PHYS 380: Molecular and Cellular Biophysics

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Cell structure and molecular composition; intermolecular interactions and hydration; protein structure and function; cytoskeletal filaments; DNA structure, packing and chromosomes; rate equations and biological dynamics (e.g., cytoskeletal polymerization); self-assembly; cell membranes; action potentials and biological electricity; molecular motors; cell motility.

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Introduction to Biophysics

Subset of Biophysics

- BIOPHYSICS: The bridge between biology and physics
    - Biology usually presents a problem
    - Physics offers quantitative, abstract, and predictive tools to solve them
- MOLECULAR BIOPHYSICS: A subset of biophysics, where biological problems presented are on the molecular scale
    - Biomolecules and interactions with other molecules
- CELLULAR BIOPHYSICS: Subset of biophysics focusing on physical principles used in cell function

Significance of Biophysics

- How does physics play a role in biology?
    - Physicists were the ones who invented ways of approaching biological problems
    - This stimulated a revolution in biological and technological advancements
- Nowadays, physicists can model and analyze complex systems, or even manipulate single molecules
- There's still a lot to discover!

From Big to Small

cellular communities → cells → molecular structure → biomolecules → simple molecules → atoms

- Molecular biophysics focuses molecular structures and biomolecules

Aspects of Life

- All living things are made of cells
    - At higher levels, there are noticeable differences [diversity]
    - Fundamentally, all cells are similar on a molecular scale [unity]
- Let's take a closer look at unity at the molecular level
    - Cells use the same chemical building blocks to build their biomolecules (DNA, protein, etc.)
    - Information flows from DNA → RNA → Protein

- Physical/mechanical properties are very similar
- The great four macromolecules
  - **Nucleic acid:** Crucial role in storing and transmitting genetic information
  - **Protein:** They do most of the work in cells and help with the structure, function, and regulation of the host
  - **Lipid:** Stores long-term energy, protects via formation of barriers and membranes
  - **Carbohydrate:** Used for energy storage and surface properties

Central Dogma of Molecular Biology

- describes the flow of genetic information within a biological system
  - DNA Replication → DNA Transcription → mRNA Translation
- Differences become information
  - Can we not assume that different links within a chain can carry information?
  - What kind of info?

Biological Complexity and Modelling

- DNA
  - we can model only one aspect at a time
    - Sequence, binding site, charged rod, elastic rod, random walk
- Proteins
  - proteins have so many different configurations; we need separate models for each type of structure (primary, secondary, tertiary, etc.)
    - We look at hydrophobic or hydrophilic amino acids in sequences
    - We look at random walks for native states
    - We look at α-helices and β-sheets for secondary structures
- Lipid Membrane
  - can be modeled as different types of surfaces, based on phospholipid properties
    - array of springs, random surface, RC circuit, semi-permeable barrier
- Cell movement
  - Receptor array, swimmer, random walk, genetic network

- Solutions
    - We will be looking at how solutions can be idealized as lattices, mean flow, a viscous medium, and more
    - Lattice solution, flow, viscous medium, diffusive landscape, hydrophilic medium, dielectric medium

Modelling as a Spring

- A lot of biological models are based on simple springs in classical mechanics
- We can then associate a simple harmonic oscillator to biological systems!!

Modelling, Chance, and Entropy

Starting Point for Modelling

- BIOPHYSICAL MODEL: A simulation of a biological system that is based upon the physical properties of that system
    - Assumptions will need to be made
    - Certain features will have to be ignored → a model will never be perfect
- One biological system could have many different models! → each with different parameters
    - We need to choose what we want to study from that system
    - EX: DNA can be viewed in different ways:
        - Chemical Structure → Sequences and Binding Sites
        - Behaviour → due to Charge, Elasticity, Thermal Undulations
- Always start with the easiest, simplest model
    - Work your way up until your model fails
    - Balance between simplicity and correctness of prediction

Wrong Models

- If you're simplifying something, how can you tell what's right from wrong?
    - Rooted in foundational or fundamental knowledge
    - If there are known & proven averages or measurements – use them!
- But, there are also several ways that you can arrive at the "wrong model"
    - No relevance to the system ->wrong parameters
    - Lack of detail → oversimplifying
    - Too many assumptions
    - The perfect model with incorrect results
        - This usually means that there is something unknown about the situation → might lead to breakthrough in science!
- Just learn from each experience

Chance in life: Multiplicity (W)

- Ex: Coin tossing → a sequence of heads or tails given x coins
    - All sequences are equally probable → Thus unpredictable
    - Is the composition (# of H's or T's) predictable? → Yes!
    - How about figuring out the most probable composition or state? → Yes!
- From Randomness to Predictability
    - Let's take a look at the randomness of coin flips
        - Assume coins are on needles and placed in boiling water
        - Movement of water can "flip" these coins from H to T, T to H randomly
    - What does the system tend towards?
        - Most probable composition is 50% Heads, 50% Tails
        - **A closed system tends towards a state of higher multiplicity**
            - Although we can't look at SEQUENCE, we look at MULTIPLICITY
            - **Multiplicity (W):** number of sequences with the same composition
                - We look at the number of H's or T's in a coin toss
                - Assume n = # of H's and N = total # of tosses
                - Then N-n = # of T's

$$W(n,N) = \frac{N!}{n!\,(N-n)!}$$

                - Each toss is random, but the composition is predictable with great precision
        - Disordered at high multiplicity

Connecting Multiplicity to Entropy

- Ludwig Boltzmann established an important link!
    - Connection between the microscopic (W) and macroscopic worlds (E)
    - Connected multiplicity with energy / entropy / equilibria

$$S = k_b \ln W$$

- S = entropy
- W = multiplicity
- $K_b = 1.38 \times 10^{-23}$ J/K
  Boltzmann constant

- For a two-state system:

$$S = k_B \ln W = k_b \ln \frac{N!}{n!\,(N-n)!}$$

Law of Increase of Entropy

- Any system tends towards a state of higher entropy!
  - The entropy after some time will be greater than before
  - This is called the "Arrow of Time"
    - We can determine when a specific event occurred, as lower entropy is in the past
- Example:
  - If there are a bunch of particles in a box... the system will tend towards a higher entropy S, so the particles will distribute and expand the box

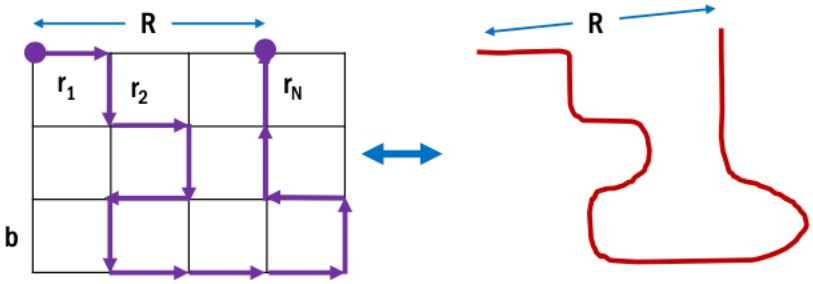
Entropic Elasticity of Polymers

- We can assume that these are entropic springs → Any chain molecule can be depicted as "beads on a string"
- that means:
  - That means there are many configurations of this polymer
  - But which types would be predictable? Less ordered?
- Consider two cases:
  - FULLY STRETCHED:
    - Very ordered, less entropy → One chain configuration only
    - L is total length

- PARTIALLY STRETCHED:
    - Disordered, more entropy → more likely since there are more
    - x is distance b/w start and end

Random Walk Model for Polymers

- We can mimic a polymer using a simple random walk model
- RANDOM WALK MODEL: random walk on a rectangular lattice, jumping to neighbouring sites of the lattice each step length (b). R is the direct length



Example: Genomic Size of Viral DNA

- What do we have here?
    - Electron microscopy image of a bacteriophage genome that has escaped its capsid
    - It's a ruptured bacterial virus with DNA spilling out!
- PHYSICAL SIZE: amount of physical space occupied by DNA
- GENOMIC SIZE: length in base pairs of the DNA
- estimate the genomic size of DNA by examining the physical size of randomly spread DNA → we need…
    - A model relating the physical size of DNA(R) to the number of base pairs (Nbp)
    - Some known values
        - Length of DNA base pair, length of mitochondrion, diameter of nucleus, thickness of lipid bilayer, etc.
    - Let's work towards that!
- If we're trying to model the size of a polymer in solution, we need some parameters
    - Contour Length (L): how much "bend" there is

- Persistence Length (ξp): max length where polymer is considered rigid
- End-to-End Distance (R): distance from tip to tip of polymer chain

$$\sqrt{\langle R \rangle^2} \approx \sqrt{2L\xi_p}$$

- Radius of Gyration (RG): average distance between monomers and center of mass of polymer

$$\sqrt{\langle R_G^2 \rangle} \approx \sqrt{\frac{L\xi_p}{3}}$$

Intramolecular / Intermolecular Interactions and Hydrogen Bonding

Intramolecular vs. Intermolecular

- INTRAMOLECULAR FORCE: a force that binds atoms together to make a molecule or compound
    - Ionic Bond vs. Covalent Bond vs. Metallic Bond
    - These are strong forces
        - Chemical bonds are considered intramolecular forces
        - Can measure bond enthalpies and bond strength to determine chemical properties
- INTERMOLECULAR FORCE: a force that is present between two molecules or atoms that are not bonded
    - Often weaker than intramolecular forces
        - Important since they help determine physical properties of molecules like:
            - Boiling Point, Melting Point, Density, Enthalpy of Fusion, etc.
    - Hydrogen Bonding
    - Ionic Bonding
    - Ion-Induced Dipole Forces vs. Ion-Dipole Forces
    - Vander Waals Forces (Keesom, Debye, London Dispersion)

Valance Shell

- The outermost electrons determine how atoms interact
- Inert gases only have filled shells, and are therefore chemically unreactive
- The outmost shell of other elements is unfilled and can participate in reactions with other atoms/molecules
- An element's chemical reactivity is based on how its outermost electron field (valence shell) is filled

Ionic Bond

- formed when electrons are transferred from one atom to another
- Ex: NaCl
    - Na has 1 electron in valence shell
    - Cl has 7 electrons in valence shell
    - When electron jumps from Na to Cl, both atoms become charged ions
    - Na+ and Cl- experience electrostatic attraction (between oppositely charged atoms), and form an ionic bond

Covalent Bond

- formed when electrons are shared between two or more atoms
- A MOLECULE is a cluster of atoms held together by covalent bonds
    - Shared electrons complete valence shells of both atoms to become more INERT
- BOND LENGTH between two nuclei is determined by attraction/repulsion
- Ex: Hydrogen atom
    - Each H has 1 electron in valence shell
    - Alone, its valence is incompletely filled
    - Together, two H atoms share 2 electrons
        - Obtain completely filled 1st shell
        - Shared electrons adapt modified orbits around the two nuclei
        - Distance between the two nuclei is optimized by electrostatics and gives us a bond length of 0.074 nm
- There can be more than one covalent bond per molecule
    - How can we determine how many bonds there will be?
        - We look at the number of missing electrons in valence shell!
- **Spatial Geometry**
    - Covalent bonds are characterized by particular geometries
    - Molecules formed by O, N, C have a precise 3D structure defined by BOND ANGLES and BOND LENGTHS
    - H2O has a V-shape due to 109° bond angle for oxygen

- **Bond Strength**
    - the amount of energy that must be supplied to break a covalent or noncovalent bond
    - Expressed in units of kcal/mole or kJ/mole
        - Kilocalorie: amount of energy needed to raise temperature of 1L of water by 1°C
            - 1kcal=4.2kJ
    - Covalent bonds are strong because
        - They become more inert as outer valence is completely filled
        - Not breakable spontaneously at room temperature
        - Responsible for the formation of small and large molecules
- **Single and Double Bond**
    - Sometimes, covalent bonds can share more than two electrons
    - SINGLE BOND:
        - sharing of one e- from each atom/molecule
    - DOUBLE BOND:
        - sharing of two e- from each atom/molecule
        - More "stiff", short, and cannot rotate (planar)
        - Major influence on 3D shape of macromolecules
- **Polar and Non-Polar**
    - Different atoms are attracted to each other to different degrees
        - C,N,O atoms attract electrons strongly, while H atom is weak
        - A POLAR structure is one where:
            - Positive charge is concentrated towards one end of the molecule (positive pole)
            - Negative charge concentrated to the other end (negative pole)
    - POLAR COVALENT BOND: covalent bonds that share electrons unequally

Hydrogen Bond

- The water molecule is a PERMANENT DIPOLE
    - Contains covalent bonds connecting atoms together
- Water molecules are held together and connected by HYDROGEN BONDS
    - It is a polarizable medium

Weak Intermolecular Forces

- COULOMB FORCE
    - electrical force between charged particles
    - Can be described using Coulomb's Law
    - Weak in water
- CHARGE-DIPOLE INTERACTIONS
    - occur in presence of atom with formal net charge and a dipole
- CHARGE-DIPOLE INTERACTIONS
    - occur in presence of atom with formal net charge and a dipole that rotates
    - We model net charge as a sphere
- DIPOLE-DIPOLE INTERACTIONS
    - occur in presence of two molecules with dipoles
- DIPOLE-DIPOLE INTERACTIONS
    - occur in presence of two molecules with dipoles that rotate
- CHARGE-INDUCED DIPOLE FORCE
    - a dipole is induced on a net-zero charge due to a neighbouring charge q
- DIPOLE-INDUCED DIPOLE FORCE
    - a dipole is induced on a net-zero charge due to a neighbouring dipole
- LONDON DISPERSION FORCE
    - attraction between two transient, quantum dipoles that arise from quantum fluctuations
- VAN DER WAALS FORCES
    - weak, short-ranged electrostatic attractive forces between uncharged molecules
    - Arise from interaction of permanent or transient electric dipole moments

Types of Hydrogen Bonds

- Hydrogen Bond (H-Bond)
    - an electrostatic force of attraction between
        - (1) an H atom that's covalently bound to a more electronegative group like O, N, F, and
        - (2) another electronegative atom (like O, N, F) bearing a lone pair of electrons
- Hydrogen bonds can be:
    - Intermolecular: occurs between separate molecules (e.g. H_2O)
    - Intramolecular: occurs within same molecule (e.g. $C_2H_4(OH)_2$) → folds into itself

Hydrogen Bond in Water

- Hydrogen bonds are formed between two adjacent H_2O molecules
    - This is because they are polarized
- Hydrogen bonds are strongest when linear
    - They have 1/20 of the strength of a covalent bond

Water Molecule

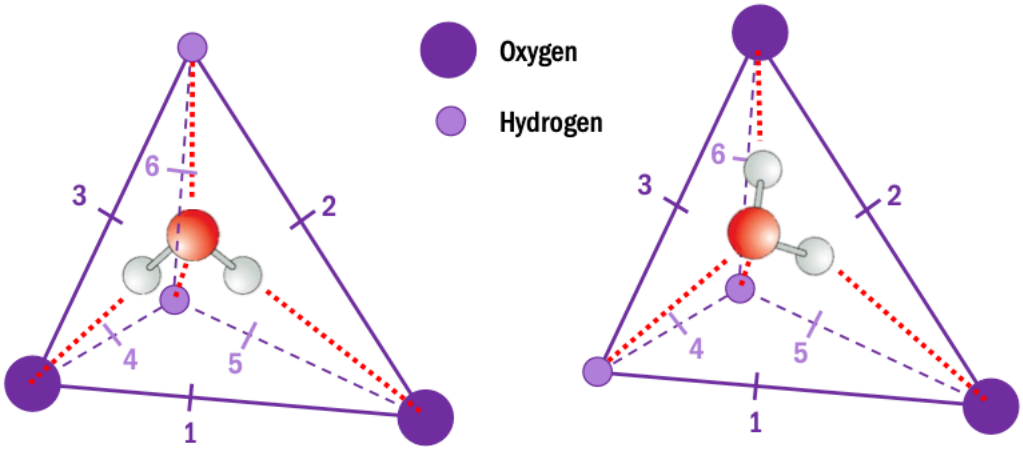
- WATER MOLECULE has net neutral charge (# protons = # electrons)
- But electrons are ASYMMETRICALLY DISTRIBUTED, making water a POLAR MOLECULE
    - Oxygen nucleus draws e- away from hydrogen nuclei
    - H becomes more positive (δ+) and O becomes negative(δ-)

Water Network

- Water molecules join together as a HYDROGEN-BONDED NETWORK
    - H-bonds constantly form, and constantly break (~33% broken at room temperature)
    - As T rises, there are less H-bonds (more breaks)
- Such COHESION is responsible for water's unique properties:
    - High surface tension
    - Specific heat
    - Heat of vaporization

Tetrahedral Bonding

- Each water molecule forms a TETRAHEDRAL BONDING arrangement with its neighbouring water molecules
- Why is this possible?
    - Oxygen is bound to 2 H and 2 lone pairs
    - Together, four pieces need to be separated equally → Forms a tetrahedral shape
- MULTIPLICITY (W): total number of fully H-bonded configurations of the central molecule
    - There are a total of 6 tetrahedral H-bonding arrangements (W=6)
    - Central H2O molecule can be H-bonded with 4 surrounding water molecules on the tetrahedral vertices, each facing towards one edge



W = # of possibilities of the 2 H's to orient towards 1 edge = 6

$$S = k_B \ln(6)$$

- Presence of other hydrophobic/hydrophilic molecules can affect H-bonding arrangements

Hydrophilic Molecules

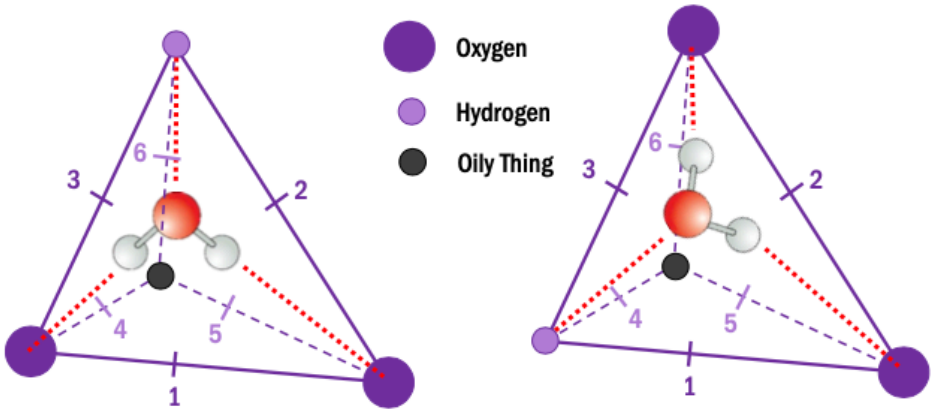
- substances that dissolve readily in water
- Composed of ions or polar molecules that attract water
- Water surrounds each ion or polar molecule and carry it into the solution

Hydrophobic Molecules

- substances that contain a majority of nonpolar bonds, rendering them insoluble in water
    - Non polarized molecules do not attract water
    - Chances of surrounding substance are slim
- Hydrocarbons that contain many C-H bonds are especially hydrophobic

Hydrophobic Molecules in Water

- What happens if we introduce an oily (hydrophobic) substance inside water?
    - It does not break the previously formed hydrogen bonds
    - H-bonds are not broken, but rather water molecules are reoriented around oily substance to remain fully H-bonded
    - Entropy decreases since reoriented molecules are more structured than before
    - But that means individual water molecule entropy remains the same
    - If temperature increases, water molecules are heated and have more movement, disrupting H-bonds
        - Become less structured, and therefore has higher entropy
- We introduce an oily substance at a vertex; so edges with this vertex are no longer possible configurations
    - Now W=3



The water molecule will reorient itself
W = 3

Hydrophobic Forces in Water

- Water forces hydrophobic groups together to minimize their disruptive effects on the H-bonded water network
- The more hydrophobic groups that are separated, the more entropy decreases for water
- Combined hydrophobic groups increase entropy

Water and Life

- Weak interactions play a crucial role in everything
- Key constant:
    - Boltzmann constant kb = 1.38 e-23 m2kgs-2K-1 or J/K
    - Temperature at T = 300K, 1 kbT = 0.6 kcal/mol = 4.14 e-21 J = 1/40 eV
    - Energy of H-atom is ~10 eV
    - Covalent bonds between atoms are ~1 eV

Hydrogen Bonds in Water

- If a molecule can form H-bonds with water, then they can form H-bonds between each other
    - H-bonds formed between molecules dissolved in water are relatively weak → due to competition with H2O

H-Bonds in DNA

- DNA is made of four nucleotide building blocks
- Two strands of DNA are held together by hydrogen bonds between base pairs
    - Bases are hydrophobic and located on inside of structure, creating helix

H-Bond in Protein

- Polypeptide chains often fold into either α-helix or β-sheet forms
    - For α-helix: H-bonds are between N-H and C=O of neighbouring peptides within one strand
    - For β-sheet: H-bonds are between peptide bonds in different strands

Self-Assembly of Lipids

- Formation of a sealed compartment that shields hydrophobic tails from water

Entropy and Free Energy

Temperature

- TEMPERATURE 1.0: what you measure with a thermometer
- TEMPERATURE 2.0: the thing that's the same for two objects after they have been in contact for a while (THERMAL EQUILIBRIUM)
    - The exchange quantity to achieve thermal equilibrium is energy
- TEMPERATURE 3.0: a measure of the tendency of an object to spontaneously give up energy to its surroundings
    - When two objects are in thermal contact, the one that tends to spontaneously lose energy is at the higher temperature

Ideal Gas Law

- PV=nRT
- In thermodynamics, PV=NkbT, N = number of molecules

Entropy

- SECOND LAW OF THERMODYNAMICS: the total entropy of an isolated system increases over time
    - Multiplicity tends to increase
- ENTROPY (S): the logarithm of the number of ways of arranging things in the system, multiplied by Boltzmann's constant
    - Unit: J/K when W (number of microstates within a given state) is unitless

$$S \equiv k_B \ln W$$

- ENTROPY is also referred to as the "disorder" of a system → the more there are ways to arrange the molecules, the higher the entropy

Chemical Thermodynamics

- The study of chemical reactions constrained by the laws of thermodynamics
    - The system does not end up in the same state where it started
- What's different in chemical reactions versus cyclic processes?
    - The system is not isolated but rather interacting with its surrounding all the time
    - There interactions can be thermal or mechanical
    - The energy of the system is usually not fixed
- We can fix some parameters

Enthalpy (H)

- the energy needed to create a system plus the work needed to make room for it

$$H \equiv U + PV$$

Helmholtz Free Energy (F)

- the energy needed to create a system, minus the energy you can get from the environment → Energy needed to create the system from scratch
    - Energy from environment is the heat you can get for free from a specific temperature

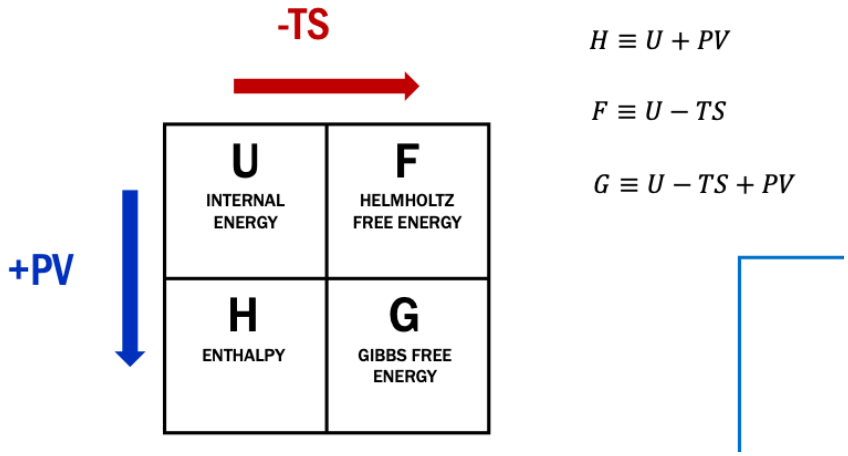
$$F \equiv U - TS$$

Gibbs Free Energy (G)

