Biomolecules **in Action**

Techniques that provide insights into the nanoworld continue to garner Nobel Prizes. However, none of those methods has made it possible to observe exactly how enzymes and other biomolecules function. **Frank Vollmer**, Leader of a Research Group at the **Max Planck Institute for the Science of Light** in Erlangen, has now changed all that – with a plasmonic nanosensor.

TEXT **CHRISTIAN MEIER**

Frank Vollmer spared no effort. Sure, scientists often put a lot of time into developing new methods, especially when they're looking for something fundamentally new. But Vollmer, a biochemist by training, spent around 20 years working as a physicist to devise the tool he needed to realize his project. Ever since his days as a biochemistry student in Hanover in the 1990s, he's always wanted to watch the machinery of life at work.

That desire still drives him today in his role as a Research Group Leader at the Max Planck Institute for the Science of Light in Erlangen and a professor at the University of Exeter. He wants to understand why the tiny machines that keep life processes running sometimes go awry, causing us to become ill. To realize his plan, Vollmer had to become an inventor. He needed a kind of camera that can record individual enzymes and the movements of their parts, but the laws of physics appeared to prohibit such a device. Nevertheless, the scientist recently caught a glimpse of one of the most delicate mechanisms of life.

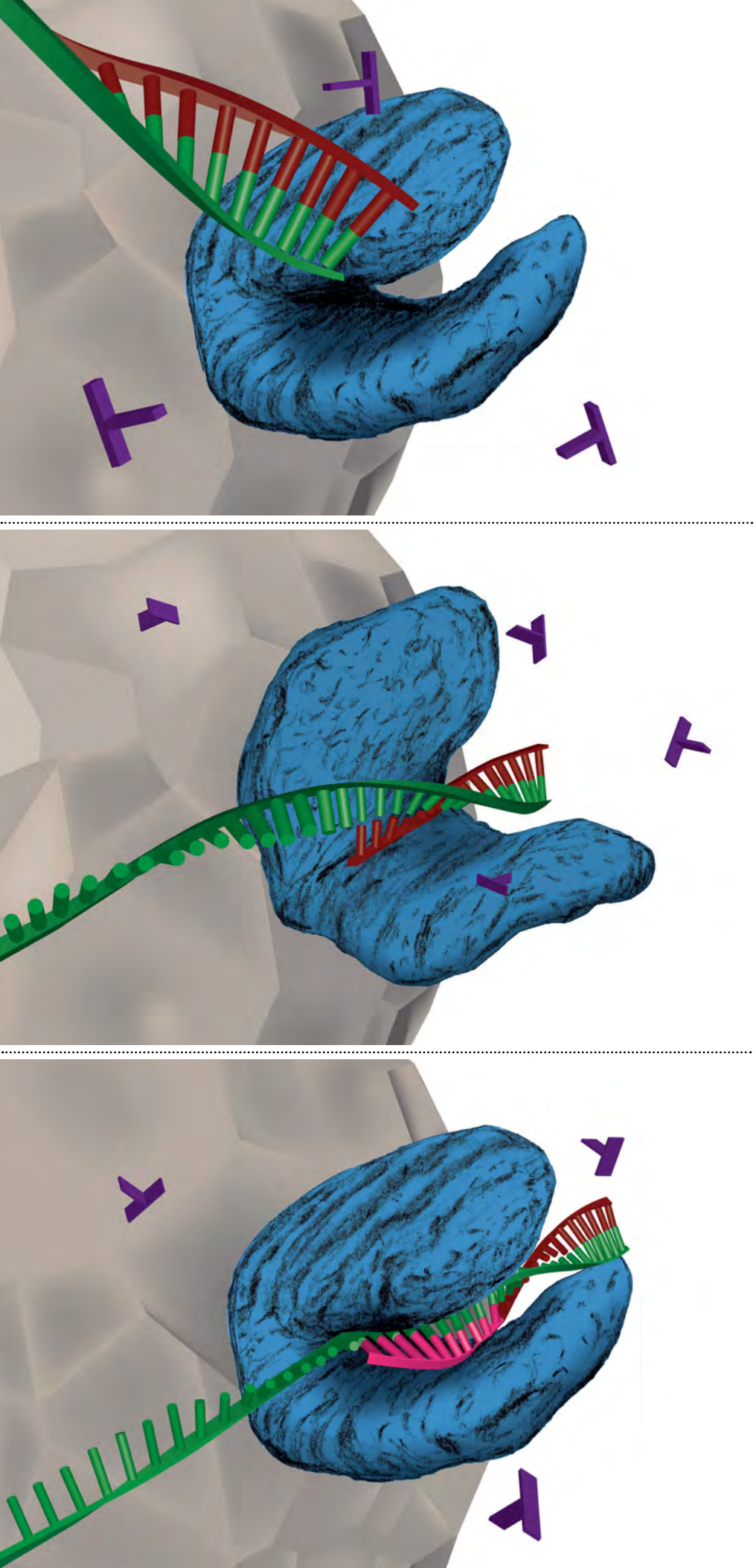
BIOLOGICAL TOOLS WORK LIKE TECHNOLOGICAL ONES

