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A nanoparticle–dendrimer conjugate prepared from a one-step chemical coupling of monofunctional nanoparticles with a dendrimer

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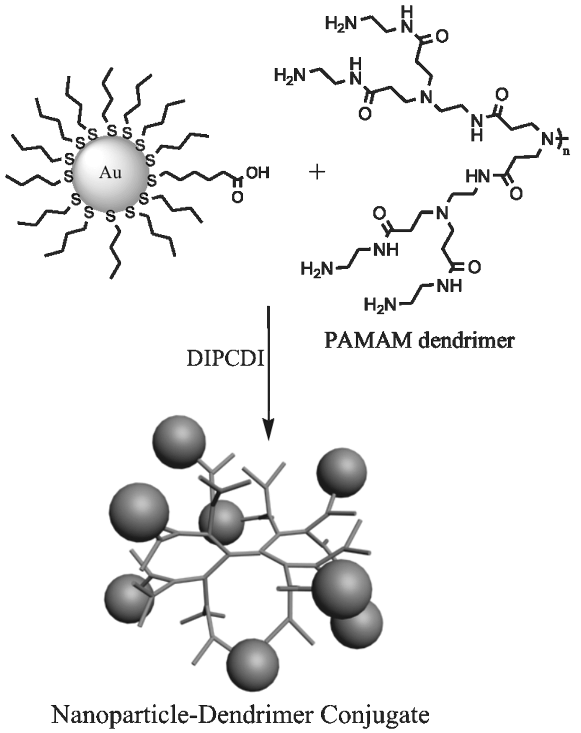
Gold nanoparticle–dendrimer conjugate clusters were prepared by a covalent coupling of monofunctional gold nanoparticles with a generation 5 PAMAM dendrimer.

Using wet chemical methods, it is now possible to prepare many different types of nanoparticles which exhibit potentially useful properties for device applications.[1,2] However, a major challenge that still remains is how to assemble these materials in a reliable manner to offer robustness and control over the materials' properties. Currently, self- or templated-assembling based on non-covalent chemical interactions is a major approach used for this purpose. In the templated-assembling aspect, polymers, both natural and synthetic, have been used as templates. As an example, poly(amidoamine) (PAMAM) dendrimers have been used as templates for nanoparticle preparation, as well as for the assembly of carboxylic acid-functionalized gold nanoparticles.[3–6] Rotello *et al.* observed that electrostatic interactions could be used for the controlled assembly of carboxylic acid-functionalized gold nanoparticles with PAMAM dendrimers.[5,6] In another example, DNA nucleotides have been used for the preparation of nanoparticle assemblies through complementary hydrogen bonding or electrostatic interactions.[7]

Despite the tremendous success and large variety of nanomaterials and nanoarchitectures developed from self- and templated-assembling, some general limitations also exist in self-assembled nanomaterials. The current assembling processes largely rely on non-covalent interactions between the templates and nanoparticles. These interactions can be disrupted or broken due to changes in pH, temperature, ionic strength or solvent polarity in the environment. Thus, the self-assembled nanomaterials may not be suitable for a wider range of applications. This lack of stability also limits how they may be processed into a final product, since a slight change in the chemical environment has a dramatic effect on the assembled nanomaterial architectures.

An alternative approach to preparing nanoparticle assemblies is to use covalent bonding. Recently, we developed a solid phase synthesis technique to prepare monofunctional gold nanoparticles, *i.e.*, nanoparticles with a single functional group attached on the surface.[8–10] We then demonstrated that covalent bonding between monofunctional gold nanoparticles and polymers could be used for the controlled preparation of nanoparticle–polymer conjugates. For example, nanoparticle rings of different sizes were prepared from the conjugation of monofunctional gold nanoparticles to polylysine of different molecular weights.[11] It was found that these nanorings remained stable, even after repeated drying and dispersing cycles, and it was also demonstrated that they exhibit non-linear optical properties.[12] Furthermore, we have shown that nanoparticle chains can be prepared using poly(acrylic acid) as the template. These examples demonstrated that sophisticated nanoarchitectures may be prepared by simple chemical reactions of monofunctional gold nanoparticles with appropriate polymers.

In this communication we further demonstrate the preparation of small gold nanoparticle–dendrimer conjugates through such an approach. Gold nanoparticles with a single carboxyl group on the surface were coupled to a generation 5 PAMAM dendrimer, as shown in Scheme 1. Monocarboxyl group-modified gold nanoparticles with an average core diameter of 2.8 nm were prepared using a previously reported solid-phase synthesis technique.†[8,9,13,14] In the presence of activation agent, di-*iso*-propylcarbodiimide (DIPCDI), the monocarboxylic–gold nanoparticles were expected to form amide bonds with the amino groups on dendrimer surface. A generation 5 PAMAM dendrimer has 128 primary amine



Scheme 1 The synthetic route used to prepare the nanoparticle–dendrimer conjugate from monofunctional gold nanoparticles and a generation 5 PAMAM dendrimer.

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groups on its surface and has an estimated diameter of 5.4 nm.[15] As a result, "giant" nanoparticle clusters consisting of about 4–10 nanoparticles per cluster were expected, as shown in Scheme 1. These nanoarchitectures, with controlled and limited numbers of particles and interparticle distances, may offer unique opportunities to study the interparticle interactions and their effect on the material properties.

The conjugation of monocarboxylic nanoparticles with the dendrimer was accomplished according to the following procedure. 400 μL of a monofunctional nanoparticle solution (0.75 mg mL^{-1}) in 1 : 1 dichloromethane/methanol solvent was mixed with 30 μL of a 5% weight dendrimer solution in methanol. 20 μL DIPCDI was added to the solution to promote amide bond formation and the solution allowed to react at room temperature for 24 h with gentle shaking. During this time, it was observed that the nanoparticles remained soluble and did not form precipitates. The samples were then centrifuged for several minutes, the nanoparticles remaining soluble in the solution. The lack of precipitates suggests that extensive nanoparticle cross-linking did not occur between multiple conjugates. Furthermore, it was found that the nanoparticle–dendrimer conjugates could be dried and redissolved back into solution with no apparent loss of solubility.

The coupling reaction product was subjected to TEM analysis without further purification. Fig. 1 shows a TEM micrograph of the nanoparticle–dendrimer conjugates. As can be seen, nanoparticles are not randomly distributed in the TEM grid, with many of them appearing as clusters ranging in size from 4–10 nanoparticles per cluster and with a diameter of 10–13 nm. This cluster size is not unreasonable, considering the diameter of the nanoparticles plus the nanoparticle protective monolayer and the estimated diameter of a generation 5 PAMAM dendrimer. A statistical analysis of over 3600 nanoparticles from multiple samples is shown in Fig. 2. The histogram indicates that the majority of the nanoparticles (55%) appear in clusters with sizes ranging from 4–10 nanoparticles per cluster, and that much smaller percentages are in the form of single particles, dimers/trimers or larger aggregates. The single nanoparticles could be those which did not react with the dendrimer or could represent dendrimers with only a single nanoparticle attached. Likewise, the dimers/trimers may be dendrimers with two or three nanoparticles attached. There are multiple possibilities which may explain the larger clusters (>10 particles per cluster). It is possible that these clusters may be dendrimers simply with a larger number of nanoparticles, but they may also be two or more nanoparticle–dendrimer conjugates in close proximity, such that they are overlapping. Finally, a small percentage of the nanoparticles may have more than one functional group, which could cause the cross-linking of two or more dendrimer molecules.

