Functional membrane-based integrated biosensing devices for detection and quantitation of specific nucleic acids and other biomolecules

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Omar will conduct most of the implementation of the project, building upon more fundamental work completed under the supervision of Dr. Lorenzo Di Michele and Prof. Pietro Cicuta, whose involvement is critical for the design. Their roles will be largely supervisory, troubleshooting the theoretical and practical considerations for the device, as well as interfacing with potential other team members and collaborators. As a Sensor CDT student, Omar is experienced with the basic electronics required in this project, and his work with microfluidics and vesicles during his PhD make him well suited for such a project.

Summary

Lipid vesicles can be functionalized with non-complementary DNA ligands that, alone, do not give rise to any adhesion. In this condition, only the addition of a third *target* DNA strand, which binds together the otherwise non-complementary ligands, will induce adhesion between vesicles (**Fig 1**). Using this principle, two electrochemical cell devices are proposed for the detection specific DNA strands.

The first is based on functional lipid mesophases, in which the porosity depends on the concentration of *target* DNA strands (1). This porosity is in turn linked to the ionic conductance of the material, which is easy to measure and quantify.

The second simply considers the probability of vesicles adhering to a supported lipid bilayer at different concentrations of *target* DNA. The number of vesicles adhering to the supported lipid bilayer will change according to *target* DNA concentration (2), and this can be quantified by surface impedance spectroscopy akin to cell counting (3).

Once optimised for DNA detection, this sensing platform could be applied and extended to the detection of other biomolecules, including antibodies/antigens and other biomarkers.

Proposal

Problem: Absorbance spectroscopy can determine the concentration of DNA samples, but it requires high concentrations (>10 ng/µl) and implies purchasing a spectrophotometer. Commercially available quantitation assays can measure concentrations down to 0.1 ng/l, but rely on fluorescent probes and require a spectrofluorimeter. Both these techniques are not sequence specific, and therefore none of the existing strategies can guarantee simultaneous detection and quantitation of oligonucleotides. Moreover, these existing technologies rely on expensive and bulky experimental setups for the readout of the results.

Instead, we propose a simple, portable, label free and inexpensive technology for quantitation and detection of specific oligonucleotides based on responsive lipid materials. Additionally, these responsive materials require low-volume/low-concentration samples (1-10 l at 1 pM), and have shown the ability to be adapted to protein sensing (using biotin-streptavidin interactions) at similar concentrations; much improved on other existing techniques. Again, as a label-free, low-cost and portable approach, this could be an interesting sensing device for medical diagnostics, and allows for easy multiplexing.

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Biological Systems: Initially the biological components of our system will primarily consist of lipid vesicles and short oligonucleotides. However, work with a collaborator Prof. E. Delcanale from the University of Parma, a specialist in supramolecular sensing technologies, could allow for different surface functionalisation to take advantage of antibody/antigen specificity, and more widespread application, but this might depend on both the efficacy of the device and the timescale to proof-of-principle with DNA strand sensing.

Design Goals for the Hardware: For the surface impedance device (**Fig 2**), a simple electrochemical cell using two ITO slides as electrodes coupled with an Arduino to generate an AC potential is to be fabricated. The output voltage will be compared with the input and also the value from a reference electrode to measure the change in impedance due to vesicle adhesion in the presence of the *target* ligand. This is an adaptation of existing work on surface impedance spectroscopy for cell counting (2).

For the ionic conductance device (**Fig 3**), we will apply work from our collaborators in the Knowles group in Chemistry to integrate electrodes in a microfluidic chip (4). In the chip we will have a network of vesicles densely packed, into which we will add our analyte through an inlet valve, and the changes in ionic conductance using the potential difference between the two electrodes, again making use of the Arduino.

Lastly, for wireless transmission (for potential application in the field) a ZigBee wireless module could also be employed, to allow transmission of results to a portable device such as a phone.

