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Center for BioMolecular Modeling

...where teachers come first

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**Center for BioMolecular Modeling**
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Abstract: The purpose of the 2005-2006 St. Dominic SMART team was to create a model of the T7 RNA Polymerase (T7 RNAP) using data from the Protein Data Bank and a visualization program called RasMol. T7 is a virus that infects bacteria, but its RNA Polymerase is a very important molecule to scientists. Scientists can use T7 RNAP to create large amounts of a specific protein for their research or to study transcription in vitro. This polymerase is also used by drug companies to produce human insulin for diabetics. Before this polymerase was used to make human insulin, people with diabetes had to use pig’s insulin. This caused problems because some of their immune systems rejected this insulin. T7 RNAP is not only useful to scientists, but also helps thousands of people affected with diabetes all around the world.

By designing this model, we were able to better understand the function and the chemical reactions of this polymerase. Our scientist mentor, Dr. Vaughn Jackson, gave us a presentation to help us understand how the molecule moves down the DNA and makes messenger RNA from the DNA template. He explained that T7 RNA Polymerase is shaped like a hand. There is a “thumb”, a “palm”, and “fingers”. The five-helix subdomain of the “fingers” domain pivots at a twenty-two degree angle. This movement threads the DNA through the hand-shaped part of the molecule and allows the enzyme to move down the DNA strand.

Dr. Jackson also showed us how the T7 RNA Polymerase makes the messenger RNA (mRNA) from the DNA. First a transcription “bubble” forms in the DNA separating the two strands and allowing the polymerase to transcribe one of the strands of the DNA. After this, one nucleoside triphosphate (NTP) floats into the T7 RNA Polymerase at a time to match with the template DNA’s nucleotide. The NTP moves into a position to be bound to the mRNA. The NTP has three phosphates contained in it that are then stabilized.

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by two magnesium atoms, an arginine side chain, and a lysine side chain. This process is called the insertion process. Our first structure of T7 RNAP, 1s76, shows the molecule during the insertion process. After the NTP has been inserted, it is bonded to the mRNA by a hydrolysis reaction in which two of its three phosphates are removed. The pyrophosphate product of this reaction leaves the active site. The polymerase then moves further down the DNA, and another NTP is brought into the active site. The second structure of T7 RNAP, 1s77, shows the molecule after the nucleotide has been added and the pyrophosphate has been detached from the NTP. The T7 RNAP molecule then continues this process until it reaches the termination point. At this point, the mRNA floats away and ribosomes attach to it. The ribosomes then produce the protein that is coded for by this mRNA copy of DNA.

**Structure of GNNQQNY, Sup35**

St. Joan Antida High School

**Students:** Joanne Chalhoub, Christine Dargis, Catherine Dornfeld, Erica Garcia

**Teacher:** Anne Staab

**Mentor:** Dr. Anita Manogaran, University of Illinois-Chicago

**Abstract:** In the study of protein function, one of the most important factors to the outcome of a molecule is the way it folds. If a protein does not fold properly, it will be unable to meet its function. The cells in our body contain ways to correct or rid our body of these misfolded proteins. In some cases, when a protein misfolds, it can aggregate and/or form fibers to create a prion, which then can induce other proteins to misfold and aggregate. Baker’s yeast has been known to have a number of prions including [PSI+], which is the prion form of the Sup35 protein. The Sup35 protein is important in translational termination, but when in the prion form, it loses the ability to efficiently perform this process. Within prion domain of Sup35, located in the N-terminus, a seven amino acid region, GNNQQNY, has been found to form a “cross-beta spine” structure, which is thought to contribute to the fibrilar structure of the prion. The researchers at University of Illinois-Chicago's Laboratory for Molecular Biology are interested in the structure of GNNQQNY because it helps in understanding the structural change of Sup35 from a normal form to the prion form. Furthermore, it can provide insight to how prions fold in human/mammalian systems.
Abstract: The SYP tyrosine phosphatase, also known as SHP-2, is an enzyme that cleans phosphates off of the amino acid tyrosine. SYP has three sections, including an amino-terminal SH2 domain, a carboxyl-terminal SH2 domain, and a phosphatase domain. The amino-terminal SH2 domain of the SYP tyrosine phosphatase is a specific section that regulates the cleaning of phosphates off of tyrosine amino acids. When resting, the amino-terminal SH2 domain of SYP binds with the phosphatase domain of the same molecule and keeps it "off." When active, the amino-terminal SH2 domain of SYP binds with phosphate-containing tyrosine amino acids on other proteins, which releases the phosphatase domain of SYP and enables it to turn "on."

