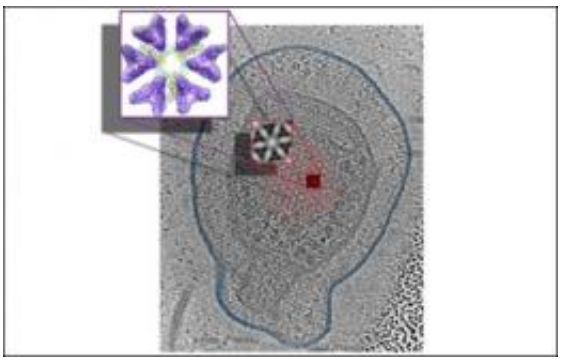
Understanding bacterial sensors: Researchers piece together model of chemoreceptor arrays

February 29 2012, By Kimm Fesenmaier



Researchers have built the first model that depicts precisely how chemoreceptors and the proteins around them are structured at the sensing tip of bacteria. The architecture, showing trimers of receptor dimers in purple, is based on ECT data (background) and new crystallography results. Credit: Briegel et al./Caltech

(PhysOrg.com) -- Nearly all motile bacteria can sense and respond to their surroundings—finding food, avoiding poisons, and targeting cells to infect, for example—through a process called chemotaxis. This allows the bacteria to move towards chemicals they are attracted to, and away from ones that repel them. Because chemotaxis plays a critical role in the first steps of bacterial infection, a better understanding of the process could pave the way for the development of new, more effective antibiotics. Researchers at Caltech are helping to reveal just how chemotaxis works.

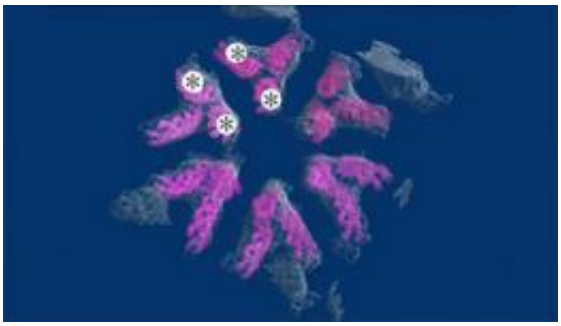
In bacteria, the sensing process begins with chemoreceptors—proteins that extend, like tiny antennae, from the cell body to the exterior of the cell. Chemoreceptors bind to attractants, like sugars and amino acids, and to repellents, like metals; they then send signals to motors controlling the whiplike flagella that steer the swimming bacterium in a particular direction.

In an effort to better understand chemotaxis, Grant Jensen, a professor of biology at Caltech, has been working with research specialist Ariane Briegel to determine the exact arrangement of these exquisitely sensitive receptors. Using advanced electron microscopy techniques and new crystallography results, Jensen and Briegel, working with researchers from Cornell University, have built the first model that depicts precisely how chemoreceptors and the proteins around them are structured at the sensing tip of bacteria. Their results appeared recently in the *Proceedings of the National Academy of Science* (*PNAS*).

The entire chemotaxis system functions with about 11 proteins, making it one of the simplest examples of a signal transduction pathway (a system in which the activation of a receptor leads to any number of chemical steps that produce a specific response—in this case, a bacterium swimming in a particular direction). In humans, signaling pathways control everything from development and tissue repair to immunity and aspects of brain function; defects in such pathways produce diseases such as diabetes and cancer. In animal cells, a signal transduction pathway might include 500 proteins. The relatively simple pathway producing the chemotaxis system, therefore, “is the best starting point to understand a full signal transduction pathway," Briegel says.

In 2009, Jensen's group was able to get the first glimpse of the chemoreceptor architecture. To see it, the researchers used a state-of-the-art electron microscope, purchased by Caltech using a gift from the Gordon and Betty Moore Foundation, that enabled them to observe

bacterial cell samples in a near-native state. Unlike traditional electron microscopy—for which samples must be fixed, embedded in plastic, sectioned, and stained—the new imaging technique, called electron cryotomography (ECT), involves freezing samples so quickly that they become trapped within a layer of transparent, glasslike ice. The microscope can then capture many high-resolution images as the sample is rotated.

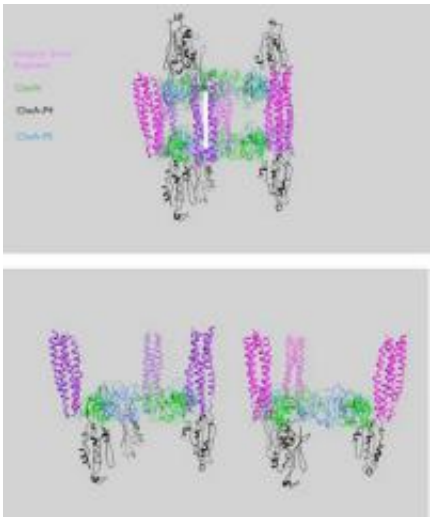


Six chemoreceptors (pink) are located at each corner of each hexagon, arranged as "trimers of dimers." In this arrangement, two dimers of each trimer face two from the next trimer.Credit: Briegel et al./Caltech

With that first look, three years ago, Jensen and Briegel discovered that chemoreceptors are arranged in a regular, repeating lattice of hexagons that are 12 nanometers apart, center-to-center.

