MOLECULAR PRESENTATIONS

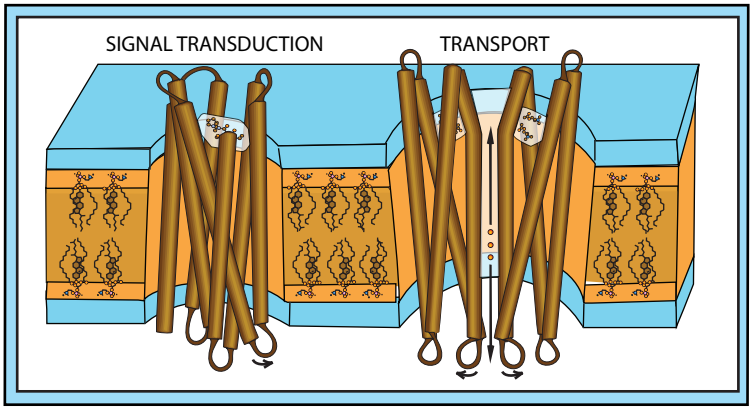
Hydration of Receptor Binding Sites

J. C. Collins, PhD

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

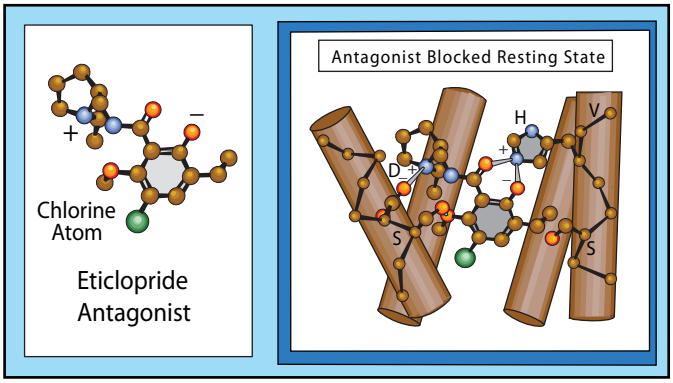
Although receptor binding sites have been studied extensively, the role water may play in regulating space within the sites or in assisting regulator molecules in and out is essentially unknown.

The problem is that most receptors are composed of protein coils which pass through dynamic cell membranes to control functions within the cell. The coil units are mobile and difficult to isolate.[1]



In their resting-states, binding sites are open to external surface water, but when neurotransmitter or hormone molecules enter, as shown above, they displace the water and one or more coils rotate to activate processes within the cell or to open a pore and permit ions or molecules in and out. In Signal Transduction proteins, a single coil usually rotates, while in Transport Proteins, two or more coils rotate to open a pore. In a third type of receptor, when an activating molecule binds, the binding portion inverts to bring the molecule into the cell.

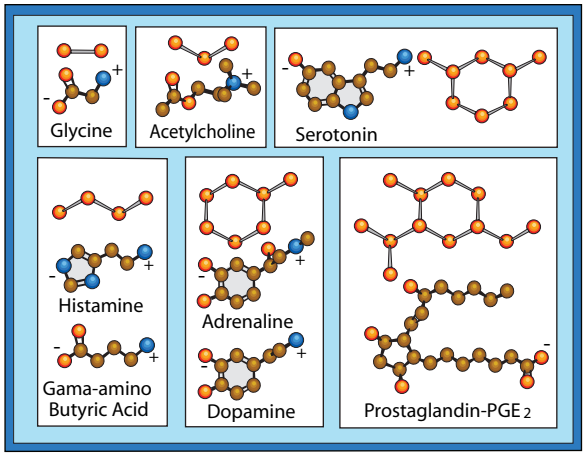
Although receptor proteins are difficult to isolate, large "antagonist" molecules, which bind to the sites in their open resting-states, are used to stabilize the protein and permit isolation. For example, in 2010, Professor Stevens and his group at the Scripps Institute, by changing several aminoacids in the coils and using a large antagonist molecule, they were able to obtain a structure for the open human receptor site for the central nervous-system agonist molecule "dopamine."[2] Dopamine is a critical receptor molecule in the brain; if depleated, causes Parkinsons Disease.



