BIONANOTECHNOLOGY

Lessons from Nature

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PREFACE

Today is the most exciting time to be working in nanotechnology, and bionanotechnology in particular. Chemistry, biology, and physics have revealed an immense amount of information on molecular structure and function, and now we are poised to make use of it for atomic-level engineering. New discoveries are being made every day, and clever people are pressing these discoveries into service in every imaginable (and unimaginable) way.

In this book, I present many of the lessons that may be learned from biology and how they are being applied to nanotechnology. The book is divided into three basic parts. In the first part, I explore the properties of the nanomachines that are available in cells. In Chapter 2, I present the unfamiliar world of bionanomachines and go on a short tour of the natural nanomachinery that is available for our use. Chapter 3 provides an overview of the techniques that are available in biotechnology for harnessing and modifying these nanomachines.

In the second part, I look to these natural nanomachines for guidance in the building of our own nanomachinery. By surveying what is known about biological molecules, we can isolate the general principles of structure and function that are used to construct functional nanomachines. These include general structural principles, presented in Chapter 4, and functional principles, described in Chapter 5.

The book finishes with two chapters on applications. Chapter 6 surveys some of the exciting applications of bionanotechnology that are currently under study. The final chapter looks to the future, speculating about what we might expect.

Bionanotechnology is a rapidly evolving field, which encompasses a diverse collection of disciplines. This book necessarily omits entire sectors of research and interest and is unavoidably biased by my own interests and

my own background as a structural biologist. Biomolecular science still holds many deep mysteries and exciting avenues for study, which should provide even more source material for bionanotechnology in the coming decades. I invite you to explore the growing literature in this field, using this book as an invitation for further reading.

I thank Arthur J. Olson for many useful discussions during the writing of this book.

DAVID S. GOODSELL

THE QUEST FOR NANOTECHNOLOGY

1

The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big.

—Richard Feynman*

Nanotechnology is available, today, to anyone with a laboratory and imagination. You can create custom nanomachines with commercially available kits and reagents. You can design and build nanoscale assemblers that synthesize interesting molecules. You can construct tiny machines that seek out cancer cells and kill them. You can build molecule-size sensors for detecting light, acidity, or trace amounts of poisonous metals. Nanotechnology is a reality today, and nanotechnology is accessible with remarkably modest resources.

What is nanotechnology? Nanotechnology is the ability to build and shape matter one atom at a time. The idea of nanotechnology was first presented by physicist Richard Feynman. In a lecture entitled "Room at the Bottom," he unveiled the possibilities available in the molecular world. Because ordinary matter is built of so many atoms, he showed that there is a

*All opening quotes are taken from Richard P. Feynman's 1959 talk at the California Institute of Technology, as published in the February 1960 issue of CalTech's *Engineering and Science*.

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remarkable amount of space within which to build. Feynman's vision spawned the discipline of nanotechnology, and we are now amassing the tools to make his dream a reality.

But atoms are almost unbelievably small; a million times smaller than objects in our familiar world. Their properties are utterly foreign, so our natural intuition and knowledge of the meter-scale world is useless at best and misleading at worst. How can we approach the problem of engineering at the atomic scale?

When men and women first restructured matter to fit their needs, an approach opposite from nanotechnology was taken. Instead of building an object from the bottom up, atom-by-atom, early craftsmen invented a top-down approach. They used tools to shape and transform existing matter. Clay, plant fibers, and metals were shaped, pounded, and carved into vessels, clothing, and weapons. With some added sophistication, this approach still accounts for the bulk of all products created by mankind. We still take raw materials from the earth and physically shape them into functional products.

Mankind did not make any concerted effort to shape the atoms in manufactured products until medieval times, when alchemists sowed the seeds of the modern science of chemistry. During their search for the secrets of immortality and the transmutation of lead to gold, they developed methods for the willful combination of atoms. Chemical reaction, purification, and characterization are all tools of the alchemists. Today, chemists build molecules of defined shape and specified properties. Chemical reactions are understood, and tailored, at the atomic level. Most of chemistry, however, is performed at a bulk level. Large quantities of pure materials are mixed and reacted, and the desired product is purified from the mixture of molecules that are formed. Nonetheless, chemistry is nanotechnology—the willful combination of atoms to form a desired molecule. But it is nanotechnology on a bulk scale, controlled by statistical mechanics rather than controlled atom-by-atom at the nanometer scale.

We are now in the midst of the second major revolution of nanotechnology. Now, scientists are attempting modify matter one atom at a time.

Some envision a nanotechnology closely modeled after our own macroscopic technology. This new field has been dubbed *molecular nanotechnology*

for its focus on creating molecules individually atom-by-atom. K. Eric Drexler has proposed methods of constructing molecules by forcibly pressing atoms together into the desired molecular shapes, in a process dubbed "mechanosynthesis" for its parallels with macroscopic machinery and engineering. With simple raw materials, he envisions building objects in an assembly-line manner by directly bonding individual atoms. The idea is compelling. The engineer retains direct control over the synthesis, through a physical connection between the atomic realm and our macroscopic world.

Central to the idea of mechanosynthesis is the construction of an *assembler*. This is a nanometer-scale machine that assembles objects atom-by-atom according to defined instructions. Nanotechnology aficionados have speculated that the creation of just a single working assembler would lead immediately to the "Two-Week Revolution." They tell us that as soon as a single assembler is built, all of the dreams of nanotechnology would be realized within days. Researchers could immediately direct this first assembler to build additional new assemblers. These assemblers would immediately allow construction of large-scale factories, filled with level upon level of assemblers for building macroscale objects. Nanotechnology would explode to fill every need and utterly change our way of life. Unfortunately, assemblers based on mechanosynthesis currently remain only an evocative idea.