- in a system at constant pressure and temperature, this is the work needed to create it
- Delta G in a creation tells us the maximum amount of work that can be obtained from a reaction

$$G \equiv U - TS + PV$$

Important Diagram



$$H \equiv U + PV$$

$$F \equiv U - TS$$

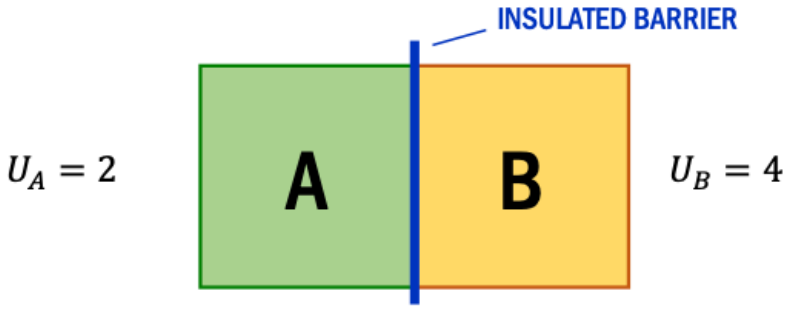
$$G \equiv U - TS + PV$$

Two-Level Systems for Energy Levels

- The more ENERGY LEVELS/SHELLS an atom has, the more energy it has (more electrons)
- We can use a TWO-LEVEL SYSTEM as a simple model
  - Focus on 2 levels: ϵ=0 (ground state), ϵ=1 (excited state)
- Total energy (U) = Sum of each individual energy state times the number
- The multiplicity (different ways that we can distribute the particles if the total energy does not change) is:

$$W_A = \frac{N!}{n_0!\, n_1!}$$

- For an INSULATED, COMBINED SYSTEM with two parts, <u>multiply the multiplicities to get the total multiplicity</u>



$$W_{\text{TOTAL}} = W_A \times W_B = 45 \times 210 = \mathbf{9{,}450}$$

- For a COMBINED SYSTEM IN THERMAL CONTACT
    - Can exchange energy in the form of heat
    - Since the system will always tend towards larger multiplicity or greater entropy
        - Utotal = the sum of the energy of two systems
        - Determine a way to distribute the multiplicity to obtain the highest overall multiplicity (the product of two multiplicity)
            - Always happens at thermal equilibrium
- Heat will always flow from a high-temperature system to a low-temperature system if both systems are in thermal contact
    - This flow will continue until the temperature is uniform, at which point the total multiplicity and total entropy is maximized

Probability and Multiplicity

- Probability is proportional to multiplicity

Let's revisit our **EQUATION FOR ENTROPY**:

$$S = k_B \ln W = k_b \ln \frac{N!}{n!\,(N-n)!}$$

We know that **PROBABILITY IS PROPORTIONAL TO MULTIPLICITY**

$$\text{Probability} \propto W = e^{\left[\frac{S}{k_B}\right]} = e^{\left[\frac{-TS}{k_B T}\right]}$$

- Minimum free energy state is the most probable state
    - The probability to be in any free-energy state is given below

If $U = 0$

$$\text{Probability} \propto e^{\left[-\frac{F}{k_B T}\right]} = e^{\left[\frac{-TS}{k_B T}\right]}$$

If $U \neq 0$

$$\text{Probability} \propto e^{\left[-\frac{F}{k_B T}\right]} = e^{\left[-\frac{U-TS}{k_B T}\right]}$$

PROBABILITY TO BE IN A CERTAIN FREE ENERGY STATE

Protein Structure and Function

Protein

- a class of macromolecules that consists of one or more chains of amino acid residues
    - They perform many functions within organisms, from molecular transport to DNA replication
- A PROTEIN SEQUENCE helps to determine a protein's 3D structure
    - This structure plays a role in determining its function
- Any MISFOLDING of proteins can cause medical issues/diseases
    - This type of disease is a proteopathy
    - Examples: Alzheimer's disease, Parkinson's disease

Protein Functions

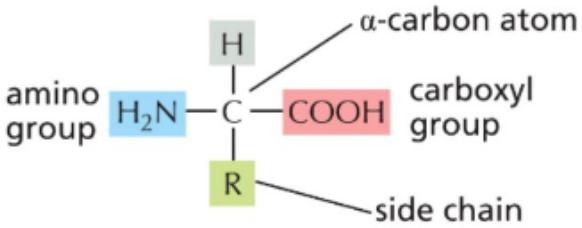
- An ENZYME catalyzes covalent bond breakage or formation
    - Living cells contain thousands of different enzymes
    - Each can catalyze (speed up) a particular reaction
    - Examples:
        - Trypotophan Synthetase - makes the amino acid tryptophan
        - Pepsin - degrades dietary proteins in the stomach
        - DNA Polymerase - copies DNA
        - Protein Kinase - adds a phosphate group to a protein molecule
- A STRUCTURAL PROTEIN provides mechanical support to cells and tissues
    - Collagen & Elastin – common constituents of the extracellular matrix [outside the cell] and form fibers in tendons and ligaments
    - Tubulin – forms long, stiff microtubules [inside the cell]
    - Actin - forms filaments that underlie and support the plasma membrane [inside the cell]
    - α-Keratin – forms fibers that reinforce epithelials (ie. hair, horn)
- A TRANSPORT PROTEIN carries small molecules or ions
    - Many proteins embedded in cell membranes transport ions or small molecules across the membrane

    - Examples:
        - Serum Albumin – carries lipids
        - Hemoglobin – carries oxygen
        - Transferrin – carries iron
        - Bacteriorhodopsin – light-activated proton pump in bacteria that transports H+ ions out of the cell
- A MOTOR PROTEIN generates movement in cells and tissues
    - Myosin – provides the motive force for humans to move
        - Located in skeletal muscle
    - Kinesin – interacts with microtubules to move organelles around the cell
    - Dynein – enables eukaryotic cilia and flagella to beat
- A STORAGE PROTEIN is a protein that stores amino acids or ions
    - Ferritin – iron binds to this small protein to be stored in the liver
    - Ovalbumin – in egg white; used as a source of amino acids for the developing bird embryo
    - Casein – in milk; used as source of amino acids for baby mammals; health supplements
- A SIGNAL PROTEIN carries extracellular signals from cell to cell
    - Many of the hormones and growth factors that coordinate physiological function in animals are signal proteins
    - Examples:
        - Insulin – small protein that controls glucose levels in the blood
        - Nerve Growth Factor (NGF) – stimulates some nerve cells to grow axons
        - Epidermal Growth Factor (EGF) – stimulates growth and division of epithelial cells
- A RECEPTOR PROTEIN detects signals and transmits them to the cell's response machinery
    - Rhodopsin – in the retina; detects light

    - Acetylcholine Receptor – activated by acetylcholine released from a nerve ending
    - Insulin Receptor – allows a cell to respond to the hormone insulin by taking up glucose
- A TRANSCRIPTION REGULATOR binds to DNA to switch genes on/off
    - Lac Repressor – in bacteria, it silences the genes for the enzymes that degrade the sugar lactose
    - DNA-binding Protein – acts as a genetic switch to control development in multicellular organisms, including humans
- A SPECIAL-PURPOSE PROTEIN is highly variable in nature
    - Organisms make many proteins with highly specialized properties
    - Examples:
        - Antifreeze Proteins – in Arctic and Antarctic fishes, they protect their blood against freezing
        - Green Fluorescent Protein – in jellyfish, they emit a green light
        - Monellin – a protein found in an African plant; sweet taste
        - Glue Proteins – allows mussels to stick to rocks in seawater

Amino Acids

- Proteins are assembled from a set of 20 different amino acids
- Each AMINO ACID has different chemical properties
    - They are linked together by covalent peptide bonds
- The general formula (R is the size chains)



Classification of Amino Acids

- By polarity: POLAR vs. NONPOLAR
    - At pH = 7, any amino and carboxyl groups will be ionized
- For POLAR amino acids, we can also subcategorize them by:
    - Acidic: negatively charged side chains
    - Basic: positively charged side chains
    - Uncharged polar: no ionization of side chains, but side chain is polar

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negatively charged
Glutamic acid	Glu	E	negatively charged
Arginine	Arg	R	positively charged
Lysine	Lys	K	positively charged
Histidine	His	H	positively charged
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

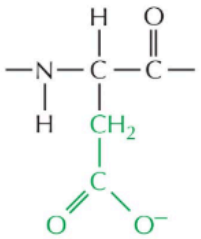
AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

FAMILY 1: ACIDIC SIDE CHAINS

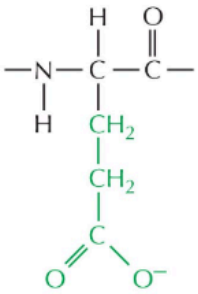
aspartic acid

(Asp, or D)

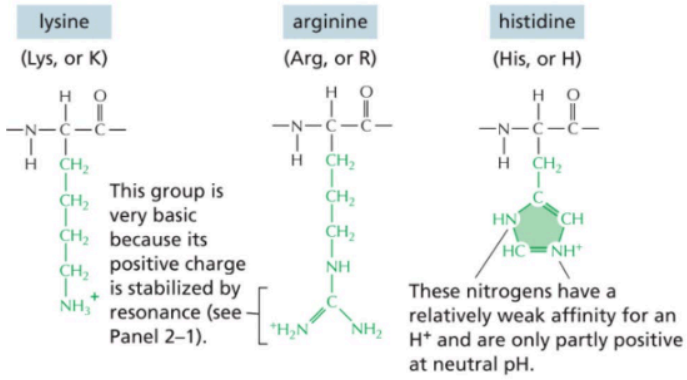


glutamic acid

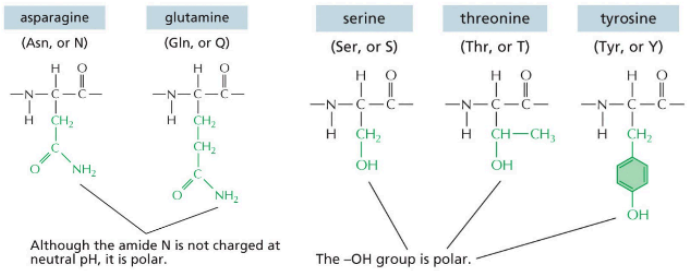
(Glu, or E)



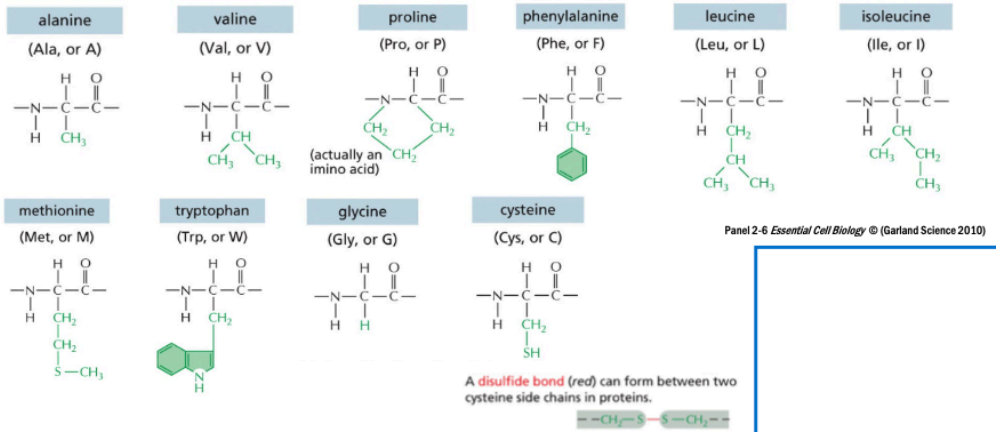
FAMILY 2: BASIC SIDE CHAINS



FAMILY 3: UNCHARGED POLAR SIDE CHAINS



FAMILY 4: NONPOLAR SIDE CHAINS



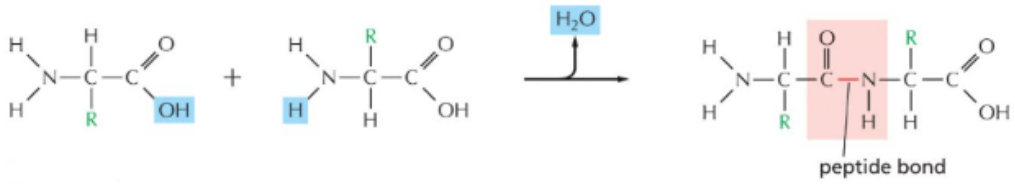
Panel 2-6 *Essential Cell Biology* © (Garland Science 2010)

Polypeptide Chain

- when amino acids are linked together in a chain
    - Different proteins will have different amino acid sequences
    - They are linked together by <u>peptide bonds</u>
- A PROTEIN is a long polymer of amino acids
    - Short (<50) amino acid chain is a PEPTIDE
    - Always write with N-terminus on left to C-terminus on right

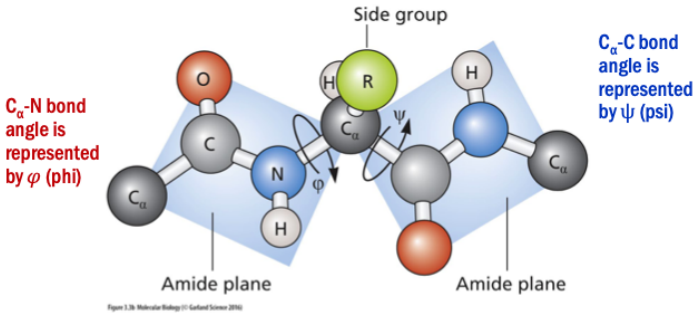
Formation of Peptide Bonds

- A CONDENSATION REACTION removes water from the system
    - Carboxyl group (-COOH) will share electrons with adjacent amino group (-NH2)
- A COVALENT BOND forms between one amino acid's AMINE NITROGEN and the other's CARBOXYL CARBON
    - A molecule of water is removed as a side product



Bond Angles in a Polypeptide Chain

- The peptide bond is PLANAR, so they lie on an AMIDE PLANE
    - The α-CARBON in between amide planes allows for rotation
- This will give us two angles:
    - Cα-N bond angle is represented by φ (phi)
    - Cα-C bond angle is represented by ψ (psi)



Configurations

- The BOND ANGLES for either ψ or φ can be from -180° to +180°
- We can define CIS or TRANS configurations of peptide bonds
    - R groups on the same side: Cis → interaction of R-groups
    - R groups on different sides: Trans
- Although there can be many bond angles for ψ and φ...
    - There are steric collisions between atoms within each amino acid
    - Most pairs of ψ and φ do not occur

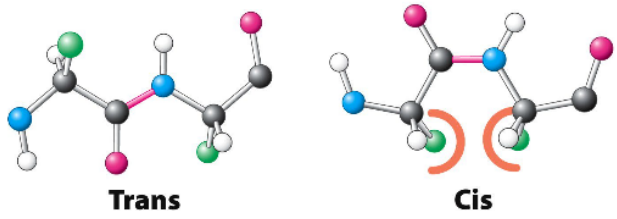


Figure 2.20
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Ramachandran Plot

- presents observed pairs of angles in a protein, allowing us to see which configurations are favoured

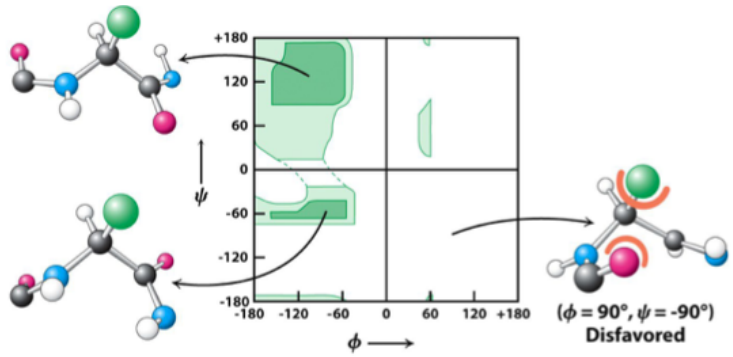

Figure 2.23
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Modelling Bond Angles

- We need to look at <u>relative</u> angles between two planes to determine how many different configurations there are
    - Define an <u>axis of rotation</u>
    - Change the angle of one plane at a time

Protein Folding: Helix-Coil Transitions

Protein Folding

- WEAK INTERACTIONS create strong bonding arrangements
- HYDROPHOBIC FORCES help proteins fold into compact conformations
    - Polar amino acid side chains tend to be on the outside of folded protein
    - Non-polar amino acid side chains are buried inside to form highly packed hydrophobic core
- Hydrogen bonds within a protein molecule help to stabilize its folded shape and conformation
- They can occur between backbones and/or side chains
    - Allow for many configurations

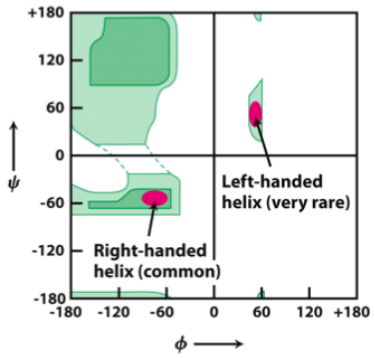
Ensuring Proper Folding

- Various CHAPERONE PROTEINS assist in the covalent folding or unfolding of macromolecules, specifically protein folding
    - They prevent newly synthesized polypeptide chains and assembled subunits from aggregating into non-functional structures
    - Many are heat shock proteins → because heat can cause stress, which increase the likelihood of unproper folding

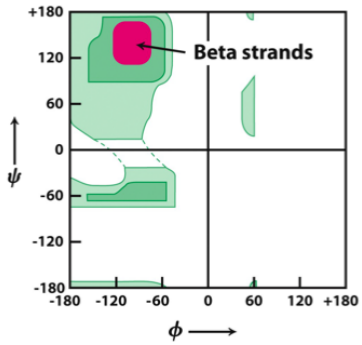
Secondary Structures

- The SECONDARY STRUCTURES of proteins are driven by weak interactions
- The α-HELIX and β-SHEET are common folding patterns
    - They result from hydrogen bonds that form between the N-H and C=O groups in the polypeptide backbone

- α-HELIX
    - can be left or right handed



    - A segment of an α-helix can CROSS A LIPID BILAYER
        - The hydrophobic side chains on the helix can insert into the hydrophobic core of the lipid bilayer
    - Helices can intertwine to create a COILED-COIL
        - The minimize exposure of hydrophobic residues
- β-SHEET



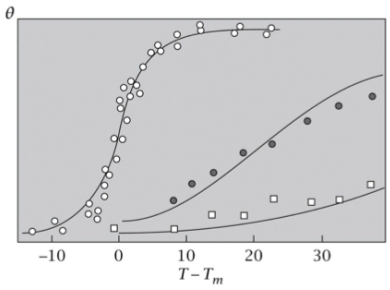
    - Can be antiparallel (H-bond with adjacent) or parallel (alternating H-bonding)

Higher level of Organization

- Many TERTIARY STRUCTURES will be composed of separate FUNCTIONAL DOMAINS
- There are both helices and sheets packed into a stable element

α-Helix COIL TRANSITION

- RIGHT-HANDED COIL is the most favourable conformation of the alpha helix
    - Stabilized by hydrogen bonds
- We can take a look at the SUBUNITS for each homogeneous polypeptide chain
    - Each subunit can either be in a helix or coil state
- Can make predictions based on states
- There are two possible states for each monomer in a HOMOGENEOUS POLYPEPTIDE CHAIN
    - Coil: Bond angles are free to rotate, larger entropy
    - Helix: bond angles are frozen, lower energy state
- We can set the monomer to be in one of the two states: H (Helix) or C (Coil)
    - Once you have an H-bond to form the H state, it becomes a cascade effect, where each additional H-bonds is easy to form
    - Hydrogen bonds are usually between the jth amino acid and the (j+4)th amino acid
        - Because there are 4 amino acids per turn
- We care about DNA and protein denaturation
    - Temperature causes transition between H and C
- These transitions are cooperative
    - Amount of helix changes in a sigmoidal way as a function of an external variable (such as temperature)
    - Sigmoidal curve steepens with increasing size of system (in this case, chain length)
- We want to calculate cooperativity, or ratio of H to C in a solution



The Lifson-Roig Model

- LR model assigns each residue in a polypeptide a helical (H) or coil (C) residue state
    - Represent polypeptide with a string of H and C
    - Total of 2^N molecular states
- We can assign individual residues a STATISTICAL WEIGHT
    - The product of these weights gives rise to a statistical weight for that particular molecular state (all residues together)
    - We can predict observable properties
    - $\boldsymbol{u}$ is residue weight assigned to coil states
    - $\boldsymbol{v}$ is residue weight assigned to helix states with coil neighbour
    - $\boldsymbol{w}$ is residue weight assigned to helix states, no coil neighbour
- The statistical weights are based on LOCAL INTERACTIONS
- The expected overall state is the sum of the probability times the value

Partition Function (Z)

- describes the statistical properties of a system in thermodynamic equilibrium
    - dimensionless
- Focus on CANONICAL partition function
    - System is allowed to exchange heat with the environment
    - Fixed temperature, volume, and number of particles
    - Assume each weight is equal to its multiplicity W and sigma equals to 1

$$Z = \sum_{i=1}^{N} e^{-\frac{F}{k_B T}} \qquad S = k_B \ln W \Rightarrow W = e^{\frac{S}{k_B}} = e^{\frac{F-E}{-k_B T}} \approx e^{\frac{-F}{k_B T}}$$

$$Z_N = \sum \frac{N!}{n!\,(N-n)!} s^n = (1+s)^N$$

    - Can also use matrix formalism (for sigma not equal to 1)

$$\begin{bmatrix} C & H \end{bmatrix} \begin{bmatrix} C & H \\ C & H \end{bmatrix}^{N-1} \begin{bmatrix} 1 \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & \sigma s \end{bmatrix} \begin{bmatrix} 1 & \sigma s \\ 1 & s \end{bmatrix}^{N-1} \begin{bmatrix} 1 \\ 1 \end{bmatrix}$$

Simpler Model

- If we divide all the states by the Weight (C), then C will become a reference point for further analysis and comparison
- Since the weight of each microstate is equal to its multiplicity
    - For each C, the weight is 1
    - For each H after an H, the weight is s
    - For each H after a C, the weight is σs
- σ is a constant related to the probability of getting H after a C
    - Much less than 1 ($10{-}4 \leqslant \sigma \leqslant 10{-}2$) since the probability to start helix is lower than getting a helix after a helix
- FRACTIONAL HELICITY: The ratio of helix states to the total number of residues or monomers in the sequence

$$\theta = \frac{\langle \text{Number of H's} \rangle}{\text{Number of Monomers}}$$

$$\boldsymbol{\theta} = \frac{\langle \text{Number of H's} \rangle}{\text{Number of Monomers}} = \frac{Ns(s+1)^{-1}}{N} = \frac{\boldsymbol{s}}{\boldsymbol{s+1}}$$

    - May also be defined using T and Tc (critical temperature)

$$\boldsymbol{\theta = \frac{e^{0.00614(T-T_C)}}{1 + e^{0.00614(T-T_C)}}}$$

$-T_C = T_M$

T_C = critical temperature

T_M = midpoint temperature

- Physical meaning of s
    - Relative weight for H

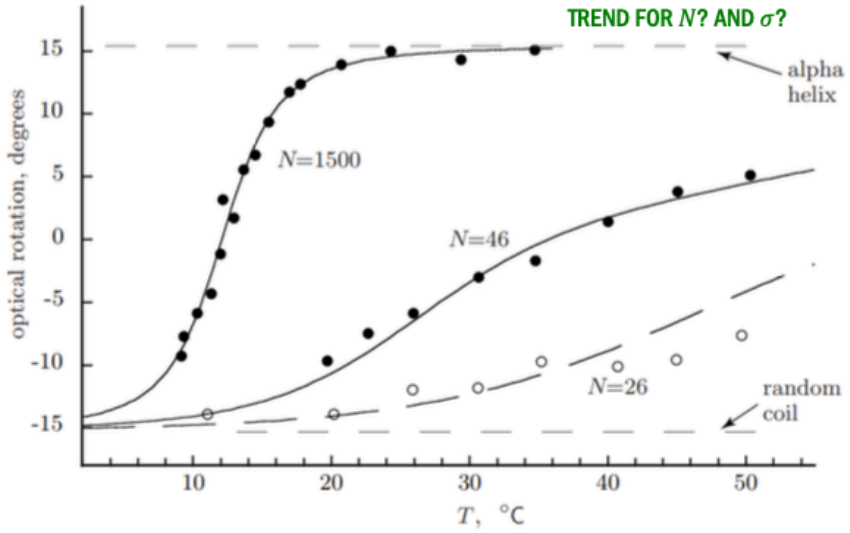
$$s = \frac{\text{weight of H}}{\text{weight of C}} = \frac{\text{Prob(H)}}{\text{Prob(C)}} = \frac{e^{-\frac{(-\varepsilon)}{k_B T}}}{W_C}$$

