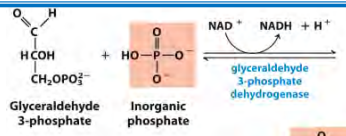
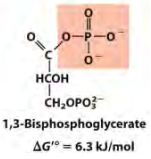
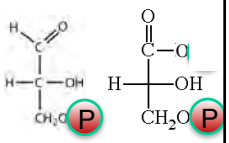
Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



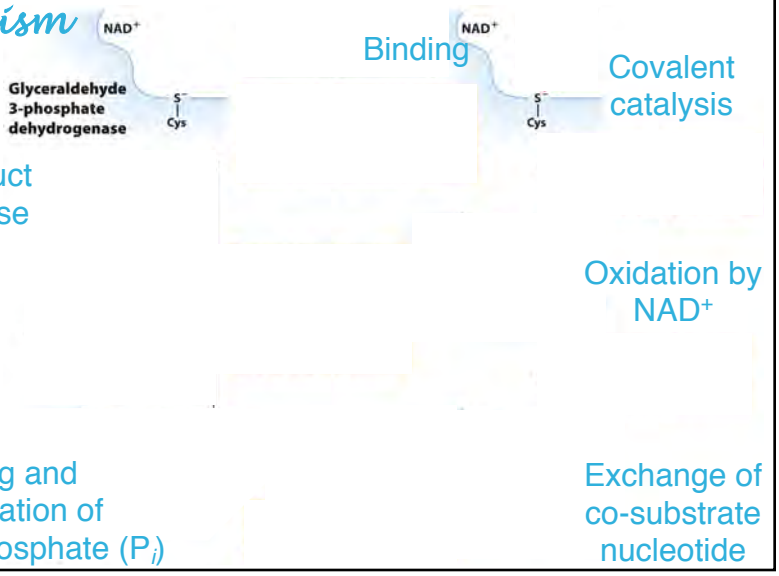




- Rationale:
  - Recall Pyruvate is an acid;
  - need to oxidize aldehyde
  - incorporates inorganic phosphate
  - generation of a high-energy phosphate compound
  - **which allows for net production of ATP via glycolysis!**
- First energy-yielding step in glycolysis
- First oxidation: aldehyde to carboxylate (ox)/NAD^+ to NADH (red).
- Active-site cysteine
  - forms high-energy thioester intermediate
  - subject to inactivation by oxidative stress
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +1.8 kcal/mol)

Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Mechanism



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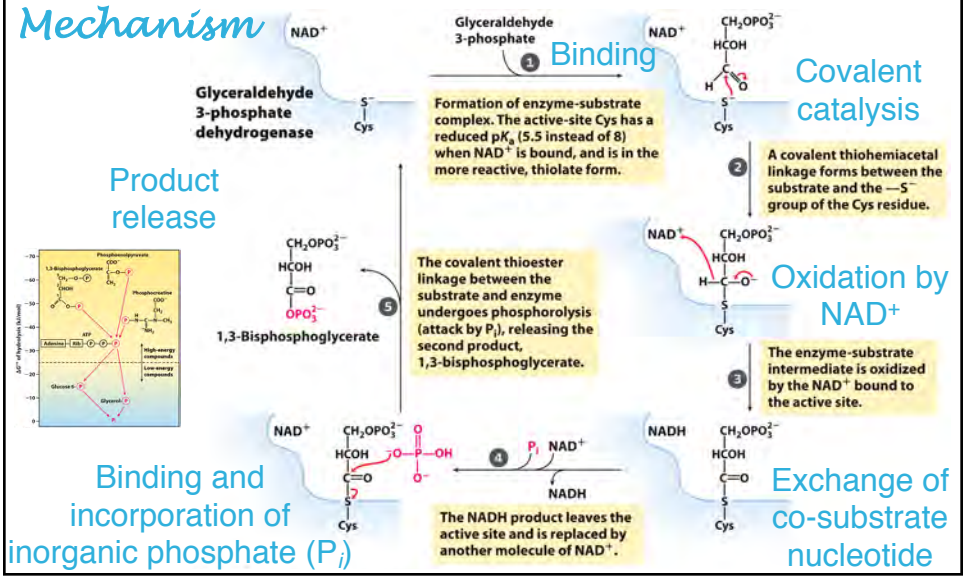
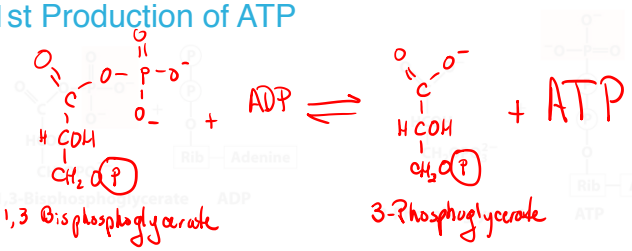
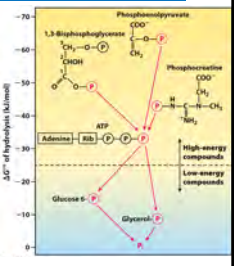


