3. Chemistry of Ca channel blockers

3.1 Pyrimidines and Dihydropyrimidines

The pyrimidines uracil, thymine, and cytosine occur widely in nature since they are components of nucleic acids, in the form of N-substituted sugar derivatives (nucleotides). The physical and chemical properties of these and related pyrimidines have been extensively studied. Several analogues have been used as compounds that interfere with the synthesis and functioning of nucleic acids; there are several other important groups of pyrimidines with medicinal uses. The barbiturates were for a long period the major group of sedative hypnotic drugs. Some diaminopyrimidines, including pyrimethamine and trimethoprim are antimalarial agents; trimethoprim is also an effective antibacterial agent when used in combination with sulphonamides. Minoxidil is a vasodilator which has been used in the treatment of hypertension.

3.2 Synthesis of pyrimidines

The most general and widely used route to pyrimidines involves the combination of reagent containing the N-C-N skeleton with one containing a C-C-C unit. These syntheses are typical examples of typical examples of ‘double nucleophile plus double electrophile’ method of constructing heterocycles. Both the nitrogen atoms of N-C-N reagents act as nucleophile and both the terminal carbon atom in C-C-C are electrophilic. Urea, thiourea, and guanidine are commonly used as N-C-N reagents. α,β unsaturated ketones or acid derivatives can also be used to provide the C-C-C fragment of the ring system. The choice depends upon the substituents required in the final product.

\[ \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{Me} \quad \text{N} \quad \text{Me} \]

\[ \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{Me} \quad \text{N} \quad \text{Me} \]

\[ \text{Conc. HCl} \quad \text{Reflux} \]

(a)

\[ \text{C}_2\text{H}_5\text{O} \cdot \text{Na}^+ \quad \text{Reflux} \]

(b)
In practice the synthesis of pyrimidine can readily be achieved by reaction of component shown in reaction (a) is carried out in concentrated HCl under reflux and (b) and (c) sodium ethoxide in ethanol under reflux. These synthetic approaches are versatile and well established. A method that is closely related to synthesis of pyrimidines is the Biginelli dihydropyrimidine synthesis shown below. In its classical form three component condensation of β-keto ester, an aromatic aldehyde and urea. The first step of the reaction probably an aldol reaction between the aldehyde and the ketoester, the method then becomes variant of the standard approach to pyrimidines.

The reaction of 1-3 dicarbonyl compounds or an equivalent reagent with formamide provides a route to several pyrimidines that are unsubstituted at the 2 position. The reactions of pyrimidines involved acetylation, bromination, electrophilic substitution and nucleophilic displacement.

**3.3 Biginelli reaction**

The combination of an aldehyde, β-keto ester, and urea under acid catalysis to give a dihydropyrimidine was first reported by Pietro Biginelli in 1893.\(^4\) Referred to as the Biginelli reaction, this one-pot condensation reaction generates compounds with pharmacological activity, including calcium channel modulation, mitotic kinesin Eg5 inhibition, and antiviral and antibacterial activity. Although the original reaction conditions suffered from poor yields and a limited substrate scope, the recent discovery of dihydropyrimidine biological activity has led to a renewed exploration of
the reaction conditions, revealing a variety of compatible solvents, acid catalysts, and an expanded substrate scope. Most recently, the development of asymmetric methods has allowed the generation of enantio enriched dihydropyrimidines. Further, the reaction manifold has been extended from its solution-phase origins to include microwave assisted, solid-phase, and fluorous-phase reactions. The gradual development of the Biginelli reaction over the past 115 years, coupled with the biological study of the resulting compounds, has provided an entryway into the relatively unexplored dihydropyrimidine compounds.

