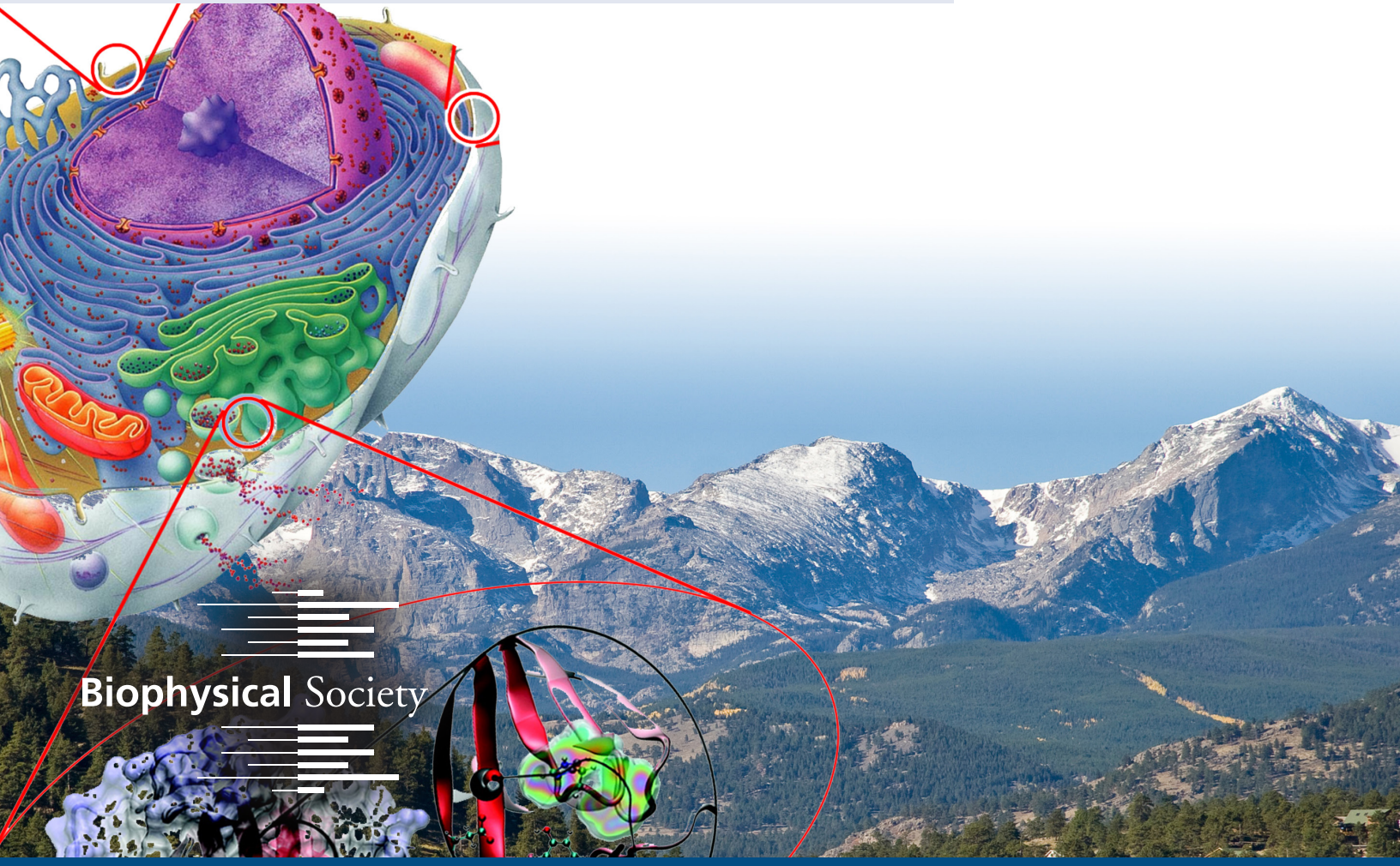
Biophysical Society Conferences

Proton Reactions: From Basic Science to Biomedical Applications

Estes Park, Colorado | August 3-8, 2025



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Organizing Committee

Elena Pohl, University of Veterinary Medicine Vienna, Austria
Gregory Voth, University of Chicago, USA

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August 2025

Dear Colleagues, Friends, and Students,

It is our great pleasure to welcome you to the Biophysical Society Conference on *"Proton Reactions: From Basic Science to Biomedical Applications,"* held at the Holiday Inn in beautiful Estes Park, Colorado.

Following the resounding success of the inaugural meeting at Granlibakken, Lake Tahoe, in the summer of 2023—expertly organized by Ana-Nicoleta Bondar and Thomas DeCoursey—we are excited to continue this growing tradition. Proton transport mechanisms remain a central topic in biophysical research, with an ever-expanding diversity of methods, models, and approaches. This year's program brings together over 23 internationally renowned invited speakers, each contributing unique insights into the science of protons. In addition, more than 16 emerging researchers will present their work in short talks selected from submitted abstracts. We have made a deliberate effort to build in ample time for open, in-depth discussions—both during sessions and in the fresh Colorado mountain air—to encourage deeper understanding and foster meaningful collaborations.

Estes Park, nestled in the Rocky Mountains of northern Colorado, is the perfect setting for our meeting. Known as the gateway to Rocky Mountain National Park, it offers stunning vistas, abundant wildlife, scenic drives, hiking trails, and a charming downtown filled with unique shops and attractions.

We extend our heartfelt thanks to all plenary and invited speakers who have made the journey to join us, despite ongoing global challenges. Your talks—and your contributions to the discussion of short talks and posters—are the backbone of this important conference. We also gratefully acknowledge the tireless efforts of our professional organizers from the Biophysical Society, Umi Zhou and Dorothy Chaconas, as well as the generous support from the Chicago Center for Theoretical Chemistry, IUPAB and SUTTER Instruments.

We look forward to a week of vibrant science, lively discussion, and new connections in one of the most inspiring locations in the country.

With warm regards,
Elena E. Pohl and Greg Voth
The Organizing Committee

Biophysical Society Code of Conduct, Anti-Harassment Policy

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all, regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit.

All BPS meetings and BPS-sponsored activities promote an environment that is free of inappropriate behavior and harassment by or toward all attendees and participants of Society events, including speakers, organizers, students, guests, media, exhibitors, staff, vendors, and other suppliers. BPS expects anyone associated with an official BPS-sponsored event to respect the rules and policies of the Society, the venue, the hotels, and the city.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Attendees or participants who are asked to stop engaging in harassing behavior are expected to comply immediately. Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed is NOT REQUIRED to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if the complainant does not feel comfortable with such an approach, they can report the behavior as detailed below.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Reporting a Violation
Violations of this Conduct Policy should be reported immediately. If you feel physically unsafe or believe a crime has been committed, you should report it to the police immediately.

To report a violation to BPS:

- You may do so in person at the Annual Meeting at the BPS Business Office in the convention center.

- You may do so in person to BPS senior staff at Thematic Meetings, BPS Conferences, or other BPS events.

- At any time (during or after an event), you can make a report through http://biophysics.ethicspoint.com or via a dedicated hotline (phone numbers listed on the website) which will collect and relay information in a secure and sensitive manner.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated per the procedure detailed below. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Investigative Procedure

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation. Please note, if a complaint is filed anonymously, BPS may be severely limited in our ability to follow-up on the allegation.

- An impartial investigative committee, consisting of the current President, President-Elect, and Executive Officer will be established. If any of these individuals were to be named in an allegation, they would be excluded from the committee.

- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.

- The committee will speak to the alleged offender and present the complaint.

- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.

- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.

- The investigative committee may seek BPS Counsel's advice.

- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

- If the severity of the allegation is high, is a possible repeat offense, or is determined to be beyond BPS's capacity to assess claims and views on either side, BPS may refer the case to the alleged offender's home institution (Office of Research Integrity of similar), employer, licensing board, or law enforcement for their investigation and decision.

Disciplinary Actions

Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a written warning to ejection from the meeting or activity in question without refund of registration fees, being banned from participating in future Society meetings or Society-sponsored activities, being expelled from membership in the Society, and reporting the behavior to their employer or calling the authorities. In the event that the individual is dissatisfied with the results of the investigation, they may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

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GENERAL INFORMATION

Registration/Information Location and Hours

Venue check-in to obtain your room key will be located at the Front Desk at Holiday Inn Estes Park, 101 South Saint Vrain Avenue, Estes Park, CO 80517.

The BPS Registration Desk, to pick up your badge and meeting materials, will be located in the Ballroom Front Foyer during the following times:

Sunday, August 3	3:00 PM – 6:00 PM
Monday, August 4	8:00 AM – 12:00 PM
Tuesday, August 5	8:00 AM – 12:30 PM
Wednesday, August 6	8:00 AM – 12:30 PM
Thursday, August 7	8:00 AM – 12:30 PM

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Salon BC. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) The poster session will be held in Salon E.

2) A display board measuring 48’’(121.92cm) in width and 36’’ (91.44cm) in height will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the E-book.

3) There will be formal poster presentations on Tuesday. Please refer to the daily schedule for your formal presentation information. 120 minutes have been allotted for poster presentations on Tuesday. Presenting authors with odd-numbered poster boards should present during the first 60 minutes, and those with even-numbered poster boards should present during the last 60 minutes.

4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be discarded.

Note Pads/Pens

Society pens will be provided, however please bring your own note pad.

Meals, Coffee Breaks, and Socials

Breakfasts and luncheons will be served in Salon A. Tuesday evening's dinner banquet will also be held in Salon A. Coffee Breaks will be held in the Ballroom Front Foyer. The evening socials, scheduled for Sunday, Monday, Wednesday, Thursday, will be held in the Salon F. Poster session on Tuesday will be held in Salon E.

Smoking

Please be advised that the Holiday Inn Estes Park is a non-smoking facility.

Name Badges

Name badges will be given to you when you check-in at the Registration Desk in the Ballroom Front Foyer. Badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

Internet

Wi-Fi will be provided at the venue.

On-Site Contact Information

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from August 3-7 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas
Phone : 301-785-0802
Email: dchaconas@biophysics.org

Proton Reactions: From Basic Science to Biomedical Applications

Estes Park, Colorado

August 3-7, 2025

All scientific sessions will be held in the Salon BC unless otherwise noted.

PROGRAM

Sunday, August 3, 2025

3:00 PM – 6:00 PM	**Registration/Information**	**Ballroom Front Foyer**
4:50 PM – 5:00 PM	**Welcome and Opening Remarks** Elena E. Pohl, University of Veterinary Medicine Vienna, Austria Gregory Voth, University of Chicago, USA	
5:00 PM – 6 :00 PM	**Plenary Talk** Chair: Gregory Voth, University of Chicago, USA Robert Stroud, University of California, San Francisco, USA ***The Conundrum of How and When Proton(s) Drive Transport?***	
6:00 PM – 7:30 PM	**Dinner on own**	
Session I	**Multiscale Aspects of Proton Transport** Chair: Emily Liman, University of Southern California, USA	
7:30 PM – 8:00 PM	Jessica Swanson, University of Utah, USA ***Bridging Simulations and Theory to Unravel Electrochemically Driven Transport***	
8:00 PM – 8:30 PM	Alexey Amunts, The Max Planck Institute of Molecular Physiology, Germany ***Structure of the II2-III2-IV2 Mitochondrial Supercomplex from A Parasite Reveals A Protein Inhibitor of Complex III***	
8:30 PM – 10:00 PM	**Get Together (Wine & Cheese)**	**Salon F**

Monday, August 4, 2025

7:00 AM – 8:30 AM	**Breakfast**	**Salon A**
8:00 AM – 12:30 PM	**Registration/Information**	**Ballroom Front Foyer**
Session II	**Proton Transport in Mitochondria** Chair: Vivek Sharma, University of Helsinki, Helsinki, Finland	
9:00 AM – 9:30 AM	Mario Vazdar, University of Chemistry and Technology in Prague, Czech Republic ***Molecular Dynamics Simulations of Proton Transfer in Mitochondrial Uncoupling Proteins***	
9:30 AM – 10:00 AM	Elena E. Pohl, University of Veterinary Medicine Vienna, Austria	