HYDROGEN BOND (FREE) ENERGY

MULTIPLICITY FOR C

Testing Coil-Helix Transition

- Will a given polypeptide assume a random coil or α-helix structure?
    - Depends on competition between conformational entropy and H-bond formation
        - Depends on composition, thermal environment, and chemical environment
- We can test the helix-coil transition using POLARIZED LIGHT
    - We can see a near-total conversion of sample from one form to the other upon temperature change



    - As the size of protein increases, it is more sensitive to change in temperature

Hydrogen Bonds in Water

- Any molecule that can form H-bonds to each other can alternatively form H-bonds to water molecules
    - The competition with water molecules means that the H-bonds formed between two molecules dissolved in water are relatively weak

Proline (Pro, P) as a Helix Breaker

- Stabilizes an H-bond between monomer j and j+2
    - Does not skip 3 monomers, so it does not form helix → helix breaks if Pro is added

Protein Folding: Toy Model

Protein Can Assemble into Shapes

- Proteins can assemble into COMPLEX STRUCTURES
    - Ex: Dimers, helices, and rings
- These complex proteins connect together through BINDING SITES
    - Different types of binding sites will result in different overall conformations
- Some proteins have elongated fibrous shapes
    - COLLAGEN is a triple helix formed by 3 protein chains
    - ELASTIN has molecules cross-linked together

Stabilization of Proteins

- DISULFIDE BONDS (S-S BONDS) help to stabilize a favoured protein conformation
    - Most common covalent cross-links
    - They don't change the conformation, they reinforce a protein's shape so that it retains its activity longer

Protein Binding Sites

- 3D structures allow proteins to interact with other molecules
- BINDING SITES allow a protein to interact with SPECIFIC LIGANDS
    - Protein folding usually creates cavities on protein surface
    - These cavities contain a set of amino acid side chains that can bond only with certain ligands
- KEY-LOCK MECHANISM of biological recognition arises from a mixture of shape and weak interactions
- Ex: Antibodies and Antigens
    - An ANTIBODY is Y-shaped and has 2 identical binding sites for its ANTIGEN, one on each arm of the Y
    - Antibody is composed of 4 polypeptide chains (2 heavy, 2 light)
        - Held together by disulfide bonds
    - Each chain has two domains: variable and constant
        - Variable domains are closest together and form an antigen-binding site

        - They differ the most to provide variety in attracting different antigens and molecular recognition
- Proteins are held together by weak bonds, but allow the FOLDED PROTEIN to interact specifically with other molecules

	OPEN (DENATURED)	COLLAPSED (FOLDED)
Entropy	Larger Multiplicity (More Chain Entropy)	Smaller Multiplicity (Less Chain Entropy)
Energy	Less Favourable Contacts (More Positive Energy)	More Favourable Contacts (More Negative Energy)
Stability	Shape-Shifting Molecule	Shape is Frozen

Protein Folding

- Proteins must be in the right shape, or else they can't function
    - Certain thresholds of multiplicity need to be met for different structures
        - For a folded protein, W is about 1
- Driving force
    - HYDROPHOBIC (NONPOLAR) AMINO ACIDS are the main driving force behind protein folding since they want to be "buried" inside

Protein as Random Walks

- PROTEIN CONFORMATIONS are compact, with solvent typically making contact only with amino acids at surface of the protein
- Use COMPACT RANDOM WALKS
    - Also Hamiltonian walks
    - Self-avoiding random walks that visit every site of lattice
- By covering all lattice sites by monomers, all solvent sites are pushed to the surface

Homo-Polymer

- HOMO means that all the monomer subunits are the same
- We can look at the number of HYDROPHOBIC CONTACTS
- We can also look at both hydrophobic (H) and hydrophilic (P) contacts to try and get a unique ground state (WN = 1)
    - A good candidate for protein structure
- Main Goal: Some sequences are good candidates for a protein if they can fold in a unique way for a specific # of H or P contacts
    - We now consider an HP Model

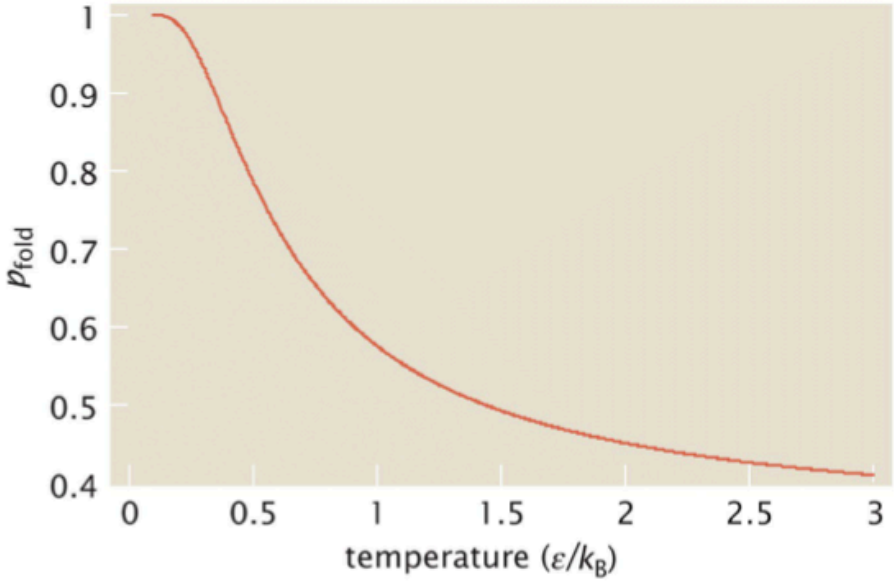
HP Model

- The HP MODEL divides 20 amino acids into 2 classes:
    - **HYDROPHOBIC (H)**: Ala, Val, Ile, Met, Leu, Phe, Trp, Cys, Tyr
    - **POLAR (P)**: Gly, Asn, Ser, Thr, Glu, His, Pro, Arg, Gln, Asp, Lys
- This model is useful because:
    - It is a drastic reduction of the complexity of the sequence
        - For a 100-mer, the number of possible sequences goes from 20^{100} to 2^{100}
        - Allows us to analyze complex problems using a simplified model
- The HYDROPHOBIC ENERGY of an HP model depends on sequence → assign an energy cost ($\boldsymbol{\varepsilon}$) for every unfavourable contact
    - The weight for the state will be e^(- $\boldsymbol{\beta}$ x cost)
- This PROTEIN FOLDING PROBLEM can be asked for each sequence:
    - Given an HP sequence, which of the possible structures minimizes the hydrophobic interaction energy
    - The lowest energy state is identified as the native state of the protein
- We can calculate the PROBABILITY of finding the chain in the native structure as follows:

$$p_{\text{fold}} = \frac{\text{Weight(Native)}}{\text{Total Weight}}$$

Probability of Finding Native State

- A SIGMOIDAL PLOT is characteristics of many real proteins



Degeneracy of the Native State

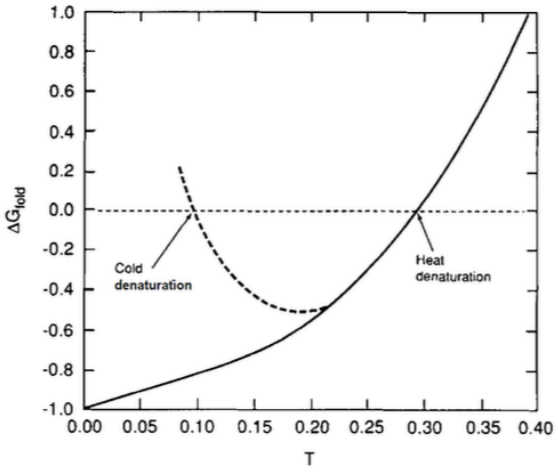
- For a given sequence, the DEGENERACY $\boldsymbol{gNN}$ (or simply multiplicity WNN) of the native state is the number of lowest energy (native) conformations
    - Good protein will have gN = 1, and the shape matters
- For a given number of monomers (N), we can try to figure out how many sequences (2-3%) are PROTEIN-LIKE
- Protein structures that have large number of protein-like sequences associated with them are called DESIGNABLE PROTEINS

Sequence Design

- We can use our knowledge of HP sequence design to choose specific conformations of proteins
    - Hydrophobic residues are usually sequestered in the interior of the bundle
    - Polar ones are on the surface facing the solvent

Chain Entropy vs. Energy

- What are the differences between an OPEN (DENATURED) and COLLAPSED (FOLDED) protein?
- Free energy of Folding
    - $U = -\varepsilon \times$ (# of favourable contacts), ε is related to T
    - HEAT DENATURATION: If $|\varepsilon|$ decreases with increasing temperature
    - COLD DENATURATION: If $|\varepsilon|$ decreases with decreasing temperature



Two Competing Views

- There are two competing views about protein folding into secondary structures, and the energetics behind it
    - Framework Model
        - The formation of secondary structure <u>precedes</u> chain collapse
        - This means that there is less chain entropy to lose
        - Folding is driven by local interactions
    - COLLAPSE MODEL:
        - Chain collapse <u>promotes</u> the formation of a secondary structure
        - Governed by hydrophobicity (non-local interactions)
        - Inside the collapsed protein, the density of water is lower
            - This means that hydrogen bonding is stronger
            - With stronger hydrogen bonding, there is a stronger tendency to from alpha-helices or beta-sheets

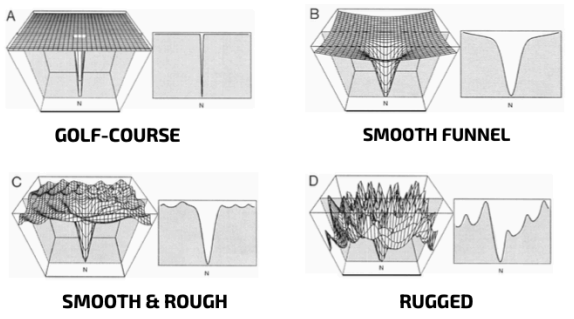
- If compact, there is less chain-entropic penalty for forming secondary structures
- As the proteins continues to collapse, there are more "hydrophobic" gaps within the protein
    - This reduces competition with polar molecules, reinforcing hydrogen bonding

Levinthal Paradox

- A rough estimate would say that 1050 conformations are possible for a polypeptide chain such as ribonuclease (124 monomers).
- Even if the molecule could try a new conformation every 10^{-13} seconds, it would still take about 10^{30} years to try a significant fraction of them.
- Is folding pathway random?
    - Theoretically, if random, it would take very long to find the protein's native state
    - But the folding times for real proteins is 10 mus to minutes

Energy Landscape

- FOLDING KINETICS can be described using ENERGY LANDSCAPES
    - Levinthal Paradox assumes golf-course landscape (2D) where everything has the same free energy (equally random)
    - A <u>smooth funnel</u> landscape takes into account different energies of different conformations
- Proteins with FUNNEL-LIKE FOLDING LANDSCAPES are said to be under <u>THERMODYNAMIC CONTROL</u>
- Proteins with RUGGED FOLDING LANDSCAPES are said to be under <u>KINETIC CONTROL</u>



Enzymes and Reaction Kinetics

Enzymes

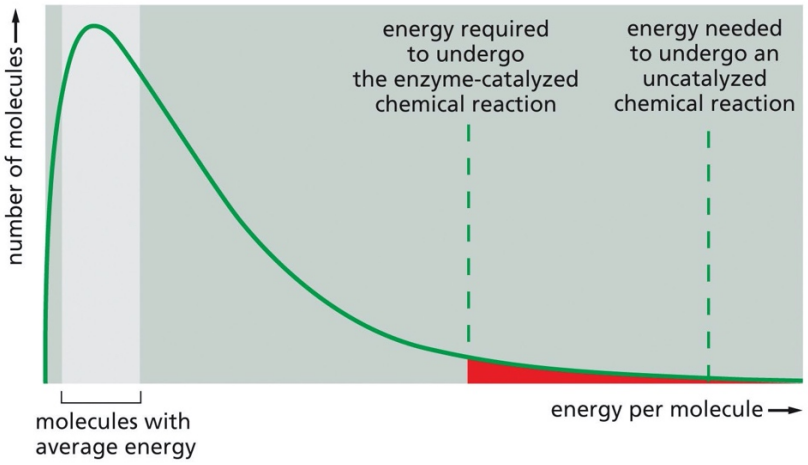
- An ENZYME helps to speed up energetically favourable reactions
    - They help produce disorder in the universe
- Biologically speaking, enzymes help to lower the barriers that block chemical reactions
    - They help lower the activation energy
- Enzymes have an ACTIVE SITE that bind to SUBSTRATE molecules
    - Reduces activation energy of a chemical reaction
    - Act as catalysts

Activation Energy

- Every reaction has a REACTANT and a PRODUCT
- Reactant must collect enough energy to surpass the activation energy for reaction to occur
- Lowering the activation energy greatly increases the probability of a reaction to occur
    - Catalysts increase the rate of chemical reaction

Catalysts vs. Temperature

- Catalysts can boost collisions to increase energy
    - Allows molecules to surpass activation energy for desired reaction



- Temperature is NOT a catalyst
    - It speeds up the reaction, but is not selective

How do enzymes work

- Each enzyme has an ACTIVE SITE which binds to SUBSTRATES
    - Forms an enzyme-substrate complex
    - Reaction occurs and produces enzyme-product complex
    - Product is then released
    - Allows enzyme to bind further substrate molecules

Reaction Rates

- Without any additional help, molecules will collide with each other due to RANDOM WALK
- We can calculate the REACTION RATE for these collisions → the collision rate

$$R = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} \propto [A][B]$$

- Forward reaction: A+B → C, requires collisions
- Backward Reaction: C → A+B, do not require collisions

$$R = k_+[A][B] - k_-[C]$$

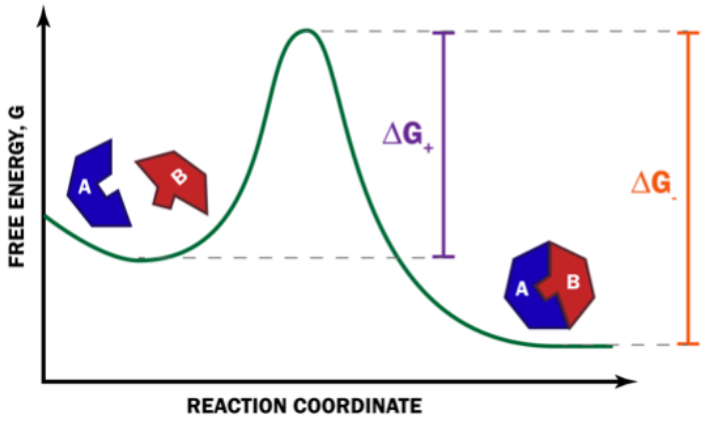
Free Energy (G) of a System

- In biochemistry, the FREE ENERGY (G) is a quantity that expresses the disorder of the universe
    - G matters only when the system undergoes changes
- The CHANGE IN G (ΔG) is critical in determining whether a reaction is energetically favourable or unfavourable
    - Favourable ones have negative delta G
- Unfavourable Reactions
    - What happens if we have an ENERGETICALLY UNFAVOURABLE REACTION with a POSITIVE ΔG (such as the formation of a peptide bond)?

- REACTION COUPLING allows an overly negative ΔG from an energetically favourable reaction to drive an energetically unfavourable reaction
    - Overall delta G is negative

Reaction Coordinate

- Catalytic reactions can be represented by using an ENERGY vs. REACTION COORDINATE graph
- In most cases, $\Delta G+$ is so high that an enzyme is required to overcome the activation energy barrier



Arrhenius Equation

- k = rate constant
- A = Arrhenius constant
- E_a = activation energy
- R = gas constant
- T = temperature

$$k = Ae^{-\frac{E_a}{RT}}$$

Can we tie this back to ΔG and k_B? Assume $\Delta G_{\pm} \gg k_B T$

$$k_{\pm} \propto e^{-\frac{\Delta G_{\pm}}{k_B T}}$$

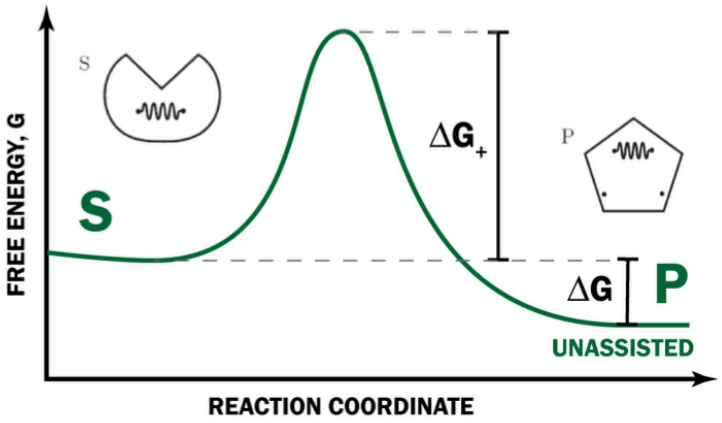
Conceptual Model of an Enzyme

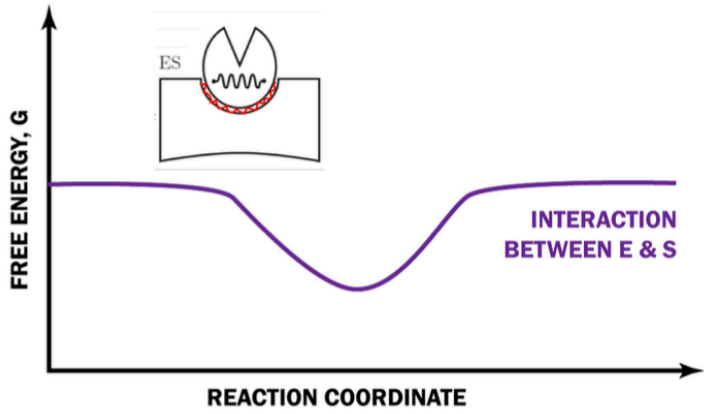
- 1. Enzyme E has binding site with a shape and distribution of charges
- 2. To match perfectly, S (or both E + S) must deform
    - Bonds can be close to breaking point
- 3. From the ES state, stretched bond can break and give rise to a so-called EP complex

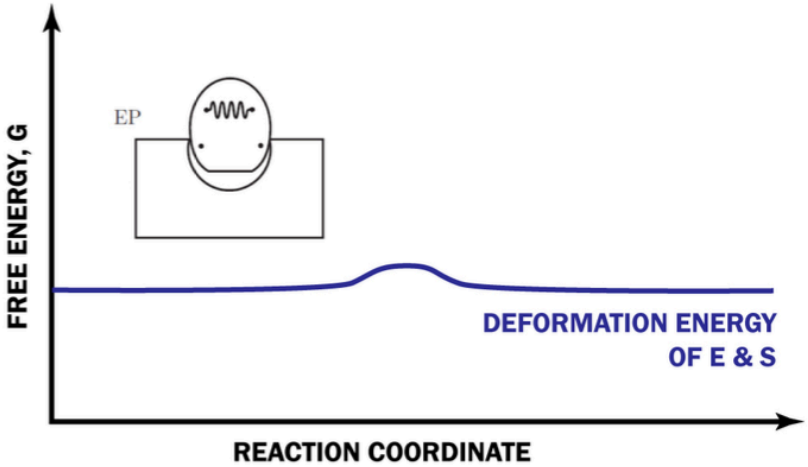
- New bond forms to stabilize product P

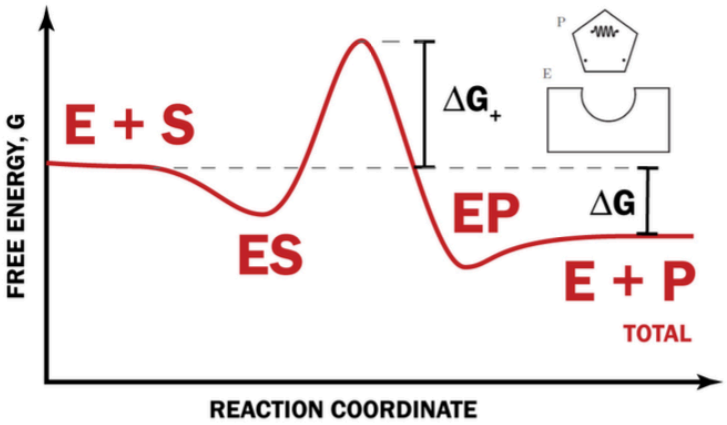
- 4. P is not a perfect fit so it unbinds and returns E to normal

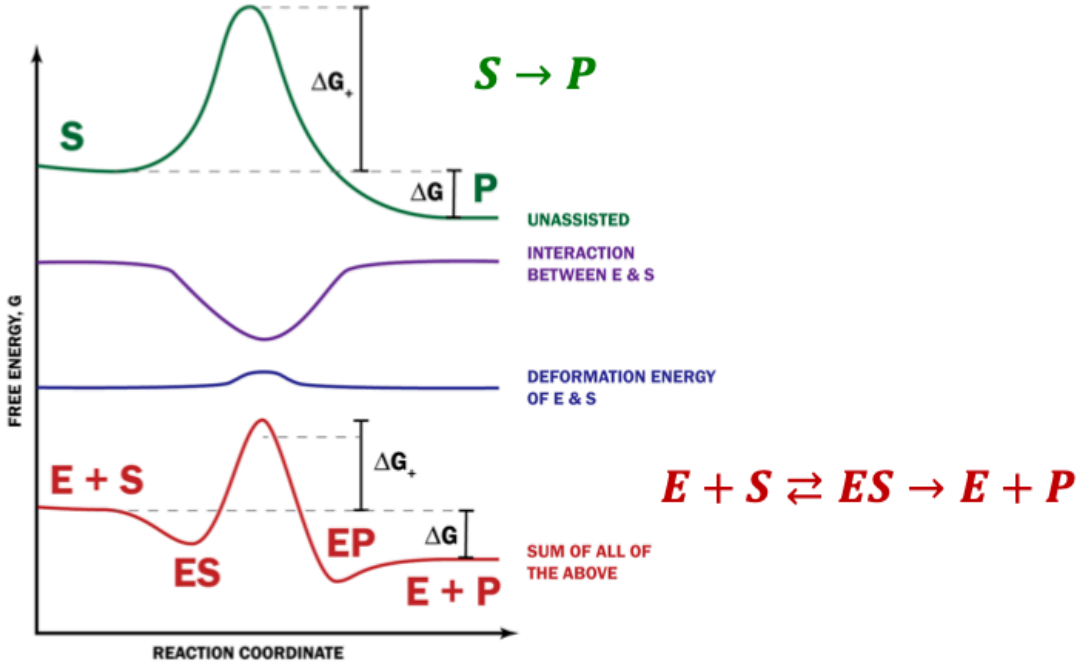
Free Energy Landscape

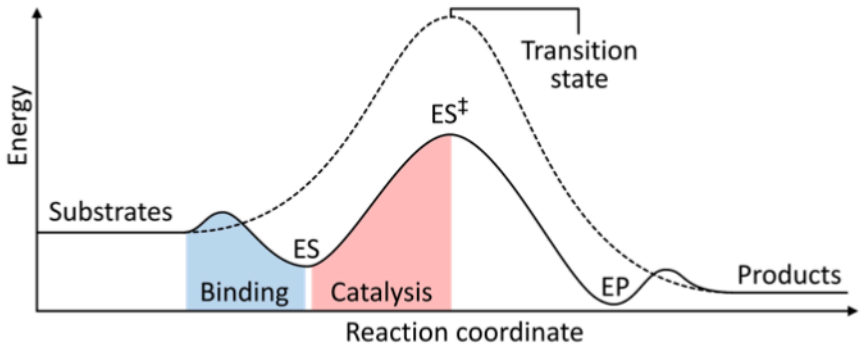







FREE ENERGY, G
E + S
ΔG‡
ES
EP
ΔG
E + P
TOTAL
REACTION COORDINATE


S → P
S
ΔG‡
ΔG
P
UNASSISTED
INTERACTION BETWEEN E & S
DEFORMATION ENERGY OF E & S
FREE ENERGY, G
E + S
ES
EP
E + P
SUM OF ALL OF THE ABOVE
E + S ⇄ ES → E + P
REACTION COORDINATE


Energy
Transition state
ES‡
Substrates
ES
Binding
Catalysis
EP
Products
Reaction coordinate

Enzyme Kinetics

- Formation of ES is fast and reversible; formation of a product is slow and irreversible

$$E + S \underset{k_-}{\overset{k_+}{\rightleftarrows}} ES \xrightarrow{r} E + P$$

$$\frac{d[E]}{dt} = -k_+[E][S] + k_-[ES] + r[ES]$$

$$\frac{d[S]}{dt} = -k_+[E][S] + k_-[ES]$$

$$\frac{d[ES]}{dt} = k_+[E][S] - k_-[ES] - r[ES]$$

$$\frac{d[P]}{dt} = r[ES]$$

INITIAL CONDITIONS ($t = 0$):

