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MINI-REVIEW

Current and potential biotechnological applications of odorant-binding proteins

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Abstract

Odorant-binding proteins (OBPs) are small soluble proteins whose biological function is believed to be facilitating olfaction by assisting the transport of volatile chemicals in both vertebrate and insect sensory organs, where they are secreted. Their capability to interact with a broad range of hydrophobic compounds combined with interesting features such as being small, stable, and easy to produce and modify, makes them suitable targets for applied research in various industrial segments, including textile, cosmetic, pesticide, and pharmaceutical, as well as for military, environmental, health, and security field applications. In addition to reviewing already established biotechnological applications of OBPs, this paper also discusses their potential use in prospecting of new technologies. The development of new products for insect population management is currently the most prevailing use for OBPs, followed by biosensor technology, an area that has recently seen a significant increase in studies evaluating their incorporation into sensing devices. Finally, less typical approaches include applications in anchorage systems and analytical tools.

Key points

- *Odorant-binding proteins (OBPs) present desired characteristics for applied research.*
- *OBPs are mainly used for developing new products for insect population control.*
- *Incorporation of OBPs into chemosensory devices is a growing area of study.*
- *Less conventional uses for OBPs include anchorage systems and analytical purposes.*

Keywords Odorant-binding protein · Review · Olfactory system · Biotechnology

Introduction

The olfactory system plays an essential role in maintaining life, representing one of the most basic principles in nature: perception of the environment in order to make sense of odorant cues that elicit behavioral responses which are crucial for survival. It is well established that, through olfaction, mammalians can constantly monitor the ever-changing chemical composition of their surroundings by detecting compounds, some of them in concentrations as low as parts per trillion (Breer 2003). Similarly, insects are able to use various semiochemicals to find one another, locate food sources, oviposition sites, mating partners, suitable hosts and identify predators (Brito et al. 2016).

Knowledge regarding the molecular mechanism involving odorant detection is essential when working towards the discovery of biotechnological applications for olfactory sensing. Despite the morphological differences between the characteristic olfactory organs of vertebrate (sensory epithelium within a nasal chamber) and invertebrate (appendages such as antennae) species, odorants are detected in olfactory neurons by similar mechanisms (Hildebrand and Shepherd 1997). Recent studies have provided more refined insights into aspects related to odorant detection as well as strategies adopted to achieve a better understanding of the olfactory system.

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Such information has been discussed in excellent reviews over the last years (Firestein 2001; Hansson and Stensmyr 2011; Leal 2011; Sankaran et al. 2012; Pelosi et al. 2014; Grabe and Sachse 2018). In short, after reaching the olfactory organ, volatile organic compounds (VOCs), which have low water solubility and polarity, are transported by odorant-binding proteins (OBPs) across the aqueous environment of mammalian nasal mucus (Zarzo 2007) or insect sensillar lymph, where chemosensory proteins (CSPs) are also found (Brito et al. 2016). They are then presented to membrane-bound olfactory receptors (ORs), where they are detected and signal transduction is initiated. It is worth pointing out that chemosensory receptor families also include gustatory receptors (GRs) and ionotropic receptors (IRs). GRs are related to the processing of information in gustatory sensilla, present on several parts of the body, such as mouthparts and ovipositors in females insects (Dahanukar et al. 2005). IRs are expressed in sensory neurons that respond to many odors but do not express neither ORs nor GRs and are believed to function as ligand-induced ion channels (Benton et al. 2009).

Both vertebrate and invertebrate OBPs are small, soluble proteins with a hydrophobic binding pocket in their compact structure. The main difference between insects and vertebrate classes of OBPs is regarding their three-dimensional structure. Vertebrate OBPs belong to the lipocalin superfamily of proteins and exhibit their typical β-barrel structure (Fig. 1a, b) (Flower 1996; Flower et al. 2000) with a calyx-shaped hydrophobic cavity combined of eight antiparallel β-sheets with a short segment of α-helix at the C-terminus (Sankaran et al. 2012). On the other hand, insects OBPs are an independent gene family composed of six α-helical domains (Fig. 1c, d) folded into very compact globular structures where six conserved cysteines form three interlocked disulfide bridges in an arrangement that provides great stability (Sandler et al. 2000; Tegoni et al. 2004; Brito et al. 2016). Moreover, another important element that should be considered when comparing both classes of OBPs is their number per species: *Culex quinquefasciatus* and *Aedes aegypti* genomes code for 109 and 111 OBPs, respectively (Manoharan et al. 2013) whereas there are only 5 known OBP genes in mice, for example (Shiao et al. 2012; Cave et al. 2019).

Despite the fact that ORs are responsible for the detection and recognition of different odorants (Mombaerts 1999; Korsching 2002; Breer 2003; Leal 2011; Suh et al. 2014; Grabe and Sachse 2018), previous reports indicated that ORs cannot be properly folded when expressed in bacteria (Wang et al. 2016), causing them to be only partly located in the membrane and also found in cell organelles and inclusion bodies (Kaiser et al. 2008). Currently, there are well-established experimental strategies which allow the functional heterologous expression of ORs in eukaryotes. In vitro systems involve cell culture platforms, among which is possible to highlight *Xenopus laevis* oocytes (Franco et al. 2018; Liu et al. 2020) and also human embryonic kidney cells (HEK)

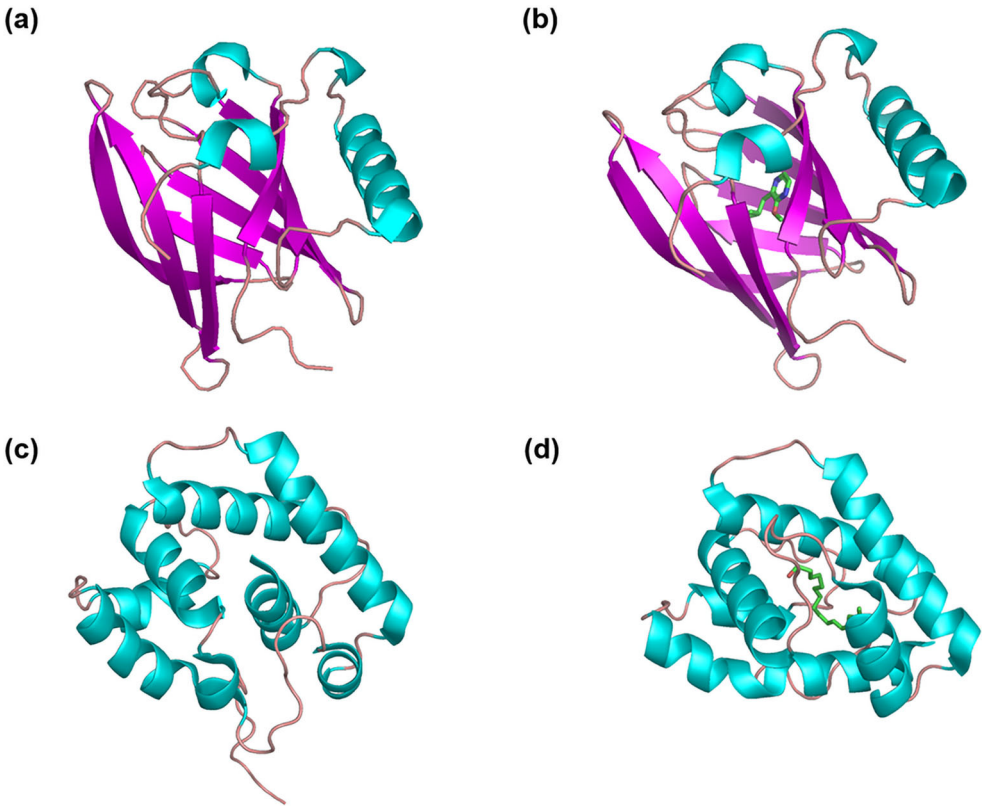

Fig. 1 Representative three-dimensional structures of vertebrate and insect OBPs. α-Helices are shown in cyan, β-barrel in magenta and loops in brown. Presented structures were obtained from the Protein Data Bank (PDB) and prepared using PyMOL software. **a** Odorant-binding protein from the nasal mucosa of a pig, pOBP (PDB ID: 1A3Y). **b** The same protein complexed with pyrazine (2-isobutyl-3-metoxypyrazine) (PDB ID: 1DZK). **c** *Bombyx mori* pheromone-binding protein (BmorPBP) in its unbound form (PDB ID: 1GM0). **d** BmorPBP1 in complex with its natural ligand bombykol (PDB ID: 1DQE)