	Understanding the Molecular Mechanism of Mitochondrial Uncoupling induced by 2,4-Dinitrophenol	
10:00 AM – 10:30 AM	Vivek Sharma, University of Helsinki, Helsinki, Finland * ***Thermodynamics and Kinetics of Proton Transfer Reactions Catalyzed by Respiratory Complex I***	
10:30 AM – 10:45 AM	Md. Raihan Uddin, Department of Biochemistry, NY, USA * ***Proton Pumping Mechanism of Photosynthetic NDH-1 and Complex I***	
10:45 AM – 11:15 AM	**Coffee Break**	**Ballroom Front Foyer**
Session III	**Session: Transporters** Chair: Peter Pohl, Johannes Kepler University Linz - JKU, Austria	
11:15 AM – 11:45 AM	Simon Newstead, University of Oxford, United Kingdom ***Understanding the Role of Protons in Drug Transport Via SLC15A2***	
11:45 AM – 12:00 PM	Zhi Yue, University of Chicago, USA* ***Protons Enable Calcium Passage through the Hydrophobic Channel in the SARS-CoV-2 Envelope Protein***	
12:00 PM – 12:15 PM	Jonathan D Colburn, University of Oxford, United Kingdom * ***From Sequence to Mechanism: Insights into Proton Coupling in Cystinosin from Multiscale Molecular Simulations***	
12:15 PM – 1:30 PM	**Lunch**	**Salon A**
1:30 PM – 5:30 PM	**Hiking/Interactions**	
5:30 PM – 6:30 PM	**Dinner on own**	
Session IV	**Light Driven Proton Transport** Chair: Satoshi Tsunoda, Nagoya Institute of Technology, Japan	
6:30 PM – 7:00 PM	Peter Pohl, Johannes Kepler University Linz, Austria ***Phototriggered Proton-Selective Carrier Currents***	
7:00 PM – 7:30 PM	Noam Agmon, The Hebrew University of Jerusalem, Israel * ***Multi-Proton Dynamics Near Membrane-Water Interface***	
7:30 PM – 8:00 PM	Yoshitaka Kato, University of Tokyo, Japan * ***Proton Transport Mechanism of Newly Discovered Light-Driven Inward Proton Pump, ELTxer***	
8:00 PM – 10:00 PM	**Get Together (Wine & Cheese)**	**Salon F**

Tuesday, August 5, 2025

7:00 AM – 8:30 AM	**Breakfast**	**Salon A**
8:00 AM – 12:30 PM	**Registration/Information**	**Ballroom Front Foyer**
Session V	**Voltage-gated Proton Channels**	

	Chair: Jessica Swanson, University of Utah, USA	
9:00 AM – 9:30 AM	Thomas DeCoursey, Rush University Medical Center, USA ***Dissecting the Signature Sequence of the Voltage-gated Proton Channel, Hv***	
9:30 AM – 10:00 AM	Boris Musset, Paracelsus Medical University, Austria ***Somatic Cancer Mutations in the S4 Transmembrane Segment of the Human Voltage-Gated Proton Channel HV1***	
10:00 AM – 10:30 AM	Akira Kawanabe, Hiroshima University, Japan ***The Functional Regulation of the Voltage-Gated Proton Channel by ATP***	
10:30 AM – 10:45 AM	Gustavo Chaves, Paracelsus Medical University, Nuremberg, Germany * ***Exploring Biophysical Divergences within the Voltage-Gated Proton Channel (Hv) Family***	
10:45 AM – 11:15 AM	**Coffee Break**	**Ballroom Front Foyer**
Session VI	**Proton Pump Rhodopsins** Chair: Rubin Liang, Texas Tech University, USA	
11:15 AM – 11:45 AM	Satoshi Tsunoda, Nagoya Institute of Technology, Japan ***Driving Force of Various Proton Pump Rhodopsins***	
11:45 AM – 12:15 PM	Keiichi Inoue, University of Tokyo, Japan ***Proton Transfer in the Photocyclic Reaction of Ion-Transporting Microbial Rhodopsins***	
12:15 PM – 12:30 PM	Masaki Tsujimura, University of Tokyo, Japan and RIKEN Pioneering Research Institute, Wako, Japan * ***Converting a Chloride-Pumping Rhodopsin into a Proton Pump***	
12:30 PM – 1:30 PM	**Lunch**	**Salon A**
1:30 PM – 5:00 PM	**Hiking/Interactions**	
5:00 PM – 6:30 PM	**Dinner Banquet**	**Salon A**
Session VII	**Proton Transport: Physiology and Disease** Chair: Simon Newstead, University of Oxford, United Kingdom	
6:30 PM – 7:00 PM	Joseph Mindell, NINDS, NIH, USA ***Mechanisms of Novel Diseases Disrupting Lysosomal Via ClC-7 Chloride Transporter Mutations***	
7:00 PM – 7:30 PM	Emily Liman, University of Southern California, USA ***Targeting OTOP Proton Channels: Insights from Chemical Modulators***	
7:30 PM – 7:45 PM	Daniel R. Sauter, Sophion Bioscience Inc., San Diego, USA * ***Automated Patch Clamp Assay for Proton-Activated HOTOP1 Channel***	
7:45 PM – 9:45 PM	**Poster Session (Wine & Cheese)**	**Salon EF**

Wednesday, August 6, 2025

7:00 AM – 8:30 AM	**Breakfast**	**Salon A**
8:00 AM – 12:30 PM	**Registration/Information**	**Ballroom Front Foyer**
Session VIII	**Origins and Effects of Proton Transport in Proteins** Chair: Thomas DeCoursey, Rush University Medical Center, USA	
9:15 AM – 9:30 AM	Jiahua Deng, Princeton University, USA * ***Role of A Tryptophan in Proton-Coupled Electron Transport in Ribonucleotide Reductase***	
9:30 AM – 10:00 AM	Ana-Nicoleta Bondar, University of Bucharest, Romania ***Protonation-Dependent Stable Pore Formation by the PHD Peptides Requires A Water- And Lipid-Mediated Hydrogen-Bond Network***	
10:00 AM – 10:30 AM	Huong Kratochvil, University of North Carolina at Chapel Hill, USA ***Designed Water and Sidechain Dynamics in De Novo Proton Channels***	
10:30 AM – 10:45 AM	Merissa Brousseau, University of Wisconsin-Madison, USA * ***Beyond Peak Currents: Solid-Supported Membrane Electrophysiology as a Means of Investigating Universal Transport Models***	
10:45 AM – 11:15 AM	**Coffee Break**	**Ballroom Front Foyer**
Session IX	**Effect of Lipid Membranes on Proton Transport** Chair: Noam Agmon, Hebrew University of Jerusalem, Israel	
11:15 AM – 11:45 AM	Jochen Hub, Saarland University, Germany ***How Complex Lipid Compositions Control Mechanical Stability of Biomembranes***	
11:45 AM – 12:00 PM	Ambili Ramanthrikkovil Variyam, Technion - Israel Institute of Technology, Israel * ***Proton Diffusion on the Surface of Mixed Lipid Membranes Highlights the Role of Membrane Composition***	
12:00 PM – 12:15 PM	Shizhen Wang, University of Missouri-Kansas City, Kansas City, USA * ***Regulation of Proton Transport by Membrane Sterols***	
12:15 PM – 1:30 PM	**Lunch**	**Salon A**
1:30 PM – 5:30 PM	**Hiking/Interactions**	
5:30 PM – 7:00 PM	**Dinner on own**	
Session X	**Proton Transport Mechanisms** Chair: Gregory Voth, University of Chicago, USA	
7:00 PM – 7:30 PM	Katharine White, University of Notre Dame, USA ***Protons as A Second Messenger: Ph-Dependent Allostery in Signaling Proteins***	

7:30 PM – 8:00 PM	Rubin Liang, Texas Tech University, USA ***Decrypting the Non-Adiabatic Photoinduced Electron Transfer Mechanism in Light-Sensing Cryptochrome***	
8:00 PM – 10:00 PM	**Get Together (Wine & Cheese)**	**Salon F**

Thursday, August 7, 2025

7:00 AM – 8:30 AM	**Breakfast**	**Salon A**
8:00 AM – 12:30 PM	**Registration/Information**	**Ballroom Front Foyer**
Session XI	**Proton Transport Tied to Structure** Chair: Ana-Nicoleta Bondar, University of Bucharest, Romania	
9:00 AM – 9:30 AM	Marylin Gunner, City College of New York, USA ***Coupled Protonation States and Hydrogen Bond Networks in Proton Pumps***	
9:30 AM – 10:00 AM	Kota Katayama, Nagoya Institute of Technology, Japan ***Structural Insights into Spectral Tuning from the Three-Dimensional Structures of Red and Green Cone Pigments***	
10:00 AM – 10:15 AM	Ruchika Bajaj, University of California, San Francisco * ***Understanding ABC Transporters to Navigate Human Diseases***	
10:15 AM – 11:15 AM	**Coffee Break**	**Ballroom Front Foyer**
Session XII	**Modelling of Charge Transport** Chair: Mario Vazdar, University of Chemistry and Technology in Prague, Czech Republic	
11:15 AM – 11:45 AM	Gregory Voth, University of Chicago, USA ***Advances in Modeling Transmembrane Proton Transport in Biomolecular Systems***	
11:45 AM – 12:00 PM	Jong Ho Choi, University of Chicago, USA * ***Water Connectivity and its Cooperative Coupling to Proton Transport in A Key Region of Complex I***	
12:00 PM – 12:15 PM	Chang Yun Son, Seoul National University, South Korea * ***Modeling Classical and Quantum Charge Transport in Large Scale with Stochastic Λ-Dynamics***	
12:15 PM – 1:30 PM	**Lunch**	**Salon A**
1:30 PM – 6:00 PM	**Hiking/Interactions**	
6:00 PM – 7:00 PM	**Dinner on own**	
7:00 PM – 8:00 PM	**Plenary Talk** Chair: Elena E. Pohl, University of Veterinary Medicine Vienna, Austria Leonid Sazanov, Institute of Science and Technology, Austria ***The Mechanism of Proton Translocation by Respiratory Complex I***	

8:00 PM – 8:15 PM	**Closing Remarks** Elena E. Pohl, University of Veterinary Medicine Vienna, Austria Gregory Voth, University of Chicago, USA	
8:15 PM – 10:00 PM	**Open Discussion to all Topics & Executive Meeting (Wine & Cheese)**	**Salon F**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

THE CONUNDRUM OF HOW AND WHEN PROTON(S) DRIVE TRANSPORT?

Robert Stroud
University of California, San Francisco, USA

No Abstract

BRIDGING SIMULATIONS AND THEORY TO UNRAVEL ELECTROCHEMICALLY DRIVEN TRANSPORT

Jessica MJ Swanson;
[1]University of Utah, Chemistry, Salt Lake City, UT, USA

Unraveling the mechanisms by which channels, transporters, and enzymes respond to electrochemical gradients, such as the proton motive force, remains an outstanding challenge in biophysics. This work explores the use of Multiscale Responsive Kinetic Modeling (MsRKM) to quantitatively bridge data from simulations and experiments to unravel channel and transporter mechanisms. We demonstrate differences between electrically- and chemically-driven flux, resulting in different transport mechanisms for systems like the Cl^-/H^+ antiporter ClC-ec1, and different off-pathway flux for systems with a single-pathway dominant mechanism like the Shaker K^+ channel. For all systems, we find that a full network description and the inclusion of off-pathway flux are essential to capture current across a range of electrochemical conditions. Conversely, capturing both responses powerfully reduces the kinetic solution space, revealing inconsistencies between simulations and experiment.

STRUCTURE OF THE II2-III2-IV2 MITOCHONDRIAL SUPERCOMPLEX FROM A PARASITE REVEALS A PROTEIN INHIBITOR OF COMPLEX III

Alexey Amunts[1,2];
[1]Munster Univeristy, Munster, Germany
[2]Max Planck Institute for Molecular Physiology, Dortmund, Germany

Wwe report a new type of a mitochondrial supercomplex in Apicomplexa-related parasites. The 1.8 megadalton supercomplex comprises 104 proteins and 114 lipids. The 2.1-Å resolution structure represents the first observation of CII in this type of supercomplex, and activity assays confirmed the presence of a complete electron transfer from succinate to molecular oxygen utilising CII. The distinctive feature is achieved via an apicomplexan subunit, which bridges two copies of CII with CIII dimer and CIV. The second finding is the identification of a negative regulator at the conserved site on CIII. We identified a heterodimer that is a protein binder found only in the parasite locking the Rieske iron-sulfur rotein head, inactivating electron transfer. From an evolutionary perspective, the structure reveals a programmed +2 frameshifts, which illuminate evolutionary adaptations at the gene expression level. Together, the data offers deeper insights into mitochondrial nanoarchitecture and its evolutionary and functional complexity.

MOLECULAR DYNAMICS SIMULATIONS OF PROTON TRANSFER IN MITOCHONDRIAL UNCOUPLING PROTEINS

Mario Vazdar[1]; Elena E Pohl[2]; Jürgen Kreiter[2]; Sanja Vojvodic[2]; Giorgia Roticiani[2];
[1]University of Chemistry and Technology, Prague, Czech Republic
[2]University of Veterinary Medicine, Vienna, Austria

Inner mitochondrial membrane proteins of the SLC25 family, including ADP/ATP carriers and uncoupling proteins, are essential for regulating the mitochondrial proton gradient. An open question is how these proteins mediate proton transfer during uncoupling—a process that requires the presence of long-chain fatty acids (FAs). Although multiple models have been proposed, the exact mechanism by which FAs contribute to proton translocation remains unresolved. Here, we propose a mechanism based on the fatty acid (FA) cycling hypothesis and expand it with new details. In this mechanism, the neutral FA shuttles the proton across the membrane. At the same time, the protein facilitates the translocation of the deprotonated FA anion back to the membrane/water interface. Using all-atom unbiased molecular dynamics (MD) simulations, we propose a multistep process in which the FA anion first binds to the matrix side of the inner mitochondrial membrane and then slides along the protein-lipid interface in the lipid environment, similar to the "credit-card" model found in scramblases. The sliding finishes in binding of the FA anion to arginine residues in the membrane interior. In the final step, the FA anion is protonated by water in the protein cavity, which is catalyzed by negatively charged amino acids in the vicinity of the FA anion binding site. The neutral FA molecule exits the protein and spontaneously diffuses outside the membrane, completing the proton transport cycle. The experimental data, including site-directed mutagenesis and membrane conductance assays, support this mechanism: disrupting these central residues impairs FA-mediated conductance, which is in line with our predictions. These results provide a mechanistic framework for how inner mitochondrial proteins may enable FA-assisted proton transport. The involvement of conserved structural features across the SLC25 family suggests this could be a widespread mechanism for controlled proton leak, with broader implications for mitochondrial bioenergetics and thermogenesis.

