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Using machines in cells

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The rapid pace of miniaturization in the semiconductor industry has resulted in faster and more powerful computing and instrumentation, which have begun to revolutionize medical diagnosis and therapy. The lithographic techniques used to fashion electric circuits in microprocessors and memory chips can also produce mechanical structures, commonly known as micro- and nano-electromechanical systems (MEMS and NEMS).

MEMS devices currently range in size from one to hundreds of micrometers and can be as simple as the singly supported cantilever beams used in atomic force microscopy or as complicated as a video projector with thousands of electronically controllable microscopic mirrors. NEMS devices exist correspondingly in the nanometer realm. The concept of using externally controllable MEMS devices to measure and manipulate biological matter (BioMEMS) on the cellular and subcellular levels has attracted much attention recently. This is because initial work has shown the ability to detect single base pair mismatches of DNA [1] and to quantifiably detect antigens [2] using cantilever systems. In addition is the ability to controllably grab and manipulate individual cells and subsequently release them unharmed (http://www.sandia.gov/media/NewsRel/NR2001/gobbler.htm).

The attraction of using ever smaller systems to analyze and alter biological systems *in vivo* and *in vitro* is evident: smaller systems are less invasive, require smaller amounts of analytes (thus are more sensitive) and have smaller volumes over which they are effective, creating the ability to localize diagnosis and therapy, and confining them to targeted regions. Although current technology has the limited ability to detect femtomolar concentrations of analytes from microliter volumes of solution in the laboratory, the grand vision is that of implantable or even autonomous mobile systems operating *in vivo*, which are able to detect the naturally occurring minute quantities of biochemicals in their native environments and respond through signaling to external instruments, dispensation of drugs or direct physical manipulation of their surroundings.

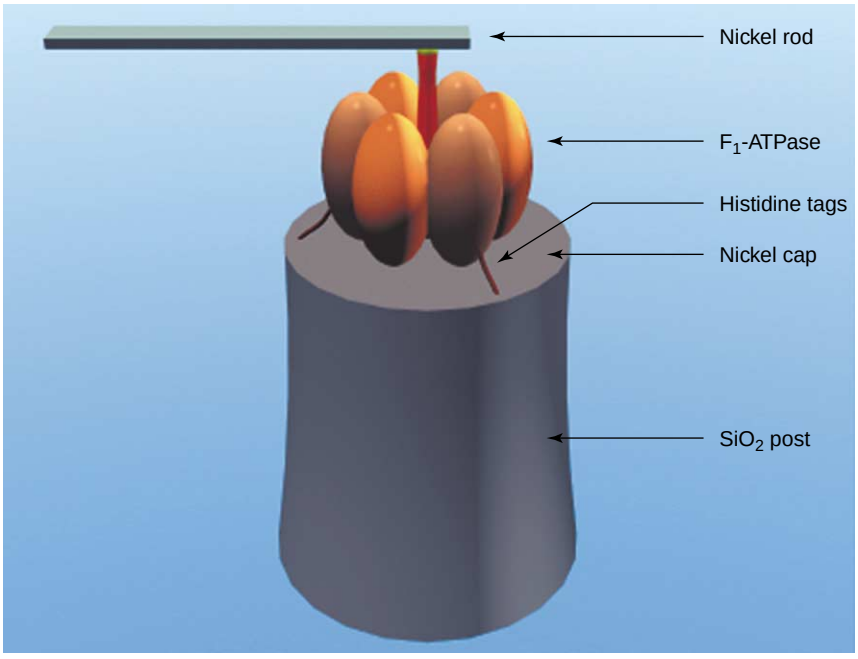

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Figure 1. A self-assembled hybrid device powered by F_1-ATPase. The genetically engineered motors are attached using histidine tags to Nickel-capped SiO_2 posts defined by electron beam lithography. Nickel rods ($75 \times 15 \times 750$ nm), also made using electron beam lithography, are attached to the rotor of the each motor. Upon the addition of ATP, rotary motion of the rods was observed using conventional bright field optical microscopy.

