Primary Metabolism, Enzyme Thermodynamics and Plant Cell Wall Structure

Lecture 3
Biofuels and Bioproducts

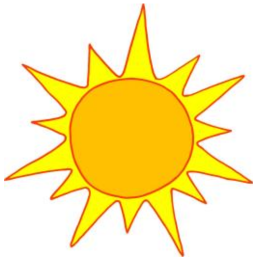
Bronx Community College - 2017
Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

Outline

- Thermodynamics/Kinetics, Redox/Enzyme Review
- Metabolic Pathways and their Drivers
- Cellular Respiration: Consumption of Sugars
- Photosynthesis: Production of Sugars
- Cell Wall Structure and Diversity
- Introduction to Biomass Conversion and 1st Generation Biofuels

Lecture notes created from Raven et al *Biology of Plants* 1999

Solar Energy



Solar Energy = 13 x 10^{23} calories (5.4 x 10^{24} J) per year

~ 20% absorbed by Earth's atmosphere

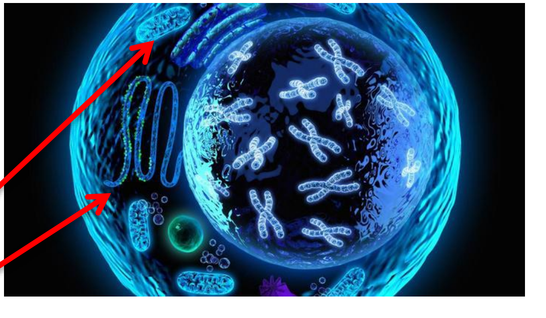
~ 30% reflected back into space as light

~ 50% is absorbed by Earth and converted to heat (evaporation, clouds, rain/snow, wind)

< 1% captured by living organisms and converted into all known biochemical energy!

1st Law of Thermodynamics

- *Energy cannot be created or destroyed, but it can change form. i.e. The total energy of a 'system' and its 'surroundings' must be the same before and after an energy exchange*
  - Chemical/Potential Energy (coal, gasoline)
  - Thermal Energy (heat, friction, loss)
  - Mechanical/Kinetic Energy (engine, boiler = work)
  - Light/Electricity
  - Nuclear Energy*



System (e.g. mitochondria)
Surroundings (e.g. cytoplasm)

2nd Law of Thermodynamics

- *In all energy conversions and exchanges, if no energy enters or exits the system, the potential energy of the final state will always be <u>less</u> than the potential energy of the initial state.*

  - Exergonic (spontaneous) reactions = Negative ΔH = energy is released from system to surroundings
  - All naturally occurring processes are exergonic

e.g. oxidation or "combustion" of glucose

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6\,H_2O + 673\text{ kcal}$$

ΔH = -673 kcal/mole of glucose

Entropic Contributions

- Entropy is the 'disorder' of a system
- Increase Entropy = Increase Free Energy (ΔG)

$$\Delta G = \Delta H - T\Delta S$$

$$C_6H_{12}O_6 \quad + \quad 6O_2 \longrightarrow 6CO_2 + 6\,H_2O$$

ΔG = -686 kcal/mole of glucose

The increased 'disorder' of converting 7 molecules (1 glucose + 6 O_2) to 12 molecules ($6CO_2$ + 6 H_2O) lends 13 kcal/mol to the overall Free Energy of the reaction

Common 'Redox' Processes in Plant Biology

- Reduction = gain of electrons (gain of H atom e-s)
- Oxidation = loss of electrons (loss of H atom e-s)

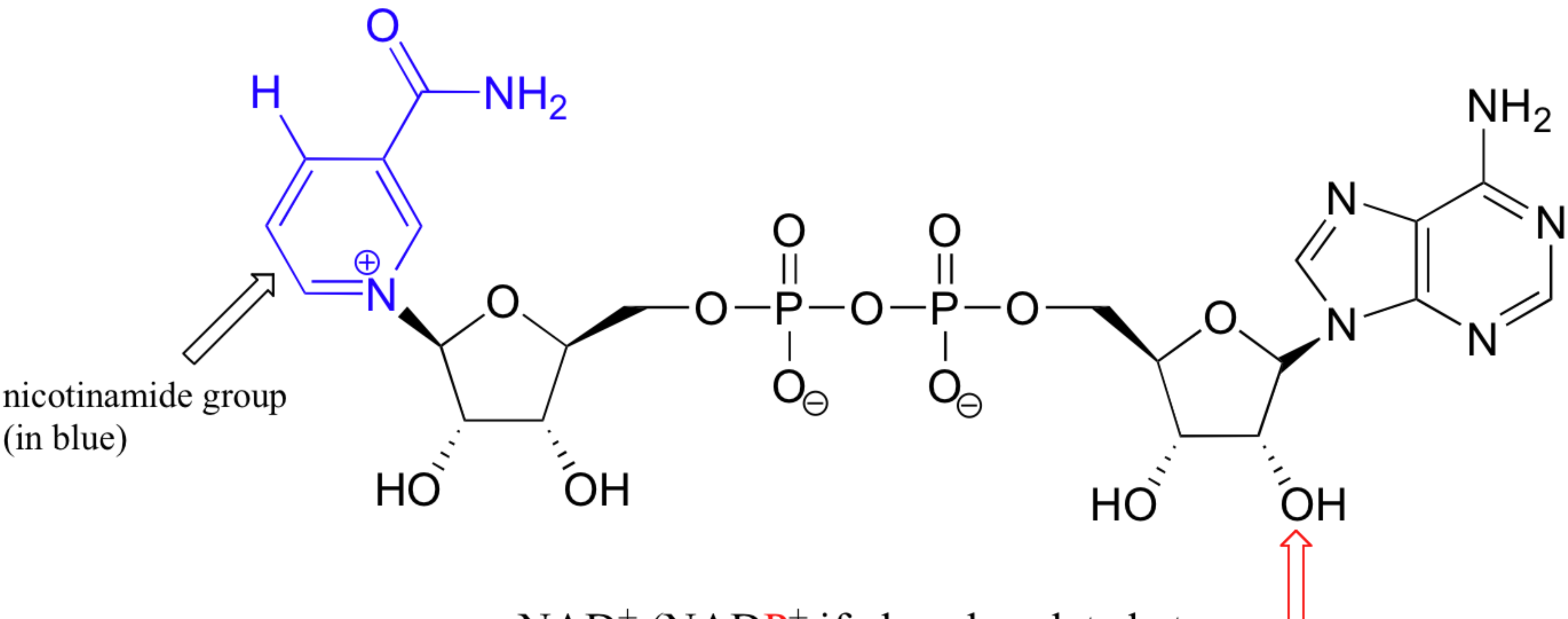
$$6CO_2 + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

