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: 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.

In the past few years, the PI's laboratory has been performing 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. These studies have resulted in numerous publications and shown potential for translation into therapeutics.

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.

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
11/2019- Associate Member, The Broad Institute of MIT and Harvard

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
Honorary Professor, Chinese Academy of Medical Sciences and Peking Union Medical College, 2016
Alumni Representative Speech, 100 year PUMC celebration, 2017
Keynote Lecture, Nature Conference "Inflammatory Diseases", 2017
National Jewish Health Distinguished Seminar Speaker, 2018
Honorary Professor, Zhejiang University, China, 2018
Dorothy Crowfoot Hodgkin Award, The Protein Society, 2019
The Seymour & Vivian Milstein Award for Excellence in Interferon and Cytokine Research, 2019
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
Fellow of the Biophysical Society Award, 2020
Elected member, the Henry Kunkel Society, 2020
Vallee Visiting Professorship, 2020

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

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

Sharif H, Wang L, Wang WL, Magupalli VG, Andreeva L, Qiao Q, Hauenstein AV, Wu Z, Nunez G, Mao Y, Wu H* (2019). Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome. ***Nature*** 570: 338-343. PMCID6774351

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

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 12/2023	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 their potential for impact on human health. As an undergraduate student, I prepared myself for a collaborative career in research by exploring several approaches including organic synthesis, biochemistry/cellular biology, and computational/structural biology. 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. Currently, I investigate how the NLRP1 and CARD8 inflammasomes—two proteins that sense pathogens and endogenous damage—remain inhibited, and how their dysregulation and constitutive activation leads to human disease. Through structural, biochemical, and cellular approaches, we seek to ultimately understand the complete mechanisms underlying these complex inflammatory circuits to design therapeutic interventions. Long-term, I aspire to pursue a career as an independent and collaborative academic scientist studying cellular signaling 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, Winter 2015
Dr. Sriram Subramaniam, National Cancer Institute

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
Graduate Mentor, Wu Lab undergraduate trainees	2019–Present
Tutor, BBS Graduate Program Qualifying Exam	Winter 2019
Teaching Assistant, BCMP 200: Principles of Molecular Biology	Fall 2018
Graduate Mentor, Wu Lab summer undergraduate trainees	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.; Zhang, Y.; Zhao, J.; Ruan, J.; Luo, X.; Lou, X.; Bai, Y.; Wang, J.; **Hollingsworth, L. R.**; Magupalli, V. G.; Zhao, L.; Lou, H. R.; Kim, J.; Lieberman, J.; Wu, H. Disulfiram Inhibits Pyroptosis by Selectively and Covalently Modifying a Reactive Cysteine in Gasdermin D. *In Press, Nature Immunology*.

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; 2nd Ed.

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 to an HIV surface glycoprotein. 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 targeting sphingosine kinases in cancer.

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 *Arachis*: Structural and functional implications, *PLoS One* **2019**, 4(11): e0222440.

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	Honors 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

APPLICANT BIOGRAPHICAL SKETCH

NAME: Humayun Sharif

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

POSITION TITLE: Instructor (Harvard Medical School) Research Fellow (Boston Childrens Hospital)

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.

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. Prior to joining Wu lab, I worked briefly in the Eck lab at Dana Farber Cancer Institute/Harvard Medical School In short period that I spent there, I was able to purify BRAF full-length in complex with MEK proteins and 14-3-3 (Park et al. *Nature* 2019). I played a crucial role in purification of this autoinhibited complex.

Ever since I joined Hao Wu's lab, I have been focusing on innate and adaptive immune system molecular pathways. Inflammasomes are large multiprotein complexes which play key roles in innate immunity by participating in the production of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. NLRP3 is the most extensively studied inflammasome sensor that responds to a broad spectrum of unrelated microbial stimuli and endogenous signals. NLRP3 dysregulation is directly related to systematic and joint inflammation that leads to conditions like gouts, rheumatoid arthritis and type II diabetes. By combining novel protein purification strategies with state of the art cryo-electron microscopy (cryo-EM) techniques, I

was able to solve the high-resolution structure of NLRP3 bound with NEK7 (Sharif et al. *Nature* 2019). Activated NLRP3-NEK7 conformation modeling based on the NLRC4 inflammasome, predicts a novel hotspot of interaction of NEK7 with neighboring NLRP3 molecule. Mutations in this surface in *in vitro* and cell-based assays abolish the NLRP3 ability to form the NLRP3 inflammasome and abrogate downstream signaling. Moreover, my ongoing efforts in understanding molecular mechanisms of inflammasome extends to newly characterized NLRP1 and CARD8 inflammasomes. I am trying to elucidate how DPP9 regulation plays critical role in inhibition and activation of NLRP1 and CARD8 inflammasomes.

