Metabolism
Metabolism

cytosol

mitochondria

ATP synthase
Basic concepts in metabolism

1 Steady state VS equilibrium

<table>
<thead>
<tr>
<th></th>
<th>system</th>
<th>$\Delta G$</th>
<th>In living organism</th>
<th>Common feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state</td>
<td>open</td>
<td>$\neq 0$</td>
<td>✔</td>
<td>Concentration of certain intermediates reaches constant</td>
</tr>
<tr>
<td>equilibrium</td>
<td>closed</td>
<td>$= 0$</td>
<td>✗</td>
<td></td>
</tr>
</tbody>
</table>

2 Reaction spontaneity *(required by steady state)*

1) Determination: $\Delta G < 0$ or $Q < K_{eq}$ ($K_{eq}$ is constant); $\Delta E > 0$ (for redox reaction)

For a general rxn:

$$aA + bB \rightarrow cC + dD$$

$$\Delta G = \Delta G_0' + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

$$Q = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

For redox rxn:

$$\Delta E = \Delta E^0 - \frac{RT}{nF} \ln \frac{[C]^c[D]^d}{[A]^a[B]^b};$$ (if $aA + bB \rightarrow cD + dD$ is a redox rxn)

$$\Delta G = -nF\Delta E$$ (n is the number of electrons transferred in the reaction);

$$\Delta E = E_{e^- \text{ acceptor}} - E_{e^- \text{ donor}}$$

($\Delta E$ is irrelevant to the number of electrons transferred in the reaction);
Basic concepts in metabolism

2 Reaction spontaneity \( (\Delta G = \Delta G^0 + RT \ln \frac{[C][D]^f}{[A][B]^t}) \)

2) how to achieve reaction spontaneity
   a. coupled to exergoic reaction (usage of energy currency):
      ATP hydrolysis (phosphoanhydride bonds); thioester bond breaking; redox rxn
      e.g.

      ![ATP hydrolysis reaction diagram]

      Try to find other examples in the reactions you’ve learned.

   b. preceded by a exergonic reaction that produces its substrate;
      followed by a exergonic reaction that consumes its product.
      e.g.

      ![Exergonic reaction diagram]
Glycolysis & Citrate Cycle

➔ Print the two charts in your mind (including enzymes)

  Energy consumption and production steps; high energy intermediate production steps; carbon tracing; regulated steps; CO₂ production steps

➔ Key steps of enzyme mechanisms

➔ Function of cofactors

➔ Regulation
Glycolysis & Citrate Cycle

**Key steps of enzyme mechanism**

### Glycolysis
1. Hexokinase
2. Phosphoglucone isomerase
3. Phosphofructokinase
4. Aldolase
5. Triose phosphate isomerase
6. Glyceraldehyde 3-phosphate dehydrogenase
7. Phosphoglycerate kinase
8. Phosphoglycerate mutase
9. Enolase
10. Pyruvate kinase

### Citrate Cycle
0. Pyruvate dehydrogenase
1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. α-ketoglutarate dehydrogenase
5. Succinyl-CoA synthase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase
Glycolysis & Citrate Cycle

Triumph over minor details first.

Glycolysis

1. Hexokinase
   Function of Mg$^{2+}$; induced conformational change
2. 1,3-Phosphoglycerate kinase
   Function of Mg$^{2+}$
3. Phosphofructokinase
   Function of Mg$^{2+}$
4. Triosephosphate isomerase
   Catalytically perfect
5. Triose phosphate isomerase
   C&atalytically perfect
6. Phosphoglycerate kinase
   Induced conformational change;
7. Phosphoglycerate kinase
   Induced conformational change;
8. Enolase
9. Enolase
10. Pyruvate kinase
    tautomerization

Citrate Cycle

1. Citrate synthase
   Induced conformational change; irreversible rate limiting step: deprotonation of Ac-CoA;
2. Aconitase
   pro-R VS. pro-S
3. Isocitrate dehydrogenase
   Difference from pyruvate DH (rxn order and cofactor)
5. Succinyl-CoA synthase
   Two Energy transfer intermediates
6. Succinate dehydrogenase
   Integral membrane enzyme; prosthetic group
7. Fumarase
8. Malate dehydrogenase
Step 1  The substrate binds.

Step 2  An enzymatic acid, probably the α-amino group of a conserved Lys residue, catalyzes ring opening.

Step 3  A base, thought to be a conserved His residue, abstracts the acidic proton from C2 to form a cis-enediolate intermediate (the proton is acidic because it is α to next carbonyl group).

Step 4  The proton is replaced on C1 in an overall proton transfer. Protons abstracted by bases rapidly exchange with solvent protons.

Step 5  The ring closes to form the product, which is subsequently released to yield free enzyme, thereby completing the catalytic cycle.

Through what mechanism does PGI open the ring, close the ring and achieve proton transfer?

Acid catalysis: open the ring

Base catalysis: transfer the acidic C-2 proton to C-1; close the ring
Step 1  Substrate binding.

Step 2  Reaction of the FBP carbonyl group with the -amino group of the active site Lys to form an iminium cation, that is, a protonated Schiff base.

Step 3  C3-C4 bond cleavage resulting in enamine formation and the release of GAP. The iminium ion is a better electron-withdrawing group than the oxygen atom of the precursor carbonyl group. Thus, catalysis occurs because the enamine intermediate is more stable than the corresponding enolate intermediate of the base-catalyzed aldol cleavage reaction.

Step 4  Protonation of the enamine to an iminium cation.

Step 5  Hydrolysis of the iminium cation to release DHAP, with regeneration of the free enzyme.

The transition state is a Schiff base. How does the formation of Schiff base contribute to catalysis?
Step 1 GAP binds to the enzyme.

