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Protein Crystallization and Biosensor Applications of Hydrogel-Based Molecularly Imprinted Polymers

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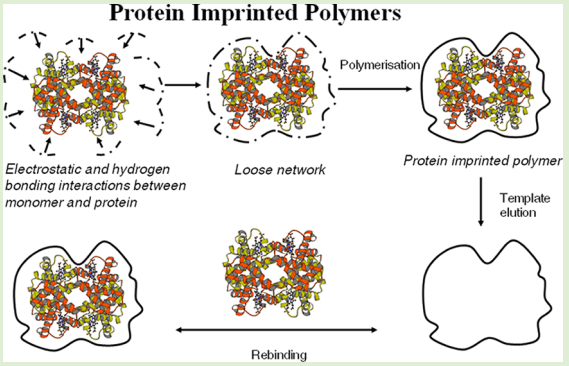
ABSTRACT: We have characterized the imprinting capability of a family of acrylamide polymer-based molecularly imprinted polymers (MIPs) for bovine hemoglobin (BHb) and trypsin (Tryp) using spectrophotometric and quartz crystal microbalance (QCM) sensor techniques. Bulk gel characterization on acrylamide (AA), *N*-hydroxymethylacrylamide (NHMA), and *N*-isopropylacrylamide (NiPAM) gave varied selectivities when compared with nonimprinted polymers. We have also harnessed the ability of the MIPs to facilitate protein crystallization as a means of evaluating their selectivity for cognate and noncognate proteins. Crystallization trials indicated improved crystal formation in the order NiPAM < AA < NHMA. QCM studies of thin film MIPs confirm this trend with *N*-hydroxymethyl acrylamide MIPs exhibiting best discrimination between MIP and NIP and also cognate/noncognate protein loading. Equivalent results for acrylamide MIPs suggested that the cavities were equally selective for both proteins, while *N*-isopropylacrylamide MIPs were not selective for either cognate BHb or noncognate BSA. All BHb MIP-QCM sensors based on AA, NHMA, or NiPAM were essentially nonresponsive to smaller, noncognate proteins. Protein crystallization studies validated the hydrophilic efficacy of MIPS indicated in the QCM studies.



1. INTRODUCTION

Molecular imprinting is an established technique for producing molecularly selective sites within polymer matrices during polymerization.[1−3] The template molecule is intimately present during the polymerization process, held there by weak intermolecular forces such as van der Waals and hydrogen bonding. Postpolymerization, the template molecule is therefore physically entrapped within the voids of the cross-linked polymer. Removal of the template molecule leaves ghost-sites within the polymer that are highly selective for the rebinding of the template molecule when compared with molecules of a similar shape and size.[3] Typically, antibodies exhibit a dissociation constant (K_d) of 17 pM.[4] Recently, there have been reports of MIPs demonstrating apparent dissociation constants for protein binding of a similar magnitude to antibodies, namely, for the peptide mellitin[5] (25 pM).

To date, molecular imprinting has been effectively used for the small molecules such as drugs, pesticides, and explosive markers.[4−6] More recently, there has been a strong surge of interest in the ability to imprint biological molecules such as proteins, DNA, and viruses.[7−10] The crucial difference in the technique between imprinting small molecules and large metastable biologicals is in the choice of monomers and solvent for MIP synthesis. Whereas, small molecules are relatively stable in an organic solvent, biomolecules such as proteins will destabilize and denature under such conditions. Therefore, to imprint for biologicals, much effort has been invested in studying water-based polymer systems, namely, hydrogels.[8−11] Hydrogel-based molecularly imprinted polymers (HydroMIPs) have predominately been based on cross-linked polyacrylamide.[9,10] Such polymer networks have shown high selectivity for template proteins when compared with a nonimprinted control polymer (NIP). However, the real acid-test in selectivity is in testing the HydroMIP against other (noncognate) proteins.

More recently, we reported the first application of molecularly imprinted polymers to facilitate protein crystallization.[12] Proteins being the major machinery of life are often the targets of drugs; their functions are determined by their three-dimensional structure, hence, detailed understanding of protein structure is essential for rational design of therapeutic treatments. The most powerful method for determining the structure of proteins is X-ray crystallography, which is totally reliant on the availability of high quality crystals. With the advent of genomics/proteomics and, hence, the exponentially increasing number of proteins that can be identified as potential

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drug targets, crystallization of proteins is becoming increasingly important.[12,13]

The ultimate means to obtain good crystals is to control their conception stage, that is, the nucleation stage, which is the first step that determines the entire crystallization process.[13] Several substances such as minerals, human hair, silicas, and other materials have been used as nucleants, but being random materials with no special affinity for proteins, they have had limited success. MIPS on the other hand have specific affinity for proteins and are therefore proving to be effective in inducing crystals of proteins that were previously difficult to crystallize.[12]

