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REVIEW

# Current Status of Nanomedicine and Medical Nanorobotics

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Nanomedicine is the process of diagnosing, 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. In the relatively near term, nanomedicine can address many important medical problems by using nanoscale-structured materials and simple nanodevices that can be manufactured today, including the interaction of nanostructured materials with biological systems. In the mid-term, biotechnology will make possible even more remarkable advances in molecular medicine and biobotics, including microbiological biorobots or engineered organisms. 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.

**Keywords:** Assembly, Nanomaterials, Nanorobot, Nanorobotics, Nanotechnology.

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## 1. NANOTECHNOLOGY AND NANOMEDICINE

Annual U.S. federal funding for nanotechnology R&D exceeded $500 million in 2002[1] reaching $849 million in FY 2004[2] 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,[3] with annual nanotechnology investment worldwide reaching approximately $3 billion in 2003. The worldwide market for nanoscale devices and molecular modeling should grow 28%/year, rising from $406 million in 2002 to $1.37 billion in 2007, with a 35%/year growth rate in revenues from biomedical nanoscale devices.[4]

In December 2002 the U.S. National Institutes of Health announced a 4-year program for nanoscience and nanotechnology in medicine.[3] Burgeoning interest in the medical applications of nanotechnology has led to the emergence of a new field called nanomedicine.[3, 5–12] Most broadly, nanomedicine is the process of diagnosing, 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.[5] 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".[13] Even state-funded programs have begun, such as New York's Alliance for Nanomedical Technologies.[14]

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.[7] Over the next 5–10 years, biotechnology will make possible even more remarkable advances in molecular medicine and biobotics–microbiological robots or engineered organisms (Section 3). 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).

## 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 Desai and Ferrari created what could be considered one of the earliest therapeutically useful nanomedical devices,[15] employing 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 which 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, secreting insulin back out through the pores while the immune system remains 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.[16] 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,[17] including encapsulated neurons which could be implanted in the brain and then be electrically stimulated to release neurotransmitters, possibly as part of a future treatment for Alzheimer's or Parkinson's diseases.

The flow of materials through nanopores can also be externally regulated.[18] The first artificial voltage-gated molecular nanosieve was fabricated by Martin and colleagues[19] 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[20] have been directed at immobilizing biochemical molecular-recognition agents such as enzymes, antibodies, other proteins and DNA inside the nanotubes as active biological nanosensors,[21] to perform drug separations,[22, 23] and to allow selected biocatalysis.[23] Others are investigating synthetic nanopore ion pumps,[24] voltage-gated nanopores embedded in artificial membranes,[25] and an ion channel switch biosensor[26] that detects changes in chemical concentration of $\sim 10^{-18}$.

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Molecular dynamics theoretical studies of viscosity[27] and diffusion[28] through nanopores are in progress.

Finally, 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.[29] 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. Current research is directed toward reliably fabricating pores with specific diameters and repeatable geometries at high precision,[30] understanding the unzipping of double-stranded DNA as one strand is pulled through the pore[31] and the recognition of folded DNA molecules passing through the pore,[32] experiments with new 3–10 nm silicon-nitride nanopores,[32] and investigating the benefits of adding electrically conducting electrodes to pores to improve longitudinal resolution "possibly to the single-base level for DNA".[32] Nanopore-based DNA-sequencing devices could allow per-pore read rates potentially up to 1000 bases per second,[33] possibly eventually providing 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[34,35] 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 $(3.8\ \text{nm})^3$ polymer block was created. Chiral separations, enzymatic transition state activity, and high receptor affinities have been demonstrated.

Molecularly imprinted polymers could be medically useful in clinical applications such as controlled drug release, drug monitoring devices, quick biochemical separations and assays,[36] recognition elements in biosensors and chemosensors,[37] and biological and receptor mimics including artificial antibodies (plastibodies) or biomimicking enzymes (plastizymes).[37] But 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.[35]

### 2.3. Quantum Dots and Nanocrystals

Fluorescent tags are commonplace in medicine and biology, found in everything from 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 which 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 long-lived 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. (www.qdots.com), 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.[38] 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.[39] Quantum dots are being investigated as chemical sensors,[40] for cancer cell detection,[38] gene expression studies,[41] gene mapping and DNA microarray analysis,[42] immunocytochemical probes,[43] intracellular organelle markers,[44] live cell labeling,[45,46] medical diagnostics and drug screening,[47] SNP (Single Nucleotide Polymorphism) genotyping,[48] vascular imaging,[49] and many other applications.[50,51] Quantum dot physics has been studied theoretically[52] and computationally using time-dependent density functional theory[53] and other methods.[54–56]

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.[57] Experiments showed that these nanocomposites not only retain the intrinsic photocatalytic capacity of $TiO_2$ and the bioactivity of the oligonucleotide DNA, but more importantly 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 to the semiconductor scaffolding a strand of DNA that matches a defective gene within a cell, 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. 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 testing in a laboratory model is at least two years away.[58]

### 2.4. Fullerenes and Nanotubes

Soluble derivatives of fullerenes such as $C_{60}$ have shown great utility as pharmaceutical agents. These derivatives, many already in clinical trials (www.csixty.com), have good biocompatibility and low toxicity even at relatively high dosages. Fullerene compounds may serve as antiviral agents (most notably against HIV,[59] where they have also been investigated computationally[60,61]), antibacterial agents (*E. coli*,[62] *Streptococcus*,[63] *Mycobacterium tuberculosis*,[64] etc.), photodynamic antitumor[65,66] and anticancer[67] therapies, antioxidants and anti-apoptosis agents which may include treatments for amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease)[68] and Parkinson's disease. Single-walled[69,70] and multi-walled[71–73] carbon nanotubes are being investigated as biosensors, for example to detect glucose,[72,74] ethanol,[74] hydrogen peroxide,[71] selected proteins such as immunoglobulins,[70] and as an electrochemical DNA hybridization biosensor.[69]

### 2.5. Nanoshells and Magnetic Nanoprobes

Halas and West at Rice University in Houston have developed a platform for nanoscale drug delivery called the nanoshell.[75,76] 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 IR 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.[77] 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.

Nanospectra Biosciences (www.nanospectra.com), a private company started by Halas and West, is developing commercial applications of nanoshell technology. Nanospectra is conducting animal studies at the MD Anderson Cancer Center at the University of Texas, specifically targeting 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. In mid-2003, Rice researchers announced the development of a point-of-care whole blood immunoassay using antibody-nanoparticle conjugates of gold nanoshells.[78] Varying the thickness of the metal shell allow precise tuning of the color of light to which the nanoshells respond; near-infrared light penetrates whole blood very well, so it is an optimal wavelength for whole blood immunoassay.[79] Successful detection of sub-nanogram-per-milliliter quantities of immunoglobulins was achieved in saline, serum, and whole blood in 10–30 minutes.[78]

An alternative approach pursued by Triton BioSystems (www.tritonbiosystems.com) 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 (whether visible or micrometastases) is covered with bioprobes after several hours, a magnetic field generated from a portable alternating magnetic field machine (similar to a miniaturized MRI machine) heats the iron particles to more than 170 degrees, killing the tumor cells in a few seconds.[80] Once the cells are destroyed, the body's excretion system removes cellular residue and nanoparticles alike. Test subjects feel no pain from the heat generated.[80] Triton BioSystems plans to start designing human tests and ask the FDA for permission to begin human clinical trials in 2006.

