mSSB UE2.3 Metabolic Engineering

Cellular metabolism and the main metabolic pathways

By
Ioana Popescu

Cellular metabolism

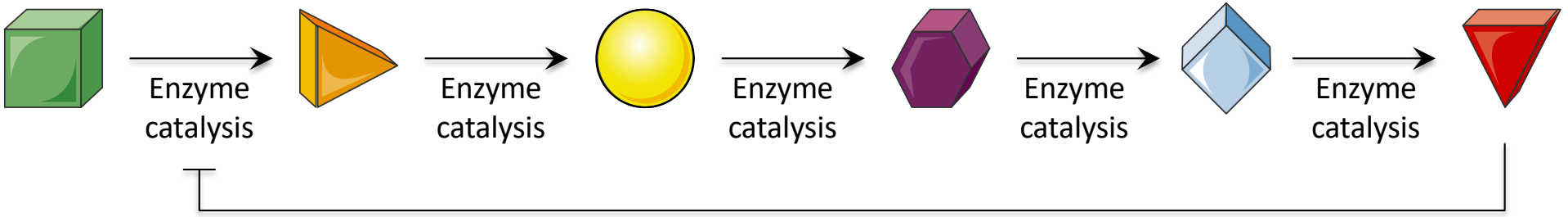
Metabolite:
- a naturally occurring molecule typically under 1000 MW

Enzyme:
- any protein which catalyzes chemical reactions involving the small molecules
- (ribozyme = catalytic RNAs)

Transporter:
- a membrane bound protein which shuttles ions, small molecules or macromolecules across membranes, into cells or out of cells.

Metabolism:
- complete set of biochemical reactions within a cell
- sum of processes involved in energy conversions in the cell

Metabolic pathways
- complex sequences of controlled chemical reactions



Cellular metabolism

Metabolite:

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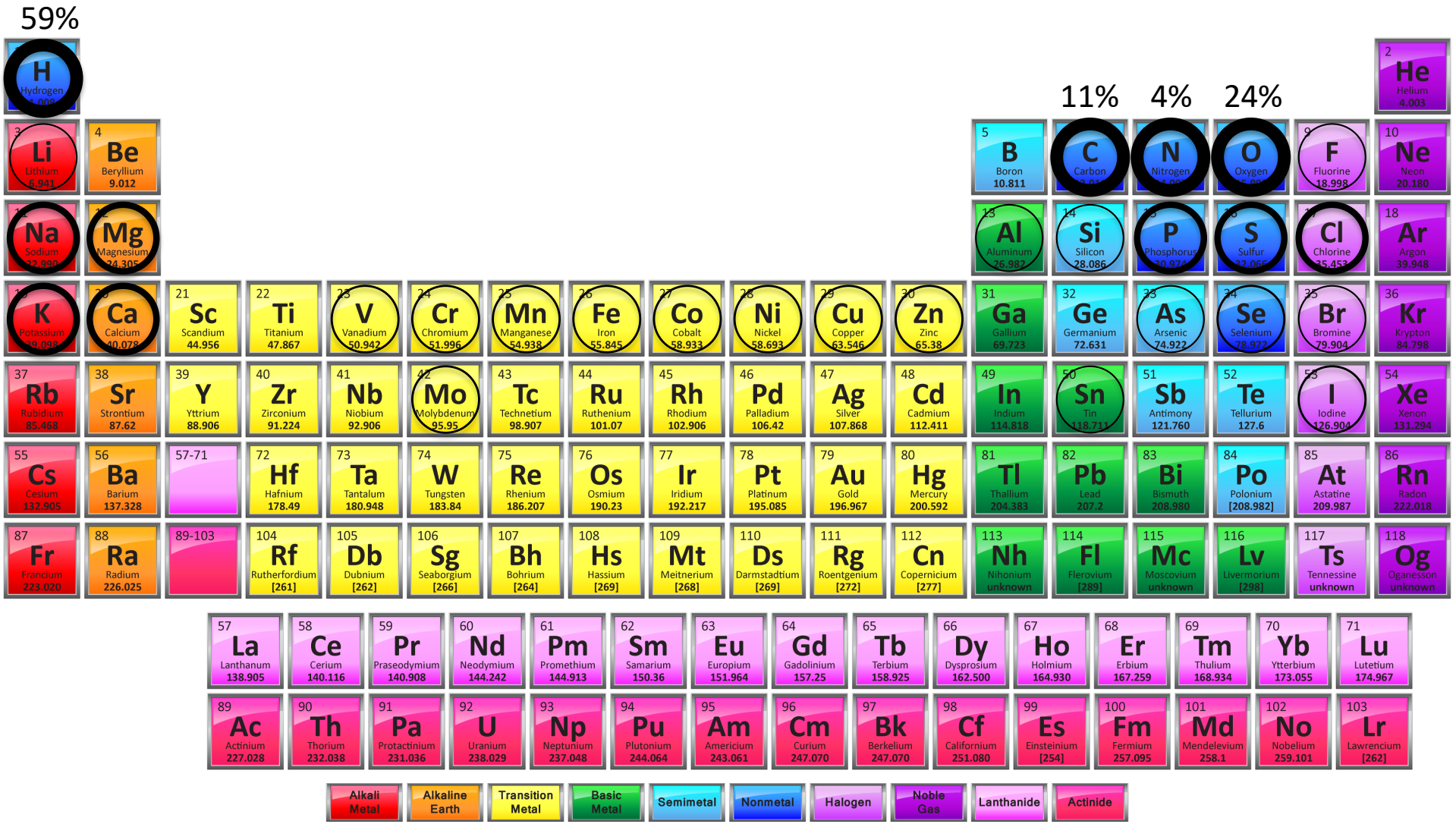
Background information from organic chemistry:

- Elements
- Bonds
- Molecules
- Isotopes
- Ions
- Nomenclature
- Stereochemistry

Chemical components of a cell



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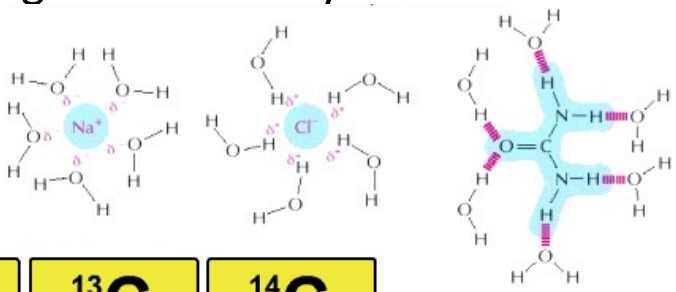
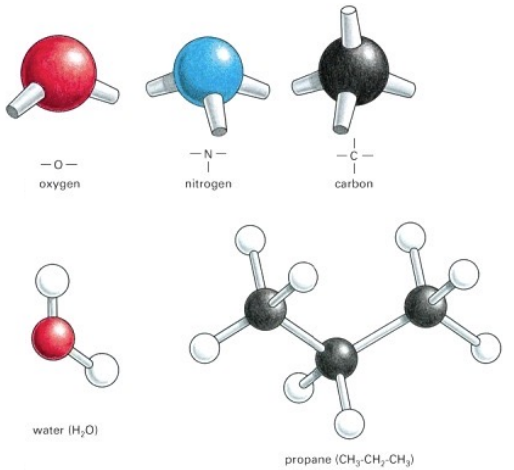
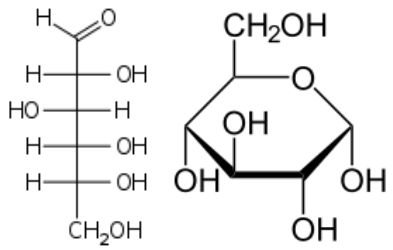
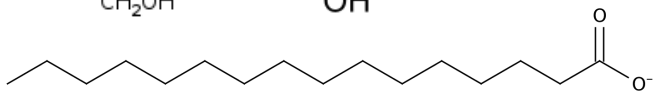


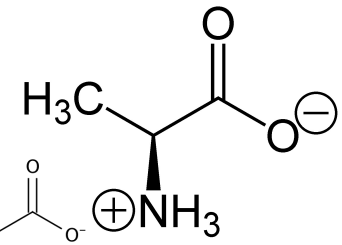


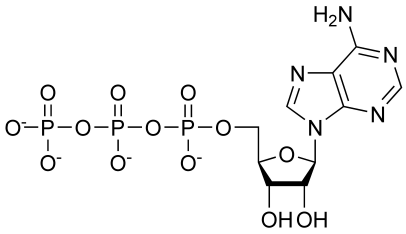
^{12}C	^{13}C	^{14}C
12.00000 98.89%	13.00335 1.11%	14.0 $t_{1/2}$ = 5715yrs
Stable	Stable	Radioactive Cosmogenic/ anthropogenic

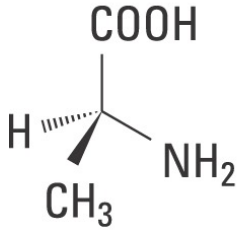
Building blocks of the cell	Larger units of the cell
SUGARS	POLYSACCHARIDES
FATTY ACIDS	FATS, LIPIDS, MEMBRANES
AMINO ACIDS	PROTEINS
NUCLEOTIDES	NUCLEIC ACIDS











Alberts et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science (2002)

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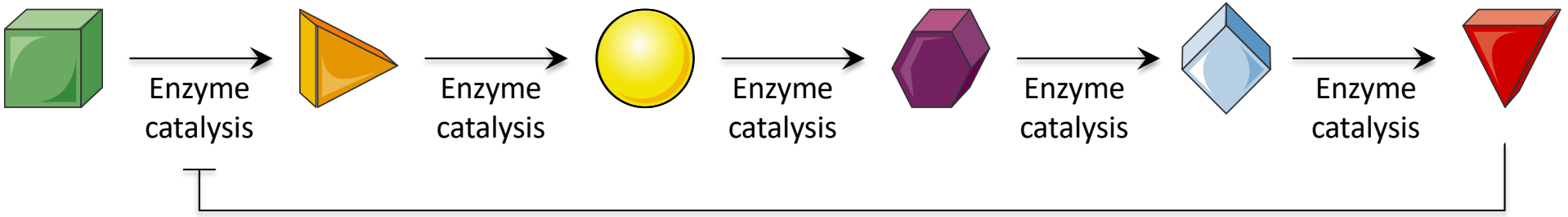
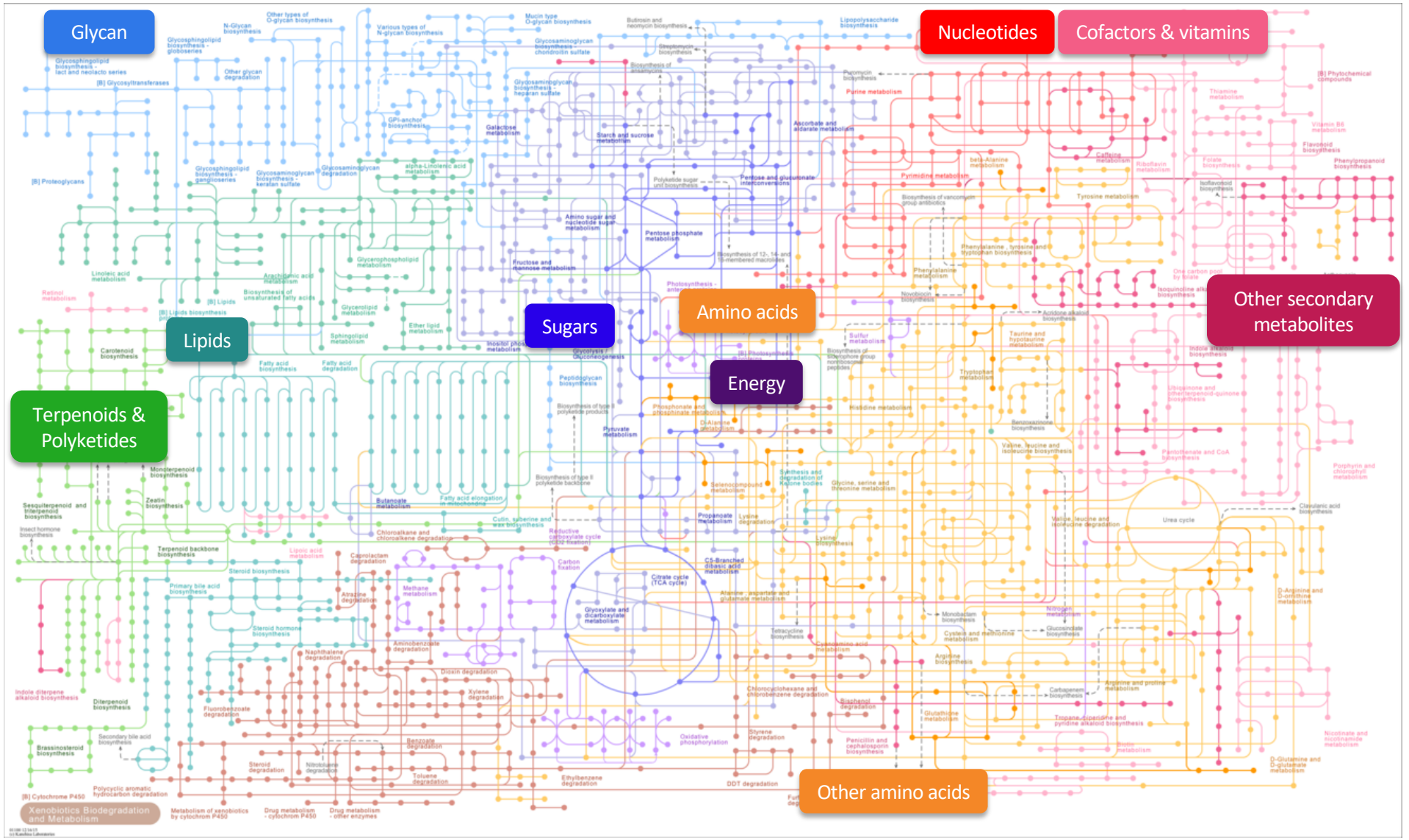


Cellular metabolism



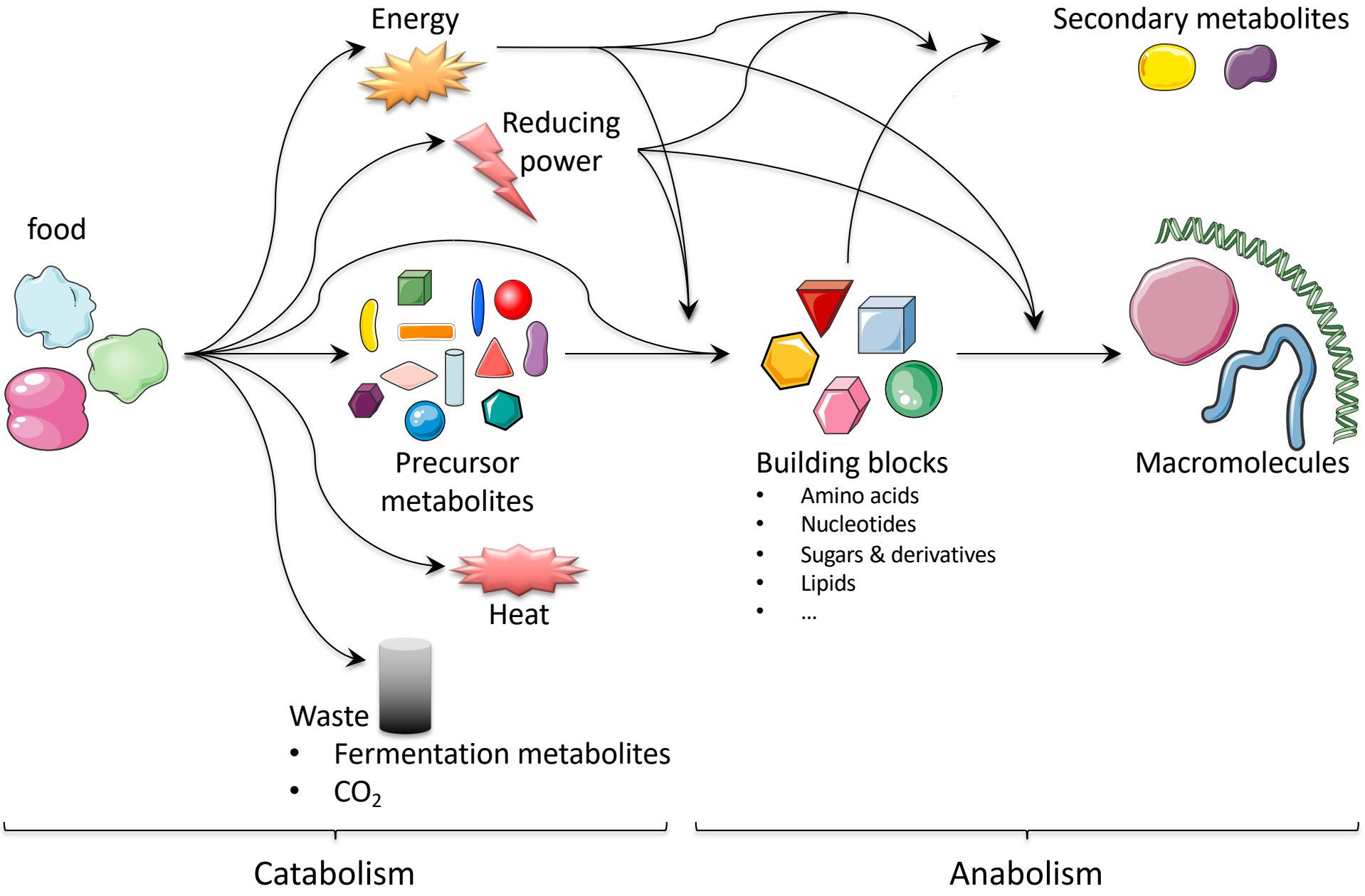
KEGG Kyoto Encyclopedia of Genes and Genomes

MetaCyc™

BRENDA The Comprehensive Enzyme Information System

Rhea

Catabolic and anabolic pathways in cellular metabolism

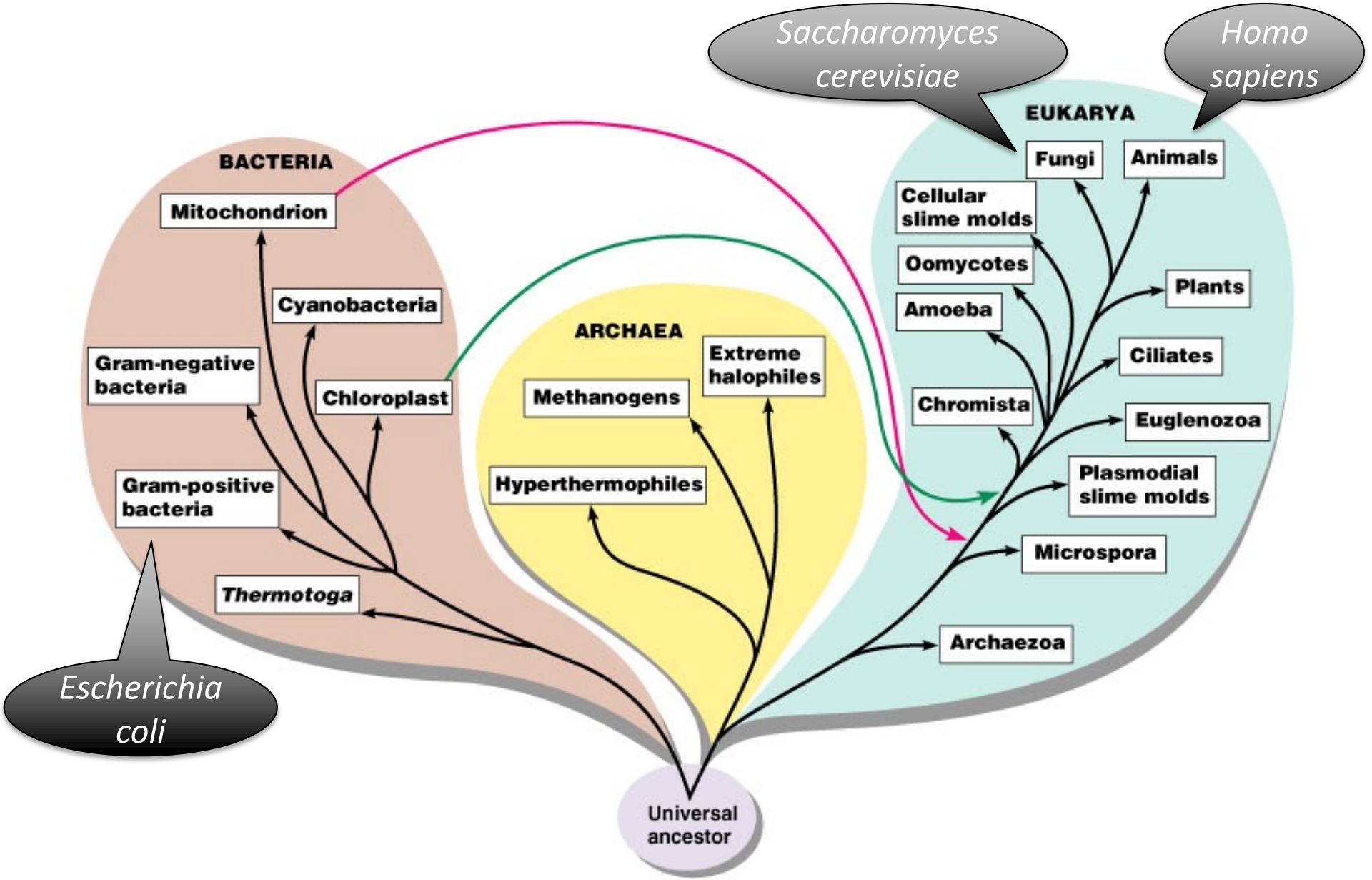


Classification of organisms

	Designation	Description
Source of Carbon	• Autotroph • Heterotroph	• CO_2 • Organic molecules (e.g. sugars)
Source of Electrons	• Organotroph • Lithotroph	• Organic molecules (e.g. sugars) • Inorganic molecules (e.g. H_2, H_2O, H_2S, NH_3, S)
Source of Energy	• Phototroph • Chemotroph • Mixotroph	• Light • Oxidation of chemical compounds • Light & Oxidation of chemical compounds
Ability to use O_2 for respiration & sensitivity to O_2	• Strict/Obligate aerobe • Strict/Obligate anaerobe • Facultative aerobe/anaerobe • Aerotolerant anaerobe	• Require O_2 for respiration – cannot survive without O_2 • Cannot survive in the presence of O_2 • Can survive in the presence or absence of O_2 • Do not respire O_2, but are able to survive in the presence of O_2

Human: Heterotroph, Organotroph, Chemotroph, Strict/Obligate aerobe
E. coli: Heterotroph, Organotroph, Chemotroph, Facultative aerobe/anaerobe
S. cerevisiae: Heterotroph, Organotroph, Chemotroph, Facultative aerobe/anaerobe
Plants & algae: Autotroph, Lithotroph, Phototroph, Strict/Obligate aerobe

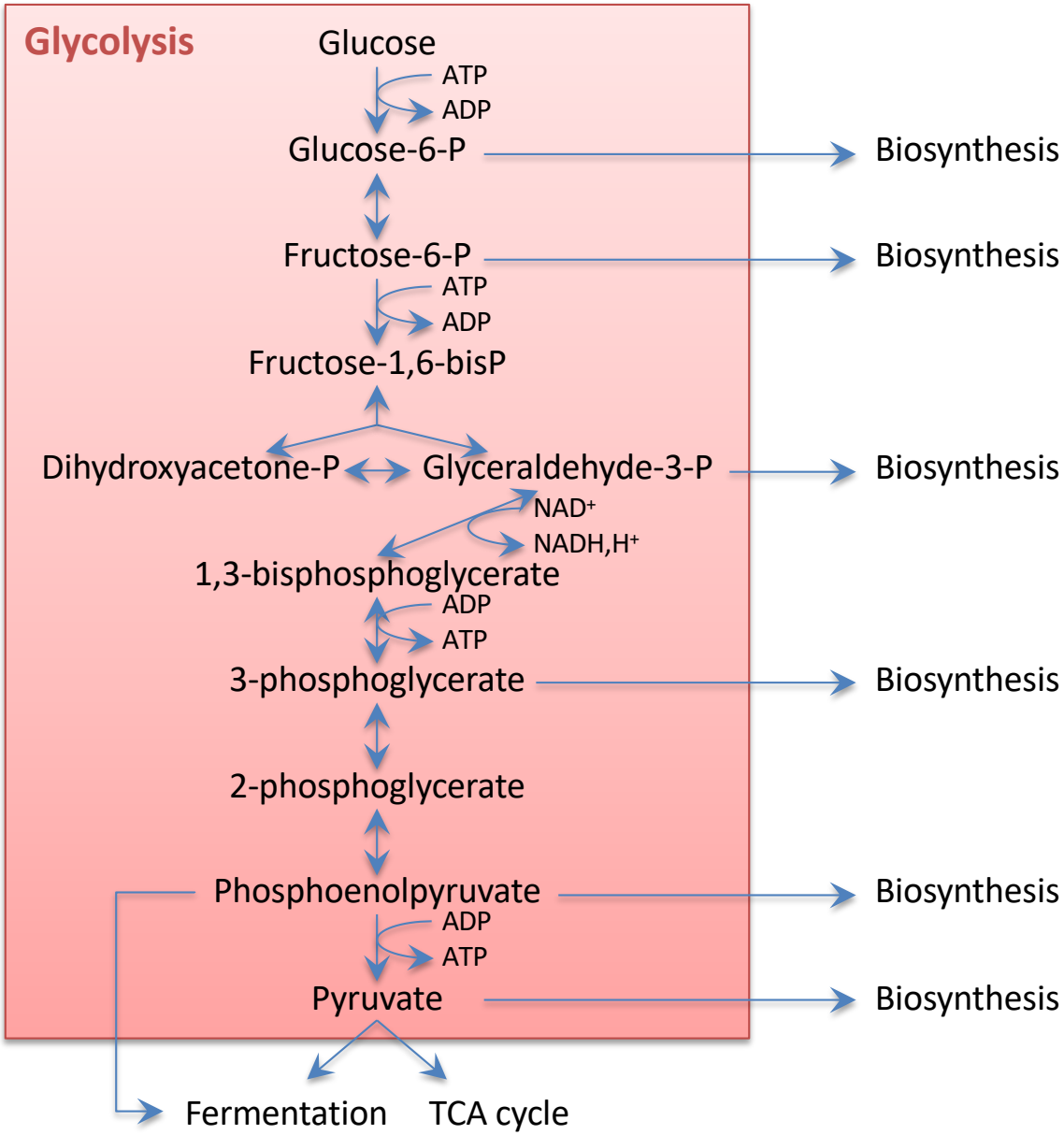
Different organisms – different cellular metabolisms



Cell content

	E. coli	*S. cerevisiae*	*H. sapiens*
Metabolites	3 755	16 042	114 100
Reactions	8 254	31 624	6 302
Pathways	1 336	9 595	49 029
Enzymes	1 204	909	2 126
Transporters	299	149	0
Proteins	1 549	1 259	5 779
Metabolite synonyms	37 982	27 947	333 312
Metabolites in reactions	3518 (93.7 %)	11 157 (69.5 %)	23 473 (20.6 %)
	http://www.ecmdb.ca	http://www.ymdb.ca	http://www.hmdb.ca
	Sajed et al. Nucleic Acids Research (2016) 44, D495-D501	Jewison et al. Nucleic Acids Research (2012) 40, D815-D820	Wishart et al. Nucleic Acids Research (2013) 41, D801-D807

