Bacteria's Achilles heel uncovered by single molecule chemistry

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Bacteria do not give up their secret weaknesses easily. Associate Professor Nikos Hatzakis, University of Copenhagen, used every trick in the nano-technological workbook to get a glimpse of an unknown Achilles heel of bacteria. Credit: Jes Andersen/University of Copenhagen.

Drug resistant bacteria are fast becoming one of the big worries of the 21 century. Now researchers at the University of Copenhagen have discovered a previously unknown weakness; an "Achilles heel", of bacteria. Their discovery, a crucial step in bacteria's energy metabolism, may be the first step in developing an entirely novel form of antibiotics.

Associate Professor Nikos Hatzakis at the nanoscience center and department of Chemistry University of Copenhagen together with Associate Professor Lars Jeuken from University of Leeds UK have published the article "Single Enzyme Experiments Reveal a Long-Lifetime Proton Leak State in a Heme-Copper Oxidase" in the periodical *Journal of the American Chemical Society* (*JACS*).

Bacteria pump protons in and out of their cells in order to maintain a finely tuned imbalance between the pH value inside and out. This imbalance, or gradient, is the power source in the microbe's production of ATP, much like a difference in water pressure is the power source for a hydroelectric generator. ATP, in turn, powers most biological processes in the bacteria, so being able to manipulate the pH imbalance could be a powerful way to disable germs.

After careful studies the team discovered what might be a way to manipulate the generated pH balance through manipulating the proton pumps. They found that when the bacteria become dangerously sour inside, the proton pumps may leak. In other words, it leaks when the imbalance between inside and out becomes too great, says Nikos Hatzakis.

"I believe the leaking mechanism acts as a safety valve in the bacteria. If we can design a drug which targets such safety valve in proton pumps, it would be a very powerful antibiotic indeed, so the leak state is a serious weakness: An Achilles heel, even if germs do not have heels", smiles Nikos Hatzakis.

The proton pump itself is an enzyme only 5 nanometers across. It sits on the surface, or membrane, of the bacteria. Getting to measure its leakage was no easy matter. In order to see it, the team "surgically" removed the pump from the bacterial membrane, and placed it on a microscope slide, but looking was not enough, explains Sune Jorgensen who performed most of the studies done in Denmark.

"We wanted to be able to control the pump: To turn it on and off. In order to do that, we constructed a miniscule electrode. We coated the microscope slide with 30 nanometers of gold. This is thin enough to see through, but electrically conductive, so it allowed us to switch the pump on and off with an electrical current", says Sune.

The discovery of the bacterial safety valve is at odds with the classical biological view, where enzymes and proteins are assumed to be either activated or de-activated. To find one that is running, but not working optimally, comes as something of a surprise, according to Hatzakis.

"This result is obviously interesting because of its possible application in antibiotics research, but it also answers a fundamental question about how enzymes work", says Hatzakis and continues:

"Deciphering this behavior required a unique combination of chemistry insights, biology and nano-technology. No one had ever looked at just one pump at work, before, but we managed to look at one or very few at a time. And that's pretty cool smiles Nikos Hatzakis.

Like many good answers in science, this one also raises new questions and paves the way for applications, says Hatzakis "Whether or not we can design drugs that exploit the weakness of the bacteria, might very well be one of the next questions to answer with the single molecule techniques of the University of Copenhagen, Department of Chemistry, Nano-Science Center."