Anal. Chem. **2009,** *81,* 4192–4199

Polymer-Oligopeptide Composite Coating for Selective Detection of Explosives in Water

Marta Cerruti,*,†,‡ Justyn Jaworski,§,|| Digvijay Raorane,‡,⊥ Chris Zueger,‡ John Varadarajan,‡ Carlo Carraro,† Seung-Wuk Lee,|| Roya Maboudian,† and Arun Majumdar‡

Department of Chemical Engineering, Department of Mechanical Engineering, and Department of Bioengineering, University of California at Berkeley, Berkeley, California 94720, and Joint Graduate Group in Bioengineering, University of California, Berkeley and University of California San Francisco, California 94720

The selective detection of a specific target molecule in a complex environment containing potential contaminants presents a significant challenge in chemical sensor development. Utilizing phage display techniques against trinitrotoluene (TNT) and dinitrotoluene (DNT) targets, peptide receptors have previously been identified with selective binding capabilities for these molecules. For practical applications, these receptors must be immobilized onto the surface of sensor platforms at high density while maintaining their ability to bind target molecules. In this paper, a polymeric matrix composed of poly(ethylene-co-glycidyl methacrylate) (PEGM) has been prepared. A high density of receptors was covalently linked through reaction of amino groups present in the receptor with epoxy groups present in the co-polymer. Using X-ray photoelectron spectroscopy (XPS) and gas-chromatography/mass spectroscopy (GC/MS), this attachment strategy is demonstrated to lead to stably bound receptors, which maintain their selective binding ability for TNT. The TNT receptor/PEGM conjugates retained 10-fold higher TNT binding ability in liquid compared to the lone PEGM surface and 3-fold higher TNT binding compared to non-specific receptor conjugates. In contrast, non-target DNT exposure yielded undetectable levels of binding. These results indicate that this polymeric construct is an effective means of facilitating selective target interaction both in an aqueous environment. Finally, real-time detection experiments were performed using a quartz crystal microbalance (QCM) as the sensing platform. Selective detection of TNT vs DNT was demonstrated using QCM crystals coated with PEGM/TNT receptor, highlighting that this receptor coating can be incorporated as a sensing element in a standard detection device for practical applications.

The detection of explosives has been the focus of many studies, both in the gas phase, to detect the presence of buried or conceived explosives from the sublimating vapors,[21,30,34,38] and in the liquid phase, to detect contamination in soil and groundwater in proximity of ammunition depots.[1,8,13,24,34,37] The latter is particularly important because explosives such as TNT and its derivatives are known to be toxic.[16]While most sensors presented in the literature are highly sensitive, often their selectivity is insufficient for performance in the field. In practical applications selectivity is critical to successful detection, where the molecule of interest exists in a complex environment together with many other species that can create false positive signals. This is primarily attributed to the lack of diversity available for sensor coatings specific for these targets,[10] as most current coating technologies rely on weak or non-specific molecular interaction.[3,7,9,11,12,14,17,18,21,27,30] In the case of explosive detection, highly selective coatings capable of discriminating between TNT, DNT, and other explosive molecules have been achieved through the use of molecule specific antibodies.[1,8] Antibodies, while often possessing high affinities for their target molecules, are limited to aqueous environments within a limited pH range, temperature range, and ionic activity to retain their active binding configuration. Further-

* To whom correspondence should be addressed. E-mail: marta.cerruti@gmail.com.

† Department of Chemical Engineering.

‡ Department of Mechanical Engineering.

§ Joint Graduate Group in Bioengineering.

|| Department of Bioengineering.

⊥ Current affiliation: Applied Materials Inc., 3050 Bowers Avenue, Santa Clara, CA 95054, U.S.A.

(1) Anderson, G. P.; Moreira, S. C.; Charles, P. T.; Medintz, I. L.; Goldman, E. R.; Zeinali, M.; Taitt, C. R. *Anal. Chem.* **2006**, *78*, 2279–2285.
(2) Arya, S. K.; Solanki, P. R.; Singh, R. P.; Pandey, M. K.; Datta, M.; Malhotra, B. D. *Talanta* **2006**, *69*, 918–926.
(3) Casalini, R.; Kilitziraki, M.; Wood, D.; Petty, M. C. *Sens. Actuators, B* **1999**, *56*, 37–44.
(4) Cerruti, M. Fissolo, S. Carraro, C. Majumdar, A., and Maboudian, R. *Langmuir* **2008**, ASAP.
(5) Clark, D. T.; Thomas, H. R. *J. Polym. Sci. Pol. Chem.* **1978**, *16*, 791–820.
(6) Clochard, M.-C.; Betz, N.; Goncalves, M.; Bittencourt, C.; Pireaux, J.-J.; Gionnet, K.; Deleris, G.; Le Moel, A. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2005**, *236*, 208–215.
(7) Echols, R. T.; Christensen, M. M.; Krisko, R. M.; Aldstadt, J. H. *Anal. Chem.* **1999**, *71*, 2739–2744.
(8) Whitley, A.; Hayhurst, J. L.; Medintz, I. L.; Mattoussi, H. *J. Am. Chem. Soc.* **2005**, *127*, 6744–6751.
(9) Hagleitner, C.; et al. *Nature* **2001a**, *414*, 293–296.
(10) Grate, J. W.; et al. *Anal. Chem.* **2001b**, *73*, 5247–5259.
(11) Giordano, M.; et al. *Appl. Phys. Lett.* **2004**, *85*, 5349–5351.
(12) Patel, S. V.; et al. *Sens. Actuators, B* **2003**, *96*, 541–553.
(13) Gao, D.; Wang, Z.; Liu, B.; Ni, L.; Wu, M.; Zhang, Z. *Anal. Chem.* **2008**, *80*, 8545–8553.
(14) Grate, J. W.; Nelson, D. A.; Skaggs, R. *Anal. Chem.* **2003**, *75*, 1868–1879.
(15) Grun, J.; Revell, J. D.; Conza, M.; Wennemers, H. *Bioorg. Med. Chem.* **2006**, *14*, 6197–6201.
(16) Hannink, N.; Rosser, S. J.; French, C. E.; Basran, A.; Murray, J. A. H.; Nicklin, S.; Bruce, N. C. *Nat. Biotechnol.* **2001**, *19*, 1168–1172.
(17) Heirlemann, A.; Ricco, A. J.; Bodenhofer, K.; Dominik, A.; Gopel, W. *Anal. Chem.* **2000**, *72*, 3696–3708.
(18) Janata, J.; Josowicz, M. *Nat. Mater.* **2003**, *2*, 19–24.

