June 2020

Molecular investigation of tight junction proteins related to the small intestines

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Abstract

The intestines are an integral part of homeostasis and vital functions that occur throughout the body via the mechanisms of the epithelial barrier. Deficiencies in the intestinal epithelial barrier corresponds with intestinal pathologies and mental disorders. Interestingly, the effectiveness of the barrier function is correlated with a family of transmembrane proteins known as claudin. Alterations of expression of claudin-3 and -23, which are known barrier-forming proteins, occur during the presentation of intestinal pathologies. Investigating how these proteins act using experimental methods presents many difficulties. Therefore, we will be using advances in computational modeling to study the assembly of claudin-3 and -23. By analyzing, the behavior of these proteins we can gain new insight on the mechanisms of transports and communication in the gut and to the rest of the body as wells as understand the molecular origins of intestinal disorders.

Molecular investigation of tight junction proteins related to the small intestines

by

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Bioengineering
B.S., Syracuse University, 2019

Thesis
Submitted in Partial Fulfillment of the Requirements of
the Master of Science in Bioengineering

Syracuse University
June 2020

Acknowledgements

I would like to thank my advisor, Shikha Nangia, Ph.D., for allowing me to participate in her lab and research. The willingness to break things down, so the fundamental concepts pertaining to the research was understood. Additionally, I am grateful for your patience and guidance, which allowed this endeavor to be possible.

Next, I would like to acknowledge Nandhini Rajagopal, doctoral student, who has helped give me direction for the project. Furthermore, she has helped me every step of the way and her willingness to answer all of my questions, has contributed greatly to the success of the project.

Moreover, I am thankful for the expertise and guidance of Jingjing Ji, a doctoral student, who has helped me with the minute details pertaining to my work and her willingness to help me correct my mistakes and comprehend the material.

Ultimately, I would not have been able to perform and carry out the work in this lab without the help of every individual in the Nangia Research Group.

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Introduction

Microbiome. The microbiome is a diverse ecosystem of bacteria, viruses, and fungi that assist digestion and absorption of necessary nutrients. Digestion usually begins in the mouth when salivary glands release preliminary enzymes and mechanisms of mechanical breakdown. Food travels to the stomach via the esophagus, where little absorption occurs. In the stomach, there are enzymes as well as other chemicals like bile that help break down the food into smaller conglomerates before entering the small intestines. The small intestines are broken down into three compartments: the duodenum, the jejunum, and the ileum. The duodenum neutralizes the acidity of the contents leaving the stomach through the production of bicarbonate ions. Furthermore, in the duodenum, many pancreatic enzymes are released in this portion of the small intestines, to break down mostly fats and proteins.[17] The main function of the other two compartments of the small intestine is to absorb nutrients from the intestinal cavity, or the lumen into circulation. Intestinal enzymes range from lipase, which break down fats, to amylases that break down carbohydrates. The body requires carbohydrates, proteins, fats, vitamins, minerals and water for cell synthesis and cell maintenance.[11] However, our body alone is not equipped to break down certain substances and produce certain vitamins and minerals. Interestingly, there are trillions of bacteria, that are present in the intestinal lumen, that supplement the digestive process, allowing the body to absorb the necessary nutrients, provided that good dieting is practiced.

Composition of bacteria present in the "gut" changes depending on the use of antibiotics, dieting habits and more. It changes depending on the types of food eaten and the presentation of disease and/or deficiencies. Gut bacteria works symbiotically with the body to break down and release nutrients, which our body can use. However, in the past decade, studies have shown that the influence of gut bacteria may not be isolated to the digestive process.

Gut-Brain Axis. In recent mice studies, researcher raised mice without gut bacteria and these mice illustrated anxious and depressive behavior. Furthermore, researchers have conducted correlation studies with intestinal deficiencies relating to changes in the microbiome composition and the presentation of neurological conditions such as schizophrenia, depression, autism, and epilepsy.[6] In an article that was released by C&EN in 2019, it proposed different possible mechanisms, by which the gut bacteria may be influencing the brain (Fig. 1). It was proposed that the gut may be influencing activity of the vagus nerve, which is the conduit between the brain and the gut. Due to the release of bacterial by-products, which may act as messengers, or neuroactive molecules on this conduit.[6] Secondly, the microbes can produce metabolites and neurotransmitters that can enter circulation. These structures have the ability to cross the blood brain barrier and cause alterations in brain chemistry. Furthermore, gut bacteria can elicit presentation of intestinal inflammation and the inflammatory cytokines, or immune cells can circulate in the blood and make their way into the brain. Correlations between these inflammatory immune cells and psychiatric disorders, such as depression have been found. Moreover, in 2018, researchers at the University of Alabama reported the presence of gut bacteria in human brain tissue, which may indicate the

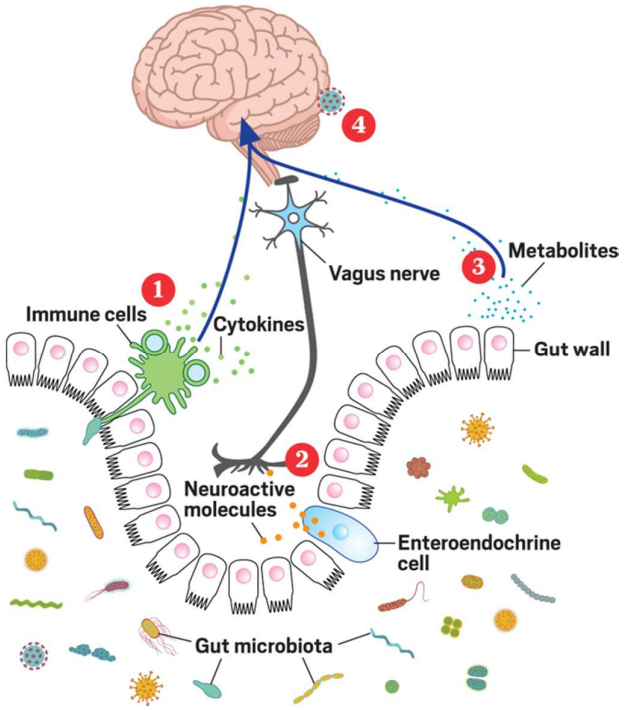

Figure 1. An illustration of proposed mechanisms of communication between the microbiota in the gut and the brain[6].

transport of microbes across the gut wall, into circulation across the blood brain barrier and into brain tissue.[6] Ultimately, there are many proposed mechanisms for this "gut-brain axis," however, the molecular understanding by which transport across the gut wall occurs and the communication between the gut and the brain remains elusive. First, we must investigate how transport across the interface, between the intestinal lumen, where the gut bacteria reside, and circulation occurs at the molecular level before insights can be determined pertaining to the communication on this axis.

Intestinal Morphology. Intestinal morphology is intricate to carry out the important function of digestion and simultaneously preventing the absorption and transport of antigens, gut bacteria or other toxins. The lining of the intestinal lumen contains circular folds and villi. The villi are present to increase the amount of surface, by which absorption and transport can take place. Increasing surface allows for increased efficiency of absorbing the nutrient the body needs to survive. In Fig. 2, there is an illustration of the cross section of the small intestines and the villi are the finger-like protrusion in the outer portion of the intestinal lumen.

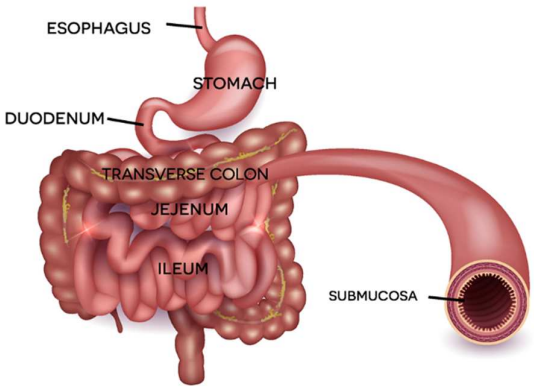

Figure 2. A depiction of stomach and the intestines and the cross-section of the small intestines, which illustrated the intestinal lumen and the villi.