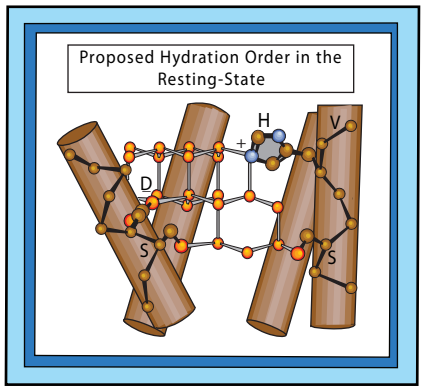
As illustrated, the eticlopride molecule essentially fills the binding site in its open form. Ionic couplings with aspartate acid (D) on the back side of the coil on the left and a histadine ring (H) on the right provide major binding. A serine (S) on the left hydrogen bonds with an oxygen atom in the molecule while, on the lower right, binding is so tight that serine (S) on the V-coil is forced out of position.

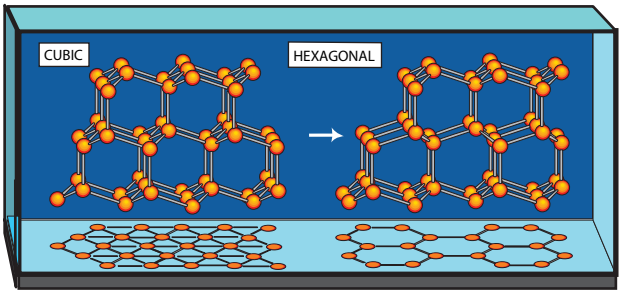
With regard to receptor site hydration: as a medicinal chemist in the 60s, a number of permanent models of neurotransmitters and hormones were constructed to see if they would reveal structural features which could be used in designing new drugs.

Much to my surprise, I found that the dimensions of the molecules appeared to correspond to linear elements of water, 2.76Å between the molecules, the same as in ice.[3] Since binding sites for these molecules are open to the surface, water must be able to move in and out. However, the sites are confining and molecular orbital studies had indicated that a trimer[4] and ordered linear elements of hydration of as many as six water molecules might form on lipid surfaces.[5]



Thus, in receptor sites, where water molecules have lower energy, linear elements must form and, although they may last only about 10^{-10} seconds,[6] by absorbing energy from neighboring water molecules, may generate the formation an ice-like cubic hydration order of the type displayed in the the dopamine site on the right. Although most water in the site would be randomly-distributed in the liquid state, an ice-like-bonded pattern of molecules may be generated kinetically to hold the site open for the stepwise entrance of receptor molecules.



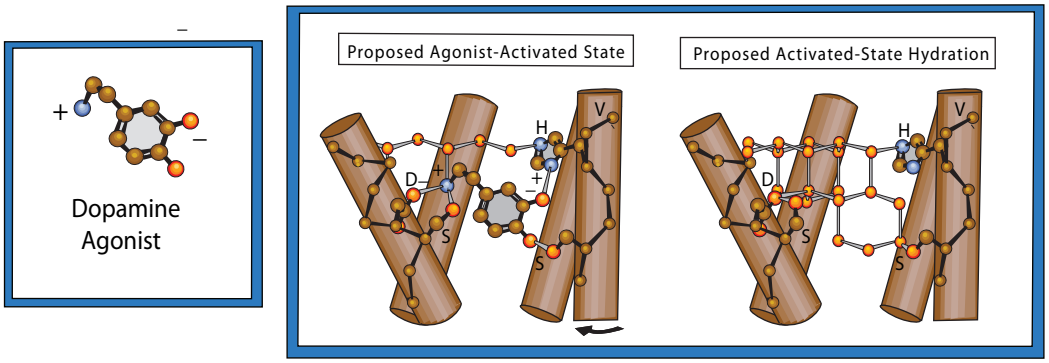


As water freezes, the cubic isomer, composed entirely of linear elements, forms most, rapidly, but then, reverts to the normal hexagonal form.[3] Although present for only an instant, it may occupy space, interact with neighboring molecules and produce ice-like spectra.[7]

In fact, when larger receptor molecules like insulin and transfer RNAs were constructed and viewed in particular orientatons, they mimicked units in cubic ice. And when the models of five water-soluble enzymes were constructed and examined, lipid polypeptides, which initially were coated with unstable ordered water when released from ribosomes,[8] had assembled to form the anhydrous cubic central cores of the proteins.

