A VISUALIZATION OF CHEMICAL SIGNALING

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Acknowledgements

The events that led to this production are associated with individuals who helped, taught, inspired, and led me in my academic pursuits. Had there not been a School of Informatics at the IUPUI campus, I would not have sought an advanced degree. The interdisciplinary nature of informatics particularly appealed to me.

In fall semester of 2001 I took Chemistry C371. Here Dr. Gary Wiggins introduced me to informatics from the perspective of a discipline in which I had some training. Dr. Kenny Lipkowitz patiently taught me how to use the online tools.

Nearly a year later I started exploring the M.S. in Chemical Informatics program. My first contact was Mary O'Neil, who continued throughout to help me navigate the administrative requirements. She also introduced me to Dr. Sam Melosivich, who was to become my first academic advisor. He really helped me stretch my brain around informatics.

Spring of 2004, I took C372, molecular modeling, with Dr. Kelsey Forsythe, who would eventually become my second academic advisor. He really expanded my mind about what was included in chemical informatics. I was also really taken by the robustness of the visual output form molecular modeling software.

Early September of 2004, as my first classes in the program were starting, William Aspry assembled a conference at IU Bloomington about the research agenda for informatics. Not only did this give me an exceptional overview of the field I was entering, but I also met Albert William, who became my gateway for exploring

visualization in chemistry. He has been helped me tremendously on my journey through this program.

Albert suggested that I take a course that in 3D modeling and animation. In fall 2005, I enrolled. It was taught by Clint Koch, who has continued to help me build on what I learned in his class.

In October of 2005, Clint loaned me a DVD of productions that were presented at SIGGRAPH (ACM) in 2003. One of the productions was a description of DNA replication by Drew Berry (WEHI). I had seen several conceptual presentations of this process in lectures before but had somehow postponed my understanding of it. In less than half a minute of watching Berry‘s video I suddenly and thoroughly comprehended it so acutely that it took my breath away. I wanted to understand how this happened to me in more depth.

I first looked to Dr. Paul Pietsch, an anatomist whom I had met decades earlier during my undergraduate work in Bloomington. He helped me understand that my answer was not in the locus of structural anatomy of the brain.

Later that year I met Dr. Karl Mac Dorman. When he joined our faculty, I sought his view on how Berry’s video provided such a cognitive leap for me. In spring of 2006 I took his Psychology of Human Computer Interaction course. He helped me understand that the exercise of construction can be a path to research just as well as analysis.

Chemical signaling within and between cells was something I had been hearing and reading about. Much of it seemed very obscure. It seemed it would be an interesting challenge to construct what chemical signaling looked like.

After a semester of looking around for someone to guide me in this, I found Dr. Grant Nicol in the School of Medicine. He helped me also recruit Dr. Theodore Cummins for my research committee. Together they provided the guidance and feedback that shaped the production and technical accuracy.

After the departure of Dr. Forsythe from the department, Dr. Matthew Palakal generously agreed to chair my committee.

I am also indebted to my family for giving me the space and support to return to being a student after so many years outside academia.

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Preface

At the orientation session for new students to the School of Informatics in the Fall of 2004 there were introductory remarks by Dr. Darryl Bailey in which he described Informatics as “Data becoming information, becoming knowledge, becoming wisdom”. Much of the coursework for Chemical Informatics focuses on the beginning step of data becoming information. I see this thesis project as the construction of a teaching tool by facilitating information to become knowledge via application of the tools of informatics.

TABLE OF CONTENTS

Tables

Figures

Chapter One: Background

Physical sciences are based on observation. Much observation cannot be directly visual because of the size, time scale, or proximity of the subject matter. For example the objects studied in the field of nuclear physics exist on a very small scale and move very fast compared to the scale and perceptual capabilities of humans. Innovative scientists have discovered methods to measure and characterize this subject matter by measures such as energy change. The data from experimentation is mostly numerical.

