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# Lipid zipper triggers bacterial invasion

**Millions of people die each year from infections both in developing and industrial countries. There is still no effective treatment for a large number of diseases caused by pathogens. In order to treat infectious diseases effectively, we need to understand the mechanisms that bacteria use to infect human cells. The cytoskeleton of the host cell usually plays a key role in this process. Researchers at the University of Freiburg have discovered a novel mechanism that is used by Pseudomonas aeruginosa to enter the cell without involving cytoskeletal or components. Dr. Thorsten Eierhoff from the Centre for Biological Signalling Studies (BIOSS) and his colleagues call it a 'lipid zipper mechanism'.**

Many invasive bacteria's ability to infect humans depends largely on their success in entering human cells. This in turn depends on their ability to induce the bending of the outer membrane of the host and endocytosis. There are two known mechanisms that bacteria use to enter cells where the cells' cytoskeleton plays a key role in bending the membrane. Most bacteria possess ligands that they use to bind to suitable membrane receptors, thus enabling them to enter a host cell by activating specific signalling pathways in the host. The receptor-ligand interactions of Listeria lead to the polymerization of actin, which is one of several components of a cytoskeleton. This mechanism is known as the zipper mechanism. In Shigella, cell entry is mediated by a so-called trigger mechanism, which requires the bacteria to inject effector proteins into the host cell. These proteins trigger the polymerization of actin fibres, which then bend the cell envelope from the inside and form vesicles that engulf the bacteria. Inside the vesicles, the bacterium is comparatively well protected against the host immune response and is able to propagate, at least for a time.

A team led by Dr. Thorsten Eierhoff and Junior Professor Dr. Winfried Römer at the Centre for Biological Signalling Studies (BIOSS) in Freiburg has identified a novel mechanism of bacterial invasion for Pseudomonas aeruginosa, where the bacteria are able to enter cells without involving actin.

## LecA and Gb3 induce wrapping

Dr. Thorsten Eierhoff specialises in research into the uptake of bacteria into cells.
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Infections with Pseudomonas aeruginosa, which is a common wound and hospital pathogen, are dangerous, especially for patients with compromised immune systems or cystic fibrosis. Such patients are particularly susceptible to Pseudomonas pneumonia, an infection caused by P. aeruginosa. The pathogen colonizes lungs and skin and is a major problem, especially for burn sufferers. It is resistant to several antibiotics and is even able to grow on disinfectants. The bacterium's appendages (pili) enable it to adhere to organic and abiotic surfaces. It has the capacity to form biofilms and is able to colonize catheters and breathing tubes.

The molecular mechanisms by which the pathogens enter cells need to be known in order to develop strategies for the prevention and therapy of Pseudomonas infections. Eierhoff and his colleagues in Römer's team have carried out in vitro experiments with synthetic membranes and lung cells and found that Pseudomonas uses a previously unknown mechanism to achieve engulfment into the host cells. "We call this a lipid zipper mechanism," says Eierhoff going on to explain that the mechanism involves an interaction between bacterial LecA proteins and so-called Gb3 lipids in the outer membrane of human cells. The glycosphingolipid Gb3

(globotriaosylceramide) located on the host cell carries three sugar molecules, consisting of one glucose molecule with two galactose molecules attached.

The receptor Gb3 in the outer membrane of human cells plays a key role in cell-cell recognition and cell proliferation processes, in particular during embryonic development. The galactose molecule of the Gb3 lipid can interact with Shigella shiga toxins and Pseudomonas aeruginosa LecA lectins. The engulfment process is induced by a pathogen that docks to the cell; the bacteria's LecA molecules and the Gb3 glycosphingolipids then interlock like the teeth of a zipper. The cell envelope gradually wraps itself around the pathogen and conveys it into the inside of the host cell. "This process is not as trivial as it may sound," says Eierhoff. "What you have to take into account is that the curvature is the complete opposite of a natural one." The vesicle membrane needs to be bent from inside at the site where the pathogen establishes contact with the cell in order to form a bubble that can take up the pathogen.