Central pathways

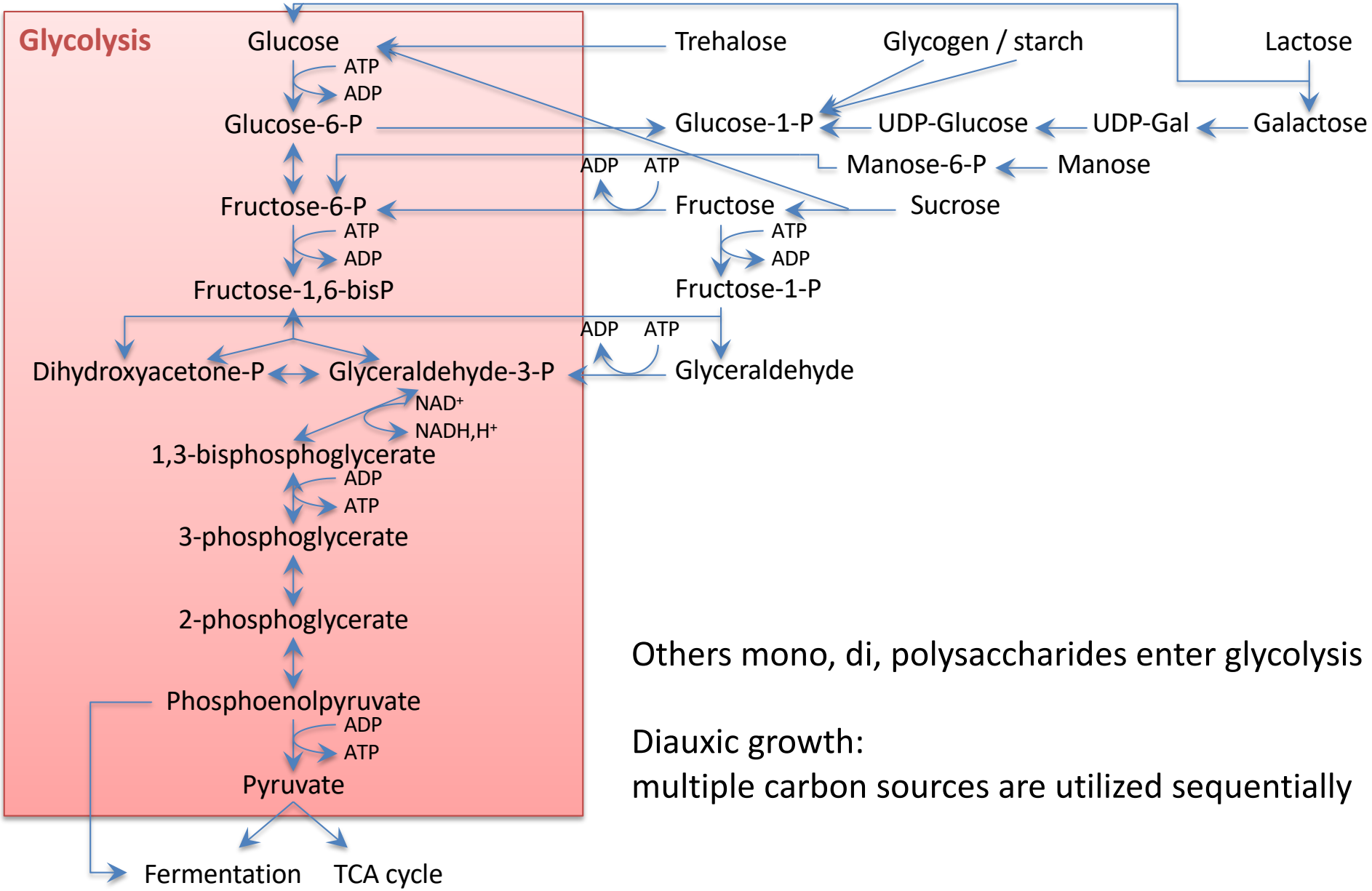


Glycolysis:

- Oxidation of 1 Glucose (6C) to 2 Pyruvate (3C)
- Production of cellular energy sources:
  - 2 ATP
  - 2 $NADH,H^+$
- Supply of 6 precursor metabolites for biosynthesis
- $\Delta G'° = -74\ kJ.mole^{-1}$
- $\Delta G' = -98\ kJ.mole^{-1}$

Central pathways

Glycolysis

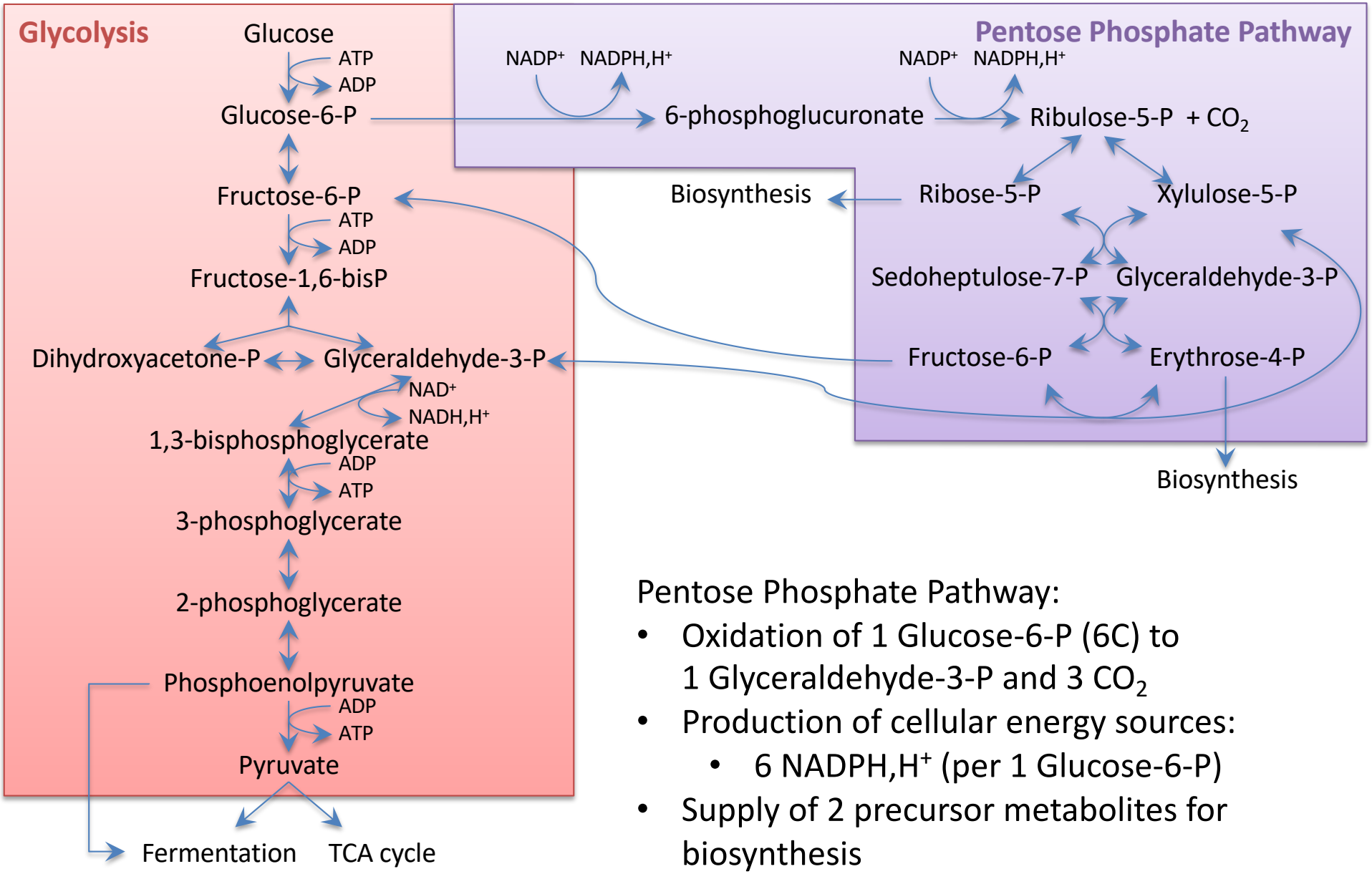


Others mono, di, polysaccharides enter glycolysis

Diauxic growth:
multiple carbon sources are utilized sequentially

Central pathways

Pentose Phosphate pathway

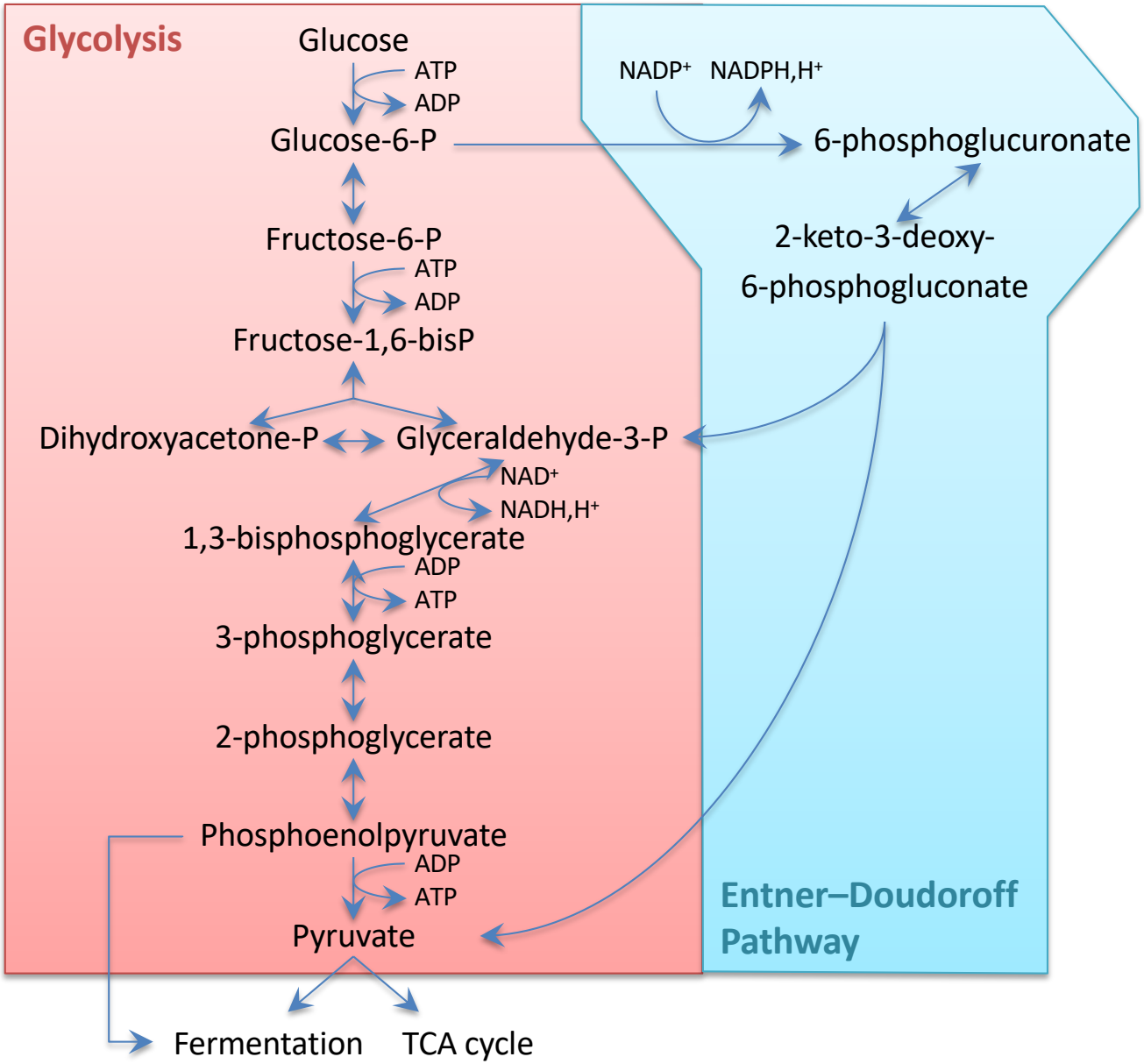


Pentose Phosphate Pathway:

- Oxidation of 1 Glucose-6-P (6C) to 1 Glyceraldehyde-3-P and 3 CO_2
- Production of cellular energy sources:
  - 6 $NADPH,H^+$ (per 1 Glucose-6-P)
- Supply of 2 precursor metabolites for biosynthesis

Central pathways

ED pathway

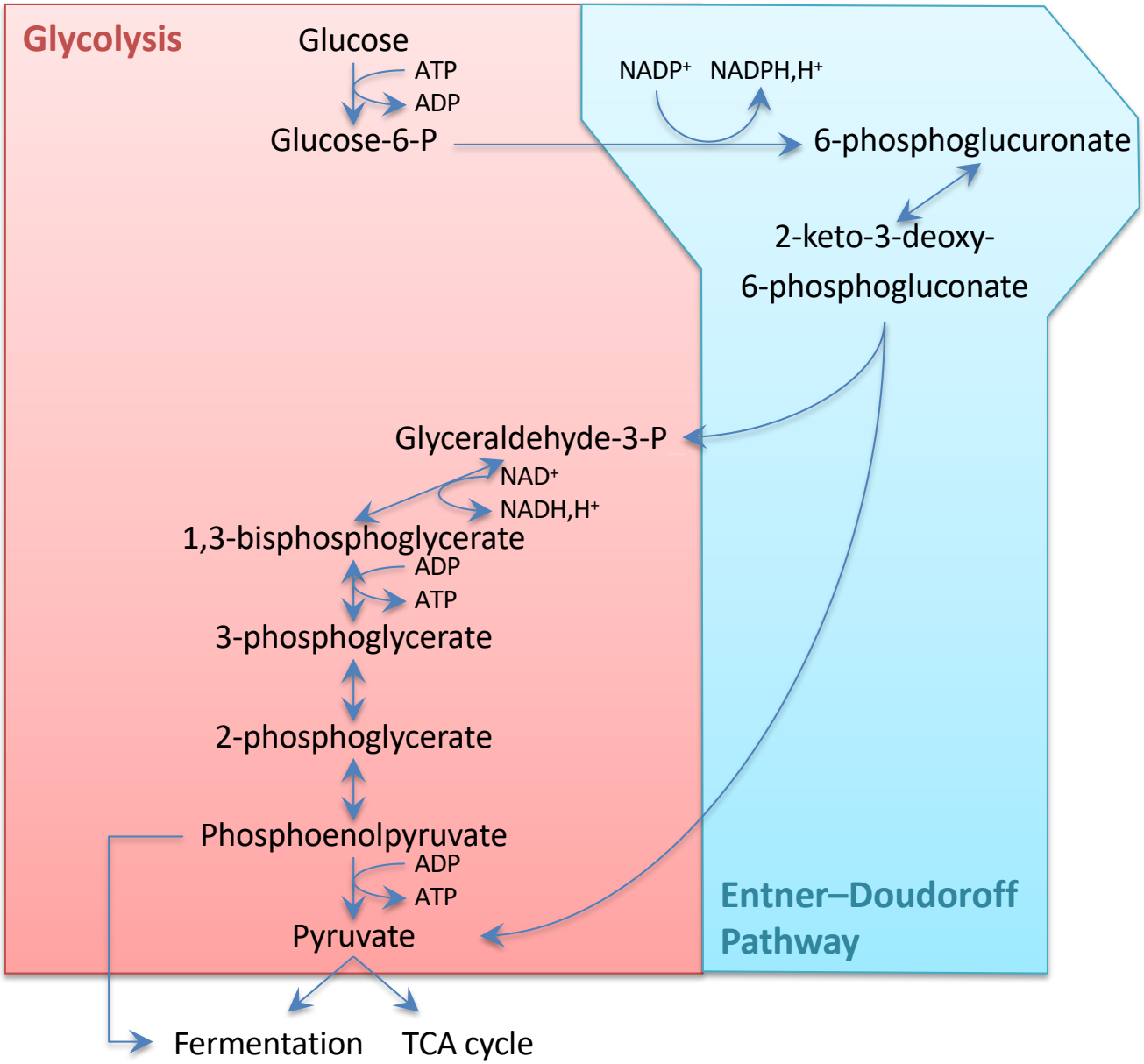


Entner–Doudoroff Pathway:

- Only in some bacteria (ex. *E. coli*), archeae and *Entamoeba histolyca, Aspergillus niger, Penicillum notatum*
- Oxidation of 1 Glucose (6C) to 2 Pyruvate (3C)
- Production of cellular energy sources:
  - 1 ATP
  - 1 $NADH,H^+$
  - 1 $NADPH,H^+$
- Supply of 5 precursor metabolites for biosynthesis

Central pathways

ED pathway



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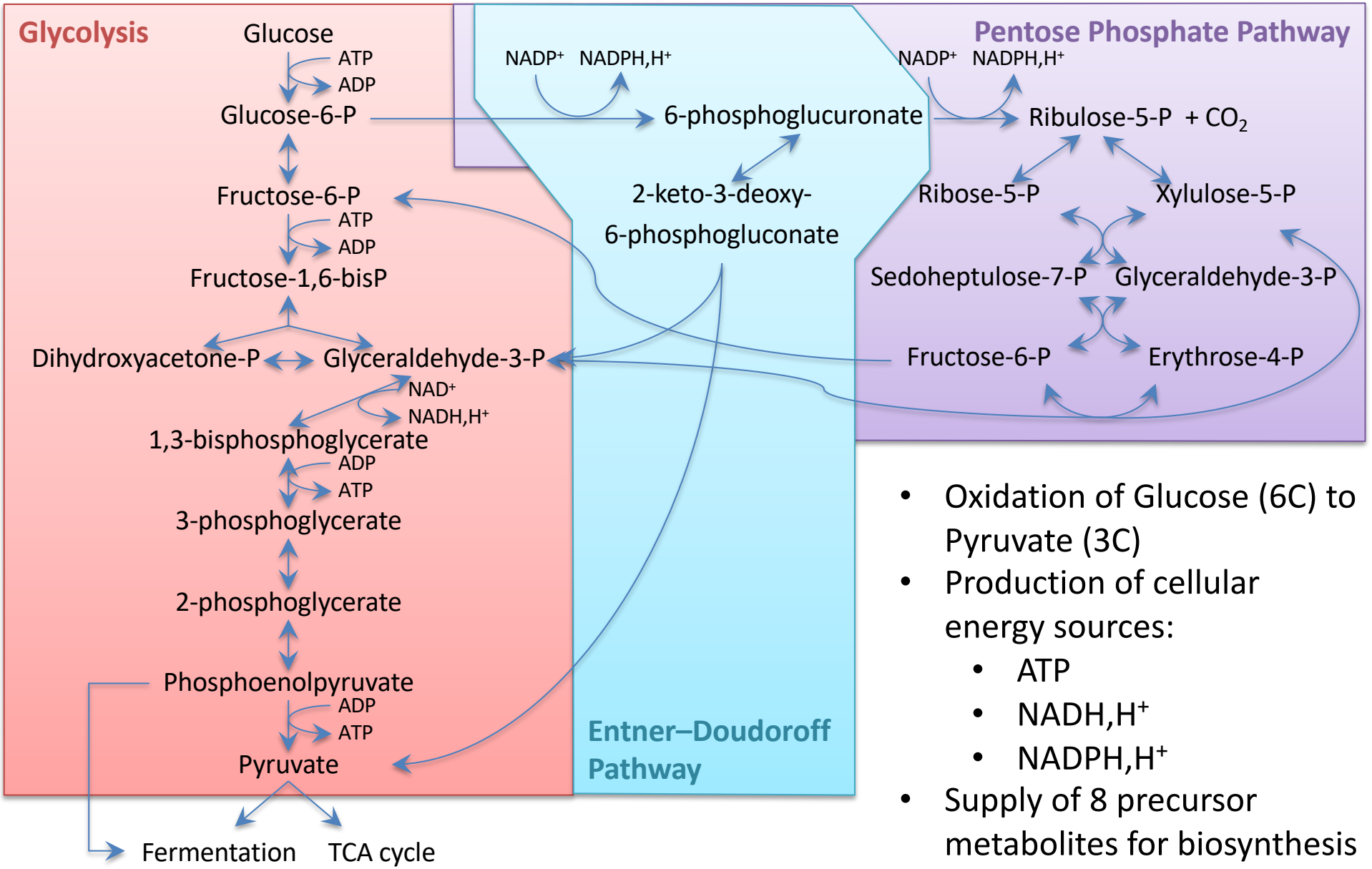
- Supply of 5 precursor metabolites for biosynthesis

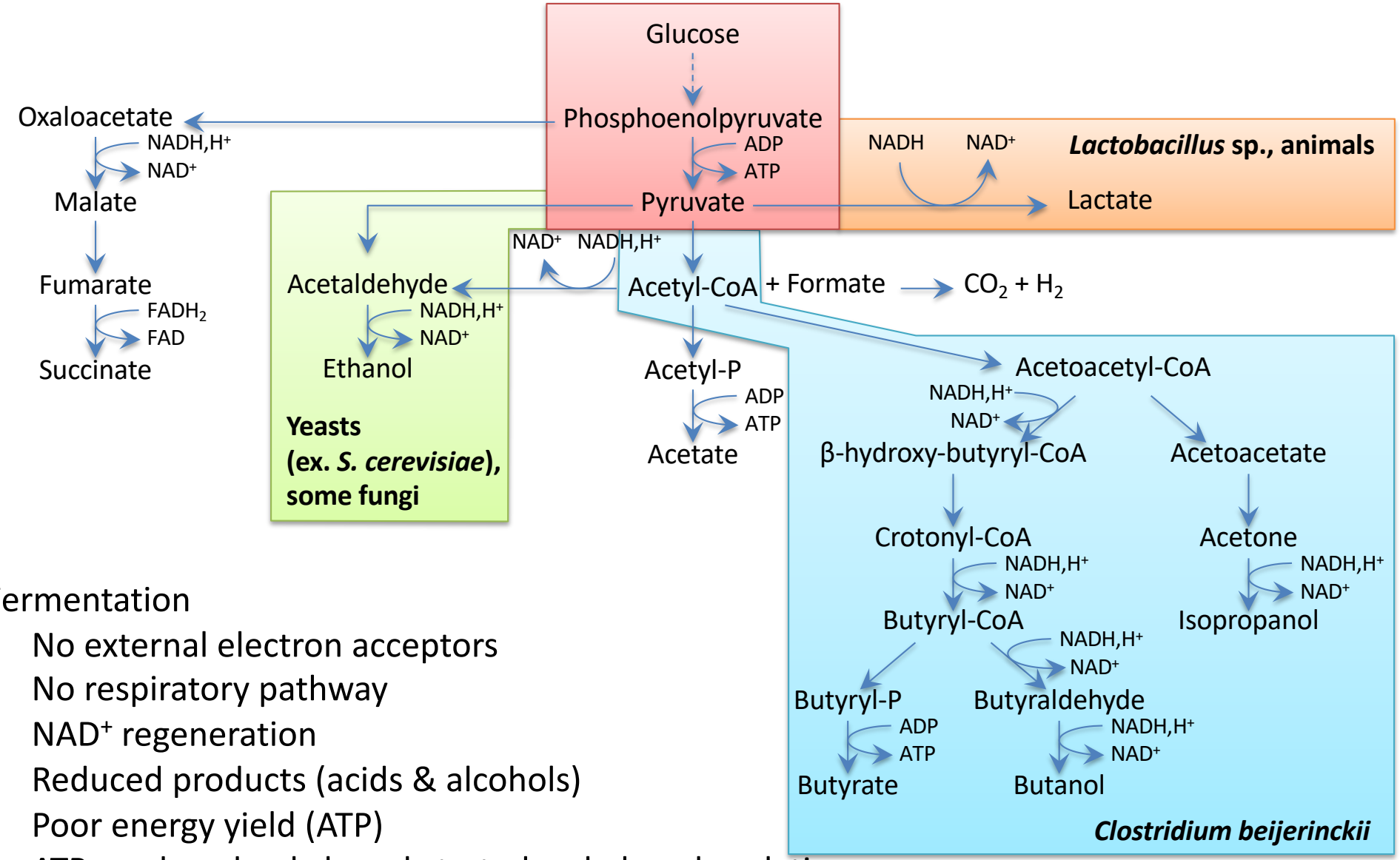
Central pathways Metabolism of carbohydrates and carboxylic acids



- Oxidation of Glucose (6C) to Pyruvate (3C)
- Production of cellular energy sources:
  - ATP
  - $NADH,H^+$
  - $NADPH,H^+$
- Supply of 8 precursor metabolites for biosynthesis

Central pathways

Fermentation

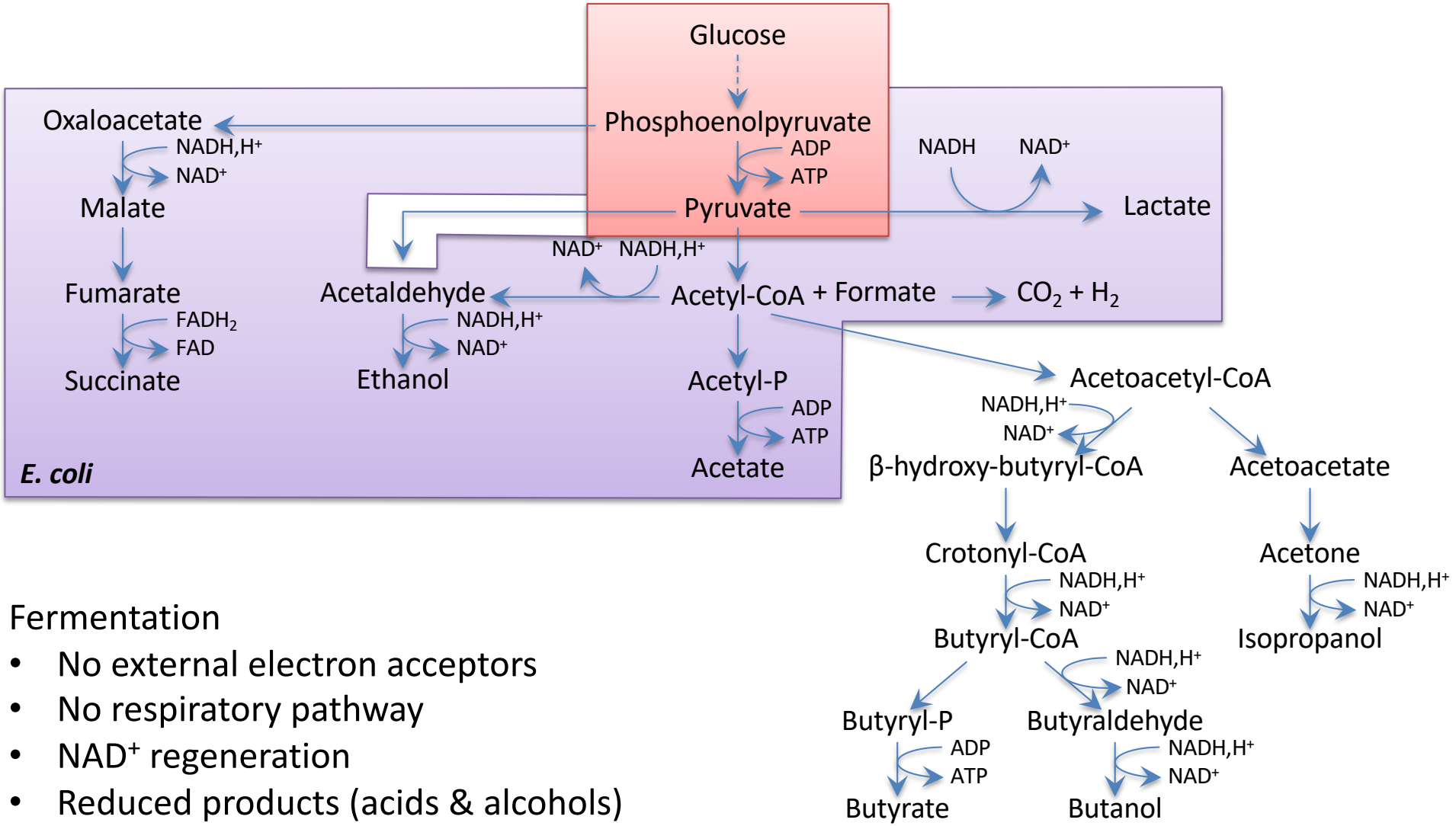


Fermentation

- No external electron acceptors
- No respiratory pathway
- NAD^+ regeneration
- Reduced products (acids & alcohols)
- Poor energy yield (ATP)
- ATP produced only by substrate-level phosphorylation

