Lecture 29: Glycolysis

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

![Glycolysis diagram]

**What you should note:**
- Reactions on the left with unfavorable energy changes (red arrows) become favorable in glycolysis due to coupling.
- The energy released (yellow highlight) from oxidation and dephosphorylations on the left is efficiently captured as NADH or ATP in glycolysis.

**Actual Gibbs energy change during glycolysis - showing coupling and energy capture**
Step 1. Hexose kinase Reaction: direct coupling

Glucose + ATP $\rightarrow$ Glucose-6-P + ADP

Group transfer reaction: Phosphoryl group is transferred from ATP to glucose.

- Favorable hydrolysis of ATP is 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 both substrates causes a large change in the structure of the enzyme to produce the catalytically competent conformation – 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 ($\Delta G<0$).

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.

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

Step 3 Phosphofructokinase (PFK):

Fructose-6-P + ATP $\rightarrow$ Fructose-1,6-bisphosphate (F16P)

- Favorable hydrolysis of ATP is directly coupled to phosphorylation of fructose-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.

\[ \Delta G = \Delta G^0 + RT \ln \left( \frac{[B]}{[S]} \right) \]

Step 6: Glyceraldehyde-3-P Dehydrogenase- energy capture

The oxidation of G3P reaction proceeds in two steps. The first is the oxidation of the aldehyde to a carboxylic acid using NAD\(^+\) as the electron acceptor. This results in the formation of a covalent enzyme-substrate intermediate. The second step is the phosphorylation of the intermediate by inorganic phosphate.

The Reaction Steps are (see diagram to right)

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

Step 7: Phosphoglycerate kinase: energy capture

The added phosphate is transferred to ADP, forming ATP.
Pyruvate will next enter into the TCA cycle (aka Krebs cycle, aka citric acid cycle) ...unless oxygen is limiting....

Under anaerobic (oxygen limited) conditions, which occurs in the muscle tissue during vigorous activity, the NADH produced in glycolysis cannot be reoxidized to NAD⁺ by electron transport because there is insufficient oxygen to accept electrons. Under these conditions, the cell runs out of NAD⁺ and glycolysis will halt and the cell can no longer produce ATP.

The levels of NAD⁺ can be restored by using pyruvate as the electron acceptor: in mammals, lactate is the product of this reaction. The reduction of pyruvate will oxidize NADH to NAD⁺, allowing glycolysis to resume.

Fermentation in yeast: because NAD⁺ is needed for the Glyceraldehyde-3-phosphate Dehydrogenase reaction, NADH must be oxidized. Pyruvate is converted to acetaldehyde, releasing CO₂. Subsequently, acetaldehyde is reduced to ethanol and NADH oxidized to NAD⁺.