Scientists use supercomputer to learn how cicada wings kill bacteria

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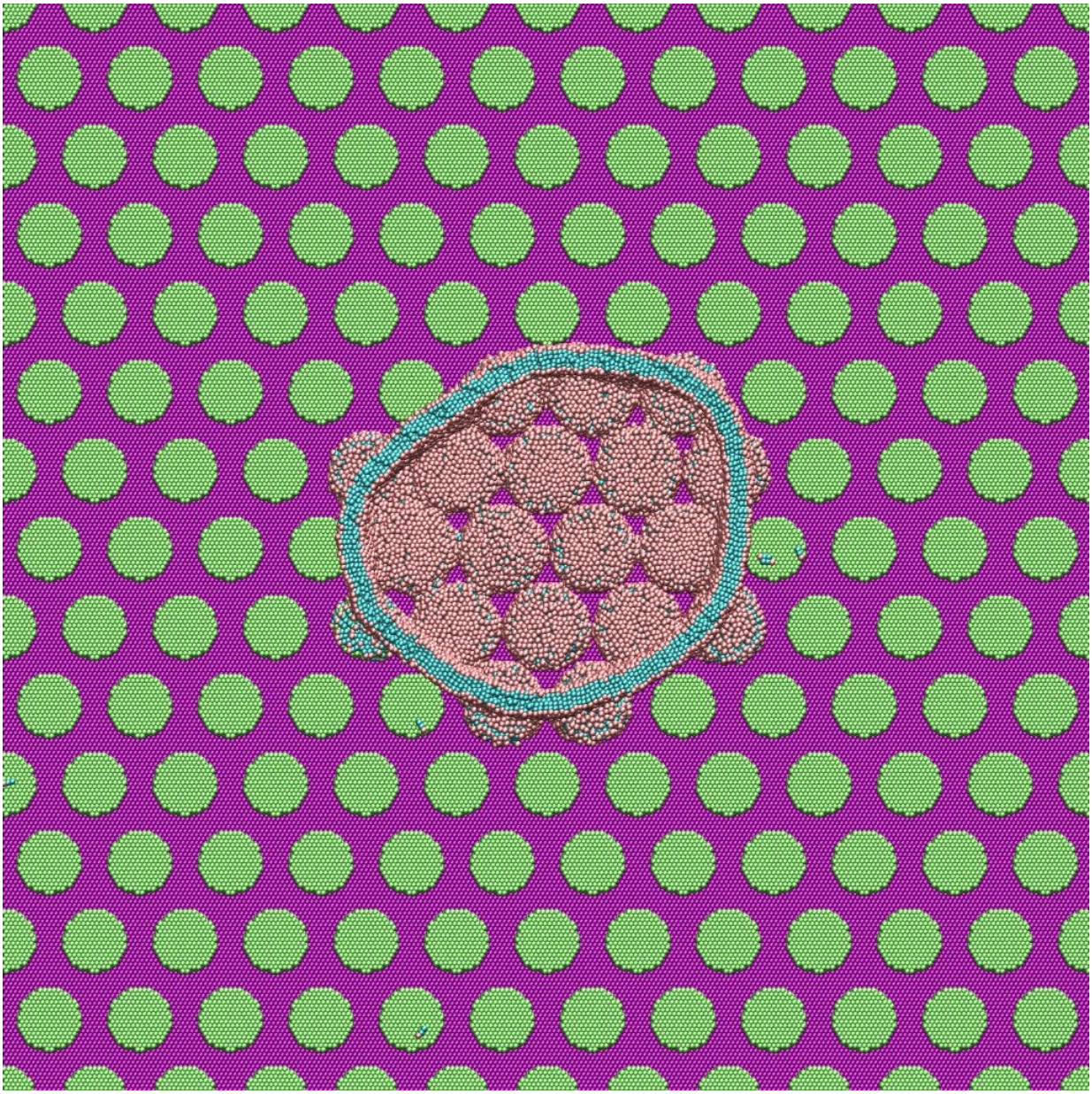


ORNL researchers simulated the nanostructure of a cicada-wing-like surface to gain insight into its antibacterial abilities. Top view cross-section: simulated lipid bilayer vesicles interact with nanopillars, showcasing the lipid arrangement and membrane rupture in high-curvature regions. Credit: Jan-Michael Carrillo/ORNL

Over the past decade, teams of engineers, chemists and biologists have analyzed the physical and chemical properties of cicada wings, hoping to unlock the secret of their ability to kill microbes on contact. If this function of nature can be replicated by science, it may lead to development of new products with inherently antibacterial surfaces that are more effective than current chemical treatments.

