Supporting Information

Highly Selective and Sensitive Chromo-fluorogenic Detection of Tetryl Explosive Using Functionalized Silica Nanoparticles

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1. Experimental procedures

A 30% suspension of Ludox silica nanoparticles AS-30 Colloidal Silica were purchased from Sigma-Aldrich and nanoparticles were used with no further purification. Solvents were of absolute grade and were purchased from Scharlab. The 3% solution in acetonitrile of the subsequently nitrated explosives 2,4,6-trinitrophenylmethylnitramine (Tetryl), 2,4,6-trinitrotoulene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), pentaerythritol tetranitrate (PETN), were purchased from SelectLab Chemicals. 2,4-Dinitrotoluene (DNT) was acquired from Aldrich Chemistry. The authors thank the "La Marañosa" Technology Institute of the Spanish Ministry of Defence for preparing octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), nitroglycerin (NG) and ethylenglycoldinitrate (EGDN). Dimethylsulfate (DS), iodomethane (IodMet), acetic anhydride (AcAn), acetyl chloride (AC), *n*-butylamine (BAm), sodium sulfate, sodium thiosulfate (STS) and copper(II) chloride were purchased from Sigma-Aldrich. Sodium chloride, sodium nitrate, hydrogen peroxide and sodium perborate (SP) were purchased from Scharlau. Triethyl amine (TAm) was purchased from J.T. Baker. Finally, sodium hydrogen phosphate was obtained from Acros Organics.

Thermogravimetric analyses were carried out on TGA/SQTA 851e Mettler Toledo equipment using an oxidant atmosphere (Air, 80 mL/min) with a heating program consisting in a heating ramp

of 10°C per minute from 293 K to 1273 K, and an isothermal heating step at this temperature for 30 minutes. Transmission Electron Microscopy (TEM) images of particles were obtained with a Philips CM10, operating at 20 KeV. TEM samples were prepared by spreading a drop of nanoparticles solution in decane onto standard carbon-coated copper grids (200 mesh). Fluorescence spectroscopy was carried out in a Felix 32 Analysis Version 1.2 (Build 56) PTI (Photon Technology International), and UV-visible spectroscopy was carried out with a Lambda 35 UV/Vis Spectrometer (Perkin Elmer Instruments).

2. Preparation of coated silica nanoparticles

Coated silica nanoparticles (**N1**, **N2**, **N3** and **N-SH**) were prepared from Ludox silica nanoparticles using the corresponding trialcoxysilyl derivatives following the procedures reported by Montalti and coworkers.[1] Nanoparticles **N1** and **N2** were prepared using Ludox silica nanoparticles AS-30 and the trialcoxysilyl derivatives 3-[2-(2-aminoethylamino)ethylamino]propyl-trimethoxysilane (**N3TS**) and mercaptopropyltrimethoxysilane (**MPTS**) in two different amine-thiol ratios (10:1 and 5:1 for **N1** and **N2** respectively). Also, hybrid nanoparticles using 3-(aminopropyl)-triethoxysilane (**N1TS**) and **MPTS** were prepared (**N3**) in order to test the selective response of the material upon changing the chemical nature of the binding site. Finally, as a model, and in order to test the role played by the binding site in the chromo-fluorogenic response observed, hybrid nanoparticles **N-SH** were prepared using only **MPTS**. As an example, the preparation of **N1** nanoparticles is described below in more detail.

Preparation of coated silica nanoparticles N1: Ludox silica nanoparticles AS-30, with an average diameter of 18 nm, (12 mL), were added to a solution containing acetic acid (60 mL), water (60 mL) and ethanol (100 mL). Then a mixture of mercaptopropyltriethoxysilane (**MPTS**, 0.1636 mmol) and 3-[2-(2-aminoethylamino)ethylamino]propyl-trimethoxysilane (**N3TS**, 1.6363 mmol) was added to the nanoparticle suspension. The crude reaction was heated at 80°C for 48 hours, ethanol was evaporated, then the acetic acid solution was neutralized with a saturated solution of ammonium acetate. Functionalized nanoparticles (**N1**) were precipitated and isolated by filtration, washed with water and acetone and dried at 40°C. This method obtained a uniform distribution of both the functional groups on the silica surface.

