iOBPdb – A Database for Experimentally Determined Functional Characterization of Odorant Binding Proteins

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Summary/Abstract

Odorant binding proteins, OBPs, are a diverse family of small, globular, extra-cellular proteins which assist in solubilizing volatile organic compounds (VOCs) so they can be internalized and transported by an organism. Since their initial discovery in early eighties [1], thousands of OBPs have been identified through genome sequencing and characterized by fluorescence ligand binding assays [2]. While a given individual OBP has been studied in the context of their role in specific organism, there has not been studies towards the understanding of the comparative structure-function relations of all known OBPs, primarily due to a lack of a centralized database that incorporates the binding affinity with the structure of all OBPs. Incorporating OBP information into a database requires not only an extensive search of all existing resources, but also creating a useful platform that relates sequence structures to target functions. Using data obtained from 215 functional studies containing 381 unique OBPs from 91 insect species we created a database, dubbed as iOBPdb, that contains OBP binding affinities for a wide range of VOC targets. We demonstrate here that the construction of this initial database provides powerful search and associative capabilities including interrogating odor binding proteins as clusters and groups by sequence similarity versus protein and target molecular weights, and by the functional groups of the VOC targets. The comparative interrogation of the probe-target recognition allows for a more comprehensive understanding of the underlying structural features of all OBPs that had not been possible by only examining the OBPs individually. We present our results in a variety of phylogenetic representations as well as providing the binding profiles of OBP groups to VOC functional group moieties. iOBPdb will have an enormous range potential applications spanning from eNOSE bionanosensors, development of novel bioassays and drug development, discovery of novel pesticides which inhibit VOC / OBP interactions, as well providing a foundational basis for the functional understanding of the logic of odor sensing and perception in the brain.

Keywords: Odor Binding Proteins, volatile organic compounds, OBP database, Insects, Olfactory Receptors, Molecular Biomimetics, Machine Learning, Bionanosensors, Odor Perception

1. Introduction and Justification

Odorant binding proteins (OBPs) are a diverse family of small, 10-20 kDa, soluble extracellular target binding proteins found in both terrestrial vertebrates and invertebrates [2]. Since their initial discovery in insects' sensillum lymph in 1981 [1], thousands of new OBPs have been identified and isolated through genome sequencing and molecular biology approaches. These studies indicate that there is no shared homology between insect and mammalian OBPs. OBPs in mammals are comprised of a beta barrel type structure, whereas the OBPs in insects are a globular structure comprised of alpha helices. Although OBPs are multifaceted in terms of their potential roles in both insects and mammals alike, they are primarily thought to act as odor transporters, solubilizing volatile organic compounds (VOCs) and pheromones from the surrounding air into the aqueous phase of the odor sensory organ, such as the mucus in the nose or sensillum lymph of an antennae. A simplified view of the odorant binding event is shown on figure 1. Once the odors from the environment are solubilized by OBPs, they can then be transported to odor sensing neurons which are coated with olfactory receptor (OR) proteins, which can recognize and bind to specific odors, thus signaling an olfactory response [2]. There are however increasing observances of non olfactory related uses of OBPs in insects as OBPs have been shown to be expressed almost ubiquitously across organs in insects, not just the olfactory organs [3]. Some additional uses include: immunity, mating, moisture detection, signaling molecule transporter, and biochemical inhibitor.

The literature uses a variety of naming schemes to describe OBP related proteins, such as but not limited to: chemosensory proteins (CSPs), pheromone binding proteins (PBPs), antennal-specific protein (ASPs) antennal binding proteins (ABPXs) and other fringe one off names. Olfactory binding proteins are often sub categorized as either being: classic olfactory binding proteins (classic OBPs), general olfactory binding proteins (GOBPs), atypical olfactory binding proteins (atypical OPBs), minus-C olfactory binding proteins (minus-C OBPs), or plus-C olfactory binding proteins (plus-C OBPs). The nomenclature used to describe OBPs is diverse, and are typically characterized by features identified in initial discovery. In conjunction with cystine count, OBPs are also characterized by other features. For example, general odorant binding proteins (GOBPs) are defined based on ubiquitous nature of their expression in both male and female insects. Pheromone binding proteins (PBPs) are a subfamily of OBPs which were identified initially due to their preferential binding to a pheromone in radio-labelled ligand binding studies in the early 1990s [4]. Chemosensory proteins (CSPs) are related to insect OBPs in terms of function, however are relatively smaller in comparison to OBPs, share very little sequence homology, and typically only contain 4 cystines instead of the classic 6 cystines. CSPs are primarily produced in chemosensory organs of insects instead of the antennae [3].

