Novel antithrombotics for the ageing population in the trauma setting

Team
Matt A. Peterson; Associate Professor
Department of Chemistry & Biochemistry
Ryan Rasmussen PhD, FNP; Associate Professor
College of Nursing
Dario Mizrachi; Assistant Professor
Department of Cell Biology and Physiology
College of Life Sciences

Increase in the ageing population has resulted in a change in the demographics of trauma patients. The elderly are at high risk of sustaining injuries with potentially serious hemorrhagic complications. Therefore, between 40-65% of adults aged 75 years old and older in the USA and Europe are subjects to antithrombic therapy. An antithrombotic agent is a drug that reduces the formation of blood clots. Anticoagulants and Antiplatelet agents prevent clots formation and growth. Current solutions like Aspirin, Warfarin, Heparin, and other antithrombotic therapies remain in the circulation for long periods of time. In the event of an emergency, the risk of bleeding for these individuals undergoing these chronic therapies is high, as is the risk of mortality.

We have uncovered a new mechanism of platelet aggregation that requires the release of intracellular **Zinc** in platelets. This finding enables us to be the first group in the world to address the above-mentioned needs with a new approach. Our proposal seeks to produce Antithrombotic agents that, when administered acutely or chronically, may enable trauma centers appropriate treatments or surgical solutions as needed. We aim to identify druggable compounds using a unique synthetic biology approach. Furthermore, we seek to establish a new method to report the need for Antiplatelet therapy based on **Zinc** content in platelets rather than plasma Zinc alone.

Plans for future funding

The National Institute of Health is the biggest contributor to funding. Using the keyword "antithrombotic" we discovered that there are over 900 active projects with 50% of the awards corresponding to the National Heart, Lung, and Blood Institute (NHLBI); and over 30% to the National Institute of Neurological Disorders and Stroke (NINDS). These active projects received awards that amount to approximately $500 million. The most common funding mechanisms are R01 and R15. An example of a successful R15 is "Effect of zinc on tPA induced thrombolysis." An example of a successful R01 is "Molecular Studies of Hemolytic Thrombosis."

Our team will apply for R15 funding to NHLBI in the upcoming June 2023 deadline with three aims: 1) Molecular Biology of Zinc in coagulation 2) Pharmacology of identified antithrombotics 3) Accuracy of Zinc testing for better understanding the need for antithrombotics. There is no record of recent awards for new antithrombotics in the last decade nor of a challenge to plasma Zinc testing.

PROJECT NARRATIVE

THE PROBLEM

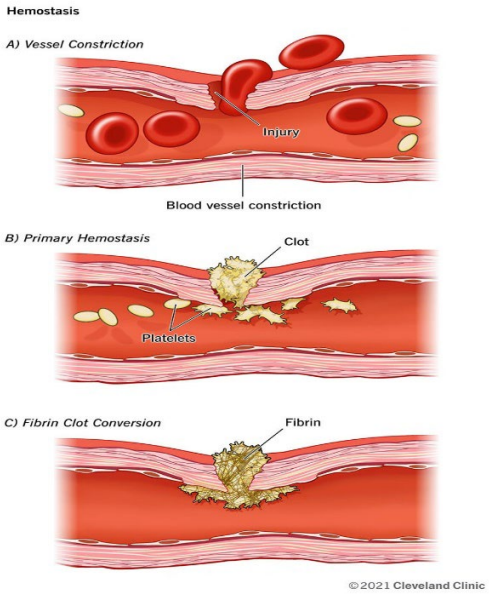
The ageing population has resulted in a change in the demographics of trauma. This includes an increase in clinically vulnerable older people with comorbid disease, functional dependence, disability and frailty syndromes. Diminished physiological reserve and increased comorbidities may mean older people are more susceptible to injury and bleeding. Timely reversal of anticoagulation in the acute setting can help restore hemostatic function and potentially reduce bleeding, prior to definitive surgery or other interventions. Initial challenge to this protocol is the often long half-life of antithrombotics in plasma. However, once hemostasis is achieved, decisions to recommence antithrombotic agents in the longer term, weighing up bleeding and thrombotic risk, can be also challenging.

In the following years, it is expected that people over 65 years will represent up to one-fifth of the world population and almost 40% of trauma admissions by 2050. In developed countries such as the United States, it is not uncommon to see patients in their 80s and 90s taking anticoagulants or antiplatelets admitted with trauma.

