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Peptide Self-Assembled Monolayers for Label-Free and Unamplified Surface Plasmon Resonance Biosensing in Crude Cell Lysate

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Short peptides, composed of polar or ionic amino acids, derived with a short organic thiol, significantly reduce nonspecific adsorption of proteins in complex biological matrices such as serum and crude cell lysate, which have nonspecific protein concentrations of 76 and 30−60 mg/mL, respectively. Minimizing these nonspecific interactions has allowed rapid and direct quantification of *β*-lactamase in a crude cell lysate using a surface plasmon resonance (SPR) biosensor. A library of short peptides with varying chain length and amino acid composition were synthesized using a solid-phase approach. A 3-mercaptopropionic acid (3-MPA) linker was covalently attached to the amino terminus of the peptides to subsequently form a monolayer on gold in the form of 3-MPA-$(AA)_n$−OH, where *n* is the length of the amino acid chain ($n = 2-5$). Leu, Phe, Ser, Asp, and His were selected to investigate the effect on nonspecific adsorption with different physicochemical properties of the sidechains; aliphatic, aromatic, polar, acid, and base. Advancing contact angles measured the hydrophobicity of each peptidic self-assembled monolayer (SAM) and showed that hydrophilicity of the gold surface improved as the chain length of the polar or ionic peptides increased, while aromatic and aliphatic peptides decreased the hydrophilicity as the chain length increased. The nonspecific adsorption of undiluted bovine serum on SPR sensors prepared with the library of 3-MPA-$(AA)_n$−OH showed that the lowest nonspecific adsorption occurred with polar or ionic amino acids with a chain length of $n = 5$. We demonstrate that a monolayer composed of 3-MPA-$(Ser)_5$−OH has significant advantages, including the following: (1) it minimizes nonspecific adsorption in undiluted bovine serum; (2) it provides a high surface concentration of immobilized antibodies; (3) it shows a great retention of activity for the antibodies; (4) it improves the response from *β*-lactamase by ∼1 order of magnitude, compared to previous experiments; and (5) it allows direct quantification of submicromolar *β*-lactamase concentration in a crude cell lysate with a nonspecific protein concentration of 30−60 mg/mL. The use of this peptide-based monolayer offers great advantages for quantitative SPR biosensing in complex biological media.

Numerous biosensing techniques rely on the measurement of chemical or biological processes occurring on surfaces to detect molecules. In particular, affinity biosensors provide a measurable signal triggered by the binding of a molecule to a surface-immobilized receptor. Multiple physicochemical phenomena can be utilized to measure molecules with affinity biosensors, such as the change in mass, impedance, current, optical output or wavelength, or a change in refractive index, among others.[1] The latter is especially interesting in the case of measuring proteins, antibodies, or enzymes, because these molecules have a large molecular weight and high refractive index, resulting in a sensitive response using a refractive index sensor. To measure this response, surface plasmon resonance (SPR) is a label-free analytical technique that allows real-time measurements of small changes of refractive index caused by the binding of a molecule with a molecular receptor such as DNA, enzymes, or antibodies.[2−4] The SPR effect occurs when a thin metallic film deposited on a dielectric material is excited in total internal reflection. SPR is sensitive to within 200−300 nm over the metallic surface, usually gold or silver. Thereby, any molecule migrating within this sensing volume with a refractive index different from the solution will cause a change in the SPR response. This results in SPR being sensitive to numerous categories of molecules, such as DNA or proteins. The broad sensitivity to many important classes of molecules makes SPR an interesting bioanalytical technology but, at the same time, greatly limits its application with real biological samples, because of nonspecific adsorption. Previous studies have demonstrated the ability of SPR affinity biosensors to efficiently detect or quantify specific biological markers in solutions such as buffers or strongly diluted biological matrices.[5] It is imperative to extend this technique to more-complex matrices such as cellular lysate, blood serum, urine, and blood.[6,7] However, for complex matrices containing high concentrations of proteins that have

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the potential to interact with the surface of the SPR biosensors, the greater potential for nonspecific responses that can mask the analytical signal has limited the scope of SPR to solutions that are pure or contain few impurities.[8,9] To extend SPR to more-complex matrices, an efficient method of reducing nonspecific interactions is required. The need to reduce nonspecific interactions in biological matrices is common to many analytical techniques, such as electrochemical biosensors,[10] surface acoustic wave biosensors,[11] or SPR biosensors.[12,13] Nonspecific adsorption is also a major cause for prosthesis and implant rejection.[14] Hence, the design of chemical layers capable of protecting the surface of SPR sensors or other surfaces against nonspecific adsorption is an important challenge to overcome for the development of biosensors that are capable of measuring molecules directly in biological samples and for improved biocompatibility of surfaces.

In the case of SPR biosensors, proteins contained in the biological solution are the major source of nonspecific adsorption. Proteins are present in the millimolar range in most biological fluids, while the analytes of interest are in the nanomolar range or less. Nonspecific adsorption of proteins can be reduced by modifying the surface chemistry of the SPR biosensor with organic self-assembled monolayers (SAMs).[15] When designing a SPR biosensor, such monolayers must excel at limiting nonspecific adsorption while also serving as the linker to the molecular receptor used for the specific analyte detection.[16] To this end, the use of polymers such as poly(ethylene glycol) (PEG)[17−20] is the most commonly used approach. Alkanethiols also provide low nonspecific adsorption of proteins on a gold surface, while allowing the immobilization of molecular receptors for SPR biosensing.[13] A further approach used to enhance the signal-to-noise ratio for the detection of lectin in the presence of nonspecific serum proteins uses carbohydrate−tri(ethylene glycol)−alkanethiol coadsorbed monolayers.[21] In this case, the monolayer incorporated elements of organic monolayer and biological components. The recent work by Jiang's group has demonstrated excellent resistance to nonspecific adsorption using a monolayer of a zwitterionic polymer (polyCBAA).[22,23] Antibodies were immobilized on free carboxylic acids of the polymer. Also, detection of streptavidin in 100% human plasma was performed with a SPR sensor coated with polyCBAA. The total protein concentration in human plasma is 60−80 mg/mL.[24]

Recent studies have suggested that monolayers based on biological building blocks, especially amino acids and peptides, could mitigate the nonspecific adsorption of proteins in biological matrices and provide an appropriate template for the immobilization of molecular receptors.[25−28] In particular, the use of peptidomimetic polymers containing lysine residues has demonstrated excellent resistance to cell adhesion.[25,26] Moreover, the use of PEG−lysine copolymer dendrons improves the signal-to-noise ratio in a solution containing bovine serum albumin.[27] Previous work conducted within our laboratory showed the potential of SAMs based on amino acids to reduce nonspecific adsorption of bovine serum proteins on SPR biosensors.[28] In that study, polar or ionic amino acids with short sidechains reduced nonspecific adsorption of bovine serum proteins more efficiently than hydrophobic amino acids with longer sidechains. These monolayers reached a level of nonspecific adsorption similar to that of previously investigated organic antibiofouling agents. To further investigate the properties and potential applications of amino-acid-based monolayers in biosensing, we extend that study to peptide-based SAMs. The investigation of peptides of differing lengths and compositions is required to determine their resistance to nonspecific adsorption and their sensitivity in bioassays.