Figure 1 – Functional domains of a odor binding protein, OBP, relevant in the capture of a volatile organic compound, VOC.

The naming convention presently used is unnecessarily complicated, unwieldy, and often detracts from understanding what the actual function of the protein in question is. The problem in consistency of naming arises from the fundamental problem of understanding what parameters makes this large family of thousands of extracellular binding proteins similar or dissimilar from each other. Certain modes of distinguishing these proteins from one another include insect tissue localization, sexual dimorphic expression, insect species of discovery, species of origin of VOC (plant or predator), cystine count in underlying amino acid sequence of binding protein, alpha helices present in folded protein, and preference of binding proteins to certain chemicals or functional groups. While sharing a similar globular structure, insect OBPs differ in terms of conformation, size, and rigidity due to variations in the underlying amino acid sequences, alpha helix count, as well as cystine count (Figure 2). The classic insect OBP contains 6 highly conserved cystine residues which form 3 disulphide bonds. However, there are insect OBP variants with fewer

than 5 cystines which only from 2 disulphide bonds and are aptly named minus-C OBPs. Conversely there are insect OBPs with 8 or more cystines which are termed as plus-C OBPs. A special variant of plus-C OBPs are atypical OBPs which typically are 20-30 amino acids longer compared to regular insect OBPs and contain 10 or more cystines. Atypical OBPs are also sometimes referred to as two domain OBPs or double domain OBPs which refers to a fusion protein consisting of two OBPs [5]. The variation in cystine count, drastically changes the underlying protein folding, survivability in the extracellular environment and definition of ligand binding pocket. This is not to be confused with dimer OBPs which are two distinct OBP proteins (can be two of the same protein or two different proteins) which pair together in order to sandwich a ligand. The difference is the dimer consists of two distinct proteins whereas the atypical OBP is one large continuous protein.

The divergent molecular conformations of insect OBPs alter their function, i.e., the ability to bind to various odors and pheromones. While it is generally agreed that OBPs share specificity for a multitude of different molecules, there are several insect OBPs which undeniably exhibit preference for certain molecular moieties and functional groups. Perhaps the most widely known example is LUSH, also known as DmelOBP76a, which was shown to be essential in the recognition of a specific pheromone known as 11-*cis* vaccenyl acetate [6]. When LUSH is knocked out, other OBPs could not compensate in terms of perpetuating signal transduction. The double domain features of atypical OBPs is also speculated to help ensnare signaling molecules in order to prevent signaling action or is potentially needed for binding to really large ligands [7]. Conversely fever cystines appear to be responsible for the relatively broad binding capabilities of CSPs to various ligands.

Figure 2 – The functional domains of OBPs and their structures. The structural depiction of a classical OBP with six alpha helices (denoted in black as as α1- α6) and three disulphide bonds. Different arrangements of OBPs and CSPs. It may be noticed that the differences between protein size, number of cysteine bonds, and number of alpha helices.

The specificity of a given OBP to a exclusive target is useful not only in the classification of the OBPs but in terms of their structure-function relationships, but also the potential of using them as sensor elements in practical applications. The binding of insect OBPs to various molecules have been widely studied using 1-NPN competitive fluorescent ligand binding assays [8]. For example, it has been found that 1-NPN is a reporter molecule which provides a continuous baseline signal as it binds to the OBP of interest [9]. Other reporter molecules such as AMA are used to study the binding of mammalian OBPs. An odor/pheromone is introduced which will then compete with 1-NPN in terms of occupation of the ligand binding slot of the OBP which confers to a decrease in signal. These types of studies typically involve individual experiments focused on a single OBP. Such studies have been accelerating for the last two decades, as the high throughput sequencing of novel insect genomes has made it much easier to identify OBPs and enable further testing via functional experiments.

In this report, we undertake two related studies, first, to provide a database from the collected and listed insect OBPs that can be classified in a variety of phylogenic representations. Secondly, we report binding profiles of OBP groups against functional groups found on target VOCs. In short, our report herein is intended to provide a new impetus in functionally classifying OBPs from other organisms providing a robust platform for odor perception neuroscience, targeted pesticides, and development of multiplexed biosensors.

2. Materials and Method

OBP binding data was obtained through an extensive survey of the OBP literature from the last 15 years (see the complete literature sources for the compiled data in the data reporting section). In total, there have been 215 functional studies which confers to 381 unique OBPs from 91 distinct insect species. These functional studies surveyed over 700 different potential binding molecules which are encoded by a CAS (Chemical Abstracts Service) number identifier. Structural information for these molecules were obtained by retrieving the respective molecular formula and SMILES (simplified molecular-input line-entry system) string associated with the CAS identifier through PubChem's (https://pubchem.ncbi.nlm.nih.gov) programmatic API, PUG REST. Functional groups present on these molecules were identified by analyzing the underlying SMILE strings. Additional information was obtained from already existing databases and additional literature, making it easier to identify potentially useful OBPs or VOCs which may be pertinent in the realm of human health and biotechnological applications. This includes VOC expression profiles in healthy humans,[10] VOC profile of various human respiratory diseases,[11-21] and PDB (Protein Data Bank) entries if OBPs exist in RCSB (https://www.rcsb.org).

Figure 3- Schematics describing the development of the OBP database for insects, *iOBPdb*. One may search the database by either providing the VOC of interest by providing either a name, CAS number, Molecular Formula, or SMILES string, or alternatively by the OBP of interest by providing either a name, or ascension number. This will return the binding affinity data of OBPs tested against various VOCs per the parameters provided. One can also filter/sort the results by various properties of the VOC such as: its functional groups, presence in healthy humans, as well as presence in diseased humans. These additional filters serve to expedite search for users that may be interested in biotechnological / therapeutic use of OBPs.

To organize information collected from the literature search, a many-to-many relational database was modeled and designed, which we have termed IOBPdb. The database was hosted through UW's clustered ovid servers. It was deployed using a combination of PHP and MySQL. The structure of the database connects binding affinities, compounds, odorant binding proteins, and diseases. An initial copy is stored on ResearchWorks, University of Washington's digital repository for scholarly works. Figure 2 shows the schema for the database. A user can either search by compound ID or OBP name to retrieve binding data of interest. This binding data can be further sorted by functional groups of VOCs, molecular weight of VOCs, prevalence of VOCs in healthy humans, prevalence of VOCs in disease and many more options.

3. Results

The acquired OBP information from the literature has been stored in a database called iOBPdb which is intended for use as a robust platform for expansion by including other OBPs, e.g., from mammalians, reptiles, and fishes. The database is available for the researchers interested in using the iOBPdb platform further expansion by including newly discovered OBPs from insects as well as other organisms. On the one hand, while the comprehensive database is envisioned for use in neuroscience studies via structure-function relationships and the study of odor perception. On the

other hand, the database would also serve as a potent biological resource for biomimetic design of the highly potent molecular probes that would function as the key capture elements in future biosensor architecture.

As examples of exploring the relative similarities of the structure-function relationships among the odor binding proteins from insects, we constructed a phylogenetic map for all OBPs contained in the database in a variety of schemes. For example, the newick tree was generated using Clustal Omega multiple sequence alignment (http://www.ebi.ac.uk/Tools/msa/clustalo/), and the resulting phylogenetic map was constructed and displayed using the iTOL web server. The results of this

Figure 4: Two types of representations of the insects OBPs: (a) Circular phylogenetic map, and (b) Unrooted phylogenetic map.

phylogenetic analysis can be seen in its circular form on Figure 4(a) and unrooted form on Figure 4(b). One can clearly observe that CSPs form a unique clade separate to the minus-C OBPs despite sharing similar cystine counts. Additionally, one can observe two distinct subfamilies of minus-C OBPs, one more closely related to CSPs and the other more closely related to classical OBPs. There is also a distinct sub family of atypical OBPs adjacent to a small sub family of plus-C OBPs. In general, however we observe minimal sequence similarity amongst plus-C OBPs as they are scattered across the phylogenetic tree. This may suggest that they are highly ligand specific as a result. On the other hand, the GOBPs and PBPs form a very distinct monophyletic clade separate form CSPs and OBPs. GOBPs also subdivide into two families termed groups 1 and 2. Classical OBPs have also been subdivided into distinct clades which are designated as clades 1-5.