Central pathways

Fermentation



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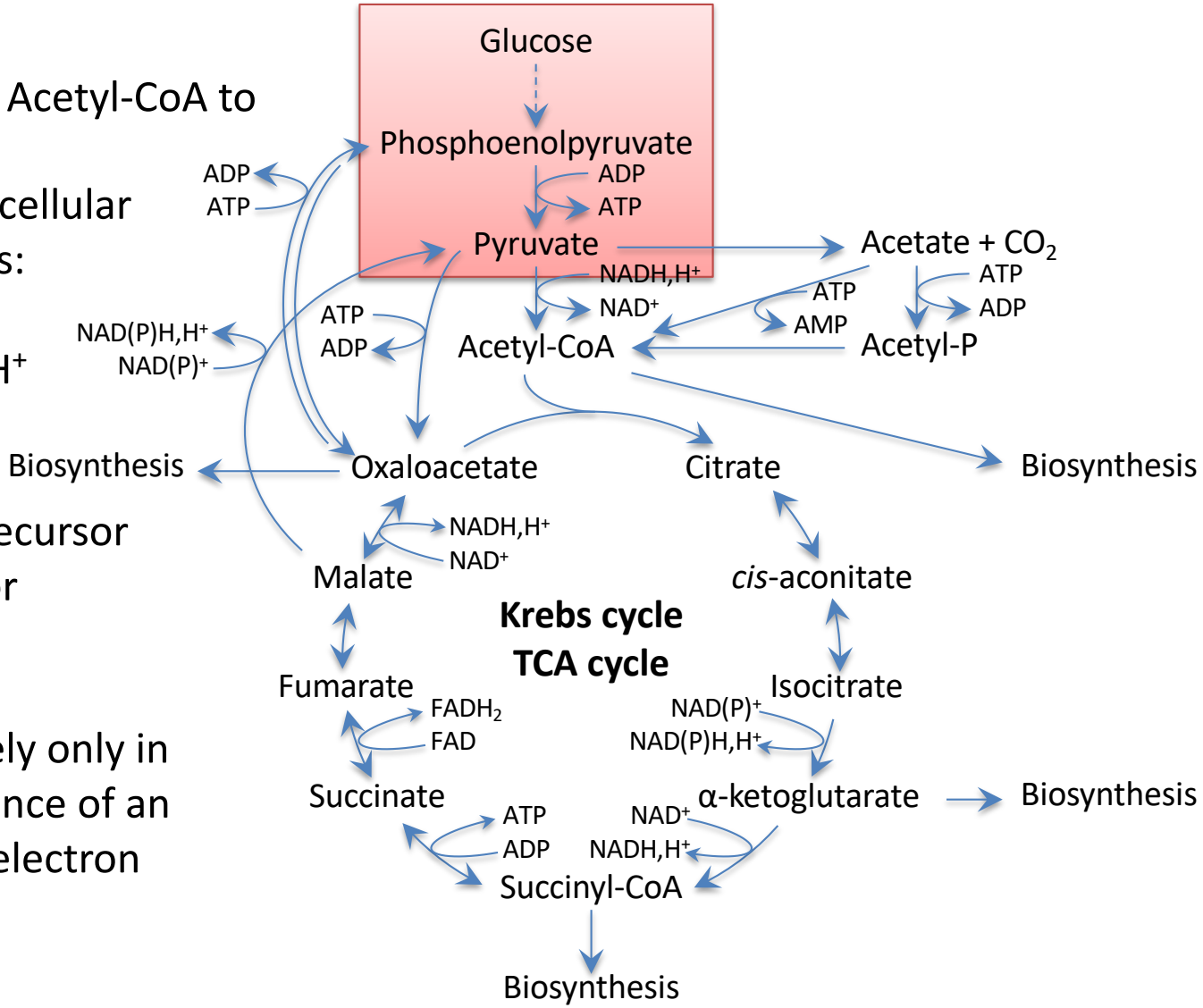
Central pathways

Krebs cycle

TCA Cycle:

- Oxidation of 1 Acetyl-CoA to 2 CO_2
- Production of cellular energy sources:
  - 1 ATP
  - 3 $NADH,H^+$
  - 1 $FADH_2$
- Supply of 4 precursor metabolites for biosynthesis
- Operates:
  - Completely only in the presence of an external electron acceptor



Central pathways

Krebs cycle

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- Production of cellular energy sources:
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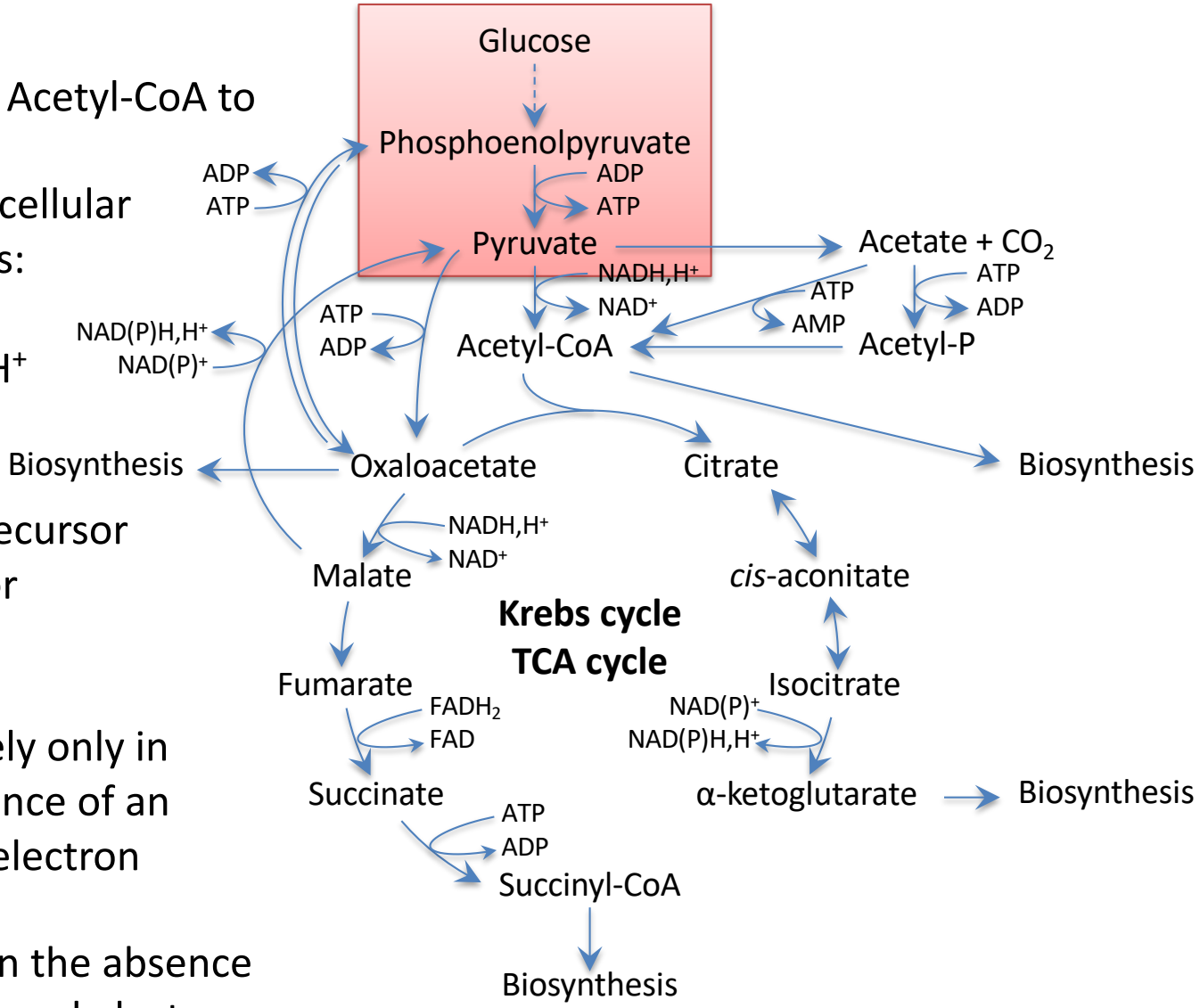
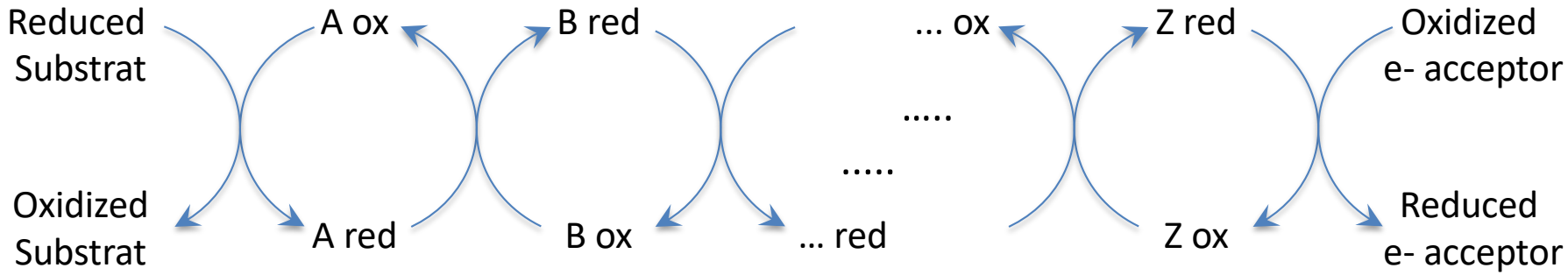
  - 3 $NADH,H^+$
  - 1 $FADH_2$
- Supply of 4 precursor metabolites for biosynthesis
- Operates:
  - Completely only in the presence of an external electron acceptor
  - Partially in the absence of an external electron acceptor



Central pathways

External terminal electron acceptors:



Type of respiration	External terminal electron acceptors	Reduced products
Aerobic respiration	O_2	H_2O
Anaerobic respiration	Fumarate: HOOC-CH=CH-COOH	Succinate: HOOC-CH_2-CH_2-COOH
	Trimethylamine N-oxide: $(CH_3)_3$-N→O	Trimethylamine: $(CH_3)_3$-N
	S, $S_2O_3^{2-}$, SO_4^{2-}	H_2S
	NO_3^-, NO_2^-, NO	N_2
	Fe^{3+}	Fe^{2+}

- A H^+ (electrochemical) gradient is produced across a membrane = proton-motrice force
- Flow of H^+ down the gradient (across the membrane) through the H^+ channel of ATP synthase: ATP synthesis = oxidative phosphorylation

Central pathways

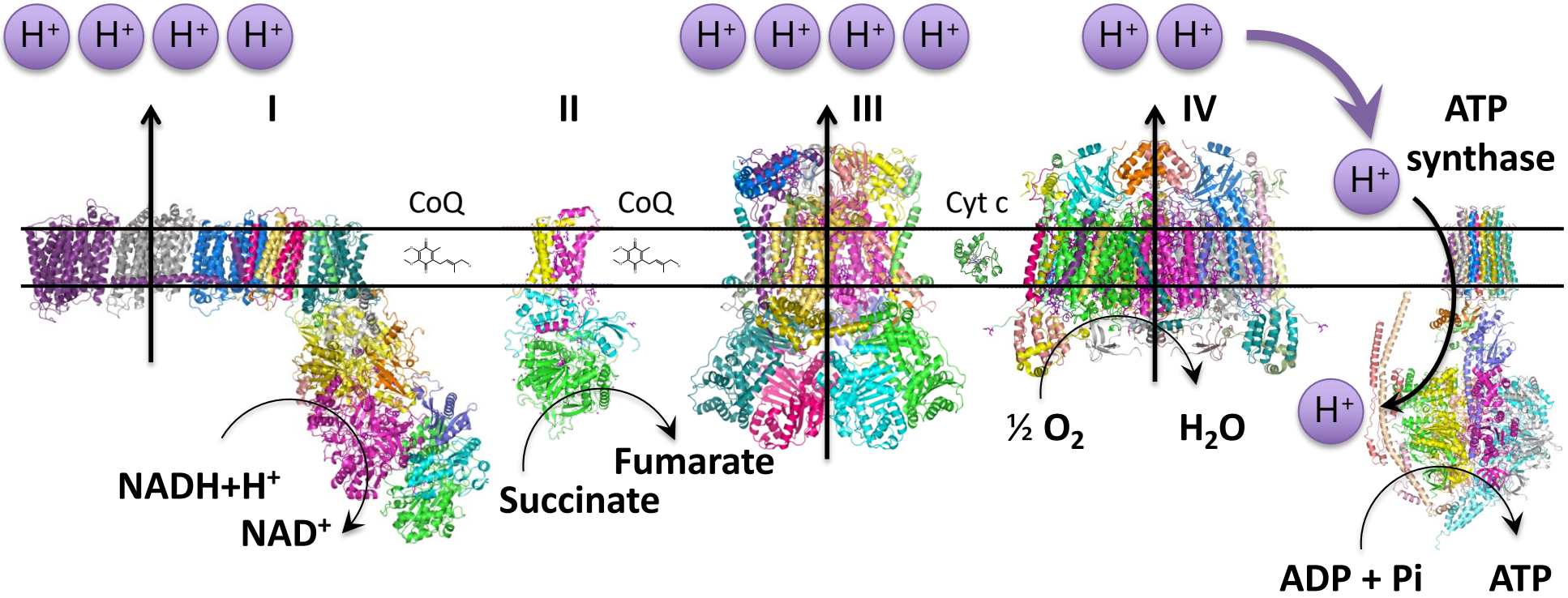
Eukaryotic aerobic respiration

- Oxidative phosphorylation
- Mitochondria – internal membrane

P/O:

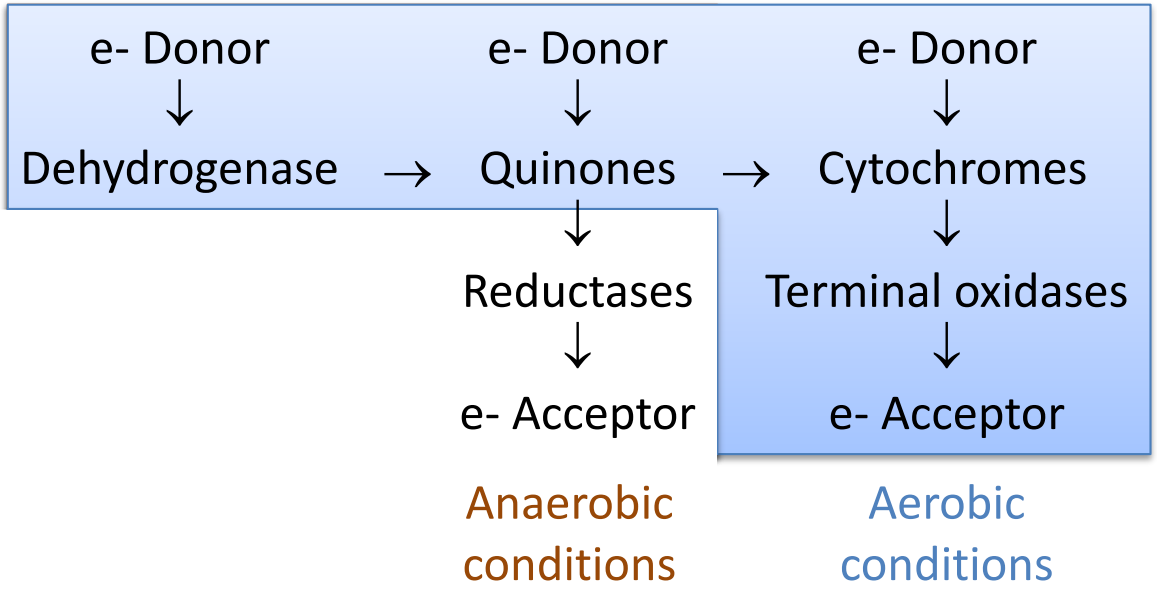
- $NADH+H^+ + ½\ 0_2 \rightarrow NAD^+ + H_2O$ $\Delta G°' = -218\ kJ.mol^{-1} \Rightarrow$ ~0,96 – 3 ATP
- $FADH_2 + ½\ 0_2 \rightarrow FAD + H_2O$ $\Delta G°' = -165\ kJ.mol^{-1} \Rightarrow$ ~0,88 – 2 ATP

} 15 to 38 ATP / Glucose



Central pathways — Prokaryotic respiration (bacteria & archaea)

- Cytoplasmic membrane
- Complexity – ability to adapt to different
  - Growth conditions
  - Electron donors: NADH, NADPH, $FADH_2$, organic substances, H_2, NH_3, NO_2^-, S, S^{2-}, Fe^{2+}
  - Electron acceptors:
    - organic compounds (fumarate, dimethyl sulfoxide, trimethylamine N-oxide)
    - inorganic compounds (O_2, NO_3^-, NO_2^-, NO, ClO_3^-, ClO_4^-, S, $S_2O_3^{2-}$, SO_4^{2-}, SeO_4^{2-}, AsO_4^{3-}, CO_2, oxidized manganese ions, gold, Fe^{3+})



Central pathways

E. coli aerobic respiration

NADH:ubiquinone oxidoreductase I

NADH:ubiquinone oxidoreductase II

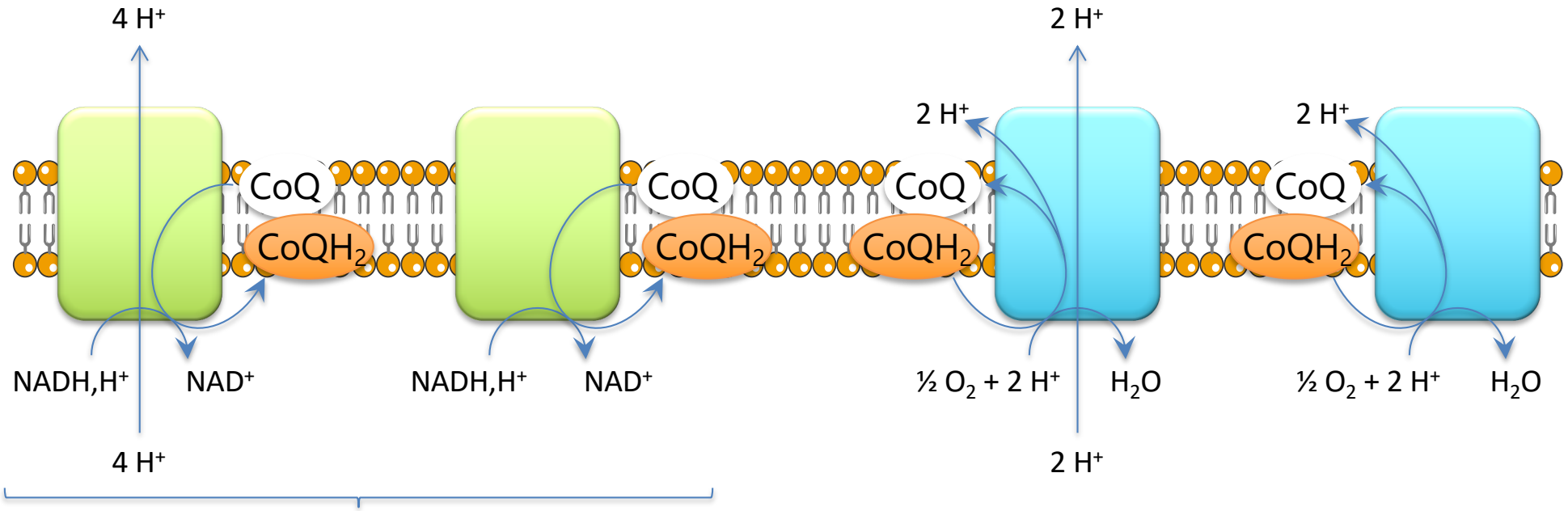
- used preferentially in aerobic and nitrate respiration

Cytochrome *bo* Terminal oxidase

- expressed at high O_2 levels

Cytochrome *bd*-I Terminal oxidase

- expressed at low O_2 levels
- Low K_m for O_2

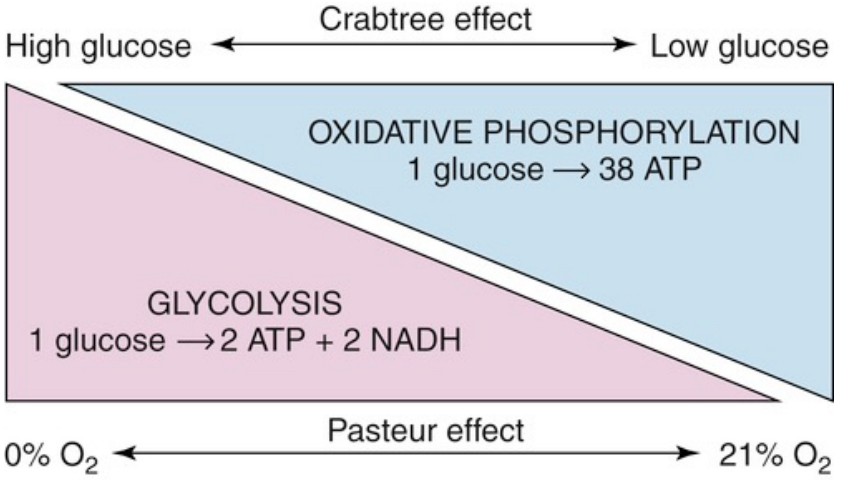


Used both during glucose limited aerobic growth

Central pathways

Metabolism of carbohydrates and carboxylic acids

- Oxidation of 1 Glucose to
  - 6 CO_2 (Complete oxidation)
  - Various fermentation products (Partial oxidation)
- Production of cellular energy sources:
  - Up to 38 ATP / glucose
  - Reducing power (NADH, NADPH, …)
- Tight regulation:
  - Redox potential
  - Energy charge
  - Presence of terminal e- acceptors
  - Growth substrate
    - Amount
    - Type
      - Catabolite repression
      - Diauxic growth: multiple carbon sources are utilized sequentially
  - Allosteric modulation of enzymes
  - Covalent modifications of enzymes (e.g. phosphorylation)
  - Transcriptional control
  - Compartmentalization & transport



Central pathways – Precursor metabolites – Building blocks

Metabolism of carbohydrates and carboxylic acids

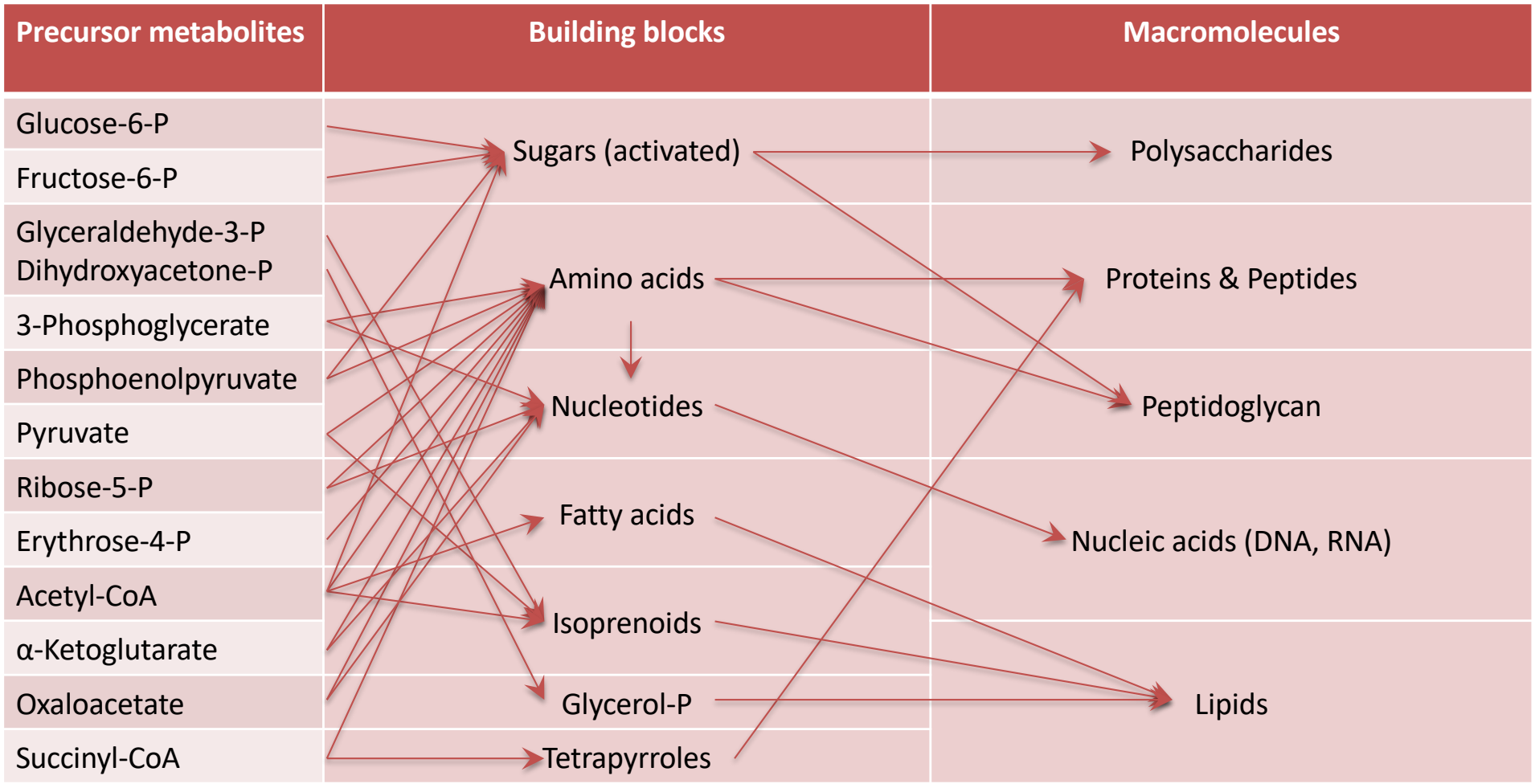
- Supply of 12 major precursor metabolites for biosynthesis
  - Used with ATP and NAD(P)H for the biosynthesis of cellular building blocks

Precursor metabolites	Pathway	Amount required for biosynthesis of *E. coli*	Building blocks produced
Glucose-6-P	Glycolysis	205 µmol / g cell	NDP-glucose
Fructose-6-P	Glycolysis	71 µmol / g cell	NDP-mannose, NDP-N-acetylglucoseamine, …
Glyceraldehyde-3-P Dihydroxyacetone-P	Glycolysis	129 µmol / g cell	Isoprenoids Glycerol-P (& lipids)
3-Phosphoglycerate	Glycolysis	1496 µmol / g cell	Gly, Ser, Cys, nucleotides (purine)
Phosphoenolpyruvate	Glycolysis	519 µmol / g cell	Phe, Tyr, Trp
Pyruvate	Glycolysis	2833 µmol / g cell	Ala, Val, Leu, Ile, Lys, Isoprenoids
Ribose-5-P	Pentose phosphate	898 µmol / g cell	His, nucleotides (pentose)
Erythrose-4-P	Pentose phosphate	361 µmol / g cell	Phe, Tyr, Trp
Acetyl-CoA	TCA cycle	3748 µmol / g cell	Leu, Fatty acids, Isoprenoids
α-Ketoglutarate	TCA cycle	1079 µmol / g cell	Glu, Gln, Pro, Arg, nucleotides (purine)
Oxaloacetate	TCA cycle	1787 µmol / g cell	Asp, Asn, Lys, Thr, Met, Ile, nucleotides
Succinyl-CoA	TCA cycle		Met, Lys, tetrapyrroles (ex. Heme)

Precursor metabolites – Building blocks – Macromolecules

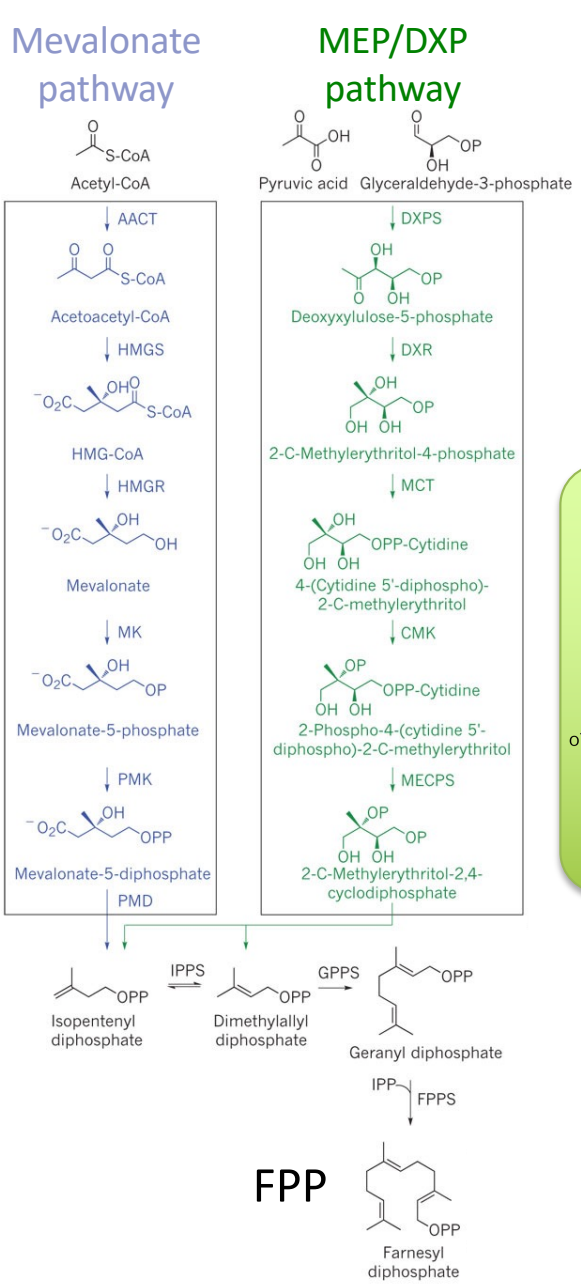
Metabolism of carbohydrates and carboxylic acids

