Inorganic Chemistry

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Stabilization of AuNPs by Monofunctional Triazole Linked to Ferrocene, Ferricenium, or Coumarin and Applications to Synthesis, Sensing, and Catalysis

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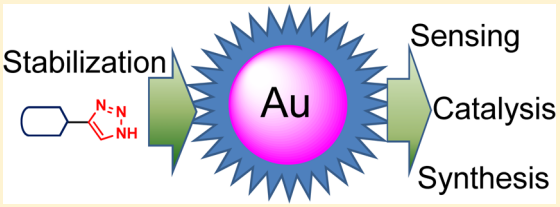
Supporting Information

ABSTRACT: Monofunctional triazoles linked to ferrocene, ferricenium, or coumarin (Cou), easily synthesized by copper-catalyzed azide alkyne (CuAAC) "click" reactions between the corresponding functional azides and (trimethylsilyl)acetylene followed by silyl group deprotection, provide a variety of convenient neutral ligands for the stabilization of functional gold nanoparticles (AuNPs) in polar organic solvents. These triazole (trz)-AuNPs are very useful toward a variety of applications to synthesis, sensing, and catalysis. Both ferrocenyl (Fc) and isostructural ferricenium linked triazoles give rise to AuNP stabilization, although by different synthetic routes. Indeed, the first direct synthesis and stabilization of AuNPs by ferricenium are obtained by the reduction of $HAuCl_4$ upon reaction with a ferrocene derivative, AuNP stabilization resulting from a synergy between electrostatic and coordination effects. The ferricenium/ferrocene trz-AuNP redox couple is fully reversible, as shown by cyclic voltammograms that were recorded with both redox forms. These trz-AuNPs are stable for weeks in various polar solvents, but at the same time, the advantage of trz-AuNPs is the easy substitution of neutral trz ligands by thiols and other ligands, giving rise to applications. Indeed, this ligand substitution of trz at the AuNP surface yields a stable Fc-terminated nanogold-cored dendrimer upon reaction with a Fc-terminated thiol dendron, substitution of Cou-linked trz with cysteine, homocysteine, and glutathione provides remarkably efficient biothiol sensing, and a ferricenium-linked trz-AuNP catalyst is effective for $NaBH_4$ reduction of 4-nitrophenol to 4-aminophenol. In this catalytic example, the additional electrostatic AuNP stabilization modulates the reaction rate and induction time.



INTRODUCTION

In the past few decades, a variety of ligands[1−9] have been synthesized and used to stabilize gold nanoparticles (AuNPs)[9] either in organic solvents or in water. The surface properties, including the ligand type, binding force of gold with other atoms, as well as ligand coverage of AuNPs, control the solubility, stability, and applications of AuNPs. For example, in thiolate-stabilized AuNPs, covalent bonding between the gold and sulfur atoms contributes to passivation of the surface of thiolate-stabilized AuNPs and makes AuNPs stable in the solid state,[1] whereas citrate-stabilized AuNPs were usually prepared in aqueous solution because of the multipackage of ionic species on the AuNP surface.[2] In addition, the influence of the surface properties of AuNPs was also observed in several examples on the coordination-induced stabilization of AuNPs with nitrogen donors, particularly dendritic supramolecules.[9] Indeed, poly(amidoamine) dendrimers show significant template effects in the formation of nanoparticles in various solvents.[3] Copper-catalyzed azide alkyne (CuAAC) "click" chemistry has generated supermolecules,[4] such as poly(ethylene glycol) (PEG)-terminated dendrimers and polymers,[5] also stabilizing AuNPs in aqueous solution.[6] Besides, other nitrogen ligands, such as imidazoles[7] and pyridines,[8] have also been utilized for the stabilization of AuNPs.[9] The dual property of triazole-AuNPs (trz-AuNPs) that, on the one hand, are stable and, on the other hand, have modest AuNP−N bond strength, allowing facile ligand substitution of triazoles by other ligands for various applications, makes this family of AuNPs particularly attractive. Indeed, upon trz ligand substitution, it is possible to synthesize various other liganded AuNPs, to use the ligand-exchange processes for sensing and to provide catalytically efficient AuNP surfaces.[10] Stabilization and uses of water-soluble PEGylated trz-AuNPs have recently been shown.[11] Here we focus on the stabilization of trz-AuNPs linked to ferrocene,[12,13] ferricenium, and coumarin (Cou) termini using these monofunctional trz-AuNPs that are not synthesized in water and are soluble in organic solvents.

