# BI/CH 422/622

**OUTLINE:**

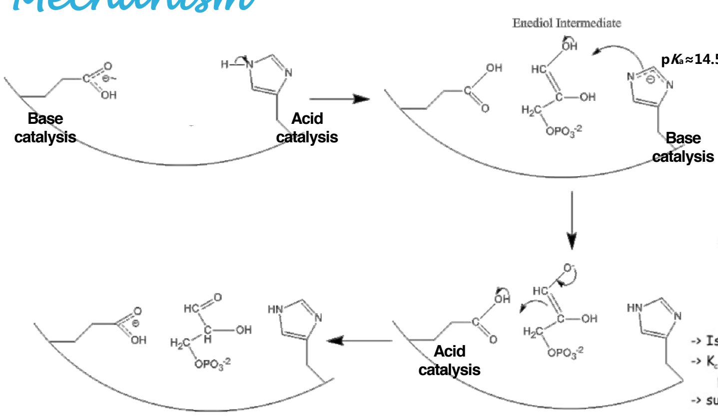
Glycogenolysis

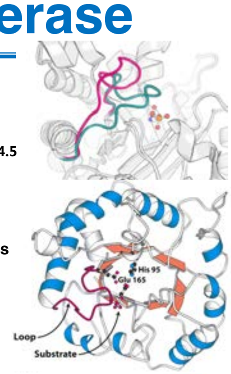
- **phosphorylase** – acid/base; carbo-cation; PLP cofactor
- debranching enzyme
- **phosphoglucomutase** – acid/base; phospho-enzyme; bisphosphate int.

Glycolysis

- Introduction & overview; 2 phases
- Phase I
  - hexokinase – phosphotransferase-coupling
  - **PGI** – endiol
  - PFK1 – phosphotransferase-coupling
  - **Aldolase** – Schiff base (electron sink to stabilize a carbanion)
  - **TPI** – endiol (fast)
- Phase II
  - **GAPDH** – oxidation
  - PG kinase – return on investment– substrate-level phosphorylation
  - **PG mutase** – acid/base phospho-enzyme
  - **Enolase** – enolate
  - Pyruvate Kinase – phosphotransferase

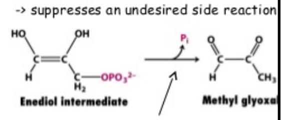
# Glycolysis: Triose-phosphate isomerase


Triose phosphate isomerase (TPI)

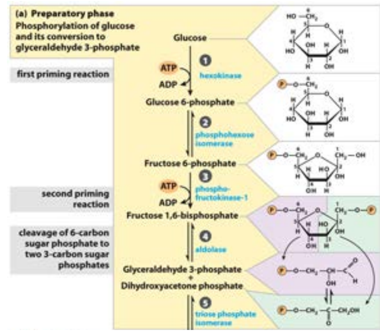
- -> Isomerisation accelerated $10^{10}$-fold
- -> $K_{cat}/K_m$ = $2*10^8$ $M^{-1}$ $s^{-1}$ -> kinetically perfect enzyme
- -> suppresses an undesired side reaction




TIM caps enediol with loop around $P_i$ so that its not a good leaving group, and the elimination reaction to form methyl glyoxal is limited.

# Glycolysis: Overview

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

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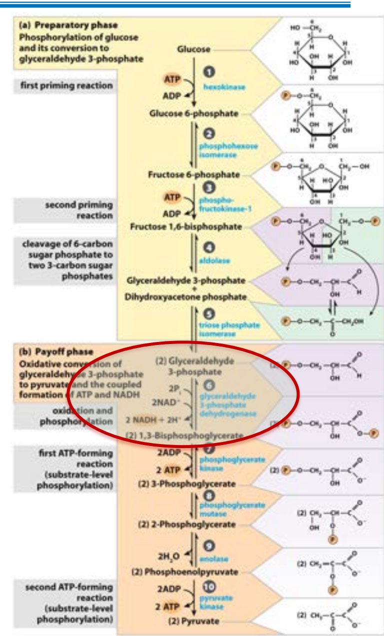
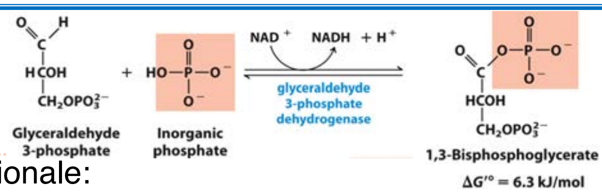
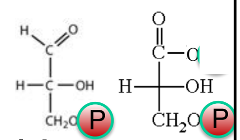


# Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)




- 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!**

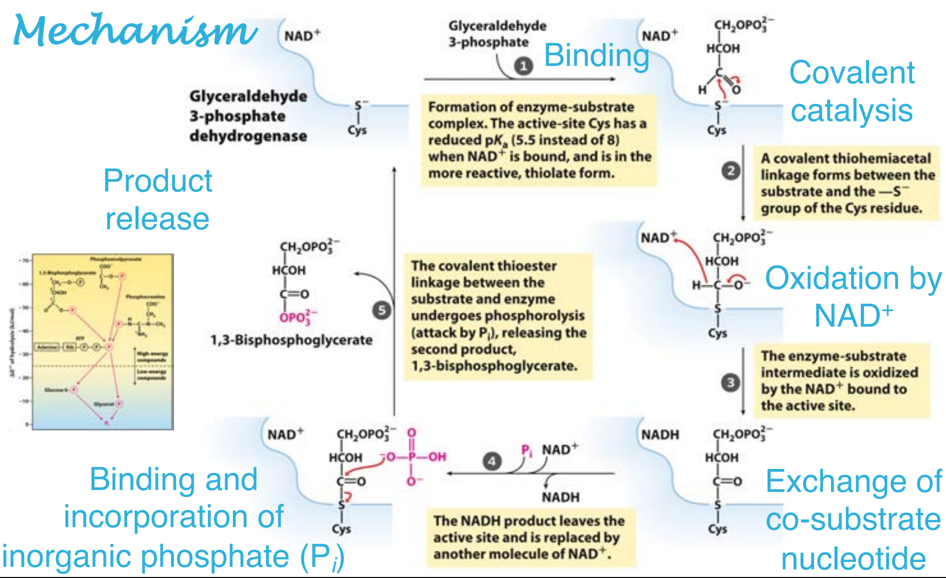


- First energy-yielding step in glycolysis
- First oxidation: aldehyde to carboxylate (ox)/$NAD^+$ to NADH (red).
- Active-site cysteine
  - forms high-energy thioester intermediate
  - subject to inactivation by oxidative stress

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

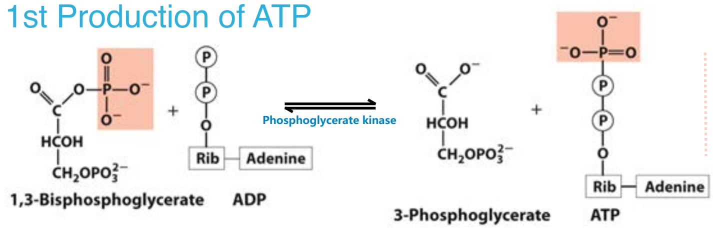
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - coupled to next reaction to pull forward

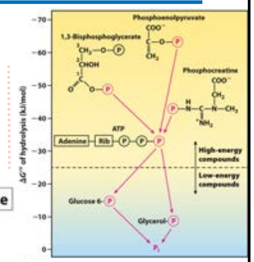
# Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



# Glycolysis: Phosphoglycerate Kinase (PGK)

## 1st Production of ATP

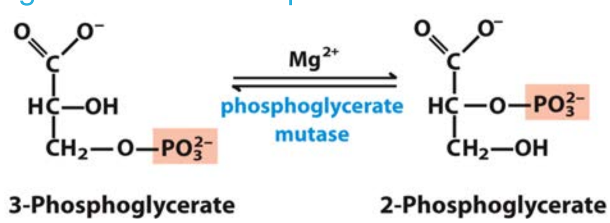


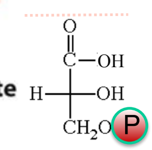



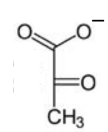
- Rationale:
  - substrate-level phosphorylation to make ATP
  - first of two “payoff” steps
- 1,3-bisphosphoglycerate is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Named for the reverse reaction; recall Kinases are enzymes that transfer phosphate groups between ATP 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 Mutase (PGM)

