

Outline of biosignaling, membrane transport, metabolic thermodynamics, biochemical redox, the electron transport chain (ETC) and oxidative phosphorylation (oxphos)

Basics of glucose metabolism

- Biosignaling (esp. via insulin, glucagon, and adrenaline pathways) is heavily involved in glucose (blood sugar) regulation
- Glucose is stored in the liver and muscles in the form of **glycogen**, a branching network of glucose molecules attached to a central glycogenin protein
 - The **liver** stores it to be able to provide glucose to the body
 - The **muscles** store it to be able to provide energy to themselves
- Key terms regarding glucose regulation (which we will encounter in greater detail in coming lectures):
 - **Glycolysis**: **breakdown** of **glucose** into pyruvate
 - **Gluconeogenesis**: making of “new” **glucose** from non-carbs
 - **Glycogenesis**: making of **glycogen** from glucose
 - **Glycogenolysis**: **breakdown** of **glycogen** into glucose 1-phosphate (which the liver can convert to glucose to ship out but the muscles keep to themselves and use for glycolysis)
- **Insulin & glucagon** are hormonal “opposites” that work to control blood sugar & anabolism vs. catabolism
 - **Glucagon** says “all” the glucose is gone
 - Released (**from pancreatic α -cells**) in response to low blood glucose
 - Increases glycogenolysis and gluconeogenesis
 - Decreases glycogenesis
 - Raises blood glucose
 - Liver has receptors, but muscles don’t
 - Adrenaline has similar effects, but can also affect muscles
 - But muscles cannot raise blood sugar since they can’t release glucose into the bloodstream since they lack G6Pase
 - Acts via GPCR pathways
 - **Insulin** says better let some glucose in!
 - Released (**from pancreatic β -cells**) in response to high blood glucose
 - Increases glycogenesis and glycolysis
 - Decreases glycogenolysis and gluconeogenesis
 - Lowers blood sugar
 - Acts via RTK pathways
 - Much of the regulation of glycogen metabolism is through phosphorylation by PKA (stimulated by glucagon & epinephrine) & dephosphorylation by PP1 (stimulated by insulin)
 - Should be able to analyze diagrams of this, don’t need to memorize
- **GLP-1 (Glucagon Like Peptide - 1)**
 - Made from same precursor as glucagon, but has opposing functions
 - Secreted by intestines & acts through GLP receptors (GLP-1Rs), which are GPCRs

- Increases insulin production → more glucose taken into cells (in response to ↑ signaling through insulin RTKs)
- Decreases glucagon production → less glucose made
- GLP-1R agonists (GLP-1RAs) like Ozempic serve as treatment for type 2 diabetes & obesity

Biosignaling Expectations (LO18)

- Because there are literally infinite numbers of potential pathways, my goals for you are to know the fundamentals of key types of components (G proteins, RTKs, etc.) and to be able to interpret signaling pathway diagrams
 - You are only responsible for knowing (without seeing a diagram) the Gs pathway through to PKA, typified by β-adrenergic and glucagon receptor pathways
 - When it comes to signaling pathways, anything that can be answered using a provided diagram alone is fair game
- The key to biosignaling is to **follow the arrows!**
 - Symbols including arrows, triangles, + signs, often green color-coding, indicate activation
 - Symbols including –| symbols, – signs, often red color-coding indicate inhibition
 - Keep an eye out for “double negatives” (e.g. inhibition of an inhibitor)
- You should know when insulin, glucagon, and adrenaline are produced, which types of pathways they act through, and their net effects on: glycogen synthesis (glycogenesis), glycogen breakdown (glycogenolysis), and blood sugar levels but **do not** need to know (without looking at a diagram) the enzymes that they stimulate and/or inhibit
- Be able to recognize and classify types of membrane transport

Be able to predict the effects of agonists, antagonists, and mutations on biosignaling pathways

- **Agonists** turn “on” a pathway, mimicking natural ligands. **Antagonists** turn “off” pathways, such as by blocking the binding of a natural ligand.
- **Mutations**
 - Mutations in receptors
 - Mutations that prevent ligand binding and/or mutations that prevent binding of intracellular proteins → decreased signaling/activity
 - Mutations that cause overexpression (making excess protein) and/or lead to constitutive activation (active “all the time,” even without needing ligand binding) → increased signaling/activity
 - Mutations in G-proteins
 - Mutations that affect the *catalytic* activity of G-proteins prevent them from inactivating themselves (hydrolyzing GTP to make GDP) → lead to increased activity
 - Mutations that affect the ability of a G protein to interact with the protein they stimulate and/or the protein that activates them (e.g. GEF) → lead to decreased activity
 - Mutations in kinases
 - Mutations in catalytic residues prevent ability to phosphorylate substrates
- Changes upstream (earlier/higher up) in a pathway will typically have a wider-reaching response than changes downstream (later/lower down) in a pathway
 - This is because upstream changes have the potential to impact more processes
- Changes downstream (later/lower down) in a pathway can overcome/cancel out upstream changes, but can only affect processes downstream of themselves.