3.3.1 Mechanistic Studies

The first mechanistic studies of the Biginelli reaction were conducted by Folkers and Johnson forty years after Biginelli’s initial report. Four possible combinations of the three reaction components were examined for the generation of dihydropyrimidine 6 (Figure 1): (A) the termolecular reaction between benzaldehyde, ethyl acetoacetate, and urea, (B) the combination of ethyl acetoacetate and benzal-bisurea, (C) the reaction of benzaldehyde and ethyl β-carbamidocrotonate, and (D) the reaction of ethyl α-benzalacetoacetate and urea. Folkers and Johnson based their mechanistic conclusions on reaction yields and visual observation. They proposed that the simultaneous combination of the three reaction components in A was improbable. D was ruled out on the basis of the low reaction yields (2%). In contrast, B and C gave high yields of 6 (80%). The authors note that B may undergo fragmentation of the benzal-bisurea, regenerating the three reaction components, which may then form the product by another pathway. Further, the authors posit that the β-carbamidocrotonate in C hydrolyzes to the original three reaction components. Therefore, they conclude that 6 are likely formed from cyclization of 5, which can be generated from either B or C. A second mechanistic proposal was suggested by Sweet and Fissekis forty years after Folkers’ pioneering work. This proposal involves an aldol condensation between benzaldehyde and ethyl acetoacetate to form a stabilized carbenium ion 7. Trapping with N-methylurea gives 8, which can cyclize to form 9 (Figure 4). The observation that independently prepared 10 reacts with N-methylurea under acidic conditions to generate 9 provides evidence in support of this mechanism. Evidence against this mechanism is provided by Kappe who found that reaction of 10 with N-methylthiourea produces thiazine 11 and not dihydropyrimine 12, which is the observed product under standard Biginelli conditions (catalytic amounts of HCl, refluxing ethanol).
Kappe further explored the mechanism of the Biginelli reaction using NMR spectroscopy and trapping experiments. He proposes the formation of N-acyliminium ion 13 from benzaldehyde and urea via an unobservable (1H NMR) hemiaminal 14 (Figure 1). Interception of 13 with the enol tautomer of ethyl acetoacetate gives 15, the precursor to dihydropyrimidine 6. Kappe suggests that the first step, formation of 14, is rate limiting, thus preventing the observation of intermediates 13 and 15 by NMR. However, evidence to support this mechanism was provided by two trapped species, 16 and 17 (Chart 1).

### 3.4 Reaction Advancements

Improved Reaction Conditions with a deeper mechanistic understanding of the Biginelli reaction, several advancements were made to address the poor and variable yields (20-70%) and limited substrate scope often associated with this reaction. Conditions that support the formation and reaction of N-acyliminium ion 13 provide one route to improving the Biginelli reaction. Hu and coworkers report consistently high yields when the reaction proceeds in the presence of BF₃·OEt₂ and CuCl in a mixture of acetic acid and THF. A Lewis acid activated acyl imine 18 is proposed to
be an intermediate in this reaction. Likewise, Kappe and Falsone report that polyphosphate ester in THF provides increased reaction yields. An activated enol phosphate 19 is the proposed intermediate for this reaction. Overall, these two methods are comparable; both providing improved yields over the original Biginelli conditions. Atwal and coworkers introduced a modification to the original Biginelli reaction that affords high product yields and the preparation of previously inaccessible dihydropyrimidines. The Atwal modification (Scheme 2) involves reaction of preformed unsaturated keto esters (i.e. 10) with a protected urea 20 to give a 2-substituted dihydropyrimidine 21. Deprotection with trifluoroacetic acid (TFA) affords the dihydropyrimidine product 6, while deprotection with ammonia or a primary amine gives the previously inaccessible amino pyrimidines 22. While this modification expands the substrate scope of the Biginelli reaction, the presynthesis of 10 and added deprotection step depart from the convenience of the one-pot reaction conditions.

The development of the Biginelli reaction has advanced considerably since its discovery 115 years ago. Mechanistic insights have provided rational modifications to the experiment protocols, allowing dihydropyrimidines to be synthesized in high yield. The interesting and diverse biological activity of dihydropyrimidines has been explored through the generation of libraries of compounds via microwave, solid-phase, and fluorous-phase technologies. Most recently, asymmetric methods have been developed to give enantio enriched dihydropyrimidines. The frontier of the Biginelli reaction will continue to be developed as new asymmetric methods are reported and as the biological importance of this class of compounds is explored in greater detail.