When researchers at Stony Brook University's Department of Materials Science and Chemical Engineering developed a simple technique to duplicate the cicada wing's nanostructure, they were still missing a key piece of information: How do the nanopillars on its surface actually eliminate bacteria? Thankfully, they knew exactly who could help them find the answer: Jan-Michael Carrillo, a researcher with the Center for Nanophase Materials Sciences at the Department of Energy's Oak Ridge National Laboratory.

For nanoscience researchers who seek computational comparisons and insights for their experiments, Carrillo provides a singular service: large-scale, high-resolution molecular dynamics (MD) simulations on the Summit supercomputer at the Oak Ridge Leadership Computing Facility at ORNL.

"We immediately contacted Jan-Michael and expressed our interest and motivation in the possibility for a simulation. Although we know how an MD simulation works, it's a complicated process, and we just don't have

much experience doing them," said Maya Endoh, a research professor at Stony Brook and co-author of the team's paper, which was published earlier this year in *ACS Applied Materials & Interfaces*.

Getting compute time on Summit isn't as easy as making a phone call, of course—nanoscience researchers must apply to receive such simulation work at the CNMS, and their projects are subject to peer review as part of the application process. But that's not the only service Carrillo facilitates. Beyond accessing CNMS's state-of-the-art equipment for nanoscience research, he is also uniquely situated to help request neutron beamtime at ORNL's Spallation Neutron Source for future experiments.

"Our techniques for lipid MD simulations are not unique. What's unique is that we're able to leverage the OLCF's resources so we can scan many parameters and do larger systems," Carrillo said. "What's also interesting is ORNL's SNS—their techniques match the time scale of the MD simulations. So, we plan to compare some of the results from MD simulations directly with the results in SNS as well as experiments here in the CNMS."

Replicating nature's microbe killer

Stony Brook's Endoh and Tadanori Koga, an associate professor, decided to investigate cicada wings after being inspired by a 2012 research article published in the journal *Small* that detailed their ability to puncture bacterial cells with lethal results. As researchers in polymer material science, Endoh and Koga sought to replicate the wings' nanopillars with directed self-assembly.

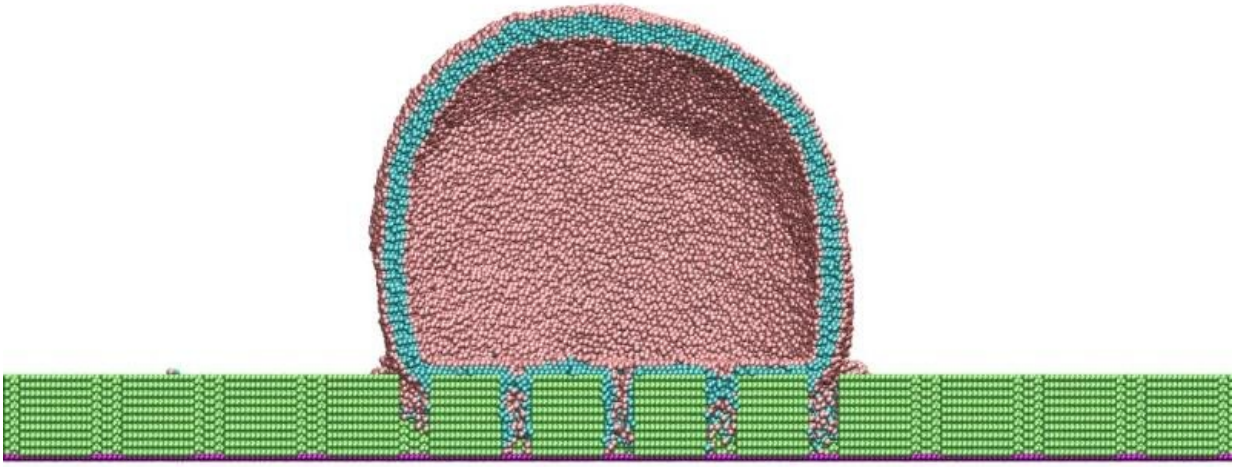
Self-assembly is a process that uses block copolymers made up of two or more chemically distinct homopolymers that are connected by a covalent bond. The materials offer a simple and effective route to fabricate dense, highly ordered periodic nanostructures with easy control of their

geometric parameters over arbitrarily large areas. For example, the nanopillars on a cicada's wings generally have a height and spacing of 150 nanometers, but varying those dimensions had interesting results.

