

Aqueous Sensing with iOR-Functionalised Carbon Nanotube Devices

Introduction

So far in this thesis, multiple design schemes have been described for the construction of biosensors with insect odorant receptors. In **?@sec-fabrication**, transducers were fabricated and characterised which used either various carbon nanotube network morphologies or a graphene thin-film for the conductive channel. The carbon nanotube network morphologies included highly bundled networks from dropcasting in an organic solution, and less bundled networks dropcast in surfactant solution either with or without steam present. The networks deposited using steam were found to have a more homogeneous morphology than the other network types, giving rise to devices with a large on-off ratio and highly consistent device-to-device electrical characteristics. Functionalisation approaches using the attachment linkers PBASE, PBA/EDC, pyrene-NTA and pyrene-biotin were discussed in **?@sec-noncovalent-functionalisation**, where linker functionalisation was investigated using electrical, spectroscopic and optical analysis techniques. Successful attachment of the pyrene group of PBASE was demonstrated by comparing device transfer characteristics and Raman spectra of the thin film before and after PBASE functionalisation. Fluorescence microscopy was also used to identify and eliminate multiple obstacles to successful PBASE functionalisation.

In this chapter, aqueous sensing using insect odorant receptor functionalised thin-film transistors was achieved through the use of two different functionalisation approaches. The first involved attaching linker to the transducer in organic solvent, and subsequently exposing the linker-modified transducer to odorant receptor nanodiscs; the second involved attaching linker to odorant receptors prior to device functionalisation, which allowed the functionalisation process to be performed entirely in aqueous solution. The first approach was similar to approaches used in previous research for thin-film functionalisation with insect odorant receptors [**@Murugathas2019a**; **@Murugathas2020**]. However, sensing with devices functionalised in this manner was not consistently reproducible. A range of slight changes to this organic solvent functionalisation procedure were trialled to identify the source of the inconsistency in biosensor behaviour. This testing indicated that the quality of functionalisation was variable, which may be attributed to surface coatings on the thin film or the protective structure of the nanodiscs interfering with odorant receptor attachment. From the results of this testing process, the second approach was developed, which was used to successfully create a functional device. This sensor could detect aqueous volatiles in the femtomolar range, and retained this level of sensitivity upon being washed and reused.

Aqueous Sensing of Ethyl Hexanoate with OR22a-Functionalised Device

OR Nanodisc Functionalisation

A carbon nanotube network field-effect transistor device, fabricated using post-Jun 2023 methods as described in [?@sec-fabrication](#), was functionalised with OR22a nanodiscs. The network used for the device was deposited using the steam-assisted surfactant method, and the device was encapsulated with AZ® 1518 using the post-Jan 2023 photolithography mask. The functionalisation was performed as follows:

1. The device was exposed to UV light for 1 minute, placed in AZ® 326 developer for 3 minutes, then rinsed with acetone, isopropanol and nitrogen dried.
2. The device was vacuum annealed for 1 hour at 150 °C.

Note: Steps 1 & 2 were added to ensure any residual photoresist on the channel was removed or passivated before functionalisation, see [?@sec-photoresist-contamination](#).

3. A solution of 1 mM PBASE (Setareh Biotech) in methanol was prepared by fully dissolving 2 mg PBASE in 5 mL methanol by vortex mixing at 1000 rpm in a dark room.

Note: PBASE was stored at –18 °C for 18 months prior to use, and was thawed under vacuum for 15 minutes in dark conditions before opening.

4. The device was then rinsed with methanol, fully submerged in ~ 1 mL of PBASE in methanol solution and left covered with parafilm for 1 hour, then rinsed with methanol for 15 s, rinsed with phosphate buffered saline (PBS) for 15 s and nitrogen dried to remove residual PBASE.
5. The device was left dry and in darkness while collecting the OR22a nanodiscs from the –80 °C freezer.
6. 10 µL OR22a nanodiscs (batch number ND-OR22a-SB018, 1.9 mg/mL, prepared 7 months earlier) were diluted in 1 mL freshly-prepared PBS.

Note: The full 1 mL was used to flush out the nanodisc vial when preparing the nanodisc solution, with successive additions and subtractions of 50 µL 1× PBS into and from the vial.

7. The device was submerged in the OR22a nanodisc solution and left covered with parafilm for 1 hour, then rinsed with 1× PBS for 15 s and gently nitrogen dried.

The liquid-gated electrical characteristics shown in Figure 1 were taken using the B1500A semiconductor device analyser of the sensing channel (channel 7) before and after functionalisation with OR22a nanodiscs. The liquid-gate electrolyte used was 1× PBS containing 0.5% v/v

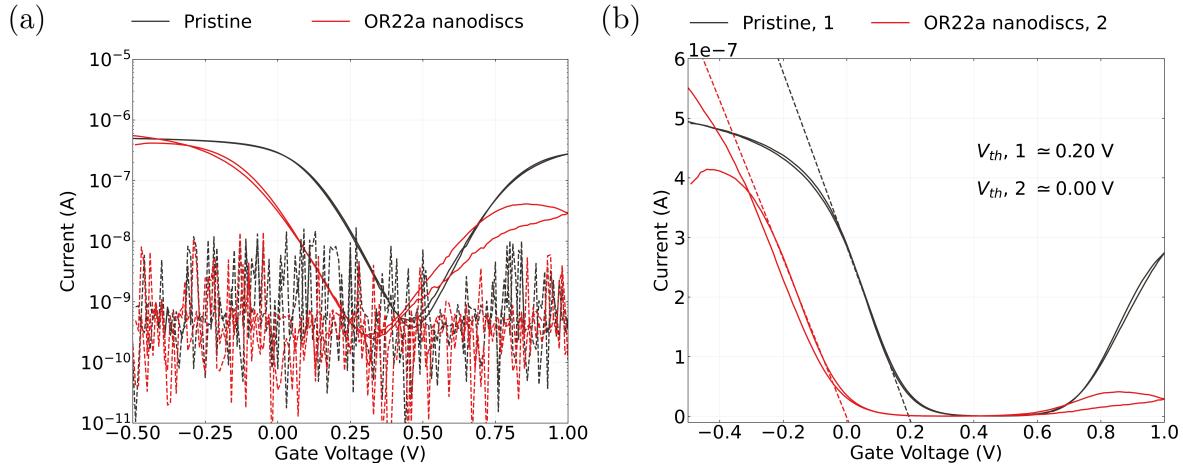


Figure 1: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain voltage was $V_{ds} = 100$ mV for both the forward and reverse sweep. In (a), the characteristics are shown on a logarithmic scale, where the gate current for each transfer curve is shown with a dashed line. In (b), the characteristics are shown on a linear scale alongside a dashed line tangent to the subthreshold slope of the characteristic curve in the forward direction. The threshold voltage corresponding to the intercept of this slope with the x-axis is shown for each transfer characteristic curve.

DMSO. The device exhibited ambipolar characteristics before functionalisation, which is typically seen for steam-deposited carbon nanotube films ([?@sec-electrical-characterisation-CNT](#); [?@sec-cnt-devices](#)). However, *p*-type behaviour dominates after device functionalisation due to a significant drop in *n*-type conductance. A slight increase in hysteresis was observed post-functionalisation. Leakage current (shown by the dashed traces) never exceeded 1×10^{-7} V, both before and after functionalisation. The significant change in electrical characteristics could be due to some combination of five possible factors: adsorption of solvent onto the network, network attachment of PBASE without subsequent protein attachment, non-specific adsorption of protein onto the network, PBASE-mediated attachment of the membrane scaffold protein (MSP) of nanodiscs to the network, and PBASE-mediated attachment of odorant receptors to the network. As the nanodisc volume is much larger than that of the odorant receptor, any direct protein adsorption most likely adsorption of the nanodisc membrane onto the carbon nanotube network. Odorant receptor attachment with PBASE is therefore the only desirable functionalisation result for sensing purposes.

Functionalisation of the channel resulted in a negative shift in threshold voltage of -0.20 ± 0.03 V. This significantly exceeds the threshold voltage shifts measured for both methanol adsorption (-0.15 ± 0.02 V) and device exposure to PBASE in methanol (-0.06 ± 0.04 V), confirming that protein has attached to the carbon nanotubes. However, both direct protein adsorption [[@Bradley2004](#); [@Heller2008](#); [@Kauffman2008](#)] and empty nanodisc membrane attachment [[@Murugathas2019a](#)] should also lead to a significant negative threshold voltage shift and therefore increased *p*-conduction in the liquid-gated transfer characteristic curve. In all three cases, the voltage shift is predominantly the result of negative charge transfer from the adsorbed proteins to the semiconducting carbon nanotubes [[@Bradley2004](#); [@Heller2008](#); [@Murugathas2019a](#)]. It is likely that the negative shift observed results from some combination of the three types of attachment. It should be noted that while the size of the functionalisation-induced threshold voltage shift can be used to determine whether an amine-tagged protein has attached to the nanodisc network, it cannot be used to specifically determine whether odorant receptors have attached to the network.

Atomic force microscope images were taken of the device channels both before functionalisation and after sensing with the functionalised device to confirm the presence of nanodiscs. The images are shown in Figure 2 (a)-(b). As far as the author knows, these are the first atomic force microscope images taken of iOR nanodiscs found on a sensing channel rather than on a separate carbon nanotube film; this was made possible by using the 20 μm wide aperture encapsulation mask discussed in [?@sec-encapsulation](#). AFM images showing iOR-nanodisc functionalised carbon nanotube networks have been reported by Murugathas *et al.*, but these images were not of channels used for sensing [[@Murugathas2019a](#)]. The visible nanodisc features in Figure 2 (b) are much smaller than the aggregated or agglomerated nanodisc features in the atomic microscope images taken by Murugathas *et al.* [[@Murugathas2019a](#)]. On the dense network morphology used here, the position of nanodisc clusters relative to the carbon nanotubes is also less distinct. To confirm whether nanodiscs have preferentially attached to the carbon nanotubes, a more quantitative approach is required.

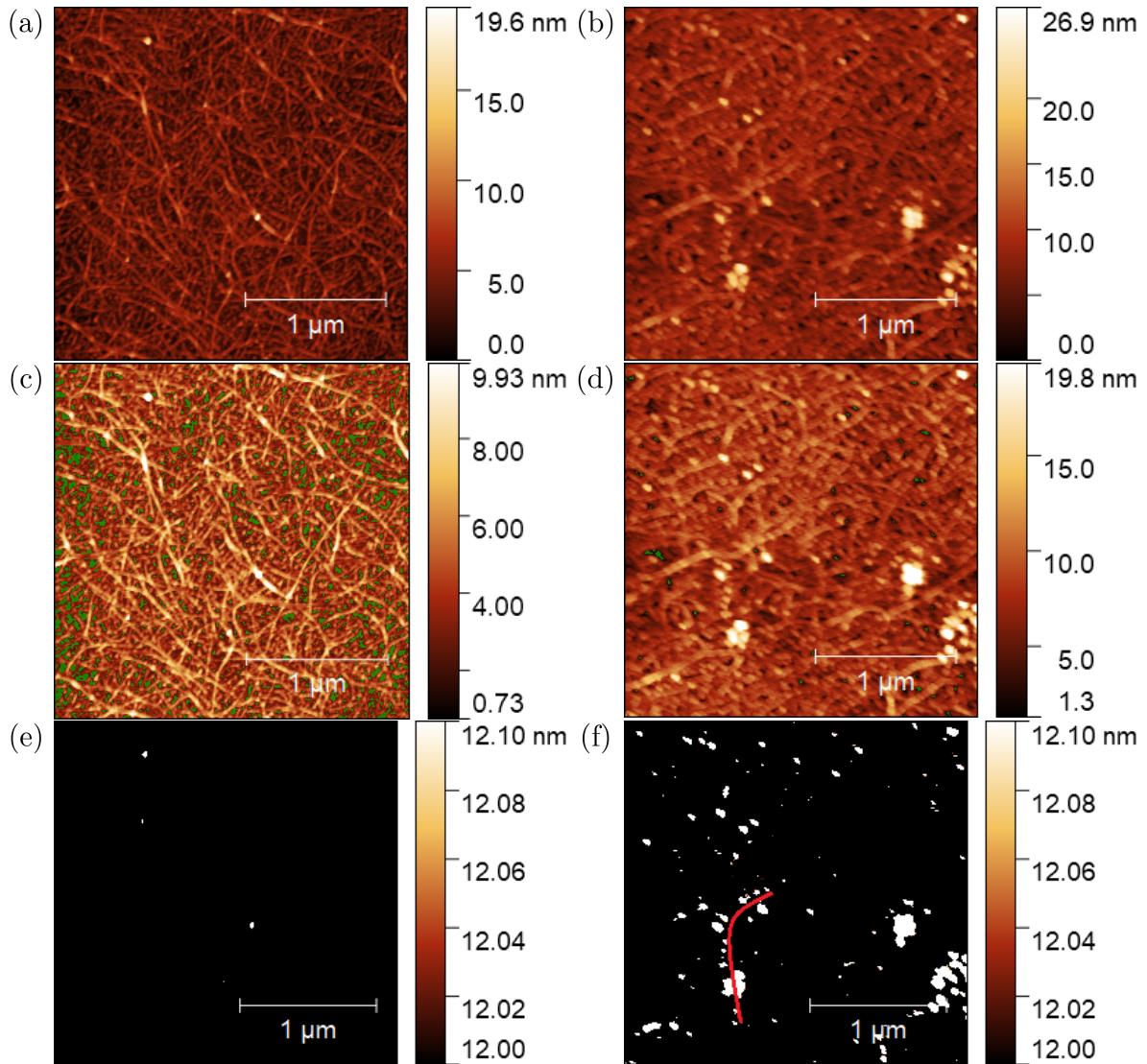


Figure 2: Atomic force microscope images of the channel region of carbon nanotube network devices before and after OR22a nanodisc functionalisation. The channel network of a pristine device is shown in (a), while (b) is of channel 7 from the sensing device functionalised in this section. The same images over a smaller height scale are shown in (c) and (d), with regions at or below the median substrate height (2 nm) highlighted green. Binary representations of the atomic force microscope images are shown in (e) for the pristine device and (f) for the functionalised device, with a threshold height of 12 nm used (10 nm above the average substrate height).

Following the discussion in ?@sec-pristine-AFM, the median substrate height in Figure 2 (b) is assumed to be ~ 2 nm as there are no visible imaging artifacts. This height is shown against each carbon nanotube network using the Gwyddion masking tool in Figure 2 (c) and (d). In Gwyddion, both images were then simplified to a binary representation, where features above a certain threshold were shown as white and features below shown as black. This representation, shown in Figure 2 (e) and (f), has the appearance of a cross-section through the network at the threshold height. The threshold was chosen as the minimum height where carbon nanotube spindles were no longer apparent in the functionalised image, 10 nm above the substrate. Figure 2 (e) shows only a few, sparsely distributed features, each with a width below 50 nm. These features may correspond to large nanotube-nanotube junctions, surfactant residue, or other surface contamination (?@sec-pristine-morphology). In Figure 2 (f), many features are over 50 nm in width. They are often found close together, and form a curved line across the network in the bottom left corner (shown in red); this arrangement of nanodisc features is similar to that reported previously for OR22a nanodiscs on sparser, more bundled networks [@Murugathas2019a].

