Towards Vapour Detection with an Insect Odorant Receptor Bioelectronic Nose

by

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Acknowledgements

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Rifat, Alex - vapour sensor Erica Cassie - FET sensing setup Rob Keyzers and Jennie Ramirez-Garcia - NMR spectra Patricia Hunt - Computational chemistry

1. Odorant Receptor Biosensors with Carbon Nanotube Network and Graphene Transistors

1.1. Introduction

In **?@sec-thin-film-transistors**, it was established that as carbon nanotubes and graphene are highly sensitive and are easily modified with biomaterials, they make an ideal platform for biosensing [1], [2]. In the early 2000s, it was established that sensitive and selective biosensors could be created by modifying a carbon nanotube field-effect transistor channel with protein receptors [1], [3]. In the following two decades, a wide range of other biological receptors have been attached to carbon nanotube FETs and graphene FETs for the creation of biosensors, including enzymes [4]–[6], antibodies [7]–[9] and aptameric DNA [10]–[12]. These miniaturised 'lab on a chip' devices are of particular interest due to their low cost, rapid use time, simple operation and small size compared with more traditional biological analysis methods [1]. It is hoped that these sensors could be deployed outside the laboratory in a range of front-line settings requiring rapid and reliable detection [13]. Specific examples include rapid diagnostic testing at a busy medical clinic [14], or the mass detection of biological threats across a large shipment of imported fruit [15], [16].

Rapid developments in this biosensor technology and parallel developments in the understanding of animal olfaction led to these transistors being used in bioelectronic nose applications from the late 2000s onwards [17]–[20]. 'Bioelectronic nose' is a general term which refers to the use of an biologically-modified electronic array to detect specific odor traces in a highly selective and sensitive manner [13]. As the name suggests, odor response signals should be highly similar to the electrochemical signals received by olfactory neurons in an animal nose [13]. Metal oxide transistors were first used to detect odorants in a mammal-like bioelectronic nose in the early 1980s [21]. A biomimetic approach to bioelectronic nose development couples the CNT FET or GFET signal-amplifying transducer element with elements of the animal olfactory system. These sensitive elements include olfactory cells [22], odorant binding proteins (OBPs) [23], [24] and odorant receptor proteins (olfactory receptors, ORs) [25], [26]. The aim for novel olfactory-based biosensors is to detect volatile odors in the air at ppb or ppt concentrations, giving it the same utility as an animal nose [13].

1.2. Odorant Receptors

1.2.1. *In vivo* Structure and Function

Odorant receptors (ORs) are an essential part of the olfactory systems of most animals, including humans. ORs let us distinguish between thousands of distinct volatile chemical traces, interpreted as odors [13], [27]. Vertebrate odorant receptors are part of a group of seven-transmembrane proteins known as G-protein coupled receptors (GPCRs). These ORs undergo a change in conformation in response to the binding of a specific range of target compounds, which leads to activation and dissociation of the G-protein within the cell [13], [27], [28]. Intracellular signalling events triggered by G-protein dissociation create an action potential, which is transmitted by neuronal pathways to the animal brain and interpreted as an odor [13], [27].

1.2.2. Artificial Membranes

Odorant receptors are transmembrane proteins, and therefore require stabilisation from a lipid membrane to preserve their structure when solubilised [13], [29]. Odorant receptors can be expressed in cells and isolated alongside with the native cell membrane for sensor use, or embedded in an artificial lipid membrane format that mimics a cell membrane. These artificial membranes include micelles, nanovesicles (including nanoliposomes), lipid cubic phases, amphipols, and nanodiscs. The insoluble transmembrane proteins can also be held in a specific detergent environment to preserve their structure and function [13], [29].

