How to very efficiently functionalize gold nanoparticles by "click" chemistry†

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Difficulties previously encountered in the very useful "click" functionalization of gold nanoparticles (AuNPs) resulting in low yields are now overcome by using specific conditions: 1 : 1 water–THF medium, stoichiometric $CuSO_4$ and sodium ascorbate, inert atmosphere at 20 °C that provide quantitative "click" reactions between azidoalkylthiolate-AuNPs with various hydrophilic (PEG-containing) and hydrophobic (organic and organometallic) alkynes.

Functionalization of gold nanoparticles (AuNPs)[1] is a key challenge given the broad range of applications of AuNPs in biology,[2] nanomedicine,[3] optics[4] and catalysis.[5] Since the seminal reports by Brust and Schiffrin[6] of the stable alkylthiolate AuNPs, considerable interest has arisen in their chemistry and physics. A large variety of functional AuNPs are now known,[1–7] but many families of AuNPs are still resistant to direct synthesis,[6] ligand exchange reactions[7] or functionalization using high-temperature or other incompatible processes.[1] Thus, in view of these multiple applications, it was essential to reconsider here the problem of the efficiency of AuNP functionalization.

The recently improved Huisgen 1,3-dipolar cycloaddition of azide with alkynes, called "click" chemistry, has opened new avenues in organic synthesis under mild conditions.[8] Thus, this strategy is potentially very useful to functionalize AuNPs and is the subject of the present report. Three recent papers reported attempts to carry out such "click" reactions on AuNPs containing thiolate ligands.[4] Brust's group introduced 2500 azide-functionalized thiolate ligands onto AuNPs of 12 nm diameter and 7 lipase groups by "click chemistry" using excess lipase, which corresponds to 0.3% of clicked azido groups.[9] Williams' group substituted 52% of the alkylthiolate ligands by reaction with bromoundecane thiols, and the substitution of the bromide by azide was achieved in 92% yield. The subsequent "click" reactions with several terminal alkynes (in excess) that were activated by a carbonyl linkage produced triazole rings with conversions mostly between 1 and 22% yields of 1,2,3-triazole formation in various solvents (54% was obtained in one specific case).[10] Simon's group assembled AuNPs on DNA templates *via* click chemistry using a 1000-fold excess of AuNPs.[11] These repeated low yields showed that difficulties are encountered in the application of "click" chemistry to the functionalization of AuNPs. They were attributed to the lack of reactivity due to solubility problems and to decomposition or aggregation of the Cu^I catalysts,[10] but they dramatically contrast with the exceedingly easy "click" reactions that are well known in organic synthesis to proceed under very mild conditions and that made these "click" reactions so popular.[8]

In addressing this problem, we reasoned that, on one hand, solubility must be an important constraint that needs to be carefully ensured for the click reaction, and that, on the other hand, a polar co-solvent such as water must be used to solubilize $CuSO_4$. We know that alkanethiol-coated AuNPs are soluble in organic solvents, not in water, which is a probable source of difficulty.[12] Thus, we investigated the possibility of using a homogeneous water–THF reaction medium obtained by adding the AuNPs in THF to the aqueous solution of a water-soluble alkyne or a THF–water solution of a purely organic alkyne. We also increased the amount of Cu^I catalyst obtained from $CuSO_4$ and sodium ascorbate in order to overcome the potential problem of aggregation of Cu^I, which was invoked as a reason for systematic low-yielding reactions. Finally, we found that it is essential to carry out the "click" reactions with AuNPs under an inert atmosphere, which inhibits re-oxidation of Cu^I, rather than the aerobic "click" reaction conditions generally used in organic synthesis.

If any of the above conditions is not fulfilled, we found that the "click" reaction proceeds in very low yields with thiolate-AuNPs, fully confirming the literature reports. If these precise conditions are fulfilled, however, we now find that the "click" reactions are virtually quantitative at room temperature in THF–water under an inert atmosphere between alkylthiolate-AuNPs terminated by azido groups and linear or dendronic alkynes, which leads to various functional AuNPs containing the 1,2,3-triazolyl groups, even including AuNP-cored dendrimers.[12,13] A variety of terminal alkynes including organic, organometallic, dendronic, polyethylene glycol (PEG), and long-chain dendronic, PEG-containg alkynes **1–6** were chosen (Chart 1) and always provided very satisfactory results despite the variety of size and hydrophilicity.