Figure 2 (b) shows that the maximum image height of the OR nanodisc-functionalised channel is 27 nm, while Figure 2 (f) indicates only nanodisc features are present at 12 nm. Therefore, nanodisc agglomerates at least 15 nm tall are present on the channel. Assuming a average carbon nanotube bundle diameter of 2 nm, with an average substrate height of 2 nm, the nanodisc agglomerates visible in Figure 2 could be up to ~ 23 nm tall. The estimated height range for nanodiscs is $\sim 10 - 20$ nm [@Nath2007; @Bayburt2010; @Murugathas2020; @Cheema2021]. It therefore appears that nanodiscs have formed a single layer on the carbon nanotube network. Height measurements of biological samples taken via AFM have been shown to underestimate actual feature height by over 50% [@Vobornik2023]. Even so – assuming this squishing effect is not significantly in excess of 50% – the agglomerates are only a few nanodiscs high at most. As the nanodisc agglomerates are up to 200 nm across at their widest point (Figure 2), comprising of at least 20 nanodiscs, it appears that the clustering behaviour is primarily across the plane rather than vertical. The observed behaviour indicates nanodiscs individually attach to preferred locations on the network instead of nucleating in solution prior to attachment.

This attachment behaviour can be contrasted with the behaviour observed in the atomic microscope image of agglomerated OR22a nanodiscs taken by Murugathas *et al.* [@Murugathas2019a]. The network is significantly sparser, so the relative extent of clustering at different points is easier to discern. There is clearly significant variation in nanodisc attachment across a single nanotube bundle, and it appears that clustering is more significant at junctions between carbon nanotube bundles. Preferred attachment locations away from junctions could result from the higher reactivity of exposed metallic CNTs [@Cao2009], or from regions which are particularly clean of contamination. Interestingly, while the OR22a nanodisc features seen by Murugathas *et al.* via AFM are similar in breadth to those seen here, OR agglomerates which are at least 53 nm tall are present [@Murugathas2020]. It is unclear why the extent of vertical agglomeration differs between this work and that of Murugathas *et al.*, but may be linked to the difference in morphology between the networks used. One possibility is that nanodiscs will preferentially attach to metallic carbon nanotubes, but prefer to attach

to each other instead of semiconducting nanotubes. Semiconducting tubes in a highly bundled network may block proteins from accessing metallic tubes in the same bundle, leading to a greater degree of self-attachment.

Fluorescence microscopy was also used to confirm the presence of odorant receptors tagged with *Aequorea Victoria* green fluorescent protein (GFP-ORs) on the carbon nanotube film. A device was functionalised with GFP-OR nanodiscs using the process described at the beginning of this section (batch number ND-GFP-OR43b-0002, prepared 12 months earlier). This functionalisation was performed in darkness, with the odorant receptor nanodisc vial transported under an opaque cover to protect it from light. After functionalisation, devices were briefly rinsed with DI water and nitrogen dried to remove salt residue left by the 1× PBS. A control device was also prepared without the use of PBASE, skipping steps 3 and 4 in the functionalisation process. Fluorescence microscope images were taken immediately after functionalisation; devices were transported to the fluorescence microscope room in a foil-wrapped container, and the fluorescence microscope room was kept dark while images were taken. Fluorescence images of the devices are shown in Figure 3. The SiO₂ regions in each image appear bright under the GFP filter, indicating attachment between the GFP-OR nanodiscs and the SiO₂ substrate has occurred. As the process from ?@sec-photoresist-contamination has been followed, it is unlikely that the GFP-OR nanodiscs are affixed to residual photoresist.

A comparison of fluorescence in the channel region between images on the left of Figure 3 (GFP-OR nanodiscs) and the images on the right (GFP-OR nanodiscs and PBASE) is given by the inset in Figure 3 (c)-(f). The inset demonstrates that the channels not incubated in PBASE are significantly less bright than those that had been incubated with PBASE. It appears that the presence of GFP-OR nanodiscs in 1× PBS is limited on these channels. However, when the carbon nanotubes are modified with PBASE, there is clear attachment of GFP-OR nanodiscs to the channel, and the channel shows up brightly under the fluorescence microscope. Importantly, since the GFP is attached to odorant receptors rather than the nanodiscs themselves, there must be odorant receptors present on the channel after functionalisation; attachment to the channel is not limited to empty nanodiscs. This trend was consistent across all conducting channels on each of the two devices. A similar comparison technique has been used previously to detect human odorant receptor attachment to a carbon nanotube network via 1,5-diaminonaphthalene [@Lee2012b]. As far as I know, this is the first time fluorescence has been used to investigate the attachment of insect odorant receptor nanodiscs to a carbon nanotube network.

Aqueous Sensing of Ethyl Hexanoate

The procedure used for biosensor detection of ethyl hexanoate in liquid was the same as the procedure outlined in ?@sec-dummy-sensing, except 0.5% v/v DMSO was present in the buffer solution (to improve ethyl hexanoate solubility [@Galvao2014]) and dilutions of ethyl hexanoate in the same 0.5% v/v DMSO 1× PBS were added during the sensing series. Dilutions of 1 fM, 1 pM, 1 nM and 1 μM ethyl hexanoate in 0.5% v/v DMSO 1× PBS were prepared

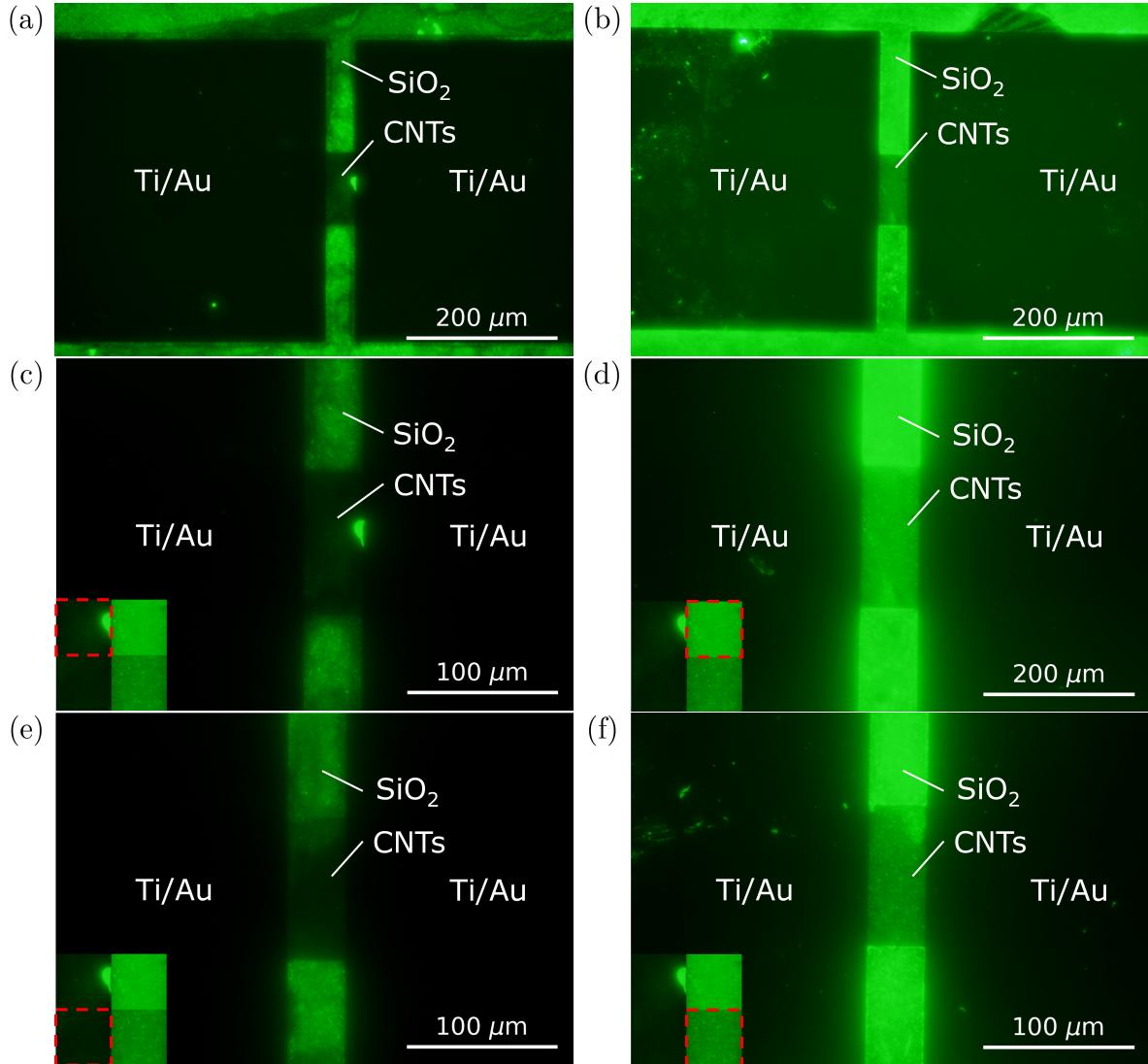


Figure 3: The fluorescence images on the left side – (a), (c) and (e) – show unencapsulated carbon nanotube network channels from a device incubated in GFP-OR nanodiscs. The rectangular dark regions to the left and right of each image are the Au electrodes. The fluorescence images on the right – (b), (d) and (f) – show the channels of a similar device after successive PBASE and GFP-OR nanodisc incubation. Images (a) and (c) are both of the same channel on the first device, and images (b) and (d) are of the same channel on the second, but (c) and (d) were taken using a greater magnification. The insets in (c)-(f) compare the central channel region of (c)-(f) more directly. All images were taken with the same microscope settings (GFP filter and 10 s exposure time), taken in quick succession, directly after functionalisation in a dark room.

before device characterisation according to Table 1, using pre-prepared concentrations of 200 fM, 200 pM, 200 nM and 200 μ M ethyl hexanoate in DMSO. The ethyl hexanoate in DMSO dilutions were prepared beforehand as a 1:10 dilution series in DMSO using 200 mM stock solution, where dilutions ranged from 20 mM to 200 fM. Sampling measurements were taken using the B1500A semiconductor device analyser, with the transfer measurement in Figure 1 (b) taken directly before sensing. The full control series plus sensing sequence is shown in Figure 4. Gate current remained negligible across the entire sensing procedure.

Table 1: Preparation of analyte dilutions in 1X PBS from solutions of analyte in DMSO. The 1X PBS used was prepared on the same day as the dilution process.

Conc. in DMSO	1XPBS added	Final volume	Final conc.
5 μ L \times 0 fM	995 μ L	1 mL	0 fM, 0.5% v/v DMSO
5 μ L \times 200 fM	995 μ L	1 mL	1 fM, 0.5% v/v DMSO
5 μ L \times 200 pM	995 μ L	1 mL	1 pM, 0.5% v/v DMSO
5 μ L \times 200 nM	995 μ L	1 mL	1 nM, 0.5% v/v DMSO
5 μ L \times 200 μ M	995 μ L	1 mL	1 μ M, 0.5% v/v DMSO

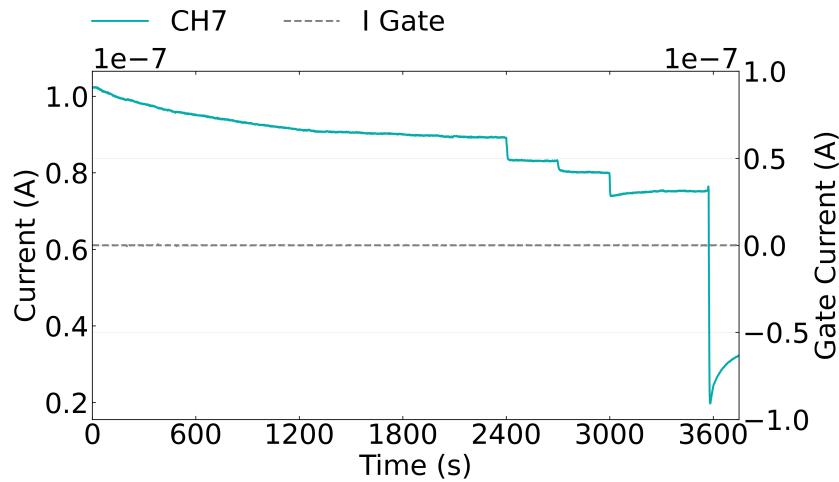


Figure 4: The control series (0–1800 s) and ethyl hexanoate sensing series (1800–3900) of the OR22a-functionalised device channel. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. Functionalisation and sensing was performed using the methods developed in this thesis by Danica Fontein, School of Chemical and Physical Sciences, Te Herenga Waka – Victoria University of Wellington.

The control series for the sensing series is shown in Figure 5 (a). No clear stepwise response is seen to PBS additions or subtractions. The functionalised device shows similar baseline drift behaviour to that of a pristine device, with a period of short-term decay quickly yielding to a more long-lived decay behaviour. A linear fit $I = c_1 t + c_2$ to the region 1200 – 1800 s had a

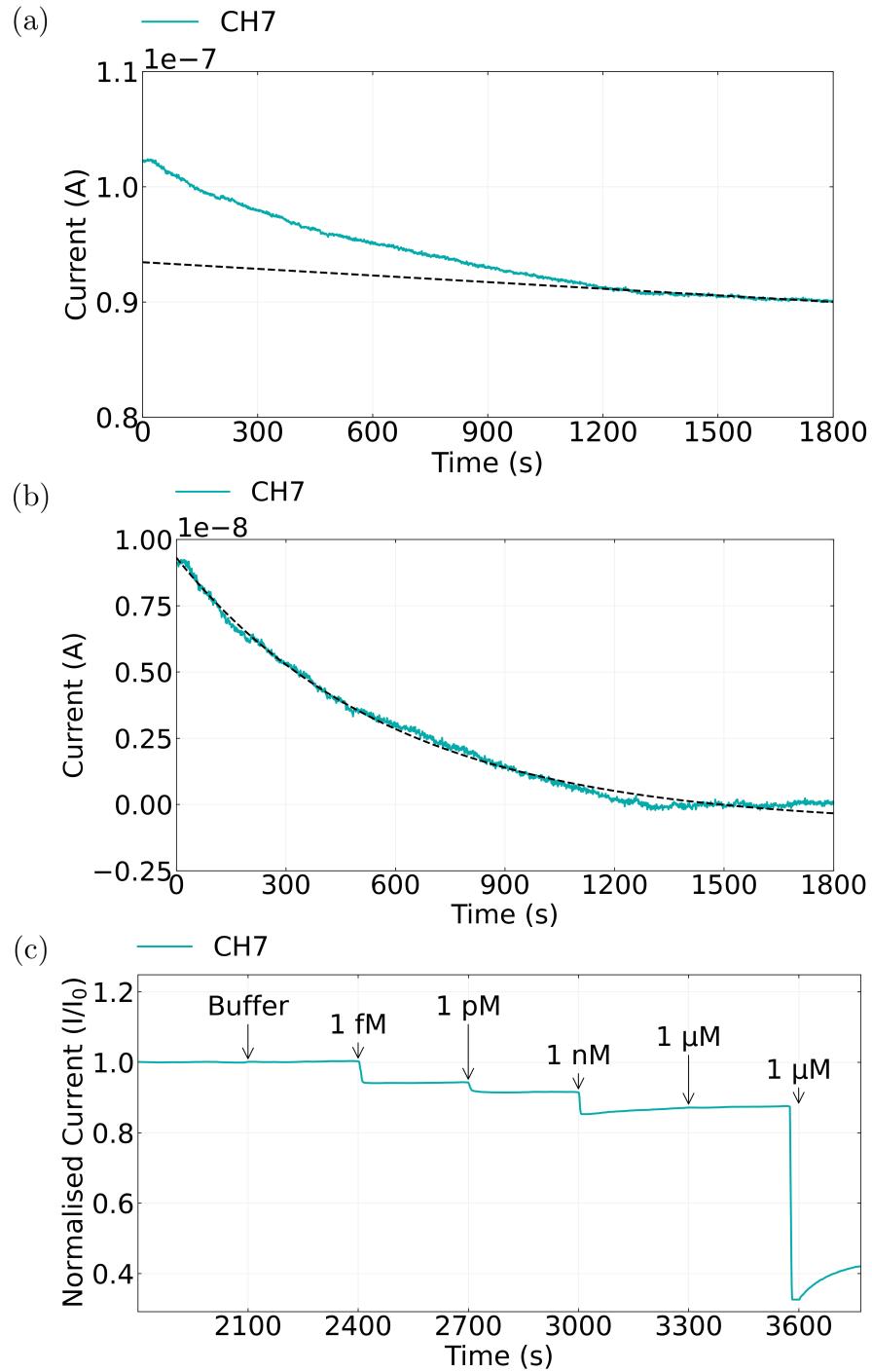


Figure 5: The control series of the OR22a-functionalised device is shown in (a) alongside an extrapolated linear fit from 1200 s onwards. The control series with the linear approximation subtracted is shown in (b), where an exponential curve has been fitted. The normalised ethyl hexanoate sensing series is shown in (c) with baseline drift subtracted and a moving median filter applied. The concentration of each 20 μ L addition is indicated above the time of addition. Across both the control series and sensing series, $V_{ds} = 100$ mV and $V_g = 0$ V.

gradient of $c_1 = -1.76 \pm 0.02$ pA/s. This gradient is smaller than the range of values found for the linear fit approximating the longer-term drift of a pristine device ([?@sec-baseline-drift](#)), but of the same order of magnitude. The linear fit was then subtracted from the control series and an exponential fit $I = I_0 \exp(-t/\tau)$ was performed on the remaining dataset from 0-1200 s, as shown in Figure 5 (b). A value of 660 ± 10 s was found for the exponential time constant, similar to those found for the channels of the pristine device. This confirms that the 1800 s control series is sufficient to avoid the presence of short-term decay during sensing.

