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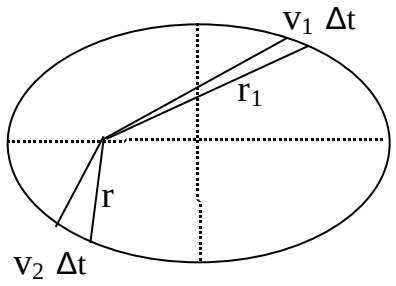
# I. Introduction

## a. Prelude

Putting together “physical forces” with “biomolecules” is still enough of a novelty that I begin with an example of how “forces” have made us think differently about the universe during the past few centuries. In his exciting biography of Johannes Kepler, The Watershed, Arthur Koestler wrote of crossing of a divide in human thinking, a crossing between the age of astrology and geometry to a time when one began to think about the cause and effect acting in the universe. The realization that came from Kepler was that by looking at the paths of the planets, one could recognize two features that today are known as his First and Second Laws.

First, bravely against the idea that the planets moving in circles, Kepler allowed himself to see that they were moving in elliptic paths and that the sun was at one of the foci of each planet’s ellipse.

Second, he looked at how fast the planets were moving relative to the distance they were from the sun, faster and slower depending on whether they were closer or further away. He measured the area of the triangle that was swept out in any given period of time $\Delta t$ -- one half the distance $v\,\Delta t$ moved along the planet’s path times its distance r from the sun. He realized that this area, $\frac{1}{2} v\,\Delta t\, r$, was the same no matter where the planet was in its trajectory, no matter how fast it was moving.




$$v_2\, r_2 = v_1$$

Apparently these observations tormented him enormously. Why? It was a classic case of applied vs. basic research.

Kepler earned his living as a court astrologer. He had to tell the prince important things like the best time to start a war or maybe kill his Chancellor. He had to pull this important information out of astronomical data that he was pondering and plotting onto his ellipses.

And there were lots of numbers for him to ponder. There was a flood of information at that time. The telescope had recently been invented. The work of Tycho Brahe was providing abundant and accurate measurements of the movements of the stars and planets.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... In fact, it was not much different from the tide of numbers we're getting nowadays from all the protein and nucleic acid crystal structures and sequences. It was quite an undertaking for Kepler with his old system of Information Resource Management. He even had to work without logarithms because they had not yet been invented. But he carried on —not only because of his need to understand but also to do something to satisfy the prince.

Painfully, reluctantly, he was beginning to realize that if the planets were moving faster and slower, closer and further away from the sun, then there was something weird going on between the sun and the planets. Something was pushing and pulling the planets! A completely alien idea.

Still he wanted to talk about the heavens with ideologically correct phrases like "the harmony of the spheres". Planets were supposed to move in circles. Irregularities like ellipses sometimes got patched together by postulating "epicycles", putting little circles on a big circle, a kind of "overtone" in the harmony of the spheres. So there he was, one foot on the side of astrology and pure geometry, the other foot on the side of cause and effect. Did he ever realize what an essential step he had taken?

It is said that several years later Isaac Newton really had to dig through Kepler's notes in order to find Kepler's "laws" buried deep within them. And what he pulled out with those laws was a frightening idea that he called "action at a distance". Those equal-area triangles brought him -- maybe compelled him -- to see the laws of gravity, momentum, and acceleration. He saw that there was a fundamental equivalence of the action by the forces bodies felt when they collided and that which was felt at a distance.

A few postulates about how bodies moved, a single possibility for the mathematical form of the gravitational "action at a distance" (whose physical basis we still do not understand!), and all those tables of numbers fell into a pattern. Even now, with all our numbers for the planets, stars, and galaxies, it is the same gravitational "action at a distance" that is foremost in how we think about our swirling, heaving universe.

Newton's realization irreversibly put mankind on the other side of the mental watershed. We reflexively think now in terms of causative forces when we think about how matter is organized. The equality of those triangular areas was enough to start of the modern age of reason.

Forward 300-plus years. Zoom from $10^{+14}$ meters to $10^{-9}$ meters. It is not planets; it is proteins, fats, nucleic acids and carbohydrates. It is not orbits; it is sequences, structures, motions,....

[picture of Hemoglobin.]



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Again the problem of how to absorb all the information, to use it to learn how the protein works. We seem to have the equivalent of Brahe's tables of numbers. We might possibly have the equivalent of Kepler's laws (if so, buried again, this time in dozens of journals and thousands of articles), but there is certainly nothing yet to compare with Newton's cut through the tangled knots of hypotheses and postulates. What we need is to know the operative forces, measure them, recognize them, see how they act on the scale of the nano-objects that compose us.[1]

## b. Qualitative features of large molecules

The first thing you can say about macromolecules is that they are big, at least compared to most other molecules. They must have their size, apparently. If they could do their work by being any smaller, by now evolution would have allowed smaller versions to take over. They are "macro" for good reasons, and we should ask what those reasons might be. The feature of size itself confers qualitative properties on any molecular system, it helps to look at those qualitative properties just for building a framework for thinking how large molecules proceed in their natural life.

