Supplementary Information

Mechanistic Studies of Fluorescent Sensors for the Detection of Reactive Oxygen Species

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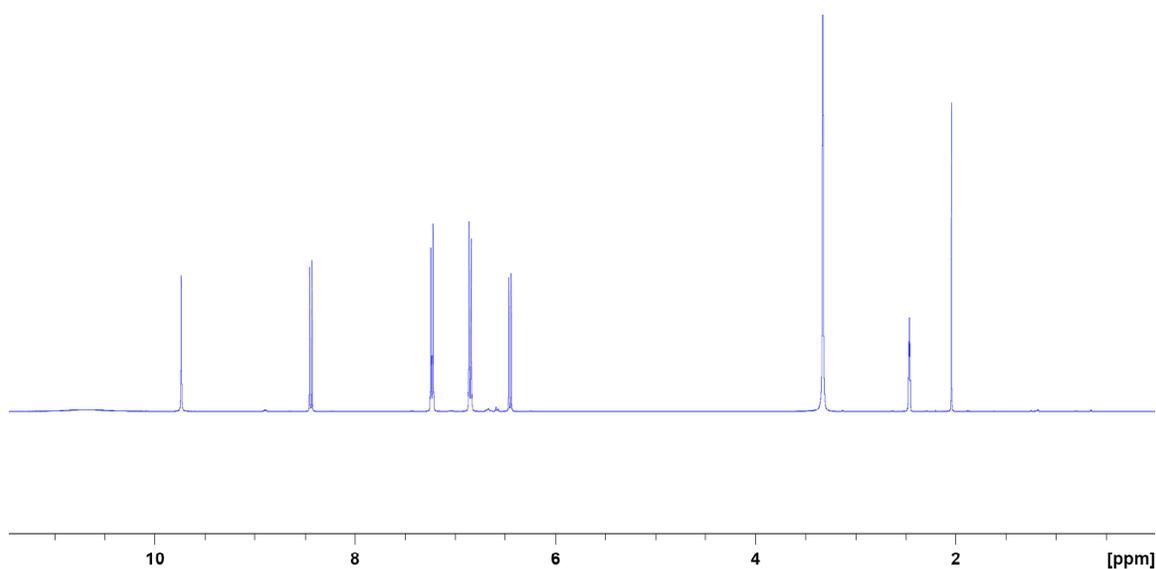## I. Nitrobenzofurazan derivatives.

a) ^{1}H NMR spectra of ABFhd compound

Figure SI 1: ^{1}H NMR of ABFhd in DMSO-d_6 at 298 K

b) ¹H NMR spectrum of 2,6-dimethyl-4-nitrosophenol.

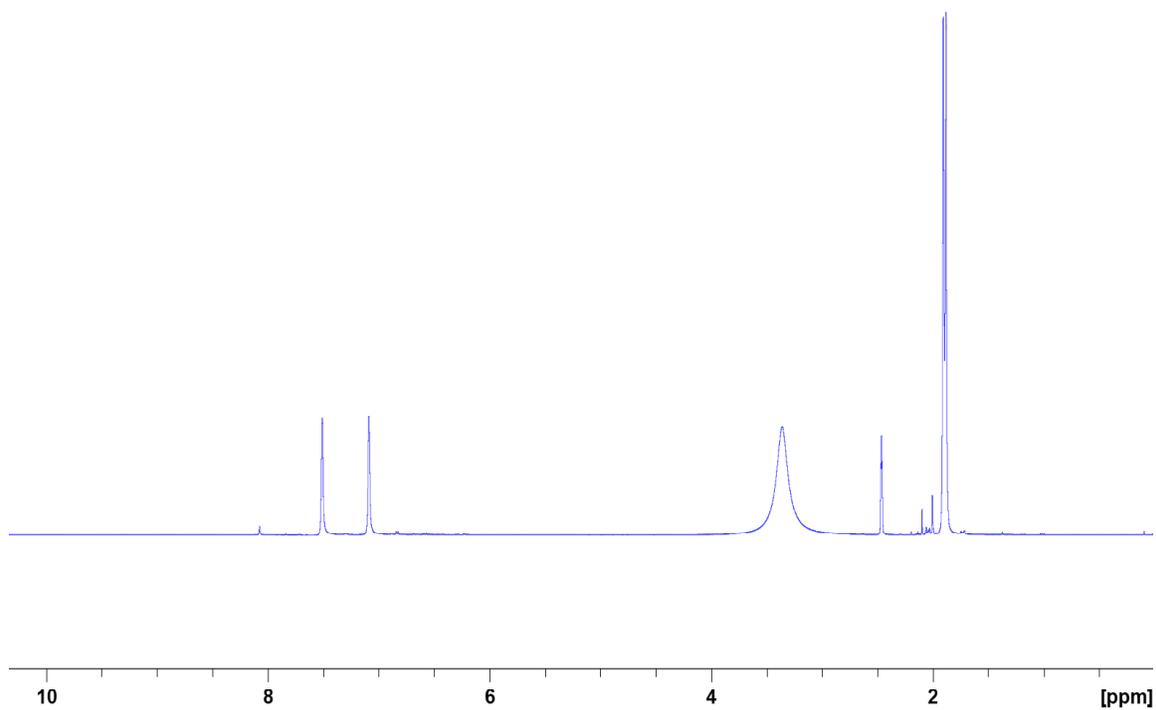**b) ¹H NMR spectrum of 2,6-dimethyl-4-nitrosophenol.**