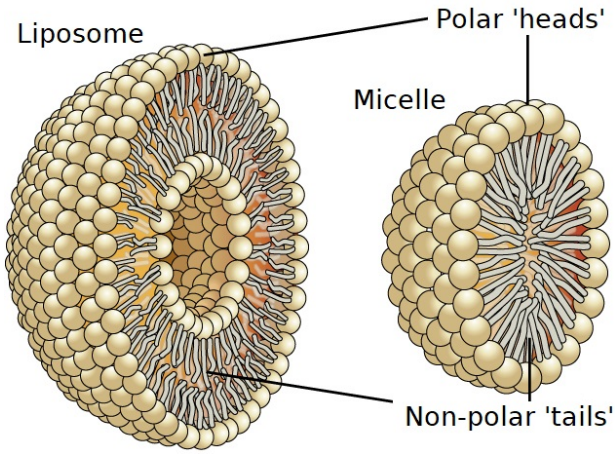

Figure 1.1.: Liposomes and micelles are made up of a lipid membrane, which acts as a substitute for the cell membrane *in vitro*. Adapted from [30].

Amphipols (Apols) are synthetic polymers with a hydrophilic backbone and grafted hydrophobic side chains. This gives the polymer an amphiphilic nature, enabling them

to stability bind the transmembrane protein into a soluble complex. This configuration has been used to non-covalently immobilise a transmembrane protein for surface plasmon resonance via strepavidin-biotin bonding [29].

Nanodiscs have a particularly high stability compared to other formats as their disc-shaped lipid bilayer is encompassed by an membrane scaffold protein (MSP) [31], [32]. The amphiphilic membrane scaffold protein protects the exposed hydrophobic side chains of the nanodisc when solubilised[29]. The relative stability of this format means it is frequently used in the fabrication of biosensor devices, which are often described as being particularly reliable and long-lived [25], [33]–[35]. Another advantage of nanodiscs is that the membrane scaffold protein can be attached to biosensor surfaces at specific affinity tags, for example, the MSP hexahistidine tag ('his-tag') [29], [32]. Unlike other artificial membranes, there is also little variation between the size of individual nanodiscs [29], [31]. Depending on the type of MSP used, a nanodisc measures between 10-20 nm across and can hold either a single or several odorant receptors [31], [32]. Nanodiscs have been found to be significantly less prone to non-specific binding (see Section 1.5) than micelles [29].

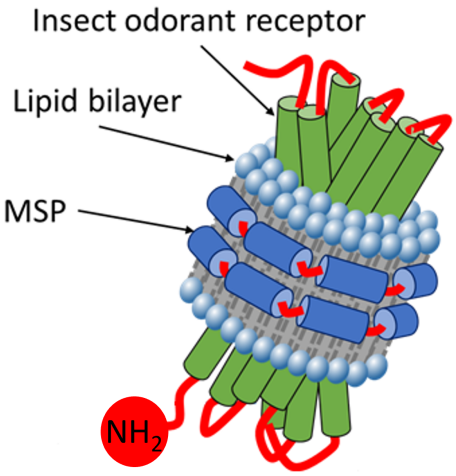

Figure 1.2.: A nanodisc containing an insect odorant receptor transmembrane protein. MSP — membrane scaffold protein. Reproduced with permission from [36].

1.3. Odorant Receptor Carbon Nanotube and Graphene Biosensors

1.3.1. Sensor Functionalisation

Odorant receptors are highly sensitive and selective, making them suitable as the biological sensing elements in a bioelectronic nose [13]. There are multiple advantages to

the use of odorant receptors over a whole olfactory cell, including their smaller size and relatively high stability [13]. Odorant receptors can be expressed and isolated for sensor use using heterologous cell systems, where a host cell replicates a protein from transfected RNA or DNA material. The most commonly used expression cells are human embryonic kidney (HEK) cells, *E. Coli* bacteria and *S. cerevisiae* (baker's yeast) [13].

For a bioelectronic nose to operate, sufficient coupling must exist between the bioreceptor element and the sensor transducer. Odorant receptors can be directly attached by physical adsorption; however, this approach is difficult to control, and can result in weak coupling between the odorant receptors and the transducer [13]. Alternatively, a bifunctional linker element may mediate the attachment between functional groups of the bioreceptor and the carbon-ring surface of the transducer in a biochemical process referred to as functionalisation [37]. In this thesis, the amino functional group is of particular interest, but there are many other nucleophilic functional groups available for binding, including carboxyls, hydroxyls, thiols/sulfhydryls, phenols, imidazoles and so on [13], [29]. The linker chemical interacts with the transducer either through stronger covalent bonding or weaker non-covalent bonding. The relative advantages and disadvantages of each type of receptor immobilisation can be found in Table 1.1, while a more thorough comparison of covalent and non-covalent linker functionalisation can be found in **?@sec-noncovalent-functionalisation**.

