CHAPTER 32

Progress in Nanomedicine and Medical Nanorobotics

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1. NANOTECHNOLOGY AND NANOMEDICINE

"There is a growing sense in the scientific and technical community that we are about to enter a golden new era," announced Richard E. Smalley, founder of the Center for Nanoscale Science and Technology at Rice University in Texas and winner of the 1996 Nobel Prize in Chemistry. In his Congressional testimony [1] on June 22, 1999, Smalley spoke in support of a new National Nanotechnology Initiative before the Subcommittee on Basic Research of the U.S. House Science Committee in Washington, DC. "We are about to be able to

ISBN: 1-58883-042-X/\$35.00 Copyright © 2005 by American Scientific Publishers All rights of reproduction in any form reserved. build things that work on the smallest possible length scales, atom by atom," Smalley said. "Over the past century we have learned about the workings of biological nanomachines to an incredible level of detail, and the benefits of this knowledge are beginning to be felt in medicine. In coming decades we will learn to modify and adapt this machinery to extend the quality and length of life. Twenty years from now, nanotechnology will have given us specially engineered drugs which are nanoscale cancer-seeking missiles, a molecular technology that specifically targets just the mutant cancer cells in the human body, and leaves everything else blissfully alone. To do this, these drug molecules will have to be big enough—thousands of atoms—so that we can code the information into them of where they should go and what they should kill. They will be examples of an exquisite, human-made nanotechnology of the future."

Following this testimony, the U.S. President, in his January 2000 State-of-the-Union speech, announced that he would seek \$475 million for nanotechnology R&D via the National Nanotechnology Initiative, effectively doubling federal nanotech funding for Fiscal Year 2001. The President never referred to "nanotechnology" by name, but he gushed about its capabilities, marveling at a technology that will someday produce "molecular computers the size of a teardrop with the power of today's fastest supercomputers." Annual U.S. federal funding for nanotechnology research and development exceeded \$500 million in 2002 [2], reaching \$849 million in fiscal year 2004 [3] and could approach \$1 billion in next year's budget; the European Commission has set aside 1.3 billion euros for nanotechnology research during 2003–2006 [4], with annual nanotechnology investment worldwide reaching approximately \$3 billion in 2003. Private-sector analysts estimate that the worldwide market for nanoscale devices and molecular modeling should experience an average annual growth rate of 28% per year, rising from \$406 million in 2002 to \$1.37 billion in 2007, with a 35% per year growth rate in revenues from biomedical nanoscale devices [5].

In December 2002, the U.S. National Institutes of Health (NIH) announced a 4-year program for nanoscience and nanotechnology in medicine [4]. Burgeoning interest in the medical applications of nanotechnology has led to the emergence of a new field called nanomedicine [4, 6–13]. Most broadly, nanomedicine is the process of diagnosing [14], treating, and preventing disease and traumatic injury; of relieving pain; and of preserving and improving human health, using molecular tools and molecular knowledge of the human body [6]. The NIH Roadmap's new Nanomedicine Initiatives, first released in late 2003, "envision that this cutting-edge area of research will begin yielding medical benefits as early as 10 years from now" and will begin with "establishing a handful of Nanomedicine Centers...staffed by a highly interdisciplinary scientific crew including biologists, physicians, mathematicians, engineers and computer scientists...gathering extensive information about how molecular machines are built," who will also develop "a new kind of vocabulary—lexicon—to define biological parts and processes in engineering terms" [15]. Even state-funded programs have begun, such as New York's Alliance for Nanomedical Technologies [16].

It is useful to regard the development path of nanomedicine as a succession of three mutually overlapping and progressively more powerful technologies.

First, in the relatively near term, over the next 5 years, nanomedicine can address many important medical problems by using nanoscale-structured materials and simple nanodevices that can be manufactured today (Section 2). This includes the interaction of nanostructured materials with biological systems [8]—the first 12 Ph.D. candidates in "nanobiotechnology" began laboratory work at Cornell University in June 2000, and many other universities have started similar programs as state, federal, and international funding has soared.

Second, over the next 5–10 years, biotechnology will make possible even more remarkable advances in molecular medicine and biobotics (microbiological robots or engineered organisms), some of which are already on the drawing boards (Section 3).

Third, in the longer term, perhaps 10–20 years from today, the earliest molecular machine systems and nanorobots may join the medical armamentarium, finally giving physicians the most potent tools imaginable to conquer human disease, ill-health, and aging (Section 4). Issues relating to future Food and Drug Administration (FDA) approval of nanomedical materials, devices, and even nanorobots are beginning to be addressed by legal writers [17, 18].

2. MEDICAL NANOMATERIALS AND NANODEVICES

2.1. Nanopores

Perhaps one of the simplest medical nanomaterials is a surface perforated with holes, or nanopores. In 1997 Tejal Desai at Boston University and Mauro Ferrari at Ohio State University created what could be considered one of the earliest therapeutically useful nanomedical devices [19]. Along with collaborators at the Biomedical Microdevices Center at the University of California at Berkeley, Desai and Ferrari employed bulk micromachining to fabricate tiny cell-containing chambers within single crystalline silicon wafers. The chambers interface with the surrounding biological environment through polycrystalline silicon filter membranes that are micromachined to present a high density of uniform nanopores as small as 20 nanometers in diameter. These pores are large enough to allow small molecules such as oxygen, glucose, and insulin to pass but are small enough to impede the passage of much larger immune system molecules such as immunoglobulins and graft-borne virus particles. Safely ensconced behind this artificial barrier, immunoisolated encapsulated rat pancreatic cells may receive nutrients and remain healthy for weeks, happily secreting insulin back out through the pores, while the immune system remains blissfully unaware of the foreign cells which it would normally attack and reject.

Microcapsules containing replacement islets of Langerhans cells—most likely easily harvested piglet islet cells—could be implanted beneath the skin of some diabetes patients [20]. This could temporarily restore the body's delicate glucose control feedback loop without the need for powerful immunosuppressants that can leave the patient at serious risk for infection. Supplying encapsulated new cells to the body could also be a valuable way to treat other enzyme- or hormone-deficiency diseases, including encapsulated neurons that could be implanted in the brain and then electrically stimulated to release neurotransmitters, possibly as part of a future treatment for Alzheimer's or Parkinson's diseases. In conjunction with the biomedical company iMEDD, Desai has been active in continuing this work for immunoisolation [21], drug delivery [22, 23], and sensing [24], with U.S. patents in process. Neurotech (Paris) [25] and others are also working on encapsulated cell technologies [26] and similar techniques.

The flow of materials through nanopores can also be externally regulated [27]. The first artificial voltage-gated molecular nanosieve was fabricated by Charles R. Martin and colleagues [28] at Colorado State University in 1995. Martin's membrane contains an array of cylindrical gold nanotubules with inside diameters as small as 1.6 nanometers. When the tubules are positively charged, positive ions are excluded and only negative ions are transported through the membrane. When the membrane receives a negative voltage, only positive ions can pass. Future similar nanodevices may combine voltage gating with pore size, shape, and charge constraints to achieve precise control of ion transport with significant molecular specificity. Martin's recent efforts [29] have been directed at immobilizing biochemical molecular-recognition agents such as enzymes, antibodies, other proteins, and DNA inside the nanotubes as active biological nanosensors [30-32], to perform drug separations [33, 34], and to allow selected biocatalysis [34]. An exquisitely sensitive ion channel switch biosensor has also been built by an Australian research group [35]. The Australian scientists estimated that their device, currently being commercialized by Ambri Biosensor, could detect a minute change in chemical concentration equivalent to a single sugar cube tossed into Sidney harbor, or roughly one part in a billion billion ($\sim 10^{-18}$). Others are also investigating synthetic nanopore ion pumps [36] and voltage-gated nanopores embedded in artificial membranes [37], and molecular dynamics theoretical studies of viscosity [38] and diffusion [39] through nanopores are in progress.

Daniel Branton's team at Harvard University has conducted an ongoing series of experiments using an electric field to drive a variety of RNA and DNA polymers through the central nanopore of an alpha-hemolysin protein channel mounted in a lipid bilayer similar to the outer membrane of a living cell [40, 41]. As early as 1996, the researchers had determined that the individual nucleotides making up the polynucleotide strands must be passing single-file through the 2.6-nm-wide nanopore, and that changes in ionic current could be used to measure polymer length. By 1998, Branton had shown that the nanopore could be used to rapidly discriminate between pyrimidine and purine segments (the two types of nucleotide bases) along a single RNA molecule. In 2000, the scientists demonstrated the ability to distinguish between DNA chains of similar length and composition that differ only in base pair sequence, and Branton continues to perfect this approach [42–46]. Current research is directed toward reliably fabricating pores with specific diameters and repeatable geometries at high precision [47–49], understanding the unzipping of double-stranded DNA as one strand is pulled through the pore [50], recognizing of folded DNA molecules passing through the pore [46], experimenting with new 3–10-nm silicon–nitride nanopores [46], and investigating the benefits of adding electrically conducting electrodes to pores to improve longitudinal resolution "possibly to the single-base level for DNA" [46]. It has been suggested that nanopore-based DNA-sequencing devices could allow per-pore read rates potentially up to 1000 bases per second [51]. Because nanopores can rapidly discriminate and characterize DNA polymers at low copy number, future refinements of this experimental approach may eventually provide a low-cost high-throughput method for very rapid genome sequencing.

2.2. Artificial Binding Sites and Molecular Imprinting

Another early goal of nanomedicine is to study how biological molecular receptors work and then to build artificial binding sites on a made-to-order basis to achieve specific medical results. Molecular imprinting [52–57] is an existing technique in which a cocktail of functionalized monomers interacts reversibly with a target molecule using only noncovalent forces. The complex is then cross-linked and polymerized in a casting procedure, leaving behind a polymer with recognition sites complementary to the target molecule in both shape and functionality. Each such site constitutes an induced molecular "memory" capable of selectively binding the target species. In one experiment involving an amino acid–derivative target, one artificial binding site per (3.8 nm)³-polymer block was created. Chiral separations, enzymatic transition state activity, and high receptor affinities have been demonstrated.

Buddy D. Ratner at the University of Washington in Seattle has researched the engineering of polymer surfaces containing arrays of artificial receptors, which are created using a radiofrequency-plasma glow-discharge process to imprint a polysaccharide-like film with nanometer-sized pits in the shape of such biologically useful protein molecules as albumin (the most common blood protein), fibrinogen (a clotting protein), lysozyme and ribonuclease (two important enzymes), and immunoglobulin (antibodies) [52]. Each protein type sticks only to a pit with the shape of that protein. Such engineered surfaces could be used for quick biochemical separations and assays [56] and as recognition elements in biosensors and chemosensors [57], because such surfaces will selectively adsorb from solution only the specific protein whose complementary shape has been imprinted, and only at the specific place on the surface where the shape is imprinted. However, molecularly imprinted polymers have limitations, such as incomplete template removal, broad guest affinities and selectivities, and slow mass transfer; imprinting inside dendrimers (Section 2.7) may allow quantitative template removal, nearly homogeneous binding sites, solubility in common organic solvents, and amenability to the incorporation of other functional groups [55]. The RESIST Group at the Welsh School of Pharmacy at Cardiff University [53] and others [57] have looked at how molecularly imprinted polymers could be medically useful in clinical applications such as controlled drug release, drug monitoring devices, and biological and receptor mimics including artificial antibodies (plastibodies) or biomimicking enzymes (plastizymes) [57].

2.3. Quantum Dots and Nanocrystals

Fluorescent tags are commonplace in medicine and biology and are found in everything from human immunodeficiency virus (HIV) tests to experiments that image the inner functions of cells, but different dye molecules must be used for each color, color-matched lasers are needed to get each dye to fluoresce, and dye colors tend to bleed together and fade quickly after one use. "Quantum dot" nanocrystals have none of these shortcomings. These dots are tiny particles measuring only a few nanometers across, about the same size as a protein molecule or a short sequence of DNA. They come in a nearly unlimited palette of sharply defined colors that can be customized by changing particle size or composition. Particles can be excited to fluorescence with white light, can be linked to biomolecules to form longlived sensitive probes to identify specific compounds up to a thousand times brighter than conventional dyes used in many biological tests, and can track biological events by simultaneously tagging each biological component (e.g., different proteins or DNA sequences) with nanodots of a specific color.

Quantum Dot Corp. (QDC) [58], the manufacturer, believes this kind of flexibility could offer a cheap and easy way to screen a blood sample for the presence of a number of different viruses at the same time. It could also give physicians a fast diagnostic tool to detect, say, the presence of a particular set of proteins that strongly indicates a person is having a heart attack or to detect known cellular cancer markers [59]. On the research front, the ability to simultaneously tag multiple biomolecules both on and inside cells could allow scientists to watch the complex cellular changes and events associated with disease, providing valuable clues for the development of future pharmaceuticals and therapeutics. Quantum dots are useful for studying genes, proteins, and drug targets in single cells, tissue specimens, and living animals [60]. In August 2003, QDC, Matsushita, and SC BioSciences agreed to develop, manufacture, and market QDC's life science detection products [61]. "Qdot nanotechnology is revolutionizing biological detection," said Takao Kanamura, Senior Managing Director of Matsushita Kotobuki Electronics. "We project products developed under this agreement to generate revenue for QDC in excess of \$100 million per year by 2007." Quantum dots are being investigated as chemical sensors [62] and for cancer cell detection [59], gene expression studies [63], gene mapping and DNA microarray analysis [64], immunocytochemical probes [65], intracellular organelle markers [66], live cell labeling [67, 68], medical diagnostics and drug screening [69], SNP genotyping [70], vascular imaging [71], and many other applications [72, 73]. Quantum dot physics has been studied theoretically [74] and computationally using time-dependent density functional theory [75] and other methods [76–78].

Researchers from Northwestern University and Argonne National Laboratory have created a hybrid "nanodevice" composed of 4.5-nm nanocrystals of biocompatible titanium dioxide semiconductor covalently attached with snippets of oligonucleotide DNA [79]. Experiments showed that these nanocomposites not only retain the intrinsic photocatalytic capacity of TiO₂ and the bioactivity of the oligonucleotide DNA but, more important, also possess the unique property of a light-inducible nucleic acid endonuclease (separating when exposed to light or X-rays). For example, researchers would attach a strand of DNA that matches a defective gene within a cell to the semiconductor scaffolding, and then introduce the nanoparticle into the cell nucleus where the attached DNA binds with its defective complementary DNA strand, whereupon exposure of the bound nanoparticle to light or X-rays snips off the defective gene. "We call it a 'Swiss army knife' because, unlike today's drugs, we can inject 10 kinds of good genes all at once and target them in extremely specific or extremely broad ways," says researcher Tatjana Paunesku [80]. Other molecules besides oligonucleotides can be attached to the titanium dioxide scaffolding, such as navigational peptides or proteins, which, like viral vectors, can help the nanoparticles home in on the cell nucleus. This simple nanocrystal nanodevice might one day be used to target defective genes that play a role in cancer, neurological disease, and other conditions, though the work is still at a preliminary stage and testing in a laboratory model is at least 2 years away [80].

2.4. Fullerenes and Nanotubes

Soluble derivatives of fullerenes such as C_{60} —a soccer-ball-shaped arrangement of 60 carbon atoms per molecule—have shown great utility as pharmaceutical agents. These derivatives, many already in clinical trials, have good biocompatibility and low toxicity even at relatively high dosages. Fullerene compounds may serve as antiviral agents (most notably against HIV [81]; they have also been investigated computationally [82, 83]), antibacterial agents (*Escherichia coli* [84], *Streptococcus* [85], *Mycobacterium tuberculosis* [86], etc.), photodynamic antitumor [87, 88] and anticancer [89] therapies, antioxidants and antiapoptosis agents that may include treatments for amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) [90] and Parkinson's disease, and other applications—most being pursued by C Sixty [91], the leading company in this area. C Sixty is also investigating possible drug-delivery "nanopills" consisting of two closed-end single-walled carbon nanotubes nested mouth-to-mouth, forming a capsule-like container [92].

Both single-walled [93, 94] and multiwalled [95–97] carbon nanotubes are also being investigated as biosensors; for example, to detect glucose [96, 98], ethanol [98], hydrogen peroxide [95], selected proteins such as immunoglobulins [94], and an electrochemical DNA hybridization biosensor [93]. NASA and the National Cancer Institute are developing a biosensor catheter to detect specific oligonucleotide sequences that serve as molecular signatures of cancer cells, with preliminary *in vitro* testing using tissue samples from patients with chronic myelogenous leukemia and acute promyelocytic leukemia, two neoplastic diseases for which molecular signatures have been well characterized [97].

2.5. Nanoshells and Magnetic Nanoprobes

Researchers Naomi Halas and Jennifer West at Rice University in Houston have developed a platform for nanoscale drug delivery called the nanoshell [99, 100]. Unlike carbon fullerenes, the slightly larger nanoshells are dielectric-metal nanospheres with a core of silica and a gold coating, whose optical resonance is a function of the relative size of the constituent layers. The nanoshells are embedded in a drug-containing tumor-targeted hydrogel polymer and injected into the body. The shells circulate through the body until they accumulate near tumor cells. When heated with an infrared laser, the nanoshells (each slightly larger than a polio virus) selectively absorb the infrared frequencies, melt the polymer, and release their drug payload at a specific site. Nanoshells offer advantages over traditional cancer treatments: earlier detection, more detailed imaging, fast noninvasive imaging, and integrated detection and treatment [101]. This technique could also prove useful in treating diabetes; Instead of taking an injection of insulin, a patient would use a ballpoint-pen-size infrared laser to heat the skin where the nanoshell polymer had been injected. The heat from nanoshells would cause the polymer to release a pulse of insulin. Unlike injections, which are taken several times a day, the nanoshell-polymer system could remain in the body for months.

In 2002, Rice University licensed its patented nanoshell technology to a private company started by Halas and West, Nanospectra Biosciences [102] of Houston, Texas, to develop commercial applications. Nanospectra is conducting animal studies at the M.D. Anderson Cancer Center at the University of Texas and specifically targets micrometastases, tiny aggregates of cancer cells too small for surgeons to find and remove with a scalpel. The company hopes to start clinical trials for the cancer treatment by 2004 and for the insulin-delivery system by 2006. Also in mid-2003, Rice researchers announced [103] the development of a point-of-care whole-blood immunoassay using antibody-nanoparticle conjugates of gold nanoshells [104]. By varying the thickness of the metal shell, researchers in Halas' group can precisely tune the color of light to which the nanoshells respond; near-infrared light penetrates whole blood very well, so it is an optimal wavelength for a whole blood immunoassay [103]. Successful detection of sub-nanogram-per-milliliter quantities of immunoglobulins was achieved in saline, serum, and whole blood in 10–30 minutes [104]. Groups at the University of Washington [105] and the Korea Advanced Institute of Science and Technology [106] are also investigating the use of gold nanoshells for biological applications.

