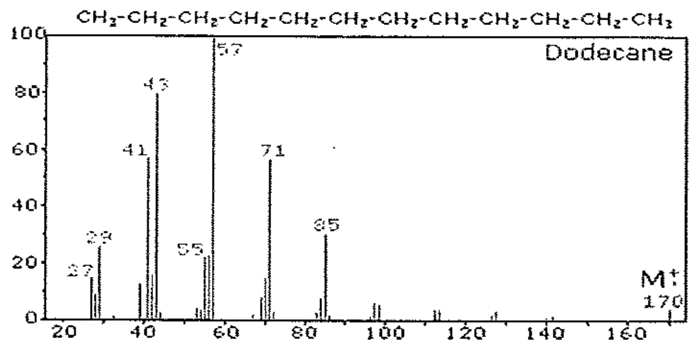
Fall '13	**CH370**	Name KEY
Hackert	**Exam III**	UTeID ________

$R = 8.314 \times 10^7$ 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 ; $\theta = [S]/(K_d + [S])$; $\nu = n - \nu K_d/[S]$;
(Note: You must show work including equations used for full credit to numerical problems.)

1. **Mass Spec:**
   a) Consider the following mass spec results obtained for positive ions from dodecane (M=170; $C_{12}H_{26}$ formula shown). Identify (by mass identity, isotope and charge) the largest peak shown having mass-to-charge ratio of 57.

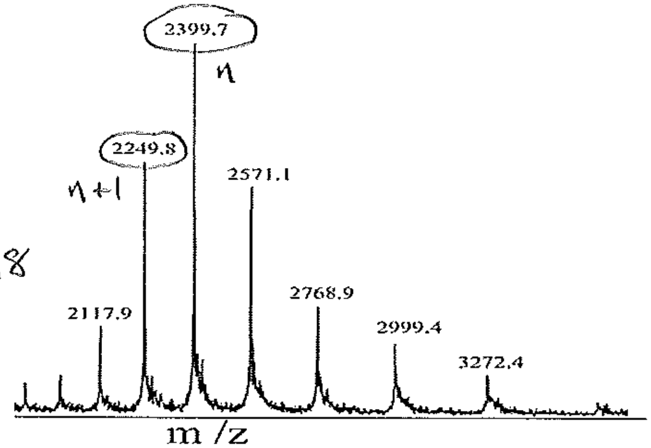


all ^{12}C and ^1H

(2) $M_{57} = [CH_3-CH_2-CH_2-CH_2]^+$ (1) (1)

b) **ESI mass spec:**
   What is the charge on the ion in the largest peak? 15 (2)

   Est. mass of protein: 35,996 (2)



(4) $\frac{M}{n} \approx 2399.7$; $\frac{M}{n+1} \approx 2249.8$

$2399.7n \approx 2249.8n + 2249.8$

$149.9n \approx 2249.8$

$n = 15$

c) Consider a time-of-flight experiment. A 56 kDa protein arrives in 28 μs in a certain time-of-flight instrument. What is the estimated mass of a protein that arrives in 24 μs but is known by other means to have twice the charge as the 56 kDa protein? 82.3 kDa (4)
   Note: kinetic energy KE = *(Ze)Es*; $(m/Z) = 2eEs(t/D)^2$, or $m = [2eEs(1/D)^2]\, Z\, t^2$.

(4) $\frac{m_2}{m_1} = \frac{z_2 \cdot t_2^2}{z_1 \cdot t_1^2} \Rightarrow m_2 = m_1 \left(\frac{z_2}{z_1}\right)\cdot\left(\frac{t_2^2}{t_1^2}\right)$ } (2)

$m_2 = 56\,kDa\left(\frac{2}{1}\right)\left(\frac{24^2}{28^2}\right) = 82.3\,kDa$

d) A major protein fragment peak in mass spec has m/z=2000 and a smaller peak at m/z=2000.20 attributed to replacing one C12 with C13, what is the actual mass of the protein (2)

(2) A) 400 B) 2000 C) 4000 D) 8000 (E) 10000 F) 20000

e) Which mass spec method takes advantage of the high absorption coefficient of aromatic materials like 2,5-dihydroxybenzoic acid? (2)

(2) A) ESI B) FAB (C) MALDI D) TOF E) SCOP.

