Review Summary – CH370 - Exam 2

Review of Nucleic Acids: Structures / Folding

Know N Bases; Primary & Secondary structure: double helix by Watson & Crick -1953

Nucleotide pairings: Watson-Crick

Conformations of nucleosides - syn / anti; Sugar pucker: endo or exo

Stabilization (destabilization) Hydrogen Bonding / Electrostatics / Stacking

Denatured DNA: Heat denaturation of DNA is called "melting," Tm / *hypo*chromism.

DNA Sequencing – Maxam-Gilbert vs. Sanger - sequencing (basics; how to read a sequencing gel)

DNA microarrays – general principles of gene-expression profiling (red / green / yellow)

Radioactivity and Counting

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

Half life: $A = A_o \exp(-kt)$ where $k = \ln 2/\text{half-life}$

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

   Liquid Scintillation Counting: Excited solvent / $1°$ "fluor" / $2°$ "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; $\eta = $ Viscosity $\sim 0.01 g/(cm\text{-}sec)$

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

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

Analytical Methods / Modern Analytical Ultracentrifuge

   Optics: **Schlieren** ($\alpha = aK(dc/dx)$; **Interference** ($\Delta J = (aK\Delta c)/\lambda$); **Abspt. optics** ($A \sim c$)

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

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

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

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 170-240 nm (proteins); 170-300 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

Light Scattering: "Static" vs. "Dynamic"

Wavelength >> particle size

Rayleigh (Static) Scattering – $i/I_o = N[8\pi^4\alpha^2 / r^2\lambda^4](1 + \cos^2\theta)$ for unpolarized radiation.

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 **$R_\theta = KCM$**

   $KC/R_\theta = 1/(M*P(\theta)) + 2 A_2 C$; Mean Square Radius (**$R_g$**) 10 nm to 150 nm

Dynamic Light Scattering –Hydrodynamic (Stokes) Radius (**R_h**) 1.5 to 1000 nm

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

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

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

Wavelength << particle size; SAXS $\rightarrow$ shape information from interference / folding, binding