Introduction

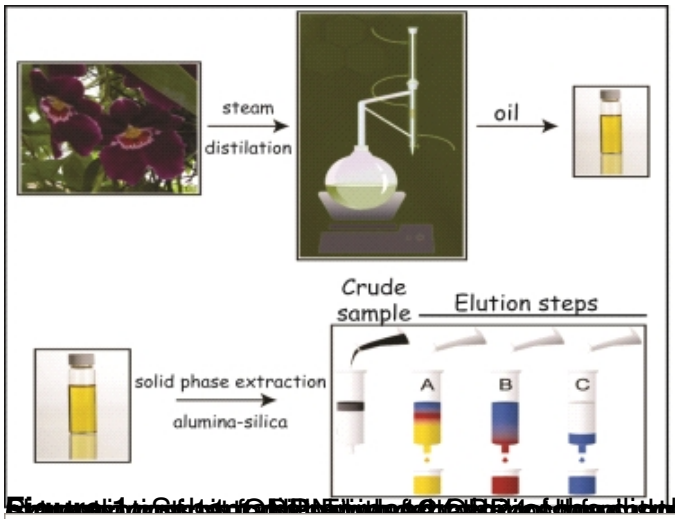
The mosquito Anopheles gambiae is the major vector for transmission of the parasite Plasmodium falciparum, which causes malaria, a disease responsible for severe illness and deaths of millions of people. Like other insects, mosquitoes rely on olfaction to find their food and mates. In addition, the blood-feeding female mosquitoes have evolved specific, odor detection-based systems for finding their mammalian hosts and obtaining the blood meal that is required for egg maturation. Understanding of mosquito olfaction, as well as discovery of new compounds capable of disrupting the normal olfactory capacity of female mosquitoes, may allow the development of new strategies for controlling the malaria parasite transmission. Accordingly, our research focuses on the study of two key classes of molecular components of the olfaction apparatus, odorant binding proteins (OBPs) and odorant receptors (ORs) and their interactions with volatiles of natural and/or synthetic origin.

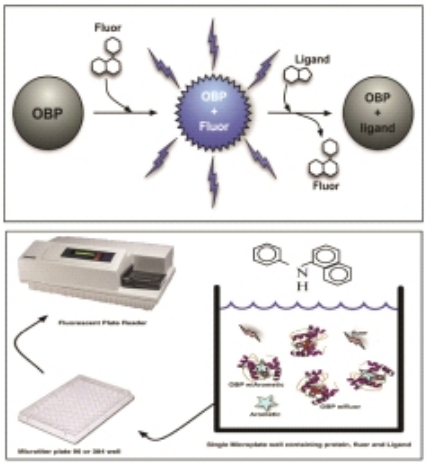
Research objectives

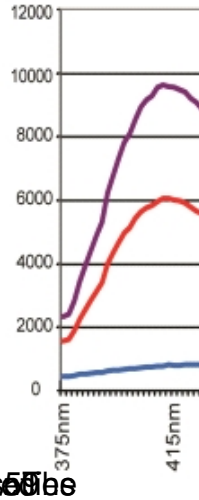
(i) Establishment and use of protein-based high throughput screening assays for identification of specific ligands for Anopheles gambiae OBPs
(ii) Establishment and use of cell-based high throughput screening platforms for identification of ligands for Anopheles gambiae ORs
(iii) Generation of plant extracts from endemic plant collection and screening for the presence of OBP and/or OR ligands

