Fall '14 **BCH 370** Name KEY
Hackert **Exam III - a** 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 a time-of-flight experiment involving two proteins (A and B). Protein B is 4X the size of protein A, but B carries 2X the charge of A. If protein A arrives at the detector in 32 μs, how long should it take for protein B to arrive?

Recall that in TOF: $(m/Z) = 2eEs(t/D)^2$, or $m = [2eEs(1/D)^2]\, Z\, t^2$

(4) $\frac{m_B}{m_A} = \frac{z_B \cdot t_B^2}{z_A \cdot t_A^2} \Rightarrow t_B^2 = \left(\frac{m_B}{m_A}\right)\cdot\left(\frac{z_A}{z_B}\right)\cdot t_A^2 = \left(\frac{4}{1}\right)\left(\frac{1}{2}\right)(32\mu s)^2$

$t_B^2 = 2048\, \mu s^2$

$t_B = 45 \mu s$

b) A major protein fragment peak in mass spec has m/z=6000 and a smaller peak at m/z=6000.20 attributed to replacing one C12 with C13, what is the actual mass of the protein? 30,000 ×5

(2) +1

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

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

2. **Ligand Binding:**

a) 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 = 0.09 μM and $[E]_o$ = 0.30 μM, and $[S]_o$ = 6.0 μM, estimate the % occupancy of the ligand (S) sites under these conditions. 98.5%

$[E]_0 = 0.30\mu M$, $[S]_0 = 6\mu M$ → since $[S]_0 >> [E]_0$ → then $[S]_f \approx [S]_0$

(4) $\theta = \frac{[S]}{K_d + [S]} = \frac{6.0\mu M}{0.09\mu M + 6.0\mu M} = 0.985$

b) A dialysis equilibrium experiment is carried out using a radiolabelled ligand with the following results being obtained: At equilibrium the total concentrations of ligand and protein inside the dialysis tubing are 4.1 microM and 3.8 microM respectively; and the concentration of ligand in buffer outside dialysis tubing is 0.6 microM. Assuming a single binding site, the value of Kd calculated from these results is $K_d = 0.051 \mu M$

(4) $[L]_{tot} = 4.1\mu M = [PL] + [L]_f \Rightarrow [PL] = 3.5\mu M$

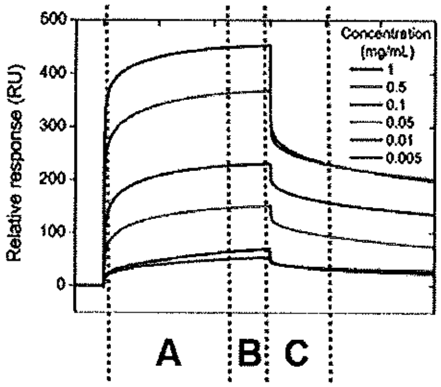
$[P]_{tot} = 3.8\mu M = [PL] + [P]_f \Rightarrow [P]_f = 0.3\mu M$

