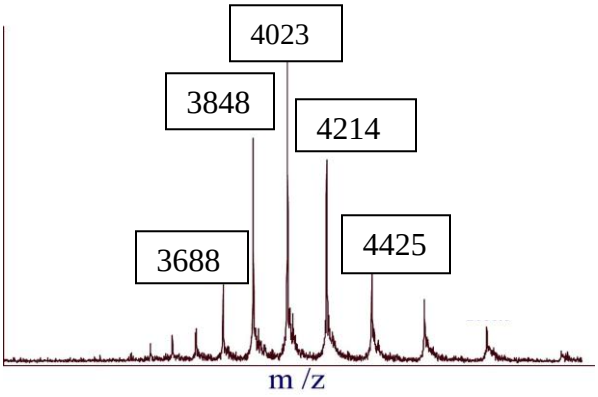
a) Consider the following mass spec obtained from methylene chloride (CH_2Cl_2). Given that there are two common isotopes of chlorine (^{35}Cl at ~75% and ^{37}Cl at ~25%). Identify the **two** ion peaks at "86" and "51" by placing the formula (composition and charge) for the corresponding **cations** on the blank lines provided.



(6)

b) ESI mass spec:

Est. mass of the protein: _____ What is the charge on the largest ion peak? ____



(6 / 1)

c) The two most common techniques for producing ions of proteins for mass spec analysis are:

(2) _____ _____

d) Consider a time-of-flight experiment. A 104 kDa protein with a single charge arrives in 32 μs in a time-of-flight experiment. What is the estimated mass of a protein that arrives in 16 μs and is known by other means to have four times the charge as the 104 kDa protein? **Note:** $m/z = 2V\,(t/D)^2$

_____ .

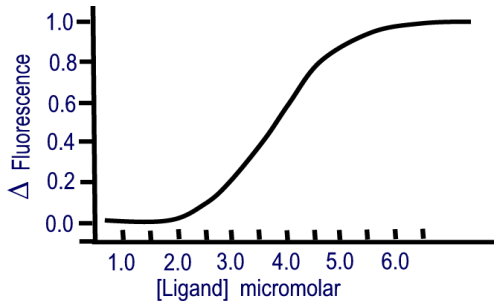
(5)

2. a) A dialysis equilibrium experiment is carried out using a radiolabelled ligand with the following results being obtained: At equilibrium the total concentrations of protein and ligand inside the dialysis tubing are 4.5 microM and 3.8 microM respectively; and the concentration of ligand in buffer outside dialysis tubing is 0.4 microM. Assuming a single binding site, the value of Kd calculated from these results is _____.

(5)

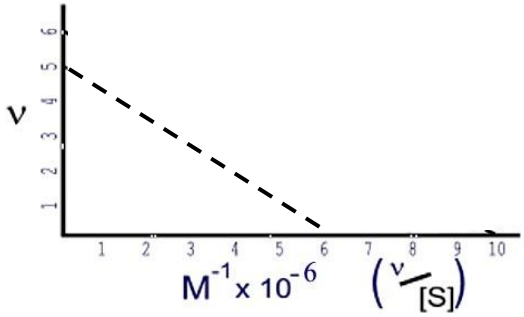
b) Consider the following fluorescence experiment that follows the change in fluorescence as the concentration of the ligand is increased and the [protein] remains fixed. Can anything be deduced about the value of K_d from this data? If so, what is the approximate K_d? _____ If not, why not?

(4)



c) Use your understanding of the Scatchard Equation to determine the number of binding sites and Kd (with units) from plot given below (Hint:see binding equation above)

(2 / 4) "n" = _____ "K_d" = _____



(Note: In this plot the raw values of ν/[S] have been multiplied by 10^{-6} and then represented on the x-axis as numbers from 1 to 10.