This synthetic procedure allows the preparation of silica nanoparticles in which the functional groups were expected to be uniformly distributed into the surface forming a compact monolayer. The only expected differences between the solids would derive from the initial concentrations of amine and thiol derivatives. The values of organic content in mmol of **MPTS** and **N3TS** or **N1TS** per gram

of SiO_2 for solids **N1**, **N2**, **N3** and **N-SH** were determined by elemental and thermogravimetric analysis, and the results are shown in Table SI-1.

Table SI-1. Total mmols of **N3TS**, **N1TS** and **MPTS** per gram of SiO_2, average coverage and interdistance between anchored groups for the functionalised nanoparticles **N1**, **N2**, **N3** and **N-SH**.

Solid	Ratio amino/thiol used (mmol/mmol)	α_{total} (mmol/g SiO_2)	α_{N3TS} (mmol/g SiO_2)	α_{N1TS} (mmol/g SiO_2)	α_{MPTS} (mmol/g SiO_2)	β_{total} (molecules/nm^2)	Distance (Å)
N1	10 / 1	0.3103	0.2707	-	0.039	0.97	10.34
N2	5 / 1	0.2906	0.2244	-	0.066	0.94	10.69
N3	10 / 1	0.3491	-	0.3406	0.008	1.05	9.75
N-SH	-	0.2593	-	-	0.259	0.88	11.31

Thermogravimetric analyses were carried out under an air flow and with a heating rate of 10ºC/ minute in the 30-1000ºC intervals. As an example typical thermograms for **N1** are described below. Three clearly defined zones were observed for **N1**; (i) from 30ºC to 200ºC, which was assigned to loss of water and organic solvents (3.03%), (ii) from 150ºC to 800ºC, which was assigned to the organic matter attached to the nanoparticle surface (8.05%), and (iii) from 800ºC to 1000ºC, a third step assigned to the condensation of silanol groups (0.41%). The silica residue amounted to 88.51%. Amine and thiol contents were determined by elemental analysis and thermogravimetric measurements, amounting to 0.271 and 0.039 mmol/g SiO_2, respectively. The diameter of the **N1** nanoparticles was determined by TEM. TEM images for all the prepared nanoparticles show a very homogenous particle size of 18.0 ± 2.6 nm (see Figure SI-1 as an example for **N1**).
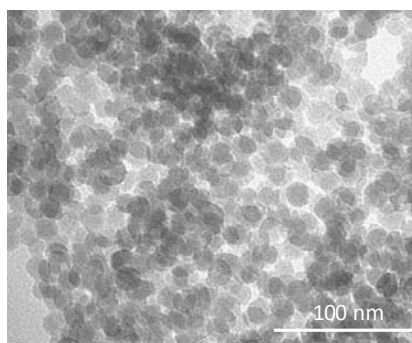

Figure SI-1. TEM images of functionalized nanoparticles **N1**, average diameter = 18.0 ± 2.6 nm.

3. Experimental signaling procedure for the detection of explosives.

3.1. Solution studies:

In a first step the reactivity of the squaraine (**I**) with the surface thiols in all the studied materials was tested. At this respect, addition of squaraine to suspensions of **N1**, **N2** and **N3**

nanoparticles in water-acetonitrile 90:10 v/v induced a marked bleaching of the solution due to the reaction of the grafted thiol moieties with the dye. Then, the response of water-acetonitrile 90:10 v/v suspensions of **N1**, **N2** and **N3** nanoparticles in the presence of nitrated explosives was tested. The best results were obtained with suspensions of **N1** nanoparticles because, of all the nitrated explosives tested, Tetryl was able to inhibit the thiol-squaraine reaction giving rise to a clear selective chromo-fluorogenic response (see bellow).