The SYP molecule is an indispensable regulator of cell division and reproduction. In animals that are SYP deficient (for example, experimental mice in which the SYP gene has been "knocked out"), cell division is so abnormal that even the embryo does not develop. Therefore, no SYP-deficient animals, either mice or humans, ever develop. In animals in which there is something wrong with SYP, such as a mutation that results in irregularity in the binding of the amino-terminal SH2 and phosphatase domains to one another, the animal will develop but will have defects due to abnormal cell growth. This is because the SYP enzyme will never rest and will continuously take phosphates off of proteins that control cell reproduction. This can lead to accelerated cell division. Mutations in the gene that encodes SYP in humans result in a condition called Noonan’s syndrome, where abnormal bone growth and leukemia result.

Our model represents the amino-terminal SH2 domain of the SYP enzyme. The model includes a piece of another protein, the PDGF receptor, which binds to the amino-terminal SH2 domain of SYP and activates it. Differently colored amino acids in our model represent differences that arise from mutations in the SYP gene in people who have Noonan's syndrome.
Kinesin and Myosin Travel Along Molecular Rails
Madison West High School

**Abstract:** Kinesin and myosin are motor proteins (driven by ATP) that walk along molecular rails in order to transport “cargo” within cells; kinesin moves along microtubules, myosin move along actin microfilaments. Their cargos include proteins, membrane vesicles, and organelles. Myosin also produces the contraction of muscle cells. Although they have similar functions, the binding sites, the ATPase sites, and the cargo binding sites differ. There are many types of kinesin and myosin throughout the body that vary in load (bound to cables) and direction of motion. Kinesin is composed of two identical “feet,” attached to a cable, which walk using a hand-over-hand motion. Myosin is found as a string of connected feet that moves by lifting one foot, planting it farther down along the microfilament, pulling its cable forward, then lifting a foot up again and repeating the motion. There are several diseases linked to mutations of kinesin and myosin including Alzheimer’s disease, blindness, Retinitis Pigmentosa, Charcot-Marie-Tooth Disease, hypertrophic cardiomyopathy, and May-Hegglin anomaly. We used rapid prototyping technology to print the RP-Rasmol derived PDB files 2KIN (kinesin) and 1B7T (myosin) in order to compare and contrast the two proteins and to visualize more specifically how they move along their respective “rails.”

Cytochrome P450 and the Metabolism of Drugs
Kettle Moraine High School

**Abstract:** Cytochrome P450 is a microsomal membrane-bound protein that metabolizes xenobiotic compounds, most commonly pollutants, environmental compounds, and drugs; (continued on page 5)
and is located primarily in the liver. CYP2D6 is one of several liver P450s, and it primarily metabolizes pharmaceuticals, such as anti-arrhythmics, anti-depressants and beta-blockers. Research on P450s is extremely valuable to the pharmaceutical industry because CYP2D6 binds as substrates and inhibitors, drugs such as: codeine, quinidine, fluoxetine, ritonavir. This binding can lead to the metabolism of the drug, or the inhibition of the metabolism of another drug through interaction. Because of this, it is possible to predict how effective drugs will be, in terms of their lifetime in the blood, based upon how they fit into the CYP2D6 binding site.

The CYP2D6 active site includes a heme group and five amino acids that impact binding. These five include: glutamate 216 which is part of the F-G helix, aspartate 301 which is along the I-helix, and phenylalanines 102, 481, and 483. The heme present is responsible for carrying out hydroxylation on substrates. If a drug can fit well into the binding site, it will be metabolized before it has done its job; if a drug does not fit easily, interactions may result because it will block the binding site when another drug molecule needs access, so the system’s concentration of that drug will spike.