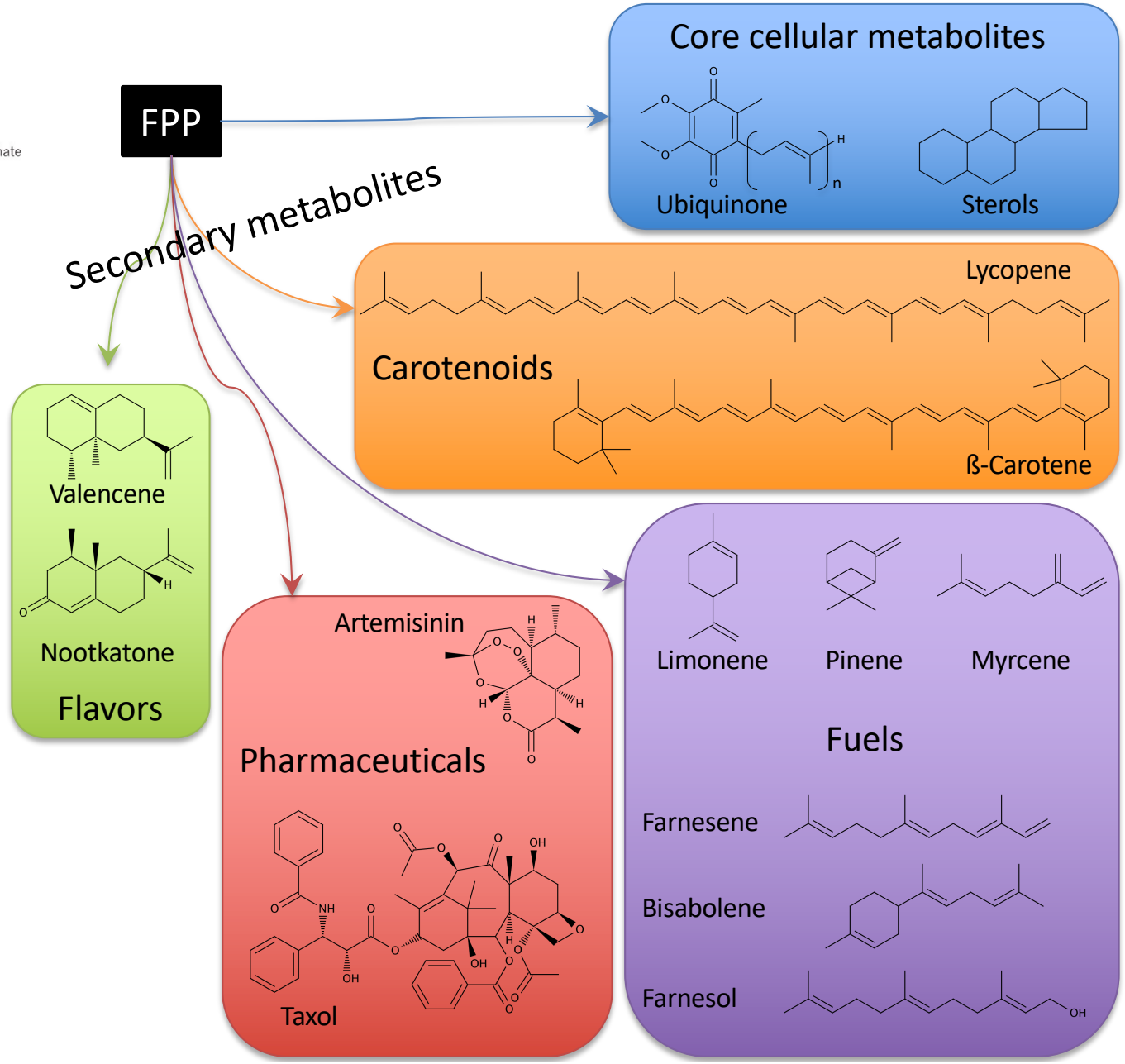
- Supply of 12 major precursor metabolites for biosynthesis
  - Used with ATP and NAD(P)H for the biosynthesis of cellular building blocks
    - Polymerization of building blocks



Isoprenoids / Terpenoids

over 25 000 known compounds





Secondary metabolism

Not essential for
- Growth
- Respiration
- Development
- Reproduction

Beneficial for the producer:
- Improve survival fitness
- Bactericides or fungicides
- Specific receptors

Produced at low titers

Complex organic compounds
- Difficult to synthetize
- Difficult to derivatize

Bioactive molecules
- Pharmaceuticals
  - Antibacterials
  - Antifungals
  - Antitumor
  - Antihelminthic
  - Antiviral
  - Herbicidal
  - Insecticidal
  - Immunosupressors

↓

Interesting for humans

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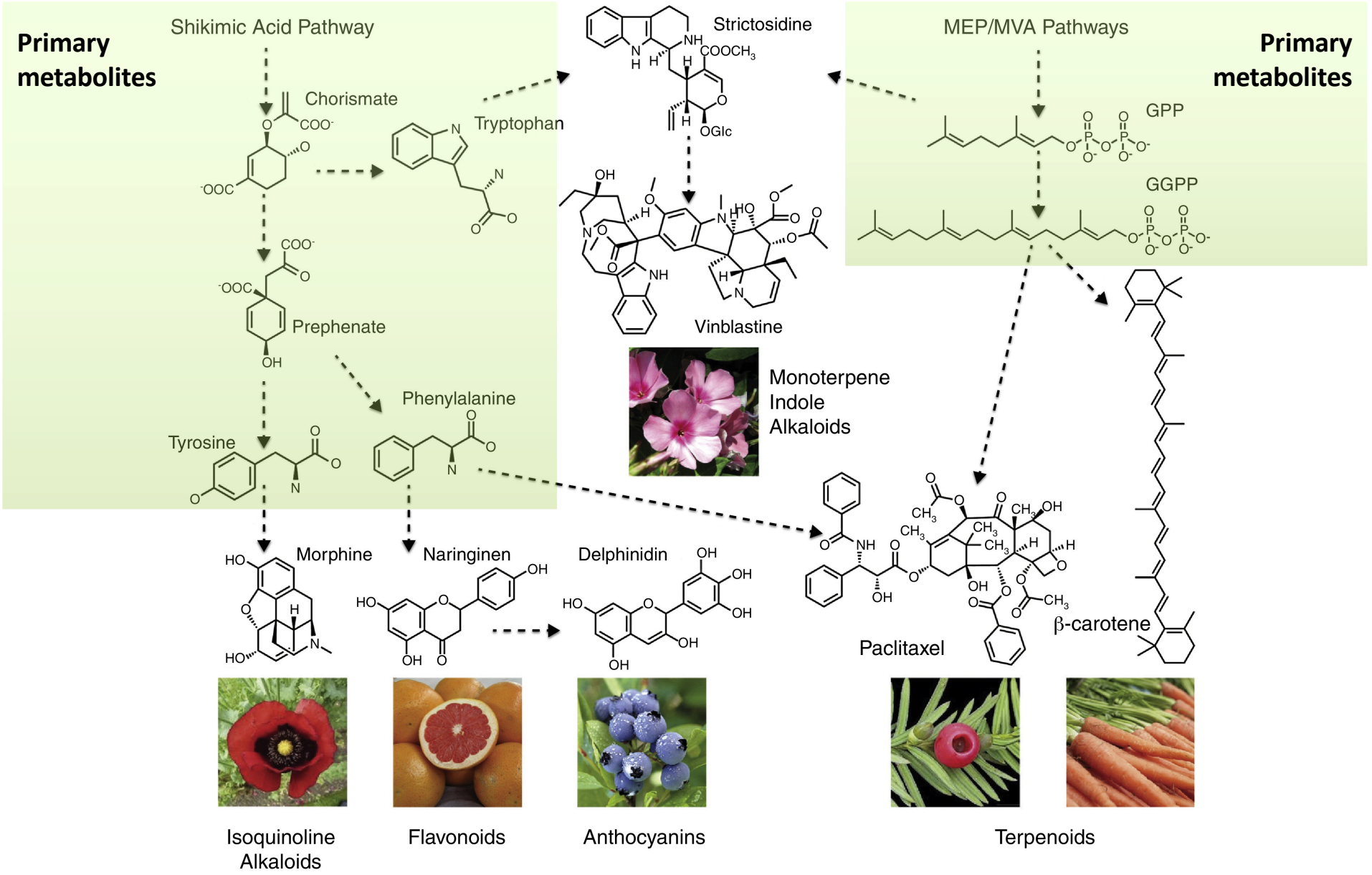
Candidates for metabolic engineering

Main classes
- Isoprenoids / Terpenoids
- Alkaloids
- Flavonoids
- Non ribosomal peptides
- Polyketides

Secondary metabolism

plants



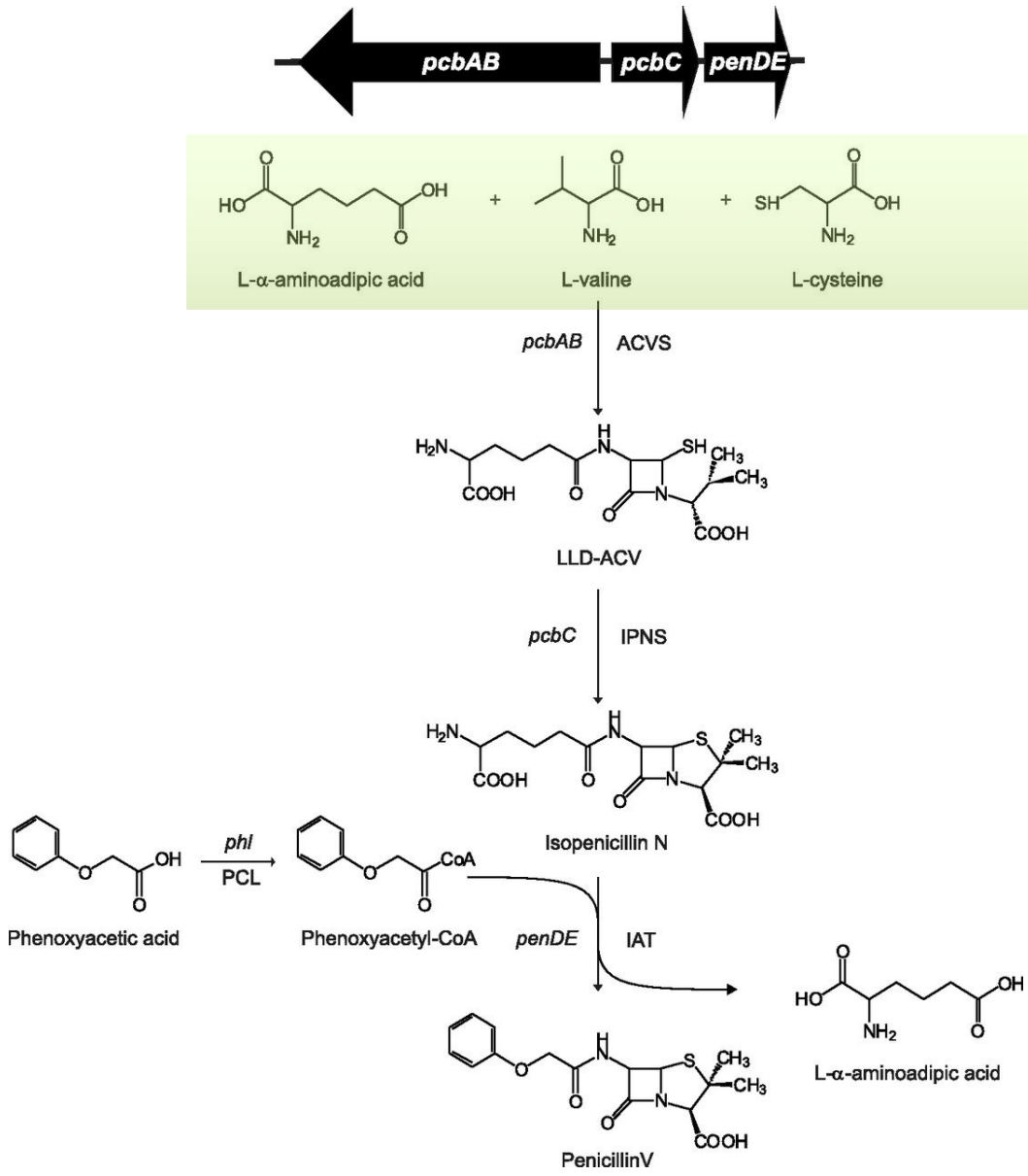
Wilson & Roberts. Current Opinion in Biotechnology (2014) 26, 174–182

Secondary metabolism

fungi

Non-ribosomal peptides

Ex. penicillin production by *Penicillium chrysogenum*

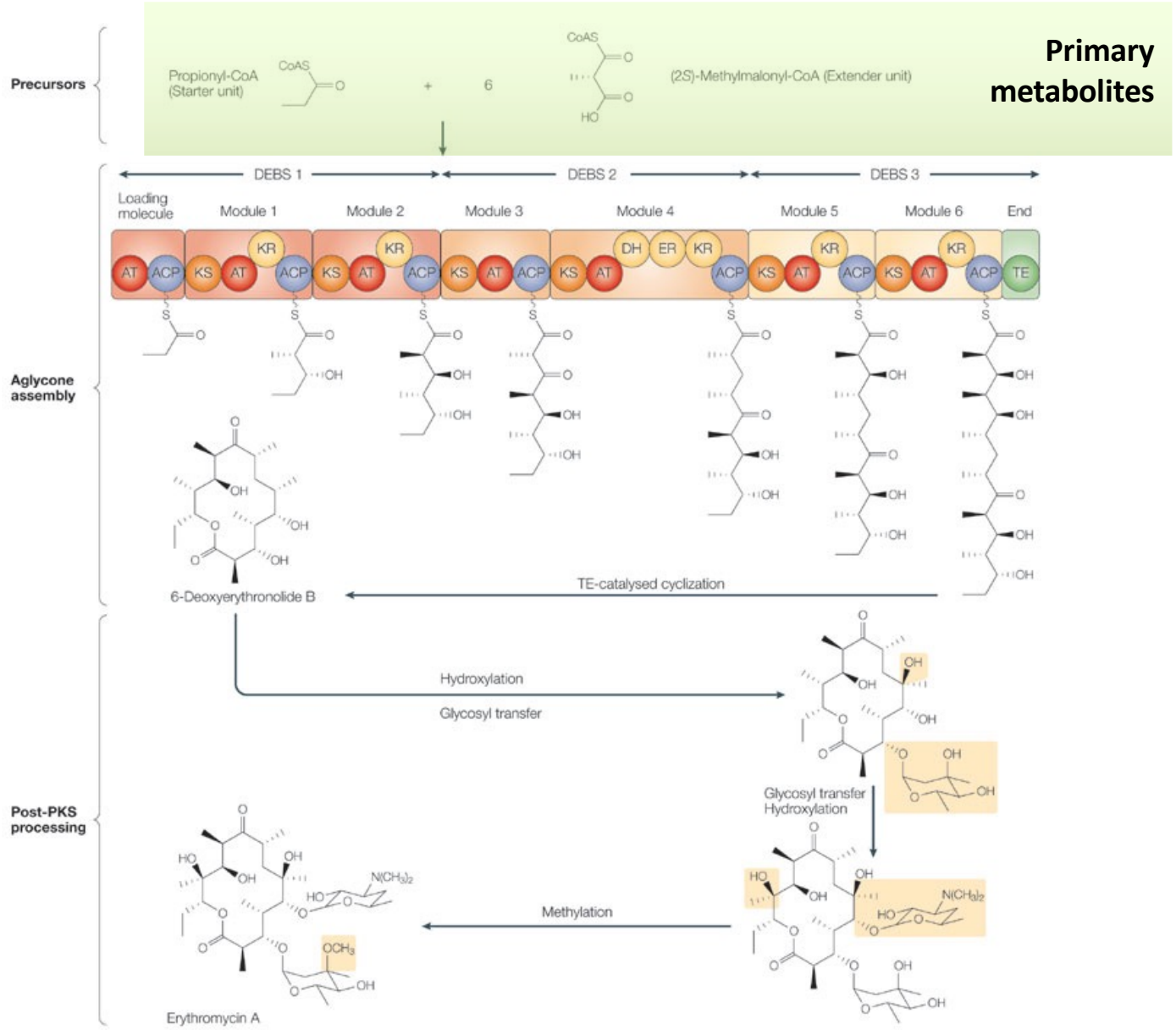


Secondary metabolism

bacteria

Polyketides

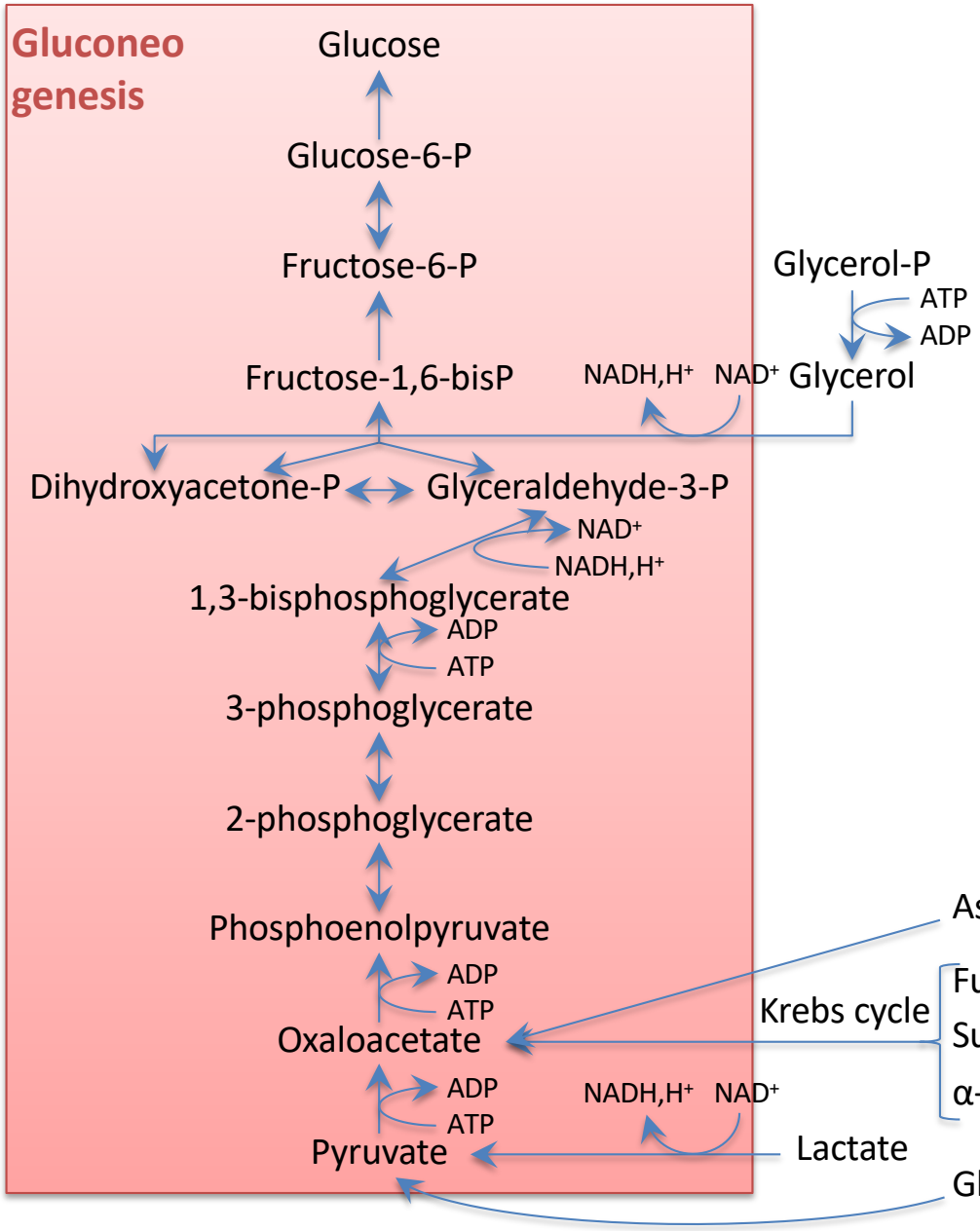
Ex. Erythromycin production by *Saccharopolyspora erythraea*



Weissman & Leadlay. Nature Reviews Microbiology (2005) 3, 925-936

Producing sugars

Gluconeogenesis

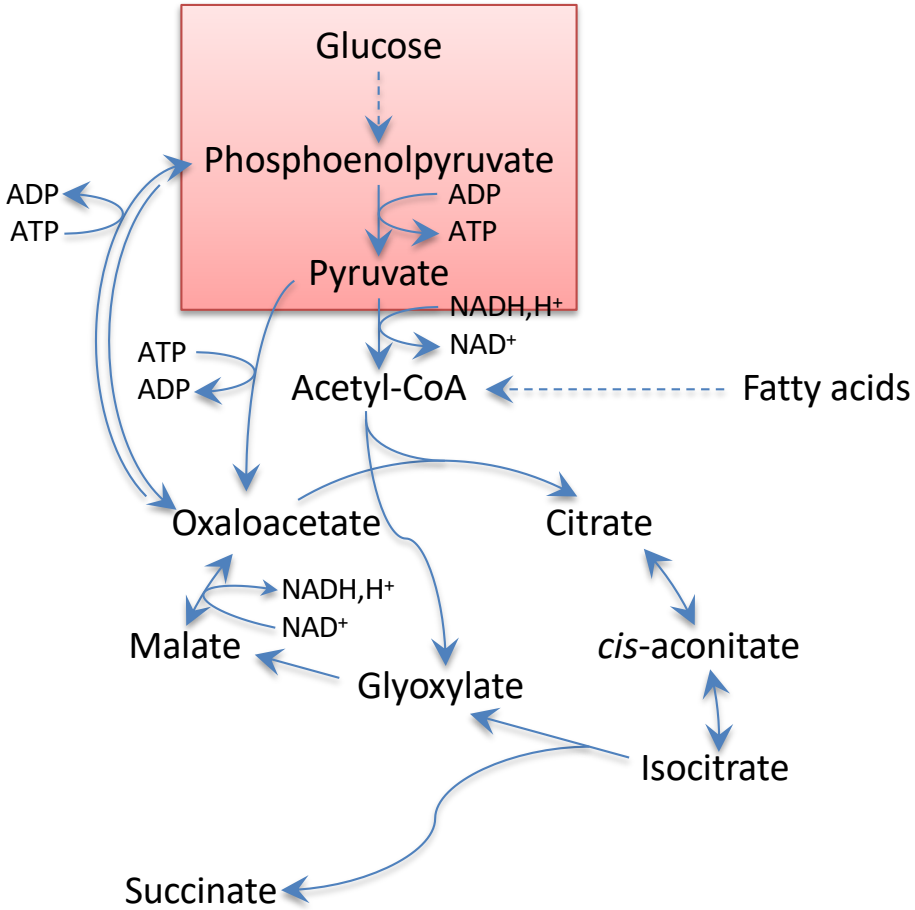


Gluconeogenesis:

- synthesis of glucose from noncarbohydrate precursors
  - Pyruvate
  - Lactate
  - Glycerol
  - Amino acids (all but Lys, Leu)
- From pyruvate:
  - 7 glycolytic enzymes
  - 4 enzymes to bypass the 3 irreversible steps of glycolysis
  - Cost:
    - 6 ATP
    - 2 $NADH,H^+$

Producing sugars

Glyoxylate bypass



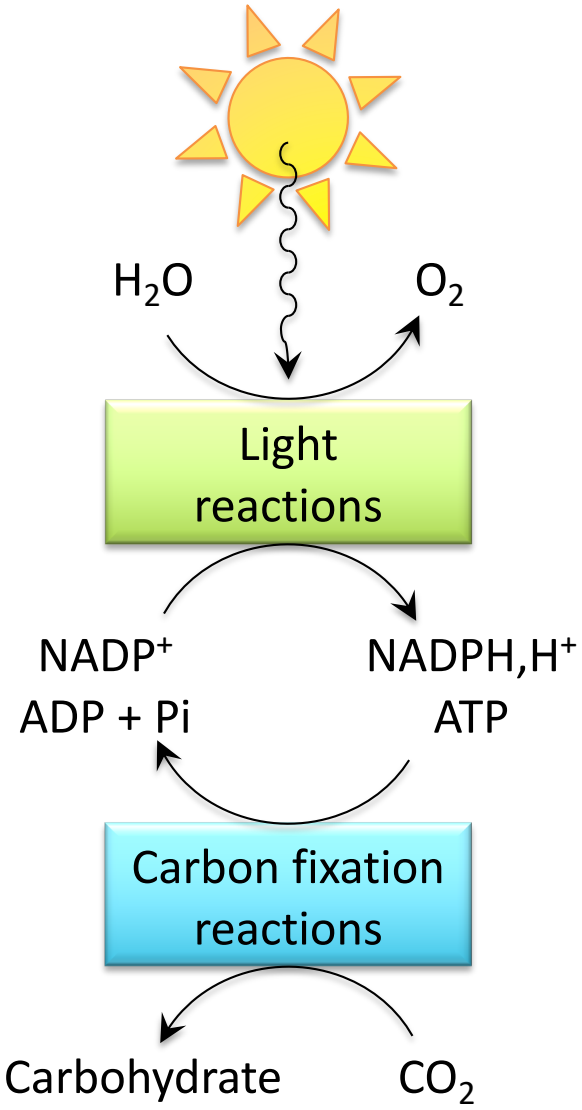
Glyoxylate by-pass:

- Germinating seeds of plants
- Some bacteria (ex. *E. coli*)
- Some invertebrates
- Glyoxysomes of eucaryotes
- Allows grow on acetate and fatty acids: 2 Acetyl-CoA $\rightarrow$ 1 Succinate
- Supply of precursor metabolites for biosynthesis

Producing sugars

- Autotroph organisms
  - CO_2 assimilation / CO_2 fixation / carbon fixation
- Phototroph
  - Energy = light

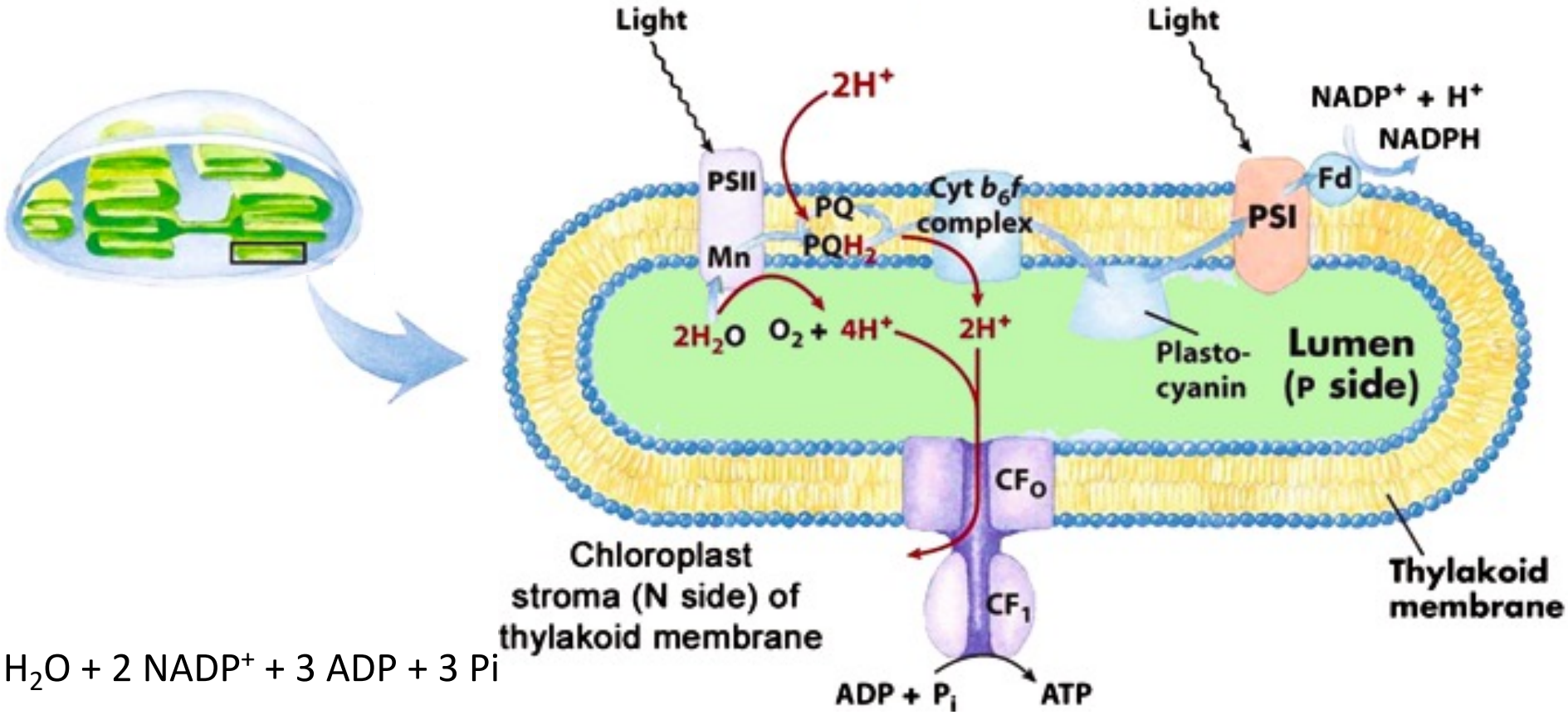
$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$



