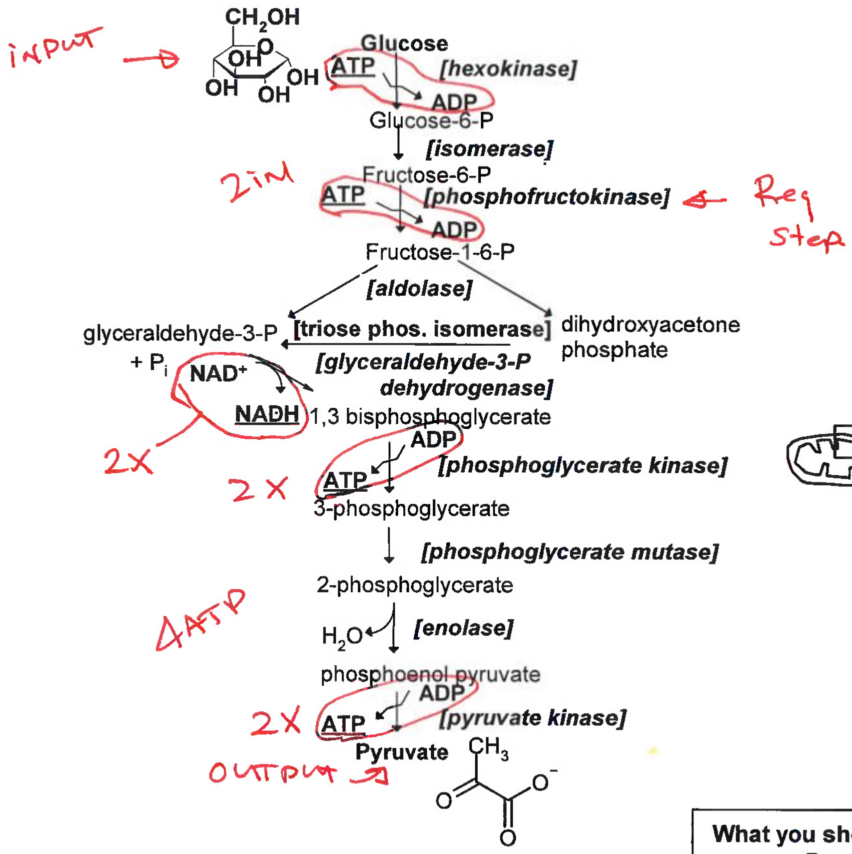
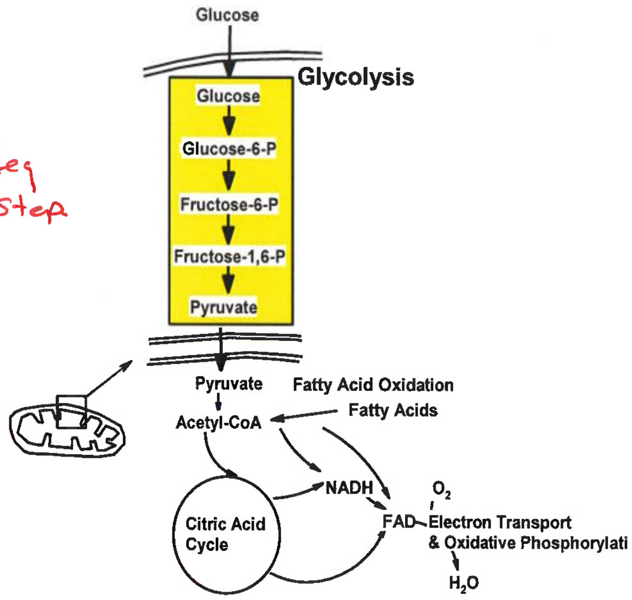
Lecture 29: Glycolysis

Location: cytosol

1. **Input:** glucose
2. **Output:** pyruvate
3. **Net energy prod.:** 2 ATP, 2 NADH

- **Key controlling step:** Phosphofructose kinase (PFK)
- **Kinase (X + ATP → X-P + ADP)** Substrate-level phosphorylation
- **Dehydrogenase (Redox)**

