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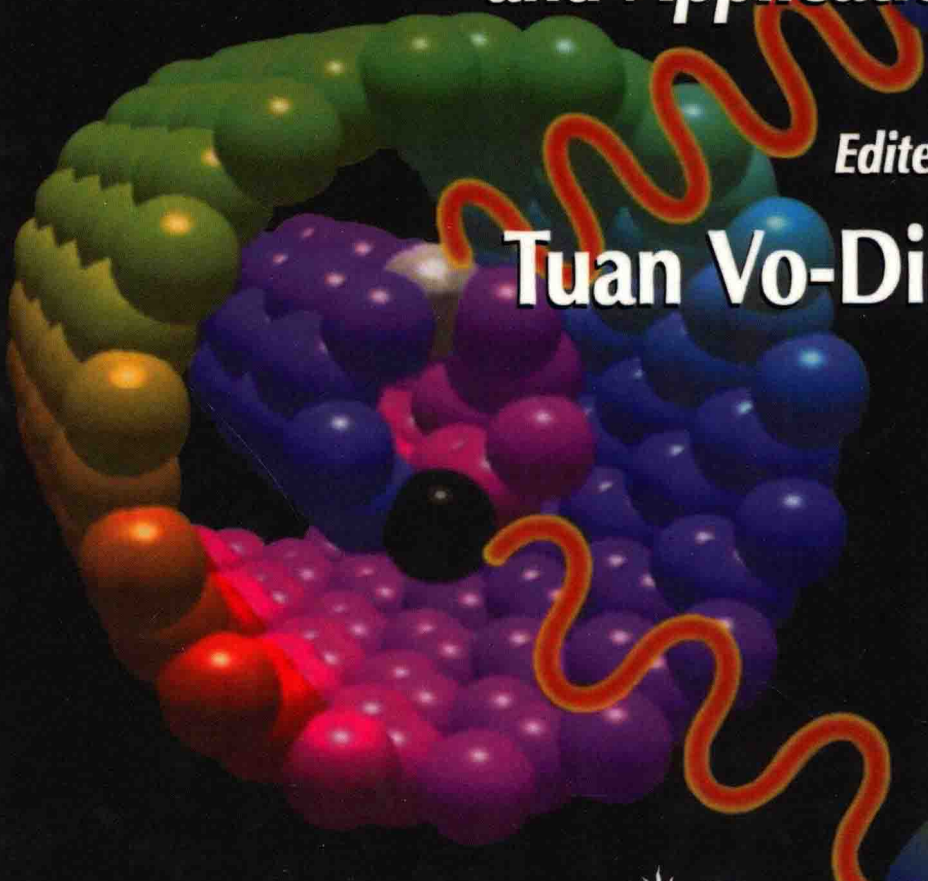
Protein Nanotechnology

Protocols, Instrumentation, and Applications



Edited by

Tuan Vo-Dinh

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Preface

Protein Nanotechnology: Protocols, Instrumentation, and Applications is intended to serve as an authoritative reference for a broad audience involved in nanotechnology research and in the teaching, learning, and practice of nano-technology for genomics, proteomics, bioengineering, and medicine. Recently, nanotechnology—which involves research on and the development of materials and species at the length scales of 1 to 100 nm—has been revolutionizing many important scientific fields, ranging from biology to medicine. This is technology on the scale of molecules, and it has the potential of developing devices smaller and more efficient than anything currently available. To understand complex biological nanosystems at the cellular level, we urgently need to develop a next-generation nanotechnology tool kit. It is believed that the new advances in genetic engineering, genomics, proteomics, medicine, and biotechnology will depend on our mastering nanotechnology in the coming decades. The combination of nanotechnology, materials sciences, and molecular biology opens the possibility of detecting and manipulating atoms and molecules using nanodevices, which have many potential applications across a wide variety of biological research topics, as well as medical uses at the cellular level.

Today, the amount of research in biomedical sciences and engineering at the molecular level is growing exponentially because of the availability of these new investigative nanotools. They are capable of probing the nanometer world and will make it possible to characterize the chemical and mechanical properties of cells, discover novel phenomena and processes, and provide researchers with a wide range of tools, materials, devices, and systems with unique characteristics.

With the completion of the sequencing of the human genome, one of the greatest impacts of proteomics is the establishment of an entirely new approach to biological and medical research. Proteins are major cellular components that play an essential role in maintaining the proper functioning of the cell. Nanotechnology promises to provide the tools for studying how the tens of thousands of proteins in a cell (the so-called proteome) work together in networks to orchestrate the chemistry of life. Specific genes and proteins have been linked to numerous diseases and disorders, including breast cancer, muscle disease, deafness, and blindness. Protein misfolding processes are believed to cause such diseases as Alzheimer's, cystic fibrosis, "mad cow" disease, an inherited form of emphysema, and even many cancers. Nanotech-

nology also has the potential to dramatically change the field of diagnostics, therapy, and drug discovery in the postgenomic area. The combination of nanotechnology and optical molecular probes are being developed to identify the molecular alterations that distinguish a diseased cell from a normal cell. Such technologies will ultimately aid in characterizing and predicting the pathologic behavior of diseased cells, as well as the responsiveness of cells to drug treatment.

