

Researchers get first 3-D glimpse of bacterial cell-wall architecture

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The bacterial cell wall that is the target of potent antibiotics such as penicillin is actually made up of a thin single layer of carbohydrate chains, linked together by peptides, which wrap around the bacterium like a belt around a person, according to research conducted by scientists at the California Institute of Technology (Caltech). This first-ever glimpse of the cell-wall structure in three dimensions was made possible by new high-tech microscopy techniques that enabled the scientists to visualize these biological structures at nanometer scales.

"This is both a technological and biological advance," says Grant Jensen, associate professor of biology at Caltech, a Howard Hughes Medical Institute investigator, and the principal investigator on the study.

Their research appears in the online early edition of the *Proceedings of the National Academy of Sciences (PNAS)*.

"Bacterial cells rely on a cage-like net that surrounds them to maintain their integrity," Jensen explains. "If it weren't for this molecular bag, the bacteria couldn't survive; they would likely rupture."

This bag, called a sacculus, is made out of peptidoglycan, a mesh-like structure of carbohydrates (glycans) and amino-acid peptides. It is the sacculus, Jensen notes, that is targeted by the antibiotic penicillin; penicillin blocks a bacterium's ability to grow and remodel the bag to fit it as the bacterium itself grows. "If the bug can't make this bag," Jensen says, "it can't multiply, and you get better."

Researchers have long been interested in understanding the precise architecture of the sacculus. In particular, Jensen and his colleagues have wondered whether the so-called glycan strands--which are cross-linked by peptides to create peptidoglycan--"wrap around the cell like a belt wraps around a person," or whether they stand up from the surface of the bacterial cell, "like grass."

The answer to this debate has eluded the scientists, however, because trying to image such tiny biological objects has been beyond their technological reach. Until now, that is.

"Six years ago, a gift from the Moore Foundation allowed us to buy what is arguably the world's best electron cryomicroscope," says Jensen. "This allowed us to take a different kind of picture of small biological objects than has ever been possible before. These pictures are 3-D images to molecular resolution--you can actually start to see individual biological molecules. Using it, we were able to see this network of glycan strands. It was just remarkable."

By pairing the electron cryotomography and a purification technique that involved removing the sacculi and flattening them in a very thin layer of water, postdoctoral scholar Lu Gan, the paper's first author, was able to image the peptidoglycan structure in three dimensions, which allows for a virtual 3-D tour of the bacterial sacculus.

"What we saw were long skinny tubes wrapping around the bag like the ribs of a person or a belt around the waist," says Jensen. "We also saw that the sacculus is just a single layer thick."

"This is a clear answer to this old question," adds Gan. "We now know what the architecture of this most basic shape-determining molecule is. We now know the right answer versus having a family of answers, some of which are wrong."

Understanding how the cell wall is built is important, says Jensen, because scientists have long been in the dark about some of the most basic physical and mechanical aspects of bacterial life, including why they are shaped the way they are. "It's hard to understand how a building is constructed unless you can see the studs," he explains. "Now that we can see the studs--now that we can see the basic architecture of the sacculus--we're closer to understanding how a bacterium could direct its own growth, and how drugs that block that process might work."

Source: California Institute of Technology

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