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A fluorescent redox sensor with tuneable oxidation potential

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ABSTRACT

A fluorescent redox sensor was prepared by attachment of hydroquinones to the fluorophore rhodamine B; fluorescence is reversibly modulated by hydroquinone-centered chemical redox reactions, and oxidation potential of the sensor is tuneable by variation of hydroquinone structure.

There is a significant current interest in the development of new fluorescent molecular probes for the characterization of redox states and processes in a micro- and nanoscale environment. Synthetic fluorescent probes which reversibly respond to changes of redox potential and are at the same time applicable to microscopic imaging of the redox status of live cells were described only recently.[1] Another potential application of such probes is the exploration of redox reactions of individual molecules at microsecond temporal resolution by the rapidly developing technology of single molecule fluorescence spectroscopy.[2] We have recently been able to follow dynamic processes at single 2,2′bipyridine (bpy) copper(II) complexes using rhodamine as a fluorescent reporter,[3] and plan to extend these mechanistic studies to metal-centred redox reactions and catalysis using redox-responsive fluorescent substrates.

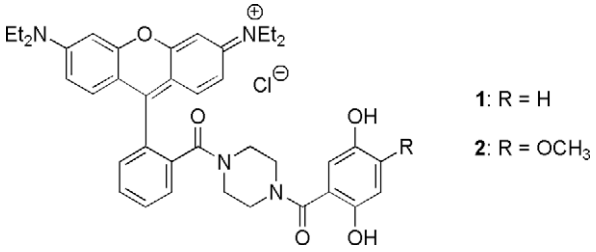
Modular probes which combine a fluorophore and a redox active moiety offer the possibility of independent optimization of redox and photophysical properties for specific applications.[4] The first probes of this type were described by the groups of Lehn[4a] and Fabrizzi[4b] who coupled a $[Ru(bpy)_3]^{2+}$ fluorophore to benzoquinone (fluorescence quencher, no quenching by the reduced hydroquinone form), or an anthracene fluorophore to a redox active copper complex (Cu(I): no quenching, Cu(II): quenching). Information provided by such probes is limited to the signalling of an oxidizing or reducing condition relative to their intrinsic redox characteristics. Nanometer-sized electrodes have been suggested to probe redox status, for example of living cells,[5] over a wider potential range, but their construction and application is technically challenging.

As a convenient way to gain more precise information on the redox properties of a specific chemical or biological microenvironment, we suggest application of a *set* of molecular probes in which the fluorophore is linked to redox-active molecules of varying redox potentials. To prove the principle, we coupled the highly fluorescent dye rhodamine B to two different hydroquinones.

Compounds **1** and **2** (Scheme 1) were obtained by amide coupling reaction between the free secondary amino function of rhodamine B piperazine amide[6] and commercially available 1,4-hydroquinone-2-carboxylic acid or readily prepared 2-methoxy-1,4-hydroquinone-5-carboxylic acid.[7] (See Ref. 13–16 for further details.)

On excitation at the absorbance maximum at about 568 nm in water at pH 7, **1** and **2** display strong 592 nm fluorescence at an intensity comparable to that of rhodamine B. Cyclic voltammetry of **1** and **2** in water/DMSO 7:1 (0.1 M KH_2PO_4-buffer adjusted to pH 7.1), using a glassy carbon electrode and Ag/AgCl/satd KCl reference electrode, reveals irreversible oxidations with cathodic peaks at 555 mV (**1**) and 450 mV (**2**), with an about 500 mV separation of cathodic and anodic peaks. This is in reasonable agreement with literature data on hydroquinones,[8] and the about 100 mV lower oxidation potential of hydroquinone **2** bearing an electron-donating $-OCH_3$ group matches well the 110 mV difference of cathodic peaks of 1,4-hydroquinone-2-carboxylic acid and 2-methoxy-1,4-hydroquinone-5-carboxylic acid under the same conditions.

Various copper(II) complexes including $[Cu(phen)_2]^{2+}$ (phen = 1,10-phenanthroline) have been reported to mediate smooth oxidation of hydroquinones to benzoquinones in aerated solution.[9] Fluorescence of **1** and **2** decreases to 17% and 26%, respectively, of the original value in the presence of excess $[Cu(phen)_2]^{2+}$ (Fig. 1), indicating formation of the quenched quinone form of **1** and **2**. The latter could be extracted with dichloromethane and identified by



Scheme 1. Fluorescent probes **1** and **2**.

