Nanoparticles

Molecular Recognition by Calix[4]arene-Modified Gold Nanoparticles in Aqueous Solution**

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The concept of exploiting metal clusters for nanotechnology applications dates back to the development of the original Au_{55} cluster by Schmid et al. in 1981.[1] Over the past decade, so-called monolayer protected clusters (MPCs) of gold and, to a lesser extent, silver have been studied extensively owing to their extreme stability and the plethora of tunable properties that are controlled by the particle size and by the ligand chemistry.[2–8] The stability is usually achieved by the use of thiolate ligands, which form a protective shell around the particles to which they are attached by the strong Au–S interaction. Typical sizes of MPCs range from 1 to approximately 40 nm depending on the preparation method used. Since most preparations lead to materials that are insoluble in water a comparatively small number of studies have been carried out in aqueous systems.[9–18] In particular, in view of future bioanalytical applications it is, however, desirable to develop new MPC systems that are not only stable and soluble in water, but also capable of molecular recognition in aqueous systems. Very stable, yet chemically versatile water-soluble MPCs are obtained when a sulfanylalkyl oligo(ethylene glycol) is used as a stabilizing ligand.[16] Herein we report the preparation and chemical properties of 14-nm gold MPCs, which are stabilized in this way and, in addition, carry in their ligand shell calix[4]arene moieties, which confer their specific molecular recognition properties to the particles. Calixarenes are host molecules which can bind cationic guests strongly and with high specificity depending on the size of the calixarene cavity.[19,20] Some of us have recently shown that, in non-aqueous systems, the incorporation of calixarenes in 1.5–4-nm MPCs can enhance their affinity to guest molecules.[20] By themselves, calixarenes are completely insoluble in water, which significantly limits their potential applications as molecular recognition units. The sulfanylalkyl oligo(ethylene glycol) ligands in the system described herein act therefore not only as stabilizers for the MPCs but also as solubilizers for the attached calix[4]arene units. It is thus now possible to demonstrate molecular recognition by an unmodified calixarene cavity in water. The particles prepared have been characterized by transmission electron microscopy (TEM) and by UV/Vis, and NMR spectroscopy. Specific recognition of immobilized cationic pyridinium moieties by the calixarene-modified MPCs in aqueous solution has been demonstrated by a simple color test and by atomic force microscopy (AFM).

The preparation of the calixarene-modified nanoparticles is schematically illustrated in Figure 1. Citrate-stabilized gold

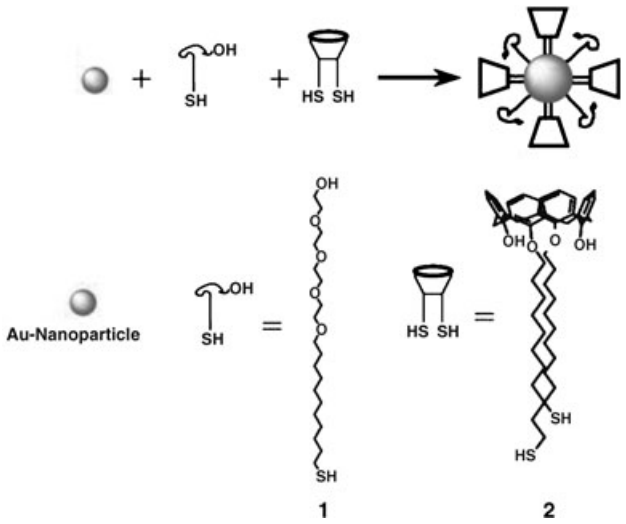

Figure 1. Reaction scheme illustrating the one-step stabilization and functionalization of gold nanoparticles with **1** and **2** carried out in a THF/water mixture.

