Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Gold Nanoparticle-based Fluorescent "Turn-On" Sensing System for the Selective Detection of Mercury Ions in Aqueous Solution

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General methods, instrumentation, and measurements

Absorption spectra were obtained on a Shimadzu UV-2501 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer using quartz cuvettes with a path length of 1 cm. Fluorometric assays with various analytes were measured by monitoring changes in fluorescence intensity using a Synergy Mx Microplate Reader (BioTek, USA). According to a previously reported method,[1] citrate-capped AuNPs (Fig. S12), and cationic BODIPY derivative **1**-PPh_3^+ were prepared.

The fluorescence quantum yield (Φ_{FL}) of the AuNP/**1**-PPh_3^+ adsorbate was calculated by measuring the integrated emission area of the corrected spectra and comparing that value to the area measured for Rhodamine 6G in ethanol when excited at 480 nm (Φ_{FL} = 0.95).[2] The quantum yields for AuNP/**1**-PPh_3^+ adsorbate in HEPES buffer (10 mM, pH 7.4) was then calculated using eq 1, where *F* represents the area under the emission spectra for the standard and samples, η is the refractive index of the solvent, and *Abs* is the absorbance at the excitation wavelength selected for the standard and samples. Emission was integrated between 495 and 800 nm (λ_{ex} = 480 nm, $Abs^{standard} \approx 0.01$, $Abs^{sample} \approx 0.06$).

$$\Phi_{FL}^{sample} = \Phi_{FL}^{standard}\left(\frac{F^{sample}}{F^{standard}}\right)\left(\frac{\eta^{sample}}{\eta^{standard}}\right)\left(\frac{Abs^{standard}}{Abs^{sample}}\right) \qquad (1)$$

Fluorometric assay studies

The AuNP/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (final concentration: 3 nM) and the cationic BODIPY dye **1**-PPh_3^+ (final concentration: 1 μM) in HEPES buffer (10 mM, pH 7.4) containing 5% ethanol. After stirring for two hours at 25 °C, the unadsorbed BODIPY dye **1**-PPh_3^+ was removed by centrifugation. The fluorometric assay conditions were optimized by monitoring the fluorescence enhancement of the AuNP/**1**-PPh_3^+ adsorbate upon addition of Hg(II) ions in assay solutions made of various buffers and at varying pHs as well as concentration of **1**-PPh_3^+ (Figs. S3-4).

For assays, each metal ion (50 μM) was added to a solution of AuNP/**1**-PPh_3^+ adsorbate suspended in phosphate buffer (50 mM, pH 8.0, 25 ^oC). Fluorescence intensity at 510 nm was measured 2 min after the addition of each metal to AuNPs/**1**-PPh_3^+ adsorbate.