The results of the protein crystallization studies can be used to inform on the use of the HydroMIP thin films for general protein recognition applications (e.g., protein extraction and protein biosensing). In this paper we show that nucleation studies can allow an interesting route to the feedback of essential information regarding whether parameters such as cross-linking density and choice of monomers can lead to better selectivity. We present data on the effect of hydrophilicity of functionalized acrylamide monomers on MIP selectivity. This in turn has the potential to help us better understand the polymer parameters crucial for development and optimization of HydroMIP-based protein biosensors. The quartz crystal microbalance (QCM) has progressively become a useful tool in the arsenal of instruments used by the analyst primarily due to its ability to give information regarding thin film mass deposition on the surface as well as changes in viscoelasticty of preadsorbed layers by measuring resonant parameters such as the series resonance frequency and crystal impedance. The theory and application of the QCM in the liquid phase as a mass and viscoelastic sensor has been extensively reviewed.[14,15] Its application to biosensing of diagnostic markers such as proteins, viruses, and DNA through biomodification with biorecognition entities such as antibodies and DNA probes has opened up many diagnostic possibilities.[16,17] Biosensors for proteins are currently expensive to develop because they require the use of expensive antibodies. HydroMIPs offer the promise of a viable synthetic receptor technology and the replacement of, for example, antibodies in the development of new diagnostic assays for biomarkers[18,19] and in the development of biosensors for analytes of significance in medicine, food, and the environment including antibiowarfare sensors.[20,21] Because of their biomimicry capabilities (and their potential to act as synthetic antibodies), HydroMIPs potentially offer a route to the development of new low-cost biosensors. We demonstrate also in this paper the application of the QCM technique to distinguish between the behavior of MIPs and NIPs in the presence of cognate and noncognate proteins.

2. EXPERIMENTAL SECTION

2.1. Materials. Acrylamide, *N*-hydroxymethylacrylamide (NHMA), *N*-isopropylacrylamide (NiPAm), *N*,*N*-methylenebisacrylamide (bis-acrylamide), ammonium persulphate (APS), *N*,*N*,*N*,*N*-tetramethylethyldiamine (TEMED), sodium dodecyl-sulfate (SDS), glacial acetic acid (AcOH), Tris base, Tris HCl, phosphate-buffered saline (PBS) tablets (137 mM L^{-1} NaCl; 27 mM L^{-1} KCl; 10 mM L^{-1} Na_2HPO_4; 1.76 mM L^{-1} KH_2PO_4), succinic acid, bovine hemoglobin (BHb), bovine serum albumin (BSA), bovine liver catalase (BCat), and equine heart myoglobin (EMb) were all purchased from Sigma-Aldrich, Poole, Dorset, U.K. Sieves (75 μm) were purchased from Endecotts Ltd. and Inoxia Ltd., U.K. Quartz crystal microbalance crystals (9 MHz fundamental resonance) with gold-on-chrome electrodes were supplied by Nihon Dempa Kogyo Company Ltd. (Tokyo, Japan) and kindly donated from Dr Aizawa's laboratory (AIST, Tsukuba, Japan).

Hen egg-white lysozyme (L 7651), thaumatin from *Thaumatococcus danielii* (T 7638), bovine pancreatic trypsin (T 4665), and bovine hemoglobin (H 2500) were obtained from Sigma-Aldrich. The Human Macrophage Migration Inhibitor Factor (MIF) was provided by G. Crichlow of Yale University and produced as described in ref 22. The 24-well crystallization Linbro trays and siliconized glass coverslips were obtained from Molecular Dimensions Ltd., U.K. All the crystallization agents, buffers, and chemicals were purchased from Sigma- Aldrich.

2.2. Methods. *2.2.1. HydroMIP Preparations.* Acrylamide, NHMA, or NiPAm were used as functional monomers. Each functional monomer (54 mg) was dissolved in 800 μL of Milli-Q water to which was added bis-acrylamide cross-linker (6 mg), 20 μL of a 10% (w/v) ammonium persulfate (APS) solution and template protein (12 mg); the final volume was made up to 980 μL using Milli-Q water to create each mononmer solution. The solution was purged with nitrogen for 5 min and 20 μL of a 5% (v/v) *N*,*N*,*N*′,*N*′-tetramethylethyldiamine (TEMED) was added to catalyze the polymerization process. Polymerization was allowed to occur overnight at room temperature giving a final cross-linking density of 10%.

For every HydroMIP created, a HydroNIP control was prepared in an identical manner for each of acrylamide, NHMA, or NiPAm, but in the absence of protein. MIP and NIP gels based on acrylamide, NHMA, or NiPAm were prepared for trypsin and hemoglobin.

