



A field effect transistor biosensor with a γ -pyrone derivative engineered lipid-sensing layer for ultrasensitive Fe^{3+} ion detection with low pH interference

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ABSTRACT

Field effect transistors have risen as one of the most promising techniques in the development of biomedical diagnosis and monitoring. In such devices, the sensitivity and specificity of the sensor rely on the properties of the active sensing layer (gate dielectric and probe layer). We propose here a new type of transistor developed for the detection of Fe^{3+} ions in which this sensing layer is made of a monolayer of lipids, engineered in such a way that it is not sensitive to pH in the acidic range, therefore making the device perfectly suitable for biomedical diagnosis. Probes are γ -pyrone derivatives that have been grafted to the lipid headgroups. Affinity constants derived for the chelator/ Fe^{3+} complexation as well as for other ions demonstrate very high sensitivity and specificity towards ferric ions with values as high as 5.10^{10} M and a detected concentration as low as 50 fM.

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1. Introduction

The development of point-of-care and self-monitoring approaches in medical practices underline the needs of reliable real time sensing devices and require the use of non-intrusive technologies such as a wireless sensor network. Indeed, up to now, most of biomedical tests are being realized in hospitals and require large and complex equipments. Operation of such complicated systems usually requires high skilled technicians and can be time consuming. All those factors lead to a high cost for medical tests that prevent a large population from having adequate health care service. This problematic has lead to the development of emerging techniques with highly specific features (Walker et al., 2000). The development of field effect transistors based sensors has risen

as one of the most promising approaches for the future of biomedical diagnosis and monitoring (Kuila et al., 2011; Li et al., 2013; Lerner et al., 2013). They fulfil the requirements in terms of fabrication cost and size, as well as, in term of easiness to use (Bernards et al., 2008; Khodagholy et al., 2012; Wijaya et al., 2010). All these features make them suitable for high throughput fabrication and individual use by patients.

Sensing field effect transistors are currently based on metal-oxide-semiconductor field effect transistors (MOSFET) technology (Chen et al., 2011a; Collet and Vuillaume, 1998; Bergveld, 1986). Instead of a metal layer used as gate in MOSFET, a BioFET sensor operates with a reference electrode dipped into an electrolyte solution (Schöning and Poghossian, 2002). Nevertheless, for both types of devices, the electrical field across the gate dielectric controls the conductivity in the transistor channel, between source and drain. In classical MOSFET, this electric field is controlled by application of a potential difference between the gate and the source (or drain). First transistor-based sensors were developed in the mid 1960s for the detection of ions and have been especially used as pH sensors (Chang et al., 2010). These so called ISFETs (Ion-Sensitive Field Effect Transistors) are usually comprised of an oxide gate dielectric bearing hydroxyl groups at the metal-solution

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interface. Their sensing properties rely on the amphoteric property of the OH functions that can depending on the pH, either gain or lose reversibly a proton into -OH_2^+ or -O^- respectively, at the metal–electrolyte interface, causing therefore strong changes of the density of surface charge of the gate dielectric. The response mechanism to changes in ion concentrations of such system is mainly attributed to the influence of ion concentration on the potential drop across the electrolyte within the diffuse layer, and it can be identified as the surface potential of the oxide layer on the electrolyte side, ψ_0 . These surface potential fluctuations will lead therefore to both a modulation of the electric field across the oxide layer and of the conductivity of the channel that can then be easily measured (Lin et al., 2013a; Presnov et al., 2013; Bae et al., 2013). More recently, graphene based ISFET have also been developed for pH sensing. In such device the sensing performances are related to the changes in graphene conductivity when the pH of the buffer is changed (Kiani et al., 2013; Ohno et al., 2010). Moreover, ISFETs turned out to be very useful in biology to measure for instance the biological activities of cells (Lee et al., 2009a) or enzymes (Lin et al., 2013b). As exemplified hereby, Sakata et al. (2011) have measured in real-time the respiration activity of fertilized ova using such device. Indeed, the carbon dioxide produced by the cell metabolism is released in the extracellular medium and combines with water to form carbonic acid which freely dissociates into $\text{H}^+ + \text{HCO}_3^-$ resulting in a protonation of the hydroxyl groups at the surface of the oxide gate dielectric and a conductance modulation. However, despite their interest of use in research labs, this device configuration has a number of potential limitations that are unsuitable for the development of sensors with high specificity and sensitivity. De facto, in monitoring applications, very small variations of the pH analyte (blood, urine, plasma), which is very likely to happen when a patient is being treated with drugs, will induce undesirable changes of the channel conductivity. Additionally, this type of device is therefore not advisable for the detection of specific objects (molecules, ions) in a solution of unknown composition and some alternatives have to be found. To overcome and solve such drawbacks, technological solutions have been envisioned and tested like the, immobilization of specific probes and/or biomolecules onto the gate-dielectric surface. This alternative approach was successfully developed for the fabrication of bio-FETs (biologically modified field-effect transistors) with the use and grafting of biomolecules (Uslu et al., 2004; Wu et al., 2011; Tatikonda et al., 2013; Hammock et al., 2013; Lee et al., 2009b), leading to sensors with higher specificity and selectivity. Although the use of such probes will lead to an increased selectivity, their unique presence is insufficient to counter-act the effect of pH as they are often themselves sensitive to pH. To circumvent these side effects and gain stability in a wide range of pH we propose to change the chemical properties of the gate dielectric. Accordingly, we demonstrate herein that the use of a monolayer of engineered lipids as gate dielectric instead of an