In a typical assay, 10 mg of **N1** nanoparticles were suspended in 15 mL of water-acetonitrile 90:10 v/v solutions (blank sample). At the same time, 10 mg of **N1** nanoparticles were suspended in 15 mL of water-acetonitrile 90:10 v/v containing the corresponding nitrated explosive. Then samples were sonicated for 15 minutes and pH was adjusted to 7-7.2 (this pH was selected because amines are partly protonated and the formation of the charge-transfer complex is partially inhibited at acid pH, see bellow). Then, 1.35 ml of the corresponding suspensions were mixed with 50 µL of an acetonitrile solution of the squaraine dye ($C = 1.0$ x 10^{-4} mol L^{-1}). After 3 minutes, an aliquot was filtered (0.2 µm Teflon filters) and the absorbance of the SQ dye at 642 nm was measured. Also kinetic measurements, monitoring the changes of the squaraine absorption band centered at 642 nm, in the presence and in the absence of nitrated explosives were carried out. For the fluorescence kinetic measurements, 1.35 mL of the initial suspension were mixed with 15 µL of an acetonitrile solution of the squaraine dye ($C = 1.0$ x 10^{-4} mol L^{-1}) in the fluorescence tray, and the fluorescence changes occurring with time were monitored at 666 nm (excitation at 642 nm).
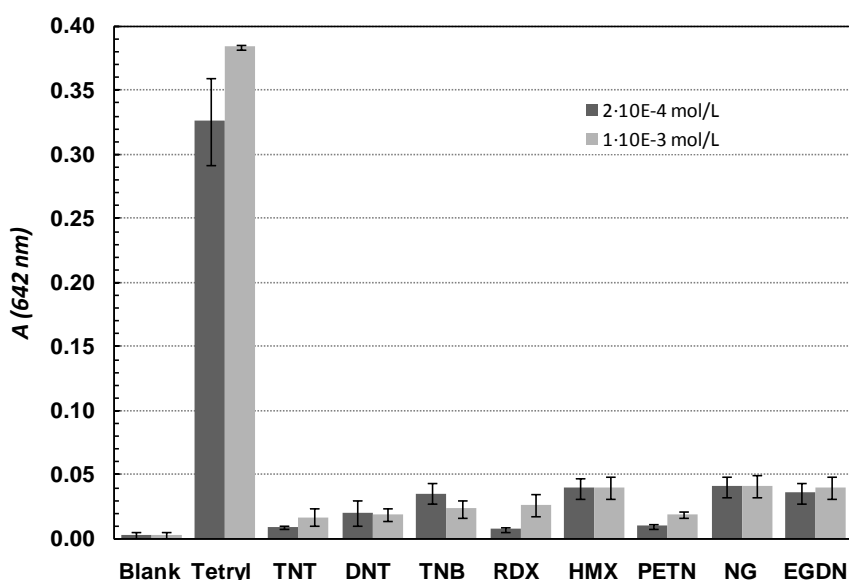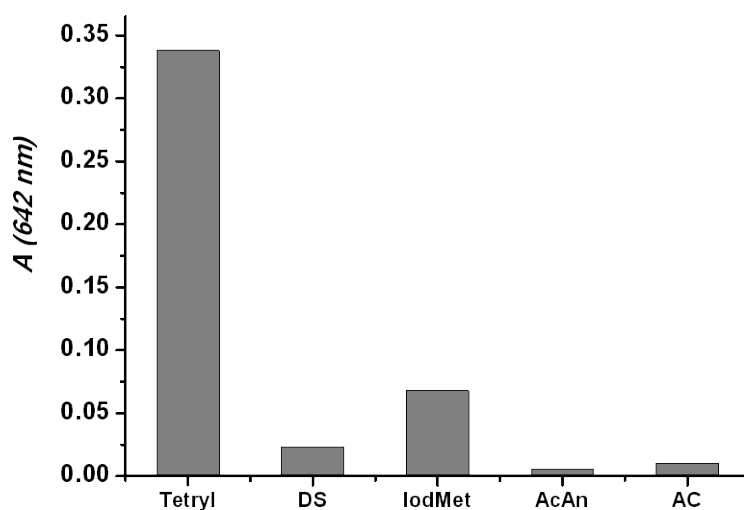