Undecanethiolate-AuNPs of 2.5 nm (by TEM) diameter containing 480 gold atoms and 230 dodecanethiolate ligands (from elemental analysis) were synthesized by the Schiffrin–Brust method,[6] then submitted to alkylthiolate ligand substitution by 1-bromoundecanethiolate using a 10-fold excess of 1-bromoundecanethiol in dichloromethane.‡ This led to AuNPs in which 72 ± 3% (*i.e.* 166 ± 6) of the

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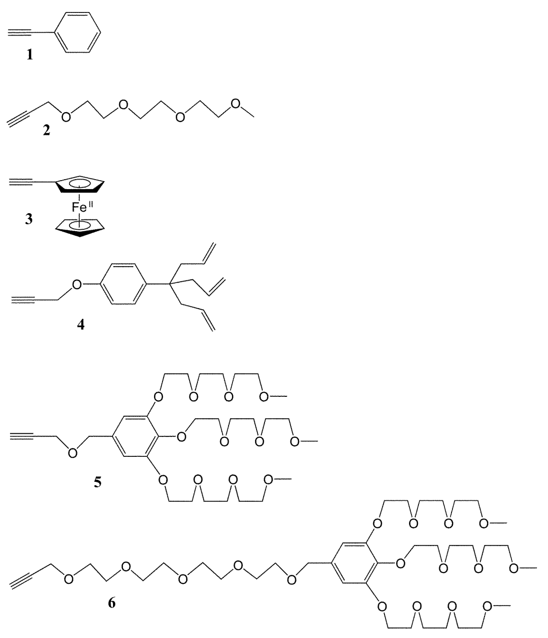
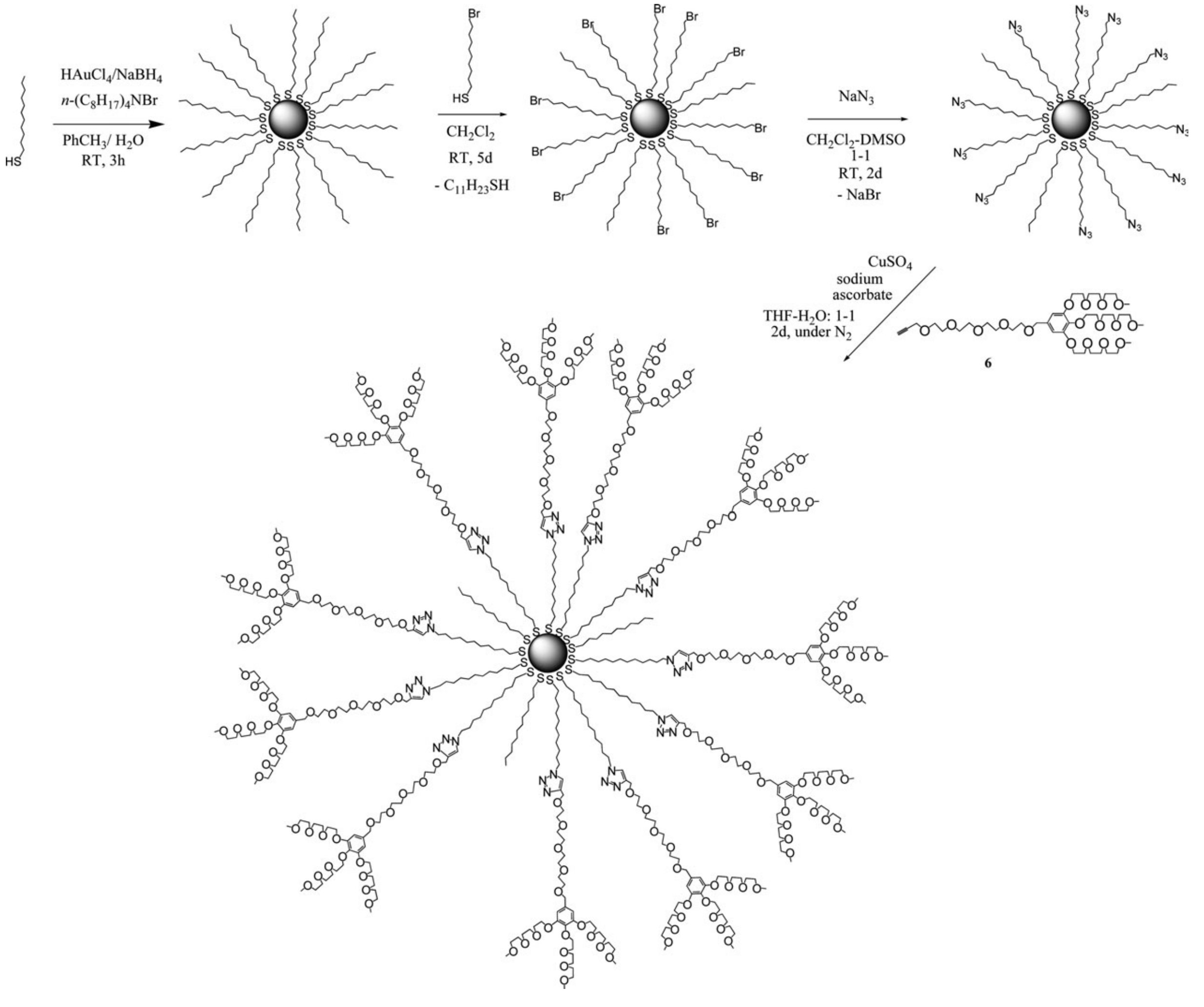


