

# THE COMPLEX FUNCTIONALITY OF KCSA

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## Abstract

The potassium crystallographically-sited activation channel (KcsA) is one of the proteins found in cell membranes of all living organisms. It is an ion channel that, through various physical and chemical means, almost exclusively permits the efflux of potassium ions through the cell membrane at speeds approaching 100 million ions per second. This protein is responsible for propagating electrical signals throughout the body. Though not all functions of this protein are currently understood, investigation of KcsA's known functions and characteristics has led to the synthesis of a physical representation constructed by the Z Corp Z406 Color 3D Printer. This model can serve as an education and research tool because it represents the structure and functions of the important parts of the protein in its activated state, namely the selectivity filter, water cavity, and stabilizing half-helices, in such a way that the learner can physically manipulate potassium ions and water molecules through the model. KcsA's functions vividly illustrate concepts in biology, chemistry, and physics; a model (or models) that can demonstrate these functions would be a beneficial instructional tool.

## 1. Background

Knowledge of the existence of ion channels can be dated back to the early 1950's, when A.L. Hodgkin and A.F. Huxley studied the squid axon, which ran from one end of the squid's body to the other [1]. They determined that something in the cell was able to control the flow of current after a certain threshold of electrical potential was reached. From this determination came the theorization and confirmation of proteins capable of 'channeling' electricity, and the concept of the action potential – the wave of current that is also known as a nerve impulse. Over the years, proteins were identified that could open and close to allow the permeation of sodium, potassium, and calcium ions into and out of cells, and thus regulate the electrical current that resulted in nervous activity. However, the parts of the proteins that actually control current flow were still unknown and the majority of knowledge about ion channel mechanisms remained a mystery for over thirty years.

This situation changed in 1987, when the cloning of a certain gene from the *Drosophila* fly resulted in the discovery of specific functional sections of the potassium ion ( $K^+$ ) channel protein [1]: the voltage sensor, which detects electrical changes in the cell and activates the channel, and the ion-specific selectivity filter. Yet, the mechanisms by which these segments actually carried out their functions required an as-of-yet unattained three-dimensional understanding of the protein.

In 1998, after several years of research and experimentation, Dr. Roderick MacKinnon determined the crystal structure of the  $K^+$  channel protein (KcsA, for potassium [K] crystallographically sited Activation channel), cloned from the *streptomyces lividans* bacterium (Figure 1). Using X-ray crystallography, he determined the spatial arrangement of atoms in the protein [2]. The discovery of a water-containing 'cavity' in the center of the protein as well as several important amino acids, in addition to the now-possible comprehension of the selectivity filter, led to MacKinnon's shared 2003 Nobel Prize in Chemistry.

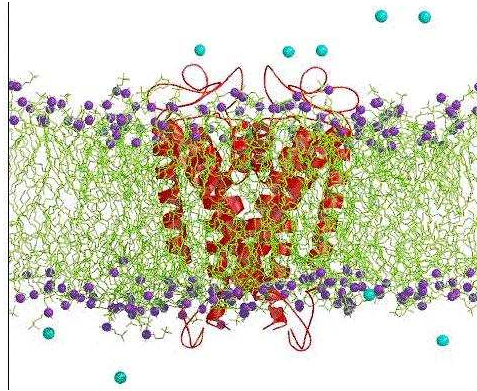


Figure 1 The protein KcsA in the lipid bilayer of the cellular membrane [3]

Though much has been discovered about KcsA, many questions and speculations still remain. The structures of alternate states of KcsA have not yet been isolated, and its exact physical conformation is still unknown. Other similar potassium ion channels have offered educated hypotheses of the closed state qualities, but only empirical evidence can confirm the actual physical conformation and mechanisms. A physical model of the protein may aid in the discovery of the more elusive properties of KcsA's structure.

## 1.1. KcsA

Although there are different types of potassium ion channels with varying shapes, KcsA, whose closed conformational structure is known (Figure 2), is the focus of this research. KcsA exists in the cellular membranes of organisms, with one opening inside the cell (cytoplasm) and the other exposed to the extracellular environment. The protein itself is composed of four identical subunits, which twist around each other to form the actual channel and complete protein. Though they are not physically linked, the subunits are able to associate with

