BIOGRAPHICAL SKETCH

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NAME: Cheng, Ao

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

POSITION TITLE: Cryo-EM manager

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)	END DATE MM/YYYY	FIELD OF STUDY
Anhui University, Hefei, Anhui	BS	07/1997	Biochemistry
Fudan University, Shanghai	MS	07/2002	Biochemistry and Molecular Biology
National University of Singapore, Singapore	PHD	01/2009	Structure Biology, Virology and Nanoscience & Nanotechnology

A. Personal Statement

As my education and research experiences shown, I was dedicated to research. I should highlight two unique facts in my research career experience. Firstly, I have relative long bench research experience and worked in at two or three labs same time during four of seven stages (total 12 labs) because of collaboration, which have brought me opportunities to learn solid research skills on cryo-EM, structural biology, biochemistry, molecular biology as well as broad knowledge on virology and cancer biology. Secondly, I was not luck in my first postdoc (with Daniel Krummel at Brandeis) and second postdoc (with Qiu-Xing Jiang at UTSWMC) research stages. The two PIs' labs that I worked in were shut down by their universities, which seriously disrupted my research and publication. But with my passion to science and persistence personality, I have overcome these career obstacles with my independent research achievements when working in UMN.

While in high school I had excellent performances in high school Mathematics and Physics competition in China national and province level, which defined my career to be a scientist. Since late 1996, I have accumulated long term research experience by working in labs, through which I acquired independent research abilities.

In my fourth year of undergraduate study, I did my graduate thesis research project advised by Professor Ruduo Huang. The project is about characteristics of the spectroscopy of mannan and polymannan. I extracted and purified mannan from yeast and glucomannan from Konjac, a wild plant., measured their structural chemistry with spectroscopy and developed a method to turn the polymannan into a type of gel as a new type of molecular carrier. I learned purification techniques and some structural approaches from the project. Professor Huang who taught my enzymology course mentored me closely. Her talk always inspired me.

In the September of 1999, I was admitted by Fudan University as a graduate student for a Master degree, major in biochemistry and molecular biology. I was co-cultured by Prof. Yu-yang Li of school of life sciences and Prof. Houyan Song of medical school. They are two famous Chinese scientists. Prof. Song is 85 years old now and is still active in research. My thesis work centered around staphylokinase, a plasminogen activator, as a thrombolytic drug. Arg-Gly-Asp sequence was introduced into staphylokinase to improve its function. I found a new gel filtration way to renature the protein. The work was published in Chinese Journal of Biotechnology. Another work on human DNA polymerase λ resulted in a co-authorship published in Sci China Ser C-Life Sci. Through 3 years training in Fudan I mastered the techniques in gene cloning, protein chemistry, fermentation, protein renaturation, CD spectra, etc. And I learned how to

find ideas and how to do research.

After I were conferred Master degree from Fudan, I joined in Prof. Jianping Ding lab as a research staff, a structural biology group of Shanghai institute of Biochemistry and Cell biology, Chinese Academy of Sciences. The institute is a top research institute in China. From here, I started to learn crystallography. At that time of SARS epidemic in 2003, I investigated SARS-CoV RNA-dependent RNA polymerase which is the central enzyme in the virus's replication complex. The work was published in Virology. During same time, I found a nature mild inhibitor of HIV-1 RT, Sodium Houttuyfonate, from Chinese Medicine herbs.

In the late of 2004, Prof. Sek-Man Wong from National University of Singapore (NUS) called me to ask if I am willing to Singapore for PhD research. I accepted the offer because my curiosity to new things is very high and NUS is a high-ranking university in the World. Singapore racial and cultural diversity impressed me firstly. NUS is an English-speaking western style university. Along my research training at NUS, I was given other career development training: public speaking, literature analysis, critical thinking, teaching and conference organization. Through my PhD research on complex structure of flu virus NS1 protein with dsRNA and structure of hibiscus chlorotic ringspot virus, I learned structural biology (crystallography) in Adam Yuan lab, virology in Sek-Man Wong lab and a bit of nanotechnology in Andrew Wee lab (physics). All three are my PhD advisor. Most of the research was published in Cell Research and Acta Crystallogr Sect F. The left will be published soon. In 2008, my last year at NUS, my department found NUS Centre for BioImaging Sciences (CBIS). Prof. Wah Chiu is a CBIS affiliate member and visiting professor. He created a cryo-EM course there. This is my starting point on cryo-EM.