10.1021/ac8019174 CCC: $40.75
Published on Web 04/30/2009

more, their large size may not prevent sufficiently high density coatings for practical sensing applications.

Recently, we have demonstrated that phage display can be used to obtain highly selective oligopeptide-based receptors for small volatile molecules, which combine high affinity with the target of interest while retaining a small size and possessing chemical robustness. In particular, by exploiting the combinatorial screening power of this technique, we showed that oligopeptides are capable of selectively binding the explosive TNT while remaining inactive toward DNT and vice versa.[19] For many sensing applications, a high density of receptor is required to yield a measurable signal when the target concentration is low. This is the case for TNT, which has a vapor pressure of $\sim 2 \times 10^{-4}$ Torr at 25 °C,[26] and water solubility of 100 mg/L.[29]Therefore, the development of coating materials containing a high density of specific receptors with intact binding efficacy is the critical next step for the fabrication of specific and sensitive sensing devices.

We have examined suitable polymers to use as matrixes to embed the receptors in, with the goal of increasing the number of bound receptors available for interactions with the target molecules. The polymeric matrixes were selected to contain reactive groups readily available for coupling with the functional groups present on the receptors. In this paper we report on a particular co-polymer meeting such requirements: poly(ethylene-co-glycidyl methacrylate), called "PEGM". This co-polymer (Figure 1A) contains an epoxy group in the glycidyl methacrylate monomer, which reacts with amino groups present in the receptor structure to form stable covalent linkages. Such bonds are not hydrolyzed in water, thus making this coating particularly suited for detection in aqueous environments. This is of relevance for DNT and TNT detection because these explosives are commonly stored in ammunition depots or weapon testing sites[24] and can seep into the surrounding ground waters. As they are known to be harmful to health,[16] their detection in liquid is especially important to monitor and regulate the contamination in groundwater.

Here, we first show the successful covalent conjugation of an oligopeptide TNT receptor (developed through phage display) to PEGM, as verified using XPS. Then, we demonstrate that the oligopeptides embedded in PEGM retain their functional activity as receptors for TNT in water. Finally, we demonstrate real-time selective detection of TNT in water using a QCM sensing platform as a proof of concept. These results indicate these receptors embedded in PEGM as promising coatings for the development of highly selective and sensitive sensors for explosive detection.

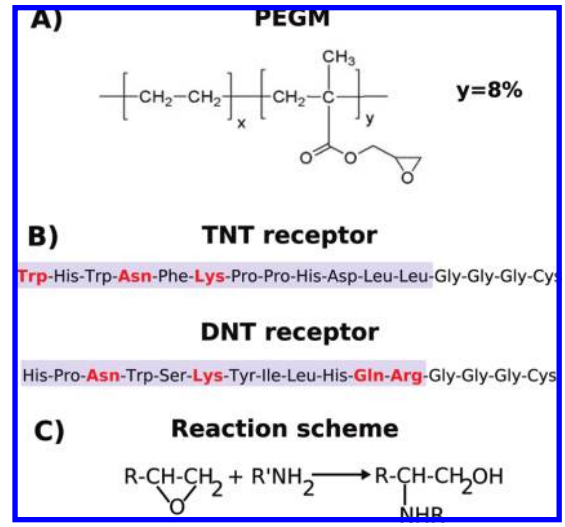

Figure 1. Polymer and receptors used in the present study, and schematics of the reaction used to bind them together. Section A: structure of poly(ethylene-co-glycidyl methacrylate), called "PEGM" in the paper. Section B: primary structure of the TNT and DNT receptors used. The first highlighted twelve amino acids are those composing the sequence of the receptors found through phage display, whereas the final three glycines and cysteine were added during the synthesis of the peptides used in this work. The amino acids written in red are those possessing a primary amino group that can react with the polymer according to the scheme presented in Section C. Section C: schematic of the reaction between PEGM and the receptors. R is the residue from PEGM, whereas R′ is from the receptors.

MATERIALS AND METHODS

Materials. A PEGM (Sigma-Aldrich (St. Louis, MO)) containing solution was prepared using xylene (Fischer Scientific (Pittsburgh, PA)) as a solvent, with a final concentration of 10 mg/mL. Mild heating to ~50 °C was required to achieve complete dissolution of PEGM. Primary structures of the receptors used in the present paper are shown in Figure 1, and their synthesis is described below. Solutions of trinitrotoluene and dinitrotoluene (Chem Service Inc. (West Chester, PA)) were prepared in ethanol for both XPS and GC/MS experiments. Pre-loaded cysteine-Wang resin, amino acids, and coupling reagents were purchased from Novabiochem (San Diego, CA) for peptide synthesis. Cleavage reagents trifluoroacetic acid, thioanisole, water, phenol, ethanedithiol, and triisopropylsilane were also obtained from Sigma-Aldrich (St. Louis, MO).