oxide (Nguyen et al., 2013 which was further stabilized by a two-dimensional polymerization in the plane of the layer (Charrier et al., 2010; El Zein et al., 2012) leads to a non-sensitive pH dielectric compared to other systems. Furthermore, electrical characteristics measurements have shown that such monolayer of thickness ~ 2.7 nm behaves as a good dielectric, with low leakage current and relatively high voltage electrical breakdown (Dumas et al., 2011). Therefore, these unique properties make this dielectric a system of choice for the fabrication of reliable and specific sensors. In this study the sensor was developed for the detection of ferric ions (Fe^{3+}), and the specificity of the detection is ensured by the grafting of specific probes to the lipids, in this case a γ -pyrone derivative chelator.

In the following, we focus on the properties of our sensor and present results on sensitivity and specificity towards Fe^{3+} ions. We first demonstrate that the engineered sensing layer constituted of the lipid layer and the hydroxyl γ -pyrone derivative is not sensitive to pH change in a wide range of pH therefore fulfilling the conditions for high sensitivity and specificity measurements. Accordingly, we show a very high sensitivity of the sensor which we attribute to a high affinity constant of the chelation process between chelators immobilized on the lipids headgroups and ferric ions. Finally, the specificity towards Fe^{3+} ions is also investigated.

2. Materials and methods

Sub-sections regarding material, device fabrication, and the formation of the lipid monolayers are to be found in the supplementary information file.

2.1. Preparation of the modified lipids with γ -pyrone

Under physiological conditions, γ -pyrone derivatives (L) are known to show high affinity towards iron ions (Kontoghiorghe, 1988; Moggia et al., 2006, 2009; Santos, 2002; Liu and Hider, 2002; Thirumurugan and Perumal, 2011). Indeed, they have the ability to form five-membered chelate rings with iron in which the metal (M) is coordinated by two vicinal oxygen atoms leading to neutral and stable [3L:1M] complexes. The synthesis and characterization of the modified lipid with γ -pyrone was described in detail elsewhere (Nguyen et al., 2013) (Fig. 1a). First, the phosphatidylcholine head-group of the commercially available DC8,9PC **1** was selectively cleaved with phospholipase C affording **2** in quantitative yield. The later was then reacted with the γ -pyrone derivative **3** leading to the end-capped lipid **4** in almost quantitative yield. Compound **3** was synthesized in 2 steps from commercially available kojic acid using standardized procedures (Imafuku et al., 1979; Ma et al., 2004; Ellis et al., 1996).

