

BI/CH 422/622

OUTLINE:

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)

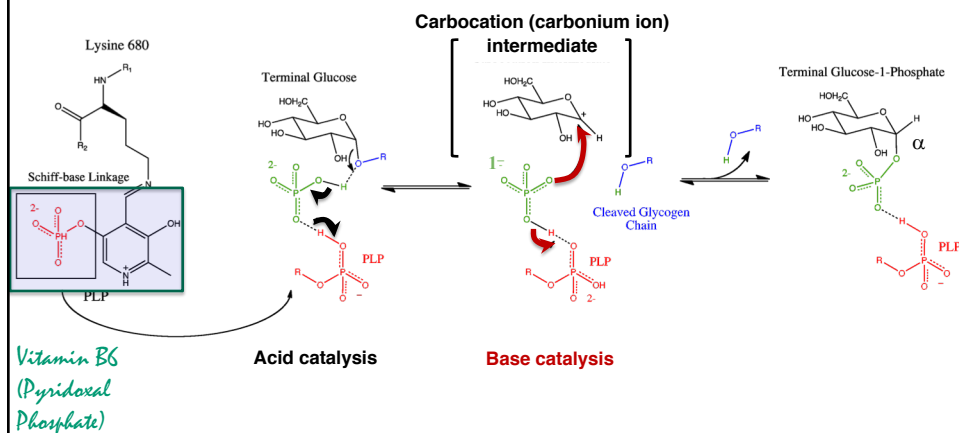
Aldolase

triose-phosphate isomerase (TPI)

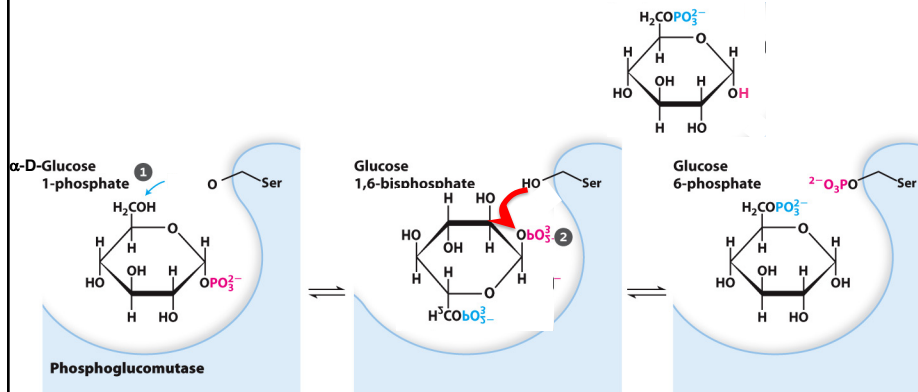
Phase II

Glycogenolysis is performed mainly by Glycogen Phosphorylase

Mechanism



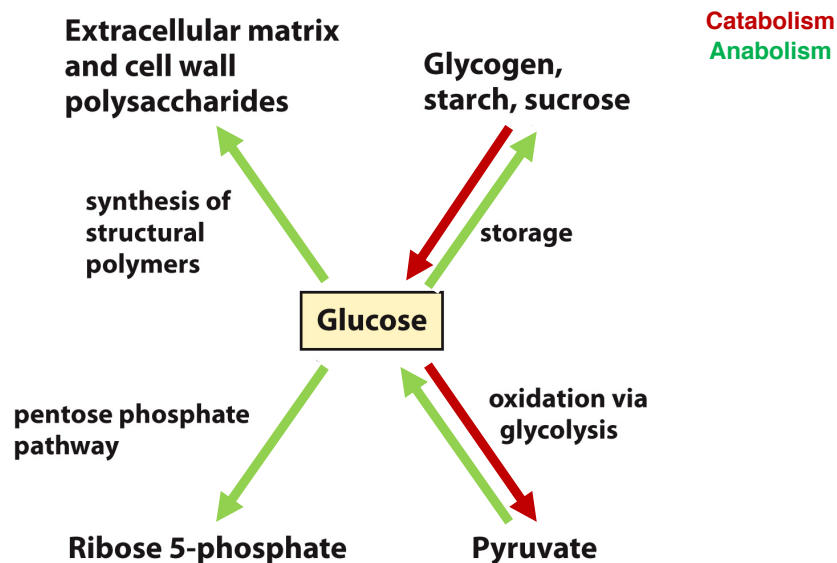
Glycogenolysis produces Glucose 1-Phosphate, which must be converted to Glucose 6-Phosphate by Phosphoglucomutase for Metabolism



