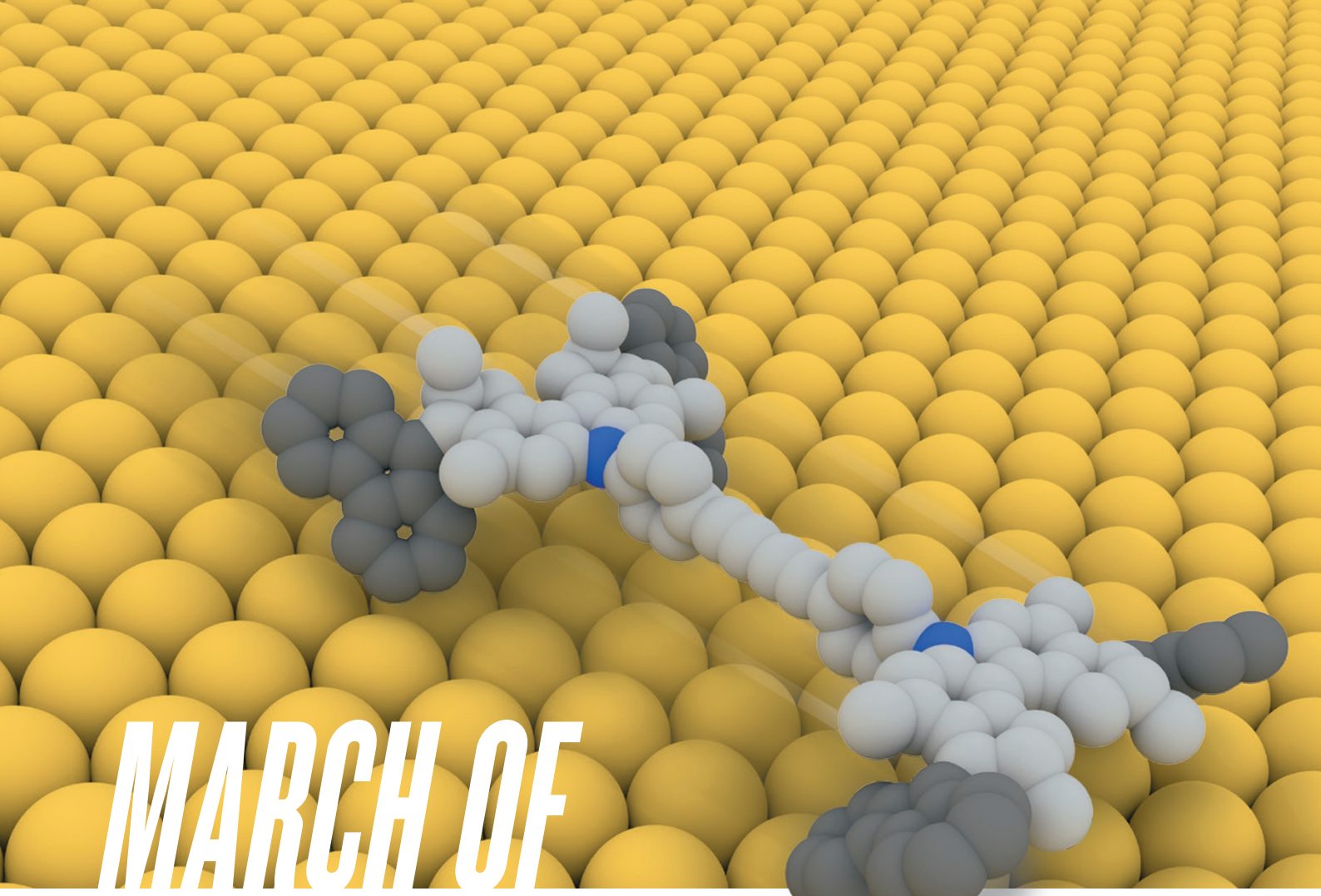


MARCH OF THE MACHINES

BY MARK PEPLOW

INSPIRED BY BIOLOGY, CHEMISTS HAVE CREATED A CORNUCOPIA OF MOLECULAR PARTS THAT ACT AS SWITCHES, MOTORS AND RATCHETS. NOW IT IS TIME TO DO SOMETHING USEFUL WITH THEM.