Science disciplines use the advantage of the visual system in gaining understanding of numerical data. Plots of data reveal relationships that are obscure when viewed as tables of numbers. For more complex data sets, visual data mining tools are gaining popularity (Simoff, 2008). In chemistry, none of the interactions on the atomic or molecular scale are visible. Yet the structure of many molecules has been determined by crystallography techniques enabling construction of geometrically accurate 3D models of molecules that make their spatial relationships and interactions readily understandable.

Animation of 3D models adds further to comprehension (Höfflerand, 2007). Moving images reveal more about complex shapes of objects (e.g., molecules) by moving the viewpoint to reveal the unfamiliar geometry.

The human visual system gives us the ability to detect fine grained features and patterns in images more quickly than in other information representations such as text (van Wijk, 2006). It has also been suggested that the use of images in teaching can substantially enhance student comprehension (Kolb, 1973).

Visual representations are useful in creating educational experiences that simulate visual observations of the objects and phenomena of science. For example, medical

illustrations have long been integral to curricula in medicine. While our internal organs are so close, they remain obscure, because flesh is opaque. Illustrations of anatomy allow a view inside. In-Silico interactive simulation can provide an even more robust substitute for real life observation with the addition of interfaces that provide tactile feedback.

One of the earliest serious efforts to create multimedia productions including visualizations of concepts of science was a series of educational films produced by Frank Capra with sponsorship from Bell Laboratories starting in 1957. The most popular of these, *Hemo The Magnificent,* was an explanation of the human circulatory system for young students. It was shown in secondary schools as part of the science curriculum throughout the United States. The production mixed film with 2D animations for science education rather than entertainment, where such media had gotten its start. Multimedia use in science education has since flourished. Many science textbooks include a disk with multimedia content to supplement comprehension by the student.

Scientists eventually took this to the level of using multimedia presentation for communicating with one another about their research. An early example of this was done at the National Institutes of Health in 1968 (Fitzhugh, 1968). The evolution of this use in communications between scientists has led to publications such as the Journal of Visualized Experiments (JoVE) for communicating complex laboratory methods in the life sciences.

While the multi-media visual representations based on non-visual scientific observations have proven useful, there is ambiguity as to where they belong in the taxonomy of science and art. ACM SIGGRAPH and IEEE have provided forums for demonstration of such productions as well as journals for discussions on this topic. Much

of the discussion about this pivots on the value of visualization. Van Wijk (van Wijk, 2006) discussed this in some depth, concluding that the value is dependent on the discipline from which it is considered. Construction of visual solutions to problems of science have brought accomplishments such as the determination of the double helix structure of DNA and the discovery of the source of the cholera epidemic in 1854 (Tufte, 1990).

The unique contribution of this project is the use of real molecular structure data in the robust audio- visual explanation of ion currents in nerves. The challenges encountered primarily surrounded how to process published molecular structural data to turn it into 3D models that could be appropriately manipulated to animate the description of the initiation and conduction of ion currents that are nerve signals. To address this for large molecules such as sodium and postassium channels, molecular modeling software was used to prepare a molecular surface depiction that could be saved as a file type that could be converted to be compatible with the 3D modeling and animation application. For smaller molecules, models were constructed in the 3D modeling application that were visually similar to those produced in the molecular modeling application but less data intensive.

Chapter Two: Production

Overview

The starting point for this visual production was the goal of using reported molecular structures in a robust explanation of the workings of some aspect of chemical signaling in living systems. The specific topic of sodium and potassium channels in initiation and propagation of nerve impulses suggested itself as an illustrative example. The objective was further defined to make a visual presentation that would be useful in teaching about the role of these ion channels in nerve signal initiation and propagation to graduate students in the School of Medicine. Defining this specific topic and target audience provided a frame of reference for the construction of the production. Figure 1 shows a schematic overview of the production steps.

In further discussions with the research committee, a general story line was developed that included: the description of how sodium and potassium channels worked in this role; what results when they do not function properly (description of a diseased state); and some historical perspective on some of the scientists and their key discoveries that led to the current understanding in the field. Figure 1 shows the steps in the production process.

