Chain-like assembly of gold nanoparticles on artificial DNA templates *via* 'click chemistry'†

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We present a new type of azide-functionalized gold nanoparticle and their coupling to an alkyne-modified DNA duplex using the copper(I)-catalyzed Huisgen cycloaddition ('click chemistry'), resulting in a chain-like assembly of nanoparticles on the DNA template.

The proceeding miniaturization in microelectronics together with the size limitations and enormous costs of conventional lithographic processes requires the development of new strategies for the fabrication of electronic devices for the near future. The DNA molecule has become a promising template for the bottom-up assembly of nanoscale electronic devices due to its well-defined chemical structure and the multitude of electrostatic and chemical binding sites utilizable for modification with molecules, metal ions or metal nanoparticles. Diverse approaches have been reported for the continuous metallization of DNA within the past years.[1]

However, the fabrication of DNA-based circuit elements requires the spatially defined immobilization of chemical building blocks to the DNA strand which, due to their electronic structure, are capable of electrical switching or charge storage. The use of metal nanoparticles with diameters below 2 nm, which allow the exploitation of single electron tunneling effects, appears very promising in this context.[2]

For the assembly of metal nanoparticles and DNA, various methods targeting different binding mechanisms between the particle and the strand have been developed, including hybridization of single stranded oligomers, attached to the nanoparticle surface, with a template strand of complementary sequence, or electrostatic binding of nanoparticles to the charged DNA backbone[3,4] In other works, the decoration of the DNA has been accomplished by the use of cisplatin as an interconnector between the DNA bases and amino groups present in the nanoparticle's ligand shell.[5]

Mostly, native DNA with a high number of potential binding sites along the DNA backbone has been applied for the formation of such nanoparticle DNA conjugates. However, the use of artificially synthesized DNA which incorporates modified DNA bases can provide spatially defined binding tags for the nanoparticles which may overcome the lack of site specificity in native DNA. Recently, we reported on the decoration of such artificial, alkyne-modified DNA strands with different azide-modified molecules by using a Cu(I)-catalyzed version of the Huisgen cycloaddition ('click chemistry').[6,7] The 'click chemistry' approach has also been applied to bind acetylene-functionalized lipase to the surface of 14 nm gold nanoparticles, while retaining the enzymatic activity of the complex.[8]

Here, we describe a new type of gold nanoparticle functionalized with an azide-modified glutathione derivative. These particles can rapidly be coupled to alkyne-modified DNA duplexes to prepare chain-like DNA–nanoparticle conjugates. Schematic representations of the nanoparticle synthesis and their immobilization to the DNA template are depicted in Fig. 1.

The synthesis of the alkyne-modified DNA was accomplished by replacing a natural triphosphate with the alkyne-modified triphosphate **2** in a PCR-reaction, as we already described in a previous work.[7a] The two selected primers amplify a 294 base pair long fragment of the polymerase η gene of yeast.

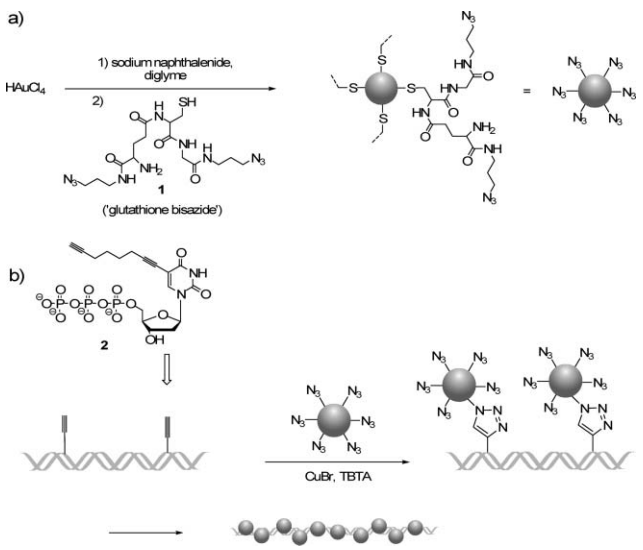

Fig. 1 (a) Schematic of the two-step cluster synthesis: (1) the reduction of the gold-precursor, dissolved in diglyme, by sodium naphthalenide; (2) the stabilization of the gold particles by the addition of azide-modified glutathione **1**. (b) Schematic of the immobilization of the azide-terminated nanoparticles to alkyne-modified DNA strands *via* a 'click' reaction. DNA was synthesized utilizing the alkyne-modified triphosphate **2**.

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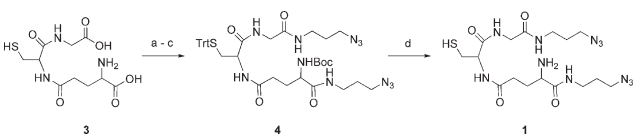
The PCR-reaction was performed with polymerase *Pwo* without additives. The sequence of the DNA template is given in the ESI†. The azide modified glutathione ligand was synthesized according to standard procedures for peptide couplings (Scheme 1).