The robot moves slowly along its track, pausing regularly to reach out an arm that carefully scoops up a component. The arm connects the component to an elaborate construction on the robot's back. Then the robot moves forward and repeats the process — systematically stringing the parts together according to a precise design.

It might be a scene from a high-tech factory — except that this assembly line is just a few nanometres long. The components are amino acids, the product is a small peptide and the robot, created by chemist David Leigh at the University of Manchester, UK, is one of the most complex molecular-scale machines ever devised.

It is not alone. Leigh is part of a growing band of molecular architects who have been inspired to emulate the machine-like biological molecules found in living cells — kinesin proteins that stride along the cell's microscopic scaffolding, or the ribosome that constructs proteins by reading genetic code. Over the past 25 years, these researchers have devised an impressive array of switches, ratchets, motors, rods, rings, propellers and more — molecular mechanisms that can be plugged together as if they were nanoscale Lego pieces. And progress is accelerating, thanks to improved analytical-chemistry tools and reactions that make it easier to build big organic molecules.

Now the field has reached a turning point. "We've made 50 or 60 different motors," says Ben Feringa, a chemist at the University of Groningen in the Netherlands. "I'm less interested in making another motor than actually using it."

That message was heard clearly in June, when one of the influential US Gordon conferences focused for the first time on molecular machines and their potential applications, a clear sign that the field

KARL-HEINZ ERNST

A molecular 'nanocar' travels across a metal surface, propelled by bonding changes.

has come of age, says the meeting's organizer, chemist Rafal Klajn of the Weizmann Institute of Science in Rehovot, Israel. "In 15 years' time," says Leigh, "I think they will be seen as a core part of chemistry and materials design."

Getting there will not be easy. Researchers must learn how to make billions of molecular machines work in concert to produce measurable macroscopic effects such as changing the shape of a material so that it acts as an artificial muscle. They must also make the machines easier to control, and ensure that they can carry out countless operations without breaking.

That is why many in the field do not expect the first applications to involve elaborate constructs. Instead, they predict that the basic components of molecular machines will be used in diverse areas of science: as light-activated switches that can release targeted drugs, for example, or as smart materials that can store energy or expand and contract in response to light. That means that molecular architects need to reach out to researchers who work in fields that might benefit from their machine parts, says Klajn. "We need to convince them that these molecules are really exciting."

SHUTTLE LAUNCH

Many of today's molecular machines trace their origins to a relatively simple device built in 1991 by Fraser Stoddart, a chemist now at Northwestern University in Evanston, Illinois. It was an arrangement known as a rotaxane, in which a ring-shaped molecule is threaded onto an 'axle', a linear molecule capped by bulky stoppers at each end. Included in this particular axle, towards either end of the chain, were two chemical groups that could bind to the ring. Stoddart found[1] that the ring could hop back and forth between these two sites, creating the first molecular shuttle.

By 1994, Stoddart had modified the design so that the axle had two different binding sites[2]. The shuttle existed in solution; changing the acidity of this liquid forced the ring to hop from one site to the other, making the shuttle into a reversible switch. Similar molecular switches could one day be used in sensors that respond to heat, light or specific chemicals, or that open the hatch of a nanoscale container to deliver a cargo of drug molecules at precisely the right time and to exactly the correct place in a person's body.

Stoddart's switches displayed two properties that would come up again and again in the molecular machines that followed. First, the links between the ring and the axle's binding sites were not the strong covalent bonds that knit atoms into molecules. Instead, they were weaker electrostatic attractions between slightly positive and negative regions of the two components. This meant that the bonds could be readily formed and broken, much like zipping and unzipping the hydrogen bonds that link the two strands of DNA. Second, the shuttles did not need an external energy source to zip back and forth. They were powered by collisions with other molecules in the solution, a jostling effect called Brownian motion.

A plethora of other switches soon followed. Some were controlled with light or changes in temperature, whereas others worked by binding specific ions or molecules from solution, in a similar way to how ion channels work in cell membranes, opening or closing in response to chemical signals.

