

Volatile Organic Compound Detection Using Insect Odorant-Receptor Functionalised Field-Effect Transistors

by

Eddyn Oswald Perkins Treacher

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1. Biosensing with Insect Odorant-Receptor Functionalised Carbon Nanotube Devices

1.1. Introduction

To test devices with the new vapour delivery system, it was important to first ensure the functionalised devices worked consistently as biosensors in the existing aqueous sensing setup.

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1.2.1. OR Nanodisc Functionalisation

A carbon nanotube network field-effect transistor device, fabricated using post-June 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.

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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 1XPBS 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 μ L OR22a nanodiscs (batch number ND-OR22a-SB018, 1.9 mg/mL, prepared 7 months earlier) were diluted in 1 mL freshly-prepared 1XPBS.

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 μ L 1XPBS 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 1XPBS for 15 s and gently nitrogen dried.

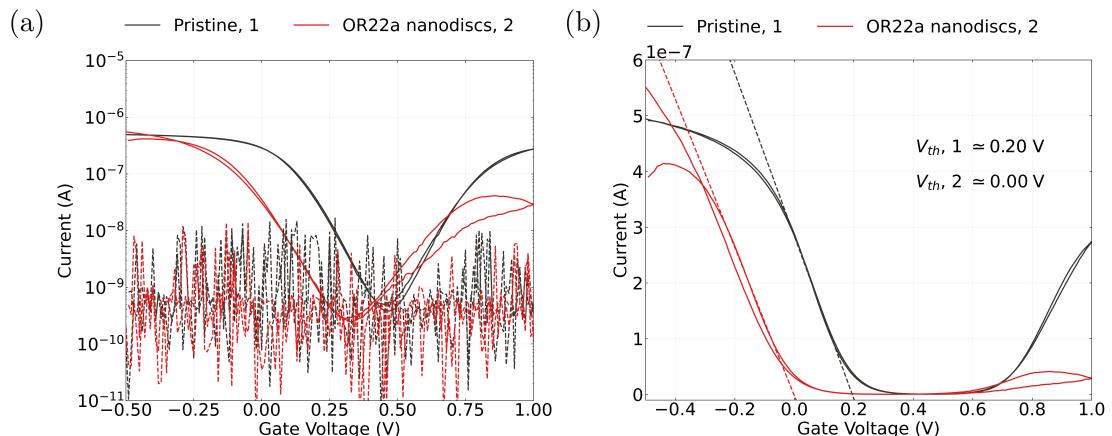


Figure 1.1.: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain current was $V_{ds} = 100$ mV for both the forward and reverse sweep. In (a), the characteristics are shown on a logarithmic scale, where the gate current 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. The threshold voltage corresponding to the intercept of this slope with the x-axis is shown for each transfer characteristic curve.

Liquid-gated electrical characteristics were taken of the sensing channel (channel 7) before and after functionalisation with OR22a nanodiscs. These electrical characteristics were taken in using a liquid gate buffer of 1XPBS containing 0.5% v/v DMSO with the

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B1500A semiconductor device analyser. These characteristics are shown in Figure 1.1, shown using both a logarithmic and linear current scale. The device exhibited ambipolar characteristics before functionalisation, which is typically seen for steam-deposited carbon nanotube films ([?@sec-cnt-devices](#)). However, *p*-type behaviour dominates after device functionalisation due to a significant drop in *n*-type conductance. There was little hysteresis present, which is also typical for these devices. A slight increase in hysteresis was observed post-functionalisation. Leakage current (shown by the dashed traces) never exceeds 1×10^{-7} V, both before and after functionalisation. The significant change in electrical characteristics observed could be due to 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. Note that as the nanodisc volume is much larger than that of the odorant receptor, any direct protein adsorption is highly likely to be adsorption of the nanodisc membrane scaffold protein onto the carbon nanotube network. Odorant receptor attachment via PBASE is therefore the only desirable functionalisation result for sensing purposes.

Only minor changes were observed in the on-off ratio when comparing the device channel before and after functionalisation. The on-off ratio for the pristine channel was 1120 ± 220 , fairly typical for a transfer curve from a steam-assisted surfactant-deposited CNT network device (see [?@sec-cnt-devices](#)). The on-off ratio increased slightly to 1830 ± 550 after functionalisation. We expect to see an increase in on-off ratio for a device successfully functionalised with OR22a nanodiscs, which may result from an increase in negative charge causing modulation of Schottky barriers between metallic and semiconducting carbon nanotubes within the network [1]. However, we also expect increased hole conductance from the attachment of PBASE, even without proteins being present ([?@sec-PBASE-electrical-characterisation](#)). It is therefore difficult to determine whether functionalisation has been successful from the on-off ratio of transfer characteristics alone.

Functionalisation of the channel resulted in a negative shift in threshold voltage of -0.20 ± 0.03 V. This is significantly in excess of threshold voltage shifts measured for both methanol adsorption (-0.15 ± 0.02 V) and after exposure to PBASE in methanol (-0.06 ± 0.04 V), confirming that protein has attached to the carbon nanotubes. However, both direct protein adsorption [2], [3] and empty nanodisc attachment [1] should also lead to a significant negative threshold voltage shift 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 [1]–[3]. 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 protein has attached to the nanodisc network, it cannot be used to specifically determine whether odorant receptors have attached to the network.

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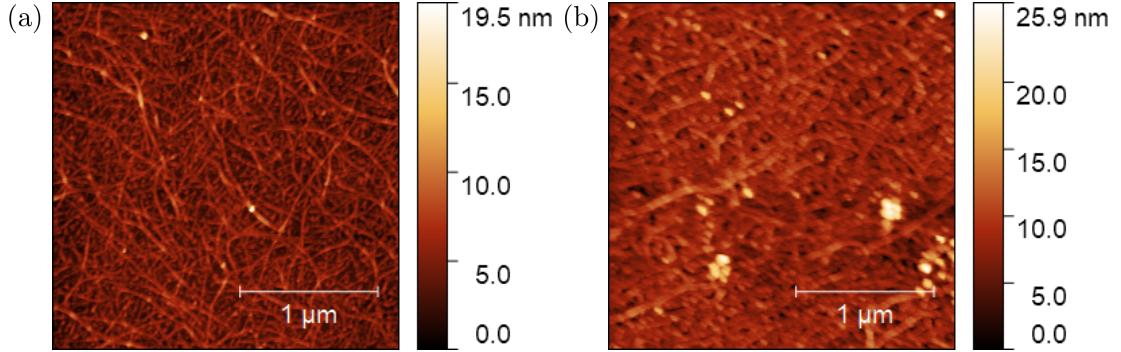


Figure 1.2.: Atomic force microscope images of the channel region of carbon nanotube network devices before and after functionalisation. The channel network of a pristine device is shown in (a), while (b) is of channel 7 from the sensing device functionalised in this section.

Atomic force microscope images of the device channels both before functionalisation and after sensing with the functionalised device to confirm the presence of nanodiscs. 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](#). These images are shown in Figure 1.2. Aggregations of nanodiscs are visible in Figure 1.2 (c)–(d). These nanodisc clusters are especially sizable in the lower half of Figure 1.2 (c), where the two largest clusters are 200 ± 10 nm across at their widest point. However, these features are still much smaller than most of the aggregated nanodisc features seen by Murugathas *et al.* [1]. Furthermore, the nanodisc features closely follow the carbon nanotube bundles across the densely bundled morphology used by Murugathas *et al.* [1]. On the dense network morphology used here, the position of nanodisc clusters relative to the carbon nanotubes is less distinct. To confirm whether nanodiscs have preferentially attached to the carbon nanotubes, a more quantitative approach is needed.

The average height of the substrate was found for both pristine and functionalised atomic force microscope images using the masking tool in Gwyddion. The masks for each image are shown in Figure 1.3. Average substrate heights of 1.9 ± 0.3 nm and 3.3 ± 0.4 nm were found for the pristine and functionalised networks respectively. In Gwyddion, both networks were 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 1.4, 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, 8.7 nm above the substrate. Figure 1.4 (a) shows only a few, sparsely distributed features, the majority of which are below 50 nm in width. These features may correspond to large nanotube-nanotube junctions, surfactant residue, or other surface contamination

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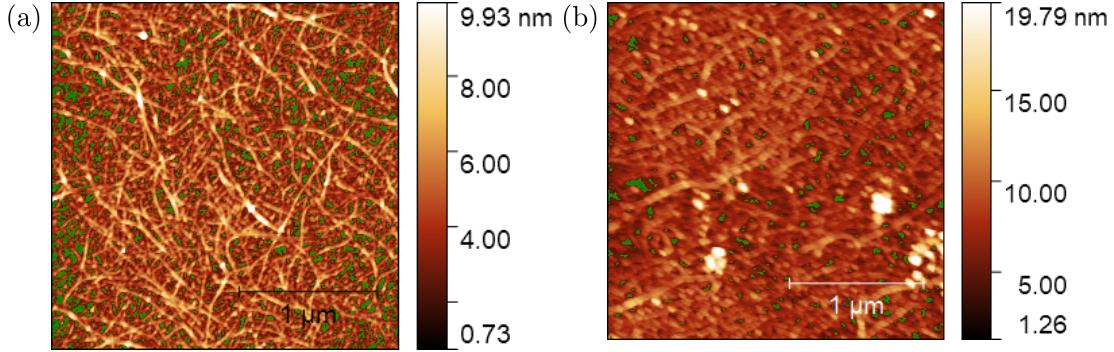


Figure 1.3.: Atomic force microscope images with the substrate background highlighted with a green mask. Here, (a) shows a device channel after functionalisation with PBASE and methanol, while (b) shows channel 7 from the sensing device functionalised with OR22a nanodiscs in this section.

(?@sec-pristine-morphology). In Figure 1.4 (b), many features are over 50 nm across. They are often found close together, and form a curved line across the network in the bottom left corner (shown in red); this curvilinear arrangement of nanodisc features gives a strong indication specific attachment to the carbon nanotubes has occurred.

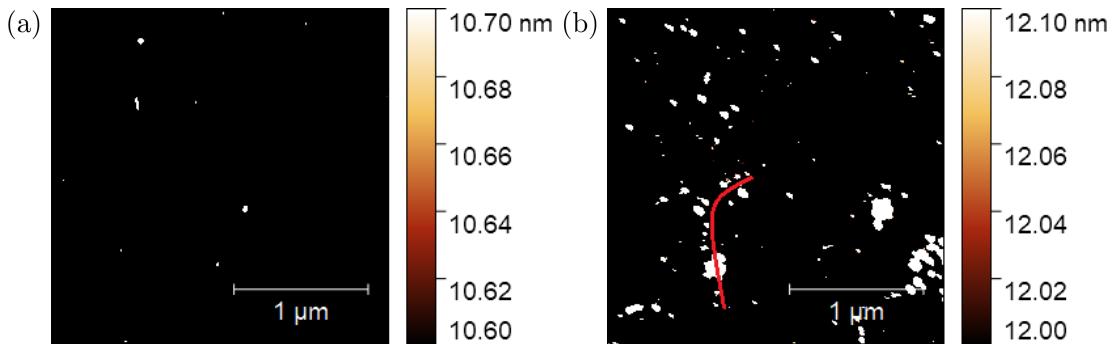


Figure 1.4.: Binary representations of the atomic force microscope images of (a) the pristine device, with a threshold height of 10.6 nm, and (b) the functionalised device, with a threshold height of 12.0 nm. Both thresholds are 8.7 nm above the substrate background of their respective images.

As the maximum height of the functionalised AFM is 25.9 nm and only nanodisc features are present at 12.0 nm, there must be nanodisc agglomerates present at least 13.9 nm tall. The estimated height range for nanodiscs is $\sim 10 - 20$ nm [4]–[6]. Assuming an average carbon nanotube height of 1.45 nm, with an average substrate height of 3.3 nm, the nanodisc agglomerations could be up to ~ 21 nm tall. Note that height measurements of biological samples taken via AFM have been shown to underestimate actual feature height by over 50% [7]. Even so, the breadths of the nanodisc features are significantly greater than their heights. While the cross-sections of the largest features

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are up to 20 nanodiscs across, they are a few nanodiscs high at most; certainly less than five. It appears that, rather than clustering together in solution and attaching as an aggregate, the nanodiscs are individually attaching to preferred locations on the network. These locations may be at junctions between two large carbon nanotubes, which have a relatively large surface area available for binding, or at locations particularly clean of contamination. AFM images showing iOR-nanodisc functionalised carbon nanotube networks with significant vertical clustering have been reported, but these images were not of channels used for sensing [1].

Fluorescence microscopy was also used to confirm the presence of GFP-tagged odorant receptors on the carbon nanotube network. A device was functionalised using nanodiscs with odorant receptors attached to *Aequorea Victoria* green fluorescent protein (GFP) using the process described earlier. When these nanodiscs were used, the functionalisation was performed in darkness, with the odorant receptor nanodisc vial transported under an opaque cover to protect it from light (batch number ND-GFP-OR43b-0002, prepared 12 months earlier). After functionalisation, devices were briefly rinsed with DI water and nitrogen dried to remove dried-down salt residue left by the 1XPBS. A control device was also prepared without the use of PBASE, skipping steps 3 and 4 in the functionalisation process. Fluorescence images of the GFP-OR functionalised and control devices are shown in Figure 1.5. Note that 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.

The silicon dioxide regions in each image appear bright under the GFP filter, indicating non-specific binding between the GFP-OR nanodiscs and the silicon dioxide substrate. As this device has been annealed, UV exposed and developed before functionalisation, the likelihood this non-specific attachment is to residual photoresist is reduced significantly (see [?@sec-photoresist-contamination](#)). The SiO₂ substrate also appears brighter in the images on the right of Figure 1.5, which are of the device initially exposed to PBASE. The discussion in [?@sec-pyrene-interactions](#) indicates that the pyrene moiety of PBASE non-specifically interacts with the silicon dioxide substrate. Assuming no significant variation in the fluorescence of GFP between nanodisc vials, the PBASE coating appears have led to more nanodiscs attaching to the silicon dioxide, giving rise to the brighter fluorescence of the silicon dioxide seen for the PBASE-incubated device on the right of Figure 1.5.