Figure 5 (c) shows the cleaned and filtered ethyl hexanoate sensing data from the OR22a-functionalised device from 1800 s onwards. The concentration of each 20 μL addition is indicated above the corresponding addition time. The actual concentration in the liquid-gate well is determined by both the concentration of analyte being added and the volume of 1X PBS 0.5% v/v DMSO already present. The concentration of ethyl hexanoate in the well after each addition is shown in Table 2. With each addition of ethyl hexanoate, a subsequent rapid drop in source-drain current across the channel was observed. This current decrease appears irreversible, as the current stabilises after each addition at a lower current level than prior to the addition. As in previous works, all responses were observed without requiring an ORCO coreceptor present [[@Murugathas2019a](#); [@Murugathas2020](#); [@Khadka2019](#); [@Cheema2021](#)].

Table 2: Comparison of ethyl hexanoate analyte in the 20 μL additions to the total ethyl hexanoate concentration in the liquid-gate well, alongside the volume in the PDMS well after each addition.

Addition #	1	2	3	4	5	6
Addition conc.	0 fM	1 fM	1 pM	1 nM	1 μM	1 μM
Volume in well	100 μL	120 μL	140 μL	160 μL	180 μL	200 μL
Conc. in well	0 fM	170 aM	140 fM	130 pM	110 nM	200 nM

The relationship between ethyl hexanoate concentration in the sensing well and the signal responses of the OR22a-functionalised device are shown in Figure 6. Both mammalian and insect odorant receptors show a logarithmic electrical response to low concentrations of target analyte, while responses saturate at higher analyte concentrations [[@Persaud1982](#); [@Jin2012](#); [@Kwon2015](#); [@Yoo2022](#); [@Khadka2019](#); [@Murugathas2020](#); [@Cheema2021](#)]. The response behaviour with concentration across the first five datapoints has the shape of a Langmuir response curve [[@Jin2012](#); [@Kwon2015](#); [@Yoo2022](#)], indicating that the interaction between analyte and the monolayer of immobilised odorant receptors forms a similar equilibrium to that of the dynamic equilibrium seen for adsorption and desorption from a adsorbent surface [[@Ayawei2017](#)]. The responses by OR22a to both methyl hexanoate and ethyl hexanoate on different sensing platforms follow a similar trend, where saturation occurs at high picomolar concentrations [[@Murugathas2020](#); [@Cheema2021](#)]. Due to the low number of datapoints in Figure 6, comparing this dataset to a physical model directly is infeasible, but the first five measurements do not conflict with previous observations of a logarithmic trend that gives way

to saturation for insect odorant receptor sensors.

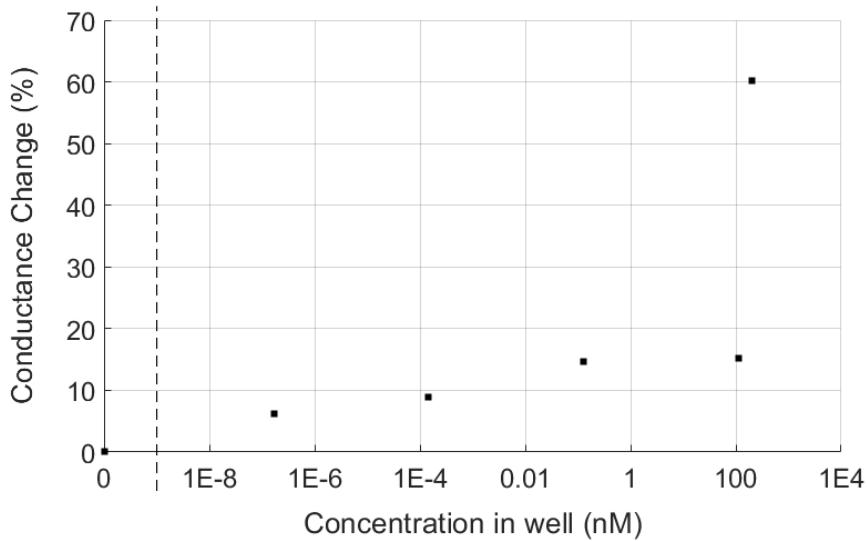


Figure 6: Cumulative device response corresponding to the 20 μL analyte additions relative to the ethyl hexanoate (EtHex) concentration present in the device liquid-gate.

However, the final addition resulted in an unexpectedly large response of $\sim 45\%$ which dramatically deviates from this well-established trend. This large response occurs when the concentration of ethyl hexanoate in the well is approximately doubled from $\sim 100 \text{ nM}$ to $\sim 200 \text{ nM}$. The size of this response well exceeds any error due to insufficient mixing in the PDMS, which is usually on the scale of $\sim 1\%$ ([?@sec-salt-conc-series](#)). The analysis in the subsequent section (Section) demonstrates that this response cannot be explained as the carbon nanotubes (or nanodiscs) responding to a relatively large concentration of ethyl hexanoate directly. No significant changes in gate current occur during the sensing series (Figure 4), so the response cannot be explained as being due to changes in the gate capacitance due to leakage current. The responses of OR22a to target analyte at liquid-gate concentrations above 1 nM have not previously been investigated for field-effect transistor sensors [[@Murugathas2019a](#); [@Murugathas2020](#)]. More research may be required to understand device behaviour at analyte concentrations well above the apparent saturation regime at high picomolar concentrations.

Addressing Biosensor Variability

Variability in Biosensor Behaviour

Despite the successful detection of ethyl hexanoate by an OR22a nanodisc-functionalised biosensor in Section , it was found that this behaviour was not readily reproducible. The

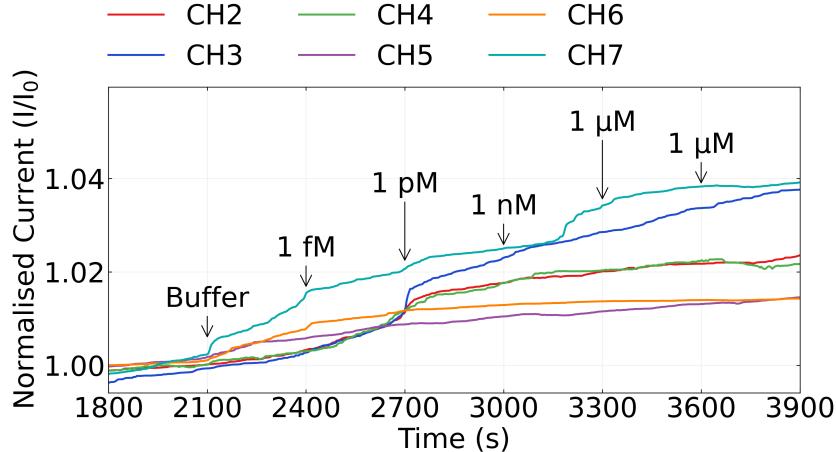


Figure 7: The normalised sensing series of another OR22a-functionalised device across six multiplexed channels, where current data has been despiked, baseline drift removed and a moving median filter applied. The concentration of each $20\text{ }\mu\text{L}$ addition is indicated above the time of addition.

results from the previous section were not repeated when using the same procedure for fabrication of devices alongside an identical functionalisation process with the same batch of OR22a nanodiscs (ND-OR22a-SB018). The ethyl hexanoate sensing sequence from six functionalised device channels is shown in Figure 7. The current response to each analyte addition is similar to that seen after the initial addition without ethyl hexanoate present. The largest contributing factor to current change appears to be drift. Unlike the clear decreases in current subsequent to ethyl hexanoate additions seen in Figure 5 (c), no decreases are seen in Figure 7 to any ethyl hexanoate solution addition. It is clear that although the same process has been followed here as in Section , the functionalised device is not responding to the presence of target analyte. The device characteristics were therefore investigated for differences from the functionalisation results seen in Section .

Liquid-gated electrical characteristics were taken of each sensing channel from this device before and after functionalisation with OR22a nanodiscs, in the same manner as in Section . These characteristics are shown in Figure 8. The average threshold shift was -0.06 ± 0.02 , the same as that of a device functionalised with PBASE in methanol without subsequent functionalisation with OR22a nanodiscs. To test whether protein was present on the channel, an atomic force microscope image was taken of channel 6. The resulting image is shown in Figure 9 (a) with the mean substrate height ($\sim 2\text{ nm}$) masked in green. A binary representation of the image was also prepared with a 12 nm threshold, shown in Figure 9 (c). As in Figure 2 (f), the surface is densely populated with non-nanotube features over 50 nm across. These features also form clusters across the network. Nanodisc agglomerates $\sim 29 - 37\text{ nm}$ in height are therefore present on channel 6 (following the discussion in Section), despite the lack of a significant threshold shift subsequent to functionalisation. The agglomerates are still

relatively small compared to those reported by Murugathas *et al.*, so the large size of nanodiscs in Figure 9 relative to those in Section 3 is unlikely to be an issue [Murugathas2019a].

Both OR nanodisc and empty nanodisc attachment via PBASE have been shown to cause significant gating of the network (Section 3). The amine group on proteins can be attached directly onto carbon nanotubes by adsorption, although this attachment is relatively weak [Bradley2004]. Figure 9 (b) shows an AFM image of a carbon nanotube network film after submersion in a 10 $\mu\text{L}/\text{mL}$ OR22a nanodisc in PBS solution for 1 hour (batch NDOR22a-0016-1), without prior exposure to PBASE in methanol. A ~ 2 nm high image artifact (see ?@sec-pristine-AFM) is present in the lower left of the image, bounded in red, and so the median substrate height of the image is found at ~ 4 nm, highlighted green in Figure 9 (b). A binary representation of this AFM at with a threshold height of 14 nm is shown in Figure 9 (d), where nanodisc agglomerations are clearly visible. Therefore, it might be reasoned that the lack of a gating effect even while nanodiscs are present results from a direct attachment mechanism which circumvents the PBASE linker.

Figure 10 (a) shows the sensing results from the device functionalised without the use of PBASE. Small, positive current responses to additions of ethyl hexanoate diluted in 1% v/v DMSO 1 \times PBS are visible. These responses may result from the weakly attached OR22a nanodiscs being mechanically removed by the pressure of each addition on the device channels. While the sensing response shows similarities with that seen in Figure 7, the electrical characteristics of the device channel are starkly different to those in Figure 8. These channel characteristics are shown in Figure 10 (b). The negative threshold shift was -0.27 V, far larger than any seen in Figure 8 and similar in size to the threshold shift after functionalisation seen for the working biosensor in Figure 1. Despite the difference in attachment mechanism, there is still significant electrical modification of the channel by the nanodiscs not seen for the unresponsive device in Figure 7. It appears that attachment by mechanisms other than PBASE cannot sufficiently explain the variability in sensor behaviour.

A new explanation is therefore required for this apparently contradictory scenario where nanodiscs can be present on a device channel without the observation of significant gating effects post-functionalisation. The most straightforward explanation is that no reliable correlation exists between the two phenomena. Given the consistent threshold shift results for various linker functionalisations seen in ?@sec-noncovalent-functionalisation, this scenario implies a significant variation in protein structure and charge behaviour within a single nanodisc batch, which seems unlikely. Another possibility is that some type of surface coating is causing nanodiscs to not attach to either PBASE or the carbon nanotubes. This surface coating might be attractive, attaching to both nanodiscs and carbon nanotubes, but forming a barrier layer between the two. Alternatively, this coating might be repulsive, causing nanodiscs to attach weakly to the substrate around the carbon nanotubes and not the nanotubes themselves. Variations in the degree to which a network is coated may then explain why the same functionalisation method might work for one device but not another. The following section investigates ways of eliminating possible sources of surface coating for more reliable functionalisation results.

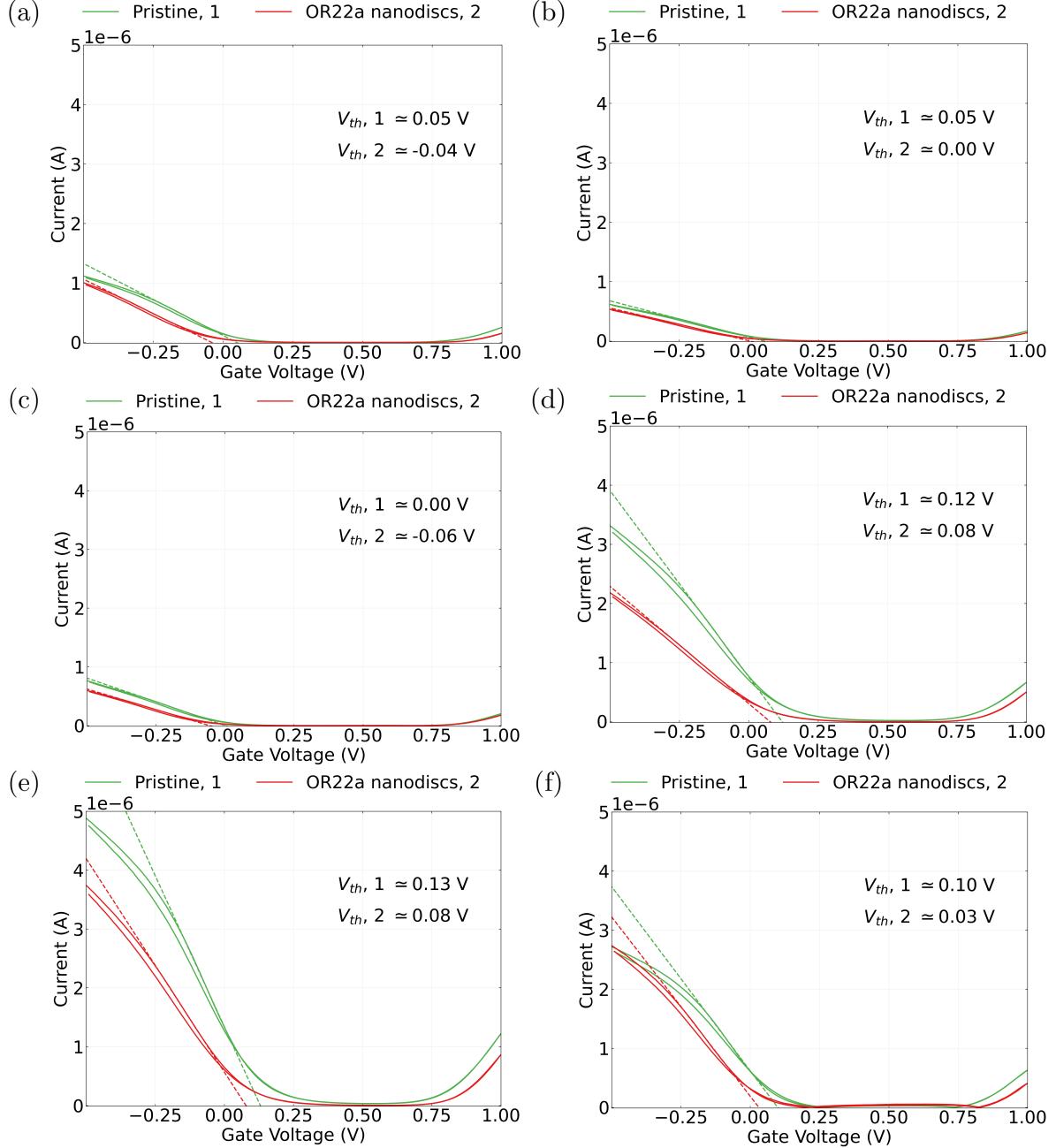


Figure 8: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain voltage was $V_{ds} = 100$ mV for both the forward and reverse sweep. Each subfigure (a)-(f) corresponds to a different channel of the functionalised device; (a) corresponds to channel 2, (b) corresponds to channel 3, (c) corresponds to channel 4, (d) corresponds to channel 5, (e) corresponds to channel 6 and (f) corresponds to channel 7. The dashed line shown is tangent to the subthreshold slope of the characteristic curve in the forward direction. The threshold voltage corresponding to the intercept of this slope with the x-axis is shown for each transfer characteristic curve.

