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Dual on–off and off–on switchable oligoaziridine biosensor

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ABSTRACT

A water-soluble biocompatible aziridine-based biosensor with pendant anthracene units was synthesized by radicalar polymerization of N-substituted aziridines in supercritical carbon dioxide. The binding ability of the sensor towards a series of metal ions was examined by comparing the fluorescence intensities of the solutions before and after the addition of 100 equivalents of a solution of the metal ion chloride salt. A fast, simple and highly optical sensitive dual behavior, "off–on" and "on–off" response, was observed after the biosensor was exposed to the metal cations in aqueous solution. Zinc presented the highest fluorescence enhancement (turn-on) and copper presented the highest fluorescence quenching (turn-off). The response time was found to be instantaneous and the detection limit was achieved even in the presence of excess metal cation competitors. By using immunofluorescence microscopy it was also shown that oligoaziridine acts as an "on–off" probe through highly sensitive (detection limit of 1.6 nM), selective and reversible binding to copper anions under physiologic conditions using living Human Fibroblast cells. The stoichiometry for the reaction of the biosensor with Cu^{2+} was determined by a Job plot and indicates the formation of an oligoaziridine-Cu^{2+} 1:2 adduct.

1. Introduction

In recent years there has been a growing need to develop highly selective and efficient chemical sensors for fast, easy and economical monitoring of a wide range of molecules in many fields such as environmental chemistry, food industry, medical diagnosis and life sciences (Bissel et al., 1992; de Silva et al., 1997, 2000; Desvergne and Czarnik, 1997; Prodi et al., 2000). Optical sensors are molecular receptors whose optical properties change upon binding to specific guests. One remarkable application of optical sensors is the sensing of toxic and carcinogenic metals in environmental samples (Valeur and Leray, 2000; Prodi et al., 2000). Copper is an essential trace element present in the human body and plays a vital role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals but can often be toxic to certain biological systems when the levels of Cu^{2+} exceed cellular needs. Fluorescent probes for Cu^{2+} have been thoroughly studied since they are able to respond through a simple fluorescence enhancement (off–on) or quenching (on–off) mechanism. Despite the fact that different complexing reagents are already known for the determination of copper via chemical sensors (Quang and Kim, 2000) only few are able to work under physiological conditions (Wang et al., 2010; Yu et al., 2009; Swamy et al., 2008). With respect to fluorescence response, anthracene and pyrene have emerged as the most effective functional groups for fluorescence signaling (Akkaya et al., 1990; Huston et al., 1990; Shiraishi et al., 2005) due to their high sensitivity and selectivity (Fabbrizzi et al., 2003). Since polyaziridines are known to be good metal chelating agents (Rivas et al., 2009), and can be easily synthesized using supercritical carbon dioxide ($scCO_2$) as a clean and sustainable technology, a fully integrated sensing system comprising both pyrene and polyaziridine's potential was envisaged.

Herein, we report the "green" synthesis of a specially designed oligoaziridine biosensor having pyrene pending units in $scCO_2$, its photophysical characterization, and dual fluorescence response (turn-on and turn-off) towards a series of metal cations in aqueous solutions under physiologic conditions. Although potentially useful for investigating zinc(II) metalloneurochemistry, in this study the biosensor was used to monitor intracellular Cu^{2+} in Human Fibroblast (HF) cells.

2. Materials and methods

2.1. Materials

N-(p-Toluenesulfonyl)-2-(anthracen-9-ylmethylene)aziridine (1) was synthesized according to literature (Branco et al., 2010).

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All the used chemicals were used as received. Carbon dioxide was supplied by Air Liquide with a purity of 99.998%.

2.2. Instrumentation

The FTIR spectrum was obtained on a Perkin Elmer Spectrum 1000 instrument and sample films were cast directly onto NaCl disks. The NMR spectra were recorded on a Bruker ARX 400 MHz equipment using D_2O as solvent. ^1H NMR chemical shifts are reported as δ (ppm=parts per million) relative to the residual solvent peak. The UV-spectra were acquired in a Perkin Elmer Lambda 25 UV/Vis Spectrometer. The fluorescence spectra were recorded at 25 °C on a Perkin Elmer LS 45 Luminescence Spectrometer using a 10 mm path quartz cell.

