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 2017-2018 SMART Team and MAPS Team programs.
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 Advisors


David Sampe  Karen Tiffany  Dan Koslakiewicz & Dean Billo  Stacey Strandberg & Scott Fleischmann  Fran Grant  Mark Arnholt  Melissa Kirby  Josh Demski  Molly Schuld & Jose Perez  Donna LaFlamme  Cindy McLinn, Katie DiFonzo  Jonathan Kao  Paula Krukar & Katie Brown
National SMART Team Program

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.

The National SMART Team program is available to schools across the US and Canada. This school year, we have 28 teams participating the National SMART Team program. In addition to this MSOE event, we have teams who will present at the Experimental Biology conference in Chicago next month, and others will present in New York City in May.

National SMART Teams

Brookfield East High School
Madison West High School
Marquette High School
Monona Grove High School
Valders High School (Entry 1 & 2)

Presentations

<table>
<thead>
<tr>
<th>Poster Number &amp; School Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brookfield East High School</td>
<td>4</td>
</tr>
<tr>
<td>2. Brown Deer High School</td>
<td>5</td>
</tr>
<tr>
<td>3. Cedarburg High School</td>
<td>6</td>
</tr>
<tr>
<td>4. Cudahy High School</td>
<td>7</td>
</tr>
<tr>
<td>5. Valders High School (Entry 1)</td>
<td>8</td>
</tr>
<tr>
<td>6. Divine Savior Holy Angels HS</td>
<td>9</td>
</tr>
<tr>
<td>7. Grafton High School</td>
<td>10</td>
</tr>
<tr>
<td>8. Greenfield High School</td>
<td>11</td>
</tr>
<tr>
<td>9. Hartford High School</td>
<td>12</td>
</tr>
<tr>
<td>10. Kettle Moraine High School</td>
<td>13</td>
</tr>
<tr>
<td>11. Laconia High School</td>
<td>14</td>
</tr>
<tr>
<td>12. Madison West High School</td>
<td>15</td>
</tr>
<tr>
<td>13. Marquette High School</td>
<td>16</td>
</tr>
<tr>
<td>14. Monona Grove High School</td>
<td>17</td>
</tr>
<tr>
<td>15. Pilgrim Park Middle School</td>
<td>18</td>
</tr>
<tr>
<td>16. Ronald Reagan High School</td>
<td>19</td>
</tr>
<tr>
<td>17. St. Dominic Middle School</td>
<td>20</td>
</tr>
<tr>
<td>18. St. Joan Antida High School</td>
<td>21</td>
</tr>
<tr>
<td>19. Valders High School (Entry 2)</td>
<td>22</td>
</tr>
<tr>
<td>20. Waukesha STEM</td>
<td>23</td>
</tr>
<tr>
<td>21. Westosha Central High School</td>
<td>24</td>
</tr>
<tr>
<td>22. Whitefish Bay High School</td>
<td>25</td>
</tr>
<tr>
<td>23. Wisconsin Virtual Learning</td>
<td>26</td>
</tr>
</tbody>
</table>
Nearly 11,285 people die each year due to complications of infections caused by the drug-resistant pathogen, Methicillin-resistant *Staphylococcus aureus* (MRSA). L-enduracididine (L-End), a rare amino acid not naturally occurring in humans, is a component of antibiotics such as teixobactin, enduracidin, and mannopeptimycin, all of which have potent activity against MRSA. The growing concern over MRSA makes these antibiotics ever more crucial, thus increasing the importance of L-End synthesis. The Brookfield East High School MSOE Center for BioMolecular Modeling SMART Team used 3D modeling and printing technology to examine the structure-function relationships of the protein, MppP, and its involvement in the L-End synthesis pathway. The pathway begins with a reaction between MppP and the amino acid L-Arginine (L-Arg) where an oxygen atom is added to the L-Arg substrate. A pyridoxal-5'-phosphate (PLP) cofactor at the active site of the enzyme, MppP (consisting of Arg352, Asp27, Asp227, Glu15, and Thr12), is covalently bound by Lys221, Ser91, Ser190, Asp188, and Asn160. When an amino group of L-Arg replaces the amino group of the active site, L-Arg is bound to PLP to form a L-Arg-PLP unit. Here, the result of the hydroxylation is 4-hydroxy-2-ketoarginine (4HKA), which is then used in a series of steps involving the enzymes MppR and MppQ to create the finished product of L-End. By understanding the role of MppP and L-Arg in the L-End synthesis pathway, L-End may be more readily available to help in the research involved with developing and improving clinically relevant antibiotics used to fight MRSA.

