The thermodynamics and kinetics content review covers the following items tested in the Physical Sciences section of the MCAT. This material includes oxidative phosphorylation, substrate level phosphorylation, and the electron transport chain. This material overlaps with some content found in the Biological Section as well as material that can be found in Content Category 5E of the AAMC MCAT Content Checklist. Download a copy from our site.

Note that the enzyme kinetics material is also listed in the Biological Sciences Section. The enzyme kinetics content review is in chapter 2.

Chapter 1

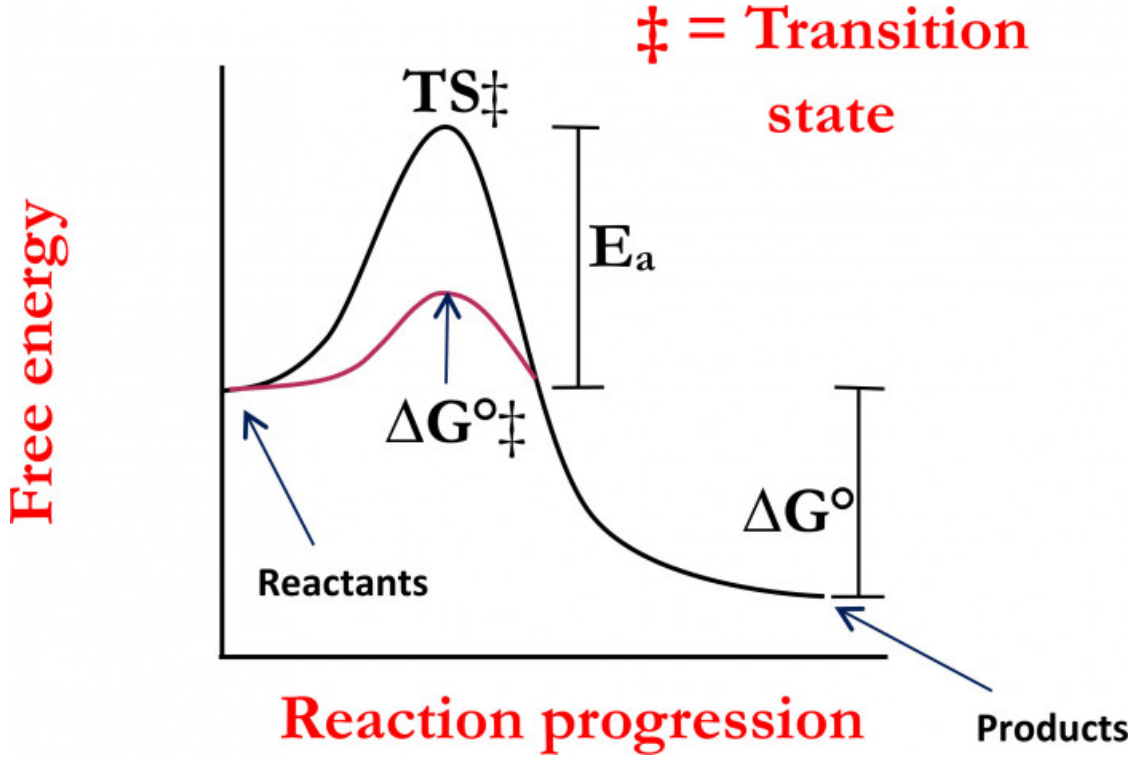
- ☐ **Thermodynamics vs Kinetics**
- ☐ **Gibbs Free Energy & Spontaneity**
- ☐ **Equilibrium & Steady State**
- ☐ **1st Law of Thermodynamics (work, heat, enthalpy)**
- ☐ **2nd Law of Thermodynamics (Entropy and the Hydrophobic effect)**
- ☐ **Coupled Reactions (Oxidative Phosphorylation and Electron transport chain)**
- ☐ **Electron Transfer Potential**
- ☐ **Heat Exchange**

INTRODUCTION

Chemical reactions involve changes in energy that result from the formation and dissociation of bonds. The energetic changes that occur during chemical reactions can be described through both thermodynamics and kinetics. For a given reaction A + B ⇒ C, thermodynamics describes the relative levels of reactants (i.e. substrate and product) at equilibrium, a point in the reaction where no further changes take place over time. Kinetics describes how fast the reaction proceeds from reactants to products. For catalysts such as enzymes, this is reflected in the rate of formation of the transition state ($TS^{\ddagger}$), the maximal point of energy in the reaction.

Thermodynamics tells us whether or not we can harvest the free energy present in chemical bonds. The Gibbs free energy (ΔG) describes the maximal amount of energy that can be harvested and used to do work from any given chemical reaction. Work can be any of a number of things including the assembly and

disassembly of cellular structures, the contraction of a muscle, and the firing of a neuron during neurotransmission. For a reaction at equilibrium (see below), there is no net change in free energy ($\Delta G = 0$). Spontaneous processes occur if the total change in Gibbs free energy is negative.



The energy difference between the products and reactants is the standard Gibbs free energy change ($\Delta G°$). The value for this is a standard for comparing one reaction to another. It is a known chemical constant. The ° symbol represents "standard" chemical conditions: 1M reactants, pressure = 1 atmosphere, temperature = 25 °C, pH = 0. The standard for biological reactions is often written as $\Delta G°'$. These conditions are temperature = 25°C, pH = 7.0, and $[H_2O]$ = 1.0 M. For simplicity, we will mostly use $\Delta G°$. Think of standard conditions as a reference point. In the generic reaction progression shown here, the products have a lower energy than the reactants, meaning they are more stable. This reflects the fact that the reaction releases more energy than it consumes and is said to be exergonic (as opposed to endergonic if the reaction consumes more energy than it releases). The energy is derived from the chemical bonds that are formed and broken during the reaction.

Reaction coordinate diagrams describe the energetic changes that occur during the progression of a chemical reaction. By graphing the relative energies of the reactants, products, and transition state ($TS^‡$), valuable information can be gained regarding the chemical reaction. E_a represents the experimental energy

of activation. This is the amount of energy required to generate $TS^{\ddagger}$, a transient intermediate that has properties of both the reactants and the products. Note that the E_a is reduced in the presence of a catalyst (enzyme) as the $\Delta G^{\circ\ddagger}$, which represents the free energy of activation, is less than the activation energy.

Kinetics deals with how to actually perform a chemical reaction on an appropriate time scale and how to find a reaction pathway that accomplishes this. Once the energy is harvested from a set of chemical bonds, it must be conserved in any of several ways, or it will be lost to the atmosphere in the form of heat or entropy. One way to achieve this desired conservation of energy is through chemical coupling of reactions. In biology, this is largely accomplished through the catalytic activity and regulation of enzymatic activity. Here an enzyme will break chemical bonds, release energy and form new products that possess chemical energy potential in the electrons of the newly formed bonds. Chemical coupling is commonly seen during the various steps of biochemical pathways.

Equilibrium and steady state

A system at equilibrium is stable as a function of time and requires no input of energy for its maintenance. One example of a biological equilibrium is the maintenance of serum pH at 7.4. However, most biological systems exist in a dynamic equilibrium, or a steady state. Such systems remain constant as a function of time, but require a constant input of energy to maintain. One example of this is the maintenance of intracellular levels of sodium and potassium. Na^+/K^+ pumps use energy in the form of ATP to maintain the electrochemical gradient between a cell and its exterior.
If an equilibrium reaction is disturbed by any of several methods (i.e. temperature, loss of product), then the system will re-set itself through an adjustment known as Le Chatlier's principle. This is also called the principle of mass action. For example, in the reaction $H_2CO_3 \Rightarrow CO_2 + H_2O$, some of the carbon dioxide is drawn off as a gas. As an adjustment the reaction will proceed to the right (dissociation of carbonic acid) to re-set the equilibrium.

The relative levels of reactants and products at equilibrium for the reaction

$A + B \Rightarrow C + D$

can be written as an equilibrium expression:

K_{eq} = [Products]/[Reactants] = [C][D]/[A][B]

Where K_{eq} is the equilibrium constant.

The actual free energy change ΔG is related to the standard free energy change through the following equation:

$\Delta G = \Delta G° + RT \ln[K_{eq}]$

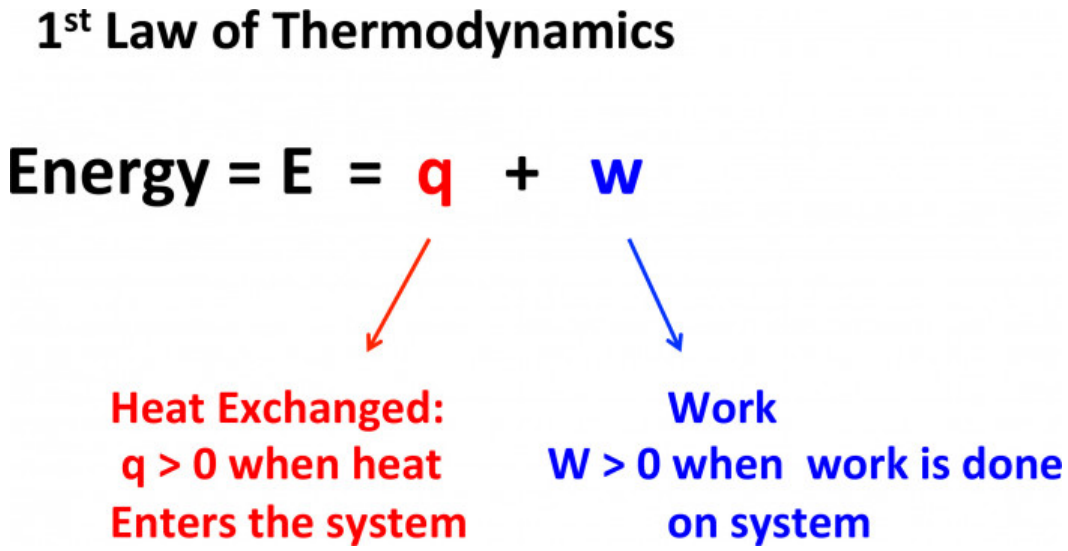
This shows that the free energy of a reaction is a function of the concentration of reactants and products at equilibrium. Importantly, reactions can go forwards and backwards through changing the concentration of reactants and products. In the equation, R = the gas constant (0.00198 kcal/mol/degree Kelvin) and T represents the temperature in Kelvin units.

At equilibrium, ΔG = 0, meaning that

ΔG° = -RT ln[Keq]

Thermodynamics

Thermodynamics is an important topic that is always on the MCAT. Think of thermodynamics as the study of energy as it occurs in the form of heat and work. The MCAT content guide specifically discusses the laws of thermodynamics. The first law of thermodynamics is also known as the principle of energy conservation and is commonly written as E = q + w as shown in the figure.



There are multiple interpretations and applications of the law. One way to imagine this is to understand that the first law states that energy can neither be created nor destroyed, meaning that the energy (E) of the universe is constant. The universe can be thought of as a system (sys) plus its surroundings (sur).

Energy can be converted from one form to another. This can be seen over and over during metabolism. One of the best examples of this is the metabolic process of glucose oxidation that occurs in tissue through pathways such as glycolysis ($C_6H_{12}O_6$) and the Krebs cycle. Oxidation is the transfer of electrons from one molecule to another. Recall that combustion reactions use molecular oxygen (O_2) to completely oxidize carbon atoms residing in chemical bonds (i.e. methylene groups) to carbon dioxide (CO_2), the highest oxidation state for carbon. During glucose oxidation, the bonds in glucose are broken and a portion of the energy is transferred to NAD^+ to generate NADH. Thus, energy is converted from the chemical bonds of glucose to potential energy in NADH. Once the NADH is oxidized in the electron transport chain, it will then be converted into the potential energy of the proton gradient, which is further converted into the energy of the phosphoanhydride bond through the generation of ATP.

During glucose oxidation, bonds are broken and energy is released. Enthalpy (H) describes the total amount of heat given off in a reaction at constant pressure, a condition in which most reactions occur. As bonds are formed and broken during chemical reactions, heat is often consumed or liberated. Recall that the formation of bonds releases energy and the breaking of bonds absorbs energy.

