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Novel surface plasmon resonance sensor for the detection of heme at biological levels via highly selective recognition by apo-hemoglobin ☆

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ABSTRACT

We have developed a novel surface plasmon resonance (SPR) biosensor for heme detection that utilizes the reconstitution of the heme cofactor with apohemoglobin (apoHb), hemoglobin from which the heme has been removed, as the sensing mechanism. The binding is highly specific, efficient and generated very strong SPR signals. This is the first report that uses immobilization of the apoprotein in a hydrophilic polymer matrix and senses the corresponding cofactor by SPR. This is also the first report of high sensitivity heme detection in real time by SPR and the sensing surface is re-generated many times without loss of sensitivity or selectivity. The sensing surface was fabricated by covalent immobilization of hemoglobin in a polyacrylic acid matrix in situ, which allowed for a high concentration of protein to be located in the plasmon detection range on the Au chip. Removal of the heme from the hemoglobin–polymer conjugate (Hb–PAA) resulted in a surface anchored apoHb–polymer conjugate. The limit of detection was approximately 2 μM or 1.30 μg/mL, which is relevant for biological heme levels (1–50 μM for hemolytic pathological conditions). This apoHb–polyacrylic acid system demonstrates a new concept in SPR detection with the use of protein cofactor binding pockets for analyte detection. The methodology that we developed here may be extended for the detection of a number of other cofactor molecules with high sensitivity, selectivity and low detection limits. In future, such sensors could be useful for the development of point-of-care devices to detect biologically important small molecules.

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1. Introduction

The growing importance of early detection presents a need for fast, inexpensive, rapid, disposable and sensitive biosensors that reach ultralow detection limits while maintaining high stability, selectivity and suitability for point-of-care use. Development of such devices allows for a widespread and preventative disease detection, epidemic control and treatment, especially in developing countries. For example, malaria plagues certain parts of the world which is partly due to the difficulty of providing prompt diagnosis, access to laboratory support and high cost of diagnosis [1]. Malaria diagnostics would greatly benefit from an immediate point of care diagnosis of this debilitating tropical disease, as the current gold standard test for malaria detection is microscopy of blood stain films to confirm the presence of malaria parasites by a trained pathologist [2]. An alternative method for malaria diagnosis can be the detection of elevated heme levels in blood serum. Initial diagnosis of malaria could greatly benefit from a quick, reliable, easy detection method, at low cost. Another motivation is to develop rapid, reliable detection of heme to investigate its role in other pathological conditions such as ischemia reperfusion, sickle cell disease and porphyria [3–5]. Biological elevated levels of heme during hemolytic processes are in the range of 1–50 μM [6–9]. Heme detection is also relevant for the investigation of a range of cellular biological functions such as cellular signaling [4].

Presently, methods exist for heme detection but require the use of toxic reagents, strict storage conditions or complex instrumentation, all of which do not relate well to reliable, low cost, high sensitivity, multiplexed, real-time testing [1,10].

Abbreviations: SPR, Surface plasmon resonance; apoHb, Apohemoglobin; Hb–PAA, Hemoglobin–polyacrylic acid conjugate; TNT, Trinitrotoluene; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NHS, N-hydroxysuccinimide; PAA, Polyacrylic acid; PEG, Polyethylene glycol; SAM, Self-assembled monolayer; μRIU, Micro refractive index unit; PBS, Phosphate buffered saline; HPLC, High performance liquid chromatography; MS, Mass spectrometry; ANS, 1-anilino-8-napthalene sulfonate

☆ *Novelty statement*: This is the first report that demonstrates a new concept in SPR detection with the use of protein cofactor binding pockets for heme detection. This is also the first report of high sensitivity heme detection in real time by SPR.

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For example, the most commonly used heme detection method, pyridine hemochrome, utilizes an excess of pyridine and heme in the reduced state to form a strongly absorbing hemochromogen complex [11]. The heme concentration is determined from the absorbance of the hemochromogen at 557 nm [9]. Spectrophotometric methods are limited by sample pureness and can require various separation requirements and also do not provide real-time, automated analysis.

