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Application of nucleic acid–lipid conjugates for the programmable organisation of liposomal modules

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ABSTRACT

We present a critical review of recent work related to the assembly of multicompartment liposome clusters using nucleic acids as a specific recognition unit to link liposomal modules. The asymmetry in nucleic acid binding to its non-self complementary strand allows the controlled association of different compartmental modules into composite systems. These biomimetic multicompartment architectures could have future applications in chemical process control, drug delivery and synthetic biology. We assess the different methods of anchoring DNA to lipid membrane surfaces and discuss how lipid and DNA properties can be tuned to control the morphology and properties of liposome superstructures. We consider different methods for chemical communication between the contents of liposomal compartments within these clusters and assess the progress towards making this chemical mixing efficient, switchable and chemically specific. Finally, given the current state of the art, we assess the outlook for future developments towards functional modular networks of liposomes.

Contents

1. Introduction

Modular compartmentalisation of chemical processes and function is central to the organisation of living systems. Multiscale assembly from macromolecular complexes to organelles, cells, tissues, organs and organisms gives rise to sophisticated function across length scales from parallel biochemical modules that are in communication with

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one another and can sense changes in their environment. On colloidal length scales this compartmental organisation is predominately derived from the use of lipid bilayer membranes as functional interfacial barriers. These two-dimensional fluid interfaces host functional protein channels and receptors that regulate the passage of specific chemicals and biochemical signalling between compartments.

Mimicry of biology's compartmentalisation of its chemistry on micrometer and sub-micrometer length scales holds promise for technology development in several fields. One example is the design of multistep micro-/nano-reactors for chemical process control; this would allow the maintenance of rare compounds, only available in small quantities, at high concentrations in self-assembled compartments while also allowing multi-step reactions in chemically incompatible environments (e.g. acidic and alkaline pH, or oxidising and reducing environments) as the reaction steps through each compartment (Fig. 1). While rational design of such sophisticated self-assembled multistep microreactors is some way from becoming a reality, the principle of single compartment, self-assembled catalytic capsules has already been demonstrated [1–3]. For example, enzymes encapsulated within polymersomes have recently been shown to be able to generate and release antibiotics in bacterial cultures [4]. The next step towards design of multicompartment nanoreactors is the controlled assembly of modular capsules within close spatial proximity that might begin to allow communication between compartments.

A further application of compartmentalised nanostructures is within the field of nanomedicine [5]. Nanomedicine aims to use soft and nanoscale materials to control the temporal and spatial distribution of therapeutics within a patient by determining the biodistribution and drug release kinetics of a particular formulation in a predictable fashion. It is also desirable to deliver multiple therapeutic compounds simultaneously and preferably within the same particle such that they arrive at their target simultaneously [6]. Possible clinical applications include combination therapies to overcome multidrug resistant bacteria, codelivery of a prodrug with an activating agent and traceable delivery of therapeutics by combining the drug with an image contrast agent. In many cases it would be desirable that these compounds are kept physically isolated from one another within the particle structure to prevent unfavourable drug–drug interactions or store each compound in different favourable environments (e.g. pH). Therefore it would not always be favourable to encapsulate multiple active agents within a single compartment: multicompartment approaches will be required.

Synthetic biology is an emerging field broadly defined as the engineering of biological parts and devices as well as the redesign of natural biological systems [7,8]. A bottom-up approach to synthetic biology refers to self-assembly approaches for engineering new systems created from biological components [9]. Within this context lies the ambitious challenge of building a functional cell from its fundamental molecular constituents. While many properties combine to define a living cell, including a metabolism, responsiveness to its environment, the ability to reproduce and to ultimately evolve [10], being able to replicate a small number of life-like properties within a synthetic system is currently considered to be a favourable outcome. For example, in vitro synthetic gene expression has been achieved in liposomes by encapsulation of a DNA plasmid with *E. coli* extract and incubating these liposomes within a "feeding solution" [10–12]. In terms of engineering cell-like materials within synthetic biology for new functional devices, reproduction and evolution of the "cell" may not be necessary for first generation technologies. However the ability to encapsulate metabolic processes that are responsive to their external environment will have many applications including environmental sensing, novel medicines and catalysis. Different functional elements could be combined in a modular fashion that is familiar to both engineering design and biological organisation, where each module is a synthetic gene network (e.g. BioBricks [13,14]) expressed within a liposomal compartment in close communication with similar such modules in a multicompartment architecture. This concept of modular compartmentalisation of function has already been demonstrated in protocell design, where light-activated release of lactose from a lipid organelle is coupled to the in vitro gene expression of green fluorescent protein within an emulsion droplet [15]. A similar photo-responsive synthetic organelle has previously been described where light-driven trans-membrane proton gradients are generated using the bacteriorhodopsin protein, which then drives F_0-F_1 ATP synthase to generate ATP, which could provide chemical potential energy to drive further downstream bioenergetic processes [16]. The advantage of designing bottom-up synthetic cells over reengineering existing organisms lies in the ability to eliminate potentially unwanted cross-talk between the host and synthetic biochemistries as well as providing a system of minimal biochemical complexity that is easier to understand, redesign and control.

The aforementioned applications in nanoreactors, drug delivery and synthetic biology provide ample motivation for the design and engineering of multicompartmental structures on the micro- and nanoscales. Several approaches towards this goal exist within the published literature. These include careful assembly protocols for encapsulation of smaller vesicles within larger ones to create multicompartmental "vesosomes" [17–19], "capsosomes" created by embedding liposomes within layer-by-layer polymer shells [20–22], encapsulation of aqueous two phase systems within single liposomes [23–25] and association of lipid vesicles via site-specific ligand–receptor interactions such as biotin–avidin bonds [26–28]. Many of these approaches were discussed in a recent review [29]. Here, the central focus will be on the use of nucleic acid functionalities on the surface of liposomes to mediate their associations. The two key advantages of nucleic acids for this purpose are [30]: (i) DNA (usually) forms an asymmetric interaction with its complement, allowing the controlled assembly of different liposomal compartments, compared with symmetric binding interactions, e.g.

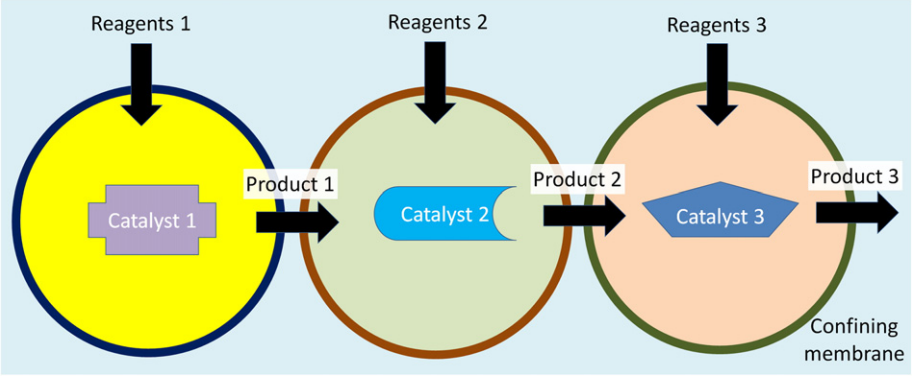

Fig. 1. Schematic cartoon depicting the concept of biomimetic chemical process control within a network of liposomal modules. Each compartment has a different chemical environment and catalytic function but is in communication with its environment and the other modules allowing transport of chemical species and a cascade of sequential reactions within consecutive compartments.