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Living droplets

Tiny droplets of water in oil can serve as miniature culture vessels for living single cells and multicellular organisms.

High-throughput assays using living cells have seen great advances, but the problem of evaporation makes reduction of volumes in microtiter plates difficult. The use of microfluidics eliminates evaporation, but other constraints of the system make it challenging to adequately isolate single living cells from one another so that each can be individually subjected to well-defined and independent assay conditions.

New research shows how combining microfluidics with emulsion technology allows manipulation and assaying of isolated single living cells and organisms. Emulsions are mixtures of two immiscible substances. In a water-in-oil emulsion, tiny droplets of water are surrounded by oil. By adding the components of a biochemical reaction to the water phase, each droplet can act as a tiny reaction vessel, allowing millions of individual reactions in a single tube.

Andrew Griffiths at Université Louis Pasteur has been using emulsions for years in directed evolution screens, but conventional emulsions have a problem. “The droplet sizes are quite polydisperse,” explains Griffiths. “This is problematic in directed evolution experiments because you can have droplets of different sizes with exactly the same gene inside but different phenotypes.” This can be problematic for other assays also, but work by others has shown that microfluidics devices can make monodisperse droplets. “Microfluidics allows you to do all sorts of clever things with droplets that are difficult to do otherwise,” says Griffiths. “For example you can split, fuse and sort droplets in a microfluidics chip and do it at very high speeds.”

Griffiths and his colleague Christoph Merten were interested in applying droplet-based microfluidics to miniaturize and increase the throughput of live-cell assays as part of the drug-discovery process. They started collaborating with David Weitz at Harvard University who was working on the physics of droplets in microfluidic systems.

Merten says: “The first interesting question was whether it is possible to encapsulate single cells and keep them alive in the droplets.” This was highly dependent on the oil and surfactant used. They found that a perfluorocarbon oil and surfactant worked well (Fig. 1). Perfluorocarbon solvents provide several advantages. They do not cause swelling of the material in the microfluidic device, and they dissolve 20 times more oxygen than water. Finally, nonfluorinated molecules, such as the drug candidates they

NEW ELECTROPHILIC PROBES SLIDE IN

Recently discovered electrophilic probes open the door to activity-based protein profiling (ABPP) studies of a broader range of proteins.

It can be extremely challenging to study enzymes. Without any knowledge of substrate or product, where do you start? For those who study enzymes in large families, this task can be especially daunting as it is that much harder to find a specific inhibitor to get on the right road.

Pioneered by Ben Cravatt’s team at the Scripps Research Institute, ABPP is a great way to do just that. In this strategy, an electrophilic or photoreactive probe is generated that covalently targets an enzyme’s conserved active site. By affinity enrichment and liquid chromatography–tandem mass spectrometry (LC-MS/MS), the active protein can be isolated and easily distinguished from its closest relatives. To get your hands on a specific and potent inhibitor, just throw in an activity-directed probe for your favorite enzyme. “This is the game,” says Cravatt.

To expand the ABPP toolbox, Cravatt’s team tested carbon electrophiles for their ability to label proteins in mouse tissue proteomes. In addition to discovering two cysteine-selective electrophiles, they also report a phenylsulfonate ester that labels several other residues, including aspartate, glutamate, histidine and tyrosine.

Taking a closer look at the residues labeled by these probes, Cravatt’s group noticed that they were not only amino acids that are critical in catalysis. The cysteine-specific probes targeted the substrate-binding domain of a few metabolic enzymes, and the phenylsulfonate ester labeled a critical regulatory tyrosine of dual-specificity tyrosine phosphorylation–regulated kinase 1A.

A key limitation of ABPP is that irreversible inhibitors are only known for a handful of enzyme classes. Existing reversible inhibitors and natural products can be adapted as ABPP probes by adding UV light–activated photoreactive groups, but such probes are restricted to studies in cell culture–based systems.

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Hence, all you need is a nucleophilic residue in an active site or in a substrate-binding pocket for the enzyme to be studied by ABPP.

There is still a lot of work to do to design that perfect activity-directed probe for your favorite enzyme. “This is the
electrophiles. RESEARCH PAPERS

Cravatt’s group noticed that one enzyme class by embedding them into scaffolds that direct probes can be further tuned to be more selective for individual nucleophile pairs you want to use,” explains Cravatt. “These probes are more selective for individual enzyme classes by embedding them into scaffolds that direct binding to specific targets.”

Electrophilic probes, however, have the potential to target more than just enzymes. Cravatt’s group noticed that one of the carbon electrophiles labeled a conserved cysteine in 3-chloride intracellular channels, opening the door to studying ion channels by ABPP.

What is next for Cravatt’s team? There are thousands of proteins that we know nothing about. By generating ABPP probes directed to large classes of such unannotated proteins, they hope to begin to characterize them.

As for all of you out there hoping for that great small-molecule tool? “Unifying [ABPP] with small-molecule screening could really provide the first general method to begin to identify targets in native systems,” says Cravatt. By generating ABPP probes that are subsequently embedded in the inhibitor of interest, the target(s) could be labeled in living cells, enriched and identified by LC-MS/MS. This is good cause for chemical biologists to be dancing in the streets.

Michelle Pflumm

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