Detecting diluteness: New experimental and theoretical approaches 'dive into the pool' of membranes organelles

June 26 2017, by Erika Ebsworth-Goold

Engineers at Washington University in St. Louis and Princeton University developed a new way to dive into the cell's tiniest and most important components. What they found inside membraneless organelles surprised them, and could lead to better understanding of fatal diseases including cancer, Huntington's and ALS. Credit: Washington University in St. Louis

Inside each and every living cell, there are miniscule structures called membraneless organelles. These tiny powerhouses use chemistry to cue the inner workings of a cell—movement, division and even self-destruction.

A collaboration between engineers at Princeton University and Washington University in St. Louis has developed a new way to observe the inner workings and material structure of these vitally important organelles. The research, published today in *Nature Chemistry*, could lead to a host of new scientific applications, as well as a better understanding of diseases such as cancer, Huntington's and ALS.

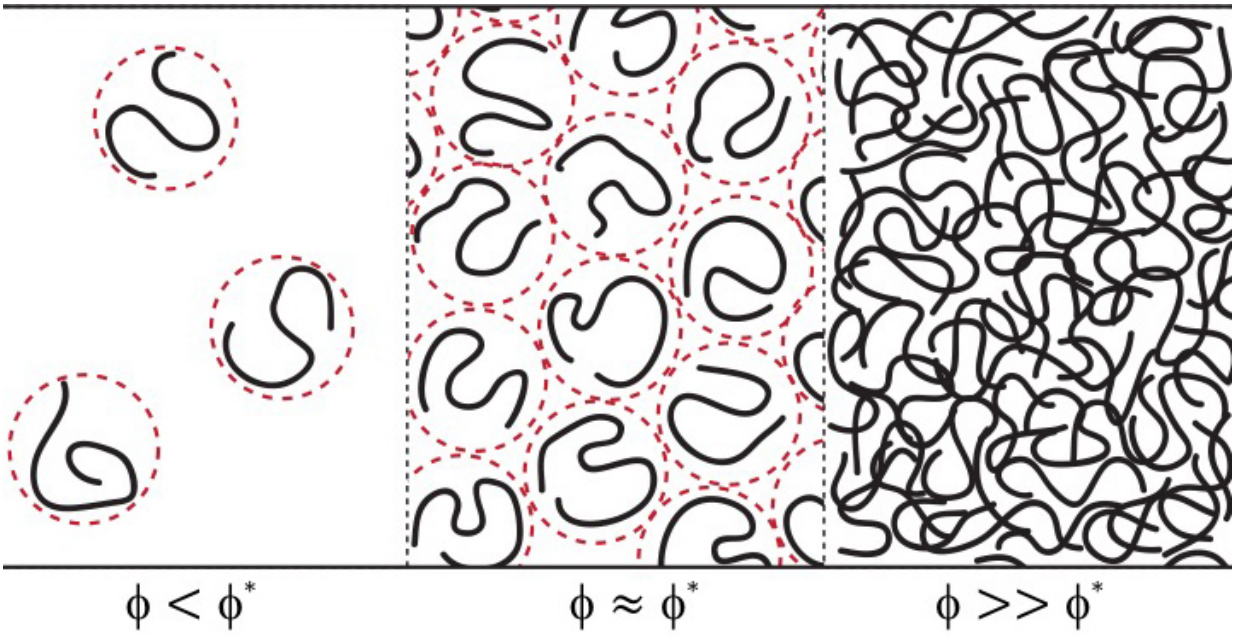
"They're like little drops of water: They flow, they have all the properties of a liquid, similar to raindrops," said Rohit Pappu, the Edwin H. Murty Professor of Engineering at Washington University's School of Engineering & Applied Science. "However, these droplets are comprised of protein that come together with RNA (ribonucleic) molecules."

In the past, peering into organelles has proven difficult, due to their tiny size. Clifford Brangwynne, associate professor in chemical and biological engineering at Princeton's School of Engineering and Applied Science, and his collaborators, developed a new technique—called ultrafast scanning fluorescence correlation spectroscopy or usFCS—to get an up-close assessment of the concentrations within and probe the porosity of facsimiles of membraneless organelles. The approach uses sound-waves to control a microscope's ability to move and then obtain calibration-free measurements of concentrations inside membraneless organelles.

In their research, Brangwynne and his team, including postdoctoral researchers Ming-Tzo Wei and Shana Elbaum-Garfinkle, used cells taken from a roundworm. With usFCS, they were able to measure protein concentrations inside organelles formed by the specific protein,

LAF-1. This protein is responsible for producing p-granules, which are protein assemblies responsible for polarizing a cell prior to division. Once the Princeton researchers were able to clearly peek into the organelles and view the LAF-1, what they found surprised them.

"We found that instead of being densely packed droplets, these are very low density, permeable structures," Brangwynne said. "It was not the expected result."



For the first time, engineers from Washington University in St. Louis and Princeton University were able to get a good look inside membraneless organelles, tiny components inside a cell. This illustration shows the differing viscosity found in them; a discovery that could bring new lab breakthroughs and disease understanding to the forefront. Credit: (Courtesy: Washington University in St. Louis)

That's when Washington University's Pappu and his graduate research assistant Alex Holehouse tried to make sense of the surprising findings from the Princeton group. Pappu's lab specializes in polymer physics and modeling of membraneless organelles.

"We were able to basically swim inside the organelles to determine how much room is actually available. While we expected to see a crowded swimming pool, we found one with plenty of room, and water. We're starting to realize that these droplets are not all going to be the same," Pappu said.

In the case of the LAF-1 organelles, the researchers found the formation of ultra-dilute droplets derives from information encoded in the intrinsically disordered regions of these protein sequences. The features of that sequence ensure that this protein is a highly floppy molecule, rather like cooked spaghetti, lacking the ability to fold into a specific, well-defined structure. In contrast, in other organelles formed by different proteins, the material properties are more like those of toothpaste or ketchup. Brangwynne and Pappu are continuing to collaborate to figure out how different protein sequences encode the ability to form droplets with very different material properties. This work has direct implications for understanding biological functions of membraneless organelles and for understanding how changes to these material properties give rise to diseases such as neurodegeneration or cancers.

"There is an explosion of engineering applications and transformations for mechanistic cell biology that are on the horizon. These advancements will be accessible as we learn more about the foundation of these organelles and how their amino acid sequence determines material properties and function," Pappu said. "These organelles are doing remarkable things inside cells, and a really neat question is: How can we mimic them?"

Pappu says one day, researchers could hack the design principles of organelles to fashion everything from intracellular chemistry labs to tiny drug delivery vehicles and imaging agents. Aside from the practical applications, there are also potential implications for understanding and diagnosing a whole host of diseases.

"It is essential to be able to understand how one can regulate the functions of these droplets," Pappu said. "If we succeed, the impact could be transformative: It's not just cancer, it's neurodegeneration, about developmental disorders, and even the fundamentals of cell biology."

More information: Phase behaviour of disordered proteins underlying low density and high permeability of liquid organelles, *Nature Chemistry* (2017). DOI: 10.1038/nchem.2803

Provided by Washington University in St. Louis