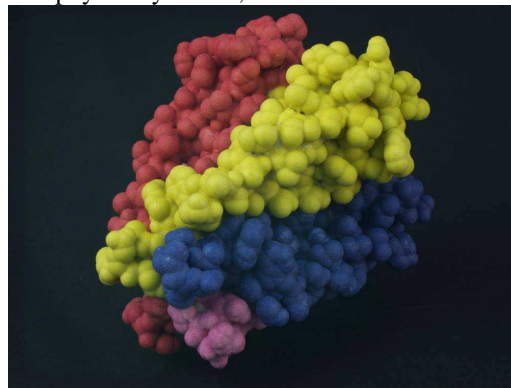


Figure 2 Three-dimensional view of KcsA, with each identical subunit shown in a different color

one another in the presence of potassium ions. Each subunit consists of almost 400 amino acids that form a continuous helical chain. Several sections of this chain comprise important parts of the protein: water cavity, the stabilizing half-helix, and the selectivity filter. The functions of these three parts of KcsA are explained in the following sections.

### 1.1.1. water cavity

Potassium ions, which are positively charged ions, are unstable in the absence of other ions or molecules. In the environment of the cell cytoplasm, each potassium ion is surrounded by a shell of water. This shell assumes a square antiprism configuration (Figure 3), a twisted cube of eight water molecules enclosing the potassium ion in the middle. In this twisted cube, the oxygen atoms of the water molecules point towards the potassium ion. The function of the water cavity (Figure 4) in KcsA is to act as an antechamber for the incoming potassium ions [4]. The cavity's shape and size allows for approximately fifty molecules of water and one ion; X-ray crystallography has shown that

water in the immediate vicinity of the potassium forms a square antiprism, keeping it as stable as possible in the center of the cavity, waiting to be pushed into the potassium-specific selectivity filter.

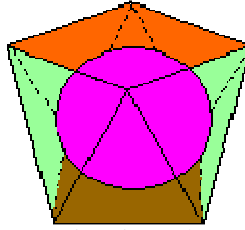


Figure 3 Square antiprism configuration of the potassium ion. The coordinating oxygen atoms of water are located at the vertices of the antiprism [5]

The water cavity serves a secondary purpose as a boundary, barring the ingress of most large molecules and ions [6].

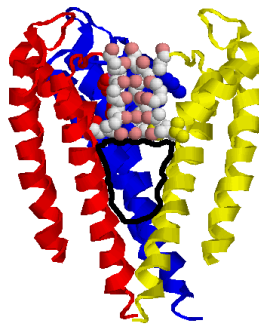


Figure 4 KcsA with the front subunit removed. The water cavity is the area enclosed by the black loop.

### 1.1.2. stabilizing half-helix

The twisted helical shape of each subunit contributes greatly to the ability of the water cavity to function. The stabilizing half-helix, named for its short length relative to the other parts of the chain, points directly at the water cavity (Figures 5). This chain of amino acids facilitates an electrically hydrophobic (water-fearing) environment for the molecules lining the cavity. This hydrophobicity prevents water from bonding with KcsA so that it can coordinate and stabilize the potassium ion in the center of the cavity [6].

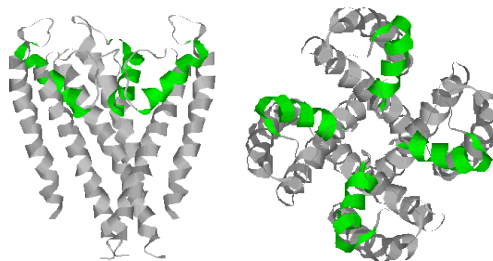


Figure 5 Side (1 subunit removed) and top views of KcsA, with half-helices colored green.

### 1.1.3. selectivity filter

The most important part of KcsA, the selectivity filter is a highly discriminating chain of amino acids that allows the passage of potassium ions and prevents the passage of particles smaller than the  $K^+$  ion. The selectivity filter forms the narrowest part of the trans-protein channel, and in the active state of KcsA, assumes a rigid, specialized conformation that exclusively permits potassium movement (Figure 6). It is the selectivity filter that has been preserved through evolution in potassium ion channels, where other sections of the protein may have changed [2].

The exclusivity of the filter can be attributed to the presence of carbonyl oxygen atoms (oxygen atoms connected to carbon with a double bond) jutting out into the channel. Each subunit distributes six oxygen atoms along the lining of the channel in a distinct shape, such that potassium is coordinated in a square antiprism configuration as it moves through. The oxygen atoms are positioned at precisely the correct distance to stabilize an ion the size of potassium. Thus potassium ions are able to move from the cytoplasm to the water cavity, through the selectivity filter, and into the aqueous extracellular environment retaining a similar electrical and energetic configuration throughout each phase of the process. This accounts for the high selectivity of KcsA. Larger particles cannot fit into the channel, and particles smaller than potassium (such as sodium ions, which are present in the cellular environment) are unable to coordinate correctly; only potassium ions can flow from inside to out without the expenditure of energy [6].