3.5 Mechanism of Biginelli reaction

The Biginelli reaction is a multiple-component chemical reaction that creates 3,4-dihydropyrimidin-2(1H)-ones 4 from ethyl acetoacetate 1, an aryl aldehyde (such
as benzaldehyde 2), and urea 3.\textsuperscript{8} It is named for the Italian chemist Pietro Biginelli.\textsuperscript{9,10}

This reaction was developed by Pietro Biginelli in 1891. The reaction can be catalyzed by Brønsted acids and/or by Lewis acids such as copper (II) trifluoroacetate hydrate and boron trifluoride. Several solid-phase protocols utilizing different linker combinations have been published.\textsuperscript{11-12} Dihydropyrimidinones, the products of the Biginelli reaction, are widely used in the pharmaceutical industry as calcium channel blockers, antihypertensive agents, and alpha-1-a-antagonists. The reaction mechanism of the Biginelli reaction is a series of bimolecular reactions leading to the desired dihydropyrimidinones.\textsuperscript{13} According to a mechanism proposed by Sweet in 1973 the aldol condensation of ethylacetacetoacetate 1 and the aryl aldehyde is the rate-limiting step leading to the carbenium ion 2. The nucleophilic addition of urea gives the intermediate 4, which quickly dehydrates to give the desired product 5.\textsuperscript{14}

This mechanism is superseded by one by Kappe in 1997:
This scheme begins with rate determining nucleophilic addition by the urea to the aldehyde.\textsuperscript{15} The ensuing condensation step is catalyzed by the addition of acid, resulting in the imine nitrogen. The β-ketoester then adds to the imine bond and consequently the ring is closed by the nucleophilic attack by the amine onto the carbonyl group. This final step ensures a second condensation and results in the Biginelli compound.

3.6 Introduction to Ion Channels

3.6.1 Cellular Membranes

Living cells are surrounded by membranes, which allow the cells to form units separated from the environment. These biological membranes are therefore important parts of the human cell and essential components of a living cell organization. They act as barriers and separate the organelles in interior of all cells (likes cytoplasm) from the surrounding outside of the cell. The basic unit of most biological membrane consists of phospholipid bilayers. An important function of the cellular membrane or plasma membrane is not only the separation of the cell but also to regulate the communication of the cell and the transport of various molecules as a selectively permeable membrane. Selected molecules or ions only are allowed to move through the membrane in a controlled way and also in both directions, in and out of a cell. Cellular membranes also play a crucial role in a variety of cellular processes, for example ion conductivity and cell signaling. The movement of molecules passing through the membrane can be either a passive or an active process. There are many mechanisms that allow the exchange of molecules in and out the cell as mentioned below.

The passive process: This movement occurs without input of cellular energy.
1. Diffusion. Some small molecules or ions are able to cross the cell membrane by diffusion.

2. Osmosis. The cell membrane behaves as a semi permeable barrier. If on both sides of the membrane different concentrations of solutes exist, then, the solvent molecules may move through the membrane into a region of higher solute concentration which leads to an equalization of the solute concentration at both sides.

**The active process:** This mechanism requires energy for transportation.

**Mediated Transport:** The movement of a solute such as sugar across a membrane occurs with the help of special proteins called transport proteins.

**Endocytosis:** It is a process where the cell absorbs the molecules from outside by engulfing and fusing.

**Exocytosis:** A process which releases the cellular substances contained in vesicles. The substances can pass into the cell by formation of a vesicle membrane with plasma membrane. After that, the substances release to the exterior of the cell.

Very important for the communication of the cell via the cellular membrane are membrane proteins.