2.3. Synthesis of the oligoaziridine biosensor

The monomer **1** (1.0 equiv.), ethanol aziridine (2) (4.0 equiv.), and initiator ($BF_3 \cdot OEt_2$, 10 wt%) were loaded into the high-pressure cell. Liquid CO_2 was loaded into the cell using a high-pressure compressor. The cell was immersed in a thermostated water bath at 65 °C and CO_2 was pumped into the system to achieve a pressure of 25 MPa. At these experimental conditions a homogeneous phase was obtained with all the reactants completely soluble in the supercritical medium. The reaction was allowed to proceed for 24 h under stirring to ensure higher yields. At the end of the reaction, the resulting polymer was slowly washed with fresh high-pressure CO_2 in order to clean the remaining residues of initiator and unreacted monomers. Upon venting oligoaziridines **3** and **4** were obtained as an inseparable mixture, as light yellow oil in quantitative yield, based on recovered starting materials. IR (film) ν_{max} (cm^{-1}): 3388, 1655, 1459, 1368 (S=O), 1154 (S=O), 1035. ^1H NMR (400 MHz, D_2O): δ 8.27, 7.94, 7.44, 6.94, 6.68 (ArH, bs), 4.00, 3.64, 3.32, 3.03-2.97, 2.74 (backbone, bs), 2.39 ($ArCH_3$, s). MS (MALDI-TOF): m/z 736.3 [$M+1$] ($m=3$, $n=1$, oligoaziridine **3**), 349.3 [$M+1$] (oligoaziridine **4**). From ^1H NMR analysis (integration of the aromatic protons relative to the protons of the oligomers backbone) the oligomers **3**:**4** ratio in the mixture is approximately 1:6.

2.4. Sensing protocol

A series of metal ions (Li^+, Mg^{2+}, Ce^{3+}, Pb^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Hg^{2+}) solutions were prepared from the corresponding chloride salts. The optical response of the biosensor ($\lambda_{ex}=370$ nm) was examined by comparing the fluorescence intensity of the solution (10 μM) before and right after the addition of 100 equivalents of the analyte. The quenching experiments were both performed in aqueous solution (pH=7.4) and in methanol.

2.5. Proliferation of HF cells in the presence of the oligoaziridine biosensor

To characterize the proliferation of HF cells (Normal Human Dermal Fibroblasts adult, criopreserved cells, PromoCell) in the presence of the oligoaziridine biosensor, cells were cultured in 24 well plates at 1×10^5 cells/well, for 24 h, with Dulbecco's Modified Eagle Medium (DMEM)-F12 supplemented with heat-inactivated fetal bovine serum (FBS, 10% v/v) and 1% antibiotic/antimycotic solution. Cells were kept in culture at 37 °C, in a humidified atmosphere with 5% CO_2. Cell growth was monitored using an Olympus CX41 inverted light microscope equipped with an Olympus SP-500 UZ digital camera.

2.6. Characterization of the cytotoxic profile of the oligoaziridine biosensor

Once HF cells attained confluence, they were subcultivated in a 96-well plate (Nunc) by a 5-min incubation in 0.18% trypsin (1:250) and 5 mM EDTA. Cells were seeded at a density of 6×10^4 cells/well and cultured with DMEM-F12 for 24 h. The plates were UV irradiated for 30 min, before cell seeding as previously described in the literature (McRae et al., 2009). After one day, the culture medium was removed and the sensor was dissolved in DMEM-F12 at a concentration of 0.1 mg/mL. Then 200 μL of this solution was added to the cells. After an incubation of over 24 and 96 h, the mitochondrial redox activity of the viable cells was assessed through the reduction of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium (MTS) (Promega) into a water-soluble brown formazan product ($n=5$). The medium of each well was then removed and replaced with a mixture of 100 μL of fresh culture medium and 20 μL of MTS/PMS reagent solution. The cells were incubated for 4 h at 37 °C, under a 5% CO_2 atmosphere. The absorbance was measured at 490 nm using a microplate reader (Sanofi, Diagnostics Pauster). Wells containing cells in the culture medium without sensor were used as negative control. Ethanol 96% was added to wells containing cells as a positive control (Ribeiro et al., 2009; Baoum et al., 2010).