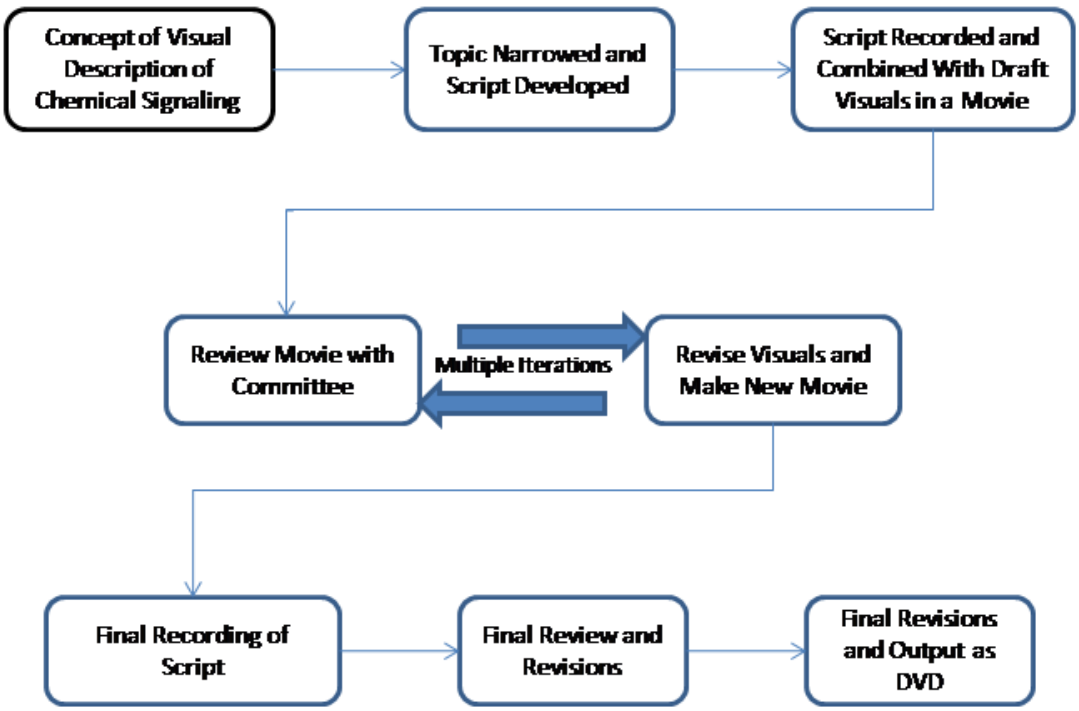

Figure 1: Schematic Overview of the Production Process

A detailed script of the narration for the production was written along with the preparation of a storyboard describing the corresponding visual components. An anamatic, or draft, of the production was assembled from the recorded script and sketches and other coarse grain representations of the visuals. The anamatic was used to determine the flow and transitions within the presentation and generate data about how long each visual component needed to be on the screen.

Rationale for Visual Components

Every image that appears on the screen has a planned purpose. The images and their related animation are coordinated with the narration to optimize communication of the subject matter. Table 1 summarizes the visual components and rationale.

