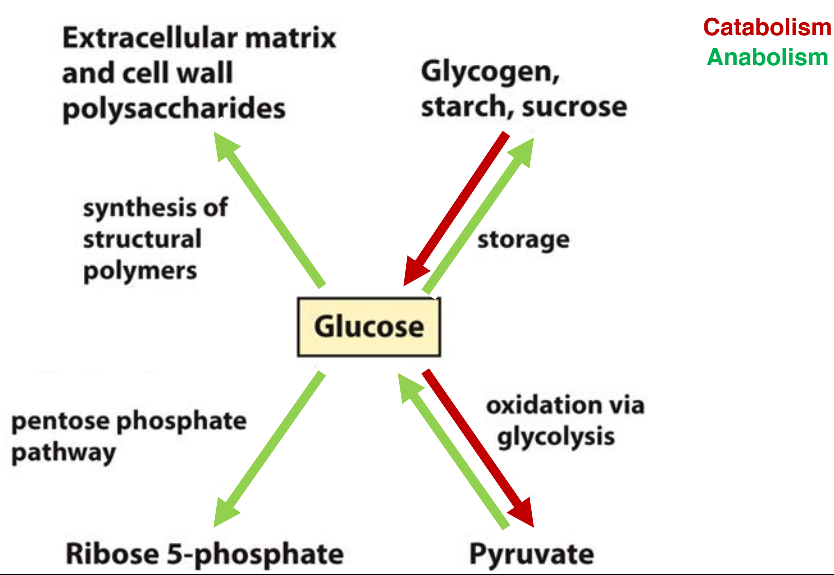
BI/CH 422/622

OUTLINE:

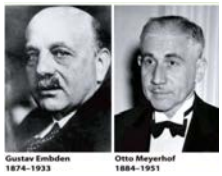
Review
Bioenergetics
Membrane Transport
Catabolism of Glucose
- Glycogenolysis
  - phosphorylase
  - debranching enzyme
  - phospho-gluco-mutase (PGM)
- Glycolysis
  - Intro & overview; 2 phases
  - Phase I
    - hexokinase
    - phospho-gluco-isomerase (PGI)
    - phospho-fructo-kinase (PFK-1)
    - Aldolase
    - triose-phosphate isomerase (TPI)
  - Phase II
    - GAPDH
    - PG kinase
    - PG mutase
    - Enolase
    - Pyruvate Kinase
- Fermentation

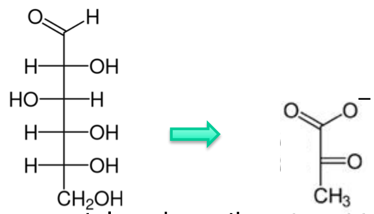
Glucose Utilization

Four Major Fates of Glucose



Glycolysis

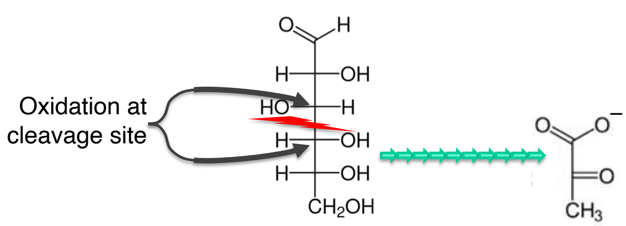




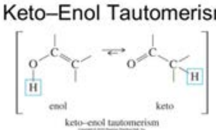
Learning outcomes for each pathway in course:
1. Understand the logic for getting from starting compound to end product
2. Know the names, structures, enzymes of each step
3. Energetics
4. Control mechanisms

- Sequence of enzyme-catalyzed reactions by which glucose is converted into pyruvate
  - Pyruvate can be further aerobically oxidized.
  - Pyruvate can be further anaerobically fermented.
  - Pyruvate can be used as a precursor in biosynthesis.
- In the evolution of life, glycolysis probably was one of the earliest energy-yielding pathways.
- Research of glycolysis played a large role in the development of modern biochemistry.
  - understanding the role of coenzymes
  - discovery of the pivotal role of ATP
  - development of methods for enzyme purification
  - inspiration for the next generations of biochemists

Glycolysis



Keto–Enol Tautomerism

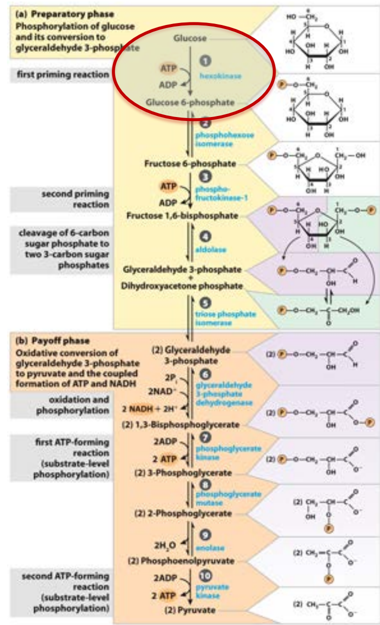


- Enols are not stable and they isomerize to the corresponding aldehyde or ketone in a process known as keto-enol tautomerism.

