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Enzyme-assisted polymer film degradation-enabled biomolecule sensing with poly (N-isopropylacrylamide)-based optical devices

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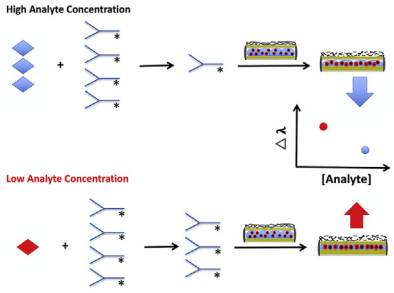
HIGHLIGHTS

- A novel biosensing motif was developed.
- Enzyme degradation of a polymer film enabled sensor response.
- Sensor could detect sub-nanomolar concentrations of IgG.
- Sensor exhibited species selectivity.

GRAPHICAL ABSTRACT



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ABSTRACT

A biosensor for mouse Immunoglobulin G (IgG) was generated from responsive polymer-based interference filters (etalons). To accomplish this, an excess amount of alkaline phosphatase-modified goat anti-mouse IgG (AP-GAM, F(ab')$_2$ fragment specific to mouse IgG) was added to mouse IgG, and allowed to react for some time. After a given reaction time, the bound AP-GAM could be isolated from the unbound, excess AP-GAM by addition of goat anti-mouse IgG (Fc fragment specific)-modified magnetic microspheres (GAM-M) that bind the mouse IgG bound to AP-GAM. After application of a magnetic field, the free, unbound AP-GAM was isolated from the mixture and exposed to an etalon that has its upper Au surface modified with phosphate-containing polymer that can be degraded by AP-GAM. By the phosphate-containing polymer being degraded by the excess AP-GAM, the cleaved phosphate groups can diffuse into the interference filter's active polymer layer that yields a change in the optical properties that can be related to the amount of IgG in the sample. This concept is extremely straightforward to implement, and can be modified to detect a variety of other analytes of interest.

Pathogens, such as bacteria and viruses, are a major class of species that can have direct negative impacts on human and animal health [1–3].Most recently, Zika virus has emerged as a major health concern in South America and other tropical areas around the world. Oftentimes, Zika virus infection has no symptoms, which makes it very difficult to diagnose. Therefore, those infected usually are not aware, [4] which can lead to extremely negative impacts on reproductive health [5].As of 2016, there is no known cure for Zika virus, and no vaccine to prevent it. Therefore, an approach that can alert people when they are infected can help circumvent the negative impacts on those infected, especially to those that are, or are planning to become, pregnant.

Due to the high specificity of antibodies for particular epitopes on corresponding antigens, antibody-based detection systems for

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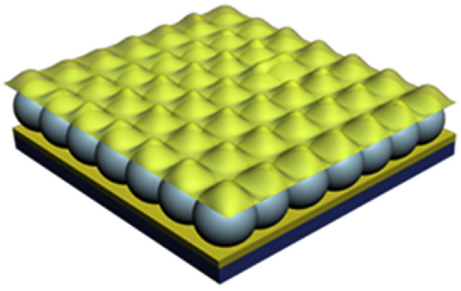
specific antigens have been used as versatile and powerful tools for bioanalysis [6,7]. Thus far, many biosensors have been developed, with one of the most commonly used approaches being enzyme-linked immunosorbent assay (ELISA) that exploits antibody-antigen interactions [8,9]. However, in many cases, ELISA requires significant time to perform, the surface chemistry needs to be carefully tuned for each specific analyte, and it requires a lab setting to achieve quantitative results. Lateral flow assays (LFA) for antibody/antigen detection have also attracted significant attention due to their ease of fabrication, the ability to use small sample volumes, while maintaining high quality figures of merit (e.g., high sensitivity and low detection limits). The most common and widely used LFA is the home pregnancy test. Although LFAs are extremely useful, there are some issues with biomolecular affinities as the reaction times between the capture and analyte biomolecules is short and there is the possibility of non-specific reactivity to the LFA substrate. Much attention has also been focused on a variety of other approaches that exploit various phenomena, e.g., surface plasmon resonance (SPR), [10–12] quartz crystal microbalance (QCM), [13] and electrochemical impedance spectroscopy [14]. With these various approaches available, there is still a need for new cost-effective sensing technologies with high specificity and sensitivity, so that people, especially those in developing countries and low-income regions, will have disease diagnosis kits more readily available to them.

