BIOGRAPHICAL SKETCH

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NAME: Gonzalez, Fabio A.

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

POSITION TITLE: Graduate Student

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
Universidad Icesi, Cali, Colombia	B.S.	08/2019	Chemistry
Universidad Icesi, Cali, Colombia	B.S.	08/2019	Pharmaceutical Chemistry

A. Personal Statement

My research studies are directed towards understanding the function of membrane proteins and protein complexes, by studying the molecular determinants of protein-to-protein interactions at the atomistic resolution level. During my undergraduate research I studied DNA to protein interactions by molecular dynamics and protein docking simulations. Followed by this, I spent two years of my graduate studies conducting large-scale simulations of membrane lipids and analyzing the dynamic properties of lipids in biological membranes. The early exposure to computational chemistry familiarized me with experimental tools for integrative modelling, such as NMR restraints, cross-linking restraints, EM density maps, etc. Complementary to my previous experience, I decided to continue my PhD research in the experimental field where I am interested in understanding the cellular role of selenoproteins, more specifically selenoprotein S, from a structural biology approach. By elucidating the structure of selenoprotein S and the mechanism of binding to protein partner we may be able to propose the function of this protein in different cellular mechanisms.
So far, I have gained experience in both experimental techniques and theoretical techniques. My list of technical skills includes working with Linux operating systems, programming languages (Python, Tk-Tcl, R) and simulation software (NAMD, Amber, Gromacs). In addition, my experimental skills encompass the ability to purify membrane proteins, characterization of biomolecules by size exclusion chromatography and intact mass spectrometry, *in vitro* pull-downs, *in vitro* cross-linking, western blotting and preparing biological samples for structural studies such as X-ray crystallography and imaging by negative staining.

B. Positions, Scientific Appointments, and Honors

Positions and scientific appointments

2019- Graduate Research Assistant, Department of Chemistry and Biochemistry, University of Delaware
2018-2019 Member, American Chemical Society
2019-2020 Member, Biophysical Society
2022- Member, American Society for Biochemistry and Molecular Biology

Honors

2019 Cum Laude (B.S in Chemistry with minor in Biochemistry), Universidad Icesi
2019 Cum Laude (B.S in Pharmaceutical Chemistry), Universidad Icesi
2020 CiSE 2020 Best Paper Award

C. Contributions to Science

- **Understanding the dynamical properties of viral envelopes through molecular dynamics simulations.** My early research involved the mechanistic characterization of full-scale viral lipid bilayers. Using molecular modelling and employing massive parallel computers, I investigated physical properties of the HIV-1 viral membrane at the coarse-grained resolution level. Using high performance computing we derived the transverse diffusion rates of lipids in a native environment.

1. Segura CP, Katyal N, **González-Arias F**, Bryer AJ, Perilla JR, Hadden-Perilla JA. Coronavirus through Delaware's Computational Microscope. Dela J Public Health. 2020 Jul;6(2):6-9

2. **Gonzalez-Arias F**, Reddy T, Stone JE, Hadden-Perilla JA, Perilla JR. Scalable analysis of authentic viral envelopes on FRONTERA. Computing in science & engineering. 2020 August; 22(6):11-20.

- **Characterization of selenoprotein S cellular functions.** Our research group is interested in understanding the different cellular roles of membrane-bound proteins, selenoprotein S in this case, as new functions have been characterized in recent years. The lack of structural information on this selenoprotein prevents understanding the primary function of selenoprotein S in different cellular processes such as ERAD, inflammation, viral replication, gene regulation, etc. Thus, our ongoing efforts are directed at characterizing the binding mechanism of selenoprotein S to molecular partners through biochemical techniques and structural techniques. So far, we have summarized the latest insights into the structure, interactome studies, and cellular roles of selenoprotein S.

