Review Summary – CH370 / 387D - Exam 3

Mass Spectrometry: produces ions / uses electric and magnetic fields to measure the mass ("weight") or mass / charge ratio of the charged particles: Parts: ion source; analyzer; detector

Source: Electron impact (EI) / Chemical Ionization (CI) / Fast atom bombardment (FAB) Field desorption (FD) /Electrospray ionization (ESI) /Laser desorption (LD)

Analyzer: ions separated according to mass. Quadrupole / Magnetic Sector / TOF

Detector which produces a signal from the separated ions.

Linked Systems: GC/MS; LC/MS; MS/MS

Source of "ions" - Applications with Biomacromolecules –

a) **Time-of Flight (TOF)** Mass Spectrometer: same kinetic energy KE = *(Ze)Es*
where"Ze" is the charge, "E" the electric field, and "s" the length of the source region
before allowed to "drift" to the detector; $(m/Z) = 2eEs(t/D)^2$, or $m = [2eEs(1/D)^2] Z t^2$
mass = (constant) x Z x t^2

b) **Matrix-Assisted Laser Desorption-Ionization** (**MALDI**) / TOF

c) **Electrospray Ionization (ESI):** nondestructive / microdroplets

d) **Capillary Electrophoresis (CE) and ESI**: very small samples – femtomole (10^{-15})

Sequence Analysis Using Mass Spectrometry: MS/MS

Ligand Binding:

a) **Basic Equations** :

$E + S \Leftrightarrow ES$; **Kd = [E][S]/[ES]** and Ka = 1/ Kd

So = S + ES; Eo = E + ES

If So >> Eo , then S ~ So then Kd [ES] = [Eo – ES][So]

[ES] = EoSo/(Kd + So); define q **= [ES]/Eo = So/(Kd + So)**

thus **Kd = So when** q **= 0.5**

when above assumptions are not true → solve quadratic equation (see KJ notes)

then q **= ((Eo + So + Kd) – SQRT((Eo + So + Kd)**2 – 4 Eo x So)) / 2**

b) Manipulations of Equation / **Plots**

1) Preferred – Non-linear regression analysis using statistical program

2) Plots:

Double reciprocal plot **1**/q **= Kd/ [S] + 1** ; plot 1/θ vs. 1 / [S] , slope = Kd

Scatchard Plot: θ = [S]/(Kd + [S]) or θKd + θ[S] = [S] or q **= 1 -** q**Kd /[S]**
plot θ vs. θ/So slope = - Kd

Multiple Binding Sites: define ν = nθ

n **= n -** n**Kd/[S]** → plot ν vs. ν/[S] ; intercept = n and slope = -Kd

c) Measurements – how do experimentally determine [S] or [ES], etc:

1) **Equilibrium Dialysis** – Measure [S] free and [S] free + bound

2) Take advantage of difference in **fluorescence signal** between bound and unbound ligand species if possible. Such systems are more convenient to work with and are also readily adaptable to using with stopped flow kinetics to measure rate constants.

3) ITC – **Isothermal Calorimetry**

- a more rigorous alternative to van't Hoff analysis to obtain thermodynamic info.
- measure ΔH directly during titration → n, ΔH and Keq from best fit to data.

Keq → ΔG; ΔH and ΔG → ΔS.

4) SPR – **Surface Plasmon Resonance** – a quantum optical-lectrical phenomena.

= Biacore (Biomolecular Interaction Analysis) systems define the characteristics of proteins in terms of their *specificity* of interaction with other molecules, the rates at which they interact *(binding and dissociation)*, and their *affinity* (how tightly they bind to another molecule).

- The detection principle relies on surface plasmon resonance (SPR), an electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions. The resonance is a result of energy and momentum being transformed from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the film from the reflected light. The angle at which the light energy can be transferred (resonance coupled) to a metal (gold) surface depends on the refractive index of the material on the opposite side of the reflected light which in turn depends on the concentrations of receptor and bound ligand at the surface. By monitoring the SPR-angle as a function of time, the kinetic events at the surface can be displayed as a "sensorgram."
- SPR can provide k(on) and k(off) data to give more than just Keq values.

