

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.

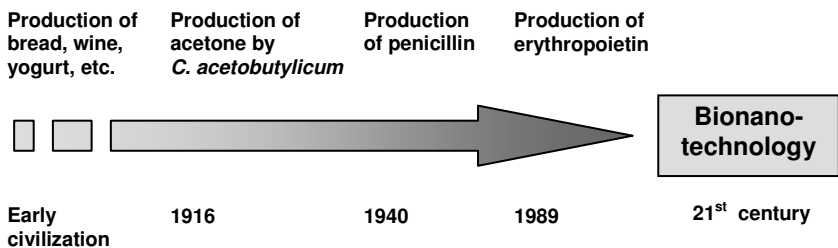


Figure 1.1: Milestones in the development of modern biotechnology into bionanotechnology. Production of bread, wine, and yogurt was practiced thousands of years before the commercial production of acetone, antibiotics, and biotechnological drugs.

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 described 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 administered 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.

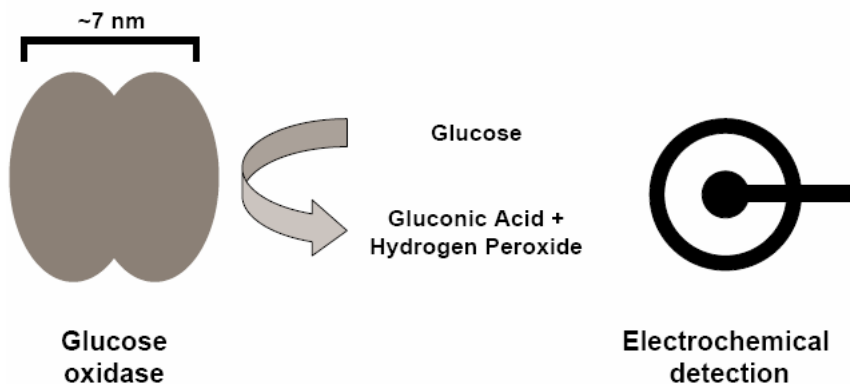


Figure 1.2: Detection of glucose level is based on the combination of enzymatic reaction and electrochemical detection. Hydrogen peroxide is being formed upon the oxidation of glucose by the glucose oxidase (GOX) enzyme. The level of the redox reaction is being determined electrochemically. In principle the reaction can be performed on the nano-scale as the size of the enzyme is less than 10 nm.

The diagnostic use of enzymatic reactions includes common instruments such as home glucometers that utilize the catalytic reaction of the glucose oxidase enzyme with glucose to produce gluconic acid and hydrogen peroxide (Jaffari and Turner, 1995). The later chemical product is being electrochemically determined and converted into a digital output (Figure 1.2). This is indeed one of the key examples of the merging between biotechnology and electronics to produce a hybrid enzyme-electronic interface. Miniaturization of such and other enzyme-based detectors is one of envisioned nanobiotechnological devices (Jaremkov and Rorstad, 1998).

DNA-based technology includes the polymerase chain reaction (PCR) process that is being extensively used in forensic science to clearly determine the source of biological samples. Another technique that utilizes the specificity of nucleic acids interaction is the DNA microarray chip technology. This technology allows us to follow the expression pattern of thousands or even tens of thousands of genes simultaneously. The DNA-array techniques are not only invaluable to basic medical studies but it also paves the way for future personal

medicine. Also these applications can be significantly advanced by the use of nanotechnology, for example by performing the reactions at very small volume using “lab-on-a-chip” settings. This should allow much smaller amounts of biological samples for the application of the nucleic acids based techniques. Such an increase in sensitivity should be especially helpful in forensic science and in the early detection of pathological processes such as early phase of malignant transformation. Other very important applications include environmental monitoring and homeland security detection of biological and chemical agents.

1.4. The Interface Between Nanotechnological and Biotechnology: Bionanotechnology

As mentioned above, nanotechnology is a relatively young scientific discipline. Chapter 2 provides some of the basic themes and principles of nanotechnology that are mainly directed towards readers with a background in the biological sciences. As a basic introduction, nanotechnology could be described as technological applications using systems and devices with orders at the nanometer scale (as most of the readers are aware, one nanometer equals to one thousandth of a micrometer or one millionth of a millimeter - 1/1,000,000,000 meter). The study of nanotechnology includes molecular systems, molecular assemblies (such as quantum dots), and organized self-assembled devices and machines. All these are considered to be part of the “bottom-up” approach in which simple components at the nanometric scale join together to form complex machines, devices, or instruments, by spontaneous processes that involve molecular recognition and self-assembly events.

Biology and biological recognition should have great importance in these directions of molecular recognition and self-assembly. Biomeolecules and biological complexes are naturally-occurring building blocks, recognition modules, and even machines (such as the ribosome, the complex protein assembly line, or molecular motor). Even more complex structures such as plant and animal viruses or bacteriophages (viruses that attack bacteria) could be formed by association at the nano-

scale. Biological recognition, such as the one attained by specific antibodies (see Chapter 4), can specifically direct the “bottom up” assembly. By the utilization of the high specificity and the spontaneity of biological association processes, biological assemblies can serve as a “smart scaffold” for the self-association of complex organic and inorganic nano-machines and nano-devices.

Other directions are the “top-down” approaches which consist of either continuous improvement of common ultraviolet (UV) lithographic processes or the use of more elaborate lithographic technologies such as electron-beam (E-beam) lithography or focused ion beam (FIB) lithography. As of 2006, industrial scale lithography, used in the field of micro-electronics, already reached a 90 nm resolution by the use 193 nm deep ultraviolet photolithography. Electromagnetic radiation of shorter wavelength was already demonstrated to be useful at even better resolution (down to 45 nm). Furthermore, the use of beam of electrons or ions instead of photons allows the fabrication of objects at even higher resolution (as low as 20 nm).

There is not doubt that these techniques, either the “bottom-up” or “top-down”, will revolutionized the field of biotechnology. The above mention biotechnological techniques and procedures and many others could be extensively benefited from their interfacing with modern nanotechnology techniques. This may be merely the miniaturization that will result in much simpler procedures that will require significantly smaller volumes of biological samples and will make the medical procedures much simpler and more pleasant to the patient. Yet, it could lead to assembly of sophisticated machines at the nano-scale that do not only measure but also perform various tasks that are based on these measurements. Moreover, the interface of biotechnology and nanotechnology can offer the attainment of sensitivities that are orders of magnitudes higher than current techniques. The ability to determine the presence of the very first cancerous cell in the body or the monitoring of minute amounts of pollutants or volatile explosives will dramatically revolutionize the medical practice as well as homeland security and environmental monitoring.

1.5. Supramolecular (Bio)Chemistry: The Theoretical Basis for Self-Assembly

Supramolecular chemistry consists of the study of the structure and function of the entities formed by association of two or more chemical species by non-covalent interactions. These type of supramolecular structure are actually type of “supermolecules” that are distinct from regular molecules that are being formed by continuous chemical covalent bonding between atoms. To distinguish between supramolecular structures and covalent chemical structures, we will usually use the term “assemblies” to describe the non-covalent structures.

The foundations of this field of science were laid down only few decades ago. The founding fathers are considered to be Donald J. Cram, Jean-Marie Lehn, and Charles J. Pederson who were awarded the Nobel Prize in Chemistry in 1987. The citation of the prize reads “for their development and use of molecules with structure-specific interactions of high selectivity”. According to one definition suggested by Lehn: “supramolecular chemistry is chemistry beyond the molecule”. It should be noted that the term “supermolecules” was already coined in the 1930s (Pfeffer, 1927), yet the exact description of the complex chemical interactions that lead to the formation of these ordered structures was yet to be described decades later.

It is also worth to note that while this notion of supramolecular assemblies is relatively new, many biological entities can consider as supramolecular assemblies. These can be as simple as functional dimers (e.g., Fos-Jun transcription factor) and as complex as molecular nano-machines such as the ribosome or molecular motors. Taken together, biology can be considered as a most advanced playground of supramolecular chemistry as complex biology functions is being achieved by the formation of complex molecular structures by non-covalent interactions (Aizenberg *et al.*, 2005). Throughout this book we will consider self-assembly and molecular-recognition (see below) as central themes in bionanotechnology and nanobiotechnology.