Glycolysis: Phosphoglycerate Kinase (PGK)

1st Production of ATP

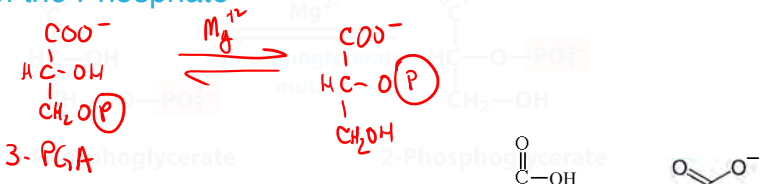




- Rationale:
  - substrate-level phosphorylation to make ATP
  - first of two "payoff" steps
- 1,3-bisphosphoglycerate is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Named for the reverse reaction; recall Kinases are enzymes that transfer phosphate groups between ATP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

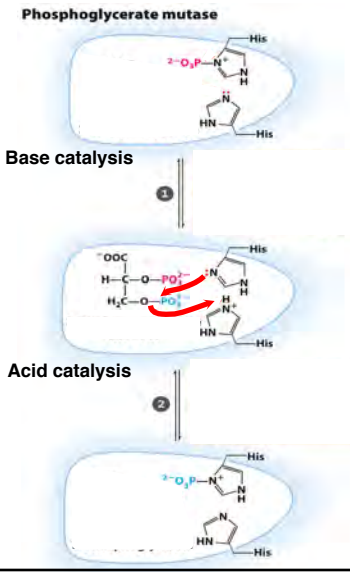
Glycolysis: Phosphoglycerate Mutase (PGM)

Migration of the Phosphate



- Rationale:
  - 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.
  - Notice that reduction of C3 and oxidation of C2 means no net redox.
- Mutases catalyze the (apparent) migration of functional groups.
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +1.1 kcal/mol)
  - reactant concentration kept high by favorability through PGK reaction.

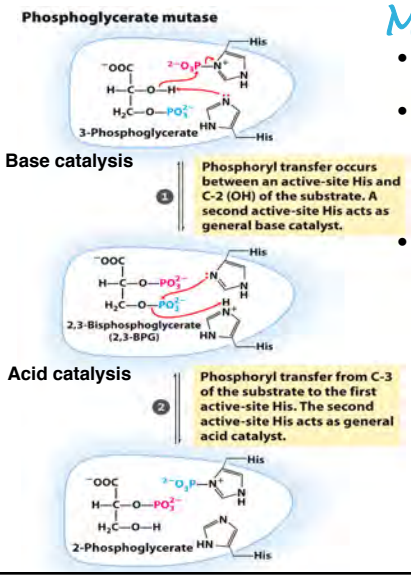
Glycolysis: Phosphoglycerate Mutase (PGM)



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)



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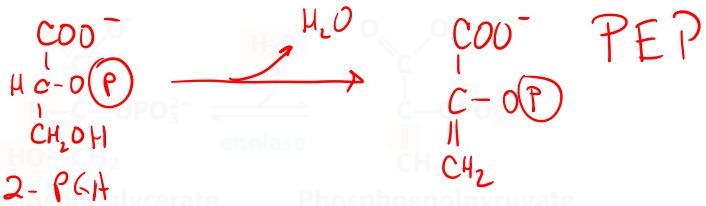
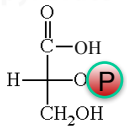
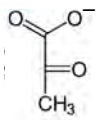
Glycolysis: Enolase



