

Developing an Insect Odorant Receptor Bioelectronic Nose for Vapour-Phase Detection

by

Eddyn Oswald Perkins Treacher

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Te Herenga Waka - Victoria University of Wellington

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1. Biosensing with Insect Odorant Receptors

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 reliability, low cost, rapid use time, simple operation and small size compared with more traditional biological analysis methods [1], [13]. It is hoped that such sensors could be deployed outside the laboratory in a range of front-line settings requiring rapid and reliable detection [14]–[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 dating back to 1961, which refers to the use of an biologically-modified electronic array to detect specific odor traces in a highly selective and sensitive manner. As the name suggests, the signals from this system mimic the electrochemical signals received by olfactory neurons in an animal nose [14], [21]. A biomimetic approach to bioelectronic nose development couples the CNT FET or GFET signal-amplifying transducer element with sensitive components of the animal olfactory system. These components include olfactory cells [22], odorant binding proteins (OBPs) [23], [24] and odorant receptor proteins (olfactory receptors, ORs) [15], [25]. An animal nose can discern specific volatile odors in the air at low parts-per-trillion concentrations, performance which is far superior to even the best available gas sensor technology. The aim for novel olfactory-based electronic biosensors is to match or surpass this level of selective accuracy [14], [16], [21], [26], [27].

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1.2. Odorant Receptors in Field-Effect Transistor Biosensors

1.2.1. Odorant Receptors

Odorant receptors (ORs) are an essential part of the olfactory systems of most animals, including humans. Vertebrate odorant receptors are part of a group of seven-transmembrane proteins known as G-protein coupled receptors (GPCRs) [14], [21], [28], [29]. Compounds entering a vertebrate nose selectively bind to specific odorant receptors, which undergo a change in conformation [14], [16]. The binding process leads to activation and dissociation of the G-protein within the neuronal cell. Intracellular signalling events triggered by G-protein dissociation are converted to an action potential which is then transmitted to the brain [21], [28], [30]. The combination of activated receptors is then interpreted as a specific odor [16], [26], [31], [32]. Odorant receptors may be activated by a few or many target (or ‘agonist’) compounds. The target compounds are determined by subtle differences in OR amino acid composition [15], [33], [34]. An ‘antagonist’ compound may inhibit the response of a receptor to other compounds [33], [35]. Compounds which trigger a strong signal from a specific odorant receptor are often referred to as ‘positive ligands’ for that receptor, while those that cause no response are ‘negative ligands’ [25], [36], [37]. ORs let us distinguish between thousands of odors [14]–[16], [28].

1.2.2. Artificial Membranes

Odorant receptors are transmembrane proteins, which are insoluble and tend to aggregate and oligomerise in solution [38]. They therefore require stabilisation from either a specific detergent environment or a membrane layer to preserve their structure and function when solubilised [14], [39]. Odorant receptors can be expressed and isolated using heterologous cell systems, where a host cell replicates a protein from transfected RNA or DNA material [14], [21]. The most commonly used expression cells are human embryonic kidney (HEK) cells [40], [41], *E. Coli* bacteria [15], [42] and *S. cerevisiae* (baker’s yeast) [27]. The cell membrane can then be used directly in a sensor [14]. Alternatively, odorant receptors can be embedded in an artificial lipid membrane format that mimics the native cell membrane [38]. These membranes can be produced in large numbers and remain in storage for much longer than live cells [43], [44]. Lipid membranes are constructed from phospholipid molecules, which comprise of a small, hydrophilic, polar ‘head’ and long, hydrophobic, non-polar ‘tail’ [45], [46]. These artificial membranes include detergent micelles, nanovesicles (including liposomes), and nanodiscs [15], [47]. The small size of these artificial membranes makes them appropriate for use with nanomaterial-based transducers [14], [44].

A nanovesicle is a nanoscopic spherical bilayer fluid sac. There are various types of artificial nanovesicles, including liposomes, ethosomes, transfersomes, niosomes and phytosomes. The type of nanovesicle depends on its chemical makeup [46]. For example, a

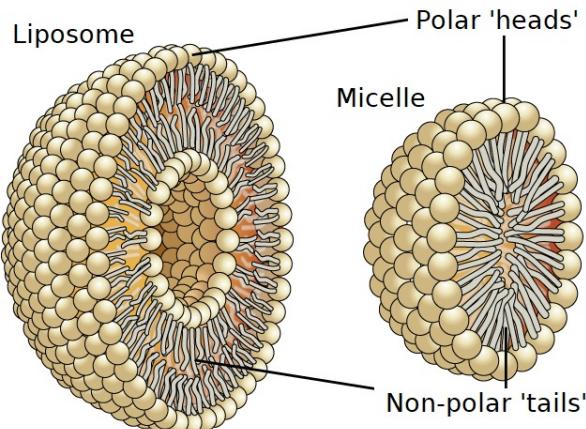


Figure 1.1.: Liposomes and micelles are made up of a lipid membrane, which acts as a substitute for the cell membrane *in vitro*. The polar, hydrophilic ‘heads’ and non-polar, hydrophobic ‘tails’ of the component phospholipids are indicated. Adapted from [48].

liposome is made up of phospholipid and cholesterol, and can consist of one or more concentric amphiphilic bilayers. The liposome can contain hydrophobic compounds within the bilayer due to hydrophobic interactions, while hydrophilic compounds are held within the vesicle core or interior [38], [46]. A nanovesicle can be used solely as a format to protect membrane proteins [25], or with the addition of integrated ion channels can mimic the operation of a cell *in vivo*, with intracellular signalling pathways triggered by the membrane proteins [44]. Nanomicelles (or simply micelles) are also nanoscopic and spherical, but unlike nanovesicles have no inner fluid sac [38], [45]. Micelles self-assemble when phospholipid is mixed with detergent. The surface of the micelle is made up of the hydrophilic detergent and phospholipid heads, while the internal core is made up of the hydrophobic phospholipid tail [38]. Hydrophobic compounds can be contained within the core of the micelle [45]. Figure 1.1 illustrates the difference between the liposome and micelle structures.

