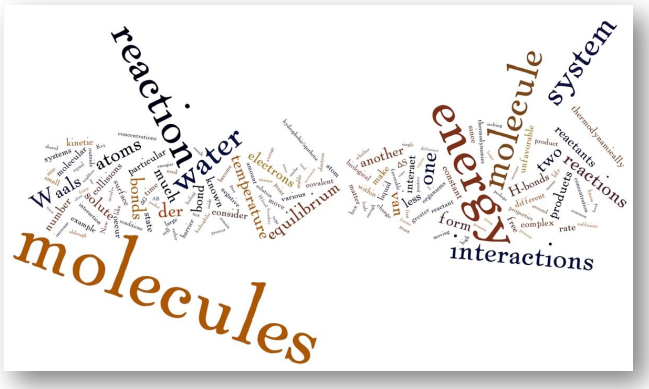
5. Molecular interactions, thermodynamics, and reaction coupling

In which we drastically change gears and move from evolutionary mechanisms to the physicochemical properties of organisms. We consider how molecules interact and react with one another and how these interactions and reactions determine the properties of substances and systems.



A very little thermodynamics

While the diversity of organisms and the unique properties of each individual organism are the products of evolutionary processes, initiated billions of years ago, it is equally important to recognize that all biological systems and processes, from growth and cell division to thoughts and feelings, obey the rules of chemistry and physics, and in particular the laws of thermodynamics. What makes biological systems unique is that, unlike simpler physicochemical systems that move toward thermodynamic equilibrium, organisms must maintain a non-equilibrium state in order to remain alive. While a chemical reaction system is easy to assemble *de novo*, every biological system has been running continuously for billions of years. So, before we continue we have be clear about what we mean and imply when we say that a system is at equilibrium versus being in a obligate non-equilibrium state.

To understand the meaning of thermodynamic equilibrium we have to learn the see the world differently, and learn new meanings for a number of words. First we have to make clear the distinction between the macroscopic world that we directly perceive and the sub-microscopic, molecular world that we can understand based on scientific observations and conclusions - it is this molecular world that is particularly important in the context of biological systems. The macroscopic and the molecular worlds behave very differently. To illustrate this point, we will use a simpler model that displays the basic behaviors that we want to consider but is not as complex as a biological system. In our case, let us consider a small, well-insulated air-filled room in which there is a table with a bar of gold (we use gold since it is chemically rather inert, that is, un-reactive. Iron bars, for example, could rust, which would complicate things). In our model the room is initially at cosy 70 ºF (~21 ºC) and the bar of gold is at 200ºC. What will happen? Can you generate a graph that describes how the system will behave over time? Our first task is the define the system – that is, that the part of the universe in which we are we interested. We could define the system as the gold bar or the room with the gold bar in it. Notice, we are not really concerned about how the system came to be the way it is, its history. We could, if we wanted to, demonstrate quite convincingly that the system's history will not influence its future behavior – this is a critical difference between biological and simple physicochemical systems. For now we will use the insulated room as the system, but it doesn't really matter as long as we clearly define what we consider the system to be.

Common sense tells us that there will be an energy transfer between the gold bar and the rest of the room and that the temperature of the gold bar will decrease over time; the behavior of system

has a temporal direction. Why do you think that is? Why doesn't the hot bar get hotter and the room get cooler? We will come back to this question shortly. What may not be quite as obvious is that the temperature of the room will increase slightly as well. Eventually the block of gold and the room will reach the same temperature and the system will be said to be at equilibrium.

Remember we defined the system as isolated from the rest of the universe, but what does that mean? Basically, no matter or energy passes into or out of the room – such a system is said to be a closed system. Because it is a closed system, once the system reaches its final temperature, NºC, no further macroscopic change will occur. This does not mean, however, that nothing is going on. If we could look at the molecular level we would see that molecules of air are moving, colliding with one another, the of bar, and the table constantly. The molecules within the bar and the table are also vibrating. These collisions can change the velocities of the colliding molecules. (What happens if there was no air in the room? How would this change your graph of the behavior of the system?) The speed of these molecular movements is a function of temperature, the higher (or lower) the temperature, the faster (or slower) these motions would be. As we will consider further on, all of the molecules in the system have kinetic energy, which is the energy of motion. Through their interactions, the kinetic energy of any one particular molecule will be constantly changing. At the molecular level the system is dynamic, even though at the macroscopic level it is static. We will come back to this insight repeatedly in our considerations of biological systems.

And this is what is important about a system at equilibrium: it is static. Even at the molecular level, while there is still movement, there is no net change. The energy of two colliding molecules is the same after a collision as before, even though the energy may be distributed differently between the colliding molecules. The system as a whole cannot really do anything. In physical terms, it cannot do work - no macroscopic changes are possible. This is a weird idea, since (at the molecular level) things are still moving. So, if we return to living systems, which are clearly able to do lots of things, including moving macroscopically, growing, thinking, and such, it is clear that they cannot be at equilibrium.

We can ask, what is necessary to keep a system from reaching equilibrium? The most obvious answer (we believe) is that unlike our imaginary closed room system, a non-equilibrium system must be open, that is, energy and matter must be able to enter and leave it. An open system is no longer isolated from the rest of the universe, it is part of it. For example, we could imagine a system in which energy, in the form of radiation, can enter and leave our room. We could maintain a difference in the temperature between the bar and the room by illuminating the bar and removing heat from the room as a whole. A temperature difference between the bar and the room could then (in theory) produce what is known as a heat engine, which can do work (that produce macroscopic change.) As long as we continue to heat one block and remove heat from the rest of the system, we can continue to do work, that is, macroscopically observable changes can happen.

Cryptobiosis: At this point, we have characterized organisms as dynamic, open, non-equilibrium systems. An apparent exception to the dynamic aspect of life are organisms that display a rather special phenotypic adaptation, known generically as cryptobiosis. Organisms, such as the tardigrad (or water bear), can be freeze-dried and persist in a “suspended animation” state for decades. What is critical, however, is to note that when in this cryptobiotic state the organism is not at equilibrium, in

much the same way that a piece of wood in air is not at equilibrium, but capable of reacting. An organism in a cryptobiotic state is certainly not dead; it can be reanimated when returned to normal conditions.[141] Cryptobiosis is an genetically-based adaptation that takes energy to produce and energy is used to emerge from the stasis state. While the behavior of tardigrads is extreme, many organisms display a range of adaptive behaviors that enable them to survive hostile environmental conditions.

