BIOGRAPHICAL SKETCH

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NAME: Eric Smith

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

POSITION TITLE: Postdoctoral Fellow

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Oakland University, Rochester MI	N/A	08/2005	04/2007	Engineering
University of Michigan- Ann Arbor	BS	05/2007	12/2009	Biochemistry
University of Michigan- Ann Arbor	MS	09/2012	05/2013	Chemical biology
University of Michigan- Ann Arbor	PhD	08/2013	08/2018	Chemical biology
University of Michigan- Ann Arbor Postdoctoral Fellow	N/A	08/2018	11/2018	Structural biology and cell biology
Northwestern University, Evanston IL Postdoctoral Fellow	N/A	12/2018	Present	Structural biology

A. Personal Statement

The goal of this project is to use structural biology and biochemistry to study the overall structure and mechanism of DNA gyrase. My graduate training revolved around studying telomeric protein-nucleic acid complexes and has provided me with an excellent background to undertake my proposed studies of DNA gyrase. During my graduate career I studied telomeres and telomerase using a combination of structural biology, biochemistry, and cell biology. I was co-first author on an article published in *Proceedings of the National Academy of the Sciences* that used X-ray crystallography, biochemistry, and cell biology to detail how a mutation altered the structure of the telomeric protein TPP1 inhibiting its ability to stimulate telomerase processivity at chromosome ends. Additionally I helped solve the crystal structure of a telomeric protein complex that is essential for chromosome segregation during meiosis, which was published in *Nature Structural and Molecular Biology*. Currently, I am co-first author on a manuscript that was accepted for publication in *Proceedings of the National Academy of the Sciences* where I performed an in-depth biochemical characterization of the residues required for telomerase recruitment to the telomere. During my graduate career I was not only successful in publishing, but I was also selected as a trainee on the NIH funded Career Training in the Biology of Aging training grant. Additionally, to further advance my training in structural biology, I was chosen to attend the CCP4 X-ray crystallography school at Argonne National Laboratory. Finally, I was the recipient of two graduate student research grants by the University of Michigan. As a postdoctoral researcher I want to build on my training in structural biology by learning how to use cryo-EM to study protein-DNA complexes. My ultimate goal is to be an independent investigator in an academic lab that focuses on the structural biology of proteins that are involved in complex DNA handling processes, such as DNA replication or repair. Dr. Mondragon has an excellent record of training students and postdoctoral fellows to have successful independent scientific careers and the proposed research plan will help me grow as an independent scientist.

a) Bisht K[#], Smith EM[#], Tesmer VM, Nandakumar J. Structural and functional consequences of a disease mutation in the telomere protein TPP1. PNAS. 2016 Nov 15;113(46):13021-13026. PMCID: PMC5135350 #denotes equal contribution

b) Pendlebury DF, Fujiwara Y, Tesmer VM, Smith EM, Shibuya H, Watanabe Y, Nandakumar J. Dissecting the telomere-inner nuclear membrane interface formed in meiosis. Nat Struct Mol Biol. 2017 Dec;24(12):1064-1072. PMCID: PMC5755706
c) Smith EM, Tesmer V, Nandakumar JK. Mapping the complete telomere and telomerase interaction. 2017 Telomeres and Telomerase meeting, Cold Spring Harbor, New York
d) Smith EM, Pendlebury DF, Nandakumar J. Structural biology of telomeres and telomerase. Cell Mol Life Sci. 2019 Nov 14. doi: 10.1007/s00018-019-03369-x. Review. PMID: 31728577

B. Positions and Honors

Positions and Employment

2010-2012 Associate Scientist II, Cayman Chemical, Ann Arbor MI
2012 - 2018 Graduate Student, University of Michigan, Ann Arbor MI
2018 - 2018 Postdoctoral Fellow, University of Michigan, Ann Arbor MI
2018 - Present Postdoctoral Fellow, Northwestern University, Evanston IL

Honors

2016-2018 Trainee on the Career Training in the Biology of Aging Grant (NIH Grant: T32-AG000114)
2018 Rackham Graduate Student Research Grant, University of Michigan
2017 Rackham Travel Grant, University of Michigan
2015 Rackham Travel Grant, University of Michigan
2015 CCP4 Crystallography School at Argonne National Laboratory
2014 Rackham Graduate Student Research Grant, University of Michigan

C. Contributions to Science

1. **Determining residues requisite for telomerase recruitment to telomeres:** Since telomerase is overexpressed in approximately 90% of all cancers, identifying the interface it uses to interact with TPP1 could provide an avenue for the development of anticancer therapeutics. Recent advances in electron microscopy have allowed for the determination of a high resolution structure of tetrahymena telomerase. Although this important structure provides insights into how human telomerase may be recruited the field still lacks any high resolution information about the human telomerase-TPP1 interface. To provide information on how telomerase interacts with TPP1 I identified specific residues in two different domains of telomerase that when mutated negatively affect telomerase recruitment to the telomere. To carry this out I performed a large scale alanine scanning mutagenesis screen. I mutated thirty residues in two different domains of the protein component of telomerase called TERT. To determine which residues were important for telomerase recruitment, but didn't affect the formation of the ribonucleoprotein I performed a series of IF-FISH experiments. After identifying residues that had a defect in telomerase recruitment I determined which mutations had perturbed interactions with TPP1 with a series of biochemical experiments. Ultimately, I identified residues in both domains that affect telomerase recruitment and interaction with TPP1. This study allowed us to create a model of how TPP1 may be interacting with telomerase. Recently, a manuscript describing this work was accepted for publication in the *Proceedings of the National Academy of the Sciences*.
    a. Smith EM, Tesmer V, Nandakumar JK. Mapping the complete telomere and telomerase interaction. 2017 Telomeres and Telomerase meeting, Cold Spring Harbor, New York
2. **Further characterizing the telomerase recruitment surface on TPP1:** Because of its importance for the continued division of stem and germ line cells, There are several diseases that arise from abnormal telomere and telomerase biology, the most notable of which is called dyskeratosis congentia (DC). My graduate work first focused on the telomeric protein TPP1, which is the only protein shown to directly recruit telomerase to chromosome ends, and was found to be mutated in a patient with DC. My study was the first to structurally characterize a DC mutation and specifically demonstrate how this mutation leads to telomere shortening in human cells. I used X-ray crystallography to show that an acidic loop in TPP1 that is critical for recruiting telomerase was altered by this mutation. I utilized biochemical experiments to show that the mutation didn't affect the ability of TPP1 to interact with other shelterin

