

Modeling Protein Dynamics of the H-RAS Molecular Switch Using Rapid Prototyping

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Abstract

The purpose of this research is to display protein dynamics of human *RAS* with the use of solid freeform fabrication, also known as rapid prototyping, and innovative shape memory materials. Such dynamics include the conformational change and resulting action of the protein. The majority of current educational models attempt to illustrate these processes with static representations. In comparison, a protein with shape memory would be dynamic, interactive and deformable. In *RAS*, the moving pieces of the model consist of two switch regions at amino acid residues 30-38 and 60-76 that undergo a large conformational change. By representing these two conformations in a single model, the active versus inactive state brought about by substrate binding may be uniquely compared. The substrates in *RAS* are energy molecules, guanosine diphosphate (GDP) and guanosine triphosphate (GTP), which binds near switch I and affects changes throughout the protein including the switch II region where effector molecules bind. Overall, such a model exhibits conformational change, substrate binding, and effector affinity. These processes are both conceptually important in the biological sciences and difficult to grasp using only text and two dimensional depictions. An educational tool that conveys a third dimension is therefore valuable. Unfortunately, due to the limited capability of materials and machinery, a fully dynamic model was not produced over the course of this project.

Keywords: protein modeling, *RAS*, conformation change, shape memory alloy, solid freeform fabrication

1. Introduction

Biochemical and molecular level sciences are increasingly popular fields of study as knowledge and technological development facilitate further discoveries. The understanding and determination of protein structures are especially important for research, understanding various biological processes, and drug design. It is therefore beneficial for students to receive a strong base of knowledge in these areas early in their educational careers. As it is, computer animations and illustrations commonly supplement lectures and text descriptions. A further compliment, such as physical models, will increase knowledge of basic principles by adding a spatial understanding to the learning process. The goal of this project is to create an educational tool that captures attention and displays a new element in rapid prototyped models – protein dynamics.

1.1. the H-RAS protein

The human *RAS* protein is used in this project for two reasons – it possesses a significant conformational, or shape, change and is of interest in current cancer research. *RAS* acts as a molecular switch – in its activated form it is complexed with guanosine triphosphate (GTP) and in its inactive form to guanosine diphosphate (GDP). The difference between the GTP and GDP complexed forms of *RAS* is a conformational change over 40 angstroms in length across the surface of the *RAS* protein. *RAS* anchors to the cell membrane via cysteine residues 181 and 184 toward the C-terminus¹ and plays an important role in a signal transduction cascade that controls cell proliferation, differentiation, and metabolism. The conformational change *RAS* undergoes alters the binding affinity of various effector molecules following *RAS* in the signal transduction pathway².

The two major regions of conformational change are the switch I and II regions consisting of amino acid residues 30-38 and 60-76 respectively. Switch I consists of loop 2 while Switch II consists of α -helix 2 and loop 4. In the GTP conformation, part of the switch II α -helix unwinds into a β -sheet. Switch II interacts with effector molecules. Of the two switch regions, residues Thr35, Gly60, and Gln61 interact with the γ -phosphate – it is the presence or absence of this phosphate that initiates other conformational changes, especially in the loop 2 region of switch I³. Figure 1 depicts the conformational change between *RAS* complexed to GDP and GTP with switch I colored pink and switch II colored orange. Ribbon and space filled depictions are shown to the left and right respectively.

