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 the 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 to characterizing the binding mechanism of selenoprotein S to binding 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

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

BIOGRAPHICAL SKETCH

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NAME: Rozovsky, Sharon

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

POSITION TITLE: Associate 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
Tel Aviv University, Tel Aviv, Israel	B.S.	08/1994	Chemistry
Columbia University, New York, NY	Ph.D.	10/2000	Physical Chemistry
University of California, Berkeley, Berkeley, CA	Postdoctoral	06/2007	Biophysical Chemistry

A. Personal Statement

My long-term interests lie in deciphering the molecular function of selenium-containing proteins, identifying the different cellular roles these proteins play and thus establishing the different ways in which selenium impacts human health. The proposed research covers significant ground on my path towards these goals, as it will map the biological functions of membrane-bound selenoproteins that contribute to the sensing and resolution of oxidative stress in cells. Related to this, we will also address questions associated with the takeover of such important membrane proteins by viral reproduction. Since starting my faculty position, my research program has been focused on selenoproteins, employing my expertise in biochemistry, structural biology, physical chemistry of biomembranes, and biophysical chemistry. More recently, we have also incorporated proteomics, chemical biology, cell biology, and computational biology to learn more about selenoproteins in their cellular context. Our ability to employ suitable techniques from a diverse toolbox has undoubtedly contributed to our successes in producing membrane-bound selenoproteins in large quantities despite their low abundance in cells. This has enabled us finally to study their biochemistry in detail, and we are the only group to characterize membrane-bound selenoproteins biochemically.
I have trained 12 Ph.D. students, who have won travel awards and training grants, as well as five M.S. students, 35 undergraduate researchers, and five postdoctoral fellows, one of whom now holds a faculty position. My research and outreach programs over the years have been funded and supported by a single principal investigator R01, several NSF grants, including a CAREER award, and large instrumentation grants. I have organized numerous symposiums and conferences on a variety of scientific topics and continue to straddle multiple fields and interdisciplinary topics.
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 was renewed several times and has become a model for other universities and programs that seek to increase integration and inclusion. As a long-time member of the Chemists with Disability Committee of the American Chemical Society, I have also built and managed an ever-growing web page that offers a large variety of resources for students with disabilities, advisors, and administrators.

Ongoing and recently completed projects that I would like to highlight include:

- R01 GM121607A

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.

- NSF 2150863

Rozovsky (PI)
09/01/22 – 08/31/25
This project focuses on the membrane-bound selenoprotein S which is part of the protein quality control pathway. The objective is to employ proteomics-based approach to identify protein partners that directly interact with selenoprotein S.

- 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 for undergraduate (REU) program aimed at increasing 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., Schaefer-Ramadan, Jun Liu, Rujin Cheng, and Sharon **Rozovsky**. (2022) Selenoprotein S: A versatile disordered protein. Archives of Biochemistry and Biophysics, vol 731, p 109427. Funding: NIH GM121607 and NSF MCB-2150863; Instrumentation: P20GM10431.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022	Professor, University of Delaware, Newark, DE
2022	Reviewer NSF Molecular Biophysics
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 in the Oncology Basic and Translation
2021	*Ad hoc* reviewer NIH F31/32 Fellowship, Special Emphasis Panel for Fellowships on Cell Biology, Developmental Biology and Bioengineering (NIH/CSR)
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	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
2003 – 2007	Postdoctoral Research Assistant, University of California, Berkeley, Berkeley, CA
2000 – 2002	Postdoctoral Research Assistant, Columbia University, New York, NY

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. *Connecting Protein Dynamics to Protein Function*

In my early work, I determined the timescale of conformational rearrangements in the enzyme triosephosphate isomerase using solid- and solution-state NMR spectroscopy. At that time, protein flexibility was a surprising and unexpected novelty, and while accumulating evidence suggested that this mobility within proteins promotes their reactivity, a cohesive and concrete demonstration of this concept was still lacking. My influential work was one of the first studies that clearly showed how protein dynamics couple to enzymatic reaction mechanisms. Starting from a creative application of solid-state NMR to probe enzyme dynamics on the appropriate timescales, the study innovatively established the connection between a protein's conformational exchange, the chemical function of the enzyme, and the energetics of the process. This pioneering work set the standard for contemporary studies of protein structure-function relationships, for which identifying the mobile structural elements and directly demonstrating their effect on function is now expected. Furthermore, I also obtained the crystal structure of the enzyme-substrate complex of triosephosphate isomerase and was able to show how the active site residues align just before the chemical reaction occurs.

a. **Rozovsky**, S. & McDermott, A.E. (2001). The time scale of the catalytic loop motion in triosephosphate isomerase. Journal of Molecular Biology, 310(1), 259-270. PMID: 11419951
b. **Rozovsky**, S., Jogl, G., Tong, L., & McDermott, A.E. (2001). Solution-state NMR investigations of triosephosphate isomerase active site loop motion: ligand release in relation to active site loop dynamics. Journal of Molecular Biology, 310(1), 271-280. PMID: 11419952
c. Jogl, G.(**), **Rozovsky**, S.(**), McDermott, A.E. & Tong L. (2003). Optimal alignment for enzymatic proton transfer: structure of the Michaelis complex of triosephosphate isomerase at 1.2-A resolution. Proceedings of the National Academy of Sciences of the United States of America, 100(1), 50-55. PMCID: PMC140880
d. **Rozovsky**, S. & McDermott, A.E. (2007). Substrate product equilibrium on a reversible enzyme, triosephosphate isomerase. Proceedings of the National Academy of Sciences of the United States of America, 104(7), 2080-2085. PMCID: PMC1794347

