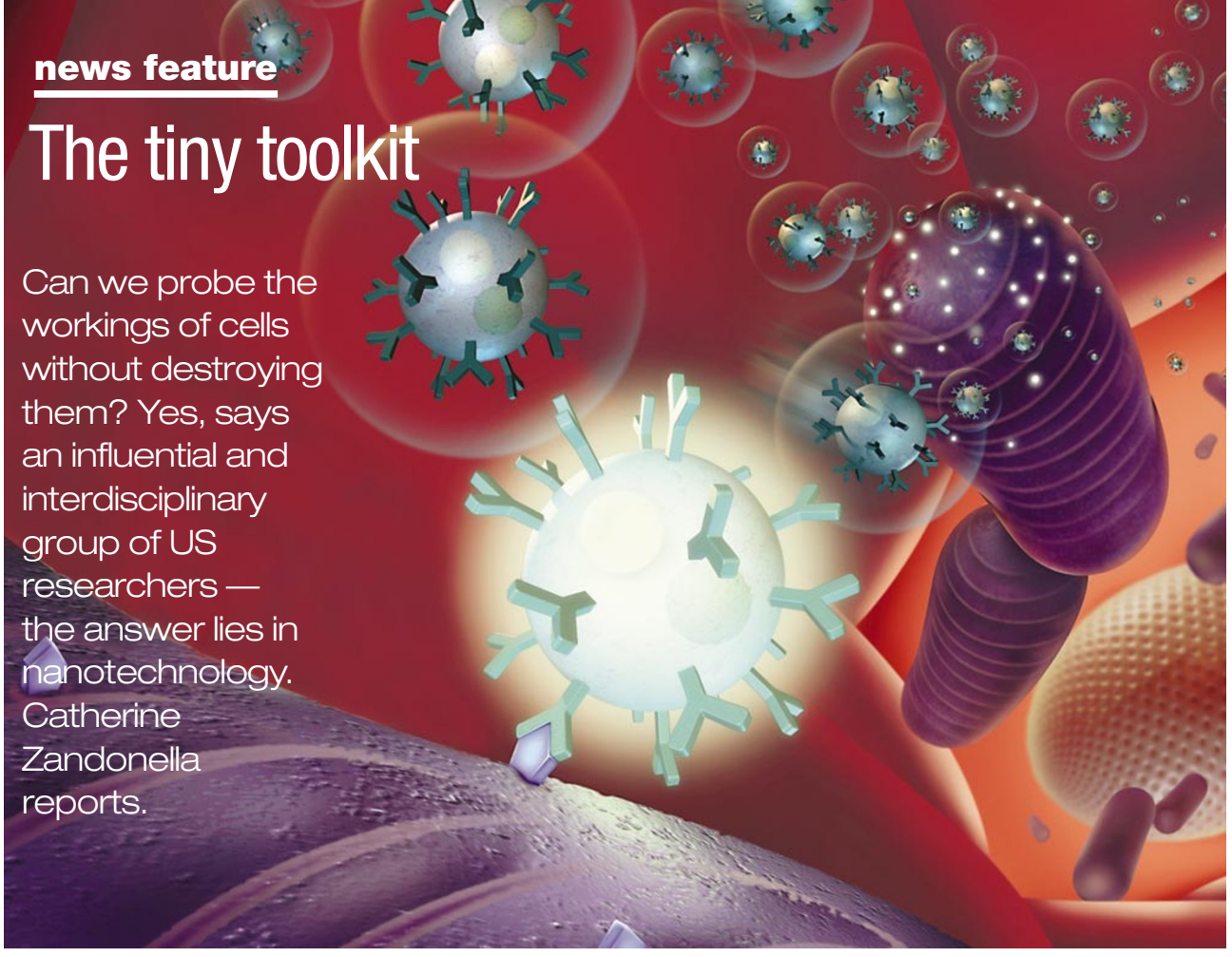
The tiny toolkit



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Can we probe the workings of cells without destroying them? Yes, says an influential and interdisciplinary group of US researchers — the answer lies in nanotechnology. Catherine Zandonella reports.

As the basic unit of life, the cell gets little respect. When we want to know what is happening inside it, we rip open its membrane and dump out its contents. And along with the cellular fluid and organelles, out of the cell leaks valuable information that is lost forever.

If we could go a little easier on cells, that information might become available to researchers. And according to the NanoSystems Biology Alliance, a group of seven scientists on the US west coast, nanotechnology could provide the means to do so. They are working on tools that, although still in their infancy, could one day be capable of tracking life within the cell in real time.