After polymerization, the MIP gels formed were conditioned using the following technique. The MIPs were granulated separately using a 75 μm sieve. The crushed gels were transferred into 1.5 mL centrifuge eppendorf tubes and conditioned by conducting a stringent washing procedure on the gels, washing the gels with five 1 mL volumes of either Milli-Q water followed by five 1 mL volumes of 10% AcOH/SDS eluant (pH 2.8). Each wash step was followed by centrifugation. The gels were centrifuged using an eppendorf mini-spin plus centrifuge for 3 min at 6000 rpm (RCF: 2419*g*), and the supernatants were collected and transferred into empty 1.5 mL eppendorfs. The gels were then centrifuged for a further 3 min at 6000 rpm (RCF: 2419*g*) to extract further supernatants from the gels. All supernatants were extracted by micropipettes and collected for analysis by spectrophotometry.

2.2.2. Rebinding Studies. Rebinding studies were performed on BHb and trypsin MIPs. After the conditioning of the gels, five washes of Milli-Q water at 1 mL volumes were performed to equilibrate the gels to remove any residual 10% AcOH SDS remaining in the gels. Once the gels were equilibrated, 1 mL of template protein (3 mg) solution prepared in Milli-Q water was added to the target MIPs and NIP controls. These were then vortexed for 5 min, left to stand for 20 min, and then centrifuged as previously described, and the supernatants were collected. The MIPs and NIPs were then washed with four 1 mL volumes of Milli-Q water, followed by five 10% AcOH/SDS washes. The supernatants for these washes were collected and analyzed using UV/vis spectrophotometry in plastic cuvettes. The wavelengths used for Hb were 405 nm for analysis of water washes and 393 nm for analysis of SDS/AcOH washes. The wavelength used for trypsin analysis in both water washes and SDS/AcOH washes was 293 nm.

2.2.3. Protein Crystallization Experiments. The crystallization drops were set up in hanging drops by mixing 1 μL protein solution with 1 μL reservoir solution. A 0.2 μL aliquot of polymer (as a viscous gel) was then dispensed with a P2 pipet (Starlab) into the drops. The same polymer but not imprinted with protein (NIP) was also dispensed at the same conditions to act as a control. An additional control without any polymer was also set up. All experiments were set up at the following conditions for each protein: (a) MIF (12 mg/mL), 1.15 M ammonium sulfate, 3% (v/v) isopropanol, and 0.1 M Tris buffer, pH 7.5; (b) lysozyme (20 mg/mL), 2.8% (w/v) NaCl, 0.1 M sodium acetate, pH 4.5; (c) thaumatin (32 mg/mL), 0.3 M Na K tartarate, 0.1 M bis tris propane buffer, pH 6.8; (d) trypsin (60 mg/mL), 13% PEG8K, 0.1 M Na cacodylate 6.5; (e) hemoglobin (60 mg/mL), 22%PEG3350, 0.2 M magnesium chloride, 0.1 M bis tris 5.5. All crystallization trials were carried out at room temperature (ca. 22 °C)

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and observed regularly over a period of 4 weeks. The three functionalized MIPS, namely, (1) polyacrylamide, (2) poly-NHMA, and (3) poly-NiPAm MIPs were tested on their cognate proteins, that is, trypsin and hemoglobin. In addition, these MIPs were tested on other noncognate proteins, namely, thaumatin, lysozyme, and macrophage inhibitory factor (MIF).

2.2.4. QCM Sensor Studies. QCM crystals were sealed and air capped (single-sided) with PVC glue, see Figure 1, in order to prevent

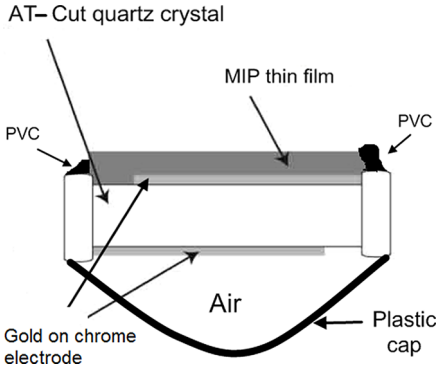

Figure 1. Quartz crystal microbalance (QCM) with a MIP thin film layer "top side" and an air-controlled cap "bottom side".

short circuiting when the QCM was submerged in solution. PA, NHMA, and NiPAm gels for BHb were synthesized using the hydrogel production procedures outlined in section 2.2. Before polymerization, MIPs and NIPs were deposited as thin films onto the capped QCM crystals, as illustrated in Figure 1b.

