NANOCAVITY NETWORK-BASED IMPEDANCE SENSOR FOR MOLECULAR DIAGNOSTICS

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Abstract
We demonstrate label-free detection of a molecular binding event using a nanocavity network-based impedance technique. A micro-affinity column of biotinylated beads is packed inside of a glass microchip, and the binding of avidin occludes the nanocavities between beads and is measured as a change of impedance through the packed column. We developed a model that indicates a ~8% increase in resistance upon saturated binding of avidin to the 1 μm diameter beads in the column. Preliminary data in 0.84 μm beads indicates a 20% increase in resistance upon saturation with avidin.

Keywords: Impedance sensor, microparticles, bead-based analysis, label-free

1. Introduction
Bead-based analysis methods are emerging as efficient techniques for molecular diagnostics due to the high surface to volume ratio, versatility in surface-chemistry, and ability to manipulate the beads with microfluidics [1, 2]. We present a unique label-free method to detect biomolecular binding based on impedance changes through a nanocavity network formed by packing microparticles or nanoparticles in microfluidic channels. This method requires no fluorescent labeling of analyte and allows a simple readout of binding through impedance at a given frequency.

Figure 1. (A) Layout of the device, (B) active region consists of dams to trap beads with integrated electrodes, (C) beads are packed and resistance is measured, (D) sample is introduced and change in resistance is measured, (E) cross section of column, which shows dam structure.
The demonstrated microfluidic integration of the nanocavity system is also advantageous, allowing easy introduction of analyte solution and measurement buffer. Previously, it has been shown that beads coated with a protein will lead to an increased resistance of a nanopore they are passing through, when compared to the uncoated bead [2]. In that situation a time dependent dynamic measurement of resistance is required, and beads must be pre-treated before analysis. However, in our system, the measurement is made in a stationary nanocavity system which is directly treated with analyte (Fig. 1). Binding of analyte then decreases the cross-sectional area of the void space and increases the measured resistance across the nanocavity system.

2. Theory

Resistance changes through nanocavity systems were modeled using as a geometric model a closely-packed array of microparticles (Fig. 2). This model will give the upper limit on expected changes since a less than crystalline pack is expected. Using the unit cell described in Figure 2A we calculated the z-dependent cross sectional areas and perimeters (Fig. 2B) and total unit cell resistance. Knowing the perimeter, and assuming saturated binding, we calculated the obstructed area of the nanocavity with binding of various size molecules. For various size microparticles and biomolecules, the resistance increase upon binding is

![Figure 2](image.png)

Figure 2. (A) Packed microparticles lead to z dependent areas and perimeters that repeat with the unit cell, defined here. Perimeters allow calculation of obstructed area with binding of biomolecules. (B) The z dependence of area and perimeter through a nanocavity system created by 3 \( \mu \)m radius microparticles. (C) Resistance ratio upon binding as a function of the bead pack radius is plotted for various sized biomolecules.

![Figure 3](image.png)

Figure 3. (A) Optical micrograph of the active region after biotinylated beads have been packed. (B) Fluorescent micrograph of beads after binding with FITC conjugated avidin.
plotted in Fig. 2C. This analysis suggests 1 µm diameter or smaller bead sizes for ~10% change in resistance for an average protein molecule of 50 Å in diameter.

3. Experimental

Dams were created in a glass substrate using a single-step wet etching process. Electrodes were patterned onto another glass substrate via electron beam evaporation, and the two pieces were aligned and thermally bonded after input and contact regions were drilled. Polystyrene beads (0.84 µm in diameter) functionalized with biotin were packed into a micro-affinity column, creating a nanocavity network. The beads were then coated with an avidin solution. The avidin was fluorescently labeled in order to confirm binding (Fig 3). Impedimetric measurements were made with a Novocontrol impedance analyzer.

4. Results and Discussion

Figure 4 shows the impedance magnitude and phase angle between electrodes ‘a’ and ‘d’ before beads are loaded, after beads are loaded, and after the beads are coated with avidin. Buffer was flushed through the device before each measurement was made. The resistance values are plotted in Figure 5 for electrodes ‘a-c’ with ‘d’ as a common reference electrode (Fig 2A).

Acknowledgments
NDSEG Fellowship for JTN, Whitaker Fellowship for DD, and GlaxoSmithKline.

References