Ultra-sensitive optical oxygen sensors for characterization of nearly anoxic systems

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Oxygen quantification in trace amounts is essential in many fields of science and technology. Optical oxygen sensors proved invaluable tools for oxygen measurements in a broad concentration range, but until now neither optical nor electrochemical oxygen sensors were able to quantify oxygen in the sub-nanomolar concentration range. Herein we present new optical oxygen-sensing materials with unmatched sensitivity. They rely on the combination of ultra-long decaying (several 100 ms lifetime) phosphorescent boron- and aluminium-chelates, and highly oxygen-permeable and chemically stable perfluorinated polymers. The sensitivity of the new sensors is improved up to 20-fold compared with state-of-the-art analogues. The limits of detection are as low as 5 p.p.b., volume in gas phase under atmospheric pressure or 7 pM in solution. The sensors enable completely new applications for monitoring of oxygen in previously inaccessible concentration ranges.
Oxygen undoubtedly belongs to the most important analytes on earth. Traditionally, most oxygen sensors were designed for the physiological range. However, numerous applications require monitoring of oxygen at significantly lower concentrations, for example, corrosion protection, surface treatment, the semiconductor industry and biological research. For instance, it was demonstrated that bacteria can show respiration and potentially aerobic growth far below the Pasteur point (~10 µM dissolved oxygen (DO)) \(^{14-16}\). Recently, sensors that quantify DO in concentration ranges of 100 nM and below have gained special interest as biologists explore aerobic life in areas very close to anoxic conditions \(^7\). Unfortunately, few sensors are capable to resolve at such low concentration \(^8,9\), and measurements below 0.5 nM are currently impossible.

Optical sensors proved to be indispensable tools for oxygen quantification that have mostly replaced the more conventional Clark electrode. Their advantages include minimal invasiveness, simplicity, suitability for miniaturization, versatility of formats (planar optodes, fibre-optic sensors, micro- and nanoparticles, paints and so on) and suitability for imaging of oxygen distribution on surfaces or in volume. Moreover, optical oxygen sensors are tuneable over a wide range of concentrations. Optical oxygen sensors rely on quenching of a phosphorescent indicator by the analyte. Both the nature of the indicator and the matrix (which acts as a solvent and support for the dye and as a permeation-selective barrier) are of great importance since the sensitivity of an oxygen sensor is roughly proportional to the permeation-selective barrier. State-of-the-art indicators are luminescence decay time of the indicator and to the oxygen sensitivity of an oxygen sensor.

The chelates are modified with perfluoroalkyl chains to ensure compatibility with highly oxygen-permeable and chemically inert polymers \(^{10}\). The new-sensing materials rely on new blue light-excitable BF\(_2\)-chelates featuring ultra-long room temperature phosphorescence. The chelates are modified with perfluoroalkyl chains to ensure compatibility with highly oxygen-permeable and chemically inert perfluorinated polymers. The resulted ultra-sensitive sensors are ideally suitable for characterization of nearly anoxic systems.

### Results

#### Photophysical properties

The new-sensing materials rely on novel difluoroboron- and aluminium chelates of 9-hydroxyphenalenone (HPhN; Fig. 1) and its benzannelated derivative 6-hydroxybenz[de]anthracene-7-on (HBAN; scheme 1; ref. 22). In contrast to the common fluorescent difluoroboron chelates of dipyromethenes and tetraarylazadipyromethenes \(^{23,24}\), the new difluoroboron-based dyes simultaneously show prompt fluorescence, delayed fluorescence and phosphorescence when embedded in polymers (Fig. 2a,b). Room temperature phosphorescence is an extremely rare phenomenon for compounds with no heavy atoms. It was previously observed for BF\(_2\)-chelates of aliphatic β-diketones coupled to polyactic acid \(^{16,17}\) or physically entrapped in the same polymer \(^{19}\). This work and our results indicate that phosphorescence of aliphatic and aromatic BF\(_2\)-chelates of β-diketones appears to be a general phenomenon. As can be seen, the extension of π-conjugation in HPhN derivatives results in bathochromic shifts of absorption, fluorescence and phosphorescence compared to the reported aliphatic diketone chelates. In fact, the absorption of the HPhN and HBAN difluoroboron chelates peaks at 450 and 459 nm, respectively, \((\varepsilon_{450} = 10.2 \times 10^{3} \text{M}^{-1} \text{cm}^{-1}) \text{ and } \varepsilon_{459} = 12.9 \times 10^{3} \text{M}^{-1} \text{cm}^{-1}) \). This enables excitation with bright blue 450 and 470 nm LEDs and with conventional white light sources. Temperature affects the quantum yields of all three emissions.
As expected, the phosphorescence QYs decrease at higher temperature and the delayed fluorescence becomes stronger (Fig. 2b). Since both phosphorescence and delayed fluorescence possess virtually identical decay times (Supplementary Fig. 1) and are quenched by oxygen to the same extent, their ratio can be used to eliminate temperature crosstalk. Importantly, prompt fluorescence is suitable for referencing purposes, as it is not affected by oxygen. However, the most outstanding property of these dyes is their extraordinary long phosphorescence decay time of 360 and 730 ms for BF₂(HPhN) and BF₂(HBAN), respectively (in PS at 20 °C), which, to the best of our knowledge, are the longest decay times recorded for visible light-excitable dyes. This makes them suitable for monitoring sub-nanomolar DO concentrations.