- For example, imagine you have a receptor A that stimulates both B (which stimulates C) and D (which stimulates E)
 - A mutation that prevents activation of A would prevent activation of B (and thus C) and D (and thus E)
 - A mutation that prevents activation of B would prevent activation C BUT NOT D (and thus not E)
 - A mutation that prevents activation of D would prevent activation E BUT NOT B (and thus not C)
 - A mutation that causes constitutive (all the time) activation of B would lead to increased C activity (but not affect A, D, or E activity)
 - A mutation that causes constitutive (all the time) activation of D would lead to increased E activity (but not affect A, B, or C activity)
 - A mutation that prevents activation of A combined with a mutation that causes constitutive activation of B, would still lead to increased C activity (but D/E activity would remain decreased due to the A mutation)

Membrane transport

- **Passive transport:** passage for you is free because you're thermodynamically "wanted" on the other side (movement along electrochemical gradient)
 - **Simple diffusion:** You just sneak in
 - Only possible for small polar molecules (e.g. water) and smallish nonpolar molecules
 - **Facilitated diffusion:** A body guard (typically in the form of a membrane-spanning protein) helps you get through
 - Selective pores have channel structures that only permit movement of specific molecules
 - Can be "gated" so that they only open in response to a stimulus such as binding of a specific molecule (ligand-gated channels) or changes in voltage across a membrane (voltage-gated channels)
- **Active transport:** you are not thermodynamically "wanted" on the other side (movement against electrochemical gradient), so your passage will need to be paid for energetically
 - **Primary active transport:** You pay for your own entry/exit at the gate
 - Often involves pumps that "spend" ATP to change the shape of the pump via phosphorylation and/or ATP hydrolysis ("burning" ATP)
 - **Secondary active transport:** Your friend pays ahead for you
 - Primary active transport of one molecule is used to create an electrochemical gradient. Subsequent movement of that molecule back down its gradient is coupled to the cotransport of a second molecule against its gradient.

Overview of signal transduction

- Some important properties of biosignaling pathways to keep an eye out for are and identify examples of: specificity, sensitivity, amplification, modularity, desensitization/adaptation, integration, divergence, and localized response
- There is a large amount of cross-talk between signaling pathways
- There are 4 main types of signal transduction: **Be ready to compare and contrast**
 - **G-protein coupled receptor (GPCR) pathways**

- These involve a membrane-spanning GPCR's ectodomain binding to a ligand and activating an intracellular G-protein that leads to production of second messenger(s) that relay the signal inside of cells
- There are a LOT of types that:
 - 1) Recognize different signals (thanks to different GPCRs)
 - 2) Set off various pathways in the cell (thanks to different G proteins (especially $G\alpha$))
- *You are only responsible for knowing (without seeing a diagram) the Gs pathway through to PKA, typified by β -adrenergic and glucagon receptor pathways*
- **Receptor enzymes (esp. receptor tyrosine kinases, RTKs)**
 - These involve RTKs' ectodomains binding to ligands, activating the kinase domains of the RTKs and leading to intracellular phosphorylation cascades
 - *We focused mainly on the insulin receptor (INSR) pathways, and this is the one you should focus on in your reviews, BUT you may be asked to interpret and/or compare diagrams of other RTK pathways*
- **Gated ion channels**
 - The messenger acts as an extracellular signal to open the gate for the ions to go in or out. It does not go in itself
 - Form of facilitated diffusion (a type of passive transport)
- **Nuclear receptors (e.g. for steroid-based signaling)**
 - This is the only case in which the primary messenger enters the cell, which it does through simple diffusion due to its largely nonpolar nature
 - A type of passive transport
- Only gated ion channels & nuclear receptors involve membrane transport
- Some (but not all) signal transduction pathways involve both primary & secondary/second messengers
 - **Primary messengers:** signals that "arise" extracellularly to provide info to a cell (e.g. hormones)
 - **Secondary/second messengers:** Signaling molecules created and/or released inside of cells in response to a extracellular binding of a primary messenger to a receptor
 - Examples:
 - cyclic AMP (cAMP) (produced by adenylyl cyclase (AC))
 - phospholipid-based:
 - from phospholipase C: IP3 & DAG
 - from PI3K: PIP3 & Ca^{2+} released from intracellular stores
 - These **do not** include proteins, phosphorylation cascades, etc.
- Some signal transduction pathways involve phosphorylation cascades in which kinases activate other kinases which activate other kinases which activate other kinases. . .
 - This is especially common with RTK pathways
 - This is frequently facilitated by scaffolding proteins that bind to multiple pathway components to keep them together
- **Scaffolding proteins** are typically long flexible proteins with binding sites for many additional proteins – they help hold pathway components nearby one another to control signaling and allow for specific signaling cascades to be efficiently turned on and off
 - Often involve phosphate-binding domains, which allow for regulated binding of signaling partners
 - SH2 domain binds to phosphorylated Tyr residues

- SH3 and PH domains bind the membrane phospholipid PIP₃
- Scaffolding proteins and signaling-responsive domains can coordinate cross-talk between pathways
- Hooking up signaling networks in different ways can allow the same cell to respond differently to the same signal at different times and/or different cells to respond to the same signal in different ways

