Mechatronics Rendering of Cellular Active Transport (Sodium-Potassium Pump)

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Submitted by:

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INTRODUCTION

Cells are the basic and the smallest unit of life. Detailed processes occurring inside a cell can only be seen with the use of high powered microscopes and in most cases are difficult to understand. Over the years, many models and animations were created to explain cell membrane structure and function. By using Mechatronics concepts learned in the SUMMIT program, we will be able to make complex biological concepts tangible for students and increase their interest in the learning experience. Working with something that moves and is highly visual can be a great motivator for students of any age.

The goal of this project is to provide a visual demonstration of the sodium-potassium pump mechanism in a cell, and to show students conformational change in membrane protein during cellular active transport.

BACKGROUND

Cell Membrane Structure and Function

As shown in **Figure 1**, cell membranes contain different types of proteins. These include:

- ***Marker proteins*** attached to carbohydrates to help other cells recognize their cell type.
- ***Receptor proteins*** recognize and bind to specific substances.
- ***Transport/Trans-membrane proteins*** aid the movement of substances in and out of the cell.

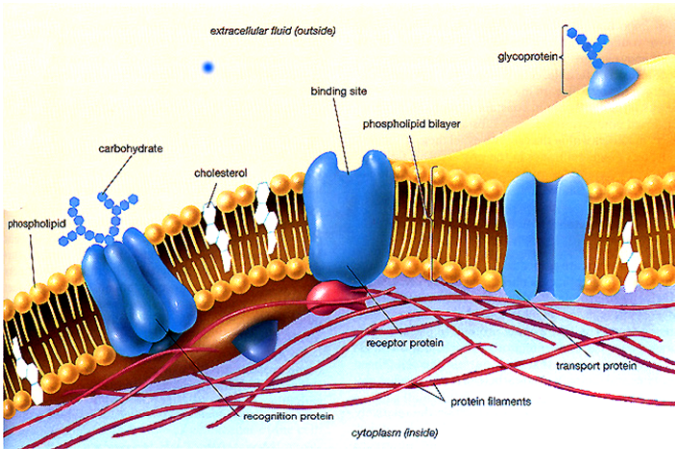


Figure 1. The Cell Membrane

Facilitated diffusion can only transport across the membrane down the concentration gradient. Cells need to transport certain amino acids and

ions. Many of these have lower concentrations outside the cell than inside. It is in this context that ACTIVE TRANSPORT is needed and this process requires energy in the form of ATP (Adenosine Tri-phosphate).

SODIUM-POTASSIUM PUMP

It is one of the most important membrane-transport systems and occurs in almost all animal cells. In a complete cycle, 3 Na+ ions are pump out of a cell as 2 K+ ions enter the cell. Usually, concentrations of Na+ ions are found outside the cell and typically higher concentrations of K+ ions are present inside the cell.

The sequence of events can be summarized as follows and is also shown in Figure 2:

- The pump, with bound ATP, binds 3 intracellular Na^+ ions.
- ATP is hydrolyzed, leading to phosphorylation of a cytoplasmic loop of the pump and release of ADP.
- A conformational change in the pump exposes the Na^+ ions to the outside, where they are released.
- The pump binds 2 extracellular K^+ ions, leading somehow to dephosphorylation of the alpha subunit of the protein.
- The pump reorients to release K^+ ions inside the cell.
- The pump is ready to go again.

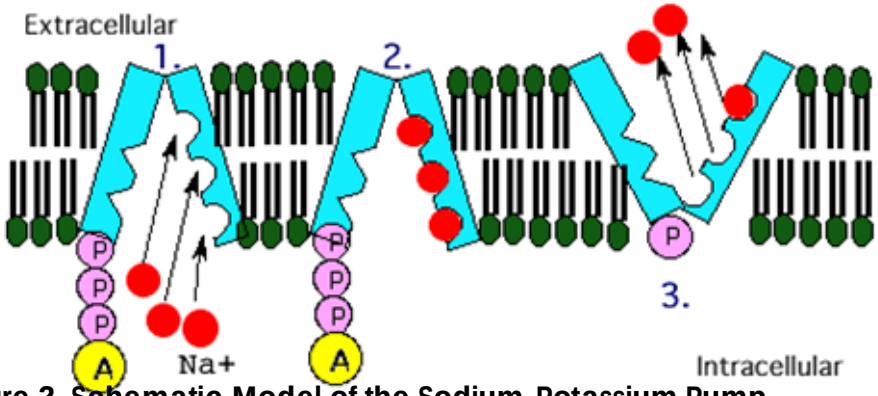


Figure 2. Schematic Model of the Sodium-Potassium Pump

IMPORTANCE OF THE SODIUM-POTASSIUM PUMP:

