AMINO ACIDS: TOPIC ONE

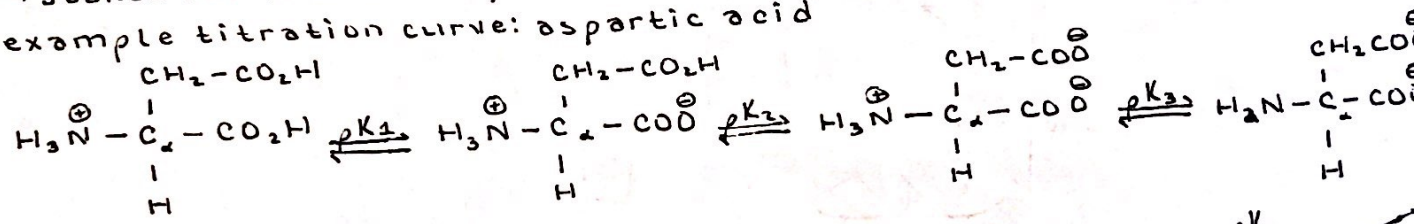
- Side Chains
  - hydrophobic: A, V, I, L, M, F, Y, W
  - hydrophilic: $\underbrace{R, H, K}_{+\text{charge}}$, $\underbrace{D, E}_{-\text{charge}}$, $\underbrace{S, T, N, Q}_{\text{polar uncharged}}$
  - borderline: G, P, C, U
  - amphipathic (in addition to other property): M, Y, K
- Transfer Free Energy
  - measures preference of solubility in water compared to nonpolar solvent

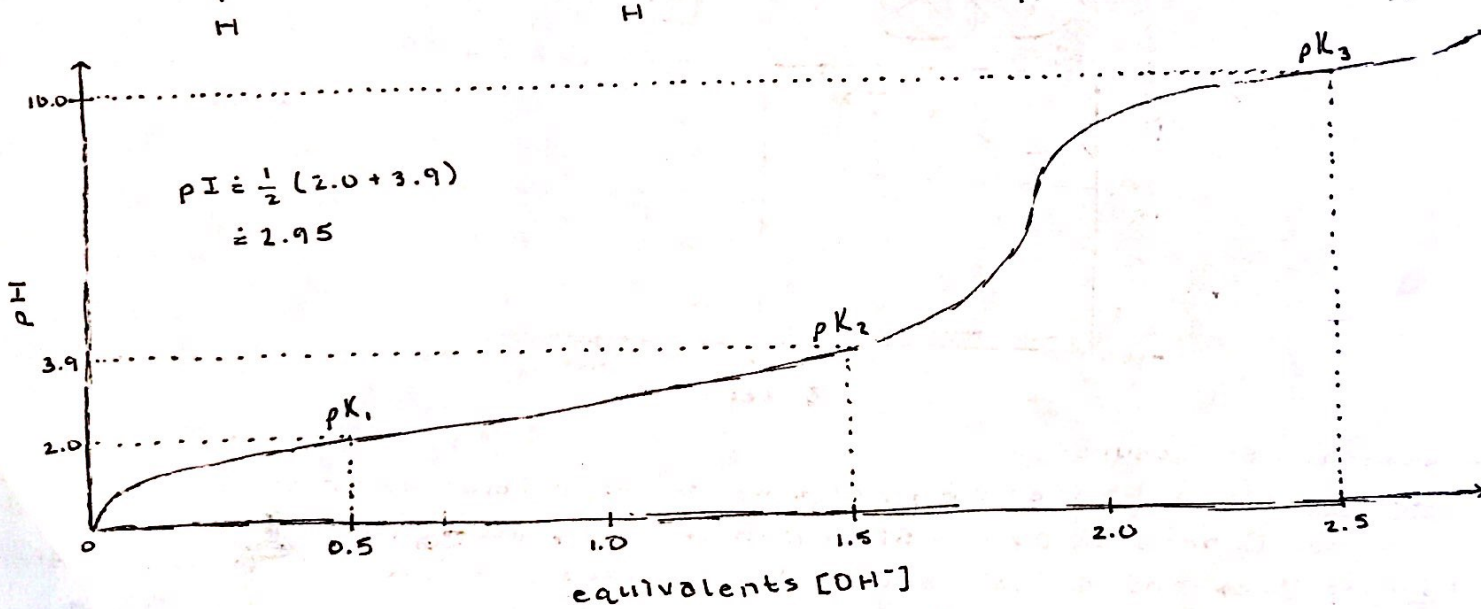
    → $K = \frac{[X]_{H_2O}}{[X]_{org}}$ → $\Delta G^\circ_{tr} = \mu^\circ_{org} - \mu^\circ_{H_2O} = -RT \ln K$

    → $\Delta G^\circ_{tr} > 0 \Rightarrow$ hydrophobic

    → $\Delta G^\circ_{tr} < 0 \Rightarrow$ hydrophilic
  - correlated w/solvent-accessible surface area + aliphaticity
- Equivalence Point + Titrations
  - $pI = \frac{1}{2}(pK_1 + pK_2)$

    → stands for *isoelectric point* (when <u>zwitterion</u> is dominant)
  - example titration curve: aspartic acid





- pKa Protein Value Influences
  1) dielectric constant

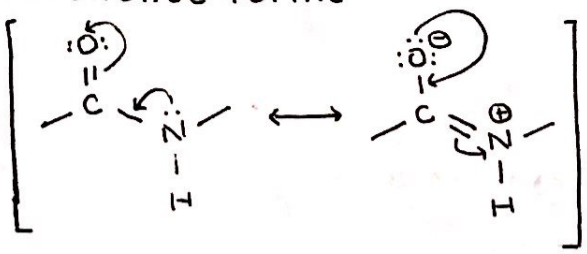
     → charged residue in hydrophobic interior protein cavity (lower dielectric constant) $\overset{pK_a}{>>>}$ charged residue exterior to protein + exposed to aqueous environment (higher dielectric constant)
  2) charge-charge/salt bridge interactions

     → interactions present $\overset{pK_a}{>>>}$ no interactions present
  3) hydrogen bonding

     → H-bond donor $\overset{pK_a}{>>>}$ H-bond acceptor

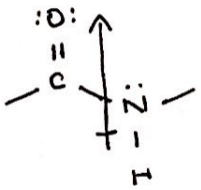
PROTEIN STRUCTURE: TOPIC TWO

- Amide Group + Peptide Bond
  - resonance forms



→ partial double bond character due to resonance
- no free rotation about peptide bond
- peptide bond is planar
- configuration of peptide bond is usually *trans*

  - peptide bond possesses a dipole

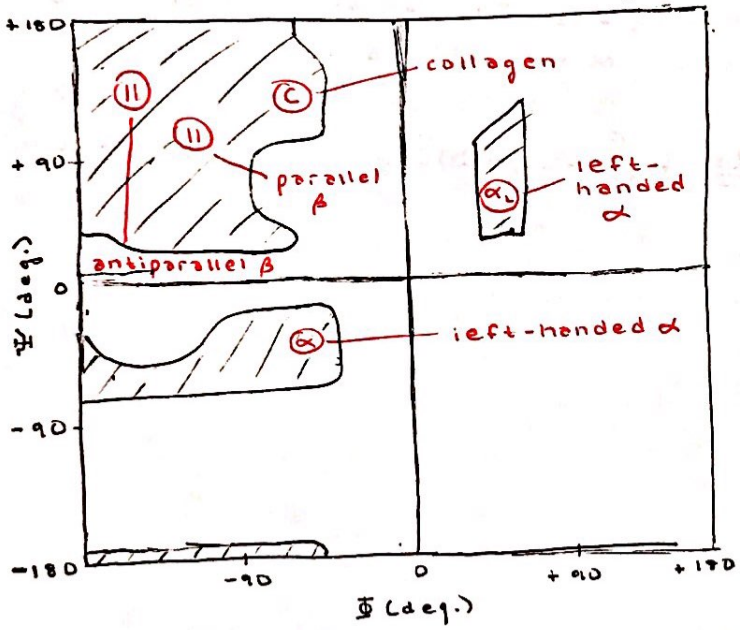


→ goes across the *amide plane* from the N, over the peptide bond, + toward the O

→ parallel to the helix axis

  - dihedral angles
    - → Ψ: N-C_α-C-N dihedral angle
    - → Φ: C-N-C_α-C dihedral angle

    } only certain combinations are allowed due to steric hindrance; shown by *Ramachandran plot*



- Secondary Structures
  - α-helix: right-handed 3.6_{13} helix w/ 1.5 Å transition per residue
    - → H-bonds between C=O of aa_i + N-H of aa_{i+4} along helical axis
  - β-sheath: extended chain segment stabilized by H-bonds between peptides on adjacent strands
    - → can be *parallel* or *antiparallel*
  - β-bend: short loop often joining antiparallel β-sheath strands
    - → *Type I* is slightly less open (carbonyl projects inward)
    - → *Type II* is slightly more open (carbonyl projects outward)
  - four main *supersecondary structural motifs*
    1) βαβ motif 2) β-hairpins 3) α-helix bundles 4) Greek key motif
- Four Protein Fold Classes
  1) α-proteins: symmetric arrangement around hydrophobic core
  2) β-proteins: strands linked by loops + turns
  3) α + β proteins: well-separated α-helices + β-strands
  4) α/β proteins: alternating α-helices + β-strands