**MAPS Team Program**

The Modeling A Protein Story (MAPS) program consists of teams of students and their teachers who learn about a specific protein theme, such as insulin, hemoglobin or aquaporin, using educational resources developed by the CBM. After the teams develop an understanding of the protein structure-function relationship, they then develop their related protein story and design and 3-D print a physical model with which they present their findings. The selected protein for 2017-18 school year was **insulin**. Teams have utilized online resources and hands-on models to investigate the unique structure-function relationships of this critical protein hormone. Teams then “MAPped” out a path to explore through which they will gain a better understanding of how scientists have engineered insulin analogs to serve as diabetes medications with specific properties based on the ability of insulin to aggregate into dimers and hexamers.

The MAPS Team program is available to schools across the US and Canada. This school year, 25 teams had a MAPS team dedicated to modeling our featured protein. In addition to this MSOE event, we have teams who will present at the Experimental Biology conference in San Diego this month, as well as in various regional events this spring in Florida, Colorado, and New York City.

**MAPS Teams**

Greenfield High School
Pilgrim Park Middle School
Waukesha STEM School
Wisconsin Virtual Learning
As of September 2017, seven states have legalized the recreational use of marijuana, while 22 states have legalized its medical use. Regardless of attitudes toward recreational use, constituents of marijuana (Cannabis sativa, C. indica) known as cannabinoids are potentially useful in treating pain and inflammation, stress and anxiety disorders, and possibly seizure disorders. Diseases such as obesity and substance abuse disorders may be treated by agents that block the actions of cannabinoids. The main receptor in the brain activated by cannabinoids is the cannabinoid receptor 1 (CB₁). CB₁ is a transmembrane protein found on presynaptic neurons throughout the brain. Seven alpha helices span the cell membrane. Various ligands bind to these alpha helices throughout the protein. Agonists such as tetrahydrocannabinol (THC) and other cannabinoids bind with CB₁ in the area of alpha helices 3, 6, and 7, at Phe268 and Phe379. Antagonists such as AM6538 or rimonabant bind with CB₁ in the area of alpha helix 2 at Phe170 and Phe174. Antagonist binding brings helices 3 and 6 close together, causing an “ionic lock” to form between Arg214 and Asp338 that prevents G protein signaling. The Brown Deer SMART Team (Students Modeling a Research Topic) has designed a model of CB₁ using 3D printing technology to investigate structure-function relationships. Research on the agonists and antagonists of the CB₁ receptor is important because it is not completely understood how much therapeutic potential they possess. Perhaps when the therapeutic potential of the CB₁ receptor is developed, quality healthcare can be given to patients using cannabinoid receptor-based therapeutics.
According to the CDC, 1 in 3 adults in the US has high cholesterol, which is linked to cardiovascular disease. Cholesterol, a hydrophobic molecule, is transported in the blood by binding a lipoprotein. Scavenger receptor class B type 1 (SR-B1), a transmembrane protein that binds high density lipoprotein (HDL) carrying cholesterol, helps transfer cholesterol from HDL into liver cells where it can be excreted in bile. This is important for lowering blood cholesterol levels and reducing the risk of heart disease and stroke. SR-B1 belongs to a larger family of scavenger receptor proteins that includes lysosome membrane protein 2 (LIMP-2), and SR-B1 and LIMP-2 share structural homology. The structure of the C-terminal transmembrane domain of SR-B1, determined through NMR, is composed of three helices with a leucine zipper motif. Mutagenesis studies implicate this leucine zipper motif in dimerization of SR-B1 and proper receptor function. A crystal structure of LIMP-2 revealed the existence of a large, primarily hydrophobic cavity that runs the entire length of the protein that suggests a role in the selective transfer of cholesterol into the liver cell. Using 3D printing technology, the Cedarburg SMART (Students Modeling A Research Topic) Team utilized the known structure of LIMP-2 to investigate structure-function relationships in SR-B1. Determining important HDL/SR-B1 interactions will increase knowledge of how SR-B1 functions in transferring cholesterol from plasma HDL to the liver for excretion and can lead to the development of medical treatments to increase cholesterol excretion by the liver and prevent heart disease and stroke.
For patients with the disease diabetes mellitus, life can be an unpredictable road of doctor’s appointments, insulin monitoring, and much more. Diabetes is the inability of an individual’s body to regulate their blood glucose levels, specifically caused by the pancreas’ inefficiency or incapability to discharge insulin. The hormone insulin is secreted by beta cells in the pancreas in reaction to rising glucose levels in the bloodstream. After this occurs, insulin enters into the bloodstream and binds to cells, signaling for glucose absorption. Complications in patients with Type 1 diabetes are due to damaged beta cells, causing an inability secrete insulin. Current treatment pathways for these patients include synthetic insulin injections or the use of an insulin pump, so as to avoid developing hyperglycemia. Likewise, difficulties related Type 2 diabetes arise due to a patient’s pancreas not producing or using insulin efficiently. Treatment pathways for Type 2 diabetes patients includes healthy food and lifestyle choices, along with diabetes medications, such as sulfonylureas and meglitinides. These specific diabetes medications stimulate the pancreas to secrete insulin; sulfonylureas are a long-acting medicine, taken 1-2 times a day, and meglitinides are a short-acting medicine, taken about 15-30 minutes before a meal (“Sulfonylureas and Meglitinides”). The MAPS Team used 3D printing to model the insulin protein, primarily highlighting amino acids Cys6, Cys7, Cys11, Cys19, and Cys20. Insulin is an indispensable factor to sustaining healthy living, making additional research needed to create a better quality of life for diabetes patients in the present, and the future.

