BIOGRAPHICAL SKETCH

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NAME: Accardi, Alessio

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Universita degli Studi di Roma "La Sapienza"	Laurea	03/1997	Physics
Istituto di Cibernetica e Biofisica-CNR (Genova)	Ph.D.	10/2001	Physics
Istituto di Cibernetica e Biofisica-CNR (Genova)		02/2002	Biophysics
Howard Hughes Medical Institute, Brandeis University		03/2007	Biochemistry

A. Personal Statement

Since establishing my research program, I employed my broad training protein biophysics, electrophysiology, and structural biology to study the mechanisms of ion channels, active transporters and phospholipid scramblases. My lab uses a combination of biophysical, biochemical, and structural approaches that is enhanced by highly productive collaborations. Over the years my group has made key contributions to the study of three biomedically important families of membrane proteins: the CLC chloride channels and exchangers, the TMEM16 Ca^{2+} activated chloride channels and lipid scramblases, and the Xkr family of apoptotic scramblases. These proteins play fundamental roles in physiology, where they control processes ranging from neuromuscular excitability and epithelial salt reabsorption to blood coagulation, membrane fusion and repair. Mutations disrupting the function of these proteins cause diseases of muscle, brain, bone and kidneys. In our work we delineated the key structural transitions underlying their activity and defined the molecular mechanisms of substrate specificity, transport and gating. In the next phase of my research, we aim to understand their regulation, how their activity is altered in disease, and the evolutionary bases of the diverse functional properties that exist within each of these families. These studies will lay the foundation to understanding mechanisms that may open new avenues for therapies. In the last 5-7 years my group has gained very significant expertise in cryoEM, which enabled us to make important contributions to our understanding of CLCs, Xkrs, and TMEM16 proteins.

Current support

- R01 AI178180-01 "Structural basis of apoptotic scrambling", Accardi A (PI), 02/01/2024–01/31/2028, $418,719
- R35 GM152012-01 "Structure and function of chloride channels, transporters and scramblases", Accardi A (PI), 01/2024-12/2028, $292,917

B. Positions and Honors

Positions:

2022-present Professor, Department of Anesthesiology, Weill Cornell Medicine, New York, NY
2016-2022 Associate Professor, Department of Anesthesiology, Weill Cornell Medicine, New York, NY
2010-2016 Assistant Professor, Department of Anesthesiology, Weill Cornell Medicine, New York, NY

2007 – 2010 Assistant Professor, Department of Molecular Physiology & Biophysics, University of Iowa, Iowa City, IA
2002 –2007 Postdoctoral Fellow with Dr. Christopher Miller, HHMI/Brandeis University
2001 – 2002 Postdoctoral Fellow with Dr. Michael Pusch, Istituto di Biofisica-CNR, Genova, Italy

Other Experience and Professional Memberships:
2022 Chair, Gordon Research Conference on "Ligand Recognition and Molecular Gating"
2019-Present Councilor, Society for General Physiologists
2018-Present Editorial board member, *Journal of General Physiology*
2018 Vice-Chair, Gordon Research Conference on "Ligand Recognition and Molecular Gating"
2017-Present Co-organizer, Erice school on Ion channel and Transporter Biophysics
2015-2016 Chair, Membrane Biophysics Subgroup of the Biophysical Society
2004-2007 Post-doctoral Councilor Society for General Physiologists
2004-Present Member, Society for General Physiologists
2000-Present Member, Biophysical Society

Honors:
2002 "Antonio Borsellino" PhD thesis prize from the Italian Society for Pure and Applied Biophysics
2019 Ad-hoc reviewer BCMB study section
2019 Ad-hoc reviewer ZNS1 study section
2020 Ad-hoc reviewer BRAIN study section
2023 Ad-hoc reviewer ZNS1 study section
2024 Ad-hoc reviewer ZRG1 study section

C. Contributions to Science
A complete list of my publications can be found at:
https://www.ncbi.nlm.nih.gov/myncbi/alessio.accardi.1/bibliography/public/

1. Basis of ion binding and selectivity in the CLCs (related to R35 GM152012)
Using a combination of ITC, electrophysiological recordings and atomic mutagenesis we showed that the basis of substrate binding and discrimination are evolutionarily conserved in CLC channels and transporters and identified backbone amides as the key determinants for the unique ability of these channels to select for Cl^- ions.
1. Leisle, L., Lam, K., Dehghani-Ghahnaviyeh, S., Fortea, E., Galpin, J., Ahern, C.A., Tajkhorshid, E., **Accardi, A.** "Backbone amides are key determinants of Cl^- selectivity in CLC ion channels", *Nat Commun, 2022 Dec 6;13(1):7508. doi: 10.1038/s41467-022-35279-1,* PMID: 36473856 PMCID: PMC9726985
2. Picollo A, Malvezzi M, **Accardi A.** "Proton block of the CLC-5 Cl-/H+ exchanger." *J Gen Physiol.* 2010 Jun;135(6):653-9. doi: 10.1085/jgp.201010428. PMID: 20513761 PMCID: PMC2888053
3. Picollo A, Xu Y, Johner N, Bernèche S, **Accardi A.** "Synergistic substrate binding determines the stoichiometry of transport of a prokaryotic H^+/Cl^- exchanger." *Nat Struct Mol Biol.,* 2012, 8;19(5):525-31 doi: 10.1038/nsmb.2277, PMID: 22484316; PMCID: PMC3348462
4. Picollo A., Malvezzi M., Houtman J., and **Accardi A.** "Basis of substrate binding and conservation of selectivity in the CLC family of channels and transporters" *Nat Struct Mol Biol.* 2009 Dec;16(12):1294-301; doi: 10.1038/nsmb.1704. Epub 2009 Nov 8. PMID: 19898476; PMCID: PMC2920496.

2. Gating mechanism of CLC exchangers and channels (related to R35 GM152012)
We used a combination of biochemical crosslinking, functional assays, X-ray crystallography and MD simulations to show that the gating in the CLC transporters involves rearrangements of helices that are distal to the permeation pathway and that these movements are coupled to the formation of an aromatic pathway that enables the movement of a protonated glutamate side chain in and out of the Cl^- permeation pathway without competing with the permeating Cl^- ions.
1. Fortea, E., Lee, S., Chadda, R., Argyros, Y., Sandal, P., Mahoney-Kruszka, R., Ciftci,H.D., Falzone, M.E., Huysmans, G., Robertson, J.L., Boudker, O.*, **Accardi, A***. "Structural basis of pH-dependent activation in a CLC transporter" *Nat Struct Mol Biol* 2024; *doi: doi.org/10.1038/s41594-023-01210-5*
*Co-corresponding authors
2. Leisle, L., Xu, Y., Fortea, E., Galpin, J., Vien, M., Ahern, C.A., **Accardi, A*** and S. Bernèche * "Divergent

Cl^- and H^+ pathways underlie transport coupling and gating in CLC exchangers and channels", *eLife*, 2020; 9:e51224 DOI: 10.7554/eLife.51224 **Co-corresponding authors*

3. Vien, M., Basilio, D., Leisle, L. and **Accardi A.** "Probing the conformation of a conserved glutamic acid within the Cl^- pathway of a CLC H^+/Cl^- exchanger" *JGP, 2017 Apr 3;149(4):523-529. doi: 10.1085/jgp.201611682 PMID: 28246117 PMCID: PMC5379918*
4. Basilio D., Noack K., Picollo A., **Accardi A.,** "Conformational changes during the CLC transport cycle", *Nat. Struc. Mol. Bio., 2014, 21(5):456-63. doi: 10.1038/nsmb.2814, PMCID: PMC4040230*

3. Molecular mechanisms of phospholipid scrambling by TMEM16 proteins (related to R35 GM152012)
Members of the TMEM16 family of Ca^{2+}-activated Cl^- channels had been proposed to be involved in Ca^{2+}-dependent scrambling, but it remained controversial whether these proteins were scramblases or channels regulating scrambling. We were the first to demonstrate that the purified and reconstituted fungal afTMEM16 homologue has intrinsic scramblase activity. We used cryo-electron microscopy to determine the structures of afTMEM16 in membrane nanodiscs in Ca^{2+}-free and Ca^{2+}-bound conformations to show that Ca^{2+}-binding induces global rearrangements of the scramblase. Remarkably, we found that scrambling by TMEM16s does not require opening of a hydrophilic groove that serves as a conduit for lipid translocation, as previously proposed. This led us to propose a novel paradigm for scrambling, where membrane thinning is the key energetic determinant of lipid scrambling.