The intestinal wall consists of four major demarcations, or layers, which are the adventitia, muscular layer, submucosa, and mucosa which are listed from farthest to closest layer to the intestinal cavity. This is depicted in Fig. 3. The adventitia is a smooth tissue membrane, that consists of two layer of mesothelium and the parietal layer, where

serous fluid is secreted.[16] The muscular layer consisted of various muscle tissues, that perform peristalsis or the contraction of smooth muscles cells that move the contents inside the lumen through the small intestines. Subsequently, the submucosa is a thin layer of extracellular matrix that is enriched with collagen. It functions to connect the muscular layer to the mucosa. Ultimately, the outer most layer is the mucosa. Mucosal layer consists of the epithelial cells, which secretes a thick protective fluid. However, the main function of the mucosa is to mediate absorption and the transport of nutrients from the lumen into circulation.[16] The barrier between the contents of the gut and circulation is largely determined by the activity of epithelial cells that make up the mucosa.

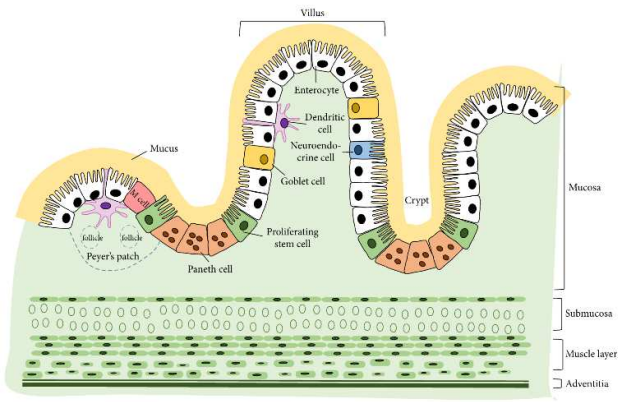

Figure 3. A cartoon illustrating the four layers of the small intestine as well the monolayer of polarized epithelial cells of the intestinal epithelium.[16]

Epithelial Barrier. The components, or cells that make up the epithelial barrier are continuously replaced approximately every four to five days via renewal and migration. The new epithelial cells originate in the crypt of the villi which is depicted in Fig. 3 from stem cells. The progenitor cells in this region differentiate into mature epithelial cells as they migrate from the crypt to the top of the villus.[16] The aged epithelial cells slough off into the lumen of the intestines to be later excreted following passage through the colon.

Intestinal epithelium is a single layer of cells lining the gut lumen and as aforementioned, it selectively filters, which allows for the translocation of essential nutrients, water, and other substances. The permeability properties vary in different portions of the intestines and the permeability is described as the movement of particles across the "gut wall." Permeability is an intrinsic property of the intestines and it is mediated via two main pathways: the transcellular pathway and the paracellular pathway (Fig. 4). The transcellular pathway is associated with transport through the cell. Transport in this pathway is largely regulated by specific transporters to aid in the absorption of amino acids, small chain fatty acids as well as short chained carbohydrates.[4] However, this pathway also includes receptor mediated endocytosis, or the binding of specific nutrients to corresponding receptors, which results in endocytosis of the nutrient into the cell and transportation of the nutrient from the cell and into circulation. Transportation via the transcellular pathway is an active process. In contrast, the paracellular pathway, is mode of transportation of nutrients between adjacent epithelial cells. Substances from the lumen are able to travel down the concentration gradient between epithelial cells and diffuse from the lumen into the circulation. However, only specific substances are allowed to move across this the gut wall via this form of transport.

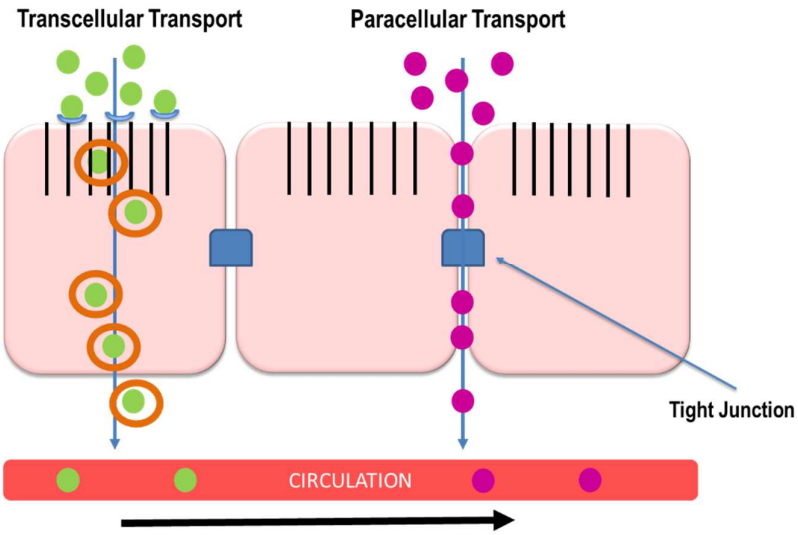

Figure 4. The transportation of nutrients from the intestinal lumen between epithelial cells (paracellular transport) and through the cell (transcellular transport).

Paracellular Pathway.

The paracellular pathway is regulated by intercellular complexes along the lateral membranes of adjacent cells. Although, a channel is present between adjacent cells, there are points of contact as well. The contact between adjacent epithelial cells includes three main components: desmosomes, adheren junctions and tight junctions. Desmosomes are highly ordered membrane domains that mediates cell-cell-adhesion and linkages between the cytoskeleton for adjacent cells. They function to mechanically integrate cells with tissues to resist mechanical stress. Another component of the paracellular pathway are the adheren junctions, otherwise known as zonula adherens, which are formed by interactions between transmembrane proteins.[4] The adheren junctions are an important factor in linking adjacent cells, maintaining cell polarity and the regulation and proliferation other adhesive complexes like desmosomes.[4] Thirdly, the tight junctions are the most apical adhesive junctional complex in the paracellular pathway in mammalian epithelial cells. Tight junctions are dynamic in nature and contain multi-protein complexes that function as semi-permeable barrier to transportation of substances in this pathway.

Tight Junction.

In the 1960s, the advances in imaging, due to the introduction of electron microscopy allowed for the illustration of apparent fusion between epithelial cells, known as kissing points.[4] The visualization of the "kissing points" is illustrated in Fig. 5. Currently, it is known that the tight junctions facilitate the passage solutes in the intercellular space. Furthermore, the tight junction aids in the prevention of translocation of

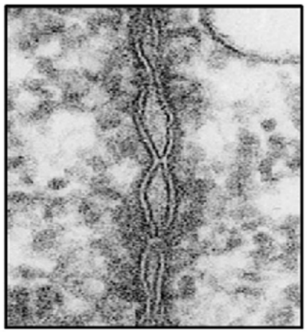

Figure 5. A freeze fracture electron microscopy image of the tight junction[24].

luminal antigens, microorganisms and other toxins.[4] The tight junction consists of many proteins, but the main characterizations for these proteins are occludins, junction adhesion molecules, and claudins as shown in the Fig. 6.

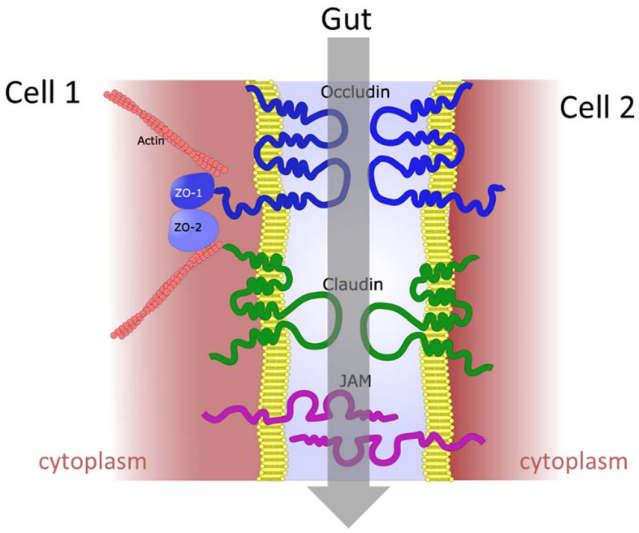

Figure 6. A cartoon of an intestinal tight junction, corresponding component proteins and the concentration gradient in the paracellular pathway.

