BI/CH 422/622

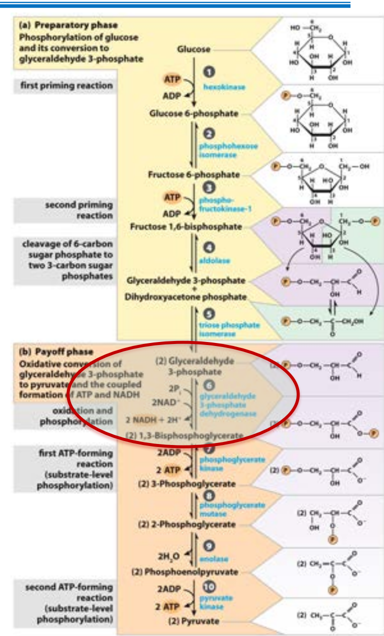
OUTLINE:
Review
Bioenergetics
Membrane Transport
Catabolism of Glucose
Glycogenolysis
- phosphorylase
- debranching enzyme
- phospho-gluco-mutase (PGM)

Glycolysis
- Introduction & overview; 2 phases
- Phase I
  - hexokinase
  - phospho-gluco-isomerase (PGI)
  - phospho-fructo-kinase (PFK-1)
  - Aldolase
  - triose-phosphate isomerase (TPI)
- Phase II
  - glyceraldehyde-3-phosphate dehydrogenase
  - PG kinase
  - PG mutase
  - Enolase
  - Pyruvate Kinase

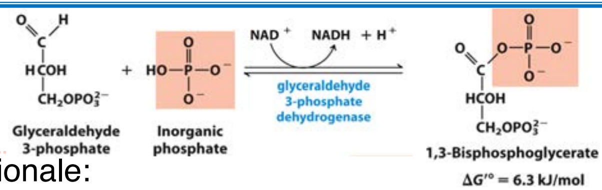
Fermentation

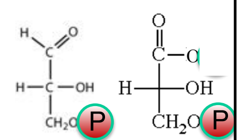
Glycolysis: Overview

- Two Phases/Four concepts
  - Preparatory phase
    - Phosphorylation by ATP
    - Cleavage
  - Payoff
    - Oxidation
    - Phosphorylation of ADP



Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



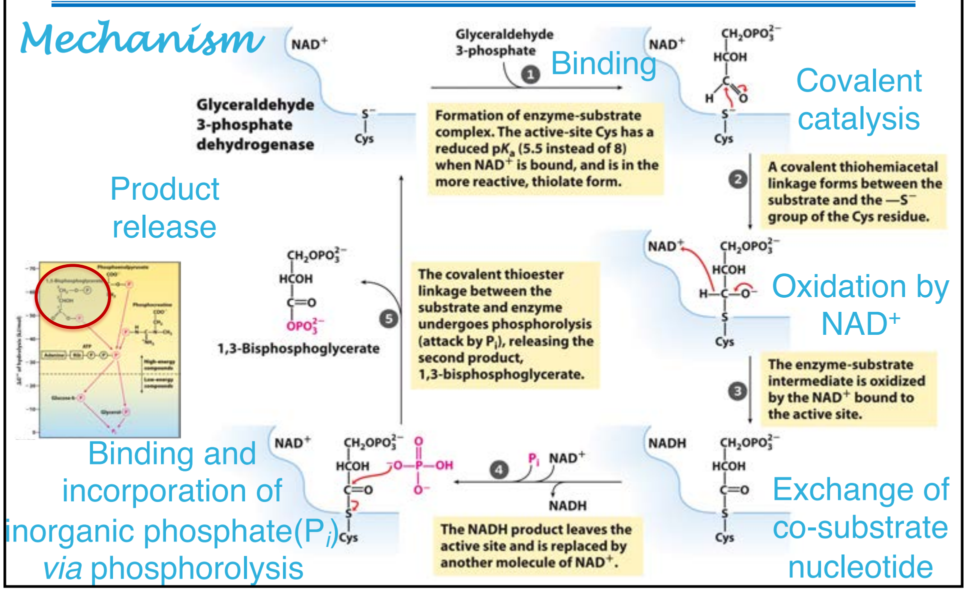


- Rationale:
  - Recall Pyruvate is an acid; need to oxidize aldehyde
  - incorporates inorganic phosphate
  - generation of a high-energy phosphate compound
  - **which allows for net production of ATP via glycolysis!**
- First energy-yielding step in glycolysis
- First oxidation: aldehyde to carboxylate (ox)/NAD^+ to NADH (red).
- Uses active-site cysteine for covalent catalysis
  - forms high-energy thioester intermediate
  - subject to inactivation by oxidative stress

The oxidation of glyceraldehyde is only slightly unfavorable, even coupled to NAD+ reduction, but the hydrolysis of bis-phosphate is even more favorable

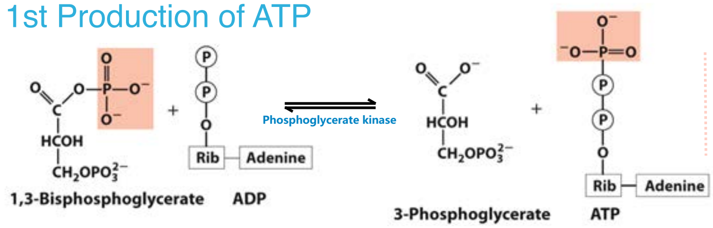
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - coupled to next reaction to pull forward

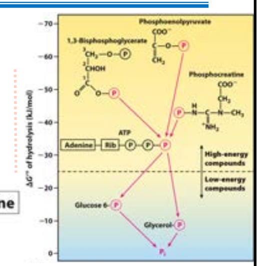
Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



Glycolysis: Phosphoglycerate Kinase (PGK)

1st Production of ATP

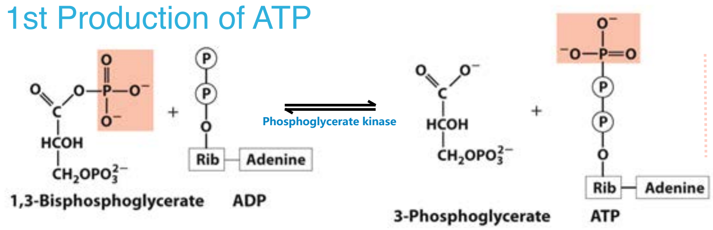


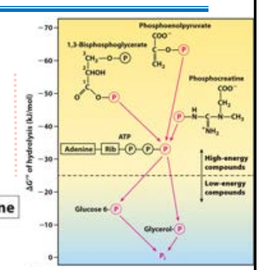


- Rationale:
  - substrate-level phosphorylation to make ATP
  - first of two “payoff” steps
- 1,3-bisphosphoglycerate is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Named for the reverse reaction; recall Kinases are enzymes that transfer phosphate groups between ATP and various substrates.
- Highly thermodynamically favorable/reversible ($\Delta G^{o\prime}$ = –5.5 kcal/mol)
  - This reaction can pull the entire pathway to this point; but only modestly favorable (–1.9 kcal/mol)

Glycolysis: Phosphoglycerate Kinase (PGK)

1st Production of ATP

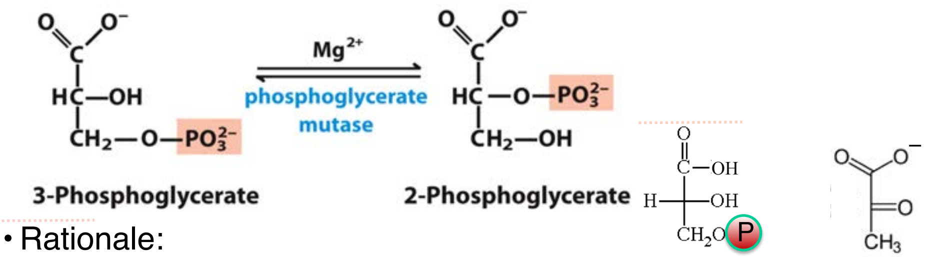




- Rationale:
  - substrate-level phosphorylation to make ATP
  - first of two “payoff” steps
- 1,3-bisphosphoglycerate is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Named for the reverse reaction; recall Kinases are enzymes that transfer phosphate groups between ATP and various substrates.
- Highly thermodynamically favorable/reversible ($\Delta G^{o\prime}$ = –5.5 kcal/mol)
  - This reaction can pull the entire pathway to this point; but only modestly favorable (–1.9 kcal/mol)