Stoddart, however, took his research in a different direction. Working with James Heath at the California Institute of Technology in Pasadena, he used millions of rotaxanes to make a memory device[3]. Sandwiched between silicon and titanium electrodes, the rotaxanes could be electrically switched from one state to another and used to record data. This molecular abacus, roughly 13 micrometres across, contained 160,000 bits, each composed of a few hundred rotaxanes — a density of roughly 100 gigabits per square centimetre, comparable to the best commercial hard drives available today.

> "I THINK THEY WILL BE SEEN AS A CORE PART OF CHEMISTRY AND MATERIALS DESIGN."

Using 24 of the best-performing bits, Stoddart's team stored and retrieved the letters 'CIT' (for the California Institute of Technology). But the switches were not very robust, typically falling apart after fewer than 100 cycles. One promising solution is to load them into tough, porous crystals known as metal-organic frameworks (MOFs), which protect the switches and organize them into a precise 3D array (see *Nature* **520,** 148–150; 2015).

Earlier this year, Robert Schurko and Stephen Loeb of the University of Windsor, Canada, showed that they could pack about 10^{21} molecular shuttles into each cubic centimetre of a MOF[4]. And last month, Stoddart unveiled[5] a different MOF that contained switchable rotaxanes. The MOF was mounted on an electrode, and the rotaxanes could be switched en masse by changing the voltage.

Researchers working on these MOFs hope that the 3D, solid scaffolds will offer a greater density of switches than conventional silicon transistors, and make the molecules easier to switch in a controllable way, potentially offering vast amounts of data storage. "The sci-fi way to think about it would be to address each molecule as a bit," says Loeb. But more realistically, he says, a speck of the MOF containing hundreds of switches could act as one bit. As long as most of the switches in the speck function properly, he says, they will collectively and reliably encode data.

Others have used rotaxanes to make switchable catalysts. In 2012, Leigh described[6] a system with a nitrogen atom in the middle of the rotaxane's axle, where it is normally covered by a ring. Add an acid, and the ring moves to one side, exposing the nitrogen atom so that it can catalyse a common chemical reaction. It goes further: last November, Leigh reported[7] a rotaxane system with two different catalytic sites. Moving the ring from one to the other allowed the chemists to switch the rotaxane's activity, so that it could stitch together a mixture of molecules in two different ways. Leigh is now working on putting several different switchable catalysts into the same solution, where they could be toggled on and off in a sequence to build target molecules into complex products, in much the same way as enzymes do in a cell.

NANO MOTORS

In 1999, after early experiments with shuttles and switches, the field took a big step forward with the creation of the first synthetic molecular motor[8]. Built by Feringa's team, it was a single molecule containing two identical 'paddle' units connected by a carbon–carbon double bond. This fixed the paddles in place until a burst of light broke part of the bond, allowing the paddles to rotate. Crucially, the shape of the paddles meant that they could turn in only one direction — and as long as there was a supply of light and some heat, the motor would just keep spinning.

Feringa went on to use similar molecular motors to create a four-wheel-drive 'nanocar'[9]. He also showed[10] that the motors could give liquid crystals enough of a twist to slowly rotate a glass rod sitting on top of them. The rod was 28 micrometres long — thousands of times the size of the motors.

Some chemists argue that although these motors are cute, they are ultimately useless by themselves. "I've always been a bit sceptical of artificial motors — they're too difficult to make, too difficult to scale up," says Dirk Trauner, a chemist at the Ludwig Maximilian University in Munich, Germany.

But the chemical principles behind them might be very useful indeed. Using the same light-activated mechanism, researchers have developed around 100 drug-like compounds that can be switched on or off in response to light.

In July, for example, a team led by Trauner

reported[11] a light-switchable version of combretastatin A-4, a potent anticancer compound that comes with some serious side effects, because it indiscriminately attacks tumour cells and healthy tissue alike. The team's switchable drug could drastically reduce system-wide side effects: it contains a nitrogen–nitrogen double bond that holds two sections of the molecule apart and renders it inactive. Only under blue light will the bond break and allow the sections to rotate into the molecule's active form. Trauner says that an area of tissue just 10 micrometres wide can be specifically targeted in this way, using light delivered through a flexible tube or by an implanted device. Trauner is planning mouse studies to test the compound's effectiveness against cancer.

