Elucidating the mechanism of a light-driven sodium pump

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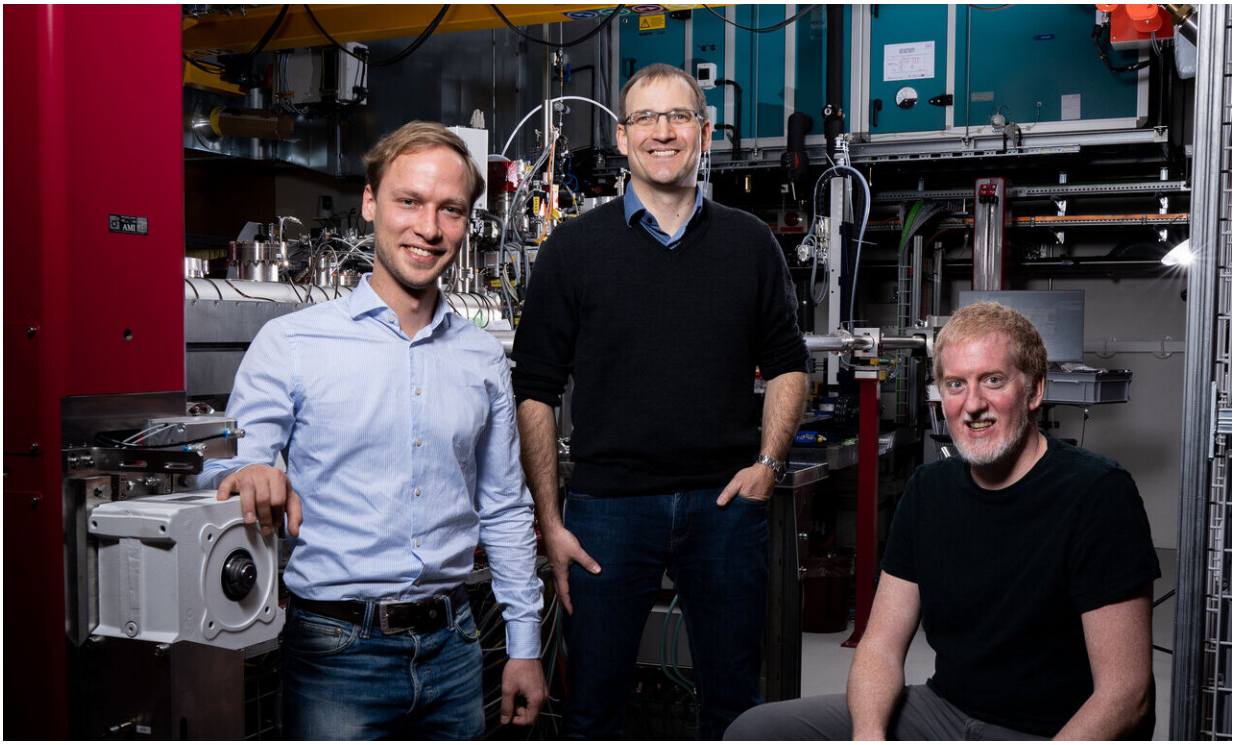

Petr Skopintsev (left), Jörg Standfuss (centre) and Christopher Milne (right) at the Alvra experimental station at the X-ray free-electron laser SwissFEL Credit: Paul Scherer Institute/Mahir Dzambegovic

Researchers at the Paul Scherrer Institute PSI have succeeded for the first time in recording a light-driven sodium pump from bacterial cells in action. The findings promise progress in the development of new

methods in neurobiology. The researchers used the new X-ray free-electron laser SwissFEL for their investigations. They have published their findings today in the journal *Nature*.

Sodium plays an essential role in the vital processes of most biological cells. Many cells build up a concentration gradient between their interior and the environment. For this purpose, special pumps in the cell membrane transport sodium out of the cell. With the help of such a concentration gradient, cells of the small intestine or the kidneys, for example, absorb certain sugars.

