



# Notes on Molecular Biophysics

## Notes on Molecular Biophysics

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- Notes on Molecular Biophysics - *Review Outline on Modern Instrumental Analysis*
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- May 5, 2021
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ElegantL<sup>A</sup>T<sub>E</sub>X

- Describe protein backbone and side-chain conformations in terms of dihedral angles and interactions
- Understand the factors that determine protein conformations
- Know common nucleic acid conformations and to understand the interactions that stabilize such ordered conformations
- Know the composition of cell membrane and understand unique features of membrane proteins
- Understand the structures of micelles and bilayers
- Understand how small molecules are transported across membranes
- Understand basic concepts of spectroscopic techniques: circular dichroism, fluorescence and nuclear magnetic resonance
- Be knowledgeable to the application of these techniques to life sciences
- Appreciate the use of statistical thermodynamical model to explain behavior of biological systems, like helix-coil transition in protein, structural transition in DNA
- Understand the models, learn the equilibrium and kinetic experimental approaches to study "states" in the protein folding pathway, and determine the constants
- Understand the *in vivo* and *in vitro* methods to study protein-ligand interaction qualitatively and quantitatively, and determine the thermodynamical and kinetical parameters.

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## Introduction

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section

### Definition

*definition*



*principle*



*I*  
*theo*



Note

Example

**Part Part 1**

**Fundamental Biochemistry and Cell  
Biology**

# Chapter 1 Protein Structure

## Introduction



### 1.1 Introduction

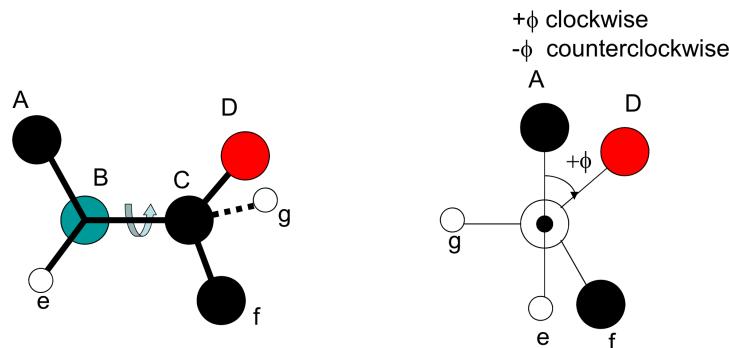
1. Only L-amino acids are found in natural proteins.  
Look along the H-C<sub>α</sub> bond, seeing CO-R-N in clockwise indicates L.
2. Nomenclature of main/side chain atoms:  $\alpha, \beta, \gamma \dots$

### 1.2 Polypeptide chain

#### 1.2.1 peptide bonds

- N is partially positive (resonance?) while O is partially negative.
- The 6 atoms are coplanar!

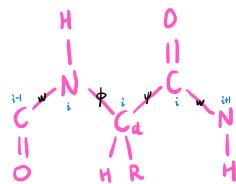
#### 1.2.2 torsion angle



- Look through B-C, if B-A (the nearer) needs to rotate clockwise to reach superposition with C-D, the angle is positive.
- But in which direction we look through doesn't matter because the result is the same.

#### 1.2.3 backbone dihedral angles

- $\phi$  : N-C<sub>α</sub>
- $\psi$  : C<sub>O</sub>-C<sub>α</sub>
- $\omega$  : C<sub>O</sub>-N

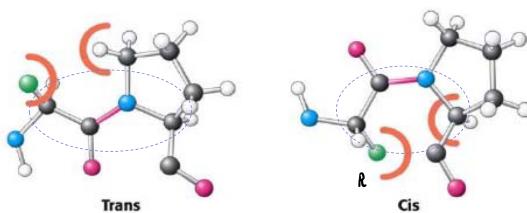


The protein structure is almost determined by these angles.

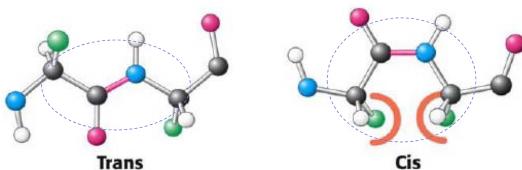
### ★ $\omega$ angle

It is usually fixed.

- For proline, groups in *trans* is less repulsive, but there's *cis*.



- For others, *trans* is almost always favored (8 kJ/mol).

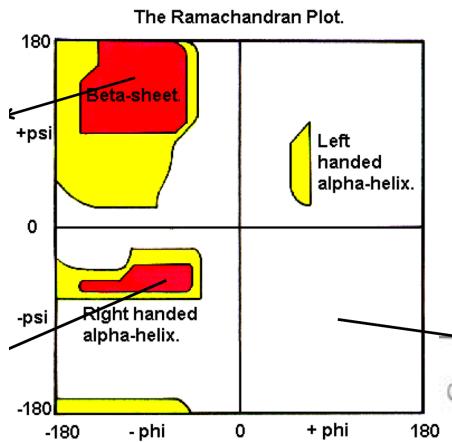


- However, some X-Pro structures are *cis*.

#### 1.2.3.1 $\phi$ and $\psi$ angles, the Ramachandran Map/Plot

Assumption: No two atoms in a molecule can come any closer than the sum of their **van der Waals radii**. Those who disobeys it are called "disallowed conformations".

- The plot is similar for most amino acids.
  - There are semi-allowed regions, where the distances are slightly less than the sum of vdw radii.
  - For a certain kind of protein, we can draw the plot according to statistical data. Those who are out of allowed region might be in linkers, turns or loops.
- two special AAs
  - The plot of Gly has a larger area and is centralsymmetric. Both are due to the R group is simply a small H atom.
  - $\phi$  of Pro is fixed to about  $60^\circ$  because of the fixed cyclopentane.  $\psi$  is most favored at about  $-60^\circ$  and  $160^\circ$



- Gly often interrupts  $\alpha$  and  $\beta$ . Pro cannot exist in anti-parallel  $\beta$  sheet. Instead, they are often located in turns and loops.