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).[81] 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

Multisegment gold/nickel nanorods are being explored by Leong's group at Johns Hopkins School of Medicine[82] 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 gene-delivery 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 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[83] 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.[84] 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 have been shown, in vitro, to specifically kill leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at becquerel (picocurie) levels,[85] with promising preliminary results against advanced ovarian cancer in mice.[86] 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,[87] 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.[88] 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. But 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:[89] "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. Yoshihisa Suzuki at Kyoto University has designed a novel drug molecule that releases antibiotic only in the presence of an infection.[90] 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,[90] "different spectra of antibiotics could be released from the same dressing material, depending on the strain of bacterium." 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.[91] Other stimulus-responsive "smart" hydrogels are being studied, including a hydrogel-composite membrane co-loaded 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."[92]

Nanoparticles with an even greater range of action are being developed by Raoul Kopelman's group at the University of Michigan. Their current 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.[93] The miniscule size of the particles—20–200 nanometers—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 magnetic resonance imaging (MRI). The particles also enhance the killing effect during the subsequent laser irradiation of brain tissue, concentrating the destructive effect only on sick cells unlike traditional chemotherapy and radiation which kills cancerous cells but also destroys healthy cells. Nanoparticles allow MRI to see a few small brain tumor cells as small as 50 microns—depending on the cancer type, tumor cells can range from 5–50 microns each and may grow in locations separate from the tumor site, hence are sometimes not visible to surgeons. Fei Yan, a postdoc in Kopelman's lab, is working on these nanodevices, called the Dynamic Nano-Platform (Fig. 1), now being commercialized as therapeutic "nanosomes" under license to Molecular Therapeutics (www.moleculartherapeutics.com). 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."

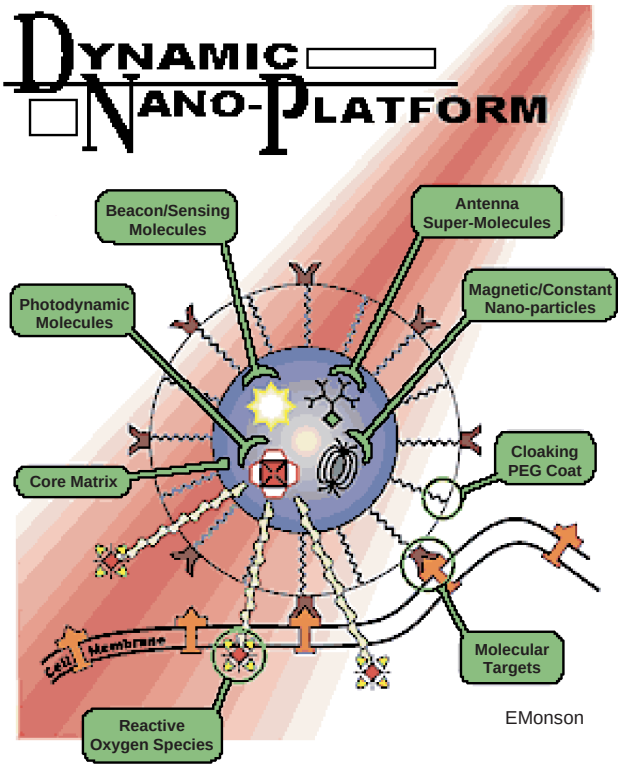


**Fig. 1.** This illustration of the Dynamic Nano-Platform (DNP) or "nanosome" shows proposed extensions of the technology, which may eventually incorporate magnetic and optical control and contrast elements to enable a number of functions from biological sensing to targeted photo dynamic cancer therapy.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

### 2.7. Dendrimers and Dendrimer-Based Devices

Dendrimers[94] represent yet another nanostructured material that may soon find its way into medical therapeutics.[95] Starburst dendrimers are tree-shaped synthetic molecules with a regular branching structure emanating outward from a core that form nanometer by nanometer, with the number of synthetic steps or "generations" dictating the exact size of the particles, typically a few nanometers in spheroidal diameter. The peripheral layer can be made to form a dense field of molecular groups that serve as hooks for attaching other useful molecules, such as DNA, which can enter cells while avoiding triggering an immune response, unlike viral vectors commonly employed today for transfection. Upon 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[96] and in animal models,[97,98] though clinical human trials of dendrimer gene therapy remain to be done. Glycodendrimer "nanodecoys" have also been used to trap and deactivate some strains of influenza virus particles.[99,100] 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. In July 2003, Starpharma (www.starpharma.com) was cleared by the U.S. FDA for human trials of their dendrimer-based anti-HIV microbicide. Their product has been successful in preventing simian-HIV. Computational simulations have also been done on some dendrimer-based nanoparticles.[101]

James Baker's group at the University of Michigan is extending this work to the synthesis of multi-component nanodevices called tecto-dendrimers built up from a number of single-molecule dendrimer components.[102–106] Tecto-dendrimers have a single core dendrimer surrounded by additional dendrimer modules of different types, each type designed to perform a function necessary to a smart therapeutic nanodevice (Fig. 2). Baker's group has built a library of dendrimeric components from which a combinatorially large number of nanodevices can be synthesized.[106]

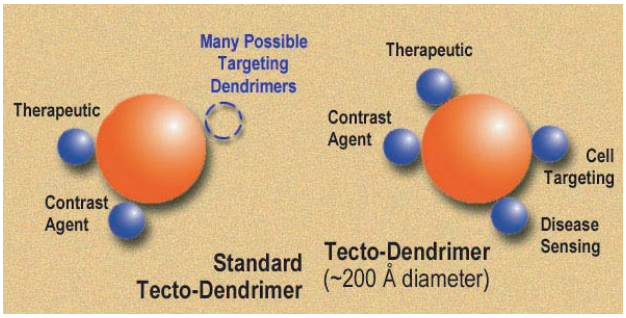


**Fig. 2.** The standard tecto-dendrimer device, which may be composed of monitoring, sensing, therapeutic, and other useful functional modules.[106] Image courtesy of James Baker, University of Michigan.

The initial library contains components which will perform the following tasks: (1) diseased cell recognition, (2) diagnosis of disease state, (3) drug delivery, (4) reporting location, and (5) reporting outcome of therapy. By using this modular architecture, an array of smart therapeutic nanodevices can be created with little effort. For instance, once apoptosis-reporting, 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 response of the cells to methotrexate 100-fold over free drug."[105] At least a half dozen cancer cell types have already been associated with at least one unique protein which 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.[106] The same cell-surface protein recognition-targeting strategy could be applied against virus-infected cells and parasites. 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.[105]

NASA and the National Cancer Institute have funded Baker's lab to produce dendrimer-based nanodevices that can detect and report cellular damage due to radiation exposure in astronauts on long-term space missions.[107] By mid-2002, the lab had built a dendrimeric nanodevice to detect and report the intracellular presence of caspase-3, one of the first enzymes released during cellular suicide or apoptosis (programmed cell death), one sign of a radiation-damaged cell. The device includes one component that identifies the dendrimer as a blood sugar so that the nanodevice is readily absorbed into a white blood cell, and a second component using fluorescence resonance energy transfer (FRET) that employs two closely bonded molecules. Before apoptosis, the FRET system stays bound together and the white cell interior remains dark upon illumination. Once apoptosis begins and caspase-3 is released, the bond is quickly broken and the white blood cell is awash in fluorescent light. If a retinal scanning device measuring the level of fluorescence inside an astronaut's body reads above a certain baseline, counteracting drugs can be taken.

