PREFACE

Throughout history of human civilization, our quest to understand *Nature* has fueled our imagination to make the necessary inventions that further our perception of *Nature*, perceptions beyond the natural limits of our senses. Our constant desire to improving our understanding of time and space has been a powerful driving force. For example, the invention of various telescopes for observing distant objects, and microscopes for studying the very small, has enabled discoveries of distant galaxies and planets light years away, and of the micrometer-size unit of life—the "Cell", and its nanometer-size subcellular organelles. The ultimate goal, however, is the understanding of cellular structure–function at atomic resolution and in real time in live cells. In the past decade, major advances in technology and approach, has come close to realizing this objective. In this book, examples of the various advancements made in recent years, to realizing the ultimate goal of understanding cellular structure–function at atomic resolution and in real time in live cells, are presented.

Throughout history, the development of new imaging tools has provided new insights into our perceptions of the living world and profoundly impacted human health. The invention of the light microscope more than 300 years ago, was the first catalyst, propelling us into the era of modern cell biology and medicine. Using the light microscope, a giant step into the gates of modern cell biology and medicine was made with the discovery of the "Cell". The structure and morphology of normal and diseased cells, and of disease-causing microorganisms, were observed for the first time using the light microscope. Then in the 1930s, with the birth of the electron microscope (EM), dawned a new era. Through the mid 1940s and 1950s, a number of subcellular organelles were discovered and their functions determined using the EM. Viruses, the new life forms, were discovered and studied for the first time, and implicated in diseases ranging from the common cold to autoimmune disease (AIDS). Despite the capability of the EM to image biological samples at near nanometer resolution, sample processing resulting in morphological alterations remained a major concern. Then in the mid 1980s, scanning probe microscopy was invented, further extending our perception of the living world to the near atomic realm. One such scanning probe microscope, the atomic force microscope (AFM), has helped overcome both limitations of light and electron microscopy, enabling determination of the structure and dynamics of live cells and biomolecules in 3D, at near angstrom resolution. The resolving power of the light microscope is dependent on the wavelength of the light used and hence, 250–300 nm in lateral and much less in depth resolution can at best be achieved using light for imaging. As a result, cellular structures measuring less then 250–300 nm in lateral

or depth dimension, had evaded visual detection until invention of the AFM. In this book, the utilization of the AFM in the discovery of a new cellular structure, the "porosome" and its structure and dynamics at nanometer resolution in real time in live cells (Chapter 1), the structure and dynamics of subcellular organelles at nanometer resolution and in real time (Chapters 2 and 3), and in Chapter 4, the examination of the intricate biomineralization in diatoms, is presented. Similarly, the AFM has been utilized to understand the molecular mechanism of membrane fusion in cells (Chapter 8), and the assembly and disassembly of membrane-associated proteins (Chapters 8 and 9). Target membrane proteins, SNAP-25 and syntaxin (t-SNARE) and secretory vesicle-associated membrane protein (VAMP or v-SNARE), are part of the conserved protein complex involved in fusion of opposing bilayers. VAMP and syntaxin are both integral membrane proteins, with the soluble SNAP-25 associating with syntaxin. Therefore, understanding of SNARE-induced membrane fusion required determination of the arrangement and interaction between membrane-associated v- and t-SNARE proteins. Ideally, the atomic coordinates of membrane-associated SNARE complex using X-ray crystallography would provide atomic details of the t-/v-SNARE complex. Such atomic details of membrane-associated t-/v-SNARE complex has not been possible, primarily due to solubility problems of membrane SNAREs, compounded with the fact that v-SNARE and t-SNAREs need to reside in opposing membranes when they meet, to form the appropriate physiologically relevant SNARE complex. The remaining option has been the use of nuclear magnetic resonance spectroscopy (NMR); however, the NMR approach too has not been successful, primarily due to the molecular size limitation of NMR application. Regardless of these set backs and limitations, AFM force spectroscopy has provided for the first time at nm resolution, an understanding of the structure, assembly, and disassembly of the membrane-associated t-/v-SNARE complex in physiological buffer solution (Chapter 8).

The above examples demonstrate the power and scope of the AFM in providing an increased understanding of the cell. Similarly in the book, examples of a number of new and novel approaches in the study of cellular structure-function, is also discussed. In chapter 10 for example, the structure and dynamics of metalloproteins in live cells, has bee investigated using X-ray absorption spectroscopy (XAS). In recent years, XAS has emerged as a new and powerful tool in investigating the structure and dynamics of metals in cells and in metal containing cellular biomolecules. Utilizing the high flux and broad energy range of X-rays supplied by synchrotron light sources, the selective excitation of core electronic transitions in each metal within cells, can be achieved. Spectroscopic signals from these electronic transitions can therefore be used to determine the chemical architecture of metals in cells, in cellular components, and in biomolecules, at varying degrees of structural resolution. Real time investigation of protein folding, structure and dynamics at high resolution in living cells, is the next major step in nano cell biology. In Chapter 14, using a QQ-reagent based protein transduction technology, investigation of protein folding, structure and dynamics, at atomic resolution *in vivo* can be investigated. Today, nano manipulation and tagging using light-activated ion channels, has made

it possible to precisely and remotely regulate activity in neurons (Chapter 11). Quantitative phase imaging (Chapter 5), and Fourier imaging correlation spectroscopy (Chapter 6), now provide novel insights into our understanding of cellular structure–function. Similarly, THz investigation of condensed phase studies, provide further understanding into the structure–function of biomolecules. Lately, a plethora of novel approaches, combined with conventional tools and techniques, provide a better understanding of cellular structure–function (Chapters 6, 8, 11, 15, and 16). Using new and improved computational resources; AFM, XAS, NMR, proteomics, genomics, and molecular dynamic simulation, have become leading and powerful tools and approaches in the study of the cell, and investigation into the new field of "*Nano Cell Biology*".

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