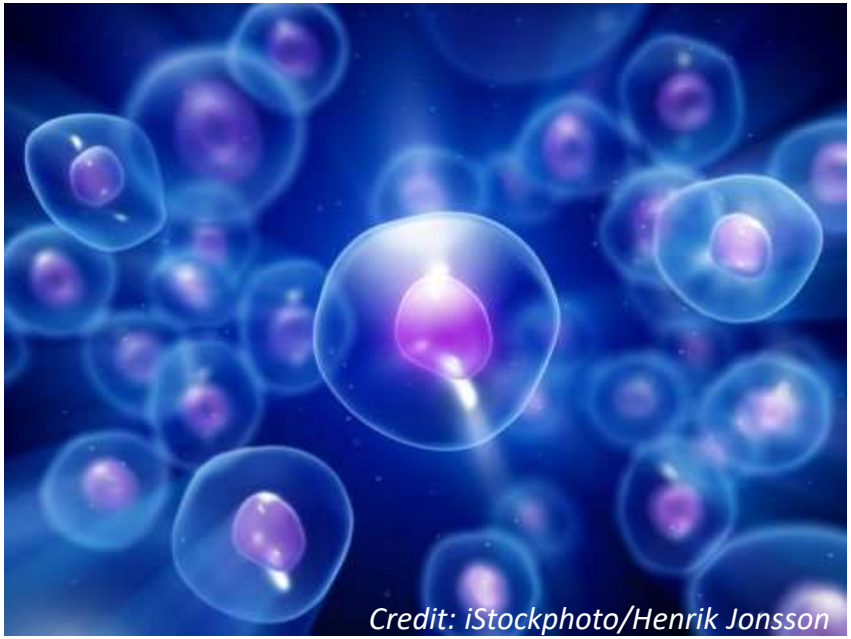
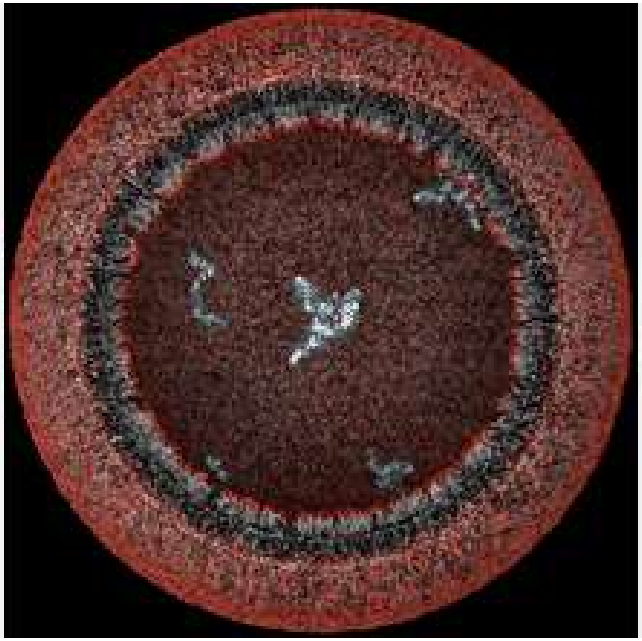
CHAPTER 3



LIPIDS

Membrane compartments

Assembly of amphiphilic monomers into protocellular compartments



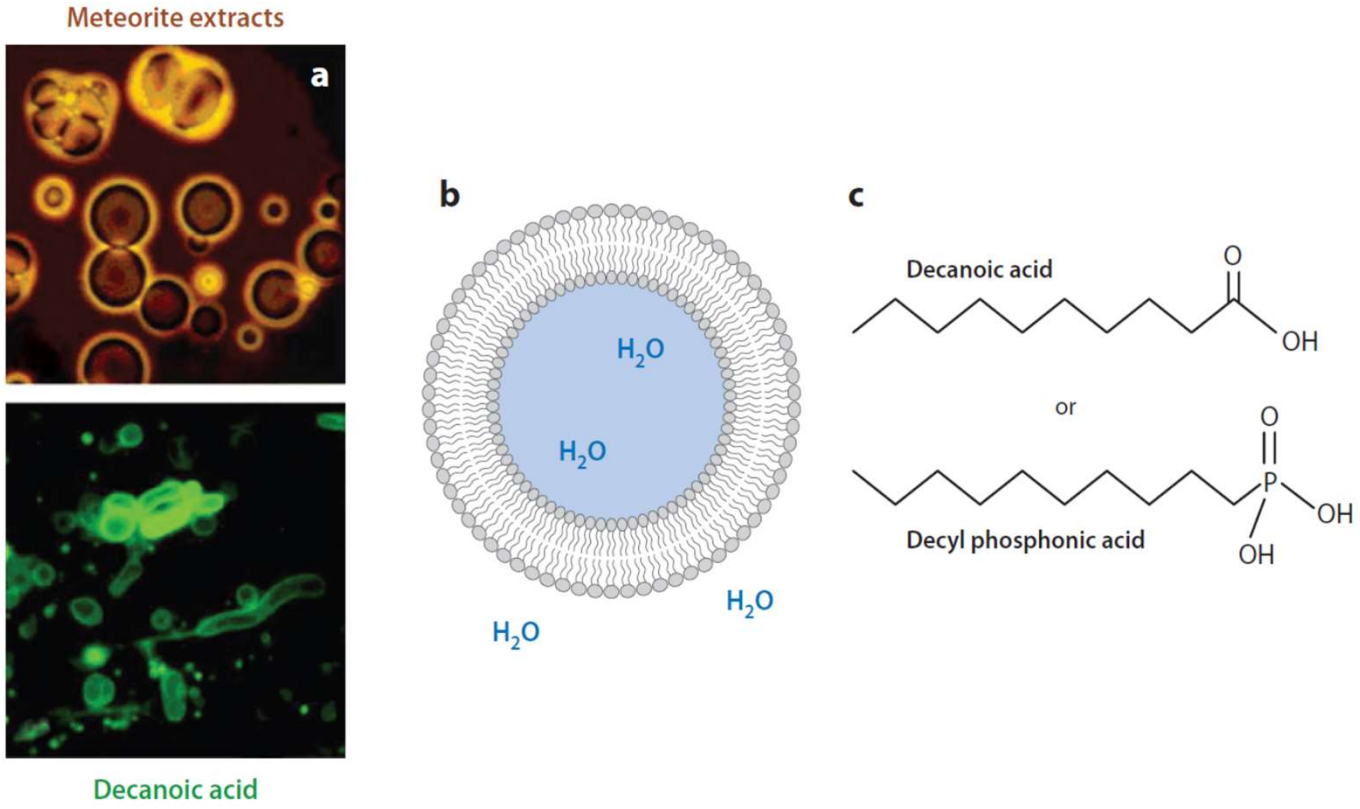
Credit: *Janet Iwasa*

A three-dimensional view of a model protocell (a primitive cell) approximately 100 nanometers in diameter.

The protocell's fatty acid membrane allows nutrients and DNA building blocks to enter the cell and participate in non-enzymatic copying of the cell's DNA. The newly formed strands of DNA remain in the protocell

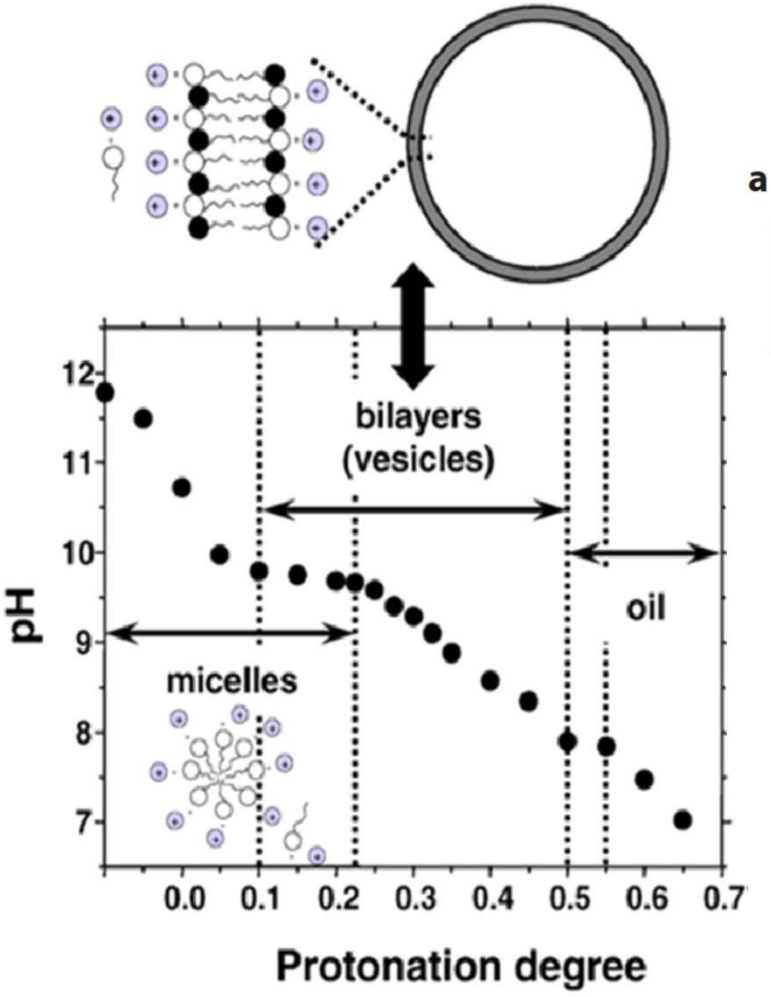
Encapsulation – essential for life

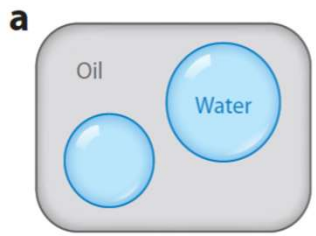
Fatty acids have been found in meteorites – plausible prebiotic synthesis pathways existed in the early Solar System

