ROYAL SOCIETY OF CHEMISTRY

Journal Name

COMMUNICATION

Extremely Robust and Post-Functionalizable Gold Nanoparticles Coated with Calix[4]arenes via Metal-Carbon Bonds

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Gold nanoparticles stabilized with a thin layer of post-functionalizable calix[4]arenes were prepared through the reductive grafting of a calix[4]arene-tetra-diazonium salt. These particles show exceptional stability towards extreme pH, F⁻, NaCl, and upon drying. Post-functionalization of the calix-layer was demonstrated, opening the way to a wide range of applications.

Due to their unique electrical, optical and catalytic properties, gold nanoparticles (AuNPs) have stimulated intense research activities dedicated to a large range of applications including catalysis,[1a] nano-electronics,[1b] medicine,[1c] optics,[1d] plasmonic devices[1e] and sensing.[1f] For many decades, gold colloids were stabilized by electrostatic repulsion (e.g. when capped with citrate) or by steric repulsion. Functionalizing ligands can be physisorbed on the AuNPs (via carboxylates) or chemisorbed, mainly using alkanethiols, even if phosphines or amines can also be used.[2] The versatility of alkanethiol chemistry allowed the grafting of a wide range of ligands on the AuNPs, enhancing their utilization in many scientific areas.[3] The dynamic nature of the Au-S bond allows the formation of dense self-assembled monolayers on gold surfaces, but also limits the stability of these layers.[4] In contrast, the reduction of aryl diazonium salts produces aryl radical species able to bind to the metal surface *via* covalent Au-C bonds, whose bond energy has been calculated to be 14.2 kJ mol⁻¹ higher than that of Au-S bonds,[5] which leads to the formation of a more robust organic layer around the AuNPs.[6]

It is, however, well known that the reductive grafting of aryl diazonium salts leads to disordered multilayer organic films because of the difficulties to control the vertical extension of the grafting.[7] Indeed, aryl radicals are highly reactive species that can react with the surface but also with the already-grafted aryl layers. Reported strategies for the controlled formation of monolayers are mainly based on the introduction of bulky protecting groups.[8] We have developed a different strategy that relies on the grafting of calix[4]arenes bearing diazonium groups at their large rim.[9] Thanks to the well pre-organized and 3D conic structure of the calix[4]arene core, densely packed monolayers and mixed binary monolayers were obtained on flat surfaces, with a good control of the surface composition and a high homogeneity of the deposit.[10] Provided that the small rim of the calix[4]arene is decorated with appending reactive arms, like carboxylic groups, the immobilized calix[4]arene moieties can be easily post-functionalized with various molecules.[9,10]

Here we report the reductive grafting of calix[4]arene-tetra-diazonium salt **2** to prepare functional and water soluble AuNPs stabilized by a highly robust organic shell involving Au-C bonds (Scheme 1).[11] These AuNPs were obtained either through a one-pot synthesis of AuNPs in the presence of **2** (AuNPs **3a**) or through a ligand-exchange process (AuNPs **3b-d**). The exceptional stability of the resulting AuNPs under various conditions is demonstrated and post-functionalization of calix-stabilized AuNPs through amide linkage was readily achieved using the carboxylic terminal groups at the small rim.

Calix[4]arene-tetra-diazonium **2** was obtained in good yield (98%) by reacting the corresponding calix[4]arene-tetra-aniline **1**[9] with NOBF₄ in acetonitrile (Scheme 1).[12] **2** was found to be thermally stable up to 120°C (Fig. S9). To our knowledge, this compound is the first isolated calix[4]arene-tetra-diazonium salt. The first type of calix-AuNPs (**3a**) were synthesized by reducing HAuCl₄ with NaBH₄ in the presence of calix[4]arene-tetra-diazonium **2** in acetonitrile (Scheme 1). After centrifugation, the calix-AuNPs **3a** were washed with NaOH and

Scheme 1. Synthesis of calix[4]arene-tetra-diazonium salt **2** and of AuNPs **3a** from HAuCl₄. AuNPs **3b-d** were obtained by grafting **2** on citrate-protected particles.

HCl solutions and water to eliminate the excess of non-grafted calix[4]arene compounds. Nanoparticles **3a** were dispersed in aqueous NaOH (1 M) and stored at 4°C for several weeks.