## Migration of the Phosphate

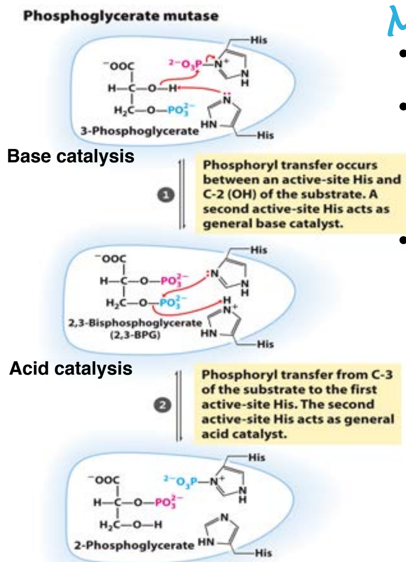




- Rationale:
  - Notice that reduction of C3 and oxidation of C2 means no net redox.
  - Need to get C3 dehydrated, so need to move phosphoryl group
  - Need to form high-energy phosphate compound to make glycolysis a net ATP producer.
- Mutases catalyze the (apparent) migration of functional groups.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

# Glycolysis: Phosphoglycerate Mutase (PGM)

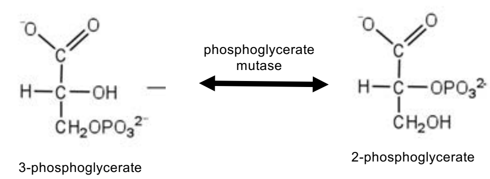



*Mechanism* — Acid/base Catalysis

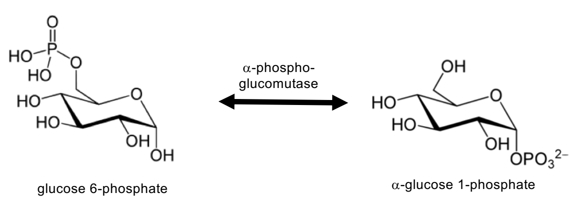
- Similar to other mutases
- One of the active-site histidines is post-translationally modified to phospho-histidine.
- Phospho-histidine donates its phosphate to 3-phosphoglycerate at the C2-oxygen before retrieving the phosphate from the 3-carbon oxygen.
  - Note that the phosphate from the substrate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

# The Mutases

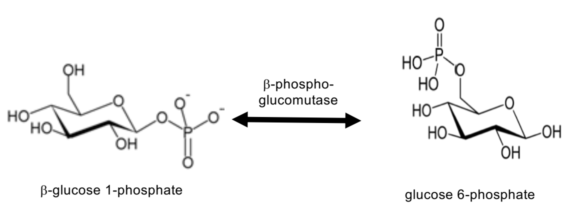
## Accomplishing Phosphomutase Activity




- One binding step
  - No reorientation required

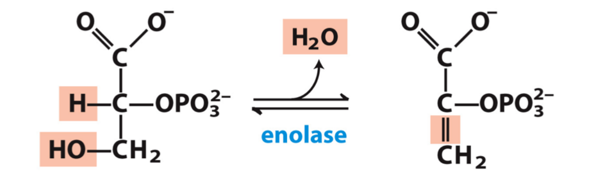



- Two binding steps
  - Glycogen metabolism
  - Bis-phosphorylated intermediate flips in "vestibule"
  - No glucose exchange

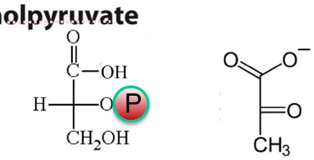



- Two binding steps
  - Free bis-phosphorylated intermediate flips outside active site
  - HAD
  - Exchange glucose
  - Micro-organisms

# Glycolysis: Enolase



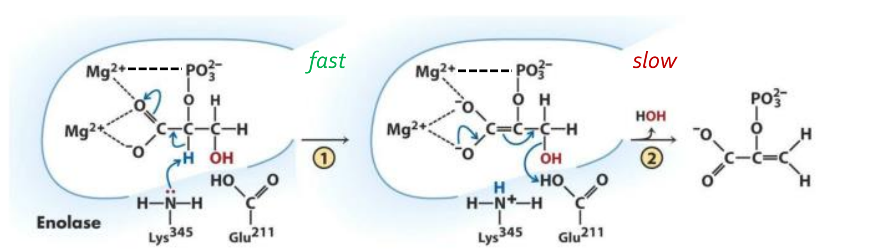

2-Phosphoglycerate Phosphoenolpyruvate



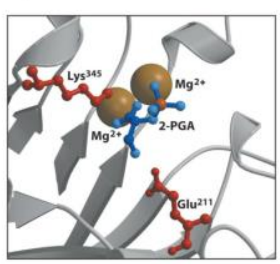
- Rationale:
  - Dehydrates C3 to reduce it like pyruvate
  - Double-bonded C2-C3 is part of an en-ol except that the C2-alcohol is in ester linkage with a phosphate
- 2-Phosphoglycerate is not a good enough phosphate donor to generate ATP.
  - loss of phosphate from 2-PG would give a secondary alcohol, which is completely stable
- Slightly thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - product concentration kept low to pull forward

