Fluorescent or not? Size-dependent fluorescence switching for polymer-stabilized gold clusters in the 1.1–1.7 nm size range†‡

Nicolas Schaeffer,[a] Bien Tan,[a] Calum Dickinson,[a] Matthew J. Rosseinsky,[a] Anna Laromaine,[b] David W. McComb,[b] Molly M. Stevens,[b] Yiqian Wang,[b] Laure Petit,[c] Catherine Barentin,[c] David G. Spiller,[d] Andrew I. Cooper*[a] and Raphaël Lévy*[d]

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The synthesis of fluorescent water-soluble gold nanoparticles by the reduction of a gold salt in the presence of a designed polymer ligand is described, the size and fluorescence of the particles being controlled by the polymer to gold ratio; the most fluorescent nanomaterial has a 3% quantum yield, a 1.1 nm gold core and a 6.9 nm hydrodynamic radius.

Nanoparticles have promising applications in optoelectronics, biological sensing devices, and as building blocks for biologically-driven self-assembly of nanostructures.[1,2] Recently, the synthesis of small metal nanoparticles that exhibit a strong visible or near-infrared fluorescence (in comparison with the bulk metal[3]) has been reported.[4] Syntheses in organic solvents include direct synthesis of small nanoparticles[5] and formation of clusters through etching the surface of a larger preformed particle.[6] Fluorescent tiopronin- and glutathione-protected gold nanocrystals have also been prepared.[7] Zheng *et al.* reported the formation of water-soluble gold clusters with an intense blue fluorescence and a 41 ± 5% quantum efficiency using PAMAM dendrimers as stabilizers.[8] The latter example is complicated by the fact that the dendrimer itself is fluorescent in the absence of gold.[9] In a previous study, we have described the one-step synthesis of monodisperse water-soluble, polymer-stabilized gold nanoparticles.[10,11] The particle size could be tuned between 1.9 nm and 5 nm by changing the polymer backbone structure, the nature of the polymer end-group, and the relative amount of gold precursor *versus* polymer.[10,11] Among all the polymers studied, pentaerythritol tetrakis(3-mercaptopropionate)-terminated polymethacrylic acid (PTMP-pMAA), was found to produce the smallest and most monodisperse gold nanoclusters.

Here, using a higher molecular weight PTMP-pMAA, we extend the size-range tunability to the sub 2 nm range and demonstrate a transition between non-fluorescent and fluorescent nanoparticles. The most fluorescent nanomaterial has a 3% quantum yield, a 1.1 nm gold core and a 6.9 nm hydrodynamic radius.

The polymer was synthesized by chain transfer mediated polymerization as described previously.[11] It comprises a linear chain of water-soluble methacrylic acid units and a hydrophobic end group consisting of, on average, three unreacted thiols and one thioether linkage.[11] A somewhat higher molecular weight was targeted in order to counterbalance the hydrophobicity of the end-group and thus to obtain higher overall solubility in water. Increasing the solubility allows a lower gold-to-ligand ratio to be achieved and should therefore lead to the formation of smaller nanoparticles. The relative amount of PTMP transfer agent with respect to methacrylic acid (MAA) monomer (1 : 50 mmol/mmol) was decreased compared to the synthesis described previously (see ESI‡).[11] ^{1}H NMR spectroscopy and mass spectroscopy data (ESI) showed that most polymer chains are formed with a single thioether link to the PTMP unit—that is, with three pendant unreacted thiols per end group.[12] Gel permeation chromatography (GPC) data (Fig. S1‡) indicated that the average polymer molecular weight was significantly higher than in our previous studies and that the molecular weight distribution was narrow (M_n = 6359 g mol^{-1}; M_w = 7524 g mol^{-1}; PDI = 1.2).[11] This PTMP-pMAA ligand was then used as a "multi-dentate" capping agent to control the growth of the gold nanoparticles. In a typical experiment, 25 μL of aqueous 20 mM $HAuCl_4$ solution was added to a 1 mL aqueous polymer solution and left for two hours. The final reduction step was achieved by adding 100 μL of a freshly prepared 5 mM $NaBH_4$ aqueous solution. The nanoparticles were allowed to form and, after 24 h, the unreacted polymer was removed by preparative-scale size exclusion chromatography (see ESI for details‡).