The heatmap on Figure 5(a) that overlays the phylogenetic map showcases the distribution of sequence lengths of OBPs. We can clearly observe that CSPs are by far the smallest sub family of proteins, whereas the plus-C and atypical proteins are the largest family. Other interesting features to note are that GOBPs and PBPs are similar in terms of size and are consistently larger than the classical OBPs. Minus-C OBPs and classic OBPs tend to be similar in terms of size.

Figure 5: Phylogenetic tree of OBPs generated with respect to the molecular weight (a) Molecular weight of the OBPs, (b) VOC Molecular weight preference.

Among a variety of ways of representing the OBP data with respect to the target VOCs, we present in Figure 5(b), another example; a heatmap denoting the preference of each OBP binding to a VOC of a particular molecular weight. In this case, the PBPs and GOBPs appear to preferentially bind with larger molecular weight VOCs. In the case of PBPs, the binding preference to larger molecular weight VOCs may be an artifact of the experimental bias for testing against larger heavier targets as pheromones tend to be larger molecules. On the other hand, the GOBPs do not have mw-based bias in terms of VOC selection, and they normally tend to preferentially bind to higher molecular weight targets. Classical OBPs, Minus-C OBPs and CSPs do not appear to have a preference in terms of the size of the VOC. Interestingly, Plus-C OBPs seem to display a preference for lower molecular weight targets. This may be a result of the increase in cystines creating more disulfide bonds that restricts the conformation of plus-C OBPs and narrows its binding domain. Similar type of phylogenetic trees could also be generated based on other biochemical or biophysical factors, such as functional groups of VOCs (examples of phylogenetic trees have been produced in the Supplementary Information).

Here, we also present tabulation of mean binding activity of the OBP subfamilies that specifically preferentially bind to certain functional groups (Table 1). Here, all the available VOCs have been considered that had binding activity associated with them. Similarly, we also tabulated the binding activities that only consider VOCs that at least weakly bind (anything below 20 Kd) to the OBPs (Table 2). The general trends in the tables are preserved in both versions of the analyses considered herein, although Table-2 more specifically captures information pertaining to likely VOC binders. Also in Table 2, one may observe that PBPs and GOBPs (group 2 in particular) have an enhanced affinity towards ketones, ethers and aldehydes. Alcohols seem to bind relatively well across several OBP clades, although minus-C OBPs appear to have an enhanced affinity compared to other OBP clades. The CSPs also do not appear to have a strong affinity to any one functional group, which may indicate that the binding activity is broader across the OBPs. An interesting feature that stands out is that phenols preferentially bind to atypical OBPs, Minus – C OBPs and clade 1 OBPs. These are all closely related groups on the phylogenetic map. Further tables of OBP group binding profiles for VOC specific targets (i.e. benzaldehyde, nonanal etc.) can be found in the Supplementary Information.

Table 1: Mean binding affinity (Ki) of functional moieties by OBP groups

Functional group		Atypical OBPs	CSPs	Classic OBPs (clade 1)	Classic OBPs (clade 2)	Classic OBPs (clade 3)	Classic OBPs (clade 4)	Classic OBPs (clade 5)	GOBPs (group 1)	GOBPs (group 2)	Minus C OBPs	PBPs	Plus C OBPs
ketone		19.190	29.479	8.787	18.039	33.943	15.372	29.294	15.163	12.116	19.086	7.850	15.215
alcohol		20.720	39.931	12.276	21.222	36.227	14.191	18.254	11.566	14.091	19.440	12.650	27.194
ether		25.105	25.951	9.393	12.691	26.937	23.726	29.965	23.484	11.506	24.009	9.915	15.951
aldehyde		17.865	28.574	8.473	24.691	18.477	13.089	19.259	9.545	14.321	16.481	8.190	21.962
ester		24.968	25.741	9.441	13.229	28.467	17.959	30.799	23.508	11.293	24.355	9.611	13.187
aromatic		18.632	26.133	12.340	24.813	29.777	23.629	18.881	20.716	22.263	17.740	19.832	17.696
phenol		1.200	27.523	11.965	15.316	24.006	9.203	22.037	26.899	32.112	17.667	16.277	41.938
carboxylic acid		-	18.052	-	39.372	82.641	6.250	20.656	5.714	3.905	7.466	4.543	-

Binding	Ki range	Color
Strong	Ki < 5	
Moderate - Strong	5 < Ki < 10	
Moderate	10 < Ki < 15	
Moderate - Weak	15 < Ki < 20	
Weak	Ki > 20	

Table-2: Mean binding affinity (Ki) of functional moieties by OBP groups (only for VOCs w/ binding affinity <20 Ki)

Functional group		Atypical OBPs	CSPs	Classic OBPs (clade 1)	Classic OBPs (clade 2)	Classic OBPs (clade 3)	Classic OBPs (clade 4)	Classic OBPs (clade 5)	GOBPs (group 1)	GOBPs (group 2)	Minus C OBPs	PBPs	Plus C OBPs
ketone		12.309	10.094	7.087	10.437	10.913	12.552	10.333	8.993	6.555	7.723	5.771	11.577
alcohol		9.117	9.728	8.158	12.791	10.572	10.388	9.187	8.966	7.629	6.740	5.357	11.179
ether		5.850	10.791	7.771	9.616	11.884	11.541	9.933	10.667	5.996	6.839	4.842	11.200
aldehyde		12.477	10.138	6.723	12.077	9.673	11.991	10.273	7.980	5.661	6.763	5.920	10.643
ester		5.300	11.030	7.819	9.821	11.813	11.352	10.034	10.667	5.996	4.923	4.898	11.177
aromatic		10.463	11.324	8.980	9.330	11.083	11.959	10.890	10.775	8.692	7.243	11.609	11.424
phenol		1.200	9.811	3.680	11.745	9.361	9.203	10.250	14.877	12.280	4.683	9.404	9.297
carboxylic acid		-	10.405	-	15.100	11.360	6.250	5.905	5.714	3.905	6.625	4.543	-

Binding	Ki range	Color
Strong	Ki < 5	
Moderate - Strong	5 < Ki < 10	
Moderate	10 < Ki < 15	
Moderate - Weak	15 < Ki < 20	
Weak	Ki > 20	

4. Discussions

In the results, we have presented the outcome of the OBP-VOC based ligand-receptor interactions and presented them in a variety of phylogenetic visualization as well as provided the database algorithm that facilitates the useful classification of these unique molecular interactions. The expectation here is that the initial version of iOBPdb would trigger, as working platform for further studies in the classification of the odor binding proteins in other organism, such as mammalians, reptiles, and fishes.

As has been detailed, it has been well known that the organismal olfactory and gustatory systems can detect and discriminate volatile and solubilized molecules at low concentrations in air and in water for smell and taste, respectively. As schematically simplified in Figures 1 and 2, the process of target detection involves capturing of the target molecule that triggers a biological sensing mechanism by which a signal is transferred to and is recognized by the brain that could discriminate even minute differences in the elemental compositions and structures of thousands of molecules all at once. Gaining insights into the detailed understanding of the processes of sensing have far reaching implications. Firstly, molecular detection has significance in the fundamental processes that underly the mechanisms of molecular recognition and signal transduction processes, e.g., in olfactory systems, specialized by a variety of classes of organisms. Secondly, a database of OBP / VOC profiles for a variety of insect species can facilitate the creation and design of pesticides which can leverage similarities in binding profiles of certain VOC functional group moieties to create OBP specific inhibitors. Finally the iOBPdb database could unlock the potential use of OBPs as molecular biomimetic lessons for the design of engineered molecular probes.

Despite the widespread functional binding studies, relatively few applications of insect OBPs in biosensors were reported [22]. OBPs are small globular proteins are robust enough to stand up to wide ranges of pH and temperatures (even to 80–100°C) for substantial mistreatments [23], without denaturing and losing their binding properties. In addition, OBPs are easier to be isolated and purified in the process of production compared with membrane protein ORs, because ORs are G protein-coupled receptors, GPCRs, and have seven transmembrane α helix [24]. All of these will greatly enhance the practicability of those materials using in sensors. Therefore, OBPs are excellent candidates as biological elements in the development of olfactory biosensors.

5. Conclusions and Future Prospects

Among the sensory cortexes in the brain, the olfactory perception could be considered as the most basic to comprehend as the ligand-receptor interactions are based on the physico-chemical interactions under a given physiological conditions that could be readily tested under practical experimental considerations. In one of the most widely studied organisms, in insects, the recognition of odor is accomplished by odor binding proteins in binding to and carrying the specific odor to olfactory membrane-bound proteins in the antenna, the first step in the odor perception. Although there are considerable studies in odor recognition during the last two decades, especially with the odor binding in insects, these studies have been mostly discrete and, in general, there is a lack of comprehensive database which makes it extraordinarily tedious to carry out comparative analysis of OBP-odor interactions. Assimilating the current knowledge in odor binding proteins in insects, here we report a comprehensive database, dubbed iOBPdb, that meets these expectations. The database provides a novel platform for a broad study of OBPs with volatile organic compounds that assimilates the existing knowledge at a singular location as an easily accessible portal. More specifically, the iOBPdb:

- For the first time, provides a comprehensive database, with compiled OBPs with binding specificity to VOC, in insects, which had not been possible before

- Provides a platform to compare multiple OBPs from different insect species, which would allow for more robust and complete comparative analysis

- Gives demonstrations of the compiled data presented in a variety of phylogenetic tree type visualization, providing trends OBP-VOC interactions in a variety of parameters

- That could now facilitate foundational studies towards discovering the functionality logic in the perception of odor

- Facilitates the search of novel pesticides that will only target an OBP of a specific pest species

- That provides basic lessons from biology as guidance in designing odor binding molecular probes in practical nanobiodevices.

In short, by providing the iOBPdb database portal, we anticipate that this study could provide substantial impetus in accelerating the understanding of the neurological basis for odor perception in the brain, discovery of novel targeted pesticides for specific insect species, and applications in practical implementations, such as biosensors for volatile organic compound identification in diseased or toxin affected individuals, designing odor specific analytical probes, and many more possibilities.

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7. Data Sharing

iOBPdb can be accessed though an online portal:
https://depts.washington.edu/dennig2/phpmyadmin/

The CSV file containing all of the data stored on iOBPdb is available here:

Citations/References cited:

1. Vogt, R. G., and Riddiford, L. M. (1981). Pheromone binding and inactivation by moth antennae. *Nature* 293, 161–163. doi: 10.1038/293161a0

2. Pelosi, P, and R Maida. "Odorant-binding proteins in insects." Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology vol. 111,3 (1995): 503-14. doi:10.1016/0305-0491(95)00019-5

3. Rihani, Karen et al. "The 40-Year Mystery of Insect Odorant-Binding Proteins." Biomolecules vol. 11,4 509. 30 Mar. 2021, doi:10.3390/biom11040509

4. Vogt, R G et al. "Molecular cloning and sequencing of general odorant-binding proteins GOBP1 and GOBP2 from the tobacco hawk moth Manduca sexta: comparisons with other insect OBPs and their signal peptides." The Journal of neuroscience : the official journal of the Society for Neuroscience vol. 11,10 (1991): 2972-84. doi:10.1523/JNEUROSCI.11-10-02972.1991

5. Costa-da-Silva, A.L., Kojin, B.B., Marinotti, O. et al. Expression and accumulation of the two-domain odorant-binding protein AaegOBP45 in the ovaries of blood-fed Aedes aegypti. Parasites Vectors 6, 364 (2013). https://doi.org/10.1186/1756-3305-6-364

6. Xu, PingXi, et al. "Drosophila OBP Lush Is Required for Activity of Pheromone-Sensitive Neurons." Neuron, vol. 45, no. 2, 2005, pp. 193–200., https://doi.org/10.1016/j.neuron.2004.12.031.

