BIOGRAPHICAL SKETCH

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NAME: Hao Wu

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

POSITION TITLE: Asa & Patricia Springer Professor of Biological Chemistry & Molecular Pharmacology

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
Perking University, Beijing, China	B.Sc. Equiv	01/1985	Biology
Peking Union Medical College, Beijing, China	MD candidate	01/1988	Medicine
Purdue University, West Lafayette, Indiana	PhD	10/1992	Biochemistry
Columbia University, New York, New York	Postdoc	06/1997	Biochemistry

A. **Personal Statement**

Since starting her laboratory in 1997, the PI has focused on structural immunology, in particular, the structural basis of intracellular signal transduction in the mammalian immune system. Her contributions began in the TNF receptor pathway, which is inappropriately activated in autoimmune states such as rheumatoid arthritis (RA) and Crohn's disease. Blockade of TNF functions with drugs like Humira, correspondingly has had major therapeutic implications. The PI's laboratory has elucidated precise structural bases for how TNF signaling occurs and, thereby, provided a rational basis for understanding the most effective therapies for these conditions. The PI also elucidated the structural basis for signal transduction of the pro-inflammatory interleukin-1 receptor (IL-1R) family (such as receptors for IL-1, IL-18 and IL-33) and the Toll-like receptor (TLR) family, which share a set of overlapping cytoplasmic signaling proteins with the TNF receptor family. Most recently, the PI's laboratory performed structural studies on inflammasomes, which are cytosolic complexes for caspase-1 activation. In all areas, a unifying theme - revealed in substantial part by the PI's contribution - has been the identification and functional characterization of large oligomeric protein complexes that mediate these signaling cascades. The PI is experienced in many aspects of structural biology, including protein crystallography, biochemistry, and biophysics. Her current work also extends to electron microscopy, cellular imaging and structure-based drug design.

The PI is an experienced mentor for over 22 years. Throughout her career, she has been committed to training students and fellows and have supervised ~55 trainees. Many of these trainees are now Professors at universities, which include: University of Bayreuth, Germany; University of University of Louisville; Institute of Biophysics, Chinese Academy of Sciences; Yeungnam University, Korea; Soongsil University, Korea; Fudan University, China; INMM, CNR, Rome, Italy; Academia Sinica, Taiwan; Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences; NC State University; Federal University of Rio de Janeiro, Brazil; Florida State University; University of Connecticut; Fisk University; Zhejiang University, China; Oregon Health & Science University; UT Southwestern Medical Center. Many of the trainees are Senior Scientists at companies and agencies such as Pfizer; Boehringer-Ingelheim; Regeneron; Morphic Therapeutic; Scholar Rock; SMOC Therapeutics; Ribon Therapeutics; Gilead Sciences; Genewiz; Food and Drug Administration; GE Healthcare and Morphotek Inc. She have been the Associate Director of the Program in Cellular and Molecular Medicine, and been serving on the fellowship committee of the Cancer Research Institute for ~15 years.

B. Positions and Honors

1997-2001 Assistant Professor of Biochemistry, Weill Medical College of Cornell University.
2001-2003 Associate Professor of Biochemistry, Weill Medical College of Cornell University.
2003-7/2012 Professor of Biochemistry, Weill Medical College of Cornell University.
7/2012- Asa and Patricia Springer Professor of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and the Program in Cellular and Molecular Medicine, Boston Children's Hospital

Rita Allen Scholar Award, 7/2002-6/2004
Mayor's Award for Excellence in Science and Technology, 2003
Margaret Dayhoff Memorial Award, Biophysical Society, 2003
NIH Merit Award, 2012-2022
Editorial Board, F1000 Research, 2012-
Editorial Board, Cancer Cell, 2012-
Elected AAAS Fellow, 2013
Purdue University Distinguished Science Alumni Award, 2013
Election to the National Academy of Sciences, 2015
Pioneer Award from the National Institute of Health, 2015
Alumni Representative Speech, 100 year PUMC celebration, 2017
Keynote Lecture, Nature Conference "Inflammatory Diseases", 2017
National Jewish Health Distinguished Seminar Speaker, 2018
National Cancer Institute Distinguished Scientist Lecture Series (DSLS), 2019
Keynote speaker, 17th International TNF Conference TNF Conference, 2019
Keynote speaker, Dana-Farber Cancer Institute cancer immunology & virology scientific retreat, 2019
Co-organizer, American Society for Biochemistry and Molecular Biology (ASBMB) annual meeting, 2019
Dorothy Crowfoot Hodgkin Award, The Protein Society, 2019
The Seymour & Vivian Milstein Award for Excellence in Interferon and Cytokine Research, 2019

C. Contributions to Science (in approximate chronological order)

Elucidation of the specificity and oligomerization mechanism of TNF receptor associated factors (TRAFs, 1/2/3/5 and 6), which are the major signaling proteins for TNF receptor family-, IL-1R family-, and TLR- family-induced NF-κB activation. When the PI started working on TRAFs, no structural information was available. The PI identified consensus motifs for different TRAFs using structural studies, which became widely used tools for biologists. The PI's work also led to understanding the ubiquitin ligase activity of TRAF6 and its dependence on dimerization and higher-order oligomerization.

