

Direct Detection of Low-Concentration NO in Physiological Solutions by a New GaAs-Based Sensor

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Abstract: Nitric oxide (NO) acts as a signal molecule in the nervous system, as a defense against infections, as a regulator of blood pressure, and as a gate keeper of blood flow to different organs. In vivo, it is thought to have a lifetime of a few seconds. Therefore, its direct detection at low concentrations is difficult. We report on a new type of hybrid, organic-semiconductor, electronic sen-

sor that makes detection of nitric oxide in physiological solution possible. The mode of action of the device is described to explain how its electrical resistivity

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changes as a result of NO binding to a layer of native hemin molecules. These molecules are self-assembled on a GaAs surface to which they are attached through a carboxylate binding group. The new sensor provides a fast and simple method for directly detecting NO at concentrations down to 1 μM in physiological aqueous (pH = 7.4) solution at room temperature.

Introduction

The Nobel Prize in Medicine in 1998 was awarded for discoveries concerning “nitric oxide as a signaling molecule in the cardiovascular system”. Nitric oxide (NO) protects the heart, stimulates the brain and kills bacteria. Further research results rapidly confirmed that NO is a signal molecule of key importance for the cardiovascular system. We know today that NO acts as a signal molecule in the nervous system, as a defense against infections, as a regulator of blood pressure, and as a gate keeper of blood flow to different organs. It plays an important role in biological systems, as the “endothelium-derived relaxation factor” (EDRF), in cytotoxic immune response to pathogen invasion and as a neurotransmission regulator of the central nervous system.^[1–3] About 1 to 10 mM NO is sufficient to activate guanylyl cyclase and permit the

signaling event. It has been reported that less than 1 μM NO, generated in endothelium cells, suffices to influence blood pressure control.^[4] Hence, direct detection of such a low concentration of NO is important for further understanding its role in physiological systems and as an indication of their malfunctioning. However, its direct detection is very difficult because of its reactivity, making it a short-lived species with a lifetime, in vivo, of a few seconds.

Here, we present a fast and simple method to directly detect NO at concentrations down to 1 μM . We do so in physiological aqueous solution (pH = 7.4) at room temperature. To achieve this, we used a GaAs-based sensor^[5, 6] for which we measured the electrical resistivity changes that resulted from NO binding to a layer of native hemin molecules self-assembled on the GaAs (100) surface, to which they were attached through a carboxylate group (see Figure 1). A special feature of the new sensor is its small size, limited only by lithography. Size is an important factor for applications requiring spatial imaging of the distribution of NO, for example, by insertion of the sensor into small organisms. Existing methods for monitoring NO are based on chemiluminescence,^[7, 8] electron paramagnetic resonance spectrometry,^[9] the Griess method,^[10] and fluorescence.^[11] With most of these, local measurements of NO in real time are impossible. Recently, an electrochemical technique was developed that overcomes this difficulty,^[12] but this method suffers from interferences in the solutions used.

As functional materials, porphyrin derivatives exhibit properties which are interesting for applications in many fields such as oxygen storage, electron transfer, redox

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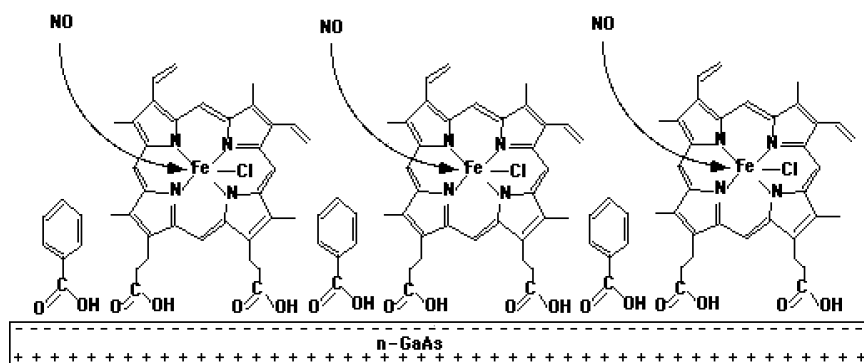


Figure 1. Binding of NO to a layer of hemin/benzoic acid molecules on a GaAs surface.

