Autonomous Systems and Synthetic Biology

HENRY HESS
Columbia University
New York, New York

Autonomous systems have proven to solve various engineering challenges; from the drastic changes they have brought to our manufacturing processes since the 1950's to, later, the way space and ocean are explored. More recently, autonomous systems have been conceived at the micro and nano-scale scale for materials and biomedical applications. These microscopic devices and their applications draw inspiration from biology, where autonomous systems operate individually and collectively. A prototypical autonomous device in biology is a Vibrio cholerae bacterium. It packs the ability to move, sense, target, adapt, and release active substances into a few cubic micrometers. A biological example of a smart material composed of autonomous systems is muscle. Muscle is hierarchically assembled from microscopic subunits, has the ability to exchange information with the environment via electrical and mechanical stimuli, and incorporates energy conversion modules and self-healing ability. Both, V. cholerae bacteria and muscle cells, are significant achievements in engineering by evolution: They operate with low power consumption, limited computing resources, and in diverse environments.

Such examples of microscopic systems with complex functionalities inspire the emerging field of synthetic biology, which covers diverse approaches. A prominent research strategy – inspired by the success of large scale integration of electronic circuits - is to focus on the design of standardized gene circuits which can serve as modules of complex programs to be executed by bacterial cells. This approach is akin to the delivery of a well-organized set of blueprints to a contract manufacturer, who manufactures equipment according to the delivered specifications and utilizes the experience of its technicians to operate the equipment and produce a chemical of interest. A second strategy, which is the topic of this talk, aims to develop the technical expertise required to rationally design complex, interacting microscopic systems (Schwille and Diez 2009). This approach builds on nanotechnology as well as the increasingly complex in vitro experiments in cell biology, where critical cellular functions are replicated to test our understanding of essential and auxiliary mechanisms and components.

The challenges in designing such biomimetic systems using nanoscale building blocks include their controlled operation in the presence of Brownian motion and other sources of noise, the proper integration of molecular information, the consideration of lifetime and reliability issues, and the anticipation and utilization of emergent phenomena.

INTRODUCTION TO KINESIN-POWERED MOLECULAR SHUTTLES

Kinesin motors are proteins that generate mechanical work using ATP molecules as fuel. (Howard 2001) For each ATP molecule it hydrolyzes, the kinesin motor takes a step of 8 nm along a microtubule, a tubular structure assembled from thousands of tubulin proteins which serves as track for the kinesin. The kinesin motor can advance against a force of about 5 pN, converting more than 50% of the free energy of ATP hydrolysis into mechanical work in the process. Within cells, kinesin is primarily responsible for the transport of molecular cargo from the center of the cell to the periphery. However, biophysicists have developed the ability to observe and manipulate kinesin motors and the associated microtubule filaments outside the cell, in so-called *in vitro* gliding assays. In these assays (Fig. 1), kinesins are adhered to a surface, and fluorescently labeled microtubules are propelled by the kinesin motors in the presence of ATP.

