Motivation Letter

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One of my most impactful experiences during college occurred during my third year molecular biology course. Almost every lecture, I was enthralled by the different macromolecular complexes that were the focus of the class. I was primarily intrigued by the simple shapes the professor had used as representations in his powerpoints. "How accurate could these simple drawings be to the actual structures of these complexes?" I would think. This curiosity eventually resulted in the development of my daily routine; I would take note of complexes that I found particularly interesting, find their structures in the Protein Data Bank, and then examine them in PyMOL.

I was fascinated by how diverse many of these macromolecules looked, both in size and shape. Something even more fascinating to me was how their structure affected their function. Once in a while, I would end up on seemingly endless searches for the structure of a protein or macromolecular complex. My inability to find a structure would lead me to conclude that this biomolecule had yet to be visualized. What surprised me most was that many of these mystery biomolecules were involved in pathways critical to human health and disease. A particular example that stood out to me was the DNA damage repair protein, BRCA2, for which I found a number of partial structures, but no complete structure. As this protein played an important role in tumor suppression, it was striking to me that it's full structure had yet to be solved. Realizing that there are many important biological complexes, such as BRCA2, that remain to be visualized, I was motivated to pursue research in the field of structural biology. I was fortunate enough to have the opportunity to conduct my bachelor's thesis research in a structural biology lab, learning a great deal along the way. Now I would like to continue my education to explore the topic further in depth. Hence, I am applying to the Master's program in Biology at ETH Zurich, with the goal of conducting structural biology research to gain a better understanding of the molecular world.

Three years of experience working in the Agrawal lab has prepared me for challenges faced in the field of structural biology. During my first two years in the lab I was tasked with the overexpression and purification of a translational factor in a bacterial overexpression system. Over the course of this project I learned a great deal of protein biochemistry, primarily protein affinity purification methods, along with basic molecular biology lab techniques, such as culturing bacteria, western blotting, SDS-PAGE, and PCR. Since graduating, I have been working full time as a research assistant in the same lab and I have had more opportunities to explore a diverse array of projects. In one of the projects, I standardized a GTPase assay to measure the differences in susceptibility of different types of ribosomes to a variety of antibiotics. I also analyzed these structures and was able to determine the molecular basis of susceptibility or resistance. In another project I processed cryo-EM data of the small ribosomal subunit from M. smegmatis grown in nutrient limiting conditions. I interpreted the different classes of density maps obtained from this dataset and identified the presence of multiple translational factors bound to the subunit. I have learned a number of key computational skills important for structural biology through each project. These include scripting in the Unix command line, cryo-EM data processing with cryoSPARC, using Chimera and ChimeraX for the visualization of maps and PDBs, and using Coot and

PHENIX for model building and refinement. In addition, I have also been learning how to vitrify and prepare grids for data collection.

Though there are many excellent structural biology research groups present at ETH Zurich, I am keen to work in the lab of Dr. Nenad Ban. My interest stems from the lab's work on the structure determination of large biomolecular assemblies, with a particular focus on the translational machinery. My prior experience in ribosome biology and cryo-EM would help me adapt quickly to the research needs of the lab. The group's history of working with the mTOR complex, which is a major upstream regulator of translation, is also of great interest to me as I am particularly curious about changes in the landscape of protein synthesis during nutrient limiting conditions.

In conclusion, I hope to further my academic training with the goal of understanding the molecular world in greater detail. My fascination for structural biology has only grown after conducting research first hand. If I am given the opportunity to enroll at ETH Zurich as a Masters student, I will make the most of it by working hard to make contributions to our understanding of how basic biological mechanisms govern human health and disease.

Structural insights into nutrient starvation and mTOR inhibition driven ribophagy

Ayush Deep

Background

Nutrient limiting conditions and mTOR inhibition result in the selective autophagy of the translational machinery in eukaryotes through a process known as ribophagy[1-3]. Specific adaptor proteins, NUFIP1 and ZNHIT3, are required for the degradation of the 60S subunit, but where these factors bind on the 60S remains unclear[1]. Data has also shown that ribosomes may be remodelled during nutrient limiting conditions to allow for ribophagy receptors to bind[1], but the exact structural basis of this remodelling has yet to be elucidated. Since translational regulation and autophagy are disrupted in a number of pathologies, structural insight into these processes is of great importance to understanding the biological basis of maladies such as ribosomopathies, metabolic disorders, neurodegeneration, and cancer[4, 5].

Objectives and goals

The primary focus of this project will be to determine the structure of the 60S pre-ribophagy complex (60S-NUFIP1-ZNHIT3), along with possible intermediate states using two complementary structural biology techniques, cryogenic electron microscopy (cryo-EM) and cross linking mass spectrometry (CLMS)[6]. This study will assist in understanding the exact structural changes that occur on the ribosome to allow for the binding of these particular ribophagy receptors. A heterogeneous sample of 60S subunits purified directly from mammalian cells grown in mTOR inhibited and/or nutrient deprived conditions will be used. This dataset will also be further analyzed for the formation of novel complexes that may only be present during mTOR inhibition or nutrient starvation.

Methods of investigation

HEK293 cells will be grown and then incubated in amino acid deprived or Torin-1 treated media for 60 minutes[1]. Ribosomes will be purified using a sucrose cushion gradient and fractionated as described in ref[7]. Chemical cross-linking with amine-reactive crosslinker disuccinimidyl diacetic urea (DSDU) can be done as necessary to maintain the integrity of native complexes during cryo-EM grid preparation[8]. The 60S fraction will be used to prepare grids and micrographs will be collected. During cryo-EM data processing, extensive heterogeneous classification will allow for the elucidation of complexes that include NUFIP1 and ZNHIT3, along with other potential factors.

The 60S sample will then be analyzed with cross-linking mass spectrometry (CLMS) to determine which proteins are bound to the large subunit[9]. This data will later be used for placement and modelling of bound proteins. Software such as cryoID[10] will assist in protein identification with cryo-EM density maps.

The final step will involve the modelling and interpretation of the cryo-EM density maps. Programs such as cryoDRGN[11] may also be used in conjunction with standard structural biology modelling software to determine the dynamics of various classes. A mechanism for assembly and biological relevance will be proposed based on interpretations of the structural ensembles.

Timetable and milestones

Milestone	**Time**
Cell culture and ribosome purification	2 to 4 weeks
cryo-EM grid preparation, screening, and data collection	1 to 2 weeks
CLMS data collection and processing	2 weeks
Data processing, map interpretation and model building	3 to 4 months

Table 1: Approximate timeline of the 6 month project.

Note: If additional time is remaining, other fractions from ribosome purification (40S, 80S, 110S, and polysomes) can be imaged and analyzed.

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