MOLECULAR PRESENTATIONS

Linear Hydration and Order in the Living Cell

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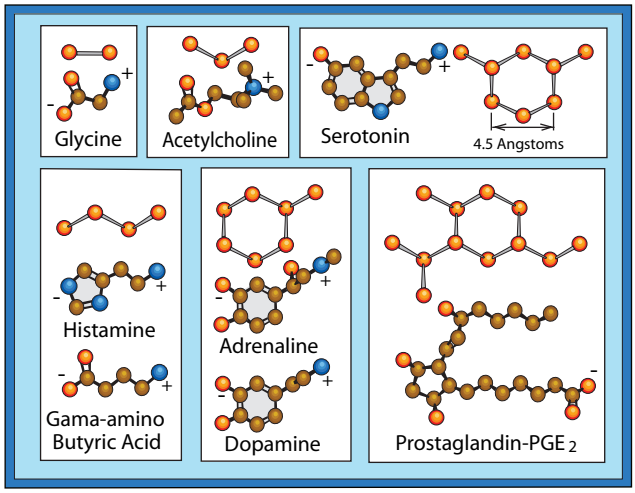
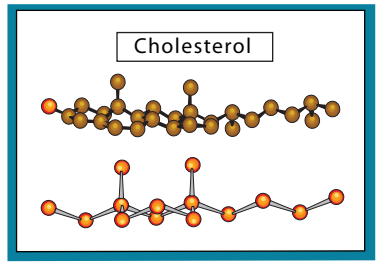
Dedicated to the late Professors Carl Djerassi and William S. Johnson of Stanford University.

In 600 BC, Thales of Miletus, who is called "The Father of Science" said:"Water is the womb in which the principles of life were given birth." In fact, recent studies have brought forth an image of how molecules in the cell produce life.

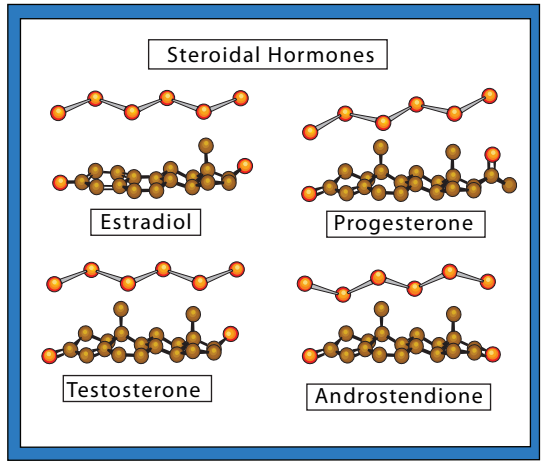
Sixty years ago, as a medicinal chemist, I began constructing permanent molecular models of neurotransmitters and hormones to see if I could find structural features which might assist in developing new drugs - only to find that the molecules appeared to differ in length by a unit of 2.25 Angstroms, the same as the distance between water molecules in the linear elements of ice. When larger molecules, like cholesterol and the steroidal hormones, (which are made from it) were analyzed, they corresponded to linear segments of six and seven ice-like water molecules.

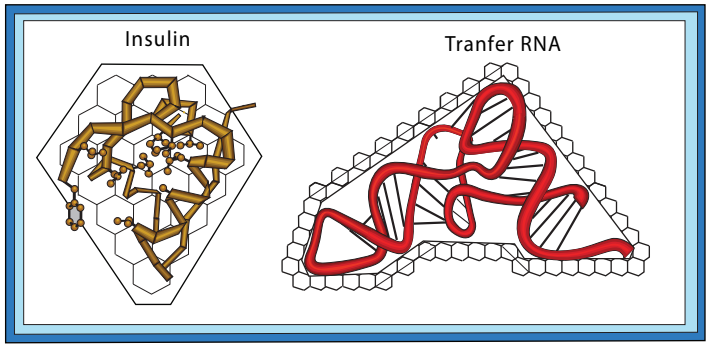




As I viewed the correlations, I felt that water, in its low-energy ice-like form might be occupying receptor sites to hold them open and assist receptor molecules in and out.

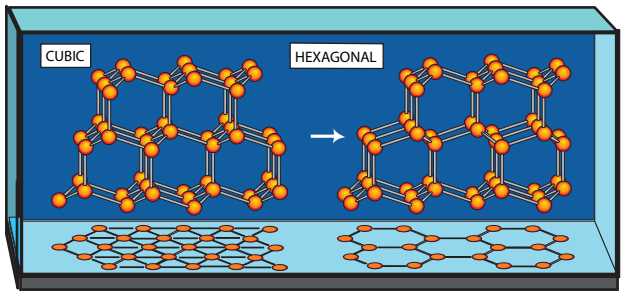
When models were made of even larger receptor molecules, like insulin and transfer-RNA, and compared to the spatial properties of normal hexagonal ice, they did not fit - however, they did fit patterns in cubic ice, which is the initial form produced when water freezes, but is too unstable to be isolated.[1]





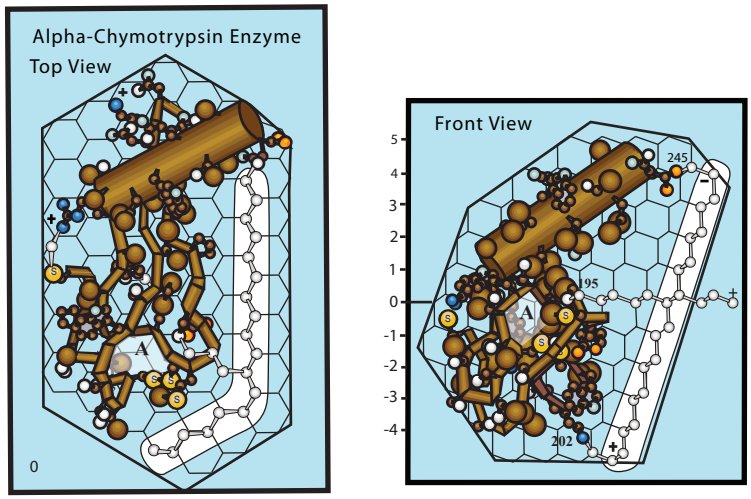
Although it is difficult to portray the three-dimensional structures of the molecules,when viewed as on the left in this parallel perspective manner, even though they are in different structural classes (insulin is a polypeptide,[2] t-RNAs are ionic polynucleotides[3]) both display cubic ice geometry.

Again, as water freezes, the isomer which forms first is cubic. It is called the "kineic product" because it forms most rapidly as electron orbitals overlap to form bonds between atoms.[4] Bonding between carbon atoms in the diamond are also cubic, but they are stable - those in ice are more stable with hexagonal units over each other. For years, ice-bonding was considered to occur only in ice but in 1969, molecular orbital calculations suggested that the trimer, with three water molecules bonded together at 2.76 Angstroms, the same as in ice, might be the most stable bonded unit in liquid water,[5] and in the following year, two experimental studies indicated that linear ice-like linear elements might be forming in liquid water. First: X-ray-analysis suggested that linear trimers and tetramers might be forming on the surface of liquid water,[6] and second, in a detailed 100-page thermodynamic study indicated that there is a rapid exchange in bond energy between enthalpy and entropy in water molecules as small molecules approach proteins.[7] Lumry and Rajender, the authors of the study, said that molecular biologists would have to realize that water, in both liquid and ice forms, are involved in regulating motions and interactions of vital molecules.



Based on those two studies, as well as an NMR study in 1973, which revealed that water surrounding proteins and nucleic acids exhibits the peaks of ice as well as liquid water,[8] numerous proposals were presented for the structuring nature of water.[9] But, when crystallographic models of proteins were examined and no ordering of hydration could be found, it was concluded that only small elements of, hydration, like the trimer, must be forming - that order in assembly and interactions must be provided by internal thermodynamics;[10] water is simply a solvent. However, in 1984, when molecular orbital calculations suggested that linear elements of hydration with 5 or 6 water molecules might form adjacent to non-water-bonding lipid surfaces,[11] I began constructing molecular models of multiple water-soluble enzymes and viewing them at angles that might reveal linear hydration adjacent to lipid surfaces.

By constructing them, one polypeptide segment after another from the amine ends and then taking photographs of Top and Front Views over cubic lattices, cubic patterning appeared, not only in outer structures, but particularly, in their anhydrous cores. For example, in the views below of the left side of the alpha-chymotripsin molecule,[12] you can see how hydration, even though present as short units, may propagate linear segments next to compressed geometric blocks of polypeptide with no water trapped inside.



