# BIOGRAPHICAL SKETCH

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NAME: Baconguis, Isabelle

eRA COMMONS USER NAME (credential, e.g., agency login): BACONGUI

POSITION TITLE: Assistant Scientist/Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

| INSTITUTION AND LOCATION | DEGREE (if applicable) | END DATE MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of Pennsylvania, Philadelphia, PA | BA | 05/2005 | Biochemistry |
| Oregon Health and Science University, Portland, OR | PHD | 03/2013 | Neuroscience |
| Cold Spring Harbor Laboratory Course, Cold Spring Harbor, New York, Cold Spring Harbor, NY | Other training | 10/2009 | Intensive laboratory/computational course focusing on the major techniques to determine three-dimensional structures of macromolecules |

## A. Personal Statement

My research group focuses on understanding the molecular mechanisms underlying channel function using biochemical and biophysical techniques. We are particularly interested in investigating the role of epithelial sodium channels (ENaC), part of the functionally diverse ENaC/Degenerin (DEG) superfamily, in regulating salt and water balance in mammals. With over 17 years of experience studying ion channels at the atomic level, my expertise spans the expression and isolation of eukaryotic membrane proteins/complexes, membrane protein crystallography, single-particle cryo-electron microscopy, and electrophysiology. My extensive expertise, training, and dedication position me to successfully steer the direction of the proposed research and ensure its successful completion.

During my training, I successfully determined the structures of acid-sensing ion channels (ASICs), which are part of the ENaC/DEG superfamily. These accomplishments involved forming complexes with various toxins that stabilize different functional states of ASICs. Additionally, I played a crucial role in mapping ion-binding sites in ASICs and proposed a model for ion selectivity in ASICs using structural techniques. My previous work provided a solid foundation for detailed structural and functional explorations of human ASIC. Over the past year, our study of human ASIC (hASIC) has produced eleven different structures under various conditions, offering new insights into hASIC function. These findings are described in a preprint.

My earlier work with ASICs was pivotal in developing strategies for comprehensive molecular studies of ENaC. I laid the groundwork for the proposed research by identifying, optimizing, and designing technology for the expression and purification of human ENaC, which was key to revealing the first structure of heteromeric ENaC. Additionally, I generated high-affinity monoclonal antibodies that specifically target three-dimensional epitopes in human ENaC subunits. These antibodies are essential for cryo-electron microscopy studies of ENaC and serve as valuable tools for cellular biology research. Their utility extends to mouse ENaC and the human δβγ ENaC complex, demonstrating broad applicability. Our research using these monoclonal antibodies was instrumental in resolving three ENaC complexes: two trimeric and one dimeric. This work, detailed in a recently published paper in *Structure*, highlights our ability to capture and preserve multiple distinct complexes, an essential capability for the proposed experiments. With our high-affinity antibodies and expertise in isolating challenging targets such as ENaC, I am well-prepared to achieve the objectives outlined in the proposed research, which involves investigating native ENaC complexes isolated from mouse lungs, kidneys, and colon. By effectively integrating selected biochemical and biophysical techniques and leveraging heterologous expression technology, my research endeavors aim to uncover fundamental principles governing

ENaC function in different epithelial tissues. All the necessary resources, equipment, and cutting-edge core facilities required to conduct this research are readily available and actively utilized in my laboratory.

1. Cahill J, Hartfield KA, Heusser SA, Poulsen MH, Yoshioka C, Pless SA, **Baconguis I**. Conformational plasticity of human acid-sensing ion channel 1a. bioRxiv 628012 [**Preprint**]. 2024 Dec 12. Available from: doi: 10.1101/2024.12.11.628012. PubMed PMID: 39713315; PubMed Central PMCID: PMC11661276.

2. Houser A, **Baconguis I**. Structural insights into subunit-dependent functional regulation in epithelial sodium channels. Structure. 2024 Dec 5;. doi: 10.1016/j.str.2024.11.013. [Epub ahead of print] PubMed PMID: 39667931; NIHMSID:NIHMS2037740.
3. Noreng S, Posert R, Bharadwaj A, Houser A, **Baconguis I**. Molecular principles of assembly, activation, and inhibition in epithelial sodium channel. Elife. 2020 Jul 30;9 PubMed Central PMCID: PMC7413742.
4. Noreng S, Bharadwaj A, Posert R, Yoshioka C, **Baconguis I**. Structure of the human epithelial sodium channel by cryo-electron microscopy. Elife. 2018 Sep 25;7 PubMed Central PMCID: PMC6197857.