**3.6.2 Membrane proteins**

Approximately 30% of the entire proteins are membrane proteins. Membrane proteins can be categorized into peripheral and integral membranes. Integral membrane proteins are permanently attached to the membrane (e.g. G-protein-coupled receptors and ion channel). Peripheral membrane proteins are temporary attached either to the lipid bilayer or the integral membrane by a combination of electrostatic or van der Waals interactions with the lipid head group (e.g. Phospholipase C and Lipoxygenases). Membrane proteins are important in all cells. They play roles in all cellular processes and mediate a wide variety of important processes such as transport of nutrients, export of toxins and cell-cell interactions. Now a day, the majority of the drugs are targeted to membrane proteins.\(^{16-17}\) Due to the limited technology to express membrane proteins; the structure and function of membrane proteins are still not well understood. Right now there are 969 proteins in the protein database. High quality crystal structures of membrane protein are required to extend the knowledge in this area. At the moment, there are new techniques applied to successfully crystallize a variety of membrane proteins the bicelle\(^{18}\) and HILIDE\(^{19}\) methods are introduced to obtain structure of membrane proteins generally, there is continuous development to improve these methods.
3.7 Ion channels

Ion channels are pore-forming proteins and their function is to facilitate the diffusion of ions across the cellular membrane. Channels are usually ion selective. Ions are able to pass through in a highly selective way the channel and they prevent the passage of others. Under physiology conditions, calcium channels allow only calcium ions to go through. The 3 pathways of selected ion flow through the channel by electrochemical gradients accounts for an ion's concentration gradient across a cell membrane. Channels are major components of the nervous system which play key roles in variety of biological processes such as cardiac, skeletal and smooth muscle contraction. Therefore, channels have been intensively studied and they are important molecular targets for the treatment of many human diseases. There are now many techniques which have been applied to study the ion channels such as voltage clamp electrophysiology, immune histochemistry, reverse transcriptase-polymerase chain reaction analysis (RT-PCR) and also large variety of computational methods.

3.7.1 Classification of the ion channels

There are several possible ways to classify ion channels into groups. For instance,

1) By gating: The conformational change between closed, open and inactivated of the channels is called gating.
   (a) Voltage-gated ion channels are controlled by the voltage gradient across the membrane. (e.g. voltage-gated calcium channels, voltage-gated potassium channels and voltage-gated sodium channels etc.).
   (b) Ligand-gated ion channels are regulated by conformation changes induced by ligands. (e.g. nicotinic acetylcholine receptor (nAchR) and neurotransmitter gamma-aminobutyric acid receptor (GABA) etc.)

2) By ion channels can be categorized by the species of ions passing through those gates. (e.g. chloride channel, sodium channel, potassium channel etc.)

3.8 Voltage-gated calcium channels (Cav)

Cav s play a major role to control calcium influx and respond to change in intracellular calcium concentrations that occur in such cellular system as cardiac muscle, neurotransmitter release and muscle contraction. Crystal structures of the transmembrane domains of Cav s are not available. Therefore, the homology modeling method was used to build Cav structure models based on voltage-gated K+ and Na+ channels (Kv and Nav). Even though, Kv and α1 subunits of Cav are composed of four basic domains, each domain formed six transmembrane but there are still
significant differences at the atomic level. The Kv channel contains four identical repeats while Cav is non-identical formed by a single polypeptide. Molecular dynamics simulations and experimental data are used to support that the four domain repeats of Cav are arrangement in a clockwise manner. Another difference between Kv and Cav are the P segments, especially selectivity filter region. For Kv, the main carbonyl oxygen atoms in the selectivity filter orient towards the lumen. In case of Cav, the side-chains of four glutamates are predicted to face the pore.

![Figure 5](image)

Figure 5. (a) Schematic cartoon of voltage-gated calcium channel (b) The α1 subunit is comprised of four homologous domains, each of which contains six transmembrane helices.

The global architecture of Cav is composed of four basic components. α1 subunit is located in the cell membrane and calcium ions can pass through. The auxiliary β, CaM and α2δ subunits bind with high affinity to the loops of domain I and II. Cav α2δ
is a single pass transmembrane subunit which is formed by two disulfide-linked proteins.\textsuperscript{26} Figure 5 shows also the transmembrane Cav which consists of four homologous repeats membranes panning domains (I–IV). Each repeat is formed by six segments (S1-S6) shown in figure 6. The first 4 segments (S1-S4) are the voltage-segment domain and the last 2 segments (S5- S6) form the calcium-selective pore domain. The S4 segment contains positively charged residues and acts as a voltage sensors controlling Cav gating.