2. Ligand Binding:

a) All of the following can be obtained from an isothermal equilibrium experiment **except (select all that apply)** : (2)

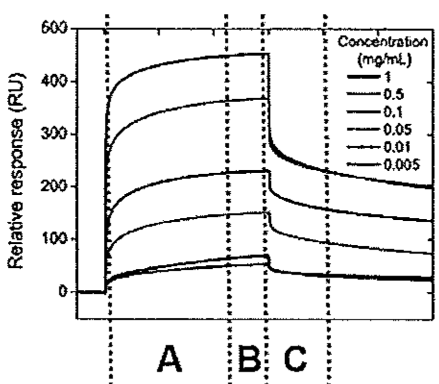
(2) A) ΔH B) Keq (C) kon D) ΔS E) ΔG

b) Consider the binding of a ligand to a single, noncooperative site on a very large protein with 777 amino acids of which 17 are tryptophans and 27 are tyrosines . If the experiment is done in Houston, and if K_d = 0.11 μM and $[E]_o$ = 0.25 μM, and $[S]_o$ = 2.0 μM, estimate the % occupancy of the ligand (S) sites under these conditions. ~95%

(4) $\theta \approx \frac{[S]}{K_d+[S]}$, since $[S]_0 >> [E]_0 \rightarrow$ assume $[S]_f \sim [S]_0$

$$\theta \approx \frac{2.0}{0.11 + 2.0} \approx .948$$

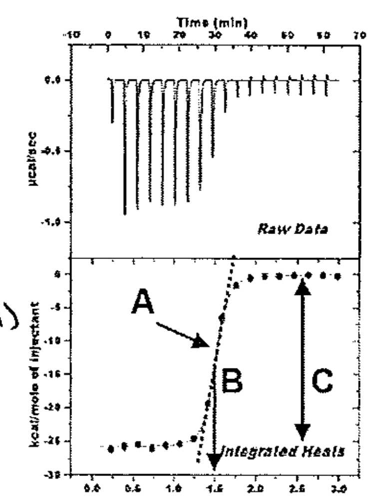
c) Consider the plot with labeled regions "A", "B" and "C", then answer the following questions:
(6)



What is the technique? SPR (1)
What is the X axis? Time (1)
What information can the A region determine? kon (1)
What information can the B region determine? n (1)
What information can the C region determine? koff (1)
Given A, B, and C, what other information can you obtain?
K / ΔG (½ each)

d) Consider the plot with labels "A", "B" and "C", then answer the following questions: (1)

(4) What is the technique? ITC
What information can the line labeled A determine? K_d (1)
What information can the arrow labeled B determine? n (1)
What information can the arrow labeled C determine? ΔH^0 (1)
Given A, B, and C, what other information can you obtain?
ΔG^0 / ΔS^0 (½ each)



e) 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 2.8 microM respectively; and the concentration of ligand in buffer outside dialysis tubing is 0.8 microM. Assuming a single binding site, the value of Kd calculated from these results is

(4) ~~$K_d = 1\mu M$~~

$PL \rightleftharpoons P + L$

$K_d = \frac{[P][L]}{[PL]}$

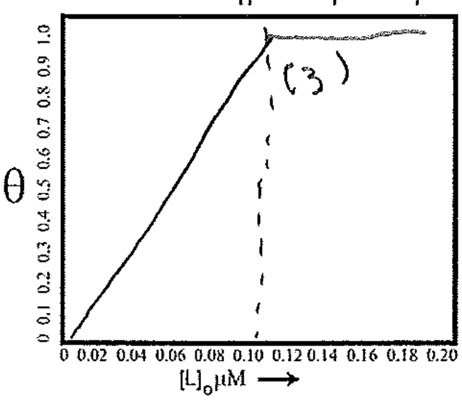
$[P]_t = [P]_f + [PL] = 4.5\mu M \Rightarrow [P]_f = 2.5\mu M$

$[L]_T = [L]_f + [PL] = 2.8\mu M \Rightarrow [PL] = 2.0\mu M$

$[L]_f = 0.8\mu M$

f) On the figure below sketch the expected binding curve of fractional occupancy vs. total ligand
(3) concentration [L] when the value of Kd = 5pM is much less than the [protein]=0.10 μM.

This an example of very tight binding. $K_d << [P]$



3. Image Formation:

a) In "seeing" objects, what are the two most important factors in determining the amount of detail or resolution that can be obtained in the resultant image? (Hint: Abbe equation)
(2) wavelength (1) (amt) angle of scattered light collected (1)

b) A crystal with a **large** unit cell will have "reflection spots":
(2) A) Far apart from each other (B) Relatively close to each other
C) Equal to the number of atoms in the unit cell
D) Large spots equal to the number of heavy atoms

c) Recalling the X-ray crystallography tutorial, if one were to use the intensities from the "Cat" image with the phases from the "Duck" image, one would most likely see:
(2) (A) Duck (2) B) Cat C) Cat without the tail D) Only the tail

