The MSOE Center for BioMolecular Modeling would like to acknowledge and thank the National Institutes of Health Science Education Partnership Award (NIH-SEPA 1R25OD010505-01) and the National Institutes of Health Clinical and Translational Science Award (NIH-CTSA UL1RR031973) for their support in funding the 2015-2016 SMART Team and MAPS Team programs.

The MSOE Center for BioMolecular Modeling would also like to thank Marquette University for hosting the SMART Team and MAPS Team Poster Session.

Phenylalanine Hydroxylase based on 3j8u.pdb

Life is Complicated...
Let’s deal with it!
The Local Mentor-Matched SMART Team Program

The Local Mentor-Matched SMART Team Program introduces students and their teachers into the “community of science” through partnering with a scientist to explore a research topic in depth. The local program consists of three phases: the Qualification Phase, the Research and Model Design Phase, and the Presentation Phase. During the Qualification Phase, MSOE staff direct the students to complete a series of tasks under the guidance of their teachers to demonstrate their knowledge of the basics of protein structure/function and computer visualization of biomolecules. Upon successful completion of the qualification exam, each team enters into the Research and Model Design Phase where they are paired with a research scientist mentor. The students work with their mentor to investigate a selected aspect of the mentor’s research topic by reading primary citations, visiting their mentor’s lab, writing an abstract and designing a molecular model. A poster session during the Presentation Phase provides the students with an authentic experience to communicate their molecular story through the poster and 3D model they have designed. These presentations encourage the SMART Team students to develop their communication skills – skills that will benefit them regardless of their future career paths.

Local Mentor-Matched SMART Teams and Their Advisors

Audubon High School       Brian Coffey & Julissa Chavez
Brookfield Academy        Robbyn Tuinstra, Ph.D.
Brookfield East High School Emily Barman捷
Brown Deer High School     David Sampe
Cedarburg High School      Karen Tiffany
Cudahy High School         Dan Koslakiewicz & Dean Billo
Divine Savior Holy Angels  Stacey Strandberg & Scott Fleischmann
Greenfield High School     Julie Fangmann
Hartford Union High School Mark Arn Holt
Laconia High School        Joshua Demski
Marquette University High School Carl Kaiser & Keith Klestinski
Messmer High School        Mark Zachar & Justin Spaeth
Saint Dominic School      Donna LaFlamme
Westosha Central High School Jonathan Kao
National SMART and MAPS Team Programs

In the National SMART (Students Modeling a Research Topic) Team program, teams of students and their teachers from across the country delve into the molecular world, explore science as a process and work closely with a scientist mentor from a research institution near their school. Together they explore and learn the structure-function relationship of a specific protein in relation to the research being done in the mentor’s laboratory. They then design a model of the protein of interest that highlights important structural features that allow them to present their molecular story using a physical representation of the protein that they developed.

Modeling A Protein Story (MAPS) is a new program that allows teams of students and teachers to model the unique structure-function relationships of a specific protein and then develop a research question to explore. The selected protein for the 2015-2016 school year was *aquaporin*. Teams have utilized online resources and hands-on models to explore the unique structural features of the water channel that allow rapid passage of water across the cell membrane while preventing the passage of small ions and other chemicals. Teams then “map” out a path to explore through which they will gain a better understanding of how specific aquaporin isoform structures confer functions unique to the needs of various tissues and organs, such as the eye, brain, kidney, skin, fat, liver, pancreas, etc. as well as exploring how specific mutations can result in dysfunction and disease.

The National SMART and MAPS team programs are available to schools across the US and Canada. New this year, we have scheduled a number of regional presentation events to serve as a capstone experience for the teams and introduce the teams to cutting edge research. In addition to teams who presented at the Experimental Biology conference in San Diego earlier this month, National SMART and MAPS teams are presenting their work this spring at events in Milwaukee, New York, Boston, and Denver.

**Presenters and Page Number**

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MAPS

Brookfield Central High
DC Everest High
Waukesha STEM School
Whitefish Bay High
Wisconsin Virtual Learning
According to the National Council on Alcoholism and Drug Dependence, 1 in every 12 adults suffers from alcohol abuse and alcoholism. Alcohol abuse is associated with impaired judgment and cognitive functions and can ultimately be lethal, killing almost 75,000 people a year. The N-methyl-D-aspartate receptor (NMDAR) protein is a major target of alcohol action in the brain. The Audubon High School SMART (Students Modeling A Research Topic) Team has designed a model of NMDAR using 3D printing technology to investigate structure-function relationships. NMDARs, responsible for multiple cognitive functions, are composed of several domains including, the amino terminal domain, a ligand binding domain, a transmembrane domain, and an intracellular domain. NMDARs bind glutamate, a major excitatory transmitter, transporting signals from neuron to neuron through the synapse. Glutamate binds to NMDAR on the postsynaptic cell, the cell that receives a signal, which then opens the ion channel allowing sodium and calcium to enter and stimulate the cell. Alcohol passes through the blood brain barrier, a filtering mechanism of capillaries in the brain, and binds to specific amino acid side chains in the NMDAR. When alcohol is present, NMDAR’s function is limited because the ion channel gate is restricted from opening. When alcohol binds to sites in the transmembrane domain, it blocks the entry of sodium and calcium into the neuron and hinders synaptic transmission via the NMDAR. Research has shown possibilities for multiple approaches to medically treat alcohol abuse and alcoholism. Understanding the structure-function relationship between NMDAR and alcohol should help us learn how to better manage alcohol abuse and alcoholism.