Thin films were achieved by beading and compressing 10 μL of the polymerizing solutions directly onto the crystals. QCM frequency and impedance measurements were taken using an Agilent 4194A Impedance Analyzer. An in-house written QBasic program was used to drive the analyzer and collect series resonance frequency and impedance data in real time. During a typical run, the thin-film-capped crystals were then submerged in various solutions such as 10% SDS/AcOH, deionized water or 3 mg/mL protein solutions (cognate BHb and noncognate BSA) for 4 min each and crystal impedance and frequency was recorded. Continuous real-time scans were conducted to assess characteristic frequency and impedance changes of the gels during surface exposure to these wash, elute, and protein rebinding conditions.

3. RESULTS AND DISCUSSION

3.1. MIP Selectivity. The molecular imprinting effect or imprinting efficiency is characterized by the rebinding capacity exhibited by the protein-specific MIP in relation to the control NIP. This can be represented as a selectivity ratio, α, using eq 1.

$$\alpha = \frac{[\text{specific binding}]_{\text{MIP}}}{[\text{specific binding}]_{\text{NIP}}} \quad (1)$$

Figure 2 clearly demonstrates the ability of the polyacrylamide MIP (Figure 2a) to uptake reloaded cognate BHb protein, whereas the NIP (Figure 2b) exhibits little or no color change after attempting to reload protein. Selectivity ratios, α, were determined through the specific-bound protein (BHb) concentrations recovered in the 10% SDS/AcOH eluted supernatant fractions. Figure 3a shows the load/wash and

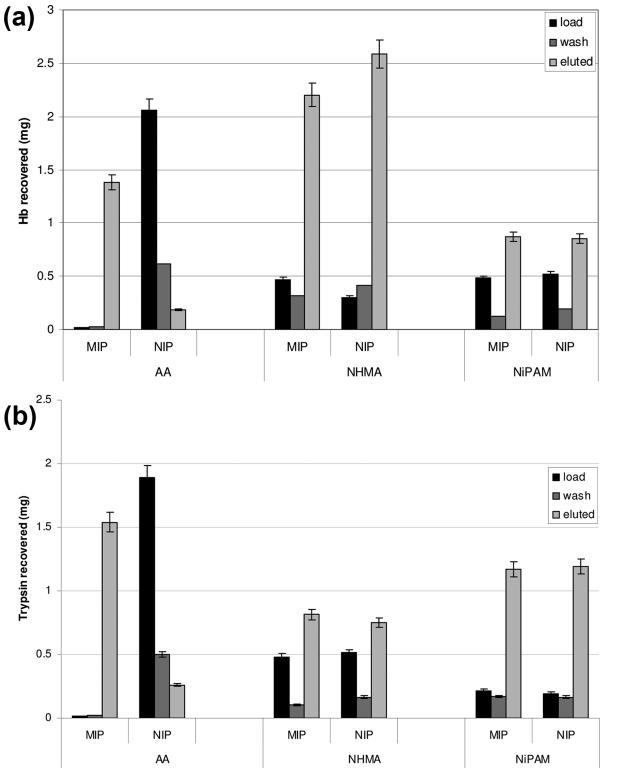

Figure 3. Amount of protein recovered after load, wash, and elution of (a) Hb and (b) trypsin in MIPs and NIPs made from acrylamide (AA), *N*-hydroxyacrylamide (NHMA), and *N*-isopropylacrylamide (NiPAM). Load, wash, and elution supernatants were analyzed spectrophotometrically.

elute profiles for BHb MIP and NIP for AA, NHMA, and NiPAM. The MIP/NIP selectivity ratios for polyacrylamide, poly-NHMA, and poly-NiPAm were 4:1, 4:1, and 3:1,

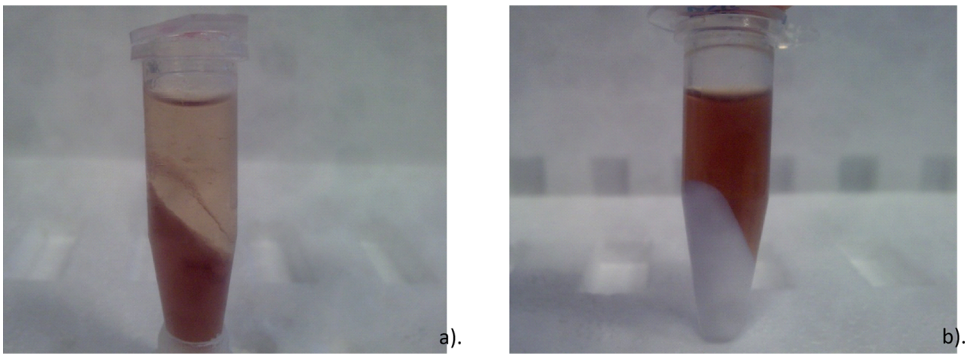

Figure 2. Bulk polyacrylamide (PA) gels at loading stages: (a) BHb-HydroMIP retaining the BHb-load; (b) control HydroNIP rejecting the BHb-load.