My earlier work in Wu lab as a postdoc lead to the very first 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 (Sharif et al. *PNAS* 2017). The DNA-PK complex reveals density for the C-terminal globular domain of Ku80 that interacts with the arm domain of DNA-PKcs. 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.

On the basis of my current result, I would like to pursue structural and functional studies of activated NLRP3 inflammasome with novel strategies to purify the complex to homogeneity, NLRP1 and CARD8 inflammasomes. In my independent role as a principal investigator I would also like to dissect molecular mechanisms of activations of other NLRs (NOD-like receptors) and Inflammasome and would like to explore the universal mechanism of activation of these NLRs.

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*** *June20, 2019 edition.*

Commentaries:

- News and Views of Nature: Nozaki K.and Miao. E. A licence to kill during inflammation. Nature June 12, 2019 https://www.nature.com/articles/d41586-019-01764-9
- News and Research: Harvard Medical School (to be published soon)
- https://www.biocentury.com/bc-innovations/translation-brief/2019-06-12/harvard-smoc-group-solving-structures-large-complexes-

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**)*

Inflammasomes are cytoplasmic supramolecular large assemblies that are formed in response to diverse endogenous and exogenous damage signals. NLRP3 is extensively studied inflammasome sensor that is activated by seemingly unrelated stimuli. NEK7 kinase licenses the assembly and activation of NLRP3 inflammasome. Our studies helped in our understanding of firstly, the molecular basis of NLRP3 interaction with its inducer protein NEK7. The earing shaped NLRP3 structure nestles NEK7 C-lobe. With the help of structure guided mutations and cellular assays we were able to propose a model of activation of NLRP3 inflammasome. Secondly, in a collaborated extensive study, we were able to identify HDAC6 mediates localization of NLRP3 and Pyrin inflammasome at microtubule-organizing center (MTOC).

Functional regulation of NLRP1 and CARD8 inflammasomes by DPP9

Hollingsworth, L.R. 4th*, Sharif, H*, Griswold, A*, Fontana, P., Ball, D.P., Bachovchin, D., Wu, H. Structural mechanisms of NLRP1 inflammasome and functional role of DPP9 peptidase in its activation. (Manuscript in preparation)

Sharif, H*., Hollingsworth, L.R. 4th*, Griswold, A*., Li, Y, Fontana, P., Ball, D.P., Bachovchin, D., Wu, H. Molecular mechanisms for activation of CARD8 inflammasome (Manuscript in preparation)

Two previously overlooked inflammasome proteins, NLRP1 and CARD8, recently gathered attention for their novel domain structures, activation mechanisms, disease relevance, and exciting biology. Activation of NLRP1 and CARD8 depends on DPP9 mediated regulation and proteasome mediated N-terminal degradation that releases the inflammatory CT, which in turn engages caspase-1 directly or indirectly, leading to inflammatory cytokine release and pyroptotic cell death. Our studies are paving way to elucidate the molecular mechanisms of NLRP1 and CARD8 inflammasome activation that is regulated by DPP9 peptidase.