Peptide Synthesis. TNT and DNT receptors found through phage display were created using solid-phase peptide synthesis to provide pure forms of the receptor for analysis and for attachment to the polymer matrix. Using standard Fmoc chemistry,[15] pre-loaded cysteine-Wang resin was used for 0.1 mmol scale synthesis of the peptides seen in Figure 1. Resins were preswelled for 30 min in NMP prior to deprotection. Deprotection steps using 3 mL of 3% DBU in NMP were carried out for 20 min on a rocking platform. Washing steps involved rinses with 4 mL of NMP, 4 mL of methanol followed by 4 mL of dichloromethane, and with 4 mL of NMP. Coupling steps of 3 mL of 0.2 M amino acid, Hobt, and DIC were carried out on a rocking platform for 2 h. Kaiser tests were performed to identify the presence of primary amines to monitor the extent of reaction.[20] Cleavage reactions were performed for 2 h with a cocktail of 82.5%

(19) Jaworski, J. W.; Raorane, D. R.; Huh, J. H.; Majumdar, A.; Lee, S. W. *Langmuir* **2008**, *24*, 4938–4943.
(20) Kaiser, E.; Colescot, R.; Bossinge, C.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595.
(21) Narayanan, A.; Varnavski, O. P.; Swager, T. M.; Goodson, T. *J. Phys. Chem. C* **2008**, *112*, 881–884.
(22) Olivares, O.; Likhanova, N. V.; Gomez, B.; Navarrete, J.; Llanos-Serrano, M. E.; Arce, E.; Hallen, J. M. *Appl. Surf. Sci.* **2006**, *252*, 2894–2909.
(23) Park, S. M.; Park, J. W.; Ha, H.-P.; Han, H.-S.; Seo, G. *J. Mol. Catal. A: Chem.* **2007**, *273*, 64–72.
(24) Pennington, J. C.; Brannon, J. M. *Thermochim. Acta* **2002**, *384*, 163–172.
(25) Provatas, A.; Matisons, J. G.; Smart, R. St. C. *Langmuir* **1998**, *14*, 1656–1663.
(26) Pushkarsky, M. B.; Dunayevskiy, I. G.; Prasanna, M.; Tsekoun, A. G.; Go, R.; Patel, C. K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19630–19634.

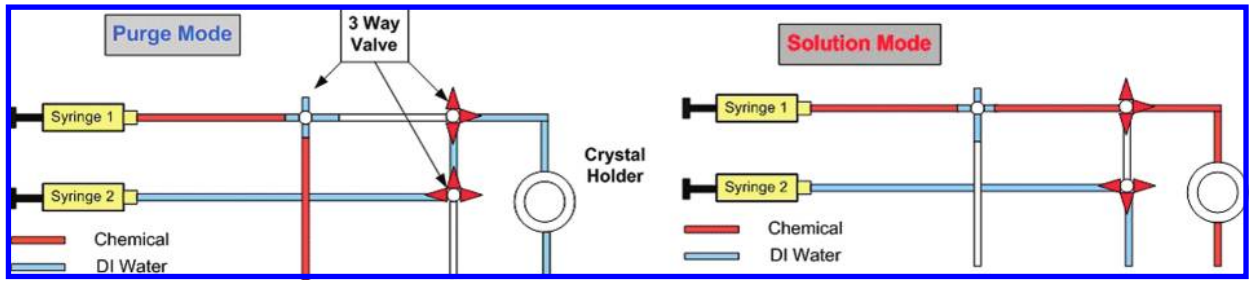

Figure 2. Schematics showing the two modes of operation of the QCM setup.

trifluoroacetic acid, 5% thioanisole, 2.5% water, 5% phenol, 2.5% ethanedithiol, and 2.5% triisopropylsilane. Samples were purified by HPLC to >95% purity.

Preparation of Polymeric/Receptor Coating. Gold substrates used to deposit the polymeric and receptor coatings were prepared by sputtering a 5 nm layer of Cr on a (100) Si wafer, followed by 25 nm of Au. They were diced to the desired dimensions (5×5 mm^2 for XPS analysis, 3×3 mm^2 for GC/MS analysis), sonicated in acetone for 15 min, and further cleaned using UV-Ozonolysis (UVO-Cleaner, model 42, Jelight Company, Inc. (Irvine, CA)) for 5 min. A droplet of PEGM solution was immediately placed onto the Au substrate and allowed to dry, leaving a fairly uniform coating on the substrate. A volume of 10 μL was used for XPS experiments and 5 μL for GC/MS experiments. The TNT or DNT receptor attachment was carried out by exposing the polymer-coated chips to solutions containing 2.5 mg/mL of receptor, in 90% acetonitrile (ACN, Fischer) and 10% ultrapure 18 MΩ water. Volumes of 15 μL for XPS experiments, and 5 μL for GC/MS were exposed to the polymer, followed by 15 and 5 μL of triethyl amine (TEA, 0.8 mg/mL in ACN) for XPS experiments and GC/MS substrates, respectively. TEA was used as a catalyst to favor the reaction between the polymer and the receptor sketched in Figure 1C.

XPS Analysis of Receptor Activity. Samples used for XPS analysis were prepared by drying 30 μL of a 22 mM solution of TNT onto gold chips coated with PEGM and receptive peptides. The samples were then evacuated in the loading-lock chamber for 30 min, and then transferred to the measurement chamber, with a base pressure of 10^{-9} Torr, at which point the spectra were acquired to quantify the amount of TNT on the samples. To identify the extent of receptor activity, samples were then washed overnight in water to remove non-specifically bound TNT, followed by nitrogen drying. XPS measurements were carried out after this wash to identify the extent of TNT that remained bound to the surface. All XPS measurements were performed with an Omicrometer EA125 electron energy analyzer and an Omicrometer DAR400 source with Al Kα X-rays at an energy of 1486.6 eV. The detector angle was 0° to the surface normal. Spectral deconvolution was performed after background subtraction with the Shirley method. The spectra were analyzed using Origin 6.0 software, and peaks were fit to Gaussians or Lorentzians to determine peak positions. For energy calibration purposes, the Au $4f_{7/2}$ peak was used from the substrate as a reference at 84 eV.[32] If the Au from the substrate was too low in intensity to read, the hydrocarbon contamination peak was used as a reference at 285 eV, as explained by Clark and Thomas.[5] It should be noted that many of the references cited in this paper did not follow this convention, and set the hydrocarbon contamination and the gold peaks at lower energies. Thus, care should be taken when comparing the energies reported in the present work with those published in previous literature.