\textbf{3.9 Classification of voltage-gated calcium channels}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{channel_classification}
\caption{The percent identity of all voltage-gated calcium channels $\alpha_1$ subunits.}
\end{figure}

The Cavs can be divided into low-voltage-actived (LVA) and high-voltage-active (HVA) calcium channels depending on the depolarization necessary to activate the channel.\textsuperscript{27} HVA calcium channels are activated at the membrane rapidly and at more negative potentials than LVA. HVA channels are all members of the Cav 1 and Cav 2 family. All members of the Cav 1 family (Cav 1.1-1.4) are of the L-type, Cav 2.1 is of the P/Q type, Cav 2.2 is of the N-type and Cav 2.3 is of the R-type. Furthermore, all members of the Cav 3 family (Cav 3.1-3.3) are in the HVA classification which is named T-type.\textsuperscript{28} The amount of the similarity of the amino acid for several of voltage-gated calcium channel is given in Fig 1.2.
Table 2: Sizes of individual α1 subunits of voltage-gated calcium channels

<table>
<thead>
<tr>
<th>Subunit / origin</th>
<th>Molecular weight (kDa)</th>
<th>Number of amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav1.1 / rabbit skeletal muscle</td>
<td>212</td>
<td>1873</td>
</tr>
<tr>
<td>Cav1.2 / rabbit heart</td>
<td>242.8</td>
<td>2171</td>
</tr>
<tr>
<td>Cav1.2 / rabbit lung</td>
<td>242.5</td>
<td>2166</td>
</tr>
<tr>
<td>Cav1.2 / rat aorta</td>
<td>243.6</td>
<td>2169</td>
</tr>
<tr>
<td>Cav1.3 / human pancreas</td>
<td>247.6</td>
<td>2181</td>
</tr>
<tr>
<td>Cav1.3 / human brain</td>
<td>245.2</td>
<td>2161</td>
</tr>
<tr>
<td>Cav1.4 / human retina</td>
<td>219.5</td>
<td>1912</td>
</tr>
<tr>
<td>Cav2.1 / rabbit brain</td>
<td>257.3</td>
<td>2273</td>
</tr>
<tr>
<td></td>
<td>273.2</td>
<td>2424</td>
</tr>
<tr>
<td>Cav2.2 / ray</td>
<td>264.5</td>
<td>2326</td>
</tr>
<tr>
<td>Cav2.2 / human brain</td>
<td>265.2</td>
<td>2339</td>
</tr>
<tr>
<td></td>
<td>251.8</td>
<td>2237</td>
</tr>
<tr>
<td>Cav2.3 / ray</td>
<td>252.2</td>
<td>2222</td>
</tr>
<tr>
<td>Cav2.3 / rat brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cav3.1 / mouse brain</td>
<td>205.2</td>
<td>1833</td>
</tr>
<tr>
<td>Cav3.2 / human heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cav3.3 / rat brain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The length of the protein chain of almost all calcium channels ranges approximately from 1870 to 2420 amino acids. A summary over all calcium channels together with the number of amino acid of the α1 subunit and the related molecular weight is given in Table 2. The similarity of LVA and HVA channels is less than 30 % of sequence homology. Two subfamilies of HVA channels L-type (Cav 1) and Neuronal types (Cav 2) have about 50% sequence homology. Individual members of each subfamilies share more than 80% of sequence homology.
3.10 Mechanism of voltage-gated calcium channels

The voltage-gated calcium channels are opened and closed by voltage of calcium ion changes as shown in figure 7. At resting closed state, the voltage-gated calcium channel is closed and ions cannot pass. Then, the membrane potential will be increased, which results that the channel can be activated to open state upon depolarization and allows ions to pass. During the depolarization, the open probability of the channel is remained briefly and then voltage dependently reduced by channel inactivation leading to inactivated closed state. Instead, inactivated closed state cannot immediately be reactivated. It requires a brief period of time to depolarization of the membrane and recovery from inactivation. On the other hand, the membrane potential at the open state also can deactivate the channel leading to the resting closed state.30