BOND	Dissociation energy kJ/mol
C-H	400
C-O	350
C-C	350
O-H	450

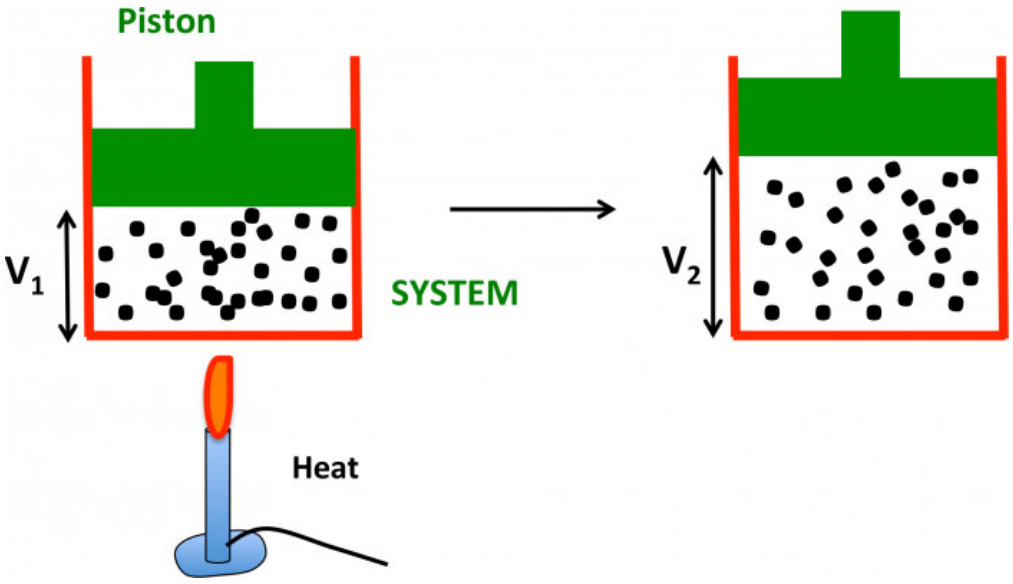
Bond dissociation energy is the energy of a single chemical bond and reflects the strength of a chemical bond. Bond energies can be used to estimate the heat of formation (ΔH_f) of compounds.

The energy released through the oxidation of carbon in the various bonds comprising glucose is converted into the electron transfer potential energy of the reducing carriers NADH and $FADH_2$. This occurs by the phenomenon of coupling, a key concept in metabolic reactions that create and use energy. During the process of mitochondrial electron transport, the reduction potential of NADH and $FADH_2$ is converted into ATP, the energy currency of the cell. This is discussed below in the context of oxidative phosphorylation, a common application of thermodynamics that you can expect to see on the MCAT.

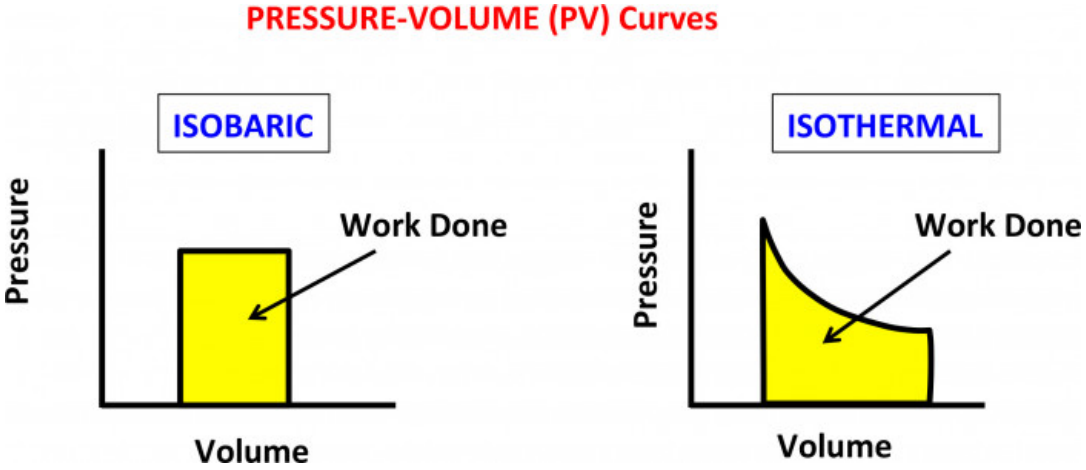
The first law of thermodynamics can also be seen as describing the changes in energy and heat as it is added to a closed system. A common example of such a system is shown in the figure with the presence of a gas confined in a cylinder by a piston. Upon heating the cylinder, the internal energy of the system increases (i.e. kinetic energy of gas molecules). As the heat is added to the system, the volume of the gas increases from V_1 to V_2. In this case, the system does work on the surroundings. The work (W) performed by the system is defined as:

$W = P\Delta V$

where P = pressure and ΔV represents the change in volume of the system. Here, the energy of the system performs work on the surroundings. Appreciate that when a system does work on the surroundings it loses energy. In this case, the energy of the system will change by $-\Delta W$. Therefore, the first law of thermodynamics can be written as $\Delta E = q - P\,\Delta V$. This can be seen in the case of a gas enclosed in a system held by a piston (see image). If a heat source is infused into the system, then the internal energy (E) of the system must increase according to the first law of thermodynamics. When heated, the system gains energy and this can be measured in the form of increased kinetic energy of the gas molecules in the system. The infused heat does work on the surroundings by pushing the piston up and increasing the volume ($V_2 > V_1$). Because the system (gas molecules) does work on the surroundings, the system loses energy in the form of work ($-\Delta W$). In this case, the heat energy is converted into another form of energy: work.



Pressure-volume (PV) curves show the changes in pressure vs volume in any of a number of processes. We show two PV curves: one at constant pressure (isobaric) and the other at constant temperature (Isothermal). The amount of work is equivalent to the area under the curve. Although PV curves are common in thermodynamics, they are also used in cardiovascular biology (work = pumping blood by the heart) and respiratory physiology (work = breathing). Expect to see these curves on the MCAT.



Second law of thermodynamics

According to the second law of thermodynamics, a reaction will proceed spontaneously in the direction that proceeds with an increase in entropy in the system (the chemical reaction) and the surroundings (the universe). Entropy

can be considered as a measurement of disorder, or the degree of randomness. One way to think about this is to consider "disorder" in the context of the number of states that something can exist in. In this manner, the number of places (i.e. states) where energy can be distributed is synonymous with entropy. For molecules this includes translational, rotational, and vibrational energy. Therefore,

$$\Delta S_{TOTAL} = \Delta S_{TRANSLATION} + \Delta S_{VIBRATION} + \Delta S_{ROTATION}$$

In order for a reaction to proceed spontaneously, $\Delta S_{system} + \Delta S_{surroundings}$ must be greater than zero. The Gibbs free energy is the maximal amount of energy available to do work and its must be negative for a reaction to proceed spontaneously. It is related to entropy through the following equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

ΔH° represents the standard change in enthalpy for a reaction. Positive and negative enthalpies are referred to as endothermic and exothermic reactions, respectively. Therefore, enthalpy is more or less the same as bond energy.

Application of the second law: The hydrophobic effect and entropy.

Because most reactions are performed in solution, solvent molecules such as water can interact with both the reactants and products. Polar solvent molecules such as water or ethanol congregate around those reactants and products that have charge (i.e. dipole moment). The interaction between the solvent and reactant, products, and even intermediates is called solvation and can greatly influence the enthalpy and entropy of a reaction.

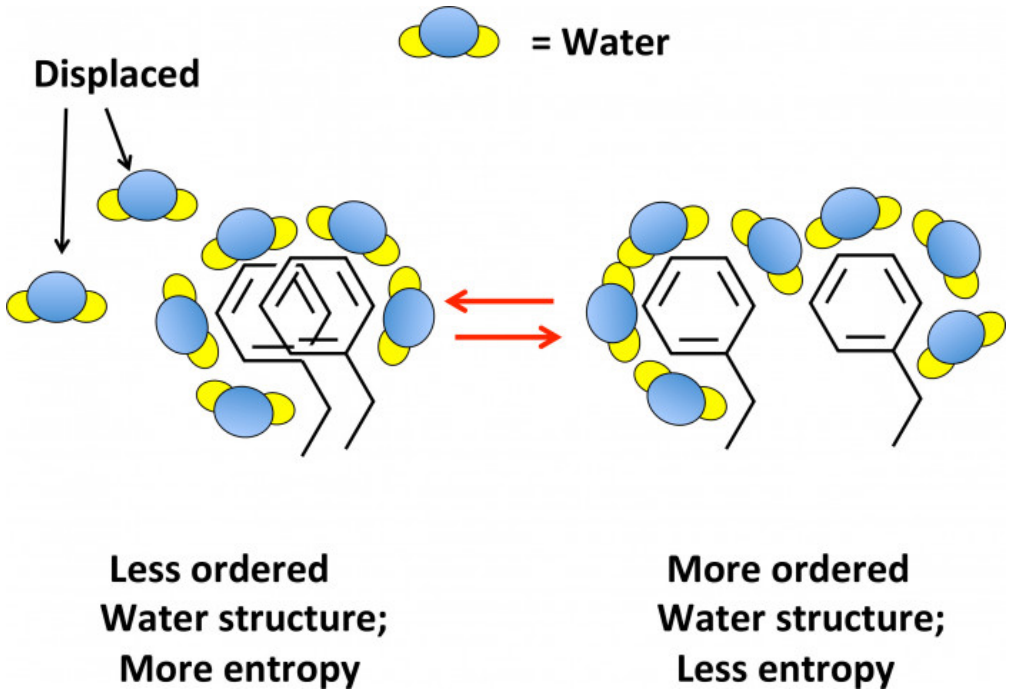
The notion of solvation can also be seen in the context of protein folding, the process of how a protein arrives at its three dimensional conformation. In the folded state, many proteins adopt a globular, compact structure composed of an inner core of hydrophobic residues (i.e. Phenylalanine) and a surface of hydrophilic, polar amino acids that interact with the aqueous solvent. Thus, hydrophobic residues limit their association with water, serving as the basis for the "hydrophobic effect". This results in a structure where water orderly surrounds hydrophobic structures. Intrinsic to the concept of order (and disorder for that matter) is entropy. Think of molecules as energy sources (i.e. kinetic energy of rotation and potential energy in chemical bonds). Entropy will therefore increase as they become more dispersed in space. As quoted from

entropysite.ocy.edu, "Entropy change is the measure of how more widely a specific quantity of energy is dispersed in a process."

Apply this concept to two interacting phenylalanine (Phe) residues in a polypeptide chain in the primary sequence (see image below). The hydrophobic effect contributes to protein folding involving these two Phe residues. By virtue of its hydrophobic benzene ring, Phe prefers to occupy the inner core of the folded protein in the final 3D configuration. Appreciate that the transfer of Phe from the hydrophobic interior of a protein to the hydrophilic exterior (or vice versa) will cause a change in the arrangement of the solvent (water) around the amino acids (i.e. dispersion). This hydrophobic effect is shown in the diagram.