7. Manoharan, Malini et al. "Comparative genomics of odorant binding proteins in Anopheles gambiae, Aedes aegypti, and Culex quinquefasciatus." Genome biology and evolution vol. 5,1 (2013): 163-80. doi:10.1093/gbe/evs131

8. Loh, B et al. "Use of the fluorescent probe 1-N-phenylnaphthylamine to study the interactions of aminoglycoside antibiotics with the outer membrane of Pseudomonas aeruginosa." Antimicrobial agents and chemotherapy vol. 26,4 (1984): 546-51. doi:10.1128/AAC.26.4.546

9. Ban, Liping et al. "Binding properties of a locust's chemosensory protein." Biochemical and biophysical research communications vol. 293,1 (2002): 50-4. doi:10.1016/S0006-291X(02)00185-

10. de Lacy Costello, B et al. "A review of the volatiles from the healthy human body." Journal of breath research vol. 8,1 (2014): 014001. doi:10.1088/1752-7155/8/1/014001

11. Ratiu, Ileana Andreea, et al. "Volatile Organic Compounds in Exhaled Breath as Fingerprints of Lung Cancer, Asthma and COPD." *Journal of Clinical Medicine*, vol. 10, no. 1, Dec. 2020, p. 32. *Crossref*, https://doi.org/10.3390/jcm10010032.

12. Belizário, José E et al. "Breath Biopsy and Discovery of Exclusive Volatile Organic Compounds for Diagnosis of Infectious Diseases." *Frontiers in cellular and infection microbiology* vol. 10 564194. 7 Jan. 2021, doi:10.3389/fcimb.2020.564194

13. Sethi, Shneh et al. "Clinical application of volatile organic compound analysis for detecting infectious diseases." *Clinical microbiology reviews* vol. 26,3 (2013): 462-75. doi:10.1128/CMR.00020-13

14. Filipiak, Wojciech et al. "Characterization of volatile metabolites taken up by or released from Streptococcus pneumoniae and Haemophilus influenzae by using GC-MS." *Microbiology (Reading, England)* vol. 158,Pt 12 (2012): 3044-3053. doi:10.1099/mic.0.062687-0

15. Traxler, S., Barkowsky, G., Saß, R. *et al.* Volatile scents of influenza A and *S. pyogenes* (co-)infected cells. *Sci Rep* **9,** 18894 (2019). https://doi.org/10.1038/s41598-019-55334-0

16. Purcaro, Giorgia et al. "Volatile fingerprinting of human respiratory viruses from cell culture." *Journal of breath research* vol. 12,2 026015. 1 Mar. 2018, doi:10.1088/1752-7163/aa9eef

17. Aksenov, Alexander A et al. "Cellular scent of influenza virus infection." *Chembiochem : a European journal of chemical biology* vol. 15,7 (2014): 1040-8. doi:10.1002/cbic.201300695

18. Schivo, Michael et al. "Volatile emanations from in vitro airway cells infected with human rhinovirus." *Journal of breath research* vol. 8,3 (2014): 037110. doi:10.1088/1752-7155/8/3/037110

19. Berna, Amalia Z et al. "Reproducible breath metabolite changes in children with SARS-CoV-2 infection." *medRxiv : the preprint server for health sciences* 2020.12.04.20230755. 7 May. 2021, doi:10.1101/2020.12.04.20230755. Preprint.

20. Ibrahim, Wadah et al. "Diagnosis of COVID-19 by exhaled breath analysis using gas chromatography-mass spectrometry." *ERJ open research* vol. 7,3 00139-2021. 5 Jul. 2021, doi:10.1183/23120541.00139-2021

21. Grassin-Delyle, Stanislas et al. "Metabolomics of exhaled breath in critically ill COVID-19 patients: A pilot study." *EBioMedicine* vol. 63 (2021): 103154. doi:10.1016/j.ebiom.2020.103154

22. Barbosa, Arménio J M et al. "Protein- and Peptide-Based Biosensors in Artificial Olfaction." *Trends in biotechnology* vol. 36,12 (2018): 1244-1258. doi:10.1016/j.tibtech.2018.07.004

23. Ahmed, Tofael, et al. "Molecular cloning, expression profile, odorant affinity, and stability of two odorant-binding proteins in Macrocentrus cingulum Brischke (Hymenoptera: Braconidae)." *Archives of insect biochemistry and physiology* 94.2 (2017): e21374.

24. Buck, L, and R Axel. "A novel multigene family may encode odorant receptors: a molecular basis for odor recognition." *Cell* vol. 65,1 (1991): 175-87. doi:10.1016/0092-8674(91)90418-x