## B. Positions, Scientific Appointments and Honors

### Positions and Scientific Appointments

| | |
|---|---|
| 2016 - | Assistant Professor, OREGON HEALTH & SCIENCE UNIVERSITY |
| 2013 - 2016 | Vollum Fellow, OREGON HEALTH & SCIENCE UNIVERSITY |
| 2008 - 2008 | Teaching Assistant, Oregon Health and Science University, Neuroscience Graduate Program, Portland, OR |
| 2007 - 2013 | Graduate Student, OREGON HEALTH & SCIENCE UNIVERSITY |

### Honors

| | |
|---|---|
| 2019 - 2022 | Transformational Project Award, American Heart Association |
| 2013 - 2018 | Early Independence Award, NIH Common Fund High-Risk High-Reward |
| 2013 | Student Paper of the Year, Oregon Health and Science University |

## C. Contribution to Science

1. Resolving the first structure of epithelial sodium channel (ENaC): Understanding the molecular basis for the function of ENaC is crucial, not only for elucidating their roles in physiological and pathophysiological processes but also for providing blueprints for future therapeutic strategies. Despite the mounting evidence of its clinical significance in diseases like hypertension, the lack of ENaC structure has held the field back. This lag is due to difficulties in protein expression, protein instability, sample heterogeneity, and inherent pseudosymmetry hampering structural studies by X-ray crystallography and cryo-electron microscopy. We successfully overcame these challenges by incorporating high affinity monoclonal antibodies to form complexes with ENaC. The work in my lab describes, for the first time, the structure of the protease-sensitive heteromeric ENaC, the founding member of the large ENaC/DEG superfamily. First, the structure reveals the functional trimeric subunit stoichiometry, an intensely debated topic of numerous studies since the cloning of the subunits in 1993 and 1994. Second, the structure demonstrates how the three homologous subunits, α, β, and γ arrange in a counterclockwise direction ultimately settling a long-standing controversy. Third, the structure reveals the architecture of key gating components that defines the unique functional property of ENaC, an ion channel that converts from closed to open by proteolysis. Importantly, the structure provides the map of the positions of inhibitory domains in α and γ subunits, providing the first blueprint for critical sites of inhibition of ENaC activity.

    a. Noreng S, Bharadwaj A, Posert R, Yoshioka C, **Baconguis I**. Structure of the human epithelial sodium channel by cryo-electron microscopy. Elife. 2018 Sep 25;7 PubMed Central PMCID: PMC6197857.

2. Understanding the molecular mechanisms of $Na^+$-mediated and protease-dependent regulation of ENaC function: ENaC activity is regulated by several factors. One such factor is extracellular $Na^+$, which inhibits

ENaC activity through a process known as $Na^+$ self-inhibition. Another factor is proteases, which can increase ENaC activity by removing inhibitory domains in the extracellular region. Interestingly, removal of the inhibitor domains eliminates $Na^+$ self-inhibition. We employed single-particle cryo-electron microscopy to reveal the structural relationship between $Na^+$ self-inhibition and the cleavage of inhibitory domains. We identified the $Na^+$ binding site and captured the different cleavage states of the α subunit of ENaC. This work also provided a model showing how the removal of inhibitory sites can disrupt the $Na^+$ binding site, thereby removing the inhibitory effects of $Na^+$.

a. Noreng S, Posert R, Bharadwaj A, Houser A, **Baconguis I**. Molecular principles of assembly, activation, and inhibition in epithelial sodium channel. Elife. 2020 Jul 30;9 PubMed Central PMCID: PMC7413742.