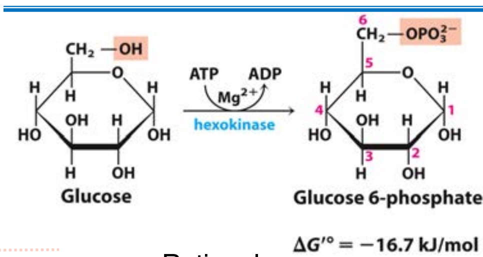
- It developed before photosynthesis, when the atmosphere was still anaerobic.
- Thus, the task upon early organisms was how to extract free energy from glucose anaerobically.
- Some of the free energy is captured in the synthesis of ATP and NADH.
- The solution:
  - First: All intermediates are phosphorylated: keeps them in the cell and "Activates" them for chemical degradation.
  - Second: Need to split in middle to make pathway convergent
  - Third: Overall oxidation (loss of only 4 e^-)
  - Fourth: Keto-enol tautomerization is key
  - Fifth: Collect energy from the high-energy metabolites.

Glycolysis: Overview

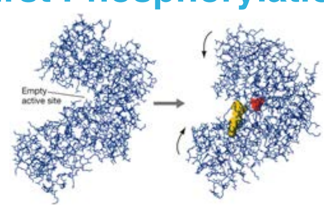
- Two Phases/Four concepts
  - Preparatory phase
    - Phosphorylation by ATP
    - Cleavage
  - Payoff
    - Oxidation
    - Phosphorylation of ATP



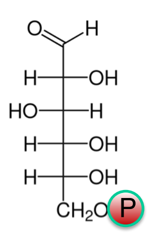
Glycolysis: Hexokinase



First Phosphorylation

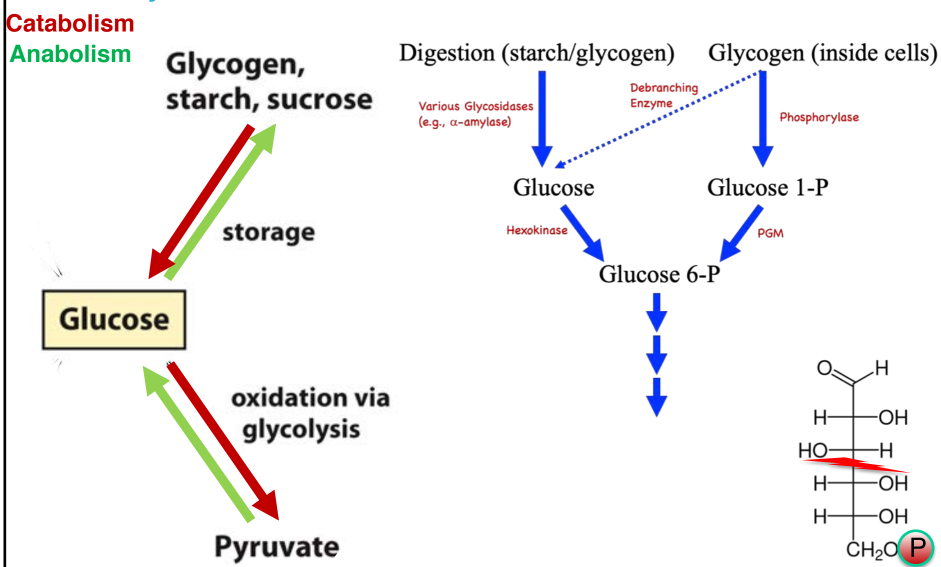


- Rationale
  - traps glucose inside the cell
  - lowers intracellular (unphosphorylated) glucose concentration to allow further uptake by facilitative diffusion through GLUTS.
- This process uses the energy of ATP (energy coupling).
- Multiple isoforms of hexokinase exist in organisms (e.g., hexokinase I, II, III, and IV (glucokinase)).
- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphate of ATP.
- ATP-bound Mg^{++} facilitates this process by shielding the negative charges on ATP.
- Highly thermodynamically favorable/irreversible
  - $\Delta G^{o'} = -4$ kcal/mol
  - regulated mainly by product inhibition