GC/MS Analysis of Receptor Activity. Samples used for GC/MS were prepared by drying 5 μL of a 1 mM ethanolic solution of TNT on gold chips coated with PEGM and receptors. The samples were then placed inside the desorption tube of a thermal desorber (Markes Intl. Inc.) and heated at 300 °C using a Unity Thermal Desorption System. The desorbed material was passed directly into an GC/MS calibrated for TNT quantification (GC model Agilent 6890N, MS model 5975 Inert Mass Selective Detector). Control experiments were performed to ensure that a consistent amount of TNT was initially exposed to each substrate. Analogous GC/MS measurements were carried out on samples exposed to TNT after soaking in water overnight. More than one chip was loaded in the thermal desorber to provide a sufficiently readable signal for samples washed overnight. This was necessary because the TNT bound to only one sample of PEGM or DNT receptor functionalized surfaces was too low to be detected. Thus, at least three chips of samples containing the DNT receptor and at least 15 samples coated with just PEGM were loaded in the thermal desorber, and the amount of TNT measured was then divided by the number of samples used to get the average amount of TNT left on each sample. A final set of experiments was carried out with GC/MS by drying 5 μL of a 1 mM ethanolic solution of DNT. GC/MS measurements were carried out on these samples before and after overnight washing in water. More than one chip was placed in the thermal desorber to obtain a detectable signal, as explained above. The amount of TNT and DNT remaining on the samples washed overnight was evaluated by comparing the GC/MS response obtained for such samples with that obtained for the samples prepared containing 5 nmol of TNT or DNT, prior to washing.

QCM Detection of TNT and DNT in Water. These experiments were performed using a Research Quartz Crystal Microbalance (Maxtek, Inc. (Cypress, CA)). The quartz crystal was placed in a Teflon crystal holder which also acted as a reaction chamber. The crystal holder equipped for liquid flow was connected by Teflon tubings to two syringe pumps, containing deionized (DI) water and a solution of either DNT or TNT, respectively. These solutions were prepared by dissolving 100 mg/L of either TNT or DNT in DI water and rocking overnight to ensure complete dissolution. A schematic of the system used for this set of experiments is shown in Figure 2.

A 1″ diameter, gold-coated quartz crystal was coated with 5 μL of PEGM solution prepared as described above. TNT receptor

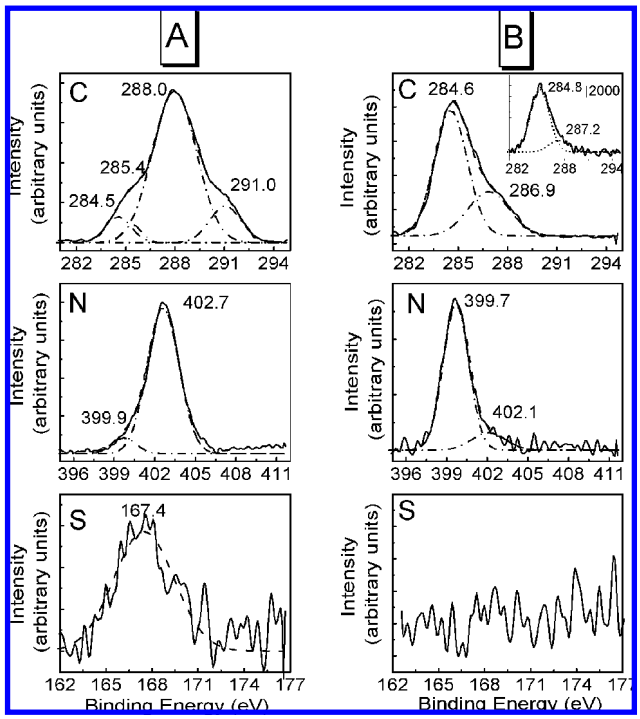

Figure 3. XPS spectra of C 1s (first row), N 1s (second row) and S 2p (third row) peaks of TNT receptor deposited on a bare Au slide before (column A) and after (column B) washing in water overnight. Experimental spectra are plotted in solid line, fitted spectra in dashed line, and fits for each peak component in dash-dotted line. The inset in the C peak, column B, represents the C 1s spectrum recorded on a bare Au substrate.

was then bound to PEGM following the same procedure described before. As shown in Figure 2, the flow rates of solutions exiting the syringes are controlled using a set of three-way valves, adjusted in such a way that only one solution flows through the sensor chamber (quartz crystal holder) at any given time. Therefore, the setup can be used in either "solution mode" or "purge mode", allowing the chamber to be flushed with a solution containing the molecule of interest (TNT or DNT), or DI water, respectively. Prior to every experiment, the resonance frequency of the quartz crystal was measured and a stable baseline was obtained in the "purge mode". Then, the system was switched to "solution mode", and the crystal coated with TNT receptor/PEGM was exposed to either TNT or DNT solutions. The change in resonance frequency of the quartz crystal was measured in real time. The system was maintained in this mode until no further changes in resonance frequency were observed. Finally, the system was switched back to "purge mode", and the crystal was rinsed with DI water, until a stable baseline was obtained again.

RESULTS AND DISCUSSION

Attachment of TNT Receptor to PEGM. XPS spectra of TNT receptor deposited on a bare Au slide before and after rinsing in water overnight are shown in Figure 3. The C 1s spectrum of the TNT receptor deposited on Au shows different components, representing the many types of carbon environments found in an oligopeptide. A first broad peak is centered at 284.5 eV and is related to aliphatic C atoms. A second peak located at 286.5 eV corresponds to carbon bonded to an amino group ($C\text{-}NH_2$).[36] A second, intense and broad peak is centered at 288.1 eV and may be assigned to carbon in amide groups.[22] This peak is typically indicative of the presence of a peptide on the surface.[28] The last peak located at 290.4 eV may be assigned to a $\pi\pi^*$ shakeup band due to the presence of aromatic groups within the peptide.[6] The N 1s spectrum shows two main peaks. The lowest peak in intensity is centered at ~400 eV and is related to non-protonated amines and amides. The peak at higher energy is indicative of the presence of a large amount of protonated N atoms,[25,33] thus indicating that most amines or imines in the lateral chains of the amino acids composing the peptide are in fact charged. The S 2p spectrum shows a peak at ~168 eV, indicative of the presence of oxidized S in the terminal cysteine present in the receptor.[35]

