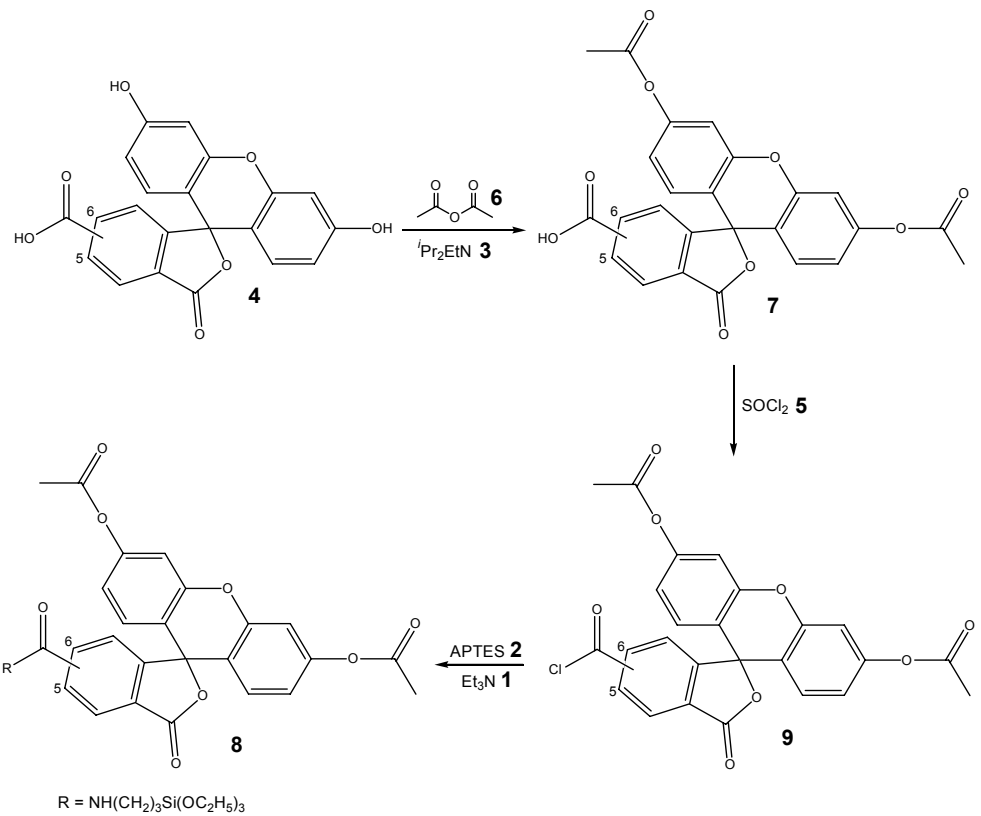
'An optical sensor for reactive oxygen species: Encapsulation of functionalised silica nanoparticles into silicate nanoprobes to reduce fluorophore leaching'

V. J. Hammond, J. W. Aylott, G. M. Greenway, A. Webster and C. Wiles

Instrumentation. Unless otherwise stated, nuclear magnetic resonance (NMR) spectra were obtained at room temperature as solutions in deuteriochloroform ($CDCl_3$) using a Jeol GX400 spectrometer. The chemical shifts are given in parts per million (ppm) with coupling constants in Hertz (Hz). Fluorescent measurements were performed using AB2 software version 4 on a Bowman Series 2 Luminescent Spectrometer (or a Zeiss LSM 510 Meta on an Axiovert 200M in the case of cellular measurements) using a continuous wave Xenon lamp (150 W), with excitation and emission wavelengths of 469 nm and 521 nm respectively (sensitivity = 575).



5(6)-Carboxyfluorescein diacetate 4.[1] δ_H (400 MHz, DMSO) 2.30 (6H, s, 2 x OCH_3), 6.96 (4H, m, ArH), 7.30 (2H, m, ArH), 7.56 (0.4H, d, *J* 8.2, ArH, 5-isomer), 7.85 (0.6H, s, ArH, 6-isomer), 8.18 (0.6H, d, *J* 7.9, 6-isomer), 8.31 (0.4H, dd, *J* 1.4 and 8.2, ArH, 5-isomer), 8.35 (0.6H, dd, *J* 1.4 and 7.9, ArH, 6-isomer), 8.64 (0.4H, m, ArH, 5-isomer) and 10.16 (1H, brs, CO_2H); δ_C 21.0 (2 x $COCH_3$), 81.7 (C_0), 110.0 (2 x CH), 115.6 (CH, 5-isomer), 115.7 (CH, 6-isomer), 117.8 (CH, 5 isomer), 117.9 (CH, 6-isomer), 124.2 (CH, 5-isomer), 124.3 (CH, 6-isomer), 124.6 (2 x C_0), 128.8 (4 x CH), 131.6 (2 x C_0), 151.4 (C_0), 151.5 (C_0), 151.6 (C_0), 152.1 (2 x C_0O), 168.7 (CO), 168.8 (2 x CO) and 169.2 (CO_2H); MALDI-TOF/MS *m/z* 460 $(M+H)^+$461 $(M+Na)^+$ 483.

