# DEVELOPMENT OF LABEL-FREE BIOSENSOR FOR THE DETECTION OF ADENOSINE DIPHOSPHATE AS A UNIVERSAL KINASE/ATPASE ASSAY USING NANOIMPRINTED FLEXIBLE TWO-DIMENSIONAL PHOTONIC CRYSTAL

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## ABSTRACT

The use of photonic crystals (PC) as biosensors for label-free detection of kinase activity is presented. Here, we exploited the unique optical properties of PC to detect enzymatic reactions using Protein Kinase A (PKA) as model system. Detection of one of the reaction products, adenosine diphosphate (ADP) was achieved with sufficient sensitivity using nanoimprinted flexible two-dimensional PC (2D-PC) without the use of tags or dyes. This assay offers a rapid, simple and cost-effective method which can be used as a universal assay procedure to detect kinase reactions.

## KEYWORDS

Photonic crystals, biosensor, kinase assay, label-free, adenosine diphosphate

## INTRODUCTION

The increasing recognition of kinases as validated drug targets has been the subject of intense research interest because of their association with broad range of diseases and disorders, including cancer [1]. The development of technologies that involve rapid, cost-effective and easy-to-use device to monitor the activity of these enzymes is therefore of utmost importance to develop novel therapeutics. The ability of PCs to detect changes in reflectance intensity without the use of tags, dyes or radiometric reagents makes them an ideal stage for simple and rapid analysis of enzyme reactions. We have previously reported the use of 2D-PC as a novel single-step label-free biosensor using insulin:anti-insulin as model system [2].  We want to pursue the application of PC not only to monitor binding but also detect enzyme reaction through changes in PC property. This can open a wide range of applications such as kinase inhibition assays that are of great interest in the field of drug development.

## EXPERIMENT

The design of the PC biosensor takes advantage of the specific optical characteristics created by the periodic nanostructures where upon ADP binding to the immobilized anti-ADP on the PC surface, alters the distance and angle of the reflected light. This interaction leads to a change in reflectance intensity in accordance to Bragg's Law. Hole-array PC (hole diameter and distance between holes: 240 nm) was fabricated via nanoimprint lithography (NIL) on cyclo-olefin polymer (COP) film (thickness: 100 μm) to produce a 2D-PC with a reflection wavelength of ~480 nm. The design of the experiment is depicted in Figure 1.

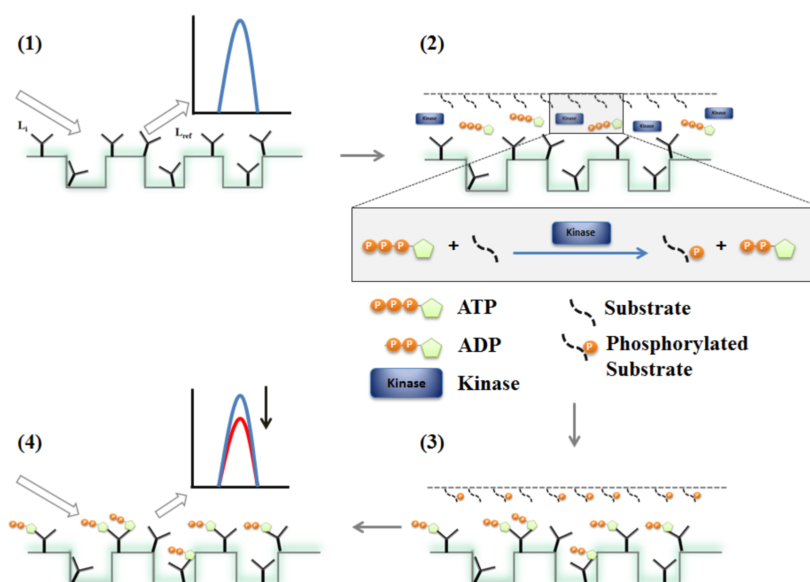


Figure 1. A schematic diagram for the label-free detection assay of ADP using nanoimprinted photonic crystal to monitor kinase/ATPase activity

Label-free detection of ADP was carried out by immobilizing polyclonal anti-ADP antibody (1 μg/ml) onto the nanoimprinted flexible 2D-PC surface (Fig1.1) through self-assembled monolayer (SAM) [3]. Selectivity of anti-ADP

was tested by incubating adenosine monophosphate (AMP), adenosine triphosphate (ATP) and ADP onto the surface-immobilized antibody. A calibration curve was constructed by incubating different concentrations of ADP onto the immobilized antibody on the PC surface for 30 mins at ambient temperature. On the other hand, PKA activity was monitored using a PKA peptide substrate. A reaction mixture containing the kinase, substrate, $MgCl_2$ and ATP was prepared in 20mM phosphate buffer, pH 6.5. The reaction was allowed to proceed at the indicated time points (1, 2, 3 hours) at ambient temperature (Fig.1.2) and ADP produced was allowed to be adsorbed onto the immobilized anti-ADP antibody (Fig.1.3). Reflection intensities were determined using dry conditions at wavelengths 350-800 nm (Fig.1.4) using a UV/VIS spectrophotometer [4].

**RESULTS AND DISCUSSION**

Protein kinases play a major role in signal transduction by phosphorylating a substrate at specific amino acid residues using ATP. This chemical reaction yields ADP as one of the products. Kinase activity assays usually monitor the production of phosphorylated substrates which make the procedure very selective. To develop a general method to monitor any kinase reaction, we devised an assay that monitors ADP using anti-ADP anti-body immobilized on a 2D-PC. We took advantage of the PC property where surface modification leads to a change in reflection intensity. Results showed that immobilized anti-ADP is selective towards ADP while discriminating other nucleotides (Figure 2).

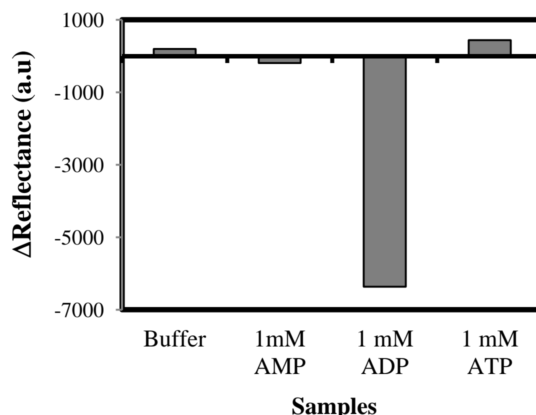


Figure 2. Selectivity of the surface immobilized anti-ADP towards ADP

A dose-dependent decrease in reflection peak intensity upon binding of different ADP concentrations to the immobilized anti-ADP on the PC surface was observed (Figure 3a, b and 4a). These results indicate that 2D-PC is amenable to detect small molecules, thus a quantifiable data can be achieved using this method.

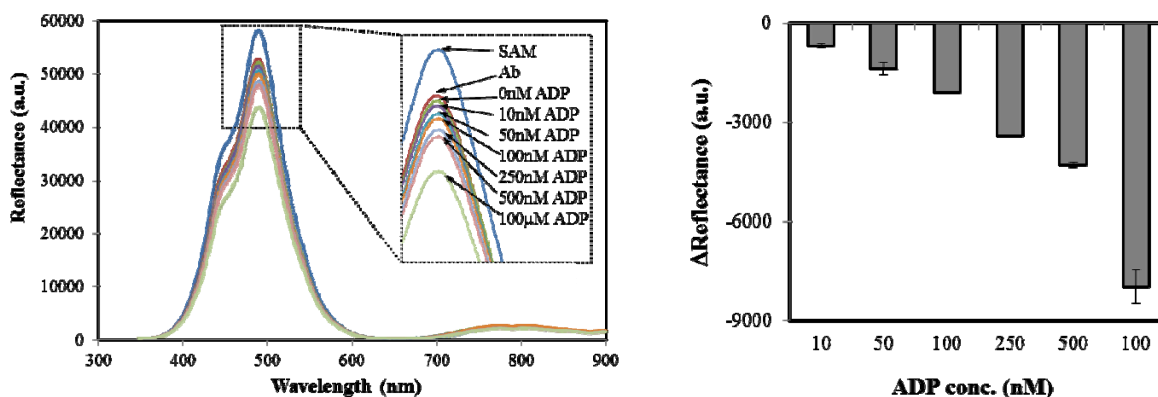


Figure 3. Change in reflectance peak intensity at 480 nm as a function of ADP concentration. (A) Raw data (B) Change in reflection peak intensity of ADP solutions upon binding to anti-ADP immobilized onto PC.

The ability of surface-immobilized anti-ADP on 2D-PC to detect ADP as product of the PKA enzymatic activity was observed through changes in reflection intensities (Figure 4). ADP production observed over time is a clear indication of a chemical reaction taking place. Moreover, this assay can detect reasonable sensitivity at low nanomolar concentrations of ADP. This study shows that 2D-PC can be used to monitor any chemical reaction involving small molecules.

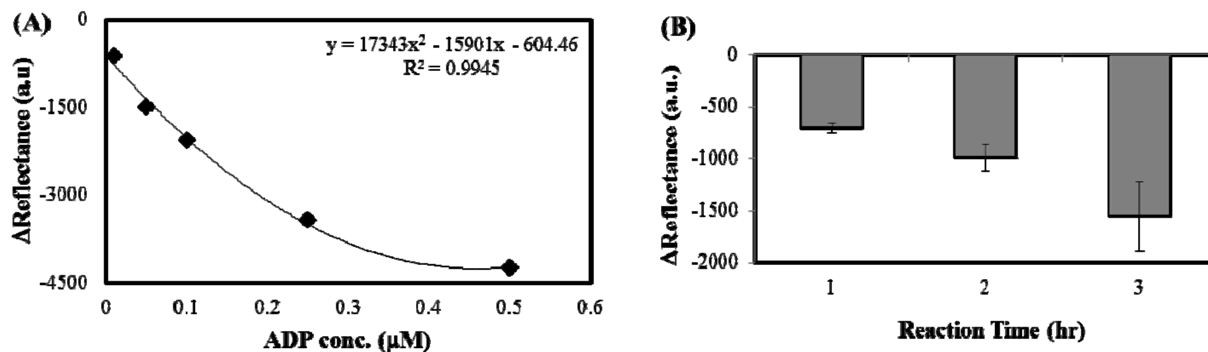


Figure 4. Quantitation of PKA kinase activity (**A**). ADP calibration curve (**B**). ADP detection from PKA kinase activity

## CONCLUSION

Existing technologies on kinase screening and profiling involve the use of specific reagent for a specific target and require radioactive isotopes to test activities. Furthermore, ADP detection assays involve the use of fluorescently labeled tags or enzyme-coupled reactions which complicate and possibly alter structure and by extension interactions. Thus, single-step, label-free assays for ADP detection using PC can be an attractive area of research, both in basic research and in drug discovery, as they provide a generic method for measuring the activity of kinases which can also be extended to ATPases and other ADP-generating enzymes. Towards this goal a single-step method will be employed by adding a solution containing the enzyme and adenosine triphosphate onto the system with immobilized antibody and substrate.

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