The World Health Organization (2017) lists cancer as a leading cause of death. Eukaryotic Initiation Factor 6 (eIF6) is critical for cancer cell growth/survival. Understanding its mechanism of action will help to develop new drug targets that can slow the cancer epidemic. One of the hallmarks of cancer is increased rate of protein synthesis, supporting the rapid growth and reproduction of cancer cells. eIF6 is crucial to maintaining these enhanced levels of protein production. Data show eIF6 is upregulated in human cancer cells, but its mode of regulation is unknown. eIF6 is essential for the synthesis of the 60S ribosomal subunit and for mediating interactions between the 60S and 40S ribosomal subunits. eIF6 binds to 60S using residues Y151, T150, N106, S102, K100, T75, and D12 to interact with 60S ribosomal protein L23 (RPL23) residues K132, N136, A137, G138, S139, V140, and V141. eIF6 was modeled by the Cudahy SMART (Students Modeling A Research Topic) Team using 3D printing technology to investigate structure and function relationships. eIF6 interactions with 60S must be regulated as the release of eIF6 from the 60S allows the 60S and 40S subunits to bind and initiate protein synthesis. C-terminus sites could functionally regulate eIF6 interaction. One model suggests that when the C-terminal tail is phosphorylated, eIF6 is released from 60S, initiating protein synthesis. Previous research suggests by reducing levels of eIF6 or by preventing its release from the 60S subunit, protein synthesis is attenuated and tumor growth is inhibited. Therefore, determining the exact mechanism of binding between eIF6 and the 60S ribosomal protein-RPL23 in cancer cells is important and could aid in the development of new treatments for cancer patients.
Authors: Trevor Wenzel, Jesse Linsmeier, Nate Griepentrog, Sylvia Streblow, Madison Storm, Jacob Pattee
Teacher: Joseph Kinscher

Lead (Pb\(^{+2}\)) displaces the cofactor zinc (Zn\(^{+2}\)) needed for 5-aminolaevulinic acid dehydratase (ALAD) catalytic activity. ALAD, a homo-octamer metalloenzyme, catalyzes porphobilinogen (PBG) synthesis; a crucial step in heme formation. Each subunit has a TIM-barrel fold with a N-terminal arm that allows subunit interactions in the homo-octamer form (shown here). Held inside each TIM-barrel subunit are at least two Zn\(^{+2}\) cofactors that act as electron pair acceptors during catalysis. ALAD cysteine 122 and 124 coordinate with a Zn\(^{+2}\) cofactor, while lysine 199 and 252 in the active site coordinate with PBG. In the first step of heme synthesis, two aminolaevulinic acid (ALA) molecules are condensed by ALAD to form porphobilinogen (PBG). When Pb\(^{+2}\) is present in the body, it takes the place of Zn\(^{+2}\) in ALAD causing a form of lead poisoning. Pb\(^{+2}\) inhibits PBG production through steric obstruction in the active site and/or its inability to act as an electron pair acceptor during catalysis. This ultimately prevents heme synthesis, leading to anemia from insufficient hemoglobin production.