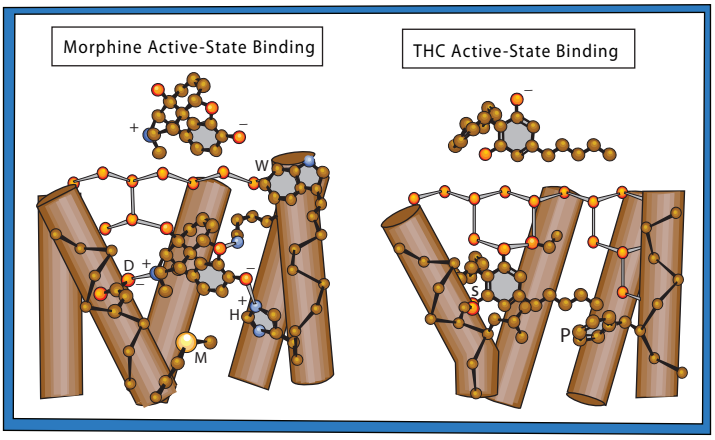
Thus, in 1991, even though the concepts were speculative, I published *The Matrix of Life* with the Transient Linear Hydration (TLH) hypothesis to explain the role of hydration order in receptor sites and as assistance in the assembly of proteins and nucleic acids.[9] Although the concepts triggered vitriolic comments from experts in water and proteins, the ideas continued to permit the deveopment of what appeared to be viable interpretations of processes which are too rapid for experimental analysis.[10]

For example, even though dopamine activation of the binding site is too labile to permit isolation, the TLH hypothsis permitted the development of a schematic model.



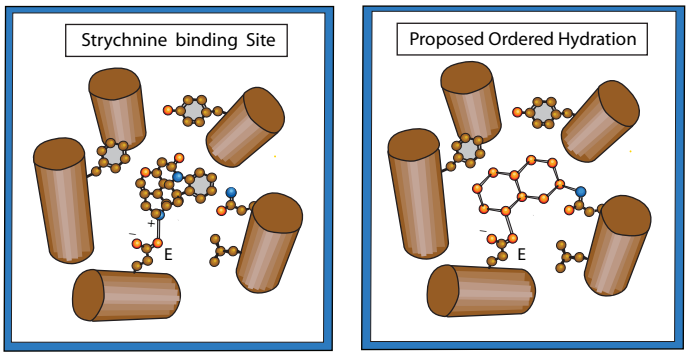
Although detailed computer analyses will be required to determine the precise structure, it must be realized, the structure is produced kinetically, not thermodynamically.[11]

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[12] and tetrahydro-cannabinol[13] are similar, but with substantially different binding peptides.

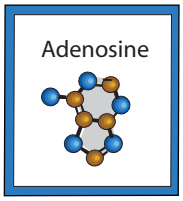


In the proposed figures on the left, notice that the phenolic oxygen of morphine bonds to the cationic ring of histidine while the aliphatic end of the tetrahyrocannabinol molecule rests on the aromatic ring of phenyl-alanine. As the morphine and THC molecules leave the binding sites, water must assist them out in quantized steps and then transiently hold the site open as more water moves in.