"We all consist of nanotechnology," says Vollmer. That's true. For one thing, proteins, enzymes and other biomolecules are just a few nanometers in size. One nanometer, a millionth of a millimeter, is as tiny as a soccer ball is in relation to planet Earth. For another thing, like man-made machines, these tiny workhorses in the body each serve a specific purpose – one protein transports oxygen, another breaks down sugar molecules, a third reads genetic information – and often have moving parts.

An enzyme that carries out a fundamental life process reveals just how similarly biological and technological tools function: DNA polymerase duplicates the DNA molecule, thus enabling cells to divide. This process underlies every form of reproduction. The enzyme is reminiscent of a hand with a thumb and fingers that literally grasps the DNA strand being copied.

Observing enzymes such as DNA polymerase in action is especially important when the biological machines develop a hitch. Catching a glimpse of these nanomachines at work could then reveal where the problem lies. When DNA is copied during cell division, errors can occur, much like typographical errors in a book. In some circumstances, such mutations can lead to

Graphic: Han and Holm, based on original material from the MPI for the Science of Light



An enzyme gets a grip: Like a hand opening and closing, DNA polymerase grasps the building blocks of our genome. When the biomolecule is bound to a gold nanowire that focuses light tightly, the overlap with the light spot changes. In this way, the movement of the enzyme can be observed.

diseases, such as cancer. In other illnesses, such as Alzheimer's, proteins change shape, causing biomolecules to lose their ability to function.

Frank Vollmer regrets that science has so far been largely blind to the movements of biomolecules. "We can't see the very thing that's so crucial for our understanding of disease," he laments. "The fact that the hand opens and closes very rapidly doesn't help matters either."

Despite the tiny size and extremely rapid nature of life processes, Frank Vollmer found a way to watch nature's nanotechnology at work. To do this, he uses visible light – just like our eyes, cameras and video cameras do. Light waves are a harmless part of the electromagnetic spectrum. They are much gentler than, say, X-rays, whose high energy can virtually pulverize molecules. In addition, they cause very little heating of a sample. In short, visible light is a practically neutral observer.

Unfortunately, a normal light microscope is unable to resolve nanostructures because visible light can't be focused to a small enough point of light. A focal point measuring half the light's wavelength is about the limit, or around 200 nanometers. Although some methods, such as STED microscopy, use physical tricks to get around this resolution limit, they can only observe cell components that are labeled with fluorescent molecules, or markers. The

» Frank Vollmer wanted to understand how RNA is read.
To answer this question, he first had to develop a suitable method.

points of fluorescent light then show how the biomolecules move about, much as car headlights at night reveal where a car is heading. But a great deal remains in the dark, such as whether the points of light belong to a car or a truck, or whether a door is opened on a stationary car. In the same way, the fluorescent molecules don't show in detail whether or how a protein changes shape. Moreover, scientists can never be sure that the labels don't interfere with the function of the biomolecule they are investigating.

LIKE A WHISPERING GALLERY

Frank Vollmer reached the limits of optical observation at the latest during his doctoral work at Rockefeller University in New York, where he was researching how the second carrier of genetic information, RNA, is read. "At that point, I realized that a suitable method had yet to be invented." The biochemist decided to devise one himself and became a physicist to do it. He had, after all, always been interested in this discipline.