BioMEMS

Because BioMEMS involves the interface of MEMS with biological environments, the biological components are crucially important. To date, they have mainly been nucleic acids [1], antibodies [2] and receptors [3] that are involved in passive aspects of detection and measurement. These molecules retain their biological activity following chemical attachment to the surfaces of MEMS structures (most commonly, thiol groups to gold) and their interactions are monitored through mechanical (deflection of a cantilever), electrical (change in voltage or current in the sensor) or optical (surface plasmon resonance) measurements. The biological components are in the nanometer range or smaller; therefore, the size of these systems is limited

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by the minimum feature sizes achievable using the fabrication techniques of the inorganic structures, currently 100 nm to 1 μm. Commercially available products resulting from further miniaturization could be problematic because of the expanding cost and complexity of optical lithography equipment and the inherent slowness of electron beam techniques. In addition to size limitations, the effects of friction have plagued multiple moving parts in inorganic MEMS, limiting device speeds and useful lifetimes. Finally, MEMS are currently powered exclusively by electricity; an electrical power supply complicates any autonomous mobile system.

Mechanical proteins and hybrid organic–inorganic devices

These constraints, and also sheer scientific curiosity, mean that recent discoveries of mechanical biological proteins have led to first generation proof-of-concept biological–inorganic hybrid devices. These devices are <50 nm in length, are powered by optical or chemical energy and are capable of operation *in vivo*. Our group's initial efforts in the construction of hybrid devices have used the molecular motor F_1-ATPase. F_1-ATPase is a component of F_0F_1-ATPase, an energy transducing protein found in bacteria, plants and animals that converts the electro-osmotic energy of a proton gradient into chemical energy stored in ATP. The F_0 and F_1 portions are mechanically coupled through the rotation of a common rotor: F_1 is a multiunit enzymatic protein of 12 nm diameter, with a 3 nm rotor that rotates as the protein consumes ATP.

Figure 1 depicts a hybrid device made by our group using the rotary motor F_1-ATPase attached to a patterned inorganic substrate that is capable of rotating a micromachined nickel rod attached to its rotor [4]. The motors were genetically engineered to express poly(histidine) on one side, which was used to attach the motors only to the nickel spots on the surface. Other genetic engineering facilitated the attachment of biotin to the rotor, which was linked to biotin-poly(histidine) on the nickel rods through a streptavidin linkage. The nickel rods were made using electron beam lithography and standard materials deposition and etching techniques, and were 75 × 15 × 750 nm in size. The length was necessary to facilitate observation of the rotation using conventional optical microscopy and, even with their large size, the rods were observed to rotate at ~8 rotations per second. Work by Yoshida and co-workers. has suggested a high efficiency for F_1-ATPase (> 80%) and have directly shown that the motors can rotate at >130 rotations per second when minimally loaded [5,6].

Other systems

Other biological molecular motor systems have also been engineered. For example, kinesin, an ATP-powered linear stepping motor, which unidirectionally 'walks' hand-over-hand on tracks of 25 nm wide microtubules, has natural functions that include intracellular cargo transport, ciliary movement and cellular architectural modification. Deposition of microtubules commonly uses an electrostatic interaction between the negatively charged tubulin and substrate functionalized with positively charged materials, such as diethylenetriaminopropyltrimethoxysilane [7]. Kinesin is often adsorbed non-specifically through a hydrophobic interaction onto various surfaces after a simple incubation. Both microtubules and kinesin have been deposited on patterned substrates, and directed transport of microtubules or other cargo by the kinesin has been achieved in a limited manner [8,9].

Possible devices using motor proteins are not limited by the choice of molecules;