Ultra-sensitive oxygen-sensing materials. Owing to the exceptionally long lifetimes of these chelates, resulting sensing materials are expected to have sensitivities orders of magnitude above those based on conventional sensing chemistries. Indeed, sensors composed of difluoroboron chelates in PS show very high sensitivities, even though PS has only moderate oxygen permeability ($P \approx 8.8 \times 10^{-10} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, ref. 25) KSV for BF₂(HPhN) and BF₂(HBAN)-based sensors was $6 \times 10^{-3}$ and $12 \times 10^{-3}$ p.p.m. v.⁻¹, respectively, (Fig. 3a), which makes them suitable for monitoring sub-nanomolar DO concentrations. The limit of detection for the BF₂(HBAN)-based material, assuming a resolution of 0.5% $I_0$, is in fact 45 p.p.b.v. (gas phase) or 60 pM (DO). Both sensing materials surpass the sensitivity of nearly all previously published sensors. Still, highly permeable matrices such as Teflon AF and poly(trimethylsilylpropyne) can potentially be used to create even more sensitive materials. Poly(trimethylsilylpropyne) has the highest known oxygen permeability ($P \approx 23,000 \times 10^{-10} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, ref. 26) but was not considered because of poor chemical stability and possession of double bonds that are prone to oxidation and thus analyte consumption. Hyflon AD 60 and Teflon AF are amorphous, glassy, perfluorinated copolymers that combine high free volume and unmatched chemical stability. In this work we used Hyflon AD 60 ($P \approx 170 \times 10^{-10} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, ref. 29) and Teflon AF 1600 ($P \approx 1,200 \times 10^{-10} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, ref. 30). Unfortunately, most conventional dyes, as well as BF₂(HPhN) and BF₂(HBAN), show extremely poor solubility in these matrices and aggregate readily. We failed to obtain usable sensors based on the BF₂-chelates due to poor luminescence in perfluorinated polymers at any relevant concentration (0.01–1% wt. of the dyes in respect to the polymer). Therefore, in order to render the indicators soluble in Hyflon and Teflon AF, HPhN and HBAN were perfluoroalkylated with perfluoroctyl iodide (scheme 1). The resulting ligands 9-hydroxy-4,7-diperfluorooctyl-1H-phenalenone (HPhNPF) and 6-hydroxy-1,4-diperfluorooctyl-7H-benzo[de]anthracen-7-one (HBANPF) bear two C₈F₁₇ chains each, although in the case of HBANPF there is some impurity of tri-substituted ligand (Supplementary Figs 2 and 3). As indicated by nuclear Overhauser effect spectroscopy (NOESY) and heteronuclear multiple-bond correlation spectroscopy (HMBC) spectra (scheme 1). The resulting ligands 9-hydroxy-4,7-diperfluorooctyl-1H-phenalenone (HPhNPF) and 6-hydroxy-1,4-diperfluorooctyl-7H-benzo[de]anthracen-7-one (HBANPF) bear two C₈F₁₇ chains each, although in the case of HBANPF there is some impurity of tri-substituted ligand (Supplementary Figs 2 and 3). As indicated by nuclear Overhauser effect spectroscopy (NOESY) and heteronuclear multiple-bond correlation spectroscopy (HMBC) spectra...
Bluish prompt fluorescence remains constant, and the intense yellow phosphorescence slowly appears for the areas covered with anaerobic sodium aluminium complexes. (Table 1) and the sensors resolve up to 10 p.p.b.v. or 12 pM DO. The sensors, the Stern–Volmer constants $K_{SV}$ decrease at higher temperature. This can be explained by a relatively strong effect of temperature on the phosphorescence decay time $t_0$ (280, 250 and 220 ms at 5, 20 and 35 °C, respectively, for Al(HPhNPF)$_3$ in Teflon AF 60) and a negligible influence on the bimolecular quenching constant $k_q$ (1.65, 1.65 and 1.69 p.p.m. v. $^{-1}$ s $^{-1}$ at 5, 20 and 35 °C, respectively). This indicates that in contrast to most polymers, the oxygen permeability of Hyflon AD 60 appears to be barely affected by temperature.