(Corcoran et al. 2014; Hou et al. 2020), while in vivo heterologous expression is based on the use of transgenic *Drosophila* techniques, including the "empty neuron" system (Dobritsa et al. 2003; Chahda et al. 2019) and the $Or67d^{GAL4}$ knock-in system (Kurtovic et al. 2007). Therefore, expression of ORs in heterologous systems are much more demanding and undermined by low yields and poor stability for both vertebrate and insect receptors (Hamana et al. 2010; Corcoran et al. 2014; Carraher et al. 2015), since it encounters problems which are inherent to their nature as seven-transmembrane proteins, frequently causing protein aggregation and toxicity (Kaiser et al. 2008; Miazzi et al. 2019). The manipulation of membrane proteins is often difficult and time consuming, which has contributed to the investigation of simpler biomolecules to be used as sensing elements (Barbosa et al. 2018). Also, insect ORs have a unique topology consisting of heterodimers assembled by a ligand-selective OR and an olfactory coreceptor (Orco) (Larsson et al. 2004; Sato et al. 2008; Nichols and Luetje 2010; Vosshall and Hansson 2011), forming gated ion channels that have already been shown to be nonfunctional without Orco expression (Zhou et al. 2014; Franco et al. 2016).

Still on this matter, CSPs belong to a different family of small soluble proteins identified in many insect orders. They are shorter and share no sequence homology with OBPs, in addition to presenting a different type of α-helical fold, containing only four conserved cysteines forming two disulfide bridges instead of three in classical OBPs (Campanacci et al. 2003). CSPs present high thermal stability and are associated with the transport of small hydrophobic compounds in chemosensory organs, which could make them suitable targets for the same biotechnological applications as OBPs. It is worth noting that Liu et al. (2016) have described CSPs from *Bemisia tabaci* to be crucial in facilitating the transport of fatty acids, while others seem to be tuned to insecticide exposure, suggesting these results could be further exploited for the development of a new generation of biosensor chips to monitor lipid blood concentration and chemical environmental pollution (Liu et al. 2017). However, CSPs have a larger tissue distribution when compared with OBPs, and their role in olfaction still is a matter of debate, since they are also expressed in nonsensory organs and proposed to be involved in a variety of other functions, such as tissue regeneration (Nomura Kitabayashi et al. 1998), development and differentiation (Maleszka et al. 2007; Zhou et al. 2013) and physiological shifts (Guo et al. 2011), among others. Also, even though a few CSPs have been reported to bind behaviorally active compounds such as brood pheromones (Briand et al. 2002), host plant volatiles (Zhou et al. 2020) and repellent compounds (Duan et al. 2019), they are way less rigid when compared with OBPs. Their conformation can be so drastically modified upon ligand binding that its pocket becomes able to accommodate three ligands (Campanacci et al. 2003). For this reason, molecular modeling and docking simulations are less reliable and ligand selectivity is affected, since such a flexible binding site can accommodate a variety of different compounds.

Finally, the small size and easy production of both insect and vertebrate OBPs place them in a favorite position for biotechnological applications. Such proteins have been efficiently expressed in bacterial systems (Wojtasek and Leal 1999; Yabuki et al. 2010; Yang et al. 2011; Klint et al. 2013), as well as in yeast (Ferrari et al. 1997; Briand et al. 2001; Lartigue et al. 2004) with satisfactory yields. Also, purification can be easily accomplished by chromatographic steps. Besides having exceptional stability to solvent denaturation and to proteolytic degradation, OBPs are particularly stable to thermal denaturation. In addition, binding of the ligand inside the hydrophobic cavity has been reported to increase their thermal stability even more (Paolini et al. 1999). OBPs have pretty compact three-dimensional structures that, when combined with structure prediction methods such as homology modeling, dynamics simulation and molecular docking, support the creation of reliable models (Venthur and Zhou 2018). The number of OBP structures resolved so far provides sufficient information regarding the architecture of the binding pocket to allow the modification of their sequences by site-directed mutagenesis with a reasonable degree of accuracy, making it possible to obtain mutants with the desired specificity and predicted behavior (Pelosi et al. 2018).

In summary, OBPs have desired features such as being small, soluble, stable, and easier to manipulate and modify, making them more accessible targets for research. Their superior workability has led to studies in different systems over recent years (Fig. 2), and this review aspires to present a contemporary unified source of information concerning current applications as well as perspectives and potential uses in biotechnology involving OBPs. At present, the development of new products for insect population control is the most widely discussed use for these proteins. However, the growing amount of research aiming to uncover new commercial applications has led to a noteworthy increase in studies evaluating the construction of biosensors as well as the potential use of OBPs for less conventional purposes, which are discussed in the following text.

Screening for behaviorally active compounds

Prospecting new attractants or repellents for population management of agricultural pests and insect vectors strongly relies on a better comprehension of the molecular mechanism underlying the olfactory system. Screenings for novel behaviorally active compounds capable of interfering in olfaction-mediated behavioral responses can be performed based on molecular interactions between candidate compounds and olfactory proteins through an approach called "reverse chemical

