

## Biochemistry II (BB 422/622)

### OUTLINE

Review of 421

Goals of 422

Review of chemical principles

Thermo

C/O cycles

Overview of Metabolism

ATP cycles

Energy Coupling

**Chemical Reactivity**

**Bioenergetics**

Membrane Transport

**membrane structure review**

**membrane proteins review**

membrane transport

Material covered  
during Snow-Day  
lecture

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# Chemical Reactivity

First lets quickly review **bond cleavage** and kinds of **nucleophiles**  
and **electrophiles** commonly used by the cell in biochemical  
reactions

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## Chemical Reactivity

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Most biochemical reactions fall within only a few categories:

- Cleavage and formation of **C–C bonds**
- Cleavage and formation of polar covalent bonds  
(**Addition-Elimination** Reactions)
  - nucleophilic substitution mechanism
  - addition–elimination mechanism
    - hydrolysis and condensation reactions
    - Eliminations (without cleavage)
- Internal **rearrangements**
- Group transfers** ( $H^+$ ,  $CH_3^+$ ,  $PO_3^{2-}$ ,  $NH_3^+$ )
- Oxidations-reductions** ( $e^-$  transfers)

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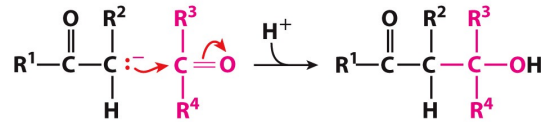
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## Chemical Reactivity

C-C bonds

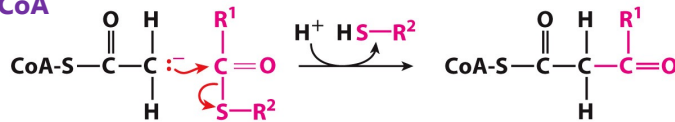
### Examples of Nucleophilic Carbon-Carbon Bond Formation Reactions

#### Ketose sugar

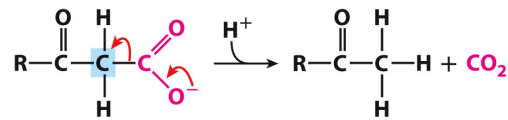


#### Aldol condensation

#### Acetyl-CoA



#### Claisen ester condensation



#### Decarboxylation of a $\beta$ -keto acid

Notice that these are all reactions beta to a carbonyl

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## Chemical Reactivity

### Nucleophilic Substitution & Addition-Elimination

- Substitution from  $sp^3$  carbon proceeds normally via the nucleophilic substitution ( $S_N1$  or  $S_N2$ ) mechanism.



- Substitution from the  $sp^2$  carbon proceeds normally via the nucleophilic addition–elimination mechanism.
  - nucleophile adds to the  $sp^2$  center, giving a **tetrahedral intermediate**
  - leaving group eliminates from the tetrahedral intermediate
  - leaving group may pick up a proton

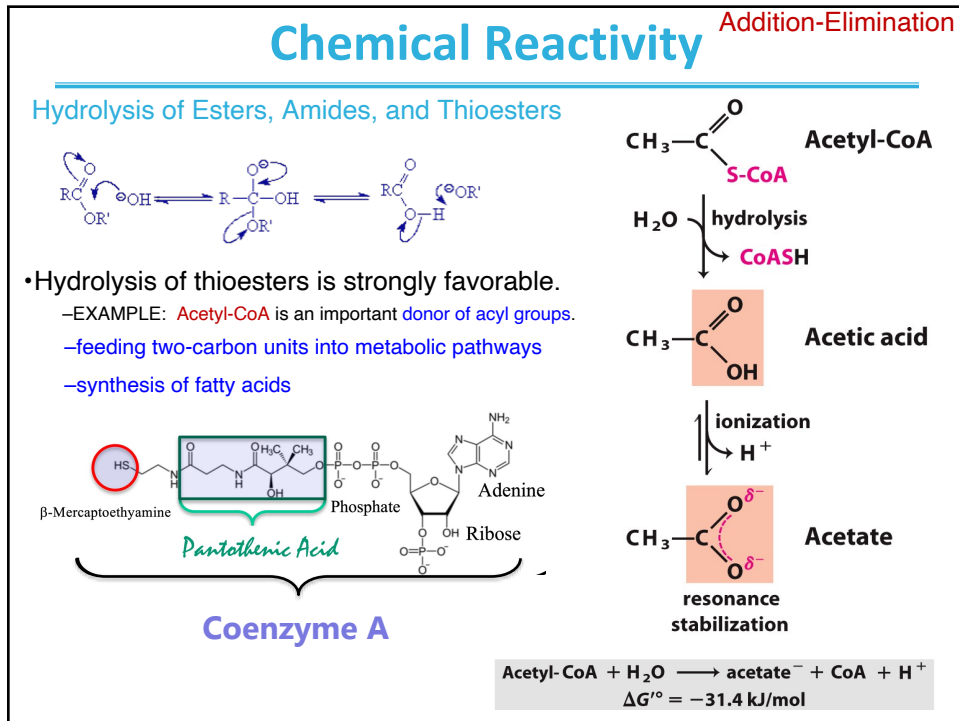
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## Chemical Reactivity