G proteins

- Small proteins that act as “self-limiting switches”
 - Switch between active, GTP-bound and inactive, GDP-bound states
 - When “active”, they can interact with, and modulate the activity of, other proteins
 - Have the (highly regulated) ability to break down GTP (GTPase activity) but not make GTP (they inactivate themselves!)
 - They hydrolyze GTP and then have GDP exchanged for a fresh GTP
 - GDP is held tightly and GTP concentrations are high, so GDP release is the slow step in activation, *not* GTP binding.
 - Once GDP is released (typically with the help of a GEF), GTP can readily bind.
 - Can be monomeric or heteromeric
 - G proteins associated with GPCRs are heterotrimeric
 - G α is the catalytic subunit, which binds GTP/GDP
- G proteins are highly regulated and dysregulation of G proteins or their regulators can cause cancer
 - **GEFs (GTP-GDP exchange factors) help turn them “on” (facilitate exchange of GDP for GTP)**
 - *GEFs turn G-proteins on by GETting GDP the F out!*
 - e.g., GPCRs, SOS
 - **GAPs (GTPase activator proteins) help turn them “off” (facilitate GTP hydrolysis)**
 - *GAPs turn G-proteins off by giving GTP a zAP!*

GPCRs (G Protein Coupled Receptors)

- Glucagon and adrenaline (aka epinephrine) commonly act through Gs-associated GPCR pathways
 - In these pathways, the G protein stimulates adenylyl cyclase to produce the second messenger cyclic AMP (cAMP)
 - They have different receptors (actual GPCRs) that bind different ligands (glucagon **or** adrenaline), but they both serve as GEFs to activate Gs
- Overview of Gs (stimulatory of PKA) pathway – used by β -AR and glucagon receptors
 - ① Binding of the agonist (stimulatory ligand) to the GPCR (a GEF) turns “on” the G protein
 - ② Once turned “on”, the α -subunit dissociates and binds to effector proteins that relay the signal further
 - Activation of adenylyl cyclase (AC) by G α generates the second messenger cAMP
 - cAMP activates Protein Kinase A (PKA)
 - Among other things, PKA activates glycogen breakdown (glycogenolysis)
 - β - and γ - subunits of the G protein also serve important roles
 - ③ GTP hydrolysis (often promoted by GAPs), turns the G protein “off” and leads the system to reset

- We focused on the Gs pathway, but some GPCRs signal via different second messengers (which you are not responsible for, but here is an example included for reference)
 - E.g. In response to signal binding to receptor, Gq activates →
 - Activates PhosphoLipase C (PLC) →
 - PLC cleaves PIP2 to inositol 1,4,5-triphosphate (IP3) & diacylglycerol (DAG) →
 - IP3 opens calcium channels →
 - DAG & Ca²⁺ activate Protein Kinase C (PKC) →
 - Lots of stuff happens
 - The Ca²⁺ can pass the message on through calmodulin; Ca²⁺ binding to calmodulin causes conformational change in calmodulin → Modulates calmodulin's interactions with other proteins

Receptor Tyrosine Kinases (RTKs)

- Unlike GPCRs, in the case of RTKs, the receptors are enzymes – more specifically, they are tyrosine kinases (enzymes that phosphorylate tyrosine residues)
- Act as dimers, but not all are constitutive (all the time) dimers
 - INSulin Receptor protein (INSR) is a constitutive dimer (always a dimer), but requires ligand binding for activation
 - Upon insulin binding, it autophosphorylates (phosphorylates itself) and thus activates
 - For some RTKs, ligand-binding causes dimerization (and subsequent activation)
 - Sometimes, these are mix-and-match to create heterodimers (e.g. HER receptors)
 - Some RTKs don't require ligand-binding for activation (e.g. HER2) and their overexpression can lead to excessive signaling, cell growth, and cancer
- Many RTKs are involved in growth factor signaling and thus mutations in them can lead to cancer
- **Insulin signaling through Ras:** You **do not** need to memorize this pathway. A figure will be provided if you need it
 - INSulin Receptor protein (INSR) is a constitutive dimer that autophosphorylates (phosphorylates itself) and thus activates upon binding of insulin (primary messenger) →
 - Phosphorylates IRS1 (Insulin Receptor Substrate 1) →
 - Interacts with molecules (e.g. Grub2, SOS) that lead to activation of a monomeric G protein called Ras →
 - Activates a kinase called Raf →
 - Sets off a phosphorylation cascade → (e.g. Raf → MEK → ERK)
 - Leads to transcriptional changes
- **Insulin signaling through PI3K and PKB/Akt:** You **do not** need to “know” this pathway, but you should be able to interpret diagrams of it and connect it to the bigger picture of how it relates insulin signals to glucose transport and glycogen breakdown
 - Involves activation of **PKB (Protein Kinase B), aka Akt**
 - “Only” in the muscles, heart, and adipocytes – PKB/Akt activation leads to the movement of “premade” membrane vesicles containing **GLUT4 glucose transporters** to the cell surface in response to insulin
 - PKB/Akt (not just in the above-listed tissues) counters the effects of PKA (thus glucagon) by activating a phosphatase called PP1 → inhibits glycogenolysis and activates glycogenesis