First, glutathione **3** was *S*-trityl and *N*-Boc protected according to literature procedures.[9] Subsequently, both free carboxyl groups were coupled with two equivalents of 3-aminopropylazide (prepared from 3-aminopropyl bromide)[10] according to a standard peptide coupling protocol to give the protected bisazide **4**. The IR spectrum of this compound showed a strong absorption at 2094 cm^{-1}, which is a diagnostic indication of the azide group. After removal of the protecting groups by treatment with trifluoroacetic acid and triethylsilane as a cation scavenger, the water soluble bisazide **1** could be obtained and purified by reversed phase HPLC.

The synthesis of the azide terminated gold nanoparticles was performed according to a two-step synthesis for nanoparticles developed by Schulz-Dobrick *et al.*[11] The gold precursor $HAuCl_4$ was dissolved in diglyme and subsequently reduced with sodium naphthalenide, yielding primary nanoparticles weakly stabilized by the solvent. Stabilizing ligands need to be added to the solution in order to generate the final ligand-stabilized nanoparticles. We used the glutathione azide ligand **1** for the final stabilization of the nanoparticles. The size of the prepared nanoparticles can be influenced by the amount of reducing agent and the rate of its addition, the interval between reduction and final ligand modification, and the ratio of the stabilizing ligand to gold. As was already demonstrated by Schulz-Dobrick *et al.*, the size of the primarily formed nanoparticles increases with the amount of reducing agent added. Furthermore, we observed an increase in particle size with increasing gold-to-ligand ratio in the stabilization step. In order to avoid a large excess of free glutathione azide molecules in the 'click'-reaction, we utilized equimolar amounts of ligand and $HAuCl_4$. With this ratio we could obtain stable nanoparticles, whereas experiments using less glutathione azide in the synthesis yielded unstable particle dispersions. The nanoparticle product was analyzed using TEM and AFM (Fig. 2) and used without further purification for the immobilization on DNA experiments.

AFM analysis of the particles immobilized on the mica surface reveal a maximum height of 2–3 nm with no particle aggregates observable (Fig. 2a and ESI†). In the TEM micrograph (Fig. 2b), the particles deposited on an amorphous carbon supporting foil appear marginally larger and a few aggregates can be observed. Statistical analysis of the particle size distribution performed on the TEM data (see ESI†) yielded a mean particle diameter of 4 nm. The tendency of the particles to aggregate on the amorphous carbon support (in the TEM study) and the absence of aggregation on the mica surface (by AFM analysis) can be explained by the



Scheme 1 (a) Ph_3CH, CH_3COOH, $BF_3{\cdot}OEt_2$, 59%; (b) Boc_2O, NaOH, H_2O, dioxane, 90%; (c) carbonyldiimidazole, THF, then 3-aminopropylazide, 68%; (d) TFA, H_2O, Et_3SiH, CH_2Cl_2, 80%. Boc = *tert*-butyloxycarbonyl, Trt = trityl.

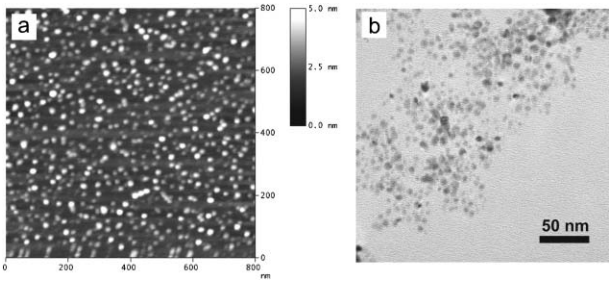


Fig. 2 (a) AFM height image of the glutathione azide-stabilized nanoparticles immobilized on mica; (b) TEM micrograph of the glutathione azide-stabilized nanoparticles (see section analysis of the AFM image and TEM-derived size distribution in ESI†).

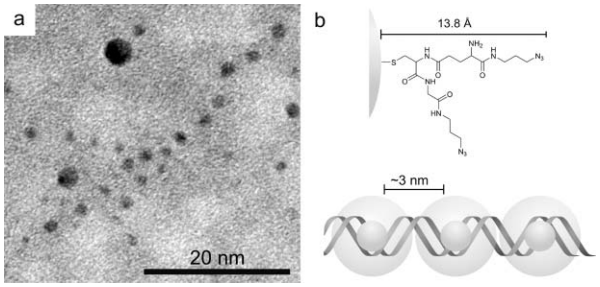


Fig. 3 (a) representative TEM micrograph of one-dimensionally and equidistantly assembled nanoparticles on DNA immobilized on the TEM foil. (b) Model showing twice the length of the long chain of the glutathione azide ligand as the crucial factor to explain the formation of the uniform arrangement.

difference in wetting and drying behavior of the water–diglyme particle dispersions on such substrates. The presence of aggregates on the TEM foils can introduce a systematic error in the statistical analysis of the TEM data and explain the shift in the mean particle size in the direction of larger diameters compared to the results of the AFM studies.