An alternative approach pursued by Triton BioSystems [107] is to bond iron nanoparticles and monoclonal antibodies into nanobioprobes about 40 nanometers long. The chemically inert probes are injected and circulate inside the body, whereupon the antibodies selectively bind to tumor cell membranes. Once the tumor is covered with bioprobes after several hours, a magnetic field generated from a portable alternating magnetic field machine (similar to a miniaturized magnetic resonance imaging (MRI) machine) heats the iron particles to more than 170 degrees, killing the tumor cells in a few seconds [108]. Once the cells are destroyed, the body's excretion system kicks in and removes cellular residue and nanoparticles alike. Test subjects seem to feel no pain from the heat generated [108]. Triton BioSystems targets both visible tumors and micrometastases. Samuel Straface, chief executive at Triton BioSystems, explained that chemotherapy is analogous to napalm, killing large swaths of tissue in hopes that all tumor cells are killed in the process, whereas the nanobioprobes function more like carefully planted explosives detonated by remote control [108]. "Chemotherapy is nasty stuff," he said. "The effects here are minimal. We expect virtually no collateral damage." Triton BioSystems began mouse trials in 2002, and tests show that the treatment itself brings no ill effects to the mice. By mid-2003, Straface was nearly finished with efficacy trials to see how the treatment actually works on tumors. Once those trials are done, the company will start designing human tests and ask the FDA for permission to begin in 2004.

Mirkin's group at Northwestern University uses magnetic microparticle probes coated with target protein-binding antibodies plus 13-nm nanoparticle probes with a similar coating but including a unique hybridized "bar-code" DNA sequence as an ultrasensitive method for detecting protein analytes such as prostate-specific antigen (PSA) [109]. After the target protein in the test sample is captured by the microparticles, magnetic separation of the complexed microparticle probes and PSA is followed by dehybridization of the bar-code oligonucleotides on the nanoparticle probe surface, allowing the determination of the presence of PSA by identifying the bar-code sequence released from the nanoparticle probe." Using polymerase chain reaction on the oligonucleotide bar codes allows PSA to be detected at 3 attomolar concentration, about a million times more sensitive than comparable clinically accepted conventional assays for detecting the same protein target.

2.6. Targeted Nanoparticles and Smart Drugs

Targeting drugs to particular organs or tissues, such as a cancer tumor, is one way to achieve some specificity of action. For example, an immunotoxin molecule [110] is an engineered hybrid of functional protein modules fabricated from two different types of proteins: a toxin and an antibody. Toxin proteins are normally produced and released by infectious bacteria. The protein binds to the surface of a host cell, penetrates it, and kills it. Toxin molecules are so potent that just a few of them can kill a cell. Antibodies are proteins produced by the immune system to recognize and bind to specific foreign materials. An immunotoxin molecule is made by fusing a part of the gene encoding a toxin with a part of the gene encoding an antibody that recognizes surface features on cancer cells. This creates a novel gene that can be used to express a new synthetic protein molecule. This new molecule will bind only to a cancer cell (via a module from the antibody protein), then penetrate it and kill it (via modules from the toxin protein). The first experiments with mice showed that these engineered proteins successfully eliminated certain tumors. Then, early in 2000, National Cancer Institute researchers confirmed that an immunotoxin made from a truncated form of Pseudomonas exotoxin was cytotoxic to malignant B-cells taken from patients with hairy cell leukemia [111]. Another set of clinic trials at the Universitate zu Koeln in Germany that were completed in 2003 also found that a ricin-based immunotoxin had moderate efficacy against Hodgkin's lymphoma in some patients [112].

Multisegment gold/nickel nanorods are being explored by Leong's group at Johns Hopkins School of Medicine [113] as tissue-targeted carriers for gene delivery into cells that "can simultaneously bind compacted DNA plasmids and targeting ligands in a spatially defined manner" and allow "precise control of composition, size and multifunctionality of the genedelivery system." The nanorods are electrodeposited into the cylindrical 100-nm-diameter pores of an alumina membrane, joining a 100-nm-length gold segment and a 100-nm-length nickel segment. After the alumina template is etched away, the nanorods are functionalized by attaching DNA plasmids to the nickel segments and attaching transferrin, a cell-targeting protein, to the gold segments, using molecular linkages that selectively bind to only one metal and thus impart biofunctionality to the nanorods in a spatially defined manner. Leong notes that extra segments could be added to the nanorods, for example, to bind additional biofunctionalities such as an endosomolytic agent, or magnetic segments could be added to allow manipulating the nanorods with an external magnetic field.

Targeted radioimmunotherapeutic agents [114] include the FDA-approved "cancer smart bombs" that deliver tumor-killing radioactive yttrium (Zevalin) or iodine (Bexxar) attached to a lymphoma-targeted (anti-CD20) antibody [115]. Other antibody-linked agents are being investigated such as the alpha-emitting actinium-based "nanogenerator" molecules that use internalizing monoclonal antibodies to penetrate the cell and that have been shown, *in vitro*, to specifically kill leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at becquerel (picocurie) levels [116], with promising preliminary results against advanced ovarian cancer in mice [117]. However, drug specificity is still no better than the targeting accuracy of the chosen antibody, and there is significant mistargeting, leading to unwanted side effects.

Enzyme-activated drugs, first developed in the 1980s and still under active investigation [118], separate the targeting and activation functions. For instance, an antibody-directed enzyme-triggered prodrug cancer therapy is being developed by researchers at the University of Gottingen in Germany [119, 120]. This targeted drug molecule turns lethal only when it reaches cancer cells while remaining harmless inside healthy cells. In tests, mice previously implanted with human tumors are given an activating targeted enzyme that sticks only to human tumor cells, mostly ignoring healthy mouse cells. Then the antitumor molecule is injected. In its activated state, this fungal-derived antibiotic molecule is a highly strained ring of three carbon atoms that is apt to burst open, becoming a reactive molecule that wreaks havoc among the nucleic acid molecules essential for normal cell function. However, the molecule is injected as a prodrug—an antibiotic lacking the strained ring and with a sugar safety-catch. Once the sugar is clipped off by the previously positioned targeted enzyme, the drug molecule rearranges itself into a three-atom ring, becoming lethally active. Notes chemist Philip Ball [121]: "The selectivity of the damage still depends on antibody's ability to hook onto the right cells, and on the absence of other enzymes in the body that also activate the prodrug."

A further improvement in enzyme-activated drugs are "smart drugs" that become medically active only in specific circumstances and in an inherently localized manner. A good example is provided by Yoshihisa Suzuki at Kyoto University, who has designed a novel drug molecule that releases antibiotic only in the presence of an infection [122]. Suzuki started with the common antibiotic molecule gentamicin and bound it to a hydrogel using a newly developed peptide linker. The linker can be cleaved by a proteinase enzyme manufactured by *Pseudomonas aeruginosa*, a Gram-negative bacillus that causes inflammation and urinary tract infection, folliculitis, and otitis externa in humans. Tests on rats show that when the hydrogel is applied to a wound site, the antibiotic is not released if no P. aeruginosa bacteria are present, but if any bacteria of this type are present, then the proteolytic enzyme that the microbes naturally produce cleaves the linker and the gentamicin is released, killing the bacteria. "If the proteinase specific to each bacterium [species] can be used for the signal," wrote Suzuki, "different spectra of antibiotics could be released from the same dressing material, depending on the strain of bacterium." This specificity of action is highly desirable because the indiscriminate prophylactic use of antibiotics is associated with the emergence of strains of drug-resistant bacteria, and most antibiotics apparently have at least some toxicity for human fibroblasts. In subsequent work, an alternative antibiotic release system triggered by thrombin activity, which accompanies Staphylococcus aureus wound infections, was successfully tested as a high-specificity stimulus-responsive controlled drug release system [123].

Other stimulus-responsive "smart" hydrogels are being studied [124], including a hydrogelcomposite membrane coloaded with insulin and glucose oxidase enzyme that exhibits a twofold increase in insulin release rate when immersed in glucose solution, demonstrating "chemically stimulated controlled release" and "the potential of such systems to function as a chemically-synthesized artificial pancreas" [125].

Nanoparticles with an even greater range of action are being developed by Raoul Kopelman's group at the University of Michigan. The first effort in this direction has produced PEBBLEs (probes encapsulated by biologically localized embedding) [126], dye-tagged nanoparticles constructed from a polyacrylamide matrix using bulk processes [127] that can be inserted into living cells as biosensors to monitor intracellular oxygen [128], calcium [126], zinc [129], and pH [126] levels; metabolism, or disease conditions. The next goal is the development of novel molecular nanodevices for the early detection and therapy of brain cancer, using silica-coated iron oxide nanoparticles with a biocompatible polyethylene glycol coating [130]. The miniscule size of the particles—20–200 nm—should allow them to penetrate into areas of the brain that would otherwise be severely damaged by invasive surgery. The particles are attached to a cancer cell antibody or other tracer molecule that adheres to cancer cells and are affixed with a nanopacket of contrast agent that makes the particles highly visible during MRI. The particles enhance the killing effect during the subsequent laser irradiation of brain tissue. Nanoparticles allow MRI to see a few small brain tumor cells as small as 50 μ —depending on the cancer type, tumor cells can range from 5 to 50 μ each and may grow in locations separate from the tumor site, and hence they are sometimes not visible to surgeons. Traditional chemotherapy and radiation kill cancerous cells but also destroy healthy cells; by using nanoparticles, the killing agents can be made to directly attack primarily the sick cells [131].

Fei Yan, a postdoctoral researcher in Kopelman's lab, is working on these nanodevices, called the Dynamic Nano-Platform (Fig. 1), in research originally funded by the Unconventional Innovations Program of the National Cancer Institute and now being commercialized as therapeutic "nanosomes" under license to Molecular Therapeutics [132]. According to the company, "the nanosome platform provides the core technology with interchangeable components that provide ultimate flexibility in targeting, imaging and treatment of cancer and cardiovascular disease indications."



Figure 1. This illustration of the Dynamic Nano-Platform or "nanosome" shows proposed extensions of the technology, which may eventually incorporate magnetic and optical control and contrast elements to enable a number of functions from biological sensing to targeted photo dynamic cancer therapy [131]. Image courtesy of Molecular Therapeutics, Inc. [132] and illustrator Eric E. Monson, who reserve all rights.

2.7. Dendrimers and Dendrimer-Based Devices

Dendrimers represent yet another nanostructured material that may soon find its way into medical therapeutics. Starburst dendrimers [133, 134] are tree-shaped synthetic molecules with a regular branching structure emanating outward from a core. Dendrimers form nanometer by nanometer, so the number of synthetic steps or "generations" dictates the exact size of the particles in a batch. Each molecule is typically a few nanometers wide, but some have been constructed up to 30 nm wide, incorporating more than 100,000 atoms. The peripheral layer of the dendrimer particle can be made to form a dense field of molecular groups that serve as hooks for attaching other useful molecules, such as DNA, which hunker down among the outermost branches. Computational studies have been done on some dendrimer-based nanoparticles [135].

In 1998, James R. Baker Jr. cofounded the Center for Biologic Nanotechnology at the University of Michigan to bring together doctors, medical researchers, chemists, and engineers to pursue the use of dendrimers [136] as a safer and more effective genetic therapy agent [134]. For Baker, these nanostructures are attractive because they can sneak DNA into cells while avoiding triggering an immune response, unlike the viral vectors commonly employed today for transfection. The dendrimer molecule is decorated with specific snippets of DNA, then injected into biological tissue. On encountering a living cell, dendrimers of a certain size trigger a process called endocytosis, in which the cell's outermost membrane deforms into a tiny bubble, or vesicle. The vesicle encloses the dendrimer, which is then admitted into the cell's interior. Once inside, the DNA is released and migrates to the nucleus, where it becomes part of the cell's genome. The technique has been tested on a variety of mammalian cell types [137] and in animal models [138, 139], though clinical human trials of dendrimer gene therapy remain to be done. Donald Tomalia at Central Michigan University, another cofounder of the Center for Biologic Nanotechnology and the original inventor of dendrimers, has reported using glycodendrimer "nanodecoys" to trap and deactivate some strains of influenza virus particles [140, 141]. The glycodendrimers present a surface that mimics the sialic acid groups normally found in the mammalian cell membrane, causing virus particles to adhere to the outer branches of the decoys instead of the natural cells. Tomalia's new company, Dendritic NanoTechnologies Inc. [142], sells a growing number of precision dendrimer molecules manufactured in bulk-at year-end 2003, the largest molecule for sale was a generation-10 ethylene diamine core PAMAM dendrimer with chemical formula C_{40,942}H_{81,888}N_{16,378}O_{8,188}. In July 2003, Starpharma was cleared by the FDA for human trials of their dendrimer-based anti-HIV microbicide [143]. Their product has been successful in preventing simian-HIV.

Baker's lab now has a more ambitious agenda, however: the synthesis of multicomponent nanodevices called tecto-dendrimers built up from a number of single-molecule dendrimer components [144–148]. Tecto-dendrimers are composed of a single core dendrimer, which may or may not contain a therapeutic agent, surrounded by additional dendrimer modules (Fig. 2). The additional dendrimer modules are of several types, with each type designed to perform a function necessary to a smart therapeutic nanodevice. Baker's group has built a library of dendrimeric components from which a combinatorially large number of nanodevices can be synthesized [148]. The initial library contains components that will perform the following tasks: diseased cell recognition, diagnosis of disease state, drug delivery, reporting location, and reporting outcome of therapy. By using this modular architecture, an array of smart therapeutic nanodevices can be created with little effort. For instance, once apoptosisreporting, contrast-enhancing, and chemotherapeutic-releasing dendrimer modules are made and attached to the core dendrimer, it should be possible to make large quantities of this tecto-dendrimer as a starting material. This framework structure can be customized to fight a particular cancer simply by substituting any one of many possible distinct cancer recognition or "targeting" dendrimers, creating a nanodevice customized to destroy a specific cancer type and no other, while also sparing the healthy normal cells. In three nanodevices synthesized using an ethylenediamine core polyamidoamine dendrimer of generation 5, with folic acid, fluorescein, and methotrexate covalently attached to the surface to provide targeting, imaging, and intracellular drug delivery capabilities, the "targeted delivery improved the cytotoxic



Figure 2. The standard tecto-dendrimer device, which may be composed of monitoring, sensing, therapeutic, and other useful functional modules [148]. Image courtesy of James Baker, University of Michigan.

response of the cells to methotrexate 100-fold over free drug" [147]. At least a half dozen cancer cell types have already been associated with at least one unique protein that targeting dendrimers could use to identify the cell as cancerous, and as the genomic revolution progresses it is likely that proteins unique to each kind of cancer will be identified, thus allowing Baker to design a recognition dendrimer for each type of cancer [148].

The tecto-dendrimer nanodevice platform is readily generalized to treat many diseases other than cancer. For instance, to cure viral infections, the body must kill virus-compromised cells, and infected cells are easy to recognize because they display nonhuman viral proteins on their surfaces. Baker envisions stocking a warehouse of viral protein-recognition dendrimers, making it possible to build any particular antiviral tecto-dendrimer by coupling the appropriate targeting dendrimer from the warehouse with the standard tecto-dendrimer. This same strategy could be applied against parasites, which also have unique nonhuman surface proteins, and even against parasites that hide inside human cells, such as malaria [148]. Tomalia's group at Central Michigan University is also pursuing tecto-dendrimer devices up to 5–100 nm in size [149]. Molecular modeling has been used to determine optimal dendrimer surface modifications for the function of tecto-dendrimer nanodevices and to suggest surface modifications that improve targeting [147].

NASA and the National Cancer Institute have funded Baker's lab to produce dendrimerbased nanodevices that can detect and report cellular damage resulting from radiation exposure in astronauts on long-term space missions [150]. By mid-2002, the lab had built a nanodevice that detects and reports the intracellular presence of caspase-3, one of the first enzymes that is released during cellular suicide or apoptosis (programmed cell death), which is one sign of a radiation-damaged cell. Baker's caspase-detecting dendrimer has two components. The first component fools white blood cells into identifying the dendrimer as a blood sugar so that the nanodevice is readily absorbed into the cell. The second component uses fluorescence resonance energy transfer (FRET) that employs two closely bound molecules. Before the cell undergoes apoptosis, the FRET system stays bound together and the interior of the white blood cell remains dark on illumination. Once apoptosis begins and caspase-3 is released, however, the bond is quickly broken and the white blood cell is awash in fluorescent light. A retinal scanning device is being devised to measure the amount of fluorescence inside an astronaut's body. If the level is above a certain baseline, then possible counteracting drugs can be taken.

2.8. Radio-Controlled Biomolecules

Although there are already many examples of nanocrystals attached to biological systems for biosensing purposes, the same nanoparticles are now being investigated as a means for directly controlling biological processes. Researchers Joseph Jacobson, Shuguang Zhang, and Kimberly Hamad-Schifferli, at the Massachusetts Institute of Technology, and their colleagues [151] have attached tiny radio frequency antennas—1.4-nm gold nanocrystals of less than 100 atoms—to DNA. When a \sim 1-GHz radio frequency magnetic field is transmitted

into the tiny antennas, the nanoparticles spin rapidly as alternating eddy currents are induced in the nanocrystals, producing inductive heating. The biological molecules to which the crystals are attached experience highly localized heating, causing the double-stranded DNA to separate into two strands in a matter of seconds in a reversible dehybridization process that leaves neighboring molecules untouched. When the magnetic field is removed, the strands rejoin immediately [151]. "Regulation of biomolecules using electronic radio frequency control represents a new dimension in biology," says Zhang. "So far, there are no tools that can do this. To be able to control one individual molecule in a crowd of molecules is very valuable."