Metabolic Thermodynamics Expectations (LO10)

- Because there are literally infinite numbers of potential pathways, my goals for you are to know the fundamentals metabolic pathways and to be able to interpret pathway diagrams
- **You do not need to memorize pathways. You will be allowed to use your laminated reference guide on CC3 and will be provided with any diagrams you need**
- At this point, you are expected to be able to
 - Recognize metabolically reversible and metabolically irreversible reactions
 - Compare metabolically reversible and irreversible reactions in terms of energetics
 - Predict whether pathways are physiologically reasonable
- I will NOT try to confuse you with ΔG° vs. $\Delta G'^\circ$ and will use those interchangeably. BUT you DO need to be able to differentiate between ΔG° and ΔG

Overview of metabolism

- Metabolic pathways are highly interconnected
- The different component molecules of metabolic pathways can be referred to as **intermediates** and/or metabolites
- Metabolism encompasses **anabolism** (building molecules) and **catabolism** (breaking down molecules).
- There are also **anapleurotic reactions** which contribute to both anabolism and catabolism by replenishing pathway intermediates.
- Catabolic pathways are often oxidative → electrons from molecules are passed on to electron carriers such as NAD⁺ and FADH (to make NADH and FADH₂) in thermodynamically-favorable redox (oxidation/reduction) reactions.
 - NADH and FADH₂ carry electrons to the electron transport chain (ETC), which drives ATP synthesis through oxidative phosphorylation
 - This involves using energy released from redox reactions to pump protons out of the mitochondrial inner matrix into the intermembrane space, creating a gradient used to drive ATP synthase
 - The bulk of NADH production occurs through the citric acid cycle (aka tricarboxylic acid cycle (TCA), Krebs cycle)) which also serves as a central coordinating hub for both catabolic and anabolic pathways
- Anabolic pathways are often reductive
- Catabolic and anabolic pathways often share many enzymes and intermediates, but they are not exact inverses of one another since each needs to be energetically-favorable

Bioenergetics and thermodynamics

- Molecules will react in a way that takes them towards equilibrium. Once reached, they have no drive to react.
- K_{eq} : Generic formula for an **equilibrium constant** (K_{eq} , or simply K) - this relates the concentrations of products & reactants at equilibrium
$$aA + bB \rightleftharpoons cC + dD$$

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

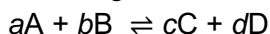
Where those are the concentrations **at equilibrium**

Often abbreviated [products]/reactants, but remember you need to take stoichiometry into account and multiply, not add, if you have multiple reactants and/or products

- $K_{eq} < 1$ if reactants are favored (there are more reactants than products at equilibrium)
- $K_{eq} = 1$ if there are equal amounts of products and reactants at equilibrium
- $K_{eq} > 1$ if products are thermodynamically favored (there are more products than reactants at equilibrium)

- **Reaction quotient (Q) - this relates the actual concentrations of products & reactants**

For the generic reaction



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

As above, sometimes this is abbreviated as [products]/[reactants]

- $Q < 1$ if there are more reactants than products
- $Q = 1$ if there are equal amounts of products and reactants
- $Q > 1$ if there are more products than reactants

Gibbs free energy change

- Tells you how much energy is released to reach equilibrium, from where you are starting
 - – if reaction goes in the forward direction as written (make more products) to reach equilibrium
 - + if reaction goes in the reverse direction as written (make more reactants) to reach equilibrium
 - 0 if the reaction is at equilibrium
- Comes down to changes in entropy and enthalpy: $\Delta G = \Delta H - T\Delta S$
 - *Note: Going back to LO3: You cannot know based on ΔG alone whether entropy (randomness/disorder) and/or enthalpy (bonds and interactions) are more favorable in products or reactants. (e.g. ΔH could be – but ΔS positive OR ΔH could be + but $-\Delta S$ positive OR ΔH could be – and ΔS negative)*
- **Interpreting ΔG :**
 - If ΔG is positive
 - The reaction is thermodynamically unfavorable in the indicated direction (forward as written unless specified)
 - The reaction is thermodynamically favorable in the non-indicated direction (reverse as written unless specified)
 - If ΔG is negative
 - The reaction is thermodynamically favorable in the indicated direction (forward as written unless specified)

- The reaction is thermodynamically unfavorable in the non-indicated direction (reverse as written unless specified)
- If ΔG is 0, the reaction is at equilibrium
- **Standard state**
 - The standard state in chemistry is defined as: all reactants and products at 1M concentrations (1 atm for gasses) & temperature = 298 K (25°C)
 - The standard state in biochemistry is defined as: pH 7.0 ($[H^+] = 10^{-7} M$); $[H_2O]$ is 55.5 M; $[Mg^{2+}]$ is 1mM
 - **STANDARD STATE DOES NOT = EQUILIBRIUM!!!! (Unless $K_{eq} = 1$ ($\Delta G^\circ = 0$))**
 - **STANDARD STATE DOES NOT = NORMAL/TYPICAL!!!!**
- **Standard free energy change (ΔG° or $\Delta G'^\circ$):** If you start with molecules at standard conditions – remember this includes equal amounts of products & reactants), how much energy is released to reach equilibrium; **physical constant of a reaction**
 - – if reaction goes in the forward direction as written (make more products) to reach equilibrium
 - + if reaction goes in the reverse direction as written (make more reactants) to reach equilibrium
 - In biochemistry, we often use the terms ΔG° & $\Delta G'^\circ$ (also can be written as $\Delta G'^\circ$) interchangeably, but there is a formal distinction
 - ΔG° = standard free-energy change under chemistry definitions of standard state
 - $\Delta G'^\circ$ = standard transformed free-energy change under biochemistry definitions of standard state
- Relating ΔG° to **equilibrium** concentrations:

$$\Delta G^\circ = -RT \ln K_{eq}$$

$$(\text{or } \Delta G'^\circ = -RT \ln K'_{eq})$$

- **Interpreting ΔG°**
 - If ΔG° is negative
 - Products are favored at equilibrium (more products than reactants at equilibrium)
 - The more the products are favored (larger the K'_{eq}), the more negative it will be
 - If ΔG° is positive
 - Reactants are favored at equilibrium (more reactants than products at equilibrium)
 - The more the reactants are favored (smaller the K'_{eq}), the more positive it will be
- **ΔG = actual free energy change under the given conditions**
 - **$\Delta G = \Delta G'^\circ + RT \ln Q$** Sometimes this is written as $\Delta G = \Delta G'^\circ + RT \ln([products]/[reactants])$
 - The concentrations of products and reactants (which can be controlled) greatly influences free energy change and thus favorability!
 - Dependent on current concentrations compared to equilibrium concentrations
 - If $Q > K'_{eq}$, ΔG will be positive $\rightarrow$ reaction will be driven towards making more reactants to reach equilibrium (reverse reaction favored)
 - If $Q < K'_{eq}$, ΔG will be negative $\rightarrow$ reaction will be driven towards making more products to reach equilibrium (forward reaction favored)
 - If $Q = K'_{eq}$, ΔG will be 0 $\rightarrow$ reaction is at equilibrium $\rightarrow$ no net change in concentrations of products or reactants

- Free energy changes are additive, so we can couple reactions together and use thermodynamically-favorable reactions to drive thermodynamically-unfavorable ones
- The energy given off in one direction is equal in magnitude to the energy required in the other direction

Equilibrium and steady-state

- The further a reaction is from equilibrium in terms of concentrations of products & reactants, the more energy will be given off to get to equilibrium
 - Thus, thermodynamic favorability is highly dependent on concentrations
 - Even inherently favorable reactions depend on concentrations (though differences in concentrations need to be more “extreme” since the proportions of products vs reactants at equilibrium are already extremely skewed)
 - Cells control concentrations of reactants and products to control thermodynamic favorability of metabolic reactions. This allows them to:
 - Control the directionality of metabolically reversible reactions
 - Control the energy released by metabolically “irreversible” reactions (e.g., keep their energy currency (ATP) valuable)
- Life is driven by the desire for equilibrium. But, once reached, life would cease to exist because there’s no thermodynamic drive → equilibrium is purposefully kept out of balance.
- **Steady state** keeps constant levels of metabolic intermediates despite being away from equilibrium
 - It happens by forming (can be via multiple routes) & consuming (can be via multiple routes) or otherwise removing these intermediates at equal rates (rather than forming & un-forming them at equal rates)
 - Unlike equilibrium, it allows concentrations of reactants and products to change
 - Think of water flowing through a pipe (steady state) vs. water just sitting in a sealed pipe (equilibrium)

Metabolic networks include both readily reversible, as well as metabolically-“irreversible” reactions

- Some reactions have a low magnitude (near equilibrium) ΔG and are thus **metabolically reversible**
 - You can control the direction of the reaction simply by altering concentrations of products & reactants (**thermodynamic regulation**)
 - Think of a swinging door where you can woo people from either side
- Some reactions are so inherently thermodynamically favorable in one direction (large ΔG°) that we call them **metabolically “irreversible.”**
 - These serve as no-going-back-easily points of metabolic pathways and thus are highly regulated
 - They often involve ATP hydrolysis (or similar reactions)
 - Think of a door with a handle only on a single side. Theoretically you could break through the door to go through in the “wrong” direction, but it would take a lot of energy!
 - Rather than reversing irreversible reactions, cells take alternative routes around them.
- Overall, pathways need to have a $-\Delta G$
 - But a $-\Delta G$ in one direction = an equally $+\Delta G$ in the other direction
 - So, anabolic & catabolic pathways cannot be simple inverses of one another
 - Rather, they share reversible reactions and have different irreversible ones

Thermodynamic vs. kinetic regulation

- Controlling the metabolic network involves controlling concentrations of substrates and products (**thermodynamic regulation**) as well as controlling the concentrations and activity of enzymes (**kinetic regulation**)
 - **Thermodynamic regulation:** Metabolically reversible reactions (but not irreversible reactions) can be controlled by controlling substrate and product concentrations
 - **Kinetic regulation:** Metabolically irreversible reactions can be controlled by regulating the enzymes that catalyze them
 - As covered in more depth in later lessons, this can involve things like:
 - Post-translational modification (often phosphorylation/dephosphorylation to activate or deactivate the enzymes)
 - Allosteric regulation (regulation through binding of small molecules such as pathway intermediates)
 - Transcriptional regulation
 - Compartmentalization