Nanodiscs have emerged as a model membrane candidate which has many advantages over the more traditional nanovesicle and micelle formats. The nanodisc is a disc-shaped lipid bilayer encompassed by an membrane scaffold protein (MSP) [15], [38], [49]. The amphiphilic membrane scaffold protein protects the exposed, strongly hydrophobic side chains of the nanodisc in an aqueous environment [15], [39]. Unlike liposomes and micelles, there is little variation between the size of individual nanodiscs due to constraints placed on the bilayer by the encompassing scaffold protein used, meaning greater consistency within and between membrane batches [38], [39]. Nanodiscs have also been found to be significantly less prone to non-specific binding (see Section 1.4) than micelles [39]. Another advantage of nanodiscs is that the membrane scaffold protein can be attached to biosensor surfaces at specific affinity tags, for example, the scaffold protein hexahistidine tag ('his-tag') [39], [49]. Depending on the type of MSP used,

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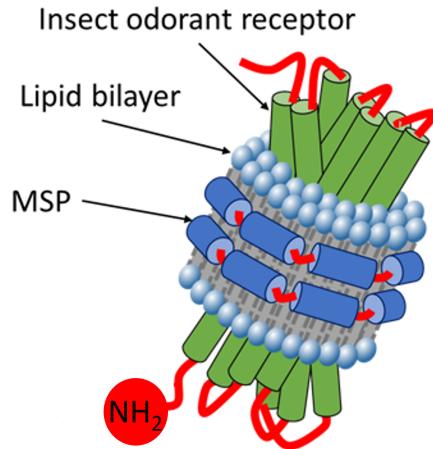


Figure 1.2.: A nanodisc containing an insect odorant receptor transmembrane protein.

MSP = membrane scaffold protein, NH₂ = the odorant receptor N-terminus.

Reproduced with permission from [36].

a nanodisc measures between 10-20 nm across and can hold either a single or several odorant receptors [38], [49]. The protein coating of the nanodisc makes it particularly stable. The stability of nanodiscs means they can be used to produce particularly reliable and long-lived biosensor devices [15], [43], [47], [50].

1.2.3. Sensor Functionalisation

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 [14], [26], [27]. 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 [51]. 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 [14], [39]. 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](#).

1.2. Odorant Receptors in Field-Effect Transistor Biosensors

Table 1.1.: A comparison of the advantages and disadvantages of different approaches for immobilising odorant receptors onto carbon nanotube or graphene transducers. (Simplicity = minimal cost, time and effort required for functionalisation; Stability = successful operation over a long time and under a range of conditions; Specificity = receptor attachment in a controlled and directional manner; Strength = strong receptor-transducer binding; Synergy = receptor attachment without negative impacts on transducer operation or 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 summarises all published odorant-receptor functionalised carbon nanotube and graphene field-effect transistor-based sensors to date. The 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 human odorant receptors, but has used a range of different covalent and non-covalent transducer immobilisation techniques when producing the sensors. Dog and mouse odorant receptors have also been used, by the Park group and by Goldsmith *et al.* respectively. As far as the author knows, the Plank group at Te Herenga Waka – Victoria University of Wellington is the only group to have produced carbon nanotube and graphene field-effect transistors functionalised with insect odorant receptors. The behaviour of insect odorant receptors is significantly different to that of vertebrate odorant receptors, and their behaviour in sensor applications is currently not well understood. The distinction between vertebrate and insect odorant receptors is discussed in more depth in Section 1.3.

Three functionalisation linkers were used by both the Park group and a secondary research group: non-covalently attached glutaraldehyde (GA)-conjugated 1,5-diaminonaphthalene (DAN) [26], [34], non-covalently attached 1-pyrenebutanoic acid N-hydroxysuccinimide ester (PBASE) [25], [37], and covalently attached nickel/nitrilotriacetic acid (Ni-NTA) modified diazonium salt [43], [52]. The bonding between the linker molecule and receptor element is typically covalent, regardless of the type of bonding that exists between linker and transducer. Interestingly, no single paper compares multiple possible functionalisation techniques directly, making it difficult to assess the relative quality of various attachment methods. The limit of detection (LOD) could be used as a rough measure of quality. The functionalisation procedure resulting in the lowest limit of detection used was non-covalent [20], though other functionalisation techniques used appear to have more consistent LOD. Furthermore, non-covalent functionalisation of odorant receptors has never been used for vapour sensing. The next section further explores the sensing behaviour of biosensors functionalised with the most commonly-used protocols.

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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	LOD
Non-covalent	Vacuum-drying	Kim, 2009. [53]	CNTFET	Human	Cell membrane	100 fM
	DMT-MM	Yoon, 2009. [17]	CNTFET	Human	Cell membrane	10 fM
	PDL	Jin, 2012. [18]	CNTFET	Human	Nanovesicles	1 fM
		Park, 2012. [54]	CNTFET	Dog	Nanovesicles	1 fM
		Lim, 2014. [40]	CNTFET	Human	Nanovesicles	10 fM
		Lim, 2015. [44]	CNTFET	Human	Nanovesicles	1 fM
		Son, 2015. [55]	CNTFET	Human	Nanovesicles	10 ng/L
		Ahn, 2015. [56]	CNTFET	Human	Nanovesicles	1 fM
	GA-conjugated DAN	Park, 2012. [20]	GFET	Human	Cell membrane	0.04 fM
		Lee, 2012. [19]	CNTFET	Human	Cell membrane	1 fM
		Kwon, 2015. [26]	GFET	Human	Cell membrane	0.1 fM
		Goodwin, 2021. [34]	GFET	Human	Cell membrane	0.5 pM
Covalent	PBASE	Murugathas, 2019. [36]	CNTFET	Insect	Nanodiscs	1 fM
		Murugathas, 2020. [25]	GFET	Insect	Nanovesicles, Nanodiscs	1 fM
		Ahn, 2020. [41]	GFET	Human	Nanovesicles	100 fM
		Yoo, 2022. [37]	CNTFET	Human	Micelles	1 fM
	Diazonium salt/Ni-NTA	Goldsmith, 2011. [43]	CNTFET	Mouse	Micelles, Nanodiscs	~7 ppb
		Son, 2017. [52]	CNTFET	Human	Micelles	10 fM
	Half-v5 mouse Ab	Lee, 2018. [57]	CNTFET	Human	Nanodiscs	1 fM