- This process prevents Na+ ions from accumulating in the cell. Too much Na+ ions is toxic to the cell and these ions continously diffuse into the cell through ion channels. The increase of these ions would then cause water to enter the cell by osmosis causing the cell to swell and burst.
- This process helps maintain the concentration gradient of Na+ ions and K+ ions across the cell membrane. Many cells use the Na+ ion concentration gradient to help transport other substances needed also by the cell, such as glucose.

    - Cells in the body absorb nutrients and eliminate wastes with the assistance of the sodium/potassium pump. Molecules such as glucose are actively transported into cells by being coupled with sodium ions moving across the membrane through open protein channels, down their concentration gradient. Similarly, certain metabolic wastes can leave the cell coupled with potassium exiting the cell.

Physiologic Significance

Controlling the sodium/potassium balance is one way the body regulates pH. When body fluids become too basic the kidneys excrete sodium, and when it is too acidic the kidneys excrete potassium. You can avoid getting kidney stones by increasing the acidity in your kidneys. When the urine is too basic, calcium and phosphorus bind together to form kidney stones. By having a surplus of potassium you will make your urine more acidic.

Sodium and potassium help keep blood pressure constant. If you don't consume foods containing much potassium you won't have much in your cells. You're more than likely to have a lot of sodium on the outside of your cells, though. Your cellular pumps have to work extra hard to keep the sodium outside your cell membrane. More sodium will get inside your cells, and with it comes water. Your cells can actually burst because of the amount of water drawn into it. The water comes from surrounding fluids and blood. This causes a decrease in blood pressure. Having enough potassium will help keep your blood pressure at its most healthful level.

The Na+/K+ pump maintains an electrochemical gradient across cell membranes producing an electrical membrane potential that is essential for nerve impulse conduction/transmission and muscle contraction.

EXPERIMENTAL PROCEDURE

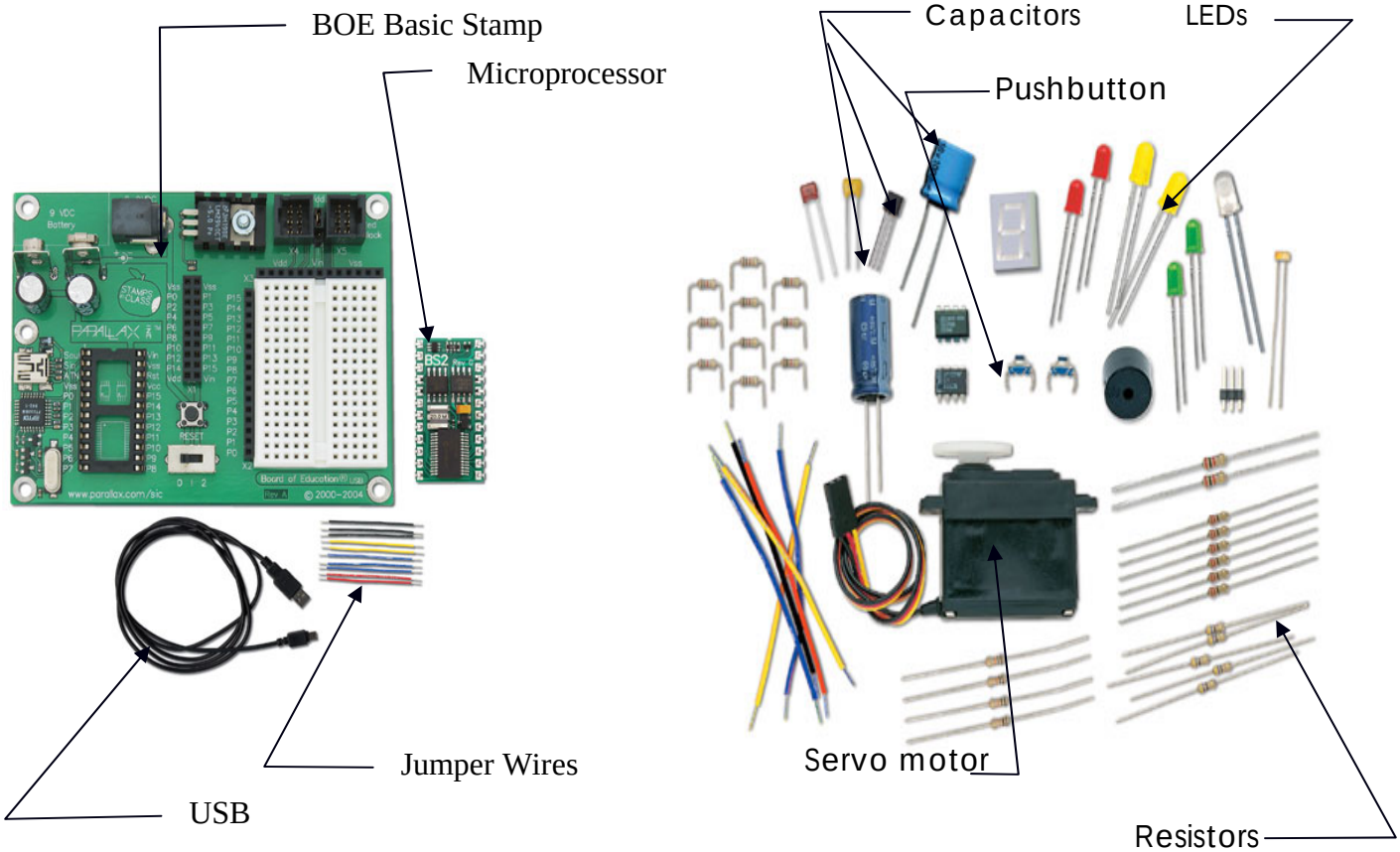
This model will show how Mechatronics was used to produce a visual simulation of a cellular process. We utilized a microcontroller, servo-motors, simple gears and levers and many LED's and electronic circuits on a decorated platform to illustrate the process, hence the name "Mechatronics Rendering of Cellular Active Transport". Computer code created in P-Basic directs the Parallax Basic Stamp 2 microprocessor to cycle the model through a series of events designed to visually illustrate the mechanism of the sodium-potassium active transport pump.