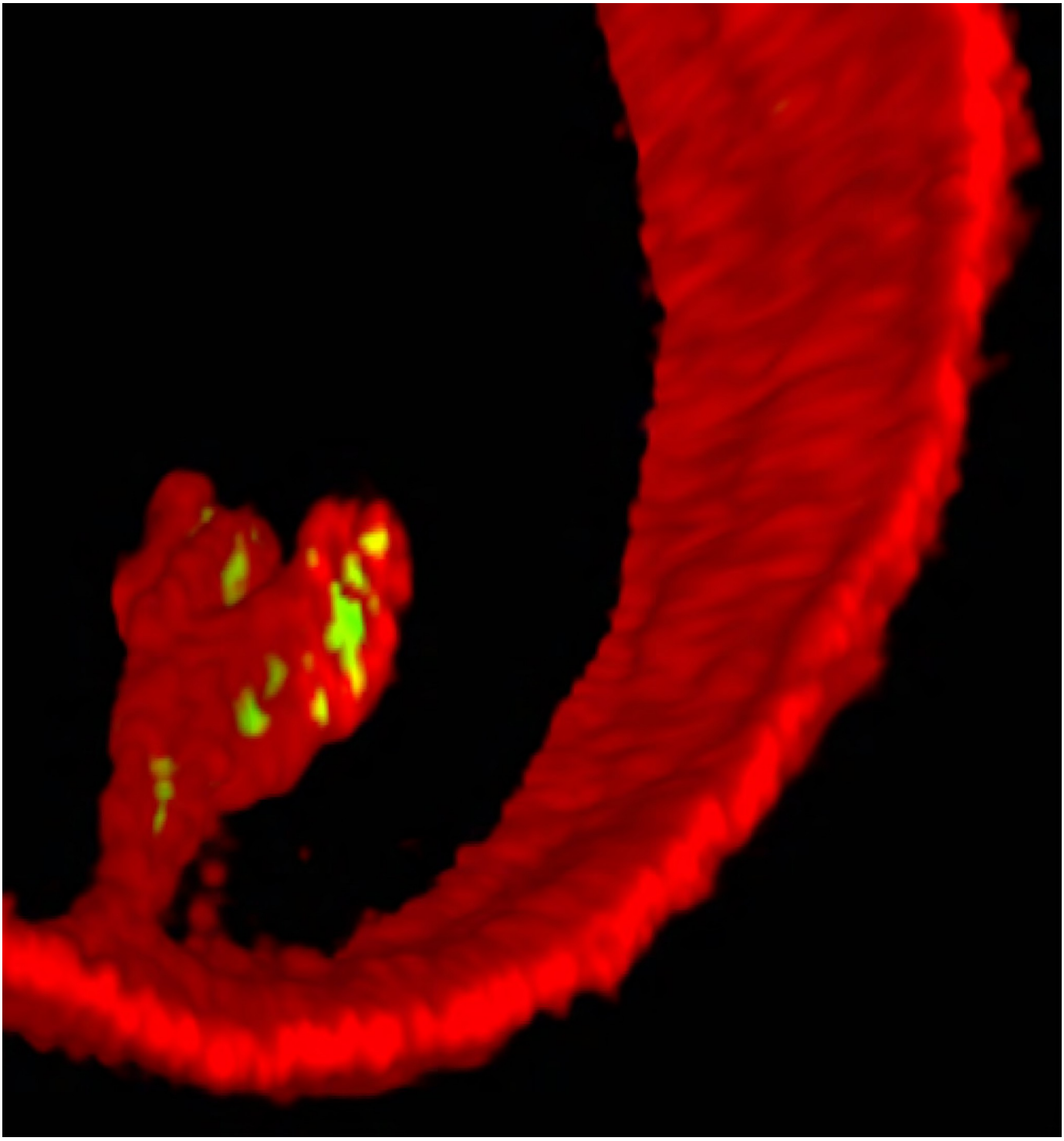
## Experiments in vitro and in cellulo

Using in vitro experiments with GUVs (giant unilamellar vesicles, synthetic lipid vesicles imitating lung epithelial cells), Eierhoff was able to show that two factors were sufficient to achieve complete engulfment of the Pseudomonas bacterium using the lipid zipper: Gb3 and LecA. It is a highly dynamic process: LecA binds to Gb3, additional Gb3 lipids are then recruited and this results in the attachment of a growing number of Gb3 molecules.

Biophysical simulations based on the in vitro GUV experiments show that the adhesion energy of the two molecules is quite sufficient to bend the membrane and induce invasion. "The bacterium basically glides through the membrane into the cell," says Eierhoff. "The lipid zipper is not only an induced structure, but it actually leads to the infection of the cell."

The pharmacological inhibition of the cytoskeleton showed that the process enables the pathogen to enter the cell without manipulating actin. In synthetic membranes, however, the invagination of the membrane did not lead to completely closed vesicles. Experiments have shown that all bacteria that are inside giant unilamellar vesicles remain connected with the outer membrane via a lipid bridge. The researchers assume that in nature there are certain enzymes or processes that cut off the bacterium's connection with the outside world, especially given that a bacterium's objective is to enter and infect cells.

## New molecule as invasion barrier



Pseudomonas aeruginosa (green) invades a synthetic vesicle (red) using the lipid zipper.


Eierhoff, Römer and their team are focusing first of all on the initial steps of bacterial invasion. In cooperation with Prof. Dr. Nicolas Winssinger, a chemist at the University of Geneva, the researchers from Freiburg managed to identify a highly affine ligand for bacterial LecA. This ligand prevents Pseudomonas from coming into contact with the glycosphingolipid Gb3, thereby preventing infection. LecA is a tetramer whose individual monomers are able to bind a Gb3 galactose residue. LecA binds predominantly to sugar molecules, which are typical components of receptors or signalling molecules. In addition, Pseudomonas is a typical wound pathogen that tends to colonize damaged soft tissues. "Structures that are hidden away in healthy tissues are exposed in wounds and can then be recognized by bacteria. Pseudomonas, for example, recognizes carbohydrate-containing structures."

The new LecA ligand is an organic molecule with two galactose molecules that are located at the right distance from each other to fit perfectly into the binding pockets of LecA. The researchers were able to show in cell cultures that Pseudomonas bacteria exposed to this particular ligand have 90% less chance of invading human lung cells. Eierhoff asks: "Might it be possible to completely block LecA with this ligand?" The medical relevance of this biologically highly active molecule is obvious: it might be able to effectively combat Pseudomonas infections.

**Further information:**

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