1.2.4. Sensing Behaviour

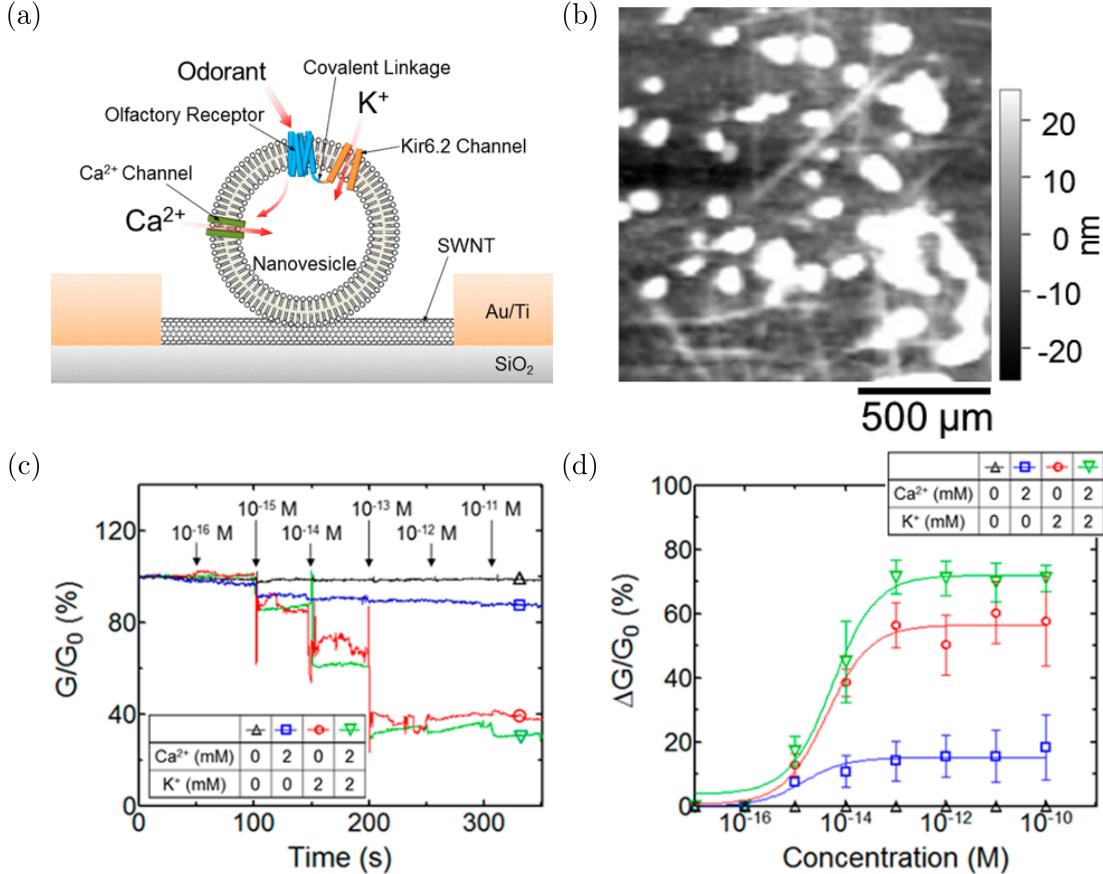


Figure 1.3.: Schematics detailing the nanovesicle-based carbon nanotube field-effect biosensor of Lim *et al.* (a) shows a schematic of the different elements and signalling pathways present in the sensor, (b) shows an atomic force microscope image of the functionalised device, (c) shows real-time conductance changes resulting from amyl butyrate additions to the electrolyte gate against relevant controls, and (d) shows the dose-dependent response pattern to amyl butyrate. Reproduced with permission from [44].

Nanovesicle-based odorant receptor biosensors can be used to couple a vertebrate odorant receptor with an ion channel, where the presence of analyte leads to a flow of ions into an olfactory cell [14], [44]. Lim *et al.* functionalised a CNT FET with nanovesicles featuring a human odorant receptor hOR2AG1 covalently coupled with a potassium ion channel, alongside an endogenous calcium ion channel, as shown in Figure 1.3 (a). These nanovesicles were attached to the random carbon nanotube network through a charge-charge interaction with poly-D-lysine, demonstrated with the atomic force microscope image shown in Figure 1.3 (b). Binding of amyl butyrate to hOR2AG1 causes the OR to change conformation, opening the coupled potassium ion channel and gating the

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transistor channel. The real-time signal responses associated with amyl butyrate binding in the electrolyte environment are shown in Figure 1.3 (c). Intracellular signalling by the odorant receptors means that target binding also opens the calcium ion channel, so a response can be seen when only calcium ions are present. Without potassium or calcium ions present, ion inflow cannot occur, so no conductance change is observed. Figure 1.3 (d) shows the dose dependent response to amyl butyrate in various electrolytes [44].

Odorant receptors can also be expressed in the native cell membrane and attached directly to the biosensor channel. Here, the changes in odorant receptor conformation that result from analyte binding cause affects the distance between charges on the odorant receptor and the transducer channel, gating the channel [14], [26]. Kwon *et al.* functionalised graphene field-effect transistors with human odorant receptors hOR2AG1 and hOR3A1 using non-covalently attached 1,5-diaminonaphthalene (DAN) modified with glutaraldehyde as a linker, as shown in Figure 1.4 (a). The odorant receptors attach to the GA-modified DAN via a Schiff-base reaction [58]. OR attachment was demonstrated by SEM imaging as well as a significant change in device resistance, shown in Figure 1.4 (b). Both hOR2AG1 and hOR3A1 showed real-time responses to their corresponding target analyte at sub-femtomolar concentrations, as shown in Figure 1.4 (c) and Figure 1.4 (d) respectively. No responses were seen from linker-modified graphene to the same analyte additions. The dose-dependent response curve of both these odorant receptor sensors is shown in Figure 1.4 (e). As in Figure 1.3 (d), a Langmuir-type dose response behaviour was seen, where a logarithmic increase in signal response is observed for sub-femtomolar or femtomolar concentration analyte additions, which gives way to saturation behaviour with picomolar additions.