Received: September 3, 2014
Published: October 14, 2014

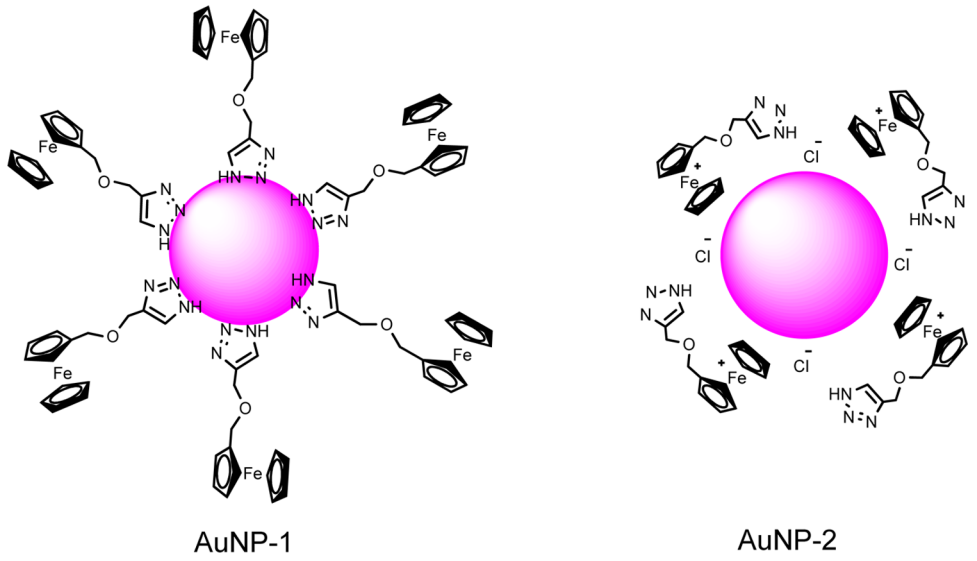


Figure 1. AuNPs stabilized by Fc-trz (**AuNP-1**) or oxidized Fc^{+}-trz-Cl^{-} (**AuNP-2**) in an organic solution.

For applications, ferrocenes have attracted attention because of the sensing and biomedical applications of the ferrocenyl (Fc) derivatives related to their reversible redox properties.[14] Catalytic properties will also be shown to result from fast triazole removal by substrates at the AuNP surface. Indeed, noble-metal nanoparticles,[15] in particular AuNPs, are excellent catalysts for the reduction of 4-nitrophenol (4-NP), which is toxic and inhibitory in nature, in order to produce 4-aminophenol (4-AP), which has properties and applications such as corrosion inhibitor, dying agent, and, in particular, intermediate for the synthesis of paracetamol.[16] Finally, Cou is a well-known fluorescent dye[17] that are linking to triazole-AuNPs for the investigation of fluorescent-sensing properties based on AuNP ligand substitution by biologically relevant thiols. Indeed, the thiols cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play key roles in the biological systems.[18] Many diseases are relevant to the abnormal contents of Cys or Hcy in the human body. For instance, an abnormal level of Cys may cause skin lesions and liver damage.[19] Furthermore, Hcy is a risk factor for Alzheimer's and cardiovascular diseases.[20] Excess Cys has been associated with neurotoxicity and many other diseases.[21] Accordingly, the development of chemosensors for biological thiol derivatives (biothiols) is of great importance, as recently indicated.[22] Sensing biological thiols using the fluorescence "turn-on" method on the surface of AuNPs results from either the formation of new fluorescent species or the release of dye adsorbed on the AuNPs, as recently reported.[23]

RESULTS AND DISCUSSION

In order to easily incorporate the redox complex and other functionalities into AuNPs in an organic solution, two kinds of small monosubstituted triazole molecules, Fc-trz and Cou-trz, were synthesized, taking into account their electrochemical or photochemical properties. Each functional unit was linked to the trz ring by an ether group that increased the flexibility of the trz ligand to make sure that the triazoles smoothly arrange on the surface of AuNPs.

Ferrocene- and Ferricenium-Triazole-Stabilized AuNPs. Late-transition-metal sandwich complexes are known for their stability in several oxidation states and their ability to effect efficient stoichiometric and catalytic electron-transfer processes[24] for multiple applications.[25,26] They are also rather easy to functionalize on the ligands,[25] and thus the introduction of alkynyl or azido groups has been reported in view of further CuAAC "click" functionalizing chemistry.[27] The Fc-linked 1,2,3-triazole (Fc-trz; see Figure 1) was synthesized using the CuAAC "click" reaction between (trimethylsilyl)azide and propargyloxymethylferrocene in a *N*,*N*-dimethylformamide (DMF)/methanol (MeOH) solution at 100 °C with CuI as the catalyst (Experimental Section). Fc-trz was characterized by ^{1}H and ^{13}C NMR and IR spectroscopies, electrospray ionization mass spectrometry (ESI-MS), elemental analysis, and cyclic voltammetry (see the Supporting Information, SI). Coumarin triazole (Cou-trz) was synthesized according to the literature,[28] but its photochemical property and applications were not reported.

