DNA-MEDIATED FUSION OF SPHEROPLASTS WITH SYNTHETIC LIPOSOMES

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The Idea

We have very good control over phospholipid liposome (vesicle) formation, transport, and fusion; we also know how to lyse the external cell membrane of gram negative bacteria, yeast and single cell algae, all of which then form a 'spheroplast' state, from which the whole cell can be recovered under appropriate culture. Removing the external cell membrane/wall is indeed a standard step in various protocols for uptake of material into the cells. The key idea of our proposal is to demonstrate a 'hybrid' system, engineering controlled adhesion and fusion of artificial liposomes to spheroplast cells. This could represent a new high throughput and selective tool for delivering cargo into cells, not limited to genetic material and very flexible in terms of size and chemical nature of the cargo.

Who We Are

The project will be carried out as a collaboration between P. Cicuta, L. Di Michele (University of Cambridge, Physics) and Martin Howard (John Innes Centre, Norwhich). LDM and PC have established expertise and a track record of successful collaboration on hybrid DNA-lipid systems, particularly on the use of synthetic DNA linkers to drive specific adhesion between artificial liposomes [1, 2, 3, 4]. This is the first time they aim to work together on living systems. PC has significant experience in singe-cell manipulation [5] and imaging [6, 7] to address cell biological questions of chromosomal packing and cell cycle control. MH and his group will support the endeavour with their skills in mathematical modelling of cellular mechanisms, genetic circuits, applied here to how we can engineer interaction with exogenous agents introduced by the liposomes; their interest also joins up on the questions of chromosomal segregation [8] and regulation of cell size [9] which can be revisited with the tools developed here.

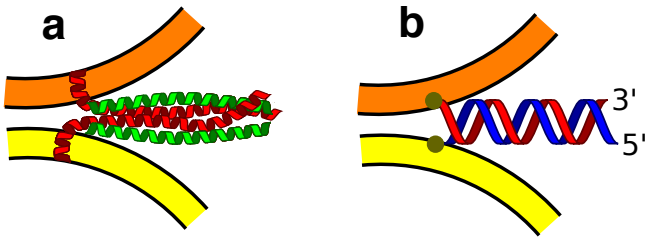

Figure 1. SNARE and DNA-mediated membrane fusion. **a**, Not-to-scale sketch of SNARE receptors binding and promoting membrane fusion. **b**, The same effect can be replicated using two complementary membrane anchored DNA oligonucleotides.

Implementation

DNA-nanotechnology enables the production of nanostructures of complex shape and functionality, by self-assembly from synthetic oligonucleotides [10]. These nanostructures can be designed to mimic a diverse range of molecular machines of key biological importance, and therefore hold great promise as tools for synthetic biology. Examples (including our work) are simple DNA linkers mimicking membrane-anchored ligands and receptors [1, 2, 11, 12], bipedal DNA motors transporting cargoes along DNA tracks [13], and artificial nanopores that mimic biological protein channels [14].

SNARE proteins (soluble N-ethylmaleimide sensitive factor (NSF) attachment protein receptors) are membrane-anchored receptors known for their ability of mediating adhesion and then fusion of lipid membranes. Fusion occurs when SNARE proteins anchored on different membranes combine

in tight α-helix bundles, bringing the bilayers in very close proximity [Fig. 1**a**] [15]. Single-stranded DNA molecules can be functionalised with a range of hydrophobic moieties (cholesterol, lipids, carbon chains etc.) that can graft the oligonucleotides to lipid bilayers by inserting into its hydrophobic core. If complementary, two of such ssDNA chains can bind to each other and promote membrane fusion by effectively mimicking the action of SNARE receptors [Fig. 1**b**] [16, 17].

