Research Statement

Diverse proteins, lipids, and other substances are involved in maintaining biological systems. Their seemingly stochastic interactions are amazingly well regulated to carry out specific functions. Biologists have studied to reveal hidden molecular pathways that account for sequential reactions occurring in biological systems and also successfully visualized major structures in cells. However, the functional states of macromolecular complexes are often difficult to be coupled to their corresponding structural states mainly due to their small sizes. My research interests are to visualize reliably the macromolecular complexes in biological materials, to build structural basis of their functions and to assess the differences in them under disease conditions to understand structural correlates of many biological functions at nanometer scale using electron tomography and computation-intensive quantitative measurements and modeling. My specific interests are in the following.

Electron Tomography Electron microscopy is a powerful imaging tool that has found broad application in the biological and material sciences. Electron tomography, which combines transmission electron microscopy and computed tomography, has made revolutionary impact on the study of biological and material samples by providing 3-dimensional (3D) structures at nanometer or sub-nanometer scale. In electron tomography, two-dimensional images of a sample are collected at 1° or 2° intervals to tilt angles of $\pm60^o$-70°. The ~100 images are then aligned using gold beads as fiducial markers and the volume of the section in the field of view is typically reconstructed by a back-projection scheme. The range of the tilt angle is ideally $\pm90^o$, which is almost impossible due to technical reasons. The missing information due to the limited angles gives rise to a loss of spatial resolution for images within a reconstructed volume. To increase the spatial resolution, 2-dimensional projection images of a specimen were collected along two orthogonal axes as needed. A section thickness of a sample was typically less than ~150 nm to obtain sufficient quality of projection images. Recently, scanning transmission electron microscope has been increasingly used for samples thicker than 200nm because entire structures of interest (several hundred nanometers in size) can be found within a reconstructed volume. Thus, with more than 15 years of electron tomography experience, I intend to collect electron tomography data using scanning transmission electron microscopy as well as conventional transmission electron microscopy to apply electron tomography on a variety of macromolecular structures in biological and non-biological materials ranging from ~10 nm to ~150 nm in size. This would combine the benefits of scanning electron microscopy and electron tomography leading to further understanding of detailed structures and functions of various cellular structures and other non-biological materials such as polymer composites of diverse applications.

EM3D The major procedural bottlenecks that limit high throughput electron tomography are image alignment, volume reconstruction and segmentation of structures of interest to generate their 3D surface models. To aid the procedures, an electron tomography software package, EM3D had been developed in the McMahan lab at Stanford University, and I have been involved in improving EM3D in order to provide convenient, general tools for processing electron tomography data. There are currently several other applications freely available for electron tomography data analysis, but they are limited in providing an integrated analysis application, gray-scale-driven 3D surface-model generation in EM3D, which is essential for generating surface models at the highest possible resolution. Using surface models of structures of interest generated by EM3D, my quantitative tools developed for structural analysis have been successfully employed to demonstrate that nanoscale biological macromolecular complexes implicated in the communication of a neuron with another neuron or a cell are dynamically variable structures. I intend to improve EM3D by implementing all of the tools to make electron tomography more accessible and convenient to people who are interested in electron tomography. Recently, I have prepared and submitted a R01 grant proposal to NIH with my colleagues at Texas A&M University, Bayer College of Medicine and Rice University in Texas. The current version of EM3D and its improvement with my colleagues and other faculty members of Texas Tech University will enhance the applicability of EM3D in ultra-structural study of non-biological materials such as self-assembled

nanocomposites and polymers as well as of biological samples to obtain their structural characteristics at nanometer scale.

Biological materials Transportation of neurotransmitters between a nerve cell and other nerve cell is critical for their communication, and the malfunction of synaptic transmission can lead to degenerative brain or muscular disease such as Alzheimer's disease. The synaptic transmission in a nerve cell occurs at a specialized region called active zone. Electron tomography on active zones at junctions between neurons and muscles of frog has first shown that the active zone material (AZM) macromolecules at active zones thought to contain key proteins for synaptic transmission form a highly ordered network of distinct classes of AZM complexes at 2-3nm spatial resolution. Several members of the classes such as ribs, pins, and etc. are connected to a synaptic vesicle (SV) containing neurotransmitters in a way that, together with certain biochemical findings, has led to the hypothesis that the AZM macromolecules contain proteins that help bring the SVs close to the active zone on the presynaptic membrane and mediate the SVs' membrane fusion with the presynaptic membrane during synaptic transmission. Accordingly, a crucial step toward a comprehensive understanding of the mechanisms regulating transmitter release at synapses is detailed structural knowledge of the spatial relationship of SVs with the presynaptic membrane and how the AZM is related with SV fusion. Using electron tomography I have examined the spatial relationships of docked SVs to the presynaptic membrane at frog and mouse neuromuscular junctions (NMJs) and discovered that there is a significant variation in the contact area of docked SVs with the presynaptic membrane and that at frog NMJs the AZM components such as ribs and pins are correlated with the contact area: the larger the contact area, the shorter the distance of the rib/pin-vesicle contact sites on their connected vesicle membrane to the presynaptic membrane. Furthermore, when frog NMJs are electrically stimulated, the contact area of docked SVs becomes smaller than that at rest suggesting that SVs having large contact areas preferentially fuses. Most of docked SVs had no indication of hemifusion, but a small portion of SVs showed significant merging at their SVs' contact sites indicating that they are hemifused with the presynaptic membrane. Hemifusion has long been thought to be a fusion intermediate promoting membrane fusion. The finding that the hemifused vesicles possess large contact areas indicates that the enlargement of the contact area contribute to the destabilization of the membranes in the contact site leading to hemifusion of the membranes in contact. The structural examination of ribs and pins using electron tomography revealed that their structural variation is closely coupled with the variation in the contact area suggesting that their shortening contributes to the regulation of their SV's contact area by generating force. The random shortening and lengthening of multiple ribs and pins connected to each docked SV are expected to generate the bell-shaped distribution of the contact area which was confirmed from the normal or Gaussian distribution of the contact area. The findings led to the AZM-mediated variable force hypothesis proposing that the extent of the contact area is a structural correlate of the fusion probability of a docked SV and that the extent of contact area is regulated by random shortening and lengthening of AZM macromolecules linking a SV and the presynaptic membrane. The hypothesis provides a simple explanation for the commonly observed random variation in the degree of neurotransmission at a single synapse level. I also found that dense-core vesicles known to be involved in brain development and maturation of neuromuscular junctions are docked, connected with the AZM and fuse at active zones suggesting that the AZM is a universal nanomachine regulating vesicle docking and fusion. I intend to study slices of mammalian brains and muscles by collaborating with other faculty members to test the hypothesis at different synapses toward building a standard model of the regulation mechanism of vesicle fusion, and I also intend to apply the developed quantitative structural analysis techniques to various cells to uncover detailed subcellular structures crucial for cellular functions and maintenance and to reveal underlying physical principles accounting for their functions.