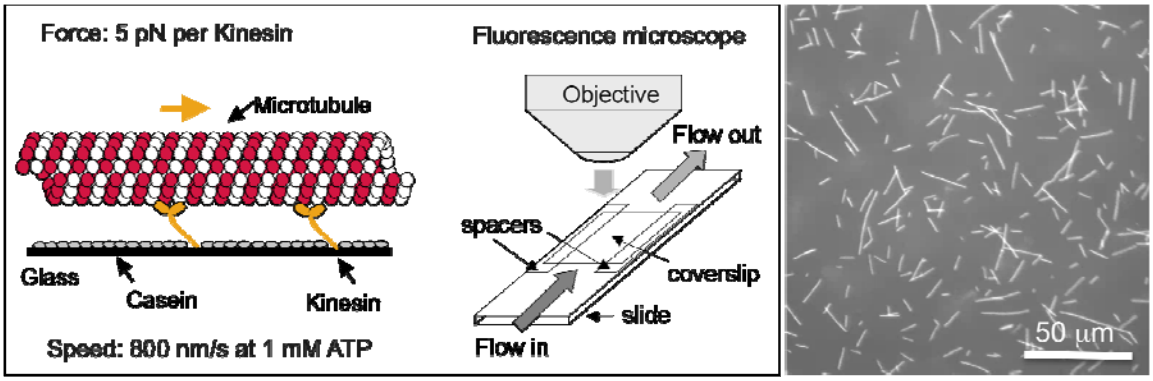

FIGURE 1 Surface-adhered kinesin motors can propel fluorescently labeled microtubules (diameter 25 nm) across a surface. A coating of casein proteins on the surface prevents undesired attachment of the kinesin motor domains to the surface. The experiment is conducted in a cell composed of a coverslip, spacers and a slide and the microtubule motion is observed with a fluorescence microscope. Microtubules appear as fluorescent rods, while the kinesin motors on the surface are invisible.

Aside from enabling the study of motor proteins, these microtubules propelled by kinesin motors can serve as a nanoscale transport system. By controlling the direction of microtubules, the attachment and detachment of cargo to microtubules, and the supply of ATP fuel, microtubules can be induced to act as nanoscale delivery trucks or “molecular shuttles” (Hess and Vogel 2001)

These molecular shuttles – assembled from biological components with unmatched functionality - can be employed to explore design concepts for nanoscale systems and devices.(Hess et al., 2002a; Hess et al., 2002b)

While molecular shuttles can be individually controlled (van den Heuvel et al., 2006), the inherent advantages of molecular devices are better exploited if costly efforts to achieve individual control are abandoned, and instead the autonomous operation of molecular shuttles within an externally directed “swarm” is accepted. A suitable analogy is an anthill: While it is in principle possible to induce an individual ant to perform a specific task, the anthill as a complex system relies on the emergence of useful actions from the autonomous decisions of individual ants. A striking demonstration of emergence

in swarms of molecular shuttles is the assembly of “nanospools” from “sticky” microtubules (Figure 2). (Hess et al., 2005)

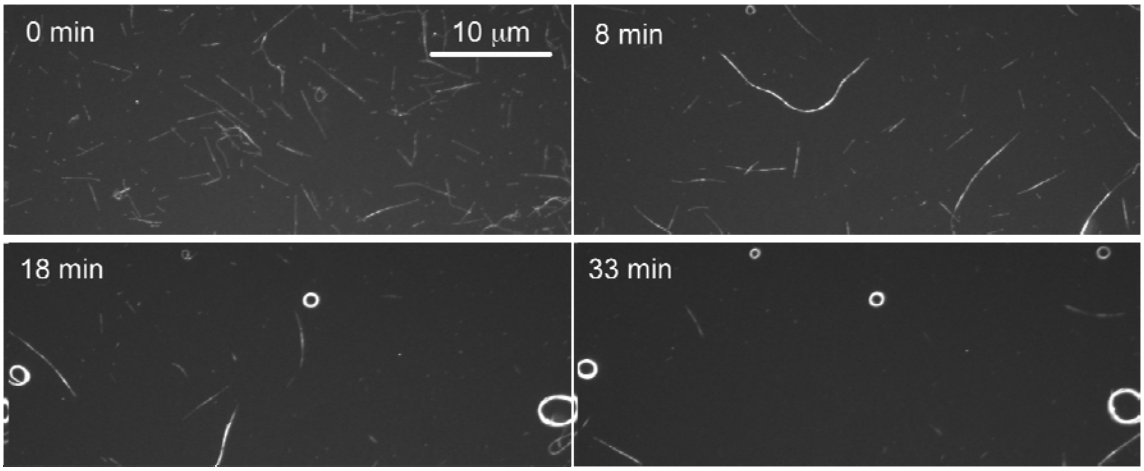

FIGURE 2 Biotin-functionalized microtubules rendered “sticky” by a partial coating of streptavidin self-assemble into wires and ultimately spools.

“SMART DUST” BIOSENSORS AS APPLICATIONS OF MOLECULAR SHUTTLES

Microorganisms excel in biosensing, having the ability to detect a variety of analytes with high specificity and sensitivity, process the incoming information, and communicate their measurements. From the perspective of an engineer, microorganisms act as microscopic sensor packages, which are immersed into the sample (their environment) and collectively respond to analytes.