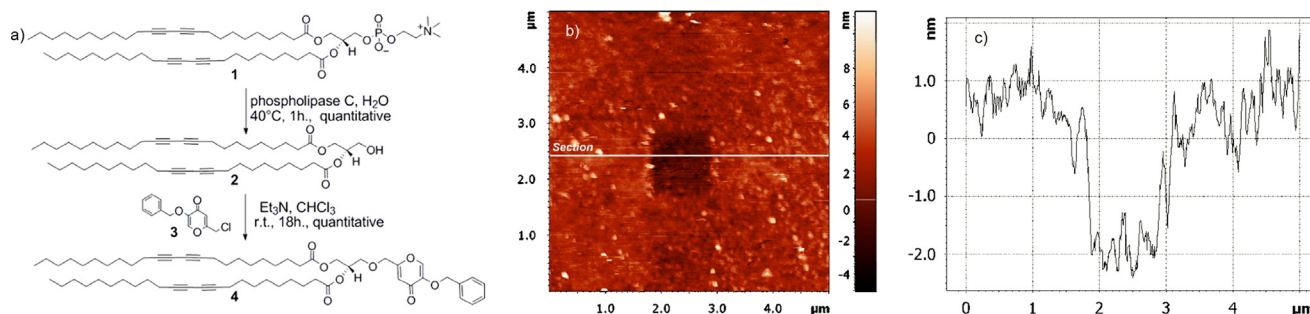


Fig. 1. (a) Synthesis of the modified lipid **4**, (b) AFM image of a lipid **4** monolayer after making a hole with an AFM tip. The cross-section of the hole (c) allows the estimation of the monolayer thickness to ~ 2.7 nm.

2.2. Electrical measurements set-up

Static electrical characteristics (output, I_{DS} – V_{DS} at a given V_{GS} , and transfer, I_{DS} – V_{GS} at a given V_{DS}) were measured using our device as depicted in Fig. S2a). A HP 4140B pico-ampereter/DC voltage source meter apparatus provides both voltage sources and current readings. Two main power supplies are used: one for the channel polarization (between source and drain) and the other for the top gate polarization. The top gate contact is an Ag/AgCl reference electrode dipped into the electrolyte solution. A third power supply unit can be arbitrarily applied to the back gate (silicon substrate); in all reported measurements, the substrate was maintained at ground level. In the described setup, the main carriers are electrons; both drain and gate are positively polarized with respect to the source. The transistors work in accumulation mode, i.e. the more positive charges accumulate at the lipid monolayer surface (gate dielectric surface), the more current will flow between source and drain.

2.3. Sensing measurements protocol

In a typical assay: 100 μ l of an aqueous solution of ions at a given concentration or a buffer solution was poured on top of the active layer (gate area) and left for 5 min. Ion sensitivity measurements have been implemented using strictly the same protocol guarantying that the electrical response (I_{DS}) comes only from the possible ions trapped on the active layer. In each case, the ion-chelator complexes were stabilized by addition of overloads of kojic acid. After two minutes reaction, excess of kojic acid and unreacted ions were removed by thorough rinsing with water. Buffer solutions at a concentration of 0.3 M, with a pH range [3–8] or [8–10.5] have been prepared using appropriate amount of citric acid/sodium phosphate or glycine/sodium hydroxide, respectively. The solution was left inside the PDMS well during measurement.

The sensitivity of the device can be determined directly from the transfer characteristics of the transistor, i.e. the current in the channel versus the gate voltage (V_{GS}). During our experiments, the drain-source voltage (V_{DS}) was maintained at a constant voltage (1.5 V), i.e. above the saturation regime of the transistor. The transistor was

used in accumulation mode and an increase of I_{DS} was expected upon capture of the positive tested ions.

Because sensors response may vary from one to another, all data reported in the manuscript are the result of three independent sets of measurement that were obtained using the same device. The reproducibility of the method is demonstrated by the standard deviations.

3. Results and discussions

3.1. Sensitivity to pH

Aforementioned pH dependence constitutes a major drawback for the development of specific and sensitive biological diagnostic devices and critical needs of non pH-sensitive devices are of crucial interest. Therefore we have undertaken the study of the pH effect on the electrical properties of our sensor. For comparison, two different devices have been fabricated using either the modified lipid with the γ -pyrone derivative (compound **4**, Fig. 1a) or the commercially available DCPC lipid (compound **1**, Fig. 1a) as the active component. In a typical experiment, 100 μ l of a buffer solution at a given pH is poured onto the active area of the transistor. The pH sensitivities were obtained from the transfer curves recorded at V_{GS} varying from 0 V to 2 V. In order to prevent of any possible degradation of the lipid monolayer under certain pH conditions, that would affect the results, sets of pH measurements were realized in random order. Two sets of transfer curves obtained from transistors, with surface gate dimensions of $600 \times 100 \mu\text{m}^2$, and with the lipids **1** (blue) and **4** (red) are shown on Fig. 2a for pH values 4.5 and 8.0, and 3.75 and 7.4, respectively. The electrical behaviours are clearly different between the two devices, even at similar pHs, emphasizing thereby the importance of the terminal groups on the obtained results. Furthermore, the device fabricated with the lipid **1** leads to a large variation of the threshold voltage with pH unlike the device made from **4** which shows very small shift and appears to be less pH-sensitive in the tested range. For both devices the threshold voltage has been extrapolated from transfer curves obtained from a pH range between 2.5 and 10.5 using Eq. (1), see Fig. 2b. The threshold voltage corresponds to the value of V_{GS} at which there is no current flowing