2. *Physical Chemistry of Membranes, their Lateral Organization, and Protein-Membrane Interactions*

During my postdoctoral training, I have studied the physical forces that govern the lateral organization of membranes. I reported periodic structures in lipid bilayers that enabled quantitative studies of membrane domains' coordination. This is the first system in which a stable domain arrangement and its spatiotemporal evolution were recorded in lipid bilayers. As such, it contributed to our understanding of biological membranes as fluid yet highly organized surfaces. Additionally, I characterized the binding of a peripheral membrane protein to membranes. Using single-molecule microscopy, I was again able to arrive at a quantitative description of the interactions of a protein with its membrane-bound target.

a. **Rozovsky**, S., Kaizuka, Y. & Groves, J.T. (2005). Formation and spatio-temporal evolution of periodic structures in lipid bilayers. Journal of the American Chemical Society 127(1), 36-37. PMID: 15631436
b. **Rozovsky**, S**.**, Forstner, M.B., Sondermann, H.H. & and Groves, J.T. (2012) Binding kinetics of Epsin N-terminal Homology (ENTH) to lipid bilayers measured by single molecule total internal reflection microscopy. *Journal of Physical Chemistry B* 116(17), 5122-5131. PMID: 22471245

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

My research group developed ^{77}Se-NMR into a new tool 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. This is because the NMR-active ^{77}Se isotope is also an excellent surrogate for sulfur, which itself has no isotope suitable for high-resolution, biological NMR spectroscopy. Such a nuclei substitution has thus enabled the use of ^{77}Se NMR spectroscopy to study 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 work solved two major problems that had 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 challenge of interpreting data. Therefore, 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, where we 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 now can be used by the community to analyze ^{77}Se-NMR spectra and unlock their information content. Expanding ^{77}Se-NMR ability further, we were recently able to map the local environment of a selenium site using distance measurements between ^{77}Se and nearby ^{13}C atoms. Based on this technique, selenomethionine can now be used as a reporter on protein interfaces, ligand binding, and conformational mobility in proteins.

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. PMID: 35320434. Funding: NSF MCB-1616178; training NIH T32GM133395; instrumentation: NIH P20GM104316 and P30GM110758

4. 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. A significant challenge to studying selenoproteins is their low cellular abundance, caused by the specialized, low-flux biosynthetic pathway that incorporates selenocysteine into proteins. One of our avenues turns the often-problematic high reactivity of selenocysteine into an asset for engineering proteins. We can exploit this property for the ligation process of expressed protein fragments to reap many advantages: It enables the preparation of challenging proteins and facilitates complicated ligations. Compared to other methods, the overall yield is significantly increased. Furthermore, the method can be applied in a stepwise fashion, which enables the preparations of large proteins by multi-step ligations where several protein fragments are combined into functional proteins. In a different conceptual approach, we utilize the expansion of the genetic code to prepare functional selenoproteins. Through this technique, we incorporate one of several unnatural amino acids

in vivo during protein expression. Once produced, these amino acids are then modified using residue-specific, bio-orthogonal chemistries. For these efforts, we developed with our collaborators several innovative, unnatural amino acids for crosslinking applications and studies of protein interactomes. In yet another effort, we developed novel reagents to trap sulfenic (S-OH) and selenenic acids (Se-OH), both of which are important short-lived signaling intermediates in proteins. While our reagents and methods were developed with selenium-centered questions in mind, most of them are not at all restricted to selenoprotein studies but, in fact, provide new tools to study a large variety of biological systems.

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. 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. 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 GM121607

5. *Towards a Mechanistic Understanding of Selenoproteins*

A major focus is studies of membrane-bound selenoproteins that lack a stable tertiary structure. These disordered selenoproteins play a central role in managing cellular stress, inflammation, and immune response. Our goal is to achieve a mechanistic understanding of these membrane-embedded enzymes that rely on selenium for executing their cellular functions, i.e., we chart their enzymatic reactions, structures, conformational changes, dynamics, interactions with their protein partners, and specific biological roles. For one, we have characterized the contribution of selenocysteine to their enzymatic activity. Furthermore, we measured the selenoproteins' redox potentials, rates at which selenylsulfide (Se-S) bonds reform, and the tendency of selenocysteine in selenoproteins to oxidize. We have also studied the cleavage of their peptide bonds to form shorter variants *in vivo* and *in vitro*. In addition, we have examined the interactions of these selenoproteins with their protein partners using mass spectrometry. In ongoing efforts, we are characterizing the role of selenoproteins in viral processes by mapping their interactions with viral protein complexes and examining which native cellular activities are disrupted or taken over by the virus for its replication.

a. Liu J., Li F. & Rozovsky S. (2013). The intrinsically disordered membrane protein selenoprotein S is a reductase Biochemistry 52(18), 3051–3061. PMCID: 2356620. 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: 25117454. Funding: NIH P30GM103519 and 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
d. Ghelichkhani, F., Gonzalez, F.A., Kapitonova, M.A., Schaefer-Ramadan, Jun Liu, Rujin Cheng, and Sharon **Rozovsky**. (2022) Selenoprotein S: A versatile disordered protein. Archives of Biochemistry and Biophysics, vol 731, p 109427. Funding: NIH GM121607 and NSF MCB-2150863; Instrumentation: P20GM10431.

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