

A test for magnetic sensitivity: When Damien Faivre holds a test tube with a culture of magnetic bacteria first parallel and then perpendicular to a magnetic field, the medium's turbidity changes. The bacteria's magnetosome chains are indeed aligning with the magnetic field.

Magnets – Made by Microbes

Their medical importance would be considerable: magnetic nanoparticles such as those produced by magnetotactic bacteria could, among other applications, help detect tumors. **Damien Faivre** and his colleagues at the **Max Planck Institute of Colloids and Interfaces** in Potsdam are studying how these microbes work in order to harness their sophisticated mechanisms.

TEXT **CHRISTIAN MEIER**

A compass has always been an indispensable tool, not only for mariners wishing to reach their destination. Certain aquatic bacteria also navigate using Earth's magnetic field. Their inner compass consists of a chain of tiny nanoparticles of the magnetic mineral magnetite.

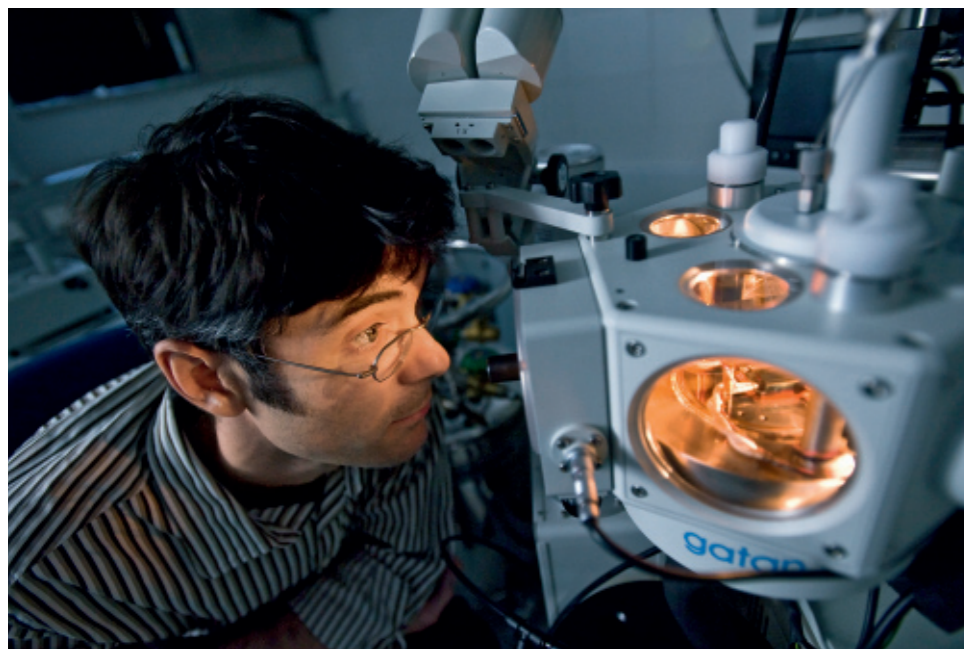
These particles are produced by the bacteria themselves and have such unique magnetic properties that they are of great interest for medical and other technology applications. To date, however, only nature knows how they are produced. Damien Faivre, a chemist working at the Max Planck Institute of Colloids and Interfaces in Potsdam, hopes to unravel the secret with the help of his seven-man team. Once the researchers understand how the bacteria produce the nanoparticles, they hope it will be possible to develop a procedure to manufacture the particles, first in the test tube and later on an industrial scale.

In 1975, American microbiologist Richard Blakemore discovered that some aquatic organisms navigate along the Earth's magnetic field, and named

them accordingly: magnetotactic bacteria. However, these microbes do not seek out the North Pole, but the deeper regions of their aquatic environment. The magnetic field lines that lie away from the equator do not run parallel to the Earth's surface but point downward. This guides the magnetotactic bacteria toward the deeper waters, where sediment and water mix. These oxygen-starved areas provide the ideal conditions for the bacteria to live and flourish. They are not able to use gravity for vertical orientation, since they are nearly as dense as water and thus do not perceive their weight.