Cataracts, the clouding of the eye, affects vision in a majority of the elderly. Specifically, 70 percent of Caucasian Americans over 80 suffer from cataracts. By studying Aquaporin-0 (AQP0), the water channel specialized to the eye, scientists can gain a better understanding of cataract formation. AQP0, located on the membranes of fiber cells in the lens of mammalian eyes, regulates water concentration in ocular lens cells. In doing so, AQP0 induces changes in the shape of the lens in a process known as accommodation. According to one prominent theory, water intake causes ciliary muscles to contract, tightening the lens and altering focal length. When aquaporin fails to correctly facilitate accommodation, structural proteins called crystallins aggregate and form cataracts. An important aspect in the regulation of AQP0 is the gateway protein calmodulin (CaM). One theory suggests CaM acts as a "plug" to inhibit water intake. A more recent theory suggests that CaM causes AQP0 to "twist" and tighten the pore, shutting off water flow. The Brookfield Central MSOE CBM MAPS Team used 3D imaging technology to examine the structural and functional relationships between AQP0 and CaM. Failure for CaM to properly manage aquaporin functioning may be a primary cause of cataract development. Much of recent research has dealt with the relationship between CaM and AQP function. Through careful examination of CaM induced effects on AQP function, key details were revealed about AQP and lens interaction which can, in turn, potentially produce vital findings in AQP-caused anomalies such as cataracts.
Imagine life threatening problems which appear when you cannot regulate water properly. Problems such as, cerebral edema, tumors, or seizures arise when the protein Aquaporin-4 (AQP4) is insufficient in the brain. AQP4 maintains the cerebral water balance, potassium uptake, movement of glial cells, and communication between astrocyte cells. These functions aid in the water transport of the brain, helping to sustain a normal amount of water. Water is important to the brain because it provides energy to produce hormones and neurotransmitters. AQP4 water channels are found in the cell membranes of astrocytes and ependymal cells, and osmosensory areas of the hypothalamus. Water transport through AQP4 depends on concentration gradients across the membrane, which is where the rate of transport is determined by astrocytes. An AQP4 deficiency would slow down the migration of astrocytes, playing a role in two types of cerebral edema called vasogenic and cytotoxic. With vasogenic brain edema, less water is transported out of the brain, while with cytotoxic brain edema, less water is transported into the brain. Someone with cytotoxic brain edema may have difficulties functioning, although the risk for brain swelling and its effects are higher with vasogenic brain edema. The Wisconsin Virtual Learning MAPS Team (Modeling a Protein Story) used 3D technology to model the structure, primarily highlighting amino acids 127, 55, 27, and 92 derived from the protein data bank file: 3GD8. More research into the function of AQP4 could possibly serve as a therapeutic source for a variety of cerebral disorders.

Throughout evolution, organisms developed differently from one another and thus defend themselves against viral attacks through the use of different systems. Animals defend themselves through complex immune systems which plants do not possess. Therefore, plants have a sophisticated silencing mechanism which detects and destroys double stranded RNA (characteristically viral) in a process called RNA interference (RNAi). Small interfering RNAs (siRNA) are cleaved from double stranded introns by an enzyme called DICER. In the plant, the role of siRNA is to target viral messenger RNA to be broken down via the RNAi pathway. P19 is a protein that is expressed by a family of viruses, tombus viruses, in order to suppress the RNA silencing pathway. P19 binds as a dimer to small RNA duplexes of a specific length and prevents them from participating in further steps of RNA silencing. The sequence-independent binding results from hydrogen-bonding and electrostatic interactions between the 8 saddle-like β-sheets of the homodimer and the sugar-phosphate backbone of the siRNA. P19 binds specifically to 21-nt siRNAs due to a higher affinity caused by two tryptophan residues located on an α helix at the N terminus of each monomer, which provide end-capping interactions to stabilize this length in the binding pocket of the dimer. In humans, small RNAs such as siRNA or microRNAs (miRNAs) regulate the expression of messenger RNA (mRNA). Therefore, P19 could be used as a tool to control gene expression by inhibiting the siRNA. Developing P19 as a biotechnological tool offers unique opportunities for increasing our understanding of small RNA biology and its role in human disease. It serves as a molecular caliper, which selects siRNAs with a high degree of size selectivity (20-22 nucleotides), but without regard for the sequence. This could be applied to detect small RNAs from biological samples such as human blood. Engineering P19 has allowed binding to human miRNA which can be used to detect human miRNAs or sequester miRNAs in human cells to allow a desired change in miRNA activity.
Comparison of Structure and Mechanism in BoNT/A1 and BoNT/A2: Implications for Therapeutic Applications
Brookfield Academy Upper School
Poster #3

Authors: Elizabeth Cinquegrani, Mark Morris, Leah Wang, Harry Kalsi, Shalini Gundamaraj, Sumeri Kohari, Lena Ding, Srimayi Mylavaramu, Alex Dortzbach, Juliana Vollmer
Teacher: Robbyn Tuinstra, Ph.D.
School: Brookfield Academy Upper School
Mentors: Madison Zuverink, Ph.D. Candidate and Joseph T. Barbieri, Ph.D.
Medical College of Wisconsin, Department of Microbiology and Molecular Genetics

