This paper presents a three-dimensional approach to look at the dynamic conformational changes in the switch I and switch II regions of the human *ras* protein using solid freeform fabrication and shape memory materials. The switch I and switch II regions consist of amino acids and is where the conformational changes occur which fall in the residue regions of 30-38 and 60-76. The energy used to cause the conformational changes in the switch regions involves the use of guanosine diphosphate (GDP) and guanosine triphosphate (GTP). GDP and GTP bind near the switch I region in which it takes affect throughout the whole protein. With the use of shape memory materials like NiTinol, and a resistive heating switch, the switch I and II regions will be able to show the conformational changes in a three-dimensional model. Shape memory materials are alloys or polymers that undergo a change of crystal structure at a certain temperature, once above that temperature whatever shape it holds, the polymer or alloy remembers it; when heated back to the temperature the alloy or polymer will slowly form back to the shape remembered. By creating this model it will be used as an educational tool to show how the *ras* protein works with respect to its on state and off state in such difficult signaling pathways. Upon conclusion of this research project there will be a three-dimensional model that shows the conformational changes of the switch I and II regions using an on/off switch simulated by the exchange of GDP to GTP.

**Keywords:** solid freeform fabrication, conformational changes, shape memory materials, ras

1. **Introduction**
   
   1.1. *ras* protein

   The human-RAS also known as *ras* p21 is a protein that plays an important role in signaling pathways. The reason this protein is being studied is to understand its shape and conformational changes. By understanding the proteins shape one will be able to understand how it works in the signaling pathway and its role in cancer.

   Ras has two forms, one which is its active state in which Guanosine Triphosphate (GTP) is bound and the other is its inactive state in which Guanosine Diphosphate (GDP) is bound. The process starts with a ligand or some other outside chemical messenger that attaches to a tyrosine kinase receptor causing it to autophosphorylate. When phosphorylated, the tyrosine kinase receptor has a binding site for a scaffold protein like DRK which sends out a son of sevenless (SOS) signal. Attached to the SOS is a nucleotide exchange factor GEF, which when activated, causes a G-protein, in this case *ras*, to releases the GDP and exchange it for a GTP which turns it into its active state. When *ras* is in its active state, it sends a signal downstream to where the signal goes through other proteins and makes its way down to the DNA causing a change in gene transcription (Figure 1).
When binding between GDP and GTP, *ras* goes through conformational changes in the switch I and II regions. Switch I and II consist of amino acids located between 30-38 and 60-76 in the primary structure of *ras*. Looking at *ras* in its GDP-bound state one can notice that the switch I, color pink in figure 2, is bent out of shape while the switch II, colored orange, consists of two loops. In its GTP-bound state, the switch I region of *ras* is bent out of shape but has slightly rotated, while the switch II region has partially unwound and has also rotated (Figure 2).1

1.2. shape memory alloy

Shape memory alloys (SMA) are metals that remember a certain shape at a given temperature(s). SMAs can be shaped in either one-way, obtaining a shape by being heated, or two-way, obtaining a shape by re-cooling. The type of SMA that was used for this project was NiTinol. NiTinol was discovered in the 1960’s and the name refers to two metals, Ni for *nickel* and Ti for *titanium*, and NOL which stands for *Naval Ordnance Laboratory* which was the place the NiTinol was discovered. NiTinol wire is used in a wide array of applications from medical procedure to cell phone antennas.
When a shape memory alloy goes through its shape change it also goes through a microscopic crystal lattice change (Figure 3). When the alloy is cooled it enters its martensite phase in which it has a start temperature, $M_S$, and an end temperature, $M_f$. When in the martensite phase the alloy is able to be easily bent and when bent it goes into the martensite deformed phase. When the alloy is heated it enters its austenite phase where it too has a start temperature, $A_S$, and an end temperature, $A_f$. Now when in the austenite phase it is the opposite of the martensite due to it is stiffness; thus it is not easily bent.

