Electrochemical transistors with ionic liquids for enzymatic sensing

Sang Yoon Yang,[a] Fabio Cicoira,[a] Robert Byrne,[b] Fernando Benito-Lopez,[b] Dermot Diamond,[b] Róisín M. Owens[c], George G. Malliaras[c]

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

We report an enzymatic sensor based on an organic electrochemical transistor that uses a room temperature ionic liquid as an integral part of its structure and as an immobilization medium for the enzyme and the mediator.

Although conducting polymer electrodes have been used in biosensing and actuation for decades, recent developments in the field of organic electronics have made available a variety of devices that bring unique capabilities at the interface with biology.[1-2] One example is organic electronic ion pumps, which are able to precisely control the flow of ions between two reservoirs, and have been used to pump neurotransmitters and stimulate cochlear cells in the inner ear of a guinea pig.[3-5] Another example is organic electrochemical transistors (OECTs) that are being developed for a variety of biosensing applications, including the detection of ions,[6-8] metabolites (such as glucose[9] and lactate[10]) and antibodies.[11]

Originally developed by Wrighton in the 80's,[12] OECTs consist of a conducting polymer film (channel of the transistor) in contact with an electrolyte. A gate electrode is immersed in the electrolyte, while source and drain electrodes make contact to the channel and allow a measurement of its conductance.[13] A polymer that is commonly used in OECTs is poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS). In a typical experiment, a positive potential is applied at the gate electrode, which causes cations from the electrolyte to enter the PEDOT:PSS film and dedope it, causing a decrease in its conductance. This manifests itself as a change in the current that flows between the source and drain electrodes.[13]

OECTs exhibit a typical feature associated with transistors, namely a small current at the gate electrode can cause a large change in the current that flows in the channel, and as such it has been used to amplify biological signals. The gate electrode, for example, can be used as a "working electrode", and the current that flows in it as a result of a redox reaction can be amplified by the OECT. This amplification manifests itself by the fact that the sensor's sensitivity increases with gate voltage,[14] and leads to devices that require very simple electronics for signal readout. Zhu *et al.* took advantage of this fact to demonstrate a simple sensor for glucose in phosphate buffered saline (PBS).[9] The sensor consisted of a PEDOT:PSS OECT with a Pt gate electrode and with the redox enzyme glucose oxidase (GOx) dissolved in the electrolyte (PBS). This work was extended to demonstrate sensors that work down to the micromolar concentration range,[14] can be made entirely out of polymers by using an appropriate mediator,[15] and operate with other redox enzymes, allowing the development of multianalyte sensors.[10]

In parallel to these developments, room temperature ionic liquids (RTILs - molten salts which are entirely composed of ions and are in the liquid state at ambient conditions[16]) have been gaining considerable interest in electrochemistry as alternatives to aqueous electrolytes such as PBS.[17] Reasons include a large electrochemical window of operation, and a high conductivity, which alleviates the need for a supporting electrolyte. For the particular case of enzymatic sensing, enzymes have been found to retain their selectivity, stability and in some cases even enhance their catalytic activity in a RTIL medium, though this last point is still a matter of debate.[16, 18-22] Consequently, RTILs have been incorporated in amperometric biosensors.[23-25]

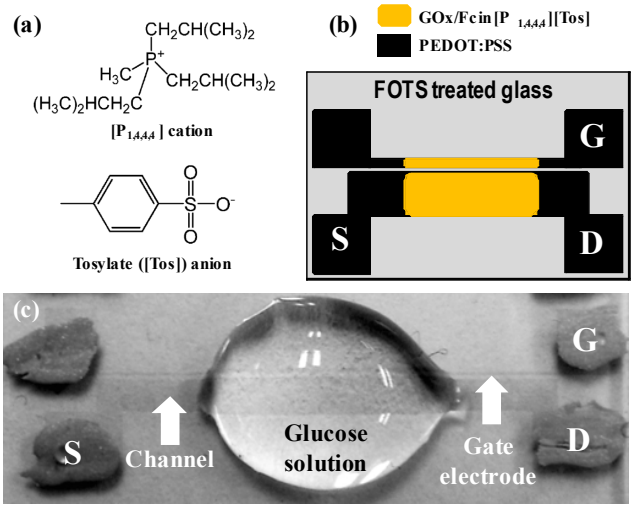

Fig. 1: (a) Chemical structure of $[P_{1,4,4,4}][Tos]$. (b) Layout of the OECT, indicating the area where the RTIL was confined. (c) Photograph of the OECT with a drop of glucose solution added. The balls at the pads are made of silver paste.