Botulinum neurotoxin type A (BoNT/A) is a potent neurotoxin, causing muscle paralysis in the host by blocking the release of the neurotransmitter, acetylcholine, from motor neurons associated with skeletal muscle. Despite this toxicity, BoNT/A is used pharmaceutically as a treatment for numerous neurological diseases, including migraines, dystonias, and as an anti-wrinkle agent in cosmetic surgery. BoNT/A is one of seven serotypes of botulinum (A-G), which, along with tetanus toxin, are produced by several species of Clostridium. All clostridial neurotoxins, such as BoNT/A, are di-chain proteins, consisting of a 50-kDa light chain, the catalytic domain, connected by a disulfide bond to a 100-kDa heavy chain. The catalytic domain is one of three major proteins, consisting of a 50-kDa light chain, the catalytic domain, connected by a disulfide bond to a 100-kDa heavy chain. The catalytic domain is a Zn-dependent protease that cleaves SNAP-25, one of three major proteins present in the SNARE complex. The SNARE protein complex is required for the fusion of synaptic vesicles with the neuronal membrane. Cleavage of SNAP-25 inhibits fusion of synaptic vesicles to the plasma membrane to inhibit acetylcholine release and muscle contraction, leading to flaccid paralysis. Brookfield Academy SMART (Students Modeling A Research Topic) Team students modeled BoNT using 3D printing technology, highlighting amino acid residues associated with protease activity in the catalytic domain, and GT1b and SV2 interactions within the receptor binding domain. This model is meant to help understand and communicate structure/function relationships of BoNT/A and promote potentially therapeutic uses of the toxin.

Modeling Aquaporin-1 Expression in Autosomal Polycystic Kidney Diseased Kidneys
Whitefish Bay High School
Poster #25

Authors: Jieun Heo, Ryan Davis, Mary Claire Potter, Kaitlyn Jackson, Gam K., Evan Davis, Mohammed Shaheer
Teachers: Katie Brown and Paula Krukar
School: Whitefish Bay High School

About 500,000 people in the United States and six million people worldwide are affected by autosomal polycystic kidney disease (ADPKD). The disease drives nearly half of ADPKD patient to end-stage renal or kidney failure. The role of the human kidney is to remove toxins from the bloodstream and other body fluids through the creation of urine. In this function, the kidney is greatly aided by aquaporin-1 (AQP1). AQP1 is the membrane-inserted water-transporting protein in cell plasma membranes in kidney proximal tubules where transepithelial water transport is facilitated, playing an important role in reabsorption of water from the renal tubular fluid. AQP1 also insures against the entry of acidic substances through the cell membrane, helping regulate the internal balance of the cell and thus the organ. The Whitefish Bay High School MAPS (Modeling A Protein Story) Team used computer programing to model AQP1 and its integral role in the movement of water across membranes. In a normal human kidney, AQP1 is localized in proximal tubules and descending thin limbs of Henle’s loop. However, as the disease progresses to an end-stage disease, AQP1 can be seen on cysts that develop on the tubules. AQP1 is most abundant in normal adult kidneys and in early stage ADPKD kidneys, but is reduced in the membranes of end-stage ADPKD kidneys. Thus, after an initial increase in AQP1 expression to combat the stresses of the disease on osmotic equilibrium, there is a significant decrease in the total water expression as ADPKD progresses because cyst size increases and AQP1 expression decreases. Therefore, study of AQP1 expression can aid in finding more targeted solutions to the highly penetrating ADPKD.

Authors: Elizabeth Cinquegrani, Mark Morris, Leah Wang, Harry Kalsi, Shalini Gundamaraj, Sumeri Kohari, Lena Ding, Srimayi Mylavaramu, Alex Dortzbach, Juliana Vollmer
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Regulating Jeans with Genes – A Protein Driven Pathway to Appetite Suppression
Westosha Central High School
Poster #24

Authors: John Dietz, Jared Holloway, Trevor Millhouse, Connor Muff, Victoria Salerno, Zach Wermeling, Lucas Wysiatko
Teacher: Jonathan Kao
School: Westosha Central High School
Mentor: SuJean Choi, Ph.D.
Marquette University, Department of Biomedical Sciences

According to the World Health Organization, over 1.9 billion people in the world are overweight. Many of these people are overweight as a direct result of overeating. Obesity could potentially be treated by inducing an anorexigenic response, or loss of appetite. Several neurotransmitters are involved in signaling for appetite suppressing or stimulating responses. BDNF, the brain-derived neurotrophin factor, works as one of many appetite regulators in the ventromedial nucleus (VMN) in the hypothalamus. Injection of BDNF into rat VMN has been shown to drastically reduce appetite, increase metabolism, and ultimately lead to weight loss. Conversely, knockout of BDNF has been shown to lead to overeating and obesity. BDNF has a high affinity for the receptor Trk-B due to neurotrophins having variable binding domains. The Westosha Central SMART (Students Modeling A Research Topic) Team modeled BDNF as it binds to Trk-B. Once bound, transduction sends a cascading signal that can stop feeding behavior. Various signals have been shown to affect BDNF concentration leading to anorexigenic and orexigenic responses. BDNF concentration correlates with increased leptin, a hormone made by adipose tissue, physical activity, and glutamate. Concentrations of BDNF decrease as the neurotransmitter GABA increases. Understanding BDNF production and signaling provides researchers with a better opportunity to control expression and elicit a specific response in an attempt to treat obesity or eating disorders.