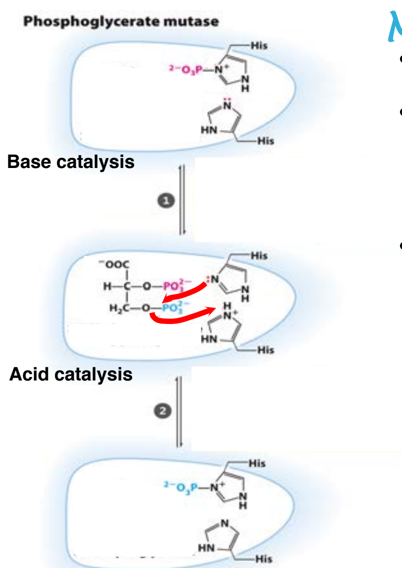
Glycolysis: Phosphoglycerate Mutase (PGM)

Migration of the Phosphate



- Rationale:
  - Notice that reduction of C3 and oxidation of C2 means no net redox.
  - Need to get C3 dehydrated, so need to move phosphoryl group
  - Need to form high-energy phosphate compound to make glycolysis a net ATP producer.
- Mutases catalyze the (apparent) migration of functional groups.
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.1 kcal/mol)
  - reactant concentration kept high by favorability through PGK reaction.

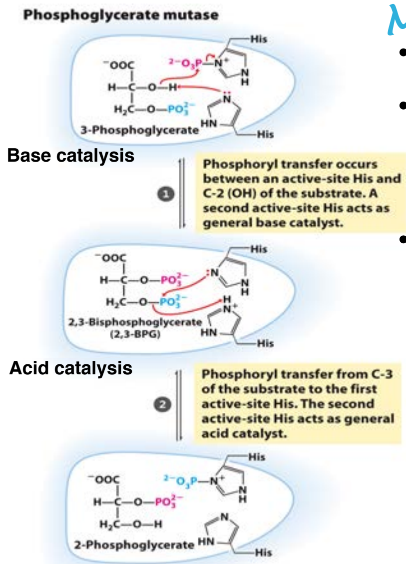
Glycolysis: Phosphoglycerate Mutase (PGM)



Mechanism Acid/base Catalysis

- Similar to other mutases
- One of the active-site histidines is post-translationally modified to phospho-histidine.
- Phospho-histidine donates its phosphate to 3-phosphoglycerate at the C2-oxygen before retrieving the phosphate from the 3-carbon oxygen.
  - Note that the phosphate from the substrate ends up bound to the same His at the end of the reaction.
  - Note that the other His acts as an acid/base catalyst

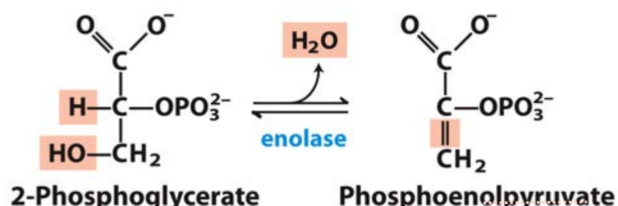
Glycolysis: Phosphoglycerate Mutase (PGM)

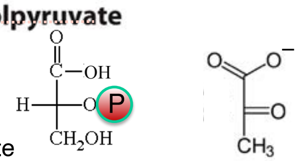


Mechanism Acid/base Catalysis

- Similar to other mutases
- One of the active-site histidines is post-translationally modified to phospho-histidine.
- Phospho-histidine donates its phosphate to 3-phosphoglycerate at the C2-oxygen before retrieving the phosphate from the 3-carbon oxygen.
  - Note that the phosphate from the substrate ends up bound to the same His at the end of the reaction.
  - Note that the other His acts as an acid/base catalyst