Description	Source	Rationale
Birds in flight	Video	The flight of birds is familiar to the audience while the underlying suggestion is that this complex behavior is possible due to sensing and signaling systems that operate the birds' specialized anatomy.
Bird in tree	Video	The focus on a single bird is coordinated with the narrator indicating that the scope is limited to part of one of the signaling systems.
Human hand	Video	Familiar part of human anatomy communicates that what follows is about human systems.
Human profile	3D model	Familiar image reinforces that human systems are the topic. Provides coarse grained overview of what is to follow.
Graph of resting potential	Composite of 2D images	Familiar image from science provides transition to finer grained explanation that follows.
Potassium channel in nerve cell membrane	Composite of 3D models and 2D images	More robust version of potassium channel image presented in textbooks is familiar to audience while animation demonstrates function described in narration.
Sodium – Potassium exchange pump in nerve cell membrane	Composite of 3D models and 2D images	More robust version of image sequence presented in textbooks is familiar to audience. Animation and text description emphasize that this is an active rather than passive process.
Nerve ending	Composite of 3D Models	This is the finest grained and most realistic of all visual components as it approaches the molecular scale.
Nerve axon with patch clamp	Composite of 3D models and 2D images	Coarser grained depiction of impulse propagation familiar to audience because similar to textbook images and laboratory experience.
Nerve axon with myelinated regions	3D model	Model is familiar to audience as it is based on textbook images. Animation emphasizes conduction rate difference from non-myelinated regions.
Sodium channels and DNA molecule	3D models	Random activity of sodium channels introduces their role in epilepsy and DNA image suggests its hereditary nature.
Brain, dendrite network and multi-trace chart	Composite of 3D model and 2D images	Images and related animation emphasize that the misbehaving Sodium channels leading to epileptic symptoms are located in the brain.
Reenactment of tonic-clonic convulsive seizure	Video	Provides realistic view of a common type of epilepsy related seizure that audience may reasonably see in the course of their careers.
Sodium channel and images of drug molecules	Composite of 3D models	Animation of drug molecules moving to different locations on the Sodium channel emphasizes that different drugs act on different locations of the channel.
Images of scientists and figures related to their research	Composite of 2D images	Images emphasize the span of time over which discoveries were made that enabled study of ion channels.
Image of Sodium channel and of publications related to more current discoveries	Composite of 2D images	Images of publications give audience a view of what they may reasonably encounter in the course of their careers.

Table 1: Visual Components and Rationale

Visual Components

Visual components were video, 2D images, and 3D models. Video was shot, captured, and edited by the author. 2D images were created with Adobe® Photoshop®CS3, taken as photographs of 3D models, or sourced from the World Wide Web as cited. With the

exception the human figure, which was purchased from turbosquid.com, 3D models were constructed in Autodesk® Maya®. Molecular 3D structures were obtained from Protein Databank and subjected to other processing steps before importing into Autodesk® Maya®2008 for texturing and inclusion in the respective scenes. Figure 2 shows a screen capture of a protein molecule after importation to Maya. Table 2 summarizes the 3D models that were constructed.