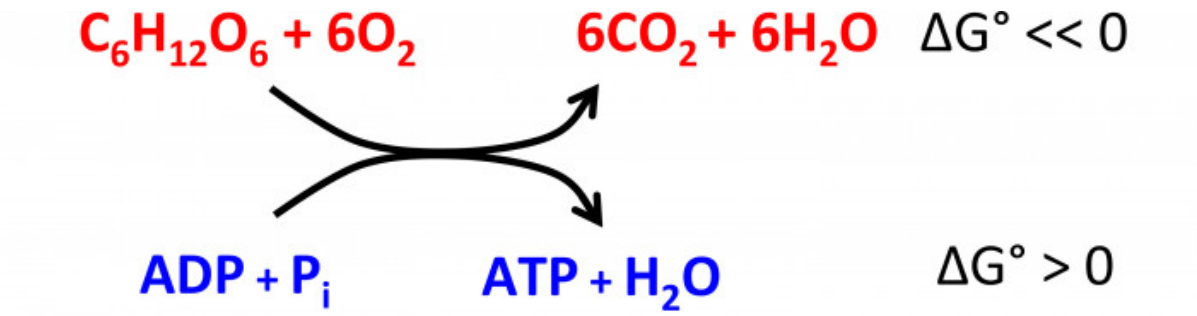
Think of the re-ordering of solvent molecules like water as an entropic effect. Because the hydrophobic Phe residues are being exposed to the aqueous solvent by virtue of translation, there would be an initial decrease in total entropy due ($\Delta S < 0$) due to the ordered solvation of the Phe side chain with water. Note that through the aromatic rings in Phe --arranged in a base-stacking configuration through π-π interactions as shown-- that water molecules are released into the bulk solvent. This makes the interaction more energetically favorable as it increases entropy, or the number of disordered states. Once again as per the law of thermodynamics, reactions proceed in the direction that results in an increase in the total entropy:

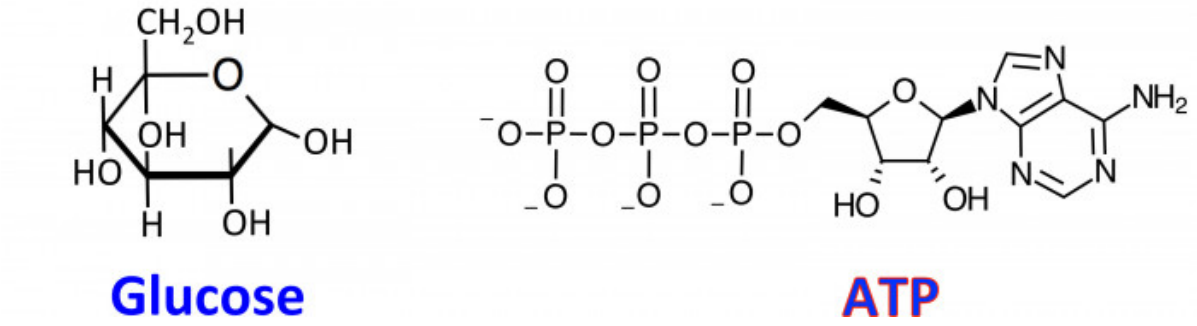
$$\Delta S_{system} + \Delta S_{surroundings} > 0$$



Thermodynamics and oxidative phosphorylation

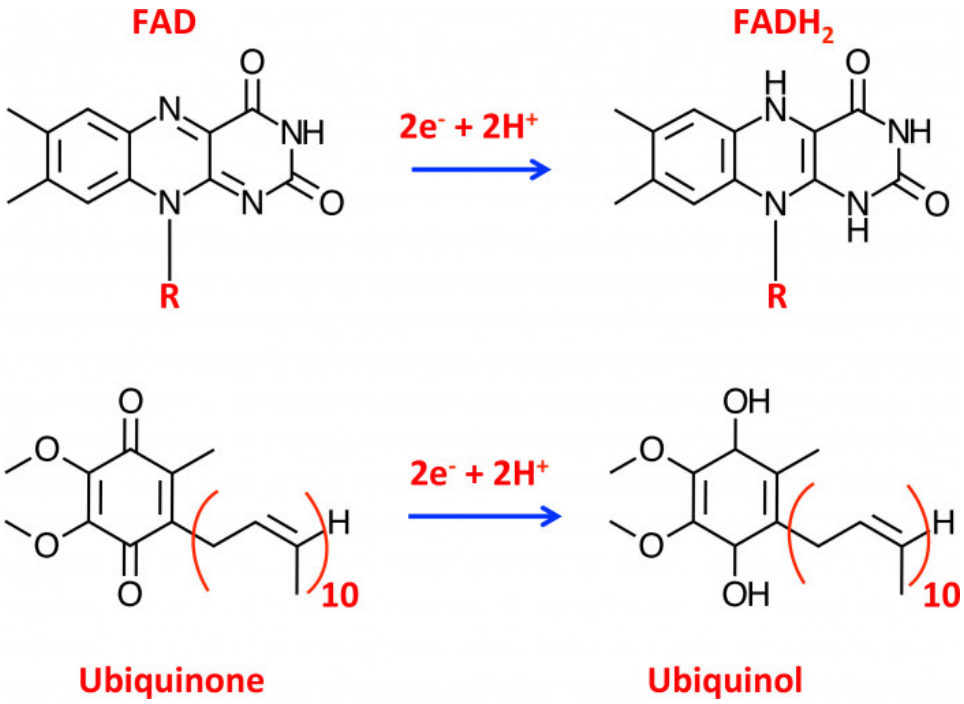
One of the best examples of coupling in biology can be seen in the process of oxidative phosphorylation. Never forget that oxidation is the loss of electrons and reduction is the gain of electrons (OIL RIG). Oxidative phosphorylation is the process by which the energy derived from the oxidation of glucose ($C_6H_{12}O_6$) is coupled to the synthesis of ATP through multiple pathways including glycolysis, Krebs cycle, and the process of electron transport. Oxidation of the C-C, C-H, and C-O bonds in glucose releases a lot of energy, particularly in the form of heat (enthalpy). As $\Delta G° << 0$ for the complete oxidation of glucose into CO_2 and H_2O, energy from this reaction can be coupled to the phosphorylation of ADP to generate ATP, the major form of energy currency in all cells. The conversion of ADP into ATP through the addition of inorganic phosphate is normally an endergonic process ($\Delta G° > 0$), but the TOTAL $\Delta G°$ for the reaction ($C_6H_{12}O_6 + 6O_2 = CO_2 + 6H_2O$) is negative when coupled to the complete oxidation of glucose. The electron bonds in glucose are ultimately converted into the high energy potential of the phosphoanhydride bonds of ATP.



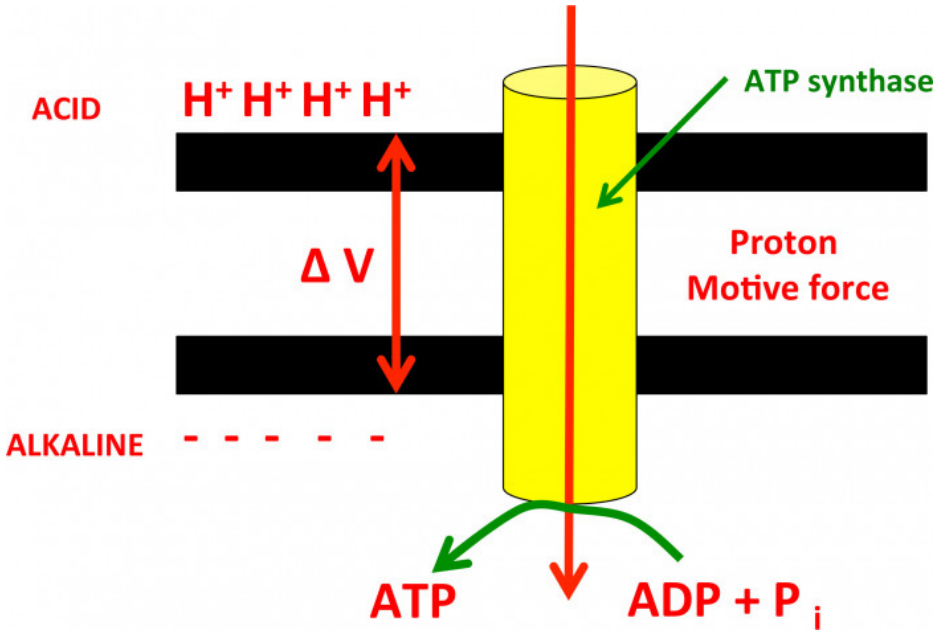


Electron transfer potential and synthesis of ATP.

The transfer of energy from glucose oxidation to the chemical bonds in ATP is not always a direct process, but it is most often conducted through chemical and electron carrier intermediates. This occurs in various biochemical pathways. The energy derived from the loss of electrons during glucose oxidation is transferred as high energy electrons to the reducing carriers NADH and $FADH_2$. These electrons are deposited into the mitochondrial electron transport chain.



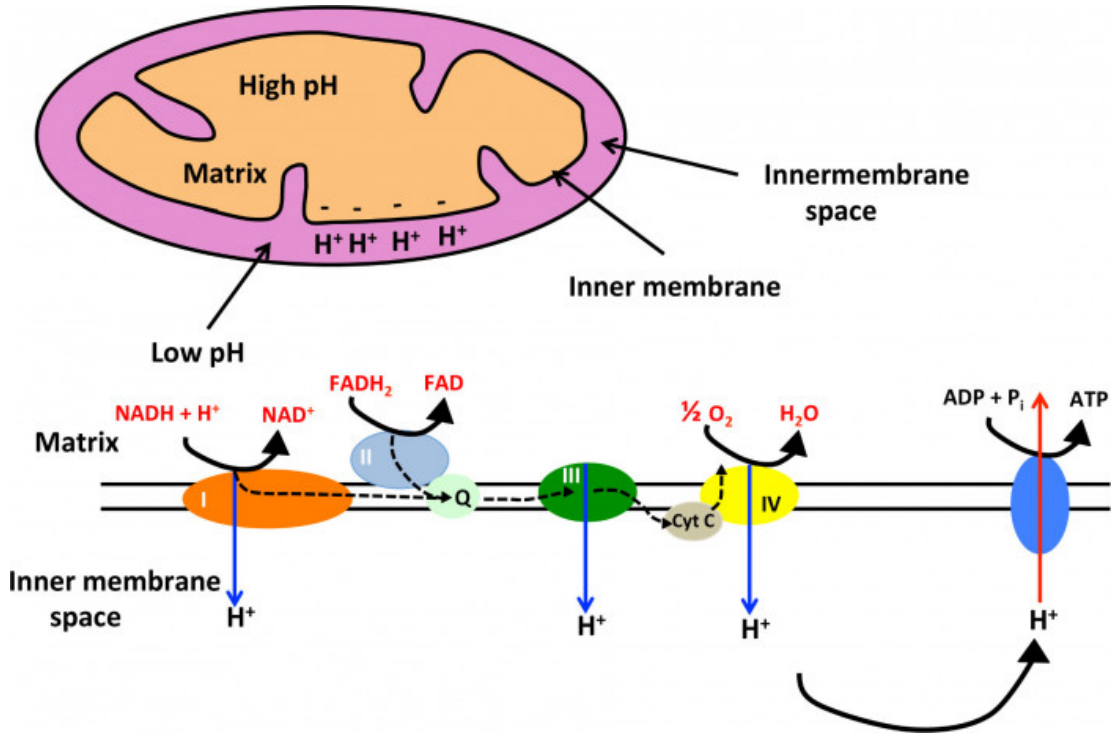
During electron transport, redox events with the hydride anion takes place (H:- = $H^+ + 2e^-$) with various electron carriers such as quinones and flavins such as $FADH_2$. Electrons flow from more reduced to less reduced carriers. Electron flow is concomitant with the directional flow of H^+ from the matrix to the inner membrane space. As electrons are transferred from carrier to carrier, protons are released into the inner-membrane space. This is because the inner mitochondrial membrane is impermeable to protons. Therefore, the reduction potential derived from the energy released upon the oxidation of carbon bonds in glucose is converted into a potential energy gradient composed of protons. Electrons are ultimately transferred to O2, creating water. The energy of the proton gradient is used in multiple processes, particularly the synthesis of ATP.

