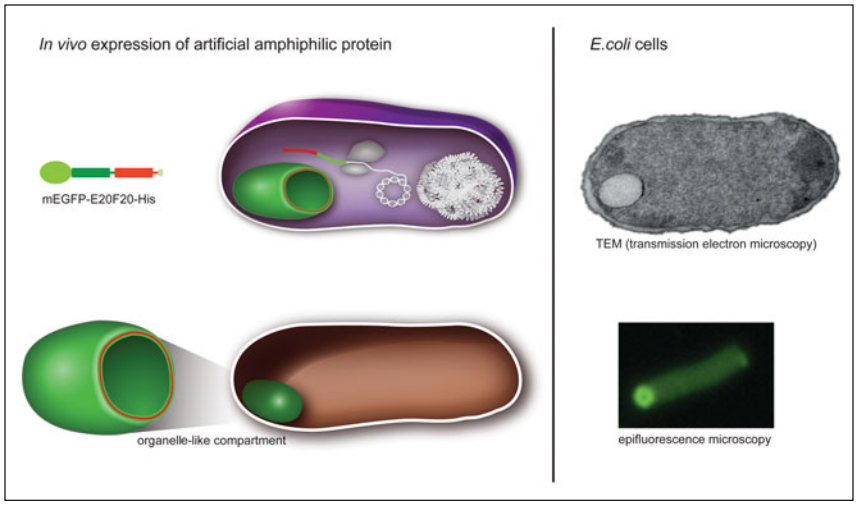
Bio Focus

Building synthetic organelles from the gene up

Membrane-bound compartments, such as lipid micelles, are essential cellular structures that have endured and diversified across millennia following their probable key role in the origin of life. Recently, micelles and related types of micro-compartments (e.g., emulsions) have been gaining attention as platforms that can enable new chemical, biological, and materials technologies. The use of both micro- and nano-compartments has been demonstrated for a wide range of applications, including drug delivery, genetic screening, and nanomaterial synthesis. This in turn has inspired a groundswell of novel approaches to compartment production. The majority of these approaches rely on physical mechanisms, such as fluid-fluid shearing in microfluidic channels. In contrast to these simple physical systems, however, biological organelles remain far superior in performing sophisticated tasks, such as generating chemical gradients, sequestering toxic substances, and dynamically sculpting polymeric or biomineral materials.

Synthetic biology offers the prospect of harnessing this natural complexity by means of genetic re-programming. One can imagine, for example, providing cells with instructions to synthesize modified organelles with tailor-made functions. However, in practice this goal has been hampered by the complexity of multi-enzyme pathways that cells employ to produce lipid membranes. Deftly bypassing this barrier, a collaborative team of researchers at the University of Freiburg, Germany, and the Hungarian Academy of Sciences has recently demonstrated the first known example of cell-programmed production of organelle-like structures, as reported in the November 2, 2014, online edition of *Nature Materials* (DOI: 10.1038/NMAT4118).



Overview of a genetic approach for producing organelle-like compartments in live cells. Amphiphilic proteins are produced by the ribosomes of *E. coli* and then self-assemble for *de novo* compartment production. Instructions for polypeptide chain length and chemistry are specified by genetic constructs transformed into the cells. The amphiphilic protein structure that led to *de novo* compartmentalization is composed of a hydrophobic part, highlighted in red and a hydrophilic part highlighted in green. The transmission electron micrograph section of a cell shown in the back reveals compartments of the modified cell architecture. © Schiller group.

The researchers harness proteins—in lieu of lipids—as a key step toward achieving controlled intracellular compartmentalization. The protein system utilized is based on a simple stretch of five amino acids that exhibits either hydrophilic (see Figure, green highlights) or hydrophobic (see Figure, red highlights) character, according to a single residue switch in the sequence (using either hydrophilic glutamine or hydrophobic phenylalanine; see Figure). Amphiphilic proteins composed of these repeat domains have previously been shown to self-assemble *in vitro*. As a major finding in their study, the researchers show that protein self-assembly can also occur in living cells. When expressed from genetic constructs in *E. coli*, the amphiphilic proteins self-assemble into compartments approximately 500 nm in diameter within the cells. Spatially resolved elemental analysis using transmission electron microscopy shows that the formed vesicles are "empty"—(i.e., filled primarily with water). Amazingly, the cells remain viable even as they generate these (relatively) large empty spaces.

The researchers demonstrate that compartmentalization is a potentially tunable process, as successful vesicle formation requires a particular polypeptide domain structure (20 hydrophilic segments followed by 20 hydrophobic segments), and variations in the order or length of these domains either abolish self-assembly or yield unique intracellular morphologies. The researchers also demonstrate that additional proteins and non-natural amino acids can be integrated, through genetic engineering, into the self-assembled membranes.

While much remains to be learned regarding design rules for vesicle formation, and new ways must be invented to stuff them full of useful things, the results suggest that a vast range of functionalized synthetic organelles might eventually be created. Thanks to this recent advance, a future with customized cellular organelles now appears closer than previously imagined.

Lukmaan A. Bawazer