1. Quaternary Structure Determination:
- Native molecular weight = size exclusion chromatography (gel filtration)
- Subunit MW from SDS-PAGE +/- BME (S-S bonds).
- Quaternary structure consistent with both.

2. X-Ray Diffraction:
- How the technique works:
  1. X-rays are scattered by electrons
  2. Intensity depends on position of atoms.
- How an electron density map is fit.
- How to evaluate the correctness of a model.

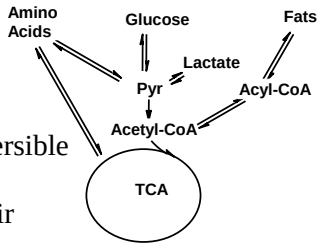
3. Thermodynamics:
- Do you know the difference between Gibbs free energy (ΔG) and standard energy (ΔG^o)?
- Predict the equilibrium position from ΔG^o.
- Predict the direction of the reaction from ΔG.
- $\Delta G = \Delta G^o + RT \ln[\text{products}]/[\text{reactants}]$.
- Indirect coupling: Change ratio of products/reactants to lower ΔG (e.g. aldolase).
- Direct coupling: Use of ATP to lower ΔG^o (e.g. hexosekinase, PFK).
- Free energy of concentration gradient, $\Delta G = RT\ln[In]/[Out]$
- Free energy of transport of a charge across a voltage difference ($\Delta G = ZF\Delta\psi$)

4. Carbohydrates:
- Ketose versus aldose.
- 3 carbon sugar-glyceraldehyde - important intermediates in glycolysis
- 5 carbon sugar –ribose, in nucleic acid
- 6 carbon sugars – glucose and fructose
- Disaccharides: nomenclature.
- Storage polysaccharides (glycogen). Shorthand nomenclature for linkage (e.g. β(1-4)).
- Structural polysaccharides: Cellulose
- Bacterial cell wall structure

5. Lipids:
- General structure of fatty acids, waxes, triglycerides, phospholipids, Sphingolipids
- Structure difference between saturated and un-saturated fatty acids
- Biochemical role of fatty acids, triglycerides, phospholipids
- Role of hydrophobic effect in spontaneous formation of micelles and bilayers, CMC.
- Properties of lipid bilayer membranes.
  1. Permeability properties, osmotic effects.
  2. Melting temperature and fatty acid composition (van der Waals interactions.)

6. Biological Membranes:
- Components of biological membranes and their function:
  1. Cholesterol, phospholipids
  2. Peripheral membrane proteins.
  3. Integral membrane proteins.
- Fluid mosaic model of biological membranes
- General structural properties of membrane proteins (hydrophobic residues & H-bonds)
- Ion channel selectivity.
- Thermodynamics of protein insertion.

7. Metabolic Pathways:



i) Pathway & location.
ii) How all pathways are tied together →
iii) Input and outputs.
iv) Why some steps are reversible while others are not.
v) Controlling step(s) & their importance.
vi) Steps that produce energy & how that energy is stored (NADH/FADH2/thioesters)
vii) Global regulation of glucose metabolism between glycogen synthesis/degradation & glycolysis/gluconeogenesis.
viii) Overall processing of nutrients (proteins, lipids, sugars). Pyr to acetyl-CoA is one way.

8. Enzyme Nomenclature.
1. Kinase: $X + ATP \leftrightarrow X\text{-}P + ADP$
2. Phosphatase: $X\text{-}P \rightarrow X + P_i$
3. Dehydrogenase: $XH_2 + NAD^+ \leftrightarrow X + NADH$

9. Electron Transport - Final elec. acceptor (O_2)
- Inorganic & organic carriers.
- Calculation of redox changes:
  i. Either by balancing: H_2O then H^+ & e^-
  ii. Electron counting
- Direction of electron flow:
  NADH oxidation (I→Q→III→cytoC→IV→O_2).
  $FADH_2$ oxidation (II→Q→III→cytoC→IV→O_2).
- Generation of a proton gradient.

10. ATP Synthesis: How energy is generated from a pH gradient using the F_1F_o ATPase (ATP synthase)
- Contains 3 β-subunits, one that binds nothing, one that binds ADP+P_i and one that stabilizes ATP over ADP+P_i, causing the spontaneous formation of ATP from bound ADP+P_i.
- Pumping of 3 H^+ causes rotation of γ-subunit. Rotation of γ-subunit changes the conformation of β-subunits, causing bound ADP+P_i→ATP.