Biosensors have also been produced where odorant receptors are held in a detergent micelle format instead of the native cell membrane. The mechanism behind sensing is the same as for odorant receptors in the cell membrane, where a conformational change in the odorant receptors leads to channel gating [14], [37]. Yoo *et al.* functionalised random-network CNT FETs with detergent micelles which contained human odorant receptor hOR2T7. PBASE was used as the linker molecule, which attaches to the odorant receptor via its amine group and non-covalently tethers it to the transducer, illustrated in Figure 1.5 (a). Successful immobilisation was demonstrated by a raised atomic force microscope height profile after receptor attachment (Figure 1.5 (b)) and an on-current drop in the liquid-gated transfer characteristics of the device (Figure 1.5 (c)). The sensor showed sharp real-time responses to the addition of DMMP concentrations, as seen in Figure 1.5 (d). The dose dependence curve for DMMP responses is shown in Figure 1.5 (e), again showing a Langmuir-type response curve to successive DMMP additions. Various analytes with a similar scent to DMMP were added at high concentrations to the liquid-gate, shown in Figure 1.5 (f). No response was seen to any these additions, demonstrating the selectivity of the sensor.

In the first study of this kind, Goldsmith *et al.* demonstrated that a single-CNT device functionalised with mOR174-9 odorant receptors in either a micelle or nanodisc format could be used as a vapour-phase biosensor. Micelle immobilisation was confirmed using

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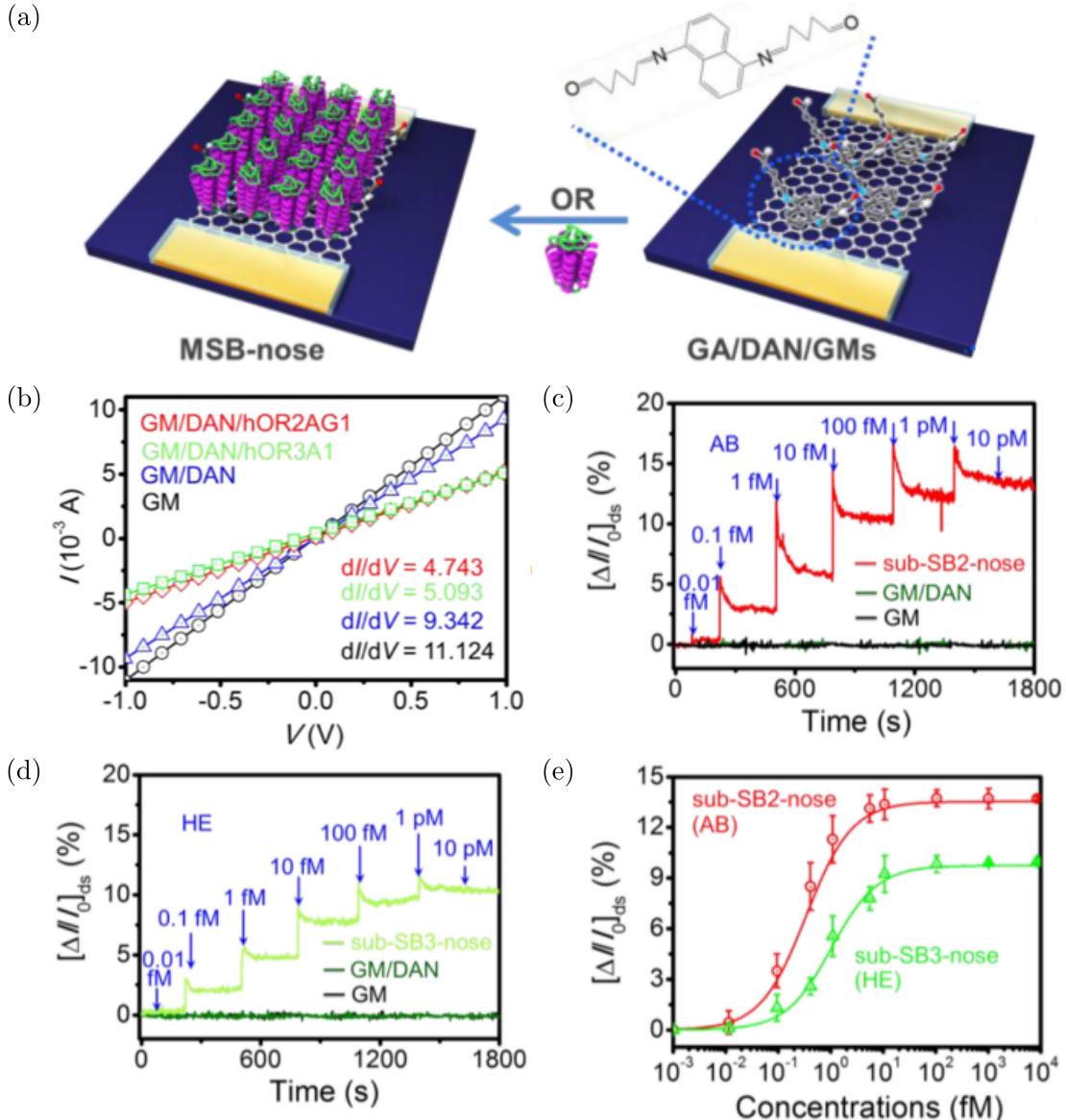


Figure 1.4.: Schematics showing the odorant receptor-functionalised graphene field-effect biosensor of Kwon *et al.* (a) shows the functionalisation of odorant receptors onto graphene using non-covalently attached GA-modified DAN linker; (b) compares transfer characteristics of the device with graphene only (GM), graphene with DAN linker (GM/DAN), and after modification with one of two different ORs (hOR2AG1, hOR3A1); (c) shows the real-time responses of the liquid-gated hOR2AG1-modified transistor (sub-SB2) to various concentrations of amyl butyrate (AB) analyte; (d) shows the real-time responses of the hOR3A1-modified transistor (sub-SB3) to various concentrations of helional (HE) analyte; and (e) shows the dose-dependent response curve corresponding to the sub-SB2 and sub-SB3 sensors. Reproduced with permission from [26].

