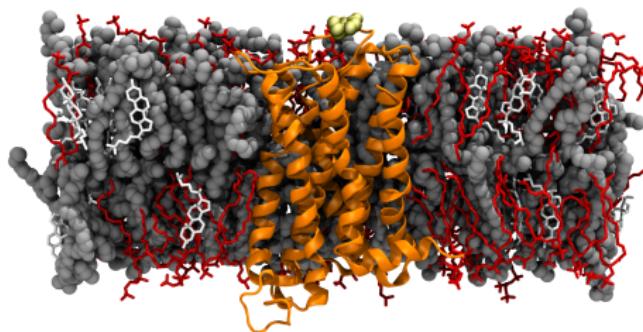


LAB 3

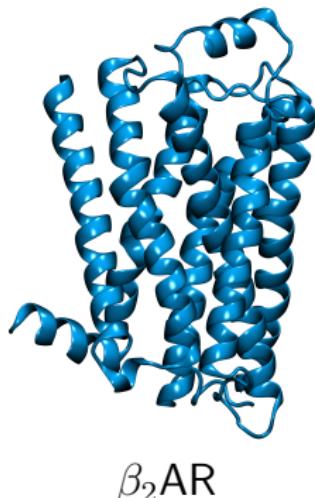
Intramembrane Protein—Lipid Interactions and Their Effect on Protein Conformation

Waldemar Kulig Giray Enkavi Matti Javanainen



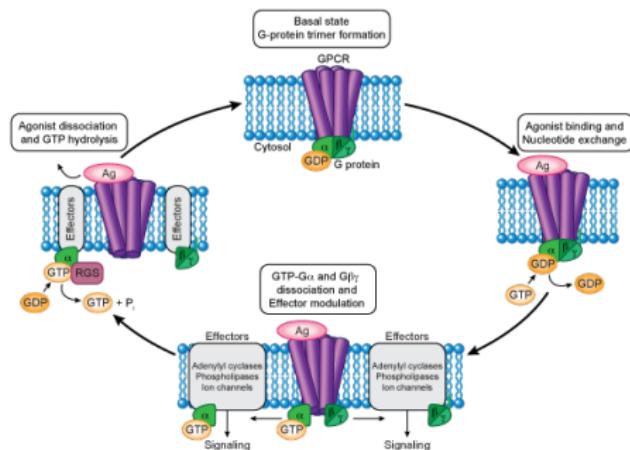
Scientific Background

- G-protein coupled receptors (GPCRs) constitute one of the largest families of plasma membrane receptors.
- They are major contributors to the cellular signal transduction and respond to a wide variety of extracellular stimuli such as light, taste, odor, peptides, neurotransmitters, and hormones.
- The human β_2 -adrenergic receptor (β_2 AR) is one of the best-characterized GPCRs. It is expressed in pulmonary and cardiac myocyte tissues and is a therapeutic target for asthma and heart failure.



Scientific Background

It is now evident that β_2 AR activation (and therefore the function) is modulated by lipids. However, the atomic-scale mechanism lipids use to regulate the receptor is unknown.



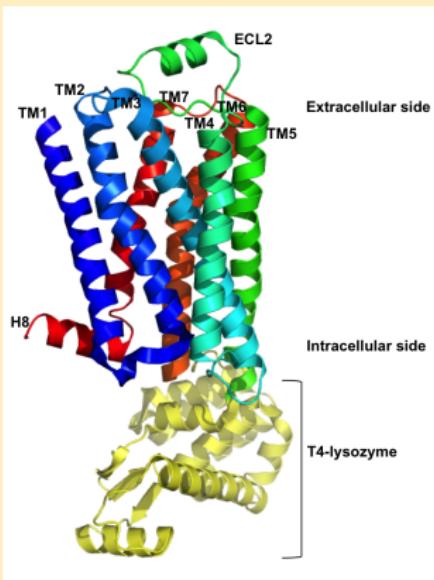
<https://mutagenetix.utsouthwestern.edu>

Let's Start!

Please open your Instruction Book. We will start from the section 3.2 on page 37.