Photosynthetic reduction = H is gained by CO_2

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

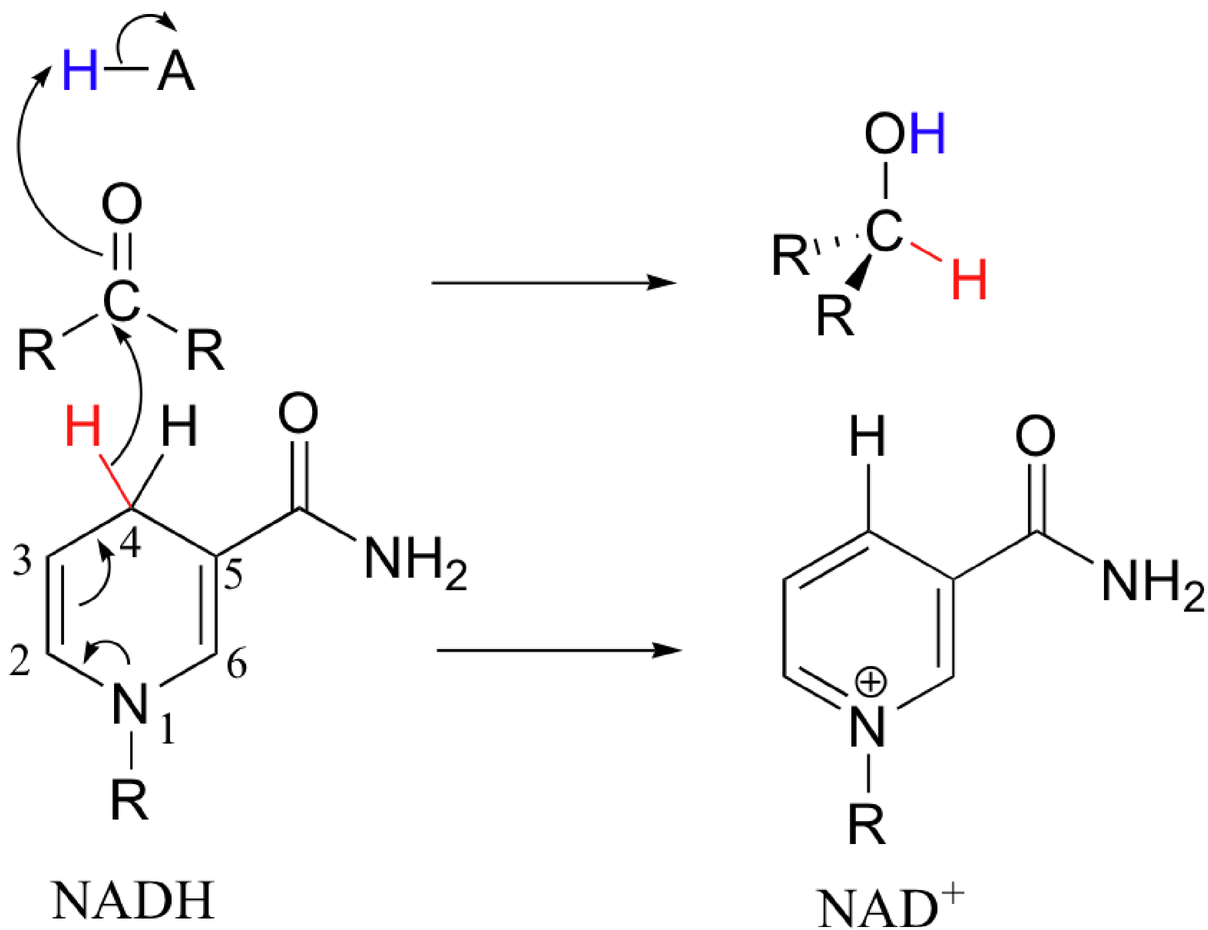
Glucose oxidation = H is lost from glucose

The Structure of NAD⁺
Can you draw NADH and NADPH?



NAD⁺ (NADP⁺ if phosphorylated at arrow position)

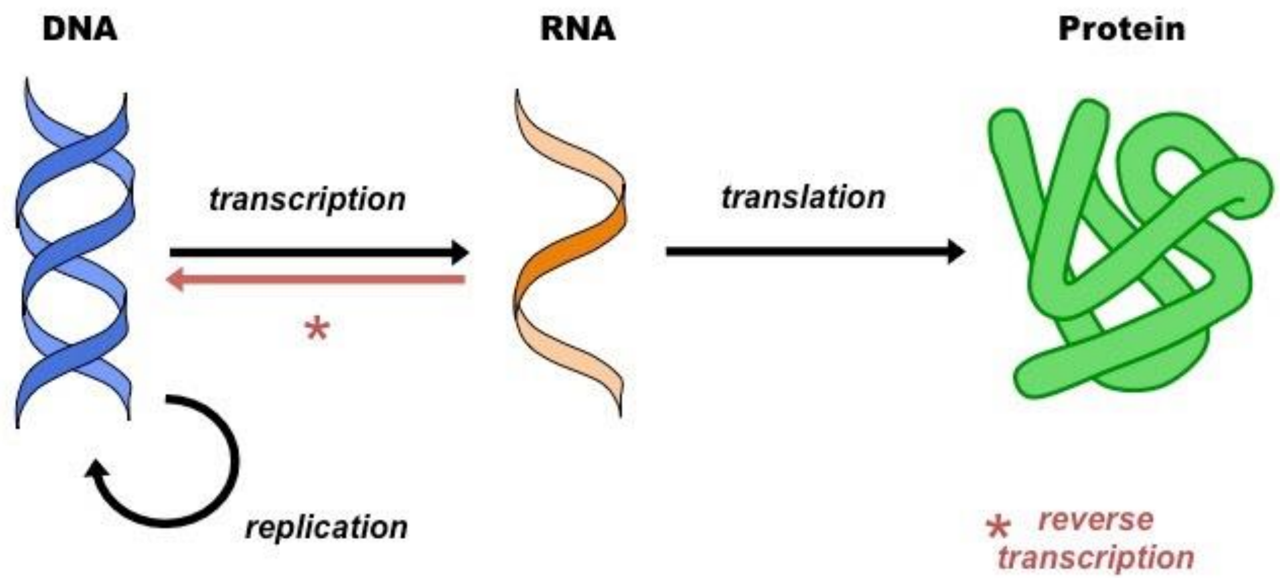
Ketone Reduction with NADH



Kinetics of Bioprocesses are Enzyme Dependent

- Activation Energy ($\Delta G^{\ddagger}$)= Determines a reaction's rate (i.e. kinetics)
- Enzymes are catalysts and lower $\Delta G^{\ddagger}$
- Approximately 80,000 known enzymes that catalyze approximately 5000 different reactions
- Most enzymes are proteins, some are RNA
- Enzyme MW range = 10,000 – 2,000,000
- Enzymes rely on co-factors (metals) and co-enzymes (small organic molecules, e.g. NADPH) to perform reactions

Central Dogma of Biology: Gene Expression



- A segment of DNA (gene) is ***transcribed*** by RNA polymerase
- Product is an antiparallel RNA strand (e.g. mRNA, tRNA, rRNA) called a *primary transcript*
- In eukaryotic cells additional processing occurs (polyadenylation, capping, splicing) to stabilize the RNA
- RNA often exits nucleus into the cytoplasm
- ***Translation*** occurs on ribosomes in the cytoplasm
- Ribosomes pair mRNA *codons* with tDNA *anti-codons* to form polypeptide chains
- Polypeptides fold and undergo other post-translational modifications (e.g. methylation, phosphorylation) to form proteins/enzymes