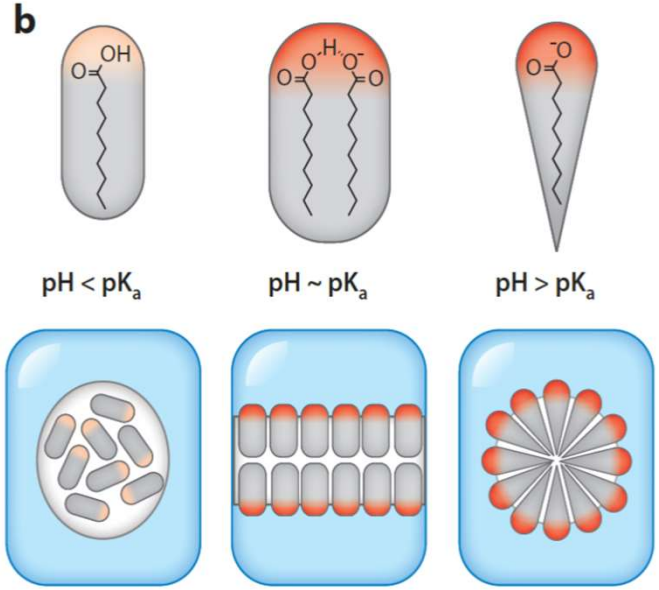


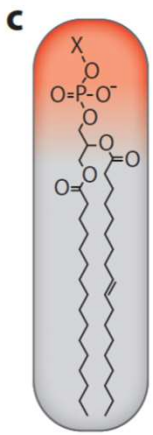
Extracts of meteorites containing these compounds spontaneously form vescicles when hydrated

pH-dependent phase behavior of fatty acids in water



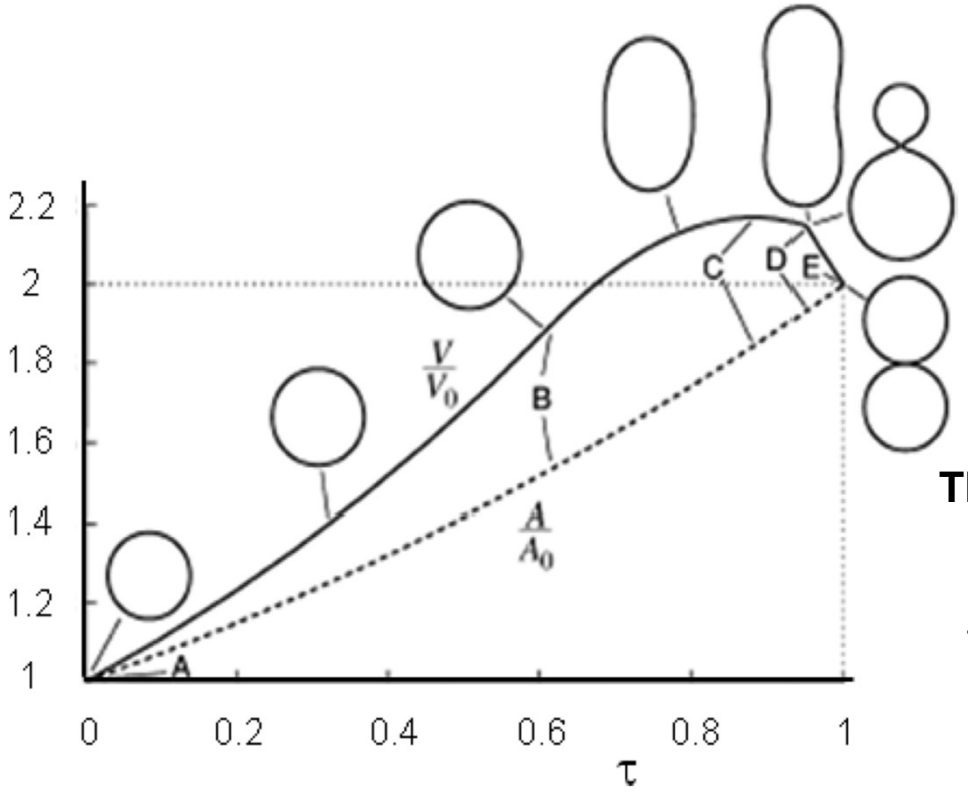


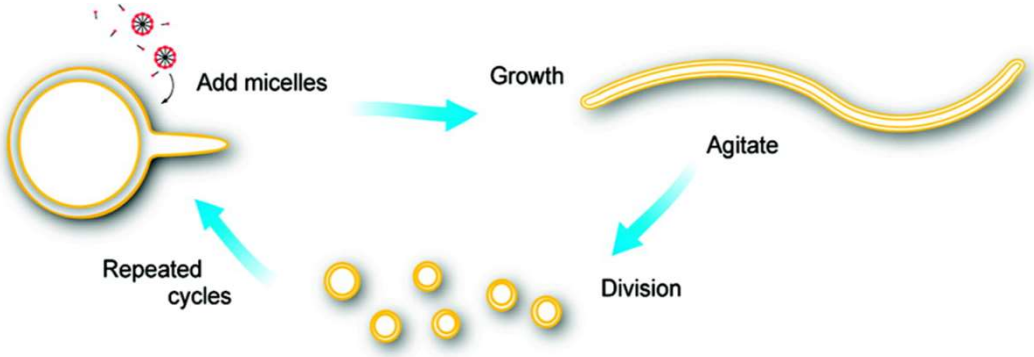




80 mM oleic acid/ sodium oleate in water

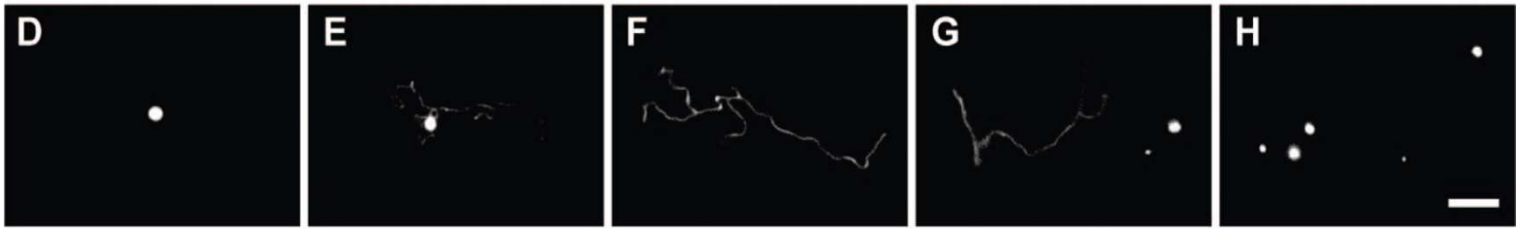
Growth and division of vesicles





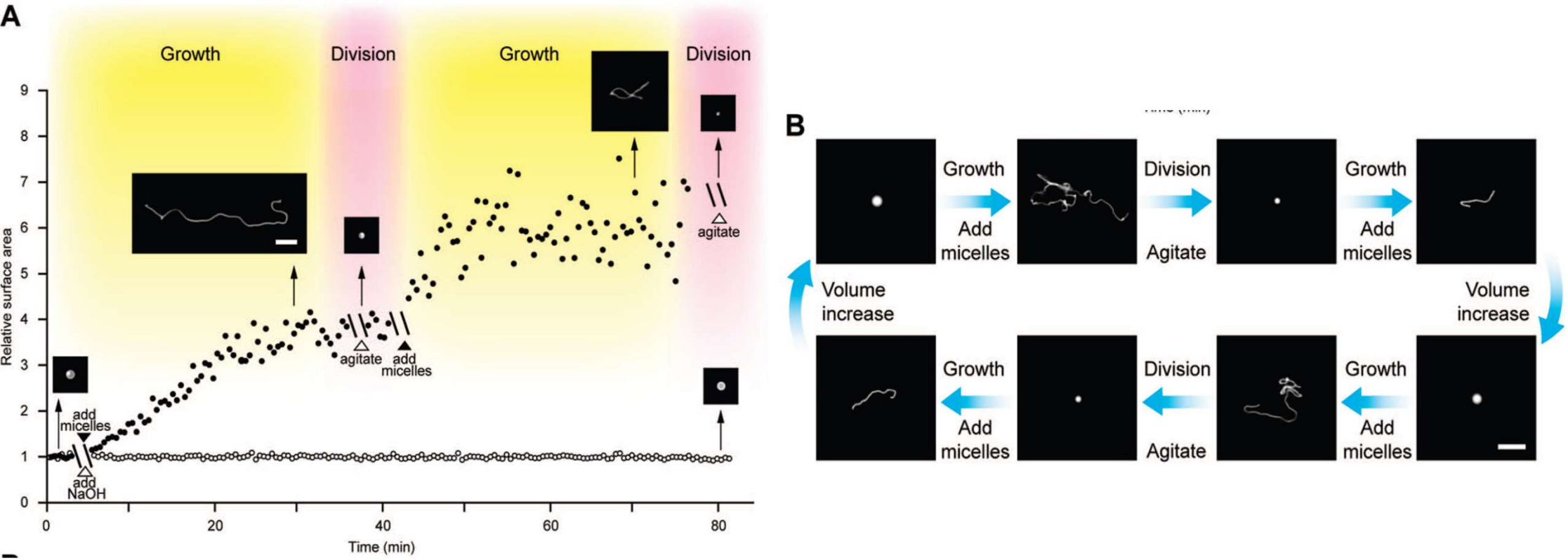
The growth of large multilamellar fatty acid vesicles fed with fatty acid micelles:

when solute permeation across the membranes is slow, the transient imbalance between surface area and volume growth causes formation of long thread-like vesicles. Modest shear forces are then sufficient to divide them into multiple daughter vesicles without loss of internal contents.