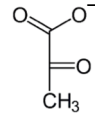
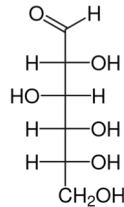
- Phosphoglucomutase must be phosphorylated at an active-site Ser.
- This is performed by a Glc 1,6-P₂ to initiate the active form.
- During turnover, the bisphosphate flips within a “vestibule”

Glucose Utilization

Four Major Fates of Glucose



Glycolysis

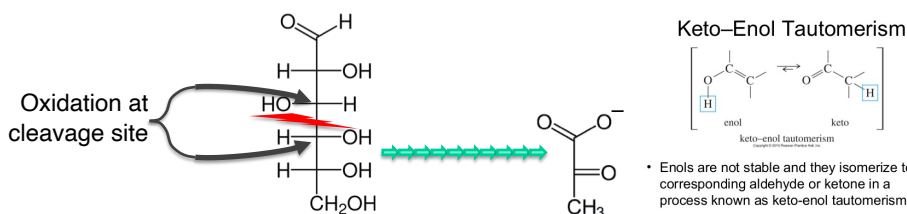


Learning outcomes for each pathway in course:

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 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

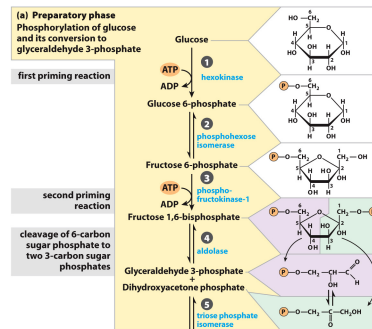


- Enols are not stable and they isomerize to the corresponding aldehyde or ketone in a process known as keto-enol tautomerism.

- 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.

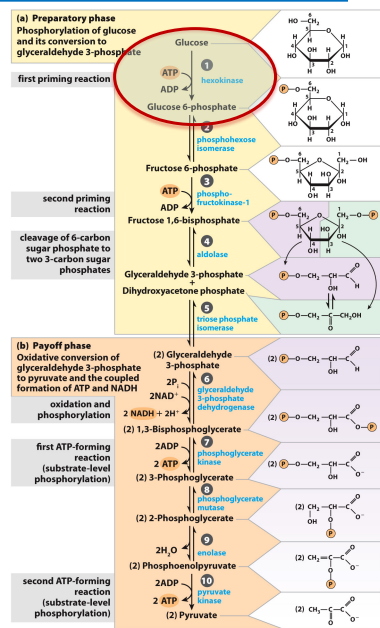
Glycolysis: Overview

- Two Phases/Four concepts
 - Preparatory phase
 - Phosphorylation **by** ATP
 - Cleavage

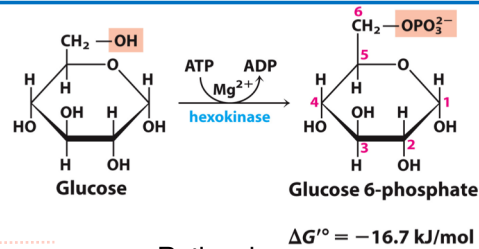


Glycolysis: Overview

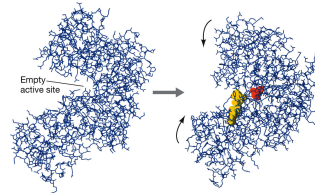
- Two Phases/Four concepts
 - Preparatory phase
 - Phosphorylation **by** ATP
 - Cleavage
 - Payoff
 - Oxidation
 - Phosphorylation **of** ATP



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).