Occludins are transmembrane protein that have a molecular weight that ranges from 60-82 kDa. This tetra-spanning integral membrane protein is comprised of two extracellular loops, short cytoplasmic N-terminus, and a long cytoplasmic C-terminus.[4] Analysis of occludins indicate that the extracellular loops aid in the regulation of permeability properties of the tight junction.[4]

In addition to the Occludin, the junction adhesion molecules, or JAMs, also influence the permeability characteristics of the tight junction. JAMs are also integral membrane proteins that belong to the immunoglobin superfamily.[4] The extracellular portions of these molecules have the ability to bind to multiple ligands via homophilic and heterophilic interactions. These interactions are proposed to aid in the regulation of cellular function as well as paracellular permeability.

Claudins are a family of proteins that have molecular weights that range from 21-34kDa. They are transmembrane proteins that are located in the lateral membrane of epithelial cells. Claudin proteins can interact with other claudins on the same cell, but the formation of the tight junction or the properties of the permeability are dependent on the extracellular interactions of claudins on adjacent epithelial cells.[5]

Recent mice knockdown studies in conjunction with the measurement of transepithelial electrical resistance, or TEER, has shown that claudins are the components of the tight junction that dictate barrier properties of the paracellular pathway.[2] Initially electrophysiological research demonstrated that the formation of the tight junction corresponded to an increase in transepithelial resistance. TEER measurements are performed by applying an AC electrical signal across electrodes placed on both side of a cell monolayer as depicted in Fig. 7. Once the electrodes are placed, voltage and current are measured to calculate epithelial resistance. Additionally, to decrease variability between the measurements obtain, the *Transwell*® - culture conditions were used. TEER is a rapid and non-invasive method of quantifying epithelial resistance.[2] Considering the aforementioned technique of quantifying epithelial resistance, researchers used knockdown studies to determine which junctional complexes in the paracellular pathway largely determined the permeability characteristics. In a study using claudin-5 with respect to the

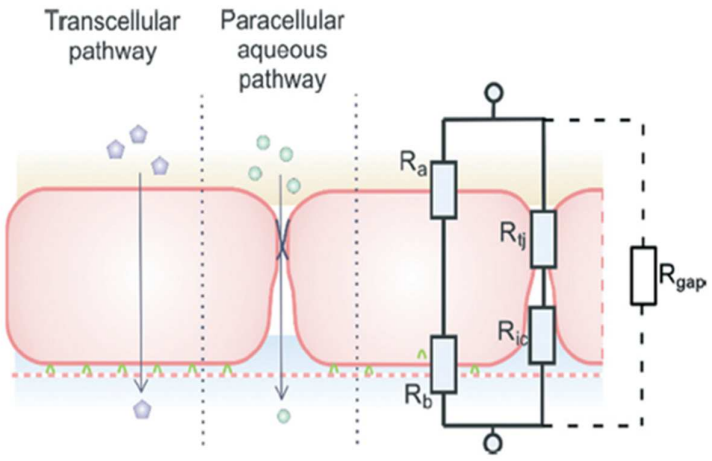

Figure 7. An illustration of an equivalent circuit model of the transcellular and paracellular route to test resistance across the cell monolayer.[2]

tight junctions of the blood-brain barrier, researchers performed mice studies that resulted in the lack of expression of occludins and subsequently, the double knockdown of occludins and claudins. Consequently, in the initial knockdown study, the transepithelial electrical resistance was quantified and minimal change in resistance was demonstrated when compared to a cell that expressed occludins. Furthermore, the pathway remains impermeable to molecules that were greater than 800 Da. In the double knockdown study, a drastic decrease in transepithelial electrical resistance was observed as well as a drastic change in the permeability characteristics in the paracellular pathway. Without the presence of occludins and claudins, molecules ranging in size from 3-10 kDa were allowed to travel down the paracellular pathway.[3] As a result, scientists were able to conclude that occludins play more of a participatory role in tight junction formation whereas the claudins are the proteins that predominately dictate the permeability characteristics of the pathway.

Claudins. Claudins are integral membrane proteins that have span from the cytoplasm of the cell through the cell membrane an into the paracellular space. In these family of proteins, there are four hydrophobic transmembrane helices that are numbered from 1 to 4 from the N-terminus to the C-terminus. Additionally, claudins possess one intracellular loop and two extracellular loops. It was not until 1998, that the claudin was first discovered. However, much is to be learned about these proteins and how they interact to determine permeability in the paracellular pathway.

Since the debut of the claudin, scientists have discovered 27 different claudins in the human body. In addition to experimental detection of differing claudins, advances in homology modeling has resulted in the elucidation of the amino acid sequences of the differing claudins. Homology modeling is a representation of the similarity of residues as they correspond to positions in the reference protein. Recent advances in this type of modeling allows for the alignment of sequence with template structure, distant homologues,

modeling of loops, side chains and more.[3] Furthermore, these advances have led to detection of errors in previous models of protein structures. In the phylogenic tree shown in Fig. 8, claudins are characterized as classic or non-classic claudins. The demarcation is dictated by similarities in the sequences of the differing claudins. Classic claudins have increasingly similar sequence relative to other classic claudins. Moreover, classic claudins have shorter sequences when compared with non-classic claudins.[3] In contrast, non-classic claudins are have increased dissimilarity with other claudins and less is known about the behavior of these proteins with relation to tight junction formation.

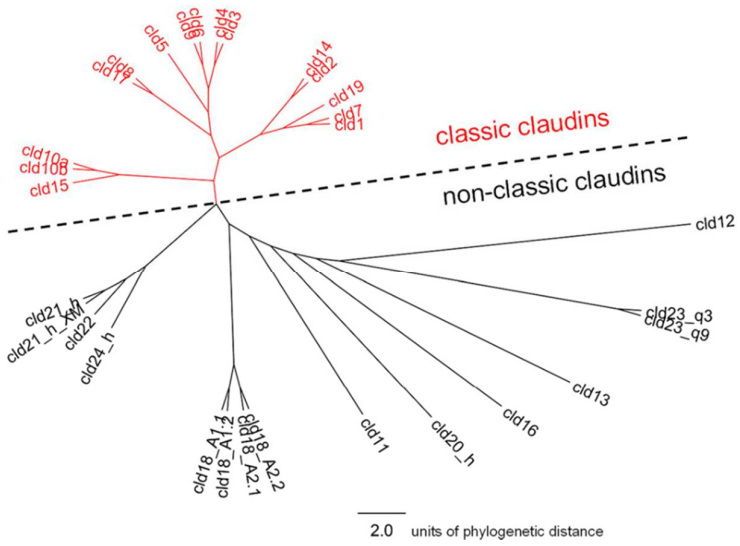

Figure 8. The phylogenic tree for claudins in the claudin family. Claudins in red are characterized as classic claudins shown in black are non-classic claudins.[5]

Claudin Expression. Claudins are expressed in tight junctions throughout the entire body. For example, tight junctions in the blood brain barrier largely express claudin-5. Furthermore, due to the lack of substance that can cross this barrier, it can be concluded that tight junctions consisting of claudin-5 correspond to increased transepithelial resistance. In addition, the stomach exhibits relatively high expression of claudin-3, claudin-4 and claudin-5 which corresponds to different permeability characteristics in the paracellular pathways located in the stomach when compared to the blood brain barrier. Recent studies have denoted that in different parts of the intestines, there are different claudins being expressed in the intestinal tight junctions.[5] The differing expression of these transmembrane proteins is an important

factor in the determination of permeability properties; therefore, it is necessary how these different proteins interact with each other.