A comparison of fluorescence in the channel region between images on the left of Figure 1.5 (GFP-OR nanodiscs) and the images on the right (GFP-OR nanodiscs and PBASE) is given by the inset in Figure 1.5 (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 1XPBS is limited on these channels. However, when the carbon nanotubes are modified with PBASE, the GFP-OR nanodiscs are able to attach to the channel, and so the channel shows up brightly under the fluorescence microscope GFP filter. Importantly, since the

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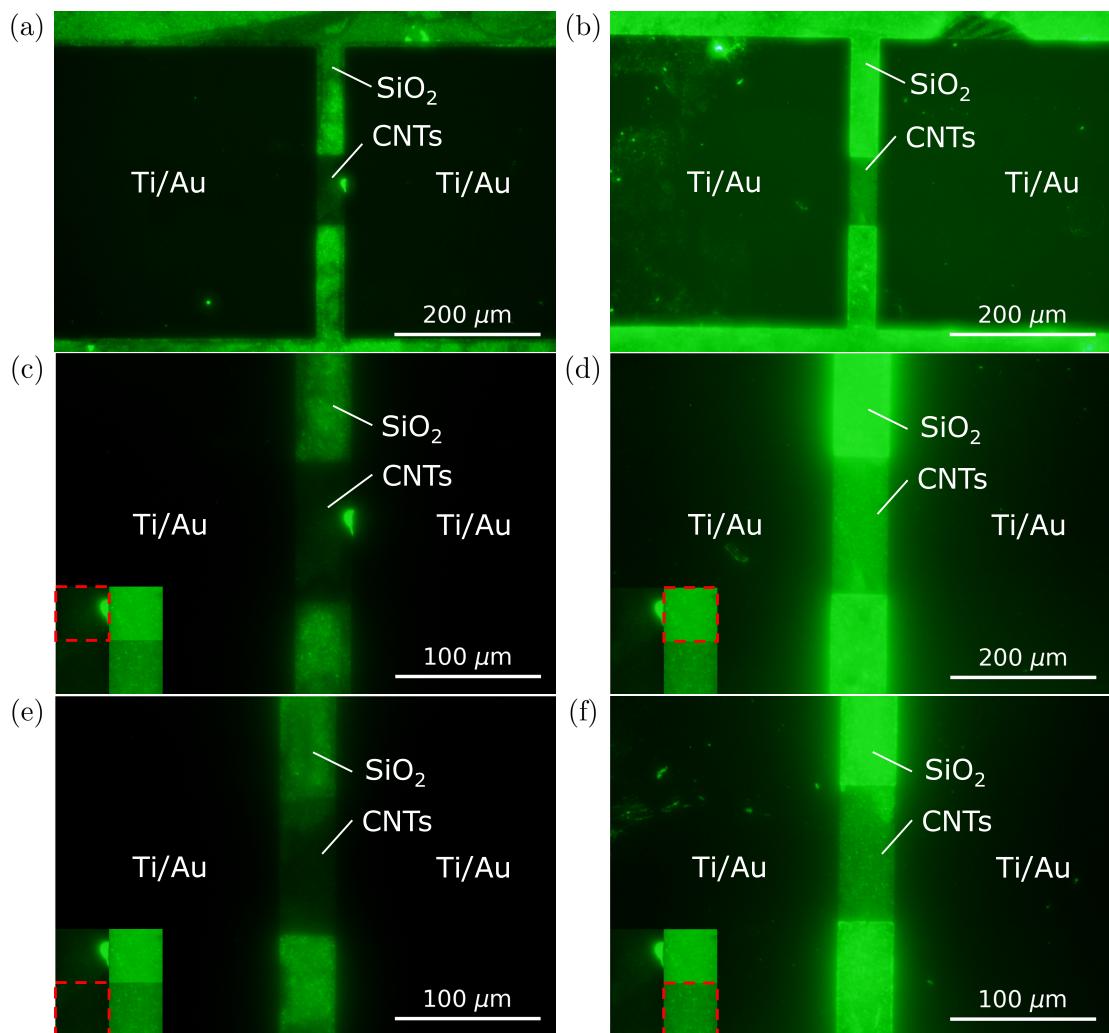


Figure 1.5.: The fluorescence images on the left side – (a), (c) and (e) – show unencapsulated carbon nanotube network channels from a device incubated in GFP-OR nanodiscs. The rectangular dark regions to the left and right of each image are the gold electrodes. The fluorescence images on the right – (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.

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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. As far as I know, this is the first time fluorescence has been used to investigate the attachment of odorant receptor nanodiscs to a carbon nanotube network.

1.2.2. Aqueous Sensing of Ethyl Hexanoate

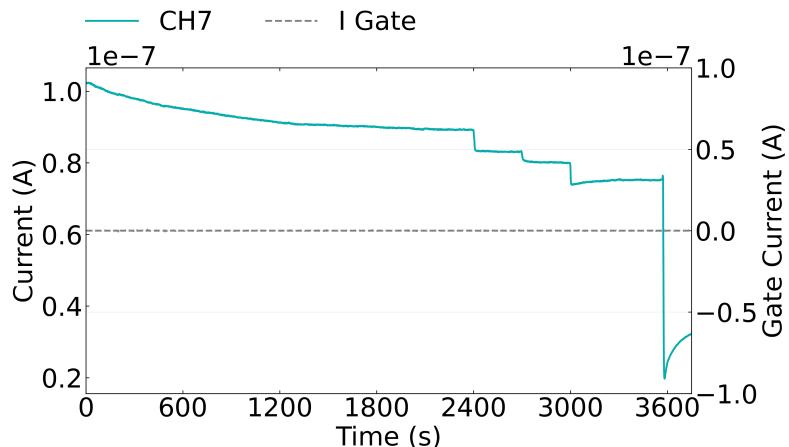


Figure 1.6.: The control series (before 1800 s) and ethyl hexanoate sensing series (after 1800 s) of the OR22a-functionalised device channel. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. No responses to 0.5% v/v DMSO 1XPBS were seen during the control series, while significant responses to additions of ethyl hexanoate diluted in 0.5% v/v DMSO 1XPBS were seen at 2400 s, 2700 s, 3000 s and 3600 s. Functionalisation and sensing was performed by Danica Fontein, School of Chemical and Physical Sciences, Te Herenga Waka - Victoria University of Wellington, using the methods developed in this thesis.

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) and dilutions of ethyl hexanoate in the same 0.5% v/v DMSO 1XPBS buffer solution were added during the sensing series. The 0.5% v/v DMSO 1XPBS was prepared by adding 5 μ L of DMSO to 995 μ L 1XPBS before device characterisation. The dilutions of ethyl hexanoate were prepared with the same 1XPBS at the same time, where 5 μ L of 200 fM, 200 pM, 200 nM and 200 μ M ethyl hexanoate in DMSO were placed into four individual vials containing 995 μ L 1XPBS each, giving 1mL vials of 1 fM, 1 pM, 1 nM and 1 μ M ethyl hexanoate in 0.5% v/v DMSO 1XPBS. The ethyl hexanoate in DMSO dilutions were prepared beforehand as a 1:10 dilution series in DMSO using 200 mM stock solution,

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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 1.1 (b) taken directly before sensing. The full control series plus sensing sequence is shown in Figure 1.6. Gate current remained negligible across the entire sensing procedure.

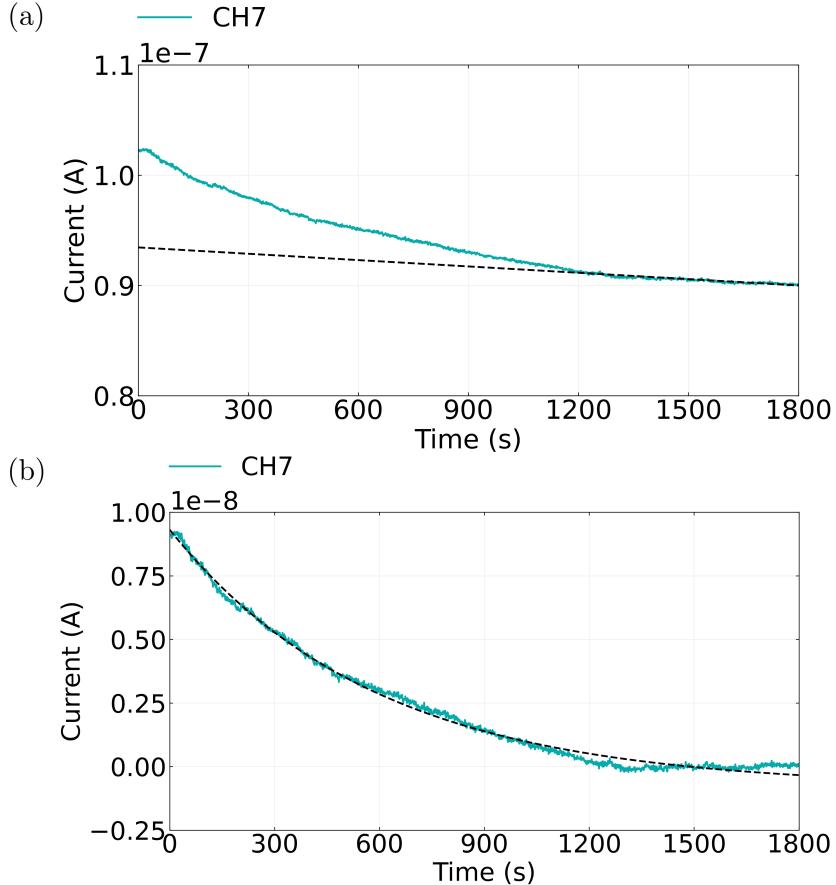


Figure 1.7.: The control series for the OR22a-functionalised device is shown in (a), alongside an extrapolated linear fit to the control series from 1200 s onwards. The control series with the linear approximation subtracted fitted to an exponential curve is shown in (b).

The control series for the sensing series is shown in Figure 1.7 (a). No clear stepwise response is seen to buffer 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 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, as shown in Figure 1.7 (b). A value of 590 ± 3

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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.

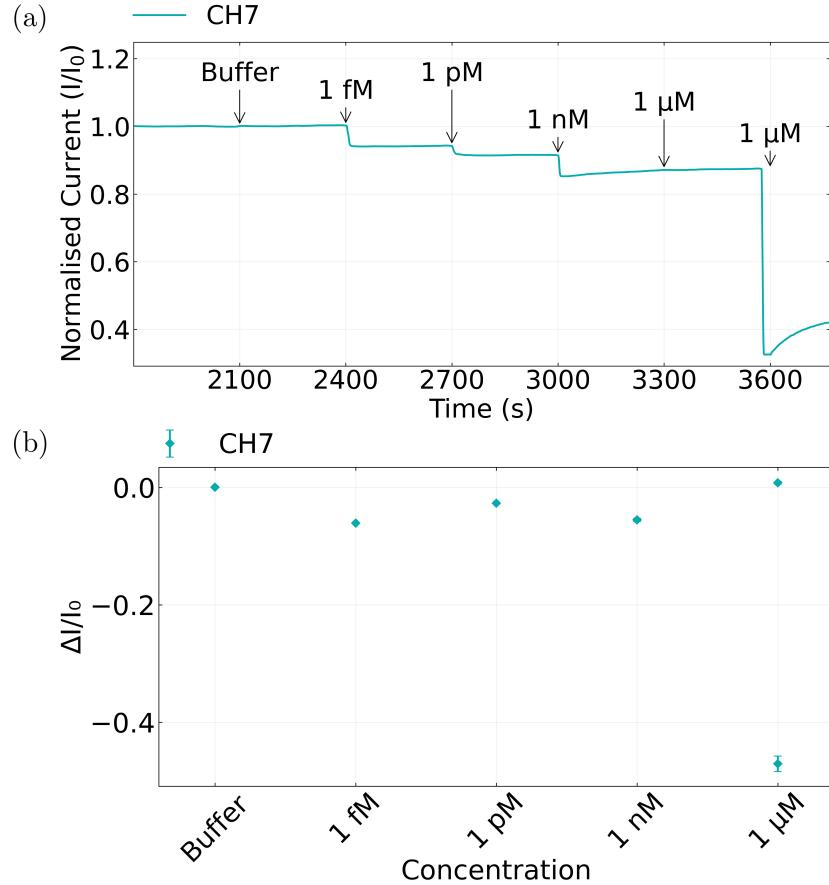


Figure 1.8.: The normalised sensing series for the OR22a-functionalised device is shown in (a). The current data has been despiked, with baseline drift removed and a moving median filter applied. The concentration of each 20 μL addition is indicated above the time of addition. The signal data corresponding to the mean difference in current before and after each addition is shown in (b).

It appears that the exponential fit overestimates current measurements between 1100 s and 1500 s and underestimates measurements between 1500 s and 1800 s. This deviation from the fit may result from the linear approximation used to represent long-term baseline drift being weaker for this channel than for those discussed previously in [?@sec-dummy-sensing](#) and [?@sec-pristine-EtHex](#). This could result from the exponential terms for long-term baseline drift having relatively short time constants, so $t \ll \tau_i$ no longer holds and higher order terms in the linear approximation are no longer negligible. This observation may indicate a relationship exists between device functionalisation and the long-lived device decay behaviour. However, it may simply result from the natural

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variation between randomly-deposited device channels. Further work may be required to confirm the existence of such a relationship, though this work is outside the scope of this thesis.

Figure 1.8 (a) 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 source-drain current across the channel decreased rapidly with each addition of ethyl hexanoate in 0.5% v/v DMSO 1XPBS solution. This current decrease appears irreversible, as the current stabilises after each addition at a lower current level than prior to the addition. This behaviour appears to be a response by OR22a to its positive ligand ethyl hexanoate, similar to the response by OR22a to methyl hexanoate seen by Murugathas *et al.*. The ORCO coreceptor was not required to be present for responses to be seen. The device showed responses to ethyl hexanoate over a wide range of concentrations, beginning with a $\sim 6\%$ response to 1 fM EtHex in 0.5% v/v DMSO 1XPBS, with a maximum response of $\sim 45\%$. Interestingly, as seen in Figure 1.8 (b), no clear dose-dependent response was observed. The behaviour seen may be explained by a decreased sensitivity to subsequent additions seen by Murugathas *et al.* [1] competing with the logarithmic increases in the concentration around the channel.

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1.3.1. Variability in Biosensor Behaviour

Despite the successful detection of ethyl hexanoate by an OR22a nanodisc-functionalised biosensor in Section 1.2, it was found that this behaviour was not readily reproducible. The 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 1.9. Figure 1.9 (a) has been left unfiltered to illustrate the variation in behaviour between channels, while Figure 1.9 (b) has been prepared in the same manner as Figure 1.8 (a). 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 1.8 (a), no decreases are seen in Figure 1.9 (b) to any ethyl hexanoate solution addition.

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 Section 1.2. These characteristics are shown in Figure 1.10. 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,

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as shown in Figure 1.11. The same image analysis as in Section 1.2 was performed, with the substrate mask shown in Figure 1.12 (a) giving an average substrate height of 3.8 ± 0.4 . A binary representation of the image with a 12.5 nm threshold, 8.7 nm above the average substrate height, is shown in Figure 1.12 (b). As with the functionalised device in Figure 1.4 (b), Figure 1.12 (b) shows clustering of features, many of which are larger than 50 nm across. It seems, therefore, that nanodisc aggregates up to 27.5 nm high are present on channel 6, despite the lack of a significant threshold shift as a result of functionalisation.