SPR-based approach for heme sensing would provide a technique that offers rapid real time measurements and they could be translated to point-of-care detection [12,13]. SPR utilizes the surface plasmon wave generated when an intense beam of laser light passes through a prism and hits gold surface (Scheme 1A). The plasmon wave propagates ~300 nm above the gold surface. Therefore, when binding events occur at the Au surface, the refractive index changes and this in turn shifts the SPR resonance angle. SPR is a highly sensitive, real-time method for the detection of a variety of ligands and proteins. Use of SPR also voids the need for labeling of the analyte, such as fluorescent tags, which can increase the number of steps required to process samples. SPR is most commonly used in the biological field for studying protein–protein interactions, protein–antibody and ligand binding kinetics [14–18]. SPR is also used as an analytical sensing technique in food monitoring, medical diagnostics and environmental testing [17,19]. Most often, an antibody or aptamer is tethered to the Au surface and SPR signal is generated upon selective binding of an antigen or the pathogen, drug, hormone or a protein [20–22]. The SPR signal is dependent on the analyte molecular size, as well as concentration.

Surface plasmon resonance has been used to reach low levels of detection (attomolar) for important biomarkers such as prostate specific antigen [23] and carcinoembryonic antigen [24], both of which are larger molecular weight analytes. However, detection of small molecules having a molecular weight less than a few thousands by SPR is difficult due to the small change in refractive index upon binding to the surface-bound biorecognition element. Often, indirect competitive immunoassays are used to detect small molecules such as trinitrotoluene (TNT) and 2-hydroxybiphenyl [13,25]. In the case of TNT detection, an antibody specific to TNT was used in conjunction with TNT–ovalbumin conjugate as a surrogate ligand. In the absence of TNT, the antibody and protein bind and give rise to a fairly large SPR signal. Although, when TNT is present the binding event is blocked and the SPR signal is decreased. [25]. Many SPR assays utilize a "sandwich method" or extra macromolecule to enhance the signal obtained from small molecule binding events. Molecularly imprinted hydrogels have also been used as a method for the development of a high sensitivity glucose SPR sensor [26]. With small molecule detection by SPR, a post-binding amplification strategy is commonly employed to enhance the SPR signal. Examples of these include nanoparticles [27–30], secondary antibodies and catalyzed precipitation reactions [13,31,32], which are coupled to the analyte binding on the SPR chip.

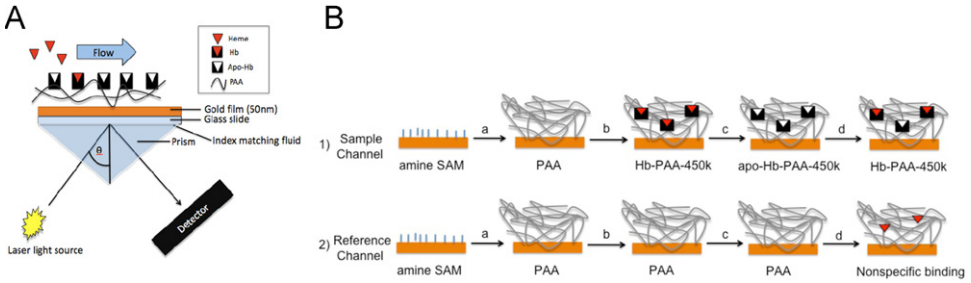
We recently demonstrated the stability and biological function of hemoglobin–polyacrylic acid (Hb–PAA) conjugates. The polymer and the protein are attached to each other randomly, which resulted in nanonetworks and these networks provided new opportunities for sensing applications. The use of PAA as an immobilization matrix for Hb is beneficial in providing multiple attachment points, improved mechanical stability and water retention within the protein–polymer film on the Au-chip surface. The use of PAA allows for a range of potential chemistries that can be used to alter PAA properties such as hydrophobic modification. We have previously shown that PAA covalently attached to Hb enhances thermal stability of Hb [33].