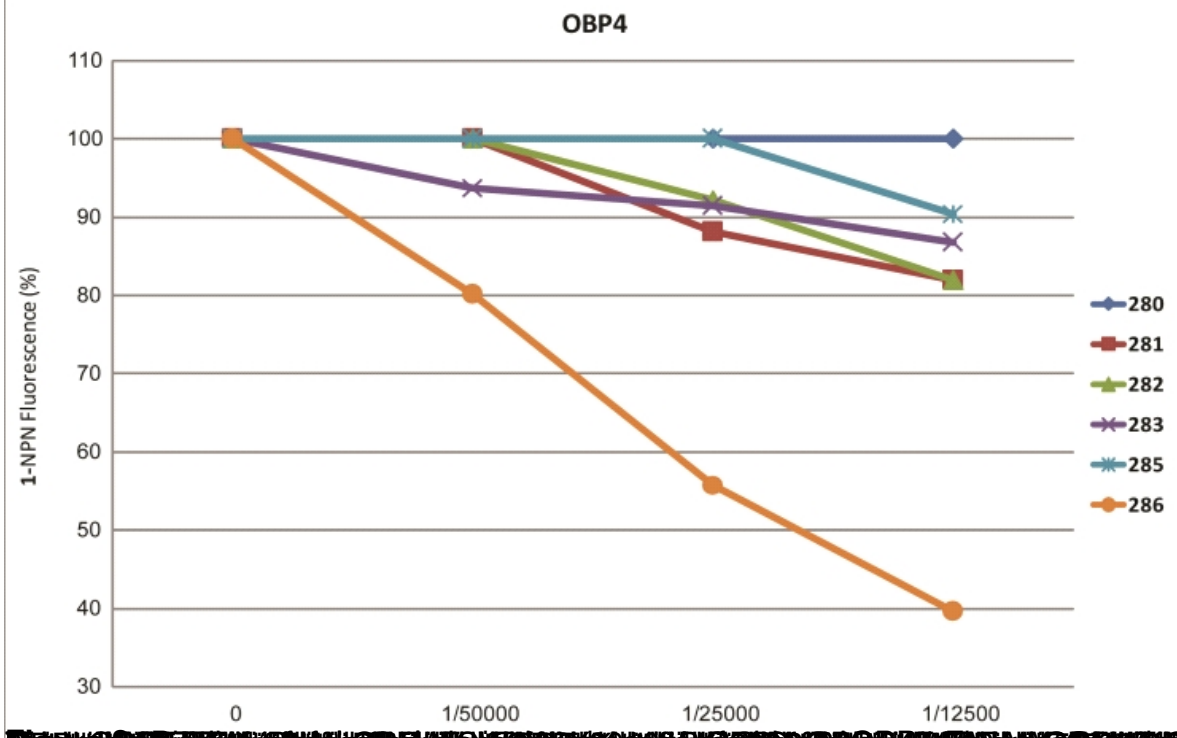
Odorant-binding proteins (OBPs)

The study of the Anopheles gambiae odorant binding proteins (OBPs) aims at the identification of new volatile ligands from endemic plants that can interfere with the olfaction system of the mosquito. OBPs are small, secreted proteins that can bind volatile ligands and transfer them to the odorant receptors. The genome of A. gambiae has 57 genes encoding OBPs, with ten of them exhibiting female-predominant expression.
Our work focuses on the use of a binding assay and its adaptation to a high throughput format for the identification of ligands present in plant extracts that bind to specific OBPs. The assay is based on the displacement of the fluorescent probe N-phenyl-1-naphthylamine (1-NPN) that binds to OBPs. Ligands can thus be identified by the displacement of OPBP-bound 1-NPN and the consequent reduction in fluorescence **(Figure 1)**.









Odorant receptors (ORs)

Insect ORs constitute a novel family of seven transmembrane (7TM) proteins, which are not (structurally and genetically) related to the mammalian GPCR family of ORs and whose biochemical and other properties (i.e. post-translational modifications, expression levels, stability, internalization etc) have not been investigated in any detail. Despite recent progress in the investigations on the mechanisms of signal transduction and the deorphanization of insect ORs, the molecular and biochemical details of OR structure-function relationships responsible for the functional properties of the receptors, particularly mosquito ones, remain unexplored. For these reasons, the availability of heterologous expression systems for members of this novel family of 7TM receptors is a crucial need, especially for the mosquito, which is a medically important vector, and should greatly facilitate the development of cell-based assays for the screening for discovery of new ligands and development of new repellents.

A comprehensive study on the expression profiles of 80 ORs in A.gambiae antennae and palps was carried out by RT-PCR and quantitative real-time PCR with the goal to identify

potential candidate receptors for human odors. On the basis of prevalence of expression in female antennae, a subset of 14 ORs were identified as the strongest candidate receptors for human odor detection by female mosquitoes (Iatrou, K., and H. Biessmann. 2008, Insect Biochem Mol Biol 38:268-74).

Furthermore, using a lepidopteran insect cell-based expression platform (Douris, V., L. Swevers, V. Labropoulou, E. Andronopoulou, Z. Georgoussi, and K. Iatrou. 2006. Adv Virus Res 68:113-56), we demonstrated efficient expression of three ORs of the mosquito malaria vector Anopheles gambiae (namely the receptors OR1 and OR2, which respond to components of human sweat, and OR7, the ortholog of Drosophila's OR83b, which is thought to represent the ubiquitous heterodimerization partner of all ORs; Figure 3).