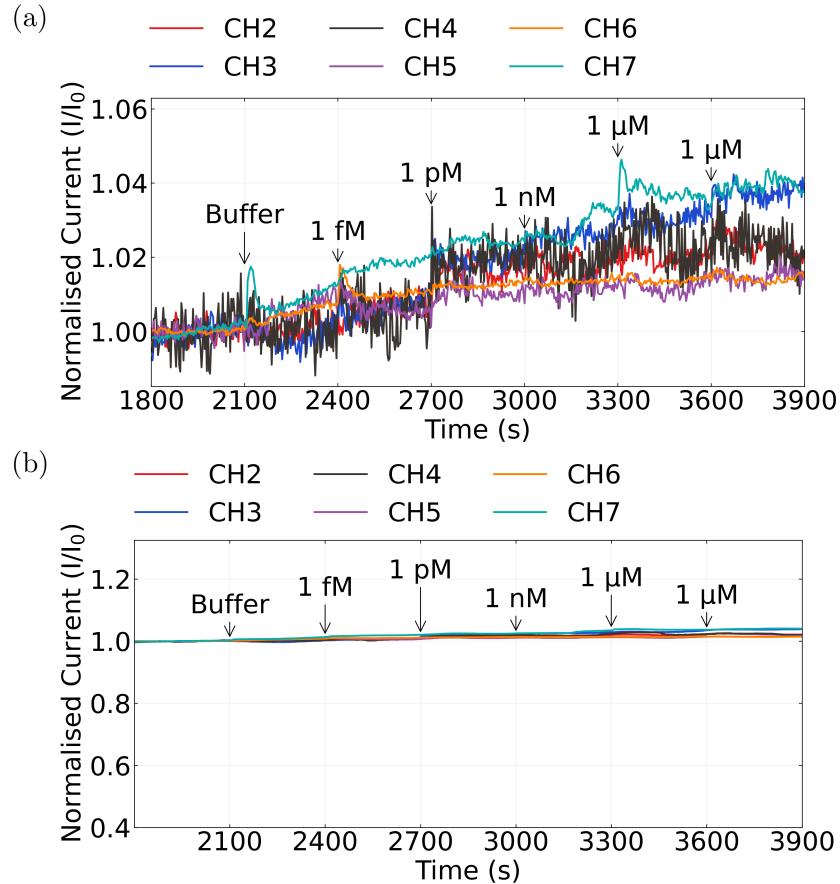


Figure 1.9.: The normalised sensing series of another OR22a-functionalised device across six multiplexed channels, where current data has been despiked and baseline drift removed. The concentration of each 20 μL addition is indicated above the time of addition. The same sensing series is shown in both (a) and (b), where a moving median filter has been applied in (b).

Both OR nanodisc and empty nanodisc attachment via PBASE have been shown to cause significant gating of the network (Section 1.2). Therefore, it might be reasoned that the lack of a gating effect, but with nanodiscs present, results from a direct attachment mechanism circumventing the PBASE linker. The amine group on proteins can

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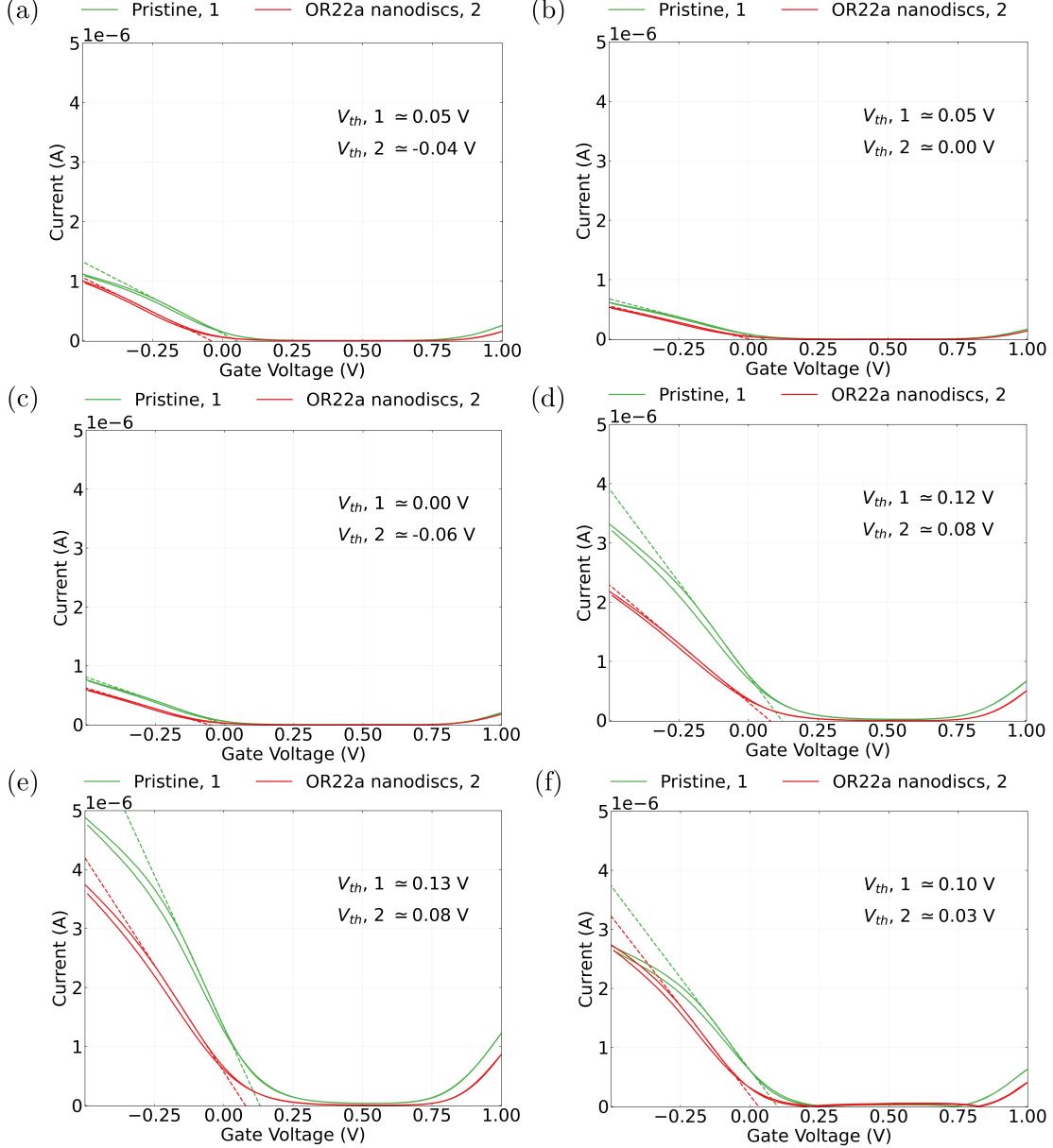


Figure 1.10.: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain current was $V_{ds} = 100$ mV for both the forward and reverse sweep. Each subfigure (a)-(f) corresponds to a different channel of the functionalised device; (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. The threshold voltage corresponding to the intercept of this slope with the x-axis is shown for each transfer characteristic curve.

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be attached directly onto carbon nanotubes by adsorption, although this attachment is relatively weak [2]. Figure 1.13 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. Figure 1.14 shows the substrate mask in (a) and a binary representation of this AFM with nanodisc aggregates clearly visible in (b). In Figure 1.15 (a), the negative threshold shift of a channel modified in this way was -0.27 V, similar in size to the shift due to functionalisation seen for the working biosensor.

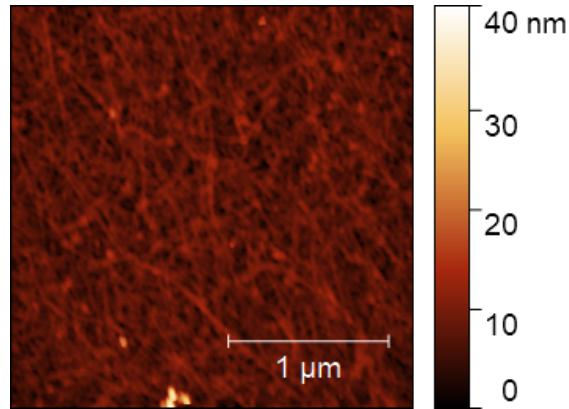


Figure 1.11.: An atomic force microscope image of channel 6 from the OR22a nanodisc functionalised device which showed no response to ethyl hexanoate.

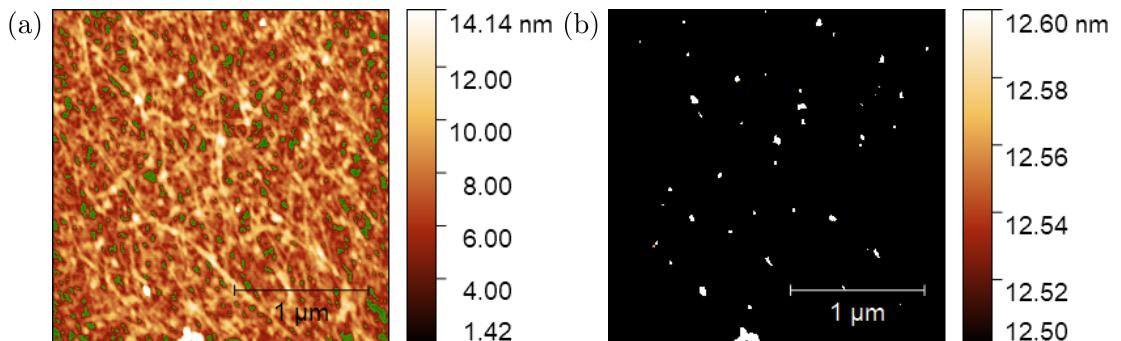


Figure 1.12.: The mask used to find the average substrate height of the functionalised channel 6 is shown in (a), with the substrate highlighted green. The bounds of the colour map have been lowered in (a), as colour mapping over the full height range makes it difficult to clearly distinguish between sub-20 nm features and the substrate. A binary representation of the atomic force microscope image with a threshold height of 12.5 nm is shown in (b).

The attachment of nanodiscs in 1XPBS may seem surprising, given that the fluorescence microscopy work in Section 1.2.1 indicates no GFP-OR present after functionalisation without PBASE. However, this device channel did not work as a sensor when tested with

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ethyl hexanoate. Figure 1.15 (b) shows a small, positive current response to additions of ethyl hexanoate diluted in 1% v/v DMSO 1XPBS, which may result from the weakly attached OR22a nanodiscs being mechanically removed by the pressure of each addition on the device channels.

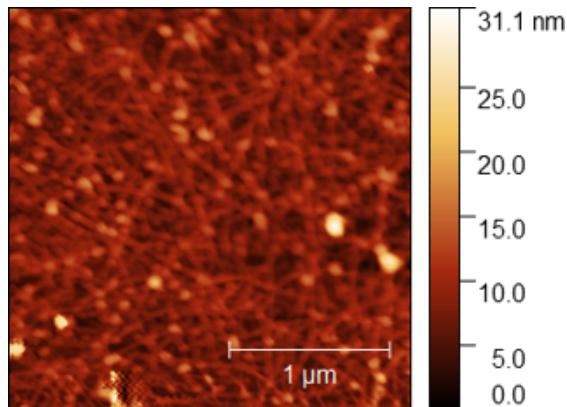


Figure 1.13.: An atomic force microscope image of a carbon nanotube film submerged in OR22a nanodiscs for 1 hour without prior exposure to PBASE or methanol.

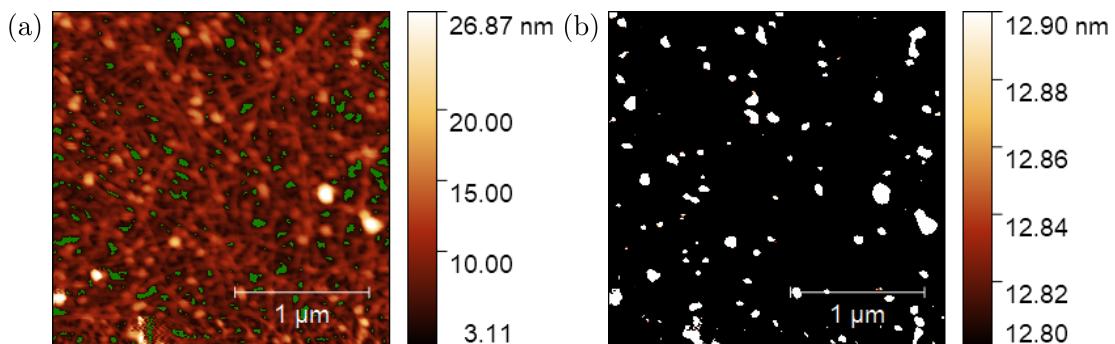


Figure 1.14.: The binary representation of the network, with a height threshold of 12.8 nm (average substrate height = 4.1 nm), is shown in (b).

An explanation is required for the seemingly contradictory situation where nanodiscs can be present on a device channel without significant gating effects. The most straightforward explanation is that no reliable correlation exist 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. Variations in the de-

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gree 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.

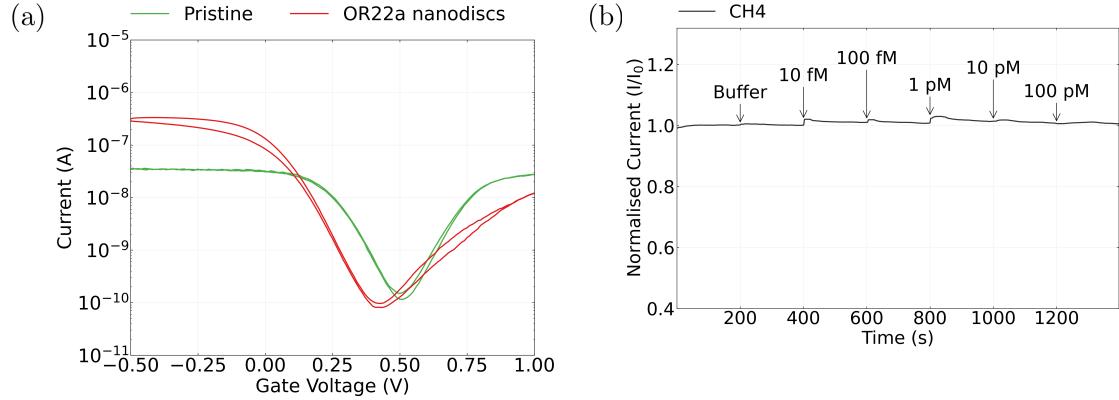


Figure 1.15.: The device characteristics in (a) are from channel 4 of the device placed in OR22a nanodisc solution for 1 hour without prior exposure to PBASE or methanol before and after functionalisation. Real-time sampling using this channel is shown in (b). A 20 μ L addition of 1% v/v DMSO 1XPBS was made at 200 s. Subsequently, 20 μ L additions of ethyl hexanoate diluted in 1% v/v DMSO 1XPBS 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.

1.3.2. 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 1.3.1. To understand which of these candidates is responsible for the significant variability in biosensor functionality,

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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

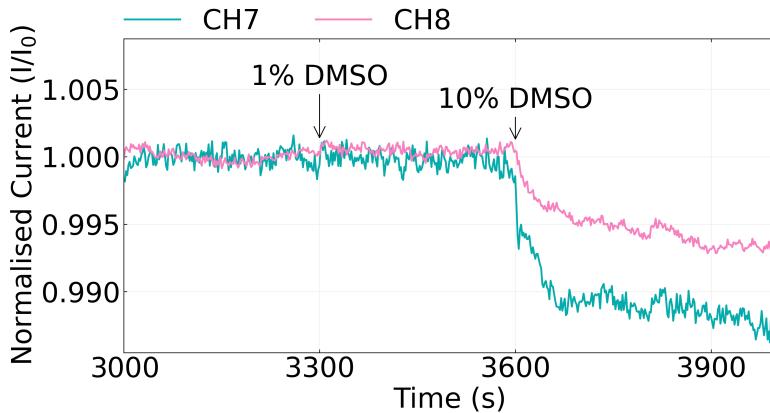


Figure 1.16.: Response to changing the concentration of DMSO in the PDMS well of a OR22a-functionalised sensor. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. The well originally contained 100 μL of 1% v/v DMSO 1XPBS. 20 μL additions of DMSO in PBS were made at 3300 s and 3600 s.