1. Ghelichkhani F, **Gonzalez FA**, Kapitonova MA, Schaefer-Ramadan S, Liu J, Cheng R, Rozovsky S. Selenoprotein S: A versatile disordered protein. Arch Biochem Biophys. 2022 Nov 30;731:109427
2. Ghelichkhani F., **Gonzalez FA**., Kapitonova MA, Rozovsky S. (2023). Selenoprotein S interacts with the replication and transcription complex of SARS-CoV-2 by binding nsp7. Journal of Molecular Biology, 435(8), 168008. PMCID: PMC9911985.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/fabio.gonzalez.1/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: **Rozovsky, Sharon**

eRA COMMONS USER NAME: **srozovsky**

POSITION TITLE: **Professor of Chemistry and Biochemistry**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Tel Aviv University, Tel Aviv, Israel	B.S.	08/1994	Chemistry
Columbia University, New York, NY	Ph.D.	10/2000	Physical Chemistry
Columbia University, New York, NY	Postdoctoral	12/2002	Biophysical Chemistry
University of California, Berkeley, Berkeley, CA	Postdoctoral	06/2007	Biophysical Chemistry

A. Personal Statement

My research interests are the functions and regulation of selenoproteins, a class of proteins that contain the essential trace element selenium in the form of selenocysteine, identifying the different cellular roles these proteins play, and thus establishing the different ways in which selenium impacts human health. I am particularly interested in a mechanistic understanding of the biological functions of membrane-bound selenoproteins that contribute to the sensing and resolution of stress in cells.

The contributions of my group to this overall area include the studies of the enzymatic activity of selenoprotein S and selenoprotein K, two selenoproteins that are disordered enzymes. We also devised novel methods for their production, which enabled us to characterize their enzymatic activity, redox properties, and the bonds formed by the selenocysteine. In addition, we explored their interactions with various protein partners, including viral proteins, and identified general principles underlying the ability of diverse protein classes to interact with specific segments of selenoproteins S and K.

Our contributions also include the development of a diverse chemical biology toolbox for the preparation and investigation of membrane selenoproteins and other redox enzymes. This includes our efforts to advance expressed protein ligation and genetic code expansion for the generation of selenoproteins, the creation of chemical probes for capturing reaction intermediates, and the development of ^{77}Se NMR spectroscopy for studying sulfur and selenium sites in macromolecules. These innovations have allowed us to examine the distinctive protein environment of selenoproteins and to identify the general themes guiding their reactivity.

For over a decade, I have been actively working to enable students with disabilities to become active participants in the scientific endeavor and thereby broaden the spectrum of students in science. The associated outreach activities over the years include a unique Research Experience for Undergraduates (REU) program at the University of Delaware for students with disabilities. This REU has become a model for other universities and programs that seek to increase integration and inclusion. As a member of the American Chemical Society's Chemists with Disabilities Committee, I have developed and maintained a comprehensive website that provides a wealth of resources for students with disabilities, academic advisors, and administrators.

Ongoing and recently completed projects that I would like to highlight include:
American Heart Association Innovative Project Award
This project focuses on calcium flux regulation through interactions of selenos with sarcoplasmic/endoplasmic reticulum calcium ATPase
7/1/2023 – 6/30/2025

- NSF 2150863

Rozovsky (PI)
09/01/22 – 08/31/25
This project aims to investigate the membrane-bound selenoprotein S and its interactions with other protein partners beyond the protein quality control pathway, using a proteomics-based approach.

- R01 GM121607

Rozovsky (PI)
07/05/17 – 04/30/23
The goal of this project is to characterize the contribution of integral membrane selenoproteins involved in the degradation of misfolded and maturation-defective proteins.

- Unidel Foundation 2020D

Rozovsky (PI)
01/01/2021– 08/31/21
The major goal is to purchase a high-end Eclipse Orbitrap mass spectrometer to accelerate discovery in life sciences, human health, and biopharmaceuticals.

- NSF 1560325

Booksh (PI); Rozovsky (co-PI)
09/01/16 – 08/31/21
This research experience for undergraduates (REU) program is aimed at increasing the participation of students with disabilities in STEM.

