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Bridging Biomolecules with Nanoelectronics

11.1 Introduction and Background

The field of nanostructures has grown out of the lithographic technology developed for integrated circuits, but is now much more than simply making smaller transistors. In the early 1980s, microstructures became small enough to observe interesting quantum effects. These structures were smaller than the inelastic scattering length of an electron so that the electrons could remain coherent as they traversed them, giving rise to interference phenomena. Studies on the Aharonov–Bohm effect and universal conductance fluctuations led to the field of “mesoscopic physics”—between macroscopic classical systems and fully quantized ones.

Now the size of the structures that can be produced is approaching the de Broglie wavelength of the electrons in the solids, leading to stronger quantum effects. In addition to interesting new physics, this drive toward smaller length scales has important practical consequences. When semiconductor devices reach about 100 nm, the essentially classical models of their behavior will no longer be valid. It is not yet clear how to make devices and circuits that will operate properly on these smaller scales. The replacement for the transistor, which must carry the technology to well below 100 nm, has not been identified. It is anticipated that the semiconductor industry will run up against this “wall” within about 10 years.

The current very large-scale integrated circuit paradigm based on complementary metal oxide semiconductor (CMOS) technology cannot be extended into a region with features smaller than 10 nm. With a gate length well below 10 nm, the sensitivity of the silicon field-effect transistor parameters may grow exponentially due to the inevitable random variations in the device size. Therefore, an alternative nanodevice concept of molecular circuits was proposed, which was a radical paradigm shift from the pure CMOS technology to the hybrid semiconductor. The concept combines the advantages of nanoscale components, such as the reliability of CMOS circuits, and the advantages of patterning techniques, which include the flexibility of traditional photolithography and the potentially low cost of nanoimprint-
one would like to stretch the DNA straight and simply read the
sequence of the base pairs. While we are still far from this goal,
nanofabrication techniques promise to bring us much closer.

During recent years, self-assembly has become one of the most
important strategies used in biology for the development of com-
plex, functional structures. Self-assembly on modified surfaces is
one of the approaches to self-assemble structures that are particu-
larly successful. By the coordination of molecules to surfaces, the
molecular systems form ordered systems—self-assembled mono-
layers (SAMs). The SAMs are reasonably well understood and are
increasingly useful technologically. A thin film of diblock copoly-
mers can be self-assembled into ordered periodic structures at
the molecular scale (∼5–50 nm) and have been used as templates
to fabricate quantum dots, nanowires, and magnetic storage
media. More recently, in epitaxial assembly of block-copolymer
films, molecular level control over the precise size, shape, and
spacing of the order domains was achieved with advanced litho-
graphic techniques. The development of methods for patterning
and immobilizing biologically active molecules with micrometer
and nanometer scale control has been proven integral to
ranges of applications such as basic research, diagnostics, and
drug discovery. Some of the most important advances have been
in the development of biochip arrays that present either DNA,10
protein,11 or carbohydrates.12 The use of patterned substrates for
components of microfluidic systems for bioanalysis is also pro-
gressing rapidly.14–15 Surface modification and patterning at the
nanoscale to anchoring protein molecules is an important strat-
egy on the way of obtaining the construction of new biocompat-
bile materials with smart bioactive properties. In fact, surfaces
patterned by protein molecules can act as active agents in a large
number of important applications including biosensors capable
capable of multifunctional biological recognition. In particular, physical
adsorption of proteins onto semiconductor surfaces makes pos-
sible to combine the simplicity of the method with the versatility
of chemical and physical properties of proteins.

In recent years, there has been substantial attention focused
on the reactions of organic compounds with silicon surfaces.
The major attraction is the incorporation of the richness of
organic chemistry with the versatility of semiconductor sci-
ence and technology. More recently, it has been demonstrated
that TEMPO,2,2,6,6-tetramethylpiperidinyloxy can bond with
a single dangling bond on hydrogen-terminated Si(100) and
Si(111) surfaces. Functional organic molecular layers were
found to self-assemble on metal2 and semiconductor surfaces.7
The technique of self-assembly is one of the few practical strate-
gies available to arrive at one to three-dimensional ensembles
of nanostructures. There are many different mechanisms by which
self-assembly of molecules and nanoclusters can be accom-
plished, such as chemical reactions, electrostatic and surface
forces, and hydrophobic and hydrophilic interactions.

