Appendix 1 - Student manual

HOW DOES AN ION CHANNEL WORK?

THEORY

In the cell specific proteins are used to transport ions across the celmembrane: the ion channels. These channels are protein complexes that are incorporated into the cell membrane thereby forming a channel between the cytoplasm and the outside of the cell.
In your body are a lot of different ion channels located. There are for example channels for sodium, chloride and potassium.

Each ion has its own channel. But what is the reason that a channel is specific for only one kind of ion?

Question 1:

A. How may an ion channel block the passage of large ions?

B. How may an ion channel block the passage of negatively charged ions?

PERFORM

Due to bioinformatics researchers can look in detail to 3D structures of proteins. Therefore the programme yasara is used. By studying the structure of the ion channel, it is possible to learn more about the function. As example you will use the potassium channel, because the structure is well known.
Start Yasara and open the file > Yasara scene: KcsA – surface only.sce
With the mouse button you can see the protein from every side.

- Push the left mouse button and move your mouse: turn the molecule.
- Push the right mouse button and move the mouse forward and backwards: zoom in and out.

The total channel is build with four identical protein molecules and are hold together with Van Der Waals interactions and hydrogen bridges.
View > Hide surface > Molecule
Select 'Sequence': B, C, en D > click OK
Check all three surfaces > click OK
There remains only one subunit A (Molecuul A)
View > Show surface > Molecule
Select 'Sequence' the molecules of which you would like to see the surface > Click OK
Check only 'Van Der Waals surface' > Click OK
The chosen surfaces appear on the screen.
The separate proteins have a specific structure that will form a hole when they are attached: the potassium channel. When the protein complex is attached to the cell membrane the hole connects the cytoplasm with the outside of the cell.

Question 2:
A potassium channel exists of different proteins, which form a protein complex. How is the structure of such a aggregated protein called?

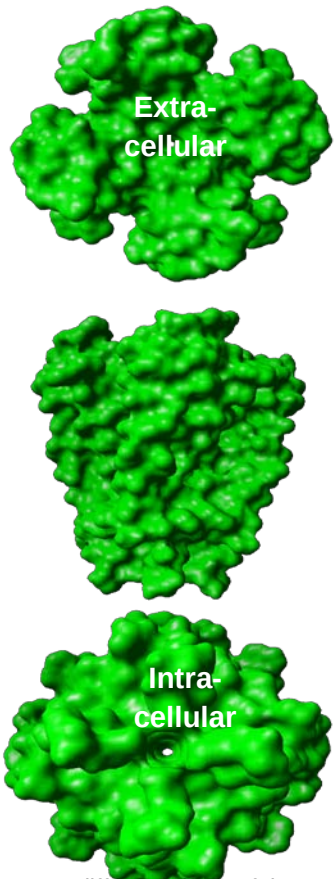

Figure 1: different views of the potassium channel

When you have to complete protein complex on your screen it looks with some imagination like a green paprika. The channel is located from the bottom to the top of the paprika. At the bottom is the intracellular site (inside of the cell) and at the top is the extracellular side (outside of the cell).

The journey that a potassium ion has travel from the inside to the outside of the cell exists of three parts:

1) The entry: The first part of the channel is wide enough for different ions and hydrogen molecules to pass.
2) The cavity: When ions pass the first part of the channel they arrive in a big hole filled with water.
3) The filter: The potassium ion has to pass tight passage to exit the channel. The filter is the selectivity part of the channel.

If you want to take the journey as the potassium channel you can 'fly' through the channel with yasara. Start at the intracellular side, turn the protein in the direction that the hole of the channel is in front of the screen and that you can see the end of the channel (when you open KcsA– surface only.sce the protein is already pointed in the right direction). By pressing the right mouse button and move it in your direction, you zoom in on the channel.

The postassium channel is potassium specific. This means that the ion channel is build in such a way that of all the ions from the cytoplasm, only the potassium passes the channel.

If we look in detail at the structure of the entrance, we see that each protein contains a negatively charges aminoacid[1] (Glutamic acid). The entrance of the channel is surrounded by four negative charges.
Choose file > new
Open file > Yasara scene > KcsA - neg residues.sce.
Now you see the secondary structure of the potassium channel, the negative residues at the entrance are the atoms displayed as balls.

These negative charged have the following effect:

- Positive ions are attracted to the channel and have the possibility to enter the channel.
- Negative ions are pushed away from the channel and don't have the possibility to leave the cell through this ion channel.

Question 3:

A. Which charge has potassium?

B. Will potassium enter/leave the potassium channel or both ways?

C. Explain why only positively charged ions are able to enter the channel.

D. Name three positively charged ions, which are located in the cytoplasm.

1.

2.

3.

The channel is build specificity for entering positively charged ions. But in the cytoplasm are a lot of different positively charged ions present. These ions all can enter the channel, but only the potassium ion

[1] Amino acids are the structural units that make up proteins. Amino acids are biologically important organic compounds made from amine (-NH2) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid. There are 20 different amino acids.

can leave the channel on the other site. Apparently somewhere in the channel another selection mechanism takes place.

