BIOGRAPHICAL SKETCH

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NAME: Zhiying Zhang

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POSITION TITLE: Research Scholar

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
Zhengzhou University	BA	08/2014	07/2018	Bioengineering
Peking University	PHD	10/2018	07/2023	Biochemistry and Molecular Biology
Memorial Sloan Kettering Cancer Center	Postdoc	10/2023	present	Structural Biology

A. Personal Statement

My academic training has provided me with an excellent background in multiple disciplines such as molecular biology, biochemistry, structural biology, microbiology, and bioengineering. During my undergraduate years in the microbiology lab at Zhengzhou University, I was involved in research on antiviral drugs targeted to hepatitis B virus (HBV), which has been marketed as a drug. During my PhD in a structural biology lab at Peking University, I was responsible for researching potent broad-spectrum neutralizing antibody drugs against SARS-CoV-2. The SA55 neutralizing antibody drug, developed in collaboration with partners, is currently the only publicly reported antibody drug lacking escape mechanisms by the novel coronavirus. SA55 has been patented and formulated into nasal spray and injectable forms and is currently undergoing clinical trials. These experiences have allowed me to accumulate expertise in virology research and drug development. My postdoctoral training has focused on bacterial anti-phage mechanisms and viral immunity. My sponsor, Dr. Dinshaw Patel, a globally recognized expert in nucleic acid structure and in the field of innate immunity, has extensive experience in training postdoctoral researchers, and has successfully trained several outstanding independent scientists. Besides providing new technologies, I have had the opportunity to engage in public presentations, lectures, laboratory management, and student guidance. These activities aim to enhance my ability to become an independent researcher. Additionally, my advisor has had many outstanding collaborators, expanding my professional network in the academic field. Currently, although I have only been engaged in postdoctoral research for less than a year, I have achieved promising results in the fields of HIV and anti-phage defense systems. I believe that with the current research environment and the research plan I have proposed, I am laying a solid foundation for my future as an excellent independent researcher in bacteriophage research and viral immunology.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 – present Research Scholar, Memorial Sloan Kettering Cancer Center
2018 – 2020 Class Monitor, Peking University.
2019 – 2020 Biochemistry Teaching Assistant, Peking University.
2019 – 2020 Laboratory Teaching Assistant, Peking University.

Honors

2014 – 2018 First-class scholarship, Zhengzhou University.
2020 – 2022 Merit Student, Peking University.
2018 – 2019 Academic Excellence Award, Peking University.
2021 – 2022 Academic Excellence Award, Peking University.
2020 – 2021 Outstanding Poster Award, Peking University Biochemistry Academic Week
2021 – 2022 Gu Wenyu Scholarship, Peking University.

C. Contributions to Science

1. Graduate Career: Molecular mechanisms of SARS-CoV-2 Nabs targeting NTD and RBD.

Prophylactic and therapeutic drugs are urgently needed to combat COVID-19 caused by SARS-CoV-2. Over the past years, SARS-CoV-2 neutralizing antibodies have been developed for preventive or therapeutic uses, especially targeting receptor binding doimain (RBD) and N-terminal domain (NTD). To investigate the molecular mechanisms of SARS-CoV-2 neutralizing antibodies (Nabs) targeting NTD and RBD, I solved two NTD-specific antibodies using X-ray crystallography and cryo-EM, which all target a single supersite, but yet can be easily escaped by Beta and Omicron variants (Cell Research, PMID: PMC8138844). I also solved six RBD-specific antibodies using cryo-EM, to study the group B, D, E, and F antibody-evasion mechanism of Omicron (Cell Research, PMID: PMC8385480). Especially, the antibody cocktail SA55+SA58 which can broadly and potently neutralize SARS-CoV-2 variants and sarbecoviruses, without available escape mechanisms, making it a valuable bsNAb drug candidate (Cell Reports, PMID: PMC9712074). To date, I have published four papers that are listed below.

1) Cao Y[#], Yisimayi A[#], Bai Y[#], Huang W[#], Li X[#], **Zhang Z[#]**, Yuan T[#], An R, Wang J, Xiao T, Du S, Ma W, Song L, Li Y, Li X, Song W, Wu J, Liu S, Li X, Zhang Y, Su B, Guo X, Wei Y, Gao C, Zhang N, Zhang Y, Dou Y, Xu X, Shi R, Lu B, Jin R, Ma Y, Qin C, Wang Y, Feng Y, Xiao J, Xie XS. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. **Cell Research**. 2021 Jul;31(7):732-741.
2) Du S[#], Liu P[#], **Zhang Z[#]**, Xiao T, Yasimayi A, Huang W, Wang Y, Cao Y, Xie XS, Xiao J. Structures of SARS-CoV-2 B.1.351 neutralizing antibodies provide insights into cocktail design against concerning variants. **Cell Research**. 2021 Oct;31(10):1130-1133.
3) Cao Y[#], Jian F[#], **Zhang Z[#]**, Yisimayi A[#], Hao X[#], Bao L[#], Yuan F, Yu Y, Du S, Wang J, Xiao T, Song W, Zhang Y, Liu P, An R, Wang P, Wang Y, Yang S, Niu X, Zhang Y, Gu Q, Shao F, Hu Y, Yin W, Zheng A, Wang Y, Qin C, Jin R, Xiao J, Xie XS. Rational identification of potent and broad sarbecovirus-neutralizing antibody cocktails from SARS convalescents. **Cell Reports**. 2022 Dec 20;41(12):111845.
4) Zhu S[#], Liu Y[#], Zhou Z[#], **Zhang Z[#]**, Xiao X, Liu Z, Chen A, Dong X, Tian F, Chen S, Xu Y, Wang C, Li Q, Niu X, Pan Q, Du S, Xiao J, Wang J, Wei W. Genome-wide CRISPR activation screen identifies candidate receptors for SARS-CoV-2 entry. **Science China Life Sciences**. 2022 Apr;65(4):701-717.

2. Postdoctoral Career:

1) Structural basis of CXCR4 mediated HIV-2 infection.

HIV (Human Immunodeficiency Virus) is a virus that attacks the body's immune system, specifically the CD4 T cells, which are crucial for the immune response and can lead to the development of AIDS (acquired immunodeficiency syndrome). During the viral entry process, the CXC chemokine receptor 4 (CXCR4) on the host cell is recognized by the HIV envelope glycoprotein gp120.

I have determined the cryo-EM structure of the CXCR4 in its apo form at 2.6 Å resolution and shown that it adopts an unanticipated tetrameric alignment. This appears to be the only known tetrameric G protein-coupled receptor (GPCR), which is unique, and its interface is strikingly different from previous models of CXCR4 dimerization that were solved using crystallography. The reason for the existence of dimeric and tetrameric forms of CXCR4 is unclear, and further exploration of its function is necessary. I next solved the 3.3 Å cryo-EM structure of ligand CXCL12 bound to CXCR4 and found that the complex adopts an 8:8 stoichiometry where four CXCL12 dimers are sandwiched between a pair of CXCR4 tetramers.

In addition, to understand the mechanism of CXCR4-mediated HIV infection, I have obtained a 3.6 Å resolution cryo-EM structure of the hCXCR4-gp120 complex. This structure reveals an important motif of gp120 (a four amino acid involved in a turn motif) that inserts into a CXCR4 groove, the same pocket that is also engaged by

its ligand CXCL12 and the small molecule inhibitor IT1t, indicating the potential for developing highly effective HIV drugs and vaccines targeting this motif.

2) Anti-phage defense system.

The arms race between bacteria and phages has led to the development of anti-phage defense systems. My postdoctoral research has primarily focused on mechanistic studies of prokaryotic defense systems against phages. This includes SMC-like Lamassu and membrane-associated Kiwa antiphage defense systems, as well as viral defense involving Brig family DNA glycosylases.

The Lamassu (Assyrian protective deity) family is a three component antiphage defense system composed of a Structural Maintenance of Chromosomes (SMC) sensor (LmuB), a putative Kleisin-like protein (LmuC), and a variable effector with diverse N-terminal cell-killing functions (LmuA). The Lamassu system is distinct from canonical SMCs in that LmuB contains a Glu to Gln substitution in the Walker B motif that can dramatically slow down ATP hydrolysis, while LmuC contains a single domain unlike the domain-linker-domain topologies of canonical Kleisins. My initial *in vitro* structure-function studies together with postdoc Arpita Chakravarti have focused on a Cap4 nuclease-containing LmuA in a LmuABC context and its complex with DNA in collaboration with Samuel Sternberg (Columbia) and Aude Bernheim (Institute Pasteur) labs who are undertaking *in vivo* functional studies. Our 3.3 Å cryo-EM structural studies on the Cap4 LmuABC complex has identified a novel SMC fold where Kleisin-like LmuC anchors kinked coiled-coil hinge segments of SMC-like LmuB and together with inserted CTD of LmuA effector adopts a compact scaffold. We are currently analyzing cryo-EM data on the Cap4 LmuABC-DNA complex towards deciphering the mechanistic basis for nuclease activity of the N-terminal domain of LmuA effector. In the longer term, we propose to study Lamassu systems with LmuA effectors exhibiting distinct hydrolase-protease, phosphodiesterase, and NADase (SIR2) activities.

Kiwa (sea guardian deity of Maori mythology) is a two-component antiphage defense system. Our collaborator Franklin Nobrega (Southampton) has proposed that membrane-spanning KwaA on sensing phage infection releases cytosolic KwaB effector from the KwaAB complex to initiate a RecBCD-dependent control of phage DNA replication. I have solved 3.6 Å cryo-EM structures of tetrameric membrane-spanning KwaA in open (C2 symmetry) and closed (C4 symmetry) states in lauryl maltose neopentyl glycol (LMNG) detergent, with the two states suggestive of distinct functions. I have also solved the 3.6 Å cryo-EM structure of a higher-order KwaAB complex composed of four closed KwaA and eight KwaB subunits, that in an oligomeric alignment, are distributed laterally within the membrane, reflective of an autoinhibited state. Current efforts are aimed at identifying specific interactions mediating DNA and Gam (a DNA mimic protein) complexes with KwaB, thereby providing insights into the mechanism of Kiwa-mediated antiphage defense.

Phages modify DNA bases through glucosylation of their genomes to counteract cleavage by restriction enzymes and CRISPR-Cas nucleases. Bacteria have evolved Brig family DNA glycosylases identified in the lab of our collaborator Luciano Marriffini (Rockefeller) to convert hydroxymethyl C (hmC) and glucosylated-hydroxymethyl C (Glc-hmC) DNA to abasic sites, thereby preventing phage replication. These DNA glycosylases include Brig2 that converts hmC-DNA to an abasic site, Brig1 that converts α- but not β-Glc-hmC-DNA to an abasic site, and BrigY (also called BapA) that converts α- and β-Glc-hmC DNA to an unknown product. hmC-DNA has been chemically prepared in the lab of our collaborator Ronald Micura (Innsbruck) and efforts are underway in the same lab to prepare chiral isomers of Glc-hmC-DNA by both enzymatic and chemical approaches in pure form for structural studies. To date, I have solved 1.6 Å crystal structures hmC-DNA bound to wild-type and catalytic D127N mutant of Brig2. In the structure of the wild-type hmC-DNA bound to Brig2, chemistry occurred to form an abasic site whose sugar ring is positioned inside the DNA duplex. In the structure of the D127N hmC-DNA bound to Brig2, the hmC base and sugar are both directed outwards and positioned in the catalytic pocket of Brig2. Critically, the hydroxyl group of hmC is hydrogen bonded to the side chain of Glu55, with this recognition explaining why Brig2 targets hmC but not mC or unmodified C. Once I obtain pure amounts of both chiral isomers of Glc-hmC-DNA from the Micura lab, the same structural approach will be undertaken to mechanistically characterize the enzymatic function of DNA gycosylases Brig1 and BapA.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2018	The Standards of Scientific Research	P
2018	Revolution of Modern Science &Technology and Marxism	A+
2018	Principles of Biology	A-
2018	Progresses in Molecular and cellular Biology	A-
2018	The Mechanism and Application of Modern Biological Techniques	A
2018	Intensive literature reading and seminars (I)	P
2018	Academic English Listening and Speaking for Graduate Students	B+
2018	Progress in Genetic and Developmental Biology	B+
2019	Principles of Modern Biology (II)	B
2019	Current topics on molecular and cellular biology	B
2019	Current topics on Genetics and Developmental Biology	B
2019	Lab Rotation	P
2019	Three Dimensional Cryo-Electron Microscopy	B
2019	Teaching Practice	P
2019	Intensive literature reading and seminars (II)	P
2020	Basic Theory and Scientific Research Practice of Graduate Students	P
2021	Laboratory Techniques of Modern Biology	P
2021	Literature Readings and Topic Discussions	P
2022	The Writing Rules of Academic Thesis	A-

Except for the scientific ethics course, Peking University graduate courses are graded P (pass) or F (fail). Passing is D or better. The scientific ethics course is using the letter grade system, A-, A, A+: Excellent; B-, B, B+: Good; C-, C, C+: Average; D, D+: Pass; F: Fail. One course credit unit is usually equivalent to 16 hours of instruction.