Table 1.1.: A comparison of the advantages and disadvantages of different approaches for immobilising odorant receptors onto carbon nanotube or graphene transducers. (Simplicity = the amount of cost, time and effort involved in functionalisation; Stability = the ability for the sensor to operate over a long time and under a range of conditions; Specificity = the ability to attach the receptor in a controlled and directional manner; Strength = the strength of attachment between receptor and transducer; Synergy = the ability for the receptor to attach without negatively impacting transducer operation or restricting receptor activity.)

Attachment Type	Simplicity	Strength	Specificity	Stability	Synergy
Direct Adsorption	High	Low	Low	Low	Medium
Linker, non-covalently tethered	Medium	Medium	Medium	Medium	High
Linker, covalently tethered	Medium	High	High	High	Low

Table 1.2.: Summary of past fabrication methods for odorant receptor-functionalised carbon nanotube and graphene biosensors. PBASE = 1-pyrenebutanoic acid N-hydroxysuccinimide ester, GA = glutaraldehyde, DAN = 1,5-diaminonaphthalene, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4 methylmorpholinium chloride, NTA = nitrilotriacetic acid, PDL = poly-D-lysine, Ab = Antibody fragments, CNTFET = carbon nanotube field-effect transistor, GFET = graphene field-effect transistor, TX = transfer characteristics.

Attachment	Attachment Method	References	Transducer	OR Type	OR Format	Verification	LOD
Direct	Vacuum-drying	Kim, 2009. [38]	CNTFET	Human	Cell membrane	TEM	100 fM
Non-covalent	GA-conjugated DAN	Park, 2012. [20]	GFET	Human	Cell membrane	TX, SEM	0.04 fM
		Lee, 2012. [19]	CNTFET	Human	Cell membrane	Fluorescence	1 fM
		Kwon, 2015. [39]	GFET	Human	Cell membrane	TEM	0.1 fM
		Goodwin, 2021. [40]	GFET	Human	Cell membrane	AFM, Raman	0.5 pM
	PBASE	Murugathas, 2019. [36]	CNTFET	*Insect*	Nanodiscs	TX, AFM	1 fM
		Murugathas, 2020. [26]	GFET	*Insect*	Nanovesicles, Nanodiscs	TX, AFM	1 fM
		Ahn, 2020. [41]	GFET	Human	Nanovesicles	TX, SEM	100 fM
		Yoo, 2022. [42]	CNTFET	Human	Micelles	TX, AFM	1 fM
	DMT-MM	Yoon, 2009. [17]	CNTFET	Human	Cell membrane	TX, SEM	10 fM
Covalent	Diazonium salt/Ni-NTA	Goldsmith, 2011. [33]	CNTFET	Mouse	Micelles, Nanodiscs	TX, AFM	~7 ppb
		Son, 2017. [43]	CNTFET	Human	Micelles	TX, AFM	10 fM
	PDL	Jin, 2012. [18]	CNTFET	Human	Nanovesicles	SEM	1 fM
		Park, 2012. [44]	CNTFET	Dog	Nanovesicles	SEM	1 fM
		Lim, 2014. [14]	CNTFET	Human	Nanovesicles	AFM	10 fM
		Lim, 2015. [45]	CNTFET	Human	Nanovesicles	AFM	1 fM
		Son, 2015. [46]	CNTFET	Human	Nanovesicles	TX, SEM	10 ng/L
		Ahn, 2015. [47]	CNTFET	Human	Nanovesicles	AFM	1 fM
	Half-v5 mouse Ab	Lee, 2018. [48]	CNTFET	Human	Nanodiscs	TX, AFM, SEM	1 fM