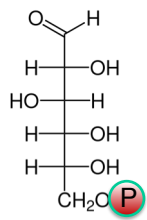
- Multiple isoforms of hexokinase exist in organisms (e.g., hexokinase I, II, III, and IV (glucokinase)).

- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphate of ATP.

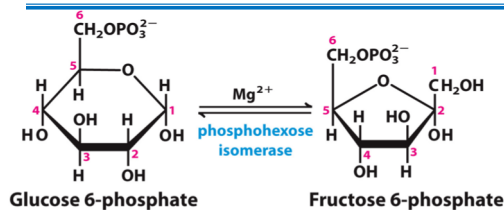
- ATP-bound Mg^{++} facilitates this process by shielding the negative charges on ATP.

- Highly thermodynamically favorable/irreversible

- $\Delta G^\circ = -4 \text{ kcal/mol}$
- regulated mainly by product inhibition



Glycolysis: Phosphoglucose isomerase (PGI)



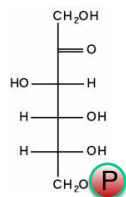
Setting up Second Phosphorylation

• Rationale

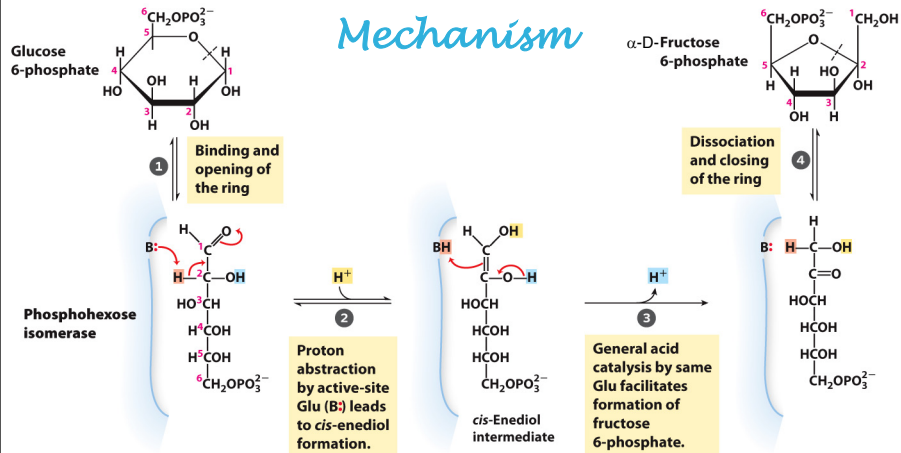
- Convert C1 from aldehyde to hydroxyl which is easier to phosphorylate

• Slightly thermodynamically unfavorable/reversible

- $\Delta G^\circ = +0.4 \text{ kcal/mol}$
- product concentration kept low by pairing with favorable next step to drive reaction forward



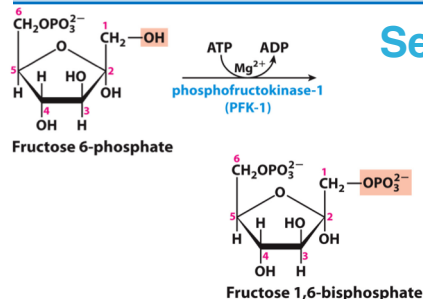
Glycolysis: Phosphoglucose isomerase (PGI)



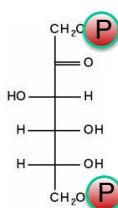
- The isomerization is catalyzed by the active-site glutamate via general acid/base catalysis.
- An **aldose (glucose)** can isomerize into a **ketose (fructose)** via an enediol intermediate.

