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Controlling transport across artificial cell membranes

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STANDFIRST:

Engineering synthetic cells faces the challenge of transferring biomolecules, such as nucleic acids and proteins, through simple lipid bilayers. Now, a new study reveals how energy-dissipating oil droplets can create reconfigurable passageways shuttling biomolecules across liposomal compartments. [39 words]

NEWS

The cellular membrane, which defines the boundary of a cell, is not a hermetically sealed barrier isolating the compartment from its extracellular surroundings. Instead, it functions as an information hub — a nexus for bi-directional exchange of molecules, materials, and signals — facilitating communication between the cell and its surroundings. Efforts to replicate these complex interfaces in synthetic cells(*1*) require a delicate balance between two opposing tendencies: isolation, which physically segregates the intracellular content, distinguishing the inside from the outside, and communication, which, by well-regulated exchanges of signals and content, provides each means to receive inputs from the other.

In modern living cells, this balance between isolation and communication is achieved through a well-coordinated interplay of membrane permeability and the activities of membrane proteins. The essential impermeability of the phospholipid bilayer to the passive movement of most solutes allows for molecular segregation whereas channels, pumps, and transporters facilitates the active transport of small molecules, e.g., amino acids, sugars, and ions. Additionally, cells use a diverse class of mechanisms – collectively termed endocytosis – to exchange large macromolecules, clusters, pathogens, and materials between the inside and the outside(*2*). Here, the cargo is trafficked in the form of membrane-bound vesicles by budding invaginations and pinching-off pieces of the plasma membrane. These morphological and topological membrane transformations are energy intensive(*3*), and typically involve coordinated, cooperative, and synergistic actions of one or more proteins(*4*).

Do cells have other less demanding options? Can the transport of content be achieved without using sophisticated protein machineries and without inducing large-scale topological breach of the plasma membrane? Are there common, non-specific, and readily available physical-chemical mechanisms that can do the job? Using cell-free liposomal models, Jia-Qi Tian, Nan-nan Deng, and colleagues(*5*) now demonstrate that they might. Using suspensions of synthetic liposomes and oil microdroplets, produced *de novo* in microfluidic channels, the researchers show remarkable wetting-dewetting transitions driving dynamic association spanning complete engulfment of the oil droplet by the

liposome on the one hand to full spatial separation of the two on the other. The authors that these dynamics arise because of the changes of interfacial energies, which can be triggered on command by heat, evaporation, or osmotic pressure gradient. They show that these recofigurable oil-based channels can bidirectionally shuttle molecular cargo – both hydrophilic and hydrophobic alike – across the liposomes without a topological breach. The findings, thus, suggest a heretofore unappreciated, new route – involving oil-based channels and hydrophobic conduits – to ferry molecules and materials across the membrane barrier.

Tian, Deng, and others begin by reporting the observations of a striking interplay – a tango at the mesoscale – between the oil microdroplets and liposomes. They prepare water-in-oil-in-water (W/O/W) double emulsion droplets in a microfluidic device(6). The oil phase they use consist of a phospholipid-laden mixture of chloroform and hexane, and the aqueous phase is a mixture of polyvinyl alcohol (PVA), polyethylene glycol (PEG), and surfactants. Consistent with previous studies(6), the authors find that the oils (*sans* lipids) dewet and break away, thereby creating two co-existing microphases: (1) liposomes or giant unilamellar vesicles and (2) oil microdroplets, the latter stabilized presumably by a monolayer of dissolved lipids.

The researchers find that this complex multiphase emulsion does not persist for long periods, being metastable. Over the time scale of several tens of minutes to hours, the oil droplets begin to re-mix with the liposomes. This reincorporation of oil gradually dissolves the membrane surface area and correspondingly elevates oil area. This remarkable reversal in phase topology – the dewetting-rewetting dynamics – suggests a non-equilibrium process. The authors propose that the driving force is the changing balance of interfacial energies(7, 8), because of the continued evaporation of chloroform in the oil droplets. Lending credence to the proposition, other environmental triggers of interfacial energy changes, namely temperature and osmotic pressure, also yield the qualitatively comparable behavior.

Seeing the possibilities of dynamically tuning the balance of interfacial energy – and thus programming the 'tango' between the oil droplets and liposomes – Tian, Deng, and coworkers next tweak the constituents of the emulsion and achieve a cyclical process of phase separation (dewetting) and encapsulation (engulfment). As expected, these cycles are accompanied by corresponding expansion and contraction of the membrane, facilitated by the lipid-laden oil droplets acting as a lipid reservoir – a process reminiscent of surface area regulation in living cells(9).