NON-COVALENT INTERACTIONS: TOPIC THREE

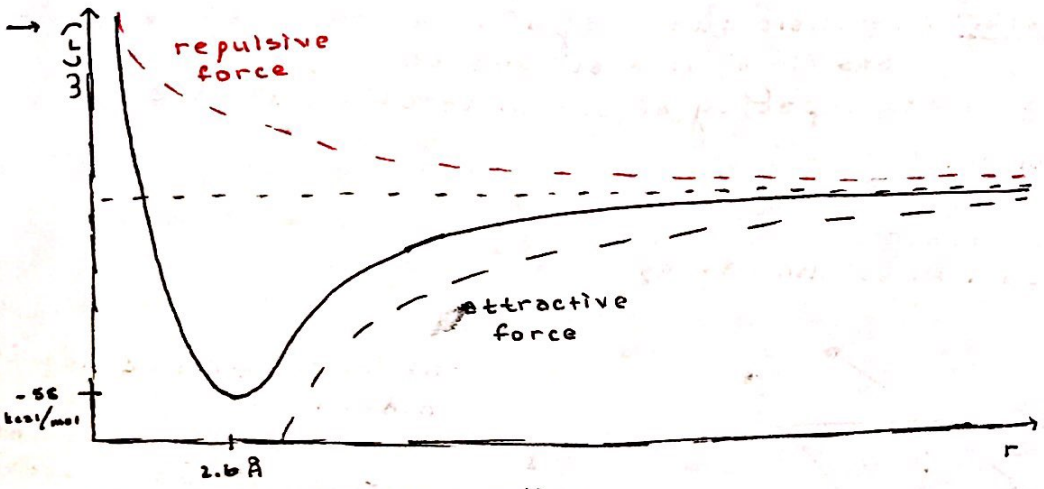
- Coulombic Interactions
  - force + interaction energy formulas

$\rightarrow F = \frac{q_1 q_2}{4\pi\varepsilon_0\varepsilon_r r^2} = \frac{z_1 z_2 e^2}{4\pi\varepsilon_0\varepsilon_r r^2}$ $\rightarrow w(r) = \frac{z_1 z_2 e^2}{4\pi\varepsilon_0\varepsilon_r r}$

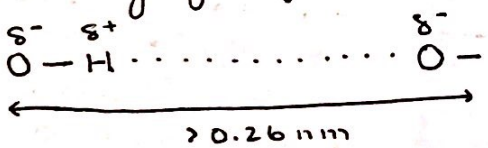
$\rightarrow$ <u>Bjerrum Length</u>: distance at which $w(r) = kT$; symbol is l_B

  - abundant in proteins along w/ ion-dipole interactions
- Hydrogen Bonding
  - energy of hydrogen bond

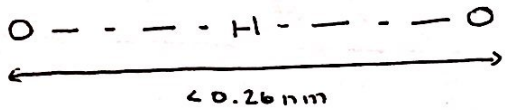
$\rightarrow w(r) = \frac{\mu_1 \mu_2}{2\pi\varepsilon_0\varepsilon_r r^3}$

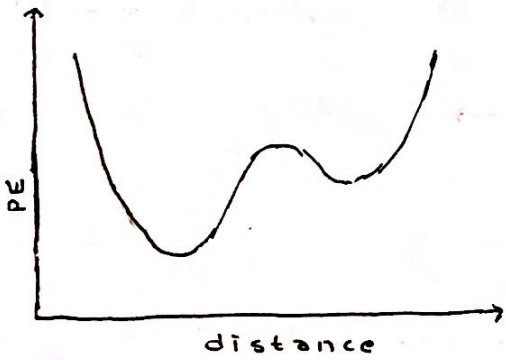


  - two types of hydrogen bonding
    1) ordinary hydrogen bond (HB)



    2) low-barrier hydrogen bond (LBHB)



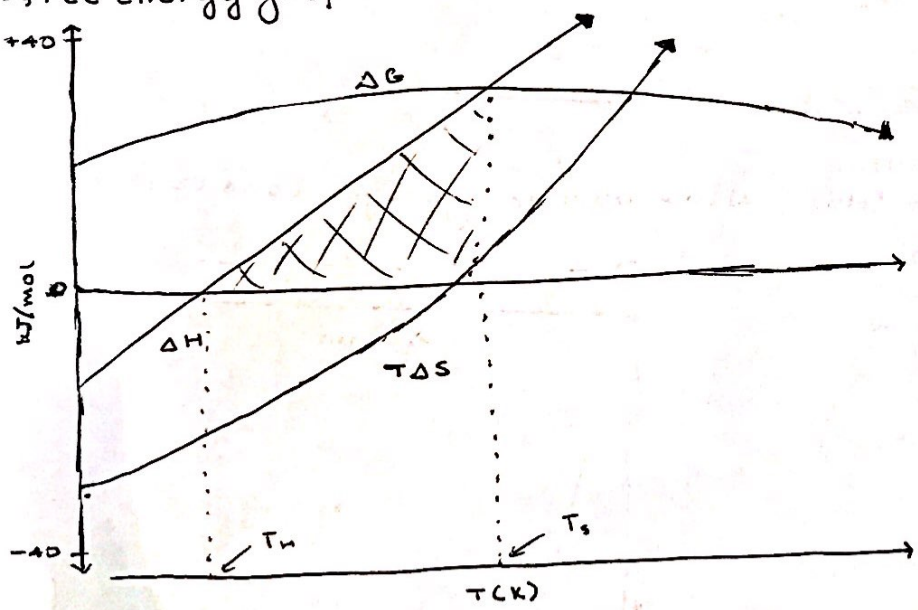


$\rightarrow$ LBHB formation requirements
  (i) matching donor + acceptor pKa
  (ii) short donor-acceptor distance
  (iii) exclusion of H_2O

$\rightarrow$ LBHB lower the activation energy barrier for formation of E·P complex

HYDROPHOBIC EFFECT: TOPIC FOUR

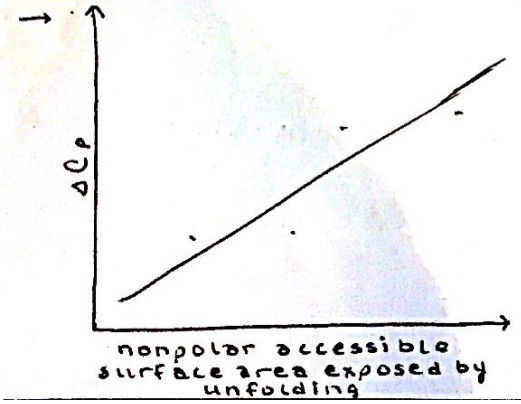
- **Hydrophobic Effect**: interaction between molecules insoluble in water resulting in association of said molecules via reorganization of water
  - is an *entropic effect*
    - → ordered water molecules called *flickering clusters* surround water-insoluble molecules
      - amount of these clusters is proportional to SASA of the amino acid residue(s)
- Heat Capacity Definitions
  - **heat capacity**: amt. heat needed to raise the temperature of a substance by 1K
  - **specific heat capacity**: intrinsic quantity of heat capacity per unit mass (in g) of a substance
  - **molar heat capacity**: heat capacity of one mole of substance
- Liquid Hydrocarbon Model
  - steps
    1) create a cavity in water
    2) add liquid hydrocarbon to the cavity
  - free energy graph



→ at $T = T_S$, ΔG reaches its maximum
- liquid hydrocarbon has compact gaseous state properties
- hydration free energy change is zero

→ at $T = T_H$, liquid hydrocarbon forms no interactions w/ H_2O
- no enthalpy change

- ΔC_p
  - arises from folding of most proteins
    - → formulas
      - $\Delta H(T_2) - \Delta H(T_1) = \Delta C_p (T_2 - T_1)$
      - $\Delta S(T_2) - \Delta S(T_1) = \Delta C_p \ln(T_2/T_1)$
  - correlates w/ SASA



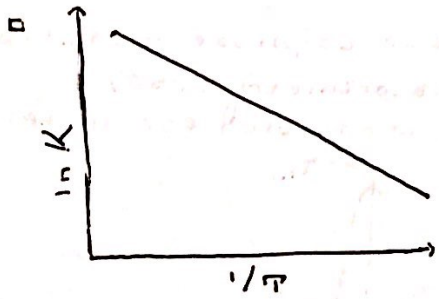
ABSORPTION + OPTICAL SPEC. AND VAN'T HOFF: TOPIC FIVE

- Two-state Protein Denaturation
  - $N \underset{}{\overset{K}{\rightleftharpoons}} D$
    - → $K = \frac{[D]}{[N]} = \frac{f_D}{f_N}$ → $f_N + f_D = 1$
    - → derivation of van't Hoff equation
      - $\Delta G_d = -RT \ln K$