Glycolysis: Enolase

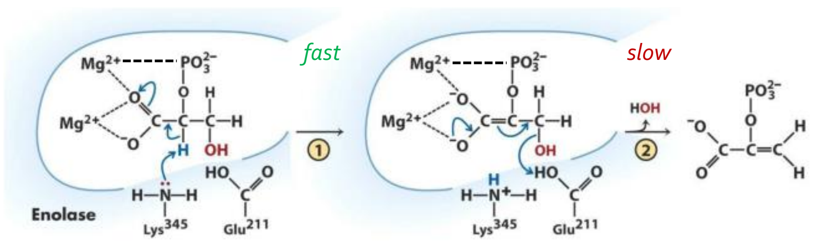




- Rationale:
  - Dehydrates C3 to remove alcohol; more like pyruvate
  - Double-bonded C2-C3 is part of an en-ol except that the C2-alcohol is in ester linkage with a phosphate; without the alcohol hydrogen, it cannot tautomerize
- 2-Phosphoglycerate is not a good enough phosphate donor to generate ATP.
  - loss of phosphate from 2-PG would give a secondary alcohol, which is completely stable
- Slightly thermodynamically unfavorable/reversible ($\Delta G^{o\prime} = +1.8$ kcal/mol)
  - product concentration kept low to pull forward

Glycolysis: Enolase

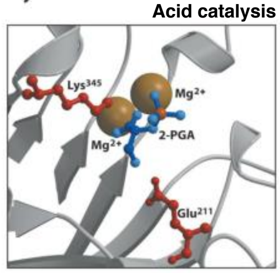
Mechanism Dehydration

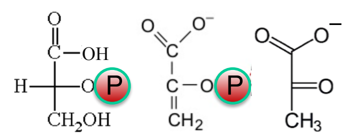


2-Phosphoglycerate bound to enzyme
Base catalysis

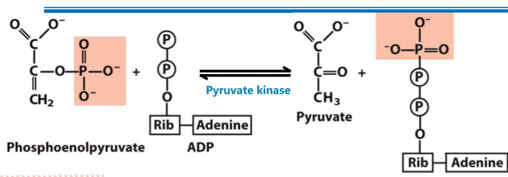
Enolic intermediate
Acid catalysis

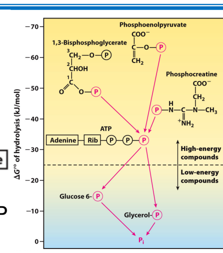
Phosphoenolpyruvate

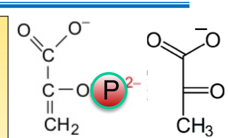


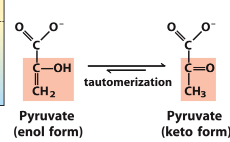


Glycolysis: Pyruvate Kinase (PK)