Application example. To illustrate potential applications of these trace oxygen sensors, the kinetics of oxygen consumption during enzymatic oxidation of glucose was monitored as it approached completion. The sensors based on Teflon AF 1600 show a twofold increase in sensitivity compared with the Hyflon-based ones. Al(HBANPF)$_3$ in Teflon AF 1600 is the most sensitive sensor material presented, with a $K_{SV}$ of 960 × 10$^{-3}$ p.p.m. v. $^{-1}$ and a detection limit of 5 p.p.b.v. or 7 pM. Potentially, even more sensitive sensor materials based on Teflon AF 2400 ($P \approx 3000 \times 10^{-10}$ mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$; ref. 30) showed signs of aggregation and were therefore not further investigated. Importantly, the Stern–Volmer plots for the phosphorescence decay time are similar to those obtained for the intensity measurements (Fig. 3b and Supplementary Fig. 10) so both read-out schemes (ratometric intensity or decay time) can be used. It should be mentioned that sensitivities are so high that obtaining reliable calibrations becomes rather challenging. Even high-purity nitrogen contains oxygen impurities in the low p.p.m. range, hence all aluminium complex-based sensor materials were calibrated using a standard addition method described in the experimental section.

Figure 3 | Oxygen sensitivity of the trace sensors at 20 °C. (a) Stern–Volmer plots for BF$_2$HPhN and BF$_2$HPhN in PS. (b) Stern–Volmer plots for the aluminium complexes. (c) Photographic images of the Al(HPhNPF)$_3$/Hyflon AD 60 sensor under illumination with a 366-nm line of a ultraviolet lamp.

Photographic images of the Al(HPhNPF)$_3$/Hyflon AD 60 sensor under illumination with a 366-nm line of a ultraviolet lamp.
Figure 4 | Real-time monitoring of oxygen levels during enzymatic oxygen consumption. Three trace sensors with overlapping ranges are used to monitor oxidation of glucose catalysed by glucose oxidase. A running average of 3 data points was used for all sensors. Whereas, the reference sensors fail to resolve below 2–10 nM; the new ultra-trace sensor can reliably monitor oxygen even at much lower concentrations (insert). The sensor, two other oxygen sensors were used: a commercially available trace sensor from Pyroscience GmbH and a sensor based on Pd(II)-5,10,15,20-meso-tetrakis-(2,3,4,5,6-pentafluorophenyl)porphyrin in Hyflon, which is comparable in sensitivity to previously published sensors. Figure 4 shows the high resolution of the new sensors in the ultra-trace region, whereas the other sensors fail to resolve oxygen concentrations below 1 nM. The insert shows a mono-exponential fit of the ultra-trace region corresponding to purely diffusion-controlled depletion of oxygen.

Precise quantification of oxygen impurity in inert gases represents another potential application of the new sensors. The Al(HPhNPf)₃/Hyflon sensor was used to quantify oxygen content in high-purity gases obtained from Linde (Austria). For example, two samples of compressed nitrogen 5.0 (99.9999%, <10 p.p.m.v. O₂) contained 3.7 and 2.5 p.p.m.v. O₂, whereas compressed nitrogen 6.0 (99.9999%, <1 p.p.m.v. O₂) contained 0.52 p.p.m.v. O₂ which is within the specifications of the gas manufacturer.

Discussion

We prepared new optical oxygen-sensing materials with unmatched sensitivity. The most sensitive shows an ~20-fold increase in sensitivity compared with state-of-the-art materials. Such extraordinary high-sensitivity results from the combination of extremely long phosphorescence decay times of the indicators and very high oxygen permeability of the matrix. Novel heavy metal-free chelates of rigid aromatic analogues of β-diketones represent unique indicators for oxygen sensors that feature absorption in the blue part of the electromagnetic spectrum, very long-decaying room temperature phosphorescence, good molar absorption coefficients and adequate quantum yields. The unique dual emission of the chelates (delayed fluorescence and phosphorescence) can be used for intrinsic temperature compensation. These sensors enable completely new applications for monitoring of oxygen in previously inaccessible concentration regions, and are likely to become invaluable tools in various fields of science and technology.