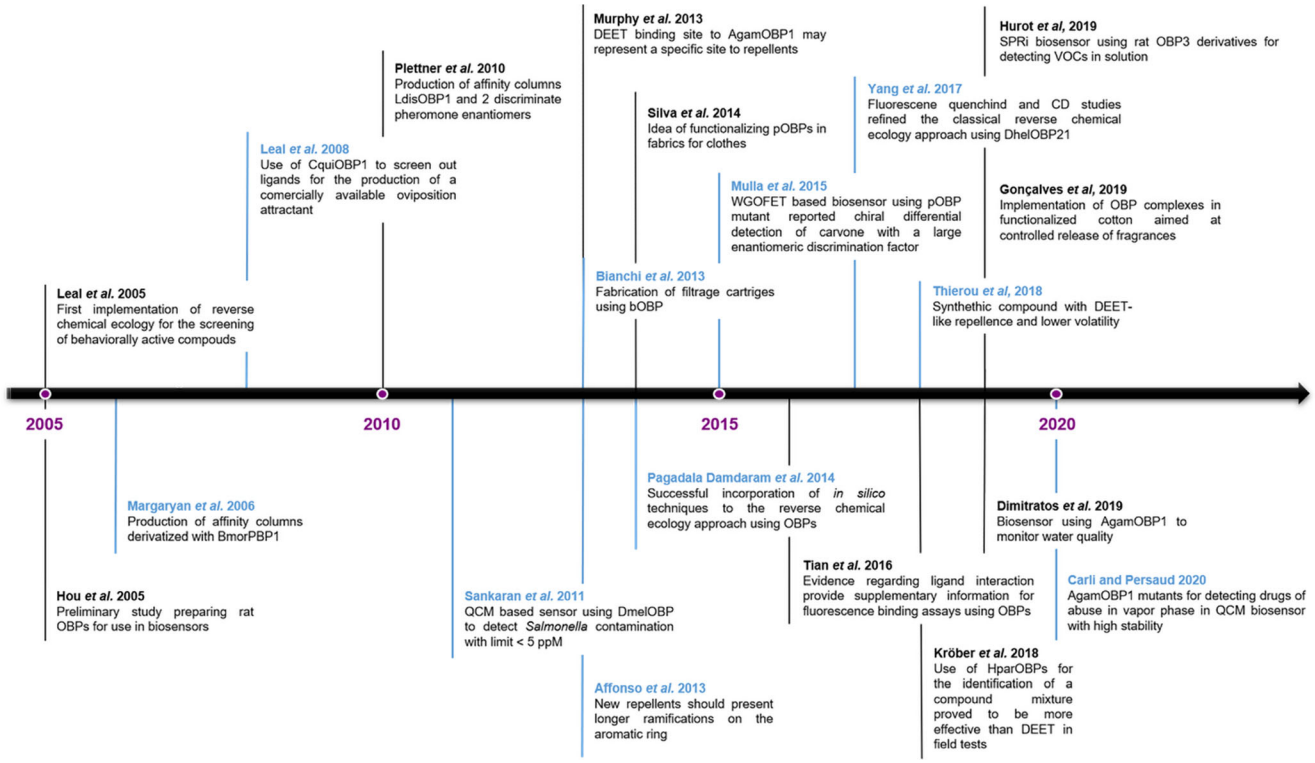

Fig. 2 Overview of biotechnological applications of OBPs over the years

ecology". This strategy is very similar to receptor-based drug discovery and has become an efficient and reliable method for such purpose (Brito et al. 2016).

In light of reverse chemical ecology and based on the principle of competitive binding between a ligand and a fluorescent probe for the hydrophobic pocket of an OBP, fluorescence binding assays (D'Onofrio et al. 2020) are the most widely adopted technique to estimate binding affinities of OBPs to relevant compounds for further use in behavioral tests. Even though other olfactory proteins such as ORs (Franco et al. 2018) and CSPs (Duan et al. 2019) have already been employed as molecular targets for the discovery of new behaviorally active ligands, OBPs are still the most utilized proteins due to the aforementioned advantages associated with their use (Table 1).

This strategy was first implemented for the identification, synthesis and field evaluation of formulations of the pheromone system of the Navel Orangeworm moth, *Amyelois transitella*, leading to the development of better lures (Leal et al. 2005). Later, a study combining reverse and conventional chemical ecology approaches made possible to screen out a strong attractant for the southern house mosquito *C. quinquefasciatus* using CquiOBP1 as a molecular target (Leal et al. 2008). The obtained results have provided significant data for the production of the oviposition attractant which is commercially available nowadays. More recently, six OBPs from *Anopheles gambiae* were employed in binding assays in order to identify mosquito repellents in essential oils extracted from aromatic plants. Their use in the initial evaluation was reported to reduce the number of samples to be examined in repellent bioassays by 57 % due to nonbinding (Kröber et al. 2018). The authors were able to identify a number of compounds and mixtures with significant spatial repellency activity, among which the combination of carvacrol plus cumin alcohol proved to be more effective than DEET (the current golden standard in insect repellents) (Antwi et al. 2008; Diaz 2016) at reducing the number of *Anopheles* species in shelters. In a similar way, two OBPs from the dark black chafer (DBC) *Holotrichia parallela* were described as displaying preferential binding to one particular green-leaf plant volatile (GLV). Both HparOBP20 and HparOBP49 strongly bind (Z)-3-hexenyl acetate, a plant volatile that is released in large amounts after damage and was previously reported to play a crucial role in insect-plant interactions. Field tests revealed that mixing this GLV with its species sex pheromone increases the number of caught insects up to 7-fold when compared with traps baited with sex pheromone alone (Ju et al. 2018). It is not unreasonable to suppose that these findings could assist in the development of new environmentally friendly strategies for DBC population control. Moreover, not long ago, CmedOBP14 from the rice leaf folder *Cnaphalocrocis medinalis* was successfully employed in fluorescence binding assays for the screening of behaviorally active compounds, of which L-limonene and nerolidol were identified as attractants and ß-ionone a repellent (Sun et al. 2019).

After a while, computational methods were shown to be reliable in order to pre-select candidate ligands and started to

Table 1 Overview of OBPs used for screening of behaviorally active compounds

OBP	Species	Ligand	Activity	Reference
AimelOBP3, AimelOBP5	*Ailuropoda melanoleuca*	β-Ionone, cedrol, citral, farnesol, safranal	Attractant	Zhu et al. (2017)
AgamOBP1, AgamOBP3, AgamOBP4, AgamOBP5, AgamOBP30, AgamOBP47	*Anopheles gambiae*	Carvacrol, cumin alcohol, butyl cinnamate, ethyl cinnamate	Repellent	Kröber et al. (2018)
BdorGOBP	*Bactrocera dorsalis*	Methyl eugenol	Attractant	Pagadala Damdaram et al. (2014)
CmedOBP14	*Cnaphalocrocis medinalis*	l-Limonene, nerolidol, ß-ionone	Attractant, attractant, repellent	Sun et al. (2019)
CquiOBP1	*Culex quinquefasciatus*	Trimethylamine, nonanal	Repellent	Leal et al. (2008)
DhelOBP21	*Dastareus helophoroides*	(+)-β-Pinene	Attractant	Yang et al. (2017b)
HparOBP20, HparOBP49	*Holotrichia parallela*	(Z)-3-Hexenyl acetate	Attractant	Ju et al. (2018)
SspOBP7	*Sclerodermus* sp.	(+)-α-Longipinene, terpinolene	Attractant	Yi et al. (2018)

be incorporated as a manner of optimizing the screening process (Brito et al. 2016). Molecular docking and molecular dynamics studies with an OBP from the fruit fly *Bactrocera dorsalis* were successfully employed for the prediction of behaviorally active semiochemicals for this agricultural pest (Pagadala Damdaram et al. 2014). Actually, studies mainly focused on establishing *in silico* screening models to preaccess the potential affinity for new ligands are becoming increasingly relevant in emerging publications, as in the case of Du et al. (2018). They used OBP3 from *Megoura viciae* in molecular docking and molecular dynamics simulations to explore its binding mode to (E)-β-farnesene (the main component of the aphid alarm pheromone) analogs. Results provide theoretical guidance for the rational design of aphid alarm pheromone-like molecules that can be used for the development of products which would be able to keep these insects away from crops.

Reverse chemical ecology has also been employed for a very particular and interesting purpose involving the giant panda, *Ailuropoda melanoleuca*, which is now considered to be a vulnerable species after many years in the list of endangered ones. The discrepancy between physiology and lifestyle of the giant panda still conceals unexplored aspects that puzzle specialists: its obligate bamboo diet is not compatible with its typical carnivorous digestive system. The low energy obtained from such a poor diet leads to a sedentary life and a very limited reproduction rate, with only a single offspring every other year, increasing the vulnerability of the species. Conventional chemical ecology approaches are based on a process of extracting secretions from semiochemical senders, separating extracts into fractions, using receptors to assist in the identification of active ingredients and, finally, by elucidating chemical structures and synthesis (Leal 2017), which is still too invasive for studying endangered or vulnerable species. As an alternative, Zhu et al. (2017) used reverse chemical ecology associated with proteomics to perform a study regarding olfaction and chemical communication in order to better elucidate the molecular mechanisms responsible for such unique and anomalous diet. Based on their abundance in the nasal mucus, AimelOBP3 and AimelOBP5 were selected for functional and structural characterization. Results indicated that AimelOBP3 has high affinity to plant volatiles such as citral, safranal, farnesol, β-ionone and cedrol; this last one being best represented in bamboo leaves collected in the spring (when the mating season of the panda occurs) and also to long-chain aldehydes, common constituents of insect sex pheromones. Although the pheromone mixture of the giant panda is yet to be identified, it is possible that these linear aldehydes might present a similar function in this species. On the other hand, AimelOBP5 binds unsaturated fatty acids but not their corresponding aldehydes or the plant volatiles which were observed as the best ligands for AimelOBP3, suggesting that these two proteins have complementary spectra of binding. It is worth emphasizing that the discovery of odorants produced by the giant panda, particularly by males, could play an important role in conservation efforts when used to enhance female-oriented behaviors, increasing sexual motivation. Additionally, compounds able to attract the giant panda to bamboo could be employed as attractants to other food sources, drawing the panda to feed on supplemental nutrition such as the panda bread (Bian et al. 2013). Therefore, it is not unexpected that reverse chemical ecology could start to be employed to a greater extent when dealing with endangered or even difficult-to-reach species for which it would be difficult to obtain enough biological samples or perform accurate behavioral observations.