The group's planned devices certainly sound impressive: arrays of nanowires that can detect thousands of proteins secreted by a cell are just one example. And beyond the alliance, other researchers are creating equally exciting inventions, including a nano-sized fibre-optic probe that can seek out target molecules, and gold particles that can be used to turn specific proteins on and off. Many of these projects may fail before they produce practical devices, but those that succeed will give biologists the chance to experiment on cells in a new way — as a system, rather than as a collection of organelles and individual processes.

To view a cell in this way — as a thriving beehive of interconnected activities such as cell signalling and the shuttling of nutrients and metabolites — researchers need to think about their experiments in a new way, says Jim Heath, a nanotechnology researcher at the California Institute of Technology, Pasadena, and a member of the nanosystems alliance. He likens the process to a child playing a video game — instead of reading the instructions, they are likely just to pick up the joystick and go for it. When treating the cell as a system, experimenters are forced to do the same. "We can't approach this by reading the instruction manual starting at page one," says Heath. "We need to jump in and play the game."

Cell block

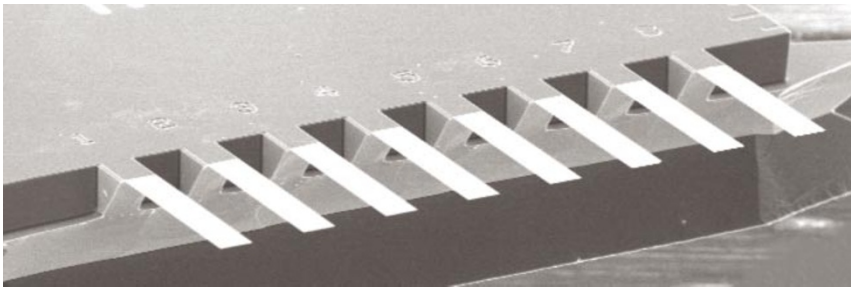
To begin with, researchers need the right controller. This could soon be available in the form of the nanolab, a device being developed by Heath and his colleagues that will combine several assays on a centimetre-square silicon chip. The chip resembles a miniature cell farm, with rows of cells, each nestling in its own well atop a tiny pore in the silicon. Fused with the cell membrane, the pore serves as a conduit between the inside of the cell and the outside world. Close to this conduit is a densely packed array of nanowires, metal rods just a few nanometres thick. Each nanowire is coated with a bimolecular probe, such as an antibody, that binds to a target protein. Proteins that diffuse through the membrane and bind to an antibody change the nanowire's electrical conductance, and this can be measured by a detector connected to the array.

Light the way: molecular-sized probes that light up when they bind to a target ion are one of several promising new tools for cellular studies.

Several groups have already reported using carbon nanotubes and nanowires to detect specific DNA sequences and proteins. What is new about Heath's approach is that 1,000 nanowire detectors can be jammed into a few square micrometres — roughly the area taken up by a single cell. To create these arrays of nanowires, Heath and his colleagues developed a new technique, called superlattice nanowire pattern transfer, which can create individual semiconductor nanowires that are as little as 8 nm in diameter with the same distance between each wire[1]. Previous methods could only produce 20-nm nanowires separated by gaps of 40 nm.

Potentially, each of Heath's nanowires could bear a different antibody or oligonucleotide, a short stretch of DNA that can be used to recognize specific RNA sequences. "On one chip, we will have 1,000 single-cell experiments," says Heath. So far, he has built chip prototypes and devised methods for coating each wire with a different antibody. Over the next few months he plans to refine these techniques, and to begin to test the chip on the proteins secreted by cancer cells.

Some barriers remain to be overcome. The



A team at the California Institute of Technology is using tiny cantilevers to probe molecular bonds.

cells need to be surrounded by an artificial fluid that mimics their normal environment. But ions in this fluid can cover the sensor and obscure biochemical events that occur more than a few nanometres away. To get around this, researchers place cells in a fluid with a low density of ions, but this stresses the cells and makes the data less reliable. Advances in the surface characteristics of nanowires may, however, give rise to more sensitive biosensors, which could be used in more natural fluids. "We haven't reached the fundamental limits of these detectors," says Scott Manalis, a nanotechnology researcher at the Massachusetts Institute of Technology (MIT) in Cambridge. "There is definitely room for improvement."

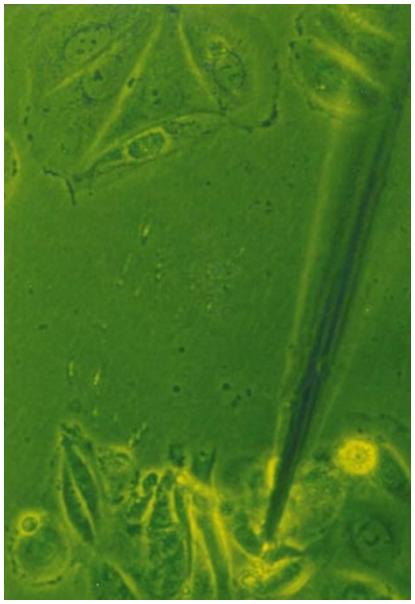
For the moment, the nanolab can measure only those proteins that are secreted by the cell. This problem can be solved — albeit in a way that destroys the cell. Stephen Quake, an alliance member and physicist at the California Institute of Technology, has added microfluidic channels — tiny liquid-filled canals — to the slab, which can be used to shunt cells to different areas. In one part of the chip, chemicals that disrupt the cell membrane will be used to release the contents onto another array of nanowires, coated with DNA probes, to provide a snapshot of gene expression at that moment.