Citations:

a Li, F., Lutz, P.B., Pepelyayeva, Y., Arnér, E.S., Bayse, C.A. & **Rozovsky**, S. (2014). Redox active motifs in selenoproteins. Proceedings of the National Academy of Sciences of the United States of America, 111(19), 6976-81. PMCID: PMC4024873. Funding: NSF CAREER MCB-1054447

b Liu, J., Chen, Q. & **Rozovsky**, S. (2017). Utilizing selenocysteine for expressed protein ligation and bioconjugation. Journal of the American Chemical Society 139(9), 3430-3437. PMCID: PMC5824972. Funding: MCB-1054447; training NIH T32GM008550; instrumentation NIH P20GM104316 and P30GM110758

c Scinto, S.L., Ekanayake O., Seneviratne, U., Pigga, J., Boyd, S.J., Taylor, M.T., Liu, J., am Ende, C.W., **Rozovsky** S. & Fox, J. (2019). Dual reactivity trans-Cyclooctenol probes for sulfenylation in live cells enable temporal control via bioorthogonal quenching. Journal of the American Chemical Society, 141(28), 10932-10937. PMCID: PMC6756850. Funding: NIH R01GM121607 and NSF MCB-1616178; instrumentation NIH P20GM104316 and P30GM110758

d Ghelichkhani, F. Gonzalez, F.A., Kapitonova, M.A. & **Rozovsky**, S. (2023). Selenoprotein S interacts with the replication and transcription complex of SARS-CoV-2 by binding nsp7. Journal of Molecular Biology, 435(8), 168008. PMCID: PMC9911985. Funding: NIH R01 GM121607 and NSF MCB-1817651

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022	Professor, University of Delaware, Newark, DE
2022	Organizer of the Franklin Award Symposium on “Intact Mass Spectrometry of Protein Assemblies”
2022	Member of the Organizing Committee of the 12th International Symposium on Selenium in Biology and Medicine, Honolulu, Hawaii, 2022
2022	*Ad hoc* reviewer NIH Basic Mechanism in Cancer Health Disparities Special Emphasis Panel, Oncology Basic and Translation
2021	*Ad hoc* reviewer NIH F31/32 Fellowship, Special Emphasis Panel for Fellowships on Cell Biology, Developmental Biology and Bioengineering
2021	Reviewer Delaware Clinical and Translational Research
2019 – present	Member of the State of Delaware Radiation Authority Oversight Committee
2019	Reviewer NSF Graduate Research Fellowships Program
2018, 2022	Reviewer NSF Molecular Biophysics
2018, 2020	Reviewer NSF Chemistry REU Program
2018 – present	Member, Franklin Institute Committee on Science and the Arts
2016 – 2022	Member, American Chemical Society Committee on Chemists with Disabilities
2016 – 2022	Associate Professor, University of Delaware, Newark, DE
2013 – 2022	Co-director of Research Experience for Undergraduate Program at the University of Delaware
2008 – 2018	Co-organizer of the annual “Delaware Membrane Protein Symposium”

2008 – 2016 Assistant Professor, University of Delaware, Newark, DE

Honors

2021	The 2021 University of Delaware's Arts and Sciences Outstanding Advocacy Award
2019	The 2019 American Chemical Society Stanley C. Israel Regional Award for Advancing Diversity in the Chemical Sciences (co-awarded with Dr. Booksh)
2011	National Science Foundation CAREER Award, National Science Foundation
2009	University of Delaware Research Foundation Innovation Award

C. Contributions to Science

1. *Selenoproteins in the Integrated Stress Response*

A major focus of my group is on membrane-bound selenoproteins that take part in the integrated stress response. In addition to managing cellular stress, these disordered selenoproteins also play a central role in inflammation and the immune response. Working towards our goal to achieve a mechanistic understanding of these membrane-embedded enzymes that rely on selenium for executing their cellular functions, we strive to chart their enzymatic reactions and interactions with their protein partners, the associated protein structures, conformational changes, and dynamics, and ultimately their specific biological roles. At this point, we have characterized the contribution of selenocysteine to the enzymatic activity of several membrane-bound selenoproteins. We were also able to measure the redox potentials of selenoproteins, the rates at which selenylsulfide (Se-S) bonds reform and were able to establish the tendency of selenoproteins' selenocysteine to oxidize. Furthermore, we have studied, *in vivo* and *in vitro,* the formation of shorter selenoprotein variants based on peptide bond cleavage. Most recently, we have recorded the interactions of selenoprotein S with the replication and transcription complex of SARS-CoV-2. In a continuing effort, we examine the interactions of these selenoproteins with their protein partners using mass spectrometry and structural biology.