3.11 Voltage-gated calcium channels molecular pharmacology

The activity of voltage-gated calcium channels is essential to couple electrical signals on the cell surface to physiological events in cells. The pharmacology of the each family of calcium channels is individual e.g. the molecular targets of Cav1.2 channels are used widely in the therapy of cardiovascular diseases (Table 2). For more detail, the summarization of molecular, physiological, and pharmacological properties of all calcium channel family are reviewed by Catterall et al (2005).31

L-type calcium channels
The four members of L-type calcium channels have specialized functions in skeletal muscle and cardiac muscle, and they are, therefore, therapeutic targets for cardiovascular disease treatment. The conformation changes of gating states (resting, open and inactivated) L-type calcium channels are accompanied by changes in drug binding affinity upon the change in membrane voltage (activation/depolarization). L-type calcium channels are sensitive to three major classes of drugs\textsuperscript{32-34} dihydropyridines (DHPs),\textsuperscript{35-37} phenylalkylamines (PAAs),\textsuperscript{38} and benzothiazepines (BZPs).\textsuperscript{39} From experimental approaches, it is known that these three classes of drugs bind to separate but overlapping regions in IIIS5, IIIS6, and IVS6 segments of the α1 subunits. The binding residues of these drugs are shown in figure 8.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{The amino acid residue interaction with dihydropyridines (DHPs), phenylalkylamines (PAAs), and benzothiazepines (BZPs). As can be seen from the Fig 7 different drugs interact at different binding sites, leading there to different interaction mechanisms which have been investigated in more detail. However, there are still questions open which conformations (open or closed) are interacting with the drug. The intention of the present work is to focus on voltage gated L-type calcium channels Cav1.2 and to investigate the mechanism and the structure of closed and open pore region.}
\end{figure}

3.12 Calcium channel blocker - Phenylalkylamines (PAAs)
Phenylalkylamines (PAAs) such as verapamil, gallopamil or devapamil, belong to the class of calcium channel blockers of L-type. The common structure of PAAs is the presence of two methoxylated aromatic rings. In this work, the structure-activity relationships of PAAs focus on varapamil and its derivative. Verapamil is a drug of this class within currently clinical use in particular for the therapy of coronary heart disease. The qualitative and quantitative structure-activity relationship of various groups of verapamil was investigated based on an analysis of the frequency dependent negative inotropic action exerted in cat papillary muscles. The inotropic effect of verapamil which relate to muscular contractions is mainly caused by the S conformation. The verapamil shows effects on the slow Ca2+ current and movement which affects cardiac excitation-contraction coupling. The quantitative results were obtained by comparing ED50 values. The PAA verapamil and its derivatives can be divided into of 3 groups. Group A concerns derivatives differently substituted at the benzene ring near the asymmetric carbon atom. In Group B the isopropyl group is exchanged. The last group C contains derivatives with different substituent at the amino group nitrogen atom.

![Chemical structure of Verapamil and its derivatives](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Molecular name</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-(OCH₃)₂</td>
<td>Verapamil</td>
</tr>
<tr>
<td>3,4,5-(OCH₃)₃</td>
<td>D600</td>
</tr>
<tr>
<td>4-OCH₃</td>
<td>D557</td>
</tr>
<tr>
<td>3,4-Cl</td>
<td>D595</td>
</tr>
<tr>
<td>3-CF₃</td>
<td>T13</td>
</tr>
<tr>
<td>3-OCH₃-4-OH</td>
<td>PR23</td>
</tr>
<tr>
<td>3,4-CH₃</td>
<td>D559</td>
</tr>
</tbody>
</table>
In summary, for substituent of group A and B it was found that the character of the substituent strongly affects the potency of various compounds, but it is not affected by the inotropic effect. The tertiary amino nitrogen of group C and two benzene rings of group A are important for frequency dependent negative inotropic. Moreover, the molecular importance of the N-methyl group C is based on steric effects.

