Expression of Insect Olfactory Receptors for Biosensing on SAW Sensors

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INTRODUCTION

Chemical-sensing and communication is crucial for insects, which consequently possess diverse and highly sensitive olfactory systems. There is currently considerable interest in the development of biosensors that can mimic the sensitivity and specificity of insect olfaction.

Odour detection occurs within the antennae of insects. Highly-tuned olfactory receptors (ORs) are located in the membranes of sensory neuron dendrites that innervate specialized sensory hairs (sensilla). Activation of the receptor protein by a specific odour compound, results in a change in the membrane potential, which in turn elicits an intracellular sequence of events encoding information regarding the stimulus. This information is then relayed to olfactory processing centres in the brain.

In this communication we describe the development of a protein expression system for insect olfactory receptors in an Sf9 cell-line, which will then be used as part of a surface acoustic wave (SAW)-based biomimetic sensor. Receptor-expressing Sf9 cells will be used as the biological component on dual SAW resonator devices, where one side of the device is coated with cells not expressing the olfactory receptors as a reference.

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THE BENEFITS OF USING Sf9 CELLS

- Sf9 cells originate from the moth *Spodoptera frugiperda* and are therefore able provide 'native' conditions for the expression and membrane targeting of insect olfactory receptors.
- Sf9 cells naturally express the co-receptor protein **Sfru\Orco,** which is required for the correct functionality of insect olfactory receptors.
- Sf9 cells are adherent, grow at 28 C and do not require constant [CO_2] making them much more adaptable to growth on SAW devices and survival during sometimes lengthy experiments.

EXPRESSION OF INSECT OLFACTORY RECEPTORS IN Sf9 CELLS

Sf9 cells have previously been used to functionally characterise insect olfactory receptors from the fruit fly *Drosophila melanogaster* [4, 5] and two species of moth [1, 3]. The full complement of over 60 olfactory receptors from *D. melanogaster* has been identified and the odour perception profiles for each receptor are known [2].

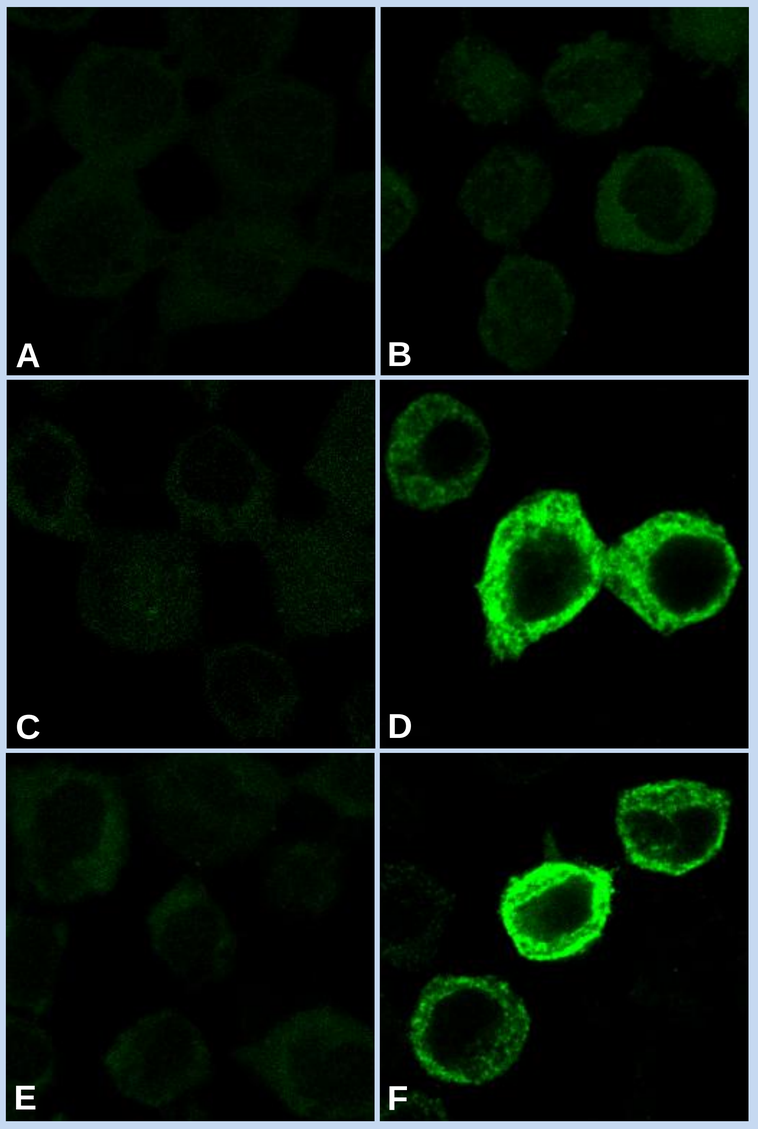

Figure 1. Fluorescent images showing expressed ORs in Sf9 cells. Sf9 cells were transfected with a control DNA construct pIB-empty (A & B) or olfactory receptor DNA constructs pIB-myc-Or22a (C & D) and pIB-myc-Or67d (E & F). In images A, C & E cells membranes were not permeabilised and hence the antibody was not able to enter the cell to detect the receptor proteins. In images B, D & F cells were permeabilised. Images are shown at 100x magnification.

