Luminescent nanobeads: attachment of surface reactive Eu(III) complexes to gold nanoparticles†

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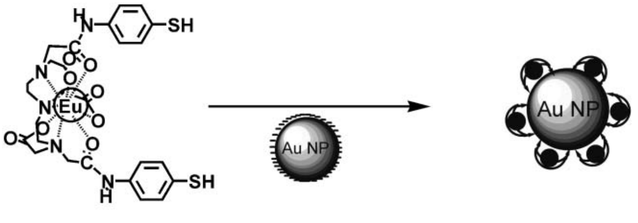
Gold nanoparticles were used as a scaffold to assemble multiple tailor-made europium(III) complexes yielding water-soluble gold nanoparticles that display red, Eu^{III}, luminescence.

Fluorescence detection is a popular method to achieve high sensitivity in bioanalytical applications.[1–4] However, single molecule luminescent labels have limited efficiency and cannot probe multiple interactions. It is a challenge for synthetic chemists to introduce multiple fluorescent labels on a reporter group without engaging in multiple step syntheses, for example, in the preparation of dendritic labels. Gold nanoparticles[5] provide an attractive scaffold for the attachment of multiple fluorescent labels.[6–8] Although gold colloids have been extensively used in colorimetric detection of biomolecules[3,9] their fluorescence applications are less well explored.[8,10,11] Lanthanide lumophores circumvent the problems associated with organic fluorescence in biological samples, due to their intrinsic photophysical properties[12] which include visible, narrow band-width luminescence with long lifetimes and large Stokes shifts. Detection of lanthanide luminescence in biological systems is easily achieved by introducing time-gating of the luminescent signal to eliminate short-lived background fluorescence. Luminescent lanthanide(III) ions have been incorporated into nanosized materials either through doping of metals[13–15] or through coating of materials such as polystyrene or latex.[16,17] We have been interested in ligand design for development of luminescent lanthanide complexes.[18,19]

In this paper we introduce the assembly of water-soluble multilabels utilising gold nanoparticles (Au-NPs) as an inert and stable scaffold on which to assemble tailor-made luminescent lanthanide labels. To anchor a luminescent complex on a surface we chose a ligand **H_3L** with two surface reactive sites to ensure a strong affinity coupled with a rigid structural framework, based on diethylene triamine pentaacetic acid (H_5DTPA) (Scheme 1). The design of **H_3L** incorporates a hard O and N binding core that gives thermodynamically stable ($\log K \approx 15$)[20] neutral lanthanide complexes, soluble in polar solvents. The pendant arms incorporate built-in phenylamide sensitisers for lanthanide luminescence with thiol groups that can bind to soft transition metals. **H_3L** was prepared by the simple condensation reaction of 4-aminothiophenol with DTPA-bis(anhydride). The formation of disulfide species was detected by ^1H NMR during ligand isolation. Reduction by hydrazine afforded the pure ligand, **H_3L**, which has been fully characterised (Supporting Information). A signature for the product formation is the appearance in the ^1H NMR spectrum of the characteristic shifts of the phenyl protons at 7.1 and 7.4 ppm (AB spin system), which integrate accordingly to the protons of the aliphatic backbone. The ligand formulation is confirmed by a peak in the ES(+) mass spectrum at 608 Da, assigned to $\{M + H\}^+$ and the elemental analysis data. The europium(III) complex (Scheme 1) was accessed through the tripotassium salt of **H_3L** by reaction of 1 : 1 **K_3L** with $EuCl_3{\cdot}6H_2O$ in water. Electrospray mass spectrometry of **EuL** shows a peak at 758 Da with a characteristic isotope pattern, which is attributed to $\{M + H\}^+$ species. The UV-vis absorption spectrum shows a peak at 266 nm, assigned to the π–π^* transition of the phenylamide groups. Methanolic solutions of **EuL** show the characteristic transitions $^5D_0 \rightarrow {}^7F_J$ where $J = 0$, 1, 2, 3, 4 at 580, 590, 613, 650 and 694 nm with a luminescence lifetime of 0.8 ms.