        $\Delta H_d - T\Delta S_d = -RT \ln K$

        $-\ln K = \frac{\Delta H_d}{RT} - \frac{T\Delta S_d}{RT}$

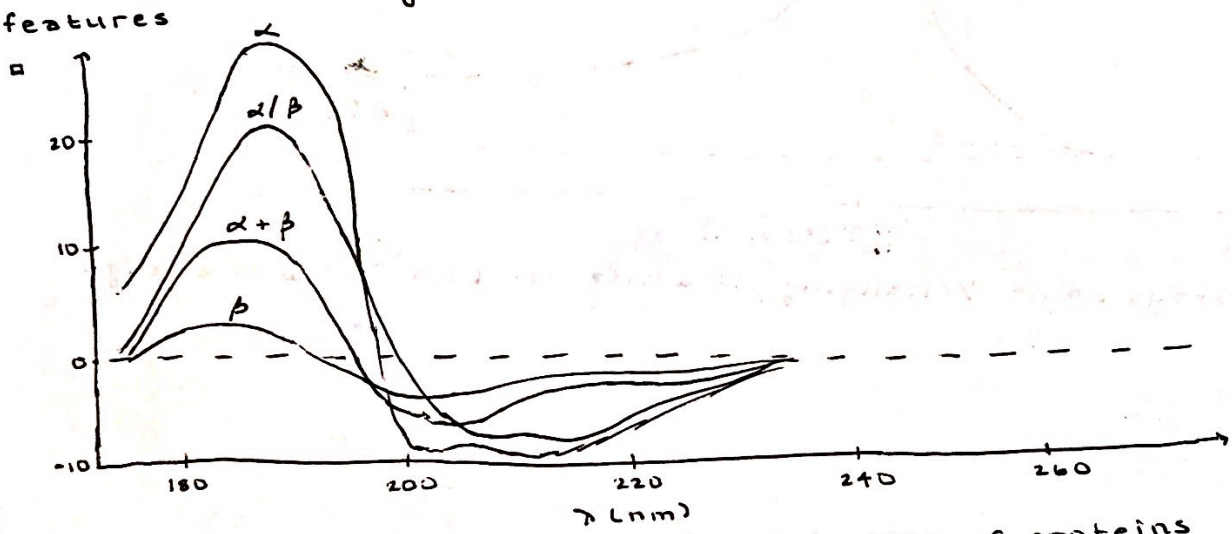
        $\ln K = \frac{\Delta H_d}{R}\left(\frac{1}{T}\right) + \frac{\Delta S_d}{R}$



* slope = $-\frac{\Delta H}{R}$

* int. = $\frac{\Delta S}{R}$

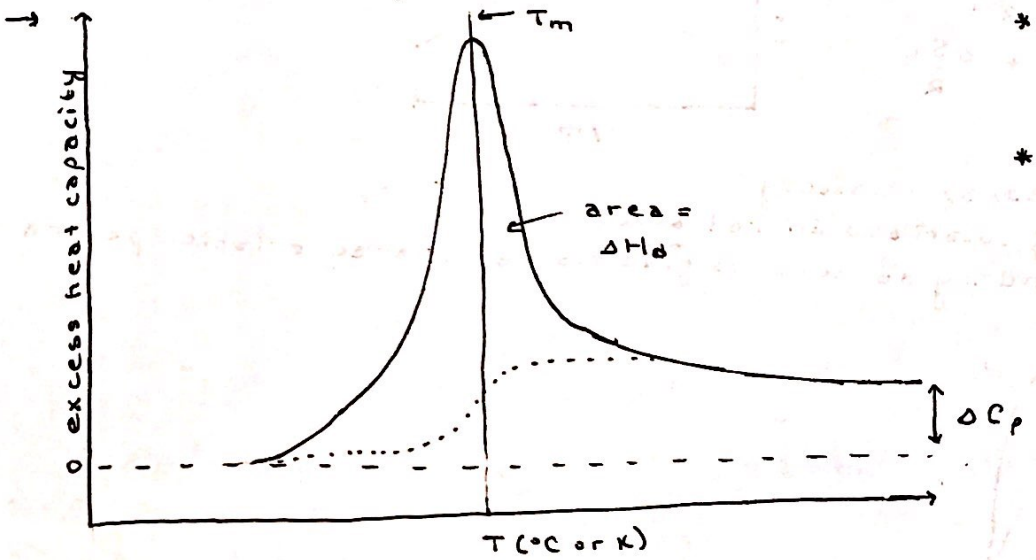
- Circular Dichroism (CD) spectroscopy
  - utilizes circularly polarized EM radiation
    - → different secondary structures give rise to characteristic spectra features



  - can be used to monitor thermal denaturation of proteins

DSC: TOPIC SIX

- C_p + C_v Relation
  - formula
    - → $C_p = C_v + VT(\alpha^2/\beta_T)$
  - C_p is easier to measure for solids + liquids (difficult to maintain constant volume)
    - → discontinuities occur at phase transitions
- Differential Scanning Calorimetry (DSC)
  - two-state unfolding transition for protein

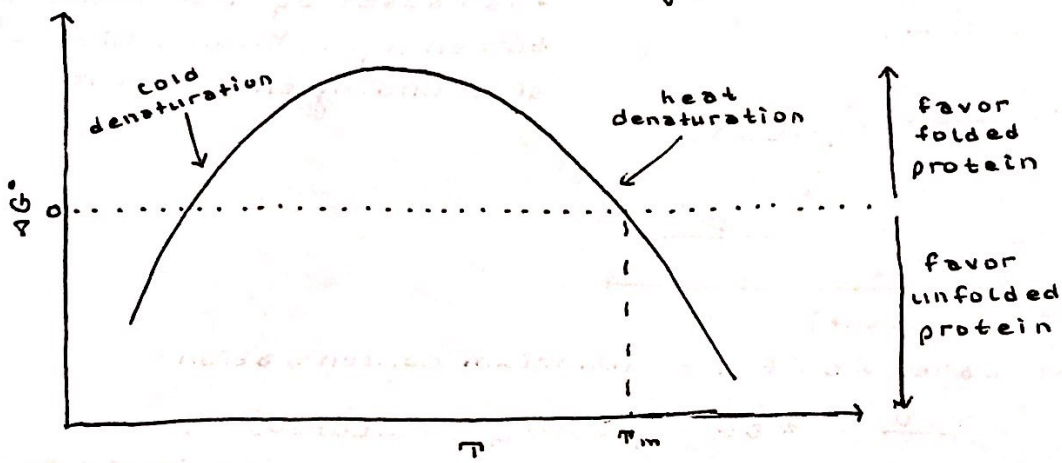


* $f_N = f_D$ at the transition temperature (T_m)
* $N \rightleftharpoons D$ as system progresses to higher temperatures

→ ΔC_p stays same w/changing pH while $\Delta H°$ (via T_m) will change

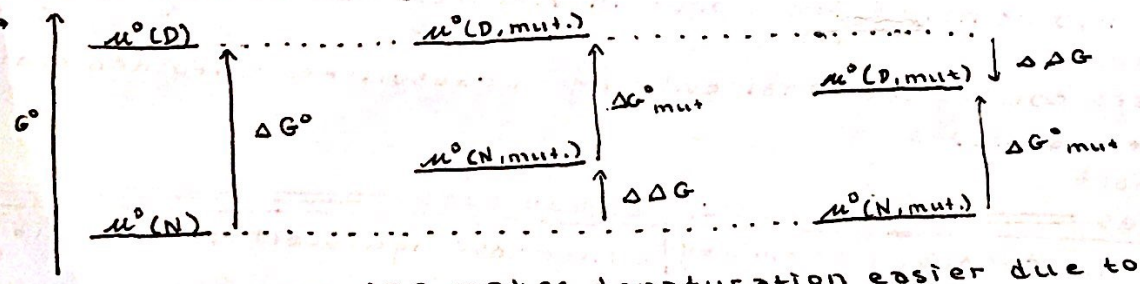
PROTEIN STABILITY + FOLDING THERMODYNAMICS: TOPIC SEVEN

- Denaturation
  - K_D can be altered by
    - → temperature → pressure
    - → denaturants
  - there is a maximum ΔG°_D for many protein denaturations

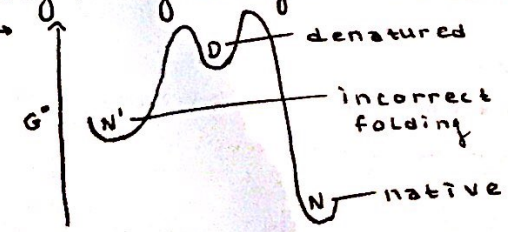


* ΔH dominates at low temp. due to energetically favorable solvation of nonpolar group
* ΔS dominates at high temp. due to greater configurational entropy + low ΔC_p from hydrophobic interactions

- Factors Influencing Protein Stability
  - hydrogen bonding
    - → lack of internal H-bond is generally destabilizing to native state
      - advantage to unfolding + forming one
  - electrostatic interactions
    - → ion/ion pairs inside of protein are generally destabilizing due to high energetic cost of dehydration
    - → ion/ion pairs on surface can be stabilizing due to ion-dipole interactions w/solvent
  - disulfide bonds
    - → reducing them generally further destabilizes the denatured state + makes folding more thermodynamically favorable
- Mutation + Mutation Studies
  - mutations can change ΔG of native or denatured state



  - → increasing loop size makes denaturation easier due to greater configurational entropy
    - $\Delta S_{conf} = -2.1 - \frac{3}{2} \ln n$
- Globular Proteins
  - only marginally more stable than denatured state