Recently, photonic materials/crystals have played important roles in various analytical and medical fields [15–19]. In 2010, the Serpe Group reported on a novel optical device (etalon) that can be fabricated by sandwiching a layer of poly (N-isopropylacrylamide) (pNIPAm)-based microgels between two thin, semi-transparent metal layers (typically Au in our case) [20]. The structure of the microgel-based etalons is shown in Scheme 1. Briefly, etalons are fabricated by painting a concentrated solution of pNIPAm-based microgels onto a Au-coated glass substrate, followed by washing away the excess microgels that are not directly attached to the Au. Then the etalon is soaked in water and further rinsed before deposition of another Au layer on top of the microgels. The etalons show visible color and exhibit unique multipeak reflectance spectra. The position of the peaks and the peak order can be predicted by equation (1):

$$\lambda m = 2nd\cos\theta \quad (1)$$

where the specific wavelength maximum (λ) of a peak depends on the peak order (m), the refractive index of the dielectric (n) and the spacing between the mirrors (d), as well as the angle of incidence (θ). As a result, the size of the microgels is one factor that can dictate the color of the devices and the position of the devices reflectance peaks. We have used this structure for a variety of applications, primarily focused on sensing, biosensing and drug delivery [21–25].



Scheme 1. The structure of a pNIPAm microgel-based etalon, which consists of two 15 nm Au mirrors (yellow layers), with 2 nm Cr adhesion layer. The mirrors sandwich a densely packed layer of microgels (blue layer) all assembled on a rigid glass substrate (dark bottom layer).

In this submission, a new sensing motif is introduced that utilizes positively charged pNIPAm-co-N-(3-Aminopropyl) methacrylamide hydrochloride (APMAH) microgel-based etalons coated with enzyme-responsive/reactive species to detect biomolecules; specifically mouse IgG in this submission. These experiments serve as a proof of concept for sensing other antigens in samples. As shown in Scheme 2, the immunoassay is performed by adding an excess amount of alkaline phosphatase-modified goat anti-mouse IgG (AP-GAM) (F(ab')2 fragment specific to mouse IgG) to mouse IgG in solution, and allowing them to interact for 30 min. After 30 min, the AP-GAM was isolated from the unbound, excess AP-GAM via addition of goat anti-mouse IgG (Fc fragment specific)-modified magnetic microparticles (GAM@M). After application of a magnetic field, the free, unbound AP-GAM could be isolated and exposed to the etalon. The etalons here were coated with a phosphate-containing polymer that is cleaved by AP-GAM and releases phosphate. The released phosphates are then available to diffuse into the microgel layer where they neutralize the charges on the microgels, which yields a shift in the position of the peaks in the etalon's reflectance spectrum. Finally, the shift in the reflectance peak can be correlated to the initial concentration of mouse IgG. While we use a reflectance spectrometer to probe the optical properties of the devices in this investigation, we are moving toward generating a hand-held device containing simplified optics (or even the light source/camera from a cellular phone) to probe the optical properties of the devices to make the measurements truly portable.

To accomplish this, pNIPAm-co-APMAH microgels were synthesized and used to generate etalons according to previously published protocol [26,27]. Briefly, NIPAm and APMAH were copolymerized at 70 °C, using ammonium persulfate (APS) as the initiator and N, N′-methylenebis(acrylamide) as the crosslinker. The approximate diameter of the prepared microgels was measured via dynamic light scattering (DLS) to be 1042 ± 9 nm. Transmission electron microscopy (TEM) was used to further characterize the microgels, and a representative image can be seen in Fig. 1. The resulting microgels were used to fabricate etalons as shown in the photograph in the insert of Fig. 1. To generate the phosphate-containing polymer, phosphoric acid 2-hydroxyethyl methacrylate ester (chemical structure shown in Fig. S1) was polymerized using APS as the initiator under N_2 atmosphere at 60 °C. Following synthesis, the polymer was purified via dialysis. The concentration of the solution after dialysis was 0.01 g/mL. The molecular weight of the synthesized polymer was measured by gel permeation chromatograph (GPC) to be 1.099×10^6 with a PDI 1.166. 200 μL of the polymer solution was then spin-coated onto the top layer of the etalon and allowed to dry. The spin-coating process was repeated another two times to make sure there was adequate polymer deposited on the top of Au surface. After deposition, the surface was rinsed with deionized water and soaked in PBS buffer solution. The thickness of resultant polymer layer was measured via ellipsometry to be 156 ± 4 nm. The deposition of the polymer layer is also evidenced by the XPS data (Fig. S2 and Fig. S3).

To perform the assay, 150 μL of GAM@M suspension, 270 μL of PBS buffer solution, 1.7 μL (dissolved in 28 μL PBS buffer) of AP-GAM were added into six separate centrifuge tubes. Then into these tubes, was added 0 μg (0 pmol), 0.1 μg (0.67 pmol), 0.2 μg (1.34 pmol), 0.3 μg (2.01 pmol), 0.4 μg (2.68 pmol) and 0.5 μg (3.35 pmol) mouse IgG, respectively. Each tube was then wrapped in aluminum foil and gently shook for 1 h at 37 °C. After 1 h, the magnetic beads were separated from the solutions by exposing each tube to an external magnet, which pulled the magnetic