a. Liu J., Li F. & **Rozovsky** S. (2013). The intrinsically disordered membrane protein selenoprotein S is a reductase. Biochemistry, 52(18), 3051–3061. PMCID: PMC2356620. Funding: NIH P30RR031160, P30GM103519, and NSF CAREER MCB-1054447
b. Liu J., Zhang Z. & **Rozovsky** S. (2014). Selenoprotein K form an intermolecular diselenide bond with unusually high redox potential. FEBS Letters, 588, 3311-3321. PMCID: PMC25117454. Funding: NIH P30GM103519 and NSF CAREER MCB-1054447
c. Ghelichkhani, F., Gonzalez, F.A., Kapitonova, M.A., Schaefer-Ramadan, S., Liu, J., Cheng, R. & **Rozovsky** S. (2022). Selenoprotein S: A versatile disordered protein. Archives of Biochemistry and Biophysics, 731, 109427. PMCID: PMC10026367. Funding: NIH R01 GM121607 and NSF MCB-1817651
d. Ghelichkhani, F. Gonzalez, F.A., Kapitonova, M.A. & **Rozovsky**, S. (2023). Selenoprotein S interacts with the replication and transcription complex of SARS-CoV-2 by binding nsp7. Journal of Molecular Biology, 435(8), 168008. PMCID: PMC9911985. Funding: NIH R01 GM121607 and NSF MCB-1817651

2. *New Tools for Studying Proteins with Reactive Cysteines and Selenocysteines*

My research team advanced our understanding of the role of selenium and sulfur in biology by developing a variety of reagents and methods that enable the investigation of reactive cysteines and selenocysteines in proteins. Together with our collaborators, we developed several innovative, unnatural amino acids for crosslinking applications and studies of protein interactomes. In yet another effort, we co-developed novel strategies and reagents to trap the short-lived intermediates sulfenic (S-OH) and selenenic acids (Se-OH), which play an important role in protein-based signaling. While our reagents and methods were developed with selenium-centered questions in mind, they have broad applicability in the study of various biological systems beyond selenoproteins.

a. Liu, J., Cheng, R. & **Rozovsky**, S. (2018). Synthesis and semi-synthesis of selenopeptides and selenoproteins. Current Opinion in Chemical Biology 46, 41-47. PMCID: PMC6195835. Funding: NIH GM121607 and NSF MCB-1616178

b. Scinto, S.L., Ekanayake O., Seneviratne, U., Pigga, J., Boyd, S.J., Taylor, M.T., Liu, J., am Ende, C.W., **Rozovsky** S. & Fox, J. (2019). Dual reactivity trans-cyclooctenol probes for sulfenylation in live cells enable temporal control via bioorthogonal quenching. Journal of the American Chemical Society, 141(28), 10932-10937. PMCID: PMC6756850. Funding: NIH R01 GM121607 and NSF MCB-1616178; instrumentation: NIH P20GM104316 and P30GM110758
c. Liu, J., Cao, L., Klauser, P. C., Cheng, R., Berdan, V. Y., Sun, W., Wang, N., Ghelichkhani, F., Yu, B., **Rozovsky**, S. & Wang, L. (2021). A genetically encoded fluorosulfonyloxybenzoyl-l-lysine for expansive covalent bonding of proteins via SuFEx chemistry. Journal of the American Chemical Society, 143(27), 10341-10351. PMCID: PMC8310613. Funding: NIH R01 GM121607

3. *Studies of Selenium and Sulfur Sites in Proteins by ^{77}Se NMR Spectroscopy*