In this chapter, we present most recent advances in the de-
development of techniques in immobilizing a single nanostructure
and producing arrays of 3D magnetic protein nanostructures
with high throughput on surface modified semiconductor sub-
strates. Well-characterized test nanostructures were prepared
using current state-of-the-art nanofabrication techniques. Both
nanointeracting and scanning probe microscopy studies (AFM,
SEM, and TEM) on semiconductor nanostructures and these
molecular self-assembly systems were performed. The combina-
tion of e-beam lithography, scanning probe microscopy imaging,
spectroscopy, and self-assembly approaches provide not only
the high throughput of producing arrays of protein nano-
structures but also with highest precision of positioning single
nanostructure and/or single molecule.

11.2 Preparation of Molecular Magnets

11.2.1 Folding of Magnetic Protein Mn,Cd-MT

Metallothionein (MT) is a metal binding protein that binds
seven divalent transition metals avidly via its twenty cysteines
(Cys). These Cys form two metal binding clusters located at the
carboxyl (α-domain) and amino (β-domain) terminals of MT.19
The two clusters were identified as α-cluster (M(S)n)3− and the
β-cluster (M(S)n)3− (Figure 11.1),20–22 where M denotes metal ions
(Zn2+, Cd2+, or others), according to both x-ray crystallographic
and NMR studies. MT binds to metals ions via metal-thiol link-
ages. As shown in Figure 11.1, the (M(S)n)3− and (M(S)n)3− have
the zinc-blende like structure that is similar to the “diluted mag-
netic semiconductor (DMS)” compounds.

In general, semiconductors are not magnetic. However, a
DMS exhibits magnetic properties by doping with Mn and Cd
or other II–VI metal ions in certain ratio. The doped metal clusters
among the semiconductors are in zinc-blende structures. Meanwhile,
these semiconductors possess magnetic property
only in low temperatures. The magnetic properties may be the
result of the d-sp^2 orbital hybridization and the alignment of
the electron spins. The bridging sulfur atoms may also contribute to
the alignment of the spins of the Mn2+ ions. Thus, by chelating
the Mn2+ and Cd2+ with MT (i.e., Mn,Cd-MT), a “magnetic pro-
tein” may be obtained.

Recently, the single molecule magnets (SMMs) have attracted
much attention. However, the Curie temperature of these mole-
cules has to be as low as 2–4K to avoid the thermal fluc-
tuation among the electron spin within the molecules.7 To be
of practical utilization, it is highly desirable to create a room
temperature molecular magnet. With this intention in mind, one
has to construct and investigate a new metal binding pro-
tein, MT, which sustained characteristic magnetic hysteresis loop
from 10 to 330 K. The protein backbone may restrain the net spin
moment of Mn2+ ions to overcome the minor thermal fluctuation.
The magnetic-metallothionein (mMT) presented may reveal a
possible approach to create high temperature molecular magnet.
In order to prepare the Mn,Cd-MT magnetic proteins, the fold-
ing mechanism of protein should be introduced.

11.2.2 Protein: A Mesoscopic System

Protein is a complex biomolecule that contains a large number
of basic residues—amino acids. Therefore, it is not possible to
analyze it completely by macroscopic approaches. Meanwhile, it
is not feasible to describe the dynamics of its polypeptide chain behavior by using conventional statistical approaches either. Thus, a protein can be thought of as a mesoscopic system. However, the conformational transition from unfolded state to the native state of a protein may be similar to the conventional phase transition model. In physics, a phase transition is the transformation of a thermodynamic system from one phase to another. In a folding process, proteins follow the thermodynamic theories and transform from the unfolded state to the folded state, where the state is defined as a region of configuration space with minimal potential. Therefore, we named this conformational change as “state transition.” Due to the complexity of a protein folding system, a single experimental study may reveal only a part of the fact of protein folding. Therefore, we should examine the protein-folding problem with multidimensional approaches and integrate the findings to reveal the true mechanism of protein folding.

Protein folding may follow a spontaneous process or a reaction-path directed process in vitro. A choice between the two may be determined by the intrinsic properties of proteins, for example, the varying folding transition boundaries. However, a general model, named “first-order-like state transition model,” in which the aggregated proteins exist within finite boundaries can encompass both processes without any confictions. According to this model, the folding path of the protein may not be unique. It can be folded, without being trapped in an aggregated state, via a carefully designed refolding path circumventing the transition boundary, that is, via an overcritical path. The intermediates, following an overcritical path, are in a molten globular state, and their behavior is consistent with both a sequential and a collapse model. However, both soluble (folded) and precipitated (unfolded) proteins can be observed in the direct folding reaction path in vitro. In terms of the “first-order-like state transition model” language, this can be described as stepping across the state transition line in the protein folding reaction phase diagram. Since in protein refolding it is important to prevent protein aggregation in vitro, similarly, in biomedical applications, the revelation of the mechanism of the formation of the two states (unfolded and folded) becomes significant.