hydrosols were treated with a 2:1 mixture of the stabilizing ligand (1-sulfanylundec-11-yl) tetraethylene glycol (**1**) and 25,27-bis(11-thio-1-oxyundecan)-26,28-dihydroxycalix[4]arene (**2**) in a water/THF solvent system to directly give the modified particles **3**, which were isolated from excess ligand material by centrifugation. Given the conformation of the relatively rigid **2** it is reasonable to assume that both thiol functionalities of **2** bind to the surface of the same gold particle. Otherwise, cross-linking of particles by bisthiol bridges and precipitation of aggregates could be expected,[21] but this has not been observed in this case. On the contrary, the particles are extremely stable and can be centrifuged, dried, and re-suspended in aqueous solution several times without loss of material. The ruby red suspensions are very robust against non-specific aggregation. For example, unlike most other hydrosols, the particles do not aggregate even in a 2 M sodium chloride solution. They exhibit a characteristic plasmon absorption band in the UV/Vis spectrum at 526 nm and their size is that of the original citrate-stabilized particles (14 ± 1 nm) as confirmed by TEM (Figure 2).

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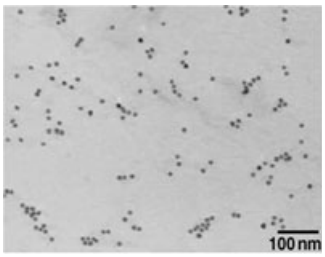

Figure 2. TEM image of the 14-nm gold nanoparticles **3**, that are stabilized and functionalized with **1** and **2**.

The molecular composition of the ligand shell has been studied by ^{1}H NMR spectroscopy (Figure 3). As expected, the spectrum of the particles shows significant peak broadening, in particular of the methylene protons adjacent or close to the sulfur atom that binds to the surface of the particle. This effect is chiefly due to the lack of mobility of the closely packed molecules in the ligand shell, which on the nanometer scale resembles a solid-state material. Several other factors contributing to line broadening in NMR spectra of MPCs have also been suggested, and the phenomenon is still subject to some debate.[5,6,14] Although the broadening of the features impedes a more detailed interpretation, it is apparent that all the main characteristic features of the two different ligands are present in the spectrum of the particles. Importantly, slightly broadened signals between $\delta = 6.6$ and 7.2 ppm, which are characteristic of the aromatic protons in the calixarene cavity are clearly present in the spectrum of the particles. This result indicates the inclusion of **2** in the ligand shell. It can be inferred from previous studies of peptide-stabilized MPCs of the same size that the number of thiol ligands is typically between 800 and 1000 molecules per gold particle.[18] Under the assumption of similar adsorption kinetics for both **1** and **2** it can thus be estimated that each gold particle carries on the order of 300 molecules of **2**. A quantitative analysis of the binding properties of the material will be required to confirm this estimate. Such experiments are underway. The binding properties of a solution-free, water-soluble model calix[4]arene derivative towards *N*-alkyl pyridinium salts in D_2O are given quantitatively in the Supporting Information. The molecular recognition properties of the particle-bound calixarene in water are qualitatively demonstrated by specific binding studies using two different chemically modified substrates (Figure 4). In the simplest case the substrate

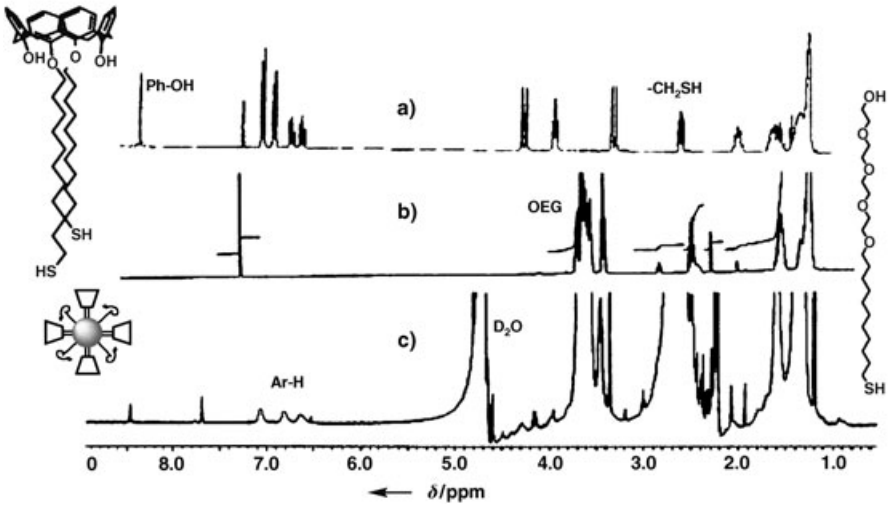

Figure 3. ^{1}H NMR spectra of **2** (a) and **1** (b) in $CDCl_3$, and of **3** (c) in D_2O showing that **1** and **2** have been attached to the gold nanoparticles (the signals of the methylene protons adjacent to the thiol and oligoethylene glycol (OEG) protons are marked). In particular, the signals of the aromatic protons (Ar-H) in spectrum (c) indicate the presence of the calixarene cavity in the ligand shell of the particles.

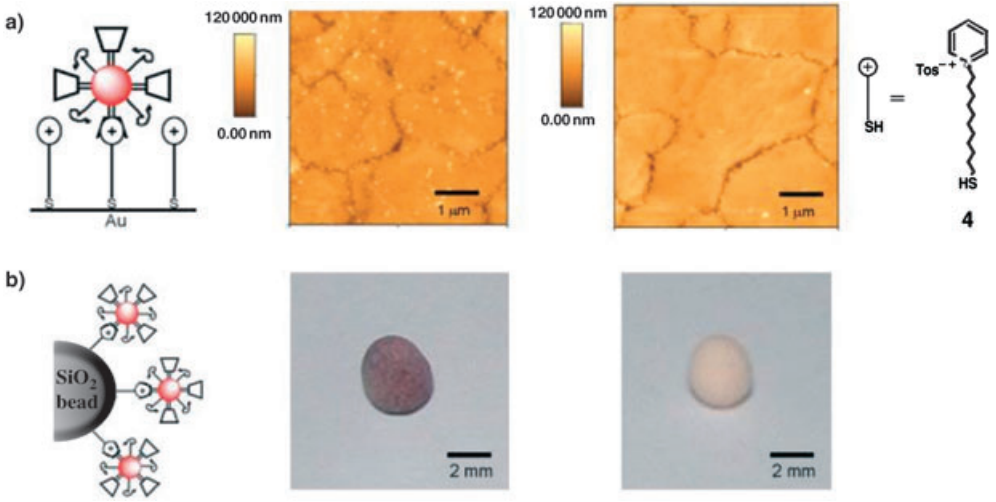

Figure 4. Specific binding of the calixarene-modified gold nanoparticles **3**, from aqueous solution to a self-assembled monolayer (SAM) of the pyridinium ions of **4** (Tos = (4-methylphenyl)sulfonyl) on a gold surface shown by AFM (a) and to a molecular sieve bead primed with **4** (b). The control experiments (right) show the nonspecific attachment of only very few particles to the SAMs (a) and no visible attachment of particles to the molecular sieve beads (b).

was prepared by the nonspecific adsorption of **4** (Figure 4) to white molecular sieve beads. For non-aqueous media it is known that the cationic pyridinium moiety is specifically recognized by calix[4]arenes.[20] The nanoparticles **3** bind specifically from aqueous solution to the molecular sieve beads primed with **4** indicating that the calixarene cavity of **2** exhibits its characteristic cation binding properties also in aqueous media and when immobilized in the ligand shell of the MPCs. This binding interaction is easily observed with the naked eye owing to the intense red color of the gold nanoparticles. A number of control experiments clearly confirmed that both the pyridinium and the calixarene moieties have to be present to achieve any attachment of MPCs to the molecular sieve beads. Further binding studies were carried out by AFM using a self-assembled monolayer (SAM) of **4** on a flat gold surface as the substrate. As demonstrated in the AFM images in Figure 4 a the calixarene-modified nanoparticles **3** were found to bind selectively to this SAM from aqueous solution, while binding to clean gold surfaces occurred only as a non-specific minority event. MPCs without calixarene in their ligand shell did not show any significant binding to the SAMs or to clean gold surfaces.