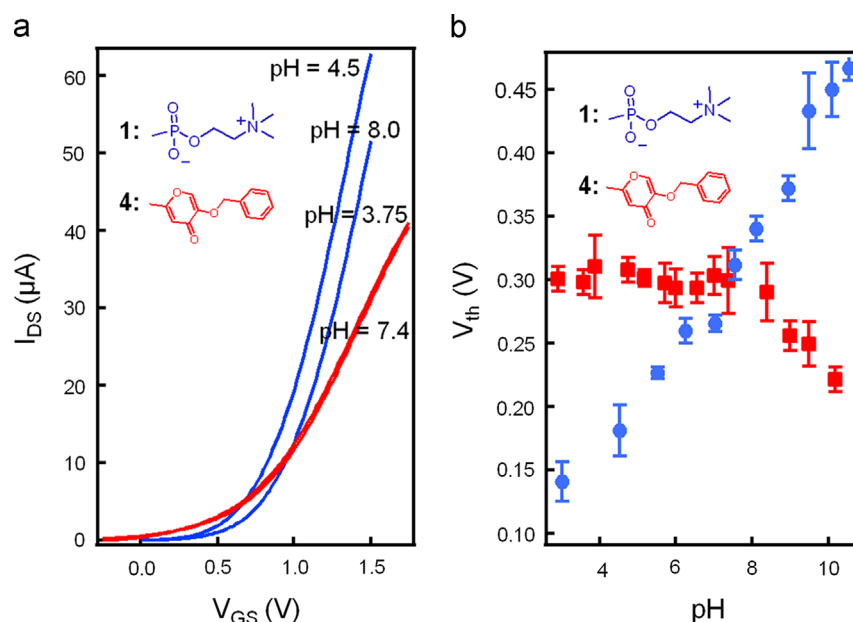


Fig. 2. (a) Transfer curves obtained with lipid **1** (blue, pH=4.5; 8.0) and lipid **4** (red, pH=3.75; 7.4) transistors while exposing the sensors to different pH solutions. (b) Threshold voltage extrapolated from transfer curves obtained with lipid **1** (blue circles) and lipid **4** (red squares) transistors while exposing the sensors to a whole range of pH solution. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in the transistor channel; it varies with the charge density at the surface of the dielectric gate.

$$\sqrt{I_{DS}} = \left(\mu \frac{C_0 W}{2 L} \right)^{1/2} (V_{GS} - V_{Th}) \quad (1)$$

A linear pH-dependence of the threshold voltage was observed for **1** (blue circles) with a slope of 46.3 mV/pH, very similar to an oxide layer (~ 59 mV/pH) (Chang et al., 2010), whereas **4** (red squares) shows no dependence over the same pH range 2.5–8.5. Interestingly, similar results for an analogous γ -pyrone derivative on pH stability was referenced in literature, i.e. the 2-palmitoyloxy 5-palmitoyloxy γ -pyrone stable over the pH range 3–10 (Besse, 2006). Afterwards a linear decrease was obtained at higher pH. As above-mentioned, these behaviours originate from the chemical edge-groups and more accurately of their pKa as well as to slight conformational changes upon pH variation. Thus, from the structures **1**, **2** and **4** the electrical changes arise essentially from the polar head groups namely the phosphatidylcholine (Moncelli et al., 1994), the hydroxyl and the γ -pyrone, respectively. The significant differences in pKa of the above polar groups are an asset and enough to characterize their pH-dependence and explain the obtained electrical output signals. Indeed, stronger is the basic character higher will be the pH sensitivity. In our case the ketone (C=O) group of the γ -pyrone (**4**) has the lowest basic character (meaning the highest acidic character and has consequently the lowest pKa value) compared to the alcohol (OH) group in **2** and the phosphate (RR'PO₄[−]) group in **1**; the latter possessing the highest basic character.

