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Molecular Investigation of the Intestinal Barrier in Health and Disease

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Abstract

The intestinal epithelial barrier contributes to the absorption of nutrients and in maintaining homeostasis. Several intestinal disorders such as Crohn's disease and Ulcerative Colitis show common pathological features corresponding to a decreased intestinal epithelial barrier function, which makes it imperative to investigate the functional units of the intestinal barriers formed by complex protein interfaces called the tight junctions. Among the diverse set of transmembrane proteins involved in the formation of tight junctions, the mal-distribution of claudin family of proteins show direct correlation to compromised barrier functioning. Interestingly, claudin-3 expression reduces drastically in compromised barriers, while an increased expression of claudin-23 is suggested to improve the barrier functions. Experimental study of membrane proteins can be time consuming and tedious. In this work, we used a computational modeling approach to investigate the nature of interfaces formed by the combination of claudin-3 and 23 to achieve a fundamental understanding of the role of claudin interfaces in intestinal barrier functions.

Acknowledgements

I would like to thank my advisor, Shikha Nangia, PhD, for allowing me to participate in her lab and research. The willingness to break things down, so the fundamental concepts pertaining to the research were understood, which helped me to take control of my understanding and goals moving forward. I am grateful for your patience and guidance because this has 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 to Radhakrishna Sureshkumar, PhD, for agreeing to be my reader for this project.

Executive Summary

The gastrointestinal tract that comprises of the small and large intestines are vital to digestion, metabolic homeostasis, and overall health. Digestive enzymes act in the small intestine to breakdown ingested food into smaller fragments that are of suitable size for absorption. Several enzymes perform the chemical process of metabolizing the ingested food; for example, enzymes such as—lipases, proteases, and amylases—breakdown fats, proteins, and carbohydrates, respectively. The absorption of digested food takes place through the layer of epithelial cells that separate the intestinal lumen from underlying mucosal tissue. The intestinal lumen has finger-like projections called villi that increase the surface area available for absorption of digested food into the bloodstream.

Beside absorption, the intestinal epithelial layer plays a quintessential role in preventing permeation of toxins and pathogens from the intestine into the bloodstream, referred to as the—epithelial barrier. The uptake of nutrients across the epithelial barrier occurs via two mechanisms: active and passive transport. The main difference between these mechanisms is that one requires energy (active) and other the does not (passive). The passive transport mainly occurs through the paracellular space between adjoining or adjacent epithelial cells. The paracellular space, however, is guarded by physical barriers known as tight junctions formed by integral membrane proteins of the adjoining cells. The tight junctions consist of a variety of membrane proteins such as—claudins, occludins, and junction adhesion molecules among others. The size and charge selectivity of the epithelial barrier in the intestinal tight junction is attributed to claudin proteins.

Claudin proteins form tight junctions in epithelial an endothelial tissues all over the body. There are 24 known claudins in humans. Each member of the claudin family is classified as

pore-forming or barrier-forming based on their individual ability to increase or decrease permeability, or the ability to allow specific substance across the barrier. In the gastrointestinal tract, claudins are expressed throughout, and some claudins are more prevalent in certain sections than others. The claudins of interest in this study are 3 and 23, and they are both readily prevalent in the duodenum or the beginning of the small intestine. claudin-3 is classified as barrier-forming tight junction protein whereas claudin-23 is known to play a role in barrier properties of the tight junction with other claudins.

The study of tight junction architecture and the role of claudins in the intestinal epithelial barrier function is vital because recent research has linked tight junction defects to the manifestation of intestinal diseases. For instance, the overexpression of claudin-23 is linked to intestinal tumors. The change in expression of claudin-3 is linked to decreasing epithelial barrier function present in celiac disease or the abnormal mucosal immune response that occurs when gluten is ingested. Moreover, inflammatory bowel diseases like Crohn's disease and Ulcerative Colitis increase the permeability thereby decreasing the efficiency of the barrier. Although inflammatory bowel diseases are characterized by inflammation, the specific cause of these diseases remains elusive. To understand the mechanism of these maladies, it is essential to understand the interaction of claudins in healthy intestinal tight junctions.

Claudin proteins that are secreted in a cell oligomerize in a cell membrane to form a strand; these are cis interactions. When these structures interact head-on with the claudin strand of a neighboring cell (trans interactions), these form the tight junction. There are four distinct interacting patterns in tight junctions, and they go as follow: (a) same type of claudin in cis (homomeric) and trans (homotypic); (b) same type of claudin in cis (homomeric) and different in trans (heterotypic); (c) different types of claudin in cis (heteromeric) and same in trans

(homotypic); and (d) different types of claudin in cis (heteromeric) and in trans (heterotypic). Claudin-3 and Claudin-23 both are expressed in epithelial cells, and they form heteromeric and heterotypic tight junctions.

We adopted coarse grain molecular modeling approach to study claudin-3 and -23 heteromeric interactions. The recently developed, Protein Association Energy Landscape (PANEL) method was used to generate exhaustive set of pair interactions followed by equilibration. Using multiple simulations (3000) in parallel, we sampled 94% of the 360°×360° rotational space formed by angles θ and θ', where $\theta = \{x|0 \leq x \leq 360°\}$ and $\theta' = \{x|0 \leq x \leq 360°\}$. The potential energy landscape profile of heteromeric claudin-3 and -23 yielded dimer conformations B and C as the low energy, stable structures. Given that dimer C is a barrier-forming, and dimer B is a pore-forming conformation, more work needs to be performed to develop the heterotypic tight junction architecture.

The results of this study lay the foundation of the molecular-level understanding of claudin-3 and -23 in the intestinal tight junctions. No work prior has focused on the fundamental interactions of claudins in the intestine using advances in computational research. The work presented here will inspire both computational and experimental study in exploring primary causes of intestinal diseases.

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Advice to Future Honors Students

There is a lot I wish I knew at the beginning of this incredible journey, but I will try to be concise. Honors is more than a distinction at graduation or something to put on your resume, it is an opportunity. It is an opportunity to meet and interact with high functioning and down-to-earth people who like and do things different from you. It is an opportunity to grow in way you can even comprehend. It is an opportunity to challenge yourself, not only in your academics but socially and spiritually. It is an opportunity to use resources that are not available to students who are not in the Honors program. More importantly, it is an opportunity to have a built in support system, because everyone in honors wants you to achieve and succeed. Use honors to spread your wings. For instance, take classes that are completely different than your area of study or ones that you don't think you will enjoy because these are the classes that tend to surprise you. In my case, I was engineering major taking a class called History of Women's Suffrage movement and this turned out to be one of the most rewarding classes in my experience at Syracuse University. My advice is this…find out all that Honors has to offer you and USE IT!

INTRODUCTION

Gastrointestinal tract: A Macroscopic Perspective. The intestines are an integral part of digestion, metabolic homeostasis, and overall health. The purpose of digestion is to break down ingested food into substances small enough to participate in absorption. By the time food reaches the duodenum, the beginning of the small intestine, (Figure 1), it is partially digested. Intestinal enzymes, such as lipases, proteases and amylases, break down fats, proteins, and carbohydrate even further into fatty acids, amino acids, and monosaccharides with molecular sizes that are ideal for facilitating absorption. The absorption of digested food occurs through the layer of epithelial cells that separate the lumen from the underlying a mucosal tissue. The wall of the lumen is lined with finger-like projections known as villi (Figure 1) that increase the surface area available for absorption. Upon absorption the nutrients are passed to the blood stream for circulation.

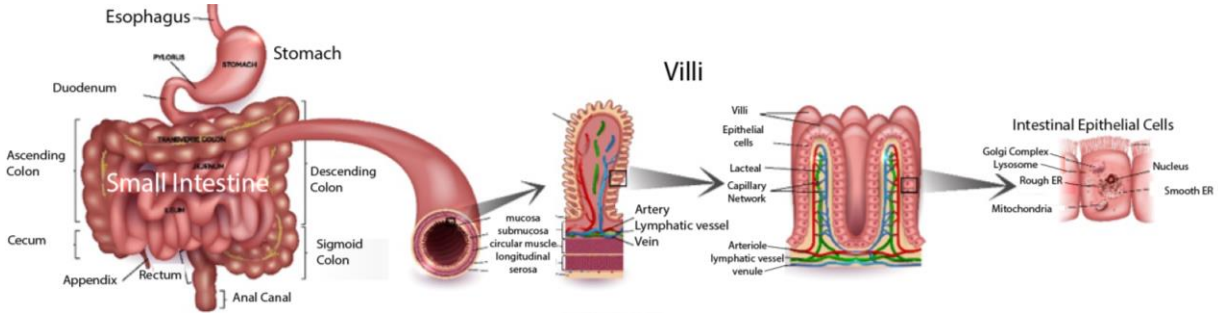


Figure 1. Gastrointestinal tract. The lumen of the small intestine shows villi and the layer of epithelial cells that form tight junctions.

Epithelial barrier. Besides absorption of nutrients, the intestinal epithelium plays a critical role in acting as a barrier for permeation of toxins and pathogen from the lumen into the mucosal tissues. Each section of the intestine absorbs digested nutrients based on the permeability of the epithelial barrier. The absorption of nutrients and water occurs via active and passive mechanism

with and without expenditure of energy, respectively (Boundless 2018). The passive transport occurs mainly through the paracellular space between adjoining epithelial cells. Lining the paracellular space, however, there are physical barriers called tight junctions constituted by a network of integral membrane proteins, such as claudins, occludins, and junction adhesion

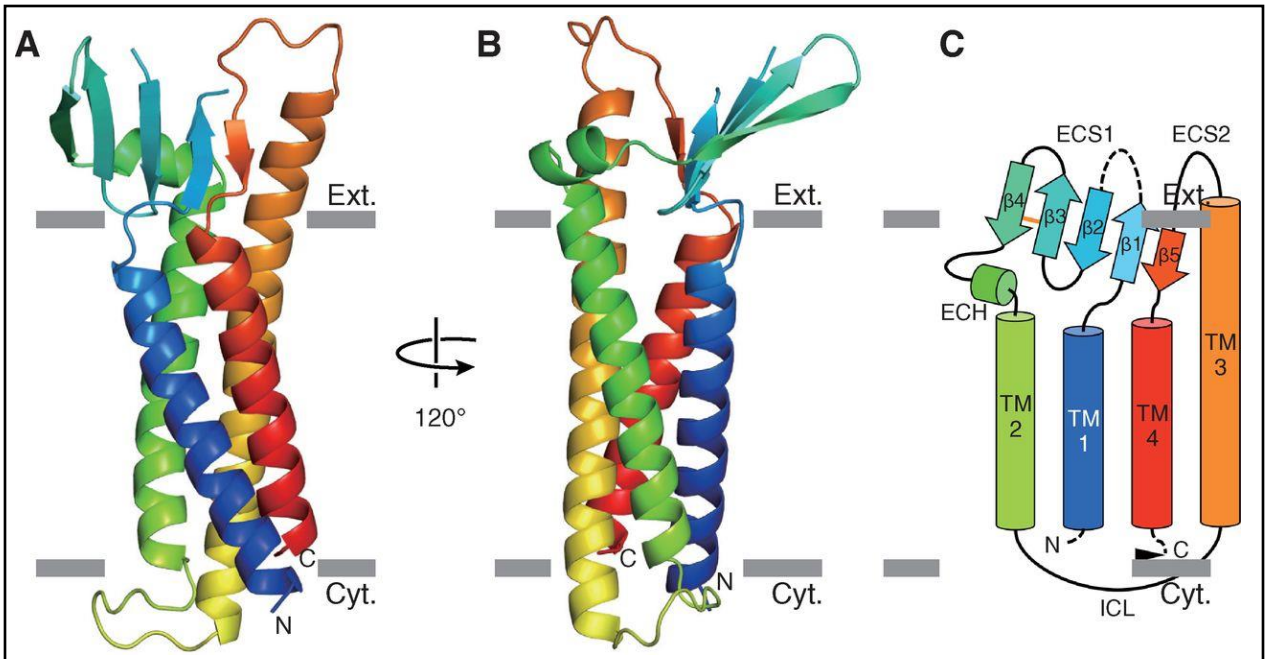


Figure 2. Claudin-5 protein structure from X-ray crystallographic data. (A) and (B) show the ribbon structure of the TM domains, ECL loops, IC loops, N-term (blue) and C-term (red) of claudin-5 embedded in the membrane (grey). Panel C is the cartoon structure showing the β-sheet folds of the ECL2 loops. (Adapted from Science. 2014 344, 304-7)

molecules. The claudins are the quintessential tight junction proteins that are responsible for the charge and size selectivity of the epithelial barrier in the intestine.

Claudin proteins. The claudin family of tight junction proteins is present in various tissues throughout the body. There are 24 known claudins in humans. Claudins are membrane proteins with four transmembrane helices (TM1−4), two extracellular loops (ECL1−2), one intracellular loop (IC) as well as N-terminal and C-terminal cytoplasmic domains (Figure 2). Each member of the claudin family is classified as pore-forming or barrier-forming based upon their individual ability to increase or decrease the permeability in a tight junction. Claudins are differentiated into

two groupsbased on their degree of sequence similarity: classic claudins (-1 to -10, -14, -15, -17, and -9) and non-classic claudins (-11 to -13, -16, -18, and -20 to -24). The expression of claudin proteins in a tissue determines the permeability or conversely—the transepithelial resistance (TER); high permeability means low transepithelial resistance, and vice-versa.

Intestinal tight junctions. Claudin family of proteins is expressed throughout the gastrointestinal tract (Table 1). Certain claudin, like claudin-12 occur throughout, while claudin-2 and -15 are predominantly expressed in the proximal parts of the gastrointestinal tract. Claudin-3, -4, -7 and -8 expression is higher in the large intestine. Claudin-3 is classified as a barrier-forming tight junction proteins.Claudin-23 plays a role in the barrier properties of the tight junction along with other claudins.

Table1. Claudin expression in intestinal segments.

Intestinal segments	Claudin expression
Duodenum	1,2,**3**,4,5,7,12,15, **23**
Ileum	2,7,8,12,15
Large intestine	1,3,4,7,8,12,15

Intestinal pathologies. Tight junction defects are linked to the manifestation of intestinal

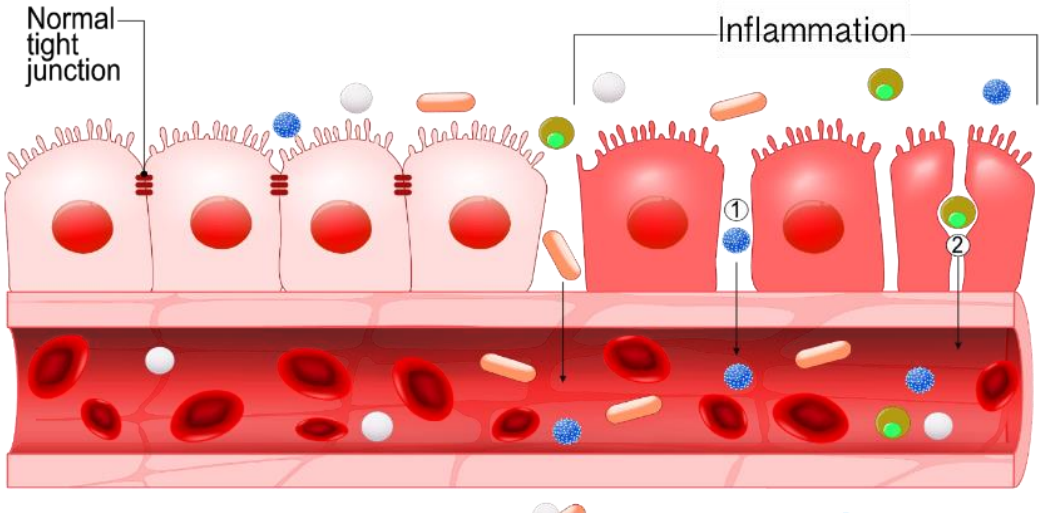


Figure 3. Normal and inflamed epithelial cell layer of the small intestine showing normal and ruptured tight junctions.

diseases, such as inflammatory bowel disease, celiac disease and more, that affect thousands of people. For instance, change in claudin-3 expression results in a decrease in the absorption efficiency in celiac disease and an abnormal mucosal immune response occurs when gluten is ingested. On the contrary, overexpression of claudin-3 increases paracellular resistance and thereby results in an increase in TER. In addition, inflammatory bowel diseases like Crohn's disease and ulcerative colitis have the ability to increase permeability and decrease in the TER. Inflammatory bowel disease presents as abdominal pain that is caused by inflammation of the intestinal walls (Figure 3). Inflammation may progress into stenosis, which may result in complete obstruction of the gut. Although both Crohn's disease and ulcerative colitis are characterized by chronic inflammation, the specific cause has remained elusive. (Vilela et al. 2012). Overexpression of claudin-23 has been tied to intestinal tumors. To understand how certain maladies manifest in the body, it is important to understand the claudin-claudin interactions in healthy intestinal tight junctions.

Tight junction architecture. The claudin proteins secreted in a cell oligomerize in a cell membrane (*cis* interactions) to form a strand, which then interacts head-on with the claudin

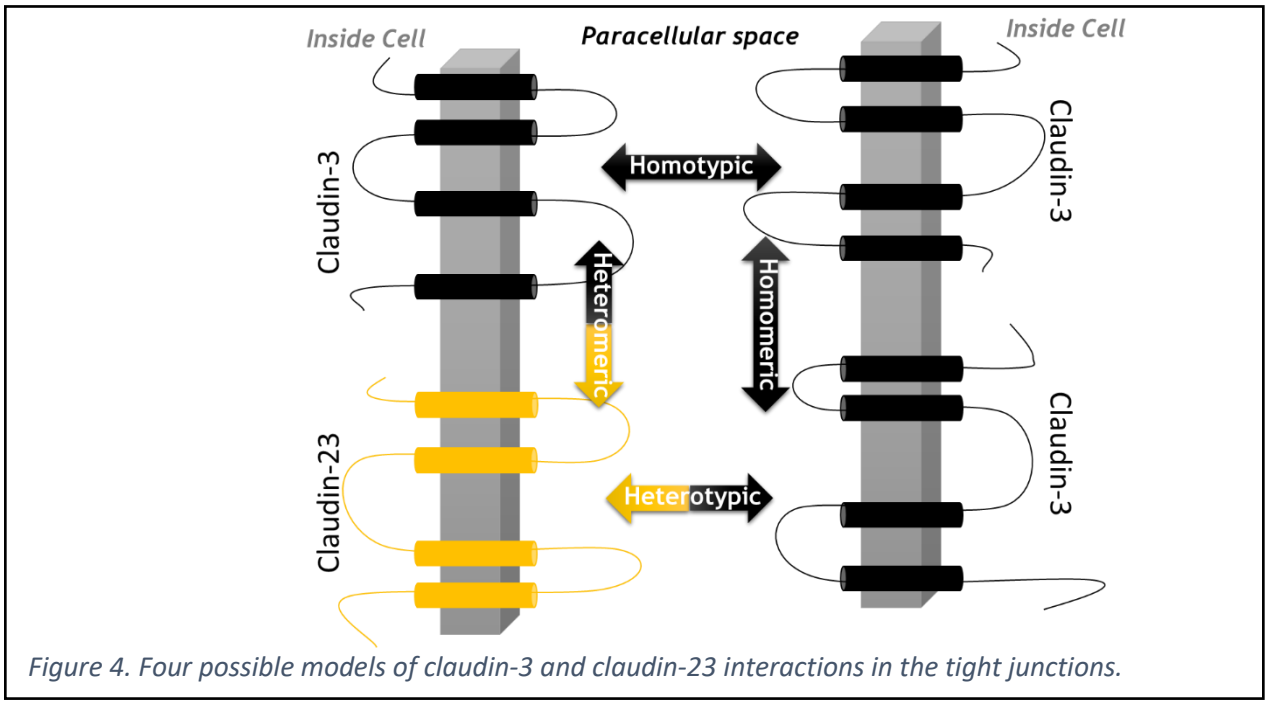


Figure 4. Four possible models of claudin-3 and claudin-23 interactions in the tight junctions.

strand of the neighboring cell (*trans*) interactions to form the tight junctions. There are often multiple claudin types expressed in the cells of a given tissue. Therefore, the tight junction architecture can have claudin that interact with each other in four distinct ways (Figure 3): (a) same type of claudin in *cis* (homomeric) and *trans* (homotypic); (b) same type of claudin in *cis* (homomeric) and different in *trans* (heterotypic); (c) different types of claudin in *cis* (heteromeric) and same in *trans* (homotypic); and (d) different types of claudin in *cis* (heteromeric) and in *trans* (heterotypic). For example in the intestine, both claudin-3 and claudin-23 are expressed in epithelial cells and they form heteromeric and heterotypic tight junctions.

The need for computational modeling. Fundamental understanding of inflammatory bowel diseases has generated avid interest among researchers; but the complexity of the tight junction strands has been a major challenge to therapeutic advances. Published experimental data on the structure of tight junctions and associated transport properties across the gut epithelial layer are sparse and inadequate for elucidating the mechanisms underlying the absorption of nutrients. As an alternative to experimental methods, we utilized advanced computational methods to provide a molecular-level description of the three-dimensional tight junction architecture and its dynamical nature as well as interfacial properties of the paracellular pathway, which collectively allow us to

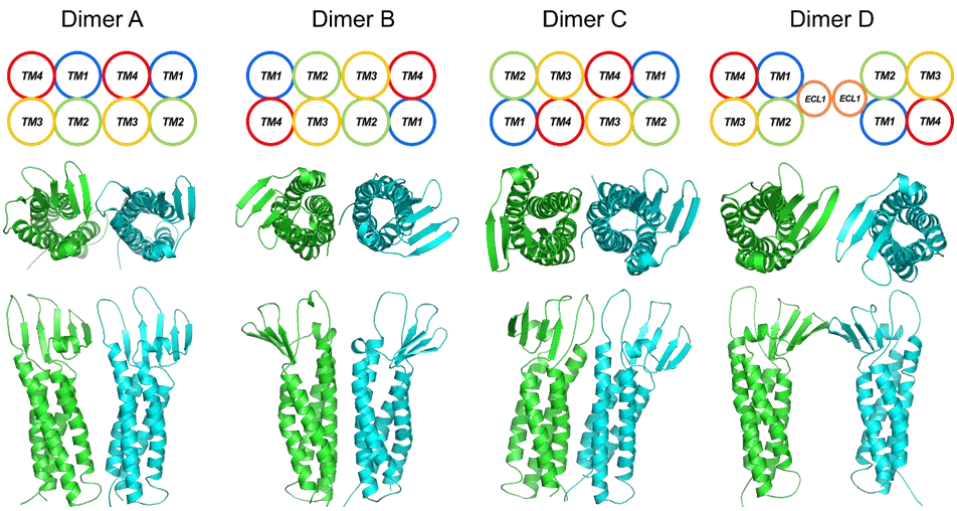


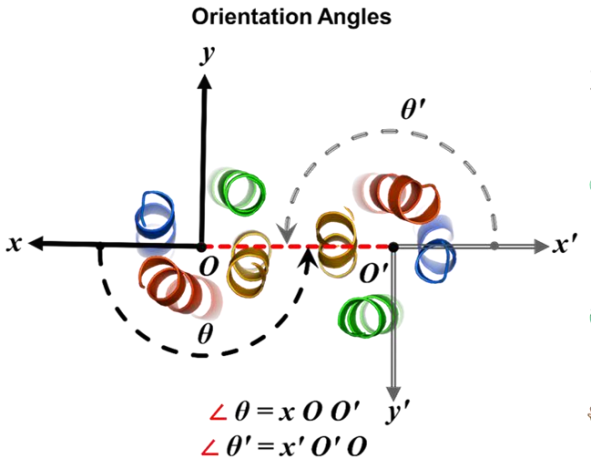
Figure 5. Orientations of A-D dimeric interfaces. (Adapted from JPCB 2018, 122, 7463–7474).

characterize transport across the gut barrier. As a first step to understanding the role of claudin-3 and claudin-23 proteins in the intestinal tight junctions, we investigated their heteromeric interactions.

BACKGROUND

The homomeric and heteromeric association of claudin family of proteins has been reported in our previous work (Rajagopal et. al 2019). In all those studies, we use standard molecular dynamics techniques to observe self-assembly of claudins. Both homomeric (1-1, 2-2, 3-3, 4-4, 5-5, and 19-19) and heteromeric (3-5) claudin interactions, revealed four ubiquitous dimeric interfaces, labeled A−D (Figure 4).

To classify the relative orientation of each monomer with respect to each other in the A−D conformation, we developed a set of dihedral like angles $\theta = \{x|0 \leq x \leq 360°\}$ and $\theta' = \{x|0 \leq x \leq 360°\}$ between dimeric claudin (Figure 6).



Dimer	θ	θ'
A	100°± 45°	275°± 45°
B	180°± 35°	180°± 35°
C	115°± 30°	115°± 30°
D	350°± 45°	350°± 45°

Figure 6. Rotational space of two claudin monomers relative to each other and the dimer A-D definitions. (Adapted from JPCB 2018, 122, 7463–7474).

METHODS

We developed coarse grain (CG) models of equilibrated claudin-3 and -23 structures using the Martini v2.2 force field. To preserve the conformations of the proteins the ElNeDyn approach was adopted. Using the *panel.py* script developed in our group, the proteins were embedded in a 10×10 nm^2 membrane patch composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) lipids. The systems were solvated with standard Martini CG water with 0.15 M NaCl salt concentration. The simulations were performed using GROMACS v2018.1 and molecular visualization was done using the VMD software. Each system was energy minimized followed by 25 ns of isothermal-isochoric (*NVT*) and 50 ns of isothermal-isobaric equilibration sets,

followed by 0.4 μs of *NPT* production MD. A temperature of 300 K was maintained using the v-rescale thermostat with a coupling constant of 1.0 ps. Pressure of 1 bar was maintained under semiisotropic pressure conditions using Parinello-Rahman barostat with a compressibility of 4.5×10^{-5} bar^{-1} and a coupling time of 12 ps. A cutoff of 1.1 nm was used of the van der Waals and electrostatic interactions.

RESULTS AND DISCUSSION

Claudin-3 and -23 interaction energy landscape. The heteromeric claudin-3 and -23 assembly was evaluated using the PANEL approach. Using 3000 starting conformations, we successfully sampled 94% of the $\theta = \{x|0 \leq x \leq 360°\}$ and $\theta' = \{x|0 \leq x \leq 360°\}$ rotational space (Figure 7). The potential energy landscape profile of the rotational space yields the relative stability of the conformations. We identified low energy conformation within in the −1100 and −1200 $kJmol^{-1}$ range. The results show that among the four established A−D dimer types, only dimer B and C are stable in claudin-3 and -23 cis assembly. Dimer conformations A and D have non-bonded potential energies in −400 and −200 $kJmol^{-1}$ range, which is much higher than the B and C

dimers.

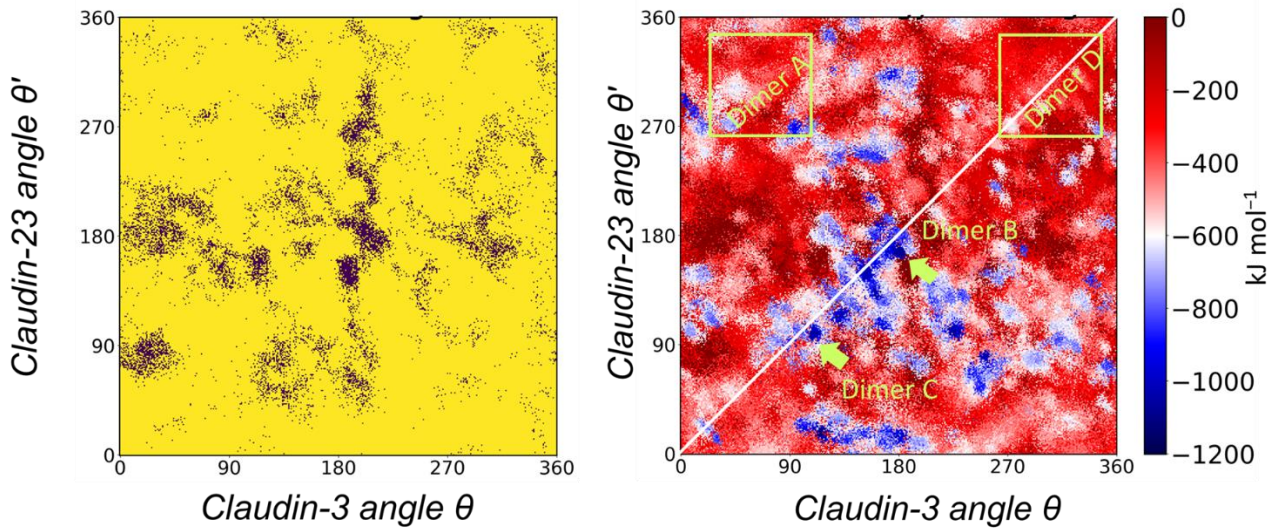


Figure 7. PANEL plots for claudin-3 and -23 homomeric interaction. (A) Grid coverage of the 360° × 360° space (yellow-sampled; brown-not sampled). (B) Contour plot of the minimum potential energy. Conformations of dimer A–D are marked.

The relative orientation of claudin-3 and -23 is also represented in the polar plots with claudin-3 in the center showing the lowest energy structure is formed via TM3/TM3 or TM3/TM4 of clauin-3/cladin-23 interactions (Figure 8).

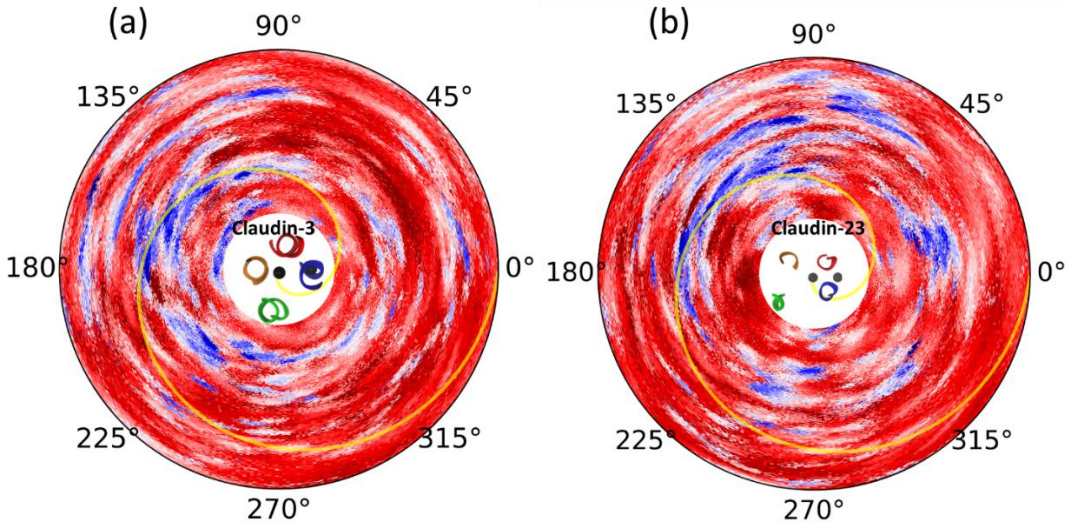


Figure 8. Radial contour plots of (a) claudin-3 (center) and claudin-23 (radially outward); (b) claudin-3 (center) and claudin-23 (radially outward). The energy scale is the same as in figure 7.

The results provide a molecular level basis for claudin-3 and claudin-23 heteromeric interactions. The lowest energy conformations of both dimers C and dimer B will be used to construct the heterotypic tight junction barrier/pore studies in the future.

FUTURE DIRECTIONS

The claudin-3 and -23 heteromeric interaction is a component of the extensive ongoing study of all intestinal claudins in Nangia's lab. Currently, analysis of homomeric claudin 2, 3, 4, 23 and the remaining heteromeric claudin pairs (2-3, 2-4, 2-23, 3-4, and 4-23) is being performed. The results of this are being incorporated into a manuscript that will be submitted for peer-review next month.

REFERENCES

Badrick, A. (2018, June 3). Absorption in the Large Intestine - Regulation. Retrieved November, 2018, from http://teachmephysiology.com/gastrointestinal-system/large intestine/absorption-large-intestine/

Barmeyer, C., Schulzke, J. D., & Fromm, M. (2015, May 19). Claudin-related intestinal diseases. Retrieved June, 2018, from https://www.sciencedirect.com/science/article/pii/S1084952115001032

“Boundless Anatomy and Physiology.” Lumen Learning, Lumen, 2018, courses.lumenlearning.com/boundless-ap/chapter/the-small-intestine/.

Groschwitz, K. R., & Hogan, S. P. (2009, July). Intestinal barrier function: Molecular regulation and disease pathogenesis. Retrieved February 20, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4266989/

Grunwald, D., Mehta, M., & Sheth, S. G. (2016, December 21). PseudomembranousCollagenous Colitis: A Case of Not-so-Microscopic Colitis. Retrieved from https://acgcasereports.gi.org/pseudomembranous-collagenous-colitis-a-case-of-not-so microscopic-colitis/

Johnson, J. (2017, July 27). Crohn's disease - A Review of Symptoms and Treatment - ppt video online download. Retrieved from https://slideplayer.com/slide/5876338/

Iravani, O., Wei-Rong, B., & Chua, P. (2013, April 1). Claudins and gastric carcinogenesis. Retrieved November, 2018, from http://journals.sagepub.com/doi/full/10.1177/1535370213477981

Nandhini Rajagopal, Flaviyan Jerome Irudayanathan, and Shikha Nangia. *Palmitoylation of Claudin-5 Proteins Influences Their Lipid Domain Affinity and Tight Junction Assembly at the Blood–Brain Barrier Interface.*The Journal of Physical Chemistry B 2019 123 (5), 983-993. DOI: 10.1021/acs.jpcb.8b09535

Shi, J., Barakat, M., Chen, D., & Chen, L. (2018, December 04). Bicellular Tight Junctions and Wound Healing. Retrieved February 25, 2019, from https://www.mdpi.com/1422 0067/19/12/3862/htm

Szklarczyk, M. D. (Ed.). (2018). Claudin 23; Plays a major role in tight junction-specific obliteration of the intercellular space, through calcium- independent cell-adhesion activity. Retrieved July, 2018, from https:// db.org/network/10090.ENSMUSP00000049725

Tieleman, D. P. (2010). Methods and Parameters for Membrane Simulations. Retrieved December, 2018, from file://hd.ad.syr.edu/03/eaa8f6/Documents/Downloads/9780854041893-c1.pdf

Tsukita, S., Furuse, M., & Itoh, M. (2001, April 01). Multifunctional strands in tight junctions. Retrieved from https://www.nature.com/articles/35067088

Uy, Karize, and A. Joseph. “What Are Epithelial Cells?” WiseGEEK, Conjecture Corporation, 7 Jan. 2019, www.wisegeek.com/what-are-epithelial-cells.htm#comments.

Van, C. M., & Anderson, J. M. (2013, July 01). Claudin interactions in and out of the tight junction. Retrieved December, 2018, from

Vilela, E. G., Torres, H. O., Martins, F. P., Ferrari, M. D., Andrade, M. M., & Cunha, A. S. (2012, March 07). Evaluation of inflammatory activity in Crohn's disease and ulcerative colitis. Retrieved February 25, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3297045/