Researchers discover molecular 'add-ons' that customize protein interfaces

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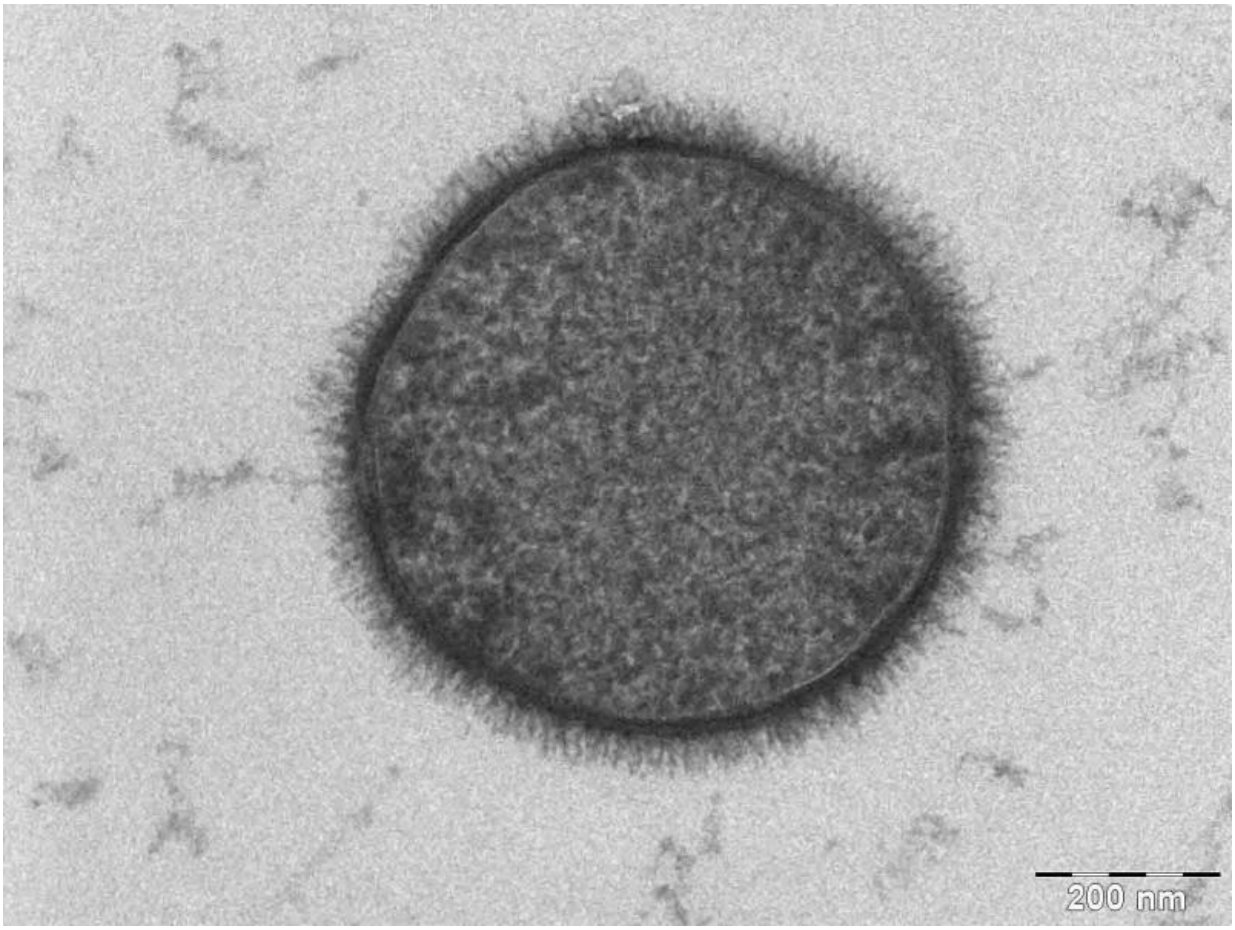

The Bacterium Bacillus subtilis taken with a Tecnai T-12 TEM. Taken by Allon Weiner, The Weizmann Institute of Science, Rehovot, Israel. 2006. Credit: Public Domain

Researchers in the United States and Germany have just discovered a previously overlooked part of protein molecules that could be key to how proteins interact with each other inside living cells to carry out specialized functions.