In conclusion, we have introduced a very simple route for the preparation of water-soluble calixarene-functionalized MPCs of gold. Importantly, it has been demonstrated that in an aqueous environment the calixarene retains its molecular recognition properties, which have been utilized herein to bind the MPCs selectively to chemically modified substrates. The MPCs also act as readily detectable markers of the specific recognition events demonstrating a high potential for simple, color-based diagnostic tests, for example, those required for many routine bioanalytical applications. The preparative method is very general and can readily be adapted to other artificial molecular recognition systems. Particularly attractive is the opportunity demonstrated, to make totally water insoluble recognition systems amenable to aqueous solutions, which again is of interest in the context of bioanalytical applications of MPCs.

Experimental Section

Citrate-stabilized gold nanoparticles of 14 nm diameter were prepared following the classical Turkevich Frens procedure.[22,23] Briefly, an aqueous solution of sodium citrate (10 mL, 17 mM) was added to a boiling aqueous solution of $HAuCl_4$ (180 mL, 0.3 mM), and the reaction mixture was heated under reflux for 30 min. It was then allowed to cool to room temperature, stirred overnight, and filtered before use (0.45 μm millipore filter). **1** was synthesized as described in [24]. **2** was synthesized as described in [20].

3: THF (12.5 mL) was added to an aqueous solution of citrate-stabilized gold nanoparticles (12.5 mL, 2.9 nM). To this mixture, solutions of **1** (10 mg, 0.025 mmol, 0.5 mL) and **2** (10 mg, 0.012 mmol, 0.5 mL) in THF were added simultaneously under stirring. It was stirred for 3 h and filtered (0.45 μm millipore filter). The particles were purified by repeated centrifugation (3 times) at 11000 rpm (Sigma 1-13 model) and re-dispersion in water. A molar absorption coefficient of $4.2\times10^{8}\,M^{-1}cm^{-1}$ (at 526 nm) based on gold nanoparticles of 15 ± 1.2 nm diameter was used to calculate a final concentration of 1.5 nM (12.5 mL).[25] For ^1H NMR spectroscopy pellets of centrifuged particles were dried under vacuum overnight and re-dissolved in D_2O.

4: Thioacetic acid S-[11-(toluene-4-sulfonyloxy)-undecyl] ester (**a**) was prepared by refluxing a mixture of toluene-4-sulfonic acid undec-10-enyl ester (5 g, 15.4 mmol) and thioacetic acid (2.3 g, 31 mmol) in toluene (200 mL) for 3 h under exclusion of oxygen and in the presence of a catalytic amount of 2,2′-azobis[(2-methyl)-propanenitrile] (AIBN) and subsequent removal of the solvent by rotary evaporation. The solid crude product was dissolved in CH_2Cl_2, washed with water and sodium hydrogen carbonate, and purified by column chromatography (silica gel, hexane 90%/ ethyl acetate 10%) to give a yellowish viscous solid (90% yield). Pyridine (0.5 g, 5 mmol) was added to a solution of **a** in acetonitrile (2 g, 5 mmol, 100 mL) and heated under reflux for 24 h. The solvent was removed by rotary evaporation and the pure white solid product was obtained by precipitation in ethyl acetate/acetone in good yield (80%). This white solid (2 g, 4 mmol) and *para*-toluene sulfonic acid (1 g, 7 mmol) were dissolved in methanol (20 mL) and heated under reflux overnight then the solvent was removed by rotary evaporation. Precipitation of the resulting mixture in CH_2Cl_2 yielded a pure white sticky solid product **4** (75% yield). SAMs of **4** on gold (gold films of approximately 250-nm thickness on quartz glass, Arrandee) were prepared by overnight immersion of the cleaned and flame-annealed substrates in a solution of **4** in $CHCl_3$ (5 mL, 1.2×10^{-4} M) and subsequent thorough rinsing with $CHCl_3$ and drying in a stream of nitrogen. Single molecular-sieve beads (4 Å type, Aldrich) were modified by overnight immersion in solutions of **4** (2.5 mL, 1.2×10^{-4} M) in chloroform. After removal from the solution, the beads were washed thoroughly with chloroform and allowed to dry at room temperature. Specific recognition experiments were carried out by overnight immersion of the substrates (SAMs and beads) in a solution of **3** (0.034 nM, 2.5 mL for SAMs and 1.2 nM, 1.5 mL for beads). Adequate control experiments were performed to exclude the possibility of nonspecific binding.

