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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

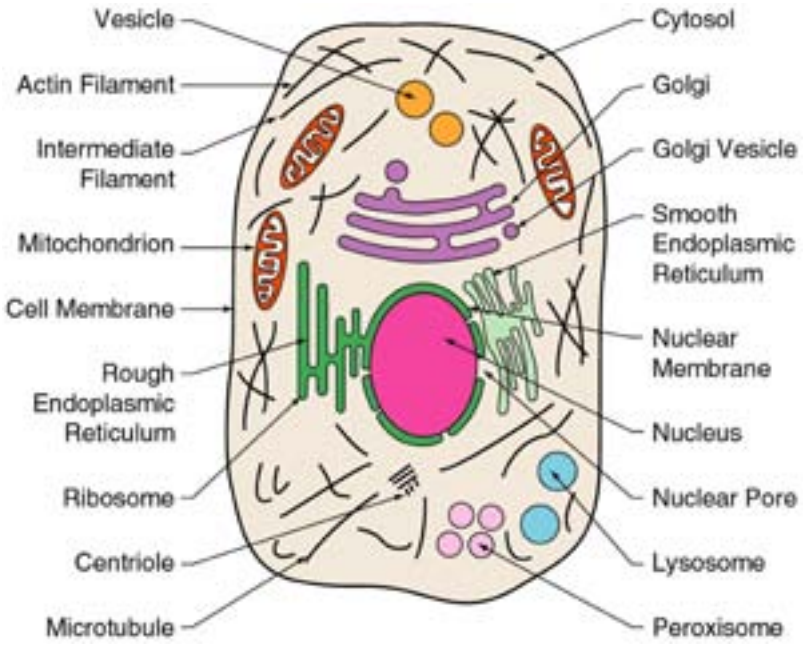

Fig. 1. Schematic diagram of a cell and its components.

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

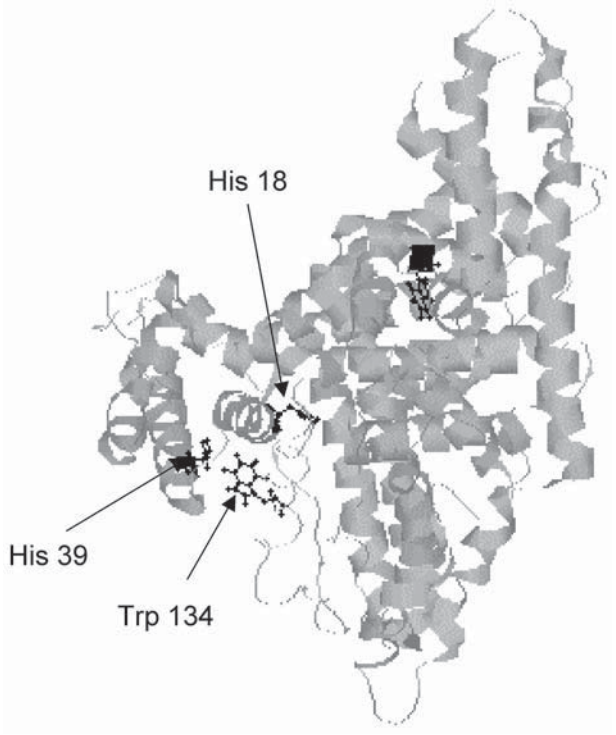

Fig. 2. Three-dimensional structure of BSA.

proteins can be denatured and renatured repeatedly; that is, they can be unfolded and refolded back to their natural configuration. Diseases such as Alzheimer's disease, cystic fibrosis, mad cow disease, an inherited form of emphysema, and even many cancers are believed to result from protein misfolding.

Extensive experimental and theoretical research efforts have been devoted to determine the structure of proteins. By using a combination of computational methods, mass spectroscopy, and nuclear magnetic resonance (NMR) techniques, researchers have identified an optimal set of small molecules for use in synthesizing novel bidentate antidotes or detection agents for clostridial neurotoxins, such as tetanus and botulinus. The crystal structure of the tetanus toxin C fragment (TetC, Protein Data Bank access code 1A8D) is shown with doxorubicin and a peptide computationally docked into sites 1 and 2, respectively, in **Fig. 3**. The structures of sialic acid and lavendustin A are shown about an NMR stack plot showing that lavendustin A binds to TetC ***(3)***.