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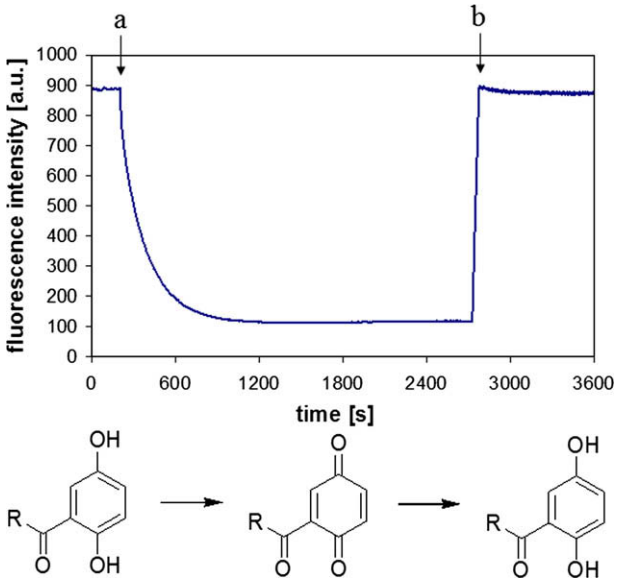

Figure 1. Decrease of 592 nm fluorescence (excitation wavelength 566 nm) of **1** (10 μM) on addition of $[Cu(phen)_2]^{2+}$ (1 mM) (a), and regeneration of fluorescence on addition of cysteine (100 mM) (b). Water, pH 7 (10 mM MOPS buffer), T = 25 °C; R = Rhodamine B-piperazine amide.

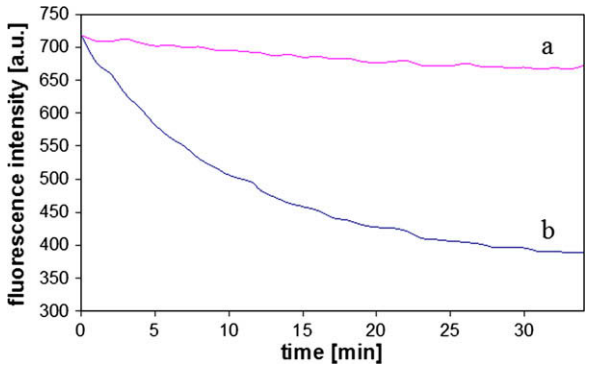

Figure 2. Decrease of 592 nm fluorescence of **2** with time in the presence of 10 μM $[Cu(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})_2]^{2+}$ (a) and $[Cu(phen)_2]^{2+}$ (b). Water, pH 7 (10 mM MOPS buffer), T = 25 °C.

electrospray mass spectrometry (**1-2** H, $[M-Cl]^+$ = 645.3; calcd. 645.3; **2-2** H, $[M-Cl]^+$ = 675.3; calcd. 675.3). Fluorescence of **1** and **2** is immediately and fully restored on addition of excess reducing agent cysteine (Fig. 1). Fluorescence of the control rhodamine B piperazine amide is unaffected by $[Cu(phen)_2]^{2+}$ or cysteine.

At 10 μM concentration, $[Cu(phen)_2]^{2+}$ becomes inefficient for the oxidation of **1**. However, the stronger reductant **2** is still effectively oxidized under these conditions, as evident from a decrease of fluorescence by 46% after 20 min (Fig. 2). The complex $[Cu(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})_2]^{2+}$, a weaker oxidant than $[Cu(phen)_2]^{2+}$,[10] is at 10 μM concentration much less effective (6% oxidation of **2** in 20 min, Fig. 2), and ineffective toward **1**. The complex $[Cu(2,9\text{-dimethylphen})_2]^{2+}$, a stronger oxidant than $[Cu(phen)_2]^{2+}$,[9,10] rapidly oxidizes both **1** and **2**. Combined application of probes **1** and **2** allows to discriminate the oxidizing power of the three Cu^{2+} complexes. This is not possible by applying only a single probe. Beside the chemical oxidation of **1** and **2**, rapid enzyme-catalyzed oxidation is achieved using horse radish peroxidase in the presence of H_2O_2.

Rhodamine-type fluorophores are widely used probes in cellular imaging. **1** and **2** are readily internalized by live HeLa cells and visualized by fluorescence microscopy (Fig. 3). Since the intracellular microenvironment is reducing[11] due to significant concentrations of the thiol glutathione, it is likely that the probes are present exclusively in the hydroquinone form. Cellular fluorescence is hardly diminished on addition of 100 μM $[Cu(phen)_2]^{2+}$, or permeabilization with saponine followed by addition of 100 μM H_2O_2 and 0.1 μM HRP, indicating that the probes are difficult to oxidise in a cellular microenvironment. A better response to changes of intracellular redox state might be achieved with naphthohydroquinone analogs of **1** which in our preliminary investigations also display strong redox-controlled fluorescence modulation. Compared with hydroquinone, the potential of naphthohydroquinone is more negative by 210 mV.[12]

In conclusion, we described a new type of rhodamine-based, water-soluble fluorescent redox sensor which allows a fine-tuning of its oxidation potential by structural variation of a redox-responsive hydroquinone moiety. By including other literature-reported, donor- and acceptor-substituted hydroquinones and naphthoquinones, the sensor might cover a potential range of about 500 mV. The probes are readily internalized by live cells and should in principle be applicable as intracellular redox sensors, although the hydroquinone derivatives used in the present work poorly match the reducing potential inside living cells. In addition, probes are highly fluorescent with a strong redox-dependent modulation of fluorescence, and thus fulfill the requirements for application in real-time mechanistic studies of metal complex or enzyme catalyzed oxidation reactions by single molecule fluorescence microscopy.