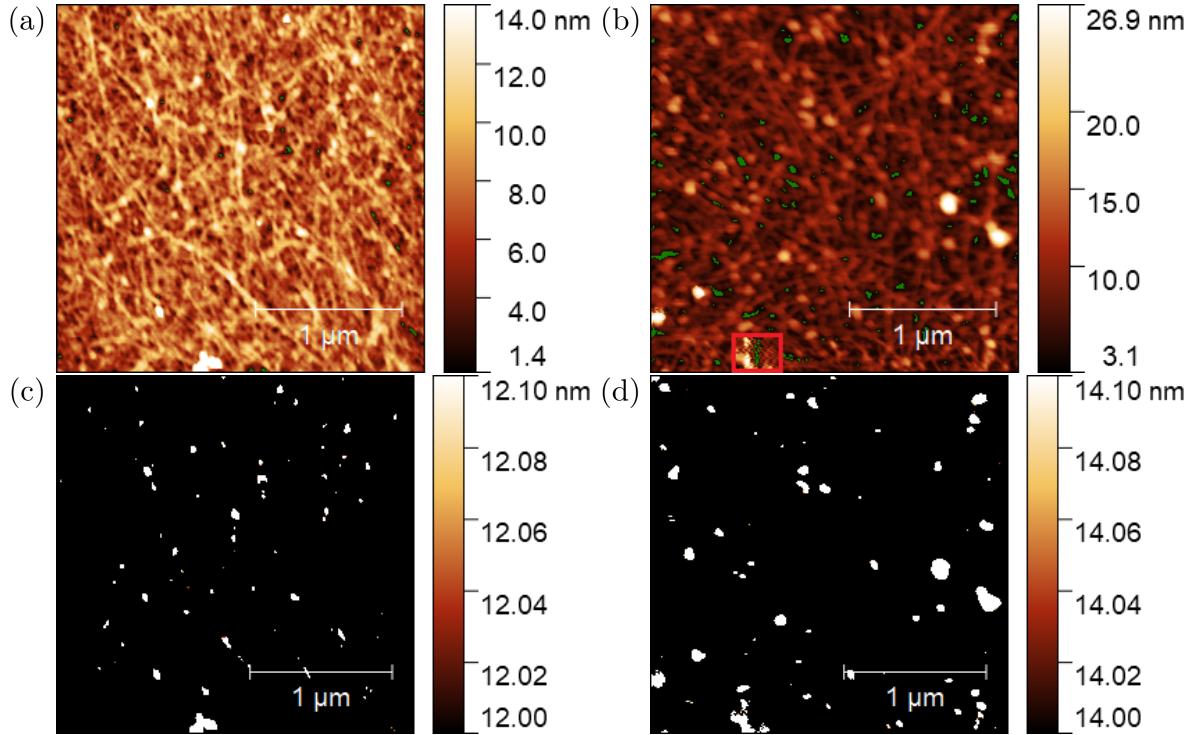


Figure 9: An scaled atomic force microscope image of channel 6 from the unresponsive device is shown in (a). An atomic force microscope image of a device modified with OR22a nanodiscs without the use of PBASE is shown in (b), with an imaging artifact bounded in red. A binary representation of the atomic force microscope image in (a) with a threshold height of 12 nm is shown in (c); a binary representation of the atomic force microscope image in (b) is shown in (d), with a threshold height of 14 nm. In (a) and (b), the average substrate height has been highlighted green with the Gwyddion software mask tool.

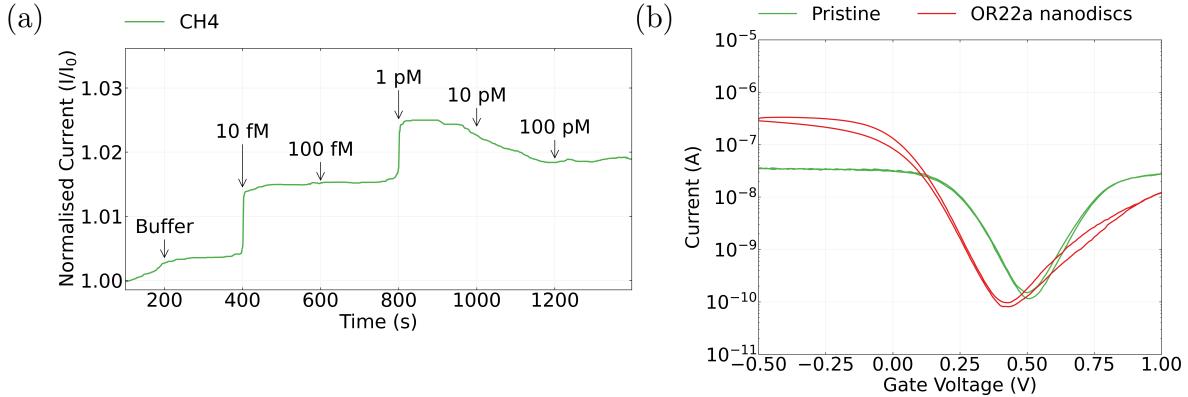


Figure 10: Real-time sampling using channel 4 of the device modified with OR22a nanodisc solution without prior exposure to PBASE or methanol shown in (a). A 20 μ L addition of 1% v/v DMSO 1 \times PBS was made at 200 s. Subsequently, 20 μ L additions of ethyl hexanoate diluted in 1% v/v DMSO 1 \times PBS were made at 400 s, 600 s, 800 s and 1000 s and 1200 s, with the concentration of each addition indicated above the time of addition. Liquid-gated carbon nanotube network device transfer characteristics of the same channel are shown in (b), before and after functionalisation. Source-drain voltage was $V_{ds} = 100$ mV.

Potential Sources of Variability

Throughout the course of this thesis, multiple potential candidates for the unwanted surface coating discussed in the previous section have been identified. These include the surfactant used in carbon nanotube deposition ([?@sec-pristine-morphology](#), [?@sec-pristine-electrical-characterisation](#)), the solvent used in functionalisation ([?@sec-PBASE-electrical-characterisation](#)), residual photoresist ([?@sec-photoresist-contamination](#)), and the hydrophobic layer which naturally forms on graphene and carbon nanotubes in air ([?@sec-hydrophobicity](#)). Another possibility is that PBASE itself is acting as a surface coating, which could result from multilayer coverage restricting access by the odorant receptors to directly attached PBASE ([?@sec-PBASE-attachment](#)). Alternatively, PBASE may have hydrolysed into PBA prior to attachment, forming an inert surface layer upon π -stacking around the carbon nanotubes. However, no threshold shift directly attributable to PBA attachment should occur ([?@sec-PBA-characterisation](#)), and this is not what was observed when characterising the non-working device in Section . To understand which of these candidates is responsible for the significant variability in biosensor functionality, the sensing procedure was performed with slight variations on the biosensor fabrication and functionalisation procedures. In each test, an individual element of one of these procedures was altered to prevent the introduction of a specific surface coating. The biosensor was then characterised and tested to see if it would respond to its target odorant.

* Spurious Responses

Before testing to see if a surface coating was responsible for the lack of response seen to ethyl hexanoate by the device in Section , it was important to investigate the possibility the signals seen in Section were false positives. The highly sensitive device channel could credibly respond to three different rapid environmental changes occurring with each addition: the mechanical action of the addition, a difference between the 0.5% v/v DMSO in 1 \times PBS containing the analyte and the 0.5% v/v DMSO in 1 \times PBS already in the well, or a direct response to analyte. It is important to eliminate the first two possibilities to be certain that the device is responding to analyte. The control series as it stands demonstrates responses cannot be explained by mechanical action. All analyte solutions are prepared simultaneously using the same 1 \times PBS, taking care to avoid cross-contamination, so responses are unlikely to result from a difference between the 1 \times PBS in the analyte solution and the 1 \times PBS in the well. It then must be verified whether a functionalised device channel will respond to a change in DMSO concentration, which is the result of the DMSO concentration in an analyte addition being different to the concentration in the PDMS well.

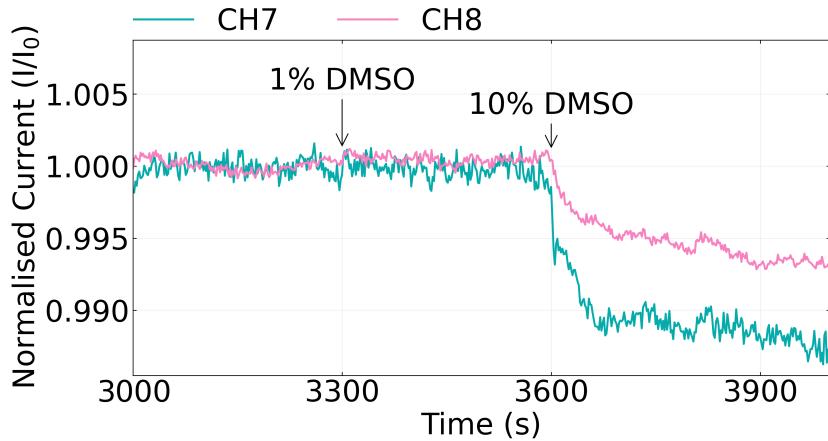


Figure 11: Response to changing the concentration of DMSO in the PDMS well of a OR22a-functionalised sensor. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. The well originally contained 100 μ L of 1% v/v DMSO 1X PBS. 20 μ L additions of DMSO in PBS were made at 3300 s and 3600 s.

Figure 11 shows that a increase in DMSO concentration in the well from 1% v/v to 2.5% v/v leads to a steep drop in current of 0.5% – 1.5% across two OR22a-functionalised device channels, significantly smaller than the >2% changes seen in Section . Furthermore, as precise measurements were used during analyte preparation, a change in proportion of DMSO in the well of this scale during a sensing sequence is unlikely. It therefore seems improbable that a change in DMSO concentration is causing the sensing responses in Section . However, the author recommends a slight modification to the control series used earlier for a more robust experimental baseline in future works. For a device with 100 μ L 0.5% v/v DMSO 1 \times PBS in the well after the control addition at 100 s, a subsequent 20 μ L addition of 1% v/v DMSO 1 \times

PBS at 200 s and 20 μ L addition of 0% v/v DMSO 1 \times PBS addition at 300 s can be used to check for spurious responses due to changing DMSO concentration, while ensuring the DMSO concentration in the well is still 0.5% v/v after the control series.

* Fabrication

Two different approaches were trialled to eliminate possible surfactant contamination, both of which drew heavily on previous methods used for iOR biosensor fabrication [@Murugathas2019a; @Murugathas2020]. Solvent-deposited carbon nanotube network and graphene devices were fabricated as described in ?@sec-qw-processing. The same functionalisation process for each device was used as described in Section with OR22a nanodiscs.

The normalised sensing behaviour for the solvent-deposited carbon nanotube device is shown in Figure 12. Gate current remained negligible across this time period (<0.1% of average drain current). No current change subsequent to each addition exceeded 2%, and current changes appear negligible on the scale of the changes seen in Section . Deviations from linear drift dominate the change in current across the sensing run, especially for channels 2 and 3. Figure 14 shows that the average threshold shift from functionalisation was -0.02 ± 0.01 V, which indicates attachment of PBASE but not nanodiscs. As the pre-2023 encapsulation mask was used, an AFM was taken of a separate film modified in the same manner before and after functionalisation shown in Figure 15 (a) and (b) respectively. Attached nanodisc aggregations are clearly visible. Rather than appearing similar to the atomic force microscope images of an OR22a-functionalised highly-bundled morphology in the literature, the functionalised film image appears closer in nature to those observed for the OR35a-functionalised and empty nanodisc functionalised highly-bundled film [@Murugathas2019a]. Figure 15 (c) and (d) show that over a larger scale these aggregations can be over 300 nm tall, forming large galaxy-like clusters which indicate mutual interaction during deposition. These results lend further support to the relationship between network morphology and vertical aggregation of nanodiscs, as discussed in Section .

The electrical characteristics of a graphene device channel before and after functionalisation with OR22a nanodiscs in the manner outlined previously are shown in Figure 13 (a), showing a significant decrease in on-current and a negatively shifted Dirac point with functionalisation, as seen previously for working OR22a nanodisc GFET biosensors [@Murugathas2020]. Figure 13 (b) shows when the process is repeated on another device without initial exposure to PBASE and methanol, a larger negative Dirac point shift is observed. It appears the initial functionalisation with PBASE and methanol decreases the charge transferred to the graphene when the nanodiscs are introduced; the difference between these two surface modifications is analogous to that seen for the carbon nanotube devices in Section . These results are a further indication that excess PBASE inhibits electrical interaction between the proteins and transistor channel. A pristine graphene surface and a OR22a-functionalised graphene surface are shown in Figure 15 (e) and (f) respectively. Graphene folds of approximately 5 nm in height are visible on the right hand side of Figure 15 (e). When functionalised with OR22a

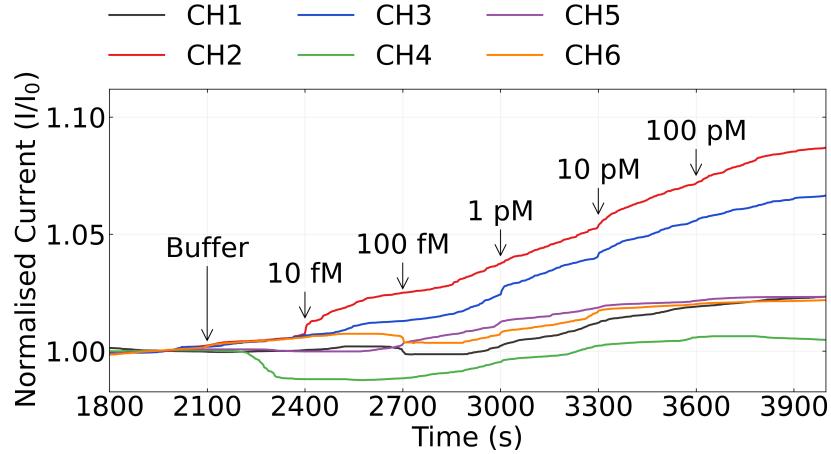


Figure 12: The normalised sensing series from the solvent-deposited, OR22a-functionalised device across six multiplexed channels, $V_{ds} = 100$ mV and $V_g = 0$ V, where current data has been despiked, baseline drift removed and a moving median filter applied.