(A) Absorption spectrum of AuNPs and emission spectrum of 1-PPh_3^+

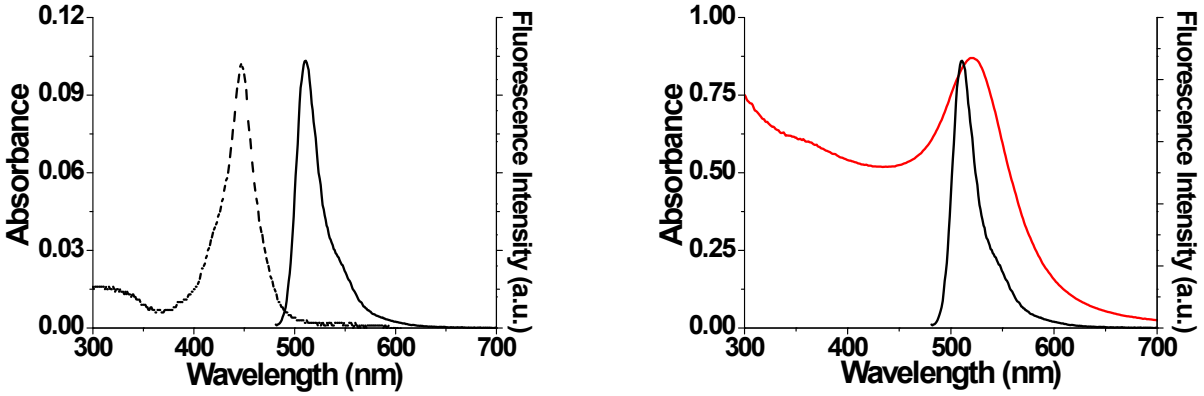


Figure S1. (left) Absorption (dashed line) and emission spectra (solid line) of **1**-PPh_3^+ in HEPES buffer (10 mM, pH = 7.4, 5% EtOH, 25 °C). Excited at 460 nm. (right) Normalized absorption spectrum of AuNPs (red line) and emission spectrum of **1**-PPh_3^+ (black line).

(B) Fluorescence quenching of 1-PPh_3^+ upon addition of AuNPs

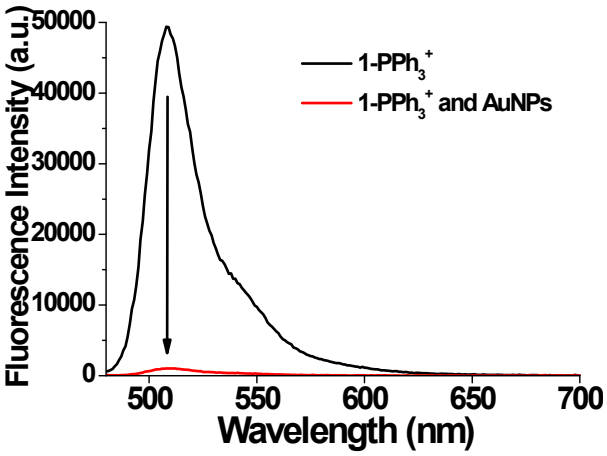


Figure S2. Emission spectra of cationic BODIPY **1**-PPh_3^+ (1 μM) in the absence (black) and the presence (red) of AuNPs (final concentration: 3 nM) in HEPES buffer (10 mM. pH 7.4) containing 5% EtOH as cosolvent at 25 °C. Excited at 460 nm.

(C) Effect of concentrations of cationic BODIPY dye 1-PPh_3^+ on the efficiency of fluorescence recovery of AuNP/1-PPh_3^+ adsorbate upon the addition of Hg(II) ions.

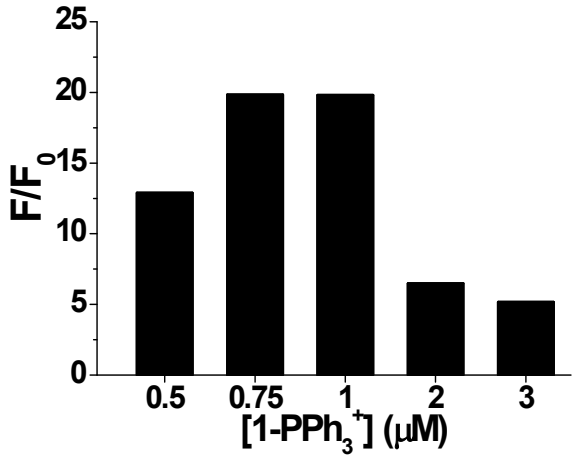


Figure S3. Relative fluorescence turn-on response of AuNP/**1**-PPh_3^+ adsorbate, which is prepared by AuNPs (3 nM) with various concentrations of **1**-PPh_3^+ (0.5, 0.75, 1, 2, and 3 μM, respectively), toward 50 μM $HgCl_2$. Excited at 460 nm. Fluorescence intensity at 510 nm was measured 2 min after the addition of $HgCl_2$ to AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 °C). F_0 and F correspond to the fluorescence intensity of AuNP/**1**-PPh_3^+ adsorbate in the absence and the presence of 50 μM $HgCl_2$, respectively. AuNP/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (3 nM) and **1**-PPh_3^+ (0.5, 0.75, 1, 2, or 3 μM) for 2 hours in HEPES buffer (10 mM, pH 7.4, 5% EtOH, 25 °C) and centrifuging.