Different kinds of forces dominate behavior depending on different sizes of the system. Gravity is important at one end of the size scale, but it is not such serious business for small particles.

For sub-atomic and sub-nuclear particles, one thinks of enormously strong forces of such short range that one can forget about them outside the atomic nucleus. Only when we climb to the level of a whole atom does it make sense to talk about electrostatic interactions, electronic orbitals, chemical bonds. When we step further up to molecules, we are already thinking of properties that no longer resemble those of the atoms that compose them.

And the behavior of macromolecules is in turn determined by features that are of little relevance to small molecules. In fact, the very size of many biological molecules creates essential properties that could not be achieved by smaller molecules.
Three of my favorite big-size properties are:

[1] Musician/physicist Joe Wolfe of Sydney, Australia recently wrote an ear-opening set of papers on "the information content of music". His point is that there is a waxing and waning of this "content", what it takes to describe the music. It begins with the themes and colorings in the composer's brain. These must be spelled out as markings on sheets of music which translate upon performance into the staggering number of numbers that spell out the vibrations in the air and the coding on a CD. After that it's a reduction back to the relatively few "bits of information" (themes, colorings) recreated in our own heads to bring us close to what the composer had in mind. Perhaps with protein structures we're at the CD stage. We're reading the sheets or the CD, but the theme of the thing -- like Newton's equations and a few important numbers -- isn't clear yet.


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**1). When a macromolecule moves, it displaces many small solvent molecules.**

Think of a 100 Angstrom - by - 100 Angstrom patch of two facing membranes. Imagine that they come together by 3 Å. Given the 30-cubic-Angstrom size of a water molecule[2], how many water molecules are squeezed out from between these membrane patches by this tiny displacement? (100 x 100 x 3)/30 = 1000 waters.

Say that these water molecules are very slightly attracted to the membrane surfaces (that are usually covered by water-soluble material). The strength of this attraction might be rather small compared to the thermal energies agitating the individual water molecules. But multiply that small energy by 1000 and you see that thermal energy might not be enough to bring the patches of membrane together.

This kind of arithmetic comes up repeatedly when one is thinking about the mutual approach of membranes or of proteins, nucleic acids, or any large molecule.

**2) Large molecules are flexible.**

The same goes for trees, skyscrapers and suspension bridges. If they could not bend with the wind, they would not stand long. Macromolecules are already big enough that one must speak of their bending, flexing, changes in conformation as energetic events -- means to respond to surroundings, means of maintaining stability through multiple configurations, ability to store energy by changing shape. In fact, a protein is designed for its power to undergo large structural changes in directed and intended ways. These large molecules are already mechanical objects with laws of stress and strain easily as interesting as those of the buildings and bridges that usually enjoy attention for their mechanical abilities. Remarkably, the bending and twisting energies of nanometer-thick bilayers and linear macromolecules can be summarized with the similar definitions of elasticities and plasticities used for mighty beams and cables. Indeed, when we speak of forces organizing biomolecules we often leap over unsolved difficulties at the atomic level to speak of large molecules in the language of macroscopic objects.

**3) Large molecules create time or, better, time scales.**

For example, if we talk about a nerve firing, we talk about events that occur reliably over milliseconds. If one watches single channels opening and closing, again, the events are best graphed with the time axes ticked in milliseconds.

Where do these time scales come from? How does one design a system whose time of response is so different from the natural vibrations and rotations of its components?

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[2] The specific volume of water is 1 cc/gram, that is, 1 $cm^3$/gram or $10^{24}$ $Å^3$/gram.
The molecular weight of water is 18, so one gram is (1/18) mole or 1/18th of Avogadro's number of molecules. The volume per water molecule is then

$$\frac{10^{24}\,Angstroms^3}{\frac{1}{18}moles} = \frac{10^{24}\,Angstroms^3}{\frac{.6 \leftrightarrow 10^{24}}{18}molecules} = \frac{18}{.6}\,Å^3/\text{molecule} = 30\,Å^3/\text{molecule}.$$


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Electrons jump around in periods of ultraviolet frequencies or periods less than femtoseconds;

Chemical bonds vibrate in the infrared, faster than picoseconds;

Computational molecular biologists feel good when they can shake things for a few hundred picoseconds;

Dielectric relaxation covers microseconds to nanoseconds.

Somehow proteins manage to stretch things out to stay in different conducting states for milliseconds. How is the system so tuned that channels specific to one ion type respond just slower enough than those of another ion type to create a desired pattern of currents? This is one of the most neglected capabilities of macromolecules. If channel proteins skittered around too fast they could not carry signals!

Biophysicists can take some pleasure realizing that the best kinetic data coming out these days on large molecules of any kind are those from the study of ionic channels. If molecular dynamicists started to respect these data rather than doing dynamics on the time scales that they are used to from studying simpler materials, I think that they would finally begin to sense the soul of the molecular machine.