### 1.2.3.2 structure features of typical conformations

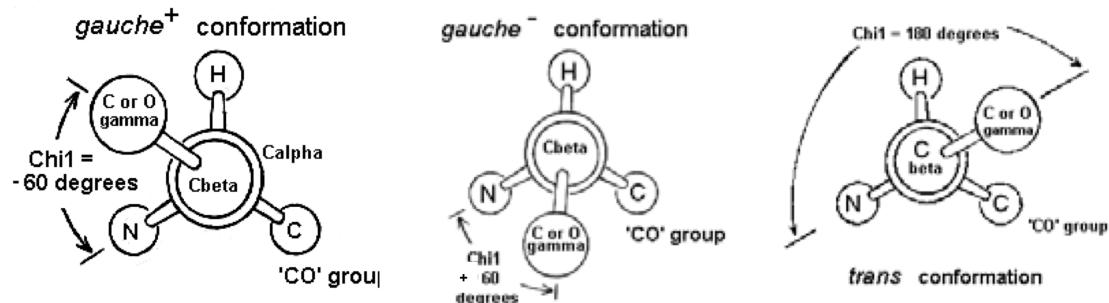
conformations	approximate $\phi$ and $\psi$	features
$\alpha$ helix	$-57^\circ, -47^\circ$	3.6 residues/turn, $100^\circ/\text{residue}$ CO of $i$ th and NH of $i+4$ th AA form H bond side chains protude radically outside  helical wheel projection: projection along the axis of $\alpha$ helix. 18 AAs, 5 cycles. Maybe nonpolar AAs are located on the same side and polar AAs on the other side
anti-parallel $\beta$ sheet	$-139^\circ, 135^\circ$	CO and NH on adjacent chains form H bond with each other to stabilize
$\beta$ turn	—	$i+1$ is usually Pro and $i+2$ usually Gly at the surface of protein (reverse the direction) nucleation center of folding

### 1.2.4 side chain dihedral angles

- $\chi_1 : N-C_\alpha-C_\beta-C_\gamma$ ,  $\chi_2 : C_\alpha-C_\beta-C_\gamma-C_\delta$ , then goes along the side chain.
- Possibilities of  $\chi_1$ . All are avoiding overlapping with the main chain. Because C=O is the bulkiest group, *gauche*<sup>+</sup> ( $\chi_1 = -60^\circ$ ) is the most favored. (trans is 2nd where  $\chi_1 = 180^\circ$ )
- $\chi$  tend to adopt staggered conformations.

- For aromatic residues,  $\chi_2$  tends to be  $\pm 90^\circ$  to minimize close contact with main chain.
- For residues who form H bond with the environment, the last  $\chi$  (Asp, Asn:  $\chi_2$ ; Glu:  $\chi_3$ ) adopts a wide range since that bond always rotates.

For those like Tyr who have two ways on the chain, both sides have the same  $\chi$  number. This is not so meaningful.



## 1.3 In addition

- tertiary structure: combination of secondary structures
- quaternary structure: specific assembly of folded subunits (3rd struct)
- examples
  - kertain: coiled coil ( $\alpha$ )
  - silkworm silk fibers:  $\beta$
  - collagen: left-handed triple helix (Pro)

# Chapter 2 Interactions in Proteins

## Introduction



### 2.1 Basic concepts

Internal energy involves:

- kinetic energy
- potential energy

Recall three thermodynamical laws.

#### To roughly remember

*Life is but a interplay of weak forces (non-bonding interactions)*

bond	energy (kJ/mol)
ionic ( $\text{COO}^-$ and $\text{NH}_3^+$ )	20~80
hydrogen	4~50, depending on distance and orientation
dipole (CO and CO)	5~10
vdw	0.5~3 per atom pair
hydrophobic	4~8 per non-polar groups

*Functions of non-bonding interactions:*

- additional strength to stabilize (high level structure)
- flexible, interact and perform functions



### 2.2 General electrostatic interactions

#### 2.2.1 ion-ion

For atoms carrying charge.

- equation

$$V = \frac{Q_i Q_j}{4\pi \epsilon_0 \epsilon_r r_{ij}}$$

•  $\epsilon_r$  : relative dielectric constant

- The force is weaker in water (larger  $\epsilon_r = 78.5$ ) than in hydrophobic core (though hard to measure)/other organic solvents.
- salt effect

$$I = \frac{1}{2} \sum c_i q_i^2$$

$$D = \sqrt{\frac{\epsilon_0 \epsilon_r k_B T}{2 N_A e^2 I}}$$

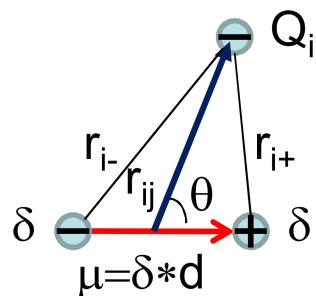
$$V = \frac{Q_i Q_j}{4\pi \epsilon_0 \epsilon_r} \frac{\exp(-r_{ij}/D)}{r_{ij}}$$

$I \uparrow, V \downarrow$ . "Neutralizing charges"

eg: N and C terminal, side chain (salt bridge)

### 2.2.2 ion-dipole

- for ion with groups carrying no formal charge
- dipole: **negative**  $\rightarrow$  **positive** point charge
- dipolar molecule: those with dipole moment ( $H_2O, CO$ )

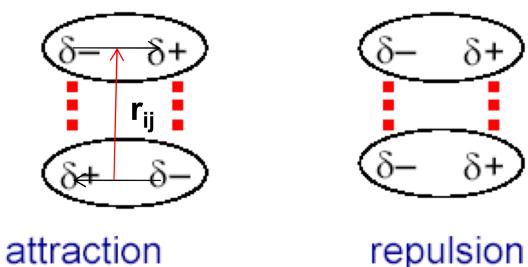


$$V = \frac{Q_i \delta}{4\pi \epsilon_0 \epsilon_r} \left( \frac{1}{r_{i-}} - \frac{1}{r_{i+}} \right) = \frac{Q_i \delta}{4\pi \epsilon_0 \epsilon_r} \cdot \frac{r_{i+} - r_{i-}}{r_i - r_{i+}} \approx \frac{Q_i \mu}{4\pi \epsilon_0 \epsilon_r r_{ij}^2}$$

eg: dissolve salt.  $\Delta H = E_{lattice} - E_{hydration}$ , hydration: ion with water.

entropy may increase.