Most elderly patients can present hypertension, cardiovascular disease and impaired sensitivity to catecholamines. In addition, they are receiving chronic medications that can affect heart rate and blood pressure, blunting the response to injury in hemodynamically compromised patients. Elderly patients are usually taking antiplatelets or anticoagulants which clearly can affect outcome, especially in patients with traumatic brain injury. The widespread use of novel anticoagulants might complicate the management and outcome in this setting.[1, 2]

THE DISCOVERY

Hemostasis is a process to prevent hemorrhage by arresting and keeping the blood within the damaged vessel walls. Hemostasis is a complex process that is contingent on the complex interaction of platelets, plasma coagulation cascades, fibrinolytic proteins, blood vasculatures and cytokine mediators.

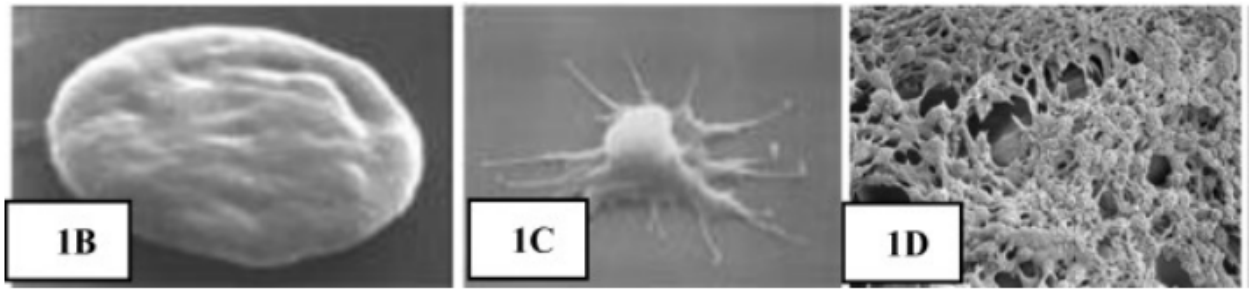


Vascular spasm occurs whenever there is an injury or damage to the blood vessels. This will trigger a vasoconstriction, which could eventually stop the blood flow. In addition, the Extra Cellular Matrix becomes highly thrombogenicity, promoting platelet adhesion and aggregation.
Following vasoconstriction, exposed collagen from the damaged surface will encourage platelets to adhere, activate and aggregate to form a platelet plug, sealing off the injured area.

The platelet adhesion mechanism is generally supported by the particular interactions between the membrane receptors and absorbed plasma proteins. These membrane proteins are the important

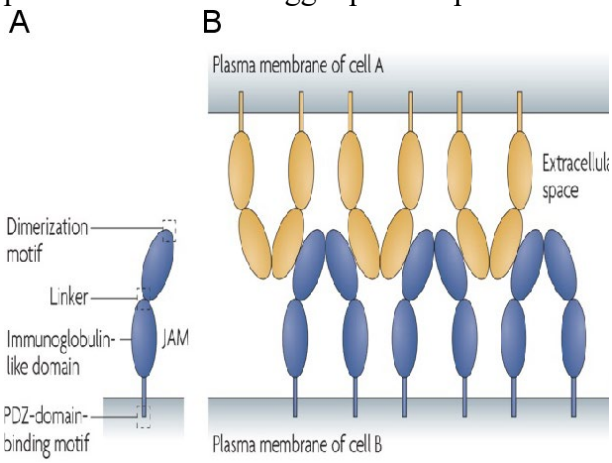
players involved to facilitate hemostatic function by mediating the interactions within cell-platelet and platelet-substrates.

A variety of stimuli can activate platelets. Platelet cells can also be activated upon biomaterial surface stimulation. Adhered platelets undergo degranulation and release cytoplasmic granules that contain serotonin, platelet activating factors, Zinc, and ADP. ADP is an important physiological agonist stored in the dense bodies of platelets that play an essential function in normal hemostasis and thrombosis. At the same time, platelets tend to synthesize and discharge thromboxane A2 (TXA2), aiding in vasoconstriction and platelet aggregation. Each activated platelet extends pseudopods, clumping and becoming aggregated. These activations are further heightened by the generation of thrombin via the hemostasis mechanism. Platelet aggregation promotes a primary platelet plug. Eventually, the formed platelet plug ought to be stabilized by the formation of fibrin.