Choose file > new

Open file > Yasara scene > KcsA – 1K bound.sce

When the ions arrived in the 'cave', they have to travel through the selectivity filter (part 3 of the journey). This is a tight passage that connects the channel to the outside.

Question 4:

In which way can the tight passage contribute to the selectivity of the potassium channel?

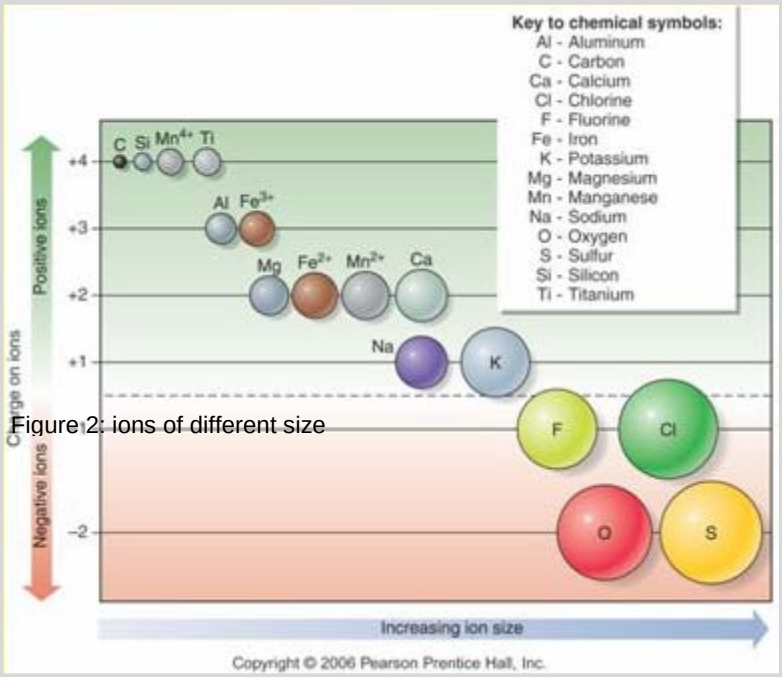

Figure 2: ions of different size

But what about ions that are smaller than potassium, for example sodium? Sodium has the same charge and is smaller and thereby should be capable of travelling through the potassium channel. Yet this does not happen. What is going on here?

From the chemistry we know that when a salt is dissolved in water, it breaks down into positive and negative charged ions. But a single positive or negative charge is energetically very unfavorable. Therefore, the positive and negative charged ions, if they become detached from each other, look for particles elevate their charge. Water molecules are there extremely suitable for, because they are a bit negative on one side and a bit positive on the other side (this is because oxygen is more electronegative then hydrogen and so electrons of the covalent O-H binding are pulled to the oxygen side) . A water molecule may partly compensate the positive charge on the ion by bringing its negative side close to it. The charge of an ion is so strong that one water molecule not enough is. Therefore there are seven water molecules simultaneous around one ion, forming a thin film of water molecules around the ion. For an ion it is energetic unfavorable to lose the water molecules around him, because thereby a free charge is created.

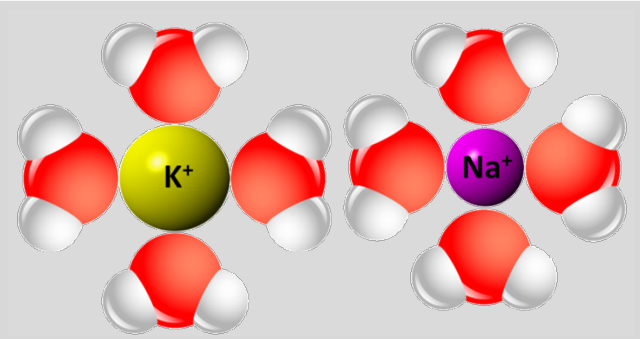

Figure 3: sodium and potassium with a thin film of water molecules

Question 5:

A. When the potassium ion travelles through the channel shall the thin film of water molecules be around him? Is the thin film of water molecules around the potassium ion when it travels through the channel?

The selectivity filter takes the function of the thin film of water molecules over.

B. Zoom in on the selectivity filter in Yasara. Which atoms are in contact with potassium ion?

C. Are these atoms a bit positively or negatively charged?

Choose file > new

Open file > Yasara scene > KcsA – 1Na bound.sce

In this representation sodium is bound in the selectivity filter.

Opdracht 6:

What is the reason that sodium does not travel through the channel?

Thick about the energy needed to lose the thin film of water molecules around ions.

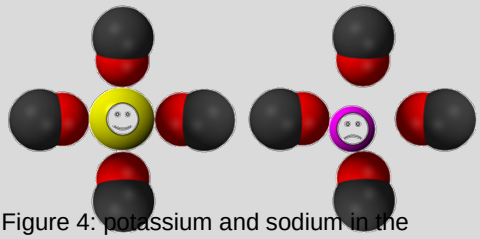

Figure 4: potassium and sodium in the selectivity filter of the potassium channel

For more information about the potassium channel you can look at this movie: http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_the_sodium_potassium_pump_works.html