Chart 1

undecanethiolate ligands have been substituted as shown by ^{1}H NMR in $CDCl_3$ upon replacement of the terminal methyl and methylene signal at $\delta = 0.83$ ppm and δ 1.22 ppm, respectively by the CH_2Br signal at $\delta = 3.40$ ppm. After purification by reprecipitation upon addition of ether, quantitative nucleophilic substitution of bromide by azide was achieved upon reaction with a 20-fold excess NaN_3 in a dichloromethane–DMSO 1 : 1 mixture at ambient temperature (20 °C) for two days as shown, after purification upon reprecipitation using ether, by the complete replacement of the CH_2Br signal at $\delta = 3.40$ ppm by the CH_2N_3 signal at $\delta = 3.25$ ppm in the ^{1}H NMR spectra. The "click" reactions were carried out with the six terminal alkynes **1–6** (Chart 1) at room temperature under N_2 in water–THF for two days using a stoichiometric amount of $CuSO_4$ and sodium ascorbate. Then, extraction with dichloromethane and purification by precipitation was carried out using ether for the reactions with **1** and **3**, and using methanol for **4**, and from methanol solutions with ether for the polyethylene glycol-containing compounds **2**, **5** and **6**. (AuNPs functionalized with **1**, **3** and **4** are soluble in dichloromethane and chloroform, and the AuNPs functionalized with **2**, **5** and **6** are also water soluble.) Subsequent washing with a 15 N



Scheme 1 Overall synthetic scheme for the efficient functionalization of AuNPs under ambient conditions using the "click" reaction with the dendronic alkyne **6**.

aerobic aqueous ammonia solution removed the Cu^{II} cations from all the functionalized AuNPs. All these "click" reactions where found to be virtually quantitative under these optimized conditions, as shown by the disappearance of the CH_2N_3 signal at $\delta = 3.25$ ppm in the ^1H NMR spectra and N_3 absorption band at $\nu = 2094\ cm^{-1}$ and alkyne band at $\nu = 2160\ cm^{-1}$ in the IR spectra. Note that all the reactions of Scheme 1 proceed at room temperature, which is also the case for all the "click" reactions of **1–6**. The AuNPs can be dissolved and reprecipitated (*vide supra*) without change in the spectra, the plasmon band at 520 nm in the UV-Vis spectrum remaining constant along the reactions, and the TEM data showed that there was no change in the AuNP core size along the reactions of Scheme 1.

