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Protein detection using hydrogel-based molecularly imprinted polymers integrated with dual polarisation interferometry

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ABSTRACT

A polyacrylamide-based molecularly imprinted polymer (MIP) was prepared for bovine haemoglobin (BHb). A 3 mg/ml solution of BHb was injected over a dual polarisation interferometer (DPI) sensor to form a physisorbed layer typically of 3.5 ± 0.5 nm thickness. Onto the pre-adsorbed protein layer, MIP and NIP (non-imprinted polymer) were separately injected to monitor the interaction of BHb MIP or NIP particles under different loading conditions with the pre-adsorbed protein layer. In the case of NIP flowing of the protein layer, there was negligible surface stripping of the pre-adsorbed protein. When a protein-eluted sample of MIP particles was flowed over a pre-adsorbed protein layer on the sensor chip, the sensor detected significant decreases in both layer thickness and mass, suggestive that protein was being selectively bound to MIP after being stripped-off from the sensor surface. We also integrated thin-film MIPS for BHb and BSA onto the DPI sensor surface and were able to show that whereas BHb bound selectively and strongly to the BHb MIP thin film (resulting in a sustained increase in thickness and mass), the BHb protein only demonstrated transient and reversible binding on the BSA MIP. MIPs were also tested after biofouling with plasma or serum at various dilutions. We found that serum at 1/100 dilution allowed the MIP to still function selectively. This is the first demonstration of MIPs being integrated with DPI in the development of synthetic receptor-based optical protein sensors.

1. Introduction

Molecularly imprinted polymers (MIPs) continue to receive much attention in research effort as they promise (and in some cases are delivering) synthetic materials capable of mimicking the selective binding function of antibodies and enzymes. The implications for such biomimicry are immense in the field of biosensor development and novel drug delivery modes. Over the past decade, there has been an exponential increase in research activity in developing hydrogel-based molecularly imprinted polymers (HydroMIPs) for the imprinting of proteins [1]. Hydrogels are insoluble, crosslinked polymer network structures composed of hydrophilic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water [2]. Monomers that have commonly been used for non-covalent molecular imprinted hydrogels are generally chosen on their ability to form weak hydrogen bonds between the monomer and the template [2–4]. Polyacrylamide hydrogels are known to be very inert, offer hydrogen bonding capabilities, and are biocompatible. For these reasons, acrylamide has been commonly used for molecular imprinting [5–9].

The use of optical sensor platforms in conjunction with imprinted polymers has been recently reported, primarily detailing the use of SPR [10] and quantum dots/array technologies [11,12]. Both applications have been reviewed in depth by Al-Kindy et al. [13].

Interferometric sensors based on dual polarisation interferometry (DPI) can be used for biological detection and the sensing is accomplished by directly monitoring a bioconjugate reaction occurring within an evanescent field extending out from the interferometer's sensing channel [14]. There are no subsequent steps, which are typical in many other sensing schemes, in which a second bioconjugate is reacted with the first to produce a sandwich complex.

The optical analytical technique has been designed specifically for the study of thin films, which uses electromagnetic evanescent wave probes to characterise the film above a planar waveguide surface. By including an optical bridge in the form of a buried reference waveguide, the sensitivity of thin film measurements is maximised with the resolutions determined by the interaction length between thin film and the evanescent field [15].

The measurement principle involves the use of a HeNe laser light (λ = 632.8 nm) source coupled to the end facet of a silicon substrate

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and a ferroelectric liquid crystal halfwave plate switches the plane of polarisation of the input beam between Transverse Electric (TE) and Transverse Magnetic (TM) at frequencies of typically 50 Hz. At the output, interference fringes in the far-field form on a digital camera screen, with the fringes being representative of the relative phase position of the sensing and reference light paths at the output. Any thin film changes thereof on the sensor surface of the waveguide will interact with its evanescent field and change its effective refractive index (RI). Such changes will move the phase of the light exiting the sensing waveguide, and the position of the fringes on the camera will move.

The phase positions refer to the two orthogonal polarisations (TE and TM) that are measured by the instrument. The absolute effective index of a waveguide mode is found by solving Maxwell's equations of electromagnetism for a system of uniform multiple dielectric layers in which the fields in the semi-infinite bounding layers are exponentially decaying solutions. The parameters required are the RI and thickness of each layer for each of two polarisations. Provided the input information is complete, an effective index value is obtained which is representative of the distribution of optical power amongst the layers. If a new layer is introduced to (or removed from) the system, it will alter the effective index. For each of the two polarisations, the new effective index can satisfy a continuous range of thickness and refractive index values. However, there will only be one unique combination that satisfies the index of the two polarisations.

Using different evanescent field profiles, various characteristics of the thin-film can be resolved. Different polarisations are used to resolve the optical density and the thin film thickness simultaneously at resolutions of <1 pg/mm^2 and <10 pm [16]. If a new layer is introduced to (or removed from) the system, it will alter the effective index. For each of the two polarisations, the new effective index can satisfy a continuous range of thickness and refractive index values. However, there will only be one unique combination that satisfies the index of the two polarisations.