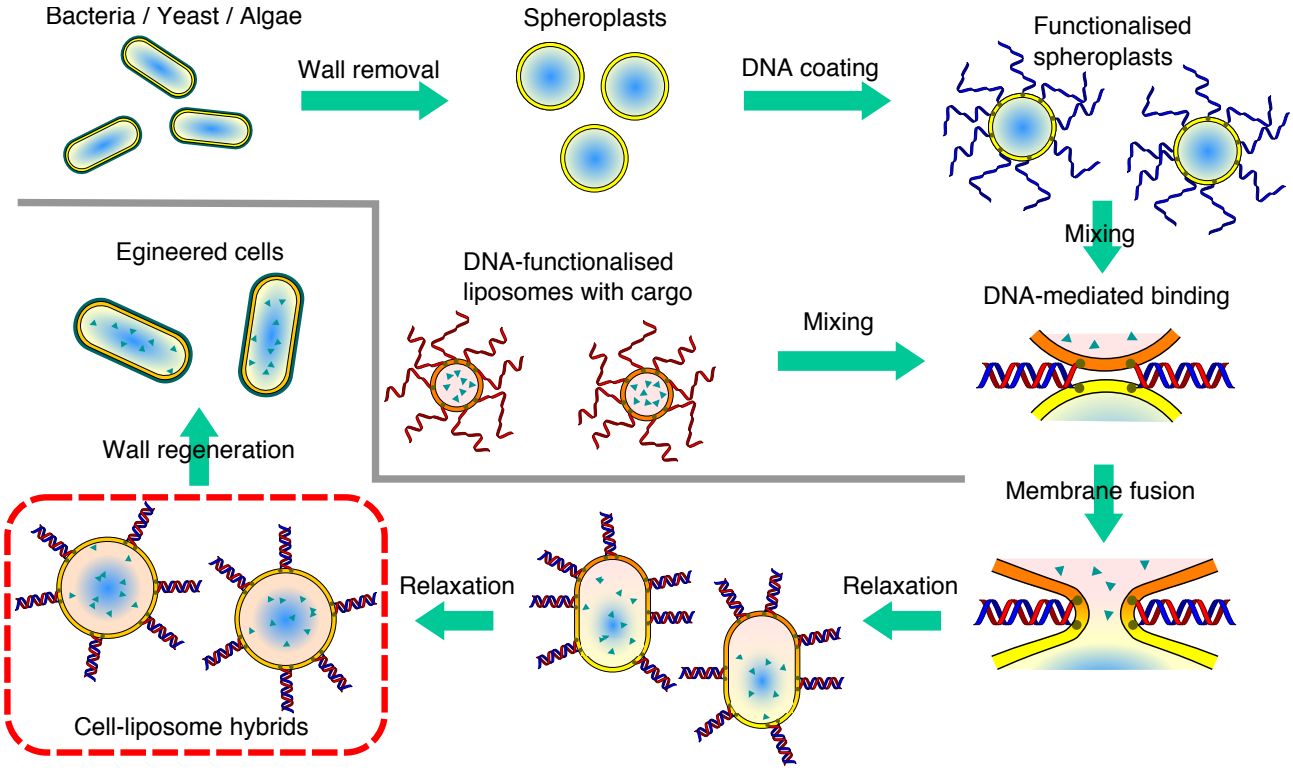

Figure 2. DNA-mediated membrane fusion applied to combine spheroplasts with artificial liposomes. Spheroplasts are prepared through wall removal from bacterial, yeast, or algal cells and functionalised with hydrophobised single-stranded DNA linkers. Likewise, cargo-loaded liposomes are decorated with the complementary oligonuceotides. Upon mixing, DNA binding promoted membrane fusion and thereby mixing of the cell and liposome contents. Eventually, cells may be able to produce a new wall and resume cell cycle.

The procedure we envisage is sketched in Fig. 2.

- Spheroplasts will be prepared first from bacteria ($E.coli$) following a standard procedure in our lab [5]. Fission yeast ($S.pombe$) and single cell algae ($Chlamydomonas\,R.$) are also of great interest to us, and will be explored. Removal of the external cell wall is carried out using established protocols [18, 19].
- Unilamellar lipid vesicles will be synthesised via extrusion (for small $< 1\,\mu$m diameters), gel-assisted formation [20], electroformation [21], or microfluidic methods [22] ($> 10\,\mu$m). Different methods are preferable depending on the lipid composition (*e.g.* charged/neutral, synthetic/natural) and will enable encapsulation of a broad range of cargoes including plasmids, proteins, passive crowding agents (PEG), fluorophores, nanoparticles/quantum dots, DNA nanostructures etc.
- Both spheroplasts and lipsomes will be functioanlised using two types hydrophobised ssDNA molecules designed *ad hoc* to bind and promote fusion. Synthetic/functionalised oligonucleotides of arbitrary sequence are commercially available.

Benefits and outcomes

Once optimised, this protocol will provide a powerful synthetic biology tool. In the most straightforward scenario liposomes could serve as large reservoirs of regulatory molecules, plasmids, or nutrients that cells would not otherwise uptake, or that would be impossible to deliver with conventional transfection methods. Yet much more ambitious experiments can be designed, where liposomes contain DNA nanomachines that directly interfere with live cell mechanisms. Specifically, we envisage the

possibility of encapsulating the components of DNA-based chemical networks, which once activated by an external stimulus (*e.g* exposure to a specific DNA *ligand*) can trigger a cascade of reaction that ultimately affects gene regulation, for instance by releasing silencing RNA.
Besides synthetic biology applications, it will also be possible to exploit liposome fusion in order to apply controlled perturbations at the single-cell level (non-genetic material), and study the response over multiple generations once spheroplasts have regenerated their wall and resumed the cell cycle [Fig 2]. This opportunity is particularly valuable to investigate phenomena such as cell-size control and how it is affected by controlled alterations in volume, area and concentration of specific molecules that could either be diluted out or further supplied by the liposomes.
Beyond the scope of this prooof of concept, the technology could be further developed and integrated as a lab-on-chip.
The project is a new interdisciplinary (physics-biology) and intercultural (experiment/modeling) interaction between Cambridge and Norwich.
We envision a mainstream grant application to develop this idea fully, using the results obtained here as preliminary data.

Sponsor for the research and cost centre

The fund-holder will be Dr Lorenzo Di Michele, Leverhulme Research Fellow at the Cavendish Laboratory. This funding complements three research projects in the Cavendish: EPSRC Programme grant CAPITALS on functional lipid systems (PC, LDM, ET); EPSRC CDT in Advanced Sensor Technology (PC, OA); HFSP grant on bacteria chromosome organisation (PC, ST).

Budget

We require limited travel funds for two in-person meetings during the project (Cambridge and Norwich): 400
Most of the funds are required for consumables and reagents: 3600. These will cover synthetic DNA and specialty phospholipids, necessary to work on the optimisation of DNA-mediated membrane fusion as a versatile tool to merge spheroplasts with artificial liposomes, in an effort of combining live cell machinery with a broad range of synthetic components, creating hybrid units. We will also make use of cell culture facilities.

Equipment including cell culture facilities, wet lab for liposome/DNA preparation, functionalisation and characterisation, and fully automated fluorescence microscopes for visualisation is available within the Physics of Medicine Building of the Cavendish Laboratoy, where experiments will take place.
Lab work will be carried out by 2nd year PhD students O. Amjad (OA), S. Takamori (ST), PDRA E. Talbot (ET) and L. Di Michele (LDM), all with significant hands on experience on closely related experimental systems.

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