1. Feng, Z., Alvarenga, O., and **Accardi A.** "Structural basis of closed groove scrambling by a TMEM16 protein", *Nat Struct Mol Biol*. 2024 Apr 29. doi: 10.1038/s41594-024-01284-9. Epub ahead of print. *PMID: 38684930*.
2. Falzone M.E., Feng Z., Alvarenga O.E., Pan Y., Lee B., Cheng X., Fortea E., Scheuring S. and **Accardi A.** "TMEM16 scramblases thin the membrane to enable lipid scrambling" *Nat Commun.* 2022 May 11;13(1):2604. doi: 10.1038/s41467-022-30300-z. *PMID: 35562175; PMCID: PMC9095706.*
3. Falzone M.E., Rheinberger J., Lee, B.C., Peyear T., Sasset L., Raczkowski A.M., Eng E.T., Di Lorenzo A., Andersen O.S., Nimigean C.M., **Accardi A.**, "Structural basis of Ca^{2+}-dependent activation and lipid transport by a TMEM16 scramblase", *eLife*, 2019;8:e43229, doi: 10.7554/eLife.43229 *PMID: 30648972 PMCID: PMC6355197*
4. Malvezzi, M., Andra, K.K., Pandey, K., Lee, B.C., Brown, A., Iqbal, R., Menon, A.K., **Accardi, A.** "Out of the groove transport of lipids by TMEM16 and GPCR scramblases", *Proc Natl Acad Sci USA,* 2018, June 20, doi: 10.1073/pnas.1806721115, *PMID: 29925604 PMCID: PMC6065010*

4. Ca^{2+} dependent ion transport by TMEM16 channels and scramblases (related to GM152012)
Using a combination of protein biochemistry and functional assays we showed that whereas some TMEM16 proteins, such as the human TMEM16A channel, are bona fide Cl^- channels, most family members, such as the human TMEM16K and the fungal nhTMEM16 and afTMEM16, have dual activity as non-selective ion channels and lipid scramblases. To understand the basis for this functional diversity we used cryoEM, functional assays and MD simulations to functionally stabilize the nhTMEM16 scramblase in a channel-like conformation, showing that the dual activity of the TMEM16 scramblases arises from the unique ability of their permeation pathway to adopt distinct conformations that enable the permeation of substrates as diverse as phospholipids and ions.

1. Khelashvili G*, Falzone ME, Cheng X, Lee BC, **Accardi A***, Weinstein H.* Dynamic modulation of the lipid translocation groove generates a conductive ion channel in Ca2+-bound nhTMEM16. *Nat Commun.* 2019 Oct 31;10(1):4972. doi: 10.1038/s41467-019-12865-4. *PMCID: PMC6823365*. **co-corresponding author*
2. Lee, B.C., Menon, A.K., and **Accardi A.** "The nhTMEM16 scramblase is also a non-selective ion channel" *Biophys J.,* 2016 Nov 1;111(9):1919-1924. doi: 10.1016/j.bpj.2016.09.032; PMCID: PMC5103024
3. Malvezzi A., Chalat M.N., Janjusevic R., Picollo A., Terashima H., Menon A.K., **Accardi A.** "Ca^{2+}-dependent phospholipid scrambling by a reconstituted TMEM16 ion channel" *Nature Commun*, 2013, 4:2367. doi: 10.1038/ncomms3367. *PMID: 23996062, PMCID: PMC3970400*
4. Terashima H., Picollo A., **Accardi A.**, "Purified TMEM16A is sufficient to form Ca^{2+} activated Cl^- channels", *Proc Natl Acad Sci USA,* 2013 Nov 26;110(48):19354-9. *PMCID: PMC3547389*

5. Structural basis of apoptotic scrambling by Xkr proteins (related to AI178180)
Members of the X-Kell related (Xkr) protein family mediate phosphatidylserine externalization on the surface of dying cells, a key signal for their recognition and clearance by macrophages. Defective Xkr-mediated scrambling impairs clearance, leading to inflammation. It was proposed that activation of the Xkr4 apoptotic scramblase

requires caspase cleavage, followed by dimerization and ligand binding. Using biochemical approaches, functional assays, cryoEM and MD simulations we showed that purified monomeric, full-length human Xkr4 (hXkr4) scrambles lipids. CryoEM imaging shows that hXkr4 adopts a novel conformation, where three conserved acidic residues create an electronegative surface embedded in the membrane. Using molecular dynamics simulations we showed this conformation induces membrane thinning, which could promote scrambling. Thinning is ablated or reduced in conditions where scrambling is abolished or reduced. Our work provided insights into the molecular mechanisms of hXkr4 scrambling and suggests the ability to thin membranes might be a general property of active scramblases.

1. Chakraborty, S., Feng, Z., Lee, S., Alvarenga, O.E., Panda, A., Bruni R., Khelashvili G., Gupta K., **Accardi A.** "Structure and function of the human apoptotic scramblase Xkr4", *bioRxiv* 2024.08.07.607004; doi: https://doi.org/10.1101/2024.08.07.607004

BIOGRAPHICAL SKETCH

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NAME: Feng, Zhang

eRA COMMONS USER NAME (credential, e.g., agency login): FENGZHANG01

POSITION TITLE: Postdoctoral Associate

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Northwest Agriculture and Forestry University	BS	06/2013	Biology
University of Science and Technology of China	PHD	06/2019	Structural Biology

A. Personal Statement

I am currently a postdoctoral researcher in Alessio Accardi's laboratory at Weill Cornell Medicine. Prior to joining this lab, I received my PhD in Biochemistry and Molecular Biology, focusing on the structure and function of membrane proteins. My research initially used X-ray crystallography, but I transitioned to cryo-EM in 2017 for projects investigating the structure and function of human and bacterial ABC transporters. In Accardi Lab, I am interested in the structure and function of a large family of membrane proteins called phospholipid scramblases. Lipid scramblases can rapidly move lipids back and forth between two leaflets of the cellular membranes and thus disrupt the membrane asymmetry. In particular, I am fascinated by the molecular mechanisms underlying how lipids interact with the protein to achieve scrambling. Using cryo-EM and nanodisc assembly approaches, I have determined the high-resolution structures of multiple fungal and mammalian TMEM16 scramblases. These structures enabled unambiguously model building for the lipids interacting with a hydrophilic gating groove at different states. Structure-based mutagenesis revealed that the lipid-protein interactions at the extracellular side are important for lipid scrambling with a closed groove, while specific interactions are not necessary for scrambling via an open groove. In addition, in collaboration with colleagues, we proposed that TMEM16 scramblases thin the membrane to enable scrambling. This hypothesis is based on the observations that lipid scrambling is strongly depends on membrane thickness and that an open groove is helpful for scrambling but not strictly required. In a recent publication, we elucidated the structural basis for lipid scrambling by a closed groove. We found that lipids interacting with the closed groove are rearranged to adopt tilted poses, resulting in a moderate level of membrane thinning. Moving forward, I am interested in imaging TMEM16 scramblases in liposome vesicles which mimic the physiological properties of a cell membrane more closely. This will allow me to gain a deeper understanding of how the membrane is deformed for lipid scrambling.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020.01 – present Postdoctoral Associate, Department of Anesthesiology, Weill Cornell Medicine, New York, NY
2020.01 – present Member, American Biophysical Society
2020.01 – present Member, New York Academy of Sciences
2023.01 – present Member, Society of General Physiologists

Honors

2024 Best Post Award, New York Aera Cryo-EM Meeting, New York, NY

C. Contributions to Science

1. My recent publications focused on the molecular mechanisms of lipid scrambling by lipid scramblases of the TMEM16 family. These publications found that opening of a hydrophilic groove which was thought to be essential for TMEM16 scramblase activity, is not necessary for lipid scrambling. Instead, we proposed that TMEM16 scramblases thin the membrane to enable lipid scrambling, which are supported by structural and functional evidence that the scrambling activity of TMEM16 depends on membrane thickness. These publications found that a closed groove is capable to scramble lipids and further we provided the structural basis for closed groove scrambling. Additionally, we highlighted the importance of careful interpretation when using cryo-EM data for mechanistic studies. We showed that both the structural conformation and the distribution of different conformations of a TMEM16 scramblase can be influenced by cryo-EM imaging conditions. This observation serves as a valuable caution not only for the field of TMEM16 scramblase but the entire biophysics community.

a. **Feng, Z**., Alvarenga, O. E., Accardi, A*. (2024). Structural basis of closed groove scrambling by a TMEM16 protein. *Nature Structural & Molecular Biology*, online, DOI: s41594-024-01284-9.
b. Falzone, M. E.[#], **Feng, Z**.[#], Alvarenga, O. E., Pan, Y., Lee, B., Cheng, X., Fortea, E., Scheuring, S., Accardi, A*. (2022). TMEM16 scramblases thin the membrane to enable lipid scrambling. *Nature Communications*, 13(1), 2604.
c. **Feng, Z**., Zanni, D. E., Alvarenga, O. E., Chakraborty, S., Rychlik, N., Accardi, A.*. (2024). In or out of the groove? Mechanisms of lipid scrambling by TMEM16 proteins. *Cell Calcium*, 121, 102896.