Previous studies have indicated that chemical environment, temperature, pH, ionic strength, dielectric constant, and pressure—considered as solvent effects collectively—could affect the fundamental structure, thermodynamics, and dynamics of polypeptides/proteins. The reaction ground state can be expressed as a dual-well potential according to the two-state transition model. The conformational energy, in general, of the unfolded state is relatively higher than that of the native state (Figure 11.1). When the system reaches thermal equilibrium, most protein molecules are found in their native state. No unfolded or intermediate states are observable. However, as described previously, if a denaturant is added, the reaction potential may change accordingly as indicated in Figure 11.1. Then, an unfolded protein may be stable, as it is now at the lowest energy under the newly established equilibrium. The energy of the system can be expressed as follows:

$$H_f = H_p + \lambda H_s$$  \hspace{1cm} (11.1)  

where $H_f$, $H_p$, and $H_s$ denote the potential energy of the interacting protein-solvent total system, the protein, and the solvent, respectively. The factor, $\lambda$, is a weighting factor of the solvent environment ($0 \leq \lambda \leq 1$). It approaches unity when a denaturant is present as pure solvent and decreases in value as the concentration of the denaturant is reduced.

When $\lambda$ of the system is changed drastically, direct folding ensues and leads to the release of some of the bound denaturant. According to the Donnan effect in a macromolecule-counter ions interactive system, the diffusion of the bound denaturant can be expressed by Fick’s first law:

![FIGURE 11.1 Metal binding clusters of MT that was modified from x-ray crystal structure (2). Where the circles denote metal ions Zn$^{2+}$, Cd$^{2+}$, or Mn$^{2+}$. Each metal ion was linked with protein via metal-thiol bonds.](image-url)
\[ \vec{j} = -D \nabla n \]  
(11.2)

where

- \( n \) denotes the concentration of the denaturant that is dissociated from the protein
- \( D \) denotes the diffusive constant
- \( \vec{j} \) denotes the flux, respectively, of the solute

According to the Einstein relation

\[ D = \frac{kT}{6\pi \eta R_{H}} \]  
(11.3)

where

- \( k \) is the Boltzmann constant
- \( T \) is the temperature in Kelvin
- \( \eta \) is the viscosity of the solvent
- \( R_{H} \) is the hydration radius of the solutes

Due to the intrinsic diffusion process, the solute exchange processes are not synchronous for all protein molecules. Therefore, the folding rate of protein may not be measured directly by a simple spectral technique, that is, the stopped-flow CD,\textsuperscript{15} continuous-flow CD,\textsuperscript{46} or fluorescence.\textsuperscript{45} However, the reaction interval of protein folding can be revealed by the autocorrelation of reaction time from these direct measurements. The detailed mechanism and an example will be discussed later.

If we look at the energy landscape funnel model of protein folding,\textsuperscript{46} it appears that proteins can be trapped in a multitude of local minima of the potential well in a complicated protein system. The native state, though, is at the lowest energy level. When thermal equilibrium is reached, most of the protein molecules are located in the lowest energy state, with a population ratio as low as \( e^{-\Delta E/kT} \), according to the Maxwell–Boltzmann distribution in thermodynamics. The \( \Delta E \) denotes the energy difference between the native state and a local minimum; \( k \) and \( T \) denote the Boltzmann constant and temperature in Kelvin, respectively. At high concentration (>0.1 mg/mL), however, considerable amount of insoluble protein has been observed in protein folding,\textsuperscript{33–37,40} indicating that insoluble proteins are at an even lower energy state than the native protein. Therefore, by considering the intermolecular interactions during the protein folding process, the reaction energy landscape may be expressed as a three-well model (Figure 11.2). As shown in Figure 11.2, the unfolded protein (U) is in the highest energy state; the native protein (N) is in the lower energy state. However, the intermediate (I) that may cause further protein aggregation/precipitation is in the lowest energy state. Although the energy state of the intermediate/aggresome is the lowest energy state, the conformational energy of the individual proteins composing the aggresome may not be lower than the native protein. Namely, in single molecular simulation, this extra potential well of intermediate (I) is nonexistent. Therefore, in the conventional energy landscape model (the single molecule simulation model), the lowest energy state “I” cannot be observed. According to the Zwanzig’s definition of state, the protein molecules in the intermediate (I) belong to an unfolded state.\textsuperscript{13} Hence, in a direct folding reaction, the soluble (N) and the insoluble parts (U) can coexist and they can be observed simultaneously, which is similar to the situation where the phase transition line is crossed in reactions congruent to the “first-order phase transition” model. Therefore, we named the protein folding reaction as “first-order like state transition model” (as shown in Figure 11.3).

The \( \Phi(n_{1}, n_{2},...) \) in Figure 11.3 denotes the folding status of protein, where \( n_{1}, n_{2},... \) represent the variables affecting the folding status, such as, temperature, concentration of denaturants, etc. The reaction curve indicates an overcritical reaction path of a quasi-static folding reaction. The gray area in Figure 11.3 indicates the state transition boundary of protein folding. The gray line and dash line indicate the reaction path of direct folding. By combining the three-well model (Figure 11.2) and the direct

![Figure 11.2](image-url)  
Three well model of multi-protein molecules folding reaction. The U denotes the unfolded state. N denotes the native state and I denotes the protein–protein complex (aggresome) intermediate.

![Figure 11.3](image-url)  
The protein folding phase diagram, where the \( \Phi(n_{1}, n_{2},...) \) denotes the folding status (the order parameter) of protein. The \( n_{1}, n_{2},... \) denote the variables that affect the folding status such as temperature, concentration of denaturants, etc.
folding reaction of the “first-order like state transition model” (Figure 11.3), we realized that those folded protein molecules along the direct folding path might fold spontaneously or form aggregates. Spontaneous folding may be driven by enthalpy–entropy compensation.

As indicated previously, the conformation of protein changed with changes of the solvent environment. It seems that the protein may fold spontaneously, such as in Anfinsen’s experiment\(^{14}\) and direct folding reactions. The protein folding reaction, similar to all chemical reactions, reaches its equilibrium by following the fundamental laws of thermodynamics. Although protein folding has been studied extensively in certain model systems for over 40 years, the driving force at the molecular level remained unclear until recently.

It is known that polymers and macromolecules may self-assemble/self-organize into a wide range of highly ordered phases/states at thermal equilibrium.\(^{49–52}\) In a condensed solvent environment, large molecules may self-organize to reduce their effective volume. Meanwhile, the number of the allowed states (\(\Omega\)) of small molecules, such as buffer salt and other counter-ions in solution, increases considerably. Therefore, the entropy of the system, \(\Delta S = R \ln(\Omega/\Omega_i)\), becomes large, where \(i\) and \(f\) denote the initial and final states, respectively. Meanwhile, the enthalpy change (\(\Delta H\)) between the unfolded and native protein is around hundreds kcal/mol.\(^{53}\) Therefore, the Gibbs free energy of the system, \(\Delta G = \Delta H - T \Delta S\), becomes more negative in this system when the large molecules self-organize.\(^{54}\) A similar entropy–enthalpy compensation mechanism has been used to solve the reaction of colloidal crystals that self-assemble spontaneously.\(^{55,56}\)

According to our studies,\(^{31,33–37}\) the effective diameter of the unfolded protein is about 1.7–2.5 fold larger than the folded protein. Therefore, with the same mechanism, those macromolecules (proteins) may tend to reduce their effective volumes and increase the system entropy when thermal equilibrium is reached. The increase in entropy may compensate for the change of the enthalpy of the system and enable the reaction to take place spontaneously. This may be the reaction molecular mechanism of spontaneous protein folding reactions. Meanwhile, a similar mechanism can be adopted into the self-assembly process of magnetic protein in nanopore arrays.

### 11.2.3 Quasi-Static Thermal Equilibrium Dialysis for Magnetic Protein Folding

Due to the intrinsic diffusion process, the solvent exchange rate is slow and thus the variation of \(\lambda\) is slow and can be thought of as quasi-static. Therefore, we named this buffer exchanging process as a quasi-static process. We manipulated the reaction direction of the protein folding through this process. Meanwhile, we can obtain stable intermediates in each thermal equilibrium state. These intermediates may help us reveal the molecular folding mechanism of protein that is to be discussed in Section 11.3. The following is an example of the stepwise folding method,\(^{31,33–37}\) and the buffers used were described in these studies.

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**Step 1:** The unfolded protein (U) was obtained by treating the precipitate or inclusion body with denaturing/unfolding buffer to make it 10 mg/mL in concentration. This solution was left at room temperature for 1 h. This process was meant to relax the protein structure by urea and pH (acidic or basic) environments. The disulfide bridges were reduced to SH groups and the protein was unfolded completely.

**Step 2:** The unfolded protein (U) in the denature/unfolding buffer was dialyzed against the folding buffer 1 for 72 h to dilute the urea concentration to 2 M, producing intermediate 1, or \(M_1\).

**Step 3:** \(M_1\) was obtained by dialyzing \(M_1\) against the folding buffer 2 for 24 h to dilute urea concentration to 1 M.

**Step 4:** \(M_1\), an intermediate without denaturant (urea) in solution, was then obtained by dialyzing \(M_2\) against the folding buffer 3 for 24 h.

**Step 5:** \(M_3\) was further dialyzed against the folding buffer 4 for 24 h, and the pH changed from 11 to 8.8 to produce \(M_4\).

**Step 6:** Finally, the chemical chaperonin mannitol was removed by dialyzing \(M_3\) against the native buffer for 8 h to yield \(M_5\).

It should be noted that all the equilibrium time of each step is longer than the conventional dialysis time. In general, for the free solvent case, the solute may exchange with the buffer completely within hours. However, it is known that the denaturant molecules interact with protein, similar to the Donnà effect, and the solute exchange may be slow and needs more time for the system to reach thermal equilibrium, especially for the first refolding stage. The folding time of each process is relatively longer than the regular solvent exchange process. Therefore, we can obtain the magnetic protein that follows a similar process. Protein microenvironment protects the net electron spin of molecules from thermal fluctuation.

The bridging ligands (i.e., sulfur atom, S) between the magnetic ions may be responsible for aligning the electron spin of magnetic ions. As indicated in Figure 11.4, the valence bonding electrons of the bridging Cys may hop between the bonded metal ions, such as Mn\(^{2+}\) and Cd\(^{2+}\); whereas the Cd\(^{2+}\) in the \(\beta\) metal cluster is rather important in restraining the orientation of the electron spins of the bridging sulfurs and in aligning the spins of Mn\(^{2+}\) in the metal binding clusters. Therefore, this electron hopping effect may turn the Mn,Cd-MT into a magnetic molecule. However, the protein backbone surrounding the \(\beta\) metal cluster may provide a strong restraining effect to overcome the thermal fluctuations from the environment. Therefore, the magnetization can be observed in room temperature. However, the geometrical symmetry of the spin arrangement in all Mn-MT may cause partial or complete cancellation of detectable magnetization. These results also indicated that the threshold temperature of the molecular magnet might rise to room temperature if the proper prosthetic environment, such as protein backbone, can be linked against the thermal fluctuation of the temperature.
Therefore, we have successfully constructed a molecular magnet, Mn,Cd-MT, that is stable from 10 to 330 K. The observed magnetic moment can be explained by the highly ordered alignment of (Mn,CdS)₃⁻ clusters embedded in the β-domain in which sulfur atoms serve as key bridging ligands. The discovery of mMT may allude to new schemes in constructing a completely different category of molecular magnets.

### 11.3 Nanostructured Semiconductor Templates: Nanofabrication and Patterning

The rapidly developing of interdisciplinary activity in nanostucturing is truly exciting. The intersections between the various disciplines are where much of the novel activity resides, and this activity is growing in importance. The basis of the field is any type of material (metal, ceramic, polymer, semiconductor, glass, and composite) created from nanoscale building blocks (clusters of nanoparticles, nanotube, nanolayers, etc.) that are themselves synthesized from atoms and molecules. Thus, the controlled synthesis of those building blocks and their subsequent assembly into nanostructures is one fundamental theme of this field. This theme draws upon all of the material-related disciplines from physics to chemistry to biology and to essentially all of the engineering disciplines as well.

The second and most fundamental important theme in this field is that the nanoscale building blocks, because of their size being below about 100 nm, impart to the nanostructures that are created from them new and improved properties and functionalities that are still unavailable in conventional materials and devices. The reason for this is that the materials in this size range can exhibit fundamentally new behavior when their sizes fall below the critical length scale associated with any given property. Thus, essentially any material property can be dramatically changed and engineered through the controlled size-selective synthesis and assembly of nanoscale building blocks. The present juncture is important in the fields of nanoscale solid-state physics, nanoelectronics, and molecular biology. The length scales and their associated physics and fabrication technology are all converging to the nanometer range.

The ability to fabricate structures with nanometer precision is of fundamental importance for any exploitation of nanotechnology. In particular, cost effective methods that are able to fabricate complex structures over large areas will be required. One of the main goals in the nanofabrication area is to develop general techniques for rapidly patterning large areas (a square centimeter, or more) with structures of nanometer sizes. Presently, electron-beam (e-beam) lithography is capable of defining patterns that are less than 10 nm. These patterns can then be transferred to a substrate using various ion milling/etching techniques. However, these are “heroic” experiments, and can only be made over a very limited area—typically a few thousand square microns, at most. While such areas are immediately useful for investigating the physics of nanostructures, the applications we would like to pursue will eventually require a faster writing scheme and much larger areas. The time and area constraints are determined by the direct e-beam writing. It is a “serial” process, defining single small regions at a time. Furthermore, the field of view for the e-beam system is typically less than 100 nm when defining the nanometer-scale structures. One simply cannot position the electron beam with nm precision over larger areas.

While e-beam lithographic methods are very general, in that essentially any shape can be written, we will also make use of “natural lithography” (tricks). Similarly, advances in the knowledge of the DNA structure has recently been applied to the fabrication of self-organized nm surface structures. There are many such “tricks” that could prove crucial to the success of the projects in allied fields. A list of current methods toward nanofabrication is given below.

#### 11.3.1 Patterned Self-Assembly for Pattern Replication

By exploiting e-beam and focused ion beam lithography, self-assembled monolayers can be patterned into 10–20 nm features that can be functionalized with single molecules or small molecular groupings. These patterned areas will then be used as templates to direct the vertical assembly of stacks of molecules or to direct the growth of polymeric molecules. Schematics of the processes and the possible templates used are shown in Figure 11.5. The initial 2D pattern will thus be translated into 3D nanosized objects. With the capability of the full control over the interfacial properties, it will be possible to release the objects from the templates and transfer them to another substrate, after which the nanopatterned surface can be used again to provide an inexpensive replication technique.

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**FIGURE 11.4** Proposed electron spin model of Mn⁺⁺ in β metal binding cluster of Mn,Cd-MT-2.
11.3.2 Fabrication by Direct Inkjet and Mold Imprinting

This part of the technique will draw on recent advances in printing techniques for direct patterning of surfaces. This involves the use of inkjet printing to deliver a functional material (semiconductor or metal) to a substrate, which is then controlled by patterning in the surface free energy of the substrate, to allow very accurate patterning of the printed material. Currently, devices show channel lengths down to 5 μm, but indications are that geometries can be reduced to submicron dimensions. The method will be concerned with the limits to resolution that can be achieved by this process, and also the structure and the associated electronic structure at polymer–polymer interfaces, such as that between semiconductor and insulator layers in the field-effect device.

In 1995, Professor Stephen Y. Chou of Princeton University invented a new fabrication method in the field of semiconductor fabrication. It is called nano-imprint lithography (NIL). Briefly speaking, this technique was demonstrated by pressing the patterned mold to contact with the polymer resist directly. The patterns on the mold will transfer to the polymer resist without any exposure source. Therefore, the diffraction effect of light can be ignored, and the limitation is dependent only on the pattern size of the mold rather than on the wavelength of the exposure light. In Figure 11.6 we show a nano-grating structure made by using the thermal imprinting technique.

NIL technology is a physical deformation process and is very different from conventional optical lithography. This technology provides a different way to fabricate nanostructures with easy processes, high throughput, and low cost. Currently, there are three main NIL techniques under investigation, namely, hot-embossing nano-imprint lithography (H-NIL), ultra-violet nano-imprint lithography (UV-NIL), and soft lithography. Those NIL technologies can be applied to many different research fields, including nano-electric devices, bio-chips, micro-optic devices, micro-fluidic channels, etc.