In the paracellular pathway, there are two main mechanisms of interaction between claudin proteins. Due to imaging via freeze fracture electron microscopy, we are able to elucidate that multiple claudins are expressed on the same cell. Additionally, studies have shown that claudins have the ability to self-associate. Initially, claudins on the same cell will

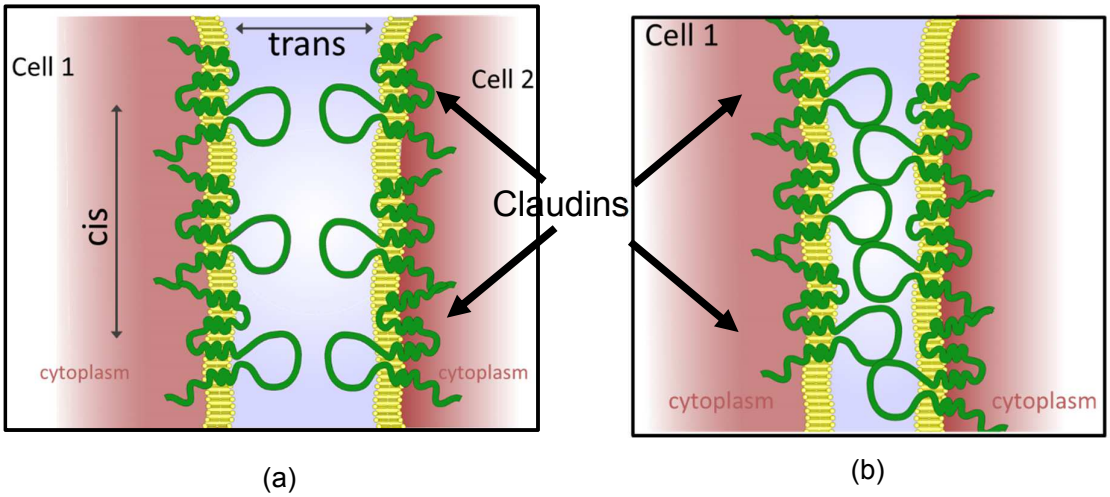

Figure 9. Cartoon illustrations of the different mechanism, by which claudins interactions to form the tight junction via *cis* and *trans* interactions (a) and the formation of a "tight" tight junction following the strong head-on interactions between the claudin proteins (b).

interact via their transmembrane helices and orientations of their extracellular loops, this is known as a *cis* interaction. The oligomerization of claudins on the same cell largely affects the orientations of these claudins and their extracellular loops in the paracellular space. The orientation of the extracellular loops dictates the interaction of the oligomerization of claudins on the adjacent cell. This head-on interaction between the extracellular loops of the claudin on adjacent epithelial cells is known as a *trans* interaction. The mechanism of interaction between claudins in a tight junction are illustrated in Fig. 9.

Significance

The differences in claudin composition in various tight junctions effect the permeability properties of corresponding paracellular pathways and are a rate limiting factor for paracellular passage. Furthermore, the expression of these proteins differs due to localization, meaning if the tight junction is present in the crypt or villus of the mucosa of the intestines or in tight junctions in different compartments of the small intestine.[15] Claudins not only influence adequate transport in the paracellular passage, but recent studies have found correlations of claudin expression with the presentation of intestinal diseases. Through experimentation, it is known that claudin-2 controls the passage of monovalent cations, and influences pore formation as well as the flux of water in this pathway. Additionally, the upregulation of claudin-2 was found to correspond with intestinal inflammation related to Crohn's disease and Ulcerative Colitis.[7] The presentation of epithelial deficiencies has been linked to other claudins as well, such as Claudin-3. Claudin-3 is present in intestinal epithelium, however, in the presence of Irritable Bowel Syndrome or IBS, a downregulation of this protein is observed.[12] In recent *in vitro* experimentation, claudin-3 demonstrated decreased expression due to pro-inflammatory cytokines.

Many experimental studies have been conducted to elucidate more information on claudins and their activity in the epithelium. However, due to the visualization techniques and the experimental techniques available at this time, it is nearly impossible to ascertain the behavior of the claudin protein in the lipid membrane and at the atomic level. First, we must understand how these proteins interact to mediate transport across the intestinal wall before we understand the molecular activity that dictates communication on the "Gut-Brain Axis". Due to the inadequacies of experimental methods and the advances in computation, we will use computational modeling to ascertain the behavior of claudin proteins embedded in lipid membranes in an environment analogous to the gut.

As a result, our lab is collaborating with an experimental methods laboratory at the University of Michigan under the charge of Dr. Asma Nusrat. In this lab, they were able to determine patterns of expression pertaining to claudin-2, -3, and -23. The experimental studies in this lab demonstrated that in the intestinal wall, there is a higher expression of claudin-3, which a is known barrier forming protein and claudin-23, which is a non-classic claudin, that is known to enhance barrier forming properties at the top of the villus. In this same location a decreased expression of claudin-2 was observed. However, at the crypt of the villus, the opposite was observed; there was an increased expression of claudin-2 and decreased expression of claudin -3 and 23. Furthermore, at the top of the villus "tighter" or "non-leaky" tight junctions were observed, whereas at the crypt, "leaky" tight junctions, or increased permeability was observed. The focus of our study is to understand how the co-expression of claudin-3 and -23 forms "tight" tight junctions. We will use simulations to understand the behavior these proteins and the orientation, in which they prefer to interact. Due to the interactions of claudins on the same cell influencing eventual tight junction formation, we must first understand how claudin-3 and -23 behave in the cis-interaction, before simulating the trans-interactions and the formation of the tight junction.

Background

Computational Modeling. Computational models are mathematical models that use mathematics, physics, and computer science in order to study complex systems.[19] The introduction of this technique allows scientists to increase efficiency of investigation through an iterative process of modeling.[19] Furthermore, this type of modeling provides unified computational environments and easier access of a broad range of modeling tools. Modeling environments are characterized by scientific concepts that relate to the modeling process, which are being investigated. Computational systems allow scientist to make non-trivial inferences based on symbolic representations, or accurate approximations. Moreover,

scientists can adjust variables or a combination of variables and observe the outcomes, which would be unrealistic in the experimental setting. Albeit, the presence of many equivalent models can exist for a single system, some techniques are computational more efficient than others.[19] A simplified schematic for the computational process begins choosing a set of concepts, then corresponding representations. Subsequently, the transformations are applied, and mappings made from the symbolic representation are analyzed.

Hierarchal Scheme of Simulation Methods. Interestingly, computation models can represent many different phenomena in accordance with hierarchy of multiscale modeling which is illustrated in Fig. 10. Multiscale modeling refers to the use of multiple models at different scales that are used simultaneously to describe a system. The systems that are represented can range from weather forecasting based on atmospheric conditions and flight simulators, which use complex equations to govern flight of the aircraft to biological systems where it is possible to study molecular processes, cell-cell-interactions and their effect with relation to tissues and organs.[1] Using a macroscale system, the simulations to be greater in size and run for longer timescales, however, important information pertaining to particle or molecule interaction is lost. In contrast, microscale simulation retains the minute details of a system, but it is not efficient computationally. Multiscale modeling consists of analysis and modeling tools that allow us to understand the relation between models at different resolutions, as well as,

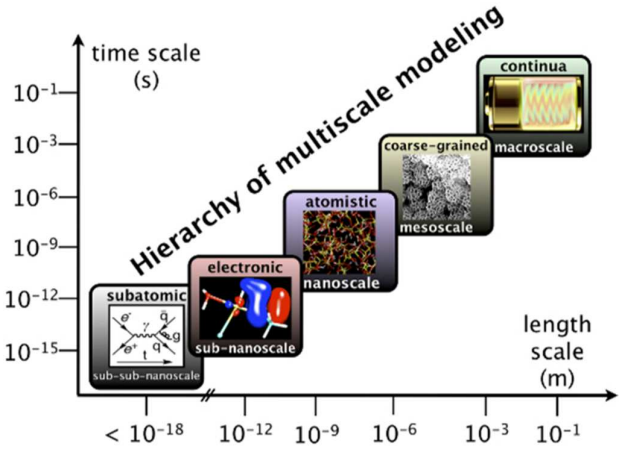

Figure 10. A depiction of the corresponding length scales in meters and timescale in seconds that is appropriate for systems of different resolutions.[23]

algorithms, which enables the realization of ideas in the computational setting.[22] When using computational modeling with respect to biological systems, there are many techniques available. However, in this work molecular dynamics will be used.

Molecular Dynamics. When studying the physical and chemical properties of solids, liquids, amorphous material and biological molecules, molecular dynamics is a powerful simulation technique. For investigating biomolecular systems, quantum mechanics is the proper theory. However, by using Newton's Laws and additional environmental parameters as the basis for particle movement in a system, we are able render accurate approximations.[10]

The movement of particles is an iterative process at each time step, or the points in the simulation when data is recorded. All the particles in a system are place in a simulated box to create a discrete environment for the particles to interact. During the simulations, each of the particles has a position (x, y, and z-coordinate) in space and corresponding velocity vectors. A particle in the system, experiences a force acting upon it from the other particles in the system. Following the calculations of the individual forces acting upon a particle, the net force is calculated. Using the pre-determined mass of the particle and the change in velocity corresponding to the time step, the computer calculates the change in position for the particle. At every time step, these calculations are executed for each particle in the system. The output position for each of the particles is the input position used for the next iteration.