### 2.8. Radio-Controlled Biomolecules

While 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. Jacobson and colleagues[108] have attached tiny radio-frequency antennas—1.4 nanometer gold nanocrystals of less than 100 atoms—to DNA. When a ~1 GHz radio-frequency magnetic field is transmitted into the tiny antennas, alternating eddy currents induced in the nanocrystals produce highly localized inductive heating, causing the double-stranded DNA to separate into two strands in a matter of seconds in a fully reversible dehybridization process that leaves neighboring molecules untouched.

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. This requires attaching gold nanoparticles to specific oligonucleotides which, 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 rf magnetic field then heats 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 which also turn genes on and off.[109] Says Gerald Joyce:[110] "You can even start to think of differential receivers–different 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."

The gold nanocrystals can be attached to proteins as well as DNA, opening up the possibility of future radio frequency biology electronically controlling 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.[111] 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-amino-acid 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 rf field, the S-peptides adopt their usual conformation and the RNAase remains active, but with the external rf field switched on, the rapidly spinning nanoparticles prevented the S-peptide from assembling with the larger protein, inactivating the enzyme.

## 3. MICROSCALE BIOLOGICAL ROBOTS

One convenient shortcut to nanorobotics is to engineer natural nanomachine systems—microscale biological viruses and bacteria—to create new, artificial biological devices.

Efforts at purely rational virus design are underway but have not yet borne much fruit. For example, Endy et al.[112] 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.[113, 114] Nevertheless, combinatorial experiments on wild type T7 by others[115–117] have produced new but immunologically indistinguishable T7 variants which have 12% of their genome deleted and which replicate twice as fast as wild type.[117] The Synthetic Biology Lab at MIT (syntheticbiology.org) is building the next generation T7, a bacteriophage with a genome size of about 40 Kbp and 56 genes. 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."

Young and Douglas[118] have chemically modified the Cowpea chlorotic mottle virus (CCMV) viral protein cage surface to allow engineering of surface-exposed functional groups. 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.[119] The researchers have investigated re-engineering the artificial virion to make a complete tumor-killing nanodevice, exploiting a gating mechanism that results from reversible structural transitions in the virus.[120] The natural viral gate of CCMV has been reengineered to allow control by redox potential; 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. 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.[119]

The rational design and synthesis of chimeric viral replicators is already possible today, and the rational design and synthesis of completely artificial viral sequences, leading to the manufacture of completely synthetic viral replicators, should eventually be possible. In a three-year project[121] 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, then injecting the synthetic RNA into a cell-free broth containing a mixture of proteins taken from cells, which then directed the synthesis of complete (and fully infectious) polio virion particles.[121]

Engineered bacterial "biorobots" are also being pursued. Mushegian[122] concludes that as few as 300 highly conserved 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. Thus a minimal synthetic microbe—a basic cellular chassis—could be specified by a genome only 150,000 nucleotide bases in length. 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, or 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.

In November 2002, J. Craig Venter, of human genome-sequencing fame, and Hamilton O. Smith, a Nobel laureate, announced[123] that their new company, Institute for Biological Energy Alternatives (IBEA), had received a $3 million, three-year grant from the Energy Department to create a minimalist organism, starting with the *Mycoplasma genitalium* microorganism. Working with a research staff of 25 people, the scientists are removing all genetic material from the organism, then synthesizing 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. 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, the cell will be deliberately hobbled to render it incapable of infecting people, and will be strictly confined and designed to die if it does manage to escape into the environment.

In 2003, Egea Biosciences (www.egeabiosciences.com) received "the first [patent][124] to include broad claims for

the chemical synthesis of entire genes and networks of genes comprising a genome, the 'operating system' of living organisms." Egea's proprietary GeneWriter™ and Protein Programming™ technology has: (1) produced libraries of more than 1,000,000 programmed proteins, (2) produced over 200 synthetic genes and proteins, (3) produced the largest gene ever chemically synthesized of over 16,000 bases, (4) engineered proteins for novel functions, (5) improved protein expression through codon optimization, and (6) 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 Egea calls "word processing for DNA".[125] The patent recites one preferred embodiment of the invention as 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.[125]

## 4. MEDICAL NANOROBOTICS

The third major development pathway of nanomedicine—molecular nanotechnology (MNT) or nanorobotics[5, 7, 126]—takes as its purview the engineering of complex nanomechanical systems for medical applications. 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, precision 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

In his remarkably prescient 1959 talk "There's Plenty of Room at the Bottom," the late Nobel physicist Richard P. Feynman proposed employing machine tools to make smaller machine tools, these to be used in turn to make still smaller machine tools, and so on all the way down to the atomic level.[127] 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[127] 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[128] 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[126] laying the foundations for molecular machine systems and molecular manufacturing, and subsequently by Freitas' technical books[5, 7] on medical nanorobotics.

### 4.2. Nanorobot Parts and Components

#### *4.2.1. Nanobearings and Nanogears*

In order to establish the feasibility of 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 upon *ab initio* structural analysis and molecular dynamics simulations. Notes Drexler:[126] "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."

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,[126] shown with end views and exploded views in Figure 3 using both ball-and-stick and space-filling representations. This bearing has 206 atoms of carbon, silicon, oxygen and hydrogen, and is composed of a small shaft that rotates within a ring sleeve measuring 2.2 nm in diameter. The

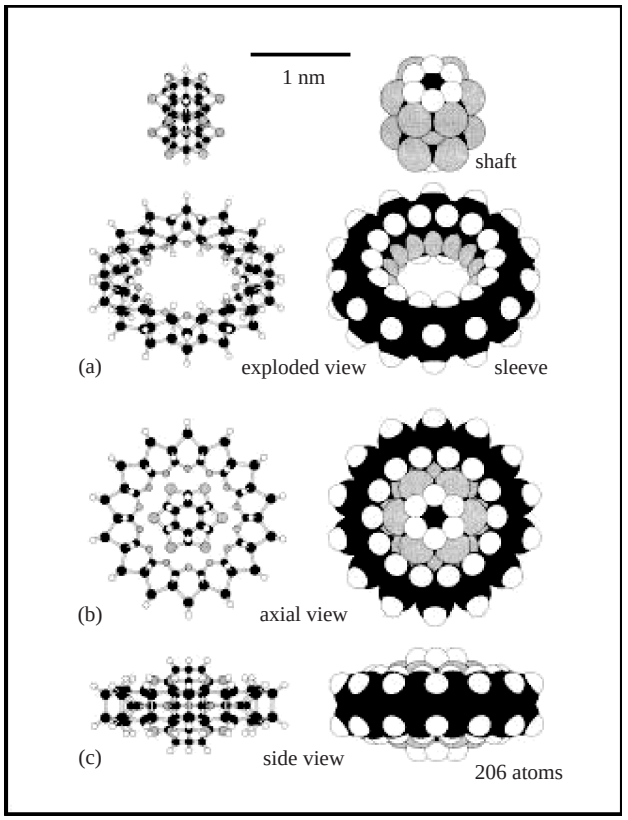


**Fig. 3.** End views and exploded views of a 206-atom overlap-repulsion bearing.[126] Image courtesy of K. Eric Drexler. © 1992, John Wiley & Sons, Inc. Used with permission.