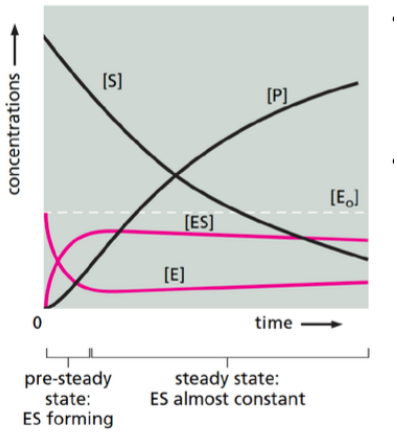
$$[E]_0 = [S]_0 \quad [ES]_0 = 0 = [P]_0$$

CONSERVATION:

$$[E]_0 = [ES] + [E]$$

$$[S]_0 = [ES] + [S] + [P]$$

- [ES] increases, but then decreases depending on enzyme efficiency
- At steady state, [ES] is almost constant since r is very small



- We can use the MICHAELIS-MENTEN MODEL to define the REACTION RATE once more

  - K_m = Michaelis constant
  - v = reaction rate
  - v_{max} = max reaction velocity

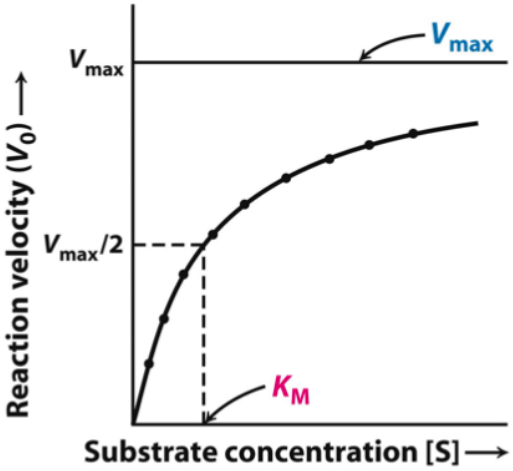
$$v = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]}$$

Approximations in Michaelis-Menten Model

- Equilibrium Approximation
    - r = 0
- Steady State Approximation
    - d[ES]/dt = 0

Michaelis-Mention Equation Curve

- Km is defined as the substrate concentration at which the reaction rate is half of Vmax



- If Km increases → binding affinity is weaker → need more enzyme to reach Vmax/2
- Sometimes, it's more informative to take the reciprocal of the Michaelis-Menten Equation to get a LINEAR GRAPH
    - Called a Lineweaver-Burk Plot

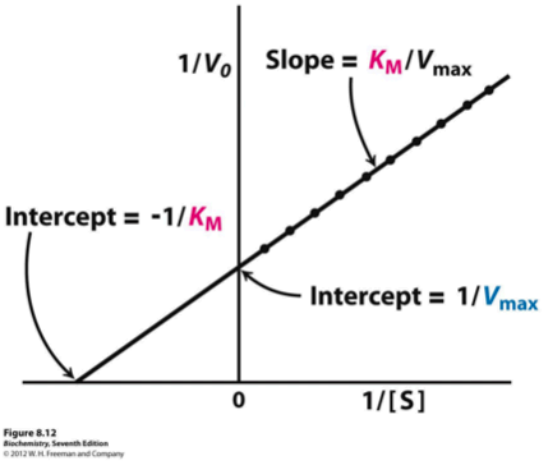


Figure 8.12
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- Sometimes, it's more informative to take the reciprocal of the Michaelis-Menten Equation to get a **LINEAR GRAPH**
    - This is called a **LINEWEAVER-BURK PLOT**

$$\frac{1}{\frac{d[P]}{dt}} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}[S]}$$

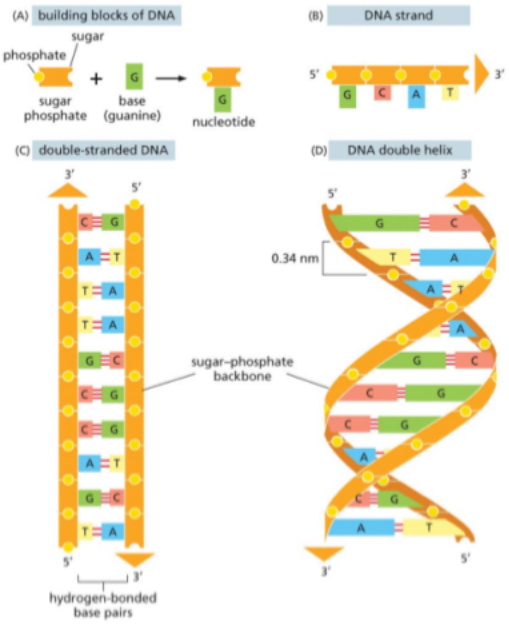
Some Additional Comments

- The Michaelis-Menten mechanism
    - Must apply both the equilibrium and steady-state approximation
    - Applies to many different kinds of enzymes including lysozyme
- Formation of ES is fast and reversible → it can be spontaneous
- The formation of a product is slow and irreversible (due to formation and break of bonds)

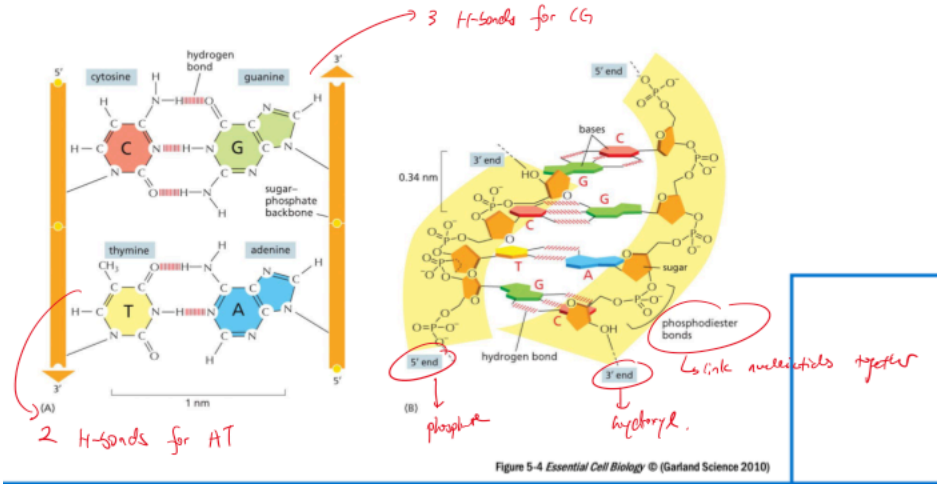
DNA Structure and Packing

Nucleotides

- DNA is made of 4 nucleotide building blocks
    - Each nucleotide has a <u>sugar</u> and <u>phosphate</u> linked to a <u>base</u>
- There are 4 different bases: A, C, G, T
    - Covalently linked together
    - Arrows represented polarity, they run <u>antiparallel</u> to each other



- There are 2 H-bonds for AT and 3 H-bonds for CG; Purine (AG) 2 rings; Pyrimidine (TC) 1 ring

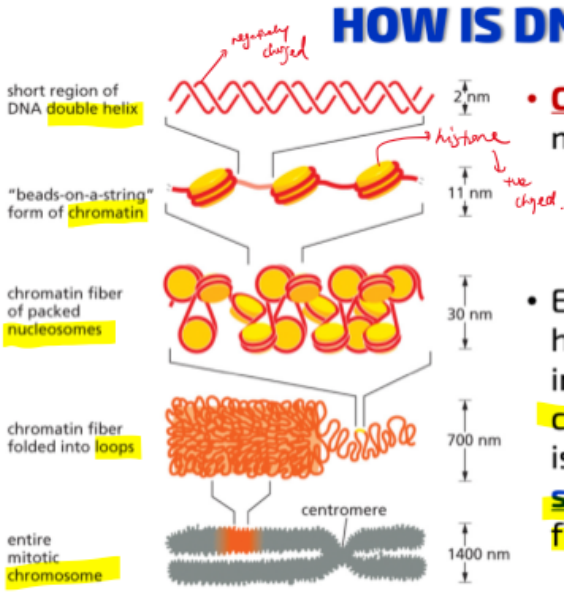


DNA Packaged into Chromosomes

- In eukaryotic cells, very long double-stranded DNA molecules are packaged into structures called chromosomes → highly condensed
- They contain long strings of genes
    - A long segment of DNA that contains instructions for making proteins
- Detailed folding process to get to a chromosome

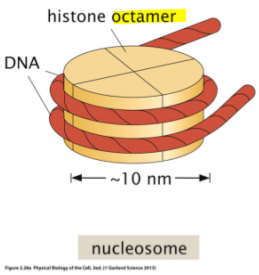
Packing of DNA

- Chromosome packing occurs on multiple levels
    - DNA wraps around histones, which then fold within themselves to become more compact
- Each DNA molecule has been packaged into a mitotic chromosome that is 10,000-fold shorter than its fully extended length
- Double helix → chromatin → nucleosomes → loops → chromosome



Nucleosome and Histone

Histone is an octamer (8 subunits) that has a diameter of about 10nm.

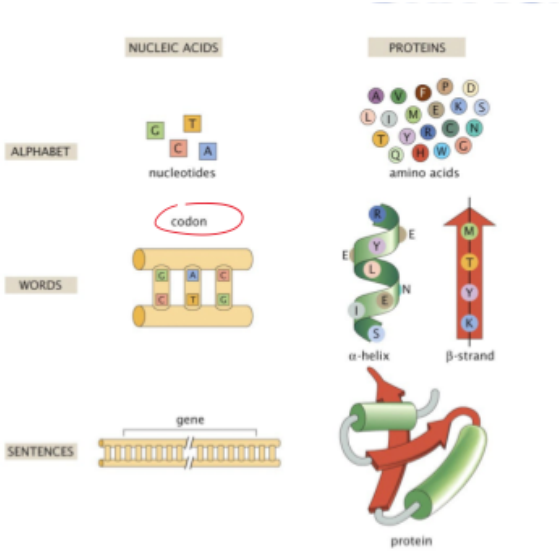


Modelling DNA

- Sequence (helix vs. coil)
- Binding Site
- Charged Rod
- Elastic Rod
- Random Walk

DNA vs. Proteins

- DNA provides instructions to create proteins
- DNA helps to translate between polymer languages
    - Genes know how to make proteins
    - Proteins are sub-robots that carry out functions

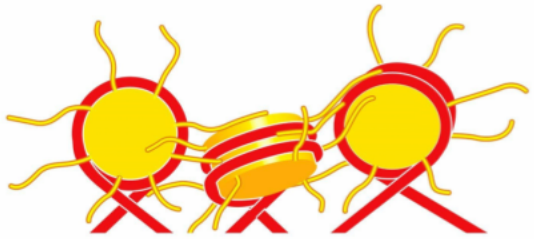


DNA Electrostatics

- DNA is <u>negatively charged</u> at approximately $-\frac{e}{1.7\ \dot{A}}$
- DNA is packaged by <u>multivalent cations</u> with 2+ or **3+ charges**
    - Can form toroidal bundles
    - In salty solutions with 3+, DBA strands can attract each other
    - Viral DNA may be packed similarly

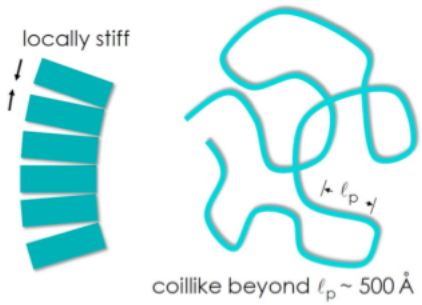
Eukaryotic DNA

- Eukaryotic (human) DNA uses electrostatics to pack itself → but electrostatic does not stabilize
    - DNA (in red) is negatively charged
    - Histone tails (in yellow) are positively charged
        - Stabilized by 200 positive charges on histone

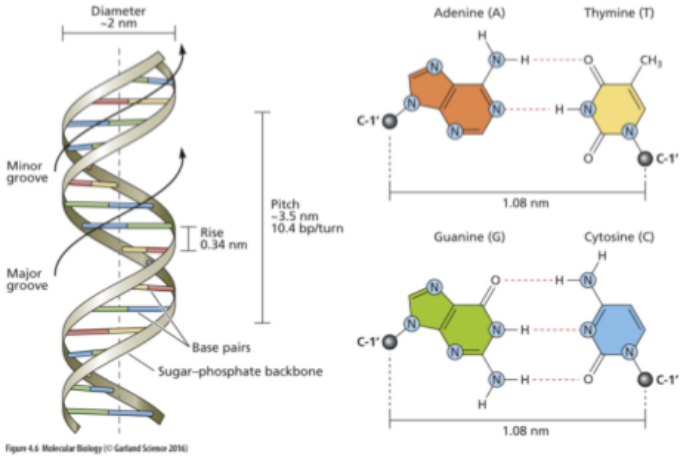


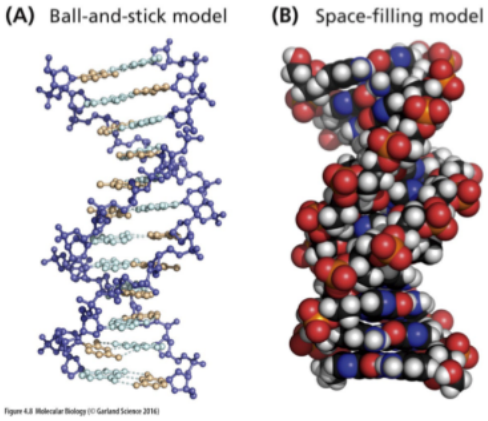
DNA as a Garden Hose

- DNA chain is locally stiff (almost linear, minimal curvature) → for a short strand
- But tends to coil up at large-length scales ($l_p \geq 500\dot{A}$) → this length is due to base pairing & H-bonding
    - Allows for wider conformational space to increase entropy
    - Resists stretching or confinement
    - l_p = "persistent length" → length of DNA that's linear



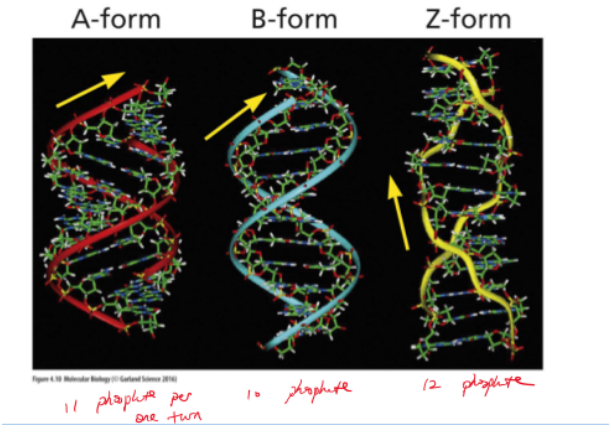
DNA is a Twisted Ladder





Three Well-Known DNA Helices

- A-Form: 11 phosphates per one turn
- B-Form: 10 phosphates per one turn
- Z-Form: 12 phosphates



DNA Bending

- We can find/calculate the energy penalty
- At short lengths, free energy wins and DNA remains straight
- At large length scales (entropy increases), DNA coils up → entropy wins over persistent length

Linking Energetics and Stiffness

- Can base stacking/pairing energetics be translated into chain stiffness or the persistence length lp?
    - We can use a worm like chain model to model this behaviour

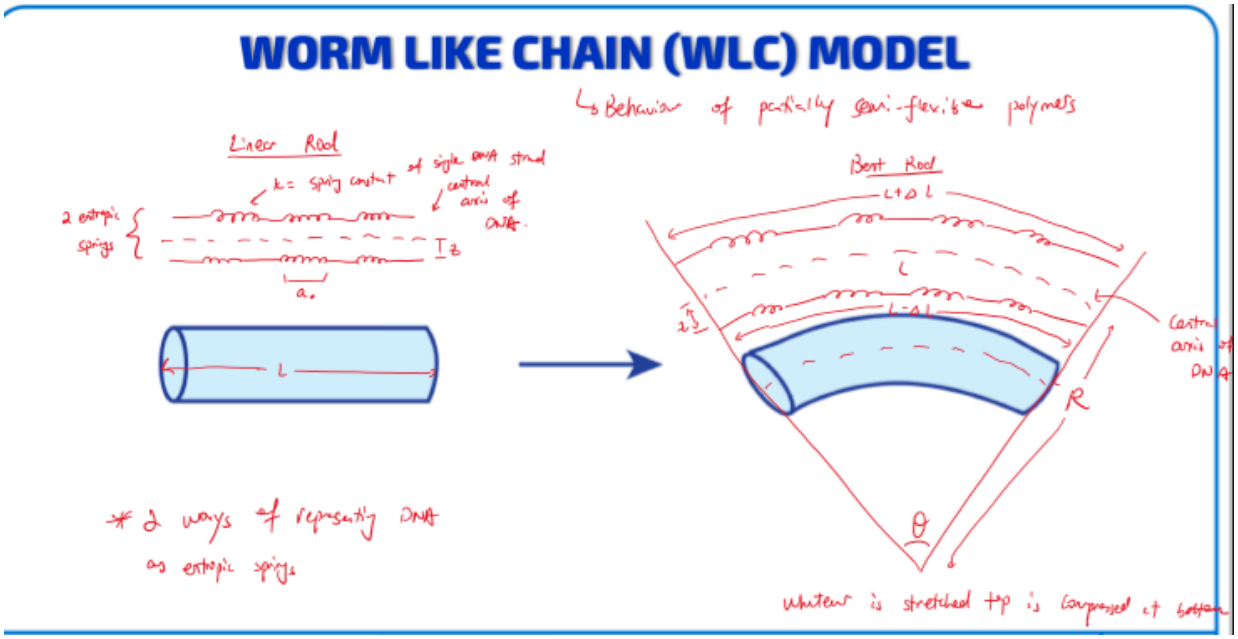
DNA Denaturation

- DNA thermal denaturation involves heating up native DNA so that it comes denatured
    - Denatured DNA is when DNA is unwound into single stranded coils
- The melting temperature Tm is directed related to DNA stability
- Zipper Model
    - DNA denatures from one end → local denature
    - Transition at Tm becomes sharper as the number of base pairs increase
- Local Denaturation
    - DNA can be locally denatured in various ways
    - Bubble formation can be catalyzed by DNA untwisting/unwinding proteins (like helicases) or binding of greasy molecules or DNA, which effectively reduces Tm (so that denature is easier)

DNA Worm Like Chain Model

WLC Model

- We make the linear rod into a bent rod



Finding the Change in Length and Spring Constant

$$\frac{L+\Delta L}{R+z} = \frac{L}{R}$$ based on similar triangles sharing θ.

$$(L+\Delta L)R = L(R+z)$$

$$LR + R\Delta L = LR + zL$$

$$\boxed{\Delta L = \frac{zL}{R}}$$

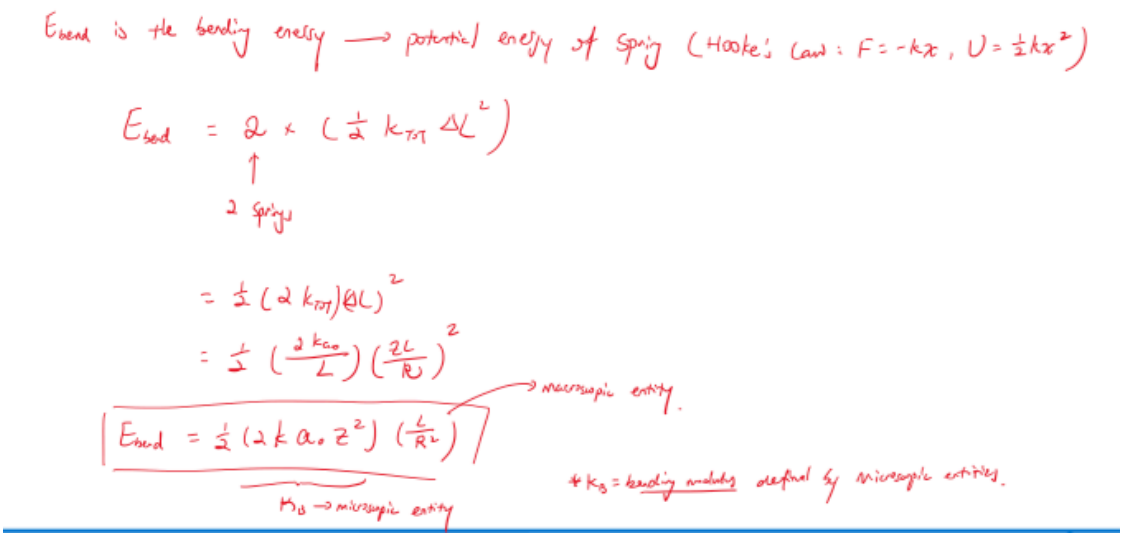
(now, look at k (DNA spring constant)

k_{TOT} = Spring constant of 1 strand of DNA

$$= \frac{k_{ind}}{\#\text{ of springs}} = \frac{k_{ind}}{L/a_0}$$

$$\therefore \boxed{k_{TOT} = \frac{k_{ind}\, a_0}{L}}$$

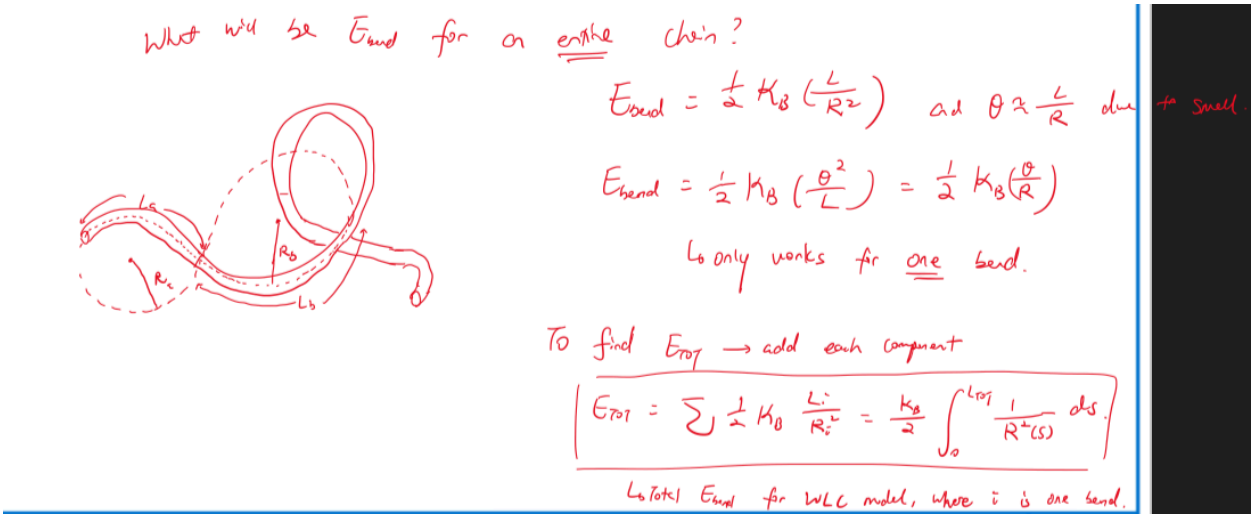
Finding the Bending Energy



Where KB is the binding modulus defined by the microscopic entities

Physical Meaning of Kappa B (KB)

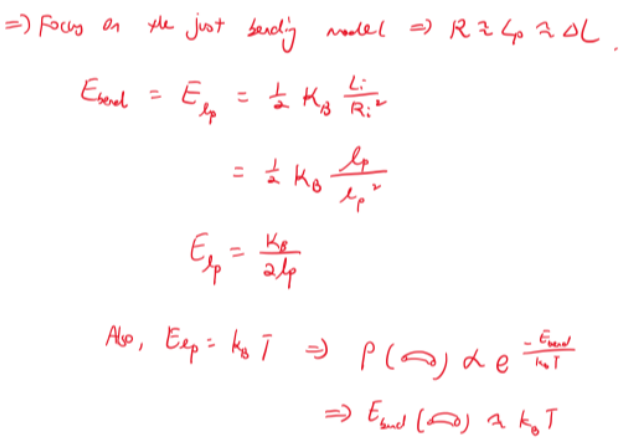
The "stiffness" of one bend of DNA



$$E_{bend\ total} = \frac{\kappa_B}{2}\int_0^L \frac{1}{R^2(s)}ds$$

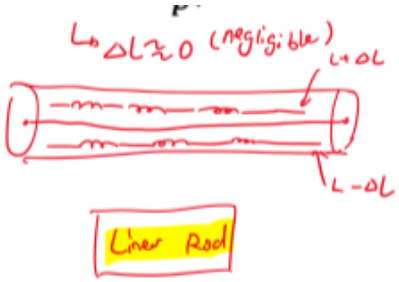
Persistent Length (lp)