catalysis, and gas sensing devices.^[13–15] Due to π – π interactions between the porphyrin aromatic rings and the ring interaction with π -active solid surfaces, differences in activity arise from differences in interfacial architecture,^[16, 17] as shown by the variation in efficiency of the electrocatalytic reduction of dioxygen. Those variations indicate that an open coordination site favors catalytic activity. Therefore, the degree of aggregation and the orientation of porphyrins on a solid surface play crucial roles in determining the binding activity of the porphyrin ring. We found that if an iron porphyrin, for example [Fe^{III}(TPP)Cl], was hinged on the oxidized GaAs surface of an electronic device by bifunctional ligands, the device was able to respond to about 30 μ M NO.^[18] In that system, the NO binding sites were originally occupied by the ligands. Here, we report on NO binding to GaAs surfaces that have been modified directly by native iron porphyrin (hemin chloride). Metalloporphyrins were chosen because theoretical calculations and experimental data show them to be much more sensitive to NO(g) than to O₂(g) or CO(g).^[18–20] A key feature of the hemin is the number and location of the carboxyl group-containing “legs” that allow it to bind directly to the oxidized GaAs surface, without intervening ligands.

Results and Discussion

The semiconductor device: The sensor is a hybrid structure of a semiconductor transducer and an organic layer of the “sensing” molecules. The transducer is based on a molecular beam epitaxy-grown GaAs/(Al,Ga)As structure called MOCSE (molecular controlled semiconductor resistor)^[5, 6, 21] (Figure 2a). The structure was designed for measuring small changes in the electric potential on its surface (with respect to a reference potential, see below). It consists of a conducting n-GaAs layer, grown on a buffer layer of (Al,Ga)As, and an ultra-thin (5 nm) insulating layer covering the conducting layer. Figure 2b shows the cross-section of the electron distribution in the semiconductor structure as obtained from a simulation based on solving the Poisson equation in one dimension for the system. As can be seen, the electrons that conduct the electrical current in this structure are localized between 20 to 50 nm from the surface by virtue of the space charge field in the n-GaAs layer, and due to the fact that the (Al,Ga)As band gap is larger than that of GaAs.

Mode of action of the device:

When molecules or ions of the analyte bind to the receptor sites of the molecules that make up the organic monolayer, the current through the device changes. The change in the current through the device must result from a change of the electric potential, Φ , on the surface of the semiconductor. In what follows, a model based on the linearized Poisson–Boltzmann (PB) approximation

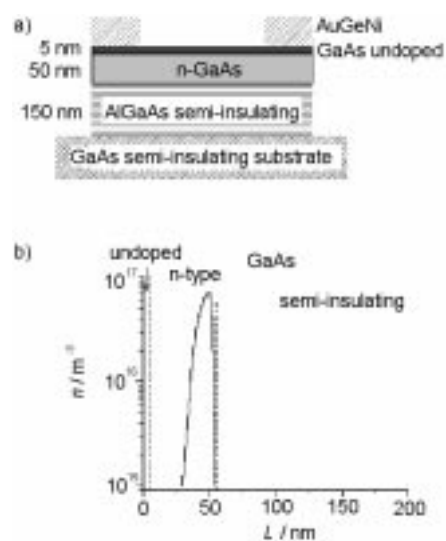


Figure 2. a) Schematic side-view representation of the GaAs-based MOCSE. The organic molecules are adsorbed between two AuGeNi ohmic contacts. b) The one-dimensional electron density distribution inside the MOCSE, as calculated by solving the Poisson equation.

is used to explain the operation of the sensor. The simplicity of the model stems from using the Debye–Hückel approximation together with a one-dimensional analysis, made possible by the fact that the horizontal dimensions of the device are much larger than the thickness of the layers, as shown in Figure 2a.