In this work, highly specific heme-binding pocket of apohemoglobin (apoHb) was chosen to develop the SPR sensor. The principle of detection is quite simple. It involves: (a) the removal of heme from Hb followed by, (b) exposure of the heme-depleted Hb (apoHb) to solutions of free heme, which resulted in the reconstitution of heme with apoHb, its natural host. This reconstitution event provides an excellent signal. Here, we show that the apoHb–PAA conjugate can be used effectively for the sensitive, selective and rapid detection of heme in aqueous solutions. This is the first report of SPR-detection of heme, where we take advantage of reversible heme binding to the apoHb–PAA conjugate.

2. Experimental

2.1. Materials and instrumentation

Poly(acrylic acid) (450 kDa), bovine hemoglobin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), hemin chloride, ethanolamine and acetone were obtained from Sigma-Aldrich and used as received. β-mercaptoethylamine hydrochloride and hydrogen peroxide were obtained from Fisher Scientific. Surface plasmon resonance (SPR) traces were recorded on an SR7000DC dual channel flow SPR spectrometer from Reichert Analytical Instruments (NY, USA) with a semi-automatic injection setup with 500 μL PEEK injection loop and Harvard Apparatus flow pump. The flow cell was setup with a 'Y' connector as to facilitate parallel flow through the sample and reference channels. The SPR data were collected at 25 °C. Phosphate buffered saline pH 7.4 (PBS, 0.1 M phosphate, 0.1 M NaCl, 2.7 mM KCl) was thoroughly degassed and used for all manipulations. Bare gold chips were obtained from Reichert (catalog #13206060). The data were analyzed using the Scrubber 2.0 software (BioLogic Software, Australia) followed by Kaleidagraph for plotting.



Scheme 1. (A) Cartoon of SPR sensor, (B 1) Sample Channel—(a) attachment of PAA to amine monolayer with EDC/NHS, (b) immobilization of Hb in PAA matrix, (c) removal of heme from Hb (sample channel only) and (d) injection of heme solution resulting in heme reconstitution (B 2) Reference Channel—(a) attachment of PAA to amine SAM with EDC/NHS, (b) channel blocked off, (c) acid/acetone wash, and (d) injection of heme solution to determine nonspecific binding [34–36].

2.2. Preparation of Au-amine sensor chip

The bare gold surface was functionalized with 100 mM β-mercaptoethylamine hydrochloride in ethanol/water (80/20 v/v). The chip was immersed in the solution, purged with nitrogen gas, sealed and allowed to sit overnight (12–18 h). The chip was then rinsed with ethanol and dried with N_2 gas. The aminothiol self-assembled monolayer covered Au chips were used immediately after fabrication [34].

2.3. SPR immunoassay procedure

The aminothiol functionalized Au chip was mounted in the Reichert SPR flow cell. PAA was attached to the amine-surface by flowing a mixture of PAA (13.9 mM COOH, 1 mg PAA/mL) EDC (100 mM) and NHS (40 mM) for 1800 s at a flow rate of 10 μL/min. The chip surface was rinsed to remove unbound PAA by flowing PBS (50 μL/min) until the baseline remained constant. The unreacted carboxylic acid groups were then activated with EDC (200 mM) and NHS (80 mM) for 1200 s at a flow rate of 10 μL/min. At this stage, the reference channel was blocked and Hb (500 μL of 100 μM in PBS pH 7.4) solution was passed over the activated PAA surface for 1800 s at a flow rate of 10 μL/min. Weakly bound Hb was washed from the chip surface using PBS at a flow rate of 50 μL/min until the baseline was constant. The reference channel was then reopened. A solution of ethanolamine (1 M, pH 8.5) was then injected through both channels for 1200 s at 10 μL/min to block any remaining activated carboxylic acid groups. An acid/acetone wash (50/50 v/v, pH 2) was injected onto the sensor surface for 300 s at a flow rate of 40 μL/min to remove the heme from the Hb–PAA conjugate anchored to the chip surface. The SPR signal was monitored to follow the progress of each step. Hemin chloride standard solutions were prepared by the dilution of a stock solution (800 μM in PBS pH 8) of hemin chloride with PBS at pH 7.4. SPR signals were then monitored as various concentrations of heme were flowed over the sensor surface. Each hemin injection was for 300 s at a flow rate of 40 μL/min. Regeneration of the sensor surface between each heme binding injection was done by flowing acid/acetone for 300 s at 40 μL/min to yield apoHb–PAA.