atoms of the shaft are arranged in a 6-fold symmetry, while the ring has 14-fold symmetry, a combination that provides low energy barriers to shaft rotation. Figure 4 shows an exploded view of a 2808-atom strained-shell sleeve bearing designed by Drexler and Merkle[126] using molecular mechanics force fields to ensure that bond lengths, bond angles, van der Waals distances, and strain energies are reasonable. This 4.8-nm diameter bearing features an interlocking-groove interface which 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.[129]

Molecular gears are another convenient component system for molecular manufacturing design-ahead. For example, Drexler and Merkle[126] designed a 3557-atom planetary gear, shown in side, end, and exploded views in Figure 5. The entire assembly has twelve moving parts and is 4.3 nm in diameter and 4.4 nm in length, with a molecular weight of 51,009.844 daltons and a molecular volume of 33.458 $nm^3$. An animation of the computer simulation shows the central shaft rotating rapidly and the peripheral output shaft rotating slowly. The small planetary gears, rotating around the central shaft, are

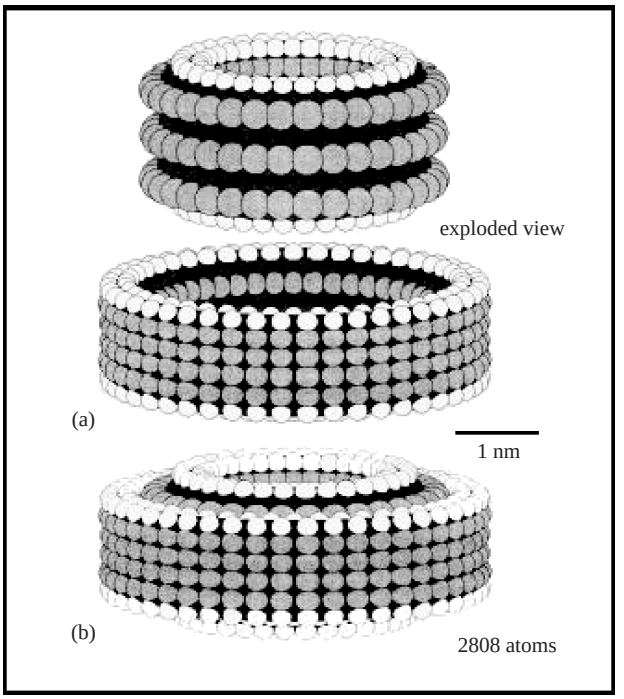


**Fig. 4.** Exploded view of a 2808-atom strained-shell sleeve bearing.[126] Image courtesy of K. Eric Drexler. © 1992, John Wiley & Sons, Inc. Used with permission.

surrounded by a ring gear that holds the planets in place and ensures that all components move in 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 $CH_2$ bridges between the parallel rings; and

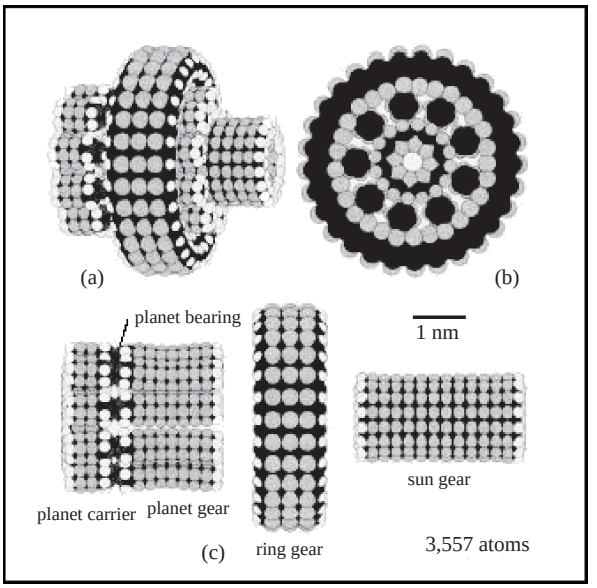


**Fig. 5.** End-, side-, and exploded-view of a 3557-atom planetary gear.[126] Image courtesy of K. Eric Drexler. © 1992, John Wiley & Sons, Inc. Used with permission.

the planet carrier is adapted from a Lomer dislocation[130] array created by R. Merkle and L. Balasubramaniam, linked to the planet gears using C—C bonded bearings.

A rotational impulse dynamics study of this first-generation planetary gear Goddard and colleagues[131] found that at the normal operational rotation rates for which this component was designed (e.g., <1 GHz for <10 m/sec interfacial velocities), the gear worked as intended and did not overheat. Started from room temperature, the gear took a few cycles to engage, then rotated thermally stably at ~400 K. Only when the gear was severely overdriven to ~100 GHz did significant instabilities appear, although the device still did not self-destruct. One run at ~80 GHz showed excess kinetic energy causing gear temperature to oscillate up to 450 K above baseline. Commenting on the ongoing design effort, Goddard[131] 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.[132] The pump and chamber wall segment shown in Figure 6 contains 6165 atoms with a molecular weight of 88,190.813 daltons and a molecular volume of 63.984 $nm^3$. The device could serve either as a pump for neon gas atoms or (if run backwards) 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. 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[131] reported that preliminary molecular dynamics simulations of the device showed that it could indeed function as a pump, although it is not very energy-efficient so further refinement of this initial design is warranted. Almost all such design research in molecular nanotechnology is restricted to theory and computer simulation,

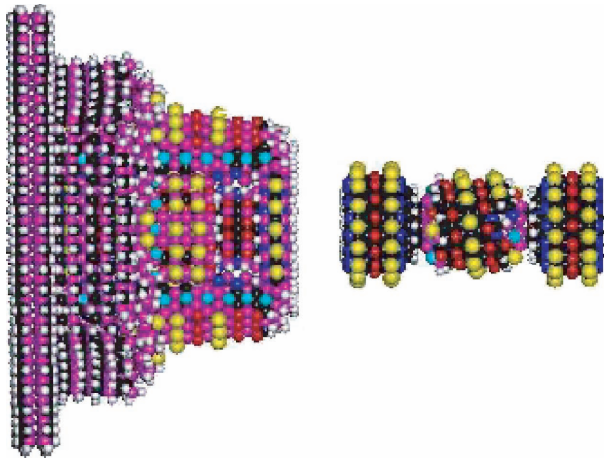


**Fig. 6.** Side views of a 6165-atom neon gas pump/motor.[132] Image courtesy of K. Eric Drexler. © Institute for Molecular Manufacturing (www.imm.org).

which allows the design and testing of large structures or complete nanomachines and the compilation of growing libraries of molecular designs. 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 multi-million atom range.[133]

On the experimental pathway, Montemagno and Bachand[134] modified a natural biomotor to incorporate nonbiological parts, creating the first artificial hybrid nanomotor. Using the tools of genetic engineering, they added metal-binding amino acid residues to ATPase, a ubiquitous enzyme whose moving part 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). Each motor molecule bonded tightly to nanoscale nickel pedestals prepared by electron beam lithography. Properly oriented motor molecules 12 nanometers in diameter were then attached to the pedestals with a precision approaching 15 nanometers, and a silicon nitride bar a hundred nanometers long was bound to the rotor subunit of each motor molecule,[135] 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, but subsequent motors have been operated for hours continuously by providing a surplus of ATP. 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, and also a chemical means of switching his hybrid motors on and off reliably:[136] 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.[137] 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. Also following the bio-nano pathway is Mavroidis's group[138] which in late 2003 received a $1 M four-year NSF 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." Others have operated chemically-powered DNA-based nanomotors (Section 4.3.2).