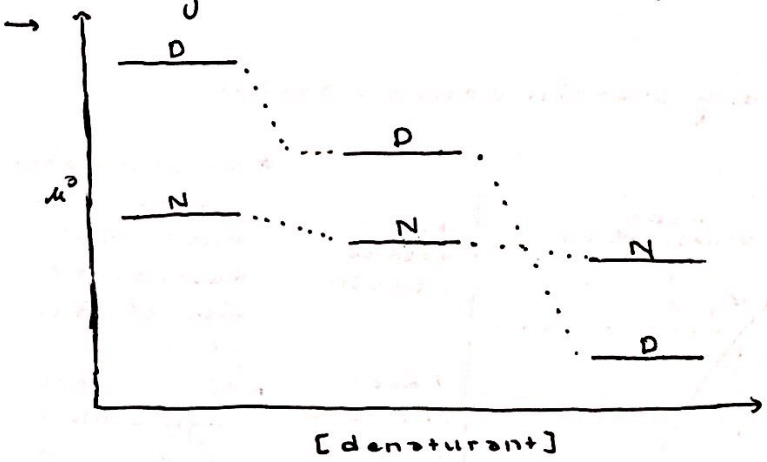


1) if ΔG is too big
   - globular protein may be too inflexible
   - can get trapped in improperly folded form

2) if ΔG is too small
   - too susceptible to unfolding
   - proteolytically unstable

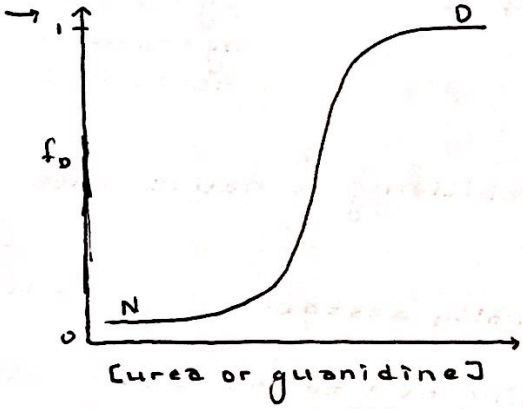
PROTEIN STABILITY + FOLDING THERMODYNAMICS: TOPIC EIGHT

- Effects of Denaturant Concentration
  - can change the conformational preference



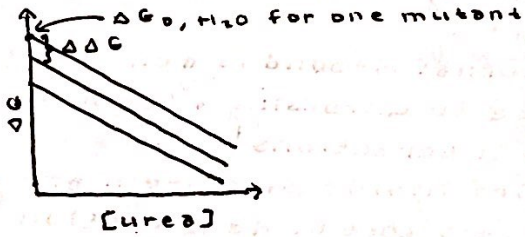
* N ⇌ D to D ⇌ N switch called *differential stabilization* (lower Tm) + is caused by interaction between hydrophobic residues + the surrounding environment

  - binds in place of water + shifts equilibrium concentration



* $\Delta G^\circ_{urea} = \Delta G^\circ_{H_2O} - m[urea]$

- ΔG_D in H_2O is given by the intercept of a ΔG_{urea} vs [urea] curve



- stabilizers + Precipitins
  - two mechanisms of action
    1) increase surface tension
       - increasing surface tension of H_2O increases the stability of the native protein by destabilizing the denatured state more (greater surface area)
    2) excluded volume effects
       - shell of H_2O removed from protein vicinity due to higher cosolvent preference
       - decreases configurational entropy + destabilizes unfolded state
- Kosmotropes + Chaotropes
  - property chart

kosmotropes ⟹	chaotropes ⟹
1) decrease hydrocarbon stability in water	1) increase hydrocarbon solubility in water
2) salt-out proteins	2) salt-in proteins
3) stabilize ordered structures	3) denature biopolymers
4) protein subunit association	4) cause dissociation of protein subunits

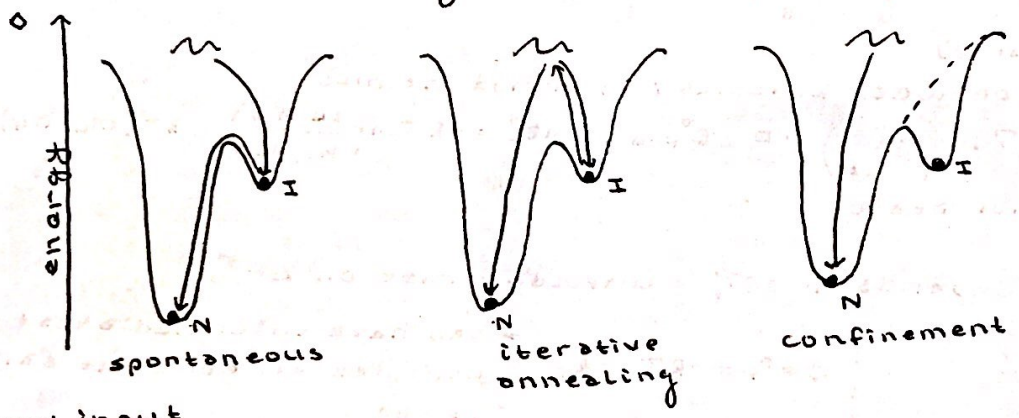
  - distinguishing them
    → ΔS_π is a function of ionic/charge radius
    - smaller ions tend to be kosmotropes + larger ions chaotropes
- optimize solubility + solvent separation of ions in pairs

PROTEIN FOLDING KINETICS: TOPIC NINE

- Factors Determining 3D Protein Structure

1) "programming" from cellular machinery
   → Anfinsen Expt.: showed all information needed to define folded/native state is contained in the AA sequence
   - also showed native state is thermodynamically most stable

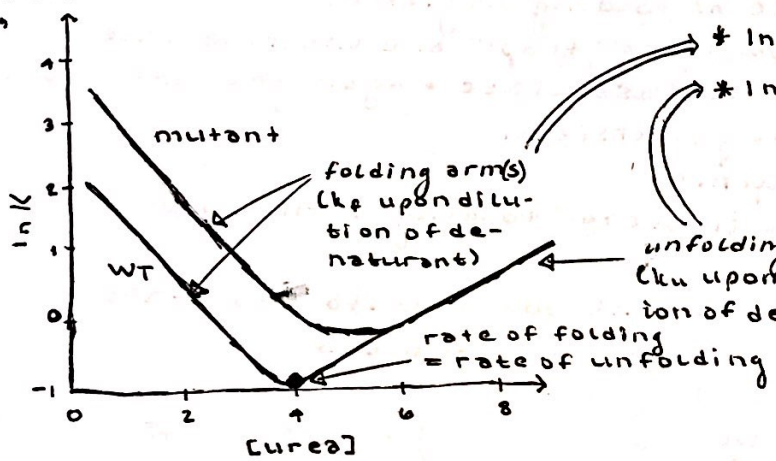
2) assistance in structural formation
   → folding pathways: proteins fold down a "folding funnel" toward the most stable/native state
   - fewer states are accessible as folding progresses
   - help is needed to avoid low-lying "traps" and intermediates
   - partially folded proteins can associate + aggregate into even more stable aggregates/oligomers/etc.
     - often biological consequences
   → chaperonins: proteins aiding in proper folding pathway of a protein
   - can increase folding rate
   - can decrease configurational entropy of unfolded state
   - can alter the folding landscapes several ways



3) energy input
   → can be coupled to ATP hydrolysis

PROTEIN FOLDING INTERMEDIATES: TOPIC TEN

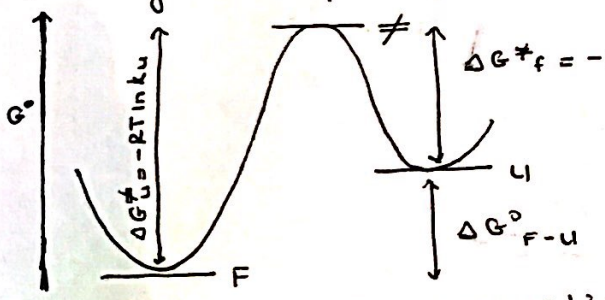
- Protein Folding + Unfolding
  - measuring rates
    - → unfolding rate (k_u) ⇒ mix protein quickly into soln. of urea or guanidinium chloride
    - → folding rate (k_f) ⇒ start w/protein in high urea or guanidinium chloride soln. + quickly dilute to reduce denaturant concentration
  - Chevron Plot
    - →



* $\ln k_f[\text{urea}] = \ln k_{f,H_2O} + m_f[\text{urea}]$
* $\ln k_u[\text{urea}] = \ln k_{u,H_2O} + m_u[\text{urea}]$

    - → relationship between kinetics + thermodynamics
      - $\Delta G^\circ_{F-U} = -RT \ln\left(\frac{k_u}{k_f}\right)$
      - $\Delta G^\circ_{F-U}[\text{urea}] = -RT\ln\left(\frac{k_{u,H_2O}}{k_{f,H_2O}}\right) + RT(m_u - m_f)[\text{urea}]$

- Folding + Transition State
  - rates
    - → folding rate depends on $\Delta G^{\neq}_f$ + unfolding rate on $\Delta G^{\neq}_u$



