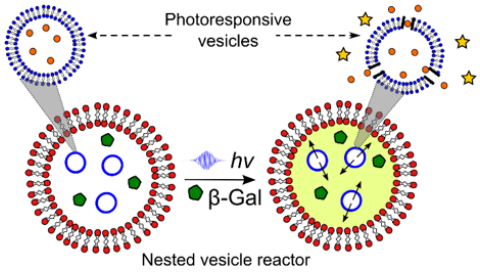
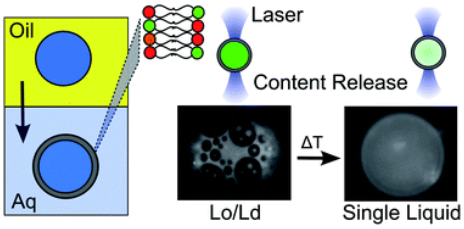
In my PhD I successfully built a built a multidisciplinary research portfolio that dovetails breakthroughs in interface science, membrane biophysics and synthetic biology to translate chemical technologies for artificial cell (AC) construction. Chemical/physical motifs can be used as tools to build self-assembled lipid vesicle 'modules' that can respond to specific internal (temperature/chemical) and external (light) stimuli. By combining these modules along different length scales and using oil/water interfaces to define the cell boundary, I have created environment sensing ACs and 'smart' nanoscale drug delivery vehicles that can respond to enzymatic components of the cancer microenvironment. Key achievements from these research projects include:

1) **Engineering light-responsive vesicle-in-vesicle (nested vesicle) systems to control enzymatic catalysis (Nat. Commun. 2018)**



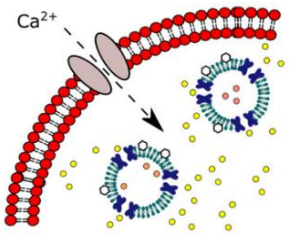
In this project, the first responsive nested vesicle system has been engineered by encapsulating the enzyme β-galactosidase (β-gal) alongside photopolymerisable vesicles loaded with a β-gal substrate into a vesicle microcompartment. UV-light can then be used to trigger photopolymerisation, release the substrate into the vesicle lumen and initiate enzyme catalysis within the cell mimic with high spatiotemporal control. These vesicles could act as a framework for the spatial control of enzyme cascades in biocatalysis, where content can be segregated and mixed with the flick of a light switch.

2) **Engineering emulsion-based ternary (three-lipid) vesicles as thermoresponsive content release platforms (Chem. Sci 2018)**



The incorporation of membrane patterning through phase separation has not been explored using the emulsion transfer method. I showed that a wide range of phase behaviour can be controllably generated via emulsion transfer. Furthermore, temperature-induced domain formation/mixing can be used to control membrane permeability with the lipid composition defining this trigger temperature. Such vesicles can be designed for molecular release / uptake at a user-specified temperature, critical in applications such as hyperthermic drug delivery.

3) **Engineering artificial signalling pathways in nested vesicle systems (PNAS, 2019)**



Here the engineering of a *de novo* signalling pathway has been demonstrated for the first time in ACs. This was achieved by encapsulating dye-loaded mechanosensitive channel (MscL) vesicles alongside deactivated phospholipase A2 ($sPLA_2$) enzymes into microscale vesicles. Addition of α-hemolysin pores to the external membrane enables calcium ion flux into the vesicle, switching on $sPLA_2$ activity. MscL-$sPLA_2$ interactions then initiate dye release through MscL, increasing AC fluorescence. As ACs can tolerate a range of cytotoxic compounds without affecting cell function, this work represents the first step in the design of synthetic signalling pathways that retain the information transduction of biological signalling processes whilst simplifying pathway design. I have further explored the phospholipase-responsive nature of mechanosensitive vesicles by utilising them as a drug delivery system for prostate cancer (PCa). Such vesicles are activated by human $sPLA_2$-IIA at concentrations found in PCa microenvironments, with greater formulation responsiveness to the enzyme than previously reported lipid-only systems. This indicates the potential for mechanosensitive channels as tools in drug delivery.