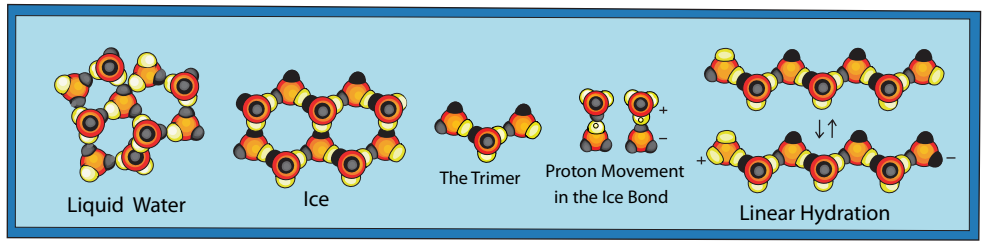
Region A is the binding site for an aromatic ring of a reaction substrate molecule, while the oxygen atom of serine 195 is in the middle of the reaction site. It serves as a seed to propagate positive charges out from the reaction site to direct the anionic/aromatic regions of polypeptides into the reaction site for cleavage. Notice that the reaction site, in the Front View, is at the side of a block of polypeptide to provide spatial control in the reaction site. Since hexagonal planes in the cubic lattice are not in the same positions above each other, layers are numbered. Layer 0 is shown behind the Top View. However, the layers are all simply composits of short linear elements which last only about 10^{-11} seconds.[13]

Since Professor Scheraga,[9] an expert on hydration, was at Cornell University nearby, I arranged a meeting to show him a number of pictures of protein assemblies. He said they were interesting but did not feel that surface water played a significant role in directing protein assembly,

In 1944, Nobel-winner Erin Schrodinger, in his little book, *What is Life?*, proposed the term "negentropy" to explain how water moved molecular evolution from randomness toward order in violation of the Second Law of Thermodynamics.[14] The concept that water forms unstable linear elements on lipid surfaces and then leaves and withdraws energy as it moves to the more random liquid state, not only explains negentropy, but how surface water absorbs and adds units of energy as polypeptides move from one configuration to another.

In spite of Professor Scheraga's and other expert's reluctance to consider the importance of surface water, I decided to publish my first edition of *The Matrix of Life*[15] in which I proposed that, as polypeptides are released from ribosomes, small peptides like glycine and serine, which bond directly with surface water, absorb energy and provide for turns in segments while segments which contain methylene (CH_2) or methyl (CH_3) groups on alpha carbons on both sides of segments, shield the chain so well from bonding with water, that, when water leaves and moves into higher-energy liquid-bonding, so much energy is removed that the chains rapidly forms coils.[16] Segments which form linear elements only on one side, simply straighten and, as the water leaves, search for other newly-exposed lipid surfaces with which to combine and form a more stable anhydrous union. As illustrated on the previous page, lipid surfaces combine tightly, while small peptides produce turns which, ultimately, produce cubic-patternerning. Once completed, the molecules are stable and in spatial harmony with the order/disorder spatial properties of thr aqueous environment and other vital molecules. Ion-tethers between protein units tie them togther in macro-structural forms in all sorts of natural symmetry patterns.

Although the proposal included a classical description of ice-bonding,[17] a more detailed description of that bonding as "covalent," the same as in carbon/carbon bonding, appeared in 1999.[18]

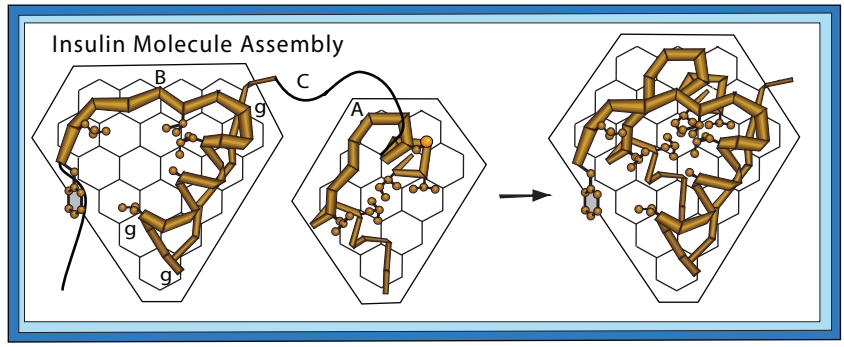


By overlapping the black negatively-charged orbital of one water molecule with the proton-containing white orbital of the neighboring water molecule, the proton is so close to the neighbor that, by moving only a fraction of an Angstrom, the neighbor can be positively charged. If that happens to triplets in liquid water, positive and negative ions are produced; but, if it happens in linear elements of hydration which form on the inner surfaces of large axonal nerve fibers, positive charges can move at almost super-conductive speeds.[19]

Based on the above properties, surface water, by rapidly changing between liquid- and ice-bonding,[7] provides for changes in chain energy and, by rapidly developing charges, neutralizes chain charges. Although molecular surfaces are too dynamic at physiological temperatures to observe structural changes in water on lipid and ionic surfaces, Professor Zewail at CIT, using a 4D ultrafast electron crystallographic procedure of water on a graphite and polyionic poly-ionic surface at sub-zero temperatures was able to visualize the formation of linear elements in cubic ice forms.[20]

Professor Zewail's studies were skillfully-designed, using solid surfaces to simulate the lipid and ionic surfaces of proteins and nucleic acids. Unfortunately, he did not live long enough following the studies to receive credit for the significance of the studies. Additional evidence for the TLH hypothsis came in 2011 from a study reported by Professor Chen and collegues at MIT who used ultra-high-speed NMR to follow hydration on the surface of a polypeptide of an enzyme as it was released from a ribosome.[21] Following release, the polypeptide was completely covered with water - but, a portion of that water was lost as the segments assembled to form the core of the finished protein - just as expected, based on the TLH hypothesis.

Since insulin was one of the first proteins to be analyzed, it will be the first for an interpretation of assembly.[2]

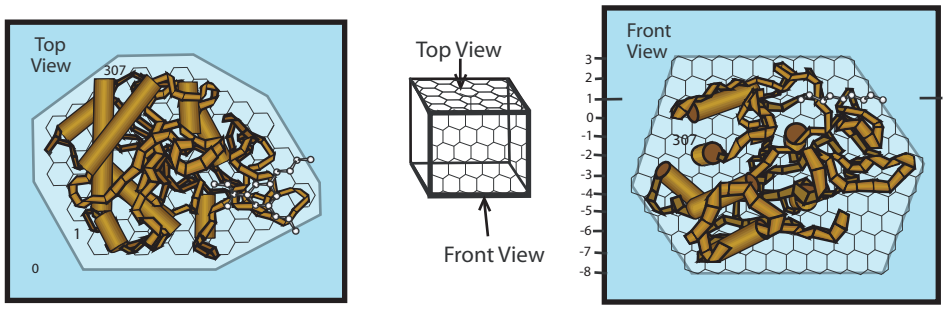


The central region of the B-segment of the insulin polypeptide is composed of so many peptides with side-chains which shield the main-chain and form linear elements of hydration on both sides, that it rapidly forms a coil as the water leaves.[16] With the 2.25Å cubic-probability pattern of hydration still forming around the coil, a glycine (g) at the bottom of the coil, by bonding directly with surface water, absorbs energy, breaks the coil and produces a turn into a short linear segment with lipid on one side which passes down to a second glycine (g). By turning abruptly, a linear segment with lipid on the front side binds into the back side of the coil into segment C. The linear segment extending from the top of the coil at the third glycine curves over to the left to position the aromatic ring of phenylalanine next the lipid side-chain of the valine on the coil.

Segment C, which contains a number of glycines serves as a mobile tether to bring unit A, composed of two short coils joined by a short segment to the back side of B. The assembled protein, by enzymatically losing its left end and segment C, is released into the blood-stream to bind with receptor sites in membranes which regulate the uptake of glucose into cells. A recent study indicates that it is the lower right side of the coil and linear segment which binds most tightly to a receptor site.[22] Based on the TLH hypothesis, the binding site most likely is periodically filled with linear elements of hydration in cubic forms when insulin is not present.

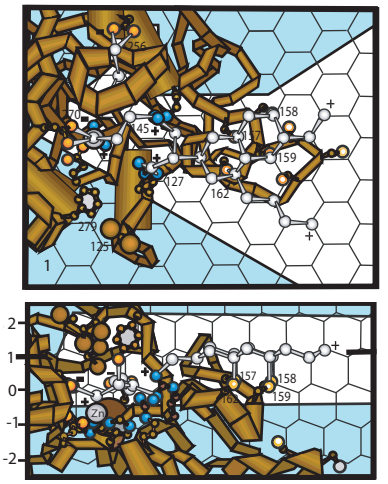
Unfortunately, hydration in binding sites is so transient and rapid, that it may be some time before it's presence can be validated experimentally.

Once again, in the following analysis of the carboxypeptidase enzyme,[23] it was oriented so it could be viewed over the cubic matrix parallel to it's longest 307 terminal coil.

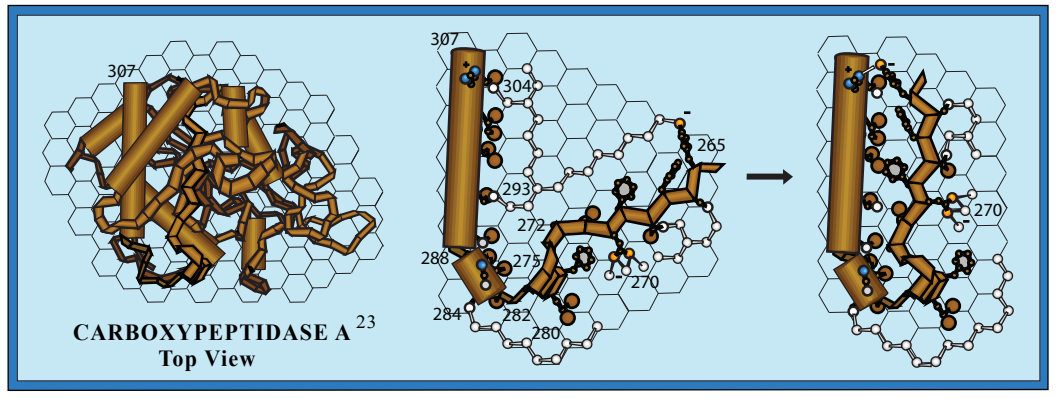


Notice in the Front View that the coils and linear segments present an entirely different appearance than in the Top View - it is as if they are floating on the planes of ice-like water molecules which are numbered to define position. However, it must be remembered - even though the cubic lattice is illustrated as a complete unit, only small linear segments are present at any instant as the polypeptide folds. The cubic lattice presented behind the protein is simply a composite of all the linear elements that were generated while the lipid regions were being folded together to form the anhydrous core of the protein.