After washing in water overnight, most of the peptide is washed away from the Au surface. The C 1s spectrum no longer displays peaks at ~288 and ~291 eV; rather it becomes similar to that measured on a bare gold substrate (shown in the inset, where only peaks relative to surface contamination are observed).[4] The peaks that most compellingly indicate the removal of the peptide from the surface are the N and S peaks: the latter has completely disappeared, and in the N spectrum, the most intense peak is now at ~400 eV with only a shoulder observed at ~403 eV. This could be indicative of the presence of a thin layer of peptide on the surface, as a similar effect was observed in the past for histidine films on Au.[39] This is confirmed by the drastic decrease in total N measured on the sample soaked in water. The ratio of the total area underlying the N peaks to that underlying the Au peaks was 23.5 before soaking in water and 0.07 afterward, which indicates that virtually all the peptide is washed away by overnight soaking in water, and the Au surface is left mostly bare.

A very different result is obtained if the Au surface is covered with PEGM, and the receptor is bound to it, as shown in Figure 4. The C 1s spectrum of just PEGM on Au (sample called "PEGM/Au" in Figure 4A) displays two main peaks, related to the presence of aliphatic carbon (peak at 285.2 eV) and carboxyl groups (peak at 289.4 eV), as expected from the molecular structure of the polymer (see Figure 1A). No N and S were detected on the polymer film. The spectra recorded on the sample containing the TNT receptor bound to PEGM (sample "TNTrec/PEGM/Au") are shown in Figure 4B. Two new peaks appear in the C 1s spectrum, located at 287.8 and 290.6 eV. These peaks are in similar positions to those observed in Figure 3A, related to amide and carboxylic groups present in the TNT receptor. In particular, the peak at ~288 eV is usually considered to be a very specific indication of the presence of peptides.[6] Also the N and S spectra

(27) Raorane, D.; Lim, S.; Majumdar, A. *Nano Lett.* **2008**, *8*, 2229–2235.
(28) Rezania, A.; Johnson, R.; Lefkow, A. R.; Healy, K. E. *Langmuir* **1999**, *15*, 6931–6939.
(29) Ro, K. S.; Venugopal, A.; Adrian, D. D.; Constant, D.; Qaisi, K.; Valsaraj, K. T.; Thibodeaux, L. J.; Roy, D. *J. Chem. Eng. Data* **1996**, *41*, 758–761.
(30) Rose, A.; Zhu, Z.; Madigan, C. F.; Swager, T. M.; Bulovic, V. *Nature* **2005**, *434*, 876–879.
(31) Sauerbray, G. *Z. Phys.* **1959**, *155*, 206–222.
(32) Seah, M. P.; Gilmore, I. S.; Beamson, G. *Surf. Interface Anal.* **1998**, *26* (7), 642–649.
(33) Tanaka, Y.; Doi, H.; Iwasaki, Y.; Hiromoto, S.; Yoneyama, T.; Asami, K.; Imai, H.; Hanawa, T. *Mater. Sci. Eng., C* **2007**, *27*, 206–212.
(34) Tu, R.; Liu, B.; Wang, A.; Gao, D.; Wang, F.; Fang, Q.; Zhang, Z. *Anal. Chem.* **2008**, *80*, 3458–3465.
(35) Willey, T. M.; Vance, A. L.; van Buuren, T.; Bostedt, C.; Terminello, L. J.; Fadley, C. S. *Surf. Sci.* **2005**, *576* (1–3), 188–196.

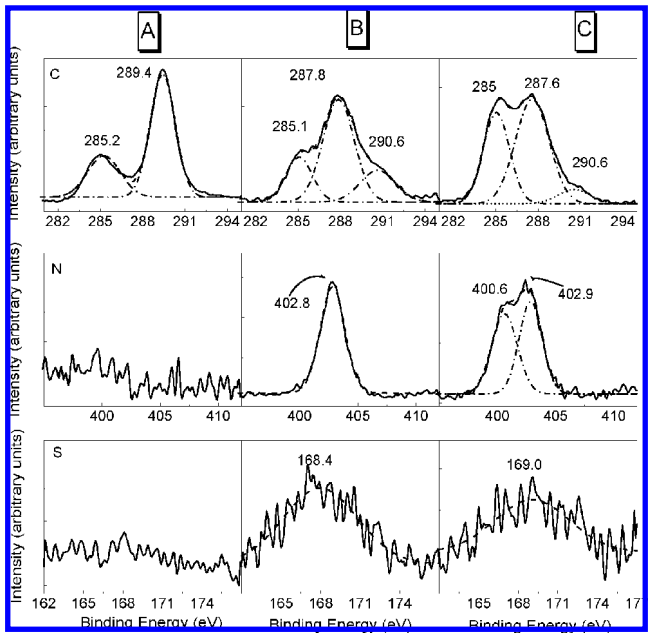

Figure 4. XPS spectra of C 1s (first row), N 1s (second row), and S 2p (third row) peaks of PEGM deposited on Au (sample "PEGM/Au") (column A), and TNT receptor bound to PEGM on Au (sample "TNTrec/PEGM/Au") before (column B), and after (column C) washing in water overnight. Experimental spectra are plotted in solid line, fitted spectra in dashed line, and fits for each peak component in dash-dotted line.

resemble those analyzed for the receptor on Au (Figure 3A). The spectra of the sample TNTrec/PEGM/Au soaked in water overnight are shown in Figure 4C. In the C 1s region, the peak at ~288 eV is still present, which is an indication that the receptor is still present on the substrate. An increase in the peak at 285 eV may be related to the adsorption of impurities on the sample surface, as well as the appearance of some of the underlying PEGM substrate, which has a carbon component at a similar position. The N 1s spectrum splits in two peaks, which is indicative of a modification in the charge of some of the amines and imines in the peptide (the lower energy component increasing in intensity is related to the presence of a larger amount of neutral species). This split may also reflect the removal of multilayers of peptide which had physisorbed onto the surface (see explanation above and ref 39). The S 2p peak remains visible on the sample and maintains the same position it had before soaking in water. All these observations indicate that most of the TNT receptor remains bound to the surface of the PEGM/Au sample. This is a different observation from the sample of TNT receptor drop-casted directly onto gold. The ratio of the total area underlying the N peaks to that underlying the Au peaks remains virtually unchanged after soaking in water, going from 2.4 measured before soaking in water to 2.8 measured after soaking, which is within sample-to-sample variability.