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Table 1. Summary of the Crystallization Results of (1) Polyacrylamide, (2) Poly-NHMA, and (3) Poly-NiPAm MIPs with Cognate and Noncognate Proteins

MIP	MIF (12.3 kDa)	lysozyme (14.5 kDa)	thaumatin (22 kDa)	trypsin (24 kDa)	hemoglobin (64.5 kDa)
Tryp MIP1	crystals	small crystals	small crystals	crystals	clear
Tryp MIP2	large crystals	small crystals	large crystals	large crystals	clear
Tryp MIP3	clear	small crystals	clear	small crystals	clear
hemoglobin MIP1	clear	clear	clear	clear	showers of small crystals
hemoglobin MIP2	clear	clear	clear	clear	crystals
hemoglobin MIP3	clear	clear	clear	clear	very tiny crystals

respectively. In the case of trypsin MIPs (Figure 3b), the MIP/NIP selectivity ratios for polyacrylamide, poly-NHMA, and poly-NiPAm were 4:1, 4:1, and 2:1, respectively. The acrylamide-based MIP/NIP selectivity ratios are in agreement with those previously published.[9,10] MIP/NIP selectivities using NHMA or NiPAM suggest that, whereas NHMA demonstrates the same selectivity as acrylamide, the NiPAM exhibits a decreased selectivity ratio, suggesting that the NiPAM MIP is tending to behave similarly to the NIP. Hence, there is no discernible selectivity. These internal measures of the selectivity of the MIP using a NIP serves to demonstrate that the MIP possesses selective cavities for the rebinding of template molecule compared with the NIP. A more rigorous measure is to test the selectivity of MIP for a noncognate protein. We therefore tested the BHb MIP against other proteins including bovine serum albumin (BSA) in this study as well as myoglobin and cytochrome C in a previous study.[9] We found that both myoglobin and cytochrome C bound with 20% affinity when compared with the target BHb protein.[9] However, we found in this study that BSA (which is a similar size to BHb) binds to the BHb acrylamide-based MIP with 70% of the target BHb binding affinity. Interestingly, though, when a polyacrylamide MIP was prepared selective for BSA, the nontarget protein BHb only bound with 6% of the target BSA binding affinity. It would appear that BSA has a high ability to bind nonspecifically to a BHb MIP, whereas BHb does not exhibit the same ability with a BSA MIP. Therefore, it is clear that MIP selectivity is not just based on size but also shape recognition. The fact that BSA binds efficiently to BHb MIP but not vice versa could be due to the widely known ability of BSA (and albumin proteins in general) to coat polymer surfaces in order to passivate them.[23]

We have also recently developed an alternative means of screening for MIP selectivity against cognate and noncognate proteins by using the ability of MIPs as novel nucleants to crystallize their target protein.[12] The ability to form crystals here relies on suitable protein-selective architecture being present in MIP cavities to induce nucleation of protein crystals. Indeed the control of testing NIPs yielded no crystals. In some cases where noncognate proteins were of a similar size to the template protein their MIPs would yield crystals. For example, trypsin-imprinted, MIP-induced crystals of lysozyme and thaumatin in addition to trypsin crystals and lysozyme-imprinted, MIP-generated crystals of thaumatin and a macrophage migration inhibitory factor. These results confirm that the MIPs can show some cross-selectivity to other proteins of similar size to the template, as observed in our spectrophotometric assessments with hemoglobin and BSA above. However, in cases where the noncognate protein is significantly different in size (e.g lysozyme imprinted MIPs and hemoglobin or catalase), no crystals are formed. The crystallization technique therefore lends itself as a tool to test for cross-selectivity of MIP for noncognate proteins. This is confirmed by the results in section 3.2.

3.2. Protein Crystallization Studies. To gain information on selectivity and cross selectivity trypsin, BHb experiments were set up with their cognate and noncognate MIPS, as well as with NIPS and controls. Crystals were not expected to form with NIPs as the correct architecture for capturing proteins does not exist. Production of crystals in MIPs for noncognate proteins of similar size points to a strong indication of cross-selectivity. Table 1 compares crystal formation data for polyacrylamide (MIP1), poly-NHMA (MIP2), and poly-NiPAm (MIP3) MIPs relating to their cognate and noncognate proteins.

Trypsin MIPs 1, 2, and 3 produced trypsin crystals within 7 days of setting up the trials (Figure 4), as well as inducing crystals of another three proteins of similar sizes, namely, MIF, lysozyme, and thaumatin.