Unfortunately, due to the application of a discrete box, or unit cell, by which the simulations occur, these boundaries can interact with the particles of the system producing artificial data and bias the system.[10] To resolve this problem, periodic boundary conditions are put in place. Application of periodic translations allowed for the infinite replication of the unit cell. Consequently, the particles move in the space filling box and pass through box borders unrestricted.[10] Furthermore, cut-off parameters pertaining to the distance, at which

the force acting between these particles are negligible have been determined and added to the system.

Simulations for Biological Systems. The technique of molecular dynamics has been revolutionary for the creation of symbolic representations of biomolecular systems. Due to the iterative process, by which the particle movement throughout the duration of the simulations, it is imperative to use understand which resolutions would render an appropriate approximation for the biological system. In this study, we will be studying how specific transmembrane proteins interact with each other while embedded in a lipid membrane. As a result, the length scale for the system will be in nanometers, and the time scale will be in microseconds. The resolutions that are compatible with the aforementioned time scale and length scale are atomistic and coarse grain, which are the resolutions readily used to represent biomolecular systems.

In conventional molecular dynamics simulations, the potential energy is described as a conservative force, which takes into non-bonded and bonded interactions. In most simulations, the electronic transitions are ignored. Most force fields are a function of atomic positions and the approximations are made from dispersion forces.[8] The dispersion forces in these simulations usually comprise of non-bonded and bonded potentials. Non-bonded potentials consist of coulomb potential, which is used to model energy between two-point charge particles and van der Waals forces, which are determine via Lennard-Jones potentials. The Lennard-Jones potentials calculated the potential energy that corresponds to the distance between interacting particle and the forces of repulsion and attraction. These parameters can be determined for atoms and particle alike, which allows researchers to create diverse system of biomolecules. Bonded potentials are used to estimate distribution of electron density; they include bond distance, valence angle, improper torsion angle at equilibrium.[8]

Atomistic Simulations. Atomistic force field are systems that are optimized through the use of quantum chemical calculations and thermophysical data.[8] Proteins, lipids, and other biological structures are represented with atomic level detail; they accurately represent and reproduce the complex surface chemistry. The details included in this level of design allow for accurate interactions between various biological structures and due to its detail, it is preferred for the use of analyzing biomolecular systems. However, due to the detail of these representation, there is an increase in the number of particles within the system and with the techniques used in molecular dynamics, this results in an increase in computational demand.[8] The system must now calculate the increased number of pairwise calculations per time step, so the time scale and the length scale must be shortened to increase computational efficiency.

Coarse-grained Simulations. Due to the increase in computational demand pertaining to the atomistic-level simulations, the coarse-grained model addresses this issue through reducing the number of particles in the simulation. Reduction occurs via the clustering multiple bonded atoms into a single interaction center known as a bead. Coarse-grain force fields average out the expensive atomistic details to simplify the biological system. As a result, it allows for the simulation and subsequent analysis for larger systems for larger time scales. This clustering method can be used for protein-protein, protein-lipid and other biological interactions.[8]

Martini Force Field. Using coarse-grained models has become increasingly popular for computational modeling of biomolecular systems. In this study, we will be using the coarse-grained force field known as the Martini force field. Initially, this force field was calibrated for the simulation of proteins and lipids; it was used to simulate multi-component membranes.[8] The way that Martini reduces the computational load using a four to one heavy atom mapping scheme. In this scheme, four heavy atoms and their corresponding hydrogen atoms are represented as an interaction center, or bead. The coarse-grained particles are assigned a specific particle or bead type: (a) charged, (b) polar, (c) non-polar, and (d) apolar.

In addition, the four major bead type are each divided into five subtypes, which are distinguished by their hydrogen bonding capability or their degree of polarity.[8] By using the twenty possible types of beads, the Martini force field allows for the representation of complex biomolecular systems consisting of various biological structures. Furthermore, the equilibrium structures of these coarse-grained models can be "reversed-mapped," or changed from the coarse-grained structure back into their atomistic structure for further analysis.

Additional parameters included in the Martini forcefield are the bonded and non-bonded interactions between the beads. The bonded interactions are derived from all-atom reference simulations, whereas the non-bonded interactions are based on the reproduction of experimental partitioning free energies.[8] The reduction in computational demand and the ability to reverse mapped equilibrium structures renders coarse-grained models increasingly desirable. Ultimately, the Martini model can predict the relative binding of peptides and lipids in close arrangements and results have been corroborated by experimental research.[21]

Protein Associated Energy Landscape. Several protein association studies embedding proteins into complex lipid bilayer and other biological environments have been executed to mimic physiological conditions. Despite the advances in computational modeling and molecular dynamics with respect to protein association studies, the quantifications, by which protein-protein association produces high degrees of uncertainty. Quantification of protein association is usually based on the final protein populations, interaction distances between proteins, or contact mapping.[14] However, in the Nangia Research Lab, a new method for quantifying protein-proteins associations was developed and it is known as the Protein Association Energy Landscapes, or PANEL. In this method, the rotational space around protein 1 and protein 2 from 0 to 360˚ was explored. The rotational space around each protein is depicted in Fig. 11. Subsequently, initial orientations of protein 1 and protein 2

associations were generated, and the proteins interacted within the van der Waals interaction radius.

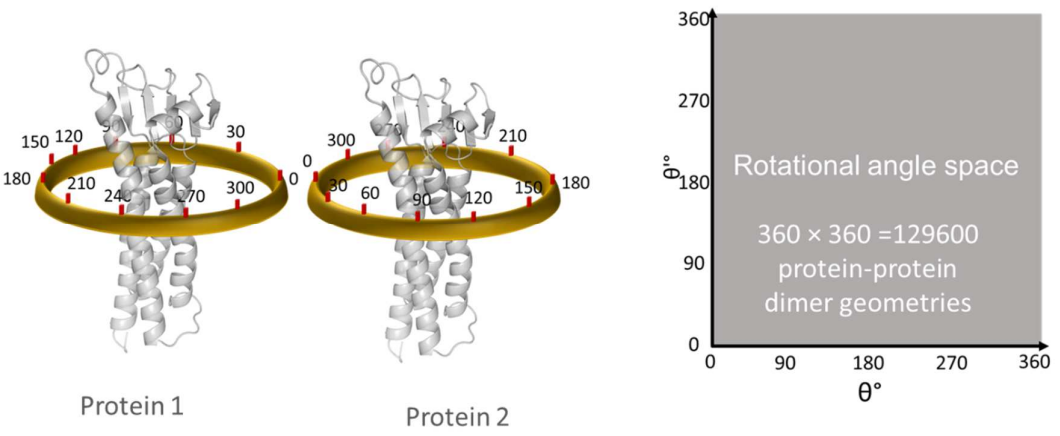

Figure 11. A visualization of the quantification of the rotational space around protein 1 and protein 2 and how it relates the 2D dimensional analysis plots, where θ is denoted by the rotational space around protein 1 and θ' denotes the rotational space of protein 2.[14]

Quantification of the stability of each protein-protein association was calculated using their non-bonded interaction energies in order to generate a potential energy profile. The energy profile was created for all orientations, regardless of likelihood of formation.[14] Each of the initial orientations, or initial seed geometries, were random and uniformly distributed in the rotational space. Each seed geometry underwent a short molecular dynamics simulation to yield a set of equilibrium conformations without constraints. Initial seed geometries were used to overcome high energy barriers that are present with other methods for protein self-assembly. Furthermore, we are able to visualize low potential energy configurations as wells as high energy configurations that lack favorable pairwise residue interaction. Ultimately, the goal of the PANEL approach is to sample the rotational space around each protein association and obtain a data set through stochastic sampling and equilibration simulations. Moreover, it can be applied any given set of proteins or transmembrane structures and is independent of limitation of specific force fields.[14]