Analysis of TNT Receptor Activity Bound to PEGM. The experiments discussed above show that the TNT receptor can be stably bound to PEGM. It is now crucial to determine if the attachment of the peptide based receptor to the polymer compromises its activity. Previous work showed that the amino acid sequence triplet, tryptophan-histidine-tryptophan, present at the N terminus of the TNT receptor is critical to the target specificity (Trp-His-Trp, see Figure 1B). The chemistry used to attach the receptor to PEGM involves the reaction of primary amino groups from the peptide with epoxy groups from the polymer. This implies that the receptor can be bound via one of the three amino acids highlighted in red in Figure 1B: the fourth amino acid, asparagine, and the sixth, lysine, both present primary amino groups in their side chain, whereas the first tryptophan has a free terminal amino group, not involved in the formation of a peptidic bond. Statistically, about one-third of the TNT receptors are bound to PEGM via this first tryptophan, which is one of the amino acids involved in TNT recognition. This may imply that the activity of the receptor could be compromised after it is bound to the polymer. To analyze the receptor activity, a series of XPS and GC/MS experiments were performed to assess and quantify the presence of TNT bound to the receptor after overnight washing in water. This amount was then compared with the results obtained for non-specific adsorption of TNT on PEGM and for adsorption on a DNT-receptor with no specific affinity to TNT, also bound to PEGM.

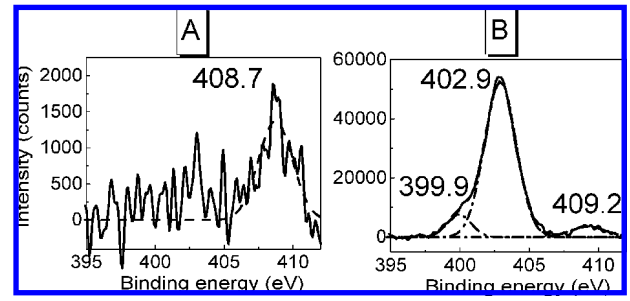

Figure 5. XPS spectra of N 1s region for samples prepared by deposition of 660 nmol of TNT on (A) PEGM/Au and (B) PEGM with TNT receptor. Experimental spectra are plotted in solid line, fitted spectra in dashed line, and fits for each peak component in dash-dotted line.

XPS Experiments. XPS was used to determine whether the TNT receptor linked to PEGM was still active toward TNT binding. The N spectrum of a droplet containing 660 nmol of TNT deposited on PEGM/Au is shown in Figure 5A. As PEGM does not contain nitrogen in its structure, the appearance of a peak at 408.7 eV after TNT deposition is readily assigned to the NO_2 groups of TNT. This is in agreement with the position observed for nitric groups in other inorganic and organic structures.[2,23] The position of NO_2 at such high binding energies allows for an effective identification of the presence of TNT also when other N-containing species are present on the sample. As an example, the spectrum of a similar TNT droplet deposited on a sample of TNTrec/PEGM/Au is shown in Figure 5B. The position of this peak is distant from other peaks relative to the peptide, thus implying that the amount of TNT can be determined without the need of laborious and somewhat uncertain spectral deconvolution.

To test the activity of the TNT receptor bound to PEGM, the area of the N peak at ~ 409 eV right after the deposition of the TNT droplet is compared with the area of the same peak after

(36) Xiao, S.-J.; Textor, M.; Spencer, N. D. *Langmuir* **1998**, *14*, 5507–5516.
(37) Xie, C.; Liu, B.; Wang, Z.; Gao, D.; Guan, G.; Zhang, Z. *Anal. Chem.* **2008**, *80*, 437–443.
(38) Yang, Y.; Swager, T. *J. Am. Chem. Soc.* **1998**, *120*, 11864–11873.
(39) Zubavichus, Y.; Zharnikov, M.; Yang, Y.; Fuchs, O.; Heske, C.; Umbach, E.; Tzvetkov, G.; Netzer, F. P.; Grunze, M. *J. Phys. Chem. B* **2005**, *109*, 884–891.

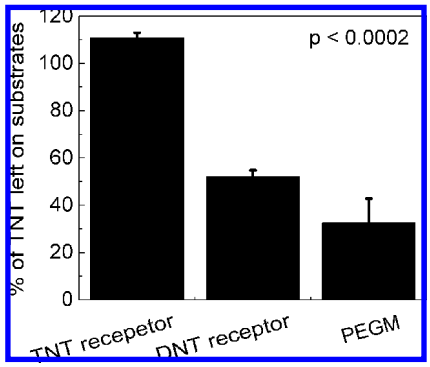

Figure 6. Percentage of TNT remaining on samples containing TNT receptor, DNT receptor, and just PEGM after overnight washing in water. The values were calculated by dividing the normalized area underlying the peak at ~409 eV relative to TNT after washing the sample in water to the normalized area of the same peak measured before washing the sample, and multiplying the result by 100, to obtain a percentage. Details about the normalization of the peak relative to TNT and *p* values calculated according to Student's *t* test are reported in the text.

soaking the TNT-containing sample in water overnight. Washing in water overnight removes only the physisorbed TNT, whereas the TNT bound to the receptor remains on the sample. As previously mentioned, the effect of TNT removal by washing overnight in water is analyzed for samples containing the TNT receptor bound to PEGM (TNTrec/PEGM/Au), samples of just PEGM/Au, and samples containing the peptide that is a receptor for DNT instead of TNT (samples DNTrec/PEGM/Au). This allows one to confirm if the specific binding of TNT to the TNT receptor is retained after washing. To be able to compare the area of the N peak at ~409 eV before and after washing, one needs to decide which components of the spectrum are to be used for normalization. The Au peaks arising from the substrate are a good choice for the sample PEGM/Au. However, the intensity of these peaks is too low for the DNTrec/PEGM/Au and TNTrec/PEGM/Au samples because of the extreme surface sensitivity of XPS and cannot be used for the normalization of the NO_2 peak. Hence, the normalization for these samples is performed using the area underlying all the components of the N 1s spectrum, which includes the peak relative to TNT and the peaks relative to the amines, imines, and amides from the peptide. As previously shown, the peptide bound to PEGM is stable to soaking in water overnight, and hence the only difference in the components of the N spectrum before and after overnight soaking in water is due to the removal of TNT. The results obtained from the percentage ratio of the normalized area underlying the N 1s peak relative to TNT before and after soaking in water overnight, multiplied by 100 to obtain a percentage, are shown in Figure 6 for all the samples analyzed.