Methods

Materials. Nitrogen (99.9999% purity) and test gases (1.000 p.p.m.v. O₂, rest N₂; and 20 p.p.m.v. O₂, rest N₂) were obtained from Linde Gas (Austria; www.linde.at), PS (molecular weight (MW) 250,000) and hydrogen peroxide (60%) were obtained from Fisher Scientific. Perfluorooctyl iodide and aquaphobe CF were bought from ABCR (Germany; www.abcr.de). Teflon AF 1600 was acquired from DuPont (USA; www.dupont.com). Hyflon AD 60 was obtained from Solvay (USA; www.solvay-plastics.com). Sodium sulphite was obtained from Carl Roth (www.carlroth.com). Boron trifluoro diethyl etherate, aluminium oxide (activated, neutral Brockmann I), aluminium(III) chloride, 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113), glucose oxidase from Aspergillus niger, catalase from bovine liver, anhydrous hydrogen fluoride (HF) and anhydrous dimethylsulphoxide were obtained from Aldrich, iron(II) sulphate heptahydrate from Merck, sodium hydroxide from Lantan (Austria; www.lantan.at) and silicon E4 from Wacker (Germany; www.wacker.de). Acetone and ethanol were acquired from Brentnag (Germany; www.brentnag.de) and other solvents including the deuterated solvents were from VWR (Austria; www.at.vwr.com). HPhNPf was obtained from Ramidus (Sweden; www.ramidus.se) and HBAN was synthesized according to the literature procedure.

Synthesis of the B₃f chelate of HPhNPf. An amount of 294 mg (1.5 mmol) of HPhNPf were dissolved in 50 ml of anhydrous THF and 1.92 g (13.5 mmol) of boron trifluoro diethyl etherate were added. The solution was stirred for 1 h at 65 °C for 2 h, the solvent was removed under vacuum and the product was purified on an aluminium oxide column using dichloromethane as an eluent. Yield: 300 mg (67%) of orange crystals. ¹H NMR (300 MHz, DMSO-d₆, δ): 8.94 (d, J = 9.1 Hz, 2H), 8.70 (d, J = 7.7 Hz, 2H), 8.03 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 9.1 Hz, 2H). Electron impact-time-of-flight (EI-DI-TOF) (m/z): 244.0499, calculated 244.0510.

Synthesis of the B₂f chelate of HBAN. A quantity of 369 mg (1.5 mmol) of HBAN were dissolved in 60 ml of anhydrous THF, and 1.7 g (12 mmol) of boron trifluoro diethyl etherate were added. The solution was stirred at 65 °C for 2 h, and 69 mg (0.3 mmol) of potassium carbonate were added and the stirring was continued for 2 h. The solvent was removed under vacuum and the product was purified on an aluminium oxide column using dichloromethane as an eluent. Yield: 340 mg (77%) of orange crystals. ¹H NMR (300 MHz, DMSO-d₆, δ): 9.26 (d, J = 7.7 Hz, 1H), 9.04 (d, J = 8.3 Hz, 1H), 8.91 (d, J = 9.2 Hz, 1H), 8.69 (d, J = 8.2 Hz, 1H), 8.50 (d, J = 7.6 Hz, 1H), 8.21 (t, J = 8.3 Hz, 1H), 7.97 (dt, J = 21.2, 7.6 Hz, 2H), 7.61 (d, J = 9.1 Hz, 1H). EI-DI-TOF (m/z): 294.0671, calculated 294.0667.

Synthesis of HPhNPf. HPhNPf (1 g, 5.1 mmol) and FeSO₄ 7 H₂O (2.5 g, 9.0 mmol) were dissolved in 120 ml dimethylsulfoxide (DMSO) and 40 ml 1:1,2-trichloro-1,2,2-trifluoroethane in a 200 ml Schlenk flask. The whole reaction was conducted under argon atmosphere. The solution was heated to 40 °C and perfluorooctyl iodide (3.4 ml, 7.03 g, 12.9 mmol) was added. 60% H₂O₂ (1.4 ml, 1.67 g, 49 mmol) was added drop-wise over 5 min. The solution turned red and formed black foam. After 30 min, 20 ml 1:1,2-trichloro-1,2,2-trifluoroethane were added and the foam dissolved. One hour after the start of the reaction, a second portion perfluorooctyl iodide (3.4 ml, 7.03 g, 12.9 mmol) was added and 60% H₂O₂ (1.4 ml, 1.67 g, 49 mmol) was added drop-wise over 5 min. The reaction was continued for 1 h, then the reaction mixture was cooled to room temperature, and the DMSO and 1,1,2-trichloro-1,2,2-trifluoroethane phases were separated. The 1,1,2-trichloro-1,2,2-trifluoroethane phase was washed three times with 36% hydrochloric acid, once with water, and was dried over Na₂SO₄. After removing the solvent, 9.3 g reddish-brown oil were obtained. The product was purified on a silica-gel column with hexane and toluene as eluents. Yield: 700 mg (13%), Rf 0.57 with toluene.