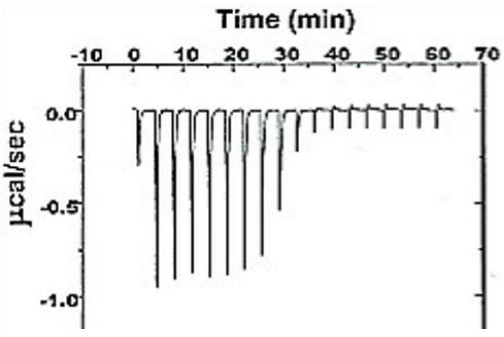
d) Below are typical data from an ITC experiment. Identify what is being measured and indicate what useful binding information can be calculated or derived from ITC data.

(6)

i) What is being measured? _____

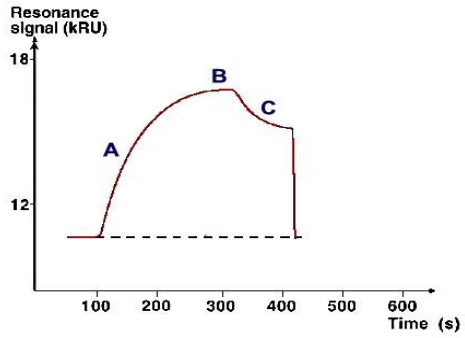
ii) List **all** the types of information that can be **calculated** or **derived** from ITC measurements?

_____



e) Consider the diagram at right. Name the technique used to produce this data and identify what information is being depicted in the diagram.

i) Technique: _____



ii) Information:

(6)

3. Image Formation:

a) In "seeing" objects, what is the most important factor in determining the amount of detail or resolution that can be obtained in the resultant image?

(2) _____

b) What does the image look like if you take the phases from the transform of a duck and combine those with the corresponding transform amplitudes collected from the transform of a cat?

(2)

4. X-rays:

a) Name two parameters that are commonly varied to screen for crystallization conditions:

(2)

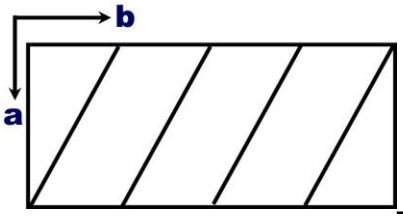
_____ _____

b) We discussed several ways of generating short wavelength radiation. List **two** major advantages of synchrotron radiation sources.

(2) Advantages: _____ _____

c) Consider the following illustration of a unit cell where the "**a**" axis is vertical and the "**b**" axis horizontal. Identify the indices associated with the Bragg planes shown in the blanks provided (assume the planes are parallel to the "**c**" axis)..

(3)



h = __ k = __ l = ___

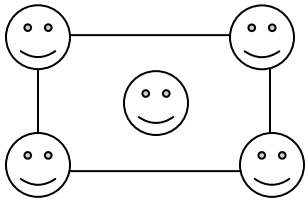
d) Write down the "Bragg Equation": _____

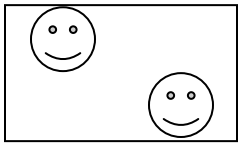
(2)

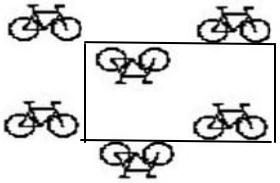
e) Calculate the **lattice constant** "**a**"and **volume** of a **cubic unit cell** the gives a measured 2θ = **18.66°** for the "18 0 18" Bragg reflection. (Assume λ = 1.5418 Å).

(6 / 2) **a** = _____ **V** = _____

f) Consider the following illustrations of unit cells. For each unit cell indentify the number of objects per unit cell and note the symmetry present, if any. If none, write "none".