These processes in which large ordered assemblies are being formed by non-covalent association of simpler building blocks are the essence of “bottom-up” design. In this approach simple building blocks are joined

together to form supermolecules or assemblies that have a distinct morphology and often also specific function or unique physicochemical properties.

1.6. The Next Steps for Self-Association at the Nano-Scale

The formation of ordered structures by self-association at the nano-scale is just the first steps toward the formation of ordered molecular structures. The next steps involve the formation of hetero-component assemblies, such as observed with the biomolecular assemblies and machines described above. The formation of such structures should be based on the combination of recognition modules that are evolve from geometrical complementary (Figure 1.3) but also on chemical recognition. Elements such as aromatic moieties that combine both properties are especially attractive elements in the self-association design schemes. This is due to the geometrically restricted nature of these interactions on the one hand, but the specific entropic and enthalpic chemical interaction on the other hand. It has already been shown that well-ordered structures, such as peptide nanotubes could self-assemble by the facilitation of such interactions (e.g., Reches and Gazit, 2003).

As will be discussed in Chapter 4, it was already demonstrated that many complex structures could be formed by the self-association of biomolecules. The pioneering work by Nadrian Seeman and co-workers had demonstrated the ability of partially complementary DNA strands, which form molecular junctions, to self-assemble into two-dimensional nanowires and three-dimensional nanocubes. Other researchers had demonstrated the ability of peptide molecules to self-assemble into tubes, spheres, plates, tapes, and hydrogels with nano-scale order (Ghadiri *et al.*, 1993, 1994; Agelli *et al.*, 1997; Altman *et al.*, 2000; Bong *et al.*, 2001; Banerjee *et al.*, 2003; Silva *et al.*, 2004). Such nano-scale peptide assemblies were already proven to have potential in diverse fields such as tissue engineering and regeneration (Holmes *et al.*, 2000; Silva *et al.*, 2004), the design of novel antibacterial agents that form nanochannels (Ghadiri *et al.*, 1994), and the fabrication of metallic nanowires (Reches and Gazit, 2003).

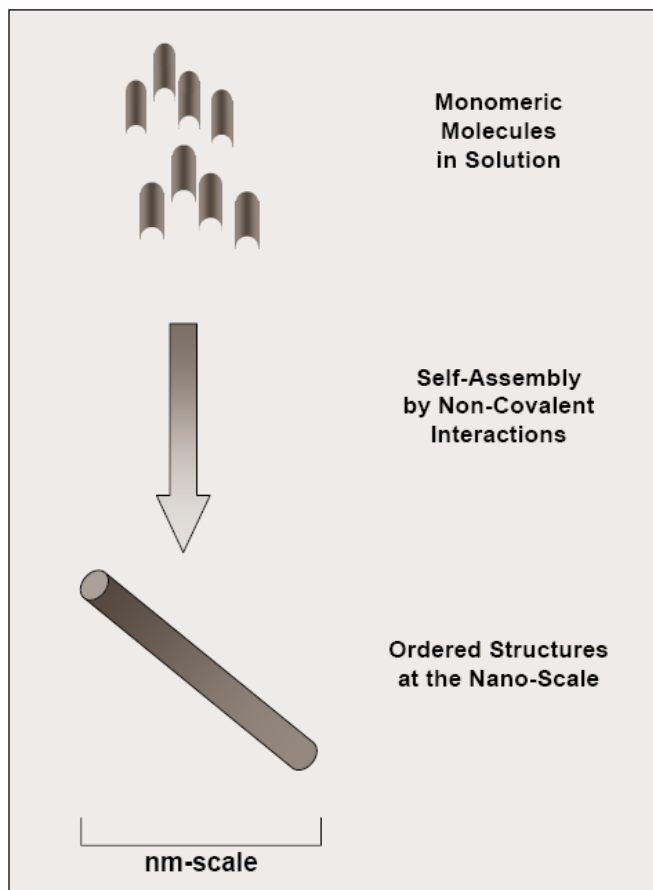


Figure 1.3: Self-assembly of molecules into ordered non-covalent structures as the nano-scale. In the current example complementary geometry is directing the association. Yet, other factors such as chemical complementary may facilitate such multi-component association.

1.7. Biology in Nanotechnology and Nano-Sciences in Biotechnology

As already mentioned above, the practice of biology or biotechnology at the nano-scale could be divided into two main categories (Figure 1.4). The first one is the technological applications of self-assembled nano-scale ordered biomolecular in various fields. These

disciplines may not have any biological relevance *per se*. The role of biology in these cases is mainly based on its remarkable specificity and diversity and its ability to facilitate the formation of very complex nanostructures by relatively simple building blocks. We would refer to this direction as bionanotechnology, the application of biology and biomolecules into technological applications at the nano-scale. These directions will be extensively described in this book. Yet, as an introduction, we would like to mention the use of biomolecules (such as DNA and proteins) for the fabrication of metallic structures at the nano-scale (Reches and Gazit, 2003; Patolsky, 2004). Such structures can serve in future computers or other devices that may have no direct association with biology. Yet, the biology may provide tools that are not currently available by any other means. This was illustrated by the practice of molecular lithography in which protein molecules of few nano-meters served as a resist for the fabrication of gold wires on DNA molecules (Keren *et al.*, 2003 - see Chapter 7). Other directions may include the formation of novel materials of unique rigidity, surface chemistry, or other physicochemical properties that may prove useful in the automobile or space industries or simple in consumer's goods.

The second direction that is quite distinct is the application of technologies that were developed at the nano-scale for the advancement of biological processes, such as tissue engineering or diagnostics. These nano-scale structures may not include any biological molecules of nano-scale ordered as they could be completely utilize silicone-based structures (such as in the case of a "lab-on-a-chip") or carbon nanostructures (such as in the case of tissue engineering on carbon nanotubes-based substrate). Such an application could actually revolutionize the field of biotechnology by allowing the early diagnosis of disease, the on-line monitoring of therapeutic procedures, and the formation of tissue in the test tubes.

It is worth mentioning that the actual use of either the terms "nanobiotechnology" or "bionanotechnology" is quite a recent one. According to the *MedLine* database, the term "nanobiotechnology" appears first in 2000, while the first hit for "bionanotechnology" is only in 2004. "Nanobiotechnology" is also a much more common term that is more than 4 times more common than "bionanotechnology". Yet, in many

of the cases the use of the later term would have been much more appropriate.

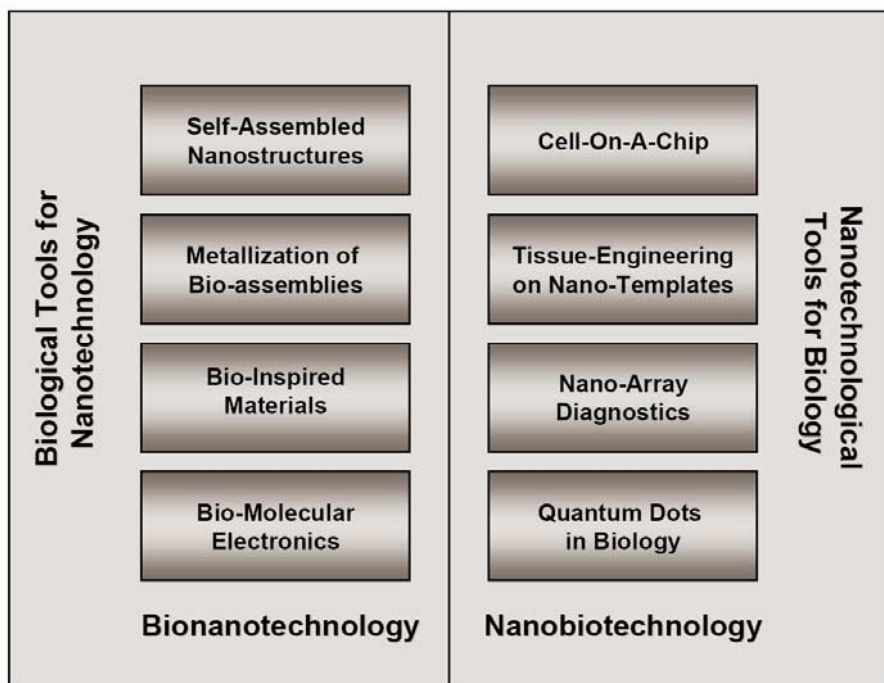


Figure 1.4: Bionanotechnology as the use of biological assemblies for various applications that may not be directionally associated with biology. Nanobiotechnology is the use of nano-science for specific biological applications.

