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Surface plasmon resonance imaging (SPRi)-based sensing: A new approach in signal sampling and management

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ABSTRACT

Surface plasmon resonance imaging (SPRi) is at the forefront of optical sensing, allowing real-time and label free simultaneous multi-analyte measurements. It represents an interesting technology for studying a broad variety of affinity interactions with impact in chemistry, both in fundamental and applied research. Signal sampling and management is a key step in SPRi measurements to achieve successful performances. This work aims to develop a strategy for selecting the sensing areas, called Regions of Interest (ROIs), to be sampled for recording SPRi signals that could results in improved sensor performances. The approach has been evaluated using antigen-antibody interaction: anti-human IgGs are immobilized on the chip surface in an array format, while the specific ligand (hIgG antigen) is in solution. This approach has general applicability and demonstrates that rational selection of sensitive areas and standard management of SPRi data has dramatic impact on sensor behaviour. The criteria of the method are: (a) creation of high density maps of ROIs, (b) evaluation of the SPRi binding signals on all the ROIs during a pre-analysis step, (c) 3D elaboration of the results, and (d) ranking of the ROIs for their final selection in further biosensor analysis. Using standard solution of antigen, three different ROIs selection approaches have been compared for their analytical performances. The proposed innovative method results to be the best one for SPRi-based sensing applications.

1. Introduction

Surface Plasmon Resonance imaging (SPRi) presently represents one of the best sensing platforms for studying a wide variety of affinity interactions (Paul et al., 2009; Scarano et al., 2010) involving nucleic acid sequences (Chen et al., 2009; Fuchs et al., 2010; Lee et al., 2005), peptides (Villiers et al., 2009), proteins (Bellon et al., 2009; Lee et al., 2008), and carbohydrates (Grant et al., 2007). In SPRi the entire biochip surface is displayed during analysis and it is possible to simultaneously monitor up to hundreds of molecular interactions. Here, the conventional SPR is coupled to micro-array format through the introduction of a Charge-Coupled Device (CCD) as detector system instead of a diode array. Open problems in this new sensing transduction approach are mainly related to the lack of standardised strategies for signal sampling and data management, these having dramatic influence on the analytical performances (i.e. sensitivity, selectivity, and reproducibility) of the biosensor, which are a key point for further application of SPRi-based sensing to real analytical problems (i.e. complex matrices). In SPRi bioreceptors are tethered on a chip gold surface in array format, as circular or square spots or even as micro-channels (D'Agata et al., 2008; Ly et al., 2007), and any portion of the biochip could be suitable for SPR signal sampling, since the only limit is represented by the lateral resolution of the CCD detector. As a consequence, any area having a diameter equal to or greater than the CCD resolution may be associated with a SPRi signal, leaving the operator completely free to select number, location and type of sensing areas undergoing signal sampling. Although this selection is a key step for the subsequent analysis, there is a lack of common criteria for doing it despite the high number of papers recently appeared in literature on SPRi. We believe that the valuable information provided by imaging data analysis would be more efficiently exploited, if coupled with well defined criteria for signal sampling and affinity binding data management. Moreover, standardisation of both procedures would eventually build up a common platform for homogenous comparison of SPRi results obtained in different contexts. With this aim in mind, we developed a method the rational and reproducible selection of sensing areas on the biochip (named Regions Of Interest, ROIs) for SPRi signal sampling carried out by a two-step approach performing a fine mapping of the array, followed by identification and selection of the best ROIs to be used for measurements. We report here how improved analytical performances can be thus achieved by using this rational design vs. conventional ROIs selection, in SPRi experiments. As proof of principle we applied