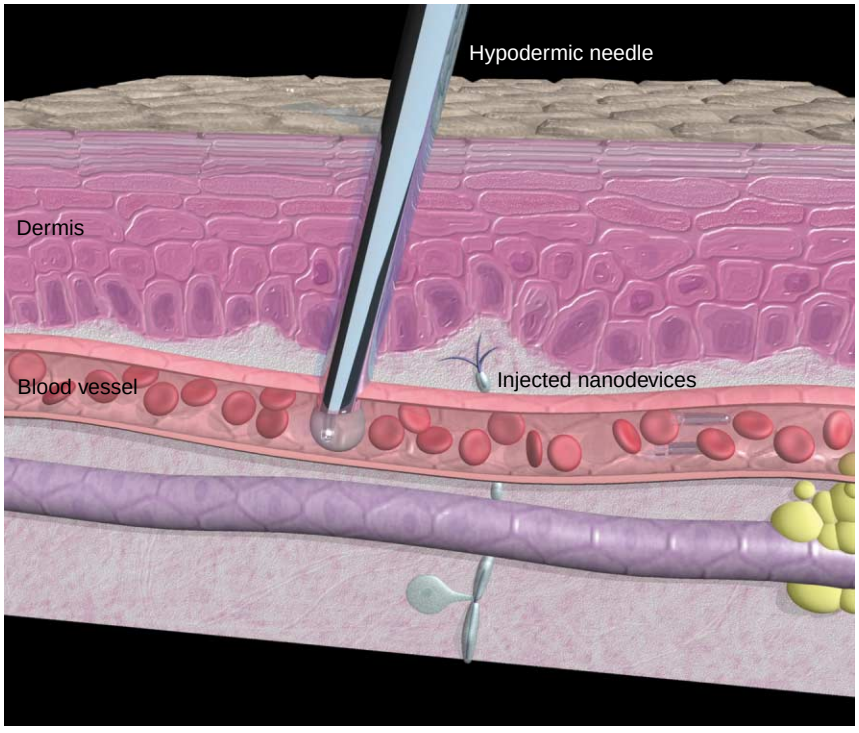

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Figure 2. Mechanical drugs. Robotic capsules are released in the bloodstream. Upon the capsules' contact with their targets, their contents are discharged into the surrounding medium or into the targets themselves.

the palette of motors and mechanical proteins is huge. Rotary and linear motors, valves and screws can be found, and even the ubiquitous ligand–receptor binding can induce an electronically detectable hinging motion [3]. To summarize, these mechanical molecules are tens of nanometers in size, can operate at high speeds (some at >50 μm sec^{-1}) and are motile by their very nature, using chemical fuel (ATP) and interacting primarily with their intended substrates. In addition, there are hundreds of different mechanical proteins in each organism, thousands among the various species of plants and animals and they are even found in the simplest prokaryote. Each motor protein in each species of organism has been adapted to certain specific tasks over millions of years of evolution. In device design, specific mechanical proteins might be optimal for certain externally directed artificial tasks, using the speed, power or processivity of a particular motor. An incredible economic advantage in device production is realized in the synthesis of these proteins. Their genes can be inserted into bacterial expression systems using standard genetic engineering techniques, such as cassette- and site-directed mutagenesis, and made in large quantities in relatively short periods of time: a typical production run in our laboratory produces 10^{16} motors in three days from start to finish, over 10^{14} motors produced per hour. These are systems that are ripe for adaptation in hybrid devices: organic and inorganic components combined in a system capable of performing tasks that are impossible by either alone.

In vivo operation

Ultimately, it will be possible for micrometer sized or smaller devices to be injected into the human body with specific intended targets (e.g. cancer cells). Current strategies to enhance drug delivery, such as conjugation with polyethylene glycol, can also help hybrid devices decrease immune response or biofouling [10]. Because of the small sizes of mechanical proteins, devices that can operate inside cells are possible. These devices can drift along with the bloodstream or provide their own locomotive power, through the attachment of a propeller instead of a rod on the device in Fig. 1, or using a more biological propulsion system, such as the bacterial flagellar motor. As these devices reach their targets, a signal is released (such as that resulting from antibody–antigen [2] or ligand–receptor [3] binding), which then activates the devices to release their contents to the environment surrounding the target, or to inject their contents into the target itself (Fig. 2). The contents of the devices can be chemicals, drugs or still smaller devices. These devices, once released, interact with their new environments, inhibiting deleterious activities or restoring lost functions. Does this sound like fantasy? It is perhaps as close as a decade away. Many of the parts of this theoretical device now exist. As feature sizes of transistors fall below 100 nm, the number of transistors and other semiconductor circuitry that can be packed inside a microscopic robot is increasing to the point of feasibility of an onboard computer. In addition, as circuit sizes shrink, their power requirements fall, permitting smaller and lighter electrical power sources. Alternatively, these services could be entirely biological in nature – the motility provided by motor proteins and computational power resulting as an emergent property [11] of many individual units acting cooperatively. Injection of drugs or devices into cells by these devices can be performed using syringes and needles of nanometer sharpness, easily made with semiconductor fabrication techniques.