GUIDED TO THE BOTTOM BY A COMPASS

The bacteria owe their compass to the magnetosomes, organelles consisting of a single particle of magnetite (Fe_3O_4) that measures less than 100 nanometers and is surrounded by a membrane to prevent the particles from clumping together. Some 20 magnetosomes form chains along protein fibers in the bacterium. They work like the needle of a



compass, turning in the direction of Earth's magnetic field, approximately in a north-south orientation. Since the magnetosomes bind to the protein fibers of the bacterium, the whole microbe turns with them. Then, when the microbes rotate their flagella, they move along the magnetic field lines toward the bottom of the body of water, as if on rails.

"The bacteria generate perfect magnetic nanoparticles," affirms Faivre. First, magnetotactic bacteria produce the particles in a uniform size – a feat

that should make chemical engineers sit up and take notice, as uniform particle size is an important mark of quality in nanoparticle production. "Not only that, but the bacteria can even control the shape of the particles," adds Faivre. Some types of magnetotactic bacteria produce bullet-shaped nanoparticles, while others make needle-shaped ones. In fact, each type of bacteria creates its particles in a uniform shape. In short, these bacteria boast perfect internal quality control in the synthesis of magnetite particles.

The magnetic properties of the particles are of enormous interest for technical applications. "They display a remanence and coercivity that cannot be matched by artificially produced crystals," says Faivre. These two physical parameters mean that the materials are magnetically hard, so their permanent magnetism remains very stable. This is a desirable property in many technical applications, for instance for magnetic data storage with unprecedented bit density.

MAGNETITE PARTICLES FOR TUMOR DETECTION

Other applications require uniform magnetic properties, and this is exactly what the bacteria's magnetic nanoparticles offer, thanks to their uniform shape and size. Artificially created elongated magnetic particles could be used as a contrast agent in magnetic resonance imaging. Tissues holding the particles would show up as darker areas on the images. If the particles could be guided to a tumor, its location could be pinpointed at an early stage. The particles could also be used to ensure that drugs target the focused area of a disease. By positioning magnets outside the body, the particles would remain in those areas. The active substances bound to the particles would thus remain in the tissue where they are needed, instead of being flushed along in the bloodstream.

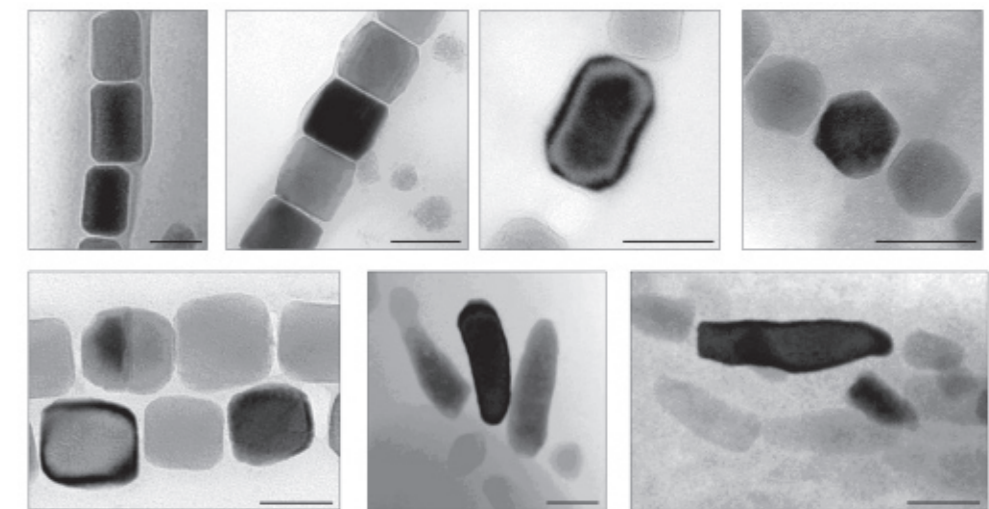
Although it is possible to create magnetite particles in the laboratory, these synthetic particles, unlike their biological counterparts, contain a small amount of oxygen. Damien Faivre's team discovered this while

- left | A close-up look at a compass: Damien Faivre inserts a sample of magnetotactic bacteria into an electron microscope and then checks that it is correctly positioned.
- right | Compass needles as a gauge: Different kinds of magnetotactic bacteria produce very specific magnetite particles of characteristic size and shape.

studying the crystal structure and chemical composition of the magnetic nanoparticles using X-ray radiation from the Berlin-based synchrotron radiation facility, BESSY.