The subject of this book is another approach to nanotechnology, which is available today to anyone with a moderately equipped laboratory. This is *bionanotechnology*, nanotechnology that looks to nature for its start. Modern cells build thousands of working nanomachines, which may be harnessed and modified to perform our own custom nanotechnological tasks. Modern cells provide us with an elaborate, efficient set of molecular machines that restructure matter atom-by-atom, exactly to our specifications. And with the well-tested techniques of biotechnology, we can extend the function of these machines for our own goals, modifying existing biomolecular nanomachines or designing entirely new ones.

BIOTECHNOLOGY AND THE TWO-WEEK REVOLUTION

The Two-Week Revolution has already occurred, although it has lasted for decades instead of weeks. Biotechnology uses the ready-made assemblers

available in living cells to build thousands of custom-designed molecules to atomic specifications, including the construction of new assemblers. This has lead to myriad applications, including commercial production of hormones and drugs, elegant methods for diagnosing and curing infectious and genetic diseases, and engineering of organisms for specialized tasks such as bioremediation and disease resistance.

Biotechnology took several decades to gather momentum. The primary impediment has been the lack of basic knowledge of biomolecular processes and mechanisms. We have been given an incredible toolbox of molecular machinery, and we are only now beginning to learn how to use it. The key enabling technology, recombinant DNA, made the natural protein assembler of the cell available for use. The subsequent years have yielded numerous refinements on the technology, and numerous ideas on how it might be exploited.

Biotechnology has grown, and is still growing, with each new discovery in molecular biology. Further research into viral biology has led to improved vectors for delivering new genetic material. An explosion of enzymes for clipping, editing, ligating, and copying DNA, as well as efficient techniques for the chemical synthesis of DNA, has allowed the creation of complicated new genetic constructs. Engineered bacteria now create large quantities of natural proteins for medicinal use, mutated proteins for research, hybrid chimeric proteins for specialized applications, and entirely new proteins, if a researcher is bold enough to design a protein from scratch.

FROM BIOTECHNOLOGY TO BIONANOTECHNOLOGY

We are now poised to extend biotechnology into bionanotechnology. What is bionanotechnology, and how is it different from biotechnology? The two terms currently share an overlapped field of topics. I will define bionanotechnology here as applications that require human design and construction at the nanoscale level and will label projects as biotechnology when nanoscale understanding and design are not necessary. Biotechnology grew from the use of natural enzymes to manipulate the genetic code, which was then used to modify entire organisms. The atomic details were not really

important—existing functionalities were combined to achieve the end goal. Today, we have the ability to work at a much finer level with a more detailed level of understanding and control. We have the tools to create biological machines atom-by-atom according to our own plans. Now, we must flex our imagination and venture into the unknown.

Bionanotechnology has many different faces, but all share a central concept: the ability to design molecular machinery to atomic specifications. Today, individual bionanomachines are being designed and created to perform specific nanoscale tasks, such as the targeting of a cancer cell or the solution of a simple computational task. Many are toy problems, designed to test our understanding and control of these tiny machines. As bionanotechnology matures, we will redesign the biomolecular machinery of the cell to perform large-scale tasks for human health and technology. Macroscopic structures will be built to atomic precision with existing biomolecular assemblers or by using biological models for assembly. Looking to cells, we can find atomically precise molecule-sized motors, girders, random-access memory, sensors, and a host of other useful mechanisms, all ready to be harnessed by bionanotechnology. And the technology for designing and constructing these machines in bulk scale is well worked out and ready for application today.

Nanomedicine will be the biggest winner. Bionanomachines work best in the environment of a living cell and so are tailored for medical applications. Complex molecules that seek out diseased or cancerous cells are already a reality. Sensors for diagnosing diseased states are under development. Replacement therapy, with custom-constructed molecules, is used today to treat diabetes and growth hormone deficiencies, with many other applications on the horizon.

Biomaterials are another major application of bionanotechnology. We already use biomaterials extensively. Look around the room and notice how much wood is used to build your shelter and furnishing and how much cotton, wool, and other natural fibers are used in your clothing and books. Biomaterials address our growing ecological sensitivity—biomaterials are strong but biodegradable. Biomaterials also integrate perfectly with living tissue, so they are ideal for medical applications.

The production of hybrid machines, part biological and part inorganic,

is another active area of research in bionanotechnology that promises to yield great fruits. Bionanomachines, such as light sensors or antibodies, are readily combined with silicon devices created by microlithography. These hybrids provide a link between the nanoscale world of bionanomachines and the macroscale world of computers, allowing direct sensing and control of nanoscale events.

Finally, Drexler and others have seen biological molecules as an avenue to reach their own goal of mechanosynthesis using nanorobots. Certainly, biology provides the tools for building objects one atom at a time. Perhaps as our understanding grows, bionanomachines will be coaxed into building objects that are completely foreign to the biological blueprint.

This book explores these bionanomachines: their properties, their design principles, and the way they have been harnessed for our own applications. An exponentially growing body of information is being amassed, revealing the structure and function of individual biomolecules and their interactions within living cells. This information is a key resource for understanding the basic principles of nanomachinery: its structure, its function, and its integration into any larger application of nanotechnology. These existing, working nanomachines provide important lessons for the construction of our own nanotechnology, whether based directly on biology or constructed completely from our own imagination.