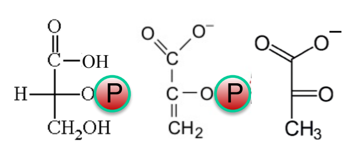
# Glycolysis: Enolase

*Mechanism* Dehydration

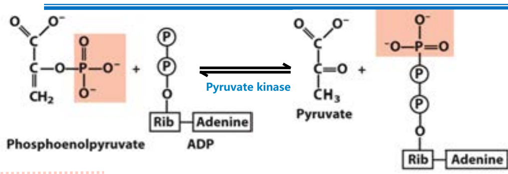


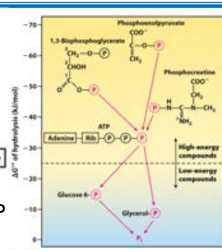

2-Phosphoglycerate bound to enzyme Enolic intermediate Phosphoenolpyruvate

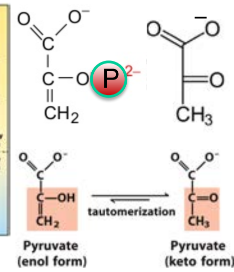


# Glycolysis: Pyruvate Kinase (PK)



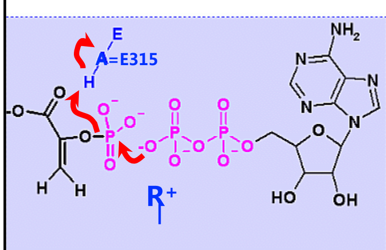





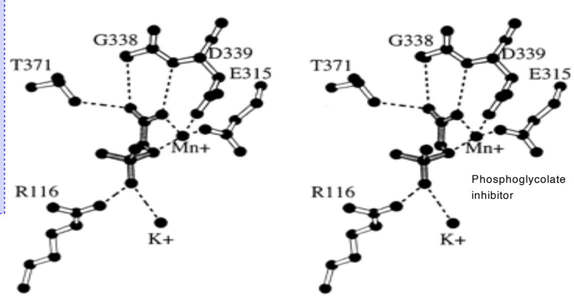

- Rationale:
  - substrate-level phosphorylation to make ATP
  - second of two "payoff" steps
  - net production of 2 ATP/glucose
- Phosphoenolpyruvate (PEP) is a high-energy compound.
  - can donate the phosphate group to ADP to make ATP
- Loss of phosphate from PEP yields an enol that tautomerizes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

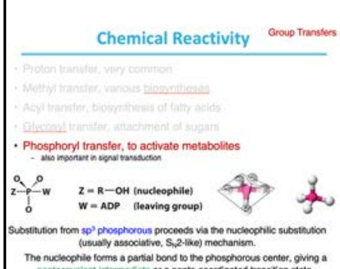
# Glycolysis: Pyruvate Kinase (PK)

*Mechanism* Phosphoryl transfer




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




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

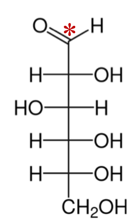
# Glycolysis: Summary

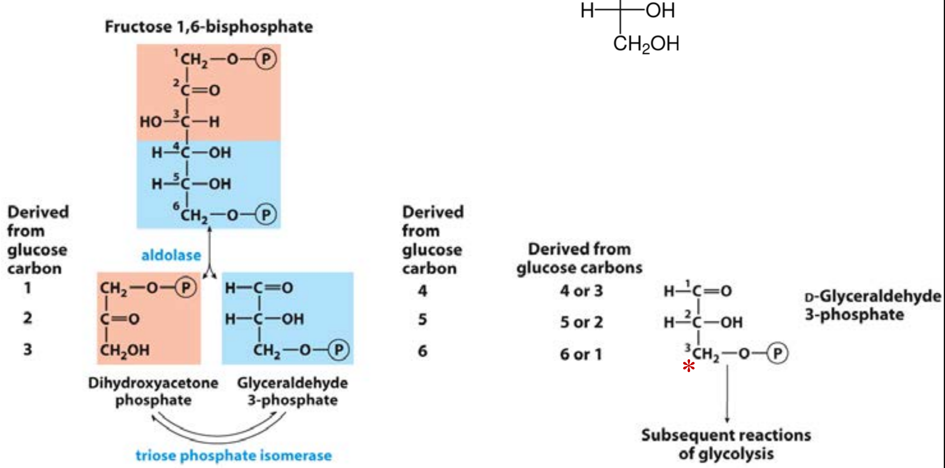
Glucose + 2 $NAD^+$ + 2 ADP + 2 $P_i$ → 2 Pyruvate + 2 NADH + 2 $H^+$ + **2 ATP**

- 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: 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?