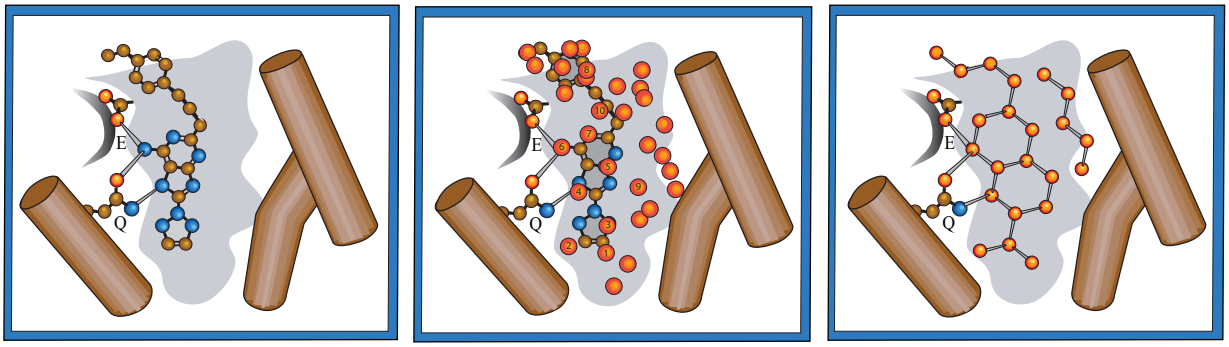
Recently, a taste receptor site which binds strychnine was reported with the figure on the left below.[14] The molecule is flat and the view is directly down into the site from the surface. Once again, notice that the cationic nitrogen in the molecule binds to an anionic glutamate anion on the coil wall and that the hexagonal TLH-patterning on the right fills the space.



Although limited studies had been reported for receptor site hydration, in 2010, Higgs, Beuming and Sherman at Schrödinger, Inc. used a *WaterMap* program to calculate the free energy of water occupying sites of the A2A receptor site of adenosine.[15] Adenosine is a major regulator molecule in the brain. Once again, the site is so fragile in its activating state that a large antagonist molecule was used to isolate the site in its resting state.



Looking down into the site, the antagonist molecule is large and appears to fill the site and bind more tightly than the much smaller adenosine molecule.



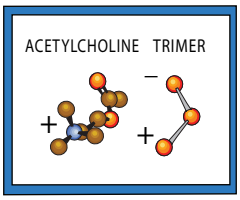
The red dots in the center figure represent the sites of higher hydration probability based on the *WaterMap* program. The numbered spots are sites of higher binding probability for the antagonist molecule. Notice that two of the blue nitrogen atoms form hydrogen-bonds to a glutamic acid (E) and glutamine (Q) on the left and that the linear element on the far right is simply a linear element which forms in the open space between the molecule and the coil.

The figure on the right displays the TLH pattern of covalently-bonding water molecules[16] which are generated by short linear elements of hydration. Again, the linear elements last only an instant, and then, by absorbing energy from neighbors, convert the neighbors into ordered units and generate the cubic pattern. Although the watermap and TLH positions of water molecules are similar, kinetics simply defines probability positions of transient water molecules, thermodynamics, the binding parameters of regulators.[15]

Although there is no current recognition or acceptance of the TLH hypothsis as valid, as more relevant information becomes available, the concept may eventually prove of value.

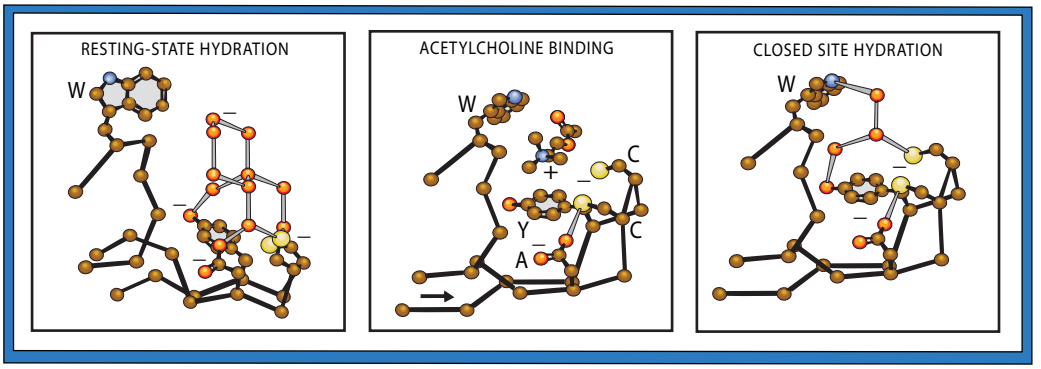
Now we will look at an ion transport receptor of the type shown on the right on page 1.