There are other difficulties with the synthetic production of magnetic nanoparticles: "So far, the available chemical processes cannot produce magnetic nanoparticles of uniform size and shape in environmentally friendly conditions," explains Faivre. In this case, environmentally friendly would mean that the particles could be produced at room temperature, normal atmospheric pressure and without harmful solvents, instead of the energy-intensive conditions of high pressure and high temperature. With this in mind, Faivre wants to understand how nature manages to produce the uniform magnetic particles. "Nature shapes material down to the smallest detail, literally down to the smallest unit, the molecule," he says. "We can learn from nature by trying to understand how natural models influence complex physicochemical and biological phenomena. As soon as the biological processes are fully understood, it should be possible to copy them in order to develop new materials."

The researchers have already made some initial discoveries about how nature produces magnetite nanoparticles. Magnetotactic bacteria control the growth of the magnetic particles through a biological process called biomineralization – another way of saying biologically controlled crystal growth. Some 20 to 30 proteins called magnetosome proteins are responsible for this process. Biologists have also discovered which sections of the bac-



terial genome contain the genetic information that encodes the magnetosome proteins.

LAB TESTS REVEAL THE FUNCTIONS OF INDIVIDUAL PROTEINS

Damien Faivre and his team now hope to identify the roles played by individual proteins and their components in biomineralization. There are essentially two methods they can use in this quest. The first involves the generation of "deletion mutants": bacteria in which a given gene has been deactivated. Except for that single inactive gene, the genome of the mutant is identical to that of the wild type. As researchers study the differences between bacteria with the inactive gene and their unaltered counterparts, they can learn about the role of the specific gene. They check whether the deletion mutant produces magnetosomes, and if so, whether they occur in the same size

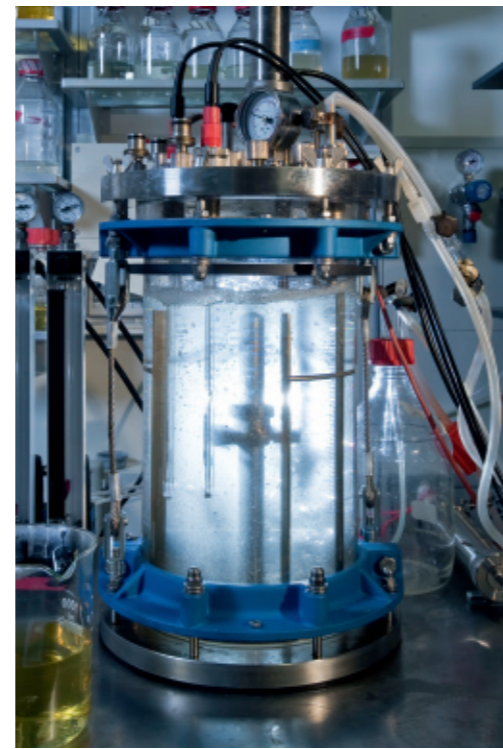
and shape as in the wild type. This method does deliver worthwhile results, but "Since magnetotactic bacteria grow very slowly, the in vivo process is a very protracted one," laments Faivre. It can take up to two years to study a single gene or protein.

For this reason, his team uses a second, more efficient method to shed light on the functions of the magnetosome proteins. They insert the gene of the relevant protein into the genome of the fast-growing bacterium *Escherichia coli*. The cell machinery of this microbe, induced by its genetic information to produce proteins, is stimulated to manufacture particularly large amounts of the implanted magnetosome protein. This is necessary so that researchers can achieve the same protein concentration in the test tube as occurs in the far smaller magnetotactic bacterium.

Finally, researchers isolate the proteins and study their properties in the test tube. To this end, they mix the pro-



Janet Andert (left) places a culture of magnetotactic bacteria in a fermenter (detail image at right). Meanwhile, Antje Reinecke adjusts the conditions to ensure optimum propagation of the microbes.



tein with iron compounds that, like magnetite, contain divalent and trivalent iron, gradually altering the pH of the solution until its components are precipitated and magnetite particles are formed. During this process, the protein influences the size or the shape of the particle being formed. "This method enables us to study a single protein in three to four months," says Faivre.