WHAT IS BIONANOTECHNOLOGY?

Nanotechnology and bionanotechnology are entirely new concepts, invented late in the twentieth century, and biotechnology has only been around for a few decades, so the scope of these fields is still being defined. With so many clever researchers working on all aspects of nanoscale structure, construction, and function, new examples that cross existing conceptual boundaries are appearing daily. Before getting started, it is worth spending a moment to compare the many technologies working at small scales and try to define the current scope of bionanotechnology.

Chemistry was the first science to manipulate molecules, starting when the first human beings cooked their food. Today, chemists design molecules and perform extensive, controlled syntheses to create them. Chemistry dif-

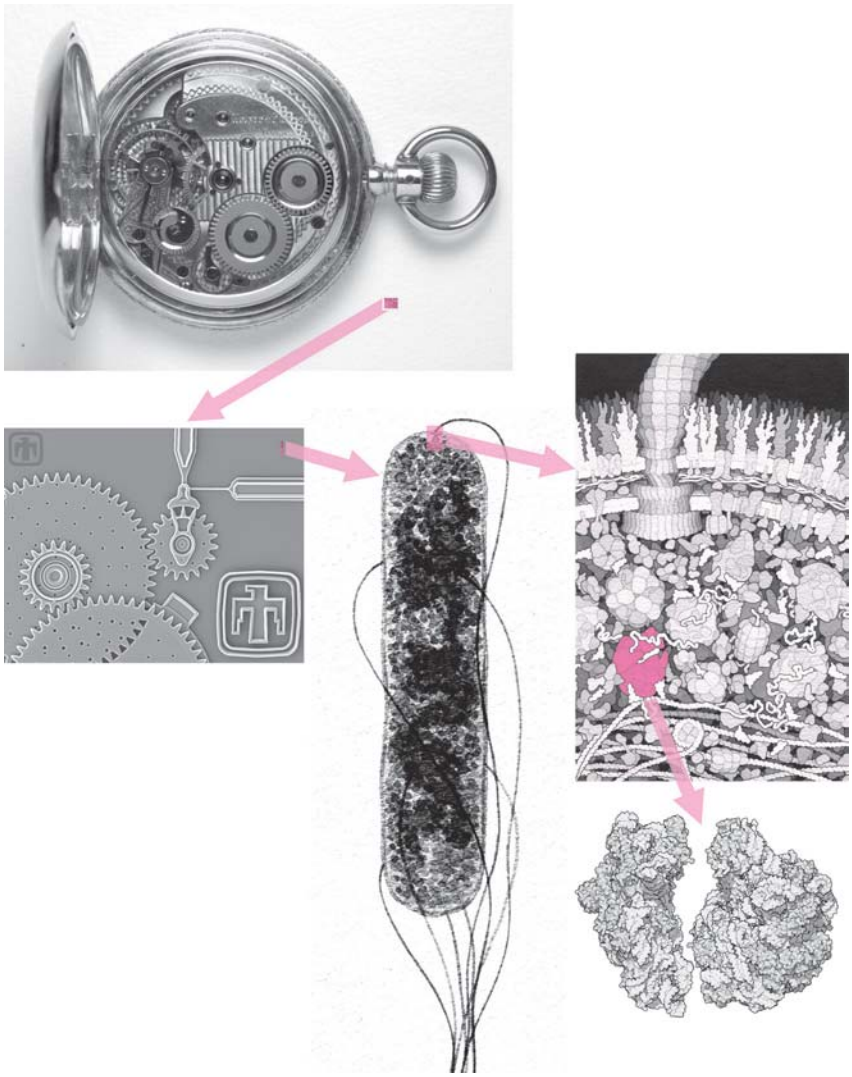

Figure 1-1 How big is bionanotechnology? Since the Industrial Revolution, scientists and engineers have constructed machines at an ever-smaller scale. Machines in our familiar world have moving parts in the range of millimeters to meters. As machining capabilities improved, tiny machines, such as the movement of a fine watch, extended the precision of machining to a fraction of a millimeter. Computer technology, with its ever-present pressure to miniaturize in order to improve performance, has driven the construction of tiny structures to even smaller ranges, with micrometer-scale construction of electronic components and tiny machines, like these tiny gears, created at the Sandia National Laboratories. Bionanotechnology operates at the smallest level, with machines in the range of 10 nm in dimension. The bacterium shown builds thousands of different bionanomachines, including a working nanoscale assembler, the ribosome, shown at lower right. Because these bionanomachines are composed of a finite, defined number of atoms, they represent a limit to the possible miniaturization of machinery. [MEMS gear photomicrograph from http://mems.sandia.gov/scripts/images.asp].

fers from bionanotechnology because it does not work at the level of individual molecules. There is no localization at the atomic level and no ability to address individual molecules. As a consequence of the bulk nature of chemistry, the molecules produced are generally limited to under a hundred atoms or so—syntheses of larger molecules are plagued by too many side reactions that form competing impurities.

Photolithography is widely used for the creation of computer hardware, and the growing field of MEMS is applying these technologies to the creation of microscale machines. Our entire information and communication technology relies on these methods. It relies on photographic techniques for reduction of scale and random deposition of atoms within the mask. Thus it is a macroscale technique scaled down to its finest limits.

Biotechnology harnesses biological processes and uses them for our own applications. In this book, I will limit the scope of biotechnology to applications that do not require atomic specification of biomolecules. For instance, researchers routinely use purified enzymes to cut and paste genetic instructions and add these back into cells. Knowledge of the atomic details are unimportant, just as knowledge of the type of ink used to print this page is not important for understanding of the words printed here.