Other experimental nanomotor research includes the 78-atom chemically-powered rotating motor synthesized in 1999 by Kelly, 139 a chemically-powered rotaxane-based linear motor exerting ~100 pN of force with a 1.9 nm throw and a ~250 sec contraction cycle by Stoddart's group,[140] a UV-driven catenane-based ring motor by Wong and Leigh,[141] and an artificial 58-atom motor molecule that spins when illuminated by solar energy by Feringa.[142] Also in 2003, the Zettl group[143] created an electrically powered 550-nm wide nanomotor by first depositing a number of multiwalled nanotubes on the flat silicon oxide surface of a silicon wafer, then using electron beam lithography to simultaneously pattern a 110–300 nm gold rotor, nanotube anchors, and opposing stators around the chosen nanotubes, then annealing the rotor to the nanotubes, after which the surface was selectively etched to provide sufficient clearance for the rotor. When the stators were alternately charged with 50 volts of direct current, the gold rotor rocked back and forth up to 20 degrees, making a torsional oscillator. A strong electrical jolt to the stators jerked the rotor and broke 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.[144] 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.

#### *4.2.3. Nanocomputers*

Truly effective medical nanorobots may require onboard computers to allow a physician to properly monitor and control their work. In 2000, a collaborative effort between UCLA 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,[145] and there has been much recent progress with nanotube- and nanorod-based molecular electronics.[146, 147] Several private companies are pursuing the first commercial molecular electronic devices including memories and other computational components of nanocomputers using techniques of self-assembly, and there is also the possibility of low-speed biology-based digital nanocomputers (Section 4.3.4).

### 4.3. Self-Assembly and Directed Parts Assembly

#### *4.3.1. Self-Assembly of Mechanical Parts*

Perhaps the best-known self-assembling molecular systems include those which form ordered monomolecular structures by the coordination of molecules to surfaces,[148] called self-assembled monolayers (SAMs),[149] self-assembling thin films or Langmuir-Blodgett films,[150] self-assembling lipidic micelles and vesicles,[151] and self-organizing nanostructures.[152, 153] 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 comprising the monolayer, although control of in-plane dimensions to <100 nm is relatively difficult.

Several attempts have been made to achieve self-assembly of small mechanical parts to avoid direct parts grasping,[154, 155] and Saitou[156] 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[157] suggests expanding the toolbox of self-assembly by including dynamic components that emulate enzymatic allostery, and presents a simple "mechanical enzyme" analog–a 2-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. As more component types are added the challenge is to avoid any undesirable local energy minima–necessitating the development of energy vs. orientation modeling tools.

The programming of engineered sequences of such conformational switches can allow the self-assembly of quite complicated mechanical structures. Saitou[156, 158] 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[156] by the Penrose[159] self-replicating blocks and by Hosokawa's self-assembling triangular parts with embedded switches.[160] Saitou claims[156] his model of self-assembling automata can also be applied to self-assembly in 2- or 3-dimensions.

Guided[161–164] or directed[165–167] self-assembly has become a growing research area. Yeh and Smith[154] have described a process of fluidic self-assembly of optoelectronic devices,

Rothemund and Winfree[168] have described a tile assembly model for pseudocrystalline self-assembly, Breivik[169] has designed and patented a set of self-replicating physical polymers, and Gracias et al.[170] have impressed electrical circuits including LEDs on the surfaces of copper-polyimide truncated octahedra each ~1 mm in diameter, then induced these octahedra to self-assemble into specified 3-D electrical networks of up to 12 devices by co-melting of opposing solder spots. (Gracias notes that hierarchical self-assembly[171] and shape-selective self-assembly using lock-and-key structures[172, 173] "offer more sophisticated strategies for the fabrication of asymmetrical networks incorporating more than one repeating unit.") Whitesides et al.[174, 175] first demonstrated capillary-force driven assembly of a simple circuit and other structures from millimeter-scale components, and electrostatic self-assembly,[176] and some of this work has since been extended to the fluidic self-assembly of microscale parts,[154, 177–180] including "micro-origami"[181, 182] or "silicon origami",[183] as well as mesoscopic nucleic acid analogs.[184] The dynamics of Brownian self-assembly,[185] the theory of designable self-assembling molecular machine structures,[156, 186] and the computational modeling of self-assembly processes are beginning to be addressed.

### *4.3.2. DNA-Directed Assembly*

Early mechanical nanorobots might be assembled, at least in part, from DNA. The idea of using DNA to build nanoscale objects has been pioneered by Nadrian Seeman at New York University.[187] 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 due to 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 comprised of 480 nucleotides each,[188] then a truncated octahedron containing 2550 nucleotides.[189] By the mid-1990s, Seeman could fabricate nanoscale DNA stick figures of almost any regular geometric shape, by the billions per batch.[190]

In 1999, Seeman reported the construction of a mechanical DNA-based device that might serve as the basis for a nanoscale robotic actuator.[191] 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 nanometers during this fully reversible structural conversion, like a hinge opening and closing. A large version of the device might function as an elbow, while smaller devices could serve as finger joints. By 2002, Seeman's group had demonstrated a mechanical DNA-based rotary motor[192] and reported the design and construction of 2-dimensional DNA arrays which might serve as templates for nanomechanical assembly.[193] 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."

In other labs: sequence-specific DNA hybridization is used to bend silicon microcantilevers,[194] Alberti and Mergny[195] 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[196] have made a single-DNA-molecule inchworm motor, and Shu and Guo[197] 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 producing 50–60 pN of force. Yurke and Turberfield[198] synthesized another DNA actuator using three single strands of artificial DNA which, when placed together, find their complementary partners and self-assemble to form a V-shaped structure. The open mouth of this nanotweezer can be made to close by adding a special "fuel" strand which binds to the single-stranded DNA dangling from the ends of the arms of the tweezers and zips them closed, moving from a ~7 nm separation to a ~1 nm separation in ~13 sec per cycle. A special "removal" strand, when added, binds to the fuel strand and pulls it away, opening the nanotweezers again. More recent work[199] has focused on a continuously running DNA nanomotor. Reif's group has 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.[200]

### *4.3.3. Protein-Directed Assembly*

While most enzymes in cells are involved in manipulating small molecules <0.25 kD, 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 ~3 kD. Molecules even larger than this are manipulated by tRNA-synthetase (a 40–100 kD enzyme that manipulates ~30 kD tRNAs), the spliceosome, the ribosome,

the proteosome, and the DNA replication complex. Many of these protein manipulators employ "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 in order to produce mRNA.[201] By designing synthetic enzymes which might be called "nanopart synthetases," possibly using synthetic amino acids, we can envision grabbing molecular parts in a solution and then, as the enzyme folds, bringing these parts into proper alignment and causing them to react, exemplifying protein-directed parts assembly. (RNA-based ribozymes[202] may prove better suited than proteins for some reactions.)

