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Highly Sensitive Nitric Oxide Detection Using X-ray Photoelectron Spectroscopy

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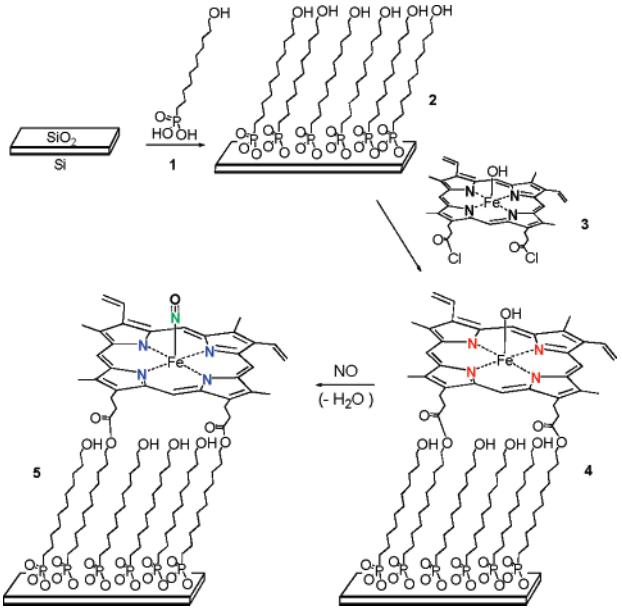
Nitric oxide (NO) is important physiologically as a gaseous messenger in the central nervous system;[1] its level is also associated with several disease states.[2] The concentration of NO in these cases is in the nanomolar range; therefore accurate and direct detection of NO at these levels is important. NO is also a decomposition product of several explosives[3] so detection of trace amounts of explosive, for example in security screening, also requires a sensitive NO assay. Thus it is not surprising that a large variety of NO detection systems have been discussed for implementation in these applications.

NO is now detected using fluorescence,[4] chemiluminescence,[5] electrochemistry,[6] electron paramagnetic resonance spectrometry,[7] and electrical[8] methods. These techniques employ surface-modified metals or semiconductors, usually as electrodes, that are treated chemically or physically to increase their interaction with NO. A range of strategies has been used for modifying these electrodes for this purpose. One common surface modification technique is to coat the electrode with a metalaporphyrin: NO binds to the iron atom of the hemelike molecule;[9] experimental and computational studies have elucidated the geometric and vibrational features of heme-NO binding.[10] Iron porphyrins are more reactive to NO than either to O_2 or CO,[11] which makes them a good choice for use in detection of NO in a competitive environment where CO or O_2 could be present. Clearly, the stability of the interface between a sensor device and the NO-binding surface heme complex is critical for device lifetime, as is the quantitative control of surface modification for measurement reproducibility. We now report a new method to stably bond monolayers of an iron heme complex onto silicon that enables direct, quantitative detection of NO at low levels.

We have described bonding self-assembled monolayers of organophosphates onto oxide surfaces;[12] these monolayer interfaces are stable to air and water.[13] In particular we have found these monolayers to be dense and ordered films on oxide terminated silicon.[14] We used a monolayer formed from 11-hydroxyundecylphosphonic acid (**1**) on SiO_2/Si as our reactive platform to attach NO-bonding molecule hematin. Several drops of aq HCl were added to a 10 μM solution of hematin (Aldrich) in CH_2Cl_2, which was then heated with a 60 μM solution of $SOCl_2$ under inert atmosphere for 24 h to give its diacyl chloride, **3**. That both carboxylic acid groups of the hematin were converted to the acyl chloride was confirmed by IR, which showed replacement of $\nu_{(C=O)-OH}$ (1710 cm^{-1}) by $\nu_{(C=O)-Cl}$ (1800 cm^{-1}) (see Supporting Information). Heme-terminated surface **4** was then obtained by treating **2** with a solution of **3** in dry CH_2Cl_2 under inert atmosphere for 24 h, followed by sonication successively with CH_2Cl_2 and water. Coupons of **4** were then transferred into ultrahigh vacuum (UHV) for X-ray photoelectron spectroscopic analysis (XPS). The survey scan of **4** showed distinct peaks in the N1s and Fe2p regions, which confirms the attachment of hematin on the surface (see Supporting Information). Surface **4** was then treated with NO to give adduct **5** (Scheme 1). Caution: NO is toxic, so care must be taken to work in a well-ventilated hood.

Scheme 1. Schematic of the Surface Modification Strategy



The quantitative interaction of NO with **4** was studied by XPS. Pure nitric oxide (MGI) was passed over **4** in a chamber that had been purged with argon to remove oxygen to avoid formation of NO_2. The resulting adduct **5** was then transferred into UHV (base pressure, 5×10^{-9} Torr) for XPS analysis. The survey spectrum of **5** was similar to that of **4** except that two new peaks appeared in the N1s region. In contrast to **4**, which shows a single peak, N2, (BE = 399.5 eV) in the N1s region attributed to the nitrogens of the hematin molecule, the N1s region of **5** has three peaks (Figure 1).