The length scale within which chain remains straight → beyond lp, the chain coils up (which gives you the worm-like chain model WLC)

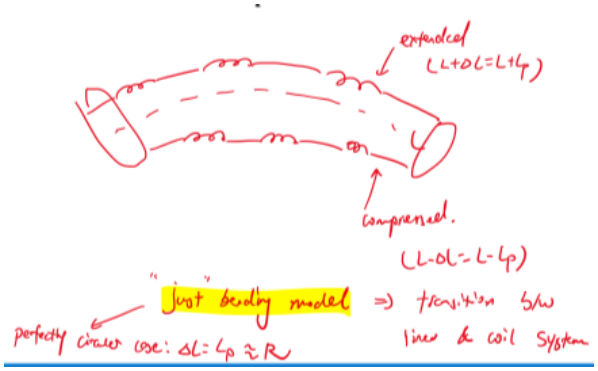


$$l_p = \frac{\kappa_B}{k_b T}$$

- For change in length ($\Delta L < l_p$): **Linear Rod**
    - Rod-like linear DNA structure (energy dominates)



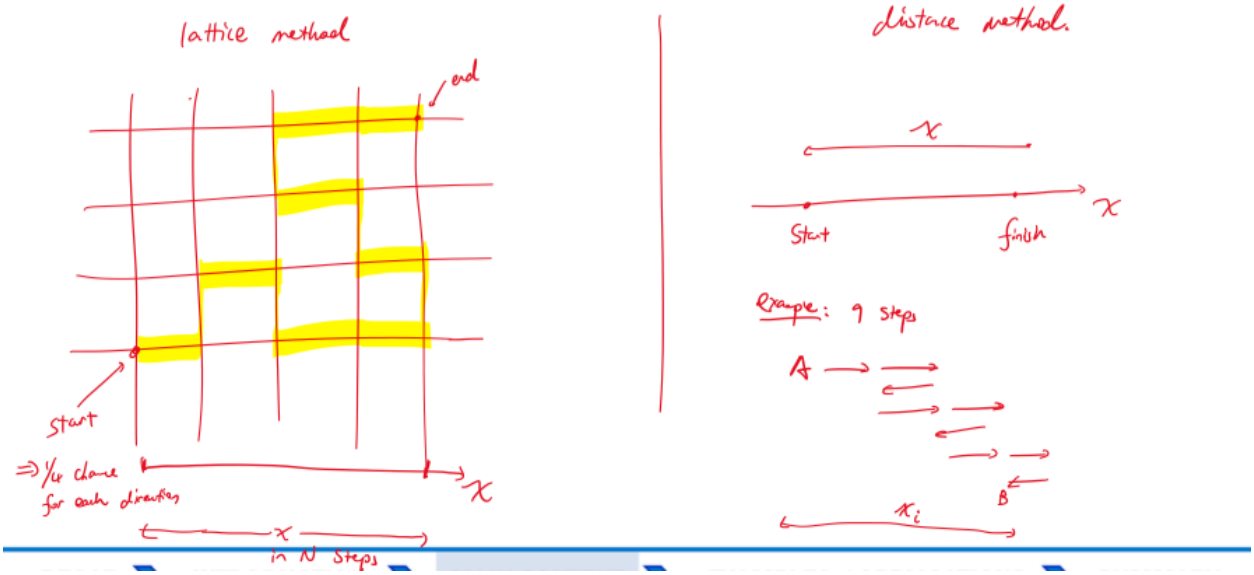
- $\Delta L \approx l_p$: **just bending model**
    - Both effects are comparative (macroscopic and microscopic) → curved rod



- $\Delta L > l_p$: **coil-like chain model (WLC)**
    - A sum of various just bending model → entropic coil

Random Walk Chain Model

The random walk (RW) chain model is given by:



Multiplicity of RW Model

If n = # of steps to right and N-n = # of steps to left

$$W(N,n) = \frac{N!}{n!(N-n)!} \qquad \text{but} \quad x = n - (N-n)$$

$$x = 2n - N$$

$$n = \frac{x+N}{2} \quad \text{and} \quad N-n = \frac{N-x}{2}$$

Suppose we have step length b & N steps,

$$W(N,n) = e^{-\frac{1}{2}\frac{x^2}{Nb^2}} = e^{\frac{-F(N,n)}{k_B T}} \qquad \text{where} \quad F(N,n) = 0 - k_B T \ln(W)$$

$$W(N,n) = \frac{N!}{\left(\frac{x+N}{2}\right)!\left(\frac{N-x}{2}\right)!} = e^{-\frac{F(N,n)}{K_b T}} = e^{-\frac{1}{2}\frac{x^2}{Nb^2}}$$

Since entropy is -kb ln(w), this gives us the effective spring constant of DNA, keff

$$k_{eff} = \frac{k_B T}{Nb^2}$$

Entropic Spring Pulled by External Force

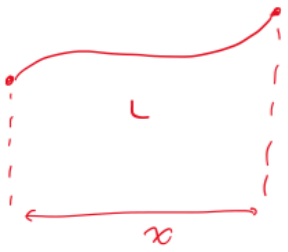
$$x = \frac{F}{k_{eff}}$$

Where x is the relative displacement with applied force and is proportional to F

WLC and Chain Elasticity

In 1995, J. Marko and E. Siggia discovered that:

$$\frac{l_p f}{k_B T} = \frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} - \frac{1}{4} + \frac{x}{L}$$



L = length of DNA chain

x = linear displacement of DNA start/end (5') (3')

f = external force applied

l_p = persistant length

For a small force applied:

- If we assume x/L is small since the stretch is small

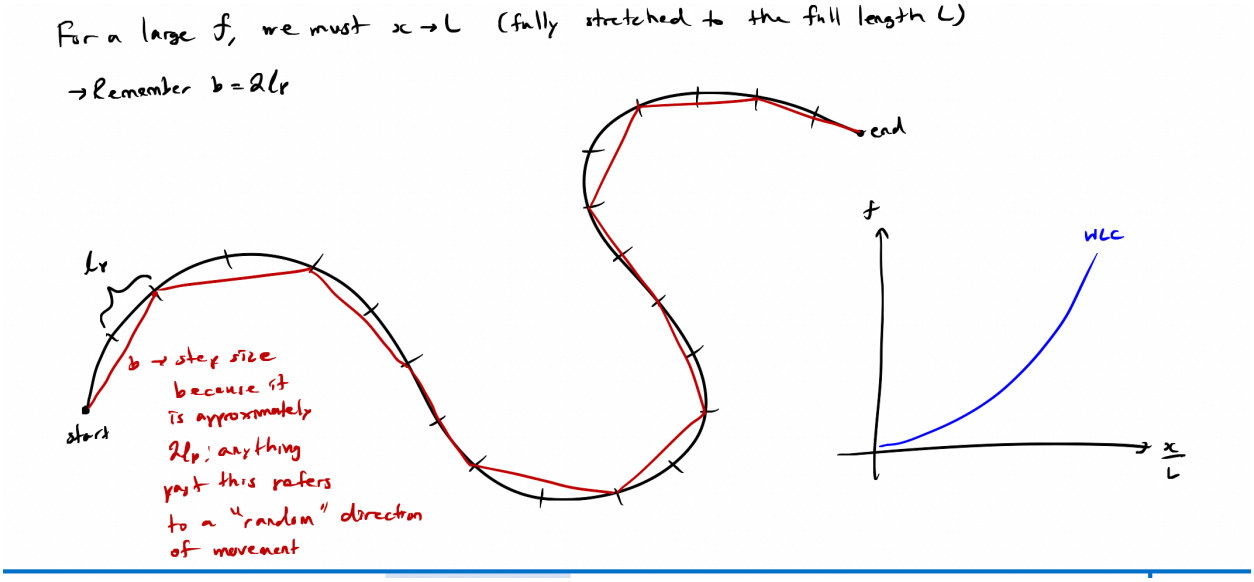
$$k_{eff} = \frac{3k_B T}{2l_p L}$$

- If b = 2lp and L = Nb, then

$$k_{eff} = \frac{3k_B T}{Lb}$$

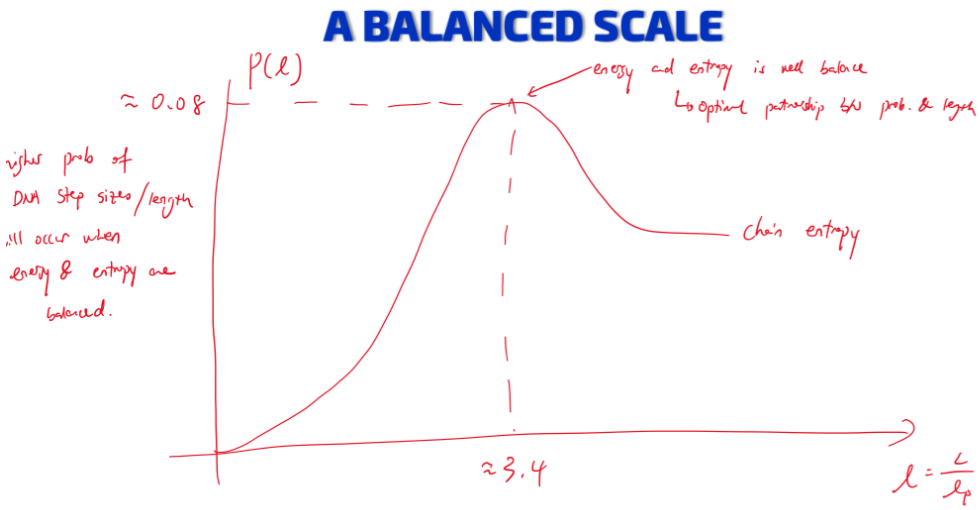
For a large force applied:

- X approaches L → fully stretched
- **b = 2lp**
    - b is the step size; anything that is greater than b refers to a "random" direction of movement



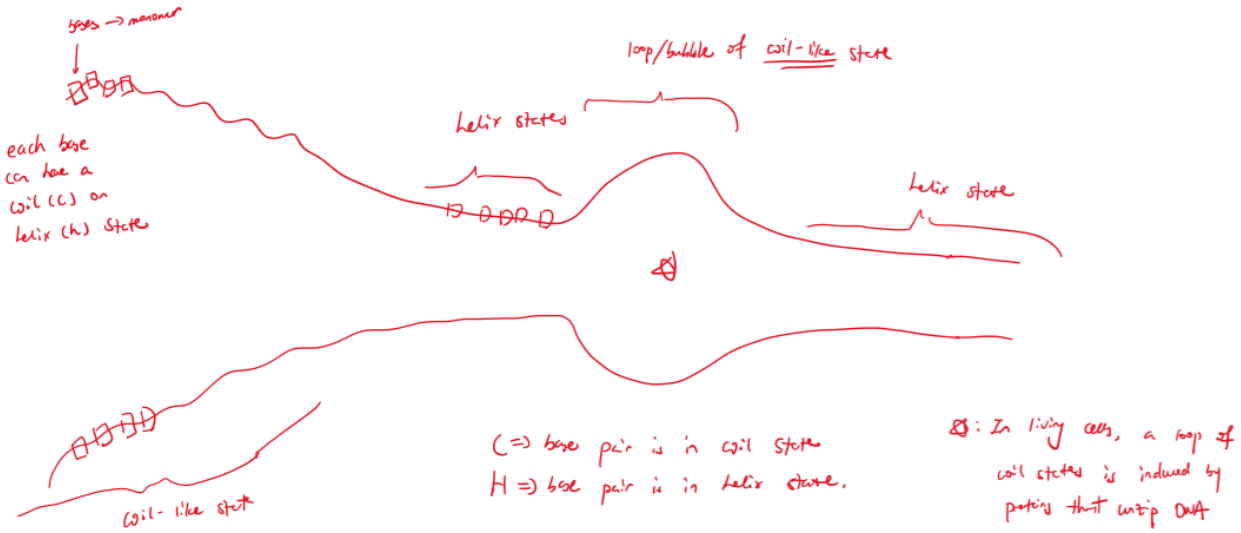
Balancing Energy and Entropy

A high probability of DNA step sizes / length will occur when energy (length) and entropy (number of configurations) are balanced



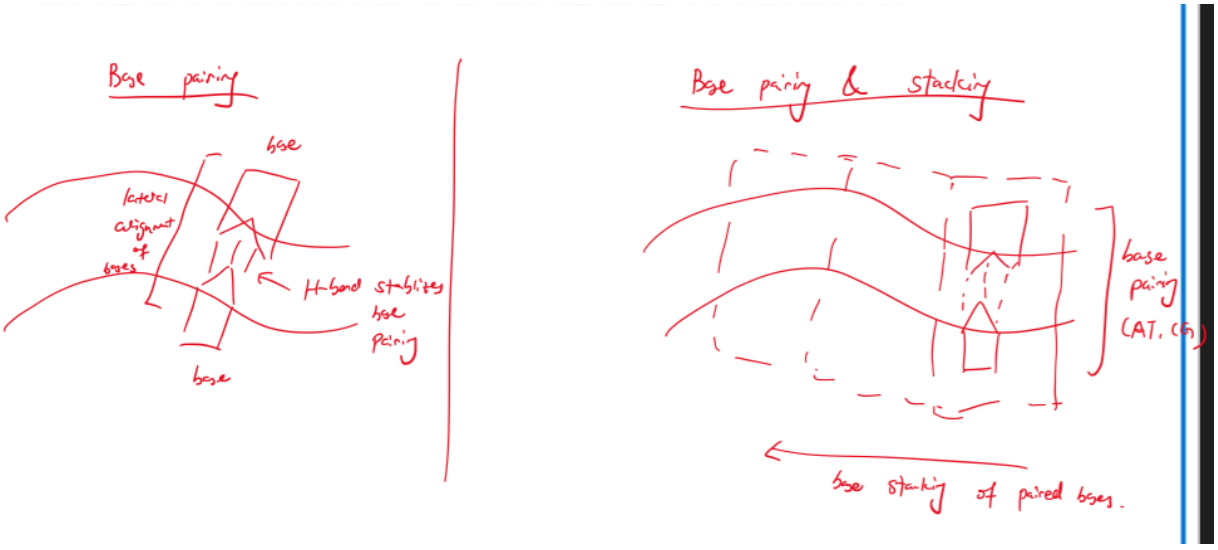
DNA Denaturation

Model of DNA Denaturation



Base Pairing and Base Stacking

Base stacking is the stacking of paired bases



Coil vs. Helix for DNA

Weights for all states:

→ 1st H: σs

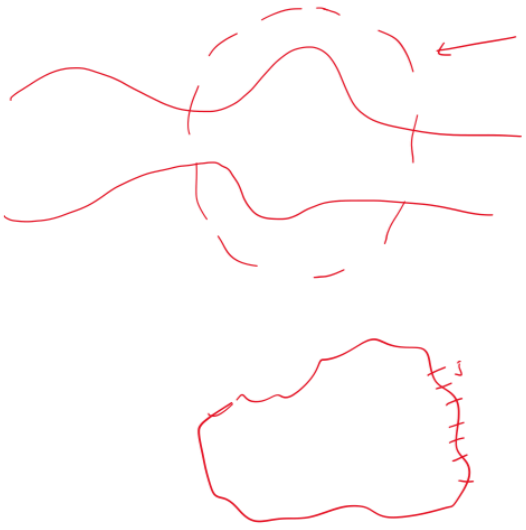
→ 2nd H: s

} $\sigma \ll 1$ b/c 1st helix state is always more difficult to achieve.

$W(HH) = \sigma s \cdot s = \sigma s^2$ → Same for proteins.

↳ exceptions: DNA looping

Looping Statistics



constrained loop that is not completely random

↳ purpose: pack DNA

$P_0 \propto \frac{1}{j^{3/2}}$, where $P_0 \approx 3\sqrt{\frac{6}{2\pi j^3}}$

We look at 6 configurations of a 3D lattice structure

↳ 6 degrees of freedom.

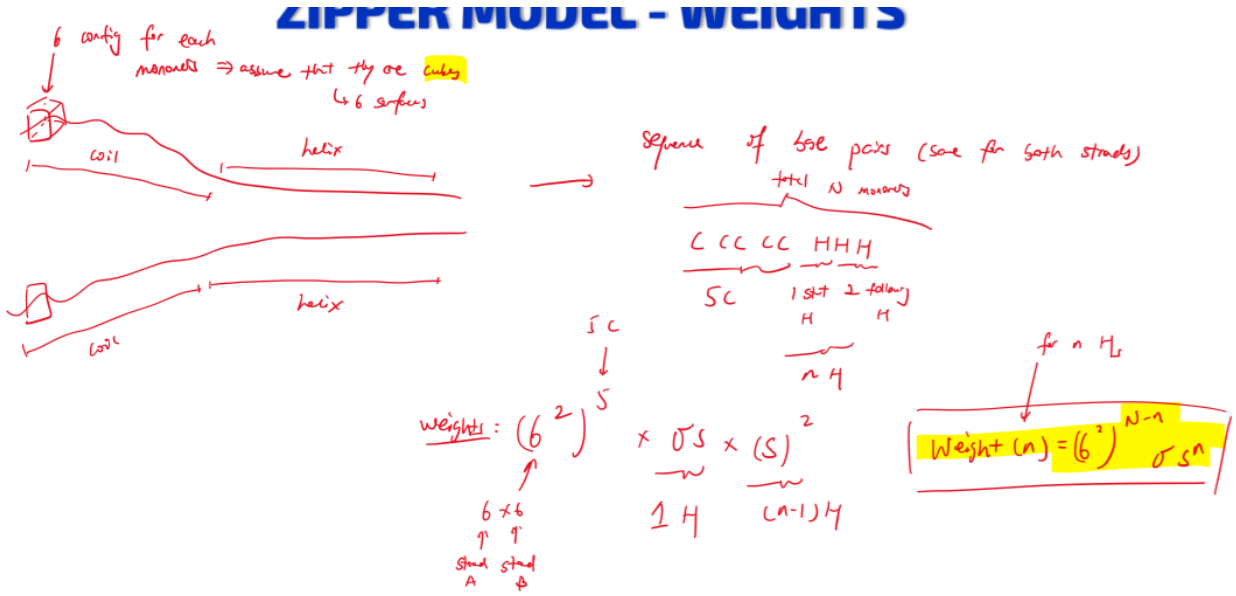
$P_0 = \frac{W(\text{loop})}{W(\text{total})}$

Zipper Model – Weights

We assume the DNA monomers are cubes with 6 surfaces and 6 degrees of freedom, then for n monomers in helical (H) states and a total of N monomers:

$$weight(n) = (6^{2(N-n)})\sigma s^n$$

Example:



Partition Function (Zn) for the Zipper Model

Consider applying the partition function

$$Z_n = \sum_{n=1}^{N} weight(n)$$

$$= \sum_{n=1}^{N} (6^2)^{N-n} \sigma s^n$$

$$= \sum_{n=1}^{N} (6^2)^{N} (6^2)^{-n} \sigma s^n$$

$$Z_n = 6^{2N}\sigma \sum_{n=1}^{N} \left(\frac{s}{6^2}\right)^n$$

Fractional Helicity for the Zipper Model

Using a sum of geometric series : $\sum ar^n = \frac{a}{1-r}$

$$Z_n = \sigma b^{2N} \left(\frac{(\frac{s}{b^2})(1-(\frac{s}{b^2})^n)}{1-\frac{s}{b^2}} \right)$$

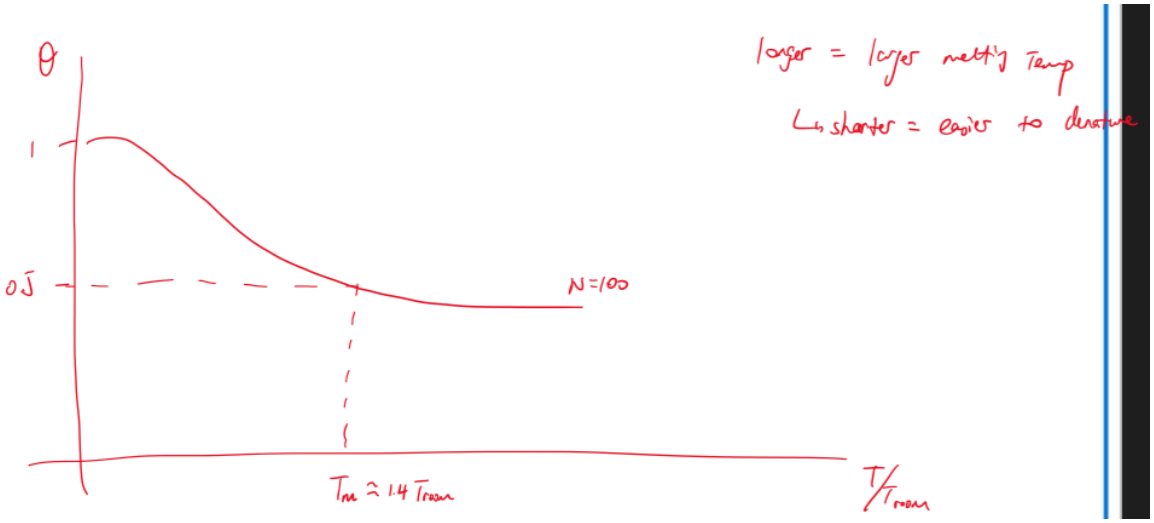
$\theta = \frac{1}{N} \langle \#\ of\ H_s \rangle$

$$\theta = \frac{1}{N}\left(\frac{s(\frac{\delta}{\delta\sigma} Z_n)}{Z_n}\right)$$

Melting Temperature

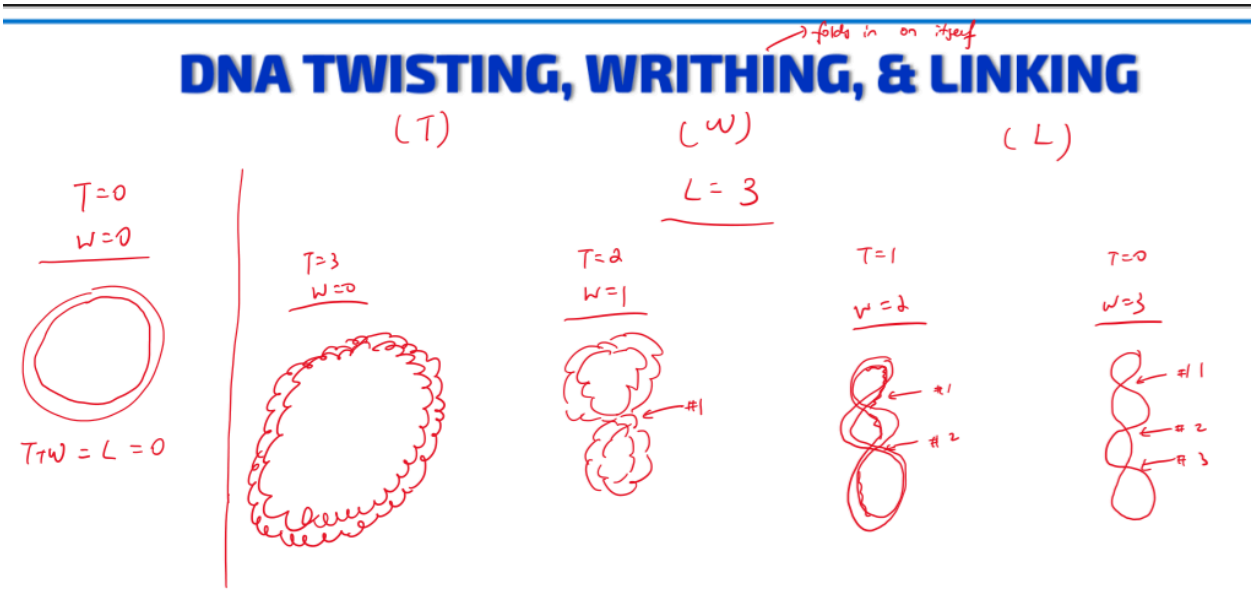
The larger the fractional helicity, the larger the melting temperature

The larger the monomer, the larger the melting temperature → shorter = easy to denature



DNA Twisting (T), Writhing (W), and Linking (L)