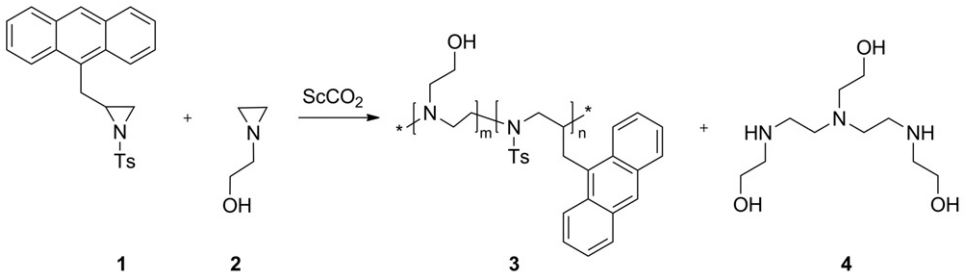
2.7. Cell incubation and imaging

HF cells were grown in DMEM-F12 containing 10% FBS in glass-bottomed coverslips with collagen. Afterwards cells were washed with phosphate-buffered saline (PBS) and then incubated overnight with 0.1 mg/mL of sensor. Finally, they were washed again with PBS before being observed with a fluorescence microscope. Fluorescence imaging of the sensor inside cells was observed using Zeiss AX10 microscope ($\lambda_{ex}=365$ nm, $\lambda_{em}=$ 420–470 nm) and images were processed with the Axio Vision Real 4.6 software. Subsequently, copper (20 μg/mL) was added to the cells and then observed during 15, 30, 60, 120 and 600 min. Finally, cells were incubated with EDTA (0.035%) and then the recovery of fluorescence was observed with time (240, 360 and 480 min). Images of HF cells without sensor were captured to be used as control.

3. Results and discussion

The use of supercritical carbon dioxide as a reaction medium, besides the many advantages of a "green" process, leads to materials with a high degree of purity, avoiding contaminants that could interfere with sensitive biological samples. The polymerization of aziridines in $scCO_2$ was previously reported using a free *NH*-aziridine monomer (Ihata et al., 2004) leading to termoresponsive polyurethanes due to the incorporation of CO_2 in the polymer backbone. Surprisingly, no incorporation of CO_2 was detected by us in the polymerization of *N*-substituted aziridine monomers. Therefore, to the best of our knowledge this is the first report of oligoaziridines obtained by polymerization in $scCO_2$. monomer **1** an aziridine bearing an anthracene pendant unit, was synthesized using a simple methodology (Branco et al., 2010) and its copolymerization with commercial *N*-ethanol aziridine (**2**) in $scCO_2$ using boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) as initiator led to a chromatographically inseparable mixture of oligoaziridines **3** ($m=4$, $n=1$) and **4** (Scheme 1).

The polymerization reaction was investigated by varying the comonomers feed ratio. We found that increasing the feed ratio of comonomer **1** in the polymerization leads to insolubility of



Scheme 1. Synthesis of an oligoaziridine biosensor by copolymerization of *N*-substituted aziridines **1** and **2** in supercritical carbon dioxide.

oligomer **3** in water and a decrease in the solubility in most of the tested organic solvents. Moreover, homopolymerization of **1** produces an insoluble material in common organic solvents. Therefore, for the synthesis of water-soluble target oligoaziridine **3** a low percentage of **1** is needed, thus fulfilling the required properties for use as a biological optical sensor: Lewis basic sites for strong coordination to metal ions and a fluorescent receptor providing a net electron transfer to the complexed metal ions. However, under the optimized conditions to obtain a water-soluble material (comonomers **1**:**2** feed ratio of 1:4), a mixture of oligoaziridines (**3** and **4**) is formed. As expected, the NMR spectrum shows the peaks corresponding to the anthracene unit in the aromatic region. The chemical shifts for oligomers **3** and **4** backbones are in accordance with data reported for the water-soluble polymer **4** obtained by a cationic ring opening polymerization in organic solvents using $BF_3 \cdot OEt_2$ as an initiator (Rivas et al., 1991). From ^1H NMR analysis the oligomers **3**:**4** ratio in the mixture is approximately 1:6. Since oligoaziridine **4** has no chromophores, and does not interfere in the fluorescence of oligoaziridine **3** (no quenching was observed), the oligomers mixture was characterized (IR, NMR and MALDI-TOF) and its sensing potential fully explored.