The UV-Visible spectra of the nanoparticles **3a** in NaOH solution (pH = 11.5) display a characteristic surface plasmon band centered at a maximum wavelength (λ_{max}) of 520 nm. The formation of mostly spherical AuNPs with an average diameter 6.1 ± 1.9 nm was further confirmed by transmission electron microscopy (TEM) measurements (Fig. 1, see ESI for further discussion). X-ray photoelectron spectroscopy (XPS) measurements on **3a** revealed (atom %) 7% Au, 69% C, 22% O and 2% Na, in agreement with what would be expected for AuNPs with calix[4]arene **2** grafted at their surface. The absence of the Au4f7/2 peak at ca. 86.0 eV[13] and of the N1s peak at 404 eV indicates the full reduction of Au³⁺ to Au⁰ and of the diazonium groups of **2**.

Calix[4]arene **2** was also used for the functionalization of citrate stabilized nanoparticles via a ligand exchange approach. Reduction of the diazonium groups occurs at the surface of the AuNPs, leading to aryl radicals that strongly bind to AuNPs through Au-C bonds.[6] For various applications, this strategy can be preferred: i) it does not require the controlled synthesis of the AuNPs, which are now commercially available in different sizes and with different stabilizing ligands, ii) it allows for a choice of size and a narrow size distribution of AuNPs optimal for the intended application, iii) it could be easier to introduce calixarenes with specific functionalities or mixtures of calixarenes[10] by the ligand exchange strategy. This strategy was thus used to graft **2** on in house synthesized citrate-stabilized

AuNPs of 15 nm and commercially available citrate-stabilized AuNPs of 10 and 5 nm.

For the large-scale functionalization of the 15 nm AuNPs (optical density ~10, in 1 mM citrate), a small amount of NaBH₄ (0.5 eqv. per **2**) was added to the AuNPs before the addition of the calixarene **2** to enhance the stability of the concentrated colloidal Au dispersion by increasing the pH and to assure efficient grafting. A solution of 10⁵ equivalents of **2** per AuNP in water was added and the suspension was stirred overnight. Calixarene functionalized AuNPs **3b** were obtained after centrifugation and multiple washing cycles with 1 mM NaOH. The functionalization of the 10 and 5 nm AuNPs was also performed by the addition of 10⁵ equivalents of **2**, but no NaBH₄ was used for these inherently more stable suspensions. Overnight stirring and subsequent washing of the AuNPs afforded calixarene functionalized AuNPs **3c** (10 nm) and **3d** (5 nm). TEM measurements indicated that the initial spherical shape and narrow size distribution of the AuNPs were maintained (Fig.S6-8).

The grafting density of calixarenes on AuNPs **3a** and **3b** was investigated by TGA experiments. The densities calculated from these TGA measurements are ~3.5 calixarenes per nm² for AuNPs **3a** and ~1.5 calixarenes per nm² for AuNPs **3b** (see ESI for details). This difference can be attributed to the larger curvature of the surface of smaller particles **3a**, allowing denser grafting of the calix[4]arenes compared to **3b**. In agreement with that, both values are higher than the density found previously for calix[4]arenes electrografted on flat surfaces (~0.53 per nm²).[10] The clear trend and reproducibility of the TGA results indicate that AuNPs **3** are protected with roughly a monolayer of calix[4]arenes.

The effectiveness of the ligand exchange and the robustness of the resulting calixarene-functionalized AuNPs is demonstrated by the way these AuNPs respond on acidic and basic conditions. In basic aqueous solutions the calix-AuNPs are well dispersed, thanks to the electrostatic repulsion from the carboxylate groups at the surface of the AuNPs. In contrast, in acidic medium the charge neutralization renders the AuNPs highly sensitive to any change of ionic strength and pH, leading to the aggregation of the calix-AuNPs **3**, changing the colour of the dispersion from red into purple or gray (depending on the size and concentration of the AuNPs used). This evolution of the color with respect to pH is shown for AuNPs **3c** in Fig. 2 and S15 and for **3a** in Fig. S16-17.
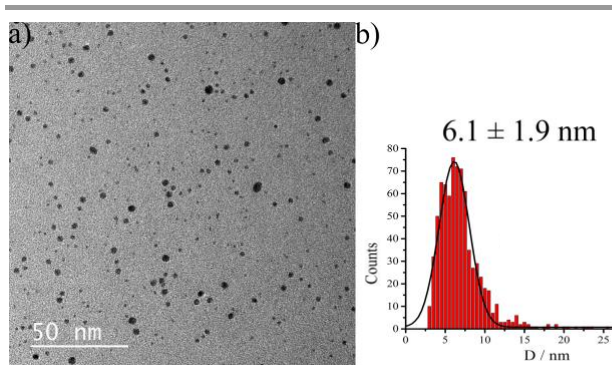