components, but that it did indeed affect the ability of TPP1 to stimulate telomerase processivity. I created a HeLa derived stable cell line that overexpressed the mutant protein. This allowed me to demonstrate by southern blot that this mutation decreased telomere length over time. I performed IF-FISH experiments with this cell line to show that telomerase recruitment was hampered by this mutation. Together all of these experiments showed that the disruption in the acidic loop of TPP1 lead to dysfunctional telomere maintance by disrupting telomerase recruitment and subsequent action at telomeres.

a. Smith EM, Tesmer V, Bisht K, Nandakumar JK. Structural Consequences of a Single Amino Acid Deletion of TPP1 that is Causative of Dyskeratosis Congenita. 2015 Telomeres and Telomerase Meeting, Cold Spring Harbor, New York

b. Bisht K[#], Smith EM[#], Tesmer VM, Nandakumar J. Structural and functional consequences of a disease mutation in the telomere protein TPP1. PNAS. 2016 Nov 15;113(46):13021-13026. PMCID: PMC5135350 # denotes equal contribution

3. **Telomeric Accessory Proteins and Human Disease:** Telomeres are nucleoprotein complexes that comprise the ends of linear chromosomes. Proteins that associate with telomeres are responsible for a variety of essential roles and mutations in those proteins can lead to serious genetic diseases. POT1 plays an essential role in protecting chromosome ends by binding specifically to single stranded telomeric DNA, thus preventing it from being misrecognized by DNA damage response machinery. However POT1 not only protects chromosome ends, but it also allows telomerase access to those same ends for end replication. Because of this dual role, a variety of cancers have been associated with mutations in the DNA binding domain of POT1. To better understand how these mutations led to replicative immortality and genome instability in cancer cells, I helped determine how telomerase action at telomeres was compromised by POT1 harboring mutations found in cancer. We found that these cancer associated mutations resulted in an increase in telomerase activity, leading to telomere instability that could initiate tumorigenesis. Although telomerase is the foremost enzyme responsible for telomere replication, a second protein complex, called the CST complex, also plays a critical role in this process. After telomerase extends the g-strand of telomeric DNA, the CST complex recruits polymerase alpha to extend the c-strands of chromosome ends. Because of this essential role, CST is a hot spot for mutations that result in telomereopathies. In a second study, I performed in cell assays to show how a disease-associated mutation in CST led to an increase in telomerase recruitment to the telomere, providing a mechanistic understanding for the elongated telomeres observed in the affected patients.

   a. Gu P, Wang Y, Bisht KK, Wu L, Kukova L, Smith EM, Xiao Y, Bailey SM, Lei M, Nandakumar J, Chang S. Pot1 OB-fold mutations unleash telomere instability to initiate tumorigenesis. Oncogene. 2017 Apr 6;36(14):1939-1951. PMCID: PMC5383532

   b. Gu P, Jia S, Takasugi T, Smith E, Nandakumar J, Hendrickson E, Chang S. CTC1-STN1 coordinates G- and C-strand synthesis to regulate telomere length. Aging Cell. 2018 May 17:e12783. PMCID: PMC6052479

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/eric.smith.4/bibliography/57682431/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Scholastic Performance

YEAR	COURSE TITLE	GRADE
2012	Cancer Biology	B+
2012	Cell Biology	A
2012	Organic Principles (Physical Organic Chemistry)	B+
2012	Critical Analysis	S
2012	Research Responsibilities and Ethics	S

YEAR	COURSE TITLE	GRADE
2013	Signal Transduction	A-
2013	Kinetics and Mechanisms	B
2013	Applied Methods of Organic Chemistry	B-
2013	Chemical Biology	B
2015	Gene Regulation	A

Courses were graded on an A, B, C, D, F Scale. Seminar classes are graded as pass/fail (S=Pass and I=Fail).