A change in the colour of the resulting nanoparticle solution was observed from red to pale yellow over a polymer concentration range of 0.05–5 mM (Fig. 1a, upper part) indicating that particles of different sizes are formed. Colourless solutions were formed at polymer concentrations above about 5 mM, most likely due to the formation of soluble gold(I) complexes.[13] UV-visible absorption spectra showed that solutions prepared with a polymer concentration of less than 0.2 mM (relatively large nanoparticles) exhibited a weak plasmon band at 520 nm, whereas solutions prepared using a higher polymer concentration (small nanoparticles) did not

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[a] *The University of Liverpool, Department of Chemistry, Crown Street, Liverpool, UK L69 3BX. E-mail: aicooper@liverpool.ac.uk*
[b] *Imperial College, Department of Materials, Exhibition Road, London, UK SW7 2AZ*
[c] *Université Claude Bernard Lyon 1, Laboratoire de Physique de la Matière Condensée et Nanostructures, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France*
[d] *The University of Liverpool, School of Biological Sciences, Crown Street, Liverpool, UK L69 3BX. E-mail: rapha@liverpool.ac.uk*

† This paper is dedicated to Professor Andrew B. Holmes FRS on the occasion of his 65th birthday.

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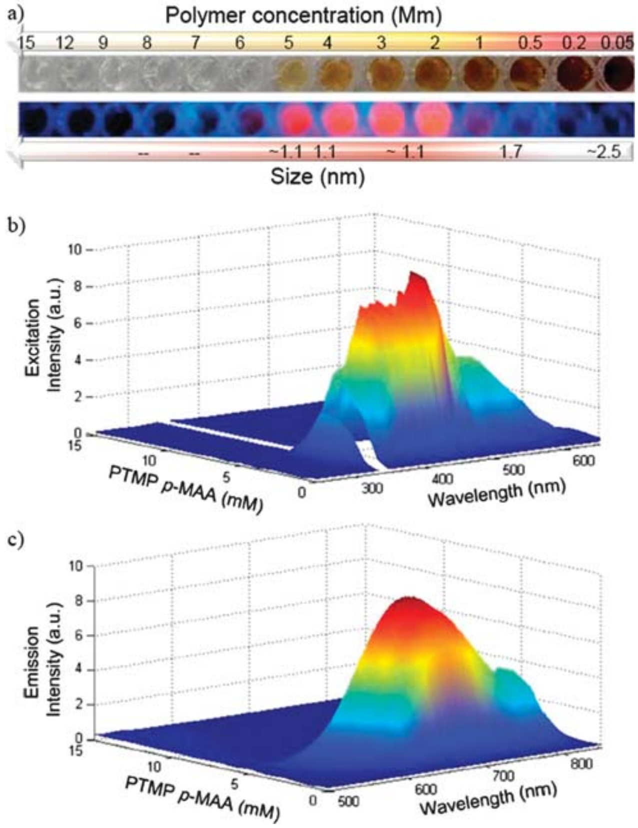

Fig. 1 (a) Colour images of gold suspensions prepared with a fixed gold concentration (0.5 mM) and various ligand concentrations under daylight (upper part) and under UV-visible light at 345 nm (lower part). (b) Excitation spectra (emission at 695 nm) of gold suspensions against ligand concentration (for a fixed concentration of 0.5 mM gold). An experimental artefact (between 340 and 360 nm) was intentionally deleted. (c) Emission spectra (excitation at 450 nm) of gold suspensions against ligand concentration (for a fixed concentration of 0.5 mM gold).