11. Anaerobic Metabolism:
Mammals: pyruvate → lactate
Yeast: pyruvate →acetylaldehyde→ethanol

12. Anabolic Pathways (gluconeogenesis, formation of oxaloacetate from pyruvate). Energy input needed, high energy steps done by a different mechanism.

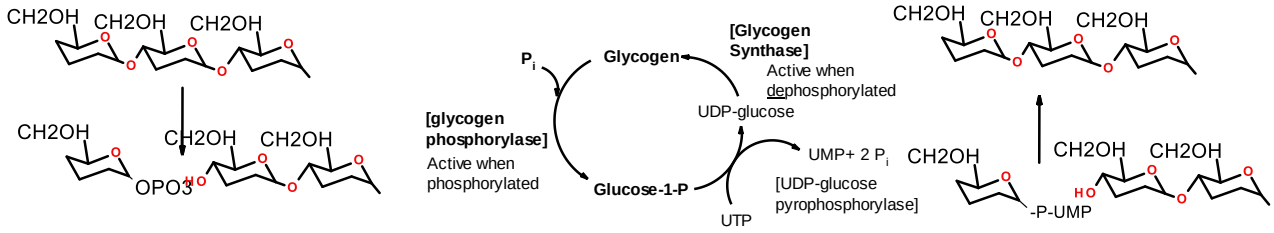
13. Amino acid metabolism:
- Transaminase accepts -NH2 from AA, replaced by C=O.

Regulation: *Use common sense.*

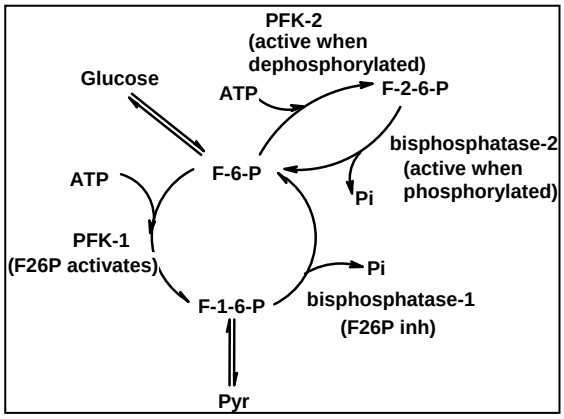
- You should try to develop a logical understanding of the way things are regulated. It usually makes good common sense. For example, high NADH & ATP levels, which are indicative of a high energy state of the cell, inhibit many enzymes in the TCA cycle. Same goes for ATP regulation of glycolysis/gluconeogeneis – glycolysis is turned off when ATP levels are high, on when AMP levels are high.
- Know how pathways are, in general, regulated by phosphorylation of enzymes, product inhibition, feedback inhibition, be able to give one example.
- Opposing pathways are regulated in a coordinated manner, both are not on at the same time (both could be off).

Regulation of Glycogen and Glucose metabolism: "*The liver is a glucose bank.*"

1. **Glucose demand:** Hormones epinephrine or glucogon are released. Binding to their receptors leads to activation of G-protein, which activates adenyl cyclase. The cAMP produced by adenyl cyclase activates protein kinases → proteins become **phosphorylated**.
2. **Glucose excess:** Hormone insulin is released. Binding to its receptor leads to protein **dephosphorylation**.
3. **Glycogen metabolism:** Syn. and degradative enzymes are *directly* controlled by protein phosphorylation.



4. **Glycolysis & Gluconeogenesis:** Directly controlled by F2-6P levels. F2-6P levels are controlled by protein phosphorylation.



- *F2-6P levels are high when insulin is present (high blood glucose) because the enzyme that makes F2-6P (PFK-2) is active when dephosphorylated*
  High blood glucose = F2-6P becomes high
- *High F2-6P levels activate PFK-1, turning on glycolysis-but only if ATP is needed.*

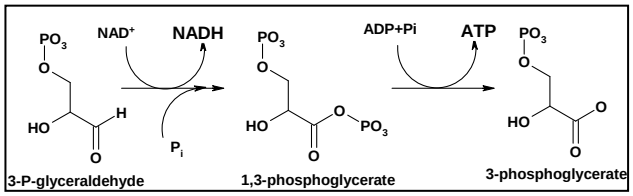
These pathways are also regulated by energy sensing:

i) High AMP activates PFK-1, turning on glycolysis to produce ATP. Bisphosphatase-1 in gluconeogenesis is inhibited by AMP since ATP is required to make glucose and high AMP = low ATP.