How Do Enzymes Work? The Lock and Key Model

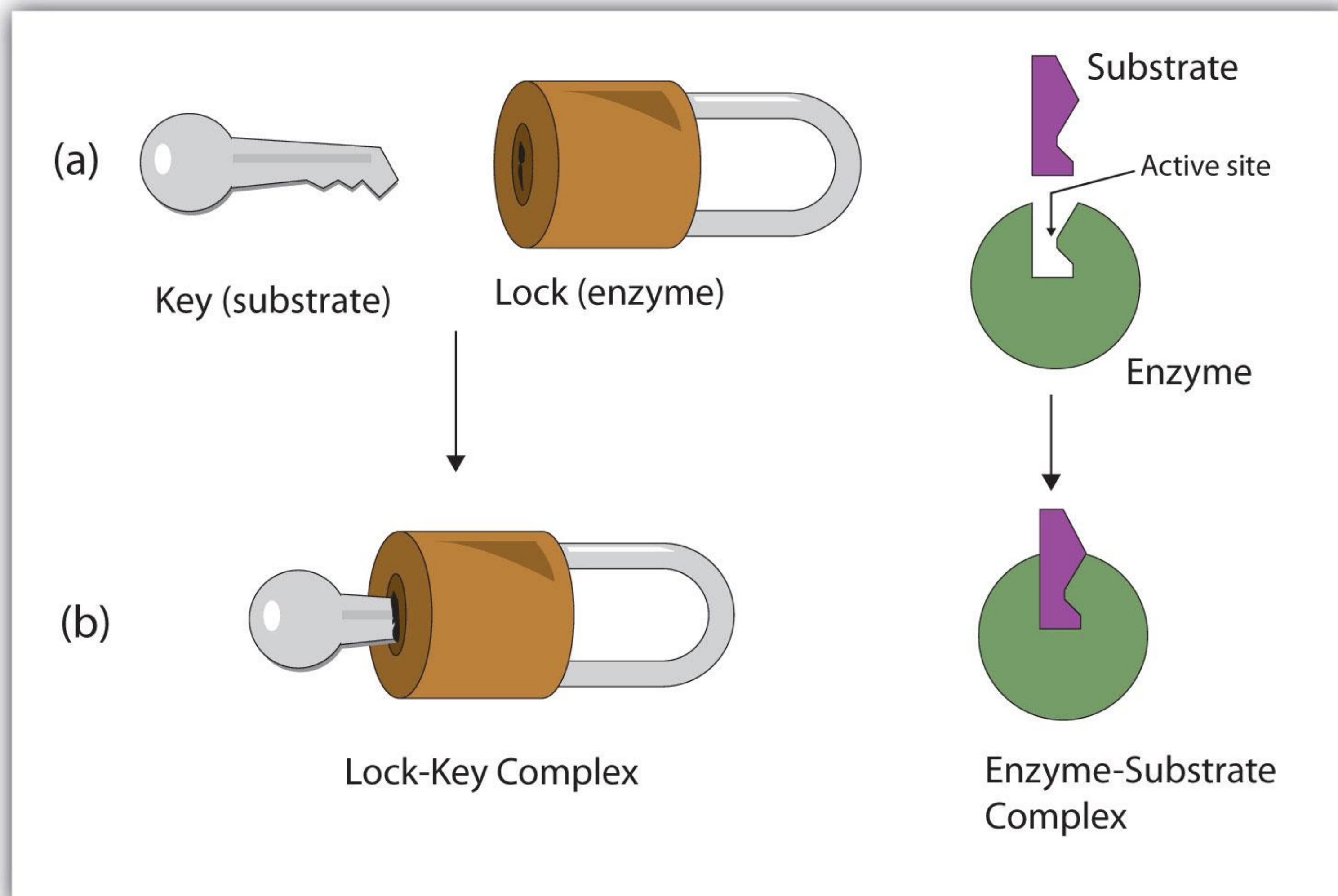
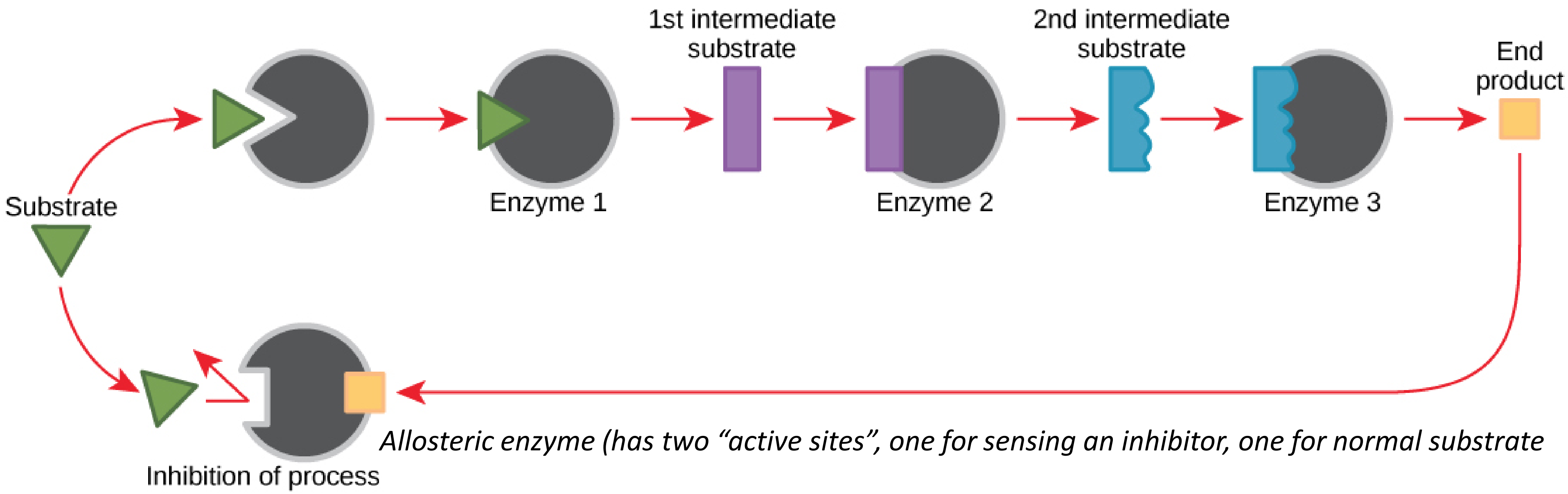


Image: Ball, et al. *Introduction to Chemistry, General, Organic and Biological*

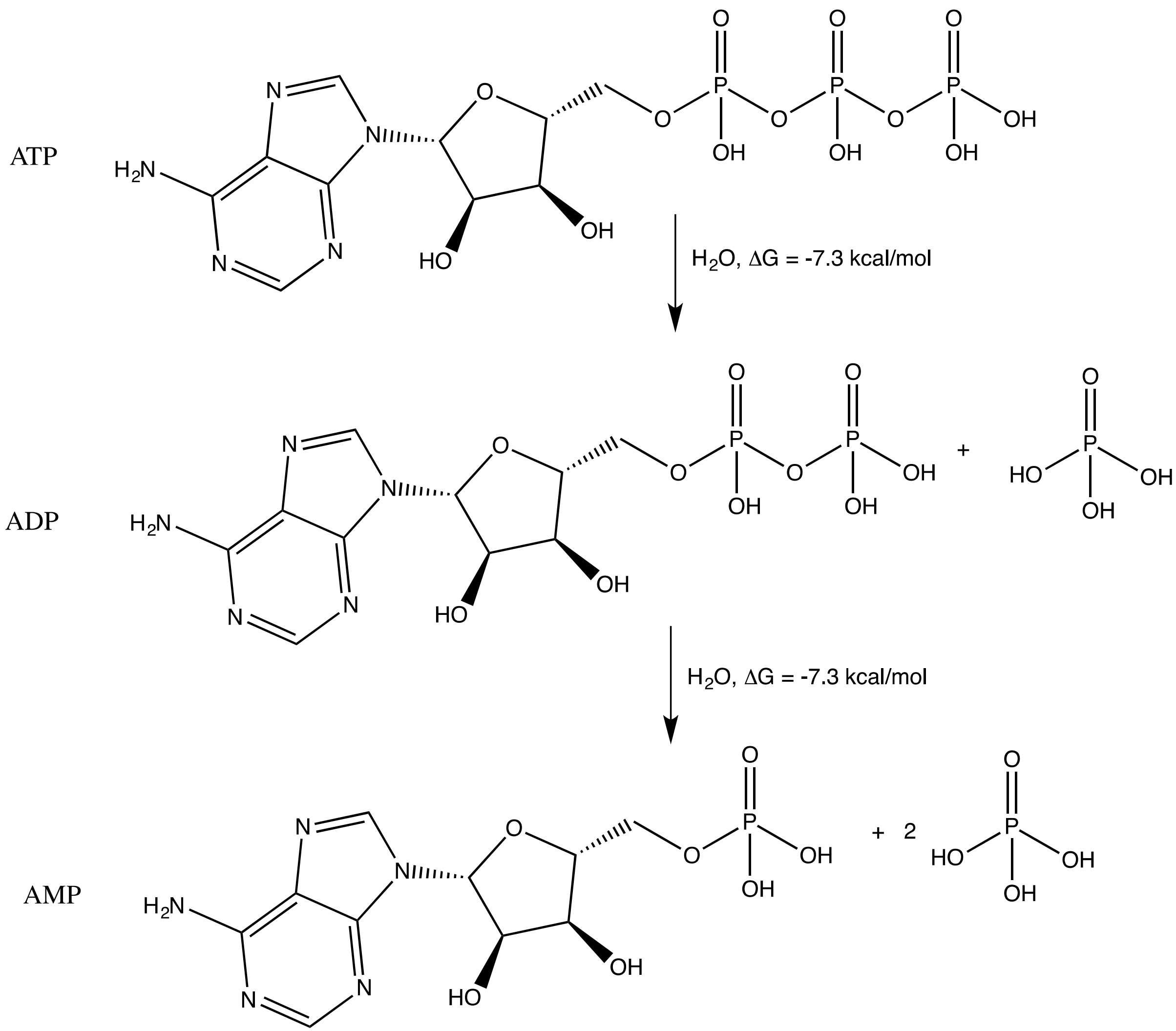
Metabolic Pathways: Enzyme Assembly Lines