The combination of biology and nanotechnology has already led to a new generation of devices for probing the cell machinery and elucidating molecular-level life processes heretofore invisible to human inquiry. Tracking biochemical processes within intracellular environments can now be performed in vivo with the use of fluorescent molecular probes and nanosensors. With powerful microscopic tools using near-field optics, scientists are now able to explore the biochemical processes and submicroscopic structures of living cells at unprecedented resolutions. It is now possible to develop nanocarriers for targeted delivery of drugs that have their outer surfaces conjugated with antibodies for targeting antigens and fluorescent chromophores for in vivo, intracellular tracking.

This volume presents the most recent scientific and technological advances of nanobiotechnology, as well as practical methods and applications, in a single source. Included are a wide variety of important topics related to protein nanotechnology. Each chapter provides introductory material with an overview of the topic of interest; a description of methods, protocols, instrumentation, and applications; and a collection of published data with an extensive list of references for further details.

The goal of *Protein Nanotechnology: Protocols, Instrumentation, and Applications* is to provide a comprehensive overview of the most recent advances in instrumentation, methods, and applications in areas of nanotechnology related to genomics and proteomics, integrating interdisciplinary research and development of interest to scientists, engineers, manufacturers, teachers, and students.

Tuan Vo-Dinh

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Contents

1

Protein Nanotechnology

The New Frontier in Biosciences

Tuan Vo-Dinh

Summary

The combination of nanotechnology and molecular biology has led to a new generation of nanoscale-based devices and methods for probing the cell machinery and elucidating intimate life processes occurring at the molecular level that were heretofore invisible to human inquiry. This chapter provides a brief overview of the field of nanotechnology and its applications to the study, design, and use of protein systems in biology and medicine.

Key Words: Nanotechnology; protein; nanosensor; nanoprobe; DNA; RNA; molecular motor.

1. Introduction: An Historical Perspective on Nanotechnology

Nanotechnology involves research and development on materials and species at length scales between 1 and 100 nm. The term *nano* is derived from the Greek word meaning "dwarf." In dimensional scaling, *nano* refers to 10^{-9}, i.e., one billionth of a unit. Thus, a nanometer is 10^{-9} m (0.000000001 m), or about the size of a molecule such as benzene. *Nanotechnology* therefore, refers to the techniques and methods for studying, designing, and fabricating things at the nanometer scale. The initial concept of investigating materials and biological systems at the nanoscale dates to more than 40 yr ago, when Richard Feynman presented a lecture in 1959 at the annual meeting of the American Physical Society at the California Institute of Technology. This lecture, entitled "There's Plenty of Room at the Bottom" ***(1)***, is generally considered to be the first look into the world of materials, species, and structures at nanoscale levels.

Nanostructures are similar in size to many biological species such as proteins. These species comprise a wide variety of basic structures such as

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polymers, carbohydrates (sugars), and lipids; thus, they have a great variety of chemical, physical, and functional properties. This structural variety and the versatility of these biological nanomaterials and systems have important implications for the design, development, and manufacturing of new and artificial assemblies (such as lipid vesicles, dendritic polymers, DNA aggregates, and nano rods or tubes) that are critical to industrial, biotechnological, and medical applications.

To understand complex biological nanosystems at the cellular level, we urgently need to develop a next-generation nanotechnology tool kit. This is technology on the scale of molecules, and it has the potential of developing devices smaller and more efficient than anything currently available. It is believed that the new advances in genetic engineering, genomics, proteomics, medicine, and biotechnology will depend on our mastering nanotechnology in the coming decades. If we can assemble biological systems and devices at the atomic and molecular levels, we will achieve a versatility in design, a precision in construction, and a control in operation heretofore hardly dreamed of. Such a dream was foreseen by Eric K. Drexler in his book *Engines of Creation (2)*, in which he envisioned that major processes in molecular technology could be based on protein engineering.

2. The Importance of Protein Nanotechnology

The living cell, with its myriad of biological components, may be considered the ultimate nanoscale device. **Figure 1** shows a schematic diagram of the cell with its various components. Some typical sizes of proteins and biological species are given in **Table 1**. Chemistry also deals with atoms and molecules, which are of nanometer sizes. However, nanotechnology differs from chemistry in a very fundamental aspect. Whereas chemistry deals with atoms and molecules at the bulk level (we do not see the molecules in chemical solutions), nanotechnology seeks to actually "manipulate" individual atoms and molecules in very specific ways.