### 2.2.3 dipole-dipole



$$V = \frac{1}{4\pi\epsilon_0\epsilon_r} \left[ \frac{\mu_i \cdot \mu_j}{r_{ij}^3} - \frac{3(\mu_i \cdot r_{ij}) \cdot (\mu_j \cdot r_{ij})}{r_{ij}^5} \right]$$

The potential depends on the relative orientation. The system tends to lower the energy.

eg:

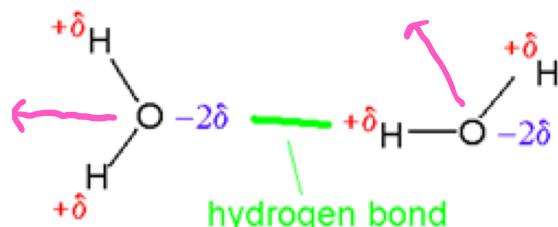
- d-d interaction:  $\text{H}_2\text{O} > \text{HCl}$
- interaction between  $\alpha$  helices (accumulation of peptide bond polarity), important for attracting charged molecules and enhances reactions.

## 2.3 van der Waals

here refers to dispersion forces

- features
  - temporary dipole, induced non-uniform  $e^-$  distribution
  - contact distance, L-J potential
- important for molecules both with and without permanent dipoles
- significant for large molecules

## 2.4 hydrogen bond



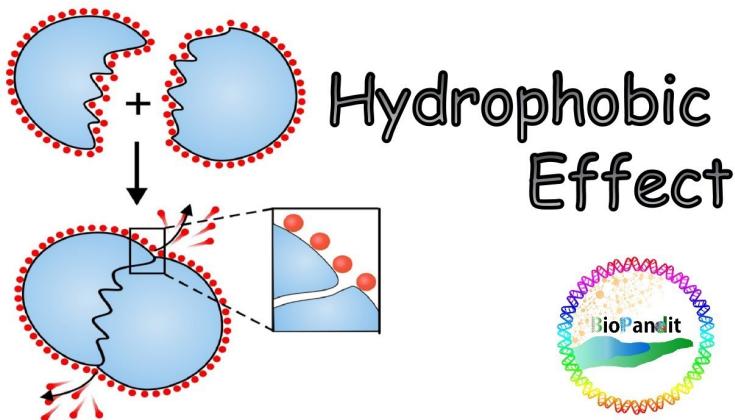
- features
  - short range:  $r_{bond} \ll r < R_{vdw}$
  - directionality:  $120\text{--}180^\circ$
- affects secondary structure & recognition, but not dominating/determining folding/assembly
- only intramolecular H bonds in the interior of a protein are favorable in presence of competition from water

actually vdW and H bond are both special cases of dipole-dipole interaction

## 2.5 hydrophobic interaction

Oil doesn't mix with water. The molecules attract themselves more than each other. Thus mixing water and oil is only a process where water molecules form a cage (more ordered, to strengthen interaction) rather than thorough mixing.

Their attraction increases ( $\Delta H < 0$ ) but the deterministic factor is  $\Delta S(H_2O) < 0$ . To minimize the entropy decrease, hydrophobic parts tend to gather to make their volume lowest and water molecules to form a cage least.



## 2.6 Disulfide bonds

Fold first, then disulfide bonds!

# Chapter 3 Week2-2 Nucleic Acid Structure

## Introduction

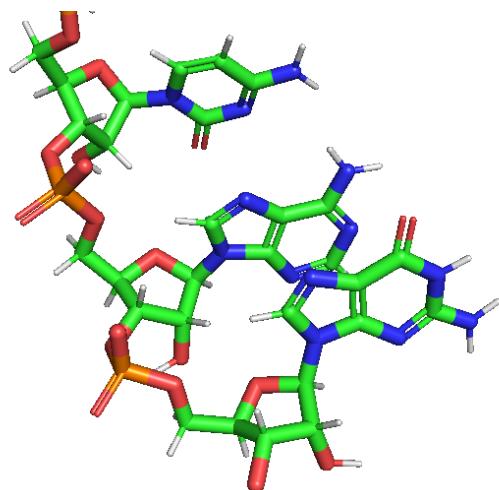


### 3.1 Components

- pentose (difference)
- base
- phosphate (phosphoester bond)

### 3.2 conformation

- bases point inwards
- plots of torsion angle
  - main chain: extended (trans, 180°)
  - $\chi$ : anti (0°)



### 3.3 Secondary structure

#### 3.3.0.1 double helix

helix	(B) DNA	( $\alpha$ ) protein
units per turn	10.5 (10)	3.6

helix	(B) DNA	( $\alpha$ ) protein
degree per unit	36°	100°
rise per unit/nm	0.34	
diameter/nm	22	

### 3.3.1 grooves

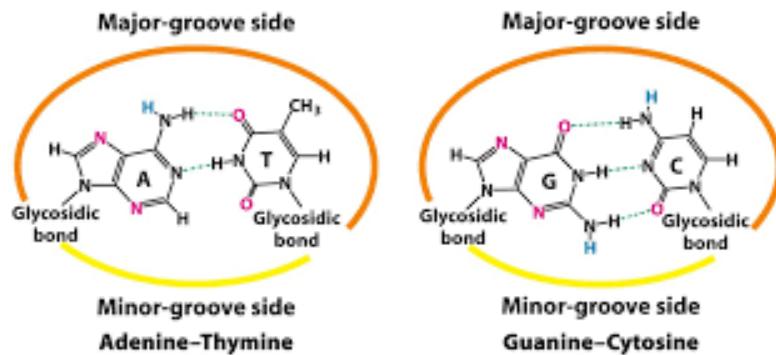


Figure 33.19  
Biochemistry: A Short Course, Second Edition  
© 2013 W. H. Freeman and Company

grooves	major	minor
part of base	more	less
actual H bonds	fewer	more
bind	proteins	drugs

Most proteins perform non-sequence-specific binding, switching on/off gene expression.