My research group develops ^{77}Se NMR spectrometry to probe the structure, dynamics, and function of biological macromolecules. While these efforts serve our interest in selenoproteins, the applications of these techniques extend much further because the NMR-active ^{77}Se isotope is also an excellent surrogate for sulfur, which itself has no isotope suitable for biological NMR spectroscopy. Because of this possibility for nuclei substitution, ^{77}Se NMR spectroscopy enables the study of the multifaceted roles of cysteine and methionine in enzymatic reactions, metal binding, and molecular recognition that underpin sulfur's critical role in proper protein structure and function. Our efforts solved both major problems that had previously stifled the routine use of ^{77}Se to study proteins: The lack of straightforward procedures to enrich proteins with the NMR-active ^{77}Se and the challenges of interpreting the NMR data. To that end, we developed several facile and cost-effective methods for isotopically enriching selenoproteins by ^{77}Se. This, in turn, enabled us to start systematic ^{77}Se-NMR studies on macromolecules that established the range of chemical shifts and the ability to follow chemical reactions in selenium-rich proteins. We built a biologically relevant library of NMR parameters of selenium-containing proteins that are now available to the community as references for the analysis of ^{77}Se-NMR spectra to unlock their information content. Further expanding the ability of ^{77}Se-NMR, we recently mapped the local environment of a selenium site using distance measurements between ^{77}Se and nearby ^{13}C atoms. Consequently, ^{77}Se NMR can now be used to study recognition, binding, and conformational mobility in biological systems.

a. Schaefer, S.A., Dong, M., Rubenstein, R.P., Wilkie, W.A., Bahnson, B.J., Thorpe, C. & **Rozovsky**, S. (2013). ^{77}Se enrichment of proteins expands the biological NMR toolbox. Journal of Molecular Biology, 425(2), 222-231. PMCID: PMC3540199. Funding: NIH P30RR031160, P30GM103519, and NSF CAREER MCB-1054447; training NIH T32GM008550; instrumentation NIH P20GM104316 and P30GM110758
b. Struppe J., Zhang Y. & **Rozovsky**, S. (2015). ^{77}Se chemical shift tensor of L-selenocystine: experimental NMR measurements and quantum chemical investigations of structural effects. Journal of Physical Chemistry B, 119(9), 3643-3650. PMCID: PMC4581879. Funding: P30GM103519 and NSF CAREER MCB-1054447
c. Chen, Q., Xu, S., Lu, X., Boeri, M., Pepelyayeva, Y., Diaz, E., Soni, S., Allaire, M., Forstner, M.B., Bahnson, B. & **Rozovsky**, S. (2020). ^{77}Se NMR probes the protein environment of selenomethionine. Journal of Physical Chemistry B, 124(4), 601-616. PMCID: PMC8088340. Funding: NSF MCB-1616178; training NIH T32GM008550; instrumentation: NIH P30GM110758 and GM110758
d. Quinn, C.M., Xu, S., Hou, G., Chen, Q., Sail, R., Byrd, A. & **Rozovsky**, S. (2022). ^{77}Se-^{13}C based dipolar correlation experiments to map selenium sites in microcrystalline proteins. Journal of Biomolecular NMR, 76(1-2), 29-37. PMCID: PMC9195563. Funding: NSF MCB-1616178; training NIH T32GM133395; instrumentation: NIH P20GM104316 and P30GM110758

4. *Methods Enabling Preparation and Modification of Selenoproteins*

A significant challenge to selenoprotein studies is their low cellular abundance due to the low flux of the specialized biosynthetic pathway that incorporates selenocysteine into proteins. Turning the often-problematic high reactivity of selenocysteine into an asset for engineering proteins, we exploited it for the ligation of expressed protein fragments. Among the many benefits of this method is its ability to prepare otherwise challenging proteins, its capacity to accomplish complicated ligations, and the significant increase in yield compared to other methods. Furthermore, because the method can be applied in a stepwise fashion, it enables the preparations of large proteins by multi-step ligations where several protein fragments are combined into

functional proteins. These preparation strategies not only permit the production of otherwise hard-to-obtain or toxic proteins but can also be used to site-specifically attach bioconjugates.
In a different approach to selenoprotein preparation and modification, unnatural amino acids are first incorporated into proteins *in vivo* and then subsequently modified via specific, bio-orthogonal chemistries. This method based on the expansion of the genetic code was fruitfully explored in my collaboration with Dr. Wang from UCSF. There a precursor of selenocysteine was developed that, once integrated into a target protein, can be turned into selenocysteine via a palladium-mediated cleavage under mild conditions. The initial protein containing an unnatural amino acid is thus turned into a proper selenoprotein. In an extension of this work also a precursor of cysteine was developed that allows for the controlled conversion into cysteine. In both systems, the generation of the desired final protein can be triggered on command and thus at any experimentally desired point in time.