BIOGRAPHICAL SKETCH

NAME: Tokars, Valerie

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

POSITION TITLE: Research Assistant Professor, Department of Pharmacology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Illinois at Chicago, Chicago, IL	B.Sc.	09/1992	Engineering
Northwestern University, Evanston, IL	Ph.D.	04/1999	Biophysics
Rosalind Franklin University, North Chicago, IL	Post-doc	05/2003	Biophysics

A. Personal Statement

My interest in macromolecular structure and function began as an undergraduate research assistant in a biological laboratory. From my perspective as an engineer, the elegance of biology became abundantly clear. Soon after, I joined the graduate school at Northwestern and during my doctoral training my formal education in biophysics was complemented with training in molecular biology as a means for investigation of cytoskeletal architecture. My interest in macromolecular structures as machines extended into my post doc years where I used structural and biochemical techniques with a hairpin ribozyme as a model system. In 2005, I assumed a research faculty position at UIC where I led an effort in developing an assay to be used for identification of non-covalent small molecules that prevented viral replication by targeting the main protease of a coronavirus. The results of this work led to the identification of a novel series of non-covalent compounds that inhibited the enzyme, and were validated as non-covalent inhibitors by structural, biophysical and kinetic methods. In 2010, I accepted a position of research assistant professor in the Department of Molecular Pharmacology and Biological Chemistry, now the Department of Molecular Pharmacology, at Northwestern University Feinberg School of Medicine to establish a platform focused on protein kinases as targets for small molecule probes and drug candidates. This included establishing protocols for facile throughput of drugs, inhibitors, and molecular fragments for the analysis and development of small molecule inhibitors as *in vivo* tools for life science research. Ultimately these compounds were developed into drug candidates that were successful in safety and toxicology trials and are targeted for first in human studies on Alzheimer's disease.

My continued interest in structural biology has recently been fueled by the advent of cryo-EM. In 2016 I became Operations Director of the Structural Biology Facility at Northwestern University. I was part of the leadership team that led a large reorganization that included merging of cryo-EM into the largely crystallography-focused facility, thus allowing the merging of two powerful atomic resolution techniques. Aside from helping users with all aspects of structural biology research and use of the instrumentation, my responsibilities include financial management, day-to-day operations, user outreach, and developing proficiency in the three branches of the facility. My expertise now includes cryo-EM, X-ray crystallography, and computational approaches. I have first-hand experience in all three techniques and I am familiar with all aspects of modern cryo-EM workflows. I am not only familiar with cryo-EM single particle analysis, but also with many other techniques and approaches, such as imaging of vesicles, lamellas, thin sections, *etc.* I work closely with users helping them design experiments, collect data and process and interpret the results.

In 2016 I obtained a Certificate in Leadership and Management in Core Facilities Management from the Kellogg Business School at Northwestern University and have participated in various workshops including management of large cryo-EM facilities at the New York Structural Biology Center and in organizing local workshops including the Third Coast Workshop in Chicago and Chicago Biomedical Symposium. I have published with a number of different research groups in high profile journals and intend to continue with this trend in the years ahead.

1. Grum (Tokars), V.L., Li, D., MacDonald,R.I. and Mondragón, A. Structures of two repeats of spectrin suggest models of flexibility. *Cell*, **98**, 523-535, 1999.

2. Grum-Tokars, V., Milovanovic, M. and Wedekind, J. (2003). Crystallization and X-ray diffraction analysis of an all-RNA U39C mutant of the minimal hairpin ribozyme. Acta Crystallographica Section D: Biological Crystallography, 59 (Pt 1): 142-145.
3. Grum-Tokars, V., Ratia, K., Begaye, A., Baker, S.C., Mesecar, A.D. (2007). Evaluating the 3C-like protease activity of SARS-Coronavirus: Recommendations for standardized assays for drug discovery. Virus Research, 133(1): 63-73. PMCID: PMC4036818.
4. McNamara, L.K., Brunzelle, J.S., Schavocky, J.P., Watterson, D.M., Grum-Tokars, V. (2011). Site-directed mutagenesis of the glycine-rich loop of death associated protein kinase (DAPK) identifies it as a key structure for catalytic activity. Biochimica et Biophysica Acta, 1813 (5):1068-1073. PMCID: PMC3101106.

B. Positions and Honors

Positions and Employment

1988 - 1992 Undergraduate research with Drs. P. Matsumura and Karl Volz, University of Illinois at Chicago
1992 - 1998 Graduate student with Dr. A. Mondragon Northwestern University
1993 - 1995 NRSA Predoctoral Fellow, Northwestern University
1999 - 2002 Post-doctoral Fellow with Dr. Joseph Wedekind. Department of Biochemistry, Rosalind Franklin University
2002 - 2005 Research Assistant with Dr. Marc Gluckman. Department of Biochemistry, Rosalind Franklin University, North Chicago, IL
2005 - 2010 Research Assistant Professor with Dr. Andrew Mesecar. Department of Pharmacognosy, University of Illinois at Chicago, Chicago, IL
2010 - 2016 Research Assistant Professor with Drs. D. Martin Watterson and Wayne Anderson. Department of Molecular Pharmacology and Biological Sciences, Northwestern University, Feinberg School of Medicine, Chicago, IL
2016 - Present Research Assistant Professor. Department of Pharmacology, Northwestern University, Feinberg School of Medicine Chicago, IL
2016 - Present Operations Director Structural Biology Facility, Northwestern University

Other Experience and Professional Memberships

2002 - 2006 Participant in scientific mentoring program for minority students
2007 Institutional Service, Co-organizer. Midwest Enzyme Chemistry Conference
2012 - 2017 Institutional Service, Poster judge. Center for Molecular Innovation and Drug Discovery
2015 - Present American Chemical Society, Member
2015 - Present Women's Faculty Organization, Member
2016 Institutional Service, Reviewer. Shared Instrumentation Program
2016 Certificate in Leadership & Management in Core Facilities, Kellogg, Northwestern University
2017 Workshop on Management of Large Cryo-EM Facilities, NYSBC, New York, NY
2017 Co-organizer. Third Coast Workshop on Biological Cryo-EM. March 2017.
2018 Co-organizer. Third Coast Workshop on Biological Cryo-EM. May 2018.
2018 Co-organizer. Chicago Biomedical Symposium. October 2108