Implementation: 1) design and build the an electrochemical cell using patterned ITO slides as electrodes, and silicone spacers, with one electrode covered in a supported lipid bilayer and order parts (~2weeks) 2) design specific DNA tethers for strands of interest (<1 week) 3) Implement surface impedance spectroscopy, including circuitry and programming Arduino for AC current as in ref.(2) (~1 week) 4) test with various DNA and vesicle concentrations to optimise changes for detection ranges desired (~2 weeks) 5) calibrate to get useful output (~1 week).

For the second device,: 1) design a microfluidic device with integrated electrode (ref.(4)), as seen in **Fig 3**, fabricate and implement circuitry for (~4 weeks) 3) Using same tethers and *target* DNA strands, adjust the input flow and tether concentrations, in order to find the right parameters to sense the concentration ranges of interest (~2 weeks) 4) calibrate to get useful output (~1 week).

Integrating a wireless protocol to the system would take <1 week, as would possibly 3D printing a more aesthetically pleasing/practical container in which to store the device.

Outcomes: The minimum outcome would be a working device-based on the surface impedance spectroscopy method, achieving the improvements on current technologies as laid out in the 'Problem' section. Ideally the responsive porous material would also be complete and functional, though this part of the project has more potential pitfalls. However, either working at the *target* strand concentrations we expect would represent a remarkable result.

Hopefully, this project will successfully demonstrate the sensing capabilities of such a system, and encourage us to devote more time to the sensing line of Omar's PhD, specifically in DNA-sensing device, as well as application to devices for medical diagnostics using antigen/antibody tethers as *targets.*

Components + Budget

Many of the components are available in our lab (PDMS, ITO slides, silicone sheets, lipids, sugars, salts etc.). Additionally, InDiAn alloy is needed for the electrodes in the integrated microfluidic device, as is money for 3D printing. Microfluidic design costs for masters can be kept to around ~£70. For the electronics outside of those in the BioMaker toolkit, including op-amps and resistors would be~£20. A wireless zigbee module would be ~£20 pounds. Cholesterol anchored DNA for the tethers can be expensive, so for the strands to be sensed and the tethers we would estimate ~£250, though we have some left-over DNA which could work. Therefore we estimate a budget, on top of the BioMaker toolkit, of <£500, though this could be reduced depending on how far into the second device we get.

References

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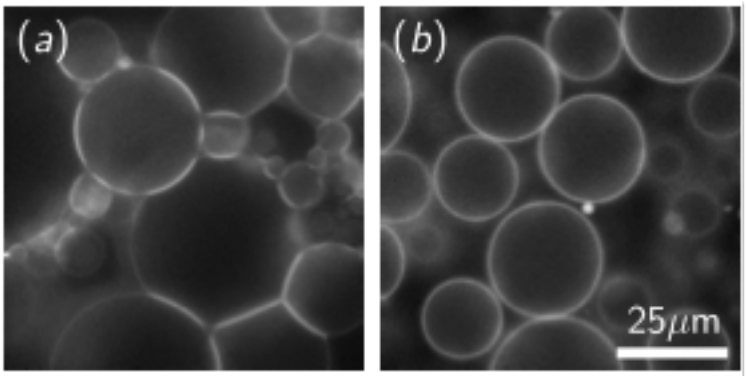


Figure 1. (a) Fluorescence microscopy image of a packing of GUVs adhering through DNA-mediated interactions. The gaps (porosity) of the material can be controlled and determine the sensing potential of the material. (b) A loose packing of GUVs decorated with non-complementary DNA, showing that there is no non-specific adhesion