1. Biosensing with Insect Odorant Receptors

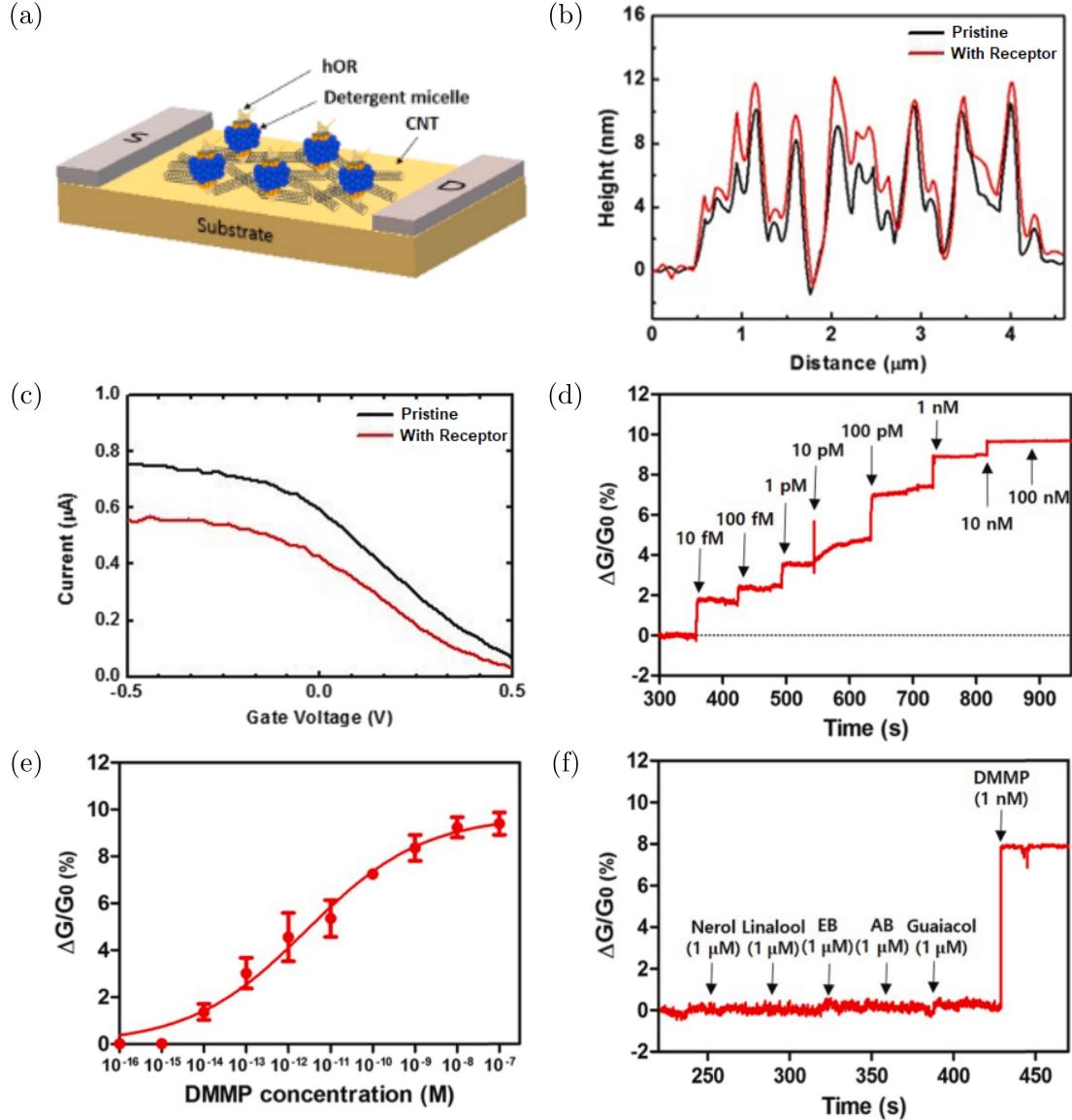


Figure 1.5.: Schematics of the micelle-based carbon nanotube field-effect transistor of Yoo *et al.* (a) shows the functionalisation of detergent micelles onto the carbon nanotube network channel; (b) shows the same height profile across an atomic force microscope image of the carbon nanotube network before and after functionalisation with micelles using PBASE; (c) shows the liquid-gated transfer characteristic of a device channel before and after functionalisation with PBASE and micelles; (d) shows real-time responses of the functionalised, liquid-gated channel to additions of dimethyl methylphosphonate (DMMP) analyte; (e) shows the dose-dependent response curve to DMMP; and (f) shows a control series demonstrating the selective behaviour of the sensor (EB = ethyl butyrate, AB = amyl butyrate). Reproduced with permission from [37].

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atomic force microscopy, as shown in Figure 1.6 (a). The mOR CNT FETs were exposed to nitrogen flow at 50% relative humidity. The conductance across the channel 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 1.6 (b) 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 persistent activity may result from the long-lived nanodisc format used [43]. As far as the author knows, no investigation up until now has investigated whether this behaviour can be replicated for insect odorant receptor devices. It is not clear that the vertebrate odorant receptors used here can simply be substituted for iORs for vapour-phase sensing. The difference between receptors is discussed further in the subsequent section.

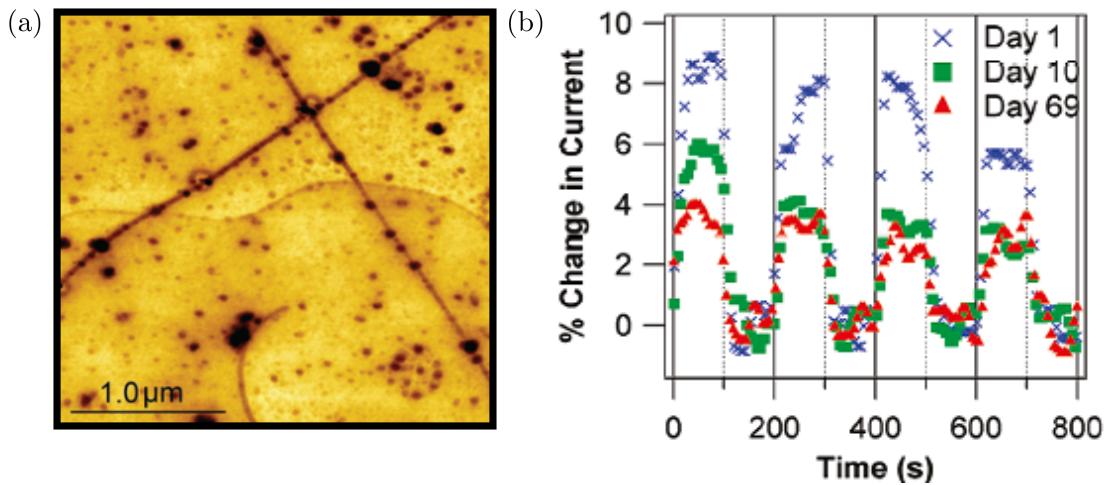


Figure 1.6.: The functionalisation of mOR174-9 nanodiscs onto single-CNT field effect transistor vapour sensing use is demonstrated with an atomic force microscope image in (a), while (b) shows real-time responses of the sensor to 2 ppm eugenol vapour. The response to eugenol on day 69 (red triangles) indicates that the device retains the ability to respond to eugenol 10 weeks after functionalisation. Reproduced with permission from [43].