C. Contribution to Science

1. **Mechanism and structure of spectrin.** As a graduate student, my project was to determine the three-dimensional structure of two repeating units of spectrin using X-ray crystallography. At the time, the structure of a single repeating unit was known. My work demonstrated the relationship between successive units and a mechanism of flexibility was proposed.
   a. Grum (Tokars), V., Li, D.L., MacDonald, R.M., and Mondragon, A. (1999). Structures of two repeats of spectrin suggest models of flexibility. Cell, 98(4): 523-535.
2. **The structure and mechanism of the hairpin ribozyme.** One of my contributions to science was in the study of the hairpin ribozyme. When I started working on the hairpin ribozyme, there was little structural information to elucidate their mechanism. As a post doc, I determined the structure of a hairpin ribozyme that allowed us to propose an atomic mechanism of action for the RNA enzyme. This proposed mechanism has served as the basis for a continued understanding of the biochemistry of RNA.
   a. Grum-Tokars, V., Milovanovic, M. and Wedekind, J. (2003). Crystallization and X-ray diffraction analysis of an all-RNA U39C mutant of the minimal hairpin ribozyme. Acta Crystallographica Section D: Biological Crystallography, 59 (Pt 1): 142-145.

b. Alam, S., Grum-Tokars, V., Krucinska, J., Kundracik, M.L., and Wedekind, J. E., (2005). Conformational heterogeneity at position U37 of an all-RNA hairpin ribozyme with implications for metal binding and the catalytic structure of the S-turn. Biochemistry, 44(44): 14396-14408.
c. Salter, J., Krucinska, J., Grum-Tokars, V., and Wedekind, J. E., (2006). Water in the active site of an all-RNA hairpin ribozyme and the effects of G8 base variants on the geometry of phosphoryl transfer. Biochemistry, 45 (3): 686-700. PMCID: PMC2546605.

3. **Ligand Identification in Targeting the Main Protease of SARS Coronavirus.** One of my key contributions to science during the process of ligand identification for the main protease of SARS coronavirus was to emphasize the importance of standardization in the scientific approach. In surveying the literature on the topic, it was apparent that larger conclusions were difficult to obtain in the efforts of ligand discovery due to a lack of assay standardization. I was able to establish the importance of the construct used as well as the assay conditions and was able to explain away apparent discrepancies in the literature as a result. This optimized assay was adapted to an HTS format to identify scaffolds to be used for development in compounds against SARS protease as well as structurally related proteins.
   a. Grum-Tokars, V., Ratia, K., Begaye, A., Baker, S.C., Mesecar, A.D. (2007). Evaluating the 3C-like protease activity of SARS-Coronavirus: Recommendations for standardized assays for drug discovery. Virus Research, 133(1): 63-73. PMCID: PMC4036818.
   b. Ghosh, A., Xi, K., Grum-Tokars, V, Xu, X., Ratia, K., Fu, W., Houser, K., Baker, S.C., Johnson, M.E., Mesecar, A.D. (2007). Structure-based design, synthesis, and biological evaluation of peptidomimetic SARS-CoV 3CLpro inhibitors. Bioorganic & Medicinal Chemistry Letters, 17(21): 5876-5880. PMCID: PMC2112940.
   c. Ghosh, A.K., Gong, G., Grum-Tokars, V., Mulhearn, D.C., Baker, S.C., Coughlin, M., Prabhakar, B.S., Sleeman, K., Johnson, M.E., Mesecar, A.D. (2008). Design, synthesis and antiviral efficacy of a series of potent chloropyridyl ester-derived SARS-CoV 3CLpro inhibitors. Bioorganic & Medicinal Chemistry Letters, 18(20): 5684-5688. PMCID: PMC2745596.
   d. Jacobs, J., Grum-Tokars V., Zhou, Y., Turlington, M., Saldanha, S.A., Chase, P., Eggler, A., Dawson, E.S., Baez-Santos, Y.M., Tomar, S., Mielech, A.M., Baker, S.C., Lindsley, C.W., Hodder, P., Mesecar, A., Stauffer, S.R. Discovery, synthesis, and structure-based optimization of a series of N-(tert-butyl-2-(N-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease. (2013) Journal of Medicinal Chemistry, 56(2): 534-546. PMCID: PMC3569073.

4. **Drug development in attenuation of the progression of Alzheimer's Disease**. In this work, I was able to assist as a team member in the development of a drug to attenuate the progression of Alzheimer's disease. My role was to use a structure based approach to validate preliminary ligand binding. Using information from biological screens coupled to the structural information derived from x-ray crystallography, the selection for ligand development could be prioritized. This approach resulted in a drug that was able to enter into Phase I toxicology and safety studies thereby reducing overall time and cost for the project.
   a. Watterson DM, Valerie L. Grum-Tokars VL, Roy SM, Schavocky JP, Bradaric BD, Bachstetter AD, Xing B, Dimayuga E, Saeed F, Zhang H, Staniszewski A, Pelletier JC, Minasov G, Anderson WF, Arancio O, and Van Eldik LJ. Development of novel in vivo chemical probes to address CNS protein kinase involvement in synaptic dysfunction. PLoS ONE 2013. 8(6): e66226. doi:10.1371/journal.pone.0066226. PMCID: PMC3694096.
   b. Roy SM, Grum-Tokars VL, Schavocky JP, Saeed F, Staniszewski A, Teich AF, Arancio O, Bachstetter AD, Webster SJ, Van Eldik LJ, Minasov G, Anderson WF, Pelletier JC, Watterson DM. Targeting human central nervous system protein kinases: An isoform selective p38MAPK inhibitor that attenuates disease progression in Alzheimer's disease mouse models. ACS Chem Neurosci. 2015 Apr 15;6(4):666-80. PMCID: PMC PMC4404319.