But Jensen and Briegel knew they were not seeing the whole picture. Arriving at the complete model required a multistep effort. First, Briegel used improved sample preparation and data-processing procedures to generate even higher resolution images of the honeycomblike chemoreceptor arrays. These new higher-resolution pictures allowed her to determine the precise arrangement of the receptors in these arrays: she discovered that six chemoreceptors are located at each corner of

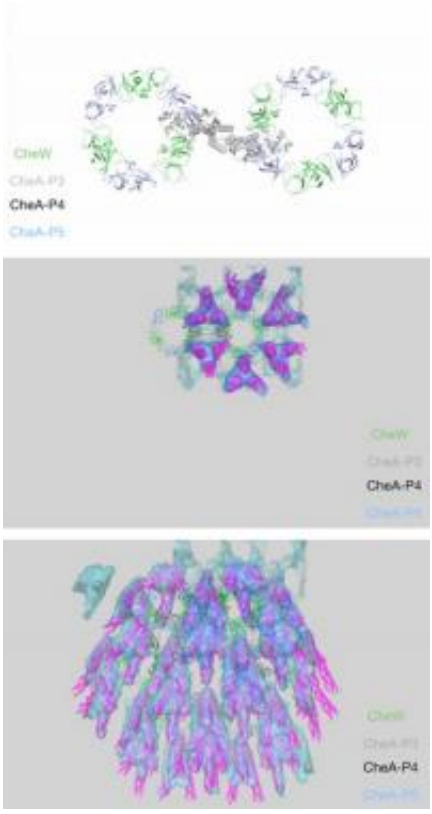
each hexagon. The chemoreceptors are arranged in a pattern that scientists call "trimers of dimers"—that is, groups of three sets of two pairs of receptors. The trimers are arranged such that each pair points toward a center of a hexagon.



Brian Crane of Cornell University solved a new crystal structure (top) featuring a double ring of chemoreceptor fragments (pink and purple) and parts of CheA (black and blue) and CheW (green). Split into two (bottom), the receptor fragments in the crystal structure lined up perfectly with Briegel's ECT images. Credit: Briegel et al./Caltech

Biologists have long known that two additional proteins, called CheA and CheW, are also found within groups of chemoreceptors. These proteins were thought to hold the receptors together and to activate a protein that then binds to the flagellar motors and causes a change in its spinning direction. But no one knew exactly how CheA, CheW, and the receptors were linked.

Understanding that, Jensen says, is "a huge step forward."



By rotating the model with two CheA proteins about part of the CheA pair,the team wound up with two connected rings (top) with the proteins and receptors in the same plane. The spacing between those rings explains the hexagonal lattice Jensen's group saw with ECT (middle and bottom). Credit: Briegel et al./Caltech

For help with the next piece, Caltech researchers teamed up with Brian Crane of Cornell University, who then solved a crystal structure featuring a double ring of chemoreceptor fragments and parts of CheA and CheW. While viewing a computer model of the structure, the researchers realized that splitting the double ring into two and then lining up the receptor fragments in each ring with the receptors in Briegel's ECT images produced a perfect match.

CheA never works alone: it forms in pairs. The ring of Crane's crystal

structure, however, only contained part of one CheA. So Crane's group used data from electron spin resonance (ESR) and crystallography experiments to build a model with two CheA proteins. The team discovered that simple rotations of part of the CheA pair brought all of the proteins and receptors into the same plane and produced two connected rings.

"The spacing between those rings explains the hexagonal lattice we see with ECT," says Briegel. "For the first time we have a very convincing model of how this whole receptor array is put together."

The group's next step is to determine what structural changes take place when an attractant binds to a chemoreceptor to send a signal to the flagella motors. Having a model for the whole receptor array, Jensen says, makes that task easier. "Seeing the arrays was one thing," he says. "Now, seeing the receptors with all the helper molecules and how they're arranged and linked together, we have a chance of understanding what happens when one of them gets activated."

Along with Briegel, Jensen, and Crane, additional authors on the *PNAS* paper, "Bacterial chemoreceptor arrays are hexagonally packed trimers of receptor dimers networked by rings of kinase and coupling proteins," are Xiaoxiao Li and Alexandrine Bilwes of Cornell, and Kelly Hughes of the University of Utah. The work was supported by the Howard Hughes Medical Institute and the National Institutes of Health.

Provided by California Institute of Technology