Y. C. Park, V. Burkitt, A. R. Villa, L. Tong and H. Wu (1999). Structural basis for self-association and receptor recognition of human TRAF2. ***Nature*** 398: 533-8

Y. C. Park, H. Ye, C. Hsia, D. Segal, R. L. Rich, H. C. Liou, D. G. Myszka and H. Wu (2000). A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. ***Cell*** 101: 777-87

H. Ye, J. R. Arron, B. Lamothe, M. Cirilli, T. Kobayashi, N. K. Shevde, D. Segal, O. K. Dzivenu, M. Vologodskaia, M. Yim, K. Du, S. Singh, J. W. Pike, B. G. Darnay, Y. Choi and H. Wu (2002). Distinct molecular mechanism for initiating TRAF6 signaling. ***Nature*** 418: 443-7

Q. Yin, S. C. Lin, B. Lamothe, M. Lu, Y. C. Lo, G. Hura, L. Zheng, R. Rich, A. D. Campos, D. G. Myszka, M. J. Lenardo, B. G. Darnay and H. Wu (2009). E2 interaction and dimerization in the crystal structure of TRAF6. ***Nat Struct Mol Biol*** 16: 658-66 PMC2834951

Elucidation of activation and inhibitory mechanisms of caspases and kinases. These enzymes are critically important for apoptotic and inflammatory signaling and were often difficult to obtain structures of. The understanding on their regulatory mechanisms revealed by work from the PI's lab is now being used for discovery of small molecule inhibitors for potential disease therapy.

Y. Huang, Y. C. Park, R. L. Rich, D. Segal, D. G. Myszka and H. Wu (2001). Structural basis of caspase inhibition by XIAP: differential roles of the linker versus the BIR domain. ***Cell*** 104: 781-90

G. Xu, M. Cirilli, Y. Huang, R. L. Rich, D. G. Myszka and H. Wu (2001). Covalent inhibition revealed by the crystal structure of the caspase-8/p35 complex. ***Nature*** 410: 494-7

G. Xu, Y. C. Lo, Q. Li, G. Napolitano, X. Wu, X. Jiang, M. Dreano, M. Karin and H. Wu (2011). Crystal structure of inhibitor of κB kinase β (IKKβ). ***Nature*** 472: 325-30 PMC3081413

Ferrao R, Zhou H, Shan Y, Liu Q, Li Q, Shaw DE, Li X and Wu H (2014). IRAK4 Dimerization and Trans- autophosphorylation are Induced by Myddosome Assembly. ***Mol Cell*** 55:891-903 PMC4169746

Identification of functional amyloid assembly in TNF-induced programmed necrosis. The PI's lab showed the surprising finding that the RHIM domain-containing proteins assemble into amyloid filaments to activate kinases and to induce cell death. These studies opened up new directions of research.

J. Li, T. McQuade, A. B. Siemer, J. Napetschnig, K. Moriwaki, Y.-S. Hsiao, E. Damko, D. Moquin, T. Walz, A. McDermott, F. K.-M. Chan, and H. Wu (2012). The RIP1/RIP3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. ***Cell*** 150: 339-50 PMC3664196

Discovery of helical signaling complexes including helical filaments formed by the death domain superfamily proteins. These protein domains were known for their tendencies to aggregate. The PI's lab elucidated that they assemble into either relatively defined helical complexes or helical filaments. These structures help to establish a new paradigm of signal transduction in innate immunity.

H. H. Park, E. Logette, S. Rauser, S. Cuenin, T. Walz, J. Tschopp and H. Wu (2007). Death domain assembly mechanism revealed by crystal structure of the oligomeric PIDDosome core complex. ***Cell*** 128: 533–46

S. C. Lin, Y. C. Lo and H. Wu (2010). Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signaling. ***Nature*** 465: 885-90 PMC2888693

Q. Qiao, C. Yang, C. Zheng, L. Fontan, L. David, X. Yu, C. Bracken, M. Rosen, A. Melnick, E. H. Egelman and H. Wu (2013). Structural Architecture of the CARMA1/Bcl10/MALT1 Signalosome: Nucleation-Induced Filamentous Assembly. ***Mol Cell*** 51: 766-79 PMC3929958

A. Lu, V. G. Magupalli, J. Ruan, Q. Yin, M. K. Atianand, M. R. Vos, G. F. Schröder, K. A. Fitzgerald, H. Wu* and E. H. Egelman (2014). Unified Polymerization Mechanism for the Assembly of ASC-Dependent Inflammasomes. ***Cell*** 156: 1193-206 PMC4000066 *Sole corresponding author

Discovery of the overarching principle of higher order assemblies and their important properties in signaling.