Perhaps, the most intriguing aspect of the present study is the realization that the microdroplet-liposome tango can be exploited to transport molecular cargo – both hydrophilic and hydrophobic alike – in and outside of the liposomes. An elegant demonstration of the movement of hydrophilic cargo involves the vectorial transport of fluorescein, a membrane impermeable molecule, which is a water and oil soluble fluorescent solute. The researchers achieve this simply by exploiting the higher affinity of fluorescein for poly(vinyl) alcohol (PVA) over poly(ethylene) glycol PEG, two water-soluble polymers. They show that the fluorescein can be pushed in the direction of higher binding

affinity (i.e., higher PVA concentration), even against the net concentration gradients of the two polymers. These programmable passageways, the authors further show, allows for the directional uptake of hydrophobic substrates, which interact with the pre-encapsulated enzyme inside the lysosomes. This "chemistry on command" enabled by the wetting-dewetting transitions in spatial confinement has a profound potential for engineering life-like behaviours in soft systems. In additional studies, the authors demonstrate the bulk transport of microdroplets could be coupled with shuttling of a diverse variety of molecular cargoes including enzyme substrates, ions and ssDNA oligomers.

This ability of dynamically reconfiguring the spatial and topological relations between the oil microdroplets and liposomes and exploiting them as oil-based channels and hybrophobic conduits to ferry molecular cargoes across the membrane barrier suggests an unusual pathway to program communication between the synthetic cells and their environment.

These findings raise a number of questions: (i) What is the ultimate, steady state fate of the oily droplets both inside and outside the liposomes? (ii) What does the dispersal of oil between the monolayer leaflets do to the interleaflet interactions? What does it do to the stability of any proteins, soluble or membrane-associated? (iii) How would these oily "organelles" inside synthetic protocells influence conformations, stabilities, and distributions (and interactions) of other biomolecular components and clusters? (iv) Would they be programmable and reconfigurable surrogates for the so-called membraneless organelles? Such questions are not confined to this particular example, but arise more broadly in the pursuit of design principles for the synthetic cell.

It is tempting to wonder if there are biological parallels to these seemingly counter-intuitive, mesoscopic oily passageways. We now know that the cytoplasmic environment is strongly emulsified: lipid droplets and related fatty body inclusions are present ubiquitously in the cytoplasm(*10*). Like the oily microdroplets here, they too have a hydrophobic core packing nonpolar molecules and are wrapped by a monolayer of amphiphilic phospholipids. Moreover, many of the recently discovered membraneless organelles or biomolecular condensates(*11*, *12*), may have hydrophobic, oil-like character. In all these cases too, specific molecules are sequestered promoting spatially localized chemistries. It remains to be established whether these organelles can (or can be programmed to) also dissipate energy and provide dynamic, reconfigurable oil-based channels for transmembrane transport.

