

# **Conclusions and Future Work**

## **Conclusions**

### **General Overview**

I started writing this thesis with one broad aim – to couple insect odorant receptors with either a carbon nanotube or graphene field-effect transistor for highly selective sensing in the vapour phase. Through this process, I hoped to gain new physical insights into the transducer and receptor elements used, through careful examination of the biosensors which resulted from their coupling. At the end of this investigation, three broad themes have emerged:

- Slight variations in transducer morphology have a substantial impact on biosensor sensitivity and power consumption.
- Intrinsic transducer hysteresis leads to biosensor current drift, which can be modelled and corrected for when interpreting sensing data.
- Surface contamination can affect transducer functionalisation and lead to significant variability in biosensor quality.

These findings and their relation to the goal of creating a reliable biosensor for a vapour-phase detection system are each detailed in this section.

### **Biosensor Performance and Transducer Morphology**

Carbon nanotube networks and graphene are used as the thin-film for sensing field-effect transistors due to their highly sensitive surface. Features present on each film were found to enhance or reduce the sensitivity of the transistor in a low-power configuration. The process used for randomly depositing carbon nanotubes influences multiple aspects of the resulting network morphology, including the density and homogeneity of the network, the diameter of bundled carbon nanotubes, the level of doping present and the relative proportion of metallic to semiconducting nanotubes. These variables were investigated atomic force microscopy, Raman spectroscopy and electrical characterisation. Devices with carbon nanotubes deposited using surfactant in the presence of steam were found to have relatively dense and homogeneous networks which gave rise to highly consistent electrical characteristics, desirable for biosensor reproducibility and multiplexing. Carbon nanotube bundle diameters were also much smaller in these networks, which gave them a relatively large on-off ratio and subthreshold slope, figures of merit which correspond to low power operation. However, steam-assisted surfactant-deposited devices were found to have significant *p*-doping. Identifying and removing the source of this *p*-doping would further enhance the performance of these devices.

## **Biosensor Drift and Transducer Hysteresis**

All devices characterised showed a degree of hysteresis, which was especially significant for solvent-deposited carbon nanotube network devices as well as any device when backgated. This hysteresis is a result of the evolving occupancy of charge traps, which are introduced to the device by gate bias or adsorbed dopants. This changing occupancy leads to a slow drift in current level when the transistor is operated with constant voltages at both the drain and gate. If left until reaching a steady state, this baseline drift can be modelled using a linear combination of three exponentials. However, it is not feasible to wait more than three hours for a biosensor to warm up with each use. A series expansion was used to model the drift over a shorter period of time, where the two longer time constant exponential terms were approximated as linear. By waiting until the first exponential approached zero, a simple linear correction was sufficient to filter out baseline drift when the time constants of the other exponentials were sufficiently large. For a pristine, liquid-gated steam-deposited carbon nanotube device, the first exponential typically took less than 30 minutes to sufficiently decay.

## **Biosensor Functionalisation and Transducer Contamination**

While the pristine surfactant-deposited carbon nanotube transistors were consistently sensitive to changes in both an aqueous and vapour environment, their selective detection ability after non-covalent functionalisation with iORs was highly variable. Using atomic force microscopy, Raman spectroscopy, electrical characterisation and fluorescence microscopy it was found that the PBASE linker was successfully attaching iOR nanodiscs to the carbon nanotube network. While functionalisation caused significant gating of the network for devices which were successful sensors, it was not seen for devices which showed no selective sensing behaviour, despite the evidence that iOR nanodiscs were present in both cases. It was therefore hypothesised that an unknown coating on the transducer was allowing the adhesion of iOR nanodiscs onto the channel region, but was preventing electrical interactions between iORs and the channel. A pristine thin-film device with a thin surface coating can still be electrostatically or capacitively gated in a highly sensitive manner. However, it has previously been noted that the primary sensing mechanism of iORs appears to be through direct charge transfer. If an insulating coating layer is present, it may prevent charge from passing between the iORs and the channel.