Reactions: favorable, unfavorable, and their dynamics

As we will see, biological systems are extremely complex and both their overall structural elements and many of their molecular components (including DNA) are the products of thermodynamically unfavorable processes and reactions. How do these reactions take place in living systems? The answer comes from the coupling of thermodynamically favorable reactions to a thermodynamically unfavorable reactions. This is a type of work, although not in the standard macroscopic physics model of work (w) = force x distance. In the case of (chemical) reaction coupling, the work involved drives thermodynamically unfavorable reactions, typically the synthesis of large and complex molecules and macromolecules (that is, very large molecules). Here we will consider the thermodynamics of these processes.

Thinking about energy: Thermodynamics is at its core about energy and changes in energy. This leads to the non-trivial question, what is energy? Energy comes in many forms. There is energy associated with the movement and vibrations of objects with mass. At the atomic and molecular level there is energy associated with the (quantum) state of electrons. There is energy associated with fields that depends upon an object's nature (for example its mass or electrical charge) and its position within the field. There is the energy associated with electromagnetic radiation, the most familiar form is visible light, but electromagnetic radiation extends from microwaves to X-rays. Finally, there is the energy that is present in the very nature of matter, such energy is described by the equation:

$$e \text{ (energy)} = m \text{ (mass)} \times c^2 \text{ (c = speed of light)}$$

To illustrate this principle, we can call on our day-to-day experiences. Energy can be used to make something move. Imagine a system of a box sitting on a rough floor. You shove the box so that it moves and then you stop pushing – the box travels a short distance and then stops. The first law of thermodynamics is that the total energy in a system is constant. So the question is where has the energy gone? One answer might be that the energy was destroyed. This is wrong. Careful observations lead us to deduce that the energy still exists but that it has been transformed. One obvious change is the transformation of energy from a mechanical force to some other form, so what are those other

[141] On dormancy strategies in tardigrades: http://www.ncbi.nlm.nih.gov/pubmed/21402076 & Towards decrypting cryptobiosis--analyzing anhydrobiosis in the tardigrade Milnesium tardigradum using transcriptome sequencing.: http://www.ncbi.nlm.nih.gov/pubmed/24651535

forms? It is unlikely that the mass of the box has increased, so we have to look at more subtle forms – the most likely is heat. The friction generated by moving the box represents an increase in the movements of molecules of the box and the floor over which the box moved. Through collisions and vibrations, this energy will, over time, be distributed throughout the system. This thermal motion can be seen in what is known as Brownian motion. In 1905, Albert Einstein explained Brownian motion in terms of the existence, size, and movements of molecules.[142]

In the system we have been considering, the concentrated energy used to move the box has been spread out throughout the system. While one could use the push to move something (to work), the diffuse thermoenergy cannot be used to do work. While the total amount of energy is conserved, its ability to do things has been decrease (almost abolished). This involves the concept of entropy, which we will turn to next.

Thinking entropically (and thermodynamically)

We certainly are in no a position to teach you (rigorously) the basics of chemistry and chemical reactions, but we can provide a short refresher that focuses on the key points we will be using over and over again.[143] The first law of thermodynamics is that while forms of energy may change, that is, can be converted between distinct forms, the total amount of energy within a closed system remains constant. Again, we need to explicitly recognize the distinction between a particular system and the universe as a whole. The universe as a whole is itself (apparently) a closed system. If we take any isolated part of the system we must define a system boundary, the boundary and what is inside it is part of the system, while the rest of the universe outside of the boundary layer is not. While we will consider the nature of the boundary in greater molecular detail in the next chapter, we can anticipate that one of the boundary's key features is its selectivity in letting energy and/or matter to pass into and out of the system, and what constraints it applies to those movements.

Assuming that you have been introduced to chemistry, you might recognize the Gibb's free energy equation: $\Delta G = \Delta H - T\Delta S$, where T is the temperature of the system.[144] From our particularly biological perspective, we can think of ΔH as the amount of heat released into (or absorbed from) the environment in the course of a reaction, and ΔS as the change in a system factor known as entropy. To place this equation in a context, let us think about a simple reaction:

oil mixed with water ⇄ oil + water (separate) ΔG is negative

[142] Albert Einstein: The Size and Existence of Atoms http://youtu.be/nrUBPO6zZ40

[143] Of course, we recommend a chemistry course sequence based on Cooper & Klymkowsky, 2014. Chemistry, Life, the Universe and Everything: here: http://tinyurl.com/onapy7k (see http://virtuallaboratory.colorado.edu/CLUE-Chemistry/)

[144] in the real world, the value of ΔG depends upon the concentrations of solute and solvent, but we will ignore that complexity for the moment.

While a typical reaction involves changes in the types and amounts of the molecules present, we can extend that view to all types of reactions, including those that involve changes in temperature of distinct parts of a system (the bar model) and the separation of different types of molecules in a liquid (the oil-water example). Every reaction is characterized by its equilibrium constant, K_{eq}, which is a function of both the reaction itself and the conditions under which the reaction is carried out. These conditions include parameters such as the initial state of the system, the concentrations of the reactants, and system temperature and pressure. In biological systems we generally ignore pressure, although pressure will be important for organisms that live on the sea floor (and perhaps mountain tops).

The equilibrium constant for a reaction is defined as the rate of the forward reaction k_f (reactants to products) divided by the rate of the reverse reaction k_r (products to reactants). At equilibrium (where nothing macroscopic is happening), k_f times the concentrations of the reactants equals k_r times the concentration of the products. For a thermodynamically favorable reaction, that is one that favors the products, k_f will be greater than k_r and K_{eq} will be greater, often much greater than one. The larger K_{eq} is, the more product and the less reactant there will be when the system is at equilibrium. If the equilibrium constant is less than 1, then at equilibrium, the concentration of reactants will be greater than the concentration of products.

$$K_{eq} = \frac{k_f}{k_r} \qquad k_f\,[\text{reactants}] = k_r\,[\text{products}]$$

While the concentration of reactants and products of a reaction at equilibrium remains constant it is a mistake to think that the system is static. If we were to peer into the system at the molecular level we would find that, at equilibrium, reactants are combining to form products and products are rearranging to form reactants at similar rates.[145] That means that the net flux, the rate of product formation minus the rate of reactant formation, will be zero. If, at equilibrium, a reaction has gone almost to completion and $K_{eq} >> 1$, there will be very little of the reactants left and lots of the products. The product of the forward rate constant times the small reactant concentrations will equal the product of the backward rate constant times the high product concentrations. Given that most reactions involve physical collisions between molecules, the changes in the frequency of productive collisions between reactants or products increases as their concentrations increase. Even improbable events can occur, albeit infrequently, if the rate of precursor events are high enough.

