Imperial College London

Department of Bioengineering
Biomedical Engineering

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Molecules, Cells and Processes

Part I

Year 1
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Lecture 1, 2: Introduction and concepts

1.1 Life

Properties:

- A high degree of chemical complexity and microscopic organization;
- Have systems for extracting, transforming and using energy from the environment;
- Have the capacity for precise self-replication and self-assembly;
- Have mechanisms for sensing and responding to alternations surroundings;
- Have defined functions for each of their components and regulated interactions among them;
- There's a history of evolutionary change.

1.2 Cells

Cells are functional units of all living organisms.

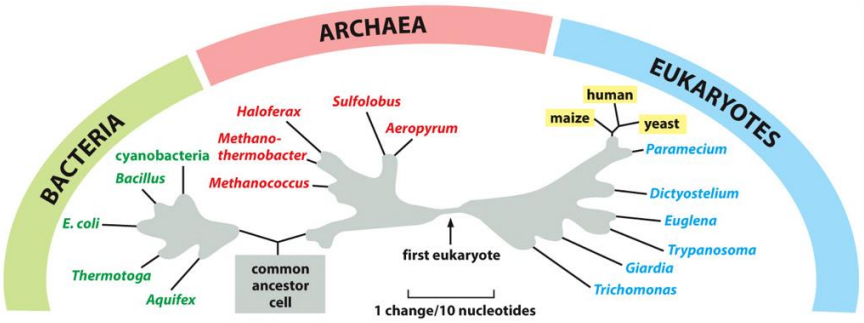
It has basic components:

- Plasma membrane: lipids and proteins
- Cytoplasm: water, metabolites, ions, coenzymes, proteins, ribosomes, DNA/RNA, organelles

1.3 Types of cells

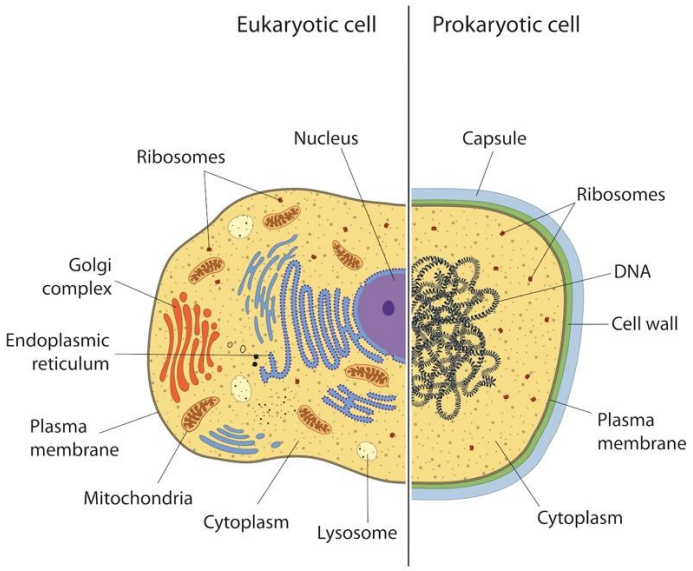
a. Classification:

- Based on evolution origin: bacteria, archaea, eukaryotes



- Based on the way of metabolism: phototrophs (energy from light), chemotrophs (energy from chemical components)

b. The difference between prokaryotic cells and eukaryotic cells:
   - No nucleus for prokaryotic cells
   - Different organelles

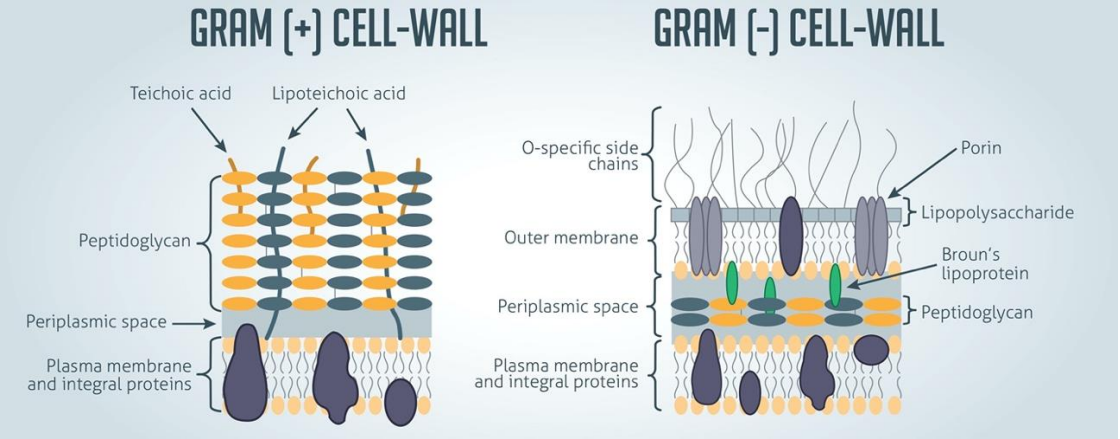


The left cell is likely to be a mammalian cell, the right cell is likely to be a E.coli cell. They are not in their actual size in this picture.

c. Bacteria

Can be classified to be either Gram-positive or Gram-negative, based on Gram staining.

- Gram-negative has outer membrane, Gram-positive does not have outer membrane
- Gram-positive has thicker peptidoglycan layer than Gram-negative



- Different color under Gram staining: blue/purple for Gram-positive, red/pink for Gram-negative
- Examples for Gram-negative is E.coli. It is used as the genetic tool since it grows fast. But not all Gram-negative bacteria are good. For example, E.coli can cause food poisoning, unary tract infections, etc.
- Example for Gram-positive is bacillus subtilis. It is also used as the genetic tool since its high protein production. Also, not all Gram-positive bacteria are good – staphylococci can cause infections.
- Cyanobacteria is a Gram-positive bacteria which can fix nitrogen and carbon dioxide, can be used to produce biofuels.

d. Eukaryotic cells

There are several types of eukaryotic cells: mammalian cells, plant cells, yeast cells, insect cells

1.4 Cell dimensions and scales

- Shapes for cells:

spherical cells e.g., *Streptococcus* | rod-shaped cells e.g., *Escherichia coli, Vibrio cholerae* | the smallest cells e.g., *Mycoplasma, Spiroplasma* | spiral cells e.g., *Treponema pallidum*

- Length scales:

Cells/organelles/bonds	**Length**
Mammalian cell	10 - 20 µm
Nucleus	5 µm
Mitochondria	2 µm
E.coli	2 µm
O-H bond	0.10 nm
C-C bond	0.15 nm
Amino acid in protein	0.40 nm
DNA base pair spacing	0.40 nm
Diameter of average protein	4 nm
Thickness of lipid bilayer	5nm

- Time scales:
  - Fick's 1st Law of Diffusion: random walk – "drunk on a hill"

$$Flux\ J = -D\left(\frac{\delta c}{\delta x}\right)$$

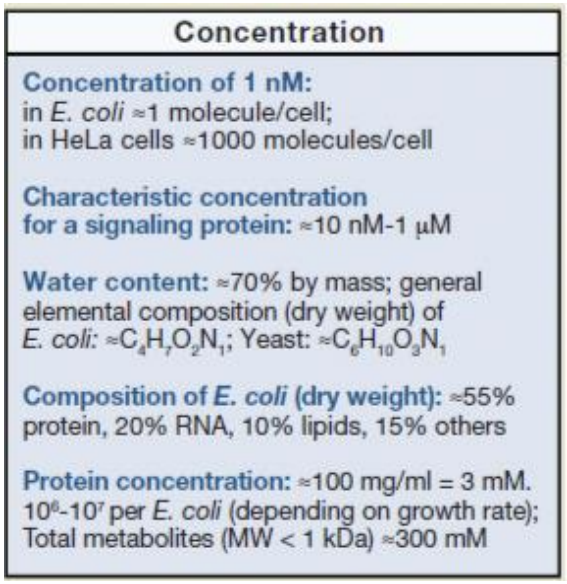
  - Einstein Smoluchowski Equation

$$t = \frac{L^2}{D}$$

    D is diffusion coefficient with unit of length^2/time

Activity	Time
Cell cycle for E.coli	20~40 min
Cell cycle for budding yeast	70~140 min
Cell cycle for HeLa cell	15~30 hr
DNA replication for E.coli	200~1000 bases/s
DNA replication for human	40 bases/s
Transcription	10~100 bases/s
Translation	10~20 aa/s

- Concentration scales



- **Molecular forces:**

  There are five primary types of forces between molecules

  ⇒ **Full covalent bonds**: Direct link between atoms by sharing electrons

  ⇒ **Electrostatic forces/Ionic Forces**: Attraction or repulsion due to –ve or +ve charged groups on molecule

⇒ **Hydrogen bonds**: 'sharing' of a hydrogen atom between two molecules

⇒ **Hydrophobic interactions**: Bunching together of hydrophobic molecules to free up water molecules

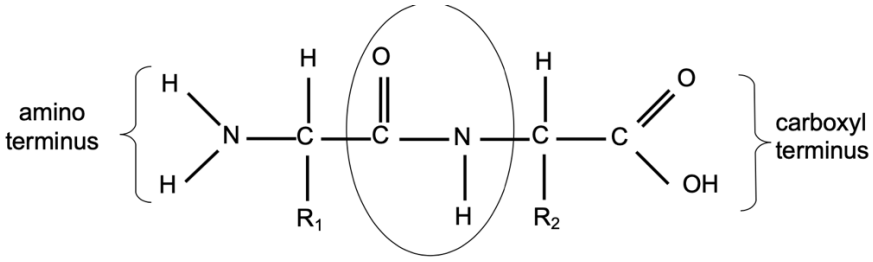
⇒ **Van der Waals forces**: Shimmering of electron clouds in one molecules give induces a net attractive force to another molecule

a. Covalent bond: sharing electrons

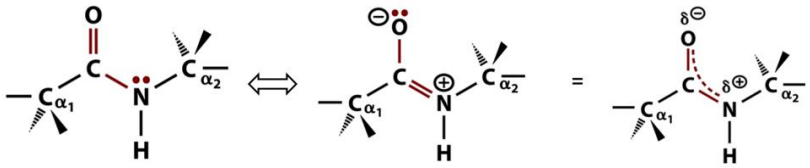
Covalent bonds are very strong, bond length is generally short.

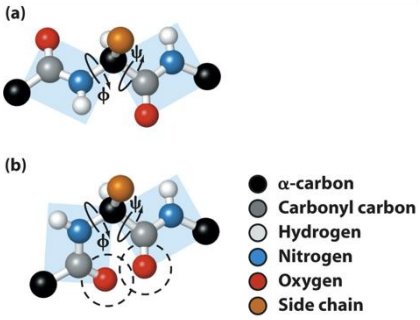
Type	Length / nm	Strength / kJ mol^{-1}	How common is it?
C–H	0.114	435	V common
C–C	0.154	368	V common
C–N	0.147	350	V common
C–N (peptide)	0.133	500	V common
S–S	0.205	252	Rare but important
C=C	0.134	720	Quite common
C=O	0.123	1076	V common

An example is the *peptide bond*.



The peptide bonds have the characteristic of rigidity.

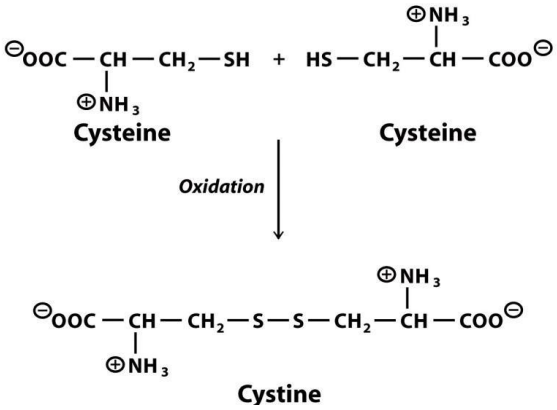




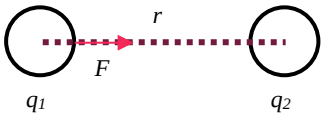
This can be seen as the "resonance" occurred. therefore, the peptide bond has a partial double bond character and hence the rigidity between C-N bond.

As the consequence, it allows the rotation of other two bonds in the polypeptide until they interfere with each other.

Another example is *disulphide bridges*. It holds the peptide chain together.



These occur when cysteine side chains with a protein are oxidized resulting in a covalent link between the two amino acids.



b. Ionic forces: electron transfer

In a vacuum, the force can be determined by:

$$F = \frac{1}{4\pi\varepsilon_0} \times \frac{q_1 q_2}{r^2}$$

where,

$\Rightarrow q_1, q_2$ are the charge on a single electron with value 1.602×10^{-19}C

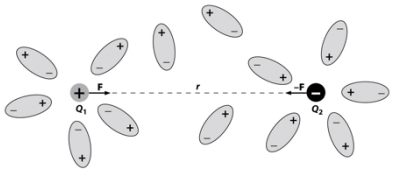
⇒ ε_0 is the permittivity of free space with value 8.85×10^{-12} F m^{-1}

⇒ Therefore, $\frac{1}{4\pi\varepsilon_0}$ has an estimated value at 9×10^{-9}N m^2 C^{-2}

In water, we need to reshape the equation to:

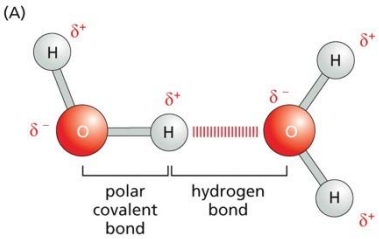
$$F = \frac{1}{4\pi\varepsilon_0 D}\times\frac{q_1 q_2}{r^2}$$

where D is the dielectric constant of water with value 80.



This is because water has an electric dipole, this align with the local electric field to reduce the ionic force.

Example: ionic forces is important for amino acids with charges chains (e.g. Glu, Asp, Lys, Arg). The majority of charged group of a folded protein are at the surface. They strong bonds when ions are within the protein interior, excluding from water and ionic solutions.

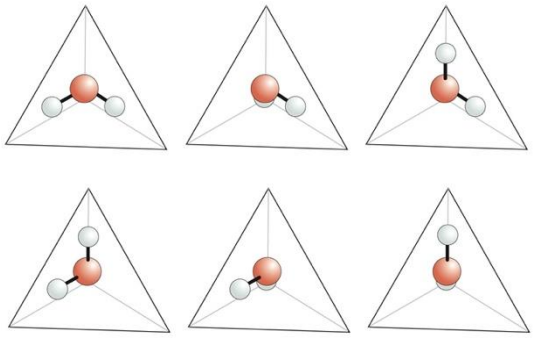


c. Hydrogen bonds

Example of H bonds is water.
Oxygen atoms in water are sp3 hybridized – 2 sigma bonds and two lone pairs. Lone pairs form H bonds with the 4 adjacent water molecules – gives a tetrahedral shape.
This gives the biological significance for proteins and DNA.

d. Hydrophobic interactions

In a network of water molecules with hydrogen bonds, a water molecule can adopt 6 different positions with 4 neighboring water molecules

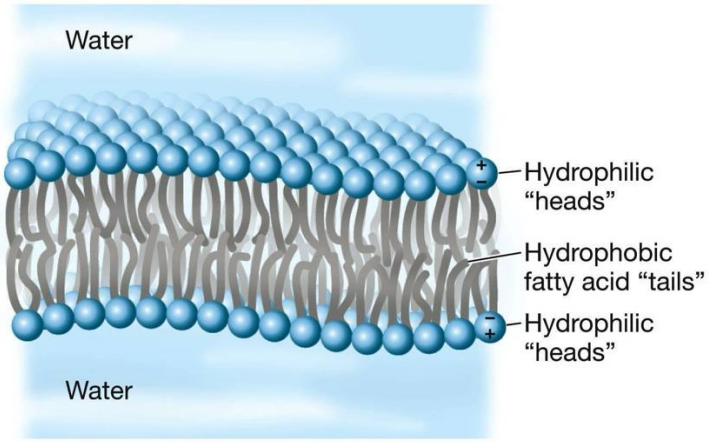


If we replace a neighboring water molecule to a non-polar molecule, the hydrogens at top 3 configurations can no longer form H bonds, this reduced the entropy.

Estimations:

Molecule	Area / nm^2	Energy per molecule / J	Energy / kJ mol^{-1}	Dissolves in water?
Oxygen O_2	0.1	0.7 k_bT	1.75	YES
Octane (C_8H_{18})	2.1	15 k_bT	37.5	NO
Protein ball 2nm radius	50.2	352 k_bT	880	Predicted NO

Examples: phospholipid bilayer

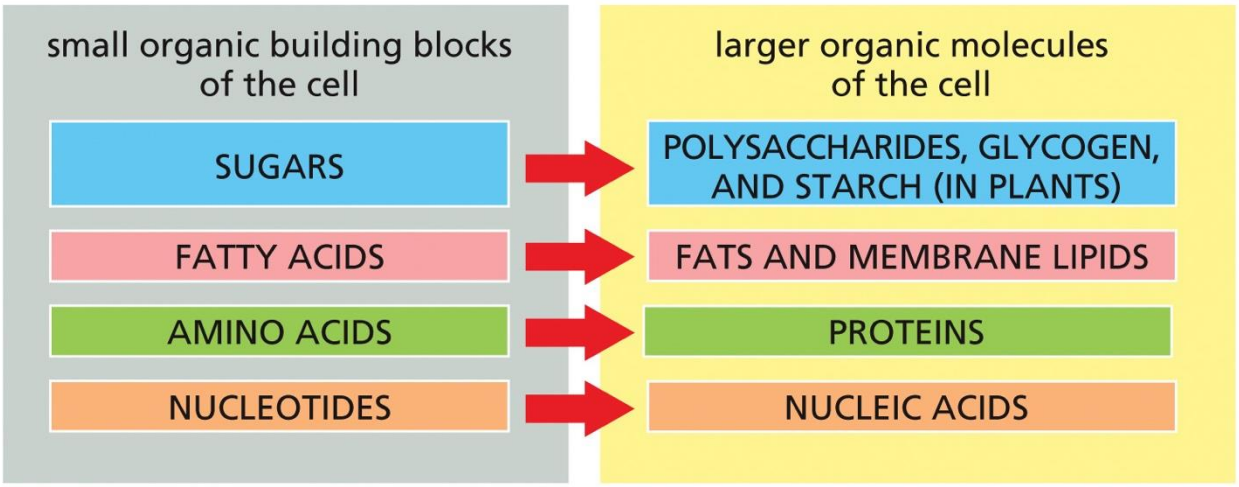


e. Van der Waals forces:
Van der Waals forces are transient, weak electrostatic attractions between two atoms. It rises due to the fluctuating electron cloud surrounding each atom which has a temporary electric dipole.