Complete List of Published Work at:
https://www.ncbi.nlm.nih.gov/sites/myncbi/valerie.tokars.1/bibliography/41167680/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Active Research Support
None

Completed Research Support
1R03MH84162-1A1 (Grum-Tokars, P.I.) 09/24/2008 – 8/31/2010

High Throughput Screening for Identifying Lead Compounds Against 3CLpro
Role: Principle Investigator
The goal of this project was to identify scaffolds to be used in ligand development against SARS coronavirus.

BIOGRAPHICAL SKETCH

NAME: Mondragón, Alfonso

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

POSITION TITLE: Ethel and John Lindgren Professor, Department of Molecular Biosciences

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Universidad Nacional Autónoma de México, México City, México	B.S	03/1981	Physics
Cambridge University Cambridge, England	Ph.D.	01/1985	Biophysics
Harvard University Boston, MA	Postdoctoral	03/1989	Biophysics

A. Personal Statement

The central theme of my work is the understanding of the atomic mechanism of crucial biological macromolecules through a combination of structural and biophysical studies. The goal of my research is to reveal paradigms for understanding fundamental biological processes common to all organisms. My expertise centers on structural biology with an emphasis in crystallographic studies, but extends to Small Angle X-ray Scattering and Transmission Electron Microscopy. All our work involves a wide range of biochemical and biophysical methodologies, such as Single Molecule studies using Magnetic Tweezers, Surface Plasmon Resonance, Analytical Ultracentrifugation, Isothermal Calorimetry, Fluorescence Polarization, Circular Dichroism, and others. I have applied these techniques to a wide range of problems, focusing in particular on proteins that interact with nucleic acids, on the structure of nucleic acids themselves, and on proteins forming the spectrin-based cytoskeleton. Specifically, our work on the structure and function of DNA topoisomerases has provided major breakthroughs in our understanding of the atomic-level mechanism of these enzymes. Our structures of Ribonuclease P (RNase P) were the first structures of large fragments of RNase P, followed by our elucidation of the structure of a ternary complex of bacterial RNase P and tRNA, studies that altered our understanding of the structure, folding, and recognition of large RNA molecules. Our studies on spectrin and spectrin-binding proteins have changed the way we understand the overall structure of spectrin and have provided the structural basis to understand its flexibility at the atomic level. Finally, our studies of metalloregulators continue our efforts to explain how proteins recognize DNA and regulate gene expression. Throughout my independent academic career, my work has been published in excellent journals, including *Blood*, *Cell*, *EMBO J., Nature*, *PNAS*, and *Science,* resulting in continuous funding by the NIH since 1994.

1. Lima,C.D., Wang,J.C. and Mondragón,A. Three-dimensional structure of the 67K N-terminal fragment of E. coli DNA topoisomerase I. Nature, 367, 138-146, 1994.
2. Grum, V.L., Li, D., MacDonald,R.I. and Mondragón, A. Structures of two repeats of spectrin suggest models of flexibility. Cell, 98, 523-535, 1999.
3. Krasilnikov AS, Yang X, Pan T, Mondragón A. 2003. Crystal structure of the specificity domain of ribonuclease P., Nature, 421. 760-764, 2003.
4. Reiter, N.J., Osterman, A., Torres-Larios, A., Swinger K. K., Pan T., Mondragón A. Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA. Nature, 468, 784-789, 2010, PMCID: PMC3058908.

B. Positions and Honors

Positions and Employment

1980 - 1981 Undergraduate research with Dr. I. Ortega-Blake, Universidad Nacional Autónoma de México
1981 - 1984 Graduate student with Drs. A.C. Bloomer and A. Klug. MRC Laboratory of Molecular Biology, University of Cambridge
1985 - 1989 Post-doctoral Fellow with Prof. S.C. Harrison. Department of Biochemistry and Molecular Biology, Harvard University
1989 - 1994 Assistant Professor. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL.

1994 - 2000 Associate Professor. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL

2000 - Professor. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL

Other Experience and Professional Memberships

1996 - 2000 Member. BBCA Study Section, NIH-NIGMS
Director. Center for Structural Biology, Northwestern University
Director. Robert H. Lurie Comprehensive Cancer Center Structural Biology Facility, Feinberg School of Medicine, Northwestern University

2007 - 2010 Member, Scientific Advisory Committee, Stanford Synchrotron Radiation Laboratory

2007 - 2013 Scientific Advisory Committee. Northeast Collaborative Access Team, Advanced Photon Source, Argonne National Laboratory

2008 - 2011 Steering Committee. APS Users Organization

2012 - 2016 Board of Scientific Counselors, National Heart, Lung, and Blood Institute, NIH

2013 - Co-Director, Synchrotron Research Center, Northwestern University
Scientific Director, Life Sciences Collaborative Access Team, Argonne National Laboratory

Honors

1981 – 1984 Overseas Research Scholarship (U.K.)

