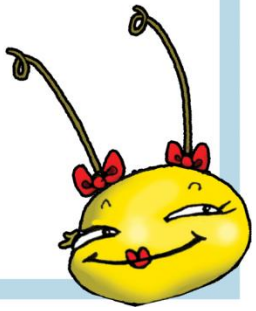
MSOE Center for BioMolecular Modeling
SMART TEAMS
2012-2013

CTSI
ADVANCING HEALTH THROUGH RESEARCH AND DISCOVERY

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
Supported by the National Institutes of Health

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 2012-2013 SMART Team program.

The MSOE Center for BioMolecular Modeling would also like to thank the Medical College of Wisconsin for hosting the SMART Team Poster Session and Final Presentations.



MSOE Center for BioMolecular Modeling
SMART TEAMS
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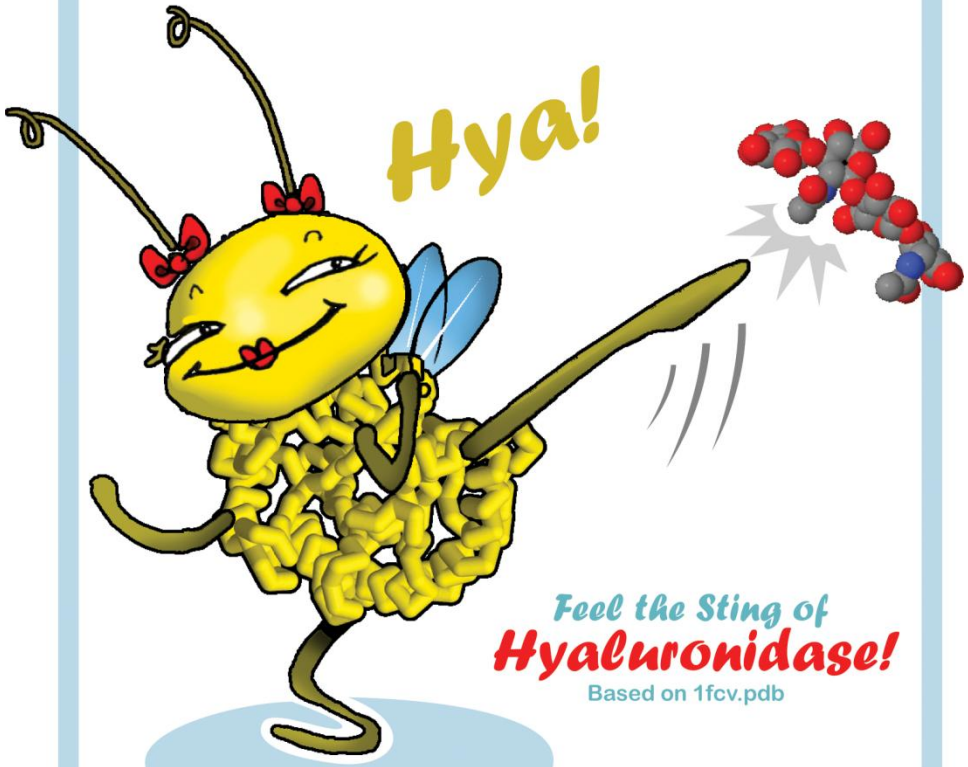
SMART Team Presentations

Session 1: 9:00 - 11:30am
Session 2: 12:00 - 2:30pm

Medical College of Wisconsin
Saturday, March 16th

http://cbm.msoe.edu/stupro/smart/local/index.html



Order of Presentations

Center for BioMolecular Modeling

Shannon Colton, Ph.D.	SMART Team Program Director
Gina Vogt, M.S.	SMART Team Program Director
Margaret Franzen, Ph.D.	Program Director
Mark Hoelzer	Lead Designer
Tim Herman, Ph.D.	CBM Director

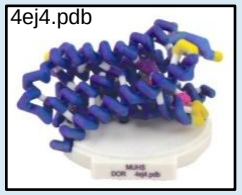
Center for BioMolecular Modeling Staff

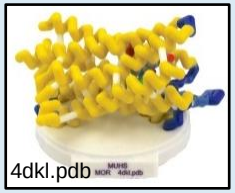
Cory Brody	Jessica Knurr
Nicole Fischer	Mike Morey
Nika Gigliotti	Ian Pavelich

Special Thanks for the time and effort from…

SMART Team Teachers	SMART Team Mentors
Fran Grant and Dan Goetz	Richard Robinson, Ph.D. and Halli Miller, M.S.
Julie Fangmann	Elizabeth Worthey, Ph.D.
Donna LaFlamme	William Jackson, Ph.D.
Jodie Garb	Rashmi Sood, Ph.D.
Robbyn Tuinstra, Ph.D.	Madhusudan Dey, Ph.D.
Joe Kinscher	Eric Singsaas, Ph.D.
Cindy McLinn	Duska Sidjanin, Ph.D.
David Sampe	Dara W. Frank, Ph.D.
Dan Koslakiewicz	Joseph Barbieri, Ph.D. and Andrew Karalewitz, Ph.D.
Paula Krukar, Katie Brown, Lisa Krueger, and Marisa Roberts	Robert Peoples, Ph.D.
Basudeb Bhattacharyya, Ph.D. Candidate	James L. Keck, Ph.D.
Becki Van Keuren	Shama P. Mirza, Ph.D.
Louise Thompson	Sanjib Bhattacharyya, Ph.D.
Steve Plum	Anil Challa, Ph.D.
Karen Tiffany	Audra Kramer, Ph.D. Candidate and Nashaat Gerges, Ph.D.
Carol Johnson and Meg Garland	Nicholas R. Silvaggi, Ph.D.
Kevin Paprocki and Tyler Reed	Vishwakanth Y. Potharla, Ph.D.
Mary Anne Haasch	Joseph McGraw, Ph.D., and Cameron Patterson, Doctor of Pharmacy Candidate
Jonathan Kao	Mark McNally, Ph.D.
Keith Klestinski and Carl Kaiser	Christopher W. Cunningham, Ph.D.