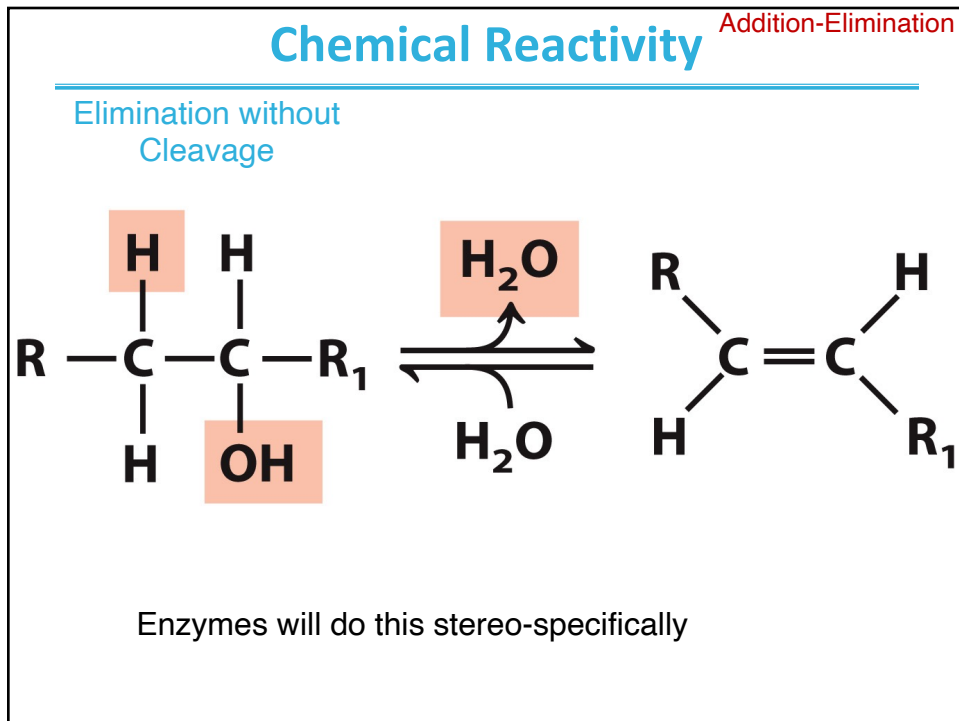
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## Chemical Reactivity

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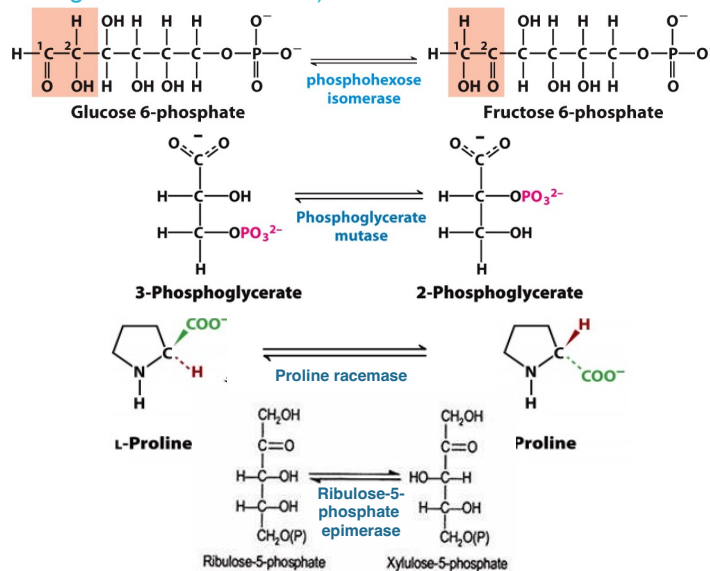
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## Chemical Reactivity