Claudin Crystal Structures. Before the PANEL method can be used, it is imperative to obtain the atomistic structures for the proteins of interest. In this study, we will be using the atomistic structures for claudin-3 and claudin-23. However, the atomistic structure for any claudin had not be experimentally elucidated until 2014. In this study, to gain insights on the atomistic structure of these transmembrane proteins, researchers used the mouse claudin-15, or mCldn 15, because it could be expressed in large amounts. Additionally, mCLDn 15 formed prominent tight junction strands when in contact with adjacent cells.[18] Researchers modified the structure by truncating residues on the c-terminus and membrane-proximal cysteines were substituted with alanine to avoid palmitoyl modification[18]. Following, the modifications, the altered protein underwent purification and crystallization. As a result, the researchers were able to obtain high resolution diffraction data from the crystals using a micro-focused x-ray beam. The resolution was determined to be approximately 2.4 angstroms, so the scientists were able to ascertain the crystal structure of claudin -15. Experimental determination of crystal structures is demanding, and expensive process and the resolutions of various visualization techniques varies. There are limitations to experimentally determine the crystal structure in biomolecular structures, especially transmembrane proteins.[18]

Due to these experimental limitations, computation structure predictions models have been created. The three main methods for computation structure predications for transmembrane proteins are ab initio modeling, threading and homology modeling.[20] *Ab initio* modeling predicts the structure of proteins based on the physical principles. However, this technique is not used because an increased demand for computational resources and low accuracy pertaining the structure of the protein. The computational demand can only be used to predict the structures of very small proteins. Threading is a technique based on close primary sequence folds into similar structures; amino acid sequence of the target protein is

"threaded" onto the structure of a suitable template. However, due to the finite number of folds, non-homologous proteins may be predicted to have similar structures. Homology is the most widely used technique for protein structure prediction. The structure of the protein is created based on the 3-D structure of an evolutionally close template protein. Close homologues can be used as the starting approximation.[20] Due to the discovery of the crystal structure of claudin-15, the template for claudin-15 will be used to predict the crystal structures for claudin-3 and claudin-23, which will be investigated in this study.

PANEL Workflow. Upon commencement of the PANEL method, crystal structures for desired proteins are elucidated via the use of homology studies. Subsequently, the newly generated crystal structures for the transmembrane proteins are converted into their coarse-grained form. The coarse-grained models for the proteins are used as the input structures and the interaction radius for each protein was determined. The interaction radius for each protein was used to determine the distance of separation between the interacting proteins, which was executed using the *P_sep.py* script. After the quantification of the interaction radius, the number of initial seed geometries are determined for the system and this information is coupled with the interaction radius to embed the proteins in the binary compositional lipid membrane. This was executed using the *P_setup.py* python script.

Docking assay for transmembrane proteins, or DAFT is a method for investigation of protein-protein and protein-lipid interaction and the goal for this technique was to allow the input of any combination of sequences, atomistic and coarse-grained structures.[21] Furthermore, a key feature of DAFT is its ability to maximally unbiased simulations and optimize the conformational space via the use of specific boundary conditions.[21] In this study, DAFT will be used with the modifications of input parameters to include number of seed geometries and distance of separation between the proteins. Moreover, each initial seed geometry will undergo energy minimization. Energy minimization is necessary because the

starting configuration can be far from equilibrium, so to reduce unfavorable interaction, energy minimization algorithms are placed to optimize the system.[8] Following the energy minimization and equilibration, the simulation undergoes a production run for certain length of time. Each production run was independent and parallel to achieve maximum computation efficiency.

The molecular dynamic production runs were carried out and the trajectories produced were processed to obtain, the combination of protein orientations in the rotational space, or the dimer angle and their corresponding interaction energies. Obtaining this information was executed through several sequential steps in the *P_analysis.py* script.[14] Additionally, the periodic boundary conditions were removed from the trajectories to extract the dimer configurations. The process trajectories were used to obtain the interaction energy between the interacting proteins using GROMACS. Subsequently, if the dimer configurations had a distance between the center of mass of each protein was greater than the interaction distance this configuration was filtered out and the remaining data was used to generate analysis landscapes.[14]

The first plot generated to analyze the proteins following the simulations was the grid coverage plot. The grid coverage was used to determine the total number of combinations of orientation of the protein 1 and protein 2 interaction. If the grid coverage was calculated to be 100%, that means all possible configurations in the rotational space were visited following the production run. The next plot was the energy contour landscape. However, to generate the energy contour landscapes, the non-bonded interaction energy of each protein pair within the

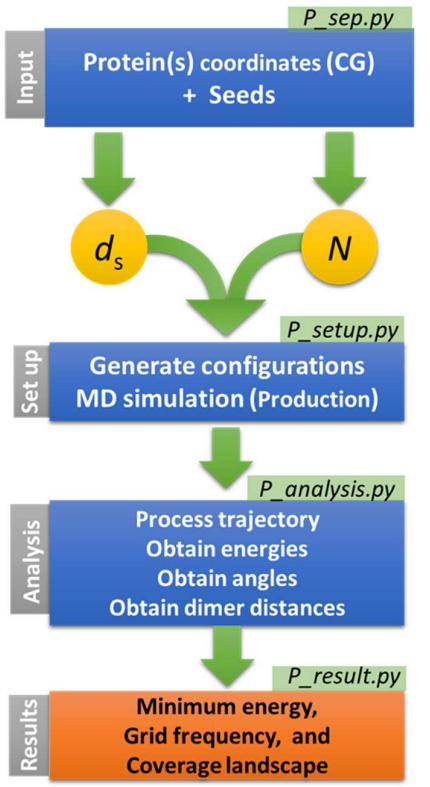

Figure 12. A schematic of the simplified workflow of python scripts used to execute the PANEL method.[14]

separation distance cutoff was recorded as a function of the rotational space (θ, θ'). The final data was used in the *P_result.py* portion of the PANEL workflow. In addition to the creation of the energy landscapes, the final data set was used to illustrate number of times each grid point, or combinations of orientations in the rotational space, was visited throughout all of the productions runs, in order to create the frequency plot. All analyzed data will be presented in two-dimensional plots. Panel workflow is illustrated in Fig. 12.

The PANEL method investigates the behavior of transmembrane in biomolecular system. In order to demonstrate the power of the approach, multiple production runs involving the same proteins, in the same biological environment, were executed using differing input parameters. The cis-interaction of claudin-5 with claudin-5 was used.

	M			
N	250	400	500	1500
500	40%	50%	54%	61%
1000	64%	73%	76%	86%
2000	85%	89%	91%	95%
3000	92%	95%	96%	97%

Table 1. The shows the percent coverage of the input parameter, where M is the duration of the run in nanoseconds and N are the number of initial seed geometries.[14]

Four sets of production runs were executed using different number of initial seed geometries as well as different lengths of time for the production runs. The different initial seed geometries were 500, 1000, 2000, and 3000 and set of geometries were carried out at the following production times: 250, 400, 500, and 1500 nanoseconds.

Following the production of all sixteen runs, the grid coverage was elucidated and quantified for each, which can be visualized in Table 1. The results of the run indicated that in spite of the increasing production time, when using 3000 initial seed geometries the grid coverage was above 92 percent and the increasing production times minimally increased the number of grid points visited. Consequently, the PANEL approach is a technique that allows for the molecular investigation of transmembrane proteins, while decreasing computational demand.

Methods

To ascertain the chemical activity of tight junctions with high expression of claudin-3 and claudin-23, the cis-interactions will be simulated and studied before any inferences can be made pertaining to overall tight junction formation. Furthermore, the ratio of expression for claudin-3 and claudin-23 can vary between adjacent cells, consequently, the cis-interaction of claudin-3 and claudin-3, claudin-23 and claudin-23 and claudin 3-23 will be investigated in separate production runs.