Authors: D. Coleman, E. Davis, L. Dragseth, A. Gent, A. Janssen, S. Koch, A. Ramirez, and B. Sevart
Teachers: Katie Brown and Paula Kruckar
Mentors: Joseph T. Barbieri Ph.D and Madison Zuverink, Medical College of Wisconsin

Ricin has a long history of use as a weapon of terrorism. Ricin attacks have been planned and thwarted by terrorist groups in the United States and across the globe. Ricin, generated from castor beans, is fatal to humans because it causes cell death. Ricin is a protein composed of two chains, A and B, linked by a disulfide bond. The A chain detaches from the B chain, and in the active site, Glu177 adenylates a base on RNA found within the 60S ribosome, thus inhibiting cellular protein synthesis. The A chain is composed of three domains: one characterized by a five-stranded beta sheet, one by five alpha helices, and the final by a disc-like shape. The B chain facilitates entry into the cell by binding to surface receptors. The B chain is composed of two nearly identically-folded domains that share high amino acid similarity. The ligands are Gal264, whose domain is defined by Asn46, Lys40, and Asp22, and Gal267, whose domain is defined by Asn255 and Asp234. The Whitefish Bay High School SMART Team (Students Modeling A Research Topic) modeled ricin using 3D printing technology to better understand its function and structure. Further research is required to develop a vaccine, although two putative formulations exist: RiVax and RiVEC. Unexpectedly, these vaccines are composed of the A chain, rather than the receptor binding B chain. A successful vaccine could be given primarily to military and national security personnel to prevent the deadly effects of biological military action using ricin.
The WHO estimates that in 2014, 422 million adults were living with diabetes, a condition which limits blood flow to the small blood vessels of the eye. To restore blood flow, the body triggers angiogenesis, or the growth of new blood vessels, using vascular endothelial growth factors (VEGF) and the VEGF receptor (VEGF-R). Blood vessels created by VEGF-R signaling are weak and leak blood and fluid into the back of the eye, causing diabetic retinopathy and eventually blindness. The VEGF-R is a dimer and each monomer is composed of seven extracellular domains and an intracellular kinase domain. Dimerization of the receptor occurs after binding VEGF, causing the correct positioning of the kinase domain to begin a signal cascade that stimulates the growth of endothelial cells into new blood vessels. Domains 1-3 are responsible for binding VEGF, with the amino acids Q263, F292, V278, R224, N259, R261, R280, Q284, and N290 forming the binding pocket for the VEGF. Domains 2-7 are responsible for the dimerization and activation of the VEGF-R. The amino acids R351, K393, A381, K379, E513, K517, T455, S346, A434, K433, and Q429 in domains 4 and 5 form homotypic interactions and control the VEGF-R activation. The Westosha Central High School SMART team designed a model of VEGF-R to study structure-function relationships. Although VEGF-R inhibitors are widely used to treat diabetic retinopathy, they are extremely expensive and not effective in all patients. Elucidation of VEGF-R's targeting and activation could lead to more effective inhibition of angiogenesis, thereby preserving vision in diabetic patients.

Propofol, a powerful anesthetic, can be used safely in medical settings, but proves deadly when used recreationally. Propofol binds to the GABA<sub>A</sub> receptor, which consists of an integral ion channel protein embedded in the membrane of neurons in the brain that is activated by the neurotransmitter molecule gamma-aminobutyric acid, or GABA. When propofol binds to the GABA<sub>A</sub> receptor, a conformational change occurs, holding the neurotransmitter GABA in its binding site and keeping the ion channel open. This allows more chloride ions to diffuse into the cell. Resting potential becomes more negative, so even with the diffusion of Na<sup>+</sup> ions during nerve stimulation, the threshold cannot be reached and the action potential is not generated. Since the neurons cannot communicate normally, a person given propofol remains unconscious. The lower part of the GABA<sub>A</sub> receptor, located in the cytoplasm of a neuron, has an unidentified molecular structure. Phe 393 variants in the A and D chains prevent propofol from acting on the GABA<sub>A</sub> receptor. The DSHA SMART team modeled the similar nicotinic receptor using 3D printing technology to better understand the structure of the GABA<sub>A</sub> receptor. A greater understanding of the structure of the GABA<sub>A</sub> receptor and the role of the Phe 393 variants in the action of propofol can lead to the development of more effective anesthetics.
Diabetes is a disease where your body fails to receive insulin signals or secrete insulin. Insulin monitors blood glucose levels and keeps them from getting to a level toxic and detrimental to the body. Analog insulins are genetically engineered versions of human insulin made using organisms like bacteria and yeast. These analog insulins are used to prevent insulin deficiency in diabetic patients. Our goal from this project was to find the structural difference between human insulin and the two of the more popular long acting insulin (degludec and glargine). Through our research we found that glargine has 2 structural differences from human insulin. The asparagine is replaced by a glycine which results in the insulin analog becoming more structurally stable than human insulin. It also does not have a threonine at B30 causing insulin substrate 1 (where insulin binds to an insulin receptor) to degrade slower. We also found that degludec has 2 structural differences from human insulin, such as having hexadecadienoic acid added to the lysine on the B29 position causing degludec monomers to form multi-hexamers, allowing it to dissolve into the bloodstream slower than regular human insulin. Degludec also does not have a threonine on its B30 position which causes insulin substrate 1 to degrade slower. From our findings, we were able to understand the structural differences that degludec and glargine have from human insulin cause them to function longer than human insulin. We were also able to understand that while these changes help in one way, they also had negative side effects such as the increased chance of cardiovascular and kidney diseases for patient who use degludec. A future research topic based off our project, would be studying how the structural differences of degludec and glargine from human insulin lead them to cause cardiovascular and kidney problems. By figuring out how these changes in structure cause problems, eventually in the future we will be able find the perfect long acting analog insulin with minimum side effects for diabetic patients.