For all the trendy talk about molecular dynamics, we still haven's a clue how to address the real features of bio-molecular time scales.

## c. Classes of molecular forces

With this perspective of largeness, what kinds of forces should we be thinking about? This course will focus on four idealized classes of interaction. I've tried to sketch them in one figure.

**Electrostatic**

the idea of an electrical charge, the observation that like charges repel and unlike charges attract, the difficulty of moving an ion from water to oil.

At first glance electrostatic forces are simple although they act in many ways depending on how charges are held and under what conditions they move, whether they are stuck onto a large surface or floating almost freely in solution. Working on the intricate structures of biomolecules, electrostatics can act in abstruse ways.

**Electrodynamic**

the fact that even though bodies can be electrically neutral they are still made out of charges, and there is motion of those charges. At any given instant, one set of charges will set up electric fields that act to perturb or to rearrange another set of charges in a way that lowers the total charge-charge interaction between the two sets. One can think of the two sets of dancing charges sending out continually changing electric fields that work on each other to create what we call "charge fluctuation" or "van der Waals" or "electrodynamic" forces.



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**Hydration and Hydrophobic**

that come from what the large molecule does to its small- molecule solvent. Much to think about here.

Water-soluble groups bound to a membrane or macromolecular surface will draw water to that surface, often holding the water tighter than it would be held to its fellow water molecules. Tenacity for this water makes it hard to squeeze it out when two such surfaces come together, a repulsive "solvation force".

Charges on a membrane or a molecular surface will hold water tightly but not so strongly as they would hold to a charge of opposite sign on an approaching surface. The water will be easily released between the two opposite charges, the work of releasing the water might be mitigated by the fact that the released waters enjoy more freedom ("entropy") than they did nailed to the surface. This drive to liberate solvent might even appear to dominate the event, to create an attractive "solvation force".

Surfaces with water-insoluble groups will not strongly attract water but will disturb or perturb it from its condition in bulk. This disturbance is usually energetically costly. The tendency will be for the water to be drawn toward the bathing solution and to have the effect of bringing the water-insoluble, or non-polar, surfaces together, a solvent-driven or "hydrophobic" attractive force between non-polar surfaces.

Similarly, solute adsorption or repulsion lowers or raises the free energy of the macromolecular surface. Changes in the surface "excess" or "deficit" if solutes that accompany changes in surface separation also act as forces between large molecules.

**Configurational-Entropic or "steric" repulsions**

from the fact that molecules like to move and they resist being pushed together in a way that restricts their freedom of motion.

This configurational interaction can be as simple as the point-size particles of an ideal gas being pressed into a smaller box or as complicated as the innards of a biological cell crowded, even deformed within the confines of a cell wall. Flexible, large molecules have natural motions that will be suppressed as they come together even to the extent that molecules can be deformed from their configurations of lowest energy.

Division of forces into these four categories is mainly for convenience. In fact, there is rarely such a clean operative distinction. For example, the collisions that confine real molecules are actually mediated by electrostatic, electrodynamic and solvation forces. The real work of organizing molecules is through a combination of interactions that is not a simple sum of each contributing kind of force in its purest form. But for learning it often helps to separate each kind of force one from the actual mix.


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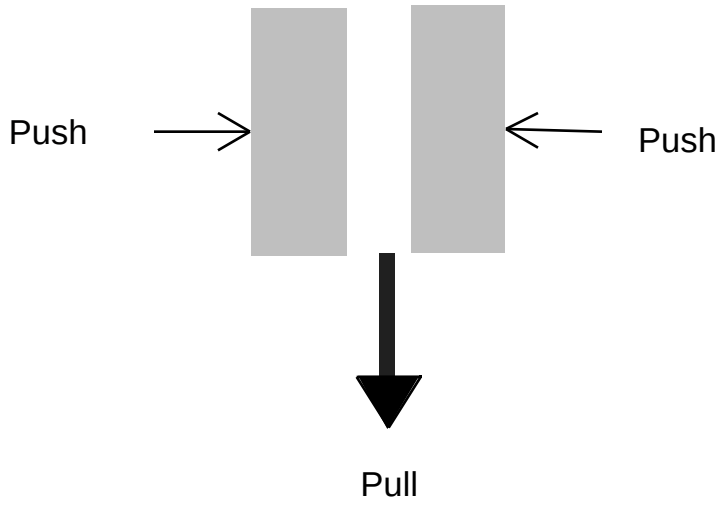
## d. Measurement of forces between large molecules

**Two ways to think about forces**

There are at least two ways to think about what happens when membranes or macromolecules are brought together in aqueous solution.

One way is that they have to be pushed. Exert a force on each of them and they move together. I imagine this as a physicist's way of thinking, directly moving bodies around and measuring what it takes to do it.