Table 1.2 gives a summary of all published odorant-receptor functionalised carbon nanotube and graphene field-effect transistor-based sensors to date. The vast majority of published works on this topic come from the Tai Hyun Park group at Seoul National University. The Park group has mainly focused on CNT FETs functionalised with nanovesicle-stabilised human odorant receptors, but has used a range of different covalent and non-covalent functionalisation techniques when producing the sensors. Three functionalisation methods have been used by both the Park group and another research group: non-covalent functionalisation of odorant receptors in the cell membrane with glutaraldehyde-conjugated 1,5-diaminonaphthalene [39], [40], non-covalent functionalisation of nanovesicle-stablised odorant receptors with 1-pyrenebutanoic acid N-hydroxysuccinimide ester [26], [42], and covalent functionalisation of micelle-stabilised odorant receptors with nickel/nitrilotriacetic acid modified diazonium salt [33], [43]. Interestingly, no single paper compares multiple possible functionalisation techniques keeping the transducer, analyte, odorant receptor and lipid membrane used the same, making it difficult to directly compare the quality of various attachment methods. The functionalisation procedure resulting in the lowest limit of detection was non-covalent [20], but covalent techniques have more consistently led to lower limits of detection. Furthermore, non-covalent functionalisation has never been used for vapour sensing with odorant receptors.

1.3.2. Sensing Behaviour

Nanovesicle-based odorant receptor biosensors can be used to mimic the natural behaviour of an odorant receptor, where the presence of analyte causes the flow of ions into the nanovesicle which the transducer detects [13], [45]. These ion-channel-coupled sensors

The biosensors using other formats receive a signal from binding of analyte to the receptor, which alters the distribution of charge relative to the transducer .

Vapour Environment

Goldsmith *et al.* have previously demonstrated it is possible to specifically detect eugenol vapour using a single-CNT device functionalised with mOR174-9 odorant receptors in either a surfactant (digitonin) or nanodisc format. In the first study of this kind, the mOR CNT FETs were exposed to nitrogen flow at 50% relative humidity. The resistance across a device gated at $V_g = 0$ V was measured while a specific concentration of the positive ligand eugenol was added to the constant flow for 100 s, then removed from the flow for 100 s. This cycle was repeated five times. Figure 1.3 (a) shows that significant real-time current increases of up to $\sim 9\%$ were observed during each cycle of exposure to eugenol. The device still responded to eugenol cycles after 69 days of storage in 25% (v/v) ethanol at 4°C. This nicely-behaved and persistent activity may result from the long-lived nanodisc format used [33]. As far as the author knows, there has been

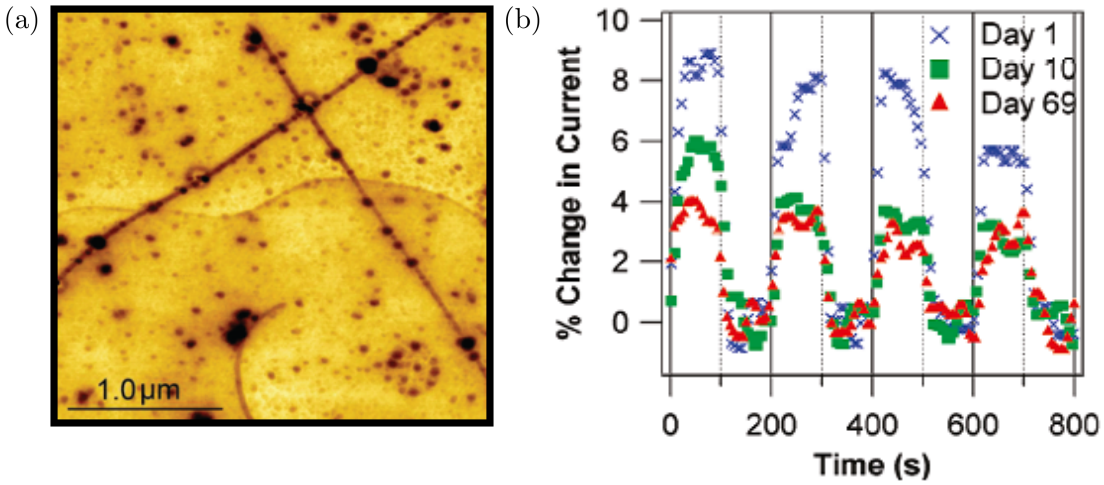

Figure 1.3.: The functionalisation of mOR174-9 nanodiscs onto single-CNT field effect transistor onto a carbon nanotube for vapour sensing application is demonstrated with an atomic force microscope image in (a), while (b) shows real-time responses of the sensor to 2 ppm eugenol vapour. The response to eugenol on day 69 (red triangles) indicates that the device retains the ability to respond to eugenol 10 weeks after functionalisation. Reproduced with permission from [33].