Figure: CUT-PASTE FROM THE ORIGINAL MANUSCRIPT

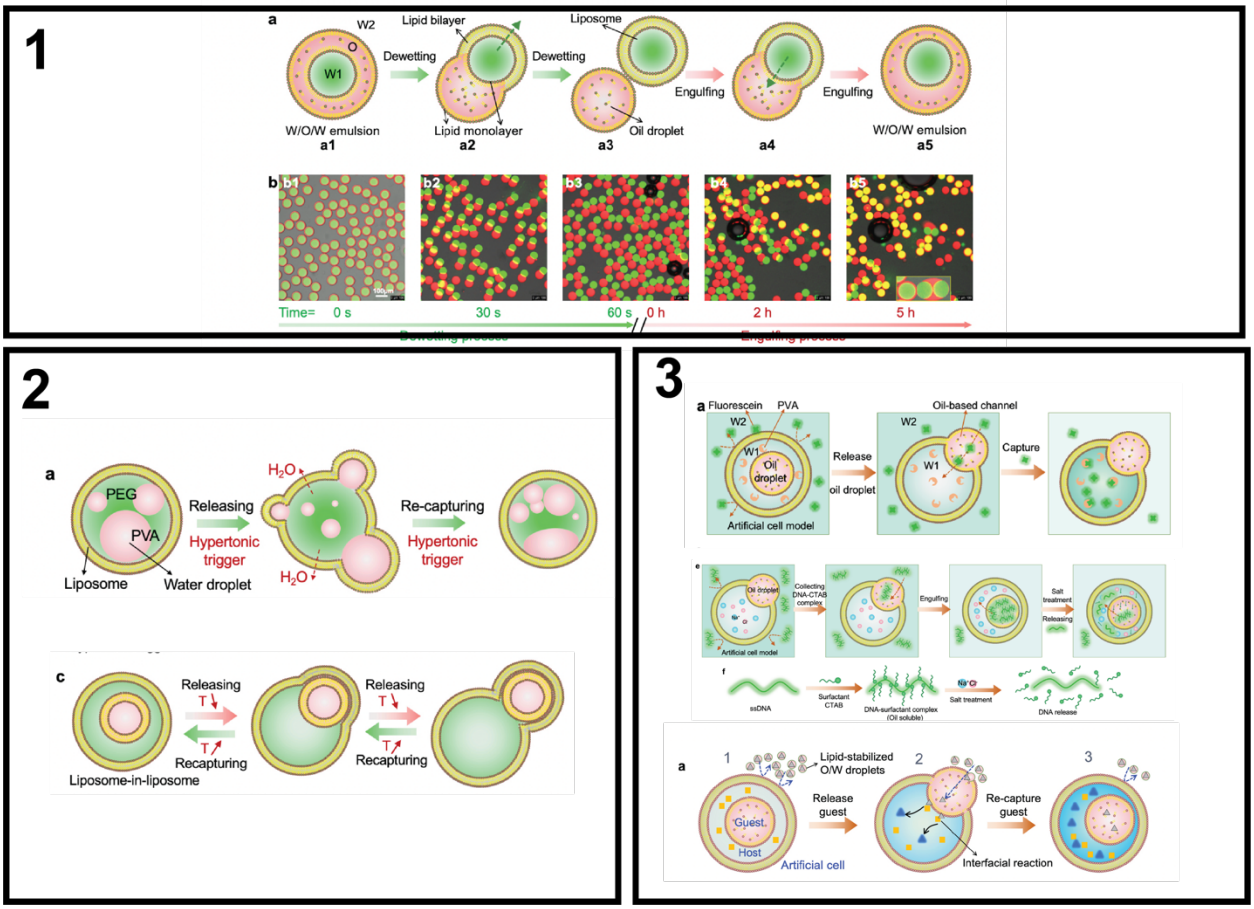

Figure Legend: **The tango between oily droplets and liposomes**: (1) the essential wetting-dewetting transition driven by evaporation-induced changing interfacial energies; (2) transporting oil droplets across liposomal membranes using osmotic and thermal stimuli; and (3) shuttling molecular cargo and prompting chemistries on command by programming the movement of the oil microdroplets within the liposomes.

The author declares no competing interests.

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References

1. J. W. Szostak, D. P. Bartel, P. L. Luisi, Synthesizing life. *Nature* **409**, 387-390 (2001).
2. S. D. Conner, S. L. Schmid, Regulated portals of entry into the cell. *Nature* **422**, 37-44 (2003).
3. M. M. Kozlov, H. T. McMahon, L. V. Chernomordik, Protein-driven membrane stresses in fusion and fission. *Trends in Biochemical Sciences* **35**, 699-706 (2010).
4. B. J. Reynwar *et al.*, Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. *Nature* **447**, 461-464 (2007).
5. M.-Y. C. Jia-Qi Tian, Chen Chen, Zhen-Hong Luo, Wilhelm T. S. Huck & Nan-Nan Deng, Interfacial energy-mediated bulk transport across artificial cell membranes. *Nature Chemical Engineering* **in press**, (2024).
6. S. Deshpande, Y. Caspi, A. E. C. Meijering, C. Dekker, Octanol-assisted liposome assembly on chip. *Nature Communications* **7**, (2016).
7. L. D. Zarzar *et al.*, Dynamically reconfigurable complex emulsions via tunable interfacial tensions. *Nature* **518**, 520-524 (2015).
8. N. N. Deng, M. Yelleswarapu, W. T. S. Huck, Monodisperse Uni- and Multicompartment Liposomes. *Journal of the American Chemical Society* **138**, 7584-7591 (2016).
9. C. E. Morris, U. Homann, Cell surface area regulation and membrane tension. *Journal of Membrane Biology* **179**, 79-102 (2001).
10. A. R. Thiam, R. V. Farese, T. C. Walther, The biophysics and cell biology of lipid droplets. *Nature Reviews Molecular Cell Biology* **14**, 775-786 (2013).
11. A. S. Lyon, W. B. Peeples, M. K. Rosen, A framework for understanding the functions of biomolecular condensates across scales. *Nature Reviews Molecular Cell Biology* **22**, 215-235 (2021).
12. J. A. Riback *et al.*, Composition-dependent thermodynamics of intracellular phase separation. *Nature* **581**, 209-+ (2020).