It is worth mentioning that even though fluorescence competition-binding assays very often provide accurate affinities of different ligands for a binding pocket, its limitations need to be considered. It has been reported that, contrary with expectations, some compounds with good binding affinities are not able to actually form a complex with the protein,

mainly because this type of experiment does not provide structural information upon ligand binding. This makes the single use of this technique sometimes less reliable because its results might include candidate compounds that do not exhibit biological activity (Siciliano et al. 2014; Yin et al. 2015; Yang et al. 2017b). Basically, the authors describe that besides high binding affinity, conformational flexibility also plays an important role in complex formation. During the collisions that happen between a protein pocket and ligands in the beginning of the binding process, suitable ligands enter and are able to fixate themselves into the pocket, leading to a desired transformation for the formation of the complex. Unsuitable ligands, on the other hand, might be able to enter the pocket but fail to bind the proteins because the conformational transformation does not occur. Recent studies have reported that a protein is able to adjust its conformation to expose its binding site, while a ligand adjusts its structure to bind the active pocket. When both these conformations are a match, stable binding occurs, on the other hand, if the conformations of the proteins and the ligand do not match, the flexibility of the protein will negatively affect ligand binding to avoid wrong ligands. In this context, more direct evidence regarding the interactions between compounds and proteins could provide supplementary information to fluorescence binding assays (Tian et al. 2016). In this regard, fluorescence quenching analysis based on the interaction between phosphor and quenching molecules have been increasingly used to study in detail what happens when a protein binds a ligand (Li et al. 2013, 2018). Briefly, that are two types of quenching modes of proteins: the static quenching, which results from stable binding between the fluorescent sample and the quencher, and the dynamic quenching resulting from collisional encounters between the fluorophore and the quencher (Sun et al. 2006). A study combining fluorescence quenching analyses and competitive binding assays have recently revealed that only six of nineteen ligands with high binding affinities were actually capable of forming complexes with SspOBP7 from the parasitoid wasp *Sclerodermus* sp. (Yi et al. 2018). Authors have hypothesized that the collision of ligands could also cause a decrease in fluorescence by colliding with the binding site of *N*-phenyl-1-naphthylamine (1-NPN) or even by affecting the stabilization of NPN-OBP complexes. Structural transformations associated with complex formation upon ligand binding can be more thoroughly evaluated by circular dichroism (CD) (Yang et al. 2017b; Li et al. 2018; Yi et al. 2018). It is worth pointing out that Yang et al. (2017a) were only able to explain why camphor, a ligand which presents high affinity and static interaction to DhelOBP21, does not elicit behavioral responses in *Dastareus helophoroides* using CD to investigate conformational changes. CD studies indicated that binding of camphor to DhelOBP21 actually destabilizes the structure because camphor could not cause the formation of a N-terminal helix necessary to stabilize the complex. Thus, it is plausible to expect the association of fluorescence binding to another technique capable of assessing the secondary structure of an OBP upon ligand binding, such as fluorescence quenching analysis or CD, to be the next update in optimizing the reverse chemical ecology approach, as computational methods once were. This new step could be used in future research to narrow down the scope of candidate behaviorally active compounds by excluding false-positive ligands that present high binding affinities to OBPs but are unable to form stable complexes.

Structure-based design of new repellents

The global market for insect repellents, especially mosquitoes, has been continuously growing and projections expect it to reach US$4.8 billion in 2022. This scenario includes a limited number of registered molecules and is largely dominated by diethyltoluamide (DEET), followed by a small number of other repellents such as icaridin and IR3535 (Devillers 2018). However, it has already been suggested that the use of such products could lead to a series of problems: limited or no efficiency in a few insects as well as the phenomena of resistance have been reported (Coleman et al. 1993; Stanczyk et al. 2013; Franco et al. 2018). Additionally, skin irritations and neurotoxic effects have been observed in humans (Antwi et al. 2008; Broschard et al. 2013; Swale et al. 2014; Wiles et al. 2014; Heng et al. 2017). Taking all that into consideration, the search efforts to find new repellents with high efficiency and little to none collateral damage to humans and the environment has become imperative. In this context, even though ligand-based computational methods have already been vastly used, structure-based modeling of new repellents presents itself as a very promising approach that can provide leads with improved binding characteristics and specificity (Table 2) but was, until recently, unfeasible due to the lack of available three-dimensional structures of olfactory proteins in complex with a repellent (Zographos et al. 2018).

The crystal structure of AgamOBP1 from *A. gambiae* in complex with DEET was demonstrated for the first time by Tsitsanou et al. (2012). The study was able to show that the repellent exploits nonpolar interactions and a unique hydrogen bond between the amide oxygen and a water molecule in order to bind at the edge of a long hydrophobic tunnel. The authors perceived such characteristics to be critical for the recognition and binding of DEET and identified desired structural features for designing new repellents. The accuracy of their methodology was evaluated and confirmed by docking simulations of 29 carboxamides and piperidine derivatives. Later, the structure of the complex between AgamOBP1 and the natural repellent 6-methyl-5-heptene-2-one (6-MH), a human sweat component, revealed that 6-MH binds to the protein at the exact same site as DEET (Murphy et al. 2013). The key interaction with a single water molecule that was previously

Table 2 Overview of OBPs used for structure-based design of new repellents

OBP	Species	In complex with	New compounds	Reference
AaegOBP1	*Aedes aegypti*	PEG	2-(4-Fluorophenyl)cyclohexanol, 1-(3-methylpiperidin-1-yl) heptan-1-one	Oliferenko et al. (2013)
AgamOBP1	*Anopheles gambiae*	DEET	6-Methyl-5-heptene-2-one	Murphy et al. (2013)
AgamOBP1	*Anopheles gambiae*	DEET	Eugenyl acetate	Affonso et al. (2013)
AgamOBP1	*Anopheles gambiae*	DEET	*p*-Anisyl hexanoate, thymol acetate, thymyl isovalerate	Da Costa et al. (2019)
AgamOBP1	*Anopheles gambiae*	Icaridin	Synthethic compound KO9	Thireou et al. (2018)

proposed to be important for DEET binding does not seem to be involved in the recognition of 6-MH. The fact that more than one compound with repellent activity but different chemical structures are able to bind to the same site supports the idea that the DEET-binding site may represent a specific binding site for repellents. Molecular dynamics simulations of AgamOBP1 in complex with 6-MH and DEET were performed to better investigate the binding of these two ligands, providing new insights on repellent recognition by AgamOBP1 (Tzotzos et al. 2018). Complexes between AgamOBP1 and DEET have also been studied by docking analysis and molecular dynamics simulation to compare the interactions of this OBP wth molecules with known attractive activities such as lactic acid, 1-octen-3-ol, indole and eugenol, and the main components of the oil of Indian clove (*Syzygium aromaticum*), with potential repellent activities, making it possible to observe the fundamental residues for an effective interaction with this protein (Affonso et al. 2013). Results showed that all tested compounds compete for the DEET binding site, interacting with the same residues. Docking analysis indicated that eugenyl acetate presents a greater affinity for AgamOBP1 than DEET. Finally, the authors suggested that new repellent derivatives from DEET should present longer ramifications on the aromatic ring in order to explore nonpolar interactions inside the long hydrophobic tunnel. More recently, the structure of DEET complexed with AgamOBP1 was used as reference to perform a structural and pharmacophoric similarity search for compounds derived from essential oils (Da Costa et al. 2019). The authors made use of several in silico techniques and reported that natural products such as thymol acetate and *p*-anisyl hexanoate binds AgamOBP1 in the same mode as DEET, forming hydrogen bonds and hydrophobic interactions, in addition to having similar physicochemical properties. This study illustrates an interesting approach, focusing on the structure of the bound repellent in order to search for compounds that interact in a similar way with the OBP. It is plausible to assume that this kind of approach could facilitate the design of new repellents with increased selectivity and affinity for the binding cavity.