A one-step process to functionalize AuNPs with Fc-trz was carried out in an organic solution. Compared to the above-mentioned ligand-substitution method, Fc-trz was used as the stabilizer instead of ferrocene thiolate ligands.[12a] The preparation of AuNPs stabilized by Fc-trz was performed using two pathways. In the first method, Fc-trz and $NaBH_4$ were dissolved in ethanol (EtOH), and a $HAuCl_4$/EtOH solution was slowly injected into this solution under a nitrogen atmosphere with stirring. After being further stirred for 2 h, **AuNP-1** was purified by dialysis against a large volume of EtOH. In this process, gold(III) was reduced by $NaBH_4$, while Fc-trz played the role of stabilizer. No oxidation of Fc was found according to UV−vis spectroscopy analysis of **AuNP-1**, which is well taken into account by the fact that $NaBH_4$ is a much stronger and faster reducing reagent than ferrocene. Indeed, as shown in Figure 2a, two bands at 321 and 430 nm belong to the ferrocene unit.

On the other hand, during the preparation of **AuNP-2**, no external reductant was involved. The Fc group works not only as the capping agent in its oxidized form but also as the reductant. In this reaction, the Fc group was oxidized to ferricenium chloride, with gold(III) being reduced to gold(0) atoms that form AuNPs. **AuNP-2** is stabilized by the ferricenium chloride triazole ligand, with stabilization resulting from the positive synergy between the electrostatic factor and

dx.doi.org/10.1021/ic5021498 | *Inorg. Chem.* 2014, 53, 11802−11808

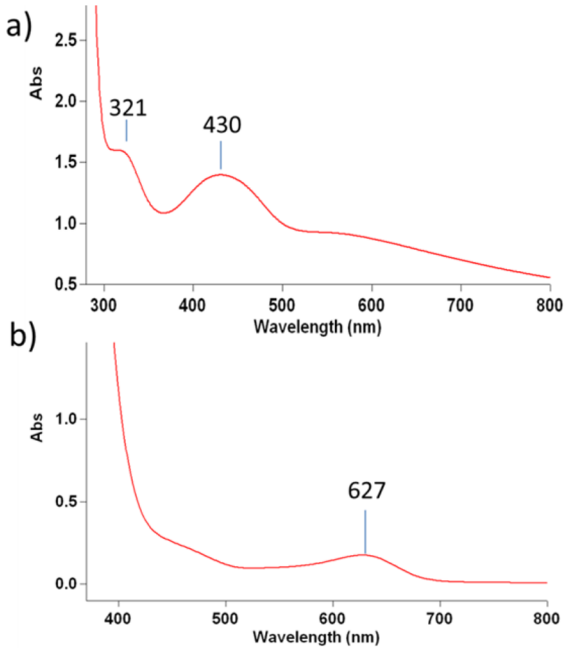


Figure 2. UV−vis spectra revealing the characteristic bands of (a) the Fc group in **AuNP-1** (321 and 430 nm) and (b) the ferricenium group in **AuNP-2** (627 nm) (both were recorded in an EtOH solution).

the trz ligand coordination to the AuNP surface (Figure 1). The oxidation of ferrocene to ferricenium was successfully recorded by UV−vis spectroscopy, with the band at 627 nm in Figure 2b corresponding to the ferricenium unit. In Figure 2a, a very weak shoulder band (because of the large ferrocene absorption) around 530−580 nm corresponds to the surface plasmon band (SPB) of **AuNP-1**. The SPB of **AuNP-2** overlaps with the ferricenium band, which is deduced from a comparison with the SPB band of **AuNP-3**. Both MeOH and tetrahydrofuran (THF) were suitable solvents in the one-step preparation of **AuNPs-1** and **AuNPs-2**.

The transmission electron microscopy (TEM) images shown in Figure 3 revealed the sizes of the Fc-trz-capped AuNPs that were prepared with or without $NaBH_4$, indicating that **AuNP-1** (5 nm) was slightly larger than **AuNP-2** (4.4 nm). The size difference between **AuNP-1** and **AuNP-2** in core size may arise from the difference of the reduction and stabilization rates in each case. Indeed, the stabilization in **AuNP-2** is stronger compared to **AuNP-1** because of the additional electrostatic stabilization of **AuNP-2**. Both **AuNP-1** and **AuNP-2** are monodispersed in an EtOH solution, as disclosed by TEM and dynamic light scattering (DLS) analyses. The multiionic layers of **AuNP-2** led to the formation of small clusters (9.8 nm) in solution compared to **AuNP-1**, which formed relatively larger clusters (12 nm). The electrochemical characterization of **AuNP-1** and **AuNP-2** was conducted by cyclic voltammetry. As shown in Figure S7 in the SI on the cyclic voltammogram of **AuNP-1**, the expected chemically and electrochemically reversible waves of the ferricenium/Fc redox system were observed. The difference between the anodic and cathodic peak potentials (ΔE) is 0.06 V, and the intensity ratio i_a/i_c is 1.0, showing the chemical reversibility of the iron(III/II) system without being marred by adsorption. The measured redox potential value of this multiferrocenyl redox system, i.e., the average of the anodic and cathodic wave potentials [$E_{1/2} = (E_{pa} + E_{pc})/2$], is 0.44 V versus decamethylferrocene.[24c] Both **AuNPs-1** and **AuNPs-2** are qualified for redox-sensing applications.