Allosteric enzyme (has two "active sites", one for sensing an inhibitor, one for normal substrate

- Enzymes within a pathway are often *segregated* with a part of the cell (e.g. cell membrane, vacuoles, mitochondria, chloroplasts)
- 'Assembly line' reaction format takes advantage of reactions' thermodynamics and equilibria
- Reaction rate increases with *temperature* up to 40^{o}C (temps > 40^{o}C = enzyme denaturing)
- Reactions are often *pH dependent*
- Regulatory (e.g. *allosteric* enzymes) allow ***feedback inhibitions*** to operate within a pathway

Metabolic Pathways are Driven by Phosphorylation/Dephosphorylation Reactions



ATPase enzymes catalyze ATP hydrolysis reactions
Kinase enzymes transfer phosphate groups between proteins and small molecules

P-OH bond is a more stable bond than P-O-P (phosphoanhydride)

Negatively charged phosphate groups repel each other

Hydrolysis is exergonic by -7.3 kcal/mol

Reverse reaction (phosphoanhyride formation) is endergonic by +7.3 kcal/mol

Thermodynamics (Hess's Law) Applied to Glucose Phosphorylation

- The synthesis of glucose-phosphate is *endergonic*

Glucose + phosphate $\longrightarrow$ glucose-phosphate + H_2O

$\Delta G^o = +3.3$ kcal/mol

- But the hydrolysis of ATP hydrolysis the is *exergonic*

ATP + H_2O $\longrightarrow$ ADP + phosphate

$\Delta G^o = -7.3$ kcal/mol

- Therefore the overall phosphorylation of glucose is *exergonic*

Glucose + ATP $\longrightarrow$ glucose-phosphate + ADP

$\Delta G^o = -4.0$ kcal/mol

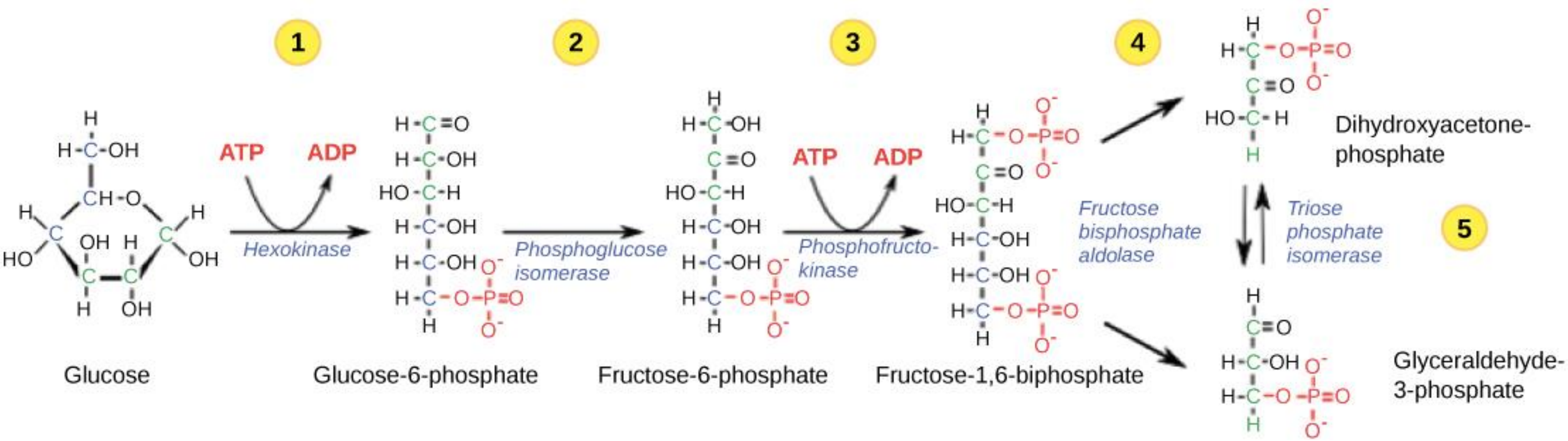
Cellular Respiration

- The breakdown of sugars and other organic molecules into CO_2 and water and can occur:
  - With oxygen present = aerobic respiration
  - Without oxygen present = anaerobic fermentation
- Divided into Four Distinct Stages
  1: Glycolysis
  2: Krebs Cycle
  3: Electron Transport Chain
  4: Oxidative Phosphorylation
- At each stage, energy is stored as ATP or lost as heat

Glycolysis

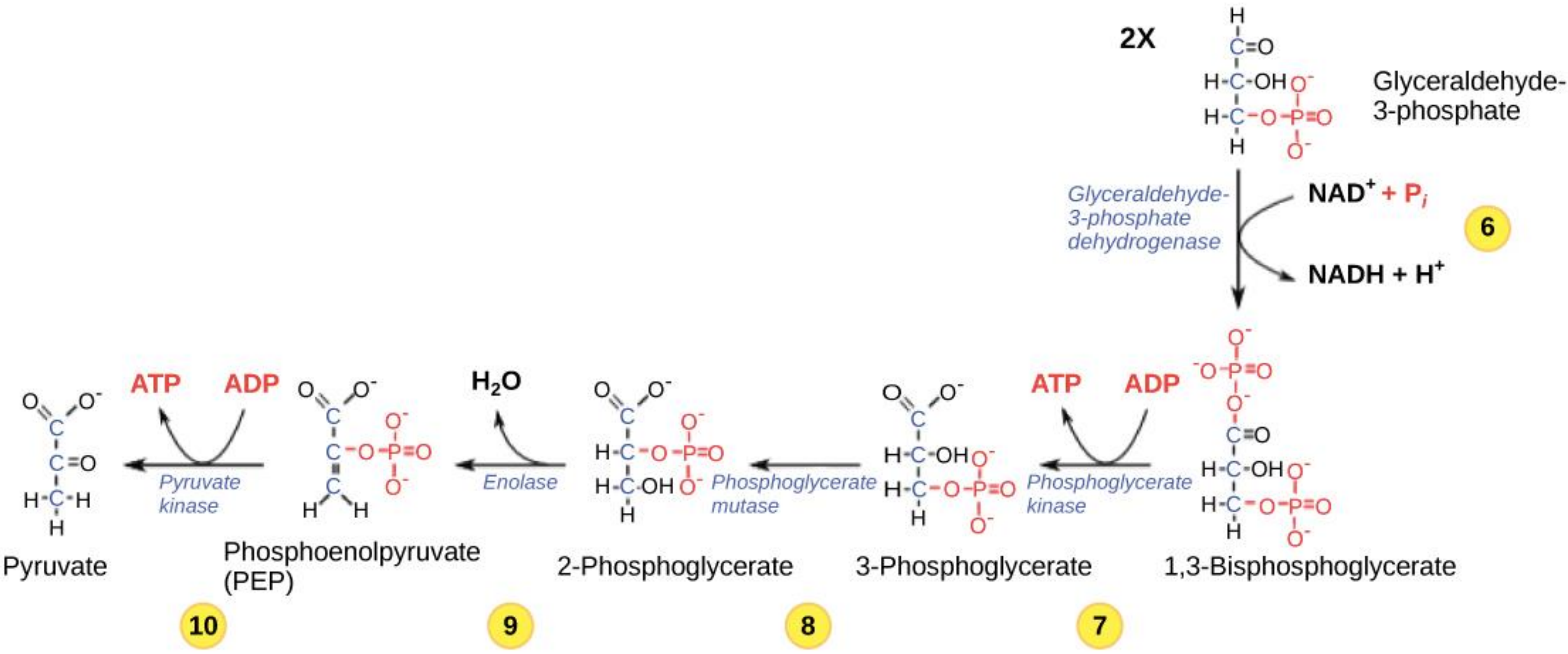
- An anaerobic process occurring in the cytosol
- 10 step reaction with each step catalyzed by separate enzymes
- Glucose (686 kcal/mol) is split into 2 pyruvate (273 kcal/mol each)
- Excess energy used to make 2 ATP from ADP and phosphate
- 4 electrons and their accompanying protons (e.g. 4 H atoms) are transferred to co-enzymes that function as electron carriers (2 NADH)

