BIOGRAPHICAL SKETCH

NAME: Cerione, Richard A.

eRA COMMONS USER NAME (credential, e.g., agency login): RAC1CORNELL

POSITION TITLE: Goldwin Smith Professor of Pharmacology and Chemical Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Rutgers College, Rutgers Univer. New Brunswick, NJ	B.A./Ph.D.	05/1979	Biochemistry
Cornell University, Ithaca, NY	Postdoc. Fellow	05/1982	Chemistry
Duke University Medical Ctr., Durham, NC	HHMI Sr. Res. Assoc.	10/1985	Biochemistry

A. Personal Statement

A major goal of my research programs has been to understand the signal transduction pathways that regulate cell growth, differentiation, and development, and how when de-regulated, they contribute to cancer progression. Our laboratories in the Department of Molecular Medicine, and the Department of Chemistry and Chemical Biology, at Cornell University have used a combination of biophysical, chemical, genetic and structural biology-based approaches to study the signaling pathways activated by the two classical GTP-binding protein families, namely, heterotrimeric (large) G-proteins that are essential to the actions of G-protein-coupled receptors (GPCRs), and the Ras-related (small) G-proteins (also known as small GTPases). These goals have been pursued by a number of postdoctoral associates, graduate students, and undergraduates, with many of my trainees (including a total of 40 PhD students and 37 postdoctoral fellows and associates) having gone on to rewarding careers in medicine, and in pharmaceutical and academic research. In our research efforts, we have taken great advantage of my involvement as the Principal Investigator at MacCHESS (Macromolecular crystallography at the Cornell High Energy Synchrotron), and the many resources provided by this facility in the areas of standard macromolecular crystallography, small angle X-ray scattering (SAXS) and now through the development of serial room temperature crystallography. Our studies of small G-proteins have primarily focused on Cdc42, related Rho GTPases and their downstream signaling targets. We have used X-ray crystallography and SAXS, in combination with cryoEM and biochemical methods, to delineate the mechanisms used to regulate these G-proteins and how they transmit signals to a host of biological targets and effectors. This helped us to discover new biological outcomes that are mediated by Cdc42 and related small GTPases important in cancer, including their ability to upregulate the expression of the GTP-binding/protein crosslinking enzyme tissue transglutaminase, which is highly expressed in aggressive cancers, leading us to study its various roles in cancer progression. Those findings then led us to discover previously unappreciated roles for Cdc42 and other Rho GTPases in promoting elevated glutamine metabolism in cancer cells, as well as in the generation and shedding of a relatively new class of signaling vesicles by cancer cells (extracellular vesicles). We further determined how a member of the Sirtuin family of NAD+-dependent deacetylases/deacylases, Sirtuin 1, regulates the formation of these vesicles, as well as how tissue transglutaminase is located on their outer surfaces and plays an important role in their ability to impact the tumor microenvironment and stimulate tumor angiogenesis. These findings motivated us to use structural methods in combination with small molecule chemistry to design new candidate drug molecules that target metabolic enzymes (e.g. the glutaminases) as well as tissue transglutaminase, given the critical roles they play in cancer progression. For the past several years, our laboratory has also been heavily invested in the study of signaling pathways triggered by GPCRs and large G-proteins that are responsible for sensory responses. A particular emphasis has been the Rhodopsin-Transducin-coupled phototransduction system operating in retinal rods that underlies the ability to see in dim light. In these studies, we are using a combination of X-ray crystallography, SAXS, and cryoEM to obtain structural pictures for the complex between light-activated Rhodopsin and its G-protein partner Transducin, as well as for Transducin bound to its biological effector, the cyclic GMP phosphoidesterase. *CryoEM has in fact become a major*

application for our studies and so we will greatly benefit from having access to the National Center for CryoEM Access and Training.