He also hopes to use photoswitchable compounds to restore vision in people with macular degeneration or retinitis pigmentosa, conditions that damage the eye's light-sensing rod and cone cells. "It's low-hanging fruit — because it's in the eye, you don't have to worry about how to get the light in," he says. Last year, he showed[12] that one injection of a photoswitchable molecule called DENAQ into the eyes of blind mice partially restored their vision for several days, allowing the animals to distinguish between light and dark. The team is now trying the same technique in primates, and hopes to begin human trials in two years' time.

Trauner and Klajn both agree that the main challenge will be to convince the cautious pharmaceutical industry that photoswitchable drugs have potential, even though they have no track record in humans. "We need to get the pharmaceutical industry excited about photopharmacy," says Trauner. "Once they see the value, we'll be in good shape."

WALK THE LINE

Long before any creature had evolved to move on dry land, cells were using legs as part of their cellular machinery. Prime examples are the two-pronged proteins called kinesins, which put one 'foot' in front of the other as they carry molecular cargo along the cell's stiff scaffolding of microtubules.

Inspired by kinesin, researchers have built artificial walkers from DNA. The molecules typically have feet that are anchored in place by binding to complementary strands of DNA laid out on a track; adding a competing DNA strand can free the foot, allowing it to take a step forward. One of the most striking examples was described[13] in 2010 by Nadrian Seeman at New York University. His DNA walker had four 'feet' and three 'hands', with which it could pick up gold nanoparticles as it moved around a tile made of folded DNA.

DNA walkers — and variants that soon trundled out of other labs — would wander aimlessly if they did not have a built-in ratchet system to stop them taking a step backward. For many walkers, that ratchet lies in the relative rates of the chemical reactions that are involved in binding and releasing their feet, with the pummelling of Brownian motion driving the released foot forward[14].

Over the past few years, detailed chemical studies and molecular dynamics simulations have shown that this 'Brownian ratchet' concept underlies all chemically driven molecular machines, including many biological motors. In 2013, for example, a team led by Nils Walter, a chemical biologist at the University of Michigan in Ann Arbor, found[15] the same mechanism at work in the spliceosome, a cellular machine that snips sections out of RNA before genetic information is translated to make proteins. "Kinesin uses it, the ribosome uses it and the spliceosome uses it," says Walter.

That shows that the same principles underlie biological machines and synthetic molecular machines, so researchers working in the two areas could share knowledge. "By and large, they're quite separate fields right now," says Walter. "I think the next breakthroughs will come if we all sit at the same table."

ROCKET SCIENCE

Meanwhile, inspired by the microscopic medical submarine of the cult 1966 film *Fantastic Voyage*, chemists have created an array of micrometre-sized particles and tubes that can zip through liquids like rockets.

Some of these motors carry a catalyst that generates thrust by producing a stream of bubbles from the liquid around them — often hydrogen peroxide. Others get their power directly from light or from external electric and magnetic fields, which can also be used to steer the vessels. "These nanomotors can go over 1,000 times their own length per second, it's incredible," says nanoengineer Joseph Wang of the University of California, San Diego. He thinks that the most promising applications lie in fast drug delivery, or low-cost clean-up of environmental pollutants — although many in the field caution that it is too early to tell whether nanomotors would trump conventional methods.

Hydrogen peroxide, a powerful oxidizing agent, is hardly conducive to *in vivo* use. "When all the work was based on peroxide there was a lot of scepticism," Wang admits. But in December last year, he reported[16] a microscale motor suitable for testing in live animals. Made of a plastic tube roughly 20 micrometres long, it contains a core of zinc that reacts with stomach acid to generate propulsive bubbles of hydrogen.

The tubes safely zipped around inside a mouse's stomach for about 10 minutes. Wang used them to carry gold nanoparticles into surrounding stomach tissue; mice dosed with plain nanoparticles ended up with three times less gold in their stomach lining than mice dosed with the tubes.