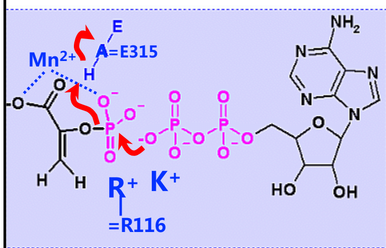


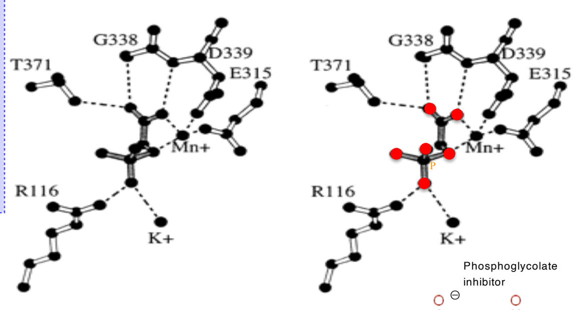
- Rationale:
  - substrate-level phosphorylation to make ATP
  - second of two "payoff" steps
  - net production of 2 ATP/glucose
- Phosphoenolpyruvate (PEP) is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Loss of phosphate from PEP yields an enol that tautomerizes into ketone.
- Tautomerization
  - effectively lowers the concentration of the reaction product
  - drives the reaction toward ATP formation
- Named for the reverse reaction; recall Kinases are enzymes that transfer phosphate groups between ATP and various substrates.
- Pyruvate kinase requires divalent metals (Mg^{++} or Mn^{++}) and K^+ for activity.
- Highly thermodynamically favorable ($\Delta G^{\circ\prime}$ = –8.2 kcal/mol)
  - This reaction pulls the entire glycolytic pathway.
  - regulated by ATP, divalent metals, and other metabolites

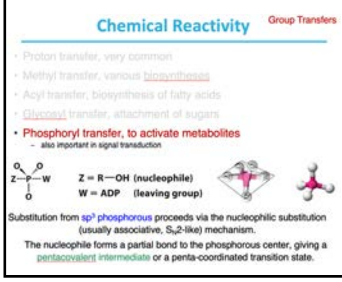
Glycolysis: Pyruvate Kinase (PK)

Mechanism

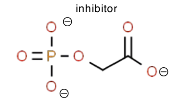
Recall Phosphoryl transfer







Phosphoglycolate inhibitor



Stereo views

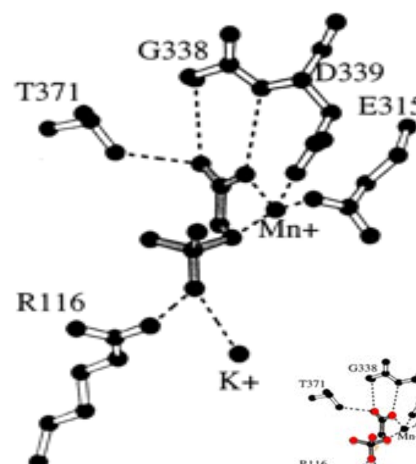
- Glu (E315) acts as an acid to protonate the enol
- Arg (R116) acts to neutralize charges of phosphates during transfer
- All kinases seem to have this Arg

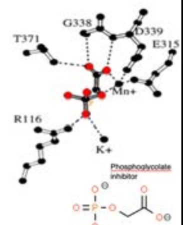
http://clfs690.alivetek.org/CLFS690/glycolglucojmol/pyruvatekinase.htm

Glycolysis: Pyruvate Kinase (PK)

Mechanism

Stereo views





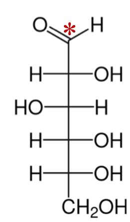
Glycolysis: Summary

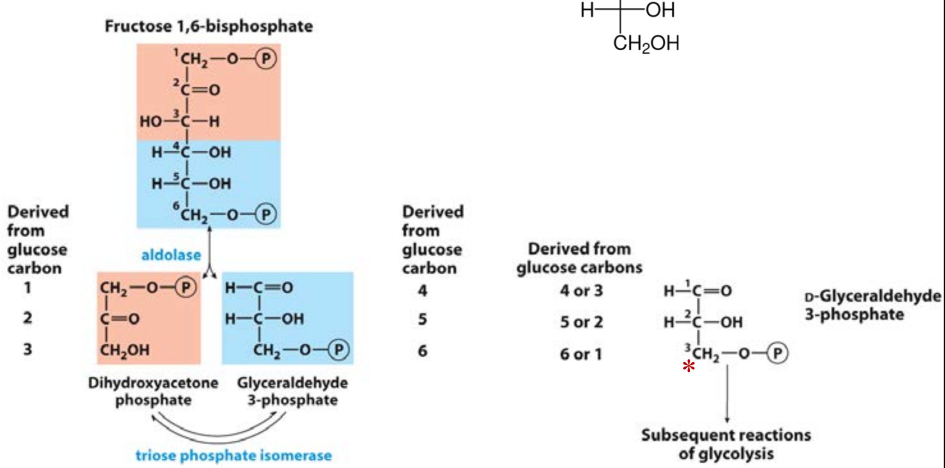
Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 H^+ + **2 ATP** + 2 H_2O