Ongoing and recently completed projects that I would like to highlight include:
R35 GM122575
Cerione (PI)
05/01/17-04/30/22
New Frontiers in Extracellular Signaling. These studies involve examining the roles of signaling activities and how they impact cellular metabolism and the generation and functions of extracellular vesicles in cancer and stem cell biology.

P30 GM124166
Cerione (PI)
08/15/19-06/30/24
MacCHESS Synchrotron Source for Structural Biology. This is a facility to provide the necessary resources and technology to support X-ray crystallography and small angle X-ray scattering experiments by the structural biology community.

R01 CA201402
Cerione (PI); Nakano (co-I)
12/04/15-4/30/25
The Unique Roles of the GTP-Binding Protein/Crosslinking Enzyme Transglutaminase and Signaling Partners in Aggressive Cancers. These studies involve examining the roles played by a unique signaling protein/crosslinking enzyme in aggressive cancers including its functions in shed microvesicles, with an emphasis on glioblastoma.

R01 CA223534
Cerione (PI); Weiss (PI); Lin (PI)
04/01/19-03/31/24
Targeting the dependency of cancer cells on the sirtuin SIRT5. These studies involve examining the roles of SIRT5 in cancer progression and developing small molecule inhibitors targeting this protein.

Citations:

1. Wang, J.B., Erickson, J.W., Fuji, R., Ramachandran, S., Gao, P., Dinavahi, R., Wilson, K.F., Ambrosio, A.L.B., Dias, S.M.G., Dang, C.V., and Cerione, R.A. (2010) Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell* **18**, 207-219. *Cover Article.* PMCID: PMC3078749
2. Antonyak, M.A., Li, B., Boroughs, L.K., Johnson, J.L., Druso, J.E., Bryant, K.L., Holowka, D.A., and Cerione, R.A. (2011) Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proc. Natl. Acad. Sci. USA* **108**, 4852-4857. PMCID: PMC3064359 *Highlight Article*
3. Latifkar, A., Ling, L., Hingorani, A., Johansen, E., Clement, A., Zhang, X., Hartman, J., Fischbach, C., Lin, H., Cerione, R.A.*, and Antonyak, M.A. (2019) Loss of Sirtuin 1 alters the secretome of breast cancer cells by impairing lysosomal integrity. *Dev. Cell* **49**, 393-408.e7. *Cover Article.* *Corresponding author. PMCID: PMC6519475
4. Gao Y., Eskici, G., Ramachandran, S., Poitevin, F., Steve, A.B., Panova, O., Skiniotis, G., and Cerione, R.A. (2020) Structure of the visual signaling complex between transducin and phosphodiesterase 6. *Mol. Cell* **80**, 237-245. PMCID: PMC759677

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021-present Distinguished Professor of Arts and Sciences in Chemistry
2002-2021 Goldwin Smith Professor of Pharmacology and Chemical Biology, Cornell University
2005-present Principal Investigator, Macromolecular Crystallography at the Cornell High Energy Synchrotron
1998-present Full Professor, Department of Chemistry and Chemical Biology,Cornell University
1993-present Full Professor**,** Department of Molecular Medicine, Cornell University
1990-1993 Associate Professor, Department of Pharmacology, Cornell University

1985-1990 Assistant Professor**,** Department of Pharmacology, Cornell University
1982-1985 Senior Research Associate at Howard Hughes Medical Institute, Duke University Medical Center, with Robert Lefkowitz, Reconstitution of Adenylyl Cyclase systems
1979-1982 Postdoctoral Fellow, Cornell University, Professor Gordon G. Hammes, Department of Chemistry, Reconstitution studies of the Chloroplast H^+-ATP Synthetase
1973-1978 Graduate Teaching Assistant, Dept. of Biochemistry, Professor Theodore Chase, Rutgers Univ.