## 3.4 Forces to stabilize

### 3.4.1 hydrogen bond

Watson-Crick base pairs vs unusual pairs

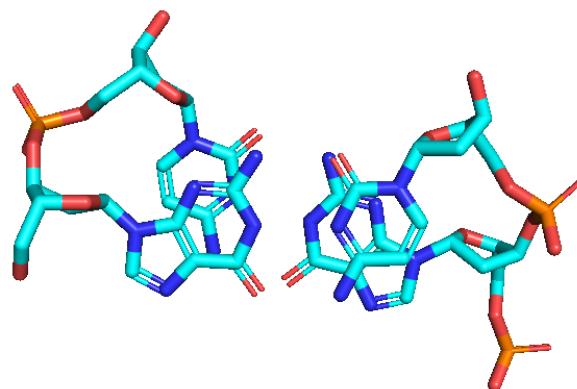
fraction of GC  $\rightarrow$  melting temperature. AT: local unwinding

### 3.4.2 stacking interaction

is a combination of

- vdW, where 0.34 nm is the optimal distance
- dipole-dipole, same direction  $\Leftrightarrow$  repel

bases on the next level rotate 36° as a balance



GC superimposition

### 3.4.3 electrostatic interaction

negative charged (-1 per bp) phosphate backbone: repulsion, controls distance between two strands

- low-salt: (phosphates) try to be trans; bases farther, prefer single strand (PCR)
- high-salt: shield them to form double strand DNA

DNA prefers to take helix to 1) form stacking 2) avoid repulsion

### 3.4.4 with water

Water forms H bond with bases, pentoses and phosphate groups. The major and minor grooves potentially form same amount of H bonds. But due to larger space, the major groove needs more water molecules. This is the cause of **entropy loss**.

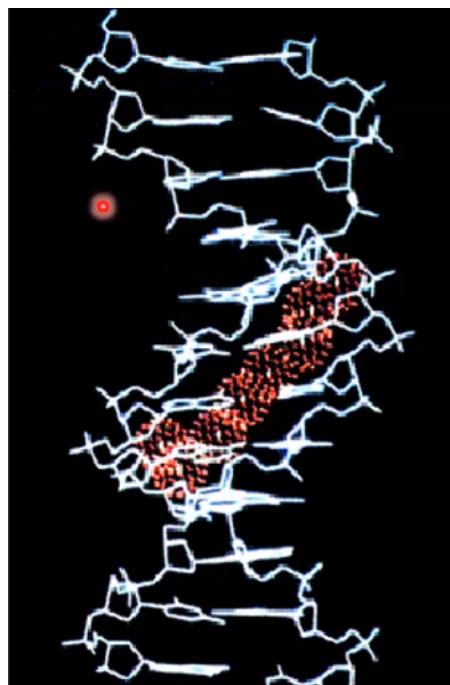
As a result, the minor groove forms **more H bonds** with water, i.e. more enthalpy gain. Thus, it can trap more water molecules (a cluster that may not form H bonds) and compensate the entropy loss. In contrast, the major groove only maintains one shallow layer of water.

## 3.5 other

- tightness: Z form (left-handed, seq-specific)>A form (seq-specific/RNA, high salt)>B form (normal)
- RNA structure

### 3.5.1 Week3-1 HW

1. interactions

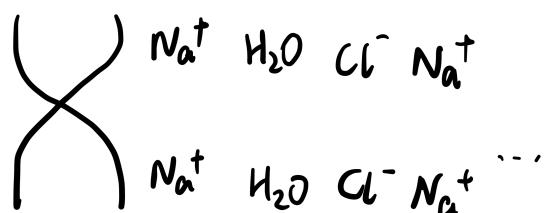


	protein	nucleic acid
interactions	covalent bond (disulfide) electrostatic (ion and dipole) vdW H bond hydrophobic	covalent bond H bond stacking (vdW, hydrophobic) electrostatic
secondary	<b>H bond</b>	<b>H bond</b> (stacking)
tertiary	H bond, <b>hydrophobic</b>	H bond, <b>stacking</b>

## 2. effect of heat and salt on DNA?

salt stabilizes the backbone, pushing the phosphate groups nearer and thus harder to be denatured by heat.

a multi-layer structure:



# Chapter 4 Structure of Cell Membrane

## Introduction



### functions of cell membranes

- compartmentalization
- selective permeable
- communication, signal transduction
- organize activities, energy production



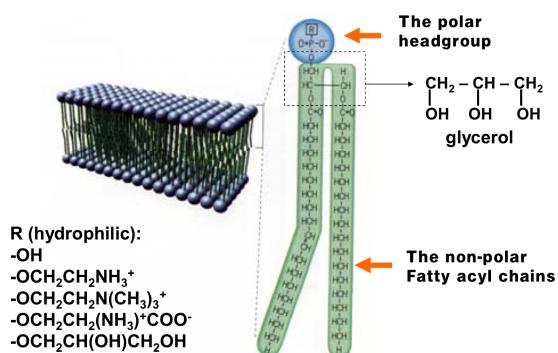
## 4.1 Lipids

### 4.1.1 types

- glycerophospholipids
- sphingolipids
- cholesterol

### 4.1.2 features

- amphiphilic
- different content in different species or organs



## 4.2 lipids on the membrane

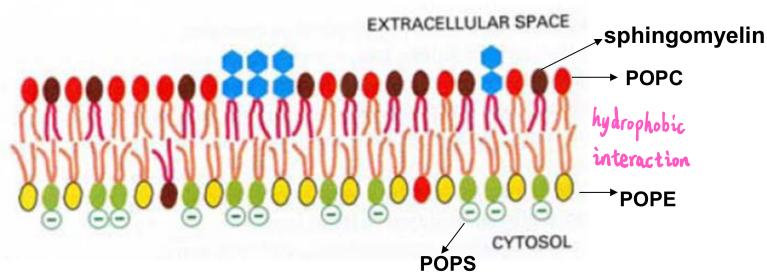
### 1. assymetric distribution (in two layers)

- negative charged phospholipids tend to point at cytosol while the neutral ones point at extracellular space

when cell undergoes apoptosis, POPS goes to the outer membrane because entropy increases.