For peptides to be used as monolayers, they must possess an amino-terminal thiol for immobilization on the gold surface of the SPR biosensor. This leaves the carboxyl terminus free for various chemistries to immobilize molecular receptors toward specific detection of a molecule. Peptides with an amino-terminal cysteine are capable of forming self-assembled monolayers on gold.[29−31] When immobilized on gold nanoparticles, they can control the aggregation of the nanoparticles.[32] Surfaces have also been modified using sulfide or disulfide conjugates of peptides,[33−35] or with alkanethiol-derived peptides.[36] Thus, metallic particles or gold surfaces have been capped with a 3-mercaptopropionic derivatized peptide[37,38] or proteins.[39] These peptides carried

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C-terminal amide functions, not allowing the immobilization of molecular receptors. Peptides with an amino-terminal thiol linker are not commercially available, but they can be readily synthesized using Fmoc-protected amino acids coupled to a phenoxy resin,[40,41] followed by reaction with a carboxylic acid thiol. These peptide conjugates should form monolayers on gold, which can then be reacted with a desired molecular receptor to construct a biosensor. To date, no study has yet established the resistance of these types of monolayers to nonspecific adsorption and their application as a chemical linker for a biosensor.

Herein, we present an application of peptide-conjugated gold monolayers for the detection of β-lactamase, which is an enzyme that is central to clinically relevant drug resistances. Because of their well-recognized importance in bacterial drug resistance to commonly prescribed penicillins and cephalosporins, a host of methods have been developed for the detection of β-lactamases. Methods for phenotypic detection include direct detection of β-lactamase activity using colorimetric compounds, which frequently rely on expensive, nonclinically relevant substrates such as nitrocefin. Most commonly used in clinical settings are bacteriological growth tests in liquid or solid media in the presence of various antibiotics. However, diagnostic times range from hours to days and reproducibility using such microbiological assays is not trivial.[42] A more rapid means for detection of β-lactamase in biological samples is urgently required as the number of β-lactamase variants continues to increase. Molecular detection of β-lactamase-encoding genes by PCR is a faster alternative to phenotypic detection but is not yet a common practice in clinical settings.[43] Immunoassays can also be performed but they provide multicomponent, indirect detection.[44] It would be advantageous to establish a more rapid, direct, sensitive, and economical method for the detection of β-lactamase in biological fluids.

We previously demonstrated that SAMs with single amino acids successfully immobilized an anti-β-lactamase antibody to the surface of SPR biosensor, providing submicromolar range detection of purified TEM1 β-lactamase.[28] Here, we demonstrate the efficiency of short peptides as SAMs resisting the nonspecific adsorption of biological fluids, while promoting the immobilization of highly active molecular receptors on SPR biosensors. A library of peptides with a length of 2−5 amino acids was synthesized using five chemically diverse amino acids to investigate the resistance of each of these peptides to nonspecific adsorption in bovine serum. Immobilization of a β-lactamase-specific antibody at the surface of a 3-MPA-$(Ser)_5$-NHS SAM allowed quantification of this antibiotic resistance indicator. A calibration of β-lactamase in phosphate-buffered saline solution (PBS) allowed submicromolar detection in cellular lysate, a complex analytical matrix. Hence, both the resistance to nonspecific adsorption and the specific response from a bioassay are established with these peptide-based SAMs.

EXPERIMENTAL SECTION

Synthesis of 3-MPA-$(AA)_n$−OH. Amino acids were bought from Novabiochem (distributed by EMD biochemicals, Ville Mont-Royal, Quebec, Canada) with the amine and sidechain protected (Fmoc−Phe−OH, Fmoc−Leu−OH, Fmoc−His(Trt)−OH, Fmoc−Asp(OtBu)−OH, Fmoc−Ser(tBu)−OH) to avoid multiple couplings and side reactions during the solid-phase synthesis. Polystyrene SynPhase lanterns[40,41] (A-Series, Mimotopes, Australia) that expose a hydroxymethylphenoxy linker were first immersed in dichloromethane (DCM) twice for 30 min, to prepare the linker for the coupling of a first amino acid to the phenoxy groups of the lantern. A solution containing 6 equiv of amino acid mixed with 3 equiv of diisopropylcarbodiimide (Sigma−Aldrich, Milwaukee, WI) was prepared in *N*,*N*-dimethylformamide (DMF) for immersion of the lanterns. Thereafter, a DMF solution that contained a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (Fluka, Milwaukee, WI) was quickly mixed with the previous solution. The reaction mixture was stored overnight, at room temperature and away from excessive light. The lanterns were then rinsed three times with DMF, three times with methanol, and three times with DCM, for 3 min at each rinse. This rinsing method was used after each of the following steps. Two pieces of the lanterns were cut to determine coupling efficiency. The first piece of lantern was used to perform a Kaiser test,[45,46] to verify that the coupling was complete. The second piece of the lantern was immersed in a 20:80 piperidine:DMF solution for 30 min to remove the N-terminal Fmoc protecting group, for peptide growth. (The piperidine was obtained from Sigma−Aldrich, Milwaukee, WI.) The Kaiser test was repeated with the deprotected lantern, to verify the completion of the deprotection reaction. The subsequent coupling reactions were performed with 3 equiv of the amino acid, 3 equiv of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium (HBTU, Novabiochem), and 9 equiv of *N*-ethyldiisopropylamine (DIEA) in DMF for 2 h. The final coupling was accomplished with 1 equiv of 3-mercaptopropionic acid (3-MPA) (Sigma−Aldrich), instead of 3 equiv of amino acid. Thereafter, a 1-h reaction in a solution of 95% trifluoroacetic acid (TFA) (EMD Biochemicals), 2.5% triethylsilane (TES) (Alfa Aesar, Ward Hill, MA), and 2.5% water cleaved the peptide from the lantern to yield the 3-MPA-$(AA)_n$−OH, where n corresponds to the number of amino acids in the peptide. TFA was evaporated and the peptide was precipitated in diethyl ether to recover the pure 3-MPA-$(AA)_n$−OH. The composition of each peptide was verified using liquid chromatography−electrospray ionization−mass spectroscopy (LC-ESI-MS). The yield varied between 35% and 85%, depending on the amino acid. Thereby, 3-MPA-$(AA)_n$−OH peptides were synthesized with $n = 1$, 2, 3, 4, and 5 for AA = Leu, Phe, Ser, Asp, His, and with $n = 8$, 10 for Ser.

Preparation of Peptidic Monolayers. Microscope slides (BK7, 22 mm × 22 mm) were coated with a 3-nm-thick titanium adhesion layer and a 50-nm-thick gold layer (purity of 99.99%, ESPI

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Metals), using a Cressington Model 308R sputter coater. These gold-coated SPR slides were immersed for at least 16 h in a 5 mM peptide solution in absolute ethanol to form a well-ordered monolayer.[7] Four replicates were prepared for each 3-MPA-$(AA)_n$−OH. Each sample was rinsed with absolute ethanol to wash away unbound peptides. Slides used to bind biological receptors were immersed in a 0.01 M HCl solution for 2 h to ensure that the C-terminal extremities were available.[28]

SPR and Contact Angle Measurements. The surface coverage for each monolayer was measured according to the change in response of the SPR sensor in PBS for the bare gold surface, relative to the peptide-coated gold surface. To measure the formation of the monolayers, each SPR sensor was mounted on a custom-made SPR instrument that was based on a dove prism with wavelength interrogation as previously described.[47] The advancing contact angles were measured for SAMs with 300 μL of PBS (CellGro, Mediatech, Inc.) with a custom-built contact angle instrument. The pH and salinity of PBS is similar to that of biological samples (i.e., bovine serum). Once mounted on the SPR instrument, the sensors were stabilized for 5 min in PBS before the acquisition of the s-polarized reference. The p-polarized real-time measurement was initiated by monitoring the baseline for 5 min in PBS, which was replaced with undiluted bovine serum for 20 min (Sigma−Aldrich) containing 76 mg/mL proteins. The serum was replaced for 5 min by PBS to quantify nonspecifically bound proteins. Raw data were processed with Matlab software. This procedure was repeated at least four times for each of the 3-MPA-$(AA)_n$−OH prepared with $n = 1-5$ residues.

