‘Metabolic flux’ describes the rate of flow of intermediates through a metabolic pathway
ΔG, determined by measuring [metabolites], reveals the rate-limiting steps of a pathway

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
<th>Δ$G^\circ'$ (kJ · mol$^{-1}$)</th>
<th>ΔG (kJ · mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexokinase</td>
<td>−20.9</td>
<td>−27.2</td>
</tr>
<tr>
<td>2</td>
<td>PGI</td>
<td>+2.2</td>
<td>−1.4</td>
</tr>
<tr>
<td>3</td>
<td>PFK</td>
<td>−17.2</td>
<td>−25.9</td>
</tr>
<tr>
<td>4</td>
<td>Aldolase</td>
<td>+22.8</td>
<td>−5.9</td>
</tr>
<tr>
<td>5</td>
<td>TIM</td>
<td>+7.9</td>
<td>~0</td>
</tr>
<tr>
<td>6 + 7</td>
<td>GAPDH + PGK</td>
<td>−16.7</td>
<td>−1.1</td>
</tr>
<tr>
<td>8</td>
<td>PGM</td>
<td>+4.7</td>
<td>−0.6</td>
</tr>
<tr>
<td>9</td>
<td>Enolase</td>
<td>−3.2</td>
<td>−2.4</td>
</tr>
<tr>
<td>10</td>
<td>PK</td>
<td>−23.0</td>
<td>−13.9</td>
</tr>
</tbody>
</table>

*Calculated from data in Newsholme, E.A. and Start, C., *Regulation in Metabolism*, p. 97, Wiley (1973).*
PFK is the major regulatory enzyme of glycolysis in muscle \((F6P + ATP \rightarrow F-1,6-BP + ADP)\)

- Homotetramer (here, 2 subunits are shown)
- Each subunit has catalytic and regulatory sites
- Positive effectors:
  - F6P (substrate)
  - ADP, AMP
  - F-2,6-BP
- Negative effectors:
  - ATP
  - citrate
ATP, ADP, or AMP can bind at the same regulatory site and influence PFK activity.
The R-state of PFK promotes binding of F6P; the T-state has low affinity for F6P

In T-state (blue), charge repulsion between Glu & F6P disfavors binding
Shift to R-state (pink) creates salt bridge between Arg & F6P, promoting binding
Positive effector; stabilizes R-state
Negative effector (non-biological); stabilizes T-state
Gluconeogenesis is a pathway in which glucose is synthesized from 2-4C precursors

- Many organisms and many cell types require a constant supply of glucose (ex: neurons, red blood cells)
- In humans, glucose can be synthesized from pyruvate (or lactate, or oxaloacetate, or certain amino acids) through this pathway (mainly occurring in the liver)
- Uses many of the same enzymes as glycolysis – those that catalyze reversible reactions
- For irreversible steps of glycolysis, uses other reactions (and other enzymes)
- Opposite regulation vs. glycolysis
Phosphatases remove the phosphoryl groups added by hexokinase and PFK
Two energy-requiring steps reverse the action of pyruvate kinase.
Pyruvate carboxylase uses the energy of ATP hydrolysis to drive a carboxylation reaction:

\[
\text{Pyruvate} + \text{HCO}_3^- + \text{ATP} \rightarrow \text{Oxaloacetate} + \text{ADP} + P_i
\]
PEPCK couples decarboxylation and NTP hydrolysis to PEP formation

\[
\text{Oxaloacetate} \quad \xrightarrow{\text{Phosphoenolpyruvate carboxykinase (PEPCK)}} \quad \text{Phosphoenolpyruvate (PEP)}
\]

\[2 \text{GTP} \rightarrow 2 \text{GDP} + \text{CO}_2\]
From glycolysis, pyruvate has multiple options for further metabolism.
Different color in muscle can reflect different levels of aerobic vs. anaerobic metabolism

- **slow-twitch muscle fiber**: Lots of heme-containing mitochondria, used in aerobic metabolism.
- **fast-twitch muscle fiber**: Fewer mitochondria; heavy reliance on anaerobic metabolism.
In homolactic fermentation, lactate DH reduces pyruvate to regenerate NAD$^+$

\[
\Delta G'^\circ = - 25.1 \text{ kJ/mol}
\]
A hydride from NADH is transferred directly to pyruvate’s carbonyl carbon.
Yeast carry out alcoholic (ethanolic) fermentation, producing $\text{CO}_2$ and ethanol.
Ethanolic fermentation converts pyruvate to ethanol in two steps.

Pyruvate $\xrightarrow{\text{pyruvate decarboxylase}}$ Acetaldehyde $\xrightarrow{\text{alcohol dehydrogenase}}$ Ethanol

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Pyruvate decarboxylase catalyzes the decarboxylation of pyruvate to acetaldehyde
Decarboxylation does not happen without catalysis

unstable carbanion intermediate
The cofactor TPP functions as an electron sink to stabilize carbanion intermediates.
TPP catalyzes the decarboxylation of α-keto acids
Resonance-stabilized carbanion
Hydroxyethylthiamine pyrophosphate

3 Protonation of carbanion

Resonance-stabilized carbanion
Pyruvate + Acetaldehyde → Hydroxyethylthiamine pyrophosphate

TPP (ylid form) → TPP (pyrophosphate form)

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Alcohol DH regenerates NAD+ through the reduction of acetaldehyde to ethanol
A hydride from NADH is transferred directly to acetaldehyde’s carbonyl carbon.
From glycolysis, pyruvate has multiple options for further metabolism.

**Anaerobic (fast, low energy yield)**

**Aerobic (slow, good energy yield)**

- Glucose → Pyruvate → NADH
- Pyruvate → Lactate (NADH) → CO₂, H₂O
- Pyruvate → Ethanol (NADH) → CO₂, NAD⁺
Oxidation of glucose releases more free energy & yields more ATP than fermentation

**Fermentation:** 2 ATP (per glucose)
- glucose → 2 lactate + 2H⁺ \(\Delta G' = -196 \text{ kJ/mol}\)
- glucose → 2CO₂ + 2 ethanol \(\Delta G'' = -235 \text{ kJ/mol}\)

**Oxidation:** up to 32 ATP (per glucose)
- glucose + 6O₂ → 6CO₂ + 6H₂O \(\Delta G'' = -2850 \text{ kJ/mol}\)