Dense liquid droplets act as cellular computers

September 14 2022

Credit: Pixabay/CC0 Public Domain

An emerging field explores how groups of molecules condense together inside cells, the way oil droplets assemble and separate from water in a vinaigrette.

In human cells, "liquid-liquid phase separation" occurs because similar, large molecules glom together into dense droplets separated from the more diluted parts of the fluid cell interior. Past work had suggested that evolution harnessed the natural formation of these "condensates" to organize cells, providing, for instance, isolated spaces for the building of cellular machines.

Furthermore, abnormal, condensed—also called "tangled"—groups of molecules in droplets are nearly always present in the cells of patients with neurodegenerative conditions, including Alzheimer's disease. While no one knows why such condensates form, one new theory argues that the biophysical properties of cell interiors change as people age—driven in part by "molecular crowding" that packs more molecules into the same spaces to affect phase separation.

Researchers compare condensates to microprocessors, computers built into circuits, because both recognize and calculate responses based on incoming information. Despite the suspected impact of physical changes on liquid processors, the field has struggled to clarify the mechanisms connecting phase separation, condensate formation, and computation based on chemical signals, which occur at much smaller scale, researchers say. This is because natural condensates have so many functions that experiments struggle to delineate them.

To address this challenge, researchers at NYU Grossman School of Medicine and the German Center for Neurodegenerative Diseases built an artificial system that revealed how the formation of condensates changes the action at the molecular level of enzymes called kinases, an example of chemical computation. Kinases are protein switches that

influence cellular processes by phosphorylating—attaching a molecule called a phosphate group—to target molecules.

The new analysis, published online September 14 in *Molecular Cell,* found that the formation of engineered condensates during phase separation offered more "sticky" regions where medically important kinases and their targets could interact and trigger phosphorylation signals.

"Our study results show that physical changes like crowding can drive condensate formation that is converted into biochemical signals, as if condensates were squishy computers," says lead study author Liam Holt, Ph.D., associate professor in the Institute for Systems Genetics at NYU Langone Health.

Among the study kinases seen to be more active in a crowded, condensed environment was Cyclin Dependent Kinase 2, known to phosphorylate the microtubule-binding protein Tau. Tangled condensates of Tau are found frequently in the brain cells of patients with Alzheimer's disease.

"Our experiments suggest that formation of more Tau condensates drives more Tau phosphorylation," adds Holt, also faculty in the Department of Biochemistry and Molecular Pharmacology. "Whether these mechanisms lead to more brain cell death, and whether reversing them could be a new treatment approach, will be important questions in our upcoming work."

Specifically, the study found that when Tau and Cyclin Dependent kinase condensed together into dense droplets, there was a three-fold acceleration of a phosphorylation at a group of sites on Tau (the AT8 epitope) linked to Alzheimer's disease.

Engineering a biosensor

In seeking to engineer useful versions of these computers, the research team tested several artificial condensates, synthesizing different scaffold molecules to see which best pulled sample kinases—MAPK3, Fus3, and Cyclin-dependent Kinase 1 (Cdk1)—together with their targets to increase signaling. Condensates form as scaffold molecules mesh together within droplets. The team found that, in their model, the gathering of large biomolecules into droplets inside one-celled living organisms called yeast made phosphorylation reactions hundreds of times faster.

The study also found that condensate formation let the included kinases phosphorylate more kinds of molecules, and without the presence of the molecular shapes usually required. This suggests that condensates in crowded cells create altered computation types, some potentially disease-related.

Moving forward, the research team seeks to build on a past study in Holt's lab, which found that a protein complex called mTORC1 controls molecular crowding by determining the number of ribosomes, "machines" that build other large proteins in cells. The team plans to study whether compounds known to inhibit mTORC1 can reduce crowding and Tau phosphorylation.

Finally, the researchers also hope that their findings advance the design of other cellular computers that react to physical forces. This could include the introduction of engineered processors into immune cells that—to attack cancer cells—would be turned on as they sought to squeeze into tissue made dense by growing tumors.

More information: Liam J. Holt, Condensed-Phase Signaling Can Expand Kinase Specificity and Respond to Macromolecular Crowding, *Molecular Cell* (2022). DOI: 10.1016/j.molcel.2022.08.016. www.cell.com/molecular-cell/fu … 1097-2765(22)00805-X