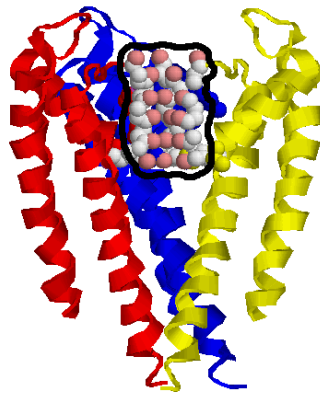


Figure 6 KcsA with the front subunit removed. The selectivity filter is enclosed in black, with the stabilizing oxygen atoms colored bright red.

## 2. Research goal

KcsA is a complex protein; as such, the most appropriate way of representing its mechanisms and functions is through a physical model. However, a physical model can only demonstrate a limited number of mechanisms. It was determined that a model should express the functionality of the selectivity filter, water cavity, stabilizing half-helices, and coordinated potassium ions in order to be meaningful. In addition, the connection of these areas with biology, chemistry, and physics must be established.

For such a representation, the following criteria need to be filled to accurately illustrate the functionality of KcsA:

1. Size-scale allowing it to be used in conjunction with the already existing **3DMD Water Kit™**
2. Color scheme enhancing functional parts of the model
3. Magnetically active lining of the water cavity designed to repel water molecules, to demonstrate the exclusive bonding to potassium ions
4. A magnetically active potassium ion, with coordinating sites arranged for square antiprism coordination
5. Removable sections of the cylindrical model, to highlight various protein areas/mechanisms
6. The ability to demonstrate why other particles cannot travel as easily through KcsA
7. Inclusion of several separate molecules which block activity of KcsA to illustrate how some diseases and toxins prevent nervous function

## 3. Z Corp Z406 Color 3D Printer

The Z Corp 3D Printer, one of the Solid Freeform Fabrication machines at MSOE's Rapid Prototyping Center, was selected to create a representative model of KcsA because of its ability to print in color, speed, and ease of use. Models can be generated on a timescale of several hours. Furthermore, the necessity to have different parts of the KcsA model in different colors created a constraint on what printer to use – no other RP machine is able to create models in color. Finally, most molecular models contain open areas not easily exposed to the surface of the model,

as well as various cavities and complex structures for which post-process cleaning becomes difficult. The Z Corp printer, however, uses powder as its building material, and any leftover powder can simply be airbrushed away. These features make the Z Corp printer the natural choice for creating models of KcsA.

## 4. Model creation process

### 4.1. overview

Designing a model involves several steps spanning various computer programs. RP-RasMol is first used to view KcsA and modify such characteristics as color, size, display type, and structural support lines (monitor lines). After such modifications, the file is exported to RP Magics and SolidWorks. These programs are used to create walls, holes for magnets, and tunnels for wires, as well as to correct design flaws. Finally, the file is taken to the Z Corp machine, where it is actually built.

### 4.2. pre-process design

The pdb file of KcsA (the file format used by RP-RasMol) was found at [www.pdb.org](http://www.pdb.org), a database website sponsored by the Research Collaboratory for Structural Bioinformatics (RCSB).

For the first two generations of models (where the complete protein was represented), RP-RasMol was used to modify several aspects of the protein including the removal of one subunit, the specified coloring to highlight important components, and the relative size of the atoms and bonds.

Creating these whole-protein models was straightforward. The pdb files can be moved from RP-RasMol to the Z Corp printer in one step. The printer is then able to create the models, in color, within a day (Figure 8). These models offered useful information on many subjects. It was established that in order to make use of a larger scale model, major portions of the protein would have to be cut away to focus on the central area of the protein, where most of the functional activity occurred. In addition, a color scheme that highlighted these areas would also be very helpful.

The following generation of models created, therefore, focused on the selectivity filter and water cavity, not the whole protein. In addition, holes for magnets as well as a supporting wall needed to be created. This required the use of RP Magics, a polygon modification program, and SolidWorks, which can create the alterations necessary for this model.

RP Magics was used to section away the outer layers of the protein, leaving only the cylindrical core in the middle. In addition, a section of the protein, corresponding to one subunit, was separated from the main model so as to be detachable (Figure 10).