Figure SI-2. Absorbance at 642 nm (squaraine band) for water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing **N1**, SQ and Tetryl, TNT, DNT, TNB, RDX, HMX, PETN, NG and EGDN at 2 x 10^{-4} mol L^{-1} and 1.0 x 10^{-3} mol L^{-1} after 3 minutes. Three independent experiments were done and the data are reported as mean ± s.
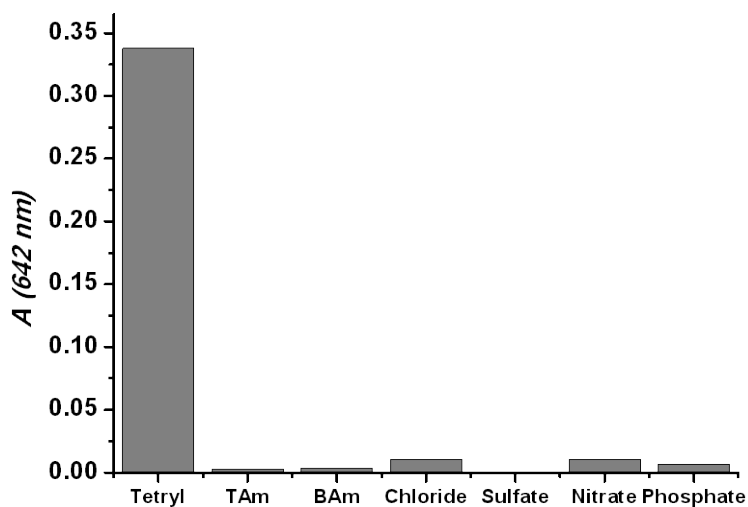
Figure SI-3. Absorbance at 642 nm (squaraine band) for water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing **N1**, SQ and Tetryl, dimethyl sulfate (DS), iodomethane (IodMet), acetic anhydride (AcAn) and acetyl chloride (AC) after 3 minutes (DS and IodMet at 1.0×10^{-5} mol L^{-1}, AcAc and AC at 2.0×10^{-4} mol L^{-1}).
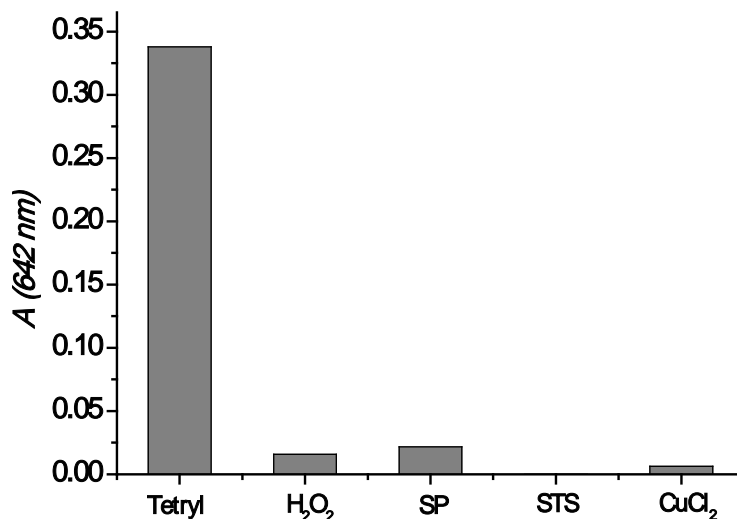

Figure SI-4. Absorbance at 642 nm (squaraine band) for water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing **N1**, SQ and Tetryl, triethylamine (Tam), *n*-butylamine (Bam), chloride, sulfate, nitrate and phosphate after 3 minutes (anions at 5.0×10^{-3} mol L^{-1}; TAm and BAm at 2.0×10^{-4} mol L^{-1}).

Figure SI-5. Absorbance at 642 nm (squaraine band) for water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing **N1**, SQ and Tetryl, hydrogen peroxide, sodium perborate (SP), sodium thiosulfate (STS) and copper(II) chloride after 3 minutes (hydrogen peroxide, SP, STS and copper(II) chloride at 5.0×10^{-5} mol L^{-1}).

Figure SI-2 shows the **N1**-SQ system's chromogenic response in the presence of two different concentrations of nitrated explosives. As shown, only Tetryl was able to inhibit the reaction on the surface of **N1** between the thiol groups and the squaraine dye. Figures SI-3, SI-4 and SI-5 depict the **N1**-SQ ensemble's response in the presence of potential interferents such as amines (triethylamine, *n*-butylamine), acylating agents (acetic anhydride, acetyl chloride), alkylating agents (dimethylsulfate, iodomethane), oxidants (hydrogen peroxide, sodium perborate, sodium thiosulfate, copper(II) chloride) and anions (chloride, sulfate, phosphate, nitrate). As observed, despite some partial inhibition noted in the presence of iodomethane, the remaining species were unable to significantly modify the reaction between squaraine and thiols on **N1**. We also observed how the presence of larger amounts of alkylating or acylating agents could easily be removed by using a basic water-acetonitrile 90:10 v/v. Then, the pH of the mixture was adjusted to pH 7-7.2, and nanoparticles **N1** and the acetonitrile solution of the squaraine dye were added as explained above to detect Tetryl. With this previous basic treatment, the alkylating or acylating agents hydrolyze and their potential interference is then eliminated.