Fig. 1 TEM micrograph and corresponding particle core size histogram of gold nanoparticles synthesized in the presence of the calix[4]arene **2** to give AuNPs **3a**.
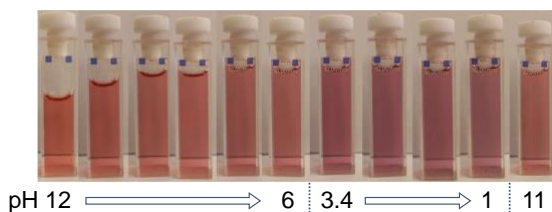
Fig. 2 The change of color of a colloidal dispersion of AuNPs **3c** in HEPES buffer adjusted to various pH values. The dispersion is stable upon addition of HCl from pH 12 down to pH 6 (λ_{max} shifts from 522 to 527 nm), while from pH 3.4 to 1 the λ_{max} shifts from 536 to 543 nm and the absorption broadens, resulting in a purple color. These effects are reversed upon addition of NaOH to get to pH 11 (λ_{max} 526 nm).

Due to the robustness of the calix-AuNPs, this pH effect on the aggregation of the AuNPs is reversible. AuNPs **3b** were precipitated in 0.1 M HCl solution and then easily resuspended in 0.1 M NaOH. This process was repeated without any noticeable change in the UV-Vis absorption spectrum (Fig. 3a). This behavior was also used as a quality test for the successful grafting of calixarene **2** on citrate-protected AuNPs, as the aggregation of citrate-protected AuNPs at low pH is irreversible (Fig. S18-19).

The extreme robustness of the calixarene-functionalized AuNPs is further shown by the complete drying of calix-AuNPs **3a** and **3b** into a gold-colored film at the bottom of the cuvette (Fig. 3b and S20), followed by resuspension in 0.1 M NaOH upon sonication to give a red colloidal suspension. Though slightly broader as compared to the original absorption spectrum of
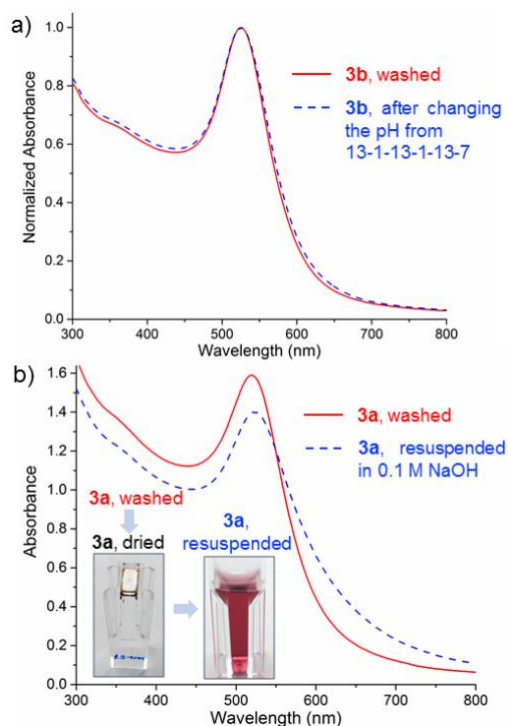

Fig. 3 (a) UV-Vis absorption spectra of calix-AuNPs **3b** after multiple mild washing steps (using 1 mM NaOH, red line), and after five subsequent centrifugation cycles using 0.1 M NaOH and 0.1 M HCl alternatingly (blue dashed line); (b) UV-Vis absorption spectra of calix-AuNPs **3a** suspended in 50 mM NaOH (red line), allowed to dry into a gold-colored film (inset), and after resuspension in 0.1 M NaOH (blue dashed line).

AuNPs **3a**, the spectrum upon resuspension still features a sharp localized surface plasmon resonance band with a λ_{max} of 522 nm and an intensity only slightly lower than the original spectrum, indicating that the vast majority of the AuNPs are successfully resuspended. Though similar results have been reported for AuNPs coated with long polymer chains,[14] this resuspendability is rather remarkable for AuNPs with an organic protection layer of no more than one nm in thickness.