It is extremely important to realize: *once cubic hydration patterning is established around the 307 coil, linear elements continue to propagate out from the core, not only to control assembly, but the reaction site and interactions with other molecules.* For example, if we look at an enlarged view of the enzymatic binding site with the zinc ion at the base, we can see that the hydroxyl groups of the serine peptides at positions 157, 158, 159 and 162 are in spatial locations to hydrogen-bond with transient linear elements of hydration which tunnel positive charges out from the reaction site to direct the negatively-charged ends of polypeptide chains into the reaction site for cleavage of terminal aromatic peptides.[23]



Another important feature involved in the formation of the anhydrous cores is that lipid regions which fold together usually are the same lengths as quantized linear elements of hydration; by folding together, all water is lost between them to produce the stable anhydrous union.



For example, as the polypeptide of the carboxypeptidase enzyme is released from the ribosome, most of the 27 aminoacids on both sides of the chain at the 307 end have hydrocarbon side chains which shield oxygen and nitrogen atoms in the chain from hydrogen-bonding with surface water.

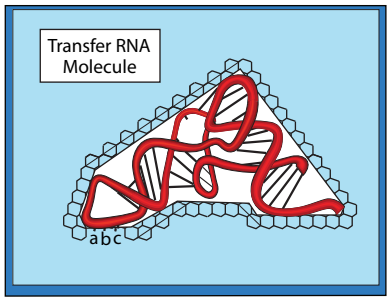
As ice-like bonding on those surfaces break and energy is transferred, the chain straightens and then rapidly forms the long coil.[16] A proline at position 288 produces a turn in the coil, and another at 282 produces another turn. Oxygens at threonines 304 and 293 are in precisely the proper positions to induce ice-like bonding of water next to the coil. From 280 to 275, the chain wraps back and forth in hydrated beta-turns so that the 272-265 segment can be at the level of the coil to rotate, bind and release an equal lengths of unstable water from both surfaces.

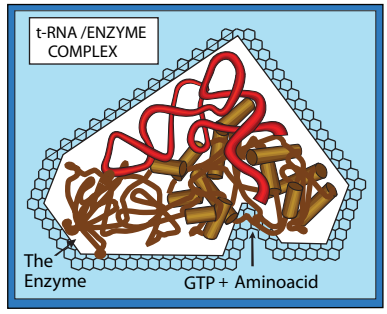
Glutamic acid, at position 270, by clustering water around it to delocalize its charge, breaks hydration order in the next hydration layer. At the same time, lipid surfaces above and below the acid continue to be covered by ordered water which, by being displaced by lipids, continue the formation of the anhydrous core. Polar and ionic side-chains of small peptides, which are left on the surface, bond with surface water and other molecules at a variety of angles.

Based on analyses of the five water-soluble proteins presented in www.cubichydration.com, *it is correlations in spatial structures of vital molecules and those that can be formed by order/disorder bonding properties of the ionic aqueous environment in which they formed and evolved, that permit them to function in such a smoth and coordinated manner in living cells.*

It is also important to realize that, as polypeptides began to be produced at random, those with sequences which could combine spontaneously to yield anhydrous stable cores survived to produce more, while those which accumulated and increased in concentration (by an enzymtic property called “feedback inhibition”) decreased production and eventually stopped. Thus, production of proteins, which could combine to produce stable useful spatial forms continued to be produced - ultimately to yield an almost limitless number of cooperativly functioning stable molecular forms..

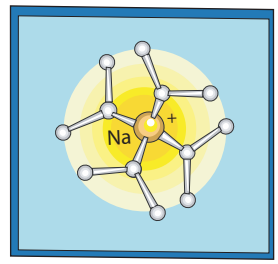
Just as small exchanges in energy between water and the polypeptide chain of insulin brought forth a new cubically-patterned form, the same energy exchanges between linear coils and loops of ribonucleic acids brought forth cubically patterned transfer-RNAs.[3]





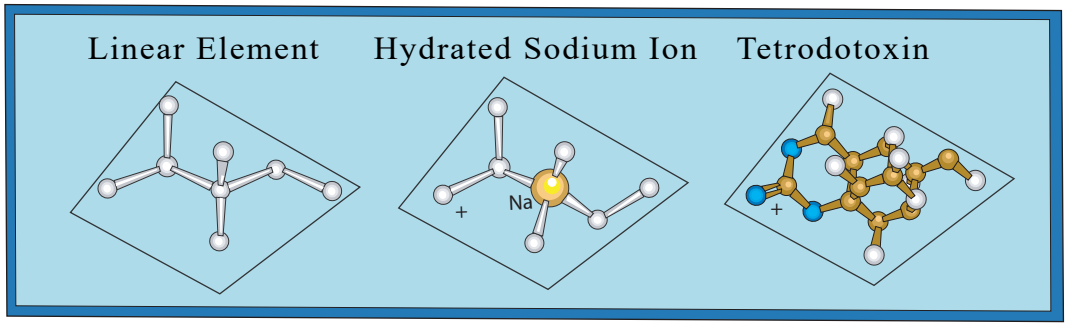
As shown on the first page, cubically patterned transfer-RNAs, like insulin, mimick spatial units in cubic ice. Details of the binding and polypeptide are included in www.cubichydration.com but it is important to realize that there are at least twenty of these cubically-ordered t-RNAs in every living cell and the same number of enzymes, with structures as shown above.[24] The enzymes bind a specific three-letter genetic code on the left end of the t-RNA molecule to a specific amino-acid on the right-hand end. As you can see, the enzymes which bind these t-RNAs have complex structures composed of both coils and linear segments, all fit together to form a structurally-stable anhydrous core.[25] In this case, not only the t-RNA molecule which binds its amino-acid into the catalytic site but the enzyme itself reflects cubic hydration patterning.

As even more complex proteins formed, it appears that they contained the same types of cubically-patterned cores but tied togther by polar or ionic tethers to form a variety of symmery forms.



Just as small ions like sodium and calcium, which bind water molecules around them in spherical orientations,[26] are held out away from the surface of helical DNA by the linearization of water around it,[27] they are held out away from the surface of tRNAs as they cluster around them to neutralize their surface negative charges. In spite of the spherical orientation of water around these ions, when they bind within proteins or pass through pores in membranal proteins, they accomodate to the linearity of water molecules in those sites. For example, at the opening of a protein pore which passes through a membrane to admit sodium ions into nerve cells, there is an extremely toxic chemical which binds within the pores and prevents sodium ion from entering. The chemical is tetrodotoxin.[28] It is produced by a bacterium in the puffer fish, and, if consumed, causes almost immediate death.

When the membranal transport site is empty, water, most-likely, is there. When sodium ions pass through, they fill the site as they pass from one water-molecule position to the



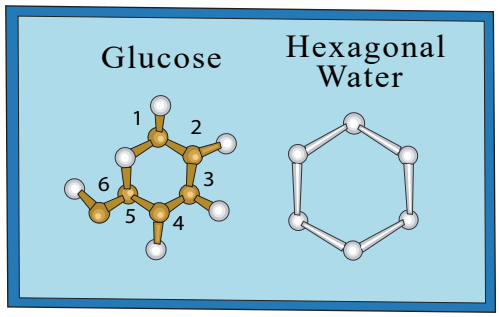
next. However, when tetrodotoxin enters, it not only fills the site but binds so tightly that sodium ions cannot pass through. Notice the extremely interesting structure of the tetrodotoxin molecule: 7 oxygen atoms and 3 nitrogens to produce an extremely toxic water-soluble chemical.