3. Results

The SPR setup cartoon (Scheme 1A) depicts how the gold surface is mounted on the prism in the Kretschmann configuration of the Reichert SPR spectrophotometer. The disposable gold surface is mounted on the prism with index matching fluid at the glass–prism interface. As the light penetrates the prism and reflected from the Au surface, the free moving electrons of the Au film generate a surface plasmon wave. The evanescent wave propagates for up to ~300 nm above the Au surface. As binding events occur on the surface, the refractive index of the medium is changed, which in turn alters the intensity of the reflected light and generates changes in SPR signal.

The protocol for the heme assay is shown schematically in Scheme 1B. The Au chip is coated with a thiolalkylamine monolayer to provide anchoring functionalities for the PAA chains. Then PAA is attached by injection of PAA/EDC/NHS solution to facilitate carbodiimide coupling of the PAA to the amine SAM via the COOH groups of PAA. The reference channel is then blocked, and Hb is immobilized only in the sample channel. Hb is attached to the PAA COOHs via its lysine side chain amino groups, facilitated by EDC coupling. After synthesis of Hb–PAA, unbound Hb is flushed with buffer, and the heme is removed from Hb–PAA by an acidic acetone wash to prepare the heme-binding pocket for analyte detection. Solutions of increasing concentration of heme in PBS were injected to determine the SPR responses.

3.1. Preparation of polymer–Hb conjugate sensing surface

The Au-surface of the sensor chip was amine functionalized overnight in an ethanolic solution of thiolalkylamine to produce the self-assembled monolayer (SAM). The sensor was rinsed, dried under nitrogen and mounted in the SPR spectrometer, and PBS was allowed to flow over the sensor until a steady baseline has been obtained. The EDC/NHS/PAA solution mixture was freshly prepared. Upon injection of the PAA/EDC/NHS solution over the amine coated Au surface (Fig. 1a), a large signal was observed. The amount of PAA attached to the chip surface was determined by the increase in the SPR response after buffer rinse, (Fig. 1b). The COOH groups of PAA were then activated by a second aliquot of EDC/NHS to ensure that most of the COOHs would be available for Hb attachment (Fig. 1c) followed by buffer rinse (Fig. 1d). To obtain quantitative information about nonspecific heme binding to PAA, the reference channel was blocked off after PAA attachment. Hemoglobin was then immobilized in the sample channel by the reaction of the EDC activated COOHs of PAA to the lysine side chains of Hb followed by buffer rinse (Fig. 1e). Hb immobilization was directly monitored by the large increase in SPR signal in the sample channel. In this case, a large amount of Hb (~3000 μRIU) has been attached to the sensor surface. The reference channel was unblocked and ethanolamine was injected through both channels to deactivate all remaining activated COOH groups.

3.2. ApoHb–PAA SPR assay

The apoHb–PAA sensor responded favorably to various heme concentrations by exploiting the reversible, yet highly specific binding of heme by the heme binding pocket of apo-Hb.