Reaction rates

Knowing whether a reaction is thermodynamically favorable and its equilibrium constant does not tell us much (or really anything) about whether the reaction actually occurs to any significant extent under the conditions with which we are concerned. To know the reaction's rate we need to know the reaction kinetics for the specific system with which we are dealing. Reaction kinetics tells us the rate at which the reaction actually occurs under a particular set of conditions. For example, consider a wooden log, which is composed mainly of the carbohydrate polymer cellulose $((CH_2O)_n$.

[145] This, of course, assumes that we have a closed system, that is, that neither the products or the reactants can leave the system, and that the volume of the system also remains constant. If the reactants can "leave the scene" of the reaction, then of course the back reaction, Products ⇆ Reactants, will be much less likely to occur.

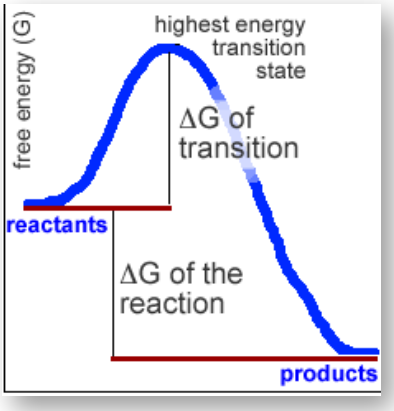
In the presence of molecular oxygen (O_2) the reaction:

$$nO_2 + \text{wooden log } ((CH_2O)_n) \leftrightarrows nCO_2 + nH_2O + \text{heat}$$

is extremely favorable thermodynamically, that is, it has a negative ΔG and a large equilibrium constant, yet the log is stable - it does not burst into flames spontaneously. The question is, of course, why ever not? Or more generally why is the world so annoyingly complex?

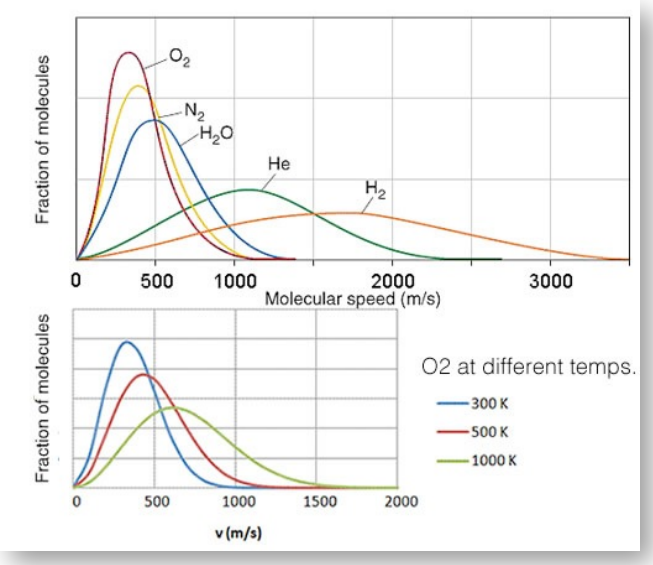
The answer lies in the details of the reaction, how exactly the reactants are converted into the products. At this point, for simplicity or perhaps better put, biologically speaking, accessibility, let us consider another non-chemical but rather widespread type of reaction. In this reaction system, there is a barrier between two compartments, specifically the barrier membrane that separates the inside from the outside of a cell. At this point, we do not need to consider the exact details of the barrier's structure (although we will in next chapter). In our particular example, outside the cell the concentration of molecule A is high, while inside the cell its concentration is low. We can write out this reaction equation as $A_{outside} \leftrightarrows A_{inside}$ (perhaps you make a prediction of the ΔG of this reaction and what it depends upon.)

The reaction consists of moving A molecules across the barrier between the inside and the outside of the cell. In our example, the concentration of A outside the cell (written $[A_{outside}]$, with the square brackets indicating concentration) is much greater than $[A_{inside}]$. At any moment in time, the number of collisions between $A_{outside}$ and the barrier will be much greater than the number of collisions between A_{inside} and the barrier. Assuming that the probability of crossing the barrier is a function of the collision frequency, there will be net movement of $A_{outside}$ to A_{inside}. The real question is how large this net flux will be. This will depend on the amount of energy a molecule needs to cross the barrier. We can represent this energy as the highest peak in a reaction graph (here we assume a simple process with a single peak, in the real world it can involve a number of sub-reactions and look more like a roller-coaster than a simple hill)[→]. In such a graph, we begin with the free energy of the reactants along the Y-axis, and plot the changing free energies of the various intermediates along the X-axis, leading to the free energy of the products. In our simplified view of the subject, the difference between the intermediate with the highest free energy and the free energy of the reactants ($\Delta G_{transition}$) roughly corresponds to the rate limiting step in the reaction and reflects the reaction's activation energy.



For a reaction to move from reactants ($A_{outside}$) to products (A_{inside}), the reactants must capture enough energy from their environment to traverse the barrier between outside and inside. In biological systems there are two major sources for this energy. The reactants can absorb electromagnetic energy, that is, light, or energy can be transferred to it from other molecules through collisions. In liquid water, molecules are moving; at room temperature they move on average at about 640 m/s. That is not to say that all molecules are moving with the same speed. If we were to look at the population of molecules, we would find a distribution of speeds known as a Boltzmann (or Maxwell-Boltzmann) distribution. As they collide with one another, they exchange kinetic energy, and one molecule can emerge from the

collision with much more energy than it entered with. Since reactions occur at temperatures well above absolute zero, there is plenty of energy available in the form of the kinetic energy of molecules, and occasionally a molecule with extremely high energy will emerge. If such an energetic A molecule gains sufficient energy and collides with the boundary layer, it could cross the boundary layer, that is, move from outside to inside. If not, it will probably loose that energy to other molecules very quickly through collisions. It is this dynamic exchange of kinetic energy that drives the movement of molecules (as well as the breaking of bonds associated with chemical reactions).



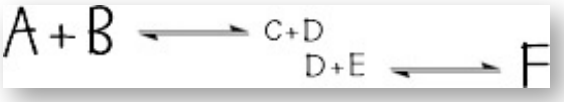
The difference between the free energies of the reactants and products ($\Delta G_{reaction}$) determines the equilibrium constant for a particular reaction system. In the case of our barrier system, since the A molecules are the same whether inside or outside the cell, the difference in the free energies of the reactants and products reflects (primarily) the difference in their concentrations. Higher concentration correlates with higher free energy (remember, we are interested in the ΔG of the $A_{outside} \leftrightarrows A_{inside}$ reaction). Clearly the more molecules of A are present, the higher the ΔG of A. One point is worth emphasizing, it is possible for a reaction to have a large $\Delta G_{reaction}$ and either a large or small $\Delta G_{transition}$. So assuming that there is enough energy in the system, and $\Delta G_{transition}$ is small enough for the reaction to proceed at a noticeable rate, you should be able to predict what happens to the system as it moves toward equilibrium. If the $\Delta G_{transition}$ is high enough, however the $A_{outside} \leftrightarrows A_{inside}$ reaction will not occur to any significant extent.