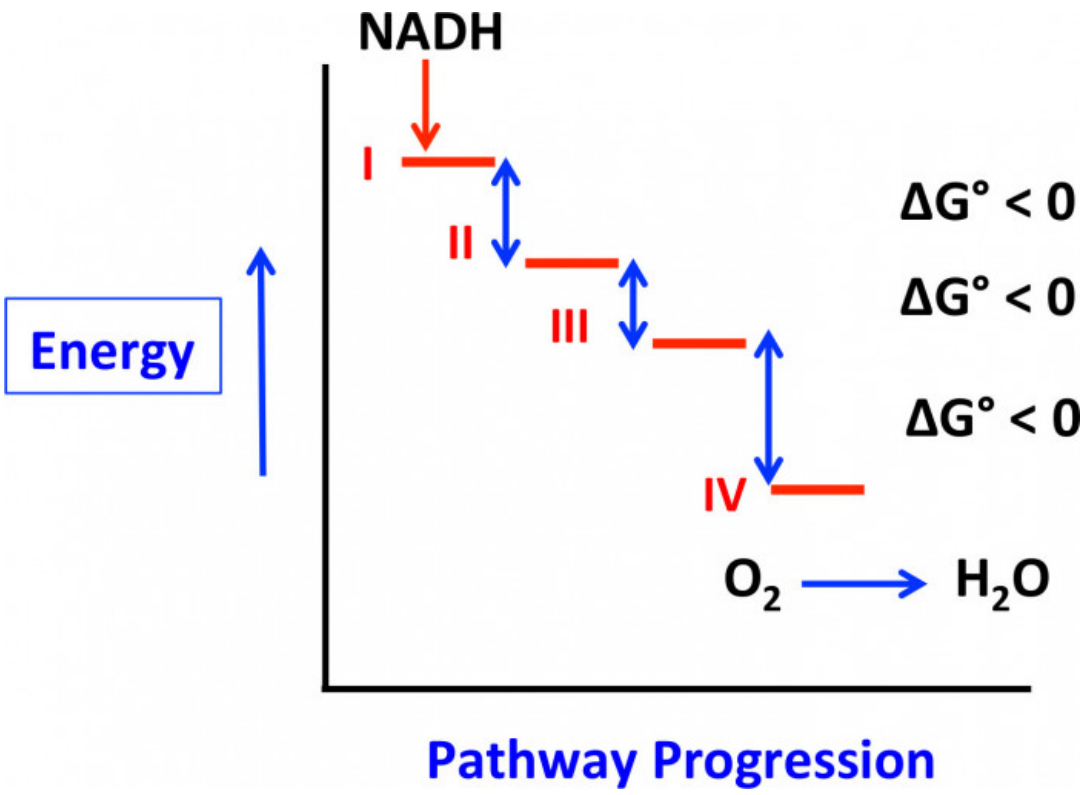


Each major step in the transfer of electrons from NADH to O2 is accompanied by large decreases in free energy as shown. The terminal acceptor in the pathway is oxygen; it is the least reduced acceptor in the entire chain and therefore is at the lowest energy point in the pathway. Electron transport centers I, III, and IV use various redox factors such as quinones (i.e. ubiquinone and ubiquinol), $FADH_2$, and cytochromes to translocate a pair of protons (proton motive force: ΔP) for the pair of electrons passing through the electron transport chain (see image).

ΔP = chemical gradient (ΔpH) + charge gradient (Δψ)

The transfer of protons generates an electrochemical gradient in the mitochondria that has enough potential to phosphorylate ADP (See figure below). A mitochondrial enzyme known as ATP synthase performs the coupling of the energy in the proton gradient with the phosphorylation of ADP. Under normal circumstances electrons will not flow down the transport chain unless ADP is being phosphorylated into ATP. Thus, the rate of ATP utilization is coupled to the rate of NADH oxidation. ATP synthase couples the conversion of the gradient into the high-energy chemical bonds of ATP. This flow of energy from reactants to products during multi-step biochemical pathways is the essence of bioenergetics and thermodynamics.


High pH
Matrix
H+ H+ H+ H+
Innermembrane space
Inner membrane
Low pH
NADH + H+
NAD+
FADH2
FAD
½ O2
H2O
ADP + Pi
ATP
Matrix
I
II
III
IV
Q
Cyt C
Inner membrane space
H+
H+
H+
H+


NADH
I
II
III
IV
ΔG° < 0
ΔG° < 0
ΔG° < 0
Energy
O2
H2O
Pathway Progression

The $\Delta G°$ of hydrolysis for phosphorylated metabolites is referred to as the transfer potential or phosphoryl group transfer potential. Approximate values for various metabolites are listed below in the Table.

Reaction Center	ENZYME	NOTES
1	NADH-Q OXIDOREDUCTASE	Lowest electron affinity in chain; uses NADH
2	SUCCINATE-Q OXIDOREDUCTASE	Enzyme is part of TCA cycle; uses $FADH_2$
3	Q-CYTOCHROME C OXIDOREDUCTASE	Oxidizes coenzyme Q and reduces cytochrome c
4	CYTOCHROME C OXIDASE	Catalyzes the reduction of O_2 to H_2O; Blocked by cyanide, carbon monoxide, azide

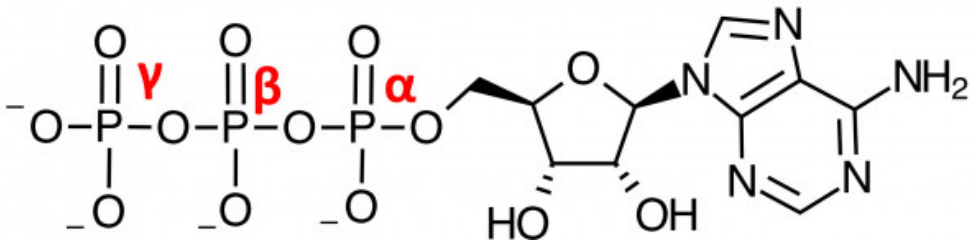
ATP hydrolysis releases approximately 7 kcal/mol. In some cases, the phosphate proximal to the ribose ring (the α phosphate) is targeted. In this case, AMP is generated and pyrophosphate (PP_i) is released. However, the hydrolysis of pyrophosphate is often coupled to the initial hydrolysis of ATP, generating a reaction that has $\Delta G° = -14$ kcal/mol. Such a large release of free energy makes the reaction essentially irreversible.

Phosphorylated Metabolite	ΔG° kcal/mol
PEP (phosphoenolpyruvate)	-15.0
Phosphocreatine	-10.0
ATP	-7.0
PP_i (Pyrophosphate)	-7.0
Glucose 6-Phosphate	-3.0

$$ATP + H_2O \longleftrightarrow AMP + PP_I \quad \Delta G° = -7.0 \text{ kcal/mol}$$

$$PP_I + H_2O \longleftrightarrow 2P_I \quad \Delta G° = -7.0 \text{ kcal/mol}$$

$$ATP + 2\,H_2O \longrightarrow AMP + 2P_I \quad \Delta G° = -14.0 \text{ kcal/mol}$$

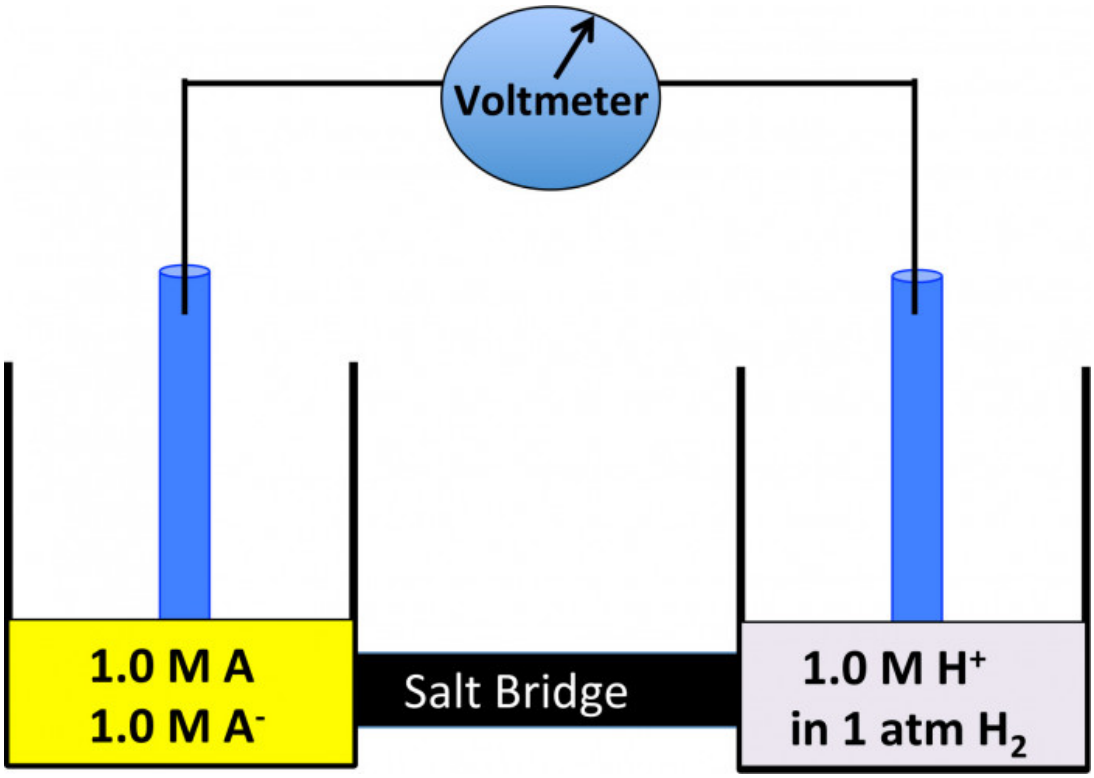


Electron transfer potential

During electron transport the transfer potential of electrons is converted into the phosphoryl chemical transfer potential that resides in the phosphoanhydride bonds of ATP. Think of the mitochondrial matrix in terms of a battery that stores charge, in this case protons generated from sequential redox reactions in the electron transport chain. The transfer potential of reactions obeys the laws of thermodynamics and can be expressed in terms of the electron reduction potential, designated E^O.

Therefore, electron transport is just an electrical circuit made up of conjugated rings (i.e. quinones and flavins) and ions (i.e. copper) that participate in oxidation/reduction reactions that allows current to flow from NADH to O_2. The proton gradient produced during this electron flow is both electrical and chemical. Stored in this battery is enthalpy.

We know that phosphoryl transfer energy is given by $\Delta G°$. However, the standard electron transfer potential, or more commonly referred to as the electron reduction potential, is given by $E°$ (or $E'°$). Take for example the redox couple A and A^-. The potential gradient across the membrane (ΔV) can be measured in millivolts (mV). Each unit change in the pH has an effect equivalent to a membrane potential of approximately 60 mV.



The reduction potential of the redox pair A and A^- is determined in a half-cell experiment by measuring the electromotive force (EMF). Such an experiment is performed with a standard half-cell reference that by convention is 1.0 M H^+ equilibrated in H_2 gas. The direction of the flow of electrons will proceed as per the laws of thermodynamics. If the reactions flow from the sample cell to the standard cell, then the sample cell electrode is arbitrarily said to be negative with respect to the electrode in the standard cell. As a convention, the reduction potential of H^+/H_2 is equal to 0 volts. Negative reduction potentials indicate that the oxidized form of the substance has a lower affinity for electrons than H_2.

The table below shows the standard reduction potentials for various biological molecules, including those involved in electron transport. E'° represents the partial reaction:

Oxidant + e^- = Reductant

Note that negative reduction potentials indicate that the oxidized form of the substance has a reduced affinity for electrons relative to the standard H_2.

Standard Reduction Potentials

Oxidant	Reductant	# e^- transferred	E'_o (Volts)
$NAD^+ + H^+$	NADH	2	- 0.32
FAD	$FADH_2$	2	- 0.22
Pyruvate	Lactate	2	- 0.19
Fumarate	Succinate	2	+ 0.03
Fe^{+3}	Fe^{+2}	1	+ 0.77
$½ O_2 + 2H^+$	H_2O	2	+ 0.82

The standard free energy change is related to the standard change in reduction potential through the following equation:

$$\Delta G° = -nF\Delta E°$$

where n = # electrons transferred; F = Faraday (23.0 kcal mol^{-1} V^{-1})

Heat exchange

The exchange of heat occurs in most chemical reactions. If heat is given off to the environment during this process, the reaction is said to be exothermic ($\Delta H° < 0$). If heat is absorbed during the reaction, then the reaction is endothermic ($\Delta H° > 0$). Because enthalpy is an indicator of heat, the standard enthalpy of formation ($\Delta H°_f$) is synonymous with the standard heat of formation. $\Delta H°_f$ is defined through the change in enthalpy during the formation of 1.0 mole of a substance in its standard state from its original elements at constant pressure.
Appreciate that heat is the transfer of energy across a system independent of matter and work. Compare this to temperature that is the average kinetic energy of matter occurring in a system. Heat can be transferred through substances in multiple ways including conduction, convection, and radiation. For conduction, heat is transferred within a stationary system with a

temperature difference. That is, heat is transferred from the high temperature region to the low temperature region. Convection involves heat transfer through gravity. For a solution, the hotter, less dense material will rise to the surface while the denser, lower temperature material will fall to the bottom. Heat is transferred during this process. Radiation is emitted through the thermal agitation of molecules. Heat is transferred through this process. The Stefan-Boltzmann Law describes the radiation as a function of time from a black body, a hypothetical reference point that absorbs ALL radiation on its surface.