"The cicada wing has a really nice pillar structure, so that's what we decided to use. But we also wanted to optimize the structure," Koga said. "At this moment, we know that the cicada wing can prevent bacteria adhesion, but the mechanism is not clear. So, we wanted to control the size and the height of the pillar and the spacing between the pillars. And then we wanted to see what geometric parameter is crucial to killing bacteria. That's the whole idea of this project."

Daniel Salatto, a guest researcher at Brookhaven National Laboratory, was tasked with constructing the nanosurfaces and conducting experiments on them. To mimic a cicada's wing, he used a polymer used widely in packaging, specifically a polystyrene-block-poly(methyl methacrylate) diblock copolymer.

"Our original approach to making the pillars bactericidal is very simple—the diblock polymer technically can create the nanostructure by itself as long as we control the environment," Endoh said. "Plus, we don't need to have a specific kind of polymer. That's why we started with polystyrene—polystyrene exists everywhere in our daily life. And even though we use a common polymer, we can have the same or similar property that the cicada wing column's bactericidal property shows."



ORNL researchers simulated the nanostructure of a cicada-wing-like surface to gain insight into its antibacterial abilities. Side view cross-section: simulated lipid bilayer vesicles interact with nanopillars, showcasing the lipid arrangement and membrane rupture in high-curvature regions. Image credit: Jan-Michael Carrillo/ORNL

Testing results experimentally, virtually

Salatto lab-tested the nanosurfaces' effectiveness against bacteria by incubating them in broths of Escherichia coli and Listeria monocytogenes. Once extracted, the samples were examined by fluorescent microscopy and Grazing-Incidence Small-Angle X-ray Scattering at Brookhaven Lab's National Synchrotron Light Source II to determine what had happened to the bacteria. Not only had the nanosurfaces killed the bacteria that touched them, but they also had not accumulated dead bacteria or debris on the surfaces.

"It's known that sometimes when bacteria cells die and they absorb onto surfaces, their debris will stay on the surface and therefore make it a

better environment for their brethren to come in and absorb on top of them," Salatto said. "That's where you see a lot of biomedical materials fail, because there's nothing that addresses debris that works well without using chemicals that more or less could be toxic to the surrounding environments."

But how did the nanosurface's pillars achieve this bacterial extermination? That's where Carrillo's simulations provide some clues to the mystery by showing how and where the bacteria's cell membrane stretched and collapsed within the local structure of the pillars.

For the Stony Brook project, Carrillo ran a MD simulation that consisted of about a million particles. The model's magnitude was due to the multiple length-scales being investigated, the size of the lipid molecule and how it arranges around the nanosurface's pillars, the dimensions of the pillars, and the length-scales of the fluctuations of the membrane.

"The simulation's results demonstrated that when there is strong interaction between the bacterium and the nanosurface substrate, the lipid heads strongly absorb onto the hydrophilic pillar surfaces and conform the shape of the membrane to the structure or curvature of the pillars," Carrillo said. "A stronger attractive interaction further encourages additional membrane attachment to the pillar surfaces. The simulations suggest that membrane rupture occurs when the pillars generate sufficient tension within the lipid bilayer clamped at the edges of pillars."

This finding came as a surprise to the Stony Brook team, which had expected that closely mimicking nature's original design would provide the best results. But their best-performing samples did not have the same structure or height as the cicada wing's nanopillars.

"We thought that the height would be important for the nanostructure

because we originally expected that the pillars' height was acting as a needle to puncture the bacteria's membrane. But it's not the way we thought. Even though the nanopillars' height is short, the bacteria still automatically died," Endoh said. "Also, unexpectedly, we didn't see any absorption on the surface, so it's self-cleaning. This was thought to be due to the insect moving its wings to shake off the debris. But with our methodology and structures, we prove that they just naturally kill and clean by themselves."

The team will continue using simulations to develop a more complete picture of the mechanisms at play, particularly the self-cleaning functionality, before applying the nanosurface to biomedical devices.

As for Carrillo, he will continue his own studies of amphiphilic lipid-like bilayer systems, while staying ready to assist other nanoscience researchers who might need the help of the CNMS, OLCF or SNS.

More information: Daniel Salatto et al, Structure-Based Design of Dual Bactericidal and Bacteria-Releasing Nanosurfaces, *ACS Applied Materials & Interfaces* (2023). DOI: 10.1021/acsami.2c18121

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