Rearrangements

Isomerizations:  
(No Change in Oxidation State)



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## Chemical Reactivity

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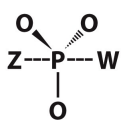
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## Chemical Reactivity

Group Transfers

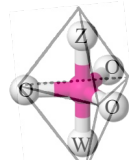
- Proton transfer, very common
- Methyl transfer, various biosyntheses
- Acyl transfer, biosynthesis of fatty acids
- Glycosyl transfer, attachment of sugars
- Phosphoryl transfer, to activate metabolites
  - also important in signal transduction
  - proceeds through a nucleophilic substitution mechanism



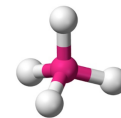
### Example of ATP hydrolysis

$Z = R-OH$  (nucleophile)

$W = ADP$  (leaving group)



Sometimes called  
“Bi-pyramidal”



Substitution from  $sp^3$  phosphorous proceeds via the nucleophilic substitution (usually associative,  $S_N2$ -like) mechanism.

The nucleophile forms a partial bond to the phosphorous center, giving a **pentavalent intermediate** or a penta-coordinated transition state.

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## Chemical Reactivity Re-dox

**Oxidation-Reduction Reactions**

Reduced organic compounds serve as **fuels** from which electrons can be stripped off during oxidation.

Chemical Structure	Compound Name	Relative $\Delta G^{\circ}$ of oxidation per carbon (kcal/mol)
$-\text{CH}_2 - \text{CH}_3$	Alkane	-196
$-\text{CH}_2 - \text{CH}_2\text{OH}$	Alcohol	-168
$-\text{CH}_2 - \text{C} \begin{matrix} \text{O} \\ // \\ \text{H(R)} \end{matrix}$	Aldehyde (ketone)	-125
$-\text{CH}_2 - \text{C} \begin{matrix} \text{O} \\ // \\ \text{OH} \end{matrix}$	Carboxylic acid	-68
$\text{O}=\text{C}=\text{O}$	Carbon dioxide	0

**Most energy** ↓ **Least energy**

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## Chemical Reactivity Re-dox

**EXAMPLE: Reversible Oxidation of a Secondary Alcohol to a Ketone**

- Many biochemical oxidation-reduction reactions involve transfer of **two electrons**.
- To keep charges in balance, proton transfer often accompanies electron transfer.
- In many **dehydrogenases**, the reaction proceeds by a stepwise transfers of **proton** ( $\text{H}^+$ ) and **hydride** ( $:\text{H}^-$ ).

$\text{CH}_3 - \text{CH}(\text{OH}) - \text{C}(=\text{O})\text{O}^- + \text{NAD}^+ \rightleftharpoons \text{CH}_3 - \text{C}(=\text{O}) - \text{C}(=\text{O})\text{O}^- + \text{NADH} + \text{H}^+$

**Lactate** **lactate dehydrogenase** **Pyruvate**

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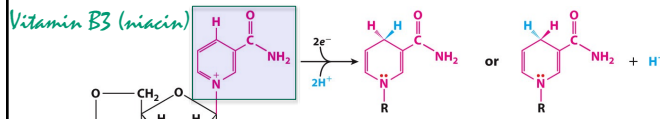
# Chemical Reactivity

Re-dox

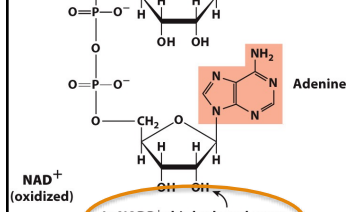
## Common Redox Cofactors: NADH, NADPH, and FADH<sub>2</sub>

