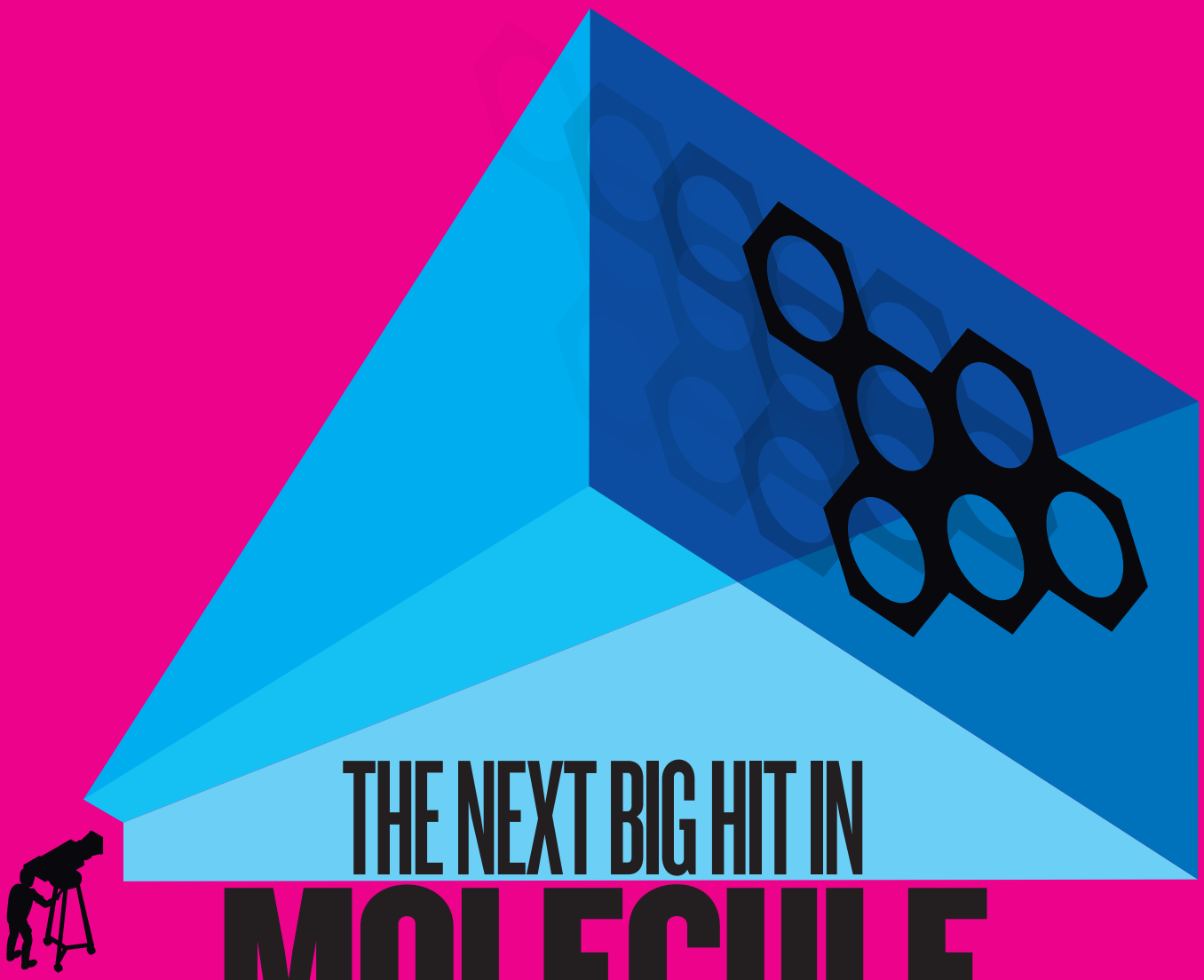

THE NEXT BIG HIT IN MOLECULE HOLLYWOOD

Superfast imaging techniques are giving researchers their best views yet of what happens in the atomic world.

