Membranal proteins are the “brains” of living cells. They hold membranes together, provide for shape, regulate flow of molecules and ions in and out and provide binding sites for regulator molecules outside to control most everything inside.

But they are extremely complex and difficult to study. In contrast to water-soluble proteins, the components are soluble in oils - they are in dynamic mixtures that are difficult to separate and usually do not crystallize. The only way they can be understood is by using hypothetical models based on physical properties and spectroscopic data.

In 1972, Singer and Nicholson proposed a “Fluid Mosaic Model” for biomembranes and, in the same year, Kirschner and Casper published the electron-scattering (ES) and neutron-scanning (NS) curves for rabbit nerve cell axon membrane. Using the Singer Model as the basic structure and the 1-to-1 complex of lecithin and cholesterol as the basic structural component, the following model was presented in 1991 based on the scattering curves.

Although plasma membranes are composed of a vast variety of phospholipids, the lecithin molecule, with two long fatty-acid chains attached to a tri-methylamino-ethyl-phosphate chain by a glycerol unit, is a major component. In a 1-to-1 complex with cholesterol, myelin surrounds large axonal nerve fibers in multiple layers as an insulating barrier.

Since much of the space within myelin membrane is generated by the spinning of the cholesterol molecules and the flipping of myelin chains, molecules and proteins with 40.5-Angstrom lipid surfaces can fairly-readily move in and out. Notice that the charged ionic groups of the phospholipids are at peaks in the electron-scattering ES curves on both sides of the membrane and that the ionic side-chains of the protein coil, which was isolated from a red blood cell, are in the same locations. Neutron scattering shows that water peaks further out on both sides of the membrane while the linear element of 18 covalently-bonded (ice-like) water molecules (2.25Å per molecule) and 27 peptides in a protein coil (1.5 Å between them) are the same length as the 40.5Å width of the lipid zone. It is no wonder that most receptor proteins in membranes are composed of coils with lengths which permit transiently-ordered linear elements of hydration to assist in conveying molecules and ions from one side to the other.
This common lengths of coils and linear elements across the membrane does not mean that all must be perpendicular to the surface but it does mean that molecules and protein complexes must have geometries and distributions of lipid surfaces compatible with those in biomembranes.

With regard to surface hydration, as illustrated in the lower left in the figure above, when positive pulses are discharged into nerve endings, polar head groups of lecithin molecules, as well as the 6 covalently-bonded water molecules next to them, align between the positive end and negative body and positively-charged protons cascade through them at incredibly high speeds. In small nerve cells, it is potassium ions which carry most of the charge - in the axon, it, most likely, is protons.

Although there is little agreement among experts regarding the ultra-high-speed conduction of charge through the axon, the recent studies clearly show that water adjacent to a poly-ionic surface forms covalent linear elements and that protonic charge readily tunnels through them. Currently, intense studies are being performed to develop materials which will permit superconductivity of electrons. The problem is that atoms must be cooled, almost to absolute zero, to hold them in particular orientations so that electrons will pass without losing energy. Needless to say, electron superconductivity at room temperature has been difficult to achieve. Although water molecules on inner surfaces of axons must align for only an instant following depolarizing discharge, it is long enough for protons to pass through with almost no loss in energy. The scientific community needs to examine a report in 1986 that protons move rapidly through water on hydration-ordering lipid surfaces and realize that superconductivity may be in the protons in the axons of our nerve cells. 

Although there are a number of types of membranal receptor proteins, the two shown above represent the major structural classes. Most proteins pass through the membrane as coils with a variety of phospholipids around them. When small transmitter molecules bind to both sides of a transport protein, as shown on the right above, coils on both sides rotate, the central pore opens and sodium ions rush in to depolarize the cell.

When a neurotransmitter or hormone molecule binds to receptor protein, like the one on the left, one or more coils in the complex rotate a few degrees and, in rotating, activate a protein inside the cell to perform a particular function. Since binding sites in receptor proteins of this type are formed by the side chains of peptides on coils around the sites, proteins with the same basic coil structures, but different peptides, are activated different regulator molecules.