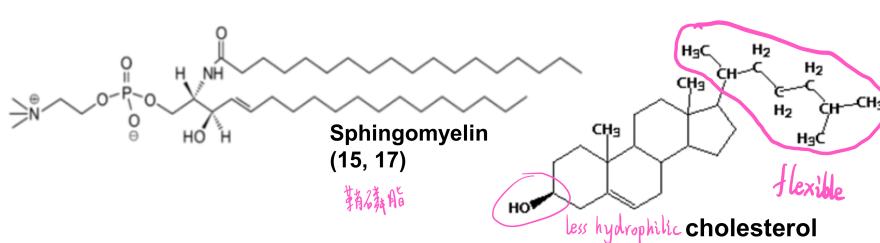
## 2. random distribution (in the same layer)

- lipids are randomly distributed, and swim.. (bulk phase)
  - vDW forces are not strong enough to hold lipids together
  - hydrophobic effect drives the lipid to assemble, so there must be water



## 4.3 lipid rafts

- sphingolipids have longer and straighter fatty acids thus have bigger vDW forces to hold themselves together (transiently)
  - glycerophospholipids: about 13?
- cholesterol can hold more lipid molecules
  - it's also very "hydrophobic"
  - it interacts with fatty acids in both sides of the plane, and has bigger surface area.
  - its ring is a rigid structure that makes lipid rafts stable.

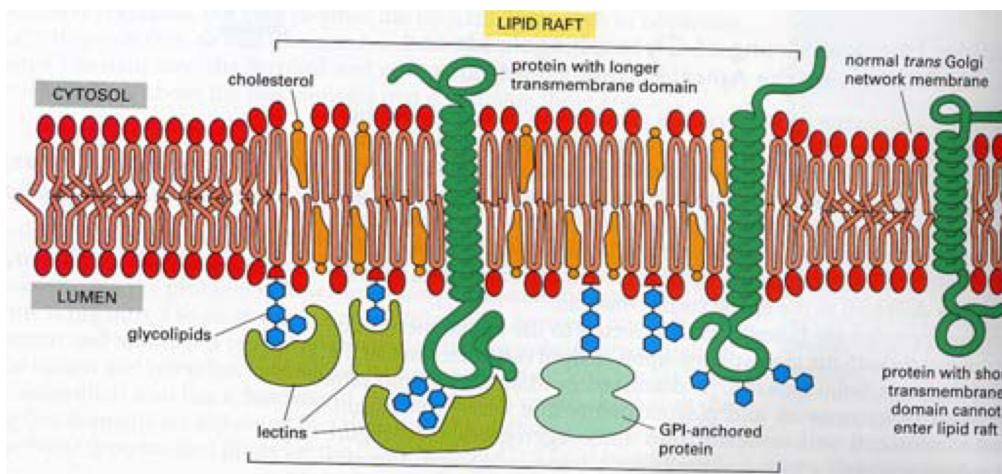


They assembly into lipid rafts, which

- are thicker (longer) than other parts of the membrane;
- are more resistant to detergents
- accommodate and gather proteins for specific functions like singaling

## 4.4 proteins

ratio ↑, function ↑



### References

*see figures*

*hydrophobicity plot tool*



Types of membrane proteins:

- integral
  - one or multiple **transmembrane** segments
  - $\alpha$  helix or  $\beta$  barrel
  - hydrophobic R chains point outwards (maybe hydrophilic inwards)
  - some are not trans-membrane
- peripheral
  - no covalent bonds, only **non-covalent** bonds
    - electrostatically: with polar head
    - terminal hydrophobic group: with bilayer core
    - or bound to an integral protein
  - either outside and inside the membrane
- anchored
  - **covalently** bond to the lipids
  - GPI-anchored proteins (G: glycosylphosphatidylinositol-linked)
    - protein-phosphoethanolamine---tetrasaccharide---inositol---
    - where lipase C functions
    - phosphate---diacylglycerol, which is a part of bilayer
  - function: enzyme, antigen, adhesion

## 4.5 micelles

[https://en.wikibooks.org/wiki/Structural\\_Biochemistry/Lipids/Micelles](https://en.wikibooks.org/wiki/Structural_Biochemistry/Lipids/Micelles)

