Review Summary – CH370 - Exam 2

Spectroscopy

Interaction of Light with Matter (induce oscillating dipoles in matter)
a) Scattered – ($\sim 10^{-16}$ sec) b) Absorption - ($\sim 10^{-15}$ sec)
Absorption Spectrum – "fingerprint" Beer-Lambert Law:
Absorbance (A); Intensity (I, I_o); Transmittance ($T = I / I_o$); $A = \log (I_o / I) = \log (1/T)$
Extinction Coefficient – E (1%), ε_M = Molar extinction coeff.

$$A = \text{O.D.} = \varepsilon \bullet c \bullet \ell \quad \text{also} \quad [\,[E^{1\%}] \bullet MW = 10 \bullet [\varepsilon_M\,]]$$

Fluorescence / Phosphorescence
Fluorescence ($\sim 10^{-4}$ 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 $k = \ln 2/\text{half-life}$
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 "v 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: $s = v/\omega^2 r = (m(1 - \nu'\rho)/f)$; → 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