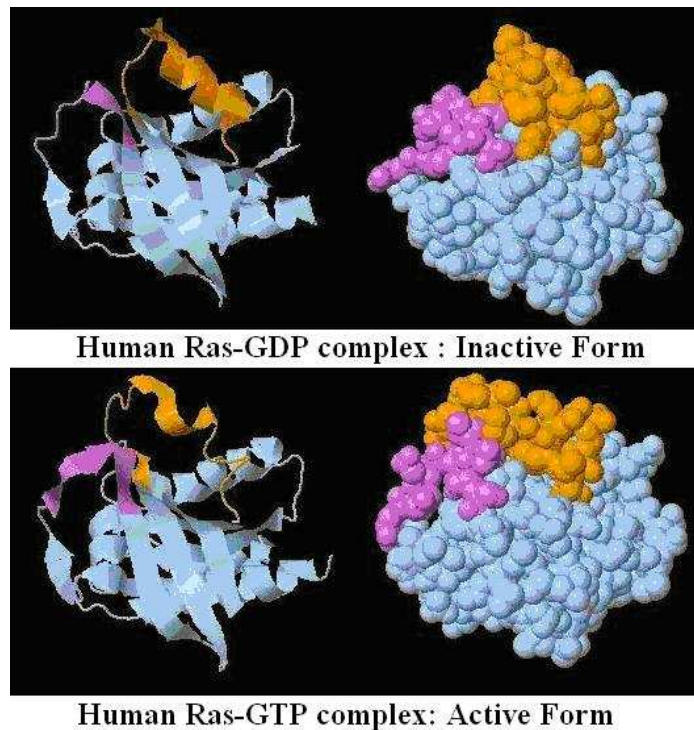


Figure 1. Ribbon and space filled depiction of two conformations⁴

RAS has a weak GTPase ability to hydrolyze the γ -phosphate, or third phosphate, of GTP transforming GTP to GDP. This means that *RAS* has the ability to switch from an on to off state. This may be accelerated by helper protein GTPase-Activating Protein (GAP) which interacts with both switch regions. In order to activate *RAS*, GDP must be replaced with GTP via the catalytic reaction of Guanine Nucleotide Exchange Factors (GEF)⁵. Figure 2 illustrates the interaction of these molecules with *RAS*. Figure 3 shows the *RAS* amino acids which interact with GTP.

Mutations in *RAS* occur in 20-30% of all human tumors and these are often point mutations at residue GLY12 and GLN61⁶. These point mutations result in the loss of *RAS*' hydrolyzing ability, i.e. *RAS*' ability to switch off. GTP becomes stuck to *RAS* keeping the protein in its activated conformation sending an unregulated signal for cell proliferation⁷. If this signal is not counteracted, the cell continuously divides resulting in tumors.

This project attempts to show the conformational changes in the switch I and II regions of *RAS* when complexed with GDP and GTP.