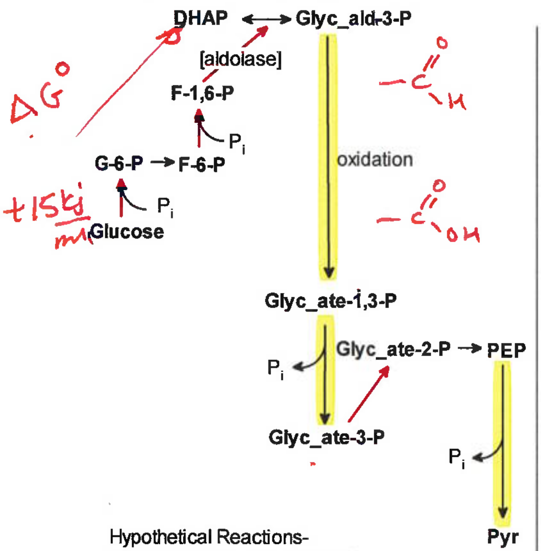




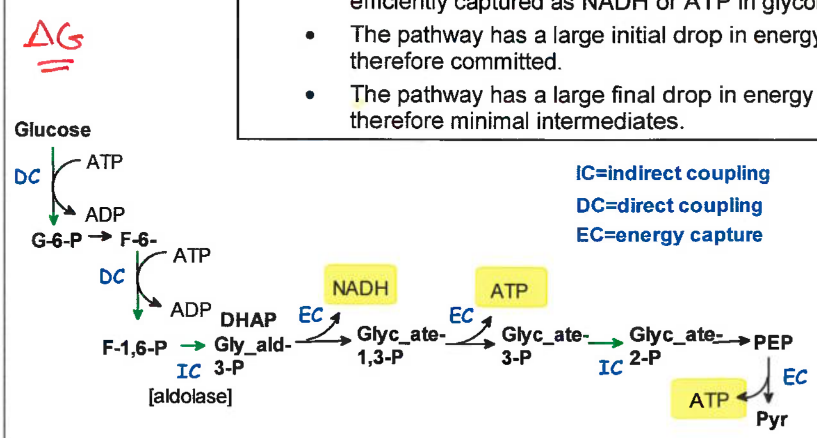
What you should note:

- Reactions on the left with unfavorable energy changes (red arrows) become favorable in glycolysis due to coupling, $\Delta G<0$ for each step.
- The energy released (yellow highlight) from oxidation and dephosphorylations on the left is efficiently captured as NADH or ATP in glycolysis.
- The pathway has a large initial drop in energy – therefore committed.
- The pathway has a large final drop in energy – therefore minimal intermediates.

Coupling and Energy Capture



Hypothetical Reactions- no coupling or energy capture



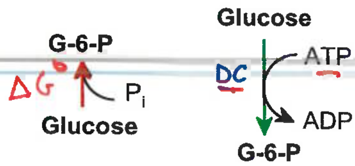
IC=indirect coupling
DC=direct coupling
EC=energy capture

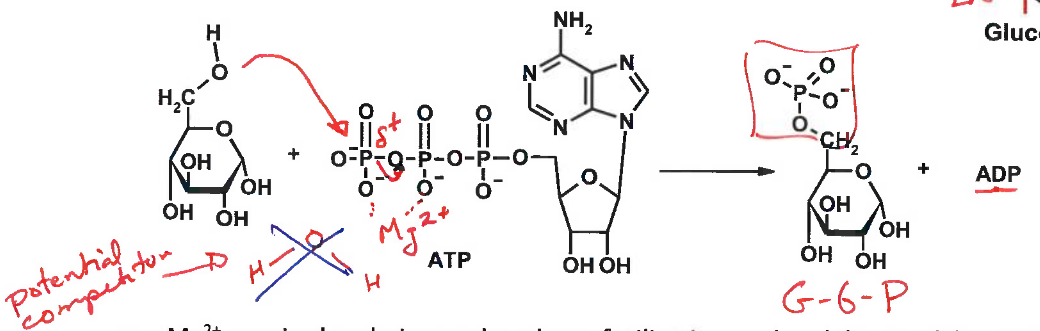
Actual Gibbs energy change during glycolysis - showing coupling and energy capture

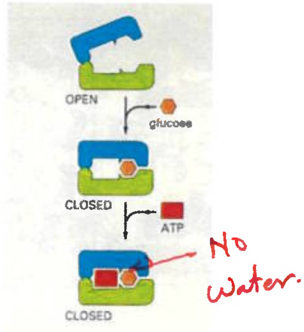
i) ΔG<0 each step
ii) Energy is captured.