Selective fluorescence quenching experiments were then performed using both methanol and water as solvents. In aqueous solutions the sensor presents three absorption bands with an absorption maximum at 370 nm. The fluorescence response of the sensor was investigated for a 10 μM concentration. Excitation of the band at 370 nm led to an emission spectrum with three bands with an emission maximum at 416 nm (methanol) or 419 nm (water). The binding ability of the sensor towards a series of metal ions was examined by comparing the fluorescence intensities of the solutions before and after the addition of 100 equivalents of a solution of the metal ion chloride salt.

The oligoaziridine sensor presents a dual behavior when exposed to different metal cations. The addition of Li^+, Mg^{2+}, Ce^{3+}, Pb^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Hg^{2+} to polymer solutions resulted in different degrees of fluorescence quenching or enhancement. In order to determine the solvent effect on the quenching/enhancement mechanism the experiments were performed both in aqueous solution (pH=7.4) and in methanol (Fig. 1).

No significant differences were found using water or methanol as solvents. A quenching of the fluorescence intensity (turn-off) was observed both in water and methanol solutions of Hg^{2+} and Cu^{2+} ions. The observed quenching effect of Cu^{2+} is slightly higher than Hg^{2+}. Surprisingly, the addition of Ce^{3+}, Pb^{2+} and Zn^{2+} ions led to an enhancement (turn-on) of the fluorescence intensity and Zn^{2+} presented the highest fluorescence turn-on (Fig. 1). Since zinc is found at high concentrations in the human body, especially in the brain, pancreas and spermatozoa, many fluorescent sensor molecules for its detection have been developed in the last years (Haas and Franz, 2009). The fluorescence quenching by Cu^{2+} has been already studied in detail in

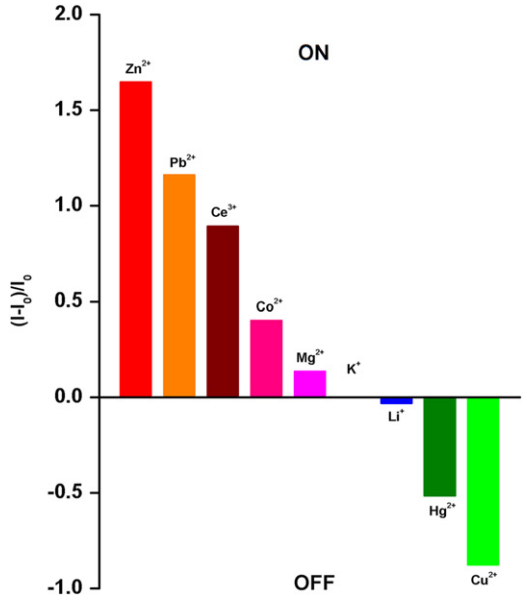


Fig. 1. Fluorescence intensity ratio $(I-I_0)/I_0$ of the oligoaziridine biosensor in methanol after the addition of 100 equivalents of metal cations (λ_{ex}=370 nm, λ_{em}=416 nm).

literature, and was found to follow a static mechanism (Rahimi et al., 2008). In the case of Zn^{2+}, the fluorescence enhancement is not yet fully understood. Although Zn^{2+} has a closed-shell d^{10} electronic configuration which is able to suppress PET, this mechanism is insufficient to describe all the studied systems so far (Kikuchi et al., 2004; Lim et al., 2005; Nolan and Lippard, 2009).

The variation of the fluorescence intensity of the sensor in the presence of other metal cations studied was not significative. We also investigated the interference of potentially complexing metal ions on the Cu^{2+} detection by measuring the fluorescense of the sensor solution having a mixture of Cu^{2+} (1 mM) and the other competing ions (20 mM). No significative changes were found in the obtained spectra which mean that the studied cations did not induce obvious interference on Cu^{2+} detection. The stoichiometry for the reaction of the sensor with Cu^{2+} was also determined, in water and in methanol, by a job plot (Connors, 1987) (see Supporting information). A maximum at 0.7 of polymer (0.3 of Cu^{2+}) indicates the formation of an oligoaziridine-Cu^{2+} 1:2 adduct. To determine the detection limit of Cu^{2+}, the amount added to the oligomer solution was gradually decreased from 20 μg/mL to 0.1 μg/mL (0.31 μM to 1.6 nM) (bottom to top) as shown in Fig. 2. We found that the oligoaziridine probe shows a detection limit of 1.6 nM for Cu^{2+}, and it can be used in the measurement of cerebrospinal fluid (CSF) copper, an indicator of the brain copper concentration in the cerebral manifestation of Wilson's disease (70–80 μg/dL; the normal value is

below 20 μg/dL, ~3 μM) (Stuerenburg, 2000) and in drinking water within the US EPA limit (1.3 ppm, ~20 μM) (EPA website, 2012).