BY MARK PEPLOW

Chemists are dreamers. Every day, they imagine molecules floating in space, with atoms moving about in a stately dance. They spin the structures mentally to examine them from all angles, perhaps twisting each molecule until a bond pops open and another snaps into place.

Such movies play inside the minds of most chemists because they offer a way to visualize how reactions happen. "The unifying thought experiment across all disciplines of chemistry is to imagine atoms moving in real time," says Dwayne Miller, a physical chemist at the Max Planck Institute for the Structure and Dynamics of Matter in Hamburg, Germany, and the University of Toronto in Canada. "This is a dream the entire field has."

Chemists have been dreaming like this for more than 150 years, ever since the idea of molecular structure was first conceived. But now these fantasies are becoming a reality. Researchers are directing molecular movies in the lab using a range of techniques, most of which illuminate the scene with incredibly brief pulses of light or electrons. Some rely on the atomic precision of scanning tunnelling microscopes (STMs), whereas others use intense bursts of X-rays to reveal their target's structure.

Their goal is to film events that take place in picoseconds (ps, 10^{-12} s) or femtoseconds (fs, 10^{-15} s), with atoms moving mere picometres (a hydrogen atom is roughly 100 pm in diameter). At this resolution, researchers can for the first time directly observe a molecule writhing in slow motion, atomic bonds vibrating and breaking, or even

ILLUSTRATION BY THOMAS POROSTOCKY

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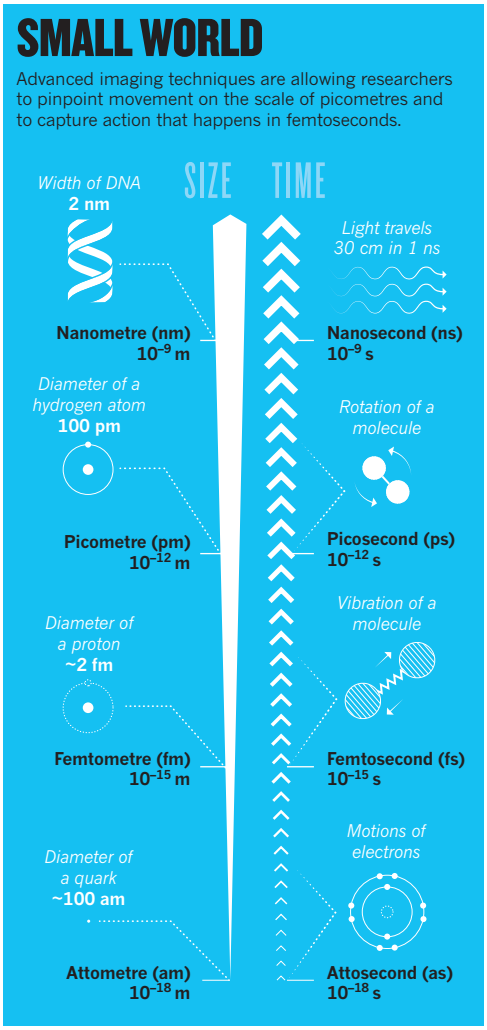
electrons washing back and forth. As these techniques become more mainstream, the pay-offs could be huge. They could provide crucial information that leads to better catalysts, artificial forms of photosynthesis or new ways to manipulate the quantum properties of molecules for computing and communication.

The stars of these movies are often composite characters, brought to life by filming ensemble casts of billions of identical molecules stacked neatly into tiny crystals. Increasingly, however, researchers are putting individual molecules in the spotlight. Single molecules are in thrall to quantum mechanics, rather than the classical, statistical laws that govern the properties of bulk materials, so imaging them in splendid isolation could give a more revealing portrait of their true nature than can a group shot.