The objective of the project is to provide a representation and demonstration for students to see the movement of the trans-membrane protein when the Na+ and K+ ions move in and out of a cell.

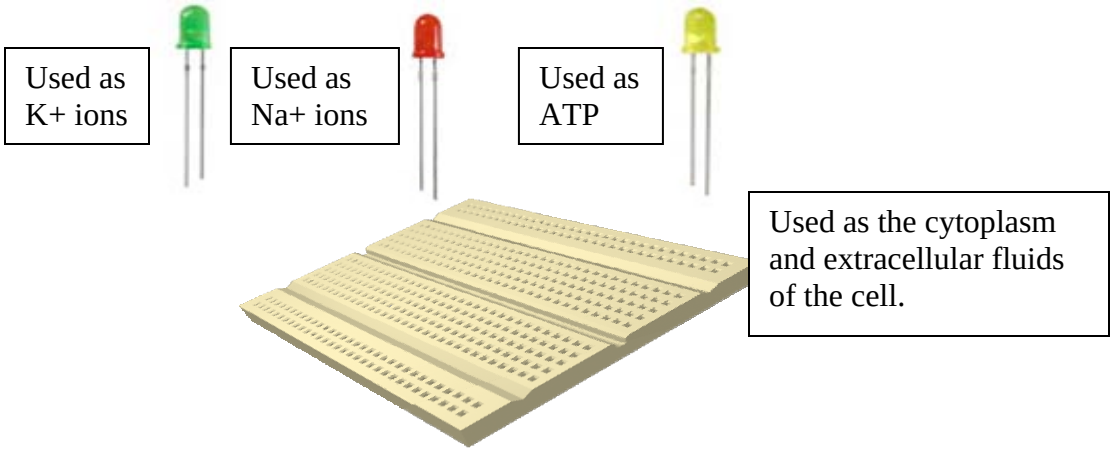
The Pump Model serves as a learning tool for students studying cellular active transport and membrane potentials in animal cells. In addition to promoting the use of technology in the classroom, this model will further demonstrate how Mechatronics can be a powerful tool to show complex processes in action and can be manipulated, hence students' interest and motivation will be increase because it's a hands-on experience.