Having looked at both dynamic and linear bonding of water on natural molecules, it should be no surprise that, as molecules approach each other in living cells, liquid-like hydration which extends from their surfaces, gives them the freedom to move from one association to the next, while linear elements of hydration of similar lengths on lipid surfaces aline next to each other, release water between them and form firm anhydrous bindings. At the same time, linear elements which form between charges, draw molecules together to reduce order. As pointed out in 1970 by Lumry and Rajender, it is rapid shifts between enthalpic and entripic binding between water molecules that provides for spontaneity in motion and interactions in living cells.[7]

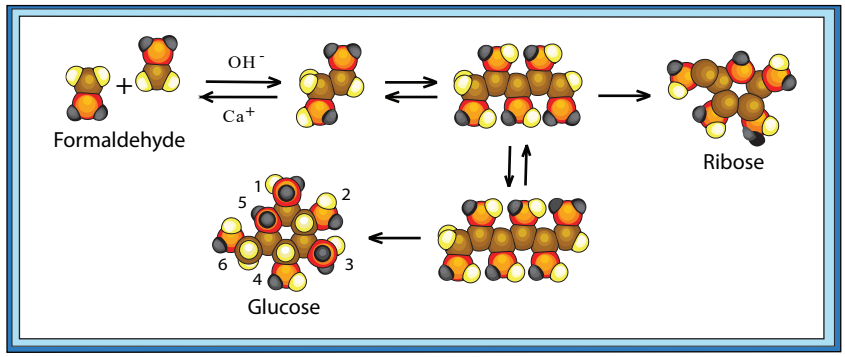
Before we consider more examples of natural molecules which mimic unis in cubic ice, we will consider some of the fundamentals that may have been involved in the formation of the early molecules of life.

Formation and Evolution of the Molecules of Life

Just as a foreign molecule, like tetrodotoxin, can bind in a site that normally is occupied by a (branched) transient linear element of hydration, there are hexagonal water-binding sites throughout the body which can bind the same small hexagonal molecule.

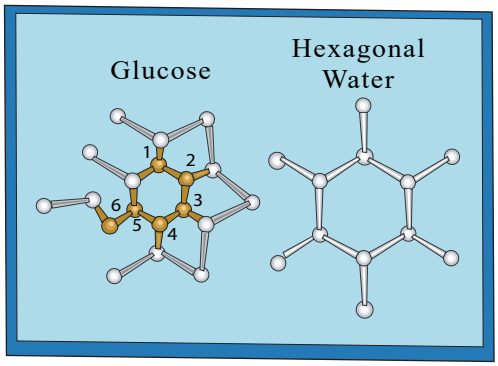


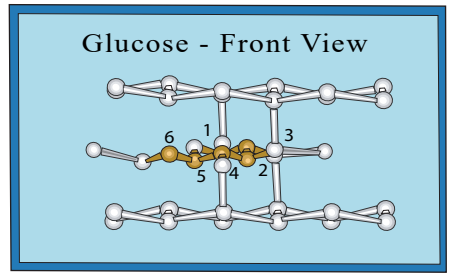
The molecule is glucose - the most abundant one on earth with four oxygens around it in the same spatial positions as water molecules in the hexagonal units in ice. The glucose molecule is produced by photosynthesis in plants and carries energy and carbon to every cell in the body. It polymerizes to make starch and cellulose and its carbon atoms are used to make almost every other molecule in the body. By mimicking the the structure of hexagonal water, it either passes smoothly into cells or is carried in by transport proteins for energy and as a raw material. In fact, based on old and recent information, it may have been one of the first vital molecules to be made on earth.



If formaldehyde molecules, which recently were found in the atmospheres of planets[29] (and are shown above with red oxygens and white hydrogens), is in weakly-basic aqueous solution, they bond together spontaneously to form five- and six-carbon units which circle around to form a variety of sugars,[30] including glucose and ribose. Since glucose mimicks the structure of hexagonal water, it bonds in numerous sites within cells. However, it differs from hexagonal water in one important respect.

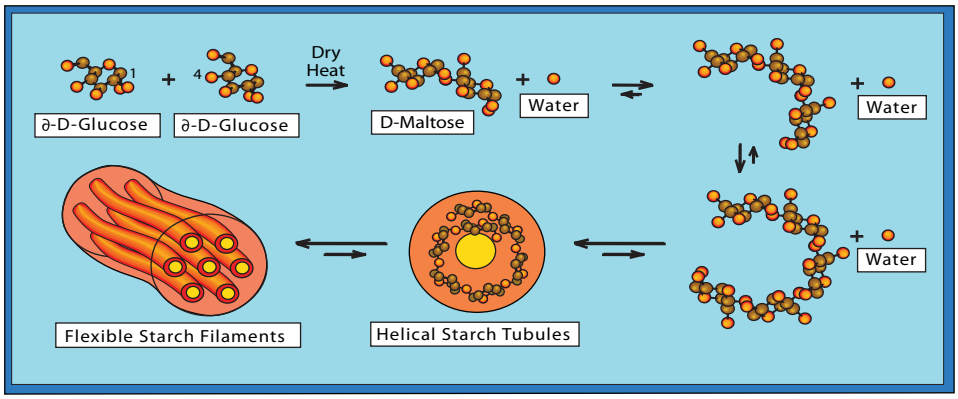
As illustrated below, it binds water molecules around it in a manner which differs from that of linear hydration bonding - it disrupts the integrated linear patterning of water around it - it produces liquid-like random-bonding to water in its plane.[31]





However, as illustrated on the right, the alcoholic oxygens at positions 1 and 3 bond vertically with water molecules in linear and hexagonal patterning, while those at positions 2 and 4, bond below. Thus, glucose hydration, by being compatible with hexagonal order above and below but produce randomness in the plane, behaves as a surfactant - it moves spontaneously to hydration-ordered surfaces, like membranes, to decrease order, and then, move rapidly along the surface in search of binding sites where it can be carried into cells.

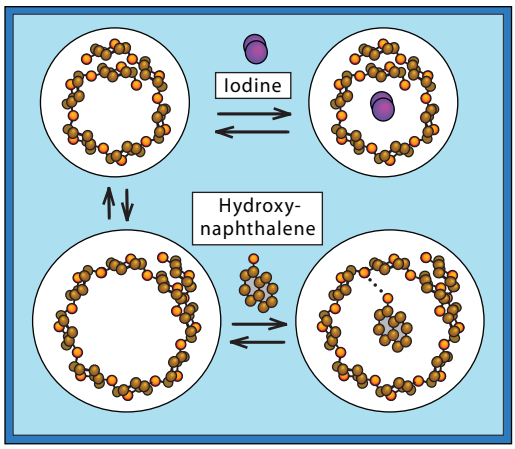
As the most abundant natural molecule on earth, with the formula of $C_6H_{12}O_6$, glucose is both the carbon and spatial analogue of hexagonal water, $H_{12}O_6$. As illusrated above, the arrangement of water molecules around it define how it moves and binds to other molecules. If it is accepted that glucose was one of the first molecules to form on the early earth, then it may have played a critical role in the formation of all others. For example, if glucose is heated, its molecules combine chemically to form a complex mixture of polysaccharides, including the coiled strands of starch molecules.[32]

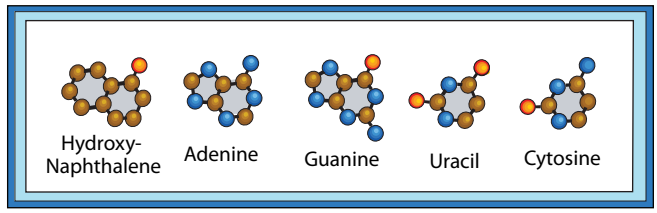


Although not shown, the OH (hydroxyl) groups extend out from the surface of the tubules to bond with water in random liquid-like fashion.[33] They solublize and suspend the coils in water and permit them to bond with each other in filaments. However, the inner cores are hydrophobic, with only the electron orbitals of oxygens binding the glucose molecules together directed toward the center.[34] If we accept the thesis that glucose was produced in abundance from formaldehyde in the first phase of biomolecular evolution, then, in the second phase, it was huge gelatinous masses, including starch coils, which filled the oceans and tidal bays.

The fact that starch coils have the property of binding small molecules, like iodine, within their cores and unwinding to increase their diameter to bind larger molecules, may have initiated the third phase in evolutionary development. If coils come in contact with molecules like hydroxy-naphthaline, they spontaneously unwind and bind them in the core.[35]

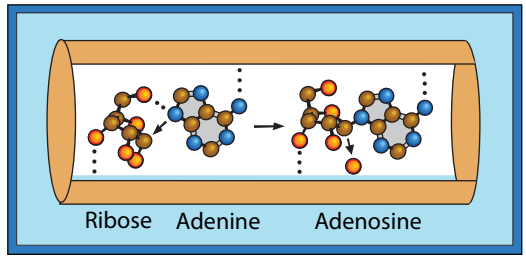
Although the report which described that property did not include the nucleoside bases, their structures are so similar to hydroxy-naphthaline that they might well also have bound within the cores.





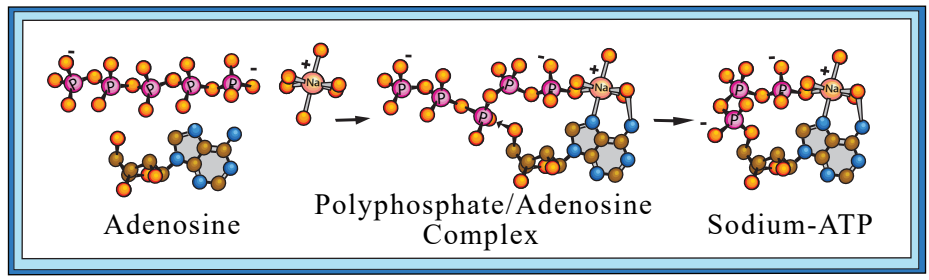
All four of the nucleoside-bases have oxygen or nitrogen atoms in positions to hydrogen-bond with oxygens in the walls to fill the cores. Of course, experiments should be performed to test the idea.