We represent the relevant part of the system by three layers ($k = 1–3$), each with a given thickness (ℓ_k) and a dielectric constant, ϵ_k (see Figure 3b). Layer 3 is the intrinsic GaAs layer bordered (on the right, in Figure 3a) by an n-GaAs layer. Layer 2 is the organic monolayer and layer 1 is an electrolyte solution. (For a device operating in air we assume that enough ions exist in the air to affect the interface between layers 1 and 2 in the same way as explained below). In addition to their dielectric response, layers 1 and 3 are characterized by Debye screening lengths,^[22] denoted λ_1 and λ_3 , respectively. The added adsorbate (here NO) is represented by a surface dipolar layer, with a surface dipole density D , located at the 1–2 interface. Such a dipole layer, if suspended in free space, would correspond to an electrical potential drop: $\Delta\Phi = 4\pi D$, but does not induce any electrostatic field outside it (but see

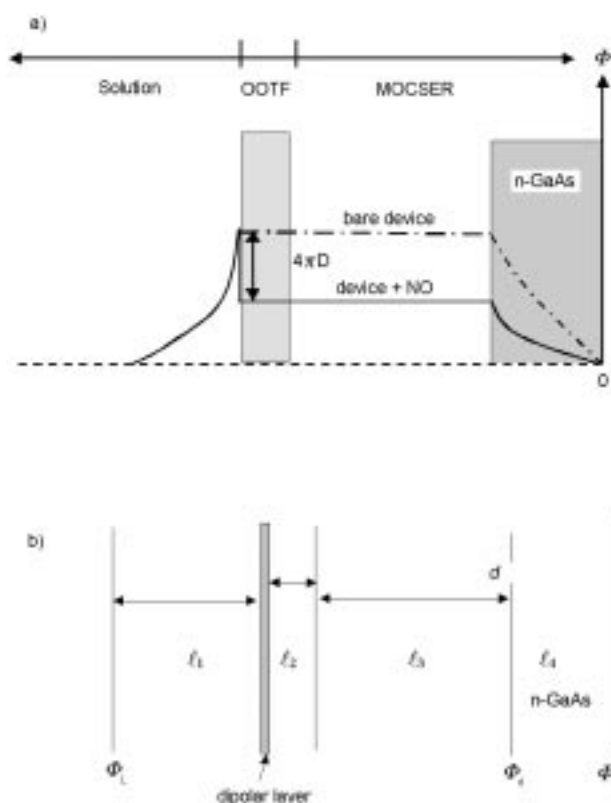


Figure 3. a) Schematic representation (not to scale) of the potential (Φ) across the MOCSEr with ligand, without any adsorbate (---), and when the analyte interacts with an organic monolayer (OOTF) that is adsorbed on the surface of the GaAs (—). D is the dipole moment density of the NO-on-organic monolayer. For simplicity's sake the situation of the monolayer without NO is not shown, as, based on the Kelvin probe data, the electrical potential changes on the n-GaAs due to only the hemin molecules are small. b) Schematic cross-section sketch of the electrolyte/molecular film (and adsorbed dipole)/GaAs system.

below and ref. [23]). Similarly, such a layer adsorbed onto a metal surface changes the metal work function by $\Delta\Phi$. The situation here is different because the structure and mode of operation of the device impose conditions in terms of the electrical potentials (Φ_L and Φ_R on the two sides of Figure 3b, to be specified below) on the system's boundaries. This results in the existence of an electrostatic field $E(d)$ at point d in region 3. It is the existence of this field that affects the current–voltage characteristics of the device. The current through the device is carried primarily in a thin region in the n-GaAs layer adjacent to the n-GaAs/(Al,Ga)As interface (cf. Figure 2b) and is controlled by the electric field across the n-GaAs layer. That electric field is determined by the electrical potential difference between the conducting region near the (Al,Ga)As interface (Φ_R) and that at the n-GaAs/undoped GaAs interface, Φ_d . A complete description of the current/dipole-layer relationship is beyond the scope of the present paper and will be discussed elsewhere. Here we limit ourselves to examining the effect of the dipole layer on the induced field $E(d)$.

For specificity we represent the dipole layer by two layers with equal but opposite surface charge densities $\pm\sigma$, one at distance δ_1 from the 1–2 interface into region 1, the other at

distance δ_2 from this interface into region 2. Electrostatic continuity relations then yield the electric potential difference between the two sides of the resulting dipole layer in the form:

$$\Delta\Phi = 4\pi D_{\text{eff}} \text{ with } D_{\text{eff}} = \sigma \left(\frac{\delta_1}{\epsilon_1} + \frac{\delta_2}{\epsilon_2} \right) \quad (1)$$

in which D_{eff} is the effective dipole density.