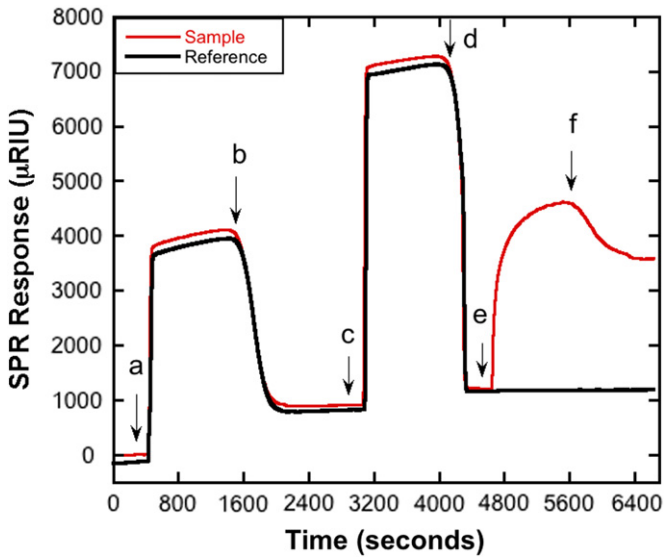


Fig. 1. Sensor construction and real time monitoring of PAA attachment and hemoglobin immobilization on the Au surface. The entire SPR run was performed in PBS buffer pH 7.4 at 25 °C. (a) injection of PAA at 10 μL/min, (b) end of injection/PBS rinse, (c) injection of EDC/NHS at 10 μL/min, (d) end of injection/PBS rinse, (e) injection of Hb into sample channel at 10 μL/min, while the reference channel was blocked off and (f) end of injection, PBS rinse of both channels. The red line represents the signal changes in the sample channel while the black line is the response from the reference channel. Arrows represent the time of specific injection points or end of injection. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The prosthetic group, heme, is easily removed by flowing an acidic acetone wash over the Hb–PAA conjugate for 300 s and rinsing with buffer, the sample channel showed a loss in signal while the reference channel which contained only the PAA, promptly returned to the original baseline. This observation clearly shows that the loss of signal in the sample channel but not in the reference channel is due to the removal of heme from Hb–PAA conjugate in the sample channel.

The sensing capability of the conjugate was tested by injecting heme solutions of increasing concentrations, while recording the SPR signals. The reference channel was used to monitor nonspecific binding of heme to PAA, while the sample channel would generate signal due to heme binding to the apoHb–PAA matrix. Therefore, subtraction of the reference channel signal from that of the sample channel provided a direct measure of heme concentration. Solutions of 2.5–30 μM hemin chloride in PBS pH 8.0 were injected for 300 s over the sensor surface at 40 μL/min flow rate and after each heme injection, the sensor surface has been regenerated for the next round by washing with acidic acetone. Signal due to the injection of 20 μM heme is shown in (Fig. 2a and b) which was followed by surface regeneration/heme removal (Fig. 2c and d). The arrow labeled (a) shows the point where heme began flowing over the sample and reference channels. The reference signal (black line) shows a slight increase due to weak, non-specific binding to PAA while the sample signal (red line) shows a rapid increase due to heme binding to the apoHb–PAA. After heme injection followed by buffer flow (b), the slight loss of signal is due to changes by the bulk solvent flowing over the sensor. The sample signal remained constant until the injection of acidic acetone to remove the trapped heme.

The resulting binding curves were analyzed with Scrubber software to align data from multiple injections, subtract the signals from the reference channel (bulk solvent effects and nonspecific binding). Fig. 3 shows the resultant curves that show solely the signal due to heme binding to apo-Hb–PAA on the sensor surface at 5 different concentrations of heme.

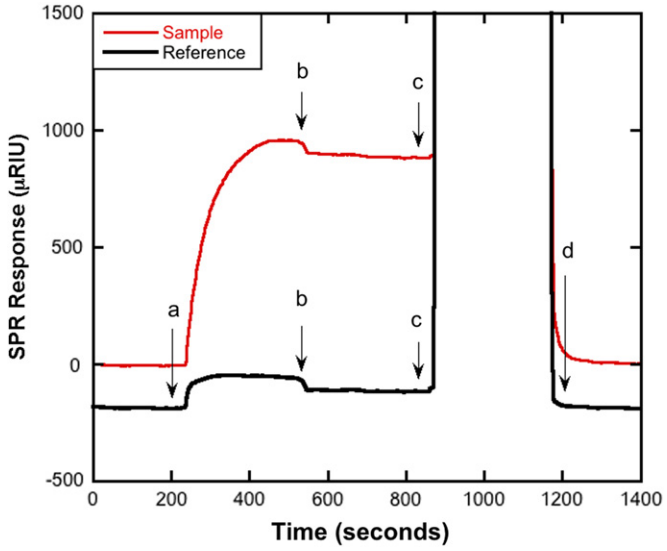


Fig. 2. Typical sensorgram illustrating the binding and regeneration properties of the Hb–PAA sensor surface. (a) Heme injection (20 μM) at 40 μl/min for 300 s, (b) end of injection/PBS rinse, (c) injection of acidic acetone wash at 40 μl/min for 300 s to regenerate apoHb, and (d) end of injection/PBS rinse. The red line represents the signal from the sample channel while the black line is the response from the reference channel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