Another way to think of what is going on recognizes the work to transfer solvent or solution from between the macromolecules to some region of pure solvent or solution far away. Unless the solution that is transferred leaves behind a vacuum, the macromolecules must come together to fill the voided space. It comes to the same net result, the large molecules come together, but now we are thinking in the chemist's language of chemical potentials or works of transfer.




In the same ways that we think about pushing molecules together, we can think about deforming molecules themselves. One can then relate molecular mechanics and molecular function by strategies developed to measure forces of interaction.

**What does one mean by a force between molecules?**

If we were thinking about forces as they are out in the universe or between atoms or molecules in a gas, with just vacuum in between, we could quite simply say that a force is connected with the work done to push bodies together or to pull them apart. But it is a little different if there is pushing aside of solvent between the large molecules and if there is the incessant thermal agitation of the molecules in this crowded space.

What do we call the push?

On what are we doing the work?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Think about creating an arrangement, a stack of bilayer membranes in a multilayer, a hexagonal array of parallel linear molecules—configurations that take a certain amount of work to create them. If you make some small change in that arrangement, for example in the separation between the membranes or the linear molecules, you can think of a force in terms of the work needed. The amount of work done is this force times the small change in separation.

But with all the jostling in the solvent, in the membranes or the macromolecules, you necessarily have to be thinking in terms of average positions and energies averaged over many microscopic arrangements.

A good physicist would call this work a "potential of mean force" to describe all that goes on when a large flexible body moves in the violently agitated world of a liquid. The idea is to recognize all the motions that add up to the total work done. A chemist might prefer to speak of a "chemical potential".

There is really split in personality here between the hypothetical physicist and chemist. One of them thinks of somehow holding on to objects and pushing them together. It just gets a little messy when the things are wriggling and there is lots of stuff in between. The chemist explicitly recognizes the material in between the two objects and talks about the effort it takes to pull out that material while the objects come together in the empirical terms of activities and generalized potentials.

Does that seem too schizophrenic? It is a difficult idea, but if we speak of a work of transfer of what is in between, we can also speak of work of pushing together.

Like so many alternatives in human thinking, it is not a good idea to make yourself choose which one is better. It is much better to try to use both ways of thinking at the same time or at least to be able to move back and forth between views and languages.

. You learn different things from being able to think either way. That dual thinking helps particularly when we come to measure forces.

**How can one measure a force between large molecules?**

Osmotic stress

How can you push a molecule with a spring that is light enough, gentle enough to act as a good scale?

Answer: You push a molecule with another molecule.

The Osmotic Stress method, which I will describe first, has that idea in it. Create an ordered array of molecules and bring that array to equilibrium with a polymer solution (Figure). There is a balance between the known "osmotic" pressure of moving water in and out of the polymer solution and moving water in and out of the ordered array. At the same time, small molecules, whose activities are set in the reservoir of the polymer solution, are able to move in and out; these activities such as pH, ionic strength are thereby controlled as the array is osmotically stressed.


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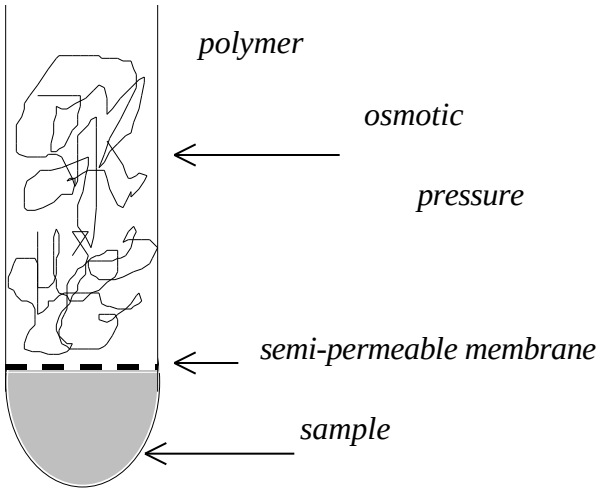
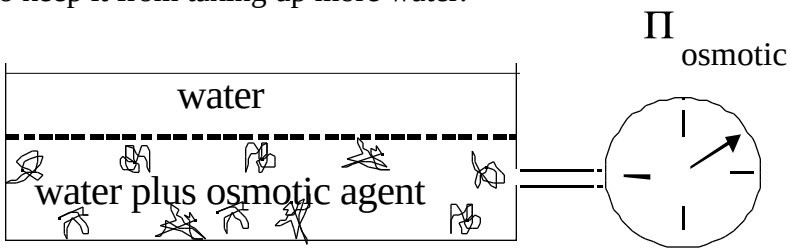



In the physical sense, the attempted spreading of the concentrated polymer solution constitutes a force against the array of molecules with which it cannot mix (either because they are kept separated by a membrane that does pass large molecules or because the large molecules are simply immiscible). As polymer concentration is increased, the polymers push harder against the array of molecules.