11.3.3 Nanopatterned SAMs as 2D Templates for 3D Fabrication

Modern lithographic techniques (e-beam or focused ion beam (FIB), SNOM lithography) are able to generate topographic (relief) patterns in the 10–50 nm size ranges. As a further step toward more complicated and functional 3D structures, chemical functionalization at a similar size scale is necessary. By exploiting e-beam, FIB, and near-field optical lithography, it is
possible to pattern SAMs directly. Using lithography, the SAMs can either be locally destroyed and refilled with other molecules, or the surface of the SAMs can be activated to allow further chemical reactions. The resulting patterned surfaces will be chemically patterned. Patterns can be introduced to incorporate H-bonding, pi–pi stacking, or chemical reactivity. These patterned areas will be used as templates to direct the vertical assembly of stacks of molecules or to direct the growth of polymeric molecules. Large, extended aromatic molecules prefer to stack on top of each other due to pi–pi stacking. These molecules have interesting electronic properties as molecular wires. When surfaces can be patterned to incorporate “seeds” for the large aromatic molecules, the stacking can be directed away from the surface. The initial 2D pattern will thus be translated into 3D nanosized objects. With the full control over the interfacial properties, it will be possible to release the objects from the templates and transfer them to another substrate, after which the nanopatterned surface can be used again to provide an inexpensive replication technique.

11.4 Self-Assembling Growth of Molecules on the Patterned Templates

The self-assembling growth of the MT-2 proteins is demonstrated as follows. One mg/mL magnetic MT in Tris. HCL buffer solution was placed onto the patterned surface, and an electric field with an intensity of 100 V/cm was then applied for 5 min to drive the MT molecules into the nanopores. The sample was then washed with DI water twice to remove the unbounded MT molecules and salts on the surface (the schematic of the process is also shown in Figure 11.7a. Figure 11.8 shows the atomic force microscopy (AFM) image of the template surface with 40 nm nanopores after they were filled by the MT-molecules. Keep in mind that most of the Si surface was still protected by photoresist after the etching processes, which has prevented the MT-molecules from forming strong OH bonds with the Si surface underneath. Therefore, the electrical field-driven MT molecules were all anchored on those areas that were not covered with photoresist. The molecules landing in each pore were then self-assembly grown vertically from the bottom of the pore into the shape of a rod (as shown in Figure 11.8). These molecular nanorods have an average height of ~120 nm above the template surface and a diameter equal to the size of the nanopore.

![Flowchart of the lithography, etching processes, and growth of protein molecules](image1)

![Three-dimensional AFM image of the patterned magnetic molecules](image2)

**FIGURE 11.7** (a) Flowchart of the lithography, etching processes, and growth of protein molecules, (b) schematics of the patterned templates with nanopores. (From Chang, C.-C. et al., Biomaterials, 28, 1941, 2007. With permission. Elsevier.)

**FIGURE 11.8** Three-dimensional AFM image of the patterned magnetic molecules. The molecules have self-assembled to grow into a rod shape. (From Chang, C.-C. et al., Biomaterials, 28, 1941, 2007. With permission. Elsevier.)
However, experiments on the templates with pore sizes larger than 100 nm gave quite different results. Figure 11.9 shows the two-dimensional AFM image of the template surface with larger pores, where we can see that the molecules did not grow vertically above the template surface. Therefore, we were not able to generate 3D images of this type of template. However, judging from the AFM phase images, the MT molecules did form a more dense structure in the larger pores compared with the case of the smaller pores. On templates with thinner photoresist and smaller pitch sizes (less than 600 nm), we also found that the molecules anchored in the pore can grow laterally toward the neighboring pores (data not shown).

By increasing both the e-beam exposure and dry etching time on the Si surface covered with a thin photoresist layer with a thickness of less than 150 nm, we were able to create a ring-type area with an exposed Si surface along the periphery of the nanopores. The SEM image of this type of template is shown in Figure 11.10. On this particular template, the molecules not only independently grew inside the pores, but they also grew along the circumference of the pores to form molecular rings on the template. Figure 11.11 shows the two-dimensional (2D) and three-dimensional (3D) AFM images of such molecular rings.

In order to gain better control of the formation of molecular nanostructures, it is important to uncover the underlying self-assembling growth mechanism. Molecular self-assembly
can be mediated by weak, noncovalent bonds—notably hydrogen bonds, ionic bonds (electrostatic interactions), hydrophobic interactions, van der Waals interactions, and water-mediated hydrogen bonds. Although these bonds are relatively insignificant in isolation, when combined together as a whole, they govern the structural conformation of all biological macromolecules and influence their interaction with other molecules. The water-mediated hydrogen bond is especially important for living systems, as all biological materials interact with water. We believe that the first layer of proteins anchored inside the nanopores was bonded with the Si surface dangling bonds. They have provided building blocks for proteins that arrived later. With the assistance of spatial confinement from the patterned nanostructures, the rest of the proteins are able to self-assemble via the van der Waals interactions and perform molecular self-assembly.

11.5 Magnetic Properties of Molecular Nanostructures

The magnetic properties of the self-assembled molecular nanorods were investigated with magnetic force microscopy (MFM). We monitored the change of contour of a particular nanorod on the template when an external magnetic field was applied. Figure 11.12a shows the MFM image of the nanorod without the external magnetic field. In Figure 11.12b, a magnetic field of 500 Oe was applied during the measurement with a field direction from the right to left. The strength of the field was kept at a minimum so as not to perturb the magnetic tip on the instrument. In Figure 11.12 we can see clearly that the contour of the nanorod has changed in shape as compared to the case with no applied field. It indicates that the molecular self-assembly carries a magnetic dipole moment that interacts with the external magnetic field.

11.6 Conclusion and Future Perspectives

Success in the synthesis of the magnetic molecules produced from metallothionein (MT-2) by replacing the Zn atoms with Mn and Cd has been demonstrated in this chapter. Hysteresis behavior in the magnetic dipole momentum measurements was observed over a wide range of temperatures when an external magnetic field was scanned. These magnetic MT molecules were also found to self-assemble into nanostructures with various shapes depending on the nanostructures patterned on the Si templates. Data from the MFM measurements indicate that these molecular self-assemblies also carry magnetic dipole momentum. Since the pore size, spacing and shape can easily and precisely be controlled by lithography and etching techniques, this work should open up a new path toward an entire class of new biomaterials that can be easily designed and prepared. The techniques developed in this particular work promise to facilitate the creation of many bio-related nanodevices and spintronics. Magnetic molecular self-assembly may find its use in data storage or magnetic recording systems, as an example. They can also act as spin biosensors and be placed at the gate of the semiconductor spin valve to control the spin current from the source to the drain. More importantly, this work should not be limited to MT-2 molecules.
and should be extended to other type of molecules and proteins as well. As mentioned in the beginning of this chapter, the various surface patterning techniques developed over the years to interface organic or biological materials with semiconductors have not only provided new tools for controlled 2D and 3D self-organized assemblies, but have also been essential to the creation and emergence of new semiconductor-molecular nanoelectronics as recently discussed by Likharev.64

In the future, it is important to develop techniques for growing and characterizing molecular self-assembly, single nanostructure, and molecule on semiconductor templates to bring a measure of control to the density, order, and size distribution of these molecular nanostructures. Using self-assembly techniques, one can routinely make molecular assembly with precise distances between them. Recent explorations of molecular self-assembly have sought to provide transverse dimensions on the mesoscopic nanometer scale. As a general—although not inviolate—rule, these attempts have led to very good local ordering (e.g., nearest neighbors).

We can anticipate, (1) the development of new (supra) molecular nanostructures via the self-assembling method (bottom up technique) and immobilization of single nanostructure or molecule; (2) the design of methods to functionalize molecular self-assembly and devices; (3) an integration of bottom-up and top-down procedures for the nano- and microfabrication of molecularly driven sensors, actuators, amplifiers, and switches; and (4) an increased understanding and appreciation of the science and engineering that lie behind nanoscale processes. All this and more is in the nature of the nanotechnology bonds as it impacts on biology and beyond. In the final analysis, however, the practice of biological synthesis that relies on molecular recognition and self-assembling processes within a very much more catholic framework than is currently being contemplated by most researchers that will dictate the pace of progress in synthesis.

The final goal is to understand this whole notion of what self-assembly is. One needs to really learn how to make use of the methods of organizing structures in more complicated ways than we can do now. On a molecular scale, the accurate and controlled application of intermolecular forces can lead to new and previously unachievable nanostructures. This is why molecular self-assembly (MSA) is a highly topical and promising field of research in nanotechnology today. MSA encompasses all structures formed by molecules selectively binding to a molecular site without external influence. With many complex examples all around us in nature (ourselves included), MSA is a widely observed phenomenon that has yet to be fully understood. Being more a physical principle than a single quantifiable property, it appears in engineering, physics, chemistry, and biochemistry, and is therefore truly interdisciplinary.

References

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