UNDERSTANDING THE MOLECULAR MECHANISM OF MITOCHONDRIAL UNCOUPLING INDUCED BY 2,4-DINITROPHENOL

Elena E. Pohl
Physiology and Biophysics, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine, Vienna, Austria

Obesity is a growing global health concern affecting approximately 20% of the population and contributing to various health complications. Although a breakthrough therapy remains elusive, one promising approach for addressing obesity involves uncoupling oxidative phosphorylation in mitochondria.
2,4-dinitrophenol (DNP), an artificial mitochondrial uncoupler, was used as an anti-obesity treatment in the 1930s, but it was withdrawn due to its toxicity and lack of an antidote. Despite these concerns, DNP's efficacy surpasses that of many contemporary anti-obesity drugs, sparking renewed interest in its potential use under controlled conditions. However, a critical gap in our understanding of the precise molecular mechanisms by which DNP and similar uncouplers operate within mitochondria has impeded the development of antidotes for their toxicity and advancement as therapeutic agents. In this talk, I provide an overview of DNP's history, explore various molecular mechanisms proposed for its action, and summarize recent results obtained using artificial bilayer membranes, mitoplasts, and MD simulations, and cryo-electron microscopy.

THERMODYNAMICS AND KINETICS OF PROTON TRANSFER REACTIONS CATALYZED BY RESPIRATORY COMPLEX I

Vivek Sharma;
[1]University of Helsinki, Department of Physics, Helsinki, Finland

One of the major unresolved questions in the field of bioenergetics is the proton pumping mechanism of respiratory complex I [1], which plays a central role in redox homeostasis in mitochondria. Several high-resolution structures of respiratory complex I in different catalytic states are available [2], but atomistic understanding of the long-range coupling between quinone reduction and proton pumping remains unclear and highly debated. By splitting the complex problem into multiple smaller parts, we study the redox-coupled proton transfer reactions with classical and hybrid QM/MM molecular dynamics simulations including free energy calculations. We show that anionic quinol (QH-) formed after two-electron reduction can drive the proton pump of respiratory complex I [3]. Our multicale simulations show low barriers of proton transfer in the membrane domain of complex I, including across antiporter-like subunits [4,5]. The emerging picture on protonation dynamics challenges some of the currently held notions on complex I mechanism.[1] Djurabekova, Amina, et al. Biochemical Journal 481.7 (2024): 499-514.[2] Ivanov, Bozhidar S., et al. Nature Communications 15.1 (2024): 9340.[3] Zdorevskyi, Oleksii, et al. bioRxiv (2024): 2024-09.[4] Zdorevskyi, Oleksii, et al. Chemical Science 14.23 (2023): 6309-6318.[5] Simsive, Luka, et al. (in preparation).

PROTON PUMPING MECHANISM OF PHOTOSYNTHETIC NDH-1 AND COMPLEX I

Md. Raihan Uddin[1,2]; Patricia Saura[6]; Umesh Khaniya[4,5]; Koreena Sookhai[3]; Junjun Mao Mao[4]; Robert L Burnap[7]; Ville R Kaila[6]; Marilyn R Gunner[2,4];
[1]City College Of New York, Department of Biochemistry, Bronx, NY, USA
[2]The Graduate Center-CUNY, Department of Biochemistry, Bronx, NY, USA
[3]City College Of New York, Bronx, NY, USA
[4]City College Of New York, Department of Physics, Bronx, NY, USA
[5]National Cancer Institute (NIH), Bethesda, MD, USA
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Proton-coupled electron transfer is a fundamental process in bioenergetic enzymes, including Complex I and its cyanobacterial analog, NDH-1. Using molecular dynamics (MD) simulations and multi-conformation continuum electrostatics (MCCE) calculations, we investigated the proton transfer mechanism in the E-channel of Thermus thermophilus Complex I and compared it to the homologous pathway in Thermosynechococcus elongatus NDH-1. Our analysis identified two extended clusters of proton-loading sites (PLS) in Complex I: an N-side uptake cluster and a Central Cluster spanning >20 Å. Microstate analysis revealed long-range coupling of protonation states, with discrete net charge transitions in the Central Cluster that correspond to loaded and unloaded states. Similarly, in NDH-1, we identified conserved protonation dynamics within the E-channel, with structural variations altering the composition of the Central Cluster while maintaining its functional charge transitions. A key distinction emerged in the P-side exit cluster. While prior studies suggested a potential PLS in Complex I, our analysis of multiple MD snapshots found no significant protonation changes in this region. However, in NDH-1, we observed an active PLS at the P-side exit, indicating that proton loading/unloading at this site depends on conformational transitions that were not captured in the Complex I simulations. This finding underscores the challenge of identifying functionally relevant PLS, as key residues may exhibit rare transitions between loaded and unloaded states. Furthermore, despite overall conservation in the E-channel, we found specific substitutions in NDH-1—including a Ser and Asn replacing a Glu and His from Complex I—as well as conserved Lys residues exhibiting reduced proton affinity, which may contribute to distinct proton loading/unloading behavior. These results provide new insights into the evolutionary adaptation of proton transport pathways in bioenergetic enzymes, emphasizing both conserved and divergent mechanisms in Complex I and NDH-1.

UNDERSTANDING THE ROLE OF PROTONS IN DRUG TRANSPORT VIA SLC15A2

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The absorption and elimination of beta-lactam antibiotics in the human body are facilitated by proton-coupled peptide transporters PepT1 (SLC15A1) and PepT2 (SLC15A2). However, the role of the proton electrochemical gradient in the mechanisms of drug recognition and discrimination still remains unclear, hampering efforts to enhance antibiotic pharmacokinetics through targeted drug design and delivery. In this presentation, I will discuss our recent cryo-electron microscopy (cryo-EM) structures of the proton-coupled peptide transporter PepT2 from Rattus norvegicus in complex with commonly used beta-lactam antibiotics such as cefadroxil, amoxicillin, and cloxacillin. Our structural data, along with pharmacophore mapping, molecular dynamics simulations, and biochemical assays, reveal critical insights into the mechanisms underpinning beta-lactam antibiotic recognition and highlight the crucial role of protonation in drug binding and transport.

PROTONS ENABLE CALCIUM PASSAGE THROUGH THE HYDROPHOBIC CHANNEL IN THE SARS-COV-2 ENVELOPE PROTEIN

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The envelope (E) protein of SARS-CoV-2 is a cation-selective channel associated with viral pathogenicity. NMR studies have provided evidence for the co-transport of H^+ and Ca^{2+} through the E channel. However, details of the ion transport mechanism, particularly how H^+ and Ca^{2+} permeate through the hydrophobic interior, remain elusive. To investigate how the E channel transports H^+ and Ca^{2+}, we combined multiscale reactive molecular dynamics (MS-RMD) with umbrella sampling to compute the free energy profiles for H^+ transport, Ca^{2+} transport, and their co-transport through the E channel's transmembrane domain. We found that the E channel is intrinsically impermeable to Ca^{2+}, whereas H^+ can permeate by creating its own water wire within the hydrophobic pore. Interesting, in the presence of H^+, simulations reveal a coupling between H^+ and Ca^{2+} and my imply that proton recruitment hydrates the otherwise dry channel to enable calcium transport. These findings highlight the unique ability of protons to generate conductive water wires in hydrophobic environments, an effect we have previously observed in systems such as carbon nanotubes and de novo channels, and offer an atomic-level view of H^+/Ca^{2+} co-transport mechanism.

FROM SEQUENCE TO MECHANISM: INSIGHTS INTO PROTON COUPLING IN CYSTINOSIN FROM MULTISCALE MOLECULAR SIMULATIONS

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Cystinosin is a proton-coupled lysosomal symporter in the SLC family. Dysfunction in this transporter leads to cystine accumulation in lysosomes, which is associated with severe metabolic disorders. Leveraging recent advances in protein structure prediction models, we explore the mechanism of Cystinosin using enhanced-sampling molecular dynamics. Our results show that AlphaFold with MSA-subsampling can generate diverse structural ensembles that include realistic occluded states and end-states. By applying simple heuristics and dimensionality reduction techniques to these synthetic ensembles, we extract valuable information such as seed-structures and collective variables for umbrella sampling. This enables the calculation of robust, hysteresis-free energetics for the full conformational cycle of the transporter. Thus, our pipeline allows us to distil out essential dynamic and functional insights from sequence alone in a computationally efficient manner, without sacrificing the accuracy of physical models. Finally, QM/MM metadynamics simulations reveal the nature and mechanism of the proton transfer process that drives substrate export in Cystinosin. We show how proton exchange between gating residues is coupled to the dynamic transition between outward- and inward-facing conformations in the alternating access cycle.

PHOTOTRIGGERED PROTON-SELECTIVE CARRIER CURRENTS

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The selective regulation of ion transport across biological membranes is a central challenge for cellular life. We present a general strategy for precise, noninvasive, and reversible photoregulation of specific ion transport across membranes using embedded azobenzene-containing photolipids as dopants that photocontrol the permeation of ion-selective carriers. Photolipid embedding and photoisomerization cause only minor changes in background conductance, consistent with classical models wherein small changes in bilayer thickness have little effect on ion permeability. Yet, in the presence of carriers, photoisomerization induces order-of-magnitude jumps in ion flux. These jumps occur with both cationic and anionic carriers; a particular protonophore shows exceptional performance, with proton currents amplified up to 200-fold by UV light and reversed by blue light. Light-triggered modulations of interfacial uptake and release reactions tentatively explain the results. Doping membranes with both photolipids and ion carriers thus offers a versatile method for reversible, millisecond-scale photoregulation of selective ion transport. The approach enables large currents, long-lasting effects, easy tuning of ion specificity via carrier choice, and does not require genetic modifications. Recent advances in photoswitch chemistry will allow shifting of the action spectrum to longer wavelengths, enabling an all-chemical, flexible strategy for regulating ion permeability in biological or synthetic cells.

MULTI-PROTON DYNAMICS NEAR MEMBRANE-WATER INTERFACE

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Protons are crucial for biological energy transduction between membrane proteins. While experiments suggest rapid proton motion over large distances at the membrane-water interface, computational studies employing a single excess proton found the proton immobilized near the lipid headgroup. To address this discrepancy, we conduct full quantum mechanical (QM) Density Functional Tight Binding (diagonal-DFTB3) simulations (up to 11 ps each), by incrementally adding protons to the simulation box, one, two and three (two trajectories for each case). We show that a single proton creates a water-wire on which it moves rapidly toward the nearest headgroup. There it is either repelled by a choline group or binds covalently to a phosphatic oxygen. The covalent bond formation, not previously observed, was confirmed by a short ab initio molecular dynamics (AIMD) simulation. With multiple protons, some are trapped by the lipid head groups, while the remaining diffuse laterally faster than in bulk water. Initially, the mobile proton(s) possesses excess energy, enabling it to jump to the center of the water slab. Then it relaxes into the third- and second-hydration shells. This is confirmed in the last simulation by by adding water, showing that the proton prefers the second-layer over the mid-water position. Lateral diffusion rates further increase (becoming an order of magnitude faster than in neat water) as the proton stabilizes in the second hydration layer. These surprising results provide insights into proton dynamics near membranes, explaining the experimental observations by Pohl and collaborators, albeit on much smaller time- and distance-scales.