Other Experience and Professional Memberships

2021 Scientific Advisory Board SHY Therapeutics-Development of small molecule therapeutics for Ras and related small GTPases
2021 External Advisory Committee; Mass. General NCI Program Project Grant
2019-present Advisory Board, P01, Glioblastoma and Extracellular Vesicles, University of Kentucky (Markey Cancer Center)
2019 Review panel member, Tumor Cell Biology Study Section
2019 Scientific Organizer, New York Academy of Sciences Workshop on Extracellular Vesicles
2016 Scientific Organizer, Inaugural Keystone Meeting, Exosomes/Microvesicles: Novel mechanisms of cell-cell communication
2016-present Member of Editorial Board, Small GTPases
2013 Co-Director of the Cancer Signaling and Cell Biology Program of the Weill/Cornell Cancer Center
2007-2010 Member, National Institutes of Health Study Section, Molecular Integrative Signal Transduction
2006-2015 Member, Editorial Advisory Board, Biochemistry
2001-2008 Member, Scientific Advisory Board Gene Network Sciences
2000-2005 Member, Editorial Board, Molecular Biology of the Cell
1998 Review Committee for Laboratory of Molecular Biology, National Cancer Institute
1997 Visiting Professor, Louisiana State University Medical Center
1997 Visiting Lecturer, Scripps Research Institute
1996-2000 Member, National Institutes of Health Study Section, Cell Biology and Physiology
1996 Member, Study Section for Department of Defense Breast Cancer Program
1992-1996, 2001-2006 Member, Editorial Board, Journal of Biological Chemistry
1991-1997 Member, Scientific Advisory Board of Cornell Biotechnology Institute

Honors

2014 Juror for Blavatnik Regional Awards for Young Scientists
2013 Plenary Presenter: Workshop on Creativity and Innovation. University of Buffalo
2013 Chancellor's Award for Excellence in Scholarship and Creative Activities
2009 American Association for Advancement of Science Fellow
2000 Frontiers Lecturer, Case Western University Medical Center
1999 Eppley Institute Distinguished Lecturer in Cancer Research, Nebraska Medical Center
1986-1990 PEW Foundation Biomedical Scholar Award
1979-1982 National Institutes of Health Postdoctoral Fellow
1979 American Cancer Society Postdoctoral Fellowship Award

C. Contributions to Science

1. Our initial efforts involved examining the signaling pathways of the EGFR and related tyrosine kinases, such as Neu/ErbB2/HER2. In attempting to identify new signaling partners for the EGF receptor, we discovered and cloned the human Cdc42 GTPase. We then identified many of its key regulators. They include the product of the Dbl oncogene, which is the founding member of an important family of guanine nucleotide exchange factors for the Rho GTPases (oncogenic Rho GEFs), as well as the Cool (for Cloned-out of library)/Pix (Pak interactive exchange factor)/ARGEFH family of GEFs, and the negative regulator, GDP dissociation factor (RhoGDI). These efforts also led us to show that heregulin activates Neu/ErbB2/HER2 by first binding to its receptor, ErbB3, which lacks detectable kinase activity but forms a heregulin-promoted heterodimer with Neu/ErbB2, stimulating Neu/ErbB2 tyrosine kinase activity.
   a. Hart, M.J., Eva, A., Evans, T., Aaronson, S.A., and Cerione, R.A. (1991) Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the dbl oncogene product. *Nature* **354**, 311-314. PMID:195638

b. Shinjo, K., Koland, J.G., Hart, M.J., Narasimhan, V., Johnson, D., Evans, T., and Cerione, R.A. (1990) Molecular cloning of the gene for the human placental GTP-binding protein, Gp (G25K): identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein CDC42. *Proc. Natl. Acad. Sci. USA* **87**, 9853-9857. PMCID: PMC55272
c. Hart, M.J., Maru, Y., Leonard, D., Witte, O.N., Evans, T., and Cerione, R.A. (1992) A GDP dissociation inhibitor that serves as a GTPase inhibitor for the Ras-like protein Cdc42Hs. *Science* **258**, 812-815. PMID: 1439791
d. Carraway, K.L. III, Sliwkowski, M.X., Akita, R., Platko, J.V., Guy, P.M., Nuijens, A., Diamonti, A.J., Vandlen, R.L., Cantley, L.C., and Cerione, R.A. (1994) The erbB3 gene product is a receptor for heregulin. *J. Biol. Chem.* **269**, 14303-14306.