Coupling reactions

There are large numbers of different types of reactions that occur within cells. As a rule of thumb, a reaction that produces smaller molecules from larger ones will be thermodynamically favored, while reactions that produce larger molecules from smaller ones will be unfavorable. Similarly a reaction that leads to a molecule moving from a region of higher concentration to a region of lower concentration will be favored. So how exactly can we build the big molecules, such as DNA and proteins, that life depends upon?

As we noted before reactions can be placed into two groups, those that are thermodynamically favored (negative ΔG, equilibrium constant is greater, typically much greater, than 1) and those that are unfavorable (positive ΔG, equilibrium constant less, often much less than 1). Thermodynamically favored reactions are typically associated with the release of energy from and the breakdown of various forms of food (known generically as catabolism), while reactions that build up biomolecules (known generically as anabolism) are typically thermodynamically unfavorable. An organism's metabolism is the sum total of all of these various reactions.

Unfavorable reactions occur when they are coupled to thermodynamically favorable reactions. This requires that the two reactions share a common intermediate. In this example [→] the two reactions share the component "D". Let us assume that the upper reaction is unfavorable while the lower reaction is favorable. What happens? Let us assume that both reactions are occurring at measurable rates, perhaps through the mediation of appropriate catalysts, which act to lower the activation energy of a reaction, and that E is present within the system. At the start of our analysis, the concentrations of A and B are high. We can then use Le Chatelier's principle to make our predictions.[146]



Let us illustrate how Le Chatelier's principle works. Assume for the moment that the reaction A + B ⇆ C + D has reached equilibrium. Now consider what happens to the reaction if, for example, we removed (somehow, do not worry about how) all of the C from the system. Alternatively, consider what happens if we add more B. The answer is that the reaction would move to the right (even though that reaction is thermodynamically unfavorable), in order to re-establish the equilibrium condition. If all C were removed, the C + D to A + B reaction could not occur, so the A + B reaction would continue in an unbalanced manner until the level of C + D increased and C + D to A + B reaction became balanced with the A + B to C + D reaction. In the second case, the addition of B would lead to the increased production of C + D, until their concentration reached a point where the C + D to A + B reaction became balanced with the A + B to C + D reaction. This type of behavior arises directly from the fact that at equilibrium reaction systems are not static at the molecular level, but dynamic – things are still occurring, they are just balanced so that no net change occurs. When you add or take something away from the system, it becomes unbalanced, that is, it is no longer at equilibrium. Because the reactions are occurring at a measurable rate, the system will return to equilibrium over time.

So back to our reaction system. As the unfavorable A + B reaction occurs and approaches equilibrium it will produce a small amount of C + D. However, the D + reaction is favorable; it will produce F while at the same time removing D from the system. As D is removed, it influences the A+B reaction (because it makes the C + D "back reaction" less probable even though the A+B "forward reaction" continues.) The result is that more C and D will be produced. Assuming that sufficient amounts of E are present, more D will be removed. The end result is that, even though it is energetically unfavorable, more and more C and D will be produced, while D will be used up to make F. It is the presence of the common component D and its utilization as a reactant in the D + E reaction that drives the synthesis of C from A and B, something that would normally not be expected to occur to any great extent. Imagine then, what happens if C is also a reactant in some other favorable reaction(s)? In this way reactions systems are linked together, and the biological system proceeds to use energy and matter from the outside world to produce the complex molecules needed for its maintenance, growth, and reproduction.[147]

---

[146] http://en.wikipedia.org/wiki/Le_Chatelier's_principle

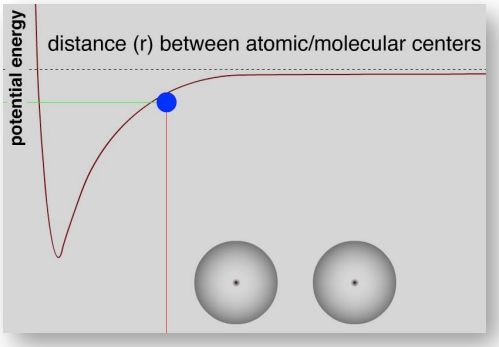
[147] http://haha.nu/science/the-amazing-human-body/

Questions to answer & to ponder:

- What are the common components of a non-equilibrium system and how does a dried out tardigrad fulfill those requirements?
- You use friction to ignite a fire. From where is the energy of the fire derived?
- A reaction is at equilibrium and we increase the amount of reactant. What happens in terms of the amount of reactant and product?
- A reaction is at equilibrium and we increase the amount of product. What happens in terms of the amount of reactant and product?
- What does the addition of a catalyst do to a system already at equilibrium?
- What does the addition of a catalyst do to a system far from equilibrium?
- Where does the energy come from to reach the activation state/reaction intermediate?
- Why does a catalyst not change the equilibrium state of a system?
- Why are catalysts required for life?

Molecules, London Dispersion Forces, and Van der Waals interactions

We have briefly (admittedly absurdly briefly) defined what energy is and begun to consider how it can be transformed from one form to another. Now we need to consider what we mean by matter, which implies an understanding of the atomic organization of the molecules that compose matter. As you hopefully know by now, all matter is composed of atoms. The internal structure of atoms is the subject of quantum physics and we will not go into it any depth. Suffice to say that each atom consists of a tiny positively charged nucleus and cloud of negatively charged electrons.[148] Typically atoms and molecules, which after all are collections of atoms, interact with one another through a number of different types of interactions. The first are Known as van der Waals interactions, which are mediated by London Dispersion Forces. These forces arise from the fact that the relatively light negatively-charged electrons are in continual movement, compared to the relatively massive and stationary positively-charged nuclei, Because charges on the protons and electrons are equal in magnitude the atom is electrically neutral, but because the electrons are moving, at any one moment, an a observer outside of the atom or molecule will experience a small fluctuating electrical field.