Glycolysis: Investment Phase



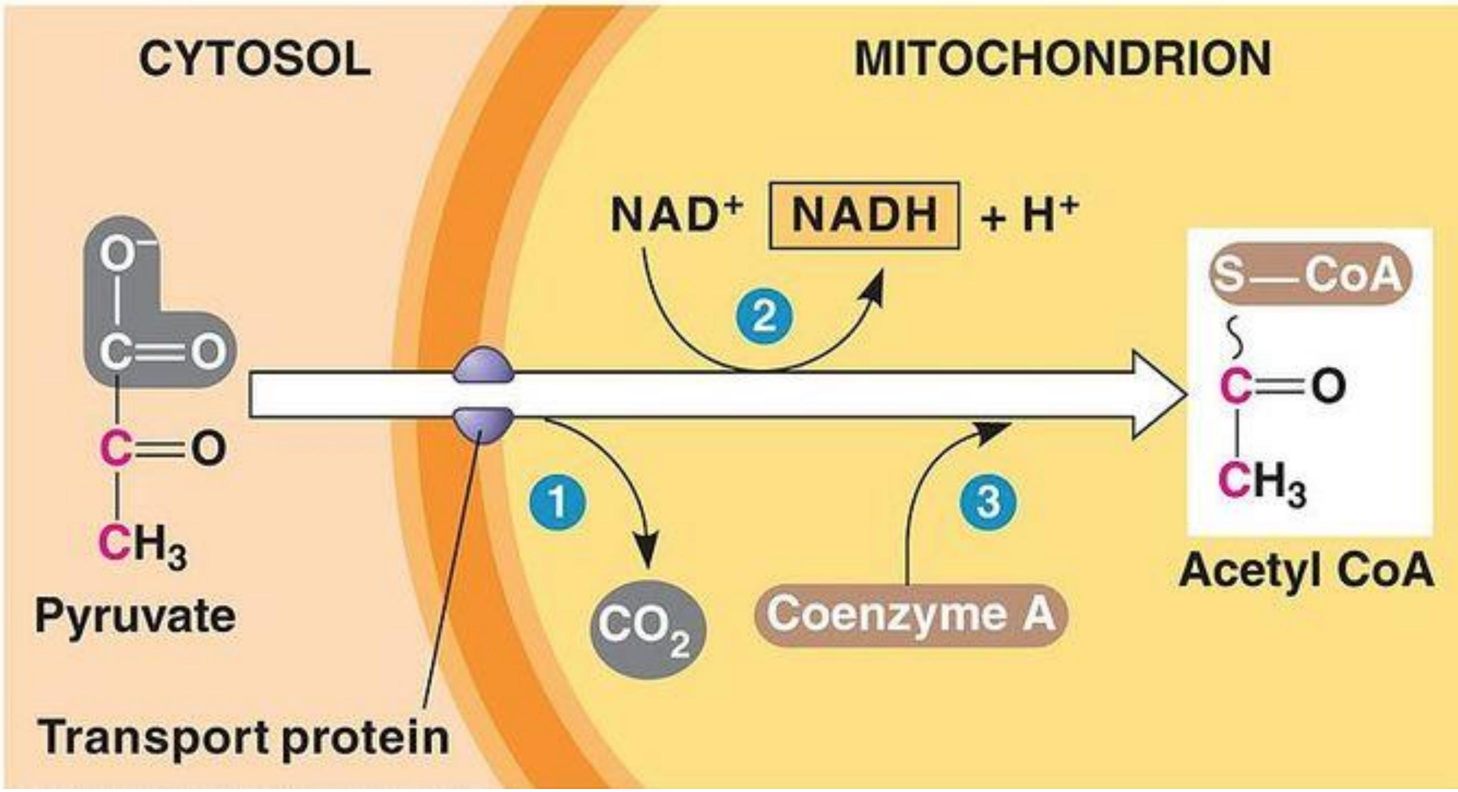
- 2 molecules of ATP consumed
- Glucose is effectually "split" into two new 3-carbon structures (both are phosphorylated)

Glycolysis: Payoff Phase

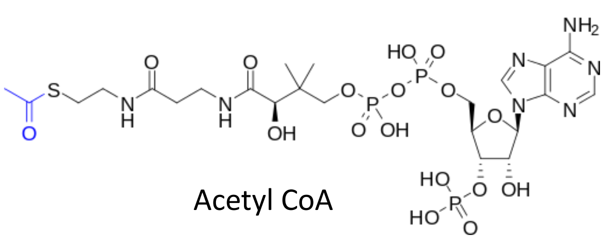


- Glyceraldehyde-3-phosphate is converted to pyruvate
- Pyruvate is a key intermediate in cellular energy metabolism
- Pyruvate can be utilized in several pathways (dependent on organism/cell type and O_2 availability)
- Overall, 2 molecules of pyruvate, 4 molecules of ATP and 2 molecules of NADH produced from 1 molecule of glucose