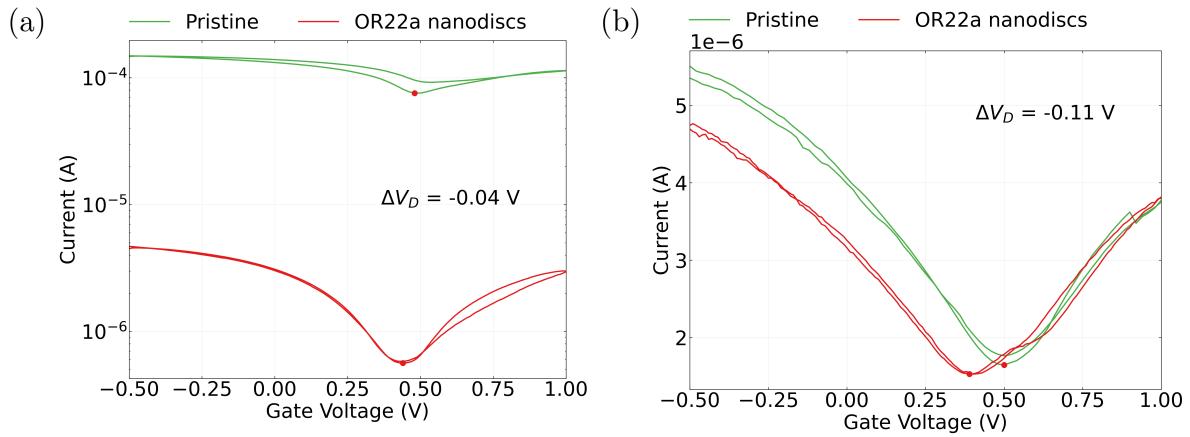


Figure 13: Liquid-gated graphene device transfer characteristics showing Dirac point voltage before and after OR22a nanodisc functionalisation, where (a) was functionalised using the standard method while (b) was functionalised without submerging the device in PBASE and methanol. Source-drain voltage was $V_{ds} = 100$ mV for both forward and reverse sweeps.

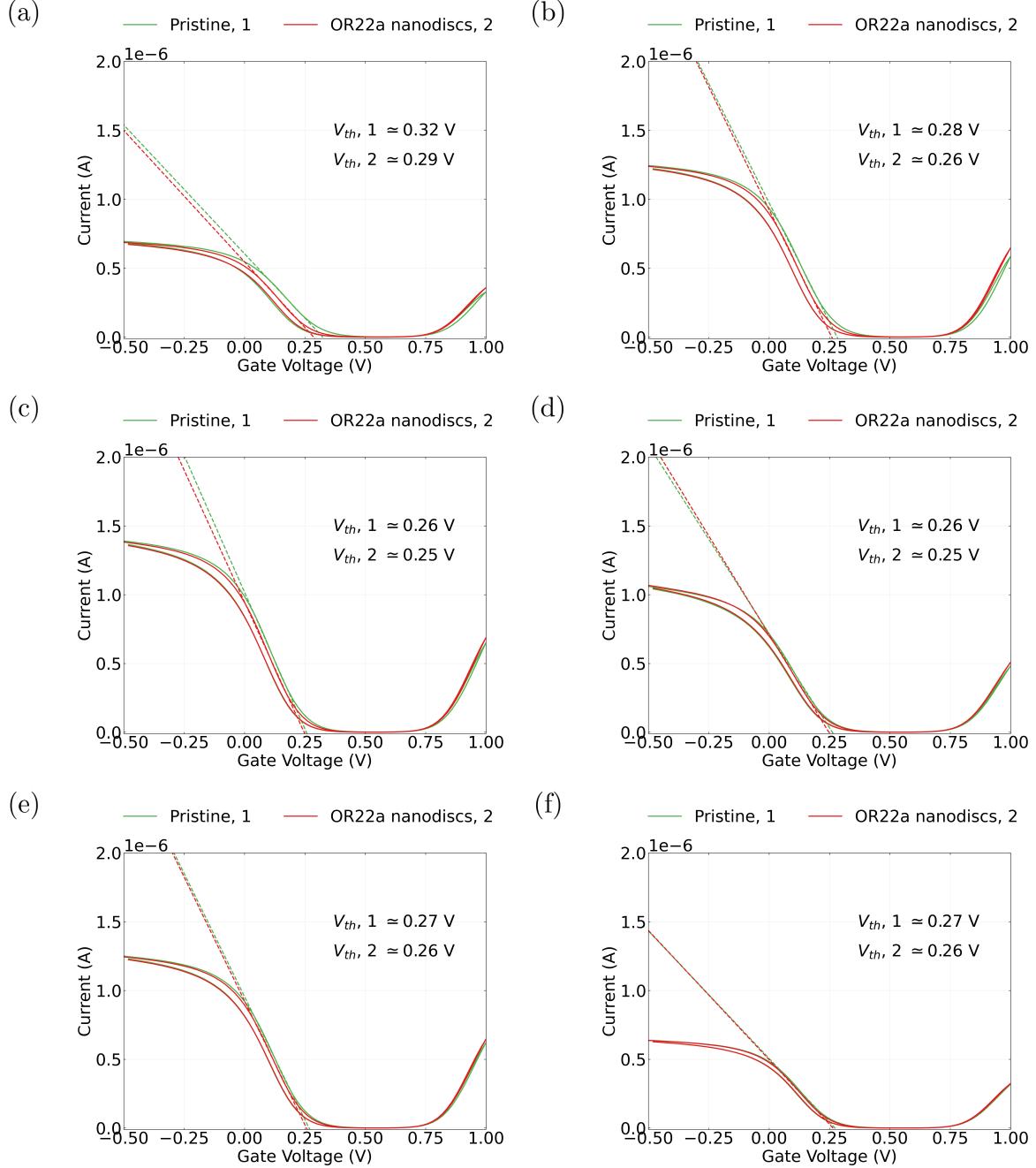


Figure 14: Liquid-gated solvent-deposited carbon nanotube device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain voltage was $V_{ds} = 100$ mV for both the forward and reverse sweep. (a) corresponds to channel 2, (b) corresponds to channel 3, (c) corresponds to channel 4, (d) corresponds to channel 5, (e) corresponds to channel 6 and (f) corresponds to channel 7. The dashed line shown is tangent to the subthreshold slope of the characteristic curve. The threshold voltage corresponding to the intercept of this slope with the x-axis is shown for each transfer characteristic curve.

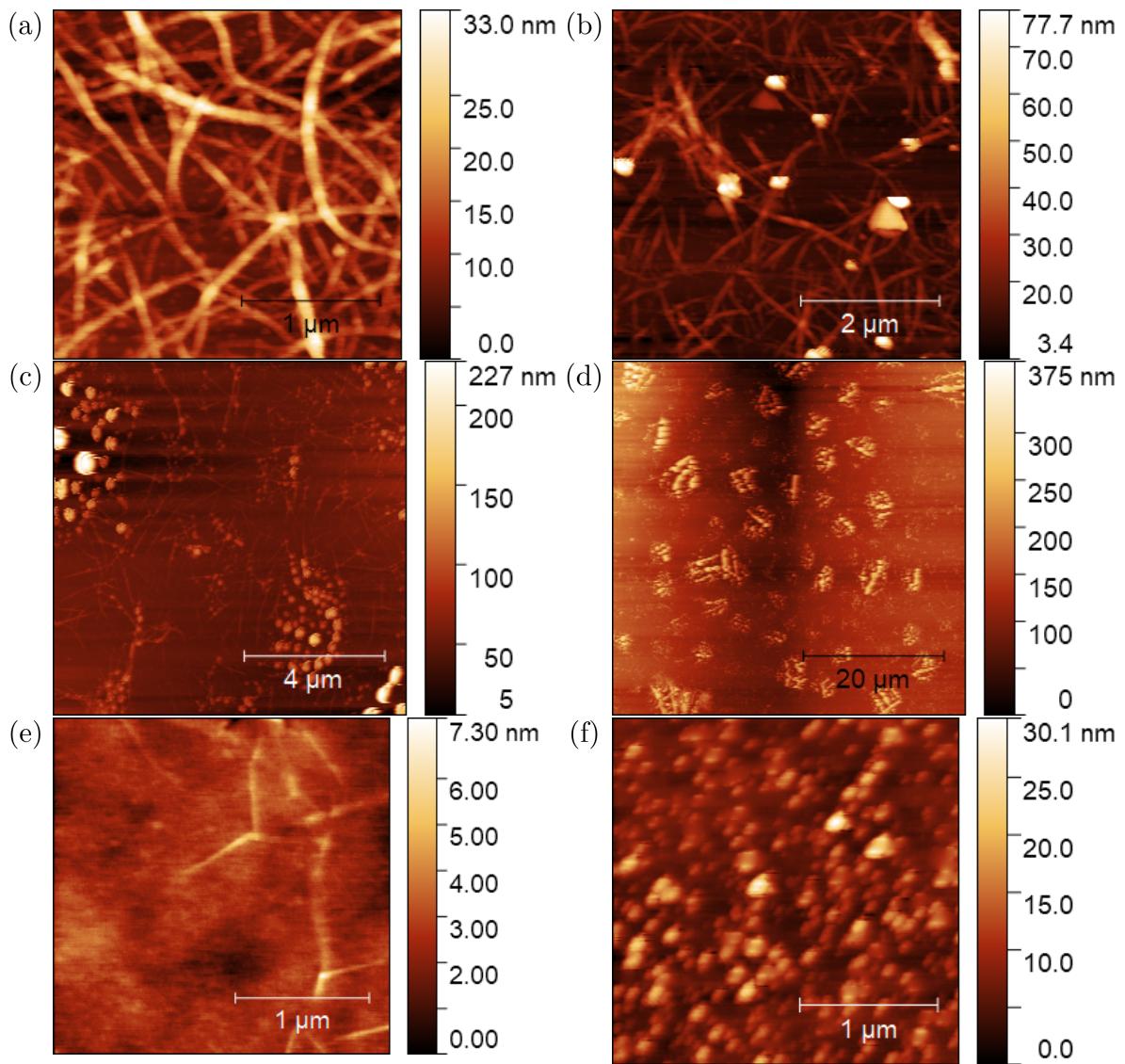


Figure 15: An $2.5 \mu\text{m} \times 2.5 \mu\text{m}$ atomic force microscope image of a solvent-deposited carbon nanotube film before functionalisation is shown in (a), and another image of the same film after OR22a nanodisc functionalisation is shown in (b). $10 \mu\text{m} \times 10 \mu\text{m}$ and $50 \mu\text{m} \times 50 \mu\text{m}$ atomic force microscope images of another film functionalised in the same manner are shown in (c) and (d) respectively. Atomic force microscope images of a monolayer graphene film on SiO₂ are also shown, before (e) and after (f) OR22a-nanodisc functionalisation.

nanodiscs, the surface is densely coated with nanodisc aggregates up to ~ 250 nm across and ~ 30 nm tall, similar in size to those observed by Murugathas *et al.* [@Murugathas2020].

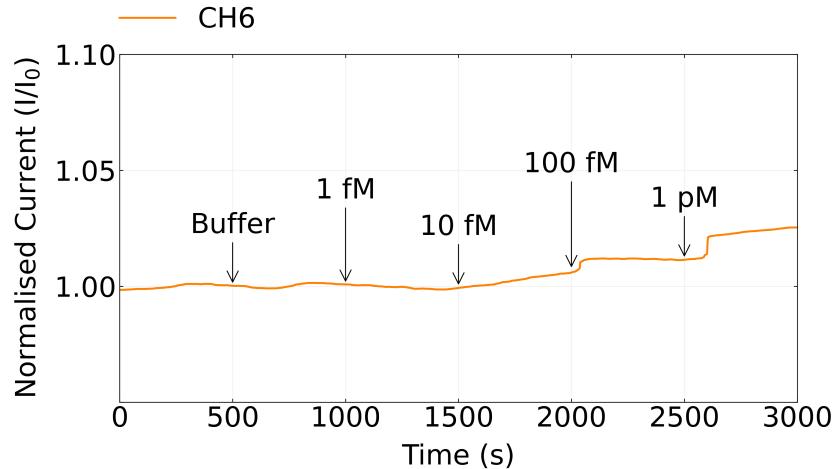


Figure 16: A normalised ethyl hexanoate sensing series taken with a OR22a-functionalised graphene device. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. The concentration of each 20 μL addition is indicated above the time of addition, with additions made at 500 s, 1000 s, 1500 s, 2000 s and 2500 s.

Both the transfer characteristics and atomic force microscope image indicates widespread attachment of nanodiscs. However, the normalised current data from a OR22a nanodisc graphene field-effect transistor in Figure 16 shows current changes of less than 1% in response to ethyl hexanoate additions. This contrasts with the OR22a nanodisc-functionalised graphene device behaviour observed by Murugathas *et al.*, where target analyte additions with concentration above 10 fM consistently caused current changes of above 2% [@Murugathas2020]. Again, there appears to be an unresolved functionalisation issue that is not clearly captured either by the atomic force microscope images or the transfer characteristics before and after functionalisation of the graphene devices. It appears that avoiding the use of surfactant on the transducer element is not sufficient to ensure consistent iOR biosensor functionalisation; it therefore seems likely that a surfactant coating is not the primary reason for the observed variability in device operation. Furthermore, the confound appears to persist across multiple transducer morphologies which show significant variations in both their active surface area and electrical properties.

* Functionalisation

Next, an investigation was made into whether the type of solvent used in functionalisation was responsible for the variability observed in device behaviour. A solvent commonly used in the literature for functionalisation with PBASE is dimethyl sulfoxide (DMSO), as discussed in

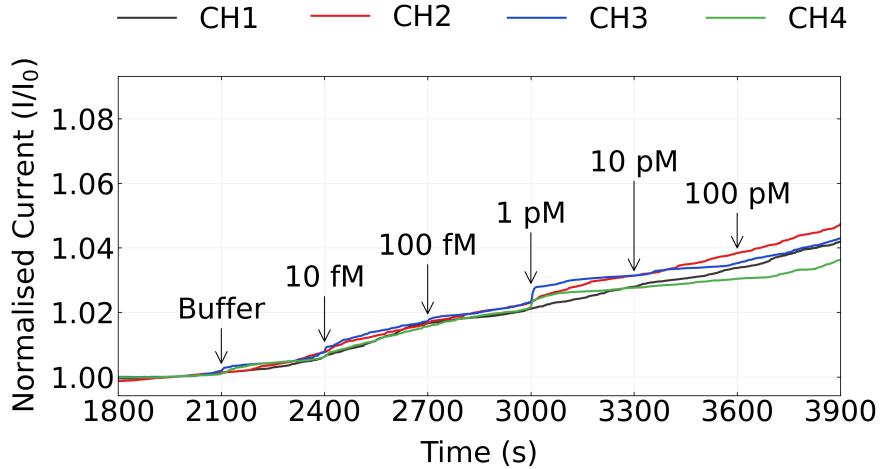


Figure 17: A normalised sensing series across four multiplexed channels from a device functionalised with PBASE in DMSO used to attach OR22a nanodiscs. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. The concentration of each 20 μL addition of methyl hexanoate in 1% v/v DMSO 1× PBS is indicated above the time of addition. Current data has been despiked, baseline drift removed and a moving median filter applied.

?@sec-PBASE-attachment. A surfactant-deposited device was functionalised with OR22a nanodiscs in the same manner as described in Section , except DMSO was used instead of methanol when preparing the PBASE solution. The sensing datasets from the four working device channels are displayed in Figure 17, where methyl hexanoate (MeHex) was used as the target compound. No channel shows a negative current response to MeHex. Current changes after each addition are consistently below 1% on all channels.