Immobilization of Anti-β-lactamase. 3-MPA-$(Ser)_5$−OH was selected for its optimal performance in limiting nonspecific protein adsorption to construct SPR biosensors specific for β-lactamase. The SAM was formed as described previously and was subsequently reacted for 30 min in an aqueous solution composed of 25 mM *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (EDC, Fluka) and 25 mM of *N*-hydroxysuccinimide (NHS, Sigma−Aldrich).[48] The NHS−ester formation was monitored using the SPR instrument with a fluidic cell constructed in-house. Each SPR biosensor was then rinsed with 18 MΩ water, followed by PBS, and placed in a Teflon reaction vessel that was designed to seal the biosensors from the external environment, limiting the evaporation of the anti-β-lactamase solution during the overnight reaction with the activated monolayer on the SPR sensor. Each step involving an antibody or an antibody-derived SPR sensor was accomplished in a refrigerated laboratory, and the sensors were kept at 4 °C until use.[49] The antibody specific for TEM-1 β-lactamase (QED Bioscience, Inc.) was diluted to a concentration of 37 μg/mL and 250 μL of this solution were reacted with the activated 3-MPA-$(Ser)_5$−OH surfaces. The samples were rinsed with PBS and reacted for 10 min in 1 M ethanolamine hydrochloride (Sigma−Aldrich) adjusted to pH 8.5 with 10 M NaOH (Fluka), to inactivate the unreacted NHS after the antibody immobilization.[50] The biosensors were rinsed and stored in PBS for at least 60 min prior to use.

β-Lactamase Expression and Purification. Wild-type TEM-1 was overexpressed in *Escherichia coli* as previously described.[51] Cells were pelleted by centrifugation (30 min, 5000 × g, 4 °C) and resuspended in 20 mL of 10 mM Tris-HCl buffer (pH 7.0) for lysis on ice using a Branson sonicator (four pulses at 200 W for 30 s with a tapered microtip). Cellular debris was pelleted by centrifugation (50 min, 20000 × g, 4 °C) and the supernatant was filtered through a 0.2 μm filter, yielding crude lysate. The purification was performed according to a two-step purification protocol using an Äkta FPLC (GE Healthcare). The first step was as previously described,[51] using a linear gradient of 10−200 mM Tris-HCl pH 7.0 to elute the enzyme. Fractions containing β-lactamase were identified using qualitative nitrocefin (Calbiochem, Mississauga, Ontario, Canada) hydrolysis assay and were confirmed via SDS−polyacrylamide (SDS-PAGE) gel electrophoresis (15% (w/v)) with Coomassie Brilliant Blue staining. For the second step, fractions containing β-lactamase were pooled (20 mL) and concentrated to 1.5 mL using an Amicon concentrator (MCWO 10000, Millipore, Billerica, MA) for injection on a Superose12 column (1.6 × 55 cm). The sample was eluted with 50 mM Tris-HCl (pH 7.0) at a flow rate of 1.0 mL/min. Fractions containing β-lactamase were identified as described previously. Enzyme purity was evaluated using separation by SDS-PAGE stained with Coomassie Brilliant Blue and the public domain image analysis software Scion Image (NIH, rsb.info.nih.gov/nih-image). Protein concentration was quantified using Bradford assay[52] (Biorad, Hercules, CA), with bovine serum albumin as the standard. Pooled fractions of purified β-lactamase were concentrated to 1 mg/mL (33 μM), as previously described.

Determination of β-Lactamase Enzyme Concentration. β-Lactamase activity for crude and purified TEM-1, as well as crude lysate from the host strain that does not overexpress TEM-1 (*E. coli* XL1-Blue), were determined by measuring the initial rate of CENTA (CalBioChem, Mississauga, Ontario, Canada) hydrolysis at 405 nm ($\Delta\varepsilon_{405}$ = 6400 M^{-1} cm^{-1}) at 25 °C in 50 mM sodium phosphate buffer (pH 7.0) under saturated enzyme conditions (700 μM CENTA)[53,54] with a Cary 100 Bio UV−visible spectrophotometer (Varian Canada, Inc., Montréal, Quebec, Canada). Concentration of β-lactamase was determined by applying the equation $[E] = V_{max}/k_{cat}$, using reported k_{cat} values[53,54] and experimentally determined V_{max} values.

Calibration of TEM-1 β-Lactamase in PBS. Solutions of different concentrations of TEM-1 β-lactamase (700, 350, 175, 88, and 44 nM) were prepared in PBS from an aliquot of the concentrated solution, prepared as previously described. These solutions were kept at 4 °C until 20 min prior to use. The SPR biosensors specific for TEM-1 β-lactamase were then mounted on the SPR instrument equipped with the fluidic cell. A delay of 10 min prior to the initial experiment was necessary for the

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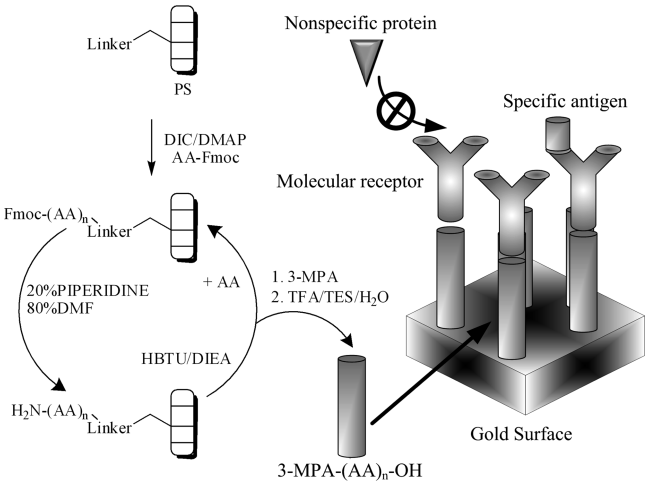

Figure 1. Synthesis of 3-MPA-$(AA)_n$−OH, where n is the number of reaction cycles prior to the termination of the reaction by grafting 3-MPA. The formation of monolayers on gold with these compounds is investigated for reduction of nonspecific adsorption. In this study, AA refers to amino acid (specifically to Leu, Phe, Ser, Asp, and His).

biosensor to stabilize before acquiring the s-polarized reference. Immediately afterward, the real-time measurement in p-polarized light was undertaken for 5 min in PBS. The solution was then replaced with β-lactamase solution for 20 min. Finally, PBS was monitored to verify whether the binding of β-lactamase was reversible.