Our goal is to look at these amino acids and two helices to determine how drugs are cleared from the system through metabolism. We are using a homology molecule model that was made using Swiss-Model with CYP2C5 as a template.

Class II Major Histocompatibility Complex
Whitefish Bay High School

Students: Mary Cieslewicz, Nate Bolyard, Jay Lim, George Chao, Tae-Eun Kim

Teachers: Judy Weiss and Marisa Roberts

Mentor: Jack Gorski

Abstract: T cells play a vital role in immune responses. Each T cell has a unique receptor (TCR) that can recognize a fragment of a pathogen such as a virus or bacteria. Class II Major Histocompatibility Complex (MHCII) proteins and antigen presenting cells (APC) are also integral to the immune response. In the first step of this immune response, the APC, typically a macrophage or dendritic cell, “swallows” the pathogen through endocytosis and digests it. Next, the MHCII accepts a digested peptide fragment of the pathogen and carries it to the surface of the APC. Here, the MHC II displays the antigen to a T cell, whose receptor examines the complex. If the T cell receptor is able to bind to the antigen-containing MHC II, the T cell notes the presence of a foreign substance. B cells in the body also have receptors that bind to a pathogen, bringing it into the cell where it is digested and displayed on the surface of the cell by an MHCII protein. The TCR may also examine the
antigen displayed by an MHCII on a B cell. If this antigen matches the fragment earlier displayed by the APC, the T cell generates growth and maturation signals to allow the B cells to become antibody factories to fight the pathogen. Using the PDB file DR1-HA-TCR.pdb to construct our physical model of a TCR, we are studying the interactions between a TCR and an MHCII protein presenting a fragment of influenza virus. Our goal is to understand how the TCR binds to the MHCII and antigen, and how the virus might try to interfere with this interaction. Researchers like Dr. Gorski study T cell receptors because understanding a T cell’s ability to detect foreign substances will help reveal insights into how our immune system fights viruses and infection.

Gap Related Domain Of Neurofibromin and H-RAS p21 Structure
Wauwatosa West High School

Students: Shazia Ali, Jessica Huffmann, Annie Davidson-Keup, Ben Schrank
Teachers: Donnie Case and Mary Anne Haasch
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Abstract: According to the American Cancer Society, an estimated 1,368,000 Americans died of cancer and related complications in 2004. Cancer, which is characterized by an uninhibited growth of cells, is caused by mutation of genes that regulate cell growth. Mutations fall into two major classes, those that cause activation of a growth activator (oncogene) or those that result in loss of function of a growth inhibitor (tumor suppressor). Our project involves the oncogene protein Ras and a tumor suppressor, NF1, which regulates Ras.

RAS is a signal transducer, a molecular switch with two states: an “on” state, which contains the GTP nucleic acid, and an “off” state, which contains the GDP nucleic acid. The “switch” is turned on by growth factor receptors such as epidermal growth factor (EGF) and results in Ras binding GTP. Once in the “on” state, RAS interacts with another growth activator, the Raf oncogene. Activation of Raf initiates a cascade of kinases that leads to an increase in gene expression and stimulation of cell growth.

To terminate the Ras activation signal, GTP is hydrolyzed to GDP resulting in Ras-GDP, the “off” state of Ras. This is accomplished with the help of a GTPase activating protein, NF1. The action of NF1 is to turn off Ras, hence it is a tumor suppressor. NF1 is also called Neurofibromin. The loss of NF1 via genetic mutations causes a cancer called neurofibromatosis. Neurofibromatosis is a genetic disorder, which is associated with the nervous system. It causes tumor growth, skin lesions, and bone deformities. Neurofibromatosis is generally inherited by birth, although 30 and 50 percent of new cases arise through spontaneous mutations. Symptoms include headache, facial pain, or facial numbness from pressure from the tumors.

Our project explores how the Ras protein interacts with NF1 to stimulate the hydrolysis of GTP to turn off the Ras oncogene protein. Scientists are interested in this interaction because it contributes to the understanding of cancer and potentially the design of drugs for Neurofibromatosis.
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