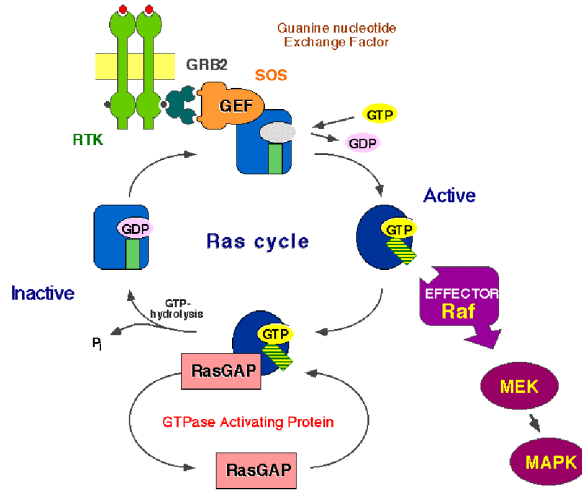


Figure 2. RAS interaction with GTP, GDP, GAP, and GEF⁸

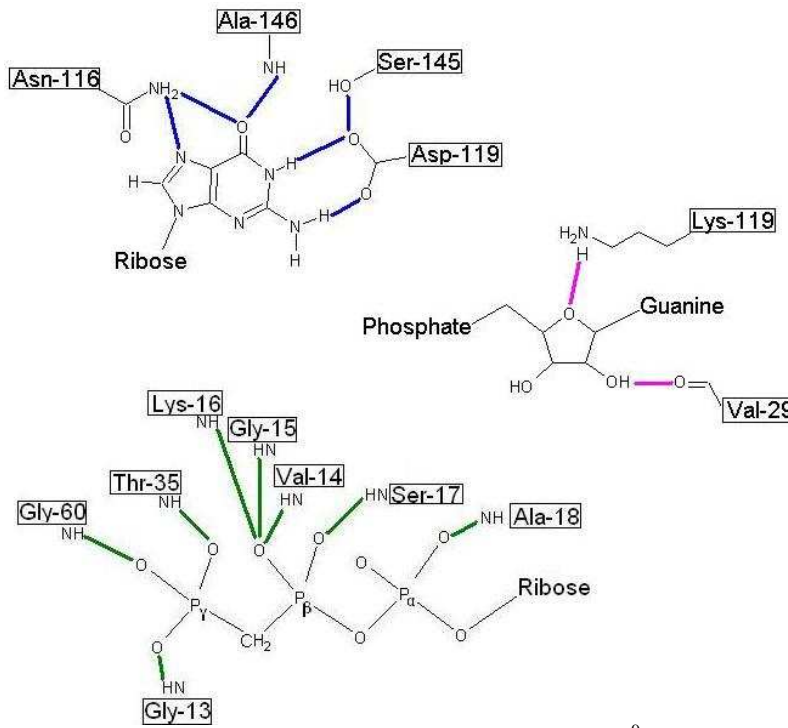


Figure 3. RAS residue and GTP interaction⁹

1.2. previous models

The Center for BioMolecular Modeling (CBM) of the Milwaukee School of Engineering (MSOE) creates unique molecular models with rapid prototyping, computer animations, and amino acid learning kits for educational purposes. Such tools help to clarify concepts with visual and spatial representations. Molecular models are extremely helpful but have thus far been static structures. Current models, due to the limits of certain rapid prototyping materials, have very little deformability and may break if dropped or subjected to excessive force. As such, conformational change is often shown by comparing two static models.

This project attempts to show this information in a single model capable of movement and deformability. Such modeling may also remedy a misconception that can be taken from the current models – that proteins are static instead of thermodynamic molecules shaped by strain energy via bonding, hydrophobic, and hydrophilic interactions.

1.3. shape memory alloys

An alloy is a combination of metal and other materials whose composition determines its physical and chemical properties. Multiple alloys show the shape memory effect with varying production costs, transformation temperatures, and susceptibility to corrosion¹⁰. Nitinol is the specific Shape Memory Alloy (SMA) of choice for this project consisting of 45% nickel and 55% titanium due to the temperature at which the memory effect is actuated¹¹.

The primary use of shape memory alloys in industry capitalizes on a superelastic property¹². This project instead takes advantage of another property, the ability of such an alloy to return to its remembered shape. This ability results from changes in the alloy's crystal structure or atomic arrangement. These crystal lattice structures can be seen in Figure 4. This rearrangement of structure is thermodynamic – the structure is the direct result of temperature and strain energy. Austenite is the high temperature form and martensite is the low temperature form. The tilt seen in the twinned martensite form alternates such that there is no visible shape change¹³.

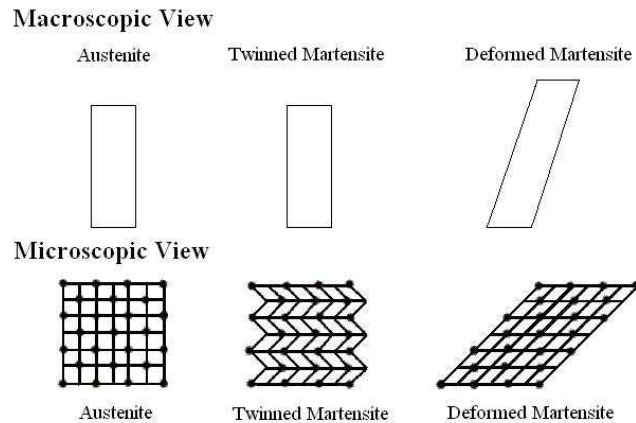


Figure 4. Crystal lattice structures which accounts for the properties of shape memory alloys

The austenite and twinned martensite states are macroscopically identical yet twinned martensite represents the structure occurring at cooler temperatures while the austenite requires higher temperature. The temperature separating the austenite from the twinned martensite state is called the transformation temperature. The martensite which has been physically deformed converts to the original austenite state by heat treatment. Figure 5 illustrates this interconversion.