Wang suggests that loading drugs or imaging compounds onto the rockets could deliver them into stomach tissue rapidly and effectively. "In the next five years we will move to practical *in vivo* applications," he says. "It really is the fantastic voyage."

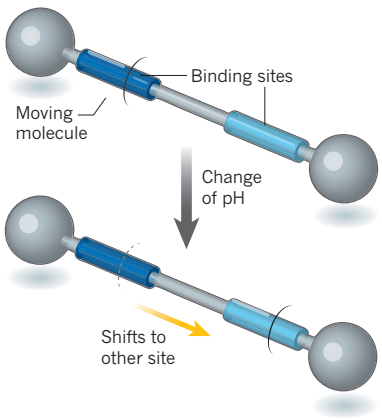
At the moment, there is limited crossover between research on these rockets and the molecular machines. "But we could bring

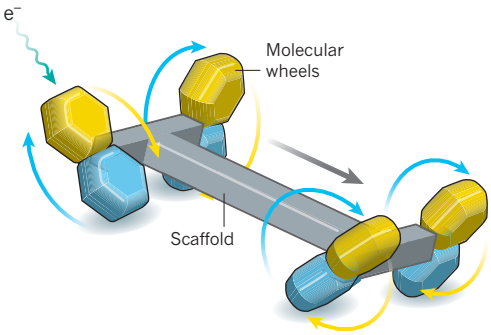
NANO MACHINES

Mechanisms the size of molecules — governed by the rules of chemistry, rather than Newtonian mechanics — could have applications ranging from drug delivery to nanoscale computer memories.

MOLECULAR SWITCH

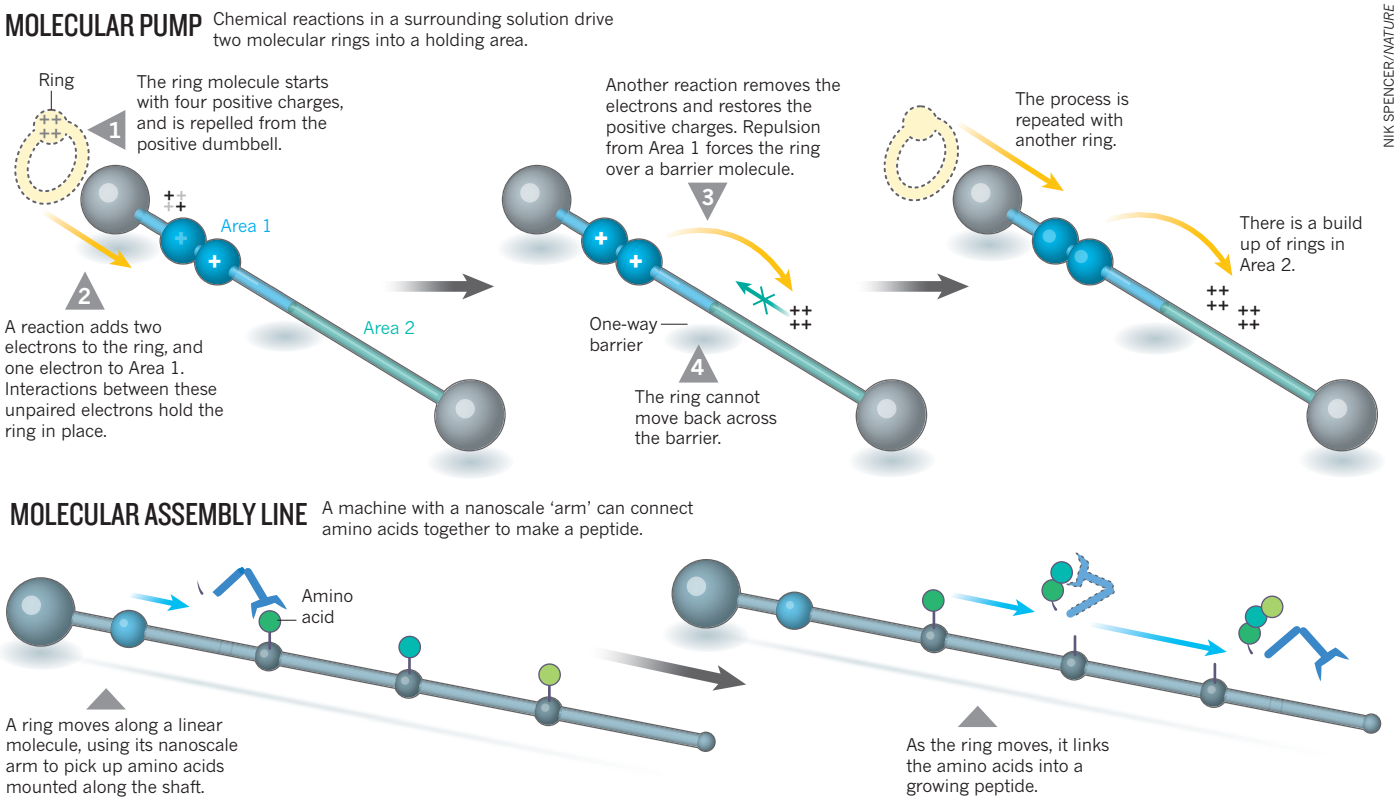
A ring-shaped molecule threaded onto a linear molecule shifts between two binding sites depending on the acidity of the surrounding solution.