ATP

- ATP acts as a cellular energy “currency”
 - It stores energy from various types of fuels and, once made, regardless of its source, it can be used by numerous enzymes to power a vast number of processes
- **Reaction coupling:** Standard free energy changes are additive → favorable reactions can be coupled to unfavorable reactions to provide the energy needed to drive them
 - ATP Usually Provides Energy by Group Transfers, Not by Simple Hydrolysis
- Energy comes from **hydrolysis** of ATP, not the physical breaking of the bond itself
 - Includes charge repulsion, greater resonance stabilization & hydration of products than ATP
 - It is inherently favorable, ($\Delta G^\circ = \sim -7.3$ kcal/mol)
 - And keeping a high [ATP]/[ADP] ratio in cells makes it even more energetically favorable!
- When the ATP level drops:
 - The *amount* of fuel decreases
 - The fuel itself loses its potency
 - So, cells need to keep high [ATP]/[ADP] (and [ATP]/[AMP]) ratios
- Other phosphorylated compounds and thioesters also have large, negative free energies of hydrolysis & some can be used to make ATP (via substrate-level phosphorylation)
- **Making and storing ATP**
 - Energy from favorable redox reactions drives the production of ATP from ADP and Pi
 - Mainly in **electron transport chain + oxidative phosphorylation** in the mitochondria, using electrons carried in by NADH & FADH₂
 - Bulk of these comes from the TCA
 - **“Substrate-level phosphorylation”:** when ATP is produced by transferring a phosphoryl group from another organic molecule (as opposed to from an inorganic phosphate, as occurs in oxidative phosphorylation)
 - Muscles store extra energy as **creatine phosphate**
 - **Adenylate kinase** lowers the ADP concentration and replenishes ATP during periods of intense demand for ATP
 - $ADP + ADP \rightleftharpoons ATP + AMP$

- **ATP, ADP, & AMP act as allosteric regulators of metabolic enzymes (often those catalyzing irreversible steps)**
 - Low ATP and/or high ADP or AMP → increase catabolism, inhibit anabolism
 - High ATP and/or low ADP or AMP → increase anabolism, inhibit catabolism
 - AMP is a more sensitive indicator of energy status than ATP or ADP
 - AMP also activates AMPK which regulates other enzymes

Redox Expectations (LO9)

- Provided reaction diagrams, recognize when redox is and isn't occurring and differentiate between oxidation and reduction
- Compare and contrast electron carriers
- Interpret reduction potentials and order electron carriers into pathways based on them
- Relate differences in reduction potentials to free energy (but won't need to do ΔG calculations)

Redox

- OIL RIG: Oxidation Is Loss of electrons or electron density. Reduction Is Gain of electrons or electron density.
 - Oxidation is often (but not always) accompanied by gain of bonds to oxygen and/or loss of bonds to hydrogen.
 - Reduction is often (but not always) accompanied by loss of bonds to oxygen and/or gain of bonds to hydrogen.
- Redox reactions involve transfer of electrons. Protons have no electrons, so protonation and deprotonation are not redox reactions.
- Electrons are often transferred in pairs in the form of **hydrides** (proton with 2 e⁻)
- **Reducing agents/reductants** donate electrons to other molecules. This reduces those molecules and oxidizes what was the reducing agent (which can now serve as an oxidizing agent/oxidant)
 - Common reducing agents our body keeps stocked up are glutathione & NADPH
- **Oxidizing agents/oxidants** take electrons from other molecules. This oxidizes those molecules and reduces what was the oxidizing agent (which can now serve as a reducing agent/oxidant)
 - The common oxidants our body keeps stocked up are NAD⁺ and FAD
- NAD(H) is used for catabolism.
 - NAD⁺ concentrations are kept higher than NADH concentrations
 - This increases thermodynamic favorability of oxidation reactions involving NAD⁺ by decreasing Q (taking things further from equilibrium)
- NADP(H) is used for anabolism.
 - NADPH concentrations are kept higher than NADP⁺ concentrations
 - This increases thermodynamic favorability of oxidation reactions involving NADPH by decreasing Q (taking things further from equilibrium)
- **Oxidation numbers**
 - The higher (more positive) the oxidation number, the more oxidized a molecule is (fewer electrons it has). The lower (less positive) the oxidation number, the more reduced a molecule is (more electrons it has).

- Redox reactions increase the oxidation number of the molecule getting oxidized (the reductant/reducing agent) and decrease the oxidation number of the molecule getting reduced (the oxidant/oxidizing agent)
- **Reduction potentials**
 - The higher the reduction potential, the more a molecule wants electrons.
 - The lower the reduction potential, the more a molecule wants to donate electrons.
 - *“Positive” vs. “Negative” is just in reference to a reference electrode, so try not to get confused by it!*
- It is thermodynamically-favorable to pass electrons from a molecule with a lower reduction potential (less positive) to one with a higher reduction potential (more positive)
 - The greater the difference in reduction potential between the giver and taker (ΔE), the more energy is released (and exactly the same amount more energy would be required to pass the electrons back).