As illustrated there, receptor sites on the outer surface of coils on both sides of the pore must be filled before the coils will rotate to admit sodium ions into the nerve cell. Although a number of types of neurotransmitter molecules open ion pores, acetylcholine, which mimicks the hydration trimer, is most important in nerve and muscle cells.

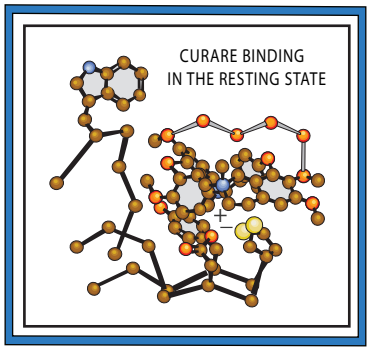


After an intensive study of receptor proteins, in 1999 Dr. Nigel Unwin published the structure of the receptor protein in the electric eel and how it functions.[17]

Since these receptor sites are open to the upper surface of the membrane, once again, water molecules must fill the void and form transient linear elements of hydration in both the resting and activating states.

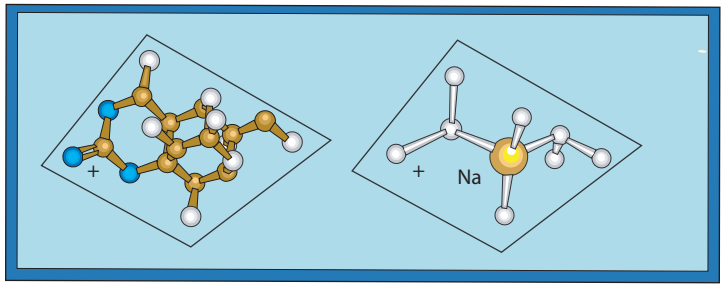


However, when the acetylcholine molecule enters, all water is displaced as the two aromatic rings and two yellow sulfur atoms are drawn in around the cationic acetylcholine molecule. When that happens, polypeptide chains attached the two central coils, rotate the coils and permit ions to enter the cell. As water enters to displace the acetylcholine molecule, it initially must bridge across the site, as shown above, and then fill the site with water to close the pore.

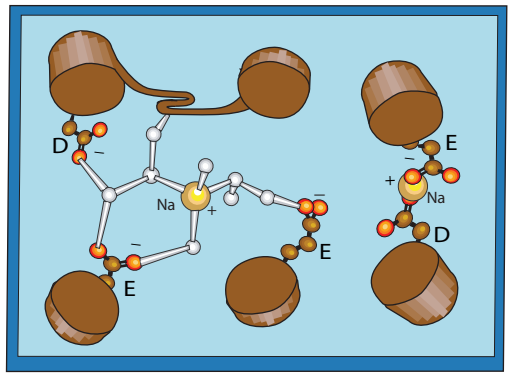


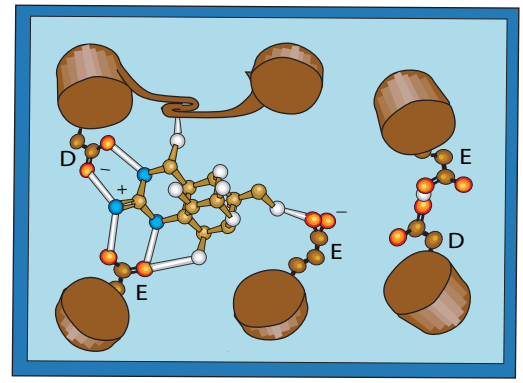
However, if an antagonist molecule like curare enters, the site is completely filled and the operating system is held in the open state; nerve traffic and muscle contraction are blocked.[17] Natives used curare-tipped arrows and darts to paralyze their prey.[18] It and similar antagonists have been used medicinally to control muscle contractions.