In this communication, we report an enzymatic sensor based on an OECT that uses a RTIL as an integral part of its structure. The strategy we follow involves patterning the RTIL over the active area of the OECT, and using it as a reservoir for the enzyme and the mediator. When the solution containing the analyte is added to the device, it mixes with the RTIL. The analyte, the enzyme, and the mediator are allowed to interact and the OECT transduces this interaction. According to this strategy, an important requirement for the RTIL is that it wets the PEDOT:PSS film, thus allowing the enzyme and the mediator to be patterned over the active area

of the device. Moreover, the RTIL should be miscible with the aqueous solution that carries the analyte (PBS). The RTIL triisobutyl(methyl)-phosphonium tosylate ([$P_{1,4,4,4}$][Tos], Fig. 1a, supplied by Cytec Industries) satisfies these requirements, as the Tos anion gives it a rather hydrophilic character. Previous studies have also shown [$P_{1,4,4,4}$][Tos] to be a biocompatible medium for glucose consumption by bacteria.[26]

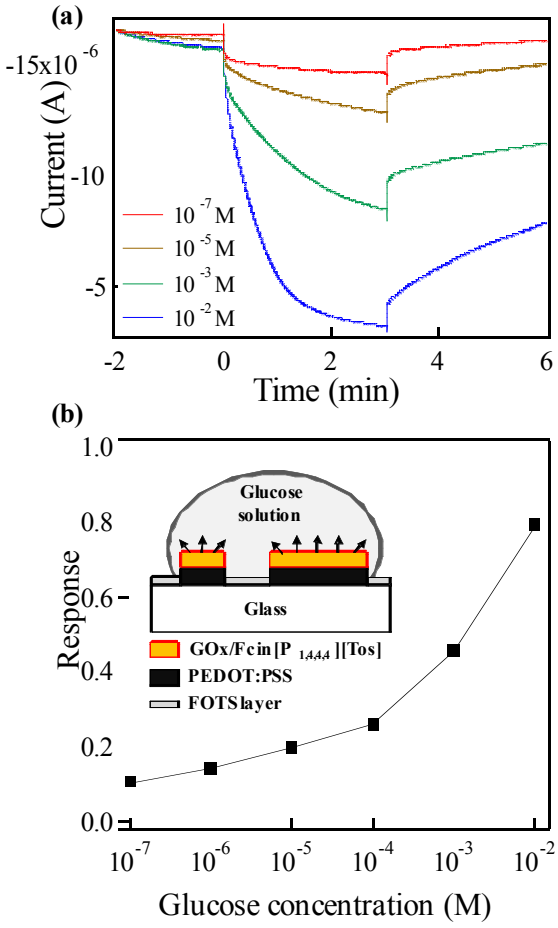

Fig. 2: (a) Transient response of the drain current of an OECT upon application of a gate voltage of 0.4 V and duration of 3 min. The drain voltage was -0.2 V. (b) Current modulation (represented as the dimensionless quantity ΔI/I) of the OECT as a function of glucose concentration. Inset shows the concept of device operation, and the arrows indicate the dissolution the RTIL carrying the enzyme and the mediator into the analyte solution.

The layout of the device is shown in Fig. 1b. Details of the fabrication process are given in *supplementary information*. Two parallel stripes of PEDOT:PSS, with widths of 100 μm and 1 mm, respectively, were patterned on a glass support using photolithography. Contact pads at the end of the stripes allowed facile electrical connection to the source-measure units. The wide stripe was used as the transistor's channel and the narrow one as the gate electrode, as it has been shown that for enzymatic sensing the area of the channel must be larger than that of the gate electrode.[27] A monolayer of (tridecafluoro-1,1,2,2-tetrahydrooctyl) trichlorosilane (FOTS) was patterned on the surface of the device leaving uncovered only a small area of the channel and of the gate electrode. These areas of PEDOT:PSS which were left uncovered by FOTS served as hydrophilic "virtual wells"[28] and were shown to be effective in confining the RTIL (and the glucose solution, when it was added) over the centre of the device.

The experiments involved placing a small amount (1.43 *μl*) of [$P_{1,4,4,4}$][Tos] that included the enzyme glucose oxidase (GOx, 500 unit/ml) and the mediator ferrocene [bis (n5-cyclopentandienyl) iron] (Fc, 10 mM) on the centre of the device and allowing it to be accommodated in the hydrophilic virtual wells. Subsequently, 50 *μl* of glucose solution in PBS were added to the device and allowed to mix with the RTIL solution. The resulting solution did not spread, but rather formed a neat droplet over the area defined by the hydrophilic virtual wells, as seen in Fig. 1c. It should be noted that we found it critical to create such wells on both the channel and the gate electrode in order to achieve reproducible control over the spread of the solution.