2. In addition to my work on TMEM16 scramblase, I have also investigated the structure and function of other membrane proteins including human vitamin B12 transporter ABCD4 and bacterial cell division membrane protein MapZ. Human ABCD4 is located in lysosome and it is responsible for translocation of vitamin B12 from the lysosome to the cytosol. Clinical investigations showed that mutations in ABCD4 have been linked to vitamin B12 deficiency in patients. My colleagues and I successfully determined the cryo-EM structure of human ABCD4 in its ATP-bound open state. By mapping disease associated mutations onto the structure, we were able to elucidate how these mutations might disrupt the vitamin B12 transport cycle. In another project focused on the bacterial cell division membrane protein MapZ. Utilizing NMR and a newly developed FtsZ polymerization assay, we identified key residues responsible for the interaction between MapZ and FtsZ. This research revealed that MapZ plays a regulatory role in the assembly of FtsZ filaments, which are essential for bacterial cell division.

a. Xu, D.[#], **Feng, Z**.[#], Hou, W.T., Jiang, Y.L., Wang, L., Sun, L.F.*, Zhou, C.Z.*, Chen, Y.*. (2019). Cryo-EM structure of human lysosomal cobalamin exporter ABCD4. *Cell Research*, 29, 1039-1041.
b. **Feng, Z.**, Zhang, J.H., Xu, D., Jiang, Y.L., Zhou, C.Z., Chen, Y.*. (2019). Multi-functional regulator MapZ controls both positioning and timing of FtsZ polymerization. *Biochemical Journal*, 476, 1433-1444.

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NAME: Lee, Sangyun

eRA COMMONS USER NAME (credential, e.g., agency login): sylee78

POSITION TITLE: Postdoctoral Researcher

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Seoul National University, Seoul, Republic of Korea	BS	07/2007	Chemistry (Biology as minor)
Seoul National University, Seoul, Republic of Korea	MS	07/2010	Physical Chemistry
University of Chicago, Chicago, IL	PhD	07/2016	Chemistry
IBM Research, Yorktown Heights, NY	Postdoctoral	07/2019	Computational Biology
Weill Cornell Medicine College, New York, NY	Postdoctoral	present	Structural Biology

A. Personal Statement

I am currently a post-doctorate researcher in Dr. Alessio Accardi lab. My research is focused on understanding the molecular mechanism of transport of small substrates through membrane proteins such as chloride channels and transporter, and lipid scramblases. Except some minor cases in leak ion channels, the substrate pathway is gated by structural rearrangements of a protein, consisting of a series of individual events occurring at various spatial and temporal scales, ranging from a single sidechain to a secondary or higher order structures in many residues, with time scale from nanoseconds to seconds. My general research interest lies on understanding the thermodynamics and kinetics of the individual rearrangements, and identify the essential step in the overall process, which eventually provides a fundamental insight of *how this protein works*. I have a broad experience in studying the structure-function relationship in a protein and the protein-substrate interaction in other protein systems, with extensive training in both computational and experimental labs, allowing me to applying various methods from both fields in one project, each of which have strengths in measuring the events in different scales. Currently I investigate conformational rearrangements underlying slow and simultaneous closure of two separate pores at individual protomers in CLC channels and transporters, which is known to be linked to several genetic diseases.

B. Positions, Scientific Appointments, and Honors

Positions:

2016 – 2019 Postdoctoral Fellow with Dr. Ruhong Zhou, IBM Research, Yorktown Heights, NY.
2020 – Postdoctoral Fellow with Dr. Alessio Accardi, Weill Cornell Medicine College, New York, NY.

Other Experience and Professional Memberships:

2017-Present Member, Biophysical Society
2021-Present Member, Society for General Physiologists

Honors:

C. Contributions to Science

A complete list of my publications can be found at:
https://scholar.google.com/citations?user=Uz7FM9kAAAAJ&hl=en

1. Molecular mechanism of CLC-ec1 transporter

I investigated the Cl^-/H^+ exchange mechanism of a bacterial CLC transporter, CLC-ec1. The protein is a homo-dimer with two independent pores at each protomer. Two glutamate residues, E148 and E203, are known to be essential for H^+ transport. I developed a new model to describe the proto- and deprotonation reactions of the glutamates in molecular dynamics simulation at high efficiency. The free energy calculation and kinetic modelling suggested the essential steps for coupling Cl^- and H^+ transports are rotation and deprotonation of E148, which are driven by Cl^- binding at two different positions in the pathway. The cryo-electron and fluorescence resonance energy transfer microscopy showed CLC-ec1 undergoes a slow, pH dependent rearrangement, opening the intracellular H^+ pathway in activating conditions, which could be related to the common gating mechanism in eukaryotic CLCs.

1. **Lee, S.**, Liang, R., Voth, G. A. & Swanson, J. M. Computationally Efficient Multiscale Reactive Molecular Dynamics to Describe Amino Acid Deprotonation in Proteins. *J. Chem. Theory Comput.* 12, 879-891 (2016).
2. **Lee, S.**, Mayes, H. B., Swanson, J. M. J. & Voth, G. A. The Origin of Coupled Chloride and Proton Transport in a Cl^-/H^+ Antiporter. *J. Am. Chem. Soc.* 138, 14923-14930 (2016).
3. Mayes, H. B., **Lee, S.**, White, A. D., Voth, G. A. & Swanson, J. M. J. Multiscale Kinetic Modeling Reveals an Ensemble of Cl^-/H^+ Exchange Pathways in ClC-ec1 Antiporter. *J. Am. Chem. Soc.* 140, 1793-1804 (2018).
4. Fortea, E., **Lee, S.**, et al. Structural basis of pH-dependent activation in a CLC transporter. *Nat. Struct. Mol. Biol.* 31, 644-656 (2024).

2. Prediction of binding affinity of a small peptide

I designed a new protocol of calculation of absolute binding free energy at high efficiency, when a short antigen peptide (epitope) binds to the major histocompatibility complex (MHC) protein or T cell receptor (TCR). I identified key residue interactions at the protein-epitope interface, determining the ligand binding affinity of native epitope sequence, then suggested some mutations in the sequence which have higher predicted binding affinity than native sequence. Our prediction was later confirmed by flow cytometry and competitive binding assay done by experimental collaborators.

1. R. Ahmed, Z. Omidian, A. Giwa, B. Cornwell, N. Majety, D. R. Bell, **Lee, S.**, *et al.* A Public BCR Present in a Unique Dual-Receptor-Expressing Lymphocyte from Type 1 Diabetes Patients Encodes a Potent T Cell Autoantigen. *Cell* **177**, 1583-1599.e1516 (2019).
2. C. A. Bessell, A. Isser, J. J. Havel, **S. Lee**, *et al.* Commensal bacteria stimulate antitumor responses via T cell cross-reactivity. *JCI Insight* **5** (2020).
3. Song, Y., **Lee, S.**, Bell, D., Goudey, B. & Zhou, R. Binding Affinity Calculations of Gluten Peptides to HLA Risk Modifiers: DQ2.5 versus DQ7.5. *J. Phys. Chem. B* **126**, 5151-5160 (2022).
4. Y. Song, D. R. Bell, R. Ahmed, K. C. Chan, **S. Lee**, *et al.* A mutagenesis study of autoantigen optimization for potential T1D vaccine design. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2214430120 (2023).