C-fos: Got Your Back!
Brookfield East High School
Poster #4

Teacher: Emily Barmantje
School: Brookfield East High School
Mentor: Audra Kramer, M.S.
Marquette University, Department of Biomedical Sciences

Climbing the stairs, speed-walking to class, jumping around the room — all are instinctually effortless. Spinal cord injuries change all of that. When a spinal column injury occurs, severed nerves prevent commands from being sent to parts of the body beyond the injury site, leaving a person with little hope of a full recovery. Our understanding of the mechanisms behind spinal injury rehabilitation is currently very limited. The transcription factor c-fos is an indicator of active neurons, making it possible to assess functional recovery in a rehabilitation program. The increased presence of c-fos within a group of cells’ nuclei can be compared in different animal rehabilitation program groups to determine ideal cells for specific spinal cord injury rehabilitation methods. C-fos works with other transcription factors such as c-jun, binding to DNA specifically on the 5'-TGAGTCA-3' sequence. For this to occur, c-fos and c-jun dimerize. Studying c-fos can aid in developing more advanced forms of spinal rehabilitation that aim on rewiring neural connections. The Brookfield East SMART (Students Modeling a Research Topic) Team designed a model of c-fos using 3D printing technology. The c-fos model focuses on the coiled-coil at the carboxy-terminal region. Further research of c-fos improves our understanding of tissues with limited regenerative abilities and allows us to utilize c-fos as a tool to assess functional tissue regeneration otherwise thought to be limited.
About 3-4% of people worldwide suffer from chronic pain. The United States spends $60 billion annually treating pain. Current treatments are ineffective; opioids are addictive and lose efficacy while pregabalin, a non-opioid neuropathic pain drug, only works in 30% of patients. Pain receptors (nociceptors) are activated chemically or mechanically. The nociceptor transient receptor potential cation channel, subfamily A, member 1 (TRPA1) is an ion channel located in the membranes of free nerve endings in the skin. TRPA1 is activated by mechanical stretching, and has many active sites where ligands (e.g. tear gas, mustard gas, and wasabi) can bind to open the channel. Once opened, Ca$^{2+}$ and Na$^{+}$ ions pass through, depolarizing the neuron. Structurally, TRPA1 is a tetramer, with each subunit containing several domains. In the pre-S1 domain, ligands bind covalently to the sulfurs at Cys621, Cys641, Cys665, and Lys710. The TRP-like domain opens for ion passage. The ankyrin repeat domain contains 16 recurring sequences of 33 residues, and may be involved with the stretch activation of TRPA1. In domains S1-S6, $\alpha$-helices hold the protein's shape and contain binding sites for agonists to open the channel and for antagonists, which prevent functioning. One antagonist, A-967079, binds in a pocket around Phe909, forming a wedge to prevent movement and function of TRPA1. Researchers aim to develop a TRPA1 antagonist that will help treat pain sufferers. The Brown Deer SMART (Students Modeling A Research Topic) Team constructed a model of TRPA1 using 3D printing technology to assist researchers in studying its structure and function.
Modeling Protein Phosphatase-1 Complexed With the Cyanobacteria Toxin Mycrocystin
Valders High School
Poster #21

Authors: Alyssa Christianson, Sanne DeBruijn, Cassidy Dehne, Abby DeMeyer, Ireland Fenlon, Jacob Krim, Jesse Linsmeier, Madison Storm, Evelyn Tapia-Campuzano, Mariah Ulness, Trevor Wenzel
Teacher: Joe Kinscher
School: Valders High School
Mentor: Rebecca Abler Ph.D, and Richard Hein Ph.D., University of Wisconsin-Manitowoc

The cyanobacteria species Microcystis aeruginosa produces microcystin (MC) toxins that can cause liver damage in animals and have resulted in the death of dogs and livestock. MC toxins are cyclic heptapeptides, and they inhibit the enzyme protein phosphatase-1 (PP1), which plays a critical role in reversing the action of protein kinases. Kinases catalyze phosphorylation, which generally promotes cellular activity. In contrast, PP1 catalyzes dephosphorylation, which can slow cellular activity. Balanced phosphorylation and dephosphorylation is critical to liver cell homeostasis, and is disrupted when PP1 is inhibited by MC toxins. MC binding to PP1 occurs through interactions at four sites on the PP1 enzyme. Two metal atoms of PP1 can coordinate via two water molecules with the glutamic acid residue of MC. The Cys273 residue of PP1 can form a covalent linkage with the Mdha side chain of MC. The hydrophobic Adda side chain of MC fits well in the hydrophobic groove of PP1. Finally, the MeAsp residue of MC hydrogen bonds with Arg96 and Tyr134 residues of PP1. The Valders SMART Team designed a model of PP1 binding MC using 3D printing technology to understand the relationship between MC toxins and PP1.