The technique allows the precise behaviour of layers to be determined in terms of both their density (absolute RI) and thickness in real time and therefore, mass, surface coverage and concentration can be calculated. Integral to this is the calibration of both the sensor chip and bulk refractive index, which allows the accurate derivation of the data, and takes into account subtle changes film parameters and variations in chip structure which may give rise to errors in sensitivity. As a result, the DPI technology presents the opportunity to further provide a rapid method for the characterisation and quantification of molecular binding events.

To date, work using dual polarisation interferometry has been focused on investigations into the studying nucleic acid interactions [17], DNA immobilisation [18], antibody–antigen interactions [19], protein characterisation [20–23], and polymer characterisation [24–26]. Herein is the first report of DPI to characterise HydroMIPs and develop thin film HydroMIP-based protein sensors.

2. Materials and methods

Acrylamide, glacial acetic acid (AcOH), ammonium persulfate (APS), N,N′-methylenebisacrylamide, ethanol (EtOH), sodium dodecyl sulphate (SDS), N,N,N′,N′-tetramethylethyldiamine (TEMED), phosphate buffered saline (PBS), succinic acid, tris base, and tris hydrochloric acid were purchased from Sigma–Aldrich. Bovine haemoglobin (BHb) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. Unmodified silicon oxynitride sensor chips were purchased from Farfield Sensors (Crewe, UK) and a programmable syringe pump (PHD 2000) was purchased from Harvard Apparatus (Holliston, MA, USA). Plastic syringes (1 ml and 5 ml with Leur Lock fittings) were purchased from Becton Dickinson UK Ltd. (Oxford, UK). Pooled plasma and serum samples were used in the biocompatibility studies.

2.1. Preparation of solutions

A solution of 10% (w/v) AcOH:SDS was prepared for use in the wash stages before and after the reloading stage of the rebinding studies. SDS (10 g) was dissolved in 90 ml of MilliQ water. 10 ml of AcOH was added and mixed thoroughly using a magnetic stirrer. A 0.3 mg/ml stock solution of BHb in RO water was prepared, as were SDS solutions to give final percentages of 10%, 5%, 2%, 0.5%, 0.1% and 0.05% (w/v). A reverse osmosis (RO) water stock solution was degassed under vacuum (with stirring) for 10 min, as was an 80% (w/w) solution of ethanol (EtOH) in degassed RO water. A stock solution of bovine haemoglobin (BHb) template solution was prepared in MilliQ water.

2.2. HydroMIP production

Molecularly imprinted (MI) hydrogels were produced using our optimised methodology [5]. It was shown that when imprinting BHb using bulk polyacrylamide hydrogels, a 10% crosslinked polyacrylamide/N,N′-methylenebisacrylamide hydrogel produced the optimal imprint for BHb in terms of specificity and rebinding efficiency of the MIP compared to the non-imprinted polymer (NIP). Hawkins et al. [5] also demonstrated that using a 10% AcOH:SDS during the elution stage of the rebinding studies performed resulted in optimal protein recovery for BHb specific MIPs.

Therefore, MI hydrogels were produced as follows for 1 ml of gel: 54 mg of functional monomer (acrylamide), 6 mg of crosslinker (N,N′-methylenebisacrylamide), and 12 mg of template protein were all dissolved in PBS or MilliQ water and added together to create the MIP solution. 20 μl of a 10% (w/v) ammonium persulfate (APS) solution was added to the MIP solution, and the solution was purged with nitrogen for 5 min. Once the solution was degassed, 20 μl of a 5% (v/v) N,N,N′,N′-tetramethylethyldiamine (TEMED) was added and the solution was then left to polymerise overnight at room temperature. We looked at freshly prepared MIP following granulation and water washing (hereafter, referred to as MIP 1). A sample of MIP 1 was washed with SDS and acetic acid followed by a further water wash to produce sample MIP 2. MIP 3 was the sample produced after MIP 2 was reloaded with target protein (bovine haemoglobin).

For every BHb MIP created a NIP was also created using the same material concentration as the MIP but without the protein template in order to serve as a control. BSA MIPs were also prepared using 12 mg of BSA template instead of BHb. The BSA MIPs were used as control polymers for BHb binding to be compared against BHb MIPs.

2.3. Dual polarisation interferometry AnaLight® Bio200 set-up

All DPI experiments were performed on the Farfield AnaLight® Bio200 instrument, which had been installed and internally calibrated by the manufacturer. The AnaLight® Bio200 instrument provides a flexible platform that incorporates a modular fluidics arrangement to enable a wide range of experimentation to be undertaken. Integral to all experimentation is the sensor chip used to exploit the technology. The sensor is a multiplayer deposited waveguide structure on a silicon wafer, and is manufactured to a high tolerance to enable accurate measurement to take place. Opening windows in the top cladding of the waveguide define the two active areas on the chip, which also define the active path length of the sensor and are lithographically produced to micron levels of precision. The precise sensitivity is dependant on the waveguide thickness and refractive index. Many variations