Platelet activation: [(B) Platelet in resting mode (C) Activated platelets change into a pseudopodia shape (D) Aggregated platelets

The role of Zinc in platelet activation is not well understood. It is believed that inability to release Zinc from the platelet granules results in coagulation impairments. The exact mechanism is unknown. Additionally, the existence of cell-cell interaction membrane proteins has long been a mystery on the surface of platelets. Junctional Adhesion Molecules type A and C are present on the surface of platelets but do not trigger platelet-platelet

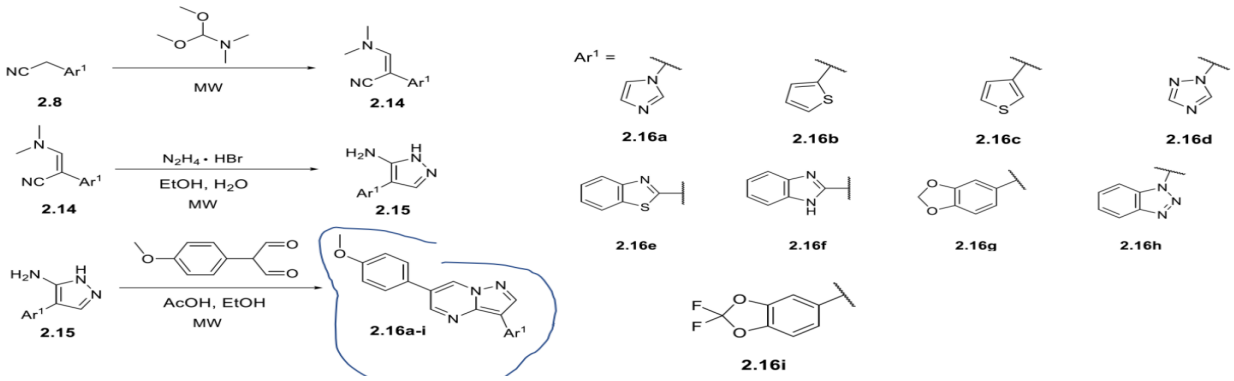


Structural features, homophilic adhesion and extracellular ligands of JAMs. **A.** JAMs structure contains two immunoglobulin-like domains in extracellular portion, a single transmembrane segment and a short cytoplasmic tail with a PDZ domain-binding motif. The dimerization motif in membrane-distal domain is essential for homodimer formation. B. Homophilic interactions of JAM-A in cis (via dimerization motif in the membrane-distal immunoglobulin domain).

The laboratory of Dr. Mizrachi recently published the effects of Zinc on JAM-A protein aggregation [3]. Furthermore, we have demonstrated that in isolated platelets the addition of 3 mM Zinc (levels comparable to those released by the platelet granules) induces aggregation to an extent greater and faster than the well-known ADP factor. The data is part of a manuscript in preparation for the journal BLOOD. Thus, we hypothesize that small molecules that can sequester Zinc would serve as agents in antithrombotic therapy.

THE TEAM

Professor Matt Peterson has generated a library of 500 compounds. The compounds in the library are intellectual property of Professor Peterson (below an example of a typical strategy). Professor Peterson will coordinate work with Chemistry undergraduate students.



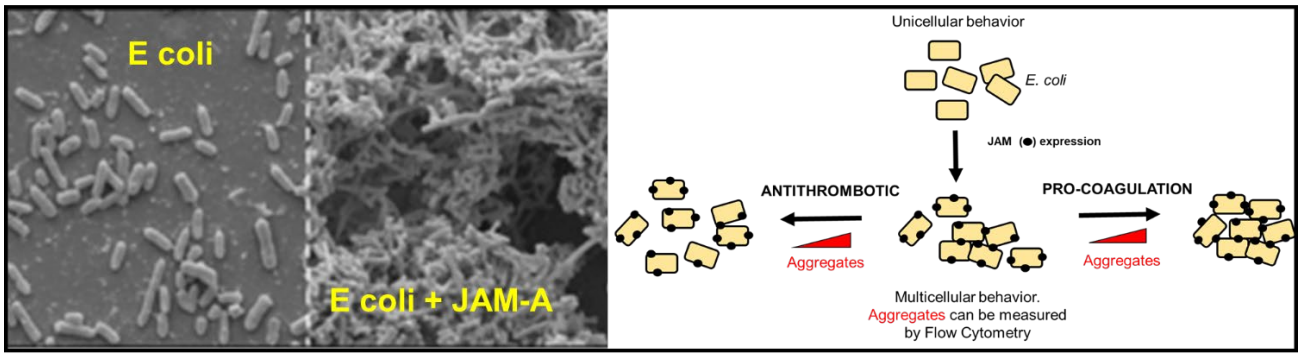
Dr. Mizrachi will provide a Synthetic Biology, high throughput technique to evaluate the effects of the library's compounds sequestering Zinc and further preventing aggregation of JAM-A. Identified compounds can be tested on aggregation assays of isolated platelets. The best compounds can be further improved through chemical synthesis under the direction of Professor Peterson. Dr. Mizrachi will coordinate work with undergraduate students of the Life Sciences College.

Professor Rasmussen is our consultant for biostatistics and also for knowledge of Trauma treatments and procedures. His expertise is invaluable in understanding the needs of Trauma treatments, endpoint goals for patient care. Professor Rasmussen will coordinate work with undergraduate students of the Nursing program.

THE APPRAOCH

Identify novel antithrombotic

Dr. Mizrachi laboratory has expressed JAM-A and JAM-C proteins on the surface (outer membrane) of E. coli. These proteins force these unicellular organisms to aggregate (cell-cell interactions). This behavior can be detected using Flow Cytometry [4]. Cells that aggregate will produce a larger slope on the linear analysis of all the cell-counting events.



We express JAM proteins in E. coli and measure the aggregation of cells using Flow Cytometry. Furthermore, compounds in 12-replicates are incubated with cell aggregates for 30 minutes prior to examining the behavior of the aggregates under the influence of the compounds. Finally, cell aggregates are exposed to 3 mM Zinc and compounds from the library in a concentration of 10 mM. If under these conditions Zinc-induced aggregates disperse with a given compound, we will assign the value of

ANTITHROMBOTIC. If under these conditions Zinc-induced aggregates demonstrate further aggregation with a given compound we will assign the value of PRO-COAGULATION agent.

This assay is high throughput and can examine the 500-compound library in 6 months under the allotted time for Flow Cytometry in the RICs facility in the Life Sciences building.
Compounds identified will be tested for performance and also will be further evaluated in future studies for half-life in plasma, as well as potential conflicts with trauma patients currently taking antithrombotics.

Zinc concentrations to establish Fitness

Alternatively, our team seeks to establish a new approach to measure Zinc levels in the coagulation pathway, and its interpretation. Levels of Zinc in plasma and in platelets that are consistent with physiological needs and retains anti-coagulation capabilities unaided pharmacologically will be categorized as in compliance or FITNESS. Zinc Fitness determined by measuring Zinc plasma levels and Zinc concentration in isolated platelets could simplify the multifactorial process by which Zinc is absorbed from food, enters circulation and ultimately is concentrated and stored in platelet granules. The number of processes and membrane proteins (e.g. Zinc transporters) responsible for physiological levels of Zinc ranges close to 20. By creating a ratio of $[Zinc]_{platelet}/[Zinc]_{plasma}$ Fitness can be easily established. This new ratio can be used to estimate the need for antithrombotic therapy in the Elderly patients.

Colorimetric kits exist for the determination of Zinc. Dr. Mizrachi's laboratory is BSL2 and can handle blood samples. Nursing students will be responsible for collecting blood samples from volunteers. Additionally, national repositories can provide samples to represent the elderly population. Professor Rasmussen is an expert in biostatistics and will supervise the interpretation of the results.
In individuals with the low Zinc Fitness ratio could indicate risk of bleeding disorders. High ratios of Zinc Fitness ratio may indicate the need for antithrombotic therapy.

BUDGET NARRATIVE

Year 1

E coli cell lines and DNA	$ 1,000
Flow Cytometry	$10,000
Platelet purification Kit	$ 3,000 (100 samples)
Zinc Colorimetric kits	$ 5,000 (100 samples)
Phlebotomy reagents	$ 1,000

Year 1

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Platelet purification Kit	$ 3,000 (100 samples)
Zinc Colorimetric kits	$ 5,000 (100 samples)
Phlebotomy reagents	$ 1,000
Chemical Synthesis to improve compounds	$ 5,000

FUNDING IDEAS

Plans for future funding

The National Institute of Health is the biggest contributor to funding. Using the keyword "antithrombotic" we discovered that there are over 900 active projects with 50% of the awards corresponding to the National Heart, Lung, and Blood Institute (NHLBI); and over 30% to the National Institute of Neurological Disorders and Stroke (NINDS). These active projects received awards that amount to approximately $500 million. The most common funding mechanisms are R01 and R15. An example of a successful R15 is "Effect of zinc on tPA induced thrombolysis." An example of a successful R01 is "Molecular Studies of Hemolytic Thrombosis."
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- R15 awards provide $450,000 for 3 years
- R01 awards provide over $1 million for 5 years
- Selected Topics in Transfusion Medicine (R21) PAR-16-441. This award is currently sponsored by NHLBI. Up to $275,000 in direct costs over two years.

Private funding:

https://www.cslbehring.com/vita/2020/seeking-researchers-in-bleeding-disorders
CSL Behring Heimburger Awards, which provide funding for coagulation research. ($22,500 US)

Pharmaceutical Companies

Bristol Myers Squibb.
Bayer AG.
Pfizer.
Johnson & Johnson.
Boehringer-Ingelheim.
These companies are main competitors in the production of pharmaceuticals related to blood. Many of them have collaborative mechanisms and few have funding available.

Future

As the work progresses the scope of the grants can broaden to study mechanism of action of the identified antithrombotic. Pharmachological evaluation of new antithrombotics. Animal trials for identified compounds that are antithrombotic as well as pro-coagulation.

References

1. Egea-Guerrero, J.J. and M. Quintana Diaz, *New oral anticoagulants in severe trauma patients: enemy at the gates?* Med Intensiva, 2015. **39**(3): p. 167-71.
2. Ho, P., et al., *Direct oral anticoagulants in frail older adults: a geriatric perspective.* Semin Thromb Hemost, 2015. **41**(4): p. 389-94.
3. Christopher Mendoza & Keegan Peterson & Dario Mizrachi & Sai Harsha Nagidi, 2022. "**Cations as Molecular Switches for Junctional Adhesion Molecule-A**," Biomedical Journal of Scientific & Technical Research, Biomedical Research Network+, LLC, vol. 42(3), pages 33558-33571, February.

4. Expression of cell-adhesion molecules in E. coli: a high-throughput method to identify paracellular modulators. Rollins J%, Worthington T%, Hooke E%, Hobson J%, Wengler J%, Hope S**, and Mizrachi D. 2021 BioRxiv doi: https://doi.org/10.1101/2021.04.08.439041 Impact Factor NA

BIOSKETCH

Matt Peterson, Ph.D.
Associate Professor
Department of Chemistry & Biochemistry (1995- present day)

Education:
BS (Chemistry), Utah State University (1987)
BS (Biology), Utah State University (1987)
Ph.D., University of Arizona (1992)
NIH Postdoctoral Fellow, Colorado State University (1993-94)

Research:
Peterson Lab studies focus on the chemical synthesis and biological evaluation of potential antitumor and/or antiviral compounds. Modification of naturally occurring nucleosides is a unifying feature in Peterson Lab research.
Current interests can be broken down into three areas: (1) synthesis and evaluation of nucleoside-based enediynes (e.g., uracil-fused and uracil-linked enediynes I and II; and adenosine mimic III); (2) synthesis and evaluation of transition-state analogue inhibitors of key enzymes involved in the pyrimidine-nucleoside manifold (e.g., potential cytidine triphosphate synthetase and cytidine deaminase inhibitors typified by structure IV), and (3) synthesis and evaluation of inhibitors of HIV integrase.

TEAM CONTRIBUTION
Professor Peterson has a unique library of ~500 small organic compounds. These compounds are small and that makes them druggable. The unique compounds are intellectual property of Professor Peterson. These compounds perform the function of binding Zinc, the requirement for the antithrombotic we are aiming to identify. Finally, his background will enable the team further modifications of the candidates from his library to improve performance.

Publications:
Singleton JD, Dass R, Neubert NR, Smith RM, Webber Z, Hansen MDH, Peterson MA. Synthesis and biological evaluation of novel pyrazolo[1,5-a]pyrimidines: Discovery of a selective inhibitor of JAK1 JH2 pseudokinase and VPS34. Bioorg Med Chem Lett. (2020) Jan 15;30(2):126813.