Before long, Vollmer came across a technique that pointed in the right direction. It is the optical analogue of a whispering gallery. In a rotunda, a word whispered toward the wall can easily be heard on the opposite side of the chamber because sound waves travel along the curvature instead of spreading out in all directions. Similarly, a light wave runs along the inside of a glass bead measuring just a few micrometers (thousandths of a millimeter), whizzing around inside it tens of thousands of times. As it circulates, the wave keeps encountering itself. At a well-defined wavelength that depends on the size of the glass bead, the wave crests travelling around the glass bead overlap precisely with the crests of previous laps. Physicists refer to this phenomenon as resonance. This is analogous to the resonant body of a musical instrument that, because of its dimensions, amplifies only certain sounds.

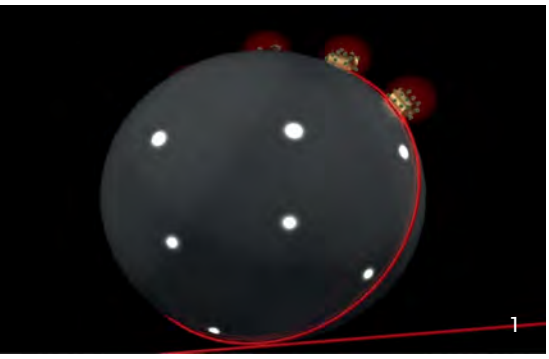
The optical whispering-gallery effect makes the glass bead a very sensitive sensor. The light wave extends somewhat beyond the surface of the bead. If a virus or protein, for example, adheres to the surface of the microbead, the particle interacts with the light wave, slowing it down a little. The effect is the equivalent of increasing the circumference of the bead's wall. Consequently, the virus or protein alters the resonant wavelength of the microbead. Although the change is minimal, it can be detected thanks to the sharpness of the resonance.

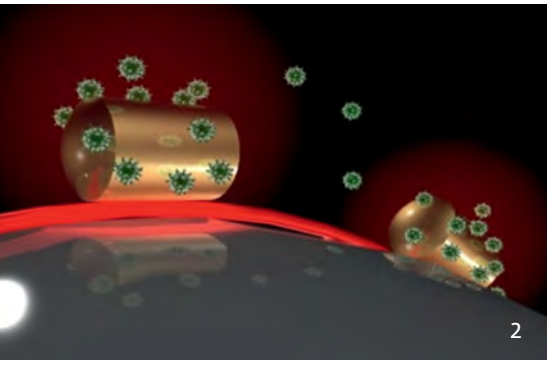
Vollmer succeeded in using the optical whispering gallery as a biosensor for the first time while still working in New York. He shone laser light into a glass bead and used it to detect proteins. At the time, though, he was able to detect only *multiple* proteins bound to the bead, as only then did the wavelength of the circulating light change appreciably. However, to understand how the nanomachines of life work, it is necessary to observe *single* biomolecules. An ensemble of proteins produces only an average value from which it is impossible to determine the behavior of individual molecules.

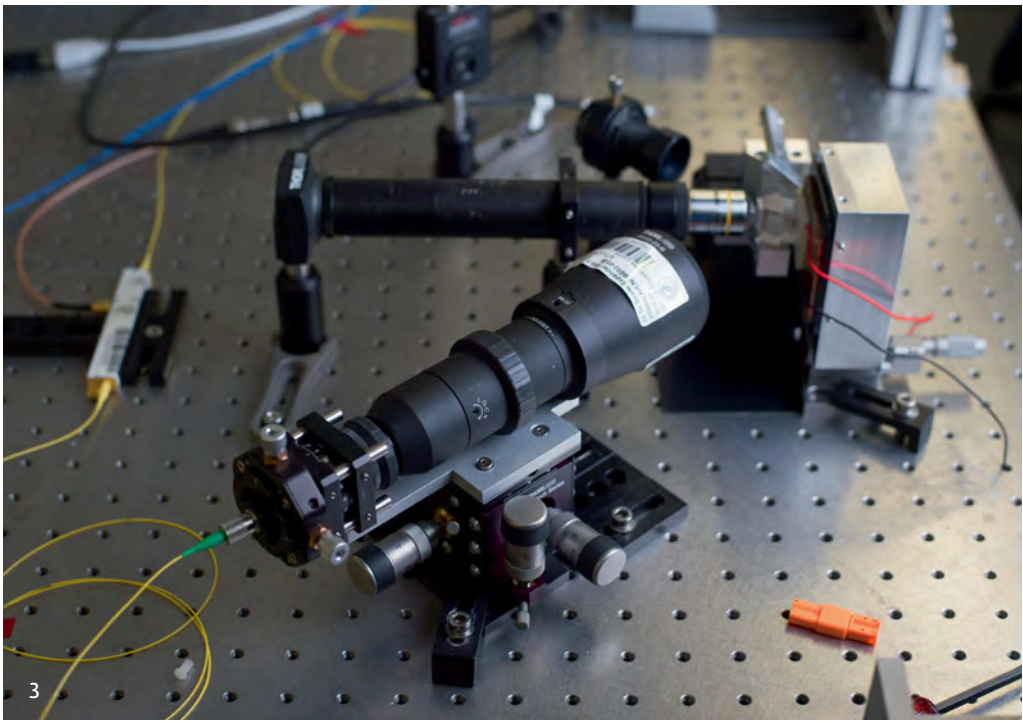
THE TRICK: A COMBINATION OF OPTICS AND PLASMONICS