In the chemical sense, the thirsty polymer solution competes with the ordered array of molecules for the water or the aqueous solution in which they both want to dissolve. As polymer concentration is increased, the thirst of the polymer solution creates a stronger pull on the water between the molecules in the array.

How strong is the push? Typically one speaks of an "osmotic pressure" of the polymer solution. All that an osmometer really does is to measure the strength with which one must squeeze on the polymer solution—through a water-permeable membrane—to keep it from taking up more water.




It is a real pressure, a work per volume, and specifically the work to transfer a volume of water from among the polymers to a polymer-free solution.

By the fact that polymer solution and ordered array are balanced in the intensity of their fight for water, one knows from the osmotic pressure calibration of the polymer solution the magnitude of the osmotic pressure—or better the "osmotic stress "—acting on the ordered array.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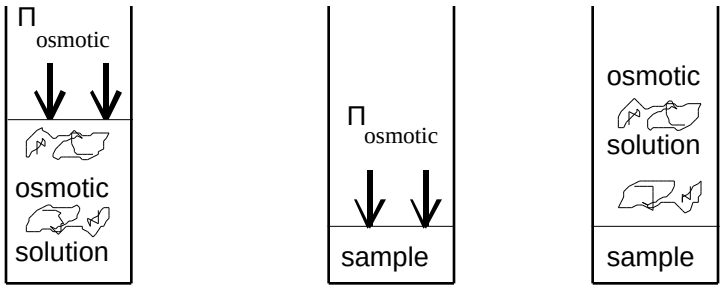


Osmometer calibration of osmotic solution

Direct force through membrane

Equilbrium between two solutions

That is the “force” part of “force vs. distance”. The second part is to measure molecular separation by some direct method. X-ray diffraction is usually the preferred choice.

Plotting the applied osmotic stress vs. the varying separation between molecules gives a continuous reading of the work it takes to bring the molecules together. If the array is a multilayer of membranes, then the pressure is exactly what is acting on the face of the membranes. Within an array of linear molecules, the applied pressure must be transformed to be expressed as the sum of forces between the molecules.

The strategy works in all kinds of systems, not only lamellar membranes or linear molecules. Non-lamellar phases of lipids and dense solutions or gels of proteins have also been observed for changes under osmotic stress. In fact, it is entirely reasonable to “map” the structures of all kinds of mixtures in terms of the osmotic work it takes to create them. It is even possible to apply osmotic stress to change the shape of single molecules, such as ionic channels, that have aqueous regions that stressing solutes cannot enter -- an exciting possibility for probing single functioning molecules in a strategy similar to that used for arrays of molecules.

Osmotic pressures of polymers often do not go high enough to measure the full range of forces. Then it is sometimes practical to squeeze on an ordered array held in a chamber with one wall that is a stiff and strong semi-permeable membrane that keeps the sample separated from water or from water solution.

To go to still higher pressures, it is possible to expose the sample to a vapor of a saturated salt solution or of a hydrochloric acid solution whose vapor pressure has been calibrated. These vapor pressure exposures are so powerful that they can virtually dry out a sample. Their main disadvantage is that one is using the air as the “semi-permeable membrane”. Salts and small solutes could move around freely in the other two procedures. Now they are trapped in the sample so that their chemical activities are not regulated. Risky for measurement of forces between the large molecules!

<u>Force</u> <u>balances:</u>

[TO BE EXPANDED]

There are also mechanical means of measuring forces



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For almost forty years, there has been steady development of 'surface force' balances. These are basically very delicate spring balances between convex lenses of quartz or glass, originally intended to measure van der Waals forces in air or vacuum. More recently there have been procedures with the surface force apparatus (SFA) to glue mica or silica sheets onto the lenses and even to coat the mica in turn by various substances adsorbed from solution.

A much newer kind of balance, the atomic force microscope (AFM), has a kind of stylus riding on a laterally movable surface. The arm of the stylus is kept at a constant force perpendicular to the surface; one measures the up-and-down motion of the stylus as it is presented with different parts of the laterally moved surface.

Although not intended for measurements between molecules, results from these force-balance methods can often be interpreted as such, for example when their surfaces are coated with lipid bilayers. One must be aware that the act of firmly immobilizing water-soluble membranes or molecules onto solid surfaces is likely to alter the properties they had in free solution.

With optical tweezers, micrometer-size beads of high refractive index can be impaled on beams of lasers. Displacement of the beam moves the bead. If there is any resistance to moving, the bead shifts off from the center of the beam. In this way it is possible to measure forces on such particles, especially when they are attached to a long molecule or embedded in a mass of material.

Pipette Aspiration (PA):

[SECTION TO BE EXPANDED]

It is possible to hold large vesicles or cell membranes with micropipettes and to manipulate them. One can suck part of the bilayer or membrane into the pipette to stretch them to measure membrane lateral compressibility (or extensibility). One can bring together two vesicles or cells to measure directly their attractive energy of contact when they flatten or otherwise deform against each other against the tension enforced by controlled sucking pressure. This "pipette aspiration" (PA) method gives very accurate information on the elasticity and plasticity of membranes as well as direct measurement of their contact energies.