The transfer characteristics of the four sensing channels are displayed in Figure 18. An average threshold shift of -0.22 ± 0.03 V is seen across these channels, significantly exceeding the expected threshold shifts of -0.06 ± 0.01 for exposure to PBASE in DMSO and -0.15 ± 0.01 for exposure to DMSO without PBASE. This functionalisation threshold shift is similar to that of the device which responded to target analyte in Section , -0.20 V. The shift is also similar to that seen for the device functionalised directly with OR22a nanodiscs without PBASE, -0.27 V. It appears that the nanodiscs are able to interact with the carbon nanotube network, yet they do not respond to the target analyte. It seems likely that the negative gating is a result of nanodisc attachment to the network, either with or without PBASE as a linker; meanwhile, there is limited or no attachment between the network and the odorant receptors themselves. This lack of connection to the odorant receptors appears to be a second factor at play in the variability seen in biosensor behaviour.

An atomic force microscope image of a film modified using DMSO in PBASE with OR22a nanodiscs is shown in Figure 19 (a). The binary representation in Figure 19 (b) shows long,

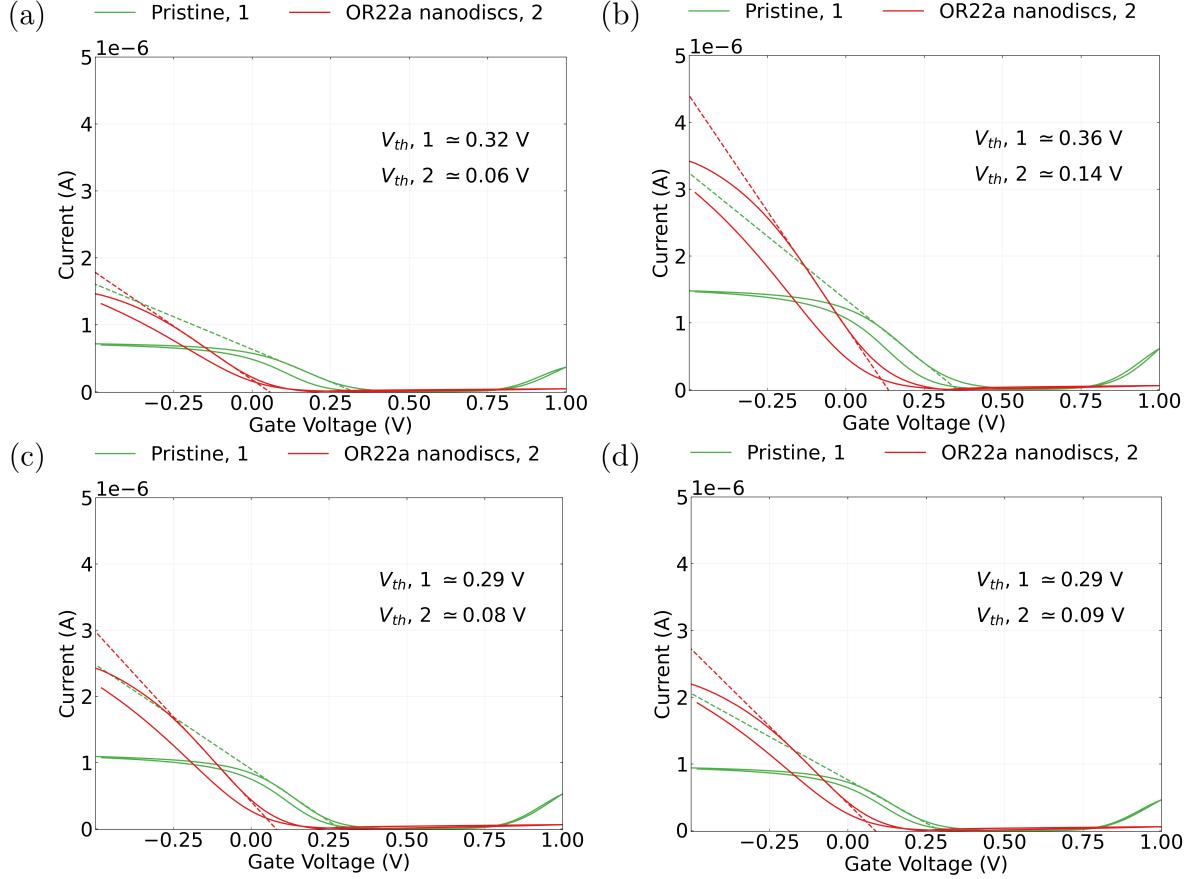


Figure 18: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation using PBASE in DMSO. Source-drain voltage was $V_{ds} = 100$ mV for both the forward and reverse sweep. Each subfigure (a)-(d) corresponds to a different channel of the functionalised device; (a) corresponds to channel 1, (b) corresponds to channel 2, (c) corresponds to channel 3, and (d) corresponds to channel 4. The dashed line shown is tangent to the subthreshold slope of the forward sweep of the characteristic curve. The threshold voltage associated with each curve is also shown.

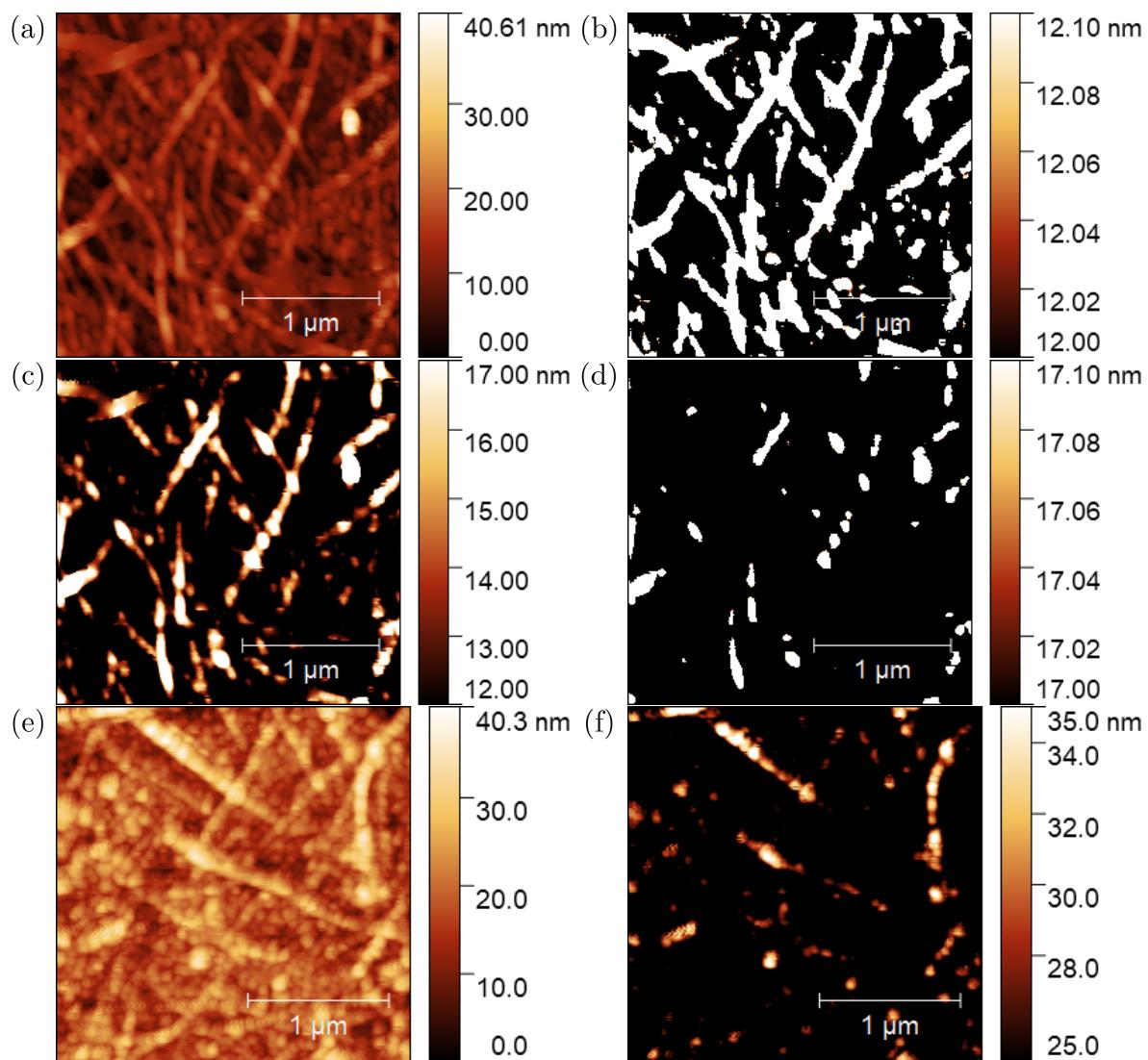


Figure 19: A $2.5 \mu\text{m} \times 2.5 \mu\text{m}$ atomic force microscope image of a carbon nanotube film functionalised with OR22a nanodiscs, using PBASE in DMSO, is shown in (a). A binary representation of the atomic force microscope image with a threshold height of 12 nm is displayed in (b). (c) shows features in the functionalised film across the height range 12 – 17 nm, while (d) shows another binary representation at 17 nm. An atomic force microscope image of a carbon nanotube film functionalised with OR22a nanodiscs, using PBA in DMSO with EDC and NHS, is shown in (e), while (f) shows the same functionalised film across the height range 25 – 35 nm.

rounded features sit directly above the above the CNT threshold height of 12 nm. By extending the threshold height range to 12 – 17 nm, it becomes apparent that these features are not simply large carbon nanotube bundles, but instead appear to be densely packed collections of nanodisc aggregates following the length of various carbon nanotube bundles. The distribution of nanodiscs along the nanotubes gives them a “string of pearls” or *Hormosira*-like appearance [NewZealandPlantConservationNetwork]. Some features are too tall for the “pearls” on the “string” to be individually distinguishable in Figure 19 (c), but Figure 19 (d) shows that by adjusting the height threshold, the rounded “pearls” along every “string” correspond to separate aggregations of nanodiscs. These images strongly suggest nanodisc aggregations are present on the network, and are more densely packed along the nanotubes than in earlier images. However, this observation does not necessarily indicate odorant receptors are interacting with the nanotube network.

As discussed in ?@sec-PBASE-purity, DMSO is a highly hygroscopic solvent. If a non-negligible amount of water is present in the DMSO during functionalisation, the PBASE is being exposed to water for more than an hour before introducing OR nanodiscs. Over this time period, the PBASE may be able to undergo ester hydrolysis [Hermanson2013-3]. To eliminate the possibility that hydrolysis has prevented the attachment of odorant receptors to PBASE on the network, an alternative method using 1-pyrenebutyric acid (PBA) in DMSO was also trialled. This method was similar to those seen in ?@sec-PBA. PBA is first attached to the carbon nanotube network, then, once the bulk of the DMSO has been rinsed off the device surface, converted to PBASE using carbodiimide (EDC) and succinimide (NHS) reagents. The PBASE is then available to attach to the OR22a nanodiscs as usual.

The functionalisation process with PBA was therefore the same as in Section , with the following steps replacing steps 3 – 4:

- A solution of 5 mM PBA (Setareh Biotech) in DMSO was prepared by fully dissolving 7 mg PBASE in 5 mL DMSO by vortex mixing at 1000 rpm.
- The device was left submerged in the 5 mM PBASE in DMSO solution for 1 hour in a parafilm-sealed container.
- A solution of 20 mM EDC and 40 mM NHS in 1× PBS was prepared by dissolving 31 mg EDC and 46 mg NHS in 10 mL 1× PBS.

Note: EDC was thawed under vacuum for 15 minutes in dark conditions before opening.

- The device was rinsed with DMSO for 15 s and 1× PBS for 15 s, then placed into the EDC/NHS solution for 30 minutes.
- The device was then rinsed with 1× PBS for 15s before OR22a nanodisc functionalisation.

These steps were designed to be broadly similar to those seen in ?@sec-PBA, with a molar excess of NHS relative to EDC to ensure full conversion of the *O*-acylisourea intermediate into PBASE. Normalised, filtered sensing series from two multiplexed channels of the functionalised

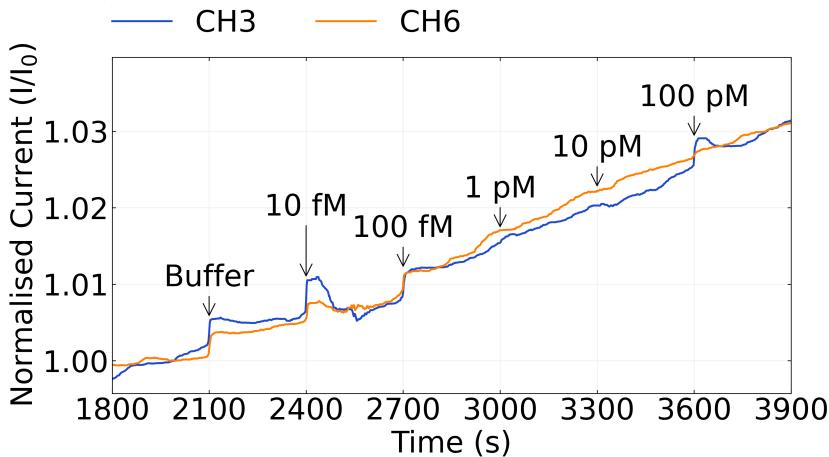


Figure 20: A normalised methyl hexanoate (MeHex) sensing series taken with a OR22a-functionalised carbon nanotube device using PBA with EDC and NHS. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. The concentration of each 20 μL addition is indicated above the time of addition.

device are shown in Figure 20. Again, any current response subsequent to additions is positive and less than 1%. An atomic force microscope image of a film modified in the same manner as the sensing device is shown in Figure 19 (e). When a suitable height range above the threshold height 12 nm is selected, as in Figure 19 (f), highly clustered nanodisc features with the appearance of pearls on a string are again visible. The transfer characteristics of each channel before and after functionalisation are shown in Figure 21, where an average threshold shift of -0.12 ± 0.01 V is observed. In [?@sec-PBA-characterisation](#), the threshold shift for PBA attachment in DMSO was -0.15 V. The difference between these two values is not large enough to convincingly conclude whether nanodiscs have attached via PBASE or not. It therefore appears that the problem identified with variability in device behaviour in Section is not resolved by the use of a different solvent or by steps to prevent PBASE hydrolysis.

From this elimination process, three possible sources of variability in device performance due to nanoscale surface contamination remain. The first is the hydrocarbonaceous layer that forms due to device exposure to air, which causes channel hydrophobicity. The second is multilayer adhesion of PBASE or PBA on the transducer surface. A further possible source of variability was also identified from the PBASE in DMSO functionalisation, where transfer measurements (Figure 18) and AFM imaging (Figure 19) each indicated that direct attachment of nanodiscs was occurring despite the device not functioning as a sensor: the possibility of minimal or no attachment of the contained odorant receptors despite nanodisc attachment. An altered non-covalent functionalisation method using pyrene-PEG-biotin was therefore developed to eliminate the confound factors identified here and investigate the resulting sensor behaviour. This novel method is trialled in the following section.