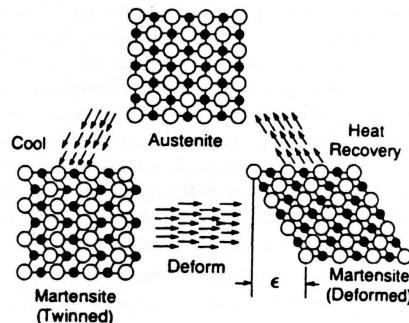


Figure 5. Change in crystal lattice structure in relation to temperature¹⁴

Nitinol must be annealed in order to fix a “remembered” state to the metal. This is done by heating the metal to 400-500°C. During the annealing process the alloy must be restrained to its desired shape during the heating process. Once annealed, Nitinol’s remembered structure can be recovered by heating the metal from 30-110°C. These temperatures vary by percent composition of the alloy¹⁵.

1.3.1. previous models using shape memory alloys

Two previous research projects at MSOE attempted the use of shape memory alloy NiTiNol to depict biomolecules – a 2001 project by Amoz Eckerson, “Shape Morphing Physical Biomolecular Models,”¹⁶ and 2002 project by

Christina M. Knihtila, “Dynamic Protein Model of the Abelson Tyrosine Kinase”¹⁷. In Christina Knihtila’s project this shape memory alloy complimented rapid prototyped biomolecules while Amoz Eckerson’s lacked the rapid prototyping element. This project differs by attempting to integrate these technologies into a single model.

2. Methodology

2.1. the protein data bank

The Protein Data Bank (PDB) is an online resource with data from published work in which the crystal structure of a biomolecular structure has been determined¹⁸. The PDB information on proteins includes files conveying three dimensions which can be manipulated and visualized using various software programs.

Research groups determine these structures using x-ray crystallography. This generally involves crystallizing a microscopic structure and scattering x-rays through the crystal as it is rotated. The result is a diffraction pattern which can be analyzed to determine a unique three dimensional atomic structure. This can be a difficult process that takes many years in isolating the desired biomolecules to be analyzed and then crystallizing the material. However, these structures are extremely useful to determine various properties, determine actions of a molecule, and design drugs. One of the most well-known examples of x-ray crystallography is Rosalind Franklin’s imaging of DNA which led to the determination of the double helix molecular structure¹⁹.

For this project, PDB files 4Q21 and 6Q21 were used²⁰. 4Q21 corresponds to human *RAS* complexed to GDP. 6Q21 corresponds to human *RAS* complexed to GTP. 4Q21 and 6Q21 were the structures found and presented by Milburn et al. in their paper “Molecular Switch for Signal Transduction: Structural Differences Between Active and Inactive Forms of Protooncogenic *ras* Proteins.”²¹

2.2. the computer model

Prior to rapid prototyping, PDB files were manipulated using various software programs. The first was RasMolTM which specifically deals with biological molecules. RasMolTM can change color, delete, add supporting structures, and change the type of depiction seen such as wireframe, backbone, electron density space filling, cartoon, and ribbon. It may also insert H-bonds, change size, provide information on amino acids, and manipulate ligands. Pieces for the static and dynamic models required RasMolTM to produce cartooned and backbone representations with wireframe ligands. RasMolTM also enabled the conversion of PDB files to different file formats required for manipulation in MagicsTM and rapid prototyping machines.