The law is written:

$$q = \sigma T4A$$

q = transfer of heat/time; σ = Stefan-Boltzmann constant (5.67×10^{-8} W/m^2K^4); T = temperature in Kelvin; A = area in m^2.

Thermal expansion

In response to heat, matter changes its size, shape, and volume. As the temperature and kinetic energy increases, solids, liquids, and gases will experience more separation at the molecular level. This can be viewed as an overall larger average distance of separation between two nuclei whose electrons form a covalent bond. In the case of solids, the area, length and volume increase. The coefficient of thermal expansion characterizes the degree of expansion for various substances in response to temperature changes and can be described for the length (L), area (A), and volume (V) of a substance as follows:

Length	Area	Volume
$\Delta L/L = \alpha_L \Delta T$	$\Delta A/A = \alpha_L \Delta T$	$\Delta V/V = \alpha_V \Delta T$

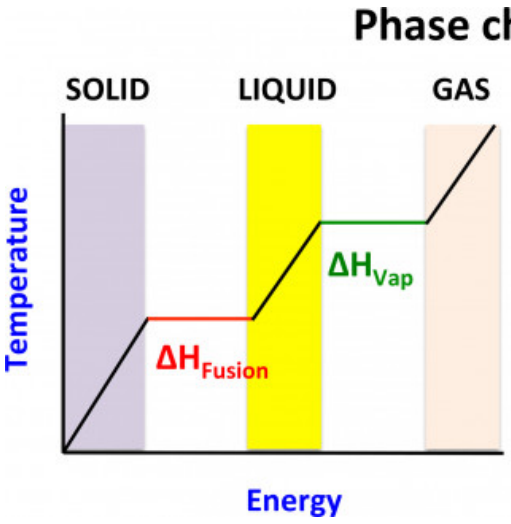
Material	α_L (°C^{-1})	α_V (°C^{-1})
Aluminum	2.4×10^{-5}	7.2×10^{-5}
Copper	1.7×10^{-5}	5.1×10^{-5}
Invar	1.0×10^{-6}	3.0×10^{-6}
Steel	1.2×10^{-5}	3.6×10^{-5}

The αL and αV values for four substances are listed in the table. Appreciate that these values are for specific temperatures. The data shown is for the temperature of 20 °C. Note from the table that the values for volume are related to those of length by a factor of three:

$$\alpha_V = 3\alpha L$$

Heat of fusion and heat of vaporization

The MCAT content outline specifically mentions the heats of fusion and vaporization. The amount of heat required to melt a substance is related to its heat of fusion (ΔH_{fusion} or ΔH_f) through the following equation: $q = m \times \Delta H_f$. In this equation, q = the amount of heat required to melt a substance, m = mass of substance in grams, and ΔH_f = the heat of fusion in cal/g. The term "heat of fusion" is synonymous with the term "enthalpy of fusion". Recall that the term "fusion" refers to the conversion of a solid to a liquid. Therefore, ΔH_f is seen as the change in enthalpy that occurs when heat (or another form of energy) is applied to a specific quantity of substance such that the state changes from solid to liquid at constant pressure. In an analogous fashion, the heat of vaporization ΔH_v refers to the amount of energy required to induce the state change from liquid to solid. The phase transitions that occur are the melting and boiling points, respectively.

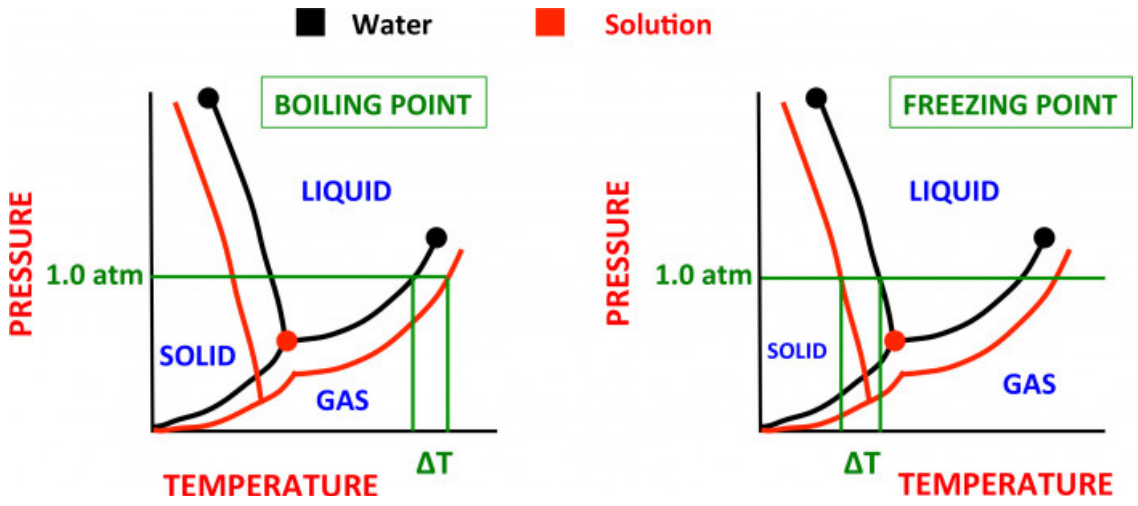


Substance	$\Delta H_{fusion(Cal/g)}$
Water	2.4×10^{-5}
Acetone	1.7×10^{-5}
Palmitic Acid	1.0×10^{-6}
Gallium	1.2×10^{-5}

Phase diagram pressure and temperature

Phase transitions occur in response to variations in pressure and temperature. The phase transition diagrams are shown for water and a typical sample such as CO_2. Compare the two samples and note that the transition line between the solid and the liquid phases have different slopes: negative for water and positive for CO_2 (a typical sample). The reason for the unique physical properties of water as shown is due to its ability to form hydrogen bonds. Recall that hydrogen bonds occur between a donor or acceptor hydrogen atom and an electronegative atom such as F, N, or O. H_2O can donate two H atoms and its oxygen atom can accept up to two H atoms by virtue of its two lone pairs of electrons.

Water is a notable substance because upon freezing, it expands due to changes that occur in its density. Water also has a very high heat capacity (4.18 J/g K), indicative of molecules with high thermal stabilities. The addition of non-volatile solutes such as NaCl to solutions of water alters the physical properties of water and therefore changes its phase transition diagram. This includes elevating the boiling point and reducing the freezing point. These physical properties are known as colligative (or "collective") properties of a solution and depend only on the number of solute particles in solution. When thinking about colligative properties appreciate that they are "intensive" properties like density and concentration, as they are independent of the size of the sample or system.



Recall that the definition of boiling point is the temperature at which the gas and liquid phases are in thermal equilibrium. The change in boiling point elevation is given by:

$$\Delta Tb = Tb\ (solution) - Tb\ (solvent) = i \times K_b \times m$$

In this equation, T_b = boiling point; K_b = ebullioscopic (or the "boiling" constant); and m = molality; i = the Van't Hoff factor, a measure of the colligative effect that a compound has upon its dissociation. The Van't Hoff factor = actual concentration of particles/concentration as calculated via mass. The Van't Hoff factor for non-electrolytes is usually 1.0, but for electrolytes such as NaCl, the value is two: $Na^+ + Cl^-$.

Appreciate that molality (mol/kg) is the amount of substance per mass of solvent, and differentiate this with molarity, which is the amount of substance per volume of solvent. In an analogous fashion, the freezing point depression is given by:

$$\Delta T_F = T_F\ (solution) - T_F\ (solvent) = i \times K_F \times m$$

where K_F is the cryoscopic constant.
Calorimetry and the measurement of heat exchange.

Calorimetry is the science of measuring heat transfer occurring during various physical and chemical processes. Calorimeters involve heating samples at defined rates and measuring the heat flow to and from the sample. As a control, all calorimeters use reference samples that have defined thermal properties.

Isothermal titration calorimetry (ITC) is a popular technique widely used in academics and in the pharmaceutical industry. ITC is a quantitative technique used to examine the affinity of ligands (i.e. chemical compounds such as inhibitors) to proteins (i.e. receptors and enzymes). Understanding the binding properties of drugs to their targets is clearly an important topic in pharmaceutical research. We think that ITC is likely to show up on the MCAT and Med-Pathway has a very insightful passage in the Protein Structure/Function and Purification Testing Module. An examination of this important technique will be elaborated on below.

Appreciate that like most reactions, ligand-protein binding events are accompanied by changes in the absorption or release of heat (ΔHB). This

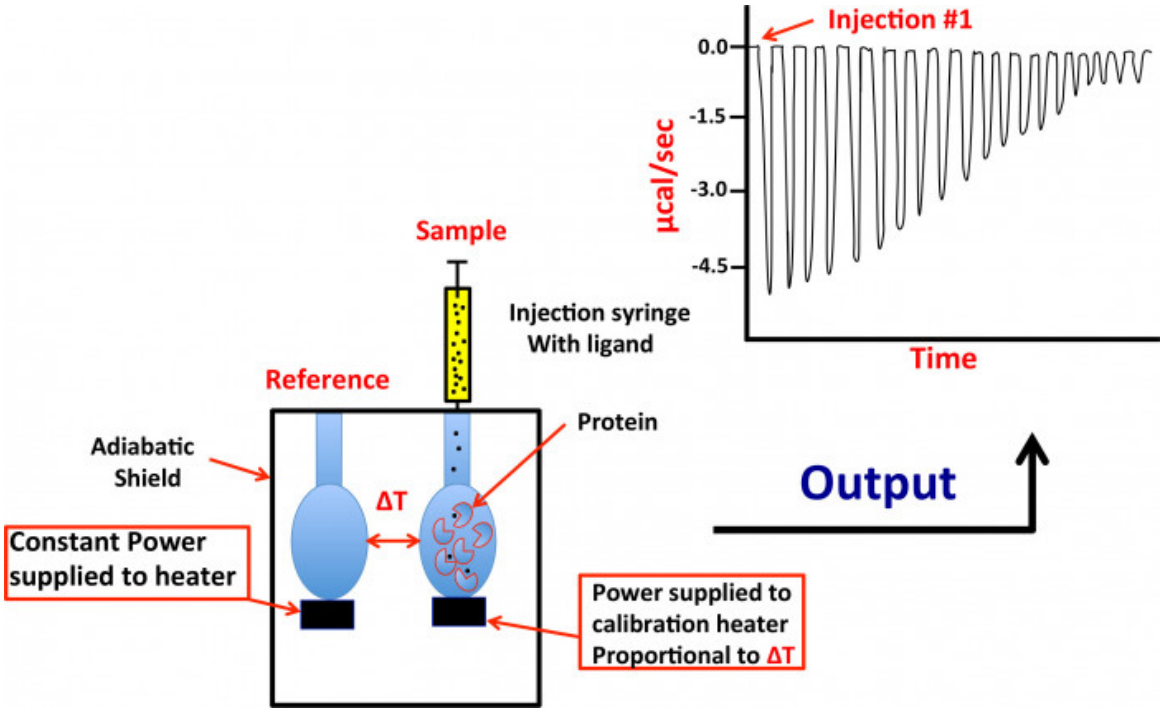
can be detected and quantified by ITC. Calorimetric signals detected by ITC represent direct measures of reaction rates. In addition to these kinetic parameters, a complete thermodynamic profile, including the determination of the Gibbs free energy, entropy, and the heat capacity (Cp, defined as $\Delta H/\Delta T$), can be achieved from the thermodynamic properties of binding events. In addition, the specific heat capacity, or "specific heat" can also be determined by dividing by the mass:

$Cp = \Delta H/(mass)\Delta T$

The calorimeter consists of two cells capable of thermal conduction surrounded by an adiabatic jacket as shown in the image. By injecting a solution of ligand into a cell containing its partner binding macromolecule, any changes in heat that occur as a result of ligand binding are detected (See figure). In the case of an exothermic reaction, the addition of ligand is detected as a positive change, the increase in temperature, in a sample cell.

