A facile *in situ* generation of dithiocarbamate ligands for stable gold nanoparticle–oligonucleotide conjugates†

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Here we demonstrate a facile strategy of preparing gold nanoparticle (AuNP) and DNA conjugates by *in situ* generation of strong metal affinity capping ligands, dithiocarbamates (DTC) modified oligonucleotides; the conjugates produced are stable at elevated temperature, resistant to ligand displacement and preserve the functionality of the conjugated oligonucleotides.

For a decade, nanomaterials have shown huge potential in the fields of biosensing, diagnostics and molecular therapeutics owing to their excellent physicochemical and optoelectronic properties.[1] Gold nanoparticles, in particular, have gained substantial interests due to their compatibility with various biosystems and that they can be easily functionalized with biomolecules. The biomolecules can bind to the target molecule with high selectivity and affinity due to inherent molecular recognition and induce a detectable optical or electrical signal change by the nanoparticles nearby. Recent research reports witnessed the applications of gold nanoparticle–oligonucleotide (AuNp–DNA) conjugates in the detection of specific DNA sequences,[2] proteins,[3] metal ions,[4] and biologically relevant small molecules.[5] More recently, AuNp–DNA conjugates have been shown to control gene expression in cells.[6] AuNp–DNA conjugates have also been used as a building block for assembling a variety of novel two and three dimensional structures and materials.[7] Lu and coworkers have utilized a phosphorothioate backbone to assemble gold nanoparticles in precisely defined positions.[8]

Oligonucleotides can easily be conjugated to gold nanoparticles by chemical adsorption of thiol-modified oligonucleotides to the surface of gold nanoparticles. Although easy to achieve, AuNp–DNA conjugates prepared in this manner are relatively unstable due to the non-covalent nature of the bond between gold and thiol. The Au–thiol bond is dynamic in nature with lower stability at elevated temperatures.[9] Thiol bonded to gold surface can exchange with free thiols in solution and can be easily oxidized by the oxidizing agents present in solution.[10] The Mirkin group has done some pioneering works in enhancing the stability of AuNp–DNA conjugates by utilizing steroid cyclic disulfide groups,[11a] multiple thiol-anchors,[11b] and triple cyclic disulfide moieties as anchors.[11c] In a recent report, thioctic acid has been used to generate stable AuNp–DNA conjugates.[12] All these strategies resulted in relatively more stable AuNp–DNA conjugates but involve complicated synthetic procedures. A strategy that is easy to work with, less time consuming and less expensive is highly desirable. In this report, we demonstrate an easy and robust way to prepare highly stable AuNp–DNA conjugates by functionalization of DNA oligonucelotides by a dithiocarbamate group (DTC–DNA), from an *in situ* reaction of CS_2 and amine-modified oligonucleotides, as bidentate capping ligands on gold nanoparticle surface.

Dithiocarbamates (DTC) are organosulfur compounds and are commonly used in agriculture as pesticides, in rubber industry as antioxidants and vulcanization accelerators.[13] Their strong affinity for the metal ions accounts for enzyme inhibition and thus, makes them biologically significant entities. DTC gained substantial attention in nanotechnology due to the ease with which they can be synthesized and the fact that small interatomic distance between two sulfur atoms of the ligand confer stronger affinity between ligands and the metal surface. DTC conjugated with small organic entities have previously been shown to bind strongly on Au nanoparticles and stabilize them.[14] Recent reports have demonstrated the utility of DTC ligands in stabilizing nanomaterials, in particular quantum dots[15a] and nanorods.[15b] These ligands were generated with ease by treating CS_2 with primary or secondary amines in an alkaline media.

In our experiment, to generate the DTC–DNA *in situ*, amine-modified oligonucleotides were reacted with an equimolar of CS_2 (100 μM) in 10 mM borate buffer (pH 9) for one hour. An aqueous solution of 13 nm Au nanoparticles was added to the reaction mixture with continued shaking (Fig. 1). An aqueous suspension of colloidal Au nanoparticles was prepared by literature methods[16] and treated with ion-exchange resin (Amberlite MB-150) to remove excess surfactants (see ESI†). To ensure a complete surface coverage of the Au nanoparticles, amine-modified oligonucleotides were added in excess (1000 : 1 ratio to AuNP) to generate sufficient amount of DTC–DNA. Aging of the AuNp–DNA conjugates was done with a stepwise addition of NaCl over 3–4 days to the final concentration of 0.3 M of NaCl (for details, see ESI†) with an aim to maximize the surface coverage of DNA on AuNps. The AuNp–DTC–DNA conjugates were centrifuged to form a pellet, washed with water and then washed with 10 mM PBS buffer (phosphate buffered saline, 0.3 M NaCl, pH 7) to remove the excess free oligonucleotides. The AuNp–DTC–DNA conjugates were finally reconstituted in PBS buffer for further analysis. AuNp–DNA conjugates *via*