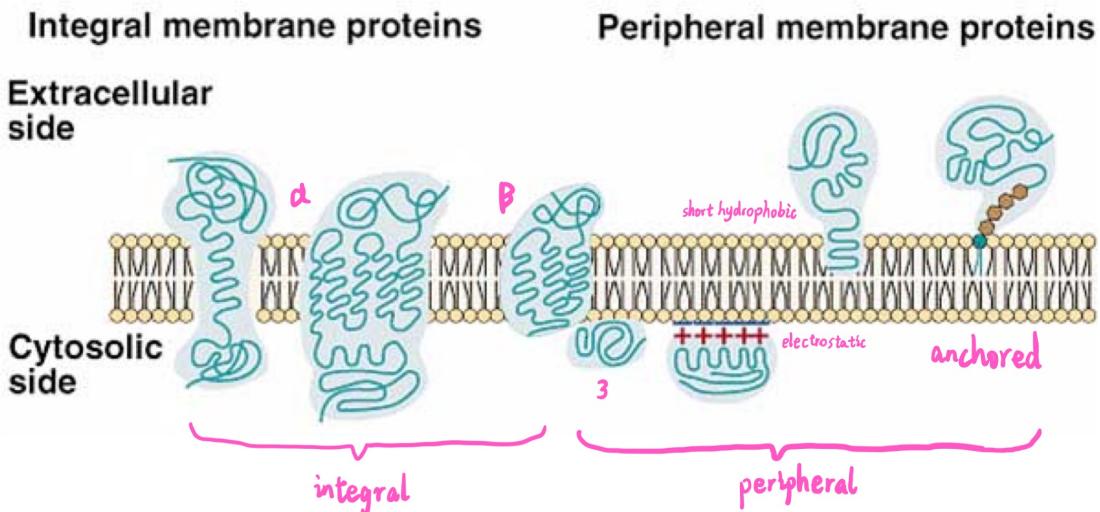


Figure 4.1

- as the concentration of detergent/lipid molecules rises (over Critical Micelle Concentration, CMC), they are no more a layer on the surface, but forms micelles () .
- structure
  - SDS can be seen as a cone () model which is due to electrostatic repulsion on the heads
    - driven by hydrophobic interaction, they form a sphere
    - when concentration get higher, the sphere get bigger and water enter inside, thus hydrophobic interaction is disrupted.
  - DPC is a zwitterion but has to be parallel (repulsion direction) to avoid contacting hydrophobic groups.
  - triglyceride may form a cylinder, which favours the formation of bilayer.

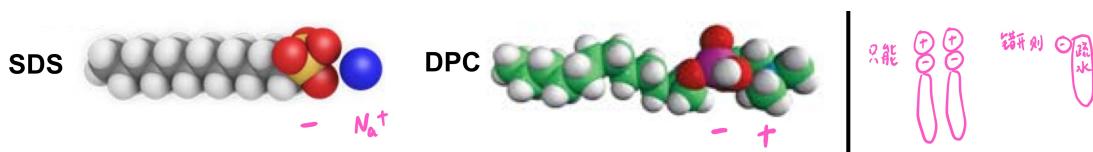


Figure 4.2

structure of molecules

structure of SDS micelles

## 4.6 lipid mobility

- the more lipids are ordered/the closer lipids are placed, fluidity  $\downarrow$ , melting point  $\uparrow$
- Bilayer has two phases. gel phase: solid; fluid phase: liquid.
- saturated FAs adopt all-trans to achieve the closest contact, but all bonds are freely rotatable, especially those which are near the center of bilayer.

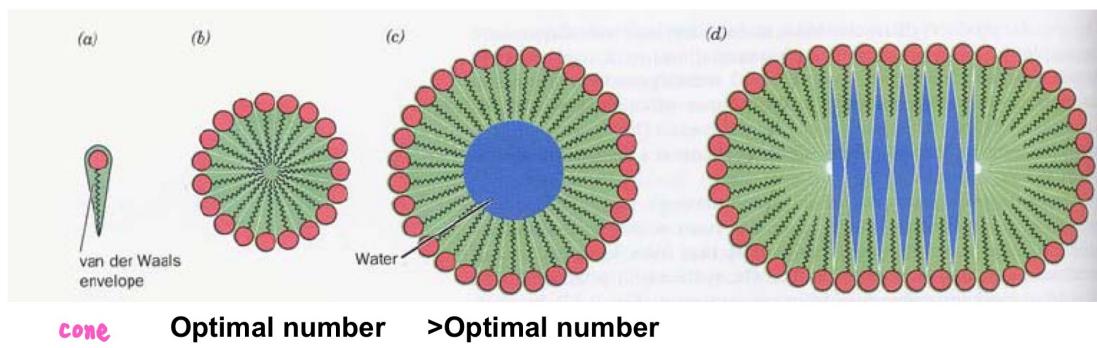


Figure 4.3

- gauche--trans--gauche makes a kink
- cis-double bond makes a bend which reduces the packing density



Figure 4.4

- cholesterol
  - block motions
  - disrupt ordered structure
which makes a balance, modulating the fluidity.

High conc cholesterol may abolish phase transition, keeping the membrane of eg. heart cells in cold blood animals functioning at a low tempearture.

- other motions
  - lateral (): much faster in fluid phase
  - flip-flop: very slow

1.3.U3 Cholesterol is a component of animal cell membranes.