NANOCAR

Electrons from a scanning tunnelling microscope tip (not shown) leap onto the molecules that form the 'wheels' of this device, causing them to change configuration, rotate and move the car forward.



NIK SPENCER/*NATURE*

a lot," says Klajn. For example, coating a micromotor with light-responsive molecular switches could offer extra control over its movement, he suggests.

PUMP IT UP

In their quest to forge molecular machines that can actually do something useful, researchers are starting to integrate several different components into a single device. In May this year, Stoddart unveiled[17] an artificial molecular pump that pulls two ring molecules out of solution onto a storage chain. Each ring slips over a stopper at one end of the chain, attracted to a switchable binding point. Flipping that switch pushes the ring over a second barrier farther along the chain, where it reaches a holding area (see 'Nano machines').

The system is not able to pump any other type of molecule, and it took a lot of trial and error to build. "It's been a long road," sighs Stoddart. But it proves that molecular machines can be used to concentrate molecules, pushing a chemical system out of equilibrium in the same way that biology can build up a store of potential energy by forcing ions or molecules up a concentration gradient. "We're learning how to design an energy ratchet," he says.

Stoddart says that such developments could enable the field to progress in two major directions: stay nano, giving the machines molecular-scale jobs that cannot be achieved in any other way; or go macro, using trillions of them together to reshape materials or move substantial cargoes, like an army of ants.

Perhaps the prime example of the nano approach is Leigh's molecular assembly line[18]. Inspired by the ribosome, it is based on a rotaxane system that picks up amino acids from its axle and adds them to a growing peptide chain. But the devices could have macro applications. Over 36 hours, 10^{18} of them working together can produce a few milligrams of peptide. "It doesn't do anything that you can't do in the lab in half an hour," says Leigh. "Yet it shows that you can have a machine that moves down a track and picks up molecular building blocks and puts them together." Leigh is now working on other versions of the machine to make sequenced polymers, with tailored material properties.

Conversely, trillions of molecular machines working together could change the properties of materials in the macroscopic world. Gels that expand or contract in response to light or chemicals, for example, could act as adjustable lenses or sensors. "In the next five years, I bet you'll get the first smart materials where you have switches incorporated," says Feringa.

Rotaxane-like molecules are already starting to see commercial applications. The Nissan Scratch Shield iPhone case, launched in 2012 and based on work by Kohzo Ito at the University of Tokyo, is made of polymer strands threaded through pairs of barrel-shaped cyclodextrin molecules connected in a figure-of-eight shape. Pressure on a normal polymer coating would break the connections between the chains, leaving a scratch. But the cyclodextrin rings act like the wheels of a pulley system, allowing the polymer strands to slip through without breaking[19]. The films can even protect a brittle screen from a sustained beating with a hammer.

For Stoddart, this shows that the components developed by molecular architects are already ripe for application. "This field has come a long way," says Stoddart. "Now we have to start showing it's useful."■

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