We used the citrate reduction of $HAuCl_4$[21] to produce water-soluble gold colloids that can be capped with our polar lanthanide complex, **EuL**, *via* displacement of the citrate shell with thiols.[22] Transmission electron microscopy (TEM) indicated a particle diameter of 10–15 nm with spherical shape for the citrate-capped clusters, consistent with the synthetic method employed. We estimated a concentration of nanoparticles of 9.4 nM using an average of 13 nm diameter per nanoparticle[23] (see Supplementary Info). The binding of **EuL** to citrate-stabilised nanoparticles was probed by UV-vis spectroscopy (Fig. 1).

The citrate stabilised Au-NPs show a broad absorption with a maximum at 520 nm, corresponding to the surface plasmon resonance (SPR).[24] Upon titration of aliquots of a methanolic solution of **EuL** (10 mM) into a solution of nanoparticles (estimated 0.2 nM in water), an increase in the intensity of the SPR band was observed, accompanied by a bathochromic shift of 7 nm. A control experiment, under the same conditions, using a lanthanide complex with no thiol groups, did not lead to any change of the SPR band (Supplementary Info). Likewise, the addition of methanol did not produce shifts in the SPR, proving



EuL

Scheme 1

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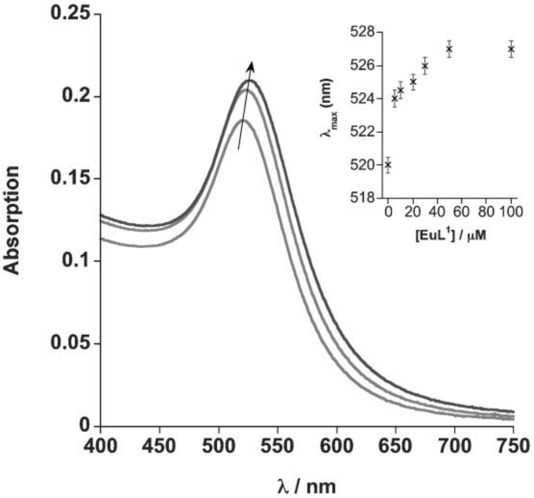

Fig. 1 UV-vis absorption spectrum of 0.2 nM aqueous citrate stabilised Au colloid, after additions of 12.5 and 125 nmol **EuL**. Inset: Plot of λ_{max} *vs.* [**EuL**].

the effect of **EuL** binding has no solvatochromic contribution. We attribute the changes in the UV-vis spectrum to a change in the surface dielectric constant of the nanoparticles, associated with adsorption of the thiol groups of **EuL** to the Au-NP surface atoms. This is consistent with previous reports of spectral SPR shifts upon adsorption of thiols on citrate-stabilised nanoparticles.[25,26] A plot of the position of λ_{max} of the SPR band *vs.* **[EuL]** (inset Fig. 1) shows a plateau which is indicative of the binding event leading to saturation of the nanoparticle surface.

To examine the size of nanoparticles following coating with **EuL** TEM was employed. The results show that **EuL** capped nanoparticles are monodisperse, unaggregated and spherical in shape with a diameter of 10–15 nm (Fig. 2).

Size exclusion chromatography of the Au-NPs[27,28] on Sephadex G-15 was used to remove any excess of **EuL** that could interfere with luminescence properties of the capped **EuL** Au-NPs. The functionalised nanoparticles rapidly elute off the column with the water solvent front as a tight, red band. Excess **EuL** is retarded by the gel. It is interesting to note that nanoparticles functionalised with **EuL** did not adsorb to the stationary phase, whereas citrate-stabilised Au-NPs were immobilised at the top of the column. The absorption spectrum post-column is identical to that observed pre-column (Supporting Information).

