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Thioalkylated tetraethylene glycol: a new ligand for water soluble monolayer protected gold clusters

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Ligand-stabilised, water-soluble gold nanoparticles of two different size ranges (2–4 and 5–8 nm) are readily prepared using monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol as a novel capping agent. These nanoparticles are as stable as alkylthiol-capped monolayer protected clusters (MPCs) and do not aggregate from aqueous solution under a wide range of stringent conditions. It is expected that this new material will be useful for a number of bio-analytical applications.

Monolayer protected clusters (MPCs) are nanometre-sized metal crystallites, which are stabilised by a covalently attached shell of ligand molecules.[1–4] MPCs of gold can be prepared following simple standard procedures that normally involve the wet-chemical reduction of a gold salt in the presence of a stabilising thiol ligand usually within a two-phase liquid–liquid system.[1–4] Owing to their extraordinary stability thiol-capped gold MPCs have in recent years facilitated a broad range of fundamental and applied studies, which would have been either exceedingly difficult, or impossible using alternative, less stable cluster preparations.[4–9] Potential applications of gold MPCs are currently being explored in many different areas including electronics,[10,11] catalysis,[12] molecular recognition,[13] gene delivery[14] and sensor science.[15] A particularly promising area for the development of new applications of MPCs is at the life-science/nanotechnology interface, since MPCs are roughly the size of small proteins, can be designed to carry almost any chemical functionality by ligand exchange reactions[16] and are optically detectable, sometimes down to the single particle level, due to their excellent light scattering properties.[17]

While MPCs are usually insoluble in water, most biological or biomedical applications require that the clusters readily dissolve in aqueous media and do not aggregate non-specifically due to electrostatic interactions. The latter is particularly critical in solutions containing charged macromolecules such as proteins. Surprisingly, these restrictions are in reality quite severe and have only been addressed recently by several groups.[18–24] Stable, water soluble gold MPCs protected by thiolated poly(ethylene glycol) (PEG-SH) of a molecular weight of 5000 have been reported by Murray and co-workers.[19] These particles showed enhanced chemical and thermal stability as well as ionic conductivity upon dissolution of a suitable electrolyte within the ligand shell. Unlike usual gold MPCs, they could, however, not be functionalised *via* ligand exchange reactions since the stabilisation by the polymeric ligand shell was found to be too effective for such reactions to occur. The same research group then developed water soluble, tiopronin (*N*-2-mercaptopropionylglycine)-capped gold MPCs, which can be readily functionalised.[20] Gittins and Caruso reported two different methods of transferring metal nanoparticles from their organic solvent into water.[21,22] The first uses non-covalent stabilisation by 4-dimethylaminopyridine (DMAP)[21] and the second covalent post-synthesis modification by various thiols containing a range of hydrophilic groups.[22] The Caruso/Gittins protocols and Murray's tiopronin method lead to high concentrations of clusters in aqueous solutions but the particles are still susceptible to electrostatic aggregation following changes in pH or ionic strength of the medium. This may, in fact, be a desirable property if one wishes to construct nanostructured solids or thin films under controllable conditions. Very recently Foos *et al.* reported water soluble gold nanoparticles capped with short chain thiolated poly(ethylene glycol).[23] These particles were amenable to ligand exchange reactions.

Here we report the synthesis of unusually robust, water-soluble gold MPCs, which are capable of further functionalisation *via* ligand exchange reactions and do not aggregate even under extreme pH and ionic strength conditions or in the presence of proteins. These properties are due to stabilisation by a thioalkylated oligo (ethylene glycol) ligand ($HS–(CH_2)_{11}–(O–CH_2CH_2–)_4–OH$), which provides a strongly bound, highly hydrophilic but uncharged shell. The novel and unusual feature of this ligand is that it combines the stability of common alkythiol-capped MPCs with the excellent water solubility of poly- or oligo (ethylene glycol)-capped particles. This is schematically illustrated in Fig. 1. We believe that these properties will be important, in particular for *in-vivo* bio-analytical applications that rely on optical detection of specific particle–particle recognition events or site-specific particle attachment *via* bio-molecular interactions.[17] MPCs of two different size ranges have been prepared; namely 2–4 nm and 5–8 nm. The larger particles were initially stabilised by tetraoctylammonium bromide and were prepared in a water/toluene two-phase system yielding a solution of *ca*. 400 mg Au dm^{-3} in toluene according to a well-established procedure.[25] This solution was separated from the aqueous phase and dried over magnesium sulfate. Then, 5 mg of monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol prepared as described by Pale-Grosdemange *et al.*[26] were dissolved in 2 ml of

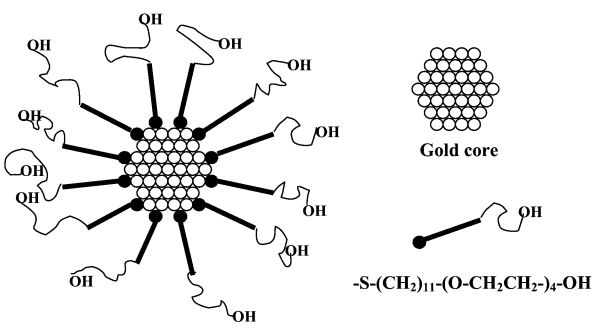

Fig. 1 Schematic representation of a gold nanoparticle protected by a monolayer of monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol. The hydrophobic C_{11}-chain confers extreme stability to the cluster, while the hydrophilic tetraethylene glycol unit ensures solubility in water.

