SYNTHETIC BIOLOGY

# Building better bubbles

**Synthetic clusters of membrane-bound droplets may provide a useful means for simulating tissues or transporting therapeutic payloads.**

They may look like nothing more than microscopic bags of marbles, but the tiny bundles of lipid-encased bubbles being assembled at the University of Oxford could become a valuable tool for diverse applications in biotechnology, basic research and even drug delivery.

Several years ago, researchers in Hagan Bayley's laboratory found that aqueous droplets added into an oil-lipid mixture self-assemble into networks, with adjacent droplets forming shared lipid bilayers. Although these proved useful for the construction of simple devices, they also suffered from some inherent limitations. "With these networks immersed in a bulk solution of oil, they couldn't interact with the outside world, which in biology is aqueous," explains Gabriel Villar, a graduate student in Bayley's group.

Villar, along with Bayley and colleague Andrew Heron, has now solved this problem. By depositing individual lipid-encased droplets into another, larger oil-lipid drop in a buffer solution, they constructed 'multisomes': networks of droplets that form lipid bilayers both with each other and as an interface with the aqueous environment surrounding the multisome.

The composition of these interior droplets can be modulated before multisome assembly. In initial experiments, the researchers used the bacterial transmembrane pore protein α-hemolysin (αHL) to allow 'communication' between compartments. Using a microelectrode, they confirmed that individual αHL molecules had properly inserted themselves into the multisome membrane, allowing the influx of ions from the surrounding environment. Treatment with an αHL inhibitor blocked this ion transport. In subsequent experiments, they generated multisomes containing two droplets—one containing a calcium-rich solution and the other containing a calcium-responsive fluorescent dye—and demonstrated diffusion through the αHL pores linking individual droplets in the multisome.

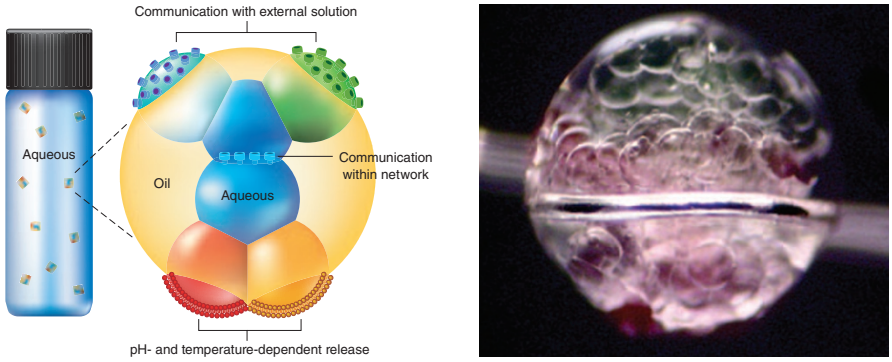


Illustration and image of a multisome suspended in buffer. Image courtesy of G. Villar and H. Bayley.

Although mammalian membrane proteins have proven far more difficult to manipulate than the bacterial proteins that Bayley's team has used to date, this platform could eventually be a powerful resource for studying membrane-spanning cellular structures such as gap junctions. Multisomes could also become a useful 'construction set' for synthetic biology, giving scientists the means to build sophisticated biochemical workflows by combining sets of droplets, each with its own cargo and cohort of membrane proteins.

Instead of simply working with individual 'protocells', the ultrasimplified simulacra of living cells currently under development by several research groups, Bayley suggests that multisomes may ultimately enable scientists to assemble far more complex multi-protocell structures that he calls 'prototissues'. "If we can make such prototissues, we believe we can interface them with electronics," he says, "and these materials might even form a good interface with tissues in the body."

Bayley acknowledges that such applications are "a bit more futuristic," but multisomes may find more immediate use in other applications, including as vehicles for controlled drug delivery. Villar and colleagues tweaked the lipid composition to produce multisomes that can only maintain bilayer integrity in a particular range of temperature or pH conditions. In the latter scenario, multisomes remained intact for 24 hours or longer under mildly basic conditions (pH 8.0) but ruptured when the pH was lowered to 5.5. "I expected that the bilayers would become somewhat permeable," says Villar, "but they just popped like soap bubbles and released their contents in an instant." This suggests that multisomes could prove suitable for either sustained delivery by diffusion through pores inserted in the bilayer or sudden release brought on by a specific physiological trigger.

Many hurdles remain to be cleared, however, and Villar and Bayley are working hard to optimize the properties of their multisomes before focusing on specific applications. Transitioning the somewhat laborious multisome manufacturing process into a more mass production–oriented workflow will be a key step in this process. "These objects would be much better made using a microfluidic system," says Bayley. "We'd like to be able to make a lot of them simultaneously, make them smaller and functionalize them in more sophisticated ways."

**Michael Eisenstein**

RESEARCH PAPERS
Villar, G. *et al.* Formation of droplet networks that function in aqueous environments. *Nat. Nanotechnol.* **6**, 803–808 (2011).