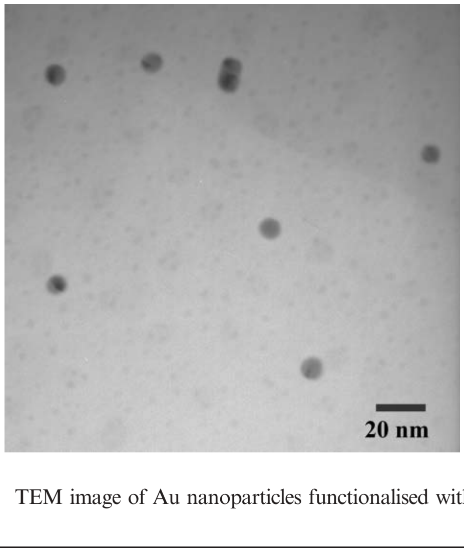

Fig. 2 TEM image of Au nanoparticles functionalised with **EuL**.

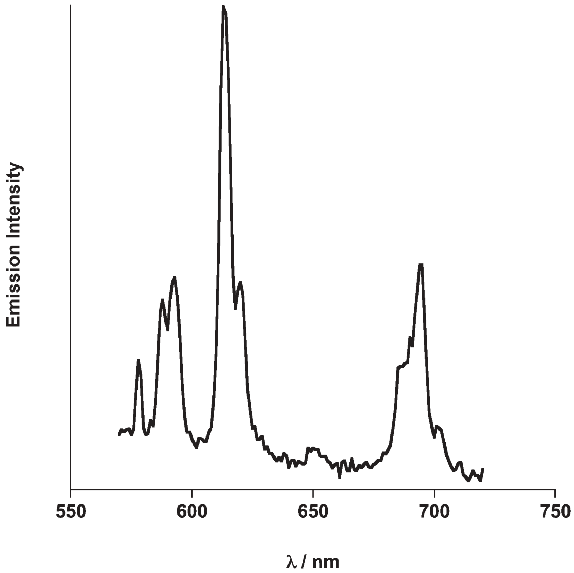

Fig. 3 Luminescence spectrum of **EuL** capped gold nanoparticles following gel filtration chromatography (λ_{exc} = 266 nm).

The purified Eu^{III} coated nanoparticles exhibit the characteristic *f–f* luminescence, upon excitation of the ligand-based absorption band at 266 nm (Fig. 3) or directly at the 394 nm *f–f* $^7F_0 \rightarrow {}^5L_6$ absorption band.[29] The relative emission intensity of the two strongest peaks at 590 and 580 nm is estimated to be 2.2 by calculation of the integrated area of the $^5D_0 \rightarrow {}^7F_2$ to $^5D_0 \rightarrow {}^7F_1$ transitions. Indirect population of the Eu^{III} state by excitation at 266 nm into the ligand $^1\pi\pi^*$ band followed by sensitisation of the lanthanide luminescent state *via* the $^3\pi\pi^*$ state of the ligand gives stronger luminescence than excitation at 394 nm due to the low absorption cross-section of the latter band, arising from the forbidden nature of the *f–f* transitions. The luminescence signal of the **EuL**-AuNP is quenched as indicated by luminescence lifetime measurements, where a single exponential fit of the signal leads to a luminescence lifetime, τ, of 0.8 μs. It has been previously reported that chromophore emission is quenched by deactivating pathways involving the excited states of the metal surface.[7] However, the appearance of the Eu^{III} signal indicates that the quenching of the *f–f* transitions is not as dramatic as one would expect for an organic fluorophore. The estimated distance of the Eu^{III} lumophore is around 1 nm from the gold surface. Previous studies that explore the correlation between chromophore distance and nanoparticle quenching of the excited states are based on fluorophores with singlet excited states[7] with one exception, where silver nanoparticles have been reported to act as both an enhancer and a quencher of Eu^{III} emission.[30]

In summary, we have synthesised water soluble Eu^{III} capped gold nanoparticles that display red luminescence. Their biological applications are currently being explored together with the development of multiple-colour luminescent nanoparticles.