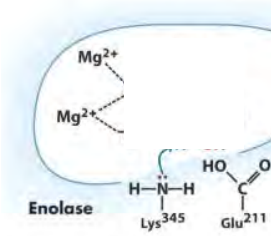




- Rationale:
  - Dehydrates C3 to reduce it like pyruvate
  - Double-bonded C2-C3 is part of an en-ol except that the hydroxyl is in ester linkage with a phosphate
- 2-Phosphoglycerate is not a good enough phosphate donor to generate ATP.
  - two negative charges in 2-PG are fairly close
  - but loss of phosphate from 2-PG would give a secondary alcohol with no further stabilization
- Slightly thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +1.8 kcal/mol)
  - product concentration kept low to pull forward

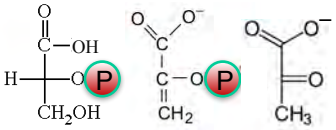
Glycolysis: Enolase

Mechanism Dehydration



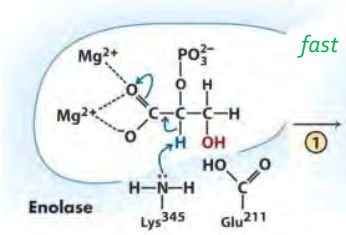
2-Phosphoglycerate bound

fast *slow*

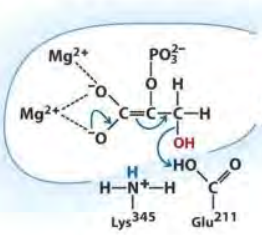


Glycolysis: Enolase

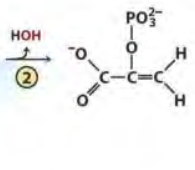
Mechanism Dehydration



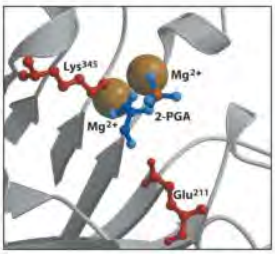
fast

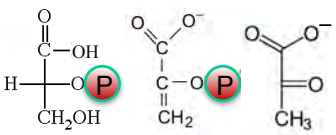


slow

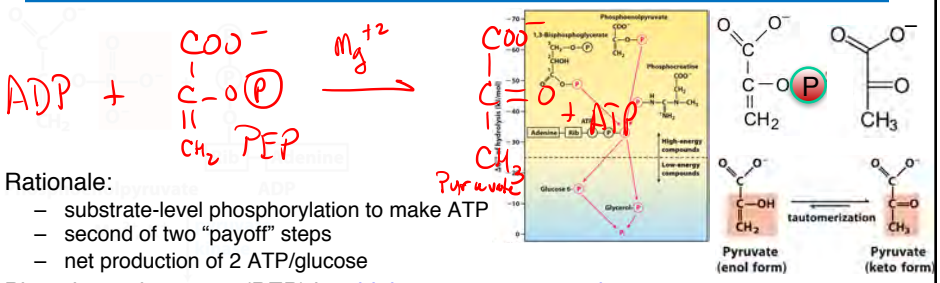


2-Phosphoglycerate bound to enzyme | Enolic intermediate | Phosphoenolpyruvate





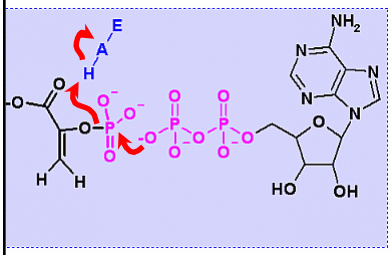
Glycolysis: Pyruvate Kinase (PK)