Figure SI 2: ^{1}H NMR of 2,6-dimethyl-4-nitrosophenol in DMSO-d$_6$ at 298 K
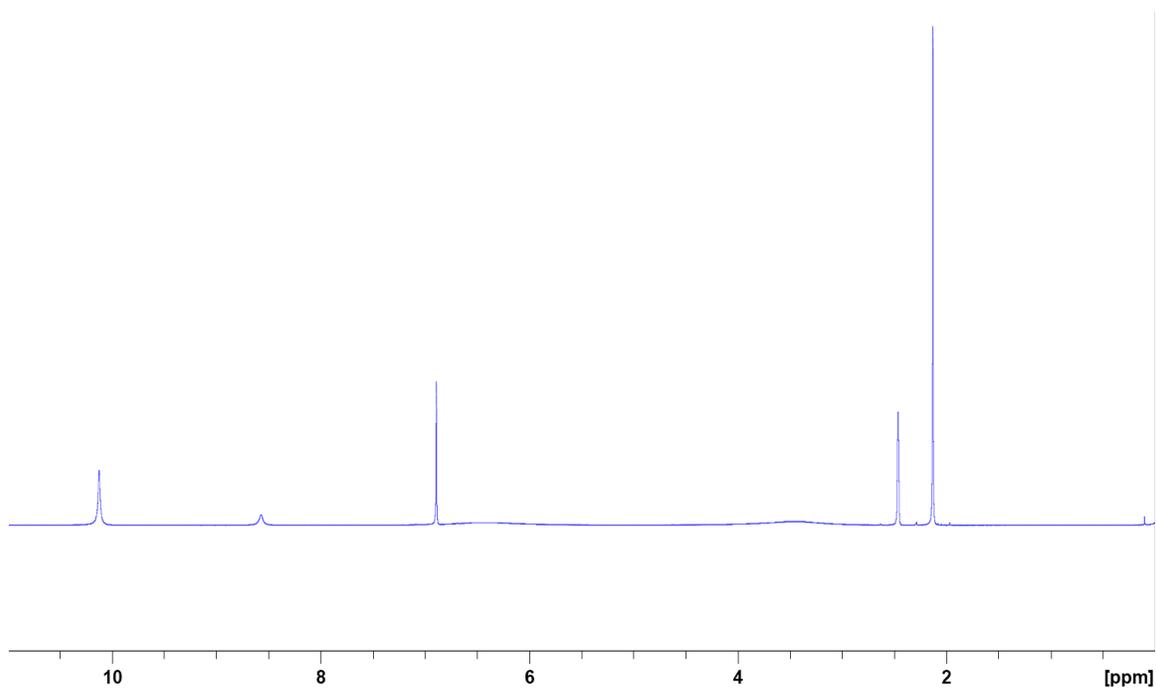
c) **1H NMR of 2,6-dimethyl-4-aminophenol hydrochloride**