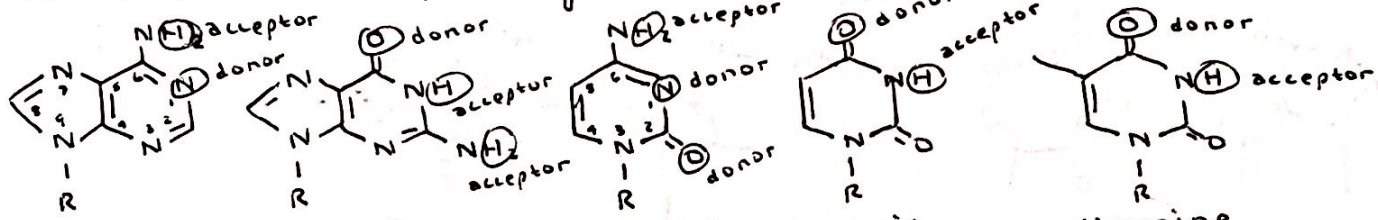
* can have intermediates + more complex kinetics but same stability

  - mutational analysis assumptions
    1) <u>specificity</u>: overall protein folding pattern not readily altered by one/few mutations
    2) <u>stability</u>: thermodynamic stability readily changed by a single mutation but folding pattern is not
    3) <u>kinetics</u>: single mutations influence folding/unfolding rates but not the pathway

- Phi Mutational Analysis
  - formula
    - → $\Phi = \Delta\Delta G^\circ_f / \Delta\Delta G^\circ_{F-U}$
      - if $\Phi = 0$, transition state is not changed but native structure is
      - if $\Phi = 1$, influence on kinetics + stability are essentially equivalent
  - patterns for mutations in proteins w/same folding pattern are conserved

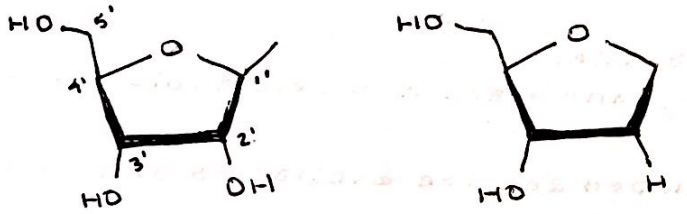
NUCLEIC ACIDS: TOPIC ELEVEN

- Structural Review
  - nucleotides + base pairing (Watson/Crick)



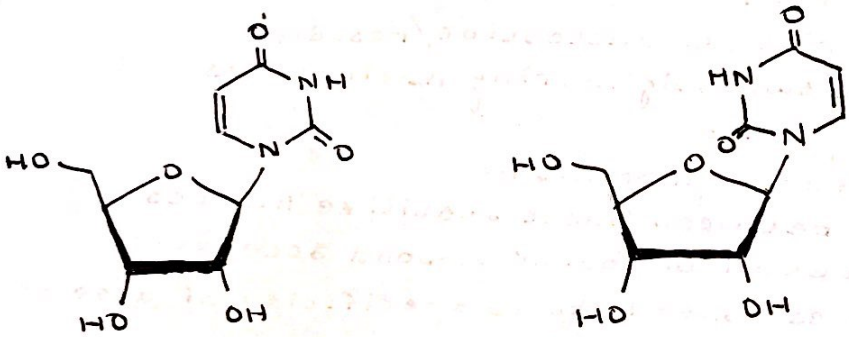
adenine guanine cytosine uracil thymine

  - sugars



ribose deoxyribose

  - *syn* + *anti* conformations of sugar + base

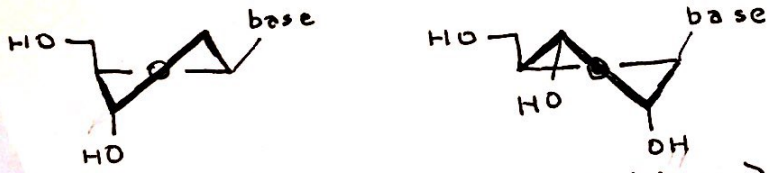


anti (required for WC base pairing) *syn*

→ differing conformations caused by rotation of the χ angle

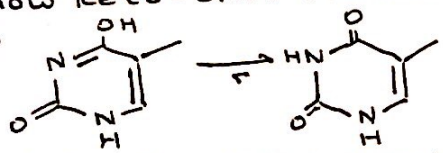
→ sugar ring itself can assume different conformations via rotation of the *pseudorotation angle*

- Confomers
  - χ + pseudorotation angles are correlated
    → anti conformation is associated w/ C_2-endo in DNA + C_3-endo in RNA



C_2-endo (deoxy.) C_3-endo (ribose)

- Nucleotide Properties
  - show keto-enol tautomerism

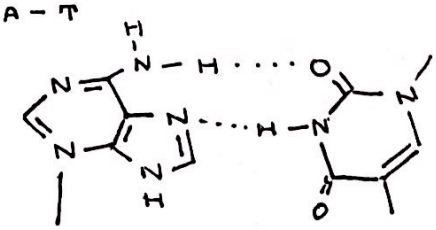
→ 

(ex. thymine)

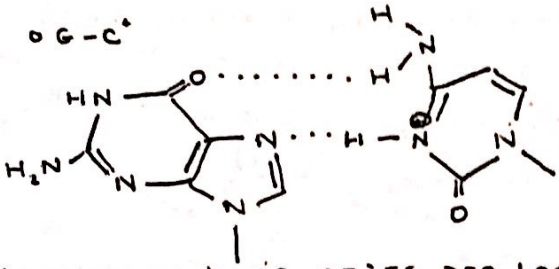
  - contain titratable functional groups
  - have large dipole moments

NUCLEIC ACIDS: TOPIC TWELVE

- Hoogstein Base Pairing
  - A – T



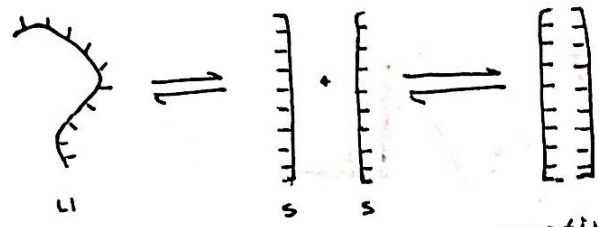
  - G – C⁺



  - distance between C_1 of Hoogsteen base pairs are less than that of Watson-Crick base pairs
- Properties of Nucleic Acids + Helix
  - properties
    - → strands can be parallel or antiparallel
    - → bases in a WC pair are not in a plane + are not perpendicular to the helical axis
    - → base pairing can remain undisturbed despite exchanges of hydrogens between bases
  - helical parameters
    - → <u>pitch</u>: (P) axial rise per turn
    - → <u>helical rise</u>: (H) helical rise per nucleotide/residue
    - → <u>twist</u>: (n) angle between two neighboring nucleotides
  - factors influencing base pairing
    - → structural changes / base polymorphisms
    - → stacking interactions between bases stabilize helices
    - → pKa can make bases better or poorer H-bond acceptors
    - → LDFs + permanent dipoles contribute to specificity of base stacking interactions

NUCLEIC ACID SECONDARY STRUCTURE: TOPIC THIRTEEN

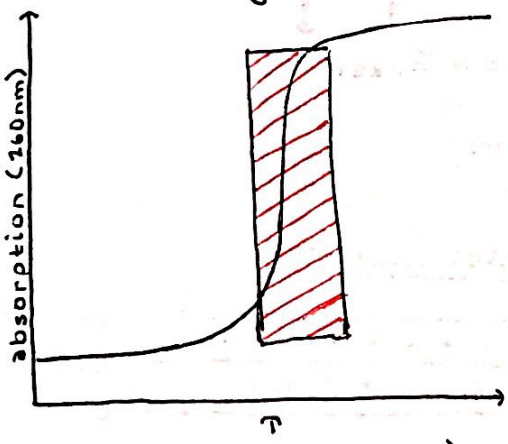
- Thermodynamics of Double Helix Formation
  - structural transitions

→ 

non-cooperative non-cooperative

* stacking interactions dominate in annealing/duplex formation

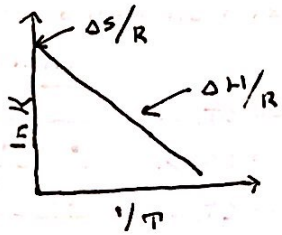
  - van't Hoff analysis can track the transition

→ 

(dsDNA melting curve)

* ΔC_p is mostly constant in the boxed range (error greater outside melting temperature range)

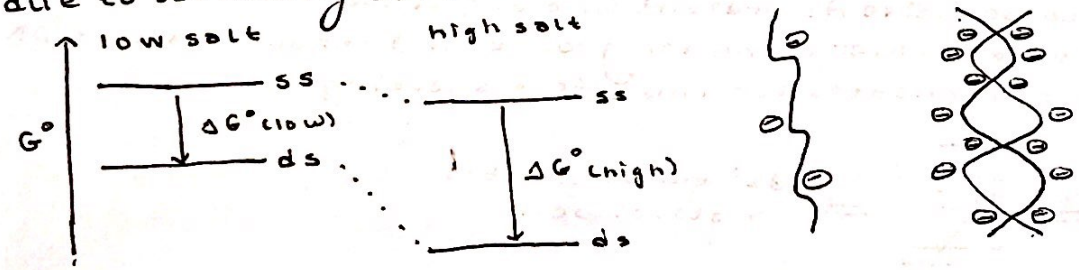
* caused by dsDNA → ssDNA → unstacked ssDNA transition