Step 2 The essential sulfhydryl group, acting as a nucleophile, attacks the aldehyde to form a thiohemiacetal.

Step 3 The thiohemiacetal undergoes oxidation to an acyl thioester by direct hydride transfer to NAD$^+$. This intermediate, which has been isolated, has a large free energy of hydrolysis. Thus, the energy of aldehyde oxidation has not been dissipated but has been conserved through the synthesis of the thioester and the reduction of NAD$^+$ to NADH.

Step 4 Pi binds to the enzyme–thioester–NADH complex.

Step 5 The thioester intermediate undergoes nucleophilic attack by Pi to form the “high-energy” mixed anhydride 1,3-BPG, which then dissociates from the enzyme followed by replacement of NADH by another molecule of NAD$^+$ to regenerate the active enzyme.
Glycolysis 8: PGM mechanism

Step 1  
3PG binds to the phosphoenzyme in which His 8 is phosphorylated.

Step 2  
The enzyme's phosphoryl group is transferred to the substrate, resulting in an intermediate 2,3-bisphosphoglycerate–enzyme complex.

Steps 3 and 4  
The complex decomposes to form the product 2PG and regenerate the phosphoenzyme.

Why isn't PGM also used to generate 2,3-BPG?

Just another link of 2,3-BPG

1. why phosphoglycerate kinase is chosen to generate 3-PG rather than through 1,3-BPG ➔ 2,3-BPG ➔ 3-PG pathway?
2. Function of 2,3-BPG in regulation of hemoglobin affinity for O₂.
Glycolysis & Citrate Cycle

⇒ Function of cofactors

- $\text{Mg}^{2+}$ (coenzyme): hexokinase, PFK
- $\text{NAD}^+$ (cosubstrate): GAPDH, etc.
- **TPP** (prosthetic group): pyruvate decarboxylase
- **TPP, lipoic acid, CoA, FAD, NAD}^+$**: pyruvate dehydrogenase complex
  \[\alpha\text{-ketoglutarate dehydrogenase}\]
Cofactor of pyruvate decarboxylase

**Active form of the cofactor:**
TPP's catalytically active functional group is the thiazolium ring. The C2-H atom of this group is relatively acidic because of the adjacent positively charged quaternary nitrogen atom, which electrostatically stabilizes the carbanion formed when the proton dissociates. This dipolar carbanion is the active form of the coenzyme.

**Function of the cofactor:**
Departure of CO$_2$ to generate a resonance-stabilized carbanion adduct in which the thiazolium ring of the coenzyme acts as an electron sink.
Cofactors of pyruvate dehydrogenase complex & α-ketoglutarate dehydrogenase (five in total)

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine pyrophosphate (TPP)</td>
<td>Bound to $E_1$</td>
<td>Decarboxylates pyruvate yielding a hydroxyethyl-TPP carbanion</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>Covalently linked to a Lys on $E_2$</td>
<td>Accepts the hydroxyethyl carbanion from TPP as an acetyl group</td>
</tr>
<tr>
<td>Coenzyme A (CoA)</td>
<td>Substrate for $E_2$</td>
<td>Accepts the acetyl group from lipoamide</td>
</tr>
<tr>
<td>Flavin adenine dinucleotide (FAD)</td>
<td>Bound to $E_3$</td>
<td>Reduced by lipoamide</td>
</tr>
<tr>
<td>Nicotinamide adenine dinucleotide ($NAD^+$)</td>
<td>Substrate for $E_3$</td>
<td>Reduced by FADH$_2$</td>
</tr>
</tbody>
</table>
Regulated step: phosphofructose kinase (PFK)
- **inhibitor:** ATP;
- **activator:** ADP, AMP, fructose-2.6-bisphosphate.
- PFK has two binding sites for ATP, one binding site for F6P.
- The inhibitor site has a lower affinity for ATP than the substrate site.

**How can the flux of this rxn change to 100-fold?**
In muscle, [ATP] is 50 times greater than [ADP] and 10 times greater than [AMP], so a 10% decrease of [ATP] can result in a 100% increase on [ADP] and 400% increase of [AMP], which increase PFK activity significantly.
Electron transport chain

<table>
<thead>
<tr>
<th>Complex</th>
<th>Full name</th>
<th>oxidized</th>
<th>reduced</th>
<th>e passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>NADH-coenzyme Q oxidoreductase</td>
<td>NADH</td>
<td>Q</td>
<td>2</td>
</tr>
<tr>
<td>Complex II</td>
<td>succinate-coenzyme Q oxidoreductase</td>
<td>FADH₂</td>
<td>Q</td>
<td>2</td>
</tr>
<tr>
<td>Complex III</td>
<td>coenzyme Q-cytochrome c oxidoreductase</td>
<td>QH₂</td>
<td>cytochrome c</td>
<td>2</td>
</tr>
<tr>
<td>Complex IV</td>
<td>cytochrome c oxidase</td>
<td>cytochrome c</td>
<td>O₂</td>
<td>2</td>
</tr>
</tbody>
</table>
Electron transport chain: Q cycle

QH₂ + 2 cyt c₁ (Fe³⁺) → Q + 2 cyt c₁ (Fe²⁺) + 4H⁺

Step 1
QH₂ + cyt c₁ (Fe³⁺) → QH + cyt c₁ (Fe²⁺) + 2H⁺

Step 2
QH₂ + QH + cyt c₁ (Fe³⁺) + 2H⁺ → QH₂ + Q + cyt c₁ (Fe²⁺) + 2H⁺
ATP synthesis

L (loose state): ADP $\cdot$ P$_i$

T (tight state): ADP $\cdot$ P$_i$ $\rightarrow$ ATP

O (open state): ATP dissociate

Energy in step 2 is provided by proton flow