

## Supporting Information

### **Silica nanoparticles functionalised with cation coordination sites and fluorophores for the differential sensing of anions in a quencher displacement assay (QDA)**

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## 1. General procedures and chemicals

### *Chemicals*

A 30 % suspension of ludox silica nanoparticles AS-30 Colloidal Silica was purchased from Sigma-Aldrich and the nanoparticles were used without any further purification. The solvents were absolute grade and were purchased from Scharlab. Anion stock solutions of fluoride, chloride, bromide, hydrogen sulphate, nitrate and dihydrogen phosphate solutions were prepared from tetrabutyl ammonium salts and, in the case of the representative divalent sulphate anion, from the bis(tetramethylammonium) salt, all obtained from Aldrich. Cations  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$  (as perchlorate salts) were also purchased from Aldrich. For the terpyridine derivative **1**, *p*-tolualdehyde, 2-acetylpyridine, ammonium acetate, *N*-bromosuccinimide and (3-mercaptopropyl)triethoxysilane, all again from Aldrich, were used. For compound **2**, sulphorhodamine B chloride (from Aldrich) and (3-aminopropyl)triethoxysilane were used.

### *General Techniques*

TG analysis, transmission electron microscopy, elemental analysis, nuclear magnetic resonance, and fluorescence spectroscopy techniques were employed to characterize the materials. Thermogravimetric analysis were carried out on a TGA/SDTA 851e Mettler Toledo equipment, using an oxidant atmosphere (Air, 80 mL/min) with a heating program consisting on a heating ramp of 10 °C per minute from 393 K to 1273 K and an isothermal heating step at this temperature during 30 minutes. Transmission Electron Microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. Samples for TEM were prepared by spreading a drop of nanoparticles solution in H<sub>2</sub>O onto standard carbon-coated copper grids (200 mesh). <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectra were acquired with Varian 300 spectrometer (Sunnyvale, CA, USA). Steady-state fluorescence measurements were carried out on a Felix 32 Analysis Version 1.2 (Build 56) PTI (Photon Technology International) and a FluoroMax-4 Spectrofluorometer from Horiba Jobin-Yvon. Fluorescence lifetimes were determined with a unique customized laser impulse fluorometer with picosecond time resolution described in detail in 1,2. The fluorescence was collected at right angles (polarizer set at 54.7°; monochromator with spectral bandwidths of 8 nm) and the fluorescence decays were recorded with a modular single photon timing unit described in 2. Typical instrumental response functions were of 25–30 ps full width at half maximum, the time division was 4.8 ps channel<sup>-1</sup> and the experimental accuracy amounted to ±

<sup>1</sup> U. Resch and K. Rurack, *Proc. SPIE-Int. Soc. Opt. Eng.*, 1997, **3105**, 96.

<sup>2</sup> Z. Shen, H. Röhr, K. Rurack, H. Uno, M. Spieles, B. Schulz, G. Reck, N. Ono, *Chem. Eur. J.*, 2004, **10**, 4853.

3 ps, respectively. The fluorescence lifetime profiles were analyzed with a PC using the software package Global Unlimited V2.2 (Laboratory for Fluorescence Dynamics, University of Illinois).

### *Synthesis of compounds 1 and 2*

**1:** A mixture of bromomethylterpyridine<sup>3</sup> (0.6 g, 1.5 mmol), potassium carbonate (2.0 g, 14 mmol) and 3-(mercaptopropyl)trimethoxysilane (381.6  $\mu$ L, 1.5 mmol) were dissolved in dichloromethane (75 mL), stirred and heated to reflux, under an Ar atmosphere, for 4 days. After cooling to room temperature, the resulting suspension was filtered, the organic solvent removed with a rotary evaporator and the crude product obtained was purified by column chromatography (silica gel, dichloromethane-ethyl acetate 9:1 v/v as eluent). The final product (**1**) was obtained as yellow oil (44 % yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>-D<sub>6</sub>):  $\delta$  (ppm) = 0.78 (t, 2H, -CH<sub>2</sub>-Si-), 1.20 (t, 9H, CH<sub>3</sub>-CH<sub>2</sub>-O-Si-), 1.84 (t, 2H, CH<sub>2</sub>-CH<sub>2</sub>-Si-), 2.70 (t, 2H, S-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-Si-), 3.81 (q, 6H, CH<sub>3</sub>-CH<sub>2</sub>-O-), 3.91 (s, 2H, -S-CH<sub>2</sub>-tpy), 7.32-7.38 (m, 2H, tpy), 7.55-7.75 (d, 4H, Ar), 7.82-7.88 (m, 2H, tpy), 8.59-8.62 (m, 2H, tpy), 8.71-8.75 (m, 2H, tpy), 8.75 (s, 2H, tpy). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>):  $\delta$  (ppm) = 8.5, 22.5, 34.2, 35.7, 50.5, 118.7, 121.3, 127.4, 129.4, 136.8, 137.0, 148.1, 149.1, 155.8, 156.6. m/z (relative intensity): 559 (5), 354 (20), 323,10 (40), 322,10 (30), 162,8 (25), 119 (20), 79,10 (100),67 (20).

