NANOBIOTECHNOLOGY
Research and applied science, as we see it today, has advanced to a place in which, instead of manipulating substances at the molecular level, we can control them at the atomic level. This exciting operational space, where the laws of physics shift from Newtonian to quantum, provides us with novel discoveries, which hold the promise of future developments that, until recently, belonged to the realm of science fiction.

Nanobiotechnology is a multidisciplinary field that covers a vast and diverse array of technologies from engineering, physics, chemistry, and biology. It is expected to have a dramatic infrastructural impact on both nanotechnology and biotechnology. Its applications could potentially be quite diverse, from building faster computers to finding cancerous tumors that are still invisible to the human eye. As nanotechnology moves forward, the development of a ‘nano-toolbox’ appears to be an inevitable outcome. This toolbox will provide new technologies and instruments that will enable molecular manipulation and fabrication via both ‘top-down’ and ‘bottom-up’ approaches.

This book is organized into five major sections; 1. Introduction, 2. Bio-templating, 3. Bionanoelectronics and Nanocomputing, 4. Nanomedicine, Nanopharmaceuticals and Nanosensing, and 5. De Novo Designed Structures. Section 1 is an introductory overview on nanobiotechnology, which briefly describes the many aspects of this field, while addressing the reader to relevant sources for broader information overviews.

Biological materials can serve as nanotemplates for ‘bottom-up’ fabrication. In fact, this is considered one of the most promising ‘bottom-up’ approaches, mainly due to the nearly infinite types of templates available. This approach is demonstrated in Section 2.

The convergence of nanotechnology and biotechnology may combine biological and man-made devices for the design and fabrication of bionanoelectronics and for their use in nanocomputing. This area is addressed in Section 3, which covers the use of biological macromolecules for electron transfer and computation.

One of the main reasons nanobiotechnology holds so much promise is that it operates at the biological size scale. Biological molecules (such as enzymes, receptors, DNA), microorganisms and individual cells in our
bodies are all nano-sized. Engineered ultrasmall particles that are made in
the exact size needed to perform specific tasks, such as drug release in par-
ticular locations in the body, drug delivery into the blood stream, or to pin-
point malfunctioning tissues (cancerous tissue, for example), are examples
of the new medical discipline termed ‘nanomedicine’. Section 4 gives a brief
look at this extensive and rapidly growing field.

The fact that nanobiotechnology embraces and attracts many different dis-
ciplines, encompassing both researchers and business leaders, has produced
many examples of bio-inspired de novo designed structures. Each scientific
group approaches the molecular level with unique skills, training, and lan-
guage, and a few examples are presented in Section 5. Cross-talk and collabor-
ative research among academic disciplines, and between the researchers and
their counterparts in business, are critical to the advancement of nanobiotech-
nology and constitute the foundation for the new material generation.

Working at the molecular or atomic level allows researchers to develop
innovations that will dramatically improve our lives. The new territory of
bionanotechnology holds the promise of improving our health, our industry,
and our society in ways that may even surpass what computers and biotech-
nology have already achieved.

Ilan Levy and Oded Shoseyov
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I

INTRODUCTION
Summary

Nanobiotechnology is a multidisciplinary field that covers a vast and diverse array of technologies coming from engineering, physics, chemistry, and biology. It is the combination of these fields that has led to the birth of a new generation of materials and methods of making them. The scope of applications is enormous and every day we discover new areas of our daily lives where they can find use. This chapter aims to provide the reader with a brief overview of nanobiotechnology by describing different aspects and approaches in research and application of this exciting field. It also provides a short list of recently published review articles and books on the different topics in nanobiotechnology.

Key Words: Nanobiotechnology; nanocomputing; nanoelectronics; nanofabrication; nanomedicine; nanotechnology.