show a plasmon band (Fig. S2‡) indicating an average particle diameter below 3 nm.[14] The gold nanoparticles prepared using a 0.5 mM gold and a 1 to 6 mM polymer concentrations exhibited visible red fluorescence when excited under UV light, as shown in Fig. 1a (lower part). Fluorescence (excitation and emission) spectra were recorded on all samples; this revealed a broad excitation band centred at 450 nm and a broad emission band at 700–750 nm (Fig. 1b and c). The magnitudes of the emission and excitation bands were strongly dependent on the polymer concentration used in the synthesis, and therefore, on the particle size, with maximum intensities observed for a polymer concentration of 4 mM. Although the photophysical mechanism leading to such optical properties is not yet completely understood, it is thought to be induced by the radiative recombination of Fermi level electrons and sp- or d-band holes.[15] Such effect is size dependent and occurs when the size of the nanoparticles is decreased and the separation of the valence energy levels is sufficiently large.[16] The excitation spectra exhibit fine structure and the emission spectra show a sharp secondary peak at 800 nm. These characteristics were observed reproducibly for excitation and emission spectra recorded for batches of nanoparticles obtained from independent syntheses. Measurement of quantum efficiency was carried out for the most highly fluorescent nanoparticle solutions (4 mM PTMP-pMAA–0.5 mM $HAuCl_4$). Tris(2,2′-bipyridyl) ruthenium hexahydrate(II) was used as a standard[17] and the quantum efficiency was found to be 3% (see ESI‡).

To test whether the observed fluorescence might be due to the polymeric ligand, excitation and emission spectra were recorded for PTMP-pMAA in aqueous solutions at all of the concentrations that were used to synthesize the particles. The same fluorescence measurements were repeated for PTMP-pMAA solutions which were oxidized (by adding 100 μL of a freshly prepared 50 mM $NaClO_4$ aqueous solution to a 1 mL PTMP-pMAA solution) and on solutions which were reduced (by adding 100 μL of a freshly prepared 50 mM $NaBH_4$ aqueous solution to a 1 mL polymer solution). No fluorescence was observed in any of these control experiments, thus showing that neither the polymer itself nor possible oxidative or reductive by-products from this ligand were responsible for the fluorescence. Size exclusion chromatography of the gold nanoparticle sample synthesized using a 4 mM polymer concentration resulted in the separation of two distinct species. The first species to elute was yellow and exhibited fluorescence when excited by UV-visible light (peak at 9 min elution time, Fig. 2). Elemental analysis of the fractions show that gold concentration correlates with fluorescence intensity. The presence of sulfur, which is attributed to the PTMP-pMAA ligand, results in two peaks, the first corresponding to the polymer stabilizing the gold particles and the second to the excess polymer (peaks at 9 min and 40 min elution time,

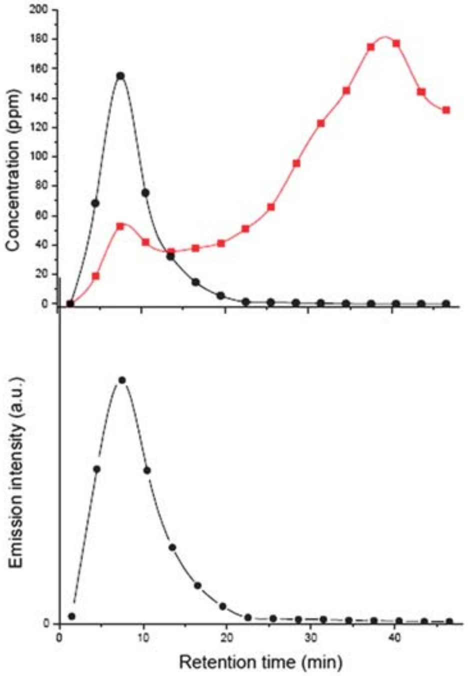

Fig. 2 Size exclusion chromatogram (Sephadex G25) of polymer-capped gold nanoparticles (4 mM PTMP-pMAA and 0.5 mM $HAuCl_4$). (a) Gold (black) and sulfur (red) concentrations determined by ICP-AES (inductively coupled plasma-atomic emission spectrometry). (b) Fluorescence emission intensity (excitation and emission wavelength are 485 nm and 645 nm respectively).

respectively, Fig. 2). Taken together, these experiments clearly demonstrate that the fluorescence observed cannot be attributed to the polymer ligand itself.