In order to study the applicability of the sensor for biomedical applications, its cytocompatibility was studied through *in vitro* studies. HF cells were seeded (6×10^4 cells/well) at the same initial density in the 96 well plates, with or without sensor to assess its cytotoxicity. After 24 and 96 h cell adhesion and proliferation was visualized using an inverted light microscope (Fig. 3).

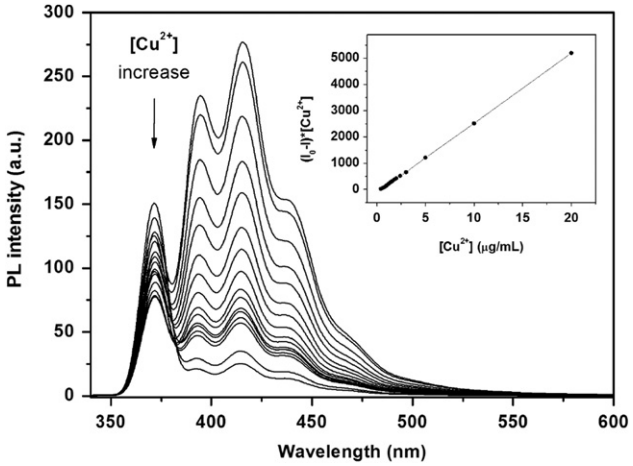


Fig. 2. Fluorescence spectra of the oligoaziridine biosensor after the addition of different concentrations of copper in methanol. The inset shows the linear relation between the copper concentration and the fluorescence turn-off.

HF cells adhered and grew in the presence of the sensor (Fig. 3a_1 and b_1) and in the negative control (Fig. 3a_2 and b_2). In the positive control, no cell adhesion or proliferation was observed. Dead cells with their typical spherical shape can be observed in Fig. 3a_3 and b_3. The observation of cell growth in the presence of the sensor showed that the sensor is biocompatible, suggesting that it does not affect cell viability.

To further evaluate the biocompatibility of the oligomers, MTS assay was also performed (Fig. 4). The MTS assay showed a significant difference between positive control ($p < 0.05$) and the negative control and cells exposed to the sensor after 24 and 96 h of incubation. Thus, suggesting that the oligomers do not change cell viability. These results show that the tested formulation does not have an acute cytotoxic effect.

Since Hg^{2+} is not present in normal living samples, the possibility of using the oligoaziridine biosensor as a fluorescent probe for monitoring intracellular Cu^{2+} was also investigated (Zeng et al., 2006; Yu et al., 2008; Zhao et al., 2009). When the sensor was added to HF cells, a fluorescence emission was observed (Fig. 5).

After incubation, the HF cells were treated with 20 μg/mL of copper, and the intracellular fluorescence decreased along with time. Subsequently, through the addition of ethylenediaminetetraacetic acid (EDTA), cells recovered the fluorescence (see