Almost no TNT is removed after overnight washing of the sample containing the TNT receptor bound to PEGM. On the contrary, when a non-specific receptor is used, such as the DNT, about 50% of the TNT is washed away by overnight soaking in water. The non-specific adsorption of TNT on the polymer PEGM is also shown in Figure 6. Student's *t* tests run on these data indicate a p value less than 0.0001 when comparing the TNT remained on PEGM coated with TNT and DNT receptors. Additionally, a *p* value of less than 0.0002 was calculated for the resultant TNT remaining on the DNT receptor/PEGM as compared to only PEGM. This indicates that the average amounts of TNT remaining on the three samples are significantly different. These results are not quantitative because of the assumption that none of the peptide was removed during overnight soaking, required for the normalization of the area underlying the TNT peak for samples containing receptors. In fact, the possible removal of some of the peptide during overnight washing may be the reason for the value of greater than 100% for the TNT on the sample containing the TNT receptor. Also, the measurements are performed in vacuum, which implies that some of the TNT might have desorbed. Still, these results clearly show a high level of selective binding of TNT molecules to the TNT receptor, compared to the binding to DNT receptor or non-specific adsorption on the polymeric matrix.

GC/MS Experiments. A quantitative analysis of the amount of TNT remaining on samples after washing in water overnight can be performed using GC/MS. The same set of experiments described in the XPS section above was conducted, using a lower amount of TNT because of the very high sensitivity of GC/MS. In particular, a droplet of a solution containing 5 nmol of TNT was exposed to each sample. The samples were then washed in water overnight, and the amount of TNT left on each sample was evaluated with GC/MS.

The results shown in Figure 7 confirm those obtained with XPS, namely, the amount of TNT retained on samples containing the receptor specific to TNT was much higher than that measured on samples containing a non-specific DNT receptor. The non-specific adsorption of TNT on PEGM was very low. The *p* values calculated from the Student's *t* test were lower than 0.0001 when comparing all data sets presented in Figure 7. Quantitavely, the ratio of TNT remaining on samples TNTrec/PEGM/Au to that left on samples DNTrec/PEGM/Au to that measured on PEGM/Au samples was 1:0.33:0.06 for GC/MS, and 1:0.46:0.29 for XPS. This shows that the trend measured with the two techniques was the same, although a higher non-specific adsorption was measured with XPS. Also, it must be noted that the absolute amounts of TNT remaining on the samples after overnight soaking measured by XPS was much higher than that measured by GC/MS. Virtually the same amount of TNT was measured by XPS before and after soaking in water overnight, on the sample containing the receptor specific for TNT. Instead, less than 1% of it was found with GC/MS, for the same type of samples. This observation is attributed to the following factors. XPS was performed in ultrahigh vacuum, and thus only the amount of TNT that resisted evacuation was detected; as such, most of the physisorbed TNT molecules were most likely removed before the spectra were collected. Hence, soaking in water overnight removed a further portion of TNT only on the samples that did not contain the receptor specific for TNT, whereas almost no changes were observed in the amount of TNT measured on samples containing the receptor specific for TNT (Figure 6). Instead, no evacuation was performed before GC/MS measurements, which implies that the physisorbed TNT was removed only during the overnight soaking. Hence, the absolute amount of TNT resisting the overnight soaking was much lower than that initially evaporated on the samples. This explains why the percentages of TNT left on the samples after overnight washing measured by GC/MS are much lower than those

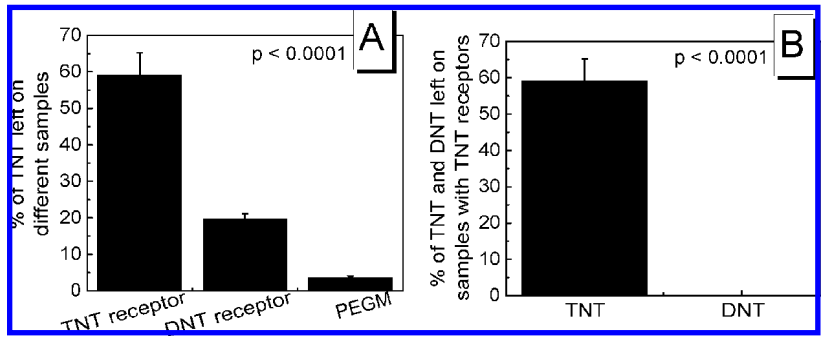

Figure 7. GC/MS measurements of (A) the amount of TNT remaining on samples containing TNT receptor, DNT receptor, and just PEGM after overnight washing in water and (B) the amount of TNT and DNT reamaining on samples containing the receptor specific for TNT, after overnight washing in water. Amounts are given in pmoles, calculated as described in the "Material and Methods" section. Details about *p* values calculated according to Student's *t* test are reported in the text.

measured by XPS (Figure 7). Moreover, only a thin, superficial layer of the solid TNT dried on the samples was in direct contact with the receptors bound to the PEGM/Au surface. This would lead to only a small amount of TNT remaining on the samples after overnight washing, as measured by GC/MS, since only a small fraction of the initial TNT could interact with the receptors, while most of the TNT was dissolved in solution. Lastly, the reason for the proportionally higher non-specific adsorption on PEGM measured by XPS compared to GC/MS is that a much higher absolute amount of TNT was dried on the samples used for XPS compared to those used for GC/MS (660 nmol vs 5 nmol), because of the very different surface sensitivity of the two techniques, which may imply that the overnight washing may not have been sufficient to completely remove all the physisorbed TNT on the samples used for XPS.