Ting F. Zhu, and Jack W. Szostak *J. Am. Chem. Soc.*, **2009**, 131 (15), 5705-5713

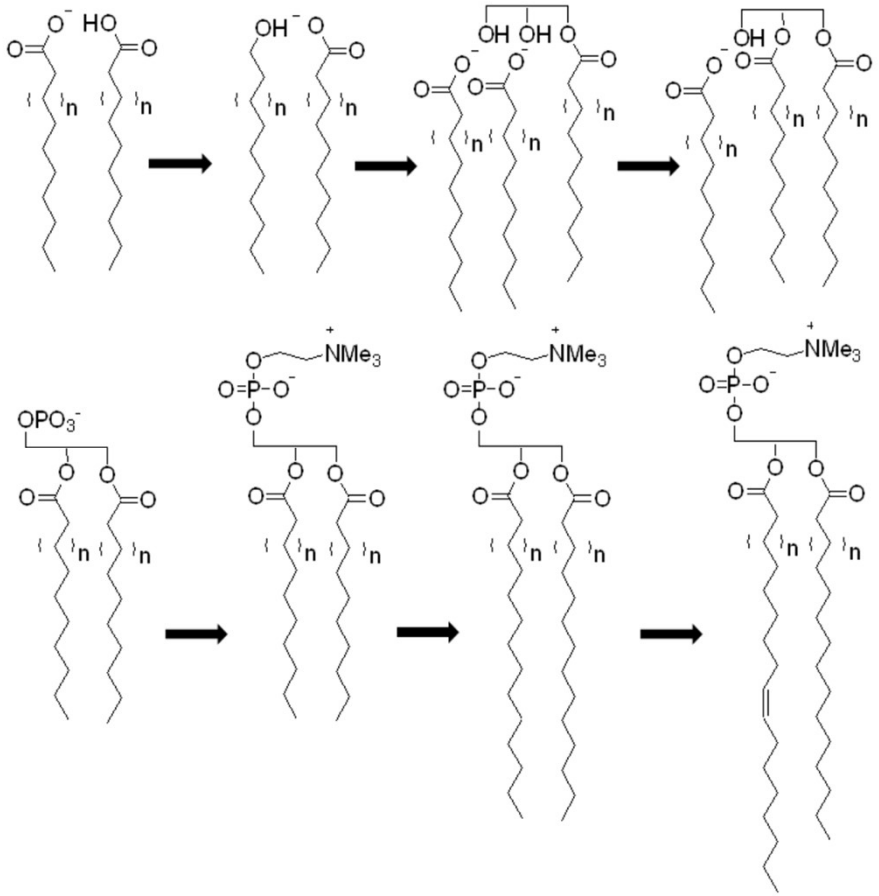
Coupled growth and division of model protocell membranes



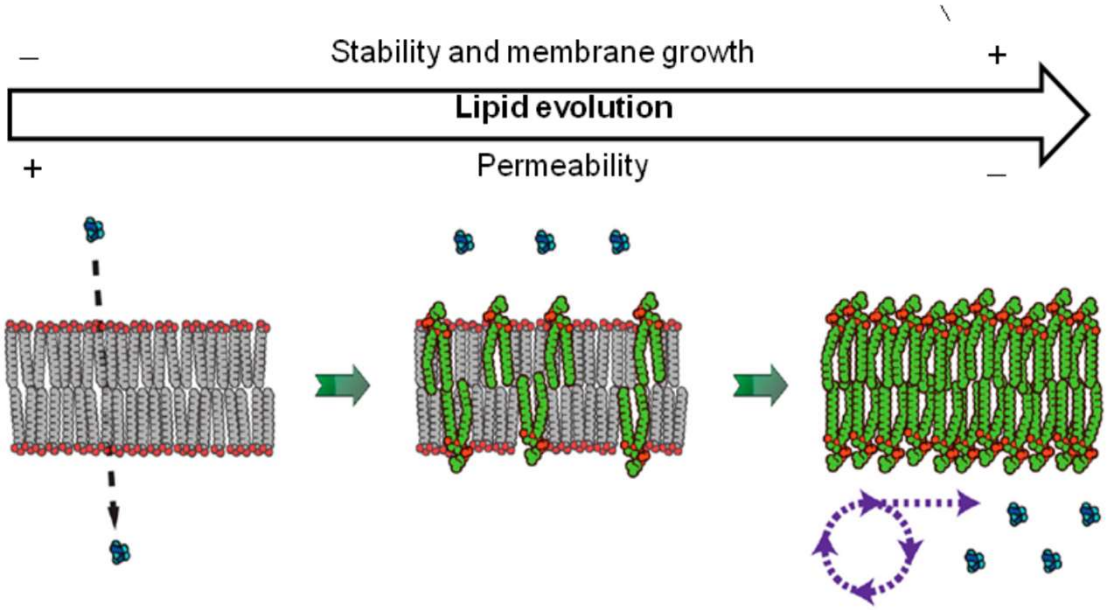
Cycles of vesicle growth and division. (A) Relative surface area after two cycles of addition of 5 equiv of oleate micelles (solid circles) or 5 equiv of NaOH (open circles) to oleate vesicles, each followed by agitation. Inset micrographs show vesicle shapes at indicated times. Scale bar, 10 μm. (B) Vesicle shapes during cycles of growth and division in a model prebiotic buffer (0.2 M Na-glycine, pH 8.5, ~1 mM initial oleic acid, vesicles contain 10 mM HPTS for fluorescence imaging). Scale bar, 20 μm.

Ting F. Zhu, and Jack W. Szostak *J. Am. Chem. Soc.*, **2009**, 131 (15), 5705-5713

Scheme of the membrane evolution

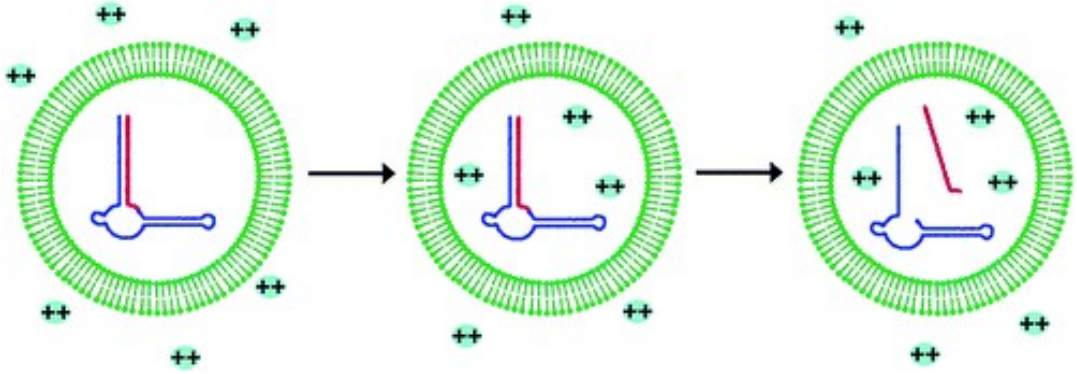


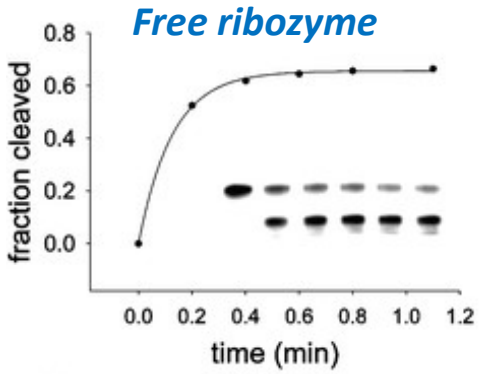
Chemical evolution of membrane components