1. INTRODUCTION TO NANOSCIENCE AND NANOTECHNOLOGY

The prefix *nano* is derived from the Greek word *nanos* meaning “dwarf,” need and today it is used as a prefix describing $10^{-9}$ (one billionth) of a measuring unit. Therefore, nanotechnology is the field of research and fabrication that is on a scale of 1 to 100 nm. The primary concept was presented on December 29, 1959, when Richard Feynman presented a lecture entitled “There’s Plenty of Room at the Bottom” at the annual meeting of the American Physical Society, the California Institute of Technology (this lecture can be found on several web sites; see ref. 1). Back then, manipulating single atoms or molecules was not possible because they were far too small for available tools. Thus, his speech was completely theoretical and seemingly far-fetched. He described how the laws of physics do not limit our ability to manipulate single atoms and molecules. Instead, it was our lack of the appropriate methods for doing so. However, he correctly predicted that the
time for the atomically precise manipulation of matter would inevitably arrive. Today, that lecture is considered to be the first landmark of science at the nanolevel.

The first 30 yr or so of the nanosciences were devoted mainly to studying and fabricating materials at the nanolevel. In those studies, much effort was devoted to shrinking the dimension of fabricated materials. It was also a time when the two basic fabrication approaches were defined: “bottom-up” and “top-down.” The bottom-up approach seeks the means and tools to build things by combining smaller components such as single molecules and atoms, which are held together by covalent forces. Theoretically, it can be exemplified by molecular assemblers, where nanomachines are programmed to build a structure one atom or molecule at a time or by self-assembly, where these structures are built spontaneously. The advantage of the bottom-up design is that the covalent bonds holding a single molecule together are far stronger than the weak interactions that hold more than one molecule together. The top-down approach refers to the molding, carving, and fabricating of small materials and components by using larger objects such as mechanical tools and lasers, such as is used today in current photolithographic approaches in silicon chip fabrication. Currently, techniques using both approaches are evolving, and many applications are likely to involve combination approaches. However, the bottom-up approach, at least theoretically, holds far more practical and applicative future potential.

Nanoscience is therefore a multidisciplinary field that seeks to integrate mature nanoscale technology of fields such as physics, biology, engineering, chemistry, computer science, and material science.

2. THE “NANO”–“BIO” INTERFACE

Biosystems are governed by nanoscale processes and structures that have been optimized over millions of years. Biologists have been operating for many years at the molecular level, in the range of nanometers (DNA and proteins) to micrometers (cells). A typical protein like hemoglobin has a diameter of about 5 nm, the DNA’s double helix is about 2 nm wide, and a mitochondrion spans a few hundred nanometers. Therefore, the study of any subcellular entity can be considered “nanobiology.” Furthermore, the living cell along with its hundreds of nanomachines is considered, today, to be the ultimate nanoscale fabrication system.

On the other hand, countless exciting questions in biology can be addressed in new ways by exploiting the rapidly growing capabilities of nanotechnological research approaches and tools. This research will form and shape the foundation for our understanding of how biological systems operate. We are exploiting nanofabrication to perform individual molecule
analyses in biological systems, to study cellular responses to structured interfaces, and to explore dynamic life processes at reduced dimensions. Our research has advanced the ability to structure materials and pattern surface chemistry at subcellular and molecular dimensions.

The groundwork of each and every biological system is nanosized molecular building blocks and machinery that cooperate to produce living entities. These elements have ignited the imagination of nanotechnologists for many years and it is the combination of these two disciplines (nano and biotechnology) that has resulted in the birth of the new science of nanobiotechnology. Nanotechnology provides the tools and technology platforms for the investigation and transformation of biological systems, and biology offers inspirational models and bio-assembled components to nanotechnology. The difference between “nanobiology” to “nanobiotechnology” resides in the technology part of the term. Anything that is “man-made” falls into the technology section of nanobiotechnology. Nearly any molecular machinery that we can think of has its analog in biological systems and as for now, it appears that the first revolutionary application of nanobiotechnology will probably be in computer science and medicine. Nanobiotechnology will lead to the design of entirely new classes of micro- and nanofabricated devices and machines, the inspiration for which will be based on bio-structured machines, the use of biomolecules as building blocks, or the use of biosystems as the fabrication machinery.