After reaching equilibrium and returning to baseline, a second injection of ligand is delivered. Continued injections ultimately result in ligand saturation and this is seen in a series of isotherms in the figure below.

ITC experiments are conducted at constant pressure and the temperature is also made constant by applying power to a heating cell. Raw calorimetric signals are equated to the amount of power applied to maintain equivalent temperatures between the two cells. Recorded temperatures are converted into



a heat change.

Hess's Law

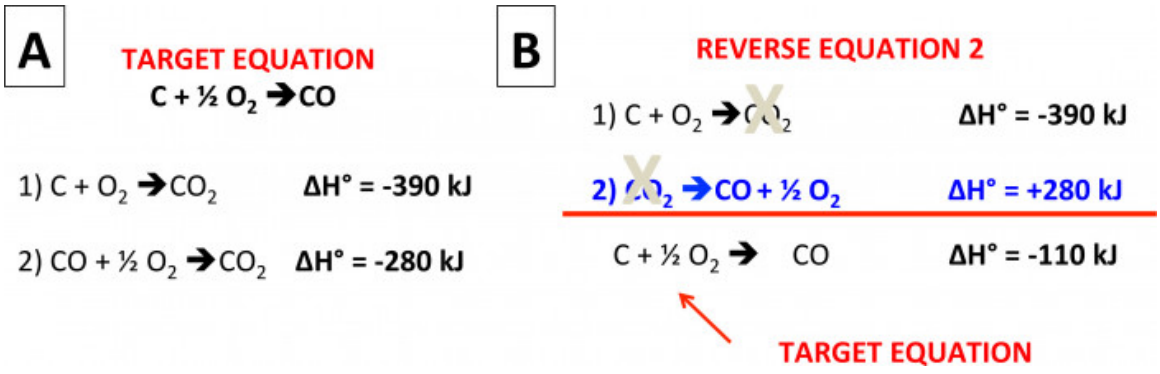
Hess' law is specifically mentioned on the MCAT content outline. In 1840, Germain Hess stated, "The heat evolved or absorbed in a given chemical process at constant pressure is always the same, whether the process takes place in several steps." Therefore, thermodynamic equations can be added or subtracted and even multiplied and divided. As the total enthalpy change for a reaction is equivalent to the sum of all individual reactions, Hess' law is a re-statement of the fact that enthalpy is a state function. That is, the heat of reaction at constant pressure is independent of the initial and final states. In mathematical terms:

$$\Delta H°_{RXN} = \Delta H°_{PRODUCTS} - \Delta H°_{REACTANTS}$$

A classic example of the use of Hess' law concerns the determination of the standard enthalpy of formation for the gas carbon monoxide (CO) from carbon (i.e. graphite) and oxygen. The reaction is given by:

$$C + \frac{1}{2} O_2 = CO$$

The approach of directly measuring the standard heat of combustion of carbon is confounded by the fact that CO_2 will also be formed as a side product in the combustion reaction. In order to avoid this issue, the heat of reaction for two separate reactions 1 and 2 can be determined as shown in Panel A of the figure. The heat of reaction can be determined through both of these equations as follows. To achieve the target equation, reverse the direction of equation 2 as shown in blue in Panel B. Note that upon reversal, the ΔH° changes from -280 kJ to + 280 kJ. Also note that the CO_2 molecules will cancel out. Because the equations can be treated as mathematical expressions,



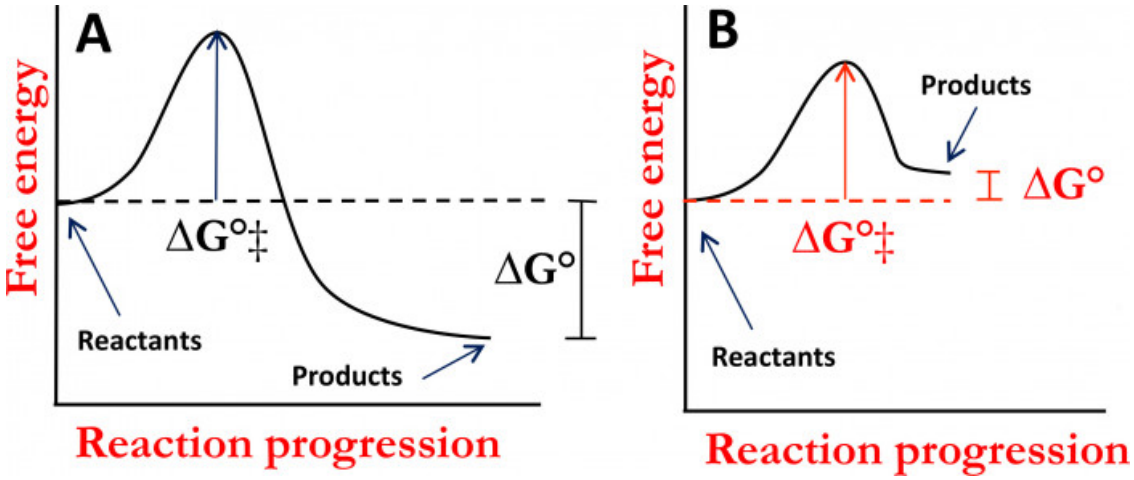
there will be a total of ½ moles O_2 as a reactant. The net $\Delta H°$ is therefore -110 kJ as shown.

Zeroth law of thermodynamics

The MCAT content guide specifically mentions the zeroth law of thermodynamics. According to Wikipedia, the Zeroth law can be stated as follows: "If two systems are each in thermal equilibrium with a third, they are also in thermal equilibrium with each other." This means that they are at the same temperature. Accordingly, Maxwell stated that, "All heat is of the same kind".

Kinetics

Whether a reaction is endergonic ($\Delta G° > 0$) or exergonic ($\Delta G° < 0$) has no bearing on how fast the reaction occurs. Rather, the rate of product formation is governed by kinetics. The thermodynamic stability is given by $\Delta G°$, but the kinetic stability is indicated by $\Delta G°‡$, the value of which correlates with the stability of the transition state intermediate. Lower $\Delta G°‡$ values represent faster reactions and more stable transition state intermediates. $\Delta G°‡$ is closely related to the experimental energy of activation (E_a). This is because $\Delta G°‡ = \Delta H°‡ - T\Delta S°‡$ and $E_a = \Delta H°‡ + RT$. That is, $\Delta G°‡$ depends on both enthalpy and entropy, but E_a only depends on an enthalpy component. As reactions have entropy and enthalpy components, $\Delta G°‡$ is therefore the true energy barrier to the formation of the intermediate; E_a is therefore an approximation.



Note that the reaction in panel A is exergonic ($\Delta G° < 0$) as the products are more stable than the reactants. This is in contrast to the endergonic reaction ($\Delta G° > 0$) shown in panel B where the reaction products are less stable than the reactants. Thus, the reaction in panel B absorbs more heat (consumes more energy than it releases) than the reaction in panel A. Therefore, in relative terms, reaction A is a slow, exergonic reaction and Panel B is a fast endergonic reaction. However, the reactants in reaction A are more kinetically stable (less reactive) than those in reaction B because of the relative magnitude of $\Delta G°^{‡}$ for each reaction.

For the reaction A + B ⇒ C + D, several conditions influence the rate of the reaction. For example molecules A and B must possess sufficient kinetic energy to overcome the energy barrier ($\Delta G°^{‡}$). Further, the rate of the reaction depends on the frequency of collisions between A and B. Although collisions are important for chemical reactions, the reactant molecules must interact in the proper orientation in order for bond formation to occur. In general, increasing the concentration of reactants and the temperature will facilitate the rate of the reaction.

The rate constant of a reaction reflects the ease at which the reaction can reach the transition state. This is not the same as the rate of the reaction, which is the amount of time required to form a given amount of product.

The Arrhenius equation describes the relationship between the rate constant of a reaction and the experimental activation energy (E_a), which was described above as an approximation to the true activation energy of the reaction. The Arrhenius equation is written as:

$$k = Ae^{-E_a/RT} \quad \text{OR} \quad \ln k = \ln A - \frac{E_a}{RT}$$

k = the rate constant; R = the gas constant (0.00198 kcal/mol/degree Kelvin) and T represents the absolute temperature in Kelvin units, and A represents what is called the "frequency factor". As can be inferred from its name, this term reflects the percentage of molecules that collide with the proper orientation to form reaction bonds.

Let's examine several types of reactions:

1) X ⇒ Y

In this first order reaction, the rate of product formation depends on the concentration of X. That is, the higher value of [X], the more product that will be formed. Therefore, the rate of the reaction is proportional to the amount of X. A rate law can be written for this reaction as:

Rate = k[X]

Here, k represents the proportionality constant, or more simply the rate constant. A first order reaction has a rate constant with units 1/seconds = s-1 (or in minutes, hours, etc).

2) A + B ⇒ C

In this case, the rate of the reaction depends on the concentration of two molecules, A and B. The rate law for such a second order reaction can be written as:

Rate = k[A][B]

The rate constant has units $M^{-1}s^{-1}$.

Not all reaction rates are apparently dependent on the concentration of various reaction components. Take the following reaction:

A + B ⇒ Product

If the [B] >>[A], then the reaction rate will not be dependent on the concentration of B. Instead the reaction appears zero order with respect to the concentration of B, and therefore behaves as if it is a first order reaction.

If a catalyst such as an enzyme is saturated with substrate, then the reaction can appear to behave as a zero order one. Take the following reaction:

A ⇒ B

One could imagine this as an isomerization reaction performed by an enzyme. If [A] is in excess, then the reaction is zero order and the rate law can be written as follows:

r = k

The integrated zero order rate law can be determined as:

$$[A]t = -k_t + [A]_O$$

where [A]t = the concentration of [A] at time = t and $[A]_O$ is the initial concentration of A.

The half life of a zero order reaction is related to the rate law through the following equation:

$$t_{1/2} = [A]_O/2k$$

The following table summarizes these reactions:

Reaction Order	Reaction	Rate Law	Integrated Rate Law	$t_{1/2}$ =
Zero	[S] → [P] Excess [S]	r = k	$[A]_t = -kt + [A]_0$	$\frac{[A]_0}{2k}$
First	X → Y	r = k[X]	$\ln[A] = -kt + \ln[A]_0$ or $A = A_0e^{-kt}$	$\frac{\ln(2)}{k}$
Second	A + B → C	r = k[A][B]	$1/[A] = 1/[A]_0 + 2kt$	$\frac{1}{k[A]_0}$

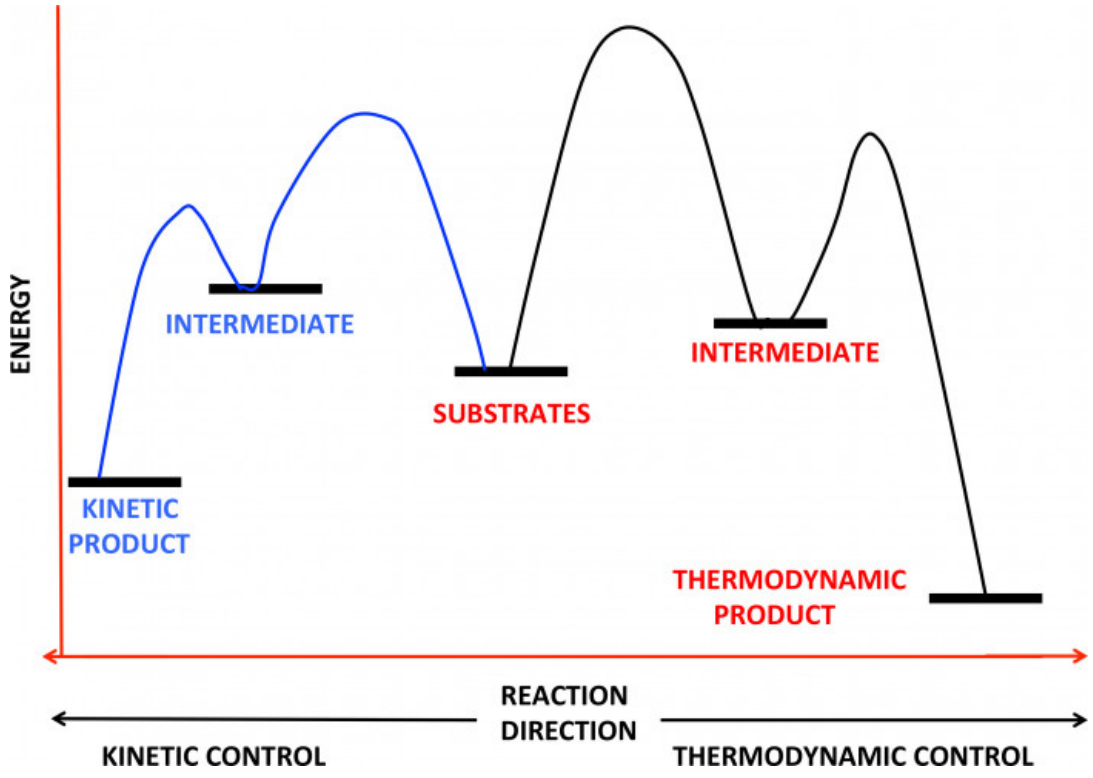
*** Note that the rate law for a reaction cannot always be determined from the reaction equation**

Kinetic control vs Thermodynamic control

MCAT Content Category 5E (Physical Sciences) lists kinetic control vs. thermodynamic control of reactions as a topic for testing. Thermodynamics is often compared to kinetics through the example of diamond turning into graphite. Although the reaction is permissible (i.e. spontaneous, $\Delta G° < 0$), it is very slow. Therefore, the reaction is kinetically controlled. Slow reactions are thought of as being kinetically stable. Differentiate this with thermodynamic stability that is a function of spontaneity.