H. Wu (2013). Higher-order assemblies in a new paradigm of signal transduction. ***Cell*** 153: 287-92 PMC3687143

Kagan JC, Magupalli V, Wu H. (2014). Supramolecular Organizing Centres (SMOCs): Site-Specific Higher Order Signalling Complexes that Control Innate Immunity. ***Nature Rev Immunol***. 14:821-6 PMC4373346

Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H*, Lieberman J* (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. ***Nature*** 535: 153-8. PMID: 27383986 PMCID: PMC5539988 *dual correspondence

Ruan J, Xia S, Liu X, Lieberman J, Wu H (2018). Cryo-EM structure of the gasdermin A3 membrane pore. ***Nature***. 557(7703):62-67. PMCID:PMC6007975

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40701424/?sort=date&direction=descending

D. Research Support:

Ongoing Support

1DP1 HD087988-01 (Wu, H) 09/30/2015-07/31/2020 NIH/NICHD (Role PI)
SMOCs: Novel Signal Transduction Complexes as New Targets for Drug Discovery
The major goal of this project is to investigate signal transduction in order to guide the development of new models for targeted drug discovery.

1R01 AI139914 (Wu, H) 06/12/18-05/31/23 NIH/NIAID (Role PI)
Elucidating the Structural Mechanism of Pore Formation by the Gasdermin (GSDM) family
The major goal of the project is to elucidate the mechanism of GSDM pore formation through biochemical and structural studies on mouse GSDMA3 and human GSDMD. No overlap with the GSMDE studies proposed in the current application.

5R37 AI050872-13 (Wu, H) 01/01/02-03/31/22 NIH/NIAID (Role PI)
Structural & Functional Studies of TLR/IL-1R Signaling
The major goal of this project is to assemble the membrane-proximal signaling complexes and to elucidate the molecular basis of this signal transduction.

1R01 AI125535 (Wu, H) 07/01/16-06/30/21
Molecular mechanisms of the RAG recombinase in V(D)J recombination and disease
The major goal of the this project is to elucidate the molecular basis of RAG in V(D)J recombination

1R01 AI124491-01A1 (Wu, H) 7/01/16-6/30/21 NIH/NIAID (Role: PI)
Mechanistic Elucidation of Inflammasome Assembly and Regulation
The major goal of this project is to elucidate structural and mechanistic information on AIM2, NLRP3 and NAIP inflammasomes

Completed Support

1R01 CA182736-01 (Gray, N) 09/26/13-08/31/18 NIH/NCI (Role: Co-Investigator)
MALT1 inhibitors for the treatment of chemo-resistant ABC-DLBCL.
The major goal of this project is to optimize MALT1 inhibitors using structure-based chemical approaches

5R01 AI045937-12 (Wu, H) 07/01/99-06/30/2017 NIH/NIAID (Role: PI)
Structural and functional elucidation of the necrosome in innate immune signaling
The major goal of this project is to elucidate the molecular basis of TNF-induced necrosis.

5R01 AI089882-05 (Wu, H) 05/01/2010 – 04/30/2015 NIH/NIAID (Role: PI)
Molecular Elucidation of the CBM complex in NF-kappaB Activation by Antigen Receptors
The major goal of the project is to elucidate the molecular basis of CBM signaling in TCR and BCR activation

APPLICANT BIOGRAPHICAL SKETCH

NAME: Humayun Sharif

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Mohammad Ali Jinnah University, Pakistan	B.S.	2007	Bioinformatics
Gwangju Institute of Science and Technology, South Korea	M.S.	2010	Structural Biology
Max Planck Institute of Biochemistry, Germany	Ph.D.	2014	Structural Biology
Harvard Medical School/Dana Farber Cancer Institute, U.S.	Postdoc	11/2014-12/2015	Structural Biology
Harvard Medical School/Boston Children's Hospital. U.S.	Postdoc	1/2016-Present	Structural Biology

A. Personal Statements

My long-term research interests involve mechanistic understanding of innate immunity and how dysregulation of the signaling pathways contribute to diseases. My academic training and research experience have provided me with an excellent background in multiple biological disciplines including biochemistry and structural biology necessary to pursue my research plans as an independent researcher. The proposed research plan is of great importance because of our limited understanding of structural and biochemical mechanisms of CARD8 Inflammasome and its interaction with DPP9.

As a doctoral student with Dr. Elena Conti, my research was focused on systematic understanding of the decapping process by combination of X-ray crystallography, biochemical and biophysical techniques. From these studies, I gained full-spectrum training in protein crystallography and accumulated good experiences in structure-function relationships of functionally vital proteins. I solved several crystal structures of protein complexes that helped in better understanding of decapping mechanism. During my undergraduate and graduate careers, I received several academic and research awards. Currently as a postdoctoral fellow I am expanding my research interests to innate immunity. Combining novel protein purification strategies with state of the art cryo-electron microscopy (cryo-EM) techniques I solved the high-resolution structure of NLRP3 bound with NEK7, that leads to downstream NLRP3 signaling, Casapse1 activation and pyroptosis. In 2017, I also solved cryo-EM structure of DNA-PK complex involved in V(D)J recombination.