Nanotechnology has been defined as engineering and manufacturing at nanometer scales, with atomic precision. The theoretical constructions popularized by K. Eric Drexler and the Foresight Institute are perhaps the most visible examples, and these are often further classified as "molecular nanotechnology." The positioning of individual argon atoms on a crystal surface by researchers at IBM is a successful example of nanotechnology.

Bionanotechnology is a subset of nanotechnology: atom-level engineering and manufacturing using biological precedents for guidance. It is also closely married to biotechnology but adds the ability to design and modify the atomic-level details of the objects created. Bionanomachines are designed to atomic specifications, they perform a well-defined three-dimensional molecular task, and, in the best applications, they contain mechanisms for individual control embedded in their structure.

BIONANOMACHINES IN ACTION

2

I am inspired by the biological phenomena in which chemical forces are used in repetitious fashion to produce all kinds of weird effects (one of which is the author).

—Richard Feynman

As you read these words, 10,000 different nanomachines are at work inside your body. These are true nanomachines. Each one is a machine built to nanoscale specifications, with each atom precisely placed and connected to its neighbors. Your body is arguably the most complex mechanism in the known universe, and most of the action occurs at the nanoscale level. These nanomachines work in concert to orchestrate the many processes of life—eating and breathing, growing and repairing, sensing danger and responding to it, and reproducing.

Remarkably, many of these nanomachines will still perform their atom-sized functions after they are isolated and purified, provided that the environment is not too harsh. They do not have to be sequestered safely inside cells. Each one is a self-sufficient molecular machine. Already, these nanomachines have been pressed into service. Natural digestive enzymes like pepsin and lysozyme are so tough that they can be added to laundry detergent to help digest away stains. Amylases are used on an industrial scale to convert powdery starch into sweet corn syrup. The entire field of genetic engineering and biotechnology is made possible by a collection of DNA-

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manipulating nanomachines, now available commercially. In general, natural bionanomachines are remarkably robust.

This chapter explores the bionanomachines made by living cells. They are different from the machines in our familiar world in many ways. They have been developed by the process of evolution (instead of intelligent design), which places unfamiliar restrictions on the process of design and the form of the final machine. Bionanomachines are also selected to perform their tasks in a very specific environment and are subject to the unfamiliar forces imposed by this environment. We must keep these differences in mind when trying to understand natural biomolecules, and we must keep these differences in mind when we use these natural bionanomachines as the starting point for our own bionanotechnology.

THE UNFAMILIAR WORLD OF BIONANOMACHINES

Biological machinery is different from anything we build with our familiar, human-sized technology. Natural biomolecules have organic, visceral, and often unbelievable shapes, unlike the tidy designs of toasters and tractors. They perform their jobs in a foreign environment, where jittery thermal motion is constantly pushing and pulling on their component parts. They are held together by a complex collection of bonding and nonbonding forces. At their small scale, bionanomachines are almost immune to the laws of gravity and inertia that dominate our machines. The world of bionanotechnology is an unfamiliar, shifting world that plays by different rules.

Gravity and Inertia are Negligible at the Nanoscale

Macroscopic objects, like bicycles and bridges, are dominated by the properties of mass. For centimeter-sized and meter-sized objects, physical properties such as friction, tensile strength, adhesion, and shear strength are comparable in magnitude to the forces imposed by inertia and gravity. So we can design picture hooks that are strong enough to hold up pictures and tires that will not fly apart when rotated at rapid speed. This balance changes, however, when we move to larger or smaller objects. As we move to larger objects, scaling laws shift the balance. Mass increases with the cube

of the size of an object, and properties such as strength and friction increase linearly or with the square of size. The increase in inertia or weight can quickly overcome the increase in strength in a large structure such as a building. These scaling laws are quite familiar, and it is common sense to add extra support as we build larger and larger structures. We do not expect to be able to build a skyscraper a mile tall.

These scaling laws also apply in the opposite direction, with the opposite effect as we go to smaller and smaller machines. Micrometer-sized objects, like individual grains of sand or individual cells, already interact differently from macroscopic objects. Inertia is no longer a relevant property, so our intuition may lead to inappropriate conclusions. For example, E. M. Purcell described the surprising properties of bacterial cells swimming in water. These cells use a long corkscrew-shaped flagellum to propel themselves through the water. When the cell stops turning the flagellum, we might expect that the cell would slowly coast to a stop, like a submarine does in the ocean. However, because of the inertia scales differently relative to the viscous forces within the surrounding water, the cell actually stops in less than the diameter of an atom.

Gravity is also a negligible force when dealing with small objects. The actions of small objects are dominated by their interaction with neighboring objects. The molecules in water and air are in constant motion, continually battering small objects from all sides. So, fine dust stays suspended in the air instead of dropping quickly to the floor, and objects in water, if you look at them with a microscope, undergo random Brownian motion. The attractive forces between small objects are also stronger than the force of gravity. Flies take advantage of these attractive forces and can crawl up walls. Similarly, water droplets can hang from the ceiling because of these attractive forces.