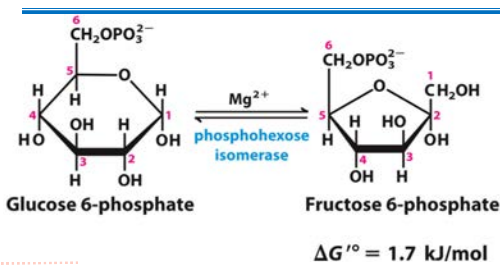


Glucose Utilization

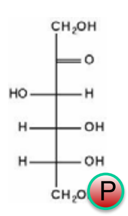
Four Major Fates of Glucose



Glycolysis: Phosphoglucose isomerase (PGI)



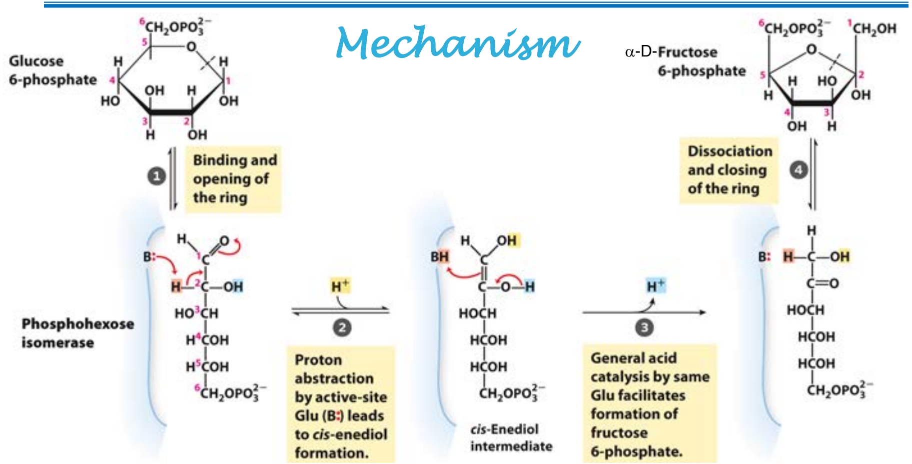
Setting up Second Phosphorylation



- Rationale
  - Convert C1 from aldehyde to hydroxyl which is easier to phosphorylate
- Slightly thermodynamically unfavorable/reversible
  - $\Delta G^{o'}$ = +0.4 kcal/mol
  - product concentration kept low by pairing with favorable next step to drive reaction forward

Glycolysis: Phosphoglucose isomerase (PGI)

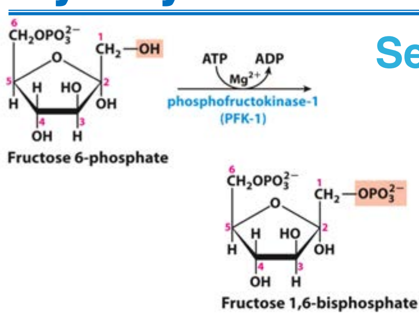
Mechanism

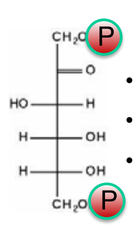


- The isomerization is catalyzed by the active-site glutamate via general acid/base catalysis.
- An aldose (glucose) can isomerize into a ketose (fructose) via an enediol intermediate.

Glycolysis: Phosphofructokinase-1

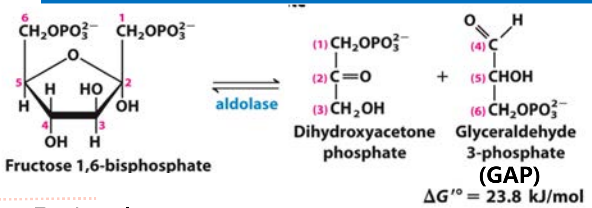
Second Phosphorylation

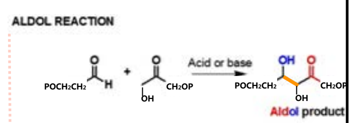




- Rationale $\Delta G'^{\circ} = -14.2$ kJ/mol
  - Symmetrical phosphorylation to set up cleavage to 3-carbon sugar
- First committed step of glycolysis
  - fructose 1,6-bisphosphate (Fru 1,6-P_2) is committed to become pyruvate and yield energy
- This process uses the energy of ATP.
- Highly thermodynamically favorable/irreversible ($\Delta G^{o'}$ = –3.4 kcal/mol)
- Phosphofructokinase-1 is highly regulated.
  - by ATP, fructose 2,6-bisphosphate, and other metabolites
  - do not burn glucose if there is plenty of ATP