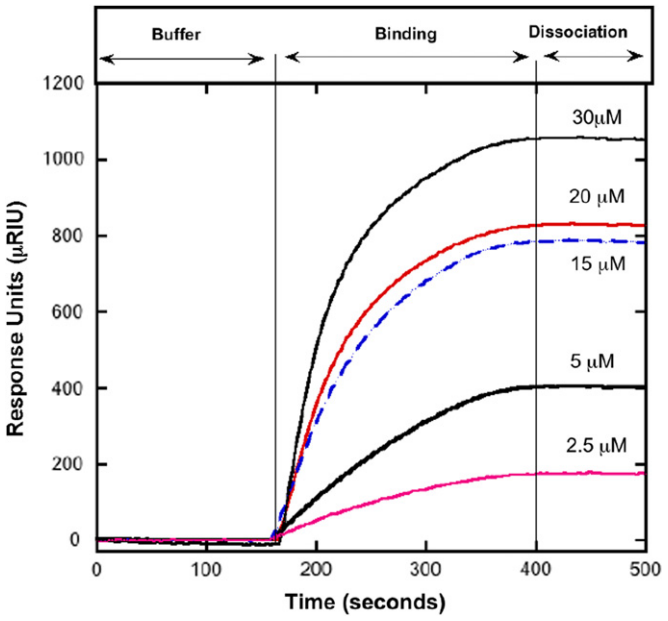


Fig. 3. Real-time responses for the binding of increasing concentrations (2.5–30 μM) of heme to apoHb–PAA conjugate immobilized on the sensor surface. The data were not smoothed. The SPR curves from the reference channel have been subtracted (as indicated) from those of the sample channel, and the curves have been aligned for best visual impact.

3.3. Sensing capability

A linear calibration plot (Fig. 4B) was constructed from SPR signals induced by the exposure of the apoHb–PAA sensor to heme solutions. Hemin stock solutions (25–300 μM) were prepared in 20 mM NaOH and diluted 10 × with PBS pH 7.4 prior to use. An extinction coefficient of $2.0 \times 10^4\ cm^{-1}\ M^{-1}$ at 368 nm for hemin in 20 mM NaOH diluted with PBS pH 7.4 was used. This value differs only slightly from previous values calculated in 100 mM NaOH [37].

4. Discussion

4.1. Au surface preparation and polymer attachment

Many sensors are limited by the use of highly specific and costly sensing surface layers. Typical commercial functionalized chips run for ~$50–200, with most sensors being developed with COOH/PEG SAMs leading to limited availability of commercially sold amine functionalized surfaces. Our ability to cheaply prefunctionalize the Au surface with β-mercaptoethylamine hydrochloride greatly lowers the cost per SPR run [34,38]. Our methodology utilizes facile, quick and routine self-assembly on bare Au chips while using simple chemistry allows the preparation of reliable sensor surfaces, in a reproducible manner. We have shown that PAA can be easily attached in situ to the amine SAM. Also, in our strategy, a long chain thiol linker (often expensive) is not needed because the PAA matrix successfully blocks the Au surface from nonspecific ligand binding. Approximately, 500–1000 μRIU of PAA was attached each time onto the Au surface, throughout the optimization of the assay procedure. This amount of PAA led to in situ covalent attachment of Hb in significant amounts. The mass of hemoglobin attached to the sensor surface was determined by comparing the baseline before the injection of Hb to the baseline after the return to buffer flow to remove unbound Hb and using the standard conversion of RIU to protein concentrations. This value consistently was