Opsin: To See or Not to See
Cedarburg High School
Poster #6

Authors: Arnholt, Abigail; Butt, Abigale; Griffin, Meggie; Janecek, Ethan; Kalmer, Isabelle; Ketelhohn, Lauren; Levy, Jake; Minerva, Nicole; Navarre, Dawson; Ratayczak, Miranda; Severe, Micaiah; Squires, Elizabeth; Wankowski, Joshua
Teacher: Karen Tiffany
School: Cedarburg High School
Mentor: Alison Huckenpahler, Ph.D. Candidate Medical College of Wisconsin, The Eye Institute

Colorblindness, a genetic disorder affecting millions of people worldwide, is caused by mutations in a retinal protein, opsin. Opisin is a G-protein coupled receptor which binds to light sensitive retinal. When light enters the cell, the retinal isomerizes from the cis form to the trans form, and opsin is activated; nerves then signal the brain. The opsin complex, sensitive to specific wavelengths of light, is identified by the wavelength that activates it; long (L), middle (M), or short (S). Mutations in opsin may lead to visual problems, including the inability to distinguish between red and green. The Cedarburg SMART (Students Modeling A Research Topic) Team has used 3D printing technology to construct a model of opsin which contains two monomers consisting of several helices that are stabilized by interactions between Lys231-Glu247 and Tyr223-Arg135. Two openings in opsin can be found in the retinal-binding pocket; one allows the cis form of retinal to enter and bind opsin, while another between allows the trans form to exit opsin. Common forms of colorblindness are characterized by mutations in amino acid residues. Three specific mutations responsible for the common red-green form of colorblindness, identified by using the single letter amino acid codes LIAVA, LIAVS, and LVAVA, involve the amino acids at positions 153, 171, 174, 178 and 180. These residues are particularly important for differentiating between M cone opsin and L cone opsin. Because the effect of different opsin mutations on the cell is unknown, a major research focus is to determine whether these mutations can be overcome to restore spectral sensitivity in colorblind people.
Antibiotic-resistant bacteria are common and hard to treat. There is potential to create synthetic antibiotics based on natural products like enduracidin and mannopeptimycin to fight drug resistant bacteria like MRSA.

MppP, an enzyme from *Streptomyces wadayamensis*, is required for the biosynthesis of L-enduracididine (L-end), a non-protein forming amino acid building block found in these and other antibiotics. MppP, the first step in the L-end synthesis pathway, functions to add an oxygen atom to the L-arginine substrate, and replace the α-amino group with a ketone to create 4-hydroxy-2-ketoarginine (4HKA). Hydroxylation is accomplished using a pyridoxal-5'-phosphate (PLP) cofactor, covalently bound by Lys221 to the enzyme, also held by Ser91, Asn160, Asp188, and Ser190 in the active site, which is modeled by the Cudahy SMART (Students Modeling A Research Topic) Team using 3D printing technology. During activity, PLP binds an L-arg substrate, held by Arg352, Asp27, and Asp227 as well as Thr12 and Glu15, performing a hydroxylation through which 4HKA product of the MppP-PLP dependent hydroxylation is synthesized. The 4HKA product of the MppP-PLP dependent hydroxylation is used in subsequent reactions in the pathway leading to the creation of the amino acid L-end. Thus, MppP is a critical part of a 3-enzyme team that is responsible for making L-end. The L-end will be incorporated into the different antibiotics by other enzymes. The conversion of L-arg to L-end will help researchers in the creation of new synthetic antibiotics to fight superbugs like MRSA.
Antibiotic Mechanisms and Resistance in Bacterial Topoisomerase II
Monona Grove High School
Poster #19

Authors: Sarah Fahlberg, Brad Hanson, Katie Klauser, and Sanaa Semia
School: Monona Grove High School
Teachers/Mentors: Tricica Windgassen, and Katarzyna Dubiel, University of Wisconsin-Madison, Department of Biomolecular Chemistry

Over the past fifteen years, a number of bacteria have displayed resistance to common antibiotics while few new antibiotics have been developed. Resistance to antibiotics is life threatening and a global issue that needs to be addressed. Because of this rise in resistance, Ciprofloxacin, a common antibiotic that binds at the active site of topoisomerase II, is becoming less effective. Topoisomerase II (TOP II) is an essential enzyme that is involved in the process of simultaneously cutting both strands of the DNA helix in order to separate replicating bacterial chromosomes and to manage DNA tangles and supercoils. When Ciprofloxacin inhibits TOP II, it does not allow the chromosomes to separate and instead the DNA breaks. Ultimately, the bacteria are not able to replicate or survive. Bacteria are prone to spread resistance to antibiotics because of their ability to replicate quickly. New research has found a drug that targets the active site of TOP II in a new way. Additional research is needed to further understand the mechanism of TOP II to help find out how drug resistance is developed. Once there is more information on drug resistance, there will be more opportunities to develop more new drugs. The Monona Grove SMART team modeled topoisomerase using 3D modeling and printing technology to use the TOP II structure to better understand the protein function. Understanding topoisomerase function can help develop new drugs and combat resistance.