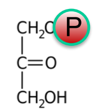
Glycolysis: Fructose-1,6-bisphosphate Aldolase

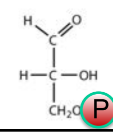




Aldol Cleavage/ condensation

- Rationale
  - Six-carbon sugar is cleaved into two three-carbon phosphorylated sugars.
- The reverse process is the familiar aldol condensation.
- Multiple isoforms of aldolase exist; aldolase A, B, C.
- Multiple mechanisms to result in same product (i.e., convergent evolution):
  - Animal and plant aldolases employ covalent catalysis.
  - Fungal and bacterial aldolases employ metal ion catalysis.
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +5.7 kcal/mol)
  - product (GAP) concentration kept low to pull reaction forward.

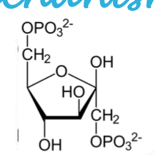


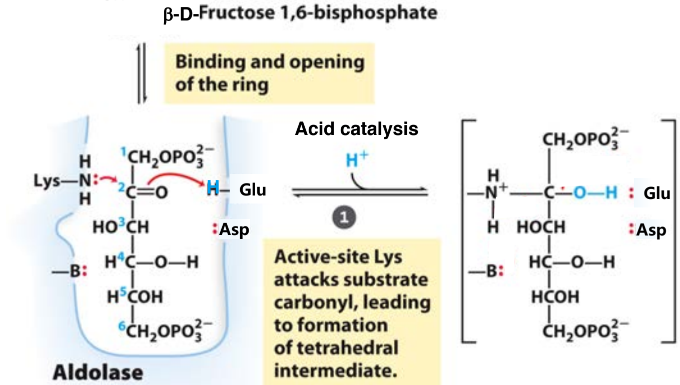


Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism Covalent Catalysis (Class I enzymes)