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2-propanol and added to 20 ml of the solution of gold particles. A barely perceptible colour change from wine red towards purple was observed due to the attachment of the thiol group to the surface of the clusters. This was reflected by a slight red shift and broadening of the plasmon absorption band around 525 nm in the UV-vis spectrum. This band is a typical feature of particles larger than *ca*. 3.5 nm. After 3 h 10 ml of water were added and the mixture was shaken vigorously. The organic phase became completely colourless within seconds while the aqueous phase turned from colourless to deep red. This indicates rapid and quantitative transfer of the clusters to the aqueous phase. The aqueous phase was separated and washed three times with diethylether. The particles were further purified by centrifugation at 13,000 rpm followed by decanting of the supernatant solution (×3). They were soluble in water, alcohols, acetone and DMSO and insoluble in chloroform, diethylether, ethylacetate and non-polar organic solvents.

MPCs in the size range of 2–4 nm were prepared following our single-phase reduction method in alcoholic solution.[27] Briefly, 30 mg of hydrogentetrachloroaurate trihydrate (chloroauric acid) were dissolved in a mixture of 50 ml 2-propanol and 0.5 ml concentrated acetic acid (to prevent possible deprotonation of thiols after addition of excess borohydride). 10 mg of monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol was added under stirring and the gold salt was reduced by rapid addition of 5 ml of a freshly prepared 0.5 M solution of sodium borohydride in methanol. The solution turned from colourless to dark brown immediately indicating the formation of particles smaller than *ca*. 3.5 nm. After further stirring for 3 h, the particles were precipitated by pouring the reaction mixture into 200 ml of hexane. The supernatant solution was decanted and the solid product was washed with diethylether and ethylacetate. The material was re-dissolved in water and further purified by centrifugation at 60,000 rpm (×2). The solubility of these particles in various solvents was qualitatively the same as that of the larger particles.

The size of the MPCs prepared by both methods was determined by transmission electron microscopy. For this purpose one drop of a dilute aqueous solution of clusters was allowed to evaporate slowly on a carbon-coated copper mesh grid. Specimens were inspected using a JEOL 2000 EX TEM operating at 200 kV. Fig. 2 shows representative micrographs of both preparations.

MPCs of both size ranges were soluble in water and did not show any signs of aggregation over the wide pH range tested (0–14). Likewise, they remained completely soluble at a salt concentration of 3.5 M NaCl and did not show any colour changes (due to aggregation) in aqueous solutions of bovine serum albumin (BSA). While appearing to be completely inert under these testing conditions, they readily underwent ligand exchange reactions with 1-dodecanethiol. To show this an ethanolic solution (10 ml) containing 3 mg of clusters and 5 mg of 1-dodecanethiol was stirred overnight. The 1-dodecanethiol-capped clusters formed by this process precipitated quantitatively but readily re-dissolved in toluene or chloroform. This shows that our water soluble MPCs will be amenable to functionalisation *via* Murray's ligand exchange route.[16]

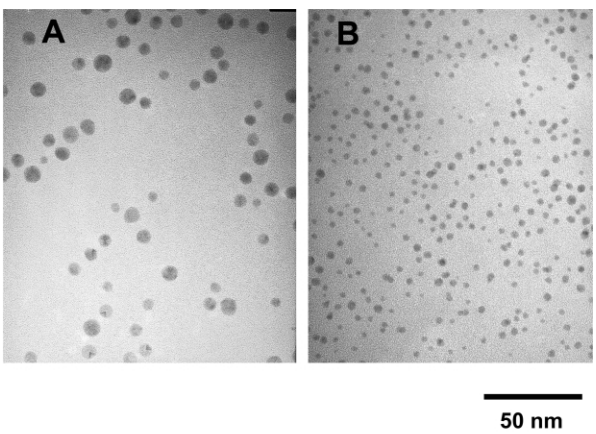

Fig. 2 TEM micrographs of monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol-capped gold nanoparticles in the size range of 4–8 nm (A) and 2–4 nm (B).

In conclusion, it has been shown that the new ligand introduced here facilitates the preparation of highly water soluble MPCs, which do not aggregate or loose stability under any conditions likely to be encountered in bio-analytical applications. The next step is now to carefully introduce a small amount of specific bio-molecular recognition functions *via* partial ligand exchange reactions. Such work is in hand.

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