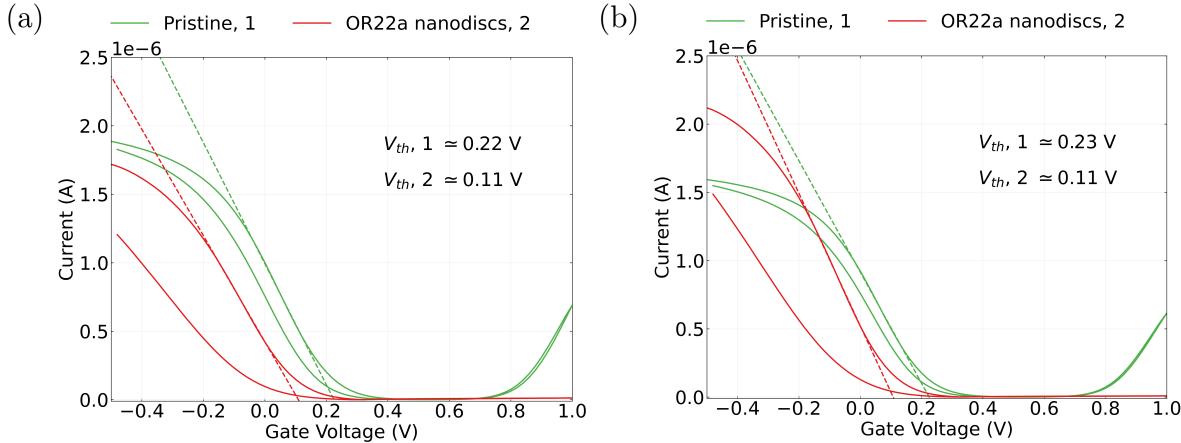


Figure 21: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation using PBA in DMSO with EDC and NHS. Source-drain voltage was $V_{ds} = 100$ mV for both the forward and reverse sweep. Each subfigure corresponds to a different device channel, where (a) is channel 3 and (b) is channel 6. The dashed line shown is tangent to the subthreshold slope of the forward sweep of the characteristic curve. The threshold voltage associated with each curve is also shown.

iOR Biosensing with Aqueous Functionalisation

Aqueous-Based Functionalisation

A carbon nanotube network field-effect transistor device, fabricated using post-Jun 2023 methods as described in ?@sec-fabrication, was functionalised with a novel method using OR10a solubilised in surfactant. The odorant receptors were not expressed in a nanodisc format to avoid nanodisc-film interaction, despite this leaving them potentially vulnerable to adverse environmental conditions [@Nath2007; @Bayburt2010]. The odorant receptors were avi-tagged, biotinylated and subsequently modified with avidin and pyrene-PEG-biotin (PPB), as described in ?@sec-NTA-biotin-PEG. This allowed the odorant receptors to attach directly from solution to the carbon nanotubes via the pyrene linker. The prior attachment of odorant receptors to linker means that this approach must be entirely performed in aqueous solution, but ensures no PBASE or PBA was needed in the functionalisation process. To eliminate the hydrocarbonaceous coating of nanotubes and ensure aqueous functionalisation was possible, a short oxygen plasma cleaning step was used.

The details of the functionalisation process, loosely based on the non-covalent functionalisation procedure used by Miki *et al.* [@Miki2019], are as follows:

1. The device was exposed to UV light for 1 minute, placed in AZ® 326 developer for 3 minutes, then rinsed with acetone, isopropanol and nitrogen dried.

2. The device was vacuum annealed for 1 hour at 150 °C.

Note: Steps 1 & 2 were added to either remove or passivate residual photoresist on the channel before functionalisation, see [?@sec-photoresist-contamination](#).

3. 10 μL surfactant-solubilised avi-tagged OR10a modified with pyrene-PEG-biotin (batch number AviHis-OR10a-001, prepared 12 months earlier) was diluted in 1 mL freshly-prepared 1 \times PBS.

Note: The full 1 mL was used to flush out the nanodisc vial when preparing the nanodisc solution, with successive additions and subtractions of 50 μL 1 \times PBS into and from the vial.

4. Device was treated with a gentle oxygen plasma, \sim 5 W at 200-300 mTorr, for 15 seconds.

Note: Oxygen plasma was very gentle, with an O₂ flow rate of <10 sccm into the plasma cleaner, to avoid excessive damage to the carbon nanotube network.

5. The device was submerged in the OR10a solution and left covered with parafilm for 15 minutes, then rinsed with 1 \times PBS for 15 s and thoroughly nitrogen dried.

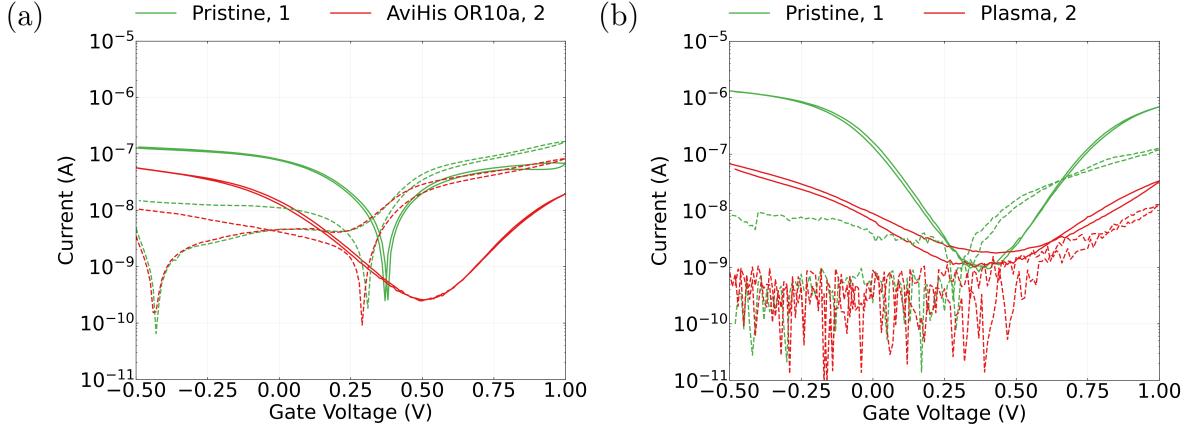


Figure 22: Liquid-gated carbon nanotube network device transfer characteristics on a logarithmic scale before and after modification with OR10a, $V_{ds} = 100$ mV, where gate current is shown with a dashed line. The change in characteristics from the functionalisation process in this section is shown in (a), while the change resulting from a 5 W plasma clean is shown in (b).

Figure 22 (a) shows the liquid-gated characteristics of a device channel before and after the functionalisation process with OR10a. The change of characteristics observed can be compared with the change of characteristics when plasma cleaned without further modification, seen in Figure 22 (b). A significant drop in channel current occurs as a result of the plasma cleaning process. This large change in mobility makes it difficult to clearly identify changes in gating

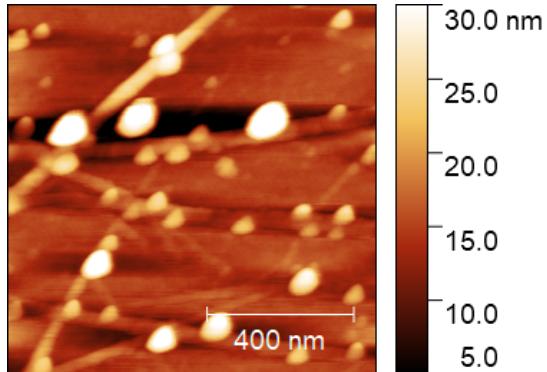


Figure 23: $1 \mu\text{m} \times 1 \mu\text{m}$ atomic force microscope image of a solvent-deposited carbon nanotube film functionalised with OR nanodiscs using Pyrene-PEG-NHS.

specifically due to the presence of the OR10a. The OR10a is not held within a nanodisc format, so the morphology of the functionalised network cannot be compared directly using previous atomic force microscope images. A solvent-deposited carbon nanotube film was prepared separately using OR nanodiscs, where the ORs were attached via their amine group using pyrene-PEG-NHS ester. Pyrene-PEG-NHS ester is very similar to PBASE but contains a PEG chain. Figure 23 shows an atomic force microscope image of the modified film. OR nanodisc aggregates very clearly follow the carbon nanotubes, indicating specific attachment between nanodiscs and carbon nanotubes can be achieved using linker containing pyrene-PEG. The presence of PEG chains may help prevent direct adsorption of proteins onto the carbon nanotubes, improving device quality and therefore sensing behaviour [@Star2003a; @Chen2004].

Aqueous Sensing of Methyl Salicylate

The procedure used for sensing methyl salicylate (MeSal) was identical to that used in Section , except using MeSal instead of EtHex. Analyte solutions in 0.5% DMSO/ $1\times$ PBS solution containing methyl salicylate concentrations at 1 fM, 1 pM, 1 nM and 1 μM were prepared beforehand. The same PBS was used for each dilution, as well as for the well, which contained 80 μL 0.5% DMSO/ $1\times$ PBS prior to sensing. Sensing measurements were taken using the NI-PXIe system. The full measurement sequence is shown in Figure 24 alongside gate current. I_g remained negligible across the full sensing procedure, and no responses were seen to PBS additions or subtractions. A linear fit to the baseline drift in the region 1200 – 1800 s, shown in Figure 25 (a), had a gradient of $c_1 = -0.38 \pm 0.01 \text{ pA/s}$, while an exponential fit to the drift minus the linear fit, shown in Figure 25 (b), had a time constant of $506 \pm 12 \text{ s}$. A deviation from the exponential fit similar to that seen for the control series in Section is observed, as observed previously.

Cleaned and filtered methyl salicylate sensing data with linear baseline drift removed is shown

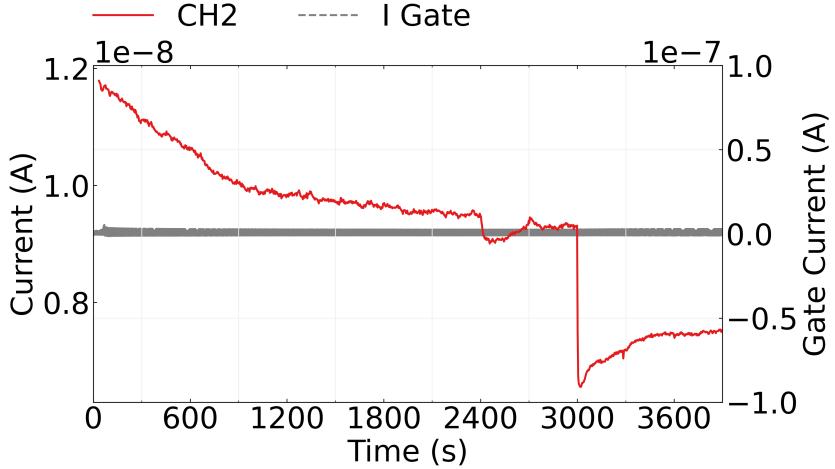


Figure 24: The control series (before 1800 s) and methyl salicylate sensing series (after 1800 s) of the OR10a-functionalised device channel. Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. No responses to 0.5% v/v DMSO 1X PBS were seen during the control series, while significant responses to additions of methyl salicylate diluted in 0.5% v/v DMSO 1X PBS were seen at 2400 s and 3000 s.

in Figure 25 (c). The concentration of each 20 μ L methyl salicylate addition is shown above each corresponding addition time. Significant and irreversible current decreases occurred directly after the 1 fM and 1 nM MeSal additions. A $\sim 3\%$ current drop occurred subsequent to the 1 fM addition and a $\sim 23\%$ current drop occurred subsequent to the 1 nM MeSal addition. No negative change in current occurred after the PBS addition, or at any of the other three analyte additions. Murugathas *et al.* also observed a similar drop-off in response with increased analyte concentration when sensing with OR10a [Murugathas2019a]. Just like the OR22a odorant receptors tested earlier, it appears that the OR10a odorant receptors saturate at the 1 nM addition, but do not show any unexplained “post-saturation” activity. Transfer characteristics of the device before and after sensing are shown in Figure 26 (a), showing a threshold shift of -0.07 V.

The device was then rinsed for 15 s in 1 \times PBS after the initial sensing series. The effect of the rinse on the transfer characteristics of the device is illustrated in Figure 26 (b). The threshold voltage before sensing was 0.16 V. The rinsing step appears to have largely restored the electrical characteristics of the device to its state prior to sensing, bringing the threshold voltage back from 0.09 V post-sensing to 0.14 V after the rinse step. In other words, it appears the gating effect due to the presence of analyte has been reversed. Assuming that the gating effect results from structural changes in the odorant receptors, the reversal of threshold shift upon rinsing indicates that without analyte present, the proteins return to their original structure. This implies that the device can be reused as a sensor. Furthermore, as adsorption of solvent leads to gating which is not reversible through rinsing (?@sec-PBASE-

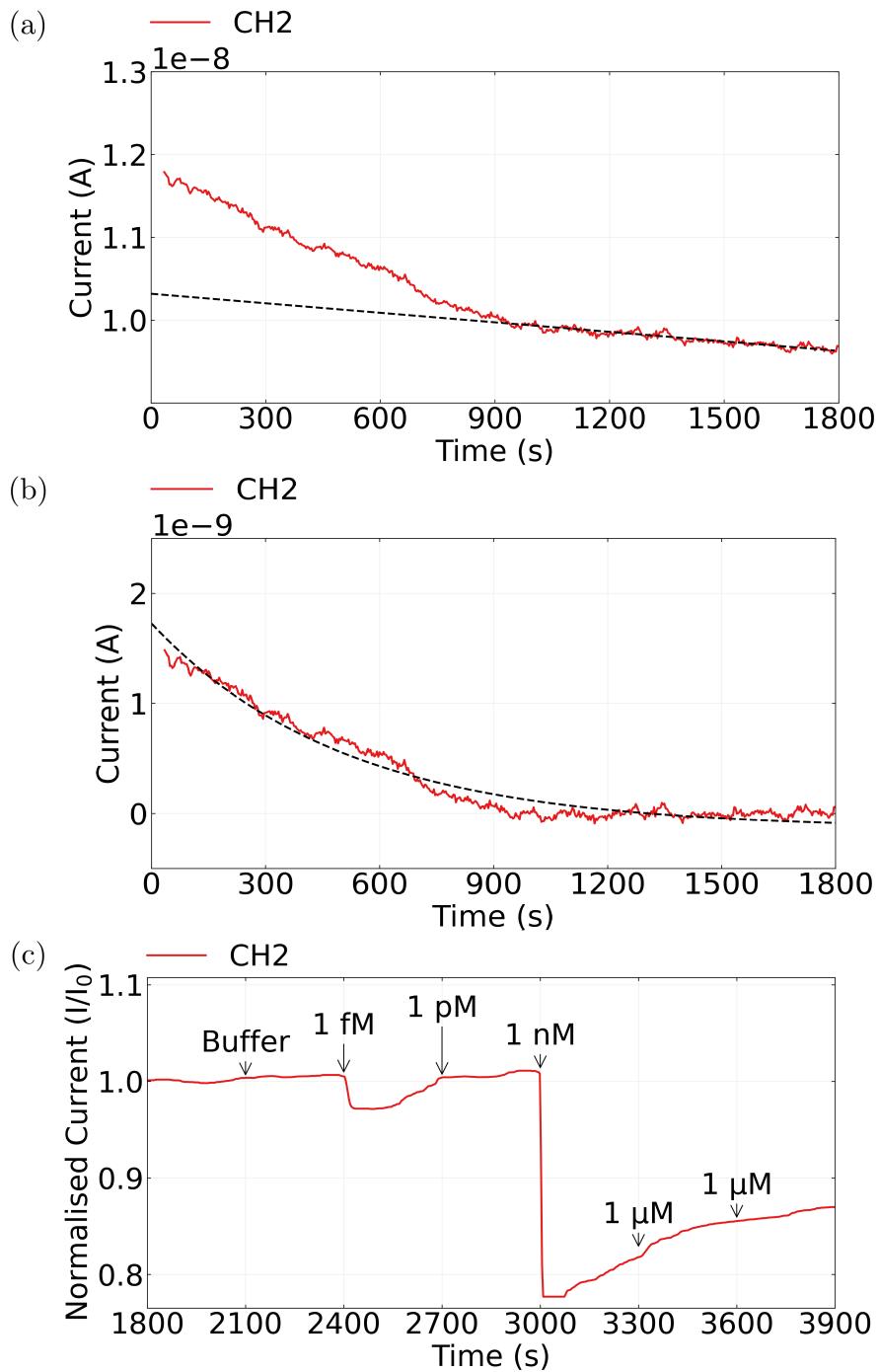


Figure 25: Control series for the OR10a-functionalised device is shown in (a) alongside a linear fit to the control series from 1200 s onwards, where the fit has been extrapolated to 0 s, shown as a black dotted line. The control series with the linear approximation subtracted is shown in (b), fitted with an exponential function shown as a black dotted line. The normalised sensing series for the OR10a-functionalised device is shown in (c), where the current data has been despiked, baseline drift subtracted and a moving median filter applied.³³