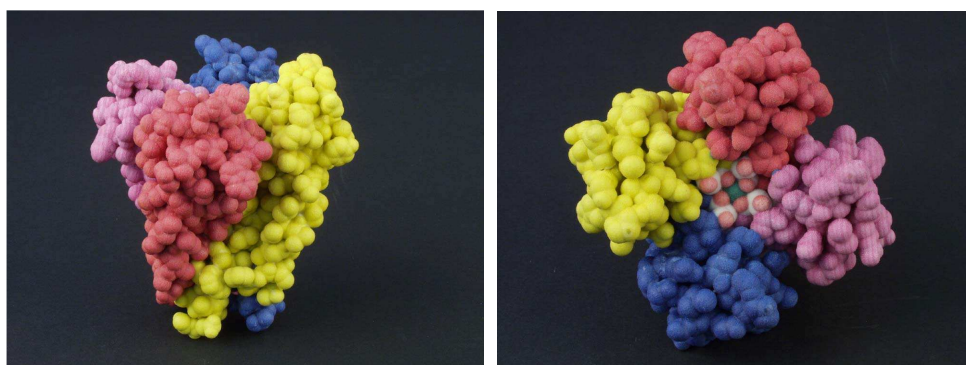


Figure 8 Side and back views of the complete KcsA protein.

At this point, two options were explored in reference to the best representation of the functionality of the selectivity filter. The first option was to create transparent oxygen atoms, on a separate SFF machine, that would be placed into the positions of the Z Corp oxygen atoms in the selectivity filter. Each individual atom would contain an electromagnetic LED (light-emitting diode) circuit that would switch on when in close proximity to a magnetic potassium ion. Thus, oxygen atoms currently coordinating a potassium ion would shine bright red to indicate that they are in use.



Figure 10 Test model of KcsA selectivity filter. The sodium ion and water molecules are included for reference

It was decided, however, that a simple magnetic system, without LED's, would be sufficient to demonstrate the coordination of potassium ions. The final channel core model, therefore, was designed to incorporate this magnetic system (Figure 11).