Multiple potential candidates for this unwanted coating were identified throughout the course of this thesis, including residual process chemicals, residual PBASE and hydrophobic alkanes. Sensing experiments were then performed that systematically eliminated possible candidates. It was found that neither avoiding the introduction of surfactant or ensuring PBASE did not hydrolyse led to consistent biosensors. Finally, a method was tested that eliminated the remaining possible candidates through the use of a oxygen plasma clean step prior to functionalisation, as well as the use of iORs in surfactant solution with linker pre-attached. This process resulted in a successful sensor in an aqueous environment, which is further indication that an unwanted surface coating is the cause of sensor variability. It should be noted that

the impact of oxygen plasma on device mobility and the vulnerability of the unprotected iORs means that this method does not reliably produce responsive vapour-phase sensors. Further optimisation of the functionalisation process is a clear priority for future works, with a detailed discussion of possible approaches found in the next section.

## Future Work

### Device Fabrication and Functionalisation

Throughout the course of this thesis, several aspects of the fabrication and functionalisation were identified as having potential for improvement. Further investigation into these approaches by future researchers could lead to improved biosensor performance and a deeper understanding of the device physics of the sensor. The encapsulation of device electrodes is critical for improving the performance of a device, preventing gate leakage through the liquid-gate and avoiding modulation of the electrode-channel Schottky barrier while sensing [@Lim2014; @Albarghouthi2022; @Heller2008]. Albarghouthi *et al.* outline an alternative encapsulation approach which leads to very low gate leakage currents which could be adapted for use with our sensing devices [@Albarghouthi2022]. Here, a multilayered encapsulation with HfO<sub>2</sub>, SU8 and PEG is used to encapsulated the device. The only change to the existing process required is the addition of a patterning step to leave the channel region exposed, similar to that seen in ?@sec-encapsulation. Furthermore, if a nonfluorescent SU8 was used instead of SU8-2150 [@Vobornik2023], devices could be characterised with fluorescence microscopy after encapsulation, which would be highly advantageous for verifying functionalisation. After passivation with PEG, highly specific attachment of fluorescence-tagged iORs could be verified in a similar manner to that used by Lee *et al.* directly prior to sensing [@Lee2012b]. If PEG passivation and linker are deposited simultaneously, as in the approach by Filipiak *et al.* [@Filipiak2018], multiplexed fluorescence microscopy could be used to determine the proportion of channel coverage by linker compared with PEG passivation.

Surface contamination was identified as one of the major factors interfering with successful device functionalisation in this thesis. To improve the reproducibility and yield of non-covalent functionalisation processes, an effective cleaning method needs to be identified which is able to remove most contaminants, reducing the size of the D-peak in the Raman spectrum of the thin-film and moving the device threshold voltage towards 0 V. Instead of reducing mobility, this approach should improve device performance. Barnett *et al.* used air oxidation and annealing to remove surfactant dopants from a carbon nanotube network, which was highly effective at restoring the threshold voltage to 0 V [@Barnett2018]. Petkov proposed oxygen radical cleaning of organic contaminants as an alternative to destructive oxygen plasma cleaning, which was shown by scanning electron microscopy (SEM) to successfully remove surface hydrocarbons [@Petkov2005]. Current annealing has also proven to be an effective means of both reducing the contact resistance of TFT devices [@Schnitzspan2020] and can be used to evaporate surface contaminants for both back-gated [@Ramamoorthy2018] and liquid-gated

[@Kireev2017] configurations. If available to the researcher, these techniques should all be explored to identify the most effective and least destructive cleaning technique.

Surface contamination becomes less significant if functionalisation methods are used which do not involve linker *pi*-stacking. For example, diazonium coupled with metal ion chelated AB-NTA (N,N -Bis(carboxymethyl)-L-lysine hydrate) has been used as a covalent linker to create sensitive vapour-phase biosensors with nanodisc-held vertebrate odorant receptors [@Goldsmith2011]. As successful covalent binding is indicated by a significant change in channel mobility, it becomes much easier to distinguish between successful and unsuccessful channel functionalisation than in the non-covalent case. Here, Cu<sup>2+</sup> could be used as the metal ion as it has a stronger binding strength than other metal ions, such as Ni<sup>2+</sup> [@Chang2017; @Aravinda2009; @Baur2010]. Another covalent approach involves tethering nanodisc-held odorant receptors to gold floating electrodes using antibody fragments [@Yang2017; @Yang2018; @Lee2018]. Non-covalent approaches which do not require direct charge transfer mechanisms for sensing could also be investigated. A similar functionalisation approach using nanovesicles (liposomes) could be taken to that of Lim *et al.* [@Lim2015], where the Kir ion channel and hORs are replaced with an ORCO ion channel and iORs [@Wicher2021]. If ion flow occurs within the Debye length, the resulting changes in charge could be detected with electrostatic gating, even if no direct electrical connection to the transducer channel exists.

