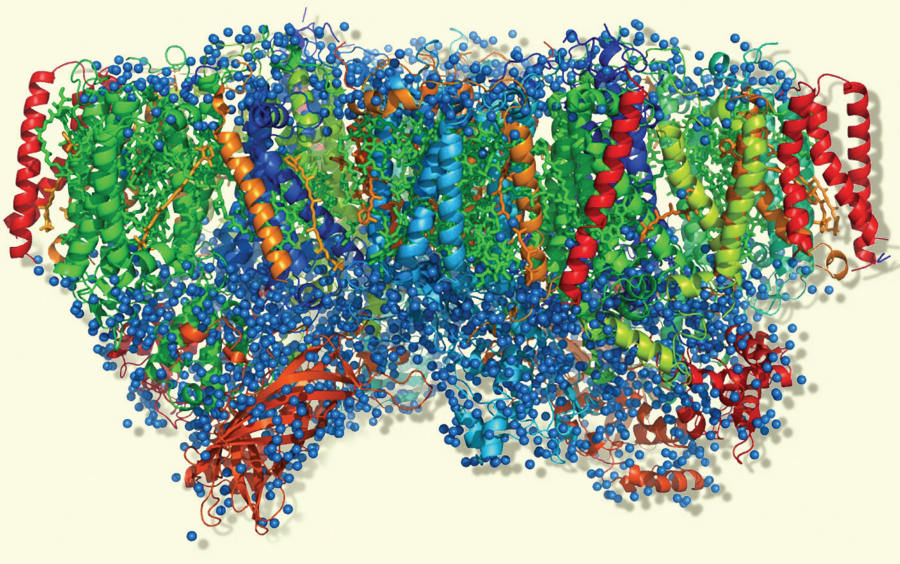
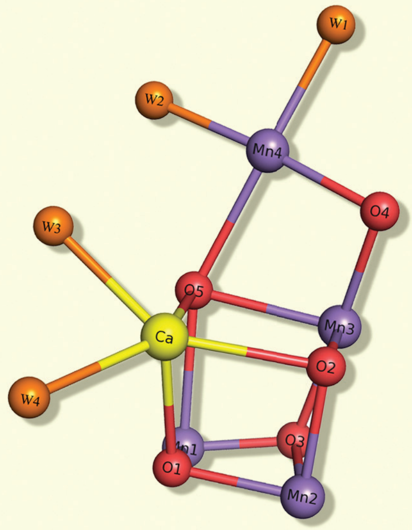
PHOTOSYNTHESIS RESEARCH at Okayama University





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PHOTOSYNTHESIS RESEARCH at Okayama University

Dean of the Graduate School of Natural Science and Technology, Professor Shen (center), with President of Okayama University, Kiyoshi Morita (right)

If it weren't for a tiny cluster of manganese, calcium, and oxygen atoms found inside the chloroplasts of plant cells, Earth would be a very different planet. Those atoms form the catalyst that allows plants to split water molecules using sunlight—the first step in photosynthesis. Without them, plants and other photosynthetic organisms wouldn't be able to use solar energy to grow, and they wouldn't give off the oxygen we breathe. Yet exactly how that key catalyst works is still a mystery.

Researchers at Okayama University in central Japan are leading the search for an answer. Two years ago, they published the most detailed images ever seen of the catalyst and the protein that carries it, drawing international attention. Now they are working to capture images of the water-splitting process in action. Their findings could help revolutionize the way we power human civilization.

"Right now we depend on fossil fuels for energy, but in dozens or hundreds of years those will eventually be used up and we will have to depend on the energy of sunlight," says Jian-Ren Shen, a professor of molecular biophysics at the university and head of a new photosynthesis research center there. "If we can understand the mechanistic basis of this reaction, then we may be able to synthesize an artificial catalyst to split water into protons and electrons. These can then be recombined to generate hydrogen gas."

The idea is to store the sun's power not in the bonds of sugar molecules, like plants do, but in fuels that humans can use to drive cars or power factories. Hydrogen is one option; methanol, produced by combining hydrogen with carbon dioxide and oxygen, is another. Both fuels are easy to store and transport, which gives "artificial photosynthesis"—and the solar fuels it generates—an advantage over solar electricity.