- Used:
  - 1 glucose; 2 ATP; 2 NAD^+, 2 ADP, 2 P_i
- Made:
  - 2 pyruvate
    - various different fates
  - 4 ATP
    - The net of 2 ATP is used for energy-requiring processes within the cell
  - 2 NADH
    - For glycolysis to continue, NADH must be re-oxidized
- Glycolysis is heavily regulated.
  - ensure proper use of nutrients
  - ensure production of ATP only when needed
  - will discuss details after we do the opposite pathway (anabolism: gluconeogenesis)

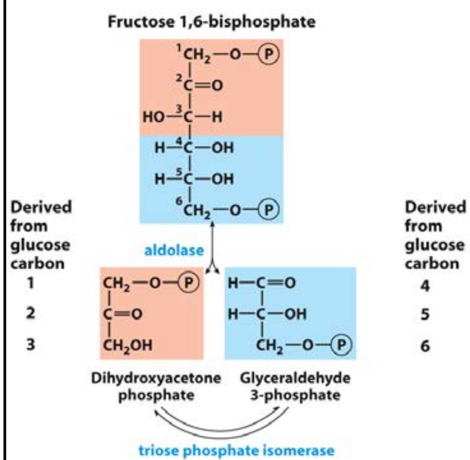
Glycolysis: Isotope-labeling studies

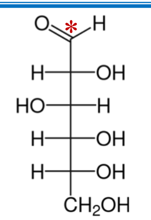
Experiment: If you give ^{14}C-glucose to bacteria, with the label only on carbon #1, where would the label appear in GAP?