“Smart Dust” transfers this concept to the engineering domain by aiming to create highly integrated microscopic sensors in large numbers for remote detection scenarios (Kahn et al., 2000; Sailor and Link 2005). A collaborative effort by five research teams with support from the DARPA Biomolecular Motors program pursued the creation of “smart dust” biosensors which rely on molecular shuttles as the core component (Bachand, Hess et al. 2009). In the sensor, antibody-functionalized molecular shuttles capture analytes, tag them with fluorescent particles, and transport them to a deposition zone for detection (Figure 3).

This design is “biomimetic” in several aspects. As outlined above, smart dust itself is inspired by organisms. The components of the molecular shuttles – kinesin motors, microtubules, and antibodies – are biological in origin. Also, the principle of operation, relying on a swarm of unsophisticated, autonomous devices to create a detectable signal is bio-inspired.

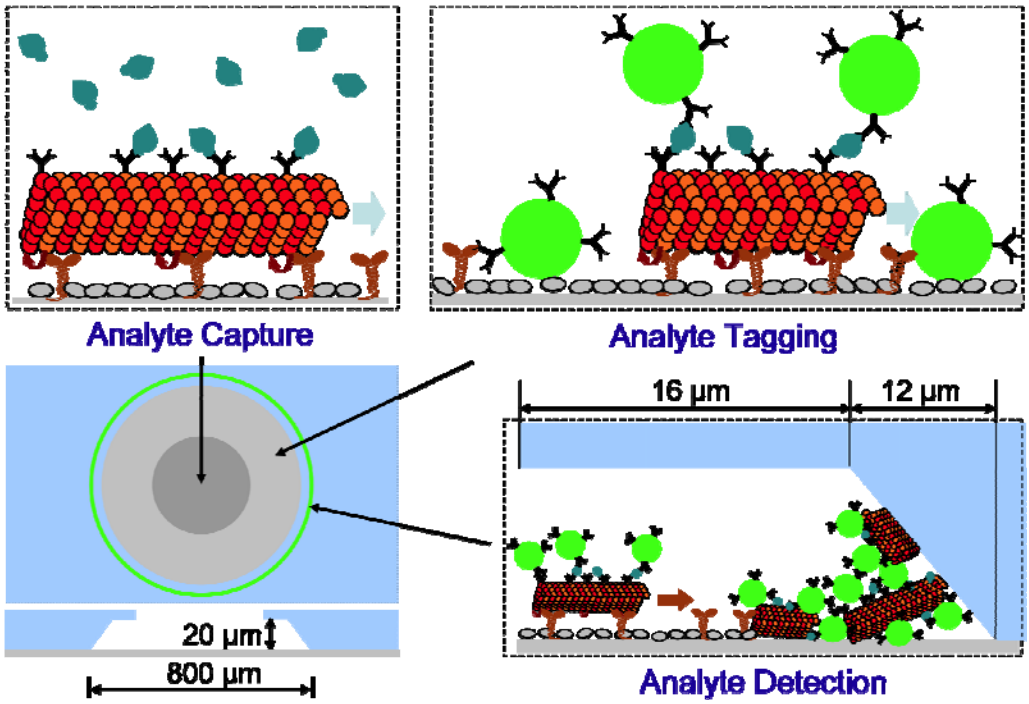

Figure 3: A “smart dust” biosensor based on molecular shuttles. Antibody-functionalized microtubules capture analyte molecules, such as protein biomarkers, in the center of a circular well, and transport them across the surface. Collisions with antibody-coated fluorescent particles leads to particle capture if analyte is present. Eventually, shuttles reach the periphery of the well, where they accumulate and their cargo of analytes and fluorescent particles is detected optically.