My sponsor Dr. Hao Wu is an internationally recognized expert in structural biology, innate/adaptive immunity and inflammation. She pioneered in mechanistic studies of caspases and tumor necrosis factor receptor associated factors (TRAFs) and has an extensive record for training postdoctoral fellows.

Prior to joining Wu lab, I worked in the Eck lab at Dana Farber Cancer Institute/Harvard Medical School for a short period. Initially, I planned to work on the structure and function of the BAF complex, a chromatin-remodeling complex that is highly mutated in cancers. However, I could not continue due to funding restrictions in the Eck lab. I ended up working on the Braf/Mek1 kinase complex. Although I made significant progress in obtaining the full-length Braf protein in complex with Mek1 within a short time (the manuscript describing this work is being written up), this project deviates from my long-term interest on protein-nucleic acid complexes. Upon talking to Professor Wu and learning about the Wu lab's interest in fundamental questions in Inflammasomes.

Positions and Honors

Positions

2/2007-7-2007	Undergraduate researcher with Dr. Joo Chuan Tong, A*STAR Institute of High Performance Computing, Singapore
8/2008-7/2010	Graduate (MS) researcher with Dr. Soo Hyun Eom, Gwangju Institute of Science and technology, South Korea
9/2010-7/2014	Graduate (PhD) researcher with Dr. Elena Conti, Max Planck Institute of Biochemistry, Germany
11/2014-12/2015	Postdoctoral fellow with Dr. Michael Eck, Harvard Medical School/Dana Farber Cancer Institute, US
01/2016-Present	Postdoctoral fellow with Dr. Hao Wu Harvard Medical School/Boston Children's Hospital, US

Academic and Professional Honors

2007	University distinction medal for outstanding undergraduate CGPA
2008-2010	Korean Govt. Scholarship award for Master's degree study and research
2010-2014	Graduate fellowship award by Max Planck Society and GRK1721 graduate school for hybrid methods in structural biology

B. Contribution to Science: Publications

Molecular and functional mechanisms of NLRP3 Inflammasome assembly

Sharif, H.*, Wang, L.*, Wang, W.L.*, Magupalli V.G., Andreeva, L., Qiao, Q., Hauenstein, A.V., Wu, Z., Nunez, G., Mao, Y., Wu., H. Structural mechanism for NEK7-induced NLRP3 inflammasome activation. *Nature (2019) (570), 338–343.*

Shen, C., **Sharif, H**., Xia, S. and Wu, H. Structural and mechanistic elucidation of inflammasome signaling by cryo-EM (Volume title: Cryo-electron microscopy: future challenges and developments). *Current Opinion in Structural Biology* (2019) (58) 18-25.

Magupalli, V.G., Negro, R., Hauenstein, A.V., Caprio, G.D., Skillern, W., Deng, Q., Tian, Y., Alam, H.B., Maliga, Z., **Sharif, H**., Hu, J.J., Schmidt, F.I., Li, Y., Kirchhausen, T. and Wu, H. HDAC6-mediated aggresome-like mechanism for NLRP3 and Pyrin inflammasome activation *(under revision in **Science**)*

The innate immune system forms an evolutionarily ancient line of defense against invading pathogens and endogenous danger signals. Within certain cells of innate immunity, including

epithelial cells and macrophages, intricate molecular machineries named inflammasomes sense a wide array of stimuli to mount inflammatory responses. Inflammasomes are large multiprotein complex which play a key role in innate immunity by participating in the production of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Dysregulation in inflammasome signaling leads to a wide range of immune disorders such as allergies, Crohn's disease and sepsis.
NLRP3 is the most extensively studied inflammasome sensor that responds to a broad spectrum of unrelated microbial stimuli and endogenous signals, uric acid crystals, extracellular ATP, including pore-forming toxins and potassium efflux. Cryoelectron microscopy (Cryo-EM) reconstruction of NLRP3 bound with NEK7 at 3.8 Å resolution reveals an earring-like structure of NLRP3 bound to NEK7.

Understanding the molecular mechanism of V(D)J recombination and NHEJ pathway through structures

Sharif H.*, Li Y.*, Dong L., Wang W., Mao Y. and Wu H. Cryo-EM structure of DNAPK holoenzyme. *PNAS* (2017) 114 (28) 7367-7372

Adaptive immunity of the vertebrate immune system relies largely on the V(D)J recombination which orchestrate the combinatorial splicing of coding segments and join them for diversification of B and T-cell receptors. During V(D)J recombination RAG1 and RAG2 proteins cleave DNA adjacent to conserved recombination signal sequence (RSS). The cleaved DNA is repaired and ligated by Non-homologous ends joining (NHEJ) repair pathway.