Nanomachines Show Atomic Granularity

Nanoscale objects are built of discrete combinations of atoms that interact through specific atom-atom interactions. We cannot design nanomachines in a smoothly graded range of sizes. They must be composed of an integral number of atoms. For instance, we cannot design a nanoscale rotary motor

like a macroscale motor, with a smooth ring surrounding an axle undergoing a smooth rotary motion. Instead, existing nanoscale rotary motors, such as ATP synthase or the bacterial flagellar motor, adopt several discrete rotary states that cycle one after the other (described in Chapter 5). There is not a smooth transition from one state to the next. Instead, the motor jumps from state to state when the appropriate chemical energy is applied. (Note that although smooth atom-scale motion is not observed in natural systems, theoretical nanoscale versions of axles and bearings have been proposed in molecular nanotechnology that take advantage of a mismatch in the number of atoms to smooth out atomic granularity.)

Because of atomic granularity, the typical continuous representations used in engineering are not appropriate. Bulk properties such as viscosity and friction are not defined for discrete atomic ensembles. Instead, individual atomic properties must be used. Quantum mechanics provides a deep understanding of the properties of atoms within biomolecules, but, fortunately, most of the basic properties may be understood qualitatively, through the use of a set of simplified rules. The central concept is the existence of covalent bonds, which connect atoms into stable molecules of defined geometry. Addition of a few rules to describe the interaction of atoms that are not bonded together—steric repulsion of nonbonded atoms, electrostatic interactions, and hydrogen bonds—allows understanding of most aspects of biomolecular structure and interaction. In general, biomolecules may be thought of as articulated chains of atoms that interact in a few well-defined ways. These qualitative rules are described in more detail in Chapter 4.

Thermal Motion is a Significant Force at the Nanoscale

Molecular nanotechnology seeks to create a "machine-phase" environment, with individual nanomachines organized like clockwork to form microscale and macroscale objects. Natural bionanomachinery takes a different approach, creating atomically precise nanomachinery but then enclosing them in a cellular space. The individual parts then interact through random motion and diffusion. In specialized applications machine-phase bionanostructures are used (two examples are presented in Chapter 5), but the bulk of

the work done in cells is performed in the context of random, diffusive motion.

Bionanomachines operate in a chaotic environment. They are bombarded continually by water molecules. They will scatter randomly if not firmly held in place. Bionanomachines operate by forming interactions with other bionanomachines, fitting together and breaking apart in the course of action. If two molecules fit closely together and have the appropriate matching of chemical groups, they will interact over long periods of time. If the interactions are weaker, they will form only a temporary interaction before moving on to the next. By careful design of the strength of these interactions, bionanomachines can form stable molecular girders that last for years or delicate biosensors that fleetingly sense trace amounts of a molecule.

Cells are complex, with millions of individual proteins, and you might wonder whether diffusive motion is sufficient to allow interaction between the proper partners amidst all the competition. At the scale of the cell, diffusive motion is remarkably fast, so once again our intuition may play us false. If you release a typical protein inside a bacterial cell, within one-hundredth of a second, it is equally likely to be found anywhere in the cell. Place two molecules on opposite sides of the cell, and they are likely to interact within one second. As articulated by Hess and Mikhailov: "This result is remarkable: It tells us that *any* two molecules within a micrometer-size cell meet each other every second."

Bionanomachines Require a Water Environment

The form and function of biomolecules is dominated by two things: the chemistry of their component atoms and the unusual properties of the water surrounding them. The energetics of this interaction are quite different from anything we experience in our macroscopic world.

Water is an unusual substance, with specific preferences. Water molecules interact strongly with one another through hydrogen bonds. They do not lightly separate and interact with other molecules, unless these other molecules have something to offer. In biomolecules, regions that carry electronic charges and regions that are rich in nitrogen and oxygen atoms interact favorably with water. These regions easily dissolve into water solution.

Regions that are rich in carbon, however, cannot form the requisite hydrogen bonds and tend to be forced together in oily drops, minimizing contact with the surrounding water. This process has been termed the "hydrophobic effect," with the term hydrophobic referring to the "water-fearing" carbon atoms that avoid contact with water. Perhaps a better image is to think of water as an exclusive social clique that has no interest in carbon-rich conversation. The hydrophobic effect is described in more detail in Chapter 4.

The hydrophobic effect strongly shapes the form and function of a biological molecule. The geometry of the molecular chain alone allows a large range of conformations to be formed. If this were the whole story, life would not be possible—chains would only rarely form a single, defined structure. But when placed in water, biomolecules respond to the environment by folding into a conformation with the hydrophobic regions tucked away inside and the surface decorated with more water-loving groups. For proteins, the chain is most often forced into a compact globule. For DNA, the base pairs are sequestered safely inside, leaving the strongly charged phosphates on the surface. For lipids, many individual molecules are forced together to form membranes, with their hydrophobic atoms sandwiched between sheets of water-loving charged atoms. If designed carefully (as are all natural biological molecules), only a single structure is formed, creating a nanoscale machine with exactly the proper conformation to perform its duty (Figure 2-1).

MODERN BIOMATERIALS

Four basic molecular plans were developed through evolution over 3 billion years ago and are still used by all living things today. Modern cells use proteins, nucleic acids (such as DNA), polysaccharides, and lipids for nearly all tasks. A handful of other small molecules are specially synthesized for particular needs, but the everyday work of the cell is performed by these four basic plans. Of course, in bionanotechnology we are not forced to stay within these existing plans, but there are many advantages to exploring them first. Most notably, we can use the thousands of working natural bionanomachines as a starting point to build our own practical nanotechnology.

