Review Summary – CH370 / 387D - 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.

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 "v bar")
Preparative Methods: RCF / Rotors / Density Gradient: Zonal vs. Isopycnic Methods
Analytical Methods / Modern **Analytical Ultracentrifuge**
Optics: **Schlieren** ($a = aK(\mathbf{dc/dx})$; **Interference (**$DJ = (aKDc)/\lambda$); **Absp. optics** ($A \sim \mathbf{c}$)
Sedimentation Velocity: $s = v/\omega^2 r = (m(1 - \nu'\rho)/f)$; → plot **(ln r) vs. t → slope = sω^2**
Sed, Vel. plus Diffusion: **$D = (kT/f) = (RT/N^o f)$** ; → **$s = MD(1 - n'r)/RT$**
Sedimentation Equil.: **$\ln c_r - \ln c_{rm} = [Mw^2(1 - n'r)/(2RT)](r^2 - r_m^2)$** → plot **ln c vs. r^2**

CD

Terms: CD / ORD / 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) or essentially looking at difference in $e_L - e_R$
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" and "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 **$R_q = KCM$**
$KC/R_\theta = 1/(M*P(\theta)) + 2A_2C$; Mean Square Radius (Rg) **10 nm to 150 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
Dynamic Light Scattering –Hydrodynamic (Stokes) Radius (R_h) **1.5 to 1000 nm**

Wavelength << particle size

SAXS – use information from the interference of scattered light from different parts of molecule
learn about **shape of the moleucle** → folding / binding (can view this on short time scales)

Mass Spectrometry

Mass spec – i) produces ions, ii) uses electric and magnetic fields to measure the mass ("weight") or mass / charge ratio of the charged particles.

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

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

Detector which produces a signal from the separated ions.

Natural isotopic abundance (1.1% C-13, etc.) / "Resolution" of mass spec

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

Names often reflect the "ion source" method then the "analyzer" method
MALDI TOF / ESI TOF

Source of "ions" - Applications with Biomacromolecules –

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

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

********** (to be continued for Exam III)