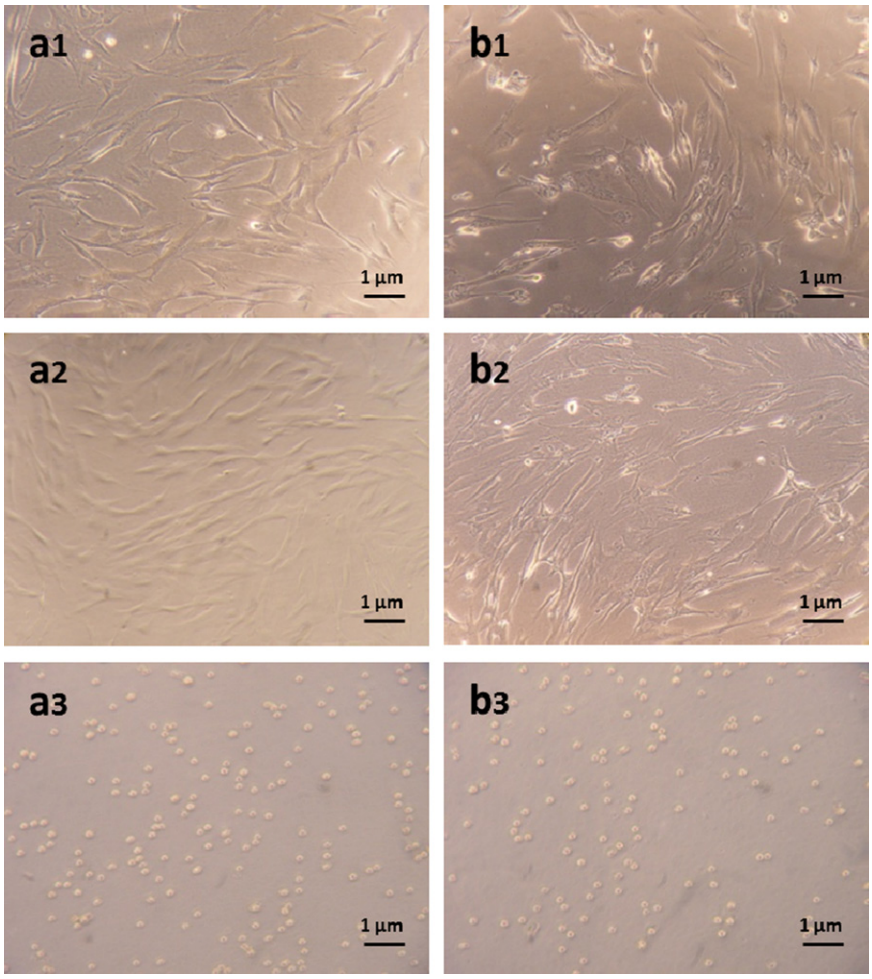


Fig. 3. Optical micrographs of HF cells after being seeded on the oligoaziridine biosensor (c=100 μg/mL) after 24 (a_1) and 96 h (b_1) of incubation; negative control after 24 (a_2) and 96 h (b_2); positive control after 24 (a_3) and 96 h (b_3). Original magnification × 100.

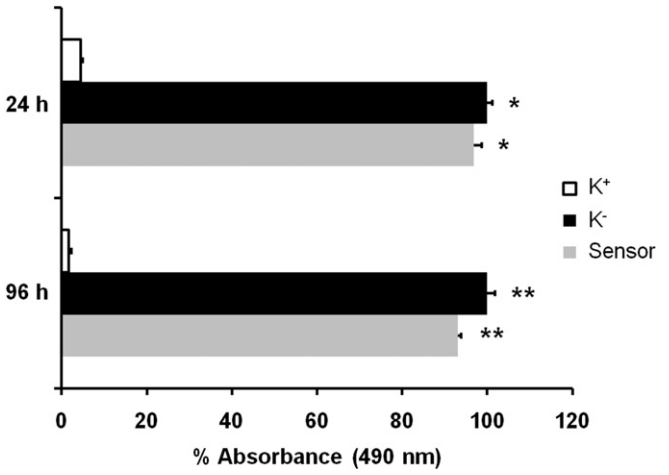


Fig. 4. Cellular activities measured by the MTS assay after 24 and 96 h in contact with the oligoaziridine biosensor. $K+$, positive control; $K-$, negative control. The results shown represent the mean ± standard error of the mean of at least three independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnet's post hoc test ($^{*}p < 0.05$ and $^{**}p < 0.05$).

Supporting information). Thus, cells are permeable to the sensor suggesting that this material can be used in physiological conditions to indicate the variation of copper concentration within living cells. Although the system is not fully selective (some metals have similar turn-off or turn-on) the biosensor is versatile enough to be used in task specific sensing protocols.

4. Conclusions

In summary, a water-soluble oligoaziridine biosensor having pendant anthracene units was synthesized in supercritical carbon dioxide and its potential as reversible chemical and biochemical (turn-off) sensor for Cu^{2+} in HF cells was demonstrated. A detection limit of 1.6 nM for Cu^{2+} was found. The response time is instantaneous and the detection limit was achieved even in the presence of excess metal ion competitors of concern as drinking water pollutants. Binding data and absorption spectroscopy suggested that the sensitivity arises from the paramagnetic behavior of Cu^{2+} in support with the binding with the oligoaziridine. Immunofluorescence microscopy experiments proved that the biosensor is able to determine the Cu^{2+} levels within living

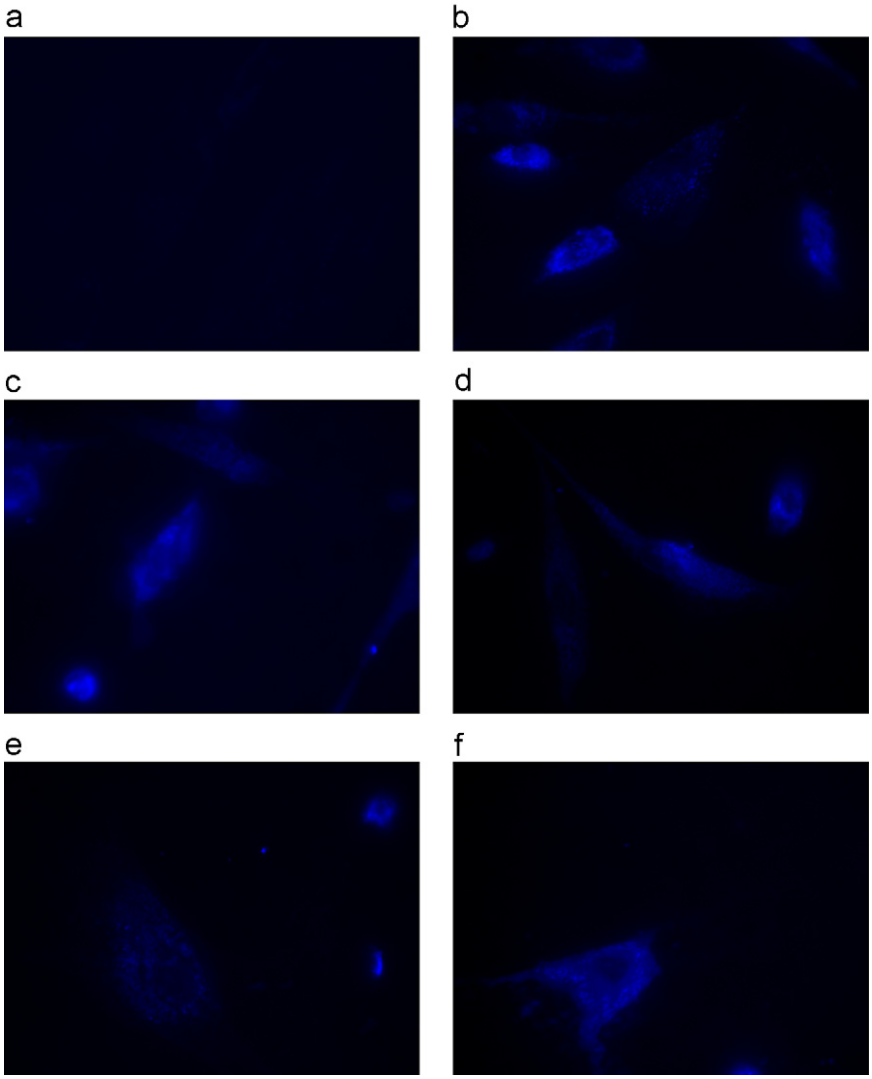


Fig. 5. Imunofluorescence assay images using HF cells and the oligoaziridine biosensor (c=100 μg/mL): (a) control; (b–d) after addition of $CuCl_2$ (c=20 μg/mL) at different times of incubation, (b) t=0 h, (c) t=0.5 h, (d) t=1 h; (e–f) fluorescence recovery after addition of an aqueous solution of 0.035% (v/v) of EDTA, (e) t=6 h, (f) t=8 h. Control experiment shows the obtained image in the absence of the sensor. EDTA=ethylenediamine tetraacetic acid. The blue color is a pseudo-color resultant from the software used in data collection. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells due to its operation under physiological conditions, and low detection limit. The study of the oligoaziridine biosensor for fluorescence microscopic imaging of Zn^{2+} in biological applications is underway.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.06.047.

