Lecture 29: Glycolysis

Key Terms:
1. Location: cytosol
2. Input: glucose
3. Output: pyruvate
4. Key energy prod.: 2 ATP, 2 NADH
5. Key controlling step: PFK
   - Kinase (X + ATP \rightarrow X-P + ADP)
   - Substrate-level phosphorylation
   - Dehydrogenase (Redox)

Glycolysis

- Glucose \rightarrow\text{Glyceraldehyde-3-P} \rightarrow\text{Pyruvate}
- NADH
- ATP

Catabolism via substrate-level phosphorylation

- Glycolysis
- TCA cycle
- Electron transport chain

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 + ATP in glycolysis.
- The pathway has a large initial drop in energy - therefore committed.
- The pathway has a large final crop in energy - therefore minimal intermediates.

To the mitochondria (TCA)

Coupling and Energy Capture

- Glyceraldehyde-3-P \rightarrow Glycine \rightarrow PEP
- NADH
- ATP

Hypothetical Reactions - no coupling or energy capture

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

1) Keeps intermediates in low conc.
2) Provides energy to unfavored steps via indirect coupling
Step 1. Hexose kinase Reaction: Glucose + ATP → Glucose-6-P + ADP

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

- 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 to produce the catalytically competent conformation — 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).

Worked 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.

\[ \Delta G = \Delta G^\circ + RT \ln \left( \frac{[G]_{\text{out}}}{[G]_{\text{in}}} \right) + RT \ln \left( \frac{[G]_{\text{in}}}{[G]_{\text{out}}} \right) \]

\[ \Delta G = -RT \ln \frac{15}{10} \]

\[ \Delta G = 0 + RT \ln \frac{15}{10} \]

\[ \Delta G \approx 0 \]

Gout ≈ Gin

Step 2 Isomerase: Glucose-6-P to fructose-6-P (ΔG=0)

Step 3 Phosphofructokinase (PFK):
Fructose-6-P + ATP → Fructose-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)
**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: Isomerase** (ketone to aldose ($\Delta G=0$)).

**Step 6: Glycerophosphate-3-P Dehydrogenase**

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 carboxylic acid by inorganic phosphate.

The Reaction Steps are (see diagram to right)

I. ES complex, active site Cys is deprotonated.
II. Thio group is a nucleophile, attacks aldehyde, proton transferred to NAD$^+$ as a hydride (H$^-$), net transfer of 2 electrons and one proton.
III. NADH released. 3-P-G remains bound to the enzyme as a stable thioester intermediate.
IV. Attack of P$_1$ producing 1,3-bisphosphoglycerate

**Step 7: Phosphoglycerate kinase**: The added phosphate is transferred to ADP, forming ATP.

Balancing conversion of glycerophosphate-3-P to thioester, demonstrating that this is a redox reaction.
Step 8: Phosphoglycerol Mutase – move phosphate from C3 to C2:

Step 9: Dehydration to create phosphoenolpyruvate

Step 10: Pyruvate Kinase

Overall \( \Delta G = -60 \text{ kJ/mol} \)

\[ \Delta G = +30 \text{ kJ/mol} \]

Net change \( \approx -30 \text{ kJ/mol} \)

Hypothetical: What happens if the phosphate transfer from C3 to C2 didn’t happen?

No large drop in energy because there is no possibility of delocalization for this product.