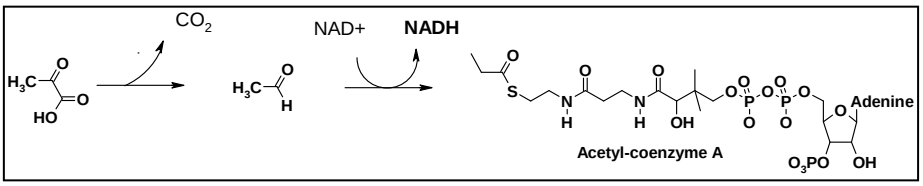
i) High ATP inhibits PFK-1 and therefore glycolysis. Gluconeogenesis can occur since AMP levels are low, indicating that there is sufficient ATP to make glucose.

Key energy generating steps:

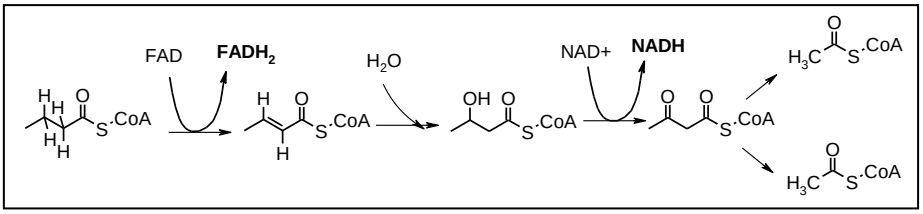
i) Glycolysis: Oxidation of an aldehyde → carboxylic Acid. One NADH is produced directly, and one ATP is produced from the phosphorylated product in the next step. 2nd ATP produced in last step.



ii) TCA Cycle: Oxidative decarboxylations, NADH and a high energy thio-ester are produced, e.g. Pyr to acetyl-CoA.



iii) β-Oxidation (TCA Cycle, fatty acid oxidation): Oxidation of an alkane → ketone (1 NADH and 1 $FADH_2$/cycle).



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iv) NADH/$FADH_2$ are oxidized in electron transport, energy stored in proton gradient (inner membrane).
v) Energy stored in proton gradient is used to generate ATP with ATP synthase (ADP + Pi → ATP)

Pathway/Location	**Input Substrate**	**Output Substrate**	**Controlling Step**	**Energetic Considerations** (Detailed numbers not required for exam.)
Glycolysis Cytosol	Glucose	Pyruvate (Pyr)	PFK-1 Inhibited by: ATP, citrate Activated by: AMP, **F26P (essential activator)**	*Small amounts of ATP generated quickly, <u>without the need for oxygen.</u>*
Gluconeogenesis Cytosol	Pyruvate	Glucose	F16bisphosphatase Inhibited by AMP Inhibited by **F26P**	Requires energy (general property of anabolic pathways).
Anerobic Met. Pyr→ Lactate/ethanol	Pyr & NADH	Lactate & NAD^+		Provides NAD^+ for glycolysis during anaerobic conditions in active muscles. Lactate is converted to glucose in the liver.
Pyr→Acetyl CoA Mito. Matrix	Pyr	Acetyl-CoA, CO_2	Inh: ATP, NADH, acetyl-CoA (product Inh)	NADH produced, high energy thio-ester formed.
TCA Cycle Mito. Matrix	Acetyl-CoA +oxaloacetate →citrate	2 x CO_2	Citrate synthase *Energy sensing:* NADH,ATP inhibit Citrate inh (product) Succinate CoA inh (feedback)	*Produces large amounts of NADH and $FADH_2$ which are converted to ATP* ***<u>if O_2 is available.</u>***
Fatty Acid Activation Cytosol.	Fatty Acid (N)	Acyl CoA (thioester)		Priming requires 2ATP equivalents.
Fatty Acid Deg. Mito. Matrix	Acyl CoA (thioester)	N/2 Acetyl-CoA		Each {(N/2)-1} cycle gives: 1 NADH, 1 $FADH_2$, 1 acetyl-CoA. Last cycle → 2 acetyl-CoA
Electron Transport Inner mito. mem.	O_2, NADH, $FADH_2$.	Water $[H^+]$ gradient		NADH → 10 protons ≈ 3ATP $FADH_2$ → 6 protons ≈ 2 ATP
Oxidative Phos Inner mito.mem.	ADP + P_i + $[H^+]$ Grad.	ATP		3 protons ≈ 1 ATP