Figure SI 3: ^{1}H NMR of 2,6-dimethyl-4-aminophenol hydrochloride salt in DMSO-d$_6$ at 298 K

d) **^{1}H NMR of dAFBhd compound**
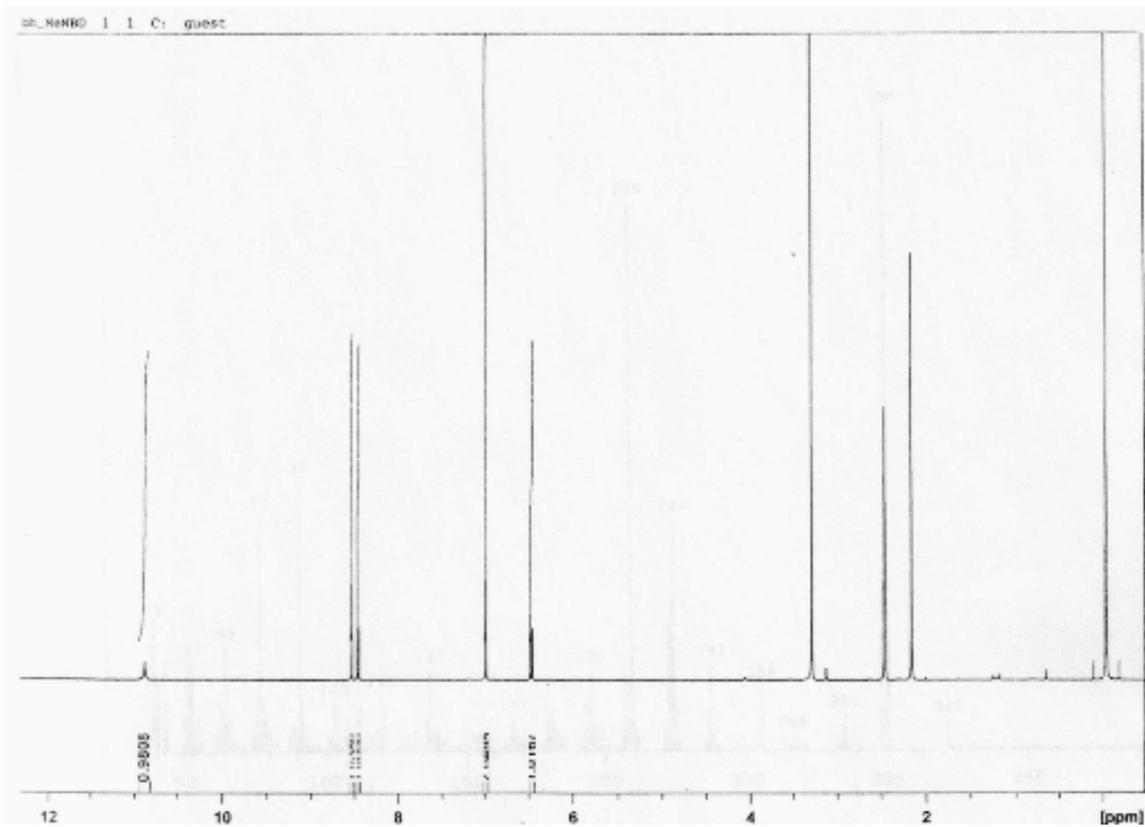

Figure SI 4: ^{1}H NMR of dAFBhd in DMSO-d$_6$ at 298 K
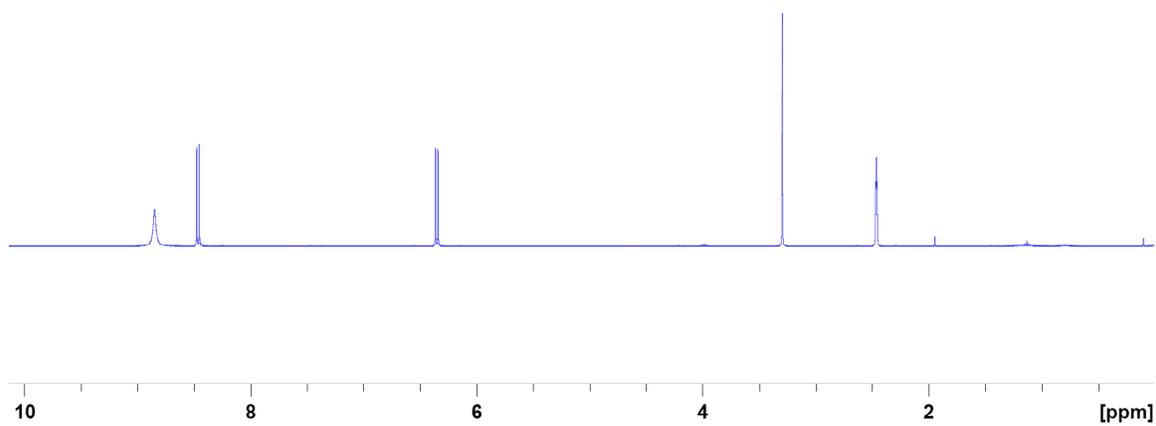
e) ^{1}H NMR of ABF compound

