Microfluidics Devices Integrating Microcavity Surface Plasmon Resonance Biosensors: Glucose Oxidase Enzymatic Activity

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***Short Abstract* — We describe the integration of 1-µm-diameter microcavity surface-plasmon-resonance sensors with microfluidics, test their sensitivity to refractive-index changes, relate their response to the *response units* (*RU*) used in Biacore instruments and demonstrate their biosensing capability by distinguishing the interaction of glucose oxidase (160kDa) with its natural substrate (βD-Glucose, 180Da) from its interactions with non-specific substrates (L-Glucose, D-Mannose or 2-DeoxyD-Glucose). The same protocol run on a Biacore-3000 reveals no interaction between glucose oxidase and the substrates. The biosensor can detect binding of about 25,000 proteins (6.5 fg), 10^6 times fewer than classical surface-plasmon-resonance biosensors.**

***Keywords* — microcavity, surface plasmon resonance sensor, stationary surface plasmon wave, microfluidics, glucose oxidase, Biacore, biosensor.**

INTRODUCTION

A central realization of modern molecular biology is that the molecular interactions responsible for biological processes define interaction networks (*e.g.*, metabolic, regulatory and signaling networks). Systems-level understanding of the function and malfunction of cells and organisms requires comprehensive models based on maps of all interactions between component parts: DNA, RNA, proteins, metabolites, hormones, ions, small molecules, *etc.*. Mapping complete interaction networks (interactomics) requires gathering kinetic information on hundreds of millions of interactions [1]. Current efforts to map important networks address yeast [1, 2], *C. elegans* [3], *Drosophila* [4], humans [5] and other organisms [6, 7].

No current biosensor technology combines the sensitivity, kinetic measurement and throughput which interactomics needs. While much current research attempts to improve biosensor sensitivity, other sensor characteristics are also important. The ideal biosensor would:

- Be sensitive and non-invasive, to permit use in a wide range of applications.
- Allow real-time, label-free detection to provide kinetic data for interaction mapping.
- Be small and integrate with microfluidics to reduce reagent usage.
- Integrate into 2-D arrays to provide high throughput for drug discovery and interaction network mapping.
- Be easy and inexpensive to manufacture.
- Be highly reproducible sensor-to-sensor and chip-to-chip.
- Allow a simple, cheap and robust instrument design.

We have developed a *micro-cavity surface-plasmon-resonance sensor* (*MSPRS*) to study the binding kinetics of unlabeled molecules based on these principles. [8] MSPRSs are much larger than standard nanoparticles (10-100nm diameter), delocalizing the *localized surface plasmon resonances* (*LSPR*s), [9] but far too small to excite the *whispering gallery modes* (*WGM*s) in 10-100µm dielectric structures. [10-12] In MSPRS, stationary surface-plasmon waves confined in a metal shell wrapped around a sub-micron dielectric nanosphere replace the propagating surface plasmon waves (*SPW*s) of planar surface plasmon resonance (*SPR*) sensors, increasing the interaction probability between the SPWs and surface adsorbate enhancing sensitivity. They also eliminate planar SPR-sensors' standard geometric and polarization requirements for SPR excitation, allowing sensor miniaturization (from ~1mm^2 to 2.5µm^2) and integration into micron-scale microfluidics.

To determine MSPRS sensitivity we examined the interaction of glucose oxidase (GOx) with βD-Glucose, L-Glucose, D-Mannose, and 2-DeoxyD-Glucose. These interactions are medically significant, well studied using other methods and fast. In addition the binding of the small βD-Glucose (180Da) with the large GOx (160kDa) is difficult to observe with other technologies.

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