The long-term goal is to apply the antennas to living systems and control DNA (e.g., gene expression, the ability to turn genes on or off) via remote electronic switching. To do that, the MIT researchers could attach gold nanoparticles to specific oligonucleotides that, when added to a sample of DNA, would bind to complementary gene sequences, blocking the activity of those genes and effectively turning them off. Applying the radio frequency magnetic field would then heat the gold particles, causing their attached DNA fragments to detach, turning the genes back on. Such a tool could give pharmaceutical researchers a way to simulate the effects of potential drugs that also turn genes on and off [152]. "It's clever to find a way to bridge two very different worlds-the biochemical world of nucleic acids and the physical world of electromagnetic waves," says biochemist Gerald Joyce of the Scripps Institute in La Jolla, California [153]. "You can even start to think of differential receiversdifferent radio receivers that respond differently to different frequencies. By dialing in the right frequency, you can turn on tags on one part of DNA but not other tags." Adds Jacobson, "Manipulation of DNA is interesting because it has been shown recently that it has potential as an actuator [Section 4.3]—a hard drive component—and can be used to perform computational operations." MIT has licensed the technology to a biotech startup, Waltham, Massachusetts-based engeneOS [154]. Von Kiedrowski's group at Ruhr-University Bochum in Germany is also developing biocompatible and thermostable gold cluster labels, tailored for nanotechnological, biomolecular, and nanomedical applications, that could be used for this purpose [155, 156].

The gold nanocrystals can be attached to proteins as well as DNA, opening up the possibility for future radio frequency biology to electronically control more complex biological processes such as enzymatic activity, protein folding and biomolecular assembly. In late 2002, Jacobson announced that his team had achieved electrical control over proteins as well [157]. The researchers separated an RNA-hydrolyzing enzyme called ribonuclease S into two pieces: a large protein segment made up of 104 amino acids and a small 18-aminoacid strand called the S-peptide. The RNAase enzyme is inactive unless the small strand sits in the mouth of the protein. Jacobson's group linked gold nanoparticles to the end of S-peptide strands and used the particles as a switch to turn the enzyme on and off: in the absence of the radio frequency field, the S-peptides adopt their usual conformation and the RNAase remains active, but with the external radio frequency field switched on, the rapidly spinning nanoparticles prevented the S-peptide from assembling with the larger protein, inactivating the enzyme. Eventually, Jacobson hopes that electronically controlled proteins and nucleotides will allow molecular biologists to cut and splice genetic information electronically, and perhaps eventually program computers to engineer new organisms [157].

Gregory Timp's group at the University of Illinois [158] is experimenting with 7- μ siliconbased microchips inserted into living cells to verify cell viability, as a precursor to testing GHz-frequency radio frequency microtransponders using carbon nanotube antennas inside cells.

Optically remote-controlled biomolecules are also being studied. For instance, researchers at the University of Washington [159] have added a reversible switch to endoglucanase (an enzyme that facilitates the breakdown of cellulose) by attaching two light-sensitive polymer chains next to the active sites. When exposed to visible light, one chain becomes hydrophilic, attracting water molecules and expanding, but when exposed to ultraviolet light, the chain becomes hydrophobic, expelling water molecules and contracting into a coil. The other chain works in reverse: under ultraviolet light it expands, and under visible light it contracts. Depending on the type of light applied, the enzyme's active site is either blocked or open;

hence enzyme activity is either off or on. The same group has also reported temperatureinduced enzyme activity switching [160].

3. MICROSCALE BIOLOGICAL ROBOTS

Biotechnology originally contemplated the application of biological systems and organisms to technical and industrial processes, but in recent times the field has expanded to include genetic engineering and the emerging fields of genomics, proteomics, transcriptomics, gene chips, artificial chromosomes, tissue engineering, and even biobotics. Biotechnology now takes as its ultimate goal no less than the engineering of all biological systems, even completely designed organic living systems, using biological instrumentalities or "wet" nanotechnology. There are many good summaries of biotechnology [161–164] and its applications to future medicine [165–167] elsewhere, so here we focus solely on efforts to engineer natural nanomachine systems—microscale biological viruses (Section 3.1) and cells (Section 3.2)—to create new, artificial biological devices.

3.1. Engineered Viruses

Bacteriophage viruses, first employed therapeutically against bacteria by d'Herelle in 1922 [168], and other viruses have recently been used as self-replicating pharmaceutical agents [169]. During the last 10–15 years, bioengineered self-replicating viruses of various types [170, 171] and certain other vectors routinely were also being used in experimental genetic therapies as "devices" to target and penetrate certain cell populations, with the objective of inserting therapeutic DNA sequences into the nuclei of human target cells *in vivo*. Inserting new sequences into viral genomes, or combining components from two different viruses to make a new hybrid or chimeric virus [172, 173], is now routine, and virotherapies using oncolytic viruses that are replication competent in tumor but not in normal cells offer a novel approach for treating neoplastic diseases [174].

Efforts at purely rational virus design are underway but have not yet borne much fruit. For example, Endy et al. [175] computationally simulated the growth rates of bacteriophage T7 mutants with altered genetic element orders and found one new genome permutation that was predicted to allow the phage to grow 31% faster than wild type; unfortunately, experiments failed to confirm the predicted speedup. Better models are clearly needed [176, 177]. Nevertheless, combinatorial experiments on wild-type T7 by others [178–180] have produced new but immunologically indistinguishable T7 variants that have 12% of their genome deleted and that replicate twice as fast as wild type [180]. The Synthetic Biology Lab at MIT [181] seeks to build the next-generation T7, a bacteriophage with a genome size of about 40 Kbp and 56 genes. With DNA synthesis becoming cheap, "we wish to redesign and rebuild the entire genome, to create the next, and hopefully better, version of T7." Considerations in the redesign process include: "adding or removing restriction sites to allow for easy manipulation of various parts, reclaiming codon usage, and eliminating parts of the genome that have no apparent function." Synthesizing a phage from scratch "will allow us to better understand how Nature has designed the existing organism."

In a three-year project [182] culminating in 2002, the 7500-base polio virus was rationally manufactured "from scratch" in the laboratory by synthesizing the known viral genetic sequence in DNA, enzymatically creating an RNA copy of the artificial DNA strand, and then injecting the synthetic RNA into a cell-free broth containing a mixture of proteins taken from cells. The synthetic polio RNA then directed the synthesis of complete (and fully infectious) polio virion particles [182], allowing the researchers to claim that the virus was made without the use of any living cells. The rational design and synthesis of chimeric viral replicators is already possible today [183–186], but the rational design and synthesis of completely artificial viral sequences, leading to the manufacture of completely synthetic viral replicators, should eventually be possible.

Mark Young and Trevor Douglas at Montana State University have chemically modified the Cowpea chlorotic mottle virus (CCMV) viral protein cage surface to allow engineering of surface-exposed functional groups [187, 188]. This includes the addition of lamanin

peptide 11 (a docking site for lamanin-binding protein generously expressed on the surface of many types of breast cancer cells) to the viral coat, and the incorporation of 180 gadolinium atoms into each 28-nm viral capsid, allowing these tumor-targeting particles to serve as tumor-selective MRI contrast agents [189]. The researchers are now attempting to reengineer the artificial virion to make a complete tumor-killing nanodevice, exploiting a gating mechanism that results from reversible structural transitions in the virus [190]. Therapeutic anticancer compounds can be placed inside the viral capsule or even manufactured *in-situ*, using the capsid as a tiny reaction vessel. The natural viral gate of CCMV has been reengineered to allow control by redox potential (the oxidation state of a local environment, which influences the tendency of a molecule to lose or gain an electron). (Although CCMV, a plant virus, does not enter human cells, the final delivery vehicle could be a reconfigured human virus that does enter human cells.) Cellular interiors have a higher redox potential than blood, so viral capsids could be shut tight in transit but would open their redox-controlled gates after entering targeted cancer cells, releasing their payload of therapeutic agents. An alternative radiation-triggered switchable gate is also being developed. The team is exploring how the modified virus capsules work in a mouse model system and is encouraged by promising initial results. In principle, the four capabilities of the engineered capsids—high-sensitivity imaging, cell targeting, drug transport, and controlled delivery-represent a potentially powerful, yet minimally toxic, way to fight metastasized cancer [189].

Scientists from Osaka University have used a protein from the hepatitis B virus to synthesize 80-nm cagelike structures whose surface is modified to include a peptide that binds with a receptor on human liver cells. In one experiment, a fluorescent dye inserted into the cages reached cancerous human liver cells (both cultured in a lab dish and transplanted into mice) without affecting other cells [191]. Alterations in the surface peptides could allow the cages to be used as vehicles to deliver drugs or genes to other tissues as well.

3.2. Engineered Bacteria

Engineered bacteria were being pursued by Vion Pharmaceuticals in collaboration with Yale University. In their "Tumor Amplified Protein Expression Therapy" program [192], antibiotic-sensitive *Salmonella typhimurium* (food poisoning) bacteria were attenuated by removing the genes that produce purines vital to bacterial growth. The tamed strain could not survive very long in healthy tissue, but quickly multiplied 1000-fold inside tumors that were rich in purines. The engineered bacteria were available in multiple serotypes to avoid potential immune response in the host, and Phase I human clinical trials were underway in 2000 using clinical dosages. The next step would be to add genes to the bacterium to produce anticancer proteins that can shrink tumors, or to modify the bacteria to deliver various enzymes, genes, or prodrugs for tumor cell growth regulation.

In 1998, Glen Evans, then at the University of Texas Southwestern Medical Center, described the possible construction of synthetic genomes and artificial organisms. His proposed strategy involved determining or designing the DNA sequence for the genome, synthesizing and assembling the genome, then introducing the synthetic DNA into an enucleated pluripotent host cell to create an artificial organism. Genome engineers could modify an existing microbe by adding a biochemical pathway borrowed from other organisms, though this remains a difficult task because tailoring an existing system to match unique requirements demands detailed knowledge about the pathway. Ultimately, says Adam P. Arkin at Lawrence Berkeley National Laboratory, "we want to learn to program cells the same way we program computers." [193] Some genome engineers have started by building the biological equivalent of the most basic switch in a computer—a digital flip-flop. "Cells switch genes on and off all the time," observes MIT's Thomas F. Knight, Jr., who has pioneered some of this research. A cellular toggle switch, made of DNA and some well-characterized regulatory proteins, might be devised to turn on a specific gene when exposed to a particular chemical. These could be used in gene therapies: Implanted genes might be controlled with single doses of specially selected drugs, one to switch the gene on, another to switch it off [193].

Arcady Mushegian of Akkadix Corporation has looked at the genes present in the genomes of fully sequenced microbes to see which ones are always conserved in nature [194]. He concludes that as few as 300 genes are all that may be required for life, constituting the minimum possible genome for a functional microbe. An organism containing this minimal gene set would be able to perform the dozen or so functions required for life: manufacturing cellular biomolecules, generating energy, repairing damage, transporting salts and other molecules, responding to environmental chemical cues, and replicating. The minimal microbe—a basic cellular chassis—could be specified by a genome only 150,000 nucleotides bases in length. By 2001, Glen Evans could already produce made-to-order DNA strands that were 10,000 nucleotide bases in length [195] and was striving to increase this length by at least a factor of ten.

An engineered full-genome DNA, once synthesized, could be placed inside an empty cell membrane—most likely a living cell from which the nuclear material had been removed. Used in medicine, these artificial biorobots could be designed to produce useful vitamins, hormones, enzymes, or cytokines in which a patient's body was deficient; to selectively absorb and metabolize into harmless end products harmful substances such as poisons, toxins, or indigestible intracellular detritus; or even to perform useful mechanical tasks. One private company, engeneOS [196], was formed in 2000 to pursue the construction of these artificial biological devices, and in 2001 another company, Robiobotics LLC, put forward a business plan to pursue "whole genome engineering" and to begin seeking funding. Several other groups may be even further along in wetware engineering.

In November 2002, J. Craig Venter, of human genome-sequencing fame, and Hamilton O. Smith, a Nobel laureate, announced [197] their new company, Institute for Biological Energy Alternatives (IBEA), had received a \$3 million, 3-year grant from the Energy Department to create a minimalist organism, starting with the *M. genitalium* microorganism. Working with a research staff of 25 people, the scientists will remove all genetic material from the organism, then synthesize an artificial string of genetic material resembling a naturally occurring chromosome that they hope will contain the minimum number of *M. genitalium* genes needed to sustain life [197]. The artificial chromosome will be inserted into the hollowed-out cell, which will then be tested for its ability to survive and reproduce. To ensure safety, Smith and Venter said the cell will be deliberately hobbled to render it incapable of infecting people; it also will be strictly confined and designed to die if it does manage to escape into the environment [197].

In 2003, Glen Evans's new company Egea Biosciences [198] vaulted into the lead, receiving the first patent [199] "to include broad claims for the chemical synthesis of entire genes and networks of genes comprising a genome, the 'operating system' of living organisms." According to the company, Egea's proprietary GeneWriter and Protein Programming technology has been proven in extensive proof of concept studies and has produced libraries of more than 1,000,000 programmed proteins, produced over 200 synthetic genes and proteins, produced the largest gene ever chemically synthesized of over 16,000 bases, engineered proteins for novel functions, improved protein expression through codon optimization, and developed custom genes for protein manufacturing in specific host cells. Egea's software allows researchers to author new DNA sequences that the company's hardware can then manufacture to specification with a base-placement error of only $\sim 10^{-4}$, which Evans calls "word processing for DNA" [200].

According to Egea's patent [199], one "preferred embodiment of the invention" would include the synthesis of "a gene of 100,000 bp...from one thousand 100-mers. The overlap between 'pairs' of plus and minus oligonucleotides is 75 bases, leaving a 25 base pair overhang. In this method, a combinatorial approach is used where corresponding pairs of partially complementary oligonucleotides are hybridized in the first step. A second round of hybridization then is undertaken with appropriately complementary pairs of products from the first round. This process is repeated a total of 10 times, each round of hybridization reducing the number of products by half. Ligation of the products then is performed." The result would be a strand of DNA 100,000 base pairs in length, long enough to make a very simple bacterial genome [200]. Evans says his prototype machine can synthesize 10,000 bases in 2 days, and that 100,000-bp strands might require "a matter of weeks" to synthesize using

a future next-generation machine [200]. "Pretty soon, we won't have to store DNA in large refrigerators," says Tom Knight. "We'll just write it when we need it" [200].

4. MEDICAL NANOROBOTICS

The third major development pathway of nanomedicine—molecular nanotechnology (MNT), or nanorobotics [6, 8, 201]—takes as its purview the engineering of all complex mechanical medical systems constructed from the molecular level. Just as biotechnology extends the range and efficacy of treatment options available from nanomaterials, the advent of molecular nanotechnology will again expand enormously the effectiveness, comfort, and speed of future medical treatments while at the same time significantly reducing their risk, cost, and invasiveness. MNT will allow doctors to perform direct *in vivo* surgery on individual human cells. The ability to design, construct, and deploy large numbers of microscopic medical nanorobots will make this possible.

4.1. Early Thinking in Medical Nanorobotics

The first and most famous scientist to voice these possibilities was the late Nobel physicist Richard P. Feynman, who worked on the Manhattan Project at Los Alamos during World War II and later taught at CalTech for most of his professorial career. In his remarkably prescient 1959 talk "There's Plenty of Room at the Bottom," Feynman proposed employing machine tools to make smaller machine tools, with these to be used in turn to make still smaller machine tools, and so on all the way down to the atomic level [202]. Feynman prophetically concluded that this is "a development which I think cannot be avoided." Such nanomachine tools, nanorobots, and nanodevices could ultimately be used to develop a wide range of atomically precise microscopic instrumentation and manufacturing tools; that is, nanotechnology.

Feynman was clearly aware of the potential medical applications of the new technology he was proposing. After discussing his ideas with a colleague, Feynman offered [202] the first known proposal for a nanomedical procedure to cure heart disease: "A friend of mine (Albert R. Hibbs) suggests a very interesting possibility for relatively small machines. He says that, although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and looks around. (Of course the information has to be fed out.) It finds out which valve is the faulty one and takes a little knife and slices it out. Other small machines might be permanently incorporated in the body to assist some inadequately functioning organ." Later in his historic lecture in 1959, Feynman urged us to consider the possibility, in connection with biological cells, "that we can manufacture an object that maneuvers at that level!"

The vision behind Feynman's remarks became a serious area of inquiry two decades later, when K. Eric Drexler, while still a graduate student at the Massachusetts Institute of Technology, published a technical paper [203] suggesting that it might be possible to construct, from biological parts, nanodevices that could inspect the cells of a living human being and carry on repairs within them. This was followed a decade later by Drexler's seminal technical book [201] laying the foundations for molecular machine systems and nanorobotics, and subsequently by Freitas's technical books [6, 8] on medical nanorobotics.

4.2. Nanorobot Parts and Components

Extending nanomedicine to molecular machine systems will probably require, among many other things, the ability to build precise structures, actuators, and motors that operate at the molecular level, thus enabling manipulation and locomotion. For example, in 1992 K. Eric Drexler of the Institute for Molecular Manufacturing theorized that an efficient nanomechanical bearing could be made by bending two graphite sheets into cylinders of different diameters, then inserting the smaller one into the larger one [201]. By 2000, John Cumings and Alex Zettl at U.C. Berkeley had demonstrated experimentally that nested carbon nanotubes do indeed make exceptionally low-friction nanobearings [204].

4.2.1. Nanobearings and Nanogears

To establish the foundation for molecular manufacturing, it is first necessary to create and to analyze possible designs for nanoscale mechanical parts that could, in principle, be manufactured. Because these components cannot yet be physically built in 2004, such designs cannot be subjected to rigorous experimental testing and validation. Designers are forced instead to rely on *ab initio* structural analysis and computer studies including molecular dynamics simulations. Notes Drexler [201]: "Our ability to model molecular machines (systems and devices) of specific kinds, designed in part for ease of modeling, has far outrun our ability to make them. Design calculations and computational experiments enable the theoretical studies of these devices, independent of the technologies needed to implement them."

In nanoscale design, building materials do not change continuously as they are cut and shaped, but rather must be treated as being formed from discrete atoms [205]. A nanoscale component is a supermolecule, not a finely divided solid. Any stray atoms or molecules within such a structure may act as dirt that can clog and disable the device, and the scaling of vibrations, electrical forces, thermal expansion, magnetic interaction, and surface tension with size lead to dramatically different phenomena as system size shrinks from the macroscale to the nanoscale [201].