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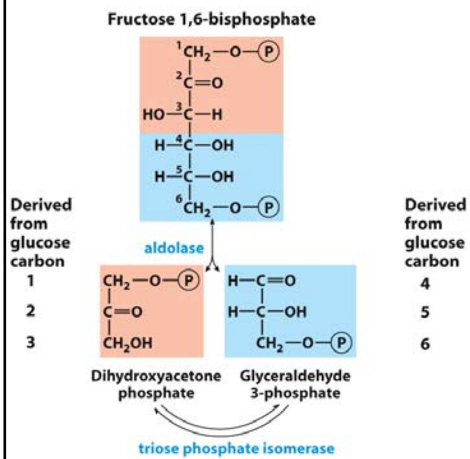
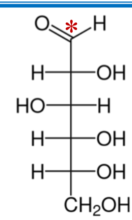
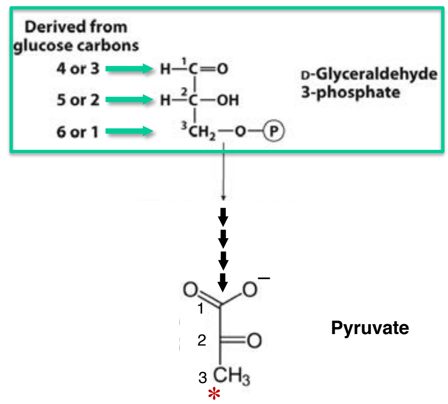
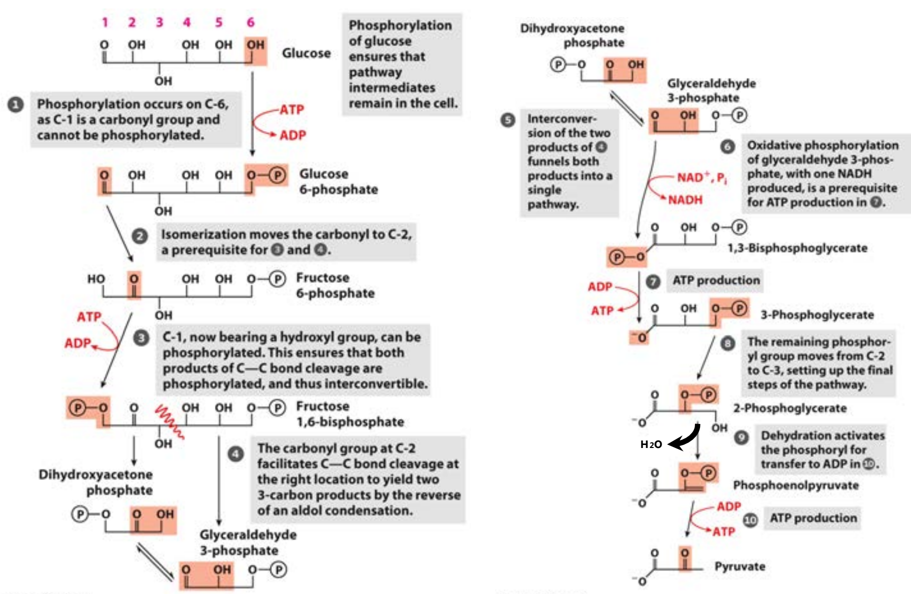


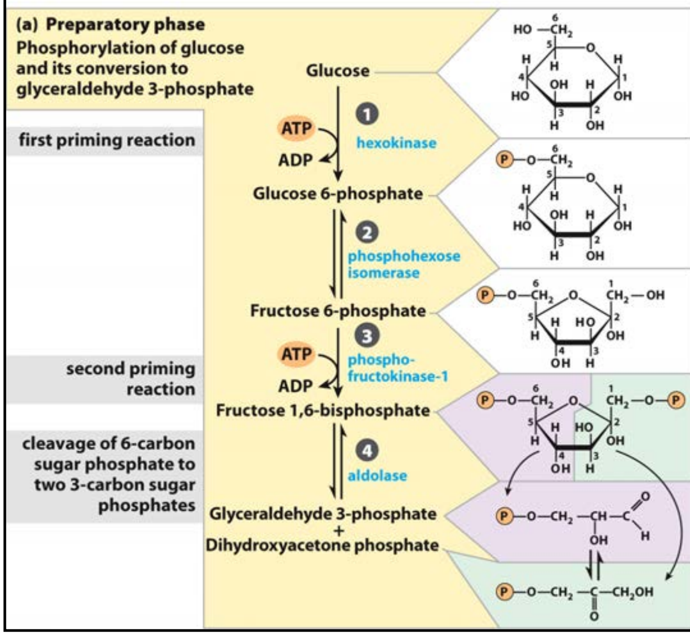
Experiment: If you give $^{14}$C-glucose to bacteria, with the label only on carbon #1, <u>where would the label appear in pyruvate</u>?