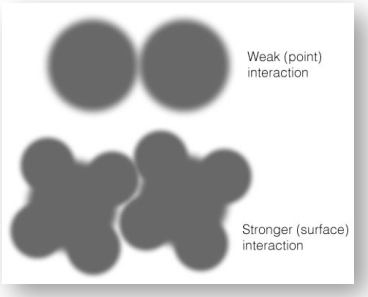


As two molecules approach one another, their fluctuating electric fields begin to interact, this interaction generates an attractive force, known as the London dispersion force (LDF), named after its discoverer Fritz Wolfgang London (1900–1954). This force varies as ~$1/R^6$ where R is the distance between the molecules; this relationship means that LDFs. The $1/R^6$ relationship means that LDFs act only over very short distances, typically less than 1 nanometer (1 nm = 10^{-9} m); as a frame of reference, a carbon atom has a radius of ~0.07 nm. The magnitude of this attractive force reaches its maximum when the two molecules are separated by what is known as the sum of their van der Waals

[148] If you are of a curious disposition you might wonder why the negatively charged electrons are not simply attracted to and become localized to the positively charged nucleus; the answer is because of quantum principles: see http://chemwiki.ucdavis.edu/Textbook_Maps/General_Chemistry_Textbook_Maps/Map%3A_Lower's_Chem1/04._Atoms_and_the_Periodic_Table/Why_Don't_Electrons_Fall_into_the_Nucleus%3F

radii (the van der Waals radius of a carbon atom is ~0.17 nm. If they move closer that this distance, the attractive LDF is quickly overwhelmed by a rapidly increasing, and extremely strong repulsive force that arises from the electrostatic interactions between the positively charged nuclei and the negatively charged electrons of the two molecules.[149] This repulsive interaction keeps atoms from fusing together and is one reason why molecule can form.

Each atom and molecule has its own characteristic van der Waals radius, although since most molecules are not spherical, it is perhaps better to refer to a molecule's van der Waals surface. This surface is the closest distance that two molecules can approach one another before repulsion kicks in and drives them back away from one another. It is common to see molecules displayed in terms of the van der Waals surfaces. Every molecule generates LDFs when it approaches another so van der Waals interactions are universal. The one exception involves pairs of small, similarly charged "ionic" molecules, that is molecules with permanent net positive or negative charge, approach each other. The strength of their electrostatic repulsion will be greater than the LDF.



The strength of the van der Waals interactions between molecules interact is determined primarily by their shapes. The greater the surface complementarity, the stronger the interaction. Compare the interaction between two monoatomic Noble atoms, such as helium, neon or argon, and two molecules with more complex shapes (figure →). The two monoatomic particles interact via LDFs at a single point, so the strength of the interaction is minimal. On the other hand, the two more complex molecules interact over extended surfaces, so the LDFs between them is greater resulting a stronger van der Waals interaction.

Covalent bonds

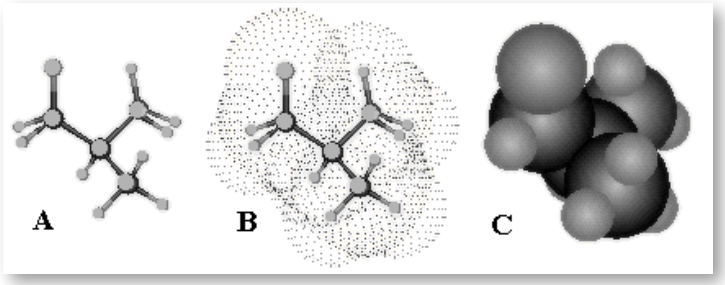
In the case of van der Waals interactions, the atoms and molecules involved retain their hold on their electrons, they remain distinct and discrete. There are cases, however, where atoms come to "share" each other's electrons. This sharing involves pairs of electrons, one from each atom. When electron pairs are shared, the atoms stop being distinct in that their shared electrons are no longer restricted to one or the other. In fact, since one electron cannot even in theory be distinguished from any other electron, they become a part of the molecule's electron system.[150] This sharing of electrons produces what is known as a covalent bond. Covalent bonds are ~20 to 50 times stronger than van der Waals interactions. What exactly does that mean? Basically, it takes much more energy to break these bonds. While the bonded form of atoms in a molecule is always more stable than the unbounded form, it may not be stable enough to withstand the energy delivered through collisions with neighboring molecules. Different bonds between different atoms in different molecular content differ in terms of bond stability; the bond energy refers the energy needed to break a particular bond. A molecule is stable if

[149] this can be explored further at http://besocratic.colorado.edu/CLUE-Chemistry/LondonDispersionForce%20copy/1.2-interactions-0.html

[150] Unlike organisms, each of which is unique in practice and theory, all electrons are identical in theory.

the bond energies associated with bonded atoms within the molecule are thigh enough to resist the energy delivered to the molecule through either collisions with neighboring molecules or the absorption of energy (light).

When atoms form a covalent bond, their individual van der Waals surfaces merge to produce a new molecular van der Waals surface. There are a number of ways to draw molecules, but the space-filling or van der Waals surface view is the most realistic (at least for our purposes). While realistic it can also be confusing, since it obscures the underlying molecular structure, that is, how the atoms in the molecule are linked together. This can be seen in this set of representations of the simple molecule 2-methylpropane.[151] As molecules become larger (as is the case with many biologically important molecules, it can become impossible to appreciate their underlying organization based on a van der Waals surface representation.



Because they form a new stable entity, it is not surprising (perhaps) that the properties of a molecule are quite distinct from, although certainly influenced by, the properties of the atoms from which they are composed. To a first order approximation, a molecule's properties are based on its shape, which is dictated by how the various atoms withjn the molecule are connected to one another. These geometries are imposed by each atom's quantum mechanical properties and (particularly as molecules get larger, as they so often do in biological systems, the interactions between different parts of the molecule. Some atoms, common to biological systems, such as hydrogen (H), can form only a single covalent bond. Others can make two (oxygen (O) and sulfur (S)), three (nitrogen (N)), four (carbon (C)), or five (phosphorus (P)) bonds.

In addition to smaller molecules, biological systems contain a number of distinct types of extremely large molecules, composed of as thousands of atoms; these are known as macromolecules. Such macromolecules are not rigid; they can often fold back on themselves leading to intramolecular interactions. There are also interactions between molecules, known as intermolecular interactions; again, these are mediated primarily by van der Waals interactions. The strength and specificity of these interactions can vary dramatically, and even small changes in molecular structure can have dramatic effects.

Molecules and molecular interactions are dynamic. Collisions with other molecules can lead to parts of a molecule rotating around a single bond.[152] The presence of a double bond restricts these kinds of movements; rotation around a double bond requires what amounts to breaking and then reforming one of the bonds. In addition, and if you have mastered some chemistry you already know this, it is often incorrect to consider bonds as distinct entities, isolated from one another and their surroundings. Adjacent bonds can interact forming what are known as resonance structures that

[151] Explicit Concepts of Molecular Topology: http://www.chem.msu.ru/eng/misc/babaev/match/top/top02.htm

[152] This could be basis of a square dance like in class activity!