Before testing to see if a surface coating was responsible for the lack of response seen to ethyl hexanoate by the device in Section 1.3.1, it was important to investigate the possibility the signals seen in Section 1.2.2 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 1XPBS containing the analyte and the 0.5% v/v DMSO in 1XPBS 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 1XPBS, taking care to avoid cross-contamination, so responses are unlikely to result from a difference between the 1XPBS in the analyte solution and the 1XPBS 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 1.16 shows that a increase in DMSO concentration in the well from 1% v/v to 2.5%

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v/v leads to a steep drop in current of 0.5% – 1.5% across two OR22a-functionalised device channels. The gate current remained negligible across this time period (<0.1% of average drain current).

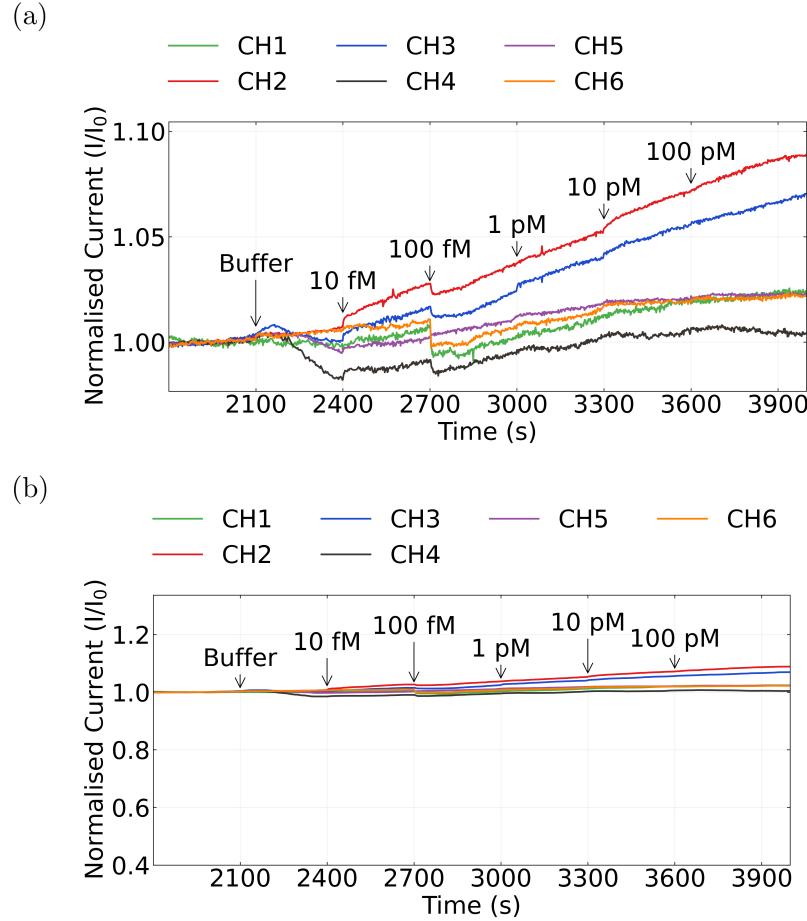


Figure 1.17.: The normalised sensing series of the solvent-deposited, OR22a-functionalised device across six multiplexed channels, where current data has been despiked and baseline drift removed. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. The concentration of each 20 μL addition is indicated above the time of addition. The same sensing series is shown in both (a) and (b), where a moving median filter has been applied in (b).

Though the current changes in Figure 1.16 share similarities with other observed responses to analyte, they are significantly smaller than the >2% changes seen in Section 1.2.2. Furthermore, precise measurement of DMSO in analyte preparations means that a change in proportion of DMSO in the well of this scale is unlikely. It therefore seems improbable that a change in DMSO concentration is the primary cause of these

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responses. However, the author recommends a slight modification to the control series for a more robust experimental baseline. For a device with 100 μL 0.5% v/v DMSO 1XPBS in the well after the first buffer addition at 100 s, a subsequent 20 μL addition of 1% v/v DMSO 1XPBS at 200 s and 20 μL addition of 0% v/v DMSO 1XPBS 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 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 [1], [6]. 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 1.2.1 with OR22a nanodiscs.

The normalised sensing behaviour for the solvent-deposited carbon nanotube device is shown in Figure 1.17. The device shows a small current increase on four channels with the 10 fM ethyl hexanoate addition, and a small decrease on five channels with the 100 fM EtHex addition. No current change exceeded 2%, and as shown in Figure 1.17 (b), the current changes appear negligible on the scale of the changes seen in Section 1.2.2. The average threshold shift from functionalisation was -0.02 ± 0.01 V, which indicates attachment of PBASE but not nanodiscs. As the device was made using the pre-2023 encapsulation mask, an AFM was taken of a separate film modified in the same manner, shown in Figure 1.19 (a). The average substrate height was 11.3 ± 0.9 nm. A binary representation of the image taken at the minimum height no spindle-like bundles were visible, 46.1 nm, is shown in Figure 1.19 (b). Attached nanodisc aggregations are clearly visible, and appear closer in nature to those observed for an OR35a-functionalised film [6] than previously seen in this work. Figure 1.19 (c) and (d) show that over a larger scale these aggregations can be at least 330 nm tall, forming large clusters which may indicate mutual interaction during deposition.

The electrical characteristics of a graphene device channel before and after functionalisation with OR22a nanodiscs in the manner outlined previously are shown in Figure 1.20 (a), showing an order of magnitude decrease in on-current and a negatively shifted Dirac point with functionalisation, as seen previously for working OR22a nanodisc GFET biosensors [6]. Figure 1.20 (b) shows when the process is repeated on another device without initial exposure to PBASE and methanol, a larger negative shift in the Dirac point 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 1.3.1. A pristine graphene surface and a OR22a-functionalised graphene surface are shown in Figure 1.21 (a) and (b) respectively. Graphene folds of up to 7.3 nm in height are visible on the right hand side of Figure 1.21

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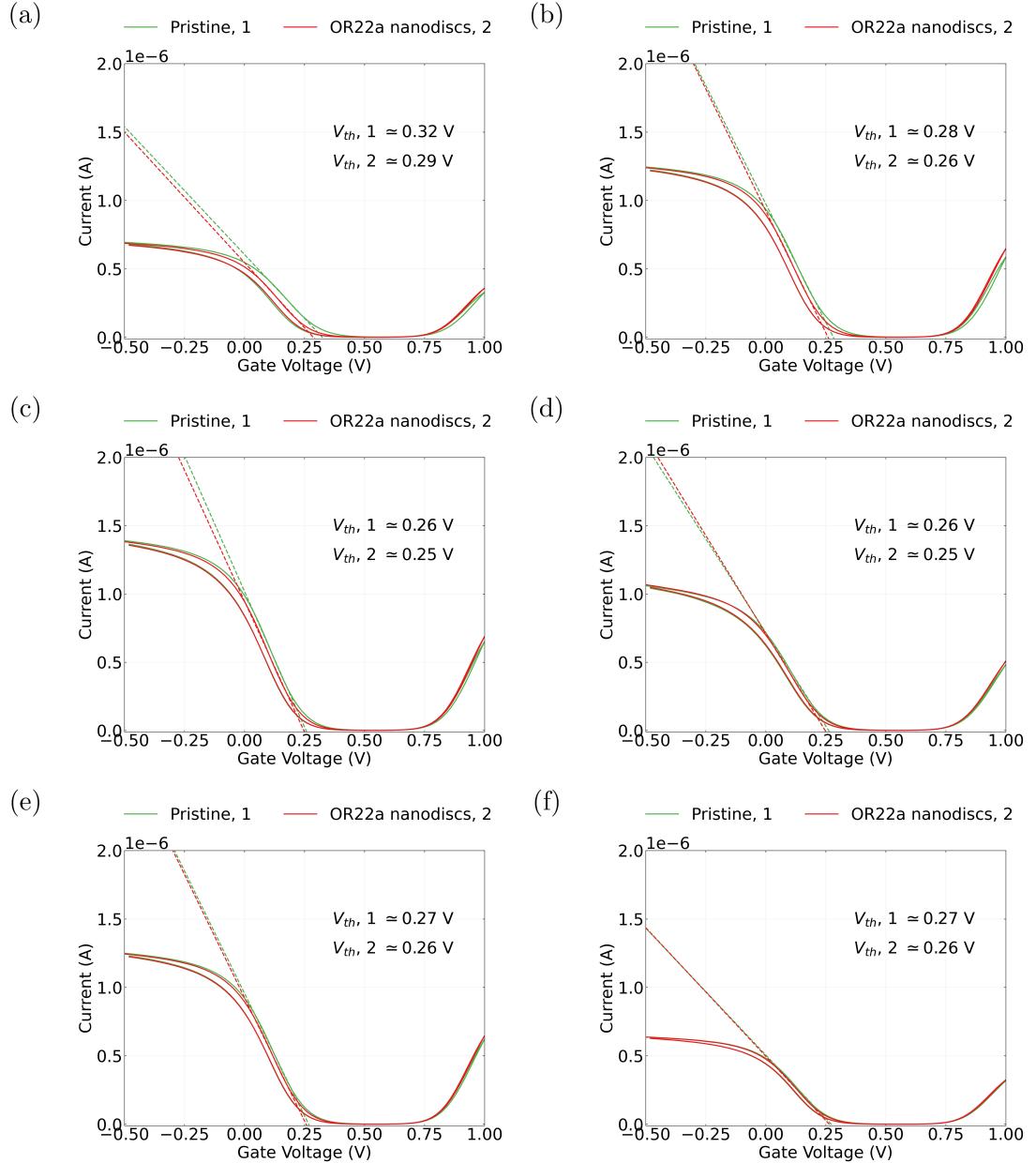


Figure 1.18.: Liquid-gated solvent-deposited carbon nanotube device transfer characteristics before and after OR22a nanodisc functionalisation. Source-drain current was $V_{ds} = 100$ mV for both the forward and reverse sweep. (a) corresponds to channel 2, (b) corresponds to channel 3, (c) corresponds 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.

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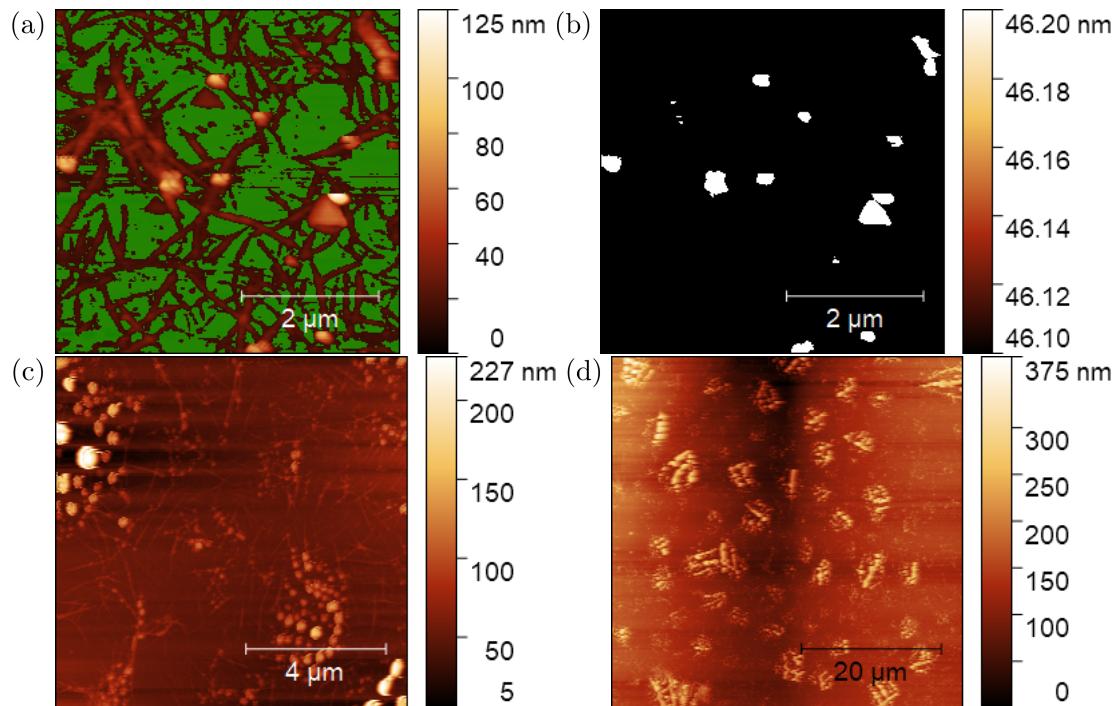


Figure 1.19.: An $2.5 \mu\text{m} \times 2.5 \mu\text{m}$ atomic force microscope image of an OR22a nanodisc functionalised solvent-deposited carbon nanotube film is shown in (a), with the average substrate height highlighted green. A binary representation of the atomic force microscope image with a threshold height of 34.7 nm 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.

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(a). When functionalised with OR22a nanodiscs, the surface is densely coated with nanodisc aggregates up to ~ 250 nm across and ~ 30 nm tall. These nanodiscs are a similar height and size to those observed by Murugathas *et al.* [6].

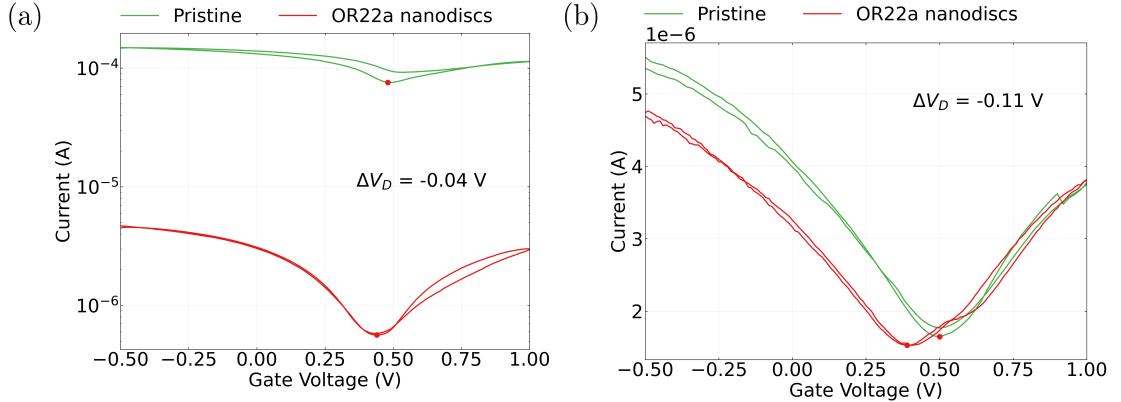


Figure 1.20.: Liquid-gated graphene device transfer characteristics before and after OR22a nanodisc functionalisation, where (a) was functionalised using the standard method while (b) was functionalised without submerging the device in PBASE and methanol. Source-drain current was $V_{ds} = 100$ mV for both forward and reverse sweeps. The shift in Dirac point resulting from functionalisation is also shown for each device.