NHEJ is initiated through the recognition and binding of broken DNA ends by the ring shaped Ku70/80 heterodimer followed by the recruitment of DNA-PKcs (DNA-dependent protein kinase catalytic subunit) to serve as a tether for the broken ends and prevent exonucleotic degradation. DNA-PKcs and Ku70/80 heterodimer makes a holoenzyme called DNA-PK, the DSBs detection complex that eventually recruits other protein sub-units to the site of DSBs for ligation. The precise mechanism of how the cleaved ends of the DNA are handed over to the NHEJ pathway is still unknown. Lack of high resolution structures prompted us to start a comprehensive effort to dissect these two interacting pathways.

In the first phase of the study, I reported the cryo-electron microscopy (cryo-EM) structures of human DNA- PKcs at 4.4 Å resolution and the DNA-PK holoenzyme at 5.8 Å resolution. The DNA-PK complex reveals density for the C-terminal globular domain of Ku80 that interacts with the arm domain of DNA-PKcs. The Ku80 interaction site with DNA-PKcs is adjacent to the previously identified density for the DNA-binding region of the Ku70/Ku80 complex, suggesting concerted DNA interaction by DNA-PKcs and the Ku complex. We were also able to generate a model based on published literature and our cryo-EM structure that explains how the DNA-PK choreographs the ligation of DSBs by holding the ends of DNAs together and making it available for processing and ligation protein complexes.

Biochemical and structural elucidation of BRAF interaction with MEK in MAPK signaling pathway

Park, E.Y.,Rawson, S., Kim, B.W., Li, K., Ficarro, S.B., **Sharif, H**., Marto, J.A., Jeon, H.S., and Eck, M.J. Architecture of an autoinhibited BRAF/MEK1/14-3-3 complex revealed by cryo-EM. *(under revision in Nature)*

The MAPK pathway plays a critical role in cellular growth, senescence and survival. This pathway proteins are frequently mutated in human cancers with many tumor harboring RAF and RAS mutations that render the pathway malfunctioning. BRAF and MEK1 kinases are

important players in this pathway. A lot of work has been focused in the context of their kinase domains only but the roles of N-terminal regulatory domains of BRAF still remains to understood at a molecular and functional level. During my short stay at Dr. Michael Eck's lab as postdoctoral fellow I was able to purify the complex of full-length BRAF and MEK1 to homogeneity. Interestingly, co-expression of BRAF full-length with MEK1 also co-purified endogenous 14-3-3 proteins. I was also successful in purifying the complexes in the presence of MEK1 inhibitor Selumetinib. After I left the Eck lab, a fellow postdoc Eun Young Park took over the project and she has made significant progress in elucidating molecular functions of the regulatory domain of BRAF.

Structural and biochemical characterization of Eukaryotic mRNA decapping activators

Sharif, H. and Conti, E. Architecture of the Lsm1-7-Pat1 complex: A conserved assembly in eukaryotic mRNA turnover. ***Cell Reports*** (2013) 5, 283–291

Sharif H., Ozgur S., Sharma K., Basquin C., Urlaub H., Conti E. Structural analysis of the yeast Dhh1-Pat1 complex reveals how Dhh1 engages Pat1, Edc3 and RNA in mutually exclusive interactions. ***Nucleic Acids Research*** (2013) 41 (17): 8377-8390.

mRNA decay is a crucial step in the eukaryotic gene expression. Translational repression and deadenylation of eukaryotic mRNAs result in their degradation or the sequestration of the transcripts in a non-translatable pool. 5′-to-3′ mRNA degradation starts with the removal of the 5′ cap structure by the help of decapping enzymes Dcp1/2 and several decapping activators. These activators are evolutionary conserved proteins that play essential role in providing the scaffold for the cap removal and translational repression but their interplay is currently unclear.
In eukaryotes, the decapping activators include DEAD-box protein Dhh1, Pat1, Edc3 and a multi-subunit protein Lsm1-7 complex.

In the first study, I was able to purify to homogeneity the octameric complex of Lsm1-7 proteins bound with C-terminal of Pat1. The high-resolution crystal structure revealed an unusual C-terminal extension of Lsm1 that plugs the exit site of the central channel and approaches the RNA-binding interface. Furthermore, I was able to elucidate the interaction basis of Pat1 with Lsm1-7 complex which is mediated by not the distinguishing cytoplasmic subunit Lsm1 but with Lsm2 and Lsm3. These high-resolution crystal structures increased our understanding of how this complex drives the degradation pathways to 5′-to-3′ degradation instead of 3′-to-5′ pathway.

In the second study, I reported the 2.8 Å resolution structure of yeast Dhh1 bound to the N-terminal domain of Pat1. The structure showed how Pat1 wraps around the C-terminal RecA domain of Dhh1, docking onto the Phe-Asp-Phe (FDF) binding site. The same binding surface on Dhh1 also recognizes Edc3. We elucidated the evolutionary conserved molecular mechanisms of the interaction between Dhh1 and Pat1, showing how Pat1 and Edc3 compete for the interaction with Dhh1 and how they impact on RNA binding. These results suggest that Dhh1 might switch protein and RNA-binding partners in the transition from translational repression to decapping.