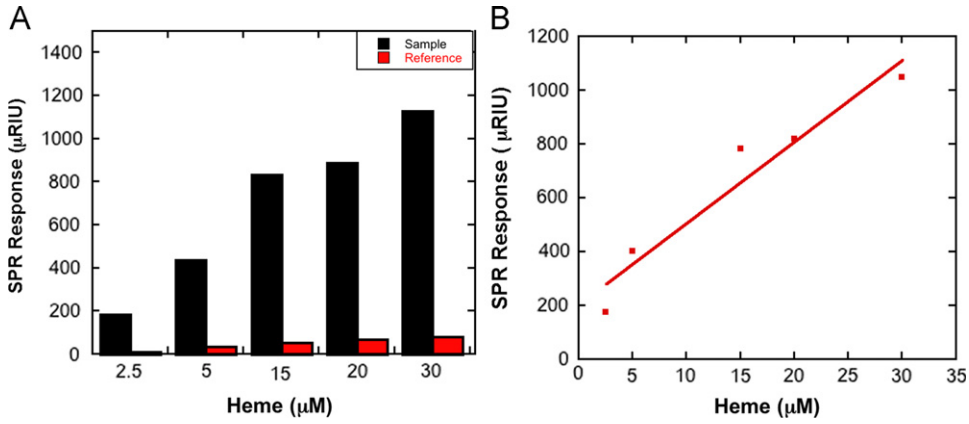


Fig. 4. (A) SPR responses in sample and reference channel for 5 heme concentrations injected over the same apoHb–PAA surface. (B) SPR calibration curve for SPR signals versus heme concentration (μM) for the same apoHb–PAA surface. Each heme response has been corrected for nonspecific binding and solvent effects by subtracting the SPR response from the reference channel (PAA only) (R^2=0.966).

~3000 μRIU or 2190 ng/mm^2 (1 μRIU=0.73 ng/mm^2) [39]. The alternative method of direct attachment of previously prepared Hb–PAA to the Au surface proved to have limitations due to the precipitation of the sample over time [33]. Current method of step-wise attachment of Hb to the sensor surface as shown in Scheme 1B could be further explored for ex situ attachment such as in a multiplexing imaging SPR array setup.

4.2. Highly specific heme binding pocket

Upon synthesis of the Hb–PAA conjugate, an acidic acetone solution was used to initially remove the heme cofactors to form the apo Hb–PAA conjugate. We found that this approach of flowing acidic acetone solution to create apoHb–PAA is reliable and can be repeatedly used to regenerate the surface [40]. Successful regeneration was confirmed by comparing the baseline signal before and after the heme injection into the heme-sensor chip. Heme-binding to the regenerated chip was consistent from run to run, and regeneration must quantitatively remove all heme bound after each analyte injection as to have a fresh surface for the next analyte injection. An acidic aqueous acetone solution (50% acetone, pH adjusted to 2) gave the best recovery of the baseline or total removal of bound heme, which is consistent with reported heme/Hb extraction procedures [35]. The acidic acetone solution also had no effect on the PAA reference channel signal.

The empty heme binding pocket in the apoHb–PAA was then used as the recognition element for highly specific heme sensing. Heme solutions, varying from 2.5–30 μM, were injected over both the channels for 300 s while monitoring the SPR signal in real time. The binding events showed a good association curve and plateaued within 200 s. As shown by the sensorgram in Fig. 2, the sample channel (red line) showed a rapidly increasing binding curve upon injection of heme (indicated by arrow labeled a). Binding of heme to apoHb is clear when compared to the response curve from the reference channel which contained only PAA (black line). Once heme binds to the pocket in the globin, the pocket rapidly collapses and allows for reorientation and formation of the iron histidine bond [41]. The binding of heme to the apoHb–PAA conjugate was extremely strong as shown by the lack of its dissociation, subsequent to the binding event, in real time (Fig. 2, red line after arrow labeled b). Upon return to PBS flow, the SPR signal remained constant and did not show any dissociation from the sensor surface (Fig. 3). This is consistent with the reported very low equilibrium dissociation constant 10^{-12}–10^{-15} M for heme dissociating from hemoglobin [41].

4.3. Sensor detection limits and reproducibility

In comparison to other heme-based sensing methods, SPR detection by reconstitution can provide a quick, reliable measurement, alleviating the need for various sample preparation steps or harmful reagents, and this approach can be readily extended for multichannel sensing in an array. In addition to malaria detection, quantification of blood plasma heme levels (1–50 μM) is useful in various other clinical efforts [5]. For example, in cardiovascular research, the pyridine hemochromogen is still used to determine heme levels after administration of hemin to hypertensive rats. The pyridine hemochromogen method has detection limits in the range of approximately 3–30 μM [42]. Relevant levels of heme in blood plasma and serum of hypertensive blood pressure range from 1–30 μM [9] which are within the range of heme concentrations tested with this SPR based sensor. The current limit of detection for this SPR biosensor is 2 μM or 1.30 μg/mL. Other detection methods, for example mass spectrometry (0.4 μM) and chemiluminescence (0.4 μM), have advantages such as high throughput and lower detection limits [43,44]. This SPR sensor has the advantage of a highly selective binding pocket. The pocket has precisely placed amino acid residues that facilitate the electrostatic, hydrophobic and hydrogen bonding with the heme cofactor. Though few other small molecules may bind in the pocket, their lower binding constants allow heme to displace them [45]. This was investigated by monitoring the SPR signal from the binding of 1-anilino-8-napthalene sulfonate (ANS) when injected with heme and by itself. Fig. S1 (Supplementary materials S1) shows the lack of SPR binding response from ANS as well as from an injection of ANS and heme.

This SPR sensor can be converted into a high throughput device when used in a multiplexed array for the detection of multiple analytes, simultaneously. The detection limit of this SPR sensor, while comparable to HPLC and MS analyses, is well-suited for current clinical needs.

We also tested the reproducibility of the data by repeated sensor regeneration followed by the injection of heme solutions, each concentration multiple times, in different sequences. The corresponding data are shown in the supplementary information. For example, a total of 12 different samples of four different concentrations, three samples at each heme concentration, were injected over the sensor surface, after regeneration of the sensing surface, and the reproducibility has been checked using multiple strategies (Figs. S2–S7). The conclusions from these data are: (1) regeneration of the sensor surface is highly reproducible and efficient and (2) signals observed with specific

heme concentrations are reproducible, independent of the order of injection. Therefore, the sensor developed here is reliable for several measurements.

The current method using the novel protein–polymer conjugates can be further expanded for the detection of other cofactors of proteins and enzymes, and therefore, this is a general strategy that can be applied for the detection of a number of key biological agents. Additionally, SPR imaging with multiplexing arrays [46], amplification labels [47] and nanoparticles can be used to further enhance the detection limits and sensitivity of this technique.

5. Conclusions

This is the first report of using Hb–PAA protein–polymer conjugate for SPR based sensing of heme via prosthetic group reconstitution. SPR has been previously used in conjunction with heme reconstitution, although the heme group was anchored by DNA conjugation to the surface and the kinetics of apoprotein binding were determined [36]. Other polymers, carboxymethyl modified dextran [48] hyaluronic acid [38], have been used on SPR surfaces to lower nonspecific binding and increase the concentration of the biorecognition element. The stability of the Hb–PAA conjugate allows for good reusability of the sensing surface as demonstrated by repeated regeneration cycles, at least 12 times. These conditions allow for a quick reconstitution time of 5 min and regeneration for reuse of the sensor. The current detection limit of 2 μM heme corresponds to the relevant biological levels in malaria and blood pressure studies [9]. This sensing method has the potential to impact the current method by which heme serum levels are determined. It also introduces a new concept in SPR detection by demonstrating the functional use of protein cofactor binding pockets.

Supplementary information available

Injection of ANS and multiple injections of a series of concentrations of heme solutions in different sequences are presented (S1–S7).

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.026.

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