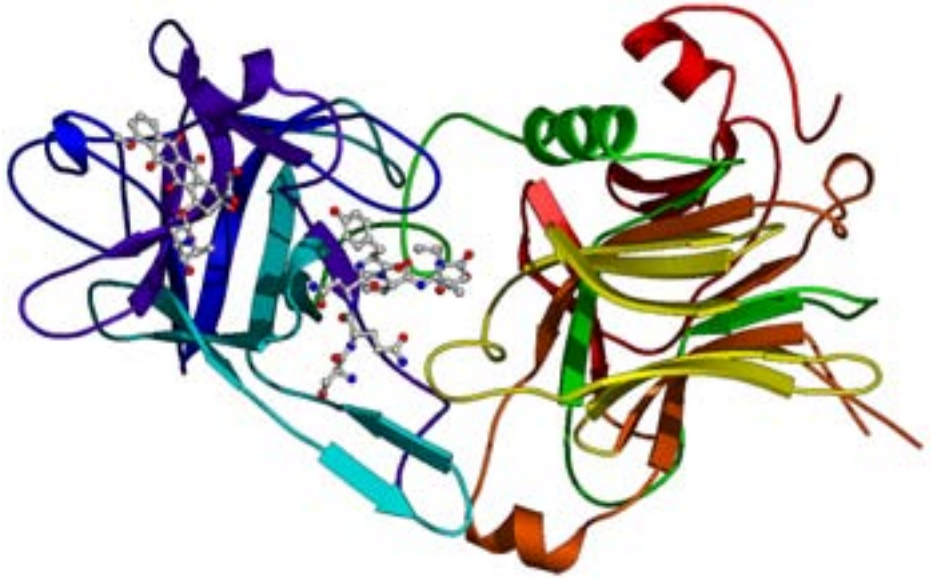

Fig. 3. Crystal structure of TetC. (Adapted from **ref. *3***.)

The goal of understanding the structure and function of proteins as integrated processes in cells, often referred to as "system biology," presents a formidable challenge, much more difficult than that associated with determination of the human genome. Therefore, proteomics, which involves determination of the structure and function of proteins in cells, could be a research area that presents more challenges than genomics. Proteomics research directions can be categorized as structural and functional. Structural proteomics, or protein expression, measures the number and types of proteins present in normal and diseased cells. This approach is useful in defining the structure of proteins in a cell. However, the role of a protein in a disease is not defined simply by knowledge of its structure. An important function of proteins is in the transmission of signals through intricate protein pathways. Proteins interact with each other and with other organic molecules to form pathways. Functional proteomics involves the identification of protein interactions and signaling pathways within cells and their relationship to disease processes. Elucidating the role that proteins play in signaling pathways allows a better understanding of their function in cellular behavior and permits diagnosis of disease and, ultimately, identification of potential drug targets for preventive treatment. As described in the various chapters of this book, protein nanotechnology holds the promise of providing the critical tools needed to obtain real-time information about the signaling processes in cells.

4. Protein Machines: Nature's Engines of Life

Life is made possible by the action of a series of biological molecular nanomachines in the cell machinery. By evolutionary modification over trillions of generations, living organisms have perfected an armory of molecular machines, structures, and processes. The simplest cells used nanoscale manipulators for building molecule-sized objects. They are now used to build proteins and other molecules atom by atom according to defined instructions encrypted in the DNA. The cellular machinery uses rotating bearings that are found in many forms; for example, some protein systems found in the simplest bacteria are used as clamps that encircle DNA and slide along its length. Human cells contain a rotary motor that is used to generate energy. A wide variety of molecule-selective pumps are used by cells to absorb ions, amino acids, sugars, vitamins, and all of the other nutrients needed for living. Cells also use molecular sensors that can detect the concentration of surrounding molecules and compute the proper functional outcome.

Consider a well-known molecular machine, the ribosome. Ribosomes are biological species that play the role of nanomachines in living cells. Ribosomes build proteins essential to the functioning of the cell. Although the size of a typical ribosome is only 8000 nm^3, this nanomachine is capable of manufacturing almost any protein by stringing together amino acids in a precise linear sequence following instructions from a messenger RNA copied from the host DNA. To perform its molecular manufacturing task, the ribosome grasps a specific transfer RNA (tRNA), which, in turn, is chemically bonded by a specific enzyme to a specific amino acid. It has the means to grasp the growing polypeptide and to cause the specific amino acid to react with, and be added to, the end of the polypeptide. In other words, DNA can be considered the biological software of the cellular machinery, ribosomes are large-scale molecular constructors, and enzymes are functional molecular-sized assemblers. These proteins are truly nature's engines of life processes.

Cells use other protein-based nanomachines to separate chromosomes during cell division, to move material, to crawl on surfaces, and to propel themselves in water. For example, during cell division in animal cells, the disassembly of the microtubule (a 25-nm-diameter hollow cylinder) moves replicated DNA to the two emerging daughter cells. The movement of another well-known molecular motor, myosin, along double helical filaments of a protein called actin (approx 10 nm across) produces the contraction of muscle cells during each heartbeat.