3. NANOBIO TECHNOLOGY

Unlike nonbiological systems that are fabricated top-down, biological systems are built up from the molecular level (bottom-up). They do this via a collection of molecular tool kits of atomic resolution that are used to fabricate micro- and macrostructure architectures. Biological nanotechnology, or nanobiotechnology, can be viewed in many ways: one way is the incorporation of nanoscale machines into biological organisms for the ultimate purpose of improving the organism’s quality of life. To date, there are a few methods for synthesizing nanodevices that have the potential to be used in an organism without risk of being rejected as antigens; another way is the use of biological “tool kits” to construct nano- to microstructures. However, the broad perspective is probably the one that will include both and will be defined as: the engineering, construction, and manipulation of entities in the 1- to 100-nm range using biologically based approaches or for the benefit of biological systems. The biological approaches can be either an inspired way of mimicking biological structures or the actual use of biological building blocks and building tools to assemble nanostructures. In a way, the first example of a nanobiotechnology system might be the production of recombinant proteins. Recombinant DNA technology can direct the ribosomal machinery
to produce designed proteins both in vivo and in vitro that can serve as components of larger molecular structures.

As already mentioned, there are two basic fabrication approaches to creating nanostructures: bottom-up and top-down. The bottom-up approach exploits biological structures and processes to create novel functional materials, biosensors, and bioelectronics for different applications. This field encompasses many disciplines, including material science, organic chemistry, chemical engineering, biochemistry, and molecular biology. In the top-down approach, nanobiotechnology applies tools and processes of nano/microfabrication to build nanostructures and nanodevices. The tools that are used often involve optical and electron beam lithography and the processing of large materials into fine structures with defined surface features. One of the major differences between nanotechnology and nanobiotechnology is that in the former, the dominant approach is top-down, whereas in the latter, it is bottom-up.

An example of the bottom-up approach is the pioneering work of two leading groups on biomolecular motor proteins (2–7). In these studies, naturally occurring motor proteins were engineered for compatibility with artificial interfaces to create new ways of joining proteins to synthetic nanomaterials. Biomolecular motors can provide chemically powered movement to micro- and nanodevices. Nanodevices utilizing motor proteins such as kinesin or F$_1$-ATPase can be used as nanoscale transporters, as probes for surface imaging, to control the movement of target substances, and to support the controlled assembly of nanostructures.

Structural properties that enable DNA to serve so effectively as genetic material can also be exploited to produce target materials with predictable three-dimensional (3D) structures in the bottom-up approach. Pioneering work using this approach is presented in studies performed by the group of Nadrian Seeman (8–12). He uses DNA motifs with specific, structurally well defined, cohesive interactions involving hydrogen bonding or covalent interactions (“sticky ends”) to produce target materials with predictable 2D and 3D structures. The complementarity that leads to the pairing of the DNA strands is the driving force for the complex assemblies with their branched structures. These efforts have generated a large number of individual species, including polyhedral catenanes, such as a cube and a truncated octahedron, a variety of single-stranded knots, and Borromean rings. The combination of these constructions with other chemical components is expected to contribute to the development of nanoelectronics, nanorobotics, and smart materials. Therefore, the organizational capabilities of structural DNA nanotechnology are just beginning to be explored, and the field is ultimately expected to be able to organize a variety of species in the material world.

Another fascinating example is the use of crystalline bacterial cell surface layer (S-layers) proteins as tools in nanofabrication and nanopatterning. The
S-layer is composed of identical protein or glycoprotein subunits that self-assemble into lattices, forming the outermost cell envelope component of many bacteria. As a result of their high degree of structural regularity, S-layers represent interesting model systems for studies on structural, functional, and dynamic aspects of supramolecular structure assembly. The nano-based approach of S-layer research was pioneered by Uwe B. Sleytr (13–16). In one of those studies, lattices of cadmium sulfide quantum dots were synthesized by using self-assembled bacterial S-layers as templates. Au and CdSe nanoparticles were also deposited directly onto the protein lattice. Given that the macroscopic electronic or magnetic properties of nanoparticle arrays are influenced by interparticle distance and geometry, it should be possible to use various natural or engineered S-layer lattices as a “tuneable” system to obtain nanoparticle assemblies with designed properties for material science. In the future, engineered S-layer proteins might be used as tool kits for the positioning of proteins or nanoparticles in nanopatterned arrays. A faster route to nanopatterns might be a top-down approach wherein S-layer proteins are assembled on nanolithographically structured substrates. Metallic or semiconductor nanoparticle assemblies generated in this way will form the basis of materials with tailored electronic or magnetic properties. Several applications have been suggested for S-layers, such as their use as templates for the nanoscale patterning of inorganic materials or as immobilization matrices for biomedical applications. However, in particular, S-layer technologies provide new approaches for biotechnology, biomimetics, molecular nanotechnology, nanopatterning of surfaces, and formation of ordered arrays of metal clusters or nanoparticles as required for nanoelectronics.

