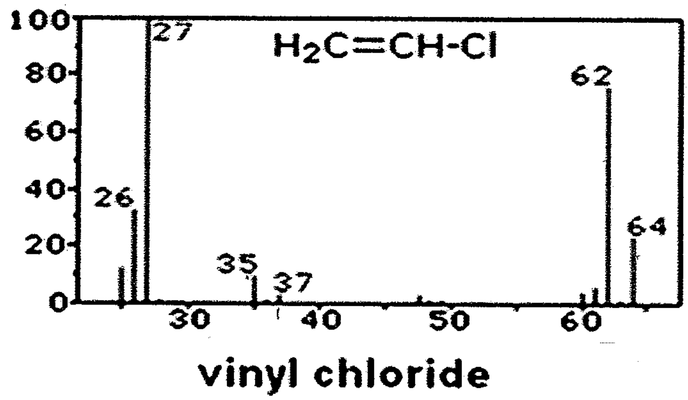
Spring '07 **CH370 / CH387D** Name KEY
Hackert **Exam III** UTeID

$R = 8.314 \times 10^7$ 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 ; $\theta = [S]/(Kd + [S])$; $\nu = n - \nu Kd/[S]$;

1. Mass Spec:

a) Consider the following mass spec results obtained for positive ions from vinyl chloride (formula shown). Identify (by mass identity, isotope and charge) the two peaks shown having mass-to-charge ratios of a) 27 and b) 64. *Hint: natural abundance of C-13 is about 1% and that Cl-37 is about 24%.*



a) m_{27} = $^1H_2\,^{12}C=^{12}C\,^1H^+$ +3

b) m_{64} = $^1H_2\,^{12}C=^{12}C\,^1H\,^{37}Cl^+$ ×3

(6) ↑+1 for $2\text{-}^{13}C$

b) Estimate the mass of a protein from the following mass spec data obtained from an ESI run. A set of eighteen peaks were obtained with the mass-to-charge ratios of peak number 9 being 5900 and that of peak number 11 (two peaks down) was 6556.

(6)

$$\frac{M+n}{n} = 6556 \approx \frac{M}{n} \;;\quad \frac{M+n+2}{n+2} = 5900 \approx \frac{M}{n+2}$$

2pts setup

$$M \approx 6556n \approx 5900n + 11800$$

$$\Rightarrow 656n \approx 11800 \quad \text{or} \quad n \approx 18$$

$$n+2 = 20$$

$$\Rightarrow M \sim 118{,}000 \text{ or } 118 \text{ kDa}$$

c) Briefly explain the basic principles of MALDI.

(5) MALDI = Matrix Assisted Laser Desorption Ionization

The protein or macromolecular sample is mixed with a matrix(2) material that strongly absorbs laser irradiation and dried. Laser(2) flash heats up matrix due to very strong absorption of light and material (matrix and macromolecule) are desorbed (1) and ionized into vapor phase (Naked!)

d) Now consider a time-of-flight experiment involving two proteins (A and B) carrying a single charge each, but protein B is twice the size of protein 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 $m = [2eEs(1/D)^2]\,Z\,t^2$*

(6) $M_A \propto Z_A t_A^2$; $M_B \propto Z_B t_B^2$

$\Rightarrow \frac{M_B}{M_A} = 2 = \frac{Z_B}{Z_A}\frac{t_B^2}{t_A^2} \Rightarrow \boxed{t_B^2 = 2 \cdot t_A^2} = 2(32\mu s)^2$ +2

$\boxed{t_B \sim 45\mu s}$ ✓

2048 μs +3

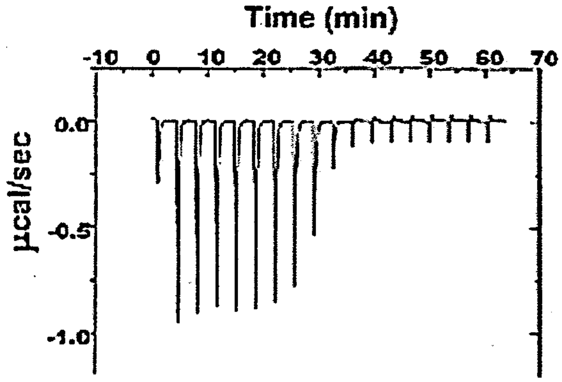
2. **Ligand Binding:**

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

(6) Measures the "heat of reaction" or binding vs. stoichiometry of titrating macromolec. with ligand. (1) → ΔH (1), binding curve

binding curve → n (1), K_d (1)

K_d → ΔG (1) ; ΔG, ΔH → ΔS (1)

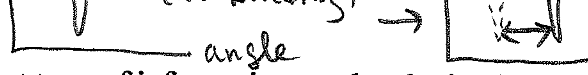


b) Now consider an SPR experiment and a typical "sensorgram" as shown below.