Pyruvate Reactions within the Mitochondrion

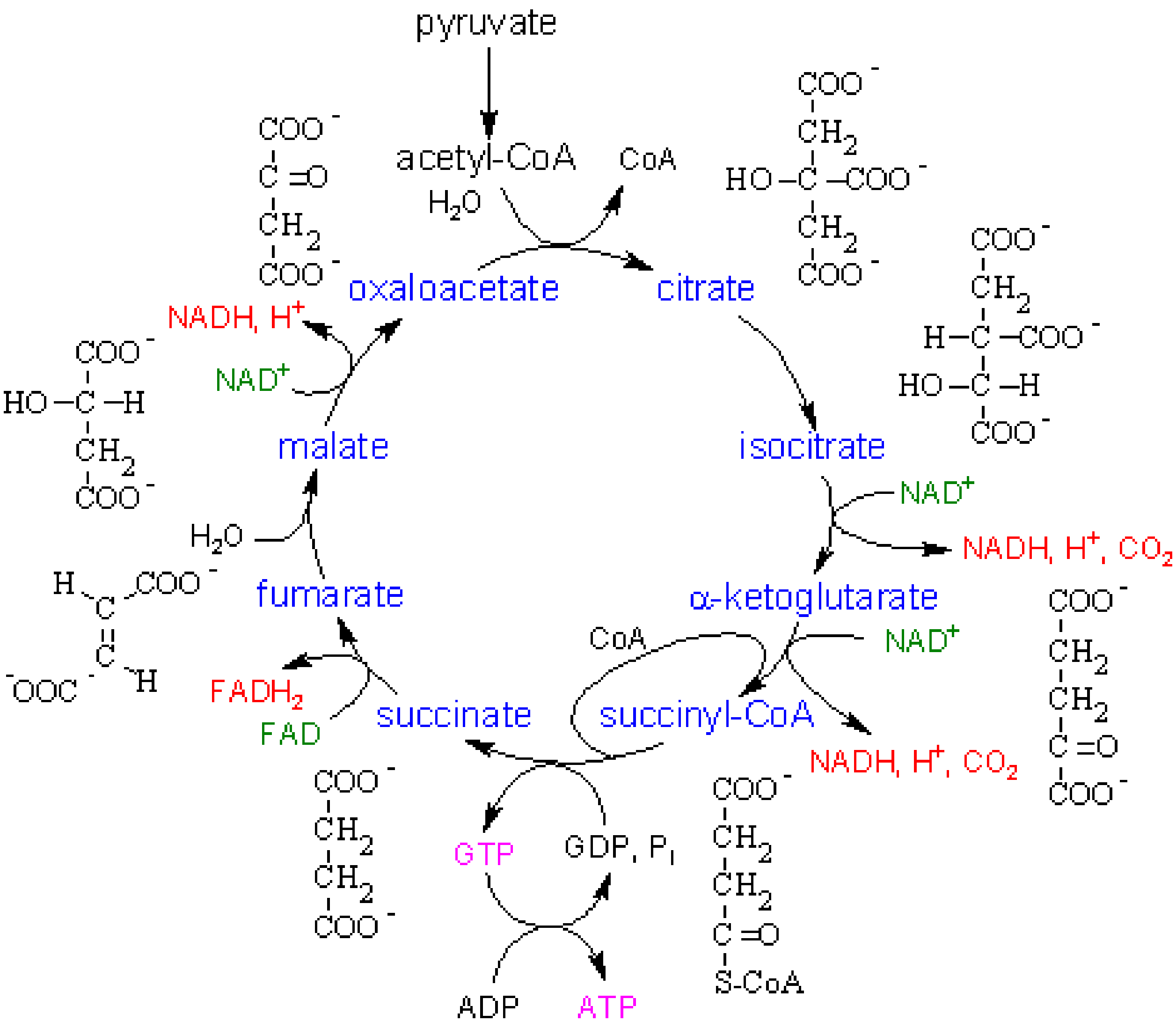


- C=O forms thioester bond to more electronegative S atom (oxidation)
- CO_2 is lost (decarboxylation)
- Gain in Free Energy drives reduction of NAD+ to NADH
- CoEnzyme A (CoA) is a nucleotide linked to pantothenic acid



Acetyl CoA

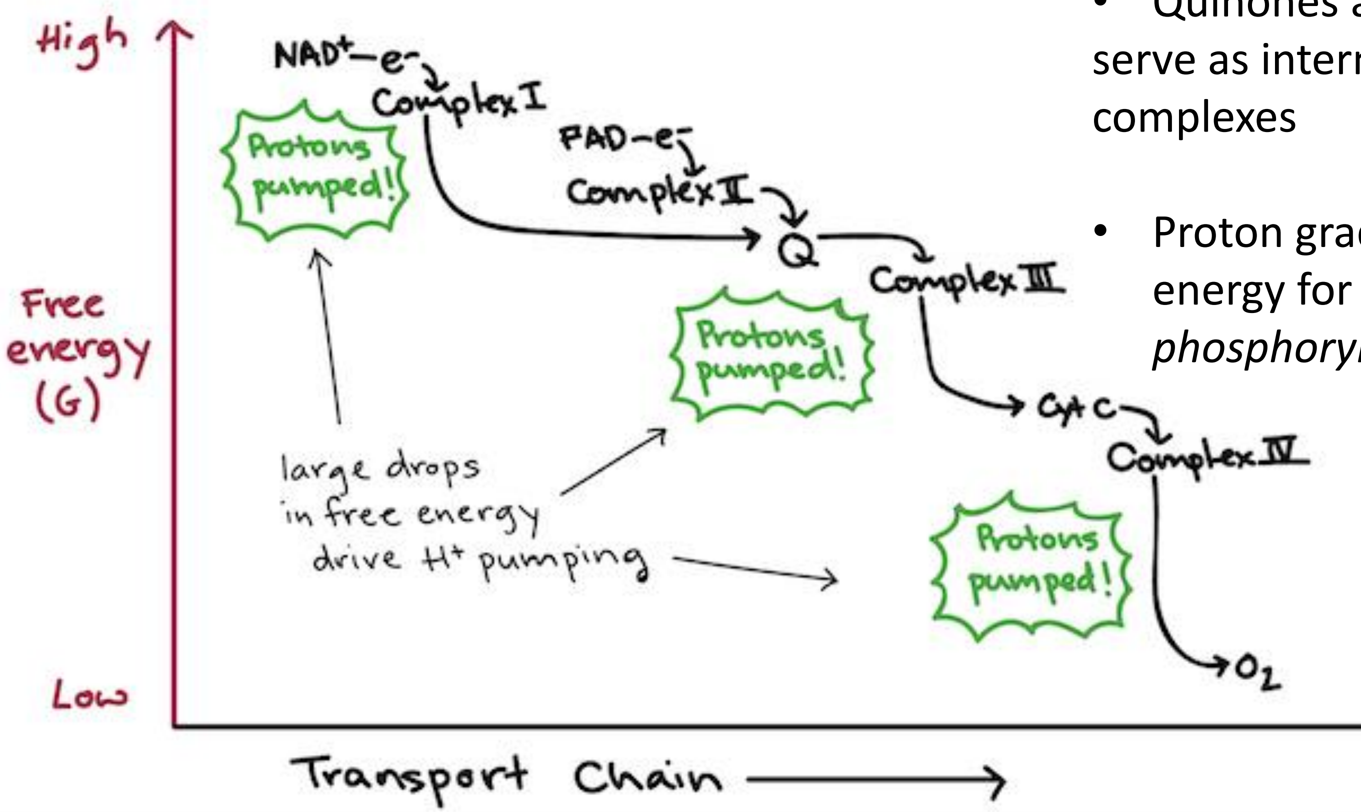
TCA Cycle (aka Krebs Cycle)



- In addition to NAD/NADH reductions, FAD/$FADH_2$ reductions also occur
- These high energy electrons then pass to inner mitochondial membrane for transport
- Intermediate molecules provide valuable starting materials for other biosynthetic pathways

Electron Transport Chains

- High energy electrons (NADH, FADH) are "stepped-down" to lower energy levels (O_2) while energy is harvested at each step
- Diverse cytochromes and iron-sulfur protein complexes can carry single electrons from H atom and reject the proton
- Quinones accept H atoms and thus serve as intermediates between complexes
- Proton gradient provides potential energy for ATP synthesis (*oxidative phosphorylation*)