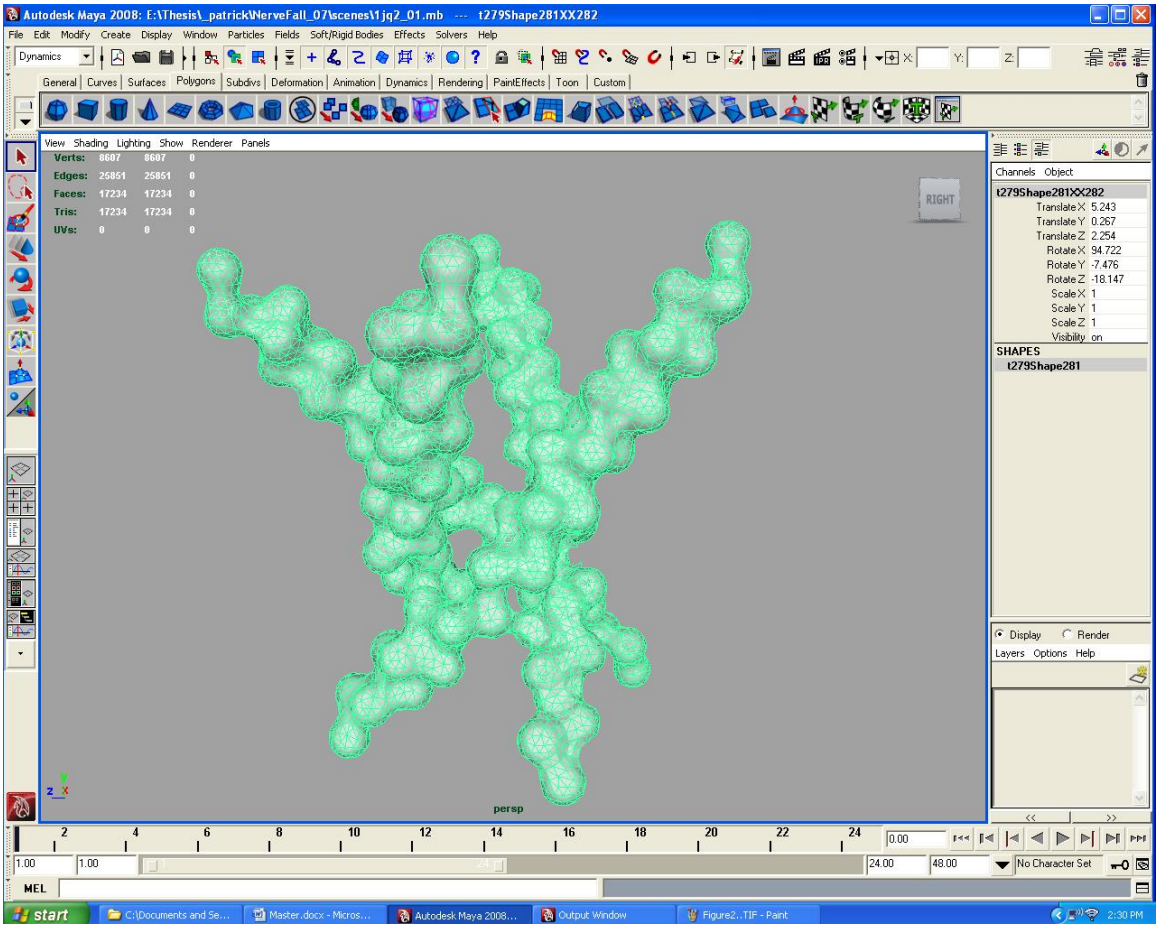

Figure 2: Ion Channel Molecular Structure from Protein Databank Imported into Maya

Model	Construction Information
Membrane Surface Texture	This texture is based on electron micrograph of neural membrane surface showing hexagonal packing of the heads of the phospholipid membrane components (Levitan, 2002). It started with a textured field of spheres captured as a 2D image that was then used as texture map on a larger sphere. The larger textured sphere was duplicated into a field of hexagonally packed spheres then captured as a 2D image. This image was made into a tileable image and used as both texture map and bump map for the membrane surfaces used in the various scenes.
Potassium Channel (cartoon image)	Based on 2D illustration (Purves, 2001), the potassium channel in cross section is a composite of two 3D models with opacity of each animated to depict opening and closing. The membrane is an arrangement of planes with texture and bump maps.
Sodium-Potassium Exchange Pump (cartoon image)	Based on 2D illustration (Nicholls, 2001) and 2D animation (McEvoy, 2003), the pump is a 3D model animated by use of a blend shape. The membrane is an arrangement of planes with texture and bump maps.
Nerve Ending	Based on micrograph of nerve ending (Levitan, 2002), the shape was revolved from a hand drawn profile. The membrane surface texture was mapped to the 3D shape.
Closed Ion Channel (molecular structure)	Started with Protein Databank (.pdb) structure imported into Chimera and defined it as a surface model. The model was then exported as a Virtual Reality Mark-up Language file. It was then converted to a Maya Ascii file using vrml2ma.exe. It was opened in Maya, textured and rescaled. It was then imported to each scene where used.
Open Ion Channel (molecular structure)	Started with Protein Databank (.pdb) structure, then subjected to the same steps as the closed ion channel
Ion Emitters	Emitters were created using Maya's dynamics options. Emitted particles were redefined as blobby surfaces and textured to represent the respective ions.
Ion Fields	Ion fields were created with Maya's particle tool and animated by applying a turbulence field. The particles were redefined as blobby surfaces and colored to represent the respective ions.
Nerve Axon	The axon was created in Maya via extrusion of a circle along a hand-drawn path. It was then textured with the membrane texture.
Myelin Sheaths	Based on 2D illustrations (Purves, 2001) and stained micrographs (Caceci), myelin sheaths were created in Maya as pipes that were sized to the axon proportional to the textbook images. A texture map was hand-drawn in Adobe Photoshop using colors sampled form stained micrographs of myelin.