2. We went on to identify many of the downstream signaling targets of Cdc42. Among these were the serine/threonine kinase PAK (p21-activated kinase)-3 which together with one of its binding partners the Cool-2/α-Pix protein has been implicated in a variety of cellular and biological processes including signaling to the the stress activated MAP kinases JNK and p38, the γ-coatomer subunit of the COP1 trafficking proteins which work together with hyperactivated mutants of Cdc42 to promote oncogenic transformation, and mTORC1 which in response to Cdc42-signaling activates the RNA cap-binding complex and cap-dependent splicing in cancer cells. We also made the surprising discovery that the Cool proteins serve both as upstream activators and downstream signaling effectors for Cdc42 and Rac, and by binding both activated Cdc42 and the E3 ubiquitin ligase c-Cbl, Cool-1 significantly influences EGF receptor signaling by extending the signaling lifetime of the receptor.
   a. Bagrodia, S., Taylor, S.J., Creasy, C.L., Chernoff, J., and Cerione, R.A. (1995) Identification of a mouse p21Cdc42/Rac activated kinase. *J. Biol. Chem.* **270**, 22731-22737. PMID: 7559398
   b. Bagrodia, S., Taylor, S.J., Jordan, K.A., Van Aelst, L., and Cerione, R.A. (1998) A novel regulator of p21-activated kinases. *J. Biol. Chem.* **273**, 23633-23636. PMID: 9726964
   c. Wu, W.J., Erickson, J.W., Lin, R., and Cerione, R.A. (2000) The γ-subunit of the coatomer complex binds Cdc42 to mediate transformation. *Nature* **405**, 800-804. PMID: 10866202
   d. Wu, W.J., Tu, S., and Cerione, R.A. (2003) Activated Cdc42 sequesters c-Cbl and prevents EGF receptor degradation. *Cell* **114**, 715-725.

3. Following the discovery of Cdc42 and its various regulators and downstream effectors, we undertook structural approaches, first X-ray crystallography and more recently cryo-electron microscopy, to obtain images of Cdc42 bound to various signaling partners. Some examples are the X-ray structure for Cdc42 bound to RhoGDI which for the first time showed how a geranyl-geranyl moiety attached to the C-terminal end of Cdc42 can fit into a hydrophobic pocket, the X-ray structure for the appendage domain of γ-COP which exhibited striking similarities to a number of adaptors involved in clathrin-mediated endocytosis, and the X-ray structure for the capped RNA-binding protein complex which is activated downstream of Cdc42 and mTORC1, bound to α- and β-importin. More recently, we have extended these approaches to probe GPCR-G protein-effector complexes, using the phototransduction signaling pathway as a starting point.
   a. Hoffman, G.R., Nassar, N., and Cerione, R.A. (2000) Structure of the Rho family GTP-binding protein Cdc42 in complex with the multifunctional regulator RhoGDI. *Cell* **100**, 345-356. *Cover Article.* PMID: 10676816
   b. Hoffman, G.R., Rahl, P.B., Collins, R.N., and Cerione, R.A. (2003) Conserved structural motifs in intracellular trafficking pathways: Structure of the γCOP appendage domain. *Mol. Cell* **12**, 615-625. PMID: 14527408
   c. Dias, S.M.G., Wilson, K.F., Rojas, K.S., Ambrosio, A.L.B., and Cerione, R.A. (2009) The molecular basis for the regulation of the cap-binding complex by the importins. *Nat. Struct. Mol. Biol.* **16**, 930-937. PMCID: PMC2782468
   d. Gao, Y., Westfield, G., Erickson, J.W., Cerione, R.A.*, Skiniotis, G., and Ramachandran, S. (2017) Isolation and structure-function characterization of a signaling-active rhodopsin-G protein complex. *J. Biol. Chem.* **292**, 14280-14289. *Highlight Article*. *Corresponding author. PMCID: PMC5572916