Detection of TEM-1 β-Lactamase in Crude Cell Lysate. The 3-MPA-$(Ser)_5$−OH-based biosensors were exposed for 12.5 min to a cellular lysate without TEM-1 β-lactamase (blank cell lysate) after a stabilizing period of 5 min in PBS. This lysate was then replaced with the lysate containing TEM-1 β-lactamase for 12.5 min for the specific detection of TEM-1 β-lactamase. The concentration of TEM-1 β-lactamase in crude lysate is high, such that saturation of the SPR biosensor was expected. Thus, the β-lactamase cell lysate was diluted with PBS by a factor of 2 and 4. Blank runs with TEM-1 β-lactamase-free crude cell lysate were accomplished with a 3-MPA-$(Ser)_5$−OH and with a 16-mercaptohexadecanoic acid (16-MHA)-based biosensor that had previously been reacted with anti-β-lactamase.

A 3-MPA-$(Ser)_5$−OH SAM with immobilized anti-β-lactamase was stabilized for 5 min in PBS prior to the injection of blank cellular lysate over a period of 12.5 min to saturate the remaining sites on the biosensor with nonspecifically adsorbed proteins. The blank lysate was then replaced with a crude cell lysate overexpressing TEM-1 β-lactamase diluted 100-fold with the blank cell lysate. The SPR response induced by this replacement allowed the quantification of the amount of β-lactamase in the crude cell lysate, using the equation obtained with the calibration using pure β-lactamase in PBS.

RESULTS AND DISCUSSION

Synthesis and Characterization of 3-MPA-$(AA)_n$−OH. Using a combinatorial approach, a library of 22 SAMs was synthesized using a solid-phase strategy where homopeptides of varying lengths were synthesized from the carboxyl to the amino terminus with covalent attachment of a 3-mercaptopropionic acid to the N-terminal amine (see Figure 1). Cleaving the final compound from the solid support produced peptides carrying thiol groups in the form of 3-MPA-$(AA)_n$−OH. Usual tests for solid-phase peptide synthesis confirmed the completeness of the reactions and the formation of the desired molecules. Kaiser tests conducted after each amino acid coupling step confirmed that the coupling reaction was successful and the terminal amine of the peptide could be deprotected from FMOC. Larger-scale syntheses were conducted for each amino acid: Leu, Phe, Ser, Asp, and His. After each coupling step, a proportion was set aside for reaction with 3-MPA to obtain 3-MPA-$(AA)_n$−OH, with $n = 2-5$. The results from $n = 1$ are from a previous investigation by Bolduc and Masson.[28] Following the cleavage of 3-MPA-$(AA)_n$−OH from the solid phase, most of the resulting compounds were analyzed using liquid chromatography−mass spectroscopy (LC-MS) with the exception of 3-MPA-$(Phe)_n$−OH, which did not elute from the HPLC column, because of its hydrophobic nature. The high-performance liquid chromatography (HPLC) purity was measured, and the mass spectroscopy (MS) confirmed the synthesis of 3-MPA-$(AA)_n$−OH with the presence of one or more of the following: the molecular ion, the molecular ion suffering one or more water losses, or from the disulfide complex of the peptide. The disulfide compound from 3-MPA-$(AA)_n$−OH was visible in the mass spectrum, confirming the presence of the thiol. The coupling reaction using HBTU/DIEA was very efficient, as assessed by the fact that only traces of shorter peptides were observed upon LC-MS analysis of 3-MPA-$(AA)_5$−OH. Thereby, the 3-MPA-$(AA)_n$−OH compounds were synthesized with $n = 2-5$ for Phe, Leu, Asp, and His and with $n = 2-5$, 8, and 10 for Ser.

Characterization of 3-MPA-$(AA)_n$−OH SAMs. SPR was used to quantify the surface coverage of the 3-MPA-$(AA)_n$−OH SAMs. Immediately after coating a BK7 glass slide with 50 nm of gold, the SPR response was recorded in PBS to determine the SPR wavelength (λ_{SPR}). Following the formation of the SAM, the SPR response was measured in PBS to calculate the change in the SPR response ($\Delta\lambda_{SPR}$) caused by the formation of the SAM on the SPR sensor. The change in SPR response was used with the equation from Jung et al. to calculate the surface concentration (Γ) of the SAMs prepared with 3-MPA-$(AA)_n$−OH:[55,56]

$$\Gamma = \rho(-0.5l_d) \ln\{1 - [\Delta\lambda_{SPR} m^{-1}(\eta_{SAM} - \eta_{PBS})^{-1}]\} \quad (1)$$

where the sensitivity (m) of the SPR instrument was measured with sucrose solutions at 1765 nm/RI. The refractive index of PBS (η_{PBS}) was determined experimentally with a high-resolution refractometer at 1.33476 ± 0.00002 RIU and the refractive index related to the SAMs was approximated to be 1.45 RIU. (Note: RIU denotes refractive index unit.) To verify this assumption, 3-MPA-$(Ser)_5$−OH is one of the only compounds tested that is an oil; thus, the refractive index could be easily and accurately measured at 1.44. The penetration depth of the plasmons (l_d) is 230 nm (2.3×10^{-5} cm in eq 1). This penetration depth is used for all calculations. The SPR substrates are always at the

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same initial wavelength (high reproducibility of manufacture) and the monolayer formation induces a change of SPR wavelength of a few nanometers. This results in no significant change in the penetration depth. The density of the molecules forming the SAMs is 0.9 g/cm^3.[15] The molecular weight of each peptide allowed the calculation of the surface coverage (in terms of molecules/cm^2). The surface concentration of 3-MPA-$(AA)_n$−OH SAMs varies, depending on the chain length of the peptide used. The average surface coverage for the homopeptides with identical chain length decreases significantly from $n = 1$ to $n = 2$, and from $n = 2$ to $n = 3$. Thereafter, the decrease is minimal from $n = 3$ to $n = 4$ and 5. The longest peptides, with $n = 5$ ($\Gamma = 1.4 \times 10^{14}$ molecules/cm^2), have a surface coverage of ~4 times less than that with $n = 1$ ($\Gamma = 5.1 \times 10^{14}$ molecules/cm^2). The lower surface concentration of the longer 3-MPA-$(AA)_n$−OH SAMs implies a less-dense monolayer. This could be explained by the secondary structure of the amino acids with longer chain length. Straight-chain alkanethiols usually have a high surface density, of $\sim 10 \times 10^{14}$ molecules/cm^2. This corresponds to a densely packed monolayer. 3-MPA-$(AA)_n$−OH compounds with small values of n likely have a surface conformation similar to that of straight-chain alkanethiols. However, peptides tend to adopt secondary structures in solution, such as helices and sheets. It was reported in a previous study that polylysine on a gold surface forms β-sheets for $n = 4$ and 10, whereas it formed α-helices for $n = 30$.[57]

The amide bands can provide structural information on the monolayers. Fourier transform infrared (FTIR) spectrum of the 3-MPA-$(AA)_1$−OH compound resulted in characteristic amide I and amide II bands at 1644 and 1560 cm^{-1}. These are the common bands that were previously observed for amides in organic monolayers.[58] FTIR analysis was also performed using a germanium-crystal attenuated total reflectance (GATR) device on each of the 3-MPA-$(AA)_5$−OH monolayers on gold. The amide bands were now observed at 1660 and 1545 cm^{-1} for Asp, Leu, Ser, and His monolayers, indicating the formation of α-helices at the surface.[57] The amide bands for Phe are located at 1673 and 1530 cm^{-1}, which is indicative of the formation of a β-sheet. Thus, the longer-chain peptide monolayers are adopting a secondary structure at the surface of the SPR sensors. This could explain the lower surface concentration for $n = 3$, 4, and 5 for 3-MPA-$(AA)_n$−OH, compared to the shorter-chain peptides of $n = 1$ and 2, because α-helices or β-sheet would not be as densely packed as straight-chain alkane thiol or short peptides in the present case.