Table 2: Models and Construction Information

Animation of the models was accomplished by setting key frames for position, opacity, distortion or other properties of the models or the virtual camera which is the viewpoint for the output of the scene. Timing of the key frames was determined from the recorded script such that what the viewer would see would fit with what the narrator was communicating at any given point. The final step in Maya® is to render the scene. In this process, Maya® calculates what each pixel should look like based on the model, lighting, special effects and other specified properties. It repeats this process for each frame in the specified range. As the final production plays at thirty frames per second, the rendered output is thirty 2D images for each second of the production.

Compositing

In the compositing process, the visual components were assembled and synchronized with the narration to create the final output, which, in this case, is a digital movie. This was done with Adobe® Premiere® Pro 2.0 and Adobe® After Effects® CS3

Summary of Software Used

Multiple software applications were used for the variety of specific tasks required in this production. Table 3 lists the role of each application used.

Application	Use
Adobe® After Effects® CS3	Compositing
Adobe® Premiere® Pro 2.0	Compositing and final video output
Adobe® Photoshop®CS3	2D Image manipulation
Autodesk® Maya®2008	Create 3D models and output image sequences for compositing
UCSF Chimera	Manipulate molecular models and convert to Virtual Reality Mark-up Language
PC Model® (Serena Software)	Build and manipulate molecular models
Sony Sound Forge	Edit sound

Table 3: Software Applications Used

Chapter 3: The Underlying Science

Ion channels are specialized protein molecules that are found in cell membranes across the entire taxonomy of flora and fauna. These channels are located in the cell membrane and function to selectively allow specific ions in or out of the cell. Through their selectivity, they advantage ion concentration differences between the inside and outside of the cell to provide the necessary driving force to rapidly move ions across the cell membrane (Nicholls, 2001). This thesis project provides a multimedia explanation of how sodium and potassium channels work in tandem in nerve cells to transport electrical charges in the form of ions that function as signals in the nervous system.

Two levels of chemical signaling are involved in the initiation and propagation of an action potential. On the molecular level, stimuli lead to a response, which is a change in conformation of the ion channel. On the cellular level, diffusion of ions through the open ion channels, driven by concentration gradients, enables the initiation and propagation of a discrete bolus of electrical charge along the length of an axon. Such electrical signals constitute information transfer within the organism. This cellular level phenomenon is the focus of this project.

When a nerve cell is at rest, it maintains a potential sixty-five millivolts lower inside its membrane compared to its surroundings. This difference in potential results from different ion concentrations inside and outside the cell, which is accomplished by potassium ions diffusing out passively through potassium channels, and sodium-

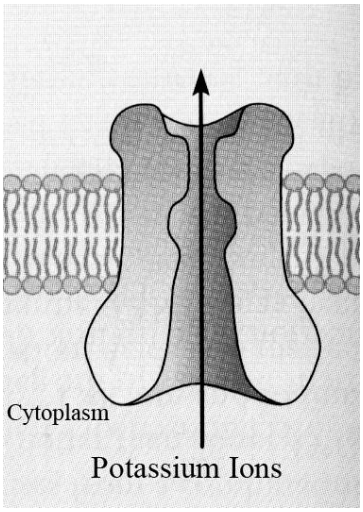

Figure 3: Potassium Channel (adapted from Nicholls, 2001)

potassium pumps moving three sodium ions out and two potassium ions back in with each cycle. These pumps move sodium and potassium ions across the cell membrane against their respective concentration gradients by an active molecular process that is powered by adenosine triphosphate, which binds to and phosphorolates the pump molecule causing a change in conformation (Nicholls, 2001).