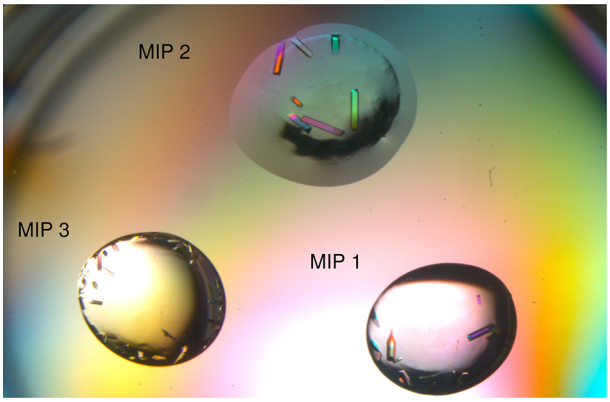

Figure 4. Crystallization of trypsin in functionalized trypsin MIPs (MIP1 = polyacrylamide; MIP2 = poly-*N*-hydroxyacrylamide; and MIP3 = poly-*N*-isopropylacrylamide.

MIPs 1, 2, and 3 of hemoglobin only produced crystals of their cognate protein after 5 days. No crystals were obtained of any of the other proteins tested. This is not surprising as hemoglobin is much larger in size than trypsin, MIF, lysozyme, and thaumatin.

In the case of lysozyme, the three polymers produced similar crystals. In all other cases where crystals were obtained, the best were in the drops containing the poly-NHMA (MIP2). This could be because the poly-NHMA (MIP2) has several hydroxyl groups that may be conducive to stronger binding of proteins. The experiments with controls and NIPs did not yield any crystals, as expected.

3.3. QCM Sensor Application. Thin film BHb MIPs were prepared on the surface of a QCM chip and the sensor was exposed sequentially to deionized water, SDS/AcOH, and 3 mg/mL BHb solution. The device was also exposed to 3 mg/

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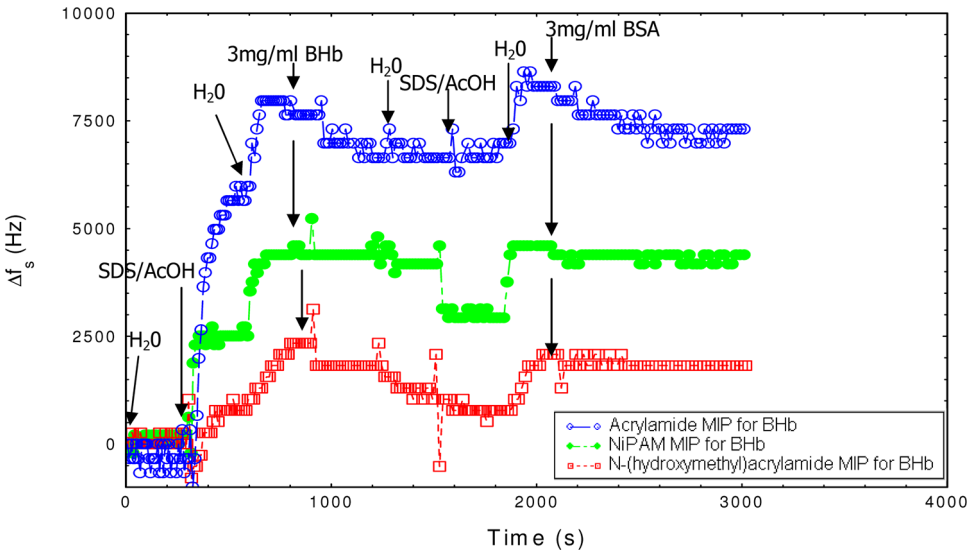

Figure 5. QCM response of functionalized acrylamide MIPs to BHb (cognate protein) and BSA (noncognate protein).

mL BSA solution. BSA is similar in size to BHb (66 and 64.5 kDa, respectively). BSA, therefore, is a good model protein to test for cross-selectivity due to competitive binding.