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 autoinhibited and active BRAF/MEK1/14-3-3 complexes. *Nature* (2019) October 13, online

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: Pietro Fontana

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

POSITION TITLE: Post doctoral 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
University of Palermo	Bachelor	10/2010	Biology
University of Palermo	Master	10/2012	Molecular Biology
University of Palermo	Master	10/2014	Biotechnology
University of Oxford	DPhil	02/2019	Pathology

A. Personal Statement

During my DPhil I have specialised in learning techniques for protein biochemistry/enzymology and structural biology by X-Ray crystallography. With this expertise I identified the role of a new component of the DNA damage response, which we named Histone Parylation Factor 1 (HPF1). I demonstrated that HPF1 can lead to a novel post-translational modification of Histone proteins named Ser-ADP-ribosylation, which consists in poly- or mono-ADP ribosylation specifically on serine residues. The discovery of Ser-ADP-ribosylation has already had a big impact in the field of ADP-ribosylation and became quickly a hot-topic amongst researchers in this field. I subsequently identified ARH3 as the only human glycohydrolase able to remove this new modification. This new discovery again garnered a lot of attention within the field, and several papers have been published on ARH3 since. Moreover, I recently translated all my findings to a *Drosophila* model, which has lead to the discovery of a new mechanism for Ser-ADP-ribosylation regulation, which is evolutionary conserved.

My long-term career goal is to become an independent group leader in immunology. Today's immunology research is mainly focussed on cell biology and animal studies, which leaves a lack of information at the mechanistic level. For this reason, I wanted to extend my previous experience in biochemistry and complement it with newly acquired skills of structural biology. The Wu lab and Harvard Medical School are providing me with the necessary assistance and training in Cryo-EM, while I am able to contribute to the lab set of skills with my expertise in biochemistry and X-Ray crystallography. This combination of techniques is enabling me to characterize NLRP1 and CARD8 inflammasomes. With my work ethic and dedication to pursuing a research career, I am fully committed to maximizing the potential of my postdoctoral training as the current essential step for me on my pathway to becoming an independent researcher.

B. Positions and Honors

10/2012 Award academic merit-based university for Bachelor's degree

10/2012 Special distinction awarded by the Master's Degree in Cellular e molecular biology committee "for the brilliant curriculum studiorum"

10/2014 Award academic merit-based university, Master's Degree in Cellular e molecular biology

10/2014 Special distinction awarded by the Master's Degree in Biotechnology for industries and scientific research committee "for the significance of the scientific work conducted"

C. Contributions to Science

Palazzotto E, Renzone G, **Fontana P**, Botta L, Scaloni A, Puglia AM, Gallo G. Tryptophan promotes morphological and physiological differentiation in Streptomyces coelicolor. *Appl Microbiol Biotechnol.* 2015 Dec;99(23):10177-89.

Gibbs-Seymour I, **Fontana P**, Rack JG, Ahel I. HPF1/C4orf27 Is a PARP-1-Interacting Protein that Regulates PARP-1 ADP- Ribosylation Activity. *Mol Cell*. 2016 May 5;62(3):432-42.

Bonfiglio JJ, **Fontana P**, Zhang Q, Colby T, Gibbs-Seymour I, Atanassov I, Bartlett E, Zaja R, Ahel I, Matic I.Serine ADP-Ribosylation Depends on HPF1. *Mol Cell*. 2017 Mar 2;65(5):932-940.

Fontana P, Bonfiglio JJ, Palazzo L, Bartlett E, Matic I, Ahel I. Serine ADP-ribosylation reversal by the hydrolase ARH3. *Elife*. 2017 Jun 26;6.

Suskiewicz MJ, Zobel F, Ogden TEH, **Fontana P**, Ariza A, Yang JC, Zhu K, Bracken L, Hawthorne WJ, Ahel D, Neuhas D, Ahel I. HPF1 completes the PARP active site for DNA damage-induced ADP-ribosylation. *Nature*. 2020 Feb.

Fontana P, Ariza A, Ahel I. Removal of Serine ADP-ribosylation in *Drosophila melanogaster*, a new mechanism. Manuscript in preparation for submission on NSMB.

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

10/2012 ERSU scholarship for academic merit.

10/2014 Medical Research Council (MRC) DPhil scholarship, Oxford University.

10/2018 Medical Research Council (MRC) post-doctoral transition fellowship, Oxford University.

