Freeze frame: Researchers solve how cells unfold proteins

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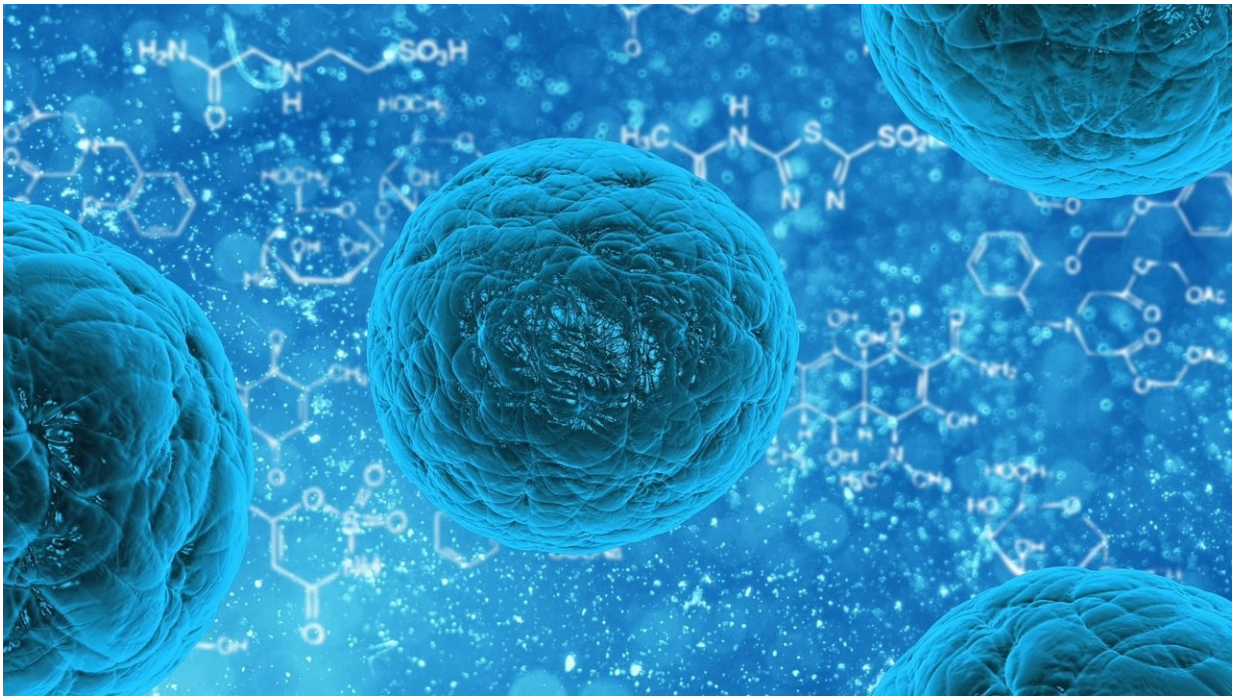

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A happy cell is a balanced cell, but for every stupendously twisted protein it creates, it must tear the old ones asunder. That means untangling a convoluted pretzel-like mass for recycling. Cdc48 plays a critical role in unraveling the spent proteins.

"Cdc48 is the swiss army knife of the cell and can interact with so many

different substrates," said Peter Shen, Ph.D., assistant professor in Biochemistry at University of Utah Health and senior author on the paper. "Until now we didn't have an understanding exactly of how it works."

Shen led a multi-institutional team of researchers to identify key structures of Cdc48 to visualize its undulations as it unfolds proteins. The results are available online in the June 27 issue of *Science*.

"We set out to do this because we care how molecular machines work," said Shen. "We decided to hone-in on Cdc48 due to clinical relevance."

For years, researchers have known that a single-point mutation in Cdc48 can cascade into serious diseases, including amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease type 2Y.

"Human Cdc48 is linked to multiple diseases and is the target of efforts to develop therapeutics for the treatment of cancers," said Christopher Hill, DPhil., distinguished professor of Biochemistry at U of U Health and co-corresponding author on the study. "The structure that we have determined can be used to advance efforts to develop more effective inhibitors and therapeutics."

In the study, the research team purified Cdc48 directly from yeast cells (Saccharomyces cerevisiae) and took snapshots of the purified particles in different configurations after it was flash frozen using cryogenic electron microscopy (cryo-EM).

"The cells are already doing the hard work for us by making these complexes," Shen said. "Because this method is so fast, we have captured Cdc48 in the act of unfolding a protein substrate."

Using this approach, the research team demonstrated how Cdc48 unfolds

the protein by threading it through a central pore of the complex, using a hand-over-hand conveyor-like movement. The recycled tangle they were imaging was a mystery until collaborators at Brigham Young University applied mass spectrometry proteomics to the same harvested complex to unmask the anonymous proteinan inactive protein phosphatase 1 complex.

Shen believes these results are applicable to human cells, because Cdc48 is highly conserved.

"We believe the structure we solved here will look very similar to what our bodies are expressing right now," he said.

The research team was unable to visualize the entire complex because Cdc48 interacts with multiple binding partners almost simultaneously. This efficient multitasking blurs the reconstruction; however, Shen wants to continue to explore how Cdc48 manages to bind with so many partners at roughly the same time.

"The coolest part is this [work] demonstrates that we can take a protein directly out of host cells and image them in their native state," Shen said. "I think this is the future of the cryo-EM field."

More information: "Structure of the Cdc48 segregase in the act of unfolding an authentic substrate" *Science* (2019). science.sciencemag.org/lookup/ … 1126/science.aax0486

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