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Abstract— Over the past three decades, electronic nose devices have gained popularity in various fields due to their great potential for detecting volatile organic compounds (VOCs). To improve the performance of such devices, proteins from the olfactory system are very attractive sensing materials. Nevertheless, conserving their biological properties when exposed to gaseous VOCs is quite challenging. Indeed, the absence of an aqueous environment can largely affect the activity of the proteins. Water molecules play a crucial role in the stabilization of the protein structure. Consequently, the hydration degree of the working milieu has a large impact on the function of the biosensors. In this study we investigated the performance of an optoelectronic nose based on odorant binding proteins and emphasized the role of humidity on the activity of the system.

Keywords— electronic nose, odorant binding protein, surface plasmon resonance imaging, volatile organic compounds

I. Introduction

Volatile organic compounds (VOCs) are small organic molecules (< 300 g/mol) that generally exhibit odorous properties. They have a high vapor pressure at ambient temperature and are often hydrophobic [1]. VOCs emanate from a wide range of biogenic and anthropogenic sources. The detection and analysis of these compounds has proven to be very useful for diverse applications such as quality control in the food and cosmetic industry, disease diagnosis, security checks, environmental monitoring, etc. Currently, the most effective and commonly employed analytical techniques are the trained human or canine nose and gas chromatography coupled with mass spectrometry (GC-MS). Although very sensitive and reliable, these techniques suffer from various drawbacks and are not suitable for large scale and industrial applications. Indeed, the biological nose is prone to fatigue, requires extensive training and can sometimes give biased results. On the other hand, GC-MS requires qualified personnel, is time consuming and often expensive. In this regard, electronic nose (eN) systems are an alternative that could address the need for an affordable, portable and high-performance device for VOC detection. By definition, an eN is a biomimetic system that consists of a set of VOC sensors having partial specificity (i.e. can interact with different classes of VOCs) combined with a transducer and a pattern recognition system. Over the past three decades, electronic nose systems have largely grown in popularity and their market size is expected to reach 34.78 M$ in 2026 [2]. Many eNs and VOC sensors based on a broad variety of transduction techniques (e.g., chemiresistive, field effect transistor (FET), gravimetric, optical, electrochemical) and sensing materials (e.g., MOS, polymers, proteins) have been developed by the research and industrial communities. Nevertheless, the performances of these devices require further improvements in terms of sensitivity and selectivity.

Designed by nature to bind VOCs, two protein families of the olfactory system are very attractive sensing materials for eN development: olfactory receptors and odorant binding proteins (OBPs). OBPs are small proteins (about 20 kDa), particularly suitable for eN systems owning to their high stability. Indeed, they can withstand large variations in temperature and pH and are resistant to proteolytic degradation. They are also soluble proteins, which facilitate their production and have a large binding spectrum that can be tuned via site-directed mutagenesis [3]. In this context, several studies have attempted to exploit the capacities of these biomolecules and have proven their effectiveness as sensing materials for VOC biosensors and eN development. Several studies have been carried out in solution (in liquid phase) but also in gas phase despite the challenges that this environment can pose. Indeed, the absence of an aqueous environment can largely affect the biological properties of the proteins and consequently their activity. To our knowledge, the impact of humidity on the performance of OBP based sensors for the detection of gaseous VOCs has not been explored.

In this work, we investigated the potential of an OBP-based optoelectronic nose for the detection of gas-phase VOCs and highlighted the effect of humidity on protein activity.

For this purpose, rat OBP3 and two mutant forms of the protein, with genetically customized binding affinities, were designed, produced and used. The OBPs were deposited on the gold surface of a prism to form a sensor microarray. The biochip was then exposed to gaseous VOCs carried by either completely dry or humid air. The performance of the sensors, under both conditions was assessed by surface plasmon resonance imaging (SPRi) which is an optical transduction technique that allows real-time, label free and multiplexed monitoring [4].