It was part of the original design that this model be interactive, allowing for the manipulation of ion concentrations that would produce corresponding visual representations along with an increase in the rate of mechanical events in the model. Unfortunately this feature was not achievable at this time primarily due to time constraints. This remains an objective for further development of this project.

MATERIALS/COMPONENTS USED:



Servo motor controls the opening and closing of the trans-membrane protein, ATP and the sequence.



PROCEDURE:

The Sodium-Potassium Pump Model consists of a representation of a region of cell membrane showing the phospholipid bi-layer and a mechanical construction simulating a trans-membrane protein channel. Numerous LED circuits in series and several servo motors are linked to the basic stamp. The voltage directed through input/output pins of the microcontroller activate a specific sequence of circuits. Which control the illumination of individual or series of colored LED's. Na+ ions and K+ ions inside and outside the cell as well as in the protein channel and a molecule of ATP are represented by many red, green or yellow LEDs. Microcontroller pins also activate the servo motors controlling the opening and closing of the protein channel, exposue of ATP binding site and forwarding of descriptive captions display. The model is activated and students must then observe and describe how the Na+ / K+ pump functions in a cell (**See Figure 3**).

Several LEDs are illuminated at the start of the process to show sodium and potassium ions in a typical cell. Red LEDs represent sodium ions, greens LEDs the potassium ions, yellow LEDs the ATP/ADP couple.

A pulse is sent to the servo motor that will turn the wheels connected to the membrane proteins to show the conformational change in shape when an ATP (Yellow LED) binds to its active site.

Another pulse is sent to another servo that will turn to show the captions of each step.

Figure 3. SODIUM-POTASSIUM SET-UP

HOW THE MODEL WORKS

- At the beginning of the process several series circuits of red and green LEDs are illuminated. These represent Na+ and K+ ions within and exterior to the cell. Electrical power is supplied by a 12 volt DC adaptor.

- Using the program code, servo No. 3 controlling the caption rod turns to display a description of cellular events in first step of the process. This servo turns at the beginning of each successive step in the process that this model will illustrate.

- Servo no. 1 controlling the protein channel positions the membrane protein open towards the inside of the cell. 3 Red LEDs below the membrane will be on showing 3 Na+ ions attracted to the binding sites on the protein. After few seconds, these will be turned off and 3 red ultra bright LED's on the membrane protein are turned on, showing the 3 Na+ ions now attached to the protein.
- Next, servo no. 2 controlling the ATP binding site will move out. While in this position, 1 yellow LED in the protein and another 2 directly below are illuminated indicating the binding of a molecule of ATP to the protein channel. A high energy phosphate group is transferred to the protein (phosphorylation), indicated in the model by the single yellow LED moving away with the protein as the two remaining yellow LED's (ADP) remain in the intracellular environment. Servo 1 turns the protein towards the outside of the cell. The 3 red ultra bright LEDs on the protein turn off at the same time as similar LEDs above the cell are illuminated, showing the Na+ ions pumped out of the cell.

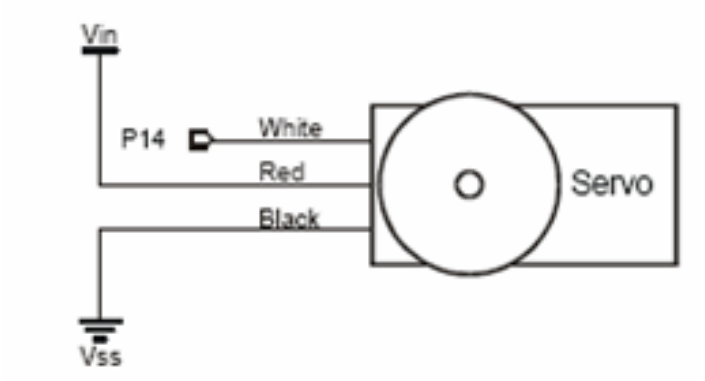
- 2 green LEDs above the cell are illuminated showing the K+ ions attracted to the membrane. These will be turned off at the same time that 2 of the same kind of LED's are illuminated in the protein, showing attachment of the these K+ ions to their binding sites on the channel protein.
- Servo no. 1 then returns the protein back to its original position towards the inside of the cell. The 2 green LEDs are still illuminated showing K+ ions attached to the protein and being drawn towards the inside of the cell. While these processes are occurring the yellow LED on the protein has been turned off to show loss of the phosphate group (de-phosphorylation) by the protein.