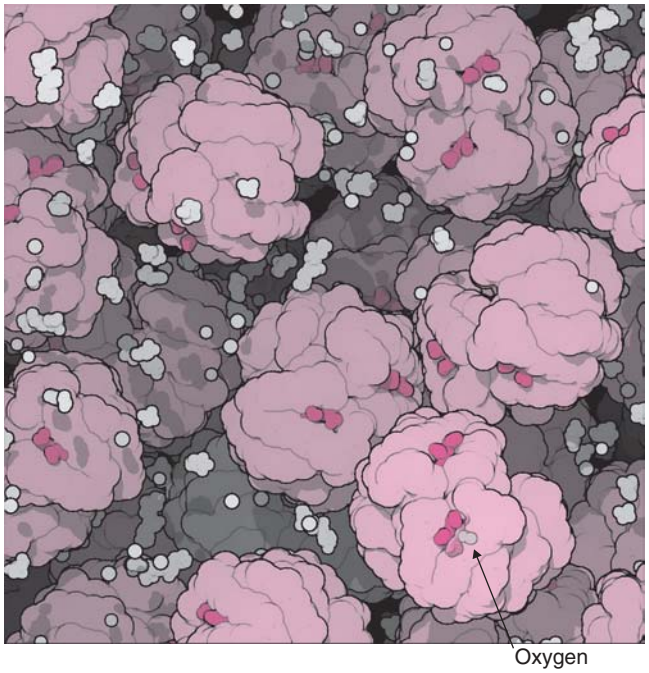

Figure 2-1 Oxygen is stored differently at the meter scale and at the nanoscale. At the meter scale, we store oxygen in high-pressure tanks. The oxygen is delivered into and out of these tanks in a continuous stream through tubes. The flow is controlled by smoothly machined valves. In contrast, at the nanoscale we transport oxygen molecule by molecule instead of in bulk. In red blood cells, the protein hemoglobin stores large amounts of oxygen at body temperature and without the need for high pressure. Individual oxygen molecules encounter hemoglobin by random diffusion, binding tightly when they meet. A complex shift in the orientation of the four subunits, mediated by the precise mating of atoms along the interface between subunits, allows hemoglobin to increase the gain on the interaction. This allows hemoglobin to gather oxygen efficiently when levels rise and to discharge all of the oxygen when levels drop.

Most Natural Bionanomachines Are Composed of Protein

Protein is the most versatile of the natural biomolecular plans. Protein is used to build nanomachines, nanostructures, and nanosensors with diverse properties. Proteins are modular, constructed of a linear chain of amino acids that folds into a defined structure, as shown in Figure 2-2. The longest protein chain (thus far) is titin with over 26,000 amino acids, and peptides with less than a dozen amino acids are used as hormones for cell signaling.

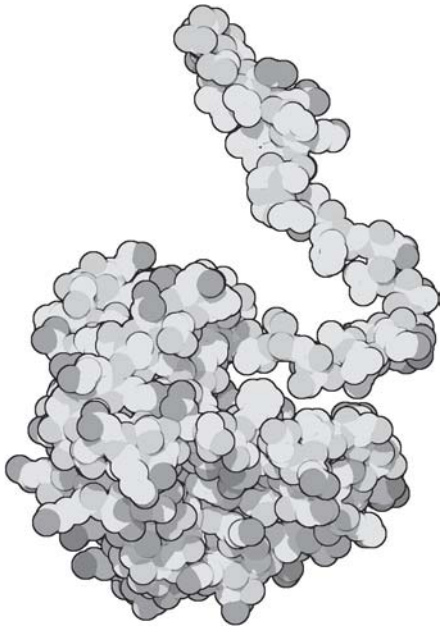

Figure 2-2 Proteins are constructed as chains of amino acids, which then fold into compact globular structures.

Typical soluble proteins have chains in the range of about 200 to 500 amino acids.

Amino acids are composed of a central α-carbon atom with three attachments: an amino group, a carboxylic acid group, and a side chain. Each successive amino acid is connected through an amide linkage between the amine of one amino acid and the carboxyl of the next amino acid in the chain. The amide linkage is rigid, strongly preferring a planar conformation of the four amide atoms and the flanking carbon atoms. The rigidity of the amide group is essential for the construction of nanomachinery with defined conformations. The rigid amide limits the number of conformations available to the chain. A more flexible chain, like the strings of aliphatic carbon atoms used in many plastics, is able to adopt many compact conformations of similar stability instead of forming a single folded structure with the desired conformation.

The combination of the rigid planar group and the exposed hydrogen

and oxygen atoms gives rise to a limited range of stable conformations of the chain. Two conformations, shown in Figure 2-3, are particularly stable. They combine minimal strain and overlap in the molecular structure with a maximal number of hydrogen bonds between the exposed amide atoms. The first is the α-helix. The chain winds like a spring so that each amide oxygen interacts with the hydrogen atom three linkages down the chain. The second is the β-sheet, composed of several adjacent strands. Each strand is fully extended, and several strands bind side by side, forming a ladder of hydrogen bonds in between.