Although many types of proteins regulate the shapes and functions of biomembranes, altering peptides in particular locations permit an amazingly few basic structures to perform an amazing number of functions. At the same time, it appears that the same basic covalent linear elements of hydration in cubic conformations provide for a common element of spatial control within the binding sites. See www.cubichydration.com for more information on covalent bonding.
Acetylcholine Receptor

For example, one of the most important neurotransmitters, which activates both nerve and muscle cells, is a small molecule with a structure which mimics the dimensions of the polarized linear trimer. Acetylcholine, even as small as it is, has had an entire class of receptors, “The Cholinergic System,” named after it. After an intensive study of the receptor in the electric eel, Dr. Nigel Unwin and his group in 1999 published the structure of one of the receptors and described how it functions. The receptor protein has the transport structure shown on the right on the previous page. Based on the transient linear hydration hypothesis, the binding site in the resting state is highly hydrated with water molecules in preferred covalently-bonded quantized positions. However, as pointed out below, they stay in those positions only about \(10^{-10}\) seconds and then gain energy from incoming water molecules and return to the liquid state.

As the acetylcholine molecule approaches the open binding site, it directs its positively-charged trimethyl-amine end toward the negatively-charged site, displaces water from it and draws both the anionic phenolic ring of the tyrosine peptide as well as both anionic sulfur atoms of the cysteine peptides up toward it. The tryptophane peptide ring (W) then closes in above to fill the void. As the peptides move upward, they draw a polypeptide chain attached to a central coil toward them, rotate coils on both sides of the receptor protein and allow sodium ions to move in and out. Since the acetylcholine molecule binds only lightly in the site, it is displaced rapidly by water, forms the hydrated site shown on the right and then hydrates further in its open resting state.

Although water in the the open site is pictured in a total cubic form, at any instant, only a few of the molecules will be in those ordered positions. Most of the water will be in random positions, either entering or leaving the site. Only by integrating over time and viewing only those in covalently-ordered positions, can we see what is really controlling the spatial properties of the site. Often we forget that water molecules on ordering surfaces and confined spaces, move in quantized steps - they jump from one position to another, exchanging quantized units of energy with other molecules as they go. As a regulator molecule approaches a binding site, it transfers its kinetic energy to water molecules in the site to move them out. As the regulator molecule leaves, it absorbs energy from entering water molecules. Quantized energy changes are so complex and so rapid, and the involvement of water molecules so transient, that they are like the electrons around atoms: they must be viewed only in theory.

With respect to the acetylcholine molecules: as they move out of the site, enzymes with a negatively-charged binding site attract them and catalytically remove the acetyl group on the end. The products, acetic acid and choline, then diffuse from the site and the nerve cell recharges, ready for the next discharge. In examining catalytic reactions and receptor binding sites, one cannot help being amazed by their rationality and amazingly orderly function.
Dopamine Receptor

Although membranal receptors are extremely difficult to isolate, Professor Chien and his group, by changing several aminoacids in the receptor protein and using a large binding receptor molecule, were able, in 2010, to obtain a structure of the receptor site for the neurotransmitter molecule dopamine.15 Dopamine is critical component in the brain - if depleated, causes Parkinson’s Disease.

As illustrated in the upper left, the Eticlopride molecule essentially fills the binding site - forming ionic couplings with an aspartate acid group (A) on the back side of the coil on the left and the cationic histadine ring (H) on V coil on the right. A serine (S) on the left coil hydrogen-bonds with an oxygen atom in the molecule. Binding on the right side in the lower region of the site is so complete, that all water is displaced by the Eticlopride molecule and by the side-chains of peptides in the coils below and behind the molecule.

Of course, in the absence of a eticlopride molecule, the receptor site in this inactive resting-state would be expected to be occupied by water molecules which continually moving in and out to produce the covalent bonding pattern shown in the figure on the upper right. Although only a limited number of ordered water molecules are shown in the site, water molecules may be ordered up to the surface to integrate with hydration order above and around the binding site.