Therefore, the low pH sensitivity for **4** is clearly a benefit for the development of highly sensitive and specific sensors fitting perfectly with the foreseen applications. Thus, to validate our statement, the engineered lipid **4** was subsequently tested in a device dedicated for specific recognition of ferric ions as well as for a set of various cations.

3.2. Sensitivity to ferric ions

The sensitivity of the device made from the modified lipid **4** was first tested for specific recognition of ferric ions using a range of solutions of Fe³⁺ at concentrations varying from 50 fM to 5 mM. The change in threshold voltage (ΔV_{th}) induced by the capture of the charges at the surface of the lipid gate dielectric is reported in Fig. 3. At low concentration a linear variation is observed up to 5 μ M showing the possibility to quantify the density of captured ions on the surface of the sensor active layer. At higher concentrations, a sub-linear behaviour appeared, suggesting a complete

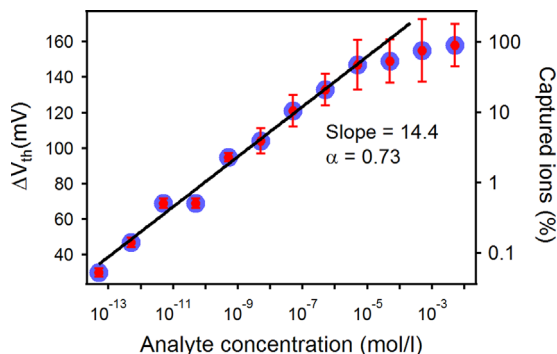


Fig. 3. Left: Variation of threshold voltage after exposing the sensor active area to ferric ions at different concentrations. At low analyte concentration, the standard deviations are too small to be observed; they remain below a value of 3 mV. Right: Percentage of captured ions with respect to the number of immobilized chelator probes on the active sensing area of the sensor.

saturation of the probes or that the maximum complexing capacity of the sensor has been reached. An extrapolation of the curve leads to the maximum change that can be obtained corresponding to a surface potential difference of 160 mV.

The sensitivity performance of the sensor can be determined using the Nernst equation (Knopfmacher et al., 2012; Chen et al., 2011b), which relates the potential of the sensitive ion electrode after capture of the ions (in our case the lipid layer surface potential on the electrolyte side) and the logarithm of the ion concentration in the bulk solution. This equation can be derived for BioFETs according to Eq. (2):

$$\Delta V_{th} = -2.3 \frac{k_B T}{ze} \alpha \times \Delta pI \quad (2)$$

with $\Delta pI = -\log [\text{Ion}]$, z the charge carried by the ion, e , the electrical charge and k_B and T the Boltzmann constant and the temperature. α is the sensitivity parameter given by $\alpha = (C_{dl}/C_s + 1)^{-1}$, with C_s the surface buffer capacitance determined by the density of active chelators on the surface, and C_{dl} the electrolyte double layer capacitance and α varies between 0 and 1. When C_{dl} is much smaller than C_s , $\alpha \sim 1$ and the sensor sensitivity approaches the Nernst limit.

For trivalent ions such as Fe³⁺, the maximum achievable theoretical sensitivity is 19.8 mV/pl. This maximum sensitivity can be inferred in our case by fitting the linear part of the experimental curve Fig. 3. With a slope of 14.4 mV/pl, the described device herein, displays a very high sensitivity with $\alpha = 0.73$. More interestingly, with a detected minimum concentration of 50 fM the device represents, to the best of our knowledge, one of the most effective sensors for the detections of ions.

