

Review Summary – CH370 - Exam 2

Nucleic Acids - Microarrays

Stabilization (destabilization) Hydrogen Bonding / Electrostatics / Stacking

Denatured DNA: Heat denaturation of DNA is called "melting," T_m / hypochromism.

DNA microarrays – general principles of gene-expression / roles of mRNA, cDNA

Profiling with fluorescent labels (red / green / yellow)

Radioactivity and Counting

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

Half life: $A = A_0 \exp(-kt)$ 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: $F_{\text{tot}} = qE - fv = ma = m(dv/dt) = 0$; $v = (qE/f)$

$f = 6\pi\eta R$ for spheres; $\eta = \text{Viscosity} \sim 0.01 \text{g}/(\text{cm}\cdot\text{sec})$

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: $F_{\text{tot}} = m_{\text{eff}}\omega^2 r - fv = m\omega^2 r(1 - v'\rho) - fv = ma = m(dv/dt) = 0$; (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: $D = (kT/f) = (RT/N^2 f)$; \rightarrow $s = MD(1 - v'\rho)/RT$

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

Light Scattering: "Static" vs. "Dynamic"

Wavelength \gg **particle size**

Rayleigh (Static) Scattering – $i/I_0 = N[8\pi^4\alpha^2 / r^2\lambda^4](1 + \cos^2\theta)$ for unpolarized radiation.

Raleigh Ratio: $R_0 = (i_0 / I_0)(r^2 / (1 + \cos^2\theta)) = [2\pi^2 n_0^2 (dn/dC)^2 / \lambda^4 N_0^2] CM$ or $R_0 = KCM$

$KC/R_0 = 1/(M*P(\theta)) + 2 A_2C$; Mean Square Radius (**R_g**) 10 nm to 150 nm

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

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)**

Wavelength \ll **particle size**; SAXS \rightarrow 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