Classic and quantum molecular simulation methods have provided an invaluable tool in the development of nanomaterials and in feasibility studies of nanotechnology designs (**Fig. 4**). For example, fundamental classic and quantum simulation studies of model nanobearings and nanomotors composed

Fig. 4. Example of nanostructures designed using classic and quantum molecular simulation methods. (Used by permission from Sumpter and Noid, personal communication.)

of concentric carbon nanotubes have shown strong effects of internal mode coupling on key aspects of the performance of the motors and bearings. Over the last several years, Noid and Sumpter have investigated the performance of nanobearings, nanomotors, and fluid flow through nanotubes using fully dynamic (molecular dynamics) simulation ***(4,5)***. Various types of molecular bearings and other mechanical devices have recently been proposed in the growing nanotechnology literature. One desired capability to be derived in the later stages of nanotechnology development, and an interesting scientific problem in its own right, is the introduction of controlled motion at the nanometer size scale. In a development closely related to molecular bearings, Noid, Tuzun, and Sumpter have simulated several model graphite nanometer-scale laser-driven motors (**Fig. 5**). The motors consist of two concentric graphite cylinders

Fig. 5. Model graphite laser-driven nanomotors designed using simulation methods. The laser-driven motors consist of two concentric graphite cylinders (shaft and sleeve) with one positive and one negative electric charge attached to opposite ends of one of the end rings of the shaft. (Used by permission from Noid et al. ***[5]***.)

(shaft and sleeve) with one positive and one negative electric charge attached to opposite ends of one of the end rings of the shaft. Rotational motion of the shaft is induced by applying one or sometimes two oscillating laser fields. The shaft cycles between periods of rotational pendulum-like behavior and unidirectional rotation (motor-like behavior). Motor performance was mapped as a function of size, field strength and frequency, and relative location of the attached positive and negative charges. Drawing on observations of multilaser-enhanced energy absorption in small molecular systems, Noid and Sumpter also mapped motor performance as a function of one- vs two-laser excitation.

5. The Nano Toolkit

Recently, nanotechnology has been revolutionizing many important areas in molecular biology, especially in the detection and manipulation of proteins and biological species at the molecular and cellular level. The combination of molecular biology and nanotechnology opens the possibility of detecting and manipulating atoms and molecules using nanodevices, with the potential for a wide variety of medical uses at the cellular level.

Today, the amount of research in biomedical science and engineering at the molecular level is growing exponentially because of the availability of new investigative nanotools based on protein nanotechnology. These new analytical tools 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 science with a wide range of tools, including materials, devices, and systems with unique characteristics. The marriage of electronics, biomaterials, and molecular biology and nanotechnology is expected to revolutionize many areas of biology and medicine in the 21st century.

The combination of nanotechnology and molecular biology 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 nanoprobes and nanosensors ***(6)***. With powerful microscopic tools using near-field optics, scientists are now able to explore the biochemical processes and nanoscale structures of living cells at unprecedented resolutions. It is now possible to develop nanocarriers for targeted delivery of drugs that have their shells conjugated with antibodies for targeting antigens and fluorescent chromophores for in vivo tracking.

The development of metallic nanoprobes that can produce a surface-enhancement effect for ultrasensitive biochemical analysis is another area of active nanoscale research. *Plasmonics* refers to the research area dealing with enhanced electromagnetic properties of metallic nanostructures. The term is derived from *plasmons,* which are the quanta associated with longitudinal waves propagating in matter through the collective motion of large numbers of electrons. Incident light irradiating these surfaces excites conduction electrons in the metal and induces excitation of surface plasmons, which, in turn, leads to enormous electromagnetic enhancement for ultrasensitive detection of spectral signatures through surface-enhanced Raman scattering (SERS) ***(7)*** and surface-enhanced fluorescence. **Figure 6** shows a scanning electron micrograph of SERS-active nanospheres (300-nm diameter coated with a 100-nm layer of silver). Nanoparticle-based SERS technology has enabled sensitive detection of a variety of compounds of medical interest. SERS nanoprobe technology has also been incorporated into the design of several fiberoptic probes for diagnostics ***(8)***. The development of a SERS gene probe technology based on solid surface–based technology has also been reported. One study demonstrated the selective detection of human immunodeficiency virus DNA and a cancer gene ***(9)***.