Figure 5 shows the QCM frequency response following sequential immersion in solutions of bovine hemoglobin, SDS/AcOH, and bovine serum albumin. First, the MIP thin film was immersed in water, followed by SDS/AcOH, in order to remove imprinted protein primarily from the surface of the polymer, but also from within the bulk of the thin film gel. After subsequent stabilization of the QCM response, the template protein was reloaded by immersing the QCM in BHb solution and the response trace recorded. Once the new response had stabilized (typically taking 15 min), the thin film was immersed in water followed by SDS/AcOH and then reconditioned in water only. The QCM was then immersed in a solution of noncognate protein to observe the effect of any nonspecific protein binding into the BHb thin film MIP. The latter procedure was followed for acrylamide, NiPAM and NHMA MIPs for BHb. We observed three distinct types of responses depending on the acrylamide used. The polyacrylamide-based BHb MIP shows a decrease in resonant frequency due to the addition of BHb. Upon washing with water and SDS/AcOH, the response returns to that before BHb addition, indicating that the effect of frequency due to BHb addition was reversed under the protein elution conditions. When the same MIP was subsequently immersed in a solution of BSA, there was a corresponding decrease in frequency similar in size to BHb addition. This suggests that the polyacrylamide BHb MIP is responding to cognate and noncognate protein alike. There would seem to be some synergy in the crystallization results where a polyacrylamide-based trypsin MIP also allowed the crystallization of thaumatin and lysozyme, proteins of similar size to trypsin. The polyacrylamide-based MIPs therefore appear to lack protein selectivity. Figure 5 also shows the QCM response of a NiPAM-based MIP for BHb. Interestingly, this MIP shows only a small frequency change to either BHb or BSA exposure (see Table 2), suggesting that the protein is not selective for either cognate or noncognate protein. Interestingly, the NHMA-based BHb MIP shows a distinct frequency decrease for BHb addition, but no change in frequency for BSA addition. This MIP appears to show selective binding of cognate BHb and nonbinding noncognate BSA. Again, referring to our data, which show the ability of MIPs to facilitate protein crystallization, there would appear to be a strong correlation between the polymer used to facilitate BHb protein crystallization and the polymer that exhibits best selectivity in the QCM results.

Table 2. QCM Response of NiPAM, AA, and NHMA Polymer MIPs and NIPs to Cognate BHb and Noncognate BSA Loading

	BHb MIP QCM response magnitude		NIP QCM response magnitude	
polymer	BHb (3 mg/mL)	BSA (3 mg/mL)	BHb (3 mg/mL)	BSA (3 mg/mL)
NiPAM	100 ± 50 Hz	100 ± 50 Hz	200 ± 50 Hz	200 ± 50 Hz
AA	1550 ± 50 Hz	2200 ± 50 Hz	350 ± 50 Hz	100 ± 50 Hz
NHMA	1600 ± 50 Hz	400 ± 50 Hz	300 ± 50 Hz	300 ± 50 Hz

Table 1 confirms that whereas small crystals are produced with polyacrylamide, the best crystals are produced with polyNHMA, and polyNIPAM only produces very tiny crystals. There would also seem to be a correlation with the hydrophilicity of the polymer. Hydrophilicity increases in the order polyNHMA > polyacrylamide > polyNIPAM, which agrees with the order of best performance of the polymers as BHb MIPs in crystallization studies and QCM studies. Table 2 compares the final QCM response to cognate and noncognate protein exposure of each MIP with its corresponding NIP. The polymers are placed in order of increasing hydrophilicity. The NIP is prepared in the absence of template protein and therefore is expected to be nonselective. Interestingly, the NiPAM MIP and NIP both show a near zero frequency response to cognate BHb and noncognate BSA. Indeed, the NIPs of all polymers exhibit only a small response to cognate and noncognate proteins. That they give similar responses indicates that the control polymers are equally unselective for both proteins. The table demonstrates the power of the imprinting technique for AA and NHMA MIPs, although it confirms that the AA MIP cavities exhibit selectivity to both cognate and noncognate proteins alike. NHMA MIP, however, does discriminate between cognate BHb and noncognate BSA with a 4:1 selectivity in QCM frequency response. There is

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approximately a 5:1 selectivity when compared with NIP. This is in agreement with the bulk polymer gel selectivities, suggesting that the thin film MIPs on the QCM surface, although in a two-dimensional format has a good population of cavities for rebinding purposes as with the three-dimensional bulk gel format.

The key difference between the polymers is their hydrophilicity dictated by the hydrophilic hydroxyl group in NHMA and the hydrophobic isopropyl group in NiPAM (Figure 6). AA

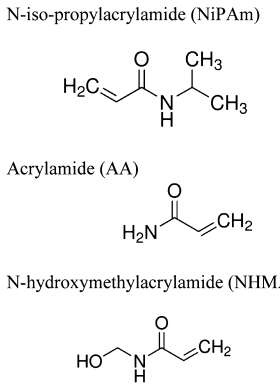

Figure 6. Chemical structures of NiPAM, AA, and NHMA.

sits between the two in degree of hydrophilicity. The nonresponse of NiPAM to either BHb or BSA suggests that there is a resistance for either protein to bind to the polymer. The hydrophilic shell of the protein appears to be a contributing feature to this lack of binding. The stark difference in selectivities between cognate and noncognate proteins for NHMA and AA suggests that the hydroxyl group in NHMA plays a significant role in the selective binding of BHb and the lack of binding of BSA. Based on the pI of the two proteins, BSA (pI = 4.6) is largely negatively charged at the pH of reloading (pH 7), whereas BHb (pI = 6.8−7.0) is electrically neutral and is still capable of forming hydrogen bonds with, for example, the hydroxy group in the NHMA MIP. That the NHMA NIP only gives small response to BHb confirms that the MIP selectivity is primarily due to cavity architecture being present in the MIP and potentially the hydroxyl group contributing to the cavity functionality. This is also confirmed by the crystallization studies. That the AA MIP is equally selective for both BHb and BSA suggests that, although cavities exist, the lack of a hydroxyl group in the cavity is reducing its ability to discriminate between cognate and noncognate protein.