One Indole Ring to Rule Them All: How Modeling of Naltrindole Bound to the Delta Opioid Receptor Can Aid the Development of Novel Analgesics



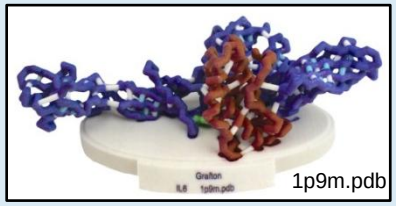


Marquette University High School

Authors: Ernst Arnhold, Nicholas Bell, Theodore Boesen, Nathan Boldt, Alexander Borden, Judson Bro, Marlon Douglas, John Fuller, Quinlan Furumo, Christian Gummin, Andrew Keuler, Daniel Kim, Alexis Martinez, Daniel Moldenhauer, Ian Mullooly, Ryan Nelsen-Freund, Daniel Ogunkunle, Luis Ortega, Scott Palmersheim, Thomas Sabatino, Benjamin Schwabe, Ryan Sung, Karsten Trzcinski and Cade Ulschmid
Teachers: Keith Klestinski and Carl Kaiser
School: Marquette University High School, Milwaukee, Wisconsin
Mentor: Christopher W. Cunningham Ph.D., School of Pharmacy, Concordia University

According to the Institute of Medicine, 116 million Americans currently suffer from chronic pain, costing our nation over $500 billion annually. As such, the use of pain-killing drugs like morphine and oxycodone has increased dramatically over the past decade. Analgesic effects are produced through agonism, or activation, of the body's mu (MOR) and delta (DOR) opioid receptors, which are G-coupled protein receptors. Tolerance, the decreased analgesic effect of MOR agonists after prolonged use, is a major problem facing opioid pain management. A drug that antagonizes, or inhibits, DOR can greatly reduce the development of tolerance to MOR agonists, offering new pain therapy potentials. One example of a selective DOR antagonist is naltrindole (NTI), which has a similar structure as morphine, except for a cyclopropylmethyl group on its nitrogen substituent and a bulky indole group. The large indole ring interacts with the W318 residue on MOR but is able to bond with W284 residue on the DOR, producing DOR-selective antagonism. Co-administration of NTI with morphine represents a potential new approach to producing analgesics with less tolerance. Understanding the structure of this ligand and enzyme may lead to structure based drug design. The Marquette University High School SMART Team is modeling naltrindole bound to DOR using 3-D printing technology.

TB or Not TB: That is Our Question The Role of Interleukin-12 Receptor in the Immune System and Preventing Tuberculosis



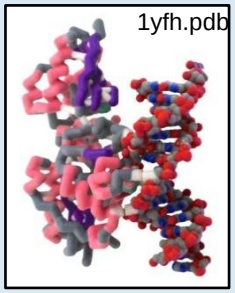
Grafton High School

Authors: Grace George, Brandon Itson-Zoske, Lelaina Evans, Mariah Fox, Megan Alascio, Molly Schmidt, Shabi Haider and Brendon Konon
Teachers: Fran Grant and Dan Goetz
School: Grafton High School, Grafton, Wisconsin
Mentors: Richard Robinson, Ph.D. and Halli Miller, M.S., Department of Microbiology and Molecular Genetics, Medical College of Wisconsin

According to the National Network for Immunization, the first tuberculosis (TB) vaccine was given in 1921 and has been administered to over 4 billion people. Unfortunately, the vaccine is not as effective as it once was because tuberculosis is becoming increasingly resistant to antibiotic treatments. As a result, new methods of treating and/or preventing this disease are underway. One method that could be used to prevent tuberculosis is to study the Interleukin-12 Receptor (IL-12R) an essential protein in initiating the immune response. When a pathogen invades the host, T-helper cells send signals to initiate an attack against the pathogen. IL-12, a cytokine, binds to IL-12R, which can signal macrophage activation to mount an attack against the invading pathogen. Some people have a mutated IL-12R which causes the patient to be infected by tuberculosis upon vaccination with BCG which contains live bacteria. IL-12 is a heterodimer with two glycoprotein subunits, p40 and p35, that are bound to the IL-12R via two disulfide bonds. If the IL-12R is mutated, the IL-12 will not bind properly to IL-12R, and the Helper T Cell's immune response to destroy the TB Cell will not commence. Although IL12R has not yet been crystallized, the Grafton SMART Team (Students Modeling A Research Topic) modeled gp130, a homolog of IL12R, using 3D printing technology. Ser 122 and Trp142 are highlighted on our model because this is where gp130 binds to IL-6, the homolog of IL-12.