Myobacterium Delirium: Inhibiting Leucine Biosynthesis to Starve M. tuberculosis
Divine Savior Holy Angels High School
Poster #8

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Teachers: Stacey Strandberg and Scott Fleischmann
School: Divine Savior Holy Angels High School
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The pathogen Mycobacterium tuberculosis represents a deadly threat to the worldwide population, especially poor, developing countries, as it kills approximately 2 million people each year according to the World Health Organization. Because of overuse and increasing resistance to current antibiotics, researchers are working to develop new drugs to more effectively treat tuberculosis. M. tuberculosis alpha-isopropylmalate synthase (IPMS) is a bacterial enzyme that catalyzes the production of leucine, an essential amino acid. When cellular leucine levels are low, the biosynthetic pathway of leucine is initiated by IPMS when three ligands; alpha-ketoisovaleric acid (alpha-KIV), acetyl-CoA which is not included in our model, and Zn$^{2+}$ interact at IPMS’s active site. IPMS has two distinct structural domains: an N-terminal alpha/beta catalytic domain which includes the active site and a C-terminal regulatory domain that includes the leucine binding site. As leucine levels increase in the organism, its biosynthetic pathway is shut down by leucine binding to an allosteric site in the C-terminal domain which inhibits the further production of additional leucine. The protein amino acids tyr554 and ala536 play a role in the interaction with the leucine ligand. The protein amino acids arg80, asp81, his287, his285, and thr254 interact with the alpha KIV. These interactions allow for the production of leucine through the initiation of the biosynthetic pathway of leucine catalyzed by IPMS. Researchers are working to design a competitive inhibitor that would interact at the allosteric leucine binding site and shut down the pathway, thus depriving M. tuberculosis of this essential amino acid. Without the ligands leucine, Zn$^{2+}$, and KIV the tuberculosis bacteria would die. The Divine Savior Holy Angel High School SMART (Students Modeling A Research Topic) Team modeled IPMS using 3D printing technology to investigate IPMS catalysis and feedback inhibition within the leucine biosynthetic pathway. The development of new drugs specific to M. tuberculosis offers a promising way to overcome the problem of antibiotic resistance and offers new tools to reduce life-threatening tuberculosis infections.
Aquaporins... Allowing (Just) Water to Go with the Flow
D.C Everest High School
Poster #9

**Teacher:** Bill Heeren
**School:** D.C Everest High School

Transporting the liquid of life, aquaporins carry out the vital function of supplying water within cells in almost all living entities. Discovered only a decade ago, aquaporins are channel proteins found in cell membranes that are responsible for the transport and filtering of water and specific molecules into and out of cells. Depending on their location and function, aquaporins may transport different varieties of compounds in addition to water, such as glycerin, to assist in certain organ functions or concentration of cells. Thus, aquaporins play a major role in diseases such as diabetes insipidus due to their regulation of intake and release of water from vital organs. There are thirteen different aquaporins expressed in humans; however, aquaporins are found in all living things, ranging from animals to plants to bacteria. Though there are several types of aquaporins, their structures are similar in composition. Aquaporin monomers consist of six long segments of helices and two short segments of helices, which encompass a narrow transmembrane channel through which water and other molecules travel in single file. Only 20 Å in diameter, this channel is just wide enough for water molecules to pass through. Within cell membranes, monomers assemble as tetramers, each monomer acting independently. They are able to transport molecules at an extremely high rate—just one human AQP1 molecule has a permeation rate of three billion molecules per second, the direction of which changes depending on the prevailing osmotic gradient. Aquaporins must be highly selective in order to perform their specific functions, and they achieve this on an atomic level. The aquaporin contains two separate filters to facilitate the selection of water molecules and the exclusion of everything else: an ar/R filter (blue), and an NPA motif (magenta). Its positively charged ar/R filter allows the aquaporin to both bind to water molecules and weaken the molecule's hydrogen bonds so it can more readily interact with the filter. Meanwhile, the NPA motif involves the local electrical fields of the atoms and amino acids along the water channel walls, which cause water molecules passing through the channel to rotate and orient themselves towards the atom’s positive charge. Because of the positive charge at the center of the channel, protons and cations are prevented from passing, thereby regulating the balance of protons inside and outside of the cell membrane.

PTP1B Inhibitors for Type 2 Diabetes Treatment
Messmer High School
Poster #18

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**School:** Messmer High School
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People with diabetes have difficulty regulating their blood sugar leading to the malfunctions of the heart, kidney, nerves, and brain if their blood sugar is too high. Most diabetics use insulin to control their condition. Insulin binds to its surface receptor in muscle and fat cells to trigger removal of sugar from the blood. Protein tyrosine phosphatase 1B (PTP1B) affects blood sugar regulation by dephosphorylating the insulin receptor and reducing its activity. Studying PTP1B can help us understand how its inhibitors can slow down the removal of phosphate from the insulin receptor, affecting the blood sugar regulation process. PTP1B normally functions to remove the phosphate group from the insulin receptor. Since PTP1B ordinarily reduces insulin receptor activity, blocking PTP1B could increase insulin sensitivity. The PTP1B active site has a highly positive binding pocket which binds to the highly negative phosphates on the phosphorylated insulin receptor. Many of the current inhibitors of PTP1B act by binding to this active site. However, these inhibitors have difficulty penetrating the cell membrane because of their formal charge, and are unable to inhibit PTP1B inside cells. LZP25 avoids this issue by not having a formal negative charge, but instead a polar area of similar size to phosphate. Binding to the PTP1B active site pocket (sites Ser216, Ala217, Ile219, Gln262, Gln266), its bulky side groups then prevent a key loop in the enzyme active site from closing. If PTP1B is inhibited in the insulin pathway by a potential drug based on LZP25, people who have type 2 diabetes as a result of insufficient insulin receptor activity could better regulate their blood sugar. If the insulin receptor would signal appropriately, the body’s normal control of blood sugar would improve, preventing problems with the heart, kidney, nerves, and brain. Messmer Catholic High School SMART (Students Modeling A Research Topic) Team has designed a model of PTP1B with LZP25 using 3D printing technology to investigate their structure/function relationships.
**Phenylalanine Hydroxylase (PheOH)**
*Marshfield High School (2) Poster #17*