3. Ligand binding induced conformational change of a protein

I predicted the binding sites of graphene and gold nanoparticle on the surface 20S proteasome and performed free energy calculation to show that binding of two ligands facilitates or inhibits opening of the substrate pathway to the active site, depending on the charge group of the ligand. This work provided an explanation for the experiment data from collaborators, showing these ligands modulate the proteasome enzyme activity. In another work, I studied Mg^{2+} binding increases the stability of RNA by calculating mean rupture force with a biased force in the simulation.

1. Ma, X., **Lee, S.** *et al.* Inhibition of the proteasome activity by graphene oxide contributes to its cytotoxicity. *Nanotoxicology*, 1-16 (2018).
2. Ma, X., **Lee, S.** *et al.* Proteasome activity regulated by charged gold nanoclusters: Implications for neurodegenerative diseases. *Nano Today* **35**, 100933 (2020).
3. Zou, A., **Lee, S.**, Li, J. & Zhou, R. Retained Stability of the RNA Structure in DNA Packaging Motor with a Single Mg^{2+} Ion Bound at the Double Mg-Clamp Structure. *J. Phys. Chem. B* **124**, 701-707 (2020).

BIOGRAPHICAL SKETCH

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NAME: Di Zanni, Eleonora

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

POSITION TITLE: Postdoctoral Fellow

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Faculty of Medical and Pharmaceutical Sciences, University of Genova	B.Sc.	2006	Biology & Biotechnology
Faculty of Medical and Pharmaceutical Sciences, University of Genova	M.Sc.	2008	Molecular biology & Biochemistry
Laboratory of Molecular Genetics, Gaslini Institute, Genova	PhD	2012	Genetics & Immunology
Institute of Biophysics (IBF), National Research Council (CNR), Genova	Postdoctoral training	2015	Biophysics
Weill Cornell Medical College, New York, NY	Postdoctoral training	present	Biophysics & Structural Biology

A. Personal Statement

I am a Postdoctoral Fellow in the Department of Anesthesiology at Weill Cornell Medical College. I have a broad research background in genetics and biophysics, with training and expertise in cell-based functional assay, cryo-electron microscopy, confocal microscopy, and electrophysiology. Over the years, my main research interest has been to comprehend molecular mechanisms underlying physiological processes in both health and disease. For this reason, during my early training, I focused on several rare diseases—including an autoinflammatory disorder, a neurodegenerative condition, a trinucleotide expansion disorder, and neuroblastoma—to develop tools for functionally characterizing transcription factors and other proteins involved in the pathophysiology of these conditions. Later in my career, I focused on investigating genetic diseases related to the dysfunction of membrane proteins, such as ion channels and scramblases. I had the opportunity to work on two major families of proteins: CLCs and TMEM16s. To this end, I moved to the Biophysics Institute and later I joined Alessio Accardi's lab at Weill Cornell Medical College to learn the latest techniques for studying and characterizing the activity of membrane proteins both *in vitro* and *in vivo*.

Investigating the role of the protein ANO5/TMEM16E in the pathogenesis of a rare inherited skeletal disease revealed that the disease-causing mutations are gain-of-function mutations, resulting in abnormal lipid transport. Our aim now is to understand the structural determinants leading to a dysfunctional protein activity in the presence of a disease-mutation. To reveal the distinct functional properties of these membrane proteins, the lipid-native environment is essential to capture their physiological functions and regulations. To this aim, our goal is to study the proteins in native vesicles where the interaction with other proteins and lipids are conserved. The combination of diverse approaches and expertise, ranging from *in vitro* studies to complex system in cells, are required to elucidate the role of these proteins in the human physiology and to develop new molecular target for pharmacological approach.

B. Positions, Scientific Appointments, and Honors

Position

2020-present Postdoctoral Fellow, Weill Cornell Medical College, New York, NY
2018-2020 Postdoctoral Fellow, Institute of Biophysics, National Research Council (CNR), Genova
2017-2018 Postdoctoral Fellow, Laboratory of Molecular Genetics, Gaslini Institute, Genova
2015-2017 Postdoctoral Fellow, Institute of Biophysics, National Research Council (CNR), Genova
2012-2015 Postdoctoral Fellow, Laboratory of Molecular Genetics, Gaslini Institute, Genova
2009-2012 PhD Fellow, Laboratory of Molecular Genetics, Gaslini Institute, Genova
2008-2009 Research Assistant, Laboratory of Molecular Genetics, Gaslini Institute, Genova, Italy

Scientific Appointments

2020 – present *Member*, The New York Academy of Sciences
2018 – present *Member*, Biophysical Society

Honors

2019 Selected speaker at 63rd Biophysical Society Annual Meeting in Baltimore, Maryland
2017 Selected Speaker at 13th A.I.S.I.C.C. Meeting in Florence, Italy
2012 Selected Speaker at 4th International Conference on Primary Central Hypoventilation in Warsaw, Poland
2011 Selected Speaker at 5th Meeting "Molecular Mechanisms of Neurodegeneration" in Milan,Italy

C. Contributions to Science

Molecular mechanisms of phospholipid scramblases of the TMEM16/Anoctamin family
Members of the TMEM16/Ano protein family in animals are functionally split into two categories: TMEM16A/Ano1 and TMEM16B/Ano2 function as Calcium-activated chloride channels, while few others, among which TMEM16F/Ano6 and TMEM16E/Ano5, have a dual function as non-selective channel and Calcium-dependent phospholipid scramblases mediating the transfer of phospholipids, like phosphatidylserine, between the leaflets of the membrane bilayer, causing the collapse of membrane asymmetry. This event triggers a variety of cellular signaling pathways which range from blood coagulation, apoptosis, bone mineralization, membrane fusion and repair. The TMEM16E member primarily localizes to the ER and is highly expressed in bone and skeletal muscle, where it plays a vital role in muscle repair and bone mineralization. Whereas loss-of-function mutations in human TMEM16E are associated with recessive forms of limb-girdle muscular dystrophy-2L and distal Miyoshi muscular dystrophy-3, gain of function mutations cause the autosomal dominant bone disease gnathodiaphyseal dysplasia (GDD). We focus our work to investigate molecular mechanisms underlying the pathogenesis of these genetic disorders. Firstly, we worked on a cell-based model, and we revealed that TMEM16E has both ion transport and lipid scrambling activity. Although a scramblase function had already been suggested by the generation of a chimeric TMEM16A-TMEM16E protein, our work proved for the first-time scramblase activity of the full length TMEM16E protein. Moreover, we investigated the role of TMEM16E mutations associated with GDD and demonstrated a gain-of-function phenotype for almost all the disease variants.
In the last few years, we worked on the purified and reconstituted human TMEM16E protein to correlate functional activity to structural changes in the protein. We obtain the first CryoEM structure of the TMEM16E and we show that it adopts the canonical dimeric butterfly fold of TMEM16 proteins, with each protomer forming an independent lipid permeation groove. Surprisingly, no structural rearrangements occur upon Calcium binding. To gain insights into the pathogenesis of GDD, we are now investigating the recently reported mutation Arg582Ile, in the extracellular TM5-TM6 loop with structural and functional experiments. The TMEM16 family is highly conserved, so studying TMEM16E will offer valuable insights into the mechanisms shared by other members of this family.

a. **Di Zanni E**, Gradogna A, Scholz-Starke J, Boccaccio A. Gain of function of TMEM16E/ANO5 scrambling activity caused by a mutation associated with gnathodiaphyseal dysplasia. Cell Mol Life Sci. 2017; 71: 4275-4283.

b. Boccaccio A, **Di Zanni E**, Gradogna A, Scholz-Starke J. Lifting the veils on TMEM16E function. Channels (Austin). 2019 Dec;13: 33-35.
c. **Di Zanni E**, Gradogna A, Picco C, Scholz-Starke J, Boccaccio A. TMEM16E/ANO5 mutations related to bone dysplasia or muscular dystrophy cause opposite effects on lipid scrambling. Hum Mutat. 2020 Jun;41: 1157-1170.
d. Boccaccio A, Picco C, **Di Zanni E**, Scholz-Starke J. Phospholipid scrambling by a TMEM16 homolog of Arabidopsis thaliana. FEBS J. 2022 May;289: 2578-2592
e. Feng Z, **Di Zanni E**, Alvarenga O, Chakraborty S, Rychlik N, Accardi A. In or out of the groove? Mechanisms of lipid scrambling by TMEM16 proteins. Cell Calcium. 2024 Jul;121:102896.

