Statement of Purpose Jim L. Zhang

Stanford Biosciences

Twenty building blocks is all it takes. From gates and chemical catalysts to motors and turbines, I have always been captivated by the diversity of protein structure and function. As an aspiring professor, I seek to continue studying these molecular complexes to contribute to our fundamental understanding of biological function. By pursuing a Ph.D. in Structural Biology, I will gain the practical and theoretical knowledge necessary to become an independent researcher within the field. Training in biophysical techniques, particularly cryo-EM, is key to this endeavor — which the faculty at Stanford University are aptly positioned to provide.

I first became interested in structural biology during my freshman year at Rice University when I began researching in the laboratory of Dr. Yizhi Jane Tao. As a first-generation, low-income student, the thought of scientific research was daunting. I had never held a pipette, but the Tao Lab provided me with incredible one-on-one mentorship. The dedicated training I received reaffirmed my interests in a scientific career, prompting me to apply and enroll in an accelerated, thesis-based B.S.-M.S. program shortly after joining. This allowed me to take graduate-level courses in advanced biochemistry, scientific writing, and research ethics; speak at regional conferences and departmental seminars; and present yearly progress reviews with a faculty-led thesis committee.

My initial project in the Tao Lab focused on developing a native-condition culture and purification scheme for Orsay, the only virus known to infect *Caenorhabditis elegans*. Purifying Orsay would provide a key reagent for structural characterization and support its potential in expanding the role of *C. elegans* as a model for eukaryotic viral infection. As the lead researcher responsible for this work, I learned to successfully apply techniques in nematode culture and density-based protein purification to obtain pure samples of the infectious virion. With these samples, I demonstrated that Orsay incorporates a spike protein into its capsid structure via immunoblotting and transmission electron microscopy. I presented these findings in an initial mid-author publication, where I was also responsible for major revisions to the manuscript. These samples were then analyzed with collaborators at Harvard University via single-particle cryo-EM, resulting in a high-resolution structure of the infectious virion — which I interpreted to reveal that the spike protein is covalently incorporated into a unique vertex about the viral capsid. Subsequent assays I performed *in vivo* demonstrated this spike protein is ultimately essential for viral infection and entry. I am currently preparing a first-author manuscript describing the composite virion structure and function of this incorporated spike protein.

My present work centers around expressing and purifying the ectodomain of a putative host-cell receptor for the Orsay spike protein via eukaryotic insect cell culture. Upon purification, I will conduct *in vitro* binding assays to confirm the expected interactions between a viral protein and its receptor and, if successful, attempt to determine a co-structure of the two interacting

proteins. I will defend this work along with previous results in my master's thesis by the end of this upcoming academic year. Alongside my graduate research project, I am also purifying and structurally analyzing an additional novel virus through cryo-EM for my undergraduate honors thesis. The virus is unique in that it contains a double-stranded RNA genome, yet has a filamentous capsid. Determining the structure of the capsid and how the virus compartmentalizes double-stranded nucleic acid into such a narrow complex will enable a structure-based analysis of viral capsid proteins. Such analysis will provide exciting clues towards the evolutionary relationships between single- and double-stranded RNA viruses, which remain ambiguous.

From my time in the Tao Lab, I have gained significant experience in conducting independent research and in practical wet-lab skills, working with multiple model organisms and biophysical techniques for the expression, purification, and analysis of proteins. My advisor's support throughout the process inspired a personal commitment to outreach and mentorship, while my enrollment in the accelerated B.S.-M.S. program provided me with early exposure to graduate study. Both have ultimately pushed me to professionally communicate my work and mentor new researchers, reaffirming my interests in an academic career.

At Stanford, I can continue to develop my practice and understanding of structural biology while shifting towards the study of cellular protein complexes. I am especially interested in working with nuclear and transmembrane proteins which, despite their fickle behavior *in vitro*, facilitate some of the most incredible physical and chemical transformations within cells. To that end, Dr. Georgios Skiniotis' work in investigating the structure and mechanics of G protein-coupled receptors greatly resonates with my interests. I am also fascinated with Dr. Liang Feng's structural investigations of translocases and the mechanisms that guide their molecular selectivity, in addition to Dr. Steven Boxer's research on electron transfer in photosynthetic reaction centers.

The Biosciences program overall offers an incredible opportunity to learn and perform cutting-edge structural biology research. The sheer size of the department provides a myriad of learning and research opportunities, which I would be able to explore with the flexibility provided by the curriculum and lab rotation system. In addition, the department's Home Program structure generates an intimate, supportive research environment that simultaneously enables interdisciplinary training and collaboration. Through Stanford, I can contribute to our understanding of cellular function through structure — gaining the skills necessary to become a professor dedicated to not only addressing the key biological questions we face today, but also to mentoring the next generation of scientists and researchers.