1.8. The Combination of Bionanotechnology and Nanobiotechnology

In spite of the distinction between bionanotechnology and nanobiotechnology, it should be stressed for future applications, especially complex ones that both fields could be merged. For example, in the field of tissue engineering, self-assembled biomolecular structures could serve as a three-dimensional template for the formation of complex organs in the test-tube. In these applications, the biomolecules serve exactly as nano-carbon structures as mentioned above. Nevertheless, the biomolecular nano-scaffold may provide unique advantages such as

biocompatibility and the ability for decoration with biological signaling motifs. In the field of nano-bio-diagnosis peptide nanotubes could serve as nano-scale elements for the improvement of the sensitivity of the signals and the specificity. Here again the biomolecular nano-scale assemblies serve in principle just as the carbon or inorganic nanostructures. Yet, the biological nanotubes have many advantages as they provide a unique anchor for the conjugation of signal-enhancing entities like enzymes or antibodies.

1.9. Nanobionics and Bio-Inspired Nanotechnology

The final introduction to the field of nanobiology (we will use this term to describe both bionanotechnology and nanobiotechnology) should also include bionics and bio-inspired technology. Bionics, which stems from [bi(o)- + (electr)onics] could be described as the application of biological principles and mechanisms to the design and fabrication of engineering systems. Although the term “bionic” was coined only in the late 1950s by the US Air Force, these principles were already explored many years before. One of the earliest examples and most significant examples should be the studies of Leonardo da Vinci at the end of the 15th century (Figure 1.5). His famous suggestions for the principles of a helicopter were based on his studies and extensive observation of the flight of bird. Other bionic applications include the use of the nose of the dolphin as a model for a pear-shaped bow protuberance of ships or self-cleaning surfaces that are based on the surface properties of lotus leaves. It should be stressed that the aim of bionics is not merely to copy nature but rather to understand its principles and use them as a stimulus and motivation for innovations. Thus, bio-inspired technology may be more suitable name to describe this activity.

As most of the bionics is based on the formation of macroscopic objects and machines, it could also include nano-scale machines. It is expected that in years to come, the same mimicking as was preformed in the macroscopic world will also be applied to the microscopic and nanoscopic world. This includes simple applications such as self-cleaning surfaces at the nano-scale but could be explored for the formation of

much more complex structures such as molecular motors or even molecular machines.

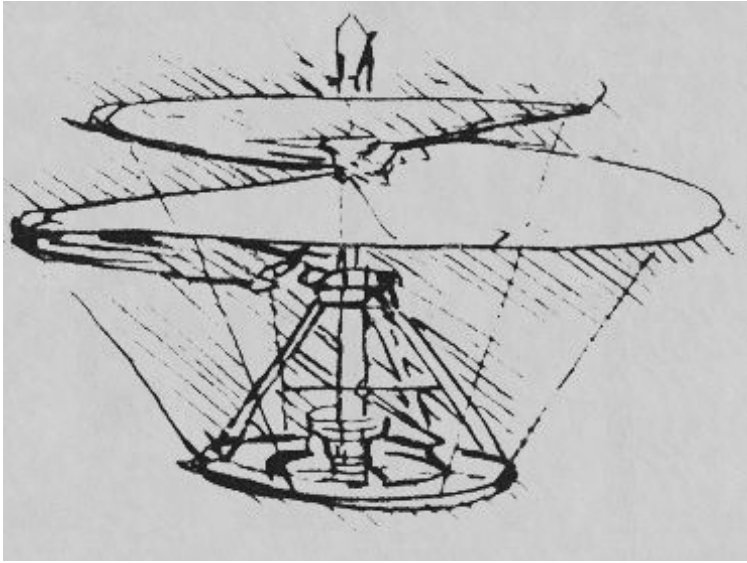


Figure 1.5: The drawing of a flying machine, based natural principles of the on flying mechanism of birds, made by Leonardo da Vinci at the end of the 15th century.