In conclusion, we have investigated the reasons causing the repeated low-yield "click" reactions with AuNPs, discovered the experimental solutions to overcome these difficulties, optimized the new "click" reactions with a variety of organic, organometallic, polyethylene glycol, and dendronic (including organic and PEG-containing) terminal alkynes and found a general procedure exemplified in Scheme 1 that works ideally in all the investigated examples. It is in particular noteworthy that PEG-containing AuNPs are biocompatible[14] and should be very useful in nanomedicine, an area that we are presently investigating.[15]

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Notes and references

‡ Experimental procedure: *Synthesis of the azidoundecenylthiolate AuNPs*: Undecanethiolate-AuNPs of 2.5 nm diameter (by TEM, see ESI†) were obtained by following the Schiffrin–Brust procedure using 0.9 mmol of $C_{11}H_{23}SH$, 0.9 mmol of hydrogen tetrachloroaurate (30 mL, aqueous solution) and 10 mmol of $NaBH_4$, after washing with ethanol and acetone followed by precipitation from ethanol. ^1H NMR ($CDCl_3$, 250 MHz): 1.22 (22H, CH_2), 0.83 (3H, CH_3). UV-Vis: plasmon band at 520 nm. TEM: diameter = 3 ± 0.4 nm. Undecanethiolate-AuNPs (130 mg) were dissolved in distilled CH_2Cl_2, and bromoundecanethiol (500 mg) was added to the solution that was further stirred for 5 d under N_2 at room temperature (RT). The solvent was then removed under vacuum, the resulting mixed AuNPs were precipitated with acetone and ethanol in order to remove the excess thiol. ^1H NMR ($CDCl_3$, 250 MHz): 3.40 (2H, CH_2-Br), 1.22 (22H, CH_2), 0.83 (3H, CH_3). UV-Vis: plasmon band at 520 nm. TEM: diameter = 3 ± 0.4 nm.
These mixed AuNPs (10 mg mL^{-1}, 150 mg) were dissolved in 15 mL CH_2Cl_2 and added to an equal volume of 0.25 M NaN_3 (244 mg) in DMSO. The solution was stirred for 48 h under N_2 at RT, water was added, and the black organic layer was isolated. This organic layer was dried over sodium sulfate and filtered, the solvent was evaporated, and the AuNPs containing the azidoundecenylthiolate ligands were washed with ethanol and dried. ^1H NMR ($CDCl_3$, 250 MHz): 3.26 (2H, CH_2-Br), 1.22 (22H, CH_2), 0.83 (3H, CH_3). UV-Vis: plasmon band at 520 nm. TEM: diameter = 3 ± 0.4 nm.
General procedure for the "click" reactions. The azidoundecenylthiolate-AuNPs (50 mg) and the alkyne substrate (0.1 mmol) were dissolved in THF. At 0 °C, $CuSO_4$ was added (2 equiv. *per* alkyne substrate, 1 M water solution), followed by the dropwise addition of a freshly prepared solution of sodium ascorbate (4 equiv. *per* branch, 1 M water solution) adjusted for a 1 : 1 THF : water ratio. The solution was allowed to stir for 2 d at 30 °C under N_2. After removing THF under vacuum, CH_2Cl_2 and an aqueous ammonia solution were added. The mixture was allowed to stir for 10 min in order to remove all the Cu^{II} trapped inside the AuNPs as $Cu(NH_3)_6^{2+}$. The organic phase was washed twice with water, dried with sodium sulfate, and the solvent was removed under vacuum. In order to remove the excess alkyne substrate, the product was precipitated with MeOH–ether for the PEG ligand, with CH_2Cl_2–MeOH for the triallyl aryl dendron, and with CH_2Cl_2–ether for the phenylacetylene and ferrocenylacetylene substrates.[14]
Example of the "click" reaction with **5**: ^1H NMR ($CDCl_3$, 250 MHz): 7.53 (1H, CH-triazole), 6.58 (2H, CH-arom.), 4.60 (2H, triazole-CH_2-0), 4.49 (2H, CH_2-CH_2-triazole), 4.14 (8H, OCH_2_arom. and arom-OCH_2CH_2), 3.66 (30H, OCH_2CH_2O), 3.37 (9H, CH_3O), 2.47 (2H, CH_2S), 1.25 (18H, $CH_2CH_2CH_2$), 0.87 (3H, CH_2CH_3). Elemental analysis: S (1.47%); Au (18.86%). ^{13}C NMR ($CDCl_3$, 62 MHz): 126.1 (CH of triazole and arom. core), 107.16 (C_qCH_2O), 70.39 (OCH_2CH_2O), 65.90 (triazole-CH_2-O), 58.91 (CH_3O). IR: disappearance of the ν_{N3}band at 2094 cm^{-1}. UV-Vis: plasmon band of the AuNPs: 520 nm in CH_2Cl_2. TEM: diameter = 2.5 nm (±0.3 nm).

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