1) What is being measured during a SPR run and how is this data manipulated to produce the "sensorgram" shown below?

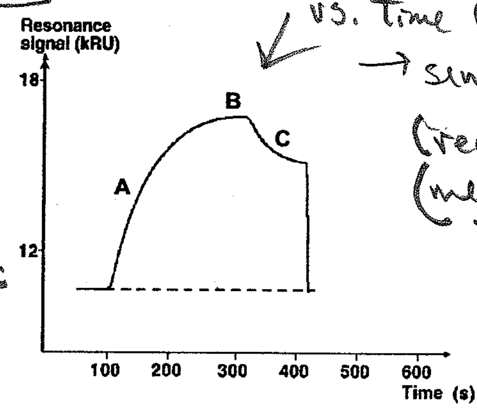
(6) In SPR, one measures the "magic" angle whereby incident laser light transfers energy to Au conducting surface (plasmon). This angle will vary with refractive index and thus mass on binding side of Au surface. More binding → more mass → more shift in angle.

Signal ∝ amt. of binding
3 pts – signal
3 pts – sensorgram



⇒ plot change in ∠ vs. time (vary conc.) → sensorgram (real time) (measurement)

2) Identify what type of information can be obtained by analyzing the regions "A", "B" and "C" shown in the sensorgram.



(6)

2 pts each.

A) Association → k_{on} info

B) Saturation → info on "n", conc. (Concentration)

C) Dissociation → k_{off} info

Note: $K_d = \frac{k_{off}}{k_{on}}$

c) Use your understanding of the Scatchard Equation to determine the number of binding sites and the dissociation constant from the Scatchard plot given below.

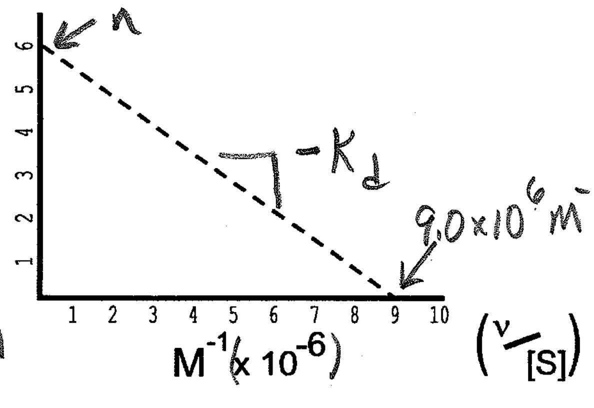
(2/4) "n" = 6 (2pts) "K_d" = 6.7×10^{-7} M (4pts)

From Given: $\nu = n - K_d \frac{\nu}{[S]}$

⇒ y-intercept = n (# of binding sites)

slope = $-K_d$

$K_d = \frac{6}{9 \times 10^6 M^{-1}} = 6.7 \times 10^{-7} M$



(−2 for K_d = .67) (−1 for 6 ± (x 10^{-7} M))

3. Microarrays: Consider a DNA microarray application to compare "normal" cells vs. "patient" cells from individuals that have been given a new drug being tested to treat prostate cancer. After 24 hours exposure, mRNA is harvested, cDNA prepared using a red-dye marker for the "normal" cell sample and a green-dye marker for the "cancer patient" samples. A competition binding experiment is then carried out using a DNA chip carrying 12000 gene markers for the human genome. Identify what can be inferred from each of the following results:

(8) a) red spot – - under-expression in cancer patient vs. "normal" cells

b) yellow spot - ≈ equal expression ⇒ no change between "normal" & "patient"

2pts each c) green spot – over-expression of gene in cancer patient

d) dark spot – no expression of gene – normal or cancer

4. 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 C-12? I = 0

(2)

b) 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 16.0 Tesla? The gyromagnetic ratio for $^1H = 26.7 \times 10^7$ rad/sec-T .