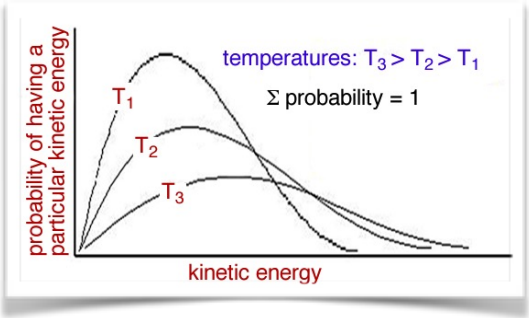
behave as mixtures of single and double bonds. Again this restricts free rotation around the bond axis and acts to constrain molecular geometry. As we seen later one the peptide bond, which occurs between a carbon (C) and a Nitrogen (N) atoms in polypeptide chains, is an example of such a resonance structure as are the ring structures found in the various "bases" found in nucleic acids. These the resonance nature of these atomic rings allows them to pack on top of one another. These various geometric complexities combine to make predicting a particular molecule's three dimensional structure increasingly difficult as its size increases.

Bond stability and thermal motion (a non-biological moment)

Molecules do not exist out of context. In the real, or at least the biological world they do not sitt alone in a vacuum. We always need to consider the system in which the molecules are found. For example, most biologically-relevant molecular interactions occur in aqueous solution. That means, biological molecules are surrounded by other molecules, mostly water molecules. As you may already know from physics there is a lowest possible temperature, known as absolute zero (0 K, –273.15 ºC , –459.67 °F). At this, biologically irrelevant temperature, molecular movements are minimal, but not apparently absent all together.[153] When we think about a system, we inevitably think about its temperature. Temperature is a concept that makes sense only at the system level. Individual molecules do not have a temperature. The temperature of a system is a measure of the average kinetic energy of the molecules within it. The average kinetic energy is:

$$E_k = 1/2 \text{ (average mass)} \times (\text{average velocity})^2$$

It does not matter whether the system is composed of only a single type of molecule or many different types of molecules, at a particular temperature the average kinetic energy of the molecules has one value. This is not to say that all molecules have the same kinetic energy, they certainly do not; they form a distribution that is characterized by its average energy, this distribution is known as the Boltzmann (or Maxwell-Boltzmann) distribution (see above)(→). The higher the temperature, the more molecules will have a higher kinetic energy.



In a gas we can largely overlook the attractive interactions between molecules, their intermolecular interactions, because the average kinetic energies of the molecules of the system are sufficient to disrupt those intermolecular interactions - that is, after all, why they are a gas. As we cool the system, we remove energy from it, and the average kinetic energy of the molecules decreases. If the average kinetic energy gets low enough, the molecules will form a liquid. In a liquid, the movement of molecules is not enough to completely disrupt the interactions between them. This is a bit of a simplification, however. Better to think of it more realistically. Consider a closed box partially filled with a substance in a liquid state. What is going on? Assuming there are no changes in temperature over time, the system

[153] https://en.wikipedia.org/wiki/Zero-point_energy

will be a equilibrium. What we will find, if we think about it, is that there is a reaction going on, that reaction is: Molecule (gas) ⇆ Molecule (liquid). At the particular temperature, the liquid phase is favored, although there will be some molecules in the system's gaseous phase. The point is that at equilibrium, the number of molecules moving from liquid to gas will be equal to the number of molecules moving from the gas to the liquid phase. If we increase or decrease the temperature of the system, we will alter this equilibrium state, that is, the relative amounts of molecules in the gaseous versus the liquid states will change. The equilibrium is dynamics, in that different molecules may be in gaseous or the liquid states, even though the level of molecules will be steady.

In a liquid, while molecules associate with one another, they can still move with respect to one another. That is why liquids can be poured, and why they assume the shape of the (solid) containers into which they are poured. This is in contrast to the container, whose shape is independent of what it contains. In a solid the molecules are tightly associated with one another and so do not translocate with respect to one another (although they can rotate and jiggle in various ways). Solids do not flow. The cell, or more specifically, the cytoplasm, acts primarily as a liquid and many biological processes take place in the liquid phase. This has a number of implications. First molecules, even very large macromolecules, can move with respect to one another. Driven by thermal motions, molecules will move in a Brownian manner, a behavior known as a random walk.

Thermal motion will influence whether and how molecules associate with one another. We can think about this process in the context of an ensemble of molecules, let us call them A and B; A and B interact to form a complex, AB. Assume that this complex is held together by van der Waals interactions. In an aqueous solution, the A:B complex is colliding with water molecules. These water molecules have various energies (from low to high), as described by the Boltzmann distribution. There is a probability that in any unit of time, one or more of these collisions will deliver sufficient energy to disrupt the interaction between A and B leading to the disassociation of the AB complex into separate A and B molecules. Assume we start with a population of 100% AB complexes, the time it takes for 50% of these molecules to dissociate into A and B is considered the half life of the complex. Now here is the tricky part, much like the situation with radioactive decay, but subtly different. While we can confidently conclude that 50% of the AB complexes will have disassembled into A and B at the half-life time, we can not predict which of these AB complexes will have disassembled and which will remain intact. Why? Because we cannot predict which collisions, by providing sufficient energy to overcome the van der Waals interaction between A and B, will lead to the disassociation of the AB complex.[154] This type of process is known as a stochastic process, since it is driven by random events. Genetic drift is another form of a stochastic process, since in a particular drifting population it is not possible to predict which alleles will be lost and which fixed, or when exactly fixation will occur. A hallmark of a stochastic process is that they are best understood in terms of probabilities.

Stochastic processes are particularly important within biological systems because, generally, cells are small and may contain only a small number of molecules of a particular type. If, for example, the expression of a gene depends upon a protein binding (reversibly) to specific sites on a DNA

[154] It should be noted that, in theory at least, we might be able to make this prediction if we mapped the movement of every water molecule. This is different from radioactive decay, where it is not even theoretically possible to predict the behavior of an individual radioactive atom.

molecule, and if there are relatively small numbers of the protein and (usually) only one or two copies of the gene (that is, the DNA molecule) present, we will find that whether or not a copy of the protein is bound to the specific DNA region is a stochastic process.[155] If there are enough cells, then the group average will be predictable, but the behavior of any one cell will not be. In an individual cell, sometimes the protein will be bound and the gene will be expressed and sometimes not, all because of thermal motion and the small numbers of interacting components involved. This noisy (stochastic) property of cells can play important roles in the control of cell and organismic behavior. It can even transform a genetically identical population of organisms into subpopulations that display two or more distinct behaviors, a property with important implications, that we will return to.