4-NP Reduction. **AuNP-2** was selected because it was the only AuNP in this series that was water-soluble, which facilitated the catalytic experiments. The lability of the AuNP−trz and AuNP−Cl^- connections on the surface of **AuNP-2** was employed to catalyze 4-NP reduction in water/EtOH (95:5) in the presence of $NaBH_4$[29] with catalyst amounts of 0.5% or 1%. This reaction was carried out in a standard quartz cuvette (path length: 1 cm) and monitored by UV−vis spectroscopy every 40 s (Figure S11 in the SI). The plots of the decrease rate of 4-NP [$-\ln(C/C_0)$] versus the reaction times (Figure 4) were collected (with C and C_0 being the 4-NP concentrations at times t and $t = 0$, respectively). The rate constant k of the catalytic reduction of 4-NP was found to be

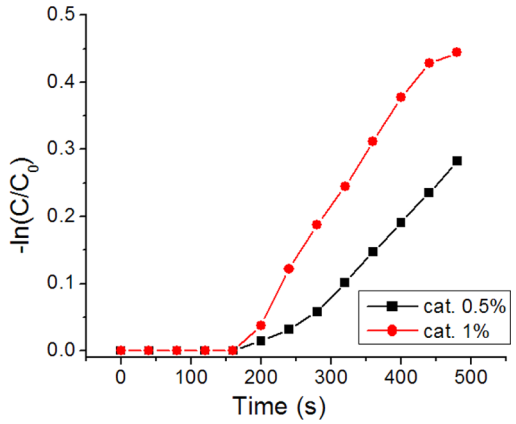


Figure 4. Plots of the decreasing rate of 4-NP [$-\ln(C/C_0)$] versus the reaction times using two distinct amounts of catalyst **AuNP-2** (0.5% catalyst in the black curve and 1% catalyst in the red curve).

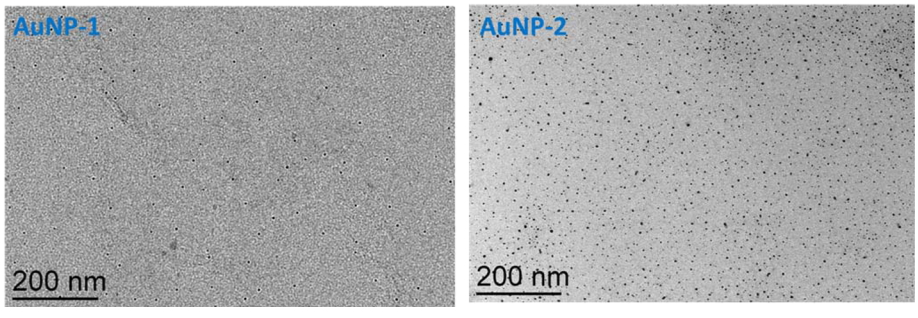


Figure 3. TEM images of **AuNP-1** (d = 5 nm) and **AuNP-2** (d = 4.4 nm) prepared using two distinct methods.

dx.doi.org/10.1021/ic5021498 | Inorg. Chem. 2014, 53, 11802−11808

1.1×10^{-3} s^{-1} with 0.5% **AuNP-2** as the catalyst, and the k value increased to 1.6×10^{-3} s^{-1} with an increase of the catalyst amount to 1%. Moreover, an induction period (200 s) was required because of the AuNP surface organization of the substrates in the mixed solvent.[30] Compared to the catalysis of 4-NP reduction with bulky water-soluble PEG-trz ligands, which provided $k = 5.2 \times 10^{-3}$ s^{-1} (0.5% catalyst),[13a] the catalytic efficiency of **AuNP-2** that was stabilized by the present triazole ligands was relatively lower (1.1×10^{-3} s^{-1}), although the core of **AuNP-2** was slightly smaller than that of AuNPs with PEG-trz ligands. However, **AuNPs-2** remained superior for 4-NP reduction to triazole-stabilized AuNPs of close size stabilized by other ligands such as thiolate and citrate because of the labile character of the AuNP−trz bonds.

Cou-trz-Stabilized AuNPs. Cou-trz-stabilized AuNPs (**AuNP-3**) were prepared in EtOH with sodium borohydride as the reductant to reduce Au^{III} to Au^0. Thus, $HAuCl_4$ and Cou-trz were dissolved together in an EtOH solution, and an EtOH solution of $NaBH_4$ was added dropwise to the mixture under a nitrogen atmosphere. The yielded solution was further stirred for 2 h. These AuNPs precipitated in EtOH in 24 h, and they were redissolved in dimethyl sulfoxide (DMSO). After precipitation−redissolution three times, excess Cou-trz was easily removed with the supernatant. The size of the **AuNP-3** core shown by TEM is 6.3 nm, i.e., slightly larger than those of **AuNP-1** and **AuNP-2**. TEM and DLS (average diameter size: d = 16 nm) analyses showed that **AuNP-3** has lower dispersion than **AuNP-1** and **AuNP-2**, presumably because of the precipitation−redissolution purification process.