Molecular bearings are perhaps the most convenient class of components to design because their structure and operation is fairly straightforward. One of the simplest examples is Drexler's overlap-repulsion bearing design [201], shown with end views and exploded views in Fig. 3 using both ball-and-stick and space-filling representations. This bearing has exactly 206 atoms including carbon, silicon, oxygen, and hydrogen, and it comprises a small shaft that rotates within a ring sleeve measuring 2.2 nm in diameter. The atoms of the shaft are arranged in a sixfold symmetry, and the ring has 14-fold symmetry, a combination that provides low-energy barriers to shaft rotation. At the atomic scale, the two opposing surfaces have periodic bumps and hollows, but the periods of these bumps are different for the two surfaces; that is, they are "incommensurate" [201, 207]. Two incommensurate surfaces cannot lock up in any particular position; hence, the barrier to free rotation is very low, on the order of $\sim 0.001 \text{ kT}$ (thermal noise at room temperature) [208]. Figure 4 shows an exploded view of a 2808-atom strained-shell sleeve bearing designed by Drexler and Merkle [201], using molecular mechanics force fields to ensure that bond lengths, bond angles, van der Waals distances, and strain energies are reasonable. Components of high rotational symmetry may consist of intrinsically curved, strained-shell, or special-case structures [201]. In the case of strained-shell structures, the bearing illustrated in Fig. 4 has bond strains of around $\sim 10\%$ $(\sim 38 \text{ zJ/atom})$, and similar hydrocarbon bearings have been designed with bond strains of \sim 5% (\sim 11 zJ/atom) [6]. For comparison, strain energies [209, 210] are <3 zJ/atom for diamond lattice, ~25 zJ/atom in C₂₄₀, ~7–27 zJ/atom in the walls of infinite carbon nanotubes of diameter 0.7–1.3 nm, up to ~59 zJ (13% strain) for some bonds around a Lomer dislocation in diamond [211], \sim 70 zJ/atom in C₆₀, and at least \sim 80 zJ/atom for C₃₆. Fullerenes are among the most highly strained natural molecules ever isolated. For symmetrical diamondoid structures with negligible hoop stress, permissible bond strains may in theory be as large as $\sim 140 \text{ zJ/atom producing a } \sim 23\%$ bond strain [201]; nanotube breaking strain is 20–30% for various chiral forms, and buckling strain is \sim 8% in axial compression. Bond strain in a simple strained-shell bearing can be lowered by making the bearing bigger, and thereby reducing the curvature. Thus, strained shell bearings are feasible, although in 2004 it remained unclear exactly how small they could be [212, 213] before becoming unstable. This 4.8-nm diameter bearing features an interlocking-groove interface that derives from a modified diamond (100) surface. Ridges on the shaft interlock with ridges on the sleeve, making a very stiff structure. Attempts to bob the shaft up or down, or rock it from side to side, or displace it in any direction (except axial rotation, wherein displacement is extremely smooth) encounter a very strong resistance [206]. Whether these bearings would have to be assembled in unitary fashion, or instead could be assembled by inserting one part into the other without damaging either part, had not been extensively studied or modeled by 2004.

Molecular gears are another convenient component system for molecular manufacturing design-ahead. For example, Drexler and Merkle [201] designed a 3557-atom planetary gear, which is shown in side, end, and exploded views in Fig. 5. The entire assembly has 12



Figure 3. End views and exploded views of a 206-atom overlap-repulsion bearing [201]. Reprinted with permission from [206], K. E. Drexler, in "Prospects in Nanotechnology: Toward Molecular Manufacturing" (M. Krummenacker and J. Lewis, Eds.), p. 1. Wiley, New York, 1995. © 1995, Wiley.

moving parts and is 4.3 nm in diameter and 4.4 nm in length, with a molecular weight of 51,009.844 Da and a molecular volume of 33.458 nm³. An animation of the computer simulation shows the central shaft rotating rapidly and the peripheral output shaft rotating slowly. The small planetary gears rotate around the central shaft, and they are surrounded by a ring gear that both holds the planets in place and ensures that all of the components move in the proper fashion. The ring gear is a strained silicon shell with sulfur atom termination; the sun gear is a structure related to an oxygen-terminated diamond (100) surface; the planet gears resemble multiple hexasterane structures with oxygen rather than CH₂ bridges between the parallel rings; and the planet carrier is adapted from a Lomer dislocation [214] array created by R. Merkle and L. Balasubramaniam and is linked to the planet gears using C—C bonded bearings. The view in Fig. 5c retains the elastic deformations that are hidden in Fig. 5a—the gears are bowed. In the macroscale world, planetary gears are used in



Figure 4. Exploded view of a 2808-atom strained-shell sleeve bearing [201]. Reprinted with permission from [206], K. E. Drexler, in "Prospects in Nanotechnology: Toward Molecular Manufacturing" (M. Krummenacker and J. Lewis, Eds.), p. 1. Wiley, New York, 1995. © 1995, Wiley.

automobiles and other machines in which it is necessary to transform the speeds of rotating shafts.

Goddard and colleagues at CalTech [205, 215] performed a rotational impulse dynamics study of this "first-generation" planetary gear. At the normal operational rotation rates for which this component was designed (e.g., <1 GHz for <10 m/s interfacial velocities), the gear worked as intended and did not overheat [215]. Started from room temperature, the



Figure 5. End-, side-, and exploded-view of a 3557-atom planetary gear [201]. Reprinted with permission from [206], K. E. Drexler, in "Prospects in Nanotechnology: Toward Molecular Manufacturing" (M. Krummenacker and J. Lewis, Eds.), p. 1. Wiley, New York, 1995. © 1995, Wiley.

gear took a few cycles to engage, then rotated thermally stably at ~ 400 K. However, when the gear was driven to ~ 100 GHz, significant instabilities appeared although the device still did not self-destruct [215]. One run at ~ 80 GHz showed excess kinetic energy causing the gear temperature to oscillate up to 450 K above baseline [205]. One animation of the simulation shows that the ring gear wiggles violently because it is rather thin. In an actual nanorobot incorporating numerous mechanical components, the ring gear would be part of a larger wall that would hold it solidly in place and would eliminate these convulsive motions that, in any case, are seen in the simulation only at unrealistically high operating frequencies.

Drexler and Merkle [216] later proposed a "second-generation" planetary gear design having 4235 atoms, a molecular weight of 72,491.947 Da, and a molecular volume of 47.586 nm³. This new version was indeed more stable but still had too much slip at the highest frequencies. Commenting on the ongoing design effort, Goddard [205] suggested that an optimal configuration could have the functionality of a planetary gear but might have an appearance completely different from the macroscopic system, and offered an example: "Because a gear tooth in the xy plane cannot be atomically smooth in the z-direction, we may develop a Vee design so that the Vee shape of the gear tooth in the z-direction nestles within a Vee notch in the race to retain stability in the z-direction as the teeth contact in the xy plane. This design would make no sense for a macroscopic gear system since the gear could never be placed inside the race. However, for a molecular system one could imagine that the gear is constructed and that the race is constructed all except for a last joining unit. The parts could be assembled and then the final connections on the face made to complete the design."

4.2.2. Nanomotors and Power Sources

Another class of theoretical nanodevice that has been designed is a gas-powered molecular motor or pump [217]. The pump and chamber wall segment shown in Fig. 6 contain 6165 atoms with a molecular weight of 88,190.813 Da and a molecular volume of 63.984 nm³. The device could serve either as a pump for neon gas atoms or (if run backward) as a motor to convert neon gas pressure into rotary power. The helical rotor has a grooved cylindrical bearing surface at each end, supporting a screw-threaded cylindrical segment in the middle.



Figure 6. Side views of a 6165-atom neon gas pump/motor [217]. Image courtesy of K. Eric Drexler. © Institute for Molecular Manufacturing (http://www.imm.org) 2nd xerox (http://nano.xerox.com).

In operation, rotation of the shaft moves a helical groove past longitudinal grooves inside the pump housing. There is room enough for small gas molecules only where facing grooves cross, and these crossing points move from one side to the other as the shaft turns, moving the neon atoms along. Goddard [205] reported that preliminary molecular dynamics simulations of the device showed that it could indeed function as a pump, although "structural deformations of the rotor can cause instabilities at low and high rotational frequencies. The forced translations show that at very low perpendicular forces due to pump action, the total energy rises significantly and again the structure deforms." Merkle acknowledges that the pump moves neon atoms at an energy cost of 185 Kcal/mole-Å (12,900 zJ/atom-nm), which is not very energy efficient, but further refinement of this crude design is clearly warranted.

Almost all such design research in molecular nanotechnology is restricted to theory and computer simulation, which allows the design and testing of large structures or complete nanomachines and the compilation of growing libraries of molecular designs. The work is relatively inexpensive and does not require the support of a large team. Of course, calculations of many-body systems are notoriously difficult, with many computer packages making a number of simplifying assumptions (e.g., nuclei as point masses, electrons treated as a continuous charge distribution, and three-dimensional potential energy functions derived semiempirically from experimental data and treated as a classical field despite their true quantum mechanical character [for ease of computation]. Goddard [218] notes that future nanosystem simulations may require 1–100 million atoms to be considered explicitly, demanding further improvements in present-day molecular dynamics methodologies, which have only relatively recently entered the multimillion atom range [219].

Other researchers are pursuing experimental approaches to developing organic nanomotors for future nanorobots. Most notably, Carlo Montemagno, currently at the University of California at Los Angeles, has modified a natural biomotor to incorporate nonbiological parts, creating the first artificial hybrid nanomotor [220, 221]. Montemagno started with natural ATPase, a ubiquitous enzyme found in virtually every living organism and that helps to convert food into usable energy in living cells. The moving part of an ATPase molecule is a central protein shaft (or rotor, in electric motor terms) that rotates in response to electrochemical reactions with each of the molecule's three proton channels (comparable to the electromagnets in the stator coil of an electric motor). ATP (adenosine triphosphate) is the fuel that powers the molecular motor's motion.

Using the tools of genetic engineering, Montemagno added metal-binding amino acid residues to the ATPase. This allowed each motor molecule to bind tightly to nanoscale nickel pedestals prepared by electron beam lithography. Properly oriented motor molecules 12 nm in diameter were then attached to the pedestals with a precision approaching 15 nm, and a silicon nitride bar 100 nm long was bound to the rotor subunit of each motor molecule [222], all by self-assembly. In a microscopic video presentation, dozens of bars could be seen spinning like a field of tiny propellers. The group's first integrated molecular motor ran for 40 minutes at 3–4 revolutions per second. Subsequent motors have been operated for hours continuously by feeding them plenty of ATP. Montemagno has been measuring things like horsepower and motor efficiency, simple tests that would be familiar to any mechanical engineer inspecting a car engine. Montemagno is also trying to build a solar-powered, biomolecular motor-driven autonomous nanodevice, wherein light energy is converted into ATP, which then serves as a fuel source for the motor. "We think we'll be able to make autonomous devices that are powered by light on a scale of 1 μ or less, smaller than bacteria," he says.

Montemagno is developing a chemical means of switching his hybrid motors on and off reliably [223]. By engineering a secondary binding site tailored to a cell's signaling cascade, he plans to use the sensory system of the living cell to control nanodevices implanted within the cell [224]. Montemagno envisions tiny chemical factories operating inside living cells. He speculates that these nanofactories could be targeted to specific cells, such as those of tumors, where they would synthesize and deliver chemotherapy agents. Within a few years, he expects to have a motor assembled within a living cell, with the cell's physiology providing the energy to run it. "My 10-year goal is to make a device that harvests single molecules within a living cell, maybe a cellular pharmacy that produces a drug, stores it

within the cell, and then based upon some signal, releases it," Montemagno said in 2000. "For a technology that wasn't expected to produce a useful device before the year 2050, I think we've made a pretty good start. But we have a long way to go before it's safe to turn these little machines loose in the human body." Also following the bio-nano pathway is Mavroidis's group at Rutgers University, which in late 2003 received a \$1Million 4-year National Science Foundation grant to produce a viral protein linear nanomotor prototype by 2007 that will "pave the way for development of complete nanorobotic assemblies" and later "make up the systems that travel the bloodstream or perform other unprecedented tasks in medicine and industry" [225].

Experimental nanomotor research is progressing in other laboratories as well. For instance, a 78-atom chemically powered rotating motor was synthesized in 1999 as a proof of principle by chemist T. Ross Kelly at Boston College [226], and chemically powered DNA-based nanomotors have also been designed and operated (Section 4.3.2). A chemically powered rotaxane-based linear motor exerting $\sim 100 \text{ pN}$ of force with a 1.9-nm throw and a ~ 250 -s contraction cycle has been demonstrated by Stoddart's group at the University of California, Los Angeles [227, 228]. Ben Feringa at the University of Groningen in the Netherlands has built an artificial 58-atom motor molecule that spins when illuminated by solar energy [229]. Wong and Leigh at the University of Edinburgh [230] synthesized a motor consisting of a catenane made of a large ring with two smaller rings and four kinds of hydrogen-bonding sites. Pulsing the rings with long-wavelength ultraviolet light causes the small rings to complete a circuit along the large ring in about 70 min, though Leigh says faster ultraviolet laser cycling should make the rings spin at millions or billions of revolutions per minute if needed. White light and a bromine solution reset the motor, returning the rings to their original positions. Another potential nanorobot power source is a modified microbial fuel cell; laboratory demonstrations of such cells have used captive bacteria or immobilized enzymes [231] that, when fed organic material such as sugar [232], convert chemical energy into electricity that could be employed to power tiny electrical motors [233]. In July 2003, U.S. patent 6,586,133 was awarded to Dale Teeters and colleagues at the University of Tulsa for their method of making micron-scale electrical batteries.

Also in 2003, the Zettl group [234] at the University of California, Berkeley, created an electrically powered 550-nm-wide nanomotor by depositing a number of multiwalled nanotubes on the flat silicon oxide surface of a silicon wafer, then using an atomic force microscope to identify the best nanotubes from the pile. The researchers used electron beam lithography to simultaneously pattern a 110–300-nm gold rotor, nanotube anchors, and opposing stators around the chosen nanotubes. The researchers then annealed the rotor to the nanotubes, after which the surface was selectively etched to provide sufficient clearance for the rotor. A third stator was already buried under the silicon oxide surface. When the stators were alternately charged with 50 V of direct current, the gold rotor rocked back and forth up to 20 degrees, making a torsional oscillator. With a strong electrical jolt to the stators, the team could jerk the rotor and break the outer wall of the nested nanotubes, allowing the outer nanotube and attached rotor to spin freely around the inner nanotubes as a nearly frictionless bearing [204]; the carbon nanotube shaft to which the rotor is attached is only a few atoms across, perhaps 5-10 nm thick. The oscillating rotor might be used to generate microwave frequency oscillations possibly up to a few gigahertz, or the spinning rotor could be used to mix liquids in microfluidic devices. Zettl's team is taking friction and conductance measurements and plans to reduce the motor size by a factor of five in the future. Other groups are investigating nanotubes for use in mechanical [235] and nanoelectromechanical [236] systems.

Another well-known proposal is for nanorobotic devices to receive all power (and some control) signals acoustically [6, 201]. Externally generated ultrasonic pressure waves would travel through the aqueous environment to the nanodevice, whereupon a piston on the device would be driven back and forth in a well-defined manner, passing energy and information simultaneously into the device. Although an acoustically actuated nanoscale piston has not yet been demonstrated experimentally, microfluidic actuators are well known [237], and there are many reasons to expect that such small pistons will work as theory [201] predicts. For example, microscale pressure sensors have already been built using conventional micro

electron mechanical systems (MEMS) fabrication techniques (e.g., a piezoresistive pressure microsensor diaphragm [238], a 250- μ medical pressure sensor that fits inside catheters [239], a 27- μ circular capacitive pressure sensor [240], and an optical pressure sensor 7 μ thick [241]. Micromachined flaps 45 μ in size have been raised from horizontal to vertical position by ultrasonic pulsing [242], demonstrating microscale acoustic actuation. Gas-filled 2–4 μ micropores insonated at 1–2 MHz may exhibit "pistonlike" or "membranelike" vibration modes [243]. At the nanoscale, pressure applied, and then released, on carbon nanotubes causes fully reversible compression [244], and experiments have shown very low frictional resistance between nested nanotubes that are externally forced in and out like pistons [204]. Masako Yudasaka, who studies C₆₀ molecules trapped inside carbon nanotubes or "peapods" at NEC, expects that "the buckyball can act like a piston" [245]. Computational simulations of acoustically driven nanopistons in a fluid environment would be useful in assessing the experimental utility of this potential nanorobot power source.

4.2.3. Nanocomputers

Truly effective medical nanorobots may require onboard computers to allow a physician to properly monitor and control their work. Molecular computing [246] has become one of the hottest research topics in nanotechnology. In 2000, a collaborative effort between the University of California, Los Angeles, and Hewlett Packard produced the first laboratory demonstration of completely reversible room-temperature molecular switches that could be employed in nanoscale memories, using mechanically interlinked ring molecules called catenanes [247], and there has been much recent progress with nanotube- and nanorod-based molecular electronics [248–250]. At least two independent companies—Molecular Electronics Corporation in Texas and California Molecular Electronics Corporation in California— have the explicit goal of building the first commercial molecular electronic devices including memories and other computational components of nanocomputers, possibly in the next few years, using techniques of self-assembly. There is also the possibility of low-speed biology-based digital nanocomputers, as briefly discussed in Section 4.3.4.

4.3. Self-Assembly and Directed Parts Assembly

4.3.1. Self-Assembly of Mechanical Parts

There is a wide range of different molecular systems that can self-assemble [251], and space does not permit more than a brief review here. Perhaps the best-known self-assembling molecular systems include those that form ordered monomolecular structures by the coordination of molecules to surfaces [252], called self-assembled monolayers (SAMs) [253], self-assembling thin films [254, 255] or Langmuir-Blodgett films [254, 256], self-assembling lipidic micelles and vesicles [257, 258], and self-organizing nanostructures [259, 260]. In many of these systems, a single layer of molecules affixed to a surface allows both thickness and composition in the vertical axis to be adjusted to 0.1 nm by controlling the structure of the molecules making up the monolayer, although control of in-plane dimensions to less than 100 nm is very difficult.