4. While studying the roles of Cdc42 in cancer progression, we discovered a surprising connection between Rho GTPase signaling pathways and the up-regulation of glutaminase C (GAC), a splice variant of GLS and member of the glutaminase family of mitochondrial metabolic enzymes, which satisfies the glutamine addiction of triple-negative breast cancer cells and various other cancer cells. This occurs through the Rho GTPase-dependent activation of the transcription factor c-Jun and through the actions of SIRT5, which protects GAC from ubiquitylation and degradation in cancer cells. We then discovered that the glutaminase family member GLS2 is essential for satisfying the glutamine addiction of luminal subtype, receptor-positive breast cancer cells. These and other efforts were aided by our development of small molecule allosteric inhibitors of these metabolic enzymes that represent anti-cancer drug candidates.
   a. Katt, W.P., Ramachandran, S., Erickson, J.W., and Cerione, R.A. (2012) Dibenzophenanthridines as inhibitors of glutaminase C and cancer cell proliferation. Mol. Cancer Ther. 11, 1269-1278. PMCID: PMC3620022
   b. Lukey, M.J., Greene, K.S., Erickson, J.W., Wilson, K.F., and Cerione, R.A. (2016) The oncogenic transcription factor c-Jun regulates glutaminase expression and sensitizes cells to glutaminase-targeted therapy. *Nat. Commun.* **7**, 11321. PMCID: PMC48374723
   c. Lukey, M.J., Cluntun, A.A., Katt, W.P., Lin, M.J., Druso, J.E., Ramachandran, S., Erickson, J.W., Le, H.H., Wang, Z.E., Blank, B., Greene, K.S., and Cerione, R.A. (2019) Liver-type glutaminase GLS2 is a druggable metabolic node in luminal-subtype breast cancer. *Cell Reports* **29**, 76-88.e7. PMCID: PMC6939472
   d. Greene, K.S., Lukey, M.J., Wang, X., Blank, B., Druso, J.E., Stalnecker, C.A., Zhang, C., Negrón Abril Y, Erickson J.W, Wilson K.F, Lin H., Weiss R.S., and Cerione R. A**. (**2019**)** SIRT5 stabilizes mitochondrial glutaminase and supports breast cancer tumorigenesis. Proc. Natl. Acad. Sci. USA 116, 26625-26632. PMCID: PMC6936584

5. We identified the GTP-binding/protein crosslinking enzyme, tissue transglutaminase (also known as TG2) as an important signaling protein, highly expressed in several aggressive cancers. We showed that it is important for a number of aspects of cancer progression including its ability to block the ubiquitination and degradation of EGF receptors by binding and disabling the E3 ubiquitin ligase, c-Cbl. We further demonstrated that is also plays key roles by crosslinking fibronectin and VEGF on the surfaces of microvesicles shed by cancer cells, enabling these vesicles to bind and alter the proliferative and survival capabilities of non-transformed cells and to stimulate tumor angiogenesis. We also made the unexpected discovery that when TGase-2 induced to adopt a particular conformational state in cells that normally occurs only after it is secreted on microvesicles (i.e. by small molecules we have developed or when expressed as a GTP-binding-defective mutant), it induces the cells to undergo cell death. This now provides a novel strategy for developing small molecule inhibitors that can induce this state in cancer cells and thus offer a potential therapeutic benefit.
   a. Li, B., Antonyak, M.A., Druso, J.E., Cheng, L., Nikitin, A.Y., and Cerione, R.A. (2010) EGF potentiated oncogenesis requires a tissue transglutaminase-dependent signaling pathway leading to Src activation. *Proc. Natl. Acad. Sci. USA* **107**, 1408-1413.PMCID: PMC2824373
   b. Zhang, J., Antonyak, M.A., Singh, G., and Cerione, R.A. (2013) A mechanism for the upregulation of EGF receptor levels in glioblastomas. *Cell Rep*. **3**, 2008-2020. PMCID: PMC3742030
   c. Feng, Q., Zhang, C., Lum, D., Druso, J.E., Blank, B., Wilson, K.F., Welm, A., Antonyak, M.A., and Cerione, R.A. (2017) A class of extracellular vesicles from breast cancer cells activates VEGF receptors and tumor angiogenesis. *Nat. Commun.* **8**, 14450. PMCID: PMC5316898
   d. Katt, W.P., Blobel, N.J., Komarova, S., Antonyak, M.A., Nakano, I., and Cerione, R.A. (2018) A small molecule regulator of tissue transglutaminase conformation inhibits the malignant phenotype of cancer cells. *Oncotarget* **9**, 34379-34397. PMCID: PMC6188150**Complete List of Published Work in**

MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/richard.cerione.1/bibliography/40596517/public/?sort=date&direction=ascending

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: Cody Aplin

eRA COMMONS USER NAME (credential, e.g., agency login): CAPLIN18

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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Minnesota Duluth	BA	05/2018	Chemistry
University of Minnesota Duluth	MS	08/2020	Chemistry
Cornell University	PhD	IP	Biophysics

A. Personal Statement

I am currently a second-year biophysics PhD student at Cornell University, where my research is focused on investigating the structure of important signaling complexes involved in cancer and other diseases. My long-term research goal is to use a structure-guided biophysical approach to study protein complexes that are important to disease progression. My academic background and research training so far have provided me with a strong foundation in protein biochemistry, time-resolved fluorescence spectroscopy, and structural biology. During my undergraduate studies, I was introduced to research through a summer research program working under the direction of Dr. Peter Grundt at the University of Minnesota, Duluth (UMD). My project consisted of synthesizing heterocyclic compounds and analyzing their oxidative properties in regioselective reactions, using a combination of 1D and 2D nuclear magnetic resonance (NMR). This experience caused me to pursue a master's degree at the same university, during which time I conducted research with Dr. Ahmed Heikal and Dr. Erin Sheets, where I characterized genetically encoded FRET probes designed to measure environmental conditions in living cells. This work resulted in several publications and gave me the opportunity to present my findings at the Biophysical Society annual meeting in San Diego, California. Here at Cornell University, I joined the research group of Dr. Richard Cerione. I have been using structural biology techniques including cryogenic electron microscopy (cryoEM), serial room temperature x-ray crystallography, and small-angle x-ray scattering (SAXS) to study the formation of signaling complexes that play important roles in disease progression. The Cerione lab is well known for their work on cell signaling and protein function, including the discovery and structural characterization of the small GTPase Cdc42, understanding the role of extracellular vesicles in cell communication, extensive characterization of the vertebrate phototransduction cascade, and structural analysis of numerous enzymes important to cancer cell growth, metabolism, and survival. Thus, I am well-positioned to perform structural, biochemical, and cellular studies to better understand these important areas of cancer biology. My thesis project will focus on the continuing the Cerione lab's work on studying tissue transglutaminase (TG2), an enzyme that plays an important role in a variety of cancers. TG2-mediated signaling has been shown to have a diverse impact on cancer growth, including encouraging cancer cell survival, growth, and migration and invasion. Using a combination of cryoEM and SAXS, I will investigate the structural basis for TG2 activation and inhibition and use this information to study how TG2 mediates its effects in aggressive cancers. These studies will further my career as an independent researcher by providing me the tools needed to ask and answer challenging biological questions.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1/2018–5/2018	Undergraduate Teaching Assistant, University of Minnesota Duluth
8/2018–8/2020	Graduate Research and Teaching Assistant, University of Minnesota Duluth
8/2020–Present	Graduate Research Assistant, Cornell University