II. Materials and Methods

A. Production of the OBPs

Three different OBPs were used for the study: the wild type rat OBP3 (rOBP3-wt) and two mutant forms of this protein that we will call rOBP3-a and rOBP3-c. The wild type protein exhibits high affinity to cyclic compounds, rOBP3-a was tuned to have higher affinity to aldehydes and finally rOBP3-c was modified to not bind any VOC and so it was used as negative control. An His-tag and a cysteine were added to the N-

terminal of the three OBPs to allow their purification and immobilization on the gold surface, respectively. The recombinant proteins were expressed in *E. coli* then purified by immobilized metal affinity chromatography. Their purity was checked by SDSPAGE and their binding properties characterized by isothermal titration calorimetry (ITC) [5].

B. Biochip fabrication

To fabricate the OBP chip, an N-BK7 right angle glass prism (Edmund optics) was used. The surface of the prism was first coated with 53 nm gold layer. Then, the prism was plasma-cleaned (Diener Electronic) for 3 minutes (75% oxygen, 25% argon, 0.6 mbar, 80 W) and left for 48h, in sealed environment, prior to OBP deposition.

Subsequently, OBP solutions (10µM, 100 mM NaH_2PO_4, pH 7.5, 5% [v/v] glycerol) were prepared. Then, using an automated, non-contact spotting robot (SciFLEXARRAYER, Scienion), a 6 nL droplet of each solution was deposited on the prism surface forming a sensor microarray. Three replicates of each sensor were randomly distributed on the microarray. It should be noted that, the parameters of the protein solutions (concentration, proportion of glycerol) were chosen on the basis of substantial optimization work.

After OBP deposition, the biochip was placed in a humid-controlled chamber (ca 96% RH) at 4°C for about 18h to allow the proteins to self-assemble. Finally, the chip was rinsed with HPLC-MS grade water to remove the unbound OBPs and installed in the SPRi measurement chamber.

Sodium dihydrogen phosphate and glycerol were purchased from Sigma Aldrich and water from Carlo-Erba.

C. Experimental Set-up and data processing

The experimental set-up consists of two parts: a VOC sampling fluidic system and an SPRi measurement part.

The first part allows to inject VOCs into the SPRi chamber at controlled flow rate (100 mL/min), pressure (50 mbar above ambient pressure), concentration and relative humidity level thanks to flow and pressure controllers (Bonkhorst). A zero-air generator (Umwelttechnik MCZ) was used to generate clean and dry (ca. 3% RH) compressed air. To perform VOC injection, the compressed carrier air was pushed through the fluidic system where it is first humidified (or not) then charged with VOCs thanks to a dynamic headspace before going into the SPRi analysis chamber. Finally, at the outlet of the chamber, the VOC concentration was measured by a photo ionization detector (PID). To perform "dry injections" a cylinder of compressed dry air (Air liquide) was used instead of the zero-air generator. A blank injection of clean air was systematically performed before each VOC analysis to make sure that the fluidic system is clean. All VOC solutions were purchased from Sigma Aldrich.

The second part (i.e., the measurement part) consists of a homemade SPRi set-up placed in an incubator at 25°C. An LED coupled to a bandpass filter was used to generate an excitation light beam at 632.8 nm wavelength. The excitation light was collimated and polarized before reaching the prism. A CCD camera was used to monitor the reflected light. Modulation in the reflectivity (ΔR) of all the sensors on the chip, upon exposure to VOCs, was monitored simultaneously at a fixed working angle. A measurement was made every three seconds. Finally, the mean response of each sensor (i.e., ΔR) at equilibrium is calculated and normalized by the root mean square then processed by principal component analysis (PCA).

III. Results and discussion

To carry out the study, different sets of experiments were performed. For each experiment, two identical chips were prepared then exposed to the same VOCs under the same experimental conditions (concentration of VOC, air flow, pressure, temperature) but different relative humidity levels (0% and 30%).

As stated earlier, rOBP3-a has been genetically customized to have strong affinity for aldehydes, in this respect, octanal and hexanal were selected in order to test the performance of the sensors. Hexanol was used as a negative control because it has a similar molecular weight to the target VOCs, namely octanal and hexanal, and does not specifically bind any of the OBPs. It is important to mention that after the synthesis and purification of the recombinant proteins, their binding affinities and activity, in solution, were validated by ITC. Additionally, in a previous study, the performance of the OBPs after immobilization on prism was examined in liquid phase. The proteins were found to be highly sensitive and selective. More importantly, the study suggested that a conformational change of the OBPs occurs upon VOC binding.

The results presented above were obtained in aqueous solution, which is known to be a favorable milieu for protein activity. Indeed, it is commonly accepted that the presence of an aqueous environment is required in order to maintain the active conformation of proteins. Water molecules play many roles with respect to protein function and in particular surface "structural" water i.e., the water molecules that interact with the proteins surface [6]. Studies on enzymes have showed that there is an improvement of their activity with increasing hydration. When working in gas phase, the hydration degree of the proteins is correlated with the relative humidity level.

In this study, we provided evidence that the level of hydration obtained at a RH of 30%, which corresponds approximately to the ambient humidity, is sufficient to obtain active OBPs for the detection of VOCs in gas phase.

Indeed, PCA of the OBPs response at equilibrium at 0% RH (Fig.1a) shows very weak discrimination between the injected VOCs. On the contrary, at 30% RH (Fig.1b), we observe a good separation between the two classes of VOCs namely alcohols and aldehydes and the sensors can even separate between hexanal from hexanol, which corresponds to a resolution of one hydrogen atom. Moreover, very satisfyingly, the loading plot indicates that the rOBP3-a sensors are the one that bind the aldehydes which is in agreement with the expected result.

Other experiments are underway to better understand the mechanisms of protein stabilization at low hydration levels. In particular, molecular docking simulations will be performed.

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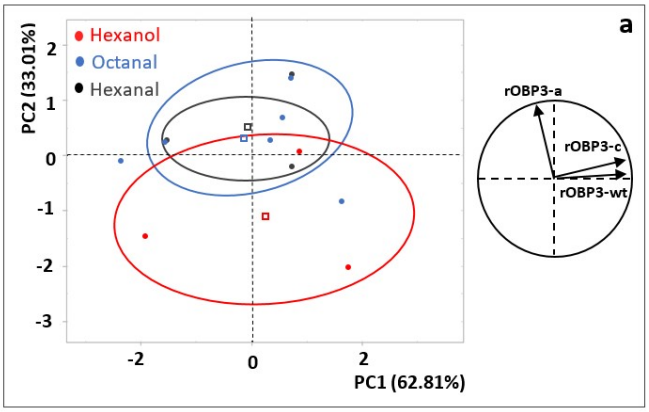
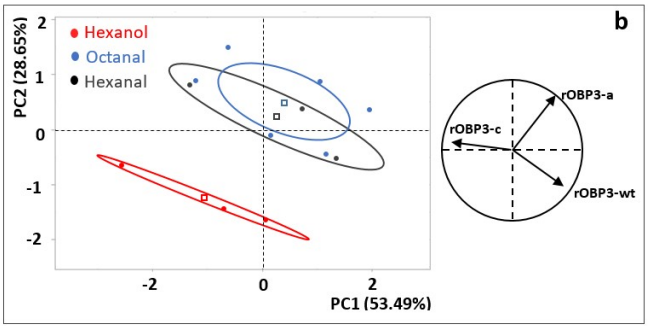
a. PCA at equilibrium with 0% RH, b. PCA at equilibrium





with 30% RH.

IV. Conclusion

Regardless of their good stability, maintaining the activity of OBPs, when immobilized on chips and exposed to a "water free" stream of gaseous odorants was found to be unachievable. However, we found that the proteins are active under ambient humidity (ca. 30%). These results confirmed that indeed water molecules plays an important role in the stabilization of protein structure and even low hydration level are sufficient to maintain protein activity. The results also showed that under the optimal conditions the OBP-based optoelectronic nose can discriminate alcohol from aldehydes with a resolution of one hydrogen atom.

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