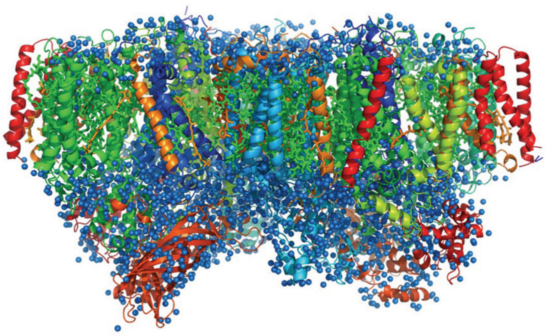
Scientists have already developed a number of artificial photosynthetic systems that work in the laboratory. So far, however, no artificial system converts solar energy to chemical energy as efficiently as plants do. (Seventy to eighty percent of the solar energy captured by chloroplasts is retained through the first step of photosynthesis, although only between 0.1 and 3 percent typically makes it to the final step of conversion to sugars.) Another problem is that the materials in many artificial catalysts are neither cheap nor abundant. Plants could inspire better alternatives. "It may be difficult to exactly mimic the natural systems, but by understanding their principals, we can make something similar to them," Dr. Shen says.

His investigation of those principals began over 20 years ago. At the time, he was studying how atmospheric pollutants affect plant growth. He noticed that pollution lowered photosynthetic activity, but when he tried to figure out exactly which step was inhibited, he encountered a problem: scientists didn't fully understand photosynthesis itself. Dr. Shen decided to shift the focus of his research to clarifying the first step in the complex chain of reactions, during which photons from sunlight enter a protein called photosystem II (PSII) and split water molecules into oxygen, hydrogen ions, and electrons. Because the bonds in water molecules are too strong to be broken by sunlight alone, the reaction requires a catalyst to lower the amount of energy that's needed. Dr. Shen wanted to identify and characterize that catalyst.

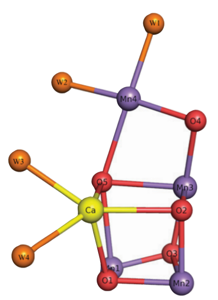
The goal would prove a difficult one to achieve. PSII is a complex of 20 different subunits, some of which are bound together only weakly; the proteins Dr. Shen was working with contained more than 50,000 atoms each (excluding hydrogen). These had to be extracted intact from cells and then purified—a "major difficulty," he says. He began experimenting with a number of combinations of detergents, salts, and pH levels to figure out which one dissolved the substances surrounding PSII most effectively without harming the protein itself.

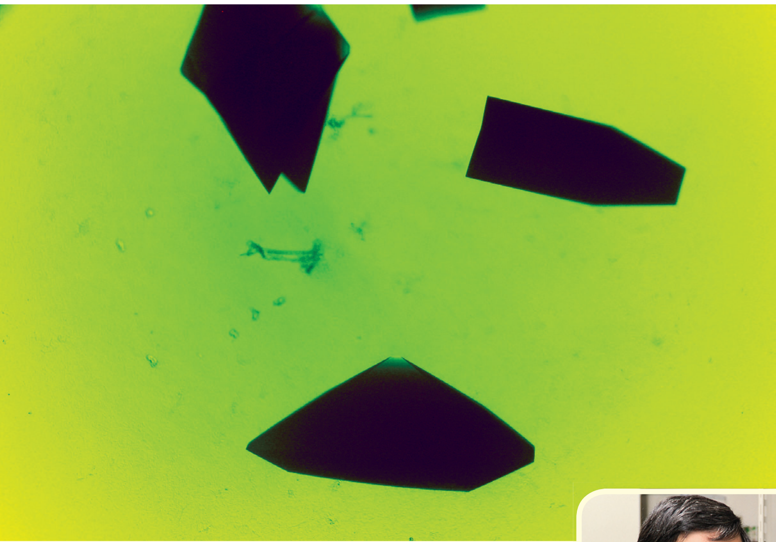
At the same time, he began a series of experiments to find the best conditions for turning the solution of proteins into high-quality crystals.