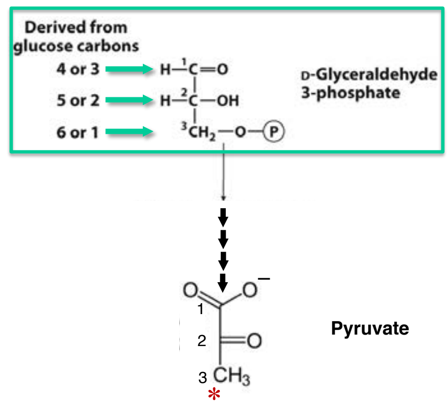


Glycolysis: Isotope-labeling studies

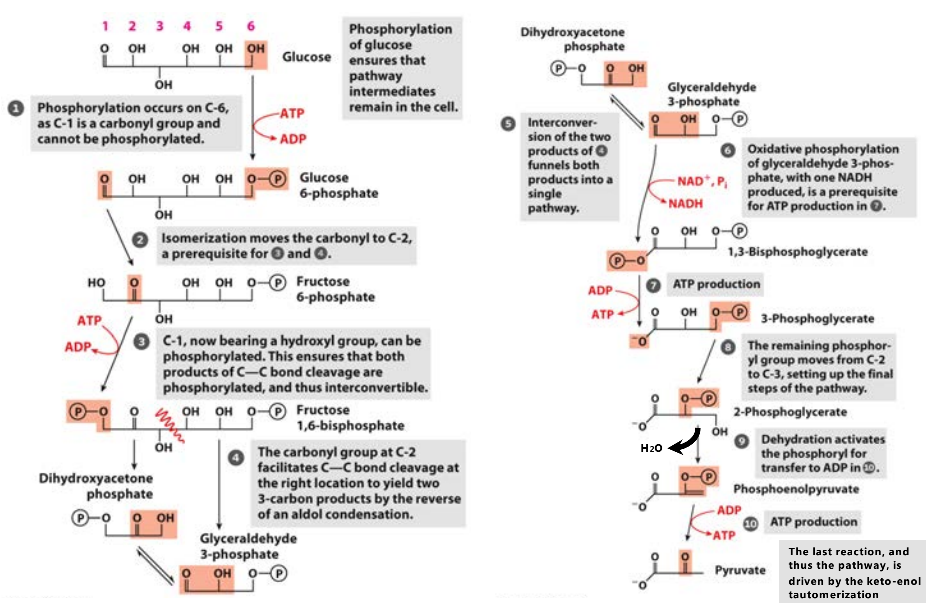




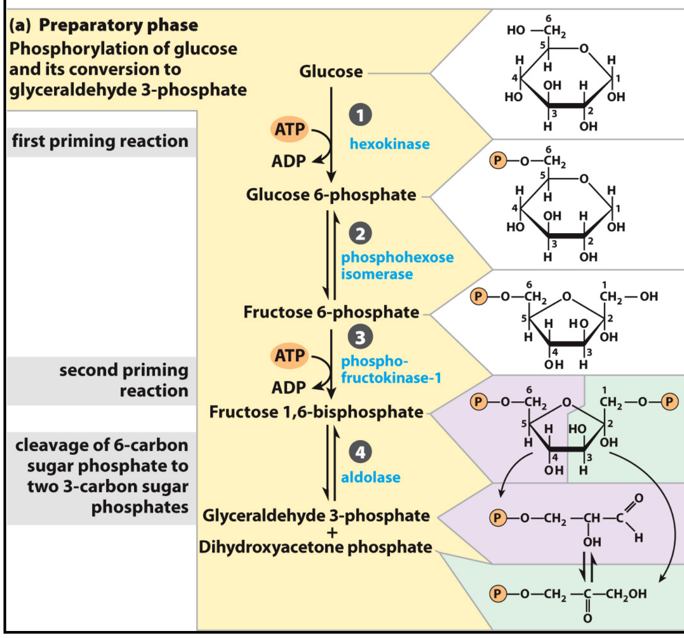
Experiment: If you give ^{14}C-glucose to bacteria, with the label only on carbon #1, where would the label appear in pyruvate?