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 1.22 shows current changes of $<1\%$ 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% [6]. 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 confounding factor 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

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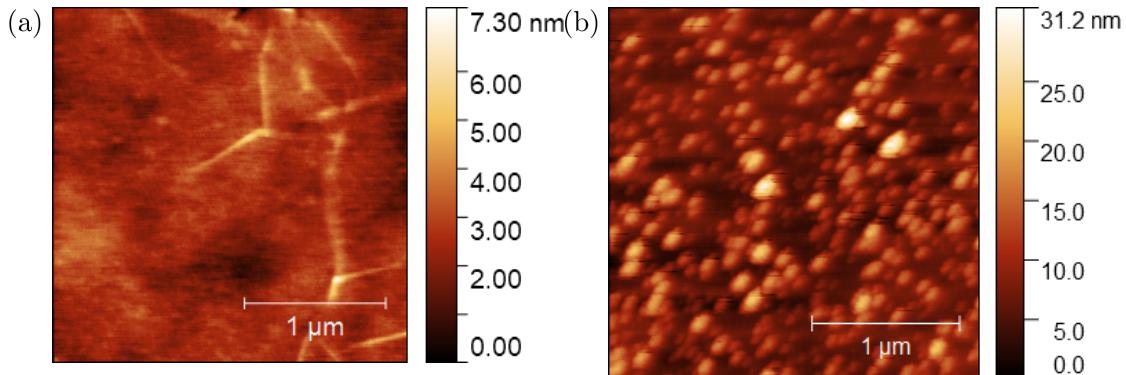


Figure 1.21.: $2.5 \mu\text{m} \times 2.5 \mu\text{m}$ atomic force microscope images of (a) a monolayer graphene film on SiO_2 and (b) a graphene film after OR22a nanodisc functionalisation.

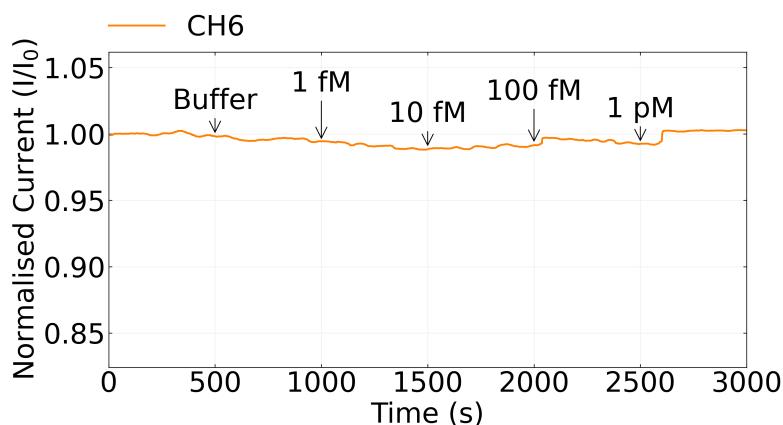


Figure 1.22.: A normalised ethyl hexanoate sensing series taken with a OR22a-functionalised graphene device. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. The concentration of each $20 \mu\text{L}$ addition is indicated above the time of addition, with additions made at 500 s, 1000 s, 1500 s, 2000 s and 2500 s.

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used in the literature for functionalisation with PBASE is dimethyl sulfoxide (DMSO), as discussed in ?@sec-PBASE-attachment. A surfactant-deposited device was functionalised with OR22a nanodiscs in the same manner as described in Section 1.2.1, except DMSO was used instead of methanol when preparing the PBASE solution. The sensing dataset from the four device channels of a suitable current level are displayed in Figure 1.23 (a), 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, and therefore appear negligible on the scale used in Figure 1.23 (b).

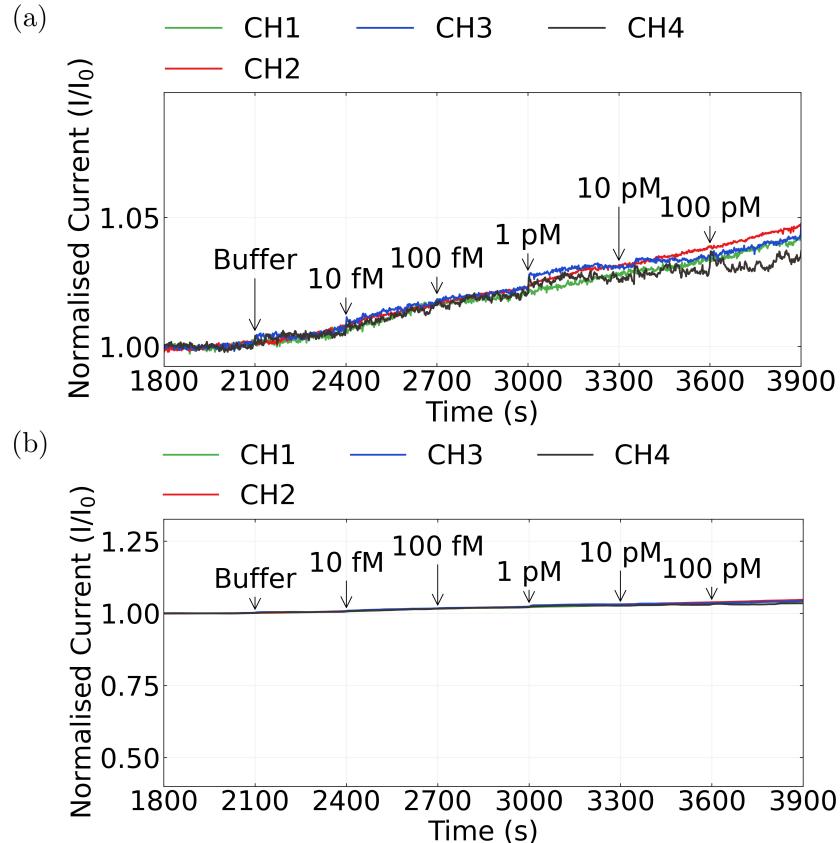


Figure 1.23.: A normalised sensing series across four multiplexed channels from a device functionalised with PBASE in DMSO to attach OR22a nanodiscs. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. The concentration of each 20 μL addition of methyl hexanoate in 1% v/v DMSO 1XPBS is indicated above the time of addition. Current data has been despiked and baseline drift removed in both (a) and (b), while a moving median filter has also been applied to the dataset shown in (b).

An atomic force microscope images of a film modified using DMSO in PBASE with OR22a nanodiscs is shown in Figure 1.24 (a), along with a mask in green indicating an

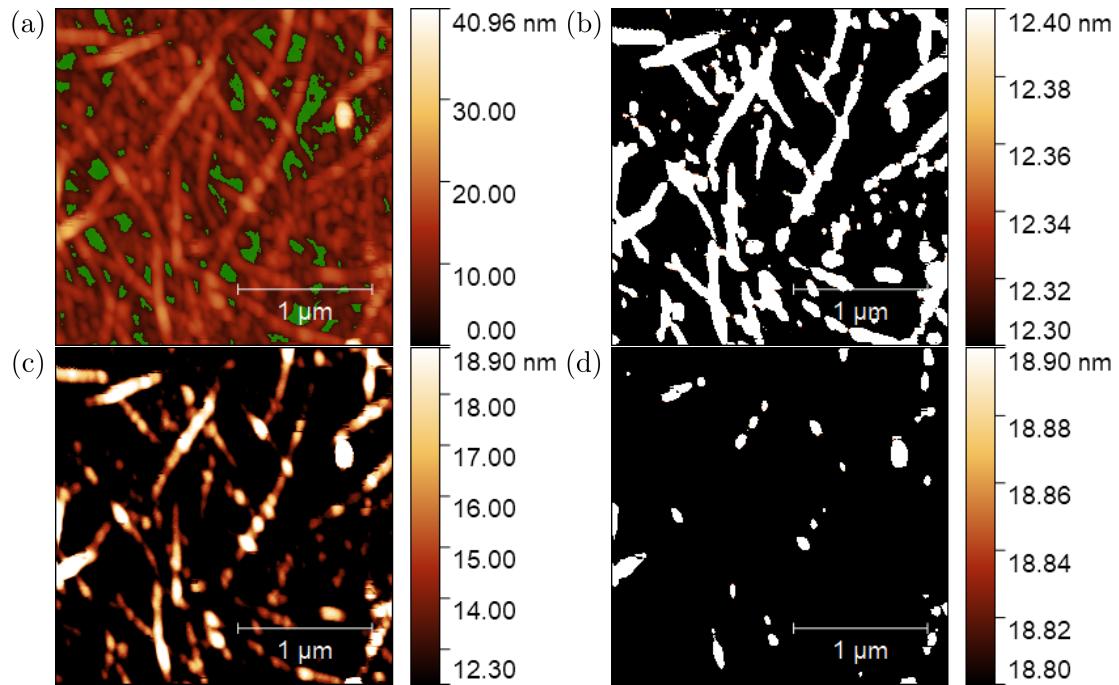


Figure 1.24.: Atomic force microscope images of a carbon nanotube film functionalised with OR22a nanodiscs using PBASE in DMSO. The average substrate height is shown in green in (a). A binary representation of the atomic force microscope image with a threshold height of 12.3 nm is displayed in (b). (c) shows features in the functionalised film across the height range 12.3 – 18.9 nm, while (d) shows another binary representation at 18.8 nm.

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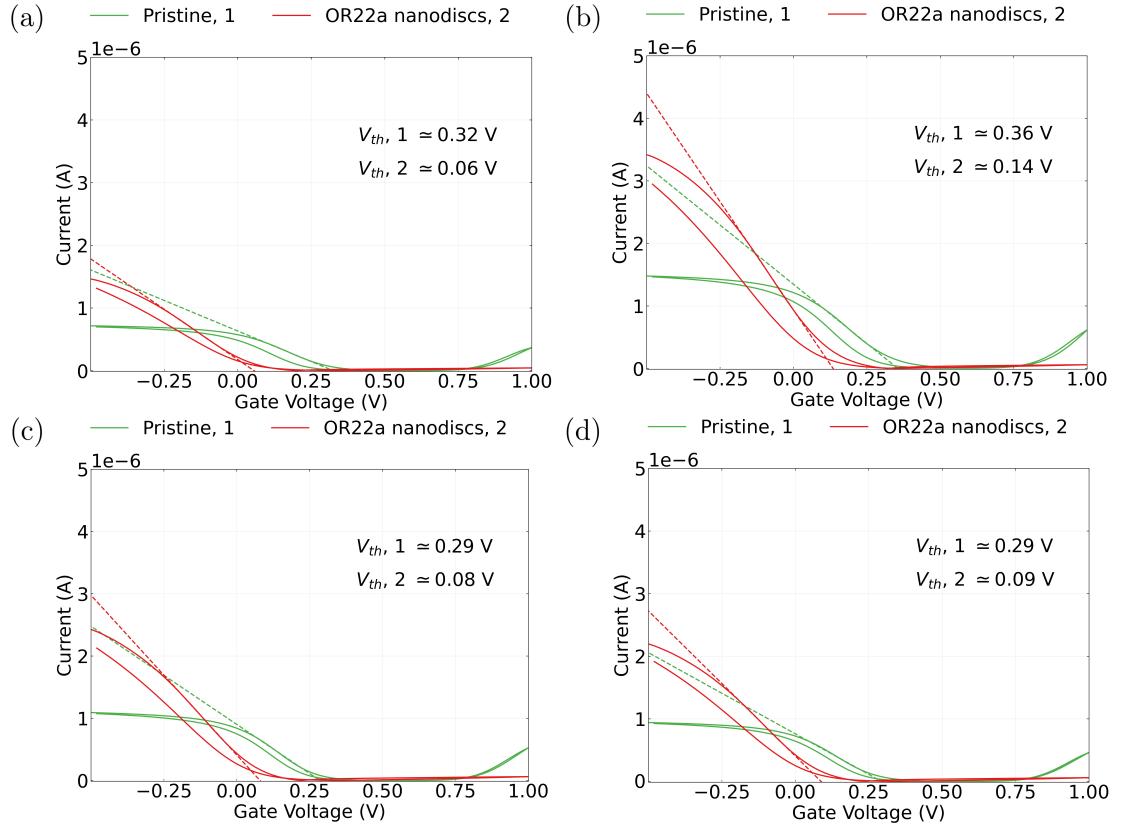


Figure 1.25.: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation using PBASE in DMSO. Source-drain current was $V_{ds} = 100$ mV for both the forward and reverse sweep. Each subfigure (a)-(d) corresponds to a different channel of the 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 characteristic curve. The threshold voltage associated with each curve is also shown.

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average substrate height of 3.6 ± 0.6 . The binary representation in Figure 1.24 (b) shows long, rounded features sit directly above the above the CNT threshold height of 12.3 nm. By extending the threshold height range to 12.3 – 18.9 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 [8]. Some features are too tall for the “pearls” on the “string” to be individually distinguished in Figure 1.24 (c), but Figure 1.24 (d) shows every rounded “pearl” along every “string” can be distinguished from one another at a taller threshold. These images strongly suggest nanodisc aggregates are present on the network, but does not necessarily indicate a direct connection between nanodiscs and the network.

The transfer characteristics of the four sensing channels are displayed in Figure 1.25. 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 1.2.2, -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 therefore seems that the nanodiscs are directly attached to 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.

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 [9]. 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 1.2.1, 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.

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- A solution of 20 mM EDC and 40 mM NHS in 1XPBS was prepared by dissolving 31 mg EDC and 46 mg NHS in 10 mL 1XPBS.

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

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

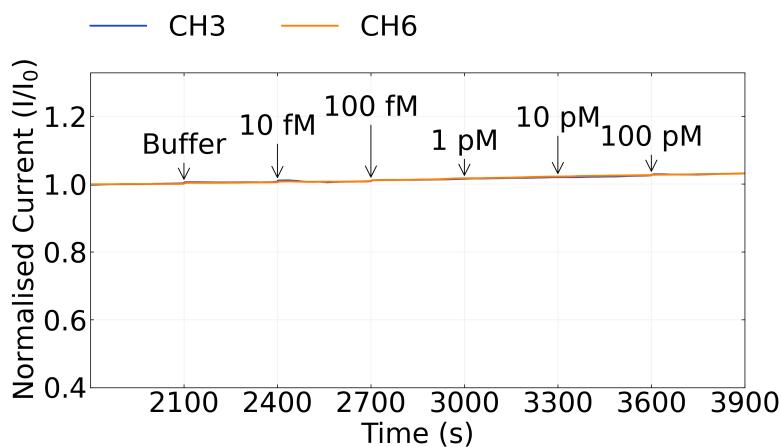


Figure 1.26.: A normalised methyl hexanoate (MeHex) sensing series taken with a OR22a-functionalised carbon nanotube device using PBA with EDC and NHS. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. The concentration of each 20 μL addition is indicated above the time of addition.

