
Complex Biological Systems

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Abstract

Biological entities range in scale and complexity from the simplest viruses to multicellular organisms with specialized cells, tissues, and organs. Within a cell of any organism, there are an enormous number of biomolecular complexes that perform physical and chemical tasks with remarkable speed and fidelity. The goals in many nanoscale engineering applications mirror the major challenges that Nature has overcome in the engineering of biomolecular systems. As such, there exist some basic paradigms of biological design that can either be mimicked or exploited for nanoengineering applications. In this chapter, several paradigms for biological control at the nanoscale are identified, and several

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key examples are leveraged to identify how these ideas can be used in a range of engineered systems.

Introduction

The natural world is teeming with examples of nanoscale engineering. Our ability to assemble, manipulate, and exploit nanoscale structures for functional purposes cannot compete with the capabilities that even the simplest life forms have established. Granted, these engineered systems required eons to design and perfect, and our patience for results is far less accommodating than is necessary for evolutionary design to serve as a paradigm for most nanoengineering applications. However, it is useful to use Nature as guidance, both as inspiration and as a source of existing tools for engineering applications.

The purpose of this chapter is to provide several key examples of biological systems that can serve as inspiration or be co-opted for nanoscale engineering. The goal is to provide examples of biological processes that are particularly amenable for this purpose. This chapter is not intended to be an exhaustive description of the potential avenues where biology can interface with nanotechnology. In fact, such an exercise would prove impossible given the remarkable potential for tapping into biological systems for their exquisite molecular control.

This chapter is particularly timely. The current state of biological sciences emboldens us to think broadly and to act quickly to utilize biological systems for various applications. Recombinant protein expression enables us to synthesize proteins with exact control over the specific amino acid sequence (Baneyx 1999). Notably, the 20 amino acids provided by Nature have been expanded to include nonnatural amino acids that introduce additional chemical functionalities that are advantageous for various applications (Langer and Tirrell 2004; Liu et al. 2007). The identification of protein function in various biological processes has been dramatically accelerated by the combination of chromatin immunoprecipitation and high-throughput DNA sequencing (i.e., ChIP-seq technology) (Park 2009). Our ability to quickly edit genes in organisms throughout the tree of life is now possible leveraging clustered regularly interspaced short palindromic repeats (or CRISPRs), resulting in facile manipulation of protein expression and introduction of non-endogenous proteins (Qi et al. 2013). These biotechnological advancements (and many others) allow us to design, synthesize, and analyze biopolymers at an unprecedented level of speed and accuracy.

Our ability to physically characterize biological processes and systems at a molecular level has also experienced a revolution in recent years. Single-molecule manipulation (AFM, optical and magnetic tweezers) has enabled the manipulation of individual biopolymers, facilitating the direct measurement of the physical forces involved in various biological processes (Bustamante et al. 2003; Greenleaf et al. 2007). Super-resolution microscopy has enabled the determination of the spatial organization of biological structures and materials within living cells at an unprecedented level of precision (Pavani et al. 2009; Shtengel et al. 2009). The

development of large-scale molecular dynamics (MD) simulations of proteins and nucleic acids has emerged as a powerful predictive tool for biological systems, culminating in the 2013 Nobel Prize in Chemistry (awarded to Karplus, Levitt, and Warshel). The development of these approaches (and many others) represents a paradigm shift in our physical understanding of biological systems.

The convergence of fundamental and applied approaches to understanding biomolecular systems leads us to a point where we can make considerable steps in leveraging a range of such systems for nanoscale engineering. This chapter contains several examples that illustrate the remarkable opportunities for this approach. The focus is on several key paradigms in Nature that can be exploited or mimicked in a variety of settings. In some cases, such systems have had a limited historical impact in engineering applications, despite the amazing potential that they have to establish an unprecedented level of molecular control of complex structures and assemblies. This illustrates the untapped potential that this course of action may have in future applications.