(5)

$\Delta E = h\nu = \frac{\gamma h \cdot H}{2\pi} \Rightarrow \nu = \frac{\gamma \cdot H}{2\pi}$ 2pts

$\nu = \frac{(26.7 \times 10^7 \text{ rad/sec·T})(16.0 T)}{2\pi \text{ rad}} = 680000000$ /sec

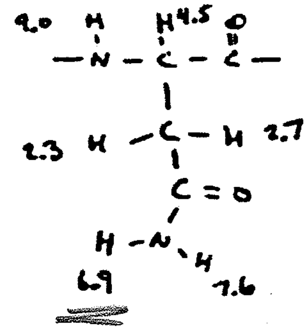
⇒ 680 MHz

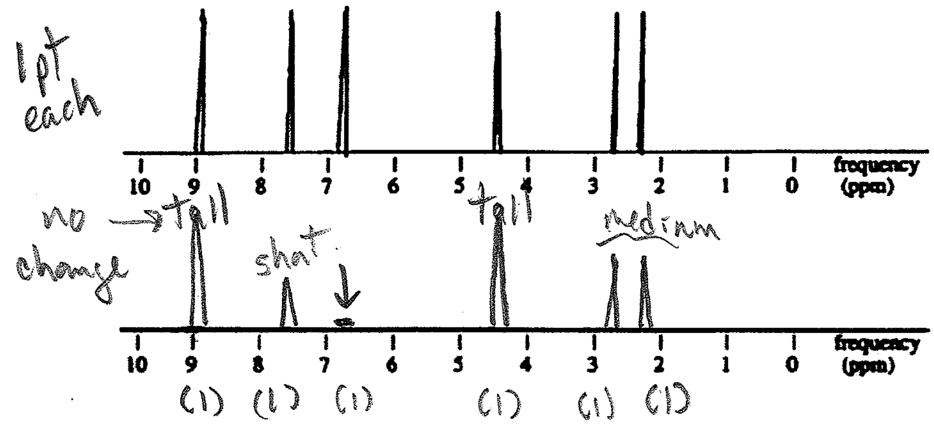
−1 for no MHz

c) (6)

NOE in NMR: Consider the NMR spectrum of asparagine. The protein sample is now irradiated with RF waves at frequency 6.9 ppm. Sketch the anticipated resulting NMR spectrum on the scale below.

asparagine





d) "Simulated annealing" is used in conjunction with the refinement of structural models determined by both X-ray and NMR methods. Briefly describe how the use of simulated annealing differs for X-ray vs. NMR structural refinements?

(6) "Simulated annealing refers to a method of refining structural models using an "energy" function to measure good agreement with expected bond angles, bond lengths, van der Waals, etc. The molecule is "heated" in the computer at "high Temp." to sample much of conformational space and then slowly "cooled".

2

In X-ray refinement there is the additional restraint of making experimentally observed F_{obs}'s match list of F_{calc}'s.

2

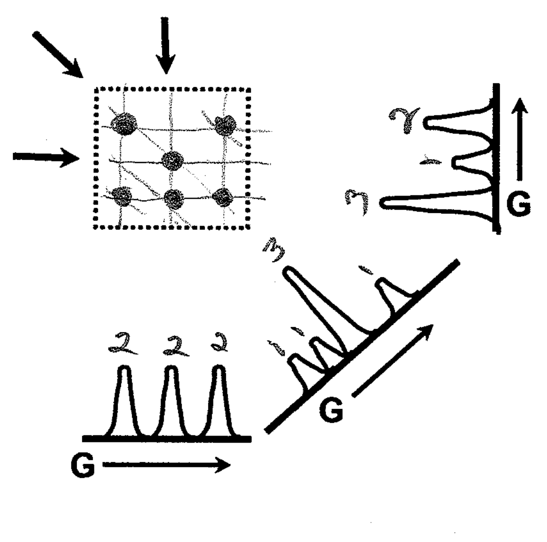
In NMR methods there is the experimental NOE list of distance restraints that need to be satisfied.