Physiology and molecular mechanism of the CLC channels and exchangers
CLCs are chloride channels and anion/proton exchangers that are essential for various physiological processes, including muscle contraction and endo-lysosome acidification. Mutations in CLC genes lead to numerous disorders, including myotonia congenita, leukoencephalopathy, osteopetrosis, epilepsy and lysosomal storage disorders. The ClC-7 localizes to lysosomes and to the osteoclasts' ruffled border in complex with its accessory protein Ostm-1. We evaluated functional effects of 13 CLCN7 mutations identified in 13 new patients with severe or mild osteopetrosis and a known ADO2 mutation. We used a combination of imaging analysis, electrophysiological measurements, and an optical assay to identify structural-functional correlations that might explain the molecular basis of disease severity.
While many CLCs have been extensively investigated for their structure and function, the mechanisms underlying their gating remain unclear. To address this, we have recently focus on identifying the structural rearrangements that occur during transport. We are investigating the CryoEM structure of CLC-1 mutants associated with gating defects to pinpoint the structural components involved in this process.

a. **Di Zanni E**, Palagano E, Lagostena L, Strina D, Rehman A, Abinun M, De Somer L, Martire B, Brown J, Kariminejad A, Balasubramaniam S, Baynam G, Gurrieri F, Pisanti MA, De Maggio I, Abboud MR, Chiesa R, Burren CP, Villa A, Sobacchi C, Picollo A. Pathobiologic Mechanisms of Neurodegeneration in Osteopetrosis Derived From Structural and Functional Analysis of 14 ClC-7 Mutants. J Bone Miner Res. 2021 Mar;36: 531-545.

Cancer: role of PHOX2B over-expression in the pathogenesis of neuroblastoma.
Neuroblastoma is the most frequent pediatric extracranial solid tumor accounting for 15% of all child deaths from cancer. The paired-like homeobox 2B (PHOX2B) gene encodes a transcription factor crucial for the early steps of autonomous nervous system development and has a crucial role in neuroblastoma. Indeed, heterozygous mutations of the PHOX2B coding region were identified in sporadic and familial cases of isolated or syndromic neuroblastoma. We investigated the role of PHOX2B untranslated regions (promoter and 3'-UTR) in the pathogenesis of neuroblastoma and applied a high-throughput drug screening approach that resulted in the identification of molecules able to down-regulate the activity of the PHOX2B promoter. We demonstrated that chloroquine and mycophenolate mofetil decreased PHOX2B expression. Ultimately, we applied a proteomics approach to identify novel proteins acting with PHOX2B in the early steps of neuroblastoma development.

a. **Di Zanni E**, Fornasari D, Ravazzolo R, Ceccherini I, Bachetti T. Identification of novel pathways and molecules able to down-regulate PHOX2B gene expression by in vitro drug screening approaches in neuroblastoma cells. Exp Cell Res. 2015; 336:43-57.
b. **Di Zanni E**, Bianchi G, Ravazzolo R, Raffaghello L, Ceccherini I, and Bachetti T. PHOX2B gene expression is a druggable target to counteract neuroblastoma cells growth: the example of mycophenolate mofetil and chloroquine. Oncotarget. 2017 Aug 4;8: 72133-72146.
c. Bachetti T, **Di Zanni E**, Ravazzolo R, Ceccherini I. miR-204 mediates post-transcriptional down-regulation of PHOX2B gene expression in neuroblastoma cells. Biochim Biophys Acta. 2015; 1849:1057-65.

Trinucleotide expansion disorder: Congenital Central Hypoventilation Sydrome, (CCHS).
Congenital Central Hypoventilation Syndrome (CCHS) is a rare neurocristopathy characterized by the absence of adequate autonomic control of respiration with decreased sensitivity to hypoxia and hypercapnia. CCHS is

caused by heterozygous in-frame polyalanine duplications within the gene encoding the paired-like homeobox 2B (PHOX2B) transcription factor involved in the regulation of neurogenesis and in the correct differentiation of the autonomic nervous system. Functional studies of the polyalanine duplications revealed a reduction in PHOX2B transcriptional activity, attributed to the formation of cytoplasmic aggregates that obstruct the protein's nuclear entry. In collaboration with clinicians, we have conducted extensive research on CCHS syndrome and the PHOX2B gene over many years. Our work has significantly advanced the understanding of the molecular mechanisms underlying the disease and has led to the initial screening of several FDA-approved drug libraries. We investigated for the first time the effects of these small molecules on the most severe PHOX2B mutations associated with CCHS. These experiments demonstrated that some molecules could rescue the correct localization and promote the clearance of aggregates formed by mutated proteins. These findings have provided a deeper insight into the molecular pathways that can be targeted, facilitating the development of therapeutic approaches for CCHS.

a. **Di Zanni E**, Bachetti T, Parodi S, Bocca P, Prigione I, Di Lascio S, Fornasari D, Ravazzolo R, Ceccherini I. In vitro drug treatments reduce the deleterious effects of aggregates containing polyAla expanded PHOX2B proteins. Neurobiol Dis. 2012; 45: 508-18.
b. **Di Zanni E**, Ceccherini I, Bachetti T. Toward a therapeutic strategy for polyalanine expansions disorders: In vivo and in vitro models for drugs analysis. Eur J Paediatr Neurol. 2011 Sep;15: 449-52.
c. **Di Zanni E**; Adamo A; Belligni E; Lerone M; Martucciello G; Mattioli G; Pini Prato A; Ravazzolo R; Silengo M; Bachetti T; Ceccherini I. Common PHOX2B poly-Alanine contractions impair RET gene transcription, predisposing to Hirschsprung disease. Biochim Biophys Acta. 2017 Jul;1863:1770-1777.

Neurodegenerative disorder: Alexander disease.
Alexander disease (AxD) is a rare, usually fatal neurodegenerative disorder, involving primarily astrocytes in the CNS, caused by dominant mutations in the gene encoding glial fibrillary acidic protein (GFAP). The molecular pathogenesis of Alexander disease appears to involve toxic oligomeric forms of mutant GFAP, but evidence suggests that overexpression of the wild-type GFAP protein can also trigger disease-related cellular events. This implies that, in patients without GFAP gene mutations or those with severe early-onset forms, the increased protein levels might contribute to the disease. We investigated a 2.2 kb sequence of the regulatory region located upstream of the GFAP gene, searching for single-nucleotide polymorphisms (SNPs) that could modulate GFAP transcription. We identified a new AP-1 binding site lying at −250 bp upstream from the GFAP transcriptional start site and we demonstrated that the two alleles of this polymorphic locus bind the AP-1 complex with different affinities, resulting in varied levels of transcriptional activity for the GFAP promoter.
Thanks to collaboration with an Alexander disease clinician who observed a halt in disease progression in a patient treated with the antibiotic ceftriaxone, we explored the molecular mechanisms underlying this treatment effect. We observed that ceftriaxone treatment resulted in a marked reduction of intra-cytoplasmic aggregates of mutant GFAP, due to elimination of GFAP mutant proteins and concurrent up-regulation of HSP27 and αB-Crystallin, polyubiquitination and autophagy. Over the years, we studied the effects of other molecules on GFAP mutations, revealing the beneficial effects of curcumin in an *in vitro* model of the disease.

a. Bachetti T, **Di Zanni E**, Lantieri F, Caroli F, Regis S, Filocamo M, Rainero I, Gallone S, Cilia R, Romano S, Savoiardo M, Pareyson D, Biancheri R, Ravazzolo R, Ceccherini I. A novel polymorphic AP-1 binding element of the GFAP promoter is associated with different allelic transcriptional activities. Ann Hum Genet. 2010: 506-15.
b. Bachetti T, **Di Zanni E**, Balbi P, Bocca P, Prigione I, Deiana GA, Rezzani A, Ceccherini I, Sechi G. In vitro treatments with ceftriaxone promote elimination of mutant glial fibrillary acidic protein and transcription down-regulation. Exp Cell Res. 2010; 316: 2152-65
c. Sechi G, Matta M, Deiana GA, Balbi P, Bachetti T, **Di Zanni E**, Ceccherini I, Serra A. Ceftriaxone has a therapeutic role in Alexander disease. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34: 416.
d. Bachetti T, **Di Zanni E**, Balbi P, Ravazzolo R, Sechi GP, Ceccherini I. Beneficial effects of curcumin on GFAP filament organization and down-regulation of GFAP expression in an in vitro model of Alexander disease. Exp Cell Res. 2012 Sep; 318: 1844-54