Figure SI 5: ^{1}H NMR of ABF in DMSO-d$_6$ at 298 K

II. Stern-Volmer Experiments

A solution of ABF 5×10^{-6}M was dissolved in methanol and kept a room temperature under air. Upon addition of either phenol or 2,6-dimethylphenol, a quenching of the fluorescence of ABF was observed. The corresponding Stern-Volmer plot are reported in figure SI 6.
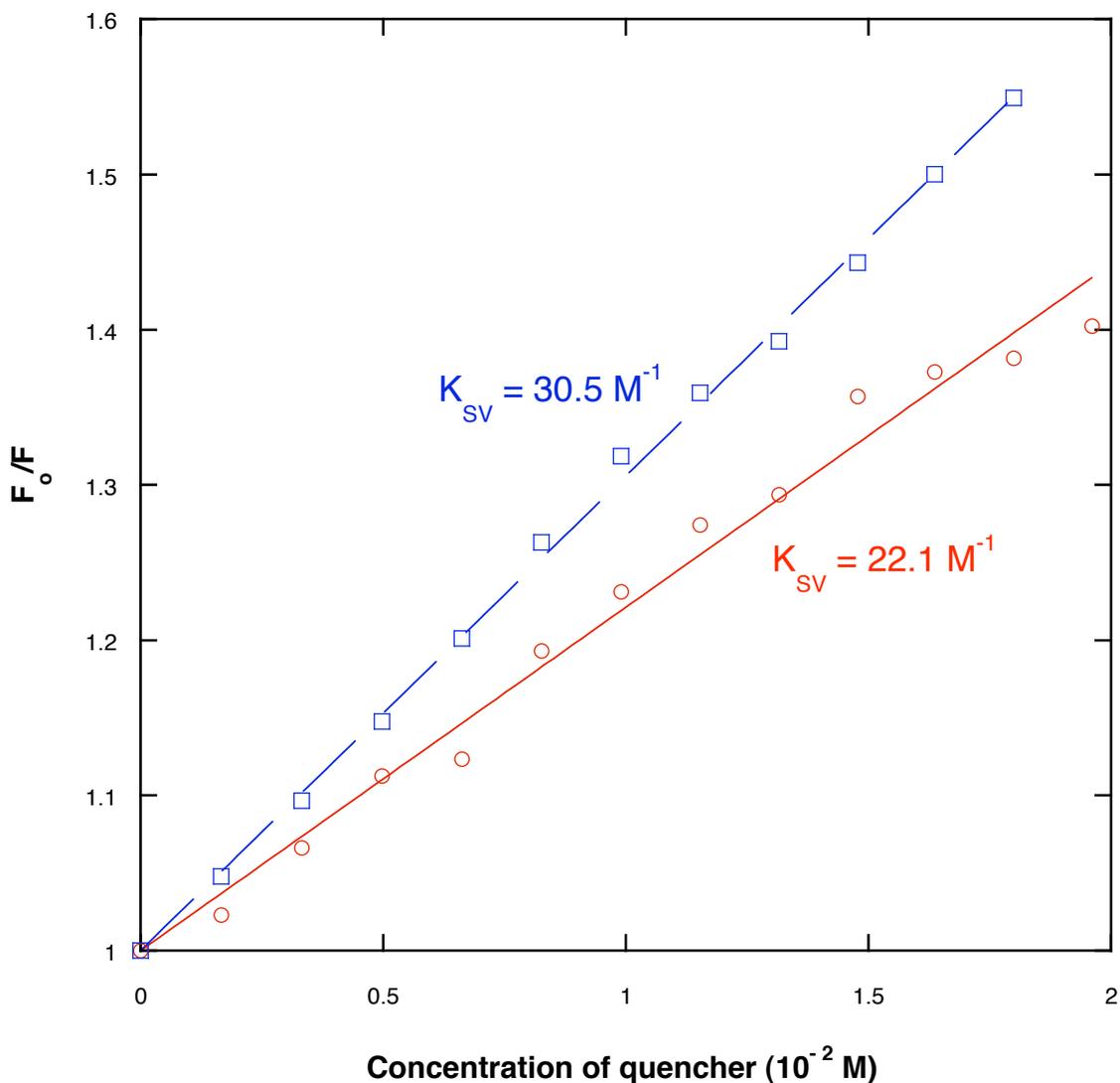