4. REVIEWING MAJOR FIELDS IN NANOBIO TECHNOLOGY

In this section, we will very briefly review the major fields of nanobiotechnology. In fact, each and every one could stand by itself as a book title. However, here we only list the different fields along with a short compilation of recent reviews from the last 5 years published on the subject. Several recently published books are also listed here (17–22).

4.1. Molecular Motors and Devices

A molecular machine can be defined as an assembly of a discrete number of molecular components designed to perform mechanical movement as a consequence of external stimulus. The concept of molecular motors is not new and in fact, every single cell contains several molecular motors as an integral part of its regular function. There are two basic types of natural molecular machines: the rotary motors, such as the F₁-ATPase of flagella, and the linear motors, such as myosin. The study of these molecular motors enables the use
and design of new molecular motors based on biomolecules or other chemical components inspired by bio-motors (23–26).

4.2. Self-Assembled Structures (Nano-Assemblies)

Self-assembly is the spontaneous organization of individual elements into ordered structures. Molecular self-assembly is a fabrication tool where engineering principles can be applied to design structures using basic principles adopted from naturally occurring self assemblies. Self-assembly’s greatest advantage is that it is energetically efficient compared to direct assembly. In recent years, considerable advances have been made in the use of peptides and proteins as building blocks to produce a wide range of biological materials for diverse applications (27–37).

4.3. Biomedical Application of Nanotechnology—Nanomedicine

Although major progress has been achieved in recent years, modern medicine is limited by both its knowledge and its treatment tools. It is only in the last 50 yr that medicine has started looking at diseases at the molecular level, and today’s drugs are thus essentially single-effect molecules. The potential impact of nanotechnology on medicine stems directly from the dimension of the devices and materials that can interact directly with cells and tissues at a molecular level. Applied nanobiotechnology in medicine is in its infancy. However, the breadth of current nanomedicine research is extraordinary. It includes three major research areas: diagnostics, pharmaceuticals, and prostheses and implants. Today, nanomedicine is one of the dominant and leading fields of nanobiotechnology (38–51).

4.4. Biological Research at the Nanoscale

Living organisms and biomolecules are far more complex than engineered materials. In the last few decades, research has focused on the connection between structure, mechanical response, and biological function at the macro- and microlevels. The introduction of research tools at the nanolevel and nanomanipulation techniques stemming from the material world has launched a new paradigm of biomolecular research. Nanoresearch tools are capable of analyzing and visualizing properties of single molecules, thereby providing the opportunity to examine bio-processes of single cells and molecular motors (52–55).

4.5. Biomimetics, Biotemplating, and De Novo-Designed Structures

One of the central goals of nanobiotechnology is the design and creation of novel materials on the nanoscale. Biomolecules, through their unique and specific interaction with other biomolecules and inorganic molecules, natively control complexed structures at the tissue and organ levels. With recent
progress in nanoscale engineering and manipulation, along with developments in molecular biology and biomolecular structures, biomimetics and de novo-designed structures are entering the molecular level. The promise in biomimetics and biotemplating lies in the potential use of inorganic surface-specific proteins for controlled material assembly in vivo or in vitro (56–67).

4.6. Nanocomputing

A comparison of biological systems to computers shows that both process information that is stored in a sequence of symbols taken from an unchanging alphabet, and both operate in a stepwise fashion. In recent years, great interest has arisen among researchers on developing new computers inspired from biological systems. Performing calculations employing biomolecules and using genetic engineering technology may soon find use as a tool for computation. The greatest promise of biological computers is that they can operate in biochemical environments (68–71).