References

Akkaya, E.U., Huston, M.E., Czarnik, A.W., 1990. Journal of the American Chemical Society 112, 3590–3593.
Baoum, A., Dhillon, N., Buch, S., Berkland, C., 2010. Journal of Pharmaceutical Sciences 99, 2413–2422.
Bissel, R.A., de Silva, A.P., Gunaratne, H.Q.N., Lynch, P.L.M., Maguire, G.E.M., Sandanayake, K.R.A.S., 1992. Chemical Society Reviews, 187–195.
Branco, P.S., Raje, V.P., Dourado, J., Gordo, J., 2010. Organic and Biomolecular Chemistry 8, 2968–2974.
Connors, K.A., 1987. Binding Constants: The Measurements of Molecular Complex Stability. John Wiley & Sons, New York.
de Silva, A.P., Fox, D.B., Huxley, A.J.M., Moody, T.S., 2000. Coordination Chemistry Reviews 205, 41–57.
de Silva, A.P., Gunaratne, H.Q.N., Gunnlaugsson, T., Huxley, A.J.M., McCoy, C.P., Rademacher, J.T., Rice, T.E., 1997. Chemical Reviews 97, 1515–1566.
Desvergne, J.P., Czarnik, A.W. (Eds.), 1997. Fluorescent Chemosensors for Ion and Molecule Recognition. Kluwer Academic Publishers, Netherlands.
EPA website. 〈http://water.epa.gov/drink/contaminants〉 (accessed 16.01.2012).
Fabbrizzi, L., Licchelli, M., Pallavicini, P., Perotti, A., Sacchi, D., 2003. Angewandte Chemie International Edition 33, 1975–1977.
Haas, K.L., Franz, K.J., 2009. Chemical Reviews 109, 4921–4960.
Huston, M.E., Engleman, C., Czarnik, A.W., 1990. Journal of the American Chemical Society 112, 7054–7056.
Ihata, O., Kayaki, Y., Ikariya, T., 2004. Angewandte Chemie International Edition 43, 717–719.
Kikuchi, K., Komatsu, K., Nagano, T., 2004. Current Opinion in Chemical Biology 8, 182–191.
Lim, N.C., Freake, H.C., Brückner, C., 2005. Chemistry—A European Journal 11, 38–49.
McRae, R., Bagchi, P., Sumalekshmy, S., Fahrni, C.J., 2009. Chemical Reviews 109, 4780–4827.
Nolan, E.M., Lippard, S.J., 2009. Accounts of Chemical Research 42, 193–203.
Prodi, L., Bolletta, F., Montalti, M., Zaccheroni, N., 2000. Coordination Chemistry Reviews 205, 59–83.
Quang, D.T., Kim, J.S., 2000. Chemical Reviews 110, 6280–6301.
Rahimi, Y., Goulding, A., Shrestha, S., Mirpuri, S., Deo, S.K., 2008. Biochemical and Biophysical Research Communications 370, 57–61.
Ribeiro, M., Espiga, A., Silva, D., Baptista, P., Henriques, J., Ferreira, C., Silva, J., Borges, J., Pires, E., Chaves, P., 2009. Wound Repair and Regeneration 17, 817–824.
Rivas, B.L., Greckeler, K.E., Bayer, E., 1991. European Polymer Journal 27, 1165–1169.
Rivas, B.L., Pereira, E., Maureira, A., 2009. Polymer International 58, 1093–1114.
Shiraishi, Y., Tokitoh, Y., Nishimura, G., Hirai, T., 2005. Organic Letters 7, 2611–2614.
Stuerenburg, H.J., 2000. Journal of Neural Transmission 107, 321–329.
Swamy, K.M.K., Ko, S.-K., Kwon, S.K., Lee, H.N., Mao, C., Kim, J.-M., Lee, K.-H., Kim, J., Shin, I., Yoon, J., 2008. Chemical Communications, 5915–5917.
Valeur, B., Leray, I., 2000. Coordination Chemistry Reviews 205, 3–40.
Wang, H.-H., Xue, L., Fang, Z.-J., Li, G.-P., Jiang, H., 2010. New Journal of Chemistry 34, 1239–1242.
Yu, F., Zhang, W., Li, P., Xing, Y., Tong, L., Ma, J., Tang, B., 2009. Analyst 134, 1826–1833.
Yu, M., Shi, M., Chen, Z., Li, F., Li, X., Gao, Y., Xu, J., Yang, H., Zhou, Z., Yi, T., Huang, C., 2008. Chemistry—A European Journal 14, 6892–6900.
Zeng, L., Miller, E.W., Pralle, A., Isacoff, E.Y., Chang, C.J., 2006. Journal of the American Chemical Society 128, 10–11.
Zhao, Y., Zhang, X.-B., Han, Z.-X., Qiao, L., Li, C.-Y., Jian, L-X., Shen, G.-L., Yu, R.-Q., 2009. Analytical Chemistry 81, 7022–7030.