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**School:** Marshfield High School

Nobel Prize winning author, Pearl S. Buck, gave birth to a daughter, Carol, in 1921. Unknown to her mother and the rest of the scientific community, Carol suffered from phenylketonuria, a genetic disorder that results in severe brain damage. This is caused by a mutation in the PAH gene, which codes for the enzyme phenylalanine hydroxylase (PheOH). Phenylketonuria is characterized by high levels of phenylalanine in the bloodstream, resulting from the inability of PheOH to hydrolyze phenylalanine into tyrosine. There are two leading theories to explain the severities of neurological damage resulting from PKU. One possible explanation is that tetrahydrobiopterin (BH$_4$) cannot hydrolyze tyrosine. The other theory examines the impact of high concentrations of phenylalanine inhibiting the transfer of tyrosine across the blood-brain barrier. Recent advancements in technology, such as newborn screening, allows geneticists to determine if the child has PKU.

**Not-So-Mighty Mitochondria: Neonatal Lethality Due to Drp1 Malfunction**
*Greenfield High School Poster #10*

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Cells require mitochondria to produce cellular energy, allowing work to be done. Defective mitochondrial function can impair proper cell function, even leading to neurodegenerative diseases, such as Parkinson’s and Alzheimer’s disease, and neonatal lethality. The defect stems from imbalances between mitochondrial fission (splitting) and fusion (combining), resulting in abnormal mitochondrial morphology. Dynamin related protein 1 (Drp1) is a GTPase (enzyme that breaks down GTP) that results in fission of mitochondria by forming tubular spirals around the outer mitochondrial membrane in order to split one mitochondrion into two. The Greenfield SMART Team modeled Drp1 using 3D printing technology to investigate its structure. The structure of Drp1 is ordered by the following four components: GTPase Domain (G Domain), Middle Domain, B-Insert, and GTPase effector domain (GED). The G Domain hydrolyzes GTP and interacts with other G Domains on neighboring Drp1s, while the Middle Domain is the primary site of dimerization. The B-Insert regulates Drp1 assembly, and the GED helps activate the G domain. Drp1 in the cytosol is a dimer; when recruited to the outer mitochondrial membrane Drp1 forms an oligomer. The specific amino acids, Met482, Glu490, Asn635, Asp638, Tyr628, and Lys642, help keep the dimer interface together due to salt bridge interactions. A mutation in Drp1, A395D, was identified as important for causing neonatal lethality due to improper assembly of Drp1 on the outer mitochondrial membrane. When Drp1 malfunctions, such as in the A395D mutation, the imbalance of fusion and fission leads to detrimental issues, such as neonatal lethality. Studying Drp1 could prevent unnecessary death and eventually lead to a better understanding of other Drp1-related neurodegenerative diseases.
Prostate cancer makes up 4.7 percent of all cancer related deaths. The exact cause of prostate cancer is unknown, but researchers have identified the leading risk factors, including excess testosterone in the body. Early treatments to reduce testosterone levels in patients involved surgical procedures. A particular heme-containing enzyme, known as CYP17, converts cholesterol-derived hormones into androgens including testosterone. Fe(III) is a key part as all reactions occur within this heme group. Pharmacological efforts, such as the drug abiraterone, decrease the efficiency of CYP17 catalysis and may present a valid treatment option for prostate cancer patients. Further efforts would be facilitated by gaining a better understanding of the mechanisms of CYP17 in the presence of its natural substrates, 17-OH progesterone and 17-OH pregnenolone. This enzyme generates an androgen precursor by catalyzing two sequential chemical conversions, the second one by an unknown process, which has been difficult to track. Using rapid-freezing, γ-ray exposure and Raman spectroscopy, the key reaction intermediates in this process are trapped and structurally defined. The Hartford Union SMART (Students Modeling A Research Topic) Team modeled the enzyme CYP17A1 with 3-D printing technology. Research efforts involving CYP17 could assist pharmaceutical companies in the development of treatments for prostate cancer.