Autonomous Self-Assembly and Self-Maintenance

Complex multicellular organisms have relatively modest beginnings. At the point of conception, humans exist as a single fertilized egg that has embedded itself in the mother's uterus. All of the instructions for development are contained within the genomic DNA of the fertilized egg. The mother provides raw materials and fuel for embryonic development. However, the assembly of molecules, cells, and tissues is conducted by the growing embryo without any outside instructions or manipulations.

A complete multicellular organism has cells that specialize to different tissues and tasks. Within each cell is a menagerie of proteins specialized to different biological processes, many of which comprise the life's work of very gifted researchers and could be discussed extensively in this chapter. However, the goal of dissecting an entire multicellular organism to illustrate its engineering potential is not possible and would be entirely overwhelming if it was possible. Instead, this section turns to a more humble creature as a whole-organism source of biological inspiration.

A bacteriophage (Latin for bacteria eater) is a virus that infects a bacterium. This simple life-form is composed of a protein shell (called a capsid) that contains a nucleic acid genome that carries the instructions for building the capsid proteins and other proteins needed for its assembly and infection. The bacteriophage $\phi 29$ has a double-stranded genome and infects *Escherichia coli* (or *E. coli*). Our understanding of the molecular-level processes involved in bacteriophage formation and infection is dramatically influenced by single-molecule experiments (Smith et al. 2001), which directly probe the forces involved in the packaging and ejection of the DNA genome within the capsid shell. The left image of Fig. 1 shows a schematic of the $\phi 29$ genomic DNA (red and blue) being packaged by the $\phi 29$ packaging motor (orange) into an assembled capsid (purple).

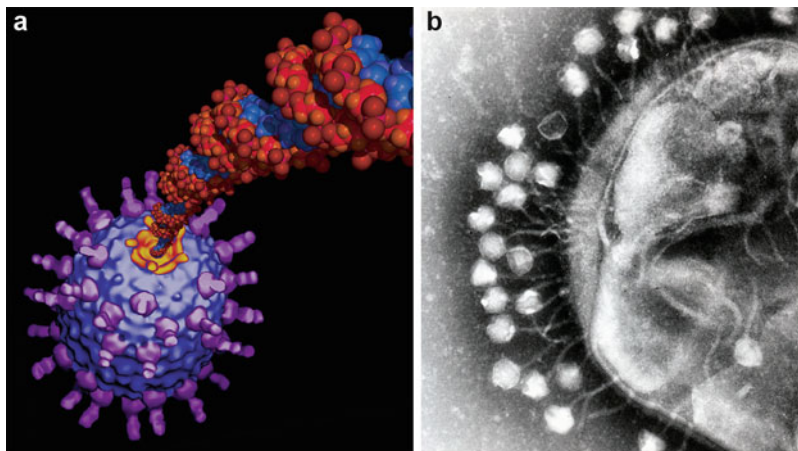


Fig. 1 Images of a virus packaging its DNA (*left*) and delivering its DNA to a bacterium (*right*), demonstrating the exquisite molecular control that even the simplest organism has acquired. *Left image* shows a bacteriophage $\phi 29$ (*purple*) packaging its genomic DNA (*red and blue double helix*) by the action of the $\phi 29$ packaging motor (*orange*) (Smith et al. 2001). The *right image* provides an electron micrograph of a bacteriophage (coliphage T1) infection of a bacterium (see http://spider.science.strath.ac.uk/sipbs/staff/Chris_van+der+Walle.htm)

Even this simple life-form exhibits remarkable self-assembly capabilities that far surpass our fabrication capabilities. Synthesis of the capsid proteins, either within the host *E. coli* cell or by *in vitro* synthesis, leads to the spontaneous assembly of empty capsids that are ready for DNA packaging. The packaging motor is able to generate forces of up to 57 pN, which makes it one of the most powerful protein motors measured. This force is necessary to overcome the considerable resistance to confining the viral DNA within the ~ 50 nm capsid. In fact, the eventual ejection of the viral genome during bacterium infection (see right image of Fig. 1) is partially driven by the internal pressure of the compacted genome. The life cycle of a bacteriophage is such that the assembly of an active virus occurs within a living cell, so the cellular machinery is available to the virus only during the initial stage of its life cycle. Many subsequent functions occur outside the cell, and the active virus must be primed and ready for infection with no external sources of materials or energy.