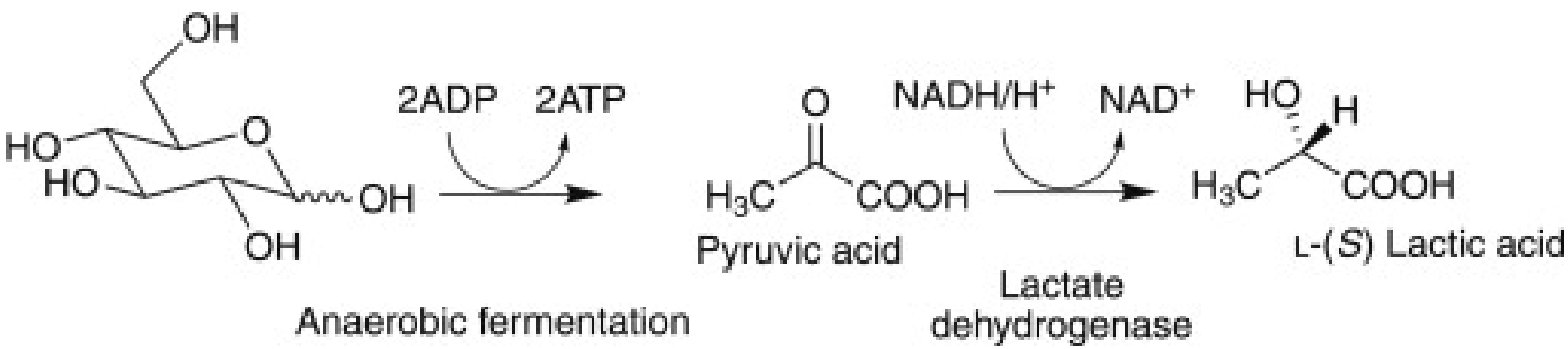


Other substrates for respiration

- In addition to polysaccharides, fats and proteins can also provide inputs for glycolysis
  - Triglycerides undergo β-oxidation of their fatty acids after glycerol is removed
  - Amino acids are deaminated and converted into Krebs cycle intermediates (α-ketoglutarate, oxaloacetate, fumarate) and then enter cycle

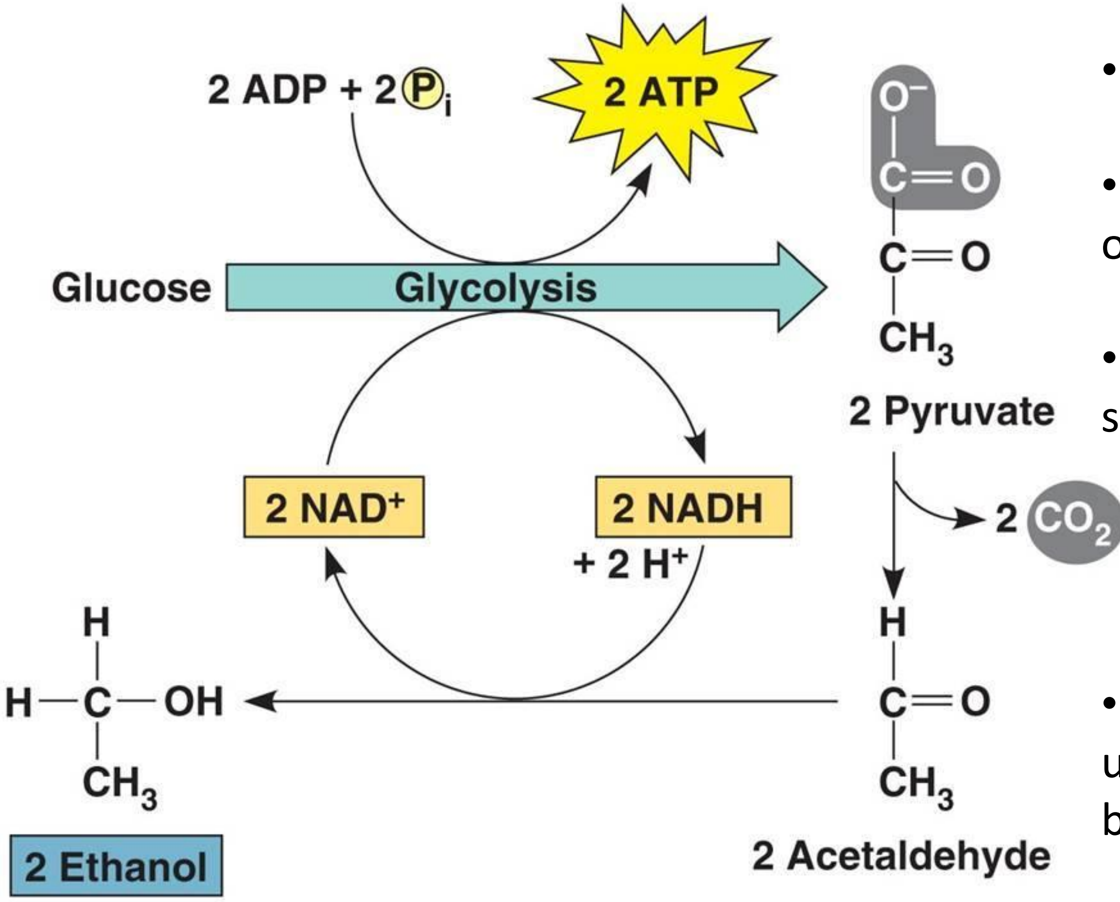
Lactate: Anaerobic Metabolism of Pyruvate

- In bacteria, fungi, protists and animal cells, lactic acid is produced from pyruvate in the absence of oxygen



- The most commercially important genus of lactic acid fermenting bacteria is *Lactobacillus*
- This organism and others can be used to add a sour flavor to beer (e.g. lambic), make kimchi, sauerkraut, yogurt)

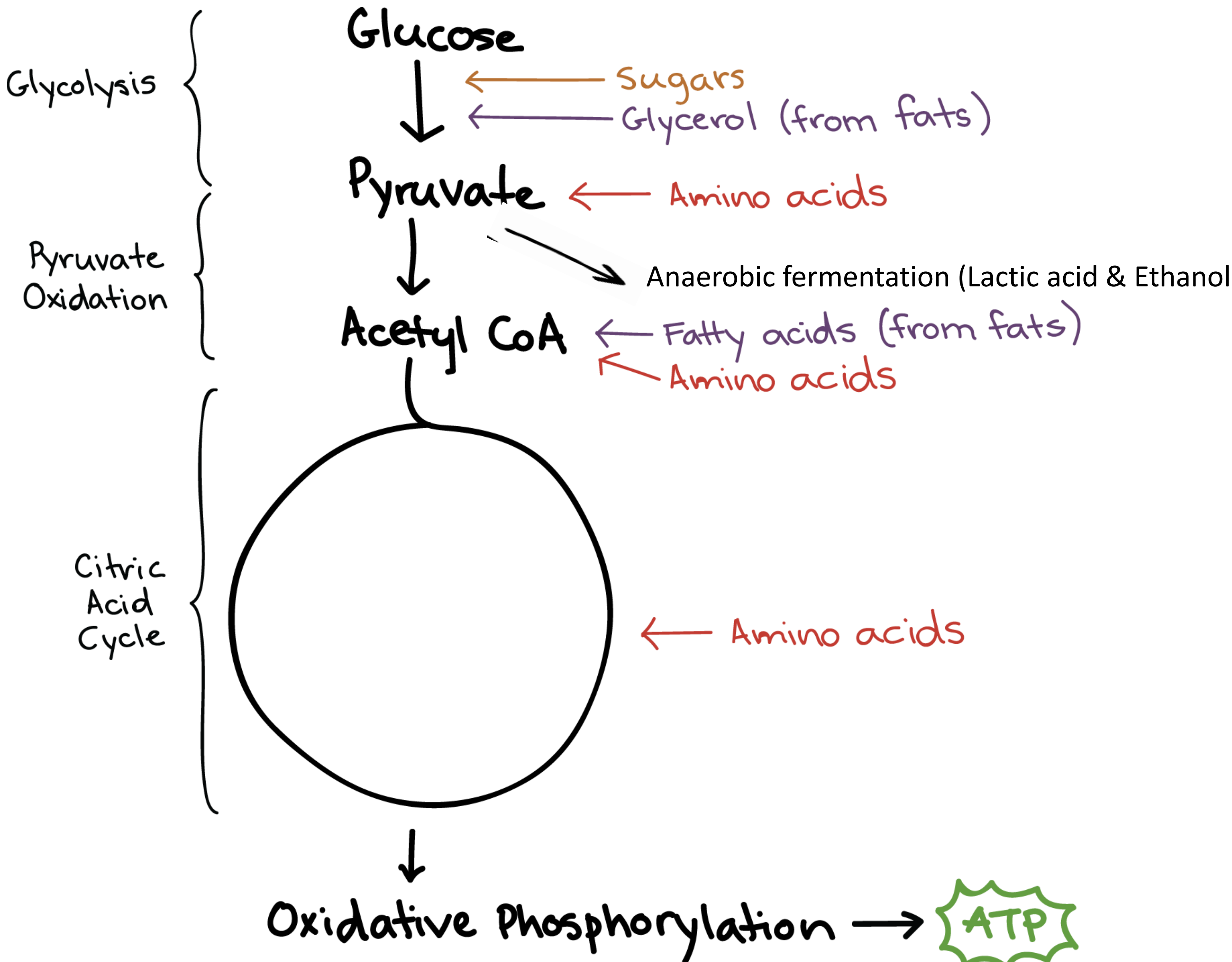
Ethanol: Anaerobic Metabolism of Pyruvate