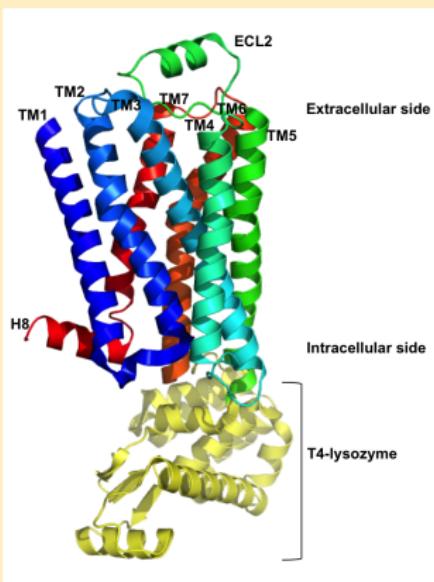
Visualization of Cholesterol Binding Sites

Question 25. How many α -helices can you see? Does the crystal structure contain all atoms? Are there any ligands bound to the protein?



Visualization of Cholesterol Binding Sites

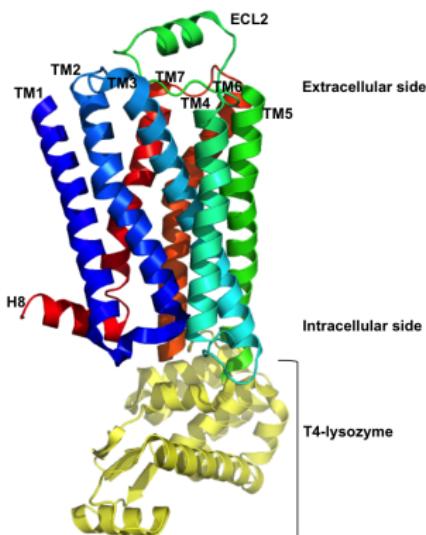
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β_2 AR contains seven membrane-spanning helices connected to each other by intracellular and extracellular loops. The crystal structure of a receptor does not contain hydrogen atoms. The receptor is co-crystallized with the ligand – timolol. The structure also contains three detergent molecules, two cholesterol molecules and number of crystallographic water molecules.

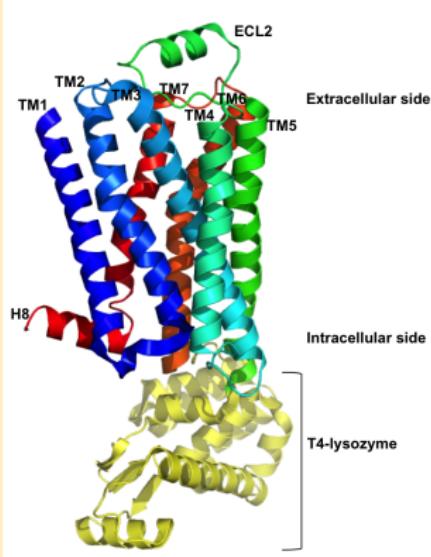
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Question 25. What is the role of lysozyme during the crystallization? Where is the cholesterol binding pocket?



Visualization of Cholesterol Binding Sites

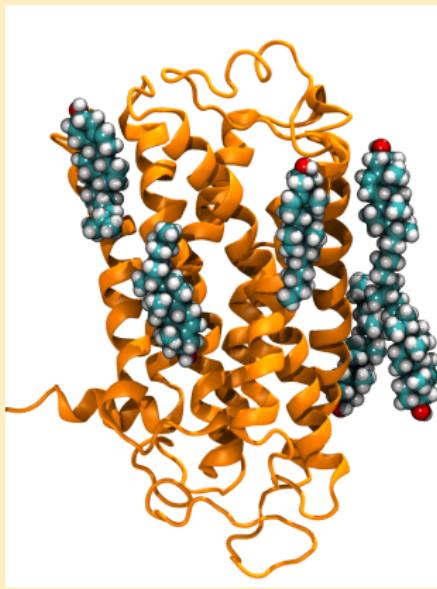
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The structure of the receptor is missing intracellular loop 3, which was substituted by T4-lysozyme between TM5 and TM6. The lysosome immobilized the protein allowing its crystallization. Cholesterol binding pocket is localized in the cleft formed by TM1-TM4.