Non-biological Materials Polymers have widespread use in many optical and electronic applications such as batteries, displays, plastic wires, optical signal processing, information storage, solar energy conversion, etc. Polymers have the advantage that they are more easily processed than metals. Large surfaces can be covered with a spin-coated polymer solution. Once the solvent has evaporated one is left with a layer of polymer chains. Most

plastics can be deformed reversibly, and they also do not break easily. A majority of my work on polymers was focused on examining the physical, structural, electrical and magnetic properties of polymers such as polyaniline. Powders of the polymers were dissolved in organic solvent such as N-methyl pyrrolidinone (NMP). After spin-coating them on a slide glass and drying them, thin polymer films were obtained. The polymer films were embedded in various solution containing cations such as H^+ and Li^+ to dope the films with such cations. The doped films exhibited varying degrees of material properties. First, I have measured the electrical conductivity of each film with lowering its temperature from room temperature down to near 4 K to investigate its temperature-dependent electrical property. To examine its structural property, X-ray diffraction was employed and there was little structural change after doping with Li^+ or H^+. X-ray photoelectron spectroscopy was also used to measure the degree of doping by examining the interaction of PAN with doping cations. Those doping cations create unpaired electron spins for each doped amine group altering the magnetic property of the cation-doped PAN films. Electron paramagnetic resonance was employed to measure the magnetic susceptibility of the films depending on the degrees of doping and the kind of doping cations. Using poly(3,4-ethylenedioxythiophene) (PEDOT) polymer film doped with poly(4-styrenesulfonate)(PSS), its electrical conductivity was significantly increased up to 10-fold by various organic solvents such as dimethyl sulfoxide (DMSO), N,N-dimethyl formamide (DMF), tetrahydrofuran(THF), and H_2O although its structure and degrees of doping had similar dependence on the doping concentration of PSS. Nanocomposites of polyaniline and Na+-montmorillonite (Na+-MMT) were synthesized by emulsion polymerization in dodecylbenzenesulfonic acid (DBSA) or camphorsulfonic acid (CSA) as dopant and emulsifier. The electrical conductivity and magnetic susceptibility of PAN-DBSA and PAN-CSA were also temperature-dependent similar to PAN films. The layer of conducting PAN-DBSA or PAN-CSA between the Na+-MMT layers were found to form in nanometer scale (~1 nm) according to X-ray diffraction. The interlayer distance of the Na+-MMT was also found to depend on the presence of the polymer layer. Electron tomography has been increasingly applied to nanoscale structures of non-biological crystalline or amorphous materials such as metals and polymer nanocomposites. Using polymer nanocomposite films provided by the Department of Materials Science & Engineering at Texas A&M University, I collected electron tomography datasets of them and observed nanometer scale variation in their orderly structures at 2-3 nm spatial resolution. I intend to apply electron tomography and the improved EM3D to various organic and inorganic materials by working together with other faculty members, which will provide valuable to obtain fine structures of materials, their structural variations, and quantitative structural analyses at nanometer scale.

Computational Modeling The broad structural variation of several AZM macromolecules led me to propose a stochastic model of AZM-medicated SV docking and priming that can be generally applicable to various synapses in brain and muscle. The computational modeling based on the model showed that the broad, unimodal distribution of the contact area of docked SVs and its correlation with its connected AZM macromolecules can be simply explained by each SV's randomly fluctuating AZM macromolecules, which are stably connected to the vesicle membrane. Furthermore, the model predicts that the distribution of contact areas of docked SVs at active zones is directly correlated with the distribution of the connection sites of AZM macromolecules on the vesicle membrane. So I intend to test the model by examining the distribution of the connection sites on vesicle membranes at various types of synapses of different species. The test will improve our understanding of how our brain and muscle function and malfunction in disease conditions. Furthermore, such modeling can be applied to various macromolecular complexes to understand their functions by quantifying their structural variations and constructing their mechanistic models with underlying physical principles. Such modeling will also help promote the interdepartmental cooperation.

Altogether, electron tomography, a powerful technique providing fine structural detail and modeling of complicated, disordered structures has broad applications, and my study using electron tomography approaches will contribute to sparking the inter-departmental joint effort of physics, biology, neuroscience, and materials science to widen our understanding of structure-function relationship and physical properties of materials and biological samples at nanometer scale.