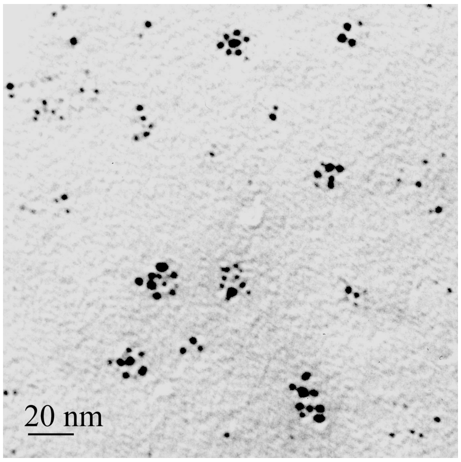

Fig. 1 TEM image of the nanoparticle–dendrimer conjugates. Samples were prepared by placing 1 μL of sample in an appropriate solvent onto a 300 mesh Formvar-coated copper grid and immediately wicking it off using filter paper. Images were acquired using a JEOL 100CX TEM at 80 KeV.

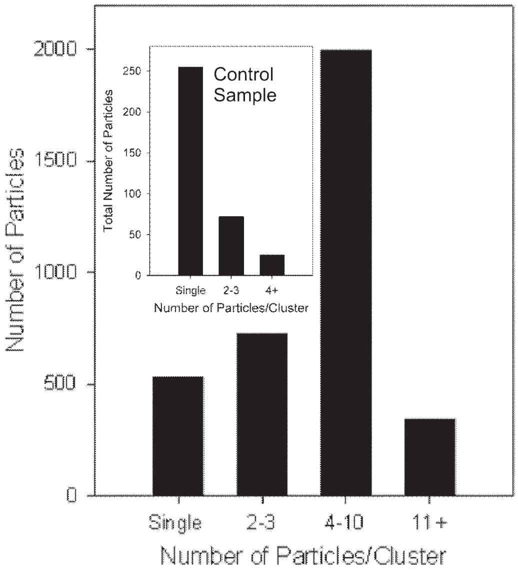

Fig. 2 Histogram showing the number of particles per cluster for various cluster sizes of nanoparticle–dendrimer conjugates and a control sample.

Other than the clustering of nanoparticles due to their conjugation with PAMAM dendrimer, we also noticed that some particles in the clusters appeared to be larger than the original monofunctional nanoparticles prior to the coupling reaction. We believe this is due to the fusion of two or three particles in proximity to each other through electron beam irradiation during the TEM imaging process. Nanoparticle fusion by laser or electron beam irradiation has been observed frequently in our nanoparticle studies and also noticed by other groups.[16] The nanoparticle size increase could not have been due to the coupling reaction because the UV-vis absorption spectroscopic study of the coupling product, as discussed below, did not show any obvious change to the surface plasmon resonance band compared to individual monofunctional gold nanoparticles.

In order to confirm that covalent bonding was responsible for the nanoparticle clusters, a control experiment was performed where the monofunctional gold nanoparticles and the dendrimer were simply mixed together, without DIPCDI being added to the reaction solution. Prior to TEM analysis, a trace amount of TFA was added to the sample to disrupt any non-covalent interactions between the dendrimer and the nanoparticles. TEM analysis showed that most of the nanoparticles were randomly distributed in the TEM grid, and that very few small aggregates were also present. A summary of the TEM results are shown in the inset to Fig. 2. These small aggregates may be the result of electrostatic

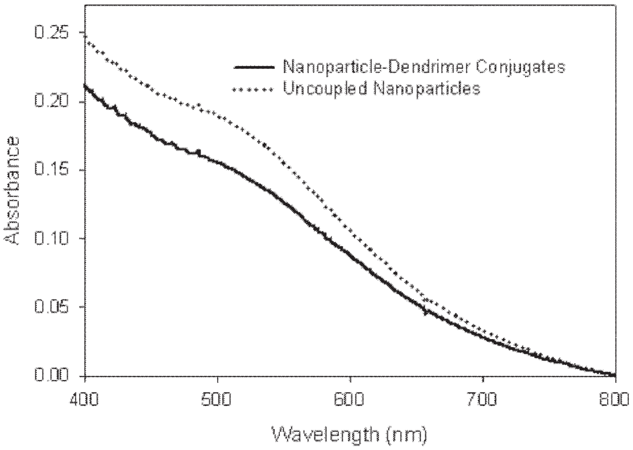

Fig. 3 UV-vis spectra of the gold nanoparticle–dendrimer conjugates and unbound nanoparticles mixed with dendrimers without DIPCDI being added. UV-vis absorption spectra were recorded using an Agilent 8453 spectrometer. The samples were prepared in a mixed solvent of 4 : 1 dichloromethane/methanol.

interactions due to the self-assembly of the nanoparticles with the dendrimer as they were dried on the TEM grid.