It is clear from the outset that not all magnetosome proteins are equally important for magnetosome production, so before they begin to study individual proteins, the researchers gather information on which ones are more likely to play key roles in biomineralization. This saves time by avoiding unnecessary test tube experiments.

One of the screening tools used for protein preselection is bioinformatics software. This enables the team to identify similarities between the genes of different magnetotactic bacteria. Any such similarities indicate important genes and thus important proteins. Another method used is bio-combinatorial engineering. Here, the researchers study which peptides, or protein portions, bind to the surface of magnetite crystals. These peptides enable direct contact between proteins and magnetic particles, and could therefore be components of important magneto-

some proteins. At the very least, the direct contact with the magnetic particle would imply that the relevant protein must have an important function. The peptides are then translated into DNA sequences – that is, the language of genetic information. Using these sequences, a computer program scans the genome of magnetotactic bacteria to identify the associated proteins.

A PROTEIN THAT CONTROLS PARTICLE SIZE

So far, Japanese and American researchers have clarified the role of one magnetosome protein in the synthesis of magnetite particles inside magnetosomes. The protein bears the unimpressive name Mms6 and is found only in magnetotactic bacteria. Mms6 is located in the membrane surrounding the magnetite particle, and scientists have discovered that it codetermines the size of the magnetic nanoparticles. To date, it is the only protein known to play a decisive role in vitro in controlling particle size. Scientists have yet to discover any proteins that determine the shape of the magnetite crystals.

In studying Mms6, researchers happened across another significant phenomenon: the peptide at one end of the protein, consisting of 25 amino ac-

ids and therefore constituting only a small portion of Mms6, influences the size of the magnetite particle. Faivre explains that this finding is vitally important, "because the artificial mass production of proteins using host organisms is limited, whereas synthetic peptides can be produced in practically unlimited quantities."

Meanwhile, the search continues for proteins involved in the formation of the magnetic nanoparticles. "Up to now, 20 protein candidates have been identified in the magnetosome membrane of the magnetotactic bacterium *M. gryphiswaldense*, and it is assumed that they have particular effects on the size and shape of magnetite crystals," explains Faivre.

Some of these candidates are currently being studied by Faivre's team at the Max Planck Institute in Potsdam. The team is also researching how individual magnetosomes link together to create a chain so that, bit by bit, the tiny compass needle is formed to guide the microbe to its food. They have shown that the formation of the chain involves a complex interaction of genetically controlled processes and magnetic forces. One of the methods used was "ferromagnetic resonance spectroscopy," which is similar to nuclear magnetic resonance. FMR, as it is called, allows the

magnetic properties of solid bodies to be examined. It can show, for example, the preferred orientation, if any, of a magnetized crystal. It allows scientists to study both the individual magnetite particles and the particle chains.

Faivre and his colleagues hope that their research will identify all the pro-

teins and other biological components (especially lipids) that direct the biomineralization of magnetite particles. The key for them is to understand the different roles the proteins play in particle synthesis. "Then it would almost be possible to make magnetic particles measuring 20, 50 or 100 nanometers in diame-

ter to order, even specifying that they should be, say, round or needle-shaped," says the biochemist. It would simply be a case of selecting the relevant proteins, like tools from a toolbox. For now, it is only a dream – but the Potsdam research team has taken the first steps toward making it a reality. ◀

GLOSSARY

Magnetosome

A magnetite particle enclosed by a membrane. Each particle measures less than 100 nanometers; different types of magnetotactic bacteria produce them in various characteristic shapes and sizes.

Magnetotaxis

The ability of some life forms to orient along the Earth's magnetic field.

Magnetic resonance imaging

Also called nuclear magnetic resonance. A procedure based on the fact that some atoms, such as hydrogen, have a magnetic moment. Their behavior in a magnetic field depends on the tissue in which they are located. This allows different tissue types to be identified. Certain magnetic substances can be used to enhance the contrast between them.

Biomineralization

Organisms use organic and inorganic substances to produce inorganic minerals and compound materials very precisely, controlling their production through biochemical processes. Notable examples, apart from magnetosome crystals, include mother of pearl and the silica skeletons of diatoms.