These steps were designed to be broadly similar to those seen in ?@sec-PBA, with an 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 device are shown in Figure 1.26. Again, any current response subsequent to additions is positive and <1%. An atomic force microscope image of a film modified in the same manner as the sensing device is shown in Figure 1.27 (a), with substrate height 3.9 ± 1.5 nm. When a suitable height range above the threshold height 12.6 nm is selected, as in Figure 1.27 (b), 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 1.28, 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

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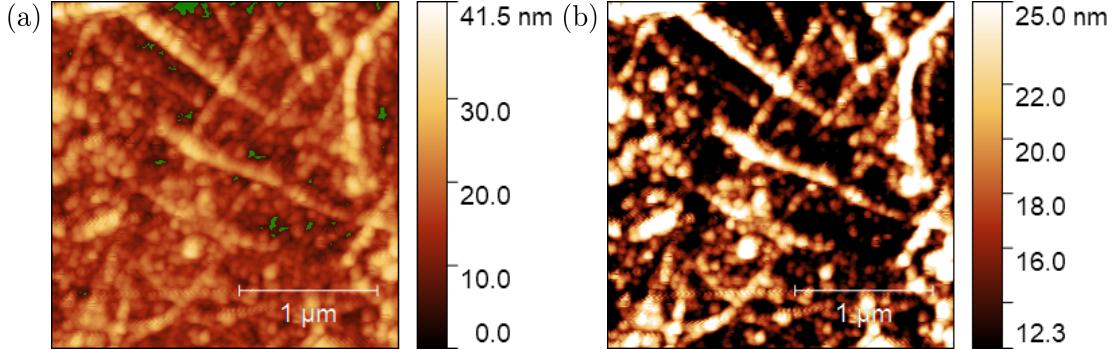


Figure 1.27.: $2.5 \mu\text{m} \times 2.5 \mu\text{m}$ atomic force microscope images of a carbon nanotube film functionalised with OR22a nanodiscs using PBA in DMSO with EDC and NHS. The average substrate height is shown in green in (a), while (b) shows features in the functionalised film across the height range $12.6 - 25.0 \text{ nm}$.

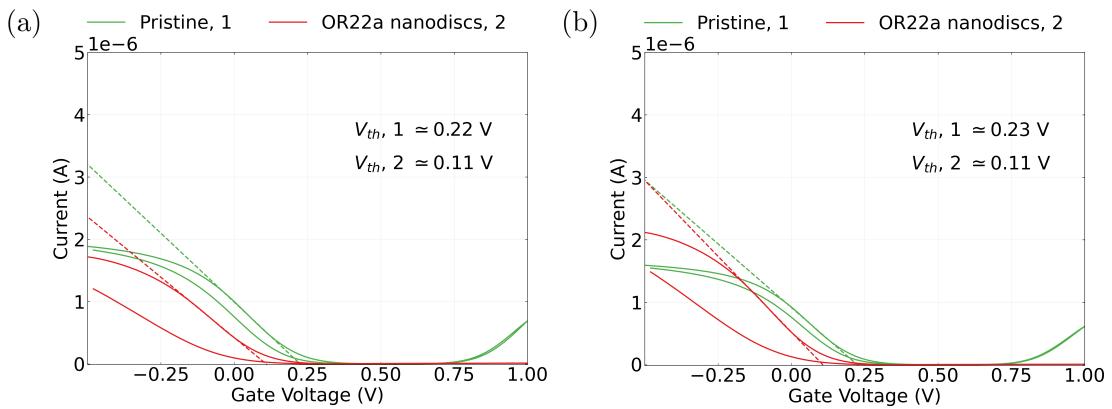


Figure 1.28.: Liquid-gated carbon nanotube network device transfer characteristics before and after OR22a nanodisc functionalisation using PBA in DMSO with EDC and NHS. Source-drain current was $V_{ds} = 100 \text{ mV}$ for both the forward and reverse sweep. Each subfigure corresponds to a different device channel, where (a) is channel 3 and (b) is channel 6. The dashed line shown is tangent to the subthreshold slope of the characteristic curve. The threshold voltage associated with each curve is also shown.

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device behaviour in Section 1.3.1 is not resolved by the use of a different solvent or 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. Finally, photoresist may remain on the device channels, even despite the use of a new method which eliminates photoresist to levels invisible under a fluorescence microscope ([?@sec-photoresist-contamination](#)). A further possible source of variability was also identified from the PBASE in DMSO functionalisation, where transfer measurements and AFM imaging both strongly suggested direct attachment of nanodiscs despite the device not functioning as a sensor: attachment of nanodiscs, but with minimal or no attachment of the contained odorant receptors. An altered non-covalent functionalisation method using pyrene-PEG-biotin was therefore developed to eliminate all of the above factors at once and investigate the resulting sensor behaviour. This novel method is trialled in the following section.

1.4. iOR Biosensing with Aqueous Functionalisation

1.4.1. Aqueous-Based Functionalisation

A carbon nanotube network field-effect transistor device, fabricated using post-June 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, and were avidin-tagged or “avi-tagged”. This eliminates the possibility of nanodiscs interfering with odorant receptor attachment, though does leave them vulnerable to harsh environmental conditions [4], [5]. Upon preparation, the odorant receptors were modified with pyrene-PEG-biotin. The pyrene-PEG-biotin linker attached to the avi-tags on the odorant receptors, as described in [?@sec-NTA-biotin-PEG](#), allowing them to attach directly from solution to the carbon nanotubes via the attached pyrene linker. This approach meant that no PBASE, PBA or organic solvent was needed in the functionalisation process. To eliminate the remaining possible sources of surface contamination, such as the hydrocarbonaceous coating of nanotubes and residual photoresist, 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.* [10], 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.

1.4. iOR Biosensing with Aqueous Functionalisation

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 1XPBS.

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 1XPBS into and from the vial.

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

Note: Oxygen plasma was very gentle, with an O_2 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 1XPBS for 15 s and thoroughly nitrogen dried.

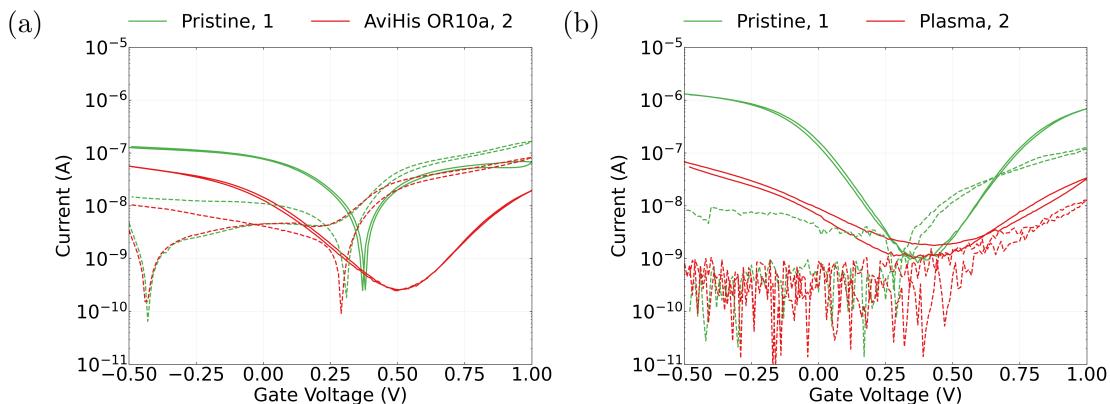


Figure 1.29.: Liquid-gated carbon nanotube network device transfer characteristics on a logarithmic scale before and after modification, where the gate current for each transfer curve is shown with a dashed line. Source-drain current was $V_{ds} = 100$ mV for both the forward and reverse sweep. The change 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 1.29 (a) shows the liquid-gated characteristics of a device channel (channel 2) before and after the functionalisation process with OR10a. The change of characteristics observed can be compared with the change of characteristics seen for a plasma clean only, seen in Figure 1.29 (b). A significant drop in channel occurs as a result of the plasma cleaning process. This large change in mobility makes it difficult to clearly identify changes in gating specifically due to the presence of the OR10a. The OR10a is not held

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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 1.30 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.

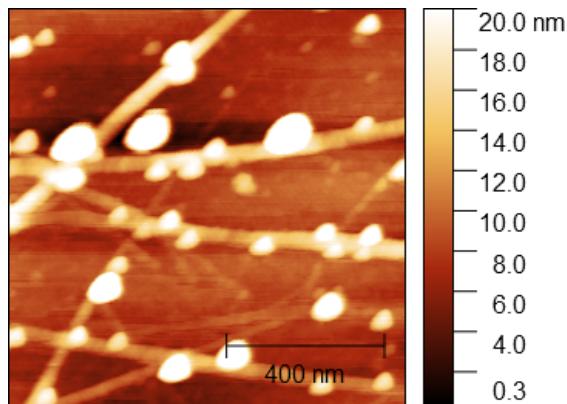


Figure 1.30.: $1 \mu\text{m} \times 1 \mu\text{m}$ atomic force microscope image of a solvent-deposited carbon nanotube film functionalised with OR nanodiscs using Pyrene-PEG-NHS.

1.4.2. Aqueous Sensing of Methyl Salicylate

The procedure used for sensing methyl salicylate (MeSal) was identical to that used in Section 1.2.2, except using MeSal instead of EtHex. Analyte solutions in 0.5% DMSO/1XPBS solution containing methyl salicylate concentrations at 1 fM, 1 pM, 1 nM and 1 μM were prepared beforehand. The same buffer was used for each dilution, as well as for the well, which contained 80 μL 0.5% DMSO/1XPBS prior to sensing. Sensing measurements were taken using the NI-PXIe system. The full control series plus sensing sequence is shown in Figure 1.31. Gate current remained negligible across the full sensing procedure, and no responses were seen to buffer additions or subtractions. A linear fit to the baseline drift in the region 1200 – 1800 s, shown in Figure 1.32 (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 1.32 (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 1.2.2 is observed, indicating that $t \ll \tau_i$ does not hold for the drift behaviour and further lending support to the possibility that functionalised devices exhibit characteristic long-term decay behaviour.

Cleaned and filtered methyl salicylate sensing data with linear baseline drift removed is shown in Figure 1.33 (a). The concentration of each 20 μL methyl salicylate addition

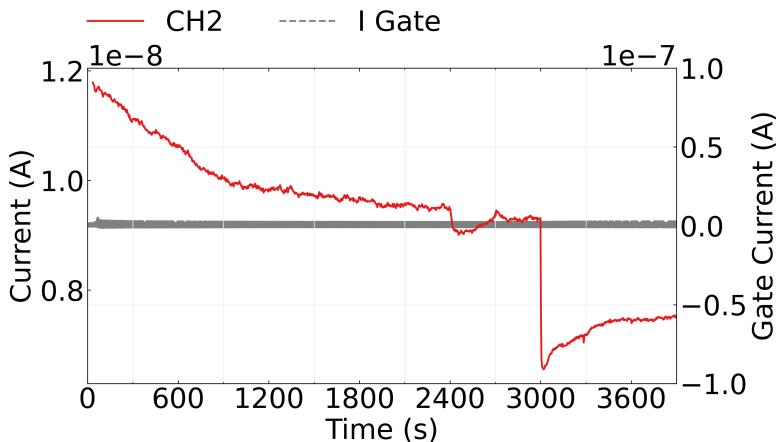


Figure 1.31.: The control series (before 1800 s) and methyl salicylate sensing series (after 1800 s) of the OR10a-functionalised device channel. Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. No responses to 0.5% v/v DMSO 1XPBS were seen during the control series, while significant responses to additions of methyl salicylate diluted in 0.5% v/v DMSO 1XPBS were seen at 2400 s and 3000 s.

is shown above each corresponding addition time. Significant and irreversible current decreases occurred directly after the 1 fM and 1 nM MeSal additions. Figure 1.33 (b) shows that 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 buffer addition, or 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 [1]. It appears that the OR10a odorant receptors saturate at a low concentration relative to the OR22a odorant receptors tested earlier. Transfer characteristics of the device before and after sensing are shown in Figure 1.34 (a), showing a threshold shift of -0.07 V.

The device was then rinsed for 15 s in 1XPBS after the initial sensing series. The effect of the rinse on the transfer characteristics of the device is illustrated in Figure 1.34 (b). The rinsing step appears to have largely restored the electrical characteristics of device to their state prior to sensing. It appears the gating effect due to the presence of analyte has been reversed. Assuming that this 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-electrical-characterisation](#)), this is also an indication that the responses are not simply due to adsorption of the DMSO present.

A second methyl salicylate sensing series was then performed with the same device,

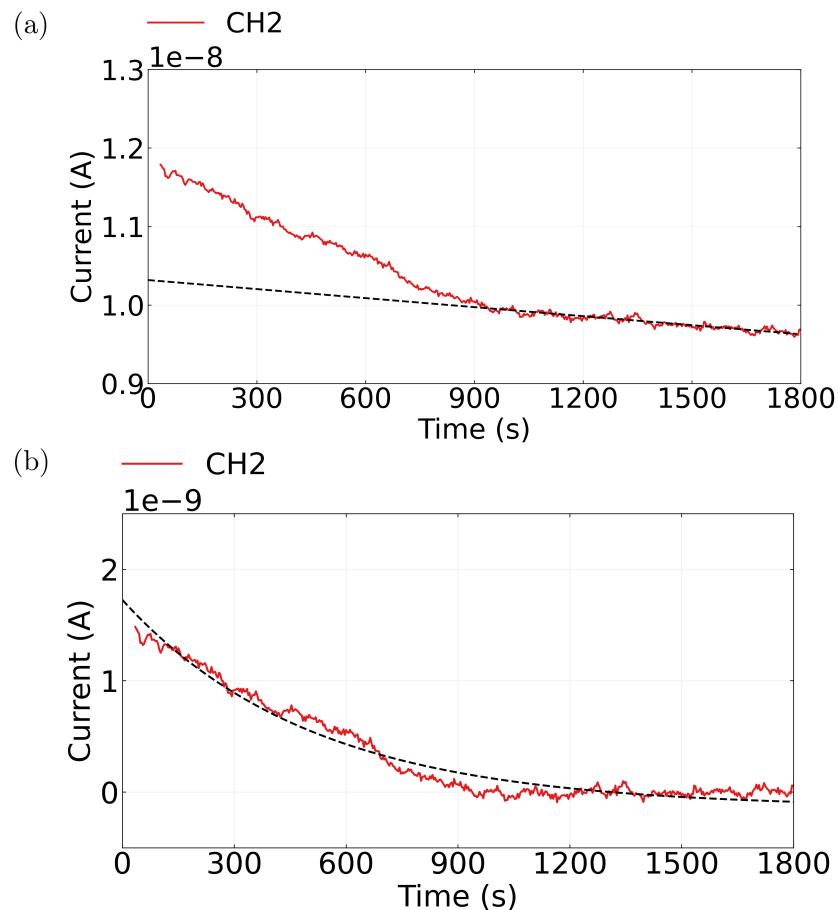


Figure 1.32.: Control series for the OR10a-functionalised device is shown in (a) alongside a linear fit to the control series from 1200 s onwards, where the fit has been extrapolated to 0 s, shown as a black dotted line. The control series with the linear approximation subtracted is shown in (b), fitted with an exponential function shown as a black dotted line.

