CELL BIOLOGY

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."

Figure 1 | Creation of water droplets containing single living cells inside a microfluidic device. Image courtesy of Christoph Merten.

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 drops." 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

CHEMICAL BIOLOGY

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 compounds. No protein purification, no substrate and no assay are required.

Most ABPP studies to date have focused on enzymes, but any protein regulated by post-translational mechanisms is fair game. "The obviously druggable portion of biological space has been the focus so far of activity-based profiling," explains Cravatt, but that is simply because "enzymes offer targets for which we have small-molecule scaffolds or affinity labels to build on."

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.

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.

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

want to screen using droplet-based microfluidics, show almost no solubility in perfluorocarbons.

Merten plans to use the system to screen for drugs that inhibit virus entry into cells. "For any cell-based assay you must guarantee that the cells survive during the assay period from a few hours to a few days," notes Merten. Their results show that cells could survive and divide for several days in a single droplet in a microfluidic device.

The tiny size of the droplets greatly reduces the amount of reagents needed for assays. "The number of viral particles we need for a 96-well plate is sufficient to screen 10^7 samples in the droplet system," remarks Merten. "We would not be able to produce enough viral particles to perform such a screen in a microtiter plate." This nicely illustrates the value of this system for assays involving limiting reagents.

To test the limits of the system the researchers encapsulated living *Caenorhabditis elegans* worms within droplets in a small piece of tubing. A worm underwent a complete lifecycle in a droplet, showing that the droplet assay could be used for assays requiring testing of progeny.

This is just the first step on the way to using these microfluidic systems for doing high-throughput cell-based or small multicellular organism screens. So far all the droplets in the assays contain the same reagents, but Griffiths says "the next step is to establish strategies to allow us to encapsulate different drug candidates in the drops."

Daniel Evanko

RESEARCH PAPERS

Clausell-Tormos, J. *et al.* Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms. *Chem. Biol.* **15**, 427–437 (2008).

early stages of scoping out the landscape of what electrophile-nucleophile pairs you want to use," explains Cravatt. "These probes can be further tuned to be 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

RESEARCH PAPERS

Weerapana, E. *et al.* Disparate proteome reactivity profiles of carbon electrophiles. *Nat. Chem. Biol.*, published online 18 May 2008.

NEWS IN BRIEF

MUTAGENESIS

A better knockout mouse

Inducible gene targeting in mice is time-consuming and can be unreliable. Zeng *et al.* now describe an improved conditional and reversible tetracycline-based system called iKO to generate knockout mice. It boasts more tightly regulated expression of the gene of interest, powered by the gene's own promoter, so there is no need to worry about leakage or tissue specificity. This should be especially welcome news to the mouse community.

Zeng, H. *et al. PloS Genet.* **4**, e1000069 (2008).

NANOTECHNOLOGY

Nanoparticle design

It can be quite a challenge to get nanoparticle-based delivery vehicles carrying cytosol-bound cargo through the cell membrane without doing any damage. Verma *et al.* now demonstrate that gold nanoparticles covered in alternating negatively charged and hydrophobic groups are safely delivered, whereas nanoparticles with these groups randomly distributed get stuck in endosomes. These findings are expected to be especially helpful to researchers designing gene and small interfering RNA–based therapies.

Verma, A. *et al. Nat. Mater.*, published online 25 May 2008.

GENOMICS

Resequencing by hybridization

Competition between next-generation sequencing technologies continues to heat up. Pihlak *et al.* describe the implementation of a sequencing-by-hybridization (SBH) approach, called shotgun SBH, that solves problems of previous SBH implementations. The genomic sample is cut into fragments, immobilized on a glass surface by rolling-circle amplification and probed sequentially with universal oligonucleotides. The high throughput and low cost of the method is appealing for simple resequencing applications.

Pihlak, A. *et al. Nat. Biotechnol.* **26**, 676–684 (2008).

MICROSCOPY

Large-scale 3D subdiffraction imaging

Many methods have been described for subdiffraction fluorescence imaging. One implementation, structured illumination microscopy (SIM), involves illumination with multiple interfering beams of structured light to double the resolution of a conventional microscope. Gustafsson *et al.* now extend SIM to imaging in three dimensions and Schermelleh *et al.* apply multicolor three-dimensional SIM to investigate the structure of the mammalian nucleus.

Gustafsson, M.G. *et al. Biophys. J.* **94**, 4957–4970 (2008).
Schermelleh, L. *et al. Science* **320**, 1332–1336 (2008).

GENOMICS

Fruit fly CNP map

It has long been suspected that copy-number changes could be influenced by natural selection. Emerson *et al.* now present evidence of just that. Using Affymetrix genomic tiling arrays, they generated a high-resolution copy-number polymorphism map for the fruit fly. They discovered that several toxin-related genes are duplicated including the cytochrome P450 gene *Cyp6g1*, critical in conferring resistance to the insecticide dichloro-diphenyl-trichloroethane, DDT.

Emerson, J.J. *et al. Science*, published online 5 June 2008.