## Cholesterol

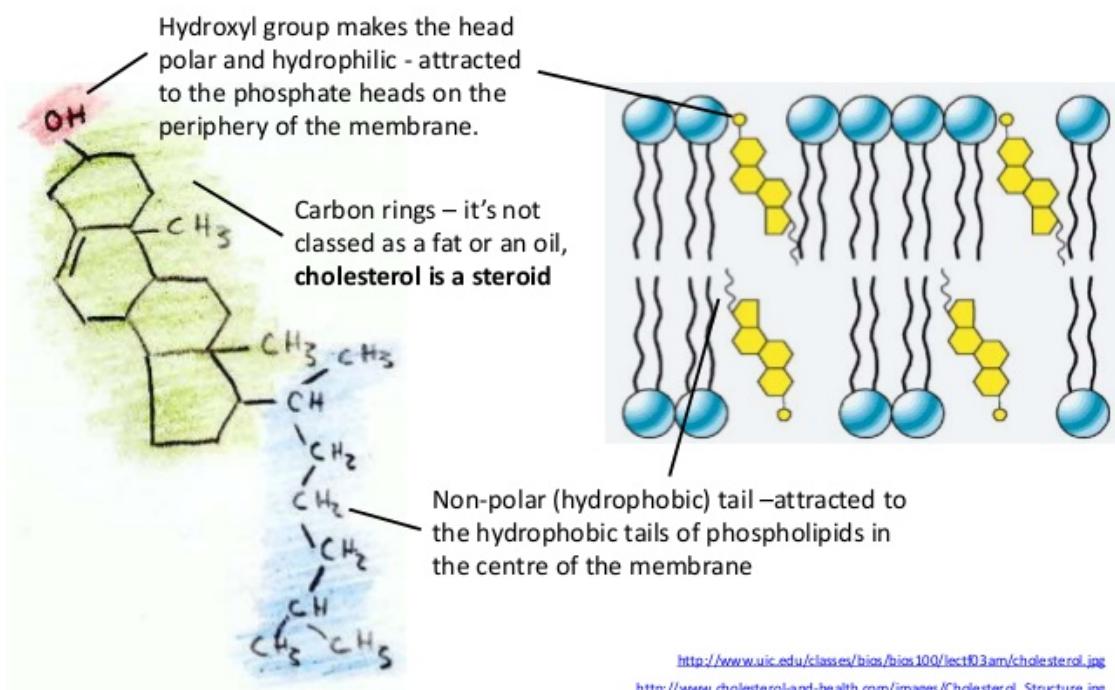


Figure 4.5

# Chapter 5 membrane-equilibrium

## 5.0.1 Week4-1 Membrane Equilibrium

### 5.0.1.1 terms

- extensive property: depend on the size or amount. follows additive rule.
- intensive property: e.g. density.
- for intensive properties, define partial molar quantity:  $\bar{Y}_i = \left( \frac{\partial Y}{\partial n_i} \right)_{T,P,n_j}$
- then we can apply the additive rule.
- chemical potential: PMQ of Gibbs free energy

#### Definition of chemical potential

$$\mu_i = \mu_i^0 + RT \ln a_i$$



### 5.0.1.2 chemical potential equilibrium

- for an open system (where  $n$  may vary but  $\mu$  doesn't vary under constant  $T, P$ )

$$dG = \sum_i \mu_i dn_i$$

- for a multiphase system in equilibrium, which is separated by semi-permeable membrane
  - for each component  $\mu_i$  should be the same and constant in all systems
  - only when this component is permeable. ( $dn_i \neq 0$ )
  - when not in equilibrium,  $\mu$  may change first. But we only calculate about the equilibrium state

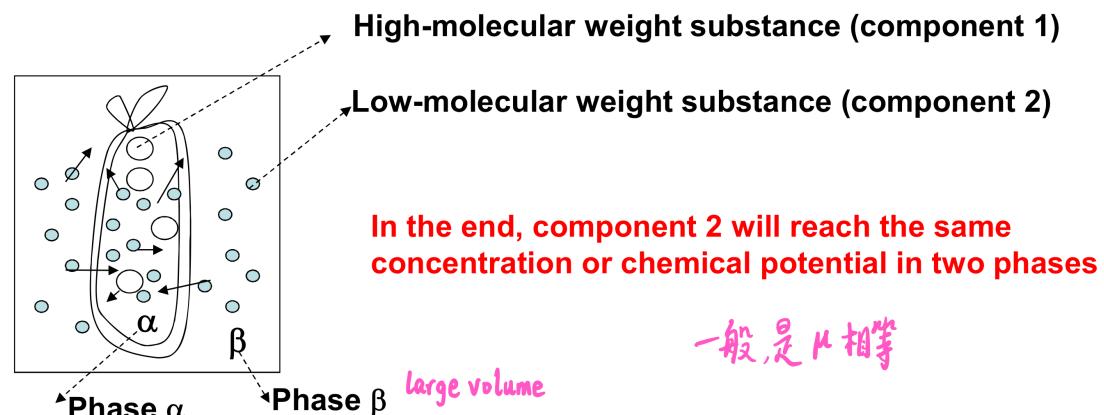


Figure 5.1

dialysis equilibrium

- when equilibrium is broken

If only one side ( $\alpha$ ) has solute A of molar concentration  $C$  that cannot pass through semi-permeable membrane. On the other side ( $\beta$ ) is pure water with the same  $T$  and  $P$  (pressure of atmosphere on the liquid).

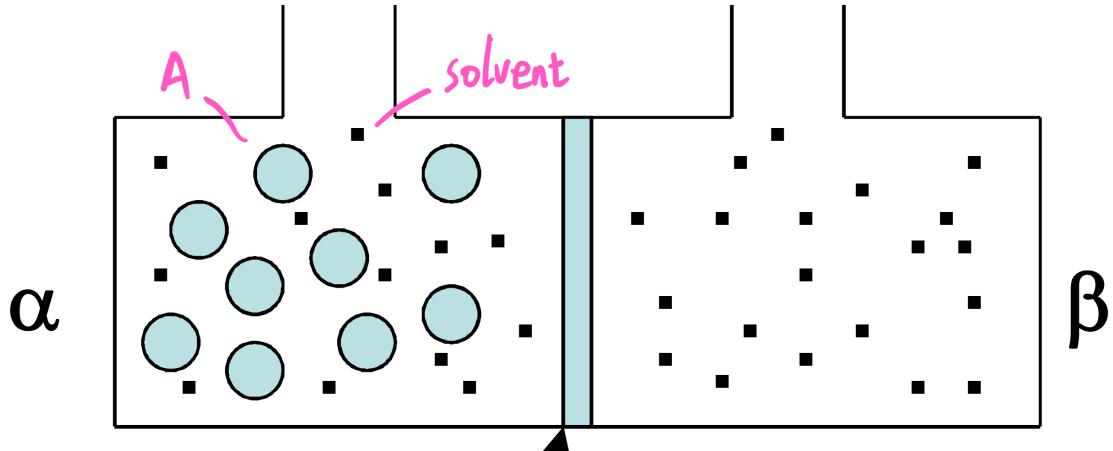


Figure 5.2: 4\_2

- equilibrium can never be reached. **Osmotic pressure** is generated.
- Imagine we change the pressure to achieve equilibrium, with approximation, we can get

$$\pi = P^\alpha - P^\beta = RTC$$

- the solute can be either macromolecules or small molecules

### 5.0.1.3 Donnan effect

the ion may start transferring, but have to keep electrical neutrality.

Donnan effect: when one side contains impermeable charged molecule, concentrations of ions A,B in different side are different.