Another physicist in the alliance, Michael Roukes, also of the California Institute of Technology, is working with Heath and Quake on a section of the nanolab chip that will measure the binding forces between individual molecules. These forces can already be assessed using atomic-force microscopes (AFM), which generate atomic-scale images of surfaces by measuring the way in which a surface attracts and repels the end of a tiny metal cantilever. The force between, say, a drug and a receptor molecule can be measured by attaching the drug to a glass slide and the receptor to the microscope's cantilever.

Roukes plans to implement something similar on the nanolab. Target receptors will be tethered to the chip, and the drug anchored on a cantilever. Roukes aims to determine the strength of the bond by measuring the change in springiness of the cantilever when the bond between the two molecules is severed. The change will be measured by a piezoelectric crystal — a device that converts mechanical pressure into electrical signals. Other groups have used cantilevers to detect biomolecules, but Roukes' devices are far smaller, and hence can generate a more detailed view of the forces between the two molecules.

To ensure that physicists such as Roukes and Heath produce genuinely useful tools, the alliance also includes three biologists. Charles Sawyers, a cancer researcher at the University of California, Los Angeles, already has plans for the nanolab. He wants to find out how chronic myeloid leukaemia cells become resistant to Gleevec, a relatively new drug that blocks an enzyme involved in the proliferation of cancerous cells. Sawyers suggests that the nanolab could be used to work out which genes are switched on when leukaemia cells are treated with the drug. "You could ask what is the first thing the cell does when it sees Gleevec," Sawyer says. He hopes that this approach could reveal how the cells develop resistance.

Another alliance member is Alan Aderem, an immunologist and cell biologist at the Institute for Systems Biology in Seattle, Washington. He is interested in immune cells, such as macrophages, that display proteins called toll-like receptors on their surfaces — these recognize various pathogens such as bacteria and viruses. The nanolab would allow Aderem to trap a single macrophage and study which of its genes are being transcribed in response to the pathogens that it encountered. "If we could understand the information embedded in macrophages, we could use them as sentinels to see what is going on in the body," he says.



Good point: an optical nanofibre can probe a cell's molecular contents without killing it.

Researchers outside the alliance are also working on new nanotechnologies. To take a closer look at a proteins, for example, it would be nice to enter the cell and poke around with a flashlight. Tuan Vo-Dinh, a chemist at Oak Ridge National Laboratory in Tennessee, is trying to do just that[2]. His device is a fibre-optic probe, about 40 nm across at the tip, capped with an antibody that binds to a target molecule. Vo-Dinh makes the nanofibre by drawing out the tip of a normal optical fibre to a very fine point. The fibre is then coated with silver to stop light escaping from the sides.

Top tip

When Vo-Dinh turns on the device, light travels down the fibre-optic probe. Because the tip of the probe is much smaller than the wavelength of light, photons cannot go all the way to the tip. Instead, the photons go as far as they can and then create evanescent fields — waves of light that decay rapidly and hence only excite molecules at the tip of the probe. To the antibody at the tip, Vo-Dinh attaches a fluorescently labelled target molecule, and then sticks the probe into the cell. There, the cell's copy of the molecule displaces the fluorescent version, causing the fluorescent signal to diminish and allowing Vo-Dinh to 'view' individual molecules.

This method has an advantage over simple fluorescent dyes, which can be used to label and track molecules, but eventually kill cells. "The nice thing is that when you pull out the fibre, the cells still survive," says Vo-Dinh. He is now using the nanofibre to investigate apoptosis, or programmed cell death.

Nanotechnology also offers researchers the chance to detect rare events or molecules that are present only at low concentrations. Quantum dots — nano-sized crystals of semiconductor metals — emit intense light at specific frequencies and can, for example, be used to label a range of biomolecules[3]. Other similar technologies are under development. A team at the University of Michigan in Ann Arbor has developed PEBBLEs, or Probes Encapsulated By Biologically Localized Embedding, which can be used to track the movement of zinc around cells and could aid studies of neurological diseases.