Structural and functional insights into the oligomerization of FtsH periplasmic domain

An J.Y.*, **Sharif H.***, Kang G.B., Kyung J.P., Lee J.G., Lee S., Jin M.S., Song, J.J., Wang J., Eom S.H. Structural insights into the oligomerization of FtsH periplasmic domain from Thermotoga maritima. ***BBRC*** (2018) 495 (1), 1201-1207.

Accumulation of misfolded membrane proteins and misassembled protein complexes pose a threat to cellular functions and cell survival and their prompt removal is essential for membrane

homeostasis. FtsH is a transmembrane protein, solely needed for the purpose of removal of misassembled membrane protein that relies on its ATP-dependent protease domain. Biochemical and structural characterization of FtsH ATPase and protease domains have been reported previously but the role of periplasmic domain was still elusive. In this study, we were able to crystalize and solve the periplasmic domain of FtsH at 1.5 Å resolution. We were able to describe, with the help of complimentary biophysical assays, the dynamic features of periplasmic domain oligomerization.

Complete List of my Bibliography
https://www.ncbi.nlm.nih.gov/pubmed/?term=humayun+sharif

C. Additional Information: Research Support: N/A

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: Louis "Bobby" Robert Hollingsworth IV

eRA COMMONS USER NAME (credential, e.g., agency login): bobbyh11

POSITION TITLE: Graduate Student

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Virginia Tech, Blacksburg, VA	B.S.	08/2013	05/2017	Chemical Engineering
Virginia Tech, Blacksburg, VA	B.S.	08/2013	05/2017	Biochemistry
Virginia Tech, Blacksburg, VA	B.A.	08/2013	05/2017	Chemistry
Harvard Medical School, Boston, MA	Ph.D.	08/2017	Expected 04/2023	Biological and Biomedical Sciences

A. Personal Statement

The *why* and *how* behind a process always piqued my interests, and quite possibly got me in trouble as a precocious and curious child. I eventually focused these interests on biology and biomedicine because of the complexity of biological systems and the potential impact of the discipline on human health. As an undergraduate student, I prepared myself for a career in collaborative research by exploring several approaches including organic synthesis, biochemistry/cellular biology, and computational/structural biology. Moreover, these experiences began to teach me how to formulate research questions, write proposals and papers, and deliver technical presentations. Equipped with this toolkit of methods and early experiences, I entered graduate school intent on investigating the molecular mechanisms underlying biological signaling pathways. In the Wu Lab I do just that, using structural and cellular methods, including cryo-EM, to study mechanism in innate immunity. The Wu Lab provides the ideal environment for my graduate education and training, as I am afforded numerous opportunities for mentorship and collaboration across a wide range of expertise, including crystallography, cryo-EM, and cellular biology. The supportive environment of the Wu Lab, coupled with my enthusiasm and grit, equip me to tackle several ambitious projects. Long-term, I aspire to pursue a career as an independent and collaborative academic scientist studying cellular signaling and mechanisms.

B. Positions and Honors

Research Experience

Graduate Research Assistant, Structural Immunology June 2018–Present
Dr. Hao Wu, Harvard Dept. of BCMP and Boston Children's Hospital PCMM

Rotation Student, Experimental and Computational Structural Biology 2017–2018
Drs. Hao Wu, Alan Brown, and Debora Marks, Harvard University

Undergraduate Researcher, Computational Protein Dynamics 2015-2017
Drs. Richard Gandour, Anne Brown, and David Bevan, Virginia Tech

Team Captain, VT Chem-E-Car 2014–2017
Department of Chemical Engineering, Virginia Tech

Undergraduate Researcher and Group Leader, Organic Synthesis 2014–2015
Dr. Richard Gandour, Virginia Tech

Amgen Scholar, Harvard University Summer 2015
Dr. Pere Puigserver, Harvard Dept. of Cell Biology and Dana-Farber Cancer Institute

Intern and Special Volunteer, National Institutes of Health Summer 2014,
Dr. Sriram Subramaniam, National Cancer Institute Winter 2015

Bioengineering Intern, George Washington University Summer 2013
Dr. Lijie Zhang, Department of Engineering

Teaching, Mentorship, and Outreach

Head Team Editor, Harvard Science in the News (SITN) Blog	2018–Present
Teaching Assistant, BCMP 200: Principles of Molecular Biology	Fall 2018
Graduate Mentor, Wu Lab summer undergraduate research students	2018
Assistant Director, Virginia Tech Graduate-Undergraduate Mentorship Program	2016–2017
Mentor, Virginia Tech Department of Chemistry "Sophomore Sibs" Program	2016–2017
Mentor, Virginia Tech Honors Residential Commons	2014–2017
Mentor, Virginia Tech Center for Enhancement of Engineering Diversity (CEED)	2014

