BIOGRAPHICAL SKETCH

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NAME: ALIREZA GHANBARPOUR

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POSITION TITLE: Assistant 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)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Shahid Beheshti University	BS	08/2008	05/2012	Chemistry
Sharif University of Technology	MS	08/2012	05/2014	Inorganic Chemistry
Michigan State University	PhD	08/2014	03/2019	Chemistry
Yale University (NRSA F32 Postdoctoral Fellow)	-	04/2019	09/2021	Cell Biology
MIT (Postdoc Associate)	-	10/2021	Present	Structural Biology/Biochemistry

A. Personal Statement

My research group focuses on elucidating the molecular determinants of substrate specificity in ATP-dependent AAA proteolytic machines and understanding how adaptors and anti-adaptors influence the specificity of AAA proteases. These molecular machines play a crucial role in removing aberrant or unneeded intracellular proteins, from bacteria to human mitochondria, thereby reshaping the proteome in response to cellular needs. Despite the critical importance of substrate specificity and adaptor-mediated degradation, the molecular mechanisms governing these processes are still only understood in a rudimentary fashion. Our research employs a multidisciplinary approach, utilizing cryo-EM, mass spectrometry, genetics, and biochemical assays to dissect the detailed molecular mechanisms underlying the specificity of these enzymes. This strategy provides a foundation for the rational design of therapeutics for human diseases associated with the dysregulation of mitochondrial AAA proteases. In the long term, we aim to leverage this knowledge to design high-throughput screening methods capable of identifying small-molecule modulators. These modulators will help develop therapeutics that regulate the interactions between AAA proteases and their adaptors, offering new treatment options for various diseases.

My training across various fields has shaped my vision for creating a lab that promotes interdisciplinary research and fosters the training of a diverse group of scientists. I trained as a chemist during my undergraduate and master's degrees, focusing on crystal engineering of small molecules and inorganic catalysis. During my PhD at Michigan State University in the Chemistry Department, I applied rational protein design to create novel model systems and molecular tools. I also gained expertise in protein crystallography, determining over 150 crystal structures that depicted the engineering steps of either a photoactive protein or an allosterically regulated protein, both of which I developed from a "naïve" protein fold. During my first postdoc in the Cell Biology Department at Yale, under the guidance of Dr. Reinisch, I expanded my expertise in cell and membrane biology. I received an F32 postdoctoral fellowship to study the de novo formation of organelles such as autophagosomes. My research led to a new model in cell biology, showing how lipid transfer proteins and scramblases cooperate in organelle biogenesis—work that has been cited over 170 times in just three years of publication. To broaden my skills in cryo-EM, mass spectrometry, and biochemistry, I pursued a postdoc at MIT, where I studied bacterial AAA proteases under the mentorship of Bob Sauer, as his last postdoc, and Joey Davis. I generated a collection of structural and biochemical data on bacterial AAA proteases, including the first structure of an adaptor-substrate

delivery complex and substrate-engaged states prior to unfolding in AAA protease field. The structures I solved, along with findings from other groups, have revealed how the core machinery of AAA+ proteases functions. However, the features that control substrate specificity and how adaptor proteins modulate this specificity, particularly in the case of mitochondrial proteases, remain less well understood. My research program aims to address this critical gap in knowledge.

Mentoring. During my Ph.D. and two postdoctoral positions, I had the opportunity to mentor eleven undergraduate students, seven of whom were women and one from a minority group. I also mentored two Ph.D. rotation students—one an international student and the other from a minority group—and supervised a technician whom I recruited and hired. Additionally, I taught nearly every semester during my Master's and Ph.D. studies, serving as a teaching assistant for General Chemistry (lab and recitation), Introductory Organic Chemistry (lab and recitation), and Analytical Chemistry (lab and recitation). My enthusiasm and work ethic as a teaching assistant were recognized with a merit award from the Chemistry Department at Michigan State University. These mentorship and teaching experiences provided me with the opportunity to work alongside individuals from diverse backgrounds and perspectives. My lab currently includes a senior scientist, a staff scientist, and a research technician, all from international backgrounds, with one female team member. They bring diverse and complementary expertise to the lab. Three Ph.D. rotation students are planning to rotate in my lab this semester and next, including two female scientists and one from a minority group. My past mentorship experiences have equipped me to train lab members in a diverse, welcoming, and productive environment. Beyond advancing my research, I am deeply committed to training the next generation of scientists from diverse backgrounds, as I believe that promoting diversity and inclusion in both the classroom and the research lab is essential for academic success.