Step 1. Hexose kinase Reaction: Glucose + ATP → Glucose-6-P + ADP

Group transfer reaction: Phosphate is transferred from ATP to glucose.







- Mg^{2+} required, polarizes γ phosphate, facilitating nucleophilic attack by -OH
- Favorable hydrolysis of ATP **directly coupled** to phosphorylation of glucose.
- Transfer of the phosphate group on ATP to water is negligible because water is excluded from the active site by a conformational change of the enzyme.
- Binding of the substrates causes a large change in the structure of the enzyme – this is referred to as **an induced fit.**

Glucose accumulation in cell – An example of indirect coupling:

Hexose kinase keeps the concentration of glucose inside the cell below its equilibrium value, making the flow of glucose into the cell spontaneous (ΔG<0).

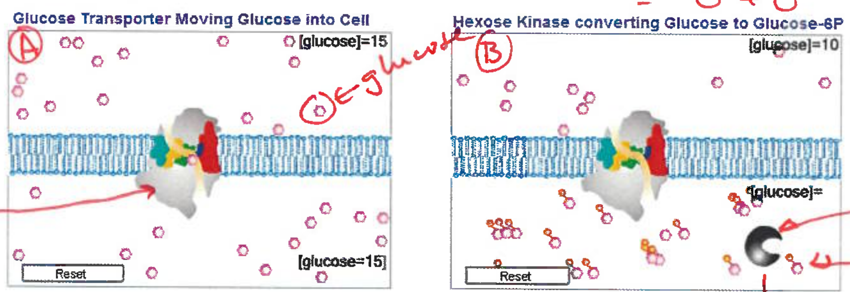
$$\Delta G = \Delta G^o + RT\ln\frac{[G]_{IN}}{[G]_{OUT}} = (\mu^o_{[IN]} - \mu^o_{[OUT]}) + RT\ln\frac{[G]_{IN}}{[G]_{OUT}}$$

Example: Calculate the sign of the Gibbs Free energy for the transport of glucose across the cell membrane in the presence (right) of hexose kinase activity to show that the flow is spontaneous into the cell.

OUT → IN

(A): $\Delta G = \Delta G^o + RT\ln\frac{15}{15} = 0 + 0$

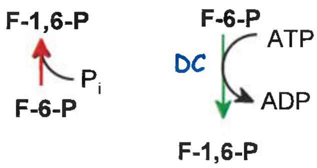
(B): $\Delta G = 0 + RT\ln\frac{1}{10}$, $\Delta G < 0$ (energy avail)



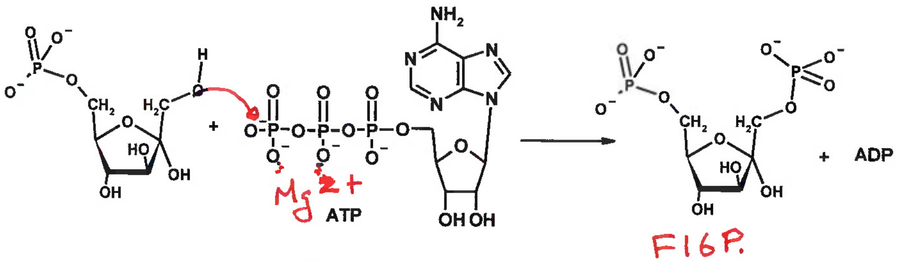
Step 2 Isomerase: Glucose-6-P to fructose-6-P (aldose to ketose, ΔG≈0)

Step 3 Phosphofructokinase (PFK):

Fructose -6-P + ATP→ Frucose-1,6-bis phosphate (F16P)