Late 2008, I was impressed by Dr. Daniel Krummel' s manuscript title" Crystal structure of human spliceosomal U1 snRNP at 5.5 Å resolution" which was published later in Nature 2009, and accepted his postdoc offer to join his new lab at Brandeis University starting on Jan 1, 2009. In June 2009. I was awarded a Training Scholarship from Argonne National Laboratory to attend APS Data collection workshop and CCP4 school. That is wonderful training to strength my structural biology skills on crystallography. My project at Brandeis is to study U1 snRNP complex with co-factors using cryo-EM in Niko Grigorieff lab at same biochemistry department. Daniel and Niko knew each other when both were in MRC for postdoc. At that time without direct detector, high resolution cryo-EM heavily relied on how you align microscopy well for good data collection and computation software. Dr. Chen Xu, Brandeis cryo-EM manager, systematically trained me cryo-EM technique from FEI Morgagni EM, Philips CM12 TEM, then to FEI F30 and F20 with SerialEM program. And those programs, IMAGIC, Frealign, EMAN2 and IMOD were run on computers there. That time Brandeis has a good cryo-EM community with a joint group meeting. When I study U1 snRNP complex with co-factors under cryo-EM, I found an exciting discovery for a co-factor itself which link to RNA granules. The discovery shifted my research direction and the research progressed well. However, on late 2011, Daniel had trouble with the new department chair, which disrupted my research and left a short time for me to find a new job.

Early 2012, I moved to UT Southwestern for cryo-EM structure of human telomerase project in Dr. Qiu-Xing Jiang lab and Shay/Wright lab. Professor Jerry Shay and Woody Wright are famous scientists on cancer biology, especially in telomerase field. UT Southwestern has excellent research and postdoc training environment. Postdoc at UT Southwestern are required to take some online courses. Although I learned critical thinking from Graduate school, while at UT Southwestern, my presentation experiences in a joint group meeting with hash criticizing style in my department implanted criticizing spirit into my habit, which I think it is one of most important academic skill I learned. Cryo-EM there is JEOL 2200 installed K2 detector. I have developed a cancer cell line with 10-20 times higher stable expression of biotin and Myc tagged telomerase, using affinity grids we developed, a 20 Å low resolution cryo-EM structure map of telomerase was achieved. However, the telomerase project is challenging since human telomerase is very low copy in cells and heterogenous. We also found that human telomerase, surprisingly, turn inactive after one processive reaction, named single-run catalysis. The part of work was published on JBC 2019. However, on August 2015, Dr. Jiang could not get tenue there, which disrupted the telomerase cryo-EM project.

On Oct. 2015, I come to Minnesota work with A research track faculty Dr. Wei Zhang at University of Minnesota. Dr. Zhang is a cryo-EM expert and UMN has a good virology program. My ongoing research is on structural mechanisms of alphavirus membrane fusion. I have taken a lead role in the project and did almost everything and made break-through discoveries on Feb. 2017 and created some new projects based on my discoveries. We have obtained exciting, unprecedented images that captured robust alphavirus membrane fusion events with target liposomes by cryo-electron microscopy. We are investigating the viral fusion protein E1 homotrimer on post-fusion membrane by cryo-ET and the post-fusion E2 homotrimer, functions as a molecular chaperon by single particle reconstruction. The E2 particles has strong preferred orientation, which we are applying Chameleon access at NYSBC. Completing this research will advance our understanding about how alphavirus glycoproteins promote membrane fusion. I expect high impactor papers come from the ongoing challenging work. However, due to funding issue, I had to move to the Hormel Institute on Nov. 2020, and late to Northwestern University on Oct. 2021.

From Nov. 2020 to Sept. 2021, I was working in Dr. Anna Sundborger lab at the Hormel Institute. The institute has Titan Krios TEM and Falcon III detector which is run by EPU program. I have collected datas on the Krios for two samples: IRGM protein and a Huntington filament bound with chaperone protein. However, Dr. Sundborger moved back to Sweden Uppsala University on June 2021.

On Oct. 2021, I took the position of cryo-EM manager at Northwestern University. I have set up our Glacios with K3 detector run by Leginon for single particle analysis, and with Ceta-CCD by EPUD for MicroED small molecular study collaborated with the Chemistry department. Managing the Glacios and Jeol1400 TEM, and other cryo-EM instruments (VitroBot, carbon coater, et al), training users, help users in their research, are my routine work. Meanwhile, I taught two courses for graduate students: Practical Training in Chemical Biology Methods and Experimental Design, The Summer Structural Biology workshop for MBTP trainees.

Most importantly, even left UMN twin cities campus on Nov. 2020 due to fund issue, I was still working on alphavirus membrane fusion project. On May 2022, we were awarded a new NIH fund for the project. Right now, I am applying Chameleon access at NYSBC to finish E2 cryo-EM structure.