(D) Effect of buffers (HEPES, phosphate, Tris) and pH of the assay solution on the efficiency of fluorescence recovery of AuNP/1-PPh_3^+ adsorbate upon the addition of Hg(II) ions

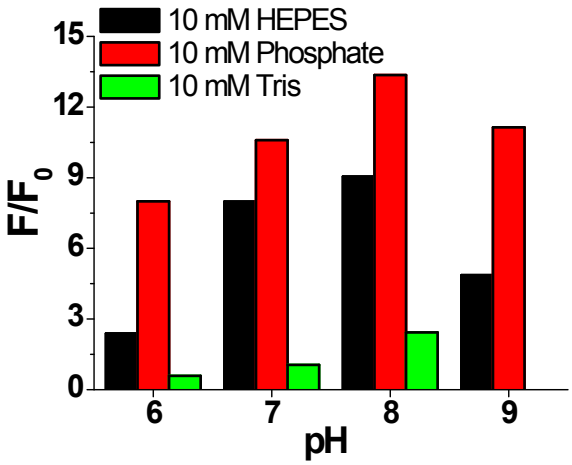


Figure S4. Relative fluorescence turn-on response of AuNP/**1**-PPh_3^+ adsorbate toward 50 μM $HgCl_2$. Excited at 460 nm. Fluorescence intensity at 510 nm was measured 2 min after the addition of Hg(II) ions to AuNP/**1**-PPh_3^+ adsorbate in various buffer systems (10 mM, 25 °C) with different pH conditions. F_0 and F correspond to the fluorescence intensity of AuNP/**1**-PPh_3^+ adsorbate in the absence and the presence of 10 μM $HgCl_2$, respectively. AuNP/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (3 nM) and **1**-PPh_3^+ (1 μM) for 2 hours in HEPES buffer (10 mM, pH 7.4, 5% EtOH, 25 ^oC) and centrifuging.: The assay performed in tris buffer showed a less efficient fluorescence turn-on signal of AuNP/**1**-PPh_3^+ upon addition of Hg(II) ions. Based on literature,[3] for the citrate-capped AuNPs, upon the addition of tris buffer, tris slowly replaces citrate on the surface of AuNPs.

(E) Effect of Hg(II) ion on emission spectrum of pure 1-PPh_3^+

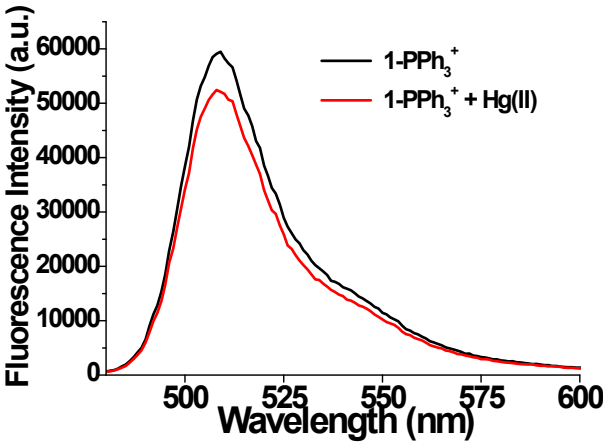


Figure S5. Emission spectra of cationic BODIPY **1**-PPh_3^+ in the absence (black) and the presence (red) of $HgCl_2$ (50 μM), respectively. Excited at 460 nm. The spectra were measured in phosphate buffer (50 mM, pH 8.0, 5% EtOH, 25 °C).

(F) Stability Study of AuNP/1-PPh_3^+ in aqueous media

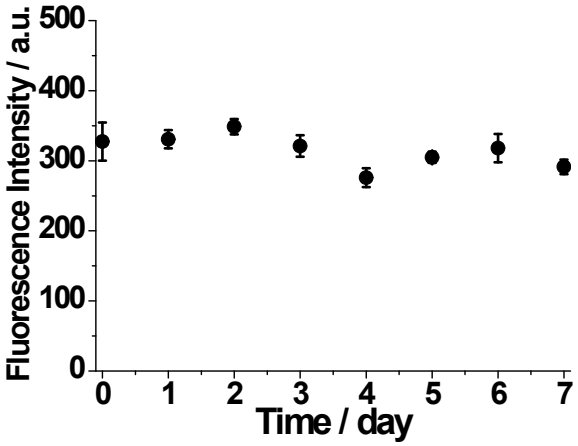


Figure S6. Chemical stability of AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 °C). Fluorescence intensity was measured at 510 nm every 1 day (0 –7 day). Excited at 460 nm.