Initially the atomistic structures for claudin 3 and 23 were converted into equilibrated coarse grain models using Martini v2.2 force field in conjunction with the ElNeDyn approach. The ElNeDyn approach, is used to preserve conformation integrity by using an elastic network as a structural scaffold to control the conformation of the protein while keeping its internal dynamics.[14] Successively, the claudins were embedded into a 10×10 mm^2 membrane, via the *uni_dist.py* python script, then through the use of the insert membrane, or INSANE script, the coarse-grained lipid templates were produced. The lipid template used was a binary lipid compositional membrane consisting of *dipalmitoylphosphatidylcholine* (DPPC) and *1,2-dioleoyl-sn-glycero-3-phosphocholine (*DOPC) in a 1:1 ratio. The system was solvated with Martini Coarse-grained water with a NaCl salt concentration of 0.15 M to increase the accuracy of protein interaction analogous to a biological environment. All systems were equilibrated, and the simulations were performed in GROMACS v2018.1 and

visualization was performed using *Visual Molecular Dynamics,* or VMD, software. Throughout the production runs continual minimization was carried out in the simulation via equilibration sets of 25 ns of isothermal-isochoric (NVT) and 50 ns of isothermal-isobaric (NPT). The time step determined for all simulation was 20 femtoseconds, which is congruent with other coarse-grain simulations of biomolecular systems. Temperature of the simulated environment was 310.15K, which is equivalent to temperature in the body and it was maintained using the v-rescale thermostat with the coupling constant of 1.0ps. In addition, semi-isotropic conditions were adopted using the Parinello-Rahman barostat. The pressure of the system was 1 bar, which was maintained with a compressibility if 4.5×10^{-5} bar^{-1}. To address the dynamic nature of the claudin proteins and the length of membrane, in which the proteins were inserted, the cutoff constant for van der Waals and electrostatic interaction was determined to be 1.1 nm. All three sets of production runs were executed and analyzed using the PANEL method.

Results and Discussion

The claudins studied in this work were claudin 3 and claudin 23. Claudin 3 is characterized a classic claudin with 220 amino acid sequence and a molecular weight of approximately 32.3 kDa. In experimental studies, it is noted that claudin-3 is known to participate in “tight” tight junction and its expression decreases in the presence of certain intestinal disorder. Whereas claudin -23 is a non-classic claudin with a 292 amino acid sequence an approximate molecular weight of 31.9 kDa, but little information is known about this protein. We investigated the interactions of claudins-3 and -3, claudins-23 and -23 and claudins-3 and -23, to discover insights on the behavior of the proteins when interacting on the same cell.

Following the processing of the trajectories produced in the runs a grid coverage plot was generated for each set of data. The grids were generated to show the conformations sampled in the $\theta = \{x|0 \leq x \leq 360°\}$ and $\theta' = \{x|0 \leq x \leq 360°\}$ rotational space. In each of the runs approximately 2500 initial seed geometries were generated to same 92% of the rotational space for claudin-3 and -3 interaction, 99% for the claudin-23 and -23 interaction

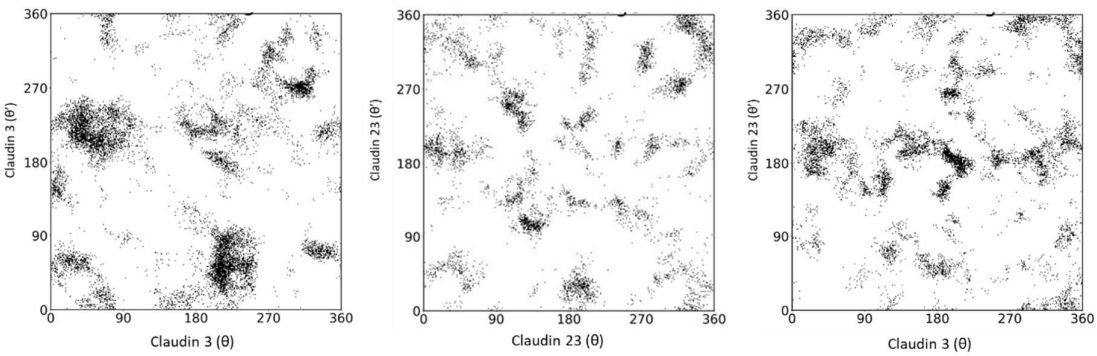

Figure 13. Shows the grid coverage for each of the production runs for the Claudins 3-3, 23-23, and 3-23; the coverage was 92, 99 and 94, respectively. White area shows conformations that were visited in the production runs whereas the black areas are conformations not visited.

and 94% for the claudin-3 and -23 interaction (Fig. 13).

Subsequently, the potential energy landscape profile of the rotational space is able to delineate the relative stability of the conformations sampled. As a result, we were able to identify global energy minima and relative energy minima of the cis assemblies. The energy

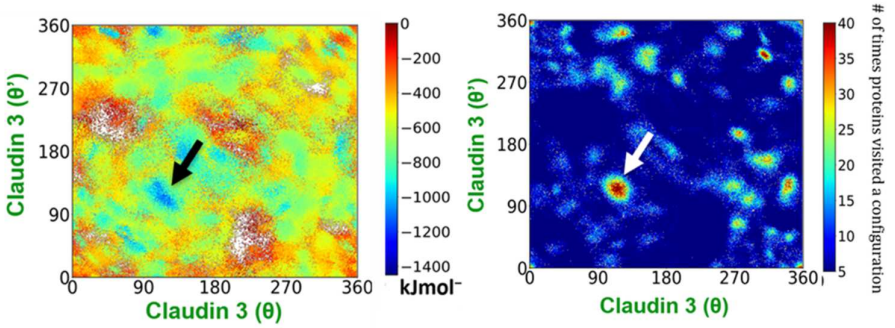

Figure 14. The energy contour and the frequency plots for the claudin 3-3 cis interaction production run. In this run, the arrow indicates a global energy minima and high frequency of combination of orientations.

potential was determined for each interaction as a summation of the non-bonded interaction

and denoted as kJ per mole. The potential energy for each orientation visited is shown in the energy landscape. In the claudin-3 and -3 interaction, a prominent low energy conformation cluster is located near (150˚, 150˚) and the corresponding energy potential is approximately -1100 kJ per mole as shown in Fig. 14. In addition to the energy plot for this interaction the frequency plot illustrates that this conformation cluster of low energy was visited several times throughout the production run (Fig. 14). Ultimately, it can be deduced that the conformations in this cluster are not only stable interactions, but viable conformations that will be used to perform the trans-interactions to understand tight junction activity.

The energy and frequency plots for the claudin-23 and claudin-23 interaction were generated (Fig. 15). The energy plot illustrated that the increase amount of low energy

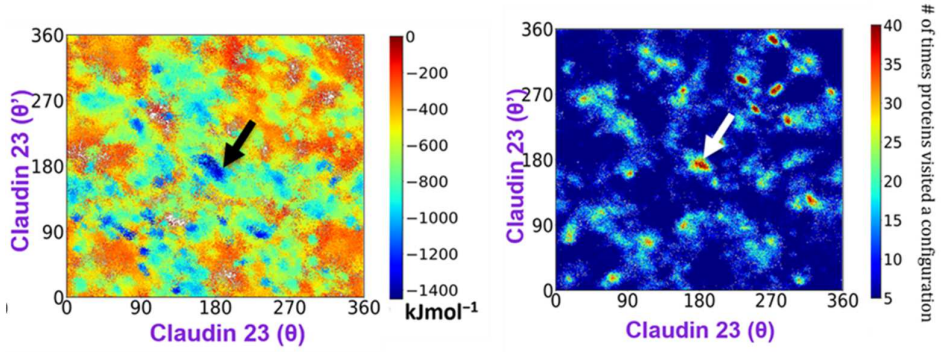

Figure 15. The energy contour and the frequency plots for the claudin 23-23 cis interaction production run. In this run, the arrow indicates a global energy minima and high frequency of combination of orientations.

clusters throughout the entire rotational space when compared with the claudin-3 and claudin-3 interaction. In addition to the increase amount of low energy clusters of conformations, the relative energy minima have a greater magnitude of energy potential, indicating that claudin 23-23 interaction is more stable than the claudin 3-3 interactions. In addition to the energy landscape, the frequency plots show areas of high frequency that corresponds with some of the clusters of relatively stable configurations. One cluster of interest in the claudin 23-23 interaction occurs near (180˚, 180˚) with an energy potential of approximately, −1500 kJ per

mole. Following the inference that claudin-23 creates more stable interactions with like protein than claudin-3, we investigated how claudin -23 might influence claudin-3 behavior.

