Electronic Supplementary Material (ESI) for Soft Matter.

Encapsulated Droplet Interface Bilayers as a Platform for High-Throughput Membrane Studies

Supplementary information

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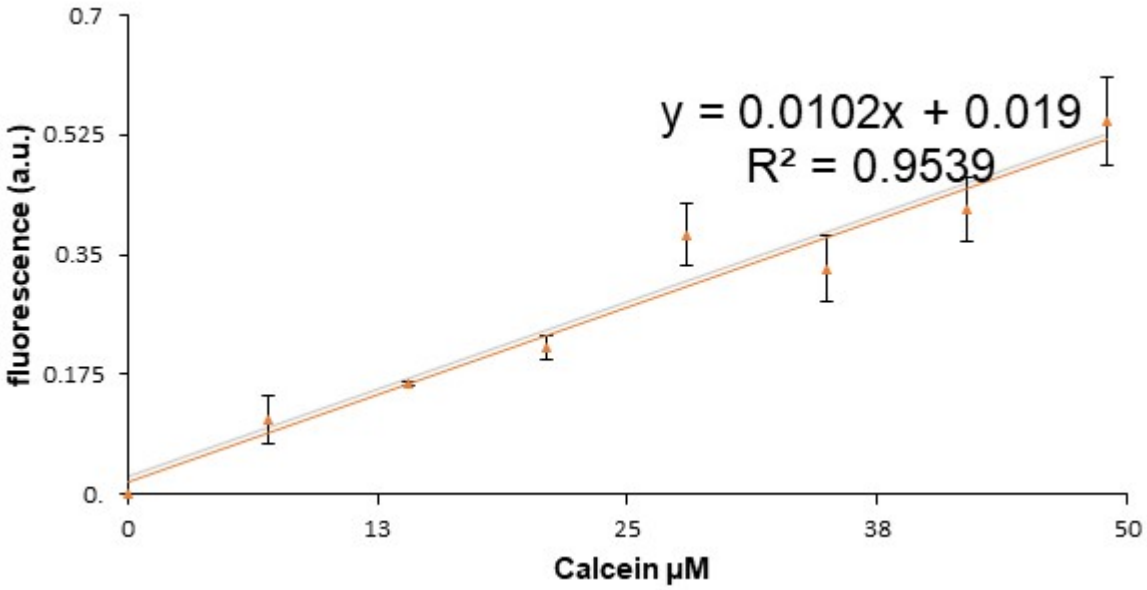
1. Quantification of calcein leakage from eDIBs exposed to SDS.

To determine the amount of calcein that had leaked from an eDIB in the well of a 96 well plate, a calibration curve was employed to link fluorescence intensity to calcein concentration. A calcein dilution series was performed in eDIB-containing wells, effectively simulating leakage from the eDIB into its surrounding buffer. The dilution series was performed with eDIBs in the well in order to account for any optical effects arising from the eDIB as well as low levels of fluorescence emitted by the quenched cores of the eDIB.

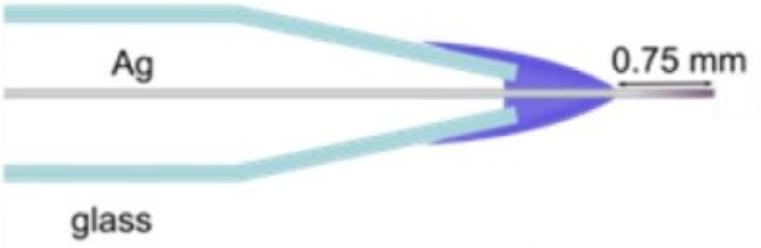


SI Figure 1. Calcein concentration and measured fluorescence in the linear, low concentration portion of the calibration curve in eDIB-containing wells.

