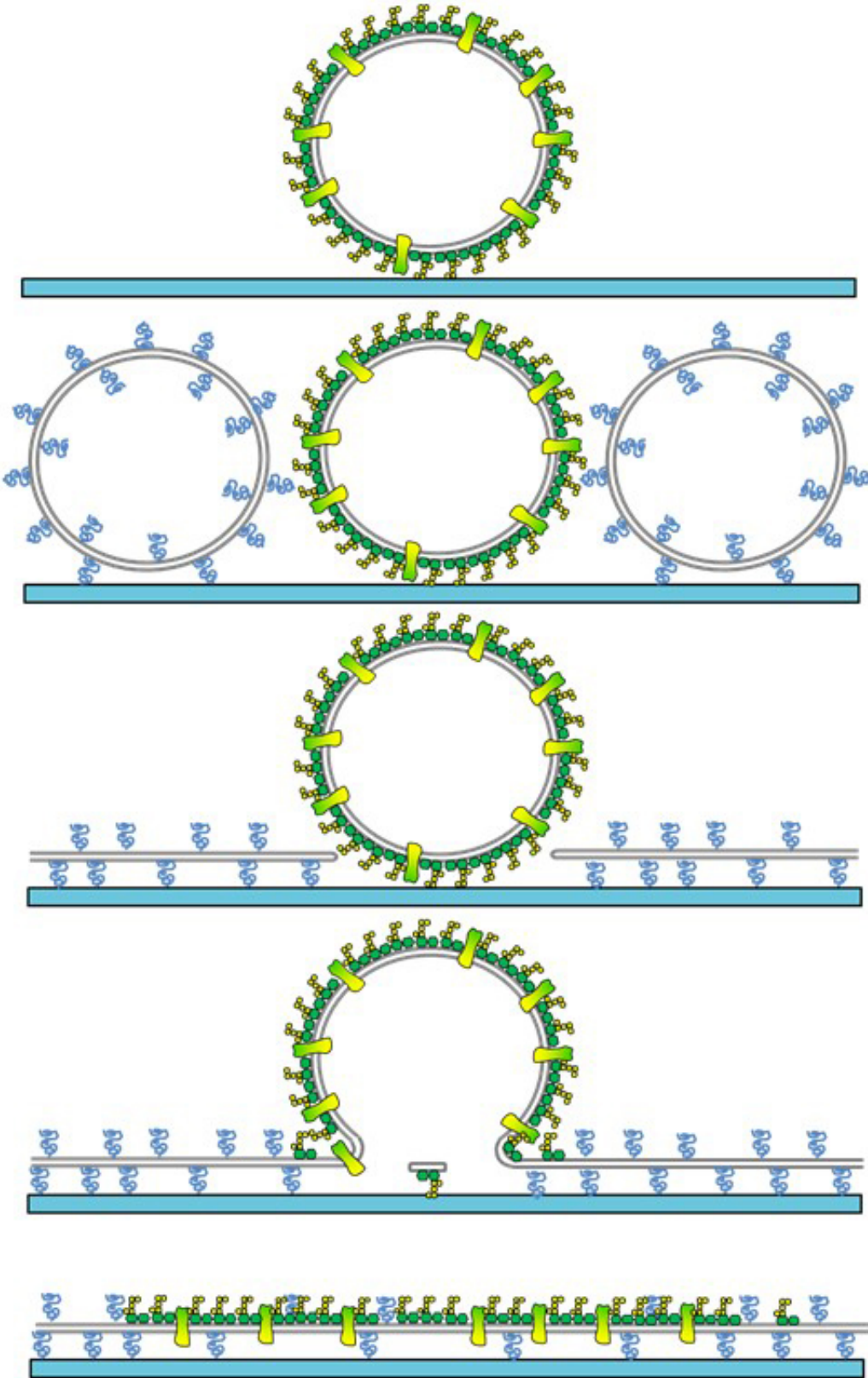


Group creates planar bacterial surface for antibacterial study

September 8 2016, by Tom Fleischman



A proposed scheme of planar bacterial OMV formation. OMVs (green) adhere

to the substrate (blue surface) and remain in vesicle form until the addition of a lipid material (white circles), which rapidly adhere to the surface and induce OMV rupturing and flattening. Credit: Susan Daniel/Provided

If you want to know if a compound is antibacterial, you can throw it into a petri dish to see if the bacteria cultured there die.

But if you want to know how that compound is actually disrupting the bacterial walls, you need to be able to examine the bacteria cell's surface. Tools that probe these details usually need a flat surface, but bacteria are ellipsoidal.

This was the dilemma facing a group of chemical and biomolecular engineers, led by Cornell associate professor Susan Daniel and colleague Matt DeLisa, the William L. Lewis Professor of Engineering. Their team devised a way to take bacterial outer membrane vesicles [OMVs] – shed from weak points on the membrane in nanometer-size particles – to create a planar representation of the bacterial outer surface.

This was achieved, Daniel said, by recent doctoral graduate Chih-Yun Hsia and is detailed in a paper, "A Molecularly Complete Planar Bacterial Outer Membrane Platform," published Sept. 7 in *Nature Scientific Reports*. Other contributors included postdoctoral associate Linxiao Chen and graduate student Rohit Singh.

The outer membrane of a bacterium is a protective barrier that contains proteins and liposaccharides important for the bacterium's function. And as bacteria evolve and become more drug resistant, gaining understanding of the outer membrane's role is crucial. OMVs are a great platform for study, Daniel said, because they are a molecularly complete representation of the cell membrane.

Their latest work was a collaboration focused on understanding the functional role of the bacterial outer membrane and its constituents in the development of novel drug designs. To gain a better understanding of OMVs, however, they needed to be able to put one under a microscope to study the surface.

For this work, the group chose E. coli, as it's among the best-understood bacterium. To flatten the OMV and study the outer surface, Hsia used a method previously reported by the group in dealing with mammalian cells: After the OMVs are deposited on a slide, a lipid material is added, which induces the OMVs to rupture into a flat sheet.

When the edges of the lipid deposits come in contact with the spherical-shaped OMVs, those edges induce the OMVs to flatten out, face up like a parachute, into [outer membrane](#)-like supported bilayers (OM-SB).

"It's fortuitous that the process happens this way because it means that the orientation of the proteins in the flattened OMV are the same as when it is still part of the original bacteria," Daniel said. "Now you have a flat bacterial surface, complete with all the constituents, and oriented properly so that you can monitor drug interactions and disruption with that surface."

Hsia said this is the first time anyone's been able to create a molecularly complete planar representation of a bacterium.

"Our approach actually contains all of the components of the E. coli, including the proteins," Hsia said. "All of our competing technologies are limited to just the lipid components of it, and that severely limits what your target is going to be."

And with the deep understanding of E. coli bacteria, researchers can modify these OM-SBs, however they want to answer very specific

questions.

"If you wanted to just express a protein in E. coli membranes," Chen said, "you could put them into these OMVs and splat them onto a surface, and now you have a membrane protein in a lipid environment, in a platform that you could then use for biomicroscopy and a host of other things."

Combining this approach with microfluidics, the group says, will lead to improved screening of compounds beneficial for future antibiotics design.

More information: Chih-Yun Hsia et al. A Molecularly Complete Planar Bacterial Outer Membrane Platform, *Scientific Reports* (2016). [DOI: 10.1038/srep32715](https://doi.org/10.1038/srep32715)

Provided by Cornell University

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