Step 1: making the Schiff Base



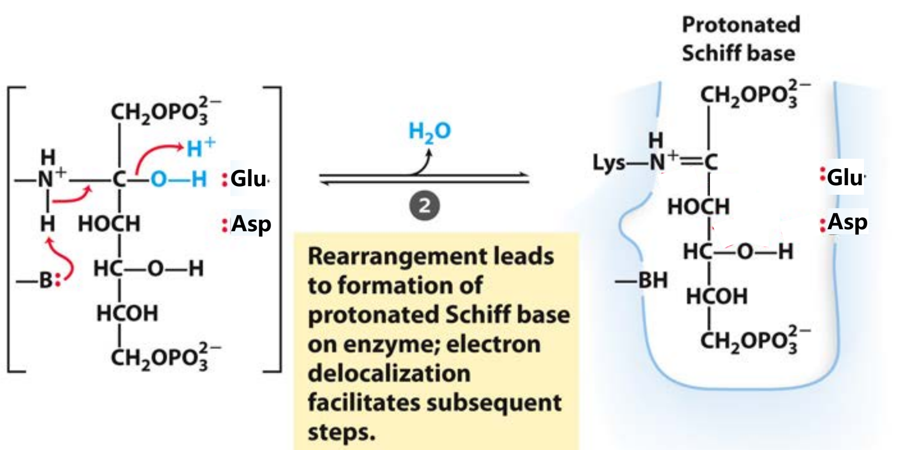


Glycolysis:
Fructose-1,6-bisphosphate aldolase

Mechanism

Step 1: making the Schiff Base

Loss of water produces Schiff base

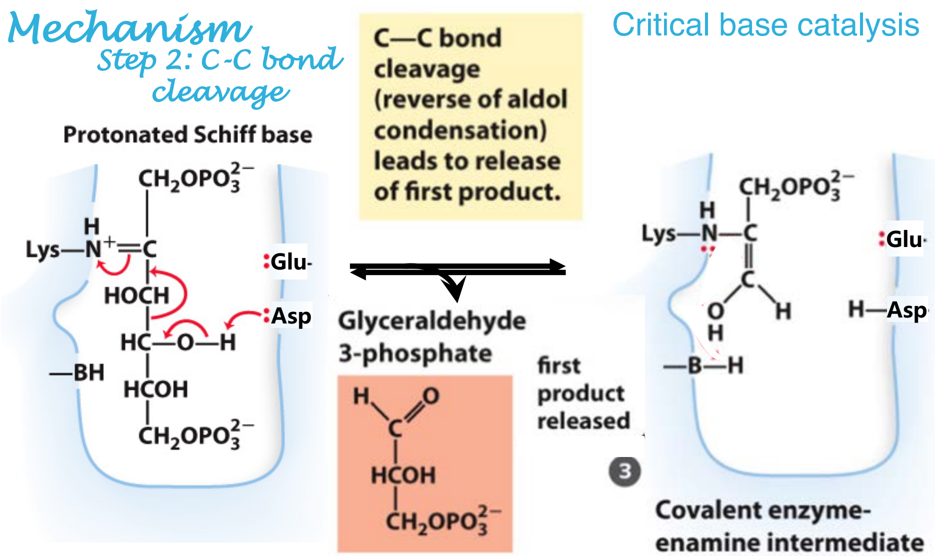


Glycolysis:
Fructose-1,6-bisphosphate aldolase

Mechanism

Step 2: C-C bond cleavage

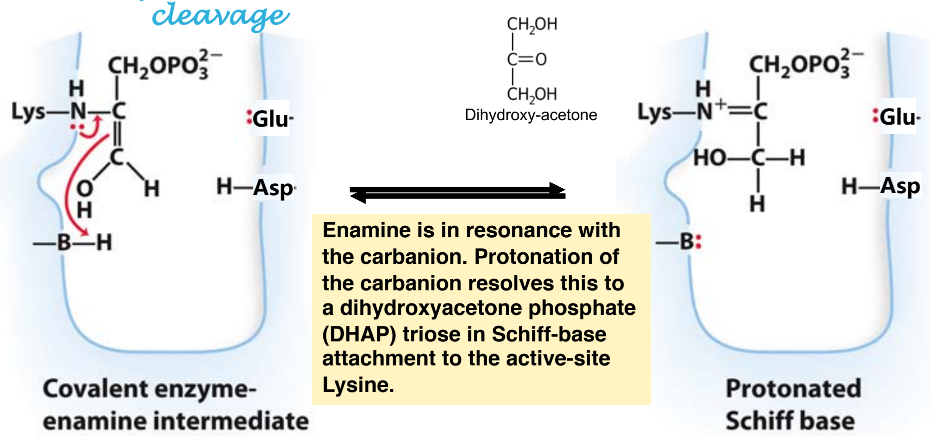
Critical base catalysis



Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism
Step 2: C-C bond cleavage