A second program, MagicsTM, was mainly used as an editing program in which files from RasMolTM were prepared for rapid prototyping. This program enabled the hollowing, scaling, cutting, and insertion of magnet holes.

2.3. rapid prototyping the physical model

Solid freeform fabrication, or Rapid Prototyping (RP), is a quick way in which to create a three-dimensional model which can then be used to create molds for industrial use, provide reference structures for surgery preparation, or aid further part design. In the two RP machines used in this project, each part is built layer by layer while the bed, upon which the part is being built, drops down for each successive layer to be created. As the part bed lowers, a second bed holding more material rises to be spread on the part bed by a roller to create the next layer of the desired part. Figure 6 demonstrates this process.

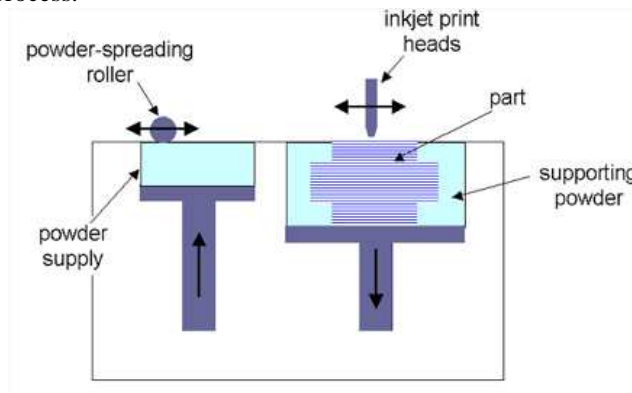


Figure 6. The Z-Corp 3D Printer²²

The two rapid prototyping machines used to model the *RAS* protein were the Selective Laser Sintering (SLS) and the Z-Corp 3D-Printer. The SLS machine was used to make a flexible backbone for the dynamic model and static models. This SLS machine fuses successive layers of plastic, metal, or ceramic powders via a CO² laser in a nitrogen environment. In the dynamic model, Somos201™ mixed with Duriflex™ materials were used to create flexible parts for the dynamic model²³. Nylon was utilized for the static models.

The Z-Corp 3D-Printer was used to produce fast, colored, and comparatively inexpensive static models and reference backbone pieces. This machine is commonly used for CBM models. Using plaster powders, a print head spreads ink and binder on each layer. The static models were built to compare structures and create structures along which the Nitinol wire was annealed. One difficulty with this machine is that it produces fragile parts²⁴.

2.4. annealing NiTiInol

In order to anneal NiTiInol, a temperature of 400-500 °C must be reached however, the more difficult part is the restraining of the alloy to the desired shape. During this heating process, the NiTiInol displays a great deal of movement and if not fully restrained, will anneal to an unspecific shape. After the annealing process the wire becomes a great deal more flexible. In order to more easily shape the wire, it was annealed once to a non-specific shape and then re-annealed by applying a greater temperature to the alloy²⁵. Restraints took multiple forms including Z-Corp 3D-Printer parts made from plaster, steel rods, aluminum wiring, and wooden dowels. One of these methods included the use of a machine called the Byron's Bender which introduces specific bends to steel rods according to information obtained from the PDB²⁶.

In order to actuate the desired temperature across the alloy, a resistive heating device was used as shown in figure 7. This effects a temperature change by running electrical current across the wire. Following difficulty with this machine, a furnace was used which is also shown in figure 7. The National wire used had a gauge of 750 micrometers.



Figure 7. Resistive heating device and furnace

2.5. the setup

The planned setup included three different elements. Switch I and II regions, as depicted earlier in pink and orange, made up the moving sections to be created with SMA. Each switch region consisted of two SMA pieces, one for each conformation. These pieces were run through soft latex tubing that had a softness of 40 ShoreA. Shore A is a standard for softness in a material – ShoreA 20-30 approximates the softness of a rubber band.

The rest of the protein was modeled with the SLS machine with the flexible Somos201™ and Duriflex™ mix. This SLS material was chosen for its flexibility, which was to support the dynamic sections of the protein, and its availability in the RP center at MSOE.