PROTON TRANSPORT MECHANISM OF NEWLY DISCOVERED LIGHT-DRIVEN INWARD PROTON PUMP, ELTXER

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Bacteriorhodopsin (BR), a light-driven outward proton pump, promotes ATP production by forming a proton gradient across the cell membrane. Since 2000, homologous genes have been discovered in many marine bacteria [1]. Furthermore, proton-pumping rhodopsins binding a light-harvesting antenna capable of utilizing broadband light have been reported [2, 3]. These findings suggest that proton-pumping rhodopsins contribute to energy production in many microorganisms. On the other hand, in 2016, we reported that xenorhodopsins (XeRs) function as light-driven inward proton pumps. Research on inward proton pumps has also contributed to a deeper understanding of the unidirectional proton transport mechanism of rhodopsin [4, 5].In this study, we discovered a novel XeR, EltXeR, which was detected in the extracellular virome of a saltern pond near Eilat, Israel, which may also include vesicles and other viral-like particles. Interestingly, it features a substitution at a highly conserved proline residue near the protonated retinal Schiff base, the photoreaction center, forming part of the proton transport pathway among in XeRs. To elucidate the molecular function and properties of EltXeR, we conducted functional and spectroscopic analyses. Our results demonstrated that EltXeR functions as an inward proton pump, similarly to other XeRs. Time-resolved spectroscopy revealed that EltXeR produces photo-intermediates similar to those of other XeRs. However, the proton inventory plot of the rate constants of photo-intermediate conversions significantly differed from that of previously reported XeR. Our results highlight the diversity of inward proton pumps and provide new insights into the molecular mechanisms of proton transport by XeRs. [1] Béjà et al., Science 289, 1902–1906, 2000 [2] Balashov et al., Science 309, 2061–2064, 2005[3] Chazan et al., Nature 615, 535–540, 2023[4] Inoue et al., Nat. Commun. 7, 13415, 2016 [5] Urui et al., Acc. Chem. Res. 57, 3292–3302, 2024

DECONSTRUCTING THE SIGNATURE SEQUENCE OF THE VOLTAGE-GATED PROTON CHANNEL, H_V

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Ion channel genes can be identified by searching amino acid sequences for "signature sequences" which are found in all members of a particular channel family. Logically, an obligatory series of amino acids would be expected to participate in some important function that is specific to the channel. For example, the K^+ channel signature sequence results in K^+ selective permeation. We identified a signature sequence in voltage-gated proton channels [Smith, S.M.E., D. Morgan, B. Musset, V.V. Cherny, A.R. Place, J.W. Hastings, and T.E. DeCoursey. (2011). Voltage-gated proton channel in a dinoflagellate. Proceedings of the National Academy of Sciences, U.S.A. 108:18162-18168] that has been used to identify this channel in a wide range of species. This sequence comprises RxWRxxR in the fourth transmembrane helix (S4), where R=arginine, W=tryptophan, and x=a neutral hydrophobic amino acid. As in most other voltage-gated ion channels, the S4 sequence of positively charged amino acids contributes of voltage gating. One further defining requirement for H_V channels is an Aspartate in the middle of the first transmembrane helix, S1. By interacting with one or more of the Arg in S4, this Asp enforces proton selectivity on the channel. Here we examine mutations and natural anomalies in the signature sequence of H_V and attempt to identify the minimum requirements and determine how much latitude exists in variants of the signature sequence.

SOMATIC CANCER MUTATIONS IN THE S4 TRANSMEMBRANE SEGMENT OF THE HUMAN VOLTAGE-GATED PROTON CHANNEL HV1

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Somatic mutations are common in cancer, with only a few driving the progression of the disease, while most are silent passengers. Some mutations may hinder or even reverse cancer progression. The voltage-gated proton channel (HV1) plays a key role in cellular pH homeostasis and shows increased expression in several malignancies. Inhibiting HV1 in cancer cells reduces invasion, migration, proton extrusion, and pH recovery, impacting tumor progression. Focusing on HVCN1, the gene coding for the human voltage-gated proton channel (hHV1), 197 mutations were identified from three databases: 134 missense mutations, 51 sense mutations, and 12 introducing stop codons. These mutations cluster in two hotspots: the central region of the N-terminal and the region coding for the S4 transmembrane domain, which contains the channel's voltage sensor. Five somatic mutations within the S4 segment (R205W, R208W, R208Q, G215E, and G215R) were selected for electrophysiological analysis and MD simulations. The findings reveal that while all mutants remain proton-selective, they all exhibit reduced effective charge displacement and proton conduction. The mutations differentially affect hHV1 kinetics, with the most pronounced effects observed in the two Arg-to-Trp substitutions. Mutation of the first voltage-sensing arginine (R1) to tryptophan (R205W) causes proton leakage in the closed state, accelerates channel activation, and diminishes the voltage dependence of gating. Except for R205W, the mutations promote the deactivated channel configuration. Altogether, these data are consistent with impairment of hHV1 function by mutations in the S4 transmembrane segment, potentially affecting pH homeostasis of tumor cells.

THE FUNCTIONAL REGULATION OF THE VOLTAGE-GATED PROTON CHANNEL BY ATP

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Voltage-gated proton channels (Hv1) are crucial membrane proteins that regulate proton flow in response to changes in membrane voltage and play essential roles in various physiological processes, including pH regulation and immune responses. While Hv1 has been considered to function independently of cytosolic adenosine-5'-triphosphate (ATP), our recent findings reveal that ATP directly modulates Hv1 activity under physiological conditions [Kawanabe et al. (2023)]. Using electrophysiological recordings (whole-cell parch clamp techniques), we demonstrated that ATP enhances Hv1 activation and increases proton conductance across the membrane under physiological ATP concentration. Notably, these effects occur independently of ATP hydrolysis, suggesting a direct, non-enzymatic interaction between Hv1 and ATP. Supporting this, additional molecular interaction assays (microscale thermophoresis) were employed to explore the possibility of direct binding. Further analyses showed that the degree of channel activation correlates with the number of phosphate groups in ATP and its analogs, implying a specific direct recognition mechanism. These findings suggest that ATP can act as a regulatory molecule that modulates ion channel function. By directly modulating Hv1 activity, ATP introduces an additional layer of control over proton channel physiological process. In this talk, I will present our experimental approach and findings, including additional supporting experiments, and discuss the mechanisms and sites at which this ATP-dependent regulation of Hv1 occurs.

EXPLORING BIOPHYSICAL DIVERGENCES WITHIN THE VOLTAGE-GATED PROTON CHANNEL (H_V) FAMILY

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Voltage-gated proton channels (HV) are typically encoded by a single gene in most species. Until recently, all 19 species examined by voltage-clamp studies follow this pattern. However, Aplysia californica represents the first known species to express multiple H_V channel genes. In heterologous expression systems, voltage-clamp recordings confirmed that three distinct Aplysia genes encode depolarization-activated, proton-selective channels, each exhibiting ΔpH-dependent gating—a hallmark of H_V channels that promotes proton extrusion by favoring opening under outward proton gradients. Two gene products, AcH_V1 and AcH_V2, displayed relatively typical behavior, though AcH_V2 activated at significantly more negative voltages, enabling inward proton current at symmetric pH. In contrast, AcH_V3 exhibited profoundly atypical features, including markedly reduced voltage dependence and persistent proton-selective leak currents at hyperpolarized potentials. These properties suggest a subset of channels remain constitutively open, raising the possibility that AcHV3 functions as a broken channel. Nonetheless, its expression in Aplysia tissues and the presence of C-terminal intracellular retention motifs suggest a physiological role, potentially in organellar membranes where leak conductance may be beneficial. Sequence analysis revealed that, unlike all previously characterized H_V channels, AcHV3 deviates from the conserved S4 helix signature sequence RxWRxxR, instead possessing LPWRxxR. This motif lacks the first arginine and replaces a conserved leucine with a helix-disrupting proline, likely contributing to AcH_V3 unusual gating behavior. This study explores the extent to which these two amino acid substitutions underlie the functional divergence of AcH_V3 from the more canonical AcH_V1 and AcH_V2 channels.

DRIVING FORCES OF VARIOUS PROTON PUMP RHODOPSINS

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Proton pump rhodopsins are photoreceptor membrane proteins which bind retinal as a chromophore, and transport protons unidirectionally even against the electrochemical potential gradient upon light absorption. An enormous number of proton pump rhodopsins have been identified in various micro-organisms, including the outward proton pumps, required for ATP production, as well as the inward proton pumps whose physiological role remains unknown. An important aspect of the vectorial transport in proton pumps is the regulation of the pump by the electrochemical gradient i.e. proton gradient and potential difference across the membrane. This is essential for the understanding of the molecular mechanism of the pump, because under physiological conditions the transmembrane potential reaches up to 280 mV. To address this issue, voltage clamp experiments on ion pumps offer the possibility of studying the influence of proton gradient and potential difference separately, which gives valuable information on the bioenergetic behavior of the pump rhodopsins. The aim of this study is to reach a deeper understanding of the regulation of the pumping process in these pumps by the electrochemical gradient. We heterologously expressed various proton pump rhodopsins including the six outward and the three inward proton pumps in mammalian cultured cells and performed voltage clamp measurements. To evaluate the performance of various rhodopsins, we determined the driving force as the maximum electrochemical load at which unidirectional proton transport is accomplished. We found significant differences in the driving forces among the tested rhodopsins, varying from 80 mV to 400 mV. We also performed the measurement under the existence of proton gradient and found different pH influence on the tested rhodopsins. We discuss the molecular origin of driving forces based on the obtained results of systematic electrophysiological measurements.

PROTON TRANSFER IN THE PHOTOCYCLIC REACTION OF ION-TRANSPORTING MICROBIAL RHODOPSINS

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Microbial rhodopsins are heptahelical transmembrane photoreceptive proteins that utilize an all-trans-retinal chromophore. They exhibit a wide range of light-dependent biological functions, including light-driven ion pumps, light-gated ion channels, phototactic sensors, and light-activated enzymes. Microbial rhodopsins are also widely utilized in optogenetics to optically control neural activity and other cellular processes. The primary event in photoactivated microbial rhodopsins is the isomerization of the all-trans-retinal chromophore to the 13-cis form. This is followed by the formation of a series of photointermediates with absorption spectra and protein structures distinct from those of the initial state, and the protein finally reverts to the initial state. This cyclic reaction is referred to as the photocycle. During the photocycles of microbial rhodopsins, these proteins exhibit their own biological functions. Notably, proton transfers are observed at many key steps of the photoreaction, where photointermediates are converted into the subsequent states. The direction and the rate of proton transfer are highly organized by amino acid residues conserved in each function class. Therefore, it is essential to understand the proton transfer events in microbial rhodopsins to elucidate the mechanisms underlying their biological functions. In this presentation, I'll present our recent findings on the significance of proton transfer events in ion-transporting rhodopsins, which have been obtained through several spectroscopic and electrophysiological methods. These results suggest that microbial rhodopsins have acquired diverse functions by evolving key residues involved in sophisticated proton transfer events essential for each function.

CONVERTING A CHLORIDE-PUMPING RHODOPSIN INTO A PROTON PUMP

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Bacteriorhodopsin (BR) and halorhodopsin (HR) are membrane proteins with a retinal chromophore covalently attached to a conserved lysine residue via a Schiff base. They function as light-driven proton and chloride pumps, respectively. BR can be converted into a chloride pump by replacing Asp85, the proton acceptor from the Schiff base, with the corresponding Thr residue found in HR. However, HR cannot be converted into a proton pump by the reverse Thr-to-Asp mutation. Even a 10-amino-acid mutant of HR from Natronomonas pharaonis (pHR_{10}), which includes residues conserved in BR-like proton pumps, does not transport protons. This study aims to convert HR into a proton pump to gain insights into the factors that are crucial for proton transport in the protein environment. The structure of pHR_{10} was modeled based on the crystal structure of the wild type using a quantum mechanical/molecular mechanical (QM/MM) approach, and its active site was compared with that of BR. This comparison revealed two key differences between BR and pHR_{10}: (i) the pK_a value of the proton acceptor from the Schiff base (Asp85 in BR), and (ii) the cavity size near the Schiff base. The active site in the QM/MM-optimized structure of a three-amino-acid mutant pHR_{10} exhibits characteristics similar to those of BR. Measurement of light-induced pH changes in the external solvent of E. coli demonstrated that this mutant pumps protons, indicating that HR was successfully converted into a proton pump. This successful conversion of a chloride pump into a proton pump provides a basis for understanding proton transport mediated by the protein environment.

MECHANISMS OF NOVEL DISEASES DISRUPTING LYSOSOMAL VIA CLC-7 CHLORIDE TRANSPORTER MUTATIONS.

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Lysosomes are highly acidic organelles that degrade macromolecules and serves as hubs for metabolic signaling. This acidic environment is generated by the V-ATPase, which promotes lysosomal function. However, other proteins are needed to create a counterion pathway that offsets an increasing membrane potential. The chloride/proton antiporter ClC-7 has been linked to controlling lysosomal pH through counterion transport. Dysregulation of ClC-7 in humans can cause osteopetrosis, neurodegeneration, and lysosomal storage disease. We recently reported two patients with a CLC-7 gain-of-function mutation, Y715C, which causes hypopigmentation and a polysystemic lysosomal storage disease. Cells from these patients contain hyperacidic lysosomes, and heterologous expression of ClC-7 with the Y715C mutation revealed strongly increased ClC-7 transport activity. Here, I will discuss the regulation of ClC-7 by a lysosomal phosphoinositide and its relation to pH regulation, giving insight into the disease mechanism. In addition, I will describe several new patients with novel ClC-7 gain-of-function mutations who demonstrate a similar disease pattern but milder symptoms. Together these results have substantial implications for understanding lysosomal pH regulation and disease therapy.