The effect of this dipole layer on the field in region 3 can be obtained analytically by solving the (one-dimensional) linearized Poisson–Boltzman equations in regions 1 and 3, and by solving the Laplace equation in region 2. Subsequently, the resulting potentials at the 1–2 and 2–3 interfaces are matched by taking into account the potential drop, $\Delta\Phi$, at the 1–2 interface. The solution is expressed in terms of an effective length:

$$\ell_{\text{eff}} = 2 \frac{\epsilon_2}{\epsilon_1 \kappa_1} \text{tgh} \left(\frac{\kappa_1 \ell_1}{2} \right) + \ell_2 + 2 \frac{\epsilon_2}{\epsilon_3 \kappa_3} \text{tgh} \left(\frac{\kappa_3 \ell_3}{2} \right) \quad (2)$$

and the electric field strength at point d is obtained in the form:

$$E(d) = \frac{\epsilon_2}{\epsilon_3 \kappa_1} \frac{\Phi_L + \Delta\Phi - \Phi_d}{\ell_{\text{eff}}} \left[\cosh(k_3 d) - \sinh(k_3 d) \text{tgh} \left(\frac{k_3 \ell_3}{2} \right) \right] \quad (3)$$

Consider now the conditions relevant to the operation of the MOCSEr. In the MOCSEr, the conducting layer (to the right of region 3) determines the ground potential. In turn, the potential Φ_R of the conducting layer is defined by the drain potential, which is set to be zero. On the other hand, the potential of the vessel in which the measurement is conducted, Φ_L is also zero (the potential in this case is defined by leaving the ground electrode on the MOCSEr (the “drain”) exposed to the electrolyte solution. Moreover, layer 3 in Figure 3a corresponds to the insulating GaAs layer and therefore $\epsilon_3 \approx 4$ and $\ell_3 \ll k_3^{-1}$, namely the thickness of this layer (5 nm) is much smaller than its screening length. Therefore, $E(d) = (\epsilon_2/\epsilon_3)(\Delta\Phi/\ell_{\text{eff}})$. Finally, since medium “2” consists of organic material, its dielectric constant is similar to that of medium 3. On the other hand, medium “1” corresponds to the electrolyte and therefore its dielectric constant is large (~ 80).

Therefore $\ell_{\text{eff}} \approx \ell_2$ and:

$$E(d) \approx \frac{\Delta\Phi}{\ell_2} = \frac{4\pi D_{\text{eff}}}{\ell_2} \quad (4)$$

From Equation (4) we see that the electric field strength at point d , which is located on the surface of the n-doped layer, depends linearly on the dipole density D and, within the given range of device dimensions, does not depend on the distance between the dipole layer and the conductive n-GaAs layer. This result relies on the fact that this distance is much smaller than the lateral dimensions of the dipole layer, which are dictated by the dimensions of the active area of the device. What is normally presented is the opposite limit, where there is no electric field associated with the dipole layer, because actually this field drops to zero beyond a distance larger than the lateral dimensions of the film. This point has been stressed recently in ref. [23] (cf. Figures 4 and 5 in that reference).

Irrespective of whether the organic molecules bound to the surface have a zero or finite dipole moment in the direction

perpendicular to the surface, if analyte binding to the receptor site changes the electron-density distribution at that site, this will change the dipole of the molecule-analyte complex as compared with that of the bound molecule alone. Therefore, the electric field $E(d)$ will change as well, as given quantitatively by Equation (4). The resulting potential profile is shown schematically in Figure 3a. The potential without the analyte is shown as a dashed-dotted line, while the potential with the adsorbed analyte is shown as a solid line. The dipole layer thus acts in a way that is equivalent to the "gate" in a regular field effect transistor, *despite the fact that no net charge is supplied to the device from an external source*. A detailed discussion of this model will be given elsewhere.

Sensor action: Typical response curves of the current through the device as a function of time, $I(t)$, are shown in Figure 4 for a device coated with the 1:1 mixed monolayer. Before immersion of the sensor into the solution, the current is constant (a). When the MOCSEER is immersed into the solution the current jumps and then stabilizes after a short time (b). Upon injection of the NO-releasing solution ($6.7 \mu\text{M}$ equivalent) into the buffer solution the current increases (c) and saturates in less than about 10 min.