no investigation up until now into whether this behaviour can be replicated for insect odorant receptor devices. It is not clear that iORs can simply be substituted for mORs for vapour sensing. The reasons for this distinction are made in the subsequent section.

1.4. Insect Odorant Receptor Biosensors

1.4.1. *In Vivo* Structure and Function

Insect odorant (or olfactory) receptors (iORs) are a diverse range of odorant-sensitive transmembrane proteins located in dendrite cells of sensory hairs, known as sensilla, on the antennae and maxillary palps of an insect [51], [52]. Insects possess a specific set of iORs tailored towards their ecological role (iORx, where the "x" denotes the OR variant), as well as a co-receptor known as "ORCO" (Odorant Receptor Co-Receptor). In the insect, a given iORx is activated by volatile compounds, while the ORCO co-receptor is insensitive to VOCs but couples with iORx to form a heteromeric complex, which activates intracellular signalling via its ion channel activity. *In vivo*, the complex is required for VOC detection and operates as a non-selective cation channel. This channel opens to allow ions to travel across the cell membrane in response to iOR interaction with VOCs [35], [49], [52]–[56].

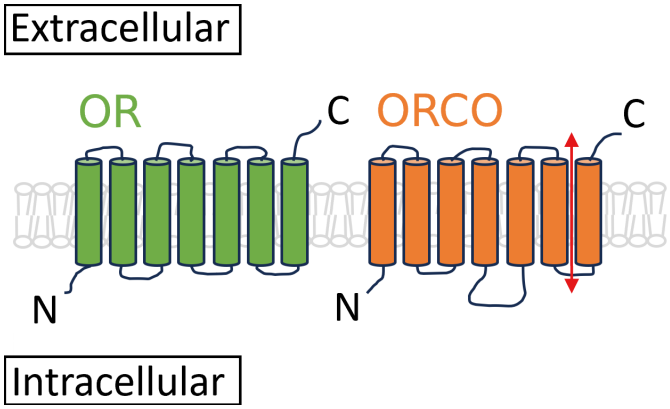

Figure 1.4.: The tuning OR and odorant receptor coreceptor (ORCO) on the native cell membrane, with C-terminus and N-terminus indicated. The red arrow indicates the location of ion transport across the membrane. Adapted from [49], [50].

iORs were initially thought to be similar in structure to vertebrate odorant receptors, but is now known that iORs have a completely different topology and mechanism to GPCRs, despite also possessing seven-transmembrane domains. Their configuration in the membrane is inverted. Equivalently, the carboxyl group or 'C terminus' of the iOR sits outside the membrane of a cell, and the amine group or 'N terminus' of the iOR sits inside the cell membrane [55]–[57]. The *in vivo* configuration of the odorant receptor on the cell membrane is illustrated in Figure 1.4.

Each odorant receptor of the *Drosophilia melanogaster* sensilla will respond to a variety of odor compound. Odors which provoke a particularly strong response from a specific odorant receptor are referred to in the literature as 'positive ligands' for that receptor [26], [36]. The strength of response by a specific OR depends on the compound being detected; furthermore, there may be no response to a compound, or one compound may inhibit the response of the receptor to other compounds. Odor compounds which provoke no response from a particular receptor are referred to as 'negative ligands' for that receptor [26], [36]. A comprehensive database that details the various *Drosophilia melanogaster* odorant receptors and their response profile to a range of volatile compounds can be consulted online [58].