Ratchet-action protein-based molecular motors are well-known in biology,[203] conformational cascades of a special genetic variant of yeast cell prions have already been used to assemble silver- and gold-particle-based nanowires,[204] 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 well-known.[205] Smith[206] has used methyltransferase-directed addressing of fusion proteins to DNA scaffolds to construct a molecular camshaft as a exemplar protein/nucleic acid biostructure. Protein-protein binding specificity can bend silicon microcantilevers.[207] Genetically engineered chaperonin protein templates (chaperone molecules) can direct the assembly of gold nanoparticles (1.4, 5, or 10 nm) and CdSe semiconductor quantum dots (4.5 nm) into nanoscale arrays.[208]

#### *4.3.4. Microbe- and Virus-Directed Assembly*

Artificial microbes might also be employed in molecular 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,[209] and microorganisms can accumulate materials and synthesize inorganic structures composed of bismuth,[210] CdS,[211,212] gold,[213] magnetite,[212,214] silica,[212] and silver.[212]

As for microbe-directed parts assembly, Kondo et al.[215] used a grooved film (created by chemically precipitating cellulose tracks less than 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 microns/minute. 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 3-dimensional collagen lattice.[216] Although ECM (extracellular matrix) 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.

To establish digital control over microorganisms, genetic circuits that can function as switches[217] or computational logic elements such as AND, NAND, and NOR gates are under active investigation. In 2000 Gardner et al.[218] 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. Elowitz and Leibler[219] 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."[220] By 2002, Weiss[221] 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.[220]

Bacteria can serve as physical system components. Tung et al.[222] are attempting to incorporate living bacteria into microelectromechanical systems (MEMS) devices to form living cell motors for pumps, valves, and conveyor belts. Turner and colleagues[223] have affixed a film of *Serratia marcescens* bacteria onto tiny beads, allowing the microbes' rotating appendages to carry the beads along, then applied the film inside tiny tubes, whereupon the gyrating bacterial arms blend fluids twice as fast as diffusion alone. Montemagno[224] 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 microns 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 ~46 microns/min with a repetition rate controlled by the spring constant of the MEMS structure.[224]

Viral shells also provide useful templates for nanoscale assembly. Belcher[165,225] employs virus capsid shells as scaffolds for the directed nanoassembly of nanoparticles such as quantum dots,[225,226] in a process of "biomimetic synthesis of nonbiological inorganic phases with novel electronic and magnetic properties directed by proteins and synthetic analogs." In one experiment,[225] a genetically engineered M13 bacteriophage with a specific recognition moiety for zinc sulfide nanocrystals assembles a ZnS-containing film having nanoscale ordering and 72-micron-sized domains. Engineered viral coat proteins can be used as scaffolds for nanomaterials synthesis[227] and self-assembly,[228] including self-assembled monolayers.[118]

### 4.4. Positional Assembly and Molecular Manufacturing

As machine structures become more complex, getting all the parts to spontaneously self-assemble in the right sequence is increasingly difficult. To build complex non-periodic structures, it makes more sense to design a mechanism that can assemble a molecular structure by what is

called positional assembly—that is, 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 this 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. (www.zyvex.com), 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." Zyvex has already demonstrated the ability to positionally assemble large numbers of MEMS-scale parts, and has 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 nanometers at near-video scan rates. Agilent Laboratories has created an ultra-high-precision micromover platform[229] capable of providing linear two-dimensional movement in steps of 1.5 nanometers, the width of about 9 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 micrometers in each direction in 2.5 milliseconds; since each micrometer is made up of 1,000 nanometers, the micromover would take approximately 20,000 steps to traverse 30 micrometers, a distance which is about half the width of a single human hair. Martel's group at MIT has worked on a similar nano-positioning device called the NanoWalker.[230]

Kim and Lieber[231] created the first general-purpose nanotweezer whose working end is a pair of electrically controlled carbon nanotubes made from a bundle of multi-walled 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. Kim and Lieber have successfully grasped organelle-sized 500-nanometer clusters of polystyrene spheres, and have removed a semiconductor wire 20 nanometers wide from a mass of entangled wires, using tweezer arms about 50 nanometers wide and 4 microns long, but the technique creates a large electric field at the tweezer tips which can alter the objects being manipulated. In 2001, Boggild's group[232] used standard micromachining processes to carve from a tiny slab of silicon an array of cantilevered micro-pliers which 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 nanometers apart, making a better-controlled nanotweezer.[233] Nanotube-based nanotweezers have since been reported by others.[234, 235]

Precise positional covalent attachment of molecules to surfaces is also being pursued. Blackledge et al.[236] used a palladium-coated SFM (Scanning Force Microscope) 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.[237] employ redox probe microscopy (RPM) in which an 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[238, 239] 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 that can 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 diamondoid 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*

There is widespread interest in the exceptional properties of diamond—it has extreme hardness and strength, high thermal conductivity, low frictional coefficient, chemical inertness, and a wide bandgap. Recent investigations have been driven by the many emerging applications for diamond in MEMS mechanical and electromechanical devices,[240, 241] optics, radiology, biochemical synthesis[242] and medicine,[243] but most especially in various electronics devices.[244–246] A method for the precise manufacture of microscopic and nanoscale diamond structures would have tremendous utility in science and industry.

In contrast to high-pressure diamond synthesis and low-pressure gas-phase diamond synthesis of diamond via chemical vapor deposition or CVD,[247] positional mechanosynthesis has been proposed by Drexler[126] 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), thus

enabling direct positional selection of reaction sites on the workpiece. The scanning tunneling electron microscope (STM) has demonstrated an ability to manipulate surface structures atom by atom, and many proposed methods involve the use of an SPM to direct chemical reactions on the surface by: (1) delivering an electric field to a sub-nanometer region of a surface to activate a chemical reaction, (2) manipulating the chemistry of the tip to make it act as a catalyst which can then be introduced precisely into the region of desired reaction, or (3) delivering mechanical energy from the tip to activate surface reactions (mechanosynthesis). The reaction selectivity of all these methods relies 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.

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 (or dimer) placement on diamond has involved many researchers including Cagin,[248] Drexler,[126] Dzegilenko,[249] Freitas,[250–252] Goddard,[248] Mann,[252] Merkle,[250–254] Peng,[251,252] Saini,[249] Srivastava,[249] and Walch.[248,253] 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.[253] 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 the insertion of a positionally controlled carbene into a surface dimer on a C(100) surface using a 9-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 6–31 G 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 $\pi$ 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.[249] 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. When planar $C_6H_2$ (methenylidene cyclopentene) 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 four-fold locations forming bonds with C atoms of two neighboring surface dimers is attached, or else a $C_4H_2$ fragment is adsorbed atop two C atoms of neighboring surface dimers, with the reaction outcome depending critically upon the initial tip-surface distances, the tip trajectory, and various allowed but undesired tip rearrangements.[249]

In 2003, Merkle and Freitas[250] 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 tooltip structures and evaluate their stability and the strength of the bond they make to the CC dimer. Considering a dimer bonded to two group IV supporting atoms (silicon, germanium, tin, or lead), this series of elements forms progressively weaker bonds to carbon, so the proposed tooltips 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 (Fig. 7). The tooltip molecule, a bi-silaadamantane dicarbon, is only the apex of a complete tool. In a full 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. Initial[252] and subsequent *ab initio* molecular dynamics simulations of these tool tips has confirmed the successful operation of the Ge tooltip at room temperatures.