- Niacin Cofactors allow Hydride (2 e<sup>-</sup>) Electron Transfers
- Flavin Cofactors allow single Electron Transfers

Vitamin B3 (niacin)

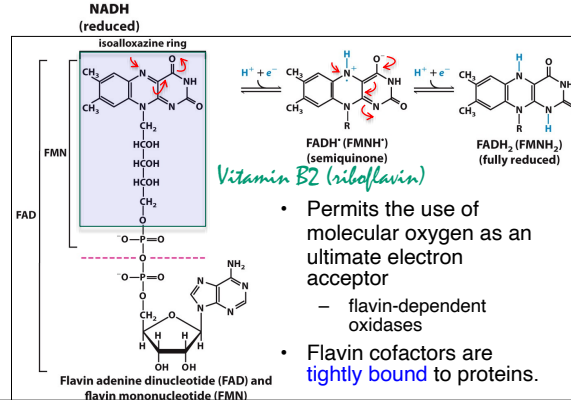


- Measure the change of absorbance at 340 nm
- Very useful signal when studying the kinetics of NAD-dependent dehydrogenases



In NADP<sup>+</sup> this hydroxyl group is esterified with phosphate.

- These are commonly called pyridine nucleotides.
- They can dissociate from the enzyme after the reaction.
- In a typical biological oxidation reaction, hydride from an alcohol is transferred to NAD<sup>+</sup>, giving NADH.



- Permits the use of molecular oxygen as an ultimate electron acceptor
  - flavin-dependent oxidases
- Flavin cofactors are tightly bound to proteins.

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# Bioenergetics

## Energetics of Biochemical Reactions:

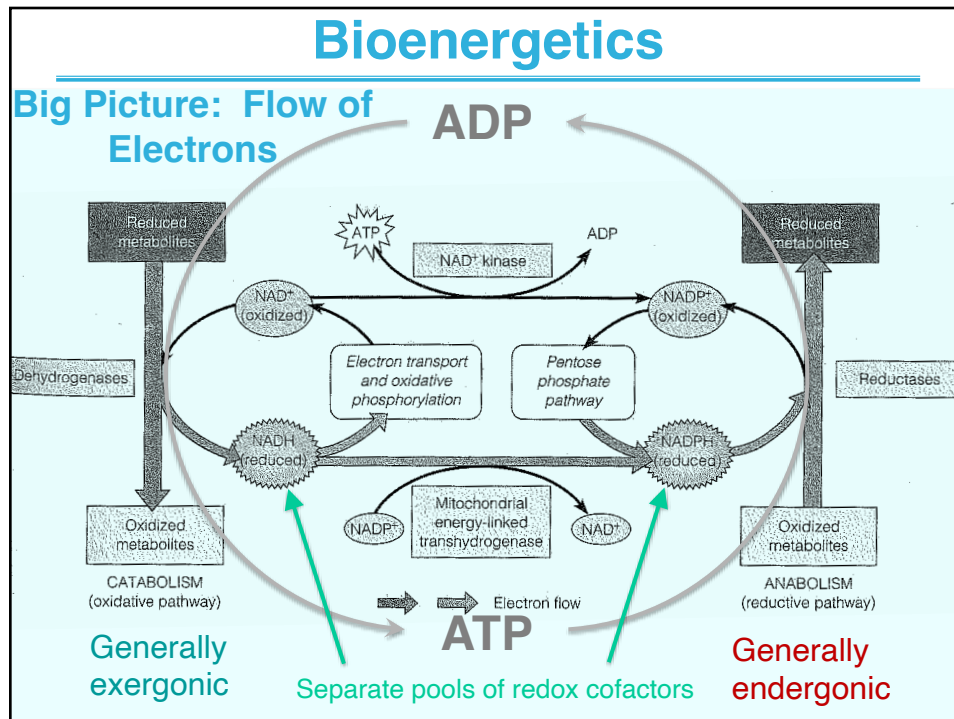
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# Bioenergetics

## Energetics of Some Chemical Reactions:

- **Hydrolysis reactions** tend to be strongly favorable (~~spontaneous~~).
  - Recall that being thermodynamically **favorable** is not the same as being kinetically rapid.
- **Isomerization reactions** have smaller free-energy changes.
  - isomerization between enantiomers:  $\Delta G^{\circ} = 0$
- Complete oxidation of reduced compounds (redox reactions) is strongly favorable.
  - This is how heterotrophs (sometimes called chemotrophs) obtain most of their energy.
  - In biochemistry, the oxidation of reduced fuels with  $O_2$  is **stepwise and controlled**.