It is already known that self-assembling molecular systems can be made to self-replicate if the components have sufficient complexity. An example in biology is offered by the bacteriophages, viruses that infect and commandeer a bacterial cell's replication mechanism to synthesize their own component molecules such as nucleic acids and proteins. Spontaneous self-assembly of the component viral proteins then occurs, which produces hundreds of virus offspring in the host cell. Whereas new viruses are formed via self-assembly of randomly arranged and randomly moving component protein molecules, the various component molecules do not associate with one another at random during the assembly process but, rather, associate in a definite sequence. Biologists believe that conformational switches in protein molecules facilitate this randomized assembly of bacteriophages. In a protein molecule with several bond sites, a conformational switch causes the formation of a bond at one site to change the conformation of another bond site. As a result, a conformational change that occurs at one assembly step provides the essential substrate for assembly at the next step [261].

Several attempts have been made to achieve self-assembly of small mechanical parts to avoid direct parts grasping [262–265], and Saitou [266] gives a simple example of "sequential random bin picking," in which a process of sequential mating of a random pair of parts drawn from a parts bin which initially contains a random assortment of parts can produce the mating of a desired pair of parts. Griffith [267] complains that existing self-assembling systems are "essentially meso- or macro-scale versions of crystallization" and suggests expanding the toolbox of self-assembly by including dynamic components that emulate enzymatic allostery. Griffith has presented a simple "mechanical enzyme" analog: a two-bit mechanical state machine that programmatically self-assembles while floating at an interface between water and poly-fluorodecalin. The mechanical state machine has a mechanical flexure that acts as the "switch" in the state machine, making a mechanical allosteric enzyme. "The problem in designing self-assembling components is to avoid undesirable metastable states, and to make the desired assembled geometries the lowest energy conformations of the system," Griffith observes. For example, "you will observe that there are no straight edges on the components to avoid local energy minima on their collision." Griffith noted that he was "making progress on a 3-state system, however it is a problem of increasing difficulty, and as more component types are added to a system the challenge is to avoid any undesirable local energy minima," necessitating the development of energy versus orientation modeling tools [266].

The programming of engineered sequences of such conformational switches can allow the self-assembly of quite complicated mechanical structures. Saitou [267–271] has presented a model of self-assembling systems in which assembly instructions are written as conformational switches—local rules that specify conformational changes of a component. The model is a self-assembling automaton explicitly inspired [267] by the Penrose [272] self-replicating blocks and by Hosokawa's self-assembling triangular parts with embedded switches [273]. It is defined as a sequential rule-based machine that operates on one-dimensional strings of symbols. An algorithm is provided for constructing a self-assembling automaton that selfassembles a one-dimensional string of distinct symbols in a particular subassembly sequence. Classes of self-assembling automata are then defined on the basis of classes of subassembly sequences in which the components are self-assemble. For each class of subassembly sequence, the minimum number of conformations is provided that is necessary to encode subassembly sequences in the class. Finally, it is shown that three conformations for each component are enough to encode any subassembly sequence of a one-dimensional assembly of distinct components and having arbitrary length. Saitou claims [267] that his model of self-assembling automata can also be applied to self-assembly in two or three dimensions (in particular, to "the assembly of micro- to mesoscale components for microelectronic applications") an extension that might eventually permit the design of physical systems capable of self-replication via self-assembly.

Guided [274–277] or directed [278–280] self-assembly has become a growing research area. Yeh and Smith [263] have described a process of fluidic self-assembly of optoelectronic devices, Rothemund and Winfree [281] have described a tile assembly model for pseudocrystalline self-assembly, Breivik [282] has designed and patented a set of self-replicating physical polymers, and Gracias et al. [283] have impressed electrical circuits including light-emitting diodes on the surfaces of copper-polyimide truncated octahedra, each ~ 1 mm in diameter, and then induced these octahedra to self-assemble into specified three-dimensional electrical networks of up to 12 devices by comelting of opposing solder spots. (Gracias notes that hierarchical self-assembly [284] and shape-selective self-assembly using lock-and-key structures [285, 286] "offer more sophisticated strategies for the fabrication of asymmetrical networks incorporating more than one repeating unit.") Whitesides et al. [287, 288] first demonstrated capillary-force-driven assembly of a simple circuit and other structures from millimeter-scale components, as well as electrostatic self-assembly [289], and some of this work has since been extended to the fluidic self-assembly of microscale parts [263, 290–293], including "micro-origami" [294, 295] or "silicon origami" [296], as well as mesoscopic nucleic acid analogs [297]. The dynamics of Brownian self-assembly [298], the theory of designable self-assembling molecular machine structures [267, 299], and the computational modeling of self-assembly processes [300] are beginning to be addressed.

4.3.2. DNA-Directed Assembly

Working from the insight that DNA could serve as an assembly jig in solution phase, Smith and Krummenacker [301] devised a possible method for the assembly and covalent linkage of protein "parts" into specific orientations and arrangements, as determined by the hybridization of DNA attached to the proteins, called DNA-Guided Assembly of Proteins (DGAP). In this method, multiple DNA sequences would be attached to specific positions on the surface of each protein, and complementary sequences would bind, forcing protein building blocks (possibly including biomolecular motors, structural protein fibers, antibodies, enzymes, or other existing functional proteins) together in specific desired combinations and configurations, which would then be stabilized by covalent interprotein linkages. This technique could also be applied to nonprotein components that can be functionalized at multiple sites with site-specific DNA sequences, although proteins, at least initially, may be more convenient building blocks because of their size, their surface chemistry, the wide variety of functions and mechanical properties they can confer on the resulting assemblies, and the many existing techniques for introducing designed or artificially evolved modifications into natural proteins of known structure. A startup company, Molecubotics, Inc. [302], has put forward a business plan to pursue the DGAP method of molecular assembly and to begin seeking funding.

Although at this writing the experimental feasibility of the DGAP approach remains unknown, others are exploring methods for covalently attaching functional (fusion) proteins to a DNA backbone in a specified manner at \sim 8.5 nm (25 bp) intervals [303]; addressable protein targeting in macromolecular (e.g., nucleoprotein) assemblies [304–307]; attaching specific DNA tag sequences to protein molecules such as enzymes to a specific region on the surface of the target protein well away from the active site [308]; laying of silver wire [309, 310], gold wire [311], and other nanowires [312] using DNA as a molecular scaffolding or possible template for circuit construction; decorating DNA with fullerenes [313] and dendrimers [314]; assembling DNA-tagged nanometer-sized gold [315, 316], magnetic [317], or other particles into larger clusters, or into spontaneous lattices or magnetic chains [318]; silicon component aggregation on DNA-directed two-dimensional array [319]; and "protein stitchery" [320]. Drexler [321] notes that evolution has not maximized the stability of natural proteins, and that substantially greater stability may be engineered by various means (e.g., increasing folding stability by >100 Kcal/mole).

Early mechanical nanorobots might be made, at least in part, of DNA. The idea of using DNA to build nanoscale objects has been pioneered by Nadrian Seeman at New York University [322]. Two decades ago, Seeman recognized that a strand of DNA has many advantages as a construction material. First, it is a relatively stiff polymer. Its intermolecular interaction with other strands can be readily predicted and programmed as a result of the base-pair complementarity of nucleotides, the fundamental building blocks of genetic material. DNA also tends to self-assemble. Arbitrary sequences are readily manufactured using conventional biotechnological techniques, and DNA is readily manipulated and modified by a large number of enzymes. During the 1980s, Seeman worked to develop strands of DNA that would zip themselves up into more and more complex shapes—first tiny squares, then three-dimensional stick-figure cubes composed of 480 nucleotides each [323], then a truncated octahedron containing 2550 nucleotides [324]. By the mid-1990s, Seeman could fabricate nanoscale DNA stick figures of almost any regular geometric shape, by the billions per batch [325].

In 1999, Seeman reported yet another breakthrough—the construction of a mechanical DNA-based device that might serve as the basis for a nanoscale robotic actuator [326]. The mechanism has two rigid double-stranded DNA arms a few nanometers long that can be made to rotate between fixed positions by introducing a positively charged cobalt compound into the solution surrounding the molecules, causing the bridge region to be converted from the normal B-DNA structure to the unusual Z-DNA structure. The free ends of the arms shift position by 2–6 nm during this fully reversible structural conversion, like a hinge opening and closing. "It's a very simple nanomachine," admits Seeman, "but in the scheme of molecular devices it's huge because it generates more than four times the amount of movement produced by typical molecular devices." A large version of the device might function

as an elbow, whereas smaller devices could serve as finger joints. By 2002, Seeman's group had demonstrated a mechanical DNA-based rotary motor [327] and reported the design and construction of two-dimensional DNA arrays that might serve as templates for nanomechanical assembly [328–330]. Seeman is now collaborating with genetic engineers and computational chemists to achieve "the design and fabrication of practical nanoscale devices" and "to make rapid progress in demonstrating DNA based nanoscale devices," including "sequence-dependent devices [that] can provide the diversity of structures necessary for nanorobotics."

Other DNA-based actuation is known. For example, sequence-specific DNA hybridization is commonly used to bend silicon microcantilevers [331]. Alberti and Mergny [332] synthesized a sequence-dependent DNA "piston" composed of a 21-base oligonucleotide that displays a 5-nm, two-stroke, linear motor-type movement. Li and Tan [333] have made a single-DNA-molecule inchworm motor. Shu and Guo [334] synthesized a 30-nm-long chimeric pRNA (DNA-packaging) motor made from six strands of RNA surrounding a center strand of DNA; in the presence of ATP, the RNA strands push the DNA axle in succession, spinning it around and producing 50–60 pN of force.

Bernard Yurke at Bell Laboratories and Andrew Turberfield at the University of Oxford synthesized another DNA actuator using three single strands of artificial DNA that, when placed together, find their complementary partners and self-assemble to form a V-shaped structure [335]. The open mouth of this nanotweezer can be made to close by adding a special "fuel" strand that binds to the single-stranded DNA dangling from the ends of the arms of the tweezers and zips them closed, moving from a \sim 7-nm separation to a \sim 1-nm separation in \sim 13 s per cycle. A special "removal" strand, when added, binds to the fuel strand and pulls it away, opening the nanotweezers again. Yurke's original sequence-dependent DNAbased actuator was cycled seven times in sequence, and more recent work [336] has focused on a continuously running DNA nanomotor. Merkle [337] has theorized a similar chemically driven DNA-based "sliding strut" actuator that demonstrates that biotechnology and selfassembly can be used to make positional devices. Hao Yan and colleagues in Reif's lab at Duke University in Durham, North Carolina, have devised X-shaped DNA tiles that link up in a square grid with some of the strands consisting of sections of DNA that can lengthen and shorten by 6.8 nm, like tiny pistons, making a net whose mesh size can expand or contract under chemical control [338]. RNA polymerase has been used as a 15-20-pN positioning motor that "can be incrementally 'walked' or positioned along DNA with nanometer scale precision" [339].

Mirkin's group [340] has created two- and three-dimensional architectures with DNA and inorganic nanoscale building blocks, including using massively parallel tip arrays, to write patterns using a DNA-based "ink"; Mavroidis's group [341] is studying combining artificial DNA structures with carbon nanotubes; and von Kiedrowski's group [342] has used synthetic three-armed tris-oligonucleotidyl building blocks to assemble three-dimensional DNA nanoscaffolds such as supramolecular tetrahedra. Reif [343] has designed (but not yet built) "the first autonomous DNA nanomechanical devices that execute cycles of motion without external environmental changes. These DNA devices translate along a circular strand of ssDNA and rotate simultaneously." Reif's first DNA device, called "walking DNA," would achieve random bidirectional motion around a circular ssDNA strand by using DNA ligase and two restriction enzymes that consume ATP as their energy source. Reif's second DNA device, called "rolling DNA," would achieve random bidirectional motion by using DNA hybridization energy in trapped states with no DNA ligase or restriction enzymes. The emerging field of DNA-based molecular construction appears quite active.

4.3.3. Protein-Directed Assembly

After noting Merkle's [337] suggestion that the positional control of nanoscale building blocks would allow the extension of the normal reactions found in biological systems to include free-radical chemistry and more recent descriptions of similar reactive chemistries being employed by enzymes [344], Bradbury [345] proposed using artificial enzymes for nanoscale parts assembly. He envisions the engineering of artificial multifunctional proteins called "robozymes" having the following properties: first, unfolded, it grabs onto molecular

building blocks [346–348], carefully keeping them separate from each other to avoid nonspecific reactions; second, using specific enzyme catalytic sites near the bound building blocks, it "activates" the molecules (perhaps producing one or more free radicals); third, induced folding brings the building blocks into relatively precise alignment, allowing the desired chemical reaction or reactions to occur; and fourth, the protein is induced to unfold, releasing the final product. With protein folding forces in the tens to hundreds of pico-Newtons, such enzymes could also provide a means of threading one molecule through another molecule, mechanically producing interlinked structures such as rotaxane and catenane [349] without using self assembly (i.e., by adding hydrophobic ring structures to the molecular parts, which will then be attracted to each other in a polar solvent such as water), and thus allowing the positional assembly of very small (<1 kDa) molecular nanoscale parts.

Note that although most enzymes in cells are involved in manipulating small molecules (<0.25 kDa), there are several classes of enzymes involved in manufacturing complex covalently bound molecules such as vitamins, enzyme cofactors, antibiotics, and toxins with masses up to \sim 3 kDa. Molecules even larger than this are manipulated by tRNA-synthetase (a 40–100-kDa enzyme that manipulates \sim 30-kDa tRNAs): the spliceosome, the ribosome, the proteosome, and the DNA replication complex. (Many of these also involve "parts insertion" or "threading" maneuvers, such as the clamp and bridge helix mechanisms in RNA polymerase II that act as a translocation ratchet to feed DNA through the enzyme interior to produce mRNA [350].) By designing synthetic enzymes consisting of synthetic amino acids, we can envision grabbing molecular parts in a solution and then, as the enzyme folds, bringing them into proper alignment and causing them to react, which might be called "nanopart synthetases" or "protein-directed parts assembly." Of course, RNA-based ribozymes [351] may prove better suited than proteins for some reactions, so we are not limited to using enzymes to form the covalent bonds required in nanoparts.

Ratchet-action protein-based molecular motors are well known in biology [352], Conformational cascades of a special genetic variant of yeast cell prions have already been used to assemble silver- and gold-particle-based nanowires [353], and the GTPase dynamin mechanoenzyme, which self-assembles into rings or spirals, wrapping around the necks of budding vesicles and squeezing, pinching them off, during cellular endocytosis, is also well known [354]. Smith [355] has used methyltransferase-directed addressing of fusion proteins to DNA scaffolds to construct a molecular camshaft as a exemplar protein/nucleic acid biostructure. Bachand and Montemagno [221] have engineered a biomolecular motor constructed of ATPase protein [220] with an attached silicon nitride "propeller" arm [222] and a reversible on/off switch [223], and other task-optimized genetically engineered molecular motors have also been synthesized by others [356]. Protein-protein binding specificity has been used to bend silicon microcantilevers [357]. Finally, molecular chaperones are a group of proteins that assists in the folding of newly synthesized proteins or in the refolding of denatured proteins. Genetically engineered chaperonin protein templates (chaperone molecules) have been used to direct the assembly of gold (1.4, 5, or 10 nm) and CdSe semiconductor quantum dots (4.5 nm) into nanoscale arrays [358].

Immunoglobulin (Ig) or antibody molecules could be used first to recognize and bind to specific faces of crystalline nanoparts, and then as handles to allow attachment of the parts into arrays at known positions, or into even more complex assemblies. As reported by Freitas [8]: Kessler et al. [359] raised monoclonal antibodies (MAbs) specific for crystals of 1,4-dinitrobenzene having well-defined molecular-level structures. These antibodies were so specific they would not bind to the same molecule when it was conjugated to a protein carrier. IgG antibodies isolated from the serum of rabbits injected with crystals of monosodium urate monohydrate or magnesium urate octahydrate evidently bear in their binding sites an imprint of the crystal surface structure because they can act as nucleating templates for crystal formation in vitro with extremely low cross-reactivity, despite the similar molecular and structural characteristics of the two crystals [360]. Antibody binding to monosodium urate crystals has been known for decades [361], and viruses have been engineered with a specific recognition moiety for ZnS nanocrystals used as quantum dots [362].

Like antigens with ordered multiple epitopes, crystals expose chemically and geometrically distinct surfaces, so different antibodies might recognize distinct faces of a crystal (possibly

including diamond crystal faces) in an interaction similar to that of antibodies for repetitive epitopes present on protein surfaces [347, 363]. For instance, one MAb to 1,4-dinitrobenzene crystals was shown to specifically interact with the molecularly flat, aromatic, and polar (101) face of these crystals, but not with other faces of the same crystal [348]. MAbs have also been elicited against cholesterol monohydrate crystals, one of which [364] was shown to specifically recognize the crystal's stepped (301) face. Here, the hydrophobic cholesterol hydrocarbon backbone is exposed on one side of the molecular steps while hydroxyl residues and water molecules are exclusively exposed on the other side. In both cases, crystal-specific antibodies were of the IgM idiotype [347]. This accords with the assumption that (unlike most commonly used antigens) crystals cannot be processed by the antigen-presenting cells, and hence antibodies must be induced through a T-cell-independent path [365]. Semiconductorbinding [278, 362] and calcite-binding [366] proteins are known that can discriminate among the various crystal faces of the given material and that can in some cases alter the pattern of crystal growth [367]. Sulfur-free gold-binding proteins recognize and noncovalently bind preferentially to the Au (111) crystal surface (gold-binding proteins use multiple repeats of 14–30-residue sequences to bind to this surface [368]).

Solubilized (derivatized) C_{60} and C_{70} fullerenes can induce the production of specific antibodies [346, 347, 369], usually by interaction with the combining sites of IgG [346]. It is speculated that highly hydrophobic pure fullerenes would be recognized by antibodies with hydrophobic amino acids in their binding sites [346, 370] or would interact with donor -NH₂ [371] and -SH [372] groups. There are several reports of antibodies being raised to single-walled carbon nanotubes [369, 370]. Computer simulations indicate that it may be possible to build antibodies that selectively bind to nanotubes of a specific diameter or chirality [370].