The researchers discovered tiny bits of molecular material—which they named "add-ons"—on the outer edges of the protein interface that customize what a protein can do. They chose the name because the add-ons customize the interface between proteins the way software add-ons customize a web interface with a user.

While it's long been known that proteins have an interface region where they connect with other proteins, it's not been clear exactly how key proteins are able to find each other within a crowded cellular environment that may contain tens of thousands of other proteins.

Now, researchers at The Ohio State University and the University of Regensburg report in the *Proceedings of the National Academy of Sciences* that it's the add-ons that enable proteins to connect exclusively with the right dedicated partner.

Florian Busch, a postdoctoral researcher in chemistry and biochemistry at Ohio State and co-author of the study, called the existence of protein add-ons "a previously unknown fundamental driving principle" to ensure that proteins interact in specific ways.

The researchers experimented with live bacteria, demonstrating the importance of add-ons to normal cellular functions. For example, they determined that in the organism *Bacillus subtilis*, in which a unique interface add-on is missing, bacteria colonies grew 80 percent less under certain conditions. The reason for this was that the missing interface add-on led to un-healthy cross-interactions of proteins in the *B. subtilis* cells.

It's difficult to overstate the importance of proteins to life as we know it. Enzymes are proteins that enable chemical reactions in cells. Antibodies are proteins that bind to foreign invaders in the body. The list goes on to include thousands of critical functions. In most cases, proteins have to connect to each other and form groups called protein complexes to perform such diverse tasks.

But exactly how proteins are able to do all that they do is a mystery—one rooted in mathematics and geometry. There are 20 known amino acids which link together in long chains and then fold up to form proteins. It's the fold that determines a protein's generic shape, or geometry. Although there are only around 1,000 known protein geometries in nature, somehow proteins are able to form complexes that perform hundreds of thousands of very specific functions.

Maximilian Plach, lead author of the paper and biochemist at the University of Regensburg, explained how the researchers knew where to look to solve the mystery.

"Much work has been put into analyzing how proteins interact with each other and what the interfaces look like, how they are constructed, and how they evolved," he said. "But the peripheral regions of interfaces have not received as much attention. I think the novelty in our approach was to look at regions that have been, as yet, regarded as less important."

The Regensburg team, led by computational biologist Rainer Merkl and protein biochemist Reinhard Sterner, analyzed the protein sequences derived from more than 15,000 bacterial and archaeal genomes on a large computer cluster. They sorted proteins that shared common evolutionary ancestors into a kind of family tree, and compared individual proteins to their protein "relatives." That's how they spotted interface structures that were present in some proteins but missing in others—the add-ons.

Busch and Vicki Wysocki, Ohio Eminent Scholar of Macromolecular Structure and Function and director of the Campus Chemical Instrument Center at Ohio State, then used native mass spectrometry to detect how the presence and absence of add-ons influenced the ability of proteins to interact with each other.

"We're really pleased that our native mass spectrometry technology could help identify the role of these interface 'add-ons'—a way for a protein to find its critical partner protein even in a crowded cellular environment with similar structures present," Wysocki said.

To Busch, one of the really exciting things about the study was the researchers' use of "big data"—in this case, entire protein and genome databases.

"I consider our work to be one important example of how to make use of publicly available data in order to understand fundamental principles in nature, and I think that data mining will become increasingly important in the biomedical field in the future," he said.

More information: Maximilian G. Plach et al, Evolutionary diversification of protein–protein interactions by interface add-ons, *Proceedings of the National Academy of Sciences* (2017). DOI: 10.1073/pnas.1707335114

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