The vesicle or membrane can be considered a very delicate spring of widely adjustable stiffness. It is possible to attach different adherent molecules A and B on apposing membranes and let them bind across the space between membranes. Then, by stiffening the spring of the membranes to which they are attached, one can pull apart A and B while measuring the force vs. separation of their binding.

Rather than choose among the various methods of force measurement, it is much wiser to try to combine them and to see them as complementary or supplementary techniques. It is best to look at the same material in as many ways as possible. For example, pipette aspiration tells us how strongly bilayers attract in solution. Osmotic stress gives us the work of pushing together lipid bilayers in a multilayer stack; there is freedom of motion so that multilayers will repel because of their thermal agitation as well


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as through their direct interaction. If the same bilayers can be immobilized without significant distortion onto a mica or silica surface, then with the additional measurement of a force balance, one can compare bilayer interaction with and without freedom of thermal motion.


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## e. A natural unit of energy for macromolecules

How does one decide what is strong and weak for organizing biomolecules? There are many criteria. It helps to keep in mind one of the most popular and practical units of energy to use almost immediately and practically in all situations.

This is thermal energy kT per molecule or RT per mole, a measure of the vigor of thermal motion. As you read this sentence, the energy of the gas molecules pinging off your nose is (3/2) kT. There is (1/2) kT fed to an object for every way it can move, every "degree of freedom". Gas molecules can move in three directions. The wriggling of large molecules is more interesting.

T is (always!) the temperature in "absolute" or Kelvin (K) units; k is Boltzmann's constant (sometimes written as $k_B$); R is also referred to as Boltzmann's constant, but it is normalized per mole rather than per molecule.

Perhaps if the human race had it to do over again and was able to improve important things, it would have recognized early on that temperature indicates the vigor of motion of the atoms and molecules composing any material. Temperature would naturally come out directly in energy units instead of being patched up with k and R. (In fact, most statistical physicists incorporate k directly into temperature and write "T" for "kT".) In any event we have kT and RT as energies which at room temperature (~20 $^{o}$C) are $4.047 \times 10^{-14}$ ergs = .583 kcal/mol = 2.437 kJ/mol (See Table).



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Table. Units of thermal energy

k or $k_B$, Boltzmann's constant

= 1.380622 x $10^{-16}$ ergs/K

= 1.380622 x $10^{-23}$ Joules/K

= 8.617093 x $10^{-5}$ electron volts/ K

R = k × Avogadro's Number

= k × (6.022169 × $10^{+23}$ molecules/mole)

= 8.31434 Joules/mole/K (1.380622 x 6.022169) × $10^{-14}$

= 8.31434 × $10^{+7}$ erg/mole/K

= 1.98717 calories/mole/K (8.31434÷(4.184 Joule/calorie))

| T ( °C) | T( °K) | kT (ergs) | RT (kcal/mole) | RT (kJ/mole) |
|---|---|---|---|---|
| 0 °C = | 273.16 K | 3.771 × $10^{-14}$ | .543 kcal/mole | 2.271 kJ/mole |
| 20 °C = | 293.16 K | 4.047 ×$10^{-14}$ | .583 kcal/mole | 2.437 kJ/mole "room temp." |
| 37 °C = | 310.16 K | 4.282 × $10^{-14}$ | .616 kcal/mole | 2.577 kJ/mole "body temp." |

1 calorie = 4.184 Joules

**Comparison with the energy of hydrolysis of ATP or GTP**

ATP or GTP hydrolysis energy

~7.5 kcal/mole

~30 kjoules/mole

~ 12.5 kT per molecule



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# Table of units

**Energy**

1 joule = $10^7$ ergs = 0.2390 calorie = 0.7376 ft lb = 6.24 x $10^{18}$ electron volts
1 calorie = 4.184 joules
1 kcalorie = $10^3$ calories = 1 food calorie
1 electron volt = 1.602 x $10^{-19}$ joules = 1.602 x $10^{-12}$ ergs ≈ 40kT (at 20 C)
1 electron volt is the energy

$h\nu$ of a photon of frequency, $\nu$ = 2.416 x $10^{14}$ Hz

$\hbar\omega$ of a photon of radial frequency $\omega$ = 1.518 x $10^{15}$ radians/second

$hc/\lambda$ of a photon of wavelength $\lambda$ = 1.242 x $10^4$ Å = 1,242 nm = 1.242 μm

1 kilowatt hour = 3.6 x $10^6$ joules
1 foot pound = 1.356 joules
1 BTU = 1.054 x $10^3$ joules

**Pressure**

**1 atmosphere** = 760 mm Hg

= 1.01325 bar

= 1.01325 × $10^{+5}$ pascals (or Newton/meter$^2$ or Joule/meter$^3$)