1981 – 1984 Scholarship from Universidad Nacional Autónoma de México

1982 University Achievement Award, Universidad Nacional Autónoma de México

1985 – 1988 Damon Runyon-Walter Winchell Cancer Fund Research Fellowship

2002 – 2004 Owen L. Coon Chair in Molecular Biology, Northwestern University

2016 - Ethel and John Lindgren Professor, Northwestern University, Evanston IL

C. Contributions to Science

1. **The structure and mechanism of topoisomerases.** One of my major scientific interests is the structure and mechanism of topoisomerases, enzymes responsible for maintaining the topological state of DNA in the cell. When I started working on topoisomerases, there was no structural information to elucidate their atomic mechanism. My laboratory determined the structure of a fragment of *E. coli* topoisomerase I that allowed us to propose the first atomic-level mechanism of action for a topoisomerase. This mechanism has been tested thoroughly by ourselves and others and has served as the basis for understanding other topoisomerases, including type II enzymes. Later, our studies of *E. coli* topoisomerase III provided the first structure of a type IA enzyme in complex with DNA and helped to explain their chemical mechanism of catalysis, which later was shown to have many parallelisms to the mechanism of type II enzymes. We also have studied type IB topoisomerases, including viral and bacterial enzymes alone and in complex with DNA. Our structural and single molecule studies on *M. kandleri* topoisomerase V were instrumental to establish a novel sub-type of type I topoisomerases, type IC. We study topoisomerases in a very comprehensive manner using a variety of techniques ranging from x-ray crystallography to single molecule methods with the goal of providing atomic level understanding of their mechanism that includes not only structural but also dynamical information. Finally, due to their role in cellular processes such as transcription, replication, and recombination, topoisomerases are the target of different chemotherapeutic agents and hence their study is not only relevant and important from a basic biological point of view, but also as it provides information that can be used to develop better antibiotics and anti-cancer drugs.
   a. Lima,C.D., Wang,J.C. and Mondragón,A. Three-dimensional structure of the 67K N-terminal fragment of *E. coli* DNA topoisomerase I. *Nature*, **367**, 138-146, 1994.
   b. Mondragón,A. and DiGate, R. Structure of *E. coli* DNA topoisomerase III, *Structure*, **7**,1373-1383,1999.
   c. Changela, A., DiGate, R. and Mondragón, A. Crystal structure of a complex of a type IA DNA topoisomerase with a single-stranded DNA molecule. *Nature*, **411**, 1077-1081, 2001.
   d. Taneja, B., Patel, A., Slesarev, A., and Mondragón, A. Structure of the N-terminal fragment of topoisomerase V reveals a new family of topoisomerases. *EMBO J.,* **25**, 398-408, 2006. PMCID: PMC138350
2. **Single molecule studies of DNA topoisomerases.** As part of our studies of the mechanism of topoisomerases, we have studied the mechanism of *E. coli* topoisomerases I and III and *M. kandleri* topoisomerase V at the single molecule level. Our initial studies were focused on topoisomerase V, a type IC enzyme, where we showed that this novel topoisomerase relaxes DNA using a constrained swiveling mechanism very similar to the one employed by type IB enzymes, even though there is no structural similarity between the two sub-types. Our single molecule studies of the *E. coli* enzymes were focused on

discovering the mechanistic differences between this highly similar topoisomerases that can both relax supercoiled DNA and catenate/decatenate DNA molecules but with different kinetic characteristics. Our studies showed that pauses between relaxation or catenation events are the major determinant of the kinetics of the process. In this way, we uncovered the kinetic characteristics that are responsible for the mechanistic differences that affect their different roles in the cell. More recently, we designed and built a novel single molecule instrument that combines magnetic tweezers and single molecule fluorescence. Using this combined instrument, we uncovered that type IA topoisomerases are constantly changing conformation while attempting to pass a DNA strand through a gate in the DNA, but only succeed in a minority of cases. This finding has changed the way we understand topoisomerases and other enzymes that change conformation during activity.

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b. Terekhova K., Gunn K.H., Marko J.F., and Mondragón A. Bacterial topoisomerase I and topoisomerase III relax supercoiled DNA via distinct pathways. *Nucleic Acids Res.* **40**, 10432-110440, 2012.
c. Terekhova K., Marko J.F., and Mondragón A. Single-molecule analysis uncovers the difference between the kinetics of DNA decatenation by bacterial topoisomerases I and III. *Nucleic Acids Res*., **42**, 11657-11667, 2014.
d. Gunn, K.H., Marko, J., and Mondragón A., Multiple-attempt dynamics of type IA topoisomerases revealed in an orthogonal single-molecule experiment. *Nat. Struc. Mol. Biol.* **24**, 484-490, 2017.