Complete List of Published Work and Scientific Abstracts in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1jyztwebdARgw0/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Shuming Zhang

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

POSITION TITLE: Postdoctoral Associate

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Weill Cornell Medicine, New York, NY, USA	Postdoctoral Associate	-	Structural biology
Howard Hughes Medical Institute, Cornell University, Ithaca, NY, USA	Postdoctoral Associate	05/2024	Biophysics
West China School of Public Health, Sichuan University, Chengdu, Sichuan, China	Doctor of Medicine	06/2022	Public health laboratory sciences
National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics in Institute of Physics, Chinese Academy of Sciences, Beijing, China	Joint-PhD training	06/2022	Biophysics
West China School of Public Health, Sichuan University, Chengdu, Sichuan, China	Bachelor of Medicine	09/2018	Preventive medicine

A. Personal Statement

I am very interested in studying the structural and mechanistic underpinnings of phospholipid scramblases using cryo-EM.

I obtained my BS degree in Preventive Medicine in 2018 and my MD degree in Public Health Laboratory Sciences in 2022 from Sichuan University, a national key university in China. During my doctoral study, I was also jointly trained in the National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics in Institute of Physics, Chinese Academy of Sciences, where I finished my doctoral research project. After that, I secured a postdoctoral position in Dr. Michelle Wang's lab at Cornell University in September 2022.

My doctoral research focused on applying single-molecule force spectroscopy techniques, such as magnetic tweezers and atomic force microscopy, to study DNA replication, and the unfolding dynamics of disease-associated nucleosomes and tetra-nucleosomes. I then learned optical tweezers and angular optical tweezers techniques during my postdoctoral period for the study of torsional stress generated by DNA polymerase.

With the deepening of my research, I realized that the experimental techniques I had learned could only provide limited resolution information in specific dimensions, which was sometimes insufficient to comprehensively explain biological problems. I also recognized that I need to see the actual structure of biomolecules because "seeing is believing". So I became more and more curious about how the structure and function of biomolecules contribute to human health and disease. Phospholipid scramblases are enzymes that facilitate the translocation of phospholipids between the two leaflets of a cell membrane. This process is crucial for various cellular functions such as blood coagulation, membrane fusion or repair, and apoptosis. Mutations of scramblases contribute to autoimmune disorders, cancer, and neurological disorders. Therefore, I decided

to join Dr. Alessio Accardi's lab to broaden my skill set in cryo-EM and reveal the mysteries of how phospholipid scramblases affect health and disease.

B. Positions, Scientific Appointments, and Honors

Positions:

2024.06–present
Postdoctoral Associate, Weill Cornell Medicine, New York, NY, USA

2022.09–2024.05
Postdoctoral Associate, Howard Hughes Medical Institute, Cornell University, Ithaca, NY, USA

C. Contributions to Science

Publications:
1. **Shuming Zhang**, Xue Xiao, Jingwei Kong, Ke Lu, Shuo-Xing Dou, Peng-Ye Wang, Lu Ma, Yuru Liu, Guohong Li, Wei Li * and Huidong Zhang *. DNA polymerase Gp90 activities and regulations on strand displacement DNA synthesis revealed at single-molecule level. ***The FASEB Journal***, 2021, 35(5): e21607.
2. **Shuming Zhang**, Bianbian Li, Ke Du, Tingting Liang, Mengyuan Dai, Wenxin Huang, Huizhi Zhang, Yihui Ling and Huidong Zhang *. Epigenetically modified N6-methyladenine inhibits DNA replication by human DNA polymerase iota. ***Biochimie***. 2020, 168: 134-143.
3. Chenyang Mi#, **Shuming Zhang#** (Co-first author), Wenxin Huang, Mengyuan Dai, Zili Chai, Wang Yang, Shanshan Deng, Lin Ao * and Huidong Zhang *. Strand displacement DNA synthesis by DNA polymerase gp90 exo- of Pseudomonas aeruginosa phage 1. ***Biochimie***, 2020, 170: 73-87.
4. Ke Du, **Shuming Zhang**, Weina Chen, Mengyuan Dai, Zhongyan Xu, Tingting Liang, Wenxin Huang, Yihui Ling and Huidong Zhang *. Epigenetic DNA modification N6-methyladenine inhibits DNA replication by Sulfolobus solfataricus Y-family DNA polymerase Dpo4. ***Archives of Biochemistry and Biophysics***, 2019, 30, 675: 108120.
5. Xiaomeng Jia, James T. Inman, **Shuming Zhang**, Yifeng Hong, Xiang Gao, Anupam Singh, Smita S. Patel, and Michelle D. Wang *. Replication under torsion. ***CSHL meetings of Eukaryotic DNA Replication Genome Maintenance***, 2023.
6. Wenyan Li#, Jie Hu#, Feng Song#, Juan Yu#, Xin Peng, **Shuming Zhang**, Lin Wang, Mingli Hu, Jia-Cheng Liu, Yu Wei, Xue Xiao, Yan Li, Dongyu Li, Hui Wang, Bing-Rui Zhou, Linchang Dai, Zongjun Mou, Min Zou, Haonan Zhang, Zheng Zhou, Huidong Zhang, Yawen Bai, Jin-Qiu Zhou, Wei Li, Guohong Li *, Ping Zhu *. Structural basis for linker histone H5-nucleosome binding and chromatin fiber compaction. ***Cell Research***, accepted.
7. Xiaomeng Jia, Xiang Gao, **Shuming Zhang**, James Inman, Yifeng Hong, Anupam Singh, Smita Patel, Michelle D. Wang *. Torsion is a Dynamic Regulator of DNA Replication Stalling and Reactivation. ***Nature***, submitted.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Alvarenga-Cruz, Omar Ernesto

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

POSITION TITLE: Graduate Student Research Assistant

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Rice University	BA	08/2015	05/2019	Biological Sciences
Weill Cornell Graduate School of Medical Sciences	PHD	08/2019	05/2025 (Expected)	Physiology, Biophysics & Systems Biology

A. Personal Statement

My first foray into understanding the dynamic nature of biology was as an undergraduate research assistant in the Warmflash Lab at Rice University. In the Warmflash lab, I studied Wnt/ β-catenin signaling dynamics in human Embryonic Stem Cells through live-cell imaging. This research experience provided me with three key outcomes: a co-authorship publication, the confidence to pursue a career in research, and a sense of purpose through passionately hunting down answers to questions I never thought I would ask. For my PhD training at Weill Cornell, I made the decision to transition from Development Biology to Molecular Biophysics. Under the mentorship of Dr. Alessio Accardi and Dr. George Khelashvili I aim to develop into a scientist who integrates advanced experimental and computational methods to understand molecular mechanisms. My tenure in both labs as a co-mentored student has provided me with a wide range of skills and research interests. For my current project, I am investigating the free energy landscape of TMEM16F through MD simulation using enhanced sampling to overcome energetic barriers. Next, I plan to computationally and experimentally probe how known and novel mutations may modify TMEM16F's landscape and in turn have functional effects. Currently I am most excited about the opportunity to acquire skills in structural biology during experimental validation. As I continue moving closer to finishing my PhD, I do so with the knowledge my research community and mentors have provided me with the knowledge and training to become an effective life-long researcher.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 – Present NSF Graduate Research Fellowship, Weill Cornell Graduate School of Medical Sciences
2020 – 2021 Molecular Biophysics Training Program, Weill Cornell Graduate School of Medical Sciences
2019 – Present Graduate Research Assistant, Weill Cornell Graduate School of Medical Sciences
2017 – 2019 Undergraduate Research Assistant, Rice University

Honors

2022 Poster Presenter, Ligand Recognition and Molecular Gating Gordon Research Conference
2019 Distinction in Research and Creative Work Award, Rice University

C. Contributions to Science

Undergraduate Research: As an undergraduate student researcher in the Warmflash Lab at Rice University, my primary research goal was to understand Wnt/ β-catenin signaling dynamics in Human Embryonic Stem Cells (hESCs). To address this, I worked alongside a PhD student to create a CRISPR-Cas generated reporter cell line containing a fusion protein between β-catenin and GFP. Through time-lapse live-cell imaging experiments of this GFP- β-catenin reporter cell line we showed that the Wnt/ β-catenin signaling response undergoes a dramatic change from a transient signal to a sustained signal in response to cellular differentiation.

1. Joseph Massey, Yida Liu, **Omar Alvarenga**, Teresa Saez, Matthew Schmerer, Aryeh Warmflash. "Synergy with TGFβ ligands switches WNT pathway dynamics from transient to sustained during human pluripotent cell differentiation" PNAS Mar 2019, 116 (11) 4989-4998; DOI: 10.1073/pnas.1815363116

Graduate Research:
My current predoctoral research in the Accardi & Khelashvili Labs is focused on investigating the molecular mechanisms and nuances of phospholipid scrambling in different TMEM16 family members, I am most interested in whether a membrane-exposed "open" hydrophilic groove is a fundamental requirement for phospholipid scrambling. In pursuit of this question, I have taken a multi-pronged approach conducting functional assays to measure scrambling activity of wild-type and mutant proteins, as well more recently performing MD simulations to investigate the free energy landscapes of different TMEM16F scramblases, as guided by the functional and structural insights I have gained from wild-type and mutant proteins.

1. Falzone, M.E., Feng, Z., **Alvarenga, O.E**. *et al.* TMEM16 scramblases thin the membrane to enable lipid scrambling. *Nat Commun* **13**, 2604 (2022). https://doi.org/10.1038/s41467-022-30300-z
2. Feng, Z., **Alvarenga, O.E.** & Accardi, A. Structural basis of closed groove scrambling by a TMEM16 protein. *Nat Struct Mol Biol* (2024). https://doi.org/10.1038/s41594-024-01284-9

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	RICE UNIVERSITY	
2015	Introductory Biology	B
2015	General Chemistry I & Lab	B
2015	Beginning Swimming	A
2015	Intro to Comparative Politics	P
2015	Introduction to Psychology	A
2016	Fundamentals of Exp Biology	B
2016	Intro to Eng Computation	A
2016	General Chemistry II & Lab	C
2016	The Science of Supplements	B
2016	Medical Internship	A
2016	Organic Chemistry I + Discussion	C
2016	Intro to Ecol & Evol Biol	A
2016	Intro to Cognitive Psychology	A
2016	Elementary Statistics	A
2017	Experimental Biosciences	B
2017	Introductory Biology II	A
2017	Single Variable Calculus II	B
2017	Biochemistry I	B
2017	Introductory Synthetic Bio	B
2017	Molecular Biology & Genetics	P
2017	Immunology	B

YEAR	COURSE TITLE	GRADE
2017	General Physics I & Lab	B
2018	Biochemistry II	B
2018	Ind Res for Bioc Undergrads	A
2018	Evolution	A
2018	Evolution of Genes & Genomes	A
2018	Intro to Stat for Biosciences	A
2018	Bioinnovation Studio	A
2018	Cell Biology	B
2018	Undergraduate Honors Research	A
2018	Biology of Infectious Diseases	A
2018	Ord Differential Equations	P
2018	Intro to the Study of Religion	A
2019	Undergraduate Honors Research	A
2019	Undergraduate Research Seminar	A
2019	Organic Chemistry II & Discussion	C
2019	14 Films Before you Graduate	A
2019	General Physics II & Lab	C
	WEILL CORNELL GRADUATE SCHOOL OF MEDICAL SCIENCES	
2019	Quantitative Understanding in Bio I	HP
2019	Contemporary PBSB 1-3	HP
2019	Faculty Research Presentations	P
2019	PBSB Seminar Series	P
2019	Responsible Conduct of Research	P
2020	Biophysics Research Seminars	P
2020	Core Prin. Of Molecular Biophysics	HP
2020	Contemporary PBSB 4-6	H
2020	PBSB Seminar Series	P
2020	Data Structures and Algorithms for CB	LP
2020	PBSB Seminar Series Yr 2	P
2021	Crit Dissection of Scientific Data	H
2021	PBSB Seminar Series Yr 2	P
2022	Crit Dissection of Scientific Data	HP

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **David Ricardo Ballesteros Gomez**

eRA COMMONS USERNAME: **NA**

POSITION TITLE: **Graduate Student Research Trainee**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Universidad Nacional de Colombia (5 years program)	BA Science	02/2014	06/2019	Biology and Cellular Biophysics
Interdisciplinary Center for Neuroscience of Valparaiso. (Research Practice)	NA	07/2019	01/2020	Neuroscience
Albert Einstein College of Medicine (Research Inership)	NA	2020/03	03/2021	Neuroscience
Weill Cornell College	Graduate Student Ph.D	2021/07	2026/09 (Expected)	Molecular Biophysics

A. Personal Statement

Science can be understood as a discipline that can learned, but also as an approach to life. It is because of this duality that I am extremely passionate about it. I firmly believe that the advancement of science not only contributes to the archives of human knowledge and the advancement of technology, but it also allows us to keep building the social fabric for humanity.

The idea of science as fundamental stone of modern society is what drove into an academic career since very early in my studies. During my undergrad I participated in two research groups. First, during my first year in undergrad I joined in Professor's Jimena Sanchez research lab where I focused in the bioprospection of extremophiles organisms. Then, enticed by the molecular mechanism that allow cells to fulfill their physiological role, I focused my studies on cellular biophysics and joined Dr Marcela Camacho's "Biology and Biophysics of the Cell Membrane" group, for my thesis research lab. Under her supervision I worked on the electrophysiological characterization of a putative CLC chloride channel protein from the parasite *Leishmania sp*. Furthermore, during my time in her lab I developed a set of basic skills in molecular biophysics. Coming out of undergrad I had a resolute passion for molecular mechanisms and was certain that I wanted to pursue a career in academia. To gain experience, I moved from my home country to perform a five-month research internship at the Interdisciplinary Institute for Neuroscience in Valparaiso, under Dr Adrian Palacios, studying the changes in activity of retinal ganglion cells upon the knockout or over expression of proteins from the Unfolded Protein Response pathway (IRE1/XB1), in a mice background model for Alzheimer (5xFAD). Next, I had the opportunity to join David Spray's lab as a Research Assistant at Albert Einstein College of Medicine in NYC. Here, I focused my work on studying the role of Piezo 1 as the main channel conferring mechanic sensitivity to shear stress in astrocytes. This resulted in a co-first authorship publication. Both experiences after undergraduate school were deeply formative, I learned how to perform independent thinking and research, especially since the topics and approaches were fundamentally different, and thus I was exposed to a wide range of challenges.

For my graduate training at Weill Cornell Medicine, I decided to narrow my focus and dived into the field of protein biophysics and structural biology. Currently I am studying under the mentorship of Dr Alessio Accardi. Dr. Accardi has contributed several pieces of seminal work in the field of CLCs physiology and biophysics and has stablished himself as one of the international leaders on the subject. His conceptual and technical mentorship are an unvaluable component of my training as graduate student. The propose training plan also encompasses several opportunities for career development including attendance to specialized courses and workshops, as well as may opportunities to attend and present at science conferences. Additionally, he supports academic grow in science outreach and social contribution to the science community. Under Dr Accardi, I am currently researching on the gating mechanism of the CLC chloride channels and transporters family. CLCs are widely expressed in humans and fulfill a large number of roles including muscle resting membrane potential, salt reabsorption in the Henle's loop, and neuronal homeostasis. Our research will elucidate the basic mechanism underlaying many of these processes as well as opening the possibility for treatment development for many diseases associated to CLC gating.

Finally, to actively participate in the construction of the science community and foster education in general, during my time in the graduate program, I have performed twice as teaching assistant in a statistic, and a biophysics class. Additionally, I have tried to support minorities in the science community by participating twice in an Institutional program (TIMIs mentor program) to mentor young students from underrepresented groups throughout their application to grad school positions. Furthermore, I have volunteer in a virtual, non-institutional extended education program, teaching pre-undergrad cellular biology classes, for low-income students in Colombia (online).