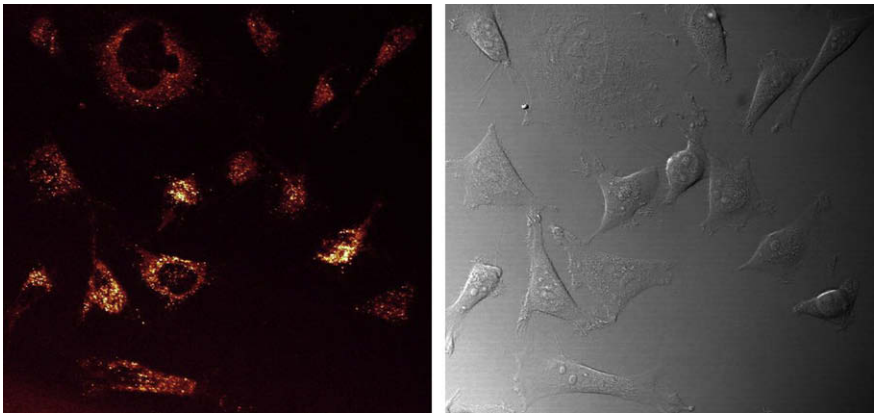

Figure 3. Fluorescence microscopy of live HeLa cells after incubation with **1** (7.5 μM for 30 min, DMEM) (fluorescence image and DIC).