Questions to answer & to ponder:

- Explain to yourself, and others, why the Boltzmann distributions is not symmetrical around the highest point.
- Based on your understanding of various types of intermolecular and intramolecular interactions, propose a model for why the effect of temperature on covalent bond stability is not generally significant in biological systems?
- How does temperature influence intermolecular interactions? How might changes in temperature influence molecular shape (particularly in a macromolecule)?
- Why are some liquids more viscous (thicker) that others? Draw a picture of your model.
- In considering generating a graph that describes radioactive decay or the dissociation of a complex (like the AB complex discussed above) as a function of time, why does population size matter?

Bond polarity, inter- and intramolecular interactions

So far, we have been considering covalent bonds in which the sharing of electrons between atoms is more or less equal, but that is not always the case. Because of their atomic structures, which arise from quantum mechanical principles (not to be discussed here), different atoms have different affinities for their own electrons. When an electron is removed or added to an atom (or molecule) that atom/molecule becomes an ion. Atoms of different elements differ in the amount of energy it takes to remove an electron from them; this is, in fact, the basis of the photoelectric effect explained by Albert Einstein, in another of his 1905 papers.[156] Each type of atom (element) has a characteristic electronegativity, the measure of how tightly the atom holds onto its electrons. If the electronegativities of the two atoms in a bond are equal or similar, then the electrons are shared more or less equally between the two atoms and the bond is said to be non-polar. There are no stable regions of net negative or positive charge on the surface of the resulting molecule. If the electronegativities of the two bonded atoms are unequal, however, then the electrons will be shared un-equally. On average, there will be more electrons more of the time around the more electronegative atom and less around the less electronegative atom. This leads to stable partially negatively and positively-charged regions to the bond; this charge separation produces an electrical field, known as a dipole. A bond between atoms of differing electronegativities is said to be polar.

---

[155] This is illustrated here (https://phet.colorado.edu/en/simulation/gene-expression-basics) and we will return to this type of behavior later on.

[156] Albert Einstein: Why Light is Quantum: http://youtu.be/LWIi7NO1tbk

In biological systems, atoms of O and N will sequester electrons when bonded to atoms of H and C, the O and N become partly negative compared to their H and C bonding partners. Because of the quantum mechanical organization of atoms, these partially negative regions are organized in a non-uniform manner, which we will return to. In contrast, there is no significant polarization of charge in bonds between C and H atoms, and such bonds are non-polar. The presence of polar bonds leads to the possibility of electrostatic interactions between molecules. Such interactions are stronger than van der Waals interactions but much weaker than covalent bonds; like covalent bonds they have a directionality to them – the three atoms involved have to be arranged more or less along a straight line. There is no similar geometric constraint on van der Waals intermolecular interactions.

Since the intermolecular forces arising from polarized bonds often involve an H atom interacting with an O or an N atom, these have become known generically (at least in biology) and perhaps unfortunately as hydrogen or H-bonds. Why unfortunate? Because H atoms can take part in covalent bonds, but H-bonds are not covalent bonds, they are very much weaker. It takes much less energy to break an H-bond between molecules or between parts of (generally macro-) molecules that it does to break a covalent bond involving a H atom.

The implications of bond polarity

Two important physical properties of molecules (although this applies primarily to small molecules and not macromolecules) are their melting and boiling points. Here we are are considering a pure sample that contains extremely large numbers of the molecule. Let us start at a temperature at which the sample is liquid. The molecules are moving with respect to one another, there are interactions between the molecules, but they are transient - the molecules are constantly switching neighbors. As we increase the temperature of the system, the energetics of collisions are now such that all interactions between neighboring molecules are broken, and the molecules fly away from one another. If they happen to collide with one another, they do not adhere; the bond that might form is not strong enough to resist the kinetic energy delivered by collision with other the molecules. The molecules are said to be a gaseous state and the transition from liquid to gas is said to be the boiling point. Similarly, starting with a liquid, when we reduce the temperature, the interactions between molecules become longer lasting until such a temperature is reached that the energy transferred through collisions is no longer sufficient to disrupt the interactions between molecules.[157] As more and more molecules interact, neighbors become permanent - the liquid has been transformed into a solid. While liquids flow and assume the shape of their containers, because neighboring molecules are free to move with respect to one another, solids maintain their shape, and neighboring molecules stay put. The temperature at which a liquid changes to a solid is known as the melting point. These temperatures mark what are known as phase transitions: solid to liquid and liquid to gas.

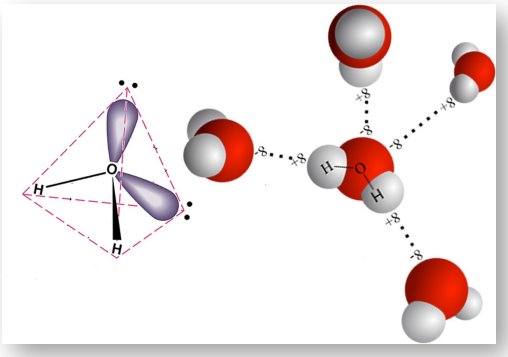
At the macroscopic level, we see the rather dramatic effects of bond polarity on melting and boiling points by comparing molecules of similar size with and without polar bonds and the ability to form H-bonds. For example, neither CH_4 (methane) and Ne (neon) contain polar bonds and cannot

[157] The nature of the geometric constrains on inter-molecular interactions will determine whether the solid is crystalline or amorphous. see: https://en.wikipedia.org/wiki/Crystal

form intra-molecular H-bond-type electrostatic interactions. In contrast, NH_3 (ammonia), H_2O (water), and FH (hydrogen fluoride) have three, two and one polar bonds, respectively, and can take part in one or more intra-molecular H-bond-type electrostatic interactions. All five compounds have the same number of electrons, ten. When we look at their melting and boiling temperatures, we see rather immediately how the presence of polar bonds influences these properties.