However, structural models suggest that the coils might, not only have bound the bases, but a ribose molecule as well. On drying, nucleosides may have formed. Once again, this is pure speculation and experiments must be performed to test the idea.

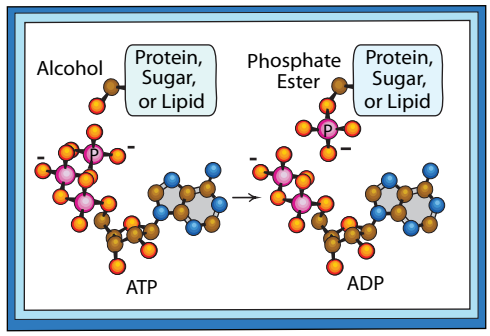


Unfortunately, we have no way of knowing the conditions within those gelatinous masses which may have produced the nucleosides. The bases could have been produced in reactions between ammonia and formaldehyde, but, nucleoside-formation, if not as proposed above, must have been by some other non-enzymatic process.

Once nucleosides began to be produced, the fourth phase must have involved phosphorylation. Although there were no enzymes to connect phosphate ions to the hydroxyl groups of glucose or the ribose rings of the nucleosides, polyphosphate ions, which are produced simply by heating phosphate ions, form cyclic structures which have extremely high energy but are surprisingly stable in water.[36] If the sodium salts of polyphosphates, as shown below, bind to the adenine ring of the nucleotide in same manner as the triphosphate binds in adenosine triphosphate,[37] they would form ATP and release the remaining polyphosphate

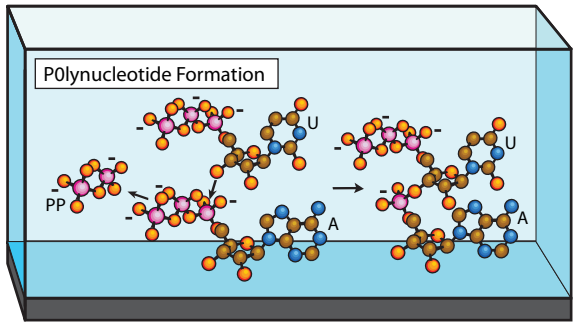


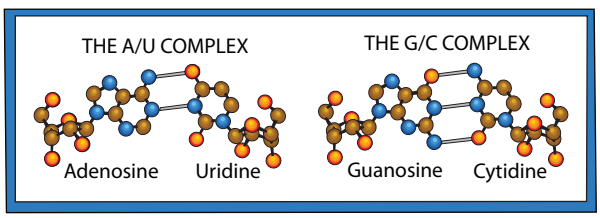
Again, we do not know, and never will know, exactly how these amazing molecules were formed in sufficient numbers and in confined areas to produce the complex systems which yield life. However, one of most amazing vital molecules is ATP.[38] With its triphosphate over the ribose ring bound to the hydrated sodium ion, water molecules are held out away from the center, unable to bond with inner surface of the phosphorus atoms to break the bonds. The result is that sodium-ATP is sufficiently stable to move throughout living cells transferring its terminal phosphate and diphosphate to millions of other molecules.



As sodium-ATP approaches a catalytic reaction site, a positive charge in the site displaces the sodium ion and binds the terminal phosphate in precisely the proper orientation, relative to the oxygen atom in another molecule, to transfer the phosphate to the other molecule. It gives the new molecule a negative charge and provides a leaving group to attach it to other molecules.

In addition to serving as sources of phosphates, the nucleoside triphosphates combined with each other, with the loss of a diphosphate, to form long strands of ribonucleic acids composed of adenosine, uridine, guanosine and cytosine nucleotides, all tied together in a variety of sequences.[39]

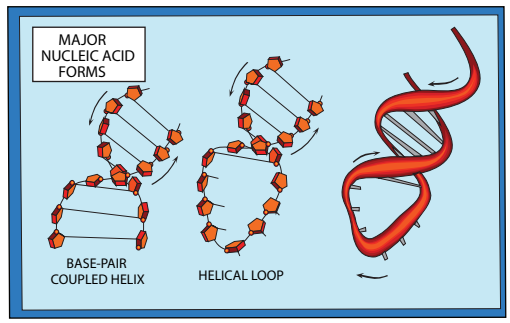


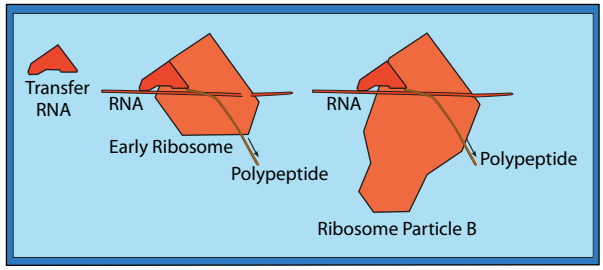


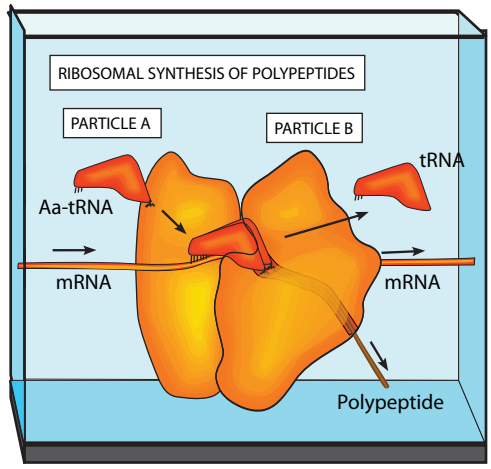
Once tied together in RNA filaments, the nucleosides began forming specific attachments between adenosine/uradine (A/U) and guanosine/cytodine (G/C).

Regions of RNA filaments which could couple A/U and G/C units together, circled each other to form a double helix, while regions which could not form proper base-pairs formed turns and loops.

As noted on page 5, some of the first stable nucleic acid units to form, most-likely, were the transfer-RNAs. They had condensed anhydrous structures which corresponded to cubic patterning with water and hydrated cations around them. Although many of the early RNAs which formed at random were unstable and hydrolyzed back to nucleosides, some survived and produced an entire world of nucleic acids with many of the properties of the proteins that were to come.[40]



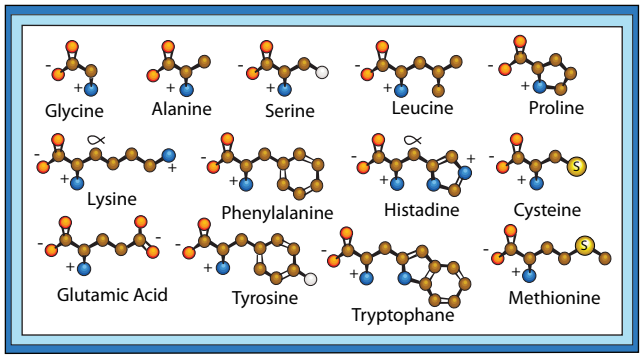


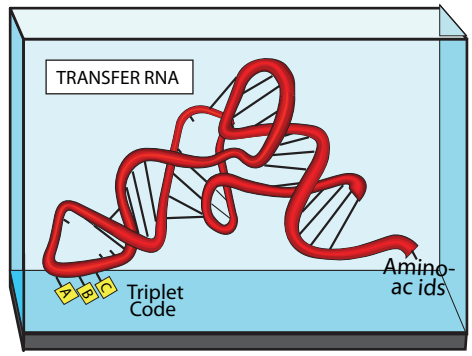


Based on correlations in structures and configurations, ribosomal particles may have grown from tRNAs with the capability of binding several t-RNAs in multiple orientations. At first, the ribosomes must have been small but, as they bound new RNAs and newly-formed proteins, they grew in size and functional capability.[39]

Obviously, we have no idea how those incredibly-complex series of events occurred, but, based on the structures of ribosomes and directions of mRNA and polypeptide channels passing through the ribosomes, cubic patterning most-likely was involved.

As each of the amino-acids in the chart below became attached to the open end of a tRNA,(as shown on the right below), with a specific sequence of three nuc-



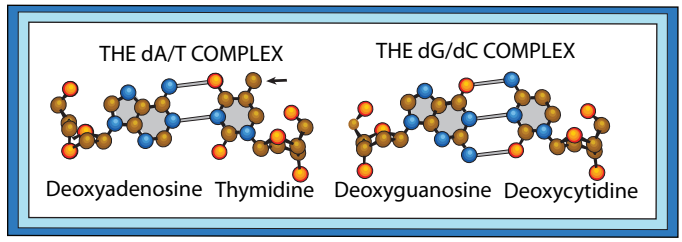


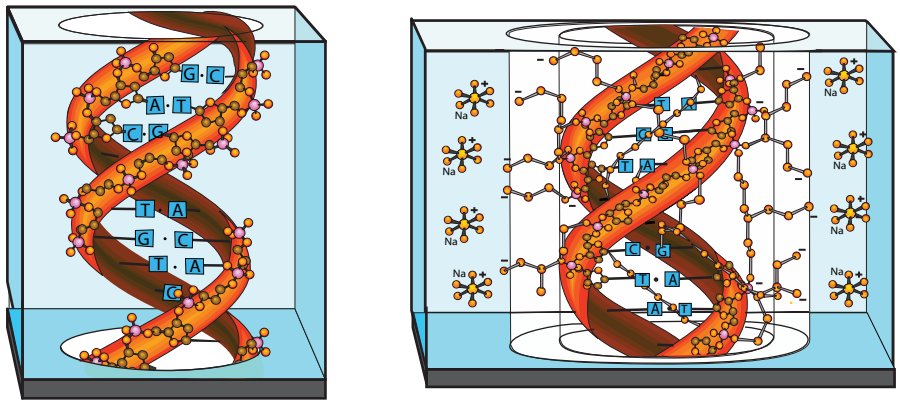
leotides on the loop, a pair of them bind to a complimentary sequence of six nucleotides on a messenger-RNA and attach the amino-acids together to form a dipeptide. By repeating the process over and over again, a polypeptide, with a specific sequence of amino-acids, is produced which passes down through a channel out into the surrounding water where it begins the process of water-directed folding and assembly.[21]

As we view the ribosome and realize how extremely complex the process is which produces polypeptides, we must wonder if there was a plan.??