The remarkable stability of calix-AuNPs is finally demonstrated by their insensitivity towards the addition of fluoride. Previous studies have shown that halide ions have affinity for metal surfaces[15] and can chemisorb on gold surface to form Au-X bonds, with a covalent character for certain halides.[16] The addition of halides to a suspension of citrate-stabilized AuNPs has been shown to lead to their aggregation.[17] The kosmotropic anions fluoride and sulfate, were also reported to destabilize AuNPs.[18] Therefore, we compared the stability of the calix[4]arene-stabilized AuNPs **3a** and **3b** to that of commercial citrate-stabilized AuNPs, AuNPs capped with dodecanethiol,[1b] and AuNPs stabilized by Au-C bonds (synthesized in the presence of 4-diazonium decylbenzene as described by Schiffrin).[19] For this, tetrabutylammonium fluoride (TBAF) in THF, or KF in water, was added to the different AuNP suspensions to obtain a fluoride concentration of 0.15 M (see ESI for details). For most AuNPs, this resulted in an immediate color change from red/pink to purple, characteristic of the aggregation of the AuNPs. However, the calix[4]arene-stabilized AuNPs **3a** and **3b** were not affected by the addition of fluoride and stable red dispersions were maintained (Fig. S21). The comparison of AuNPs **3** with the AuNPs synthesized using Schiffrin's method shows that the chemical stability of **3** is not only due to the formation of robust Au-C bonds, but that it is specific for calix[4]arene-capped AuNPs. AuNPs **3a** and **3b** were also shown to be stable in physiologically relevant conditions of 0.15 M NaCl and 0.05 M phosphate buffer, in sharp contrast to the analogous citrate-protected AuNPs (Fig. S22-23). These experiments clearly show that AuNPs decorated with calix[4]arenes through Au-C bonds are efficiently protected against physiological salt concentrations and aggressive chemical treatment.

The terminal carboxylic acid functions at the calix[4]arene small rim could be used as reactive units, allowing a further post-functionalization of the nanoparticles by a wide range of molecules or biomolecules through the formation of amide bonds. As proof-of-concept, mild conditions using EDC and NHS were chosen to achieve the post-functionalization of AuNPs **3a** either with dodecylamine or with undec-10-ynyl-1-amine (see ESI for details). The resulting nanoparticles were no longer water soluble, indicating effective post-functionalization. For both sets of post-functionalized AuNPs, analysis by FTIR spectroscopy showed a strong band at 3240 cm^{-1}, which is characteristic of secondary amide N-H stretching vibration (Fig. 4).[20] In addition, clear C-H stretching bands at 2851 and 2920 cm^{-1} appeared upon post-functionalization with both amines, corresponding to the symmetric and asymmetric stretching vibrations in aliphatic acyclic CH$_2$ groups.[20] An additional band at 2960 cm^{-1} corresponds to the C-H vibrational stretching of the
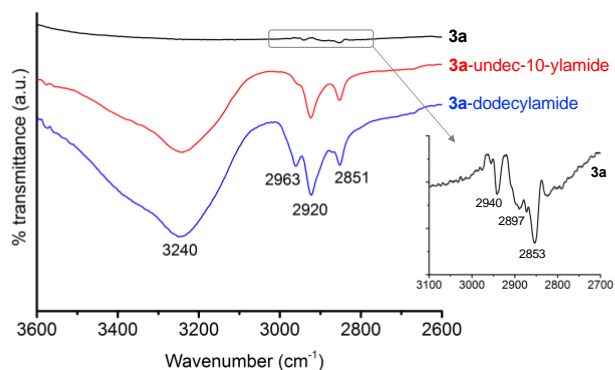
Fig. 4 FTIR spectra in the N-H and C-H stretching region of AuNPs **3a** post-functionalized with dodecylamine (blue curve) and with undec-10-ynyl-1-amine (red curve), compared to AuNPs **3a** before post-functionalization (black curve and inset).

terminal CH$_3$ groups and can be observed only for the AuNPs coupled with dodecylamine. These results demonstrate that the post-functionalization of the calix[4]arene-stabilized AuNPs is particularly easy to operate and permits to tailor the properties of the AuNPs for different applications.

In conclusion, the AuNPs coated with a layer of calix[4]arenes, attached via the formation of multiple Au-C bonds, show exceptional robustness, allowing the resuspension upon drying or treatment with strong acid. The AuNPs are stable in the presence of fluoride, NaCl, and under strong basic conditions. This allows their application both in physiological and harsh chemical conditions. Thanks to the presence of carboxylic groups on the small rim of the calix[4]arenes, post-functionalization of the AuNPs was demonstrated, opening the way to the preparation of highly stable gold nanoparticles bearing a wide range of molecules or biomolecules. We envision that the use of mixtures of differently functionalized calix[4]arenes or of calix[4]arenes with different appending arms will open new opportunities for the design of mixed binary monolayers on gold nanoparticles, with controllable levels of post-functionalizable groups.

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