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# Bioenergetics

## Reduction Potentials with higher affinity at bottom

Standard reduction potentials of some reactions

Oxidant	Reductant	n	$E^{\circ}$ (V)
Succinate + CO <sub>2</sub>	$\alpha$ -Ketoglutarate	2	-0.67
Acetate	Acetaldehyde	2	-0.60
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	-0.43
2 H <sup>+</sup>	H <sub>2</sub>	2	-0.42
NAD <sup>+</sup>	NADH + H <sup>+</sup>	2	-0.32
NADP <sup>+</sup>	NADPH + H <sup>+</sup>	2	-0.32
Lipoate (oxidized)	Lipoate (reduced)	2	-0.29
Glutathione (oxidized)	Glutathione (reduced)	2	-0.23
Acetaldehyde	Ethanol	2	-0.20
Pyruvate	Lactate	2	-0.19
Fumarate	Succinate	2	0.03
Cytochrome <i>b</i> (+3)	Cytochrome <i>b</i> (+2)	1	0.07
Dehydroascorbate	Ascorbate	2	0.08
Ubiquinone (oxidized)	Ubiquinone (reduced)	2	0.10
Cytochrome <i>c</i> (+3)	Cytochrome <i>c</i> (+2)	1	0.22
Fe (+3)	Fe (+2)	1	0.77
$\frac{1}{2}$ O <sub>2</sub> + 2 H <sup>+</sup>	H <sub>2</sub> O	2	0.82

Hates e<sup>-</sup>

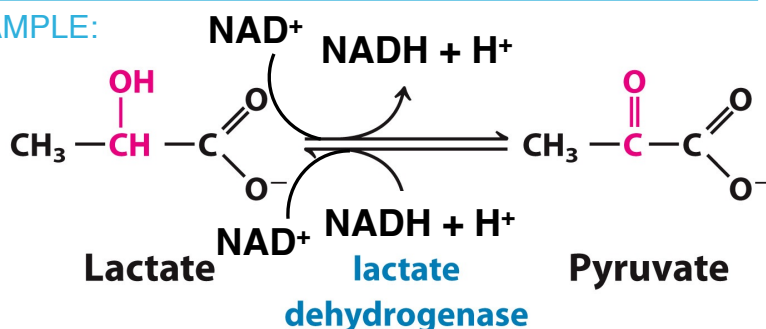
Loves e<sup>-</sup>

Oxidant + e<sup>-</sup> → reductant

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# Bioenergetics

EXAMPLE:



$$\Delta E^{\circ} = E^{\circ}_{(\text{reduction})} - E^{\circ}_{(\text{oxidation})}$$

$$= -0.32 \text{ V} - (-0.19 \text{ V})$$

$$= -0.13 \text{ V}$$

$$\Delta G^{\circ} = -n \mathcal{F} \Delta E^{\circ}$$

$$= -(2)(23.06 \text{ kcal V}^{-1} \text{ mol}^{-1})(-0.13 \text{ V})$$

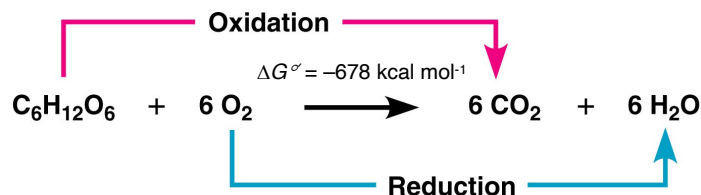
$$= +6 \text{ kcal mol}^{-1}$$

This is why the reaction is performed the other way, as you did for malate dehydrogenase by adding malate!