4.7. DNA-Based Nanotechnology and Nanoelectronics

DNA-based nanotechnology is intrinsic to all of the nanotechnological approaches mentioned thus far. An increasing number of scientists within nanoscience are using nucleic acids as building blocks in the bottom-up fabrication approach in order to produce novel structures and devices. The basic drive of this application is the well established Watson-Crick hybridization of complementary nucleic-acid strands. This force has been shown to be efficient in the construction of nanodevices, nanomachines, DNA-based nanoassemblies, DNA–protein conjugated structures, and DNA-based computation (72–88).

5. CURRENT STATUS AND FUTURE TRENDS

Nanobiotechnology is still in the early stages of development; however, its development is multidirectional and fast-paced. Nanobiotechnology research centers are being founded and funded at a high frequency, and the numbers of papers and patent applications is also rising rapidly. In addition, the nanobiotechnology “tool box” is being rapidly filled with new and viable tools for bio-nanomanipulations that will speed up new applications. Finally, an analysis of the total investment in nanobiotechnology start-ups reveals that nearly 50% of the venture capital investments in nanotechnology is addressed to nanobiotechnology (89).

One of the strongest driving forces in this research area is the semiconductor industry. Computer chips are rapidly shrinking according to Moore’s law, i.e., by a factor of four every 3 yr. However, this simple shrinking law cannot continue for much longer, and computer scientists are therefore looking for solutions. One approach is moving to single-molecule transistors (90–93). This
shift is critically dependent on molecular nanomanipulations to form molecular computation that will write, process, store, and read information within the single molecule where proteins and DNA are some of the alternatives (94–99).

As medical research and diagnostics steadily progresses based on the use of molecular biomarkers and specific therapies aimed at molecular markers and multiplexed analysis, the necessity for molecular-level devices increases. Technology platforms that are reliable, rapid, low-cost, portable, and that can handle large quantities are evolving and will provide the future foundation for personalized medicine. These new technologies are especially important in cases of early detection, such as in cancer. Future applications of nanobiotechnology will probably include nanosized devices and sensors that will be injected into, or ingested by, our bodies. These instruments could be used as indicators for the transmission of information outside of our bodies or they could actively perform repairs or maintenance. Nanotechnology-based platforms will secure the future realization of multiple goals in biomarker analysis. Examples for such platforms are the use of cantilevers, nanomechanical systems (NEMS), nanoelectronics (biologically gated nanowire), and nanoparticles in diagnostics imaging and therapy (100–106).

The art of nanomanipulating materials and biosystems is converging with information technology, medicine, and computer sciences to create entirely new science and technology platforms. These technologies will include imaging diagnostics, genome pharmaceutics, biosystems on a chip, regenerative medicine, on-line multiplexed diagnostics, and food systems. It is clear that biology has much to offer the physical world in demonstrating how to recognize, organize, functionalize, and assemble new materials and devices. In fact, almost any device, tool, or active system known today can be either mimicked by biological systems or constructed using techniques originating in the bio-world. Therefore, it is plausible that in the future, biological systems will be used as building blocks for the construction of the material and mechanical fabric of our daily lives.

REFERENCES
II

BIOTEMPLATING
Experimental Strategies Toward the Use of the Porin MspA as a Nanotemplate and for Biosensors

Stefan H. Bossmann, Katharine Janik, Megh Raj Pokhrel, and Michael Niederweis

Summary
The porin MspA from Mycobacterium smegmatis has many unique properties, one being that it is the longest and the most stable porin identified to date. It is formed by supramolecular interaction of eight identical monomers of 184 amino acid residues (m = 20,000 Da). With dimensions of approx 10 nm in length and a diameter ranging from 1 nm (constriction zone) to 4.8 nm (opening of the MspA-goblet), it is ideal for bio-nanotechnological applications. The porin possesses a hydrophobic “docking zone,” which enables it to reconstitute not only in lipid membranes, but also in numerous artificial (mono)membranes and hydrophobic, water-soluble polymer layers. Furthermore, we demonstrate here the design and proof-of-principle of an MspA porin-based biosensor for the TB-antibiotic isoniazid.