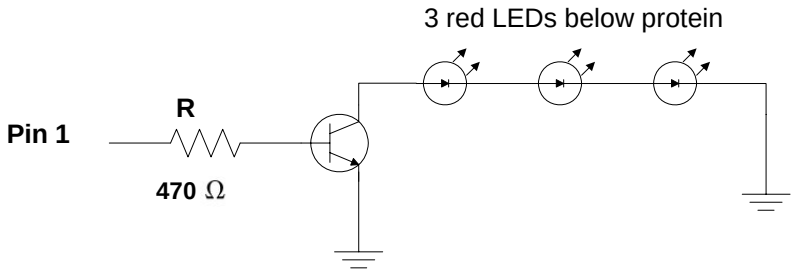
- The 2 green LEDs on the protein are then turned off and similar LEDs below the protein are turned on showing K+ ions being released into the cell. At the same time the 2 yellow LEDs below the membrane are turned off to suggest the recycling of ADP molecules.
- The entire sequence will repeat until the model is deactivated.

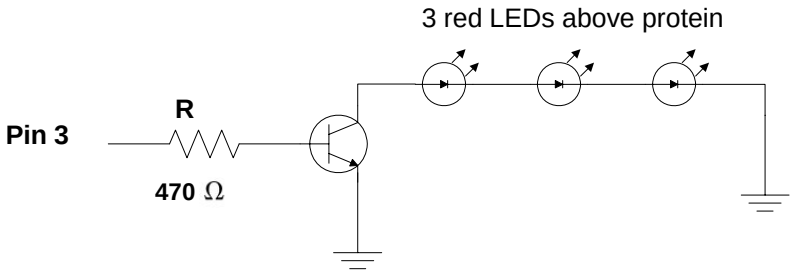
CIRCUITRY SCHEMATIC DIAGRAMS

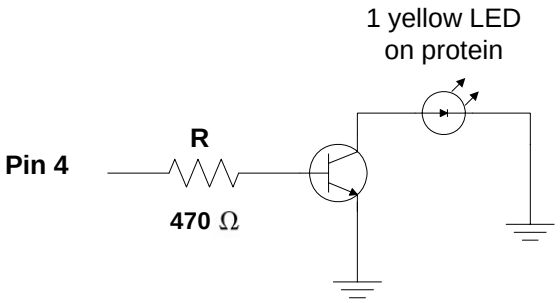
SERVO CIRCUIT:

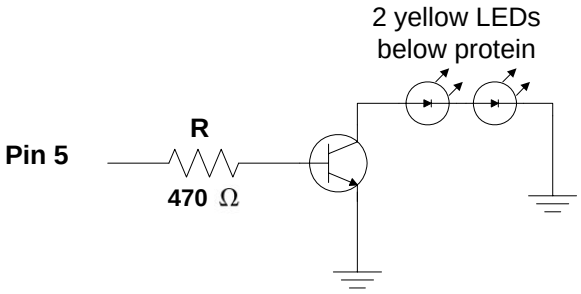
- Pin 13 servo 2 ATP binding site on protein
- Pin 14 servo 1 Protein channel
- Pin 15 servo 3 Caption- steps of the process