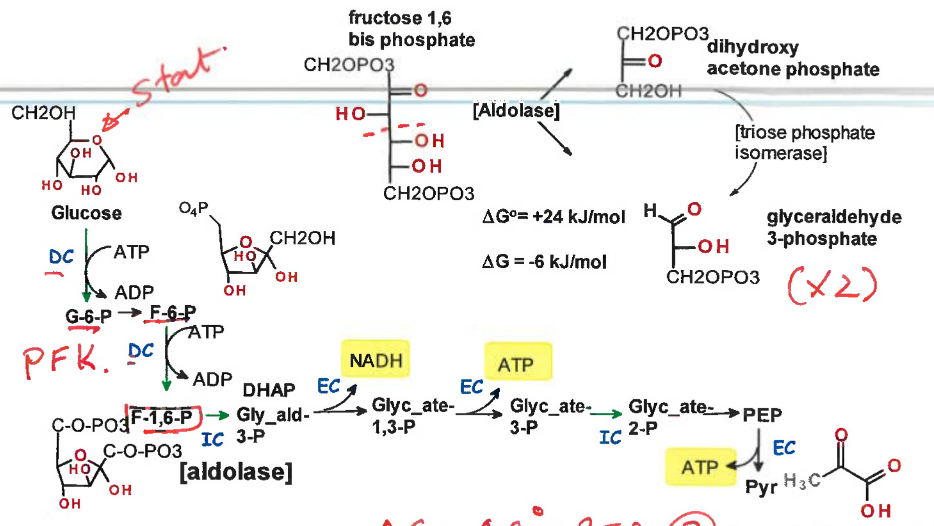


- Favorable hydrolysis of ATP **directly coupled** to phosphorylation of fructose-6-P. Phosphate transferred from ATP to F-6-P, generating F-1,6-P.
- Regulated by a large number of compounds (ATP, ADP, AMP, F26P, citrate)



direct coupling

Step 4: Aldolase Reaction (Indirect Coupling). The large free energy change in the last step of glycolysis (PEP to pyruvate) keeps the concentration of all previous intermediates low, allowing the aldolase reaction to proceed spontaneously.



Step 5: Triose phosphate Isomerase (ketose to aldose (ΔG≈0)).

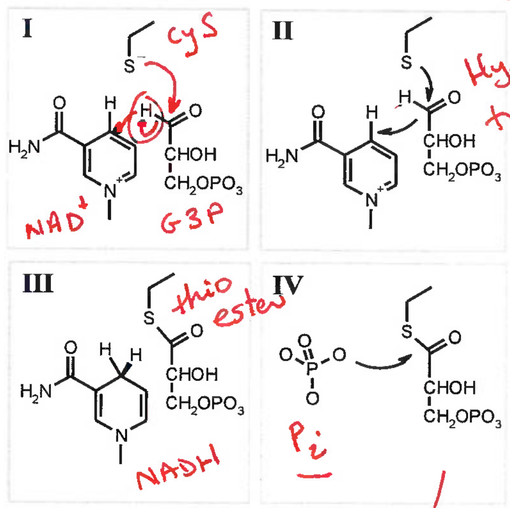
$\Delta G = \Delta G^\circ + RT\ln\frac{B}{A}$ ← [B] << [B]eq; +24; large & neg

Step 6: Glyceraldehyde-3-P Dehydrogenase (Redox)

The oxidation of G3P reaction proceeds in two steps. The first is the oxidation of the aldehyde to the carboxylic acid using NAD as the electron acceptor. This results in the formation of a covalent enzyme intermediate. The second step is the phosphorylation of the thiol ester by inorganic phosphate, generating phosphor-carboxylic acid:

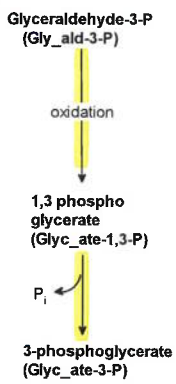
Energy released ∴ likely oxidation

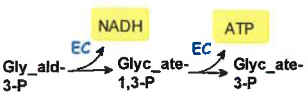
I. ES complex, active site Cys is deprotonated.
II. Thio group is a nucleophil, attacks aldehyde, proton is transferred to NAD^+ as a hydride (H^-), net transfer of 2 electrons and one proton.
III. NADH is released. 3-P-G remains bound to the enzyme as a stable thioester intermediate.
IV. Attach of P_i, producing 1,3-bisphosphoglycerate