The chemical diversity of the different side chains provides the real ad-

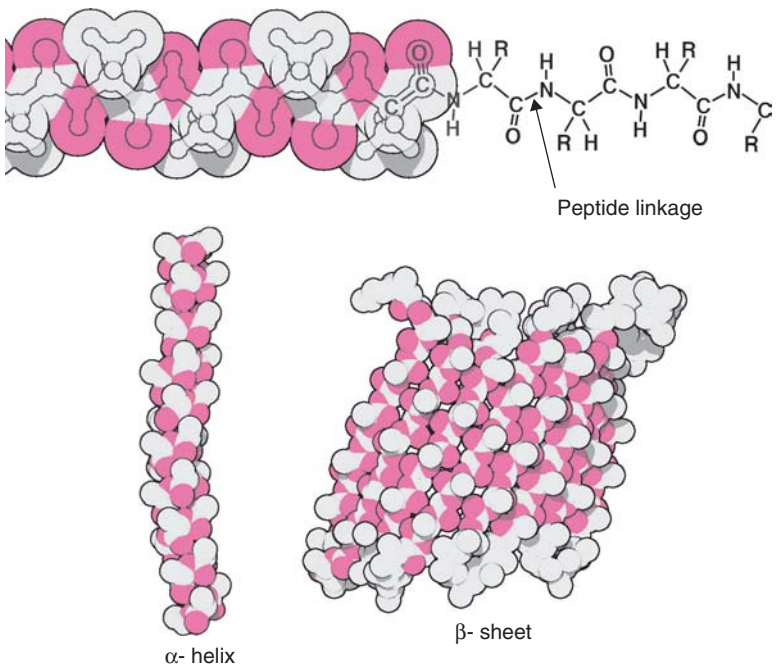

Figure 2-3 The peptide linkage connecting amino acids contains a hydrogen bond donor, the H–N group, and a hydrogen bond acceptor, the O=C group. The remaining carbon in the protein chain carries a hydrogen and one of 20 different side chains, shown with an R here. Two conformations of protein chains, the α-helix and the β-sheet, are particularly stable, because the chain is in a relatively unstrained position and all of the possible hydrogen bonds between the amide groups are formed. This β-sheet, taken from the bacterial protein porin, has alternate strands running in opposite directions.

vantage of proteins as a structural material, allowing them to be used for many different functions. The 20 side chains (shown in Figure 2-4) used in natural proteins are chemically and structurally diverse. By arranging them in the proper order, the structure of the protein may be shaped and stabilized. Then particularly reactive side chains may be placed at key locations to perform the desired function.

A variety of modified amino acids are also used for specialized tasks. Some, like selenocysteine, are added directly to protein chains as they are synthesized, using alternate translations of the normal genetic code. Most, however, are created by modifying the natural 20 amino acids after they are incorporated into proteins. For instance, a hydroxyl group may be added to proline, which allows additional levels of hydrogen bonding that are important in the structure of collagen. In blood clotting proteins, an additional carboxylic acid group is added to glutamate amino acids, allowing them to bind more tightly to calcium ions.

The error rate of biological protein synthesis limits the size of individual chains that may be constructed consistently and accurately. In bacterial

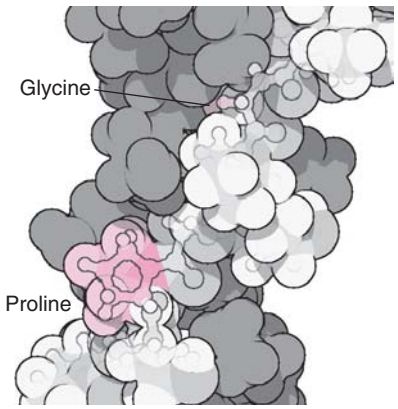

Figure 2-4A Glycine and proline play special structural roles. Glycine is the smallest amino acid, with no side chain. Because it lacks a side chain, the backbone is not as constrained, making the protein chain more flexible at sites that incorporate glycine. It is used in regions that require tight conformational turns that are not possible for other amino acids and in crowded regions with strong steric blocking constraints, such as in the tight collagen triple helix shown here. Proline is the only cyclic amino acid, with two covalent bonds to the protein backbone. It forms a rigid kink in the protein chain. In collagen, this kink allows the chain to adopt a tight triple helix.

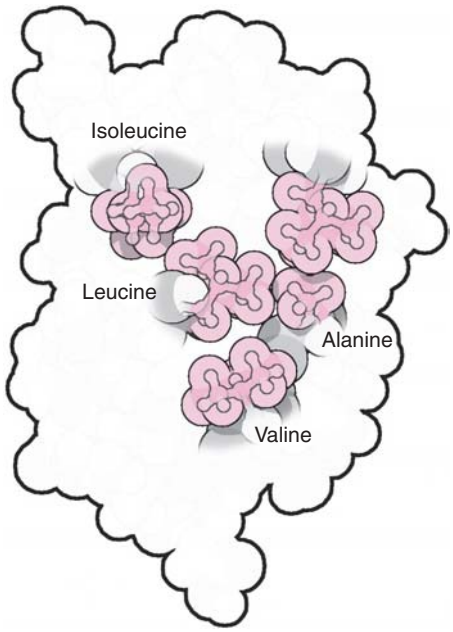

Figure 2-4B Alanine, valine, leucine, and isoleucine are carbon-rich amino acids with a range of sizes and shapes. They are relatively inflexible and strongly favor environments sheltered from water. Often, these hydrophobic residues drive folding of protein chains. The collection shown here are on the inside of insulin, forming a closely packed cluster inside the protein. Note that a variety of other short-chain carbon-rich chains are possible in this size range, such as a two-carbon chain and straight chains of three or four amino acids. However, only the four variations included here are genetically encoded in natural organisms.

cells, the genetic sequence is misread in about 1 in 2000 amino acids, substituting an improper amino acid at that location in the chain. However, these occasional errors are often tolerated and the misplaced amino acid has little effect on the function of the protein. However, processivity errors, in which synthesis of the protein terminates early and produces a truncated chain, are more serious. The frequency of processivity errors has been estimated at about 1 in 3000 amino acids. In response to these intrinsic limits, average protein chains fall in the range of 200–500 amino acids, although spectacular exceptions, such as the muscle protein titin, are synthesized for specialized tasks.

