Synthetic cells have senses too

What defines a living cell? How to capture the molecular essence of life? These fundamental questions underpin the collaborative research programme led by Prof Allen Liu at the University of Minnesota and Prof Vincent Noireaux at the University of Minnesota. The pair uses molecular components to construct prototypes of synthetic cells displaying the minimal characteristics of life. Their “cell analogs” shed light on basic biological processes, and also provide new tools for biotechnology and medicine.

Biology | Professor Allen Liu

Phospholipid bilayers are direct analogues of the membranes surrounding cells in nature

in a DNA sequence, metabolism (energy generation, building blocks synthesis and cycling), and self-organisation (encapsulate information and metabolism within a physical boundary). All three are included in Liu and Noireaux’s National Science Foundation (NSF) project, “Construction of DNA Programmed Minimal Cells with Membrane Mechanosensitive Functions.”

Although construction of synthetic biological cells now appears plausible, no functional minimal cells have yet been constructed from basic molecules. Liu and Noireaux believe that their modular approach should enable the integration of multiple molecular components through carefully characterised connections, to create the artificial cells capable of incorporating multiple cellular functions.

INFORMATION IS POWER

Using elements of the molecular machineries of the bacterium Escherichia coli, Liu and Noireaux have developed a cell-free system that can transcribe and translate the information in a DNA sequence into the proteins that carry out cellular functions. Transcription (the copying of the DNA code onto a template RNA molecule) and translation (the construction of proteins using this RNA template) are both achieved in the team’s unique cell-free transcription-translation (TXTL) system, which forms the basis for their efforts. Once the information is in the TXTL machinery is encased in a small, cell-sized compartment called a liposome. Liposomes make use of the simple fact that fats (lipids) do not dissolve in water. A double layer of molecules, each comprising a hydrophilic (water-loving) group such as a phosphate and a hydrophobic (lipid) component, when placed in water, will spontaneously orient itself to form a membrane-bound sphere, with the lipids pointing into the centre of the membrane, shielded from the surrounding water and the inside of the liposomes. The lipid bilayer is, it opens and closes in response to osmotic pressure (the pressure created by the difference in concentrations of the dissolved molecules in the surrounding water and the inside of the liposomes).

To test the function of the Mscl channel, they also encoded in the DNA of their minimal cell an engineered protein called G-GECO, which fluoresces in response to calcium ions. Thus, when calcium molecules present are mechanical (osmotic pressure) and chemical (calcium ions). When osmotic pressure causes the Mscl channel to

ORGANISATION IS KEY

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MAKING METABOLISM POSSIBLE

Having encased the information system inside a liposome, the final, and perhaps most challenging part of building a minimal cell is to enable it to communicate with the outside world. To metabolise, grow, and reproduce, the minimal cell must be able to take in sources of energy across its membrane, exel waste products in the opposite direction, and respond to signals received from the surrounding environment.

Liu and Noireaux’s current work is focused on integrating molecular channels in the membrane of the liposome, which respond to environmental stimuli by opening or closing. Modelled on another system found in Escherichia coli, they have built a DNA sequence that encodes a membrane channel protein called Mscl. Mscl is ‘mechano-sensitive,’ that is, it opens and closes in response to osmotic pressure (the pressure created by the difference in concentrations of the dissolved molecules in the surrounding water and the inside of the liposomes).

A BIOLOGICAL ‘AND GATE’

Liu and Noireaux describe this minimal cell system as the synthetic, biosensitive ‘AND gate’ (a system that requires two inputs to produce a response). In this case, the two inputs are mechanical (osmotic pressure) and chemical (calcium ions). When osmotic pressure causes the Mscl channel to
open, calcium ions flow through the liposome and activate G-GEÇO. Thus, say the pair, “We have generated a DNA-programmed cell-sized artificial cell.”

Their aim now is to couple this activation to the physical growth of the cell by linking it to the genetically controlled synthesis of lipids from smaller precursor molecules. In this way, the mechanosensitive membrane will facilitate key linkages between the cell’s structure and its DNA, a crucial further development towards true synthetic life.

**BETTER THAN BIOLOGY?**

Liu and Noireaux’s research paves the way for the creation of minimal cells capable of sensing and responding to their environment, a central step in synthetic biology that has been until now hard to achieve. Their system provides an experimental platform enabling rapid and reliable quantification of the effect of manipulating any aspect of the minimal cell system: the membrane, surrounding solution, and genetic machinery. This should speed up future advances in minimal cell engineering, which may ultimately be able to expand upon the properties found in the cells of natural organisms.

This project relies heavily upon collaboration between laboratories, institutions, and disciplines, to solve what Noireaux terms the “challenging but conceivable goal” of synthesising the minimal cell. Lying at the interface of biology and engineering, this research increases our basic knowledge of life, stimulates advances in medicine – such as drug delivery – and biotechnology, and provides future scientists studying across these institutions with an exciting introduction to cutting-edge research based on fundamental biological and physical principles. It is not, says Noireaux, “just an exercise,” but a “forward engineering approach.”

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**Behind the Bench**

**Research Objectives**

Prof Liu and Prof Noireaux’s project aim is to construct synthetic cells, consisting of a cell-free expression system encapsulated into a cell-sized phospholipid vesicle, capable of sensing the environment through its lipid bilayer by expressing mechanosensitive channels.

**Funding**

National Science Foundation (NSF)

**Collaborators**

- Sagarajy Majumder, PhD student, University of Michigan
- Jonathan Garamella, PhD student, University of Minnesota

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**Bio**

Allen Liu received his PhD in Biophysics in 2007 from the University of California - Berkeley. Since 2012, he has been an Assistant Professor in the Department of Mechanical Engineering at the University of Michigan.

Vincent Noireaux received his PhD in Biophysics in 2000 from the University Paris XI. Since 2005, he has been an Assistant, Associate and now Full Professor in the Physics Department at the University of Minnesota.

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