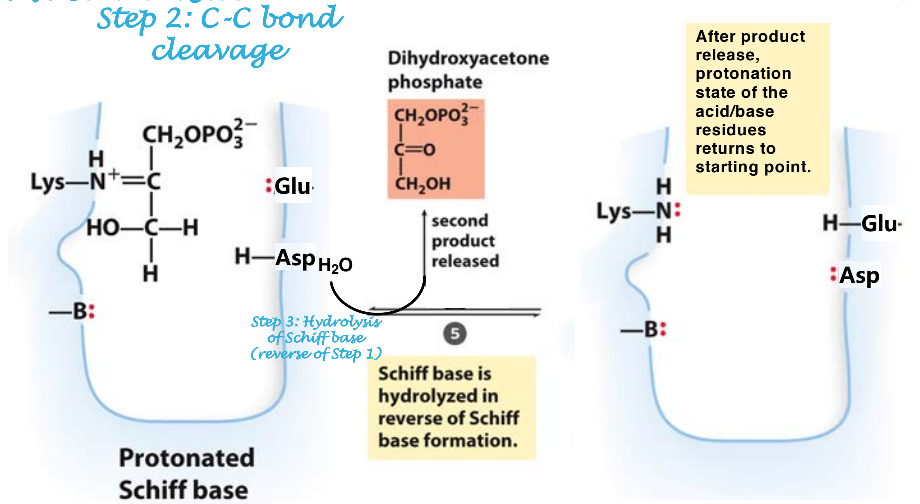
Acid catalysis protonates the carbanion



Glycolysis: Fructose-1,6-bisphosphate aldolase

Mechanism
Step 2: C-C bond cleavage

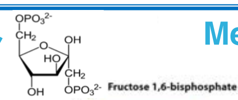
Product Release is the slow step

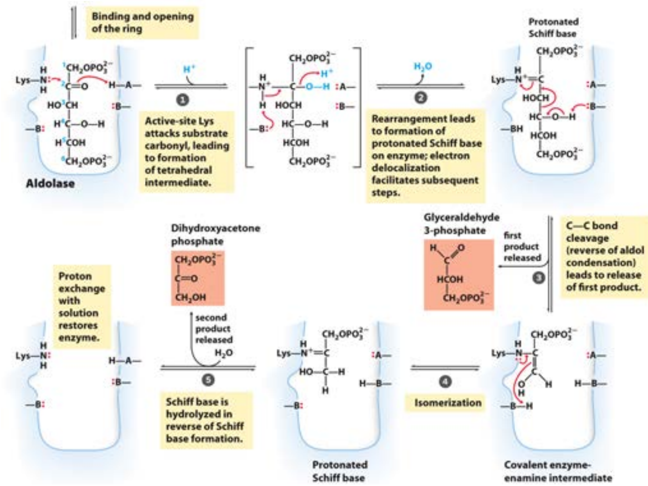


Glycolysis:
Fructose-1,6-bisphosphate aldolase

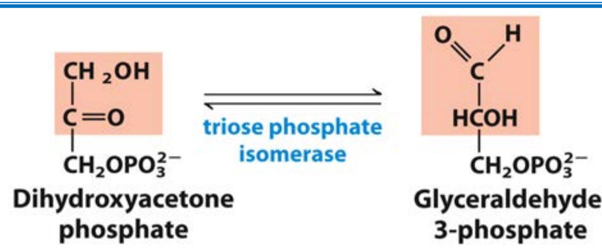
Mechanism Summary

Mechanism of Class I Aldolases

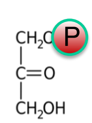


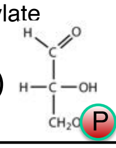


Glycolysis:
Triose-phosphate isomerase (TIM)

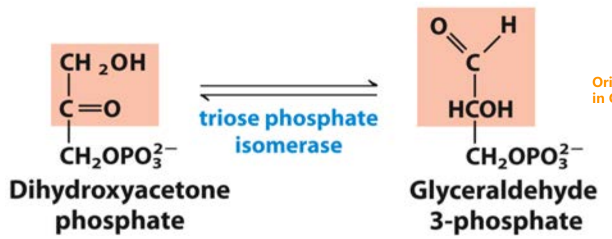


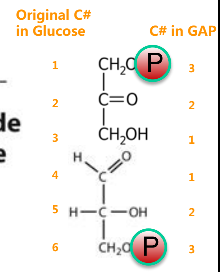
- Aldolase creates two triose phosphates:
  - glyceraldehyde-3-phosphate (GAP) – an aldose
  - dihydroxyacetone phosphate (DHAP) – a ketose
- TIM completes preparatory phase of glycolysis
- Rationale:
  - allows convergence to a single chemical pathway (the payoff in phase II)
  - Need to oxidize C1 of GAP (formerly C3 & C4 of glucose) to carboxylate
- TIM uses same enediol intermediate mechanism as PGI
- Thermodynamically unfavorable/reversible ($\Delta G^{o\prime}$ = +1.8 kcal/mol)
  - GAP concentration kept low to pull reaction forward





Glycolysis: Triose-phosphate isomerase (TIM)





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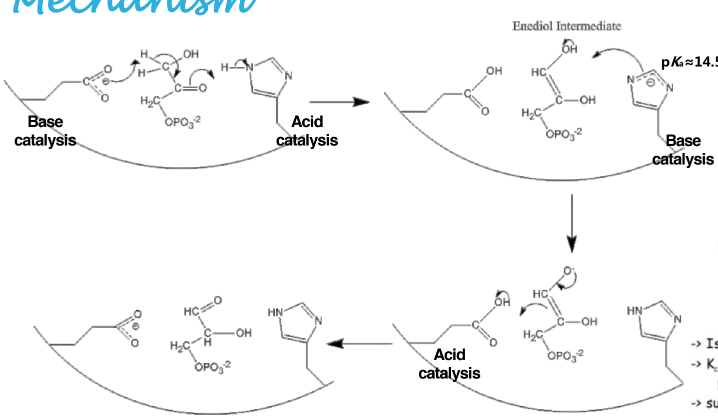
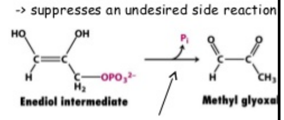
Glycolysis: Triose-phosphate isomerase

Mechanism



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



Reaction 100 times faster

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