Further, we also looked at cross-selectivity of BHb MIPs for thaumatin, lysozyme, and trypsin. Figure 7 shows that the BHb MIPs based on all three polymers (AA, NHMA, and NiPAM) are essentially nonresponsive in the frequency domain to the addition of thaumatin, lysozyme, and trypsin. This concurs with the qualitative data in Table 1 where none of the latter proteins were able to crystallize on a BHb MIP and confirms that these small proteins exhibit little or no selective-binding characteristics to the BHb-imprinted MIP.

4. CONCLUSIONS

MIPs have been characterized for the imprint efficiency using spectrophotometric and QCM sensor techniques. Their ability to facilitate protein crystallization has added an extra dimension to inform on their selectivity for cognate protein. The thin-film,

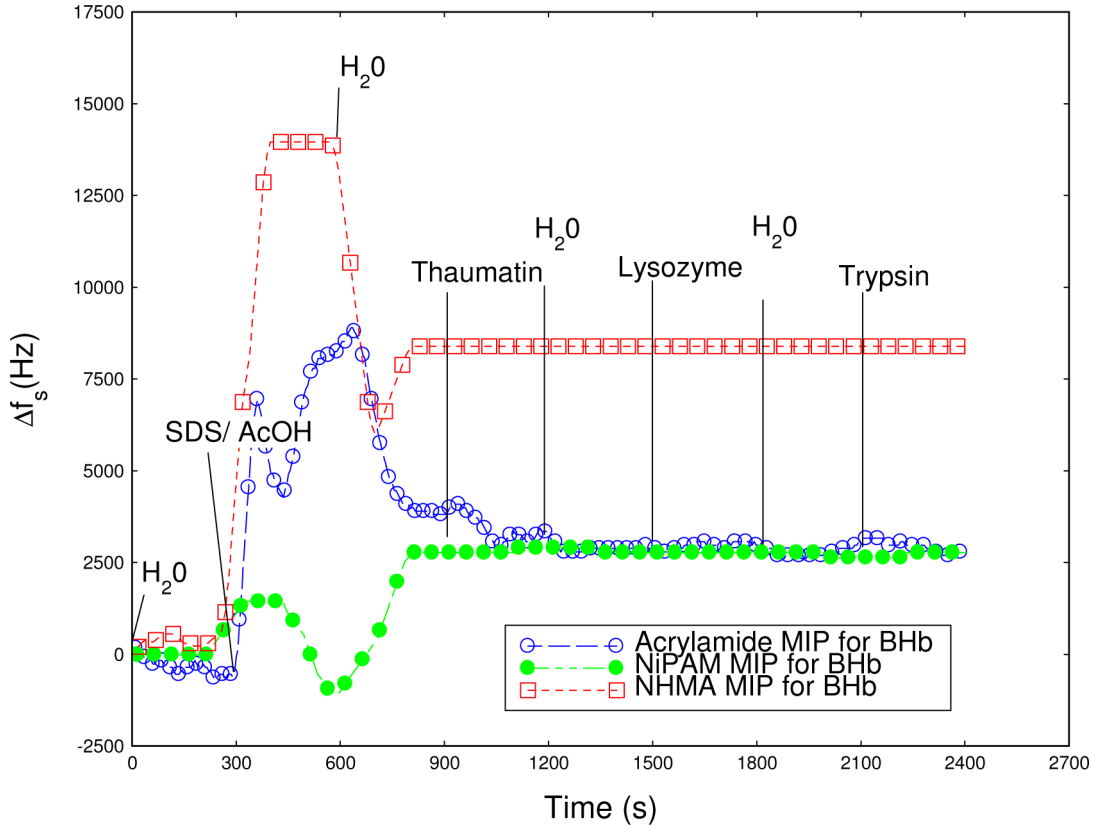

Figure 7. QCM response of functionalized acrylamide MIPs to the noncognate proteins thaumatin, lysozyme, and trypsin.

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MIP-based QCM sensors offer the promise for a simple diagnostic system for the detection of protein in, for example, disease diagnostics. We have demonstrated that MIP selectivity is a function of the hydrophilicity of the base acrylamide used to form the MIP.

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Notes

The authors declare no competing financial interest.

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