As research teams around the world develop new ways to capture individual molecules in motion, they are discovering that each technique has the potential to bring a different view of molecular behaviour into focus. Some are better at pinpointing atoms in space; others glimpse molecules in vanishingly small moments of time.

"The idea of a molecular movie covers a vast landscape," says Louis DiMauro, a physicist at Ohio State University in Columbus. "It's the difference between making an action movie or a Woody Allen picture." Yet together, he says, the methods promise to show how chemistry works in unprecedented detail. "A combination of these techniques — that's the way to produce a true molecular movie."



NIK SPENCER/*NATURE*

LIGHTS, CAMERA, ACTION!

Molecular cinematography traces its origins back to methods that emerged in the 1980s to capture snapshots of molecules. The leading technique — called pump–probe spectroscopy — uses a pulse of laser light lasting mere femtoseconds to trigger a chemical reaction (see 'Small world'). An instant later, a second femtosecond pulse arrives and interacts with the molecules in the sample, mid-reaction. This changes the light in ways that can be measured by a detector and turned into a 'picture' of the molecule. And by repeating the experiment over and over again while varying the delay between the two pulses, researchers can build a flip book of pictures showing each stage of a chemical transformation.

This technique, a form of femtochemistry, exposed the inner workings of chemical reactions as never before, revealing the identities of fleeting intermediates formed as one molecule transformed into another[1]. But the laser light used in femtochemistry has a wavelength much larger than the distance between individual atoms, so it cannot directly pick out the positions of atoms in molecules.

To get clear pictures of individual atoms, scientists have long relied on X-ray crystallography or electron diffraction, which study how photons or electrons scatter as they pass through molecules. Meanwhile, instruments such as STMs and atomic force microscopes (AFMs) offer even more detailed images of atoms in individual molecules and the shrouds of electrons around them. But those techniques usually take milliseconds or longer to acquire an image, much too slow to see atoms moving back and forth.

So in the past few years, molecular film-makers have combined various aspects of femtochemistry, diffraction and atomic imaging to create a toolbox of hybrid techniques that offer the best of different worlds, uniting temporal and spatial resolution to show atoms and molecules in their natural habitats.

Last year, researchers at the University of Regensburg in Germany used laser pulses to dramatically improve the shutter speed of an STM[2]. This kind of microscope relies on a sharp tip — narrowed to a single atom at its apex — that moves over a molecule stuck to a surface. Thanks to quantum behaviour at short distances, electrons can bleed, or 'tunnel', between the molecule and the tip, creating an electric current. As the tip moves, changes in the size of that current reveal the topography of electrons smeared around the molecule.

In the Regensburg experiments, the researchers triggered each snapshot by firing a laser pulse of terahertz (THz) radiation — between microwaves and infrared — at the STM tip. That created just enough difference in voltage between the tip and the target molecule, called pentacene, to allow an electron to tunnel out of the molecule. This passageway opened within a single cycle of the THz pulse, giving the STM a shutter speed of about 100 fs — short enough to produce a freeze-frame of pentacene's electron orbitals at that instant.

After it lost the electron, the pentacene molecule was yanked towards the surface by electrical forces, causing it to wobble up and down. The researchers used further THz pulses at various intervals to witness this kind of vibration for the first time. "There is no other way to see this oscillation in a single molecule," says physicist Jascha Repp, one of the leaders of the research team.

Although that experiment was essentially a proof of concept, Repp thinks his team can shrink the time resolution of THz-STM down to 10 fs, which could reveal even faster processes: electrons gliding across a molecule after it has absorbed light, or hydrogen ions hopping back and forth between different sites, a process called tautomerism that affects the reactivity of many biological molecules. "It could be transformational," says DiMauro. "You could watch reactions on a surface with atomic specificity."

Repp and physicist Leo Gross at IBM Research in Zurich, Switzerland, also hope to throw an AFM into the mix. This instrument has a sharp tip that acts like a record stylus as it skims over a sample, its quivering affected by slight attractions to — and repulsions from — the atoms and bonds beneath it, which offers sharper pictures of the molecule than an STM alone.