2. eDIB Electrophysiology

Fabrication of electrodes

It was required to produce bespoke electrodes in order to be able to pierce the eDIB hydrogel shell and access the internal aqueous compartments, whilst avoiding any electrical contact with the hydrogel shell which would lead to short-circuiting. In order to do this, a pulled glass capillary (1 mm ID, CM Scientific, UK) was employed to sheath a 0.1 mm silver wire, inserted through the glass capillary through the non-tapered side of the glass sheath. 0.75 mm of wire is left exposed at the tapered end of the glass capillary, and fixed in position using polydimethoxysilane (PDMS) elastomer (Sylgard 184, DOW Corning, UK). This also provided a seal so that liquid was not taken into the glass capillary. The tip of the exposed wire is then chlorinated in a 3% sodium hypochlorite solution for 30 minutes to produce the Ag/AgCl electrode. A schematic of the electrode is shown in Figure X. Finally, the tip of the electrode is coated with a droplet of 2 % agarose, to aid its insertion into aqueous droplets.



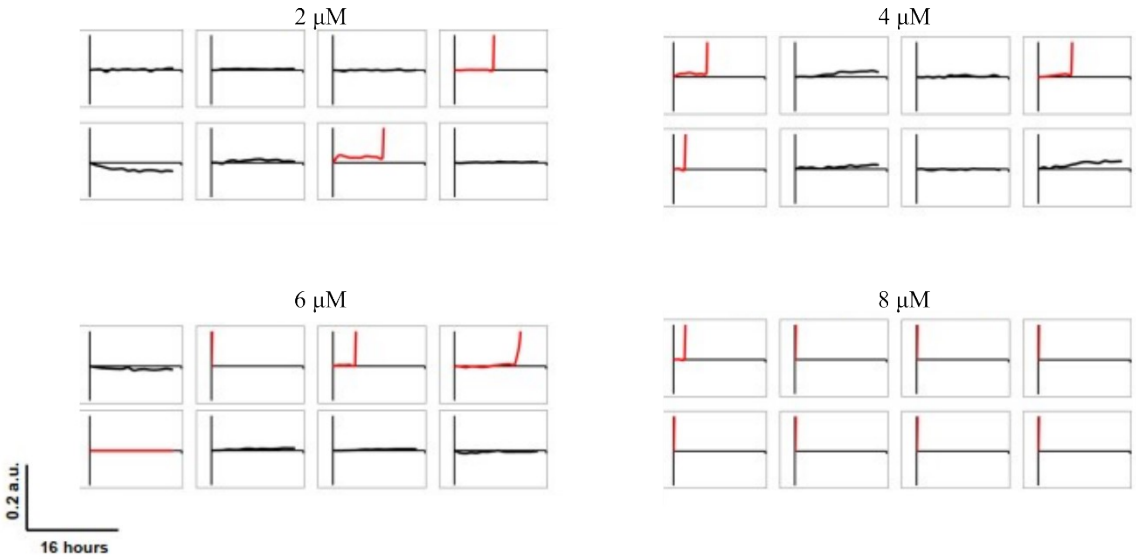
SI Figure 2. Diagram of the tip of a fabricated electrode used to perform electrophysiology on eDIBs by piercing into the alginate shell and into the aqueous droplets within.

Performance of electrophysiology

In order to perform *in situ* electrophysiology in the wells, the bespoke electrode is employed as the internal eDIB electrode, whilst a second, simpler electrode is employed to probe the aqueous media in the well. This electrode consists of a 1 mm silver wire with an Ag/AgCl tip and does not require any additional fabrication steps as the eDIB electrode does. The electrodes are mounted on micromanipulators (Narishige, Japan). The external media electrode is submerged below the oil phase in the well, whilst the eDIB electrode is gently lowered into the eDIB to push it against the bottom of the well allowing it to be pierced. Once pierced, the electrode could be manipulated to enter an internal aqueous core aided by the agarose bead suspended on the end of the electrode tip.

3. Peptide fluorescence traces

Individual fluorescence traces, taken for eDIBs exposed to increasing concentrations of a 1:1 ratio of Magainin 2 and PGLa (concentration expressed as total peptide concentration). This data, as opposed to the majority of data for the peptides shown in the main manuscript, is obtained using a well plate reader. The data shows no discernible calcein leakage prior to bilayer rupture for all peptide concentrations, informing the decision to use a binary system for peptide activity detection.



SI Figure 3. Individual fluorescence traces of eDIBs exposed to increasing concentration of total peptide (1:1 Magainin 2: PGLa)