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1.3.1. Insect Odorant Receptors

Insect odorant (or olfactory) receptors (iORs) are a diverse range of odorant-sensitive seven-transmembrane proteins located in the dendrite cells of insect sensory hairs, known as sensilla [29], [33], [59], [60]. When volatile compounds enter the sensilla, they are

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carried by odorant binding proteins (OBPs) through an aqueous environment to the dendrite cells [29], [33], [60]. These cells possess a insect-specific set of ‘tuning’ iORs alongside a generic co-receptor known as ‘ORCO’ (Odorant Receptor Co-Receptor) [29], [33], [61], [62]. The ORCO co-receptor is insensitive to target compounds (aside from synthetic compounds like VUAA1). Instead, it couples with the tuning iOR to form a non-selective, permeable ion channel [29], [61]. When a compound binds to a tuning iOR, the ion channel opens to allow cations to travel across the cell membrane, activating intracellular signalling [29], [33], [60], [61], [63]–[65]. The combination of resulting OR signals is sent to the insect brain for interpretation as an odor. The tuning iORs respond to (or are inhibited by) a huge variety of odors [29], [33], [66]. A database describing the various odorant receptors of *Drosophila melanogaster* and their corresponding target analytes can be consulted online [67].

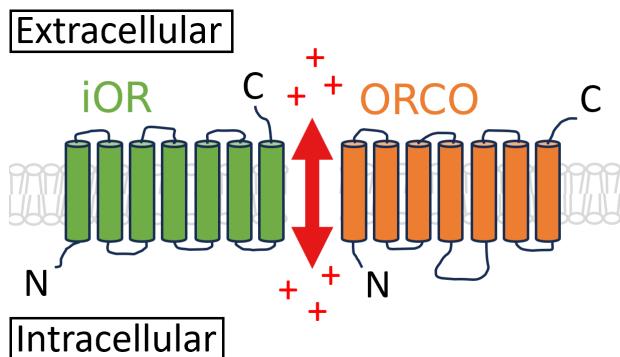


Figure 1.7.: The tuning OR and odorant receptor coreceptor (ORCO) on the native cell membrane, with C-terminus and N-terminus indicated. The red arrow indicates the location of ion transport across the membrane. Adapted from [29], [60].

Vertebrate odorant receptor proteins are terminated with an amine group outside the cell membrane, known as the N-terminus, and terminated with a carboxyl group inside the cell membrane, known as the C-terminus. Initially, iORs were thought to be similar in structure to vertebrate GPCRs [59], but is now known that iORs have a completely different topology and mechanism, despite also being a seven-transmembrane protein. The terminus configuration is inverted: the C-terminus of the iOR is extracellular, and the N-terminus is intracellular [21], [29], [33], [60], [63]. Furthermore, there is no sequence similarity between iORs and GPCRs. Evolutionarily, insect odorant receptors are thought to be closely related to insect gustatory receptors (GRs), while they bear no relation to GPCRs [21], [29], [33]. However, despite iORs not being GPCRs, some interaction between the iOR complex and the G-protein of the olfactory cell plays a role in odor detection *in vivo* [29], [64]. The *in vivo* configuration of the odorant receptor on the cell membrane, showing the terminus configuration and location of ORCO ion channel, is illustrated in Figure 1.7.

1.3.2. Sensor Functionalisation

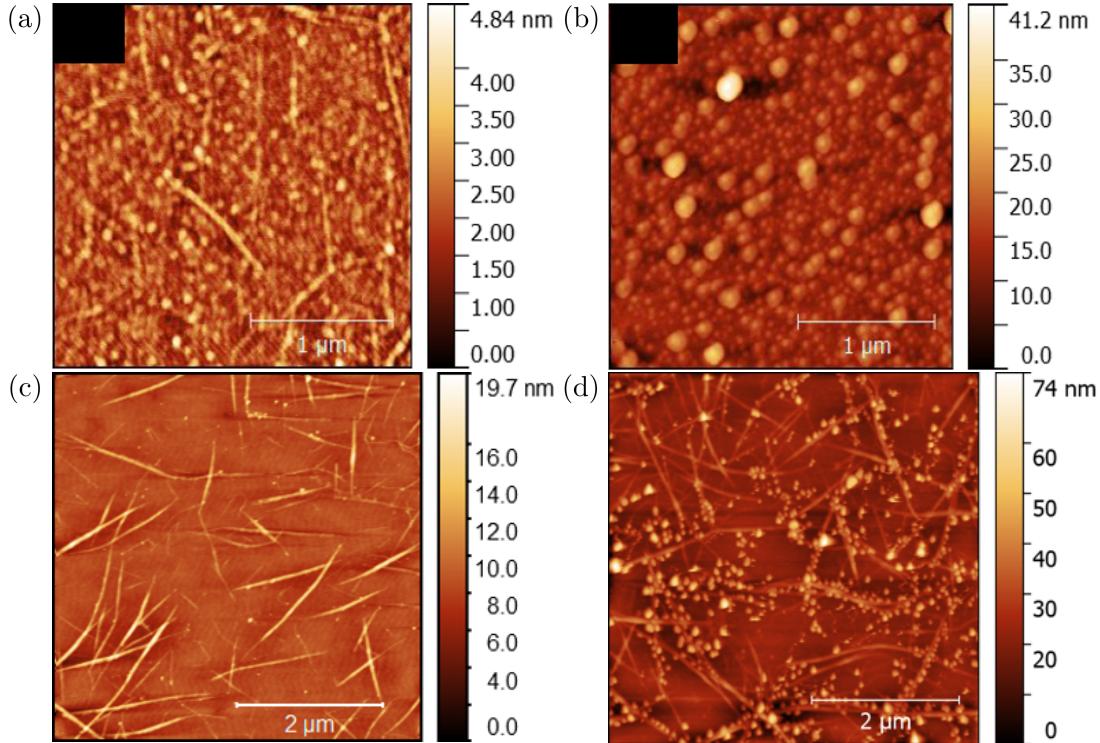


Figure 1.8.: Atomic force microscope images of (a) a pristine graphene monolayer, (b) a OR22a nanodisc-functionalised graphene monolayer, (c) a pristine carbon nanotube network, and (d) an OR22a nanodisc-functionalised carbon nanotube network. Reproduced with permission from [25], [36].