1.4.2. Sensing Behaviour

As in the case of vertebrate ORs, recent studies have shown that iORs each interact with a specific VOC or a specific range of VOCs and can also be used in bioelectronic nose applications. However, the sensing mechanisms underlying their use *in vitro* are not currently well-understood [26], [36], [59]. From further development and examination of iOR-based biosensors, new insights into the mechanisms at play may emerge. Previously,

the literature has primarily focused on the operation of iOR-FET biosensors in an aqueous environment. Here, carbon nanotube or graphene FETs have been non-covalently functionalised with insect odorant receptors in either a nanodisc or liposome format. The high surface-to-volume ratio of carbon nanotubes and graphene allow for the odorant receptors to be densely immobilised across the channel surface. The functionalised channel is placed in a liquid-gated environment contained in polydimethylsiloxane (PDMS) and gated with a Ag-AgCl reference electrode (see **?@sec-gating**). Phosphate buffered saline (PBS) is used as the liquid gate electrolyte [26], [36]. A small amount of DMSO is also added to the electrolyte, a dipolar solvent which is widely used to solubilise poorly soluble analytes in a biological setting [60].

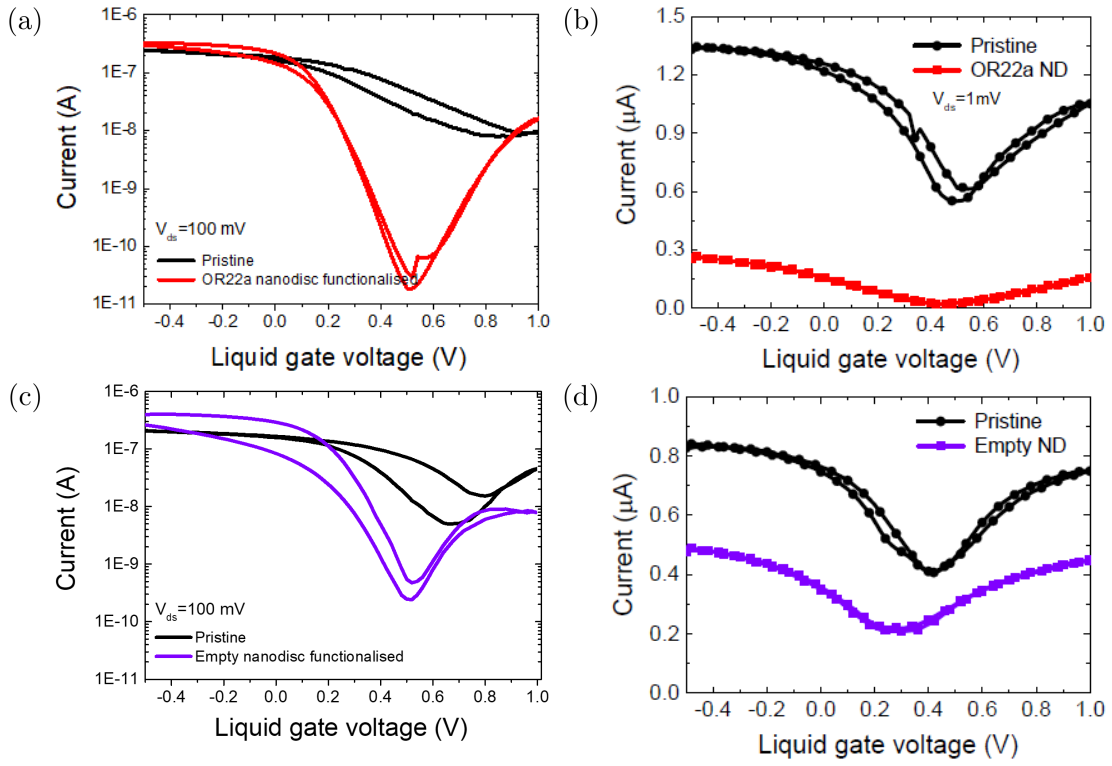

Figure 1.5.: Transfer characteristic curves before and after functionalisation of (a) an OR22a nanodisc-functionalised CNT network FET, (b) an OR22a nanodisc-functionalised graphene FET, (c) an empty nanodisc-functionalised CNT network FET and (d) an empty nanodisc-functionalised graphene FET. Reproduced with permission from [26], [36].