When coded polypepeptide synthesis first began, some of the earliest proteins to emerge most likely bound to open sites in the ribosomes to increase stability, productivity and specificity. Undoubtedly, many of them are still there. However, the production of enzymes which could readily hydrolyze unprotected mRNAs back to nucleosides, threatened the entire coding process. Only the formation of enzymes which could remove one of the hydroxy groups from ribose units of the RNAs was it possible for deoxy-ribonucleic acids to be produced, which were so stable in their double-helix forms, that enzymes were required to separate the strands and expose the coded sequences.

By replacing uridine with thymidine, with a methyl group on the ring, a destabilizing water molecule in the RNA helix was displaced to increase stability. But, double-helix DNA is not only stabilized internally, it requires at least 13 water molecules around and within it to stabilize it in its natural hydrated form.[27]

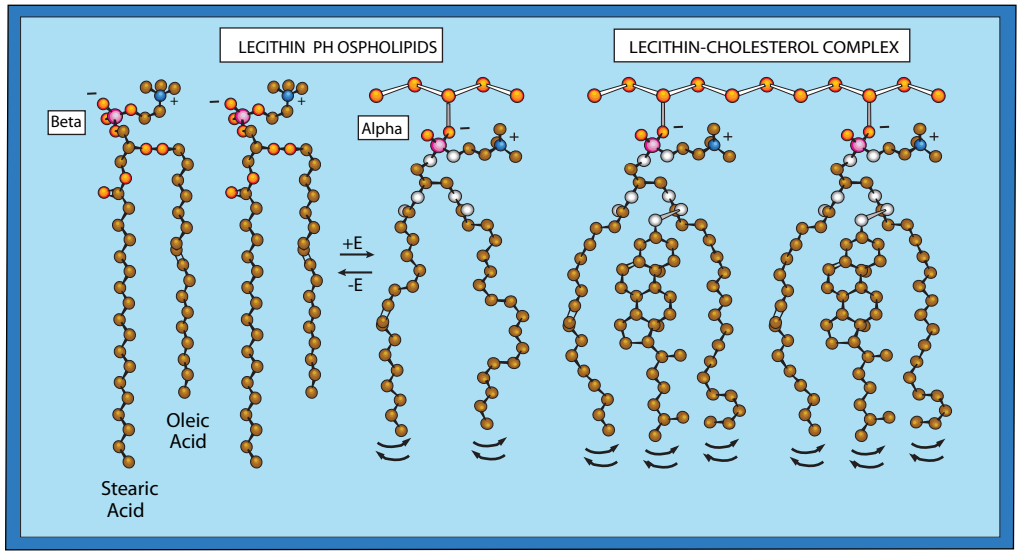




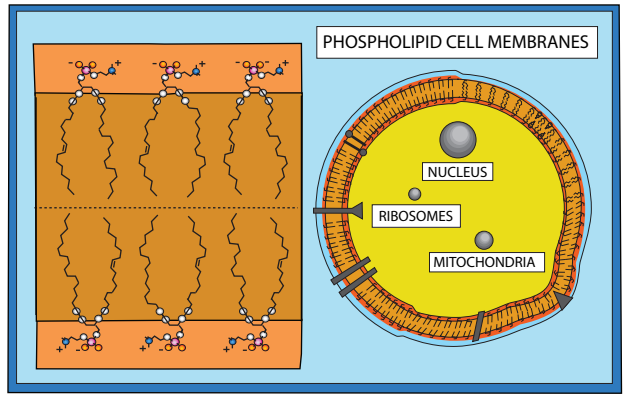
First: a linear element of hydration passes around the helix in the narrow minor groove.[41] Next: short linear elements continually bridge between the strands,[42] and last,but not least, (based on model-building and the linear hydration hypothesis), water continually produces linear elements of hydration which span between phosphates in adjacent strands to delocalize negative charges and distribute them out to sodium and calcium ions around the helix.[27] Although water is never shown around the helix in the classical model of DNA,[43] it is linear elements of hydration which periodically fill the space and provide uniform spacing between the strands. However,(once again), linear elements last for only about 10^{-10} seconds;[13] they can be "seen"spectroscopically as an ice-like cage surrounding the double helix, but cannot be identfied structurally.[8] Of course, when the anionic phosphates of DNA bind directly to the cationic groups of proteins, surface water is released and the strands can separate, either to produce new DNA or RNA with the base-codes, or bind around spherical histone proteins for storage. Nucleic acids, particularly those in microbes, have been studied extensively, but much more should be done to evaluate the role of water in the living cell.[7]

Part of the problem of not recognizing the importance of surface water on DNA came when Watson and Crick reported the structure of DNA in 1953.[43] There was so much antagonism between Roselind Franklin and Watson and Crick with regard to the X-ray diffraction data for hydrated DNA,[44] which was taken from Roseline's notebook without her permission and no credit was given to her in Watson and Crick's classical paper, that the fact that water had been used to obtain satisfactory X-ray difraction pattern was lost in the publication.

Once enzymatic proteins began to be produced, electrolysis of water increased, oxidation of glucose to acetic acid increased, fatty-acid and phospholipid sythesis began and they assembled side-by-side, (based on chain-length),[45] to produce bio-membranes. An important new stage in cellular life had begun with one of the most abundant phospholipids named "lecithin." Composed of fatty-acid chains of 18 carbons, stearic acid, with a saturate chain and oleic acid with one double-double-bond formed cellular membranes with ionic heads which formed hydrogen bonds with linearzed surface water to hold them at uniform distaces apart. Lecithin/cholesterol complexes were held a mean ofsix water molecules apart.[46]



At low temperatures, the chains lay side-by-side as shown above, but at normal temperatures, they have too much energy to remain straight - they twist and spin and occupy more space.[47] As shown above, the mean distance between phosphates in phospholipid/ cholesterol complexes, which compose axonal myelin membranes of large nerve cells, is about six linearly-bonded water molecules.[45]

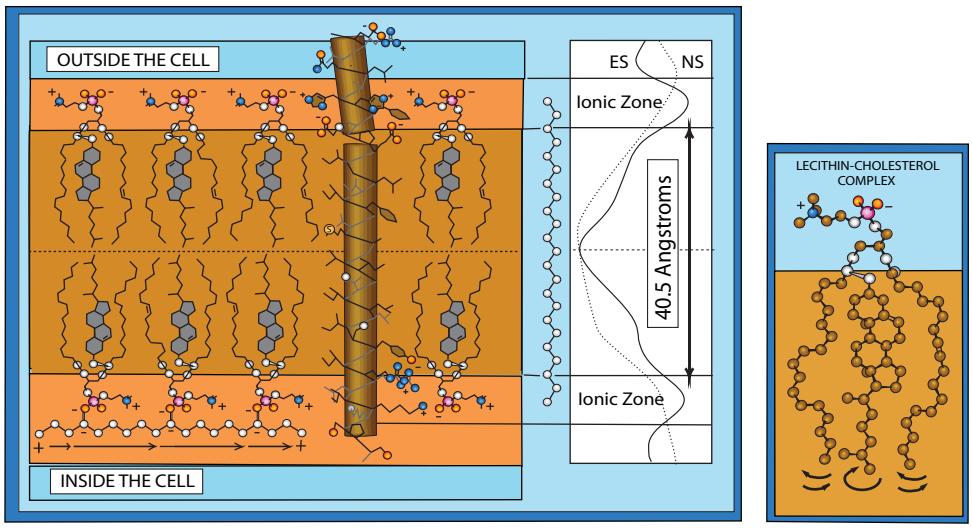


As shown on the right, phospholipids form a double-layer with their dynamic tails in contact in the middle. Although they have a variety of head-groups, the major one in nerve and muscle cells has a trimethylamino group on the phosphate.[46] Proteins, with lipid surfaces of proper length on two or three sides, began assembling in membranes to form pores. Those with conical shapes and lipids on all sides, altered membrane shapes to produce binding sites for proteins and regulator molecules.

Once phospholipid membranes developed in evolution, functional components, like nuclei, ribosomes and mitochondria, no longer had to be held as aggregates in gelatinous masses. Bacteria, molds and virus, which most likely were the earliest forms of life, by combining in numerous coordinated ways, assisted in bringing-forth the living cell.