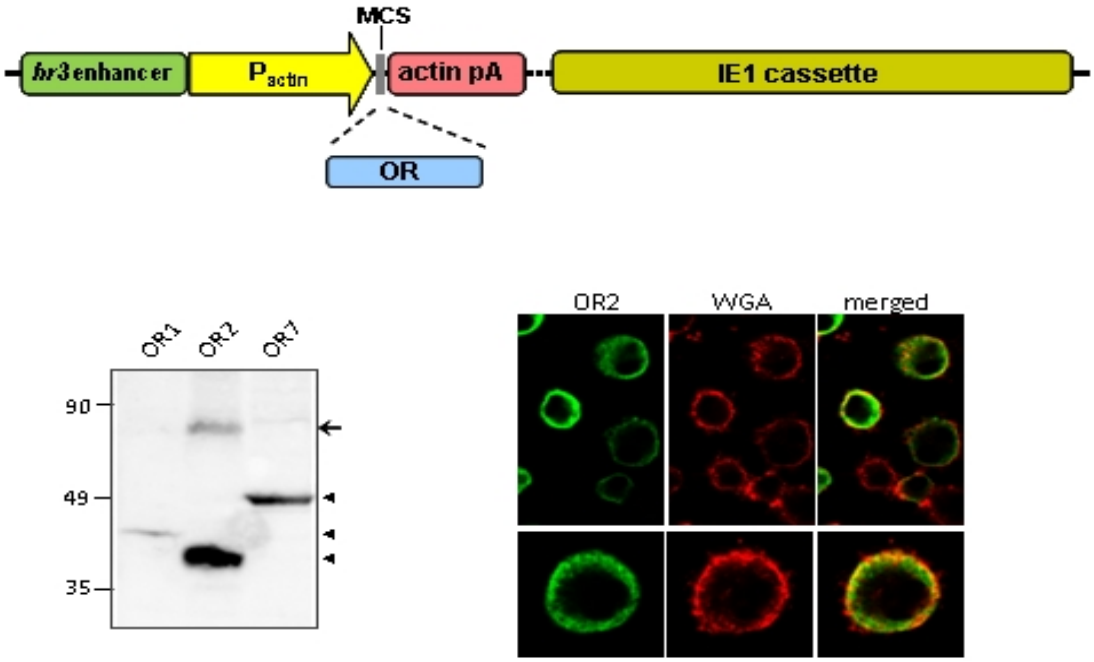


Figure 3: Expression of A. gambiae odorant receptors in lepidopteran insect cells. Shown here are the schematic representation of the OR expression vector and representative images from western blot analysis and immunofluorescence of OR-expressing cells.

Through the use of a novel "topology screen" assay developed by our group, we established that, as in the case with the ORs of Drosophila melanogaster, mosquito ORs have an inverse topology on the plasma membrane relative to GPCRs and uncovered additional and potentially important intrinsic properties, which deserve further investigation (*Tsitoura, P., E. Andronopoulou, D. Tsikou, A. Agalou, M. P. Papakonstantinou, G. A. Kotzia, V. Labropoulou, L. Swevers, Z. Georgoussi, and K. Iatrou. 2010, PLoS One 5(11):e15428*
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Having established that the mosquito ORs are non-GPCR 7TM receptors and, as is the case with their Drosophila counterparts, likely function as ionotropic channels, our current research is focussed on an effort to develop robust cell-based screening platforms that will be based on the ionotropic channel function, be amenable to adaptation to high throughput formats and used for the identification of new natural and/or synthetic ligands, leads for the development of new

mosquito attractants and repellents.

Links

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Selected publications

1. Andronopoulou, E., V. Labropoulou, V. Douris, D. F. Woods, H. Biessmann, and K. Iatrou. 2006. Specific interactions among odorant-binding proteins of the African malaria vector Anopheles gambiae. Insect Mol Biol 15:797-811.
2. Douris, V., L. Swevers, V. Labropoulou, E. Andronopoulou, Z. Georgoussi, and K. Iatrou. 2006. Stably transformed insect cell lines: tools for expression of secreted and membrane-anchored proteins and high-throughput screening platforms for drug and insecticide discovery. Adv Virus Res 68:113-156.
3. Iatrou, K., and H. Biessmann. 2008. Sex-biased expression of odorant receptors in antennae and palps of the African malaria vector Anopheles gambiae. Insect Biochem Mol Biol 38:268-274.
4. Tsitoura, P., E. Andronopoulou, D. Tsikou, A. Agalou, M. P. Papakonstantinou, G. A. Kotzia, V. Labropoulou, L. Swevers, Z. Georgoussi, and K. Iatrou. 2010. Expression and membrane topology of Anopheles gambiae odorant receptors in lepidopteran insect cell. PLoS One 5(11):e15428.
5. Biessmann, H., E. Andronopoulou, M. R. Biessmann, V. Douris, S. D. Dimitratos, E. Eliopoulos, P. M. Guerin, K. Iatrou, R. W. Justice, T. Krober, O. Marinotti, P. Tsitoura, D. F. Woods, and M. F. Walter. 2010. The Anopheles gambiae odorant binding protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. PLoS One 5(3):e9471.