While head of a research group at Harvard University, Vollmer thus worked on refining the method. He ultimately succeeded in using the glass bead to detect *single* viruses. It was a remarkable triumph, but he had not yet achieved the actual goal of observing single biomolecules. After all, a virus is still ten times larger than a protein.

"We soon realized that it would be far more difficult to detect individual biomolecules," Vollmer says. The light path changes in relation to the volume of a detected nanoparticle. The change caused by a single molecule is therefore only about one-thousandth the magnitude of that caused by a virus, putting it well below the detection limit. Fellow







Graphic: Frank Vollmer/Science Advances (left), Steven Haywood (right)

physicists advised Vollmer to try other methods. "But such methods would have required much more complicated experimental setups," Vollmer explains. He wanted a tool that biologists can use relatively easily.

In 2010 he came up with an idea that would lead to a breakthrough. The trick was to combine optics with a very different branch of physics: plasmonics. Plasmonics exploits the fact that electrons in a metal form a sort of collective, similar to a liquid, that moves freely past the firmly fixed atomic cores in the crystal lattice. Because the negatively charged electrons are attracted to the positively charged atomic cores as though they were attached to them by coil springs, the collective oscillates back and forth. The oscillations of the electric charge are called plasmons.

Plasmons produce an electrical wave that extends beyond the metal surface, just like light waves extend beyond the surface of the optical whispering gallery. The fact that such plasmons can interact with light was also crucial for Vollmer's plans. The researchers reasoned that if they attached a nanowire about the size of a single biomolecule to an optical whispering gallery, the light waves that extend beyond the surface would excite plasmons in the metal. That, in turn, would generate light waves on the surface of the wire.

A HOTSPOT AMPLIFIES THE INTERACTION

Thus, a nanowire would effectively draw the light wave out of the glass bead and focus it on a spot about the size of a protein. This hotspot focusing, as Vollmer calls it, would amplify the interaction between light and the object under investigation. In this way, even a single biomolecule could lengthen the path of the light to the extent that the change would be measurable.

Vollmer set about implementing the idea at the Max Planck Institute for the Science of Light in Erlangen. DNA

An optical whispering gallery as a nanosensor: When laser light of a suitable color (here red light) is guided through a glass fiber into a glass microbead, it circulates within the bead like sound in a rotunda (1). Gold nanowires attached to the bead concentrate the light by means of a plasmonic effect. Proteins and other molecules that bind to the nanowires (2) change the wavelength of the light coupled to the bead, thus allowing the molecules and their movements to be detected. Such nanosensors for proteins and their movements were successfully tested for the first time in an experimental setup at the Max Planck Institute for the Science of Light (3).

» A nanowire draws the light wave out of the glass bead and concentrates it on a spot the size of a protein.

polymerase served as a good test enzyme because its opening and closing motions resembling those of a "hand" should result in a periodic increase and decrease of the overlap between the enzyme and the light at the hotspot, much like the shadow of a hand changes as it opens and closes in front of a light source.

First, Isabel Schuldes, a master's degree student in Frank Vollmer's group, attached gold nanowires with a diameter of just 10 nanometers and a length of 40 nanometers to a glass bead with a diameter of 80 microns. Normally, electrostatic forces would bind the gold to the glass. In Erlangen, however, this mechanism conflicted with the chemical conditions required by the polymerase. Its preferred pH tended to break the electrostatic bond. After an elaborate search, Schuldes found a linker molecule that binds the nanowire to the glass bead: "That gave me a great sense of achievement," she says.