(G) Absorption spectra of AuNP/1-PPh_3^+ upon the addition of Hg(II) ions

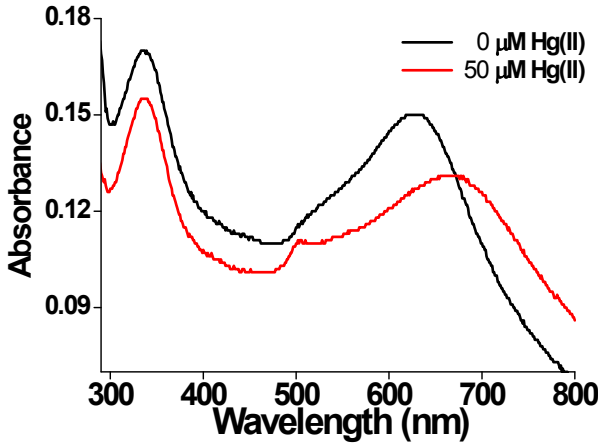


Figure S7 Absorption spectra of AuNP/**1**-PPh_3^+ adsorbate in the absence (black) and the presence (red) of 50 μM Hg(II) ions. The spectra were recorded 30 min after the addition of Hg(II) ions to AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 ^oC). AuNPs/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (3 nM) and **1**-PPh_3^+ (1 μM) for 2 hours in HEPES buffer (10 mM, pH 7.4, 5% EtOH, 25 ^oC) and centrifuging.

(H) Determination of detection limit of AuNP/1-PPh_3^+adsorbate for Hg(II) ions

The fluorescence emission spectra of AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 °C) was collected for 30 times to determine the background noise σ. Then fluorescence turn-on response of the AuNP/**1**-PPh_3^+ adsorbate to concentrations of $HgCl_2$ ranging from 0 to 50×10^{-6} mol L^{-1} upon incubation for 2 min were monitored. A linear regression curve was fitted (R^2=0.992) according fluorescence intensities at 510 nm as a function of $HgCl_2$ concentrations in the range of 0.01×10^{-6} mol L^{-1} – 10×10^{-6} mol L^{-1}, and the slope of the curve was obtained. The detection limit (3σ/slope) was determined to be 0.057 μM.

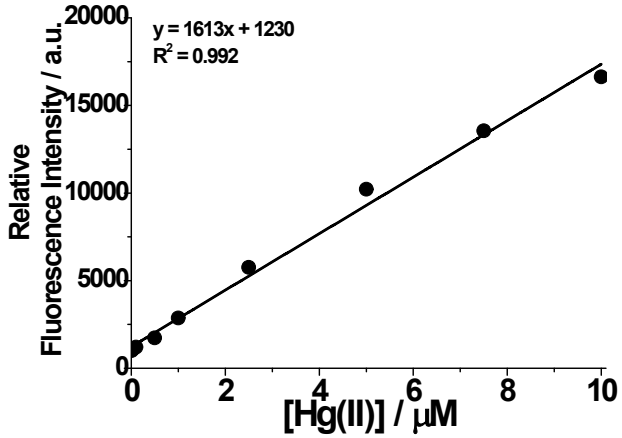


Figure S8. A linear relationship between fluorescence intensity at 510 nm and concentrations of Hg(II) ions (0.01-10 μM). Fluorescence intensity was measured 2 min after the addition of each Hg(II) ions to AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 °C). Excited at 460 nm. AuNP/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (3 nM) and **1**-PPh_3^+ (1 μM) for 2 hours in HEPES buffer (10 mM. pH 7.4, 5% EtOH, 25 °C) and centrifuging.