The magnitude is proportional to $1/r^7$ – falls very fast.

Very common in proteins – the sheer number of the interactions.

1.5 the chemistry of life



1.6 Cell processes

- Examples: replicate, metabolism, communication, specialization, differentiation

1.7 From cells to tissues

- Microbial communities – specific bacteria produce specific compounds

- Cell differentiation: same genome but different phenotypes.

1.8 Evolution

- DNA changes during evolution – mutation, duplication, segment shuffling, horizontal transfer

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Lecture 3: Lipids, membranes and transport

3.1 Lipids

- Organic molecules, low solubility in water, relative hydrophobic
- **Diverse functions:**

  ⇒ Storage of energy
    - Reduced compounds: lots of available energy
    - Hydrophobicity: good packing

  ⇒ Insulation from the environment
    - Low thermal conductivity
    - High heat capacity – absorb energy
    - Mechanical protection – absorb shocks

  ⇒ Water repellant – hydrophobicity - keep the surface of organisms dry
    - Prevent excessive wetting (birds)
    - Prevent the loss of water from evaporation

  ⇒ Buoyancy control and acoustics in marine mammals

  ⇒ Provide the main structure of cell membranes

  ⇒ Be cofactors of enzymes
    - Vitamin K: blood clot formation
    - Coenzyme Q: ATP synthesis in mitochondria

  ⇒ Be signaling molecules

- Paracrine hormones (locally)
- Steroid hormones (body-wide)
- Growth factors
- Vitamin A and D (hormone precursors)

⇒ Be pigments

- the color of tomatoes, carrots, pumpkins, birds

⇒ Be antioxidants

- Vitamin E

- **The classification of lipids:**

⇒ The lipid contains fatty acids:

- Storage lipids: triacylglycerols
- Membrane lipids: phospholipid, glycolipids, archaeal ether lipids

⇒ The lipid does not contain fatty acids: cholesterols, vitamins, pigments, etc.

- **Fatty acids:**

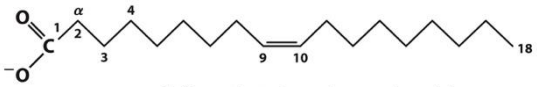
⇒ Carboxylic acids with 4-36 carbons (usually even number), mostly unbranched

⇒ 3 types:

- Saturated: no double bonds between C
- Unsaturated: 1 double bonds between C in alkyl chain
- Polyunsaturated: more than 1 double bonds

⇒ The names:

- Systematic name: *cis-9-octadecanoinc acid*
- Common name: *oleic acid*



18:1(Δ^9) *cis*-9-Octadecenoic acid

Delta numbering of carbon skeleton: $18{:}1^{\Delta 9}$ (describes location of the first carbon of the alkene in relationship to the carbonyl carbon)

- Omega numbering of carbon skeleton: $18{:}1^{\omega 9}$

(describes location of the first carbon of the alkene in relationship to the terminal methyl)

⇒ The solubility: decreases with the increase of chain

⇒ The melting point:

decreases with the decrease of the chain

decreases with the increase of double bonds

- Saturated fatty acids pack in a fairly orderly way, giving extensive favorable interactions
- Unsaturated cis fatty acids pack less orderly due to the kink, less-extensive favorable interactions
- Thus, it takes less thermal energy to disrupt disordered packing of unsaturated fatty acids – low melting point

- **Structural lipids in membrane**

⇒ a polar head group, a non-polar tail group

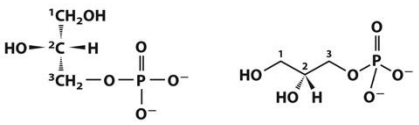
⇒ diversification come from: backbones, fatty acids, head groups

⇒ the surface properties of membrane are determined by the head group

- different organisms, different head group compositions
- different tissue, different head group compositions

- **glycerophospholipids**

⇒ primary constituents of cell membranes

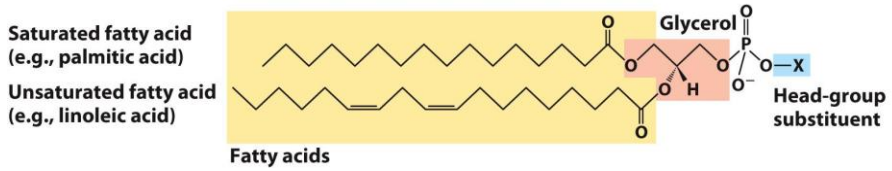

L-Glycerol 3-phosphate (*sn*-glycerol 3-phosphate)

⇒ two fatty acids form ester linkages with the first and the second hydroxyl groups of L-glycerol-3-phosphate

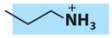
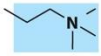
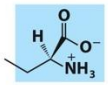
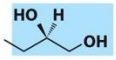
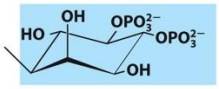
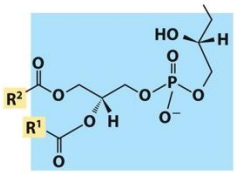
⇒ The phosphate group is charged at physiological pH

⇒ The structure:

- Unsaturated – commonly connect to C2 of L-glycerol-3-phosphate
- The highly polar phosphate group may be further esterified by an alcohol; the substituent groups are known as the head groups



⇒ Examples of glycerophospholipids:

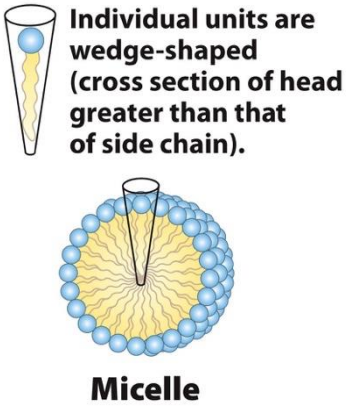
Name of glycerophospholipid	Name of X—O	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	—H	−2
Phosphatidylethanolamine	Ethanolamine		0
Phosphatidylcholine	Choline		0
Phosphatidylserine	Serine		−1
Phosphatidylglycerol	Glycerol		−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4*
Cardiolipin	Phosphatidyl-glycerol		−2

- **Phosphatidylcholine** is the major component of most eukaryotic cell membranes

3.2 The lipid bilayer – membranes

- 3 major structures - the formation depends on type of lipids and concentration:

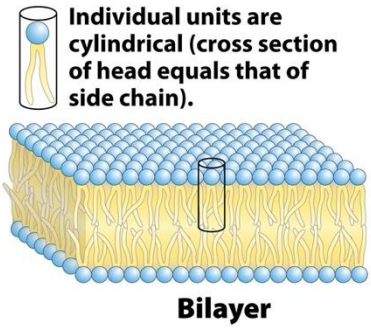
  ⇒ micelles:



- forms in the solution of amphipathic molecules that have larger, more polar head than tail
- composed of a few dozen to a few thousand lipid molecules
- aggregation of individual lipids into micelles is concentration dependent

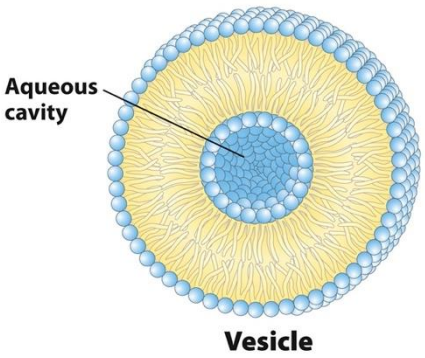
⇒ bilayers

- consists of two leaflets
- forms when lipid with polar heads and more than 1 lipid tail are in aqueous solution
- hydrophilic head groups interact with water on both sides of the bilayer
- hydrophobic tails are packed inside



⇒ liposomes (vesicle)

- small bilayers spontaneously seal into vesicles in a concentration-dependent way
- synthetic vesicle membranes can be made into vitro and can contain artificially inserted proteins
- the central aqueous cavity can enclose dissolved molecules
- useful artificial carriers of molecules (e.g. drug)
- fuse readily with cell membranes or others



- **What are membranes?**

  ⇒ Complex lipid-based structures that form pliable sheets Composed of a variety of lipids and proteins

  ⇒ Separates the cell from its surrounding

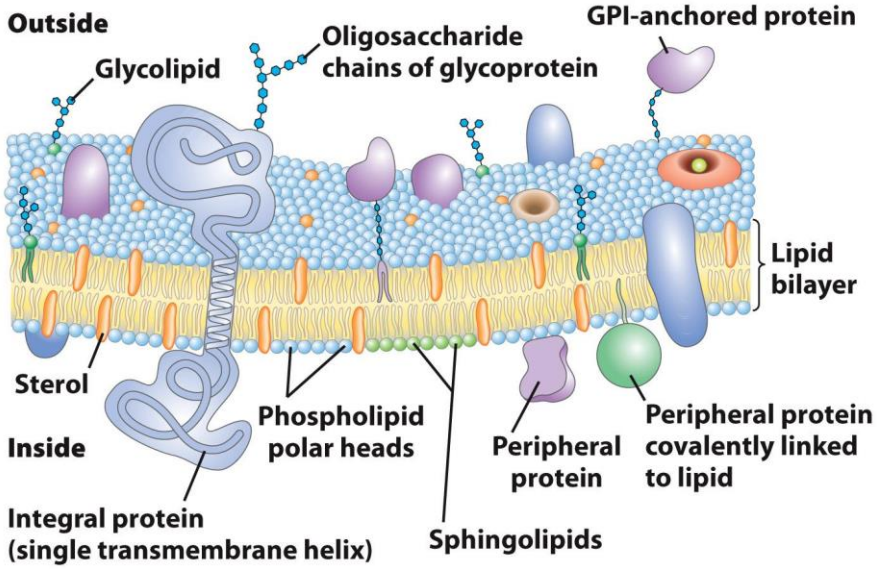
  ⇒ Eukaryotic cells have various internal membranes that divide the internal space into compartments

- **Functions:**
  - ⇒ Define the boundaries of the cell
  - ⇒ Allow import and export
    - Selective import of nutrients (e.g. lactose)
    - Selective export of waste and toxins (e.g. antibiotics)
  - ⇒ Retain metabolites and ions within the cell
  - ⇒ Sense external signals and transmit information into the cell
  - ⇒ Provide compartmentalization within the cell
    - separate energy-producing reactions from energy-consuming ones
    - keep proteolytic enzymes away from important cellular proteins
  - ⇒ Store energy as a proton gradient
  - ⇒ Support synthesis of ATP
- **Common features:**
  - ⇒ Sheet-like flexible structure, 3-10nm thick
  - ⇒ Main structure is composed of two leaflets of lipids
  - ⇒ Form spontaneously in aqueous solution and are stabilized by non-covalent forces, especially hydrophobic effect
  - ⇒ Protein molecules span the lipid bilayer
  - ⇒ Asymmetric:
    - Some lipids are found commonly “inside”
    - Some lipids are found commonly “outside”
    - Carbohydrate moieties are attached on the outer leaflet
    - Can be electrically polarized
  - ⇒ Fluid structures: two-dimensional solution of oriented lipids
- **Fluid mosaic model of membranes:**

⇒ Lipids from a viscous, two-dimensional solvent into which proteins are inserted and integrated more or less deeply

⇒ Proteins can either be embedded in or associated with the membrane

- Integral proteins are firmly associated with the membrane, often spanning the bilayer
- Peripheral proteins are weakly associated and can be removed easily – some are non-covalently attached, some are linked to the membrane lipids



- **The composition of membranes**

⇒ Varies by organisms, tissues, organelles

⇒ Ratio of lipid to proteins varies

⇒ Two leaflets have different lipid compositions, the outer leaflet is often more positively charged

3.3 Membrane proteins

- **Function of lipid protein:**

⇒ Receptors: detecting signals from outside

- Light (opsin)
- Hormones (insulin receptor)

- Neurotransmitters (acetylcholine receptors)
- Pheromones (taste and smell receptors)

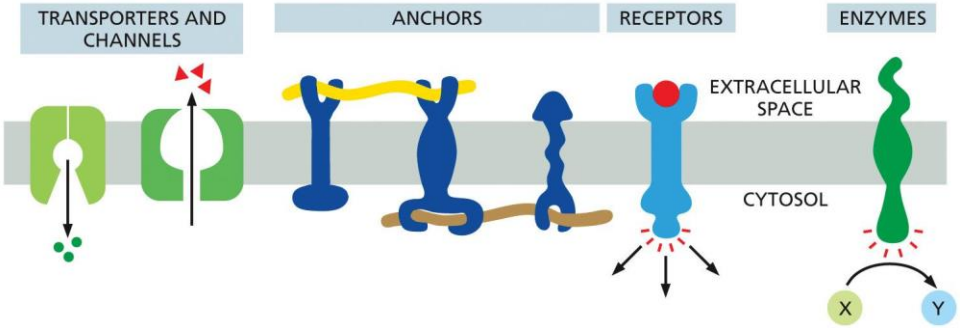
⇒ Channels, gates, pumps

- Nutrients (maltoporin)
- Ions (K-channel)
- Neurotransmitters (serotonin reuptake protein)

⇒ Enzymes

- Lipid biosynthesis (some acyltransferases)
- ATP synthesis (F_0F_1 ATPase/ATP synthase)

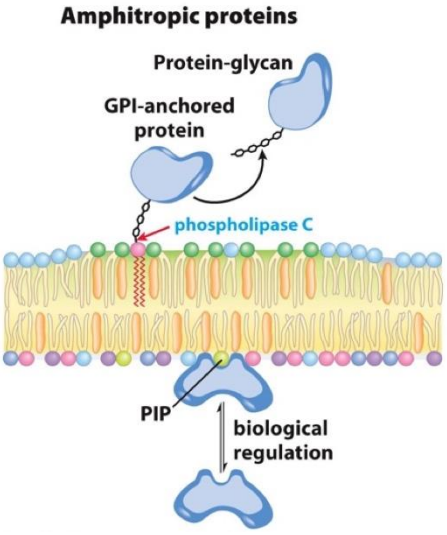
⇒ Anchors



- **Types of membrane proteins:**

⇒ **Peripheral (non-GPI lined) membrane proteins**

- Electrostatic/H-bond interaction with lipids or integral proteins
- Associate with the polar head groups of membrane
- Relatively loosely associate with the membrane
- Removed by disrupting ionic interactions either with high salt or change in pH
- Purified peripheral membrane proteins are no longer associated with any lipids

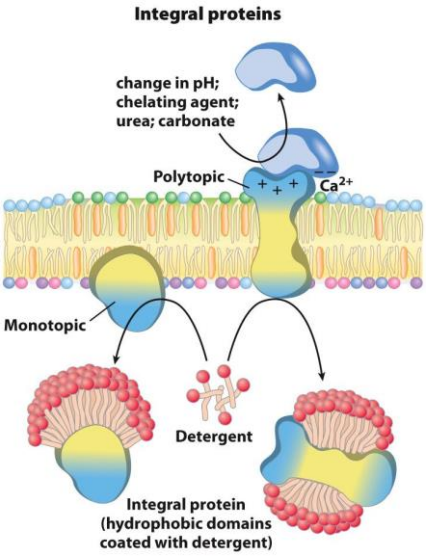


⇒ **Amphitrophic and GPI-linked proteins**

- Reversibly linked to the membrane

- Can be conditionally attached to the membrane by covalent interaction with lipid or carbohydrates attached to lipids
- Can be linked non-covalently with proteins or lipids

⇒ **Integral membrane proteins**



- Embedded within the bilayer, span the entire membrane
- Can be monotopic – interact with one leaflet OR polytopic, interact with both
- Have asymmetry like the membrane – different domains in different compartment
- Tightly associated with the 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 phospholipid associated with them

- **Lipid anchors**

  ⇒ some membrane proteins are lipoproteins

  ⇒ they contain a covalently linked lipid molecule

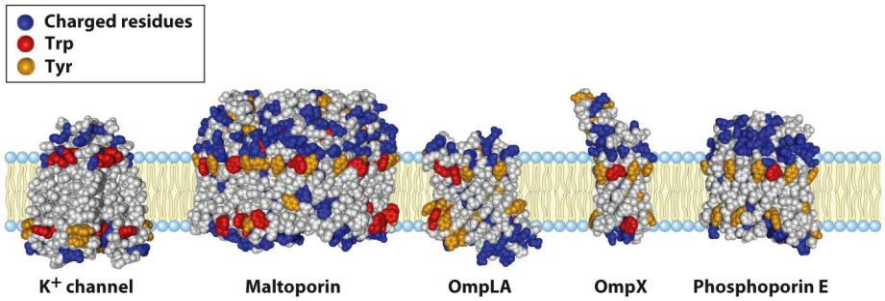
  ⇒ the lipid part can become part of the membrane

  ⇒ the protein is anchored to the membrane

  - reversible
  - allows targeting of proteins
  - some (e.g. GPI anchors) are found only on the outer surface

- Membrane proteins also contain β-sheets

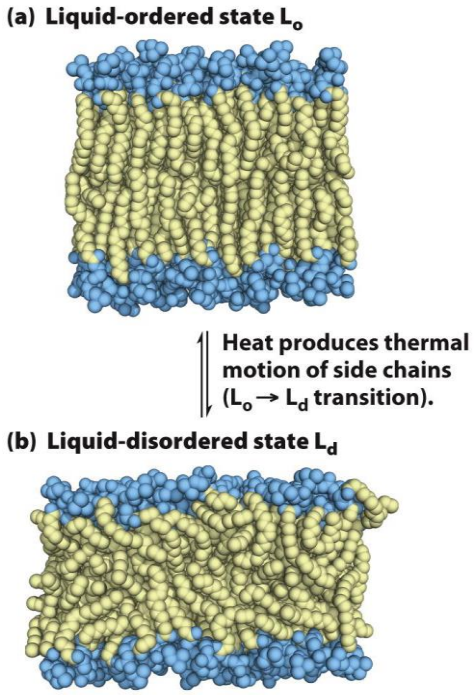
- Amino acids in membrane protein cluster in distinct regions
  - ⇒ transmembrane segments are predominantly hydrophobic
  - ⇒ Tyr and Trp cluster at non-polar/polar interface



  - ⇒ Charged amino acids are only found in aqueous domains

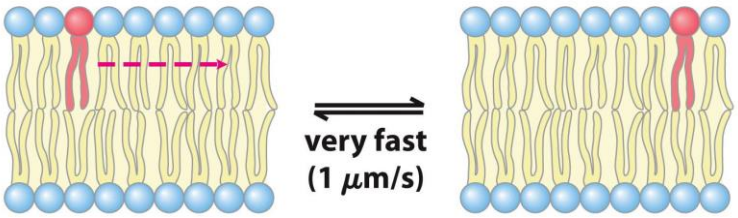
3.4 Membrane dynamics

- **Physical properties**
  - ⇒ Dynamic and flexible structure
  - ⇒ Exist in various phases and undergo phase transitions
  - ⇒ Not permeable to large polar solutes and non-polar compounds
  - ⇒ Permeability can be artificially increased by chemical treatment
- **Membrane phases**
  - ⇒ Depending on their composition and the temperature, the lipid phase can be in gel or fluid phase
    - Gel phase: individual molecules do not move around
    - Fluid phase: individual molecules can move around
  - ⇒ Heating causes gel to fluid
  - ⇒ Under physiological conditions, membranes are more fluid-like than gel-like



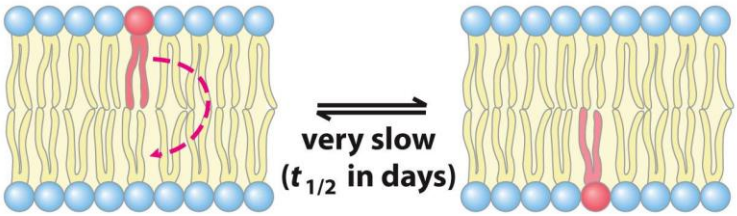
- **Organisms can adjust the membrane composition**
  - ⇒ Membrane fluidity is determined by the fatty acid composition and melting point
  - ⇒ More fluid membranes require shorter and more unsaturated fatty acids
    - Melting point decreases with the increase of the double bonds
    - Melting point increases with the increase of the length of saturated fatty acids
  - ⇒ At higher temperature, cells need more long, saturated fatty acids
  - ⇒ At lower temperature, cells need more unsaturated fatty acids
- **Diffusion mechanisms**
  - ⇒ Lateral diffusion: with one leaflet

Uncatalyzed lateral diffusion



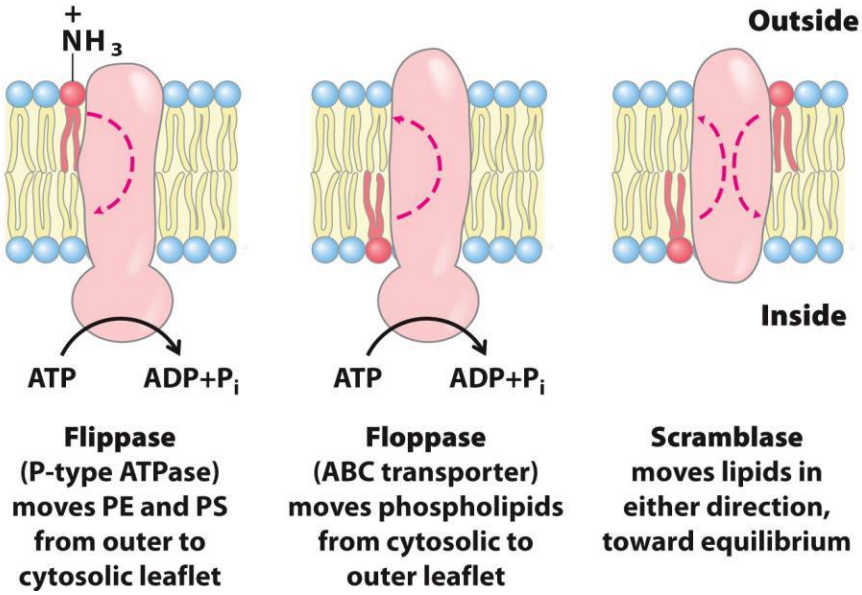
⇒ Transverse diffusion: rare, the charges heads must transverse the hydrophobic tail region

Uncatalyzed transbilayer ("flip-flop") diffusion



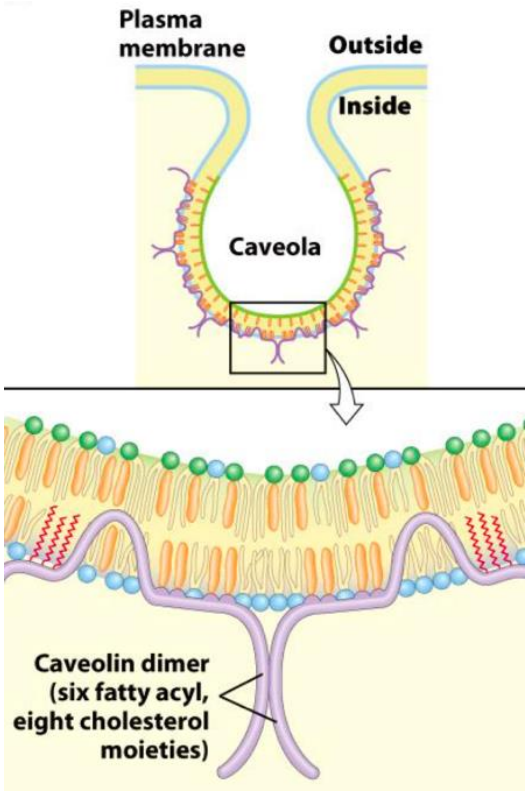
⇒ Filppases: enzyme-catalyzed transverse diffusion, use of ATP against concentration gradient

Catalyzed transbilayer translocations



- **Membrane curvature**

  ⇒ Caveolin



⇒ Other mechanisms: due to charge, lipid, proteins, etc.

- **Membrane fusion**

  ⇒ Membranes can fuse with each other without exposure of lipids to aqueous solvent

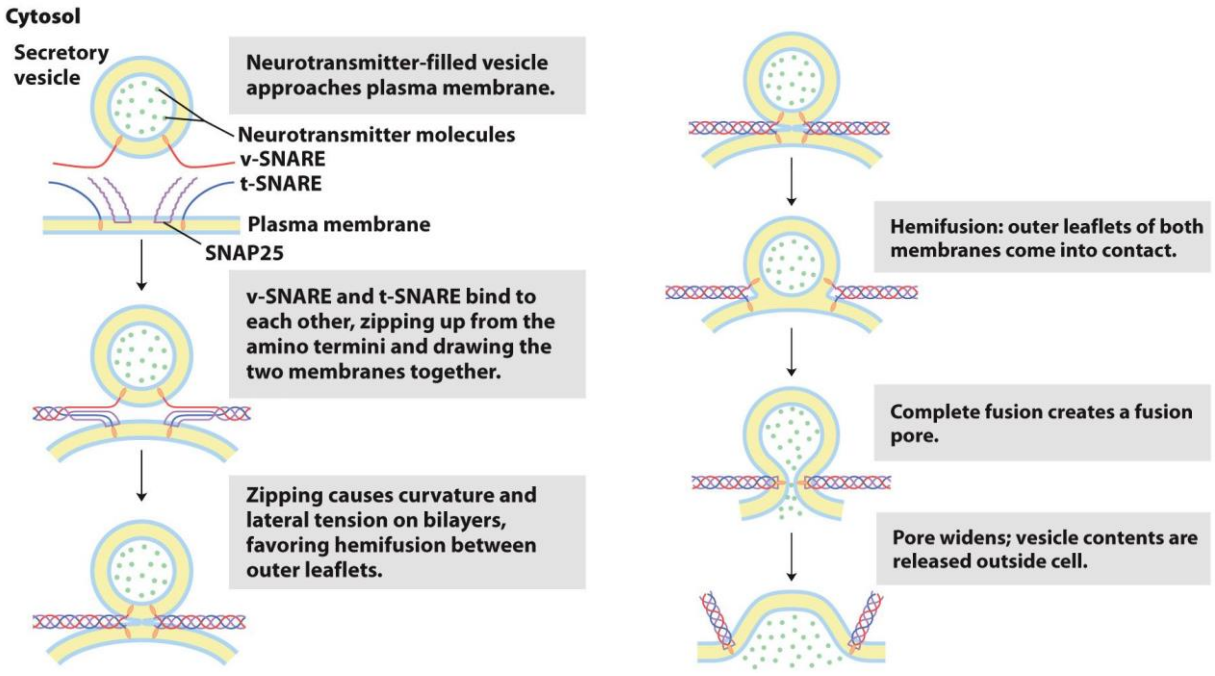
  ⇒ Can be spontaneous or protein mediated

- **Neurotransmitter release**

⇒ Mediated by SNARE-type proteins

⇒ Three types of SNARE proteins:

  - T-SNARE: assemble on the target membrane
  - V-SNARE: assemble on the vesicle membrane
  - Q-SNARE (e.g. SNAP-25): Ca^{2+} induced regulatory proteins



3.5 Transport across membranes

⇒ Cell membranes are permeable to small non-polar molecules that passively diffuse through the membrane

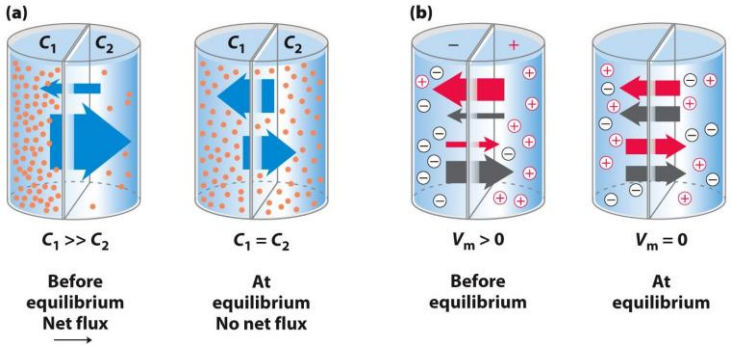
⇒ Passive diffusion of polar molecules involves desolvation and thus has a high activation barrier

⇒ Can be facilitate by proteins that provide an alternative diffusion path. These proteins are known as transporters or permeases

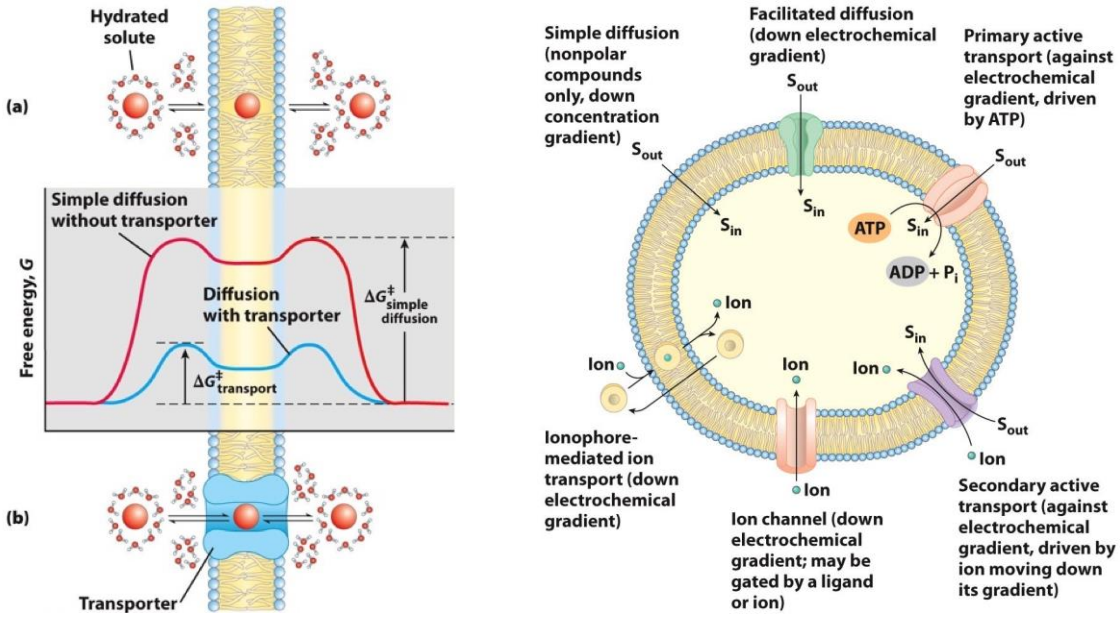
- Energetically favorable:

  ⇒ Concentration dependence: move towards equilibrium

  ⇒ Electrochemical dependence: move towards electrical equilibrium



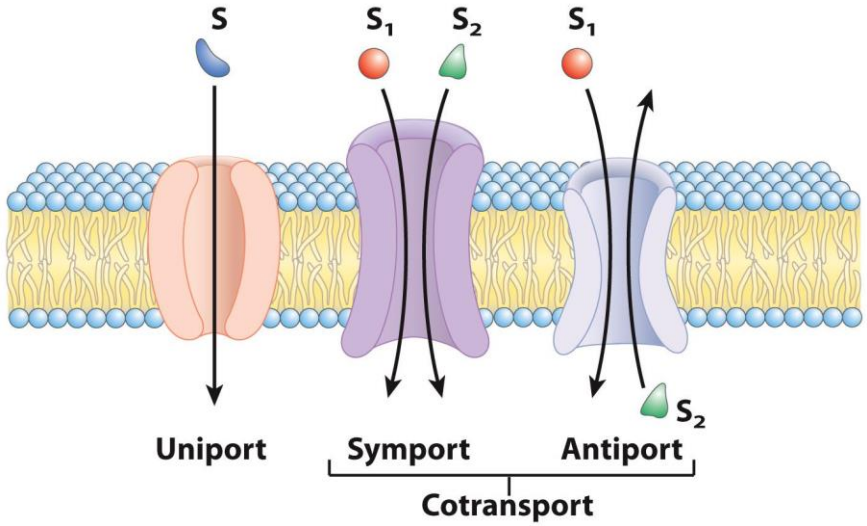
- Polar solutes need alternative paths across cell membranes
  - ⇒ High free energy needed for the molecule passes through the membrane *(a)*
  - ⇒ Need of transporters, reduce the free energy *(b)*



- **Types of transport:** *(the second diagram above)*
  - ⇒ Simple diffusion
    - Non-polar compounds ONLY
    - By concentration gradient
  - ⇒ Facilitated diffusion
    - By electrochemical gradient
  - ⇒ Primary active transport
    - Driven by ATP, against electrochemical gradient
  - ⇒ Secondary active transport
    - Driven by ion moving down the gradient, against electrochemical gradient
  - ⇒ Ionophore mediated ion transport
    - By electrochemical gradient
  - ⇒ Ion channel

- by electrochemical gradient
- may be gated by a ligand or ion

- **3 classes of transport systems:**

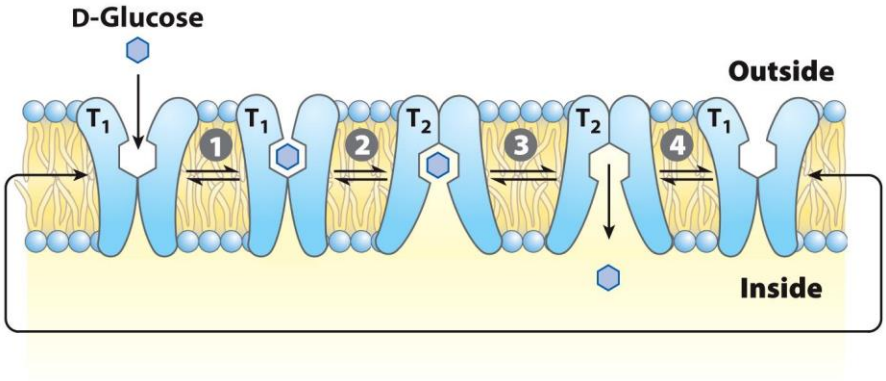


⇒ Uniport: transport of one metabolite

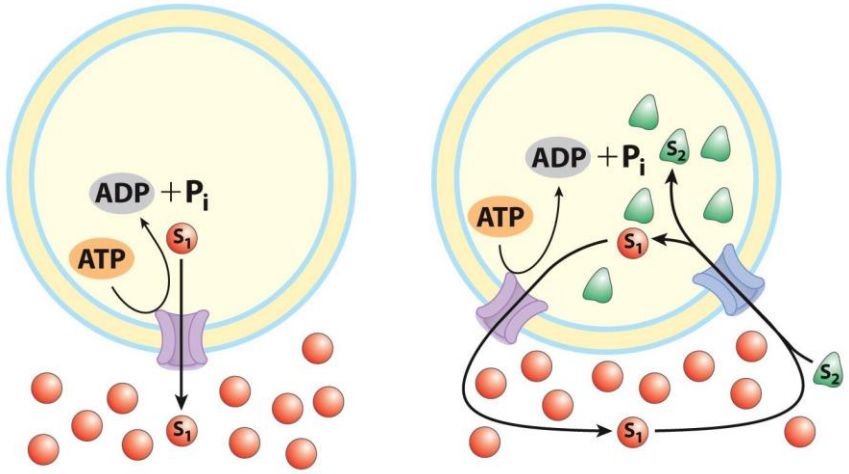
⇒ Cotransport: transport more than one metabolites

- Symport: metabolites transported to the same side
- Antiport: metabolites transported to the opposite sides

⇒ The modal for glucose transport:

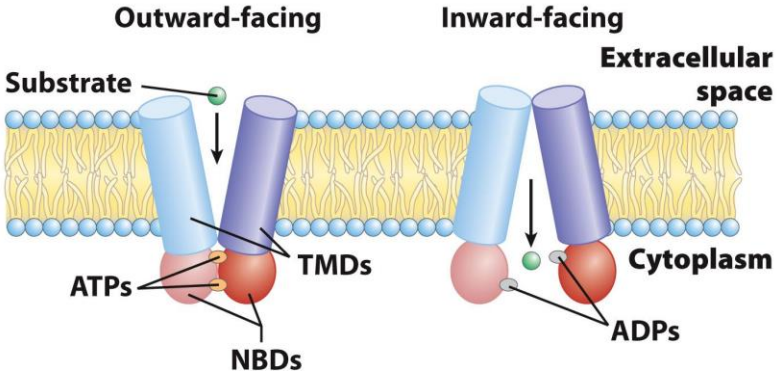


- **2 types of active transport:** against the electrochemical gradient



(a) Primary active transport (b) Secondary active transport

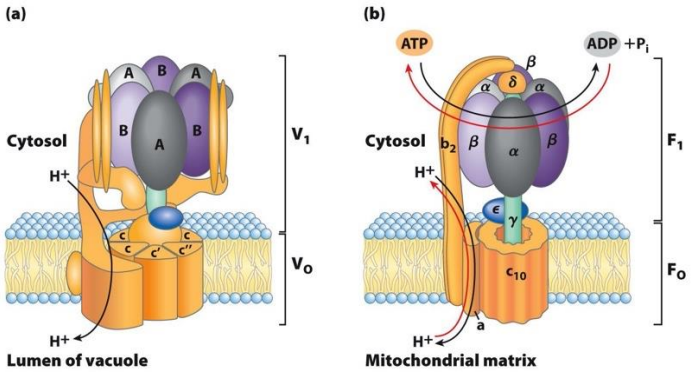
⇒ Primary active transport: ABC transporters use ATP hydrolysis to drive transport of substrates



- Mutation of human CRTF transporter (a type of ABC transporter) would result in cystic fibrosis

⇒ Energy of ATP hydrolysis can be used to drive protons through the membrane; energy of the proton gradient can be used to synthesis ATP (chloroplast, mitochondrial membrane)

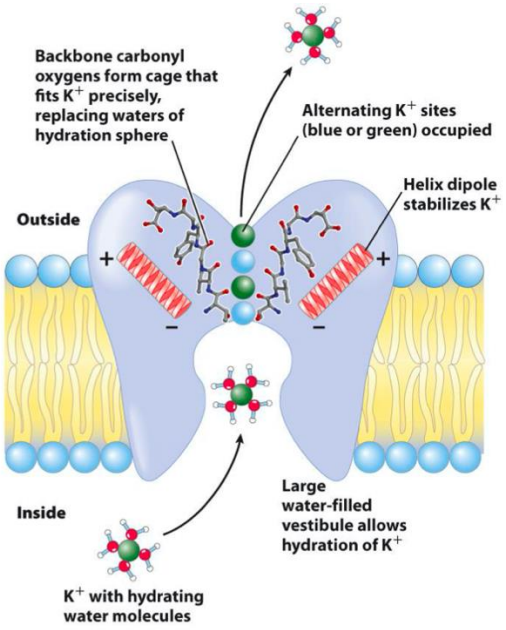
⇒ Proton driven ATPase can function in both directions:



- *(a)* The V_oV_1 H^+ ATPase uses ATP to pump protons into vacuoles and lysosomes
- *(b)* The F_oF_1 ATPase, the protons flow down the electrochemical gradient

- Ion channels:

  ⇒ Maintain the gradient concentration for active transport: replace of water to carbonyl group, allowing ions to transport



  ⇒ Can respond to cellular voltage changes

Lecture 4: Enzymes and catalysis

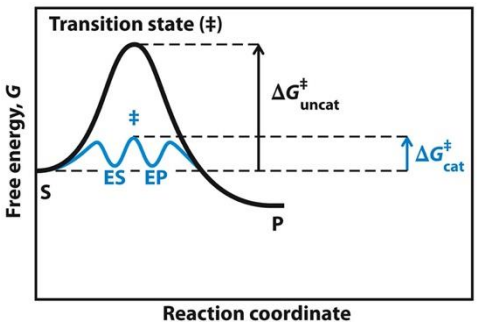
4.1 Introduction

- **Features of enzymes**
  - ⇒ Catalysts
  - ⇒ Mostly globular proteins
    - Exception: RNA
  - ⇒ Has selectivity
  - ⇒ Sometimes need additional requirements
    - Cofactors: inorganic ions (prosthetic group), can be covalently linked (e.g. Cu, Fe, Mg, Zn)
    - Coenzymes: organic or metalloorganic molecules (e.g. coenzyme A, NADH)
    - Holoenzyme: a catalytically active enzyme bound to its cofactor
    - Apoenzyme: a protein part
- Biocatalysts is better than inorganic catalysts:
  - ⇒ Greater reaction specificity
  - ⇒ Milder reaction conditions
  - ⇒ Higher rate
  - ⇒ Capacity of regulation
- **Classification:** based on the type of reaction

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

4.2 Enzyme catalysis

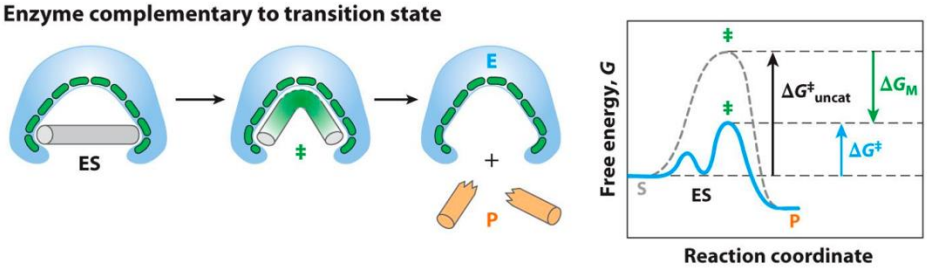
$$rate\ k = \left(\frac{k_B T}{h}\right) e^{\frac{-\Delta G^{\ddagger}}{RT}}$$



⇒ Enzymes do not affect chemical equilibrium - does not affect the free energy of reaction

⇒ Enzymes decrease the activation barrier $\Delta G^{‡}$ to increase the rate

- Enzymes organize reactive groups into close proximity and proper orientation to reduce $\Delta G^{‡}$



⇒ Uncatalyzed: transition state conversion is entropically unfavorable

⇒ Catalyzed: enzyme uses the binding energy of substrate to organize the reaction to *ES complex*

- Entropy cost is paid during binding
- Reactant complex to transition state is entropically neutral

⇒ Enzymes bind transition states best

- **Catalytic mechanisms:**

  Enzymes may use one or a combination of:

  ⇒ Acid-base catalysis: give and take protons

  ⇒ Covalent catalysis: change reaction paths, form of covalent bonds with substrates

  ⇒ Metal-ion catalysis: use redox cofactors

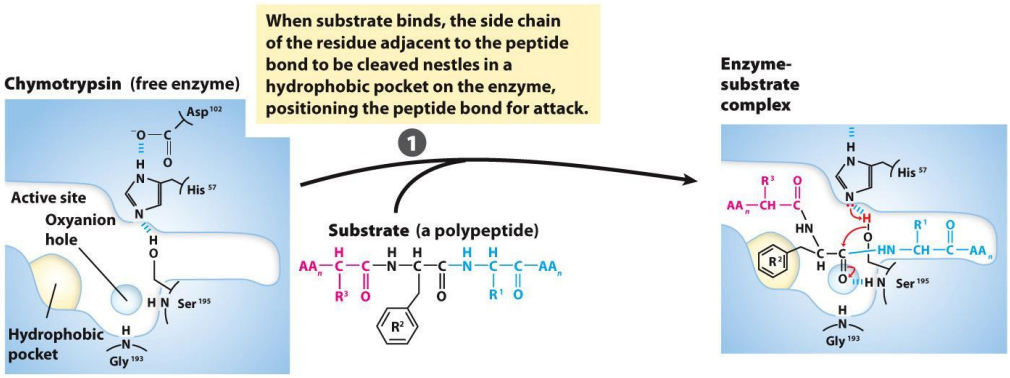
  - Involves a metal ion, binds to the enzyme
  - Interact with substrate to facilitate binding

- Stabilize negative charges
- Participate in oxidation reactions

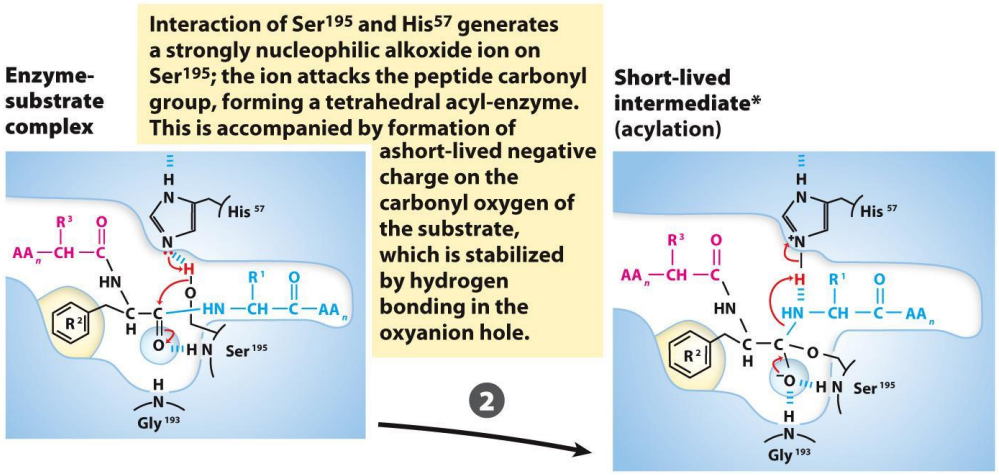
- **Catalytic example: chymotrypsin**

  ⇒ Chymotrypsin is one of the proteases cuts peptides at specific location during digestion of proteins

  - **Step 1: substrate binding**

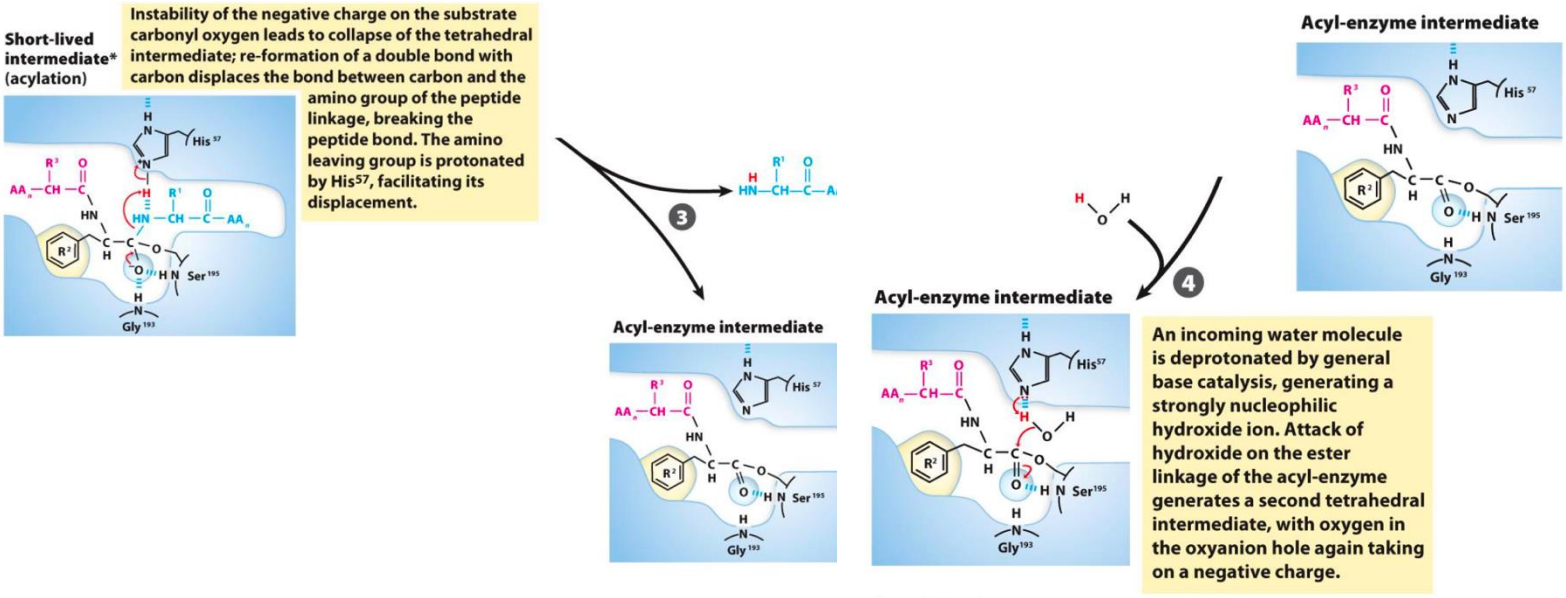


  - **Step 2: nucleophilic attack**

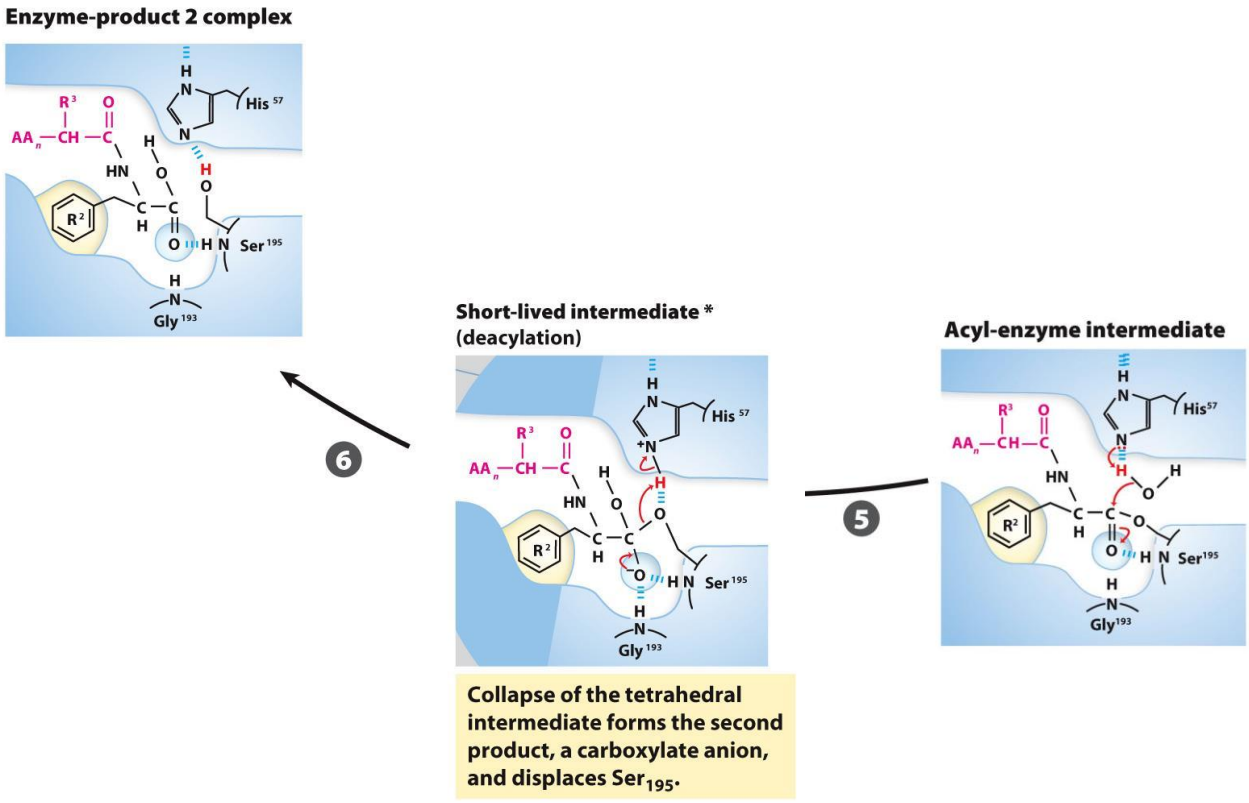


  - **Step 3: substrate cleavage**

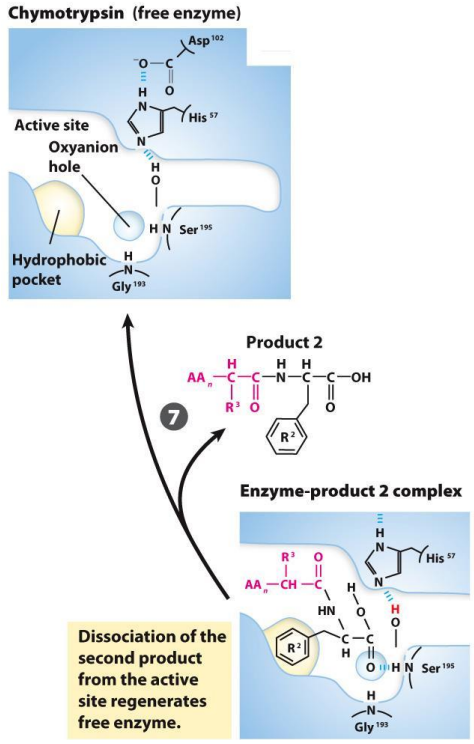
- **Step 4: water comes in**



- **Step 5: water attacks**
- **Step 6: break-off from the enzyme**



- **Step 7: product dissociates**



4.3 Enzyme kinetics

⇒ The rate at which compound react

⇒ Catalytic rate affected by

- Enzyme
- Substrate
- Effectors
- Temperature

$$E + S \underset{K\text{-}1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} E + P$$

⇒ The assumptions and constraints:

- The concentration of enzyme is constant: S_{Tot} = [S] + [ES] ≈ [S]
- Steady state assumption: rate of formation of ES = rate of breakdown of ES = 0
- The observed rate is $v_{net} = \frac{dP}{dt} = k[ES]$

⇒ The final form is a single substrate is

$$v = \frac{k_{cat}[E_{tot}][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

Known as the *Michaelis-Menten equation*

- K_{cat} is the turnover number. It describes how many substrate molecules one enzyme molecule can convert in 1 second
- K_m is the Michaelis constant. It describes an approximate measure of a substrate's affinity for an enzyme

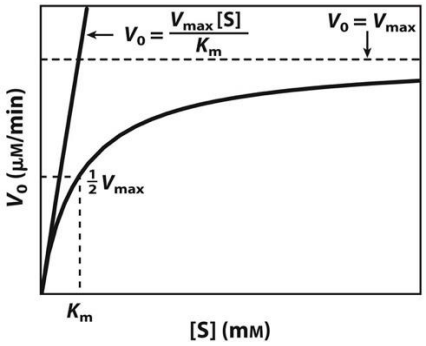
$$k_m = \frac{k_{-1}k_2}{k_1}$$

- v_{max} occurs at all enzyme is in the ES complex and depend on k[ES]

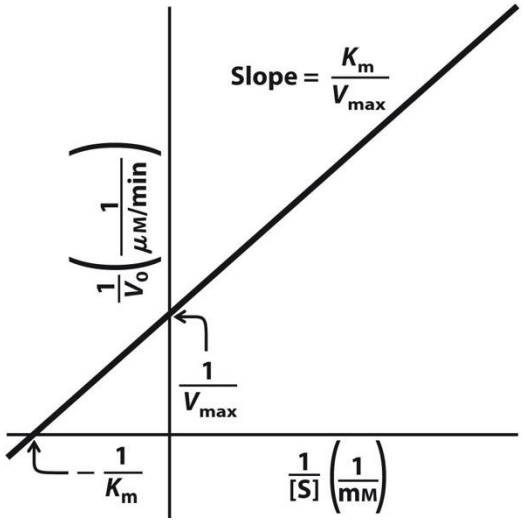
$\Rightarrow$ ideal rate $v = \frac{v_{max}[S]}{k_m + S}$, the deviations may due to:

- limitations of measurements
- substrate inhibition
- substrate prep containing inhibitors
- enzyme prep containing inhibitors

$\Rightarrow$ v is not proportional to [S] at high concentration of substrate:



$\Rightarrow$ K_m and v_{max} can be determined from Lineweaver-Buck plot:



- linearized, double-reciprocal

4.4 Enzyme inhibition

- Inhibitors are compounds that decrease the enzyme activity

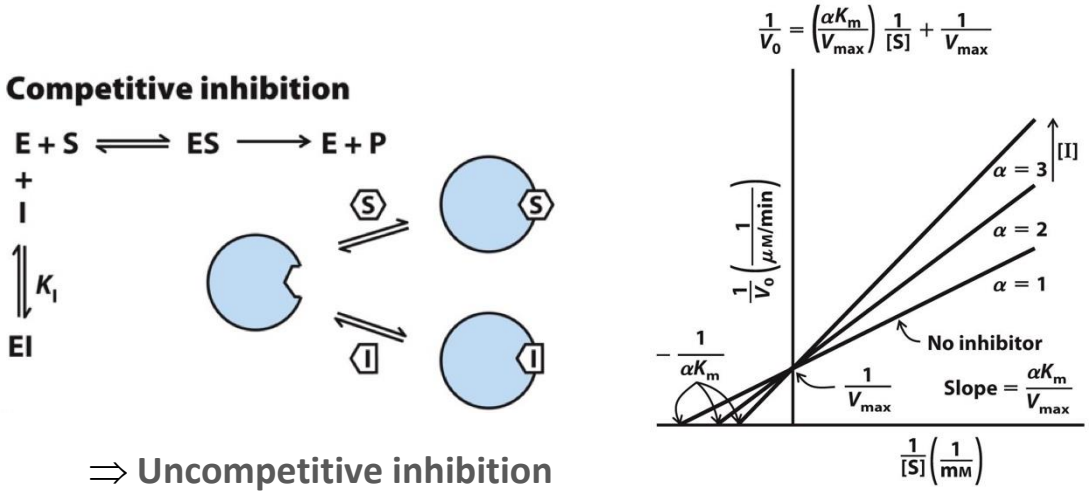
  ⇒ Irreversible inhibitor:
  - One inhibitor shut off one enzyme permanently
  - Often powerful toxins, can be used as drugs

  ⇒ Reversible inhibitor:
  - Often structural analogs of substrate or products
  - Can be used as drugs to slow down a specific enzyme
  - Can bind to the free enzyme, preventing the binding of substrate
  - Can bind to the ES, preventing the reaction

- 3 types inhibition:

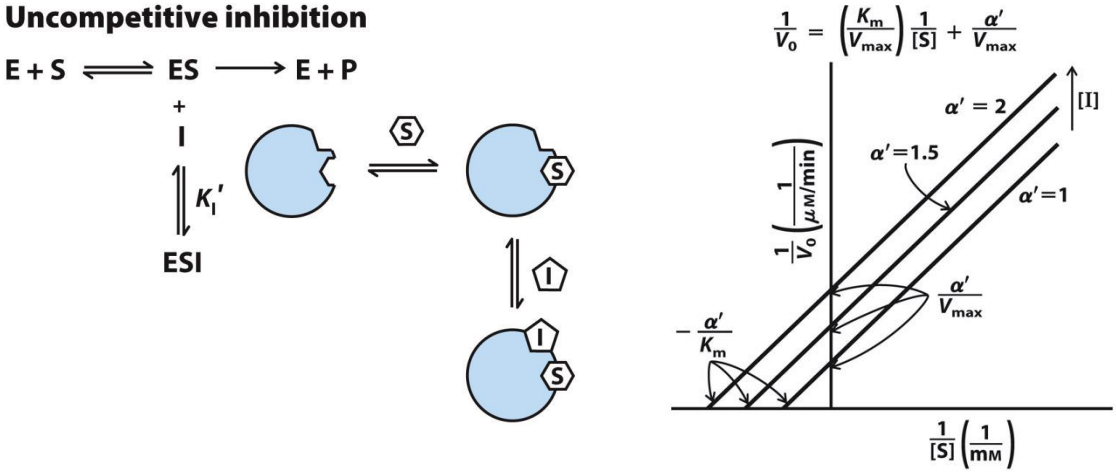
  ⇒ **Competitive inhibition**
  - Competes with substrate for binding
  - Does not change v_{max}, increase of K_m

- Lines interact with y-axis in Lineweaver-Buck plot



⇒ **Uncompetitive inhibition**

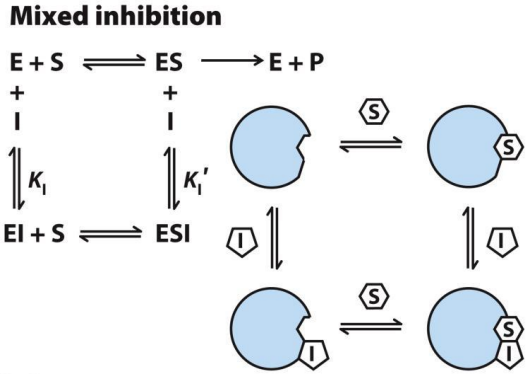
- Only binds with ES complex
- Decrease of v_{max}, decrease of K_m
- K_m/v_{max} remains constant
- Lines are parallel in Lineweaver-Buck plot

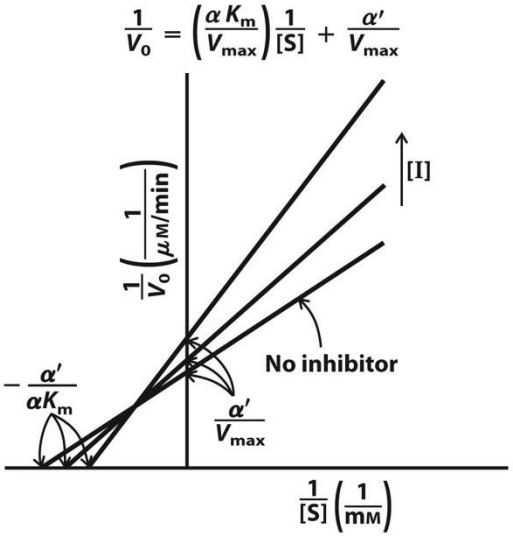


⇒ **Mixed inhibition**

- Binds enzyme with or without substrate
- Decrease of v_{max}, change of K_m

- Lines intersect left from y-axis in Lineweaver-Buck plot



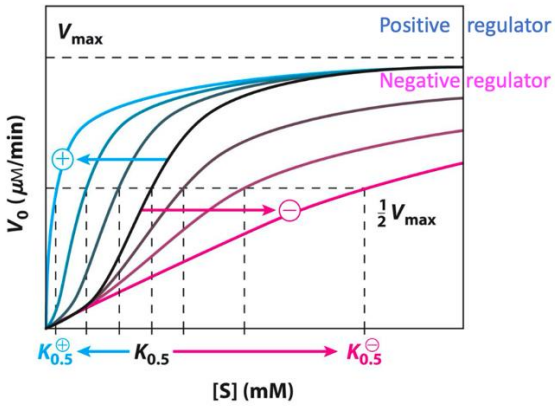


4.5 Enzyme regulation

- Enzyme can be regulated by:

  ⇒ Non-covalent modification (allosteric regulators)

  - Have +ve/-ve effectors
  - Generally small chemicals



  ⇒ Covalent modification

  ⇒ Irreversible modification

  ⇒ Reversible modification

Overall…

⇒ Control of enzyme abundances: gene expression, enzyme degradation

⇒ Control of substrate concentration: transporters, metabolism

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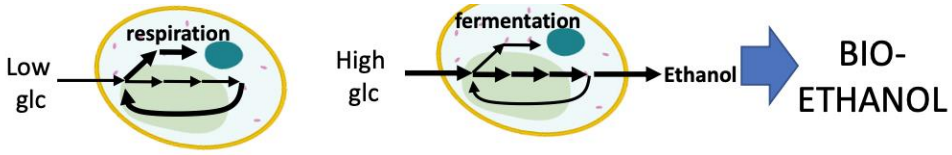
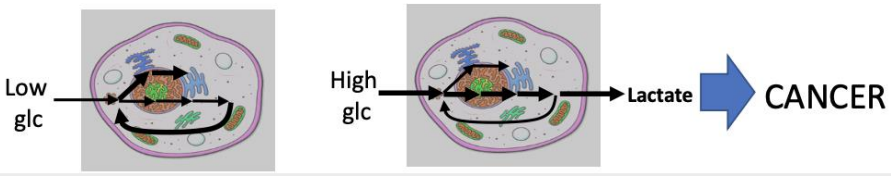
Introduction of metabolism

- Metabolism is all chemical reaction that occur in cells and organisms.
- Overall, the input is nutrients, the output is building blocks, energy and waste products
- A dynamic system and highly regulated
- Responsible for cell behavior, differentiation, metabolic state:
  For example:

  ⇒ Crabtree effect



  ⇒ Warburg effect



  ⇒ Negative feedback…

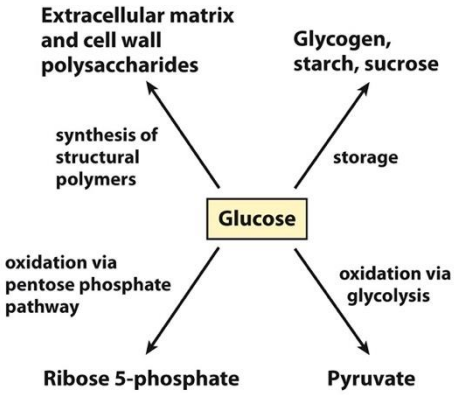
- *What is the difference between catabolism and anabolism?*

  ⇒ Catabolism deals with the breakdown of large molecules into smaller ones; anabolism is related to the synthesis of complex molecules from simpler ones.

  ⇒ Catabolism and anabolism together form metabolism, they are both energy-using processes.

Lecture 5: Catabolism

5.1 Sugar catabolism



- Four pathways for glucose catabolism:

⇒ Oxidation via glycolysis to pyruvate

⇒ Oxidation via pentose phosphate pathway to ribose 5-phosphate

⇒ Synthesis of structural polymers to extracellular matrix and cell wall polysaccharides

⇒ Storage to glycogen, starch, sucrose

- We pay attention to ***glycolysis***: the process that the 1 mole of 6-carbon glucose turns to 2 mole 3-carbon pyruvates

  ⇒ Needs no oxygen – anaerobic, happens in cytoplasm

  ⇒ Two phases:

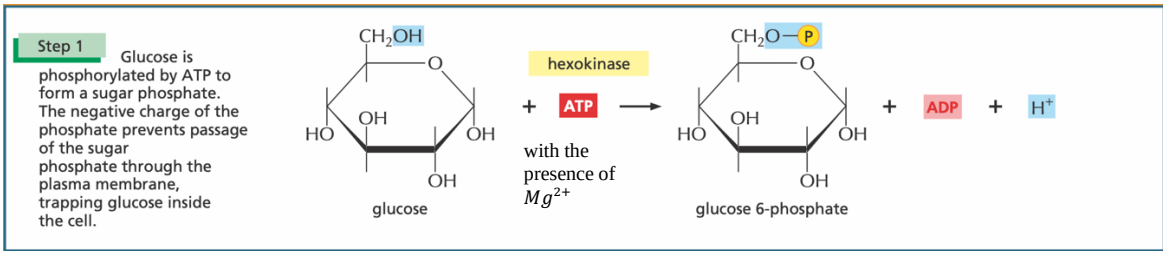
  - the preparatory phase: phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
  - the payoff phase: oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH

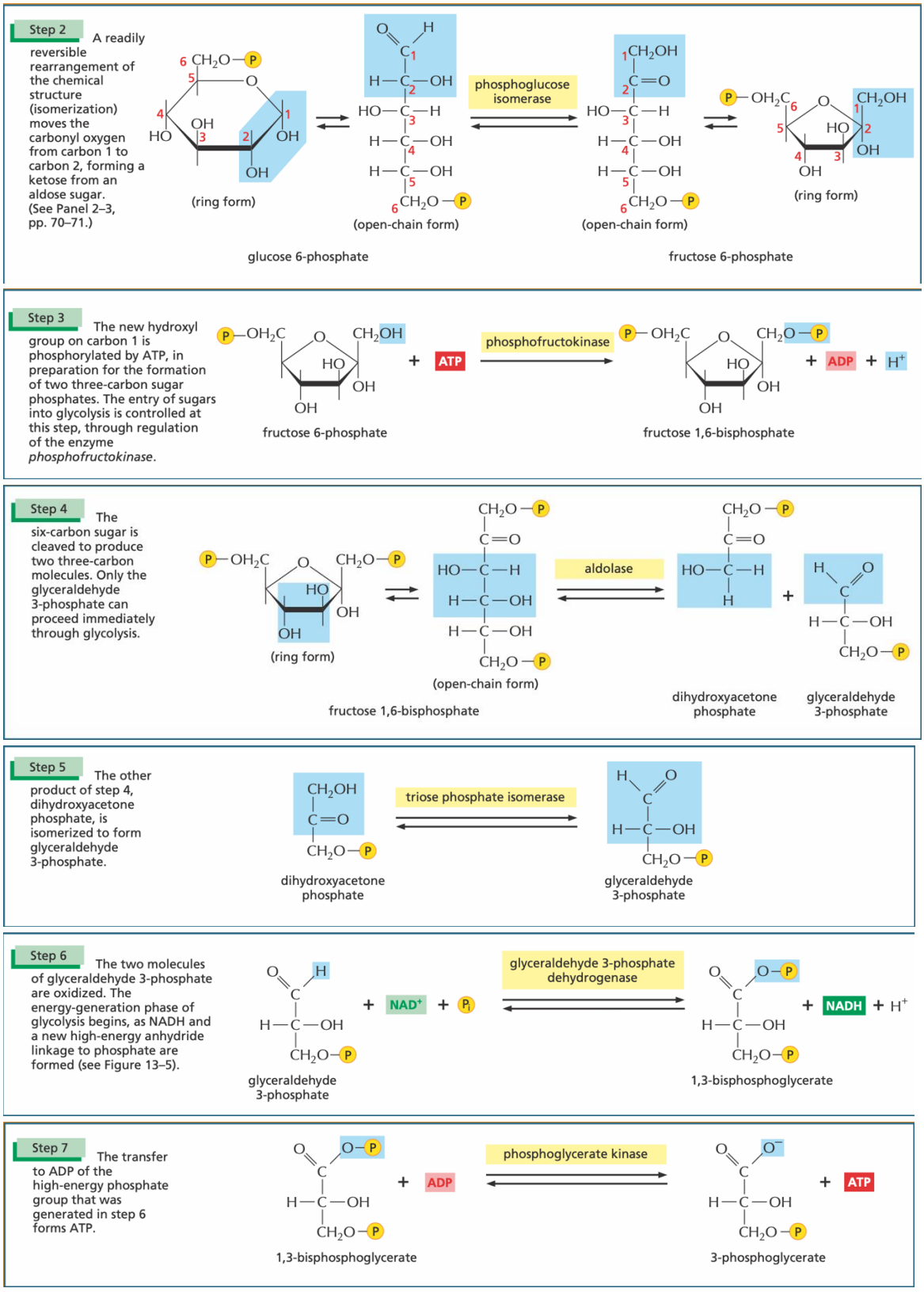
  ⇒ the overall reaction:

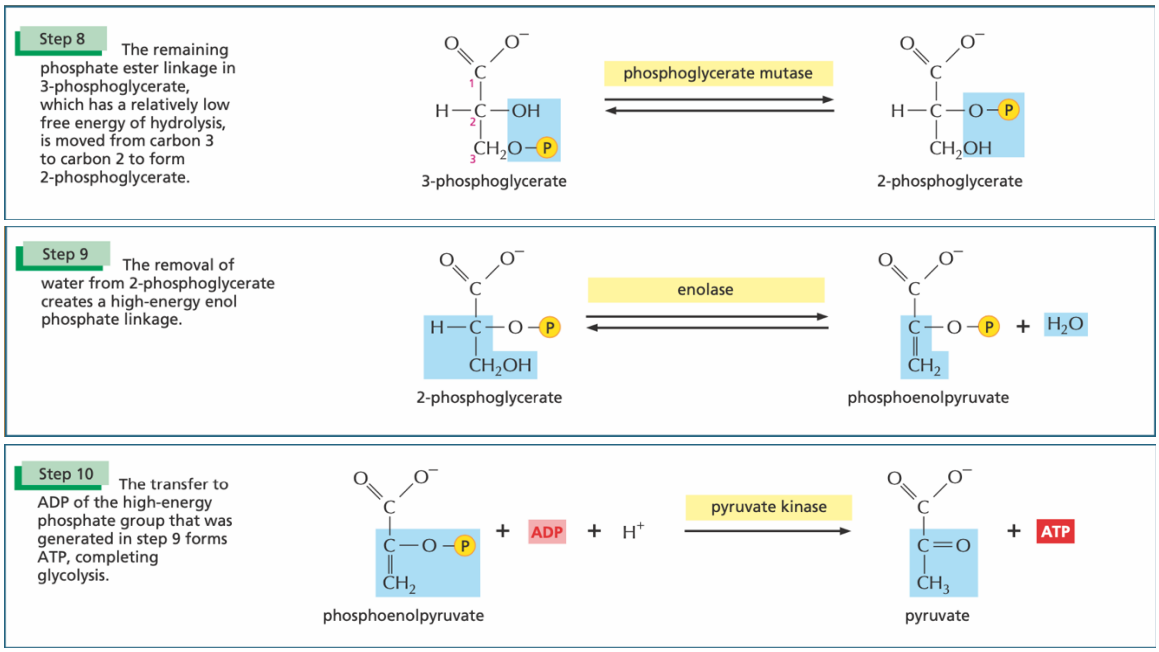
$$\boldsymbol{glucose + 2NAD^{+} + 2ADP + 2P_i \rightarrow 2\ pyryvate + 2NADH + 2H^{+} + 2ATP}$$

  - Made: 2 pyruvate, 4 ATP, 2 NADH
  - Used: 1 glucose, 2 ATP, 2 NAD^{+}

  ⇒ 10 steps involved in glycolysis:




Step 2
A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–3, pp. 70–71.)
(ring form)
(open-chain form)
phosphoglucose isomerase
(open-chain form)
(ring form)
glucose 6-phosphate
fructose 6-phosphate
Step 3
The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme phosphofructokinase.
ATP
phosphofructokinase
ADP
H+
fructose 6-phosphate
fructose 1,6-bisphosphate
Step 4
The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.
(ring form)
aldolase
(open-chain form)
fructose 1,6-bisphosphate
dihydroxyacetone phosphate
glyceraldehyde 3-phosphate
Step 5
The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.
triose phosphate isomerase
dihydroxyacetone phosphate
glyceraldehyde 3-phosphate
Step 6
The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy-generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 13–5).
NAD+
Pi
glyceraldehyde 3-phosphate dehydrogenase
NADH
H+
glyceraldehyde 3-phosphate
1,3-bisphosphoglycerate
Step 7
The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.
ADP
phosphoglycerate kinase
ATP
1,3-bisphosphoglycerate
3-phosphoglycerate



- Step 1 – step 5 are the preparatory phase
- Step 6 – step 10 are the payoff phase

⇒ The role of enzymes:

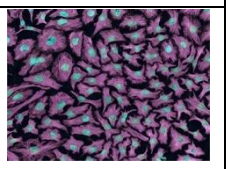
Type of Enzyme	***Function***	***Step***
Kinase	Catalyzes the addition of a phosphate group	1,3,7,10
Isomerase	Catalyzes the rearrangement of bonds in a single molecule	2,5
Dehydrogenase	Catalyzes the oxidation of a molecule by removing a hydrogen atom plus an electron	6
Mutase	Catalyzes the shifting of a chemical group from one position to another in a molecule	8,10

⇒ The energy change for each step:

Step	ΔG^{θ} *(kJ/mol)*	*Step*	ΔG^{θ} *(kJ/mol)*
1	-16.74	**6**	+6.28
2	+1.74	**7**	-18.83
3	-14.23	**8**	+4.60
4	+23.85	**9**	+1.67
5	+7.53	**10**	-31.38

- From the aspect of energy, the glycolysis can be seen as two stages. The breakdown of glucose into pyruvate releases energy while the synthesis of ATP from ADP absorbs energy
- From calculation, ΔG^{θ} =-135.56 kJ/mol, which indicates that this process releases energy

$\Rightarrow$ The fate of pyruvate:

Condition	***Product***	
Hypoxic or anaerobic (yeast)	lactate	
Hypoxic or anaerobic (vigorously contracting muscle, erythrocytes, some microorganisms)	ethanol, CO_2	 *(muscle)*
Aerobic (animals, plants, microbial cells)	Acetyl-CoA (then to TCA cycle)	

5.2 TCA cycle

- ***The link reaction***: Under aerobic condition, 3-cabon pyruvates are decarboxylated to 2-carbon acetyl-CoA in mitochondria with a wasted CO_2.

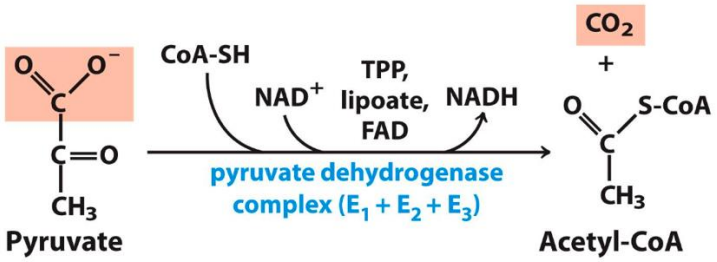
  ⇒ Net reaction:

  - Oxidative decarboxylation of pyruvate
  - First carbon of glucose to be fully oxidized

  ⇒ Catalyzed by the pyruvate dehydrogenase complex:

  - The complex contains 5 enzymes
  - *TPP, lipoyllysine, FAD* are prosthetic groups
  - *NAD^+, CoA-SH* are co-substrates

  ⇒ The products including acetyl-CoA, NADH and CO_2

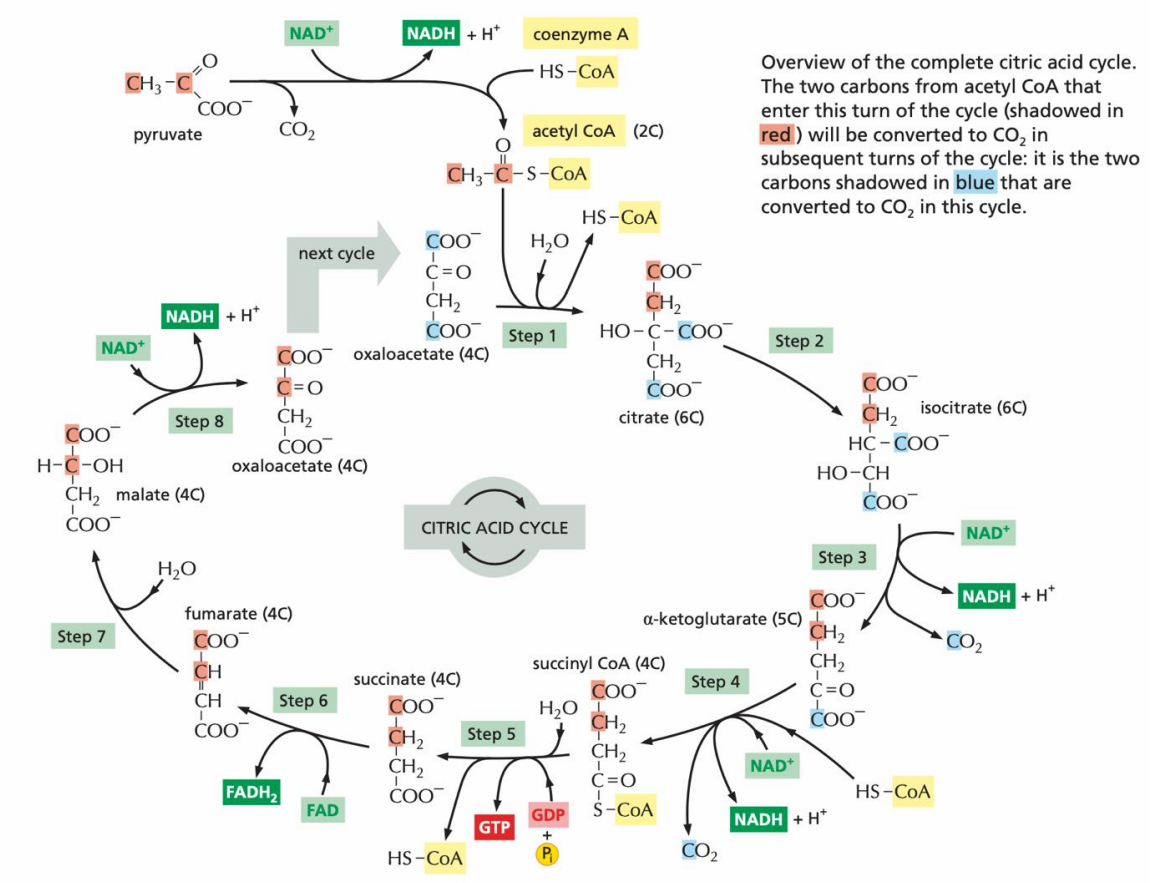


- **TCA cycle/Citric acid cycle/Krebs cycle**

  ⇒ The net reaction is:

  $$Acetyl\text{-}CoA + 3NAD^+ + FAD + GDP + P_i + 2H_20 \rightarrow 2CO_2 + 3NADH + FADH_2 + GTP + CoA + 3H^+$$

  - Net oxidation of two carbons to CO_2 equivalent to two carbons of acetyl-CoA but not the exact same carbons
  - Energy captured by electron transfer to NADH and FADH2
  - Generates 1 GTP which can be converted to ATP (i.e. generate a small amount of ATP)

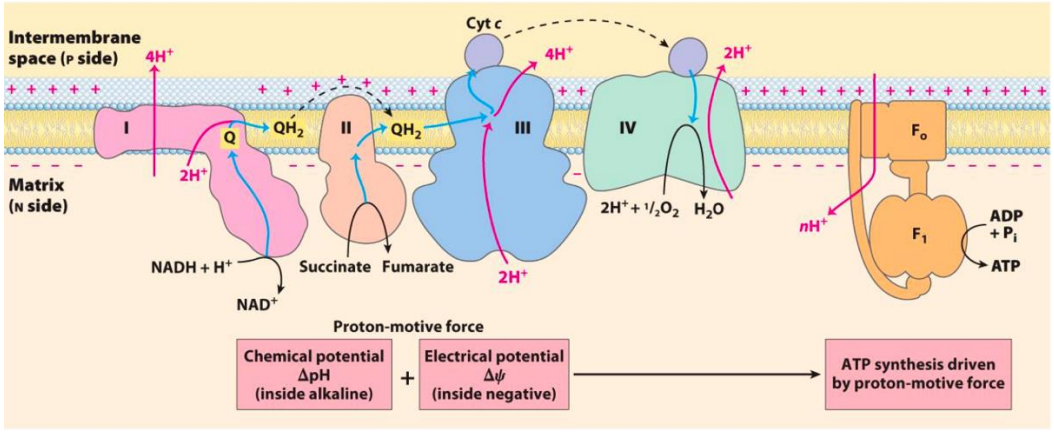


⇒ We pay attention to the change of number of carbons:

- The 2-C *acetyl-CoA* and the 4-C *oxaloacetate* form the 6-C *citrate*
- The 6-C *citrate* forms the 5-C *α-ketoglutarate* by removing a 1-C CO_2, producing 1 NADH
- the 5-C *α-ketoglutarate* forms the 4-C *succinyl-CoA* by removing a 1-C CO_2, producing 1 NADH
- the 4-C compound converted back in the final step, to the re-start the cycle, producing 1 NADH and 1 $FADH_2$

5.3 Oxidative phosphorylation

- The reduced coenzymes – NADH and $FADH_2$ – deliver their electrons to the inner mitochondria membrane to make ATP, known as electron transfer chain.



⇒ How ATP is made:

- The reduced coenzymes deliver their electrons to the first protein in the electron transfer chain, and the protein is reduced
- The electron passes to the second protein and reduce the protein again. This happens all along the electron transfer chain, release energy
- This energy is used to pump the H^+ into the intermembrane space
- Intermembrane contains a higher concentration of H^+, creating a diffusion gradient; intermembrane is more positive-charged due to the protons, creating an electrochemical gradient
- H^+ diffuses back to the mitochondria through the *ATP synthesis enzyme*. The flow of H^+ causes the enzyme makes ATP from ADP and P_i, known as *chemiosmosis*

⇒ $\boldsymbol{1\ NHDH + 11\ H^+ + ½\ O_2 \rightarrow 10\ H^+ + H_2O + NAD^+}$

$\boldsymbol{1\ FADH_2 + 6\ H^+ + ½\ O_2 \rightarrow FAD + 6\ H^+ + H_2O}$

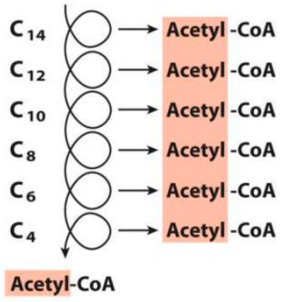
- NADH and $FADH_2$ transfer different number of protons – the number of ATP they synthesized is different.

⇒ 1 glucose forms 2 $FADH_2$ and 10 NADH.

- given 1 NADH forms 3 ATP, 1 $FADH_2$ forms 2 ATP
- the total ATP formed is 34 moles.

5.4 Lipid catabolism: beta-oxidation

- During fatty acid oxidation, a 2-carbon unit is removed from the fatty acid chain once. The removal of carbon initially starts from the beta carbon on the chain. Therefore, the process is known as the beta-oxidation.
- Beta-oxidation has a general formula:

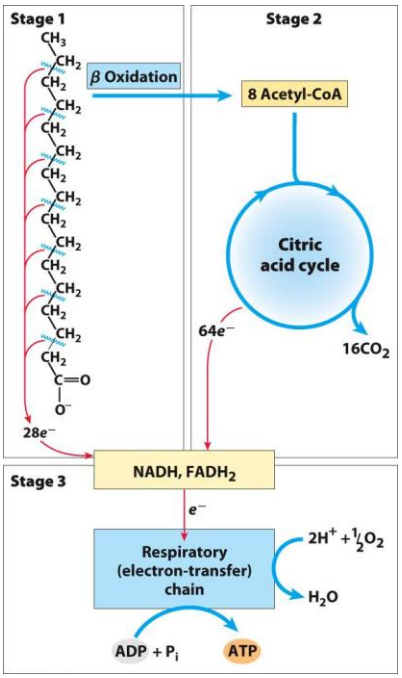


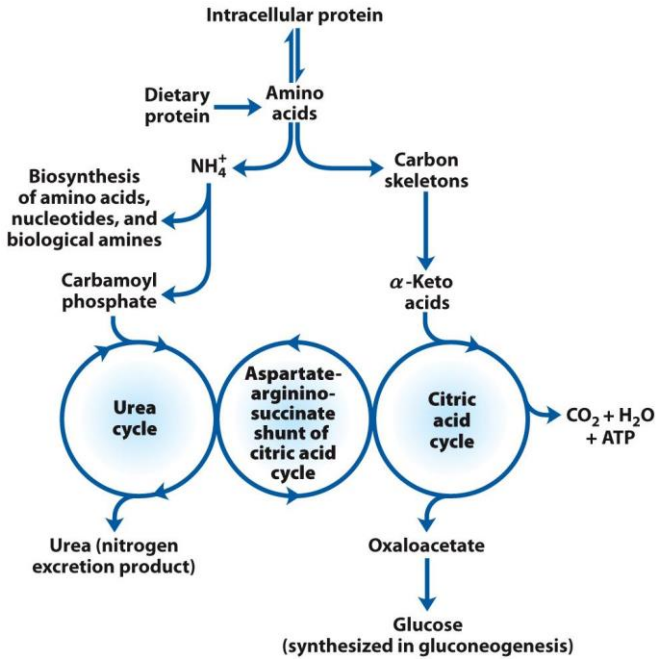
$$C_n\text{-acyl-CoA} + FAD + NAD + H_20 + CoA \rightarrow$$
$$C_{n-2}\text{-acyl-CoA} + FADH_2 + NADH + H^+ + \text{acetyl-CoA}$$

⇒ 1 acetyl-CoA, 1 $FADH_2$, 1 NADH are formed by removing two carbons from the fatty acid chain.

⇒ We take a 16-C fatty acid as an example (*see left*), it can generate 8 acetyl-CoA

- After beta-oxidation, acetyl-CoA are coming to the TCA cycle.
- The NADH, $FADH_2$ produced from the beta-oxidation and the TCA cycle are then converted to ATP by oxidative phosphorylation.



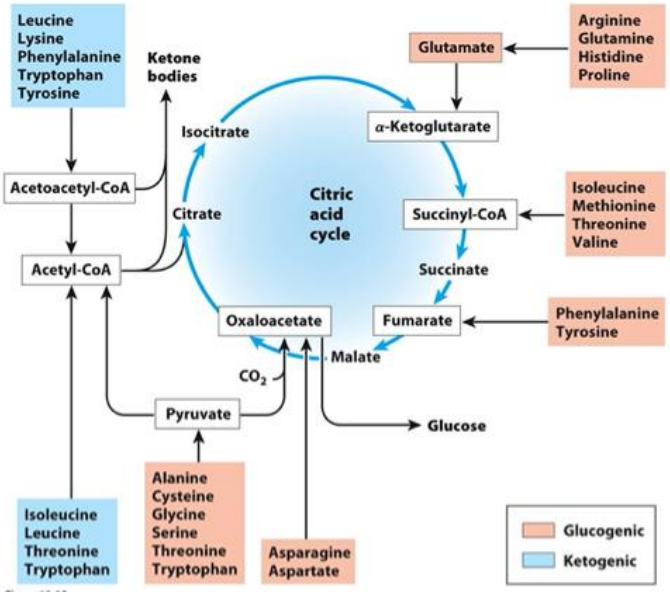


5.5 Amino acid catabolism

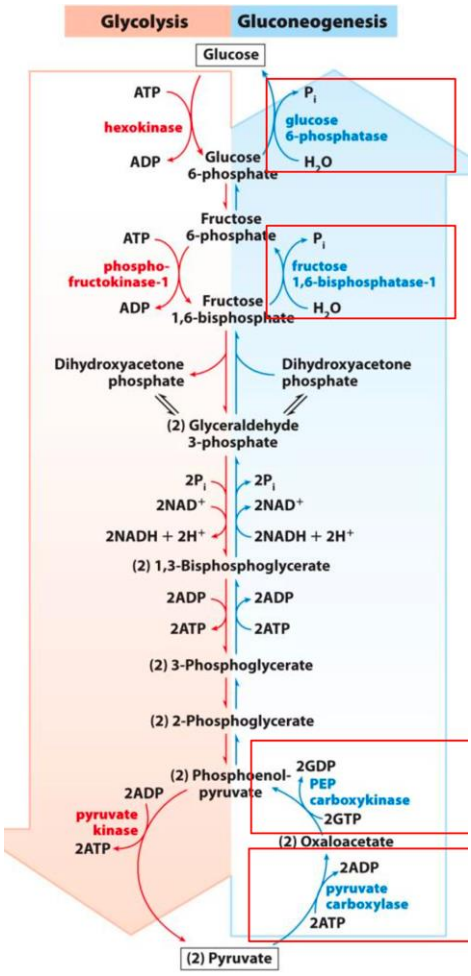
- A complex mechanism, 2 different pathways *(see the second diagram above)*
- Briefly, an amino acid will be degraded into an ammonia group (NH_4^+) and the carbon skeleton.

  ⇒ the ammonia group is fitted into the urea cycle to produce urea

  ⇒ the carbon skeleton is fitted into the TCA cycle to generate energy
- The carbon skeletons of different amino acids enter the TCA cycle at different stages. note: glucogenic and ketogenic are two groups of amino acids with different roles.



Lecture 6: Anabolism



6.1 Sugar synthesis

- Gluconeogenesis

⇒ synthesis of glucose from pyruvate

⇒ an opposite reaction of glycolysis, requires energy

⇒ most enzymes are the same (reversible reactions), but four enzymes are different, these steps require energy

⇒ typical organ: liver

⇒ "expensive": great energy input

2 pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 2 H_2O → glucose + 4 ADP + 2 GDP + 6 P_i + 2 NAD^+

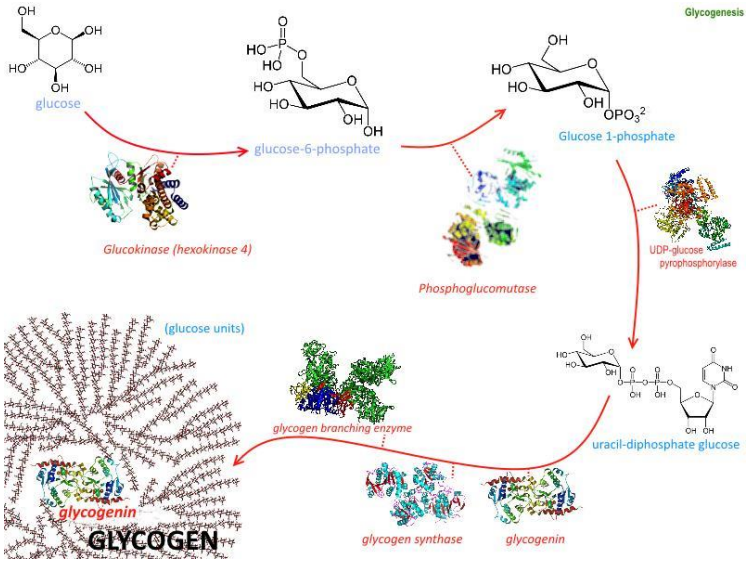
⇒ glucose is generated when glycogen stores are depleted: starvation/ vigorous exercise

⇒ can generate glucose from amino acids, not fatty acids

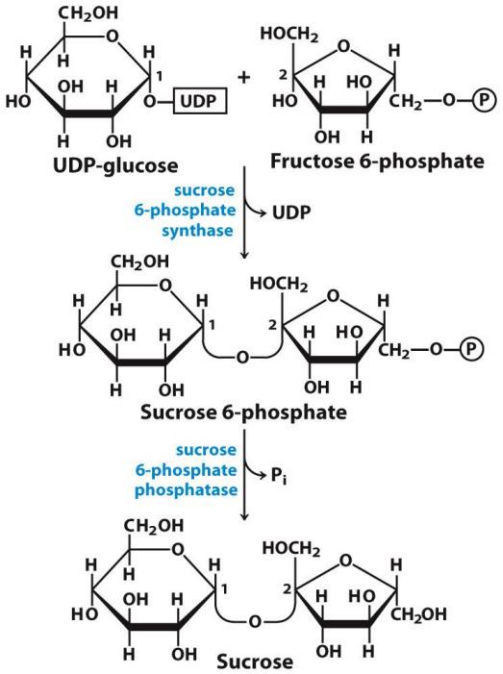
- Glycogen synthesis

⇒ polymer, made of glucose

⇒ typical organ: liver, skeletal muscle; also in yeast and other microbes

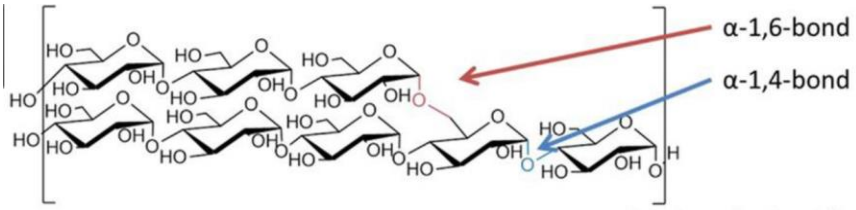


- Sucrose synthesis

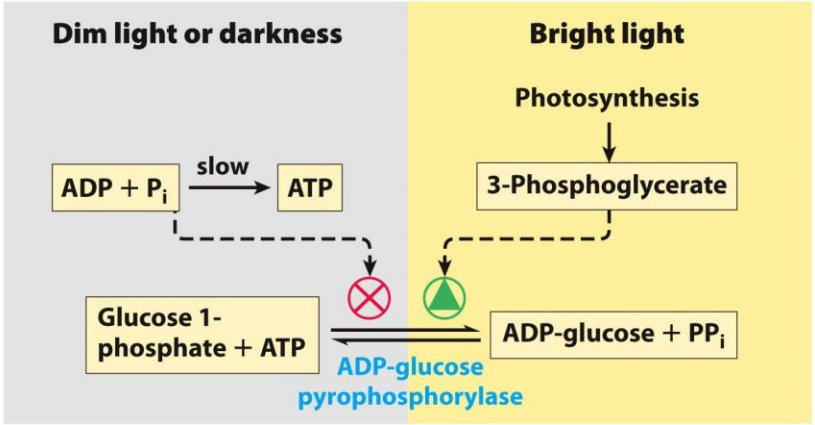


- Starch synthesis

⇒ sugar units linked by two types of bonds in a compact way (specific enzyme for degradation)



⇒ needs ADP at darkness, no need of ADP under light

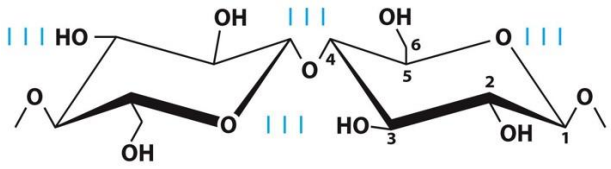


- Cellulose synthesis

  ⇒ the main component of cell walls

  ⇒ vert strong (H bonds)

  ⇒ glucose monomers linked by β(1→4) linkages



(β1→4)-linked D-glucose units

  ⇒ bacteria also make cellulose

6.2 Lipid synthesis

- **Fatty acid synthesis**

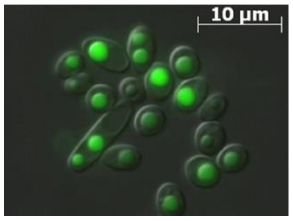
  ⇒ Similar to the beta-oxidation, fatty acid synthesis starts from acetyl-CoA, adding two carbons to the chain at each cycle (that's why most fatty acids have even number of carbons)

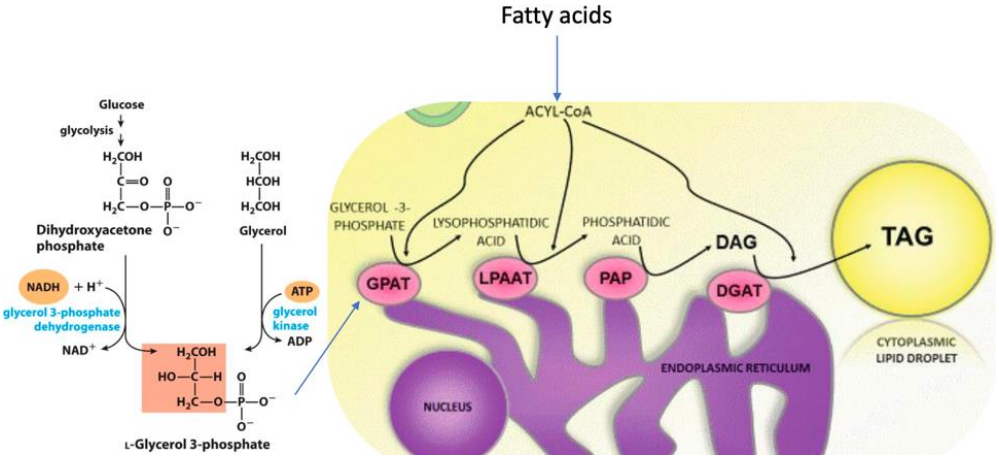
  ⇒ The process consumes NADPH

  ⇒ The cycle has four enzyme-catalyzed steps:

  - Condensation of the growing chain with activated acetate
  - Reduction of carbonyl to hydroxyl
  - Dehydration of alcohol to trans-alkene
  - Reduction of alkene to alkane

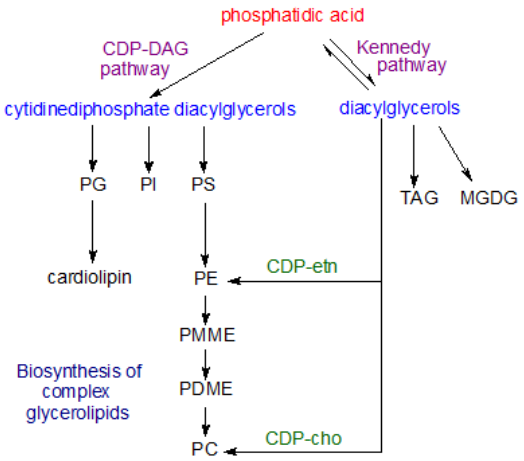
- **Triacylglycerol synthesis**





⇒ Needs three different fatty acids

⇒ Glycerol 3-phosphate is normally stored in lipid droplets (see left)



- **Phospholipid synthesis**

⇒ Two pathways depend on their roles:

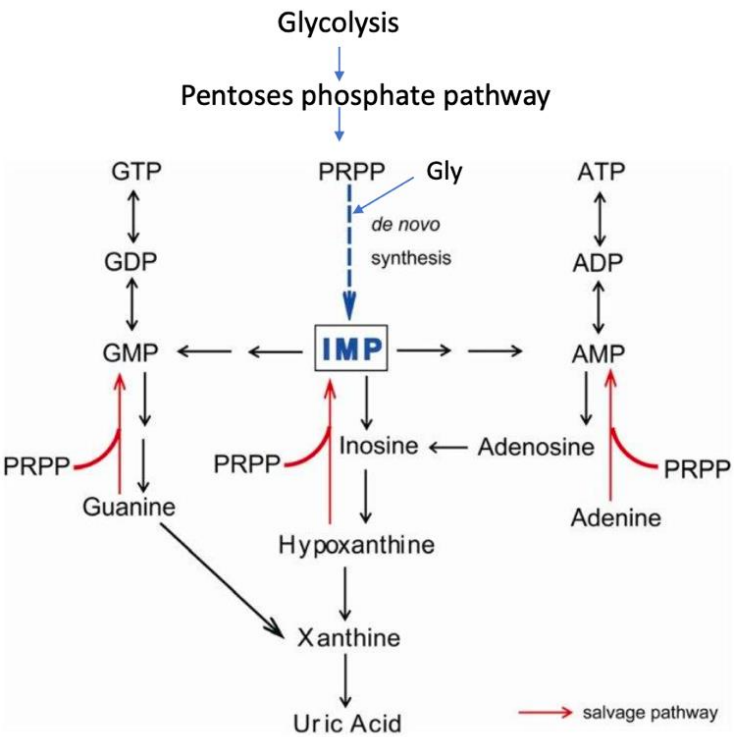
- CDP-DAG pathway
- Kennedy pathway

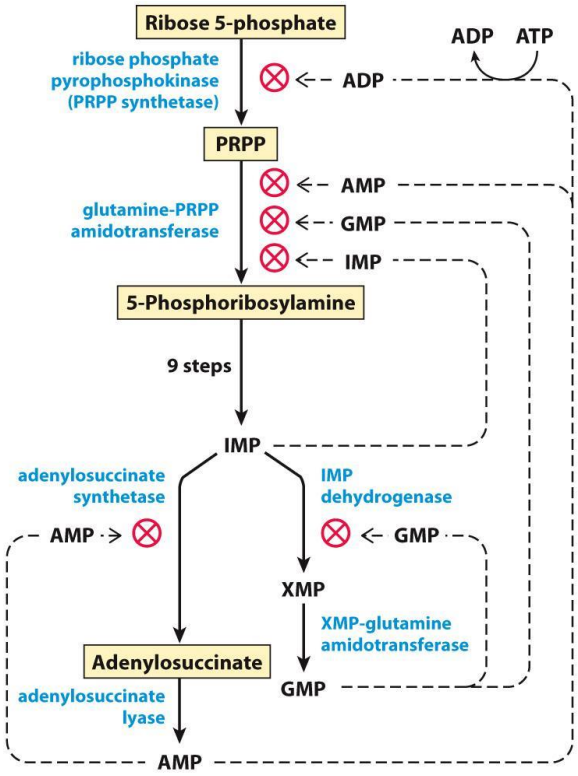
6.3 Nucleotide synthesis

- Two pathways:

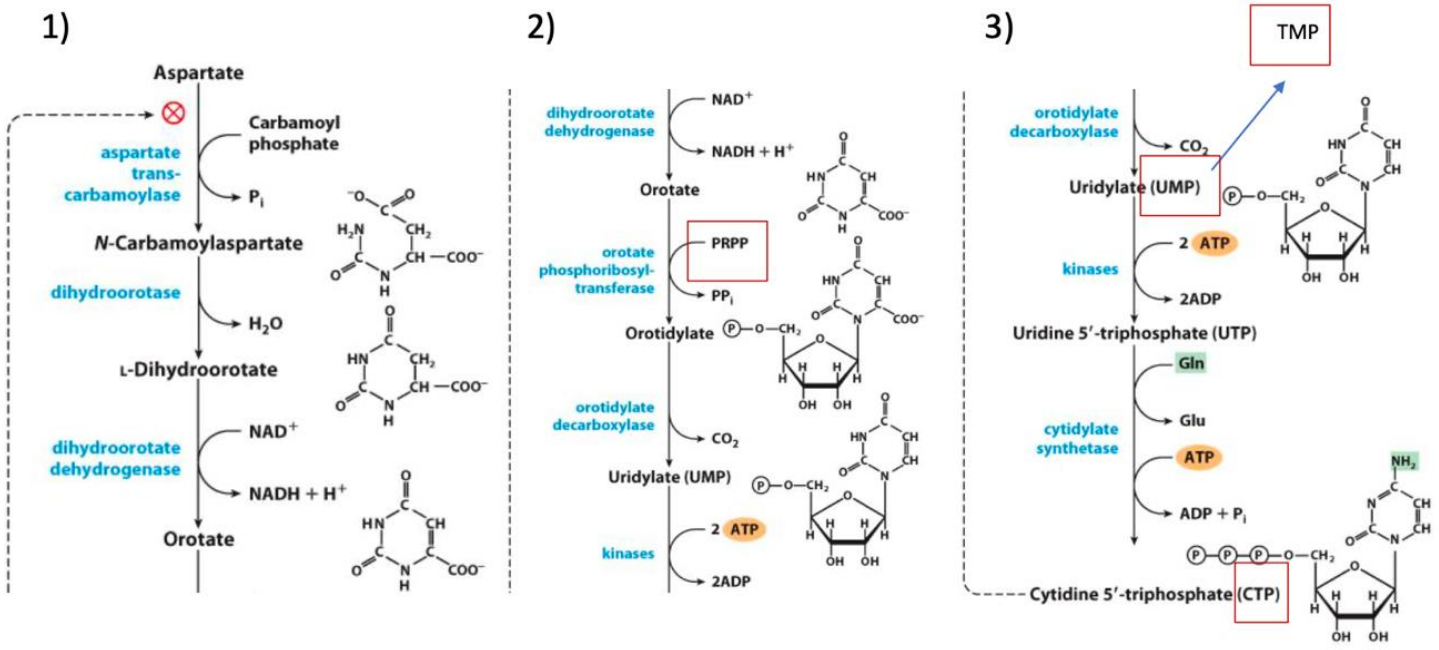
⇒ Purine (adenine, guanine) pathway:

- negatively regulated





⇒ Pyrimidine (cytosine, thymine, uracil) pathway:

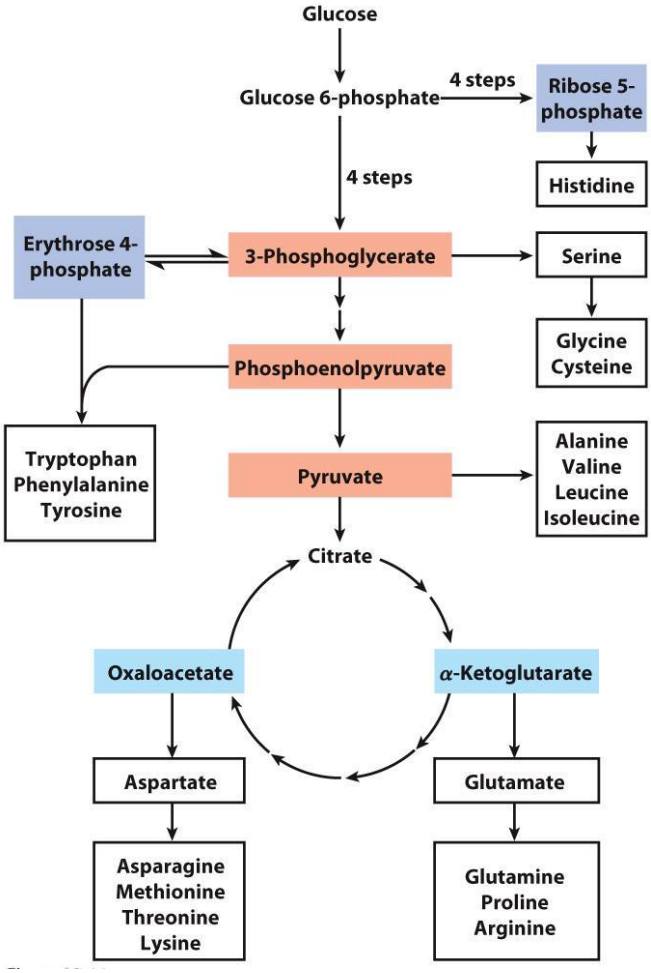


- Glu provides most of the amino groups
- Gly is the precursor of purines

- Asp is the precursor of pyrimidines

6.4 Amino acid synthesis

- Bacteria can synthesis the 20 amino acids from the intermediates in glycolysis and TCA cycle
- Mammals need require them from diet



Lecture 7, 8: Biotechnology

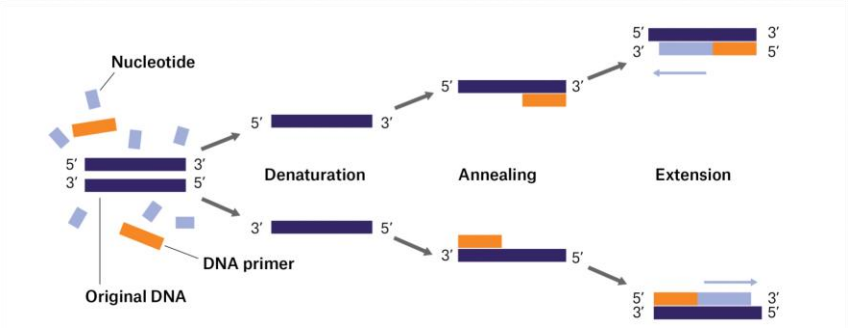
7.1 DNA

- DNA edit: DNA synthesis, chemical synthesis
- DNA copy: PCR polymerase

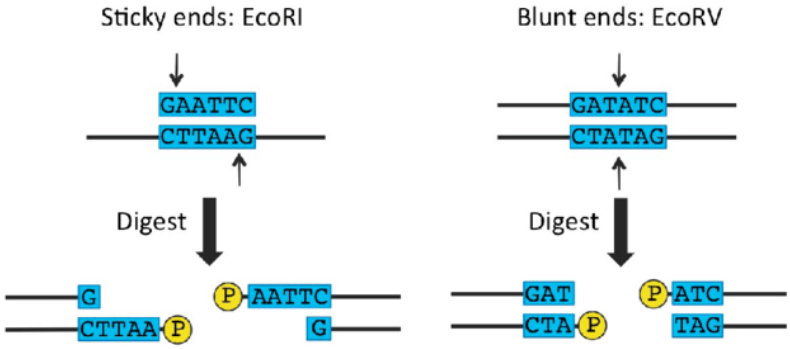
⇒ Denaturation

⇒ Annealing

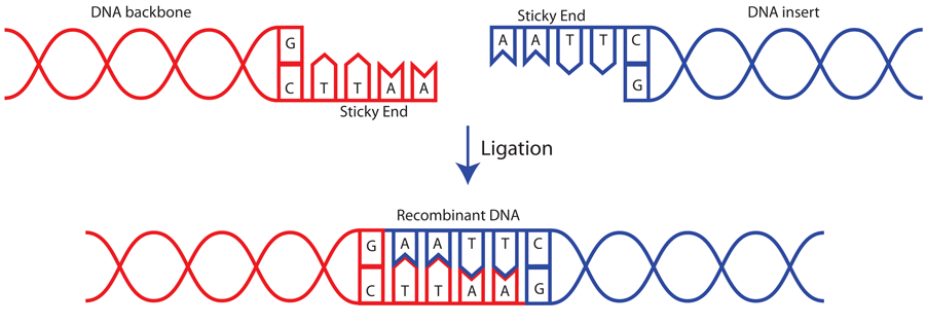
⇒ Extension



- DNA cut: restriction enzymes



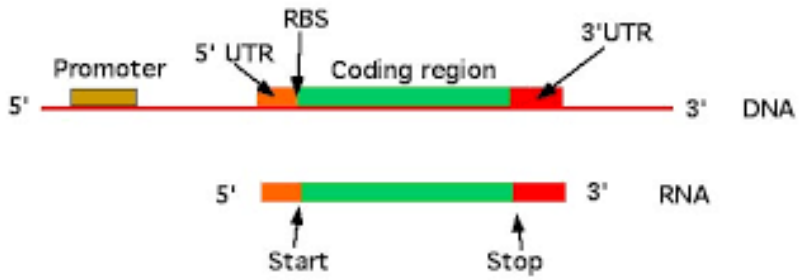
- DNA paste: DNA ligase



- DNA read: DNA sequencing

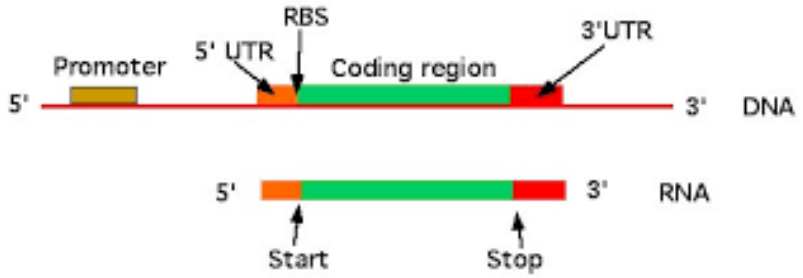
7.2 RNA

Transcription engineering



7.3 Proteins

Translation engineering



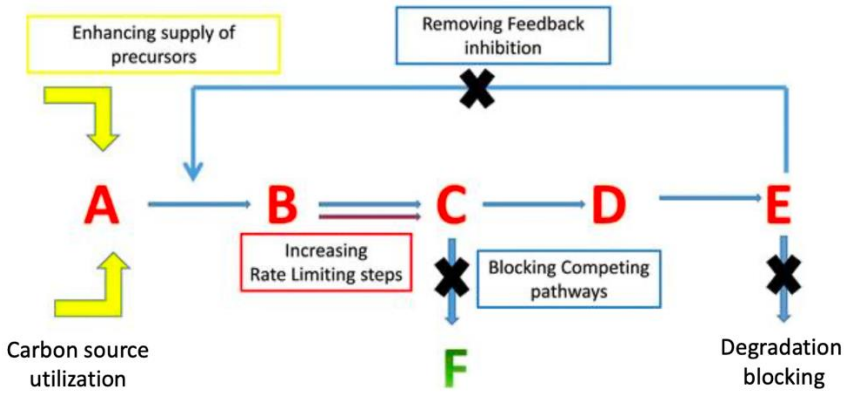
7.4 Enzymes

Enzyme engineering

- Rational design
- Evolution-based design
- Semi-rational design
- Mutation-selection methods

7.5 Metabolism

Metabolic engineering



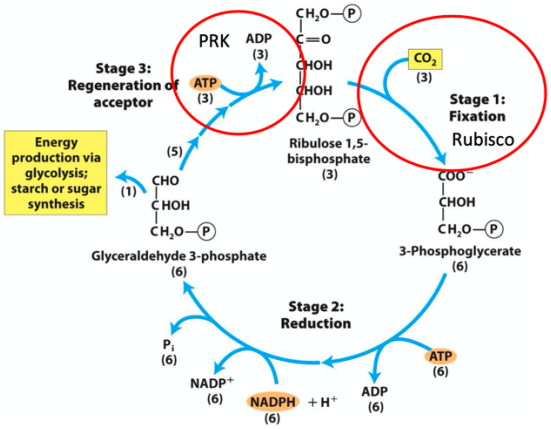
7.6 Engineering CO_2 fixation

Use E.coli to fix CO_2 since plants, algae and cyanobacteria are difficult to engineer.

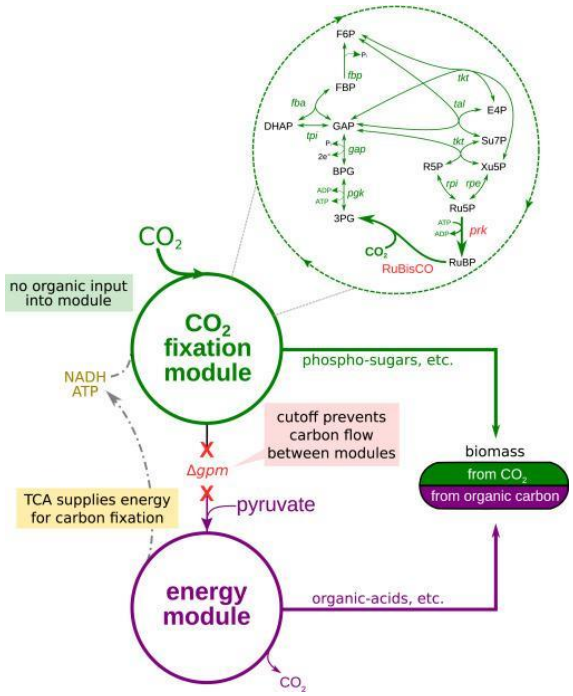
⇒ Need of knowing the Calvin cycle:

3 CO_2 + 6 NADPH + 5 H_20 + 9 ATP →

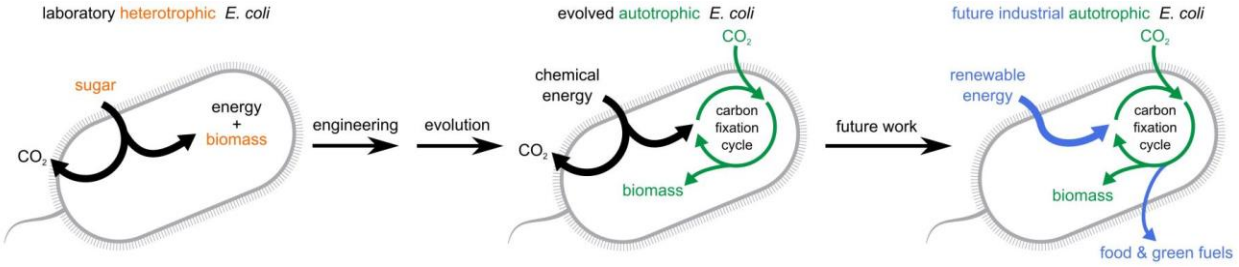
glyceraldehyde 3-phosphate + 6 $NADP^+$ + 9 ADP + 8 P_i



⇒ One example: hemi-autotrophic – using two pathways

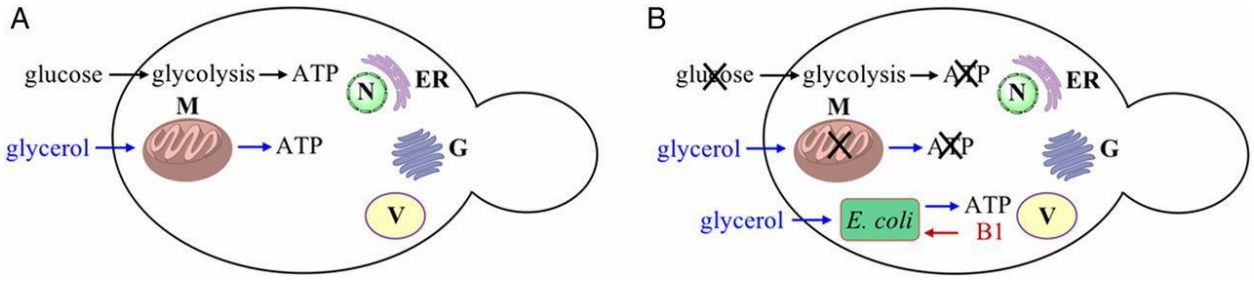


- Cut the enzyme for pyruvate, separate the two pathways
- After evolution



7.7 Engineering endosymbionts

Example: engineering yeast



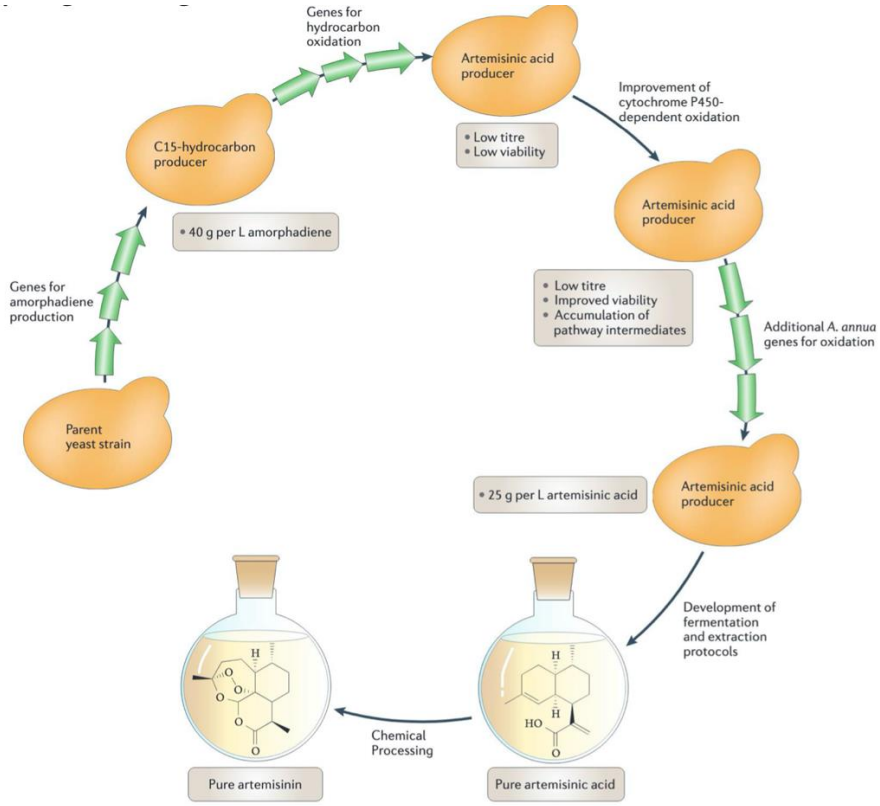
Cut some genes for glycolysis out

Cut the genes for mitochondria out

Introduce E.coli which is able to produce ATP from glycerol, to the yeast

7.8 Metabolic engineering

Example: production of antimalaria drugs by engineering yeast



7.9 Biofuel engineering

⇒ Microbial oil is the oil derived from microbial sources

⇒ Substrate can be turned into oil from microbial fermentation

⇒ Example: the microorganisms Yarrowia with starch as substrate to produce biofuels (in this case, 3 acid glycerols)

- α-amylase and glucoamylase breakdown the starch for the microorganism to use