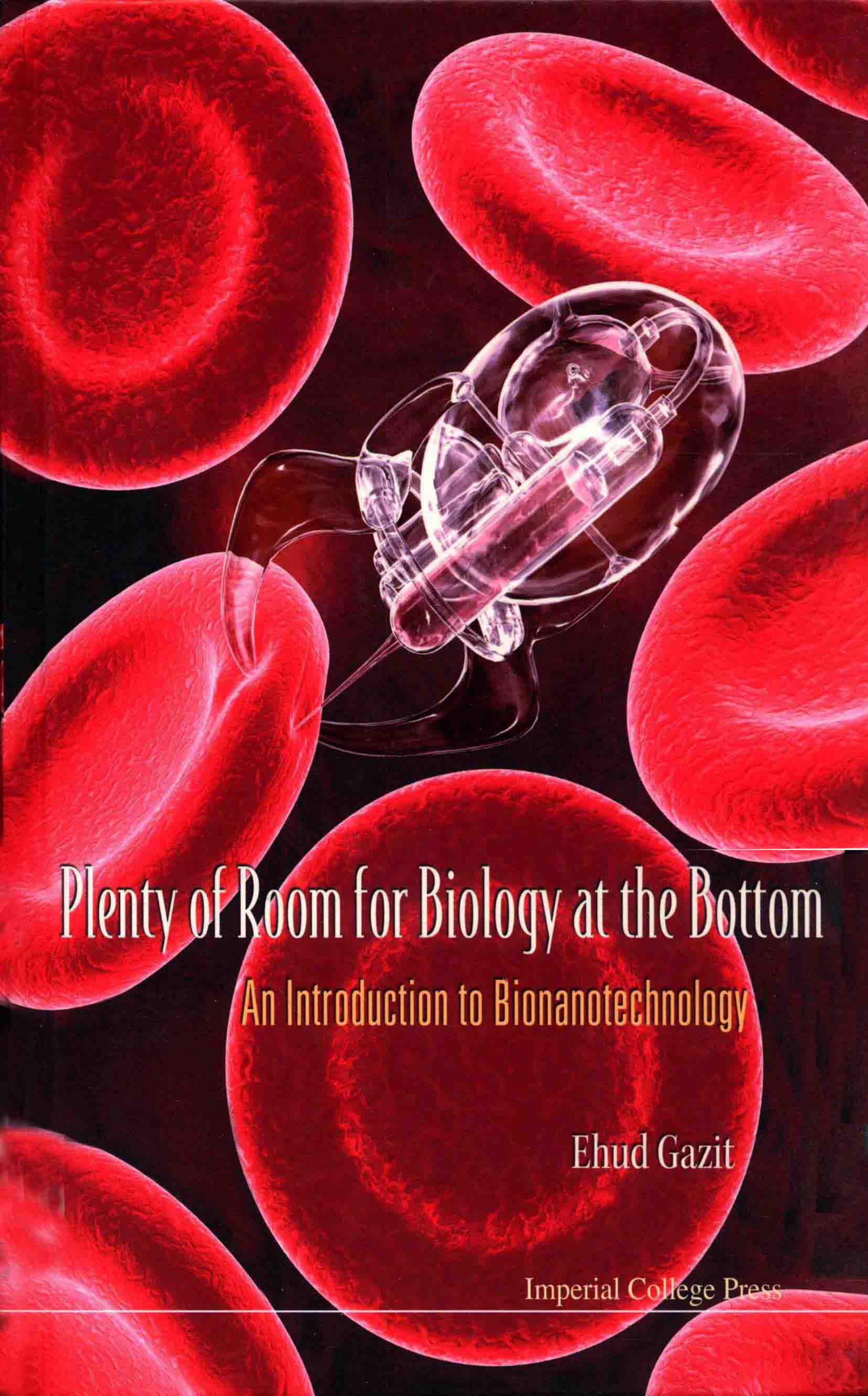

Plenty of Room for Biology at the Bottom

An Introduction to Bionanotechnology

Ehud Gazit

Imperial College Press

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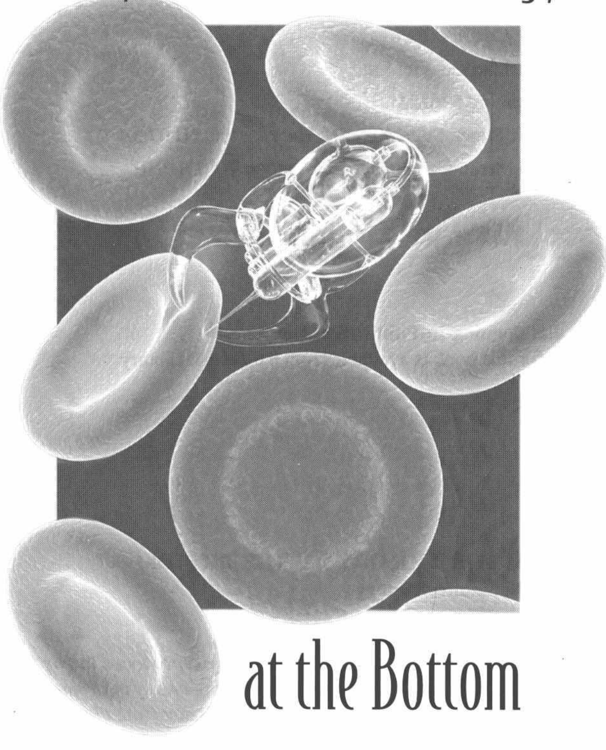

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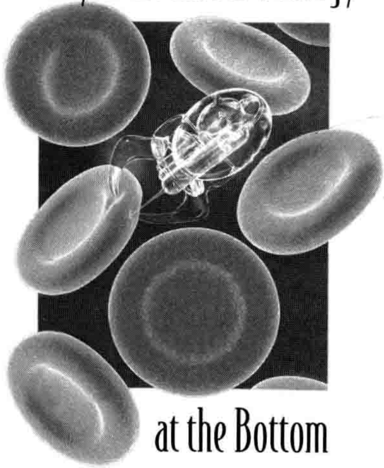

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An Introduction to Bionanotechnology

This book is dedicated to my family with love

Preface

This book represents a great challenge: It introduces the readers to the integration of two of the most important disciplines of the 21^{st} century, biotechnology and nanotechnology. A special effort was made to expose readers from diverse backgrounds to the basic principles, techniques, applications, and great prospects at the intersection of these two key fields. All of this is done without assuming a strong background in either of the two subjects. We will discuss two related fields: "nanobiotechnology" which relates to the application nanotechnological principles and tools to biology, but also "bionanotechnology" which concerns the use of biological principles such as recognition and assembly for nanotechnological applications.

While the 20^{th} century was the century of physics, electronics, and communication, the years to come are considered to be mainly dominated by the biological revolution that already started in the second half of the 20^{th} century as well as nanotechnology, a new and exciting new front that deals with minuscule nanometer-scaled assemblies and devices. Traditionally, miniaturization was a process in which devices became smaller and smaller by continuous improvement of existing techniques. Yet, we rapidly approach the limits of miniaturization using the conventional top-down fabrication tools. One of the key futuristic ways of making tiny machines will be to scale them up from the molecular level. For that purpose, many lessons could be learned about the arrangement of nano-scale machines as occurs in each and every biological system. The living cell is actually the only place in which genuine functional molecular machines, as often described in futuristic presentations of nanotechnology, could actually be found. Molecular motors, ultrasensitive nano-scale sensors, DNA replication machines, protein synthesis machines, and many other miniature devices exist even in the very simple early pre-bacterial cells that evolved more than 3 billion years ago. When we climb higher on the evolution tree, the nano-machines of course become more sophisticated and powerful. Yet, only billions of years after life emerged, we are beginning to utilize the concepts of recognition and assembly that lead to the formation of nano-scale machines for our technological needs. On the other hand many principles and applications of nano-technology that were developed for non-biological systems could be very useful for immediate biological applications such as advanced sensors and molecular scaffolds for tissue engineering as well as long-term prospects such as *in situ* modifications at the protein and DNA levels.

While the fields of nanobiotechnology and bionanotechnology are very new their prospects are immense. The marriage between biotechnology and nanotechnology could lead to a dramatic advancement in the medical sciences. It may well be a place in which many of the current diseases and human disorders will be eradicated. In a reasonable time-scale, cancer and AIDS may be regarded in the same way that polio and tuberculosis are being considered now. Genetic defects could be identified and corrected already even before birth. Nano-scale robots that may be inserted into our body could perform very complicated surgical tasks such as a brain surgery. Nano-machines may even be able to solve problems on the cellular level. Manipulation of the genetic information inside the body in real-time is only one example.

The principles of biological recognition could also serve as the basis for applications that seem to be very far from living systems as we identify them. One of the most obvious directions in the field of nanotechnology will be molecular electronics. The use of the tools of molecular recognition between biomolecules and their ability to self-assemble into elaborated structures could actually serve as an excellent model system for the 21st century nano-engineers. This course of study will link us directly to the one of the most important areas of research and development of the second half of the 20th century, the silicone-based microelectronics. Biology-based nanotechnology may facilitate our ability to overcome many of the limitations of the silicon-based world as we know them today. The field of electronics could be transformed to have complete new and exciting prospects when electronic devices will be made by nano-machines and by govern by molecular self-assembly principles. The world after this bionanotechnologic revolution may be a place where the molecular mechanism of thinking and reasoning will be understood and truly artificial intelligence machines could be manufactured.

It should always be remembered that the new era of nanobiotechnology and bionanotechnology may also lead to a situation in which there will be a very fine line between prosperity and devastation. The role of the scientists and technologists in the years to come will be to ensure the proper usage of these hardly imaginable abilities. We must make sure this revolution will be used for the benefit of mankind and not for its destruction. As those tools are so powerful, their misuse may actually lead to grave consequences.

E. Gazit

Contents

Chapter 1

Introduction: Nanobiotechnology and Bionanotechnology

The convergence between the scientific disciplines of biotechnology and nanotechnology is a relatively recent one. Yet, the combination of these highly important areas of research has already resulted in remarkable achievements. This chapter will provide basic background on classical and modern biotechnology and its interface with nanotechnology, a much less mature scientific discipline. Throughout this book we will use the term "nanobiotechnology" to describe the applications of nanotechnology techniques for the development and improvement of biotechnological process and products. This includes the use of fabrication and manipulation techniques at the nano-scale to form more sensitive and accurate diagnosis methods such as a "lab-on-chip" and real-time nanosensors. This also includes the use of matrixes with nano-scale order for the controlled release of drug as well as tissue engineering and regeneration. Later on in the book, we will describe more advance futuristic directions such the development of manipulation devices at the nano-scale ("nano-robots") to perform medical procedures in patients or nano-machines that will serve as an artificial alternative to functional biological organs.

The term "bionanotechnology" will be employed to describe the use of biological building blocks and the utilization of biological specificity and activity for the development of modern technology at the nano-scale. Such bionanotechnology practice is obviously not limited to biological applications but have much wider scope. For instance, future applications of bionanotechnology could include the use of DNA oligomers, peptide nanotubes, or protein fibrils for the fabrication of metal nanowires, interconnects or other physical elements at the nano-scale, that could be used in molecular electronics and nano-electromechanically applications and devices (Braun *et al.*, 1998; Reches and Gazit, 2003; Zhang, 2003; Patolsky *et al.*, 2004a,b).

1.1. Classical Biotechnology: Industrial Production Using Biological Systems

Biotechnology is a well-established scientific discipline. The practice of classical biotechnology, defined by the American Heritage Dictionary as "The use of microorganisms, such as bacteria or yeasts, or biological substances, such as enzymes, to perform specific industrial or manufacturing processes", goes back to the first half of the 20th century (Figure 1.1). The large-scale industrial application of biological processes, such as the production of acetone by the fermentation of starch with the bacterium *Clostridium acetobutylicum*, was already performed in 1916. The industrial production of penicillin antibiotic from *Penicillium notatum* bacteria for practical medical use was already achieved in the early 1940s.

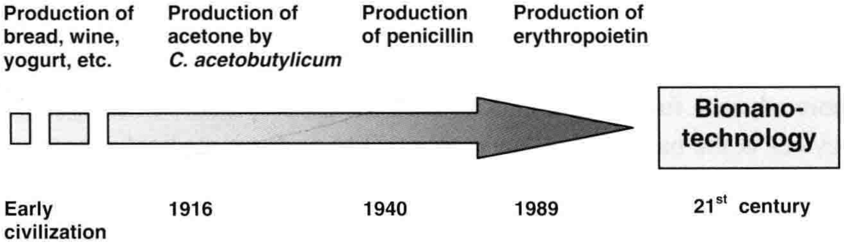

Figure 1.1: Milestones in the development of modern biotechnology into bionanotechnology. Production of bread, wine, and yogurt was practiced thousands of years before the commercial production of acetone, antibiotics, and biotechnological drugs.

Other scholars attribute the use of biotechnology practice to a much earlier era as a variety of food products, such as wine, beer, cheese and bread had already been produced with the help of yeast and bacteria for few thousands of years from the early days of civilization (Figure 1.1).

1.2. Modern Biotechnology: From Industrial Processes to Novel Therapeutics

Over the years, the practical definition of biotechnology became much more vague. Much of the activity of the modern biotechnology practice, both in the academic settings as well as the industrial ones, developed into applied biological sciences rather than a well-defined scientific discipline. The activity that is descried as "biotechnology" ranges from the production of biomolecules (e.g. functional proteins or antibodies) to serve as drugs to the development of novel diagnostic tools that are based on the interaction of specific biomolecules (such as antibody-antigen interactions as in immunodiagnostic kits or complementary nucleic acids interactions as in DNA-array microchips). The somewhat practical distinction between the pharmaceutical industry and the modern biotechnology industry is the production of large biomolecules such as functional proteins and antibodies by the latter as compared to small molecule drugs by the former.

For example, the first product of the largest biotechnology company, *Amgen*, is an industrially produced naturally occurring protein called erythropoietin (commercial name: EPOGEN®) that stimulates the production of red blood cells. This product was approved for human use in 1989 by the Food and Drug Administration (FDA) and represents one of the very first modern biotechnology products. Other biotechnology products include recombinant human insulin, human interferon, human and bovine growth hormones, and therapeutic antibodies. The production of therapeutic antibodies is especially intriguing and represents a relatively new area of research. These therapeutic agents utilize the remarkably affinity and specificity of antibodies (as described in Chapter 4) to specifically block harmful biological processes by a directed and extremely specific manner. A recent example is the humanized recombinant monoclonal antibody that binds to and inhibits the biochemical activity of the vascular endothelial growth factor (commercial name: AVASTIN™). This antibody-based treatment significantly increases the survival rates of patients with metastatic carcinoma of the colon that is incurable by conventional chemotherapy. Another major biotechnological product that was mentioned above is human interferon, a protein that is a central role in the response of the human body to viral infection, and human growth hormone, a key regulator of normal growth of children and adolescents.

Many more proteins are now been developed for their use as potential drug. One of the limitation factors for a much more common use of protein and peptide drug is their unavailability in oral formulation. Unlike small molecule drugs, which are commonly being administrated in the form of tablets or syrup, protein and peptide drug are usually being degraded during their passage in the digestive track. Therefore, the administration of the protein and peptide drugs as mentioned above is limited to injections that can not easily be performed by the patients outside of medical institutions. Another application of peptide and protein drug is topical, which again is very limited in its therapeutic scope. Actually, in this case nanotechnology can provide a very helpful and important solution. For example, arrays of hundreds or thousands of tiny nano-syringes are used for dermal application of biomolecular agents without any associated pain. Other applications include various nano-carriers are being studied for their ability to safely transfer drugs throughout most of the digestive track, but release them at the intestines. Nano-carriers are also being studied for their ability to allow the transfer of protein and peptide drugs through the blood-brain barrier (BBB) to treat disease such as brain tumors and various neurodegenerative disorders. The nano-carriers themselves can be formed by the self-assembly of biomaterials (such as peptide nano-spheres) but can also be composed of non-biological materials. At any rate, this is an example for the way in which nanotechnology can help biotechnology to reach much wider use.

1.3. Modern Biotechnology: Immunological, Enzymatic, and Nucleic Acids-Based Technology

The diagnostic aspect of biotechnology principally consists of the detection and quantification of biological materials using biochemical techniques such immunological recognition assays, enzymatic reactions, and DNA- or RNA-based technology. As will be discussed in this book, the use of nano-scale assemblies and fabrication may help to significantly improve the sensitivity as well as the specificity of the diagnosis process.

Diagnostic immunoassays include products such as the home pregnancy test kits that contain antibodies that detect minute traces of the human chorionic gonadotropin (hCG) hormone (Cole, 1997). Other products include diagnostic kits for the determination of HIV or hepatitis virus infections. In all these cases a specific high affinity and specificity molecular recognition processes facilitate the diagnosis process. The chemical basis for this recognition, specificity and affinity will be further elaborated in this book. Understanding and miniaturization of the detectors and molecular markers of the recognition process will lead to better utilization of these interactions for various diagnostic applications. The increased sensitivity of such devices will result in the need for much lower volume of blood to determine specific key biological parameters such as the glucose levels (Figure 1.2). Actually this lower amount of blood, that could be reduced from micro-liters to nano-liters, can be extracted by nano-syringes that were mentioned above that will significantly reduce the discomfort that is associated with the collection of blood samples for glucose levels determination. A more futuristic instrument may include a nano-device that measures the glucose levels using electrochemical reaction and nano-electrodes on a chip that are connected to a controlled delivery system that releases insulin according to a programmed profile. Such a nano-device will be able to mimic some of the functions of the pancreas for acute Type I and chronic Type II diabetes patients.