(I) Fluorescence "turn-on" response of AuNP/1-PPh_3^+ to different mercury ion species

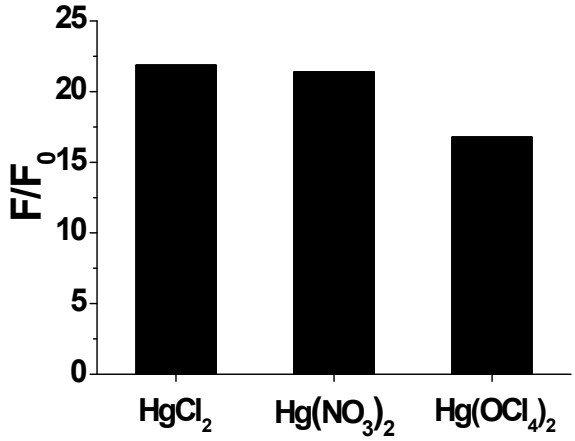


Figure S9. Relative fluorescence turn-on response of AuNP/**1**-PPh_3^+ adsorbate toward 50 μM $HgCl_2$, $Hg(NO_3)_2$, and $Hg(OCl_4)_2$. Excited at 460 nm. Fluorescence intensity at 510 nm was measured 2 min after the addition of each Hg(II) ions to AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer (50 mM, pH 8.0, 25 °C). F_0 and F correspond to the fluorescence intensity of AuNP/**1**-PPh_3^+ adsorbate in the absence and the presence of Hg(II) ions. AuNP/**1**-PPh_3^+ adsorbate was prepared by mixing AuNPs (3 nM) and **1**-PPh_3^+ (1 μM) for 2 hours in HEPES buffer (10 mM. pH 7.4, 5% EtOH, 25 °C) and centrifuging.

(J) Application

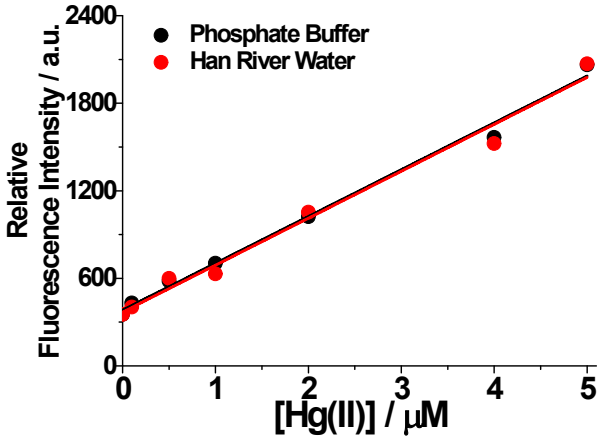


Figure S10. Comparison of fluorometric assay between Han river water samples and phosphate buffer. Fluorescence intensity at 510 nm was measured 2 min after the addition of Hg(II) ions (0, 0.1, 0.5, 1, 2, 4, 5 μM) to AuNP/**1**-PPh_3^+ adsorbate in phosphate buffer and Han river water, respectively. Excited at 460 nm.

(K) Tight Interactions of citrate-capped AuNPs and 1-PPh_3^+ in AuNP/1-PPh_3^+ adsorbate

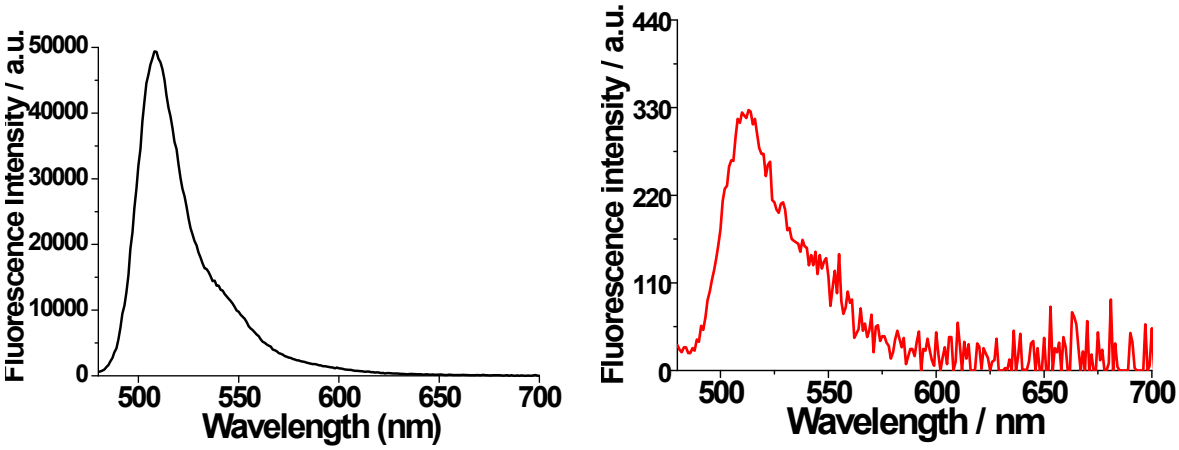


Figure S11. Comparison of emission spectra of (left) **1**-PPh_3^+ (1 μM) and (right) the supernatant taken from a dispersion of AuNP/**1**-PPh_3^+ adsorbate after centrifugation (phosphate buffer, pH 8.0, 25 °C).

(L) TEM image of citrate-capped gold nanoparticles

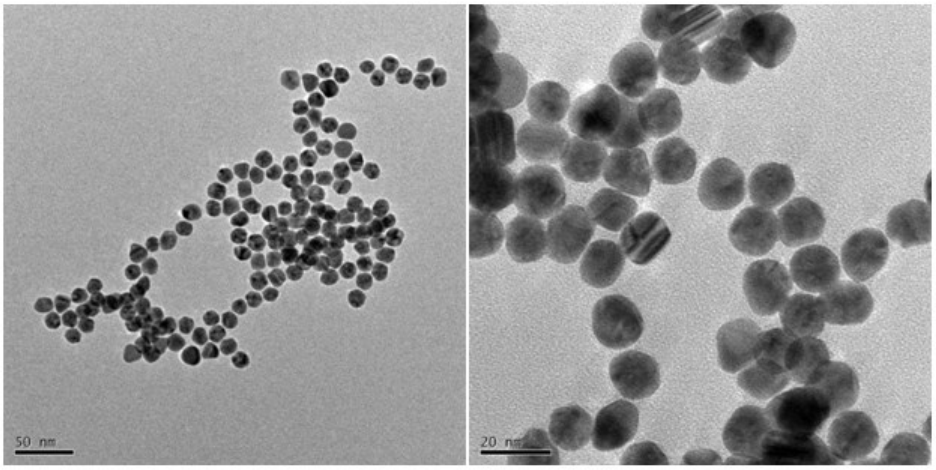
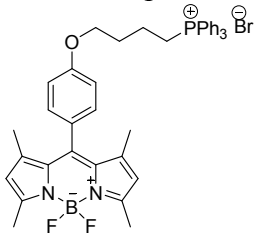
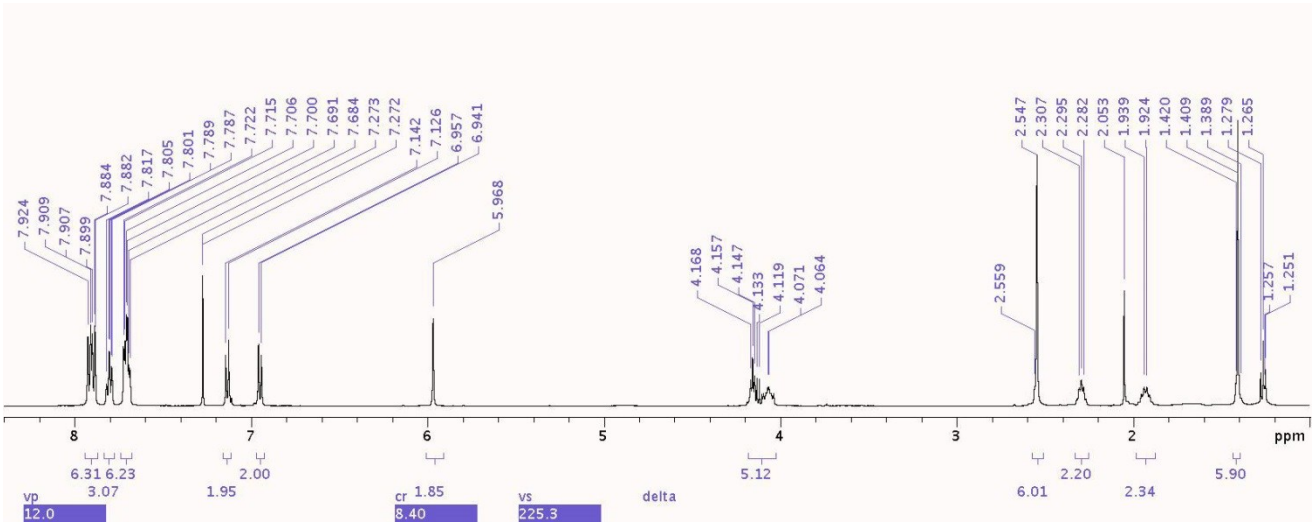


