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Recent advancements in synthetic cells research

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Abstract

This paper describes our recent investigations on the construction of synthetic cells. By following a bottom-up synthetic biology approach, we aim at constructing minimal synthetic cells based on the encapsulation of DNA, RNA and proteins within liposomes. We will firstly comment on the physics of solute entrapment inside liposomes, giving emphasis on a remarkable self-concentration effect discovered by us (Luisi et al. 2010, Souza et al. 2011, 2012). Next we will show how it is possible to exploit this phenomenon to reveal the formation of primitive-like, metabolically active cells starting from diluted macromolecular solutions (Stano et al., submitted). In conditions where a protein-synthesis reaction does not proceed at a significant rate, lipid vesicles can entrap all required solutes allowing intraliposome protein production.
The second topic deals with the formation of simple, rudimentary primitive cell communities based on giant vesicles (GVs). Oleate-containing GVs associate between each other in the presence of poly-L-arginine to form clusters that might be taken as model of primitive cell communities. Their formation, driven by simple primitive electrostatic interactions bring about a series of distinctive features (stability, enhanced permeability, solute capture, fusion) that might emphasize the role of cooperation in origin of life scenarios, flanking the usual competition issues (Carrara et al., 2012).

Synthetic Cells

One of the goals of synthetic biology is the construction of synthetic cells. In addition to the classical synthetic biology approach, based on the manipulation of living organisms, synthetic cells can be constructed by assembling separated molecular parts like lipids, DNA, RNA, proteins in cell-like systems called semi-synthetic minimal cells (Luisi et al., 2006). Born within the community of origin of life, modern semi-synthetic minimal cell research also looks for possible biotechnological applications (but whereas self-organization, spontaneous assembly, and primitiveness are key features of synthetic cell studies in origin of life context, controlled assembly, efficiency and reproducibility are distinctive facets of biotechnology).
Here we will firstly show how a large number of macromolecules self-concentrate inside lipid vesicles (liposomes), bringing about a remarkable rate acceleration of a complex biochemical reaction. Then, we will present our first attempt to build protocellular communities based on the physical association of giant vesicles (GVs).

Solute entrapment and "super-concentration"

Semi-synthetic minimal cells are defined as those cell-like particles containing the minimal and sufficient number of biological compounds (nucleic acids, protein, lipids) that would allow self-maintenance, self-reproduction, and the possibility to evolve (Luisi et al., 2006). Protein synthesis is a key module of semi-synthetic minimal cell, representing not only a necessary function of the synthetic cell, but also a good model for metabolic complexity. Following our initial observation of protein synthesis in "small" conventional liposomes (200 nm diameter) (Souza et al., 2009) we started a systematic electronmicroscopy study that revealed how macromolecules like ferritin (Luisi et al., 2010), ribosomes (Souza et al., 2011) and ribo-peptidic complexes (Souza et al., 2012) were spontaneously encapsulated within liposomes (Figure 1).

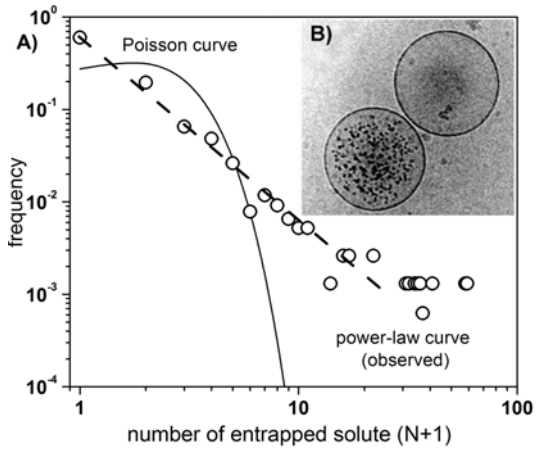

Figure 1. (A) Poisson curve versus power-law. (B) ferritin-containing and empty liposomes. Reproduced from Luisi et al. (2010) with permission from Wiley.