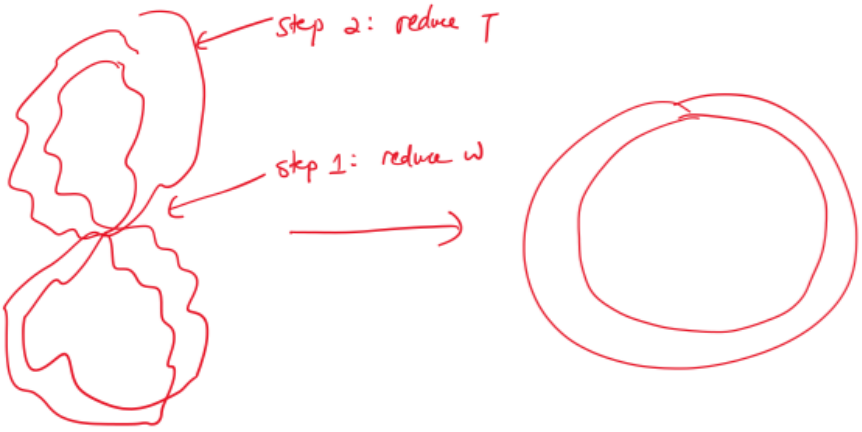
T + W = L



Untwisting DNA for Transcription

When transcription occurs, W < 0 and T < 0 → We need to reduce w first before we reduce T

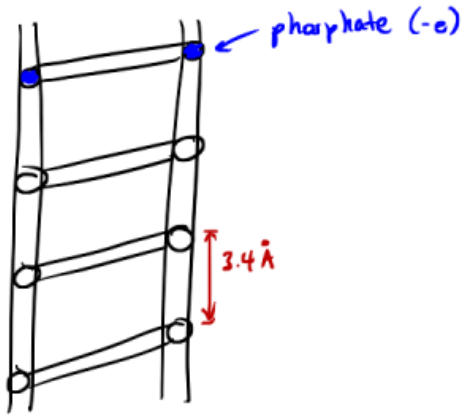
- It facilitates the untwisting for the transcription process as RNA polymerase will attach and unwind DNA



DNA as a Giant Charge

Overview

DNA is the most highly-charged molecule in nature → we can define a charge per turn of the DNA



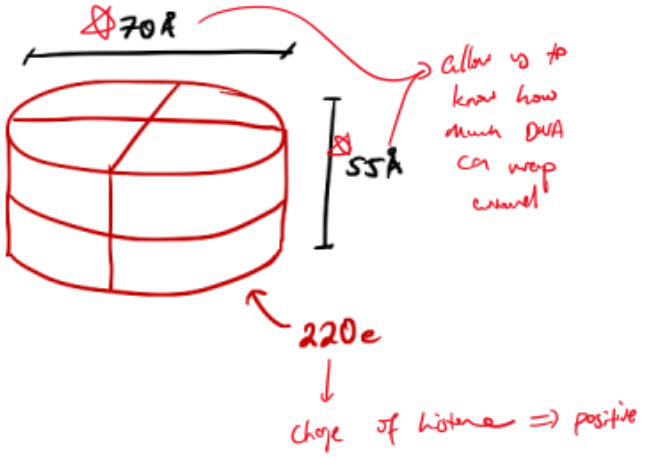
$$\text{Charge} = \frac{-2e}{3.4\text{Å}} = \frac{-e}{1.7\text{Å}}$$

This is called the charge per turn of DNA

Histone

The histone octamer has:

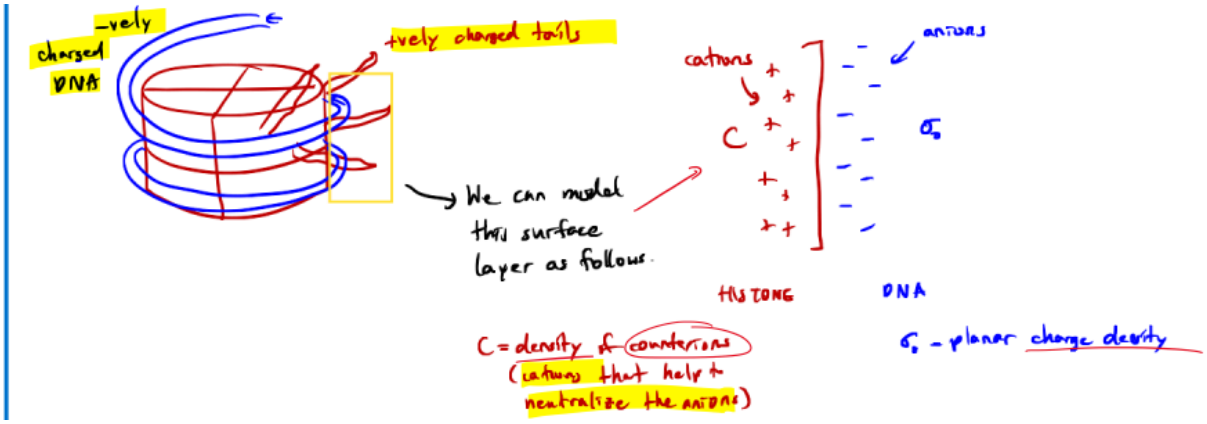
- 2 copies of (H4+H3+H2A+H2B)
- A positive charge (220 e) so it is a major player in DNA packing
    - It attracts (electrostatic) the negatively charged DNA and want to neutralize (stability) both charges



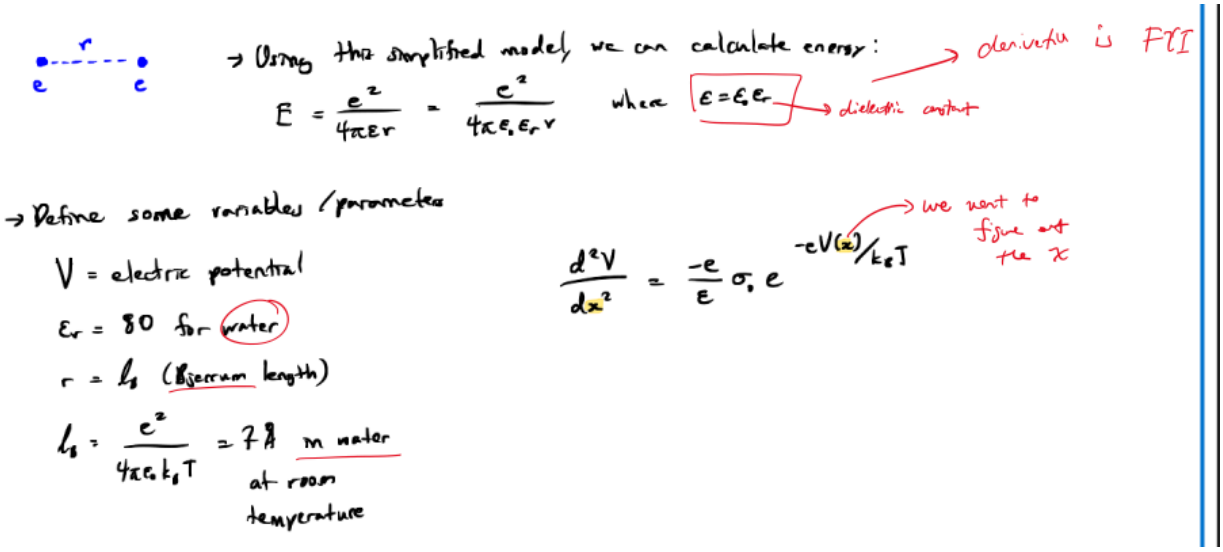
Neutralization of Charge

The histone tails are positively charged while DNA is negatively charge, so when they come together, the charges will neutralize via counter ions

- There is competition between entropy and energy

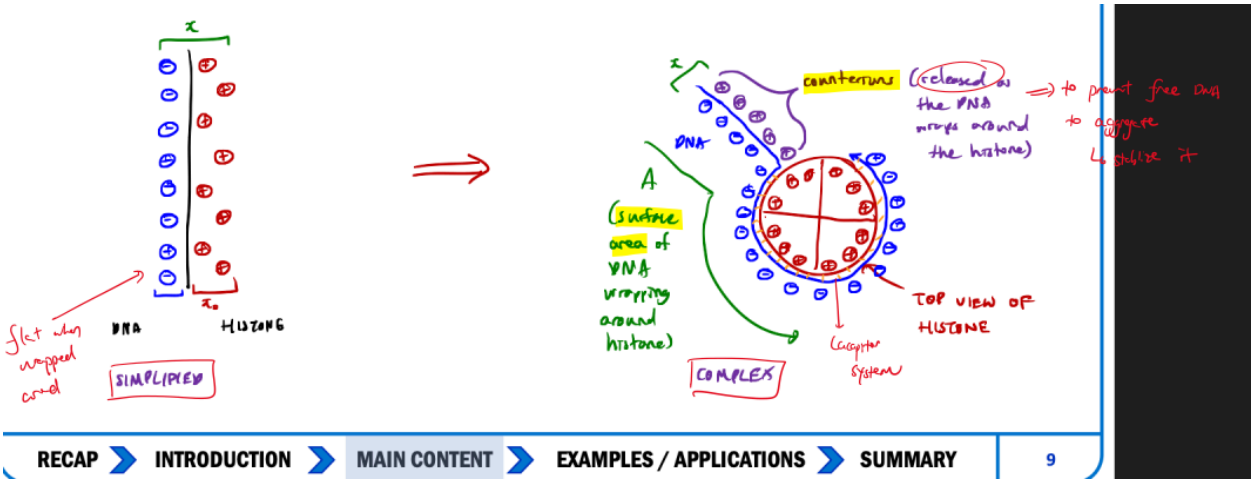


Energy and Potential

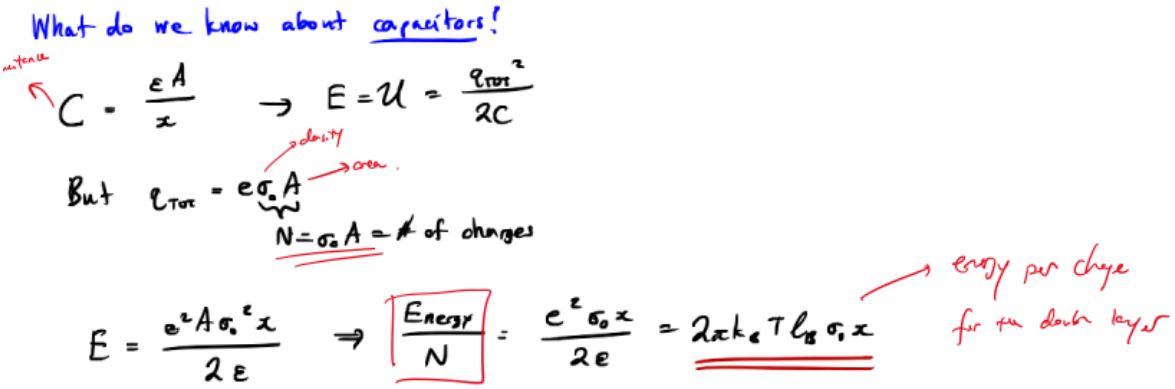


Capacitor Model for Double Layer Model

The double layer model is between a diffusive layer and a backbone charge



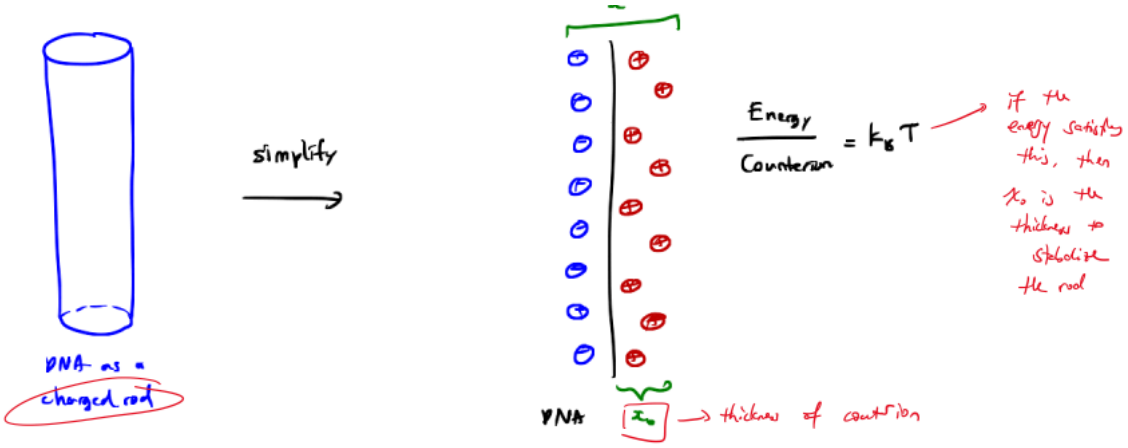
Key Equations:



Looking at a Charged Rod

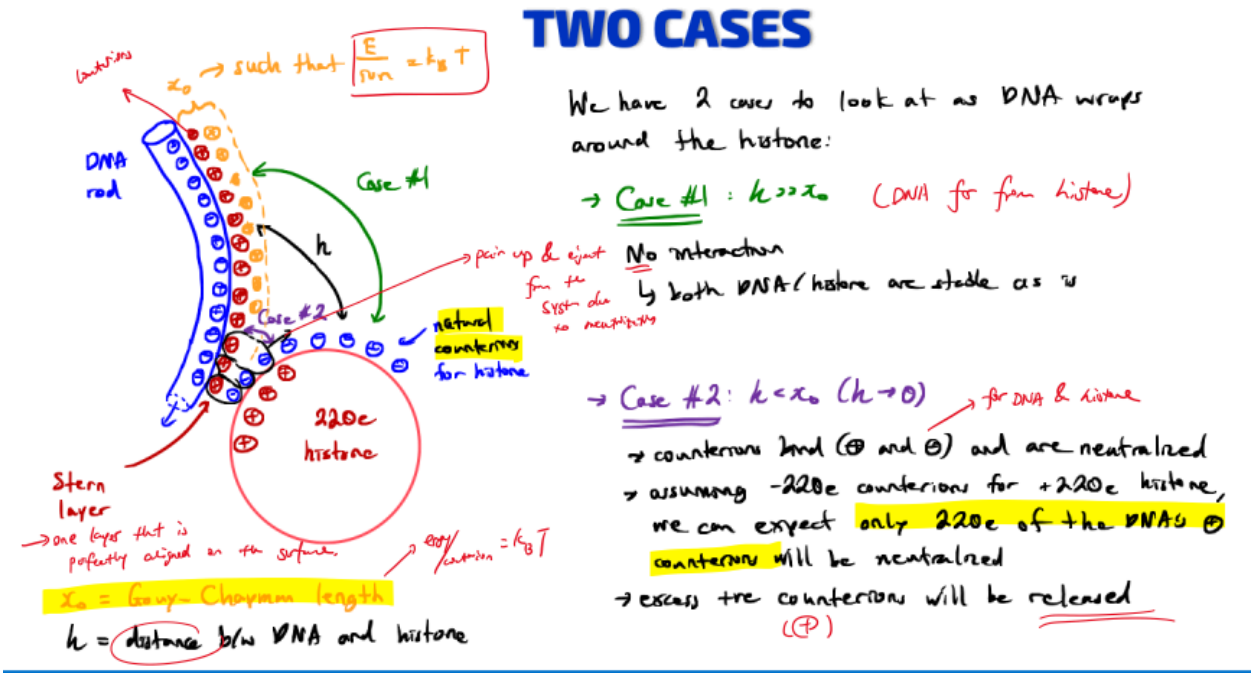
Xo is the thickness of the counter ion → The Gouy-Chapmm Length

- If energy/counterion = KbT, xo is the thickness to stabalize the rod



Two cases:

1. If h (distance between DNA and histone) >> xo
   - DNA is a far from histone → no interactions between → DNA is stabilized as it is
2. If h < xo (and h approaches 0)
   - Counterions bind (+ for DNA and – for histone) and are neutralized
   - Assume -220e counterions for +220e histone, we can expect only 220e of the DNA positive counterions will be neutralized
   - Excess positive counterions from DNA will be released



Energy Difference

The energy difference can be quantified as:

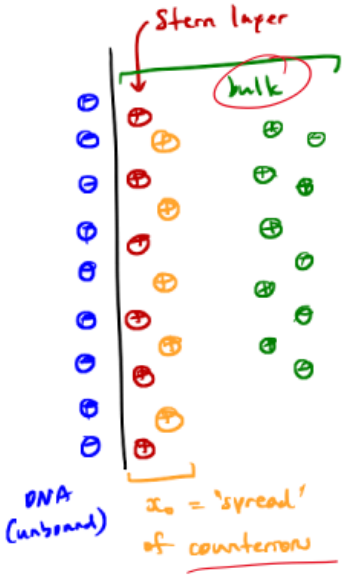
$$\frac{\Delta E}{\text{Counterion - Plate}} = -k_B T \quad \text{from electrostatics}$$

(Size of plate)

But, there is entropic effect associated with the release of positive counterions → we need to determine whether they are favourable

Entropic Effect of Counterions

Case 1: We now have a Stern Layer when close to the DNA



We can find the ENTROPY per counterion!

$-TS = -k_B T \ln W$

Concentration of counterions

$\Rightarrow S = k_B T (\ln [C]_{surface} - 1)$ ①

↳ per counterion C, per plate

Case 2: in bulk (ie: no DNA)

In bulk, we need to modify S:

$S = k_B T (\ln [C]_{bulk} - 1)$ ②

*factor both electrostatic & entropy when asked about why DNA wraps around histone

When we look at ② − ①, we get:

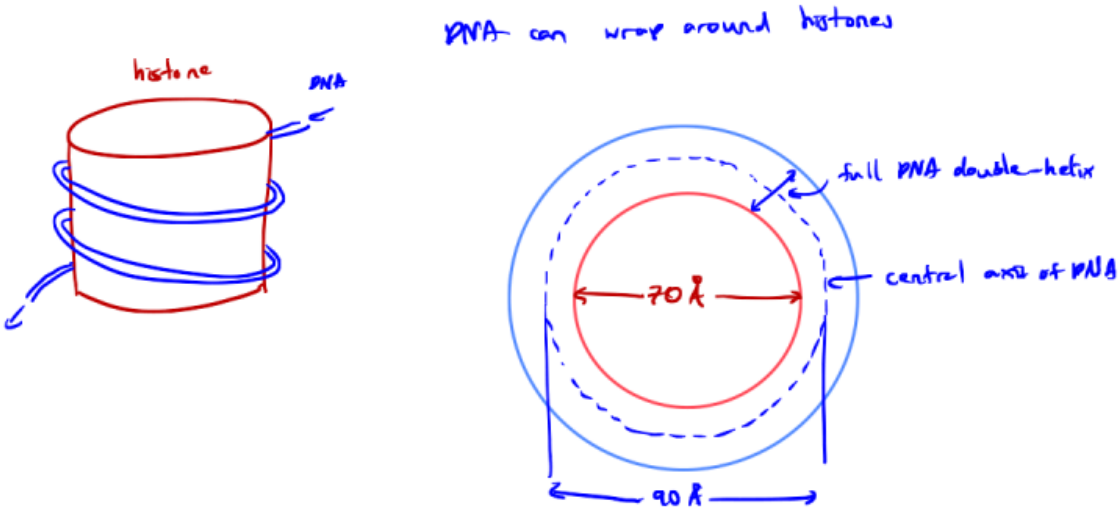
$$\Delta S = -k_B T \ln \left(\frac{[C]_{surface}}{[C]_{bulk}} \right)$$

entropic effect

entropic contribution to DNA packing

DNA Histone: Number of DNA turns

DNA can wrap around histones



Total charge on histone is 220e

- We need -220e on DNA to neutralize histone
- Since we know that the charge per turn is e/1.7A → each charge takes up to 1.7A, therefore, -220e will need:

$$220 \times 1.7A = 374A$$

- Hence, number of turns is about 1.323 turns per histone, which is close to reality of 1.4 turns

$$\#\ \text{of turns} = \frac{374\text{Å}}{\pi(90\text{Å})} = 1.323\ \text{turns} \approx 1.4\ \text{turns}$$

reality

$\Rightarrow$ model is fairly accurate

Binding Energy of DNA Histone

Allow to show change of energy and entropy

When bind:

$$E_{bend} = k_B T \frac{1}{2} l_p \int_1^L \frac{1}{R^2(s)}\, ds \longrightarrow \frac{1}{K^2}(2\pi R)\cdot 1.4$$

$$E_{bend} = k_B T \frac{1}{2} l_p \left[\frac{1}{K^2} 2\pi K (1.4)\right]$$

$$= k_B T \frac{1}{2} l_p \frac{2\pi}{K} \cdot 1.4$$

$\approx 50\, k_B T \Longrightarrow$ This allows to show change in energy and entropy

Energy vs. Entropy

Energy Change → electrostatic

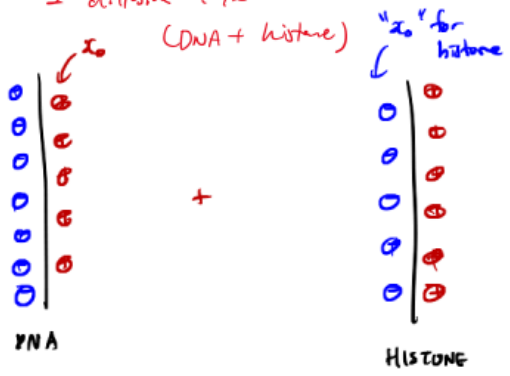
$\Delta E = -k_B T (220 \times 2)$

↓

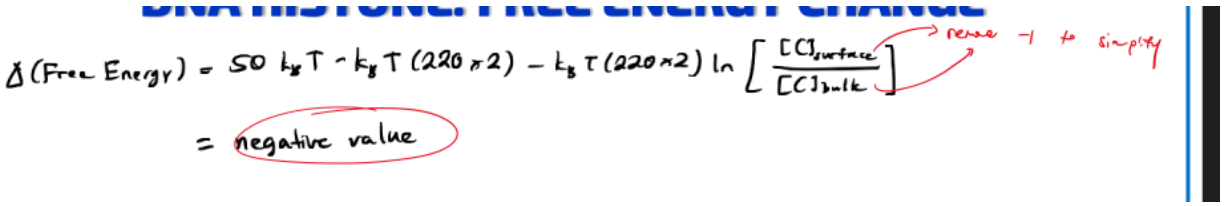
2 diffusive layer (DNA + histone)

Entropic Change → release of (+)

$\Delta(-TS) = -k_B T (220 \times 2) \ln\left[\frac{[C]_{surface}}{[C]_{bulk}}\right]$



We can then calculate the free-energy change



This means that:

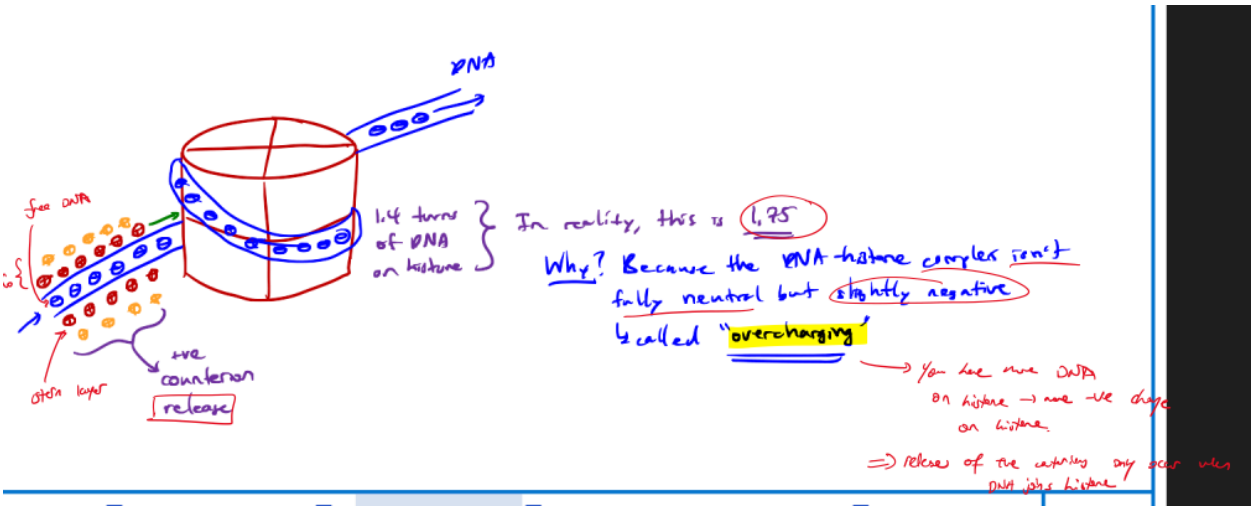
- Wrapping DNA around a histone takes place spontaneously → energetically and entropically favourable
- Overall, net favourable reaction is taking place
    - Despite a slight decrease in entropy due to a more compacted structure, since energy dropped a lot, it is still overall favourable