Key Words: Antibiotics; biosensor; HOPG; isoniazid; luminescence quenching; MspA; Mycobacterium smegmatis; photoinduced electron transfer; ruthenium-catenane; sensitizer-relay assembly.

1. INTRODUCTION

1.1. The Mycobacterial Cell Envelope
The mycobacterial cell envelope forms an exceptionally strong barrier, rendering mycobacteria naturally impermeable to a wide variety of antimicrobial agents because of its unique structure (1). In Fig. 1, the various layers of the mycobacterial cell envelope are shown schematically. The cytoplasmic membrane is the innermost layer of the envelope and has a thickness of approx 4 nm. Surrounding this membrane is the “cell-wall skeleton,” a giant macromolecule consisting of peptidoglycan (a structure of oligosaccharides and...
formed from disaccharide units of N-acetylglucosamine and N-glycolyl-
muramic acid cross-linked by short peptides), arabinogalactan (a complex
branched polysaccharide) and mycolic acids (long-chain, 2-alkyl-3-hydroxy
fatty acids). Connected with the cell-wall skeleton, but not covalently
attached to it, are a large variety of other lipids. Nikaido et al. have shown
substantial evidence for the organization of the mycolic acids in a second
lipid bilayer in addition to the cytoplasmic membrane (2). Their X-ray diffrac-
tion measurements on purified mycobacterial envelopes, free of plasma
membranes (<2% contamination or less), showed a strong reflection at 4.2 Å
and a weaker, more diffuse one at 4.5 Å. These types of reflections are char-
acteristic of ordered fatty acyl chains and were interpreted as indicating the
presence of highly ordered and less ordered regions, respectively. By cen-
trifuging a sample of cell walls onto a flat surface, measurements were
obtained showing that the acyl chains were aligned perpendicular to the
planes of the walls. The nature of the mycolic acids establishes the high-
temperature phase change, which was discovered by studying purified walls
and verifying that most of the associated lipids were previously removed with
the detergent Triton X-114. Corynebacteria have a lower-temperature phase
change and their mycolic acids are much shorter than those of mycobacteria.
Chain length is another important factor, and the configuration of the double

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**Fig. 1.** Schematic representation of the mycobacterial cell envelope. (From ref. 6, with permission.)
bond or cyclopropyl group proximal to the carboxyl group of the mycolate seems to be important as well, because a higher-temperature phase change correlates with a higher proportion of trans configuration. Some environmental mycobacteria can adjust the composition of their mycolates according to temperature, attempting to attain the required behavior of their outer permeability barrier (3). Taking all of this into account, we conclude that Mycobacterium smegmatis becomes less permeable to lipophilic drugs when grown at higher temperatures.

Measurements by continuous-wave (CW)-electron paramagnetic resonance (EPR) of lipophilic probes—spin-labeled fatty acids—“dissolved” in purified walls or whole bacteria show that these enter only a less ordered and more fluid region (4). This region may be that which is occupied by the alkyl chains of the associated lipids forming the exterior half of a bilayer, or that where these associated lipids intercalate into the part of the mycolate monolayer where the longer of the two alkyl chains of each mycolate is present. The insertion depth of the spin label determines the measured mobility, where the nitroxide type spin label is in the position of the carbon atom in the fatty acids. This effect has already been observed with conventional bilayers, but the change of mobility with depth was different in the case of mycobacterial walls, confirming the unusual nature of the mycobacterial outer permeability barrier. EPR spectra using whole cells were similar to those spectra using highly purified walls, telling us that the nitroxide-labeled fatty acids entered the outer part of the barrier only (5).

The inner leaflet of the outer membrane (OM) is composed of mycolic acids (MA), which are covalently linked to the arabinogalactan (AG)-peptidoglycan (PG) copolymer. The outer leaflet is formed by a variety of extractable lipids such as trehalose-dimycolate ("cord factor"), lipo-oligosaccharides, sulfolipids, glycopeptidolipids, phenolic glycolipids, and glycerophospholipids. The diameters of the inner and outer membranes are rather poorly defined estimates from electron microscopic images of mycobacterial cell envelopes and are drawn to scale. Two general pathways through the mycobacterial OM exist: small and hydrophilic compounds diffuse through water-filled protein channels, the porins, whereas hydrophobic compounds use the lipid pathway by penetrating the OM directly (Fig. 1).