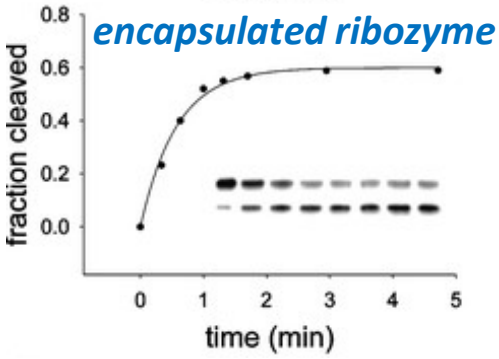


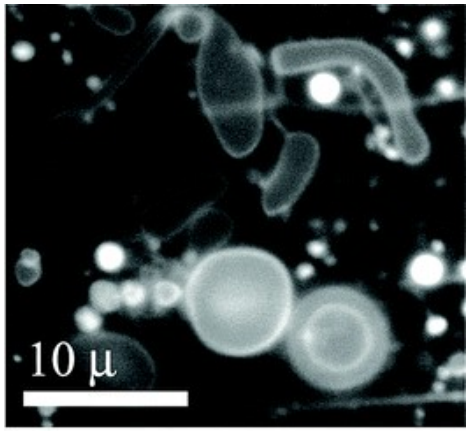
More complex components lead to slower amphiphile desorption and thus faster growth of the protocell. Decreasing permeability is a selective pressure for the emergence of internalized metabolic and transport machinery in the system

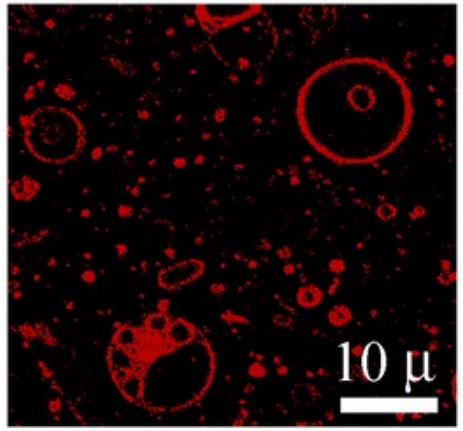
RNA Catalysis in Model Protocell Vesicles











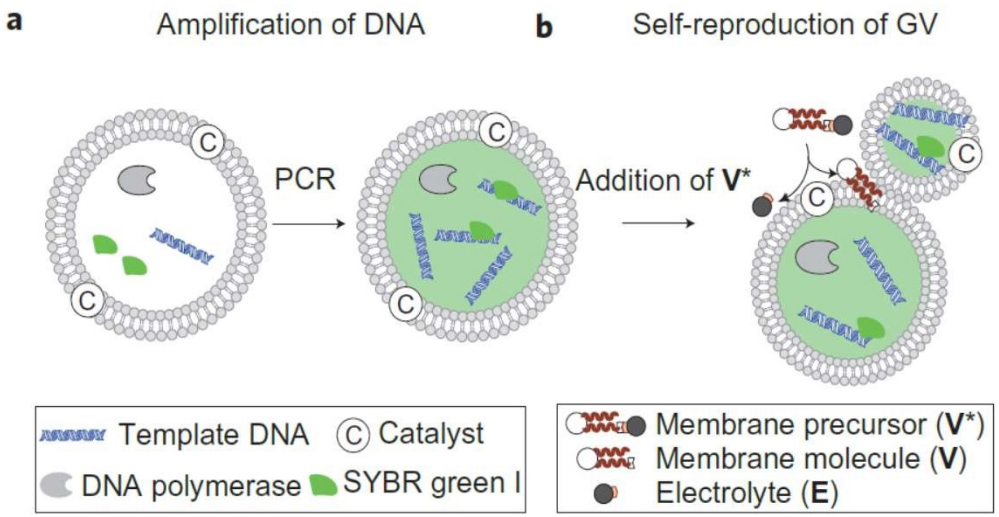
A mixture of myristoleic acid and its glycerol monoester forms vesicles that were Mg^{2+}-tolerant. Mg^{2+} cations can permeate the membrane and equilibrate within a few minutes.

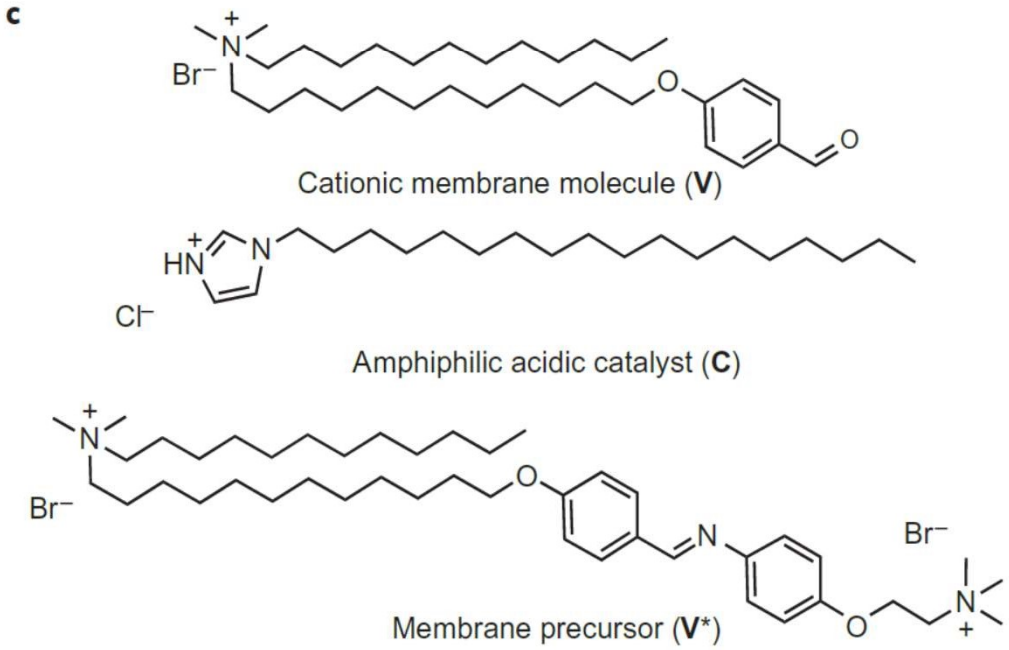
In vesicles encapsulating a hammerhead ribozyme, the addition of external Mg^{2+} led to the activation and self-cleavage of the ribozyme molecules. These vesicles can grow upon addition of micelles. It demonstrates that membranes made from simple amphiphiles can form vesicles that are stable enough to retain encapsulated RNAs in the presence of divalent cations.

Fluorescence microscopy of 2:1:0.3 MA:GMM:dodecane vesicles containing hammerhead ribozyme in the presence of 3 mM $MgCl_2$,

I. A. Chen, K. Salehi-Ashtiani, and J. W. Szostak *J. Am. Chem. Soc.*, **2009**, 127, 13213-13219

Self-reproduction of giant vesicles combined with the amplification of DNA





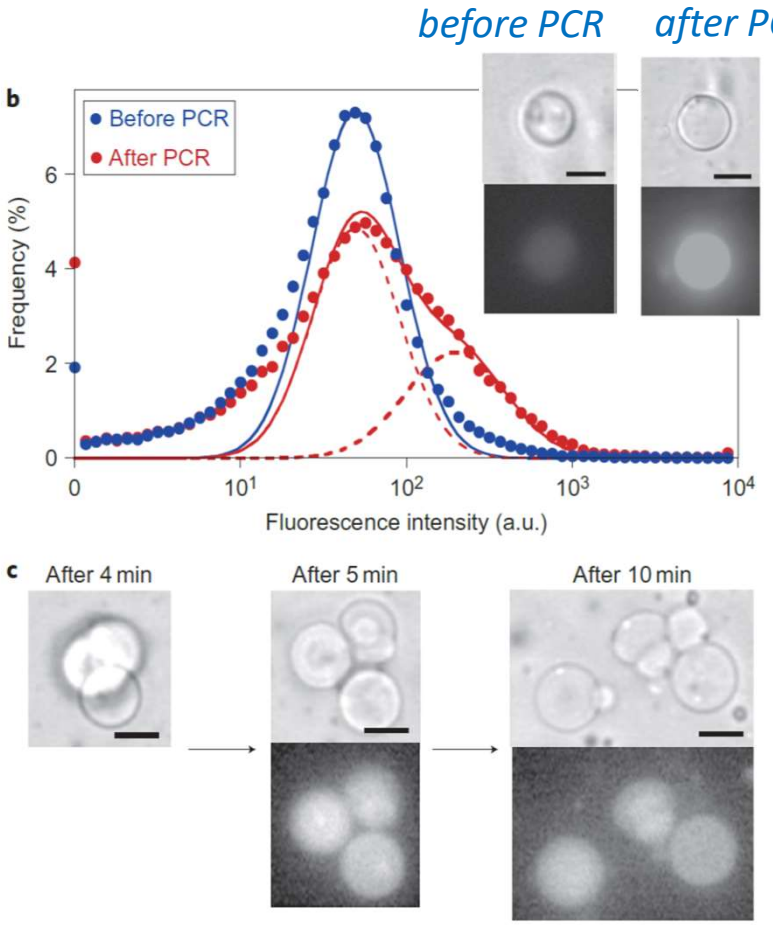
a, ***Amplification of DNA within a GV***. An aqueous dispersion of GVs containing PCR reagents was prepared using a film-swelling method with a buffered solution containing template DNA, primers, fluorescent tag SYBR Green I, deoxynucleoside triphosphates, DNA polymerase and Mg^{2+}.

b, ***Vesicular self-reproduction induced by adding membrane precursor V*.*** Addition of V* produces membrane molecules and electrolytes through hydrolysis assisted by an amphiphilic catalyst. Adhesion of the amplified DNA to the inner leaflet accelerates vesicular growth and division.

c, Chemical structures of membrane molecule V, amphiphile catalyst C and membrane precursor V*.