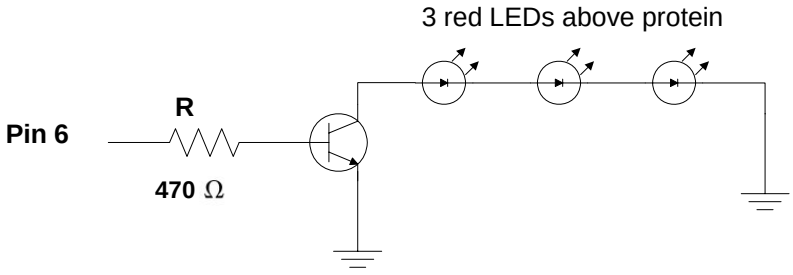


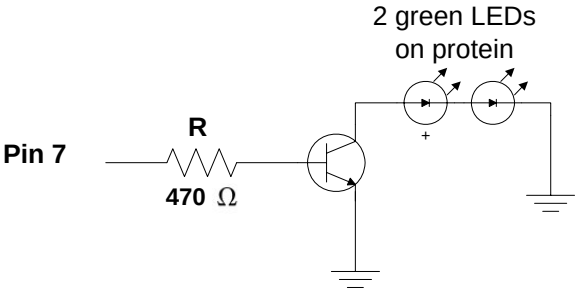


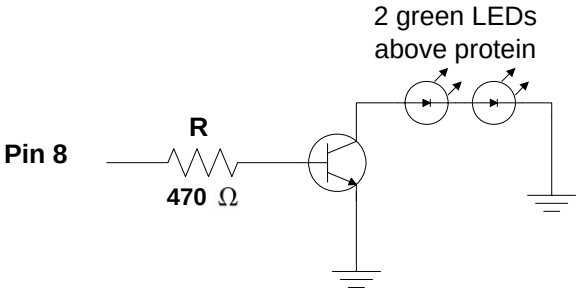


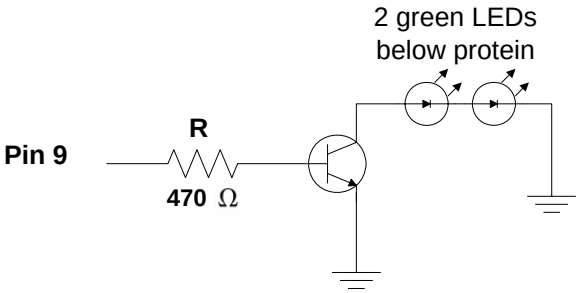

1 yellow LED
on protein
Pin 4
R
470 Ω


2 yellow LEDs
below protein
Pin 5
R
470 Ω


3 red LEDs above protein
Pin 6
R
470 Ω


2 green LEDs
on protein
Pin 7
R
470 Ω


2 green LEDs
above protein
Pin 8
R
470 Ω


2 green LEDs
below protein
Pin 9
R
470 Ω

Program code:

```
' {$STAMP BS2}
' {$PBASIC 2.5}

counter1 VAR Word
counter2 VAR Word
counter3 VAR Word
counter4 VAR Word
counter5 VAR Word
counter6 VAR Word


DO
PAUSE 1000

FOR counter4 = 1 TO 150
PULSOUT 14, 500
PAUSE 20
NEXT


PAUSE 2000
HIGH 1                  '3 red below protein on
HIGH 8                  '2 green above protein on
PAUSE 1000


LOW 1                   '3 red below off
HIGH 3                  '3 red in protein on
PAUSE 3000


FOR counter5 =1 TO 4          'caption servo
PULSOUT 15, 500
PAUSE 20
 NEXT


FOR counter3 = 1 TO 50          'servo #2 move back
PULSOUT 13,500
PAUSE 40
NEXT
```

```
FOR counter5 =1 TO 4
PULSOUT 15, 500
PAUSE 20
 NEXT


HIGH 4                  '1 yellow in protein led on
HIGH 5                  '2 yellow below on
PAUSE 2500


FOR counter2= 1 TO 30
PULSOUT 13, 1000
PAUSE 40
NEXT


FOR counter5 =1 TO 5
PULSOUT 15, 500
PAUSE 20
 NEXT


FOR counter1 = 1 TO 150          'servo# 1 close
PULSOUT 14, 1000
PAUSE 20
NEXT
PAUSE 10
LOW 3                   '3 red led in protein off
HIGH 6                  '3 red led above on
PAUSE 2500


FOR counter5 =1 TO 8
PULSOUT 15, 500
PAUSE 20
 NEXT


LOW  8                    '2 green above protein off
PAUSE 20
HIGH 7                  '2 green in protein on
PAUSE 2500
```

```
FOR counter5 =1 TO 6
PULSOUT 15, 500
PAUSE 20
 NEXT

PAUSE 500
 FOR counter5 =1 TO 7
PULSOUT 15, 500
PAUSE 20
 NEXT

LOW 4
PAUSE 2500                    '1 yellow led  in protein off
FOR counter4 = 1 TO 150          'servo #1 open
PULSOUT 14, 500
PAUSE 20
NEXT
PAUSE 2500
LOW 7                      ' 2 green in protein off
HIGH 9                     ' 2 green led below protein on
PAUSE 9000
LOW 5
LOW 6
FOR counter2= 1 TO 28
PULSOUT 13, 1000
PAUSE 40
NEXT
LOW 9

FOR counter6 =1 TO 26
PULSOUT 15, 1000
PAUSE 40
NEXT

PAUSE 7000                     'end of process
LOOP
```

RESULTS AND CONCLUSIONS:

- The Mechantronics rendering of the Na+ / K+ pump simulates how this complex cellular process functions in a simplified but effective manner..