We can find examples of proteins everywhere we look. Most proteins are soluble structures, performing their jobs in solution. Egg white exempli-

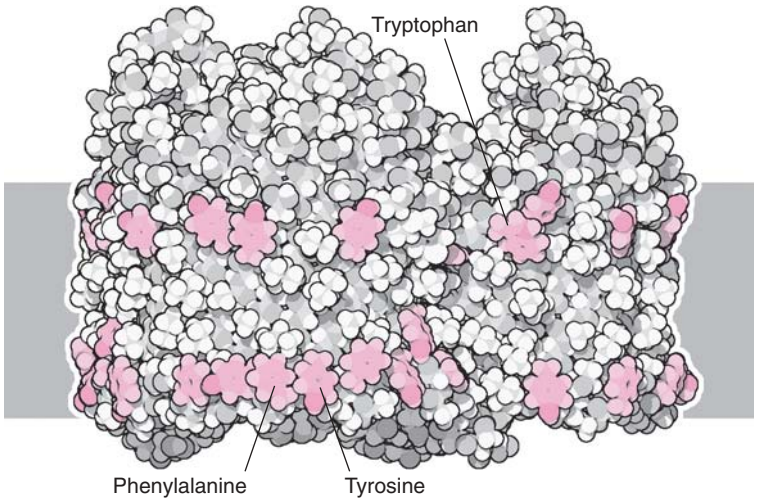

Figure 2-4C Phenylalanine, tyrosine, and tryptophan have large aromatic side chains. They favor environments sheltered from water, and, along with the carbon-rich amino acids shown in Figure 2-4B, they drive the folding of protein chains. These rings often stack on top of one another or on top of DNA bases and are used to provide specificity for aromatic rings binding in active sites. Tyrosine is a special case, with an aromatic phenyl ring and a hydroxyl group at the end. This provides a perfect mix of properties for interacting with small organic molecules, so tyrosine is often used in protein binding sites both to stabilize the carbon-rich portions of a ligand and to hydrogen bond with the ligand. Porin, a bacterial protein that spans a lipid membrane, is shown here. The membrane is shown schematically as the dark stripe. Note how these aromatic amino acids are arranged around the perimeter of the molecule, forming a belt that interacts with the carbon-rich membrane.

fies the macroscopic properties of a concentrated solution of soluble proteins: a viscous solution that denatures, turning opaque, when heated. Freeze-drying yields a deliquescent powder, which for many proteins may be dissolved in water to yield an active protein. Large protein biomaterials are also built. The rubbery material in tendons is largely composed of the protein collagen, and the tough but flexible material of hair and fingernails is largely composed of the protein keratin. These proteins are extensively cross-linked for additional strength.

Bionanotechnology is exploiting the potential of proteins in every way imaginable. Powerful methods for creating custom proteins are available, as described in Chapter 3. The major current limitation is basic knowledge. We

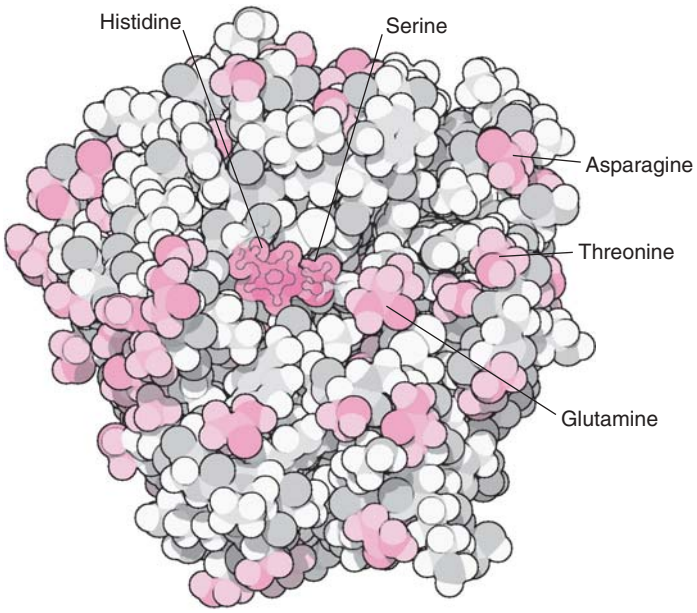

Figure 2-4D Serine, threonine, histidine, asparagine, and glutamine are amino acids with diverse hydrogen-bonding groups. They are very common on protein surfaces, where they interact favorably with the surrounding water. They are often used to glue protein structures together and to form specific interactions with other molecules. Histidine is a special case. It contains an imidazole group, which may adopt neutral and charged forms under slightly different conditions. In the neutral form, it combines a protonated secondary amine, which is electrophilic and may donate a hydrogen bond, with a tertiary amine, which is strongly nucleophilic and can accept a hydrogen bond. Histidine is used infrequently in proteins, being incorporated mainly for specialized catalytic tasks. For instance, it is being used here in the protein-cutting enzyme trypsin to activate a serine amino acid. Normally the hydroxyl group on serine is unreactive, but when activated in the proper environment it is an effective catalysts for reactions that require addition or abstraction of hydrogen atoms. Histidine also coordinates strongly with metal ions and is used to construct specific metal-binding sites.

need to understand and be able to predict the processes by which proteins fold into their stable, globular structure.

Nucleic Acids Carry Information

Nucleic acids are modular, linear chains of nucleotides, ranging up to hundreds of millions of nucleotides in length. Two forms are commonly used: