Data Article

Data on preparation and characterization of an insect odorant receptor based biosensor

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A R T I C L E   I N F O

A B S T R A C T

Insect Odorant receptors (OrXs) can be used as the recognition element in a biosensor as they demonstrate high levels of sensitivity and selectivity towards volatile organic compounds. Herein, we describe a method to express and purify insect odorant receptors and reconstitute them into artificial lipid bilayers (liposomes). These OrX/liposomes were covalently attached to a gold surface and characterized using quartz crystal microbalance with dissipation monitoring (QCM-D). The interaction of OrX/ liposomes immobilized on a gold surface to positive and negative odorants were studied by means of electrochemical impedance spectroscopy (EIS) and QCM-D. The data presented in this article are related to the research article titled "An ultrasensitive electrochemical impedance-based biosensor using insect odorant receptors to detect odorants" [1].

Specifications table

Subject area	*Chemistry, Material Science*
More specific subject area	*Biosensors*
Type of data	*Table, graph, and figure*
How data was acquired	*QCM-D graphs (Q-sense analyzer (Biolin Scientific)), EIS graphs (Palm-Sens potentiostat)*
Data format	*Analyzed*
Experimental factors	*Gold electrodes functionalization, and electrical properties of OrX/liposome immobilization and ligand binding.*
Experimental features	*OrX/liposomes were covalently attached to gold surface and QCM-D and EIS studies were utilized to investigate ligand-binding.*
Data source location	*The University of Auckland, Auckland, New Zealand*
Data accessibility	*Data are presented in this article*
Related research article	*Khadka, R., Aydemir, N., Carraher, C., Hamiaux, C., Colbert, D., Cheema, J., Malmström, J., Kralicek, A., Travas-Sejdic, J.,* 2018 [1].

Value of the data

- The method used here to purify OrX subunits and integrating into lipid bilayers of liposomes to provide a platform for biosensing studies.
- The QCM-D data after covalent attachment of OrX/liposomes on the gold surface followed by the binding of ligand is very useful to understand the mechanism of signal transduction.
- The EIS capacitance data can be used to compare the sensitivity of this novel insect OrX based biosensor with other relevant OrX based biosensors.

1. Data

The western blot analysis of purified OrX subunits are shown in Fig. 1. QCM-D data showing the real time covalent attachment of Or22a/liposomes and Or71a/liposomes on gold sensors followed by the binding of methyl hexanoate and 4-ethylguaiacol respectively, Figs. 2–5. The equivalent electrical circuit model used for fitting the impedance data is shown in Fig. 6 and the calibration plots in terms of change in normalized capacitance values for three EIS sensors (Or10a, Or22a, and Or71a) are presented in Figs. 7–9.

2. Experimental design, materials, and methods

2.1. Preparation of purified OrX subunits

The purification procedure is a variation on the one detailed in Carraher et al. [2]. To his-tag affinity purify recombinantly expressed OrX subunits from baculovirus-infected Sf9 cells, 500 mL of Sf9 cells at a concentration of at 2×10^6 mL^{-1} (measured with a haemocytometer), were infected with baculovirus at a multiplicity of infection (MOI) of 0.1, and incubated at 27 °C for 72 h with shaking at 110 RPM. The cell pellet was collected by centrifugation at 3,800 g for 10 min at room temperature and then resuspended in 40 mL of resuspension buffer A (20 mM Tris/HCl pH 7.5, 100 mM NaCl, 1x protease inhibitor cocktail (Roche Diagnostics GmbH, Germany)), with 25 U/mL Benzonase, then lysed by two passes on an Emulsiflex C5 emulsifier (Avestin, Germany) at 10,000–15,000 psi. The sample was then centrifuged at 1,000 g for 5 min to remove whole cells and nuclei. The supernatant was removed and spun at 100,000 g for 1 h at 4 °C. The membrane pellet was resuspended in 40 mL of buffer A with 1% w/v detergent (Zwittergent 3–16) and rotated for 1 h at room temperature at 10 rpm. The sample was then centrifuged
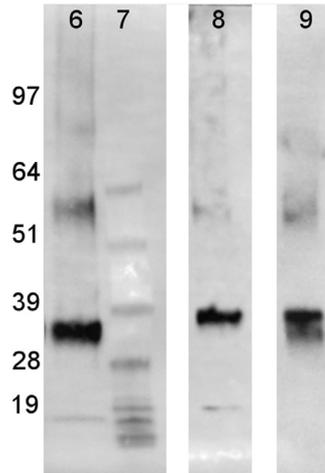
Fig. 1. Western Blot analysis of OrX/liposomes. Lane 6: Or71a/liposomes (Anti-FLAG). Lane 7: Molecular weight marker. Lane 8: Or10a/liposomes (Anti-FLAG). Lane 9: Or22a/liposomes (Anti-FLAG). Molecular weight of the markers (kDa) are indicated.
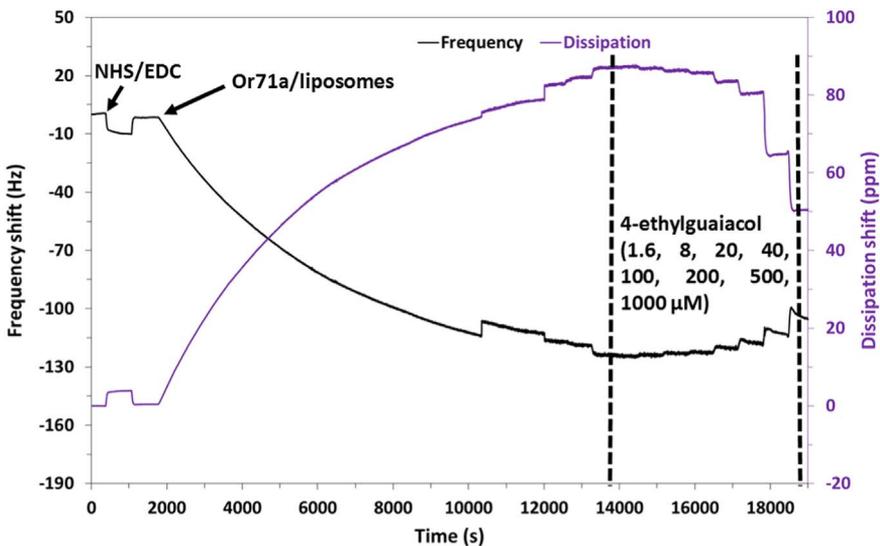

Fig. 2. Change in frequency and dissipation of a gold surface with self-assembled monolayers (SAMs) of 6-mercaptohexanoic acid (MHA) and N-hydroxysuccinimide (NHS)/1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) modification, followed by Or71a/liposome immobilization on the QCM sensor and then binding of the target ligand 4-ethylguaiacol.

at 100,000 g for 1 h at 18 °C. The supernatant was removed and loaded onto a 1-mL NiNTA column (GE Healthcare) where the zwittergent 3–16 detergent was exchanged to Fos-Choline 14 (FC-14). The column was washed in ten column volumes of buffer B (20 mM Tris/HCl pH 7.5, 0.36 mM FC-14) with 300 mM NaCl and 20 mM imidazole, and a further ten column volumes of buffer B with 100 mM NaCl and 50 mM imidazole. Protein was eluted with 4 column volumes of buffer B with 100 mM NaCl and 500 mM imidazole. Purity was assessed on Coomassie stained SDS–PAGE gels and by Western blotting.

Purification was completed with a final size exclusion chromatography (SEC) step. The elution fractions from the NiNTA purification were pooled and centrifuged at 20,000 g for 5 min to remove aggregates and contaminants. Then 5 mL of sample was injected onto a Superdex 200 16/60 column
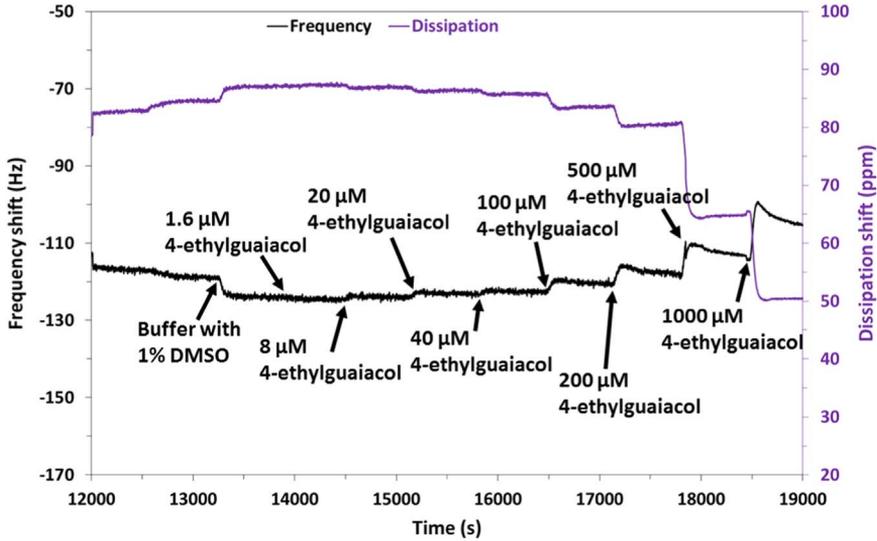
Fig. 3. Close up view of the change in frequency and dissipation with increasing concentrations of 4-ethylguaiacol (0, 1.6, 8, 20, 40, 100, 200, 500 and 1000 μM) for the Or71a/liposome QCM sensor.
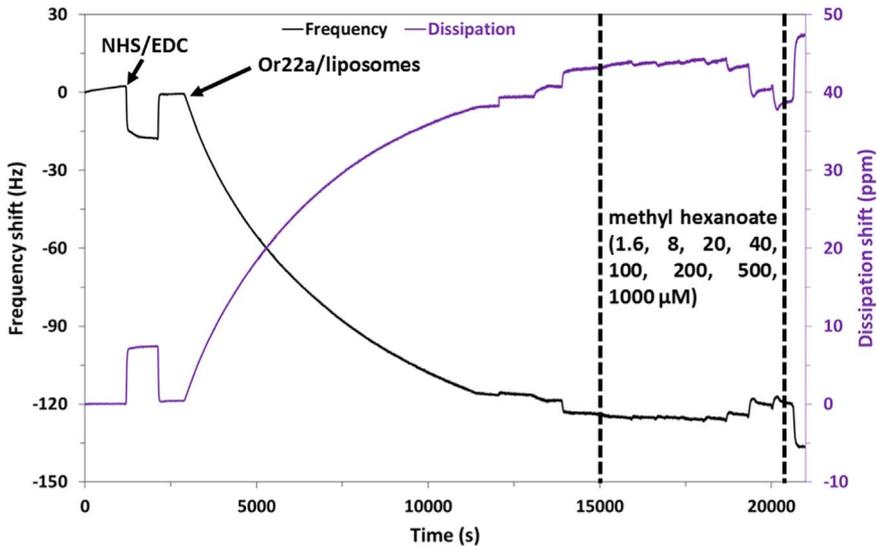

Fig. 4. Change in frequency and dissipation of gold surface with SAM and NHS/EDC modification, followed by Or22a/liposome immobilization on the QCM sensor and then binding of the target ligand methyl hexanoate.

(GE Healthcare) attached to an Akta-Pure chromatography system (GE Healthcare). The sample was run at 1 mL/min in buffer B with 100 mM NaCl, and 2 mL fractions were collected and concentrated using a 100 kDa MWCO Vivaspin filter unit (Sartorius, Goettingen Germany) and stored at −80 °C.

2.2. Preparation of liposome integrated OrX subunits

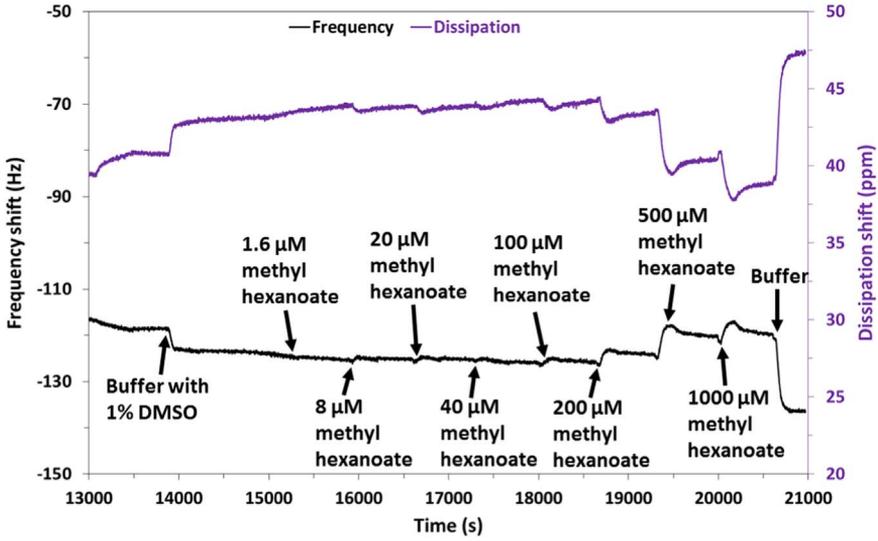Liposomes were prepared as outlined in Carraher et al. [2].

Fig. 5. Close up view of the change in frequency and dissipation with increasing concentrations of methyl hexanoate (0, 1.6, 8, 20, 40, 100, 200, 500 and 1000 μM) for the Or22a/liposome QCM sensor.
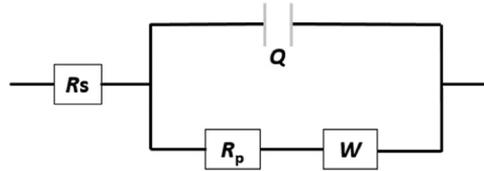

Fig. 6. Equivalent circuit diagram (Randles model) for fitting the non-Faradaic impedance curves where R_s is solution resistance, Q refers to capacitance, R_p is called polarization resistance and W refers to Warburg diffusion.
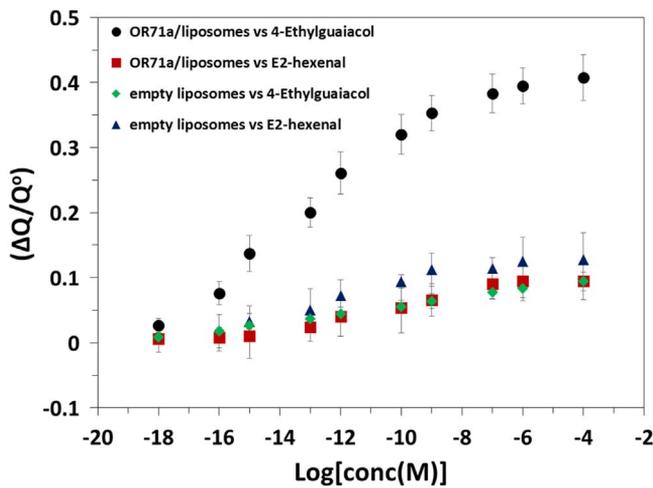

Fig. 7. Dose response curve in terms of change in normalized capacitance values for the Or71a/liposome EIS based sensor showing detection of the specific ligand 4-ethylguaiacol with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.
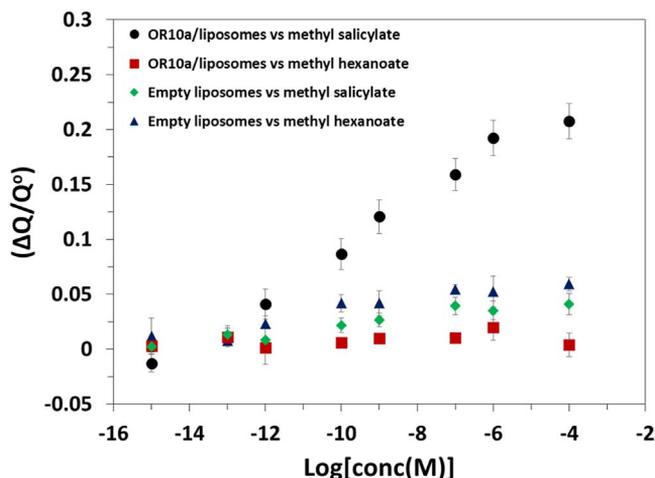
Fig. 8. Dose response curve in terms of change in normalized capacitance values for Or10a/liposomes on an EIS based sensor showing detection of its specific ligand methyl salicylate with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.
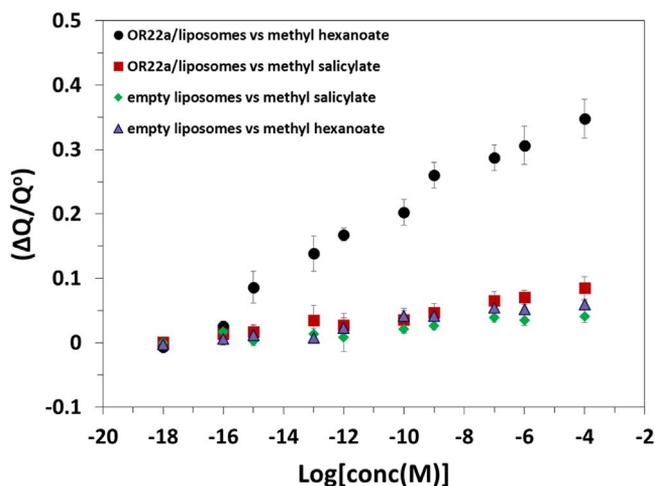

Fig. 9. Dose response curve in terms of change in normalized capacitance values for Or22a/liposomes on an EIS based sensor showing detection of its specific ligand methyl hexanoate with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.

2.3. EIS and QCM-D measurements

For QCM-D experiments, 100 nm thick gold sensor crystals were functionalized with self-assembled monolayers (SAMs) of 6-mercapto hexanoic acid (MHA) by incubating them in MHA solution overnight. MHA functionalized crystals were placed in the QCM-D chamber and real time measurements of NHS/EDC activation followed by binding of the OrX/liposomes was monitored. Different concentrations of positive and negative odorants were flowed into the chamber and changes in frequency (∆f) and dissipation (∆D) values were recorded.

For EIS experiments, a gold disk as a working electrode, platinum coil as counter electrode and Ag/AgCl (3 M NaCl, 0.209 V vs. SHE) as a reference electrode was used. EIS measurements were performed at a fixed voltage of -0.7 using a PalmSens potentiostat with degassed PBS solution as an electrolyte.

Acknowledgments

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.11.018.

References

[1] R. Khadka, N. Aydemir, C. Carraher, C. Hamiaux, D. Colbert, J. Cheema, J. Malmström, A. Kralicek, J. Travas-Sejdic, An ultrasensitive electrochemical impedance-based biosensor using insect odorant receptors to detect odorants, Biosens. Bioelectron. (2018).
[2] C. Carraher, A.R. Nazmi, R.D. Newcomb, A. Kralicek, Recombinant expression, detergent solubilisation and purification of insect odorant receptor subunits, Protein Express. Purif. 90 (2) (2013) 160–169.