Review Summary – BCH370 - Exam 2

Spectroscopy

Fluorescence / Phosphorescence

Fluorescence (~ 10^{-4} sec to 10^{-9} sec) / Phosphorescence (> 10^{-3} sec)

FRET (Fluor. Res. Energy Transfer) Eff. = $1/[1 + (R/Ro)^6]$ – needs "spectral overlap"

Radioactivity and Counting

Radioactive decay processes (α / β+ / β- / E.C.); Radioactivity rays (γ-rays)

Half life: A = Ao exp(-kt) where k = ln2/half-life

Measurement of Radioactivity: Geiger Counter / Film / PhosphorImagers / LSC

Liquid Scintillation Counting: Excited solvent / 1^o "fluor" / 2^o "fluor" / PM

Electrophoresis – transport of charged particle in an electric field.

Theory: $F_{tot} = qE - fv = ma = m(dv/dt) = 0$; $v = (qE/f)$

$f = 6\pi\eta R$ for spheres; η = Viscosity ~ 0.01g/(cm-sec)

Ferguson Plots: electrophoretic mobility reflects both charge and size/shape

Methods: slab / tube / seq. gels / (native; denatured) / Disc. Gel / PAGE / PFGE / IEF / CE

SDS-PAGE (subunit MW) / buffer system / stains; IEF gels / 2D-PAGE

Centrifugation

Theory: $F_{tot} = m_{eff}\omega^2 r - fv = m\omega^2 r(1 - \nu'\rho) - fv = ma = m(dv/dt) = 0$; ($\nu'$ is "ν bar")

Preparative Methods: RCF / Rotors / Density Gradient: Zonal vs. Isopycnic Methods

Analytical Methods / Modern Analytical Ultracentrifuge

Optics: **Schlieren** (α = aK(**dc/dx**); **Interference (**Δ**J** = (aKΔ**c**)/λ); **Abspt. optics** (A ~ **c**)

Sedimentation Velocity: $s = \nu/\omega^2 r = (m(1 - \nu'\rho)/f)$; → plot **(ln r) vs. t** → **slope = sω^2**

Sed, Vel. plus Diffusion: **D = (kT/*f*) = (RT/ $N^o f$)** ; → **s = MD(1 - $\nu'\rho$)/RT**

Sedimentation Equil.: $\mathbf{\ln c_r - \ln c_{rm} = [M\omega^2(1 - \nu'\rho)/(2RT)](r^2 - r_m^2)}$ → plot **ln c vs. r^2**

Light Scattering: "Static" vs. "Dynamic"

Wavelength >> particle size; Rayleigh (Static) Scattering – $i/I_o = N[8\pi^4\alpha^2 / r^2\lambda^4](1 + \cos^2\theta)$

Raleigh Ratio: $R_\theta = (i_\theta / I_\theta)(r^2 / (1 + \cos^2\theta)) = [2\pi^2 n_o^2(dn/dC)^2 / \lambda^4 N_o^2]$ *CM* or $\mathbf{R_\theta = KCM}$

$KC/R_\theta = 1/(M*P(\theta)) + 2A_2C$; Mean Square Radius (**Rg**) 10 nm to 150 nm

Polydispersity (*Mw/Mn*); If normalized, LS = RI for monomer but LS = 2*RI for dimer

Dynamic Light Scattering –Hydrodynamic (Stokes) Radius ($\mathbf{R_h}$) 1.5 to 1000 nm

Experimental (Use of LS and RI); $\mathbf{LS = K_{LS}CM(dn/dC)^2}$: $\mathbf{RI = K_{RI}C(dn/dC)}$

or $\mathbf{LS/RI = M[(K_{LS}/K_{RI})(dn/dC)]}$ **or** $\mathbf{M = K'(LS)/(RI)}$

Wavelength << particle size; SAXS → shape information from interference / folding, binding

CD

Terms: CD / plane polarized light vs. circularly polarized light, etc.

Special type of spectroscopy - meas. the difference in left and right handed absorbance **A(l)- A(r).**

The instrument: measurements in far **UV 180-240 nm (proteins); 180-300 nm (nucleic acids)**.

CD spectra can distinguish types of secondary structure (helix, sheet, r.coil / B-DNA, A-DNA) etc.

Applications: Folding / Secondary Structure / Denaturation / Thermal Stability / Binding

Mass Spectrometry

Produce ions / uses electric and magnetic fields to measure the 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

Time-of Flight (TOF) Mass Spectrometer: particles have same kinetic energy KE = zV or *(Ze)Es where"Ze" is the charge, "E" the electric field, and "s" the length of the source region* before particle is allowed to "drift" to the detector;

$$(m/Z) = 2eEs(t/D)^2 \text{ , or } m = [2eEs(1/D)^2]\ Z\ t^2$$

$$\textbf{mass = (constant) x } \boldsymbol{Z} \textbf{ x } \boldsymbol{t^2}$$

Source of "ions" - Applications with Biomacromolecules –

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

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

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

Sequence Analysis Using Mass Spectrometry: MS/MS