Derivatised 5(6)-carboxyfluorescein diacetate 8. δ_H (400 MHz, DMSO) 0.66 (1H, t, *J* 7.5, C*H*HSi), 0.75 (1H, t, *J* 7.5, CH*H*Si), 1.14 (9H, t, *J* 7.0, 3 x OCH_2CH_3), 1.72 (2H, m, CH_2CH_2Si), 2.32 (6H, s, 2 x $COCH_3$), 3.42 (1H, m, NC*H*HCH_2), 3.54 (1H, m, NCH*H*CH_2), 3.87 (6H, t, *J* 7.0, 3 x OCH_2CH_3), 6.95 (4H, m, ArH), 7.30 (2H, m, ArH), 7.54 (0.6H, d, *J* 8.2, ArH 5-isomer), 7.84 (0.4H, s, ArH, 6-isomer), 8.16 (0.6H, d, *J* 7.9, ArH, 6-isomer), 8.26 (0.4H, dd, *J* 1.4 and 8.2, ArH, 5-isomer), 8.33 (0.6H, dd, *J* 1.4 and 7.9, ArH, 6-isomer) and 8.45 (0.4H, m, ArH, 5-isomer); δ_C (100 MHz, DMSO) 10.1 (CH_2Si), 18.1 (3 x OCH_2CH_3), 20.5 (2 x CO_2CH_3), 22.1 (CH_2CH_2), 53.6 (NCH_2), 56.4 (3 x OCH_2CH_3), 78.2 (C_0), 110.0 (2 x CH), 115.8 (CH, 6-isomer), 116.1 (CH, 5-isomer), 117.7 (2 x CH), 122.5 (CH, 5-isomer), 123.0 (CH, 6-isomer), 124.5 (CH, 6-isomer), 125.6 (CH, 5-isomer), 128.4 (2 x CH, 6-isomer), 128.9 (2 x CH, 5-isomer), 135.0 (C_0), 135.2 (C_0), 150.8 (2 x C_0), 150.9 (2 x C_0), 151.7 (2 x C_0), 154.1 (2 x C_0), 164.0 (CO, 5-isomer), 164.2 (CO, 6-isomer), 167.8 (CO) and 168.1 (2 x CO); MALDI-TOF/MS *m/z* 664 $(M+H)^+$.686 $(M+Na)^+$.

Evaluation of dye leaching from silicate sol-gel nanoprobes containing functionalised silica nanoparticles. Leaching experiments were carried out by firstly suspending PEBBLE (200 mg) in phosphate buffer (10 ml), followed by activation of the PEBBLE with aqueous NaOH (100 µl, 0.04 M) in phosphate buffer (20 ml). After a period of 2 hr, the solution was neutralised with aqueous HCl (0.04 M) and made up to 100 ml with phosphate buffer. To determine the percentage of dye in the leachate, at 2 hr intervals, a 5 ml aliquot of the PEBBLE solution was firstly filtered under suction and then the fluorescence of the solution determined with excitation and emission wavelengths of 469 and 521 nm respectively and a sensitivity of 575.

Photobleaching investigation. For comparative purposes the photobleaching of PEBBLE, functionalised silica nanoparticles **10** and the free dye **4** was evaluated. Employing phosphate buffer solutions (2 ml) of the material (40 mg) under investigation, samples were allowed to settle in the dark for 1 hr prior to analysis. Under constant excitation at 469 nm, fluorescence data was collected over a period of 1 hr and subsequently repeated for the remaining materials.

Protein interference studies. Employing phosphate buffered solution of PEBBLE, functionalised silica nanoparticles **10** and the free dye **4** (40 mg in 2 ml), settled over a period of 1 hr in the dark, fluorescence measurements were taken for 2 min at 469 nm, prior to the addition of BSA (1 mg ml^{-1} phosphate buffer) and continued for a further 5 min, or until no further increase in fluorescence was observed. This procedure was subsequently repeated for the remaining materials and the change in fluorescence at the point of protein addition determined.

Determination of limit of detection (LOD).[2] To determine the limits of detection for the PEBBLE with respect to a series of analytes, the following equations were employed:

$$LOD = Y_B + 3S_{Y/X} \qquad S_{Y/X} = \sqrt{\frac{\sum_i \left(Y_i - \hat{Y}_i\right)^2}{n-2}}$$

1. M. Adamczyk, C. M. Chan, J. R. Fino and P. G. Mattingly, *J. Org. Chem.,* 2000, **65**, 596-601.
2. J.C. Miller and J.N. Miller, in Statistics for analytical chemistry 3rd Edition, New York, Ellis Horwood, PTR Prentice Hall, 1993.