Compounds	CH_4	NH_3	OH_2	FH	Ne
molecular weight	16.04	17.02	18.02	20.01	20.18
bond electronegativity	0.45	0.94	1.34	1.88	N/A
# of electrons	10	10	10	10	10
# of bonds	4	3	2	1	0
melting point	-182°C	-77.7°C	**0°C**	-83°C	-248.6°C
boiling point	-161.5°C	-33.4°C	**100°C**	19.5°C	-246.1°C

In particular water stands out as dramatically different from the rest of the molecules, with significantly higher (> 70°C) melting and boiling point than its neighbors. So why is water different? Well, in addition to the presence of polar covalent bonds, we have to consider the molecule's geometry. Each water molecule can take part in four hydrogen bonding interactions with neighboring molecules - it has two partially positive Hs and two partially negative sites on its O. These sites of potential H-bond-type electrostatic interactions are arranged in a nearly tetragonal geometry. Because of this arrangement, each water molecule can interact through H-bond-type electrostatic interactions with four neighboring water molecules. To remove a molecule from its neighbors, four H-bond-type electrostatic interactions must be broken, which is relatively easy since they are each rather weak. In the liquid state, molecules jostle one another and change their H-bond-type electrostatic interaction partners constantly. Yet, even if one is broken, the water molecule remains linked to multiple neighbors via H-bond-type electrostatic interactions.



This molecular hand-holding leads to water's high melting and boiling points as well as its high surface tension. We can measure the strength of surface tension in various ways. The most obvious is the weight that the surface can support. Water's surface tension has to be dealt with by those organisms that interact with a liquid-gas interface. Some, like the water strider, use it to cruise along the surface of ponds. As the strider walks on the surface of the water, the molecules of its feet do not form H-bond-type electrostatic interactions with water molecules, they are said to be **hydrophobic**, although that is clearly a bad name - they are not afraid of water, rather they are simply apathetic to it. They interact with other molecules, including water molecules, only through van der Waals interactions. Molecules that can make H-bonds with water are termed **hydrophilic**. As molecules' increase in size they can have regions that are hydrophilic and regions that are

hydrophobic (or hydroapathetic). Molecules that have distinct hydrophobic and hydrophilic regions are termed amphipathic and we will consider them in greater detail in the next chapter.

Interacting with water

We can get an idea of the hydrophilic, hydrophobic/hydroapathetic, and amphipathic nature of molecules through their behaviors when we try to dissolve them in water. Molecules like sugars (carbohydrates), alcohols, and most amino acids are primarily hydrophilic. They dissolve readily in water. Molecules like fats are highly hydrophobic/hydroapathetic, and they do not dissolve significantly in water. So why the difference? To answer this question we have to be clear what we mean when we say that a molecule is soluble in water. We will consider this from two perspectives. The first is what the solution looks like at the molecular level, the second is how the solution behaves over time. To begin, we need to understand what water alone looks like. Because of its ability to make and donate multiple H-bond-type electrostatic interactions in a tetrahedral arrangement, water molecules form a dynamic three-dimensional intermolecular interaction network. In liquid water the H-bond-type electrostatic interactions between the molecules break and form rapidly.

To insert a molecule A, known as a solute, into this network you have to break some of the H-bond-type electrostatic interactions between the water molecules, known as the solvent. If the A molecules can make H-bond-type electrostatic interactions with water molecules, that is, if it is hydrophilic, then there is little net effect on the free energy of the system. Such a molecule is soluble in water. So what determines how soluble the solute is. As a first order estimate, each solute molecule will need to have at least one layer of water molecules around it, otherwise it will be forced to interact with other solute molecules. If the number of these interacting solute molecules is large enough, the solute will no longer be in solution. In some cases, aggregates of solute molecule can, because they are small enough, remain suspended in the solution. This is a situation known as a colloid. While a solution consists of individual solute molecules surrounded by solvent molecules, a colloid consists of aggregates of solute molecules in a solvent. We might predict that all other things being equal (a unrealistic assumption), the larger the solute molecule the lower its solubility. You might be able to generate a similar rule for the size of particles in a colloid.

Now we can turn to a conceptually trickier situation, the behavior of a hydrophobic/apathetic solute molecule in water. Such a molecule cannot make H-bond-type electrostatic interactions with water, so when it is inserted into water the total number of H-bond-type electrostatic interactions in the system decreases - the energy of the system increases (remember, bond forming lowers potential energy). However, it turns out that much of this “enthalpy” change, conventionally indicated as ΔH, is compensated for by van der Waals interactions (that is, non-H-bond-type electrostatic interactions) between the molecules. Generally, the net enthalpic effect is minimal. Something else must be going on to explain the insolubility of such molecules.

Turning to entropy: In a liquid water molecules will typically be found in a state that maximizes the number of H-bond-type electrostatic interactions present. And because these interactions have a distinct, roughly tetragonal geometry, their presence constrains the possible orientations of molecules with respect to one another. This constraint is captured when water freezes; it is the basis for ice crystal

formation, why the density of water increases before freezing, and why ice floats in liquid water.[158] In the absence of the hydrophobic/hydroapathetic solute molecule there are many many equivalent ways that liquid water molecules can interact to produce these geometrically specified orientations. But the presence of a solute molecule that cannot form H-bond-type electrostatic interactions restricts this number to a much smaller number of configurations that result in maximizing H-bond formation between water molecules. The end result is that the water molecules become arranged in a limited number of ways around each solute molecule; they are in a more ordered, that is, a more improbable state, than they would be in the absence of solute. The end result is that there will be a decrease in entropy (indicated as ΔS), the measure of the probability of a state. ΔS will be negative compared to arrangement of water molecules in the absence of the solute.

How does this influence whether dissolving a molecule into water is thermodynamically favorable or unfavorable. It turns out that the interaction energy (ΔH) of placing most solutes into the solvent is near 0, so that it is the ΔS that makes the difference. Keeping in mind that $\Delta G = \Delta H - T\Delta S$, if ΔS is negative, then $-T\Delta S$ will be positive. The ΔG of a thermodynamically favorable reaction is, by definition, negative. This implies that the reaction:

water + solute ⇆ solution (water + solute)

will be thermodynamically unfavorable; the reaction will move to the left. That is, if we start with a solution, it will separate so that the solute is removed from the water. How does this happen? The solute molecules aggregate with one another. This reduces their effects on water, and so the ΔS for aggregation is positive. If the solute is oil, and we mix it into water, the oil will separate from the water, driven by the increase in entropy associated with minimizing solute-water interactions. This same basic process plays a critical influence on macromolecular structures.

Questions to answer & to ponder:

- Given what you know about water, why is ice less dense than liquid water?
- Make of model relating the solubility of a molecule with a hydrophilic surface to the volume of the molecule?
- Use your model to predict the effect on solubility if your molecule with a hydrophilic surface had a hydrophobic/apathetic interior.
- Under what conditions might entropic effects influence the interactions between two solute molecules?

[158] http://youtu.be/UukRgqzk-KE