X-ray: (see **review sheet** – and **graded homework** for sample questions)

a) Image Formation - Resolution / Wavelength (role of amplitude and phase)
b) Crystal Growth / Crystal Lattices / Lattice Constants / Space Groups
c) X-ray Sources – Sealed Tube / Rotation Anode vs. Synchrotron
d) Diffraction - Bragg Equation - $n\lambda = 2\,d\sin(\theta)$
e) Real Space Lattice/cell *vs*. Reciprocal Space Lattice/cell
f) Data Collection / Structure Factors: $F(hkl) = SQRT\,[cI(hkl)]$
g). Phase Problem: MIR / MR / MAD
Multiple Isomorphous Replacement (Heavy Atom Method)
Molecular Replacement (Rotation and Translation of Model Structure)
Multi-Wavelength Anomolous Dispersion Methods – 3 wavelengths absorption edge
h) Refinement – simulated annealing using known bond distances, angles, etc. plus the agreement between calculated and observed structure factors for all reflections.

NMR:

a) **Spin:** Spin is a fundamental property of nature like electrical charge or mass (for a proton H; I = ½ and m = ±1/2). When a group of spins is placed in a magnetic field, each spin aligns in one of the two possible orientations (±1/2). Magnetic field strength is measured in Tesla (T), vs. the earth's magnetic field in New York of approximately $5x10^{-5}$ T. A typical NMR spectrophometer has a magnetic field of 11.7 T. Nuclei with I ? 0 will interact with an applied magnetic field, giving rise to quantized states where E = ? h m H / 2p or ?E = ? h H / 2p.

b) **NMR spectroscopy**: At room temperature, the number of spins in the lower energy level, N^+, slightly outnumbers the number in the upper level, N^-. **Boltzmann statistics** tells us that

$$N^-/N^+ = e^{-?E/kT}.$$

?E is the energy difference between the spin states; k is Boltzmann's constant, $1.3805x10^{-23}$ J/Kelvin; and T is the temperature in Kelvin.

The signal in NMR spectroscopy results from the difference between the energy absorbed by the spins which make a transition from the lower energy state to the higher energy state, and

the energy emitted by the spins which simultaneously make a transition from the higher energy state to the lower energy state. The signal is thus proportional to the population difference between the states. It is the resonance, or exchange of energy at a specific frequency between the spins and the spectrometer, which gives NMR its sensitivity.

$$\Delta E = \gamma h H / 2p. = h \nu \quad \text{or transitions at } \nu = \gamma H / 2p$$

c) **Chemical Shifts**: The total magnetic field felt by a nucleus is equal to the field applied plus any local field effects ($H_{tot} = H_{applied} + H_{local}$). Thus protons are first separated by general type (-CH_3 vs. –CH_2 - vs. aromatic vs. –N-H, etc.) by different chemical shifts (ppm).

d) **Spin-spin coupling / Nuclear Overhauser Effect; NOEs**:: Nuclei experiencing different environment or having different chemical shifts are nonequivalent. Nuclei which are close to one another exert an influence on each other's effective magnetic field. This effect shows up in the NMR spectrum when the nuclei are nonequivalent. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable. This effect is called spin-spin coupling or J coupling. Nuclei can also show a through space coupling interaction. 2D spectra - irradiate at the frequency of one nuclei and look for an effect in another, nearby nucleus. Presence of **NOE peak indicates two nuclei that are within about 6Å** of each other. Need to be able to identify the peaks to use this information ("Assignment Problem" – see below).

e) **Assignment Problem**: 3D spectra - can use ^{13}C and ^{15}N isotopically enriched proteins to spread out peaks to aid in making peak assignments. Make a list of assigned NOE peaks with labeled nuclei identified. This works for proteins up to about 250-300 amino acids.

f) **Refinement by Simulated annealing:** Use molecular dynamics to minimize an "energy" function to arrive at a structure with lowest "energy" that gives good geometry for bond distances and bond angles, etc. plus good agreement with assigned list of NOE's for close contacts.

g) **Presentation of NMR results**: NMR spectroscopists usually start with several starting models and "solve" or calculate an ensemble of final structural models. These are reported in two ways: 1) "Blur-o-gram" and 2) a plot of rmsd's vs. amino acid residue number.