Burt, S. R., Machicao, P. A., Christensen, R. K., Lohner, N. B., Singleton, J. D., Peterson, M. A. (2017). An Efficient Microwave Assisted Synthesis of N'-Aryl/(alkyl)- substituted N-(4-hydroxy-6-phenylpyrimidin-2-yl)guanidines: Scope and Limita-tions. Tetrahedron Letters. (2017).

Machicao, P. A., Peterson, M. A., Schols, D. (2015). An efficient one-pot conversion of Boc-protected adenines to N6-ureas. Paulo A. Machicaoa, Matt A. Petersona, , , Dominique Scholsb, 56(47), 6574–6576. (2015).

J. R. Shelton, J. Balzarini, and M. A. Peterson* "Discovery of a nanomolar inhibitor of lung adenocarcinoma in vitro" Bioorg. Med. Chem. Lett. 24,5107–5110 (2014).

J. R. Shelton and M. A. Peterson* "Efficient Synthesis of 5'-O-(N)-Carbamyl and Polycarbamyl Nucleosides" Tetrahedron Lett. 54, 6882-6885 (2013).

J. Balzarini, F. Gago, W. Kulik, A. B. P. van Kuilenburg, A. Karlsson, M. A. Peterson, and M. J. Robins "Introduction of a Fluorine Atom at C3 of 3-Deazauridine Shifts Its Antimetabolic Activity from Inhibition of CTP Synthetase to Inhibition of Orotidylate Decarboxylase, an Early Event in the de Novo Pyrimidine Nucleotide Biosynthesis Pathway" J. Biol. Chem.287, 30444–30454 (2012).

Ryan Rasmussen Ph.D., FNP;
Associate Professor
Global Health and International Studies Coordinator
College of Nursing (2013-present day)

Education
PhD, Nursing , Informatics, University of Arizona (2019)
Master of Science Nursinng, Nursing , Brigham Young University (2011)
Bachelor of Science Nursing, Nursing , Utah Valley University (2008)
Associate of Science , Nursing , Utah Valley University (2001)

Research
Emergency Department, Trauma, Communication, Orthopedics, Informatics, and Pediatrics

Team Contribution
Professor Rasmussen is an expert in emergency medicine and trauma. His contribution will be to monitor nursing students in the study of Zinc determination in plasma and in platelets. With his expertise in biomedical informatics he can interpret our results and evaluate the fitness of Zinc in patients of the different group ages as hypothesized by this study.

Publications
Rasmussen RJ, Carrington JM. 2017. Exploring Barriers to the use of Electronic Health Records in the Trauma Room Using a Cognitive Work Analysis. [place unknown]: American Medical Informatics Association Annual Symposium.

Rasmussen RJ, Carrington JM. 2017. State of the Science Exploring Communication in the Trauma Room. [place unknown]: Western Institute of Nursing .
Journal Article, Academic Journal

Beckstrand RL, Corbett EM, Macintosh JLB, Luthy KE(, Rasmussen RJ. 2019. Emergency Nurses' Department Design Recommendations for Improved End-of-Life Care. Journal of Emergency Nursing. 45(3):286-294.

Beckstrand RL, Rohwer J, Luthy KE, Macintosh JLB, Rasmussen RJ. 2017. Rural emergency nurses'end-of-life care obstacle experiences: Stories from the last frontier. Journal of Emergency Nursing. 43(1):40-48.

Rasmussen RJ, Nuttall C. "You flipped What?". NONPF 40 Annual Meeting. Denver, Colorado. 2014 .

Dario Mizrachi, Ph.D.
Assistant Professor
Department of Cell Biology and Physiology
College of Life Sciences (2017- present day)

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
University of Santiago, CHILE	B.Sc.	12/1993	Biochemistry
University of Santiago, CHILE	M.Sc.	12/1994	Immunology
Hebrew University of Jerusalem, ISRAEL	Ph.D.	05/2002	Physiology
University of Iowa, USA	Postdoctoral	11/2004	Molecular and Structural Biology
Cornell University, USA	Postdoctoral	12/2016	Synthetic Biology

Research

My research has continuously involved membrane proteins. As an independent researcher, I have aligned my work with the field of Barriology, the study of structures that form barriers in tissues (e.g. blood-brain barrier) and control the paracellular space and thus permeability.
Tight junctions play a principal role in mechanocoupling in cells. The impact of mechanocoupling in the clinical setting is only starting to be appreciated.
My training in physiology, molecular and structural biology, synthetic biology, and biochemistry has been extensive and fruitful. My dealings with membrane protein research has ignited my desire to generalize methods for their study. Our most recent seven publications include 25 undergraduates and 2 graduate students. Our work has contributed to the fields of Synthetic biology, membrane proteins, Protein Engineering, Barriology, Physiology, Cancer, and Developmental Biology.