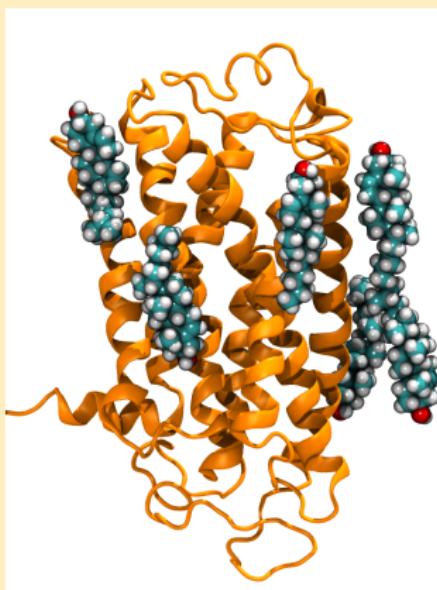
Visualization of MD Trajectories

Question 26. Do you see any lipids (cholesterol, cholesteryl hemisuccinate, DOPC) interacting with the protein surface? Where are they localized?



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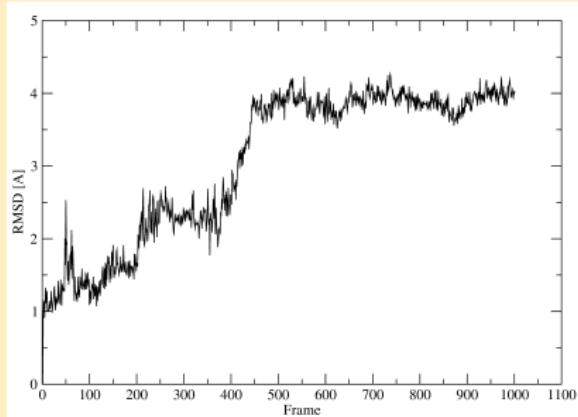


The answer here depends on the system you were working with.

System4: There are six cholesterol molecules within 5 Å from protein surface but only two (in the cleft formed by TM1-TM4) are there during the whole trajectory.

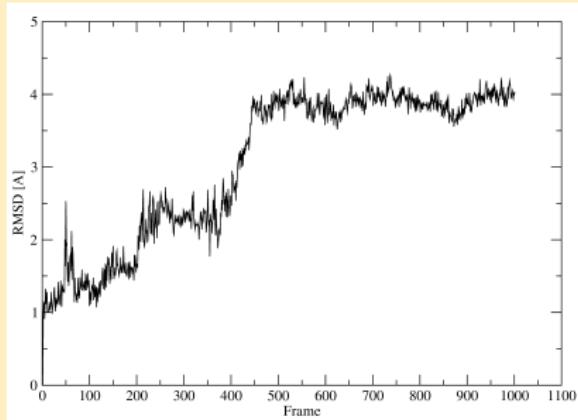
Visualization of MD Trajectories

Question 27. Is the structure of the protein stable? Which part of the protein does move the most?



Visualization of MD Trajectories

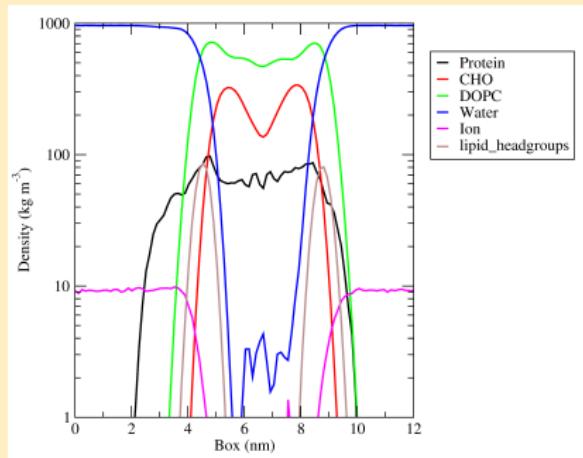
Question 27. Is the structure of the protein stable? Which part of the protein does move the most?