PEBBLEs consist of fluorescent dyes trapped inside larger cage molecules, 20–200 nm in diameter. The dyes glow when they bond with zinc and, through a microscope, the PEBBLEs can be seen winking on and off like fireflies, allowing researchers to track how and where zinc is stored and released in the cell. Abnormal zinc regulation is a feature of Alzheimer's disease, but current tools are

T. VO-DINH ET AL. NATURE BIOTECHNOL. 18, 764–767 (2000)

unable to reveal how zinc is stored and released, as these events are rare and hard to detect, says toxicologist Martin Philbert, who helped to develop the probes together with chemists Raoul Kopelman and Anne-Marie Sastry and their colleagues.

The potential of these and other nanoparticle tools is huge. James Baker, who studies such systems at the University of Michigan, points out that nanoparticles can be equipped with homing devices such as antibodies, reporting units such as tags that fluoresce when exposed to light, and even molecular systems for delivering drugs to a cell. Baker has, for example, used sphere-shaped polymers known as dendrimers to ferry a targeting molecule, fluorescent dye and methotrexate — a drug that attacks certain types of cancer cell — into a cell. In laboratory studies using tumour cells, the methotrexate killed 100 times more cancer cells when it was delivered by nanoparticles rather than simply being added to the cell culture[4].

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Anne-Marie Sastry and her colleagues aim to use tiny fluorescent probes to shed light on brain disease.

Gold prospect

Taking Heath's video-game analogy to its logical conclusion, nanotechnology could one day allow researchers to control the cell rather than just navigate around it. MIT bioengineers Kimberly Hamad-Schifferli and Joseph Jacobson can, for example, control the activity of proteins and DNA using gold nanoparticles. First, they embed the particles in either a protein or a DNA strand. Then they apply a radio-frequency magnetic field to a sample containing the nanoparticles and biomolecules, which causes the gold particles to heat up and disrupt the proteins' activity. Removing the field restores the proteins' function.

Last year, the pair used the technique to prise open a strand of DNA that had been twisted into a hairpin shape[5]. The system has not yet been used in cells, but the experiment was an impressive proof of principle. Other groups have used light-sensitive molecules to control protein function, but light cannot travel as far through living tissue as a magnetic field can. "We wanted something that could be used in a really complex system like a cell or an organism," says Hamad-Schifferli.

So how good will these nanogadgets be at generating useful data for biologists? As more and more physicists become interested in these tools, there is a risk that they will churn out cool devices that seem great in principle, but have little practical use. "Until we get all the bugs worked out, a big challenge will be getting biologists to buy into it," acknowledges Carl Batt, a food-science researcher and co-director of the Nanobiotechnology Center at Cornell University in Ithaca, New York.

Basic questions, such as the degree to which intracellular probes disrupt the workings of the cell, remain unanswered. "How do you know you aren't measuring a physiological reaction to having a piece of junk inside the cell?" asks Batt. Adding to the scepticism is the phenomenon of nano-fatigue. "The 'nano' word is over-used and over-hyped," says John Ryan, director of the Nanobiotechnology Interdisciplinary Research Collaboration at the University of Oxford, UK.

The close partnership between physicists and biologists in the Alliance for NanoSystems Biology does, however, suggest that some of these fears are being addressed. And most researchers contacted by *Nature* were excited by this coming together of disciplines. As nanotechnology brings more tools to the biologist's bench, Ryan asserts, the divisions between the fields of science will begin to break down. Universities are also helping by making courses more interdisciplinary. The approaches to problems may vary between biologists, chemists, engineers and physicists, but their common interests far outweigh their differences. "At the molecular scale," says Ryan, "all these fields reduce to the same basic science."

In this case, the science concerns the fast-paced activities of daily life in the cell. And for those in the nanosystems alliance, nanotechnology is the best way to get a grip on the many fleeting processes involved. Alliance member Leroy Hood, a molecular biologist at the Institute for Systems Biology in Seattle, predicts that nanotechnology will reveal as much new information about the cell as did the automated DNA sequencer — a device that he invented. "The combination of microfluidics and nanotechnology," Hood asserts, "will transform how biologists do everything." ■

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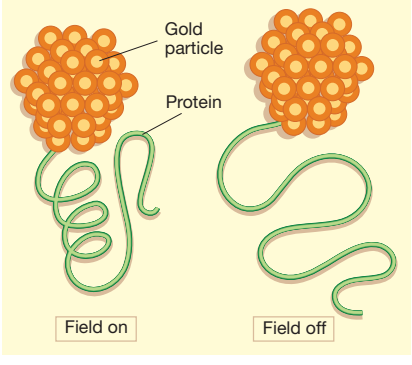

Kimberly Hamad-Schifferli (right) hopes to control proteins by attaching tiny gold particles to them — in a radio field the particle heats up, altering the protein's structure and inactivating it.

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Alliance for NanoSystems Biology
www.nanosysbio.org