The fluorescence of gold(I) complexes has been previously reported.[18] To elucidate whether the observed fluorescence could be ascribed to a Au(I) complex, a series of solutions containing PTMP-pMAA and $HAuCl_4$ in different ratios was prepared. These solutions were identical to those used for nanoparticle synthesis except that no reducing agent was added. All of the solutions were pale yellow after addition of the gold salt and turned colourless rapidly after polymer addition, indicating that gold(III) had been reduced to gold(I) by the polymer ligand.[13] Emission and excitation spectra were recorded for these solutions and no fluorescence was detected. The fluorescent samples were further characterized by HR-TEM revealing the presence of gold nanoparticles with average diameters of approximately 1.1 nm (Fig. 3 and S5a‡). The particles appeared to be spherical and exhibited a narrow size distribution (±0.2 nm). A non-fluorescent sample (0.5 mM PTMP-pMAA–0.5 mM $HAuCl_4$) was also imaged by HR-TEM and nanoparticles of an average diameter of around 1.7 nm were observed (Fig. S5b‡). In addition, one of the colourless non-fluorescent samples (8 mM PTMP-pMAA–0.5 mM $HAuCl_4$) was also analyzed by HR-TEM and no nanoparticles could be imaged.

A film of fluorescent nanoparticles was prepared and imaged by confocal microscopy. Upon intense illumination, photobleaching was observed (Fig. S6‡). To our knowledge, this is the first observation of photobleaching of gold nanoparticles, and it could be due for example to heat-induced structural changes in the particle—indeed, gold nanoparticles with diameters less than 5 nm have melting temperatures that are much lower than bulk gold (~300–500 °C *versus* 1063 °C) and this has been used in laser curing of printed gold nanoparticle inks.[19] Building on this observation, fluorescence recovery after photobleaching (FRAP) in a capillary was used to measure the hydrodynamic radius of the fluorescent species. A hydrodynamic radius of 3.5 nm was measured for the most fluorescent nanoparticle solution corresponding to a polymer layer thickness of 2.9 nm.

Drastic changes in the electronic behaviour of gold nanoparticles have been reported previously when the average core size was decreased from 1.4 to 1.2 nm, the larger clusters being in a metallic state and the smaller clusters in an insulating molecular state.[20] The appearance of fluorescence in our samples occurs in a similar size regime.

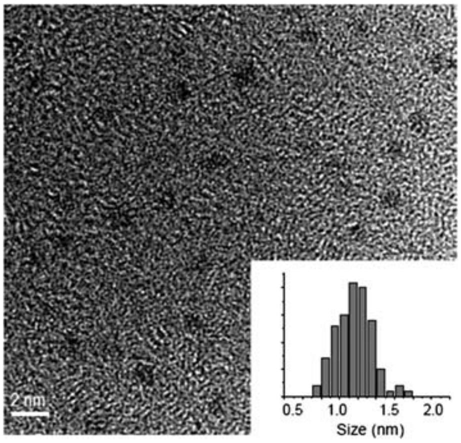

Fig. 3 Representative HR-TEM picture and particle size histogram of sizes of approximately 1.1 nm gold nanoparticles (4 mM PTMP-pMAA and 0.5 mM $HAuCl_4$).

In summary, a simple protocol is reported for the synthesis and purification of fluorescent polymer-capped gold nanoparticles. By varying systematically the polymer to gold ratio, the size of the nanoparticles can be finely tuned and a transition from non-fluorescent to fluorescent nanoparticles is observed for core diameters between 1.7 nm and 1.1 nm.

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