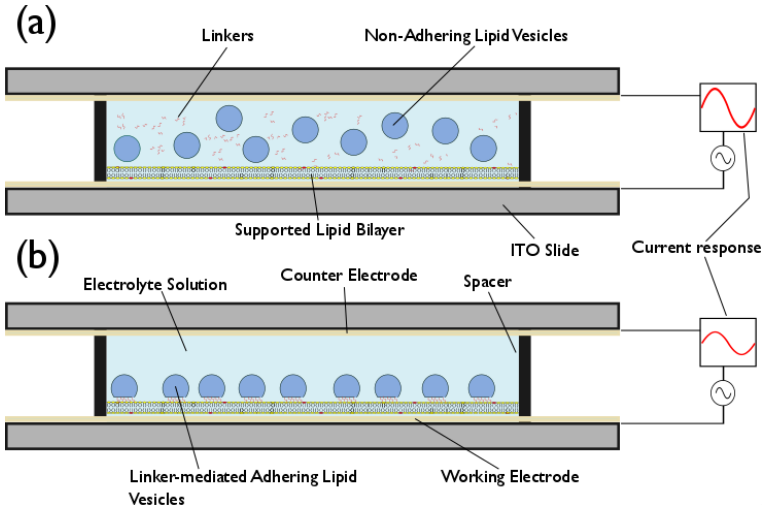
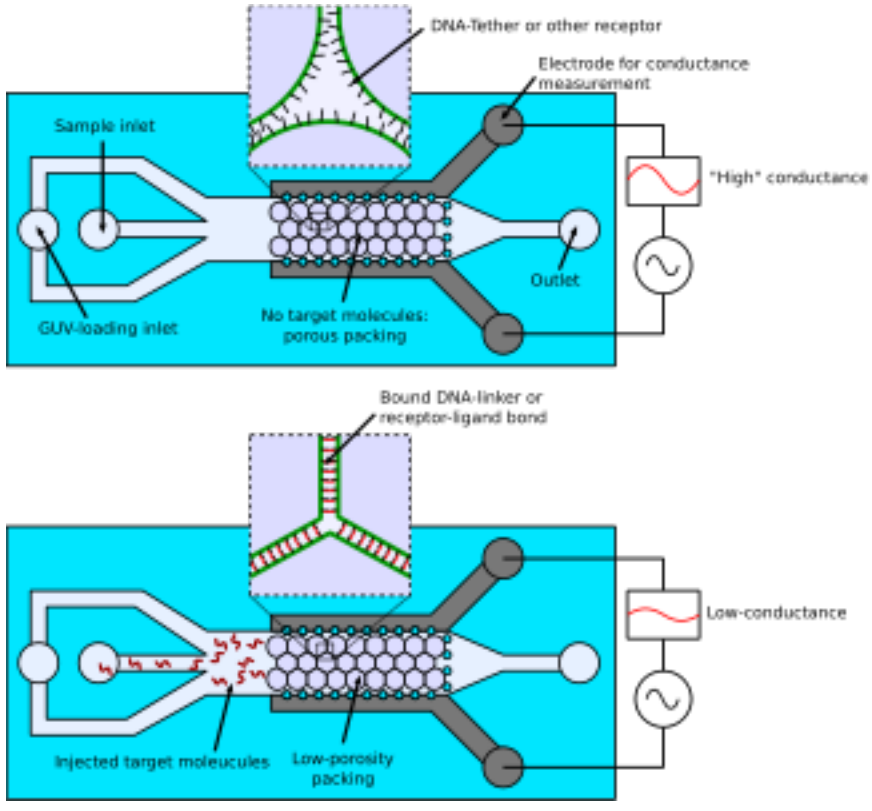


Figure 2: A schematic of two electrochemical cells to probe the linker-mediated adhesion of lipid vesicles to lipid bilayers in chambers formed using ITO slides and silicone rubber spacers, where (a) presents non-adhering vesicles and (b) adhering vesicles. This leads to different current responses of each cell, which would result in different impedance measurements, indicating a difference in the portion of vesicles adhering and therefore the linker concentration.



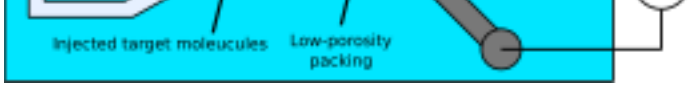


Figure 3: Not-to-scale design of the sensing device. Top: a microfluidic cell filled with a porous packing of GUVs decorated with non-sticky DNA or other receptor molecules. Bottom: the same packing after exposure to the target ligand that links the GUVs, causes the closure of the pores and a drop in the ionic current measured across the packing.