![Figure 3: A phase diagram of shape memory alloy that demonstrates the change in the crystal lattice structure when cooled and heated.](image)

1.3. protein data bank

The Protein Data Bank (PDB) is an online resource that contains data on published work on large molecules like protein and nucleic acids. The way the molecular structures are determined is through the use of x-ray crystallography by research groups. This is done by crystallizing a molecule and diffracting x-rays to determine the relative position of atoms in the molecular structure. The PDB files that were used for this project are 4Q21 and 6Q21. 4Q21 show the human RAS in its GDP bound state while the 6Q21 shows RAS in its GTP bound state. Both structural files were found and presented by Melburn et al.

2. Methodology

2.1. rapids prototyping the model

In order to create the physical model using rapid prototyping, first the PDB files were manipulated using Jmol. Jmol is an open source program to open molecules to color, shape, delete, and add supporting structures. The shapes that can be used in Jmol are wireframe, backbone, ribbon and cartoon. For this project a backbone with the size of 1.5 was used along with CPK coloring. CPK coloring is named after Robert Corey, Pauling, and was improved by Walter Koltun in which certain molecules have certain colors. Grey is for carbon, blue is nitrogen, red is oxygen, white for hydrogen, and yellow is for sulfur. The RAS molecule was colored all grey except for the switch I and II areas which were colored pink and orange to show the positions. After the use of Jmol the file was exported as a .jpeg file and sent to the Center for Biomolecular Modeling to be converted into a .ply file that could
be opened in the rapid prototyping center (RPC) program Magics. Magics is used to scale, hollow, cut, and allow for magnet holes to be inserted. Once the files were uploaded into Magics, holes were created to allow the SMA to attach to the model. The machine used to create the static parts of ras was the selective laser sintering (SLS) machine in the RPC with the Duraform PA plastic material. The SLS machine works by using a CO\textsubscript{2} laser that fuses together plastic, metal, or ceramic powers that are rolled over a platform layer by layer (Figure 4).

Figure 4: A picture representation of how the SLS machine works.

2.2. annealing NiTinol

There are three ways to anneal NiTinol, one-way, two-way shape memory effect (SME), and two-way stress induced martensite (SIM). One-way consists of bending the NiTinol to the desired shape and placing the alloy in 150 degrees Celsius water for 15 minutes, then immediately placing it in cold tap water. This allows for the alloy to take its set shape at a higher temperature and once cool go back to the original shape (Figure 5). Two-way SME is where the alloy is cooled below martensite finish temperature and bent to the desired shape, then placed in 150 degrees Celsius water until it is in its original shape. Two-way SIM is where the alloy is bent to desired shape and placed in cold tap water for 15 minutes and then immediately in 150 degrees Celsius water until it is in its original shape. The steps for the SME and SIM are repeated 15-20 times in order for two shapes to take place. The set shape takes effect in the SIM and SME when at a higher temperature and another at a cooler temperature (Figure 5).
3. Results and Conclusion

Unfortunately a model with the shape memory material was unable to be created. It seems that there may be too much titanium in the NiTinol, as it did not keep its shape when bent, but instead sprung right back to its original shape without a temperature change. However, there were full static models that were created successfully of ras along with the switch areas made alone which were used as a visual model to bend the NiTinol by hand (Figure 6). Along with the switch areas made and the full static model, a model with the switch areas removed was made in which it had hole for the NiTinol to fit in when fully annealed. In order for the NiTinol to go under a temperature change a switch box was also successfully made with the use of AA batteries, a battery holder with wires, and resistive wire. Also throughout the project shape memory polymers (SMP) were worked with but were found unusable, because they melted when heated with a wire. The shape memory effect did work with the use of hot water in the SMPs.

For future work there should be more testing on the SMA with respect to its nickel/titanium ratio by conducting temperature tests to see which proportions would work best for the model. Further testing with SMPs and different ways for it to be heated other than water should be looked into also.

Figure 6: The top left picture represents the full static model of ras, while the top right picture represents ras with the switch areas removed. The bottom left picture is the switch I area and the bottom right is the switch II area.

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5. References