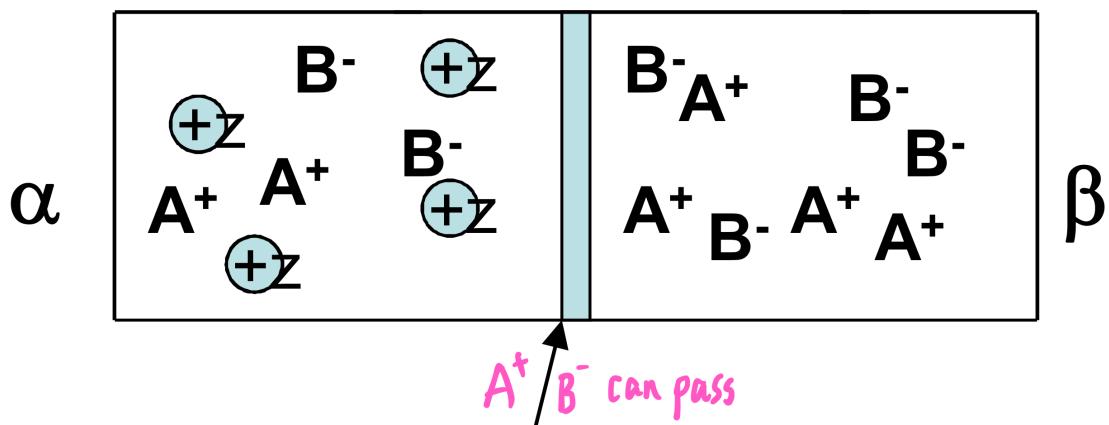


Figure 5.3: 4\_3

Write the chemical potential

$$\mu_1 = \mu_A^0 + \mu_B^0 + RT \ln C_A^\alpha + RT \ln C_B^\alpha$$

$$\mu_2 = \mu_A^0 + \mu_B^0 + RT \ln C_A^\beta + RT \ln C_B^\beta$$

At equilibrium,  $\mu_1 = \mu_2$

$$C_A^\alpha \cdot C_B^\alpha = C_A^\beta \cdot C_B^\beta$$

Note  $z$  is the charge and  $C_M$  is concentration of the macromolecule. According to electrical neutrality, let

$$C_A^\alpha + zC_M = C_B^\alpha = aC_A^\beta = C_B^\beta = br = \frac{zC_M}{2C_A^\beta}$$

then solve it

$$r_D = \frac{C_A^\beta}{C_A^\alpha} = \frac{C_B^\alpha}{C_B^\beta} = r + (r^2 + 1)^{0.5}$$

If A is  $H^+$ , then  $pH^\alpha - pH^\beta = \log r_D$

#### 5.0.1.4 combined effect: membrane potential

Consider this situation.  $Na^+$  won't pass through because of the electric potential. Chemical potential equilibrium can never be reached, as in the example about osmotic pressure. This system looks like the cell where no ions can freely pass through.

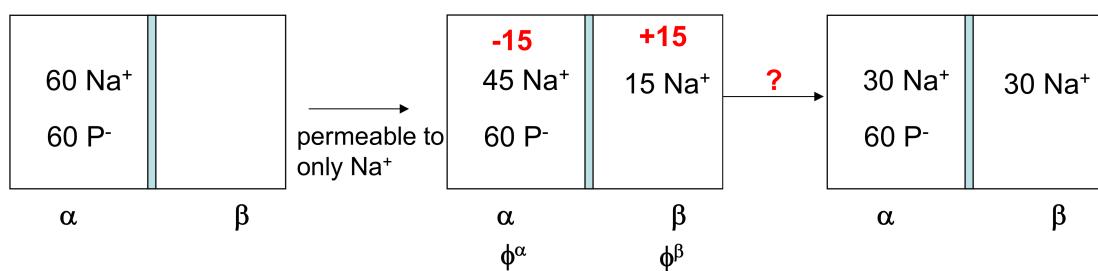


Figure 5.4

Part two of this section refers to the effect of different concentration, while part three refers to the effect of charged molecules.

Define electrochemical potential as their combination

$$\mu' = \mu + Zf\phi$$

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where  $Z$  is charge,  $f$  is Faraday constant,  $\phi$  is the relative electric potential.

At equilibrium, for single ion A on one side:

$$\Delta\varphi = \varphi^{in} - \varphi^{out} = -\frac{RT}{Zf} \ln \frac{c^{in}}{c^{out}}$$

Ionic concentration difference and membrane potential are generated simultaneously. Maintaining a concentration difference, we get membrane potential. Still, those which cannot pass through the membrane don't contribute.

For multiple ions:

$$\Delta\varphi = \varphi^{in} - \varphi^{out} = -\frac{RT}{Zf} \ln \frac{\sum_{positive} p_i c_i^{in} + \sum_{negative} p_j c_j^{out}}{\sum_{positive} p_i c_i^{out} + \sum_{negative} p_j c_j^{in}}$$

where  $p_i$  is permeability. The cell mainly regulates permeability and sometimes concentration.

summary:

By putting charged macromolecules and selectively letting ions pass through, the cell sets up the difference of concentration of ions as well as the electrical potential, acting as the foundation of activities like neural signaling.

### 5.0.1.5 in addition

inference of osmotic pressure

$$\mu_1^\alpha = \mu_1^0(T, P^\alpha) + RT \ln c_1^\alpha y_1^\alpha \mu_2^\beta = \mu_2^0(T, P^\beta) + RT \ln c_2^\beta y_2^\beta$$

where  $c$  is the molar concentration of water. Here it's approximately the molar ratio (?)

Due to  $G = U + PV - TS$ ,  $\mu = V_0 P$ ,  $V_0$  is molar volume of water.

When equilibrium,  $\mu_1^\alpha = \mu_2^\beta$ . Assume  $y = 1$  So

$$\mu_1^0(T, P^\alpha) - \mu_2^0(T, P^\beta) = V_0(P^\alpha - P^\beta) = -RT \ln \frac{c_1^\alpha}{c_2^\beta}$$

# Chapter 6

## Introduction

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## **Part Part 2**

# **Spectroscopic Tools in Structural Study**

## Chapter 7 Circular Dichroism

### Introduction

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# Chapter 8

## Introduction

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# Chapter 9

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## **Part Part 3**

# **Structural Transition and Interactions: Models and Experimental Tools**

# Chapter 10 Circular Dichroism

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# Chapter 11

## Introduction

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# Chapter 12

## Introduction

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