3. Understanding the molecular diversity of ENaC assemblies: Four ENaC subunits have been identified--α, β, γ, and δ. Previous studies have shown that different tissues express a variety of ENaC complexes with different subunit compositions, resulting in distinct channel properties. In vitro electrophysiology experiments measuring channel activity have demonstrated that the αβγ and δβγ combinations produce strong $Na^+$ currents, which can be blocked by amiloride. Channels formed by expressing only two ENaC subunits exhibit smaller $Na^+$ currents. To understand the arrangement of ENaC subunits, we used cryo-electron microscopy. Our results show that the α and δ subunits occupy the same positions in the αβγ and δβγ trimers, respectively. Additionally, we observed a ββγ trimer, providing evidence of how β and γ subunits can assemble into a trimer. We also identified a βγ dimer, likely representing an intermediate assembly state, offering insights into the assembly process of ENaC channels.

a. Houser A, **Baconguis I**. Structural insights into subunit-dependent functional regulation in epithelial sodium channels. Structure. 2024 Dec 5;. doi: 10.1016/j.str.2024.11.013. [Epub ahead of print] PubMed PMID: 39667931; NIHMSID:NIHMS2037740.

b. Noreng S, Posert R, Bharadwaj A, Houser A, **Baconguis I**. Molecular principles of assembly, activation, and inhibition in epithelial sodium channel. Elife. 2020 Jul 30;9 PubMed Central PMCID: PMC7413742.

4. Identification of key domains crucial for channel gating in ASICs: Previously, studies of ion channel function using X-ray crystallography to understand channel gating involved determination of channel structures using different orthologs. As a graduate student, I resolved structures of two different open conformations of ASIC using the chicken ASIC1a, the same target utilized to solve the first crystal structure of ASIC trapped in the ligand-bound non-conducting state and exploiting the potent modulatory effects of the spider toxin, PcTx1. The work illustrated key domains involved in toxin binding. More importantly, it provided the foundation for understanding channel function by demonstrating how ASIC opens and closes. Furthermore, the work showed how the same ion channel can adopt two different open conformations, a physiological event that is observed across different families of ion channels.

a. **Baconguis I**, Gouaux E. Structural plasticity and dynamic selectivity of acid-sensing ion channel-spider toxin complexes. Nature. 2012 Sep 20;489(7416):400-5. PubMed Central PMCID: PMC3725952.

b. Cahill J, Hartfield KA, Heusser SA, Poulsen MH, Yoshioka C, Pless SA, **Baconguis I**. Conformational plasticity of human acid-sensing ion channel 1a. bioRxiv 628012 [**Preprint**]. 2024 Dec 12. Available from: doi: 10.1101/2024.12.11.628012. PubMed PMID: 39713315; PubMed Central PMCID: PMC11661276.

5. Elucidation of the mechanism of ion selectivity in ASICs: Numerous studies of members of the ENaC/DEG have indirectly shown key residues, which are involved in ion selectivity, called the ‘GAS’ selectivity filter. The lack of high-resolution structures of any member precluded understanding of how these residues serve as barriers only allowing sodium ions over potassium ions to move across the bilayer. Utilizing the potent effects of the snake toxin, MitTx, to trap ASIC in the sodium-selective open state and obtain high-resolution crystal structures of the channel, my work, for the first time, showed how the ‘GAS’ selectivity filter adopts an unforeseen arrangement in the middle of the bilayer explaining the mechanism of sodium-selectivity in ASICs.

a. **Baconguis I**, Bohlen CJ, Goehring A, Julius D, Gouaux E. X-ray structure of acid-sensing ion channel 1-snake toxin complex reveals open state of a $Na^+$-selective channel. Cell. 2014 Feb 13;156(4):717-29. PubMed Central PMCID: PMC4190031.

b. Cahill J, Hartfield KA, Heusser SA, Poulsen MH, Yoshioka C, Pless SA, **Baconguis I**. Conformational plasticity of human acid-sensing ion channel 1a. bioRxiv 628012 [**Preprint**]. 2024 Dec 12. Available from: doi: 10.1101/2024.12.11.628012. PubMed PMID: 39713315; PubMed Central PMCID: PMC11661276.

**Published Works in MyBibliography:**

https://www.ncbi.nlm.nih.gov/myncbi/1rcOHoaUYwsQl/bibliography/public/