1. **Ghanbarpour, A.**; Sauer, R. T; Davis, J. H.; *A proteolytic AAA+ machine poised to unfold a protein substrate, BioRxiv.* ***Nat. Commun****.* (under revision).
2. **Ghanbarpour, A*.;** Telusma, B.; Powell, B.; Zhang, J. J.; Bolstad, I.; Vargas, C.; Keller, s.; Baker, T.A; Sauer, R. T; Davis, J. H.; *An asymmetric nautilus-like HflK/C assembly controls FtsH proteolysis of membrane proteins.* ***EMBO*** (under revision) ***** *corresponding author*
3. **Ghanbarpour, A.**; Cohen, S.; Fei. X.; Kinman, L.; Zhang, J. J.; Bell, T.; Baker, Tania A.; Davis, J. H.; Sauer, R. T. A closed translocation channel in the substrate-free AAA+ ClpXP protease diminishes rogue degradation. ***Nat. Commun***, 14, 7281 (2023)
4. **Ghanbarpour, A.***; Fei. X.*; Baker, Tania A.; Davis, J. H.; Sauer, R. T.; The SspB adaptor drives structural changes in the AAA+ ClpXP protease during ssrA-tagged substrate delivery. ***PNAS****,120,* e2219044120 *(*2023). *Equal Contributors
5. **Ghanbarpour, A.***; Valverde D. P.*; Melia, T. J.; Reinisch, K. M., A Model for a Partnership of Lipid Transfer Proteins and Scramblases Partner in Membrane Expansion and Organelle Biogenesis. ***PNAS****, 118, e2101562118 (2021).* *Equal Contributors,

Service to the scientific community:

- 2024 Editorial Advisory Board, Protein Science Journal.
- 2024 Ad hoc reviewer for *Biochemical Society Transactions* journal
- 2022-2024 DEI postdoc representative, Department of Biology.
- 2021 and 2022 Instructor at cryo-EM workshops for MIT outreach program.
- 2022 Session chair at 8th New England Cryo-EM Symposium (MIT).
- 2022 Undergraduate mentor at MIT summer outreach program for minority groups.
- 2017 Mentor for a STEM summer outreach program (MSU).

B. Positions and Honors

Positions

TITLE/POSITION	START DATE	END DATE	FIELD	INSTITUTION	SUPERVISOR
Graduate Student Teaching and Research Assistant	08/2014	03/2019	Chemistry	Michigan State University	Prof. James H. Geiger

Postdoctoral Associate/Fellow	04/2019	09/2021	Cell Biology	Yale University	Prof. Karin M. Reinisch
Postdoctoral Associate	10/2021	08/2024	Biology	Massachusetts Institute of Technology	Profs. Robert T. Sauer and Joseph H. Davis
Assistant Professor	08/2024	Present	Biochemistry and Molecular Biophysics	Washington University School of Medicine	-

Honors and Awards

2021 Poster Presentation Award, Molecular Membrane Biology Conference, GRC (online)
2020 National Research Service F32 Postdoc Award (Grant number 1F32GM137568-01)
2018 Ph.D. Continuation Fellowship, Faculty of Natural Science, Michigan State University.
2018 Education Merit Award for Excellence in Teaching (Superb Award), Department of Chemistry, Michigan State University
2018 Travel Award Fellowship, Biophysical Society Conference, Spring 2018
2017 Ph.D. Continuation Fellowship, Faculty of Natural Science, Michigan State University

C. Contribution to Science

Undergraduate studies (2008-2012)

As an undergraduate at Shahid Beheshti University, I worked under Professors Amini and Khavasi, focusing on small molecule crystallography and crystal engineering. My research aimed to understand the interactions driving the crystal packing of amidic ligands and use this knowledge to design crystals with desired properties. I synthesized amidic ligands with halogen substituents and found that halogen-halogen interactions play a key role in crystal packing, even with stronger interactions like hydrogen bonding. I also showed that by substituting quinoline for naphthalene, the interaction shifted to halogen-π, tuning the crystal lattice. Additionally, I collaborated on using metal-organic frameworks for ion separation and lead(II) ion absorption from water samples.

1. Khavasi, H. R.; **Ghanbarpour, A.**; Tehrani, A. A. Synthon crossover between halogen-π and halogen-halogen interaction. *Cryst. Eng. Comm*. 2014, *16*, 749-752.
2. Salarian, M.; **Ghanbarpour, A.**; Behbahani, M.; Bagheri, S.; Begheri, A. Analytical application of metal-organic framework sustained by nanosized Ag_{12} cuboctahedral node as a novel sorbent for selective solid-phase extraction of ultra-traces lead ions*. Microchemica Acta*., 2014, *181*, 999-1007.
3. Mohammadnezhad, G.; **Ghanbarpour, A.**, Amini M. M., Ng, S. W.; Di-μ-azido-κ4*N*1:*N*1-bis[(1,10-phenanthroline-κ2*N*,*N*')(thiocyanato-κ*N*)lead(II)] *Acta Cryst*., E66, m1120 (2010).
4. Mohammadnezhad, G.; **Ghanbarpour, A.**; Amini, M.M.; Ng, S.W; *catena*-Poly[[(1,10-phenanthroline-2*N*,*N*')lead(II)]–azido-2*N*1:*N*3–nitrito-3*O*,*O*':*O*'-[(1,10-phenanthroline-2*N*,*N*')lead(II)]-di–azido-4*N*1:*N*1, *Acta Cryst.*, E66, m821 (2010).

Master's studies (2012-2014)

I continued my research as a master's student in the field of catalysis at Sharif University of Technology in Dr. Bagherzadeh's lab, where I finished two key projects. First, I synthesized new Mn (II) complexes, which were structurally characterized by X-ray crystallography for the aerobic epoxidation of olefins. This method developed a greener and cheaper system for producing epoxides with various industrial interests. Second, I determined the effects of supramolecular interactions, such as the halogen….halogen interaction, in governing the crystal packing of inorganic compounds, such as Mn(II) complexes based on amidic ligands.

1. Khavasi, H. R.; **Ghanbarpour, A.,** Tehrani, A. A. The role of intermolecular interactions involving halogens in the supramolecular architecture of a series of Mn (II) coordination compounds. ***RSC Advances***, 2016, *6*, 2422-2430
2. Bagherzadeh, M.**; Ghanbarpour, A.;** Khavasi, H. R. Highly efficient aerobic epoxidation of cyclic olefins in mild conditions by a novel binuclear manganese (II) complex containing *N*-(4-nitrophenyl) picolinamide ligand. ***Catalysis Communication****s,* 2015, *65*, 72-75.

3. Bagherzadeh, M.; Haghdoost, M. M.; **Ghanbarpour, A.**; Payab, M.; Khavasi, H. R.; Ellern, A.; Woo, L. K. New Molybdenum (VI) Catalyst for the Epoxidation of Alkenes and Oxidation of Sulfides: an Experimental and Theoretical Study. ***Inorg. Chim. Acta.***, *411*, 62-66 (2014).

Ph.D. studies (2014-2019)

At Michigan State University (MSU), I shifted my research interests from inorganic chemistry to structural biology and protein engineering, under the supervision of Professor Geiger. I employed a "protein redesign" strategy to develop a model system to mimic microbial rhodopsin isomerization in a single crystal using human cellular retinoic acid binding protein II as a template. A long-standing question was how rhodopsins manage the photoisomerization of retinal selectively around one double bond, given that the photoisomerization of retinal in solution leads to a broad distribution of isomers. I created a new protein-based model system that could drive photoisomerization of retinal from all-*trans* to 13-*cis* and dark isomerization back to all-*trans* retinal in a single crystal and solution. I also discovered a novel protein photo-switch that does not require chromophore isomerization. Instead, the conformation of a single amino acid alters upon irradiation inside the binding cavity, leading to a pK_a change in the chromophore and subsequent shifting of the absorption wavelength by more than 100 nm. In a second study, I generated a new class of allosterically regulated metalloproteins by redesigning the domain-swapped dimer of human cellular retinol binding protein II to allow that triggering by ligand binding or the reduction potential of the environment. In this study, I created a new allosteric protein and confirmed all of the changes and engineering steps by atomic resolution structures, CD spectroscopy, and binding assays.

I also discovered that some variants of hCRBPII can generate a domain-swapped trimer. I devised a novel method to control the folding pathway in domain-swapped proteins by installing a strategic disulfide bond. This work was highlighted on the front cover of *ChemBioChem*. In a third project, I investigated the role of amino-acid insertion in the hinge loop region on the overall conformations of domain-swapped dimers of human cellular retinol binding protein II. Since in 3D domain swapping, the swapped region adopts its native conformation, the only part of the domain-swapped structure that undergoes a large structural change is the “hinge loop” region that links the two structurally similar domains. I demonstrated that small changes in the hinge loop sequence can have profound effects on the resulting structure, illustrating the potential for creating significantly altered protein structures with small changes in sequence. During my work as a Ph.D. student, I mentored nine undergraduate MSU students and two high school students (in local STEM programs), which allowed me to further develop my mentorship and leadership skills. I also was a teaching assistant for several chemistry courses at MSU, which enabled me to hone my teaching skills in preparation for my future career.

1. Santos, M.*; Sheng, W.*; Salmani, R. E.; Nick, S.T., **Ghanbarpour, A.**, Gholami, H.; Vasileiou, C.; Geiger, J.; Borhan, B. Design of Large Stokes Shift Fluorescent Proteins Based on Excited State Proton Transfer of an Engineered Photobase, ***J. Am. Chem. Soc.***, 143, 15091 (2021). *Equal Contributors
2. **Ghanbarpour, A.**; Santos, E. M.; Pinger, C.; Assar, Z.; Hossaini Nasr, S.; Vasileiou, C.; Spence, D.; Borhan, B.; Geiger, J. H. Human Cellular Retinol Binding Protein II Forms a Domain-Swapped Trimer Representing a Novel Fold and a New Template for Protein Engineering, ***ChemBioChem***, 21, 3192-3196. (2020). ***Highlighted on the cover.***
3. **Ghanbarpour, A.**; Pinger, C.; Xiangshu, J.; Assar, Z., Santos, E.; Nosrati, M.; Pawlowski, K.; Spence, D.; Vasileiou, C.; Borhan, B.; Geiger, J. H. Engineering the hCRBPII Domain-Swapped Dimer into a New Class of Protein Switches, ***J. Am. Chem. Soc.,*** 141, 43, 17125-17132 (2019).
4. **Ghanbarpour, A.**; Nairat, M.; Nosrati, M.; Santos, E. M.; Vasileiou, C.; Dantus, M.; Borhan, B.; Geiger, J. H. Mimicking Microbial Rhodopsin Isomerization in a Single Crystal. ***J. Am. Chem. Soc.,*** 141,1735-1741 (2019).

Post-doctoral studies: Yale (2019-2021)

I joined Prof. Karin Reinisch’s lab at Yale, where I was awarded an NRSA F32 fellowship to investigate how autophagosomes form at contact sites in the ER. ATG2A is a lipid-transfer protein tethering between the ER and autophagosomes and transferring bulk lipids between organelles. I determined that ATG2A does not work in isolation but is part of a larger machinery. Notably, I identified integral-membrane proteins present at contact sites, such as TMEM41B and VMP1 in the ER and ATG2 interactors. I determined that all these membrane proteins act as lipid scramblases, and proposed a model wherein ATG2, cooperating with the scramblases, transports lipids in bulk from the ER to the nascent autophagosome. ATG2 permits lipid transport from the ER to the seeding vesicle, with TMEM41B and VMP1 re-equilibrating the leaflets of the ER as lipids are extracted, and ATG9 in the acceptor seed scrambling ER lipids as they are inserted. My first-author *PNAS* paper describing

this study has been cited more than 170 times. I also verified that bacterial homologs of TMEM41B and VMP1, containing the VTT domain, act as scramblases. This work led to assigning a new function to a relatively large and unknown family of membrane proteins carrying the VTT domain.

1. **Ghanbarpour, A.***; Valverde D. P.*; Melia, T. J.; Reinisch, K. M., A Model: Lipid Transfer Proteins and Scramblases Partner in Membrane Expansion and Organelle Biogenesis. ***PNAS**, 118, e2101562118 (2021).* *Equal Contributors, ***Recommended in Faculty Opinions.***
2. Huang, D.; Xu, B.; Liu, L.; Wu, L.; Zhu, Y.; **Ghanbarpour, A.**; Wang, Y.; Chen, F-J.; Lyu, J.; Hu, Y.; Kang, Y.; Zhou, W.; Wang, X.; Ding, W.; Li, X.; Jiang, Z.; Chen, J.; Zhang, X.; Zhou, H.; Zhong Li, J.; Guo, C.; Zheng, W.; Zhang, X.; Li, P.; Melia, T.; Reinisch, K.; Chen, X-W. TMEM41B acts as an ER scramblase required for lipoprotein biogenesis and lipid homeostasis**. *Cell Metabolism**, 33, 1, (2021).* *Equal Contributors

Post-doctoral studies: MIT (2021-2024)

During my time in the Sauer and Davis labs at MIT, I determined more than 15 cryo-EM structures to investigate the molecular mechanisms of soluble and membrane bound AAA proteases from *E.coli* that led to new insight into the mechanism of substrate specificity and adaptor interactions in bacterial AAA protease family. For example, I obtained the first high-resolution structure of a AAA+ protease poised to unfold a stable native substrate showing a large conformational change to potentially amplify the interaction of the enzyme with the substrate's folded domain, thereby averting its premature release prior to complete unfolding. Additionally, I have visualized the role of a single-layer adaptor system in modulating *E. coli* ClpXP structure during substrate delivery and have also explored how mutations in the axial channel of *E. coli* ClpXP alter substrate specificity. These studies open multiple avenues for further research into substrate specificity, delivery, and engagement, which I aim to continue exploring in my lab.

1. **Ghanbarpour, A.**; *Sauer, R. T; Davis, J. H.; A proteolytic AAA+ machine poised to unfold a protein substrate, BioRxiv**. *Nat. Commun**. (under revision).*
2. **Ghanbarpour, A.***; Fei. X.*; Baker, Tania A.; Davis, J. H.; Sauer, R. T.; The SspB adaptor drives structural changes in the AAA+ ClpXP protease during ssrA-tagged substrate delivery. ***PNAS**,120,* e2219044120 *(*2023). *Equal Contributors
3. **Ghanbarpour, A.**; Cohen, S.; Fei. X.; Kinman, L.; Zhang, J. J.; Bell, T.; Baker, Tania A.; Davis, J. H.; Sauer, R. T. A closed translocation channel in the substrate-free AAA+ ClpXP protease diminishes rogue degradation. ***Nat. Commun***, 14, 7281 (2023)

Assistant Professor: Washington University School of Medicine (2024-present).

My lab at WashU is investigating the molecular mechanisms that govern substrate specificity in bacterial and mitochondrial AAA proteases. Our goal is to understand how accessory adaptor proteins and environmental conditions influence substrate recognition by these enzymes. Recently, we solved the cryo-EM structure of a nautilus-like assembly of membrane proteins surrounding the membrane-bound FtsH AAA protease extracted in an endogenous level from *E.coli*. This assembly includes an aperture, which may serve as an entry point for membrane protein substrates to diffuse into the cage, unveiling a novel mechanism for maintaining specificity. We have shown that this complex can reshape the membrane, an activity I have linked to an increased lipid flip-flop rate, potentially facilitating the efficient recognition of both membrane and soluble substrates by AAA proteases within the complex microdomain.

Ghanbarpour, A*.; Telusma, B.; Powell, B.; Zhang, J. J.; Bolstad, I.; Vargas, C.; Keller, s.; Baker, T.A; Sauer, R. T; Davis, J. H.; *An asymmetric nautilus-like HflK/C assembly controls FtsH proteolysis of membrane proteins. **EMBO** (under revision) * corresponding author*

Book Chapter

Kermani, A. A.; Agarwal, S., **Ghanbarpour, A.**; Advances in X-ray crystallography methods to study structural dynamics of macromolecules, *Academic Press.* Elsevier publication (2023). Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1z7o7oIomKHwHx/bibliography/public/