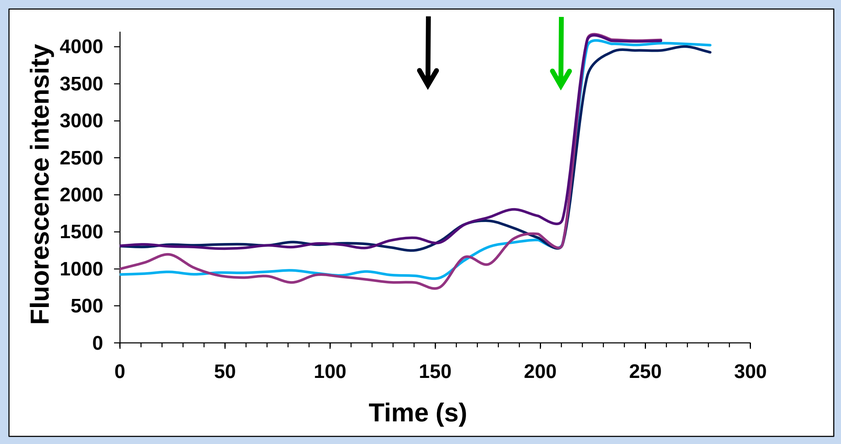

Figure 2. Ca^{2+} imaging of Sf9 cells expressing Or22a and stimulated with 10μM ethyl butyrate. On activation, the receptor facilitates the release of intracellular Ca^{2+} stores. Ethyl butyrate addition is indicated by the black arrow. Ionomycin is added at the end of the experiment (green arrow) to induce a maximal change in cytoplasmic Ca^{2+}.

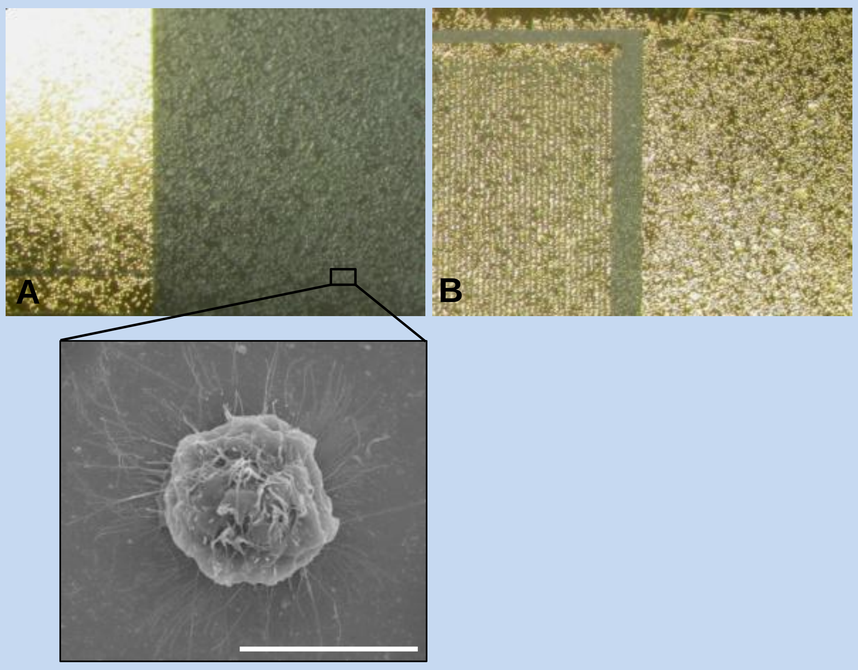

Figure 3. Sf9 cell adherence to varying SAW device surfaces. Sf9 cells adhere, within 1hr, to both $LiTaO_3$ (A) and gold (B) SAW device surfaces. Images were captured using a stereo microscope at 50x magnification. Inset image - scanning electron microscopy of an Sf9 cell adhered to the surface of a $LiTaO_3$ SAW device after 1 hr. Scale bar 10μm.

DNA constructs comprising an insect-specific vector (pIB-V5/His, Invitrogen) containing intact genes for the *D. melanogaster* olfactory receptors **Or22a** or **Or67d** were made. This genetic material was then introduced into Sf9 cells using a technique (transfection) which employs lipid vesicles that fuse to the membrane of the cells and deliver the genetic material into the cell. The novel gene products are then processed and transported to their correct location (in the case of olfactory receptors, the cell membrane) by the cells endogenous machinery.

Assessment of successful membrane expression of the two olfactory receptors was carried out by fluorescence methods. A fluorescently-labelled antibody was used to detect the location of the receptor proteins and revealed an expression restricted to the Sf9 cell membrane (Figure 1).

Fluorescent single-cell Ca^{2+} imaging is being used to assess the functionality of these olfactory receptors. Sf9 cells are loaded with a fluorescent Ca^{2+} indicator, (Fluo-4 AM, Invitrogen), and are then stimulated with the respective odorant ligands; the fruit volatile, ethyl butyrate, (for **Or22a**) and the pheromone, 11-*cis*-vaccenyl acetate, (for **Or67d**). Odorant-specific receptor activation results in an increase in the intracellular Ca^{2+} concentration (observed as an increase in fluorescence intensity). Ca^{2+} imaging results for the olfactory receptor **Or22a** are shown in Figure 2.

Sf9 CELL COMPATIBILITY WITH SAW SENSORS

The adherence and growth of Sf9 cells on the surface of SAW devices was assessed. The piezoelectric material tested was lithium tantalate ($LiTaO_3$) with and without a gold surface layer. Lithium tantalate is the substrate material for the shear horizontal SAW devices used for liquid phase and cell-based measurements and is therefore of most importance in this study. We found that the Sf9 cells were able to form a tightly adherent monolayer to the surface of the two different devices within 60 min (Figure 3). Furthermore, Sf9 cells were able to grow on these SAW devices over extended periods of time (at least three days).

Scanning electron microscopy was also carried out on Sf9 cells that had been incubated on the surface of SAW devices for 60 min. It revealed that in a short period of time Sf9 cells form strong attachments to the device surface through multiple filopodia (cell projections) (Figure 3, inset). We are therefore confident that Sf9 cells are suitable for use as a biological functional layer on SAW resonator devices.

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