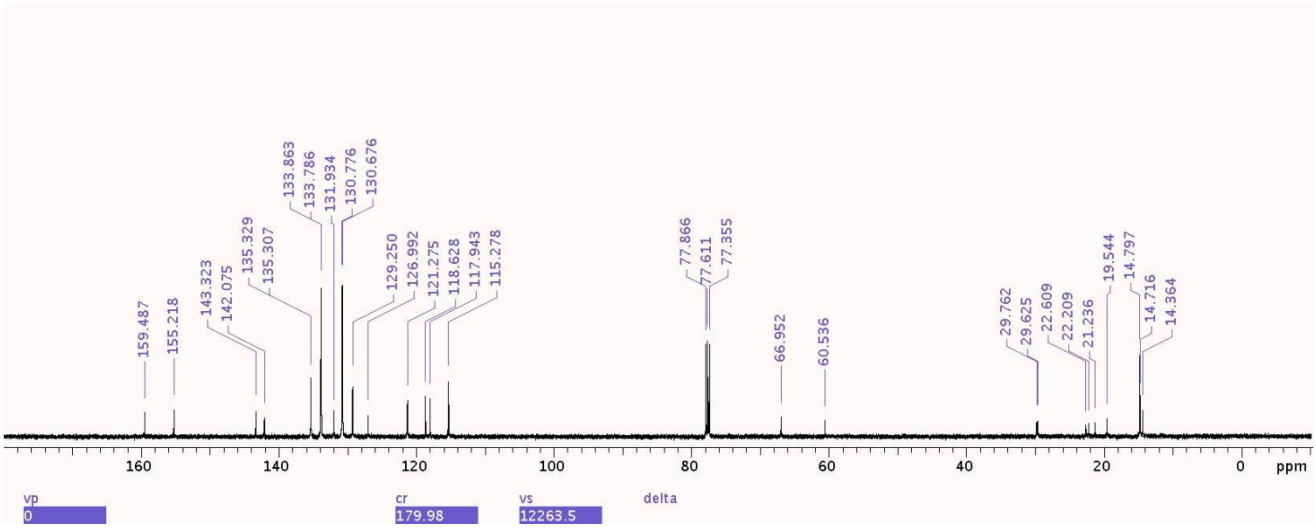
Figure S12. TEM images of gold nanoparticles

^{1}H-NMR Spectrum of **1**-PPh_3^+ in $CDCl_3$ (500 MHz):





^{13}C-NMR Spectrum of **1**-PPh_3^+ in $CDCl_3$ (125 MHz):



References

1. T.-I. Kim, J. Park and Y. Kim, *Chem.-Eur. J.,* 2011, **17**, 11978.
2. R. F. Kubin and A. N. Fletcher, *J. Luminescence,* 1982, **27**, 455.
3. X. Chen, Y. Zu, H. Xie, A.M. Kemasa and Z. Gao, *Analyst*, 2011, **136**, 1690.