Positions and Employment

Biophysical Society, Student Member

Honors

8/2015-5/2018	Best in Class Scholarship: Half-off tuition awarded based on class rank #1 in high school
8/2015–5/2018	Alworth Scholarship: $20,000 regional scholarship for high achieving students pursuing a degree in the sciences, awarded by the Alworth Memorial Foundation
6/2017–8/2017	Swenson Summer Undergraduate Research Project: 400 hour summer undergraduate research project, including a poster presentation of results, UMD
5/2020	Outstanding Graduate Teaching Assistant Award from Swenson College of Science and Engineering, UMD
8/2020-present	NIH Molecular Biophysics Predoctoral Training Program

C. Contributions to Science

1. **Undergraduate research**: Under the direction of Dr. Peter Grundt, I studied the heterocyclic indole derivative, isatin, a small molecule that is commonly used as a building block in designing a variety of biologically active compounds including antiviral and antitumor drugs. Using 1D and 2D NMR, I characterized the regioselectivity of the oxidation of N-substituted isatins and discovered that the identity of the substituted group plays an important role in regioselective addition of an oxygen to the heterocyclic molecule. This study created new insights into how to modify isatin and opened the door to design new drugs with vastly different chemical properties than its precursors. I presented my results in a poster session for the Department of Chemistry at the University of Minnesota Duluth.

2. **Masters research:** Working with Dr. Ahmed Heikal and Dr. Erin Sheets, I used a variety of fluorescence techniques including time-resolved fluorescence lifetime, time-resolved fluorescence anisotropy, and fluorescence correlation spectroscopy to characterize the ability of novel genetically encoded biosensors to sense changes in their environment. Excitedly, we were able to show that using fluorescence lifetime and anisotropy approaches, the sensors were able to detect changes in the local ionic strength or macromolecular crowding depending on the sensor design. These open new possibilities to probe the dynamic environment of living cells in response to stress and disease. Additionally, we developed new techniques to measure FRET using a combination of molecular brightness and fluorescence fluctuation analysis using fluorescence correlation spectroscopy. This technique allows for a single molecule approach to measure the environmental conditions in living cells, which has the added advantage of being able to investigate local heterogeneity in the intracellular environment by capturing events that are washed out in large ensemble studies.

a) Schwarz, J., Leopold, H., Leighton, R., Miller, R. C., Aplin, C. P., Boersma, A. J., Heikal, A. A., and Sheets, E. D. (2019). Macromolecular crowding effects on energy transfer efficiency and donor-acceptor distance of hetero-FRET sensors using time-resolved fluorescence. Methods Appl Fluoresc 7, 025002.
b) Miller, R. C.*, Aplin, C. P.*, Kay, T. M., Leighton, R., Libal, C., Simonet, R., Cembran, A., Heikal, A. A., Boersma, A. J., and Sheets, E. D. (2020). FRET Analysis of Ionic Strength Sensors in the Hofmeister Series of Salt Solutions Using Fluorescence Lifetime Measurements. The Journal of Physical Chemistry B 124, 34
c) Aplin, C. P., Miller, R. C., Kay, T. M., Heikal, A. A., Boersma, A. J., and Sheets, E. D. (2021). Fluorescence depolarization dynamics of ionic strength sensors using time-resolved anisotropy. Biophysical Journal 120, 1417-1430.

d) Kay, T. M., Aplin, C. P., Simonet, R., Beenken, J., Miller, R. C., Libal, C., Boersma, A. J., Sheets, E. D., and Heikal, A. A. (2021) Molecular Brightness Approach for FRET Analysis of Donor-Linker-Acceptor Constructs at the Single Molecule Level: A Concept. Front Mol Biosci 8, 730394.
*Indicates co-first authors