Acknowledgments

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References and notes

1. (a) Miller, E. W.; Bian, S. X.; Chang, C. J. *J. Am. Chem. Soc.* **2007**, *129*, 3458; (b) Yamada, Y.; Tomiyama, Y.; Morita, A.; Ikekita, M.; Aoki, S. *ChemBioChem* **2008**, *9*, 853.
2. (a) Roeffaers, M. B. J.; De Cremer, G.; Uji-I, H.; Muls, B.; Sels, B. F.; Jacobs, P. A.; Pierre, A.; De Schryver, F. C.; De Vos, D. E.; Hofkens, J. *Proc. Natl. Acad. Sci.* **2007**, *104*, 12603; (b) Lei, C.; Hu, D.; Ackerman, E. J. *Chem. Commun.* **2008**, 5490.
3. Kiel, A.; Kovacs, J.; Mokhir, A.; Krämer, R.; Herten, D.-P. *Angew. Chem., Int. Ed.* **2007**, *46*, 3363; The system was previously characterized by ensemble fluorescence spectroscopy, see: Brunner, J.; Mokhir, A.; Krämer, R. *J. Am. Chem. Soc.* **2003**, *125*, 12410.
4. (a) Goulle, V.; Harriman, A.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, *12*, 1034; (b) De Santis, G.; Fabbrizzi, L.; Licchelli, M.; Mangano, C.; Sacchi, D. *Inorg. Chem.* **1995**, *34*, 3581; (c) Benniston, A. C.; Copley, G.; Elliot, K. J.; Harrington, R. W.; Clegg, W. *Eur. J. Org. Chem.* **2008**, *16*, 2705.
5. Sun, P.; Laforge, F. O.; Abeyweera, T. P.; Rotenberg, S. A.; Carpino, J.; Mirkin, M. V. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 443.
6. Nguyen, T.; Francis, M. B. *Org. Lett.* **2003**, *5*, 3245.
7. (a) Evans, J. C.; Klix, R. C.; Bach, R. D. *J. Org. Chem.* **1988**, *53*, 5519; (b) Essers, M.; Haufe, G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2719.
8. Quan, M.; Sanchez, D.; Wasylkiw, M. F.; Smith, D. K. *J. Am. Chem. Soc.* **2007**, *129*, 12847.
9. Mandal, S.; Kazmi, N. H.; Sayre, L. M. *Arch. Biochem. Biophys.* **2005**, *435*, 21.
10. James, B. R.; Williams, R. J. P. *J. Chem. Soc.* **1961**, 2007.
11. Martinovich, G. G.; Cherenkevich, S. N.; Sauer, H. *Eur. Biophys. J.* **2005**, *34*, 937.
12. Bailey, S. I.; Richter, L. M. *Electrochim. Acta* **1985**, *30*, 3.
13. *Synthetic procedures for* **1**: 2,5-Dihydroxybenzoic acid (141 mg, 0.91 mmol), HBTU (345 mg, 0.91 mmol) and *N,N*-diisopropylethylamine (145 μl, 1.14 mmol) were dissolved in dry *N,N*-dimethylformamide. The reaction mixture was stirred in an atmosphere of nitrogen for 30 min, then rhodamine B-piperazineamide (500 mg, 0.91 mmol) was added and the mixed solution stirred over night. The reaction mixture was quenched with water, saturated with potassium chloride, extracted with chloroform, dried with magnesium sulfate and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica with chloroform-methanol (7:1) as the eluent to give **1** (0.16 g, 25%) as pink solid.
14. *Synthetic procedures for* **2**: Synthesis according to Ref. 13 gives **2** (61 mg, 9%) as pink solid.
15. *Analytical data for* **1**: $\lambda_{max}(H_2O)$/nm 566 (ε $dm^3\,mol^{-1}\,cm^{-1}$ 68, 000); δ_H (200 MHz; MeOD; Me_4Si) 1.30 (12H, t, *J* 6.4, H16), 3.35 (4H, br s, H17 or H18), 3.47 (4H, br s, H17 or H18), 3.68 (8H, q, *J* 6.9, H15), 6.55 (1H, d, *J* 1.6, H22), 6.68 (1H, m, H23), 6.94 (2H, d, *J* 2.4, H2), 7.04 (2H, dd, *J* 9.8 and 2.0, H4), 7.27 (2H, d, *J* 9.4, H5), 7.50 (2H, m, H25 and H9) and 7.74 (3H, m, H-10, H11 and H12); δ_C(50 MHz; MeOD; Me_4Si) 12.9 (CH_3, C16), 42.2 (CH_2, C17 or C18), 47.0 (CH_2, C15), 48.9 (CH_2, C17 or C18), 97.4 (CH, C2), 111.5 (CH, C12), 114.9 (CH, C22), 115.2 (C_q, C6), 115.4 (CH, C4), 118.7 (CH, C23), 124.2 (CH, C20), 127.2 (CH, C25), 128.3 (CH, C11), 129.6 (C_q, C13), 131.3 (CH, C10), 131.8 (C_q, C7), 132.3 (CH, C9), 133.2 (CH, C5), 136.5 (C_q, C8), 147.5 (C_q, C24), 151.6 (C_q, C21), 157.2 (C_q, C3), 159.3 (C_q, C1), 169.6 (C_q, C14), 170.5 (C_q, C19); *m/z* (ESI^+-HR) 647.3206 ($[M-Cl]^+$. $C_{39}H_{43}N_4O_5$ requires 647.3233).
16. *Analytical data for* **2**: $\lambda_{max}(H_2O)$/nm 566 (ε $dm^3\,mol^{-1}\,cm^{-1}$ 91, 500); δ_H (400 MHz; MeOD; Me_4Si) 1.30 (12H, t, *J* 7.0, H16), 3.38 (4H, br s, H17 or H18), 3.47 (4H, br s, H17 or H18), 3.70 (8H, q, *J* 7.0, H15), 3.81 (3H, s, H26), 6.42 (1H, s, H22), 6.59 (1H, s, H25), 6.96 (2H, d, *J* 2.4, H2), 7.06 (2H, dd, *J* 9.4 and 2.2, H4), 7.29 (2H, d, *J* 9.4, H5), 7.52 (1H, m, H10), 7.70 (1H, m, H11), 7.77 (2H, m, H9 and H12); δ_C(100 MHz; MeOD; Me_4Si) 12.9 (CH_3, C16), 43.0 (CH_2, C17 or C18), 47.0 (CH_2, C15), 49.1 (CH_2, C17 or C18), 56.4 (CH_3, C26), 97.4 (CH, C2), 101.1 (CH, C22), 114.3 (C_q, C6), 114.9 (CH, C25), 115.5 (CH, C4), 129.0 (C_q, C20), 131.3 (CH, C11), 131.4 (CH, C12), 131.8 (C_q, C9), 132.3 (CH, C10), 133.2 (CH, C5), 136.6 (C_q, C13), 140.8 (C_q, C7), 148.9 (CH, C8), 151.6 (C_q, C24), 157.1 (C_q, C21), 157.2 (C_q, C1), 159.3 (C_q, C3), 164.9 (C_q, C23), 169.6 (C_q, C19), 170.9 (C_q, C14); *m/z* (ESI^+-HR) 677.3275 ($[M-Cl]^+$. $C_{40}H_{45}N_4O_6$ requires 677.3258).