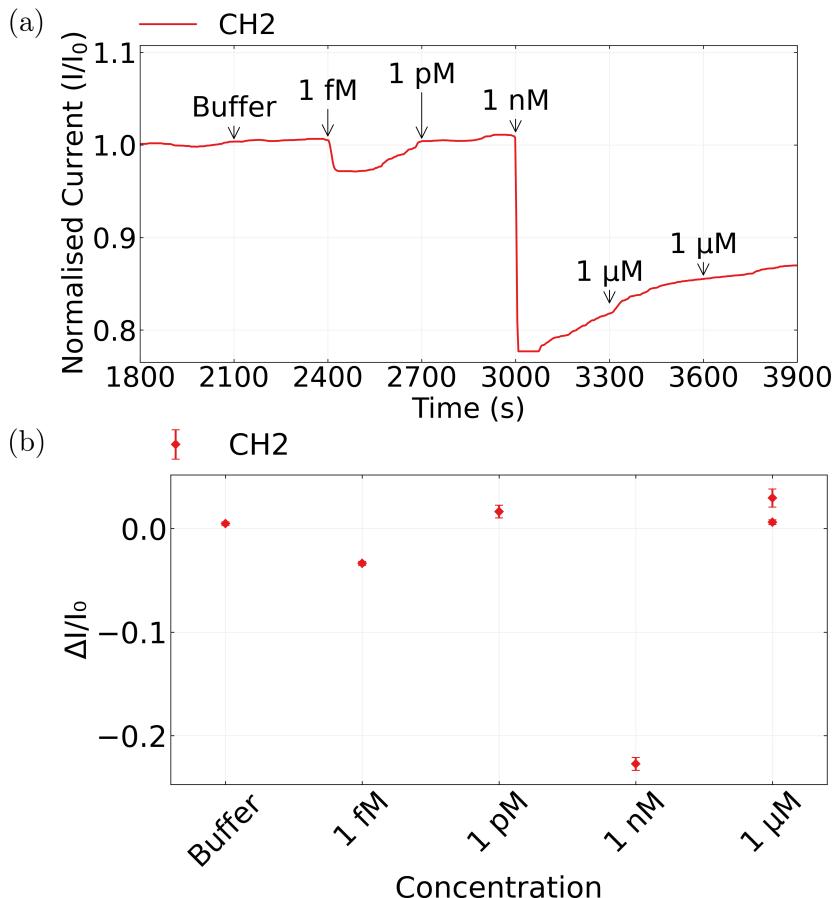


Figure 1.33.: The normalised sensing series for the OR10a-functionalised device is shown in (a), alongside the concentration of each 20 μ L addition. The current data has been despiked, with baseline drift subtracted and a moving median filter applied. The signal data corresponding to the mean difference in current before and after each addition is shown in (b).

1. Biosensing with Insect Odorant-Receptor Functionalised Carbon Nanotube Devices

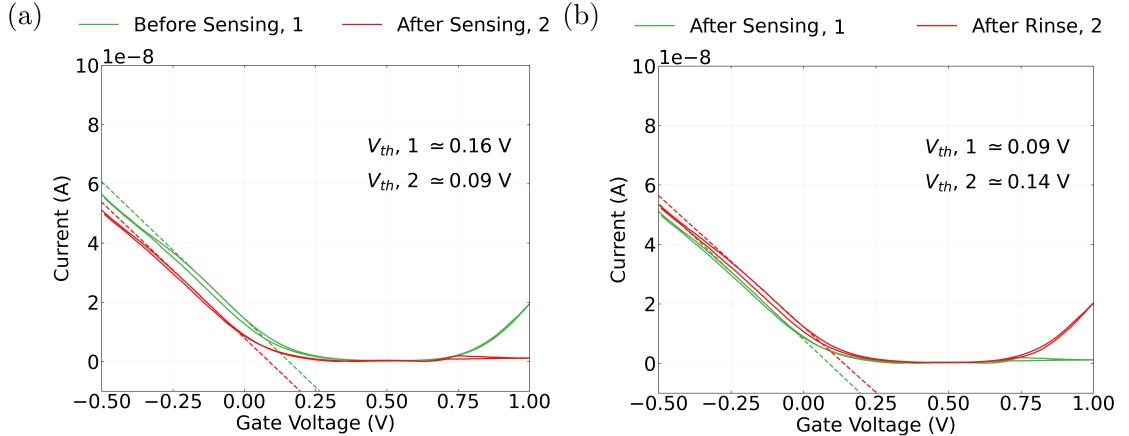


Figure 1.34.: Transfer characteristics of the device after the 1st sensing series, where $V_{ds} = 100$ mV. The transfer characteristics before and after sensing are shown in (a), while (b) shows the channel characteristics before and after being rinsed with 1XPBS.

shown in Figure 1.35 (a). The linear component of baseline drift from 1200 – 1800 s had a gradient of $c_1 = -0.31 \pm 0.01$ pA/s, similar that seen in the previous sensing run. A current response was seen directly after all five analyte additions, shown in Figure 1.35 (b). Only nanomolar additions of methyl salicylate were used to avoid saturating the proteins and reducing the sensitivity of the sensor. Interestingly, the initial 1 nM analyte addition was similar in size (24%) to the current change resulting from the 1 nM analyte addition in Figure 1.33 (23%). This result indicates that the size of responses may correspond to the well concentration in a reproducible manner, where there is a specific change in well concentration that gives the largest response. Five successive 20 μ L 1 nM methyl salicylate additions to the 100 μ L buffer in the well results in a well concentration of 500 pM MeSal. Given the steady decrease in analyte response size with successive additions in Figure 1.35, it appears that the sensor saturates at close to 500 pM, which may explain the lack of response to micromolar additions in Figure 1.33.

It also appears that the second sensing series resulted in a similar shift in transfer characteristics to that of the first series. Figure 1.36 shows a shift in threshold voltage of -0.08 V between the transfer characteristics directly before and directly after the nanomolar sensing series. This is another indication that nanomolar additions are enough to cause device saturation. The similarity between Figure 1.34 (a) and Figure 1.36 suggests a similar phenomenon has occurred during both sensing series, 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 buffer solution, without the need for the nanodisc format.

This method has a number of advantages over the OR nanodisc functionalisation method

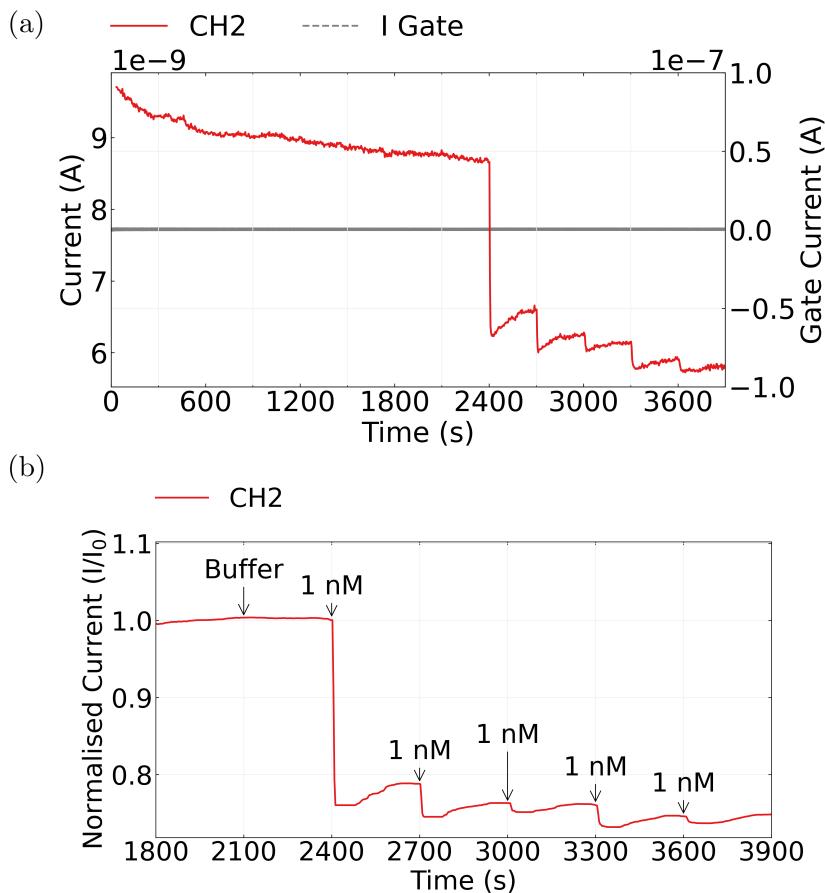


Figure 1.35.: A second methyl salicylate sensing series was performed with the OR10a-functionalised device channel subsequent to rinsing, shown in Figure 1.35 (a). Source-drain current was set at $V_{ds} = 100$ mV while gate current was set at $V_g = 0$ V. No responses to 0.5% v/v DMSO 1XPBS additions or significant gate current leakage were observed in (a). A clear response is observed after each nanomolar addition of methyl salicylate in (b). The current data in (b) has had a moving median filter applied and linear baseline drift removed.

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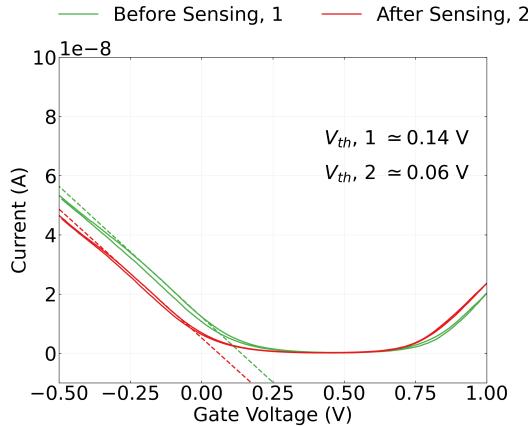


Figure 1.36.: Transfer characteristics of the device directly before and after the 2nd sensing series, where $V_{ds} = 100$ mV.

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 drawbacks. Oxygen plasma cleaning, even at low power, had a invasive 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 (**?@sec-non-covalent-bonding**), this approach seems self-contradictory. It appears a less invasive but solvent-free approach should be identified for non-covalent functionalisation [11], or a well-established covalent approach should instead be used for attaching odorant receptors (**?@sec-sensor-types**).

1.4.3. Vapour Sensing of *trans*-2-hexen-1-al

Despite the drawbacks outlined previously, the functionalisation method in this section was also used to trial vapour phase sensing for the odorant receptor devices. As the vapour pressure of methyl salicylate was too low for reliable vapour system delivery, OR35a (batch number AviHis-OR35a-001, prepared 12 months earlier) was used in the functionalisation process instead of OR10a. OR35a responds to *trans*-2-hexen-1-al (E2Hex) [1], which is suitable for use in the vapour delivery system. Back-gated transfer characteristics of a OR35a-functionalised before and after functionalisation are shown in Figure 1.37 (a), alongside characteristics from a device before and after plasma cleaning at 5 W without subsequent functionalisation in Figure 1.37 (b). As expected, a significant drop in mobility is seen as a result of the plasma cleaning step. Unlike

1.4. iOR Biosensing with Aqueous Functionalisation

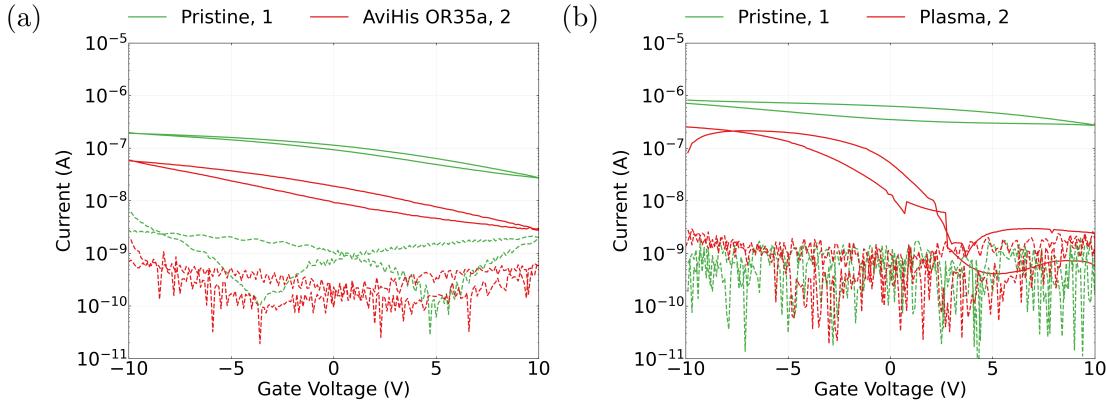


Figure 1.37.: Back-gated carbon nanotube network device transfer characteristics on a logarithmic scale before and after modification, where the gate current for each transfer curve is shown with a dashed line. Source-drain current was $V_{ds} = 100$ mV for each of the forward and reverse sweep. The change in characteristics from avi-tagged OR35a functionalisation is shown in (a), while the change resulting from a 5 W plasma clean is shown in (b).

the functionalised device, the device plasma cleaned without functionalisation turns off entirely at $V_g \sim 3$ V, with this difference possibly due to variability in the results of plasma etching for different carbon nanotube devices.

In a similar manner as in ?@sec-pristine-EtHex, the functionalised device was back-gated in the vapour delivery system and a series of exposures to E2Hex was performed. Another pristine device channel was measured during the same sensing series for a baseline comparison. Before each series was performed, the vapour system was purged of vapour, the total dilution flow was set at 200 sccm (nominal) and flow to the PID was set to 150 sccm on the flowmeter. In line with the discussion in ?@sec-vapour-drift, a 2500 s control series was used. The baseline drift is shown in Figure 1.38, with a back-gate of $V_g = 0$ V and a source-drain voltage of $V_{ds} = 100$ mV across the channel during each measurement. During this period, 200 sccm of nitrogen flow was placed through the device chamber. A linear fit $I = c_1 t + c_2$ was performed on the measurements from each channel between 2150 – 3350 s. For the pristine device, $c_1 = -1.29 \pm 0.01$ pAs $^{-1}$, while for the OR35a-functionalised device, $c_1 = -0.55 \pm 0.01$ pAs $^{-1}$. Figure 1.38 (b) shows the exponential fit $I = I_0 \exp(-t/\tau)$ to each dataset with linear drift subtracted, where the time constant for the pristine device was $\tau = 963 \pm 25$ s and for the OR35a-functionalised device was $\tau = 704 \pm 4$ s.

After the 2500 s control series, each device was exposed to three intervals of *trans*-2-hexen-1-al vapour flow from the carrier line in the same manner. A total of 5 mL of the analyte of interest was present in the analyte bottle before each sensing series. A total flow of 200 sccm was kept through the chamber at all times. During each interval, 150 sccm of this total flow passed through the carrier line. Except during intervals, no flow

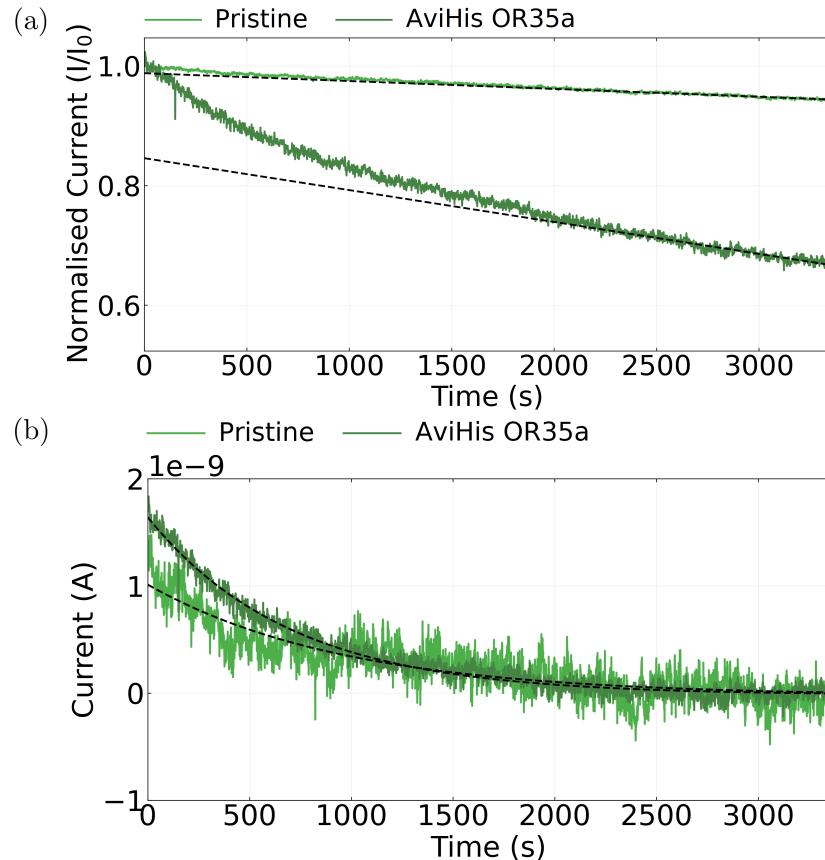


Figure 1.38.: The baseline drift data across the first 2500 s of measurement for both a pristine and OR35a-functionalised device is shown in (a), normalised with respect to the initial datapoint for a clear comparison. A linear fit to the baseline drift data between 2150 – 3350 s is indicated in (a) with a black dashed line. The difference between the raw dataset and linear fit is shown in (b), with an exponential fit to the data indicated with a black dotted line.