3. **The structure and mechanism of action of RNase P.** A second area of great interest to me is the structure and mechanism of long non-coding RNA molecules, in particular RNase P. RNase P is the ribozyme responsible for processing several RNA molecules, most notably pre-tRNA. It is found in almost all organisms in all three domains of life. When we initiated our studies of RNase P, there was virtually no structural information on these molecules. Initially, my laboratory elucidated the structure of the specificity domain of RNase P from two different types and showed that RNase P has a common structural core that is stabilized through the use of different peripheral elements, an observation that has now been extended to other large RNAs. Later, we elucidated the structure of the entire RNA component from a thermophilic bacterium. This structure represented one of the largest RNA-only structures known at the time. More recently, we elucidated the structure of a ternary complex formed by the RNA component, the protein component, and tRNA. This structure provided a wealth of information on the way one RNA molecule recognizes another RNA molecule, the mechanism of RNA cleavage by RNase P, and the overall architecture of all RNase Ps. Our structural work has helped make RNase P one of the best characterized catalytic RNA molecules and continues to make RNase P a paradigm for understanding large ribozymes that recognize their substrate in *trans* and show multiple turnover. The structures also have provided information on general RNA architecture and folding motifs. Moreover, RNase P is thought to be a relic from the RNA world and its complexity increases in higher organisms as more proteins form part of the complex, making RNase P an ideal model to study the evolution from RNA-only molecules to sophisticated ribonucleoprotein complexes. For this reason, we have expanded our studies of RNase P to include examples from archaea and eukarya with the long-term goal of understanding the evolution of catalytic function. Thus, understanding the mechanism, structure, and architecture of RNase P promises to provide information on many areas of biology ranging from the structure of long non-coding RNAs to the evolution of molecules from the primordial RNA world.

a. Krasilnikov AS, Yang X, Pan T, Mondragón A. Crystal structure of the specificity domain of ribonuclease P., Nature, 421. 760-764, 2003.
b. Krasilnikov, A.S., Xiao,Y., Pan,T. and Mondragón,A. Basis for Stuctural Diversity in Homologous RNAs, Science, 306, 104-107, 2004.
c. Torres-Larios A., Swinger K. K., Krasilnikov A. S., Pan T., Mondragón A. Crystal structure of the RNA component of bacterial ribonuclease P. Nature. 437, 584-587, 2005.
d. Reiter, N.J., Osterman, A., Torres-Larios, A., Swinger K. K., Pan T., Mondragón A. Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA. Nature, 468, 784-789, 2010. PMCID: PMC3058908.

4. **Structure-function analysis of the spectrin-based cytoskeleton.** I have been interested on the structural basis for the flexibility of the spectrin-based cytoskeleton for a number of years. Spectrin is a long molecule found in the plasma membrane of eukaryotes and links the membrane to the actin-based cytoskeleton. In addition, spectrin is closely associated with the flexibility of the red blood cell. The molecular basis for the extreme flexibility of the erythrocyte has been an intriguing problem, especially as many blood disorders are directly associated with defects in spectrin and/or its interacting partners.

Spectrin is comprised of tandem repeats forming a long chain. Each repeat is a three-helix bundle and for many years it was thought that the linker region connecting the repeats was disordered and many models of spectrin flexibility were based on this assumption. My laboratory provided the first structure of two repeats of spectrin and showed that the linker region is ordered and helical. Furthermore, the initial structures allowed us to propose atomic models to explain flexibility. Additional structures of unusual repeats or multiple repeats confirmed the initial observation that the linker regions are ordered and move independently, and allowed refinement of models of spectrin flexibility. Our structures have served as paradigms for understanding the structure and architecture of the entire spectrin superfamily of proteins and also to interpret many types of experiments testing spectrin properties. Moreover, spectrin is attached to the membrane through specific interactions with ankyrin, which serves as a mediator protein. My laboratory identified the regions in spectrin and ankyrin responsible for the interaction. We showed that a small domain of ankyrin, the ZU5 domain, is responsible for recognition of a particular spectrin repeat. A structure of the ZU5/spectrin complex elucidated the atomic basis for recognition. Both spectrin and ankyrin are associated with many human diseases, including cardiac arrhythmias and blood disorders. My work has helped to map a subset of the mutations to the structures and provide possible explanations for the atomic basis of some of the diseases. Thus, our work on spectrin and ankyrin has not only provided the molecular basis to understand spectrin flexibility and the mechanism of ankyrin/spectrin recognition, it has also served to understand the underlying causes of important human pathologies.

a. Grum, V.L., Li, D., MacDonald, R.I. and Mondragón, A. Structures of two repeats of spectrin suggest models of flexibility. *Cell*, **98**, 523-535, 1999.
b. Kusunoki, H., Minasov, G., MacDonald, R.I., and Mondragón, A. Independent Movement, Dimerization and Stability of Tandem Repeats of Chicken Brain α-Spectrin, *J. Mol. Biol.*, **344**, 495-511, 2004.
c. Ipsaro JJ, Huang L, and Mondragón, A. Structures of the spectrin-ankyrin interaction binding domains. *Blood*, 113, 5385-5393, 2009. PMCID: PMC2689041.
d. Ipsaro, J.J. and Mondragón, A. Structural basis for spectrin recognition by ankyrin. *Blood,* **115**, 4098-5101, 2010. PMCID: PMC2875089.

5. **Structural basis for protein-nucleic acid interactions.** I have a long-standing interest in protein-nucleic acids interactions. In particular, I have been interested on the atomic basis for the recognition of DNA by repressor proteins since I was a post-doctoral fellow working on phage 434 Cro and repressor proteins. In recent years, I have focused my attention on a different family of proteins, metalloregulators. One family of metalloregulators that has been particularly intriguing is the MerR family of proteins, as they regulate gene expression in response to metal levels in the cell in an exquisite fashion. An excellent example is CueR, a protein that responds to copper levels in the cell. Initial structural and biochemical work explained the extreme sensitivity of this protein and the way it recognizes metals. More recently, structures of CueR with and without metal in complex with DNA have uncovered the molecular basis for DNA recognition. These structures represent the first examples of a metalloregulator in complex with DNA in both the activator and repressor form and explain clearly the molecular basis for recognition and function. In addition, studies of another metalloregulator, Zur, a zinc sensor member of the Fur family of proteins, showed how members of this family of proteins recognize and bind DNA. Interestingly, although both proteins are metalloregulators, the structures are not related and the mechanism of DNA and metal recognition is completely different. In our collaboration with the O'Halloran Laboratory, my role has been to solve all the crystal structures and help with the design of the experiments and interpretation of all the results.