Lipid interaction of the channel: The interaction of membrane lipids with integral membrane proteins is important and influences the membrane protein structure and function. However, the mechanisms between lipid composition and membranes are not well understood yet. The next part of the introduction gives an overview of various lipid compositions and how they modulate the membrane protein.

The cell membrane or plasma membrane is a selectively permeable lipid bilayer. It separates the cytoplasm (intracellular fluid) from the extracellular moiety. It contains a variety of biological molecules such as phospholipids, glycolipids and cholesterol.
Phospholipids are the most abundant compound in the cell membrane. The Mammalian cell membrane basically consists of thin layers of phospholipids which are arranged in such a way that the polar head groups point toward the intra- and extra-cellular areas. The phospholipids have a hydrophilic head which are exposed to water and two hydrophobic tails at the center of bilayer. Moreover, cholesterol is located inside the membrane (figure 9). The cell membrane contains approximately 30-50 mol % of cholesterol.

The effects of lipid composition on the membrane protein and subsequent activity are very complex. Evidently, the molecular composition of lipids strongly affects the protein structure and function. Up to now, the molecular mechanisms of protein-lipid coupling are still poorly understood. The changes of lipid characteristics, for example head group type, the length of the tail, additional presence of cholesterol are factors which modulate the protein functions. In case of ion channels, the molecular mechanisms of protein-lipid interactions can influence directly and indirectly the protein function which may occur in different ways.

1. Alteration of the protein structure and function: The membrane proteins folding are dictated by their primary structure and their environment. The residues in the interior membrane protein are less hydrophobic than the residues in contact with the tail of lipid bilayer. The hydrophobic and hydrophilic properties of the bilayer play a crucial role for protein folding and activity. However, not all domains of the channels are in contact with the bilayer, particularly the extracellular domains, mostly non-helical, which are located outside the membrane.
2. Alteration of membrane fluidity: The fluidity is a measurement of the packing efficiency of the lipid. The movement of a tightly packed membrane is more difficult than the movement in more fluid environment. Thus, membrane fluidity is affecting the dynamics of the membrane protein as observed in the nAchR studies.\textsuperscript{45}

3. Alteration of bilayer thickness: Normally, the hydrophobic region transmembrane (TM) domain interacts with the acyl chain of bilayer while polar, aromatic and charged residues are in contact with the interface region on each side of bilayer. The short hydrophobic TM is usually expelled from the membrane, due to the fact that it is unfavorable for hydrophobic region of the TM and bilayer hydrophobic thickness to be mismatched. Furthermore, for the long hydrophobic TM in contact with the acyl chain of bilayer will tend to tilt to match with the membrane thickness. Then, this rearrangement may affect the protein function.\textsuperscript{46-48} Lipid rafts are specialized regions that are enriched of cholesterol in membrane microdomains (figure 10). These membrane regions influence the membrane fluidity, they are more tightly packed and form higher ordered ensembles than the surrounding bilayer.

![Lipid Raft Structure](image)

Figure 10. Lipid Raft Structure

3.13 Cholesterol effect

Cholesterol plays a crucial role in regulating the properties of membranes. As already mentioned it regulates the fluidity of the membrane and modulates the function of membrane protein.\textsuperscript{49} In case of ion channels, it has been described that numerous ion channels are affected in different way by cholesterol. For example, the suppression of channel activity is affected by an increase in membrane cholesterol. This effect happens in several types of K+ channel, voltage-gated Ca$^2+$ and Na$^+$ channels etc. In contrast, there are several types of ion channels, such as transient receptor potential (Trp) channel which are inhibited by cholesterol depletion. Moreover, in some case changing in membrane cholesterol affects the biophysical properties such as the voltage dependence of active or inactive channels. The mechanisms for cholesterol
modulates of ion channel are not well understood. The regulation of ion channels by cholesterol have been proposed that cholesterol might interact directly or via indirectly with ion channel. Three different mechanisms of channel regulation have been suggested

1. Specific interactions of cholesterol with the channel protein.
2. Changes of the physical properties of the membrane bilayer (i.e. fluidity).
3. Importance of cholesterol for maintaining the scaffolds for protein-protein interactions (“lipid-rafts”).
References:


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