According to the fluorescence quenching effect of the AuNP core of **AuNP-3**, the very high molar extinction coefficients and broad energy bandwidths of AuNPs result in emission−extinction of a fluorescent dye that is relatively close to the surface of **AuNP-3**. As shown in Figure 5, in which the fluorescence emission spectra of Cou-trz and **AuNP-3** are presented, the emission band (λ_{max}) of Cou-trz at 537 nm is of high intensity (λ_{ex} = 405 nm in a DMSO/water solution). On the other hand, a dramatic decrease was observed after capping AuNPs with Cou-trz ligands (see the red curve in Figure 5); that is, the emission of Cou-trz was quenched by the AuNP core of **AuNP-3**.

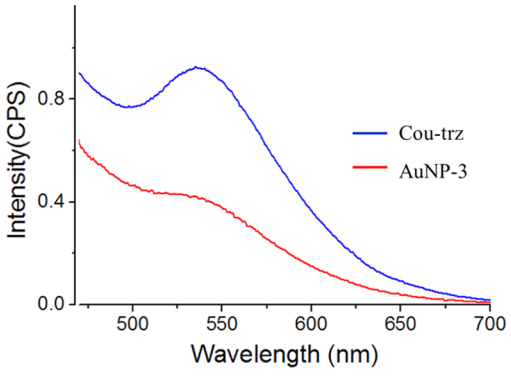


Figure 5. Fluorescent emission spectra of Cou-trz (0.2 mM) and Cou-trz-capped **AuNP-3** (both in a DMSO/water solution; λ_{ex} = 405 nm, recorded at 22 °C).

Given the combination of the fluorescence quenching effect of AuNPs and the flexible bonding of AuNPs to trz rings facilitating trz substitution at the AuNP surface, it was reasoned that sensing by a dramatic change of the fluorescence intensity upon ligand substitution should be efficient. It was indeed possible to easily detect the presence in solution of thiol or sulfide compounds in this way. Three biological thiols that are known to cause illness in the human body were taken as models to verify the sensing property of **AuNP-3**. As illustrated in Scheme 1, an aqueous solution of biothiol (Hcy, Cys, or GSH) was progressively titrated upon an increase in the concentration in a DMSO/water solution of **AuNP-3** in a standard quartz cuvette (path length = 1 cm). Along with the progress of ligand substitution, Cou-trz−**AuNP-3** was removed from the solution and replaced by AuNPs bound to biothiols. The recovery of photochemical emission of Cou-trz was consequently observed. The emission intensity of **AuNP-3** increased distinctly with an increase of the biothiols (up to 10 equiv) after being mixed for 10 min after titration (Figure 6; see full-scale fluorescence emission spectra in Figure S12 in the SI). The same phenomena were observed in sensing Cys and GSH (Figures S13 and S14 in the SI), demonstrating the photochemical application of **AuNP-3** in the sensing of biothiols.

Synthesis of a Nanogold-Cored Fc-Terminated Dendrimer. Another way to utilize the lability of trz in monosubstituted trz-stabilized AuNPs for the synthesis of Fc-containing AuNPs was achieved by the facile reaction of trz-AuNPs with Fc-terminated dendrons containing a thiol focal point as the potential AuNP thiolate ligand. Thus, the nonabranched Fc-terminated thiol dendron **6** was synthesized and utilized to substitute for the trz ligand from the surface of AuNPs. This method allows the formation of very stable AuNP thiolates containing a bulky Fc-loaded surface.[13a]