## Vapour Sensing

Further work could also be done to further improve the vapour delivery system. This work could include changes to the system configuration overall, as well as changes to the setup of the device in the characterisation chamber.

The maximum flow rates of the mass flow controllers currently present in the system are too low to carry some of the volatile compounds of interest for testing with the iORs. For example, the vapour pressure of methyl salicylate, agonist for OR10a, was found to be too low to be successfully delivered by the existing vapour delivery system [@MeSal]. All three mass controllers could be replaced two MFCs which have an identical maximum flow of either 1000 or 10000 sccm. This may require the PVC tubing to be replaced with stainless steel to cope with higher pressures, with check valves added to avoid the system being over-pressurised. These two MFCs could be operated in the manner outlined by Goldsmith *et al.* for a constant and uninterrupted flow of nitrogen [@Goldsmith2011]. In this setup, the first MFC maintains a constant flow rate through the dilution line at all times during a sensing experiment. The second MFC is also kept at a constant flow rate at all times, but feeds into a computer-automated solenoid valve which is able to switch the flow between the dilution and carrier line. The second MFC is then only directed through the carrier line during the relevant intervals of a sensing series.

Exposing iORs to vapour-phase compounds outside of a protective lipid membrane environment may cause them significant damage [@Sato2014; @Warden2019]. A capture layer can be

used to mimic the mucus within the insect sensillum lymph, which protects the dendrite cells *in vivo* [@Shkodra2021; @Sato2014; @Hurot2020; @Cali2020]. A variety of possible capture layer solutions for vapour-phase field-effect transistor sensors can be found in the literature. Lee *et al.* and Kuznetsov *et al.* integrated an aqueous membrane held by polycarbonate and  $\text{SiO}_2/\text{Si}_3\text{N}_4/\text{SiO}_2$  into their sensor, which prevented electrolyte evaporation [@Lee2015; @Kuznetsov2018]. Bio-gels or ionic liquids (ILs) are other potential candidates for mucus-like capture layers. Sato *et al.* and Hirata *et al.* used an agarose hydrogel in a microchamber array as a capture layer [@Sato2014; @Hirata2021]. Ionic liquids have low vapour pressure, high capacitance, as well as high electrochemical stability. Both 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (BMIM) [@Hwang2014; @White2015] and 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIM) [@Inaba2014; @Kiga2012] have been used as the electrolytic gate for thin-film field-effect transistors. However, alternative ionic liquid candidates may need to be identified which are compatible with the biological elements of the sensor [@Gomes2019; @Shukla2020]. Ionic liquids also show a degree of selectivity towards volatile compounds, which needs to be accounted for when used as a capture layer [@Kulkarni2007].

The capture layer could also be used as a liquid-gate to maximise device sensitivity. A gate electrode can be photolithographically patterned onto the device surface alongside the source and drain electrodes for this purpose, making contact with the capture layer through an additional gap in the device encapsulation [@Shkodra2021]. Only slight alterations would have to be made to the device design outlined in this thesis for implementation. A potential difficulty with this approach might be ensuring rapid odorant diffusion through the layer to the iOR-FET surface, especially when compounds are insoluble [@Yamada2021; @Hurot2020; @Warden2019; @Cali2020; @Lee2015; @Spencer2021]. Yamada *et al.* used hydrophobic microslits at the liquid-vapour interface to promote mixing of compounds within an aqueous environment [@Yamada2021]. It may also be possible to use odorant binding proteins to carry volatile compounds to the odorant receptors, which is how volatiles are carried from the surface of the sensillum and through the mucus membrane to the odorant receptors *in vivo* [@Larisika2015; @Kotlowski2018; @Brito2016; @Cali2020; @Pelosi2018; @Sankaran2011].