DNA Histone Summary

In reality, the turn of DNA per histone is 1.75

- Because the DNA-Histone complex isn't fully neutral but slightly negative
    - "Overcharging" → you have more DNA on histone and therefore more negative charge
- The release of counterions only occur when DNA joins histone

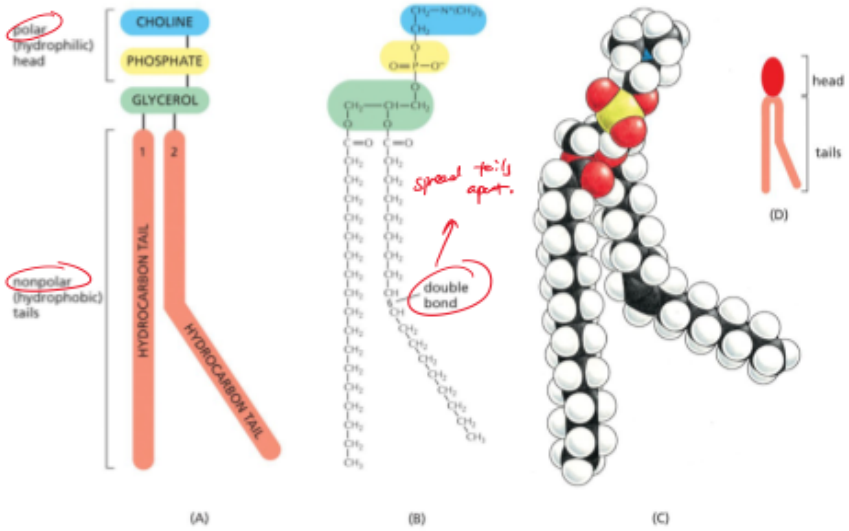
The counterion effect helps balance charges and promote stability in DNA packing



Lipids and Self-Assembly

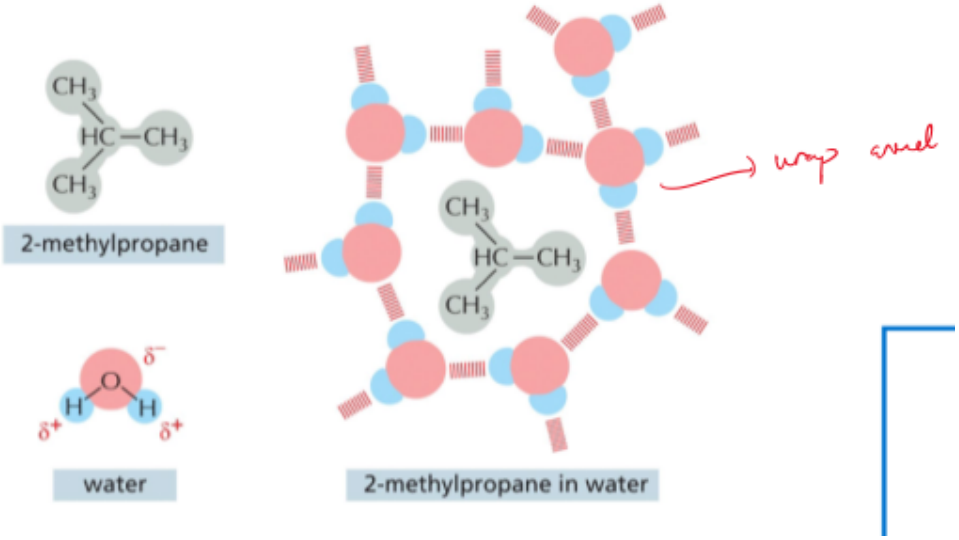
Structure of A Lipid

Polar head + non-polar tail, with potential double bonds in tails

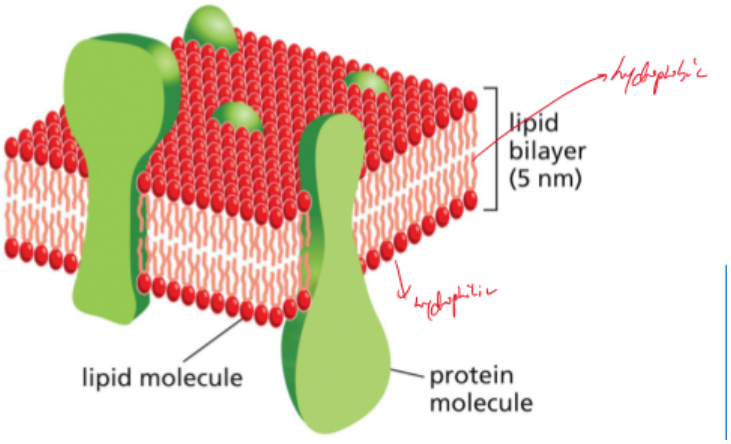


In water

Water molecules wraps around

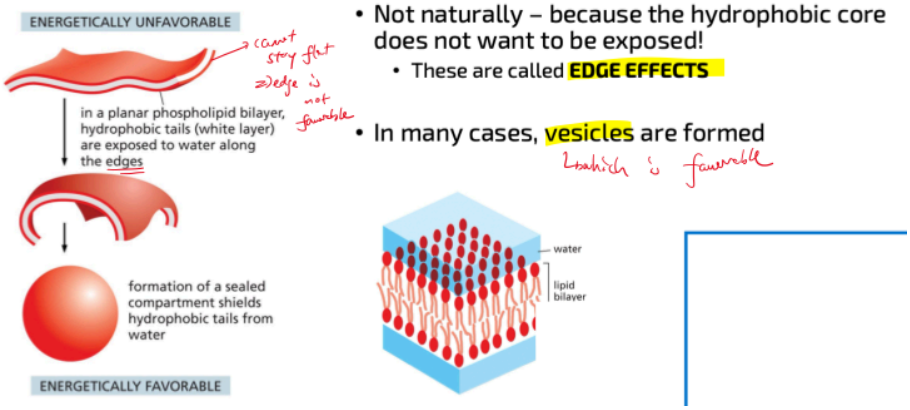


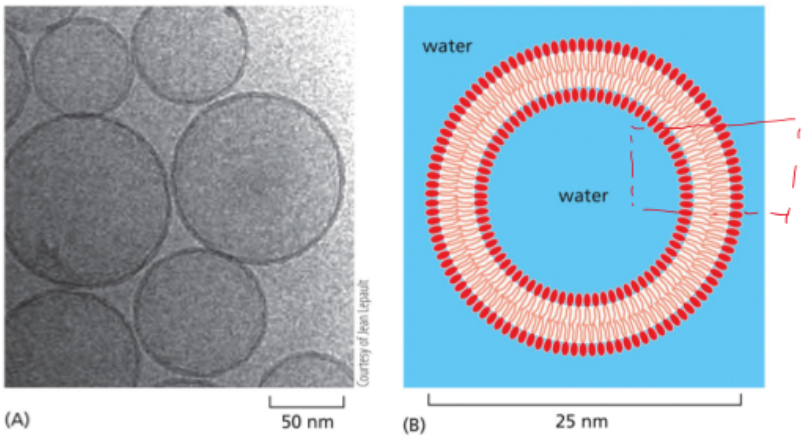
Lipid Bilayer



The bilayer does not stay flat naturally

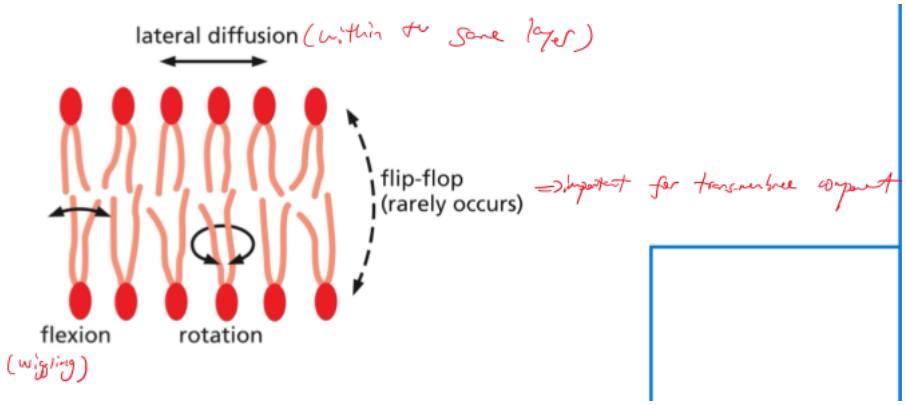
- Due to Edge effects → hydrophobic core does not want to be exposed
- In many cases, vesicles are formed

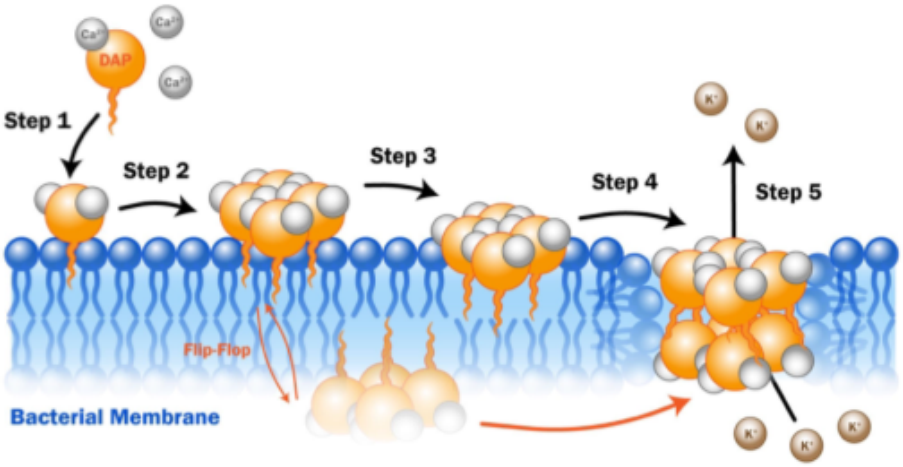




Within the Bilayer

Lateral diffusion, flexion, rotation, flip-flops (example: daptomycin)





Cholesterol promotes fluidity due to increased space → but too much can create too much space and cause the membrane to break (5 – 30% is good for a fluid membrane)

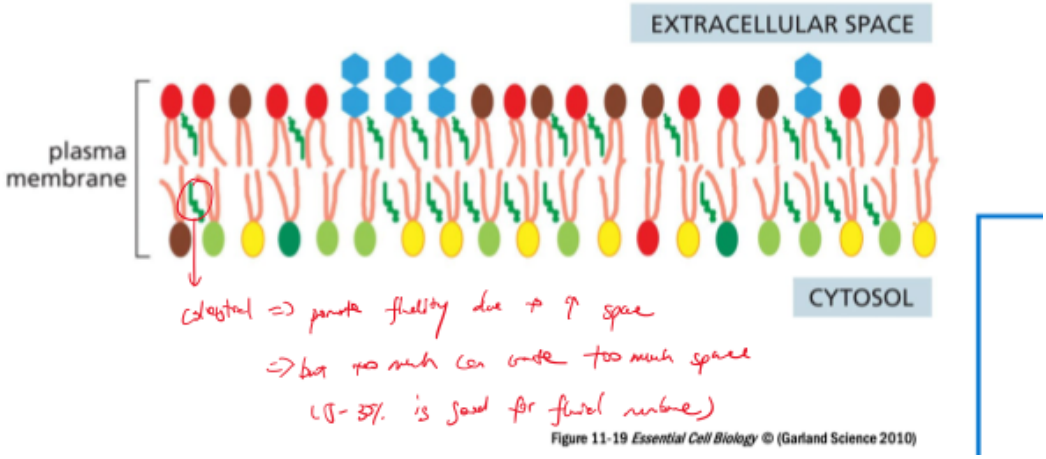
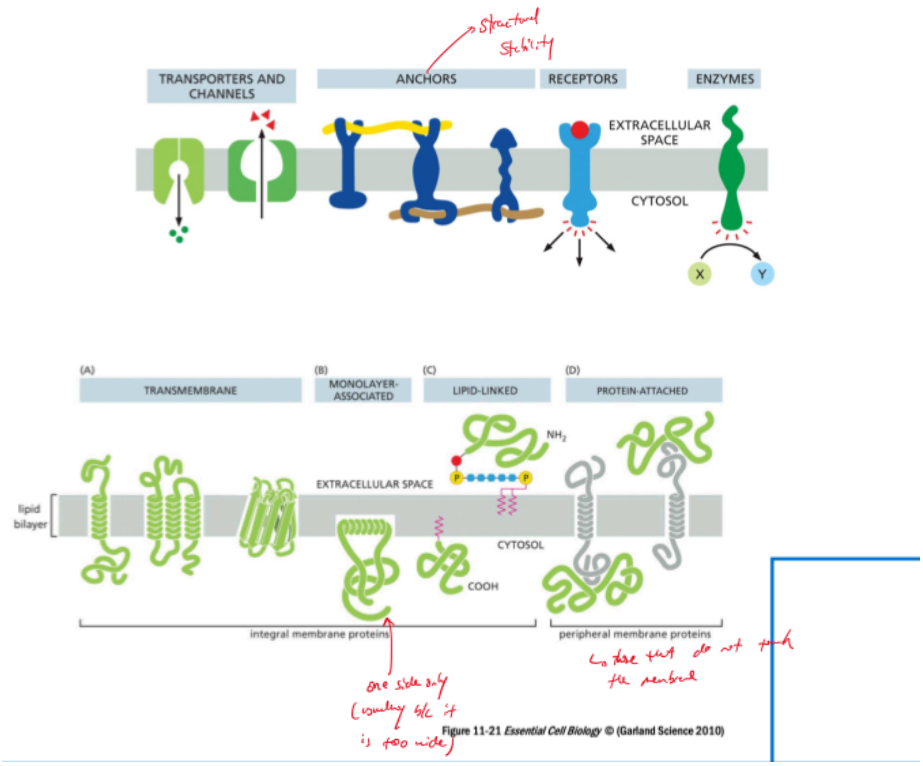


Figure 11-19 *Essential Cell Biology* © (Garland Science 2010)

Proteins and Other Things on the Lipid Membrane

Transporters and channels, anchors, receptors, enzymes

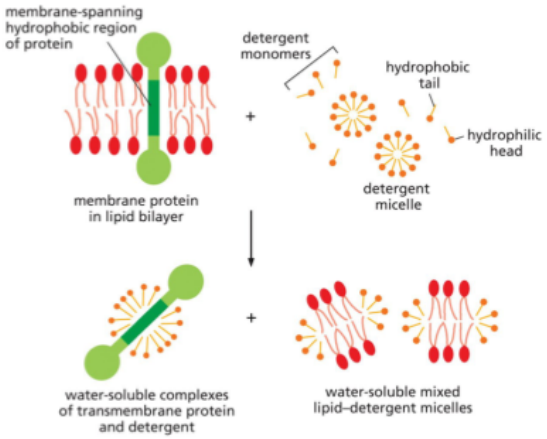
Transmembrane proteins, monolayer-associated, lipid-linked, protein attached



Solubilizing Membrane Proteins

Membrane proteins can be solubilized by a mild detergent such as Triton X-100

- Detergent molecules disrupt the bilayer and brings proteins into the solution as protein-detergent complexes



Overview of Self-Assembly

- A micelle (one layer sphere) or vesicle (bi-layer sphere) can be formed via self-assembly
- Amphiphilic molecules have both hydrophobic and hydrophilic areas
    - Two classes of amphiphiles: <u>phospholipid</u> and <u>strong detergent</u>
- Self-assembly can occur at oil-water interface or emulsion

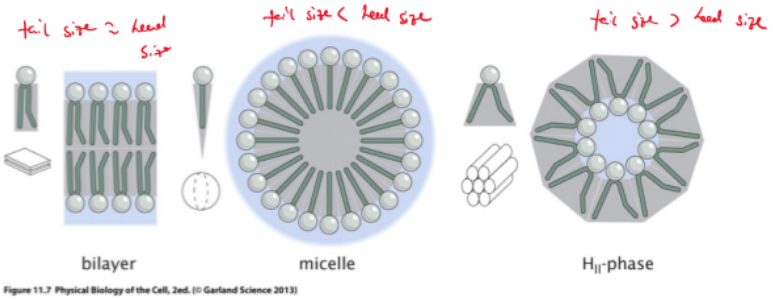
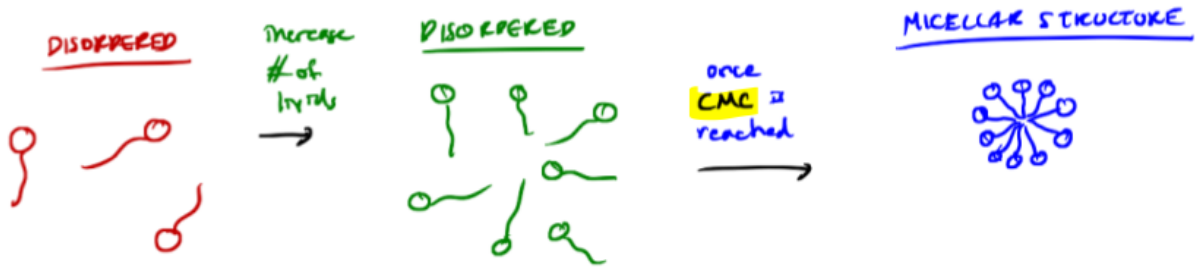


Figure 11.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Soap Bubble and CMC

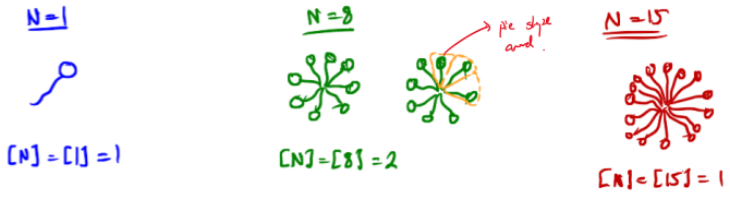
The critical micelle concentration (CMC) is the concentration of amphiphiles that is required to form a micelle → you have to have enough for self-assembly to occur



Thermodynamics of Self-Assembly

Parameters:

- N = aggregation number (number of lipids in 1 aggregate)
- [N] = concentration of amphiphiles in aggregates of aggregation number N (how many N-mers / how many aggregates of size N)



Entropic Chemical Potential

Depend on the concentration of N-mer

We define this to be:

$$\mu_i = \mu_i^0 + k_B T \ln [i]$$ for each monomer (each $N = i$)

N-mer
(ie. N = 8)

$\Rightarrow$ $k_B T \ln$ []

concentration of N-mer = $\frac{\#\text{ of N-mer}}{\text{volume}}$

Individual Chemical Potential

Here, [N] means the number of lipids present (not the number of aggregates present)

$\frac{[N]}{N}$ = concentration of amphiphiles in an N-mer state

[N]: # of ligands present (different def than phase space)

N: aggregate number (ie. N = 8)

$$\mu_N = \mu_N^\circ + \frac{k_B T}{N} \ln \frac{[N]}{N}$$

for individual amphiphiles in N-mer state

Dimerization (A+b<->C)

→ Define chemical potentials as: $\mu_i = \mu_i^\circ + k_B T \ln [i]$ where $i = A, B, C$

→ We can determine what is at equilibrium:

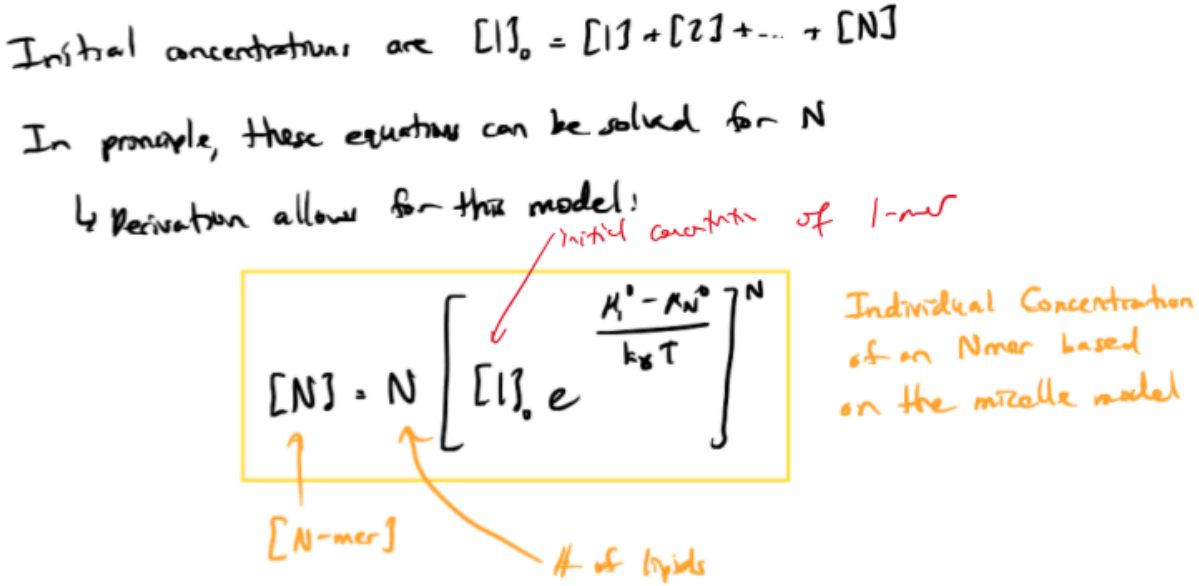
$$\mu_A + \mu_B = \mu_C$$

If $A = B$, then $2\mu_A = \mu_C = \mu^\circ + k_B T \ln [C]$ (μ°: $-\varepsilon$)

dimer means that $[C] = \frac{N}{2}$ (N: # of ligands)

$$\therefore \mu_A = \frac{\mu_C}{2} \Rightarrow \mu_A = \frac{-\varepsilon}{2} + \frac{k_B T}{2} \ln \frac{N}{2}$$

Determining the concentration of N-mer



Two different cases:

Case 1: As the number of lipids goes to infinity → the chemical potential is a constant after saturation point

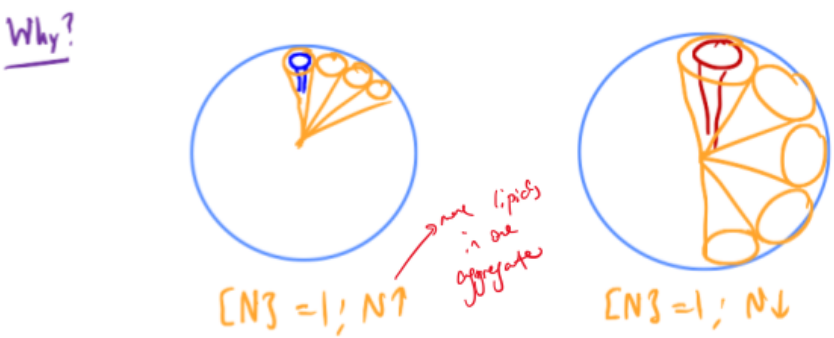
Case 2: if we have [1] only → no 2-mers are possible or most lipids exist in disordered state

Therefore, if you do not have enough lipids for a small structure / micelle, then you surely do not have enough to form larger N-mer structure

Events at CMC

At CMC, we have just enough lipids to form [N] = 1

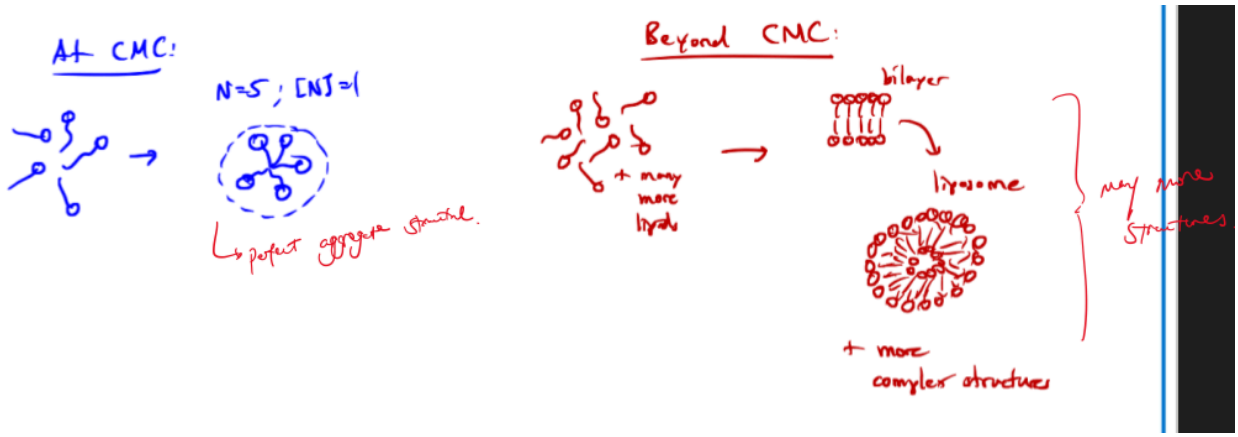
There are different starting thresholds for different types/shapes of lipids



