Fall '14
Hackert

BCH370
HW- 3 (due 12/3)

Name KEY
UTeID

Mass Spec (2)

a) Consider a time-of-flight experiment. A $\overset{m_1}{\text{35 kDa}}$ protein with a $\overset{z_1}{\text{single}}$ charge arrives in $\overset{t_1}{20\ \mu s}$ in a time-of-flight experiment. What is the estimated mass of a protein that arrives in $\underset{t_2}{14\ \mu s}$ and is known by other means to have $\overset{z_2}{\text{two times}}$ the charge as the 35 kDa protein?

Note: kinetic energy KE = *(Ze)Es or zV;* $(m/z) = 2V(t/D)^2$, or $m = [2V(1/D)^2]\,z\,t^2$.

$$\left.\frac{m_2}{m_1} = \frac{z_2 \cdot t_2^2}{z_1 \cdot t_1^2}\right\} \rightarrow m_2 = m_1\left(\frac{z_2}{z_1}\right)\frac{t_2^2}{t_1^2} = 35\,\text{kDa}\left(\frac{2}{1}\right)\left(\frac{(14\,\mu s)^2}{(20\,\mu s)^2}\right)$$

$$\boxed{m_2 = 34.3\ \text{kDa}}$$

Ligand Binding (2/1)

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 3.7 microM and 4.0 microM respectively; and the concentration of ligand in buffer outside dialysis tubing is 0.80 microM. Assuming a single binding site, the value of Kd calculated from these results is $K_d = 0.125\,\mu M$.

Inside dialysis: $[P]_{Tot} = [PL] + [P]_f = 3.7\,\mu M$; $[L]_{Tot} = [PL] + [L]_f = 4.0\,\mu M$

Outside dialysis: $[L]_{out} = [L]_f = 0.8\,\mu M$

3.2 µM

$$K_d = \frac{[P]_f[L]_f}{[PL]} = \frac{(0.5\,\mu M)(0.8\,\mu M)}{3.2\,\mu M} = 0.125\,\mu M$$

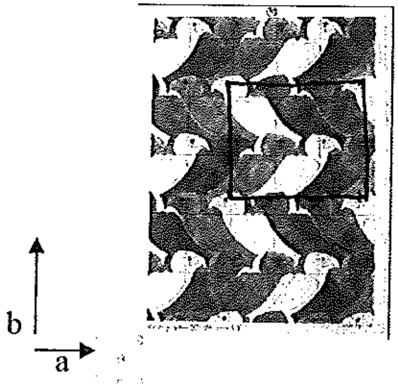
b) Which ligand binding method can produce a binding constant and information about "on" rates?

A) Equilibrium dialysis B) Pulse chase [✓ C) SPR] D) ITC [✓ E) Fast kinetics]

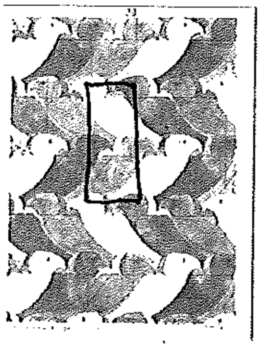
X-ray

a) Consider the following two illustrations of packed birds. In Figure A consider the three types of birds (different colors or shades of gray) to be different. In Figure B consider all the birds to be identical. Indicate by **drawing solid lines on each figure** the "best" choice for the **smallest unit cell** in each system, and then answer the questions below.

(4) **A)**



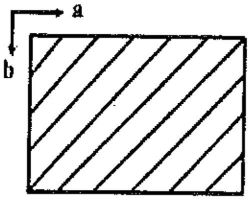
B)



	A	B
Number of birds / unit cell:	6	2
Number of birds / asym unit	6	1 (2-fold screw vertical, along b)

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.

(1)



h = 6 k = 4

c) Given that the reciprocal lattice shows three, orthogonal reciprocal lattice vectors, and based on the following data using copper K_α radiation ($\lambda = 1.5418$Å), calculate the lattice constants and volume of the unit cell.

$$d = \frac{\lambda}{2\sin\theta}$$

h k l	2θ	θ	d
14 0 0	10.25°	5.125	8.63Å
0 8 0	11.87°	5.93	7.46Å
0 0 16	11.67°	5.83	7.58Å

$a = 14 \times d_{1400}$

$b = 8 \times d_{080}$

$c = 16 \times d_{0016}$

(4) a = 120.8Å; b = 59.7Å; c = 121.3Å; V = 874,800 Å^3

d) Estimate the **maximum** number of independent reflections that could be measured for this system for i) a 3.0Å resolution data set, and ii) a 1.8Å data set? (Hint: reciprocal space)

(2)

$$V^* = \tfrac{4}{3}\pi\left(\tfrac{1}{3Å}\right)^3$$

$$V^*_{sph} = 0.1551\ Å^{-3}$$

$$\rightarrow \#_{refl} \sim \frac{0.1551\ Å^{-3}}{1.143\cdot10^{-6}\ Å^{-3}} = \underline{135,700}$$

$$V^* = \tfrac{4}{3}\pi\left(\tfrac{1}{1.8Å}\right)^3$$

$$V^*_{sph} = 0.7183\ Å^{-3}$$

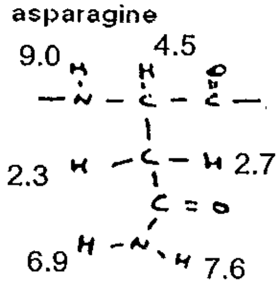
$$\#_{refl.} = \frac{0.7183\ Å^{-3}}{1.143\cdot10^{-6}\ Å^{-3}} = \underline{628,400}$$

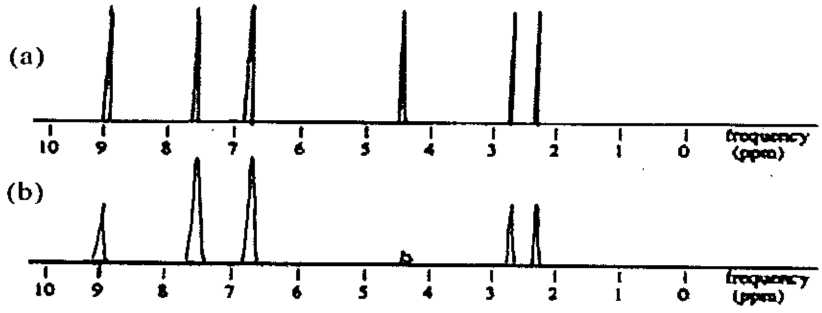
$$1/V_{cell} = \frac{1}{874,800\ Å^3}$$

$$V^*_{cell} = 1.143\cdot10^{-6}\ Å^{-3}$$

NMR (2)

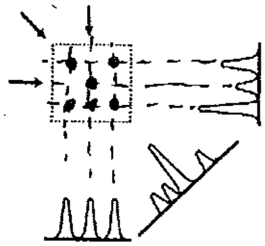
NOE: Consider the NMR spectrum of asparagine shown below in part (a). The sample is then irradiated with RF waves at 4.5 ppm. In part (b) sketch the resulting NMR spectrum expected using the same scale as in part (a).





EM (2)

Image Reconstruction: Many forms of microscopy use projection images at different angles to reconstruct 2D and 3D spatial arrangements. Consider the following TEM experiments to produce the three projections shown, and then analyze these results to reconstruct the distribution of matter within the box shown at right.



I hereby declare that I did this homework independently ______