System4: The structure of the protein changes slightly at the beginning of the simulation but later it is stable. The most mobile part of the receptor are intacellular and extracellular loops.

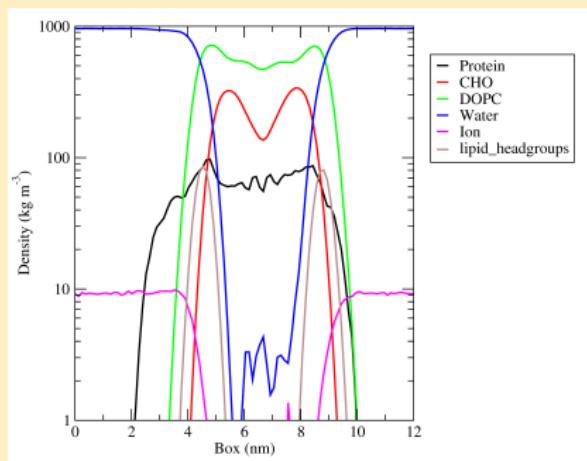
Density Profiles

Question 28. Where is the protein located as compared to the lipid bilayer? Where are the ions? Is water inside the lipid bilayer? Why?



Density Profiles

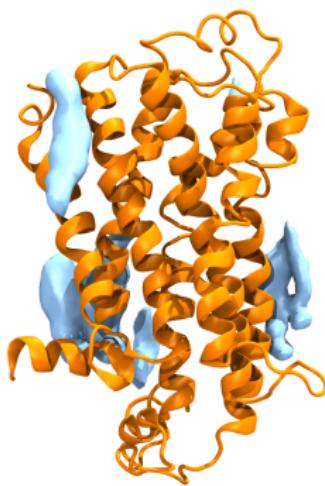
Question 28. Where is the protein located as compared to the lipid bilayer? Where are the ions? Is water inside the lipid bilayer? Why?



The answer here depends on the system you were working with.
System4: It is evident that the protein is localized inside the lipid bilayer. Ions are localized in the aqueous phase. Interestingly, water and at least one ion penetrate receptor interior. This can be explained by an opening from the extracellular side in the receptor structure which is a ligand binding site.

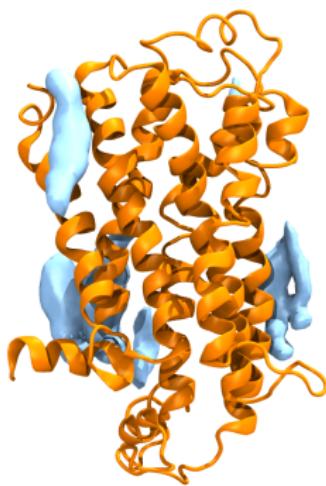
Volumetric Maps Generation Using VMD

Question 29. Do you see any lipid-binding sites on the surface of the protein? Where are they? Are they similar to the ones you saw in the crystal structure?



Volumetric Maps Generation Using VMD

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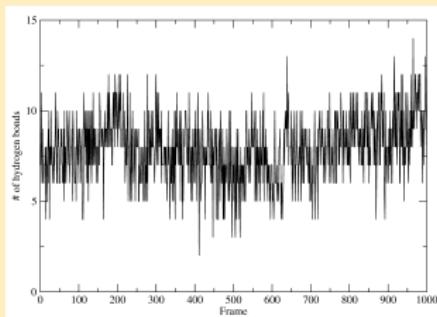


The answer here depends on the system you were working with.

System5: We see several lipid-binding sites next to the protein surface. None of them exists in the crystal structure though one site is really similar to the one we saw for cholesterol in the System4 on the previous figure.