Fig. 2a shows the transient response of the drain current of an OECT for different concentrations of glucose solution, upon the application of a 0.4 V pulse at the gate electrode with a duration of 3 minutes. The drain voltage was -0.2 V. The data shows the characteristic decrease of drain current upon gating,[14] which has been understood on the basis of the reactions shown in Fig. 3. As glucose in the solution is oxidised, the enzyme (GOx) itself is reduced, and cycles back with the help of the Fc/ferricenium ion (Fc^+) couple, which shuttles electrons to the gate electrode (Fig. 3a). For example, for 10^{-2} M of glucose, this cascade of reactions causes a current of 8×10^{-8} A to flow to the gate electrode. At the same time, cations from the solution (M^+) enter the PEDOT:PSS channel and dedope it (Fig. 3b),[29] thereby decreasing the drain current to a degree that depends on glucose concentration.[14] Due to the amplification inherent in the OECT, the change in the drain current is much larger than the gate current itself (for 10^{-2} M of glucose the drain current changes by 1.2×10^{-5} A, as shown in Fig. 2a).

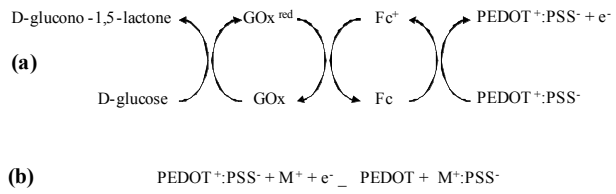

Fig. 3: Reactions at the gate electrode (a) and at the channel (b) of the OECT.

Fig. 2b shows the response of the OECT, in terms of change in drain current (ΔI/I), to glucose concentration. The detection range is shown to be at least from 10^{-7} to 10^{-2} M, and covers the clinical glucose level in human saliva (0.008 ~ 0.21 mM), suggesting that this device could be used as a glucose detector for monitoring glucose both in blood (2~30 mM) and in saliva.[30] It should be noted that in order to avoid fouling and dilution effects, a new device was used for each glucose solution that was measured (each data point in Fig. 2b was taken from a different device). This is consistent with the mode of operation of single-use sensors, which is particularly suitable to organic electronic devices as they can be produced

using low-cost techniques. The device-to-device reproducibility was found to be better than 10%.

It is important to note that, contrary to Fc, which dissolved in $[P_{1,4,4,4}][Tos]$, GOx was present in a dispersed state in $[P_{1,4,4,4}][Tos]$, and it dissolved only when the glucose solution was added to the OECT. It is well known that the dissolution of enzymes in a RTIL can result in a change of the secondary and higher enzyme structure and causes the loss of enzyme activity.[31] Therefore, a heterogeneous state in which GOx is dispersed in the RTIL can protect it from denaturation and help maintain its activity. In the same context, dispersion rather than dissolution can be used as a way to enhance the long-term stability of biosensors. Although we did not investigate this matter in any depth, we tested an OECT stored at ambient temperature 30 days after its fabrication. When a 10^{-2} M glucose solution was added the response was the same (~0.8) as that of a freshly fabricated device.

In summary, we demonstrated the integration of an organic electrochemical transistor with a room temperature ionic liquid to yield an enzymatic sensor. The ionic liquid was confined on the surface of the transistor using a photolithographically patterned hydrophobic monolayer, which defined hydrophilic virtual wells. An enzyme and a mediator were immobilized in the ionic liquid and, when the aqueous solution which carried the analyte was added, they dissolved in it. The enzyme was in a dispersed state in the ionic liquid, which may prove to be a good strategy for improving long-term storage. Using the glucose/glucose oxidase pair as a model, we demonstrated analyte detection in the 10^{-7} to 10^{-2} M concentration range.

Notes and references

[a] *Materials Science and Engineering, Cornell University, Ithaca, NY 14853, USA. Fax: +1-607-255-2365; Tel:1-607-255-1956*

[b] *Clarity: Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland. Tel: +353-1-7005404*

[c] *Centre Microelectronique de Provence, Ecole Nationale Superieure des Mines de Saint Etienne, 880, route de Mimet, 13541 Gardanne, France. Fax: +33-4-42-61-65-97; Tel: +33-4-42-61-66-44; E-mail: malliaras@emse.fr*

† Electronic Supplementary Information (ESI) available: [device fabrication procedure]. See DOI: 10.1039/b000000x/

[1] M. Berggren, A. Richter-Dahlfors, Advanced Materials 2007, 19, 3201.

[2] R. M. Owens, G. G. Malliaras, Mrs Bull 2010, 35, 449.