The advancing contact angle of PBS for different chain lengths of 3-MPA-$(AA)_n$−OH SAMs are an indication of the hydrophobicity of the surface, hence the physicochemical properties of the SAMs. The advancing contact angles indicate that 3-MPA-$(Leu)_n$−OH are generally the most hydrophobic layers tested (see Figure 2). Moreover, the hydrophobicity of 3-MPA-$(Leu)_n$−OH and 3-MPA-$(Phe)_n$−OH increases as the chain length of the peptide increases. These peptides are composed of hydrophobic aliphatic and aromatic sidechains, respectively. The observed increase in contact angle, relative to increasing chain length, indicates an increased contribution of the sidechain physicochemical property toward the overall properties of the monolayer. This tendency was similar for the SAMs prepared with Ser, Asp, and His, because the contact angle decreased as the chain lengths for those hydrophilic peptides increased, indicating an increased hydrophilicity of the SAMs.

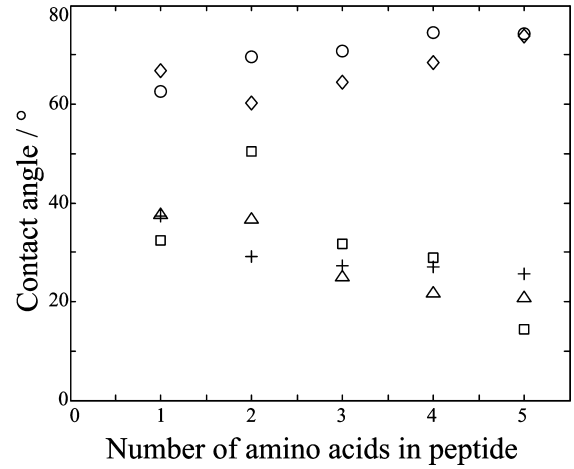

Figure 2. Changes in contact angle of the 3-MPA-$(AA)_n$−OH with increasing chain length of the peptide ($n = 3$ measurements). The symbols refer to (○) leucine, (◇) phenylalanine, (+) histidine, (△) serine, and (□) aspartic acid.

Adsorption of Bovine Serum Proteins on 3-MPA-Peptide SAMs. Determining the surface coverage of nonspecifically bound proteins with a standard solution is essential in assessing the potential use of monolayers as a chemical template for a bioassay in complex biological fluids. In the case of SPR biosensors, these nonspecifically bound proteins resulted in a response that cannot be distinguished from the response of interest, such that few studies of detection in relevant biological media have been reported. To determine the resistance of the 3-MPA-$(AA)_n$−OH SAMs to nonspecific adsorption, the monolayers on gold were exposed to undiluted bovine serum. Bovine serum contains proteins that are similar to, but slightly more concentrated than, human serum, thus providing a perfect test for the monolayers. Each 3-MPA-$(AA)_n$−OH monolayer was exposed successively to PBS for 5 min, to bovine serum for 20 min, and to PBS for an additional 5 min. An overlay of typical kinetic curves expressing the bovine serum protein adsorption (Figure 3) for each 3-MPA-$(AA)_5$−OH shows notable differences in nonspecific protein adsorption, relative to the type of amino acid present. Polar and ionic amino acids have a tendency to perform better than hydrophobic ones, as noted by the smaller changes in the SPR responses with these peptides. A second overlay of typical kinetic curves shows that the nonspecific adsorption of proteins is dependent on the length of the peptide present. Monolayers prepared with 3-MPA-$(Ser)_n$−OH of increasing chain length showed a marked decrease in the SPR response with bovine serum, corresponding to lower nonspecific protein adsorption on the surface of the SPR sensor. This corresponds to an improved performance of the monolayer in complex biological fluids.

The comparison of each 3-MPA-$(AA)_n$−OH compound with increasing chain length shows interesting trends. First, the surface concentration of proteins adsorbed on each SAM was

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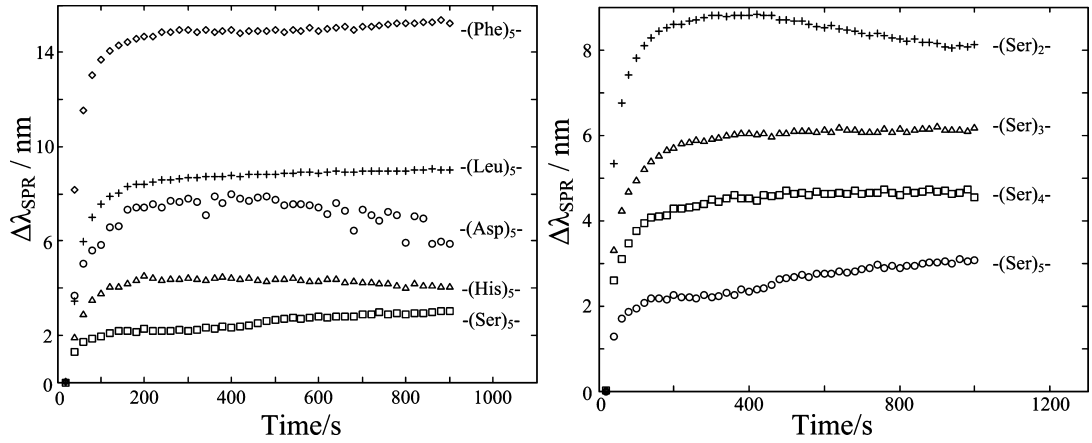

Figure 3. Sensorgrams of nonspecific adsorption of bovine serum protein for five 3-MPA-$(AA)_5$−OH compounds prepared with different amino acids (left) and for 3-MPA-$(AA)_n$−OH compounds with different numbers of Ser units in the peptide chain (right).

Table 1. Surface Concentration of 3-MPA-$(AA)_n$−OH SAMs Immobilized on the Gold Surface of SPR Biosensor

	Surface Concentration of 3-MPA-$(AA)_n$−OH SAMs ($\times 10^{14}$ molecules/cm^2)					
n	Leu	Phe	Ser	Asp	His	average
1	7.9	5.2	2.9	6.1	3.4	*5.1*
2	3.0	2.4	4.9	2.6	2.0	*3.0*
3	3.1	1.3	1.6	1.3	1.5	*1.8*
4	3.1	1.3	1.6	2.3	2.8	*2.2*
5	1.5	1.9	0.98	1.4	1.3	*1.4*

[a] Data for $n = 1$ are taken from Bolduc and Masson.[28] Measurements obtained in triplicate.