TARGETING OTOP PROTON CHANNELS: INSIGHTS FROM CHEMICAL MODULATORS

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Ion channels selective for protons are relatively rare in nature, with only a few families identified. In 2018, we showed that Otopetrin proteins, conserved across vertebrates and invertebrates, form a family of proton-selective ion channels. The founding member, vertebrate OTOP1, acts as a sensor for sour and ammonium taste by the gustatory system, while the roles of other OTOP channels remain unclear. To investigate OTOP channel function and structure, we sought chemical modulators that could activate or inhibit their activity. We first demonstrated that OTOP channels expressed in HEK-293 cells are both activated and inhibited by Zn^{2+} in a subtype-specific manner, allowing us to identify structural elements for Zn^{2+}-dependent activation. To find more specific modulators, we performed a structure-guided virtual screen which led to the identification of a series of small molecules that inhibit zebrafish OTOP1. These compounds offer insight into OTOP channel gating and provide tools for further functional and pharmacological studies.

AUTOMATED PATCH CLAMP ASSAY FOR PROTON-ACTIVATED HOTOP1 CHANNEL

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Manual patch clamp, invented in the late 1970s, remains the gold standard for studying ion channels due to its capability to directly measure ion channel function. However, this technique is low-throughput, labor-intensive, and requires a high level of expertise. Automated patch clamp (APC) systems address these limitations by enabling higher throughput and standardized recordings. The QPatch Compact (QPC) is an 8-well APC platform, featuring microfluidic flow channels, which allows for rapid extracellular solution exchange—an essential requirement for studying proton-activated ion channels. OTOP1 (Otopetrin 1) is a proton-selective ion channel expressed in many cell types and tissues, including the inner ear and taste receptor cells, where it plays a critical role in otoconia formation and acid sensing. In taste cells, OTOP1 functions as a sour taste receptor and ammonium sensor, transducing changes in pH and ammonium into action potentials that signal to gustatory nerves. OTOP1 is gated by extracellular pH changes and exhibits high proton selectivity, allowing it to mediate responses to acidic environments. Understanding OTOP1's biophysical properties is crucial for elucidating its physiological roles and therapeutic potential. We have developed an assay to study human OTOP1 recombinantly expressed in HEK293 cells using the QPatch Compact platform. Our recordings demonstrate that OTOP1 currents can be robustly activated by extracellular protons in a dose-dependent manner, confirming the channel's pH-dependent activation. Additionally, application of extracellular ammonium evoked inward proton currents, consistent with OTOP1's role as a sensor for ammonium. Furthermore, we confirmed that Zn^{2+} inhibits human OTOP1, demonstrating that this assay can be used to evaluate novel channel inhibitors. This study serves as a proof of principle that OTOP1 can be reliably recorded using the QPatch Compact system. Our assay is well-suited for identifying novel modulators of OTOP1 that could facilitate future investigations into its physiological role and used for therapeutic applications.

ROLE OF A TRYPTOPHAN IN PROTON-COUPLED ELECTRON TRANSPORT IN RIBONUCLEOTIDE REDUCTASE

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Ribonucleotide reductases (RNRs) are essential enzymes that convert ribonucleotides into deoxyribonucleotides, sustaining DNA synthesis and repair in all living organisms. Central to RNR activity is a long-range radical transport pathway that spans approximately 32 Å across the α and β subunits by a series of proton-coupled electron transfer (PCET) reactions. Although the four collinear PCET events in the α subunit have been extensively studied, the corresponding pathways and the mechanistic contributions of key residues and hydration dynamics in the β subunit remain poorly understood. In particular, multisite and orthogonal PCET processes in the β subunit demand further elucidation. In this work, we focus on the redox-active tryptophan residue and its local hydration environment within the β subunit. By combining classical molecular dynamics, grand canonical Monte Carlo (GCMC) simulations, and finite-temperature string QM/MM free energy calculations, we quantify the thermodynamic driving force for the proton transfer step and reveal its coupling to the long-distance electron transfer reaction. The results demonstrate how hydrogen-bonding networks and site-specific hydration modulate PCET reactions. Overall, our work provides mechanistic insights into the biological multisite PCET process and has general applicability to a wide range of complex biomolecular systems to further guide therapeutic and engineering applications.

PROTONATION-DEPENDENT STABLE PORE FORMATION BY THE PHD PEPTIDES REQUIRES A WATER- AND LIPID-MEDIATED HYDROGEN-BOND NETWORK

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Peptides of the pHD series form large water-filled macromolecular pores at pH < 6. The peptides are nontoxic to healthy cells, and pores form even at low peptide concentration (peptide:lipid ratio < 1:1000). A key open question is how specific amino acid residues influence the propensity of these peptides to form pores in lipid membrane environments. To study the reaction coordinate of pore formation we perform atomic level-simulations of pHD peptides in aqueous solution, at the membrane interface, and in the membrane. We apply graph-based algorithms to characterize the dynamics of the hydrogen-bond networks of the peptides. We find that lipids associated with the pore have highly unusual geometries with phosphate groups visiting the center of the membrane and acyl chains splayed or largely parallel to the membrane surface. Short hydrogen-bonded water bridges inter-connect peptides with each other and with lipid headgroups. The organization of the peptides and the stability of the periphery of the pore depend strongly on the protonation states of the carboxylic sidechains. Research was supported in part by the National Institute of Health award no. 1R01GM151326-01 and by the computing time allocations PHDPORES and TOXINTOCURE from the JURECA-DC Supercomputing Cluster of the Forschungszentrum Jülich.

DESIGNED WATER AND SIDECHAIN DYNAMICS IN DE NOVO PROTON CHANNELS

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The controlled movement of protons across cellular membranes underpins a wide range of essential processes, from energy conversion to signal transduction. In nature, proton channels achieve this task with remarkable selectivity and efficiency, enabling proton flux while excluding other ions and preserving membrane integrity. A key challenge in the field of bioinspired design is understanding and replicating the precise molecular features that enable this selective transport. Central to this function are the dynamic interactions between interfacial water molecules and pore-lining sidechains, which together create a transient but continuous hydrogen-bonded network for proton conduction.Here, we employ de novo protein design to construct self-assembling helical peptide bundles that mimic the core features of natural proton channels. By systematically varying sidechain chemistry and conformational flexibility, we uncover how specific residues contribute to the formation and modulation of water wires within the channel lumen. These synthetic systems enable direct testing of the mechanistic roles of sterics, polarity, and dynamics in proton transport.Our designed channels reveal critical principles of selective proton conduction, demonstrating that optimal function arises not from static structural features alone, but from a finely tuned interplay between backbone packing, sidechain dynamics, and pore hydration. This work establishes a new platform for probing the fundamental biophysics of proton transport and lays the foundation for engineering robust, proton-selective materials for applications in synthetic biology, energy conversion, and nanofluidics. More broadly, these findings highlight the importance of integrating structural and dynamic considerations into the design of functional biomolecular assemblies.

BEYOND PEAK CURRENTS: SOLID-SUPPORTED MEMBRANE ELECTROPHYSIOLOGY AS A MEANS OF INVESTIGATING UNIVERSAL TRANSPORT MODELS

Merissa Brousseau[1]; Katherine A Henzler-Wildman[1,2];
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The small multidrug resistance (SMR) family transporters contribute to antibiotic resistance through proton-coupled efflux of toxic compounds. Recent mechanistic studies of the model E. coli SMR transporter EmrE suggest this minimalistic efflux pump is regulated by a network of allosteric interactions that can be modulated by small molecules. The dramatic influence of substrates on the rate limiting steps in the transport cycle result in changes in the dominant mode of transport for different substrates and requires a more thorough understanding of the transport mechanism. Recent advances in solid-supported membrane electrophysiology (SSME) techniques have facilitated higher-throughput investigations of the mechanistic complexity of transporters in near physiological conditions. However, the influence of transport mechanisms on the experimental signal, contributions of pre-steady state and steady state processes, and applicability of controls in SSME experiments remains underappreciated. Here we provide a bottom-up approach for characterizing the interactions of novel substrates with loosely-coupled, promiscuous transporters. As an archetype for the SMR family for decades, the extensive biophysical characterizations that have been performed on EmrE, and more recently on functional mutants, provide a case study in how in-vitro biophysical studies can complement liposomal assays performed under a gradient to inform on mechanism. Our analyses of EmrE and mutants in the presence of varying absolute pH and substrate concentrations support the necessity of additional states in the universal transport model for EmrE while also demonstrating the importance of peak currents, signal decay, and integrated currents in mechanistic characterization of complex transport behavior.

HOW COMPLEX LIPID COMPOSITIONS CONTROL MECHANICAL STABILITY OF BIOMEMBRANES

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Biomembranes have evolved to meet seemingly conflicting demands. On one hand, they enable compartmentalization within cells and organelles, necessitating membrane integrity and mechanical stability. Damage to membranes, such as the formation of transmembrane pores, can lead to uncontrolled flux of protons or other ions and is linked to senescence and neurodegenerative diseases. On the other hand, membranes must be highly plastic to facilitate ongoing remodeling for signaling, trafficking, and morphogenesis. The mechanisms by which cells use complex lipid compositions to achieve both stability and plasticity are not well understood. We employ molecular dynamics simulations and free energy calculations, linked to second-harmonic generation spectroscopy to uncover how lipid composition, lipid geometry, and lateral sorting orchestrate membrane stability. Our findings suggest that the widely accepted continuum model for membrane electroporation needs revision. We identify a previously undetected ion permeation mechanism involving localized membrane electroporation through ion binding to headgroups. We demonstrate that two properties—pore nucleation free energy and edge tension along the pore rim—quantify membrane stability and are controlled by distinct lipid properties. Together, our work provides atomistic, energetic, and mechanistic insights into the mechanical stability of complex membranes.

PROTON DIFFUSION ON THE SURFACE OF MIXED LIPID MEMBRANES HIGHLIGHTS THE ROLE OF MEMBRANE COMPOSITION

Ambili Ramanthrikkovil Variyam[1]; Mateusz Rzycki[2]; Anna Yucknovsky[1]; Dominik Drabik[2]; Alexei A. Stuchebrukhov[3]; Nadav Amdursky[1,4];
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[2]Wroclaw University of Science and Technology, Department of Biomedical Engineering, Wroclaw, Poland
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Proton circuits within biological membranes, the foundation of natural bioenergetic systems, are significantly influenced by the lipid compositions of different biological membranes. In this study, we investigate the influence of mixed lipid membrane composition on the proton transfer (PT) properties on the surface of the membrane. We track the excited-state PT (ESPT) process from a tethered probe to the membrane with time-scales and length-scales of PT relevant to bioenergetic systems. Two processes can happen during ESPT: the initial PT from the probe to the membrane at short timescales, followed by diffusion of dissociated protons around the probe on the membrane, and the possible geminate recombination with the probe at longer timescales. Here, we use membranes composed of mixtures of phosphatidylcholine (PC) and phosphatidic acid (PA). We show that the changes in the ESPT properties are not monotonous with the concentration of the lipid mixture; at low concentration of PA in PC, we find that the membrane is a poor proton acceptor. Molecular dynamics simulations indicate that the membrane is more structured at this specific lipid mixture with the least defects. Accordingly, we suggest that the structure of the membrane is an important factor in facilitating PT. We further show that the composition of the membrane affects the geminate proton diffusion around the probe, whereas, on a time-scale of tens of nanoseconds, the dissociated proton is mostly lateral restricted to the membrane plane in PA membranes, while in PC, the diffusion is less restricted by the membrane.

REGULATION OF PROTON TRANSPORT BY MEMBRANE STEROLS

Shuo Han[1]; Sarah Applewhite[1]; **Shizhen Wang**[1];
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Voltage-gated proton (Hv) channels are standalone voltage sensors without separate pore-forming domains. They open upon depolarized voltages and conduct protons with almost perfect selectivity. Hv channels usually mediate proton efflux in most cell types, for their function is enhanced by acidic pH at the intracellular side while suppressed by extracellular acidic pH. In addition to voltage and pH, Hvs are also modulated by multiple ligands, such as zinc, polyunsaturated fatty arachidonic acid and ATP. Recently, with purified hHv1 channels reconstituted into liposomes, we showed that membrane cholesterol inhibits human Hv1 (hHv1) channels, but the underlying mechanisms are still far from clear. In the present work, we further determined the effects of multiple cholesterol analogs on the structure and function of hHv1 channels and identified key residues in hHv1 channels that are essential for cholesterol inhibition. Intriguingly, the cholesterol precursor, desmosterol, does not inhibit hHv1 channels. Instead, it strongly attenuates cholesterol inhibition, suggesting a potential role of cholesterol metabolism in regulating cellular pH homeostasis through hHv1 channels. Using the single-molecule fluorescence resonance energy transfer (smFRET) technique, we showed that while cholesterol stabilizes the hHv1 channel at resting conformations, desmosterol, however, enriches opening conformations, even in the presence of cholesterol. The smFRET results agree well with desmosterol's effect on attenuating cholesterol inhibition. Through extensive mutagenesis analyses, we also identified mutations in hHv1 channels that shift its cholesterol sensitivity by 4 folds, indicating a potential binding site at the transmembrane domain in the hHv1 channel. In summary, our studies established the important roles of membrane sterols in shaping cellular pH homeostasis by regulating the hHv1 channel and also shed light on its underlying molecular mechanisms.

PROTONS AS A SECOND MESSENGER: PH-DEPENDENT ALLOSTERY IN SIGNALING PROTEINS

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Transient changes in intracellular pH (ranging from 6.8 to 7.6) drive various cellular behaviors. Furthermore, pHi dynamics are dysregulated in cancer, contributing to tumorigenic cell behaviors. However, the specific pH-sensitive proteins (pH sensors) responsible for these cell behaviors remain largely unknown. To identify candidate pH sensitive proteins, we developed an in silico computational pipeline that uses structural data to predict residues with pKa shifts that may function as pH sensing nodes in proteins. We applied this approach to SH2 domain-containing signaling proteins, a class of proteins that are dysregulated in cancer. Our method showed that modular SH2 proteins have predicted pH sensitive networks clustering at the interface between regulatory SH2 domains and catalytic domains. Using biophysical, biochemical, and cell biological approaches, we validated the role of pH in tuning the wild-type signaling activity of two oncogenes, c-Src and Src homology region 2 domain-containing phosphatase-2 (SHP2). At low pHi, these wild-type proteins have high activity with decreasing activity as pHi increases. Additionally, we validated the predictive power of our computational pipeline by mutating the identified residues to abrogate pH dependent activity. Importantly, when we analyzed databases of somatic mutations in cancer, we found that recurrent cancer-associated mutations in these signaling proteins cluster at these pH sensitive residues, highlighting their importance in regulating these crucial signaling proteins. Together, our computational, biophysical, and cellular analyses reveal a conserved role for modular SH2 proteins as mediators of pH-dependent signaling. Moreover, our data suggest that cancer mutations cluster in these conserved networks to abrogate pH-sensitive regulation of activity and contribute to the hyper-activation of cell signaling in cancer.

DECRYPTING THE NON-ADIABATIC PHOTOINDUCED ELECTRON TRANSFER MECHANISM IN LIGHT-SENSING CRYPTOCHROME

Gustavo J Costa[1]; **Ruibin Liang**;
[1]Texas Tech University, Chemistry and Biochemistry, Lubbock, TX, USA

Cryptochromes are blue light photoreceptors found in organisms from plants to animals, playing various critical roles in life processes such as circadian rhythms, phototropism and magnetoreception. In light-sensing cryptochromes, the photoexcitation of the flavin adenine dinucleotide (FAD) cofactor triggers a cascade of electron transfer events via a tryptophan chain, eventually generating a radical pair crucial for signaling. Despite extensive studies, the initial photoinduced electron transfer (ET) from a neighboring tryptophan residue to FAD remains unclear due to the complexity of simulating all-atom dynamics in excited states, particularly regarding the roles of non-adiabatic pathways and protein environment on the reaction kinetics and quantum efficiency of the ET. To address this gap, we performed extensive non-adiabatic and adiabatic dynamics simulations with on-the-fly multireference ab initio electronic structure calculations of Arabidopsis thaliana cryptochrome 1 (AtCRY1). Our results reveal a novel photoinduced electron transfer mechanism involving non-radiative decay from higher-lying singlet states, which proceeds much faster than the adiabatic electron transfer on the S_1 state. The adiabatic process is hindered by a newly discovered low-energy S_1 local excitation minimum. In contrast, non-adiabatic relaxation can rapidly reach a dynamically stable S_1 charge-transfer minimum, setting the stage for subsequent electron transfer steps. Additionally, the protein environment stabilizes the orientation of tryptophan residues, facilitating later ET events between them while hindering the initial FAD-W400 transfer. These new insights greatly enhance our fundamental understanding of photoinduced electron transfer in cryptochromes and the structure-function relationships in photoreceptors in general.

COUPLED PROTONATION STATES AND HYDHYDROGEN BOND NETWORKS IN PROTON PUMPS.

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[4]CUNY Graduate Center, Physics, New York, NY, USA
[5]Stockholm University, Biochemistry and Biophysics, Stockholm , Sweden
[6]Oklahoma State University, Microbiology and Molecular Genetics, Stillwater, OK, USA

Proton pumps move protons uphill from the side of the membrane with low proton concentration (N-side) to the side at high concentration (P-side). The energy of downhill redox chemistry fuels this process. Protons move via hydrogen bonded chains of waters and Grotthuss competent amino acids. In different reaction intermediates the connections to the N- and P-side differ. Also, the proton affinity of residues within the protein change through the reaction cycle to load and release protons. MCCE uses Monte Carlo sampling of residue and water orientation and residue protonation states. A microstate is one position and charge for each group. By saving all the millions of microstates we can find the residues that make up the PLS and proton transfer paths. Thus, the persistence of hydrogen bonds oriented for proton transfers are found. Analysis of microstates also shows the residues whose protonation states are coupled together to form multi-residue proton loading sites (PLS). Proton transfer paths and PLS are compared in Complex I from T. thermophilus, E. coli and T. elongatus (NDH). The E channel, closest to the peripheral arm is unique to Complex I. The proton transfer paths are complex, with multiple competing routes for protons to travel. Residues along these proton transfer paths can play a role in PLS. Comparing the protonation of snapshots from MD trajectories show clusters in the channel whose overall protonation states differs by one proton being bound. However, the Monte Carlo ensemble shows that it is a group of residues stretching over 20Å, whose protonation states are coupled together to load and unload. The comparison of the three proteins shows conservation of many but not all PLS residues. Thus, the microstate analysis shows how residues play different roles to maintain function in related proteins. We acknowledge the funding of NSF MCB- 2141824.

STRUCTURAL INSIGHTS INTO SPECTRAL TUNING FROM THE THREE-DIMENSIONAL STRUCTURES OF RED AND GREEN CONE PIGMENTS

Kota Katayama[1,2];
[1]Nagoya Institute of Technology, Life Science and Applied Chemistry, Nagoya, Japan
[2]Nagoya Institute of Technology, OptoBioTechnology Research Center, Nagoya, Japan

To understand the photoreaction processes underlying the functional expression of cone pigments, which regulate the spectral tuning of light absorption and signal transduction, it is essential to: (1) determine the energy levels of the excited states of the chromophore, governed by quantum chemistry, and (2) elucidate the dynamic structural changes described by Newtonian mechanics. To date, we have clarified the subtle changes in amino acid functional groups involved in wavelength regulation, as well as the binding mechanisms of water molecules and ions, through light-induced FTIR difference spectroscopy. In this study, we report the results of our efforts to determine the three-dimensional structure of cone pigments using single-particle analysis with cryo-electron microscopy (cryo-EM). It has been a quarter-century since the structure of rhodopsin, responsible for scotopic vision, was elucidated in 2000. Now, the atomic-resolution structure of cone pigments is finally about to be revealed. What insights will the structural analysis of cone pigments provide into the molecular mechanisms underlying our perception of color? In this presentation, we will explore these questions in depth.

UNDERSTANDING ABC TRANSPORTERS TO NAVIGATE HUMAN DISEASES

Ruchika Bajaj[1];
[1]University of California San Francisco, Bioengineering and therapeutics sciences, San Francisco, CA, USA

Membrane proteins constitute 30% of the genome in organisms and are involved in numerous physiological processes. ABC transporters is a class of membrane proteins which are ubiquitously present in all organisms, bind and hydrolyze ATP to power the solute transport and are associated with several human diseases like multidrug resistance in cancer, macular degeneration, cystic fibrosis, retinitis pigmentosa etc. ABC transporters consists of two transmembrane domains (TMDs), which form the permeation pathway and nucleotide binding domains (NBDs) to bind and hydrolyze ATP and follow alternating access mechanism. Bacterial ABC transporters like binding-protein-independent mutant of maltose transporter, MalG511 from E.coli and FtsEX-PcsB from S. pneumoniae have been characterized biochemically and biophysically to study mechanism and future higher resolution studies. Structure-function relationships were studied in mammalian ABC transporters, bovine MRP4 and human P-glycoprotein. High resolution cryoEM structures of bovine MRP4 in three different states (apo state, nucleotide bound state and substrate bound state) are determined which revealed the architecture, asymmetry of NBDs, interpreted functional effects of genetic variants, located substrate binding site, deciphered associated conformational changes in catalytic cycle of bovineMRP4. Structure based drug designing and targeting MRP4 in context of cancer and cardiac diseases will be helpful to the field of medicine. Oral excipients were screened against P-gp using calceinAM fluorescence assay and digoxin flux assay were found to be inert for their effect on P-glycoprotein. beta-Cyclodextrin and light green SF yellowish were found to be inhibitory at high macromolecular range in digoxin flux assay. This information will be helpful in preparing novel generic formulations. Additionally, the meta-analysis study provides an overview of the revolutionizing field of structural biology of ABC transporters.

ADVANCES IN MODELING TRANSMEMBRANE PROTON TRANSPORT IN BIOMOLECULAR SYSTEMS

Gregory A Voth[1];
[1]Department of Chemistry, Chicago Center for Theoretical Chemistry, Institute for Biophysical Dynamics, and James Franck Institute, The University of Chicago, Chicago, IL, USA

In this presentation, the results of our longstanding efforts to characterize transmembrane proton transport by proteins will be described. These studies employ a novel, accurate, and computationally efficient multiscale reactive molecular dynamics method combined with large scale computer simulation. The methodology allows for the treatment of explicit (Grotthuss) proton shuttling and charge defect delocalization, which strongly influences proton solvation and transport in transmembrane proton channels, pumps, and transporters/antiporters. The intrinsically coupled nature of the excess proton translocation and the water hydration can also be elaborated through these computer simulations and theoretical analysis via graph theory. It is found that a prior existing "water wire", e.g., one seen in an experimental structure or in an MD simulation without an explicit translocating excess proton, is not necessary for protons to transport through hydrophobic spaces in proteins via water mediated Grotthuss shuttling. The proton translocation process can sometimes create its own transient water wire as needed and this process can be quantified via the definition of a proper collective variable. Specific simulation results will be given for coupled proton transport in the ClC Cl^-/H^+ antiporter, the PiPT phosphate transporter, and the Hv1 voltage gated proton channel, as time allows. In the case of PiPT, a complete "bottom-up" kinetic model can be formulated with multiple inputs from the molecular scale calculations. In the case of Hv1, a solution to the long-standing mystery of the origin of the proton selectivity by that channel can be provided by the simulations. If time allows (and if the project is satisfactory completed), the coupled co-transport of protons and calcium cations by the SARS-CoV-2 E-channel will also be described.

WATER CONNECTIVITY AND ITS COOPERATIVE COUPLING TO PROTON TRANSPORT IN A KEY REGION OF COMPLEX I

Jong Ho Choi; Gregory A Voth[1];
[1]University of Chicago, Chemistry, Chicago, IL, USA

Respiratory complex I (NADH:ubiquinone oxidoreductase) is the largest protein complex in the mitochondrial respiratory chain, coupling the oxidation of NADH and the reduction of ubiquinone to proton pumping across the inner mitochondrial membrane. Proton pumping depends on efficient proton transfer (PT) within the membrane domain, but how protons move across subunit interfaces remains unclear. Modeling PT in such large protein complexes is challenging: classical molecular dynamics cannot capture proton hopping, while quantum mechanical methods are too computationally expensive for systems of this size. To overcome these limitations, we employed multi-state reactive molecular dynamics (MS-RMD), a fast and reactive simulation framework based on empirical valence bond (EVB) theory that enables efficient modeling of Grotthuss-type proton hopping in complex biomolecular environments. We first performed a 1-μs classical MD simulation and characterized the hydration profile along a ~40 Å-long channel spanning ND1 E202, ND3 D66, and ND4L E34. This analysis revealed that only the segment between ND3 D66 and ND4L E34 exhibits poor hydration. Based on this observation, we applied MS-RMD simulations to explore PT along the channel: using two-dimensional umbrella sampling (2D US) in the poorly hydrated segment and one-dimensional umbrella sampling (1D US) elsewhere. The 2D US employed collective variables representing proton position and water wire connectivity to resolve the impact of hydration on PT energetics. The resulting free energy landscape shows that sufficient hydration is needed to PT in the ND3–ND4L segment. These findings highlight the critical role of local hydration in enabling efficient proton transfer across subunits within the membrane domain of respiratory complex I.

MODELING CLASSICAL AND QUANTUM CHARGE TRANSPORT IN LARGE SCALE WITH STOCHASTIC Λ-DYNAMICS

Chang Yun Son[1];
[1]Seoul National University, Chemistry, Seoul, South Korea

Charge transport plays a fundamental role in a wide range of biological processes, governing their efficiency and functionality. From energy storage and conversion to biological processes, charge carriers—ions, protons, and electrons—move through complex environments where their transport properties are dictated by molecular interactions, structural heterogeneity, and quantum effects. In this talk, I will present our efforts to model charge transport across diverse systems using advanced computational techniques, bridging classical and quantum descriptions to capture the underlying mechanisms. Particularly, I will discuss our ongoing development efforts of a novel hybrid Monte Carlo (MC)/molecular dynamics (MD) simulation approach to efficiently model proton transfer (PT) and electron transfer event in large scale condensed phase systems applicable to membrane fuel cell and biophysical channel proteins. Our primary focus is on mimicking proper PT dynamics at microsecond timescale, which then can be used for in-situ correlation analysis of protein / membrane dynamics associated with pH response or protonation state changes. For the PT model, we apply zero-energy-barrier bias at the desirable PT partner state, reproducing experimental proton diffusion in aqueous solutions with temperature transferability. We also develop similar hybrid MC/MD scheme to model electron transfer (ET) reactions in highly conjugated systems, allowing precise optimization of thermoelectric and photocatalytic properties of conjugated polymers. Through this, we offer novel insights for designing functional electronic materials and biological systems in highly charged environments through advanced simulation techniques.

THE MECHANISM OF PROTON TRANSLOCATION BY RESPIRATORY COMPLEX I

Leonid Sazanov[1];
[1]IST Austria, Klosterneuburg, Austria

Respiratory chain of mitochondria comprises five inner membrane-embedded protein complexes — complexes I to IV and ATP synthase. We study the structure and mechanism of these enzymes and their supercomplexes using cryo-electron microscopy and functional assays. Complex I couples NADH:ubiquinone oxidoreduction to the translocation of protons across the membrane by a mechanism which is still debated. Recently we presented high-resolution cryo-EM structures of ovine and E. coli complex I in different conditions, including catalytic turnover and proposed the detailed coupling mechanism. We have shown that, unexpectedly, out of three antiporter-like subunits, only the distal ND5 is capable of ejecting protons into intermembrane space (IMS). Dramatic conformational changes around the quinone (Q) binding cavity couple the redox reaction to proton translocation during "open" to "closed" state transitions of the enzyme. In the "open" state Q cavity is widely open, allowing quinone to come in/out. In the "closed" state the cavity is tightly enclosed around bound quinone so the protons needed to complete quinone reduction have to come from the central axis of the membrane domain. This initiates a "domino effect"-like cascade of electrostatic interactions within the antiporter-like subunits, ultimately resulting in the ejection of four protons per catalytic cycle from subunit ND5. Thus, the mechanism of complex I is an unexpected combination of conformational changes and electrostatic interactions. Now we show that all the features of open-to-closed state transitions during turnover are fully reproduced in further species of mitochondrial and bacterial complex I, confirming the universal applicability of our mechanism. We also assign the observed open-ready sub-state to a particular step in the catalytic cycle.

POSTER ABSTRACTS

Tuesday, August 5
POSTER SESSION
8:45 PM – 9:45 PM
Salon E

Posters are available for viewing only during the entire meeting. However, below are the formal presentation time. Presenting authors with odd-numbered poster boards should present from 7:45 PM – 8:45 PM and those with even-numbered poster boards should present from 8:45 PM – 9:45 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 7:45 PM – 8:45 PM | Even-Numbered Boards 8:45 PM – 9:45 PM

Agmon, Noam	**1-POS**	**Board 1**
Brousseau, Merissa	**2-POS**	**Board 2**
Chaves, Gustavo	**3-POS**	**Board 3**
Kato, Yoshitaka	**4-POS**	**Board 4**
Lee, Sangmin	**5-POS**	**Board 5**
Parola, Abraham	**6-POS**	**Board 6**
Sauter, Daniel	**7-POS**	**Board 7**
Sharma, Vivek	**8-POS**	**Board 8**
Sookhai, Koreena	**9-POS**	**Board 9**
Tsujimura, Masaki	**10-POS**	**Board 10**
Uddin, Md. Raihan	**11-POS**	**Board 11**
Wang, Shizhen	**12-POS**	**Board 12**
Zhang, Hezhou	**13-POS**	**Board 13**

Posters should be set up on the evening of Sunday, August 3 and removed by the event of Thursday, August 7. All uncollected posters will be discarded.

1-POS Board 1

MULTI-PROTON DYNAMICS NEAR MEMBRANE-WATER INTERFACE

Subhasish Mallick[1]; **Noam Agmon**[1];
[1]The Hebrew University of Jerusalem, Chemistry, Jerusalem, Israel

Protons are crucial for biological energy transduction between membrane proteins. While experiments suggest rapid proton motion over large distances at the membrane-water interface, computational studies employing a single excess proton found the proton immobilized near the lipid headgroup. To address this discrepancy, we conduct full quantum mechanical (QM) Density Functional Tight Binding (diagonal-DFTB3) simulations (up to 11 ps each), by incrementally adding protons to the simulation box, one, two and three (two trajectories for each case). We show that a single proton creates a water-wire on which it moves rapidly toward the nearest headgroup. There it is either repelled by a choline group or binds covalently to a phosphatic oxygen. The covalent bond formation, not previously observed, was confirmed by a short ab initio molecular dynamics (AIMD) simulation. With multiple protons, some are trapped by the lipid head groups, while the remaining diffuse laterally faster than in bulk water. Initially, the mobile proton(s) possesses excess energy, enabling it to jump to the center of the water slab. Then it relaxes into the third- and second-hydration shells. This is confirmed in the last simulation by adding water, showing that the proton prefers the second-layer over the mid-water position. Lateral diffusion rates further increase (becoming an order of magnitude faster than in neat water) as the proton stabilizes in the second hydration layer. These surprising results provide insights into proton dynamics near membranes, explaining the experimental observations by Pohl and collaborators, albeit on much smaller time- and distance-scales.

2-POS Board 2

BEYOND PEAK CURRENTS: SOLID-SUPPORTED MEMBRANE ELECTROPHYSIOLOGY AS A MEANS OF INVESTIGATING UNIVERSAL TRANSPORT MODELS

Merissa Brousseau[1]; Katherine A Henzler-Wildman[1,2];
[1]University of Wisconsin-Madison, Biochemistry, Madison, WI, USA
[2]National Magnetic Resonance Facility at Madison, Madison, WI, USA

The small multidrug resistance (SMR) family transporters contribute to antibiotic resistance through proton-coupled efflux of toxic compounds. Recent mechanistic studies of the model E. coli SMR transporter EmrE suggest this minimalistic efflux pump is regulated by a network of allosteric interactions that can be modulated by small molecules. The dramatic influence of substrates on the rate limiting steps in the transport cycle result in changes in the dominant mode of transport for different substrates and requires a more thorough understanding of the transport mechanism. Recent advances in solid-supported membrane electrophysiology (SSME) techniques have facilitated higher-throughput investigations of the mechanistic complexity of transporters in near physiological conditions. However, the influence of transport mechanisms on the experimental signal, contributions of pre-steady state and steady state processes, and applicability of controls in SSME experiments remains underappreciated. Here we provide a bottom-up approach for characterizing the interactions of novel substrates with loosely-coupled, promiscuous transporters. As an archetype for the SMR family for decades, the extensive biophysical characterizations that have been performed on EmrE, and more recently on functional mutants, provide a case study in how in-vitro biophysical studies can complement liposomal assays performed under a gradient to inform on mechanism. Our analyses of EmrE and mutants in the presence of varying absolute pH and substrate concentrations support the necessity of additional states in the universal transport model for EmrE while also demonstrating the importance of peak currents, signal decay, and integrated currents in mechanistic characterization of complex transport behavior.

3-POS Board 3

EXPLORING BIOPHYSICAL DIVERGENCES WITHIN THE VOLTAGE-GATED PROTON CHANNEL (H_V) FAMILY

Gustavo Chaves[1]; Artem Ayuyan[2]; Vladimir Cherny[2]; Arne Franzen[3]; Christophe Jardin[1]; Christian Derst[1]; Thomas E DeCoursey[2]; Boris Musset[1];
[1]Paracelsus Medical University, Center of Physiology, Pathophysiology and Biophysics, Nuremberg, Germany
[2]Rush University, Department of Physiology & Biophysics, Chicago, IL, USA
[3]Forschungszentrum Jülich, Institut für Biologische Informationsprozesse, Molekular- und Zellphysiologie (IBI-1), Jülich, Germany

Voltage-gated proton channels (HV) are typically encoded by a single gene in most species. Until recently, all 19 species examined by voltage-clamp studies follow this pattern. However, Aplysia californica represents the first known species to express multiple H_V channel genes. In heterologous expression systems, voltage-clamp recordings confirmed that three distinct Aplysia genes encode depolarization-activated, proton-selective channels, each exhibiting ΔpH-dependent gating—a hallmark of H_V channels that promotes proton extrusion by favoring opening under outward proton gradients. Two gene products, AcH_V1 and AcH_V2, displayed relatively typical behavior, though AcH_V2 activated at significantly more negative voltages, enabling inward proton current at symmetric pH. In contrast, AcH_V3 exhibited profoundly atypical features, including markedly reduced voltage dependence and persistent proton-selective leak currents at hyperpolarized potentials. These properties suggest a subset of channels remain constitutively open, raising the possibility that AcHV3 functions as a broken channel. Nonetheless, its expression in Aplysia tissues and the presence of C-terminal intracellular retention motifs suggest a physiological role, potentially in organellar membranes where leak conductance may be beneficial. Sequence analysis revealed that, unlike all previously characterized H_V channels, AcHV3 deviates from the conserved S4 helix signature sequence RxWRxxR, instead possessing LPWRxxR. This motif lacks the first arginine and replaces a conserved leucine with a helix-disrupting proline, likely contributing to AcH_V3 unusual gating behavior. This study explores the extent to which these two amino acid substitutions underlie the functional divergence of AcH_V3 from the more canonical AcH_V1 and AcH_V2 channels.

4-POS Board 4

PROTON TRANSPORT MECHANISM OF NEWLY DISCOVERED LIGHT-DRIVEN INWARD PROTON PUMP, ELTXER

Yoshitaka Kato[1]; Borja Aldeguer-Riquelme[2]; Taito Urui[3]; Yasuhisa Mizutani[3]; Oded Béjà[4,5]; Josefa Antón[2]; Keiichi Inoue[1];
[1]The University of Tokyo, The Institute for Solid State Physics, Kashiwa, Japan
[2]University of Alicante, Department of Physiology, Genetics and Microbiology, Alicante, Spain
[3]The University of Osaka, Department of Chemistry, Graduate School of Science, Suita, Japan
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Bacteriorhodopsin (BR), a light-driven outward proton pump, promotes ATP production by forming a proton gradient across the cell membrane. Since 2000, homologous genes have been discovered in many marine bacteria [1]. Furthermore, proton-pumping rhodopsins binding a light-harvesting antenna capable of utilizing broadband light have been reported [2, 3]. These findings suggest that proton-pumping rhodopsins contribute to energy production in many microorganisms. On the other hand, in 2016, we reported that xenorhodopsins (XeRs) function as light-driven inward proton pumps. Research on inward proton pumps has also contributed to a deeper understanding of the unidirectional proton transport mechanism of rhodopsin [4, 5].In this study, we discovered a novel XeR, EltXeR, which was detected in the extracellular virome of a saltern pond near Eilat, Israel, which may also include vesicles and other viral-like particles. Interestingly, it features a substitution at a highly conserved proline residue near the protonated retinal Schiff base, the photoreaction center, forming part of the proton transport pathway among in XeRs. To elucidate the molecular function and properties of EltXeR, we conducted functional and spectroscopic analyses. Our results demonstrated that EltXeR functions as an inward proton pump, similarly to other XeRs. Time-resolved spectroscopy revealed that EltXeR produces photo-intermediates similar to those of other XeRs. However, the proton inventory plot of the rate constants of photo-intermediate conversions significantly differed from that of previously reported XeR. Our results highlight the diversity of inward proton pumps and provide new insights into the molecular mechanisms of proton transport by XeRs. [1] Béjà et al., Science 289, 1902–1906, 2000 [2] Balashov et al., Science 309, 2061–2064, 2005[3] Chazan et al., Nature 615, 535–540, 2023[4] Inoue et al., Nat. Commun. 7, 13415, 2016 [5] Urui et al., Acc. Chem. Res. 57, 3292–3302, 2024

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5-POS Board 5

CLASSICAL MODEL FOR AQUEOUS PROTON TRANSPORT

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Proton transport is essential for the most important biological processes such as proton-coupled transport across membranes, pH regulation, etc. A large body of experimental and theoretical work supports that irreversible proton transport necessarily involves a complex and concerted rearrangement of the local hydrogen bond network, involving hydrogen bond cleavage to the acceptor water molecule and hydrogen bond formation to the donor molecule. Although the hydrogen bond network structures vary considerably at interfaces or even in confined systems, the aqueous systems studied have mostly focused on proton transport in homogeneous bulk media. Despite the importance of proton transport at/across biological membranes, relatively little attention has been paid to the influence of environmental factors on proton solvation and transport properties. In this work, we develop and test a novel hybrid Monte Carlo/MD method to describe proton transport in classical molecular simulations. The coupling parameter λ is used to alchemically transform hydronium and water molecules into water and hydronium molecules. At regular intervals during MD simulations, Monte Carlo moves involve a change in the value of λ to describe the proton transport event. We simulate an excess proton in bulk water, air/water interface, and confined water systems to investigate the protonation dynamics in different environments. Our simulation results provide important insights into the reorientation dynamics of interfacial water molecules on proton transport properties.