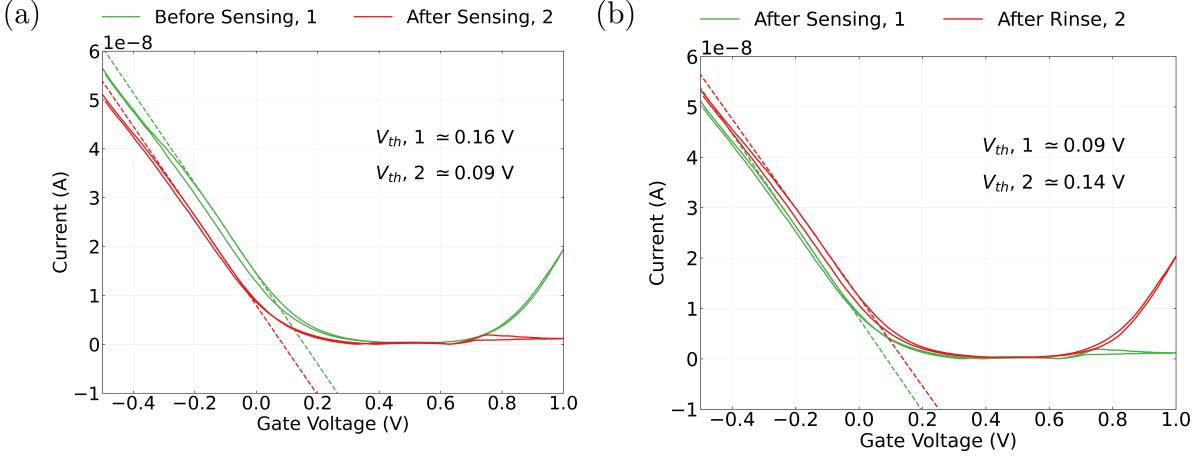


Figure 26: Transfer characteristics of the OR10a-functionalised device after the 1st sensing series, where $V_{ds} = 100$ mV. The transfer characteristics before and after sensing are shown in (a), while (b) shows the channel characteristics before and after being rinsed with $1\times$ PBS.

electrical-characterisation), this is also an indication that the responses are not simply due to adsorption of the DMSO present.

The same OR10a-functionalised device channel was then reused as a sensor subsequent to rinsing, with the full sensing run alongside gate current shown in Figure 27 (a). In this second sensing series, only 1 nM additions of methyl salicylate were made, to explore the device behaviour at concentrations close to saturation. An irreversible current response was seen directly after all five methyl salicylate additions; the concentration in the well after each addition is shown in Table 3. The expected logarithmic relationship $R = \alpha \log C$ between odorant receptor response R and low concentrations of analyte C is seen in Figure 28 (a), with a relationship constant of $\alpha = 16.8 \pm 5.7$. As the relationship holds across all additions, it appears the presence of 500 pM MeSal does not bring odorant receptors to their saturation limit. The initial 1 nM analyte addition, bringing well concentration to 170 pM, was similar in size (24%) to the current change resulting from the 1 nM analyte addition bringing MeSal concentration to 130 pM in Figure 25 (23%). This result indicates that the relationship between response size and concentration is reproducible when the sensing channel is reused.

Table 3: Comparison of methyl salicylate concentration in the $20\text{ }\mu\text{L}$ additions to the total concentration of analyte in the liquid-gate electrolyte and the volume of electrolyte in the PDMS well after each addition.

Addition #	1	2	3	4	5	6
Addition conc.	0 fM	1 nM				
Volume in well	$100\text{ }\mu\text{L}$	$120\text{ }\mu\text{L}$	$140\text{ }\mu\text{L}$	$160\text{ }\mu\text{L}$	$180\text{ }\mu\text{L}$	$200\text{ }\mu\text{L}$

Conc. in well	0 fM	170 pM	290 pM	380 pM	440 pM	500 pM
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It also appears that the second sensing series resulted in a similar shift in transfer characteristics to that of the first sensing series. Figure 28 (b) shows a shift in threshold voltage of -0.08 V between the transfer characteristics directly before and directly after the second sensing series. The similarity between Figure 26 (a) and Figure 28 (b) suggests the device gating and associated behaviour of odorant receptors was broadly similar when the sensor was used a second time, indicating a odorant receptor-functionalised device can be reused. This is surprising, given the odorant receptors lack a protective membrane. This discussion indicates that the attached odorant receptors can remain viable for at least several hours in room temperature PBS solution, without the need for the nanodisc format. The similar size of the threshold shifts seen in Figure 26 (a) and Figure 28 (b) also indicates that even though it appears the odorant receptors did not saturate with the nanomolar additions of MeSal, the saturation limit was being approached. It should be noted, however, that this discussion does not account for the impact of hysteresis and current annealing on the device transfer characteristics, both of which impact the reliability of the transfer characteristic curve as an indicator of sensing.

The functionalisation method used in this section has a number of advantages over the OR nanodisc functionalisation method outlined earlier. The plasma cleaning step eliminates uncertainty stemming from a variety of possible surface coatings on the carbon nanotubes. Using detergent-solubilised odorant receptors removes the possibility that the nanodiscs are impeding direct attachment to odorant receptors in the functionalisation procedure. However, it has its own difficulties. Oxygen plasma cleaning, even at low power, had a fairly destructive effect on the carbon nanotube network. A large drop in mobility resulted from plasma cleaning; for sparser carbon nanotube morphologies, a sizable current drop could leave a device unsuitable for sensing. Since the main advantage of non-covalent functionalisation over covalent functionalisation is its minimal impact on mobility (see [?@sec-sensor-types](#)), this approach seems contradictory. It appears a less destructive but solvent-free approach should be identified for non-covalent functionalisation [\[@Ashraf2014\]](#), or a well-established covalent approach should instead be used for attaching odorant receptors.

Conclusion

A carbon nanotube device was non-covalently functionalised using PBASE in methanol with OR22a nanodiscs and operated as a biosensor in an aqueous environment. Real-time conductance decreases of up to $\sim 45\%$ across the sensor channel were observed in response to analyte additions, which varied logarithmically low analyte concentrations. Short-term drift behaviour was found to be similar to that of pristine devices, but it appears functionalisation may affect long-term baseline drift. Other changes resulting from successful sensor functionalisation included a device threshold voltage shift of -0.20 ± 0.03 V and an increase in network height of over 15 nm, both resulting from the presence of nanodiscs on the network. Additionally,

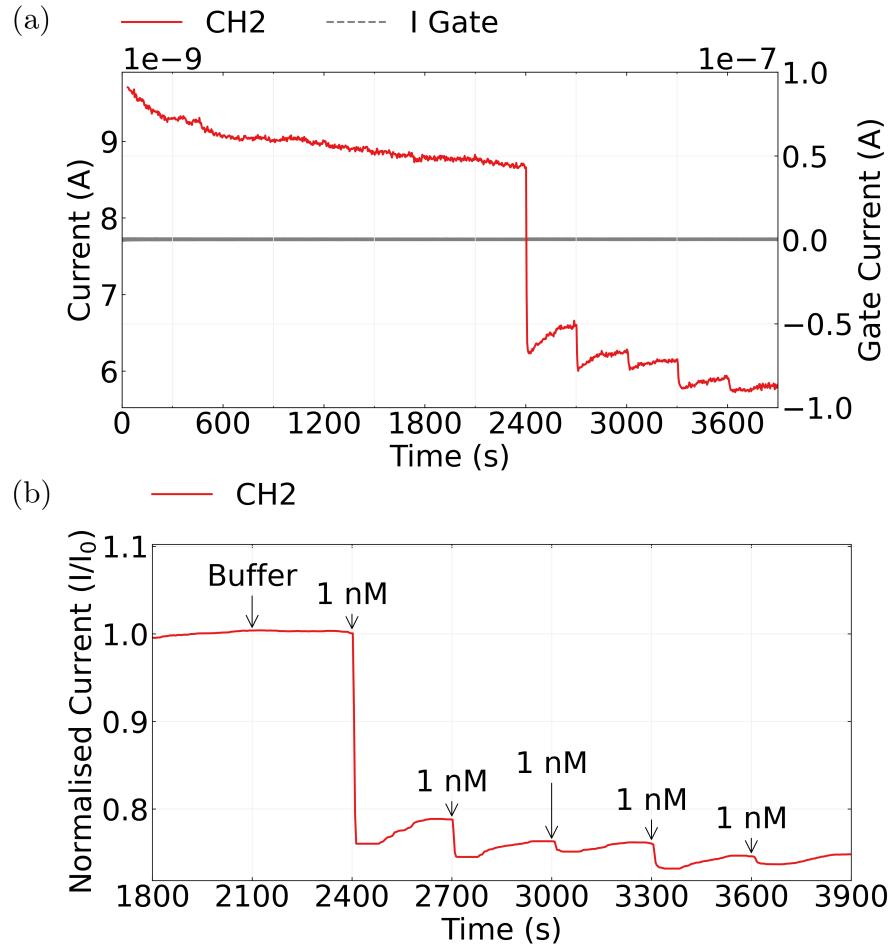


Figure 27: The rinsed OR10a-functionalised device channel was reused to collect a second methyl salicylate sensing series, shown in Figure 27 (a). Source-drain voltage was set at $V_{ds} = 100$ mV while gate voltage was set at $V_g = 0$ V. No responses to 0.5% v/v DMSO 1× PBS additions or significant gate current leakage was observed. The sensing series from 1800–3900 s is shown in (b), where a moving median filter applied and linear baseline drift removed.

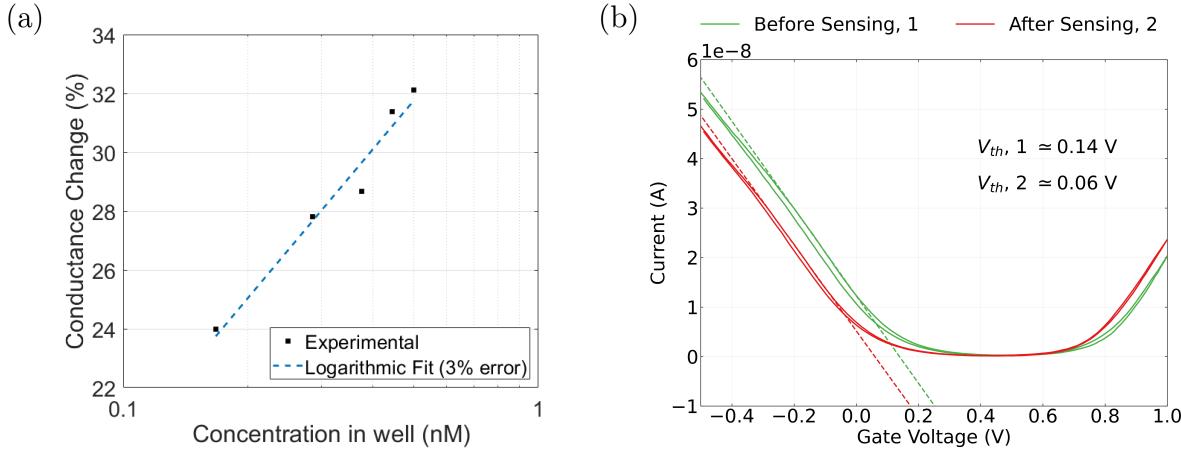


Figure 28: Device responses against methyl salicylate (MeSal) concentration present in the device liquid-gate during the 2nd sensing series are shown in (a), while transfer characteristics of the device directly before and after the 2nd sensing series are shown in (b), where $V_{ds} = 100$ mV.

to confirm the presence of odorant receptors on device channels post-functionalisation, ORs were tagged with green fluorescent protein and fluorescence microscope images taken. The functionalised channels showed significantly more green fluorescence than the control, which indicates that protein attachment to the channel is not limited to empty nanodiscs.

However, the use of the functionalisation procedure with PBASE in methanol and OR22a in nanodiscs was not readily reproducible. A repeat of the functionalisation procedure and sensing procedure across six different device channels showed no decrease in current subsequent to additions ranging from 1 fM to 1 μ M. Further control testing indicated that the successful sensing seen earlier was not spurious, meaning that the lack of reproducibility could be attributed to the functionalisation procedure. Device characterisation showed that although nanodiscs appeared to be present on the channel, with a change in network height of over 20 nm, the average threshold shift was the same as that of a device functionalised using PBASE in methanol without subsequent exposure to nanodiscs. A control taken without using PBASE also showed nanodiscs present on the channel, but resulted in a threshold shift of ~ -0.27 V. It therefore appears that while nanodiscs are present, they are blocked off from the network in a way that prevents them from altering the electrical characteristics of the channel.

Potential confounding variables identified in the functionalisation process included coatings of surfactant, solvent, photoresist, multilayered or hydrolysed PBASE, and the atmospheric long-chain alkanes which cause carbon nanotube hydrophobicity. Several possible confounding variables were individually eliminated by making slight changes to the fabrication and functionalisation of the device. Neither changing the morphology of the carbon nanotube network or using a graphene device led to a operational sensor. Furthermore, the average threshold shift for the highly-bundled device after functionalisation was -0.02 ± 0.01 V, indi-

cating a lack of electrical contact between the channel and nanodiscs even without surfactant present. By changing the solvent used for functionalisation from methanol to DMSO, the average threshold shift was -0.22 ± 0.03 V, which demonstrates electrical contact with the nanodiscs. However, no negative current changes were observed subsequent to analyte additions, suggesting other confounding factors are also present. To ensure that the hygroscopic DMSO was not hydrolysing the PBASE and preventing odorant receptor attachment, a PBA with EDC/NHS functionalisation method was used. However, the average threshold shift observed was -0.12 ± 0.01 V, similar to the -0.15 V shift seen for attachment of PBA to DMSO without subsequent modification. The confounding factor is therefore not resolved by either changing the solvent or through the prevention of hydrolysis.

Table 4: Summary of approaches used for sensor functionalisation in this chapter.

Thin-Film	Linker	Linker Solvent	iOR	Membrane format	Responds?
Steam-deposited CNT	PBASE	Methanol	OR22a	Nanodisc	Rarely
Steam-deposited CNT	No linker	Methanol	OR22a	Nanodisc	No
Solvent-deposited CNT	PBASE	Methanol	OR22a	Nanodisc	No
Graphene	PBASE	Methanol	OR22a	Nanodisc	No
Steam-deposited CNT	PBASE	DMSO	OR22a	Nanodisc	No
Steam-deposited CNT	PBA/EDC	DMSO	OR22a	Nanodisc	No
Steam-deposited CNT	PPB	Aqueous	OR10a	Not used	Yes

The remaining possible sources of surface contamination, including alkane hydrocarbons, multilayer linker and photoresist, were then addressed by making significant changes to the functionalisation procedure. To avoid multilayer coverage, pyrene-PEG-biotin was attached to avi-tagged odorant receptors before functionalisation. To remove hydrocarbons and residual photoresist, the transducer device was oxygen plasma cleaned before functionalisation in the most gentle manner available to the author. Furthermore, no nanodiscs were used, to ensure direct attachment of odorant receptors. Although there are also issues with reliable reproducibility of this method due to the plasma cleaning step, when a device was functionalised with OR10a in this manner, device current decreases of up to $\sim 24\%$ were observed in response to methyl salicylate additions. It was found that rinsing the device meant the sensors transfer characteristics were restored, and the device could be reused. When reusing the sensor, a logarithmic relationship was observed between picomolar concentrations of methyl salicylate in the well and the sensor current response. A summary of the different functionalisation processes trialled in this section can be found in Table 4.