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Electronic Supplementary Information

Programming membrane permeability using integrated membrane pores and blockers as molecular regulators

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SI1) Materials

All lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Calcein dye, buffers, oils, agar, proteins, blockers, and all other reagents were purchased from Sigma Aldrich (UK) unless otherwise stated.

SI2) Emulsion Phase Transfer

GUVs were prepared via the emulsion phase transfer technique,[1, 2] which uses emulsion droplets as templates around which a bilayer is assembled. POPC lipid (40 mg) was first dissolved in chloroform (400 μL) and mineral oil (4 mL, 10 mg mL^{-1}) and placed in an oven at 60°C for 1 h for the chloroform to evaporate off.

Glucose (200 mM) was dissolved in a buffer containing Tris-HCl (25 mM), KCl (500 mM) and DI water at pH 8. This was the external vesicle solution. Sucrose (200 mM) and calcein (1 mM) were dissolved in the same buffer to make up the internal vesicle solution. TRIMEB (1, 3, 5, 7, 10, 20 or 50 mM) or gamma-CD (20 mM) was also dissolved in the internal vesicle solution when necessary.

The lipid-in-oil solution (250 μL) and the sucrose solution (25 μL) were added to an Eppendorf and the mixture was vortexed for 30 s to create a lipid stabilised water-in-oil droplet suspension. Glucose solution (150 μL) was added to a separate Eppendorf and the droplet suspension was gently added as a layer on top of this. An Eppendorf Centrifuge 5415D was used to centrifuge the Eppendorf at 9000 g for 30 min. Droplets transformed into vesicles as they crossed from the oil to the aqueous phase, and a vesicle pellet was formed. The top oil phase and most of the lower aqueous phase was then removed to leave ca. 20 μL remaining in the Eppendorf. Glucose solution (150 μL) was used to re-suspend the pellet. A second centrifugation step at 6000 g for 10 min was conducted, followed by resuspension with glucose (150 μL) to ensure unencapsulated material was removed.

Fresh glucose solution (100 μL) and the GUV suspension (20 μL) were placed in circular PDMS chambers adhered to a glass substrate. α-HL (40 μL, 0.5 mg μL^{-1}) was added to the external solution and a glass coverslip was placed on top.

SI3) Imaging

GUVs were imaged on an inverted fluorescent microscope illuminated with a mercury arc lamp. A standard FITC filter was used to image the calcein dye. An exposure time of 200 ms was used, with images were taken every 60 s. ImageJ was used for all image analysis. Vesicle boundaries were found using the threshold function and the mean grey value represented the average fluorescence. The background signal originating as a result of unencapsulated calcein was subtracted from the vesicle signal. Fluorescence decay data was fit to an exponential decay function using Origin data analysis software.

SI4) DIB formation and electrical measurements

DIBs were formed using the lipid-in approach, where lipids (DPhPC, 10 mg ml^{-1}) were present in TRIS buffer as 100 nm Small Unilamellar Vesicles (SUVs) formed by extruding the hydrated lipid suspension 11 times through a polycarbonate membrane with 100 nm pores. These droplets were incubated in a well containing hexadecane, allowing a monolayer to form at the water-oil interface.[3]

5 mm thick Acrylic wells (ø = 10 mm) were formed by bonding to PDMS coated microscope slides using double-sided adhesive and filled with hexadecane. 1.5 µL droplets of vesicles (and blocker or α-HL) in buffer were deposited onto the agar coated tips of Ag/AgCl electrodes and lowered into the well using micromanipulators. The electrodes were connected to an Axon AxoPatch 200B Amplifier via a CV-203BU headstage (Molecular Devices) and data acquisition was controlled by a PC using an Axon Digidata 1440A digitiser (Molecular Devices). The droplets were incubated for 5 minutes before being brought into contact. DIB formation was confirmed by applying a linear voltage ramp where the measured current response (e.g., 500 pA) can be interpreted as the bilayer capacitance (e.g., 500 pF) when dV/dT = 1, as I = dV/dT*C. Bilayer area can be approximated using a specific capacitance of 0.5 µF cm^{-2}. [4] Bilayer current measurements were performed under a holding potential of 100 mV using a sampling rate of 50 kHz and a low-pass Bessel filter of 5 kHz. All traces were digitally filtered using a low-pass 1 kHz Gaussian filter and analysed in CampFit (Molecular Devices).

ESI References

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