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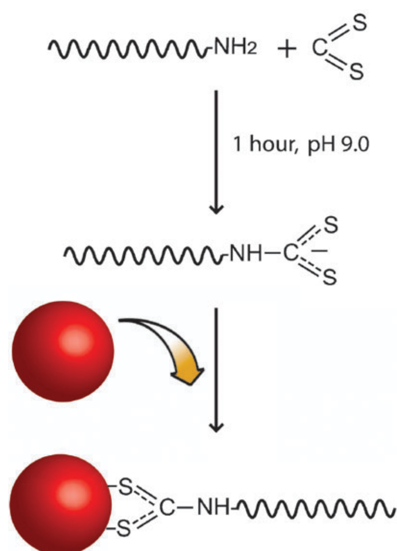

Fig. 1 Schematic of the process to generate dithiocarbamate–DNA ligands and AuNp–DTC–DNA conjugates. In the first step, CS_2 and amine-modified oligonucleotides are reacted for an hour at pH 9 to generate the DNA oligos modified with a dithiocarbamate group. In the second step, a colloidal solution of Au nanoparticles was added; capping the Au nanoparticle surface with the DTC–DNA leading to stable AuNp–DNA conjugates.

conventional chemical adsorption of monothiol-modified oligonucleotides were also prepared (referred to AuNP–T-DNA, see ESI†), and this was used as a control to test the stability of AuNp–DTC–DNA conjugates.

Spectroscopic analysis in the presence of Cu^{2+} salts was used to confirm the generation of DTC ligands in the reaction mixtures.[16] A solution of the DTC–DNA prepared turned yellow when an aliquot of $CuSO_4$ solution (1 mM) was added, showing a UV-vis absorbance peak at 390 nm, which is typical for a DTC–Cu^{2+} complex[17] (see ESI Fig. S2†). Control experiments of CS_2 or amine-modified oligonucleotides alone with copper salts showed no peak at 390 nm. These confirmed that DTC–DNA ligands were generated.

The dithiothreitol (DTT) displacement reaction was used to assess the stability of both AuNp–DNA conjugates. DTT is known to be able to displace the thiolated oligonucleotides from Au nanoparticle surface and results in irreversible aggregation.[11b] UV-Vis absorbance was used to monitor the aggregation process after addition of DTT at 40 °C for different time intervals (Fig. 2). AuNp–DTC–DNA conjugates showed no sign of aggregation even after one hour of the DTT addition, in 2 hrs aggregation started and eventually got completed in 4 hrs as evidenced by red shifting of the spectrum and broadening of surface plasmon band (Fig. 2A). In contrast, the AuNp–T-DNA exhibited aggregation within 1–2 mins of DTT addition (Fig. 2A). Au nanoparticles treated with CS_2 alone or amine-modified oligonucleotides alone did not show any sign of stability against DTT addition and got aggregated immediately. The enhanced stability of AuNp–DTC–DNA conjugates is due to stronger binding of bidentate DTC ligands towards Au nanoparticles surface. Additionally, to clarify whether the incurred stability of AuNp–DTC–DNA conjugates is a result of stronger binding of DTC ligands but not because of the difference in the number of surface ligands, the number of adsorbed DNA was quantified and compared to that of AuNp–T-DNA conjugates (see ESI† for details). We found that the surface coverage of AuNps was almost same in both conjugates, $\sim$31 pmol/cm^2 for DTC–DNA and $\sim$33 pmol/cm^2 for monothiol capped T-DNA, thus confirming that the stability achieved was due only to the stronger binding ligands. Furthermore, AuNp–DTC–DNA conjugates display high stability in elevated temperatures, with no sign of aggregation even after keeping at 90 °C for 7–8 hrs in 10 mM PBS buffer.

To confirm that oligonucleotides in AuNp–DTC–DNA conjugates retain their hybridization properties even after prolonged treatment with DTT, two sets of conjugates were prepared with amine-modified oligonucleotides DTC1 and DTC2 with complementary sequences (Fig. 3A). These two AuNp–DNA conjugates were kept in 10 mM DTT in PBS buffer for one hour before mixing. UV-Vis spectrum (Fig. 3B) showed a red-shifted broad surface plasmon band, typical of