Honors

DoD-NDSEG Alternate	2019
NSF-GRFP Honorable Mention	2019
Albert J. Ryan Fellowship	2019
Barry M. Goldwater Scholarship, Sophomore	2015, 2016
Harry S. Truman Scholarship Finalist	2016
Phi Kappa Phi Marcus L. Urann Graduate Fellowship	2017
Outstanding Senior, Virginia Tech College of Engineering	2017
Outstanding Undergraduate Researcher, Virginia Tech College of Science	2017
Outstanding Senior, Virginia Tech Departments of Chemistry and Chemical Engineering	2017
PEARC17 Conference Travel Award	2017
Howe Award for Outstanding Senior, Blue Ridge ACS Section	2017
Biophysical Society Conference: 2nd Place Poster	2017
Curiosity Aspire! Award, Virginia Tech Division of Student Affairs	2017
ACS National Symposium COMP Workshop and Travel Grant	2016
High Performance Computing Day: 1st Place Poster	2016
University Honors Class of 1954 Odyssey Fellowship	2015
Virginia Tech Illuminator Award	2015
Atlantic Coast Conference (ACC) Creativity and Innovation Fellowship	2015
Institute for Creativity, Arts, and Technology (ICAT) Student Research Grant	2015
Chem-E-Car National Poster Competition: 1st Place, 2nd Place	2015-2016
Chem-E-Car National Design Competition: 4th Place, 6th Place, 7th Place	2014-2016

C. Contributions to Science

Graduate Research:

My current projects in the Wu Lab focus on the structure and mechanism of proteins and protein complexes involved in the inflammasome pathway, which is responsible for sensing and responding to both exogenous and endogenous danger signals. Dysregulation of inflammasome pathway proteins leads to a plethora of autoimmune diseases; therefore, understanding the molecular mechanisms governing inflammasome regulation and assembly can provide new specific therapeutic options. Through my research in the Wu Lab I am training to design experiments, solve and interpret cryo-EM structures, and conduct a variety of cellular, biophysical, and

biochemical assays.

1. Hun, J. J.*; Liu, X.*; Xia, S.; Zhang, Z.; Zhao, J.; Ruan, J.; Luo, X.; Lou, X.; **Hollingsworth, L. R. IV**; Magupalli, V. G.; Kim, J.; Lieberman, J.; Wu, H. Disulfiram Inhibits Pyroptosis by Selectively and Covalently Modifying a Reactive Cysteine in Gasdermin D. *Submitted*.

2. Xia, S.*; **Hollingsworth, L. R. IV***; Wu, H. Mechanism and Regulation of Gasdermin-mediated Cell Death. In Cell Survival & Cell Death; K. Newton, J. Murphy, E. Miao; CSHL Press, 2019; Vol. 2. *In press*.

3. **Hollingsworth, L. R. IV;** Veeraraghavan, P.; Wu, K. J; McCoy, D. E.; Van Dervort, A.; Gunther, K. E. Speak Out Against Tuition Waiver Taxes. *Science* **2017**, *358*, *1395*.

Undergraduate Research:

I synthesized polyethylene glycol linkers in the Richard Gandour Lab at Virginia Tech during my freshman and sophomore years of college. During my junior year, I initiated a collaboration with Drs. David Bevan and Anne Brown at Virginia Tech, where I employed molecular docking and molecular dynamics simulations to elucidate the binding properties of an anti-HIV copolymer. Following the conclusion of this study, I conducted more complex simulations of transmembrane regions of the trimeric HIV fusion protein, gp41, embedded in an asymmetric bilayer to mimic its native lipid environment. We found that the dynamics of the transmembrane domain correlated to water and ion permeation into a membrane channel formed by gp41. In addition, I collaborated with Dr. Khidir Hilu at Virginia Tech on several projects related to peanut allergenicity and Dr. Webster Santos for computational drug design.

4. Hilu, K. W.; Friend, S.; Vallanadu, V.; Brown, A. M.; **Hollingsworth, L. R. IV**; Bevan, D. R. Molecular evolution of genes encoding allergen proteins in the peanuts genus I: Structural and functional implications. *In press, PLoS One.*

5. **Hollingsworth, L. R. IV**; Lemkul, J. A.; Bevan, D. R.; Brown, A. M.; The HIV-1 Transmembrane Domain Modulates Membrane Stability and Water Permeation, *Biophys J.* **2018**, *115*, *84-94*.

6. **Hollingsworth, L. R. IV**; Brown, A. M.; Gandour, R. D.; Bevan, D. R. Computational Study of HIV gp120 as a Target for Polyanionic Entry Inhibitors: Exploiting the V3 Loop Regions, *PLoS One* **2018**, *13*, *e0190658*.