The third element was a series of insulated wires running throughout the rapid prototyped SLS part which connected the shape memory alloy, a nine volt battery, and on-off-on switch. This on-off-on switch completes the circuit to one SMA pair at a time, one switch I and one switch II wire, so that electrical current can be run across the alloy and create the shape memory effect with a temperature change of roughly 30-40 °C.

3. Conclusion

Multiple static models were successfully created with magnet ports for energy molecules. These were created as reference structures for the dynamic model. Figure 8 displays the Z-Corp 3D-Printer plaster models.

In the dynamic model, due to malfunctions and difficulty with technology, a moving model was not completed over the course of the project. One factor was the SLS part from Somos201™ and Duriflex™ which, after multiple attempts, failed to build. In order to create a hollow SLS part, the *RAS* protein was cut into twelve pieces which did not dependably align due to severe warping of material. These pieces were instead built with nylon which is inflexible. Figure 9 displays the SLS static nylon models. Durable™ material alone may be explored upon

availability in the future.

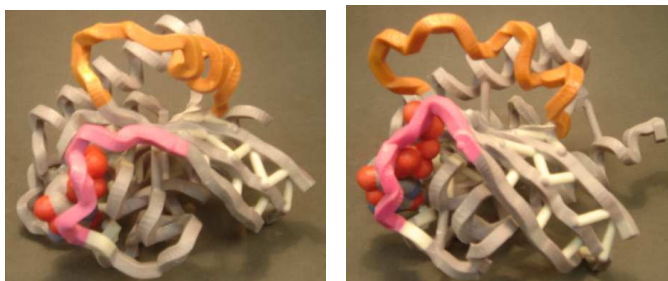


Figure 8. Plaster models of GDP-RAS complex to the left and GTP-RAS complex to the right

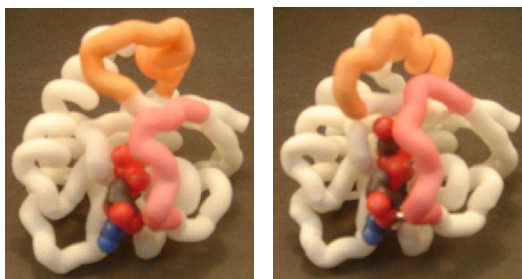


Figure 9. Nylon models of GDP-RAS complex to the left and GTP-RAS complex to the right

In addition, the resistive heating device failed late in the course of this project leading to the use of a furnace for NiTiInol annealing. A successful method with which to restrain the SMA wires in the furnace was not reached but, with more time, could be created by resizing the protein to accommodate the use of Byron's Bender for a steel jig.

Of concern was also the precise return of the shape memory alloy. Less severe conformational changes may not model well with this shape memory technology. Regardless, such a model can be created – the process is one that only requires patience and time. It is my hope that such a model may effectively be created at some point in a quick process so that it may be used to create multiple educational models.

4. Further work

Aside from creating the functional model, possibly with Duriflex™ SLS parts, an improvement to the RAS protein would be the use of further SMA to show all the conformational changes, including smaller changes, through the use of hinges or an extended shape memory backbone.

A very interesting idea that came about in the course of this project was modeling with the use of shape memory polymers (SMP) which could be injection molded – a much faster process. SMPs can be actuated with heat or magnets. Current research is investigating the actuation of SMPs with light. However, SMPs are still being heavily researched and are only currently available as raw material from a few companies that have accumulated enough patented material.

Though two-way memory may not be possible with SMPs it would likely offer unique possibilities such as a low transformation temperature that could be actuated by merely holding the molecule. Another possibility is to create a model which can be pulled out into a linear sequence representing primary protein structure and utilizing a single shape memory to refold the protein. This would be of great benefit if this could be controlled by varying temperatures to preferentially fold into the secondary structure followed by the tertiary structure of the protein.

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