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Label-free affinity biosensor arrays: novel technology for molecular diagnostics

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1. Introduction

The enzyme-linked immunosorbent assay (ELISA) has been the cornerstone of *in vitro* protein diagnostics for over 40 years, and remains critical throughout healthcare for the detection and quantification of disease biomarkers in clinical samples [1]. However, the emergence of personalized and stratified medicine with their inherent requirement for multiplexed, high-throughput diagnostics, together with the ease-of-use and low cost requirements imposed by the drive towards point-of-care diagnostics are changing the landscape. Innovative diagnostic devices are essential for realizing these emerging trends.

Label-free affinity biosensors are widely considered to be the technological solution to address these challenges. These analytical devices consist of a highly specific, high affinity probe molecule, most commonly an antibody, that is immobilized on the surface of a physiochemical transducer. The transducer monitors any binding event directly, i.e. without the need for an additional antibody, enzymatic, fluorescent or radioactive label, or any other amplification method, to provide a response that is proportional to the concentration of bound molecules. Possibly the best known biosensor of this type is the surface plasmon resonance (SPR) sensor for monitoring of antibody-antigen interactions [2]. SPR exploits the high sensitivity of surface plasmons to refractive index changes close to a metal surface, which leads to a change in resonance angle or wavelength. A local change in refractive index, for example due to the binding of an antigen to an antibody immobilized on the metal surface, can thus be detected in real-time and label-free by the corresponding shift in resonance. Today, SPR is a well-established biophysical technique that underpins fundamental research across the biological and biomedical sciences. SPR is however rarely used in clinical diagnostics, mainly due to high instrument and sensor costs. Current biosensor research is therefore focused on alternative technologies that can provide highly sensitive and selective protein detection but on a platform that is easy to use, low cost and

amenable for use in the clinic, at point-of-care or even in the home. Innovative biosensor research is flourishing; 2898 articles with ‘biosensor’ in the title were published in 2015 compared to 504 in 2000 (data from Web of Science). Much of the research to date has focused on the development and optimization of transducer materials, including a recent emphasis on carbon-based biosensors [3,4], and physiochemical transduction strategies, with transducers that are sensitive to the optical, electrochemical and mechanical properties of biological molecules receiving most attention. A number of comprehensive review articles have been published that focus on biosensor transduction strategies [5-7]. There is also a significant body of work on optimized surface chemistries for antibody immobilization and alternative high-affinity binding agents such as antibody fragments, DNA and peptide aptamers, and molecularly imprinted polymers [8]. This editorial provides an overview of two major trends of biosensor research, namely the move towards high-density biosensor arrays and the development of multi-modal biosensor technologies.

2. The fabrication of high-density biosensor arrays for multiplexed diagnostics

A comprehensive understanding of complex biological processes, both in health and disease, will require high-throughput quantification of molecular expression profiles, ideally in a single test. This multiplexed approached to protein monitoring is not only critical for fundamental biomedical research, but also underpins strategies for personalized medicine and the discovery of novel disease biomarkers. The multiplexed detection of tens, hundreds or even thousands of protein species in a small clinical sample is a significant technological challenge that requires new technological solutions. To this end, many types of photonic and electrochemical biosensors already use the low-cost, yet high volume and high precision mass-manufacturing techniques developed by the semiconductor industry that is capable of producing biosensors with a sub-micrometer footprint, ideal for high-density integration. Unfortunately, the biochemical functionalisation technology required to place different probe molecules onto each sensor does not offer the same level of integration. For example, dot-printing approaches for creating antibody arrays are currently spatially limited to around 0.1 mm. Although the resolution of these printing techniques is likely to improve, the feature sizes required for high-density biosensor arrays capable

of handling extremely small sample volumes are likely to remain beyond the scope of such systems. While high spatial resolution molecular immobilization has been demonstrated in the laboratory, for example using nanografting [9] or dip-pen nanolithography [10], these serial approaches do not provide the high-throughput required for mass-fabrication of high-density protein arrays.

Electrochemically controlled functionalization is a promising approach to spatially direct the immobilization of biomolecules, that simultaneously meets the requirements of resolution, speed, and the ability to coat each biosensor within an array with a different probe molecule. For example, electrochemical cleavage of the gold-thiol bond has been used to direct the immobilization of different proteins onto 20 μm diameter metallic electrodes separated by 15 μm [11]. The electrode microarray was subsequently used to demonstrate label-free, electronic detection of cyclin-dependent protein kinases in whole-cell lysates, with no measurable cross-talk between functionalized electrodes. A similar spatial resolution (1 μm) was also demonstrated using electrochemical grafting of diazonium salts onto conductive surfaces [12]. The use of diazonium salts is applicable to a wide variety of conductive surfaces, including silicon, and has recently been exploited to create a label-free, silicon photonic biosensor array with the potential for highly-multiplexed molecular detection [13]. These studies demonstrate the great potential of electrochemically-directed approaches for site-selective functionalization. Further research will determine the ultimate spatial resolution achievable and explore the limits of functionalization across a high-density array in terms of speed, specificity and efficiency and whether this is sufficient for production at a scale viable for commercialization.

3. Multi-modal, label-free biosensors.

The majority of existing label-free biosensors are single mode; detection is provided by a transducer sensitive to single physiochemical property. For example, the formation of an antibody-antigen complex is detected in SPR by measuring the corresponding change in refractive index, while capacitive biosensors detect the change in impedance of an antibody layer upon antigen binding. Biosensors that combine multiple *transduction* mechanisms are becoming increasing

popular. Such truly *multi-modal* assays can simultaneously probe multiple biomolecular properties, e.g. in the optical, electronic and/or chemical domains to provide greater insight and broaden the range of systems accessible for analysis. A prominent example of such multi-modal biosensing is electrochemical SPR (EC-SPR), where the gold surface that supports the optical mode is also used as the working electrode in an electrochemical cell. The complementary information contained in both the electrochemical and optical domains not only provides deeper insight into the biomolecular processes occurring at surfaces, but has also been used to improve biosensor accuracy. For example, a glucose biosensor based on an EC-SPR has been demonstrated in which the simultaneous electrochemical and optical measurements were combined to differentiate between the enzymatic activity of glucose oxidase and the unwanted background of non-enzymatic reactions [14]. Novel insights into enzymatic activity have also been reported using a multi-modal biosensor that combines electrochemical measurements with quartz crystal microbalance (QCM), which is sensitive to changes in both the mass and viscoelasticity of an immobilized molecular layer [15].

Ideally, one would like to combine the best of both worlds, i.e. multiplexing large arrays of sensors on a tiny surface area capable of profiling biomarker content in a small sample volume, with each sensing region performing multiple types of analysis. So far, this is not possible: existing microarrays can perform many tests in parallel, but they require a relatively large volume due to their macoscopic size; SPR and QCMD can perform multimodal analysis, but only on a single or a very small number of spots. We believe that the route towards true multimodal multiplexed detection is silicon technology. To this end, the electrophotonic silicon biosensor combines photonic and electrochemical detection in a single, microfabricated biosensor array. The ability to combine electrochemical and optical detection in silicon was achieved by controlling the doping density profile of a silicon photonic microring resonator such that the dopants were located in a thin layer at the silicon surface. The doped surface layer can thus be optimized to be sufficiently conductive to support electrochemical processes while thin enough to minimize losses of the optical mode confined within the resonant structure. The same platform can also be used to

electrochemically direct the immobilization of probe molecules, thereby achieving both multimodal and highly multiplexed detection.

4. Conclusions and outlook

Changes in approaches to healthcare require a new generation of diagnostics that are able to detect large numbers of proteins simultaneously, with high sensitivity and selectivity, and in a platform that is simple, cheap and portable enabling tests to be performed in clinic, at point-of-care, or even in the home. Label-free affinity biosensors are an emerging technology with the potential to meet these requirements and significant progress has been made, especially by exploiting the technological advances of the silicon microelectronics industry. While the translation of this new diagnostic technology and associated biomarkers into clinical practice still pose a number of technical, scientific and regulatory challenges, the opportunities to transform healthcare practice and patient outcomes are substantial.

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