4. Symmetry/X-ray:

a) Consider the following letters, circle all the letters that exhibit 2-fold symmetry.
(2) (A) (D) (E) F K (M) P (S)

(2) S
(2) A, D, E, M, S
−1

b) What is the angle between the **incident beam** and the **diffracted beam** for a 2.2Å resolution reflection/X-ray spot when using copper Kα radiation with λ = 1.5418Å? $2\theta = 41.0^\circ$

(3)

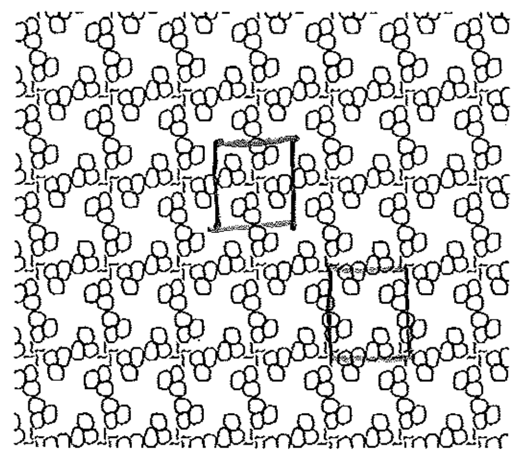
$\lambda = 2d\sin\theta$

$\sin\theta = \frac{\lambda}{2d} = \frac{1.5418}{2(2.2)} = 0.35$

$\theta = 20.5^\circ$ (1) $2\theta = 41.0^\circ$ (2)

c) Consider the following illustration showing an arrangement of "blobs" and "lines". Identify and **draw the best unit cell** on the figure then answer the following questions.

(6) 2 pts all / 2 pts sym
2 pts (1+1 content)



Either cell OK

Number of "blobs" per unit cell: 12

Number of "blobs" in the asymmetric unit: 3

Number of "lines" per unit cell: 4

Number of "lines" in the asymmetric unit: 1

d) All of the following are generally true about the h k l = 0 2 5 reflection, except (circle all):

(2) A) Its Bragg planes do not intersect a axis ✓

B) Its Bragg planes intersect the c axis at 0, 1/5, 2/5, 3/5, 4/5, 1 ✓

(1) (C) It is the same Bragg angle as the 5 2 0 reflection

D) It is the same Bragg angle as the 0 -2 -5 reflection ✓

(1) (E) Its Bragg planes intersect the b axis at 0, 1/4, 1/2, 3/4, 1

e) Calculate the interplanar spacing for the "1 1 1" Bragg planes of a **cubic unit cell** given that the Bragg reflection for the "1 1 1" is measured at $2\theta_{111}$ = **1.54°**. (Assume λ = 1.5418 Å). (d = 57.36 Å) (4)

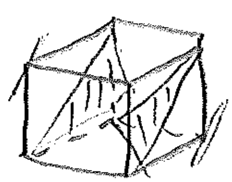
(4)

$$\lambda = 2d_{111}\sin\theta_{111} \Rightarrow d_{111} = \frac{\lambda}{2\cdot\sin 0.77^\circ} = \frac{1.5418}{2(0.01344)}$$

$$d_{111} = 57.36\ \text{Å}$$

f) Recalling that the body diagonal (bd) of a cube is of length bd=SQRT(3)*a, calculate the lattice constant "a" of the **cubic unit cell** given that the Bragg reflection for the "1 1 1" is measured at $2\theta_{111}$ = **1.54°**. (Assume λ = 1.5418 Å). (a = 100 Å)

(4)



3 pts

$$\text{Body diagonal} = 3\cdot d_{111} = 172\ \text{Å} = \sqrt{3}\cdot a$$

$$a = \frac{172}{\sqrt{3}} = \frac{172}{1.73} = 100\ \text{Å}$$

-2 for good attempt at bd rel. to d_{111}

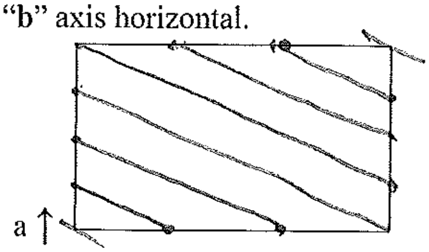
g) All of the following refer to different methods crystallographers use to solve phase problem, **except**:

(2) A) MR (B) SPR C) MIR D) MAD

(2)

h) Consider the following illustration of a unit cell. Sketch the "Bragg planes" that correspond to the Miller indices given below the cell. The "**a**" axis is vertical and the "**b**" axis horizontal.