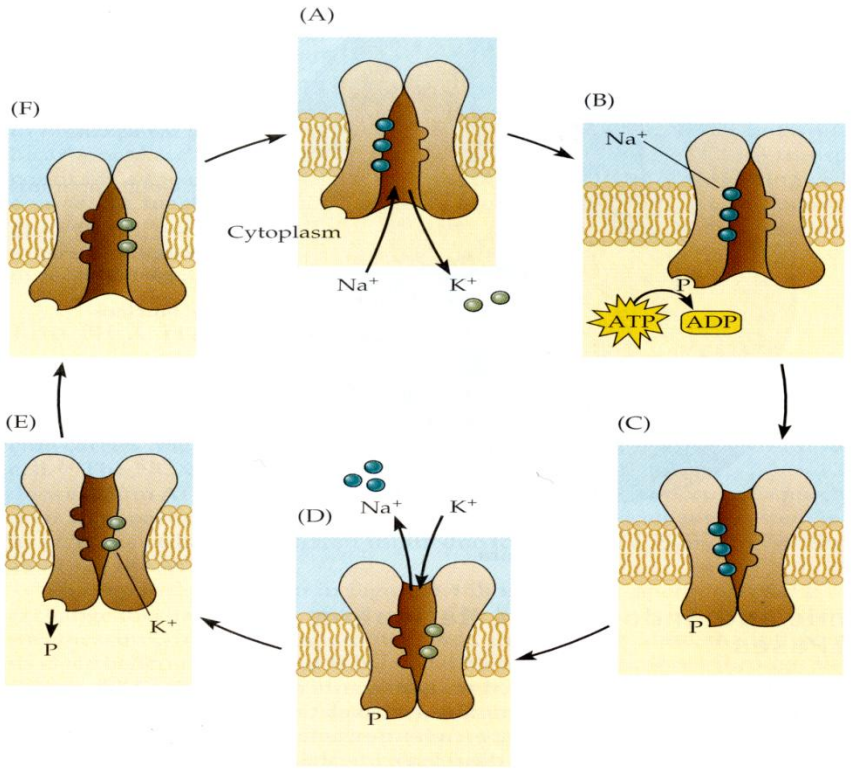

Figure 4: The Stages of the Cycle of a Sodium-Potassium Exchange Pump (adapted from Nicholls, 2001)

Nerve signaling starts with a stimulus from an organism's environment. In this case, it is a sharp pinpoint pressed to the skin. This mechanical deformation causes stretch-activated sodium channels in underlying nerve endings to open. This, in turn, allows a rapid inflow of positively charged sodium ions, changing the electrical charge in the nerve endings.

As the electrical charge in a nerve ending becomes more positive and exceeds a threshold level, proximal voltage-activated sodium channels open in response, resulting in a large local concentration of electrical charge, or action potential, in that local region of the axon. This action potential continues to activate more sodium channels along the length of the axon. As the charge is reaching its maximum, local potassium channels respond allowing an outflow of positively charged potassium ions. This outflow and

corresponding change of the charge in the axon, starts a return to the original transmembrane potential of about -65 millivolts following the action potential. This local event repeats continuously along the length of the axon, ultimately delivering the action potential to a synapse.

Some axons have regions wrapped tightly with Schwann cells that form an electrical insulating membrane called a myelin sheath (Levitan, 2002). Action potentials move through these regions differently. The charge passively flows at very high velocities through the myelinated regions and is recharged at the nodes of Ranvier, which are gaps between the regions. Nodes of Ranvier have high concentrations of sodium channels that rapidly recharge ~~to~~ the action potential before it proceeds through the next myelinated region (Nicholls, 2001). Thus, myelinated regions p faster movement of the action potential to its destination.

The scope of this project is to describe only the initiating steps in nerve signaling. Many other chemical signaling pathways are involved in the process of sensing and responding to signals making their way through the nervous system.

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CURRICULUM VITAE

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