Table 2. Surface Coverage Due to Nonspecific Adsorption of Undiluted Bovine Serum with Increasing Chain Length of Different 3-MPA-$(AA)_n$−OH on the Gold Surface of SPR Biosensor

	Surface Coverage (ng/cm^2)				
n	Leu	Phe	Ser	Asp	His
1	706 ± 19	575 ± 202	419 ± 33	416 ± 121	486 ± 149
2	598 ± 84	328 ± 67	302 ± 20	430 ± 54	329 ± 75
3	488 ± 122	388 ± 117	226 ± 20	337 ± 54	213 ± 38
4	404 ± 112	369 ± 96	165 ± 10	291 ± 122	185 ± 30
5	345 ± 78	619 ± 66	132 ± 33	290 ± 22	188 ± 71
8			246 ± 111		
10			259 ± 33		

[a] Data for $n = 1$ are taken from Bolduc and Masson.[28] Measurements in triplicate and the error represents two standard deviations on the mean.

obtained from the sensorgrams shown in Figure 3 and is reported in units of nanograms of nonspecifically bound proteins per square centimeter, using eq 1. In calculating the surface coverage of nonspecifically bound proteins, the parameters of eq 1 are determined to be identical, with the exception of the density of proteins being 1.3 g/cm^3 and the refractive index being equal to 1.57 RIU. Nonspecific adsorption generally decreased as the chain length of the 3-MPA-$(AA)_n$−OH compounds increased, in the presence of bovine serum, with the exception of 3-MPA-$(Phe)_5$−OH, which increased considerably in comparison with the other 3-MPA-$(Phe)_n$−OH compounds (see Table 2). Otherwise, this reduced nonspecific adsorption was notable in the case of 3-MPA-$(Ser)_n$−OH, which showed an ~4-fold decrease with the longer chains, compared to the already excellent properties of 3-MPA-$(Ser)_1$−OH. Overall, the results generally indicate that increasing the chain length resulted in more "peptide-like" monolayers possessing significantly improved performance toward nonspecifically adsorbed proteins from bovine serum. Further increasing the chain length of 3-MPA-$(Ser)_n$−OH to $n = 8$ and 10 resulted in decreased performance of the monolayer with the surface coverage of nonspecifically adsorbed proteins increasing to ~250 ng/cm^2 for $n = 8$ and 10 versus 132 ng/cm^2 when $n = 5$. Thus, the performance of 3-MPA-$(Ser)_n$−OH seems to be optimal at $n = 5$.

Among the 3-MPA-$(AA)_n$−OH compounds that have been tested, the 3-MPA-$(Phe)_n$−OH and 3-MPA-$(Leu)_n$−OH compounds were determined to be the worst, because these hydrophobic monolayers adsorbed a significantly greater amount of serum proteins. Comparatively, 3-MPA-$(Leu)_n$−OH formed SAMs resulting in greater nonspecific adsorption at shorter chain lengths, while 3-MPA-$(Phe)_n$−OH resulted in poor results at longer chain lengths. The performance of the other peptides improved upon testing of the 3-MPA-$(Asp)_n$−OH, 3-MPA-$(His)_n$−OH, and 3-MPA-$(Ser)_n$−OH compounds, with the latter exhibiting the best resistance to nonspecific adsorption of bovine serum proteins. Note that both the 3-MPA-$(His)_n$−OH and 3-MPA-$(Ser)_n$−OH showed improved resistance to nonspecific adsorption by at least a factor of 2, compared to the other 3-MPA-$(AA)_n$−OH. Thus, it appears that the more polar and ionic peptides SAMs are the most efficient at reducing nonspecific interactions at the surface of biosensors, in good agreement with our previous study using short ($n = 1$) monolayers and data from other SAM monolayers.[28,59] Based on these results, 3-MPA-$(Ser)_5$−OH was selected for the further validation of a bioassay using a 3-MPA-$(AA)_n$−OH SAM to immobilize a target molecular receptor and to minimize nonspecific adsorption in a complex biological fluid.

Nonspecific Adsorption on PEG Monolayer. Currently, the use of poly(ethylene glycol) (PEG) monolayers is a common approach to limit nonspecific adsorption for biosensors. A monolayer of 11-mercaptoundecane(ethylene glycol)$_3$-COOH (C11EG-

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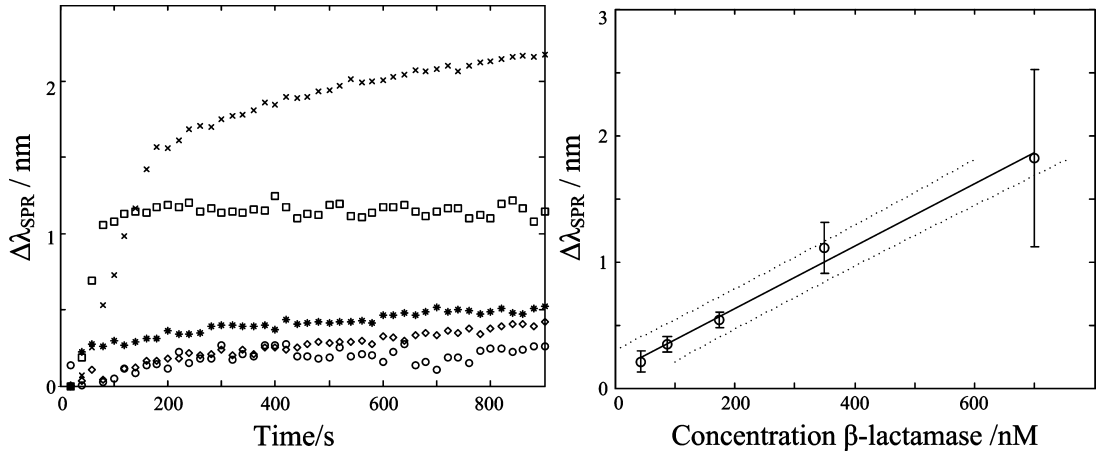

Figure 4. (Left) Sensorgrams of β-lactamase in PBS for different concentration solutions using a SPR affinity biosensor prepared with 3-MPA-$(Ser)_5$-(anti-β-lactamase). (β-lactamase concentration of the solutions: (×) 700 nM, (□) 350 nM, (*) 175 nM, (◇) 88 nM, and (○) 44 nM. (Right) Calibration of β-lactamase in PBS with the SPR sensor; the error bars represent two standard deviations on the mean ($n = 3$ measurements), whereas the light gray line represents two standard deviations on the regression. The equation of the linear regression is $\Delta\lambda_{SPR} = 0.0025$ nm/nM × [β-lactamase] + 0.13 nm, with $R^2 = 0.991$.

3COOH) has been immobilized to the gold surface of the SPR sensor. The monolayer formation resulted in a surface concentration of 1.1×10^{15} molecules/cm^2, which corresponds to a densely packed monolayer. The contact angle of this PEG is at 42° ± 3° with PBS, which is slightly greater than that for the polar and ionic 3-MPA-$(AA)_5$−OH compounds. The nonspecific adsorption of bovine serum on the PEG monolayer is very close to the best performing 3-MPA-$(Ser)_5$−OH at 100 ± 27 ng/cm^2. This value is similar to the nonspecific adsorption of 180 ng/cm^2 of PEG in human plasma reported by the Jiang group.[22] Human plasma has a similar concentration of nonspecific proteins as bovine serum.

Calibration of β-Lactamase in PBS. To validate the use of 3-MPA-$(Ser)_5$−OH monolayers, a β-lactamase model system was used to demonstrate the efficiency of SPR biosensors in a complex biological medium with these types of monolayers. No commercial biosensor is currently available to quantify β-lactamase. This enzyme constitutes a logical choice for a test system, both due to its clinical importance and because it is a well-characterized "benchmark" protein. Anti-TEM-1 β-lactamase was purchased from a commercial source. No information on the affinity constant for this antibody with β-lactamase is currently available. The molecular weight of anti-TEM-1 β-lactamase is ~155 kDa. The immobilization of anti-β-lactamase from a 37 μg/mL solution in PBS resulted in a change of the SPR response of 8.25 nm from multiple measurements ($n = 3$ measurements). Equation 1 provides an estimate of the surface coverage of the antibody on the SPR sensor. Using standard parameters for proteins, the surface concentration of anti-β-lactamase is 0.86 pmol/cm^2, or 5.6×10^{11} molecules/cm^2. The maximum surface coverage for a molecule of 160 kDa should be ~2×10^{12} molecules/cm^2, assuming a diameter of 7 nm for the molecule.[60,61] Thus, it is observed here that ~25% of the available binding sites are occupied by anti-β-lactamase. A fraction of ~25% of the bound antibodies is typical for protein coverage on a monolayer using NHS chemistry. However, it does not reflect the fraction of active antibodies at the surface that, because of the multiple orientations, the antibody can take at the surface.[62] Some orientations of the antibodies at the surface lead to hindrance or deactivation of the binding sites. Assuming that, typically, 75% of the bound antibodies are active at the surface,[63] the fraction of sites occupied by active antibodies is closer to 20%. A previous report has confirmed this value, using an enzyme assay on a gold electrode. With a warm enzyme solution (37 °C), a total enzyme coverage of 36% of the possible sites was obtained for glucose oxidase, which is an enzyme with a molecular weight similar to that of the antibodies.[61] An active fraction of 71% was obtained, with regard to the the conversion of glucose to gluconic acid and hydrogen peroxide, which was oxidized at the electrode. Improving the surface concentration of antibody should further improve the sensitivity of SPR biosensor, although further increasing the amount of antibodies sometimes results in an antigen saturation response.[62] This was characterized by a plateau in the response of an antigen, even when the amount of bound antibodies is increased.

β-Lactamase was purified to ~90% from *E. coli* overexpressing TEM-1 β-lactamase and concentrated to 1.0 mg/mL, as determined according to the catalytic activity toward the chromogenic substrate CENTA. The concentrated enzyme solution was diluted with PBS and injected manually over a β-lactamase-specific SPR biosensor, using a fluidic cell built in-house. The fluidic cell was very useful in reducing the impact of the experimental conditions on the analytical signal, especially the change of temperature. Hence, minimal drift that was due to temperature changes was observed on the sensorgrams, allowing the observation of smaller changes due to specific interactions. An overlay of typical kinetic curves obtained for solutions containing nanomolar concentrations of this enzyme clearly shows that this type of biosensor is sensitive to the concentration of the analyte in solution (see Figure 4). The

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sensitivity of the SPR biosensor is limited to nanomolar concentrations by the poor affinity of the anti-β-lactamase antibody. No highly specific antibodies are commercially available for this biological system. The biological concentration range that is expected to make a β-lactamase biosensor viable remains unknown. However, a previous study with other variants of β-lactamase was able to detect 1−10 ng of β-lactamase.[44] This detection limit was sufficient to monitor the biologically relevant range in clinical samples.

Triplicate measurements of each concentration allowed the calibration of the system to quantify the amount of β-lactamase in more-complex solutions (see Figure 4). The limit of detection was determined to be near 10 nM for β-lactamase. Considering the fluidic cell volume of ~100 μL, this corresponds to a mass of 30 ng of β-lactamase in the fluidic cell. This corresponds to a mass detection limit on the sensor of ~10 pg with our 1.9 cm^2 sensor. Therefore, this SPR sensor is comparable, with regard to the detection limit, to the ELISA techniques for other variants of β-lactamase.[44] The calibration curve for the SPR response induced by β-lactamase in PBS is linear, but the linear regression does not cross the origin, which suggests that there is a nonlinear domain for superior concentrations. This is usual with site-limited surface techniques such as SPR biosensors, where the signal is only linear for a limited concentration range. Using a Langmuir calibration isotherm allows for the compensation of these variations and also allows us to extrapolate important thermodynamic information on anti-β-lactamase, as demonstrated with eq 2:

$$\Delta\lambda_{SPR}{}^{-1} = (KC\Delta\lambda_{SPR,max})^{-1} + \lambda_{SPR,max}{}^{-1} \qquad (2)$$

where K is the affinity constant of the antibody for the antigen, $\Delta\lambda_{SPR}$ the change of the SPR response for a concentration C, and $\Delta\lambda_{SPR,max}$ the change of the SPR response at saturation of the antibody. Thus, by plotting the inverse x and y axes of Figure 4, an affinity constant of 2.5×10^6 M^{-1} is obtained, which is significantly lower than that for many antibodies. Antibodies with an affinity constant in the range of 10^8−10^9 M^{-1} are regularly used with SPR biosensors, to obtain lower detection limits. Hence, the detection limit and linearity range could be improved with an antibody engineered to have a higher affinity constant. As explained previously, an ELISA test for another variant of β-lactamase had similar a mass-detection limit, indicating that the affinity is near that which has been reported herein. The $\Delta\lambda_{SPR,max}$ value for this sensor is 2.7 nm, which corresponds to a surface concentration of 1.5 pmol/cm^2 or 9.0 $\times$ 10^{11} molecules/cm^2. This signifies that, at saturation, a β-lactamase:antibody ratio of 1.7:1 is achieved. Typically, an antigen:antibody ratio of 1.5:1 is obtained with NHS chemistry.[62] Thus, multiple binding per antibody occurs with this system, which is consistent with antibodies having 2 binding sites per molecule. It also indicates that the fraction of active antibody is close to 87%, which is slightly higher than the typical 70%−80%.[61,64] This result suggests a greater retention of activity for the antibody immobilized on 3-MPA-(Ser)$_5$−OH, although the uncertainty on the literature value of the typical active fraction at the surface prohibits statistical analysis to confirm this theory.

The SAM that is based on 3-MPA-(Ser)$_5$−OH not only provides improved resistance to nonspecific adsorption in serum and greater retention of activity at the surface, but also yields a greater antibody density immobilized onto the surface of the SPR biosensor. The average $\Delta\lambda_{SPR}$ induced by the immobilization of TEM-1 anti-β-lactamase on a NHS-MHA is 2.15 nm, according to our previous studies.[47] However, with 3-MPA-(Ser)$_5$−OH-based SAMs, the average SPR response induced by the immobilization of anti-β-lactamase is 8.35 nm, which is a 4-fold improvement, relative to NHS-MHA. This demonstrates that 3-MPA-(Ser)$_5$−OH SAMs present a significant number of available −COOH groups at the surface and thus increase the amount of biological receptors attached to the SPR surface. Coupled with the increased retention of activity, this significantly improves the performance of the SPR sensor. An improvement of ~1 order of magnitude is observed in the response obtained with either a 700-nM β-lactamase solution, using NHS-MHA ($\Delta\lambda_{SPR}$ = 0.13 nm),[47] or a 909-nM β-lactamase solution, using a 3-MPA-Gly−OH SAM ($\Delta\lambda_{SPR}$ = 0.25 nm),[28] relative to 700-nM β-lactamase solution using 3-MPA-(Ser)$_5$−OH ($\Delta\lambda_{SPR}$ = 1.8 nm). These responses were obtained using the identical experimental conditions but different SAMs to immobilize anti-β-lactamase. This significant improvement is explained with the improved retention of activity and the greater density of immobilized antibody molecules on the 3-MPA-(Ser)$_5$−OH SAM.