(2)



6. NMR:

a) Al-27 has a nuclear spin quantum number of I = 5/2, H-1 has a nuclear spin quantum number of 1/2. What is the nuclear spin quantum number of O-16? I = 0

(2)

b) A sample containing 1,000,000 identical nuclei (I = 1) is placed in a magnetic field of 10 Tesla at a temperature of 20 °C. The gyromagnetic ratio for this nucleus is 3.333×10^7 rad/sec-T . Which of the numbers below would be the best guess as to approximate the number of nuclei in the **upper-most energy state?** 2n+1

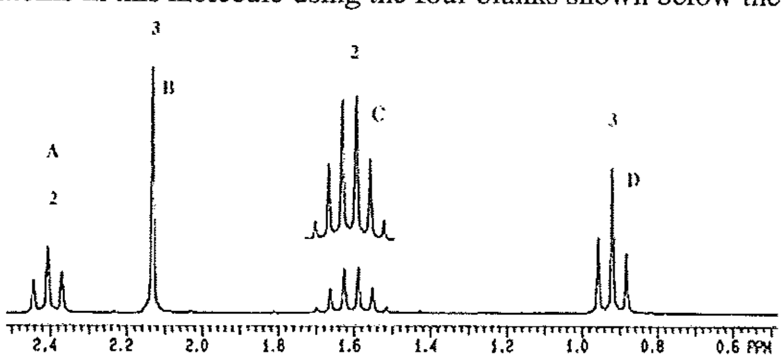
(2) A) 0 B) 1,000,000 C) 500,000 D) 499,950
E) 500,050 (F) 333,000 G) 249,950 H) 166,000

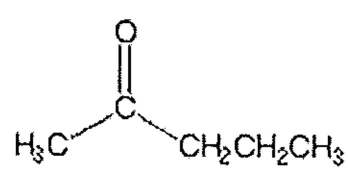
~ 1/3

c) "Simulated annealing" refers to:

(2) A) Trying to fit together protein domains that are positioned far apart
B) Spraying heavy metals on proteins in preparation for electron microscopy
(C) "Heating" the protein model in the computer in order to help it explore conformational space and then "cooling" it slowly in order to find its minimum energy conformation.
D) Creating large number of "blur-o-grams" in order to visualize molecular motion in solution

d) Consider the following proton NMR spectrum that arises from the molecule shown on the right. Match the letters (A-D) assigned to the four types of NMR peaks with the four types of hydrogen atoms in this molecule using the four blanks shown below the molecule.





(4) B A C D

e) What is the approximate limiting molecular weight for protein structure determination by the methods of multi-dimensional NMR?

(2) A) 5,000 B) 15,000 (C) 50,000 D) 250,000 E) 800,000

f) 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) NOESY

7. EM

a) If you wanted to obtain information about the relative levels of dopamine in various parts of the brain, you would want to use the following image technique.

(2) A) CAT scan (B) PET scan C) MRI scan D) SEM Imager E) AFM

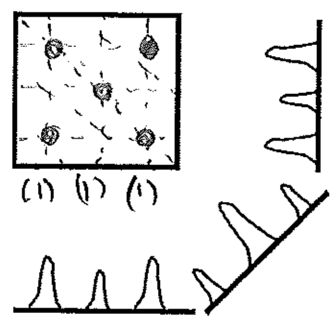
b) Discuss the differences in image production using the following microscopy techniques:

(6) i) TEM Transmission Electron Microscopy – true imaging technique but generates a 2D shadow-like image. Combine many such projection images → 3D image

2 pts each ii) SEM - Indirect method – bright and dark features based on # of scattered e⁻ (depends on position of detector)

iii) AFM (Atomic Force Microscopy) – "Touch" method ; A constant Force is maintained as a needle tip is scanned across surface of material to generate a detailed topographic image of the surface.

c) Cryo EM takes advantage of projection image averages at different angles to reconstruct 2D and 3D spatial arrangements. Consider the TEM results that produced the images shown, and then analyze these results to reconstruct the distribution of matter within the box shown at right (assume relative peak heights of 1:2:3).



(3)

True/False

T You can obtain dynamic structural information from NMR.

F In contrast to CAT scans, a PET scans uses a positron beam of radiation instead of X-rays to irradiate the body but otherwise the two imaging techniques are similar. external / internal source

F SPR is a method of visualizing brain tumors in inoperable cancer patients.

(5) F Knowing the crystal structure of a protein, we can determine the NMR spectrum for it. only approx.

T NMR spectrum offers information about the local electronic environment of the different atomic species in the protein molecule.

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

**** *GOOD LUCK on your other FINALS - We hope to have grades posted by Friday* ****