The glutathione azide-modified nanoparticles were bound to the alkyne-modified DNA duplexes *via* the formation of triazole linkages in the copper(I)-catalyzed 'click' reaction.[12] We recently developed a 'click' method for the highly efficient post-synthetic labelling of PCR fragments carrying multiple alkyne nucleobases with a variety of azides.[7a] As a catalyst, Cu(I) complexes of the ligand TBTA (tris((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amine) were used.[13] Incubation of the alkyne-tagged 300mer double strands with the nanoparticle solutions and the aforementioned catalyst system in a diglyme–water mixture indeed yielded DNA strands decorated with gold clusters. In order to avoid cross-linking of the DNA strands due to multiple surface functionalization of the azide-terminated gold nanoparticles, about a 1000-fold excess of nanoparticle material was used in the experiments. After completion of the 'click' reaction, the product was immobilized on amorphous carbon substrates and studied by TEM methods. Furthermore, AFM analysis of the reaction product on mica surfaces was performed (ESI†). In order to exclude the possibility that the immobilization of the particles on the DNA template is a result of electrostatic interactions, a control experiment was performed in which the particles were incubated with the DNA template without the addition of the Cu^{1+}-catalyst. In this case, no

cluster decorated DNA strands, but only a few aggregated structures without nanoparticles visible in the AFM phase image could be found on the mica surfaces. This effect might be caused by the hydrophobic surface of the alkyne-modified DNA strands.

Fig. 3a shows a representative TEM micrograph of the product of the 'click' reaction. A linear arrangement of nanoparticles assembled on the DNA template is clearly visualized in these images. It should be noted that the DNA template as well as the ligand shell of the nanoparticles is invisible due to a lack of contrast for organic material in TEM. As can be deduced from this and further TEM micrographs (see ESI†) of the 'click' reaction product, the one dimensional nanoparticle arrangement exhibits a nearly equidistant interparticle spacing of approximately 2.8 $\pm$ 0.5 nm. The high density of the particle coverage on the strands may result from the small distance between the alkyne tags in the DNA strands, which in the present case is the distance between two modified thymine bases.

Owing to the statistical distribution of the four DNA bases over the length of the DNA strand and the fact that we replaced all thymine bases in the strands with the alkyne-modified derivatives, a frequency of one alkyne group per four bases in the single strand and one alkyne group per two base pairs in the DNA duplex can be expected. Thus, we can estimate a distance of approximately 0.68 nm between two adjacent binding sites, which theoretically provides more than one alkyne tag per particle. The measured interparticle distance of 2.8 $\pm$ 0.5 nm (determined from the TEM micrographs) is presumably affected by the steric hindrance of the clusters due to their organic ligand shell. In a simple model, the space required by the ligand shell, *i.e.* the thickness of the ligand shell, can be estimated by calculating the binding lengths from the thiol group to the azide group in the longer chain of the glutathione molecule. For the fully extended conformation of the ligand, this calculation results in a value of approximately 1.4 nm (Fig. 3b). If the DNA template is regarded as a rigid rod in the simplified model, the average interparticle distance will be twice the thickness of the ligand shell, approximately 2.8 nm. Though this model is strongly simplified and disregards the flexible helix structure of the DNA, repulsion effects or interlocking of the ligand shells of two adjacent nanoparticles, the theoretical value matches the experimental value observed by TEM extremely well.

Besides the regular interparticle spacing, the TEM micrographs reveal that the one dimensional array consists of nanoparticles of a very homogeneous size of 1.6 nm without the ligand shell. Assuming that the metal cluster is enclosed by the major groove of the DNA due to multiple triazole formation, a structure guiding effect of the DNA on the array is possible, which could explain this uniform particle size.

Summarizing, we have reported on the synthesis of a new type of gold nanoparticle which is functionalized with a glutathione azide derivative. We have demonstrated that these particles can rapidly be coupled to artificial, alkyne-modified DNA duplexes using the copper(I)-catalyzed Huisgen cycloaddition reaction ('click chemistry'). As a result, we could obtain one-dimensional nanoparticle arrangements showing dense coverage of the DNA with particles with highly regular interparticle distances. The potential for this method is based on the versatility of this coupling chemistry to bind the nanoparticles to different alkyne-functionalized targets, as well as on the high degree of spatial control for the immobilization which can be influenced by the defined incorporation of the alkyne-modified bases in the synthesis of the DNA duplexes. Furthermore, this approach to covalent immobilization of metal clusters on a highly programmable template holds great promise for the development of novel nanoscale electrical circuit elements.

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