For spectroscopic data and elemental analyses see the Supporting Information.

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[1] G. Schmid, R. Pfeil, R. Boese, F. Bandermann, S. Meyers, G. H. M. Calis, W. A. Vandervelden, *Chem. Ber.* **1981**, *114*, 3634.
[2] M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *J. Chem. Soc. Chem. Commun.* **1994**, 801.
[3] R. L. Whetten, M. N. Shafigullin, J. T. Khoury, T. G. Schaaff, I. Vezmar, M. M. Alvarez, A. Wilkinson, *Acc. Chem. Res.* **1999**, *32*, 397.
[4] G. Markovich, C. P. Collier, S. E. Henrichs, F. Remacle, R. D. Levine, J. R. Heath, *Acc. Chem. Res.* **1999**, *32*, 415.
[5] A. C. Templeton, W. P. Wuelfing, R. W. Murray, *Acc. Chem. Res.* **2000**, *33*, 27.
[6] A. Badia, R. B. Lennox, L. Reven, *Acc. Chem. Res.* **2000**, *33*, 475.
[7] M. Brust, C. J. Kiely, *Colloids Surf. A* **2002**, *202*, 175.
[8] M. C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293.
[9] W. P. Wuelfing, S. M. Gross, D. T. Miles, R. W. Murray, *J. Am. Chem. Soc.* **1998**, *120*, 12696.
[10] T. G. Schaaff, G. Knight, M. N. Shafigullin, R. F. Borkman, R. L. Whetten, *J. Phys. Chem. B* **1998**, *102*, 10643.
[11] M. Bartz, J. Kuther, G. Nelles, N. Weber, R. Seshadri, W. Tremel, *J. Mater. Chem.* **1999**, *9*, 1121.
[12] J. Liu, S. Mendoza, E. Roman, M. J. Lynn, R. Xu, A. E. Kaifer, *J. Am. Chem. Soc.* **1999**, *121*, 4304.
[13] A. C. Templeton, S. W. Chen, S. M. Gross, R. W. Murray, *Langmuir* **1999**, *15*,66.

[14] O. K. Kohlmann, W. E. Steinmetz, X. Mao, W. P. Wuelfing, A. C. Templeton, R. W. Murray, C. S. Johnson, Jr., *J. Phys. Chem. B* **2001**, *105*, 8801.
[15] E. E. Foos, A. W. Snow, M. E. Twigg, M. G. Ancona, *Chem. Mater.* **2002**, *14*, 2401.
[16] A. G. Kanaras, F. S. Kamounah, K. Schaumburg, C. J. Kiely, M. Brust, *Chem. Commun.* **2002**, 2294.
[17] P. Pengo, S. Polizzi, M. Battagliarin, L. Pasquato, P. Scrimin, *J. Mater. Chem.* **2003**, *13*, 2471.
[18] R. Levy, N. T. K. Thanh, R. C. Doty, I. Hussain, R. J. Nichols, D. J. Schiffrin, M. Brust and D. G. Fernig, *J. Am. Chem. Soc.* **2004**, *126*, 10076.
[19] a) W. Abraham, *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *43*, 159; b) A. Arduni, E. Brindani, G. Giorgi, A. Pochini, A. Secchi, *J. Org. Chem.* **2002**, *67*, 6188.
[20] A. Arduini, D. Demuru, A. Pochini, A. Secchi, *Chem. Commun.* **2005**, 645.
[21] M. Brust, D. Bethell, D. J. Schiffrin, C. J. Kiely, *Adv. Mater.* **1995**, *7*, 795.
[22] J. Turkevich, P. C. Stevenson, J. Hillier, *Discuss. Faraday Soc.* **1951**, *11*, 55.
[23] G. Frens, *Nature Phys. Sci.* **1973**, *241*, 20.
[24] C. Pale-Grosdemange, E. S. Simon, K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **1991**, *113*, 12.
[25] L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian, G. Viswanadham, *Anal. Chem.* **2000**, *72*, 5535.

