Research programs in my laboratory

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Overall summary:

My laboratory is working in two general directions. One is the structural basis for the function of macromolecular complexes. We are studying on membrane proteins including the voltage-gated potassium (Kv) channels and the inositol 1,4,5-trisphosphate receptors (IP_3R) (1-6) as well as intracellular RNA-binding complexes(7,8). The gating mechanisms for the ion channels we are studying are well conserved and of general importance. Currently we are working on a new finding my laboratory made a couple of years ago, which we named the "lipid-dependent gating" of voltage-gated channels. We are advancing this new direction through both functional and structural studies, and will study its relation to neurological diseases caused by lipid metabolic defects in animal models and in cells derived from human patients. Our work on the IP_3R is important because calcium signaling is required for various biological processes. We have developed new methods to purify IP_3R to biochemical homogeneity and retain them in different conformations for structure determination by cryo-electron microscopy (cryoEM). Our work on intracellular signaling complexes, mainly viral RNA-sensing (RIG-I/MAVS) pathway and human telomerase holoenzyme, are important for innate immunity and cancer biology. We therefore are working on different signaling proteins closely related to important biological questions.

The second general direction in my laboratory is the development of new techniques for both single particle cryoEM and for studying eukaryotic membrane proteins in lipid environments. CryoEM is becoming a revolutionary technology for Structural Biology as well as for Cell Science. Progresses are being made in a rapid pace and will generate novel insights into various biological processes that are critically important for Life Sciences and for public health. With a NIGMS EUREKA award, my group developed a new chemical engineering method to present bioactive ligands on the surface of nano-meter thick carbon films, which we named ChemiC films(8). With an AHA National Innovative Award, we invented a bead-supported unilamellar membrane (bSUM) for studying membrane proteins in bilayers that mimic eukaryotic membranes (revised paper in review). We are advancing these two technologies. My plan is to combine the chemiC method with a new Gatan K2 Summit Direct Electron Detector in order to achieve near-atomic resolution structures for the IP_3R and small biological complexes of 150-400 kDa in mass(9-17). It will enhance our capacity in working on important molecular complexes.

1. Structural and functional studies of membrane proteins and intracellular signaling complexes

For our studies of membrane proteins, we are investigating the gating mechanisms of two types of ion channels(4,5). For Kv channels, we focus on lipid-dependent gating(2). We found that nonphospholipids stabilize the voltage sensor domains in the resting state without change in transmembrane electrostatic potential. Nonphospholipids, including cholesterol, glycolipids, many sphingolipids, etc. are abundant molecules in cell membranes, especially in neurons and glial cells. We therefore are working on understanding the general importance of these native nonphospholipids to the gating behavior of eukaryotic Kv channels. Even though lipids have been known to exert effects on membrane proteins, no one else had seen as strong effects as we did. More interestingly, a majority of monogenic lipid metabolic diseases are related to the dysregulated homeostasis of nonphospholipids. The significant neurological phenotypes in human patients are epilepsy and seizure, both of which result from hyper-excitability in the specific areas of central nervous system. Because of the cross-talks between lipid metabolic disorder and chronic inflammatory diseases as well as the altered lipid metabolism in other diseased cells such as cancer cells, we think that the lipid-dependent gating plays an important role in these diseases, and will test this hypothesis experimentally. A neuroscience EUREKA award I prepared for NINDS earlier this year was ranked at the 13th position out of a few hundreds, and the reviewers all liked this research direction and requested animal-based studies to be added to my research plan. On the biophysical front, the lipid-dependent gating in conjunction with conformation-specific binders from phage-displayed peptide library has led us to three novel conditions that stabilize a Kv channel in the resting state for structural studies.

Technical limitations in conventional membrane systems have made it difficult to study lipid-dependent gating effects in nonphospholipid-containing membranes. We developed the bSUM system to overcome these

limitations. It will allow us to measure the physicochemical properties of bilayer membranes and quantify the voltage-gated activities of Kv channels in composite lipid environments. We will focus our study on human neuronal Kv4.3 channels and Kv2.1 channels because of their importance in generating action potentials in neurons and muscles and also because of published studies suggesting that these channels are delivered into specific lipid microenvironments. To understand the clinical importance of lipid-dependent gating, we will combine our *in vitro* studies with results from patient-derived fibroblasts and neurons from animal models for specific lipid-metabolic diseases to advance the neuronal hypothesis for defects caused by dysregulated homeostasis of nonphospholipids in cells and tissues. Our studies will develop new concepts on how voltage-gated ion channels are organized in cell membranes and how lipids change *in vivo* activities of cells that harbor defects in lipid metabolism. In general, detailed mechanisms for the lipid-dependent gating effects will be important for our understanding the reported roles of voltage-gated ion channels in human diseases because of the metabolic shift usually seen in affected cells.