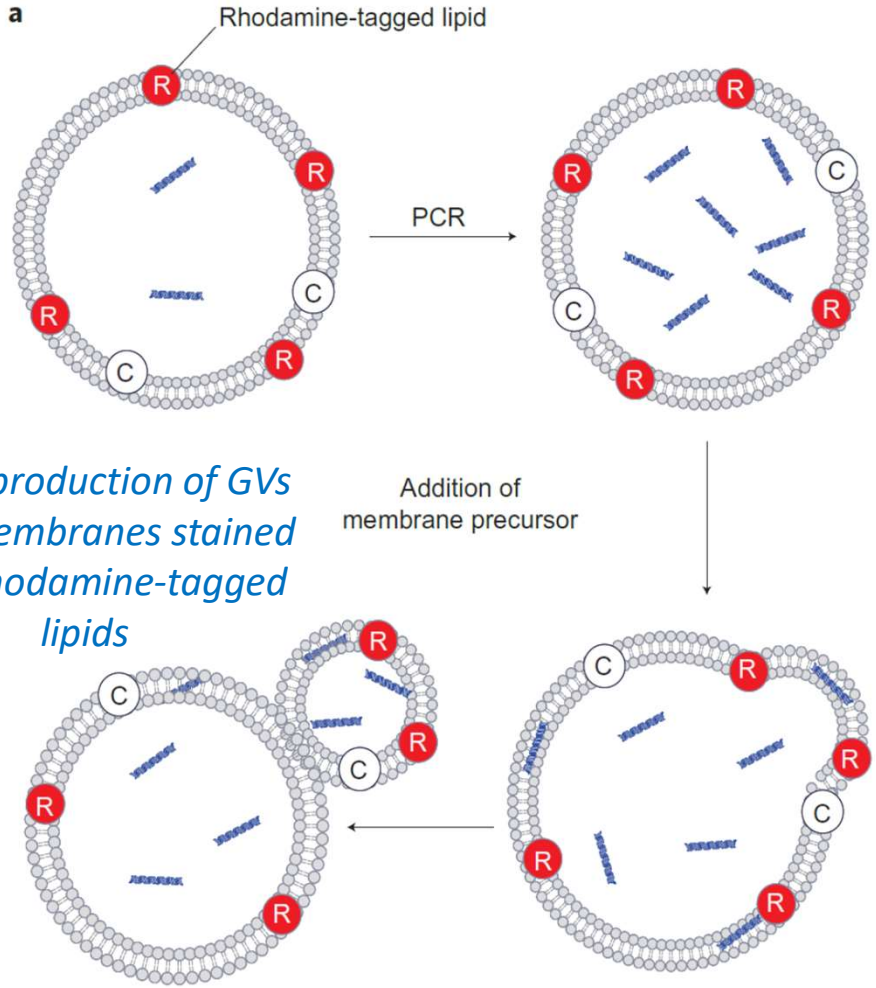
K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781

Self-reproduction of giant vesicles combined with the amplification of DNA



Real-time observation of morphological changes of DNA-amplified GVs after addition of V* .

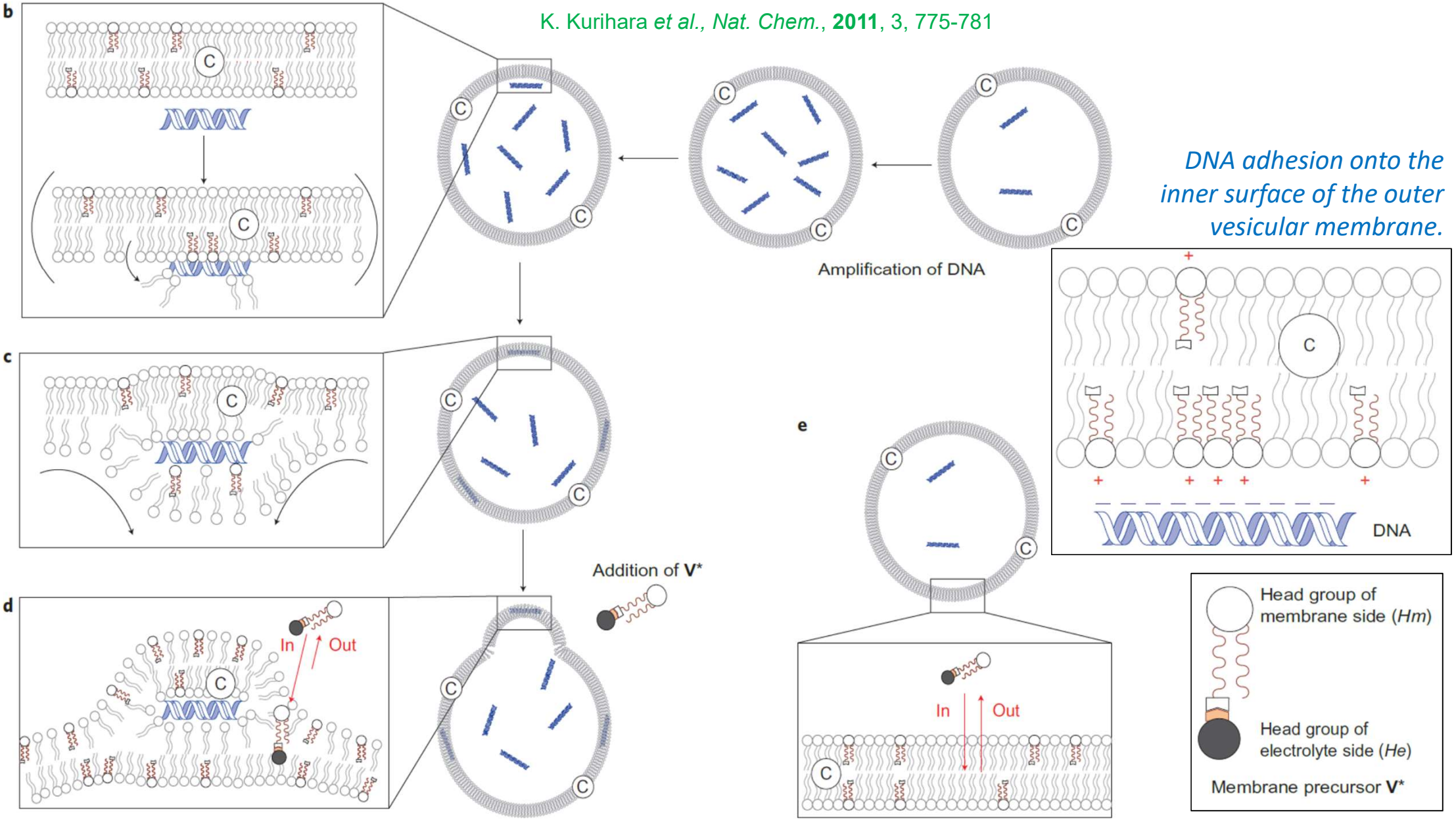
Original GVs began to grow and divide 4 min after adding V. Complete division into four GVs occurred at 5.5 min, and separation occurred at 7 min. Scale bars, 10 μm.*