2

5. Image Reconstruction: Both MRI and EM use multiple projection images at different angles to reconstruct spatial arrangements. Consider the following MRI experiments using the magnetic gradients as indicated to produce the three projections shown, and then analyze these results to reconstruct the distribution of H_2O within the box shown at right.

(5)

1pt each mass

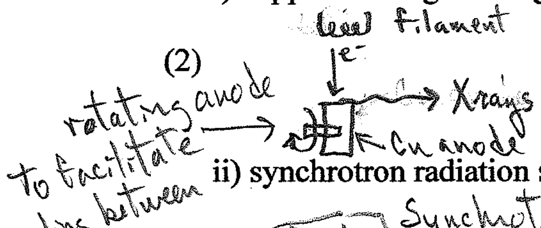


6. X-rays:

a) We discussed several ways of generating short wavelength radiation. Briefly state how such radiation is produced (or the defining characteristics of such radiation) by each of the following methods.

i) copper rotating anode generator [electronic transitions] Electrons are accelerated from filament to Cu anode, striking the Cu atoms and causing some inner shell e^- to be ejected. Characteristic X-rays given off when outer shell e^- fill vacancies.

(2)

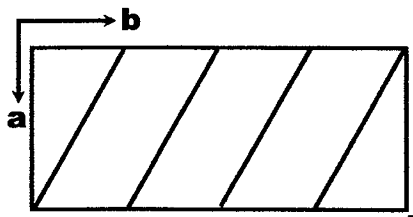


ii) synchrotron radiation source

(2) [high speed deceleration] Synchrotron radiation is produced as high energy e^- moving near the speed of light are forced to turn when going around synchrotron. → Very intense, very broad set of λs (not char. λ)

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

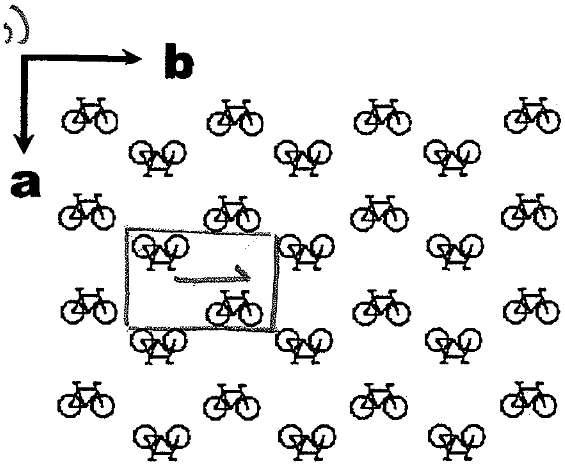
(3) 1 pt each



h = 1 k = 4 l = 0

c) Consider the following illustration of packed bicycles. Indicate by **drawing solid lines on the figure** the "best" choice for the **smallest unit cell** in each system, and then answer the questions below. (2)

(2/2) Number of bicycles / unit cell: 2 (2 pts)



(3) *Symmetry (check all that apply):*

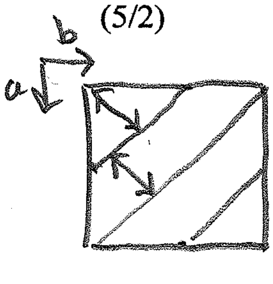
No symmetry	
2-fold parallel to a-axis	
2-fold parallel to b-axis	
2-fold parallel to c-axis	
2-fold screw axis parallel to a-axis	
2-fold screw axis parallel to b-axis	✓
2-fold screw axis parallel to c-axis	

(3)

d) Calculate the **lattice constant "a"** and **volume** of a **cubic cell** the gives a measured 2θ **= 5.78°** for the "2 2 0" Bragg reflection. (Assume λ = 1.5418 Å).

$\theta = 2.89$

(5/2) **a** = 43.25 Å (5) **V** = 80,900 Å^3 (2 pts)



$V = a^3$

$\lambda = 2 d_{220} \sin\theta_{220}$

$d_{220} = \frac{1.5418 Å}{2 \sin(2.89)} = 15.29 Å$ (2)

$\Rightarrow 2a^2 = [4(d_{220})]^2 = (61.16 Å)^2 \Rightarrow a = 43.25 Å$