Based on my research experience, my long-term research interests involve in the three parts: 1. Mechanistic and structural study on viral fusion, and viral proteins involved in virus-host interaction, viral replication machinery; and may extend fusion study to cell fusion; 2. The structural basis for the function of telomerase and other cancer-related protein particles especially from virus-related cancers; 3. RNA granule-like structures in vitro formed by potential scaffold proteins, and structures of RNA granules related proteins.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 -	Cryo-EM manager, Northwestern University, Evanston, IL
2020 - 2021	Research Associate, The Hormel Institute, University of Minnesota, Austin, MN
2015 - 2020	Research Associate, University of Minnesota, Minneapolis, MN
2012 - 2015	Postdoctoral Associate and Research Scientist , UT Southwestern Medical Center at Dallas, Dallas, TX
2009 - 2012	Postdoctoral Research Fellow , Brandeis University, Waltham, MA
2002 - 2004	Research Staff , Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences , Shanghai

Honors

2009 - 2009	Training Scholarship, Argonne National Laboratory, Chicago, IL

C. Contribution to Science

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1. At the time of SARS epidemic in 2003, I investigated SARS-CoV RNA-dependent RNA polymerase (RdRp) which is the central enzyme in the virus's replication complex. The SARS-RdRp gene was cloned from Cultured Vero cells that were infected with SARS-CoV BJO1 isolate, expressing, purifying and characterizing the unstable polymerase. This led to the first-ever creation of an active recombinant SARS-CoV RdRp to study and enabled drug developers to use RdRp as a target for developing control strategies.

    a. Cheng A, Zhang W, Xie Y, Jiang W, Arnold E, Sarafianos SG, Ding J. Expression, purification, and characterization of SARS coronavirus RNA polymerase. Virology. 2005 May 10;335(2):165-76. PubMed Central PMCID: PMC7111802.

2. Influenza infection often leads to pneumonia. My research into the structure and the role of the influenza NS1 protein pinpoint specific mechanisms of immune suppression. NS1A draws its power from its binding ability to double-stranded RNA (dsRNA), which prevents the body's natural antiviral response to the infections they are causing. My crystal structure for the wild type NS1A RBD bound to a dsRNA revealed that NS1A RBD recognizes dsRNA in a major groove binding mode with a pair of invariable Arg38 and that the recognition is entirely with the dsRNA backbone.

    a. Cheng A, Wong SM, Yuan YA. Structural basis for dsRNA recognition by NS1 protein of influenza A virus. Cell Res. 2009 Feb;19(2):187-95. PubMed PMID: 18813227.

3. The hibiscus chlorotic ringspot virus infects the hibiscus family, including kenaf, an annual crop that is harvested for use in the wood-pulp industry in North America and Asia. I achieved a visualization of the crystal structure at 3.2 Å of this plant virus by growing a type of crystal that yielded to high quality data set, from which crystal structure was able to be determined. My key findings were that calcium ion and β-annulus were integral components of the virus's structural stability. Now we also determined the cryo-EM structure at 3.3 Å and compared interesting differences of the virus structure at different pH. New publication will be added.

    a. Cheng A, Speir JA, Yuan YA, Johnson JE, Wong SM. Preliminary X-ray data analysis of crystalline hibiscus chlorotic ringspot virus. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2009 Jun 1;65(Pt 6):589-93. PubMed Central PMCID: PMC2688417.

4. In my research during the time at UT Southwestern Medical Center at Dallas, I tried to understand the structural basis for the function of telomerase by cryo-EM. Telomerase is a unique RNA-protein complex and has its own template RNA which is even bigger than TERT and used as scaffold for the whole complex, which cause the complex flexible in structure. Such flexibility is a challenge for structure study. Another challenge is that telomerase is low copy in cells, around 100 per cell. So, it is biochemistry difficult to get enough pure telomerase for enzymatic and structural study. I have developed a cancer cell line with 10-20 times higher stable expression of biotin and Myc tagged telomerase, the telomerase gene insert was carried by retrovirus and integrated with the cancer cell genome. The much efficient expression and purification system made the sample preparation much easier. Using affinity grids, a 20 Å low resolution cryo-EM structure map of telomerase was achieved. By using droplet digital TRAP assay to analyze both endogenous and the recombinant telomerase holoenzymes, we found that human telomerase, surprisingly, turn inactive after one processive reaction, named single-run catalysis. Telomerase loses its activity after one run of catalysis. Single-run catalysis is a built-in brake for telomerase. telomerase process a number activity cycle then it will be dead, although the TERT and hTR are still there. The part of work was published on JBC 2019. I am co-first author.

    a. Sayed ME, Cheng A, Yadav GP, Ludlow AT, Shay JW, Wright WE, Jiang QX. Catalysis-dependent inactivation of human telomerase and its reactivation by intracellular telomerase-activating factors (iTAFs). J Biol Chem. 2019 Jul 26;294(30):11579-11596. PubMed Central

PMCID: PMC6663873.

5. Staphylokinase, a plasminogen activator, as a thrombolytic drug. Arg-Gly-Asp sequence was introduced into staphylokinase to improve its function. I found a new gel filtration way to renature the protein.

    a. Cheng A, Song G, Su HB, Yu M, Li YY, Song HY. [The renaturation and purification of RGD-staphylokinase by gel filtration]. Sheng Wu Gong Cheng Xue Bao. 2002 Nov;18(6):693-7. PubMed PMID: 12674639.