The peaks at 398 (N1) and 399.5 eV are attributed to nitrogens from **5** and from unreacted **4**, respectively, and the peak at 405.2 eV (N3) is attributed to the N1s of iron bound NO. The shift to lower binding energy for the heme nitrogens of **5** as compared to **4** can be explained by noting that NO, a free radical, bonds to iron as the nitrosyl ligand by a formal 1 e^- reduction of the metal.[15] A similar decrease in the binding energy of the Fe2p peak was also observed (Figure 2).

The ratio of peak areas for N3 and N1 (Figure 1) is 1:4, consistent with the expected stoichiometry of the hematin−NO complex **5**. The sum of areas under the N1 and N2 peaks of **5** equals the area of the peak under the N1s peak of **4**, which confirms that the peak at 405.2 eV is in fact due to NO attachment and not to the degradation of the hematin complex (see Supporting Information).

Quartz crystal microgravimmetry (QCM) studies[16] showed that the coverage of **1** on SiO_2/Si is 850 pmol/cm^2 (±5%), and the loading of **3** on **1** is 300 pmol/cm^2 (±6%). Before we could estimate

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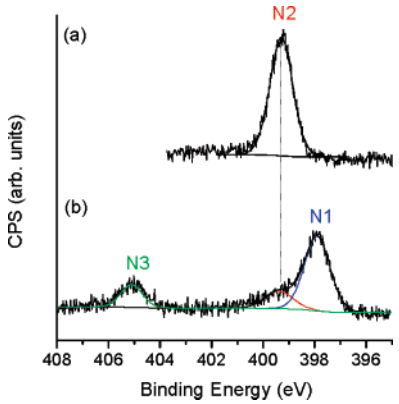

Figure 1. Detailed scan in the N1s region of (a) **4** and (b) **5**. N1 is attributed to reacted hematin, N2 is attributed to unreacted hematin, and N3 is from hematin-bound NO (Scheme 1).

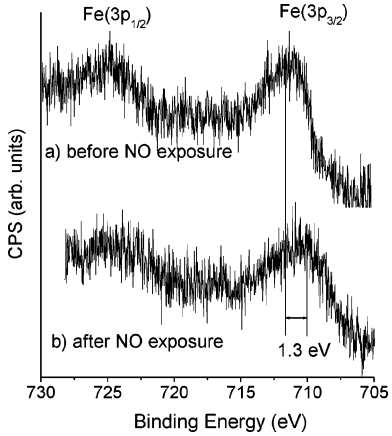

Figure 2. Detailed scan in the Fe2p region of (a) **4** and (b) **5**. A shift similar to the shift in nitrogen signal is observed.

the minimum sensitivity for NO detection by XPS from N1s peak areas of **3**, it was important to determine how accurate XPS was for quantitatively determining the loading of this complex on SiO_2/Si. To this end, the N/P ratio was measured from XPS data for **4** to be 4:3, or one hematin molecule is present for every 3 hydroxyundecylphosphonate molecules. Phosphorus peak intensities were corrected for attenuation by monolayer **4**, based on the attenuation of the Si2p signal,[14b,c] in determining this ratio. Thus the surface coverage by hematin was determined by XPS to be about 283 pmol/cm^2, in good agreement with that measured by QCM. The area sampled by our XPS spectrometer is about 0.2 cm^2. Thus the total amount of hematin present in this region is about 58 pmol. The ratio of area under nitrogen peak N2 in **5** and **4** can be used to calculate the yield for conversion to **5** adduct, which is approximately 75%; therefore the amount of NO detected here as hematin adduct **5** is 44 pmol. Sensitivity of detection is limited by the signal-to-noise ratio in the XPS; here, $S/N \approx 4$, so NO as low as 11 pmol should be detectable using our spectrometer. This detection is comparable to that of commercial NO detectors based on mass spectrometry strategies[17] and is about one-tenth as sensitive (in terms of absolute quantity detected) as the most sensitive systems, such as those based on resonance enhanced multiphoton ionization mass spectrometry[18] or frequency modulation spectrometry.[19]

We have shown that direct detection of NO can be achieved by XPS as a surface-attached hematin complex on the native oxide of silicon. Given the silicon platform used for this study, it is possible that our surface-modification strategy might be implemented on a silicon-based device for direct electrical detection of NO; indeed, this strategy is currently under investigation.

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Supporting Information Available: A detailed description of monolayer formation of **2**; XPS analysis procedure; IR spectra of hematin and **3**; XPS survey spectrum of **4**; calculation of relative areas of N1s peaks of **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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