= 1.01325 × $10^{+6}$ dyne/cm$^2$ (or erg/cm$^3$)

= 14.7 lb/in$^2$

**1 bar** ≡ $10^5$ pascals (or N/m$^2$)
≡ $10^6$ dyn/cm$^2$
**1 torr** ≡ 1 mm Hg = 133.3 pascals
**1 pascal** = 10 dyn/cm$^2$ (or erg/cm$^3$) = 7.501 x $10^{-4}$ cm Hg
**1 cm Hg =** 1.333 x $10^4$ dyn/cm$^2$ = 1.316 x $10^{-2}$ atmospheres = 1.333 x $10^3$ pascals
**1 lb/ft$^2$ =** 47.88 pascals
**1 psi (lb/in$^2$)** = 6.895 x $10^3$ pascals
**1 inch water =** 1.868 mm Hg = 249.1 pascals

Standard Volume of ideal gas 22.4136 m$^3$/kmole = 22.4136 liters/mole Standard Temperature = 273.16 K (0 $^o$C!)


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## f. The world's most popular equation

[SECTION TITLE SOUND TOO HYPED?]

To get an idea how kT and RT work, look at what might be the world's favorite equation (considering the number of ways it is implicitly or explicitly used)

$$A \Leftrightarrow B$$

This relation can refer to conversion between two chemical species. It can go for two different forms of a molecule, "tense" and "relaxed" forms of an "allosteric" [Greek 'other-formed'] protein, "open" and "closed" state of an ionic channel. Or one can simply think of two different energetic states, say vibrational states, of a molecule.

If species (or forms or states) A and B occur in the violent world of thermal agitation, then there is an immediate connection between the temperature-energy RT and the free energy difference $\Delta G^o_{BA}$, or work that it takes to convert a mole of the material from A to B.

In a reversible chemical reaction, with the assumption that the molecules don't interact with each other more than being forced to share the same container, the concentration [B] relative to [A] is

$$\frac{[B]}{[A]} = e^{-\Delta G^o_{BA}/RT}$$

"Low" temperatures are those such that thermal energy per mole RT is small compared to the work of conversion $\Delta G^o_{BA}$, it costs too much to make B by thermal jostling. At really "high" temperature (assuming nothing else happens!), there is so much thermal energy that in comparison $\Delta G^o_{BA}$ is effectively zero; concentrations of B and A are equal.

Take the natural log of both sides and multiply by RT, to see $\Delta G^o_{BA}$ another way

$$\Delta G^o_{BA} = -RT \ln([B]/[A]) = - RT \ln(K_{BA})$$

with the equilibrium constant $K_{BA} = [B]/[A]$.

Looking at it this way, the measured concentrations [B] and [A] at thermal equilibrium between forms A and B, tell you the work it would take to go between the two forms if there were no thermal bombardment. If you find exactly equal amounts of A and B, so that $\ln([B]/[A]) = 0$, then you know that the work of conversion $\Delta G^o_{BA}$ is effectively zero. If there is virtually no A left after the reaction is over, then you know that the work of formation $\Delta G^o_{BA}$ is negative and of very large magnitude compared to thermal energy RT (or kT per molecule).

Think of $\Delta G^o_{BA}$ as though you were a god, like the *deus ex machina* of ancient drama, able to hold a molecule A and turn it into B. $\Delta G^o_{BA}$ would be the work you would have to do. To the chemist, this is the "standard free energy of conversion" between forms or the "free energy of conversion between standard states". It is perhaps more familiar when the difference $\Delta G^o_{BA}$ is written in the completely equivalent language of chemical potentials $\mu^o_B - \mu^o_A$. The superscript o reminds us that these are energies *without* the part that counts the volume available to each molecule as though it were a point-size particle; the concentration terms take care of that.



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These relations between concentration and work of conversion are equally appropriate also for examining one molecule in different states that have different molecular free energies $g_A$ and $g_B$. The ratio of the amount of time the molecule spends in the two states or, equivalently, the ratio Pr(B)/Pr(A) of the probabilities of molecule being in one of the two states at any given instant, can again be written in the form of a Boltzmann distribution

$$\frac{\Pr(B)}{\Pr(A)} = e^{-(g_B - g_A)/kT}$$

with the molecular unit kT rather than the molar energy unit RT.

Different forms of a protein in solution, different configurations of a flexing membrane of molecule, or the open and closed states of an ionic channel, we can look at the probabilities of the different forms in terms of the "Boltzmann distribution", the exponential dependence on a difference in energies.