BLOCKBUSTER PRODUCTIONS

One of the attractions of STMs and AFMs is that the equipment — clusters of stainless-steel vacuum chambers and probes — can fit in a small laboratory. The techniques are the indie studios of molecular film-making, relatively accessible to many researchers.

At the opposite end of the scale are the blockbusters produced at facilities such as the US$414-million Linac Coherent Light Source (LCLS) at the SLAC National Accelerator Laboratory in Menlo Park, California. This gigantic X-ray free-electron laser (XFEL) produces bright, coherent pulses to reveal stunning protein structures. Competition for experimental time on the machine is fierce.

Last year, an international team of researchers reported using the

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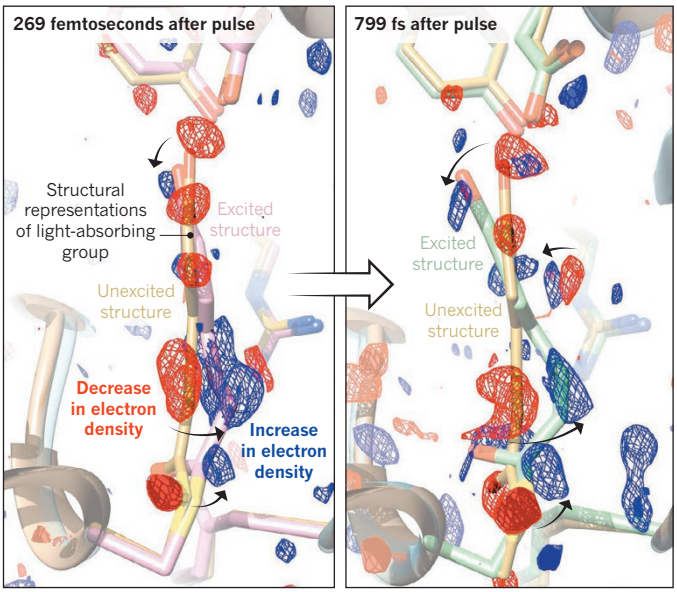
EXCITING LIGHT

X-ray pulses can capture structural changes in molecules such as photoactive yellow protein. Blue laser pulses excite the molecule and cause it to twist; this can be visualized by looking at changes in electron density.



LCLS's X-ray pulses to watch a key biological process for the first time. The team's target was photoactive yellow protein (PYP), a light sensor used by some bacteria[3]. At the heart of PYP is a light-absorbing region containing a rigid carbon–carbon double bond that cannot twist freely. The bulky groups at either end of the double bond usually point in opposite directions — a configuration called *trans*. But the team used a blue laser pulse to temporarily break one of the bonds, allowing the bulky groups to twist into a '*cis*' configuration, pointing in the same direction (see 'Exciting light'). This kind of *trans–cis* isomerism happens often in biological systems, such as the chemical process underlying vision.

The team followed the initial laser blast with a string of 40-fs-long X-ray pulses, which produced diffraction patterns that revealed the locations of the atoms. Stringing these together into a movie showed that the isomerization took place about 550 fs after light excited the PYP. "The big surprise is that it's not instant," says biochemist Petra Fromme of Arizona State University in Tempe, who was part of the team. "It completely changed our view of how this reaction happened."

This experiment targeted micrometre-scale crystals floating in solution, but other researchers have managed to use the LCLS to film individual molecules in a gas. In 2015, they produced a movie of a ring-shaped molecule breaking open[4] — a classic reaction in chemistry and biochemistry. The wavelength of the X-rays was too long to resolve atoms directly, so the team relied on theoretical simulations to sharpen the images into a 16-frame molecular movie. But a $1-billion upgrade called LCLS-II is now under construction and should offer shorter-wavelength X-rays, in briefer, more frequent pulses, which will improve the time and spatial resolution of the movies.