6-POS Board 6

HUMANIN INHIBITS NECROTIC CELL DEATH IN NEURONS

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Humanin, a 24-amino acid mitochondrial peptide, discovered in 2001 by its protective effect against amyloid beta toxicity in Alzheimer disease. We identified a novel function of the humanin-derivative AGA(C8R)-HNG17 namely, protection against cellular necrosis. Necrosis was until recently considered an unmoderated process. Recent findings suggest the opposite. We have found that AGA(C8R)-HNG17 confers protection against necrosis in neuronal cell lines PC-12 and NSC-34, where necrosis is induced in glucose-free medium by either chemohypoxia or by shift from apoptosis to necrosis. Our studies in traumatic brain injury (TBI) models in mise, where necrosis is the main mode of neuronal cell death have shown that AGA(C8R)-HNG17 has a protective effect. This was demonstrated by a decrease in neuronal severity score and by reduction in brain edema, measured by MRI. The peptide's Antinecrotic mechanism was unraveled through measurements of cellular ATP levels in PC-12 cells under necrotic conditions, showing that the peptide mitigates necrosis-associated decrease in ATP levels. Further, we demonstrated the peptide's direct enhancement of the activity of ATP synthase activity, isolated from rat liver mitochondria, suggesting that AGA(C8R)-HNG17 targets the mitochondria and regulates ATP levels. This was confirmed by time-resolved fluorescence imaging studies. AGA(C8R)-HNG17 has potential use for the development of drug therapies for necrosis related diseases e.g., TBI, stroke MI, and neurodegenerative diseases for which no efficient drug-based treatment is currently available. Humanin is naturally found in human and its levels decrease with age, suggesting that humanin can serve as treatment for age associated Alzheimer's disease.

7-POS Board 7

AUTOMATED PATCH CLAMP ASSAY FOR PROTON-ACTIVATED HOTOP1 CHANNEL

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Manual patch clamp, invented in the late 1970s, remains the gold standard for studying ion channels due to its capability to directly measure ion channel function. However, this technique is low-throughput, labor-intensive, and requires a high level of expertise. Automated patch clamp (APC) systems address these limitations by enabling higher throughput and standardized recordings. The QPatch Compact (QPC) is an 8-well APC platform, featuring microfluidic flow channels, which allows for rapid extracellular solution exchange—an essential requirement for studying proton-activated ion channels. OTOP1 (Otopetrin 1) is a proton-selective ion channel expressed in many cell types and tissues, including the inner ear and taste receptor cells, where it plays a critical role in otoconia formation and acid sensing. In taste cells, OTOP1 functions as a sour taste receptor and ammonium sensor, transducing changes in pH and ammonium into action potentials that signal to gustatory nerves. OTOP1 is gated by extracellular pH changes and exhibits high proton selectivity, allowing it to mediate responses to acidic environments. Understanding OTOP1's biophysical properties is crucial for elucidating its physiological roles and therapeutic potential. We have developed an assay to study human OTOP1 recombinantly expressed in HEK293 cells using the QPatch Compact platform. Our recordings demonstrate that OTOP1 currents can be robustly activated by extracellular protons in a dose-dependent manner, confirming the channel's pH-dependent activation. Additionally, application of extracellular ammonium evoked inward proton currents, consistent with OTOP1's role as a sensor for ammonium. Furthermore, we confirmed that Zn^{2+} inhibits human OTOP1, demonstrating that this assay can be used to evaluate novel channel inhibitors. This study serves as a proof of principle that OTOP1 can be reliably recorded using the QPatch Compact system. Our assay is well-suited for identifying novel modulators of OTOP1 that could facilitate future investigations into its physiological role and used for therapeutic applications.

8-POS Board 8

THERMODYNAMICS AND KINETICS OF PROTON TRANSFER REACTIONS CATALYZED BY RESPIRATORY COMPLEX I

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One of the major unresolved questions in the field of bioenergetics is the proton pumping mechanism of respiratory complex I [1], which plays a central role in redox homeostasis in mitochondria. Several high-resolution structures of respiratory complex I in different catalytic states are available [2], but atomistic understanding of the long-range coupling between quinone reduction and proton pumping remains unclear and highly debated. By splitting the complex problem into multiple smaller parts, we study the redox-coupled proton transfer reactions with classical and hybrid QM/MM molecular dynamics simulations including free energy calculations. We show that anionic quinol (QH-) formed after two-electron reduction can drive the proton pump of respiratory complex I [3]. Our multicale simulations show low barriers of proton transfer in the membrane domain of complex I, including across antiporter-like subunits [4,5]. The emerging picture on protonation dynamics challenges some of the currently held notions on complex I mechanism.[1] Djurabekova, Amina, et al. Biochemical Journal 481.7 (2024): 499-514.[2] Ivanov, Bozhidar S., et al. Nature Communications 15.1 (2024): 9340.[3] Zdorevskyi, Oleksii, et al. bioRxiv (2024): 2024-09.[4] Zdorevskyi, Oleksii, et al. Chemical Science 14.23 (2023): 6309-6318.[5] Simsive, Luka, et al. (in preparation).

9-POS Board 9

PROTON TRANSFER PATHWAYS OF COMPLEX I

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Complex I (NADH-ubiquinone oxidoreductase) is the first protein in the aerobic electron transport chain. It contributes to the proton gradient through coupled proton electron transport to make ATP further down the chain. For every two electrons transferred to ubiquinone, four protons are pumped across the membrane from an area of low proton concentration (N-side) to high concentration (P-side). These protons travel through three antiporter-like subunits and one E-channel comprised of multiple subunits around the interface of the peripheral and membrane arms of the protein. The exact proton transfer mechanism is still a topic of debate in the field. In Complex I of Thermus thermophilus there are six identified highly interconnected clusters whose residues make up the E-channel highlighting a path for proton transfer from the N-side to the P-side. For the proton to be pumped from the low to high concentration side of the membrane, the connectivity of the pathway needs to change to open and close the gates to the correct side of the membrane in each reaction intermediate. Three cryoEm structures of Escherichia coli Complex I prepared in different states[1] and three structures solved in different spatial conformations[2] were studied. Hydrogen bond networks were traced using C-Graphs and MCCE (Multi-Conformation Continuum Electrostatics) to identify the E-channel pathway in Complex I of E. coli. The results are compared with findings from Thermus thermophilus Complex I and Thermosynechococcus elongatus NDH-1. Funded by NSF MCB-2141824[1] Kravchuk, V., Petrova, O., Kampjut, D. et al. A universal coupling mechanism of respiratory complex I. Nature 609, 808–814 (2022). https://doi.org/10.1038/s41586-022-05199-7[2] Piotr Kolata, Rouslan G Efremov (2021) Structure of Escherichia coli respiratory complex I reconstituted into lipid nanodiscs reveals an uncoupled conformation eLife 10:e68710 https://doi.org/10.7554/eLife.68710.

10-POS Board 10

CONVERTING A CHLORIDE-PUMPING RHODOPSIN INTO A PROTON PUMP

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Bacteriorhodopsin (BR) and halorhodopsin (HR) are membrane proteins with a retinal chromophore covalently attached to a conserved lysine residue via a Schiff base. They function as light-driven proton and chloride pumps, respectively. BR can be converted into a chloride pump by replacing Asp85, the proton acceptor from the Schiff base, with the corresponding Thr residue found in HR. However, HR cannot be converted into a proton pump by the reverse Thr-to-Asp mutation. Even a 10-amino-acid mutant of HR from Natronomonas pharaonis (pHR_{10}), which includes residues conserved in BR-like proton pumps, does not transport protons. This study aims to convert HR into a proton pump to gain insights into the factors that are crucial for proton transport in the protein environment. The structure of pHR_{10} was modeled based on the crystal structure of the wild type using a quantum mechanical/molecular mechanical (QM/MM) approach, and its active site was compared with that of BR. This comparison revealed two key differences between BR and pHR_{10}: (i) the pK_a value of the proton acceptor from the Schiff base (Asp85 in BR), and (ii) the cavity size near the Schiff base. The active site in the QM/MM-optimized structure of a three-amino-acid mutant pHR_{10} exhibits characteristics similar to those of BR. Measurement of light-induced pH changes in the external solvent of E. coli demonstrated that this mutant pumps protons, indicating that HR was successfully converted into a proton pump. This successful conversion of a chloride pump into a proton pump provides a basis for understanding proton transport mediated by the protein environment.

11-POS Board 11

PROTON PUMPING MECHANISM OF PHOTOSYNTHETIC NDH-1 AND COMPLEX I

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Proton-coupled electron transfer is a fundamental process in bioenergetic enzymes, including Complex I and its cyanobacterial analog, NDH-1. Using molecular dynamics (MD) simulations and multi-conformation continuum electrostatics (MCCE) calculations, we investigated the proton transfer mechanism in the E-channel of Thermus thermophilus Complex I and compared it to the homologous pathway in Thermosynechococcus elongatus NDH-1. Our analysis identified two extended clusters of proton-loading sites (PLS) in Complex I: an N-side uptake cluster and a Central Cluster spanning >20 Å. Microstate analysis revealed long-range coupling of protonation states, with discrete net charge transitions in the Central Cluster that correspond to loaded and unloaded states. Similarly, in NDH-1, we identified conserved protonation dynamics within the E-channel, with structural variations altering the composition of the Central Cluster while maintaining its functional charge transitions. A key distinction emerged in the P-side exit cluster. While prior studies suggested a potential PLS in Complex I, our analysis of multiple MD snapshots found no significant protonation changes in this region. However, in NDH-1, we observed an active PLS at the P-side exit, indicating that proton loading/unloading at this site depends on conformational transitions that were not captured in the Complex I simulations. This finding underscores the challenge of identifying functionally relevant PLS, as key residues may exhibit rare transitions between loaded and unloaded states. Furthermore, despite overall conservation in the E-channel, we found specific substitutions in NDH-1—including a Ser and Asn replacing a Glu and His from Complex I—as well as conserved Lys residues exhibiting reduced proton affinity, which may contribute to distinct proton loading/unloading behavior. These results provide new insights into the evolutionary adaptation of proton transport pathways in bioenergetic enzymes, emphasizing both conserved and divergent mechanisms in Complex I and NDH-1.

12-POS Board 12

REGULATION OF PROTON TRANSPORT BY MEMBRANE STEROLS

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Voltage-gated proton (Hv) channels are standalone voltage sensors without separate pore-forming domains. They open upon depolarized voltages and conduct protons with almost perfect selectivity. Hv channels usually mediate proton efflux in most cell types, for their function is enhanced by acidic pH at the intracellular side while suppressed by extracellular acidic pH. In addition to voltage and pH, Hvs are also modulated by multiple ligands, such as zinc, polyunsaturated fatty arachidonic acid and ATP. Recently, with purified hHv1 channels reconstituted into liposomes, we showed that membrane cholesterol inhibits human Hv1 (hHv1) channels, but the underlying mechanisms are still far from clear. In the present work, we further determined the effects of multiple cholesterol analogs on the structure and function of hHv1 channels and identified key residues in hHv1 channels that are essential for cholesterol inhibition. Intriguingly, the cholesterol precursor, desmosterol, does not inhibit hHv1 channels. Instead, it strongly attenuates cholesterol inhibition, suggesting a potential role of cholesterol metabolism in regulating cellular pH homeostasis through hHv1 channels. Using the single-molecule fluorescence resonance energy transfer (smFRET) technique, we showed that while cholesterol stabilizes the hHv1 channel at resting conformations, desmosterol, however, enriches opening conformations, even in the presence of cholesterol. The smFRET results agree well with desmosterol's effect on attenuating cholesterol inhibition. Through extensive mutagenesis analyses, we also identified mutations in hHv1 channels that shift its cholesterol sensitivity by 4 folds, indicating a potential binding site at the transmembrane domain in the hHv1 channel. In summary, our studies established the important roles of membrane sterols in shaping cellular pH homeostasis by regulating the hHv1 channel and also shed light on its underlying molecular mechanisms.

13-POS **Board 13**

SYSTEMATIC DEVELOPMENT OF REACTIVE MOLECULAR DYNAMICS MODELS WITH MINIMUM BIAS BY EXPERIMENTAL DATA

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Reactive Molecular Dynamics (RMD) is a powerful alternative to quantum mechanics (QM)-based molecular dynamics (MD) methods. It can simulate key reactive features with a fraction of QM computational cost, allowing sufficient sampling across a much greater spatiotemporal scale. However, the parametrization of RMD force fields from purely bottom-up QM calculations has been reliant on trial-and-error tuning processes, which limits its rigor and practicality. In this work, we use a generalized variation of Experiment Directed Simulation (EDS) based on the maximum entropy principle to find a unique and minimum bias to systematically develop RMD force fields based on either simple experimental observables. We present glutamate and histidine models developed under this scheme, biased only by their experimental pK_a in water. With the physical and minimum bias, the scheme inherits the characteristic transferability from the diabatic matching method and the resulting models can successfully capture the amino acid residues' pK_a shifts in staphylococcal nuclease (SNase).