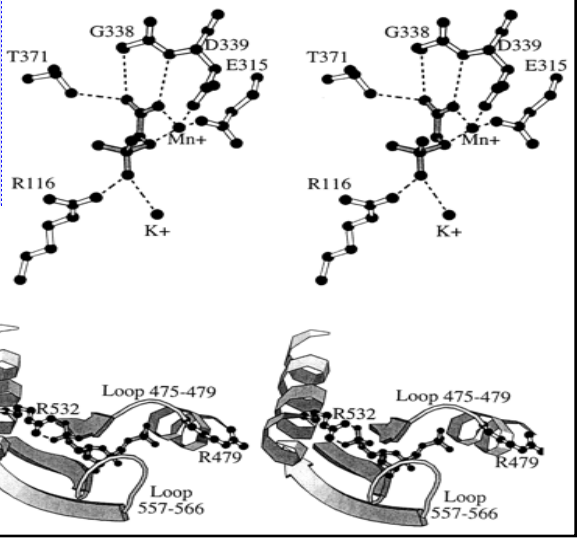


- Rationale:
  - substrate-level phosphorylation to make ATP
  - second of two "payoff" steps
  - net production of 2 ATP/glucose
- Phosphoenolpyruvate (PEP) is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Loss of phosphate from PEP yields an enol that tautomerizes 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^{++}) for activity.
- Highly thermodynamically favorable/reversible ($\Delta G^{\circ\prime} = -8.2$ kcal/mol)
  - This reaction pulls the entire glycolytic pathway.
  - regulated by ATP, divalent metals, and other metabolites

Glycolysis: Pyruvate Kinase (PK)

Mechanism Phosphoryl transfer





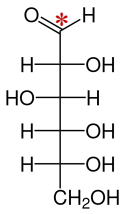
Glycolysis: Summary

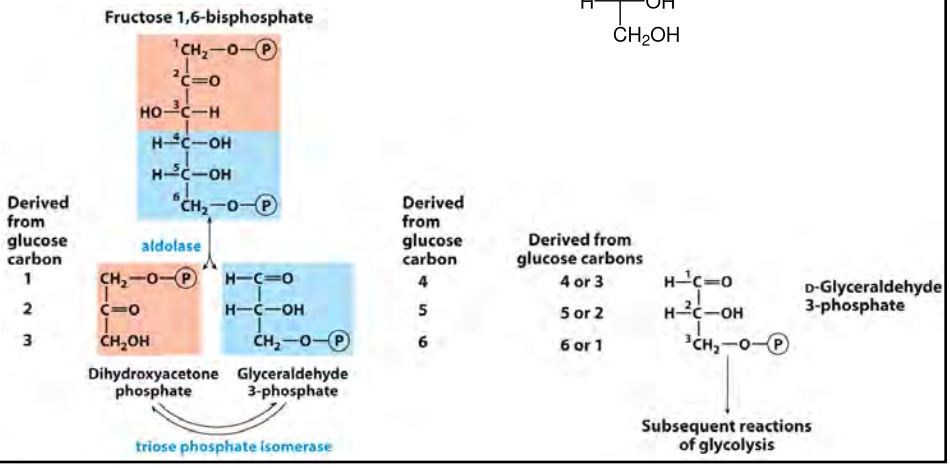
Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 H^+ + **2 ATP**

- Used:
  - 1 glucose; 2 ATP; 2 NAD^+, 2 ADP
- 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)

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 DHAP?