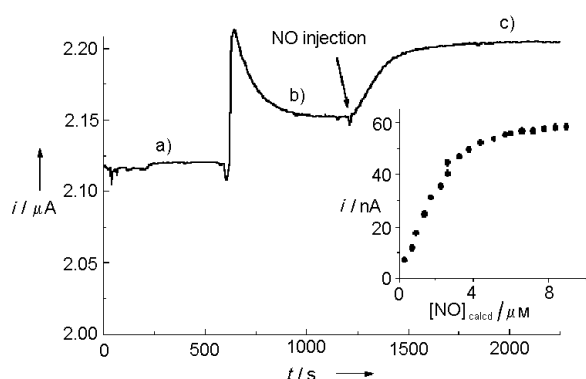


Figure 4. Current through the MOCSEER against time, when the MOCSEER is a) dry, under N_2 ; b) in anaerobic buffer solution ($\text{pH} = 7.4$); c) when NO-releasing solution is injected ($6.7 \mu\text{M}$). Inset: The relation between the current through the device and the calculated NO concentration, as derived from Equation (5).

In order to reveal the dependence of the current through the MOCSEER on the NO concentration, we calculated the concentration of NO at different times (from Figure 4) by using the following relation:^[24]

$$[\text{NO}] = 2C_0(1 - e^{-1.16 \times 10^{-3}t}) \quad (5)$$

in which $[\text{NO}]$ is the concentration of NO at time t (seconds) and C_0 is the total concentration of the NO adduct in the buffer solution. The dependence of the current on the NO concentration is shown in the inset in Figure 4. It is evident that the device can respond to a concentration as low as $1 \mu\text{M}$ of NO. This result is consistent with other direct measurements, which indicate that the device can indeed respond to less than $2.6 \mu\text{M}$ NO (see Figure 5c, at ca. 10 min). For the measurements shown in Figure 5, a new device was

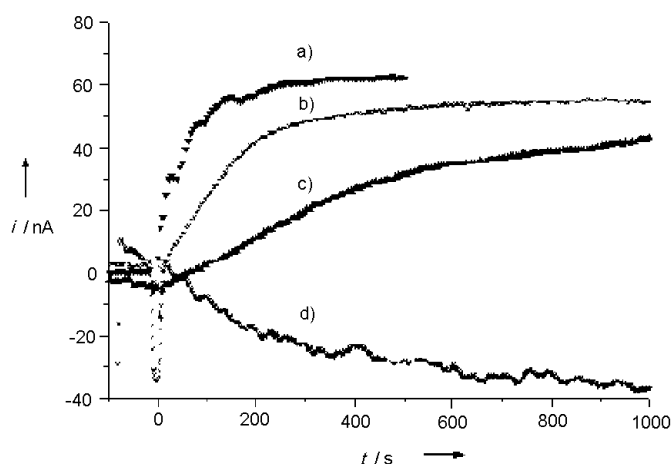


Figure 5. Current through the MOCSEER, as a function of time, for different NO-releasing solutions. The concentrations used are a) $16 \mu\text{M}$; b) $6.7 \mu\text{M}$; c) $2.6 \mu\text{M}$; d) $847 \mu\text{M}$ (bare device, without hemin molecules). The curves are shifted along the time and current axes for clarity. All measurements were done at room temperature, in $\text{pH} = 7.4$ buffer solution, and under N_2 .

used for each NO concentration. The response time of the current varies proportionally to the NO concentrations. For example, when the current reaches "steady state", the response time is about 5, 10 or 20 minutes for injecting a) 16, b) 6.7 or c) $2.6 \mu\text{M}$ NO-releasing solution, respectively. The response of the bare device to NO was also measured under the same conditions. Here, the current through the device slightly decreased when the NO-releasing solution was injected.

The response of the device when covered with hemin is surprisingly stable, notwithstanding the notorious instability of bare GaAs surfaces under such conditions (Figure 5d). Comparing curves a and b to d, shows the relative instability of the bare device in buffer solution. In separate experiments in buffer solutions no significant change in response over one hour was seen for molecule-covered devices, while bare devices deteriorated within 30 minutes to half the original response. We note that the NO concentration used for Figure 5d is about two orders of magnitude higher than that used for Figure 5b; this indicates that the system responds to NO because of the hemin and not due to direct contact between the NO and the GaAs surface.