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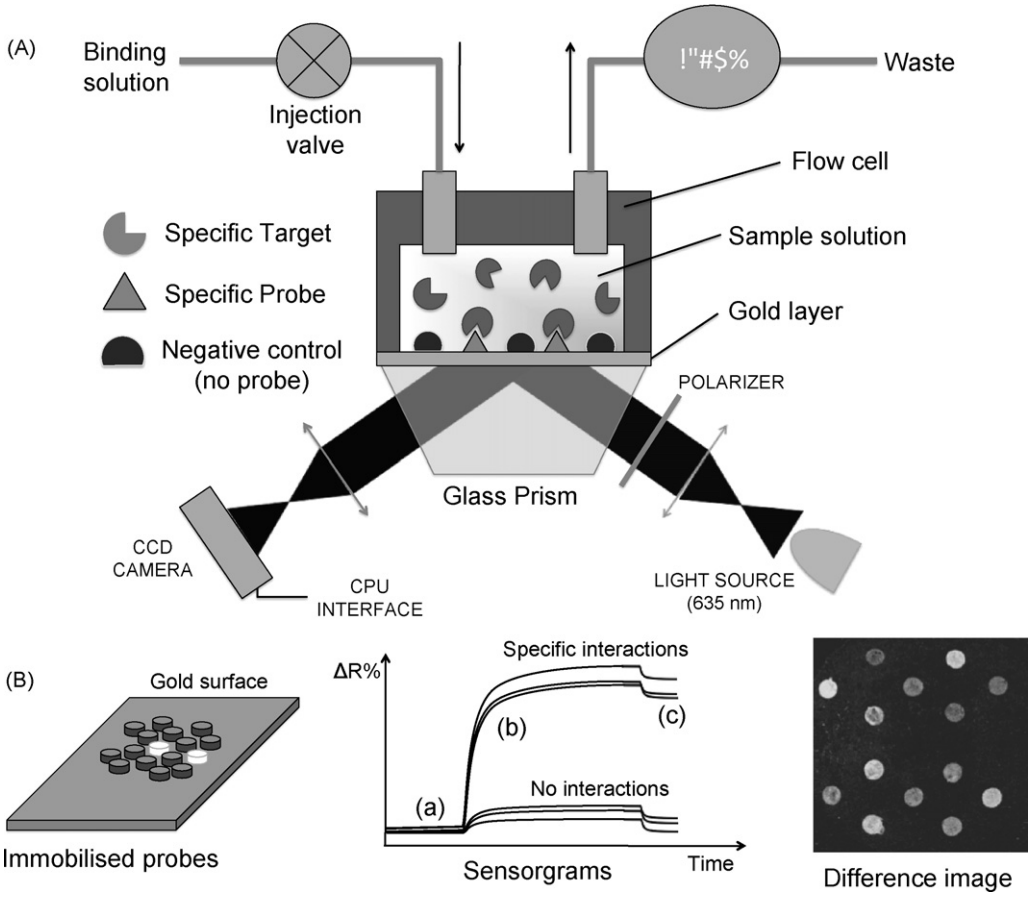

Fig. 1. (A) Scheme of the SPRi apparatus. (B) On the left, an array of probe deposited onto the biochip gold surface is represented; in the centre, a typical shape of a measuring SPRi cycle in which (a) is the signal at the baseline (carrier), (b) is the binding curve, and (c) is the washing step before the regeneration; on the right the difference image as displayed after the binding.

this approach in a paradigmatic immunosensing example involving anti-human IgG antibodies (anti-hIgG) covalently immobilized on gold chip surfaces by a dedicated chemistry, tested with the relative human IgG specific target. However, this approach can be applied to any bio-interaction and demonstrates that rational selection of sensitive areas and standardised management of SPRi data has dramatic impact on sensor behaviour.

2. Material and methods

2.1. Materials

11-Mercaptoundecanoic acid (MUA), 11-mercaptoundecanol, 1-mercaptohexanol, streptavidin, N-hydroxysuccinimide (NHS), sodium acetate trihydrate, ethanolamine hydrochloride (EA), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), Tween 20, biotin, as well as purified human IgG, and the relative biotinylated anti-human IgG were purchased from Sigma–Aldrich (Italy). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) was from Calbiochem (USA). Glycine and hydrogen peroxide 35% were from Merck KGaA (Germany). Ammonia solution was purchased from VWR International (France). Absolute ethanol was form Carlo Erba Reagenti (Italy). 184 Silicone Elastomer kit was from Dow Corning Corporation (USA). Probes for immobilisation were in an immobilisation solution containing 10 mM sodium acetate, pH 5.0 in water. Binding buffer contained HEPES 10 mM, Tween20 (Sigma–Aldrich) 0.1%, pH 7.4. To regenerate the biochip surface after the binding with the analyte a 10 mM glycine solution, pH 1.9, was used. Absolute ethanol, deionised water (18.2 MΩ cm) and all solutions were filtered (Millipore, 0.45 μm) before use. SPR imaging measurements were conducted on SF-10 glass prisms with a 5 nm-layer of chromium coated under a 50 nm gold layer (Genoptics-Horiba Scientific, Orsay, France).

2.2. SPR imaging measurements

SPRi experiments were performed on SPRi-Lab+ instrument (Genoptics-Horiba Scientific, Orsay, France) based on intensity modulation, measuring the reflectivity of monochromatic incident p-polarized light (635 nm) at fixed angle. The optical asset is based on Kretschmann configuration; a high refractive index glass prism is used as resonance coupler. A peristaltic pump (Minipuls 3 M312, Gilson) governs the 6-μl flow cell and the working flow for all measurements was 20 μl/min. Fig. 1A represents the scheme of the SPRi instrumentation.

Analyte solutions were all prepared in a binding solution (used also as carrier) and injected (175 μl) into by using a Rheodyne 7125 valve (IDEX Health & Science Group, Wertheim-Mondfeld, Germany). The measurement procedure is described all follows (Fig. 1B, centre): The solution flowing on the surface (carrier solution) is taken as reference for the baseline signal (a). Then the chip with immobilised probes was exposed to target solutions for 10 min (b). All SPRi signals were taken after washing the surface with the carrier solution to remove the unbound analyte (c). After each measuring cycle, the biochip surface was regenerated by injecting for 30 s a 10 mM glycine solution, pH 1.9. SPRi signals are recorded in real-time for each area on the biochip surface previously selected as Region Of Interest (ROI), and visualised both as