- The use of different colored LEDs and brightness intensity, movement, and attachment in the protein channel make it easier for students to understand the movement of NA+ ions, K+ ions and phosphate in and out of a cell.

- The opening and closing of the protein channel, as well as the movement of the ATP-binding site on this channel make it easier to visualize and describe the conformational changes that protein channels undergo when energy is supplied by an addition of phosphate.

REFERENCES

- Ewart HS, Klip A: Hormonal regulation of the Na(+)-K(+)-ATPase: mechanisms underlying rapid and sustained changes in pump activity. Am J Physiol 269:C295, 1995.

- Johnson, G, Raven, P. Biology: Principles and Exploration. Active Transport. Holt, Rinehart and Winston. P.59-60, 81-82, 2001.

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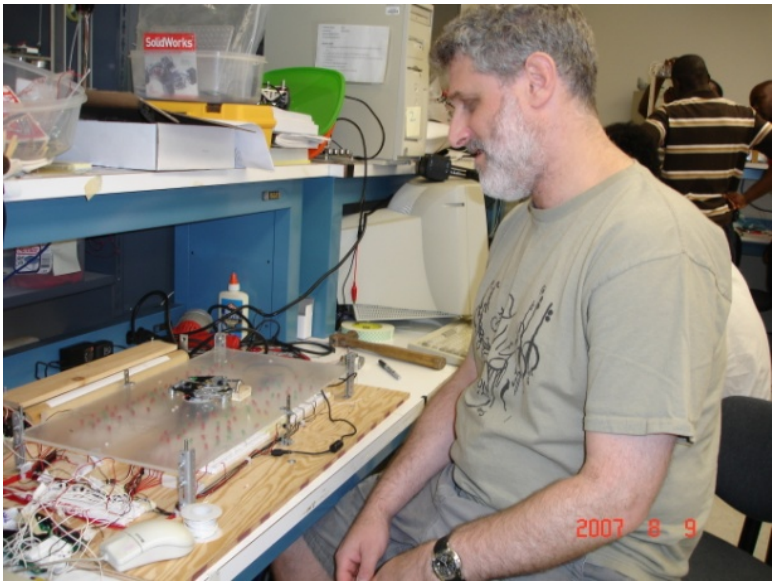
We would like to acknowledge the immeasurable contributions of several people without whom our completion of this challenging but valuable program and this project would have been unlikely:

- Praises and respect is accorded to **Professor Vikram Kapila** of the Mechanical Engineering department of Polytechnic University who posed the challenge and provided constant reminders. We have found the SUMMIT Program an excellent program for our own professional development.
- **Padmini Vijaykumar**: Words are not enough to thank her for her patience, for remaining with us even until 8 o'clock in the evening, Always around, anytime we need help, for the immeasurable assistance she gave us in understanding our program code, with the large number of circuits and for being there to constantly inspire, support, and encourage us.
- **Anshuman Pranda** who presented the topics to us, making them as simple as possible especially for most of us struggling to understand and apply them in the Lab. He was very accommodating, and saw to it that everybody had the materials they required to complete their project. You can be an excellent Professor in the future.
- **Nathan (Sang-Hoon) Lee** for the motivation and encouragement that we would be successful in this program.
- We are very grateful to each of the **TAs, Keith, Daniel**, Shing, Billy, Jared for their help and technical assistance during the short 7 days working with our project.
- **Mr. Alex Betti,** machinist extraordinaire, for refining our protein channel to improve its function.
- To **Elbert Narca** for his help in making our project visually attractive, painting our cell membrane layer, and improving our Powerpoint presentation.
- To those whose names have not been mentioned but who rendered numerous forms of assistance, thank you very much.
- Finally, we thank **Polytechnic University**, which was a most gracious host to us this summer, and the **New York State Department of Education** for funding this very valuable and expensive program. We will surely work to implement this in our classroom. Engineering can and should be introduced as early as possible to our students.
- To our families for their love, support, and understanding during the days we arrived home late while completing this project.
- Finally to **God** whose grace and strength surpass human understanding.

PHOTOS:



Why this servo is not moving the way it should be?

Its almost 8:00 PM and still smiling?

With Padmini our beautiful mentor.

Harold soldering the wires.

PHOTOS DURING THE PRESENTATION: