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REVIEW

Nanomanufacturing and Characterization Modalities for Bio-Nano-Informatics Systems

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In the next decade or two, the feature size of microelectronic devices will continue to decrease and is eventually expected to reach fabrication and material limits. With the field of microelectronics rapidly approaching the end of its roadmap, the National Nanotechnology Initiative (NNI) was created for the purpose of creating new technologies and to maintain the momentum of continuous scientific and technological progress. Primarily, the fields of nanoscience and nanotechnology aim to synthesize, characterize, apply, and control macro functional molecules and consist of three areas. First, the area of bio-nanotechnologies concerns that of biological molecules such as DNA, the molecule that serves as the blueprint of all living organisms. Harnessing the intrinsic functionality of these nano-sized biological molecules, i.e., DNA/RNA and proteins, will yield enormous potential for a wide array of applications (biomedical, energy, sensing, etc.) Second, diminishing electronic device feature sizes has spurred the development of new techniques for nanoelectronics and has emerged as a critical area of research. Third, these macro functional molecules possess rich potential for various new nanomaterials that have applications in bio-nano and nanoelectronics industries. Given the range of devices and applications that may be generated and addressed, respectively, through the fruition of these areas, development of novel and advanced core characterization and nanomanufacturing technologies will serve as a requisite strategy toward the realization of the potential underlying nanotechnological development. As such, this review will address how these novel technologies will be used to achieve a true coalescence of nanoscience and nanotechnology. This, in turn, will ultimately benefit the human condition by using the building blocks and fundamental findings of nanoscience to develop systems based on the fusion of biology, nanotechnology, and informatics, with embedded intelligence and emergent behavior.

Keywords: Bionanotechnology, Nanomanufacturing, Characterization, Materials, Medicine, Protein, DNA, RNA, Atomic Force Microscopy.

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1. INTRODUCTION

A universal goal of technological development, including nanotechnology, is the enrichment of human life. A disparity of nine orders of magnitude separates the length scales of a human (a meter) and functional molecules (nanometers) which in turn presents significant technical challenges. This goal will ultimately be achieved through the development of a definitive pathway that uses existing and future technology to paint the new roadmap from the nanoscale to benefitting human life.

The human body is an extremely intelligent and complex adaptive system. The DNA/RNA and protein molecules, which drive its natural processes, possess dimensions on the nanometer range. The challenges in

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exploring the governing mechanisms across a wide span of length scales is best stated by Anderson in his paper published in Science (1972) as "at each level of complexity entirely new properties appear, and the understanding of the new behaviors requires research which I think is as fundamental in its nature as any other." For example, a cell fuses genetic informatics with nanoscale sensors and actuators to result in perhaps one of the most efficient and autonomous micron-scale "factories." The richness in the science within the three orders of magnitude difference in length scale is far beyond our full understanding. The question of how we will span the length scales of these nano-scale capabilities which will eventually enable us to enrich human lives is not an obvious, but a key task. These basic processes that occur at the molecular level have opened up a world where the integration of individual components can eventually derive higher-order functionalities, or *emergent properties*. This leads us towards a compelling approach by fusing biotechnology, nanotechnology, and information science, which will enrich the development of revolutionary application-specific technologies.

Nanotechnology has driven the production of molecular-scale devices towards the functionalizing of materials, production of electricity or other biocompatible energies, or a novel framework by which nanosystems can be assembled. Biotechnological advancements have enabled scientists to physically manipulate genetic pathways to generate nutrients and fuels from waste products, or engineer strains of proteins to possess novel functionalities. Information technology then serves as the catalyst to organize and to functionalize the bio-nano systems. Bio-nano-informatics fusion will culminate in systemic architectures that will rival those that have taken millions of years to come to fruition in nature. With this will come the hope of achieving a fundamental comprehension of how the interplay of

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Dan Garcia received his M.S. in 2005 from the UCLA Bioengineering Department. He is currently pursuing his Ph.D. in the Department of Bioengineering and working in the laboratory of Professor Chih-Ming Ho. He is actively researching the development of the actin muscle protein for a bottom-up/top-down hybrid nanofabrication process to construct two- and three-dimensional nanostructures as defined by Micro/Nano-Electrical-Mechanical Systems (MEMS/NEMS) and micro/nanofluidics technology. Prior to joining Dr. Ho's group, Dan conducted immunology research in the laboratory of Dr. Genhong Cheng, specifically in the area of CD40 signal transduction. In 2003, Dan graduated Phi Beta Kappa from UCLA with a degree in Microbiology, Immunology, and Molecular Genetics.

Dr. Chih-Ming Ho received his Ph.D. from The Johns Hopkins University and currently serves as the Ben Rich-Lockheed Martin Chair Professor in the UCLA School of Engineering. He is the Director of the Institute for Cell Mimetic Space Exploration (CMISE). He is an internationally-renowned scientist in micro/nano fluidics, bio-nano technology and turbulence. He was ranked by ISI as one of the top 250 most cited researchers worldwide in the entire engineering category and by ECI as number 7 in the field of Micro-electrical-mechanical-systems (MEMS). In 1997, Dr. Ho was inducted as a member of the National Academy of Engineering. In the next year, he was elected as an Academician of Academia Sinica. Dr. Ho holds five honorary professorships. He has published 240 papers and presented over 100 keynote talks in international conferences. Dr. Ho was elected Fellow of the American Physical Society as well as American Institute of Aeronautics and Astronautics for his contributions in a wide spectrum of technical areas.

these three areas can be manipulated on the molecular level to produce enhanced, emergent properties and will address the question of how much further we are able to push the envelope.

In order to forge the birth of a nano-industry, we need to further our understanding in the *nanosciences*. During the past decades, generations of distinguished scientists in biology, physics, and chemistry have made tremendous contributions towards the comprehension of nanoscale molecules and their functionalities. The initiation of the NNI has further increased the momentum of new progress in these areas. *Nanotechnology*, on the other hand, is still in its infancy but it is the key to realize the advent of a new industry. The following definitive roadmap outlines requisite components towards the fruition of nanotechnology:

- Core technologies (Section 2) must be developed to enable us to visualize, pick, place, manipulate, and characterize nanoparticles with a high degree of precision.
- We then need to establish technologies for manufacturing (Section 3) and systemic integration aimed towards larger length scales with higher information content embedded within the composite structures.
- We need to explore capabilities of displaying emergent behavior (Section 4) for increasing functionalities across all of the length scales.

Instead of providing a comprehensive review, this analysis will use examples primarily based on our experiences to illustrate the challenges and the potential associated with the impacting field of bio-nano-information fusion.

2. CORE TECHNOLOGIES FOR HANDLING NANOPARTICLES

The first step towards the understanding or manufacturing of a nanoscale subject is to pick, place and see the subject. Given the dimensions of these nanoscale particles, a new set of interrogative techniques needs to be developed. Beyond the development of novel technologies, existing methodologies have found emerging applications in investigating nanoscale phenomena. For example, the atomic force microscope, once used primarily for surface topology studies, is now a foundational method for nanoscale manufacturing and molecular manipulation. A set of exploratory tools comprised of existing and maturing approaches will enable the handling of nanoparticles and the gleaning of information never before possible, both of which will play a key role in realizing the developmental goals of nanotechnology.

2.1. Visualizing

Perhaps one of the most beneficial tools afforded to the process of realizing the full potential of nanotechnology will be the ability to visualize nanoscale functioning in real time. Continually advancing imaging methods have pushed the boundaries of nanoscale resolution. Transmission Electron Microscopy (TEM), one of the more applicable approaches to nanoscale imagery, uses a highly focused beam of electrons to examine a thin sample, characteristically less than 200 nm thick. Photons produced by deflected, non-deflected transmitted electrons, backscattered and secondary electrons provide information regarding the interior of the measured sample. Furthermore, material shape, size, and phase distribution can be explored. Structural analysis can be conducted using crystallography to determine the crystal structure of the phases and the character of the crystal defects. Limitations of TEM, however, prevent it from being used to analyze live samples. As such, novel methods are evolving towards the ability to accomplish such a task.

Recently, Fang and Zhang utilized a material with a negative index of refraction to amplify a near field optical wave, creating a superlens that supplies a resolution of around 60 nm which is far below the optical diffraction limit.[1–6] In doing this, Zhang exploited a 36 nm silver, left-handed metamaterial (LHM) film with both a negative effective permittivity and permeability. A metamaterial film proves effective towards designing a superlens by facilitating the precise positioning of atoms and molecules as well as the manipulation of the lattice structure. The critical component of LHM materials lies in the resonance, and consequent excitation, of the surface plasmon on one surface of the material with that of another surface, thereby leading to the amplification of the near field optical wave.[2] These metamaterials displayed a THz magnetic response using a planar structure that is comprised of nonmagnetic conductive resonant elements. Another key factor concerning these materials is that the scaling of the device structures can be used to tune the effect of the material throughout the terahertz regime. This design enables the achievement of a high range of activity for negative-index materials. Through the use of this material, Zhang and colleagues have essentially developed the core technology for the first optical nanoscope. The continued enhancement of the resolution of this novel technology will enable discoveries into complex processes including receptor-ligand interactions, DNA translocation across cellular membranes, and beyond. Such a capability will revolutionize the ability to directly observe reactions upon which the functionality of future nanotechnological devices will be based.

2.2. Picking and Placing

Another important element of nanomanipulation involves the ability to pick and place nanoparticles in specific locations.[7,8] Scanning Tunnelling Microscopy (STM) has been well explored by Binnig and colleagues as an imaging methodology with unparalleled resolution.[9] In addition, STM studies have successfully demonstrated particle to surface transfer.[10] Eigler and Schweizer utilized the STM to position single xenon atoms on a single-crystal nickel

surface to form letters with 50 Å top to bottom dimensions.[11] As such, their methodology was also, in principle, applicable to single molecule picking and placing. STM tips are known to exert a force upon interacting atoms. Adjustment of the tip position and voltage can be used to modify the magnitude and direction of this exerted force. For the purposes of this study it was easier to demonstrate atomic placement through dragging with the STM as this required less force to accomplish than physically lifting the atoms off of the nickel surface. By positioning the tip over a specific atom, an increase in the tunnel current (to values ranging from $1-6 \times 10^{-8}$ A) resulted in the linkage between the two, signaled by the observation of a new tunneling current. The particle was subsequently dragged to a desired location and the tunneling current in the STM tip was reduced to separate the particle from the tip. Figure 1 shows the sequence of construction events used to assemble xenon atoms into a desired pattern on a nickel surface. Figure 2 shows how a linear multimer of xenon atoms was constructed by sequentially picking and placing the atoms

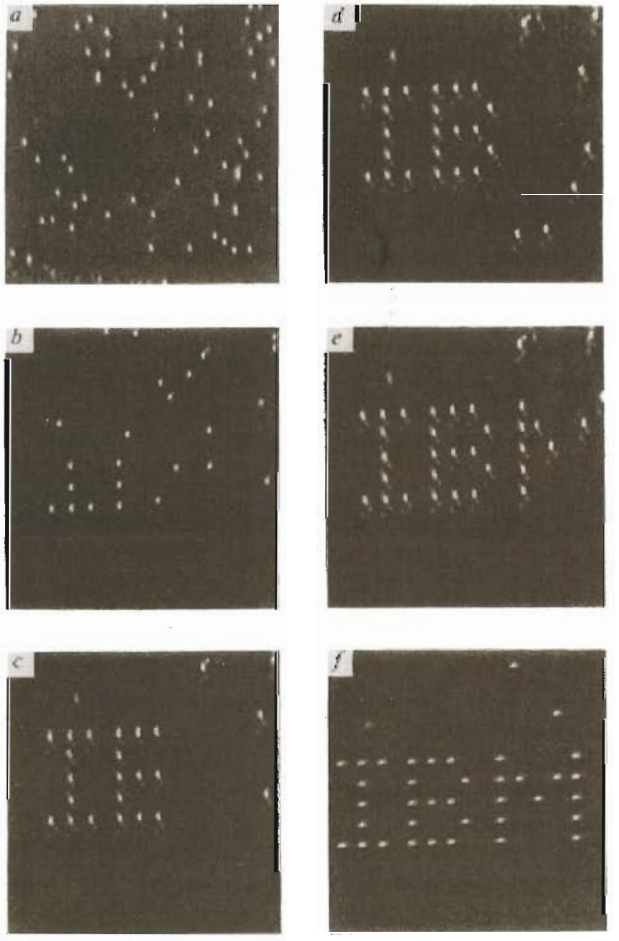

Fig. 1. (a) A nickel surface characterization with randomized xenon atoms. (b–f) A sequential assembly of the xenon atoms to acquire a desired pattern. Letters are 50 Å from top to bottom. Reprinted with permission from [11], D. M. Eigler and E. K. Schweizer, *Nature* 344, 524 (**1990**). © 1990, Nature Publishing Group.

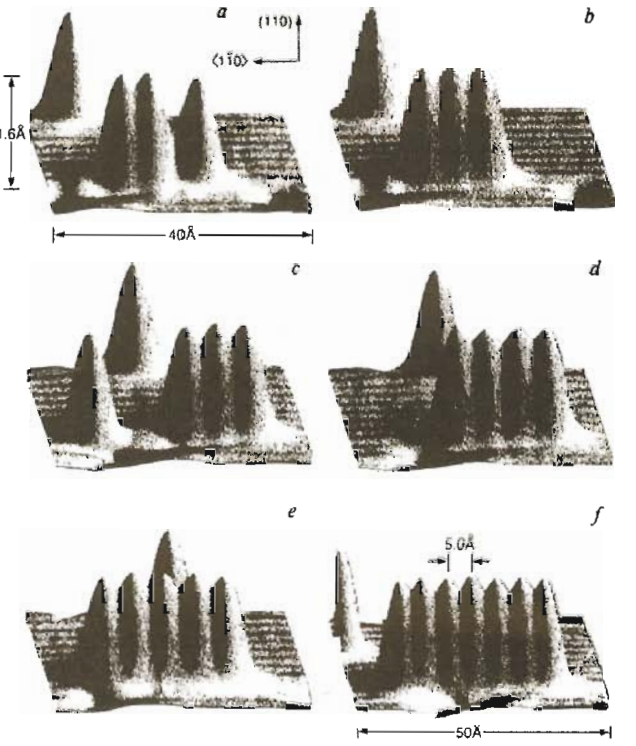

Fig. 2. Assembly of xenon multimer is shown here. A xenon dimer is observed in (a), while (b–f) demonstrates the sequential additions of xenon to yield a multimeric structure. The xenon atoms are visible at 1.6 Å protrusions while the nickel atoms are represented by the light/dark stripes. Reprinted with permission from [11], D. M. Eigler and E. K. Schweizer, *Nature* 344, 524 (**1990**). © 1990, Nature Publishing Group.

in a juxtaposed fashion to form a continuous row. The images clearly show that the STM tip was able to identify a specific row of nickel surface atoms, shown by the black and white lines on the base of the scan. The STM was subsequently used to position the xenon atoms along this specific nickel row to form dimers, and a continuous addition of atoms resulted in a multimeric aligned structure.

This STM-based atomic positioning study was compelling in the sense that it achieved picking/placing with such a degree of accuracy that the xenon atoms could be placed within 5 Å of each other in a highly ordered arrangement. Bio-nanotechnologies based on protein-protein interactions, for example, or nano or atomic-scale electronics and logic gates may all rely critically on molecular positioning. As such, this method represents an approach based on the derivation of novel applications from existing technologies. An obvious limitation would be the speed of fabrication, though continued development in the area of nanomanipulation will seek to enhance future system performance.

While initial fundamental studies were able to demonstrate the ability to accurately place molecules through precise positioning, eventual applications of this technology include directed chemical reactions, as well as molecular orientation capabilities.[11] More specifically, biomolecules could be placed in a fashion to induce coupling reactions

commonly found in natural bioenergetic processes. This would allow for the fabrication of truly biomimetic systems that harness these processes for practical uses. In addition, molecular orientation capabilities would play an important role in nanosystem scale-up manufacturing processes. Developing these capabilities does not necessarily require the invention of novel technologies, however, as existing methods have also been applied to the study of nanoscale phenomena, resulting in important findings.

2.3. Manipulating

Taking advantage of the properties of biological molecules on the nanoscale will require the successful manipulation of particles according to pre-designed parameters. Currently, technology harnesses the ability to deform individual molecules such as proteins or DNA. Not only will these methodologies result in the fusion of biology with nanotechnology, but it will also foster an increased understanding of biological macromolecules and their properties on the nanoscale. The exploitation of biomolecules will inherently involve the application of their natural characteristics. For example, a self-assembling biological system could be employed in a bottom-up fabrication scheme. Well known is the fact that the majority of living systems grow according to external forces. For instance, neurons have been shown to grow along electrical field lines. Using electrokinetics, neurons could be cultivated across electrodes to produce a hybrid circuit, composed of biological and synthetic components. The fusion of biology with nanotechnology will not only result in technological innovation, but will also encourage future research into natural biological nanosystems.

For instance, in the realm of nucleic acid detection with a fluorescence detection scheme, it is optimal to minimize the detection limit. This could be accomplished by maximizing the fluorescence intensity of the nucleic acid probe which would effectively increase the signal-to-noise ratio, thereby raising the sensitivity of the system. However, for this to occur, the particles must be focused to a minimal region, a process that requires the successful manipulation of the DNA molecules. Entropy favors the coiling of DNA molecules at equilibrium such that the probe binding sites are not easily accessible. Thus, an additional way to facilitate nucleic acid detection would be to decrease the entropic state of the DNA molecule by stretching it into a long, linear polymer.

DNA is a nanoscale long-chain macromolecule, and behaves like a mass-spring system. The manipulation of the DNA molecules would obviously prove critical in the stretching of the molecules. Placing the DNA molecule inside of a viscous flow and subjecting it to hydrodynamic focusing will stretch the DNA molecule from its coiled position at equilibrium by applying a velocity shear stress across the length of the molecule.[12, 13] To utilize the principles of hydrodynamic focusing to stretch DNA molecules,

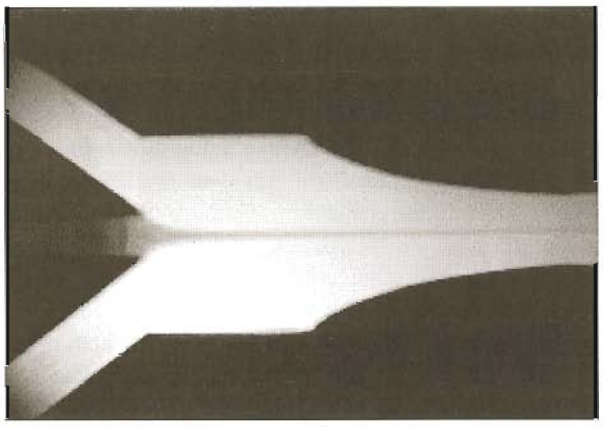

Fig. 3. A microchannel that enables the convergence of 2 buffer streams and a centerline DNA stream is shown here. This design allows for single molecule extensional control based upon the manipulation of outer channel flow rates. Reprinted with permission from [16], P. K. Wong et al., *J. Fluid. Mech.* 497, 55 (**2003**). © 2003, Cambridge University Press.

Wong and colleagues designed microfluidic channels with two buffer streams that converge to sandwich a middle stream of DNA solution (Fig. 3).[14–16] Advantages possessed by hydrodynamic focusing included the fact that DNA extensional rates were dependent upon the flow rate of the outer buffer streams. As such, pre-shear of the DNA at the centerline could be minimized, and the low mixing among the streams enabled independent DNA control (Fig. 4).

An important aspect of molecular manipulation includes the minimization of harmful conditions that may impede molecular function. Hydrodynamic focusing of DNA serves as an example of a modality that applies fundamental principles to advanced molecular manipulation and can achieve single molecule applicability under favorable conditions. Nanotechnological development will rely on continued creation of molecular manipulation methods that meet several of these requirements.

2.4. Biomolecular Characterization

A precursor to the usage of nanoparticles for practical, beneficial purposes will be the ability to characterize their properties with respects to composition, structure, etc. Biomolecular characterization, with respects to proteins,

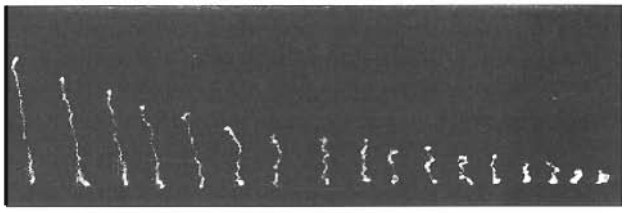

Fig. 4. Relaxation of a DNA molecule after hydrodynamic stretching is shown here in 2.5 second increments. Reprinted with permission from [16], P. K. Wong et al., *J. Fluid. Mech.* 497, 55 (**2003**). © 2003, Cambridge University Press.

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for example, has provided key information into three-dimensional structure, as well as mechanistic behavior. Information gleaned from these studies serves as the foundation for current development of devices based purely on biomolecular function.[17–20] Another important application of characterization technologies involves the investigation of DNA. Towards the realization of improving the human condition through nanotechnology, a full-scale characterization of DNA will elicit a broader understanding of the body of embedded information that governs this condition.

In the evolving fields of medical genetics and medical diagnostics, physicians rely heavily upon nucleic acid detection as a source for evaluating their patients and making devisions. Unfortunately, however, nucleic acid detection suffers the drawback of requiring an amplification procedure in order for the nucleic acid concentration to reach the detection limit of current DNA diagnostic systems. Most commonly, this amplification is satisfied through the Polymerase Chain Reaction (PCR). The incorporation of PCR into the procedure aggrandizes the expenses by necessitating the hiring of additional laboratory personal and augmenting the overall time of the medical test. Of further consequence, the amplification process introduces errors into the nucleic acid sequence, thereby complicating data interpretation. Thus, the elimination of PCR from medical diagnostics would reduce cost, personnel numbers, and increase the reliability of the tests. The exclusion of PCR would facilitate another goal of medical diagnostics, that is, to make the tests portable and real-time. A growing theme in the world of Micro-Electrical-Mechanical Systems (MEMS) is the development of integrated, or lab-on-a-chip systems, whereby an entire medical test may be conducted on a small, portable chip.[21,22] Eliminating the need for DNA amplification will bring that goal one step closer to reality.

Wang and colleagues have previously used a Laser Induced Fluorescence (LIF) technique to recognize a single base pair mutation using a single reporter molecule.[23–26] By integrating an electrokinetic focusing technique with laser-induced fluorescence detection they have pushed DNA/RNA sensor performance to the ultimate limit, that of a single base pair of a single molecule. Furthermore, three-dimensional electrodes were used to effectively concentrate the DNA/RNA molecule such that the biomolecule can be detected in very diluted concentrations (Fig. 5). As the distances between electrodes ranged from 20–100 μm, an applied voltage as low as 1 V resulted in non-hydrolysis of water as well as electric fields in excess of 100 V/cm. This design enabled DNA to be confined to a limited laser beam focal region with volumes as low as 100 fL (Fig. 6). As such, solvent-induced Rayleigh and Raman scattering of the laser beam was minimized due to the small solvent/sample volume, enabling increased signal to noise ratios and the detection of single molecule fluorescence bursts above this background.

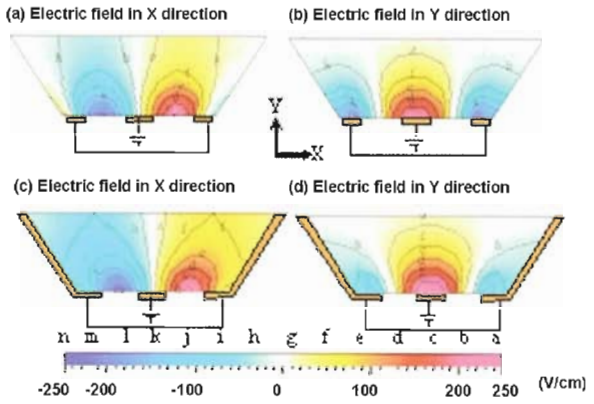

Fig. 5. 2-D electrode-induced electric fields are shown in (a) and (b), while 3-D electrode-induced electric fields are shown in (c) and (d), both with applied voltages of 1 V. Reprinted with permission from [23], T. H. Wang et al., *The 15th IEEE International Conference on Micro Electro Mechanical Systems* 15, 15 (**2002**). © 2002, IEEE.

With respects to rapid DNA sequencing, the Human Genome project took ten years to accomplish. The ability to rapidly sequence an individual person's DNA would revolutionize biomedical progress, and its potential for health maintenance and drug development would be enormous.[27–36] A pioneering methodology with specific respects to the interrogation of composition/structure has been the use of nanoscale pores to characterize DNA molecules. Meller and colleagues have utilized both an α-hemolysin channel from Staphylococcus aureus, as well as ion beam-sculpted nanopores fabricated in silicon

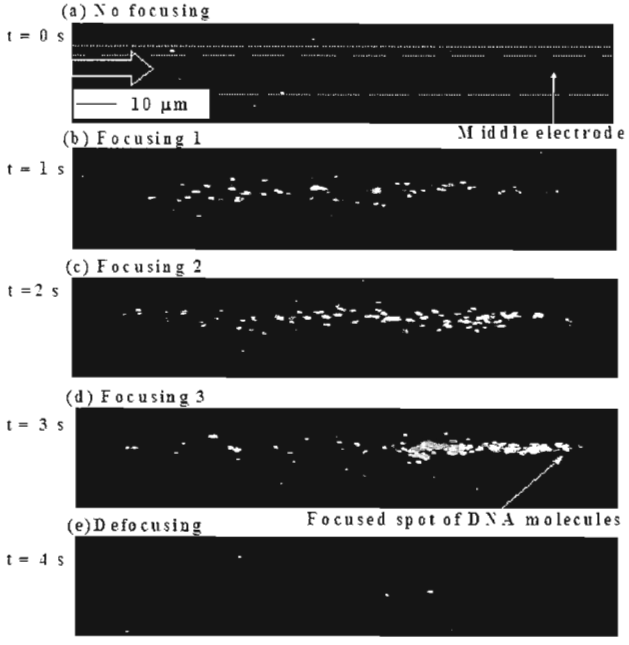

Fig. 6. CCD images of the focusing and de-focusing of DNA molecules are shown here. (d) shows the focused spots of DNA which immediately disperse upon defocusing shown in (e). Reprinted with permission from [23], T. H. Wang et al., *The 15th IEEE International Conference on Micro Electro Mechanical Systems* 15, 15 (**2002**). © 2002, IEEE.

nitride (Si_3N_4).[31] Monitoring of changing conductance values between Ag/AgCl electrodes separated by the nanopore produced important information regarding the nucleotide composition, and the effects of temperature on DNA transport, etc.

The technique of using α-hemolysin provided an important basis for the use of conventional single-channel measurement methods to determine DNA translocation events across the channels, and hence served as a major advance in the field of nanoscience. Reconstitution of the membrane protein in a lipid membrane enabled investigators to utilize a voltage bias to drive DNA molecules through the pore of the protein (1.5–2 nm diameter). Individual transport events could be observed through the transient decrease/increases in conductance across the pore that corresponded to transport/exit of the DNA strand. Based upon varying conductance characteristics of traversal events, specific nucleotides could be distinguished. Current tracing results were able to discern between transport events involving adenine polymers (poly dA_{100}) as well as cytosine polymers (poly dC_{100}) (Fig. 7). As such, these studies provided the capabilities of investigating several characteristics of the analyzed molecule, from chain length, to exact composition and structure. Information extracted from these tests provided insight into the complexities of biomolecule transport across cellular membranes, or even phage-induced DNA injection into cells.

The use of membrane proteins to examine DNA strands possessed certain limitations, including the range of conditions in which characterizations could be performed, as the α-hemolysin, and lipid membrane possessed their own range of optimal conditions for preserved activity. For example, the high temperatures and denaturants necessary to preserve single-stranded DNA in solution were often disrupting towards hemolysin stability. Furthermore, these limitations in turn confined the types of molecules that could be analyzed as the hemolysin protein pore dimensions were set at 1.5–2 nm. In addition, as the length of the hemolysin channel was approximately 5 nm, anywhere between 10–15 nucleotides could have resided within the channel at a single traversal moment, decreased resolution was often encountered. To build upon the groundwork that was established by protein/lipid-based characterization, Chen and colleagues utilized Si_3N_4-based nanopores to serve as solid-state characterization systems.[37] Not confined to the same set of limitations observed with a protein-based setup, the solid-state nanopores were able to successfully detect DNA translocation events (Fig. 8) while possessing the versatility of possessing varied pore diameters to decrease steric hindrance to DNA translocation, etc. Translocation measurements were able to provide insight into the electrophoretic mobility of the molecules as a function of voltage bias. Tests utilizing varying bias

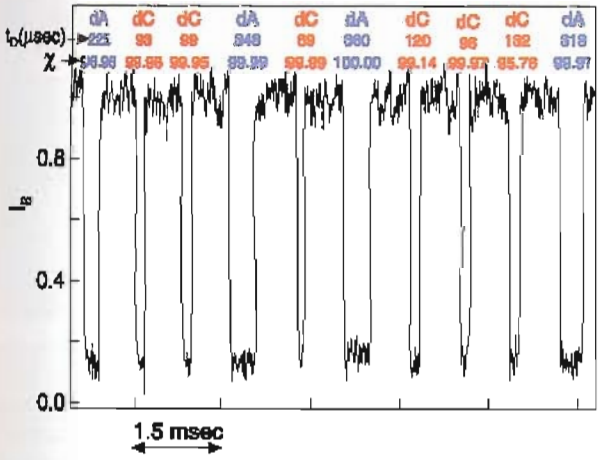

Fig. 7. Translocation events of both poly$(dA)_{100}$ and poly$(dC)_{100}$ are shown here. Differentiation between dA and dC translocation was determined using t_D provided in μs. Reprinted with permission from [31], A. Meller et al., *Proc. Natl. Acad. Sci. USA* 97, 1079 (**2000**). © 2000, The National Academy of Sciences (USA).

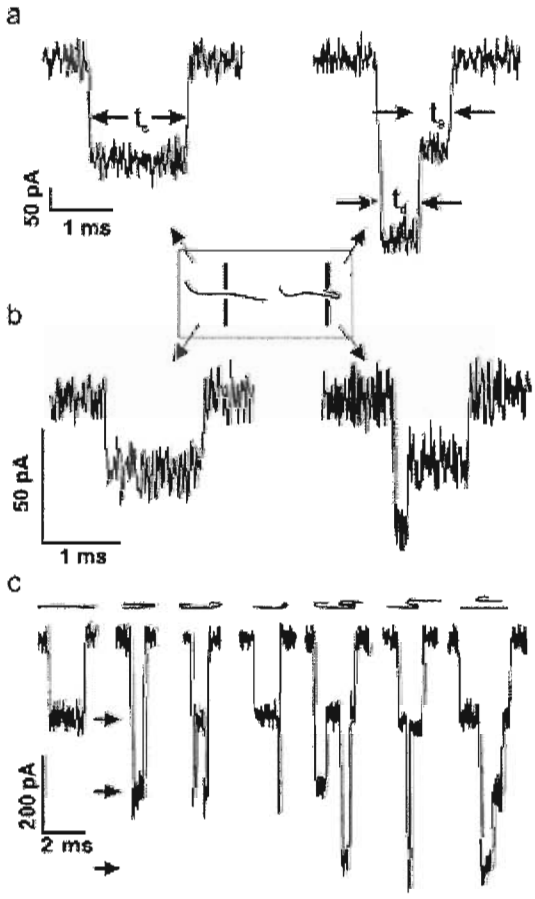

Fig. 8. Translocation across fabricated nanopores is shown here along with graphical interpretation of translocation geometries. (a) Blockade events are shown with DNA translocating either with an unfolded conformation, or with the leading section of molecule folded upon itself shown graphically. (b) Blockade events through nanochannels demonstrate similar behavior in terms of translocation conformations of the DNA. (c) Seven translocation events are observed here, each with the corresponding conformations above. Reprinted with permission from [37], P. Chen et al., *Nano. Lett.* 4, 2293 (**2004**). © 2004, The American Chemical Society.

voltages were able to show that an increased in bias voltage plays a role in linearizing double-stranded DNA to result in an increase in translocation of unfolded DNA. The evolution of nanopore-based molecular interrogation towards the solid-state will require an advanced methodology where pore length, diameter, and surface properties can be customized based on desired applications.[38–41] Furthermore, solid-state nanopores will withstand broader ranges of pH, temperature, pressure, as well as voltages that would normally impair the protein/lipid assembly to allow for the testing of a broader class of molecules in a wider range of environments.

The use of membrane proteins for DNA analysis served as a compelling methodology of investigating DNA molecules, and in a larger sense, represents an established characterization method using conventional techniques. These techniques are continually evolving, however, and are serving as the basis for transitioning towards solid-state nanopore sensors that will ultimately enhance fast sequencing DNA capabilities.

Atomic force microscopy (AFM) initially evolved as a valuable method in evaluating surface topography, largely due to its incorporation of a flexible cantilever containing a nano-sized tip, constructed of silicon nitride in the case of contact AFM or silicon in the case of tapping AFM. More recently, however, and occurring with greater frequency, is the utilization of AFM as a tool for the manipulation of nanoscale particles. For example, the field of AFM lithography exploits AFM to pattern photoresist on the nanoscale. The unparelled achievable resolutions using AFM-based techniques make it an ideal tool for nanolithography. Furthermore, AFM has also been applied towards the studies involving biomolecules, such as membrane and motor proteins. Possessing the capabilities for intimate exploration of these biological systems, atomic force microscopy has been used for protein-folding measurements. The importance in understanding protein folding mechanisms lies in the fact that protein engineering, protein-based device engineering, and even potential applications in folding-based health monitoring relies on fundamental understanding of the protein-folding quandary.[42,43]

As a consequence of their amino acid composition, proteins fold into a pre-defined shape, critical to their biological function. Interactions between their amino acid side chains dictate their folding process such that a modification to the amino acid sequence of a protein, depending on the nature of the substitution, has the potential to impede its natural function. While many of these studies could be performed using chemical or thermal-based denaturing tests, other proteins' properties, particular those that are biomechanical in nature, require direct measurement of structural characteristics. Studies performed by Rief and colleagues utilized AFM to probe reversible folding events in individual Titin Immunoglobulin domains.[44] By attaching these proteins to AFM tips, they were able to observe the unfolding of the protein to measure the restoring force as a function of deformation distance. In addition, findings demonstrated that individual unraveling events of the individual domains were observed, and that these domains could refold properly upon AFM tip relaxation.

Researching the nature of side chain interactions will foster a more complete understanding of the protein folding mechanism, which is one of the most fundamental self-assembly processes in nature. A firm comprehension of this process will provide insight into how proteins, considered the engines that drive cellular activity, assume these roles through autonomous, three-dimensional development. The use of atomic force microscopy is a prime example that demonstrates how existing technologies have been applied towards the understanding of a requisite component of future nanotechnological systems.

3. MANUFACTURING TECHNOLOGIES

Human benefit from advances in nanotechnology will stem from the transition from single nanoparticle handling to large-scale manufacturing capabilities. The spanning from nanometer to macroscales is a necessary process for transitioning the nanosciences to industry. Artificial fabrication technologies are usually considered "top-down," meaning that they involve manipulations at the macroscale, such as cutting or etching. Nature, on the other hand, relies upon bottom-up fabrication, involving molecule-by-molecule assembly to create a pre-designed subject. As such, the goal of artificial bottom-up fabrication involves the directed, and orderly integration of molecules at the nanoscale to form a macroscale device.

As time has progressed, technological innovation has produced functional systems of diminishing size. For instance, in the late 1980's, taking advantage of top-down photolithography technology from the Integrated Circuit (IC) industry, Fan, Tai and Muller (Fig. 9) produced the first micromachine with a moving part.[45] The electrostatic micromotor with a diameter on the order of one hundred microns could rotate at approximately 2 kHz. Another ten years later, Soong and colleagues developed a nanomotor, utilizing the ATPase molecule from the inner membrane of the mitochondria[46] (Fig. 10). Over time, we have successfully managed to fabricate and manipulate subjects of increasingly smaller size.[47–50]

An example of bottom-up fabrication is the self-assembling monolayer (SAM) used by chemists, such as the attachment of nano-sized motor molecules to cantilever beams to form actuators.[51,52] Various types of motor molecules have been explored. Among them, the rotaxane motor molecule, shown in Figure 11, has two separate recognition sites, each associated with a different energy potential.[53–79] Placing the rotaxane molecule in an oxidant, oxidizes the TTF site, thereby raising the energy level associated with the site.[80] As a consequence, the energy

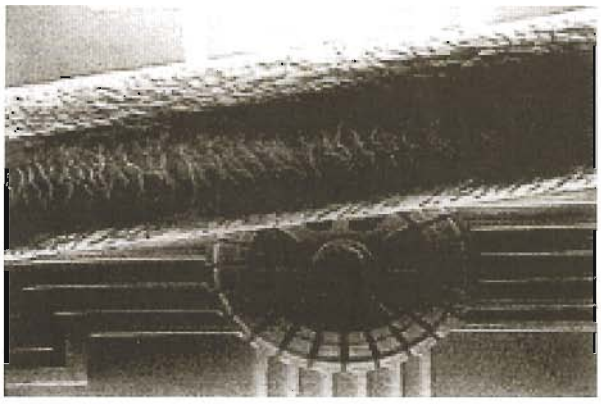

Fig. 9. Image of the first polysilicon-based electrostatic micromotor. Top down fabrication techniques included surface micromachining with polysilicon as the structural layer and silicon oxide as the sacrificial layer. Rotation was achieved by using electrostatic force field. Reprinted with permission from [45], L. S. Fan et al., *Technical Digest, IEDM* (**1988**), p. 666. © 1988, IEEE.

profile of the rotaxane molecule is altered such that the ring prefers association with the other recognition site, causing the ring to move. Applying a reductant will return the energy profile to its original state, forcing the ring back to its original position on the TTF site. As such, a molecular motor is created.

The molecules in solution can be transferred to a solid substrate using the Langmuir-Blodgett (LB) technique.[51, 52, 81] By using LB, the rotaxane molecules form a highly-packed, ordered film that can be used to

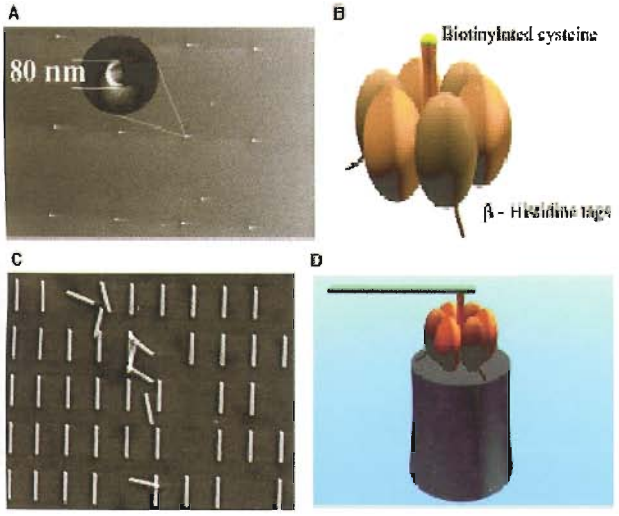

Fig. 10. An F1-ATPase biomolecular motor-powered nanomechanical device is shown here. (A) represented the nanoposts that were used for protein binding to the surface. (B) is a depiction of the engineered protein. The Fo component was removed from the engineered construct, and a cysteine residue was added to the γ-subunit for bioconjugation with the nanofabricated nickel rods found in (C). (D) is a graphical depiction of the fully-assembled system of the F1-ATPase interfaced with the nickel rods while bound to the nanoposts. Reprinted with permission from [46], R. K. Soong et al., *Science* 290, 1555 (**2000**). © 2000, The American Association for the Advancement of Science.

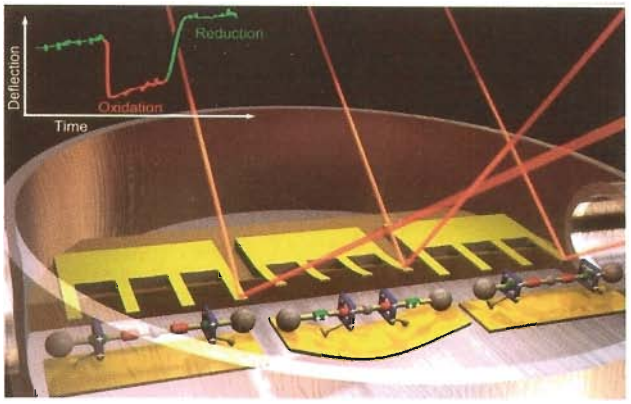

Fig. 11. Rotaxane molecule graphical representation. Alternating oxidation–reduction of the molecule results in movement of the ring structures. Reprinted with permission from [54], T. J. Huang et al., *Appl. Phys. Lett.* 85, 5391 (**2004**). © 2004, The American Institute of Physics.

harness the energy transfer that occurs during the movement of the ring.[82] To make an LB film, the rotaxane molecule is modified to make it amphiphilic, meaning that one end is made hydrophilic and another end is made hydrophobic. These molecules can then be deposited on a subphase of water to form monolayers with thicknesses of single molecules. The hydrophobic components of the molecules effectively preclude their solubilization into the subphase to maintain stable films at the air/water interface. Studying the isotherm curve of the rotaxane molecule reveals the surface pressures at which molecules form a highly-packed, ordered film on the surface of water. To transfer the rotaxane molecules to a substrate, a hydrophilic substrate is inserted into the water. Through the Langmuir-Blodgett technique, closely-packed ordered rotaxane monolayers can be transferred from a vapor–liquid interface to a vapor–solid interface.

To prove that the rotaxane molecules have formed a closely-packed, ordered film and still retain full-functionality as motor molecules, X-Ray Photoelectron Spectroscopy (XPS) is employed.[82] An X-ray projected at a certain wavelength will eject electrons of specific energies that are related to the distance of the atoms from the surface. In other words, when the atom is far from the surface, the amplitude will be low and when the atom is close to the surface, the amplitude will be high. Nitrogen atoms are located only in the ring that moves along the rotaxane molecule. Thus, the nitrogen atom may be used as a marker to determine the location of the ring along the rotaxane molecule. When an oxidant is added to a solution of rotaxane molecules attached to a solid substrate, an XPS peak is observed, confirming that rotaxane can be packed in a highly ordered, compact structure on a solid substrate.[82]

Further modifying the rotaxane molecule, a derivative was created that possessed two rings and four recognition sites. Anchoring the two rings to a gold surface created a molecular muscle that when placed in an oxidant, caused

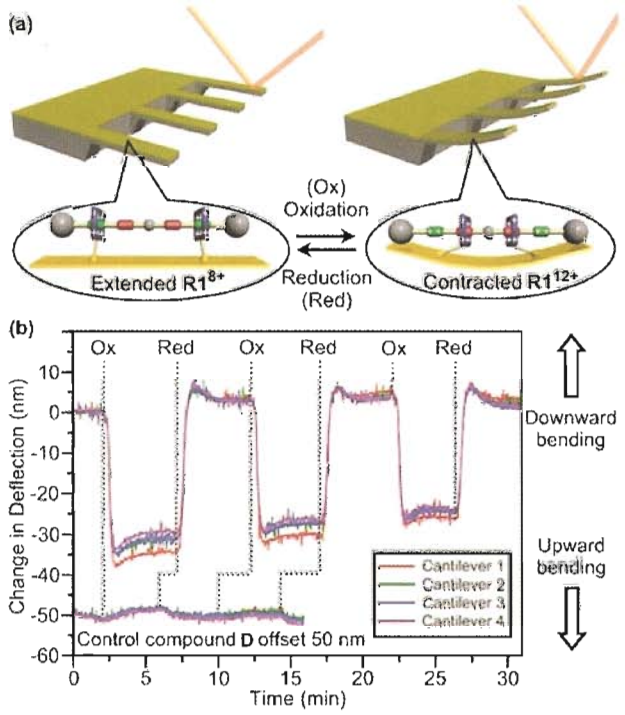

Fig. 12. (Top) A micro cantilever beam activated by nanoscale motor molecules is depicted. (Bottom) Injection of oxidation and reduction agents resulted in cantilever deflection due to contraction and extension, respectively, of the rotaxane molecule. Reprinted with permission from [54], T. J. Huang et al., *Appl. Phys. Lett.* 85, 5391 (**2004**). © 2004, The American Institute of Physics.

the two rings to move to the center of the molecule and bend the beam, a movement that can be detected by a laser beam. An array of molecular muscles attached to cantilever beams was created, as shown in Figure 12. Through alternating the application of oxidant and reductant, the beam bent upwards and returned to its original position. This experiment illustrated how the combination of top-down and the bottom-up fabrication techniques could be applied towards the formation of an integrated nano-micro mechanical system.

Organismal behavior possesses the ability to self-assemble inorganic and organic materials with awe-inspring degrees of perfection, producing flawless orientation alignment, shape, etc. A biological approach towards top-down/bottom-up integration would then serve as an ideal means for autonomous fabrication of macroscale devices.[83–92] Flynn and colleagues have investigated the M13 bacteriophage that infects bacteria in an effort to take advantage of this natural mechanism and apply it to the formation of inorganic nanowires.[93] A circular single-stranded molecule of DNA is contained inside of a flexible cylinder covered by around 2700 copies of the major coat protein pVIII. Additionally, at one end of the virus particle are five copies of the minor coat protein pIII. At the other end of the particle are five copies of the minor coat protein pIX.

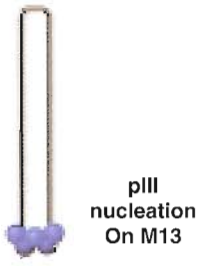

Fig. 13. Nucleation of M13 pIII minor coat protein with nanoparticles. Reprinted with permission from [93], C. E. Flynn et al., *Acta Materialia* 51, 5867 (**2003**). © 2003, Elsevier (Publisher)/Acta Materialia Inc.

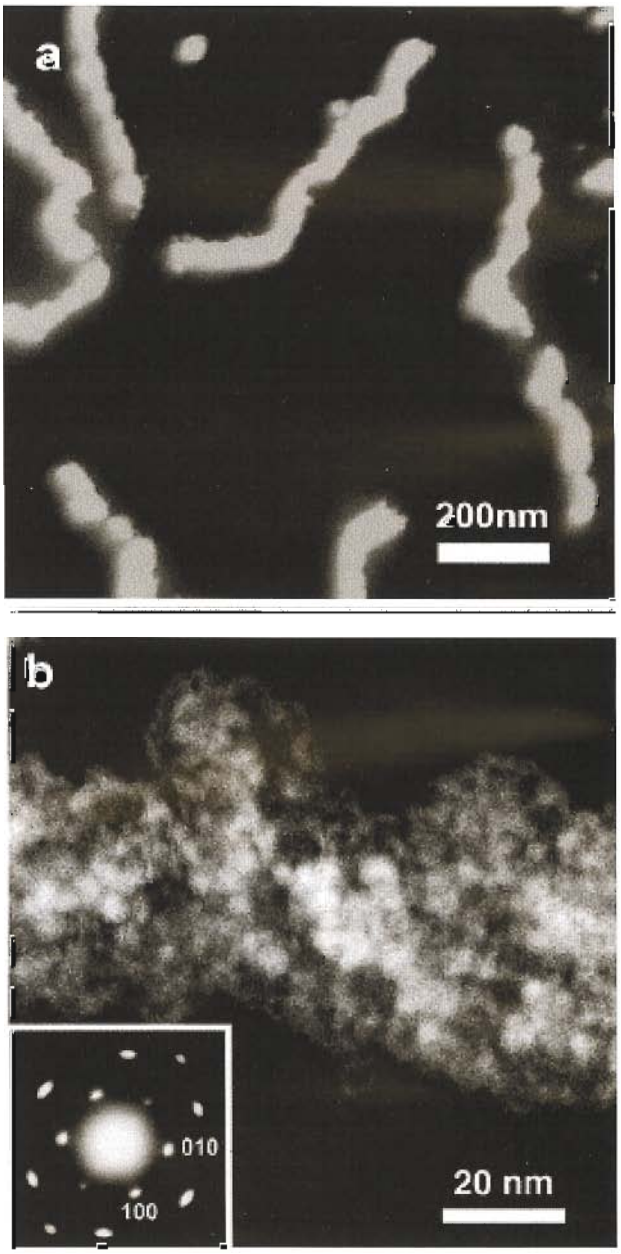

Fig. 14. (a) Nanowires created via nucleation of pIII minor coat protein with ZnS nanoparticles (b) image showing many nanoparticles on viral coat surface. Reprinted with permission from [93], C. E. Flynn et al., *Acta Materialia* 51, 5867 (**2003**). © 2003, Elsevier/Acta Materialia Inc.

Genetic engineering has made possible the modification of the minor and major coat proteins to produce virus particles with affinities for different substrates. Combinatorial libraries of M13 viruses used native M13 genomes containing random inserts into a specific gene, such as the one for a minor coat protein, to produce close to 1×10^9 different random peptide sequences as N-terminal fusions on the pIII viral coat protein, for example, on different M13 particles. Using a peptide combinatorial library, it is possible to select for phage peptides that have a binding specificity for different substrates.

Flynn and colleagues utilized viral-displayed peptide libraries with inorganic targets to engineer M13 bacteriophage particles that bind specifically to III–V semiconductor materials such as GaAs and InP as well as II–VI semiconductor materials such as ZnS and CdS, diagrammed in Figure 13.[93] Using the M13 bacteriophage with a genetically-engineered pIII protein, its possible to create self-assembling thin films of M13 phage on a specific substrate. Furthermore, it is also possible to nucleate the pIII protein sites on the M13 phage with nanocrystals, such as ZnS. The major coat protein pVIII can also be engineered to express fusion proteins that self-assemble into the ordered viral coat structure. Peptide sequences can be selected for the pVIII protein that have an affinity to bind ZnS or CdS and influence the nucleation and growth of nanoparticles. This foundational example demonstrated how biomanufacturing processes can be applied towards the fabrication of novel nanowire technology, as shown in Figures 14a and 14b, that had been formed by nucleation of ZnS nanocrystals via specific nucleation peptide expression on the pVIII protein of M13.

4. BIO-NANO-INFORMATION FUSED SYSTEMS

A human cell serves as a culmination of what nature has taken millions of years to evolve as it is an autonomously responsive system of sensors and actuators that operate based upon commands from an embedded and distributed intelligence. It is a self-regulating, self-governing unit with its nucleus serving as its central information-processor, and hence represents a fusion-based system. The transfer and transduction of information using various signal pathways found in cells serve to process and apportion this information towards a concerted action. For example, a chemical signal results in a sensory response that elicits mechanical movement or actuation from the cytoskeletal network which are characteristic mechanisms involved with chemotaxis, or chemical-induced cellular movement. As a composite system, a cell exemplifies the concept of emergence, where inputs result in a coordinated feedback. Such a feedback can be differentiated from one found in an integrated system, where individual components are synchronized through an outside driver to produce a desired

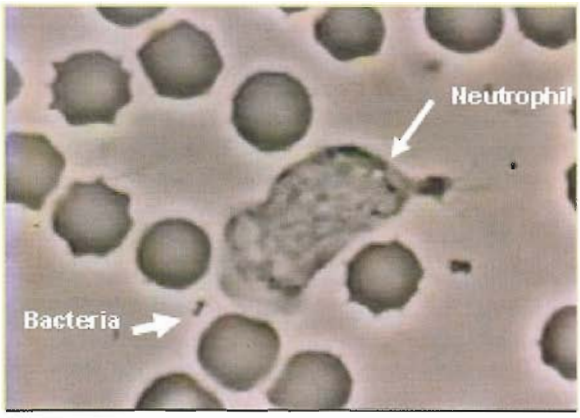

Fig. 15. A neutrophil is observed chasing a *Staphylococcus aureus* bacteria. The bacteria emits a chemical which is in turn sensed by the neutrophil which then coordinates an autonomous actuation directed towards the phagocytosis of the bacteria. Reprinted with permission from [94], D. Rogers, *Neutrophil Crawling*. Movie on a neutrophil chasing a chemoattractant-producing bacteria (Staphylococcus aureus) **(1950)**, http://www.biochemweb.org/fenteany/research/cell_migration/neutrophil.html. © 1950, Courtesy of G. Fenteany, University of Illinois, Chicago.

activity. If one considers an automobile, its core component, the engine, requires an external information processor, or driver, to derive its utility. This can be clearly distinguished from fusion, where the example of a neutrophil hunting down and encircling the Staphylococcus aureas bacteria (Fig. 15) demonstrates concerted, self-determining behavior. For example, a neutrophil tries to catch a bacterium.[94] The bacterium emits a chemical gradient that is sensed by a cell. The cell, able to follow a complex path of the chemical, moves towards the bacterium and eventually surrounds the bacteria to phagocytose it. In this process, the chemical is sensed by the chemical sensors inside the neutrophil and processed by the signal pathways. Eventually, the neutrophil acts as a whole to capture the bacterium.

A key element of manufacturing large scale molecular systems will be the derivation of emergence, or true mimicry towards applications in energy production, nanoscale medicine, etc. Continued progress in nanotechnological development possesses promising approaches whereby the input of stimuli has induced systemic behavior not previously present towards the realization of emergence.

The field of biomedical diagnostics would benefit greatly from the development of nanoscale machines capable of moving towards and detecting a specific target, biological or chemical, and then conveying a message to a macroscale monitoring device such as computer. Showing particular promise are micrometer-sized photonic crystals of porous silicon, presented by Link and Sailor, whose optical properties change upon the absorption of chemicals, the fabrication of which is illustrated in Figure 16.[95]

To fabricate the crystals called "smart dust," a substrate of single-crystal silicon(100) is electrochemically etched

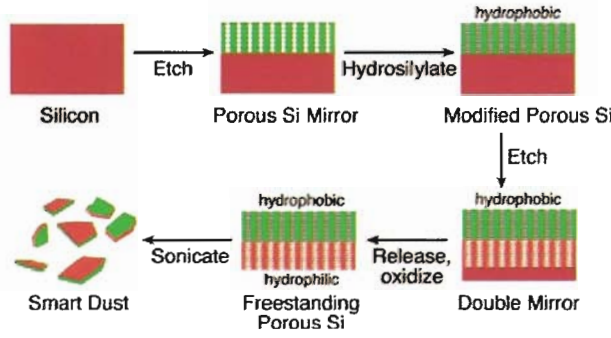

Fig. 16. Fabrication scheme of smart dust particles. Conventional microfabrication techniques enable asymmetric compositions on both sides of the silicon dust particles. An initial porous silicon is engineered to possess a hydrophobic side through the hydrosilylation. A subsequent hydrophilic porous silicon surface is developed on the other side to produce asymmetric smart dust. Reprinted with permission from [95], J. R. Link and M. J. Sailor, *Proc. Natl. Acad. Sci. USA* 100, 10607 (**2003**). © 2003, The National Academy of Sciences (USA).

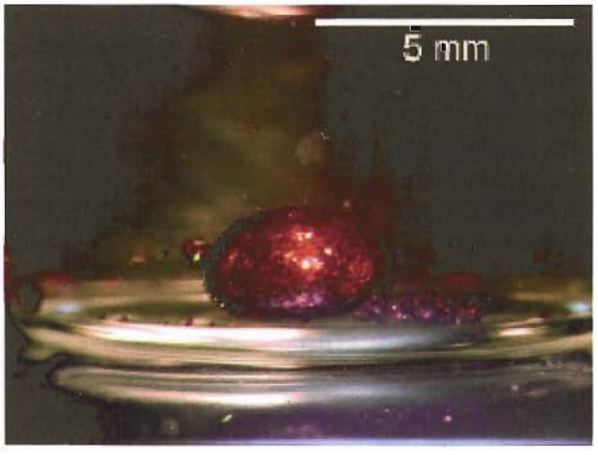

Fig. 17. Surrounding of dicholoromethane drop by self-orienting, self-assembling "smart dust" particles. This is accomplished through hydrophobic–hydrophobic interaction between the dichloromethane and the hydrophobic porous silicon. Reprinted with permission from [95], J. R. Link and M. J. Sailor, *Proc. Natl. Acad. Sci. USA* 100, 10607 (**2003**). © 2003, The National Academy of Sciences (USA).

with a mixture of hydrofluoric acid (HF) and ethanol and then a sinusoidal current density waveform is applied to make the first porous silicon mirror. The first porous silicon layer is then chemical modified through a thermal hydrosilylation with 1-dodecene, thereby making the surface hydrophobic. The second mirror is then fabricated as before with an electrochemical etch, effectively producing a double mirror made of porous silicon. The structure is then subsequently released from the silicon substrate by applying a sinusoidal current pulse. A thermal oxidation

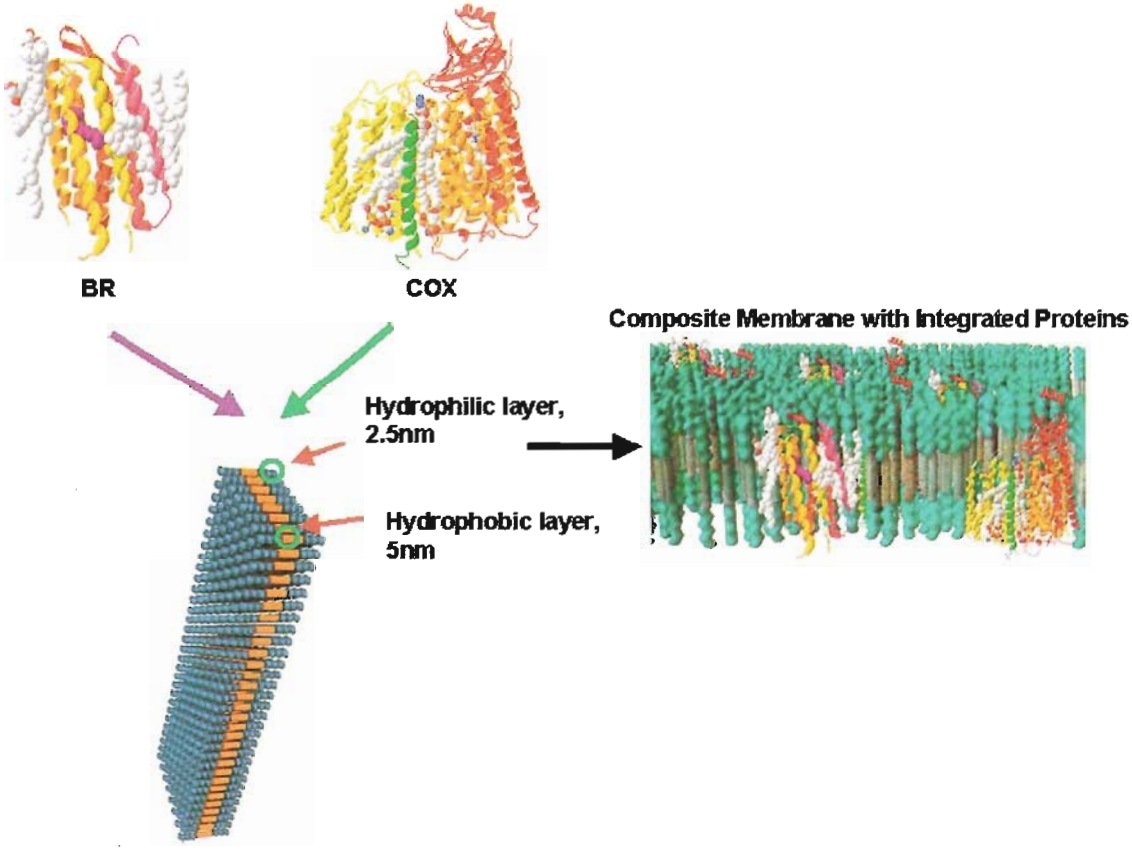

Fig. 18. The fabrication scheme of the protein-functionalized copolymer membranes depicts the integration of Bacteriorhodopsin (BR) and Cytochrome C Oxidase (COX) into ABA block copolymers which serve as biomimetic membranes. Reprinted with permission from [115], J. Xi et al., *Adv. Func. Mater.* 15, 1233 (**2005**). © 2005, Wiley-VCH.

step then imparts a hydrophilic character to the second mirror. Next, the film is placed into water and, through ultrasonication, fractured into bifunctional, micrometer-sized particles.

The "smart dust" particles are composed of two sides: a hydrophobic side that is green and a hydrophilic side that is red. Furthermore, due to their amphiphilic nature, the "smart dust" particles will orient themselves spontaneously at a water surface to form a monolayer such that the hydrophilic side faces the water and hydrophobic side faces the air. Each phase of the bifunctional particle produces a signal in a reflectivity spectrum that is indicative of an interaction with a surface. For example, the hydrophobic side displays a spectral wavelength maximum of 530 nm when it is in air. However, when exposed to heptane, the spectral wavelength maximum shifts to 560 nm. No shift is observed when exposed to water. The hydrophilic side of the film has a spectral wavelength maximum of 702 nm that shifts to 762 nm when exposed to water. Interestingly, no significant shift occurs upon exposure to heptane in a two-phase mixture.

If porous silicon particles are incubated in water with a drop of dicholoromethane, the "smart dust" particles will self-assemble and orient themselves around the drop of dicholoromethane such that the hydrophilic side (red) faces the water, as shown in Figure 17. The individual particles aggregate together to form a large, macroscopic collection with a novel reflectivity spectrum that *emerges* as a result of the particle self-assembly. As demonstrated by Link and Sailor, the "smart dust" particles proved useful in the detection of a chemical such as dichloromethane. The modification of the particles with recognition elements may add further use to the particles through *b* facilitating the detection and isolation of pathogenic organisms in food or water. Beyond the direct applicability of their work towards detection, it also represents a compelling approach to derive an intrinsic, higher-order behavior out of the system through the addition of a new condition to the solution, represented by the drop of dichloromethane.

The advent of cytomimetic systems displaying higher-order behavior based upon biotic-abiotic integration, or more specifically, the integration of proteins with synthetic materials has produced a new generation of devices with potential applications in nanoscale medicine, as well as bioenergetics.[96–100] Foundational studies have previously utilized lipid-based membranes as matrices for protein reconstitution and structural/mechanistic studies.[101–106] More recently, however, copolymeric membranes that possess structures similar to the hydrophilic-hydrophobic-hydrophilic environments observed in biological membranes have been explored as next-generation materials for interfacing with proteins due to their enhanced robustness.[107–114] Work by Ho and colleagues has functionalized these copolymer materials with cooperative protein function to exemplify a progression towards the realization of fused systems whereby the input of a specific stimuli, in this case, light, resulted in a current output due to biomolecular interactions within a composite protein-functionalized membrane.[115,116] More specifically, the bacterial membrane-bound proteins Bacteriorhodopsin and Cytochrome C Oxidase were both inserted into a block copolymeric membrane where their functionalities were coupled to produce higher-order behavior (Fig. 18). Bacteriorhodopsin and Cytochrome C Oxidase, individually speaking, both serve as proton pump components of energy conversion pathways of the *Halobacterium halobium*, and *Rhodobacter sphaeroides* bacteria, respectively. Bacteriorhodopsin pumps protons based upon light-dependent protein functionality, making it an ideal

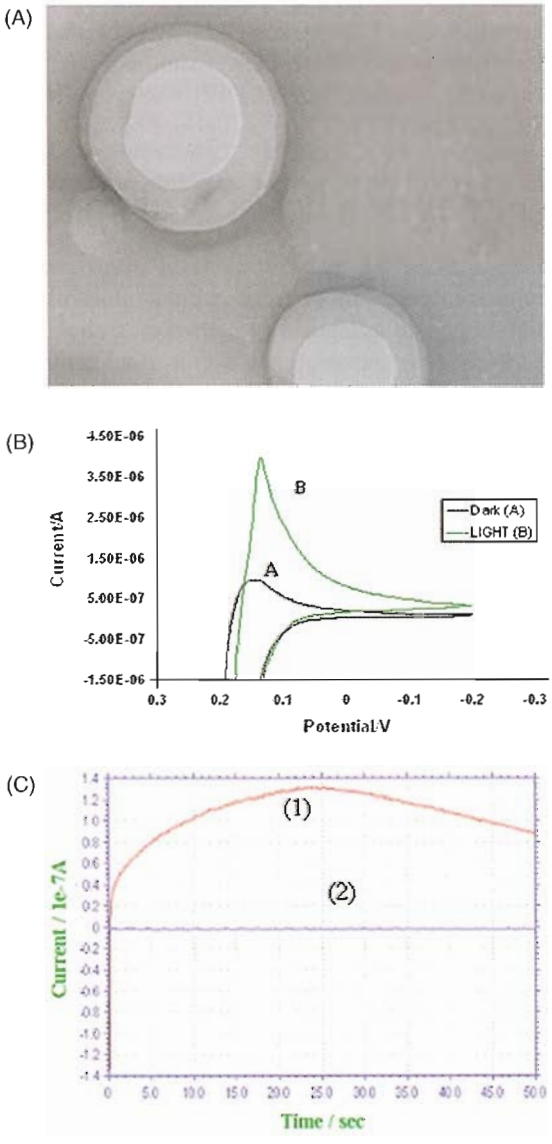

Fig. 19. Light-dependent electron release from biofunctionalized polymers was observed using both direct current measurements and cyclic voltammetry. (A) TEM image of copolymer vesicles; (B) Cyclic voltammetry of light-dependent electron release; (C) Current versus time measurements of light-dependent electron release. Reprinted with permission from [115], J. Xi et al., *Adv. Func. Mater.* 15, 1233 (**2005**). © 2005, Wiley-VCH.

candidate to serve as a light-dependent switch for biosolar applications. Cytochrome C Oxidase has previously been shown to exhibit a partial reversal of its proton pumping activity due to large proton gradients and high concentrations of its Cytochrome C mediator which results in a release of electrons observed and the reduction of Cytochrome C.[117] As such, the light-dependent proton pumping of bacteriorhodopsin effectively served as a switch to induce electron release from Cytochrome C Oxidase with estimated power/area output efficiencies as high as 6% (Fig. 19).[115,116] While continued development and addressing of key issues such as durability and robustness of biologically-active devices will further enhance their potential of finding a wide-range of applications, this demonstration of cooperative activity between energy-transducting proteins towards the derivation of useful work has served as another example of observing intrinsic, higher order behavior out of a composite system.

5. CONTROL OF A COMPLEX SYSTEM

Cellular functions are manifestations of intra- and intermolecular transports, motions of cellular molecules, and networks of signaling/regulatory pathways. Cellular activities such as gene expression are typically regulated by an extremely complex and diverse range of signal cascade reactions. This complexity is enhanced by the fact that the outcomes of these reactions are often the product of interactive events between cascades.

When the cell experiences perturbations in the form of activated genetic defects or environmental assaults, the networks are altered from equilibrium. Deviations from homeostasis and health caused by invading foreign organisms, pathologic accumulations of cholesterol and lipids, or uncontrolled cell growth and resistance to death provide the underlying basis for most morbid and mortal illness. Despite advances in molecular biology and molecular medicine, the key mechanisms and molecules for normal and pathologic cell responses remain largely obscure. A full understanding of the functions of complex networks, especially the nonequilibrium state and the possible recovering process, is the ultimate goal to targeted prevention and therapy for specific infections and cancers.

The exploration and understanding of such a complex system and its deviation from equilibrium are extremely time consuming and laborious tasks and warrants a search for alternative approaches. Bypassing the reductionist approach of applying novel interrogative modalities for understanding the entire collection of pathways, the strategy of controlling the cell functionalities, both genotypically and phenotypically, as a whole system, or a black box, towards a desired outcome of a living cell would clearly be a different but interesting route. In fact, the *control* and the *understanding* scenario can be a push-pull relationship to accelerate the exploration process.

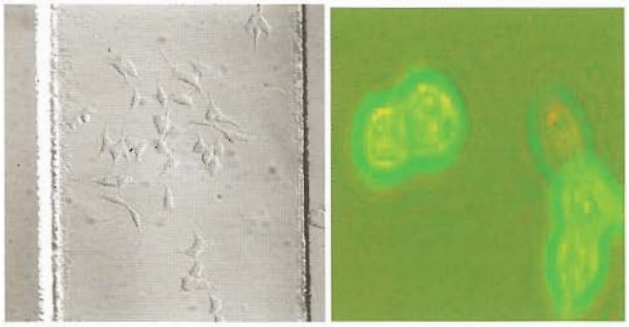

Fig. 20. (Left): HEK 293T cells are shown here and have been cultured in the microfluidic testing platform for over 80 hours. (Right): Green fluorescent protein expression in 293T cells to indicate cellular response to cytokine combinations is shown here. Reprinted with permission from [119], P. K. Wong, Ph.D. Dissertation, Department of Mechanical and Aerospace Engineering, University of California at Los Angeles **(2005)**. © 2005, University of California at Los Angeles.

In order to bring a diseased cell back to a healthy state, cocktails of agonists in many cases, are more efficient in regulating cellular activity than a single agonist. However, combinations of the stimuli and their concentrations may result in a large number of trials for identifying the optimal combination. Work by Wong and colleagues has presented a new approach to alleviate the challenge of controlling a complex system compounded by a large testing parameter space.[118,119] Using nuclear factor kappa B (NF-κB) transcription factor in the 293T kidney cell as an indicator for drug response, a green fluorescent protein-containing plasmid was utilized as the reporter molecule to measure NF-κB activity (Fig. 20). With the green flouresecent light intensity as the output of a feedback loop to search for the optimum combination of cytokines, Wong and colleagues have demonstrated the ability to reduce the 10^6 possible trials to only tens of tests. Such an approach has presented a compelling modality towards the control and understanding of complex biological systems and will continue to elucidate the mechanisms that underlie the embedded intelligence and complex nature of living systems that will eventually be engineered into synthetic, fused technologies.

6. CONCLUDING REMARKS

This work has outlined the requisite strategies for realizing the ultimate goal of nanotechnology, benefiting the human condition. The vision of fusing biology, nanotechnology, and information technology is based upon key developmental milestones including the bridging of nanoscience with nanotechnological translation through the spanning of length scales from the nano- to macroscale; generation of systems with embedded intelligence and higher order behavior; the coevolution of nanomanufacturing and interrogative modalities to investigate natural and resultant fused systems; and finally, the utilization of the collective

information gleaned from these milestones towards the control of one of nature's most complex biostructures, the cell. While fundamental studies in the nanosciences have elucidated the building blocks and foundations of mechanistic and structural behavior of nanoscale systems, the advent of nanotechnology will utilize their foundational processes to result in a class of novel devices with systemic functionality towards applications in energetics, electronics, materials, medicine, and beyond. These systems will serve as a fundamental departure from being solely integrative, or based on a conglomeration of separate components functioning simultaneously. Instead, they will possess embedded intelligence through a series of nanoscale sensors and actuators, with a coordinating behavior towards emergent, or higher order behavior. As such, the aforementioned examples involving progress in coordinated smart dust activity, or output of electricity through biological energy transduction provide a promising demonstration of basic emergent behavior. Future work will seek to dramatically increase the amounts of information contained within fabricated systems to provide progressively advanced outputs in response to various impulses.

Translating the fundamental findings of nanoscience to nanotechnology development will also parallel current and continued advancements in nanoscale interrogative modalities. These have included revolutionary visualization capabilities such as superlensing to enable direct visualization of nanoscale objects, where the amplification of near-field optical waves has enabled a resolution of around 60 nm which is far below the optical diffraction limit. Nanoscale manipulation studies have utilized scanning tunneling microscopy (STM) to pick and place xenon atoms into defined arrangements by using the tunneling current through the STM tip to make contact with the particles where they could then be dragged across a single-crystal nickel surface to create letters with 50 Å top to bottom dimensions. In addition, electrokinetic/hydrodynamic methodologies were utilized to stretch strands of DNA to optimize nucleic acid detection conditions. Because biodetection capabilities are dependent upon the minimization of the detection limit that can be accomplished through maximizing the fluorescence intensity of the nucleic acid probe, the focusing of DNA to a minimal region served to effectively increase the signal-to-noise ratio, thereby raising the sensitivity of the detection scheme. As entropy favored the coiling of DNA molecules at equilibrium such that the detection probe binding sites were not easily accessible, the entropic state of the DNA molecule was reduced by stretching it into a long, linear polymer. These outlined nanoscale manipulation strategies have collectively established a clear path towards advanced biomolecular characterization using a multitude of foundational techniques such as Laser Induced Fluorescence detection of single base pair mutations, or the use of α-hemolysin membrane proteins as high throughput DNA sequencing elements. Beyond these outlined modalities, the range of interrogative capabilities is being continually expanded and new domains are being forged that will ultimately realize new limits in nanoscale visualization, manipulation, and characterization. In addition, novel nanomanufacturing strategies will transition single molecule studies towards large-scale fabrication of systems possessing increasing information content towards that will transform what was a discipline, into a pioneering industry that will make immeasurable impacts on society.

The realization of systems based upon the fusion of biology, nanotechnology, and informatics will result in true emergence or mimicry and devices that combine sensors and actuators that respond to specific stimuli. Ultimately, the comprehensive understanding gleaned from the simulation of bio-systemic complexity will enable scientists and engineers to further transition from a mimetic vision, to a revolutionary vision of complex system control towards the ability to induce desired phenotypic and genotypic traits in biosystems, and beyond. This capability will inevitably result in the elucidation of the most compelling biomedical, materials, energy quandaries that currently, and will confront mankind.

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