Improved Specificity with 3-MPA-(Ser)$_5$−OH-Based SPR Biosensors. The analytical response is significantly improved for β-lactamase using a 3-MPA-(Ser)$_5$−OH SAM and a minimized nonspecific adsorption is obtained in serum relative to 16-MHA SAM. A more stringent test for performance of the biosensors is to measure the concentration of TEM-1 β-lactamase in a crude cell lysate. The crude cell lysate generated here contained 30−60 mg/mL of nonspecific proteins, compared to 76 mg/mL for bovine serum, thus constituting a good system for comparison. Thus, to validate the performance of the SPR sensor in crude cell lysate, a measurement of the blank cell lysate was performed on the SPR sensor with 3-MPA-(Ser)$_5$−anti-β-lactamase or with 16-MHA−anti-β-lactamase. The sensorgrams were compared to a measurement with crude cell lysate overexpressing TEM-1 β-lactamase (see Figure 5). Nonspecific adsorption was much more significant with 16-MHA−anti-β-lactamase, compared to 3-MPA-(Ser)$_5$−anti-β-lactamase. To verify reproducibility and rule out any physical effect from the replacement of a solution in the fluidic cell, a second portion of the blank cell lysate was applied to both SAMs. For 3-MPA-(Ser)$_5$−anti-β-lactamase, there was no change in signal, showing that no more nonspecific interaction occurs after a 12.5 min exposure to cell lysate, whereas for 16-MHA−anti-β-lactamase SAMs, nonspecific adsorption continued to occur at a steady rate.

Following the conditioning of the sensor with the blank cell lysate on 3-MPA-(Ser)$_5$−anti-β-lactamase, the cell lysate with β-lactamase was injected. As shown in Figure 5, the kinetic curve demonstrated an important shift of λ_{SPR}, illustrating the excellent

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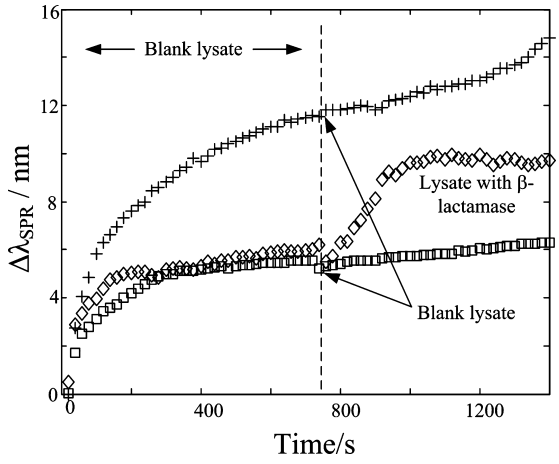

Figure 5. Detection of β-lactamase ($\diamond$) in crude cell lysate and blank measurements for two different SAMs ((+) 16-MHA and ($\square$) 3-MPA-$(Ser)_5$−OH). The dashed line represents the replacement of blank crude cell lysate with a second sample of blank lysate for (+) 16-MHA−anti-β-lactamase and ($\square$) 3-MPA-$(Ser)_5$−anti-β-lactamase or with crude cell lysate containing β-lactamase, in the case of detection measurement with 3-MPA-$(Ser)_5$−anti-β-lactamase. Nonspecific adsorption reached a maximal value after ~400 s for 3-MPA-$(Ser)_5$−OH, allowing the detection of β-lactamase in the crude cell lysate, while nonspecific adsorption was more important and occurred over a longer period for 16-MHA.

specificity of 3-MPA-$(Ser)_5$−OH-based SPR biosensors. Comparing the kinetic measurement using 16-MHA performed under the same experimental conditions demonstrated that serine-based SAMs reduce nonspecific interactions by a factor of 2, relative to 16-MHA. Thus, a direct measurement is possible with 3-MPA-$(Ser)_5$−anti-β-lactamase, following a short conditioning of the sensor with the biological fluid in which the analyte is dissolved.

Quantification of β-Lactamase in Crude Cell Lysate. To demonstrate the efficiency of the 3-MPA-$(Ser)_5$−OH monolayer on a SPR biosensor, an experimental procedure was designed for the quantification of β-lactamase in crude bacterial cell lysate. Controls using the blank bacterial cell lysate (not overexpressing β-lactamase) were performed to correct for background signal, because *E. coli* strains naturally express β-lactamases at very low levels; the resulting signal was negligible. Then, *E. coli* cells that overexpress TEM-1 β-lactamase were lysed. The β-lactamase activity assay and the Bradford assay[52] of the complex solution determined a β-lactamase concentration of 0.39 mg/mL (12.8 μM) and a total protein concentration of the primary lysate of ~30−60 mg/mL. To validate the biosensor, it was necessary to dilute the crude cell lysate 100-fold, because a micromolar-scale concentration of β-lactamase in the lysate is within the saturation range of the SPR sensor. The blank cell lysate provided an appropriate medium to dilute the overexpressed β-lactamase sample without otherwise changing the composition of the solution. The diluted β-lactamase sample was injected onto the biosensor, using the same procedure as the solutions prepared in PBS. The nonspecific interactions reached a maximum within the first few minutes of exposure of the biosensor to the crude cellular lysate. The concentration of TEM-1 β-lactamase was calculated according to the linear regression of the previous calibration of β-lactamase in PBS. A value of 56 μM was obtained for the undiluted cell lysate, which is in good agreement with the values obtained by measurement of the enzyme activity (12.8 μM). Hence, the actual concentration measured in the solution is in the submicromolar range, taking into account the 100-fold dilution into blank cell lysate. This indicates that the SPR sensor can detect submicromolar concentrations of β-lactamase in a relevant, biologically complex solution.

CONCLUSIONS

This article reports the use of 3-MPA-$(AA)_n$−OH-based self-assembled monolayers (SAMs) to reduce nonspecific protein adsorption on surface plasmon resonance (SPR) biosensors. The synthesis of SAMs with amino acids with a chain length of $n = 2-5$, attached to 3-mercaptopropionic acid (3-MPA), was accomplished using a solid-phase approach on phenoxy resin lanterns, allowing combinatorial-type synthesis. The molecules synthesized were characterized by liquid chromatography−mass spectroscopy (LC-MS) before forming a SAM on thin glass slides coated with a thin film of gold. The surface concentration of the SAMs on gold varied between 98×10^{12} molecules/cm^2 and 486×10^{12} molecules/cm^2, with longer peptide chains forming less-dense monolayers. The advancing contact angles showed that longer peptidic chains on the SAM enhanced the hydrophobic or the hydrophilic properties of the SPR surface, depending on the peptide. 3-MPA-$(AA)_n$−OH prepared with the polar and ionic amino acids Ser, His, and Asp, showed better performances, with regard to limiting the nonspecific signal induced by bovine serum proteins by a factor of 2−3, in comparison to the 3-MPA-$(AA)_n$−OH compound prepared from the more-hydrophobic amino acids Leu and Phe. The attachment of a biological receptor to 3-MPA-$(Ser)_5$−OH, using *N*-hydroxysuccinimide ester chemistry, allowed the calibration of TEM-1 β-lactamase in PBS that led to the quantification of submicromolar concentrations of this enzyme in crude cell lysate. 3-MPA-$(Ser)_5$−OH increased the amount of antibodies available at the surface of a SPR biosensor and provided a greater retention of activity and a lower nonspecific signal induced by proteins from crude bacterial lysate. This increased the analytical signal observed by a factor of ~10, compared to the results obtained for alkanethiol or 3-MPA-amino acid-based biosensors. Thus, these 3-MPA-$(AA)_n$−OH SAMs exhibited numerous advantages for SPR biosensing in complex biological fluids.

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