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Selection of peptide ligands for piezoelectric peptide based gas sensors arrays using a virtual screening approach

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ABSTRACT

Virtual and experimental affinity binding properties of 5 different peptides (cysteinylglycine, glutathione, Cys–Ile–His–Asn–Pro, Cys–Ile–Gln–Pro–Val, Cys–Arg–Gln–Val–Phe) vs. 14 volatile compounds belonging to relevant chemical classes were evaluated. The peptides were selected in order to have a large variability in physicochemical characteristics (including length).

In virtual screening a rapid and cost-effective computational methodology for predicting binding scores of small peptide receptors vs. volatile compounds is proposed. Flexibility was considered for both ligands and peptides and each peptide conformer was treated as a possible receptor, generating a dedicated box and then running a docking process vs. all possible conformers of the 14 volatile compounds.

The 5 peptides were covalently bound to gold nanoparticles and deposited onto 20 MHz quartz crystal microbalances to realize gas sensors. Gas sensing confirmed that each of the peptide conferred to the gold nanoparticles a particular selectivity pattern able to discriminate the 14 volatile compounds. The largest response was obtained for the pentapeptides Cys–Ile–His–Asn–Pro and Cys–Ile–Gln–Pro–Val while low response was achieved for the dipeptide. The comparative study, carried using a two-tailed *T* test, demonstrated that virtual screening was able to predict reliably the sensing ability of the pentapeptides. The dipeptide receptor exhibited 29% of virtual-experimental matching vs. 71% of glutathione and up to 93% for the pentapeptides. This virtual screening approach was proved to be a promising tool in predicting the behaviour of sensors array for gas detection.

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1. Introduction

In last years the use of oligopeptide modified piezoelectric sensors has been demonstrated to be a very useful tool for many different applications. Gold electrodes on the quartz crystal microbalances (QCMs) resulted particularly useful to immobilise peptides via self-assembled monolayer, using cysteine as terminal aminoacid or, alternatively, a thiolated spacer (Lucarelli et al., 2008; Serra et al., 2008; Tombelli et al., 2005). Different approaches for different purposes have been proposed with classical piezoelectric biosensors operating in liquid. It is worth to mention the evaluation of affinity properties of protein (Okada et al., 2007) protein purification (Melles et al., 2005) or detection (Drouvalakis et al., 2008; Luo et al., 2007) carried out using peptides selected with a computational-combinatorial approach (Mascini et al., 2006).

The use of oligopeptide based sensors in gas phase has been attempted for the realization of gas sensor arrays (e-noses). The immobilisation of purified olphactory receptor proteins or peptides on QCMs has been a logical step to attempt in this respect (Aono, 2012; Lu et al., 2009; Wu, 1999; Wu et al., 2001). An e-nose based on adsorbed peptides for the diagnosis of uraemia has been also proposed on the base of olphactory receptor proteins (Lin et al., 2001). A computational approach for gas sensing of alcohols (3-methyl-1-butanol and 1-hexanol) associated with the presence of Salmonella contamination in meat was recently reported (Sankaran et al., 2011a, 2011b). In the past years molecular modelling was used to rationally design synthetic receptors for a variety of analytical applications (Bini et al., 2011; Chianella et al., 2002; Mascini et al., 2008; Romero Guerra et al., 2009). Virtual docking is currently an important tool in drug discovery and significant progresses were achieved over the last decade (Elokely and Doerksen, 2013; Sousa et al., 2013; Yuriev and Ramsland, 2013). Potential predictions by simulating ligand–receptor interaction have been demonstrated to have a strong impact in developing experimental procedures (Papaleo et al., 2010; Perez et al., 2013; Xu and Russu, 2013). Moreover, trial and

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error analytical protocols can be optimized by the introduction of predictive models minimizing experimental problems as non-specific recognition, reagent stability and separation procedures (Bini et al., 2011; Mascini et al., 2013; Narcisi et al., 2011).

A comparison between a simple virtual screening procedure and experimental data for the development of peptide based gas sensor arrays is reported in this work. Piezoelectric sensors assembled using gold-nanoparticles (GNPs) bearing peptides have been recently demonstrated to be potentially useful for the development of gas sensor arrays (Compagnone et al., 2013). 5 Different sensors having a dipeptide, a tripeptide and three pentapeptides have been assembled and tested vs. different classes of volatile compounds. The data confirms that molecular virtual screening can be used as convenient tool in predicting the behaviour of peptide ligands.

2. Materials and methods

2.1. Virtual screening

All calculations concerning the virtual screening process, including all molecular modelling experiments and data preparation were performed on a 3.4 GHz Intel Core I7-2600 Desktop PC with 8 GBytes RAM and 1333 MHz bus, running Microsoft Windows 7 Professional 64 Bits.