Protein Structure of Photosystem II



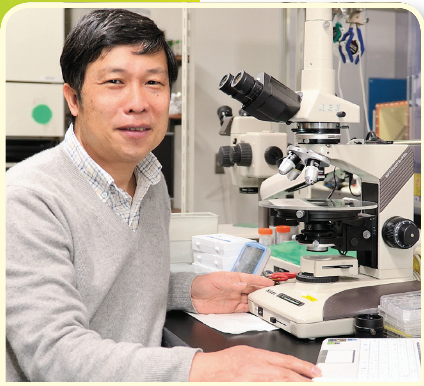
Catalyst for Water Splitting





Crystals of Photosystem II

Electromagnetic radiation was fired at the crystals to reveal the arrangement of atoms within them, a process called X-ray crystallography. But the larger the protein, the more imperfections its crystals tend to have, and the lower the resolution of images. PSII is enormous with a molecular weight of around 700 kDa. It took Dr. Shen and his colleagues 13 years to grow crystals that yielded images at a resolution of 3.7 Å. However, the bonds separating atoms in PSII's catalytic center are as short as 1.8 Å, so Dr. Shen still couldn't decipher its structure.



Professor Shen in the Lab

In 2003, he joined the faculty at Okayama University and continued his experiments there. The school was an ideal place for him to do the work, with its history of pioneering photosynthesis research reaching back to the 1960s. But in the United Kingdom and Germany, research teams were also homing in on PSII's catalytic center. By 2009, German scientists had captured images of the protein at a resolution of 2.9 Å.

That same summer Dr. Shen and his graduate students grew a batch of crystals that seemed promising. They brought them to SPring-8, one of the world's top synchrotron facilities for X-ray crystallography, located just an hour and a half from Okayama University. There they gathered data on the protein's structure in collaboration with members of a team led by Nobuo Kamiya, a professor of structural biology at Osaka City University. Dr. Kamiya took the data back to his lab for analysis.

What came out of the data was the most detailed map of PSII ever seen, making PSII the largest membrane-protein complex with its structure solved at an atomic resolution. At a resolution of 1.9 Å, the exact arrangement and number of atoms in the catalytic core became clearly visible. One calcium, four manganese, and five oxygen atoms were arranged in what Dr. Shen describes as a "distorted chair" shape surrounded by four water molecules. One back corner of the "seat" appeared slightly raised, which meant the bonds between it and the atoms adjacent to it were longer—and probably weaker—than other bonds in the structure. "These bonds can move and even be easily cleaved," Dr. Shen explains. He suspects this flexibility is important during the catalytic reaction.

Drs. Shen and Kamiya published their findings in *Nature* in 2011, to international acclaim. *Science* magazine named the research one of the top ten breakthroughs of the year, and in 2012 they won a prestigious Asahi Prize for their work. But by then Dr. Shen was already focused on the next step forward: investigating how the PSII catalyst functions during the water-splitting reaction. So far, he has a snapshot of the opening scene. He wants a feature film. His strategy is to illuminate the protein with a single photon, allow the reaction to reach an intermediate stage, and then quickly lower the temperature to freeze the catalyst in action for imaging.

Other researchers around the world are racing to do the same thing. Dr. Shen's group, however, has the advantage of excellent crystals as well as the use of a new imaging facility called SACLA that opened within the campus of SPring-8 in March 2012. SACLA is an X-ray Free Electron Laser that can reveal the movement of atoms and molecules in real time—something even the extremely powerful SPring-8 X-ray beams cannot do. That technology will be key to achieving Dr. Shen's current research goal.

Okayama University has thrown its full support behind the groundbreaking research. In April of 2013, the school established a new Photosynthesis Research Center focused on three core projects, one of which belongs to Dr. Shen. The second project, led by molecular biologist Yuichiro Takahashi, is looking at how environmental stresses like drought or excessive sunlight impact photosynthesis, work that holds important implications as climate change begins to affect agricultural productivity. The center's third project is led by inorganic chemist Takayoshi Suzuki, who is applying Dr. Shen's findings to research into synthetic compounds that split water with sunlight. Together, these projects promise not only to keep Okayama University at the forefront of fundamental research, but also to advance the practical applications that are key to a sustainable future.

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