* 

$$-RT\ln K = \left(\frac{\Delta H}{R}\right)\frac{1}{T} + \left(\frac{\Delta S}{R}\right)$$

↑ A ↑ A_{260}

  - factors affecting melting temperature
    - → Tm increases w/ionic strength
      - higher salt concentrations stabilize dsDNA more than ssDNA due to screening effect



    - → Tm increases w/chain length
    - → Tm increases w/GC content due to greater stacking interactions

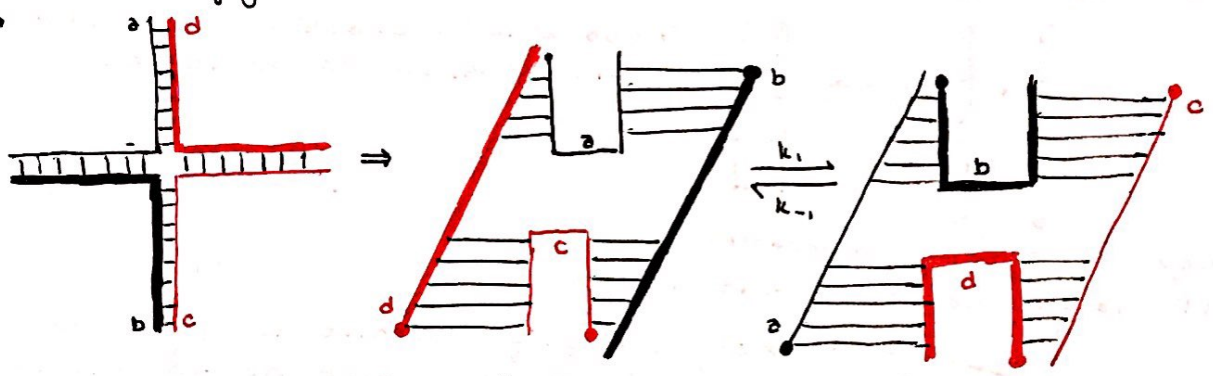
- Calculation of Approximate Tm
  - utilizes the nearest neighbor model
    - → formulas
      - $T_m = \sum_i f^i_{MN} \cdot T^i_{MN}$
      - $\Delta G = \Delta G_{initiation} + \Delta G_{terminal} + \Delta G_{symmetry} + \sum \Delta G_{stack}$
        (1.96) (0.05 if seq. ends w/AT) (0.43 if seq. is palindromic) (table)

- DNA stability is not very dependent on hydrophobicity
  - → ΔC_p is not significant

HIGHER-ORDER DNA/RNA STRUCTURES: TOPIC FOURTEEN

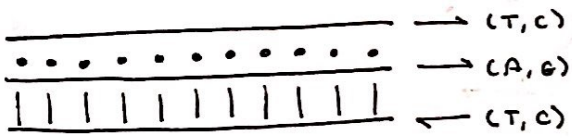
- Holliday Junctions
  - formed during genetic recombination + can take different structures

→

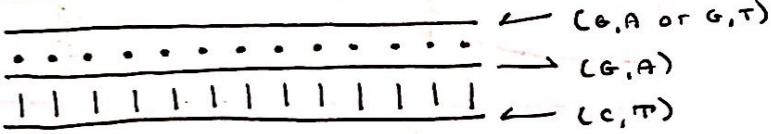


high E_{FRET} ⟷ low E_{FRET}
(can be changed by a metalloregulator)

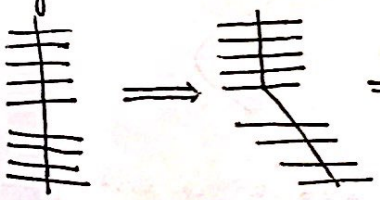
- Triple Helices
  - two forms/motifs — *parallel + antiparallel*
    - → parallel motif



antiparallel motif



  - ▫ Hoogsten BP represented by • and watson-Crick BP by |
  - formation is pH-sensitive + sometimes dependent on the presence of base modifications
- DNA Bends
  - A-tract: > 5 consecutive As present in a sequence
    - → compression at center of minor groove in A-tracts causes DNA bending (helix compensates for imperfect stacking)
    - ▫

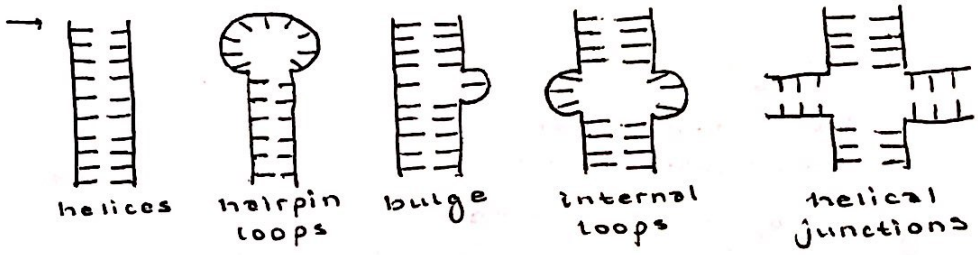


36° twist creates a global bend

  - cancel out if A-tracts are out-of-phase
    - → if spaced every 10-11 BP, bending is strong
    - → if spaced every 9 or 12 BP, bending is weak + helix is almost straight
- DNA Supercoiling
  - terminology
    - → linking number: (L) number of turns one DNA strand wraps around one another
    - ▫ $L = T + W$
    - → twist: (T) number of turns one strand of DNA makes about the helix axis ($T = BP/10.4$)
    - → writhe: (W) number of turns the duplex makes about the superhelix axis (=0 for relaxed DNA)
  - can be positive or negative
    - → right-handed is underwound
    - → left-handed is overwound
  - $\Delta L = 0$ for closed/circular DNA

RNA FOLDING + STRUCTURE: TOPIC FIFTEEN

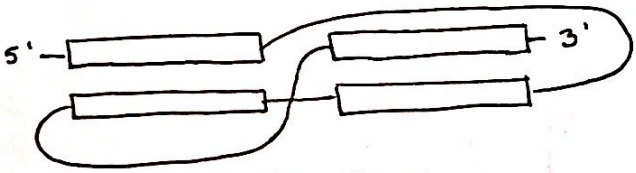
- RNA secondary structure Elements
  - five common motifs



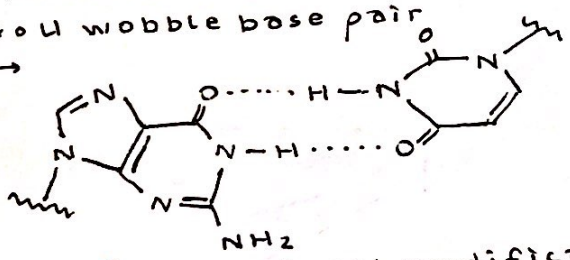
- Tertiary Structure Elements
  - <u>kink-turn motif</u>: introduces tight kinks into helical axis; standard turn has a 3-nucleotide bulge

```
→ 5' X X X   X X X   G A X X
     | | |           | | | |
  3' X X X ———————— A G X X
```

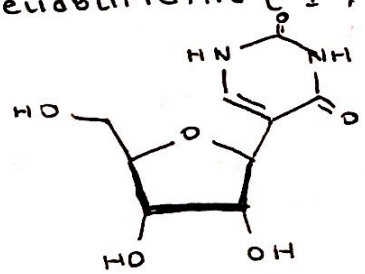
  - <u>right-angle motif</u>: introduces 90° angles in adjacent helices (usually around A) + stabilized by stacking interactions
  - <u>coaxial stacking</u>: terminal bases between two adjacent helices are stacked upon one another
  - <u>kissing loops</u>: loops from two hairpins interact w/one another
  - <u>pseudoknots</u>: formation of base pairs between loop regions + ss regions of same RNA or base pairing of alternate ssRNA regions



- Base Pairs + Modifications
  - G o U wobble base pair



  - post-transcriptional modifications
    - → methylation is the most abundant class
    - → pseudouridine (Ψ) is the most common modification

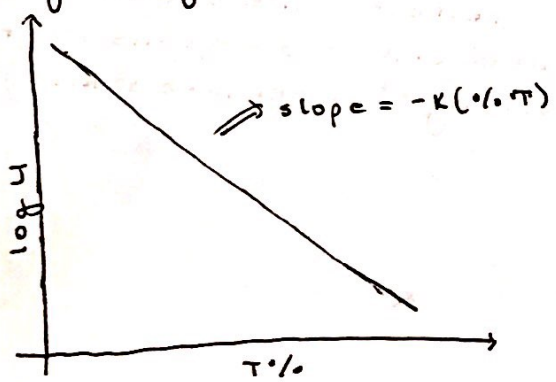


* may stabilize ssRNA base stacking + helical orientations near bulged residues

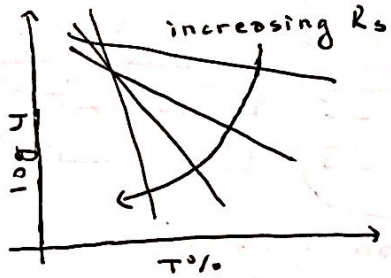
  - metal ions can influence free energy of hydration
    - → higher concentrations near the major groove