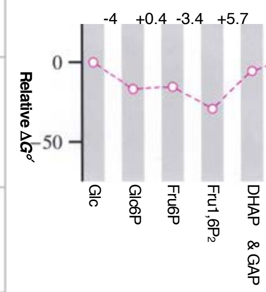

# Glycolysis: Chemical Logic



# Glycolysis: Energetics




Preparatory Phase

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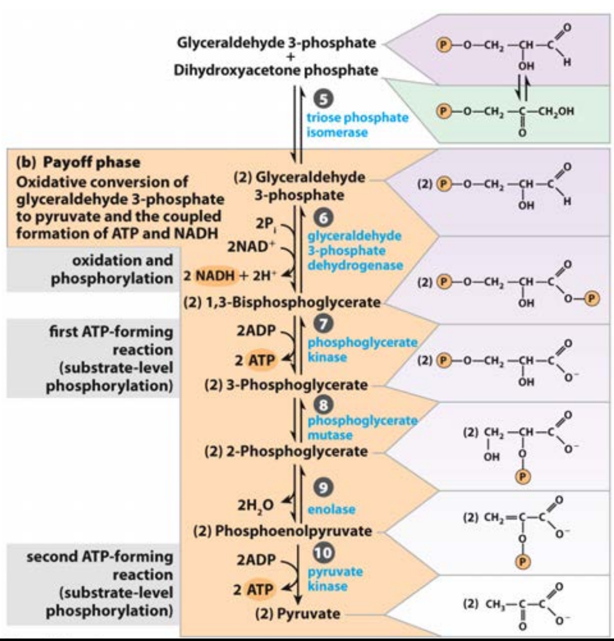
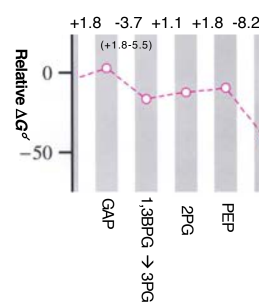
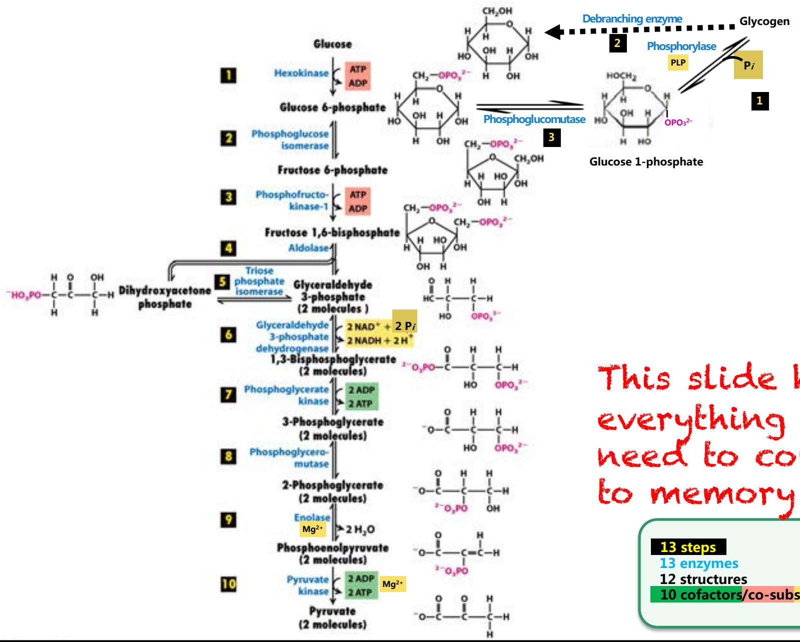



The Payoff Phase




Total for 1 Glc → 2 Pyruvate is –35 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