(a) Alcohol fermentation

- Yeast convert pyruvate to ethanol
- This can be performed in anaerobic or aerobic conditions
- First-Generation BioEthanol utilizes sucrose from corn or sugar cane
- Second-Generation BioEthanol utilizes "cellulosic" or "lignocellulosic" biomass

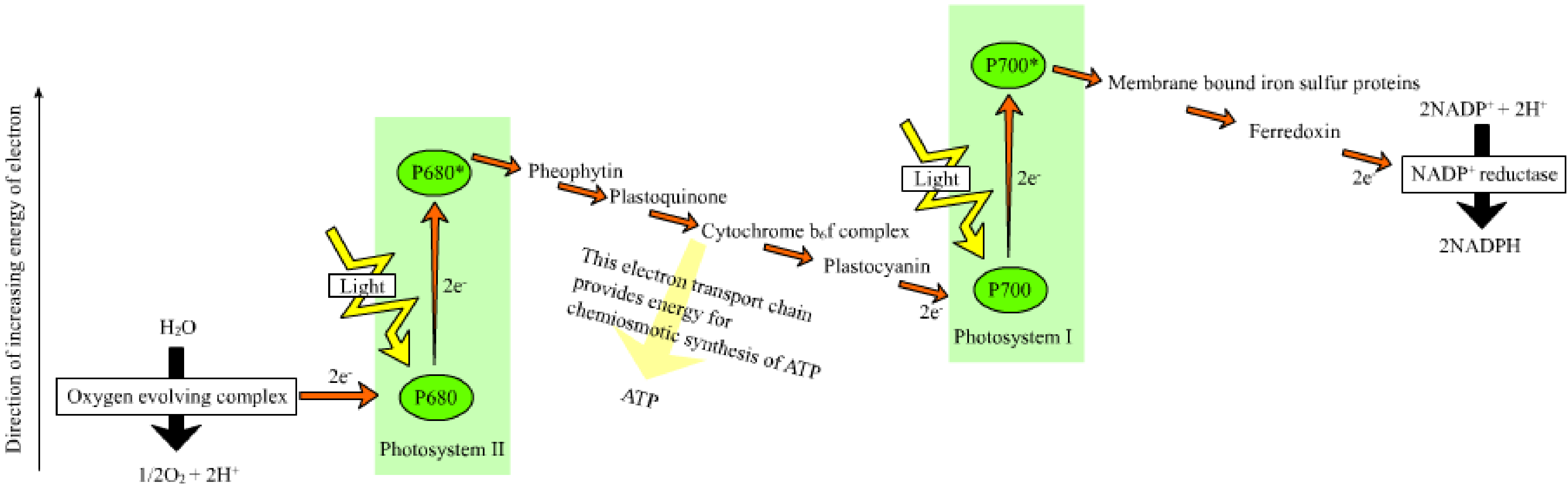
All Together Now



Photosynthesis Part 1

Light-dependent Reactions:

2 H_2O + 2 $NADP^+$ + 3 ADP + 3 P_i + light ⟶ 2 NADPH + 2 H^+ + 3 ATP + O_2



- Pigment molecules (chlorophylls a-d, carotenoids, phycobiliproteins): absorb photon, lose e-
- Electron transport causes reduction of NADP to NADPH and a H^+ gradient
- H^+ gradient is used to convert ADP and inorganic phosphate (P_i) to ATP

Image: Wikipedia

Photosynthesis Part 2

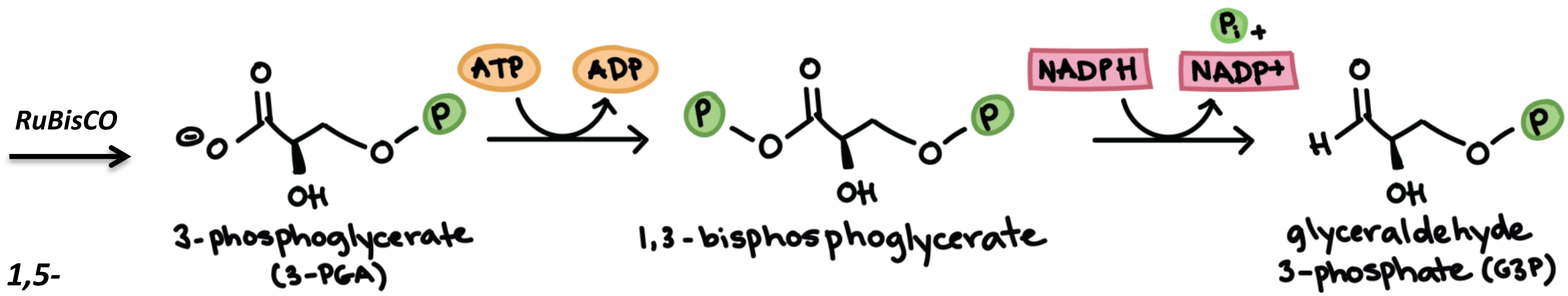
<u>Light-independent Reactions</u>: Carbon Fixation = The Calvin Cycle

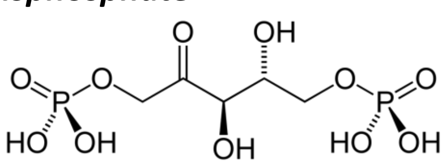
3 CO_2 + 9 ATP + 6 NADPH + 6 H^+ ⟶ $C_3H_6O_3$-phosphate + 9 ADP + 8 P_i + 6 $NADP^+$ + 3 H_2O

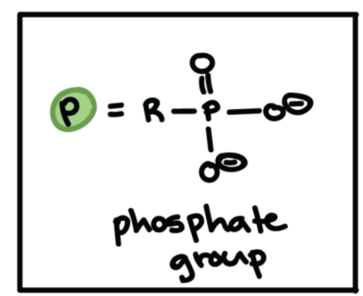
CO_2

+

ribulose 1,5-bisphosphate







- CO_2 is combined with ribulose 1,5-bisphosphate by the enzyme RuBisCO
- Two molecules of 3-PGA are formed
- ATP and NADPH are used to form another 3-carbon (triose) phosphate called G3P
- Approx. 80% of G3P is recycled to make more ribulose 1,5-bisphosphate
- ***The other 20% is used to make cellulose and starches***

Image: Khan Academy