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## Bioenergetics

EXAMPLE:



What is the  $\Delta E^\circ$  for the complete oxidation of glucose?

$$\Delta G^\circ = -n \mathcal{F} \Delta E^\circ$$

$$-678 \text{ kcal mol}^{-1} = -(24)(23.06 \text{ kcal V}^{-1}\text{mol}^{-1})(x \text{ V})$$

$$\Delta E^\circ = +1.25 \text{ V}$$

What is the reduction potential ( $E^\circ$ ) for Glc  $\rightarrow$  CO<sub>2</sub>?

$$\Delta E^\circ = E^\circ_{(\text{reduction})} - E^\circ_{(\text{oxidation})}$$

$$+1.25 \text{ V} = +0.82 \text{ V} - (E^\circ_{(\text{Glc oxidation})})$$

$$E^\circ_{(\text{Glc oxidation})} = -0.43 \text{ V}$$

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## Summary

We learned that:

- The rules of thermodynamics and organic chemistry still apply to living systems
- **Reactions** are favorable when the free energy of products is much lower than the free energy of reactants
- Biochemical phosphoryl transfer reactions are favorable when:
  - the phosphate donors are destabilized by electrostatic repulsion
  - the reaction products are often stabilized by resonance
- **Unfavorable reactions can be made possible** by chemically coupling a highly favorable reaction to the unfavorable reaction
- **Oxidation-reduction reactions** commonly involve **transfer of electrons** from reduced organic compounds to specialized redox cofactors in generally favored reactions
  - Reduced cofactors can be used in biosynthesis or may serve as a source of energy for ATP synthesis.

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# Biological Membrane Transport

Reminder of membrane structure, membrane proteins, and dynamics

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## Physical Properties of Membranes

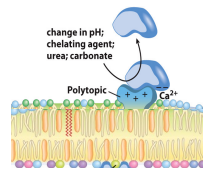
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- Not permeable to large polar solutes and ions
- Permeable to nonpolar compounds and some small polar compounds (e.g., water)
- Permeability can be artificially increased by chemical treatment.
  - when we want to get DNA into the cell
- Very stable yet dynamic and flexible structures
- Can exist in various phases and undergo phase transitions
- Can fuse
- Additional functionality provided by embedded proteins
  - recall peripheral, integral, and amphitropic proteins
  - recall that membrane proteins have hydrophobic regions

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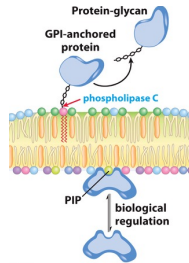
# Three Types of Membrane Proteins

## Peripheral



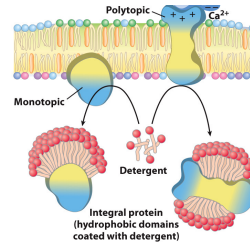
- Associate with the polar head groups of membranes
- Relatively loosely associated with membrane
  - through ionic interactions with the lipids or aqueous domains of integral membrane proteins
- Removed by disrupting ionic interactions either with high salt or change in pH
- Purified peripheral membrane proteins are no longer associated with any lipids.

## Amphitropic



- Amphitropic proteins can be conditionally attached to the membrane by covalent interaction with lipids or carbohydrates attached to lipids.
- Biological regulation results in attachment to, or cleavage from, lipids.

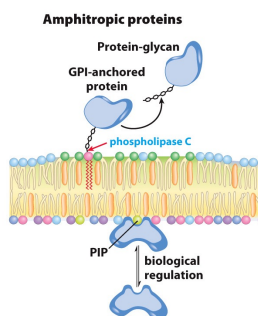
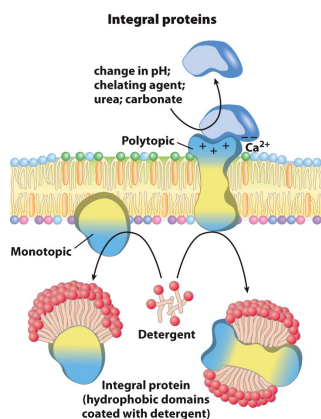
## Integral



- Span the entire membrane
- Have asymmetry like the membrane
  - different domains in different compartments
- Tightly associated with membrane
  - Hydrophobic stretches in the protein interact with the hydrophobic regions of the membrane.
- Removed by detergents that disrupt the membrane
- Purified integral membrane proteins still have phospholipids associated with them.