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: Liudmila Andreeva

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

POSITION TITLE: research 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
Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow, Russia	M. Sc. Equiv. (diploma)	06/2013	Bioengineering and Bioinformatics
Ludwig-Maximilians-Universität Munich, Gene Center Munich, Munich, Germany	PhD	04/2018	Biochemistry

A. Personal Statement

I have been interested in innate immunity, inflammation and cancer ever since my university studies. My earlier work was focused on the influence of nonsteroidal anti-inflammatory drugs on the functions of glioma C6-cells to evaluate their potential in anti-cancer therapy. After switching later to a different field where I aimed to characterize a cellular function of eIF2D – a non-canonical eukaryotic translation initiation factor, I returned to my primary field of interest studying the interaction of viral and host defense systems during my diploma thesis. In this work I found that unlike other security proteins of Mengo virus that inhibit effector caspases, security protein 2A counteracts apoptotic cell death at the initiation stage by interfering with the activation of an initiator caspase, caspase-9.

During my PhD studies I further deepened my knowledge of the innate immune system by investigating the cytosolic DNA sensor cGAS. I found out that similar to other innate immune sensors like inflammasomes or RNA sensors RIG-I and MDA5, cGAS undergoes oligomerization upon binding of long DNA species. Such oligomerization is a crucial event for cGAS activation and explains selective recognition of long DNA species by cGAS. The structure obtained during my PhD additionally suggested DNA-bending proteins like mitochondrial transcription factor A (TFAM) or high-mobility group box protein 1 (HMGB1) as cGAS co-factors. Together with my contribution to the research of other cGAS ligands, such as Y-shaped short DNA with G-overhangs and RNA:DNA hybrids, these findings broaden the understanding of biologically relevant cGAS ligands to structured rather than linear nucleic acid species and offer a set of novel and more potent cGAS activators for cancer therapies.

My current interest is to structurally and mechanistically understand the process of NLRP3 inflammasome activation and signaling in healthy state and disease, and to establish a platform for searching potential NLRP3-binding molecules for cancer prevention and treatment.

During my education and research experience I gained extensive training in biochemical and biophysical methods of protein characterization and structural biology including crystallography and cryo-EM. My post-doctoral research will further expand and sharpen my skills in studying macromolecular assemblies of immune sensors including among others such techniques as cryo-electron microscopy, fluorescent microscopy and cell-based assays.

B. Positions and Honors

Positions:

2012-2013 Master student and research assistant, M.P. Chumakov Institute of Poliomyelitis and Viral

	Encephalitis, Department of Biochemistry, Moskovskaya region, Russia
2013-2018	PhD-candidate, Gene Center Munich, LMU Munich, Munich, Germany
2018-present	Postdoctoral Research Fellow, the Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston MA, USA

Fellowships and awards:

2013-2018	International Max Planck Research School for Molecular and Cellular Life Sciences: From Biology to Medicine (IMPRS-LS), Munich, Germany
2017	Römer-Prize in PhD category, Munich, Germany
2019-present	Damon Runyon Fellowship Award

C. Contributions to Science

I participated in discoveries of physiological ligands of cGAS like Y-shaped DNA, RNA:DNA hybrids and U-shaped DNA. I also found and characterized a cooperative recognition of long DNA species by cGAS, as well as contributed to the uncovering of the first structure of NLRP3 in complex with its co-factor NEK7.

Sharif H., Wang L., Wang W. L., Magupalli V. G., **Andreeva L.**, Qiao Q., Hauenstein A. V., Wu Z., Núñez G., Mao Y. and Wu H. (2019). "Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome." *Nature* 570(7761): 338-343, doi: 10.1038/s41586-019-1295-z

Andreeva L., Hiller B., Kostrewa D., Lässig C., de Oliveira Mann C.C., Drexler D.J., Maiser A., Gaidt M., Leonhardt H., Hornung V., Hopfner K.-P. (2017) "cGAS senses long and HMGB/TFAM bound U-turn DNA by forming protein-DNA ladders." *Nature* 549(7672): 394-398, doi: 10.1038/nature23890. Non-NIH support.

Herzner A.M., Hagmann C.A., Goldeck M., Wolter S., Kübler K., Wittmann S., Gramberg T., **Andreeva L.**, Hopfner K.-P., Mertens C., Zillinger T., Jin T., Xiao T.S., Bartok E., Coch C., Ackermann D., Hornung V., Ludwig J., Barchet W., Hartmann G., Schlee M. (2015) "Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA." *Nat. Immunol.* 16(10):1025-33, doi: 10.1038/ni.3267. PMCID:PMC4669199

Mankan A.K., Schmidt T., Chauhan D., Goldeck M., Höning K., Gaidt M., Kubarenko A.V., **Andreeva L.**, Hopfner K.-P., Hornung V. (2014) "Cytosolic RNA:DNA hybrids activate the cGAS–STING axis." *EMBO J.* 33(24):2937-46, doi: 10.15252/embj.201488726. PMCID:PMC4282641.