As the dopamine agonist molecule moves into the binding site, helical coil V rotates clockwise to permit histadine and serine to bind to the phenolic oxygens of dopamine while maintaining its cationic nitrogen hydrogen-bonded to the anionic aspartate on the left-hand coil. Notice that the activating molecule is extremely small - so small that water molecules readily penetrate into the site and compete for binding. As the dopamine molecule leaves, water molecules enter and form the ordered hydration pattern proposed on the lower right which, by rotating the V coil, returns the site to its resting-state conformation with ordered hydration as shown on the upper right.
When we think of pain, we think of the analgesics that are take to relieve that pain without realizing that there are polypeptides which continuously bind to receptors in our nervous system to regulate both pain and pleasure. Molecules like morphine and heroin mimic the actions of these central nervous systems endorphins. Opiates are important in regulating pain but highly addicting and have for many years presented a major social problem.

Met-enkephalin is one of the smallest of these central opiates. It is a pentapeptide which binds to a receptor protein with a structure that is similar to that described for dopamine. It has been studied intently but, as yet, details of binding are not available. Model-building, based on the linear hydration hypothesis, have been used below to visualize how opiates may bind and function. In aqueous solution, NMR studies indicate that the molecule exists in a cyclic form, as shown on the left. Since it is compact and does not include internal water, it was put into the binding site. For clarity, front and back sections are shown with peptides identified.

As illustrated below, the met-enkephalin molecule effectively fills the proposed binding site with a phenylalanine peptide (P) filling space on the right below the molecule.

Although aspartic acid (A) is in the same positions on left coil as in the dopamine receptor, histamine (H), lysine (L) and phenylalanine (P) are on the right-hand coils. Of course, other peptides surround the met-enkephalin molecule to provide a firm anhydrous fit. The phenylalanine (P) on the V coil fills space next to the methionine sulfur atom. Phenylalanine at the top of the met-enkephalin molecule serves the interesting function of ordering water around it and stabilizing the molecule in the site. Larger opiate polypeptides occupy the upper region of the site more completely and block pain signals much longer.

In the absence of the opiates, it is proposed that the receptor would be occupied by water in short linear elements in preferred cubic locations in its active state and then revert to its more stable resting state. As you will soon see, the most effective molecule in holding in that non-activating resting state is a relative of morphine.
Although the morphine molecule has been modified chemically to produce hundreds of structural relatives, only one has been developed and used as an effective agent to bind to opiate receptor proteins in their resting states and prevent activation by morphine and structural relatives. The drug is naloxone - sold under the trade name “Narcan”.

Its structure is similar to morphine but differs in three important ways. First, it has a ketone at the position A rather than the alcohol. Second: the double bond in morphine at B is saturated with an alcohol inserted at C. Third, the methyl group at D is converted to an allyl group. Although, there is no accepted receptor explanation for actions of either morphine or naloxone, here is one based on the Linear Hydration Hypothesis.

Since morphine is just one of a number of synthetic compounds which exhibit opiate analgesic activity, illustrations of how morphine and several others may bind in the met-enkephalin binding site are presented on the next page.

Naloxone, most likely draws the receptor into its activating conformation as shown on the left. However, in contrast to the morphine molecule, which remains in this activating conformation, the lysine chain on the right-hand coil, with a turn of the coil, binds its nitrogen atom directly to the carbonyl carbon of the ketone, forms an hydration adduct with an hydroxyl group hydrogen-bonded to the one on the molecule as well as in a probability position for ordered hydration. This holds the receptor in its inactive resting-state and prevents the endorphins, morphine and analogs from activating the receptor. The reaction to produce the adduct is reversible but nitrogen atoms react readily with ketones and the nitrogen bond is relatively stable. Naloxone’s most important property is that it binds so firmly with the site that it reverses the actions of even extremely potent opiate drugs which have been taken in lethal overdoses.

Of course, the above description is full of speculations which only detailed studies will be able to evaluate but it provides a rational conceptual image of the way naloxone may produce its effective and useful blocking activity. Now we will look at how several other opiate analgesics may bind to the receptor in its activating conformation.
Although a variety of molecular structures exhibit analgesic opiate activity, morphine, demerol and methadone represent three of the major structural classes. All of them are competitive with each other and, thus, appear to act on the same met-enkephalin receptor protein which is shown on the upper right in its hydrated activating state.