Beyond CMC

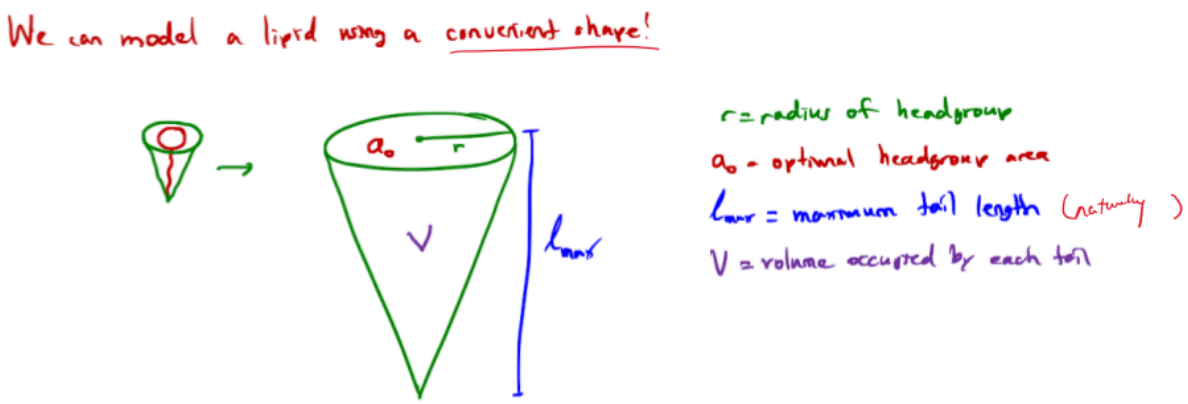
At CMC → perfect aggregate structure

Beyond CMC → many more possible structures formed at the same time

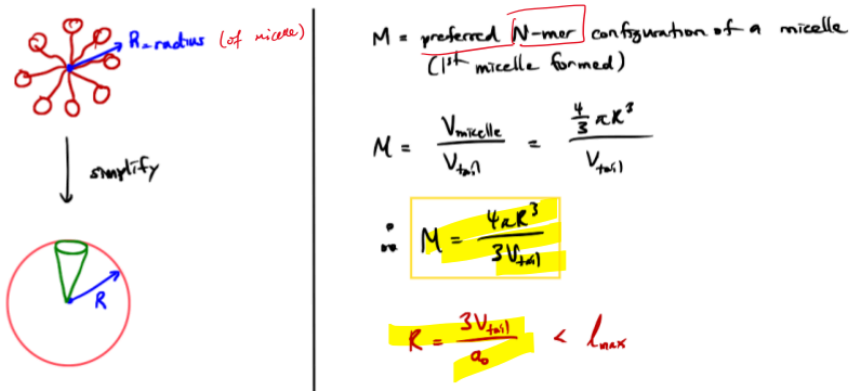


Modelling Lipid Aggregates

We use a convenient shape → a cone



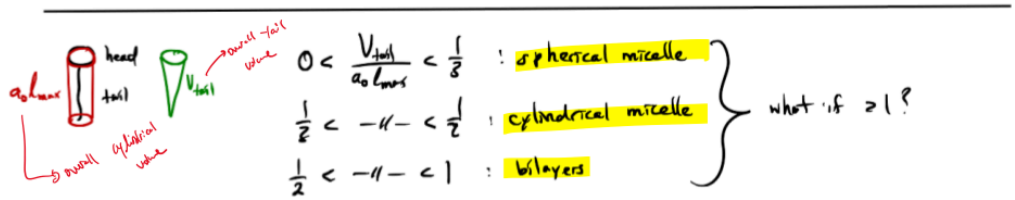
Spherical Micelles

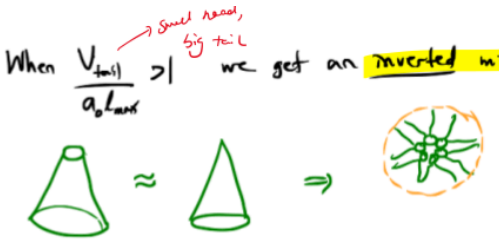


$$R = \frac{3V_{tail}}{a_o} < l_{max}$$

Different Structures

The type of structure formed will depend on the R-value





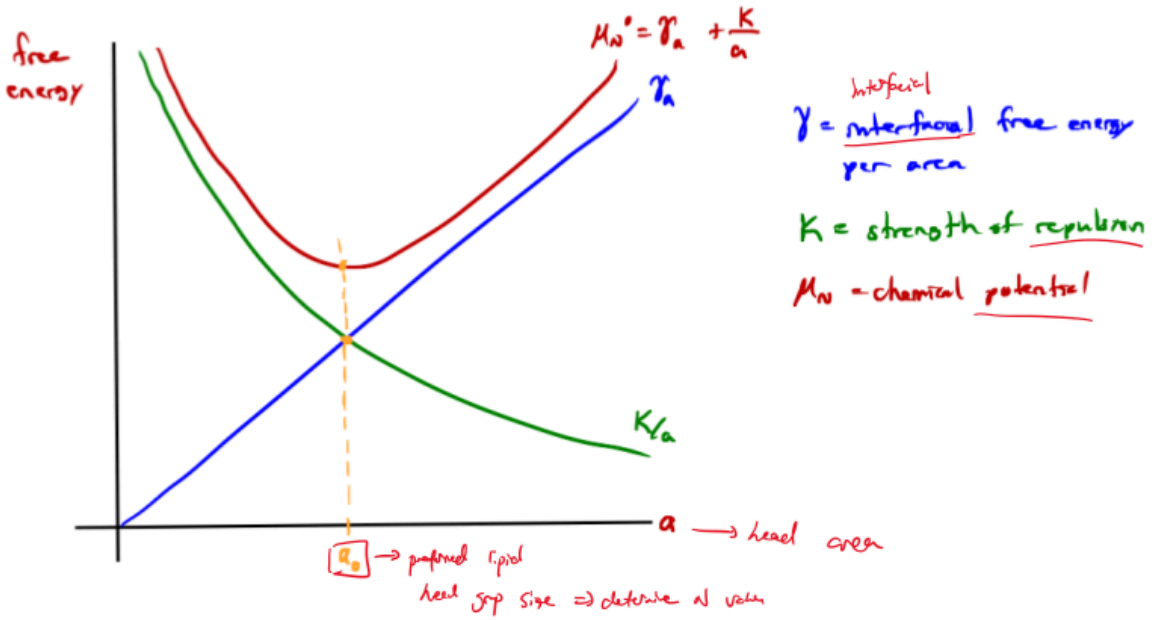
In a living cell, typical values:

$l_{max} \approx 0.126$ nm per C in tail

$V_{tail} = 26.9(n_c) \times 10^{-3}$ nm^3

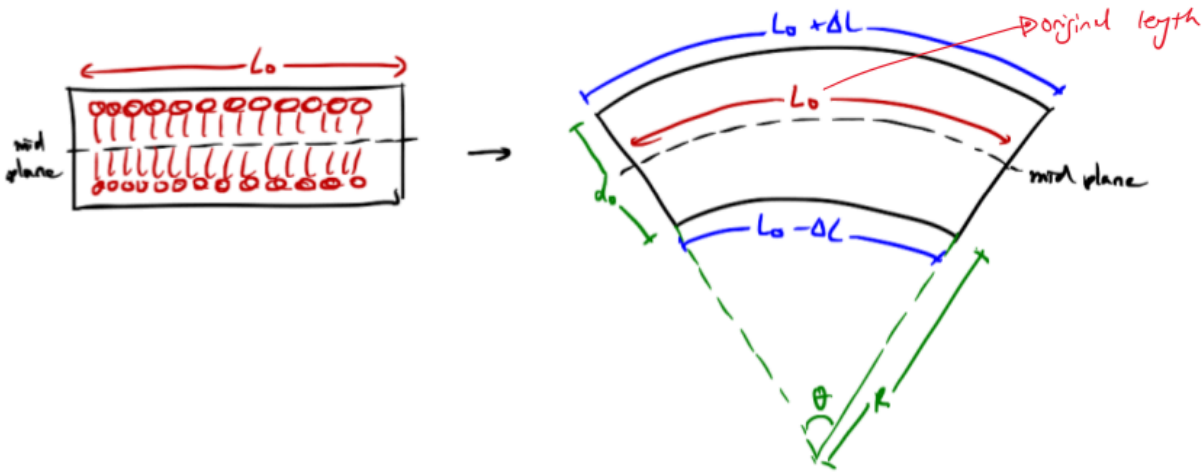
$\frac{V_{tail}}{a_o l_{max}} \approx$ 0.21 nm^2 for micelle; 0.42 nm^2 for bilayer

Interaction Between Lipids



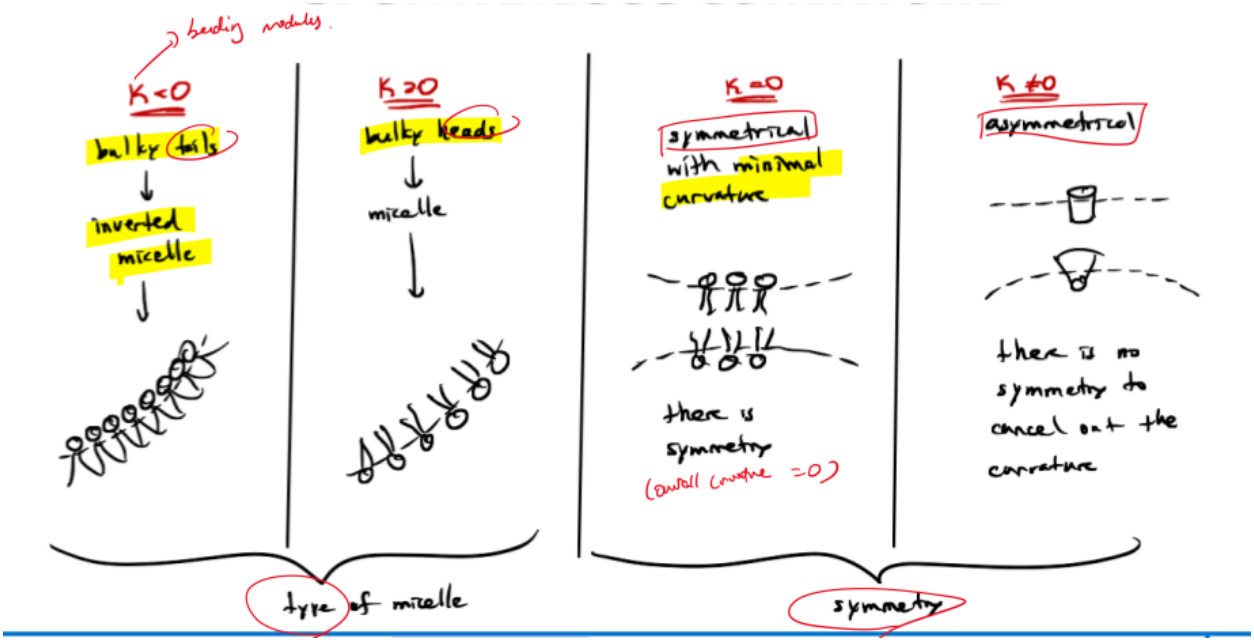
ao is the preferred lipid head group size → minimized free energy → determine N values

Bending of Bilayer / Elastic Sheet



Spontaneous Curvature for Micelle

Determines the type and symmetry of curvature based on Kappa (curvature)



Modelling Lipid Membranes

- Array of Springs
- Random Surface
- RC Circuit
- Semi-Permeable Barrier

Membrane Deformation

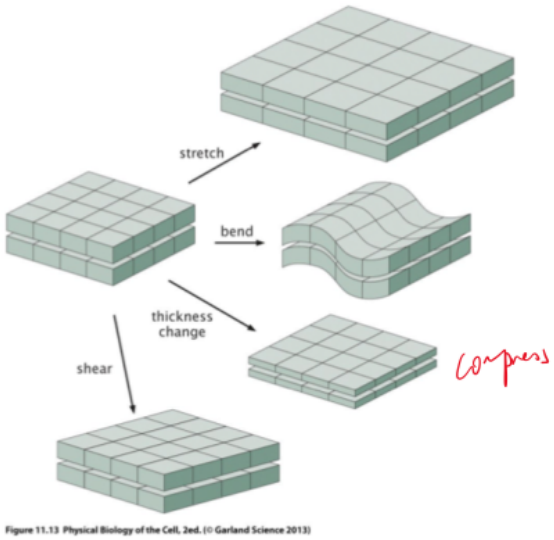


Figure 11.13 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Height Function for Membrane Geometry

A height function h(x,y) can describe membrane bending geometry

- Surface of membrane is characterized by height at each coordinate point
- Height function tells us how much the membrane is locally distributed from a flat reference point

By creating coordinate patches on the membrane surface, we can apply the height function on the membrane

- Work for any surface, including complex ones
- You can use a simplified lone coordinate patch if your surface can be represented by one function only
    - No overhangs
    - No multilayers

To calculate curvature, we make a best fit circle to the point where we are computing the curvature

- We need to tangent planes for the best fit circle → h(x) and h(y)

- We can evaluate the **CURVATURE (κ)** by:
    - Picking a point of interest and creating a tangent plane
    - Expand the height in powers of x and y
    - This creates a quadratic function:

$$h(x,y) = \sum_{i,j=1}^{2} \kappa_{ij} x_i y_i$$

- We can then define κ as: curvature matrix

$$\kappa = \begin{pmatrix} \kappa_{11} & \kappa_{12} \\ \kappa_{21} & \kappa_{22} \end{pmatrix} \quad \text{where} \quad \kappa_{ij} = \frac{\partial^2 h}{\partial x_i \partial y_j}$$

- The eigenvalues of the matrix are the two principal curvatures

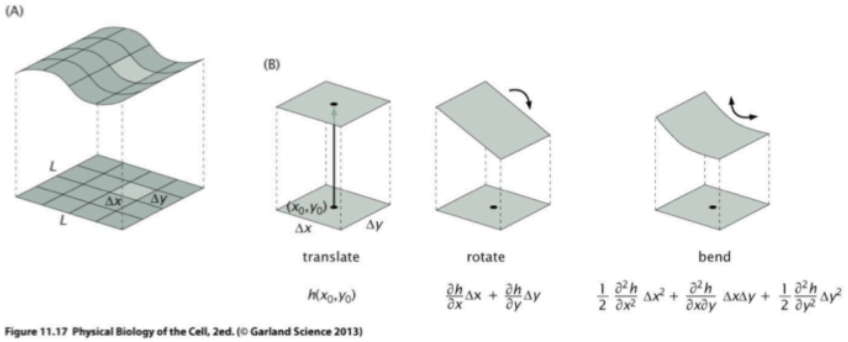


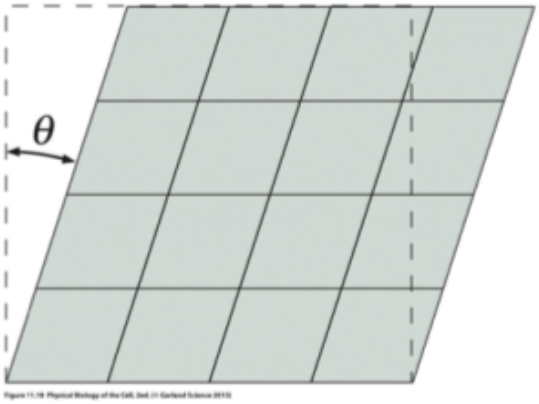
Figure 11.17 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Calculating Compression and Shearing

To determine compression, we use a thickness function w(x,y)

To determine shearing (how much sliding), we can only describe it by an angle variable theta

- Since lipid bilayers are fluid, they can really support a shear deformity
- But cell membranes are usually attached to an elastic network, which offers some rigidity
- We then need to determine the angle theta changed



Elastic Network of Lipids

Model as elstic springs

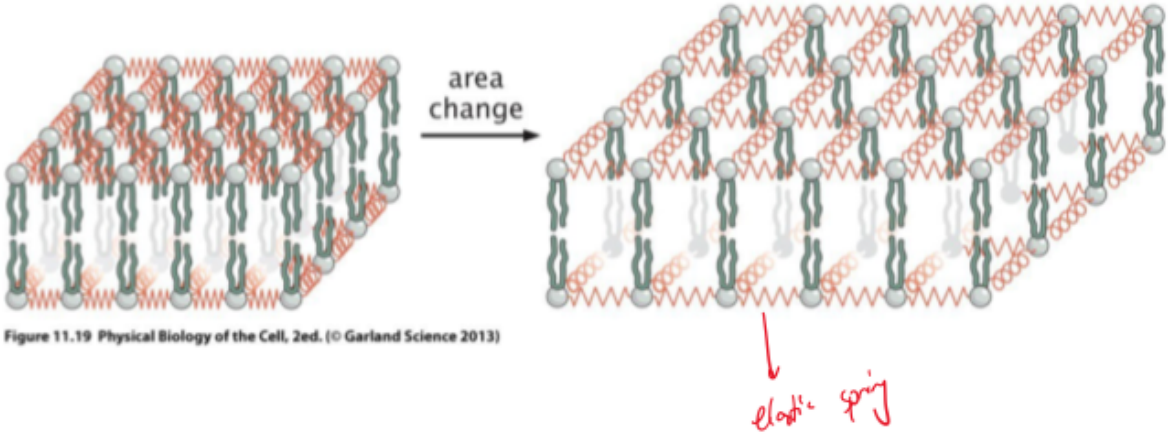


Figure 11.19 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Free Energy of Membrane Stretch

- We can call $G_{stretch}$ the **free energy of a stretched membrane**
  - Δa is the change in area
  - a_0 is the reference area
  - K_a is the **stretch modulus**

$$G_{stretch} = \frac{K_a}{2} \int \left(\frac{\Delta a}{a_0} \right)^2 da$$

- If we have uniform stretch, we can simplify the equation to:

$$G_{stretch} = \frac{K_a}{2} \frac{\Delta a^2}{a_0}$$

Modelling Bending

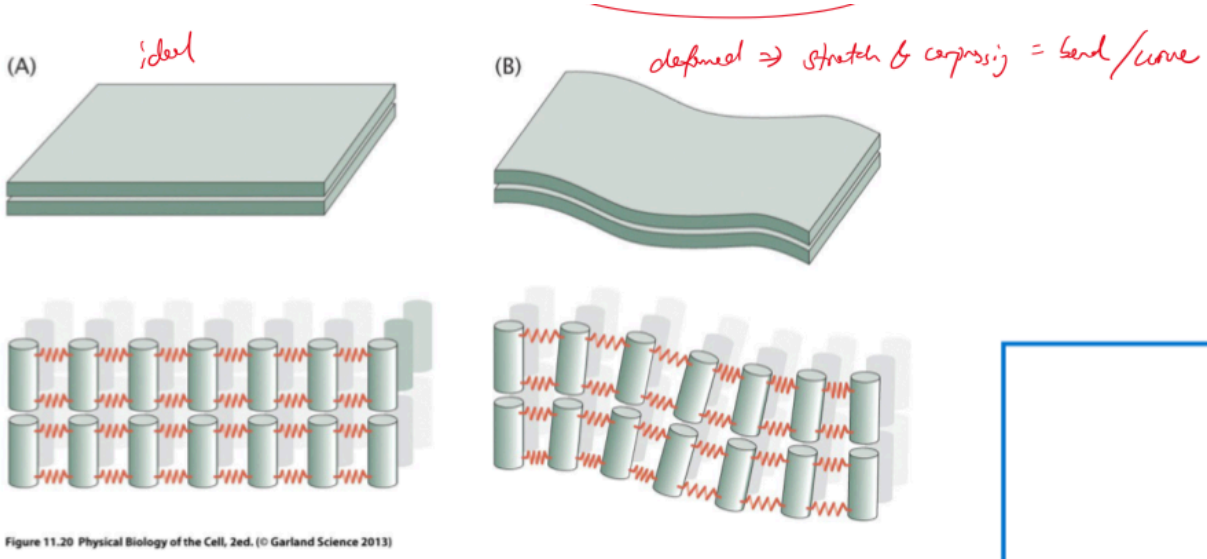
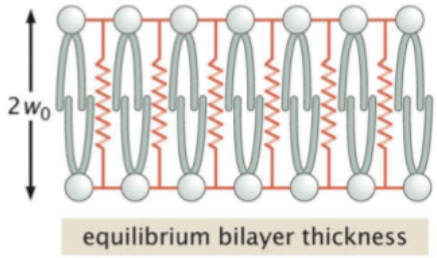


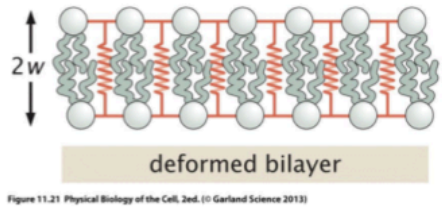
Figure 11.20 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Modelling Thickness



$$G_{bend}[h(x,y)] = \frac{K_b}{2}\int da\left(\kappa_1(x,y)+\kappa_2(x,y)\right)^2$$

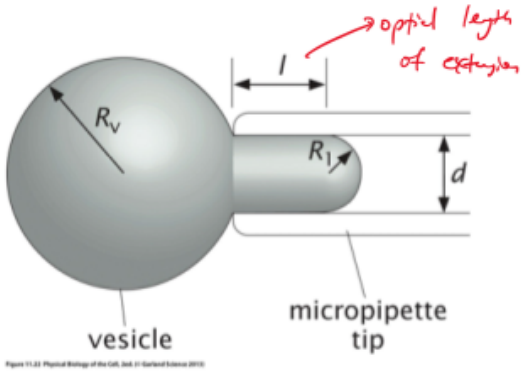
$$G_{thick}[w(x,y)] = \frac{K_t}{2}\int da\left(\frac{w(x,y)-w_0}{w_0}\right)^2$$



Membrane Stiffness

We can measure membrane stiffness via pipette aspiration

- Micropipette grabs lipid bilayer vesicle
- Sunction pressure pulls a portion of the membrane into tip



- Need the following parameters:
  - τ for tension, K_a for stretch modulus, $\frac{\Delta a}{a_0}$ for areal strain
  - R_1 for vesicle cap in micropipette; l for tether length
  - R_v for vesicle radius

change area / original area

$$\frac{\Delta a}{a_0} = \frac{R_1^2\left(1+\frac{l}{R_1}\right)}{2R_v^2}$$

Bending Free Energy, Stretching Free Energy, Free Energy of Pressure Difference, Work of Applied Load, Total Free energy

- The **bending free energy** must come from multiple components:
  - Spherical, cylindrical, hemispherical end cap

$$G_{bend} = 8\pi K_b + \frac{\pi K_b L}{r} + 4\pi K_b = 12\pi K_b + \frac{\pi K_b L}{r}$$

- The **stretching free energy** can be modelled with:

$$G_{stretch} = \frac{K_a}{2}\frac{(a-a_0)^2}{a_0}$$

- The **free energy of pressure difference** needs to be accounted for as well!

$$G_{pV} = -\Delta p\left(\frac{4}{3}\pi R^3 + r^2\pi L\right)$$

- The **work of applied load** is represented by:

$$G_{load} = -fL$$

- We can finally find G_{total}!

- We can calculate the **total free energy**:

$$G_{tot} = 12\pi K_b + \frac{\pi K_b L}{r} + \frac{K_a}{2}\frac{(a-a_0)^2}{a_0} - \Delta p\left(\frac{4}{3}\pi R^3 + \pi r^2 L\right) - fL$$

- To find f at equilibrium, we minimize G_{total} and eventually get:

$$f = 2\pi\sqrt{2K_b\tau}$$

⇒ how much force is required to pull out a bit membrane

- This relates the force applied by the load to the tension and bending modulus of the membrane

Can reflect on how tight the original membrane is