BIOGRAPHICAL SKETCH

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NAME: Liu, Yujue

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Qingdao University, Qingdao, China	B.S.	6/2017	Chemical Engineering
Nankai University, Tianjin, China	M.S.	6/2020	Medicinal Chemistry

A. Personal Statement

Throughout my research career, I have been dedicated to understanding the structure and function of proteins and complexes involved in ubiquitination and deubiquitination. During my master's degree research, I focused on viral nucleoprotein and glycoprotein purification, crystal screening, and inhibitor screening. This foundational experience honed my skills in experimental and theoretical techniques essential for structural determination. Following this, I delved into generating activity-based ubiquitin (Ub) probes targeting deubiquitinases (DUBs) and reader proteins within the ubiquitin system. During this period, I spent two years investigating the interactions between ubiquitin, DUBs, and reader proteins, as well as their structures. This research further deepened my understanding of the ubiquitin system and equipped me with advanced skills in structural biology. Currently, my focus is on elucidating the cellular roles of USP9X in various biological processes using structural biology tools. I aim to solve the structure of the USP9X-K48 diUb complex and unravel the mechanism by which USP9X recognizes diUb substrates. This research is pivotal in advancing our understanding of USP9X's role in processing polyubiquitin chains and providing a foundation for future inhibitor development targeting USP9X.

Throughout my graduate research, I have acquired a diverse set of experimental techniques, including protein purification, crystal growing and screening, enzyme inhibitor screening, activity-based probe generation, and intact protein mass spectrometry. I have also gained proficiency in in vitro pull-downs, in vitro cross-linking, western blotting, and sample preparation for imaging by negative stain electron microscopy. My experiences provide me with a robust foundation in structural biology investigation using cryo-EM. I am eager to continue exploring the molecular mechanisms governing protein interactions and functions within the ubiquitin system.

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

2021- Graduate Teaching Assistant/Research Assistant, Department of Chemistry and Biochemistry, University of Delaware

Honors

2017-2020, Graduate Scholarship in Nankai University
2014-2017, Undergraduate Literary and Ethical Scholarship

C. Contributions to Science

Understanding the cellular functions of Crimean Congo Hemorrhagic Fever Virus (CCHFV) glycoprotein Gn, Gc and GP38

During my master's research, I concentrated on the structural and functional characterization of Crimean-Congo hemorrhagic fever virus (CCHFV) glycoproteins. This project led to the successful stable expression of these glycoproteins using both the Bac-to-Bac Baculovirus Expression System and the Drosophila Expression System. I performed deglycosylation assays to explore the glycosylation mechanisms of viral proteins, providing insights into their structural and functional roles. Additionally, I successfully grew and screened crystals of these glycoproteins, obtaining crystals with well-defined shapes suitable for further structural analysis. To identify potential therapeutic agents, I conducted a thermal shift assay using the MCE compound library against the CCHFV Gc glycoprotein. Through this screening process, I identified six small molecules as initial inhibitors, marking a significant step towards developing antiviral strategies against CCHFV. These experiences have equipped me with a solid foundation in protein expression systems, glycosylation assays, crystallography, and high-throughput screening techniques.

Investigation of polyubiquitin chain recognition by reader proteins and deubiquitinases (DUBs)
My current research is focused on generating ubiquitin activity-based probes and profiling deubiquitinases (DUBs), with a special emphasis on USP9X. The functions, regulatory mechanisms, and structural details of full-length USP9X remain largely unexplored. To address this, I am developing probes that target distinct ubiquitin binding sites within USP9X, providing essential tools to study its functions. To investigate potential binding sites and linkage preferences of the full-length USP9X, I have generated a set of diUb activity-based probes (ABPs) with various linkage types, each containing a warhead capable of labeling USP9X. Among these, the K48-diUb-PA probe efficiently labels USP9X. My ongoing efforts are centered on elucidating the structure of the USP9X-K48 diUb probe complex through advanced structural techniques. Given the large size of USP9X, cryo-EM is best suited for the structural elucidation of the USP9X-diUb complex. Understanding this complex structure is crucial as it may reveal the underlying mechanisms of USP9X in recognizing cognate polyubiquitin chain structures. This research not only aims to uncover the detailed workings of USP9X but also contributes to the broader knowledge of ubiquitination and deubiquitination processes. My work in this area has strengthened my expertise in activity-based probe generation, DUB profiling, and structural biology investigation of an important family of human proteins, namely ubiquitin-specific proteases.

1. Paudel P, Banos CM, **Liu Y**, Zhuang Z. Triubiquitin Probes for Identification of Reader and Eraser Proteins of Branched Polyubiquitin Chains. ACS Chem Biol. 2023 Apr 21;18(4):837-847.

BIOGRAPHICAL SKETCH

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NAME: Zhihao Zhuang

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Sichuan University, Chengdu, China	B.S.	07/1997	Chemistry
University of New Mexico, Albuquerque, NM	Ph.D.	07/2003	Biochemistry
Pennsylvania State University, State College, PA	Postdoctoral	07/2007	Biochemistry

A. Personal Statement

The overarching goal of my research program is to understand protein ubiquitination and deubiquitination using chemical-biological, biochemical and biophysical approaches. We develop and use new tools to identify reader proteins in ubiquitin signaling, probe deubiquitinase (DUB) activities and specificities, and exploit ubiquitination machineries for targeted protein degradation. My research group has been supported by NIH over the past 14 years and we made important contributions to the ubiquitin field by developing novel chemical approaches for protein ubiquitination, activity-based DUB probes and small molecule inhibitors against DUBs. Our research does not stop at tool development. We have used the newly developed tools in obtaining in-depth biochemical and structural understanding of the regulatory roles of protein mono- and poly-ubiquitination, particularly in DNA damage tolerance and mitophagy that are heavily regulated by reversible protein ubiquitination. Currently we have projects focusing on the structure and mechanism of DUBs in complex with their native protein substrates. These DUBs are usually large in size, which makes them ideal candidates for cryo-EM structural determination. Leveraging our unique set of diubiquitin activity-based probes, we can capture the target DUBs with the diUb probes and form a stable complex for structural elucidation. Our preliminary EM images collected using a Talos L120C microscope in the University of Delaware Bioimaging Center provides a strong support for the proposed training at NCCAT. Through this training my lab will be better equipped to carry out cryo-EM studies of protein complexes in the ubiquitination and deubiquitination system. Our gained expertise in cryo-EM will also catalyze the adoption of this powerful technique on the University of Delaware campus by other researchers and groups.

Below are ongoing and recently completed projects that I would like to highlight

NIH R21AG077189 Zhuang (PI) 05/01/2022 – 04/30/2025
Title: Investigating autophagic degradation of tau mediated by polyubiquitination
The major goal of this proposal is to identify autophagy receptors of tau and characterize their interactions and identify deubiquitinase of ubiquitinated tau.

NIH R01GM129468 Zhuang (PI) 04/01/2019 – 03/31/2024
Title: Decoding the non-canonical polyubiquitin chains using chemical approaches
The major goal of this proposal is to understand the non-proteolytical function of polyubiquitination of PCNA in DNA damage tolerance using well-defined ubiquitinated PCNA species generated using chemical approaches.

NIH R21NS123322 Zhuang (PI) 07/01/2021 – 12/31/2023
Title: Developing a cell-based high throughput screening for USP15 deubiquitinase inhibitor discovery
The major goal of this proposal is to develop a ubiquitin probe-based AlphaLISA deubiquitinase HTS assay that enables cell-based and high throughput screening for human deubiquitinase USP15.

NSF 2215833 Zhuang (PI) (Grimes Co-PI) 10/01/2022 – 09/30/2025
Title: MRI: Acquisition of BioLayer Interferometer Octet RH16 for Label-Free Detection of Biomolecular Interactions
The goal of the proposal is to acquire BioLayer Interferometer (BLI) Octet RH16 for investigating macromolecular interactions.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023	NSF Review Panel Division of Chemistry
2022	NIH study section *ZRG1 CB-Q(55)R ad hoc* member
2021	NSF Review Panel Division of Chemistry
2021	NIH study section *ZRG1 CB-Q(55)R ad hoc* member
2019	Guest editor for *Frontiers in Chemistry*
2019-	Professor, University of Delaware, Dept. of Chemistry & Biochemistry
2018-	*Molecules*, journal editorial board and guest editor
2018	NIH study section *ZRG1 BCMB-H ad hoc* member
2016	NSF Review Panel Division of Chemistry
2014	NIH study section SBCA *ad hoc* member
2013-2019	Associate Professor, University of Delaware, Dept. of Chemistry & Biochemistry
2011	Faculty of 1000 member in Chemical Biology
2009-	Member, Nemours Center for Childhood Cancer Research, Nemours/Alfred I. duPont Hospital for Children
2007-	Affiliated Faculty Member, Delaware Biotechnology Institute (DBI)
2007-2013	Assistant Professor, University of Delaware, Dept. of Chemistry & Biochemistry
2003-2007	Postdoctoral fellow, Pennsylvania State University, Dept. of Chemistry

Honors

2014	American Cancer Society Research Scholar
2010	NSF Faculty Early Career Development (CAREER) Award

C. Contributions to Science

My most significant contribution to science is the understanding of protein ubiquitination and deubiquitination in eukaryotic systems through development of new chemical and biochemical approaches for deciphering the biological functions of protein ubiquitination. Below I summarize the work from my lab in three main areas of research.

Developing chemical approaches for protein ubiquitination and ubiquitin-based probes for DUBs. The rapid growth in ubiquitin biology demands efficient and facile approaches for preparing homogeneously ubiquitinated protein in an amount sufficient for in-depth biochemical and biophysical investigations. Chemical ubiquitination circumvents the requirement of ubiquitin cascade enzymes and can be readily generalized for modifying different target proteins. My group has engaged in developing chemical methods for site-specific protein ubiquitination. We use proliferating cell nuclear antigen (PCNA) as a model protein for method development. We reported semisynthetic approaches for protein ubiquitination exploiting intein chemistry to monoubiquitinate a target protein through thioether and disulfide linkages. We also achieved protein polyubiquitination with defined chain length and linkage. Our approaches represent a major improvement over the conventional enzymatic method with a large increase in the yield of ubiquitinated protein. The availability of ubiquitinated PCNA species facilitated our investigation into the regulation of eukaryotic DNA damage tolerance pathways by both mono- and polyubiquitination of PCNA and other DNA damage response proteins. Furthermore, the chemical ubiquitination approach was also used to generate novel substrates and probes to study the ubiquitination and deubiquitination processes *in vitro* and in live cells. We recently reported new DUB linkage-

specific probes and their utility in understanding the specificity and function of DUBs in conjunction with X-ray crystal structure determination of an important human DUB USP9X. We also generated a target protein (PCNA)-specific DUB probe that contains a Michael acceptor warhead that allows the identification of PCNA-specific DUBs in S. cerevisiae. My lab has developed an approach of delivering monoubiquitin activity-based DUB probes into human cells using a cell-penetrating peptide (CPP), which allowed us to carry out profiling of cellular DUB activities using label-free quantitative mass spectrometry analysis. We also developed a tetrazole-based photocaged monoubiquitin DUB probe that allows temporal control of the cellular DUB profiling.

Chen J, Ai Y, Wang J, Haracska L, Zhuang Z. Chemically ubiquitylated PCNA as a probe for eukaryotic translesion DNA synthesis. (2010) *Nature Chemical Biology*, 6(4):270-272. PMID: 20208521.

Gong P, Davidson GA, Gui W, Yang K, Bozza WP, Zhuang Z. Activity-based ubiquitin-protein probes reveal target protein specificity of deubiquitinating enzymes. *Chemical Science*. (2018), 9(40):7859-7865. PMID: 30429995; PMCID: PMC6194582.

Paudel P, Zhang Q, Leung C, Greenberg HC, Guo Y, Chern YH, Dong A, Li Y, Vedadi M, Zhuang Z, Tong Y. Crystal structure and activity-based labeling reveal the mechanisms for linkage-specific substrate recognition by deubiquitinase USP9X. *PNAS* (2019), 116(15):7288-7297. PMID: 30914461; PMCID: PMC6462090.

Gui W, Shen S, Zhuang Z, Photocaged Cell-Permeable Ubiquitin Probe for Temporal Profiling of Deubiquitinating Enzymes *J. Am. Chem. Soc.* (2020) 142(46):19493-19501. PMID: 33141564; PMCID: PMC8462974.

Developing small molecule inhibitors of deubiquitinating enzymes. Abnormalities in the ubiquitin-mediated processes have been linked to many human diseases. Human USPs are emerging as promising targets for pharmacological intervention. The advantage of inhibiting USPs lies in the potential specificities of therapeutic intervention that can lead to better efficacy and reduced nonspecific side effects. Among the human USPs, USP1 and USP11 occupy a special position as being implicated in DNA damage response. We have carried out high-throughput screening against human USP1 and USP11 and identified a number of promising leads. We also demonstrated the on-target effect of the USP1 inhibitors in cells. Besides the known bioactive compounds, we have also identified and optimized novel compounds through extensive SAR that display strong (low nanomolar) inhibition against USP1. These compounds showed good solubility, microsomal stability and favorable PD/PK property. A US patent was obtained for this series of N-benzyl-2-phenylpyrimidin-4-amine derivatives as novel USP1 inhibitors. We are also engaged in determining the mechanism of actions of the inhibitors and the catalysis and regulation of the human USPs. We are also developing inhibitors against other human DUBs, including USP15. USP15 plays an important role in mitophagy and is implicated in Parkinson's disease. USP15 small molecule inhibitor provides a promising way of restoring normal mitophagy and preventing neuronal cell death in PD patients. My lab is actively developing novel HTS-compatible DUB assays in cell lysates and live cells for inhibitor discovery against human DUBs. We have reported a cell lysate-based AlphaLISA deubiquitinase assay platform for DUB inhibitors discovery. We are currently working on developing a live cell-based DUB assay using the cell-permeable activity-based DUB probe developed in my lab.

Dexheimer TS, Rosenthal AS, Luci DK, Liang Q, Villamil MA, Chen J, Sun H, Kerns EH, Simeonov A, Jadhav A, Zhuang Z, Maloney DJ. Synthesis and structure-activity relationship studies of N-benzyl-2-phenylpyrimidin-4-amine derivatives as potent USP1/UAF1 deubiquitinase inhibitors with anticancer activity against nonsmall cell lung cancer. (2014) *J. Med. Chem.* 57(19):8099-110. PMID: 25229643; PMCID: PMC4191588.

Liang Q, Dexheimer TS, Zhang P, Rosenthal AS, Villamil MA, You C, Zhang Q, Chen J, Ott CA, Sun H, Luci DK, Yuan B, Simeonov A, Jadhav A, Xiao H, Wang Y, Maloney DJ, Zhuang Z. A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. (2014) *Nature Chemical Biology*. 10(4):298-304. PMID: 24531842; PMCID: PMC4144829.

Richard A. Burkhart, Yu Peng, Zoë A. Norris, Renée Tholey, Vanessa A. Talbott, Qin Liang, Yongxing Ai, Kathy Miller, Shruti Lal, Joseph A. Cozzitorto, Agnieska K. Witkiewicz, Charles J. Yeo, Matthew Gehrmann, Andrew Napper, Jordan M. Winter, Janet A. Sawicki, Zhuang Z, and Jonathan R. Brody. Mitoxantrone targets human ubiquitin-specific peptidase 11 (USP11) and is a potent inhibitor of pancreatic cancer cell survival (2013) *Molecular Cancer Research,* 11(8):901-11. PMID: 23696131.

Ott C, Baljinnyam B, Zakharov A, Jadhav A, Simeonov A, Zhuang Z. Cell lysate-based AlphaLISA deubiquitinase assay platform for identification of small molecule inhibitors. (2017) *ACS Chem Biol*. 12(9):2399-2407. PMID: 28836754; PMCID: PMC5947317.

Molecular mechanism of eukaryotic DNA damage tolerance. Posttranslational modification of proteins represents a crucial way of regulating cellular functions and pathways. Modification of cellular proteins by ubiquitin and ubiquitin-like proteins plays an essential role in a myriad of biological processes, particularly the DNA damage tolerance. We are investigating two essential DNA damage tolerance pathways: DNA translesion synthesis (TLS) and error-free lesion bypass, and their regulation by reversible ubiquitination. We found that monoubiquitination of PCNA serves to recruit the specialized DNA polymerase η to the stalling site and promote its switch with the replicative DNA polymerase δ. We identified Ubp10 as a deubiquitinase of K164-monoubiquitinated PCNA in S. cerevisiae. We also revealed novel modes of interaction between the Polη and monoubiquitinated PCNA using photocrosslinking and mass spectrometry. We are using cryo-EM to obtain structural information on the TLS polymerase complex containing Polη and Ub-PCNA. We are also investigating another yeast protein Mgs1 and its role in the error-free lesion bypass. We have generated polyubiquitinated PCNA that contain *p*-benzoyl-L-phenylalanine (*p*Bpa) as a photocrosslinker and are using the probes to identify reader proteins that recognize the K63-polyubiquitinated PCNA. This study is crucial for our understanding of the error-free lesion bypass pathway regulated by the K63-linked polyubiquitin chain.

Shen S, Davidson GA, Yang K, Zhuang Z. Photo-activatable Ub-PCNA probes reveal new structural features of the Saccharomyces cerevisiae Polη/PCNA complex. *Nucleic Acids Res.* (2021) 49(16):9374-9388. PMID: 34390346; PMCID: PMC8450101.

Yang K, Li G, Gong P, Gui W, Yuan L, Zhuang Z. Chemical Protein Ubiquitylation with Preservation of the Native Cysteine Residues. *ChemBioChem*. (2016) 17(11):995-998. PMID: 27113245; PMCID: PMC5298353.

Yang K, Gong P, Gokhale P, Zhuang Z. Chemical Protein Polyubiquitination Reveals the Role of a Noncanonical Polyubiquitin Chain in DNA Damage Tolerance. (2014) *ACS Chemical Biology*. 9(8):1685-1691. PMID: 24918305.

Tsutakawa SE, van Wynsberghe AW, Freudenthal BD, Weinacht CP, Gakhar L, Washington MT, Zhuang Z, Tainer JA, Ivanov I. Solution X-ray scattering combined with computational modeling reveals multiple conformations of covalently bound ubiquitin on PCNA. (2011) *PNAS*, 108(43):17672-77. PMID: 22006297; PMCID: PMC3203759.

Complete List of Published Work in MyBibliography:
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