The Future of Whole-Genome Sequencing: MGMT Mutations in a Family Could be Linked to Cervical Cancer

Greenfield High School

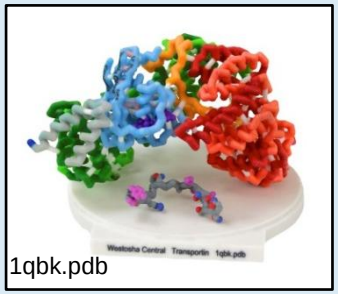


Authors: Panfua Thao, Robin Sandner, Hannah Flees, Joey Krasovich, Morgan Borchardt, Francis DeLeon-Camacho, Alyssa Gerwig, Zoe Osberg and Mary Wojczulis
Teacher: Julie Fangmann
School: Greenfield High School, Greenfield, Wisconsin
Mentor: Elizabeth Worthey, Ph.D., Human and Molecular Genetics Center, Medical College of Wisconsin

In 2008 the CDC reported 4,008 cervical cancer-related deaths in the US. Researchers at MCW used Whole Genome Sequencing technology to sequence the DNA of a mother and daughter diagnosed with a rapidly progressing form of cervical cancer. Identifying the genetic underpinnings could explain how their cancer developed and progresses, and help develop a specific treatment for this cancer. One candidate gene,O-6-methylguanine-DNA-methyltransferase (MGMT), is a DNA repair enzyme that removes a methyl group from methylated guanines via a cysteine residue (Cys145), creating a normal guanine that properly pairs with cytosine in DNA. Alteration of MGMT, such as the mutations found in this family (Ile143Val and Lys178Arg), may prevent this reaction, leaving improperly pairing methylguanines. Altered MGMT may lead to an increased rate of DNA mutations, which may lead to accumulation of mutations altering molecules responsible for regulation of cell growth, leading to cancer development. Understanding MGMT's structure, specifically at these altered positions, will assist MCW researchers in determining whether the mutated MGMT is a likely cause of disease. The Greenfield SMART Team (Students Modeling A Research Topic) modeled MGMT using 3D printing technology to analyze the likely effect of these mutations to understand its possible role in the development of cervical cancer.

Transportin' with Transportin (Trn1): A Nuclear Import Mechanism

Westosha Central High School

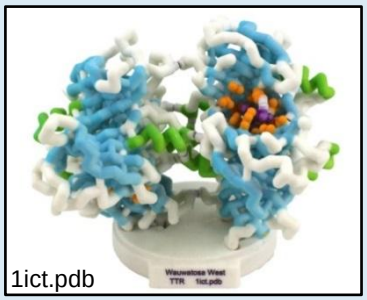


Authors: Julia Alberth, Jonah Arbet, Nick Bielski, Monica Ceisel, Sam Colletti, Evan Kirsch, Mitchell Kirsch, AJ Reeves and Julia Williams
Teacher: Jonathan Kao
School: Westosha Central High School, Salem, Wisconsin
Mentor: Mark McNally Ph.D., Department of Microbiology and Molecular Genetics, Medical College of Wisconsin

Proteins manufactured in the cytoplasm play an important role in nuclear processes such as RNA splicing. Immediately after transcription, precursor (pre-) mRNA contains introns that are removed in making mature mRNA. Splicing proteins like hnRNP A1 (A1), manufactured in the cytoplasm, are transported into the nucleus and influence RNA splicing decisions. Some proteins in eukaryotic cells use the receptor Transportin (Trn1) for import. Cytoplasmic Trn1 is found in a configuration that allows for the pick-up of cargo proteins. A1 has a nuclear localization signal (NLS) to which Trn1 can bind. Once bound, the Trn1/A1 complex enters the nucleus through a nuclear pore. The protein Ran, when associated with GTP, binds to the complex and causes a loop of approximately 60 amino acids to move and expel the cargo. With the cargo delivered, Trn1 returns to the cytoplasm as a Trn1/RanGTP complex. GTP is then hydrolyzed into GDP signaling Ran to release Trn1. The amino acid loop returns to its original position allowing for another round of transport. The model constructed using 3D printing technology by the Westosha Central HS Smart team in cooperation with MSOE features Trn1 in complex with RanGTP, the mechanical state of cargo unloading. The NLS of A1 can be modified such that Trn1 cannot bind and deliver A1 to the nucleus. Failure of A1 to reach the nucleus results in altered splicing of mRNA, which can lead to diseases like cancer. Therefore, targeting the interaction between Trn1 and its cargo may provide an option for treating diseases.

Transthyretin (TTR): Carrier of Thyroxine and Its Evil Twin (Environmental Pollutants)

Wauwatosa West High School



Authors: Zaynab Hassan, Madeline Jordan, Leah Rogers, Zoe Stack, Kayla Thao, Cheung Wongtam and Aleksandra Zielonka
Teacher: Mary Anne Haasch
School: Wauwatosa West High School, Wauwatosa, Wisconsin
Mentors: Cameron Patterson and Joseph McGraw, Ph.D., School of Pharmacy, Concordia University

Transthyretin (TTR) is a carrier protein in the blood that binds to and transports the thyroid hormone thyroxine throughout the human body. The thyroid hormone is necessary for fetal development and metabolism regulation. TTR is a tetramer formed from two dimers. Ala-108, Ser-117, Thr-119, Lys-15, Leu-17, Thr-106, and Val-121 all play a role in binding the thyroxine in a hydrophobic channel formed where the two dimers come together. Polybrominated diphenyl ethers (PBDEs), found in flame retardants, which are in numerous household products, are converted in the body to hydroxy-PBDEs. Hydroxy-PBDEs mimic the shape of thyroid hormones allowing them to bind with TTR. Hydroxy-PBDEs can have a stronger affinity to bind to TTR, disrupting the transport of the thyroid hormone necessary for developmental and metabolic processes. An initial study shows a possible correlation between high levels of PBDEs and hypoplastic left-heart syndrome, a condition found in four out of 10,000 newborns (Lucile Packard Children's Hospital at Stanford) in which the left side of the heart does not fully develop. Wauwatosa West SMART Team (Students Modeling A Research Topic) modeled TTR using 3D printing technology.

2A Protease from Human Rhinovirus 2

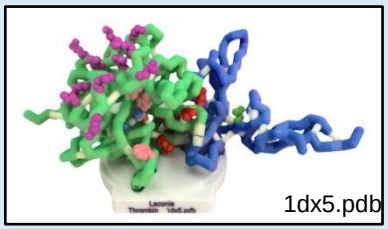
Saint Dominic Middle School

Authors: Sara Achatz, Luke Brown, Thomas Brzozowski, Jessica Diez, Danny Drees, Maddie Illman, Brian Jerke, Andy Kahler, Lauren Kohl, Vincent Marchese, Harrison Ott, John Otten, Kyle Phelps, Elizabeth Rowen, Brianne Sherman, Taylor Venuti and Elena Valentyn
Teacher: Donna LaFlamme
School: Saint Dominic Middle School, Brookfield, Wisconsin
Mentor: William Jackson, Ph.D., Department of Microbiology and Molecular Genetics, Medical College of Wisconsin

Human rhinoviruses (HRVs), a major cause of the common cold, usually produce mild illness, but in children they can trigger serious asthma exacerbations requiring hospitalization. HRVs belong to the Picornavirus family and like their close relative, the poliovirus, their short single-stranded positive-sense RNA genomes code for one polyprotein that is cleaved by the virus' two proteases into several viral proteins. The St. Dominic S.M.A.R.T. Team has modeled the 2a protease ($2a^{pro}$) of HRV2 using 3D-printing technology. $2a^{pro}$ is a homodimer with an active site and structurally essential zinc ion in each chain. $2a^{pro}$ promotes viral replication in host cells by shutting down both protein synthesis and nuclear-cytoplasmic import and signaling. By cleaving eIF4G (eukaryotic initiation factor 4G), $2a^{pro}$ prevents localization of host mRNAs to ribosomes, which are then free to synthesize the viral polyprotein using the viral RNA's IRES (internal ribosome entry site). Nuclear-cytoplasmic signaling and import is inhibited when $2a^{pro}$ cleaves specific nucleoporins (nups) in the nuclear pore complex (NPC). Proteolytic damage to Nup 62, Nup153, and Nup98 prevents the host cell's first responder, activated NFκB (Nuclear Factor kappa B), from being imported into the nucleus where it turns on stress genes needed to signal warnings to immune system. The HRV-A, HRV-B, and HRV-C species differ in their abilities to cleave both NPC nups and eIF4G. HRV-A and HRV-C 2a proteases are more efficient at cleaving Nup 62 and eIF4G *in vitro* than HRV-B 2a proteases. Since HRV-A and HRV-C are also significantly better at triggering asthma exacerbations in children than HRV-B, it has been proposed that the effectiveness of a virus strain's 2a protease can predict the likelihood of that strain to cause asthma exacerbations.

Cascading into the Thrombin-Thrombomodulin Complex: Comprehending a Substitution in Thrombomodulin

Laconia High School



Authors: Cole Tidemann, Brady Beekman, Amanda Leichtfuss, Alex Parsons, Jonathan Opheim, Ashley Garb and Noah Henke
Teacher: Jodie Garb
School: Laconia High School, Rosendale, Wisconsin
Mentor: Rashmi Sood, Ph.D., Department of Pathology, Medical College of Wisconsin

According to the Centers for Disease Control every year more than 60,000 Americans die of excessive blood clots or thrombophilia, and many more suffer from this disorder. Clotting disorders pose unique problems for women because of their impact on reproductive health and pregnancy. Thrombin, a protease that cleaves fibrinogen and activates platelets, plays a key role in the generation of blood clots. Several mechanisms prevent excessive thrombin activity. Thrombin that diffuses away from the clot binds to its receptor, thrombomodulin. Thrombomodulin-bound thrombin can no longer generate blood clots. Instead, it cleaves and activates Protein C which shuts down further thrombin generation. The substitution Gln387Pro in thrombomodulin inhibits Protein C activation and interferes with this feed-back inhibition. The Laconia SMART (Students Modeling A Research Topic) Team used 3D printing technology to model thrombin in association with thrombomodulin. Amino acid residues within the catalytic site (Ser195, His57, and Asp102) and the two substrate binding sites, exosite I (Lys36-Arg77), and exosite II (Arg 93-Lys240 of thrombin, involved in these interactions are highlighted. Understanding the relation between structure and function of the thrombin-thrombomodulin complex may lead to new therapeutics for clotting disorders.

Cut, Copy, & Mutate: EcoRI and its Function in Genetic Engineering

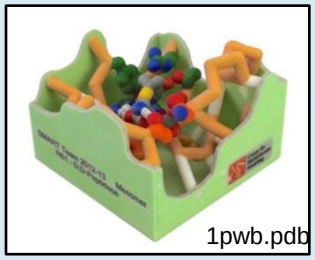
Milwaukee Academy of Science

Authors: Cameron Bester, Norris Campbell, Jonte Jackson, Jessie Jones, Tim Jones, Jailyn Kendrick, Stephon Phillips and Eddie Walls
Teachers: Kevin Paprocki and Tyler Reed
School: Milwaukee Academy of Science, Milwaukee, Wisconsin
Mentor: Vishwakanth Y. Potharla, Ph.D., Department of Biological Sciences, University of Wisconsin-Milwaukee

While farmers plant insect resistant corn, millions with diabetes inject themselves with the hormone, insulin. Despite the extraordinary differences between these practices, they have a common root: genetic engineering. Genetic engineering allows genes of interest to be moved from one species to another to create a desired protein or trait. This is accomplished through using restriction enzymes to cut DNA at a specific recognized sequence. Bacteria naturally use restriction enzymes to shred and destroy viral DNA. One of these restriction enzymes, EcoR1 endonuclease, is commonly used to genetically engineer insulin. In the early 1900s, insulin extraction and purification from a cow's pancreas was a time consuming and expensive process that yielded only a small amount of the hormone. More efficient production of insulin occurred in the 1950s when EcoR1 was used to cut the insulin gene from the human genome. Insertion of the gene into the genome of the bacteria, Escherichia coli did not disrupt normal bacterial division but did reprogram the bacteria to produce human insulin which could be collected for use. To investigate the structure of EcoR1, the Milwaukee Academy of Science SMART (Students Modeling A Research Topic) Team used 3D printing technology to design a model of the protein. EcoR1 is a homodimer composed of two polypeptide chains. Amino acids Asp91, Glu111, and Lys113 bind to the DNA sequence, GAATTC and cut between the guanine and adenine, allowing for gene insertion. Successfully engineered bacteria will now be able to use the inserted DNA sequence to create the desired protein. Genetic engineering with EcoRI is used to transfer genes between two organisms, whether it be bacteria, corn or even humans, thereby unlocking a variety of useful genetic combinations.

R61 D,D-peptidase bound to a Helen-1 Penicillin Substrate or One “Hel”-en of an Antibiotic

Messmer High School



Authors: Sonia Sosa-Gonzalez, Michaun Cobb, Ngozi Osadame, Isioma Osademe, Kyler Campbell, Merari Marin, Jhordy Rios Llamosa, Brigitte Rios Llamosa and Kasaundra Jones
Teachers: Carol Johnson and Meg Garland
School: Messmer High School, Milwaukee, Wisconsin
Mentor: Nicholas R. Silvaggi, Ph.D., Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee

Although antibiotics like penicillin save lives, antibiotic-resistant bacteria are a growing issue. According to Purdom (2007), over 70% of infections acquired by hospital patients post-admission, are resistant to at least one prescribed antibiotic. Penicillin, a β-lactam antibiotic, treats bacterial infections caused by bacteria producing toxins within a host. Many pathogenic bacteria need a peptidoglycan cell wall for normal functionality. Enzymes in the cell membrane help form this cell wall by cross-linking peptidoglycan units. β-lactam antibiotics hinder bacterial cell wall biosynthesis by competing with the peptide substrate for the active site in these enzymes. While not the main enzyme used to produce bacterial cell walls, R61 DD-peptidase, a cytoplasmic enzyme, is easily crystallized to show bacterial enzyme chemistry. The active site of R61 consists of amino acid residues Ser62, Lys65, Lys159, Arg285, Thr299, and Thr301. The Messmer SMART Team modeled R61 complexed with Helen-1, a species-specific β-lactam, highlighting the functionality and chemistry of the active site amino acids and their interaction with the beta-lactam. Understanding the structure and function of the active site of penicillin binding proteins, like R61, could lead to new, species-specific antibiotics that could prevent antibiotic resistance in bacteria.

Basis of Prokaryotic Selectivity in the Antibiotic Paromomycin

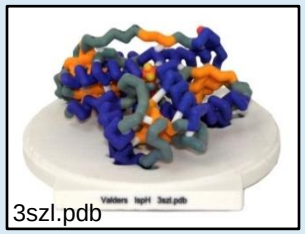
Brookfield Academy

Authors: Simar Puri, Satvir Kalsi, Ricky Singh, Neil Chand, Vipul Singh, Sangwoo Park, Chi Aguwa, Moid Ali, Anshul Dhingra, Justin Zhu, Griffin Gill and Mike Sportiello
Teacher: Robbyn Tuinstra, Ph.D.
School: Brookfield Academy, Brookfield, Wisconsin
Mentor: Madhusudan Dey, Ph.D., Department of Biological Sciences, University of Wisconsin-Milwaukee

Ribosomes are responsible for protein synthesis and are a major target of antibiotics. While translation is a universally conserved cellular process, the ability of drugs to target prokaryotic ribosomes depends on subtle variations from eukaryotic ribosomes. The ribosome is composed of ribosomal RNA (rRNA) and protein. The small ribosomal subunit, called 30s in prokaryotes, contains 21 proteins and one rRNA (16S) and the large subunit, called 50S, contains 31 proteins and two rRNAs (23S and 5S). Recent crystal structures reveal that the rRNAs adopt a 3D fold generating (I) decoding center for codon-anticodon recognition, (II) a peptidyl-transfer center (PTC) for a peptide bond formation and (III) an exit tunnel through which the nascent protein emerges. Paromomycin, used in the treatment of intestinal infections, inhibits prokaryotic ribosomes at the decoding site. Paromomycin physically restructures helix H44 of the 16S rRNA, preventing proper rotation of A1492 and A1493 during anticodon:codon recognition, decreasing tRNA selection accuracy in prokaryotic ribosomes. However, paromomycin fails to affect eukaryotes due to an A to G transition at position 1408. The Brookfield Academy SMART Team (Students Modeling A Research Topic) modeled a prokaryotic ribosome, highlighting nucleotides responsible for the prokaryotic specificity of paromomycin.

“Two Birds, One Stone”: Reduction of HMBPP by the Iron-Sulfur Protein (IspH) for Isoprene Synthesis

Valders High School

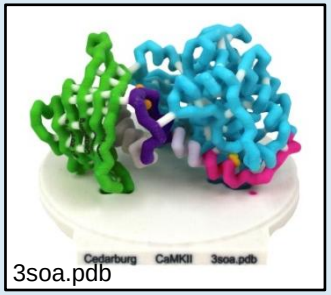


Authors: Rebecca Ansorge, Angie Brandl, Grace Ebert, Elizabeth Evans, Theresa Evenson, Brianna Glaeser, Phoenix Kaufmann, Zach Leschke, Mitchel Meissen, Paige Neumeyer, Alexis Patynski, Ian Schmidt and Christopher Singer
Teacher: Joe Kinscher
School: Valders High School, Valders, Wisconsin
Mentor: Eric Singsaas, Ph.D., Associate Professor of Biology, University of Wisconsin-Stevens Point, Research Director, Wisconsin Institute for Sustainable Technology

A global natural rubber shortage may exceed one million tons by 2020, according to the International Rubber Study Group. While global demand is increasing for rubber, a fungus *Microcyclus ulei*, is killing rubber trees in South America and reducing supply of rubber globally. This has resulted in new monoculture plantations being developed in Southeast Asia where rainforests once existed. In order to satisfy demand for rubber in the future, renewable isoprene, a hydrocarbon produced by the nonmevalonate pathway used by bacteria, may be polymerized and used to create natural rubber and biofuels. Renewable isoprene production requires the iron-sulfur protein IspH, which reduces the substrate HMBPP (1-hydroxy-2-methylbut-2-enyl-4-diphosphate) to produce IPP (isopentyl diphosphate) and DMAPP (dimethylallyl diphosphate), the two precursors in the nonmevalonate pathway. The Valders SMART Team used 3D printing technology to model IspH. The substrate HMBPP enters the active site, and the rotation of HMBPP’s hydroxymethyl group allows a complex to form with the Fe_4-S_4 cluster. The complex is further stabilized by the IspH amino acid Thr167 and HMBPP hydrogen bonding with Glu126. Electron transfer from the Fe_4-S_4 cluster and the protonation of HMBPP by Glu126, results in IPP or DMAPP in an approximate ratio of 6:1 at equilibrium. Industrial production of isoprene would be favored by shifting the IPP/DMAPP equilibrium towards DMAPP, as DMAPP is directly catalyzed into isoprene. By understanding how DMAPP production may be increased, the mass production of isoprene could be made more efficient to generate rubber and biofuels.

Calcium-calmodulin Dependent Protein Kinase II: An Unforgettable Story

Cedarburg High School

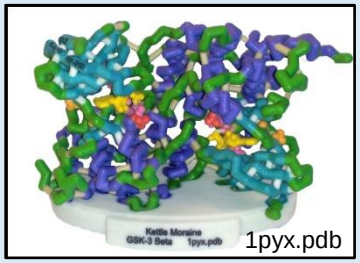


Authors: Laura Tiffany, Alex Bothe, Sarah Dyke, Theresa Eggleston, Savannah Kenny, Erin Kuhn, Alex Satchie, Kathryn Tiffany and Emily Zietlow
Teacher: Karen Tiffany
School: Cedarburg High School, Cedarburg, Wisconsin
Mentors: Audra Kramer, Ph.D. Candidate and Nashaat Gerges, Ph.D., Department of Cell Biology, Neurology and Anatomy, Medical College of Wisconsin

According to the National Institutes of Health, 5.1 million Americans have Alzheimer’s disease (AD), which affects memory and the ability to learn. In long-term potentiation (LTP), a correlate of learning and memory, the number of receptors at the synapse increases. Calcium/calmodulin dependent protein kinase II (CaMKII), a large dodecameric enzyme comprising 1-2% of all proteins in the brain, is part of a signaling pathway implicated in LTP. In this pathway, Ca^{+2} binds calmodulin (CaM) and the Ca^{+2}/CaM complex activates CaMKII, which then phosphorylates other proteins in the cell, like α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. To investigate the role of CaMKII, the Cedarburg SMART (Students Modeling A Research Topic) Team used 3D printing technology to design a CamKII model, highlighting the catalytic, self-association, and autoinhibitory domains. The Ca^{+2}/CaM complex activates CaMKII by displacing a portion of the autoinhibitory domain that blocks the active site of the enzyme, exposing both the catalytic base and Thr286, the residue involved in autophosphorylation. When CaMKII phosphorylates AMPA receptors, their numbers increase in the post synaptic neuron and they are more sensitive to glutamate. Impaired LTP may lead to the cognitive decline seen in AD.

Gone with the Wnt: Role of GSK-3

Kettle Moraine High School



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Mentor: Anil Challa, Ph.D., Biotechnology and Bioengineering Center, Medical College of Wisconsin

The Wnt/β-catenin pathway is a crucial cell signaling mechanism regulating gene expression throughout embryonic development and adult life. Inhibition of the pathway can have both desirable and undesirable effects. For example, inhibition during embryogenesis can lead to developmental defects, while later in life inhibiting this pathway provides a promising method of treating certain types of cancers and neurological disorders like Alzheimer's disease and bipolar disorder. One avenue of inhibition is the Glycogen Synthase Kinase 3 (GSK-3). Inhibition of GSK-3 during embryogenesis results in developmental defects due to altered expression of genes related to cell proliferation and cell death. Lithium chloride, a simple salt, and Phosphoaminophosphonic Acid-Adenylate Ester (AMP-PNP or ANP), a nucleotide, are two effective inhibitors of GSK-3. Both of these molecules inhibit GSK-3 function by interacting with two different sites of the protein tertiary structure. By studying how these and other molecules structurally interact with GSK-3, it is possible to not only inhibit the Wnt pathway but also treat various diseases.

Three Blind Mice: A Mutation in ADAM17 is Responsible for Embryonic Eyelid Closure Defect in *woe* Mice

Saint Joan Antida High School

1bkc.pdb

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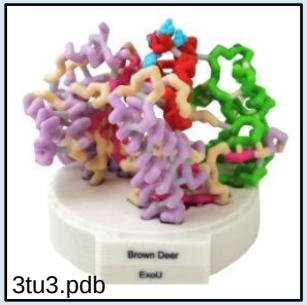
During embryogenesis, in all mammals, the eyelids grow across the eye anterior, fuse together, and subsequently reopen. This process is essential for proper eye development. ADAM17 is a Zn2+ metalloprotease that has a role in cleaving numerous proteins including growth factors involved in EGFR signaling, a molecular pathway essential for cell migration. Mice with mutations in genes encoding ADAM17, EGFR, and EGFR ligands exhibit defects in embryonic eyelid closure. Recently, woe (wavy with open eyelids) mice, also exhibiting defects in embryonic eyelid closure, were identified. Genetic analysis of woe mice identified a mutation in ADAM17 leading to three different ADAM17 mutant proteins. Two of these mutant proteins were catalytically inactive; however, the third mutant protein exhibited normal ADAM17 catalytic activity. Further molecular analysis showed that this catalytically active mutant protein exhibited T265M substitution and was expressed at very low levels. The T265 residue is within the ADAM17 Zn2+ catalytic domain (215–473 aa). The T265M mutant protein exhibits normal ADAM17 catalytic activity most likely because T265 residue is not in the Zn2+ active site. The Saint Joan Antida SMART Team modeled ADAM17's catalytic core using 3-D printing technology. In addition SMART Team identified the location of the T265 amino acid residue within the catalytic core and its position relative to the active site. A better understanding of which amino acids are essential for the ADAM17 catalytic function will ultimately lead to a better understanding of ADAM17-mediated EGFR pathway and the role of this pathway in cell migration and organ development.

Feel the Burn, then Feel the Death. ExoU as a Phospholipase

Brown Deer High School



3tu3.pdb

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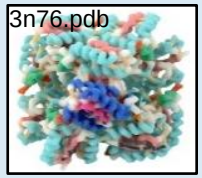
A major cause of infection-related deaths in immunocompromised patients is the protein toxin ExoU, encoded by the bacterium *Pseudomonas aeruginosa*. The Brown Deer SMART (Students Modeling A Research Topic) Team has modeled ExoU using 3D printing technology to have a better grasp on how the toxin interacts with eukaryotic cells. *P. aeruginosa* uses a type 3 secretion system (T3SS) to inject toxins including ExoU into the cell to disrupt its functionality. The T3SS is a needle-like structure comprised of proteins that allow the bacterium to transfer effector proteins into innate immune cells. ExoU travels through the T3SS using a chaperone protein (SpcU). Once inside the eukaryotic cell, ExoU interacts with ubiquitin, where it refolds into an active potent phospholipase that breaks down cellular membranes using Ser142 and Asp344 as the catalytic amino acids. The exact mechanism is unknown but the C-terminus (residues 580-683) helps in targeting the membrane, allowing ExoU to break it down. *P. aeruginosa* is able to freely reproduce inside the environment of the host organism as the immune system is not able to compensate for the infected cells and bacterium. Left unchecked, this infection will prove fatal. Research is being conducted to create an ExoU inhibitor to reduce the deaths it causes in patients with compromised immune systems.

The Inhibition Mission: DHQase and the Shikimate Pathway of *Mycobacterium tuberculosis*

Brookfield Central High School


3n59.pdb


3n76.pdb

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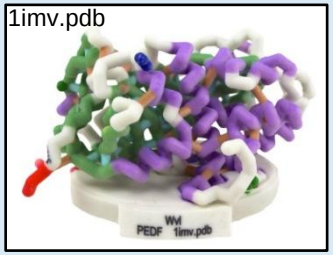
In 2011, nearly 9 million people became sick with tuberculosis, of which 1.4 million died worldwide, according to the World Health Organization. Tuberculosis (TB) is an infectious, airborne disease caused by a pathogenic bacterium, *Mycobacterium tuberculosis.* This bacterium primarily attacks the lungs and is often fatal if not treated promptly. 3-dehydroquinate dehydratase (3-DHQase) is an enzyme that catalyzes the third step of the shikimate pathway, which is essential to *M. tuberculosis*. The shikimate pathway creates a precursor to the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Inhibition of 3-DHQase will block the shikimate pathway and the TB bacteria will die. Inhibitors can be used for drug development to treat tuberculosis, especially people affected by multi-drug-resistant strains, called MDR TB. Since DHQase is absent in human cells, the drug will only affect bacteria cells, where the enzyme is inactive until substrate binds to its active site. 3-dehydroshikimate, a natural ligand, and six inhibitors can interact with DHQase. Effectively inhibiting this enzyme would render tuberculosis harmless. 3-DHQase has a flexible catalytic loop at residues 19–24. Arg^{19} and Tyr^{24} are the two key conserved residues. The ligand binding induces closure of the loop through its interaction with the side-chain atoms of loop residues: Tyr^{24} and Arg^{19}. 3-DHQase may hold the key to saving the lives of those infected by MDR TB. The Brookfield Central High School SMART Team (Students Modeling a Research Topic) created physical models of 3-DHQase dodecamer and monomer to show active-site binding molecules and inhibitors using 3-D modeling printing technology.

PEDF: An Angiogenesis Inhibitor and Its Role in Glioblastoma Multiforme

Wisconsin Virtual Learning



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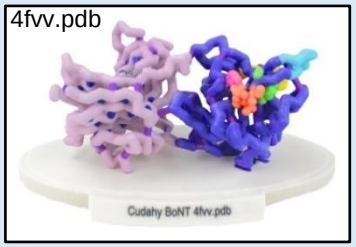
Glioblastoma multiforme (GBM) is a cancerous brain tumor with almost 100% recurrence rate even after surgery, radiation and chemotherapy. Pigment epithelium-derived factor (PEDF) has been found in areas where these tumors do not grow as aggressively. PEDF slows the growth of tumors by inhibiting angiogenesis, a physiological process involving growth of new capillaries from pre-existing blood vessels in the body. Restricting blood flow to the tumor starves it of oxygen and nutrients. The mechanism of PEDF-mediated inhibition of angiogenesis is unknown. Research has shown that PEDF undergoes posttranslational modifications (PTM), chemical changes to a protein after translation, such as the addition of carbohydrates (glycosylation) or phosphate groups (phosphorylation), which may occur during various cellular events in tumors. PEDF is phosphorylated at Ser227, Ser114 and Ser24 and glycosylated at Asn285. Glycosylation may also occur on amino acids within a specific region of the protein (amino acids 371-383). The Wisconsin Virtual Learning SMART (Students Modeling A Research Topic) Team modeled PEDF using 3D printing technology. Identifying the PTMs of PEDF in GBM tumors and plasma samples may further the understanding of angiogenesis inhibition and in turn, may lead to the development of treatments for these lethal cancers.

Wrinkle Release: The Entry Mechanism of Botulinum Neurotoxin

Cudahy High School



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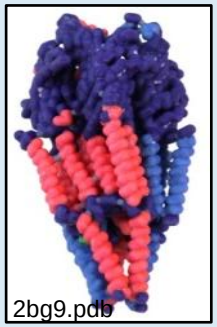
Botulism is a potentially fatal disease or therapeutic for muscular disorders, which results from intoxication of cells by the protein botulinum neurotoxin (BoNT). BoNT, produced by the bacteria *Clostridium botulinum,* paralyzes humans through inhibition of neuromuscular synaptic transmission. BoNT cleaves the Soluble NSF Attachment Protein Receptor (SNARE) proteins responsible for guiding synaptic vesicles carrying neurotransmitters for muscle stimulation to the neural plasma membrane, resulting in the muscle relaxation. BoNTs must first gain entry to the neuron using a ganglioside binding domain (GBP) that recognizes a specified ganglioside, a complex of carbohydrates and sialic acid within the neural plasma membrane. The sialic acid region of the ganglioside binds with specific residues on the BoNT GBP2: Tyr1115, Ser1275, Ile1240, Tyr1243, and Ser1242, modeled by the Cudahy SMART (Students Modeling A Research Topic) Team using 3D printing technology. After binding, the toxin is able to access a vesicle, crossing into the cytoplasm of the neuron, where the light chain of the toxin can cleave the SNARE proteins, causing a loss of muscular function due to lack of neural stimulation. In the case of a systemic BoNT intoxication, lack of muscle function can lead to respiratory failure but when used as a therapeutic BoNT relaxes specific muscles. While the action of the neurotoxin BoNT is well understood inside the neuron, the mechanism of entry is not well known. Understanding how BoNT recognizes and enters the neuron allows researchers to develop better treatments for infections and improved therapies to treat spastic muscle disorders.

$GABA_A$ Receptor: Knocked Out

Whitefish Bay High School



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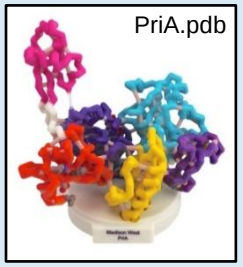
Today, surgeons and dentists would not consider operating without the use of anesthetics, drugs that help numb pain and induce unconsciousness by inhibiting the transmission of signals in nerve cells. The $GABA_A$ receptor is a transmembrane receptor protein activated by the neurotransmitter gamma-aminobutyric acid (GABA) that plays a crucial role in the action of proprofol as an anesthetic. The Whitefish Bay SMART (Students Modeling A Research Topic) Team is modeling the $GABA_A$ receptor protein using 3D printing technology. When activated, the $GABA_A$ receptor selectively allows chloride ions to pass through the membrane and into the cell, creating a more negative overall charge inside the cell. When a nerve cell is excited, a sudden change in ion concentrations triggers an electrochemical potential across the cell membrane, ultimately resulting in the passage of the original signal to the next neuron. However, the $GABA_A$ receptor acts as an inhibitor, and impedes the spreading of the message by making the cell less likely to be in an excited state, causing the cell to relay a neural signal less frequently. Propofol produces its effects by enhancing the activity of the $GABA_A$ receptor. Currently, researchers are trying to pinpoint how propofol acts on the $GABA_A$ receptor protein because its molecular mechanism is not fully understood. It has been found that a phenylalanine at position 385 on the $GABA_A$ receptor is necessary for proprofol to produce its effects. Research targeting how propofol alters the function of the $GABA_A$ receptor may lead to the development of more effective anesthetics with fewer side effects.

Indispensable Replication Restart Helicase PriA Aids Bacterial Survival: A SMART Team Story

Madison West High School



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Mentors: James L. Keck, Ph.D., Department of Biomolecular Chemistry, University of Wisconsin-Madison

Approximately one disruption in DNA replication occur every cell cycle in bacteria leading to partially duplicated chromosomes. Since unfinished replication can result in genome instability and cell death, bacteria need a mechanism to reload the replication machinery onto the genome. Known as the replication restart primosome (RRP), several proteins function to reload the replicative helicase onto abandoned replication forks, restarting DNA replication. PriA is the most conserved member of the RRP, initiating the dominant replication restart pathway. A helicase, PriA remodels collapsed forks and serves as a platform for binding of other primosomal proteins. The Madison West High School Students Modeling a Research Topic (SMART) Team modeled PriA using 3D printing technology. PriA is a multi-domain protein and residues important for DNA binding, ATP hydrolysis, and helicase activity are modeled. Since DNA replication restart pathways are essential in preserving genomic integrity and cell viability in bacteria, studies of PriA offer an approach to developing novel antibacterial compounds.