$[L]_f = 0.6\mu M$

$K_d = \frac{[P]_f [L]_f}{[PL]} = \frac{(0.3\mu M)(0.6\mu M)}{3.5\mu M}$

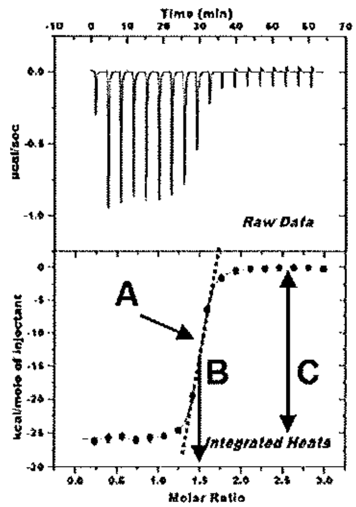
15

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



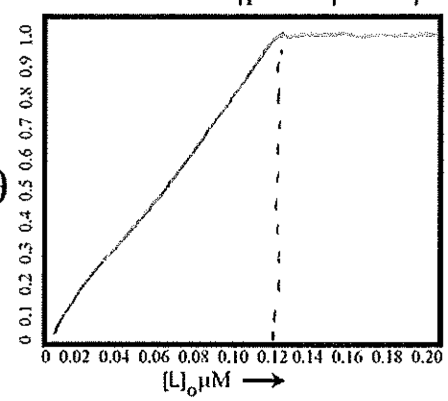
What is the technique? SPR (Surface Plasmon Res.)
What is the X axis? time
What information can the A region determine? k_{on}
What information can the B region determine? n
What information can the C region determine? k_{off}
Given A, B, and C, what other information can you obtain?
K_d / ΔG^o

d) Consider the plot with labels "A", "B" and "C", then answer the following questions:
(5) What is the technique? ITC (Isothermal Cal.)
What information can the line labeled A determine? K_d
What information can the arrow labeled B determine? n
What information can the arrow labeled C determine? ΔH^o
Given A, B, and C, what other information can you obtain?
ΔG^0 / ΔS^0



f) On the figure below sketch the expected binding curve of fractional occupancy vs. total ligand
(3) concentration [L] when the value of Kd = 12pM is much less than the [protein]=0.12 μM.

K_d is very tight
⇒ titrate 1:1 up
to [P]



14

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?

(2) ___wavelength___ ___angle of data collected___

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 B) Cat C) Cat without the tail D) Only the tail

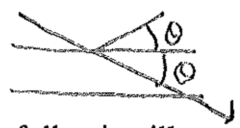
4. Symmetry/X-ray:

a) Consider the following letters, circle all the letters that exhibit 2-fold symmetry.

(2) (B) F K Q (M) P (N) (S)

b) What is the **angle between the incident beam and the diffracted beam** for a 2.4 Å resolution reflection/X-ray spot when using copper Kα radiation with λ = 1.5418Å? ___$2\theta = 37.5°$___

(3) $\lambda = 2d\sin\theta$; $\sin\theta = \frac{\lambda}{2d} = 0.32$



$\theta = 18.74°$ or $2\theta = 37.5°$

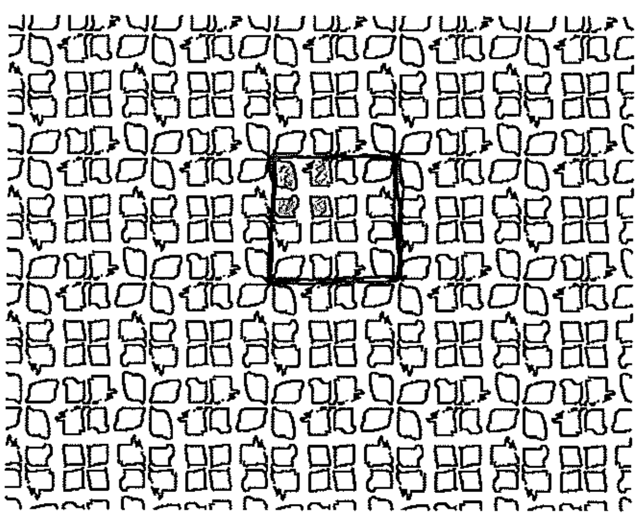
c) Consider the following illustration of packed objects. Indicate by **drawing solid lines on the figure** the "best" choice for the **smallest unit cell**, and then answer the questions below (use shading to indicate the different objects in the asymmetric unit).

(2) Draw your best choice for a unit cell on the figure.

(1) Number of "objects" per unit cell: ___16___

(2) Number of "objects" per asymmetric unit ___4___

4-fold rot. sym.



