Ultra-Sensitive and Selective Detection of DNA and Protein Biomarkers Using Frequency-Locked Microtoroid Optical Resonators

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Abstract: We use frequency-locked microtoroid optical resonators to detect protein and DNA biomarkers at attomolar to femtomolar concentrations, depending on the target. We measure binding affinities and validate our results against existing technologies.

1. Introduction

Ultra-sensitive, label-free, and rapid biochemical detection is needed for a wide variety of applications including small molecule drug discovery, environmental monitoring [1], and medical diagnostics, among others [2]. We have developed a sensing platform known as FLOWER (frequency locked optical whispering evanescent resonator), which is based on combining whispering gallery mode optical resonator technology with noise reduction techniques and data processing [3–6]. FLOWER is capable of label-free single macromolecule detection. Here, we combine FLOWER with microtoroids coated with (1) G-protein coupled receptors (GPCRs) embedded in a lipid bilayer, (2) antibodies embedded in a lipid bilayer, and (3) specific DNA sequences for specific as well as sensitive label-free and rapid biodetection. In some cases, we demonstrate detection over a wide (8 orders of magnitude) dynamic range, including the attomolar range, and validate our results with existing technology. We discuss matrix effects as well as how the large capture area of a microresonator such as a microtoroid enables orders of magnitude faster detection than nanoscale sensors such as plasmonic nanorods.

2. Results

In our first study, we examine the binding of Dynorphin A binding to kappa-opioid receptors embedded in lipid bilayer on a toroidal sensor (Figure 1).

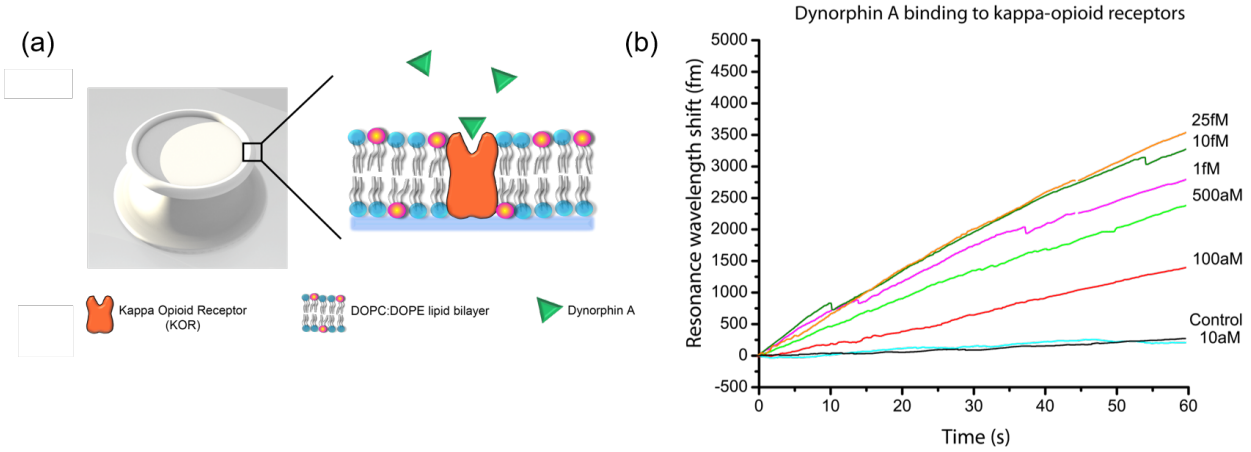

Figure 1. Dynorphin A binding to kappa-opioid receptors embedded in lipid bilayer on a toroidal sensor. **(a)** Schematic of the lipid bilayer that encapsulates our toroidal sensor. **(b)** Our results indicate that we can detect 100 aM of Dynorphin A binding to kappa-opioid receptors in a lipid bilayer on a microtoroid optical resonator with a high signal-to-noise ratio. The resonator response is not linear with concentration in this figure because the sensor begins to saturate at higher concentrations. Regenerating the surface with a low pH buffer overcomes this.

In addition, we look at the binding of the Alzheimer's biomarker amyloid-β42 in both buffer and serum solutions as well examine the effect of serum concentration on our signal. These experiments are performed by embedding antibodies for amyloid-β42 in a lipid bilayer that is tethered to the surface of the microtoroid. We discover that using an antibody embedded in a lipid bilayer functionalization approach versus a silane-based functionalization approach enables us to have lower limits of detection (< 100 aM). In addition, the lipid bilayer functionalization approach provides better resistance to non-specific binding making it more ideal for screening patient samples. We also examine the binding of prostate cancer relevant DNA sequences to the surface of our sensor.

Finally, we perform flow visualization experiments to determine the delivery time of analyte to the surface of our sensor. We examine three different injection conditions, including a stopped flow condition (Figure 2), and achieve agreement with COMSOL simulations.

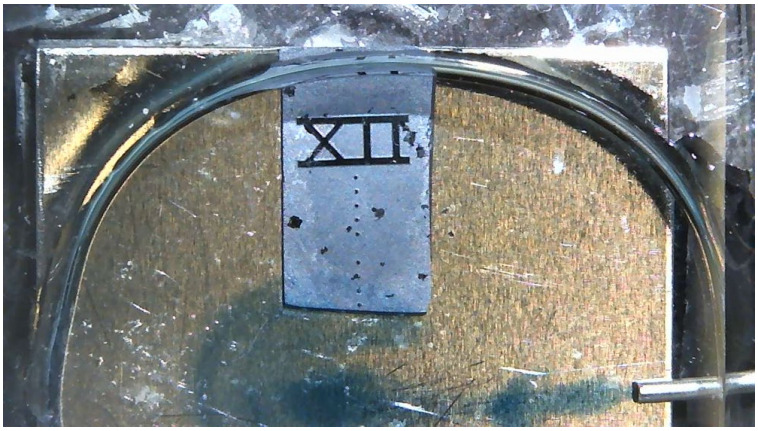

Figure 2. Top view of a flow visualization experiment performed to determine delivery time to microtoroids (small black vertical line of dots underneath the number XII). The sample chamber used in these experiments is identical to the one used in a standard microtoroid biosensing system. A silicon chip containing microtoroids is adhered to a stainless-steel substrate and a open sample chamber is formed by cantilevering a glass coverslip above the steel substrate using a 2 mm glass spacer. This image is taken 8 seconds after injection from the tube in the bottom-right corner of the image. The sample is rapidly delivered very close to the surface of the toroid.

3. Conclusions

We demonstrate attomolar detection of a variety of protein and DNA targets, calculate binding affinities, and validate our results with existing technology. In addition, we experimentally and theoretically investigate analyte delivery time. We believe our results enable a wide variety of experiments that were previously difficult to be done such as small molecule discovery for targets that are unable or difficult to be labeled, among other applications such as medical diagnostics and prognostics.

4. References

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