ELECTROPHORESIS: TOPIC SIXTEEN

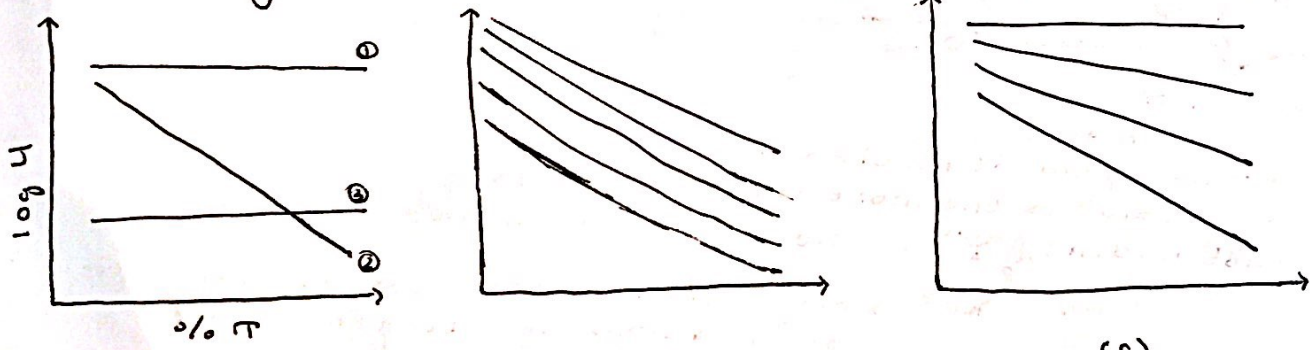
- Retardation Force
  - proportional to the *stokes radius*
    - → formulas
      - $F_{drag} = 6\pi\eta as$
        (η = viscosity)
        (a = sphere rad.)
        (s = drift velocity)
      - $R_s = \frac{k_B T}{6\pi\eta D}$
        (k_B = Boltzmann constant)
        (D = diffusion constant)
    - → higher molecular weight = larger Stokes radius
- <u>Electrophoretic Mobility</u>: velocity per unit of electric field
  - formula
    - → $\mu = \frac{Q}{f} = \frac{v}{E}$
  - depends on particle size, charge, + shape
    - → matrix enables consideration of all three factors
    - → sensitivity to R_s can be modulated by changing matrix pore sizes
    - $\log \mu = \log \mu_0 - K_R(\%T)$ ⇒ T = acrylamide



* gradient of the plot (<u>Ferguson plot</u>) gives retardation coefficient



- Info from Ferguson Plots



(A) (B) (C)

→ (A) ⇒ ① small size, high Q
② large size, high Q
③ small size, small Q

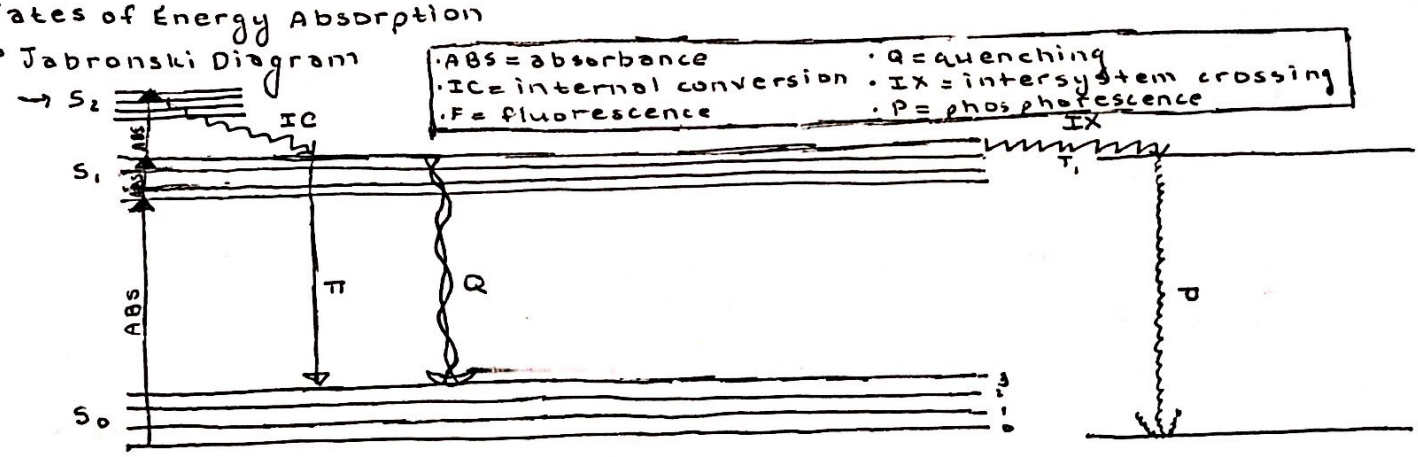
→ (B) ⇒ isozymes w/ similar sizes but different charges

→ (C) ⇒ diff. sizes but similar charges

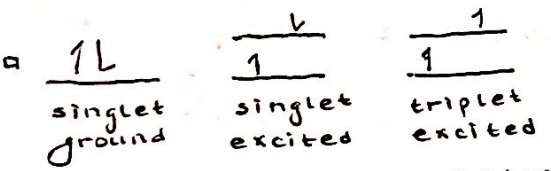
- <u>Isoelectric Focusing</u>: separation of proteins in a pH gradient according to isoelectric point
  - advantages of *gradient PAGE*
    1) increased molecular weight range
    2) increased resolution of proteins

FLUORESCENCE SPECTROSCOPY: TOPIC SEVENTEEN

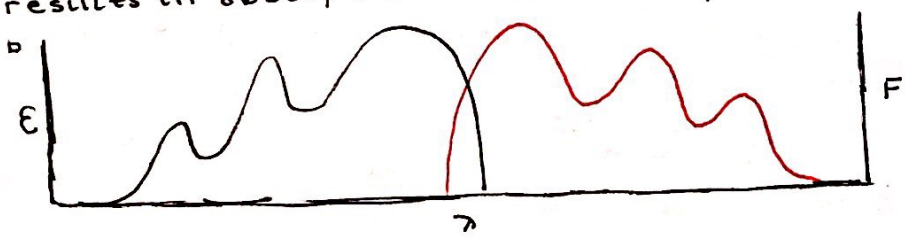
- Fates of Energy Absorption
  - Jabronski Diagram



- definitions
  - → fluorescence: emission of radiation energy
  - → internal conversion: result of collisions between molecules; energy is given off to vibrational states while molecular spin stays the same
  - → quenching: result of collisions between molecules; energy is given off as thermal energy or radiation to another molecule via excimer complex formation
  - → intersystem crossing: result of collisions between molecules; energy is given off to vibrational states but molecular spin does not stay the same
  - → phosphorescence: occurs through intersystem crossing; result of slow emission of energy from "forbidden" energy state(s) transitions (often a triplet excited state)



- Stokes shift: difference between absorption + emission wavelengths
  - → results in absorption + emission spectra looking symmetric

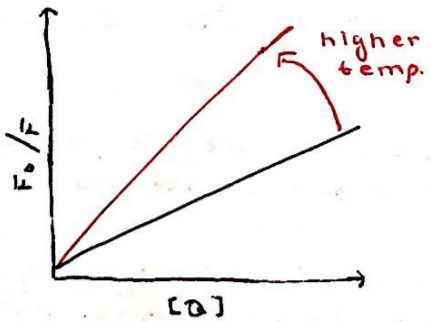


* called Franck-Condon overlap factors

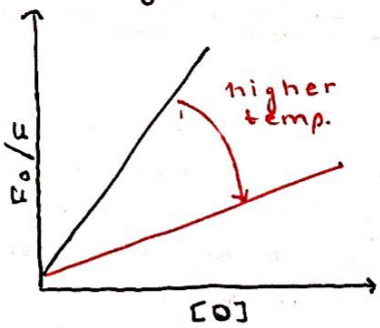
- quantum yield: (Φ) fraction of molecules that relax down to ground state via fluorescence
  - → proportional to lifetime of excited state
  - → $\Phi = \frac{N^* \cdot k_f}{I_0} = \frac{\cancel{N^*} \cdot k_f}{\cancel{N^*}(k_f + k_I)} = \frac{k_f}{k_f + k_I}$
  - → higher Φ is desirable for biological applications

FLUORESCENCE BIOAPPLICATIONS: TOPICS EIGHTEEN + NINETEEN

- Fluorescence Quenching
  - two types
    1) <u>collisional</u>: results from collisional encounters between fluorophore + quencher
    2) <u>static</u>: results from formation of a non-fluorescent fluorophore: quencher complex
  - Stern-Volmer equation
    - → $\frac{F_0}{F} = 1 + K_D[Q]$
    - temperature dependence changes based on type of Q



collisional q.



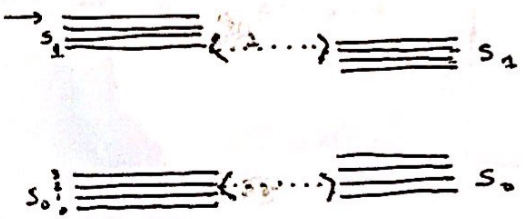
static q.