Glycolysis: Phosphofructokinase-1

Second Phosphorylation

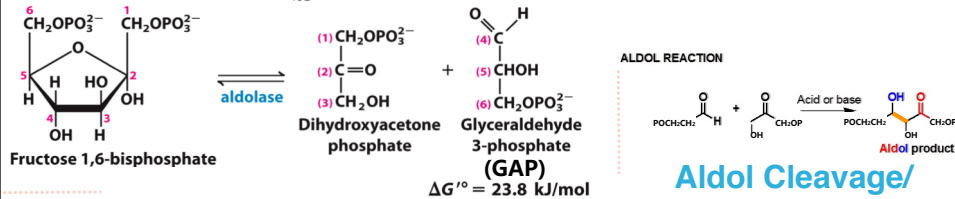


- Rationale** $\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$
 - Symmetrical phosphorylation to set up cleavage to 3-carbon sugar
- First committed step** of glycolysis
 - fructose 1,6-bisphosphate (Fru 1,6-P₂) is committed to become pyruvate and yield energy
- This process uses the energy of ATP.**
- Highly thermodynamically favorable/irreversible ($\Delta G'^{\circ} = -3.4 \text{ kcal/mol}$)
- Phosphofructokinase-1 is highly regulated.
 - by ATP, fructose 2,6-bisphosphate, and other metabolites
 - do not burn glucose if there is plenty of ATP

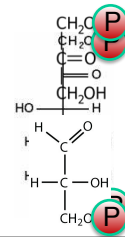


Glycolysis:

Fructose-1,6-bisphosphate Aldolase



- **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 ($\Delta G'^{\circ} = +5.7 \text{ kcal/mol}$)
 - product (GAP) concentration kept low to pull reaction forward.



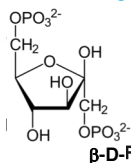
Glycolysis:

Fructose-1,6-bisphosphate aldolase

Mechanism

Covalent Catalysis (Class I enzymes)

Step 1: making the Schiff Base



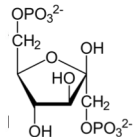
Glycolysis:

Fructose-1,6-bisphosphate aldolase

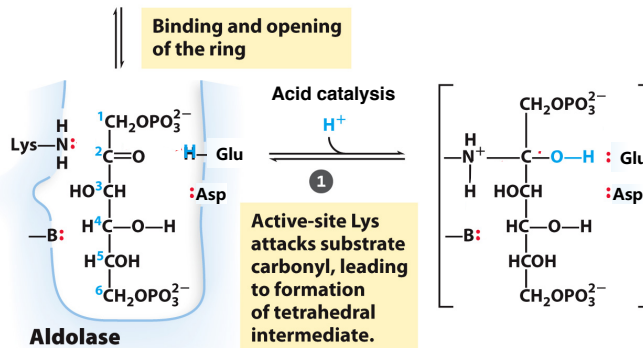
Mechanism

Covalent Catalysis (Class I enzymes)

Step 1: making the Schiff Base



β -D-Fructose 1,6-bisphosphate



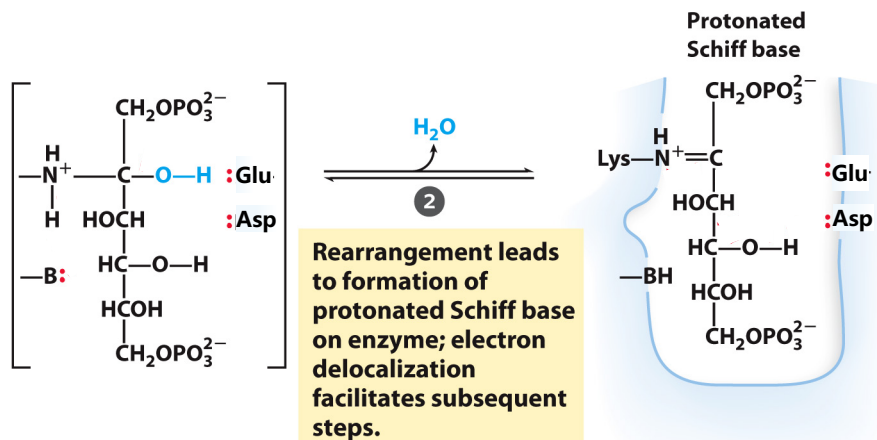
Glycolysis:

Fructose-1,6-bisphosphate aldolase

Mechanism

Loss of water produces Schiff base

Step 1: making the Schiff Base



Glycolysis:

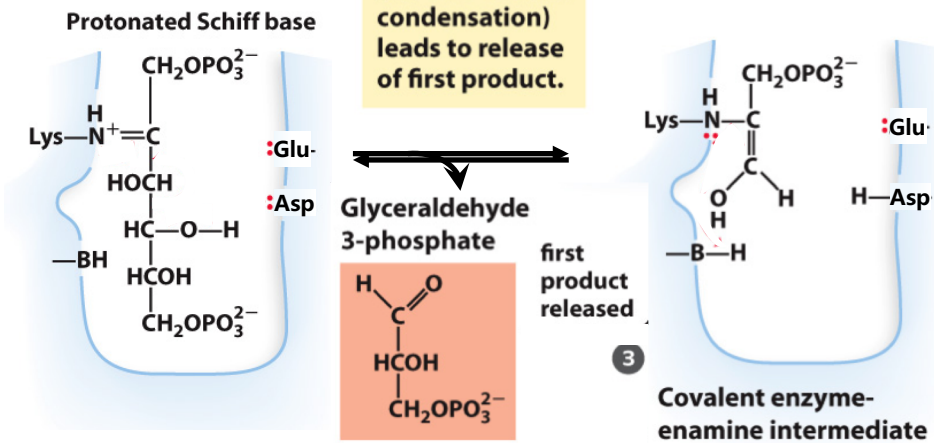
Fructose-1,6-bisphosphate aldolase

Mechanism

Step 2: C-C bond cleavage

C—C bond cleavage (reverse of aldol condensation) leads to release of first product.

Critical base catalysis



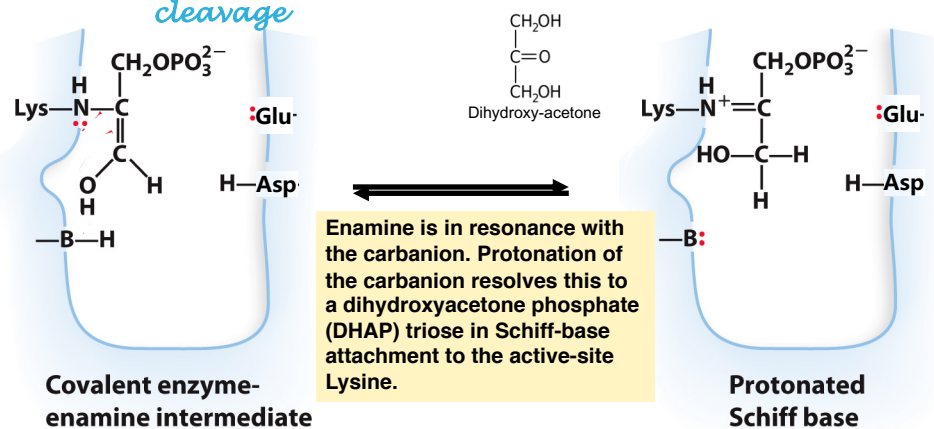
Glycolysis:

Fructose-1,6-bisphosphate aldolase

Mechanism

Step 2: C-C bond cleavage

Acid catalysis protonates the carbanion



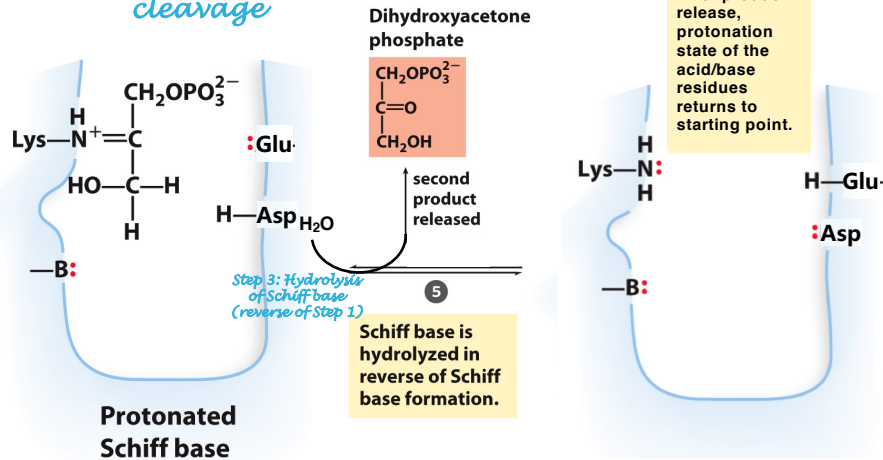
Glycolysis:

Fructose-1,6-bisphosphate aldolase

Mechanism

Step 2: C-C bond cleavage

Product Release is the slow step

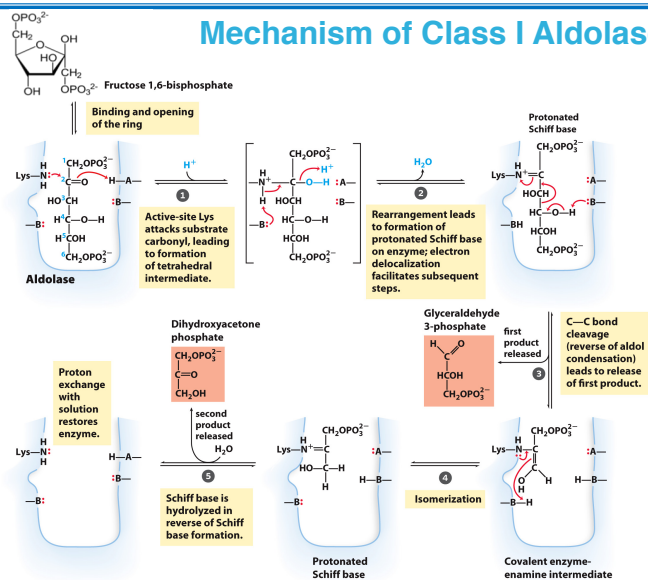


Glycolysis:

Fructose-1,6-bisphosphate aldolase

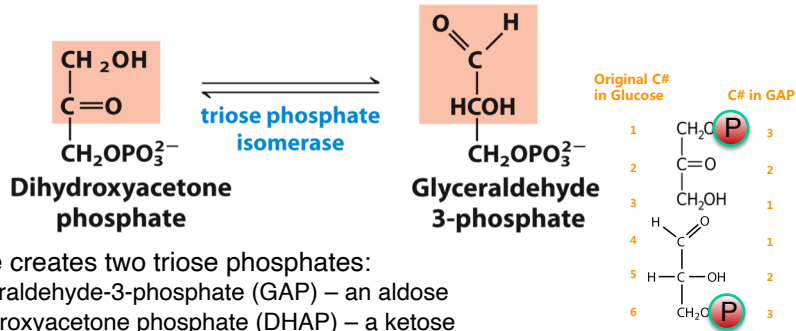
Mechanism Summary

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 ($\Delta G^{\circ'} = +1.8 \text{ kcal/mol}$)
 - GAP concentration kept low to pull reaction forward