Our structural studies of Kv channels are focused on obtaining 3D structure of a Kv channel in the resting state, which has been a long-standing question in the field. Our work on the 2D crystals of Kv channels has been much slower than expected because environmental flaws in our EM room have prevented us from collecting high-quality data in our in-house cryoEM. I have had to rely on my collaborators off campus, and thus have shifted our attention to the study of a Kv channel in the resting state by X-ray crystallography. Because of the difficulty in applying electrostatic potential (20 kV per mm is 80 mV per 4 nm) to any experimental specimens in order to stabilize voltage-sensors in the resting state, we took advantage of the lipid-dependent gating effects on KvAP channels, and identified three different conditions that serve the same purpose without transmembrane potential. Our peptide fusion proteins are able to lock the KvAP in the resting state reliably, and we are currently using crystallographic methods for structure determination. In the future, our new ChemiC technology will be applied to the single particle cryoEM study of human voltage-gated sodium channels (~400 kDa in mass). These channels are controlled by voltage and lipids. But we still do not know how these gating modes function together and whether alteration of lipid metabolism in different human cells changes the channel function. Our new ChemiC method can overcome the difficulty in overexpressing eukaryotic voltage-gated sodium channels for structural investigations. We will pursue subnanometer resolution structures in order to uncover the structural basis for the two gating modes. The combination of our new technologies with fine protein biochemistry should make this goal feasible.

Our interest in the IP_3Rs centers on the ligand-induced conformational changes(4). For the past few years, our study has been supported continuously by a Welch Foundation grant. There have been disagreements in the structural studies of the receptors in the closed state. It appears that part of the problem came from the difficulty in the biochemical purification of the receptors. We have thus developed a new procedure to purify the receptors from rat cerebellum and sf9 cells. Our biochemical preparations of the IP_3R are stable and monodispersed, suitable for crystallography and single particle cryoEM. We have resolved the structures of the IP_3R in ligand-free and ligand-bound states by single particle reconstruction at 10.5 and 12.5 Å, respectively. Because of the current disagreements in the field, we are improving the resolutions before publication. We are analyzing large datasets collected with the Titan Krios facility at the HHMI Janelia Farm Research Campus. Our immediate goal is to define the ligand-induced structural rearrangements of the transmembrane helices in our cryoEM maps. In parallel, we are developing conditions to stabilize the receptors in the open state and make them suitable for structural studies. In the long run we will investigate the interaction of the receptor with various cytosolic and luminal proteins that are known to modulate IP_3R function (18-21). These studies will reveal how IP_3Rs are controlled in physiological environment and how deregulated calcium signaling contributes to human diseases. Ultimately we will need to resolve the receptor structures in membrane. We will further develop the spherically constrained reconstruction method, which was proposed by me(16) and has recently been further improved (22,23). We will apply our new surface engineering techniques to expand the scope of this method. Due to its large size and our sub-mg level prep, IP_3Rs will be suitable as our model system to advance these technologies.

Our work on the RNA-binding complexes is taking advantage of the RNA-presenting grids. These grids have 500-1000 RNA oligos per μm^2 on the surface of the nanometer-thick carbon films. We have been working on the intracellular RNA-sensing mechanism for innate immunity. The general idea is that our grid-technology will allow us to assemble the active RIG-I/RNA complex in a controlled fashion, and can then study the interaction between the complex and the silent MAVS directly on the surface. The program is aimed at understanding the RNA-triggered RIG-I activation, the subsequent activation of MAVS, and the signal amplification through the formation of prion-like MAVS filaments on the surface of mitochondria. We prepared specific RNA-ligands that are suitable for preparing the active RIG-I dimes and can prepare these complexes

for structural and functional studies. We have purified full-length MAVS from mitochondria in an auto-inhibited state, and have solved the structures of two MAVS filaments and shed light on the chemical basis for the filament formation. There have been two different MAVS CARD filament structures. It turns out that the sorting of the data into two categories could derive two different structures. Unlike what our competitor, Dr. Egelman and his colleagues, concluded, we found that a majority of our original datasets agreed with the C3 filament, and a small portion (~40%) appears to be the C1 filament. We have obtained a 4.2 Å map for the C1 filament and are sorting the more heterogeneous C3 filaments in order to obtain a structure at a better resolution. In the next step, we will need to figure out which of the two forms or both are important for *in vivo* signaling. For the structural studies of RIG-I/RNA complexes and the MAVS dimers, we will combine our ChemiC technology with the biochemical preparations for single particle reconstruction. We will optimize our conditions in order to resolve the structures of these ~200 kDa proteins.