* slope of plot indicates solvent exposure of a fluorophore

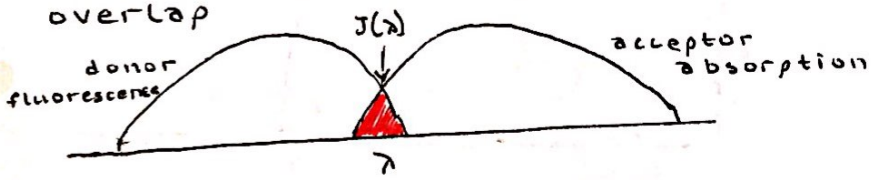
- molecular beacons are based on guanine quenching (fluorescence decreases upon target binding)
- base stacking results in greater quenching

- Fluoresce Anisotropy
  - looks at molecular tumbling through polarized light
    - → slow-tumbling molecules show more fluorescence polarization
      - tend to be smaller molecules
    - → large-tumbling molecules show less fluorescence polarization
      - tend to be larger molecules
  - increases w/protein binding + decreases w/strand separation

- FRET
  - mechanism of resonance energy transfer from donor to acceptor



→ donor + acceptor absorption spectra overlap



  - efficiency depends on donor + acceptor distance
    - → useful as a "molecular ruler"
    - → $E = \frac{R_0^6}{R_0^6 + r^6}$ → $FRET = \frac{I_A}{I_A - I_D}$

MASS SPECTROMETRY: TOPIC TWENTY

- Mass Spec Components

1) ion source

→ MALDI: matrix-assisted laser desorption ionization

- use steps
  - protein cocrystallized w/matrix
  - electric field applied between sample + TOF entrance
  - laser pulses excite matrix + protein into gas state
  - biomolecule ions like M^+ + $(M+H)^+$ are produced + detected
- pros/cons
  - pros
    - ~ mass range to 300,000 Da
    - ~ femtomole sensitivity
    - ~ soft ionization
    - ~ tolerates millimolar salts
    - ~ can analyze complex mixtures
  - cons
    - ~ coupling not as easy as LC/ESI MS
- two sample prep methods
  - (i) dried droplet: protein premixed w/matrix + soln. spotted + dried on MALDI sample plate
  - (ii) fast evaporation: water-insoluble matrix spotted + allowed to dry before protein sample applied + absorbed onto matrix crystals

→ ESI: electrospray ionization

- use steps
  - dry gas/heat applied to evaporate solvent from droplets
  - decreasing droplet size increases charge density, causing droplets to break up + eject ions
  - electric field creates Taylor cone + fine spray of highly charged droplets
- produces multiply charged ions
  - $z_n = \frac{m_{n+1} - 1.008}{m_n - m_{n+1}}$
- pros/cons
  - pros
    - ~ mass range to 70,000 Da
    - ~ femtomole sensitivity
    - ~ softest ionization method (can generate non-covalent complexes)
    - ~ no matrix interference
    - ~ LC/ESI MS coupling is easy
    - ~ can analyze high m.m. ions
  - cons
    - ~ sensitive to salts
    - ~ cannot easily analyze complex mixtures

2) mass analyzer

→ quadrupole: oscillating electric fields stabilize/destabilize ion paths or trap ions w/specific m/z ratios

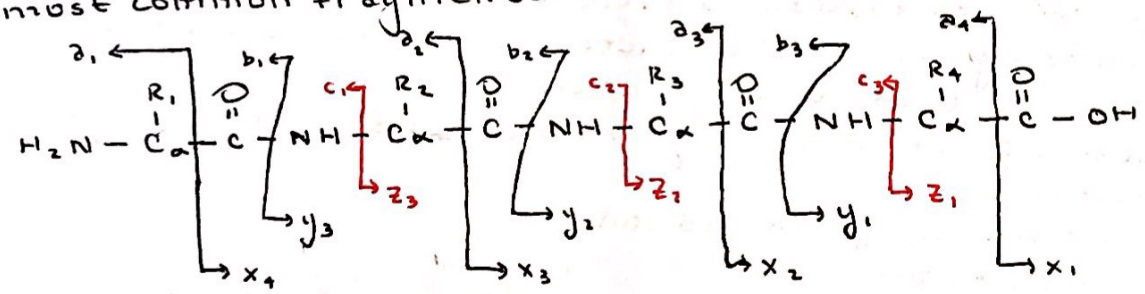
→ TOF: accelerate ions through a potential then measure time of flight in a drift tube

3) detector

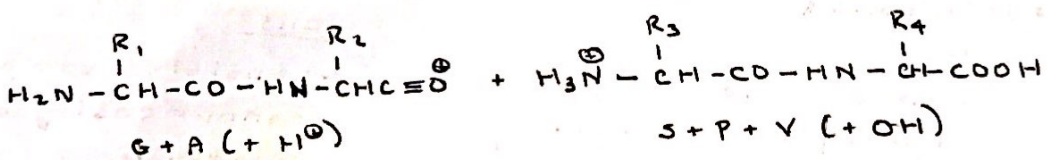
→ record charge induced or current induced when ion passes/hits detector

MASS SPECTROMETRY BIOAPPLICATIONS: TOPIC TWENTY-ONE

- **Mass Resolution:** ability to detect two neighboring peaks
  - formula
    - → $R_s = \frac{m}{\Delta m} = \frac{m_1}{m_2 - m_1}$
- accuracy + range
  - → **mass accuracy:** ratio of difference between measured + true m/z
  - → **mass range:** range of m/z that can be analyzed by a given mass analyzer
    - greatest for MALDI
- Mass Definitions
  - **average molecular mass:** weighted average of naturally occurring isotope masses
  - **monoisotopic mass:** mass of molecule using masses of most abundant isotope for each element
  - **nominal mass:** mass of molecule using integer mass of most abundant isotope for each element
  - **most abundant mass:** mass of most abundant ion in the isotope distribution

- Peptide Sequencing
  - can be done w/ tandem MS
    - → proteins digested w/ trypsin enzyme
    - → **CID:** collision-induced dissociation
      - most common fragmentation method
      - 

⇓ ex. cut at b_2/y_2



⇓

calculate fragment masses using monoisotopic mass table

MOLECULAR INTERACTIONS: TOPIC TWENTY-TWO

- Classes of Molecular Interactions
  1) <u>micro-associations</u>: small ligands interacting w/a macromolecule
     (i) substrate – enzyme binding
     (ii) cofactor – enzyme binding
     (iii) allosteric effector binding
     (iv) metals/dyes/etc. binding to proteins, nucleic acids, or membranes
  2) <u>macro-associations</u>: interactions between macromolecules
     (i) protein subunit association
     (ii) multi-enzyme complexes
     (iii) protein binding to membranes
     (iv) polynucleotide interactions
     (v) protein-nucleic acid binding
- Collisions
  - must consider diffusion of both molecules
    → $k_{coll} = \frac{4\pi R_{12} D_{12} N}{1000}$
  - only collisions w/in binding site are effective
    → $\frac{\text{binding site area}}{\text{total area}}$ = fraction of b.s. surface area (f_n) → effective collision rate = (f_n)(collision rate)
- Thermodynamic Considerations
  - most stability comes from hydrophobic interactions
    → <u>favorable components</u>
    1) hydrophobic interactions give ~25 cal/Å^{2} buried nonpolar surface area
    2) hydrogen bonds give ~0.5-0.6 kcal/mol depending on neutral or charged pair
    3) van der Waals interactions

    <u>unfavorable components</u>
    1) loss of translational + rotational entropy (-20 kcal/mol)
    2) loss of entropy upon complex formation is not size-dependent so multiple contact sites are favorable
- Adair Equation
  - relates ligands bound to total protein concentration

$$\rightarrow \bar{v} = \frac{A_b}{P_T} = \frac{[PA] + 2[PA_2] + 3[PA_3] + \dots N[PA_N]}{P_f + [PA] + [PA_2] + [PA_3] + \dots [PA_N]}$$

$$= \frac{K_1[A] + 2K_1K_2[A^2] + 3K_1K_2K_3[A^3] + \dots NK_1K_2\dots K_N[A^N]}{1 + K_1[A] + K_1K_2[A^2] + K_1K_2K_3[A^3] + \dots K_1K_2\dots K_N[A^N]}$$

→ single binding-site derivation

- $S + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SA$

$$\{[S](1-\theta)\}k_1 = \{[S](\theta)\}k_{-1}$$

$$\left(\frac{\theta}{1-\theta}\right) = K_0[A]$$

$$\frac{\bar{v}}{N-\bar{v}} = K_0[A]$$

⇓

* infinitely cooperative systems show all-or-none binding*

⇓ (assume four sites)

$$\bar{v} = \frac{4K_1K_2K_3K_4[A^4]}{1 + K_1K_2K_3K_4[A^4]}$$

⇓

*general version is called the Hill equation

- Binding Plots
  1) direct ($\bar{v}$ vs. [A])
  2) Bjerrum ($\bar{v}$ vs. log[A]
  3) Hill ($\frac{\theta}{1-\theta}$ vs. log[A])
  4) reciprocal ($1/\bar{v}$ vs. $1/[A]$)
  5) Scatchard ($\bar{v}/[A]$ vs. $\bar{v}$)
     → cooperativity gives rise to curving in these plots