Self-reproduction of GVs with membranes stained with rhodamine-tagged lipids

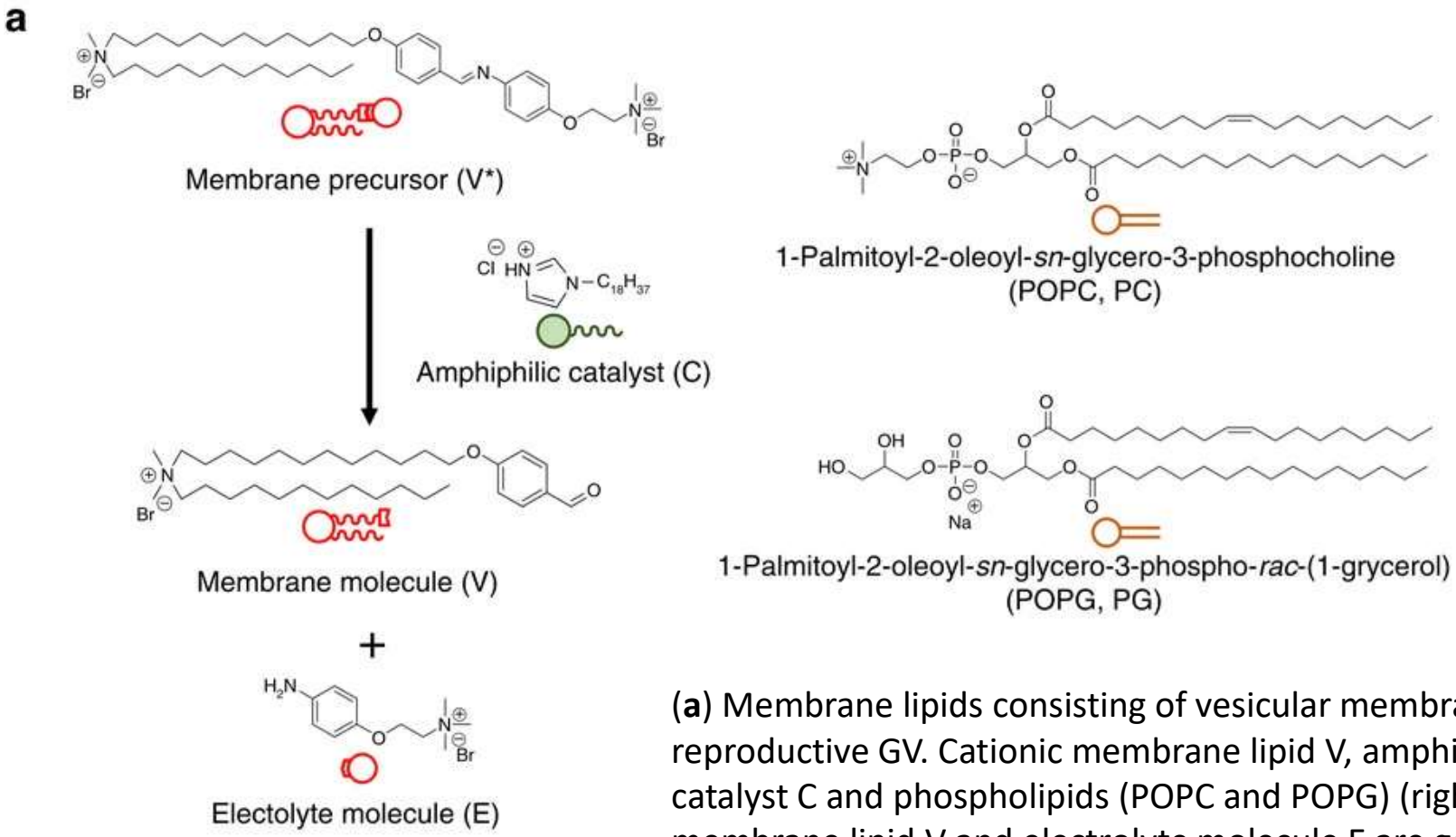
K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781

K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781



DNA adhesion onto the inner surface of the outer vesicular membrane.

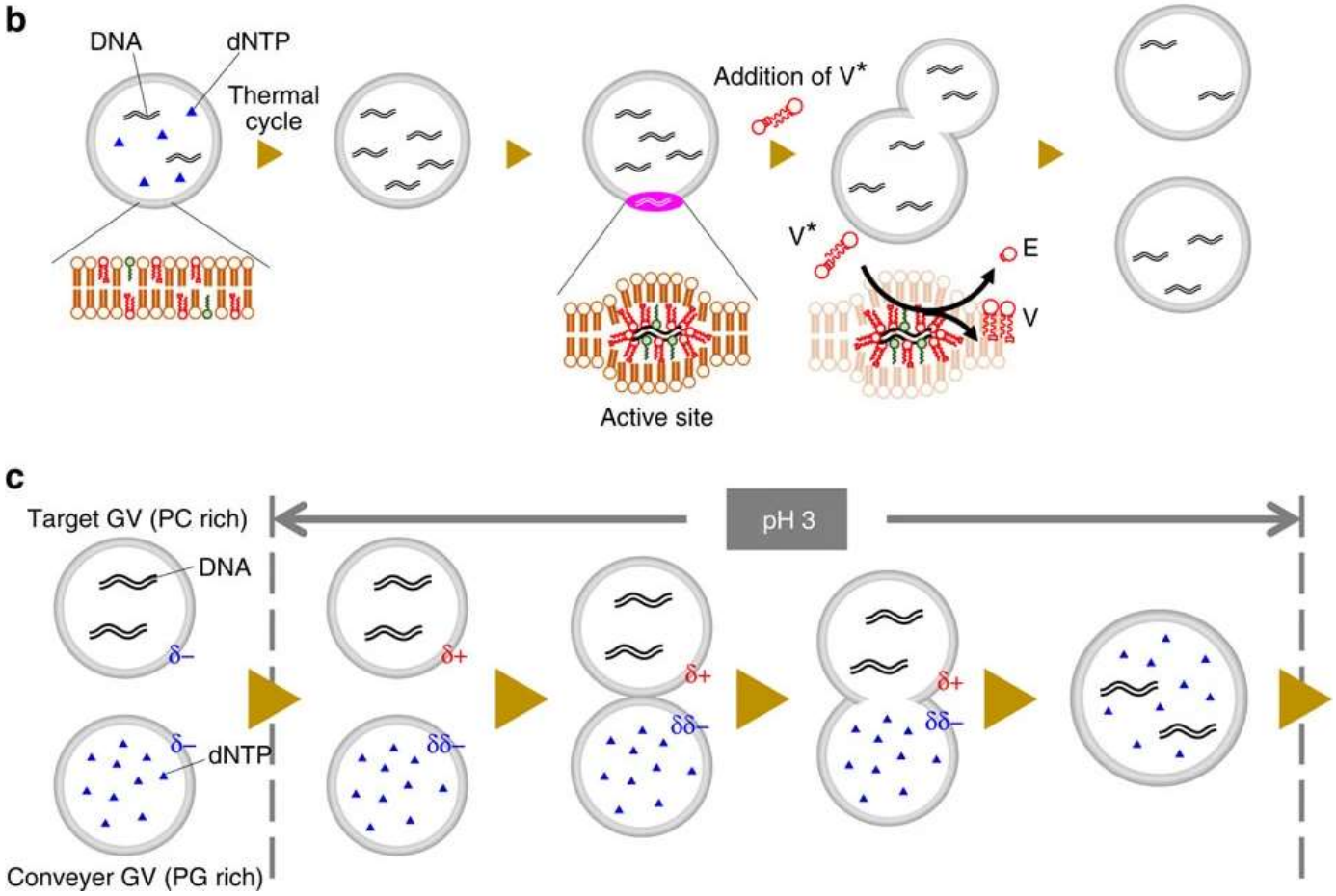
A protocell model with a primitive cell cycle



(**a**) Membrane lipids consisting of vesicular membrane of self-reproductive GV. Cationic membrane lipid V, amphiphilic catalyst C and phospholipids (POPC and POPG) (right). The membrane lipid V and electrolyte molecule E are generated through the hydrolysis of the membrane lipid precursor V*.

K. Kurihara *et al., Nat. Commun.*, **2016**, 6:8352 | DOI: 10.1038/ncomms9352

A protocell model with a primitive cell cycle



(**b**) The production of cationic membrane lipid V from its precursor V*. The cationic membrane V is produced together with the electrolyte E at an active site comprised of amplified DNA and amphiphilic catalyst C in the giant vesicular membrane.

(**c**) pH lowering induced adhesion and fusion between the target GV and the conveyer GV. The surface charge of the target GV changes to cationic due to the protonation of the POPC as well as the increase of the cationic membrane lipid V from its precursor, and the target GV adheres to the conveyer GV with a negative surface charge at pH=3. These two types of GVs fuse, and the transport of dNTP from the conveyer GV to the target GV proceeds.

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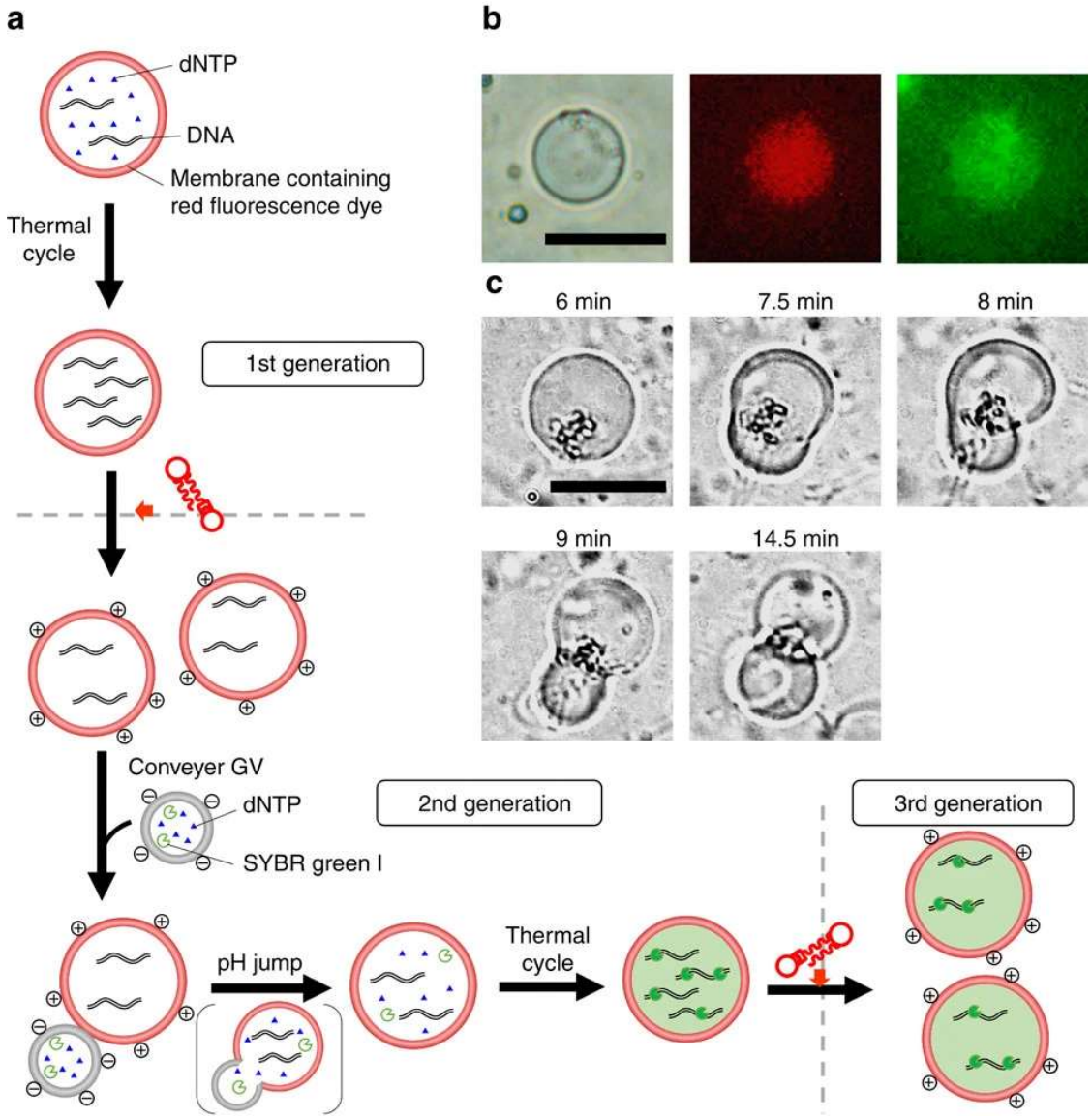
A protocell model with a primitive cell cycle