The second target we are actively working on is the human telomerase holoenzyme, a hallmark for almost 90% of human cancers. Telomerase holoenzyme is the molecular engine responsible for maintaining telomere length equilibrium. These physiological functions make telomerase an important target for basic and translational research. Chemical inhibitors against the telomerase are already in clinical trials, but the structural details on its enzymatic action are still missing. Recently published studies by EM revealed significant heterogeneity in specimens. We therefore decided to use new technologies we developed in the recent years to dissect the kinetic behavior of the enzyme and stabilize the enzyme at specific conformational states for cryoEM imaging and 3D reconstruction. With the CPRIT-funding for the past two and half years, we have made important findings of the kinetic property of the holoenzyme, which has made it possible for us to secure the complexes in specific conformational state and image them under cryoEM. Our plan is to obtain the subnanometer resolution structures of the holoenzyme and understand the conformational changes that endow the unique kinetic properties to the telomerase.

2. Developing new methods for cryoEM imaging and membrane biology.

Our ChemiC films have various applications, and can be tailored for different biological questions (9,11,13-15,24). Despite the expected difficulties when we started from scratch, we have in the past four years successfully implemented what we proposed for the NIH EUREKA award. Technically, I am planning to combine the ChemiC technology with recent progresses in cryoEM imaging and data analysis in order to achieve high-resolution structures of biological complexes that are 200-400 kDa in mass. The ChemiC films allow us to control vitrified ice to be 25-40 nm thick and significantly decrease noise from ice scattering. In addition, the chemiC films enable us to study proteineous complexes at lower concentrations because the high surface density of biological ligands will selectively enrich (sub)nanogram materials. To demonstrate the potential of the new ChemiC technology, we have been applying it to protein complexes other than the ones my laboratory is working on. Our study of the 220 kDa C3PO uncovered a new enzymatic cycle and allowed us to evaluate the feasibility of studying small complexes by single particle method. Our work on the human telomerase holoenzyme as described above is fulfilling the ChemiC's potential in the structural study of a low-abundance complex. It is becoming feasible to study complexes that are ~50 copies per cell. Our work on the RIG-I/MAVS signaling is demonstrating the assembly of specific biological process on the ChemiC surfaces (7,11,13). These aspects together will demonstrate the high potential of our ChemiC method, and make it useful for us to work on smaller, less abundant, proteineous complexes.

To further develop our technology in the coming years, I will combine the chemiC films with nanometer-scale microfabrication in order to advance the sample preparation technology in cryoEM. We have started working with materials scientists in microelectronics to implement this new platform. The other technical direction will develop a novel strategy to study mature eukaryotic membrane proteins on cell surfaces. Many membrane proteins undergo a complicated maturation process, but the current biochemical technology does not allow effective separation of mature proteins on cell surfaces from immature ones inside. Our strategy will utilize ChemiC films to select fully functional membrane proteins directly from cell surface, or from enriched fractions of surface proteins. The enriched mature proteins will be preserved for structure determination by cryoEM. These two new methods may potentially change the way things are done in single particle cryoEM.

The bSUM system we developed recently has two possible applications that are of practical use. One is that it can be expanded into bi-specific targeted delivery systems. Bi-specific antibodies have been well developed and are suitable for delivering specific chemical reagents to specific cells, such as cancer cells. These antibodies will be presented to the surface of the bSUMs that are made from bio-degradable porous beads and have the capacity to load chemical molecules. The targeted delivery of these beads will make unique reagents for cell-specific treatment in the future. On the second direction, the bSUMs can be prepared

on 50-100 nm polystyrene beads, where the electron scattering power of the beads is similar to ice. These supported vesicles will be used to reconstitute 4-6 copies of membrane proteins. The constraints of the vesicles will help the determination of the Euler angles and the lipid membranes will provide proper environment for keeping the membrane proteins in a more native condition. We will therefore be able to obtain cryoEM images of these proteins for 3D reconstruction. In general, more applications of the bSUMs will become clear when new questions emerge in the future.

In summary, the research programs in my laboratory have been centered on key questions in the fields and will continue evolving around the structural and functional studies of biologically important macromolecular complexes as well as the development of new technologies. We pursue independent projects as well as collaborative investigations. Major efforts are invested in those projects that can take good advantage of our newly invented technologies and offer new opportunities for us to improve our technologies or invent new ones.

There are multiple opportunities for graduate students to join these research programs and make their own contributions in the two general directions. Please don't hesitate to contact me for any question or further details.

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