4.3.4. Microbe- and Virus-Directed Assembly

Artificial microbes might also be employed in molecular construction. A variety of biological molecular machines are already known that display linear motions; movements related to opening, closing, and translocation functions; rotary movements; and threading–dethreading movements [373]. Gerald J. Sussman at MIT claims that when computer parts are reduced to the size of single molecules, engineered microbes could be directed to lay down complex electronic circuits [193]. "Bacteria are like little workhorses for nanotechnology; they're wonderful at manipulating things in the chemical and ultramicroscopic worlds," he says. "You could train them to become electricians and plumbers, hire them with sugar and harness them to build structures for you." Regarding microbe-directed parts fabrication, one strain of bacteria (*Pseudomonas stutzeri AG259*) is known to fabricate single crystals of pure silver in specific geometric shapes, such as equilateral triangles and hexagons, up to 200 nm in size [374], and microorganisms can accumulate materials and synthesize inorganic structures composed of bismuth [375], CdS [376, 377], gold [378], magnetite [377, 379], silica [377], and silver [377].

As for microbe-directed parts assembly, Kondo et al. [380] used a grooved film (created by chemically precipitating cellulose tracks <1 nm apart onto a copper base) to train the bacterium *Acetobacter xylinum* to exude neat ribbons of cellulose along the prepared track at a rate of 4 μ /minute; the group is attempting to genetically modify the organism to secrete alternative sugar molecules that might better resist natural degradation. Natural fibroblasts in human tissue construct complex three-dimensional collagenous fiber networks of extracellular matrix (ECM) during wound healing, fibrillogenesis and fibroplasia. Although ECM strand positioning is stochastic in natural fibroblasts, cell functionality, and ECM network characteristics can be altered by chemotactic factors, contact guidance and orientation, hypoxia, and local mechanical stress. Fibroblasts can be genetically engineered, are capable of crosslinking collagen fibers (a "covalent parts joining" type of operation), and can apply ~100 pN forces while embedded in a three-dimensional collagen lattice [381].

To establish digital control over microorganisms, genetic circuits that can function as switches [382] or computational logic elements such as AND, NAND, and NOR gates (Ref. [6], Section 10.2.3.1) are under active investigation [383–386]. For example, in 2000, Gardner et al. [384] added a memory device to an *E. coli* bacterium using two inverters for which the output protein of each is the input protein of the other, and Elowitz and Leibler [383]

made an oscillator with three inverters connected in a loop; in one test of their system, "a fluorescent protein became active whenever one of the proteins was in its low state... the result was a population of gently twinkling cells like flashing holiday lights" [386]. By 2002, Weiss [385] had created a five-gene circuit in *E. coli* that could detect a specific chemical in its surroundings and turn on a fluorescent protein when the chemical concentration falls within preselected bounds [386].

The Synthetic Biology Lab at MIT is similarly trying to create cells that are "engineered genetic blinkers" [387] and that use light as a faster means of cellular input–output than chemical-mediated signals [388]. They are also creating a set of "BioBricks" [388, 389] that are "a [standardized] set of [building block] components that have been designed for use as logic functions within a cell. The members of this family are designed to be compatible, composable, interchangeable, and independent so that logic circuits may be constructed with little knowledge or concern for the origins, construction, or biological activities of the components."

Boston University bioengineer Timothy Gardner explains [386] that the eventual goal "is to produce genetic 'applets', little programs you could download into a cell simply by sticking DNA into it, the way you download Java applets from the Internet." Bacterial memory has also been demonstrated: 150-base-long messages encoded as artificial DNA have been stored within the genomes of multiplying *E. coli* and *Deinococcus radiodurans* bacteria and then accurately retrieved [390]. Jacobson's team [151] has demonstrated remote electronic control over the hybridization behavior of DNA molecules by inductive coupling of a radio frequency magnetic field to a gold nanocrystal covalently linked to DNA (Section 2.8), offering the prospect of remote-controlled enzymes and "radio-controlled bacteria."

Bacteria can also be used as physical system components. For example, Kim et al. [391] are attempting to incorporate living bacteria into MEMS to form living cell motors for pumps and valves. The bacteria will be completely sealed inside the bioMEMS device. "When its flagellum is attached to a surface, the bacterium moves in a circular fashion, and always in the same direction," explains Tung. "A single bacterium can become a flagellar motor or pump, but a number of bacteria, all rotating in the same direction, could become a conveyor belt." Similarly, Linda Turner and colleagues at the Rowland Institute at Harvard have affixed a film of Serratia marcescens bacteria onto tiny beads, allowing the microbes' rotating appendages to carry the beads along. When the film is applied inside tiny tubes, the gyrating bacterial arms blend fluids twice as fast as diffusion alone [392]. Carlo Montemagno at the University of California, Los Angeles, has combined living cells with isolated MEMS structures to create cell-powered mechanical motors. In one experiment in 2003, a lithographically produced U-shaped structure 230 μ wide is attached to a cardiac muscle cell like a tiny prosthesis. When presented with glucose solution, the muscle cell contracts repeatedly, causing the mechanical structure to "walk" at a speed of $\sim 46 \ \mu/\text{min}$ with a repetition rate controlled by the spring constant of the MEMS structure [393]. Sequeira and Copik [394] also proposed using bacteria as power units for microscale mechanical systems.

Viral shells also provide useful templates for nanoscale assembly. Belcher [278, 362] employs virus capsid shells as scaffolds for the directed nanoassembly of nanoparticles such as quantum dots [362, 395] in a process she describes as the "biomimetic synthesis of nonbiological inorganic phases with novel electronic and magnetic properties directed by proteins and synthetic analogs." In one experiment [362], a genetically engineered M13 bacteriophage with a specific recognition moiety for zinc sulfide nanocrystals was used to assemble a ZnS-containing film having nanoscale ordering and 72- μ -sized domains. Viral coat proteins can be engineered by various techniques and have been used by others as scaffolds for nanomaterials synthesis [187] and self-assembly [396], including self-assembled monolayers [188].

4.4. Positional Assembly and Molecular Manufacturing

As machine structures become more complex, getting all the parts to spontaneously selfassemble in the right sequence will be increasingly difficult. To build such complex structures, it makes more sense to design a mechanism that can assemble a molecular structure by what is called positional assembly (i.e., picking and placing molecular parts). A device capable of positional assembly would work much like the robot arms that manufacture cars on automobile assembly lines in Detroit, or that insert electronic components onto computer circuit boards with blinding speed in Silicon Valley. Using the positional assembly approach, the robot manipulator picks up a part, moves it to the workpiece, installs it, then repeats the procedure over and over with many different parts until the final product is fully assembled.

One of the leading proponents of positional assembly at the molecular scale is Zyvex Corp., a privately held molecular nanotechnology company headquartered in Richardson, Texas [397]. Zyvex is the first engineering firm to espouse an explicit goal of using positional assembly to manufacture atomically precise structures, or more specifically, "a user-controlled fabrication tool capable of creating molecularly precise structures with 3-dimensional capability in an economically viable manner." As a first step toward this goal, in 1998 Zyvex demonstrated the ability to use three independently controlled inch-long robotic arms to manipulate tiny carbon nanotubes in three dimensions under the watchful eye of a scanning electron microscope that can monitor objects and motions as small as 6 nm at near-video-scan rates. Zyvex has already demonstrated the ability to positionally assemble large numbers of MEMS-scale parts and still has a very long way to go before it can assemble nanoscale parts into useful machines, but its work is a step in the right direction, and the research continues today (Section 4.4.2).

Microscale devices could also be used to pick and place nanoscale parts. Agilent Laboratories has created an ultra-high-precision micromover platform [398] capable of providing linear two-dimensional movement in steps of 1.5 nm, the width of about nine bonded carbon atoms. The core of the micromover is a stepper actuator or linear motor that does not rotate, but instead steps right to left or front to back. The platform can travel a total of 30 μ m in each direction in 2.5 ms; because each micrometer is made up of 1000 nanometers, the micromover would take approximately 20,000 steps to traverse 30 micrometers, a distance about half the width of a single human hair. Another group led by Sylvain Martel, formerly at the Bio-Instrumentation Laboratory at MIT, is working on a similar nanopositioning device called the NanoWalker [399, 400].

Others have begun to explore the realm of submicon manipulation of objects. For example, Philip Kim and Charles Lieber at Harvard University created the first general-purpose nanotweezer [401]. Its working end is a pair of electrically controlled carbon nanotubes made from a bundle of multiwalled carbon nanotubes. To operate the tweezers, a voltage is applied across the electrodes, causing one nanotube arm to develop a positive electrostatic charge and the other to develop a negative charge. The attractive force can be increased or decreased by varying the applied voltage: 8.5 V completely closes the arms, whereas lower voltages give different degrees of grip. Using the tool, Kim and Lieber have successfully grasped 500-nm clusters of polystyrene spheres, or clusters about the same size scale as cellular substructures. Kim and Lieber were also able to remove a semiconductor wire 20 nm wide from a mass of entangled wires, using tweezer arms about 50 nm wide and 4 μ long. The early hope was that by growing single-walled nanotubes directly onto the electrodes, the researchers could produce nanotweezers small enough to grab individual macromolecules.

The Kim–Lieber nanotweezer is very good at pinching and releasing objects, but the technique creates a large electric field at the tweezer tips that can alter the objects being manipulated, and the tweezers must be constructed one at a time, which makes the manipulation of large numbers of nano-objects a slow and tedious process. To try to improve on this, in 2001 a group led by Peter Boggild [402] of the Technical University of Denmark in Lyngby used standard micromachining processes to carve from a tiny slab of silicon an array of cantilevered micropliers that could be opened and closed electrically. Boggild then used an electron beam to grow a tiny carbon nanotweezer arm from the end of each cantilever, angled so that the tips were only 25 nm apart, making a better-controlled nanotweezer [403]. Other nanotube-based nanotweezers have since been reported by other groups [404, 405].

Precise covalent attachment of molecules to surfaces is also being pursued. Blackledge et al. [406] used a palladium-coated SFM tip to chemically modify terminal functional groups on an organosiloxane-coated surface to create biotin-streptavidin assemblies in patterns with minimum 33-nm line widths. Diaz et al. [407] use redox probe microscopy (RPM), in which a SFM tip is modified with redox-active materials, whereupon the interactions between tip

and an adsorbate or between tip and a surface are modulated by the electrode potential. This system has also been used as a microtweezer to manipulate and position objects. Hla and Rieder [408, 409] have reviewed recent progress in using scanning, tunneling microscopy (STM) to manipulate and synthesize individual molecules.

The ultimate goal of molecular nanotechnology is to develop a manufacturing technology able to inexpensively manufacture most arrangements of atoms that can be specified in molecular detail, including complex arrangements involving millions or billions of atoms per product object, as in the hypothesized medical nanorobots (Section 4.5). This will provide the ultimate manufacturing technology in terms of its precision, flexibility, and low cost. Two central mechanisms have been proposed to achieve these goals at the molecular scale: programmable positional assembly including fabrication of diamond structures using molecular feedstock (Section 4.4.1), and massive parallelism of all fabrication and assembly processes (Section 4.4.2).

4.4.1. Diamond Mechanosynthesis

Programmable positional assembly at the molecular scale is the central mechanism for achieving both great flexibility in manufacturing and the ultimate in precision. While ubiquitous at the scale of centimeters and meters, positional assembly at the molecular scale is still rudimentary, but its promise is immense. At the same time, a full analysis of how to use positional assembly to synthesize most arrangements of atoms permitted by physical law would be, at present, prohibitively complex. The 100 plus elements of the periodic table each have their own unique chemical properties, and the various combinations and permutations of these elements creates a combinatorial explosion of possibilities whose full analysis may occupy nanotechnologists worldwide for the rest of this century. A more manageable project is the analysis of a small set of positionally controlled tool tips that could be used in the mechanosynthesis of stiff hydrocarbons, and the analysis of a significant class of stiff hydrocarbons (in particular, diamond) that could be synthesized by the use of these tool tips.

Why diamond? There are four principle reasons. First, although the basic crystal structure of diamond was first elucidated in 1913 and the cleavage of diamond crystals has been seriously studied since at least 1928, it was not until the 1980s and especially the 1990s that the molecular surface characteristics of diamond were extensively investigated both theoretically and experimentally. Many practical questions about the molecular structure of diamond have now been resolved.

Second, the development since the 1950s of a significant experimental specialty known as adamantane chemistry now allows the convenient bulk synthesis of small molecules of pure diamond crystal in specific isomeric forms containing up to 50 atoms (including up to 22 carbon atoms) in size, and in some cases allows the rational regioselective functionalization of these molecules. Over 20,000 adamantane variants have been synthesized.

Third, an even more active experimental field (of similar vintage) is devoted to the synthesis, by means of chemical vapor deposition (CVD) [410], of macroscale thin films of diamond and related stiff hydrocarbon structures. These structures have practical, commercial applications today. The field of diamond CVD provides a wealth of understanding, both experimental and theoretical, of the myriad reaction mechanisms that can contribute to the growth of diamond.

Fourth, there is widespread interest in the exceptional properties of diamond. Among other desirable properties, it has extreme hardness, high thermal conductivity, low frictional coefficient, chemical inertness, and a wide bandgap. It is the strongest and stiffest material presently known at ordinary pressures. Recent investigations have been driven by the many emerging applications for diamond in MEMS mechanical and electromechanical devices [411, 412], optics, radiology, biochemical synthesis [413], and medicine [414–416], but most especially in various electronics devices [417–419]. A method for the precise manufacture of microscopic and nanoscale diamond structures would have tremendous utility in science and industry.

Useful conclusions can already be drawn from the CVD literature [410]. First, growth occurs on all major surfaces of diamond (albeit at widely differing rates, depending on reaction conditions). Second, even on a specific surface, growth occurs under a wide range

of experimental conditions and can use a wide range of different feedstocks because there is a great diversity of reaction mechanisms by which growth can occur. These two conclusions are important because they imply the availability of a wide range of synthetic methods by which diamond growth can take place. No single roadblock, nor any one theoretical or experimental obstacle, is likely to prevent progress because there are multiple alternative routes for achieving the synthesis of any specific stiff hydrocarbon. Thus, if any particular proposal for a specific tool tip, or any specific reaction mechanism should, on closer analysis, prove to be flawed or simply impossible, it can be discarded without fear of failure in the larger endeavor.

In contrast to high-pressure diamond synthesis [420] and low-pressure gas-phase diamond synthesis of diamond via CVD [410], positional mechanosynthesis has been proposed by Drexler [201] for the precise manufacture of diamond structures. Mechanosynthesis aims to achieve site-specific chemical synthesis by inducing chemical transformations controlled by positional systems operating with atomic-scale precision (e.g., the tip of a scanning probe microscope, or SPM [421]), thus enabling direct positional selection of reaction sites on the workpiece. STM has demonstrated an ability to manipulate surface structures atom by atom, and many proposed methods involve the use of STM to direct chemical reactions on the surface by: delivering an electric field to a subnanometer region of a surface to activate a chemical reaction, manipulating the chemistry of the tip to make it act as a catalyst that can then be introduced precisely into the region of desired reaction, or delivering mechanical energy from the tip to activate surface reactions. The reaction selectivity of all these methods relys on the exponential dependence of reaction rates on the activation barrier, which is lowered for surface reactions in a precisely defined area of the surface during mechanosynthesis [422].

Mechanosynthesis may be distinguished from simple piezochemistry, a general term that describes solution-phase chemical processes in which homogeneous, isotropic, slowly varying mechanical pressures (e.g., ~0.1–2 GPa in commercially available laboratory equipment) modifies chemical reactivity [423]. Under proper conditions, 2 GPa at room temperature might suffice to convert graphite to diamond [424]. Unlike forces resulting from hydrostatic pressure, forces applied by mechanosynthetic tool tips can be highly anisotropic and inhomogeneous on a molecular scale: Large loads (including compression, tension, shear, and torsion) can be applied to specific atoms and bonds in a controlled manner [201]. Stress is a scale-independent parameter [201], so tool tips for mechanosynthesis built of diamond could apply pressures equaling those in macroscale diamond-anvil pressure cells (e.g., >550 GPa [425]), with substantial effects on bonding (e.g., H₂ becomes metallic at ~150 GPa [426]). For example, a localized pressure of ~50 GPa applied to an activation volume of ~0.006 nm³/C-atom in diamond yields a ~300 zJ/C-atom reduction in activation energy, slightly greater than the C—C covalent bond energy of a 278 zJ/C-atom (or 556 zJ/bond) in neopentane [427].

Undoped diamond normally consists of a rigid lattice of carbon atoms surface-passivated by hydrogen atoms, so a necessary aspect of diamond mechanosynthesis involves positionally controlled hydrogen abstraction [428–430] and donation [201, 431] at the surface of the diamond crystal lattice. The extensive theoretical analysis of the hydrogen abstraction tool has involved many people, including Donald W. Brenner [430, 432, 433], Richard J. Colton [432], K. Eric Drexler [201], William A. Goddard III [434], J. A. Harrison [433], Jason K. Perry [434], Ralph C. Merkle [431, 434], Charles B. Musgrave [434], Michael Page [430], O. A. Shenderova [433], Susan B. Sinnott [432, 433], and Carter T. White [432]. The institutions involved include the Materials and Process Simulation Center at Caltech; the Department of Materials Science and Engineering at North Carolina State University; the Institute for Molecular Manufacturing; the Department of Chemicals and Materials Engineering at the University of Kentucky; the Chemistry Department of the United States Naval Academy; the U.S. Naval Research Laboratories, Surface Chemistry Branch; and the Xerox Palo Alto Research Center.

Site-specific hydrogen abstraction from crystal surfaces has recently been achieved experimentally. For instance, Musgrave et al. [429] and Lyding et al. [435–437] have demonstrated the ability to abstract an individual hydrogen atom from a specific atomic position in a covalently bound hydrogen monolayer on a flat Si(100) surface, using electrically pulsed STM tip in ultrahigh vacuum. He's group [438] has also demonstrated single-atom hydrogen abstraction experimentally, using STM. Hydrogen-donation tools have been described theoretically [201, 431] but have not yet been as extensively studied. However, there has been experimental demonstration of highly localized STM-catalyzed rehydrogenation of dehydrogenated hydrocarbon clusters adsorbed to the Pt(111) surface [439], and of hydrogen donation to a prepared azide-coated surface, producing highly localized amines, using a Pt-coated AFM tip [440]. In the following analysis, we assume that site-specific hydrogen abstraction and donation tools for mechanosynthesis are available and can be used to abstract or donate hydrogen atoms at will on diamond surfaces.