Although a bacteriophage typically packages its DNA genome, a bacteriophage can be coaxed to package other charged and uncharged substrates (Karunakaran 2007). This permissive motor is shown to reliably package synthetic DNA substrates that differ in charge, chemical structure, and sequence. In this regard, $\phi 29$ may be a useful engineering platform for packaging different chemical moieties within a nanoscale compartment. Furthermore, chemical treatments can be used to induce the internalized DNA to spontaneously release from active viruses, suggesting a method to release the sequestered contents of the filled capsids. The wealth of biochemical manipulations that have been established for the study of bacteriophage promotes the remarkable potential for redesigning viruses for a range of engineering purposes.

Design and Assembly of Complex Macromolecular Architectures

DNA is a remarkable molecule. The molecular structure of DNA results in the preferred formation of a double helix such that complementary base pairs (i.e., adenine-thymine and cytosine-guanine) form hydrogen bonds in the interior of the phosphate backbone double helix. Our genome is composed of a 3.2 billion base sequence of DNA, and its complementary strand zips up to form a double helical strand that is approximately 1 m in contour length. Thus, this long stretch of complementary bases results in the formation of a continuous double helix. However, disruptions in the sequence can lead to the formation of more complex three-dimensional structures that can be designed to assemble into specific architectures.

This ability to form complex secondary structures can be exploited to assemble extremely complex nanoscale structures. Figure 2 shows a series of images of self-assembled nanostructures composed entirely of DNA (Rothemund 2006) along with the computational designs of these structures. As can be seen, the structures can be designed computationally, with specific DNA sequences determined *in silico* such that the unique structure is assembled upon mixing the DNA strands in solution. The structures can be geometric and repeating, as in the lower images. However, complex non-repeating nanostructures can also be designed and assembled. For example, the image of the world map includes details at multiple length scales, including features of continental shapes and interior structures (e.g., Lake Titicaca in Peru is visible in the AFM image).

Base pairing has several advantages as a self-assembly mechanism. Unlike block copolymers that rely on soft nonspecific interactions, DNA nanostructures can be formed with non-repeating structures with specifically designed registry of individual molecules. This hinges on the sequence identity driving the assembly, leading to specific DNA strands assembling into specific locations in the assembly. DNA can be reliably synthesized, either through recombinant methods or by solid-phase synthesis. Thus, thousands of DNA molecules with distinct sequence identities can be synthesized for assembly. The design of such structures has been aided by computational approaches (Rothemund 2006) that mitigate issues of structural defects by choosing sequences that reliably form specific structures with minimal undesired products. Thus, this paradigm for controlled assembly of complex nanoscale architectures has the potential to reliably assemble complex structures with specifically tailored features.

Responsive and Reconfigurable Structures and Materials

A hallmark feature of biological self-assembly is the ability to form nanoscale assemblages with remarkable structural specificity through the orchestrated coordination of multiple subunits. These characteristics are desirable for many technological applications, including nanoscale materials for photovoltaic and energy-storage devices and biosensors for the recognition of rare cancer cells within a sample. Furthermore, soft interactions and molecular elasticity can be exploited to