In order to determine the detection limit for Tetryl, fluorescence measurements with the **N1**-SQ systems in the presence of this explosive at different concentrations were made. Figure SI-6 illustrates the changes in the emission band at 666 nm in the presence of different concentrations of Tetryl in water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing nanoparticles **N1** with

further addition of SQ. A detection limit of 0.14 ppm Tetryl in the mixed aqueous solution was established.
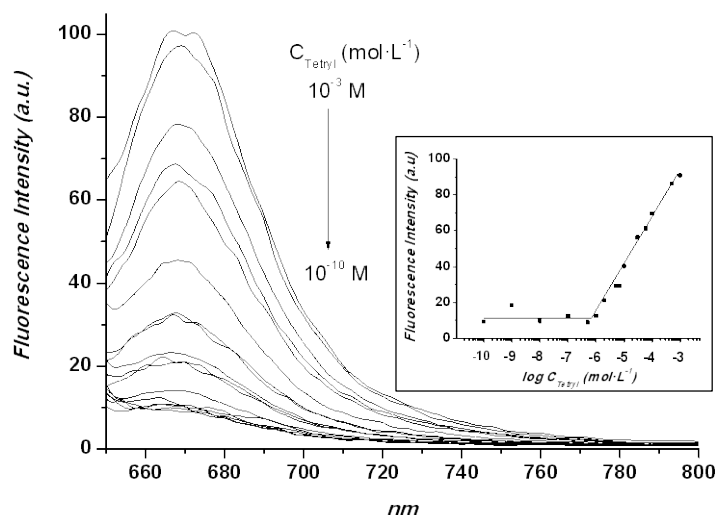

Figure SI-6. Fluorescence spectra of SQ (excitation at 642 nm) in the presence of different Tetryl concentrations in water-acetonitrile 90:10 v/v at pH 7.2, suspensions containing nanoparticles **N1** with further addition of SQ. The inset shows the calibration curve for Tetryl using the **N1**-SQ ensemble in water-acetonitrile 90:10 v/v mixtures at pH 7.2.

The response observed with water-acetonitrile 90:10 v/v suspensions of **N2** nanoparticles was different from the obtained for **N1** suspensions; i.e. the difference between the absorbance at 644 nm (SQ band) in the absence and in the presence of Tetryl, for a certain time, was lower for nanoparticles **N2** than that observed in the same conditions for **N1**. This different response should be related with the different polyamine-thiol ration in both nanoparticles. In the same line nanoparticles **N3** displayed a poorer response to Tetril when compared to **N1** both in terms if selectivity and sensitivity.

The optimal pH range for the optical detection of Tetryl with the **N1**-SQ system was determined to be 7-9. At acidic pH the amines are partly (or totally) protonated and the formation of charge-transfer complexes between the electron-rich nitrogen atoms and the electron-deficient Tetryl was observed to be highly inhibited, whereas more basic pH values were avoided in order to prevent degradation of the silica support.
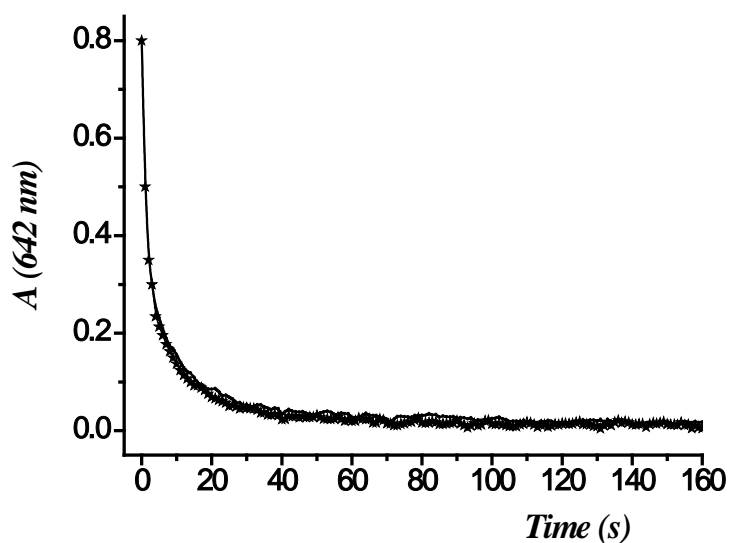
Figure SI-7. Absorbance at 642 nm (squaraine band) as a function of time for water-acetonitrile 90:10 v/v suspensions containing **N-SH** and SQ in the absence and in the presence of Tetryl (1×10^{-3} mol L^{-1}).

