Summary of Chapter 14a

1. Names of molecules and enzymes in glycolysis should be familiar.
2. Two ATPs are utilized in stage I of glycolysis to produce two glyceraldehyde 3-phosphates from one glucose.
3. Four ATPs, two NADHs and two pyruvates are produced in stage II. The overall glycolysis reaction is:
   \[
   \text{Glucose} + 2 \text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \rightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}_2\text{O} + 4\text{H}^+
   \]
4. Some important questions to be addressed are:
   1. Why does hexokinase catalyze the phosphorylation of C6 of glucose, instead of hydrolysis of ATP?
   2. Why is glucose (aldose) converted to fructose (ketose)?
   3. Why is [GAP] kept to be relatively low?
   4. Why is Hb O2 affinity increase if hexokinase is inhibited?
   5. Why is Hb O2 affinity decreased if pyruvatekinase is inhibited?
   6. Why is 3-phosphoglycerate not directly converted to pyruvate?
   7. Why does F− inhibit enolase catalytic activity?
   8. Why is TIM called a perfect enzyme?
5. Pyruvates and NADH produced by glycolysis are converted to three ways, and NAD+ is recycled:
   1. Aerobic oxidation: CO₂, H₂O, NAD⁺ and ATP
   2. Anaerobic homolactic fermentation in muscle: lactate, NAD⁺ and heat
   3. Anaerobic alcoholic fermentation in yeast: ethanol, CO₂, NAD⁺, and heat.
6. Overall process of anaerobic glycolysis in muscle:
   \[
   \text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{Lactate} + 2\text{ATP} + 2\text{H}_2\text{O} + 2\text{H}^+ + \text{heat}
   \]
   Overall process of anaerobic glycolysis in yeast:
   \[
   \text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{Ethanol} + 2\text{CO}_2 + 2\text{ATP} + \text{heat}
   \]
7. Many decarboxylation reactions including pyruvate decarboxylase require a cofactor thiamine pyrophosphate (TPP). Note: Many carboxylation reactions require a cofactor biotin.
8. ATP production of anaerobic glycolysis is 100 times faster than that of oxidative phosphorylation. Thus, ATP for tissues such as muscle consuming ATP rapidly is regenerated by anaerobic homolactic fermentation. For this reason, vigorous exercise produces a large amount of heat.
9. Lactate is aerobically regenerated to glucose in liver. Thus the homolactic fermentation does not really waste glucose.
10. Enzymes that involve in glycolytic metabolism are very stereospecific. For example, examine aldolase, lactate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase.
11. Some unique reaction mechanisms are:
   - Neutral Lys and negative charged Tyr in aldolase.
   - Essential sulfhydryl group in GAPDH.
   - Phosphorylated enzyme in PGM.
   - Mg²⁺ in enolase.
12. Under steady state,
   \[
   S \xrightarrow{J} A \xrightleftharpoons{v_f}{v_r} B \xrightarrow{J} P
   \]
   the relation between flux change (ΔJ) and concentration change (Δ[A]) is:
   \[
   \frac{\Delta J}{J} = \frac{\Delta [A]}{[A]} \frac{v_f}{v_f - v_r}
   \]
   1. Under irreversible condition, i.e., \(v_r \rightarrow 0\) and \(\frac{v_f}{v_f - v_r} \rightarrow 1\). Thus \(\frac{\Delta J}{J} = \frac{\Delta [A]}{[A]}\)
   The flux change is proportional to the concentration change of substrate.
   2. Near equilibrium condition, i.e., \(v_f \rightarrow v_r\) and \(\frac{v_f}{v_f - v_r} \rightarrow \infty\). Thus, \(\Delta J\) change effects very little on \(\Delta [A]\) change since \(\Delta [A]\) must be very small. Note: we assume that flux \(J\) and \([A]\) are constant.
Therefore, $\Delta J$ can communicate with $\Delta [A]$ in irreversible process, but not in near equilibrium condition. For this reason, the substrate concentration in irreversible process directly controls the flux of overall reaction pathways. Such a process is a rate-determining step of multiple reaction processes, such as glycolysis.

13. Rate-determining step is the slowest step in a entire pathway and has a large negative free energy change.

14. The major flux-controlling enzyme of glycolysis (i.e., rate-determining enzyme) is phosphofructokinase (PFK). Although reactions catalyzed by hexokinase and pyruvatekinase have a large negative free energy changes, those enzymes are not real rate-determining enzymes.

- Some reactants enter the glycolysis by skipping the hexokinase catalyzing reaction.
- Pyruvatekinase catalyzes the last reaction of the glycolysis.

15. PFK is regulated allosterically.

- ATP is both a substrate and an allosteric inhibitor.
- PFK has two ATP binding sites in each subunit --- catalytic site and allosteric inhibitory site.
- AMP which is produced by adenylate kinase activity from ADP is a strong PFK activator and relieve the ATP inhibition.
- Another strong activator of PFK is fructose-2,6-bisphosphate (F2,6F).

16. [ATP] is regulated by two enzymes.

1. Creatine kinase: Creatine + ATP $\leftrightarrow$ Phosphocreatine + ADP
2. Adenylate kinase: 2ADP $\leftrightarrow$ ATP + AMP

17. Adenylate kinase produces not only ATP but also PFK activator AMP.

18. Under physiological condition,

F6P + ATP $\rightarrow$ FBP + ADP (catalyzed by PFK)

FBP + H2O $\rightarrow$ F6P + Pi (catalyzed by fructose-1,6-bisphosphatase (FBPase))

Overall reaction is: ATP + H2O $\rightarrow$ ADP + Pi, (Simply wasting ATP).

- This cycle is called substrate cycle or futile cycle.
- The substrate cycle plays an important role in the regulation of glycolysis and generates body heat.

19. Flux of glycolysis changes 100-folds between rest and vigorous exertion (exercise), but [ATP] changes only ~10%. Because:

1. In resting time --- [ATP] is high, i.e., need less ATP synthesis, and [ADP] is low.
   - ATP inhibits PFK, thus glycolytic flux is reduced.
   - FBP is converted to F6P by FBPase in the substrate cycle, thus glycolytic flux is further reduced.
2. In vigorous excise --- [ATP] is low, i.e., need more ATP synthesis, and [ADP] is high.
   - PFK activity is high since [ATP] is low, thus glycolytic flux is increased.
   - ADP is converted to ATP and AMP by adenylate kinase since [ADP] is high.
   - Since AMP is a strong activator, AMP produced by adenylate kinase catalyzed reaction relieves ATP inhibition on PFK and activates PFK activity, thus glycolytic flux is increased.
   - AMP inhibits FBPase and reduces the substrate cycle activity, thus glycolytic flux is not reduced.

20. Fructose metabolisms in muscle and liver are different.

- In muscle: Fructose $\rightarrow$ Fructose-6-phosphate (by hexokinase) $\rightarrow$ glycolysis.
- In liver: Fructose $\rightarrow$ Fructose-1-phosphate $\rightarrow$ Glyceraldehyde-3-phosphate (since no hexokinase in liver) $\rightarrow$ glycolysis.

21. Galactose $\rightarrow$ UDP-galactose $\rightarrow$ UDP-glucose $\rightarrow$ Glucose-6-phosphate $\rightarrow$ glycolysis

22. Mannose $\rightarrow$ Mannose-6-phosphate (by hexokinase) $\rightarrow$ Fructose-6-phosphate $\rightarrow$ glycolysis