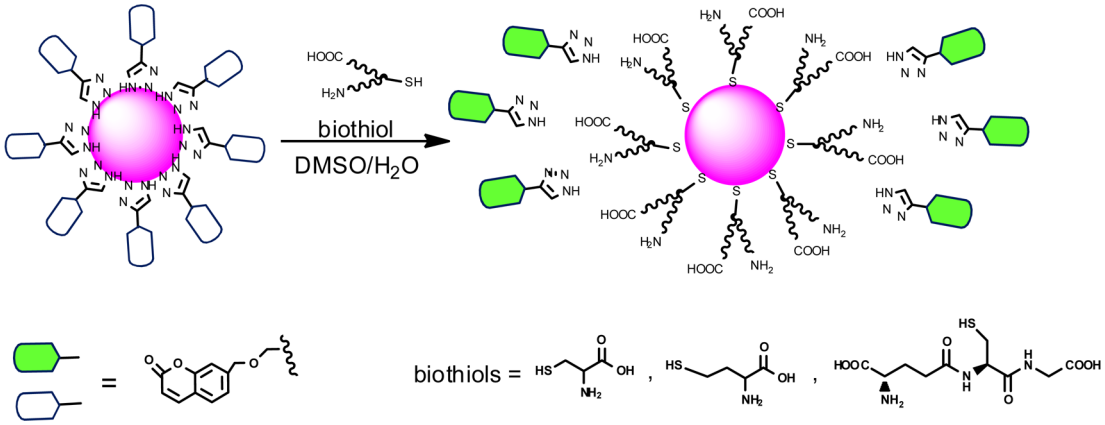
The nonaferrocene dendron **6** with 1 → 3 directionalities[31] was synthesized via the CuAAC "click" reaction of azidomethylferrocenyl with a nonaalkyne dendron.[13a] This dendron was easily grafted onto the surface of AuNPs by fast and quantitative trz ligand substitution to give **AuNP-5** (Scheme 2). In order to limit the Fc bulk at the dendrimer periphery, the dendron **6** was introduced together with linear dodecanethiol, which were found in 70% of the total of 540 ± 80[32] thiol per **AuNP-5**. **AuNP-5** retained its core size and monodispersity during the reaction in an organic solvent, which resulted in a AuNP-cored dendrimer containing about 140 ± 25 thiol dendrons, i.e., 1260 ± 180 Fc termini, and displayed chemical and electrochemical reversibility together with significant adsorption in its cyclic voltammogram (see the SI).[33]

CONCLUSION

In this work, the first trz-AuNPs that are soluble in organic media have been synthesized in a size range of 4.4−6.3 nm with narrow dispersities in EtOH with ferrocene, ferricenium, and Cou groups linked to the trz ligands. The first ferricenium-stabilized AuNPs have been synthesized by direct redox reaction between the trz-linked Fc and $HAuCl_4$ without any other reductant. The excellent stabilization of these AuNPs results from synergistic stabilization by both the electrostatic effect and trz coordination. This excellence of stabilization is confirmed by the slower catalysis of 4-NP reduction by $NaBH_4$ than with other trz-AuNP catalysts. In addition, the retention time characterized here was not found with other trz-AuNPs, indicating a higher reorganization energy at the AuNP surface than with neutral trz ligands. The two Fe^{II} and Fe^{III} redox forms of the trz-AuNPs with closely related AuNP core sizes were independently synthesized and shown to reversibly interconvert by cyclic voltammetry. Easy trz ligand substitution has been shown to usefully apply to dendrimer synthesis, biothiol

dx.doi.org/10.1021/ic5021498 | Inorg. Chem. 2014, 53, 11802−11808

Scheme 1. General Illustration of Fluorescent Sensing of Biothiols (Cys, Hcy, and GSH) with AuNP-3 via Ligand Substitution on the Surface of AuNPs Inducing Fluorescence "Turn-On" Phenomenon[a]



[a]The green color represents the recovery of the Cou fluorescence property.

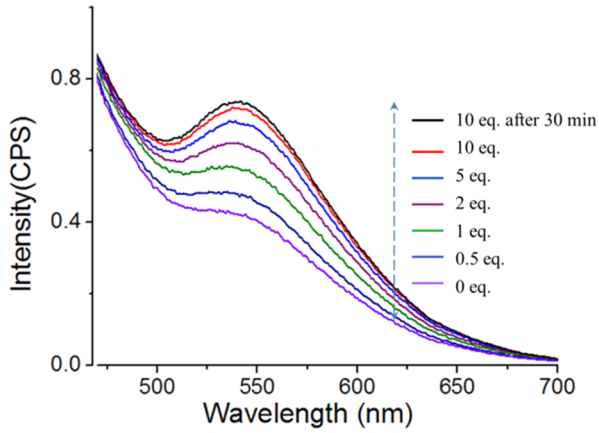


Figure 6. Fluorescent emission spectra of **AuNP-3** mixed with various amounts of Hcy (1 equiv of analyte = 0.05 mM in the solution; λ_{ex} = 405 nm; standard quartz cuvette; path length = 1 cm, recorded at 25 °C; see full-scale spectra in Figure S12 in the SI).

fluorescence "turn-on" sensing assay, and nitrophenol reduction by $NaBH_4$. Finally, concerning the synthetic aspect, facile AuNP-cored dendrimer construction is demonstrated upon triazole substitution by a thiol dendron. These remarkable applications show the versatility and applicability of trz-AuNP in organic media. The strategy that consists of stabilizing stable AuNPs by easily synthesized 1,2,3-triazoles is conclusively powerful because of the complementarity between the stability of these AuNPs and the multiple applications resulting from facile triazole substitution at the AuNP surfaces.