In order to elucidate the binding mode between AgamOBP1 and icaridin, Drakou et al. (2016) determined the crystal structure of the complex. Results showed that icaridin actually binds to two different regions: a new binding site located at the C-terminal region and also to the DEET-binding site in two different orientations. Which binding mode is preferably adopted seems to be strongly related to the conformation of the sec-butyl moiety of icaridin. A very interesting outcome of this work is the observed capability of AgamOBP1 crystals to stereoselectively bind the most active 1R,2S-isomer of icaridin's equimolar diastereoisomeric mixture. Such result provides structural evidence for the possible contribution of AgamOBP1 to the stereoselectivity of this chemical in mosquitoes. A virtual screening protocol based on structural properties was then proposed for the discovery of novel bioinspired synthetic scaffolds that exhibit lower volatility and increased protection time compared with their plant-derived parental compounds (Thireou et al. 2018). After screening a library of 42 755 synthetic molecules, sixteen were selected and further evaluated for their binding affinity to AgamOBP1 as well as their repellent properties against female *A. gambiae* mosquitoes. Four of these compounds were found to exhibit repellency indexes between 69 and 79%. The novel synthetic compound KO9 displayed DEET-like repellence (91%) while having a significantly lower volatility when compared with DEET and its plant-derived parental cuminic acid. Also, an approach combining quantitative structure-activity relationship (QSAR) modeling, molecular docking and bioassays was employed by Oliferenko et al. (2013) for the identification of potential repellents with activity against *Aedes aegypti*. QSAR analysis revealed structural molecular determinants of repellent action against the mosquito. This information was used to identify highly promising scaffolds and individual compounds with mosquito repellent activity.

Knowledge regarding the specific mechanism of binding to the OBP is crucial for the success of structure-based discovery of novel repellents. However, AgamOBP1•DEET and AgamOBP1•icaridin crystal structures are the only available experimental OBP•repellent models for structure-based approaches to this date. More experimental studies on binding

specificities of known repellents to OBPs from various insects' species are still necessary, providing data that would be extremely beneficial for this kind of approach.

Biosensors

In recent years, a higher understanding of mechanisms underlying odor recognition in biological systems has led to an increased progress in biosensor technology. This area of study is primarily focused on making the fabricated biosensor reach parameters close to its biological counterpart, such as high sensitivity, repeatability, specificity, short response time and wide spectrum detection, a goal that could be achieved through the utilization of biological components from naturally occurring chemosensory systems. Furthermore, thanks to advances in biology, biotechnology, genetical engineering and nanotechnology, it is currently possible to use biological materials for the manufacturing of such devices (Wasilewski et al. 2018; Cave et al. 2019).

The general architecture of an artificial chemosensory device consists of a system with two basic components: a chemical sensor and a pattern recognition engine, which converts the chemical input into an electrical signal (Gardner and Bartlett 1994), and it is in the chemical sensor component where development efforts have been concentrated over the last decades. As far as sensor design goes, it is possible to separate them in two major categories: the ones which employ biological components and those which rely on inanimate components, like metal oxides and polymers (Zohora et al. 2013), for the detection of target compounds. With regard to sensors which incorporate biological materials, even though there are reports of whole tissues (Strauch et al. 2014; Ibarra-Soria et al. 2017), cultured cells (Ko and Park 2005; Lee et al. 2009), olfactory neurons (Du et al. 2015), ORs (Khadka et al. 2019; Yang et al. 2017a), OR peptides (Lee et al. 2018), and CSPs (Liu et al. 2013) being used as biosensing elements, OBPs present a series of already discussed advantages that make them the most promising sensors and have prompted the exploration of these proteins associated with different detection systems in chemosensory design (Table 3).

The potential for the development of biosensors using OBPs started to be reported almost two decades ago, when a preliminary study first demonstrated the preparation of ultra-thin Langmuir-Blodgett (LB) films incorporating a recombinant rat OBP1, OBP-1F (Hou et al. 2005). Years later, Sankaran et al. (2011) focused on mimicking an insect OBP to detect VOCs indicative of *Salmonella* contamination, such as 3-methyl-1-butanol and 1-hexanol, in packaged beef. Quartz crystal microbalance (QCM) based sensors in conjunction with a synthetic peptide derived from the amino acid sequence of *Drosophila melanogaster* OBP LUSH as the sensing material were developed (Fig. 3a) and shown to be sensitive to alcohols, with estimated lower detection limits of < 5 ppm. A more recent study regarding *Salmonella* contamination has also fabricated a peptide-based biosensor using a LUSH-derived peptide, but with carbon nanotube field-effect transistor (CNT-FET) as the detection method (Son et al. 2016). In order to verify if the designed biosensor could be used for the assessment of food contamination, fresh and *Salmonella*-contaminated ham samples were presented to the biosensor. While the sensor did not elicit any response to the fresh sample, a significant signal was observed with a response time of less than 2 s for the contaminated one. Results demonstrated that the sensor succeeded in detecting 3-methyl-1-butanol at a concentration of femtomolars. It also selectively distinguished the target odor molecule from other compounds with similar structures without a complex pretreatment process. On this topic, one could easily expect this kind of approach to be more and more employed for the quality control of various food samples by using different OBPs from different organisms. Still on the matter of food quality, a proposed sensor system based on an array configuration of five surface acoustic wave (SAW) resonators coated with three types of OBPs: the wild-type OBP from bovine (wtbOBP), a double mutant of the OBP from bovine (dmbOBP) and the wild-type OBP from pig (wtpOBP), has been described for the recognition of R-(−)-1-octen-3-ol (octenol) and R-(−)-carvone (carvone) vapors, two odorants largely used in the food industry. The fabricated device was tested in nitrogen atmosphere and results showed low detection limits for the tested compounds such as 0.48 ppm for octenol and 0.74 ppm for carvone (Di Pietrantonio et al. 2013, 2015; Palla-Papavlu et al. 2014). The authors also mention that the biosensing system was able to discriminate the octenol molecules from the carvone, making it pertinent for the assessment of food contamination by molds, or for the evaluation of indoor air quality in buildings. Monitoring of air quality has also been a matter of interest for Cennamo et al. (2015). According to them, low molecular mass aldehydes are some of the main toxic compounds present in the environment and their characterization could be crucial in order to elucidate reactions that occur in the atmosphere. With that in mind, the group reported the construction of a new biosensor based on surface plasmon resonance (SPR) in a plastic optical fiber using porcine OBP (pOBP) as the sensing element. The device was able to detect butanal in solution in the range of micromolars.

As well as for air quality, water supply safety has always been a major concern to public health. Taking that into consideration, a very recent study described a novel sensor technology encompassing an olfactory protein from *A. gambiae*, AgamOBP1, as the sensing element (Dimitratos et al. 2019). This OBP binds analytes associated with coliform bacteria such as indole, a major *Escherichia coli* metabolite, and does so with high specificity and sensitivity. Taking advantage of

Table 3 Overview of biosensors incorporating odorant-binding proteins as sensing elements

OBP	Derived from	Detection method	Detection limit	Detected compounds	Reference
Acer-ASP2	*Apis mellifera*	EIS	μM	Floral odor and pheromones	Lu et al. (2014)
Acer-ASP2	*Apis mellifera*	SPR	pM	Floral odor and pheromones	Zhang et al. (2015)
AgamOBP1	*Anopheles gambiae*	Colorimetric	ppm	Indole	Dimitratos et al. (2019)
AgamOBP1-S82P	*Anopheles gambiae*	QCM	μM	Cocaine-HCl	Cali and Persaud (2020)
AgamOBP7	*Anopheles gambiae*	SiNW	ppb	Human-derived odorants	Gao et al. (2020)
AmelOBP14	*Apis mellifera*	rGO-FET	pM	Bee attractants	Larisika et al. (2015), Reiner-Rozman et al. (2016), Kotlowski et al. (2018)
LUSH	*Drosophila melanogaster*	QCM	ppm	3-Methyl-1-butanol and 1-hexanol	Sankaran et al. (2011)
LUSH	*Drosophila melanogaster*	CNT-FET	fM	3-Methyl-1-butanol	Son et al. (2016)
BdorOBP2	*Bactrocera dorsalis*	EIS	pM	Isoamyl acetate, β-ionone and benzaldehyde	Lu et al. (2015)
dmbOBP	*bovine*	SAW resonators	ppm	Octenol and carvone	Di Pietrantonio et al. (2013, 2015)
wtbOBP	*bovine*	SAW resonators	ppm	Octenol and carvone	Di Pietrantonio et al. (2013, 2015)
wtpOBP	*porcine*	SAW resonators	ppm	Octenol and carvone	Di Pietrantonio et al. (2013, 2015)
pOBP-F88W	*porcine*	WGOFET	pM	Carvone enantiomers	Mulla et al. (2015)
pOBP	*porcine*	PCD cantilevers	μM	IBMP and DNT	Manai et al. (2014)
pOBP	*porcine*	SPR	μM	Butanal	Cennamo et al. (2015)
pOBP	*porcine*	PCD cantilevers	–	IBMP	Possas-Abreu et al. (2017)
OBP-1F	*rat*	EIS	–	Isoamyl acetate	Hou et al. (2005)
OBP derivatives	*rat*	SPR	pM	Hexanal	Hurot et al. (2019)

that fact, the authors have decided to incorporate an AgamOBP1-based biosensor into a lateral flow device which is similar in application to commercially available pregnancy tests, in order to obtain faster results for the detection of indole. The apparatus was supported on a plastic substrate and assembled as nitrocellulose strips with a paper sample pad. This implementation presents a test line containing a known ligand for AgamOBP1 and a control line where an antibody for this protein is placed. Right after the sample pad and before the control and test strips, there is also a strip with a recombinant AgamOBP1-colloidal gold conjugate. The purpose of the gold, in this case, is to make the protein visible to the naked eye. When AgamOBP1 binds the synthetic ligand in the control line, it will deposit colloidal gold and create a color change. If the biosensor is working properly, the control line will always be visible, which demonstrates that AgamOBP1 was able to travel along the entire device. The AgamOBP1-colloidal gold complex is laterally carried across the device's surface when an aqueous analyte is put in the sample pad and the detection is based on the competition between the ligand in the test line and any indole present in the sample. If AgamOBP1 binds to the analyte, it will not bind the synthetic ligand in the test strip and the device will display only the control line for a positive result. On the other hand, if indole is not present in the sample, the AgamOBP1-colloidal gold is free to bind to the test strip and to the control strip, exhibiting two visible lines for a negative result.

Exploring a different field of endeavor, Ramoni et al. (2007) have investigated the potential of vertebrate OBPs for the development of biosensors for the detection of explosives. Their results showed pOBP to have high affinity towards the TNT simulant 2,4-dinitrotoluene (DNT), dimethylformamide (DMF) and phenylbenzenamine (DFA), suggesting the lipocalin scaffold could be an appropriate model for the detection of these hazardous compounds. Later, Manai et al. (2014) reported the manufacture of this type of device, chemically grafting pOBPs onto polycrystalline diamond (PCD) microcantilevers to that end. They were capable of producing sensors with sensitivity for both IBMP, a reference compound commonly used for the characterization of OBPs affinity constants, and DNT, in micromolar concentrations. However, it is also mentioned that low specificity still is an inconvenience to overcome in this case. Another interesting approach for the manufacture of biosensors has been described by Mulla et al. (2015), whose work reported on the binding of carvone enantiomers to a pOBP mutant, pOBP-F88W, detected by means

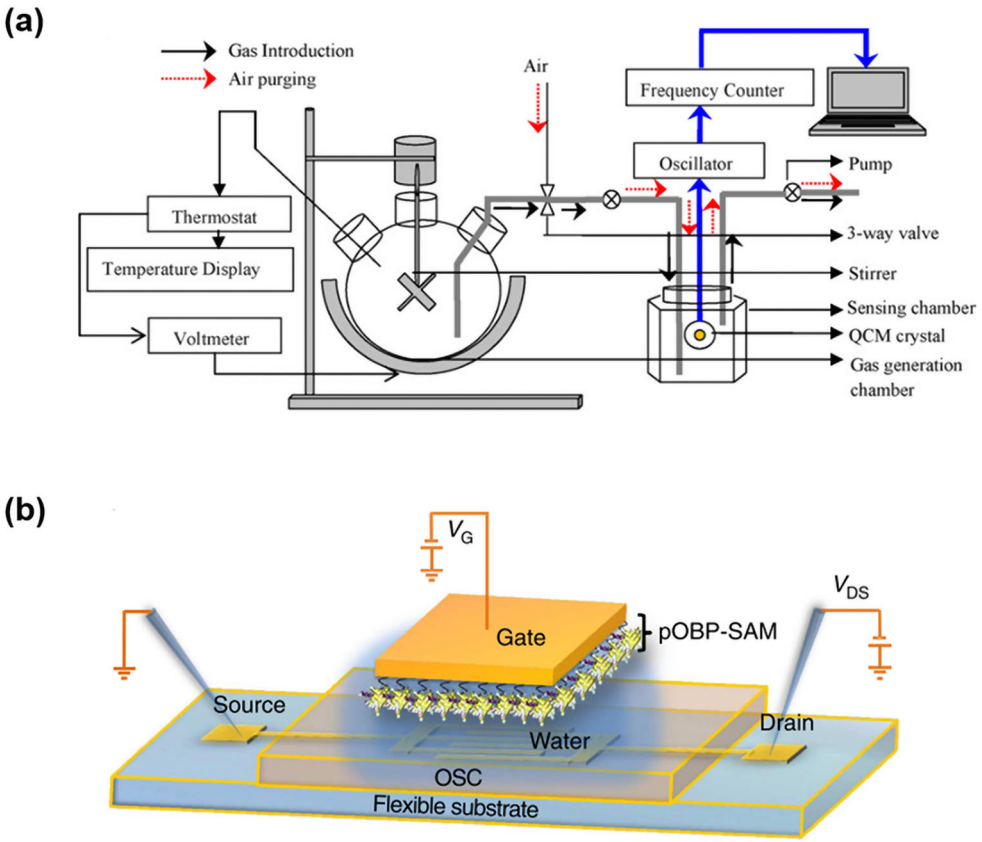

Fig. 3 Schematic view of biosensors incorporating OBPs. **a** Experimental set-up for a QCM-based sensor using a Dmel LUSH synthetic peptide. Modified from Sankaran et al. (2011). **b** Structure of a WGOFET device comprising a pOBP immobilized through a self-assembled monolayer (SAM). Modified from Mulla et al. (2015)