Functionalisation of a FET device channel with iORs significantly alters the transfer characteristics of that channel. Murugathas *et al.* found that successful functionalisation of a CNTFET device with iORs would typically increase the device on-current, increase its on-off ratio and cause a significant negative shift in threshold voltage, as shown in Figure 1.5 (a) [36]. Meanwhile, successful functionalisation of a graphene device with

iORs would typically dramatically decrease the device on-current and cause a negative shift in Dirac voltage, as seen in Figure 1.5 (b) [26]. These changes are not simply the result of linker attachment to the channel surface [36]. It is thought that the negative shift of both threshold and Dirac voltages are caused by the N-terminus amine groups on the odorant receptors or amine groups on the nanodisc membrane scaffold proteins donating electrons to the device channel, which has a similar effect to doping the channel with impurities [26], [36], [61]. Note that very similar changes occur when functionalising with empty nanodiscs which contain no odorant receptors, shown in Figure 1.5 (c) and Figure 1.5 (d). Unless the odorant receptors attach preferentially to the network over nanodiscs, it appears the gating effect is predominantly due to the large-scale attachment of nanodisc membranes.

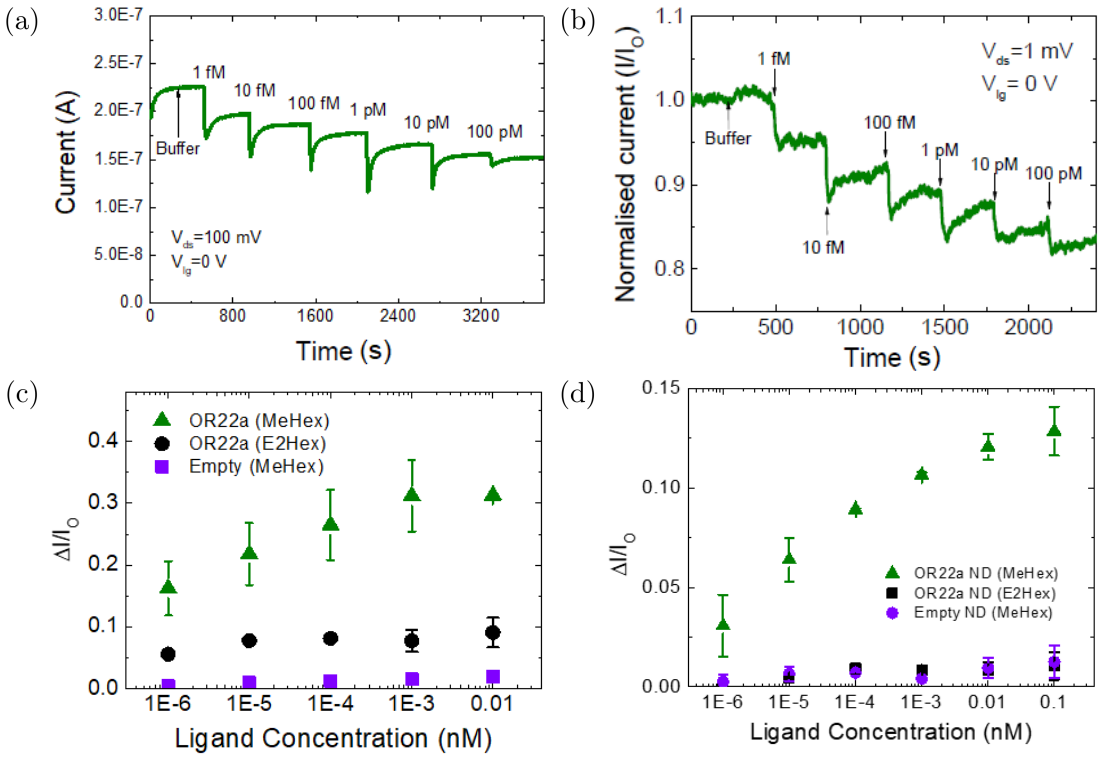

Figure 1.6.: Real-time responses to concentrations of methyl hexanoate in 1× phosphate buffer saline (PBS) with 1% v/v DMSO by (a) an OR22a nanodisc-functionalised CNT network FET and (b) an OR22a nanodisc-functionalised graphene FET, alongside the normalised signal response curves corresponding to (c) CNT network FETs and (d) graphene FETs. The response curves show the cumulative responses of OR22a-functionalised devices to both the positive ligand methyl hexanoate (green) and negative ligand *trans*-2-hexan-1-al (black). They also show the cumulative response of a empty nanodisc functionalised device to methyl hexanoate (purple). Reproduced with permission from [26], [36].