According to the Gouy–Chapman theory, surface charge density of the lipid monolayer on the electrolyte side and surface potential can be related assuming that the total charge of the double layer in the electrolyte must be equal and opposite of the surface charge σ_i . This relation is given by the Grahame Eq. (3) (Uno et al., 2007):

$$\sigma_i = \sqrt{8 \epsilon_{elect} \epsilon_0 k_B T n_0} \sinh \left(\frac{ze \psi_i}{2 k_B T} \right) \quad (3)$$

with: ϵ_{elect} , the dielectric constant of the electrolyte, ϵ_0 , the vacuum permittivity, n_0 the ionic strength of the solution and ψ_i , the surface potential. According to the site-binding model (Van Hal et al., 1995; Yates et al., 1974), the density of captured ions at saturation regime must be identical to the density of immobilized probes, i.e. in our case to the density of lipids. Since all measurements are realized in water after washing off carefully the solutions containing ions, both electrolyte dielectric constant and ionic strength are assumed to be unchanged during sensing measurement. The density of surface charges before capture of the ions can therefore be calculated from Eq. (4) as:

$$\sigma_0 = C_1 \sinh \left[\text{arcsinh} \left(\frac{\sigma_{max}}{C_1} \right) - \frac{\Delta \psi_{max}}{C_2} \right] \quad (4)$$

with

$$C_1 = (8 \epsilon_{elect} \epsilon_0 k_B T n_0)^{1/2}, \quad C_2 = \frac{ze}{2 k_B T}, \quad \text{and } \Delta \psi_{max} = \psi_{max} - \psi_0 = \Delta V_{max}$$

Considering a dense monolayer with an approximate molecular area of 63 Å² (Caffrey et al., 1991), and assuming that all probes can be complexed, a maximum density of ions of 1.4.10¹⁴ ions/cm² can be detected corresponding in our measurement to a maximum change in surface potential of 160 mV. With $T = 300$ K, $\epsilon_{elect} = 78$, $n_0 = 5.6$ μ M, and $z = 3$, Eq. (4) leads to an initial surface charge of 21 μ C/m², i.e. 1.31×10^{10} charges/cm² which is small compared to the maximum surface charge of 0.67 C/m².

Similarly, the percentage of captured ions with respect to the maximum capacity of the sensor can then be extrapolated from experimental data using Eq. (4) and replacing σ_{max} and ψ_{max} by σ_i

and ψ_i for the case of a sub-saturation of the chelator sites. The result shown in Fig. 3 (right scale) indicates that the sensor is highly sensitive at very low concentrations with the linear range of the change in surface potential corresponding to the capture of 0.05% ($336 \mu\text{C}/\text{m}^2$) to 50% ($0.33 \text{ C}/\text{m}^2$) of the maximum density of capturable ions. This behaviour can be explained insofar as the surface charge is initially low, even small amounts of captured ions will induce a measurable shift of the surface potential. The sub-linear behaviour above 50% of probe/ion complexation can be explained by (i) the reduction of available sensing sites and (ii) the charge screening induced by the presence of the ions on the surface leading to electrostatic repulsion, which is unfavourable for the capture of other ions. At each concentration, the ability to form a ligand-analyte complex is related to the affinity constant of the reaction. The determination of these constants can be done using the site-binding model (Van Hal et al., 1995; Yates et al., 1974) which describes the complexation mechanism as the equilibrium between the chelator surface sites and the Fe^{3+} ions in the bulk of the solution as follows:



With C_hFe^{3+} , the chelator/ion complexes, C_h , the available chelator sites on the surface, and, Fe_B^{3+} , the ferric ions in the bulk of the solution. K_a and K_d refer to the affinity and dissociation constant, respectively. The equilibrium condition is:

$$K_d = \frac{\nu_{\text{C}_h} \times a_{\text{Fe}_B^{3+}}}{\nu_{\text{C}_h\text{Fe}^{3+}}} \quad (6)$$

ν_{C_h} , $\nu_{\text{C}_h\text{Fe}^{3+}}$ are the numbers of chelator and chelator/ion complex sites per unit area respectively and $a_{\text{Fe}_B^{3+}}$ is the activity of the Fe^{3+} directly at the sensing layer surface. It is related to the bulk activity of Fe_B^{3+} by the Nernst equation:

$$a_{\text{Fe}_B^{3+}} = a_{\text{Fe}^{3+}} \exp\left(\frac{-e\psi}{k_B T}\right) \quad (7)$$