of a water-gated field-effect transistor (WGOFET) (Fig. 3b) in the picomolar concentration range. With this approach, analysis of the free-energy balance and degree of capacitance decreases provided strong support for the hypothesis that, on S-(+)-carvone ligand binding, the pOBP undergoes a conformational change that is not seen with the R-(−)-enantiomer, allowing for chiral differential detection with a large enantiomeric discrimination factor, which could lead to the development of a unique analytical tool with wide applicability. In a like manner, aiming to implement a portable tool that could be applied to situations such as the detection of toxic chemicals and narcotics control, as well as noninvasive diagnosis, Lim et al. (2017) used OBP LUSH from *Drosophila* as a sensing substrate for estimating ethanol concentration. The LUSH protein was fused with silicon-on-insulator (SOI)-based ion-sensitive field-effect transistors (ISFET) and workability as an olfactory sensor was investigated. In the results, LUSH OBP-fused ISFET sensor was able to detect ethanol at concentration ranges of 0.001–1%. Still on the subject of biosensors as potential analytical tools, although at a very preliminary stage, a more recent study developed a competitive fluorescence resonance energy transfer (FRET) assay to monitor benzene presence in atmospheric environment using pOBP, which displayed high affinity for benzene detection with a limit of 0.05 μM (Capo et al. 2018). These results might suggest that pOBP could eventually be employed in the design of a robust optical biosensor to continuously monitor the level of benzene in atmosphere.

Research groups have also conducted OBP-based biosensor studies with the purpose of exploring molecular recognition processes and providing a better understanding of chemical communication systems in insects. This is the case of a paper which focuses on establishing an impedance biosensor with Acer-ASP2 from the honeybee *Apis mellifera* and exploring the binding properties of the protein to its ligands (Lu et al. 2014). To do so, Acer-ASP2 was immobilized on interdigitated gold electrodes and the binding of test ligands such as queen pheromone (methyl-p-hydroxyl benzoate), alarm pheromone (isoamyl acetate) and floral odors (linalool, geraniol, ß-ionone, 4-allyl veratrole, phenylacetaldehyde, dibutyl phthalate) was detected by electrochemical impedance spectroscopy (EIS). Specific floral odors and pheromones produced linear impedance variations to different concentrations, with a detection limit of millimolars. The authors were then able to improve their work by developing a novel biosensor based on nanocup arrays (nanoCA) in order to monitor the binding of small ligands through SPR (Zhang et al. 2015). This time, the detection limit for the device was in the order of picomolars, showing that SPR detection (Bozdogan et al. 2020) using nanoCA and OBPs provides greater sensitivity than conventional electrochemical methods (Szunerits et al. 2020). The same group also investigated affinities between

an OBP from the oriental fruit fly *Bactrocera dorsalis* and different ligands of interest. Using the same manufacturing approach, BdorOBP2 was immobilized on the interdigitated electrodes to establish an olfactory biosensor and the detection of semiochemicals such as isoamyl acetate, β-ionone and benzaldehyde, which are emitted by the fly's host plants, was accomplished by EIS in the millimolar range (Lu et al. 2015).

Regarding the application of biosensors for estimating binding affinity, after demonstrating the feasibility of the method (Larisika et al. 2015) and discussing their efforts in designing, bio-functionalizing and characterizing biosensors based on reduced graphene oxide (rGO) as the gate material (Reiner-Rozman et al. 2016), Kotlowski et al. (2018) have finally described the fabrication and performance of a biosensor designed for in situ and real-time monitoring of a broad spectrum of odorants known to be attractants for bees, such as homovanillic acid (also a cancer marker for neuroblastoma), citral, methyl eugenol and geraniol. Wild-type and engineered mutants of OBP14 from the Italian honeybee *Apis mellifera ligustica* were immobilized onto a rGO field-effect transistor (rGO-FET) and used to achieve such result. Authors demonstrated the binding properties of the protein when immobilized on the biosensor to be similar to those measured in solution. Ligands were able to be detected at the picomolar scale, which could lead to the development of a method for measuring affinities to small molecules as an alternative to the currently used fluorescence assay.

One of the most interesting advantages when using OBPs as sensing elements is that their binding properties can be tailored through site-directed mutagenesis. This possibility has recently been further explored by groups such as in the study conducted by Hurot et al. (2019), where three OBP derivatives of the OBP3 from rat in association with SPR imaging (SPRi), were used to develop olfactory biosensors for the detection of VOCs in solution. The manufactured biosensors showed promising results with detection limits in the picomolar range. Optimizing binding affinity has also been a subject of interest for Wasilewski et al. (2019), whose research concentrated on determining the optimal length of a peptide chain based on the binding site of HarmOBP7 from *Helicoverpa armigera* moth to effectively bind octanal molecules. The authors initially believed that the chain length could have substantial influence on the type and effectiveness of peptide–ligand interactions. Results showed that longer peptides indeed presented the most effective interaction with the ligand. It is also worth mentioning that, although strictly not an OBP, the mouse major urinary protein (MUP) is very similar to one in its conserved lipocalin ß-barrel structure. Ricatti et al. (2019) were interested in designing MUP to bind a variety of non-natural ligands in a way that it could be used as recognition elements in biosensors. For that purpose, the authors have employed in silico techniques to select point mutations without disturbing the binding pocket as well as to choose mutants with the best theoretical binding profiles to the ligands. They were then able to express seven mutants and verify their stability and selectivity. This is a perfect example of how molecular modeling and docking techniques can be of service when designing point mutations with the intent of modifying ligand-binding affinity. It is possible to estimate mutant conformational and structural stability, which makes it easier to produce an actual stable protein with good repeatability. Also, the computational assessment of binding affinity for the ligands of interest reduces the number of candidates for heterologous expression and purification, accelerating the process by giving experimental focus only to promising mutants. This is also the case of a recent study involving AgamOBP1, where the authors analyzed its binding pocket using in silico modeling to investigate the possibility of creating mutants capable of detecting drugs of abuse in vapor phase (Cali and Persaud 2020). They were able to show that single amino acid substitutions in the binding pocket, which were modeled in silico and later tested on expressed proteins, cause dramatic changes to binding affinity. In fact, mutant AgamOBP1-S82P was found to have high affinities to cannabidiol, MDMA and cocaine hydrochloride. After immobilization on a QCM, saturated cocaine hydrochloride vapor was actually detected, something that would be impossible to achieve using wild-type OBP because the ligand was not able to access its binding cavity the same way it fits into the designed mutant's. Using such techniques, the authors were able to produce an effective mutation that changes binding characteristics of AgamOBP1 while retaining the structural stability of the protein.

It cannot be denied that important challenges remain in the development of OBP-based chemosensors, such as their lifespan, which is intrinsically limited by the use of proteins. However, recent studies have indicated the feasibility of manufacturing stable devices using OBPs as sensing elements. Possas-Abreu et al. (2017) have validated their new approach of grafting pOBPs onto synthetic diamond microcantilevers, exhibiting results of great relevance concerning stability, since a biosensing interface using synthetic diamond has been perceived for many years as desirable due to its carbon nature, which ensures good stability of a wide range of bioreceptors via covalent C–C binding (Vanhove et al. 2007; Manai et al. 2014). Also, Cali and Persaud (2020) have developed an AgamOBP1-based biosensor that was still able to detect the target analyte over the period of 10 months in laboratory conditions. Still on the matter of difficulties, sensitivity probably remains the main issue regarding artificial olfaction. Yet, a novel study has reported the fabrication of an ultrasensitive biosensor for human-derived odorants based on free-standing silicon nanowire (SiNW) arrays functionalized with AgamOBP7, achieving detection down to parts per billion (Gao et al. 2020) and

contributing with promising results to the field. This way, facilitating the production of portable, light-weight, robust, sensitive devices that can benefit several industrial, military, environmental and security field applications seems very likely to be in the potential of biosensor technology using OBPs.

Capture and release systems

OBPs have been shown to participate in biological processes other than the transport of odorant molecules to the ORs, such as storing pheromones for delayed emission (Brito et al. 2016; Pelosi et al. 2017). The broad range of hydrophobic ligands bound by OBPs and their ability in performing a dual role of engaging in the detection and release of semiochemicals suggest an array of biotechnological applications for these proteins, from the removal of pollutants and unpleasant odors from the environment to the controlled release of fragrances, drugs and chemicals, for example.

Bianchi et al. (2013) investigated the possibility of using a bovine OBP to scavenge herbicides from aqueous samples and were able to fabricate filtering cartridges for removing triazine and similar compounds from water. Another interesting application has been proposed by Silva et al. (2014), who explored the idea of functionalizing the pig OBP in fabrics for clothes. The immobilized protein would play a dual role in this scenario by slowly releasing pleasant fragrances while removing cigarette smell. More recently, the use of pig OBP fused with cell-penetrating peptides for the capture and transduction of a model molecule, 1-aminoanthracene (AMA), into liposome reservoirs has been evaluated. Results were promising: the functionalized liposomes remained stable during time and the binding capacity of OBP constructs was not compromised after anchorage (Gonçalves et al. 2018b; Gonçalves et al. 2018a). Authors suggested the possibility of employing these systems as functional nanodevices for the retention and release of molecules in textile and cosmetic industries and, a while later, reported the actual implementation of OBP complexes in functionalized cotton aimed at controlled release of fragrances (Goncalves et al. 2019).

The use of OBPs in the development of capture and release systems still is a new subject regarding biotechnological applications of such proteins, but it is pretty reasonable to imagine that new researches in this little-explored field tend to increase over the years. The multitask properties of OBPs observed in nature indicate they can execute tasks currently performed by other methods with additional advantages, such as providing better selectivity, for instance. Also, the implementation of OBP-based anchorage systems is feasible in a wide range of segments, including pharmaceutical, cosmetic and specially textile (Fig. 4), where odor control technology (McQueen and Vaezafshar 2020) represents a promising field of application for such proteins.

Analytical purposes

An interesting property presented by OBPs that can be further exploited for a variety of analytical applications is their affinity and specificity for small organic molecules. The derivatization of affinity columns with OBPs is somewhat easy to achieve and should be stable after several uses and washes with buffers. In fact, Margaryan et al. (2006) reported the synthesis and characterization of affinity columns derivatized with pheromone-binding protein (PBP) 1 from *Bombyx mori* and OBP1 from *C. quinquefasciatus*. Authors demonstrated that binding capacity was not affected by immobilization and OBPs also retained their pH-dependent ligand affinity, a mechanism which could be employed for the gradual release of tightly bound ligands from the column. It is worth noting that the use of OBPs in the preparation of affinity columns opens a wide range of possibilities, since proteins would be the ideal substrates to differentiate enantiomers, but their poor stability to temperature and solvents seems to be a limiting factor for their application in analytical columns. However, OBPs might be able to present a solution to this matter due to their exceptional stability to thermal denaturation and proteolytic degradation. As a matter of fact, pheromones and other compounds racemates represent natural ligands for these proteins, suggesting derivatized columns could be directly used in discriminating enantiomers. It has been

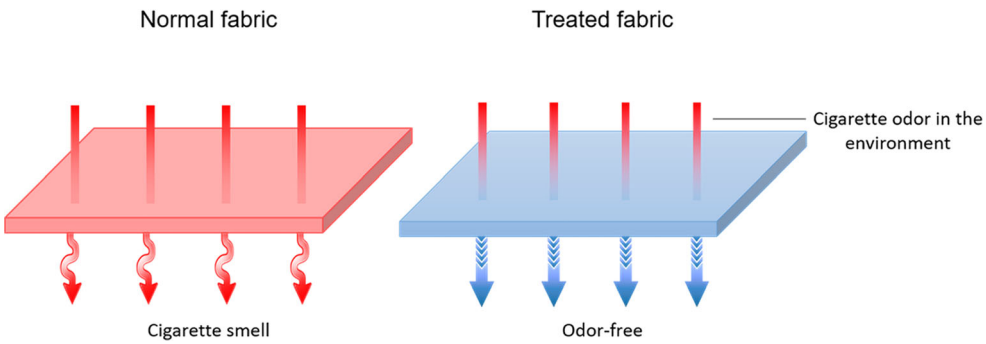

Fig. 4 Schematic view of a potential application of OBPs in odor control technology for the textile industry

described that PBP1 and PBP2 from the gypsy moth *Lymantria dispar* are able to discriminate pheromone enantiomers (Plettner et al. 2000). Studies regarding chiral interactions of a pOBP with carvone enantiomers have also been published (Mulla et al. 2015). Further, it has already been discussed that structures of OBP binding pockets can be modified to meet specific analytical requirements (Pelosi et al. 2018). Considering all this information, it is fairly possible that, if properly used as active chromatographic phases, OBPs could not only be powerful tools for affinity purification of a number of ligands, as well as for resolving racemic mixtures.

Measuring the affinity of OBPs to volatile ligands such as pheromones and other odorants found in the environment is crucial for the analysis and characterization of these proteins. As mentioned before, even though several techniques have been employed in order to measure the affinity and the specificity of OBPs to different ligands, by far the most widely adopted method uses a fluorescent reporter, whose optical properties are markedly different when in an aqueous environment and when bound to a protein into a hydrophobic pocket (Pelosi et al. 2017). However, a recent study (Tan et al. 2019) has brought to light that different fluorescent reporters, in this case, AMA and 1-NPN, seem to generate drastic differences in the calculated affinities, which was quite unexpected and started to raise doubt on the reliability of the fluorescent binding assay. Authors even recommended that the results of fluorescent binding experiments with OBPs should be confirmed by using two different probes or alternative methods while the scientific community still deals with the strong demand for label-free methodologies to measure binding constants of small ligands that could be simple, fast and free from the use of radioactive tracers. In this context, one could raise an interesting question: would it be possible to use biosensors in monitoring this affinity and actually provide an alternative method to the fluorescent binding assays that are currently in use? Although today's technology is not yet sophisticated enough to meet the reproducibility requirements of biosensors, it seems quite likely that the future of biosensors could actually find applications for such analytical purposes.

Concluding remarks

Properties such as stability to thermal and proteolytic degradation, small size, easy production and purification, added to the fact that available structural information allows for the design and synthesis of mutants, make OBPs interesting research targets for a wide range of biotechnological applications. Currently, insect population management is the most discussed use for OBPs. These proteins are vastly utilized for the screening of novel behaviorally active compounds as well as structure-based design of new repellents. However, advances in the field of insect olfaction and nanotechnology combined with their desired features have rapidly transformed OBPs in the best candidates to be employed as biosensing elements in order to meet the growing demand for versatile technologies that can be used as sensors for the detection of volatile chemicals in various areas, including environmental pollution control, health care, quality control and homeland security. Also, even though thorough scientific research still needs to be carried out in this field, the possibility of using OBPs in anchorage systems is starting to be explored and has already shown interesting results, especially for textile, cosmetic and pharmaceutical industries. Besides, their affinity for small hydrophobic compounds have been further exploited for analytical purposes and results indicate that, if properly employed, OBPs could become effective tools for affinity purification as well as for resolving racemic mixtures.

Authors' contributions A.C.A.M. and N.F.B. conceived the presented concept for a review article. N.F.B. was responsible for bibliographic search, critical discussion, article design, illustrations and writing of the manuscript. D.S.O. and T.C.S. contributed to article design, writing and bibliographic enrichment. A.C.A.M. and M.F.M. contributed with critical discussion and provided writing support. All authors provided input, read and approved the final version of the manuscript.

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Compliance with ethical standards

Coonflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Code availability Not applicable.

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