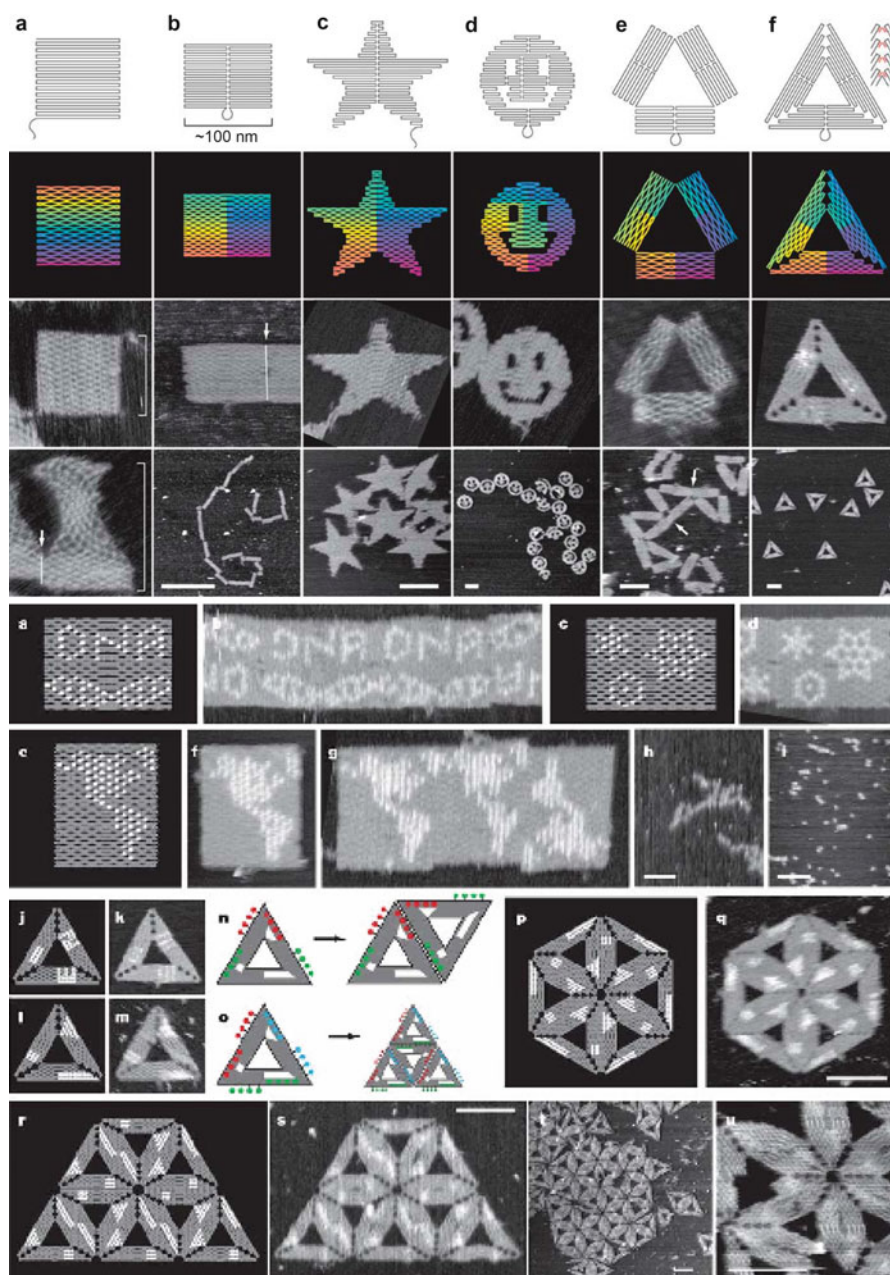


Fig. 2 Self-assembled nanostructures composed of DNA showing precise nanometer-scale control of structural features (Rothemund 2006). Scale bars for top AFM images: (b) 1 μm ; (c–f) 100 nm. Scale bars for top image: (h, i) 1 μm ; (q, s–u) 100 nm

enable structural transitions, resulting in nanostructured materials that are reconfigurable by external chemical or physical cues.

For example, clathrin is a protein that plays a major role in the creation of transport vesicles in cells. Clathrin is an essential component of the common intracellular trafficking mechanism known as clathrin-mediated endocytosis (CME). A monomer unit of clathrin is a massive protein complex (trimer of dimers) that adopts a pinwheel (or triskelion) structure, shown in the upper left image of Fig. 3. Patchy charged on the triskelion legs lead to favorable interactions between triskelia, driving collections of clathrin triskelia to assemble into cagelike lattices, an example of which is found in the upper right image of Fig. 3. During CME, these cagelike clathrin lattices stabilize highly curved membrane buds that grow into vesicles as they engulf associated cargo (see bottom image of Fig. 3 for a schematic of this process). *In vitro* assembly of clathrin within a solution results in closed, nanoscale cages with various shapes and sizes.

This remarkable process hinges on the clathrin and membrane being able to respond to the surrounding conditions by undergoing a range of physical transformations. Upon binding to the cell membrane, the cargo elicits a response on the cell surface, leading to recognition of the cargo and clathrin recruitment on the interior of the cell. At this stage, the clathrin must reorganize to form a cagelike assembly as the membrane wraps around the cargo. The cell must accommodate cargoes of varying size (~40 nm to several hundred nanometers) and shape; thus, the growing invagination must be sufficiently flexible to enable transport of a broad spectrum of vesicles. In this regard, the clathrin lattice must be sufficiently fluid in order to reorganize its connectivity to match the local shape of the cargo.

Although clathrin locally prefers six-membered rings (as in a honeycomb), the lattice is able to form five-membered and seven-membered defects by deforming the clathrin legs. For a closed polyhedral structure, Euler's polyhedron formula $V - E + F = 2$ dictates the relationship between the number of vertices V , edges E , and faces F (Euler, 1752–1753). For example, a cube has $V = 8$, $E = 12$, and $F = 6$, and Euler's polyhedron formula gives $8 - 12 + 6 = 2$. Similarly, a soccer ball is geometrically defined as a truncated icosahedron, which has 60 vertices ($V = 60$), 90 edges ($E = 90$), 12 pentagonal faces, and 20 hexagonal faces (total faces $F = 32$). Thus, the truncated icosahedron also satisfies Euler's polyhedron formula $60 - 90 + 32 = 2$. Clathrin's ability to form ringlike structures with different number of edges is essential in its ability to form closed structures that wrap around cargo of varying sizes and shapes.

Once the cagelike lattice is established, the clathrin lattice plays a role in stabilizing the invagination. Thus, the lattice must undergo a transformation to a solid-like structure after the connectivity is set to match the cargo shape and size. It is not clear whether this transformation is through some chemical signal or if the transformation is purely physical. However, modulating membrane fluctuations and local curvatures can induce fluid-solid transformations in a model clathrin system (Cordella et al. 2015). In this regard, one can envision using subtle changes to the local environment to induce transformations. These designed transformations

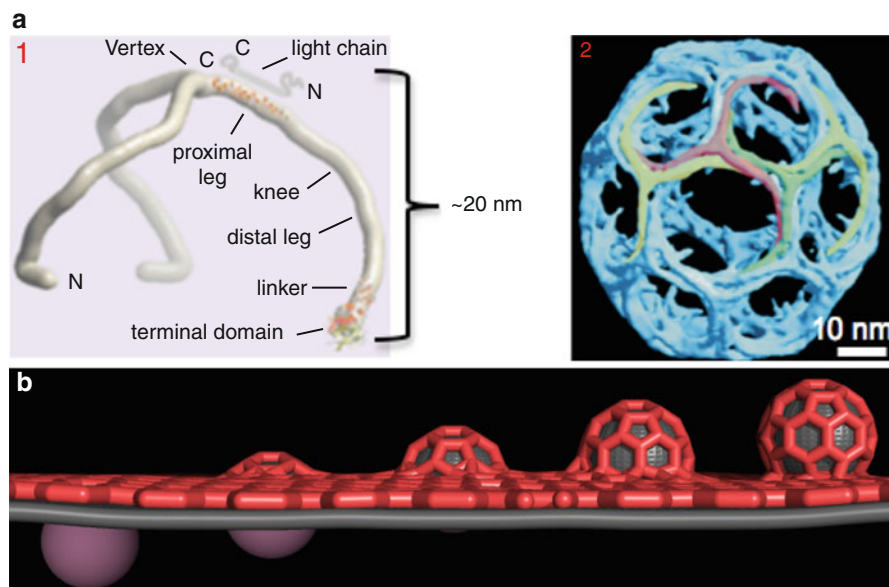


Fig. 3 Images of the protein clathrin, which spontaneously assembles and disassembles into nanoscale cages during endocytosis. The *upper left image* shows the structure of a clathrin triskelion, which is the monomer unit of clathrin that assembles into the cagelike lattices. The *upper right image* shows an example of a cagelike lattice that spontaneously assembles in vitro. The *bottom image* shows the progression of the events involved in endocytosis – the process that a cell uses to bring cargo from its exterior to its interior

would be desirable in a range of applications, including self-healing and controlled-release materials.

DNA nanostructures, as shown in Fig. 2, offer a pathway for the design of molecular architectures that undergo large-scale transformations. This idea of making molecular “transformers” is exploited to make DNA-based structures that undergo controlled nanoscale motions (Marras et al. 2015). The basic principle exploits the precise control over nanoscale assembly that is afforded by designed DNA sequences along with existing understanding of engineering design of mechanical structures. This culmination of macroscale engineering principles with molecular-level design provides a new approach to the formation of responsive and reconfigurable structures at the nanoscale.

Controlling Polymer Entanglement and Topology

The behavior of polymeric materials is strongly influenced by a range of physical effects that arise from the large molecular weight of the comprising polymer chains (Doi and Edwards 1999). Perhaps most significant for the dynamic behavior of polymeric materials is the influence of entanglements on the motion and relaxation

of polymer chains within their spaghetti-like environment. The conformation of a chain in a highly concentrated polymer solution is almost unaffected by the interactions with their neighboring chains (Flory 1953). However, the motion of a polymer through the surrounding chains involves a fluctuating, sliding-type mechanism dubbed reptation (de Gennes 1971). This dynamic mechanism is extremely sensitive to the molecular weight of the polymer chains. For example, the simplest prediction for the scaling of the diffusivity D versus the molecular weight M results in the relationship $D \sim M^{-2}$ (Doi and Edwards 1999), which is close to the experimentally observed behavior. This extreme sensitivity of the chain motion to entanglements manifests a range of physical behaviors that are common characteristics of polymeric materials, including elastic response, viscoelasticity, and plasticity.

These issues of entanglement are also relevant to the dynamics of DNA within a prokaryotic cell and within the nucleus of a eukaryotic cell. Imagine, for example, the process of disentangling two meter-long strings that are wrapped up within a small enclosure. In the case of our genomic DNA, this enclosure is several microns across, yet the DNA “string” is still a full meter in length. Furthermore, the replication of the DNA results in the parent and daughter strands exhibiting similar final states after replication completes, which implies that they would be highly entangled. Thus, separating these strands into separate cells during cell division is complicated by the daunting task of disentangling these massively long polymers within an extremely small, crowded space.

The cell has devised a remarkable solution to this problem. There exists a family of proteins called topoisomerases that perform a range of manipulations on DNA (Wang and Giaever 1988). One such protein (called topoisomerase IV (topo IV)) is able to identify local crossings of DNA that constitute an entanglement, cut one of the crossing DNA strands, pass it across the crossing, and reconnect the DNA on the other side. This process effectively eliminates the entanglement and allows the DNA to undergo unhindered motion that is necessary for a number of life cycle processes, including segregation. Single-molecule measurements of the tension response of chromosomal DNA extracted from a frog egg (i.e., *Xenopus* egg cell) show a mechanical response that is much more compliant and fluid if topoisomerase IV is present than if it is absent (Marko 2008). In this regard, one can imagine utilizing topoisomerase IV to modulate the entanglements within a DNA material, which would alter its viscoelastic properties dramatically.