7. **Hollingsworth, L. R. IV**; Brown, A. M.; Bevan, D. R. **2017**. In *Proceedings of Practice & Experience in Advanced Research Computing conference, New Orleans, Louisiana USA, July 2017 (PEARC17)*, 4 pages. http://dx.doi.org/10.1145/3093338.3104154

Undergraduate Summer Research:

I interned in the Sriram Subramaniam Lab at the NIH in between my freshman and sophomore years. I investigated the structure and regulation of the metabolic enzyme pyruvate kinase, particularly its M2 isoform (PKM2), which is differentially expressed in cancer. I spent the following summer in the Pere Puigserver Lab at Harvard University through the Amgen Scholars Program. Through our unbiased CRISPR screen we discovered that deletions in the BRD4 gene led to significant cell rescue when TCA-deficient cells were challenged with galactose media, a condition that requires TCA metabolism. Simultaneously, I-BET 525762A, a known BRD4 inhibitor, led to increased cell survival in a large chemical library screen. We characterized the mechanistic link between this compound and increased metabolic activity in cells with a defective mitochondrial complex I, wherein the inhibition of BRD4 bypassed the deficiency through an increase in complex II gene expression.

8. Barrow, J. J.*; Balsa, E.*; Verdeguer, F.; Tavares, C. D. J.; Soustek, M. S.; **Hollingsworth, L. R. IV**, Jedrychowski, M.; Vogel, R.; Paulo, J. A.; Smeitink, J.; Gygi, S. P.; Doench, J.; Root D. E.; Puigserver, P. Bromodomain Inhibitors Correct Bioenergetic Deficiency Caused by Mitochondrial Disease Complex I Mutations. *Mol Cell* **2016**, *64*, 163-175.

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

Scholastic Performance

YEAR	COURSE TITLE	GRADE
	VIRGINIA TECH: 219 CREDIT HOURS, GPA = 3.95/4.0	
2013	Mass and Energy Balances	A
2013	Organic Chemistry I	A
2013	Organic Chemistry Laboratory I	A
2013	Engineering Exploration (Intro Engineering I)	A
2013	First-Year Writing	A
2013	Intro Differential Equations	A
2013	Freshman Honors Residential Commons (HRC) Seminar	P
2014	International Perspectives on the Nanoscience of Macromolecules (Winter)	A
2014	Honors Biology II	A
2014	Independent Study (Chem-E-Car)	A
2014	Organic Chemistry II	A
2014	Organic Chemistry Laboratory II	A
2014	Undergraduate Research	A
2014	Exploration Engineering Design (Intro Engineering II)	A
2014	Vector Geometry	A
2014	Operational Methods (Laplace and Fourier Transforms)	A
2014	Honors HRC Seminar	P
2014	Honors Biology I	A
2014	Fluid Transport	A
2014	Independent Study (Chem-E-Car)	A
2014	Undergraduate Research	A
2014	Physical Chemistry I	A
2014	Survey of Chemical Literature	A
2014	Statistical Methods for Engineers	A
2014	Honors Residential College Seminar	P
2015	Computational Biochemistry and Bioinformatics (Graduate course)	A
2015	Genetics	A-
2015	Chemical Engineering Sophomore Seminar	P
2015	Chemical Engineering Simulations	A
2015	Chemical Engineering Thermodynamics	A
2015	Independent Study (Chem-E-Car)	A
2015	Physical Chemistry Laboratory I	A
2015	Physical Chemistry for Life Sciences II	A
2015	Undergraduate Research	A
2015	Topics in Honors House Seminar	P
2016	Technical Writing (Winter)	A
2016	Undergraduate Research	A
2016	Process Measurement & Control	A
2016	Heat Transfer	A
2016	Mass Transfer	B
2016	Chemical Reactor Analysis & Design	A
2016	Chemical Process Modeling	A-
2016	Independent Study (Chem-E-Car)	A
2016	Topics in Honors House Seminar	P
2016	Chemical Engineering Unit Operations Laboratory (Summer, DTU)	T (Transfer A)

YEAR	COURSE TITLE	GRADE
2016	General Biochemistry I	A
2016	Undergraduate Research	A
2016	Process and Plant Design	A
2016	Process Materials	A-
2016	Independent Study (Chem-E-Car)	A
2016	Music Appreciation	A
2017	General Biochemistry II	A-
2017	Biochemistry Laboratory	A
2017	Undergraduate Research	A
2017	Process and Plant Design	A
2017	Independent Study	A
2017	Descriptive Inorganic Chemistry	A-
	HARVARD MEDICAL SCHOOL	
2017	Analysis of the Biological Literature (including statistics and programming in R)	A-
2017	Principles of Genetics	B+
2017	Molecular Biology	A
2018	Principles of Cell Biology	A
2018	Biophysical and Biochemical Mechanisms of Protein Function	A
2018	Critical Thinking and Research (proposal writing)	P
2018	Teaching Practicum (TA position and pedagogy course)	P
2018	Conduct of Science	P
2019	Structural Biology, from Molecules to Cells	A

Ongoing Support

MCB190086 (Hollingsworth, L. R. IV) 06/17/2019-06/16/2020 NSF (Role: PI)
XSEDE Startup Allocation

Completed Support

5T32GM007226-43 (Van Vactor, D) 07/01/2018-06/30/2019 NIH (Role: Trainee)
Molecular, Cellular, & Developmental Dynamics PhD Program