**2:** The synthesis of rhodamine derivative **2** from 3-(aminopropyl)triethoxysilane and sulforhodamine B acid chloride was published elsewhere.<sup>4</sup>

## **2. Preparation and characterization of coated silica nanoparticles**

Coated silica nanoparticles **TSNP** were prepared using the corresponding trialkoxysilyl derivatives (**1** and **2**) following reported procedures by Montalti and coworkers.<sup>5</sup> Ludox silica nanoparticles AS-30, 20 nm average diameter, (5 mL) were added to a solution containing acetic acid (20 mL), water (40 mL) and ethanol (40 mL). Then a mixture of **1** (1 mmol) and **2** (0.125 mmol) were added to the nanoparticle suspension. The crude reaction was heated at 80 °C for 48 hours, ethanol was evaporated and the acetic acid solution was neutralized with a saturated solution of ammonium acetate. Solvent was removed and nanoparticles were washed with acetone. The functionalized nanoparticles (**TSNP**) were precipitated and isolated by filtration, washed with water and acetone and dried at 70 °C. This method would allow obtaining a uniform distribution of both functional groups on the silica surface.

<sup>3</sup> W. Spahni and G. Calzaferri, *Helv. Chim. Acta*, 1984, **67**, 450.

<sup>4</sup> M. Ganschow, M. Wark, D. Wöhrle and G. Schulz-Ekloff, *Angew. Chem. Int. Ed.*, 2000, **39**, 161.

<sup>5</sup> M. Montalti, L. Prodi, N. Zacheronni and G. Falini, *J. Am. Chem. Soc.*, 2002, **124**, 13540.

Thermogravimetric analyses were carried out under a flow of air with a heating rate of  $10\text{ }^{\circ}\text{C min}^{-1}$  in the  $30\text{--}1000\text{ }^{\circ}\text{C}$  interval. The final solid was maintained at  $1000\text{ }^{\circ}\text{C}$  for 30 min. In the thermograms of **TSNP** obtained three clearly defined zones were observed; (i) from  $30\text{--}150\text{ }^{\circ}\text{C}$  which was assigned to loss of water and organic solvents (2.032 %), (ii) from  $150\text{--}800\text{ }^{\circ}\text{C}$  which was assigned to the organic matter attached onto the nanoparticle surface (19.001 %) and, finally, (iii) from  $800\text{--}1000\text{ }^{\circ}\text{C}$  a third step was assigned to the condensation of silanol groups (0.330 %). The diameter of **TSNP** nanoparticles was determined to  $20 \pm 2\text{ nm}$  by TEM; the particle size was very homogenous.

Coated silica nanoparticles **SNP** were prepared using trialkoxysilyl derivative **2** following reported procedures by Montalti and coworkers.<sup>5</sup> Ludox silica nanoparticles AS-30, 20 nm average diameter, (5 mL) were added to a solution containing acetic acid (20 mL), water (40 mL) and ethanol (40 mL). Then **2** (0.175 mmol) was added to the nanoparticle suspension. The crude reaction was heated at  $80\text{ }^{\circ}\text{C}$  for 48 hours, ethanol was evaporated and the acetic acid solution was neutralized with a saturated solution of ammonium acetate. Solvent was removed and nanoparticles were washed with acetone. The functionalized nanoparticles (**SNP**) were precipitated and isolated by filtration, washed with water and acetone and dried at  $70\text{ }^{\circ}\text{C}$ . This method would allow obtaining a uniform distribution of the functional group on the silica surface.

Thermogravimetric analyses were carried out under a flow of air with a heating rate of  $10\text{ }^{\circ}\text{C min}^{-1}$  in the  $30\text{--}1000\text{ }^{\circ}\text{C}$  interval. The final solid was maintained at  $1000\text{ }^{\circ}\text{C}$  for 30 min. In the thermograms of **SNP** obtained three clearly defined zones were observed; (i) from  $30\text{--}150\text{ }^{\circ}\text{C}$  which was assigned to loss of water and organic solvents (1.7398 %), (ii) from  $150\text{--}800\text{ }^{\circ}\text{C}$  which was assigned to the organic matter attached onto the nanoparticle surface (7.5896 %) and, finally, (iii) from  $800\text{--}1000\text{ }^{\circ}\text{C}$  a third step was assigned to condensation of silanol groups (0.806 %). The diameter of **SNP** nanoparticles was determined to  $20 \pm 2\text{ nm}$  by TEM; the particle size was very homogenous.

### 3. Fluorescence studies with **TSNP** in the presence of metal cations and anions

**TSNP** nanoparticles (2.5 mg) were suspended in acetonitrile (20 mL) and then ultrasonicated during 5–8 min in order to obtain a homogeneous suspension. At the same time acetonitrile solutions of the metal cations ( $1.0 \times 10^{-2}\text{ mol dm}^{-3}$ ) and anions ( $2.0 \times 10^{-2}\text{ mol dm}^{-3}$ ) were prepared. The emission intensity of **TSNP** nanoparticles at 581 nm (excitation at 550 nm) was first measured in the presence of increasing quantities of metal cations ( $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$ ). A complete

quenching was observed upon addition of 1 equivalent of metal cation (with respect to the content of **1**). Then, 20  $\mu\text{L}$  ( $2.0 \times 10^{-4}$  mmols) of the acetonitrile solutions of metal cations were added to 3 mL of **TSNP** suspensions (containing  $1.9 \times 10^{-4}$  mmols of **1**). To this suspension, increasing quantities of the corresponding anions were added and the emission intensity at 581 nm measured (excitation at 550 nm).