Murugathas *et al.* attached a variety of insect odorant receptors to carbon nanotubes and graphene field-effect transistors using a nanodisc format. Atomic force microscope images of a graphene monolayer before and after immobilisation of OR22a nanodiscs with PBASE linker are shown in Figure 1.8 (a) and (b) respectively, while atomic force microscope images of a randomly deposited carbon nanotube network before and after OR22a nanodisc immobilisation with PBASE are shown in Figure 1.8 (c) and (d) respectively. Blobs are seen across the surface of the post-functionalisation image which are tens of nanometers in height. On the nanotube network, these blobs are seen directly next to nanotube bundles, indicating selective attachment to the nanotubes over the silicon dioxide substrate. As nanodiscs are only 10-20 nm in height, it appears that these blobs are large agglomerates of nanodiscs [25], [36], [38], [49]. As seen previously for a carbon nanotube network FET in Section 1.2, functionalisation occurs by non-covalent attachment of PBASE to the channel, and covalent attachment of the PBASE linker to the odorant receptor amine group. The nanodisc membrane also possesses amine residues [49], so in some cases immobilisation may be directly between the PBASE linker and

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nanodisc membrane.

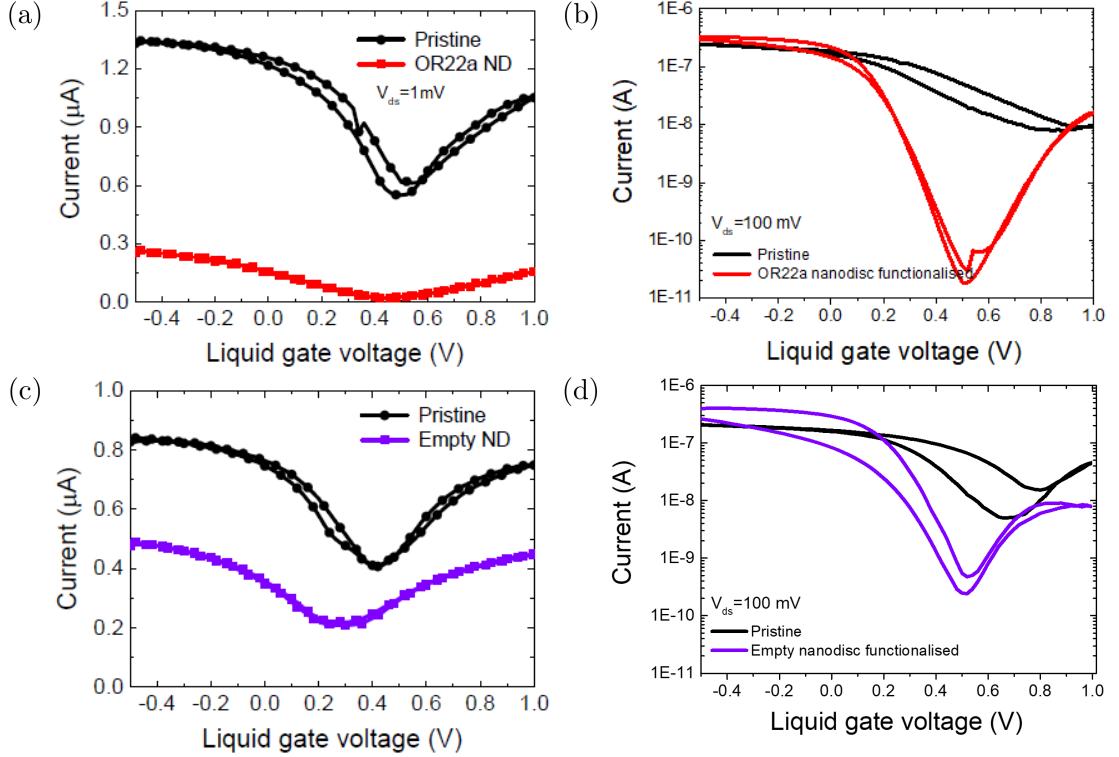


Figure 1.9.: Transfer characteristic curves before and after functionalisation of (a) an OR22a nanodisc-functionalised graphene FET, (b) an OR22a nanodisc-functionalised CNT network FET, (c) an empty nanodisc-functionalised graphene FET and (d) an empty nanodisc-functionalised CNT network FET. Reproduced with permission from [25], [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 1.9 (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 1.9 (b) [25]. 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 [25], [36], [68]. Note that very similar changes occur when functionalising with empty nanodisks which contain no odorant receptors, shown in Figure 1.9 (c) and Figure 1.9 (d). Unless the odorant receptors attach preferentially to the network over

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nanodiscs, it appears the gating effect is predominantly due to the large-scale attachment of nanodisc membranes.