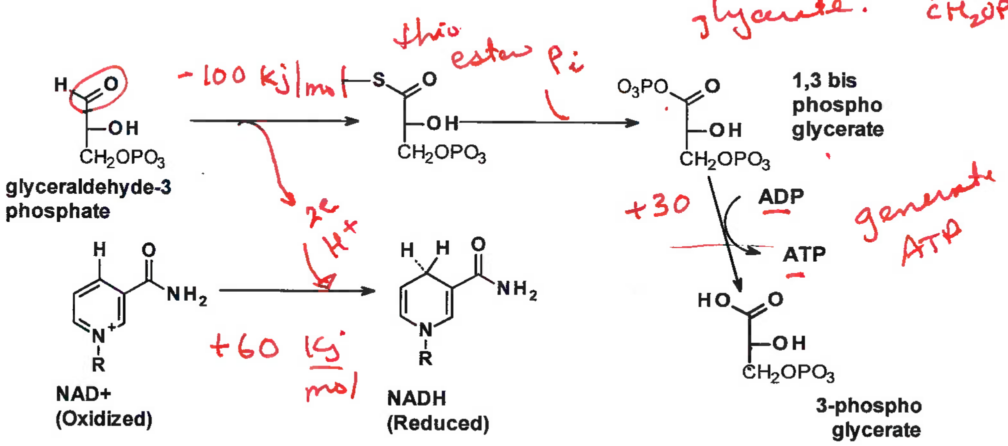


Step 7: Phosphoglycerate kinase: The added phosphate is transferred to ADP, forming ATP (***substrate*** level phosphorylation)

No capture

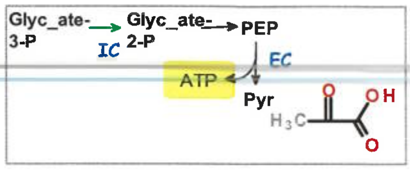




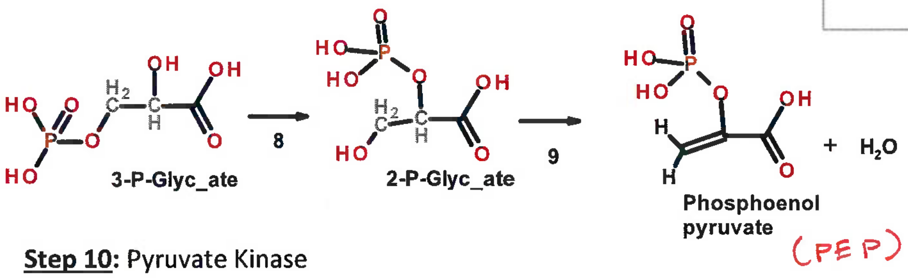


Net storage ~ 90%
~10% waste (heat)

Step 8: Phosphoglycerol Mutase – move phosphate from C3 to C2:

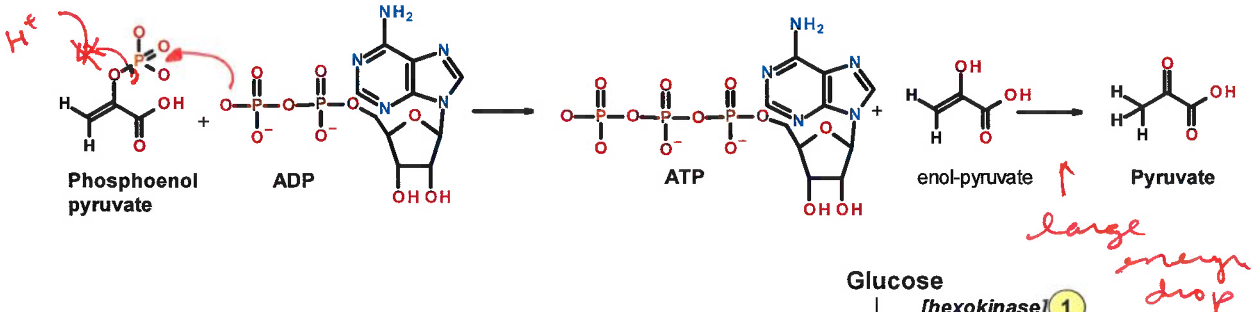


Step 9: Dehydration to create phosphoenolpyruvate



Step 10: Pyruvate Kinase

- Substrate level phosphorylation
- Indirect coupling.



Summary of Glycolysis:

Cellular location: cytosol.

Input: glucose

Output: Pyruvate

Energy generating step: 6 only oxidation

Energy capture steps: 7, 10, 6

Examples of direct coupling: 1, 3
(ATP used)

Reaction that provide energy via indirect

coupling: 10

Key Reg. step: 3 (PFK)