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[255] used voltage pulses on an STM tip to extract a single germanium atom from the (111) surface of a sample. STMs have been used to bind silicon atoms to the tip, first pulling the atoms off the surface of a Si(111) crystal face and then re-inserting them back

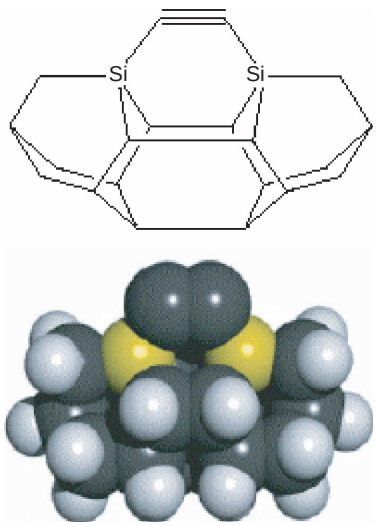


**Fig. 7.** DCB6-Si dimer placement tool tip for diamond mechanosynthesis.[250] © 2003, Ralph C. Merkle and Robert A. Freitas, Jr.

into the crystal,[256, 257] 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 one dimer in width.[258] Ho and Lee[259] have demonstrated the first repeatable site-specific mechanosynthetic covalent bonding operation of a diatomic carbon-containing molecule, $Fe(CO)_2$, on a crystal surface, albeit electrically-mediated. Most recently, a near contact atomic force microscope operated at low-temperature has been used for the vertical manipulation of selected single silicon atoms from the Si(111)–(7 × 7) surface, the first experimental demonstration of pure mechanosynthesis involving the solely mechanical 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 mechanosynthetic deposition of a single atom on a created surface vacancy.[260]

These results, both theoretical and experimental, 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 vacuum conditions. Repeated application of these basic operations should allow building up complex and atomically precise molecular structures, permitting the manufacture of a wide range of nanoscale diamond structures with atomically precise features.

### *4.4.2. Massively Parallel Manufacturing*

Massively parallel assembly is the key to the economic viability of molecular manufacturing. Biology provides perhaps the best example of the power of massive parallelism in assembly, such as polysomes in living cells (multiple ribosomes translating a single mRNA strand simultaneously). The difference between serial and parallel processing is similarly crucial in molecular manufacturing, where the basic parts are very small. If a typical molecularly precise simple component is 1 $nm^3$ in volume, then to manufacture a 1 $cm^3$ volume of molecularly precise product requires the assembly of 1000 billion billion ($10^{21}$) individual simple molecular components—even at a 1 GHz operating frequency, serial atom-by-atom manufacturing of a single object would take many thousands of years, clearly not economically viable. But with parallel manufacturing, vast numbers of molecular components can be processed simultaneously, reducing batch processing times to days, hours, or even less. At least two such techniques for performing massively parallel positional assembly have been identified: (1) massively parallel manipulator arrays and (2) 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. In order to fabricate large numbers of nanoparts and nanoassemblies, massively parallel scanning probe microscopes (SPM) arrays[261, 262] and microscale SPMs[263–266] would be most convenient. Force-sensing devices such as piezoelectric, piezoresistive and capacitive microcantilevers make it possible to construct microscale AFMs on chips without an external deflection sensor. For example, in 1995 Itoh and colleagues[267] fabricated an experimental piezoelectric $ZnO_2$-on-$SiO_2$ microcantilever array of ten tips on a single silicon chip. Each cantilever tip lay ~70 microns from its neighbor, and measured 150 microns long, 50 microns wide and 3.5 microns thick, or ~26,000 $micron^3$/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 ~20 nm/volt–for instance, 0.3-nm resolution at 125 KHz. Parallel probe scanning and lithography was been achieved by Quate's group, progressing from simple piezoresistive microcantilever arrays with 5 tips spaced 100 microns apart and 0.04-nm resolution at 1 KHz but only one $z$-axis actuator for the whole array,[268] 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/sec using a piezoresistive sensor,[269] and by 1998 to arrays of 50–100 independently controllable AFM probe tips mounted in 2-D patterns with 60 KHz resonances, including a 10 × 10 cantilevered tip array fabricated in closely spaced rows using throughwafer interconnects on a single chip.[270] Similarly, by 1997 MacDonald's group[271] had built and tested an array of micro-STMs on the surface of an ordinary silicon chip, with each tip on a cantilever 150 microns long with 3-D sensing and control—the largest prototype array had 144 probes, arranged in a square consisting of 12 rows of 12 probes each, with individual probe needles about 200 microns apart. Researchers in the Millipede project at IBM's Zurich Research Laboratory used conventional microlithography to fabricate scanning probe tip arrays of up to 1024 individual tips to achieve terabit-per-square-inch data storage densities,[272] Mirkin's group constructed an array of 10,000 microscope tips with each capable of acting independently from the others,[273] and at least one "electronic nose" microcantilever array has been fabricated with millions of interdigitated cantilevers on a single chip.[274]

Yet another alternative is Zyvex's patented Rotapod™ exponential assembly design concept,[275] 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, etc., 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 which can subsequently undertake the desired massively parallel manufacturing operations. In 2001, Zyvex was awarded a $25 million, five-year, National Institute of Standards and Technology (NIST) Advanced Technology Program government contract to develop prototype microscale assemblers using microelectromechanical systems (MEMS) and nanoelectromechanical systems (NEMS) for prototype nanoscale assemblers.

**Self-replicating systems** would achieve massively parallel assembly first by fabricating copies of themselves, then 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: (1) the widespread but erroneous perception of great technical difficulty, and (2) 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,[276] and the well-known 1980 NASA engineering study of self-replicating lunar factories,[277] 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, fully autonomous, or various combinations in between.

In the last few years there has been renewed research interest in the challenge of mechanical self-replicating systems,[278] in part due to the realization that replication can be a fundamentally simple process. Today there are several ongoing university research programs, both theoretical and experimental, on mechanical (nonbiological) self-replicating machines.[278] The biotechnology and molecular engineering communities are just beginning to seriously study mechanical replicators operating in the nanoscale size domain. Note that for the foreseeable future it is likely that onboard storage of information will not be required by nanomechanical replicators. One example of an inherently safe and flexible approach is the broadcast architecture, wherein control information is broadcast by any of several means to the replicating component. 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. Conceptual system designs for molecular manufacturing are extensively reviewed by Freitas and Merkle.[278]

### 4.5. Medical Nanorobot 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 ten to one.[5] Many bacteria move by whipping around a tiny tail, or flagellum, that is driven by a 30-nanometer 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 microns in size.[5] 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.[7]

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 Magnetic Resonance Imaging (MRI) system as a means of propulsion for a microrobot in the blood vessels.[279] 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,[280] including the magnetically-controlled "cytobots" and "karyobots" proposed by Chrusch et al.[281] for performing wireless intracellular surgery.

There are preliminary proposals for hybrid bionanorobots that could be constructed using currently foreseeable technologies. For example, Montemagno[282, 283] 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, which 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 (Sections 4.2 and 4.4.1), and to build them cheaply enough and in sufficiently large numbers to be useful therapeutically (Section 4.4.2), then what are the medical implications?