Now the scientists were able to focus their nanospotlight on biological machines. They attached DNA strands to the fine wires and dipped their nanosensor into a polymerase solution. The enzyme then copied the attached DNA. In another experiment, the researchers attached the polymerase to the nanowire and mixed DNA into the solution. Schuldes had previously confirmed that attaching the enzyme to the gold doesn't affect its activity. In both cases, during the copying process, the polymerase was inside the light spot focused by the nanowire and, it was hoped, would change the measurement signal as the enzyme hand opened and closed.

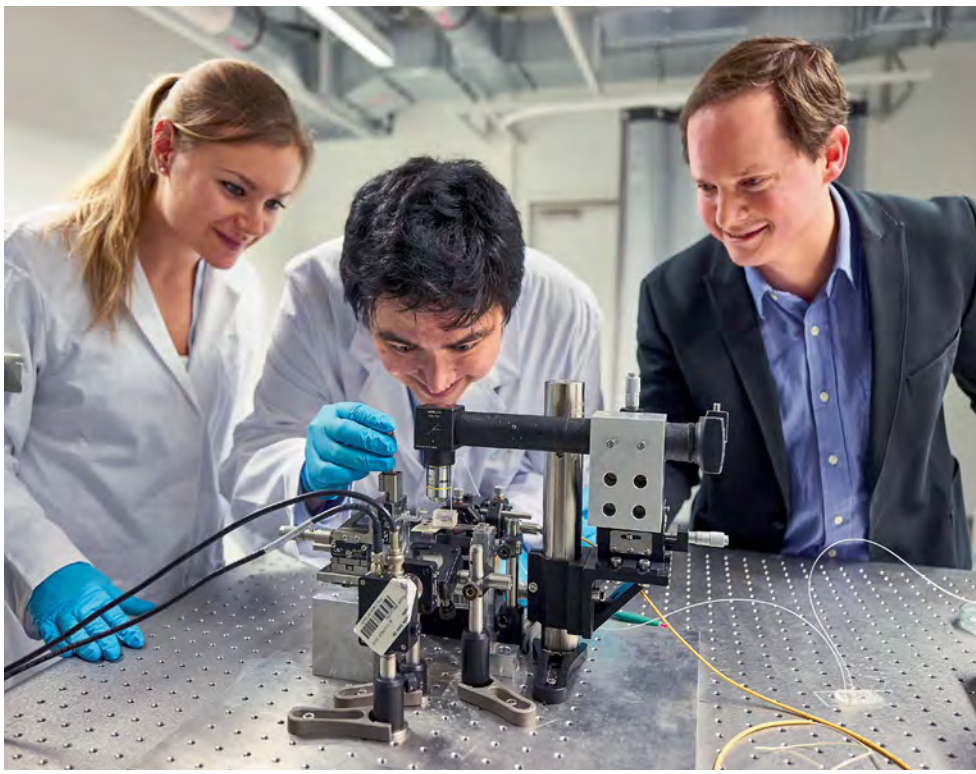
THE RESEARCHERS EVEN OBSERVED SINGLE IONS