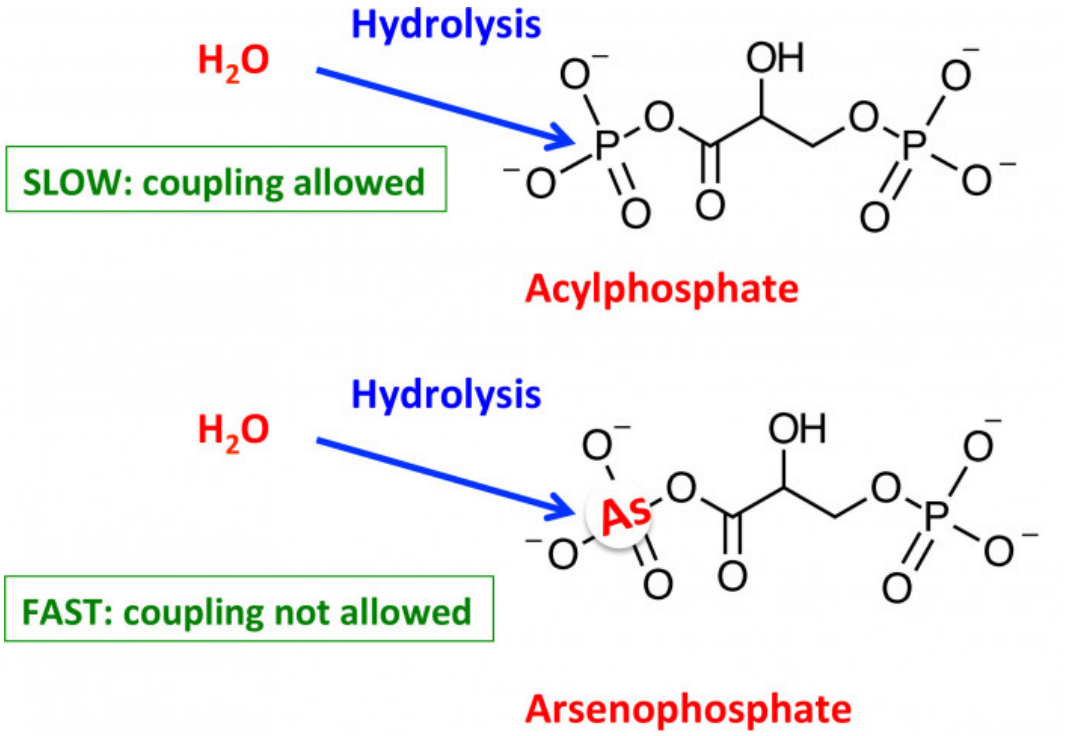
Reactions that produce multiple products can have both kinetically and thermodynamically controlled products. The most rapidly formed product is the kinetic product and the most stably formed product is the thermodynamic product. Through controlling various parameters (i.e. temperature, ionic strength, buffer, etc.), the reaction can be either kinetically or thermodynamically controlled.

For reactions that can produce more than one product, each reaction direction has a distinct activation barrier (see image). Many reactions occur where the fastest product is also the most thermodynamically stable one too. However, if a reaction occurs where the properties are not equal, then the specific reaction conditions will determine which products predominate. In general, reaction conditions that are irreversible (i.e. low temperature) tend to favor kinetically controlled products. Clearly, at low temperatures there will be a population of molecules capable of overcoming the activation barrier. In contrast, those reactions that are conducted under reversible conditions (i.e. high temperature) tend to favor formation of the thermodynamic product.



There are numerous examples of kinetic vs thermodynamic control. One interesting example of this is the role that phosphate and arsenate play in the glycolytic pathway. We all know that arsenate is poisonous. Let's see how this plays out through its effect on glycolysis. Recall that in the glycolytic pathway,

glucose is partially oxidized to pyruvate, a three-carbon keto acid. As shown in the image, during glycolysis the enzyme glyceraldehyde 3-phosphate (G-3P) dehydrogenase oxidizes glyceraldehyde-3 phosphate into 1, 3 biphosphoglyceric acid (1, 3-BPG) in a step that is thermodynamically favorable ($\Delta G^{\circ\prime} = -12$ kcal/mol). 1, 3-BPG is a high energy intermediate that contains a phosphoanyhydride bond. As shown in the figure, G-3P dehydrogenase performs a second step by coupling the conversion of 1, 3-BPG, ADP, and Pi into the synthesis of 3-phosphoglyceric acid (3-PGA) and ATP. The synthesis of ATP is normally not spontaneous, but through a coupling mechanism, ATP formation occurs through a phosphoryl transfer mechanism with 1, 3-BPG.



KINETICALLY	THERMODYNAMICALLY (ΔG)	
FAST	< 0	Aresnate
SLOW	< 0	Phosphate

The stability of the acylphosphate bond in 1, 3-BPG is critical to the formation of the first ATP molecule in glycolysis. Although ADP is the normal nucleophile for attacking the acyl phosphate during the formation of ATP, water is also a natural competitor. If water outcompetes ADP, then the high energy acylphosphate bond is hydrolyzed and no ATP product is generated. The consequence of this is that the high energy bond is broken, but energy is released in the form of heat to the environment. Consequently, there is no coupling; there is no conversion of the energy in the acyl phosphate of 1, 3-BPG to the high energy phosphoanhydride bond of ATP. Fortunately, under normal circumstances, the rate of hydrolysis is slow and coupling to ATP synthesis occurs. However, in the presence of arsenate, a competitor of phosphate for the active site of G-3P dehydrogenase, the arsenophosphate bond is rapidly hydrolyzed. As a consequence, coupling does not occur and no ATP is synthesized. Therefore, although the reactions of both phosphate and arsenate are thermodynamically favorable ($\Delta G^\circ < O$), the slow kinetic hydrolysis reaction allows coupling while the fast kinetic hydrolysis reaction with arsenate prevents it. The net result is the failure to generate ATP in the presence of arsenate.

Allosteric regulation of enzymes

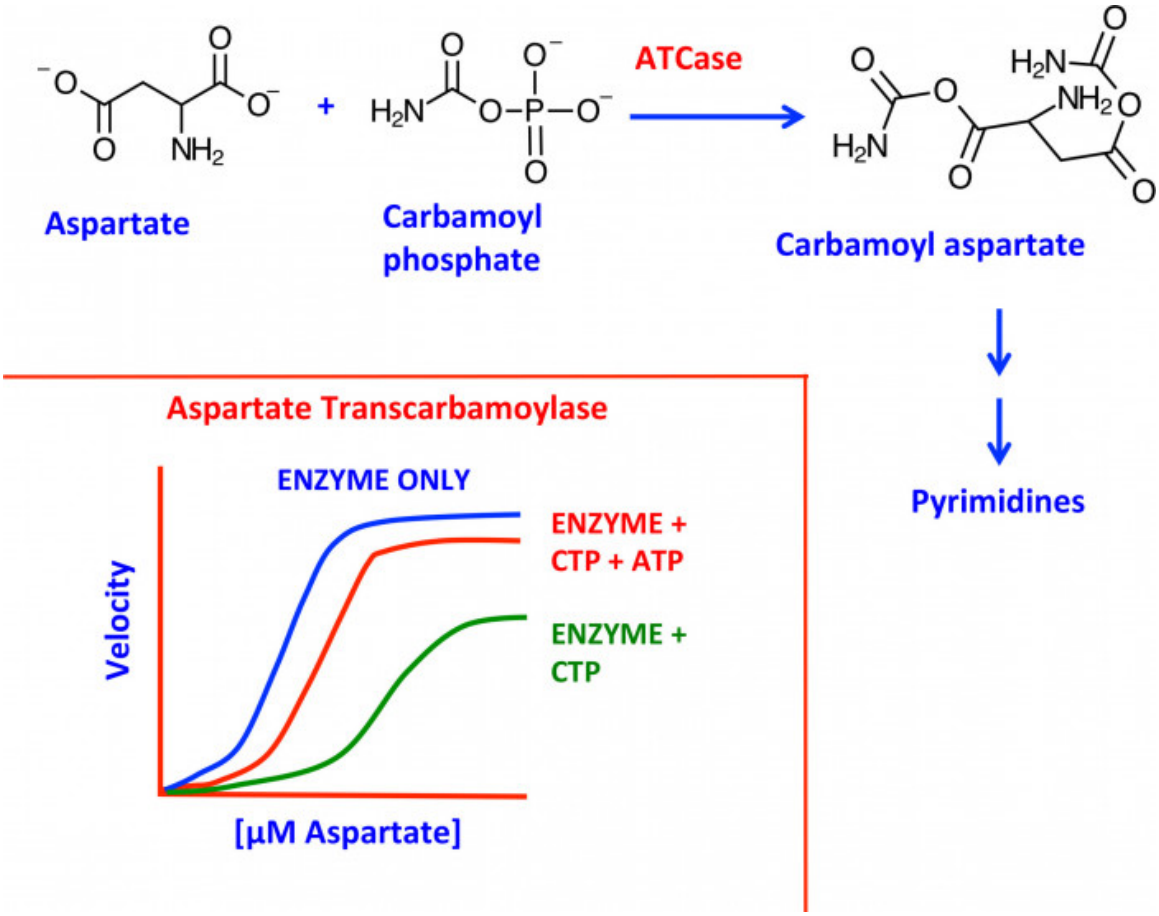
Aspartate transcarbamoylase (ATcase) and allosterism. Allosterism occurs when molecules regulate enzyme activity through binding outside of the active site. The binding events alter the conformation of the enzyme and modulate its activity.

A classic example of allosterism occurs in the multimeric enzyme aspartate transcarbamoylase (ATcase). This enzyme catalyzes the committed step in pyrimidine biosynthesis. ATcase is composed of 12 total subunits: six catalytic and six regulatory subunits (C_6R_6). ATcase catalyzes the formation of carbamoyl aspartate from aspartate and carbamoyl phosphate (see image). The structure and activity of ATcase is regulated by allosterism, the phenomenon where small molecule regulators alter protein (i.e. enzyme) function through binding to a site distinct from the active site. Allosteric regulation can be either positive or negative.

A kinetic analysis of ATcase is shown and reveals that enzyme activity is altered in the presence of ATP and CTP. The reaction is conducted with increasing amounts of the substrate aspartate in the presence of saturating levels of carbamoyl phosphate, a second substrate. The effects of ATP and CTP on enzyme activity can be seen through measuring the K_M, the concentration of

aspartate at maximal velocity (V_{max}). Such regulatory modulation of enzyme activity via nucleotides ensures balance in the production of nucleotides.