TRANSITIONING TO SYNTHETIC MATERIALS

The utilization of biological components to enable advanced “hybrid” nanodevices is an excellent approach to explore concepts and demonstrate the proof-of-principle, but hybrid devices carry with themselves the Achilles heel of a limited lifetime of the biological components. Lifetime limitations and storage requirements are often seen as the primary obstacles to a deployment of hybrid devices. This motivates efforts to recreate biological functionality, such as the ability to harvest energy from the environment, with synthetic components only.

A successful approach has been the mimicking of bacterial motility by platinum-gold nanorods in a hydrogen peroxide solution (Hong et al., 2007). The nanorods catalyze the decomposition of the hydrogen peroxide, which in turn propels them forward. Surprisingly, these nanorods have a distinctly “chemotactic” response, moving towards higher hydrogen peroxide concentrations. The analysis of the process has informed our understanding of the mechanism supporting chemotaxis in bacteria.

On the basis of the same combination of a compartmentless flow cell and electroosmotic pumping, we were able to engineer a fully synthetic membrane which mimics the ability of cellular membranes to actively transport solutes using chemical energy harvested from the solution (Jun and Hess 2010). A platinum and a gold electrode on the surface of a polycarbonate membrane were electrically connected; when placed in a hydrogen peroxide solution, fluid was pumped across the membrane at a velocity of about 1 nL cm^{-2} s^{-1} .

In a sense, these efforts to create biomimetic functional materials and bio-inspired nanodevices follow the arc of human flight: First the study of biological systems, followed by the creation of hybrid systems to elucidate the key principles, and ultimately the development of synthetic flying machines.

CONCLUSIONS

The engineering of autonomous systems such as molecular shuttles is an exciting theme in nanotechnology, where progress has relied heavily on the utilization of biological components. The assembly of the biological building blocks into newly designed structures is a very stringent test of our assumptions about these biological nanomachines and their interactions, or as Richard Feynman said: “What I cannot create, I do not understand”. The design process forces us to see biology through the eyes of an engineer, and in the process to ask questions which are related to the advanced topics - including friction, wear and fatigure - in the back of the engineering textbooks.

Bachand, G. D., H. Hess, B. Ratna, P. Satir, V. Vogel. 2009. "Smart dust" biosensors powered by biomolecular motors. Lab on a Chip 9(12): 1661-1666.

Hess, H., J. Clemmens, C. Brunner, R. Doot, S. Luna, K.H. Ernst, V. Vogel. 2005. Molecular self-assembly of "Nanowires" and "Nanospools" using active transport. Nano Letters 5(4): 629-633.

Hess, H., J. Clemmens, J. Howard, V. Vogel. 2002a. Surface Imaging by Self-Propelled Nanoscale Probes. Nano Letters 2(2): 113-116.

Hess, H., J. Howard, V. Vogel. 2002b. A Piconewton Forcemeter assembled from Microtubules and Kinesins. Nano Letters 2(10): 1113-1115.

Hess, H. and V. Vogel. 2001. Molecular shuttles based on motor proteins: Active transport in synthetic environments. Reviews in Molecular Biotechnology 82(1): 67-85.

Hong, Y., N. M. K. Blackman, N. D. Kopp, A. Sen, D. Velegol. 2007. Chemotaxis of nonbiological colloidal rods. Physical Review Letters 99(17): 178103.

Howard, J. 2001. Mechanics of Motor Proteins and the Cytoskeleton. Sunderland, MA, Sinauer.

Jun, I. K. and H. Hess. 2010. A Biomimetic, Self-pumping Membrane. Advanced Materials, in press.

Kahn, J. M., R. H. Katz, K. S. J. Pister. 2000. Emerging challenges: Mobile networking for "Smart Dust". Journal of Communications and Networks 2(3): 188-196.

Sailor, M. J. and J. R. Link. 2005. "Smart dust": nanostructured devices in a grain of sand. Chemical Communications(11): 1375-1383.

Schwille, P. and S. Diez. 2009. Synthetic biology of minimal systems. Critical Reviews in Biochemistry and Molecular Biology 44(4): 223-242.

van den Heuvel, M. G. L., M. P. De Graaff, C. Dekker. 2006. Molecular sorting by electrical steering of microtubules in kinesin-coated channels. Science 312(5775): 910-914.