There are many possibilities[5–7, 284–288] but the development pathway will be long and arduous. First, theoretical scaling studies 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,[285–288] two of which are 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. Issues related to biocompatibility of medical nanorobots are extensively discussed elsewhere.[7]

### 4.5.1. Respirocytes

The artificial mechanical red blood cell or "respirocyte"[285] is a bloodborne spherical 1-micron diamondoid 1000-atmosphere 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

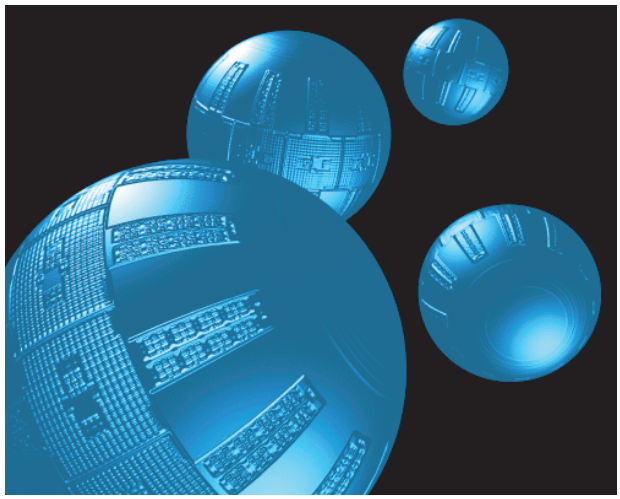

**Fig. 8.** An artificial red cell—the respirocyte.[285] Designer Robert A. Freitas, Jr. © 1999, Forrest Bishop. Used with permission.

in a diamondoid pressure tank that can be pumped full of up to 3 billion oxygen ($O_2$) and carbon dioxide ($CO_2$) molecules. Later on, these gases can be released from the tank in a controlled manner using the same molecular pumps. Respirocytes mimic 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 liters of blood. If up to 1 liter of respirocyte suspension could safely be added to the human bloodstream,[7] this could keep a patient's tissues safely oxygenated for up to 4 hours in the event a heart attack caused the heart to stop beating, even in the absence of respiration. 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 to destroy microbiological pathogens found in the human

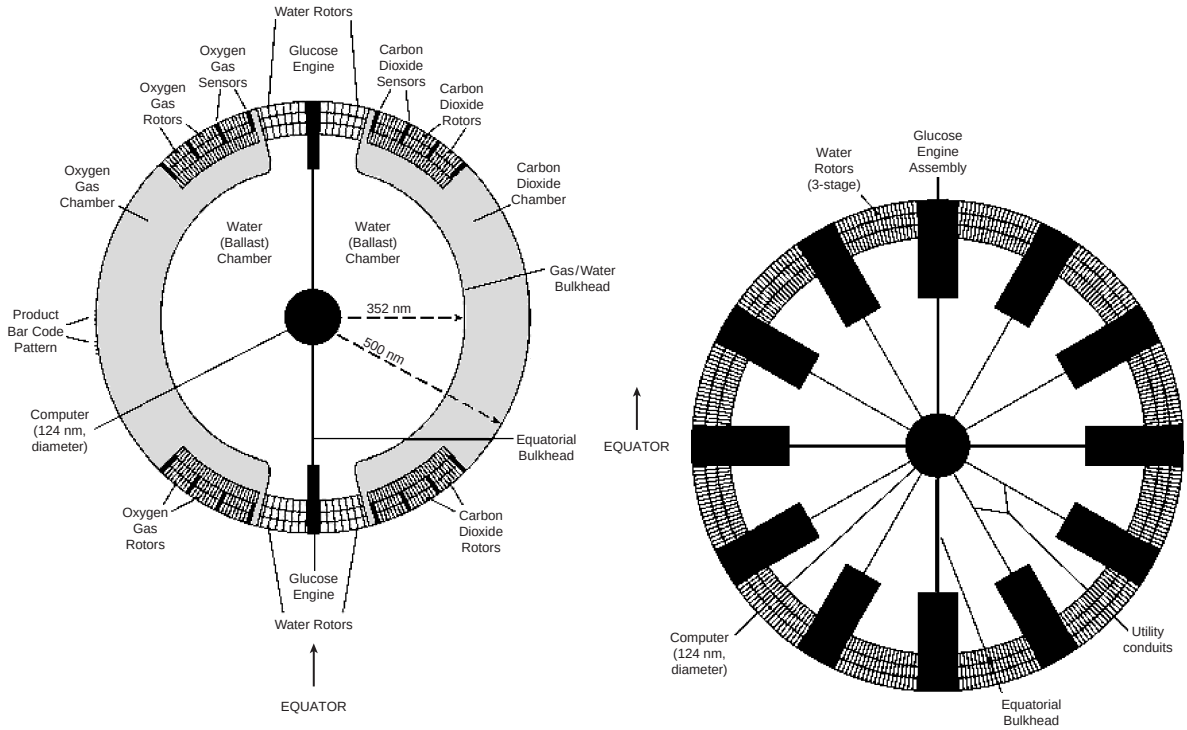


**Fig. 9.** Internal cutaway view of respirocyte—equatorial (left) and polar (right) view.[285] © 1996, Robert A. Freitas, Jr.

bloodstream using a digest and discharge protocol.[286] The microbivore is an oblate spheroidal nanomedical device (Fig. 10) measuring 3.4 microns in diameter along its major axis and 2.0 microns in diameter along its minor axis, consisting of 610 billion precisely arranged structural atoms in a gross geometric volume of 12.1 micron$^3$ and a dry mass of 12.2 picograms. The device may consume up to 200 pW of continuous power while completely digesting trapped microbes at a maximum throughput of 2 micron$^3$ of organic material per 30-second 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/sec digested per unit volume of phagocytic agent, and would have far larger maximum lifetime capacity for phagocytosis than natural white blood cells. 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 antibiotic-assisted biological phagocytic defenses, and able to extend the therapeutic competence of the physician to the entire range of potential bacterial threats, including locally dense infections.

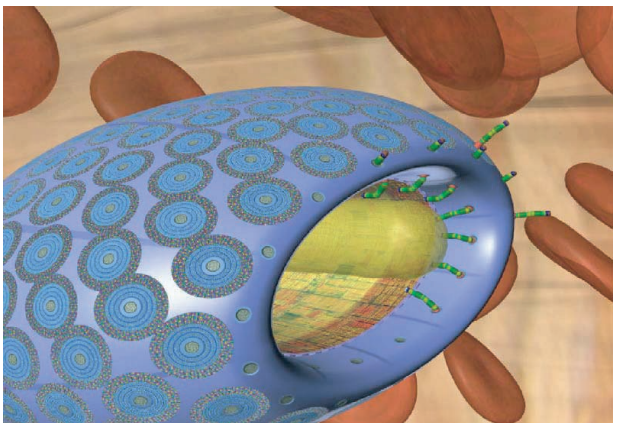

**Fig. 10.** An artificial white cell—the microbivore.[286] Designer Robert A. Freitas, Jr., illustrator Forrest Bishop. © 2001, Zyvex Corp.

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.[5] Telescoping robotic grapples emerge from silos in the device surface, establish secure anchorage to the microbe's plasma membrane, then transport the pathogen to the ingestion port at the front of the device where the pathogen cell is internalized into a 2 micron$^3$ morcellation chamber. After sufficient mechanical mincing, the morcellated remains of the cell are pistoned into a separate 2 micron$^3$ digestion chamber where a preprogrammed sequence of 40 engineered enzymes are 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-second digestion cycle. This "digest and discharge" protocol[5] 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,[289] whereas well-designed microbivores need only release biologically inactive effluent.

In the first half of the 21st century, nanomedicine should eliminate virtually all common diseases of the 20th century, and virtually all medical pain and suffering as well. It is a bright future that lies ahead for nanomedicine, but we shall all have to work very long and very hard to make it come to pass.

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