The total number of surface sites, N_s , can be described as:

$$N_s = \nu_{\text{C}_h} + \nu_{\text{C}_h\text{Fe}^{3+}} \quad (8)$$

The surface charge density is given by:

$$\sigma_i = z\nu_{\text{C}_h\text{Fe}^{3+}} \quad (9)$$

and can be expressed using Eqs. (5) and (6) as:

$$\sigma_i = zN_s \frac{a_{\text{Fe}_B^{3+}}}{K_d + a_{\text{Fe}_B^{3+}}} \quad (10)$$

From Eqs. (6) and (9), the dissociation constant can then be expressed as:

$$K_d = K_a^{-1} = a_{\text{Fe}_B^{3+}} \exp\left(\frac{-q\psi}{k_B T}\right) \frac{N_s - \sigma_i}{\sigma_i} \quad (11)$$

For each tested concentration, the dissociation constant can be calculated using the experimental values of the surface potential after trapping ions, and the corresponding densities of surface charges. Fig. 4 gives the corresponding affinity constant calculated for each tested concentration. The first observation is that the values are very high therefore reflecting the high stability of Fe^{3+} /chelator complexes, even after rinsing with water. Such high values have already been reported previously for 3D systems of such type complexes (Santos et al., 2012; Sadeghi-aliabadi et al., 2013) but these are among the highest reported so far for 2D systems (Hanaoka et al., 2008) in which complexation is limited by steric and electrostatic repulsion. This conclusion leads to the second observation that the affinity constant decreases with increasing concentrations from $5 \cdot 10^{10} \text{ mol/l}$ for a ferric ion solution

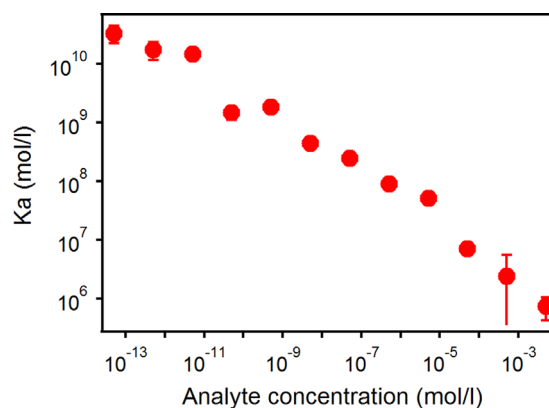


Fig. 4. Affinity constant for ferric ions/chelators complexation determined using Eq. (11) for different concentrations of ferric ions in the analyte. Standard deviations are mostly too small to be seen on a logarithm scale; they are typically of the same order of magnitude as the values of the affinity constants.

of 50 fM to $8 \cdot 10^5 \text{ mol/l}$ at 5 mM , i.e. with increasing ion surface coverage, increasing surface charge and therefore electrostatic repulsion.

3.3. Specificity of the chelator and unspecific adsorption

In a BioFET sensor, sensitivity and specificity are important key parameters that strongly depend on pH, unspecific adsorption (molecules, ions) and the probe itself. Design of specific sensors for ferric ions led us to test the lipid **4** as a monolayer in the transistor channel. In order to demonstrate the role of the probe, comparative sensing measurement of Fe^{3+} have been conducted, with the compounds **1** and **4** respectively, at a concentration of $5 \times 10^{-7} \text{ M}$. All experiments were strictly performed following the same procedure i.e. deposition of $100 \mu\text{l}$ of an aqueous solution of ferric ions left stand for 5 min, followed by addition of an excess of kojic acid prior to thoroughly rinse with DI water. Electrical characteristics, before (square) and after (circle) addition of the ferric solution, are depicted in Fig. 5a. As expected, device made from compound **4** (red, lower curves), leads to a relatively large shift up to 160 mV upon addition of the ferric ions solution while only 8 mV was observed for **1** (blue, upper curves). These results evidence the low unspecific adsorption of the deposited monolayer that is in good agreement with our expectations. Finally, the specificity of the device was tested over a set of putative competitive ions such as Fe^{2+} , Al^{3+} , K^+ , Cu^{2+} , Pb^{2+} at high concentration (Fig. 5b). Even at high concentration, up to 10^{-3} M , none of the competitive ions have given a threshold shift higher than 25 mV suggesting a high specificity and low unspecific adsorption for our device. Accordingly, from Eq. (10), low affinity constants have been determined with values remaining below 1 mol/l (except for Fe^{2+} at low concentration), highlighting the high specificity towards Fe^{3+} .