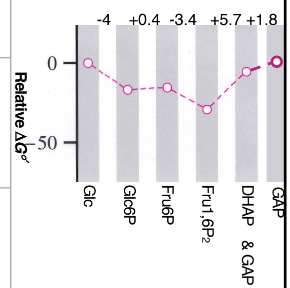
Glycolysis: Chemical Logic



Glycolysis: Energetics

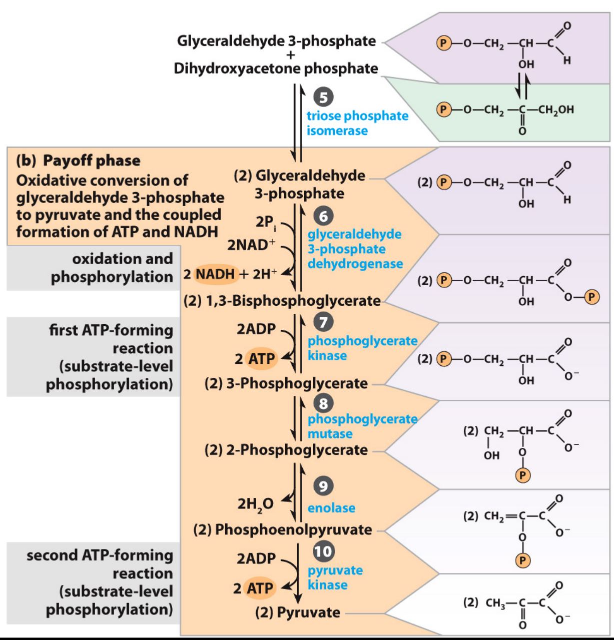


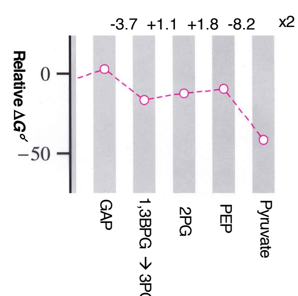
Preparatory Phase



Glycolysis: Energetics

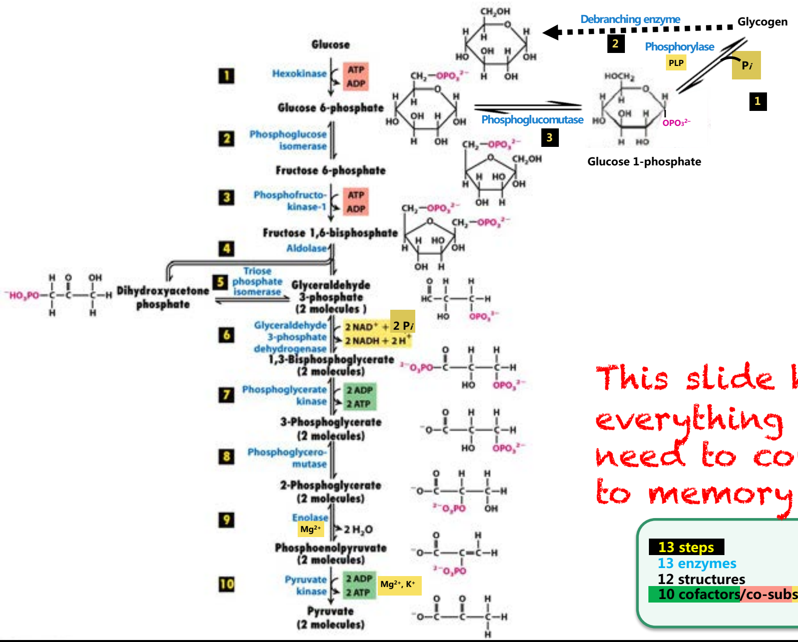
The Payoff Phase





Total for 1 Glc → 2 Pyruvate is –18 kcal/mol

Glycogenolysis & Glycolysis: Summary

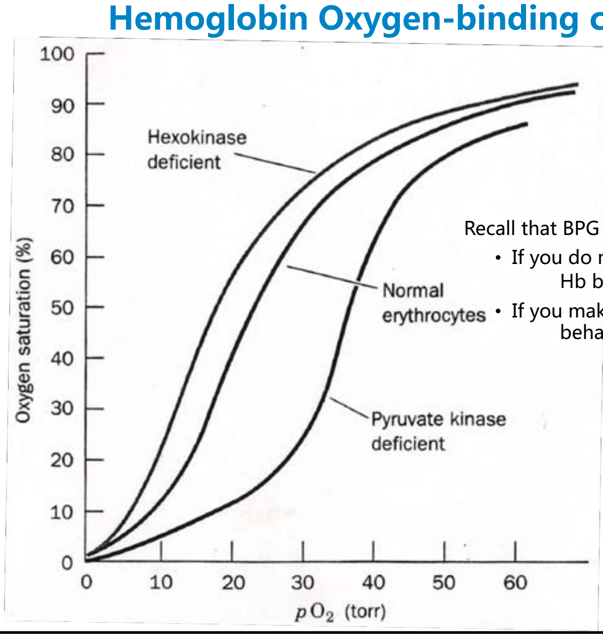


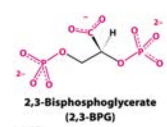
This slide has everything you need to commit to memory

13 steps
13 enzymes
12 structures
10 cofactors/co-substrates

Glycolysis: Enzyme Deficiencies

Hemoglobin Oxygen-binding curves





Recall that BPG is the key allosteric effector of Hb:

- If you do not make enough (HK deficiency), Hb behaves less cooperatively
- If you make too much (PK deficiency), HB behaves more cooperatively

Catabolism of Other Sugars

- Ingestion yields free glucose from glycogen and starch by α–amylase, maltase, and isomaltase
- In the cell, glucose molecules are cleaved from glycogen and starch by glycogen phosphorylase.
  - yielding glucose 1-phosphate (and a little free Glc)
  - uses inorganic phosphate for lysis (phosphoro-lysis)
- Disaccharides are hydrolyzed.
  - lactose: glucose and galactose
  - sucrose: glucose and fructose
  - trehalose: glucose
  - Monosaccharides fructose, galactose, and mannose enter glycolysis at different points.

Catabolism of Other Sugars