In order to quantify the relationship between the response time of the current through the MOCSEER and the NO concentration, we assumed that the response follows the relation:

$$I(t) = I_0 - B e^{-t/\tau} \quad (6)$$

which can be written as:

$$\ln(I_0 - I(t)) = -(1/\tau)t + \ln B \quad (7)$$

Here $I(t)$ is the current at time t , I_0 is the steady state current,^[25] τ is the time constant for the given concentration C_0 , and B is a constant. Based on the assumed kinetics we expect a linear dependence of $\{\ln(I_0 - I(t))\}$ against time, as indeed was obtained experimentally. Hence, the time con-

stants of the response of the sensor for each initial concentration of NO can be extracted. Figure 6 shows the linear correlation between $\log(\tau)$ and calculated NO concentration.

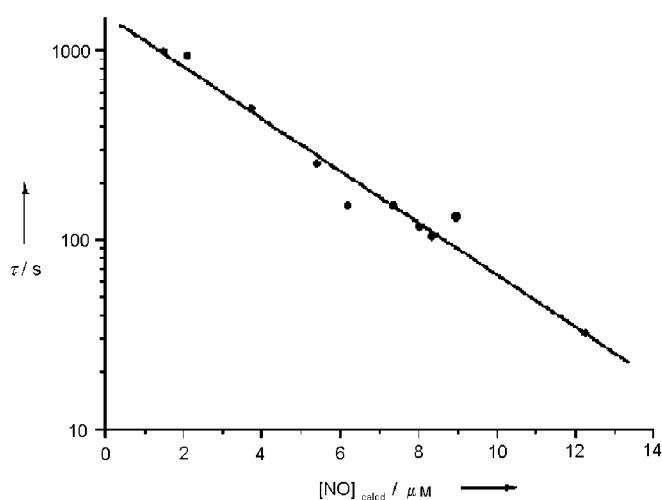


Figure 6. The time constants for change in MOCSEER current (on logarithmic scale), as a function of NO concentration, to which the device with adsorbed hemin monolayer is exposed. Each point was taken with a different device.

Several control experiments were performed to verify that the device responds only to NO and not to the NO-releasing molecule (*N,N*-dimethyl-1,3-propylamine). One experiment was performed in a buffer solution containing $60\ \mu\text{M}$ NO-releasing molecule, from which NO was removed by bubbling pure N_2 through it. There was no detectable response from the device. Another test was performed in a basic aqueous solution ($\text{pH}=10\text{--}11$), since in basic solution NO is not released from the adduct.^[26] Again, the device did not show any change in current when $100\ \mu\text{M}$ NO-releasing solution was injected. The same device, dried by N_2 , was used in a buffer solution with the same NO concentration. In this case it showed a very large current change, indicating that the device indeed responds only to NO. The device with a pure hemin monolayer adsorbed on it was checked too. Such a device also responded to NO, but the signal was smaller and the time constant longer than what was found under the same conditions with a 1:1 mixed monolayer; this indicated that adding the carboxylate spacer indeed enhances the NO binding rate to the hemin. The system of tetracarboxylate-porphyrin (hemin without the iron ion),^[27] adsorbed on the device, was also measured. That system did not respond to NO, which demonstrated that the iron ion is critical for the NO binding event.

To probe the re-usability of the sensor, sequential measurements were made on a sample, first exposed to a $90\ \mu\text{M}$ NO-releasing solution, then taken out, dried with a nitrogen gas stream and finally brought back to a neat buffer solution. Following this procedure, the device was again exposed to $90\ \mu\text{M}$ NO-releasing solution. This procedure was repeated five times (Figure 7). The saturated current decreased from