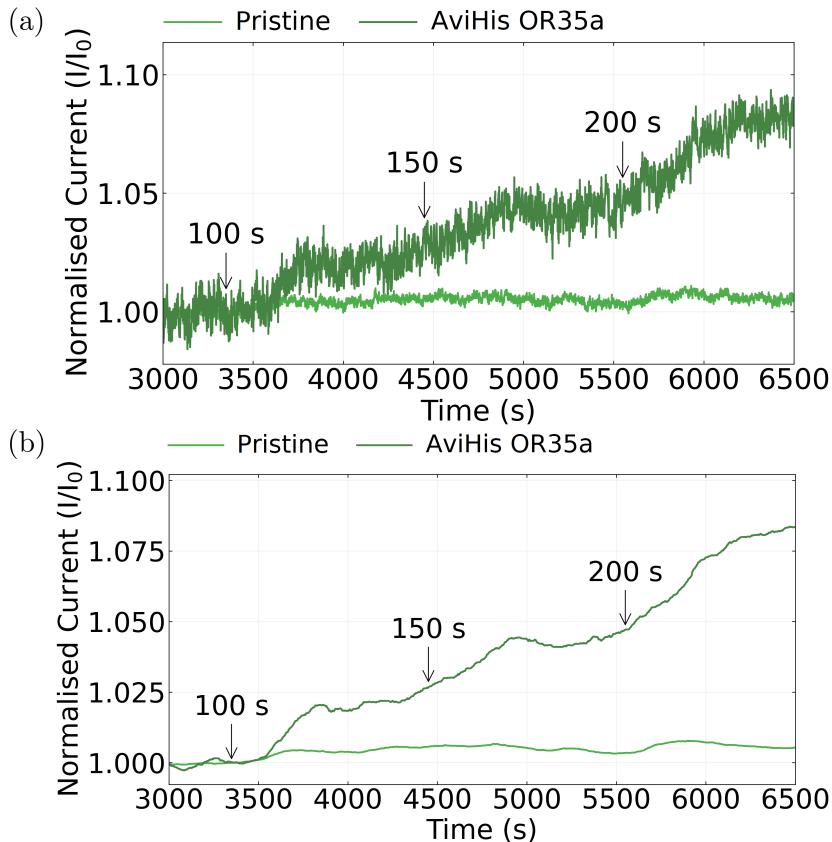


Figure 1.39.: Device channel responses to intervals of flow from the carrier line into the vapour delivery system chamber, showing responses by both the pristine and OR35a-functionalised devices. Intervals begin at 3350 s, 4450 s and 5600 s. The length of each interval is indicated above the corresponding normalised current response to *trans*-2-hexen-1-al (E2Hex) in (a). The dataset after applying a moving median filter is shown in (b).

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was placed through the carrier line. A 1000 s recovery period was used between each interval. The temperature was $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during each series. These sensing series, which have been normalised, despiked and corrected for drift, are shown side-by-side in Figure 1.39 (a), while Figure 1.39 (b) shows these series after a moving median filter was applied. A larger current change is shown by the functionalised device than the pristine device after each addition. The PID indicated a similar concentration of E2Hex was present in the device chamber with each addition, shown in Figure 1.40. Note that measurements were taken two days apart; the $\pm 50\%$ variation in chamber concentration reached with each interval is largely due to PID span drift ([?@sec-responses-to-vapour](#)).

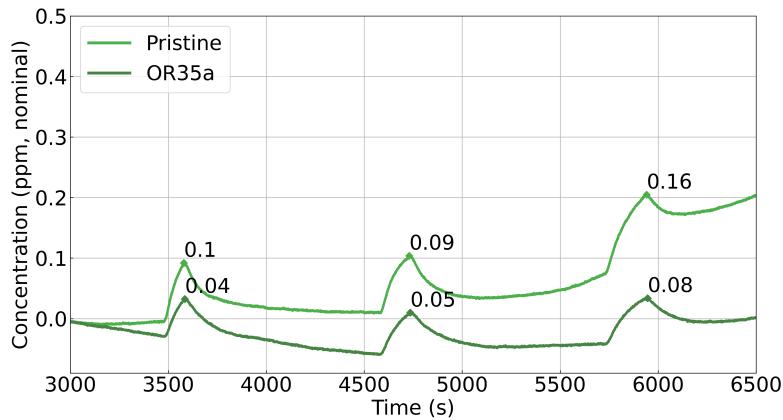


Figure 1.40.: Nominal concentration measurements by the photoionisation detector taken from the device chamber during the vapour sensing series for both pristine and OR35a-functionalised devices. The maximum nominal concentration reached during each interval of carrier flow is indicated above each peak.

The current responses seen are not the sharp, clear responses to analyte of the liquid-gated device seen in Section 1.4.2. Instead, they seem more similar to the responses by bare carbon nanotubes to vapour in [?@sec-vapour-series](#). There are also alternative explanations for the current changes observed in Figure 1.39 which do not involve specific detection of analyte. The functionalisation may have altered the drift behaviour so that the linear approximation used here for removing baseline drift is no longer useful. The breakdown in this approximation has then led to current increases that do not directly correspond to analyte being added. The functionalisation process may also have caused the device to become more sensitive to changes in vapour concentration, increasing the signal-to-noise ratio of the sensor. Regardless of their origin, the current increases observed cannot fairly be described as clear or convincing sensing responses. A working sensor should exhibit responses more similar to those seen in Section 1.4.2.

It may be necessary to ensure the sensing environment is similar to that used for aqueous sensing to achieve reliable sensing. A buffered environment may help to stabilise the

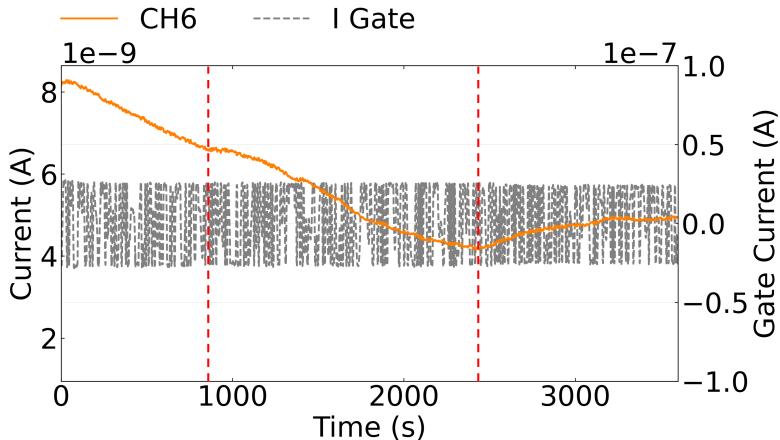


Figure 1.41.: Electrical samples from a carbon nanotube transistor in the vapour delivery system device chamber. Transistor voltages were $V_g = 0$ V and $V_{ds} = 100$ mV. During the measurement, a PDMS well containing 50 μL 0.5% v/v DMSO 1XPBS was present on the device surface. Significant changes in baseline drift behaviour are indicated with red dotted lines.

unprotected odorant receptors, and so a PDMS well containing 50 μL 0.5% v/v DMSO 1XPBS was attached to the device for vapour sensing. Despite the well being upside-down in the vapour delivery system, the hydrophobicity of the PDMS was sufficient to store the buffer securely. Current sampling over a 1 hour period in this configuration, with 200 sccm constant flow across the surface of the well, is presented in Figure 1.41. If the well solution was leaking out of the well, the conductive liquid could form a short between the channel electrodes and backgate, prompting a large increase in gate current. However, gate current remains stable across the entire measurement period, indicating the well remains secure. Conversely, fluctuations in drain current became apparent after ~ 800 s, and at ~ 2400 s, the direction of baseline drift was reversed entirely. These undesirable changes appear to result from rapid evaporation of liquid in the well; when the device chamber was opened, only $\sim 10\text{-}20 \mu\text{L}$ of buffer was present.

For readily reproducible vapour sensing, therefore, one of two approaches present themselves. The first approach is to identify a covalent attachment mechanism that allows for specific attachment to odorant receptors via a histidine or avidin tag. This approach allows for a protective format like nanodiscs to be used in the sensing setup while eliminating concerns around non-specific binding of the protective membrane protein. This is the more traditional approach, previously used for vapour sensing of eugenol with mammalian ORs [12]. Alternatively, a similar process to the one investigated here could be used. This non-covalent approach would require the development of a highly reproducible cleaning process, able to remove a variety of organic contaminants from the carbon nanotube surface prior to functionalisation. Furthermore, a liquid capture layer which is both biofriendly and has a low vapour pressure may need to be identified and used. Ideally, this liquid would be miscible with a wide range of substances, but repel

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PDMS. Further discussion of potential capture layers can be found in [?@sec-future-work-vapour](#).

1.5. 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 decreases in current level across the sensor channel by up to $\sim 45\%$ were observed in response to analyte additions. Short-term drift behaviour was found to be similar to that of pristine devices, where the fast-decay exponential had a time constant of 590 ± 3 s, but it appears functionalisation may affect long-term baseline drift. Quantitative changes resulting from sensor functionalisation included a device threshold voltage shift of -0.20 ± 0.03 V and a change in network height above the substrate by up to ~ 5 nm, both resulting from the presence of nanodiscs on the network. Additionally, 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. This result indicates protein attachment to the channel is not limited to empty nanodiscs. As far as the author is aware, this is the first time fluorescence has been used to verify the presence of odorant receptors on a carbon nanotube network.

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 above the substrate of up to ~ 20 nm relative to a pristine film, the average threshold shift was -0.06 ± 0.02 V, 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, with a change in network height above the substrate of up to ~ 10 nm relative to a pristine film, but resulted in a threshold shift of ~ -0.27 V. It therefore appears that nanodiscs are sometimes being blocked from attaching to the carbon nanotube network in a manner that alters the electrical characteristics of the carbon nanotube network.

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 hanging the morphology of the carbon nanotube network and using a graphene device led to consistent negative

1.5. Conclusion

current responses. Furthermore, the average threshold shift for the highly-bundled device after functionalisation was -0.02 ± 0.01 V, indicating 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 multiple confounding factors are at play. 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 preventing hydrolysis.

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 gently oxygen plasma cleaned before functionalisation. 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 appears the long-term baseline drift was affected by the functionalisation of the device, where the linear approximation used was relatively weak.

This aqueous functionalisation approach was also used for vapour sensing with OR35a odorant receptors. Clear responses consistent with those seen in an aqueous environment were not observed. The changes in current seen appear more likely to result from a breakdown of the linear approximation of baseline drift for functionalised devices, or from increased non-specific sensitivity of the device post-functionalisation. It was concluded that reproducible vapour sensing would require either a robust covalent functionalisation method which used a protective membrane-like format, or alternatively from using a capture layer to protect non-covalently odorant receptors from the harsh chamber environment. However, it was found 0.5% v/v DMSO 1XPBS could not act as this capture layer due to rapid evaporation of the layer having a significant effect on baseline drift from ~ 800 s onwards. An alternative liquid should therefore be identified which is biofriendly and miscible while having a low vapour pressure. It should also repel PDMS, to remain contained in the PDMS well upside down in the device chamber. These approaches significantly expand the scope of this work, and are therefore not addressed extensively in this thesis. However, some preliminary work on this topic is discussed in **?@sec-future-work**.

A. Vapour System Hardware

Table A.1.: Major components used in construction of the vapour delivery system described in this thesis.

Description	Part No.	Manufacturer
Mass flow controller, 20 sccm full scale	GE50A013201SBV020	MKS Instruments
Mass flow controller, 200 sccm full scale	GE50A013202SBV020	MKS Instruments
Mass flow controller, 500 sccm full scale	FC-2901V	Tylan
Analogue flowmeter, 240 sccm max. flow	116261-30	Dwyer
Micro diaphragm pump	P200-B3C5V-35000	Xavitech
Analogue flow controller, for micro diaphragm pump	X3000450	Xavitech
10 mL Schott bottle	218010802	Duran
PTFE connection cap system	Z742273	Duran
Baseline VOC-TRAQ flow cell, red	043-951	Mocon
Humidity and temperature sensor	T9602	Telaire
Enclosure, for humidity and temperature sensor	MC001189	Multicomp Pro

B. Python Code for Data Analysis

B.1. Code Repository

The code used for general analysis of field-effect transistor devices in this thesis was written with Python 3.8.8. Contributors to the code used include Erica Cassie, Erica Happe, Marissa Dierkes and Leo Browning. The code is located on GitHub and the research group OneDrive, and is available on request.

B.2. Atomic Force Microscope Histogram Analysis

The purpose of this code is to analyse atomic force microscope (AFM) images of carbon nanotube networks in .xyz format taken using an atomic force microscope and processed in Gwyddion (see [?@sec-afm-characterisation](#)). It was originally designed by Erica Happe in Matlab, and adapted by Marissa Dierkes and myself for use in Python. The code imports the .xyz data and sorts it into bins 0.15 nm in size for processing. To perform skew-normal distribution fits, both *scipy.optimize.curve_fit* and *scipy.stats.skewnorm* modules are used in this code.

B.3. Raman Spectroscopy Analysis

The purpose of this code is to analyse a series of Raman spectra taken at different points on a single film (see [?@sec-raman-characterisation](#)). Data is imported in a series of tab-delimited text files, with the low wavenumber spectrum ($100\text{ cm}^{-1} - 650\text{ cm}^{-1}$) and high wavenumber spectrum ($1300\text{ cm}^{-1} - 1650\text{ cm}^{-1}$) imported in separate datafiles for each scan location.

B.4. Field-Effect Transistor Analysis

The purpose of this code is to analyse electrical measurements taken of field-effect transistor (FET) devices. Electrical measurements were either taken from the Keysight 4156C Semiconductor Parameter Analyser, National Instruments NI-PXIe or Keysight B1500A Semiconductor Device Analyser as discussed in [?@sec-electrical-characterisation](#);

B. Python Code for Data Analysis

the code is able to analyse data in .csv format taken from all three measurement setups. The main Python file in the code base consists of three related but independent modules: the first analyses and plots sensing data from the FET devices, the second analyses and plots transfer characteristics from channels across a device, and the third compares individual channel characteristics before and after a modification or after each of several modifications. The code base also features a separate config file and style sheet which govern the behaviour of the main code. The code base was designed collaboratively by myself and Erica Cassie over GitHub using the Sourcetree Git GUI.

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