Obesity is a risk factor for cancer, diabetes, and strokes, causing 300,000 deaths annually in the U.S. Facets of obesity, such as adipose-tissue mass, hunger, and energy use are, in part, regulated by leptin, which binds to the leptin receptor (LR) within the hypothalamus, regulating mammalian feeding behaviors and promoting metabolic homeostasis. The LR is a homodimer belonging to the cytokine family. It binds leptin at ten residues between amino acids 433 and 617. Leptin binding activates the JAK-STAT signaling cascade, which promotes gene transcription altering feeding behavior and metabolism. The putative structure of the LR is composed of 206 amino acids, containing two exposed tryptophan residues, four-helical bundle cytokines, and four antiparallel α-helices. Understanding LR activation is crucial for obesity as studies have shown that defects in LR binding may disrupt normal metabolic function and overeating leading to metabolic disorders such as obesity. Overeating also occurs when PACAP (pituitary adenylate cyclase activating polypeptide) which functions similarly to leptin, is blocked. Importantly, leptin is blocked from producing its anorexic effects when PACAP receptors are blocked. Understanding how leptin receptors function with PACAP receptors would advance the understanding of leptin signaling in obesity. The Grafton High School SMART (Students Modeling A Research Topic) Team designed a leptin receptor model using 3D printing technology to specifically explore the structure-function relationship between PACAP and leptin, and their shared common pathway. To date, effective treatments for obesity are lacking, therefore research focused on the relationship between PACAP and LR activation could lead to the development of new therapeutic medications.
In 2015, 4.6% of children tested for lead poisoning in Wisconsin under the age of 6 were positive. Lead ions (Pb^{2+}) will displace calcium ions and bind to calmodulin (CAM) protein causing neurotoxicity. Calmodulin is a dumbbell shaped calcium modulated protein found in the cytoplasm of eukaryotic cells, and is made up of 148 amino acids. There are four calcium ion (Ca^{2+}) binding motifs or EF-hand sequences on calmodulin. An EF-hand consists of two alpha helices connected by a loop. Negative residues in the EF-hands consisting of three aspartic acids and one glutamic acid bind Ca^{2+}. Ca^{2+} activation of CAM exposes nonpolar methionine amino acids allowing CAM to bind to nonpolar regions of target kinases, activating phosphorylation cascades, which in turn activates cellular responses. Large amounts of lead can cause CAM to deactivate and small amounts of lead can cause CAM to hyperactivate. One specific target protein for CAM is Calcium/Calmodulin-Dependent Protein Kinase II (CAMKII). CAMKII is implicated to be significant in both long term potentiation and synaptic plasticity; affecting nerve cells of the brain which can be detrimental to learning and memory. The Valders SMART (students modeling a research topic) team designed a model of CAM using 3D printing technology to investigate lead poisoning.

According to the National Institutes of Health, in 2013 about one in 300 people developed type 1 diabetes by age 18 in the U.S. Type 1 diabetes patients experience high blood pressure, kidney damage, ketoacidosis, nerve damage, and other potentially fatal symptoms. Scientists have synthesized artificial insulin, a hexameric protein normally secreted by the pancreas to regulate blood glucose. In the bloodstream, insulin hexamers eventually dissociate into six active monomers, which bind to insulin receptors (IRs) on liver and muscle cells. Binding activates glucose transporters, allowing insulin to enter the cells and reduce blood sugar levels. The structure of insulin consists of two chains (A and B) connected by two disulfide bonds (A7 to B7 and A20 to B19), with the A chain having an intramolecular disulfide bond (A6 to A11). Type 1 diabetes can result from mutations, which alter the structure of insulin. These mutations prevent insulin from binding to IRs, inhibiting glucose uptake by cells and keeping blood glucose elevated. An example of such a mutation is Cys7Tyr on the A chain. The disulfide bond is disrupted, changing insulin’s shape and preventing insulin from binding to IRs. While some mutations can cause type 1 diabetes, researchers are intentionally altering insulin, creating analogs to treat diabetes patients. An analog called Humalog (LysB28-ProB29-human insulin), disrupts the primarily hydrophobic interface connecting the dimer, leading to quicker uptake of glucose into cells. By switching two amino acids on the C-terminus of the B chain (Pro28 and Lys29), an improved insulin is synthesized. Scientists are striving to enhance the efficacy of insulin analogs to increase life quality for type 1 patients.
Inhibiting pyruvate carboxylase could lead to future treatments for cancer by preventing cancer cells from multiplying. Pyruvate carboxylase is an enzyme found in the mitochondria of animal cells and contributes to the mitochondrial tricarboxylic acid (TCA) cycle by converting pyruvate into oxaloacetate that the cycle needs to generate precursors used to make lipids, amino acids, and nucleotides. Cancer cells rely heavily on many of these precursors to divide. Pyruvate carboxylase is a homotetramer with four domains and about 1200 amino acids. A truncated construct of pyruvate carboxylase from *Rhizobium etli* has 632 amino acids and is primarily comprised of the carboxyltransferase domain. It has 32 helices and 16 beta sheets. Thr882 shuttles a proton between the biotin cofactor and pyruvate, while Arg 548, Gln552 and Arg621 stabilize the enolpyruvate intermediate. Asp590 and Tyr628 form a binding pocket where carboxybiotin cofactor is inserted into the active site. All of these amino acids may react with experimental inhibitors. The active site is centered on the ligand Zinc $^{2+}$, and while pyruvate does not directly bind to Zn$^{2+}$, the ion affects the orientation of the pyruvate in the binding site. The Saint Joan Antida High School SMART (Students Modeling A Research Topic) team designed a model of the carboxyltransferase domain of pyruvate carboxylase using 3D printing technology to study structure-function relationships and model novel inhibitors. Ongoing research should continue to seek inhibitors that will shut down pyruvate carboxylase, potentially leading to new cancer treatments.

Teacher: Donna LaFlamme

Mentor: Matt Scaglione, Ph.D, Medical College of Wisconsin

According to the U.S. Centers for Disease Control, Alzheimer’s disease (AD) is the sixth leading cause of American deaths, affecting 5.5 million people and striking one in ten people over age 65. The dementia characteristic of AD is associated with the over-production and aggregation of amyloid beta (Aβ), a 40-42 amino acid peptide clipped from the trans-membrane portion of the amyloid precursor protein (APP); a large membrane protein important for neural growth and repair. In a healthy brain, Aβ is recycled; while in AD, Aβ strands form neurotoxic aggregates outside neurons called senile plaques. A major component of plaques, Aβ fibrils are homodimers consisting of two protofilaments. The protofilaments are stacked, parallel, LS-shaped amyloid-β peptides connected to each other in a cross-beta sheet structure. Interactions of three groups of hydrophobic sidechains on neighboring Aβ strands stabilize each protofilament and maintain the N-terminus L-shape and C-terminus C shape. While over 50 mutations in the APP protein are associated with early onset AD, our model of the Aβ fibril highlights the six located on Aβ: Glu22Gln, Ala21Gly, Glu22Lys, Leu34Val, Ala2Thr, Glu22Gly, Asp23Asn, and Ala2Val. The St. Dominic Middle School SMART (Students Modeling A Research Topic) Team designed a model of an Aβ fibril using 3D printing technology to investigate structure-function relationships. Current research investigates peptides that can prevent the aggregation of Aβ. Research on Aβ could lead to the discovery treatments that prevent Aβ plaques from forming and killing neurons, which could greatly impact millions of lives.

Authors: Ellena Hein, Ethan Helfenstein, Ava Lirette, Kelsi Morris, Jack Mulcahy, Liam Mulcahy, Divyank Sharma, and Aaron Smet

Teacher: Melissa Kirby

Mentors: Ashley Cowie, Francie Moehring, and Sarah Langer of the Medical College of Wisconsin

According to The Migraine Research Foundation, more than 39 million people in the U.S. suffer from migraines; some using addictive drugs like codeine to relieve them. Understanding the inflammasome NLRP3 could lead to the development of more effective pain medications with fewer side-effects. NLRP3 is part of the innate immune system and is a type of inflammasome found in the trigeminal ganglia, a nerve structure that contributes to migraine pain. ATP and other damage-associated molecular patterns released from injured cells start a signaling cascade, causing the activation of TLR4 and P2X7 receptors on the cell membrane of non-damaged cells, like the trigeminal ganglion neurons. This triggers the formation of the NLRP3 inflammasome. NLRP3 initiates production of active IL-1β (which leads to migraine pain) by inducing the activation of caspase-1. NLRP3 contains seven subunits and forms a multiprotein oligomer with ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD) and caspase-1. Each subunit of NLRP3 is a small, 91 amino acid peptide composed of 6 alpha helices. Amino acids Lys24, Asp29 and Arg41 of NLRP3 allow the molecule to bind to the ASC. The Kettle Moraine High School SMART (Students Modeling A Research Topic) Team has designed a model of one NLRP3 subunit using 3D printing technology to further understand its structure and role in pain. Further research into this protein could lead to a deeper understanding of NLRP3’s role in inflammation and cell damage, and result in more effective treatments for pain.
MiD51: Research Points to Human Dimer

Laconia High School

Poster #11

Authors: Alexis Coffeen, Theodore Holdmann, Alyssa Jodarski, Sarah Laudolff, Logan Meyer, Theresa Oliver, Chloe Smith, Bekah Tilstra, Elizabeth Walters, Cora Williams

Teacher: Joshua L Demski

Mentors: John Egner and Amber Bakkum, Medical College of Wisconsin

Disruption of Mitochondrial Dynamics Protein of 51 kDa (MiD51) has been linked to neurological diseases, such as Parkinson’s, Alzheimer’s, and Huntington’s disease. MiD51 recruits Dynamin Related Protein 1 (Drp1) from the cytosol to bring about mitochondrial fission. MiD51 belongs to the nucleotidyltransferase fold superfamily of proteins. MiD51 also interacts with ligands ADP and GDP. MiD51 is a globular protein anchored on the mitochondrial outer membrane, and is organized into three domains; a disordered domain, an N-terminal domain containing a binding pocket for ADP/GDP and a recruitment region for Drp1, and a C-terminal domain. The soluble N- and C-terminal structured domains of MiD51 contain 11 alpha helices, 9 beta sheets, and 335 amino acid residues. Residues 215-251 make up the Drp1 recruitment region (beta 3, beta 4, alpha 4). ADP and GDP ligands are present, but research shows that Drp1 recruitment occurs in humans whether ligands are present or not. Human dimeric MiD51 has never been crystallized, but is believed to exist based on the dimeric crystal structure of the mouse homolog. This is further supported by SAXS data collection and modeling. Modeling suggests that R169, R182, and D183 are important for mediating dimer formation. The Laconia High School SMART (Students Modeling A Research Topic) Team modeled MiD51 using 3D printing technology to investigate structure-function relationships. MiD51 research is important because if the structure of the protein and its function of mitochondrial fission could be understood, it could possibly be used to prevent and treat neurological diseases in the future.

ABCD: The Language of Replication Protein A (RPA)

Ronald Reagan High School

Poster #16


Teachers: Molly Schuld & Jose Perez

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RPA is a ssDNA binding protein whose function is essential to DNA replication, recombination, and repair. RPA coordinates DNA repair processes responsible for maintaining genomic integrity, and hence is an attractive target for oncology. RPA interacts with ssDNA, and recruits other proteins onto ssDNA. RPA binds to ssDNA very tightly. Current research focuses on determining how an ssDNA-RPA complex is removed from the DNA by weaker DNA binding enzymes. Structurally, RPA is a heterotrimer made up of three subunits (RPA70, RPA32 and RPA14). The DNA binding function of RPA is carried out by four distinct DNA binding domains (DBDs), A (residues 182 - 305), B (residues 306 - 424), C (residues 425 - 623) and D (residues 46 - 175), that directly attach to the single stranded DNA (ssDNA). DBDs A, B and C reside in the RPA70 subunit and are connected by flexible linkers. DBD-D resides in the RPA32 subunit. The heterotrimer is held together as a complex through interactions between DBD-C (RPA70), DBD-D (RPA32) and the RPA14 subunit. Scientists use unnatural amino acids and chemical fluorophores to capture how each DBD binds and disconnects from ssDNA. This approach shows that DBD-A binds rapidly to ssDNA, but detaches quickly, while DBD-D binds more slowly to ssDNA, but is stable. Ronald Reagan High School’s SMART (Students Modeling A Research Topic) Team has designed a model of RPA using 3D printing technology to investigate its structure and function. Additional research on RPA structure and mutations may prove helpful in determining cause and risk for cancer and developing potential treatments.
We have spent the year researching the protein insulin, and have developed a model of the engineered N-lithocholyl analog because we think it would assist people like Akello, living with Type 2 diabetes in underdeveloped countries such as Afghanistan, Bangladesh, and Cambodia. In most third-world countries, insulin medications can deteriorate due to lack of refrigeration, rendering them useless. N-lithocholyl insulin is more stable and also stays in a patient’s bloodstream longer, so patients don’t have to worry about taking several shots of insulin per day, instead they can just take one. The difference between N-lithocholyl insulin and human insulin is that it provides a more constant level in the blood. This is due to the fact that N-lithocholyl insulin contains 2 zinc ions with 2 m-cresol molecules bound at each dimer-dimer interface stabilizing an R(6) conformation. Another unique feature of this analog is that it possesses is that it has 6 fatty acids (N-Lithocholic acid) attached to the B-chains of the hexamer. The fatty acids attract other hexamers, which stick together, and slowly break apart from one another. This makes the insulin longer acting. Another benefit of N-lithocholyl insulin is that the fatty acids attached the insulin B-chains can bind to the albumin proteins in the bloodstream. This is important because it allows the insulin to remain in the bloodstream rather than being degraded. These features allow this insulin analog to be consistently absorbed and used, as opposed to other analogs with many peaks and valleys in absorption rates. This reduces the risk of hypoglycemia or hyperglycemia. This insulin analog also boasts the benefits of a stabilized R(6) structure. The Pilgrim Park Middle School MAPS team 3D printed a model of 1UZ9 to elucidate the zinc ions as well as to highlight its unique structure with the attachment of the lithocholyl groups.

Teacher: Megan Peterson
All cells rely on the information in their DNA to carry out necessary cellular processes. As a result, every time a new cell is generated, copies of the DNA must be made in a process called DNA replication. During the replication of double-stranded DNA, the strands are first separated by a replicative helicase. DNA is a bacterial replicative helicase that separates the two parental strands so that DNA polymerase can synthesize the complementary strands. Strycharska, et al. showed that nucleotide binding to DnaB can cause the formation of two structurally and functionally distinct DnaB N-terminal domain conformations: dilated and constricted. The Monona Grove High School SMART (Students Modeling A Research Topic) Team designed models of the DnaB helicase with constricted and dilated N-terminal domains using 3D printing technology to illustrate their structural and functional diversity. The two different configurations of DnaB’s N-terminal domain modulate the helicase’s interactions with replisomal partner proteins in the overall biochemical process of bacterial replication. These different conformations of DnaB in bacterial species reveal unexpected similarities to multifaceted eukaryotic replicative helicases.

Immunotherapy, in treating solid tumors, has seen increased efficacy due to the development of novel strategies of implementation. One innovative strategy, in development by Dr. Weiguo Cui and his colleagues, combines adoptive cell transfer (ACT) with pathogen-based vaccine techniques in what is called Reenergized ACT (ReACT). Tumor specific CD8 T-Cells that already have a tumor specific T-Cell Receptor (TCR), are genetically modified to express a second TCR that will recognize an outside introduced bacterial antigen. These newly created, dual specific T-Cells, when introduced to mice, show a significantly larger decrease in tumor volume than their mono-specific T-Cell counterparts. The Marquette High SMART (Students Modeling a Research Topic) Team used 3D printing technology to model the TCR complex in order to study its structure and function to better understand ReACT. TCR is a heterodimeric protein consisting of highly variable alpha and beta chains. Both chains are composed of a variable region and a constant region, both of which form antiparallel beta sheets. The variable region binds to the MHC complex whereas the constant region is near the cell membrane of the T-Cell. The constant domains of the TCR consists of cysteine residues that form disulfide bonds, which form a link between the two chains. Further research into immunotherapy, TCR structure and function, and the implementation of Reenergized ACT has major promise in increasing the efficacy of treating disease in human beings now and in the future to come.