ETC and Oxphos Expectations (LO9)

- You **should know**:
 - The path of electrons between complexes and soluble carriers from NADH and from FADH₂ to oxygen
 - Which complexes pump protons
 - The # of protons pumped and thus the amount of ATP that can be produced if you enter the pathway at different places
- The effects of perturbations to the ETC (via uncoupling agents, artificial electron acceptors, low ADP or O₂, inhibitors, etc..) on electron flow, ATP production, and H⁺ gradients
- How many H⁺ are required per ATP and why/where they are used
 - 4 total: 3 for ATP synthase + 1 to bring in a new Pi
- You **DO NOT need to know**:
 - The mechanism of ATP synthase (in terms of which parts are which, catalytic mechanism etc.)
 - The flow of electrons within complexes
 - The Q cycle

Overview

- Much of catabolism involves the breakdown of molecules through oxidative reactions
 - In these oxidations, electrons are passed off to carriers like NAD⁺ and FAD, which take them to the ETC
 - In the electron transport chain (ETC), electrons are passed off through a series of carriers, with the energy from the transfers used to power ATP production
 - The bulk of the NADH & FADH₂ generated in previous steps comes from the TCA (TriCarboxylicAcid/citric acid/Kreb cycle), but can also come from other sources (e.g. lipid catabolism)
- Energy from electron pass-offs (redox) powers the synthesis of ATP – overview
 - Released energy from favorable redox reactions (passing electrons from molecules with lower to higher reduction potential) can be used to power energetically-costly processes like the production of ATP
 - Rather than passing directly from something with a really low reduction potential to something with a really high reduction potential (big burst of energy), you can pass electrons (and thus let

off small bursts of energy) stepwise through pass-offs between molecules with smaller differences in reduction potential

- Electrons can be carried by carriers such as NADH, FADH₂, ubiquinol (QH₂), and metals held in proteins
- A common final acceptor of electrons is oxygen → end result is water
- **Chemiosmotic model:** Passing electrons stepwise to oxygen in the electron transport chain (ETC) is used to pump protons into a mitochondrial compartment, creating a proton gradient that is used to power the production of ATP

Electron carriers:

- Be prepared to compare and contrast
- **NADP(H): small soluble carrier**
 - NAD(P)⁺ = oxidized form (still negatively charged overall, but there's a + charge on the N)
 - NAD(P)H = reduced form
 - Transfer hydrides
 - Can pass e⁻ to Q in CI
 - Many sources in both cytoplasm & mitochondria, but can't cross mitochondrial membrane so electrons from it have to be shuttled in (more to come in upcoming lessons!)
- **FAD, FMN:** organic groups held tightly in proteins called flavoproteins, typically have a higher reduction potential than NAD, but potential depends on protein carrier
 - Since they have a higher reduction potential than NAD, you get more energy passing electrons to them, but less energy passing electrons from them
 - Often used for more energetically costly redox reactions, like C-C double bond formation
 - But this leaves less energy when passing to Q, so not protons are pumped when they do
 - Can pass e to Q (in CII or through other enzymes)
- **Coenzyme Q:** Q(H): Q=ubiquinone (oxidized form), QH₂=ubiquinol (fully reduced form): small molecule, but nonpolar so diffuses back and forth in membrane
 - CoQ (ubiquinone) can accept 1 or 2 electrons (and protons)
 - 1 of each → ubisemiquinone radical
 - 2 of each → ubiquinol
 - Serves as sort of "universal" carrier
 - There's a "pool" of it with contributions from many sources (CI, CII, other enzymes . . .)
- **Cytochromes:** proteins w/Fe-containing hemes
 - Cytochrome C (cytC): small protein that associates with the inner mitochondrial membrane in the intermembrane space
- **Metals** (mainly iron and copper)
 - Held in complexes in ETC
 - E.g. iron-sulfur (Fe-S) proteins

Mitochondrial Electron Transport Chain (ETC)

- Comprised of Complex I (CI) - CIV (CV is ATP synthase)
- NADH through the ETC in terms of complexes:
 - NADH → CI → Q → CIII → cytC → CIV → O₂
 - NADH → Q (through CI) → cytochrome C (cytC) (through CIII) → O₂ (through CIV)