Proteins are major cellular components that play an essential role in maintaining the functioning of the cell. Proteins have a number of functions. They can function as enzymes, which are the driving force for biochemical reactions. Also, they can serve as antibodies that recognize invading elements and allow the immune system to neutralize and eliminate unwanted invaders. Proteins have functions within physiological as well as pathophysiological processes in a cell or organism. Because diseases, therapy, and drugs can alter protein profiles, a determination of protein profiles can provide useful information for understanding disease and designing therapy. Therefore, understanding the structure, metabolism, and function of proteins at the molecular (i.e., nanoscale) level is absolutely critical to our understanding of biological

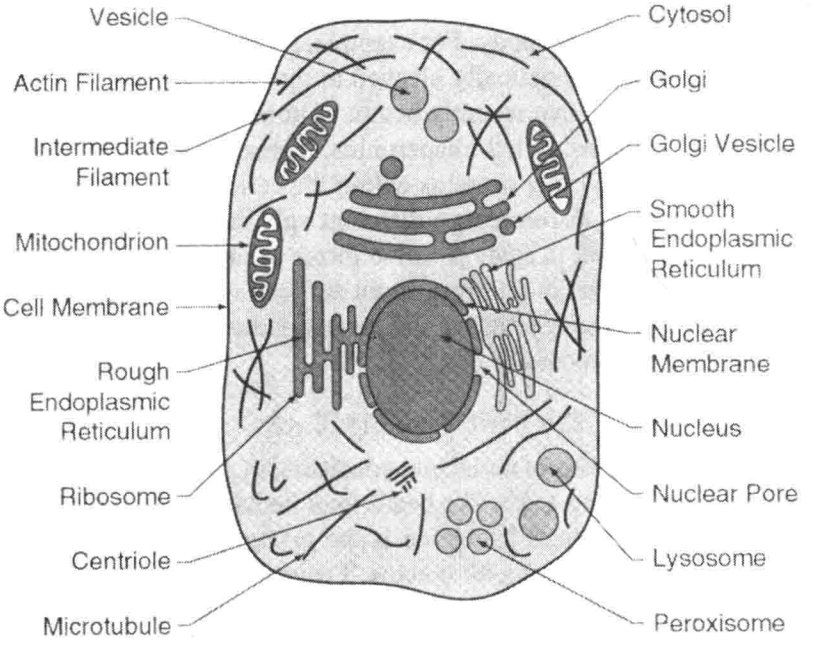

Fig. 1. Schematic diagram of a cell and its components.

Table 1
Typical Sizes of Proteins and Biological Species

Biological species	Example	Typical size	Typical mol wt (Daltons)
Small proteins	Chymotrypsin	4-nm sphere	10^4–10^5
Large proteins	Aspartate transcarbamoylase	7-nm sphere	10^5–10^7
Small assemblies	Ribosome	20-nm sphere	10^5–10^7
Large assemblies	Viruses	100-nm sphere	10^7–10^{12}
Nucleic acids	tRNA	10-nm rod	10^4–10^5

processes. This knowledge will contribute to improving our ability to manipulate biological species in molecular manufacturing, enhancing energy production using biofuel-based microbial systems, or detecting the health status of a living organism in order to effectively diagnose and ultimately prevent disease.

Proteins and genes are closely related. Briefly, DNA, the genetic code encrypted in chromosomes, is translated into a corresponding sequence of RNA, which is then read by the ribosome to fabricate a sequence of amino acids. These amino acid chains fold up into a three-dimensional (3D) shape

and become a specific protein, which is designed to perform a particular role in some part of the cell or the body. For example, some proteins are created in an inactive form, then enzymatically cleaved at the site of activity to become a new, active form. We have recently begun to understand the importance of a special type of proteins called chaperonins. These proteins are designed to assist in the folding of other proteins within the cell into their final shape and function. A gene can also undergo different splicings, and posttranslational modifications can result in several active forms of proteins. Thus, knowledge of the sequence information in genes is not sufficient to describe life. It is also critical to determine the function of the corresponding proteins, which are the actual players in the process of life.

3. Protein Structure: The Basic Building Blocks

Proteins are long chains of molecules consisting of polymers assembled from a large number of amino acids like beads on a necklace. The sequence of the amino acids in the polymer backbone is the *primary structure* of any given protein. There are 20 normal amino acids. Typical polypeptide chains contain about 100 to 600 amino acid molecules and have a molecular weight of about 15,000 to 70,000. Since amino acids have hydrophilic, hydrophobic, and amphilic groups, in an aqueous environment they tend to fold to form a locally ordered, 3D structure, called the *secondary structure*, that is characterized by a low-energy configuration with the hydrophilic groups outside and the hydrophobic groups inside. In general, simple proteins have a natural α-helix configuration. Another natural secondary configuration is a β-sheet. These two secondary configurations (α-helix and β-sheet) are the building blocks that assemble to form the final *tertiary structure*, which is held together by extensive–secondary interactions, such as van der Waals bonding. The tertiary structure is the complete 3D structure of one indivisible protein unit (i.e., one single covalent species). Sometimes, several proteins are bound together to form supramolecular aggregates that make up a *quarternary structure*. The quarternary structure, which is the highest level of structure, is formed by the noncovalent association of independent tertiary structure units.

Knowing the 3D structure of proteins is essential in understanding their function. The sequence (primary structure) provides little information about the function of proteins. To carry out their function, proteins must take on a particular shape, often referred to as an active form, through the folding process. **Figure 2** shows an example of the 3D structure of bovine serum albumin (BSA). Folded proteins, such as egg albumin, can be unfolded by heating. Following heating, the albumin, which has undergone an irreversible folding conformation change, turns white. In this form albumin is said to be denatured. Denatured albumin cannot be reversed into its natural state. However, some