These sets of experiments demonstrate the selectivity of TNT binding on the receptor specific for TNT compared to the binding to a non-specific DNT receptor or the lone PEGM polymeric matrix. Another set of experiments was performed with GC/MS to analyze the selectivity for TNT when the receptors were embedded in the PEGM matrix. To identify this selectivity, the same concentration of DNT solution was exposed to samples containing TNT receptors bound to PEGM, followed by washing in water overnight. GC/MS spectra were collected before and after the wash to measure the DNT signal and compare it to that obtained with TNT in the experiments described above. After soaking in water overnight, virtually no DNT could be detected on the samples, as shown in Figure 7B, where this result is compared to that obtained when samples with TNT receptors were exposed to TNT. This analysis shows that only TNT was strictly bound to the TNT receptors and resisted overnight soaking, whereas DNT was not, and the amount of DNT remaining on the samples was below the detection limit of GC/MS.

Real-Time Sensing Experiment: QCM in Liquid Phase. In this final section, the application of the receptor/polymeric coating is demonstrated on a well-known realistic sensing platform, namely, using QCM, in liquid phase. In typical QCM experiments, the resonance frequency of a quartz crystal is measured, which decreases as the mass adsorbed on the crystal increases.[31]

PEGM was deposited on the gold-coated QCM crystal, and TNT receptor was bound to PEGM. After reaching a stable baseline by flowing just DI water, the crystal was exposed to solutions containing 100 mg/L of either TNT or DNT. As seen

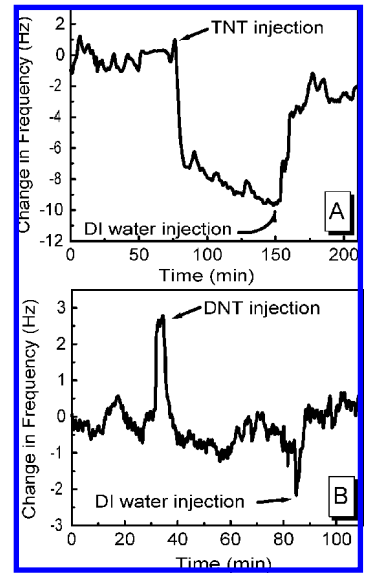

Figure 8. Change in QCM resonance frequency measured on a crystal coated with PEGM/TNT receptor, after exposure of a solution containing TNT (A) and DNT (B), respectively.

from the graph shown in Figure 8A, the resonance frequency measured with QCM decreased noticeably when the crystal was exposed to TNT solution. No appreciable changes in resonance frequency were observed after less than 30 min. After switching back to flowing DI water, the resonance frequency returned to its previous value before the exposure. The decrease in frequency of ~6 Hz observed after exposure to TNT solution is related to an increase in mass adsorbed on the crystal because of the interaction between TNT and TNT receptor. The same coating exposed to a solution of DNT (Figure 8B) shows no appreciable changes in the resonance frequency of the crystal, thus indicating that DNT molecules did not bind appreciably to the TNT receptor. This level of selectivity for TNT and DNT in the liquid phase is superior to many detection techniques previously used,[13,24,34,37] remaining comparable to results obtained using TNT antibodies.[1,8] These results clearly illustrate that the PEGM/TNT receptor coating maintained its high selectivity for TNT toward DNT also in liquid phase, and that it can be used on a real-time sensing platform.

CONCLUSIONS

We have demonstrated a strategy for the preparation of a selective coating for explosive detection. Additionally, we utilized an oligopeptide, identified from phage display to be specific for TNT, which we stably bound to a co-polymer, PEGM. The attachment was easily performed by reacting the epoxy groups present in one of the monomers of PEGM to primary amino groups present in the receptor. The attachment was stable to overnight washing with water, as shown by XPS. Additionally, we have shown that the oligopeptides bound to PEGM maintain their receptor activity. To prove this, we exposed PEGM/TNTrec to TNT solution, soaked the samples overnight in water to remove unbound TNT, and analyzed the amount of TNT which remained bound. Both XPS and GC/MS showed that the amount of TNT left on samples containing the specific TNT receptor was much higher than that remaining on samples containing a non-specific receptor or the lone PEGM matrix. In particular, the quantitative GC/MS results proved that the specific adsorption of TNT in solution on a TNT receptor was approximately 3 times larger than that of a non-specific receptor and about 10 times larger than the physisorbed amount on PEGM. GC/MS experiments also showed the selectivity of TNT over DNT binding in solution for the TNT receptors was retained when the receptors were conjugated to the PEGM matrix. A real-time highly selective explosive detection in water was also demonstrated, using QCM as a sensing platform, and coating the QCM crystal with PEGM/TNT receptor. A decrease in resonance frequency of the QCM was observed only in the presence of TNT solution, whereas no change in resonance frequency was noted when the crystal was exposed to DNT solution. The potential application of this sensitive and selective liquid detection of TNT is particularly appealing for analysis of contamination of ground waters near ammunition depots, for example.

In conclusion, we developed a sensing coating for detecting explosive molecules in water. We embedded oligopeptide based receptors, with high affinity for explosives molecules such as TNT and DNT, into a polymeric matrix. We envision that one could apply this coating to different sensing platforms, and therefore control both surface properties and receptor density by changing the polymeric matrix, surface area, and porosity.

ACKNOWLEDGMENT

This research was performed under the supports of the Office of Naval Research, the Center of Integrated Nanomechanical Systems (COINS) of the National Science Foundation (Grant No. EEC-0425914), the National Science Foundation (EXP-SA: Award No. ECCS- 0731309), and the Office of Basic Energy Sciences, U.S. Department of Energy (Contract No. DE-AC02-05CH11231).

Received for review September 9, 2008. Accepted April 18, 2009.

AC8019174