Interactions between iORs attached to the channel, such as OR22a, and positive ligands added to the electrolyte environment, such as methyl hexanoate (MeHex), can alter the current flowing through the channel. These current changes can be monitored over time and interpreted as real-time sensor responses. Figure 1.6 (a) and (b) show the respective responses of the OR22a-functionalised CNT FET and graphene FET in Figure 1.5 to methyl hexanoate in real-time. This result demonstrates that iOR-FETs are sensitive down to the femtomolar scale in an aqueous environment. Figure 1.6 (c) and (d) compare the average methyl hexanoate responses of multiple devices to that of relevant controls. It was verified that the OR22a-functionalised devices would not respond to *trans*-2-hexan-1-al, the negative ligand for OR22a; it was also verified that empty nanodiscs would not respond non-selectively to the positive ligand [26], [36].

The reduction in the channel current of a functionalised FET upon exposure to a positive ligand is notably different to the signal transduction mechanism of iORs *in vivo*, since ORCO does not appear to be required for an iOR bioelectronic nose to function. It has been proposed that the signal response results from the positive ligand binding to the iOR protein, causing it to change shape. Cheema *et al.* used neutron reflectometry to demonstrate that OR22a nanodiscs undergo a 1 nm height change after ethyl hexanoate exposure, likely resulting from a structural change [35]. This change most likely affects the channel in one of two ways. The first involves transfer of charge from the iOR to the channel, reducing I_d and causing a negative threshold voltage (or Dirac point) shift. Another could be a more indirect electrostatic gating effect, due to the movement of charge within the Debye screening length of the channel. The Debye length of 1× PBS buffer is typically much shorter than the height of a single nanodisc [36]. However, if structural changes in the iOR were primarily occurring at its base, it is still possible that the electrostatic gating could be the primary sensing mechanism.

1.5. Non-Specific Binding

Non-specific binding (NSB) refers to any attachment to the sensor channel not related to the specific analyte of interest which could interfere with sensing. Liquid-gated graphene and carbon nanotube devices are highly sensitive to the approach of charge within the Debye length of the device channel [62]. Non-specific adsorption of the analyte or ambient contamination from numerous elements used in fabricating the biosensor can lead to signal responses not attributable to the mechanism of interest (see **?@sec-CNT-sensing-mechanisms**), leading to false positives when sensing [29], [37], [62], [63]. Non-specific adsorption can occur on the channel as well as the the source-drain and gate electrodes [63]. A variety of measures can be taken to prevent NSB from occurring. Once bioreceptors have been attached to the channel, remaining exposed carbon nanotubes can be passivated with chemical coatings such as Tween-20 [63], PEG [19], [37], and ethanolamine [10], [64]. If measuring multiple channels in a multiplexed array, one channel can be left uncoated to compare the sensing response in a process known as 'internal referencing' [62].

Non-specific binding is particularly significant for protein-functionalised devices. Proteins may be spontaneously adsorbed onto carbon nanotube or graphene surfaces during functionalisation in a manner which is not linker-mediated [37], [61], [63]. Non-covalently bound proteins may also detach and reattach to available surfaces in a non-specific manner when exposed to a high ionic strength electrolyte post-functionalisation [13]. Such adsorption is known to reduce the conductance of device channels containing semiconducting carbon nanotubes, which may result from electron transfer between amino acid residues and the nanotube network. The significance of this effect is increased when electrodes are not sufficiently passivated or encapsulated due to modulation of the Schottky barrier at the metal-carbon nanotube interface [63].

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 ($100\ \mathrm{cm}^{-1} - 650\ \mathrm{cm}^{-1}$) and high wavenumber spectrum ($1300\ \mathrm{cm}^{-1} - 1650\ \mathrm{cm}^{-1}$) 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**;

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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