In many instances, DNA exists in a circular form such that the ends are married together into a continuous ring with no breaks. Closed rings exhibit several properties that cannot be altered regardless of how you reshape the ring. For example, imagine that you tie a knot in a string and then connect the ends. No matter how you deform the knotted string, the knot will not go away unless you cut the string. Thus, there is a property of the knotted ring that remains the same regardless of how you deform it. Another useful example is a telephone cord. If you twist a telephone cord and set the receiver down, the telephone cord will form interwound coils. Upon flattening the coiled cord without picking up the receiver, the shape of the cord now adopts a flattened conformation with no coils. The coils reform if you release the

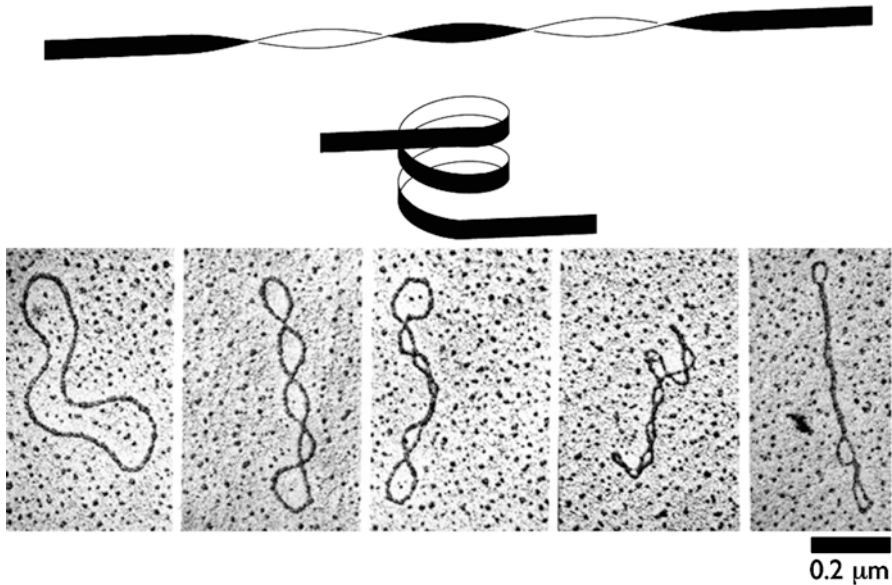


Fig. 4 Images showing the topological constraints that arise in a twisted DNA strand, resulting in a supercoiled structure. *Top image* shows a schematic of the interconversion of twist Tw and writhe Wr , resulting in the topological invariant linking number $Lk = Tw + Wr$. The *bottom image* presents electron micrographs of plasmid DNA with increasing linking number Lk . The writhed coils of DNA are called supercoils and are akin to the coiling of a twisted telephone cord

telephone cord, and they cannot be eliminated unless you pick up the receiver (breaking the loop) and untwist the cord by letting the receiver rotate until all the twists are eliminated.

The two examples in the previous paragraph represent properties of a closed ring that are analyzed in a branch of mathematics called topology. Topological invariants arise in many systems. Various examples can be used as curious and peculiar thought puzzles. For example, a teacup can be deformed into a donut, since both cases are described by a topological invariant of surfaces that contain a single hole.

Similarly, there is a topological invariant called the linking number Lk (White 1969) that applies to a closed ring (e.g., a DNA plasmid). The linking number Lk is defined as the sum of two topological quantities called twist Tw and writhe Wr ; thus, $Lk = Tw + Wr$. The twist Tw is the number of turns of twist that is introduced into the ring, and the writhe Wr is a quantity that gives the average number of times the ring crosses itself in three-dimensional space. Figure 4 shows a schematic of the interconversion of twist and writhe. The top image has two turns of twist ($Tw = 2$) and no writhe ($Wr = 0$), and the bottom image is untwisted ($Tw = 0$) and has a writhe $Wr = 2$. Thus, the linking number remains $Lk = 2$ regardless of how the twisted ribbon is deformed. The bottom image of Fig. 4 shows five electron

micrographs of plasmid DNA with increasing amounts of linking number, resulting in an increasing writhe in the form of tighter coiled structures (or supercoils).

The topoisomerase family of proteins includes members that twist and untwist the DNA strand (Wang and Giaever 1988). The various topoisomerases function by a range of mechanisms to introduce negative or positive turns of twist into the DNA. For example, the topoisomerase called gyrase binds to DNA, forms a small loop, cuts the DNA, and passes the DNA through itself (Reece et al. 1991). This process introduces two turns of negative twist per cycle. The balance of the various topoisomerases acting on the DNA dictates the degree of supercoiling throughout the cell cycle.

As indicated in Fig. 4, the degree of supercoiling dramatically influences the conformation of the DNA strand. Increasing the linking number by twisting the strand leads to tighter supercoiled structures and a more compact conformation. Since topoisomerases can control the degree of supercoiling over a broad range, these proteins have a remarkable capacity to control the global conformational properties of large DNA strands through the local process of introducing twist into the strand. These types of manipulations are very difficult to introduce into conventional polymer systems. However, topoisomerases provide an avenue for using polymer topology to dramatically manipulate the conformational properties of polymeric materials, resulting in materials that could undergo local and global shape transformations.

Interfacing Biomolecules and Inorganic Materials

Proteins have a range of accessible chemical moieties, including the 20 natural amino acids and numerous nonnatural amino acids (Langer and Tirrell 2004; Liu et al. 2007). However, a pure protein material is predominantly composed of elements in the organic region of the periodic table. Thus, the formation of inorganic materials based on biomolecular assemblies requires interfacing organic biopolymers with inorganic elements.

The natural world has numerous examples where inorganic elements interface with biomolecules Weiner et al. (2003). For example, the Great Barrier Reef (see Fig. 5) is a massive structure composed largely of calcium carbonate that is produced and maintained entirely by living organisms. This structure, which is viewable from space, is the largest example of such a structure. As such, it is reasonable to assume that interfacing biomolecules with inorganic compounds can result in the mass production of materials.

Several protein sequences have been identified to nucleate the formation of inorganic materials using solution-based chemistry (Seeman and Belcher 2002). Such materials open the door to the formation of a range of ionic, metal, and metal oxide materials using biomolecular structures as a template. For example, clathrin (discussed in the previous section) has been used as a template for the formation of nanoparticles of several metal oxides (Schoen et al. 2011). The approach is to



Fig. 5 The Great Barrier Reef is the world's largest structure that is produced and maintained entirely by living organisms. This structure demonstrates the ability for biomolecules to template and grow inorganic materials

develop peptide sequences that have one section that binds specifically to clathrin proteins and a second section that nucleates the inorganic material. This allows the facile incorporation of inorganic compounds at specific positions on the biomolecular nanostructure.

This concept is not limited to clathrin (or proteins in general). In a previous section, the concept of DNA-based assembly is introduced as a potential pathway for the assembly of complex nanostructures. The incorporation of specific DNA-protein sequences that bind to specific points on the nanostructure and nucleate inorganic materials may serve as a powerful framework for using such nanostructures for the formation of complex inorganic structures.

Conclusions

This chapter provides a general picture of how biological systems can be exploited for nanoscale engineering applications. The basic paradigms that are outlined within this chapter are illustrated using several key biological examples that are either currently exploited for specific applications or are ripe for translation to future technologies. Two main strategies are discussed for leveraging biomolecular systems – mimicking and co-opting. Both of these strategies have the potential to dramatically influence how we design, synthesize, and exploit molecules for the assembly of nanoscale structures. Following this path may lead to engineered systems that begin to incorporate the remarkable capabilities that biological systems have evolved for a range of chemical and physical processes.

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