Producing sugars

Light reactions

- e- flow: from H_2O to $NADP^+$
- A H^+ (electrochemical) gradient is produced across a membrane = proton-motrice force
- Flow of H^+ down the gradient (across the membrane) through the H^+ channel of ATP synthase: ATP synthesis = oxidative phosphorylation



2 H_2O + 2 $NADP^+$ + 3 ADP + 3 Pi

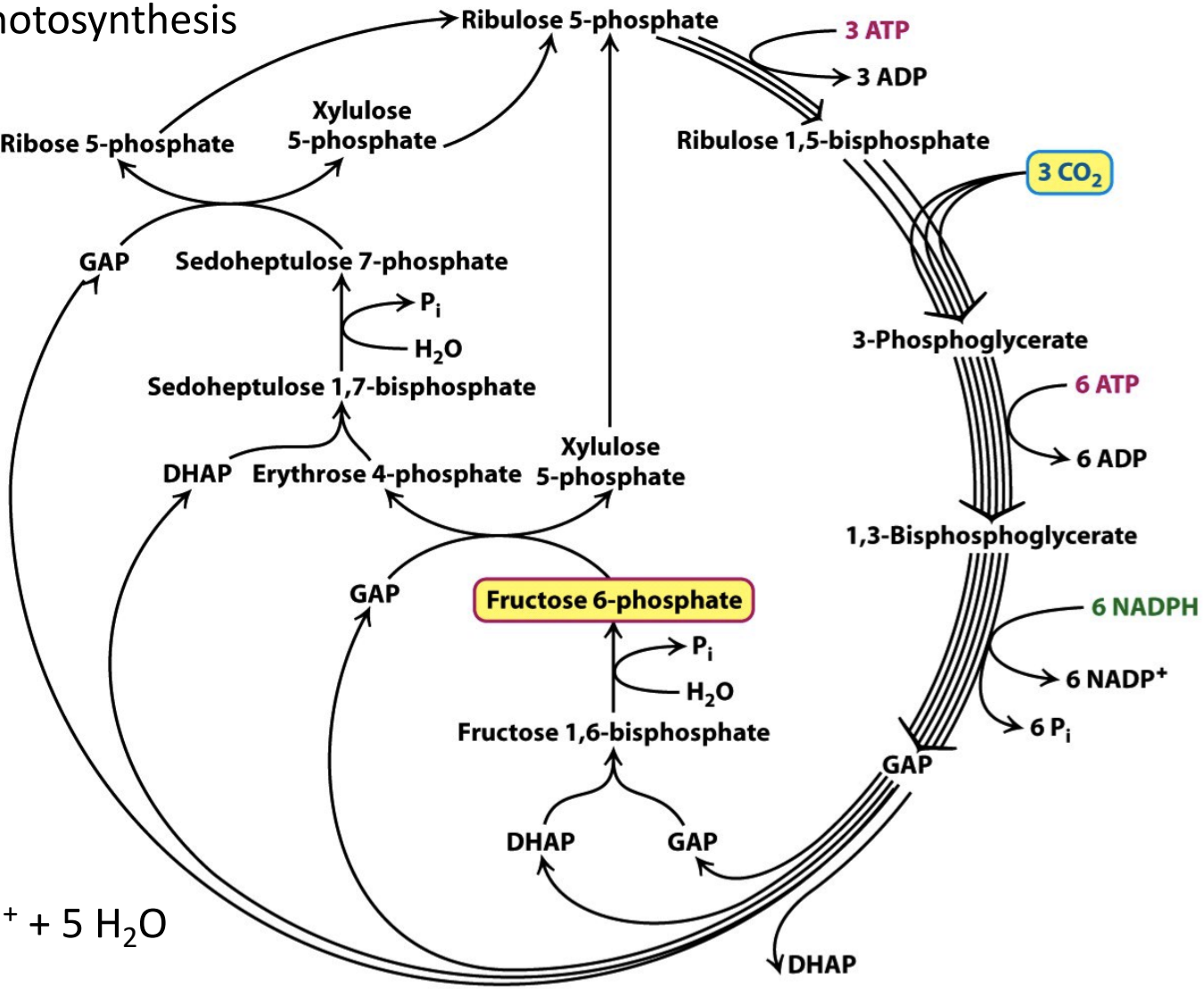
↓

O_2 + 2 $NADPH,H^+$ + 3 ATP

Producing sugars

Calvin cycle

- The dark reactions of photosynthesis
- In stoma of chloroplasts



$3\ CO_2 + 9\ ATP + 6\ NADPH,H^+ + 5\ H_2O$

↓

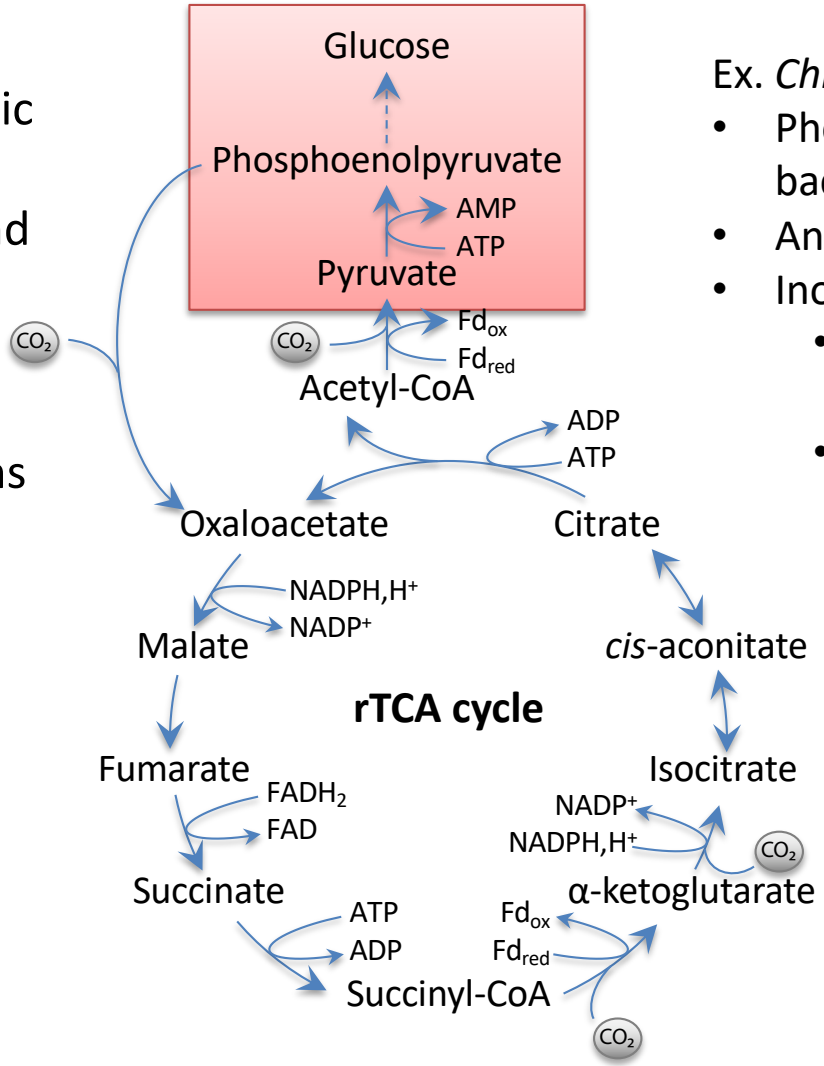
Glyceraldehyde-3-P + 9 ADP + 8 Pi + 6 $NADP^+$

Producing sugars

Reverse/reductive TCA cycle

Arnon-Buchanan cycle:

- Used by some autotrophic anaerobic and microaerobic bacteria and archaea for CO_2 assimilation
- two ferredoxin-linked (Fd) CO_2-fixation reactions are O_2 sensitive



Ex. *Chlorobium thiosulfatophilum*

- Photosynthetic green sulfur bacterium
- Anaerobic
- Inorganic medium:
  - sulfide and thiosulfate as e- donors
  - CO_2 as an obligatory carbon source = Strictly autotrophic

Berg. Applied and Environmental Microbiology (2011) 77, 1925-1936

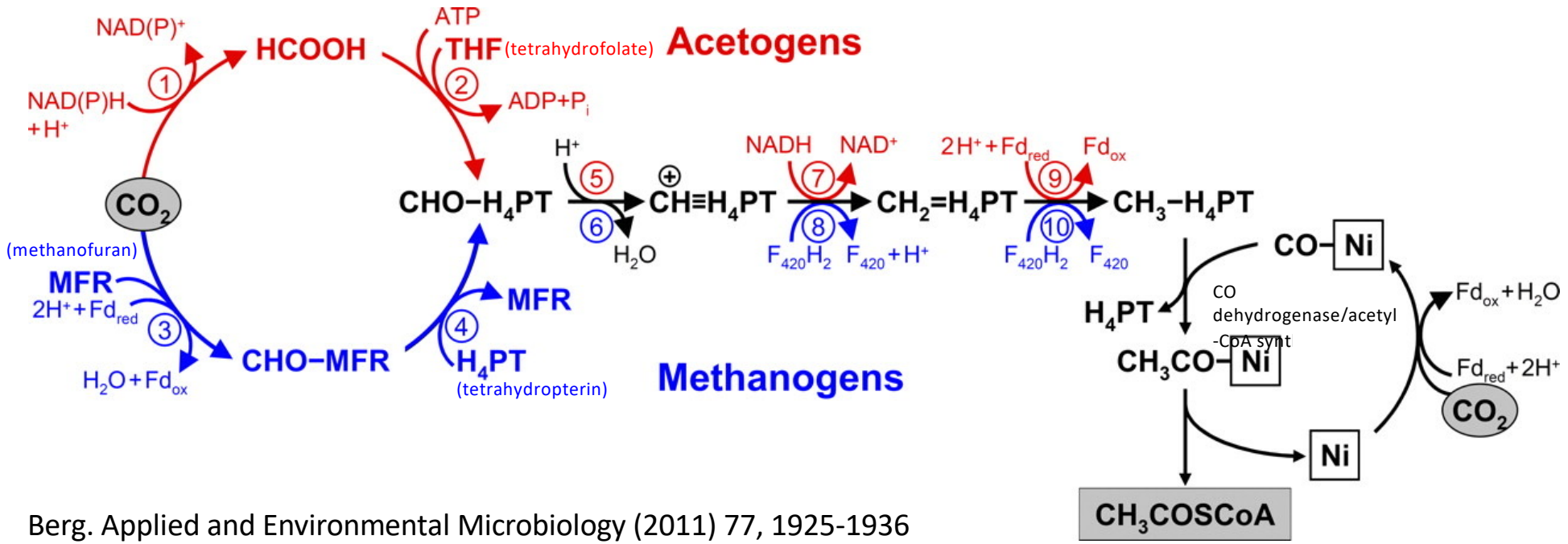
Producing sugars

Reductive Acetyl-CoA pathway

Wood-Ljungdahl pathway:

- Used by some autotrophic anaerobic bacteria and archaea for CO_2 assimilation
  - Acetogenic bacteria
  - Methanogenic archeae
  - …
- One $CO_2 \rightarrow CH_3$
- One $CO_2 \rightarrow CO$

- High energetic efficiency
- Limited to a few ecological niches
  - Anaerobiosis
  - Metals: Mo or W, Co, Ni, and Fe
  - Cofactors: tetrahydropterin and cobalamin



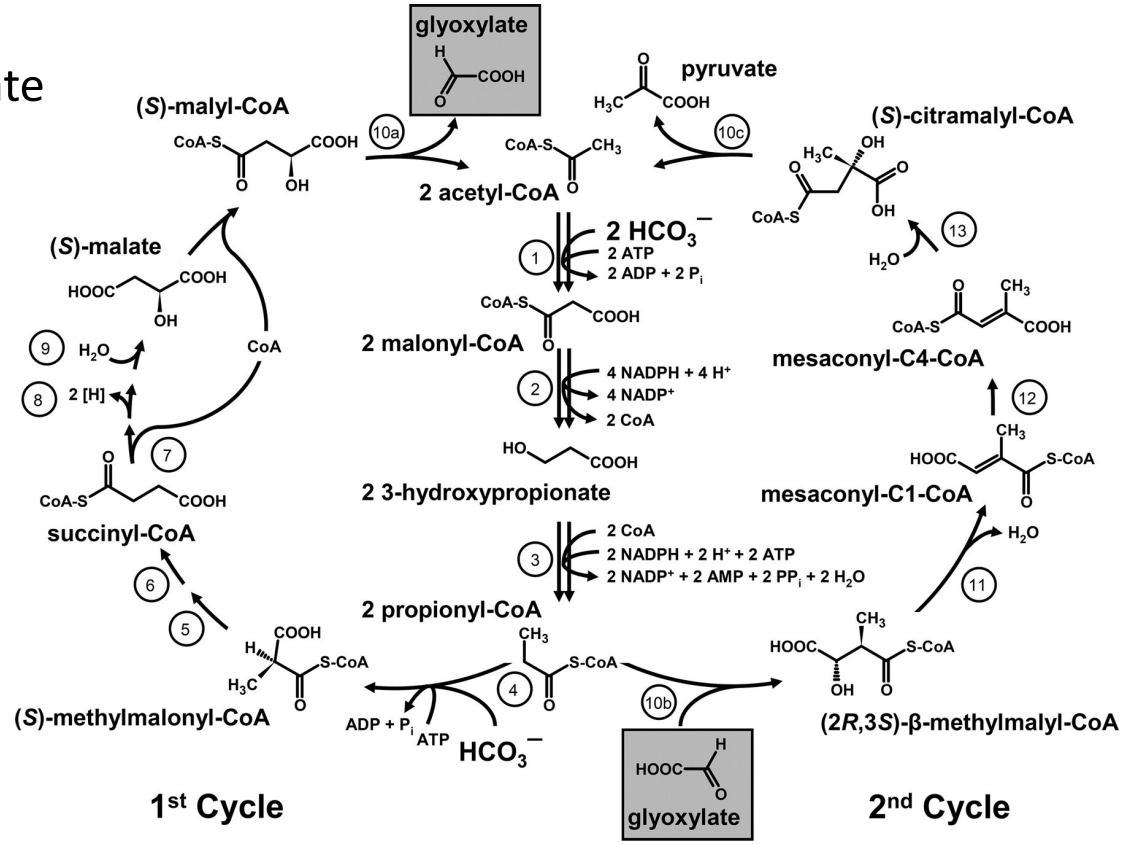
Berg. Applied and Environmental Microbiology (2011) 77, 1925-1936

Producing sugars

3-hydroxypropionate bi-cycle

Fuchs-Holo bi-cycle

- Used only by green nonsulfur bacteria of *Chloroflexaceae* family
  - Phototroph, chemotroph & mixotroph
  - Autotroph & heterotroph
  - Anoxygenic
- Co-assimilation of CO_2 & numerous compounds (e.g., fermentation products) = mixotrophy
- High energy requirements
  - 7 ATP for Acetyl-CoA to Pyruvate
- No oxygen-sensitive steps



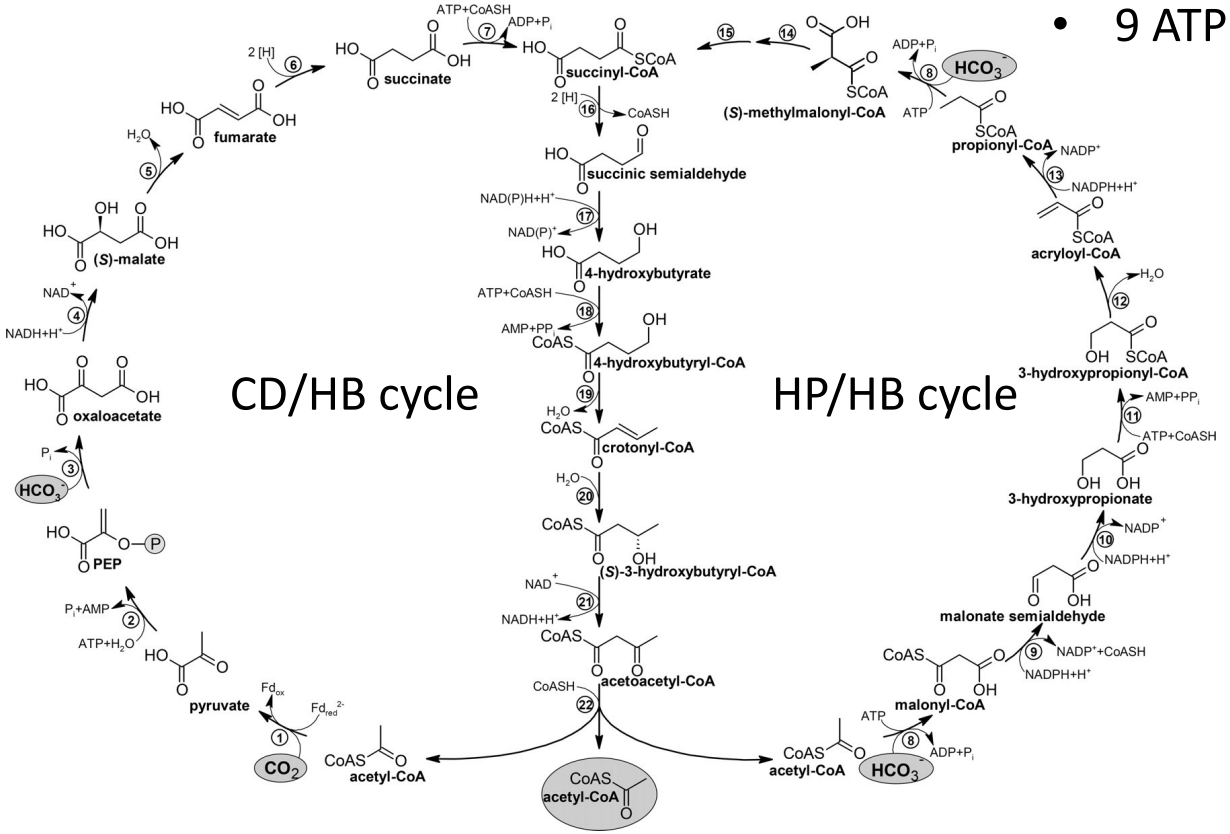
Berg. Applied and Environmental Microbiology (2011) 77, 1925-1936

Producing sugars

4-hydroxybutyrate cycles

4-hydroxybutyrate cycles

- Used only by the hyperthermophilic archaea of *Crenarchaeota* family
- dicarboxylate/4-hydroxybutyrate (DC/HB) cycle
  - *Desulfurococcales* and *Thermoproteales*
  - Anaerobic
  - 5 ATP
- 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle
  - *Sulfolobales*
  - Aerobic
  - 9 ATP



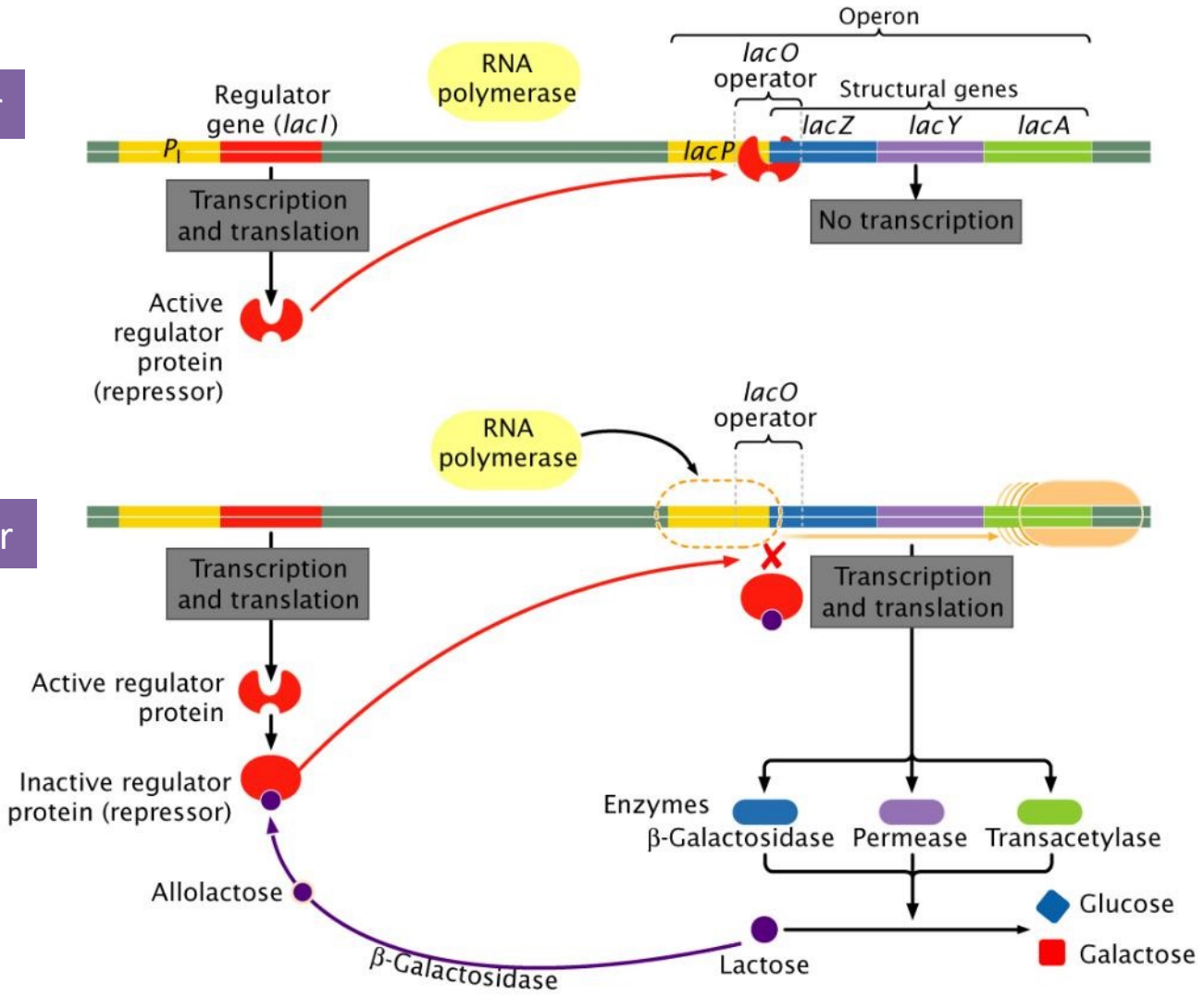
Berg. Applied and Environmental Microbiology (2011) 77, 1925-1936

Transport & Metabolism: linked processes in the cell

Ex: the *lac* operon

Absence of Inducer

Presence of Inducer



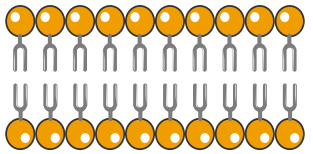
Pierce. Genetics (2nd Ed.) Freeman & Co (2005)

Transport across membranes

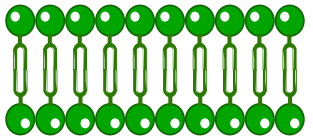
Membranes:

- Semi-permeable structure
- Dynamic
- Barrier with selective permeability
- Controlled entry & exit
  - Entry:
    - Nutriments
    - Signaling substances
    - Nucleic acids
    - Metal ions
  - Exit:
    - Products generated by metabolism
    - Secreted substances
      - Lipids
      - Carbohydrates
      - Proteins
      - Nucleic acids
    - Toxic substances
    - Metal ions
- Damaged → cell death

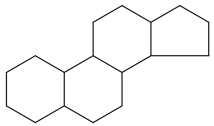
- Phospholipid bilayer :
  - Esters of glycerol + fatty acids + phosphate
    - Eukaryotes
    - Bacteria



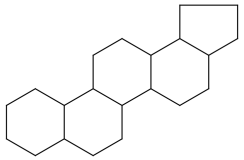
- Phospholipid monolayer :
  - Tetraethers of glycerol + fatty alcohols (+ phosphate esters)
    - Archaea



- Other lipids – for rigidity
  - Sterols
    - All eukaryotes
    - Bacteria: methanotrophs & mycoplasma



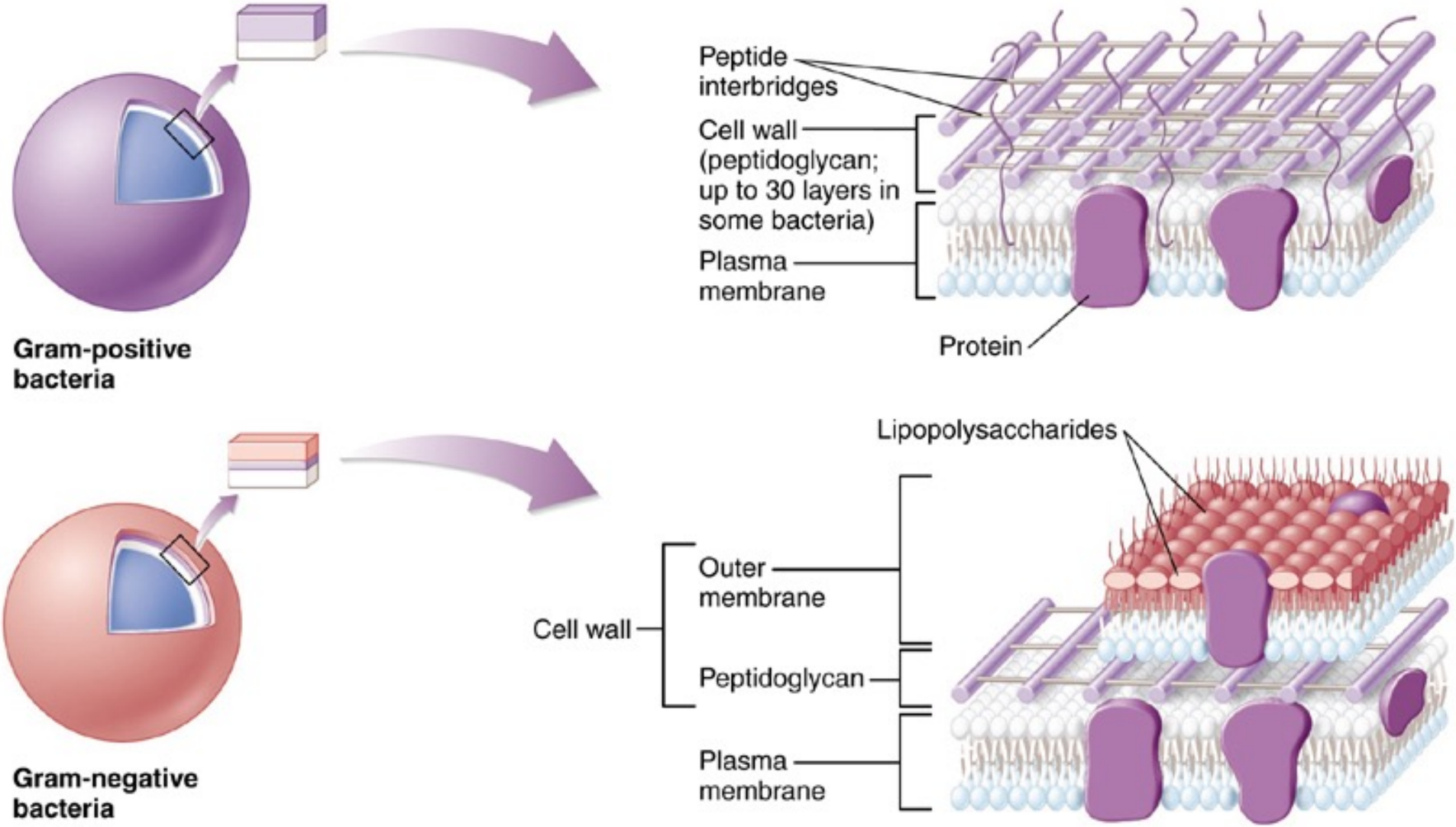
  - Hopanes
    - Bacteria



- Proteins = 75 % of membrane mass

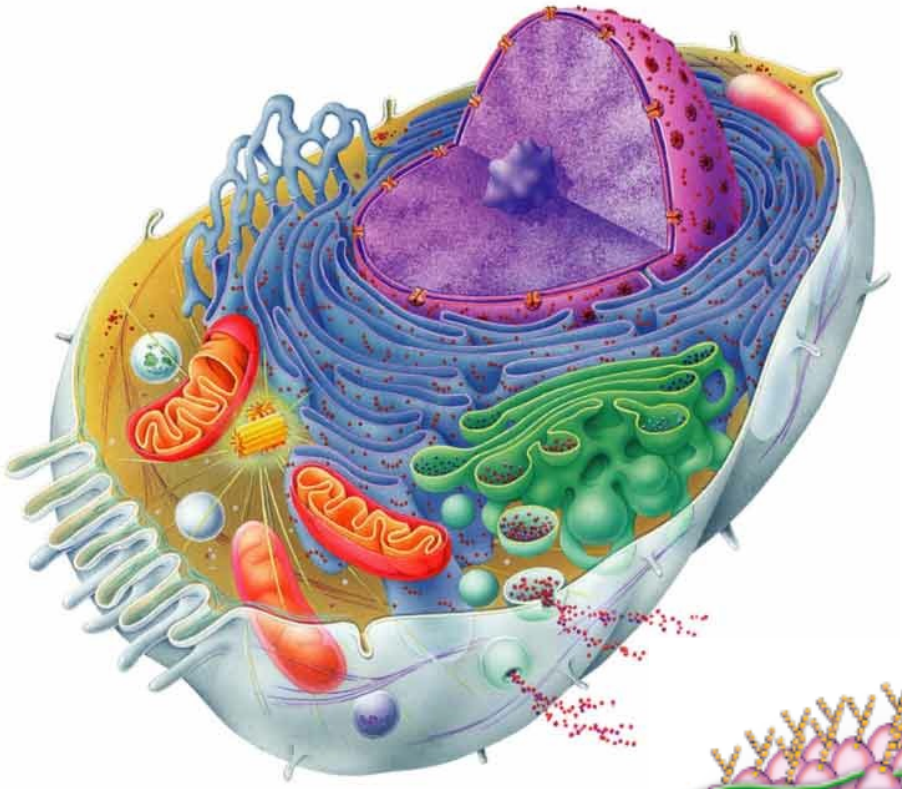
Transport across membranes

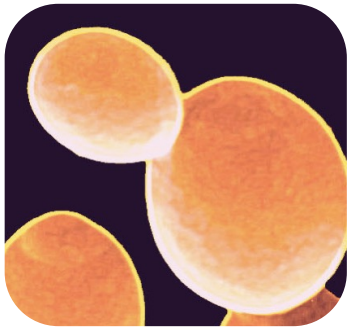
Bacteria



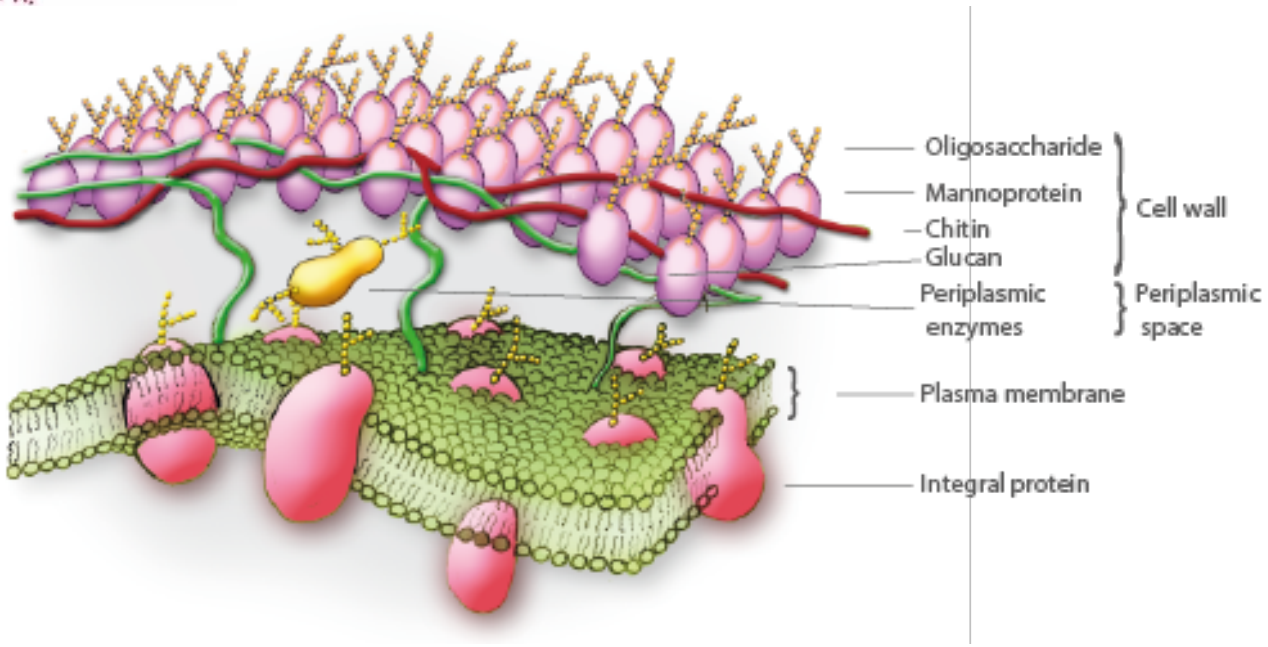
Transport across membranes

Eucaryotes





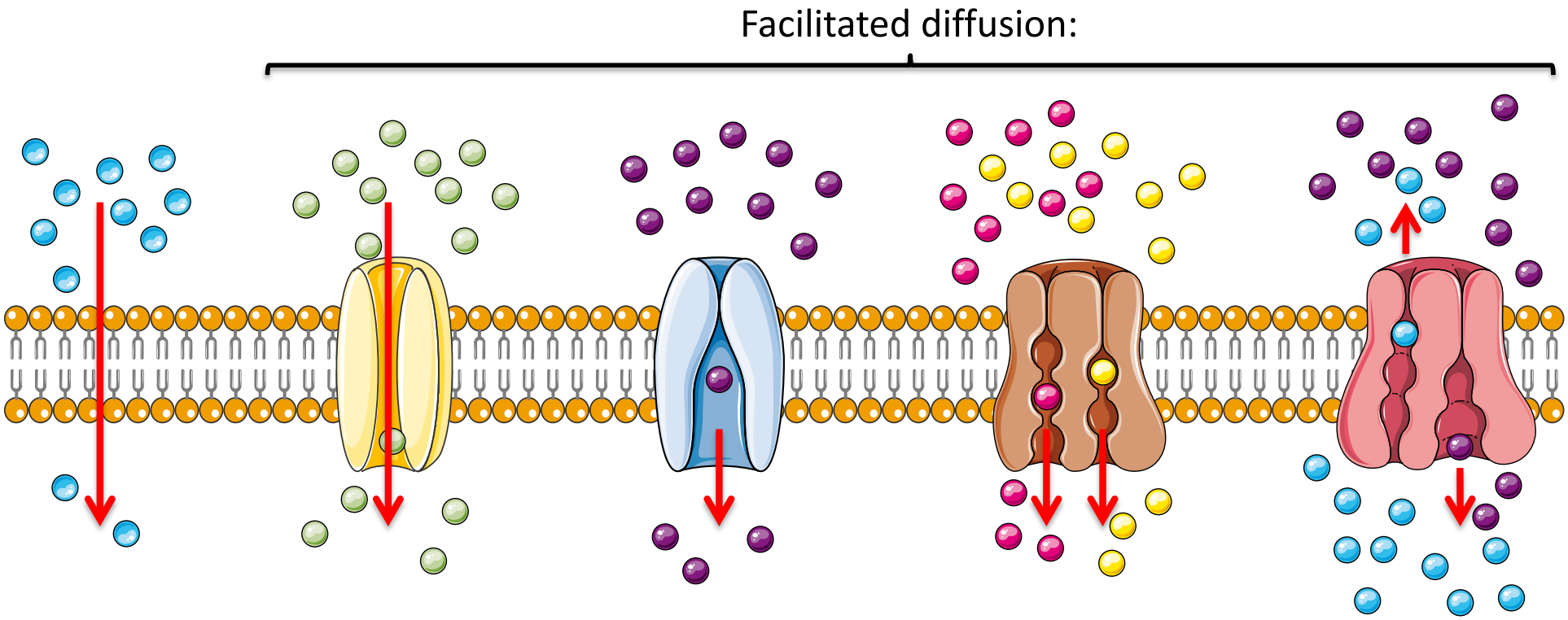
Saccharomyces cerevisiae



Transport classification: TC system

Passive transport:

- No energy required
- Occurs only in the downhill direction of concentration gradient



Diffusion	Pores & channels	Uniporters	Symporters	Antiporters
H_2O , O_2 , CO_2 , non polar organic molecules	TC#1	TC#2	TC#2	TC#2

http://www.tcdb.org

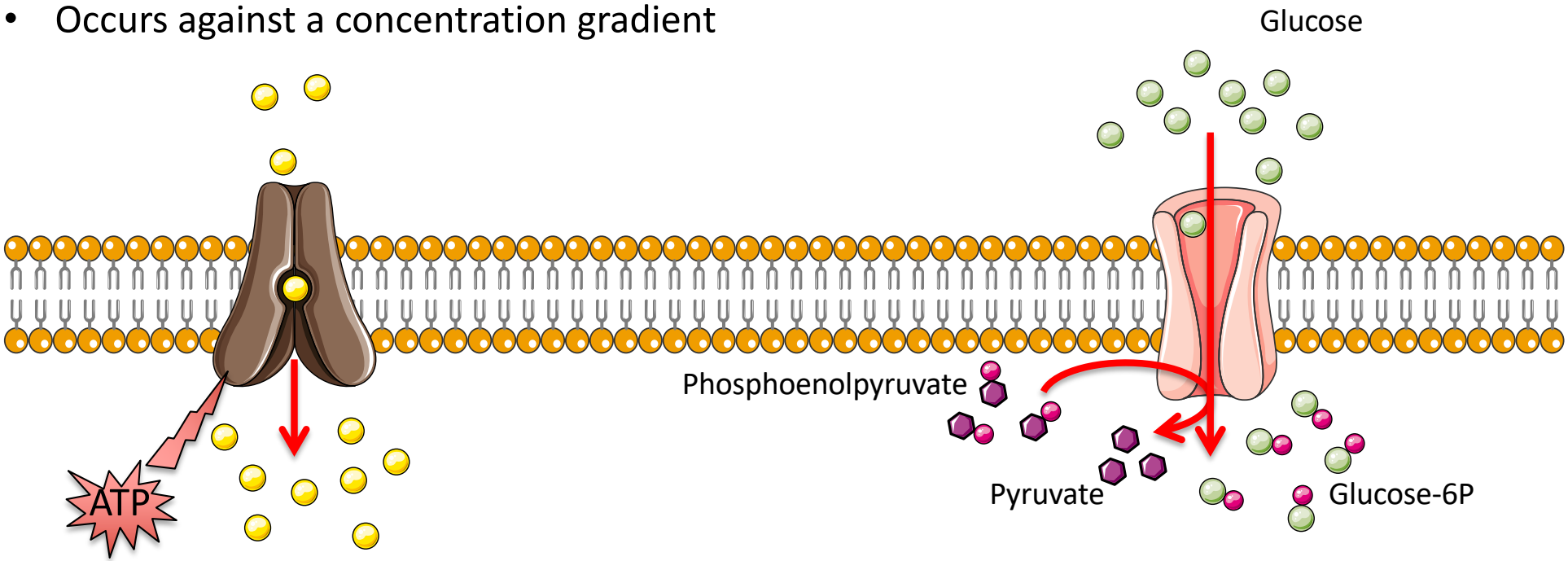
Transport classification: TC system

Active transport:

- Primary energy required
  - Chemical (ATP)
  - Electrical
  - Solar
- Occurs against a concentration gradient

Group translocators:

- substrate is modified during the transport process



Primary Active Transporters
TC#3

Group translocators
TC#4

TC#5: Transmembrane electron flow systems

http://www.tcdb.org

Kinetics of Transport processes — Diffusion

Movement of S_x is described by its flux JS_X:

- number of moles of S_x crossing a unit area of cell membrane per unit of time (moles.cm^{-2}s^{-1})
- Depends on :
  - permeability coefficient PS_X:
    - S_x solubility in lipids
    - S_x diffusion coefficient in lipids
    - Membrane thickness
  - Gradient across membrane
- Decomposed into:
  - Unidirectional influx $JS_X^{0\rightarrow i}$ – proportional to the outside concentration
  - Unidirectional efflux $JS_X^{i\rightarrow 0}$ – proportional to the inside concentration
- at equilibrium $[S_X]_0 = [S_X]_i$ - if S_x is not charged

Fick's Law:

$$JS_X = \underbrace{PS_X\,[S_X]_0}_{\text{influx}} - \underbrace{PS_X\,[S_X]_i}_{\text{efflux}}$$

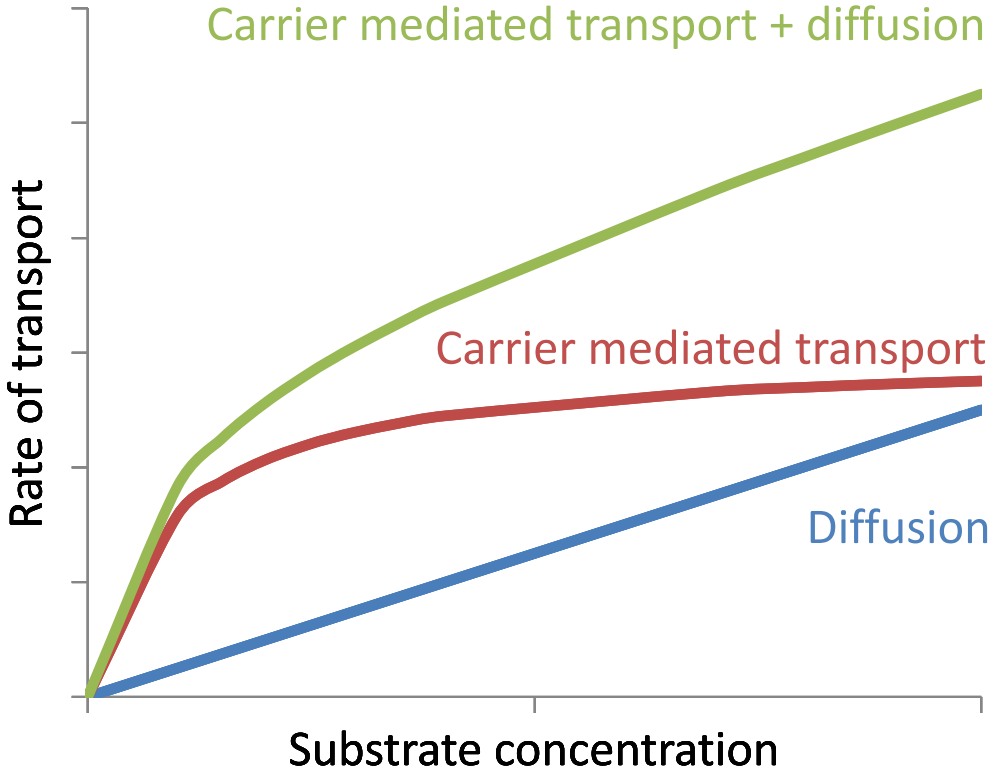
If S_x is charged: voltage difference across membrane should be considered

Electrochemical energy:

$$\Delta\mu S_X = RT \ln ([S_X]_i / [S_X]_0) + z_X F (\Psi_i - \Psi_0)$$

R = gas constant = 8,315 J.mol^{-1}K^{-1}
T = temperature °K
z_X = charge of S_X
F = Faraday constant = 96500 C
$\Psi_i - \Psi_0$ = voltage difference across membrane

Kinetics of Transport processes



Carrier mediated transport:

- Affinity
- Specificity
- Energy availability
- Regulation
- Localization

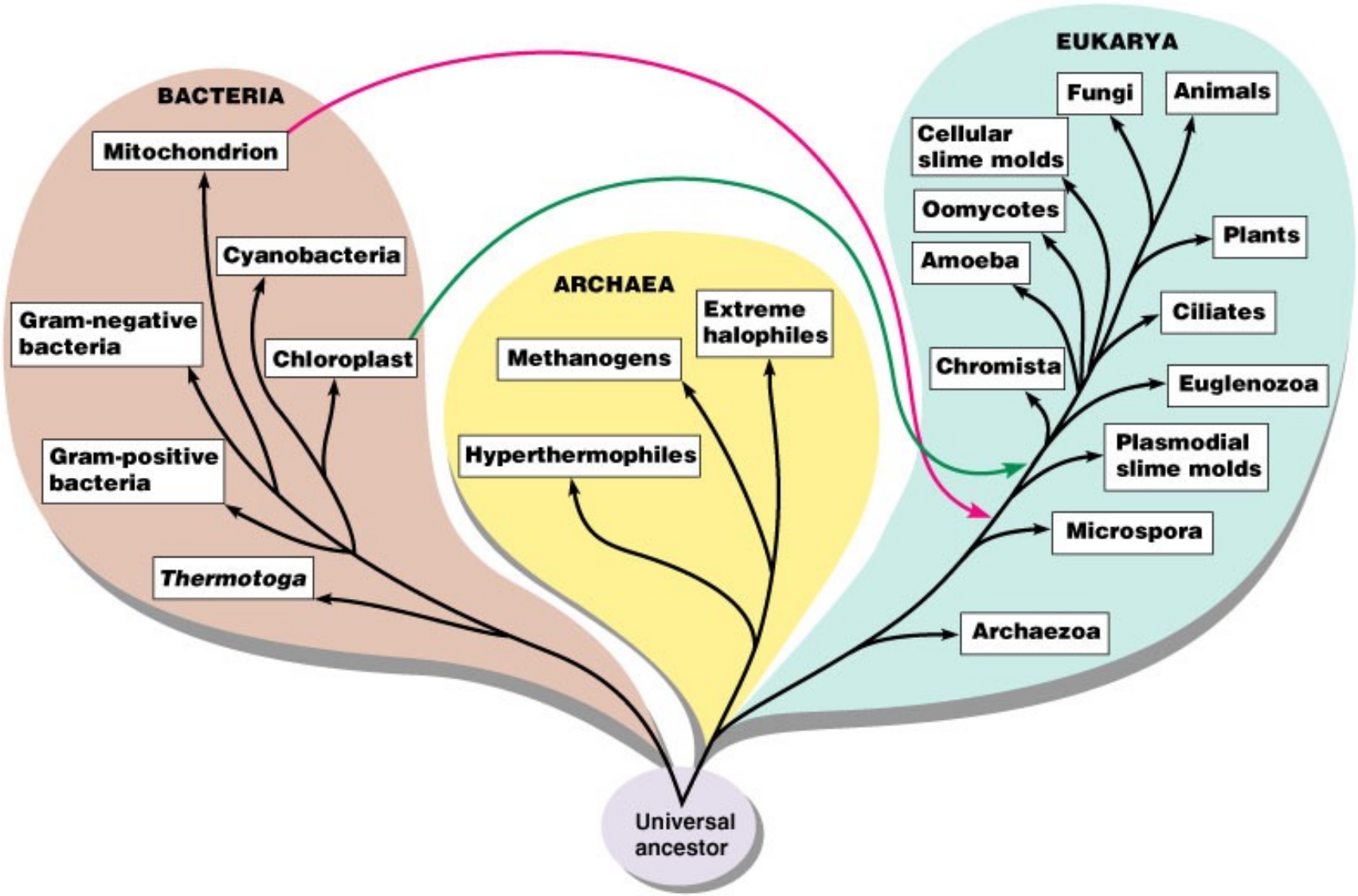
Major conformational changes

Steps: bind – transport – release
… like enzymes

Michaelis-Menten transport kinetics

$$V = V_{max} \frac{[S_X]_0}{K_m + [S_X]_0}$$

Different organisms – different cellular metabolisms



Cellular metabolism

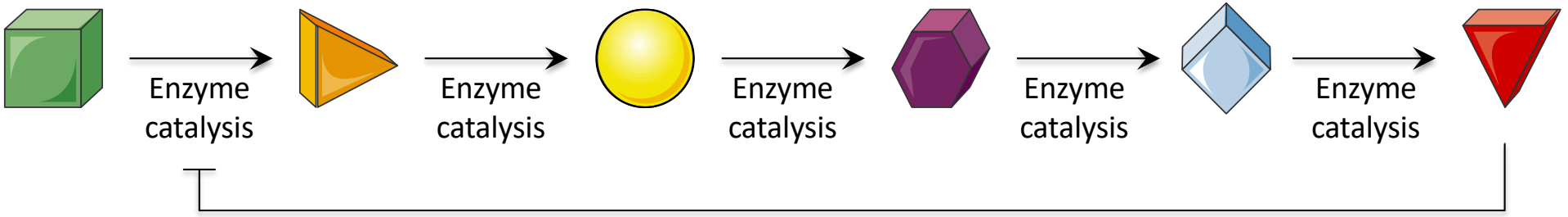
Metabolite:
- a naturally occurring molecule typically under 1000 MW

Enzyme:
- any protein which catalyzes chemical reactions involving the small molecules
- (ribozyme = catalytic RNAs)

Transporter:
- a membrane bound protein which shuttles ions, small molecules or macromolecules across membranes, into cells or out of cells.

Metabolism:
- complete set of biochemical reactions within a cell
- sum of processes involved in energy conversions in the cell

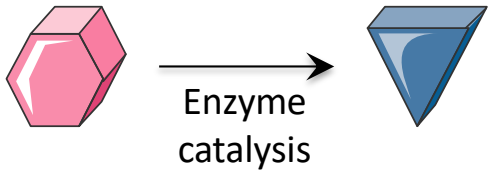
Metabolic pathways
- complex sequences of controlled chemical reactions



Cellular metabolism

Enzyme:

- Any protein which catalyzes chemical reactions involving the small molecules
- Catalysis = acceleration of chemical reactions – useful time scale
- Efficiency
- Selectivity
- Specificity / Promiscuity
- Mild conditions of temperature and pH
- Regulation – for coordination of different metabolic pathways



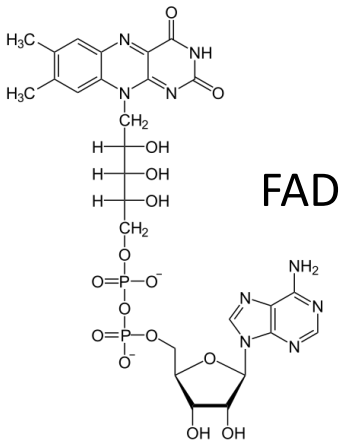
History:

- 1752: René Antoine Ferchault de Réaumur - first recognition and description biological catalysis = digestion of meat by secretions of stomach
- 1877: Wilhelm Kühne - first used the term enzyme = the unformed or not organized ferments, whose action can occur without the presence of organisms and outside of the same
- 1926: James B. Sumner - showed that the enzyme urease was a pure protein
- 1982: Thomas R. Cech and Sidney Altma - discovery of ribozymes (Nobel Prize in Chemistry 1989)

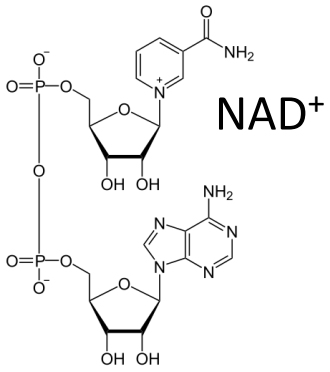
Enzymes

- Primary, secondary, tertiary, quaternary structure = essential for activity
- Some require post-translational modifications (phosphorylation, glycosylation, …)
- Some require cofactors:
  - Inorganic ions: Na, K, Mg, Cu, Fe, Mn, Mo, Zn, Ni, Se, …
  - Complex organic molecules = coenzymes
- Prostetic group = cofactor very tightly or even covalently bound

Coenzyme	Dietary precursor in mammals
Thiamine pyrophosphate	Vitamin B1 = thiamine
Flavin adenine dinucleotide (FAD)	Vitamin B2 = riboflavin
Nicotinamide ademine dinucleotide (NAD)	Vitamin B3 = nicotinic acid (niacin)
Coenzyme A	Vitamin B5 = panthothenic acid
Pyridoxal phosphate	Vitamin B6 = pyridoxine
Biocytin	Vitamin B8 (H) = biotin
Tetrahydropholate	Vitamin B9 (M) = folic acid
5'deoxyadenosylcobalamin	Vitamin B12 = cobalamin
Ascorbate	Vitamin C = ascorbic acid
Menaquinone	Vitamin K
Lipoate	Not required in diet
Coenzyme Q	Not required in diet



FAD

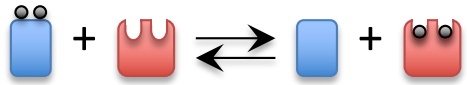
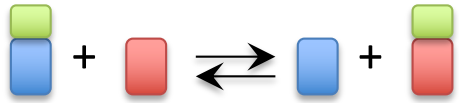
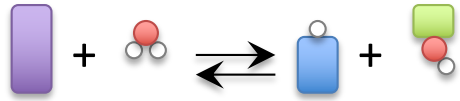
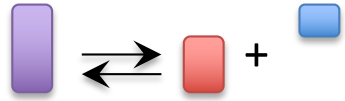
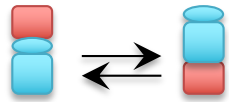
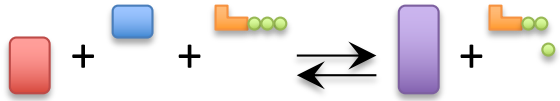


NAD^+

Enzyme classification: EC system

EC X.Y.Z.W

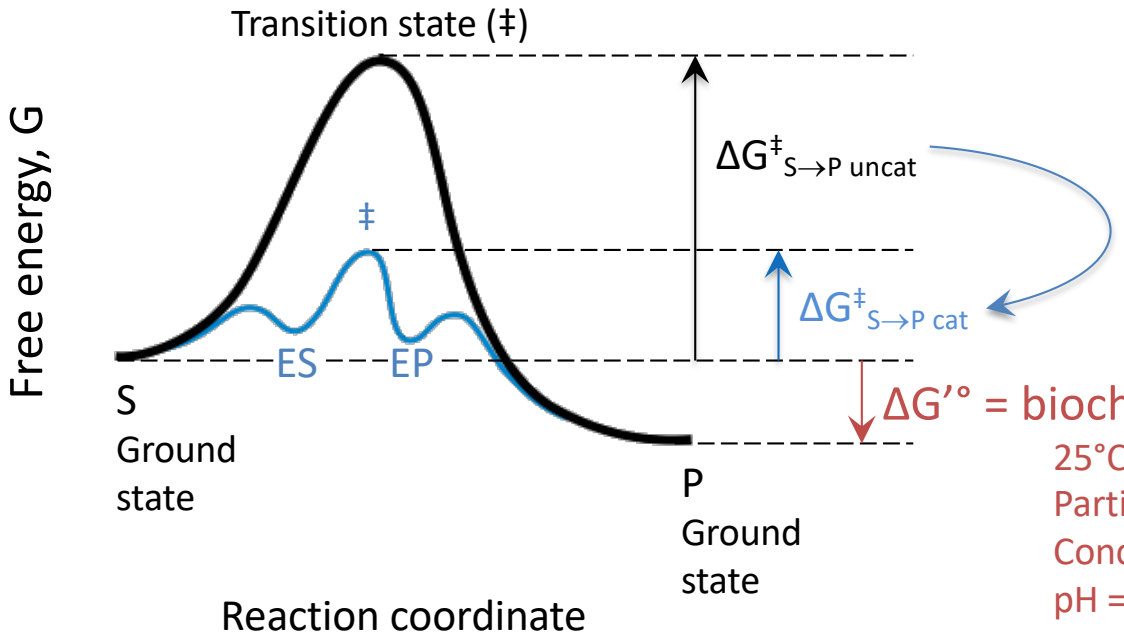
X = class
Y = subclass
Z = sub-subclass
W = the serial number of the enzyme in its sub-subclass

n°	Class		Type of reaction catalyzed
EC 1	Oxidoreductases		Transfer of e- = oxidation/reduction reactions
EC 2	Transferases		Transfer of a functional group (e.g. methyl, glycosyl or phosphate group)
EC 3	Hydrolases		Hydrolysis of various bonds
EC 4	Lyases		Cleavage of various bonds by means other than hydrolysis and oxidation
EC 5	Isomerases		Isomerization changes within a single molecule
EC 6	Ligases		Formation of covalent bonds coupled with the hydrolysis of a diphosphate bond in NTP
EC 7	Translocases		Movement of ions / molecules across membranes or their separation within membranes

How enzymes work ?

- Create an energetically favorable environment for the reaction to take place
- Active site

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



Enzymes lower the activation energy

ΔG'° = biochemical standard free energy

25°C (298°K)
Partial pressure of each gas = 1 atm
Concentration of each solute = 1 M
pH = 7

$$\Delta G'^\circ = -RT \ln K'_{eq} \qquad K'_{eq} = \frac{[P]}{[S]}$$

R = gas constant = 8,315 $J.mol^{-1}K^{-1}$
T = temperature °K

Enzymes do not change

- K'_{eq}
- $\Delta G'^\circ$
- $\Delta G'$

Free energy

$\Delta G'^\circ$ are additive

$A \rightleftharpoons B \quad \Delta G'^\circ_1$

$B \rightleftharpoons C \quad \Delta G'^\circ_2$

$A \rightleftharpoons C \quad \Delta G'^\circ = \Delta G'^\circ_1 + \Delta G'^\circ_2$

For redox reactions: standard reduction potential E'° is used to calculate $\Delta G'^\circ$

$A + 2\ e^- + 2\ H^+ \rightleftharpoons AH_2 \quad E'^\circ_A$

$BH_2 \rightleftharpoons B + 2\ e^- + 2\ H^+ \quad -E'^\circ_B$

$A + BH_2 \rightleftharpoons AH_2 + B \quad \Delta E'^\circ = E'^\circ_A - E'^\circ_B$

$\Delta G'^\circ = -nF\,\Delta E'^\circ$

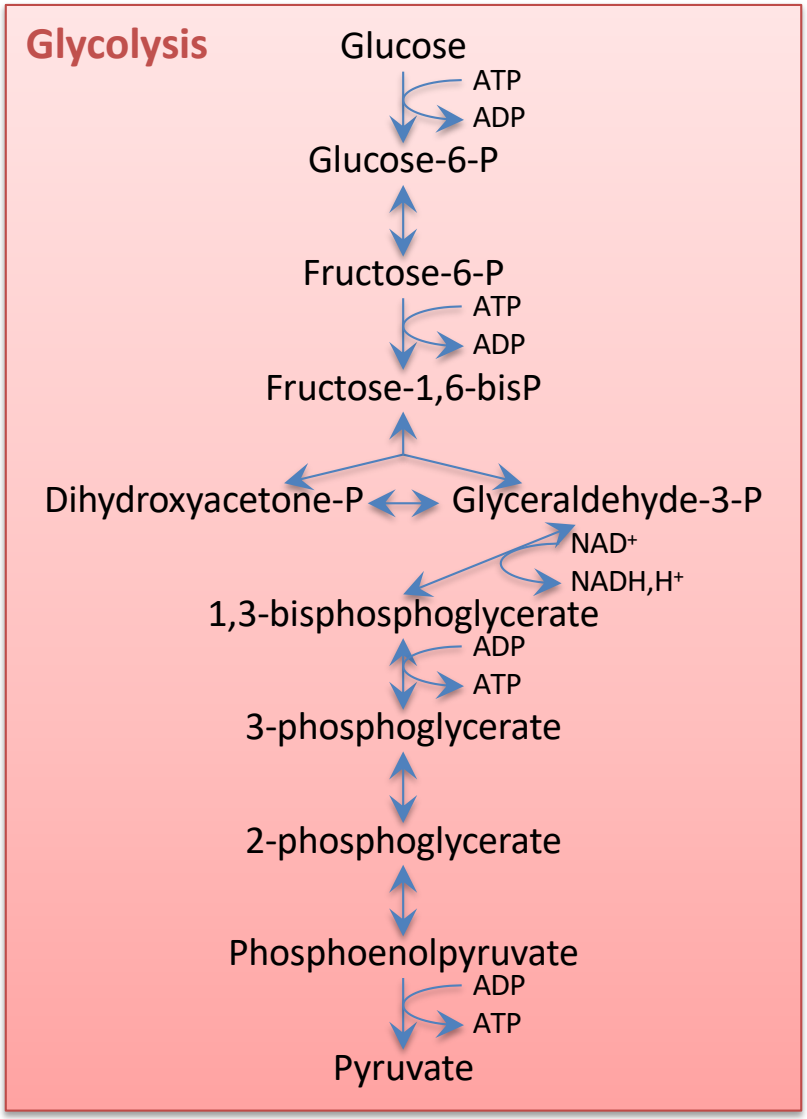
n = number of e- transferred
F = Faraday constant = 96500 C

Actual free energy of a reaction changes depends on reactant and product concentrations

$aA + bB \rightleftharpoons cC + dD \qquad \Delta G' = \Delta G'^\circ + RT \ln Q \qquad Q = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$ = reaction quotient

$\Delta G'$	Reaction	
< 0	Proceeds spontaneously forward	Exergonic
= 0	Is at equilibrium	
> 0	Cannot occur spontaneously – an input of free energy is required Proceeds spontaneously in reverse	Endergonic

Free energy changes over reactions of Glycolysis in erythrocytes



5000 µM	ΔG'° (kJ.mol^{-1})	ΔG' (kJ.mol^{-1})
	-16.7	-34
83 µM		
	1.67	-2.9
14 µM		
	-14.2	-19
31 µM		
	23.9	-0.23
140µM 19 µM	7.56	2.4
	6.3	-1.29
1 µM		
	-18.9	0.09
120 µM		
	4.4	0.83
30 µM		
	1.8	1.1
23 µM		
	-31.7	-23
51 µM		
	TOTAL:	TOTAL:
ATP: 1850 µM ADP: 140 µM Pi: 1000 µM	-73.97	-98.27

large negative free energy changes = reactions thermodynamically irreversible

No reaction is at equilibrium

Concentrations are at steady state

How enzymes work ?

- Create an energetically favorable environment for the reaction to take place
- Lower the activation energy
- Enzymes do not change
  - Reaction equilibrium (K'_{eq})
  - Thermodynamic feasibility of the reaction ($\Delta G'$)
- Enhance reaction rate (velocity)

$$\text{reaction rate} = \frac{d[P]}{dt}$$

Reaction rate is proportional
- Concentration of substrates (reactants)
- Rate constant *k*

First order reactions

$S \longrightarrow P (+ Q)$

$V = k [S]$

EC 3: hydrolases
EC 4: lyases
EC 5: isomerases

Second order reactions

$S_1 + S_2 \longrightarrow P_1 + \ldots$

$V = k [S_1] [S_2]$

EC 1: oxidoreductases
EC 2: transferases

Third order reactions

$S_1 + S_2 + S_3 \longrightarrow P_1 + \ldots$

$V = k [S_1] [S_2] [S_3]$

EC 1: oxidoreductases
EC 2: transferases
EC 6: ligases

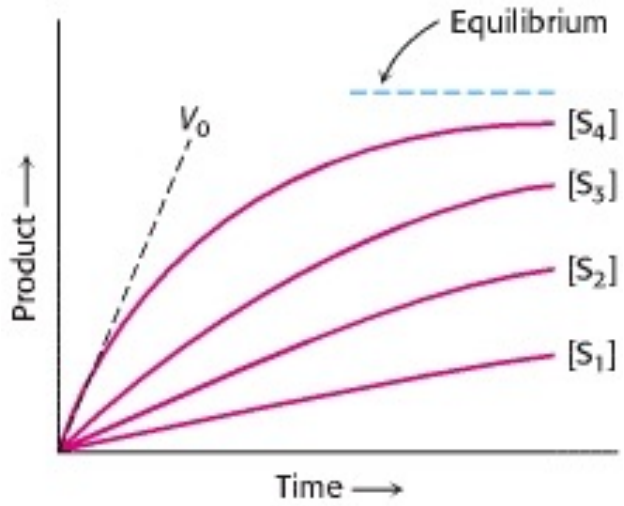
Initial Velocity

First order reactions

- the amount of product formed increases with time
- a time is reached when there is *no net change* in the concentration of S or P
- the enzyme is still actively converting S into P and *visa versa*, but the reaction equilibrium has been attained

S ⟶ P

$$\text{reaction rate} = \frac{d[P]}{dt}$$



- the initial velocity (V_0) for each substrate concentration is determined from the slope of the curve at the beginning of a reaction, *when the reverse reaction is insignificant*

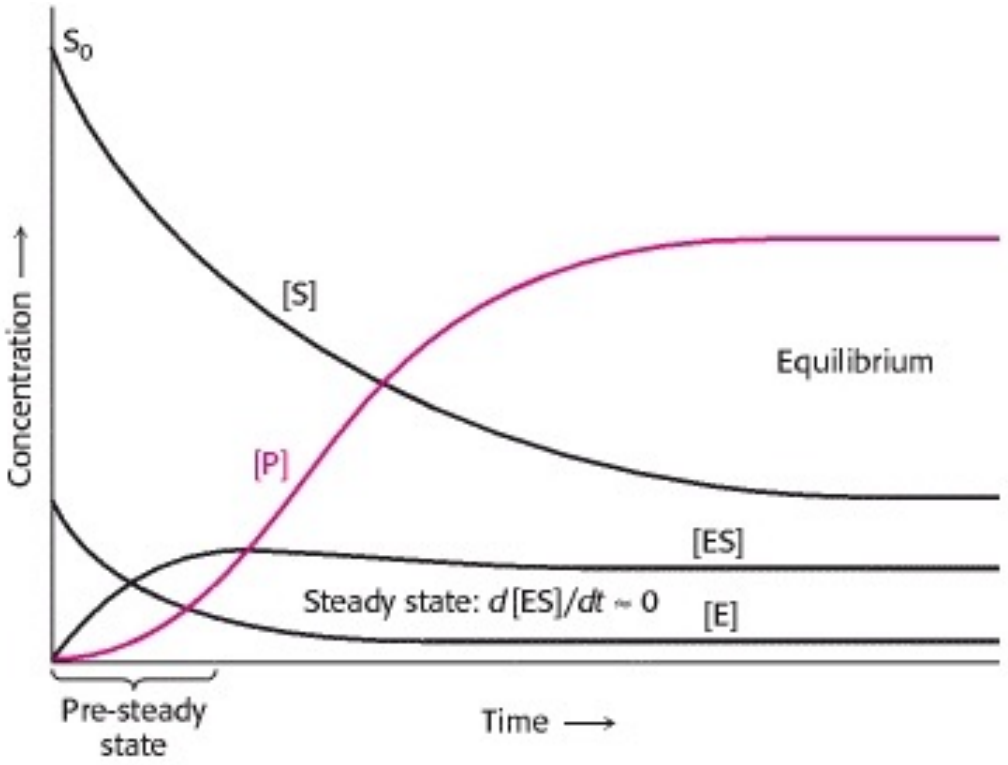
= Simplifying approach

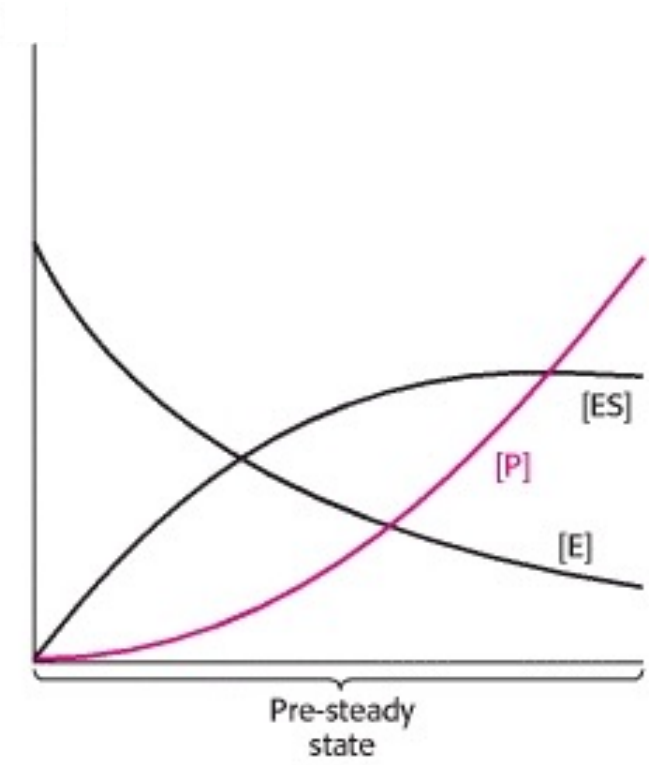
Initial velocity represents best the enzyme activity – no factors that can decrease it:

- Inhibition products
- pH changes
- Denaturation of enzyme

Steady-state

- Pre-Steady state: very short to be observed
- Steady state:
  - [ES] constant
  - V_0 reflects the steady state = approximation





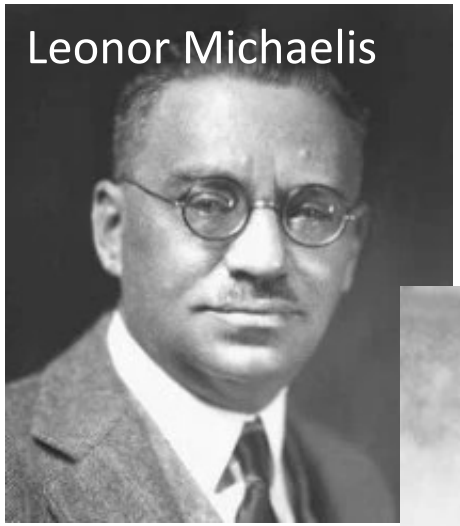
Michaelis-Menten Kinetics

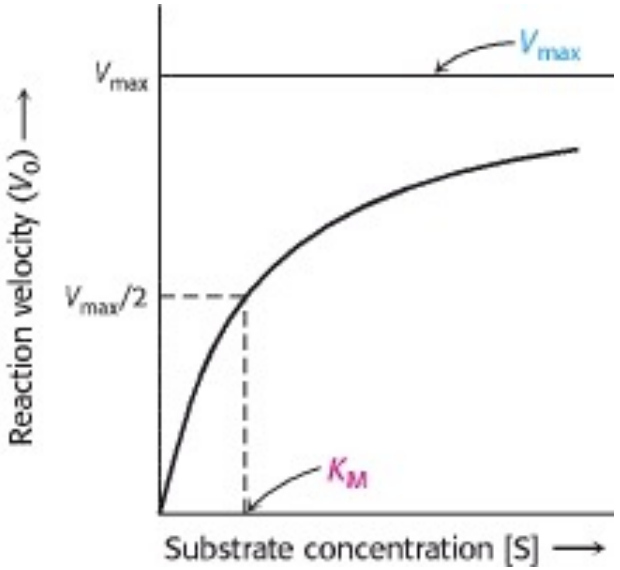
First order reactions

- simple model that accounts for most of the features of enzyme – catalyzed reactions

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

- Postulation of the reversible formation of ES





$$V_0 = V_{max} \frac{[S_0]}{K_m + [S_0]}$$

Michaelis-Menten Kinetics

First order reactions

Changes in the Concentration of Reaction Participants = formation rates – breakdown rates

Rate of [S] evolution = $\frac{d[S]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES]$

Rate of [E] evolution = $\frac{d[E]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES] + k_2\,[ES] - k_{-2}\,[P]\,[E]$

Rate of [ES] evolution = $\frac{d[ES]}{dt} = k_1\,[E]\,[S] - k_{-1}\,[ES] - k_2\,[ES] + k_{-2}\,[P]\,[E]$

Rate of [P] evolution = $\frac{d[P]}{dt} = k_2\,[ES] - k_{-2}\,[P]\,[E]$
= reaction rate

Michaelis-Menten Kinetics

First order reactions

Changes in the Concentration of Reaction Participants = formation rates – breakdown rates

Rate of [S] evolution = $\frac{d[S]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES]$

Rate of [E] evolution = $\frac{d[E]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES] + k_2\,[ES]$

Rate of [ES] evolution = $\frac{d[ES]}{dt} = k_1\,[E]\,[S] - k_{-1}\,[ES] - k_2\,[ES]$

Rate of [P] evolution = $\frac{d[P]}{dt} = k_2\,[ES]$
= reaction rate = V_0

1st assumption: initial rate
$[P] \cong 0$
$k_{-2}\,[P]\,[E] \cong 0$

Michaelis-Menten Kinetics

First order reactions

Changes in the Concentration of Reaction Participants = formation rates – breakdown rates

Rate of [S] evolution = $\frac{d[S]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES]$

Rate of [E] evolution = $\frac{d[E]}{dt} = -k_1\,[E]\,[S] + k_{-1}\,[ES] + k_2\,[ES]$

Rate of [ES] evolution = $\frac{d[ES]}{dt} = k_1\,[E]\,[S] - k_{-1}\,[ES] - k_2\,[ES] = 0$

Rate of [P] evolution = $\frac{d[P]}{dt} = k_2\,[ES]$
= reaction rate = V_0

2nd assumption: steady state
[ES] ≅ constant
$\frac{d[ES]}{dt} = 0$

Michaelis-Menten Kinetics

First order reactions

$k_1 [E] [S] - k_{-1} [ES] - k_2 [ES] = 0$

$k_1 ([E_t] - [ES]) [S_0] - k_{-1} [ES] - k_2 [ES] = 0$

3rd & 4th assumption

- Total enzyme concentration is constant:

  $[E_t] = [E] + [ES] \longrightarrow [E] = [E_t] - [ES]$

- $[S] >>> [E] \longrightarrow [S_0] = [S] + [ES] \longrightarrow [S_0] \cong [S]$

$$[ES] = \frac{k_1 [S_0] [E_t]}{k_1 [S_0] + k_{-1} + k_2}$$

$$K_m = \frac{k_{-1} + k_2}{k_1} \longrightarrow [ES] = \frac{[S_0] [E_t]}{[S_0] + K_m}$$

$$\frac{d[P]}{dt} = k_2 [ES] = V_0 \longrightarrow V_0 = k_2 [E_t] \frac{[S_0]}{[S_0] + K_m}$$

The maximum velocity occurs when the enzyme is saturated

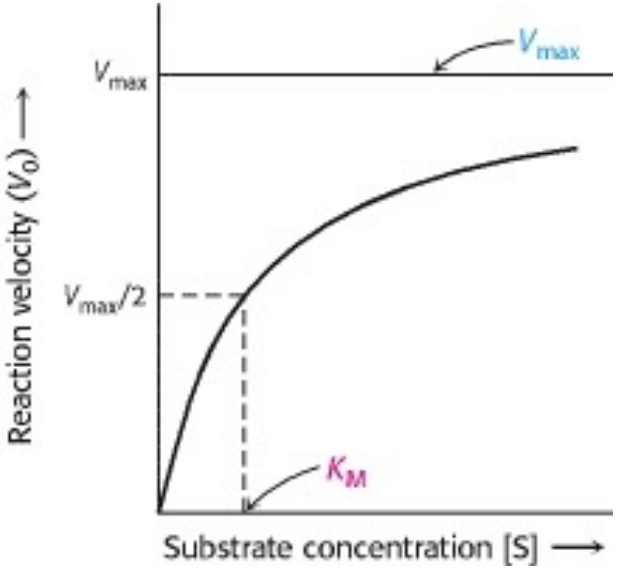
$V_{max} = k_2 [E_t]$

$$V_0 = V_{max} \frac{[S_0]}{[S_0] + K_m} = \text{Michaelis-Menten equation}$$

$k_2 = k_{cat}$ = turnover number

Michaelis-Menten Kinetics

First order reactions



$$V_0 = V_{max} \frac{[S_0]}{K_m + [S_0]}$$

$$V_{max} = k_{cat} [E_t]$$

- If [S] >> K_m : the rate of product formation is maximum

$$\frac{d[P]}{dt} = V_{max} = k_{cat} [E_t]$$

- If [S] = K_m : the rate of product formation is half of the maximum

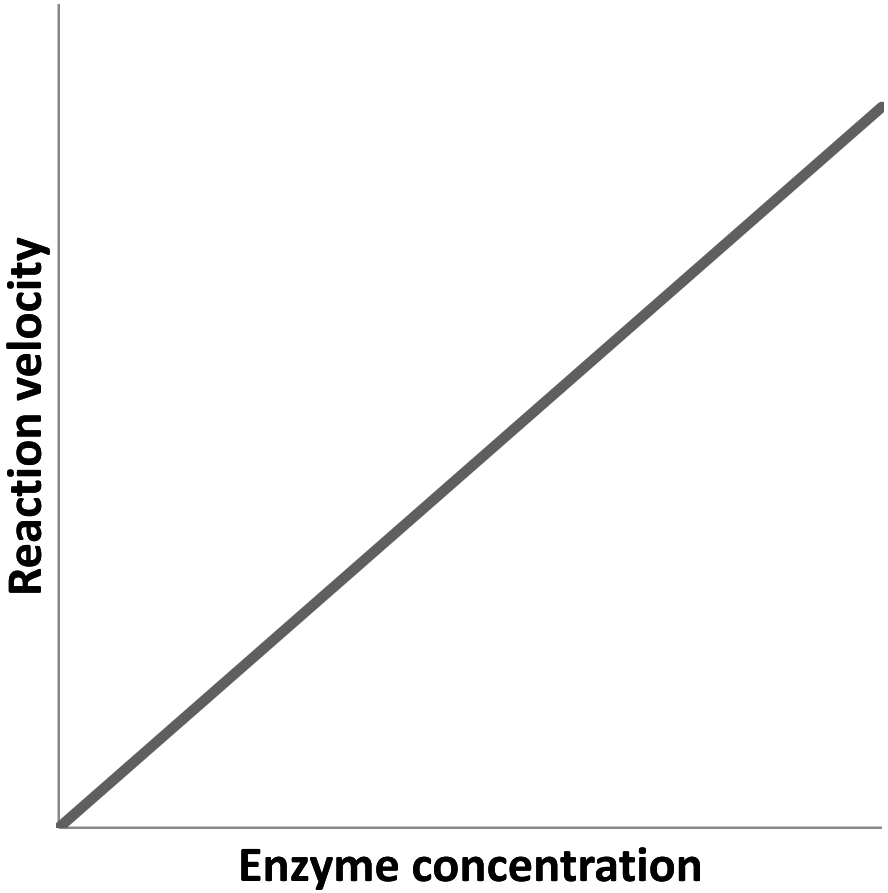
$$\frac{d[P]}{dt} = \frac{V_{max}}{2} = \frac{k_{cat} [E_t]}{2}$$

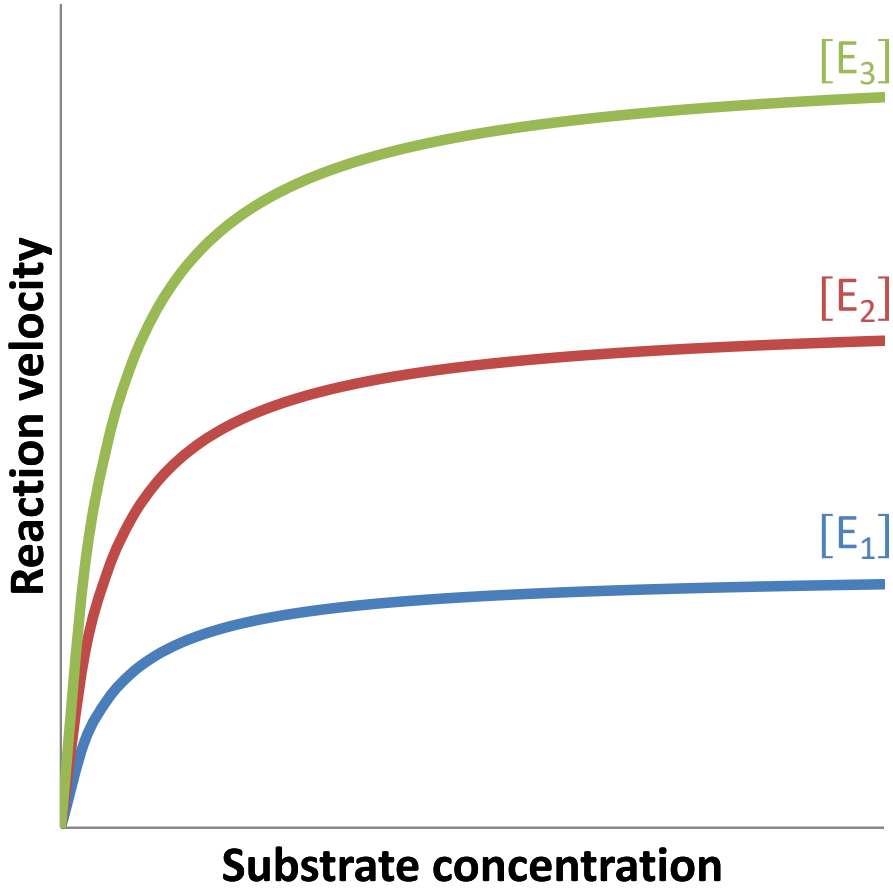
- If [S] << K_m : the rate of product formation depends on both [E] ≅ [E_t] and [S] through the efficiency constant k_{cat}/K_m

$$\frac{d[P]}{dt} = V_{max} \frac{[S]}{K_m} = [E_t] [S] \frac{k_{cat}}{K_m}$$

Michaelis-Menten Kinetics

First order reactions





$[E_1] < [E_2] < [E_3]$

V_{max} increases

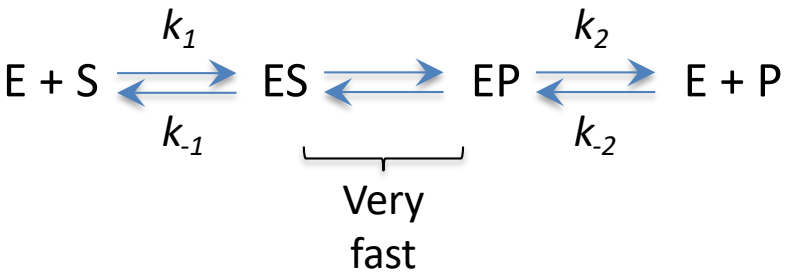
K_m is constant

Michaelis-Menten Kinetics

First order reactions

One substrate – one product

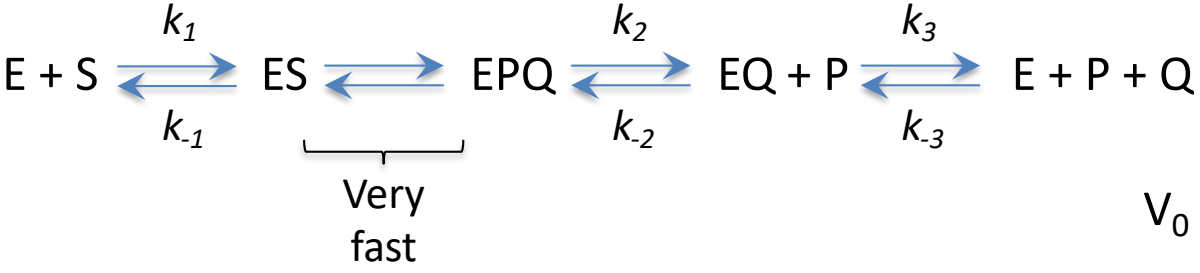
- EC 5: isomerases



$$V_0 = [E_t] \underbrace{k_2}_{k_{cat}} \underbrace{\frac{[S_0]}{[S_0] + \frac{k_{-1} + k_2}{k_1}}}_{K_m}$$

One substrate – 2 products

- EC 3: hydrolases
- EC 4: lyases



$$V_0 = [E_t] \underbrace{\frac{k_2\, k_3}{k_2 + k_3}}_{k_{cat}} \underbrace{\frac{[S_0]}{[S_0] + \frac{k_{-1}\, k_3\, (k_{-1} + k_2)}{k_1^2\, (k_2 + k_3)}}}_{K_m}$$

Michaelis-Menten Kinetics

Second order reactions

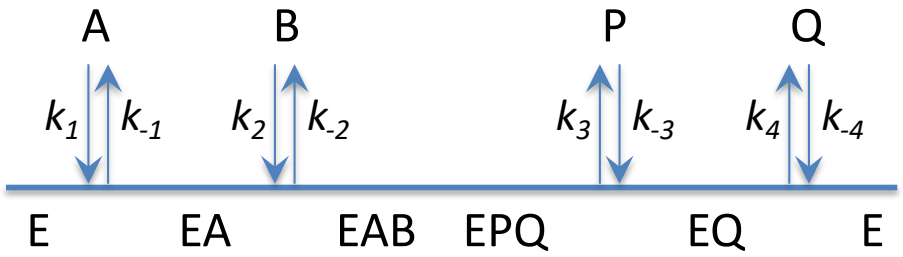
2 substrates – 2 products $A + B \rightleftharpoons P + Q$

- EC 1: oxidoreductases
- EC 2: transferases

Sequential Mechanisms

$$E + A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EAB \rightleftharpoons EPQ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EQ + P \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P + Q$$

Very fast



Random: either A or B may bind to the enzyme first, followed by the other substrate

Ex: EC 2.7.4.3 adenylate kinase:

ATP + AMP ↔ 2 ADP

Ordered: A (the *leading substrate*) must bind to the enzyme first before B can be bound

Ex: EC 1.1.1.1 alcohol deshydrogenase:

$NAD^+ + CH_3\text{-}CH_2\text{-}OH \leftrightarrow NADH,H^+ + CH_3\text{-}CHO$

$$V_0 = [E_t] \underbrace{\frac{k_3\,k_4}{k_3 + k_4}}_{k_{cat}} \frac{[A]\,[B]}{[A]\,[B] + [B]\underbrace{\frac{k_3\,k_4}{k_1\,(k_3 + k_4)}}_{K_m^A} + [A]\underbrace{\frac{k_4\,(k_{-2} + k_3)}{k_2\,(k_3 + k_4)}}_{K_m^B} + \frac{k_{-1}\,k_4\,(k_{-2} + k_3)}{k_1\,k_2\,(k_3 + k_4)}}$$