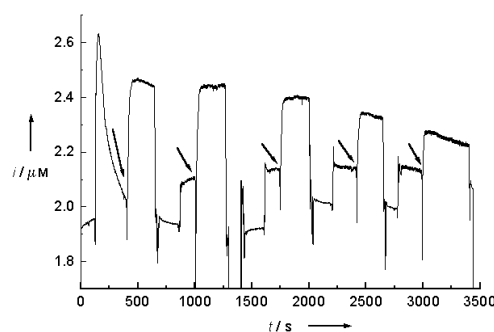


Figure 7. Sequential measurements on a single device, first exposed to a $90\ \mu\text{M}$ NO-releasing solution, then taken out, dried with a nitrogen stream and brought back to a neat buffer solution. Following this procedure the device was exposed again (arrow) to a (fresh) $90\ \mu\text{M}$ NO-releasing solution. This procedure was repeated five times.

one exposure to another in the order $1.00 > 0.75 > 0.55 > 0.45 > 0.30$. These results demonstrate that with high NO concentration and short flushing time the sensor does not recover completely between two measurements. However, for lower concentrations of NO the sensor can function for a long time without being saturated. For example, after a device that was exposed to $10\ \mu\text{M}$ NO reached the saturation current, it was dried in a stream of dry N_2 for less than 15 seconds and re-immersed in the same concentration of NO. The resulting change in current until saturation was reached, was then about 98% of the original change; this demonstrated reasonable reversibility of the system under these conditions. Exposing the NO-bound sample to a short (10 ns) 532 nm laser pulse regenerated the sensor without affecting the device, in particular the semiconductor surface, in any other way. The mechanism in that case is similar to photo-induced cleavage of NO from cytochrome *c*.^[28]

To help understand the effects of the adsorbed hemin molecules, to which the NO is expected to bind, on the device's electronic properties, the electrical contact potential difference (CPD) between the n-GaAs surface, modified by the 1:1 mixed monolayer, and a reference Au grid was measured by a Kelvin probe^[29] in ambient conditions. We found that hemin adsorption increases the effective electron affinity (χ) and decreases the band bending (V_s) of the samples studied. For example, for bare n-GaAs $\chi = 4.4 \pm 0.1\ \text{V}$ and $V_s = 0.35 \pm 0.05\ \text{V}$, whilst after adsorption of hemin $\chi = 4.6 \pm 0.1\ \text{V}$ and $V_s = 0.32 \pm 0.05\ \text{V}$. The electron affinity data indicate that the dipole of the adsorbed molecules is oriented with the negative pole pointing away from the surface. The changes in band bending are within the experimental error; this suggests that binding of the carboxylic acid groups to form carboxylates (Ga-carboxylate, according to ref. [29]) causes no change (or at most a minor decrease) of the net surface charge.^[30] The increase in current through the device upon NO binding is consistent with a (further) decrease in net surface charge and, by Poisson's relation, in surface potential, as discussed above. The slight decrease of the current in the bare device upon adsorption of NO may result from the interaction of NO with the device surface; this can lead to the release of surface-bound water molecules and induce net removal of negative charge from the n-doped layer.

The sensor described here provides a new approach to bio-sensing since it combines the simplicity of the operation of a semiconductor device and electrical monitoring with chemical selectivity and sensitivity, as well as potential high spatial resolution. We note that the device appears to be compatible with at least some biological environments, as in preliminary experiments with slides of rat brain in pH 7.4 buffer solution, a reasonable response is seen when that system is stimulated to produce NO. Further work in this direction is under way and will be the subject of future reports.

Experimental Section

MOCSEs were fabricated by using substrates with a donor (Si) concentration of 5×10^{17} atoms/cm³ in the n-GaAs layer. They were made, based on our design, by IQE Inc. (formerly QED Inc., Pa, USA). The dimensions of the sensors used in the present study were 1.5 mm \times 6.0 mm, with a sensing area of about 0.3 mm². In principle, the sensor can be made smaller by a few orders of magnitude without loss of sensitivity. In order to prevent leakage of the electrical current through the conducting solution, the device was encapsulated by using epoxy resin, leaving the “sensing window” uncovered.

Preparation of the sensor and the mode of measurement: Prior to each adsorption experiment, GaAs (100) surfaces of either single crystal substrates or of the devices were cleaned by boiling them successively in trichloroethylene, acetone, and absolute ethanol for 15 min each, etched for ten seconds in NH₃/H₂O (1:9, v/v) solution, washed in deionized water, and dried by N₂. They were then immersed overnight in a solution of hemin (15 mM) in DMF. The devices were taken out, carefully rinsed with CHCl₃/hexane (5% v/v) and blown dry with a stream of nitrogen gas.