¹H NMR (300 MHz, CDCl₃ + Freon 113 (1:1 v/v), δ): 16.75 (s, 1H), 8.77 (s, 1H), 8.15-8.00 (m, 2H), 7.86 (d, J = 7.9 Hz, 2H), 7.24 (d, J = 9.3 Hz, 2H). EI-DI (m/z): 1,031.98 found, 1,031.98 calculated.

Synthesis of HBANPF. HBANPF was prepared using the same procedure as HPhNPf, but with a fivefold excess of perfluorooctyl iodide, and the reaction mixture was stirred stirred for 2 h at 40 °C and then overnight at room temperature. Yield 500 mg (23%) starting from 0.5 g HBAN. Rf 0.69 with hexane/toluene 2:1. ¹H NMR (300 MHz, CDCl₃ + Freon 113 (1:1 v/v), δ): 17.01 (s, 1H), 8.94 (s, 1H), 8.79 (d, J = 8.2 Hz, 1H), 8.71 (d, J = 7.9 Hz, 1H), 8.52 (d, J = 8.3 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.89 (t, J = 7.6 Hz, 1H), 7.73 (t, J = 7.5 Hz, 1H). EI-DI (m/z): 1,081.99 found, 1,081.99 calculated.

Synthesis of the aluminium complex of HPiNPF. HPiNPF (100 mg, 0.097 mmol) was dissolved in 20 ml THF and 20 ml ethanol. NaOH (5.9 mg, 0.15 mmol) and AlCl₃ (3.9 mg, 29 μmol) were added and the reaction mixture was stirred for 2.5 h at 40 °C. Then the reaction was stopped the product was extracted with 1,1,2-trichloro-1,2,2-trifluoroethane and the organic phase was washed twice with water. The organic layer was dried over Na₂SO₄ and evaporated to a vacuum. Five millilitres THF were added to the raw product and the mixture was placed in the freezer for a few hours. The THF phase was removed and new THF was added. The product was washed seven times following this procedure. Yield: 68.4 mg (74%). ¹H NMR (300 MHz, CDCl₃ + Freon 113 (1:1 v/v), δ): 8.87-8.35 (m, 3H), 7.84-7.86 (m, 3H), 7.42-7.54 (m, 3H); matrix assisted laser desorption ionisation (MALDI) (m/z): [M + Na]+: 3,142.93 found, 3,142.90 calculated.
Preparation of the PS-based sensing materials. The sensor ‘cocktails’ contained the dyes (0.02% w/w in respect to the polymer), PS (5% w/w in respect to the solvent) and chloroform. These were knife-coated on a glass slide using a 175-µm spacer. The resulting thickness for the dried layer is estimated to be 6 µm.

Preparation of the Hyflon AD 60-based sensing materials. Before coating, the glass slides were rendered hydrophobic with aquaphobe CF. The dyes (0.05% w/w in respect to the polymer) and the polymer (6% w/w in respect to the solvent) were dissolved in octafluorotoluene. Knife-coating on the glass slides using a 175-µm spacer yielded a layer thickness of ~12 µm. After a second coating with a 275-µm spacer and evaporation of the solvent an ~31-µm-thick layer was obtained.

Preparation of the Teflon AF 1600-based sensing materials. The sensors were prepared analogously to those based on Hyflon, but a 5% w/w solution of the polymer in octafluorotoluene was used. The thickness of the sensing layer was estimated to be 24 µm.

Measurements. 1H NMR spectra were recorded on a 300 MHz instrument (Bruker). NOESY and HMBC spectra were acquired on an INOVA 500 MHz NMR spectrometer (Varian). DMSO-d6 was used for HPhN and HBAN and the (Bruker). NOESY and HMBC spectra were acquired on an INOVA 500 MHz NMR spectrometer (Varian). DMSO-d6 was used.

Supplementary Information

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Author contributions

P.L. designed the measurement setup, characterized the boron chelates and wrote the paper; C.S. synthesized the aluminium complexes, discovered their interesting properties and performed the initial perfluorooalkylation studies; and I.K. supervised the project. All authors contributed substantially to discussion and planning.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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