(**a**) Self-proliferation of GV-based model protocell from 1st generation to 3rd generation. DNA amplification in mother GV was followed by the first division to give rise to daughter GVs. Ingestion of dNTP in conveyer GV by daughter GVs and DNA amplification in daughter GV led the second division to give granddaughter GVs (bottom).

(**b**) Differential interface contrast microscope image of DNA-amplified daughter GV (left). Fluorescence microscope images of the red fluorescence emitted from the vesicular membrane (center) and the green fluorescence from inside the daughter GV (right). Scale bar, 10 µm.

(**c**) Division of the daughter GV to afford granddaughter GVs by the addition of precursor V* of the membrane lipid. Scale bar, 20 µm.

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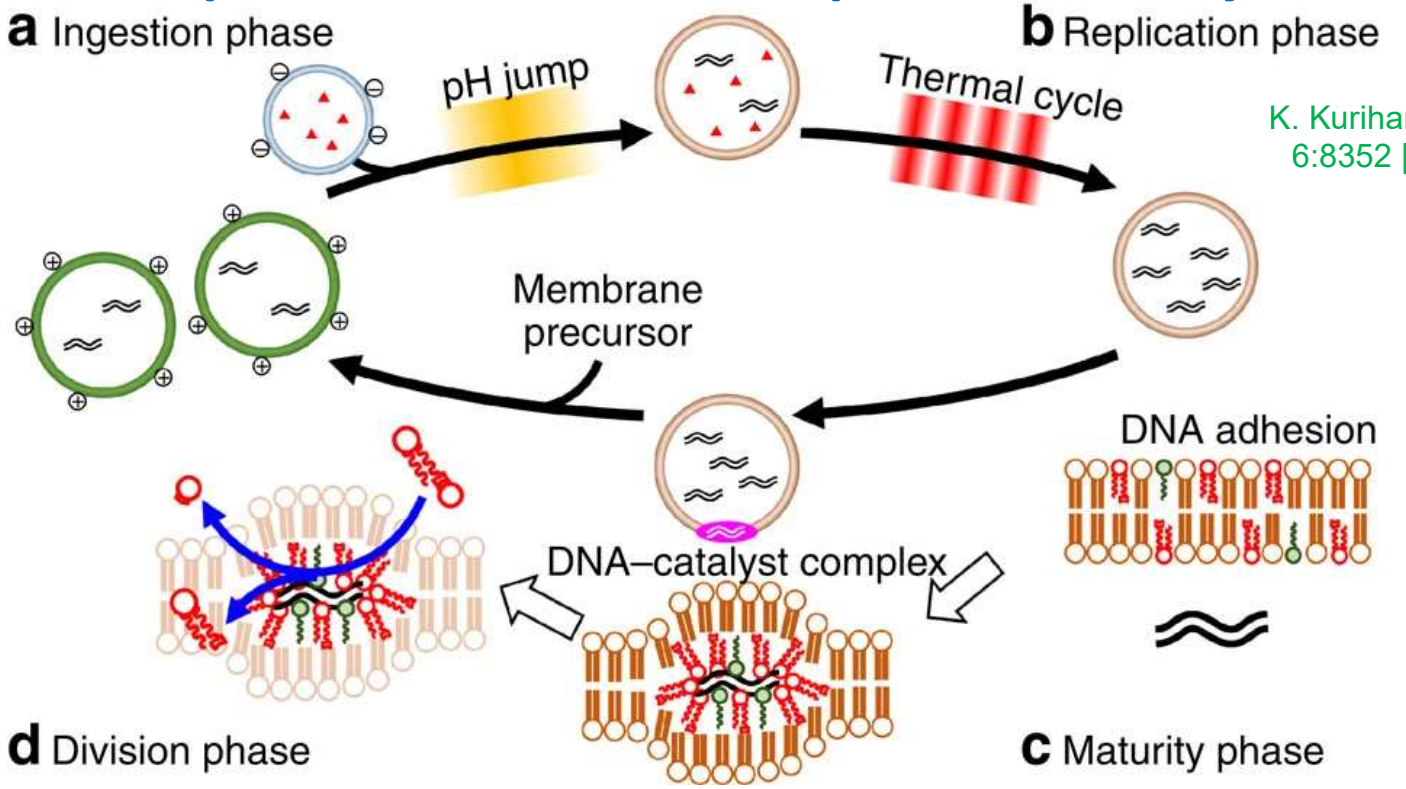
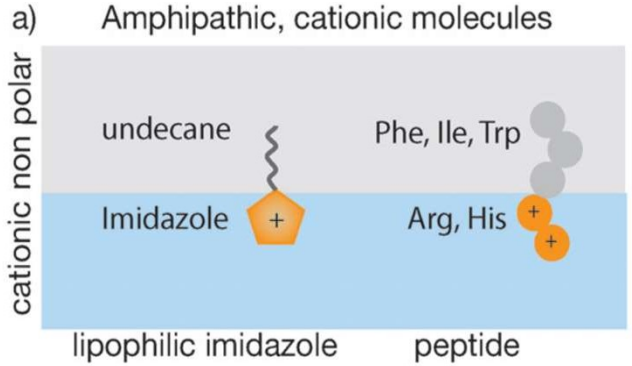
A protocell model with a primitive cell cycle

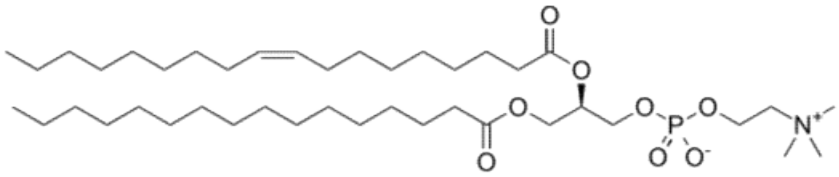


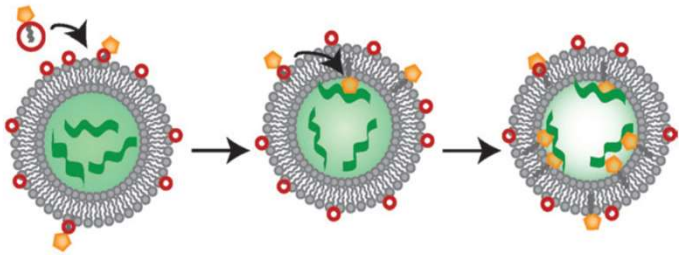
K. Kurihara *et al., Nat. Commun.*, **2016**, 6:8352 | DOI: 10.1038/ncomms9352

(**a**) In the ingestion phase, the GV of the next generation ingests substrates through vesicular fusion with conveyer GV containing dNTP, triggered by a pH jump. (**b**) In the replication phase, the replication of DNA in the next-generation GV proceeds using ingested dNTP. (**c**) In the maturity phase, the catalytic ability of the vesicular membrane matures in a sense that a complex between amplified DNA, amphiphilic catalyst C and cationic lipids V intrudes into the vesicular membrane, forming an active site for converting membrane precursor V* to lipid membrane V. (**d**) In the division phase, the self-proliferative GV grows and exhibits a budding deformation and an equivolume division when the precursor V* of the membrane lipid is added to the exterior of GVs.