In order to avoid π - π electronic interactions between neighboring adsorbates, in some experiments a spacer was introduced between the hemin molecules. In that case, GaAs substrates or the devices were directly immersed in a solution of hemin/benzoic acid (1:1) in DMF. We chose benzoic acid as spacer because it adsorbs onto GaAs through its carboxyl group,^[29] as does hemin, and its molecular height should not influence any interaction of NO molecules with hemin. The monolayers were characterized on single crystal GaAs by FTIR by using the bare, etched, oxidized GaAs surface as a reference. After chemisorption of the mixed molecular film on the GaAs substrate, a strong peak appeared at 1710 cm⁻¹ ($\nu_{\text{C=O}}^{\text{Hemin}}$ of hemin), while the peaks, indicative of the free carboxylic groups (ν_{COOH} vibrations, 1747 and 1675 cm⁻¹ for hemin and benzoic acid in DMF, respectively), disappeared. This verifies that the carboxyl groups do indeed bind to the GaAs surface.^[29, 31] The intensity of the $\nu_{\text{C=O}}^{\text{Hemin}}$ absorbance peak was about 1.0 – 1.5×10^{-3} ; this indicated that a film of about one monolayer is formed on the GaAs substrate.^[32]

AFM images (Figure 8) of the films formed on mica show that the film thickness is about 1.5–1.7 nm. This result is consistent with the value reported in ref. [33] and indicates that, at least on mica, the hemin molecules do indeed “stand” rather than lie on the substrate. In Figure 8, we see that the surface of the mixed monolayer (Figure 8a) is more corrugated than the one made from pure hemin (Figure 8b). If such a corrugation also occurs on GaAs,^[34] then it will allow an easy approach of the analyte, the NO molecules, to the hemin binding site. This would explain the enhanced sensitivity observed when the sensor is exposed to the benzoic acid/hemin mixture rather than to pure hemin.

The device response to NO molecules was determined at room temperature under anaerobic conditions (N₂) to prevent formation of the undesired NO₂. During the experiment, a constant voltage of 100 mV was applied between the ohmic contacts of the device. The change in the current with time, $I(t)$, was monitored in a buffer solution (pH = 7.4), while NO was released from an organic precursor 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxotriaz-1-ene (Sigma) in an aqueous solution of NaOH (0.01 M) with first order kinetics and $t_{1/2} = 10.1$ min at room temperature.^[26, 35, 36] A constant flow of pure N₂ over the surface of the buffer solution was maintained during the measurement.

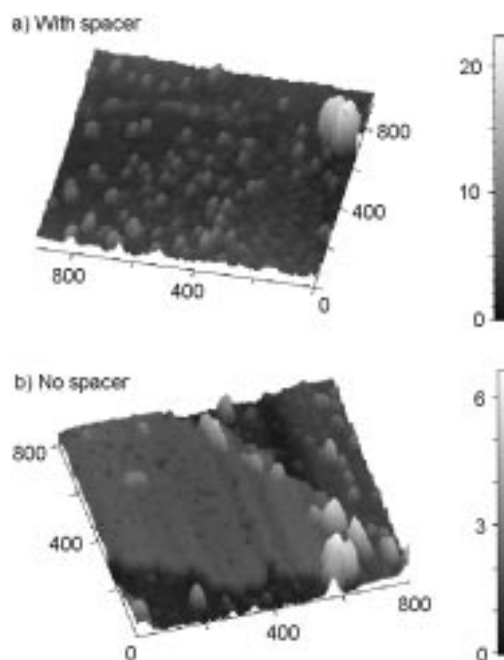


Figure 8. AFM images of molecular self-assembled films on mica surfaces: a) hemin/benzoic acid (1:1) mixed monolayer on mica; b) pure hemin monolayer on mica. The film thickness is about 1.5–1.7 nm, indicating that the hemin molecules “stand” on this substrate.

The measurements were performed with a Keithley Model236 Source-Measure Unit. The current was first measured for the dry device under N₂ and then in the buffer solution.

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