Such sodium pumps are also found in the membranes of bacteria. They belong to the family of the so-called rhodopsins. These are special proteins that are activated by light. For example, rhodopsins transport sodium out of the cell in the case of bacteria living in the ocean, such as Krokinobacter eikastus. The crucial component of rhodopsin is the so-called retinal, a form of vitamin A. It is of central importance for humans, animals, certain algae and many bacteria. In the retina of the human eye, for example, retinal initiates the visual process when it changes shape under the influence of light.

Lightning-fast movie making

Researchers at the Paul Scherrer Institute PSI have now succeeded capturing images of the sodium pump of Krokinobacter eikastus in action and documenting the molecular changes necessary for sodium transport. To do this, they used a technique called serial femtosecond crystallography. A femtosecond is one-quadrillionth of a second; a millisecond is the thousandth part. The sample to be examined—in this case a crystallised sodium pump—is struck first by a laser and then by an X-ray beam. In the case of bacterial rhodopsin, the laser activates the retinal, and the subsequent X-ray beam provides data on structural changes within the entire protein molecule. Since SwissFEL produces

100 of these femtosecond X-ray pulses per second, recordings can be made with high temporal resolution. "We can only achieve temporal resolution in the femtosecond range at PSI with the help of SwissFEL," says Christopher Milne, who helped to develop the Alvra experimental station where the recordings were made. "One of the challenges is to inject the crystals into the setup so that they meet the pulses of the laser and the X-ray beam with pinpoint accuracy."

Pump in action

In the current experiment, the time intervals between the laser and X-ray pulses were between 800 femtoseconds and 20 milliseconds. Each X-ray pulse creates a single image of a protein crystal. And just as a cinema film ultimately consists of a large number of individual photographs that are strung together in a series and played back rapidly, the individual pictures obtained with the help of SwissFEL can be put together to form a kind of film.

"The process that we were able to observe in our experiment, and which roughly corresponds to the transport of a sodium ion through a cell membrane, takes a total of 20 milliseconds," explains Jörg Standfuss, who heads the group for time-resolved crystallography in the Biology and Chemistry Division at PSI . "Besides elucidating the transport process, we were also able to show how the sodium pump achieves its specificity for sodium through small changes in its structure." This ensures that only sodium ions, and no other positively charged ions, are transported. With these investigations, the researchers also revealed the molecular changes through which the pump prevents sodium ions that have been transported out of the cell from flowing back into it.

Advances in optogenetics and neurobiology

Since sodium concentration differences also play a special role in the way nerve cells conduct stimuli, neurons have powerful sodium pumps in their membranes. If more sodium flows into the cell's interior, a stimulus is transmitted. These pumps then transport the excess sodium in the cell to the outside again.

Since the sodium pump of Krokinobacter eikastus is driven by light, researchers can now use it for so-called optogenetics. With this technology, cells, in this case nerve cells, are genetically modified in such a way that they can be controlled by light. The pump is installed in nerve cells using methods of molecular genetics. If it is then activated by light, a neuron can no longer transmit stimuli, for example, since this would require an increase in the sodium concentration in the nerve cell. However, bacterial rhodopsin prevents this by continuously transporting sodium out of the cell. Thus active sodium pumps render a neuron inactive.

"If we understand exactly what is going on in the sodium pump of the bacterium, it can help to improve experiments in optogenetics," says Petr Skopintsev, a Ph.D. candidate in the time-resolved crystallography group. "For example, it can be used to identify variants of bacterial rhodopsin that work more effectively than the form that is usually found in Krokinobacter." In addition, the researchers hope to gain insights into how individual mutations can change the ion pumps so that they then transport ions other than sodium.

More information: Femtosecond-to-millisecond structural changes in a light-driven sodium pump, *Nature* (2020). DOI: 10.1038/s41586-020-2307-8 , www.nature.com/articles/s41586-020-2307-8

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