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# Three Types of Membrane Proteins



Peripheral (non-GPI linked) membrane proteins can be dissociated from the membrane fairly easily during changes in ionic strength like pH changes.

Amphitropic and GPI-linked proteins are linked to the membrane during specific regulatory events and can be reversibly removed.

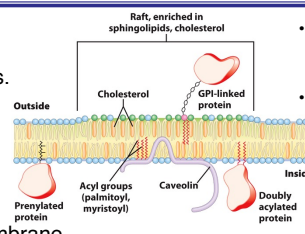
In the presence of strong detergents, integral membrane proteins can be removed from the membrane.

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# Amphitropic Membrane Proteins

## Lipid-linked Membrane Proteins & Lipid Anchors

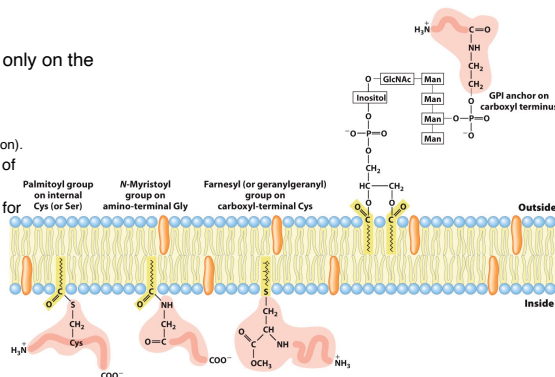
- Some membrane proteins are lipoproteins.
- They contain a covalently linked lipid.
  - long-chain fatty acids
  - isoprenoids
  - sterols
  - glycosylated phosphatidylinositol (PGI)
- The lipid part can become part of the membrane.
- The protein is now anchored to the membrane.
  - reversible process
  - allows targeting of proteins
  - Some, such as GPI anchors are found only on the outer face of plasma membrane.



- Lipid distribution in a single leaflet is not random or uniform.
- Lipid rafts:
  - contain clusters of glycosphingolipids with longer-than-usual tails
  - are more ordered
  - contain specific doubly or triply acylated proteins
  - allow segregation of proteins in the membrane

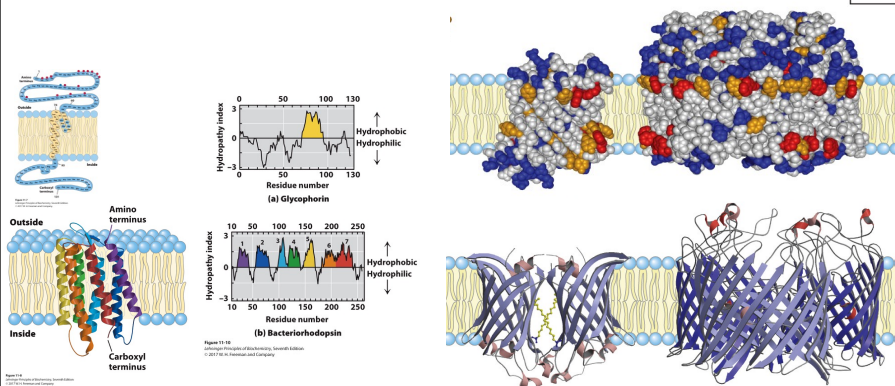
## Example:

- Proteins can be targeted to the inner leaflet of the plasma membrane by **farnesylation** (one type of prenylation).
- Farnesylation can be an intermediate in the lipidation of proteins.
- Primary sequence of the protein contains a signature for farnesylation: CaaX. (the "aaX" is removed)
  - C is a conserved Cys.
  - "a" is usually an aliphatic amino acid.
  - "X" is Met, Ser, Glu, or Ala.
- This reaction is catalyzed by **farnesyl transferase**.
- Nonfarnesylated proteins do not go to the membrane and are inactive.
  - promising cancer therapy (onco-Ras)



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# Examples of Integral Membrane Proteins



OmpLA

Maltoporin

- Transmembrane segments are predominantly hydrophobic.
- Tyr and Trp cluster at nonpolar/polar interface.
- Charged amino acids are only found in aqueous domains.

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