D. Research Support:

Ongoing: Damon Runyon Fellowship Award

Completed: None

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: Chen Shen

eRA COMMONS USER NAME (credential, e.g., agency login): wait for approval

POSITION TITLE: Postdoctoral Fellow in Boston Children's Hospital/Harvard Medical School

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
Yangzhou University, Yangzhou, China	B.Sc.	2010.7	Chemical Education
Peking University Shenzhen Graduate School, Shenzhen, China	PhD	2015.7	Physical Chemistry
Peking University Shenzhen Graduate School, Shenzhen, China	Postdoc	2017.1	Structural Biology

A. Personal Statement

After receiving his B.S. degree in chemical education, Chen was accepted into Peking University for graduate studies. From then on, He was attracted to the mysterious biological world and his Ph.D. research was aimed at structures and functions of regulatory proteins involved in tumor cell proliferation and tumor cell death. He solved the crystal structures of both caspase-8 death effector domain monomer and its domain-swapped dimer, which provide implications for the activation mechanism of this important initiator caspase. He received the Ph.D. degree in July of 2015 but stayed in his Ph.D. lab for a short period to complete a few manuscripts. After one year and a half, he arrived in Professor Hao Wu's laboratory in Boston in February of 2017. Here, he got good scientific environment to continue his studies related with innate immune research and death domain signaling. He extensively collaborated with postdocs and students in the lab to accomplish several papers involved in TLR signaling and NLR signaling. He initiated a new project on mechanistic studies for the activation of the multifaceted innate immune regulator NLRP6. He majorly focused on the structural study of auto-inhibited and activated NLRP6 inflammasome, as well as the molecular mechanisms of ligand and NLRP6 interaction in innate immune system.

B. Positions and Honors

2015-2017 Postdoctoral Fellow, Peking University Shenzhen Graduate School, Shenzhen, China
2017- Postdoctoral Fellow, Boston Children's Hospital and Harvard Medical School, Boston, MA

Honors

The progress of IKA scholarship (2014)
Outstanding Student of Peking University (2013)
First-class scholarship in YZU (2006-2009)
CRI Irvington Postdoctoral Fellowship (2019.1)

C. Contributions to Science

1. The Ph.D. period

Caspase-8 is a central molecule involved in different innate immune signaling pathways like extrinsic apoptosis, necroptosis, and inflammation. It is activated through a proximity-induced dimerization mechanism. Although the catalytic domain of caspase-8 is solved decades ago, the structural information of the pro-domain (tandem death effector domain - DED) is hindered by its highly aggregation propensity.

a. Chen solved the high-resolution crystal structure of the caspase-8 DED monomer. Based on sequence alignment with DED homologs like MC159 from the poxvirus Molluscum contagiosum *virus and* further analysis of solubility using bioinformatics tools, He performed rational site-directed mutagenesis on the caspase-8 DED domain. He identified the I128D mutant out of four candidate mutants that dramatically changed the solubility of caspase-8. With an additional F122A mutation, He was able to obtain high quality crystals to solve the monomeric caspase-8 DED structure. Besides its implication in death inducing signaling complex assembly, this work provided a general solution for dealing with the crystallization of low-solubility death domain superfamily proteins.

b. He solved the crystal structure of the caspase-8 DED domain-swapped dimer. Because a dimerized or oligomerized form of caspase-8 DED can provide insights into caspase-8 activation, He tried to figure out how the N-terminal DED induces proximity of the C-terminal caspase-8 catalytic domain. Despite the low expression level for the DED mutant construct F122A, He successfully obtained diffraction-quality crystals by extensive optimization of purification and crystallization steps. The dimeric structure of caspase-8 DED revealed an unprecedented domain-swapped form of a death effector domain, which may represent a new paradigm for activation of initiator caspases.