A drug which binds most-tightly to nerve entrance sites is tetrodotoxin. It is produced by bacteria in the salt-water puffer fish and is one of the most potent toxins known to man. If absorbed into the blood-stream, it produces almost immediate death.[19]

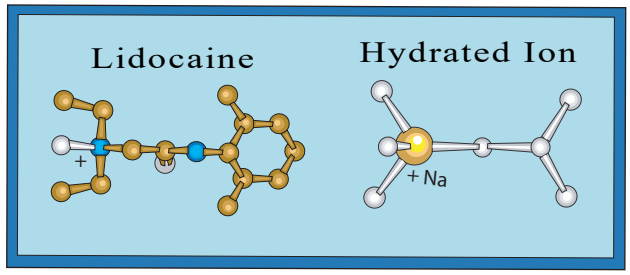


The structure of the molecule is absolutely unique. It is as if one set out to produce a molecule which would mimic the structure of a hydrated sodium ion. As illustrated above, it has five alcoholic oxygens, two ether oxygens in a bridge that comes toward you and a guandine group to give the molecule a positive charge. Eight water molecules and a sodium ion bind within sites composed of four central coils with two glutamic acids (E), an aspartic acid (D) and a lysine around the site which is shown schematically below.



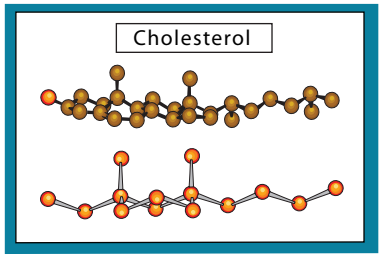


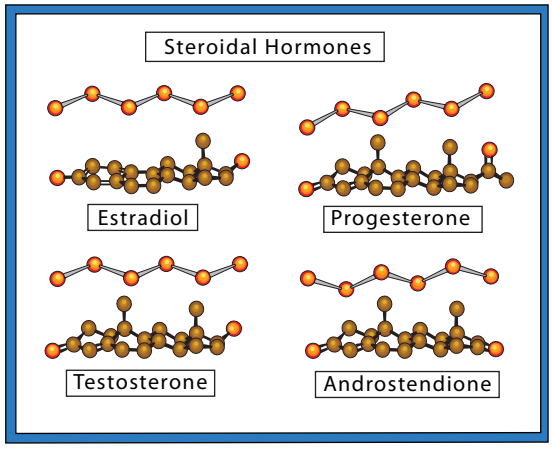
As the site opens, a hydrated sodium ion jumps in between the three acid groups, and then, as shown on the right, passes between two acids in a narrow pore into the cell. Hydrating water is left behind to prevent bloating in the cell. However, if tetrodotoxin is present, as shown on the right, the entrance site is blocked; only a single water molecule is left in the narrow open pore. This illustration of the way tetrodotoxin may function, gives us a view of how ions may move.

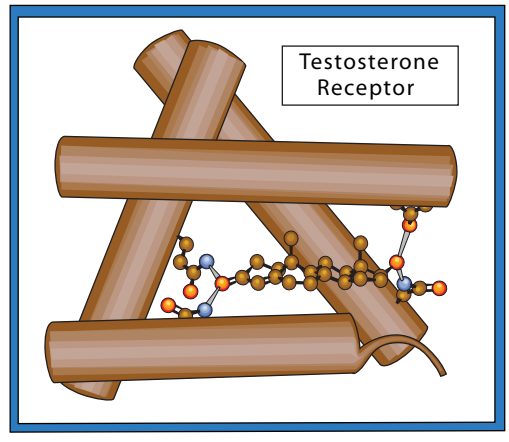


And yet, there are other drugs, like the local anaesthetic lidocaine, which mimic hydrated ions.[20] They bind in hydration spaces in ion channels to slow ion conduction by filling water spaces with non-hydrogen-bonding CH^3 groups.