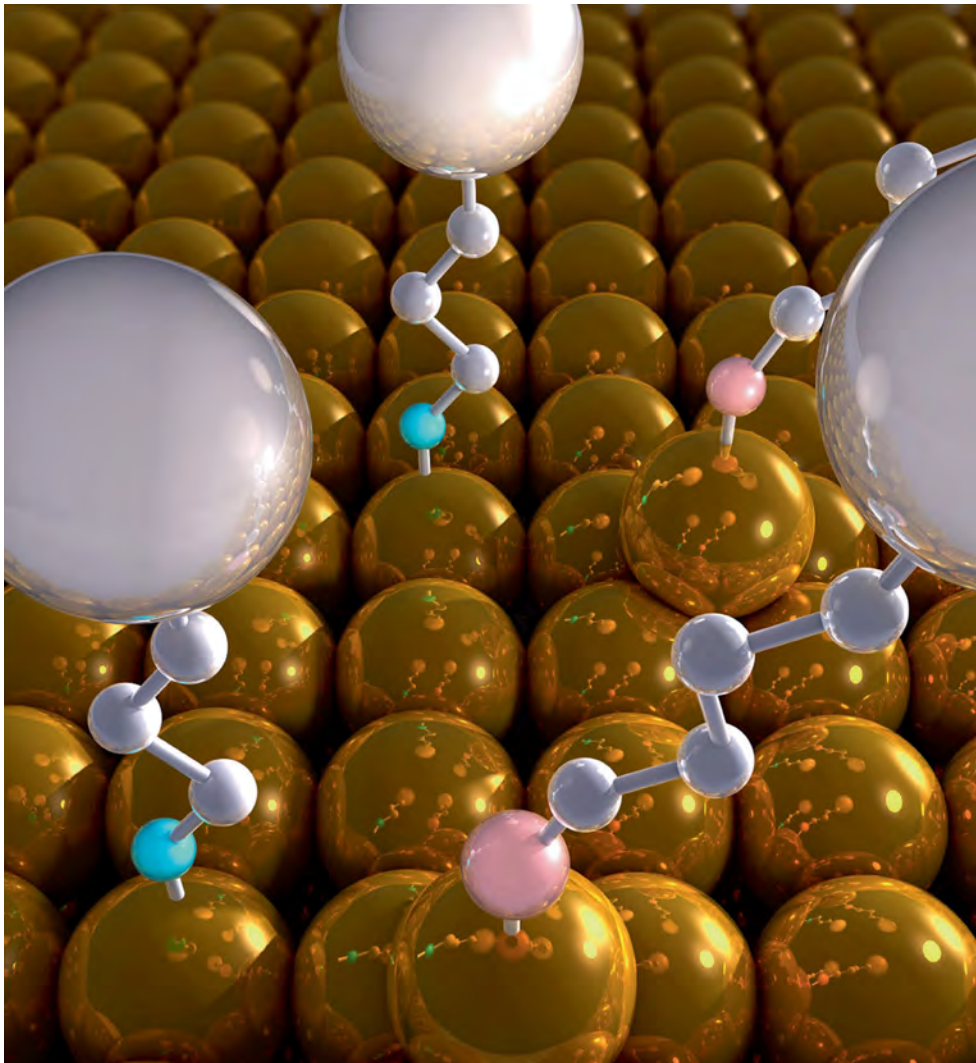
And the researchers did indeed observe their signal waxing and waning in cycles of about 20 to 50 thousandths of a second. "For the first time, we were able to observe protein dynamics without using markers," says Vollmer. And they were able to do so live, in such conditions as occur in nature. Vollmer even thinks it is possible to record the sequence of the DNA letters as the polymerase is reading them. Copying errors could be detected this way, too. "It would also be a very simple and inexpensive method for studying DNA," Vollmer says.

After this success, the researchers wondered about the limits of their new method. To their own astonishment, they were even able to observe *single* ions – that is, electrically charged atoms. The zinc and mercury ions they used are one hundred times smaller in diameter than a protein. It helped the researchers that the gold nanowires tapered to a single gold atom at the tip. The plasmonic light spot therefore focuses on an extremely small spot at the very tip, causing the electrons to gain more energy than they would normally have in gold. Because of their energy gain, the charge carriers activate a reaction between the gold atoms and the mercury ions. The researchers in Erlangen were able to observe this reaction.

"It's not about detecting single mercury ions," Vollmer stresses. The sensitivity for single ions can be used, for example, to investigate the function of ion channels, he says. These channels are embedded in the membranes of neural cells, for instance, and help conduct electrical stimuli through nerves.

Thanks to its ability to track reactions such as that between gold atoms and mercury ions, the nanosensor is also suitable as a chemical tool, the bio-





Top Isabel Schuldes, Ying-Jen Chen and Frank Vollmer (left to right) have made the plasmonic nanosensor so sensitive that it can even detect substances that form during cell death. It takes a fine touch to couple the laser light to the sensor.

Bottom The nanosensor is also suitable for analyzing chemical reactions. The Max Planck researchers used it, for example, to show that molecules with amino groups (pink) adhere to gold atoms protruding from the surface of the nanowire. Molecules with thiol groups (blue), in contrast, bind to gold atoms embedded in the surface.

physicist explains. It can be used to test and optimize conditions to *control* reactions to a certain extent. The team in Erlangen even showed how to do this. By increasing the light intensity in the focused light spot, they caused the electrons in the gold tip to reach a very high energy level. This allows a particularly strong type of chemical bond, known as a covalent bond, to form. In this way, the researchers created an amalgam between gold and mercury as if by turning a light dimmer switch.

In an experiment with two types of molecules, the researchers demonstrated the precision with which the mechanisms of chemical reactions can be observed using the Erlangen method. One type of molecule bonded with the gold via an amine group, the other via a thiol group. “It turned out that the two groups react with the gold surface through two different mechanisms,” Vollmer explains. Whereas the amines combine only with gold atoms that protrude above the surface, the thiols bind only to atoms that are fully embedded in the surface.