The principal challenge in diamond mechanosynthesis is the controlled addition of carbon atoms to the growth surface of the diamond crystal lattice. The theoretical analysis of carbon atom insertion and carbon dimer placement on diamond has also involved many people including Tahir Cagin [218], K. Eric Drexler [201], Fedor N. Dzegilenko [441], Robert A. Freitas Jr. [442–444], William A. Goddard III [218], David J. Mann [444], Ralph C. Merkle [431, 442–445], Charles B. Musgrave [446], Jingping Peng [443, 444], Subhash Saini [441], Deepak Srivastava [441], and Stephen P. Walch [218, 445]. The institutions involved include the Materials Simulation Center at Caltech; ELORET; the Institute for Molecular Manufacturing; the IT Modeling and Simulation Group at NAS/MRJ, NASA Ames Research Center; Department of Chemical Engineering, Stanford University; the Thermosciences Institute, NASA Ames Research Center; the Xerox Palo Alto Research Center; and Zyvex Corporation.

The feasibility of precisely inserting individual carbon atoms, small hydrocarbon species, or small clusters of carbon atoms on a C(111) or C(100) diamond surface at specific sites was initially supported first by the computational work of Walch and Merkle [445]. Walch and Merkle analyzed several mechanosynthetic reactions, including placement of a carbon dimer onto a C(111) surface, insertion of a positionally controlled carbene into that dimer, and insertion of a positionally controlled carbene into a surface dimer on a C(100) surface using a nine-atom cluster to model the diamond surface. The latter insertion can take place with no barrier (according to computational results based on *ab initio* calculations using Gaussian with a 6-31G basis set and B3LYP density functional), provided the approach trajectory is appropriate. Subsequent removal of the mechanosynthetic tool tip using an appropriate withdrawal trajectory (e.g., including a 90° rotation of the tool to break the π bond of the double bond) is predicted to leave a single carbon atom in the bridged position on the dimer. Classical molecular dynamics simulations by Dzegilenko et al. [441] showed that a single weakly bonded carbon dimer could be selectively removed from the upper terrace of a reconstructed diamond $C(100-(2 \times 1))$ surface by a carbon nanotube tip chemically modified with a C_2 carbene radical species strongly bonded to the end cap of the tip, but that this tool was not particularly useful for adding a carbon to the diamond surface. The carbone prefers to bond to a single radical (top) site on the C(111) diamond surface rather than at a bridged or fourfold hollow site, in agreement with the results of Walch and Merkle [445]. When planar C_6H_2 (methenylidene cyclopentene) which is brought up to the C(100) surface, either a C_3H moiety with two lower C atoms of the tip initially deposited onto the fourfold locations forming bonds with C atoms of two neighboring surface dimers is attached or else a C4H2 fragment is adsorbed atop two C atoms of neighboring surface dimers, with the reaction outcome depending critically on the initial tip-surface distances, the tip trajectory, and various allowed but undesired tip rearrangements [441]. Additional theoretical work by Musgrave's group [446] investigating the effects of highly localized electrical fields (such as might be generated by a STM tip) on the growth of diamond using tert-butyl and $C_{13}H_{22}$ cluster models indicates that although the isolated CH_2 radical is unstable (having two dangling bonds that force a ring opening on the cluster), the ethynyl (C_3H) and methyl (CH_3) radicals appear most promising for direct addition reactions.

In 2003, Merkle and Freitas [442] proposed a new family of mechanosynthetic tools intended to be employed for the placement of two carbon atoms—a CC dimer—onto a

growing diamond surface at a specific site. Their analysis used density-functional theory with Gaussian 98 to focus on specific group IV-substituted biadamantane tool tip structures and to evaluate their stability and the strength of the bond they make with the CC dimer. These tools should be stable in vacuum and should be able to hold and position a CC dimer in a manner suitable for positionally controlled diamond mechanosynthesis at room temperature. The function of a dimer placement tool is to position the dimer, then bond the dimer to a precisely chosen location on a growing molecular structure, and finally to withdraw the tool, leaving the dimer behind on the growing structure. To achieve this, the dimer is required to be both bonded relatively weakly to the tool and highly strained, and thus highly reactive, so it will easily bond to the growing molecular structure to which it is added.

There is a large combinatorial space of possible tools that might satisfy both requirements. Merkle and Freitas attempted to satisfy the two basic requirements by bonding the dimer to two group IV supporting atoms: carbon, silicon, germanium, tin, or lead. This series of elements forms progressively weaker bonds to carbon, so the proposed tools will likewise be progressively more weakly bound to the carbon-carbon (CC) dimer. The supporting group IV atoms are part of two substituted adamantane ($C_{10}H_{16}$) frameworks that position and orient them. The two substituted adamantane frameworks are rotated and fused together to make a biadamantane [447] structure (Fig. 7), creating very high angle strain in the bonds between the two supporting atoms and the dimer. This molecule, a bi-silaadamantane dicarbon, is only the tip of a complete tool. In a complete mechanosynthetic apparatus, a somewhat larger version of this molecule would likely be required so that the active tip could be held and positioned via a rigid handle structure. Subsequent molecular dynamics simulations of these tool tips [444] indicated a $\sim 20\%$ deposition success for early experimental proof-of-concept demonstrations.

Although pick-and-place of individual carbon atoms or carbon dimers has not yet been demonstrated experimentally using scanning probe microscope tips, in 1985 Becker and Golovchencko [448] used voltage pulses on an STM tip to extract a single germanium atom from the (111) surface of a sample. STM has been used to bind silicon atoms to the tip, first pulling the atoms off the surface of a Si(111) crystal face and then reinserting them back into the crystal [449, 450], and segments of individual dimer rows of silicon atoms have been extracted from the Si(100) face to create structures with atomically straight edges and lateral features that are only 1 dimer in width [451]. Other researchers have moved clusters and single atoms of silicon across a silicon surface at room temperature [450–455]; mechanosynthesis of the Si(111) lattice has been studied theoretically by Herman [456–458] and appears feasible. A near-contact atomic force microscope operated at low temperature has been



Figure 7. DCB6-Si dimer placement tool tip for diamond mechanosynthesis [441]. © 2003, Ralph C. Merkle and Robert A. Freitas, Jr.

used for the vertical manipulation of selected single silicon atoms from the Si(111)– (7×7) surface, demonstrating removal of a selected silicon atom from its equilibrium position at the surface without otherwise perturbing the (7×7) unit cell, as well as the deposition of a single atom on a created vacancy at the surface [459]. The authors note, "These manipulation processes are purely mechanical, since neither bias voltage nor voltage pulse is applied between probe and sample."

As for carbon atoms, a STM tip has manipulated individual C_{60} molecules along terraces on a grooved copper plate [460], and the pick-and-place of C_{60} on silicon [461] surfaces has been demonstrated experimentally using an SPM. Other researchers have reported positioning carbon monoxide molecules on platinum surfaces [462]. More recently, Ho and Lee [463] have demonstrated the first repeatable site-specific mechanosynthetic covalent bonding operation of a diatomic carbon-containing molecule on a crystal surface, albeit electrically mediated. These researchers used an STM tip to locate two carbon monoxide molecules and one iron atom adsorbed on a silver surface in vacuum at 13 K. Next, they lowered the tip over one CO molecule and increased the voltage and current flow of the instrument to pick up the molecule; then they moved the tip-bound molecule over the surface-bound Fe atom and reversed the current flow, causing the CO molecule to covalently bond to the Fe atom, forming an iron carbonyl Fe(CO) molecule on the surface. Finally, the researchers repeated the procedure, returning to the exact site of the first Fe(CO) and adding a second CO molecule to the Fe(CO), forming a molecule of $Fe(CO)_2$, which in subsequent images of the surface appeared as a tiny "rabbit ears" structure, covalently bound to the silver surface [463].

These results, both experimental and theoretical, support the general feasibility of molecular positional operations that can modify a diamond workpiece, adding or removing small hydrocarbon clusters on that workpiece or even adding and removing single atoms or dimers under appropriate conditions. Repeated application of these basic operations should allow building up complex and atomically precise molecular structures. The mechanosynthetic strategy considered here is based on three principal assumptions:

- Highly reactive tools: The reactions generally assumed to occur in the CVD synthesis
 of diamond involve highly reactive species. The molecular tools described here are
 likewise highly reactive, and for the same reason: synthesis of diamond structures is
 facilitated by the use of such tools.
- 2. Inert environment: Because the molecular tools can be highly reactive, they must be used in an inert environment. It is assumed that these tools will be used in vacuum, so that contact with solvent or gas molecules will not occur. More specifically, it is assumed that the mechanosynthetic work environment has no uncontrolled structures or molecules, and no free gas-phase molecules (e.g., contaminant molecules such as oxygen, nitrogen, hydrogen gas, or water, or undesired by-products of internal chemical reactions) that might react with the molecular tools or otherwise interfere with or disrupt mechanochemical operations. At the $\sim 10^{-12}$ atm pressure commonly achieved in laboratory UHV systems, a working volume smaller than ~ 4000 micron³ would be more than 90% likely to contain no free gas-phase molecules.
- 3. Controlled trajectories: The molecular tools are positionally controlled at all times. They are not free to move at random. Their controlled trajectories are such that undesired encounters with the exterior surface of the tool tip holder or its housing, other molecular tools, or any other nonworkpiece structures are prevented. Undesired reactions between radicals and other physical structures is prevented either by keeping the radicals at a sufficient distance from surfaces with which they can react or by allowing radicals to contact with surfaces that are specifically designed to be inert and to resist attack by those radicals.

The assumption of positionally controlled highly reactive tools operating in an inert environment permits the use of novel and relatively simple reaction pathways. Although the ability to achieve an inert environment using present methods might lead to one possible implementation pathway, the primary purpose of this discussion has been to establish that, given a suitable vacuum environment, a relatively simple set of reactions and a relatively simple set of molecular tools should be sufficient to allow the manufacture of a wide range of nanoscale diamond structures with atomically precise features.

4.4.2. Massively Parallel Manufacturing

Complex objects assembled from simpler components may be manufactured either serially or in parallel. In serial assembly, objects are manufactured one at a time by a stepwise manufacturing process. Examples include handcrafted unique items such as an antique pocket watch, classical industrial "mass production" items such as automobiles that emerge only one by one at the end of an assembly line, or the traditional serial digital computer that executes instructions one by one in a linear sequence. In parallel assembly, objects are manufactured along many pathways simultaneously or at many different sites, such as polysomes in living cells (multiple ribosomes translating a single mRNA strand simultaneously), mask lithography deposition of multiple circuits simultaneously on a single semiconductor wafer, or the modern parallel computer that at any moment is executing different instructions on thousands or even tens of thousands of independent processors in a highly parallel manner. Parallel manufacturing systems could have many possible control/configuration architectures, analogous to a SIMD (Single Instruction Multiple Data) approach; convergent [284, 464] or fractal [465, 466] assembly; agoric algorithms [467]; stigmergy [468], swarm [469, 470], or agent-based [467] approaches; or other manufacturing analogs taken from high-performance parallel computing.

Biology provides perhaps the best example of the power of massive parallelism in assembly. A single ribosome, able to make a single protein as directed by a single molecule of messenger RNA, is a marvelous manufacturing system. Yet, by itself, it would have little economic effect. However, billions of ribosomes operating together in each living cell can make all the proteins in a tree or—even more quickly—all the proteins in, for example, a rapidly growing kelp plant, which can literally grow 6 in per day.

The difference between serial and parallel processing is equally crucial in molecular manufacturing, where the basic parts are very small. If a typical molecularly precise simple component is 1 nm³ in volume, then to manufacture a 1 cm³ volume of molecularly precise product requires the assembly of 1000 billion billion (10^{21}) individual simple molecular components. With serial manufacturing, just one molecular component is handled at a time—even at a 1 GHz operating frequency it would take many thousands of years, which is clearly not economically viable. With parallel manufacturing, however, vast numbers of molecular components can be processed simultaneously, reducing batch processing times to days, hours, or even less. Massively parallel assembly is the key to the economic viability of molecular manufacturing.

There are two principal pathways for achieving massively parallel assembly of molecularly precise physical structures: self-assembly and positional assembly. In commercial chemical synthesis, self-assembly usually takes place in fluid phase among mole ($\sim 10^{23}$) quantities of reactant molecules, which interact to produce mole quantities of product molecules. In Seeman's experiments (Section 4.3.2), producing DNA-based structures, and in other related experiments, involving supramolecular or biomolecular self-assembly, the number of product objects produced per batch is vastly less than mole quantities but is still very large by conventional standards in macroscale manufacturing. The inherent parallelism of self-assembly is the main advantage of this pathway over positional assembly in manufacturing.

To overcome this advantage and reap the full benefits of flexibility, precision, and quality in 21st century molecular manufacturing using positional assembly—also known (in the context of molecular manufacturing) as machine-phase nanotechnology—new techniques for massively parallel positional assembly must be developed. At least two such techniques for performing position assembly have already been clearly identified: massively parallel manipulator arrays and self-replicating systems.

Massively parallel manipulator arrays would use a very large array of independently actuated manipulation devices (e.g., scanning probe tips, robot arms, etc.) to process a very large number of molecular precise components simultaneously to build a larger product object. To produce large numbers of nanoparts and nanoassemblies, massively parallel scanning probe microscopes (SPM) arrays [471, 472], and microscale SPMs [473–476] would be most convenient. Force-sensing devices such as piezoelectric, piezoresistive, and capacitive microcantilevers made it possible to construct microscale AFMs on chips without an external deflection sensor. For example, in 1995 Itoh and colleagues [477] at the University of Tokyo fabricated an experimental piezoelectric ZnO₂-on-SiO₂ microcantilever array of 10 tips on a single silicon chip. Each cantilever tip lay $\sim 70 \ \mu$ from its neighbor and measured 150 μ long, 50 μ wide, and 3.5 μ thick, or $\sim 26,000 \ \mu^3$ /device, and each of the devices could be operated independently in the z-axis (e.g., vertically) up to near their mechanical resonance frequencies of 145–147 KHz at an actuation sensitivity of $\sim 20 \ \text{nm/volt}$ (e.g., 0.3-nm resolution at 125 KHz).

Parallel probe scanning and lithography has been achieved by Quate's group at Stanford University, which has progressed from simple piezoresistive microcantilever arrays with five tips spaced 100 μ apart and 0.04-nm resolution at 1 KHz but only one z-axis actuator for the whole array [478], to arrays with integrated sensors and actuators that allow parallel imaging and lithography with feedback and independent control of each of up to 16 tips, with scanning speeds up to 3 mm/s using a piezoresistive sensor [479]. By 1998, Quate's group had demonstrated [480] arrays of 50–100 independently controllable AFM probe tips mounted in two-dimensional patterns with 60 KHz resonances, including a 10 × 10 cantilevered tip array fabricated in closely spaced rows using throughwafer interconnects on a single chip; this work continues [481–483].

MacDonald's group at the Cornell Nanofabrication Facility pursued similar goals. In 1991, the team fabricated their first submicron stylus, driven in the xy plane using interdigitating MEMS comb drives [484], including the first opposable tip pair. By 1993, they had produced a 25-tip array on one xyz actuator, and by 1995 a complete working micro-STM (including xy comb drives) measuring 200 μ on an edge, and a micro-AFM measuring 2 mm on an edge including a 1-mm-long cantilever with a 20-nm-diameter integrated tip on a $6-\mu$ high by 1- μ -diameter support shaft [485]. MacDonald's group demonstrated tip arrays with 5- μ spacings, exploiting the same process used to make the working micro-STM. With the same technology tips or small arrays of tips could be spaced 25–50 μ apart and integrated with individual z-axis microactuators, so that one xy-axis manipulator could support many tips, with each tip having a separate z actuator. By 1997, the group [486] had built and tested an array of micro-STMs on the surface of an ordinary silicon chip, with each tip on a cantilever 150 μ long with three-dimensional sensing and control. The largest prototype array has 144 probes, arranged in a square consisting of 12 rows of 12 probes each, with individual probe needles about 200 μ apart. Further development was to focus on increasing the range of movement and on fitting more and smaller probes into the same space.

Using conventional microlithography, researchers in the Millipede project at IBM's Zurich Research Laboratory [487] have fabricated scanning probe tip arrays of up to 1024 individual tips to achieve terabit-per-square-inch data storage densities [488]. Millipede project manager Peter Vettiger predicts low manufacturing costs and the ability to "build hundreds of these arrays on the same wafer" [489]. Simpler mechanical ciliary arrays consisting of 10,000 independent microactuators on a 1 cm² chip have been fabricated at the Cornell National Nanofabrication Laboratory for microscale parts transport applications [490], and other ciliary array systems for parts presentation in microscale manufacturing have been reported by the Fujita group [491]; Bohringer and colleagues [492]; Will's group [493]; Darling, Suh, and Kovacs [494]; and others. Microcantilever arrays for "electronic nose" [495] and related applications [496] have also been constructed, in at least one case having millions of interdigitated cantilevers on a single chip [497].

Active probe arrays for dip-pen nanolithography using DNA-based "ink" have been developed by Mirkin's group [498]. At a Materials Research Society meeting in late 2002, Mirkin reported that his group had constructed an array of 10,000 microscope tips, each capable of acting independently from the others: By using 10 tips in concert, they can draw essentially any desired shape. "The goal is to use dip pen nanolithography to generate [DNA-based] templates on surfaces that guide the assembly of nanoscale building blocks," Mirkin says. "It opens the door to placing electronic particles right where you want them. We think it's ultimately going to be a production tool.... This is not going to stop at 10,000. We can make arrays of arrays." The dip-pen lithography work of Li et al. [499] allows the direct on-surface fabrication of metal, semiconductor, and chemically diverse nanostructures made from both organic and inorganic materials.

Another approach is the use of independently mobile multiple manipulator platforms such as the NanoWalker system under investigation by Martel's group [500–503]. Martel envisions a fleet [504] of such wireless instrumented microrobots collectively forming a nanofactory system that could be used for positional nanomanufacturing operations.