Team Contribution

Doctor Mizrachi will provide Synthetic Biology tools for the examination of the small molecules ability to interfere with platelet aggregation due to Zinc. Dr. Mizrachi will coordinate and analyze the data generated by undergraduates using Professor Peterson's library. Dr. Mizrachi will coordinate with Professor Rasmussen the measurement of Zinc in plasma and platelets performed by nursing students. Doctor Mizrachi will coordinate the usage of data collected to apply for funding to multiple institutions.

Publications

1. Insulin receptor-inspired soluble insulin binder. Christopher Mendoza, Alek Sperry%, Cameron Hanegan%, Logan Vargas%, Trevor Case%, Benjamin Bikman ** and Dario Mizrachi (European Journal of Cell Biology-Manuscript Number: ACCEPTED 2023) Impact Factor 4.5
2. The role of individual tight junction membrane proteins in chicken neural tube defects. Allen W%., Morris A. %, May R. %, Wengler, J. %, Thacker, S. % Stark M.**, Mizrachi (UCUR publication of short communications, Peer Reviewed- ACCEPTED 2023) Impact Factor NA.

3. A universal glycoenzyme biosynthesis pipeline that enables efficient cell-free remodeling of glycans. Jaroentomeechai, T., Liu, Y., Young, O., Bhawal, R., Wilson, J., Mizrachi, D., and DeLisa, M.P. Nature Communications (In press 2022) Impact Factor 17.7.
4. Cations as Molecular Switches for Junctional Adhesion Molecule-A. Mendoza C#, Nagidi SH%, Peterson K%, Mizrachi D. Biomed J Sci & Tech Res (March 2022) DOI: 10.26717/BJSTR.2022.42.006742. Impact Factor 1.2
5. Overexpression of a constitutively active death receptor induces apoptosis in cancer cells. Peterson K. %, Palmer B. %, Christensen C. %, Bailey S. %, Mizrachi D. (Archives of Molecular Biology and Genetics. Arch Mol Biol Genet. 2022; 1(1):20-28) Impact Factor NA
6. Using the Power of Junctional Adhesion Molecules Combined with the Target of CAR-T to Inhibit Cancer Proliferation, Metastasis and Eradicate Tumors. Mendoza C.# and Mizrachi D. (Biomedicines 2022 Feb 4;10(2):381) Impact Factor 5.2

7. Calcium regulates the interplay between tight junction and epithelial adherens junction at the plasma membrane. Mendoza C.#, Nagidi S.H. %, Collett K. %, Mckell J. % and Mizrachi D. FEBS Letters 2021 (Dec 9. doi: 10.1002/1873-3468.14252. Online ahead of print. PMID: 34882783) Impact Factor 4.1
8. Chimeric Claudins: A New Tool to Study Tight Junction Structure and Function. Taylor A%, Warner M%, Mendoza C#, Memmott C%, LeCheminant T%, Bailey S%, Christensen C%, Keller J%, Suli A**, Mizrachi D. Int J Mol Sci. 2021 May 6;22(9):4947 Impact Factor 6.2
9. Molecular Characterization of the Extracellular Domain of Human Junctional Adhesion Proteins. Mendoza C#, Nagidi SH%, Mizrachi D. Int J Mol Sci. 2021 Mar 27;22(7):3482 Impact Factor 6.2
10. Expression of cell-adhesion molecules in E. coli: a high-throughput method to identify paracellular modulators. Rollins J%, Worthington T%, Hooke E%, Hobson J%, Wengler J%, Hope S**, and Mizrachi D. 2021 BioRxiv doi: https://doi.org/10.1101/2021.04.08.439041 Impact Factor NA

Current and pending support

None of the faculty in the team are currently receiving funds.