Future work and outlook

Before such fantastic devices can be constructed, control over the synthesis, manufacture and operation of individual elements of these systems must be enhanced and refined. In our previous work, the number of functioning hybrid devices produced was limited by the quantities of the inorganic components. Future work is focusing on the fabrication of these pieces in amounts commensurate with their organic counterparts. This will enable the production of micromole amounts and of more hybrid nanomachines. Other efforts are directed towards the control of the nanomachines. Biological motors that rely solely on chemical fuel, such as ATP, for power also have an additional shortcoming – control of catalytic activity. In their most primitive form, these motors will hydrolyze ATP until the supply is exhausted. Of course, Nature is not so wasteful that she would let these motors consume ATP unnecessarily. Many proteins have natural built-in regulatory mechanisms controlling their activity. Examples of these in motor proteins include the δ and ε subunits in F_1F_0-ATPase [12], as well as the phosphorylation of the tail domain in myosin-V [13]. In some applications, these natural controls will not be applicable or available. Our group has recently completed work in which an artificial control has been introduced into F_1-ATPase. Metal-ion binding sites genetically engineered into the protein control its motion by forcing different moving parts to bind to ions in solution, thus immobilizing it. The introduction of a chelator, such as 1,10-phenanthroline, removes the bound ions from the motors, restoring motility. This can be repeated many times, demonstrating repeatable control over these hybrid machines at the level of a single molecule (H. Liu *et al.*, unpublished data).

Bionanotechnology is a nascent field, the locus of physics, chemistry, biology and materials science at the nanoscale. Until now, most of the engineering at these length scales has been non-biological inorganic micro- and nanomachined pieces, often studied in high vacuum or low temperatures. Using the machinery of life, Nature has created highly efficient manufacturing systems that are capable

of producing exquisite machines of nanometer size that can operate at room temperature in aqueous environments. Our current understanding of these processes and the products made by them is not yet at the point where we are able to fully design or customize any but the simplest proteins; however, our knowledge is rapidly improving and integration of these biological molecules in crucial functional roles in NEMS devices has already begun. Advances in technology are quickening the pace of discoveries emerging from academic and industrial laboratories, which in turn lead to more technical innovation. This feedback loop will result in future devices and techniques that are presently unimaginable: autonomously operating mobile robots *in vivo* that sense and respond to their environments and dispense drugs that are mechanical in nature.

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The design of combinatorial libraries ▼

Combinatorial chemistry has an enormous impact on classical organic chemistry. Solution-phase syntheses are supported by polymeric reagents and scavengers. Automation of parallel syntheses and new purification methods, such as solid phase extraction (SPE) and automated column chromatography, are widely accepted by organic chemists. But is there a corresponding impact on success in drug discovery? When combinatorial chemistry started, huge libraries of mixtures of (impure) compounds dominated synthetic strategies. Screening results of such libraries were frustrating – hits could not be confirmed or biological activities 'disappeared' after deconvolution. Roger Lahana hit the nail on the head by formulating: 'When trying to find a needle in a haystack, the best strategy might not be to increase the size of the haystack' [1].

Therefore, better methods had to be developed to discover the needle. As long as we depend on HTS with all its inherent problems, rational design of targeted or focused libraries is the better alternative to the synthesis of huge, random libraries [2–4]. Lipinski's rule-of-five for the identification of analogs, which most probably lack sufficient oral bioavailability, was a first step in this direction. A further step forward resulted from the training of neural nets with drugs and chemicals, performed in parallel at BASF (Ludwigshafen, Germany) and Vertex (Cambridge, MA, USA), and later also at several other places [3,4]. The trained nets differentiate libraries with a higher percentage of biologically active compounds from those that include 'mere' chemicals. Genetic algorithms aid in the efficient selection of drug-like, chemically diverse libraries that can be produced from cheap building blocks [3,4].

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