Lastly, the energy landscape and frequency landscape were generated following the production runs (Fig. 16). The energy landscape illustrated a greater number of low energy conformation clusters when compared with the energy landscape for the claudin 3-3 interactions. Furthermore, the energy potential of the low energy conformation clusters was

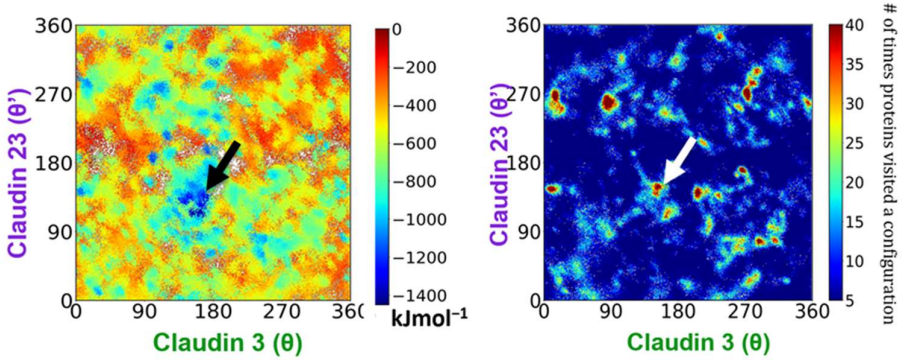

Figure 16. The energy contour and the frequency plots for the claudin 3-23 cis interaction production run. In this run, the arrow indicates a global energy minima and high frequency of combination of orientations.

approximately 1400 kJ per mole, indicating that an interaction between claudin-3 and 23 was more stable than an interaction between claudin-3 and claudin-3. Moreover, the frequency plots depict high frequency areas that correspond with low energy configurations. One conformational cluster of interest is the cluster located near (138˚, 138˚). In this region, the conformations have relatively high frequency and global energy minima.

Coarse-grained structures of conformations of relative stability and high frequency were obtained for each run. The coarse-grained structures were reverse-mapped, or changed from the coarse-grained structure into their atomistic structure, to study the interaction and the orientation of the proteins at the atomistic level resolution. In Fig. 17, isolated stable conformation of the interacting partner for each production run are shown.

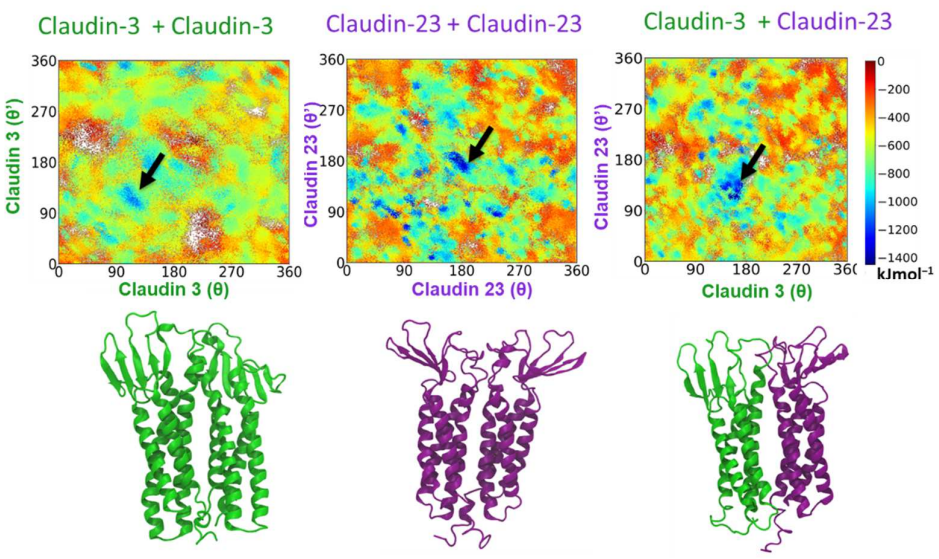

Figure 17. For the low energy and high frequency configurations, the corresponding the coarse-grained configurations for each run were isolated and reverse mapped. The green structures depict the atomistic structure of claudin 3 and the purple structures depict claudin 23.

Conclusions

The PANEL method is a robust approach to investigating the behavior of transmembrane proteins in an analogous biological environment. In this study, we were able to investigate all the combinations of binary cis-interaction of claudin-3 and claudin-23. Initially, we were able to sample over 90% of all conformation in the $\theta = \{x|0 \leq x \leq 360°\}$ and $\theta' = \{x|0 \leq x \leq 360°\}$ rotational space. As a result, we were able to adequately infer on the behavior of the aforementioned claudins. Claudin-3, which is a classic claudin and known to participate in the formation of "tight" tight junctions has the ability to create stable cis-interactions when interacting with another claudin-3 protein. However, through this work, it was determined that claudin-3 creates more viable conformations when interacting with claudin 23. Furthermore, the various landscapes depicted that claudin-23 creates relatively more stable conformations when interacting with another claudin-23 protein, than a claudin-3 protein. Following the discovery, of these viable conformations, we were able to reverse-map, or converted these conformations from their coarse-grained structure into their atomistic

structure, to investigate the protein interactions at the atomistic level. Unfortunately, due to the dynamic nature of the ratio of claudin expression for each individual cell, it is not yet possible to conclude which interactions occur more often at these tight junctions located at the villus.

Future Directions.

Going forward, the viable configuration isolated in this study will be used to for the trans-interaction studies. Following the trans-interaction studies, we will be able to deduce the activity of these proteins and identify the molecular explanations, for the correlation of high claudin-3 and claudin-23 expression and increase tightness of the tight junction. Furthermore, we will perform similar cis and trans interaction studies, including claudin 2, claudin 4, with respect to claudin 23, which are all claudins present in tight junctions located in the gut wall. Claudins are the proteins that largely dictate the permeability characteristics of the tight junction, which dictates transport in the paracellular pathway. If we can understand how these proteins behave at the molecular level, we can obtain molecular reasoning for the permeability characteristics corresponding with different compositions of co-expression present in tight junction along the intestinal wall. By understanding the mechanism, by which transport occurs, we will be able to ascertain new molecular insights about the mechanisms of communication on the gut-brain axis.

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Vita

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Education

Syracuse University, College of Engineering and Computer Science

Bioengineering on the Pre-Med Track, Bachelor of Science, May 2019

Bioengineering, Master of Science, May 2020

Syracuse University, College of Arts and Sciences

French and Francophone Studies Minor, May 2019

Syracuse University, Renée Crown University Honors Program Graduate

Presentations

SBI Stevenson Poster Session, March 2019 & March 2020

Honors Program Thesis Presentations, May 2019

Bioengineering Senior Design Presentations, April 2019

Additional Research Experience

Internship at the *Institut Nationale des Sciences Appliquées*, in Strasbourg, France Summer 2017

I created compositions to produce synthetic hydroxyapatite and analyze electrodeposition coating of synthetic hydroxyapatite on titanium alloys with respect to hip implants.

Data Organization for Research Project at Upstate Medical

I used Matlab to organize the data collected pertaining to knee loading forces and produce graphs that normalized the data for further analysis

Honors and Awards

Fulbright Finalist for Open Research/Study Award in France – 2020

University Scholar – Syracuse University – 2019

Recipient of the Richard A. Bernard Award – Syracuse University School of Engineering and Computer Science - 2019

Bioengineering Department Founder's Award – 2019

ACC Scholar-Athlete of the Year – Volleyball – 2019

NCAA & ACC Postgraduate Scholar – 2019

Honorable Mention All-American – Volleyball - 2018

Professional Membership

Phi Beta Kappa – Member – 2018

National Society of Collegiate Scholar – 2016

Tau Beta Pi – Engineering Honors Society – 2019

Work Experience

Medical Scribe (2018-2020) – Work in the Emergency Room and go with the ED physician to see patient and performed the detail documentation necessary for legal and billing purposes.

AEW Facilitator (2019) – I helped students understand the course objectives and principles learned in Calculus II.

AEW Graduate Intern (2019-2020) – I help with the organization and execution of responsibilities that help the AEW organizers keep the program running smoothly.

Teacher's Assistant (2019-2020) – I was a TA for Bioinstrumentation and Senior Design II.

Syracuse University Women's Volleyball – Volunteer Assistant Coach (2020)

Volunteer Experience

Volunteer Escort at the VA Medical Center – 2017-Present

Student Athlete Advisory Committee – 2016-2020, President 2018-2019

Honors Program Peer Mentor - I advised and mentored incoming freshman and help them assimilate to college life.

Young Scholars – At Northside Learning Center, I tutored kids, who were Somalia refugees, ranging from Elementary school to college. I helped them with completion of applications and understanding their assignments