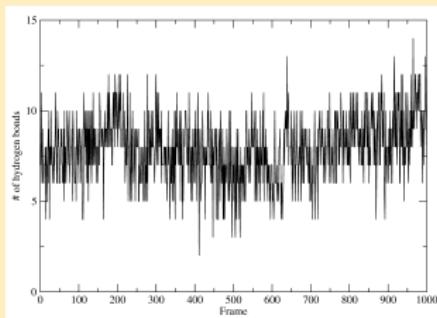
Hydrogen Bonds Analysis

Question 30. How many hydrogen bonds (on average) are formed between the protein and different lipid types? Is this number stable over time? What does it mean?



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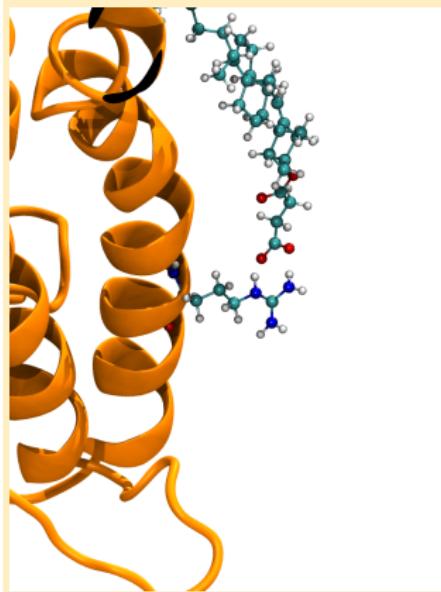


The answer here depends on the system you were working with.

System5: We see that on average there is about 7 hydrogen bonds created between the protein and CHS molecules. This number is relatively stable during the whole simulation showing that a) system is stable and well equilibrated; b) interactions between CHS and protein are strong and stable.

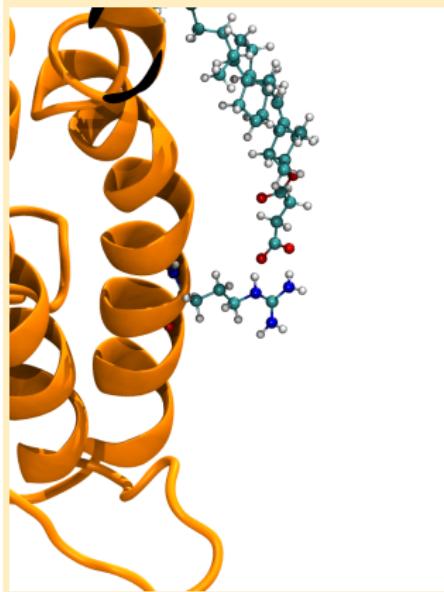
Hydrogen Bonds Analysis

Question 31. Which hydrogen bonds are the most stable? Visualize them. What chemical groups are involved in hydrogen bond formation?



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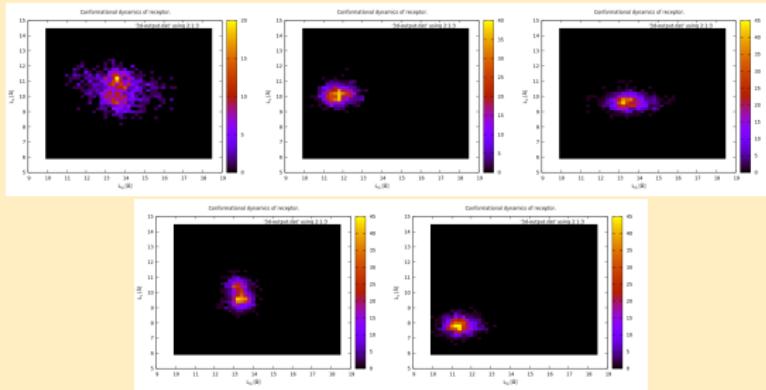


The answer here depends on the system you were working with.

System5: We see that hydrogen bond between -NH₂ group of arginine 221 and oxygen atom (O3) from carboxyl group of cholesteryl hemisuccinate 675 is the most stable hydrogen bond in this simulation. Occupancy time equals 43.51% meaning that this bond is present in the system for almost 50% of time.