Intriguingly, it was found that the intra-liposome solute distribution function does not follow – as expected – the Poisson law, but it is rather shaped as a power law (Figure 1a). In particular, although the vast majority of liposomes encapsulate a limited number of solutes, a very small fraction of liposomes (typically around 0.1%) contains instead a very high number of solutes, so that their internal concentration can

be also one order of magnitude higher than the concentration of the solutes in the environment (Figure 1b). In other words, during liposome formation, solutes can self-concentrate within the liposome cavity. We reasoned that the "super-concentration" effect could drive the formation of functional cells, for instance "protein-producing" ones, even if the low concentration of the solutes in the environment does not allow it. By diluting the transcription-translation kit at a level where no protein synthesis takes place, we simulated a primitive scenario where solutes were already present in diluted form in a sea or fresh-water lagoon. We then formed liposomes followed the course of protein production (the green fluorescent protein was used) inside and outside liposomes. Surprisingly, we found that some vesicles were able to synthesize the protein despite the low solute concentration, revealing that even a complex metabolic pathway spontaneously self-concentrates inside liposomes (Stano et al., submitted). These results provide a physical explanation to the origin of early functional cells, thanks to a favorable micro-environment created by liposomes, which can concentrate solutes inside their aqueous cavity.

Vesicles assemblies

Current research on the origin of life typically focuses on the self-organization of molecular components in individual cell-like compartments, whereas no attempts have been made to investigate communities of compartments. Here we present our study on vesicles assemblies as a model of primitive cell communities.

At this aim, we firstly prepared giant vesicles (GVs) composed by a primitive lipid, namely oleic acid, which is negatively charged. GVs were made stable by the addition of a neutral stabilizing phospholipid (POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine). Oleate/POPC GVs were prepared after the optimization of the "droplet transfer" method, which allows the facile entrapment of solutes like enzymes and DNA.

The addition of poly-L-arginine (PLA), a positively charged polypeptide, brings about the association of negatively charged oleate/POPC GVs in form of small clusters first, that in turn grow to give rise to very large clusters. The formation of these GVs "colonies" is stochastic but depends on the GVs numerical density as well as on the PLA/GVs ratio. Typically, these clusters contain up to 100 GVs. GVs are also destroyed after the addition of PLA (due to high local PLA concentration), and the maximal GVs to clusters conversion is about 50%.

Once formed, we characterize the GVs clusters properties. Phosphate bearing water-soluble solutes, such as ADP, fluorescent diphosphate and even t-RNA, present in the environment, readily penetrate into clusterized GVs much faster than isolated GVs. This is probably due to the help of PLA adsorbed between GVs, which acts here like the well-known "cell penetrating peptides". GVs in the cluster can fuse with each other (fusion yield ca. 0.5-1%). Fusion was revealed by preparing clusters from two GVs population, each containing a fluorescently labeled macromolecule. It is remarkable that vesicle fusion might provide a mechanism for increasing the internal molecular complexity thanks to the acquisition of new molecules originally entrapped in another vesicle. Finally, GVs clusters can capture new GVs from the solution and grow. The new GVs attach to the cluster border, demonstrating that PLA is still present in the outer GVs cluster surface.

GVs clusters are firmly attached to the solid support (an hydrophobic plastic polymer, in this case, and resist to hydraulic flow.

In summary, GVs clusters display several interesting features, mimicking in very simple way bacterial colonies at the lowest possible complexity level. Intriguingly, bacterial L-forms (bacteria without cell walls) and GVs display some common physico-mechanical behavior.

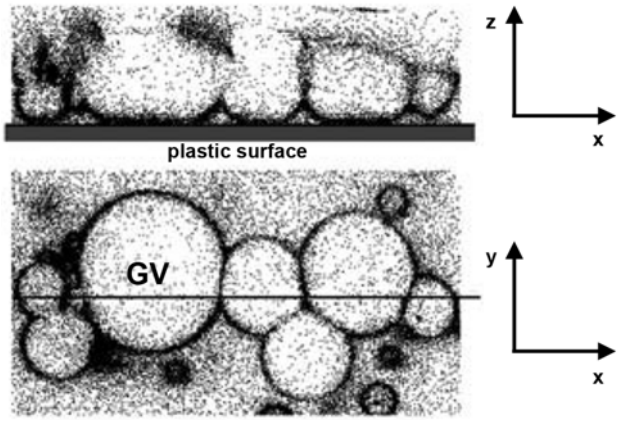

Figure 2. Sagittal (*xz*) (130×30 μm) and horizontal (*xy*) (130×60 μm) confocal images of GVs associated to form "colonies". The GV membranes have been stained by octadecyl rhodamine. Note the flattening of GVs on the plastic surface. Reproduced from Carrara et al. (2012) with permission from Wiley.

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