If we look more closely at biomembranes, we find that they are so loosely tied together that they cannot be isolated and must be constructed primaily based on spectroscopic data.

In fact, it was in 1972 when Singer and Nicolson proposed the "Fluid Moseic Model" with the bilayer structure shown on the last page[47] and a year earlier that Caspar and Kirschner published the electron-scattering (ES) and neutron-scattering (NS) curves for rabbit nerve cell lecithin/cholesterol membrane.[48]

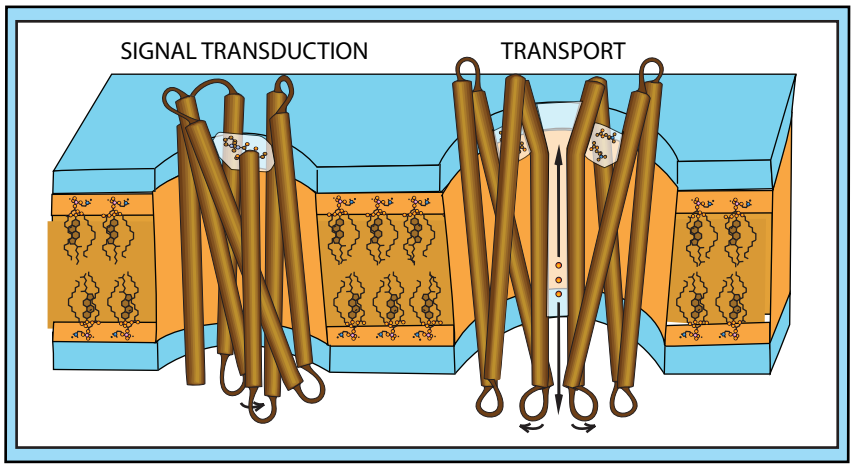


If the amino-phosphates of the phospholipids are positioned at the peaks of the electron scattering curves on both sides of the membrane, (in the same locations as the ionic and polar groups on the protein coil which was isolated from a red-blood cell),[49] the lipid zone corresponds to a width of 40.5 Angstroms - 18 ice-like-bonded water molecules (2.25Å per water molecule), lecithin/cholesterol molecules which meet in the middle and 27 peptides in the coil (1.5Å between them). Surface water, which is shown by the neutron-scattering curve, peaks in a layer on the outside of the cell and increases slowly inside. Once again, water appears to have played a major role in defining the width, as well as the surface dimensions of membranes.

Furthermore, this idealized model provides an answer to a question that has plagued physiologists for years.[50] In small nerves, when neurotransmitters like acetyl-choline open pores in a nerve endings to permit sodium ions into negatively-charged nerves, positively-charged potassium ions carry the charge to the other end of the nerve. However, in large axonal nerves, it is too far from end to end for potassium ions to carry the charge. In fact, charges pass through axonal nerves at a much higher speed. The difference is that the inner walls of axons are composed, almost entirely, of lecithin/cholesterol complexes.[46] Positive charges generated in the nerve ending aline the polar heads of the lecithin molecules and, as illustrated in the figure above, positively-charged protons in adjacent linearly-bonded elements of hydration carry the charge at almost super-conductive speeds from anionic phosphate to phosphate and amplifying node-to-node to the end.[19] For many years, molecular biologists have searched for a mechanism by which the positive pulse could be carried with very little loss of energy.[50] Based on the Transient Linear Hydration Hypothesis, the answer is with protons through linear elements of hydration which last for about 10^{-11} seconds.

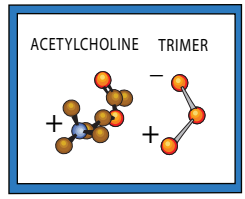
Currently, there is a search for materials which can carry the negative charges of electrons at super-conductive speeds. The problem is that electrons must be cooled to extremely low temperatures or held in conductive orientations in complex media. That's not a problem for protons in transient linear elements of hydration. It fact, it appears that nature may have been the first to utilize superconductivity to perform communications.

Now let us look at the functional proteins in membrane. On the right below is an illustration of the type of protein that is involved in producing the positive charge in afferent nerve ends. It is composed of a pair of coiled proteins with lipid peptides on their outer surfaces in contact with the phospholipids and polar and ionic ones on the inside forming an ion-conducting pore.[45]



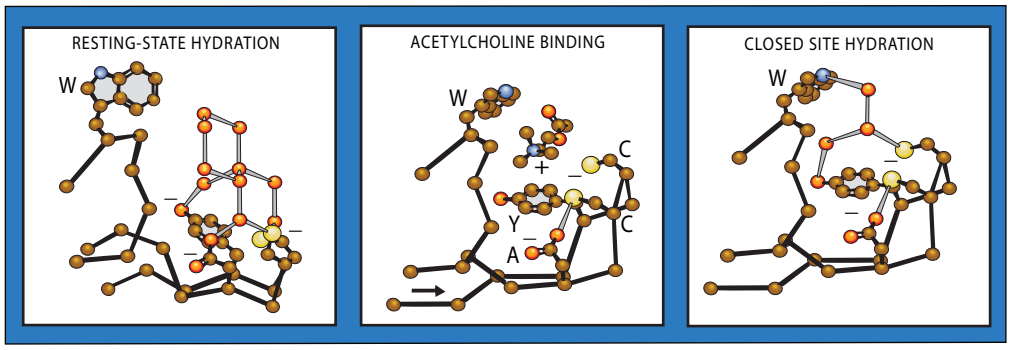
When a neurotransmitter, like acetyl-choline shown below, binds to sites near the outer surface, the inner coils turn, open the pore and permit sodium ions in to trigger the discharge.

Although a number of neurotransmitters open ion pores, acetyl-choline is the most important in nerve and muscle cells. Notice that it is one of the smallest in mimicking the trimer of water.



After an intensive study of the receptor protein in the electric eel, Dr. Nigel Unwin, in 1999, published the structure of the receptor protein and how it functions.[51]

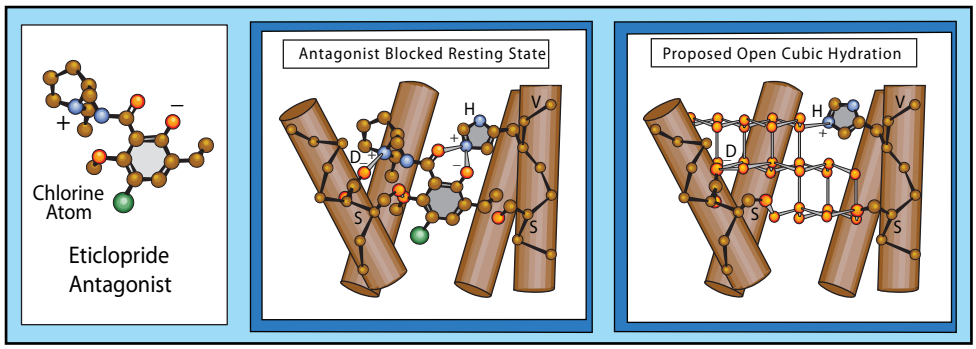
Based on the Transient Linear Hydration Hypothesis, the receptor site is most likely highly hydrated in the resting state with linear elements of hydration forming in preferred probability locations. When the acetyl-choline molecule enters, water is displaced and



two aromatic rings and two yellow sulfur atoms are drawn around it, displacing all water from the site. When that happens, a polypeptide chain attached to a central coil rotates the coil. As water enters to displace acetylcholine from the site, (as shown on the right), it fills with water and opens. Once again, it appears that linearly-ordered water provides for quantized spatial order and spontaneous function for this receptor.

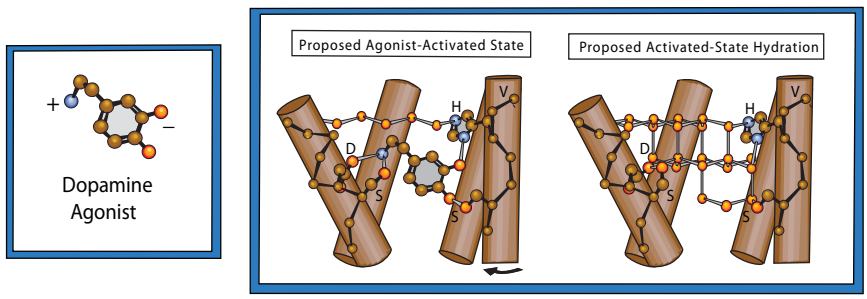
In the transduction protein above, the regulator molecule binds to the receptor site near the outer surface, and in bonding, turns one or more of the coils to activate enzymatic functions within the cell. The problem is that the receptor in its activated form is so labile, that it cannot be isolated.

In fact, large "antagonist" molecules are often used to bind sites in their resting states to provide stabilization for isolation. For example, in 2010, Professor Stevens and and his group at the Scripps Institute, by changing several amino-acids in the coils and using a large antagonist molecule, were able to obtain a structure for the resting-state human receptor site for the central nervous-system "agonist" molecule dopamine.[52] Dopamine is a critical receptor molecule in the brain; if depleted, causes parkinsons disease.