a Liu, J., Chen, Q. & **Rozovsky**, S. (2017). Utilizing selenocysteine for expressed protein ligation and bioconjugation. Journal of the American Chemical Society, 139(9), 3430-3437. PMCID: PMC5824972. Funding: NSF CAREER MCB-1054447 and MCB-1616178; training NIH T32GM008550; instrumentation NIH P20GM104316 and P30GM110758
b Liu, J., Zheng, F., Cheng, R., Li, S., **Rozovsky**, S., Wang, Q. & Wang, L. (2018). Site-specific incorporation of selenocysteine using an expanded genetic code and palladium-mediated chemical deprotection. Journal of the American Chemical Society, 140(28), 8807-8816. PMCID: PMC6082430. Funding: NSF MCB-1616178
c Liu, J., Cheng, R., Wu, H., Li, S., Wang, P.G., DeGrado, W.F, **Rozovsky**, S. & L. Wang. (2018). Building and breaking bonds via a compact S-propargyl-cysteine to chemically control enzymes and modify proteins. Angewandte Chemie, 57(39) 12702-12706. PMCID: PMC6169525. Funding: NIH R01 GM121607 and NSF MCB-1616178
d Cheng, R., Liu J., Daithankar, V. & **Rozovsky**, S. (2022). Applying selenocysteine-mediated expressed protein ligation to prepare the membrane enzyme selenoprotein S. Methods in Enzymology 662, 159-185. PMCID: PMC9126641 Funding: NIH R01 GM121607 and NSF MCB-1616178; instrumentation NIH P20GM104316 and P30GM110758

5. *Gaining a Mechanistic Understanding of the Fundamental Properties of Selenoproteins*

Selenoproteins are crucial for the survival of organisms under stress, despite their low numbers in any genome. Their unique physiochemical properties, such as low pKa, high nucleophilicity, high polarizability, and low redox potential, give them high catalytic efficiencies. However, most selenoproteins have Cys-containing homologues, and the advantages of selenocysteine in proteins remain unknown. Our group's research aims to understand selenoproteins' behavior and elucidate general properties that drive their functionality. We compare selenocysteine's behavior in different protein environments, activation by redox motifs, ability to resist damage by oxidants, and the rate of conformational switching through the formation of selenylsulfide (Se-S) or diselenide (Se-Se) bonds. Through a systematic approach to these comparisons, we are beginning to see more general themes and attributes of selenoproteins emerge.

a Liu J. & **Rozovsky** S. (2013). The contribution of selenocysteine to the peroxidase activity of selenoprotein S. Biochemistry **52** (33), 5514–5516. PMCID: PMC3809988. Funding: NSF CAREER MCB-1054447
b Li, F., Lutz, P.B., Pepelyayeva, Y., Arnér, E.S., Bayse, C.A. & **Rozovsky**, S. (2014). Redox active motifs in selenoproteins. Proceedings of the National Academy of Sciences of the United States of America, 111(19), 6976-81. PMCID: PMC4024873. Funding: NSF CAREER MCB-1054447
c Fredericks, G.K., Hoffmann, F.W., Hondal, R.J., **Rozovsky**, S., Urschitz, J. & Hoffmann, P.R. (2018). Selenoprotein K increases efficiency of DHHC6 catalyzed protein palmitoylation by stabilizing the acyl-DHHC6 intermediate. Antioxidants 7(1), 4. PMCID: PMC5789314. Funding: N/A

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/sharon.rozovsky.1/bibliography/public/