And Fromme hopes that a new generation of compact XFELs, potentially costing less than $15 million each, could open up the technique to many more scientists. She is currently working on two prototypes with collaborators, and says that next year could see the completion of the first one — called AXSIS and located at the German Electron Synchrotron (DESY) in Hamburg. These tabletop XFELs will produce X-ray pulses just a few hundred attoseconds (10^{-18} s) long, so brief that they will not destroy the target molecule.

Attosecond X-ray pulses from compact XFELs would not contain enough photons to produce clear pictures of single molecules; using them would be like taking a photograph in low light. But one idea under discussion is that a compact XFEL could feed its big brother at a facility such as SLAC with X-rays, electrons or both, to hone its brighter bursts.

If this did enable truly single-molecule XFEL imaging, Fromme would like to train the new camera on one of the most fundamental processes of nature: the moment a photon is absorbed by a biomolecule and forms an excited state. "Nobody has ever been able to see how fast that process is," she says.

A MOLECULAR SELFIE

The most energetic X-rays at the LCLS currently have a wavelength of 150 pm, slightly too long to pick out individual carbon or hydrogen atoms. To zoom in even more, researchers can use fast-moving electrons, which have much shorter wavelengths and offer better spatial resolution as they diffract through a molecule. This is the principle behind cryo-electron microscopy, which is currently revolutionizing the world of structural biology — not least because it provides detailed structures of proteins in frozen samples without the need to coax them to form crystals.

Although cryo-electron microscopy provides crowd shots of many molecules together, other techniques use electrons to image single molecules. Last year, a team led by Jens Biegert, head of research at the Institute of Photonic Sciences in Barcelona, Spain, reported[5] using laser-induced electron diffraction (LIED) to study individual molecules of acetylene (C_2H_2). In this technique, an infrared pulse lines up the molecule in a defined direction, and then a second pulse knocks two electrons out of it, breaking one of acetylene's carbon–hydrogen bonds.

Just like any form of light, these laser pulses are made of oscillating electric and magnetic fields. The electric field of the second pulse picks up one of the liberated electrons and sends it slamming back towards the molecule. The electron arrives about 9 fs after it first escaped, travelling fast enough to pass right through the fragmenting molecule. As it does so, it diffracts like a wave breaking over a rocky seashore, forming a pattern that reveals the positions of the atoms with a shutter speed of less than 1 fs. It is perhaps the ultimate molecular selfie.

Every time this happens, the electron diffracts in a slightly different direction, so Biegert's team had to run the process over and over again to gather enough data to build up clear pictures of the acetylene fragment and the hydrogen ion that splinters off it. After about one billion repetitions, each imaging a fresh molecule drawn from a gas supply, the team had made a few frames of a molecular movie that showed the bond breaking. The group hopes soon to increase the number of frames and to tackle more-complex molecules.

By imaging each molecule with just one of its own electrons, Biegert says, this LIED technique avoids key problems with conventional electron diffraction, which uses an 'electron gun' to fire bunches of electrons at ensemble samples of molecules. Those electrons repel each other as they fly, increasing the length of the pulse and making it difficult to set the shutter speed below 10 fs, he says.

In the next stage in molecular moviemaking, other researchers hope that moving from femtosecond to attosecond laser pulses will produce unprecedented slow-motion sequences. At those shutter speeds, atoms seem to move at a glacial pace, and electron movement comes into clear view. This will be a crucial step, says DiMauro, because the behaviour of electrons ultimately controls the motion of atoms in a molecule. "We've developed good techniques to watch atomic actors," he says. "But to watch the real movie, we also need to watch the electrons."

Most of the researchers involved also agree that it is high time to move on from demonstration projects, and to apply the techniques to research problems across a range of fields.

"If the people who are developing these tools can convince chemists and materials scientists, it will really give it a boost," says Biegert. After all, "the first step to understanding is seeing". ■

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