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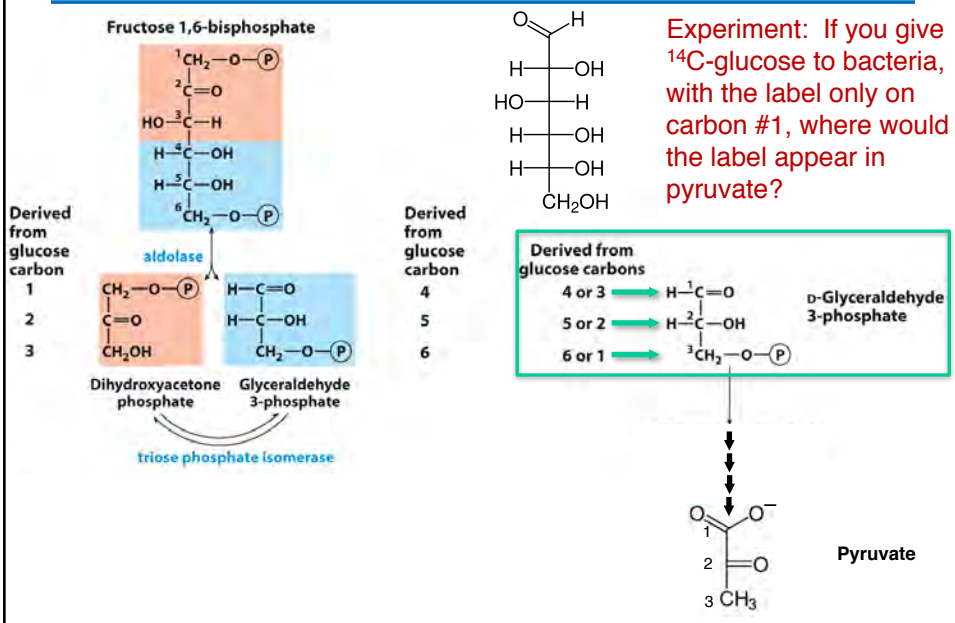
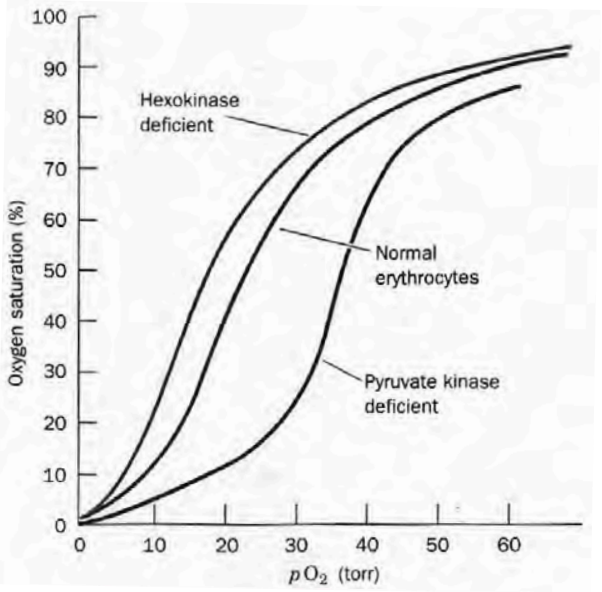
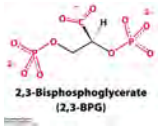


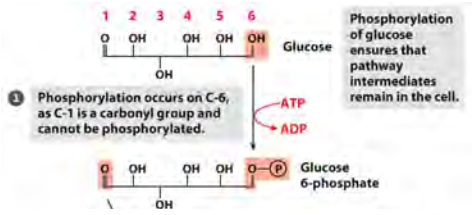
Experiment: If you give ^{14}C-glucose to bacteria, with the label only on carbon #1, where would the label appear in pyruvate?