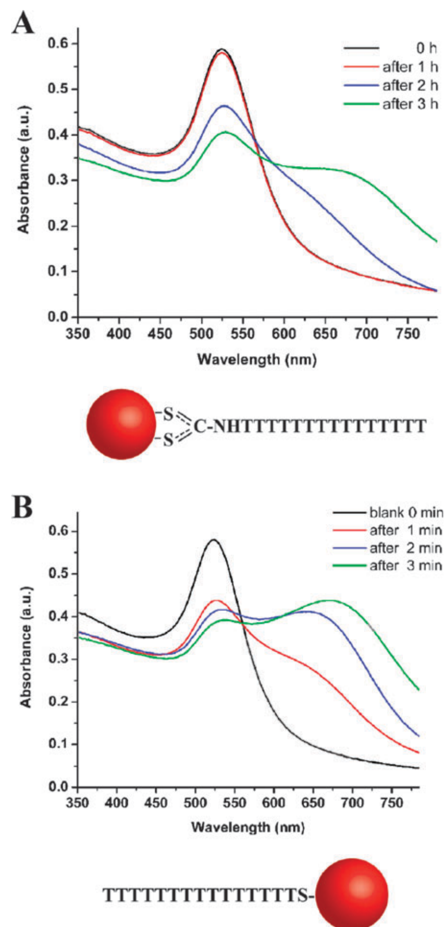

Fig. 2 UV-Vis absorbance assay after addition of DTT (10 mM). (A) AuNp–DTC–DNA conjugates; (B) AuNp–T-DNA conjugates. AuNp–DTC–DNA conjugates clearly show a significantly improved stability. In contrast, conjugates of AuNp–T-DNA started showing aggregation instantly upon the addition of DTT and completely aggregated within 2–3 minutes. Sequences of the oligonucleotides used in the preparation of AuNp–DNA conjugates are shown below each graph.

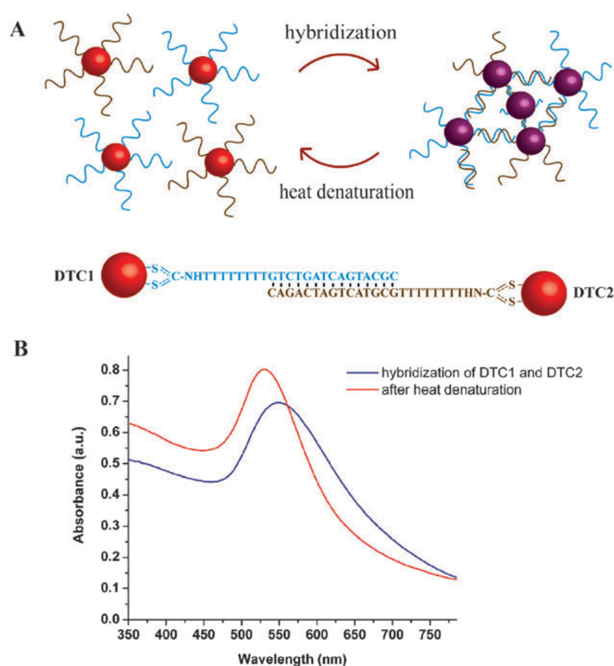

Fig. 3 Hybridization assay of the AuNp–DTC–DNA conjugates. (A) A schematic of the colorimetric assay showing reversible hybridization of AuNp–DNA conjugates. Two sets of AuNp–DTC–DNA conjugates with complementary amine-modified oligonucleotides, namely DTC1 and DTC2 were prepared. Sequences of the oligonucleotides used in the hybridization assay are also shown. (B) UV-Vis absorbance spectrum shows the broad red shifted surface plasmon band corresponding to the aggregation of AuNp–DNA conjugates. To check whether the aggregation is reversible, the aggregates were heat denaturated at 90 °C for 5 minutes and UV-vis spectrum was taken again after thermal denaturation. The blue shifted band at ∼520 nm clearly revealed the reversible nature of the aggregates, therefore providing clear evidence that aggregation of conjugates is because of the hybridization of the complementary oligonucleotides and not by the irreversible ligand displacement by DTT.

the aggregation pattern obtained by mixing AuNp–DNA conjugates tagged with complementary oligonucleotides. Heat denaturation of the aggregates yielded the blue-shifted absorption maximum corresponding to that of the individual AuNp–DNA conjugates before hybridization. This indicated that these conjugates retain their hybridization properties even in the presence of DTT for a long time.

We also evaluated the stability of AuNp–DTC–DNA conjugates in different pH (for details, see ESI†). It was observed that in solutions of low pHs from 2 to 4, these AuNp–DTC–DNA conjugates exhibited less stability and were aggregated within few hours, however they were very stable in solutions of pH 6 to pH 10 for weeks to months with no sign of aggregation.

In summary, we have demonstrated a method for enhancing the stability of AuNp–DNA conjugates by *in situ* generation of dithiocarbamate ligands by reacting CS_2 with amine-modified oligonucleotides. This strategy not only increases the robustness of the AuNp–DNA conjugates but also is inexpensive and less tedious. This strategy may contribute to improve Au nanoparticle based bio-sensing systems particularly in biological buffers where small molecules may interfere with the diagnostic assays and stable AuNp–DNA conjugates are highly desirable. The increased robustness combined with easiness to generate these ligands will witness wider applications of AuNp–DNA conjugates in diagnostic assays and their use as building blocks for assembling nanostructures with nanoparticle-oligonucleotide conjugates.

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