Cibelli, A., **Ballesteros-Gomez, D**., McCutcheon, S., Yang, G. L., Bispo, A., Krawchuk, M., Piedra, G., & Spray, D. C. (2024). Astrocyte sense glymphatic-level shear stress through the interaction of sphingosine 1 phosphate with Piezo1. iScience.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 – Present Graduate Research Assistant, Weill Cornell Medicine.
2024 – 2024 Teaching Assistant (Core Principles in Molecular Biophysics)
2022 – 2022 Teaching Assistant (Quantitative Biology I)
2022 – 2022 Recruitment Chair Grad School Program
2020 – 2021 Research Assistant, Albert Einstein College of Medicine.
2019 – 2020 Research Internship, Interdisciplinary Center for Neuroscience of Valparaiso, Chile.

C. Contributions to Science

Undergraduate Research 1: I was part of a project in the laboratory of Dr. Sanchez at Universidad Nacional de Colombia. Dr. Sanchez laboratory focuses on bioprospection of extremophiles microorganisms. During my time in his lab, I was working on the isolation and characterization of oligotrophic organism from desertic environments. What I learned from my work was included in a chapter of a book published by the research group.

- **Book Chapter**: Case Study and Application in Microbial Ecology. Chp. Microorganisms of extreme environments: The desert as case study. **Ballesteros D**, and Miranda L. Group of Astrobiology and Microbial Ecology, National University of Colombia. ISBN: 9789587832242

Undergraduate Research 2: During my time in Dr Camacho Lab at Universidad Nacional de Colombia I focused my research in the electrical characterization of two new putative CLCs from Leishmania Braziliencis (LbCLC-A and LbCLC-B) using a stable transfection CHO cell line. Our results suggest that CLC-A is a cl^- channel but work on CLC-B was inconclusive.

- Poster: Electrophysiological study of the exchanger LbCLC-B (LbrM32_V2.3670) of *Leishmania braziliensis* expressed in tow heterologous systems: Chinese Hamster Ovary (CHO) cells and *Xenopus laevis oocytes*. **Ballesteros D**, Cárdenas S, García M, Quintero N, Camacho M. III Colombian Congress of Biochemistry and Molecular Biology.

- Poster: "Electorphysiological characterization of the mutant LbCLC-A". Cárdenas S, **Ballesteros-Gómez D**, Osorno T, Echeverri A, García M, Camacho M. III Colombian Congress of Biochemistry and Molecular Biology.

- Poster: Study of LbCLC-A and LbCLC-B putative proteins expressed in Leishmania braziliensis opens a new path for understanding the function and role of the CLC exchanger CLC-6 in neuropathies. Cardenas S*, **Ballesteros D***, Camacho M. XV Annual Meeting of the Neuroscience Societyof Chile.

Research Assistant: One of the focusses of Dr Spray lab is the physiological function of neuroglia. My research focused on understanding the mechanosensitivity of astrocytes. Using primary cultured astrocytes on shear stress microfluidic devices. We discovered that the Piezo 1 is the main mechanosensitive channel involved in shear stress response, and that modulation by sphingomyelin 1 is necessary to finetune the astrocyte to sense physiological levels of shear stress. This research was particularly exciting because for the first time we were reporting mechanical responses in the context of physiological ranges of shear stress and albumin, underlying the high relevance of our findings.

- Research Article: Cibelli, A., **Ballesteros-Gomez, D**., McCutcheon, S., Yang, G. L., Bispo, A., Krawchuk, M., Piedra, G., & Spray, D. C. (2024). Astrocyte sense glymphatic-level shear stress through the interaction of sphingosine 1 phosphate with Piezo1. iScience.

- Poster: Astrocyte mechanosensitivity is amplified by albumin. **David R Ballesteros-Gomez**, Sean McCutcheon, David C Spray. Cold Spring Harbor meeting: Brain Barriers (Virtual).

Graduate Research: My ongoing research at Accardi lab focuses understanding the molecular determinants of gating in CLC channels. To this end, we have purified the structure of CLC-1 in conditions that stabilize the common gate in the open state. Additionally, I have focus on developing and optimizing a protocol for CLC-0 purification, and Cryo-EM imaging. Our interest in CLC-0 lies in its suitability for single channel recordings allowing us to investigate individually the kinetic and energetic characteristic of the common gating. Given that human CLCs play diverse roles in physiology, our research provides the basic substrate from which we can understand the mechanism of CLC activation in physiology, but more importantly, its affectation in disease. I believe that our research can potentially set up the ground for the development of potentials drugs and treatments, making our research of high relevance for human health.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	NATIONAL UNIVERSITY OF COLOMBIA - BOGOTA	
2014	(L) Foreign Language (English) I	Pass
2014	(L) Foreign Language (English) II	Pass
2014	(L) Foreign Language (English) Ill	Pass
2014	(L) Developmental Biology	A
2014	(L) Foreign Language (English) IV	Pass
2014	(L) Basic Chemical Techniques Lab	B
2014	(L) Principles of Chemistry	C
2014	(L) Earth Science	A
2014	(L) Ecology	A
2014	(L) Principles of Organic Chemistry Lab	B

2014	(L) Animal and Human Behavioral Biology	A
2014	(L) Plant Biology	A
2014	(L) Principles of Organic Chemistry	C

2015	(L)Principles of Biochemistry Lab	A
2015	(L) Differential Calculus	A
2015	(L) Animal Biology	C
2015	(L) Ethology	A
2015	(L) Principles of Biochemistry	A
2015	(L) Fundamentals of Research	A
2015	(U) Biology of Microorganisms	A
2015	(U) Animal Communication	A
2015	(U) Molecular Biology of Cells	A
2015	(L} Fundamental Biostatistics	A

2016	(U) Genetics	A
2016	(U) Cellular and Molecular Immunology	A
2016	(U) Mechanics and Waves for Bioscience	A
2016	(U) Art History and Appreciation: From Baroque to Impressionism	A
2016	(U) Cellular Biology	A
2016	(U) Vertebrata	A
2016	(U) Neurobiology of Sensory Transduction	A

2017	(U) Introduction to Cellular Biophysics	A
2017	(U) Animal Physiology	A
2017	(U) Analysis and Modeling of Biological Systems	A
2017	(U) Fundamentals of Population Economics	A
2017	(U) Fluids and Electromagnetism for Bioscience	A
2017	(U) Physiology of Animal Systems	A
2017	(U) Biophysics of Membranes	A

YEAR	COURSE TITLE	GRADE
2018	(U) Research Practice	A
2018	(U) Selected Topics in Biology	A
2018	(U) Multivariate Analysis	B
2018	(U) Scientific Research Methodology	A
2018	(U) Molecular Biology	A
2018	(U) Experimental Design	A
2018	(U) Biology of Protists and Algae	A
2018	(U) Special Topics in Biology	A
2018	(U) Developmental Biology	A
2018	(U) Fundamentals of Ecology of Landscape	A

2019	(U) Introduction to Conservation Biology	A
2019	(U) Degree Project	A
2019	(U) Evolution	A

*Universidad Nacional de Colombia's Foreign Language courses are graded as Pass or Fail not with a quantitative scale.

WEILL CORNELL MEDICINE

2022	Core Prin. of Molecular Biophysics	H
2022	Contemporary PBSB 4-6	HP
2022	PBSB Seminar Series*	Pass
2022	Crit Dissection of Scientific Data	H
2022	Contemporary PBSB 1-3	HP
2022	Faculty Research Presetations*	Pass
2022	PBSB Seminar Series*	Pass
2022	Responsible Conduct of Research*	Pass
2022	Quantitative Understanding in Bioll	H
2022	Quantitative Understanding in Bio I	H
2022	Lab Rotation 1 *	Pass
2022	Lab Rotation 2 *	Pass
2022	Lab Rotation 3 *	Pass
2022	Lab Rotation 4 *	Pass

2023	Cellular & Organismal Metabolism*	Pass
2023	Current Topics in Neurodegeneration	H
2023	PBSB Seminar Series Yr. 2*	Pass
2023	Crit Dissection of Scientific Data	HP
2023	Intro Programming for Life Sciences *	Pass
2023	Data Structures & Algorithms for CB	A

WCM program uses the grade system: Fail (F), Low Pass(LP), High Pass (HP) and Honors (H). *Graduate courses that are labeled with the word ‘Pass’ and not with a single letter grade are graded just as Pass or Fail.