Yet another alternative is Zyvex's patented Rotapod exponential assembly design concept [505], in which a single robotic arm on a wafer makes a second robotic arm on a facing surface by picking up micron-size lithographically produced parts, carefully laid out in advance in exactly the right locations so the tiny robotic arm can find them, and assembling them. The two robotic arms then make two more robotic arms, one on each of the two facing surfaces. These four robotic arms, two on each surface, then make four more robotic arms. This process continues with the number of robotic arms steadily increasing in the pattern 1, 2, 4, 8, 16, 32, 64, and so forth until some manufacturing limit is reached (e.g., both surfaces are completely covered with tiny robotic arms). Thus, a single manipulator uses supplied parts to build a large manipulator array that can subsequently undertake the desired massively parallel manufacturing operations. However, the present Rotapod manipulator design is still under development, as it requires more precision to achieve flexible and molecularly precise fabrication. In 2001, Zyvex was awarded a \$25 million, 5-year, National Institute of Standards and Technology (NIST) Advanced Technology Program government contract to develop prototype microscale assemblers using MEMS and nanoelectromechanical systems (NEMS) for prototype nanoscale assemblers [397].

(2) Self-replicating systems would achieve massively parallel assembly first by fabricating copies of themselves, and then by allowing those copies to fabricate further copies, resulting in a rapid increase in the total number of systems. Once the population of replicated manipulator systems was deemed large enough, the manipulator population would be redirected to produce useful product objects, rather than more copies of itself. Self-replicating systems are widely found in natural biological systems but have not been pursued explicitly in macroscale manufacturing for at least two reasons: the widespread but erroneous perception of great technical difficulty, and the correct perception that such massive parallelism is unnecessary for traditional macroscale manufacturing. Nevertheless, ever since John von Neumann's theoretical studies of replicating systems in the 1940s and 1950s [506], and the well-known 1980 NASA engineering study of self-replicating lunar factories [507], manufacturing automation has been slowly progressing toward the goal of the fully self-replicating factory, including most notably Fujitsu Fanuc's nearly "unmanned" robot factory in Yamanashi Prefecture that uses robot arms to make robot arms. It is worth noting that self-replicating systems can be fully remote-controlled [508], fully autonomous [509], or various combinations in between [510, 511].

Note also that parallel manipulator arrays and individual replicators are both "self-replicating—the parallel arrays build more parallel arrays, after which the collective population of array manipulators builds useful products; similarly, the individual replicators build more individual replicators, then the collective population of replicators builds useful products. Each approach offers certain advantages and disadvantages—array systems can be more efficient due to positional specialization of workflow processing, while individual replicators are more tolerant of component failures—but both approaches exemplify "self-replication."

In the last few years there has been renewed research interest in the challenge of mechanical self-replicating systems [512], in part due to the realization that replication can be a fundamentally simple process. For example, Joseph Jacobson at the MIT Media Lab suggests that "to be useful for many applications, engineered systems must be able to manufacture multiple copies. Self-replicating systems may be useful in attaining that goal and it represents a new discipline in engineering." Today there are several ongoing university research programs, both theoretical and experimental, on mechanical (nonbiological) self-replicating machines [512]. The biotechnology and molecular engineering communities are just beginning to seriously study mechanical replicators operating in the nanoscale size domain. Current methods of self-assembly, although allowing massively parallel assembly, lack the flexibility, precision, and quality that are needed for twenty-first-century molecular manufacturing. Current methods of positional assembly, including massively parallel manipulator arrays and self-replicating systems, should allow molecularly precise massively parallel assembly, although further theoretical and experimental work will be required to fully realize this capability.

Note that for the "foreseeable future," it is likely that on-board storage of information will not be required by nanomechanical replicators. One example of an inherently safer and more flexible approach is the broadcast architecture [201, Section 16.3.2(a)] [513]. In this approach, information is broadcast by any of several means to the replicating component. The replicator can be built with an internal "dead man switch" that is automatically off unless activated by an encrypted broadcast signal [514]. The physical replicator becomes, in essence, a remote-controlled manipulator receiving instructions from the outside that guide it, step by step, in assembling a second remote-controlled manipulator. After some number of repeat cycles, the result is a large number of identical remote-controlled manipulators. These manipulators can then be used to assemble large numbers of useful product objects by altering the stream of instructions sent to the population of replicated manipulator devices. Acoustic broadcast (mentioned earlier) can be used to combine both power and information transmission in one convenient mechanism.

Conceptual systems designs for molecular manufacturing have been offered by Drexler [201, 515, 516], Freitas [512], Hall [517], Merkle [208, 431, 464, 512, 513, 518, 519], and Phoenix [520], as extensively reviewed in Freitas and Merkle [512]. Though none of these conceptual designs has yet been subjected to rigorous computational simulation, as proposed by Goddard [218], several components such as gears and pumps have been simulated (Section 4.2) and have been found to perform largely as anticipated, though further design improvements are clearly needed.

4.5. Nanorobot Applications Designs and Scaling Studies

The idea of placing autonomous self-powered nanorobots inside of us might seem a bit odd, but actually the human body already teems with such nanodevices. For instance, more than 40 trillion single-celled microbes swim through our colon, outnumbering our tissue cells almost 10 to 1 [6]. Many bacteria move by whipping around a tiny tail, or flagellum, that is driven by a 30-nm biological ionic nanomotor powered by pH differences between the inside and the outside of the bacterial cell. Our bodies also maintain a population of more than a trillion motile biological nanodevices called fibroblasts and white cells such as neutrophils and lymphocytes, each measuring perhaps 10 μ in size [6]. These beneficial natural nanorobots are constantly crawling around inside of us, repairing damaged tissues, attacking invading microbes, and gathering up foreign particles and transporting them to various organs for disposal from the body [8].

There are ongoing attempts to build MEMS-based microrobots intended for *in vivo* use. For example, the "MR-Sub" project of the NanoRobotics Laboratory of Ecole Polytechnique in Montreal will use a MRI system as a means of propulsion for a microrobot in the blood vessels [521–523]. In this approach, a variable MRI magnetic field would generate a magnetic force on a robot containing ferromagnetic particles, providing a miniaturized system of propulsion able to develop sufficient power to direct a small device through the human body. Applications of the first-generation prototype might include targeted drug release, the reopening of blocked arteries, or taking biopsies. The project is currently gathering necessary information to define design rules for this type of microrobot, with a long-term goal "to further miniaturize the system and to create a robot made up of nanometric parts," making it "possible to carry out medical applications in the blood vessels which are still inaccessible." Other approaches to MEMS-based microrobots intended for *in vivo* use have been described in the literature [524, 525], including the magnetically controlled "cytobots" and "karyobots" proposed by Chrusch et al. [526] for performing wireless intracellular surgery.

There are even preliminary proposals for hybrid bionanorobots that could be constructed using currently foreseeable technologies. For example, Carlo Montemagno [527, 528] plans to use his modified ATPase motors (Section 4.2.2) to create a nanorobot that acts as a "pharmacy in a cell" by entering a cell, grabbing proteins produced by the cell that will not be used, and storing them until they are needed later by the patient. The device would consist of a tiny nickel drum, attached to the ATP-powered biological motor, that is coated with antibodies that adsorb the target molecules, whereupon an electric field pulls the molecules to a storage chamber and holds them in place.

The greatest power of nanomedicine will emerge in a decade or two, when we learn to design and construct complete artificial nanorobots using diamondoid nanometer-scale parts and subsystems including sensors, motors, manipulators, power plants, and molecular computers. If we make the reasonable assumption that we will someday be able to build these complex diamondoid medical nanorobots (Section 4.2), and to build them cheaply enough and in sufficiently large numbers to be useful therapeutically (Section 4.4), then what are the medical implications?

There are many possibilities [6–8, 529–535], but the development pathway will be long and arduous. First, theoretical scaling studies [530–535] are used to assess basic concept feasibility. These initial studies would then be followed by more detailed computational simulations of specific nanorobot components and assemblies, and ultimately full systems simulations, all thoroughly integrated with additional simulations of massively parallel manufacturing processes from start to finish, consistent with a design-for-assembly engineering philosophy. Once molecular manufacturing capabilities become available, experimental efforts may progress from component fabrication and testing to component assembly, and finally to prototypes and mass manufacture, ultimately leading to clinical trials. In 2004, progress in medical nanorobotics remains largely at the concept feasibility stage: Since 1998, the author has published four theoretical nanorobot scaling studies [530–535], summarized briefly below. Note that these studies are not intended to produce an actual engineering design for a future nanomedical product. Rather, the purpose is merely to examine a set of appropriate design constraints, scaling issues, and reference designs to assess whether or not the basic idea might be feasible, and to determine key limitations of such designs.

4.5.1. Respirocytes

The artificial mechanical red blood cell or "respirocyte" [530] is a bloodborne spherical 1- μ diamondoid 1000-atm pressure vessel (Fig. 8) with active pumping powered by endogenous serum glucose, able to deliver 236 times more oxygen to the tissues per unit volume than natural red cells and to manage carbonic acidity. The nanorobot is made of 18 billion atoms precisely arranged in a diamondoid pressure tank that can be pumped full of up to 3 billion oxygen (O₂) and carbon dioxide (CO₂) molecules. Later on, these gases can be released from the tank in a controlled manner, using the same molecular pumps. Respirocytes mimic



Figure 8. An artificial red cell: the respirocyte [530]. Reprinted with permission from [530], R. A. Freitas, Jr., Artif. Cells, Blood Subst. Immobil. Biotech. 26, 411 (1998). © 1998, Forrest Bishop.

the action of the natural hemoglobin-filled red blood cells. Gas concentration sensors on the outside of each device let the nanorobot know when it is time to load O_2 and unload CO_2 (at the lungs), or vice versa (at the tissues) (Fig. 9). An onboard nanocomputer and numerous chemical and pressure sensors enable complex device behaviors remotely reprogrammable by the physician via externally applied acoustic signals.

Each respirocyte can store and transport 236 times as much gas per unit volume as a natural red cell, so the injection of a 5-cc therapeutic dose of 50% respirocyte saline suspension, a total of 5 trillion individual nanorobots, into the human bloodstream can exactly replace the gas carrying capacity of the patient's entire 5.4 L of blood. If up to 1 L of respirocyte suspension could safely be added to the human bloodstream [8], this could keep a patient's tissues safely oxygenated for up to 4 h in the event a heart attack caused the heart to stop beating, or it would enable a healthy person to sit quietly at the bottom of a swimming pool for 4h, holding his breath, or to sprint at top speed for at least 15 min without breathing. Primary medical applications of respirocytes will include transfusable blood substitution; partial treatment for anemia, perinatal/neonatal, and lung disorders; enhancement of cardiovascular/neurovascular procedures, tumor therapies, and diagnostics; prevention of asphyxia; artificial breathing; and a variety of sports, veterinary, battlefield, and other uses.

4.5.2. Microbivores

An artificial mechanical white cell of microscopic size, called a "microbivore," has as its primary function destroying microbiological pathogens found in the human bloodstream, using a digest and discharge protocol [531, 532]. The microbivore is an oblate spheroidal nanomedical device (Fig. 10) measuring 3.4 μ in diameter along its major axis and 2.0 μ in diameter along its minor axis, consisting of 610 billion precisely arranged structural atoms in a gross geometric volume of 12.1 μ^3 and a dry mass of 12.2 pg. The device may consume up to 200 pW of continuous power while completely digesting trapped microbes at a maximum throughput of 2 μ^3 of organic material per 30-s cycle, which is large enough to internalize a single microbe from virtually any major bacteremic species in a single gulp. The nanorobots would be ~80 times more efficient as phagocytic agents than macrophages in terms of volume/s digested per unit volume of phagocytosis than natural white blood cells.



Figure 9. Internal cutaway view of respirocyte: equatorial (left) and polar (right) view [530]. © 1996, Robert A. Freitas, Jr.



Figure 10. An artificial white cell: the microbivore [531]. Designed by Robert A. Freitas, Jr., illustrator Forrest Bishop. © 2001, Zyvex Corp.

Microbivores would fully eliminate septicemic infections in minutes to hours, whereas natural phagocytic defenses—even when aided by antibiotics—can often require weeks or months to achieve complete clearance of target bacteria from the bloodstream. Hence, microbivores appear to be up to ~ 1000 times faster-acting than either unaided natural or antibioticassisted biological phagocytic defenses and are able to extend the therapeutic competence of the physician to the entire range of potential bacterial threats, including locally dense infections.

During each cycle of nanorobot operation, the target bacterium is bound to the surface of the bloodborne microbivore like a fly on flypaper, via species-specific reversible binding sites [6]. Telescoping robotic grapples emerge from silos in the device surface, establish secure anchorage to the microbe's plasma membrane, and then transport the pathogen to the ingestion port at the front of the device, where the pathogen cell is internalized into a 2 μ^3 morcellation chamber. After sufficient mechanical mincing, the morcellated remains of the cell are pistoned into a separate 2 μ^3 digestion chamber, where a preprogrammed sequence of 40 engineered enzymes is successively injected and extracted six times, progressively reducing the morcellate ultimately to monoresidue amino acids, mononucleotides, glycerol, free fatty acids, and simple sugars. These simple molecules are then harmlessly discharged back into the bloodstream through an exhaust port at the rear of the device, completing the 30-s digestion cycle. This "digest and discharge" protocol [6] is conceptually similar to the internalization and digestion process practiced by natural phagocytes, except that the artificial process should be much faster and cleaner. For example, it is well known that macrophages release biologically active compounds during bacteriophagy [536], whereas well-designed microbivores need only release biologically inactive effluent.

4.5.3. Clottocytes

The artificial mechanical blood platelet or clottocyte [533] may allow complete hemostasis in as little as ~1 s, even in moderately large wounds. This response time is on the order of 100–1000 times faster than the natural system. The baseline clottocyte is conceived as a serum oxyglucose-powered spherical nanorobot ~2 μ in diameter (~4 μ^3 in volume) containing a compactly folded biodegradable fiber mesh. On command from its control computer, the device promptly unfurls its mesh packet in the immediate vicinity of an injured blood vessel following, say, a cut through the skin. Soluble thin films coating certain parts of the mesh dissolve on contact with plasma water, revealing sticky sections (e.g., sections complementary to blood group antigens unique to red cell surfaces) in desired patterns. Blood cells are immediately trapped in the overlapping artificial nettings released by multiple neighboring activated clottocytes, and bleeding halts at once.

Although up to 300 natural platelets might be broken and still be insufficient to initiate a self-perpetuating clotting cascade, even a single clottocyte, on reliably detecting a blood vessel break, can rapidly communicate this fact to its neighboring devices [6], immediately triggering a progressive, carefully controlled mesh-release cascade. Clottocytes may perform a clotting function that is equivalent in its essentials to that performed by biological platelets, but at only 0.01% of the bloodstream concentration of those cells or about 20 nanorobots per cubic millimeter of serum. Hence, clottocytes appear to be about 10,000 times more effective as clotting agents than an equal volume of natural platelets.

4.5.4. Vasculoid

Once a mature molecular nanotechnology becomes available, could blood be replaced with a single, complex robot? Purely as a design feasibility exercise, a robotic device that replaces and extends the entire human vascular system has been the subject of a preliminary scaling study [534, 535] and is properly called a "vasculoid," or vascular-like machine. Such a robot would duplicate all essential thermal and biochemical transport functions of the blood, including circulation of respiratory gases, glucose, hormones, cytokines, waste products, and all necessary cellular components. The artificial vascular system would conform to the shape of existing blood vessels, replacing natural blood so thoroughly that the rest of the body would remain, at least physiochemically, essentially unaffected. The vasculoid appliance would be extremely complex, having \sim 500 trillion independent cooperating nanorobots. In simplest terms, the vasculoid is a watertight coating of nanomachinery distributed across the luminal surface of the entire human vascular tree. This nanomachinery constitutes a $\sim 300 \text{ m}^2$ two-dimensional vascular-surface-conforming mosaic of conjoined square nanorobot plates, equipped with a mechanical ciliary array system that helps transport important nutrients and biological cells to the tissues in containerized form. Molecule-conveying docking bays makeup 16% of all vasculoid plates, and nanotankers containing molecules for distribution can dock at these bays and load or unload their cargo. The entire appliance weighs $\sim 2 \text{ kg}$ and releases ~ 30 W of waste heat at a basal activity level and a maximum of ~ 200 W of power at peak activity level.

4.5.5. Chromosome Replacement and Antiaging Therapies

Medical nanorobots will also be able to intervene at the cellular level, performing *in vivo* cytosurgery. The most likely site of pathological function in the cell is the nucleus, and more specifically, the chromosomes. In one simple cytosurgical procedure called "chromosome replacement therapy," a nanorobot controlled by a physician would extract existing chromosomes from a diseased cell and insert new ones in their place [10, 529]. The replacement chromosomes will be manufactured to order, outside of the patient's body, in a laboratory benchtop production device that includes a molecular assembly line, using the patient's individual genome as the blueprint. The replacement chromosomes are appropriately demethylated, thus expressing only the appropriate exons that are active in the cell type to which the nanorobot has been targeted. If the patient chooses, inherited defective genes could be replaced with nondefective base-pair sequences, permanently curing a genetic disease. Given the speed with which nanorobots can be administered and their potential rapidity of action, it is possible that an entire whole-body procedure could be completed in 1 hr or less.

In the first half of the twenty-first century, nanomedicine should eliminate virtually all common diseases of the twentieth century, and virtually all medical pain and suffering as well. Because aging is believed to be the result of a number of interrelated molecular processes and malfunctions in cells, and because cellular malfunctions will be largely reversible, middle-aged and older people who gain access to an advanced nanomedicine can expect to have most of their youthful health and beauty restored. The end result of all these nanomedical advances will be to enable a process called "dechronification," which will first arrest biological aging, then reduce biological age by performing three kinds of procedures on each one of the 4 trillion tissue cells in the body.

First, a microbivore-class nanorobot device will be sent to enter every tissue cell, to remove accumulating metabolic toxins and undegradable material. Afterward, these toxins will continue to slowly reaccumulate, requiring a whole-body maintenance cleanout, perhaps annually, to reverse further aging.

Second, chromosome replacement therapy can be used to correct accumulated genetic damage and mutations in every one of the body's nucleus-bearing cells, with this therapy also perhaps repeated annually.

Third, persistent cellular structural damage that the cell cannot repair by itself such as enlarged or disabled mitochondria can be reversed as required, on a cell-by-cell basis, using cellular repair nanorobot devices.

By means of these annual checkups and cleanouts, and some occasional major repairs, biological age could be restored once a year to the more or less constant physiological age that the patient selects. Only conditions that involve a permanent loss of personality and memory information in the brain—such as an advanced case of Alzheimer's disease or a massive head trauma—may remain incurable in the nanomedical era.

It is a bright future that lies ahead for nanomedicine, but we shall all have to work very long and very hard to bring it to fruition.

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