Optical nanosensors, which have dimensions on the nanometer size scale, have been developed to probe individual chemical species in specific locations

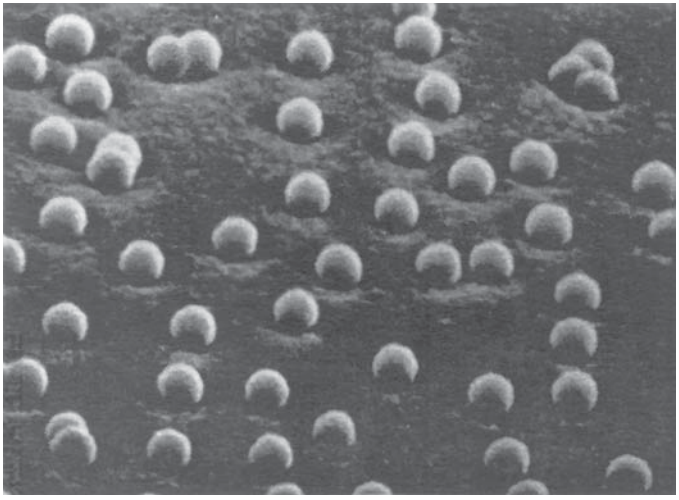

Fig. 6. Nanoprobes consisting of nanospheres coated with nanoshells of silver.

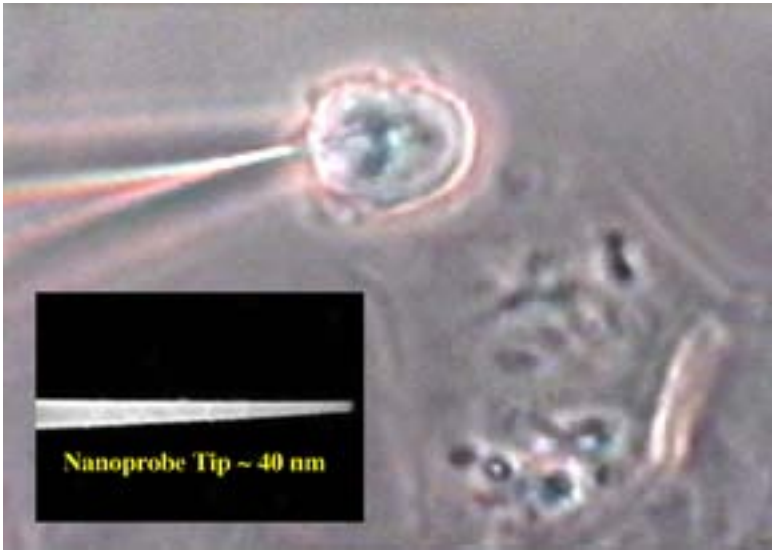

Fig. 7. Fiberoptics nanosensor for single-cell analysis. (Adapted from **ref. *10***.)

throughout a living cell ***(10)***. **Figure 7** is a photograph of a fiberoptic nanoprobe with an enzyme substrate designed to detect caspase 9, a protein expressed during apoptosis in a single cell ***(11)***. An important advantage of the optical sensing modality is its capability to measure biological parameters in a

noninvasive or minimally invasive manner owing to the extremely small size of the nanoprobe. Following measurements using the nanobiosensor, cells have been shown to survive and undergo mitosis ***(12)***. Biomedical nanosensors, both in vivo and ex vivo, will play an important role in the future of medicine. The capability to detect important biological molecules at ultratrace concentrations in vivo is central to many advanced diagnostic techniques. Early detection of diseases will be made possible by tracking down trace amounts of biomarkers in tissue.

The development of nanotechnology-based devices and techniques has allowed measurements of fundamental parameters of proteins and biological species at the molecular level. With "optical tweezer" techniques, small particles may be trapped by radiation pressure in the focal volume of a high-intensity, focused beam of light. This technique, also called "optical trapping," may be used to move small cells or subcellular organelles around at will by the use of a guided, focused beam ***(13)***. Ingenious optical-trapping systems have also been used to measure the force exerted by individual motor proteins ***(14)***. A bead coated with an immobilized, caged bioactive probe was inserted into tissue or even a cell and moved to a strategic location by an optical-trapping system. The cage could then be photolyzed by multiphoton uncaging in order to release and activate the bioactive probe. Optical tweezers can also be used to determine the precise mechanical properties of single molecules of collagen, an important tissue component and a critical factor in diagnosing cancer and the aging process ***(15)***. The optical tweezer method uses the momentum of focused laser beams to hold and stretch single collagen molecules bound to polystyrene beads. The collagen molecules are stretched through the beads using the optical laser tweezer system, and the deformation of the bound collagen molecules is measured as the relative displacement of the microbeads, which are examined by optical microscopy.

The study of biological applications of nanotechnology will be important to the future of biological research and medical science. Medical applications of nanomaterials will revolutionize health care in much the same way that materials science changed medicine 30 yr ago with the introduction of synthetic heart valves, nylon arteries, and artificial joints. The protein nanotechnologies discussed previously are just some examples of a new generation of nanotools that have the potential to detect, identify, and manipulate single proteins in vivo and drastically change our fundamental understanding of the life process itself. They could ultimately lead to the development of new modalities of early diagnostics and medical treatment and prevention beyond the cellular level to that of individual proteins, the building blocks of the life process.

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