As illustrated, the eticlopride molecule essentially fills the binding site in its open form. Couplings with aspartate (D) behind the coil on the left and the histadine ring (H) on the right provide major binding. A serine (S) on the left bonds with an oxygen atom in the molecule while serine (S) on the V-coil is pushed out of position. As dopamine or an antagonist move in and out of the binding site, water in random liquid form moves in to fill it with low-energy linear elements which last about 10^{-11} seconds, but which continually propagate new linear elements on the ends to produce a cubic pattern of ordered water to fill the open space.

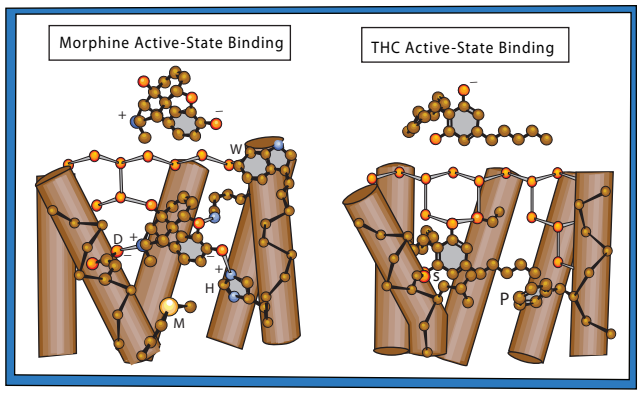
Although dopamine activation of the receptor site is too labile to be isolated, the TLH hypothesis permits development of a schematic model.



As the dopamine molecule moves into the site, ordering water is displaced and the V-coil is rotated few degrees anti-clockwise to activate enzymes within the cell. Agonist binding fills much of the lower region of the site but leaves a space for linear segments to bridge over the molecule. However, as dopamine leaves, the site initilly fills with water and, as the V-coil rotates back to resting state, hydration fills the binding site.

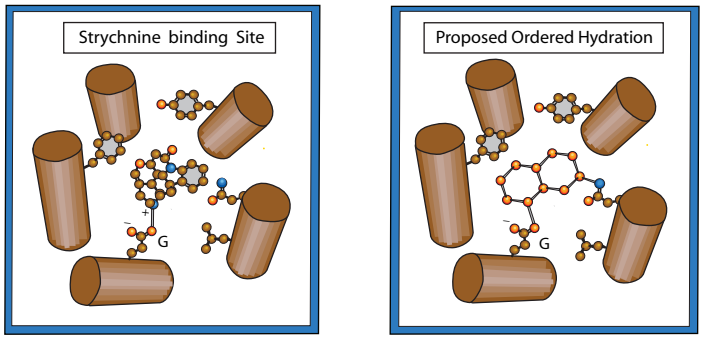
As might be expected, transduction receptor sites vary tremendously in structure based on binding peptides but most have similar coil structures and activating mechanisms.

For example, the receptor sites derived for morphine[53] and tetrahydrocannabinol[54] have similar structures but different binding peptides.

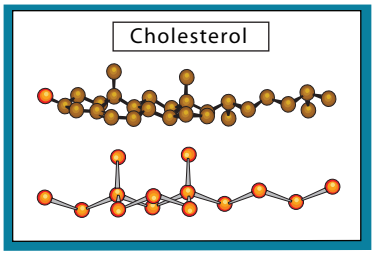


In the proposed figures on the left, the phenolic oxygen of morphine bonds to the cationic ring of histadine while the end of the tetrahyrocannabinol molecule rests on the aromatic ring of phenylanaline. As morphine and the THC molecule leave the binding sites, water, in its liquid-binding form most likely assists them in quantized steps.

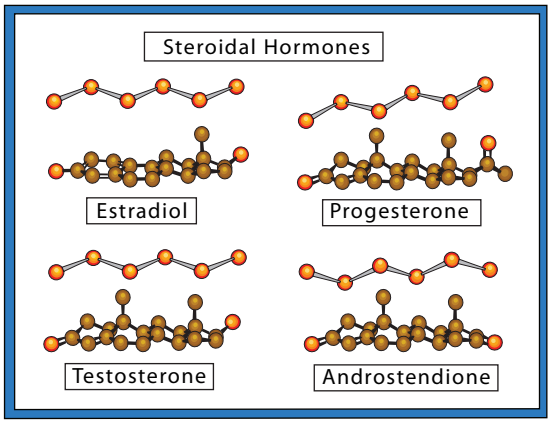
Recently, a taste receptor site which binds strychnine was reported with the figure on the left below.[55] The view is directly down into the site from the surface. The molecule is flat and spans across the receptor space, just as hexagonal water most-likely does when the site is empty.

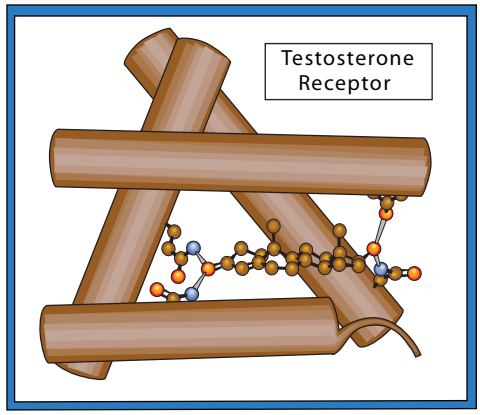


Looking back at the images of hormone and neurotransmitter molecules on page 1, where they appeared to mimic linear elements of hydration, one of the most important studies that convince me that I was on the right track was published by Professor Breton in 2006.[56] It was of the receptor site for testosterone. My graduate work had invoved the synthesis of aldosterone, a steroidal hormone produced from cholesterol, and I had always wondered why so many hormones have similar lengths.



Looking at the testosterone receptor, you can clearly see that the linear element of hydration shown in the lower left-hand corner might well bind in the same site. Whether the Transient Linear Hydration hypothesis is valid or not, only time will tell, but the correlations presented here are interesting.





Recently, the Deep Mind organization,[57] and David Baker,[58] reported the development of programs which, by storing strctural information on thousands of proteins, have been able to derive spatial structures for new proteins based solely on their aminoacid sequences. They are, indeed, breakthroughs which should make it possible to advance medical science.

However, they do not address the role of water in the assembly process. On the other hand, Irena Roterman, Barbara Kalinowska and co-workers have begun publishing on the water as an active participant in protein folding and the role of the protein core in providing functional properties.[59] Hopefully, their studies will continue and influence other leaders in the field of molecular biology to accept the importance of water, not only in the formation and function of vital molecules, but in the role it may have played, and continues to play, in the selection of the molecules of life.

Unfortunately, hydration of open receptot sites is so dynamic and rapid that it has not been possible to visualize what happens. As electrons "orbit" the nuclei of atoms and define bonding angles and distances between atoms in molecules, positions of electrons can be defined only by probabilites. Perhaps, in order to understand how vital molecules function in such an amazing way to give us life, spatial positions of ordering water molecules will have to be illustrated in probability positions as they have been in this article and in "The Matrix of Life: Second Edition" published in 2023.

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The Author

Dr. Collins received his degrees in Chemistry from Wayne University and the University of Wisconsin. After employment at General Motors Research, Central Research at E. I. Dupont in Wilmington, Delaware and Sterling Winthrop Research Institute in Rensselaer, NY, he accepted a position as Associate Professor and Chairman of the Chemistry Department at Illinois Wesleyan University. In 1967, he returned to Sterling Winthrop to direct Medicinal Chemistry Research and then handle Technical Affairs for the Corporation until 1987 when he retired to devote full time to his study of the role of water in the living cell.

He has a number of publications and patents to his credit and, while at Illinois Wesleyan, developed a reagent for selectively oxidizing primary alcohols to aldehydes which, subsequently, was named *The Collins Reagent*. However, it was during his first employment at Sterling Winthrop that he began constructing permanent models of vital molecules and drugs and noticed that distances between polar atoms on their ends corresponded those of linear elements of water molecules in ice and that the anhydrous spatial polypeptide units in the inner cores of a number of water-soluble enzymes correspond to those in cubic ice. Since ice-like bonding between water molecules would be unstable above $0^{o}C$ and last only about 10^{-10} seconds, it would explain why they have not been isolated or detected in receptor sites or on surfaces of polypeptides, proteins and nucleic acids.

Furthermore, when other classes of vital molecules were examined and dimensions of linear elements of water appeared over and over again, it suggested that low-energy ice-like elements of water, which most likely formed on lipid surfaces and between ions and molecules as they evolved may have influenced their the formation and selection as functional units in the living cell. In fact, it appeared that molecules within the cell function so smoothly and spontaneously becaause they are spatial analogues of order/disorder units of the environment in which they evolved. Based on that view of water in the living cell, he retired in 1987, took courses in computer graphics and, in 1991, published his first book, *The Matrix of Life*,

Since he was interested in getting responses regarding the concept, a number of copies were sent to experts in molecular biology. Most responses were extremely negative and demeaning but one from Linus Pauling, whom he respected more than any of the others, responded: “You are on the right track, but I think your concepts are too simple.” The other positive response was from Dr. Michael New, a lead investigator at NASA, who reviewed a preprint of my third book “Biomolecular Evolution from Water to the Molecules of Life:” :”
“Your concepts of Transient Linear Hyhdration and Cubic Hydration Patterning are valuable contributions to our understanding the unique role of water in origin of life research.”