1.3.3. Sensing Behaviour

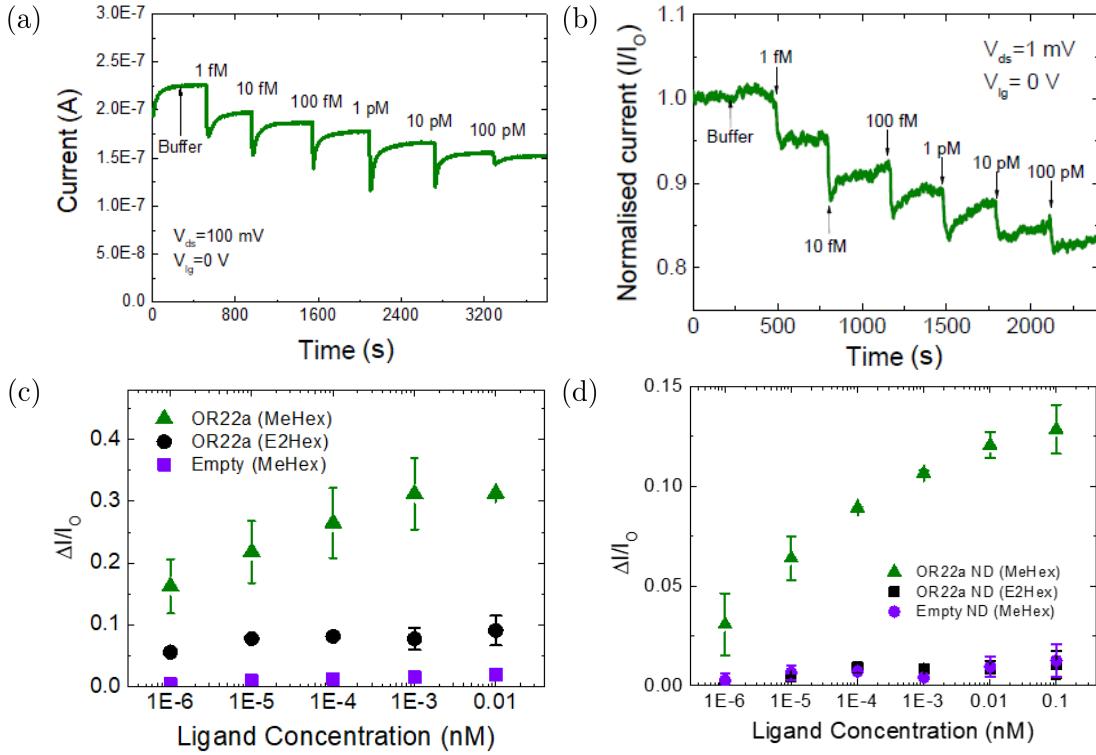


Figure 1.10.: Real-time responses to concentrations of methyl hexanoate in 1× phosphate buffer saline (PBS) with 1% v/v DMSO by (a) an OR22a nanodisc-functionalised CNT network FET and (b) an OR22a nanodisc-functionalised graphene FET, alongside the normalised signal response curves corresponding to (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 [25], [36].

Figure 1.10 (a) and (b) show the respective responses of the OR22a-functionalised CNT FET and graphene FET to various concentrations of methyl hexanoate in real-time. This result demonstrates that iOR-FETs are sensitive down to the femtomolar scale in an aqueous environment. Figure 1.10 (c) and (d) compares the dose dependent responses to methyl hexanoate from multiple OR22a-functionalised devices to that of

1. Biosensing with Insect Odorant Receptors

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 [25], [36]. It is notable that unlike iORs *in vivo*, ORCO does not appear to be required for the bioelectronic nose to function [25], [36], [50], [62]. Furthermore, G-protein signaling pathways are not required [31]. It has been proposed that the signal response results from the positive ligand binding to the iOR protein, causing a change in conformation, the same mechanism underpinning the behaviour of many of the vertebrate odorant receptor sensors seen in Section 1.2. 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 [50].

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 from 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. From further development and examination of iOR-based biosensors, new insights into the mechanisms underlying the nanodisc signal transduction may emerge [21]. As discussed here, the literature has primarily focused on the operation of iOR carbon nanotube and graphene FET biosensors in an aqueous environment [25], [36]. It is as yet unknown whether insect odorant receptors can operate in a vapour-phase environment, but this possibility is explored in this thesis.

1.4. Non-Specific Binding

Non-specific binding (NSB) refers to any attachment within the sensing environment not related to the specific analyte of interest [69], [70]. 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 [51], [68], [71]. Non-covalently bound proteins may detach and reattach to available surfaces in a non-specific manner when exposed to a high ionic strength electrolyte post-functionalisation [14]. Non-specific binding may also result from protein-protein interactions, misoriented attachment of proteins, attachment to a sticky substrate and electrostatic binding to any charged surface present, such as the reference electrode [69], [71], [72]. Liquid-gated graphene and carbon nanotube devices are highly sensitive to the approach of charge within the Debye length of the device channel, and so non-specific adsorption can lead to spurious signals when sensing [51], [69]–[71]. 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 [71], PEG [19], [51], and ethanolamine [10], [73].

1.5. Summary

Odorant receptors can be used to fabricate highly sensitive and selective biosensors using carbon nanotube and graphene field-effect transistors as the transducer element. Both vertebrate and insect odorant receptors are seven-transmembrane proteins, but each has a different sequence and have inverted terminus positions relative to the cell membrane wall *in vivo*. Insect odorant receptor detection *in vivo* differs significantly from vertebrate OR detection, with an ORCO-mediated ion channel involved. ORs can be held in the native cell membrane for sensor applications, but artificial lipid bilayer formats such as micelles, nanovesicles or nanodiscs are generally preferred due to their enhanced stability. Mammalian odorant receptors have been thoroughly explored in carbon nanotube and graphene field-effect transistor sensing applications, with both non-covalent and covalent functionalisation mechanisms used to create sensors which detect analyte at sub-femtomolar concentrations. The mechanisms behind sensing rely on transistor gating either due to ion flow into a nanovesicle format, or a conformational change in the odorant receptor. Covalently-attached mammalian odorant receptors have also been used for vapour-phase detection with a single carbon nanotube field-effect transistor device at concentrations down to ~ 7 ppb by Goldsmith *et al.*.

Femtomolar detection of analyte has also been achieved with an insect odorant receptor functionalised device. However, the exact mechanism behind detection is unclear, as the presence of ORCO is not required for successful sensor behaviour. It is possible that the mechanism results from a change in conformation of the odorant receptor, similar to the mammalian odorant receptor. Due to the possible difference in mechanism between mammalian odorant receptor detection and insect odorant receptor detection, it is not clear that vapour-phase detection can be achieved using insect odorant receptors in the same format as used by Goldsmith *et al.* The subsequent chapters explore this possibility in further detail. **?@sec-noncovalent-functionalisation** looks further at various non-covalent functionalisation approaches for the creation of a insect odorant receptor-based field-effect transistor biosensor, while **?@sec-biosensing-iORs** tests sensor behaviour in both aqueous and vapour-phase environments.

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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