Those individual energies $g_A$, $g_B$,.. themselves will be a function of all the system variables -- temperature, pressure, pH, the chemical potentials of solutes and solvent, applied electric fields, ... . Every standard free energy $G^o$ per mole or g per molecule, or a chemical potential $\mu^o$, for any molecule, molecular state, or form "i", depends on conditions in the preparation:

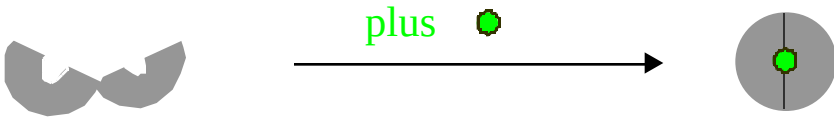
$G^o = G^o$(T, p, pH, salts, solutes,solvent, fields,.etc.).

g= g(T, p, pH, salts, solutes,solvent, fields,.etc.).

$\mu^o = \mu^o$(T, p, pH, salts, solutes,solvent, fields,.etc.).

In an actual experiment we observe changes in concentrations or probabilities with changes in these variables. We learn what energies and components it takes to create different molecules or different states of macromolecules. Imagine, for example, that two forms **A** and **B** of a protein have different affinities for a ligand (Latin *ligare*, to tie) **L** but that in the absence of **L** the difference between $g_A$ and $g_B$ favors form **A** ($g_A < g_B$). But now start adding **L**, and say it sticks better to form **B**.

The fact of sticking automatically implies a lowering of energy. Otherwise **L** wouldn't stick. There is a greater possibility of this favorable act occurring with form **B** than with form **A**. The extent of this lowering is in proportion to the number of **L**'s bound. As the concentration or availability of **L** increases, the free energy $g_B$ goes down faster than $g_A$. In the presence of added **L**, form A will therefore become relatively less favored compared to form **B**.




By watching the change the ratio of **B** to **A** with added **L**, we learn the relative amount of **L** associated with each form at each concentration of **L**.



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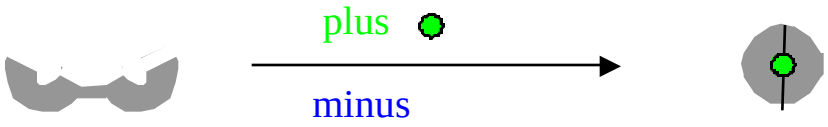
At the same time that the molecule goes from form **A** to **B** it may also change its association with species other than **L**. For example there may be a decrease in molecular solvation. Any lowering of the chemical potential of water, for example from the presence of solutes in the bath, will act to favor the less hydrated form **B**. The change in the probability of **A** vs. **B** will reflect the competition between the actions of ligand **L** and 'ligand' water.

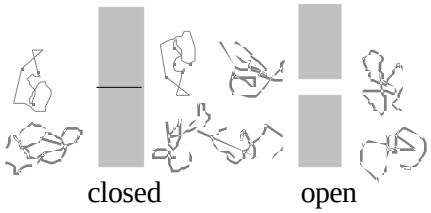



**Example: ionic channel under the osmotic stress of excluded molecules**

Consider "open" and "closed" forms of the channel which must draw a volume

$$v^w_{open} - v^w_{closed}$$

from a bathing solution whose channel excluded species exert an osmotic pressure $\Pi_{osmotic}$ .




With addition of osmotic stress the free energy of the open form will go up as $v^w_{open} \leftrightarrow \Pi_{osmotic}$ while that of the closed form will go up as $v^w_{closed} \leftrightarrow \Pi_{osmotic}$ . The ratio of open versus closed states will go down with $\Pi_{osmotic}$.

$$\left.\frac{[Open]}{[Closed]}\right|_{\Pi_{osmotic}} = \left.\frac{[Open]}{[Closed]}\right|_{\Pi_{osmotic}=0} e^{-\Pi(v^w_{open} - v^w_{open})/kT} .$$

Plotting ln([Open]/[Closed]) vs. $\Pi_{osmotic}$ will give a slope $-(v^w_{open} - v^w_{open}) / kT$ . That is, the open/closed ratio versus osmotic strength gives the difference in solute-inaccessible volumes of water associated with the two forms of the channel.

[ADD MORE EXAMPLES HERE?]



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## g. Why kT?

When there are such huge energies as the 7.5 kcal/mole of ATP hydrolysis or even the 5 to 10 kcal/mole of H-bonds, why would any self-respecting bio-scientist, cell or macromolecule worry about a puny .6 kcal/mole thermal energy?

One answer has to do with control.

All the mighty forces of synthesis and stress often bring large molecules to energetic near-balance between differently functioning forms. Only when the free energies needed to assume the alternate states are very closely spaced can these molecules be sensitive to small changes in their surroundings, be able to respond to these changes -- in pH, salt, ligand, voltage, etc. -- by shifting their numbers between the alternative forms.

Molecules can be committed to one enduring form, but they will not be very lively creations.

From this perspective, ask how would a hormone or an effector or ligand exert continually varied influence if the sites of their action were not occasionally empty, were not able to load and unload in order to gauge the concentration or the activity of these controlling molecules in the surrounding region?

A permanently bound effector would not show a degree of binding that reflects its solution activity. .

It is thus that the small-energy sensitivities are seen in alternations between states during which mousy little kT kicks the big guys around.



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