EXPERIMENTAL SECTION

General Data. All solvents and chemicals were used as purchased. Dialysis was performed with a Spectra/Por 6 dialysis membrane. NMR spectra were recorded at 25 °C with a Bruker 300 (300 MHz) spectrometer. All of the chemical shifts are reported in parts per million (δ, ppm) with reference to Me_4Si for the ^1H and ^{13}C NMR spectra. IR spectra were recorded on an ATI Mattson Genesis series Fourier transform infrared spectrophotometer. UV−vis absorption spectra were measured with a PerkinElmer Lambda 19 UV−vis spectrometer. DLS measurements were made using a Malvern Zetasizer 3000 HSA instrument at 25 °C at an angle of 90°. Fluorescence emission spectra were recorded by a Spex FluoroLog 2 spectrofluorimeter. Cyclic voltammetry measurements: All electrochemical measurements were recorded under a nitrogen atmosphere at 25 °C.

Synthesis of the 1,2,3-Triazoles. Synthesis of Fc-trz: trimethylsilyl azide (0.23 mL, 1.8 mmol) was added to a DMF and MeOH solution (3 mL, 9:1) of CuI (7.5 mg, 0.04 mmol) and propargyloxymethylferrocene (300 mg, 1.18 mmol) under nitrogen atmosphere in a pressure vial. The reaction mixture was stirred at 100 °C for 12 h. The mixture was cooled to room temperature, then filtered, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 5:1 to 2:1) to afford Fc-trz in 60% yield (210 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 7.66 (1H, CH_{trz}), 4.65 (2H, CH_2-trz), 4.35 (2H, CH_2-Fc), 4.24 (2H, CH_{Cp}), 4.15 (2H, CH_{Cp}), 4.13 (5H, CH_{Cp}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145, 122.2, 82.7, 69.1, 68.9, 62.4, 50.2. IR: ν_{Cp} at 819 cm^{-1}, disappearance of the $\sigma_{C\equiv C}$ band at 2100 cm^{-1}. ESI-MS. Calcd for $C_{14}H_{15}FeN_3O$ [M + Na^+]: m/z 320.12. Found m/z 320.0. Anal. (calcd, found for $C_{14}H_{15}FeN_3O$): C (56.59, 56.70), H (5.09, 5.34), N (14.14, 13.87) .

Coumarin triazole (Cou-trz) was synthesized according to the literature.[28]

Synthesis of the AuNPs. *AuNP-1*. Fc-trz (19 mg, 0.06 mmol) and $NaBH_4$ (2.5 mg, 0.06 mmol) were dissolved in 20 mL of EtOH in a Schlenk flask, and a $HAuCl_4$ (4.3 mg, 0.012 mmol)/EtOH (10 mL) solution was then injected dropwise into the flask in a nitrogen atmosphere with vigorous stirring. The solution was further stirred for 1 h and purified by dialysis against a large volume of EtOH before analysis. AuNPs were prepared in MeOH or THF with the same procedure.

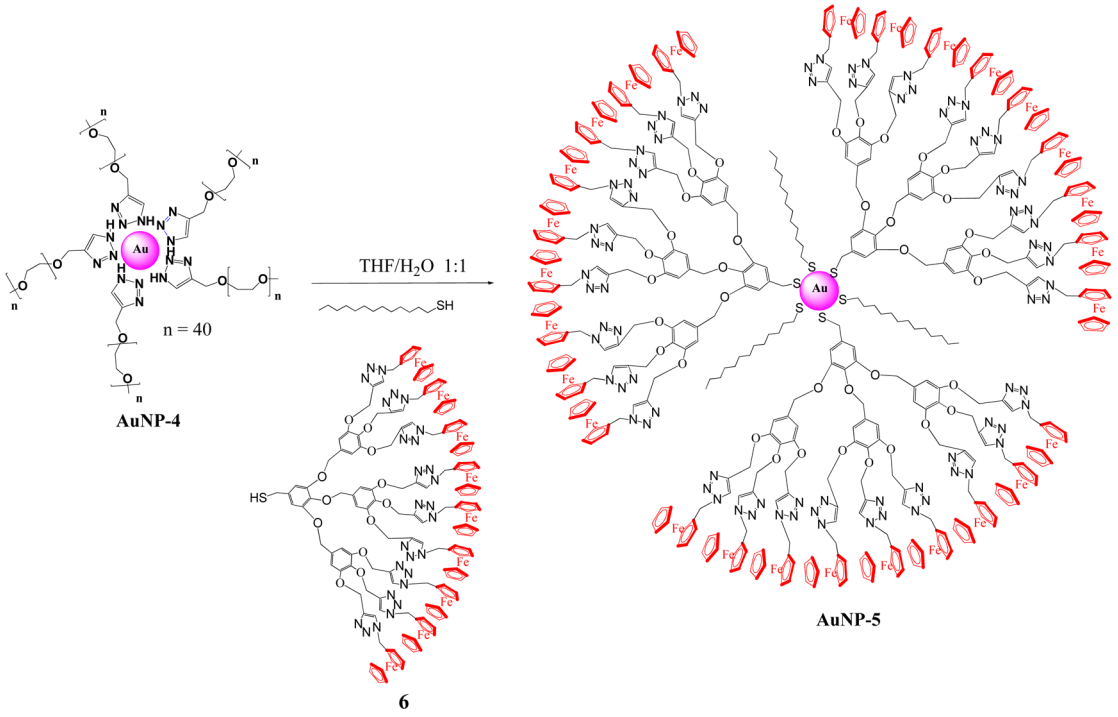
***AuNP-2*.** Fc-trz (11.5 mg, 0.04 mmol) was dissolved in 20 mL of EtOH in a Schlenk flask, and a $HAuCl_4$ (4.3 mg, 0.012 mmol)/EtOH (10 mL) solution was then injected into the flask under a nitrogen atmosphere with stirring. The solution was further stirred for 1 h and purified by dialysis against a large volume of EtOH before analysis. AuNPs were prepared in MeOH or THF with the same procedure.