- Flow of electrons from NADH through the ETC in terms of carriers: $\text{NADH} \rightarrow \text{Q} \rightarrow \text{cytochrome b} \rightarrow \text{cytochrome c1} \rightarrow \text{cytochrome c} \rightarrow \text{cytochrome a} \rightarrow \text{cytochrome a}_3 \rightarrow \text{O}_2$
- Flow of electrons from FADH_2 through the ETC in terms of complexes:
 - $\text{FADH}_2 \rightarrow \text{CII} \rightarrow \text{Q} \rightarrow \text{CIII} \rightarrow \text{cytC} \rightarrow \text{CIV} \rightarrow \text{O}_2$
 - $\text{FADH}_2 \rightarrow \text{Q}$ (through CI) $\rightarrow$ cytochrome C (cytC) (through CIII) $\rightarrow \text{O}_2$ (through CIV)
 - Flow of electrons from FADH_2 through the ETC in terms of carriers: $\text{FADH}_2 \rightarrow \text{Q} \rightarrow \text{cytochrome b} \rightarrow \text{cytochrome c1} \rightarrow \text{cytochrome c} \rightarrow \text{cytochrome a} \rightarrow \text{cytochrome a}_3 \rightarrow \text{O}_2$
- First: Get e to Q:
 - CI: NADH:ubiquinone oxidoreductase, NADH dehydrogenase
 - Takes e from NADH (variety of sources) and passes to Q
 - Pumps out 4 H^+
 - CII = succinate dehydrogenase (SDH) – part of citric acid cycle!!!!
 - Takes e from succinate to FADH_2 to Q
 - NO H^+ pumped
 - Not enough energy - FAD has a higher standard reduction potential than NAD^+ , so less difference between its reduction potential and that of Q
 - **YOU DO NOT GO FROM CI to CII!!!!!! YOU GO FROM CI TO Q OR FROM CII TO Q!**
 - You can also get e to Q from other sources (e.g. fatty acid catabolism)
- Next: Get e from Q to CIII (Cytochrome bc1 complex, ubiquinone: cytochrome c oxidoreductase)
 - Electrons passed one at a time from Q to cytC (which can only take 1 at a time) in “Q cycle” (don’t worry about it)
 - Pumps out 4 H^+
- Then: Get e from cytC to CIV: Cytochrome oxidase
 - cytC just takes them there it’s a small protein that can move through the intermembrane space
- Then then: Get e through CIV (Cytochrome oxidase) to O_2 to make H_2O
 - 2 H^+ pumped per NADH or FADH_2
- Net sum of protons pumped:
 - From NADH: $4 \text{H}^+ + 4 \text{H}^+ + 2 \text{H}^+ = 10 \text{H}^+$
 - From FADH_2 : $4 \text{H}^+ + 2 \text{H}^+ = 6 \text{H}^+$

Oxidative phosphorylation (oxphos)

- Oxidative phosphorylation uses the proton gradient created by the electron transport chain to power the synthesis of ATP
- ATP synthase mechanism
 - ATP synthase (Complex V) is an incredible molecular machine
 - 2 distinct (multisubunit) components:
 - F_0 : integral membrane protein with a proton pore
 - includes a, b, c, d, F6, OSCP (non-Greek letters)
 - F1 (originally F1 ATPase): a peripheral membrane protein
 - includes 3α , 3β , δ , γ , ϵ (Greek letters)
 - **Rotational catalysis**: mechanism by which the flow of protons through F_0 causes its c ring to rotate and, in turn, trigger the subunit conformational changes in F1

- ATP **release** is the hard part
 - Proton flow causes conformational changes that help kick it out
 - Models include binding-change model: the active site of each β subunit of F₁ cycles through the 3 conformations that take turns catalyzing ATP synthesis
 - Each complete rotation synthesizes 3 ATP
 - Connecting the movement of protons to the movement of F_o
 - The a subunit is stationary and the c ring rotates
 - Transient protonation of a key Glu or Asp residue in each c subunit elicits conformation changes that drive rotation & transmit protons
 - Requires 4 H⁺ per ATP
 - Translocation of three protons fuels synthesis of one ATP
 - But you have to spend a 4th H⁺ to bring in another Pi
 - For each NADH that goes in, ~10 protons get pumped out, and it takes 4 protons coming in for 1 ATP to be made. $10/4 = 2.5$, so you get ~2.5 ATP per NADH
 - For each FADH₂ that goes in, ~6 protons get pumped out, $6/4 = 1.5$, so you get ~1.5 ATP per FADH₂
 - An adenine nucleotide translocase serves as an antiporter to take out the newly-made ATP in exchange for another ADP to phosphorylate
- **Uncoupling agents**
 - Uncoupling agents and uncoupling proteins let protons flow freely through - energy lost as heat rather than used to make ATP
 - These weak acids move protons into the matrix, where the higher pH causes them to deprotonate
 - ETC activity increases because it gets easier to pass electrons since it's easier to pump protons and the 2 are coupled, but ATP synthesis goes down since H⁺ don't have to go through ATP synthase to flow back in
 - Ex. DNP, (2,4-dinitrophenol), FCCP, uncoupling protein in brown fat
 - **Artificial electron acceptors**
 - Molecules that can accept electrons without needing natural electron acceptors
 - **Reactive oxygen species (ROS)**
 - Holdups in ETC can lead to the formation of Reactive Oxygen Species (ROS)
 - Low amounts can play important signaling roles
 - High amounts can be highly problematic
 - There are mechanisms to protect against it
 - Oxphos is regulated by availability of O₂ & ADP
 - Inhibited by low O₂ &/or ADP
 - Inhibition of oxphos leads to buildup of NADH that inhibits catabolic pathways via allosteric regulation of enzymes