[3] J. Isaksson, P. Kjall, D. Nilsson, N. D. Robinson, M. Berggren, A. Richter-Dahlfors, Nature Materials 2007, 6, 673.

[4] K. Tybrandt, K. C. Larsson, S. Kurup, D. T. Simon, P. Kjall, J. Isaksson, M. Sandberg, E. W. H. Jager, A. Richter-Dahlfors, M. Berggren, Advanced Materials 2009, 21, 4442.

[5] D. T. Simon, S. Kurup, K. C. Larsson, R. Hori, K. Tybrandt, M. Goiny, E. H. Jager, M. Berggren, B. Canlon, A. Richter-Dahlfors, Nature Materials 2009, 8, 742.

[6] J. T. Mabeck, J. A. DeFranco, D. A. Bernards, G. G. Malliaras, S. Hocde, C. J. Chase, Applied Physics Letters 2005, 87, 013503.

[7] D. A. Bernards, G. G. Malliaras, G. E. S. Toombes, S. M. Gruner, Applied Physics Letters 2006, 89.

[8] P. Lin, F. Yan, H. L. W. Chan, ACS Applied Materials & Interfaces 2010, 2, 1637.

[9] Z. T. Zhu, J. T. Mabeck, C. C. Zhu, N. C. Cady, C. A. Batt, G. G. Malliaras, Chemical Communications 2004, 1556.

[10]S. Y. Yang, J. A. DeFranco, Y. A. Sylvester, T. J. Gobert, D. J. Macaya, R. M. Owens, G. G. Malliaras, Lab on a Chip 2009, 9, 704.

[11]D.-J. Kim, N.-E. Lee, J.-S. Park, I.-J. Park, J.-G. Kim, H. J. Cho, Biosensors and Bioelectronics 2010, 25, 2477.

[12]H. S. White, G. P. Kittlesen, M. S. Wrighton, Journal of the American Chemical Society 1984, 106, 5375.

[13]D. A. Bernards, G. G. Malliaras, Advanced Functional Materials 2007, 17, 3538.

[14]D. A. Bernards, D. J. Macaya, M. Nikolou, J. A. DeFranco, S. Takamatsu, G. G. Malliaras, Journal of Materials Chemistry 2008, 18, 116.

[15]N. Y. Shim, D. A. Bernards, D. J. Macaya, J. A. DeFranco, M. Nikolou, R. M. Owens, G. G. Malliaras, Sensors-Basel 2009, 9, 9896.

[16]M. Armand, F. Endres, D. R. MacFarlane, H. Ohno, B. Scrosati, Nature Materials 2009, 8, 621.

[17]M. C. Buzzeo, R. G. Evans, R. G. Compton, Chemphyschem 2004, 5, 1106.

[18]K. Fujita, D. R. MacFarlane, M. Forsyth, Chemical Communications 2005, 4804.

[19]K. Fujita, D. R. MacFarlane, M. Forsyth, M. Yoshizawa-Fujita, K. Murata, N. Nakamura, H. Ohno, Biomacromolecules 2007, 8, 2080.

[20]S. Park, R. J. Kazlauskas, Curr Opin Biotech 2003, 14, 432.

[21]H. Zhao, O. Olubajo, Z. Y. Song, A. L. Sims, T. E. Person, R. A. Lawal, L. A. Holley, Bioorg Chem 2006, 34, 15.

[22]Z. Yang, J Biotechnol 2009, 144, 12.

[23]Y. Liu, L. Liu, S. J. Dong, Electroanalysis 2007, 19, 55.

[24]X. Shangguan, H. F. Zhang, J. B. Zheng, Electrochemistry Communications 2008, 10, 1140.

[25]X. M. Wu, B. Zhao, P. Wu, H. Zhang, C. X. Cai, Journal of Physical Chemistry B 2009, 113, 13365.

[26] D. Baumann, A.J. Daugulis and P.G. Jessop, Appl. Microbiol. Biotechnol 2005, 67, 131.

[27]F. Cicoira, M. Sessolo, O. Yaghmazadeh, J. A. DeFranco, S. Y. Yang, G. G. Malliaras, Advanced Materials 2010, 22, 1012.

[28]B. Zhao, J. S. Moore, D. J. Beebe, Science 2001, 291, 1023.

[29]D. Nilsson, T. Kugler, P. O. Svensson, M. Berggren, Sensors and Actuators B-Chemical 2002, 86, 193.

[30]M. Yamaguchi, M. Mitsumori, Y. Kano, Ieee Eng Med Biol 1998, 17, 59.

[31]R. M. Lau, M. J. Sorgedrager, G. Carrea, F. van Rantwijk, F. Secundo, R. A. Sheldon, Green Chem 2004, 6, 483.