ATcase exists in two major forms: R (relaxed) and T (taught). The R form is an open, active form with high affinity for substrate (aspartate). In contrast, the T form is a low activity form with low affinity for substrate. Conversion from the T to the R form is accompanied by structural changes (i.e. van der Waals forces and hydrogen bonds).



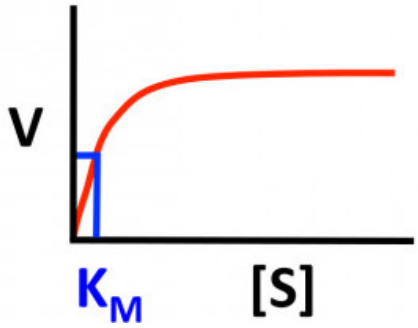
CHAPTER 2

Enzyme kinetics and inhibitors

As many drugs are inhibitors of enzymes, enzyme kinetics and inhibition is an important topic in medicine and is frequently found on the MCAT. The most commonly encountered type of enzyme reaction will be a single substrate reaction:

I $E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$

II $V = \dfrac{V_{max}[S]}{K_M + [S]}$



Where E = enzyme, S = substrate, ES = enzyme-substrate complex, and P = product

The rate constants for each step are shown as k_{on}, k_{off}, and k_{cat}.
In the beginning of the reaction, the velocity is linear, but as the substrate concentration increases, the enzyme becomes saturated where the maximum velocity is reached (V_{max}). The K_M is defined as the concentration of substrate required to reach one half the maximal velocity:

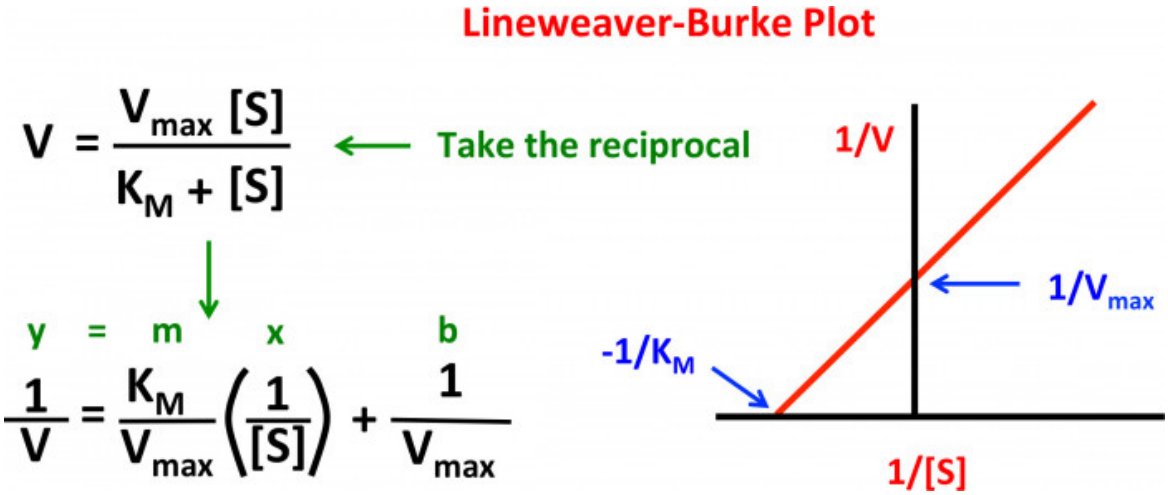
$$K_M = V_{max}/2$$

The K_M also reflects the affinity of the enzyme for the substrate: the higher the K_M, the lower the affinity for the enzyme and substrate. The Michaelis equation (II) relates the initial velocity (V) to the V_{max}, [S], and K_M as shown.
The catalytic efficiency is defined by the ratio of k_{cat}/K_M. This can often be seen as the rate limiting step of the reaction. Note that the units for this are

min^{-1} M^{-1}. This is the rate constant when $K_M >> [S]$ for the reaction E + S ⇒ ES. This first order rate constant reflects contributions from both the rate of reaction catalysis (k_{cat}) and the affinity between the enzyme and substrate (K_M) as shown in Panel II of the figure (see above).

The k_{cat} is also known as the turnover number and represents the amount of times an enzyme can perform a given reaction per unit time. This is related to the catalytic efficiency, a common MCAT subject. The preference of an enzyme for a given substrate can be determined by comparing the k_{cat}/K_M values for various substrates. Further, in theory the physical limits on enzyme efficiency are determined by the limits of diffusion, which is approximately 10^9 s^{-1} M^{-1}.

There are three major types of enzyme inhibition: competitive, noncompetitive, and uncompetitve. In addition, some inhibitors display mixed inhibition. You should be very familiar with each type of inhibition, particularly with respect to consequence of enzyme function. This includes understanding how the V_{max} and the Michaelis constant (K_M) are altered by the presence of the inhibitor.



Double reciprocal plots

Double reciprocal plots, also known as the Lineweaver Burke plot, are often used to examine enzyme kinetics and inhibition. You should be familiar with these plots and expect to see them on the MCAT. Through rearrangement of

the equation that relates velocity to V_{max}, K_M, and substrate concentrations (see image), a linear relationship can be derived. By plotting 1/V vs 1/S, a line can be drawn such that the X and Y intercepts are $1/V_{max}$ and $-1/K_M$ respectively.

Competitive inhibition

Competitive inhibitors bind to the active sites of enzymes as they are structurally similar enough to the real substrate. The reaction is always reversible. Since the inhibitor is similar in structure to the real substrate, the K_M of the reaction increases. This reflects a lower affinity for the enzyme and its true substrate. Note, however, that the V_{max} of a competitive inhibitor does not change. As the concentration of substrate increases, the relative amount of inhibitor decreases and as the reaction is reversible, the increased substrate amount will overwhelm the inhibitor for substrate binding.

Competitive inhibition can be schematically written as:

E + I = EI
EI + S = E + P

Noncompetitive inhibition

In noncompetitive inhibition, the inhibitor can bind to the enzyme irrespective of the presence of the substrate. This reduces the V_{max} but not the K_M. This is because the substrate can still bind to the enzyme (same K_M), but reduces the ability to form product (reduces V_{max}).

Noncompetitive inhibition can be schematically written as:

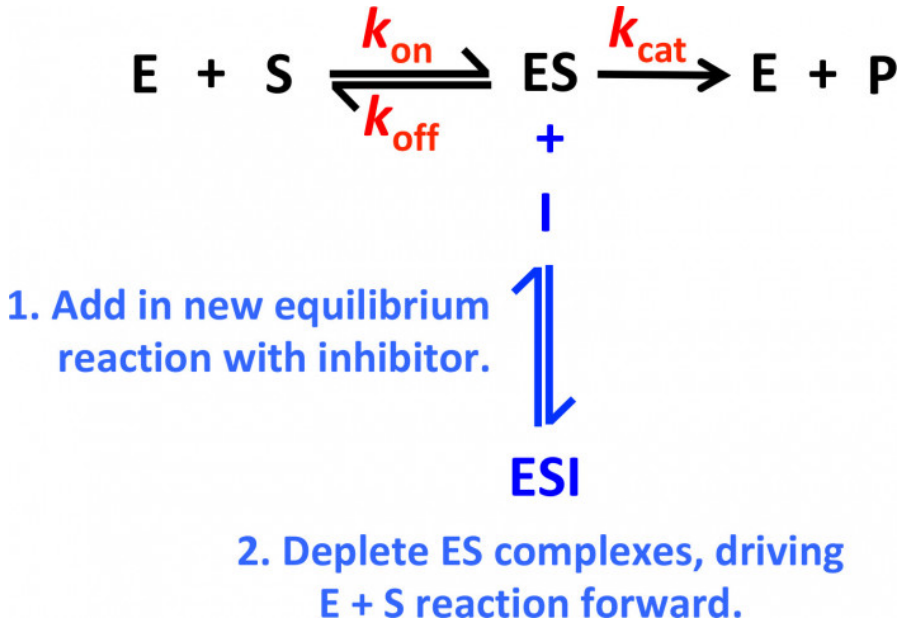
E + I = EI
EI + S = ESI
E + I + S = E + P

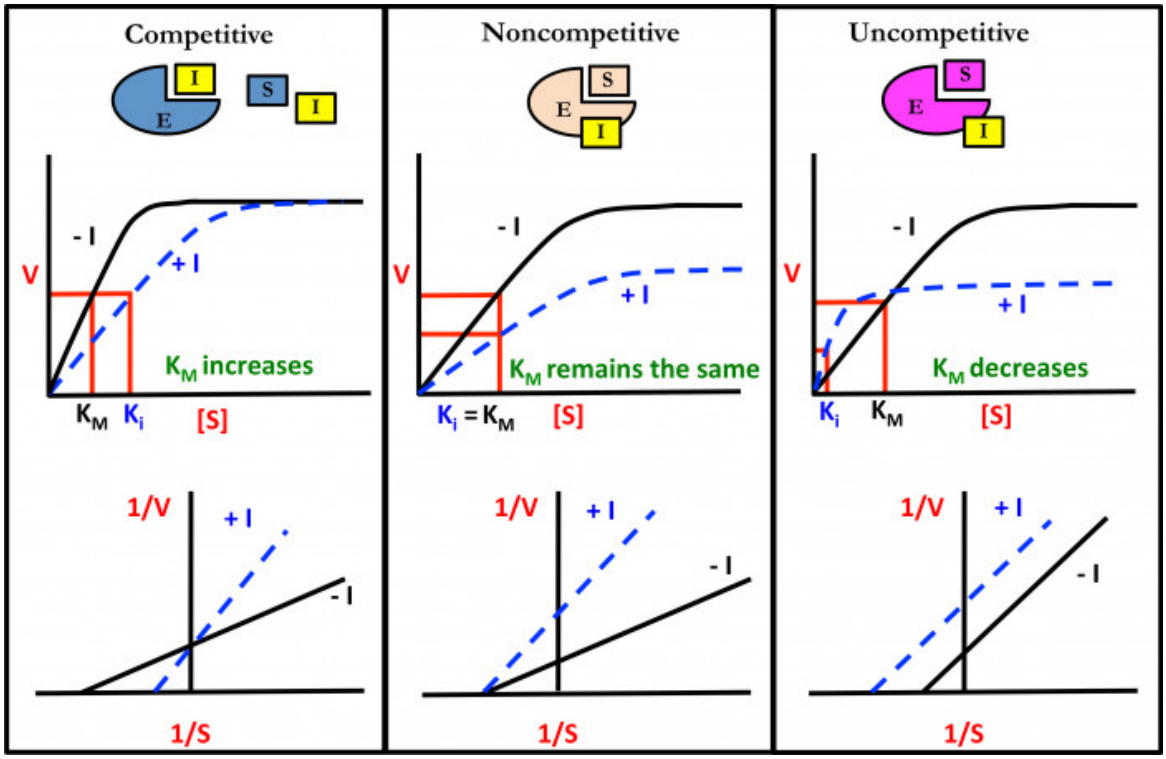
Uncompetitive inhibition

Uncompetitive inhibitors can only bind to the enzyme when the substrate is also bound. Therefore, the ES complex allows for the formation of the binding site for the inhibitor. In uncompetitive inhibition, ES complexes become depleted due to the formation of the ESI complexes. In order to preserve the original equilibrium between substrate (S) and enzyme (E), more substrate will bind to enzyme, driving the formation of new ES complexes via

mass action (LeChatlier's principle as per image below). As a result, a lower concentration of substrate is required to reach $V_{max}/2$. In other words the K_M is lowered. Because the ESI complex cannot form product, the V_{max} is reduced in uncompetitive inhibition. Therefore, uncompetitive inhibition can be written as:

ES + I = (ES)I





Mixed inhibition

The fourth type of enzymatic inhibition is a mixture of both competitive and uncompetitive. It is therefore called "mixed" inhibition. In some cases, the inhibitor interacts with the enzyme irrespective of the presence of substrate. Binding in the absence of substrate is akin to competitive inhibition, and binding in the presence is akin to uncompetitive inhibition.