Michaelis-Menten Kinetics

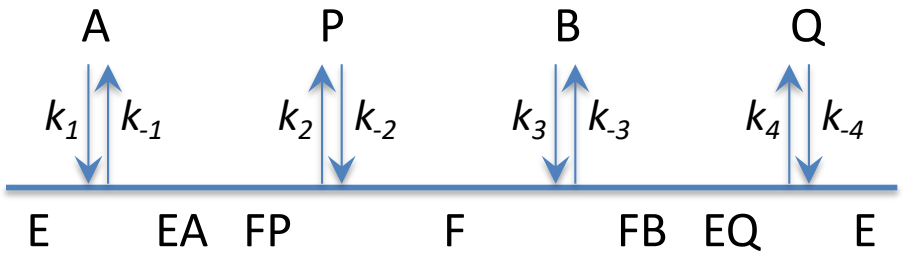
Second order reactions

2 substrates – 2 products $A + B \rightleftharpoons P + Q$

- EC 1: oxidoreductases
- EC 2: transferases

Ping-Pong Mechanisms

$$E + A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA + B \rightleftharpoons \underbrace{FP + B}_{\text{Very fast}} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} F + P + B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} FB + P \rightleftharpoons \underbrace{EQ + P}_{\text{Very fast}} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P + Q$$



Ping: The leading substrate A binds to E, change it to a chemically different form F and the first product P is released

Pong: The second substrate B can be bound by F, the second product Q is formed and the enzyme is regenerated to the original form E

Ex. EC 2.6.1.x transaminases

amino acid$_1$ + keto acid$_2$ ↔ keto acid$_1$ + amino acid$_2$

$$V_0 = [E_t] \underbrace{\frac{k_2\,k_4}{k_2 + k_4}}_{k_{cat}} \frac{[A]\,[B]}{[A]\,[B] + [B]\underbrace{\frac{k_4\,(k_{-1} + k_2)}{k_1\,(k_2 + k_4)}}_{K_m^{\,A}} + [A]\underbrace{\frac{k_2\,(k_{-3} + k_4)}{k_3\,(k_2 + k_4)}}_{K_m^{\,B}}}$$

Michaelis-Menten Kinetics

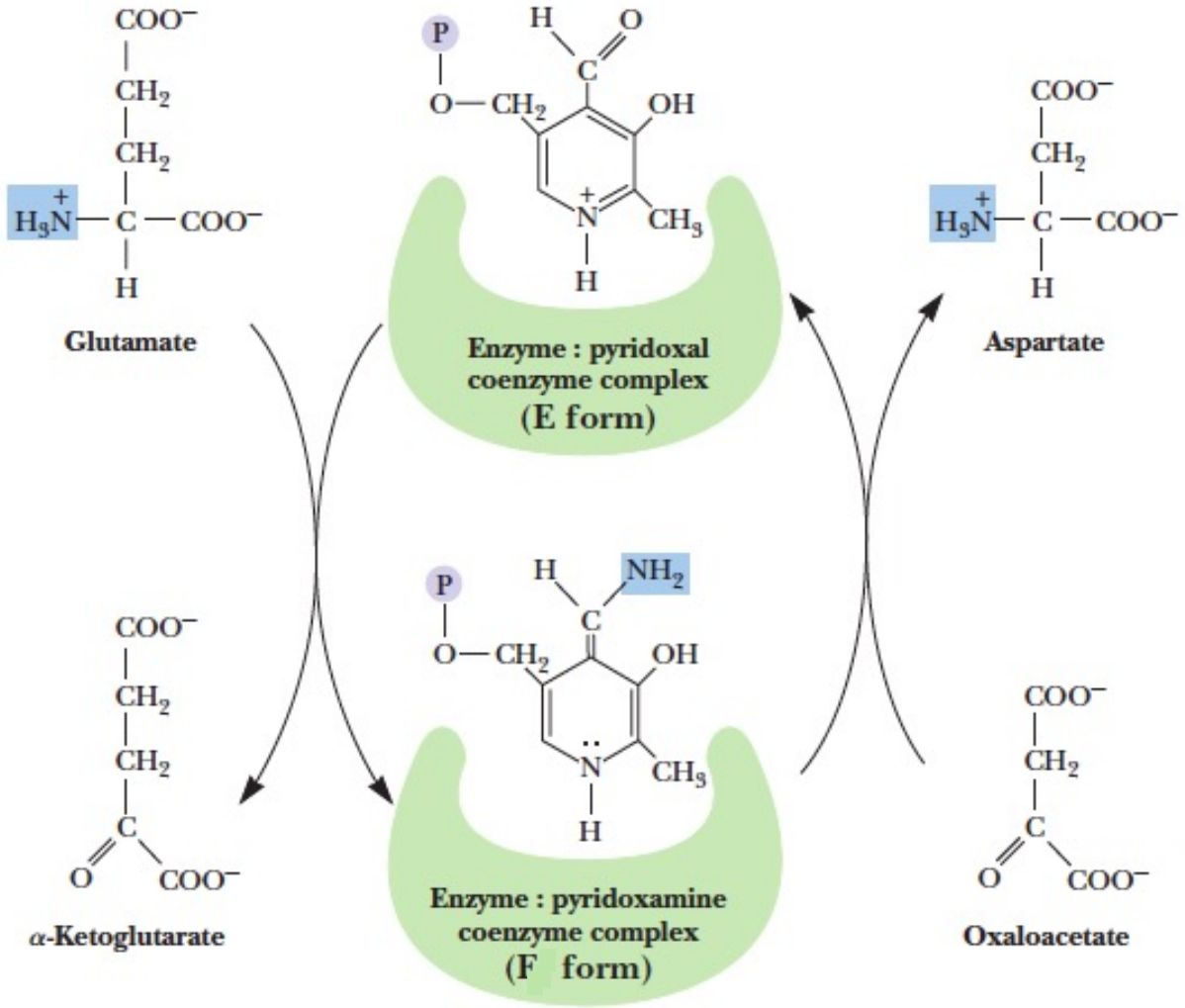
2 substrates – 2 products

$A + B \rightleftharpoons P + Q$

Ping-Pong Mechanisms

EC 2.6.1.1
Glutamate aspartate aminotransferase

L-glutamate + oxaloacetate ↔
2-oxoglutarate + L-aspartate



Michaelis-Menten Kinetics

Third order reactions

3 substrates – n products $A + B + C \rightleftharpoons P + Q + \ldots$

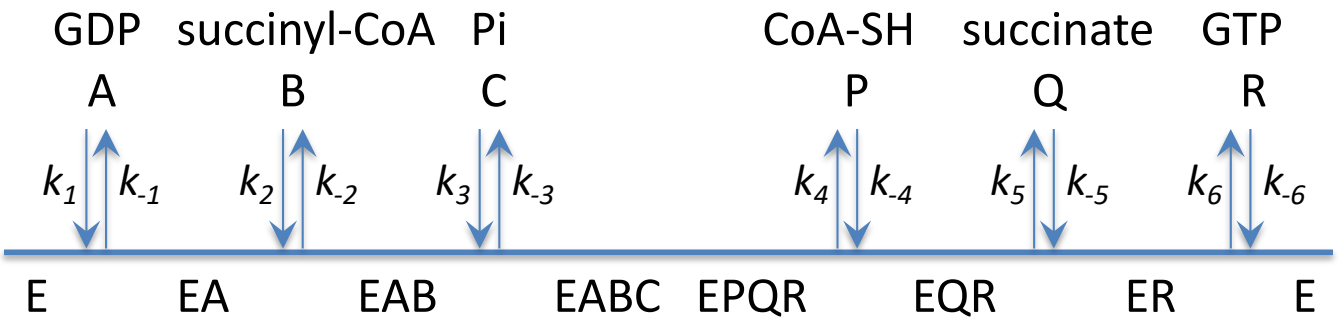
- EC 1: oxidoreductases
- EC 2: transferases
- EC 6: ligases

Many possibilities & combinations:

- Sequential Mechanisms
  - Random
  - Ordered
- Ping-Pong Mechanisms

Ex. ordered ter–ter mechanism: EC 6.2.1.4 succinyl-CoA synthetase

$$\underset{B}{\text{succinyl-CoA}} + \underset{A}{\text{GDP}} + \underset{C}{\text{Pi}} \leftrightarrow \underset{Q}{\text{succinate}} + \underset{R}{\text{GTP}} + \underset{P}{\text{CoA-SH}}$$



Michaelis-Menten Kinetics

Third order reactions

3 substrates – n products $A + B + C \rightleftharpoons P + Q + \ldots$

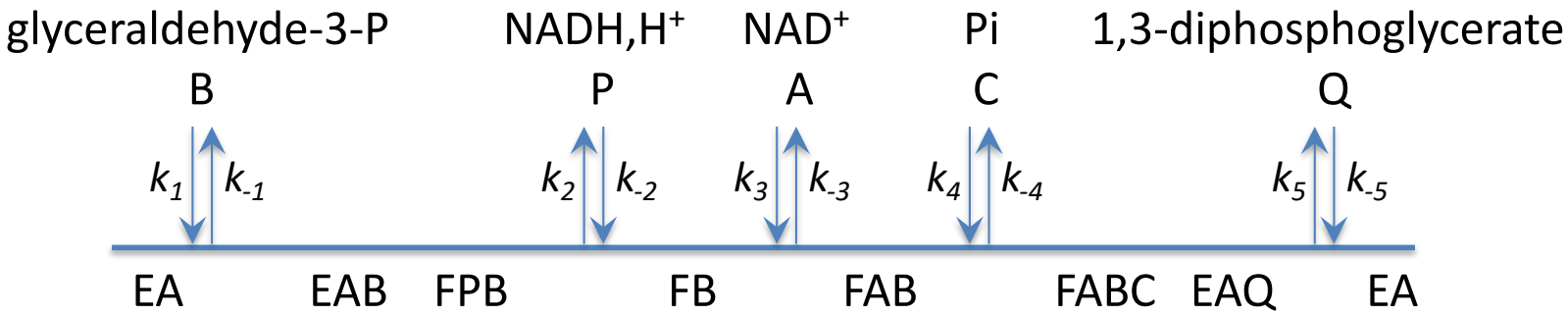
- EC 1: oxidoreductases
- EC 2: transferases
- EC 6: ligases

Many possibilities & combinations:

- Sequential Mechanisms
  - Random
  - Ordered
- Ping-Pong Mechanisms

Ex. Ping-pong mechanism: EC 1.2.1.12 glyceraldehyde-3-P dehydrogenase

$$\underset{B}{\text{glyceraldehyde-3-P}} + \underset{A}{NAD^+} + \underset{C}{Pi} \leftrightarrow \underset{Q}{\text{1,3-diphosphoglycerate}} + \underset{P}{NADH,H^+}$$



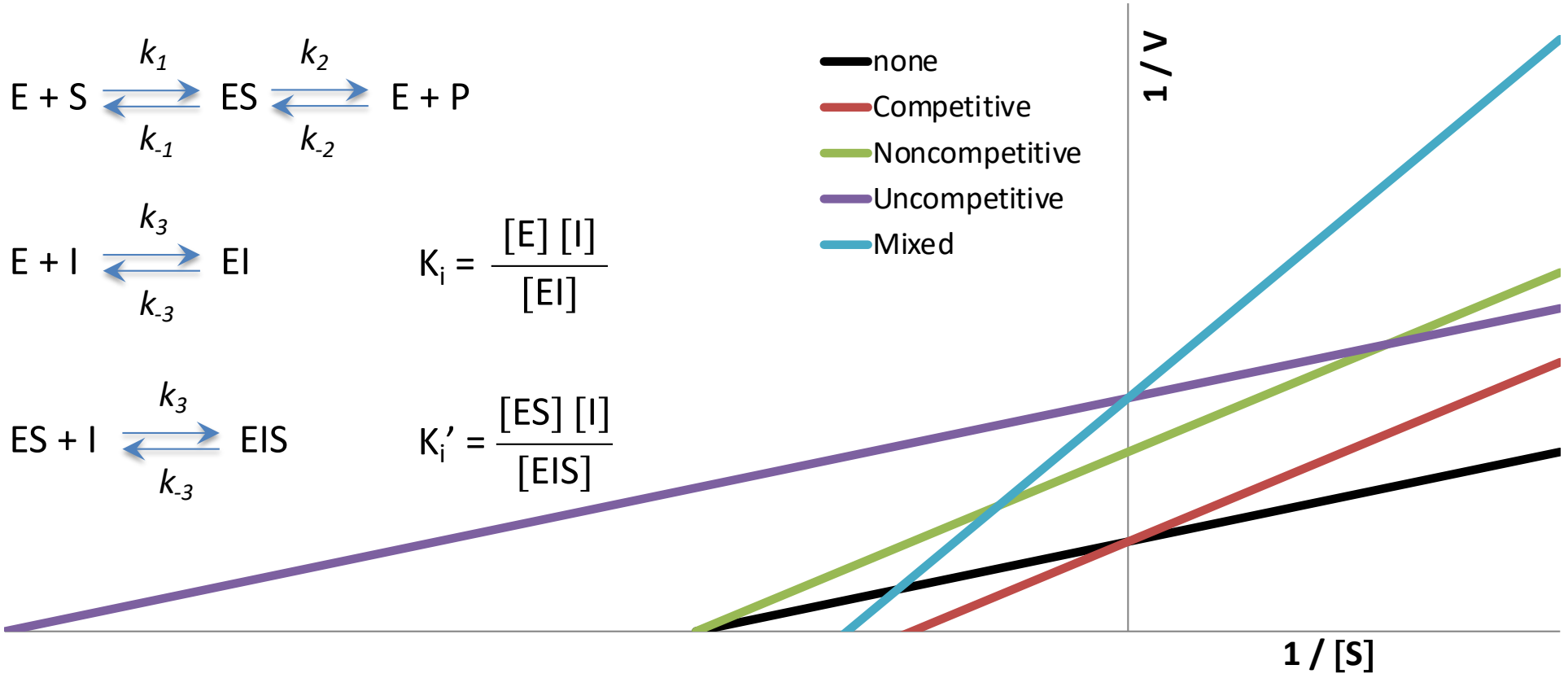
Effect of Various Types of Inhibitors

Inhibition Type	Rate Equation	Apparent Km	Apparent V_{max}
None	$V = V_{max}[S]/([S] + K_m)$	K_m	V_{max}
Competitive	$V = V_{max}[S]/([S] + K_m(1 + [I]/K_i))$	$K_m(1 + [I]/K_i)$	V_{max}
Noncompetitive	$V = V_{max}[S]/((1 + [I]/K_i)([S] + K_m))$	K_m	$V_{max}/(1 + [I]/K_i)$
Uncompetitive	$V = V_{max}[S]/(K_m + [S](1 + [I]/K_i'))$	$K_m/(1 + [I]/K_i')$	$V_{max}/(1 + [I]/K_i')$
Mixed	$V = V_{max}[S]/((1 + [I]/K_i)K_m + (1 + [I]K_i'[S])$	$K_m(1 + [I]/K_i)/(1 + [I]/K_i')$	$V_{max}/(1 + [I]/K_i')$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI \qquad K_i = \frac{[E][I]}{[EI]}$$

$$ES + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EIS \qquad K_i' = \frac{[ES][I]}{[EIS]}$$



Non Michaelis-Menten Kinetics enzymes

- Enzymes that regulate the metabolism = regulatory enzymes
- Display increased or decreased activity in response to certain signals
- At key point on the metabolic map
  - At the start of metabolic pathways
  - At junctions between metabolic pathways
  - Control rates
  - Channel metabolites

Allosteric enzymes

- Reversible & Non-covalent binding of regulatory compounds
- Homotropic:
  - modulator = substrate
- Heterotropic:
  - modulator ≠ substrate
  - = allosteric modulator
  - binds at sites different than the active site
- Conformational changes
- Large proteins
- Multimeric protein (often)

Covalently modulated enzymes

- Reversible & covalent modification by other enzymes
  - Phosphorylation
  - Methylation
  - Adenylation
  - Ribosylation
  - …
- Irreversible & covalent modification by other enzymes
  - Proteolysis

Other mechanisms

- Interaction with regulatory proteins
- Degradation

Allosteric enzymes

Cooperative behavior = binding of a certain ligand influences the affinity of the protein to a further ligand of the same (homotropic) or another type (heterotropic)

- Positive

$E + S \longrightarrow ES$

$ES + S \xrightarrow{Fast} ES_2$

$ES_2 + S \xrightarrow{Faster} ES_3$

…

- Negative

$E + S \longrightarrow ES$

$ES + S \xrightarrow{Slow} ES_2$

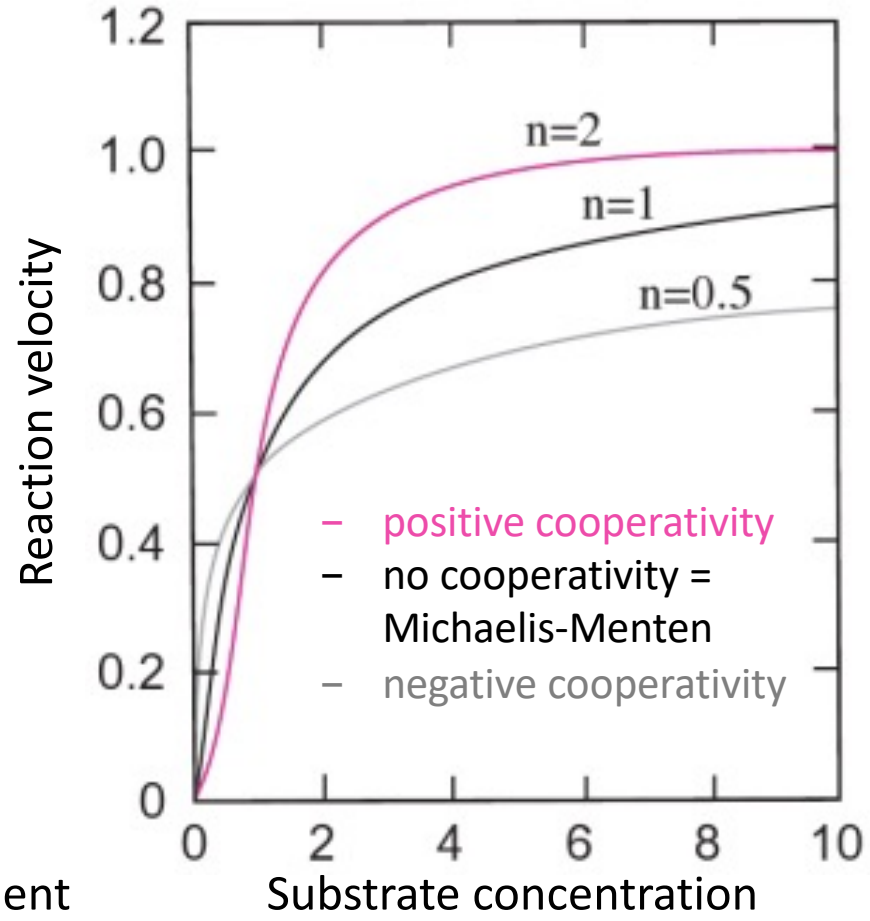
$ES_2 + S \xrightarrow{Slower} ES_3$

…

The binding constant $K_B = \dfrac{[ES_n]}{[E]\,[S]^n}$

$V_0 = V_{max} \dfrac{K_B\,[S]^n}{1 + K_B\,[S]^n}$

n = Hill coefficient



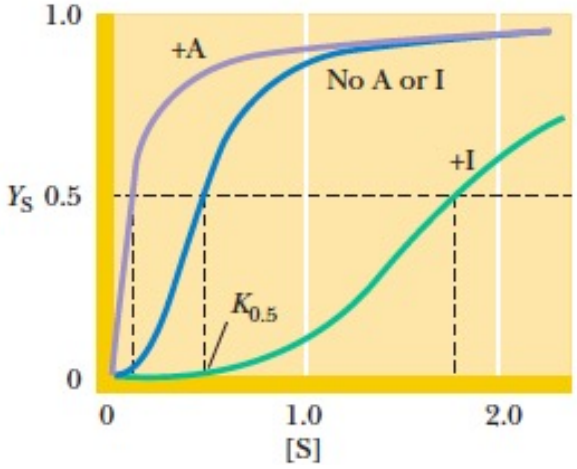
Non Michaelis-Menten Kinetics enzymes

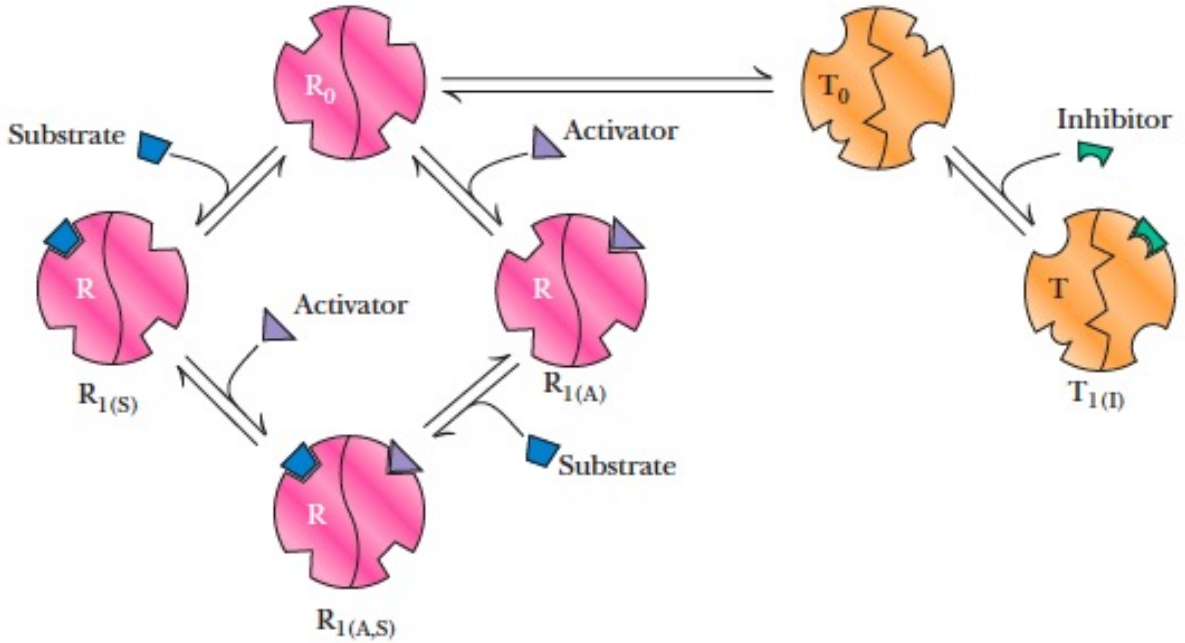
A dimeric protein that can exist in either of two states: R_0 or T_0.
This protein can bind three ligands:

1) Substrate (S): Binds only to R at site S

2) Activator (A): A positive effector that binds only to R at site F

3) Inhibitor (I): A negative effector that binds only to T at site F





Effects of A:
$A + R_0 \rightarrow R_{1(A)}$
Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

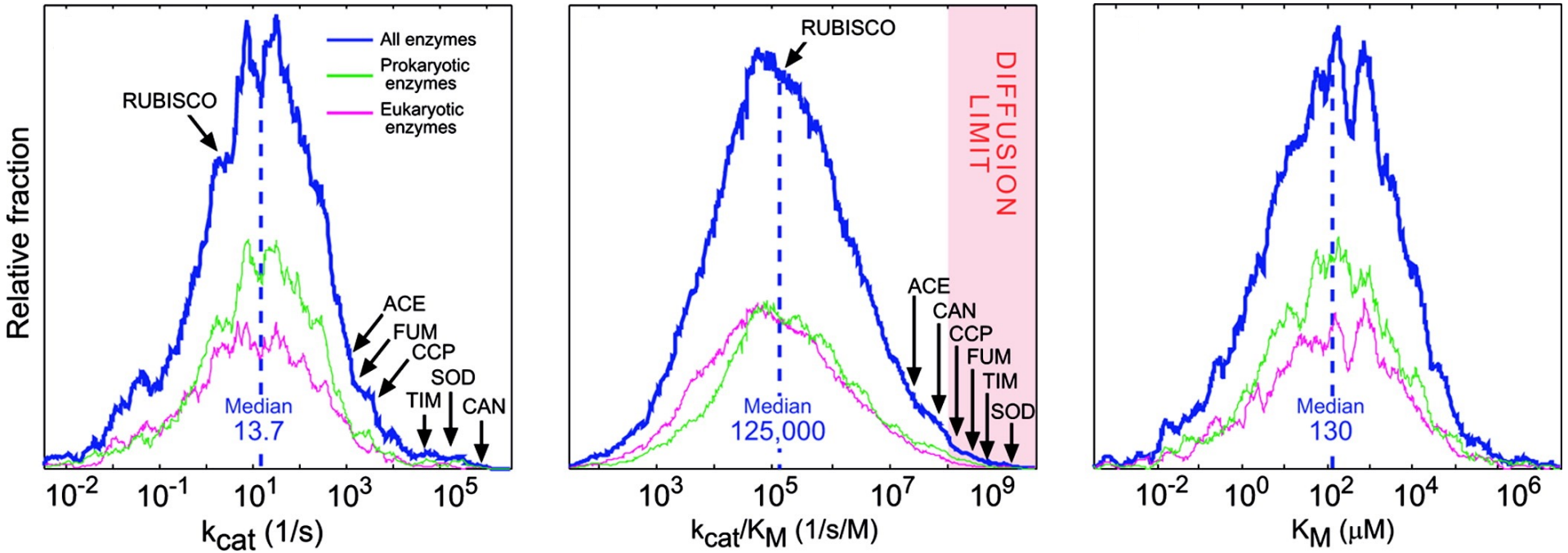
(1) More binding sites for S made available.

(2) Decrease in cooperativity of substrate saturation curve.

Effects of I:
$I + T_0 \rightarrow T_{1(I)}$
Increase in number of T-conformers (decrease in R_0 as $R_0 \rightarrow T_0$ to restore equilibrium)

Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve.

Kinetic parameters of enzymes



- ACE, acetylcholine esterase
- CAN, carbonic anhydrase
- CCP, cytochrome *c* peroxidase
- FUM, fumarase
- Rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase
- SOD, superoxide dismutase
- TIM, triosephosphate isomerase

- enzymes operating in secondary metabolism are, on average, ~30-fold slower than those of central metabolism
- Substrate low molecular mass and hydrophobicity appear to limit K_M optimization

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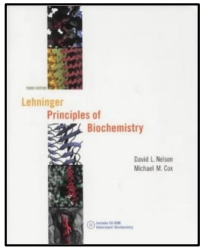
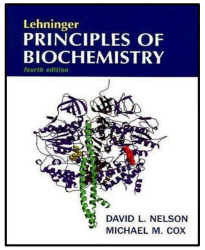

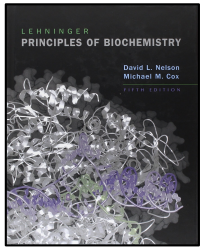

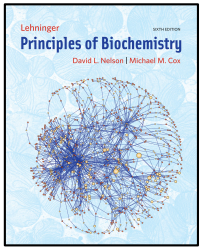

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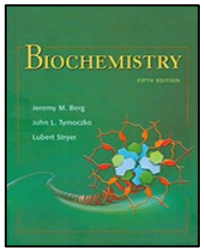

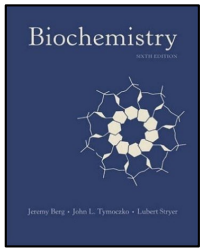

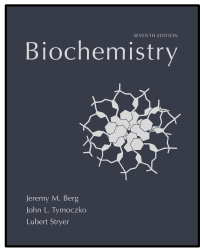

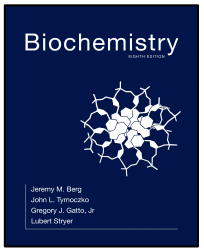

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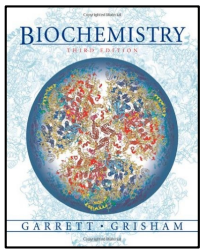

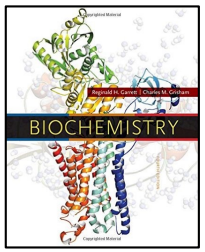

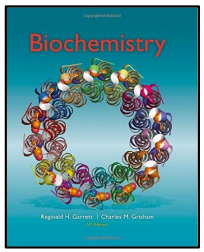

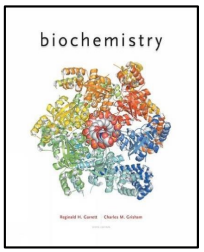

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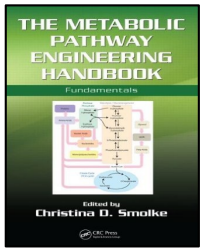

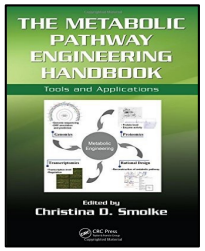

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