BIOGRAPHICAL SKETCH

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NAME: You, Yu

eRA : COMMONS USER NAME (credential, e.g., agency login): YUYOU3

POSITION TITLE: Senior Research Scientist

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
Jilin University, Changchun, China	BS	09/2006	07/2010	Life Science
Tsinghua University, Beijing, China	PHD	09/2012	01/2017	Molecular Biology
Memorial Sloan Kettering Cancer Center	Researh Fellow	06/2017	05/2021	Structural Biology
Memorial Sloan Kettering Cancer Center	Research Associate	05/2021	06/2022	Structural Biology
Memorial Sloan Kettering Cancer Center	Senior Research Scientist	06/2022	Current	Structural Biology

A. PERSONAL STATEMENT

After completing my B.Sc and M.Sc in interdisciplinary backgrounds from New Delhi, India, I decided to pursue my PhD in Biochemistry from the University of Illinois at Urbana-Champaign. My interest in defensive strategies utilized by eukaryotes and prokaryotes throughout evolution enabled me to take up multiple projects revolving around antiviral and antibacterial proteins including the virus-induced protein, Viperin, the antibacterial protein of the Toxin-antitoxin system, RtcB and the much more recently characterized proteins of the antiphage CBASS systems. With the aim of deciphering more of such defensive strategies employed by bacteria and eukaryotes, I decided to pursue my post doctorate from Memorial Sloan Kettering Cancer Center in Structural Biology, which has provided me with a platform to undertake structure-guided approaches towards uncovering various mechanisms along with an excellent collaboration with leading scientists in the field. These mechanisms are part of a recently characterized universe of novel and elusive antiphage protective operons present in the dynamic pan-genomic defense islands of bacteria. By employing innovative structural and functional studies both at MSKCC and with its collaborators, I seek to add to my repertoire of uncovering mechanisms of phage-induced robust measures employed by bacteria which might have evolutionary links with the eukaryotic immune system.

During my early period Ph.D. training in Tsinghua University, I conducted studies to investigate the structure of Nickel/Cobalt ion specific ECF transporter, an ECF transporter consist of membrane protein NikM, CbiT and soluble ATPase protein CbiO, which is essential for Nickel/Cobalt ion uptake. During my late period of Ph.D. I gained rich experience in studying membrane protein-drug molecular complex structures and functions. NADH-

ubiquinone oxidoreductase of Plasmodium falciparum (PfNDH2) represents a viable target for antimalarial drug development. Structural and functional studies revealed the RRL-552 exhibits excellent potency against both drug-resistant strains in vitro and parasite-infected mice in vivo via a potential allosteric mechanism. I also worked on structural and functional study on acid-sensing ion channel (ASIC) with mambalgin1, which is toxin isolated from black mamba snake. I reported a cryo-EM structure of chicken ASIC in complex with mambalgin1 at 5.4 Å resolution. The structure provided the first experimental evidence that mambalgin-1 interacts directly with the extracellular thumb domain of cASIC1a, rather than inserting into the acid-sensing pocket, as previously reported. Binding of mambalgin-1 leads to relocation of the thumb domain that could disrupt the acidic pocket of cASIC, illustrating an unusual inhibition mechanism of toxins on ASIC channels through an allosteric effect.
In June 2017 I joined in Dr. Dinshaw Patel's laboratory at Memorial Sloan Kettering Cancer Center, I was trained to apply electron microscopy techniques to examine SMC complexes with DNA for structural study. I learned the Smc5/6 complex, MRN complex and Spo11 complex with DNA. These works suggested SMCs' DNA-clamp mechanisms and allowed us to understand the detailed interactions among the subunits and DNA. I also investigated the role of cyclic-oligoadenylate-activated membrane protein 1 (Cam1) during the type III CRISPR immunity. Structural and biochemical analysis reveals that the CARF domain of Cam1 binds cyclic tetra-adenylate second messengers. *In vivo*, Cam1 localizes to the membrane, is predicted to form a tetrameric transmembrane pore, and provides defense against viral infection through the induction of membrane depolarization and growth arrest.