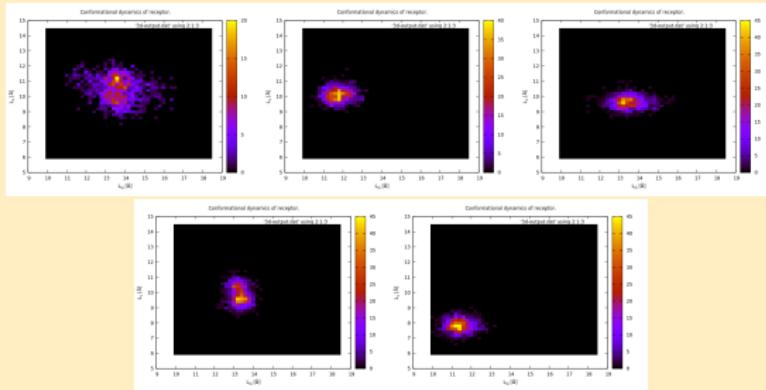
Effect of Cholesterol on Receptor Conformation

Question 32. Are these plots the same? What is the main difference? What is the conformation of the protein? Do the results depend on the cholesterol concentration?



Effect of Cholesterol on Receptor Conformation

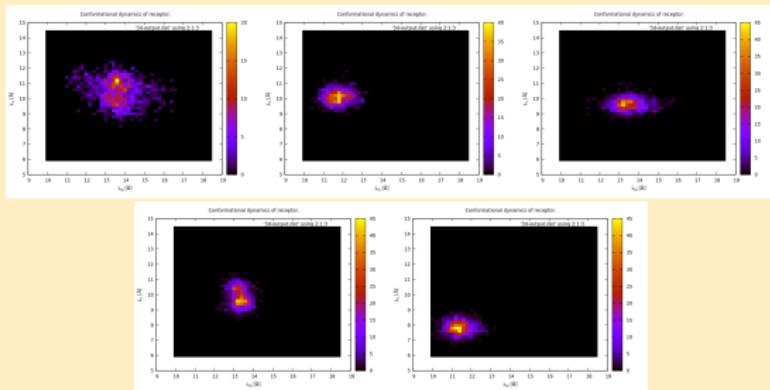
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No, plots are not the same. In a cholesterol-free membrane the receptor samples multiple conformational states. The presence of cholesterol in high densities impedes the dynamic nature of the receptor and the overall structural flexibility of the receptor.

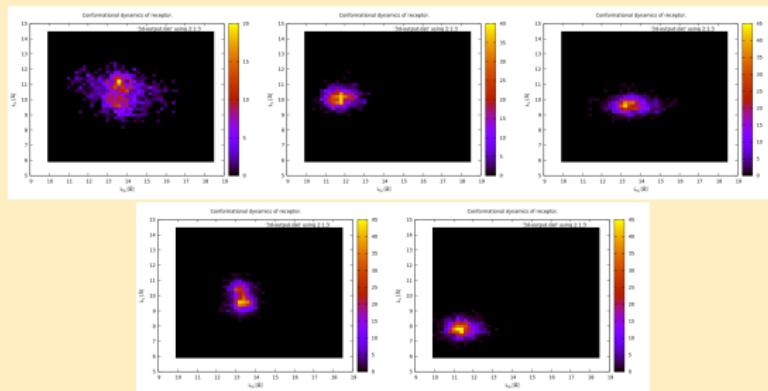
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Question 32. Does cholesteryl hemisuccinate have the same effect as cholesterol?



Effect of Cholesterol on Receptor Conformation

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Cholesteryl hemisuccinate seems to have similar effect as cholesterol. Cholesterol or cholesterol-like molecules bound at the inter-helical clefts can confine the movement of the respective helices to a substantial degree, thus dampening the overall conformational dynamics of the receptor.

Conclusions

We performed extensive atomistic molecular dynamics simulations to clarify the mechanism responsible for the modulatory role of cholesterol and cholesterol derivatives on β_2 -adrenergic receptor. In essence, we show that as cholesterol concentration reaches ~ 10 mol%, the conformational distribution of β_2 AR is drastically altered. The mechanism of action is based on the binding of cholesterol at specific high-affinity sites of the receptor (Manna *et al.* eLife 2016;5:e18432).