The extreme sensitivity of the nanosensor that Vollmer’s team developed isn’t its only distinguishing feature. As

Photo: Tilman Weishart; graphic: Frank Vollmer, design by Ella Maru Studio/Advanced Materials

A sensor with many senses: At the Living Systems Institute of the University of Exeter, Hsin-Yu Wu, Serge Vincent, Jolly Xavier, Frank Vollmer, Tom Constant and Sivaraman Subramanian (left to right) built an experimental setup that combines various approaches: the effect of an optical whispering gallery in a glass bead, the plasmonic concentration of the light that circulates inside the bead, and microscopic and spectroscopic methods. The researchers' aim is to analyze the movements of biomolecules in as much detail as possible.

an added benefit, even non-physicists can use it without the need for costly special equipment, Vollmer points out. It also works in aqueous milieus, giving biologists and physicians an eye, as it were, that they can place right at the center of microbiological life.

Vollmer's doctoral student Ying-Jen Chen, for example, uses the sensor to observe the death struggle of cells. Although this process, called apoptosis, is an intracellular process, Chen managed to observe it from outside the cell without influencing the process itself. Using the microbead method, Chen detected a chemical marker of cell death: the protein cytochrome c. To do this, Chen coated the sensor with an antibody that binds exclusively to cytochrome c.

CHIPS FOR RAPID CANCER DRUG SCREENING

Chen is also trying to shrink the microbead method down to chip size. Individual cells are held in tiny channels in plastic tiles barely larger than a fingernail. A toxin flows through the channels, causing the cells to initiate apoptosis. The released cytochrome c is then flushed out and sent to the sensor setup, which still occupies most of the

Photo: Steven Haywood

surface of a lab bench. The goal, however, is to shrink the measuring apparatus so that it, too, fits on a chip.

"The chips could be used, for example, to screen cancer drugs much faster than is normally possible today," Chen says. Many of those drugs trigger apoptosis in cancer cells to kill them. "Our method is suitable for use as an organ-on-a-chip," Vollmer adds, referring to chips that simulate entire organs by connecting cell cultures via complex microchannels or mechanisms.

Vollmer has since reached a point in his research career where he is able to return to biology after his excursion into the world of physics. He now wants to use the plasmonic nanosensor in a biological and medical setting, namely at the Living Systems Institute at the University of Exeter. "This is the ideal place for it," he says. There, he plans to investigate pathologically deformed proteins. It is possible to visualize such defective proteins by using light to stimulate the biomolecules to vibrate.

But Frank Vollmer also wants to further develop the nanosensor – into a laser scanner for single molecules. Using multiple focused light spots, it should be possible to scan molecules atom by atom and reconstruct an accurate 3-D atomic image from the data at several million frames per second. "This could finally reveal the secrets of the nanotechnology of life," Vollmer says. He may soon be recording entire movies of biomolecular machines – lights … camera … action! ◂

TO THE POINT

- **Max Planck researchers working with Frank Vollmer have developed a plasmonic nanosensor that makes it possible for the first time to observe enzymes and other biological molecules in action and even to record the movements of their parts.**
- **The nanosensor consists of a glass microbead with a gold nanowire attached to it. It exploits the effect of an optical whispering gallery, with light of a defined wavelength circulating inside the bead tens of thousands of times. At the same time, it uses plasmons to focus the light on a point just a few nanometers wide. Whenever a biological molecule binds to the gold wire or a bound nanoparticle moves, the wavelength of the light coupled to the glass bead changes.**
- **The researchers have used the sensor to track the movements of DNA polymerase, the process of cell death, and reactions of mercury ions and organic molecules with gold atoms in the nanowire.**

GLOSSARY

DNA polymerase: An enzyme that makes a copy of DNA during cell division.

Plasmon: Oscillations of the electrons in a metal can produce electrical waves. These are known as plasmons, and they can be excited with light.

STED microscopy: In fluorescence microscopy, fluorescent markers on a nano-object are excited with a focused laser. According to the diffraction limit, the light spot can be focused no more tightly than half the wavelength of the light, so to an area measuring around 200 nanometers across. A STED (stimulated emission depletion) microscope circumvents this limitation by extinguishing part of the emission in a controlled manner.