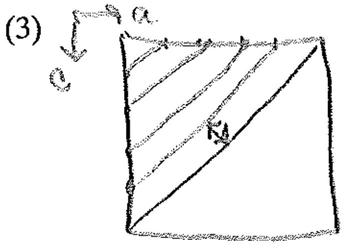
d) All of the following are generally true about the h k l = 0 2 5 reflection, except (circle all):

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

18

e) Calculate the interplanar spacing for the "0 5 5" Bragg planes of a **cubic unit cell** given that the Bragg reflection for the "0 5 5" is measured at $2\theta_{055}$ = **15.42°.** (Assume λ = 1.5418 Å). (d_{055} = 5.75 Å)

(3)

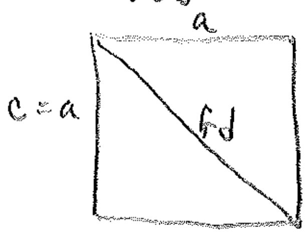


$$d_{055} = \frac{\lambda}{2\sin\theta_{055}} = \frac{1.5418\,Å}{2(\sin 7.71°)} = 5.75\,Å$$

f) Now calculate the lattice constants and volume of the **cubic unit cell from the problem above in 4e.**
(a = b = c = 40.6 Å ; **Volume =** 66,900 Å^3)

(5)

$$10 \cdot d_{055} = \text{face diagonal} \Rightarrow 2a^2 = (10 \cdot d_{055})^2 = (57.5\,Å)^2$$

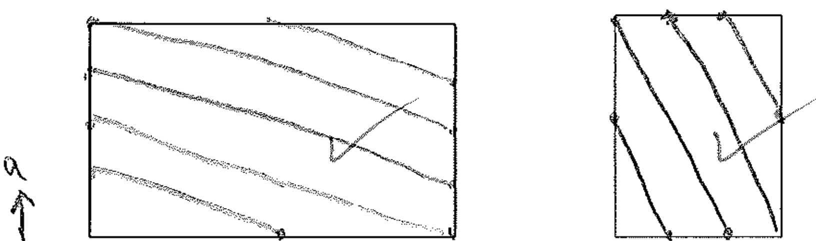


g) All of the following refer to different methods crystallographers use to solve phase problem, **except**:

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

h) Consider the following illustrations of two unit cells. Sketch the "Bragg planes" that correspond to the Miller indices given below each cell. Assume the "**a**" axis is vertical and the "**b**" axis horizontal in each case.

(4)



(4 2 0) (2 3 0)

5. 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 = _____ even/even 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**?

(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 0 −1) 3 states ~ 1/3 each

18

c) What is the **frequency** (in MHz) of a photon that has the energy required to induce a transition between two adjacent proton states in a magnetic field with a strength of 21.2 Tesla? The gyromagnetic ratio for $^1H = 26.7 \times 10^7$ rad/sec-T .

(3) $E = \frac{\gamma \cdot h \cdot m \cdot H}{2\pi}$; $\Delta E = \frac{\gamma \cdot h \cdot H}{2\pi} = h \cdot \nu$

$\rightarrow \nu = \frac{\gamma \cdot H}{2\pi} = \frac{(26.7 \cdot 10^7/\text{sec-T})(21.2\,T)}{2\pi} = 901 \cdot 10^6/\text{sec}$

≈ 900 MHz

d) "Simulated annealing" refers to:

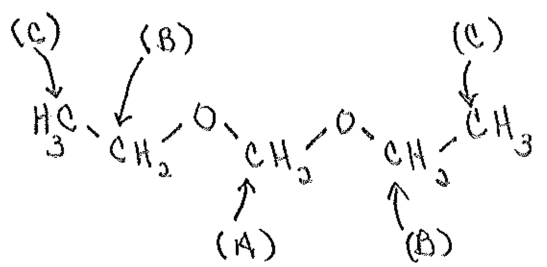
(2) A) Trying to fit together protein domains that are positioned far apart

B) Spraying heavy metals on proteins in preparation for electron microscopy

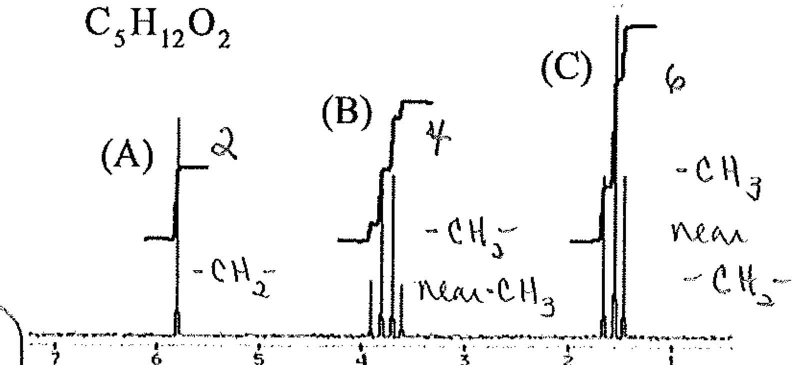
(C) "Heating" the protein model in the computer in order to help it explore conformational space and then "cooling" it slowly in order to find its minimum energy conformation.

D) Creating large number of "blur-o-grams" in order to visualize molecular motion in solution

e) Consider the proton NMR spectra and the empirical formula given below, and given that the integrated intensities for peaks (A), (B) and (C) have a ratio 1:2:3, draw the expected structure of the compound and label each of your carbons with A,B, or C corresponding to its peak in the NMR spectra.



$C_5H_{12}O_2$



(5) also $[CH_3-CH_2-C(OH)(OH)-CH_2-CH_3]$

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

(2) A) 1000 B) 5,000 (C) 35,000 D) 250,000 E) 800,000

g) We discussed the roles of Correlation Spectroscopy (COSY) and the Nuclear Overhauser Effect Spectroscopy (NOSEY) to determine protein structures by multidimensional NMR methods. What is the value/role of each method in determining structures by multidimensional NMR?

(4) COSY: Assignment of resonances to nuclei based on connectivity thru bonds.

NOSEY: Distance restraints — correlations due to interactions thru space.

16

6. EM

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

(6) i) TEM   Transmission EM – ~ slide projector ; e^- beam passes thru sample. Can enhance contrast with metal stain. Cryo EM helps preserve sample. Many images → 3D image

ii) SEM   Surface method / thicker specimens / coat surface with thin layer metal / collect back scattered electrons / scan surface to get light & dark areas depending on position of detector.

iii) AFM (Atomic Force Microscopy)

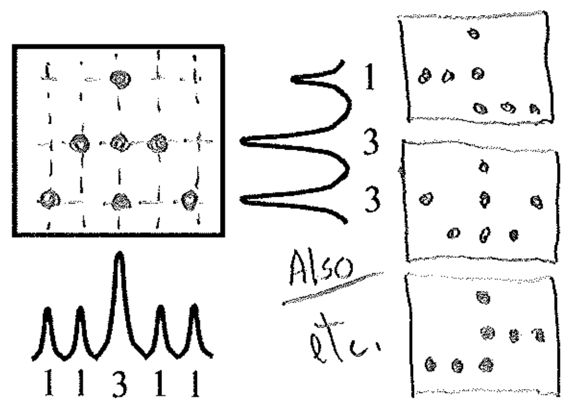
"Touch" method – fine tipped probe maintained at constant force to generate detailed topographic image of surface.

b) 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

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 perpendicular images shown, and then analyze these results to reconstruct the distribution of matter within the box shown at right.

(3)



d) Briefly define the different basis for medical imaging by CAT vs. PET scan methods:

(4)

CAT: Computerized Axial Tomography – uses an external X-ray source / computerized assembly of images at different angles.

PET: Positron Emission Tomography – patient given radioisotope, Measure emissions from internal source. Radioisotope concentrates in designated tissues. → Image metabolic act.

e) **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.

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

(4) 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 will email when the grades are posted*** ****

19