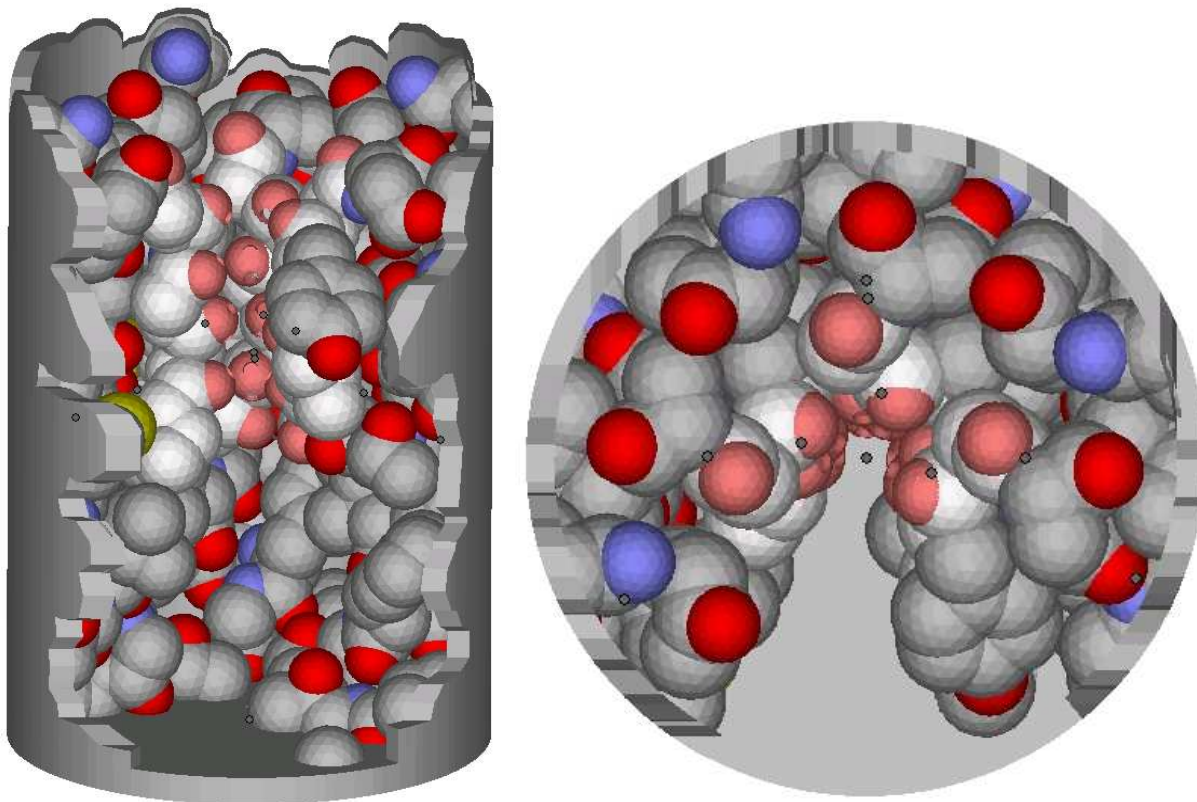


Figure 11 Front and top view of the channel core model, composed of the selectivity filter and water cavity (made in RP Magics). Note the bright red oxygen atoms of the filter; the holes in each one are designed for magnets

### 4.3 KcsA's potassium ion and representation

Because potassium is coordinated in a square antiprism configuration, the magnets in its ionic model must be placed specifically to reflect this quality. SolidWorks was used to design the potassium ion model so that it would be able

to coordinate correctly with water molecules, as well as oxygen atoms in the selectivity filter (Figure 12a).

The ion model was also designed to slide on a rod placed through KcsA's channel, to represent the protein's ability to restrict potassium movement to a straight line through the channel (Figure 12b).

This, however, presented a problem due to the inherent fault of creating solid spheres to represent atoms; real atoms are not solid, but rather nuclei surrounded by a 'cloud' of electrons. When illustrating the interaction of potassium ions with oxygen atoms in the selectivity filter, where spatial and electrical factors play a major mechanistic role, the classic guidelines for physical modeling cannot be used. Therefore, the decision was made to reduce the sizes of the potassium ions and coordinating oxygen atoms with respect to the rest of the protein. This permitted a more applicable physical representation.

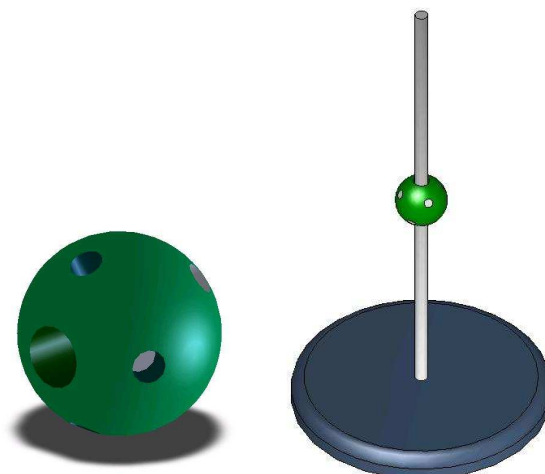


Figure 12 SolidWorks design of potassium ion. A) Side view of ion. B) Ion placed on rod, which is positioned in the central axis of the channel. The ion can slide up and down, but will be held in place by oxygen atoms.

## 5. Considerations for Future Work

Though this research aims to model the most fundamental functions of KcsA's open state, many additional models are possible. All contribute to the understanding of this important protein, and would be a great resource to both high school and college instructors. Some possible future models might include:

- Incorporation of active circuits with LED's that turn on when the selectivity filter is being occupied by potassium ions
- A moveable model of the entire KcsA protein demonstrating the differences in conformation of the open, closed, and inactivated states
- the voltage gate mechanism
- other gate types
- other types of potassium ion channels, such as MthK, as a comparison to KcsA
- the protein-FAB complex (locking KcsA in its open state) used by R. MacKinnon to obtain a crystal
- of the lipid bilayer incorporating sodium, potassium, and calcium ion channels demonstrating the process of generating and propagating an action potential
- molecules that block K transport

## 6. Conclusion

Though much investigation is still required in order to fully understand KcsA, current literature offers a comprehensive understanding of the basic functions of the protein. Two models of KcsA were created with the purpose of modeling these mechanisms and functions. The complete protein model makes use of color to illustrate the 3-dimensional structure of KcsA: its symmetry, and the layout of the channel and selectivity filter. The channel core model highlights the individual passage of potassium ions and their energetically favorable coordination by oxygen atoms in the selectivity filter. These models focus on the protein's features that are instrumental to the understanding of not only KcsA, but also to the better understanding of biology and chemistry in general.

## 7. Acknowledgements

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Note: All unreferenced pictures of KcsA have been created using RasMol.

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