**Fall '13**
**Hackert**

**CH370**
**HW- 3 (due 12/5)**

Name KEY
UTeID

**Mass Spec** (2/2 - pts)

a) **ESI mass spec:**

Est. mass of the protein: ~76,120 What is the charge on the ion peak with m/z = 3460? 22

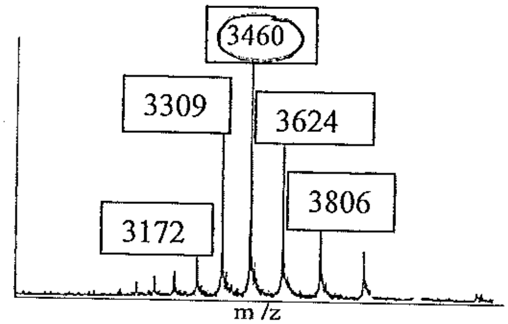
$\frac{M}{n} \sim 3460$; $\frac{M}{n+1} \sim 3309$

$\rightarrow M \sim 3460n = 3309n + 3309$

$151n = 3309$

$n = 22$

$M = 22 \times 3460 = 76,120$




b) Consider a time-of-flight experiment. A 35 kDa protein with a single charge arrives in 20 μs in a time-of-flight experiment. What is the estimated mass of a protein that arrives in 14 μs and is known by other means to have two times the charge as the 35 kDa protein? 34.3 kDa

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

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

$m_2 \sim 34.3$ 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 3.8 microM and 4.2 microM respectively; and the concentration of ligand in buffer outside dialysis tubing is 0.70 microM. Assuming a single binding site, the value of Kd calculated from these results is $K_d = 0.06\mu M = 60\,nM$

Inside: $[P]_T = [P]_f + [PL] = 3.8\mu M \Rightarrow [P]_f = 0.3\mu M$

$[L]_T = [L]_f + [PL] = 4.2\mu M \Rightarrow [PL] = 3.5\mu M$

Outside: $[L] = [L]_f = 0.70\mu M$

$K_d = \frac{[P]_f [L]_f}{[PL]} = 0.06\mu M$

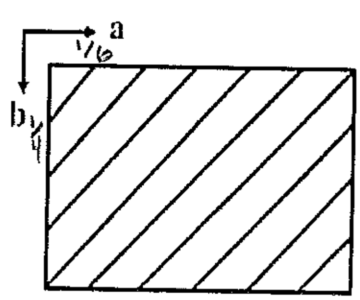
b) Which ligand binding method can produce a binding constant and information about "on" rates?

A) Equilibrium dialysis B) Pulse chase (C) SPR D) ITC (E) Fast kinetics

**X-ray** (1/4/3)

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

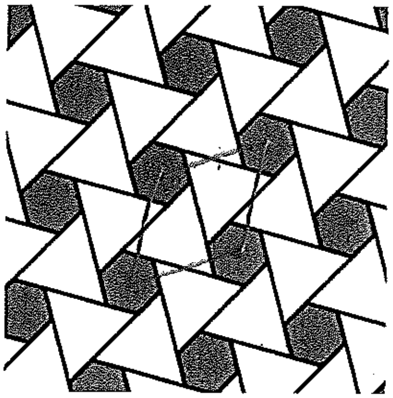
(1)




h = 6 k = 4

b) Consider the following packing pattern. Indicate by **drawing solid lines on the figure** the "best" choice for the **smallest unit cell** and then answer the questions below.

(2)



6 fold symmetry

(1) Number of triangles per unit cell / per asym. unit: 2 / 1/3

(1) Number of hexagons per unit cell / per asym. unit: 1 / 1/6

c) Given that a "reciprocal lattice" shows three, orthogonal reciprocal lattice vectors belonging to a tetragonal space group with the following diffraction data measurements, calculate the lattice constants and volume of this tetragonal unit cell. (Assume $\lambda = 1.54$ Å).

a = b

| *hkl* | *2θ* |
|---|---|
| 0 0 20 | 20.00° |
| 10 10 0 | 20.00° |

(3)

a = 62.7 Å  b = 62.7 Å  c = 88.7 Å  V = 3.49 10⁵ Å³

$$d_{hkl} = \frac{\lambda}{2\sin\theta_{hkl}} = \frac{1.54 Å}{2(0.1736)} = 4.43 Å$$

$10° \lambda$

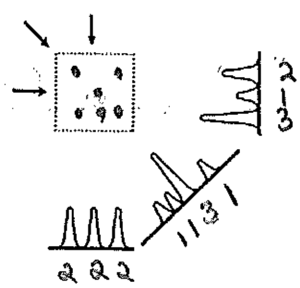
$c = 20(4.43 Å) = 88.7 Å$

$a^2 + b^2 = 2a^2 = (20 \times 4.43 Å)^2$

$a = 62.7 Å$

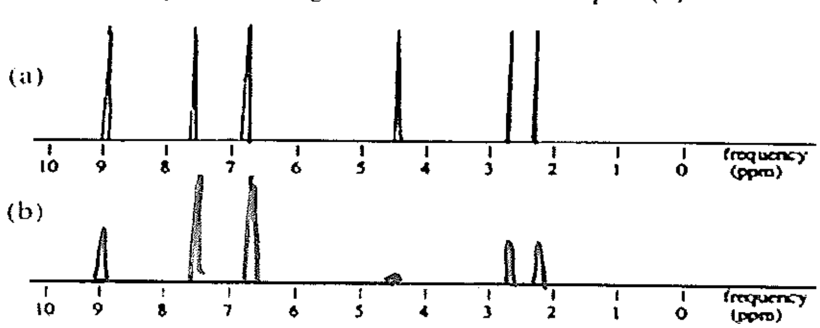
**EM** (2)

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.




**NMR** (3)

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).




asparagine