B. Positions, Scientific Appointments and Honors

2013 National Scholarship for Graduate Students, Ministry of Education of PRC.

2014 National Scholarship for Graduate Students, Ministry of Education of PRC.

2016 Innovation Award of Beijing Advanced Innovation Center for Structural Biology, 2016, Tsinghua University

2018 Outstanding Contribution in Reviewing, Elsevier Publishing Company.

C. Contributions to Science (* denotes co-first authors)

1) 2010-2014 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Nickel/Cobalt specific ECF transporter.

•We purified Ecf-S (NikM, membrane protein) and Ecf-A (NikO, soluble protein) components from Nickel/Cobalt specific ECF transporter. We crystallized NikM with Nickel/Cobalt ion and NikO in complex with DDM detergent. X-ray data collection and data procession results showed the first time structures of NikM at 1.83 Å resolution and NikA+DDM at 2.30 Å resolution.

•We identified the ion types in NikM through X-ray fluorescence spectrum and the anomalous scattering signal screening. Structural analysis and comparison indicated that NkiM unique N terminal loop is required for ion binding.

•Nickel ion uptake activity analysis and quantum chemical analysis identified the substrate selection of NikM and key residues for nickel ion binding of NikM.

•Structural analysis of NikO indicated that NikO forms a C terminal-mediated homodimer, with unexpected DDM binding in the N terminal canonical nucleotide-binding domain (NBD)

a. **Yu, Y**., Zhou, M., Kirsch, F., Xu, C., Zhang, L., Wang, Y., Jiang, Z., Wang, N., Li, J., Eitinger, T., and Yang, M. (2014) Planar substrate-binding site dictates the specificity of ECF-type nickel/cobalt transporters. ***Cell research*** 24, 267-277
b. Chai, C.*, **Yu, Y.***, Zhuo, W.*, Zhao, H., Li, X., Wang, N., Chai, J., and Yang, M. (2013) Structural basis for a homodimeric ATPase subunit of an ECF transporter. ***Protein & cell*** 4, 793-801.

2) 2014-2016 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Protein complex between Pathogenic bacteria/HIV protein and human host

•We cooperated with the Yongqiang Jiang lab to purify, crystallize and determine the structure of the complex structure of *Streptococcus suis* adhesin factor H-binding protein (Fhb) with Gb2 (host cellular receptor glycolipid GbO3 analog), which provided structural insight into Fhb-mediated host-pathogen interactions of *S. suis*.

•Cooperated with the Zhiwei Huang lab to solve the complex structure of the Vif–CBF-β–CUL5–ELOB–ELOC complex from HIV and human host, which revealed the structural basis for Vif(HIV) hijacking of the CBF-β(human host) and CUL5 E3 ligase complex (human host).

a. Guo, Y. Y.*, Dong, L. Y.*, Qiu, X. L.*, Wang, Y. S., Zhang, B. L., Liu, H. N., **Yu, Y.**, Zang, Y., Yang, M. J., and Huang, Z. W. (2014) Structural basis for hijacking CBF-beta and CUL5 E3 ligase complex by HIV-1 Vif. ***Nature*** 505, 229-233
b. Zhang, C., **Yu, Y.**, Yang, M., and Jiang, Y. (2015) Expression, purification, crystallization and structure determination of the N terminal domain of Fhb, a factor H binding protein from Streptococcus suis. ***Biochemical and biophysical research communications*** 466, 413-417
c. Zhang, C., Hao, H., Zhao, j., **Yu, Y.**, Kong, D., Chen, S., Jiang, H., Yuan, Y., Zheng, Y., Yang, M., Jiang, Y. (2016) Structural basis of the interaction between the meningitis pathogen Streptococcus suis adhesin Fhb and its human receptor. ***FEBS Lett.*** 590,1384-1392