The UV-vis absorption spectra of the nanoparticle–dendrimer conjugates and the nanoparticles from the control experiment were compared, and are shown in Fig. 3. After reacting with the dendrimer, it was found that there was no apparent change in the wavelength or intensity of the SPR band. It is possible that the distance between adjacent particles after coupling with the dendrimer was great enough such that they acted like individual particles. Previously, it has been demonstrated that the SPR band of gold nanoparticle–dendrimer assemblies could be tuned depending on the dendrimer generation. However, in those experiments, the nanoparticles were much larger in diameter (6–7 nm) and had many carboxylic acid groups on their surface, which lead to much larger aggregates and more closely packed nanoparticles.[6] The nanoparticles used in the present case are much smaller, and it is known that the SPR band of such nanoparticles is very broad.

In summary, this work further demonstrates that covalent bonding can be used in a bottom-up approach for the controlled assembly of nanomaterials. These nanoparticle assemblies with limited numbers of particles provide a potential opportunity to examine nanoparticle–nanoparticle interactions and the quantitative effect of such interactions on their optical and electrical properties. Currently, we are studying the relationship between non-linear optical properties *vs.* cluster size and interparticle distance using the conjugates of nanoparticles and different generations of dendrimers. The results will be reported in due course.

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Notes and references

† *Experimental*: All solvents and organic chemicals (ACS reagents) were purchased from Aldrich (Milwaukee, WI) or VWR (West Chester, PA), except the following items: The gold salt $HAuCl_4 \cdot xH_2O$ was purchased from Strem Chemicals (Newburyport, MA). 1% divinylbenzene (DVB) cross-linked Wang resin, with particle size around 100–200 mesh and a hydroxyl group density of 1.4–3.2 mmol g^{-1}, was obtained from Advanced ChemTech (Louisville, KY). The Sephadex gel used in gel permeation chromatography (GPC) is a lipophilic dextrin gel LH-20 from Sigma with a separation limit of 7000 Dalton molecular weight. The C_{18}-modified silica gel for reverse-phase chromatography (RPC) was purchased from Sorbent Technology (Atlanta, GA) with a pore size of 100 Å. The acetyl-protected 6-mercaptohexanoic acid was prepared according to the reported procedure.[17] Butanethiol-protected gold nanoparticles were prepared according to the Brust–Schiffrin reaction.[18,19] The TEM images of nanoparticles and conjugates were obtained using a JEOL 100CX Transmission Electron Microscope at 80 KeV.

The synthesis of monofunctional nanoparticles was undertaken as follows: Briefly, acetyl-protected 6-acetylmercaptohexanoic acid was attached to the Wang resin through ester bond formation.[13,14] After acetyl group deprotection using a solution of 33% piperidine in DMF, the thiol groups were allowed to undergo a place exchange reaction with butanethiolate gold nanoparticles in a solvent mixture of 2 : 1 hexane/dichloromethane at 40°C for 8 h. After washing off any unexchanged nanoparticles, the resin-bound nanoparticles were cleaved from the resin using a solution of 20% trifluroacetic acid (TFA) in dichloromethane for 2–4 h. The nanoparticles were then dried using a stream of nitrogen gas and washed several times with petroleum ether. The sample was further purified by passing the sample through a column of Sephadex LH-20 gel using a solvent mixture of 9 : 1 dichloromethane/methanol. The final product was then obtained by passing the sample through a column of C_{18} reverse-phase silica gel using a solvent mixture of 4 : 1 dichloromethane/methanol.

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