Peptides were generated with Hyperchem 8.0.5 software in zwitterionic form. Different tools from OpenEye Scientific Software package under academic licence were used (OpenEye Scientific Software). Ligands library was designed by converting standard IUPAC names into structures by LEXICHEM 2.1.0 package. Geometry optimization was carried out using SZYBKI 1.5.7 in default parameters. Conformers for receptors or ligands were generated with OMEGA 2.4.6. The boxes and rigid body docking process were performed using OEDOCKING 3.0.0 facilities in default parameters. VIDA 4.2.1 was used for structures visualization, molecular surfaces generation and physicochemical properties analysis. Peptides net charge at pH 7, isoelectric points and hydropathy were calculated using an internet peptide calculator (http://www.innovagen.se/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator.asp). The entire process was scripted, automated and executed using AutoIT V3, a freeware BASIC-like scripting language.

All structures were visualized and checked to guarantee their accuracy in terms of valence, bond order, bond angles and geometrical arrangement of atoms. All molecules were energy minimized and then possible stable conformers were generated in order to consider molecule flexibility. Boxes defining the docking active site were generated for each peptide conformer. The peptide was inside the box considering the whole receptor structure as possible binding site for ligands. In docking, scoring function was chemgauss4, a modification of chemgauss3, with hydrogen bonding and metal chelator terms improved. The scoring function was given by the sum of different terms like shape, hydrogen bond, aromatic, desolvation and others. None of them had intramolecular terms. The time required for each peptide conformer, from the initial design to final docking results, was about 3 min. Once the run completed the binding scores together with a molecule file containing all docked scoring ligands was generated. The binding score average of each peptide receptor was calculated over chemegauss 4 score of 10 peptide conformers.

2.2. Gas sensing

2.2.1. Chemicals and sensors

All reagents and volatile compounds used were purchased from Sigma-Aldrich (Italy). All volatile compounds were of analytical grade. 20 MHz QCM sensors, were from Elbatech (Italy). GNPs were synthetized as described in a previous article by adapted sodium borohydride reduction method (Aryal et al., 2006; Compagnone et al., 2013). 100mL aqueous solution of tetrachloroauric acid (10^{-4} M) was reduced by 0.01 g of $NaBH_4$ at room temperature resulting in the formation of ruby-red gold hydrosol containing GNPs of 2nm average diameter.

GNPs were then capped by self-assembly incubating 10^{-4} M aqueous solution of coating thiolated compounds at room temperature for 4 h. GNPs were functionalized with glutathione, cysteinylglycine (CG), and the three pentapeptides Cys–Ile–His–Asn–Pro (CIHNP), Cys–Ile–Gln–Pro–Val (CIQPV) and Cys–Arg–Gln–Val–Phe (CRQVF). The pentapeptides were synthesized using a Fluorenylmethyloxycarbonyl (F-moc) chemistry on solid phase starting, respectively, from Fmoc-Proline-Wang resin, Fmoc-Valine-Wang resin and Fmoc-Phenilalanine-Wang resin (Mascini et al., 2008); the different physico-chemical characteristics of the peptides are reported in Table 1. Ter- and tri-butyl groups were used to protect lateral aminoacid groups during the synthesis. Purity, assayed by high pressure liquid chromatography (HPLC), was $>80\%$ for all the three pentapeptides.

GNPs deposition on 20 MHz QCM sensors was achieved by drop casting of 50 μL of the GNP suspension on each side of the crystal and drying in desiccator at room temperature. Immobilised amount of nanoparticles were checked as reported in other work (Compagnone et al., 2013). The addition of 50 μL of GNPs suspension on each side of the sensor leads to a variation in the 18–22 kHz range. QCMs were stored at room temperature in the dark when not in use.

2.2.2. Measurement set-up

Changes in frequency were measured using a gas sensor array set up previously developed and used in flow. The system allowed allocation of up to 8 different sensors in the same measuring chamber.

Measurements on the 14 volatile compounds were carried out by using the whole head-space: 500 μL of sample were introduced in a 100 mL sealed glass laboratory bottle, connected through two three-way stop-cocks to the N_2 stream (66 mL min^{-1}). Three-way stop-cocks were open for 2 min to completely remove the air in the bottle (pre-opening). The stop-cocks were then closed for 10 min to equilibrate the head-space at 25 °C in stationary conditions. Finally stop-cocks were open to carry the head-space to the measuring chamber.

The frequency shift (ΔF), taken as the analytical signal, was the difference between the average of the last 10 measurements before injecting the gas sample (baseline) and the average of the last 10 values before closing the stop-cocks (after equilibration time).

3. Results and discussion

3.1. Virtual screening results

In virtual screening, the relative binding affinities of the 5 peptide receptors towards 14 volatile compounds were evaluated using a docking process. The 5 receptors included one dipeptide (CG), glutathione (tripeptide) and three pentapeptides (CIHNP, CIQPV and CRQVF) that were selected in order to have a wide range of structural and chemical properties and, then, to have more robust comparison with the experimental data.

In Table 1 the structural and physicochemical properties of the receptors and ligands are reported. The smallest receptor, CG, had a log P value (solubility ratio in octane/water) of -3.89, thus hydrophilic proprieties, and the lowest polar surface area (PSA) that represents the total surface area taken on all polar atoms including attached hydrogens. Glutathione presented the lowest

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