***AuNP-3*.** Cou-trz (30 mg, 0.06 mmol) and $HAuCl_4$ (4.3 mg, 0.012 mmol) were dissolved in 20 mL of EtOH in a Schlenk flask that is refilled with nitrogen. A $NaBH_4$ (2.5 mg, 0.06 mmol)/EtOH (10 mL) solutionwas then added dropwise into the flask under a nitrogen atmosphere with stirring. The solution was further stirred for 1 h. **AuNP-3** was purified by precipitation in EtOH and redissolution in DMSO three times.

Catalytic Reduction of 4-Nitrophenol (4-NP). A typical procedure is as follows: An aqueous solution (2.5 mL) containing $NaBH_4$ (7.2 μmol) and 4-NP (0.09 μmol) was prepared in a 3 mL standard quartz cuvette (path length: 1 cm). **AuNP-2** (0.5%, 0.45 × 10^{-3} μmol; 1%, 0.9 × 10^{-3} μmol) in EtOH was injected into the cuvette. The reaction progress was detected by UV−vis spectroscopy every 40 s.

dx.doi.org/10.1021/ic5021498 | *Inorg. Chem.* 2014, 53, 11802−11808

Scheme 2. Ligand Substitution of the trz-Capped AuNP-4 by a Nonaferrocenylthiol Dendron on the AuNP Surface, Yielding the AuNP Thiolate Poly-Fc-Terminated Dendrimer (AuNP-5)



Sensing of Homocysteine (Hcy), Cysteine (Cys), and Glutathione (GSH). A gradually increasing amount of Hcy (40 mM, 0.5, 1, 2, 5, and 10 equiv) in water was added to a 2.5 mL solution of **AuNP-3** (0.05 mM; 3:7 water/DMSO) in a standard quartz cuvette (path length: 1 cm). The solution was monitored using a fluorimeter 10 min after mixing. Titration of Cys and GSH was conducted according to the same process.

Ligand-Substitution Reaction of AuNP-4 by a Mixture of Nonaferrocenyl Thiolate Dendron 6 and Dodecanethiol To Synthesize AuNP-5. A total of 3 mL of a THF solution of the nonaferrocenylthiol dendron[13a] (150 mg, 0.25 mmol) and dodecanethiol (10 mg, 0.05 mmol, 12 μL) was added into 3 mL (1 mM) of a mPEG-trz-stabilized AuNP (**AuNP-4**)[13a] and stirred for 10 min. Then 10 mL of dichloromethane was added to the mixed solution, and the organic phase was then separated and dried over Na_2SO_4. After evaporation of the solvent, AuNPs were washed with acetone and then EtOH followed by precipitation in dichloromethane/MeOH. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.61 (9H, C*H* in triazole), 6.71 (8H, H_{Ar}), 5.28, 5.16 (24 H, ArOCH_2−), 4.91 (18H, −CH_2Cp), 4.24−4.12 (81H, CH_{Cp}), 1.83, 1.59. 1.24 (22H, CH_2− in the alkyl chain), 0.86 (3H, CH_3CH_2−). UV−vis spectroscopy: plasmon band at 535 nm. DLS: 16.3 ± 3 nm.

Using Leff's method,[32] the number of AuNP atoms in **AuNP-5** and the number of ligands, 540 thiol ligands on the surface of **AuNP-5**, were determined. From the integration of H-1 (CH_3 in dodecanethiolate) and H-2 (C*H* in Cp) in the ^{1}H NMR spectrum (Figure S14 in the SI) of **AuNP-5**, the ratio of the numbers of the two ligands (Fc-dendron/dodecanethiolate) was calculated to be about 1:2.8; that is, 70% of the thiolates on the surface of **AuNP-5** are dodecanethiolate ligands (about 400 ± 70 dodecanethiolates per AuNP). Meanwhile, approximately 140 ± 25 Fc dendrons on average are located on each AuNP surface. The calculations indicate that there are on average about 1260 ± 180 Fc units surrounding every **AuNP-5** (see the SI).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Characterization and data of compounds and AuNPs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the China Scholarship Council (Ph.D. grants to N.L.), the Univ. Bordeaux, the CNRS, and L'Oréal is gratefully acknowledged.

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