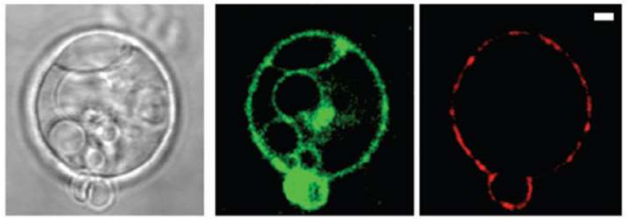
Noncovalent nucleotide association with membranes

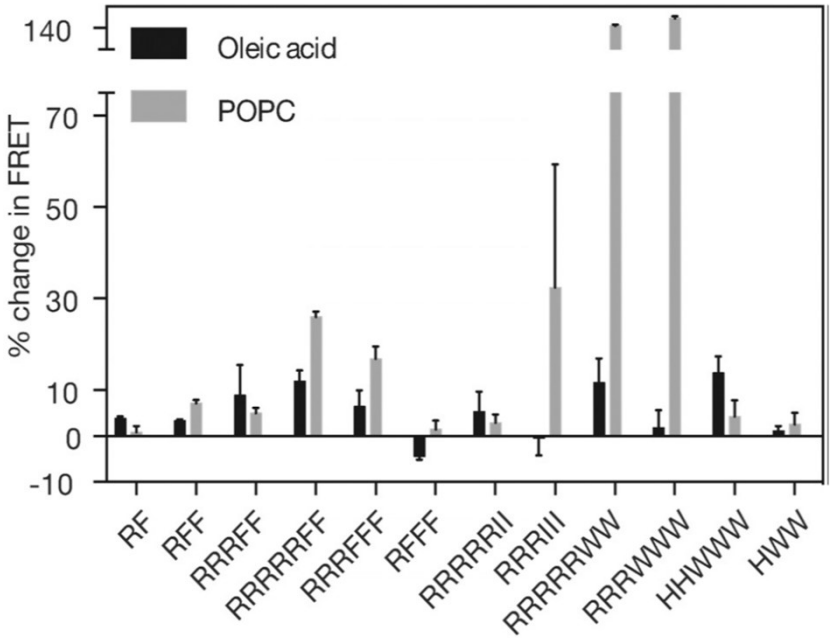






+POPC SUVs with undecylimidazole





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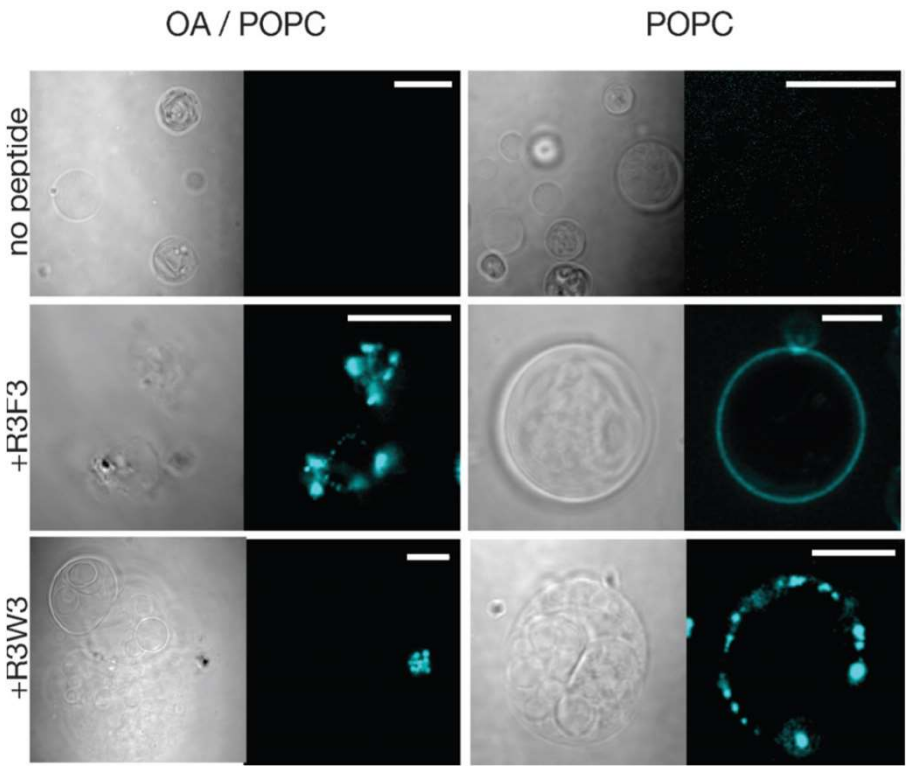
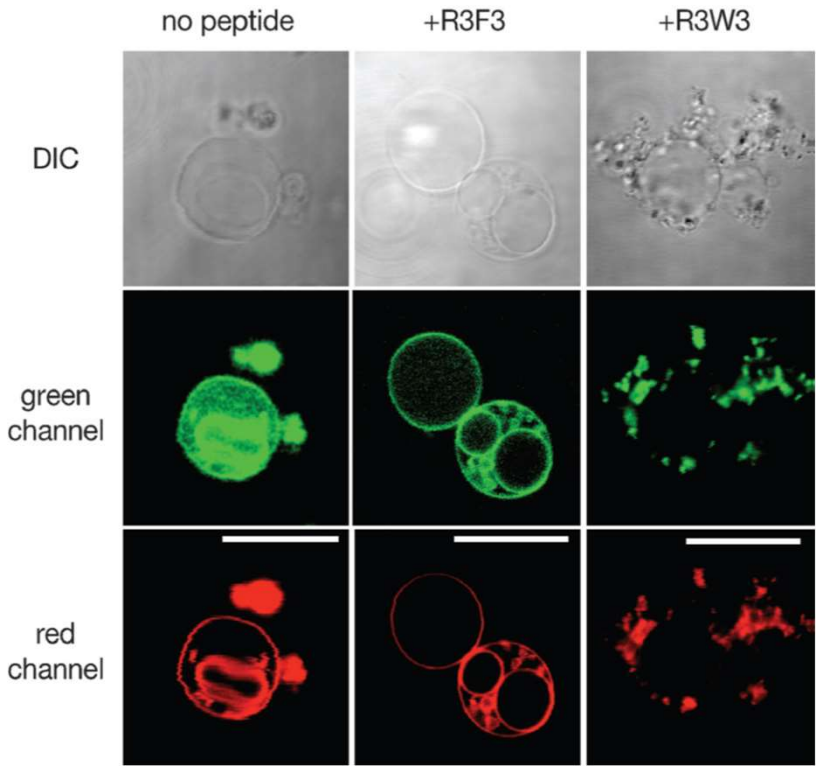
Noncovalent nucleotide association with membranes





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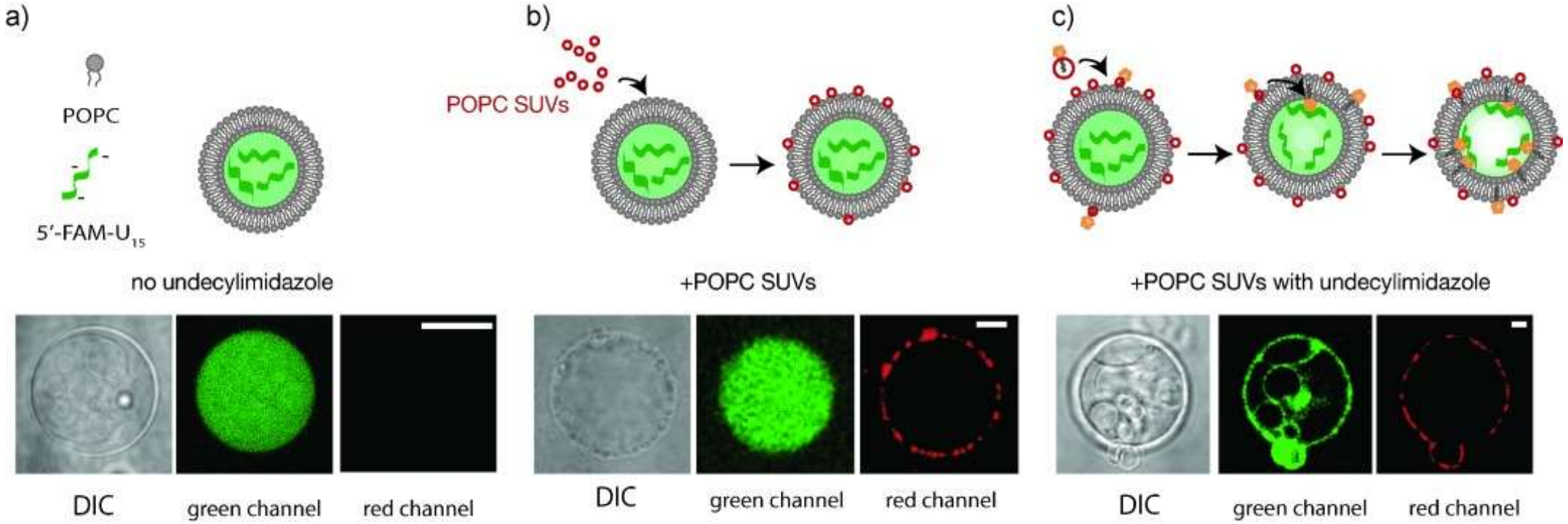

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