Cholesterol, which mimics the linear element of water molecules and combines with phospholipids to form cell membranes, by enzymatically losing its tail, produces a variety of steroidal hormones which mimic six or seven linearly-ordered water molecules. Although the molecules have slightly different structures, they appear to simulate the same transient linear elements of hydration which periodically occupy the binding site, again as kinetically-generated units.

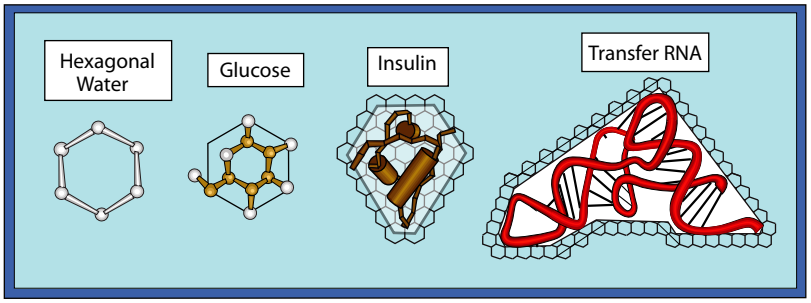






In 2006, Breton published the structure of testosterone in the binding site shown above.[21] It is not hard to imagine how water, in the linear form shown above, may occupy the site, even for a short period of time as the testosterone molecule enters and leaves.

In 1944, Erwin Schrödinger proposed that there must be some sort of order in water to have directed the evolution of the molecules of life from randomness toward order and that there might be a law, other than thermodynamics, to regulate space in the living cell.[22] Based on the above studies, it appears that cellular molecules function spontaneously and orderly to produce life because they are all spatial analogs of the dynamic and ordered spatial properties of the environment in which they evolved.[9] Only time will tell whether this concept is valid or not.



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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 Improvements 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 technique for selectively oxidizing primary alcohols to aldehydes which was subsequently named *The Collins Reagent*. However, it was during his first employment at Sterling Winthrop that he began constructing permanent models of hormone and neurotransmitter molecules and found that distances between polar atoms on the ends corresponded those of water molecules hydrogen-bonded together as they are in ice. Could it be that water, in orderly low-energy forms, occupies binding sites in receptor proteins when regulator molecules are not there? Since bonding between water molecules would be unstable in binding sites above $0^{o}C$, it would explain why water has never been detected there. However, when models of a number of water-soluble enzymatic proteins were examined, it was found that the polar and ionic atoms of peptide side-chains leading into catalytic binding sites were in positions to support transient linear elements of hydration.

Furthermore, when these enzymatic proteins were examined more closely, it was found that the geometries of their anhydrous cores corresponded to units of water molecules in cubic ice. Since cubic ice is composed entirely of linear elements of water molecules and is the initial form produced on lipid surfaces as water freezes, it might well be that they form as unstable linear elements in cubic forms on the lipid surfaces of polypeptides as they are released from ribosomes. On the other hand, small peptides which hydrogen-bond directly with surface water are in precise positions in polypeptides to provide for bends and turns. While regions of polypeptides which form adjacent linear elements of hydration lose water and produce anhydrous cores, regions which hydrogen-bond directly with surface water end up on the surface, increasing the solubility and stability of finished proteins. Only small surface regions continue to induce hydration order to provide sites for binding other proteins, regulator molecules and substrates for reactions. These were the concepts presented in 1991 in his first book, *The Matrix of Life*,

Although two more books and a number of web sites were published after that, only two positive comments were received regarding the concepts. One was from Linus Pauling who received a copy of the *Matrix* book. "You are on the right track, but I think your concepts are too simple." The other was from Dr. Michael New, a lead investigator at NASA who was asked to review a preprint of "Biomolecular Evolution from Water to the Molecules of Life." His comment was: "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." Now, the second edition of *The Matrix of Life* has been published with validating studies and additional applications. Perhaps the hydration concepts may be valid. What do you think? Send opinions to jcbjh2o@aol.com.