(6)

Number / cell: _____ _____ _____

Symmetry: _____ _____ _____

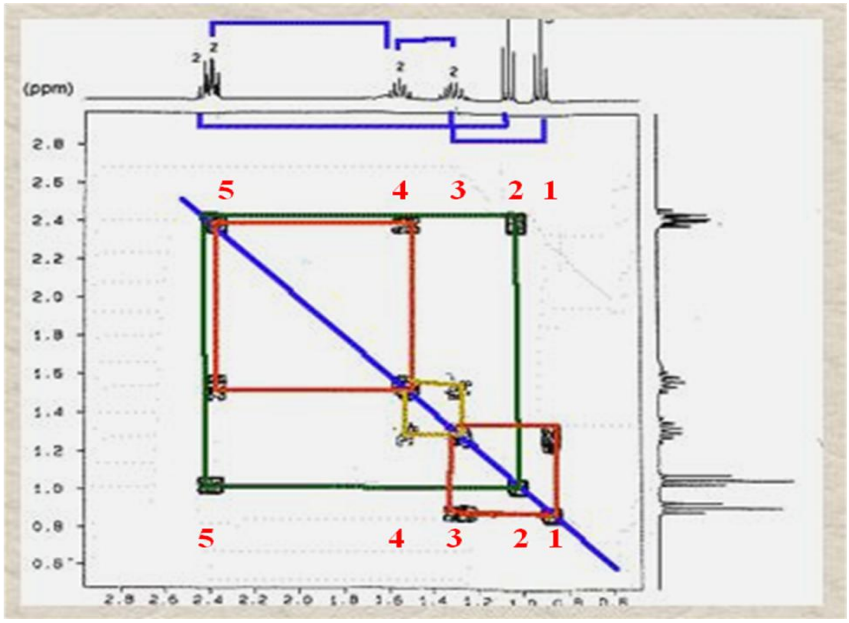
4. NMR:

a) Consider the following NMR COSY spectrum resulting in 5 peaks (labeled 1 thru 5) for a compound found to have the empirical formula $\mathbf{C_7H_{14}O}$. What type of groups are associated with peaks "1" and 3? Peak "1" _____; Peak "3" _____

(4)

b) Now identify the chemical formula for the compound. _____

(4)



b) What type of NMR experiment gives rise to the list of distance restraints needed to "solve" the structure of a protein using multidimensional NMR methods?

_____

(2)

c) Name **two advantages** and **two disadvantages** of using multidimensional NMR methods to obtain structural information compared to determining structural information by X-ray crystallography..

(4) Advantages:

1)

2)

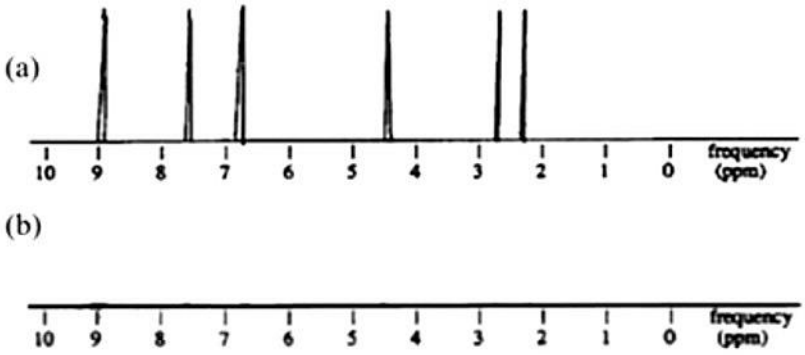
Disadvantages:

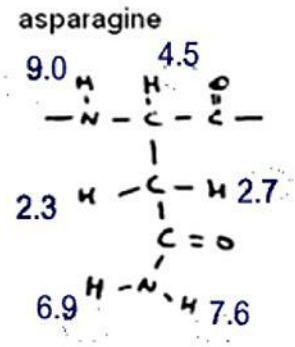
1)

2)

d) (6)

NOE: Consider the NMR spectrum of asparagine shown below in part (a). The sample is then irradiated with RF waves at 6.9 ppm. In part (b) sketch the resulting NMR spectrum expected using the same scale as in part (a).





e) Briefly (*4 sentences or less*) describe what is involved in the process of "simulated annealing" as used in the refinement of models of protein structures by multidimensional NMR methods.

(6)

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(Please **sign your name** on the **back** of this exam **near the top** in a manner that you can recognize for returning.)