As illustrated below, the cationic amine and phenolic oxygen of the morphine molecule bind to the same aspartate (A) and histamine (H) peptide as the enkephalins with the alcoholic oxygen bonded to lysine (L). In fact, all three analgesics bind their cationic nitrogen atoms to the same anionic oxygen of aspartate D and histamine H, even though in slightly different spatial positions.

In contrast to morphine, the aromatic ring of demerol, which has no anionic phenolic group, is held down below the lysine amine close to the aromatic ring of phenylalanine on the V coil. In fact, multiple layers of water molecules, many in preferred cubically-ordered positions, may form above the molecule to stabilize it in the binding site. Again, the ethyl ester of the of the molecule is hydrogen bonded to the histamine below.

Methadone’s amine hydrogen bonds to the same aspartate acid with its two aromatic rings flanking the aromatic ring of phenylalanine P on the right. The ketonic oxygen of the molecule can be seen, just below the lower aromatic ring], hydrogen-bonding with the histadine.

As mentioned above, the models presented in this article are all hypothetical but they present an internally consistent view of how they may function. It also attempts to present the role transiently-ordered water may play in providing the guiding cubic matrix of preferred positions of water molecules as they guide the motions and interactions of the molecules.

Although opiates have recently become a major concern because of addiction, another class of CNS-active drugs have been a subject of concern for a number of years.
Only recently have cannabinoids been accepted as useful medicinals. Since limited information is available regarding their receptor-binding properties, it seemed reasonable to include them in this article. Of course, extracts from plants that produce psychological highs have been used for centuries, but it wasn't until 1992 that Professor Mechoulam and his group at the Hebrew University in Jerusalem isolated and determined the structure of a molecule in the brain which regulates the function of cannabinoid receptors. The substance was named “Anandamide” and, later, found to regulate receptor CB1.

Tetrahydrocannabinol has been used for its psychological affects for many years and CBD, which is a component of hemp oil, has recently become an important medicinal oil. In 1999, Rimanabant and a number of synthetic analogues were found to block the effects of THC and CBD on the central CB1 receptor and submitted to FDA for approval as therapeutics but were turned down because of side-effects.

As anandamide approaches the receptor in its resting-state with water molecules periodically in their preferred covalently-bonded positions, almost all of the water, except for that on the upper left, is displaced by the molecule. Its terminal alcohol hydrogen bonds behind the alcohol of serine on the left coil and its amide oxygen hydrogen-bonds to the serine on the back coil. In the process, the coil on the right has been rotated counter-clockwise to permit the lower double-bond of the anadamide molecule to rest on the aromatic ring of the phenylalanine. By rotating the right-hand (and possibly a front) coil, an enzyme has been activated within the cell to perform a series of actions. With the binding site almost completely filled by the circular molecule, the only way binding can be reversed is for water to enter from the upper left and compete for binding with the serine alcohol on the left-hand coil.

As anandamide leaves, water moves in, fills the site and the right-hand coil rotates back into a more stable position. Remember, at any instant only a limited number of the linear elements which compose the hexagonal pattern are in position, most water molecules are in random positions entering or leaving.
Alternatively, the anandamide may be displaced from the binding site the rimanabant molecule. By filling critical positions in the site with larger stronger-binding chlorine atoms, it holds the right-hand coil in its resting-state conformation with the right-hand chlorine atom coordinated by charge-transfer with the aromatic ring.

THC and CBD interact in a similar fashion to the right-hand coil but their pentagonal tails are too flexible to force the coil to turn. On the other hand, both molecules have hydrocarbon side-chains and rings on the left which fill an open space between the front and back coils.

Since neither compound fills the binding space like the anandamide molecule, water can enter more freely into the polar binding sites to release the molecules. Both THC and CBD produce the same physiological responses as anandamide but much shorter duration.

Once-again, water, in transient linear elements in preferred orientations - even though present for only about $10^{-10}$ seconds appears to define preferred quantized spatial positions for water molecules that provides for both order and spontaneity of function. For more information on the Transient Linear Hydration Hypothesis, check out www.cubichydration.com.

References
References