The mycolate monolayer can be formed even though the mycolate residues are covalently attached to the polysaccharide. This probably requires that the cross-linked glycan strands and the arabinogalactan strands run in a direction perpendicular to the cytoplasmic membrane (5). The mycolates occur as esters of terminal arabinose units on the polysaccharide. The arabinosyl mycolate units are covalently linked to the galactan backbone, which is attached to the peptidoglycan. The whole polysaccharide is composed of sugars in their
furanose form, giving additional flexibility to the chain and in all probability allowing the structure to accommodate itself to the close packing of the mycolate units through this characteristic-repeating motif (5). These observations support the assumption that an asymmetric bilayer comprises the mycobacterial outer permeability barrier, with an inner leaflet of essentially “frozen” mycolate residues and an outer leaflet of more mobile lipids.

This outer membrane has unique properties: (1) it has a very low fluidity and will not melt at temperatures up to 70°C, in contrast to cytoplasmic membranes of other mesophilic organisms, which begin to disintegrate at 20°C; (2) it is thicker than all other known membranes, although it should be noted that the widely accepted thickness of about 10 nm is a rather poorly defined estimate from various electron microscopy pictures of mycobacteria and does not correspond to the length of the hydrophobic domain of MspA (3.7 nm; 7); (3) it provides a very hydrophobic cell surface, which causes the bacteria to clump in a hydrophilic environment; and (4) its fluidity decreases toward the periplasmatic side of the membrane in contrast to that of the OM of Gram-negative bacteria (8).

1.2. **MspA from M. smegmatis is the Prototype of a New Family of Bacterial Porins**

Hydrophilic molecules enter the mycobacteria by diffusing through channel-forming proteins, known as porins (6). MspA is the major porin in the OM of *M. smegmatis* mediating the exchange of hydrophilic solutes between the environment and the periplasm (9). Electron microscopy and crosslinking experiments indicated that MspA is a tetrameric protein with one central channel of 10 nm in length with a minimum inner diameter (constriction zone) of 1.0 nm (7). The MspA crystal structure revealed a homo-octameric goblet-like conformation with a single channel and constitutes the first structure of a mycobacterial OM protein (Fig. 2B) (7). MspA contains two consecutive 16-stranded β-barrels with nonpolar outer surfaces that confirm the very existence of an outer membrane in *M. smegmatis*. The length of the two membrane-spanning and pore-forming β-barrels is 3.7 nm, and the outer diameter of the 16-stranded β-barrel is 4.9 nm. The channel diameter varies between 4.8 nm and 1.0 nm at the pore eyelet, which is completely defined by two rings of aspartates. The β-sheet content is similar to that determined earlier by circular dichroism and infrared spectroscopy (9). This makes MspA the membrane protein with the longest membrane-spanning domain known to date. These properties are drastically different from those of the trimeric porins of Gram-negative bacteria and classify MspA as the prototype of a new family of channel proteins.
1.3. The Advantages of MspA in Nanotechnology Compared to Other Proteins

Proteins are macromolecules with dimensions in the nanometer range and can be tailored to specific needs by site-directed mutagenesis. Their use in nanotechnology has been severely hampered by the problem that most proteins lose their structural integrity in a nonnative environment, impeding their use in most technical processes. The MspA porin from *M. smegmatis* is an extremely stable protein, retaining its channel structure even after boiling in 2% sodium dodecyl sulfate (SDS) or extraction with organic solvents. This creates an extremely stable and adaptable environment and allows the use of MspA as a template for small molecules and nanoparticles in well defined arrangements on a nanometer scale. Ostwald processes, e.g., the coagulation of nanoparticles to bigger particles and, finally, precipitation, are prohibited or extremely decelerated when MspA is used as a template. The same principles are true for using the MspA channel for specific sensor functions. Longer template channels also allow the synthesis of longer nanowires, extending their application potential. The hydrophobic surface of MspA allows its assembly into biomimetic membranes. An additional useful feature is the tendency of MspA to self-assemble into ordered structures on