3. **Doctoral research**: As a second year biophysics student in the laboratory of Dr. Richard Cerione at Cornell University, I am studying the formation of protein-protein complexes and the roles that they play in mediating important cellular effects. In particular, I am focused on studying how a GTP binding/protein-crosslinking enzyme, referred to as type II or tissue transglutaminase (TG2), promotes cell growth, survival, and migration. Previous work in the Cerione Lab discovered that TG2 increases the levels of the epidermal growth factor receptor (EGFR) in glioma cells by sequestering the E3 ubiquitin ligase, c-Cbl, preventing it from marking the receptor for degradation. My thesis project will focus on using cryoEM to investigate the structural basis for TG2-c-Cbl complex to better understand the mechanism through which TG2 mediates its effects. Moreover, we have developed drugs that target TG2 and kill cancer cells, however the mechanism through which these drugs work is not well understood. By combining cryoEM and SAXS analysis, I will determine how these drugs impart structural changes to TG2 and use high resolution characterization of the TG2-drug complex to design better drugs to target TG2. These studies will advance our understanding of how this important enzyme mediates its role in cancer and determine new ways to treat aggressive brain cancers.

D. Scholastic Performance

YEAR	INSTITUTION	COURSE TITLE (* denotes graduate level)	GRADE (C or better is passing)
2014	University of Minnesota Duluth (UMD)	Calculus I	A
2015	UMD	Economics and Society	B
2015	UMD	College Writing	A-
2015	UMD	General Chemistry I	B
2015	UMD	General Chemistry Lab I	A-
2015	UMD	Calculus II	A-
2015	UMD	Concert Band	A
2015	UMD	Survey of American Music	A
2015	UMD	Intermediate Spanish I	A
2016	UMD	General Biology I	A-
2016	UMD	General Chemistry II	A-
2016	UMD	General Chemistry Lab II	A-
2016	UMD	Calculus III	B-
2016	UMD	Concert Band	A
2016	UMD	Chamber Music	A
2016	UMD	General Biology II	B-
2016	UMD	Organic Chemistry I	C
2016	UMD	Organic Chemistry Lab I	A
2016	UMD	Differential Equations	C
2016	UMD	Concert Band	A
2016	UMD	Ethics, Society	A-
2017	UMD	Quantitative Analysis	B
2017	UMD	Quantitative Analysis Lab	B+

2017	UMD	Organic Chemistry II	B
2017	UMD	Organic Chemistry Lab II	A-
2017	UMD	World Regional Geography	A-
2017	UMD	Chemistry Undergrad Research	S (Pass)
2017	UMD	Concert Band	A
2017	UMD	Advanced Writing: Science	A
2017	UMD	General Physics I	A
2017	UMD	General Physics Lab I	A-
2017	UMD	General Physics II	B+
2017	UMD	General Physics Lab II	B
2017	UMD	Physical Chemistry	B
2017	UMD	Physical Chemistry Lab	B+
2017	UMD	Principles of Economics: Macro	B-
2017	UMD	Discrete Math	B
2017	UMD	Intro Probability, Statisitics	C+
2018	UMD	Biochemistry	A-
2018	UMD	Biochemistry Lab	A
2018	UMD	Descriptive Inorganic Chemistry	B-
2018	UMD	Intro to Criminology	A
2018	UMD	Principles of Economics: Micro	A-
2018	UMD	Death of the Universe	B+
2018	UMD	Inorganic Chemistry	C
2018	UMD	Advanced Physical Chemistry I*	A
2018	UMD	Advanced Analytical Chemistry I*	B+
2019	UMD	Physical Chemistry II	B+
2019	UMD	Computational Chemistry*	A
2019	UMD	Methods Molecular Biosciences*	A
2019	UMD	Ecological Statistics*	A
2020	UMD	Teaching Life Sciences*	S (Pass)
2020	UMD	Biophotonics*	A
2020	Cornell University	Frontiers in Biophysics*	SX (Pass)
2020	Cornell University	Protein Structure and Function*	A
2020	Cornell University	Statistical Mechanics*	A
2021	Cornell University	Structural Methods in Biochem*	A