Glycolysis: Enzyme Deficiencies





Glycolysis: Chemical Logic



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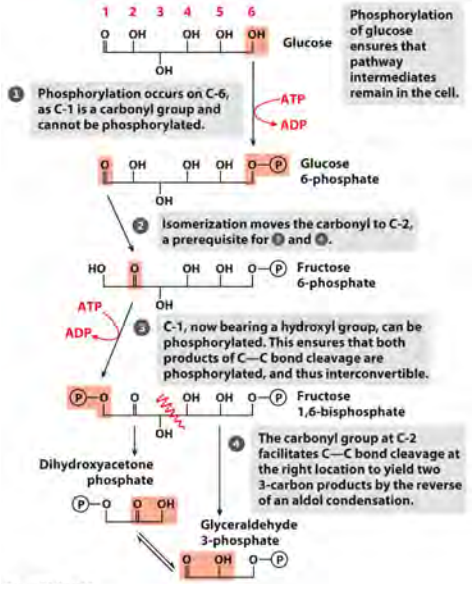
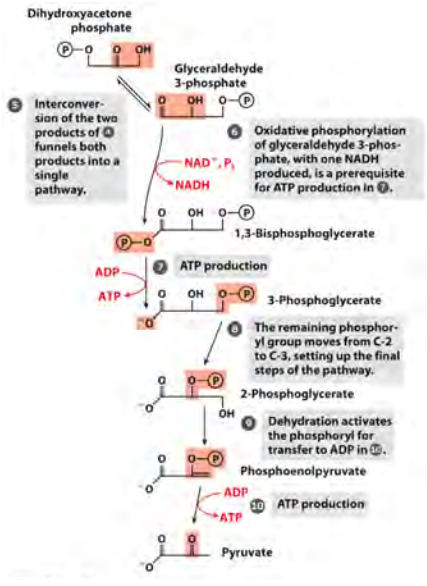
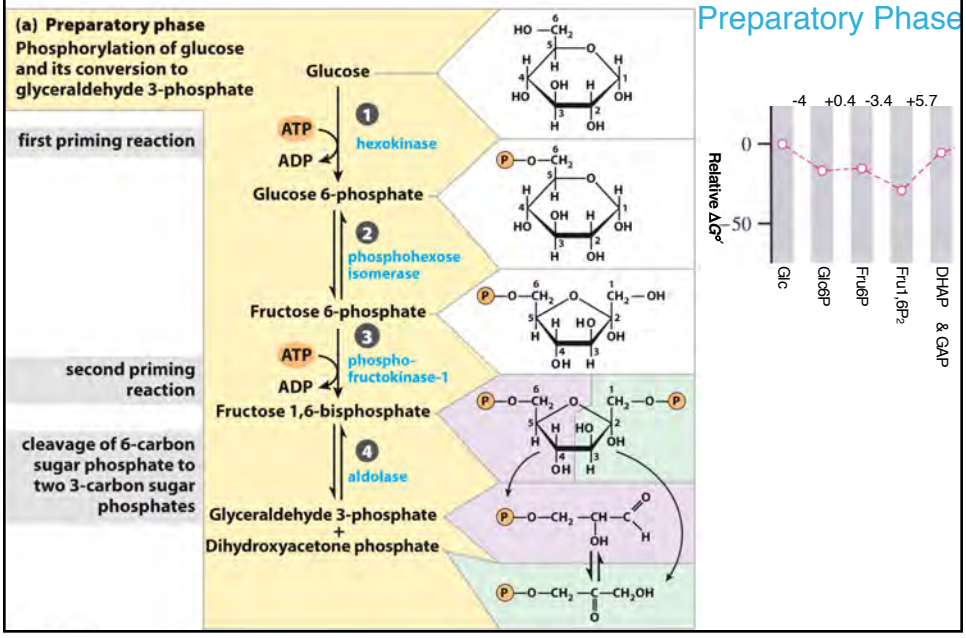


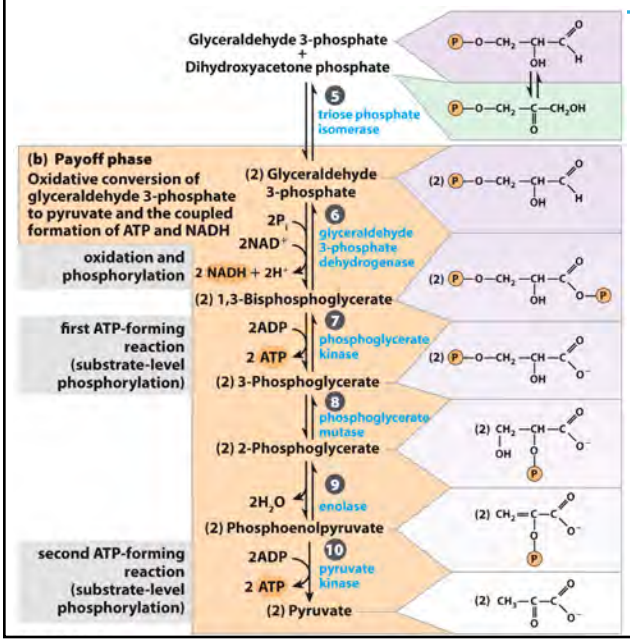


Glycolysis: Energetics

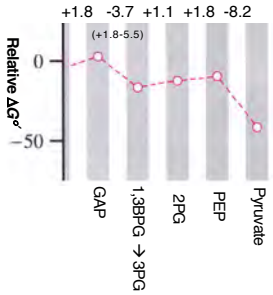


Preparatory Phase

Glycolysis: Energetics



The Payoff Phase



Total for 1 Glc → 2 Pyruvate is –35 kcal/mol