**Figure SI 6**: Stern-Volmer plot of the quenching of fluorescence of a 5×10^{-6}M ABF solution in methanol by phenol (circle) and 2,6-dimethylphenol (square), respectively. The excitation wavelength was set at 460 nm and the emission wavelength was monitored at 540 nm.

III. Chromatographic analysis of the interaction between peroxyl radical and ABFhd and dABFhd.

Released of ABF from the interaction between peroxyl radical and ABFhd or dABFhd.

The interaction between peroxyl radical produced by the thermal decomposition of AAPH at 40°C and ABFhd and dABFhd respectively was performed by HPLC (Jasco) with fluorescence detection (model FP-1520). Briefly, a solution of ABFhd (5×10^{-6} M) or dABFhd dissolved in PBS (15 mM NaH$_2$PO$_4$/K$_2$HPO$_4$, pH 7.4) in presence of AAPH (10^{-2} M) was kept under air at 40°C in a water bath. The mixture was then injected without any treatment on a C-18 reverse phase column (Zorbax, SB-C18 4.6×250 mm, Agilent). An isocratic elution with a mixture of methanol and acetonitrile (30:70) was carried out at a flow rate of 1 ml/min for 6 min. The excitation wavelength of the fluorescence detector was set at 460 nm, while the emission wavelength was 540 nm.
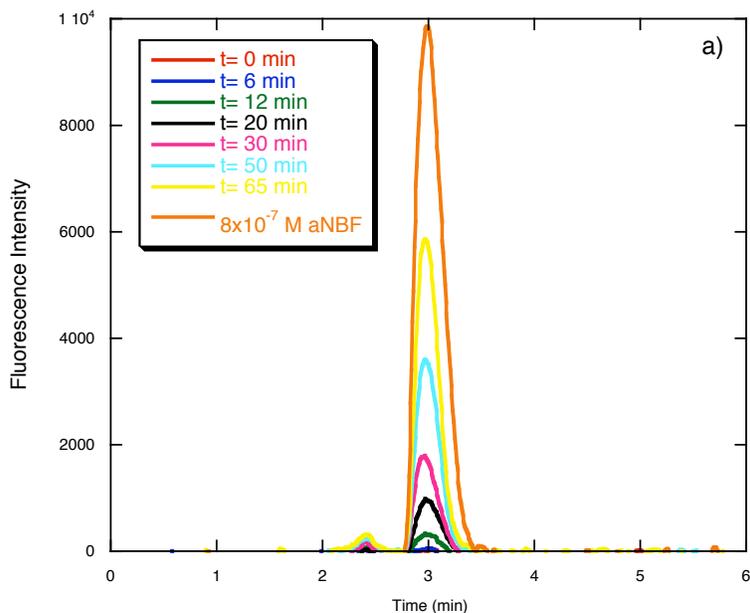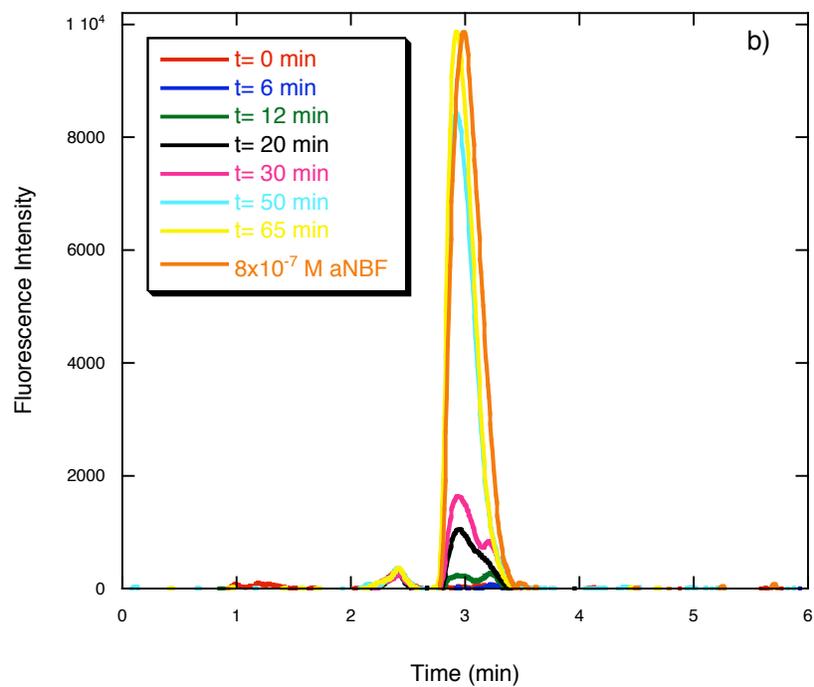
Figure SI 7: HPLC chromatograph with fluorescence detection of a solution of ABF 8×10^{-7} M and: a) ABFhd 5×10^{-6} M incubated with AAPH 10^{-2} M in PBS pH 7.4 or b) dABFhd 5×10^{-6} M incubated with AAPH 10^{-2} M in PBS pH 7.4