4. Conclusion

To summarize, we reported herein a detailed study on the performances of a novel BioFet based on the use of an engineered lipid monolayer as gate dielectric. Commercial lipids were specifically cleaved with an enzyme then tethered to a γ -pyrone derivative exhibiting high specificity and sensitivity towards ferric ions as well as a low sensitivity over a large pH range. The low sensitivity to pH in the acidic range is nearly null, and is certainly an asset for specific biosensing measurements in the biomedical field. The high sensor sensitivity was demonstrated with the

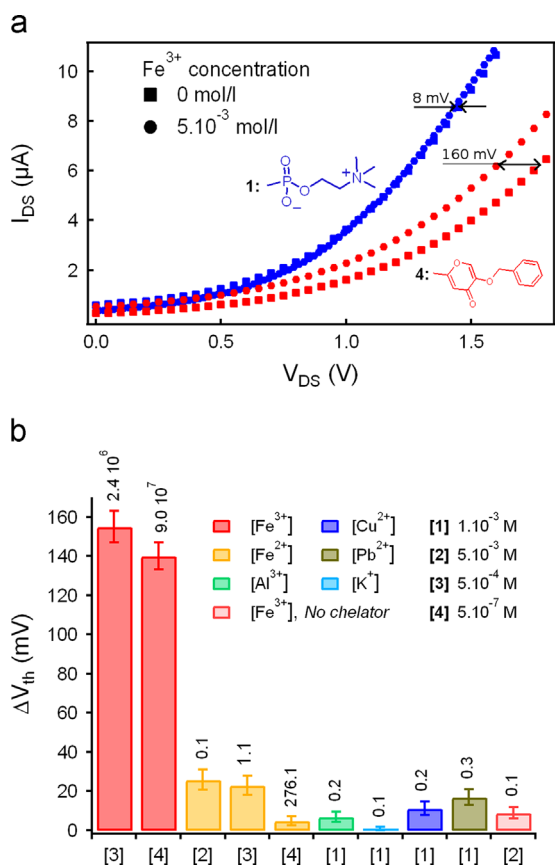


Fig. 5. (a) Transfer curves obtained with lipid compound 1 (blue upper curves) and lipid compound 4 (red lower curves) transistors before and after exposing the sensor to 5.10^{-3} mol/l ferric ions. (b) Threshold voltage shift after exposing the lipid compound 4 sensor to a whole variety of ions at high concentration and lipid compound 1 transistor (No chelators case) to 5.10^{-3} mol/l ferric ions. The values indicated on the graph are the corresponding affinity constant in mol/l. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

detection of ferric ions at concentrations as low as 50 fM. To the best of our knowledge, this detection limit is referred to be among the best values ever reported in literature for detecting ions with a FET-biosensor. We believe that the high sensitivity gained with such device is related to the low density of surface state at the gate dielectric surface, therefore making the transistor sensitive to very small changes of the density of captured charges. For this concentration a very high affinity constant of 5.10^{10} mol/l has been determined. Such type of high value was already reported for γ -pyrone derivatives molecules but never for 2D systems in which surface charging and steric repulsion are both limiting factors to chelator/ions complexation. This affinity constant decreases with increasing concentration from 5.10^{10} to 8.10^5 mol/l due to charge screening as the surface of the monolayer gets saturated with ferric ions, and to the decrease of accessible free chelator sites. However these values still remain at least 10^6 times higher than for other tested ions therefore demonstrating very high specificity towards ferric ions and also that non-specific adsorption of ions on the lipid layer is very low. Hence, the lipid monolayer does not require any passivation layer to prevent this adsorption.

These results are very promising, and we believe that because of their handiness, such type of sensors is very promising for point of care biosensing diagnostic applications. In addition, the system shows potential for high versatility. Indeed, numerous chelators have already been developed for other kinds of ions [Cu, Cl, K,...] and it would be relatively easy to engineer those and graft them to the lipids. Finally such type of sensor could be developed to realize

multiplexing measurements using microfluidic systems and make cross-sensing detection for high precision diagnostic purposes.

We are confident that the strategy described in this paper is valuable and worth pursuing because this unique combination of properties in heterogeneous phase, makes this new probe a system of choice for the foreseen applications in integrated devices.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2013.11.051>.

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