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Notes and references

1 J. Miller, *Analyst*, 2005, **130**, 265.
2 X. Zhao, L. Hilliard, S. Mechery, Y. Wang, R. Bagwe, S. Jin and W. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 15027.
3 S. G. Penn, L. He and M. J. Natan, *Curr. Opin. Chem. Biol.*, 2003, **7**, 609.
4 M. Powers, S. Koev, A. Schleunitz, H. Yi, V. Hodzic, W. Bentley, G. Payne, G. Rubloff and R. Ghodssi, *Lab Chip*, 2005, **5**, 583.
5 M. C. Daniel and D. Astruc, *Chem. Rev.*, 2004, **104**, 293.
6 F. Stellacci, C. A. Bauer, T. Meyer-Friedrichsen, W. Wenseleers, S. R. Marder and J. W. Perry, *J. Am. Chem. Soc.*, 2003, **125**, 328.
7 K. G. Thomas and P. V. Kamat, *Acc. Chem. Res.*, 2003, **36**, 888.
8 R. D. Powell, C. M. R. Halsey, D. L. Spector, S. L. Kaurin, J. McCann and J. F. Hainfeld, *J. Histochem. Cytochem.*, 1997, **45**, 947.
9 R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, *Science*, 1997, 1078.
10 H. Wang, T. B. Huff, D. A. Zweifel, W. He, P. S. Low, A. Wei and J. X. Cheng, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 15752.
11 D. J. Maxwell, J. R. Taylor and S. Nie, *J. Am. Chem. Soc.*, 2002, **124**, 9606.
12 J.-C. G. Bunzli, *Lanthanide Probes in Life, Chemical and Earth Sciences*, Elsevier, Amsterdam, 1989.
13 D. A. Chengelis, A. M. Yingling, P. D. Badger, C. M. Shade and S. Petoud, *J. Am. Chem. Soc.*, 2005, **127**, 16752.
14 G. A. Hebbink, J. W. Stouwdam, D. N. Reinhoudt and F. C. J. M. van Veggel, *Adv. Mater.*, 2002, **14**, 1147.
15 C. Tiseanu, R. K. Mehra, R. Kho and M. Kumke, *J. Phys. Chem. B*, 2003, **107**, 12153.
16 P. Huhtinen, J. Vaarno, T. Soukka, T. Lovgren and H. Harma, *Nanotechnology*, 2004, **15**, 1708.
17 V. Vaisanen, H. Harma, H. Lilja and A. Bjartell, *Luminescence*, 2000, **15**, 389.
18 A. P. Bassett, S. W. Magennis, P. B. Glover, D. J. Lewis, N. Spencer, S. Parsons, R. M. Williams, L. De Cola and Z. Pikramenou, *J. Am. Chem. Soc.*, 2004, **126**, 9413.
19 P. B. Glover, P. R. Ashton, L. J. Childs, A. Rodger, M. Kercher, R. M. Williams, L. De Cola and Z. Pikramenou, *J. Am. Chem. Soc.*, 2003, **125**, 9918.
20 C. F. G. C. Geraldes, A. M. Urbano, M. C. Alpoim, A. D. Sherry, K.-T. Kuan, R. Rajagopalan, F. Maton and R. N. Muller, *Magn. Reson. Imaging*, 1995, **13**, 401.
21 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.
22 J. H. Fendler, *Chem. Mater.*, 2001, **13**, 3196.
23 R. L. Johnston, *Atomic and Molecular Clusters*, Taylor & Francis, London, 2002.
24 S. Link and M. A. El-Sayed, *J. Phys. Chem. B*, 1999, **103**, 8410.
25 P. Mulvaney, *Stud. Surf. Sci. Catal.*, 1997, **103**, 99.
26 C. Mangeney, F. Ferrage, I. Aujard, V. Marchi-Artzner, L. Jullien, O. Ouari, E. D. Rekai, A. Laschewsky, I. Vikholm and J. W. Sadowski, *J. Am. Chem. Soc.*, 2002, **124**, 5811.
27 G. T. Wei, F. K. Liu and C. R. Chris Wang, *Anal. Chem.*, 1999, **71**, 2085.
28 G. T. Wei and F. K. Liu, *J. Chromatogr., A*, 1999, **836**, 253.
29 W. T. Carnall, P. R. Fields and K. Rajnak, *J. Chem. Phys.*, 1968, **49**, 4450.
30 H. Nabika and S. Deki, *J. Phys. Chem. B*, 2003, **107**, 9161.