Chen Shen, Hong Yue, Jianwen Pei, Xiaomin Guo, Tao Wang, Junmin Quan. Crystal structure of the death effector domains of caspase-8. *BBRC* (Biochemical and Biophysical Research Communications). 2015, **463**, 297-302.

Chen Shen*, Jianwen Pei*, Xiaomin Guo*, Lu Zhou, Qinkai Li, Junmin Quan. Structural basis for the dimerization of caspase-8 death effector domains. *Sci Rep*. 2018, **8**, 16723.

2. The postdoctoral period

The nucleotide-binding domain and leucine rich repeat containing (NLR) family Pyrin domain (PYD) containing protein 6 (NLRP6) is a multifunctional protein in innate immune signaling. NLRP6 has been shown to sense metabolites from microbiota for inflammasome activation to shape the intestinal microenvironment. This protein can also regulate anti-viral signaling through sensing viral RNA with co-factor Dhx15. However the molecular mechanism still remains elusive due to the limited structural and biochemical studies of this protein. He solved the cryo-EM structure of the NLRP6 PYD domain filament. The study showed the full length NLRP6 inflammasome activation relies on the filamentous assembly of the N-terminal PYD domain. Simultaneously, N-terminal PYD also has symmetry compatibility with ASC Pyrin domain, indicating a unified assembly mechanism for NLRP inflammasomes. On the other hand, He was curious about the different assembly mode among different NLR proteins. He took part in the study of elucidating the molecular details of NLRC4 CARD and ASC CARD assembly.

Chen Shen, Alvin Lu, Wenjun Xie, Jianbin Ruan, Roberto Negro, Tian-Min Fu and Hao Wu. Molecular mechanisms for NLRP6 inflammasome assembly and activation. *PNAS*. 2019, **116**, 2052-2057.

Chen Shen, Humayun Sharif, Shiyu Xia, Hao Wu. Structural and mechanistic elucidation of inflammasome signaling by cryo-EM. *Curr. Opin. Struc. Biol*. 2019, https://doi.org/10.1016/j.sbi.2019.03.033.

Yang Li*, Tian-Min Fu*, Alvin Lu, Kristen Witt, Jianbin Ruan, **Chen Shen** and Hao Wu. Cryo-EM structures of ASC and NLRC4 CARD filaments reveal a unified mechanism of nucleation and activation of caspase-1. *PNAS*. 2018, **115**, 10845-10852.

Elucidation the signal transduction mechanism of Toll-like receptor pathway. Chen joined the structural study of the assembly of full length Myddosome. Meanwhile, he tried different biochemical approached to understand the ubiquitin transfer mechanisms facilitated by TRAF6.

Li Wang, Qi Qiao, Ryan Ferrao, **Chen Shen**, John M. Hatcher, Sara J. Buhrlage, Nathanael S. Gray and Hao Wu. Crystal structure of human IRAK1. *PNAS*. 2017, **114**, 13507-13512.

Tianmin Fu*, **Chen Shen***, Qiubai Li, Pengfei Zhang and Hao Wu. Mechanism of Ubiquitin Transfer Promoted by TRAF6. *PNAS*. 2018, **115**, 1783-1788. (* contribute equally)

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1DgCi89OgUJQW/bibliography/public/

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: Rawson, Shaun

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

POSITION TITLE: EM IT Coordinator

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of York	MChem	07/2012	Chemistry
University of Leeds	PhD	09/2016	Structural Biology
University of Leeds	Postdoctoral	09/2017	Postdoctoral Training

A. Personal Statement

I am responsible for supporting all aspects of electron microscopy computation within the HMS Electron microscopy (EM) facility. This includes the design, implementation and maintenance of EM IT resources. I liaise with the SBGrid Core IT team and the EM facility regarding deployment of hardware, software and data management efforts. In addition, I assist local users in all areas of EM computation including; data collection, data management, structure determination, and data deposition.

B. Positions and Honors

Positions and Employment

2012-2016 Wellcome Trust PhD Program, University of Leeds
2016-2017 ISSF Postdoctoral Research Fellow, University of Leeds
2017 – 2018 CryoEM Support Scientist, Astubry BioStructure Laboratory, University of Leeds
2018 – Present EM IT Coordinator, SBGrid, Harvard Medical School

Other Experience and Professional Memberships

Honors

2014 – Leeds Faculty of Biological Sciences Postgraduate Symposium Poster 1st Prize
2015 – Leeds Faculty of Biological Sciences Postgraduate Symposium Poster 1st Prize
2016 – Professor Steve Baldwin Prize for the Best Thesis in the Area of Molecular or Cellular Biology

C. Contributions to Science

1. My PhD studies primarily focused on EM as a platform for structure-based inhibitor design. I aimed to assess the utility of EM as a tool for studying inhibitor binding through direct and indirect visualization methods. When my research began X-ray crystallography and NMR were the primary structural tools for inhibitor design as the resolution of EM was not deemed sufficient to be of use. During my studies we developed a tagging methodology for identifying ligand binding even at low resolutions before going on to use high resolution EM to directly visualize inhibitor binding and rationalize differences in inhibitor potency across species.

a. Muench SP, Rawson S, Eyraud V, Delmas AF, Da Silva P, Phillips C, Trinick J, Harrison MA, Gressent F, Huss M. PA1b inhibitor binding to subunits c and e of the vacuolar ATPase reveals its insecticidal mechanism. The Journal of biological chemistry. 2014; 289(23):16399-408. PMCID: PMC4047407

b. Rawson S, McPhillie MJ, Johnson RM, Fishwick CWG, Muench SP. The potential use of single-particle electron microscopy as a tool for structure-based inhibitor design. Acta crystallographica. Section D, Structural biology. 2017; 73(Pt 6):534-540. PMCID: PMC5458495

c. Rawson S, Bisson C, Hurdiss DL, Fazal A, McPhillie MJ, Sedelnikova SE, Baker PJ, Rice DW, Muench SP. Elucidating the structural basis for differing enzyme inhibitor potency by cryo-EM. Proc Natl Acad Sci U S A. 2018; 115(8):1795-1800. PMCID: PMC5828572

d. Amporndanai K, Johnson RM, O'Neill PM, Fishwick CWG, Jamson AH, Rawson S, Muench SP, Hasnain SS, Antonyuk SV. X-ray and cryo-EM structures of inhibitor-bound cytochrome bc1 complexes for structure-based drug discovery. IUCrJ. 2018; 5(Pt 2):200-210. PMCID: PMC5947725

2. Much of our work involved the structural study of membrane proteins, primarily the V-ATPase. Our work revealed new insights into the flexibility of this system, indicating potential mechanistic detail. In addition, we studied the suitability of alternative solubilization methods for EM. This provided the first examples of the use of styrene-maleic acid lipid particles for both negative stain and sub-nanometre cryoEM studies.

a. Postis V, Rawson S, Mitchell JK, Lee SC, Parslow RA, Dafforn TR, Baldwin SA, Muench SP. The use of SMALPs as a novel membrane protein scaffold for structure study by negative stain electron microscopy. Biochimica et biophysica acta. 2015; 1848(2):496-501. PMCID: PMC4331651

b. Rawson S, Phillips C, Huss M, Tiburcy F, Wieczorek H, Trinick J, Harrison MA, Muench SP. Structure of the vacuolar H+-ATPase rotary motor reveals new mechanistic insights. Structure. 2015; 23(3):461-471. PMCID: PMC4353692

c. Rawson S, Davies S, Lippiat JD, Muench SP. The changing landscape of membrane protein structural biology through developments in electron microscopy. Molecular membrane biology. 2016; 33(1-2):12-22. PMCID: PMC5206964

d. Parmar M, Rawson S, Scarff CA, Goldman A, Dafforn TR, Muench SP, Postis VLG. Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure. Biochimica et biophysica acta. 2018; 1860(2):378-383. PMCID: PMC5780298

Complete list of published work in my bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/shaun.rawson.1/bibliography/55040894/public/?sort=date&direction=ascending

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

Ongoing Research Support
None.

Completed Research Support
Wellcome Trust Institutional Strategic Support Fund Rawson and Muench (PI) 09/2016 – 09/2017
Wellcome Trust
ISSF Postdoctoral Research Fellow
CryoEM as used to investigate the structure of the cholesterol transport protein NCR1, a 130 kDa membrane protein, to understand its mechanism and potential as a therapeutic target.
Role: Research Fellow