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3). 2016-2017 (Graduate Research Assistant), College of Life Science, Tsinghua University
Project: Drug target (Membrane protein) complex with anti-malarial chemicals and molecular mechanisms of pain release

•Structural and functional studies of type-II NADH dehydrogenases (NDH2) from plasmodium falciparum (*Pf*NDH2). X-ray crystallography to determine the high resolution (higher than 3.0 Å) structures of *Pf*NDH2 with different substrates and three inhibitors, respectively. Our study revealed a novel mechanism for inhibition of *Pf*NDH2 with allosteric inhibitors and affirms *Pf*NDH2 as a valid drug target for anti-malarial treatment.

•Acid-sensing ion channels (ASICs) are neuronal voltage-independent Na^+ channels, which are new potential therapeutic targets in the management of psychiatric disorders, neurodegenerative diseases and pain. Mambalgin-1 (isolated from black mamba venom) specifically inhibits ASICs to exert strong analgesic effects and are thought to have therapeutic potential against pain. We purified the complex and solved the cryo-EM structure of chicken ASIC1a (cASIC1a) in complex with mambalgin-1, established a structural basis for its channel inhibition mechanism and provided crucial insights for the development of new optimized inhibitors of ASICs.

a. Yang, Y*., **Yu, Y***., Li, X*., Li, J., Wu, Y., Yu, J., Ge, J., Huang, Z., Jiang, L., Rao, Y. and Yang, M., (2017) Target Elucidation by Cocrystal Structures of NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (Pf NDH2) with Small Molecule To Eliminate Drug-Resistant Malaria. ***Journal of medicinal chemistry***, *60*(5), pp.1994-2005.
b. Sun, D.*, **Yu, Y*.,** Xue, X*., Pan, M*., Wen, M., Li, S., Qu, Q., Li, X., Zhang, L., Li, X., Liu, L., Yang, M., Tian, C., (2018) Cryo-EM structure of the ASIC1a–mambalgin-1 complex reveals that the peptide toxin mambalgin-1 inhibits acid-sensing ion channels through an unusual allosteric effect**.** ***Cell Discovery*** 4:27. *****

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4) 2017-current (Senior Research Scientist), Structural Biology Program, Memorial Sloan Kettering Cancer Center
Project: Structural and functional study on SMC5/6 complex and MRN complex.

•The Smc5/6 complex plays multiple roles in DNA replication and repair. Its genome-protecting functions rely on its interaction with DNA. Human Smc5/6 is uniquely emerging amongst SMC complexes as a critical viral restriction factor. Further, Smc5/6 inhibits the transcription and/or replication of several viruses including hepatitis B, herpes simplex (HSV-1), human papillomavirus (HPV), Epstein-Barr virus (EBV), and unintegrated human immunodeficiency virus (HIV). We first solved the cryo-EM structure of Nse5-6 subcomplex at 3.2 Å resolution, which is a unique feature of the SMC5/6 complex distinct from other SMC complexes. We purified SMC5/6 core complex (hexamer: Smc5-Smc6-Nse2-Nse3-Nse4) and solved the cryo-EM structue of SMC5/6 core complex with ATP and dsDNA. Finally, we got the cryo-EM structure of SMC5/6-ATP-DNA complex at the resolution 3.8 Å resolution. Our studies revealed the structures of all SMC5/6 subunits at atomic-level resolution for the first time and claimed that the SMC5/6 subunits form a clamp to encircle a double helical

DNA. We identified subunit transformations upon DNA capture and functional impact of multiple DNA contact sites. These studies laid the foundation for an in-depth under-standing of how Smc5/6 fulfill unique roles in genome protection and anti-virus process.
systems including viperin, CdnG, Cap5 and others and contributed to the discovery of novel small molecule termed AIPP, the generation of which is predicted to be the mechanism through which viperin executes its antiviral functions. Viperin is known to be induced in a variety of viral infections including Dengue, HIV and Influenza and the discovery of AIPP by fungal, archaeal and human viperin potentially added to the list of ways in which viperin inhibits viruses. Additionally, my work on the CdnG-Cap5 cyclic-oligonucleotide driven CBASS system led to the discovery of a novel small molecule with a unique linkage specificity termed as 3',2'-cGAMP which was 10,000-fold more potent in activating the operonically linked Cap5 nuclease effector as compared to other similarly reported cyclic-dinucleotide ligands. This led us to publish our manuscript in a top journal where it was selected as a featured article. Furthermore, I have also worked on cyclic mononucleotide based antiphage systems which are different from CBASS.

a. Li, S.*, Yu, Y.*, Zheng, J., Miller-Browne, Victoria., Zheng, S., Kuang, H., Patel, D.J., and Zhao, X.(Submitted). Molecular basis for Nse5-6 mediated regulation of Smc5/6 functions. ***Proceedings of the National Academy of Sciences***.
b. **Yu, Y**. *****, Li, S. *****, Ser, Z., Kuang, H.H., Than, Thane., Guan, D.Y., Zhao, X and Patel, D.J., (2022). Cryo-EM structure of DNA-bound Smc5/6 reveals DNA 5 clamping enabled by multi-subunit conformational changes. ***Proceedings of the National Academy of Sciences***, 119(23).
c. **Yu, Y**. *****, Li, S. *****, Ser, Z. *****, Sanyal, T. *****, Choi, K., Wan, B., Kuang, H., Sali, A., Kentsis, A., Patel, D.J. and Zhao, X., (2021). Integrative analysis reveals unique structural and functional features of the Smc5/6 complex. ***Proceedings of the National Academy of Sciences***, 118(19).

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5) 2020-current (Senior Research Scientist), Structural Biology Program, Memorial Sloan Kettering Cancer Center

Project: Type III CRISPR-Cas immune response by Cam1 mediates membrane depolarization.
Prokaryotic type III CRISPR-Cas systems provide immunity against viruses and plasmids using CARF protein effectors. Recognition of specific sequences of these invaders by crRNA guides leads to the production of cyclic oligoadenylate second messengers, which bind CARF domains and trigger the activity of an effector domain. While most effectors degrade host and invader nucleic acids, some are predicted to contain transmembrane helices (TMH) without an enzymatic function. Whether and how these CARF-TMH fusion proteins facilitate the type III CRISPR-Cas immune response has not been studied. Here we investigate the role of cyclic-oligoadenylate-activated membrane protein 1 (Cam1) during the type III CRISPR immunity. Structural and biochemical analysis reveals that the CARF domain of Cam1 binds cyclic tetra-adenylate second messengers. In vivo, Cam1 localizes to the membrane, is predicted to form a tetrameric transmembrane pore, and provides defense against viral infection through the induction of membrane depolarization and growth arrest. These results reveal that CRISPR immunity do not always operate through the degradation of nucleic acids, as initially thought, but through a wider range of cellular responses.

a. Baca, C.F. *, **Yu, Y. *,** Rostol, J.T.*, Majumder, P., Patel, D.J. and Marraffini, L.A., (2023). Cam1 mediates membrane depolarization to provide phage defense during the Type III CRISPR-Cas immune response. ***Nature, (in revised)***.
b. Patel, D.J., **Yu, Y.**, Xie, W., (2023) cGAMP-activated cGAS-STING signaling: its bacterial origins and evolutionary adaptation by metazoans. ***Nature Structural & Molecular Biology***, 352(2), 1-16.
c. Patel, D.J., **Yu, Y.**, Jia, N., (2022) Bacterial origins of cyclic nucleotide-activated antiviral immune signaling. ***Molecular Cell***, 82(24) 4591-4610.