Finally, and in order to check the role played by the polyamine binding site in the chromo-fluorogenic response, the behavior of water-acetonitrile 90:10 v/v suspensions of **N-SH** was tested in the absence and in the presence of Tetryl. Exactly the same response was obtained in both cases, i.e. the bleaching of the solution due to the free access of the SQ to the surface grafted with thiols (see Figure SI-7). This fact strongly suggests that there is not interaction between the thiol groups and Tetryl and points out the critical role played by the formation of amine-explosive interactions in the observed response.

3.1. Soil extraction studies:

The extraction of Tetryl from soil samples was carried out by means of a simple method in which soil was grinded and sifted to obtain a homogeneous soil sample. Then 30 g of soil sample was doped with a certain amount of Tetryl. The sample was stirred for 15 min with 25 mL of acetonitrile to extract the explosive traces to be then filtered. The extract obtained was concentrated, in a rotary evaporator until the solvent was eliminated. The crude extract was dissolved in 1.5 mL of a water-acetonitrile 90:10 v/v mixture (pH 7.2) to which 10 mg of **N1** were added. The resultant crude was sonicated for 15 minutes to obtain an adequate suspension of nanoparticles. Next, this suspension was mixed with 15 μL of an acetonitrile solution of the squaraine dye ($C = 1.0 \times 10^{-4}$ mol L^{-1}), and the fluorescence emission at 666 nm (excitation at 642 nm) was measured after 5 minutes. The experimental procedure was repeated for different samples containing differing amounts of Tetryl. Figure SI-9 shows the fluorescence emission spectra of the squaraine dye in final solutions for selected Tetryl concentrations.
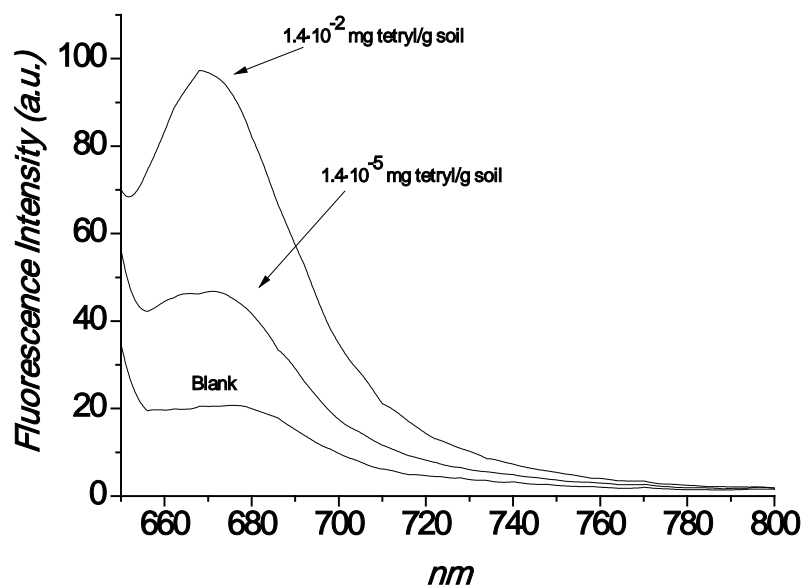
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Figure SI-9. Changes in the SQ emission band centred at 666 nm (excitation at 642 nm) in the absence and presence of Tetryl after soil extraction with acetonitrile.

4. References.

1. M. Montalti, L. Prodi, N. Zacheronni, G. Falini, *J. Am. Chem. Soc.*, 2002, **124**, 13540.