a. Mondragón,A. and Harrison,S.C. The Phage 434 Cro/OR1 Complex at 2.5 Å Resolution. *J. Mol. Biol*., **219**, 321-334, (1991).
b. Changela A., Chen K., Xue Y., Holschen J., Outten C.E., O'Halloran T.V. and Mondragón A. Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. *Science*, **301**, 1383-1387, 2003.
c. Gilston, B.A., Wang, S., Marcus, M.D., Canalizo-Hernández, M.A., Swindell, E.P., Xue, Y., Mondragón, A., and O'Halloran, T.V. Structural and mechanistic basis of zinc regulation across the E. coli Zur regulon. *PLoS Biol.* **12**, e1001987. 2014. PMCID: PMC4219657.
d. Philips, S.J., Canalizo-Hernandez, M., Yildirim, I., Schatz, G.C., Mondragón, A., and O'Halloran, T.V., Allosteric transcriptional regulation via changes in the overall topology of the core promoter, *Science***,** **349**, 877-881, 2015. PMCID: PMC4617686.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/alfonso.mondragon.1/bibliography/41140052/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance
Ongoing Research Support

R35GM118108 (Mondragón, PI) 9/30/17 – 8/31/22 1.69 academic
National Institutes of Health (NIGMS) Annual direct costs: $358,549 2.5 summer
Structural and biophysical studies of proteins, nucleic acids, and their complexes
Role: PI
The goal of this proposal is to study the structure and mechanism of DNA topoisomerases from a structural and biophysical perspective. The work involves crystallography, cryo-EM, and single molecule experiments and is focused on type I topoisomerases and also bacterial gyrase. In addition, studies of the structure of bacterial, archaeal, and eukaryotic RNase P are also part of the proposal. The RNase P studies seek to provide structural information on this universally conserved ribonucleoprotein complexes and try to understand the evolution of RNase P. Finally, the third area of interest is type IIIA CRISPR complexes, where we are seeking to understand this important system using a combination of crystallographic and EM studies.

P30CA060553 (Platanias, PI) 8/10/18 – 07/31/23 0.90 academic
National Institutes of Health (NCI) ($14,220 salary for director) 0.00 summer
The Robert H. Lurie Comprehensive Cancer Center - Structural Biology Core Facility
Role: Director, Structural Biology Core Facility
The goals of this Cancer Center Support Grant are to conduct and support cancer research and to integrate cancer-related research throughout the university; to coordinate and integrate cancer-related activities of the University including community outreach initiatives; to develop and conduct cancer education programs; to promote and participate in state-of-the-art care of cancer patients at the affiliated hospitals of the McGaw Medical Center of Northwestern University and; to develop and implement the initiatives in cancer prevention and control research. These goals are accomplished through the activities of the established programs and shared resources.

Completed Research Support

NIGMS, NIH (R01-GM057692) (A. Mondragón, PI) 12/1/2010 – 11/30/2016
National Institute of Health Structural studies of a spectrin/ankyrin complex. Role: Principal Investigator
The major goal of this project was to understand the molecular basis of ankyrin recognition by spectrin. The focus on the project was on the spectrin binding domain of ankyrin and the interactions of different domains to form a supramodule. A combination of crystallographic and biophysical studies were used to fully characterize the binding domain and to form a complex of spectrin and ankyrin.

Searle Funds at the Chicago Community Trust (L-005A) (Alfonso Mondragón, co-P.I) 1/1/2014 – 12/31/2016
Chicago Biomedical Consortium. Role: Co-Principal Investigator
This proposal funds new efforts to raise antibodies against RNA loops and RNase P. The antibodies will be produced at the University of Chicago Synthetic Antibody Facility. It is a collaboration with Prof. J. Piccirrilli and the long term goal is to develop tools for crystallization of RNA molecules.

NIGMS, NIH (R01GM058443) (Alfonso Mondragon, P.I.) 5/1/2012 – 2/28/2017
National Institutes of Health Structural Studies of the RNA component of RNase P. Role: Principal Investigator
The major goal of this project is to solve the structure of bacterial RNase P holoenzyme and its complexes with substrate and product s well as to study RNase P from other organisms.

NIGMS/NIH (R01GM051350) (Mondragón, PI) 1/1/2017 – 11/30/2017 (NCE until 11/30/2018)
National Institute of Health. Structure/Function Studies of Type I Topoisomerases. Role: Principal Investigator
The goal of this project is to study the structure and function of topoisomerases, including *E. coli* topoisomerase I and III, *M. kandleri* topoisomerase V, and bacterial gyrase in complex with DNA. The proposal encompasses crystallographic, single molecule, EM, and biophysical studies.

C-075 (Mondragón, co-PI) 2/1/2017 – 1/31/2019
Chicago Biomedical Consortium. Role: Co-Principal Investigator
This proposal funds a new project to study the conformational dynamics of bacterial T-box riboswitches by a combination of biochemical and single molecule experiments. It is a collaboration with Prof. Jingyi Fei at The University of Chicago.