IV. Estimation of the number of peroxyl radical required for inducing the release of ABF.

According to the literature[1], the rate of production of peroxyl radicals associated to AAPH pyrolysis can be obtain as follows:

$$R_{ini} = \frac{n*[Trolox]}{\tau},$$

where R_{ini} refers to the rate of production of peroxyl radical, n is the number of peroxyl radical trap by one molecule of antioxidant (n=2 for Trolox), and τ is the induction time. Under our experimental conditions, the rate of peroxyl radical production was estimated to be 13.3×10^{-7} M/ min.
The rate of consumption of dABFhd was estimated form the absorption spectrum (figure SI 8) to be 5.8×10^{-7} M/min.
By comparison of this rate with the rate of peroxyl radical production, it can be concluded that, on the average, 2 radical peroxyl are scavenged by each dABFhd.
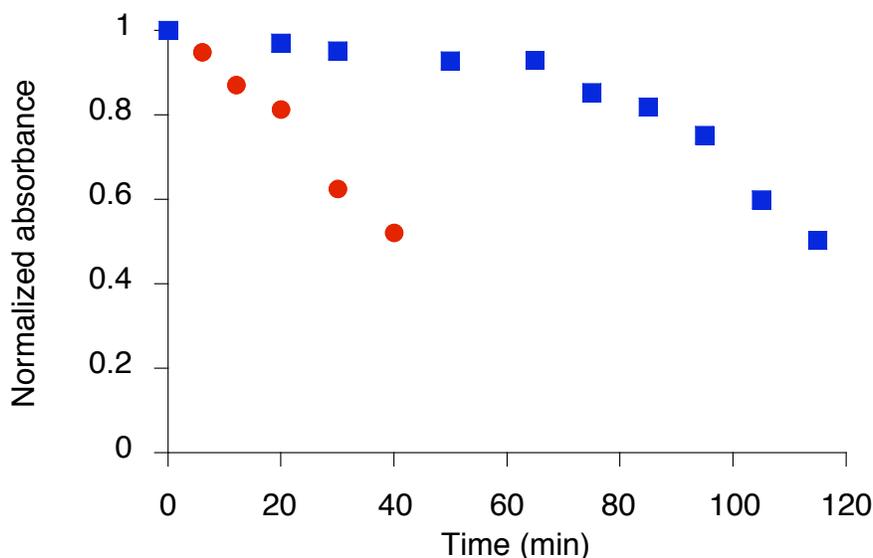

Figure SI 8: Effect of the addition of Trolox 5×10^{-5} M on a phosphate buffer solution pH 7.4 containing AAPH 10^{-2} M under air at 40C and dABFhd 5×10^{-5} M. The circles represent the maximum of absorption of the sensor in absence of Trolox and the squares in presence of Trolox.

V. Synthesis of quinoneimine in organic solvent by reaction with lead dioxide.

10 mg of ABFhd were dissolved in 150 ml of ether. 5 mg of lead dioxide were added to the solution and were mixed during 3 hours at room temperature under air until the solution became yellow. The lead dioxide was filtered and the solution was evaporated under vacuum. The resulting crystal was analyzed by EI and a mass of 270 m/z corresponding to the mass of the desired compound was found.

[1]   E. Pino, A. M. Campos, and E. Lissi, *Int. J. Chem. Kinet.*, 2003, **35**, 525.