## Multiplexing

A scent in nature contains a mixture of many different volatile organic compounds in vapour at different concentrations. A future vapour-phase iOR biosensor should therefore be able to perform multiplexed measurements with different iORs in different channels, to simultaneously measure and distinguish between mixtures of volatile organic compounds [@Kwon2015; @Hurot2020; @Hirata2021]. A similar approach could be taken to that of Kwon *et al.* and Son *et al.*, but for insect odorant receptors [@Kwon2015; @Son2017]. Care should be taken to avoid the coffee-ring effect when functionalising individual channels of a device (?@sec-coffee-ring). The use of small, deep microchannels in clean PDMS, which keep functionalisation material close to the channel, may be essential for consistent functionalisation. A difficulty that could

arise when multiplexing is that iORs can specifically bind with multiple VOCs. When interacting with an iOR, different volatiles can ‘antagonise’ each other; some iORs will not respond to a positive ligand if another specific ligand is present [@Kwon2015; @Son2017; @Munch2016; @Hirata2021]. This means a multiplexed iOR biosensor would first need to be tested individually against different volatile compounds, and then with mixtures of volatile compounds to detect any antagonistic behaviours in a systematic manner.

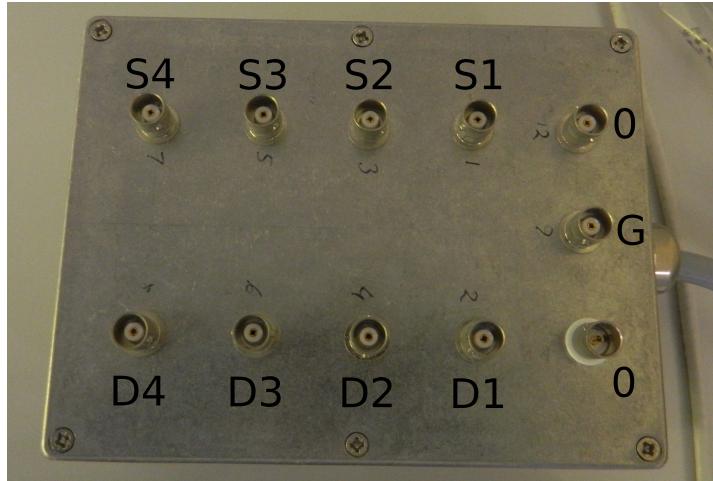


Figure 1: Breakout board for vapour delivery system device. Source electrode terminals are labelled S, drain electrode terminals are labelled D, the gate electrode terminal is labelled G and disconnected terminals are labelled 0. Each number corresponds to a different device channel.

This thesis used an existing setup for aqueous sensing which could already be used for eight-channel multiplexed measurements. For the vapour delivery system chamber, it is already possible to connect four channels at once through a custom breakout board, shown in Figure 1. Both setups should be sufficient for testing three iOR-functionalised channels simultaneously alongside an empty iOR-functionalised channel. To switch between analytes in a single vapour-phase sensing run with uninterrupted flow, a second analyte bottle could be installed which branches off from the existing carrier line. A solenoid-controlled 4-way valve could be placed after these analyte bottles, which runs the vapour flow from one analyte bottle to the exhaust and one into chamber at all times, as illustrated in Figure 2. In this way, the amount of flow passing into the chamber is largely unchanged across a sensing run. An advantage of this setup is that each carrier line only ever contains one analyte to prevent cross-contamination between lines. For multiplexing with more than two analytes, more analyte bottles could be added and a 16-way or 32-way valve used. This system could also be used to deeply investigate the effects of different exposure (sniff) times on the temporal resolution of sensing devices [@Spencer2021; @Wu2024].

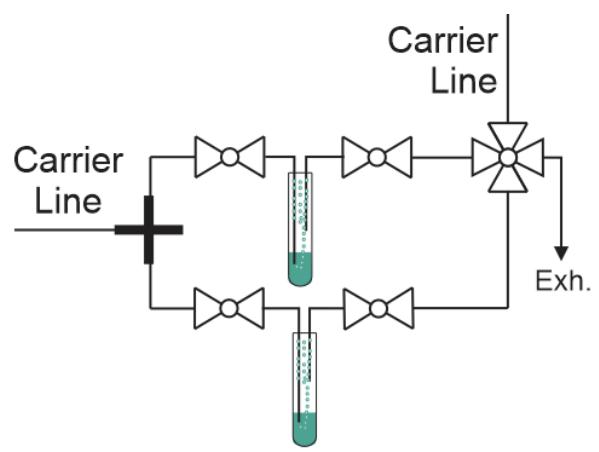


Figure 2: Multiplexing with a second analyte bottle and four-way valve.