The worldwide proliferation of bacteria resistant to common antimicrobials has been a cause for concern throughout the global medical community. Both overuse (during surgical procedures) and often misuse (in case of viral infections) of prescription antibiotics in developed and underdeveloped countries have been cited as main reasons for the development of antibiotic resistance in bacteria. The spread of the gene, New Delhi metallo-beta-lactamase-1, via the transfer of plasmids among bacteria is a prime example of the consequence of overuse of carbapenems in the Indian subcontinent. Although considered to be originated from a hospital in New Delhi, India in 2009, it has spread to European and North American hospitals. Structurally, the uniquely large and flexible active site of NDM-1 contributes to its ability to catalyze a wide array of antibiotics including beta-lactams and more specifically, carbapenems. The flexible loops of the protein allow extended spectrum activity. The enzyme inhibits these antibiotics by hydrolyzing the beta-lactam ring through a zinc-dependent multi-step process involving a bulk water molecule. Since carbapenems are generally considered last resort antibiotics to treat many Gram-negative pathogens, bacteria that possess the \( \text{blaNDM-1} \) gene are especially difficult to treat. Consequently, it is crucial to develop new antimicrobials to treat these newly emerged 'superbugs.'
Cardiovascular disease and its complications are the leading cause of morbidity and mortality in the United States. A myocardial infarction (MI) is caused by a blood clot in a coronary artery and results in loss of blood flow to the heart (ischemia). To prevent necrosis or cell death of heart tissue, ischemia is treated with reperfusion therapy, in which blood flow is restored. Reperfusion therapy paradoxically causes damage to the heart muscle cells via the activation of Nuclear Factor Kappa Beta (NFκB) which can trigger apoptosis or programed cell death, or via the generation of reactive oxygen species and subsequently further necrosis. The extent to which the transcription factor NFκB promotes cell death or even promotes survival is unknown. However, it is known to be a key inflammatory mediator that coordinates cell homeostasis under bodily stress, as with an MI or ischemia reperfusion (IR) injury. NFκB is complexed with Inhibitor Kappa Beta (IκB) and exists as a larger IκB/NFκB complex within the cytosol of the cell until inflammatory mediators activate an IκB kinase. Phosphorylation of this larger complex separates IκB from NFκB, which, in the heart, is composed of the subunits, p50 and p65, and complexes with DNA. To study the effect of NFκB inhibition on IR-induced apoptosis and necrosis, cultured cardiac myocytes received two hours of ischemia, and were reperfused with or without the NFκB inhibitor, N4-[2-(4-phenoxyphenyl)ethyl]-4,6-quinazolinediamine (QNZ). Caspase 3 activation and annexin V staining, measurements of apoptosis, revealed that inhibiting NF-κB caused a decrease in IR-induced apoptosis. Propidium iodide staining, a measurement of cell necrosis, revealed that inhibiting NF-κB caused an increase in IR-induced necrosis. Further studies into understanding the effect of NFκB inhibition on IR injury may help provide novel therapies.
Understanding α-Galactosidase A to Improve Fabry Disease Treatment
Laconia High School
Poster #13

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Fabry disease is a debilitating lysosomal storage disease. Early in life, patients with Fabry disease experience extreme pain, especially in the extremities (acroparesthesias). Patients are eventually at risk of developing kidney disease, heart failure, and strokes, all of which may lead to a premature death. The prevalence of Fabry disease in its severest form is estimated at 1:40,000; however, the prevalence of milder, later-onset forms may be as high as 1:3,600. Fabry disease is caused by mutations in the X-chromosomal GLA gene, which encodes the lysosomal enzyme, α-galactosidase A (α-Gal A). When α-Gal A activity is deficient, glycolipid substrates accumulate within lysosomes, leading to cellular, tissue, and organ pathology. To treat Fabry disease, patients are given enzyme replacement therapy (i.e., bi-weekly intravenous infusion of recombinant α-Gal A). Enzyme replacement therapy costs up to $300,000 per patient per year and commonly causes dangerous immune reactions in patients. A more cost-effective and safer treatment is desperately needed. The Laconia SMART (Students Modeling A Research Topic) Team used 3D printing technology to generate a model of α-Gal A. To be trafficked to lysosomes and to optimally degrade glycolipids, α-Gal A must be glycosylated and must dimerize. Therefore, critical residues are highlighted in the model, such as those involved in catalysis (Asp170, Asp231), N-linked glycosylation (Asn139, Asn192, Asn215), and dimerization (Phe273). Disulfide bonds, which stabilize α-Gal A tertiary structure, are also shown. Studying the structure of α-Gal A is critical in the design of a more potent therapy, which has the potential to reduce the cost and immunogenicity of Fabry disease treatment.

Investigating the Single-Stranded DNA Binding Protein Tetramer Interface
Madison West High School
Poster #14

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School: Madison West High School
Teachers/Mentors: Tricia Windgassen, and Katarzyna Dubiel, University of Wisconsin, Madison Department of Biomolecular Chemistry

All cellular life that has been discovered contains DNA, an essential molecule that carries the genetic instructions for life. Single-stranded DNA binding proteins (SSBs) play a crucial role in the replication, recombination, and repair of DNA. During replication, SSB coats single-stranded DNA (ssDNA) to protect it from degradation or prevent secondary structures that impede genome maintenance processes. Research suggests that SSB proteins perform their functions in three different binding modes: (SSB)$_{65}$, (SSB)$_{35}$, and (SSB)$_{56}$. Of the different binding modes, the SSB$_{65}$ binding mode is thought to be used during recombination and repair while the SSB$_{35}$ binding mode may be used for replication. However, due to lack of experimental data there is debate on the exact roles of the binding modes. In bacteria, as well as humans, SSB is an essential protein. Replication Protein A (RPA) is a homologue of bacterial SSB protein and studies in mice have shown that a dysfunction of RPA can lead to DNA damage and ultimately cancer. More importantly, the structures of bacterial SSB and eukaryotic RPA are very different, making bacterial SSB a possible antibiotic target. The Madison West High School SMART (Students Modeling A Research Topic) Team modeled single-stranded DNA binding proteins using 3D printing technology to further investigate the SSB binding modes and the bacterial SSB interface. By utilizing the properties of SSB proteins, scientists can develop antibiotics to target bacterial SSB proteins and create drugs to effectively combat cancer. Because of SSBs vital role, more research should be conducted so that various disorders can be combated.