Supplementary Material (ESI) for Chemical Communications

A Facile In situ Generation of Dithiocarbamate Ligands for Stable Gold Nanoparticle-Oligonucleotide Conjugates

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Experimental Section:

Preparation of 13 nm Gold nanoparticles. Gold nanoparticles were prepared following literature methods.[1] In a typical experiment, 100 mL of 1 mM of $HAuCl_4$ (Sigma Aldrich) was taken in round bottomed flask and brought to boiling with vigorous stirring followed by the addition of 10 mL of 38.8 mM sodium citrate (Sigma Aldrich) to the above boiling solution. Boiling was continued for additional 20 minutes. Heating was removed and solution was brought to room temperature with continuous stirring. Gold nanoparticles prepared thus were treated with mixed bed ion-exchange resin (Amberlite MB-150, Sigma Aldrich) to remove free surfactants and were used for further experiments.

Preparation of monothiol modified AuNp-DNA conjugates. DNA oligonucleotide (T_{15}-mer) functionalized with 5'-disulfide moiety was purchased from Integrated DNA Technologies (IDT Coralville, IA) and purified by denaturing polyacrylamide gel electrophoresis. The DNA oligonucleotide was treated with 10 mM Tris (2-Carboxyethyl) phosphine Hydrochloride (TCEP.HCl, Sigma Aldrich) for 30 minutes at pH 7 to reduce the dithiol group to monothiol followed by desalting with G-25 columns (Sigma Aldrich). Monothiolated DNA oligonucleotide was then mixed with 13 nm gold nanoparticles in a molar ratio of 1000:1 (oligonucleotide:nanoparticle) and the mixture was kept shaking for 12 hours. To the above conjugates, an aliquot of stock solution of NaCl was added to bring the salt concentration to 100 mM with continuous shaking for another 12 hrs. Subsequent addition of 100 mM NaCl was done to the above mixture and stirring was continued for additional 12 hrs. Finally, the salt concentration was increased to 300 mM and stirring was continued for another 12 hrs. The aging of the conjugates was done to enhance the surface coverage of the Au nanoparticles with DNA oligonucleotides. The conjugates thus prepared were centrifuged and washed with 10 mM PBS

buffer (0.3 M NaCl, pH 7). The conjugates were washed 2-3 times to remove unbound oligonucleotides and stored in 10 mM PBS buffer for further use.

Preparation of dithiocarbamate-capped AuNp-DNA conjugates. Amine-modified oligonucleotides (T_{15}-mer, DTC1 and DTC2) were purchased from IDT and purified by denaturing PAGE gel. CS_2 was dissolved in water to give a saturated solution (28 mM). Amine-modified oligonucleotides were treated with an equimolar concentration (100 μM) of CS_2 aqueous solution in borate buffer (pH 9). The reaction was continued for one hour to generate the dithiocarbamate ligands. Gold nanoparticles (13 nm) were then added to the above mixture and kept shaking for overnight. Aging of the AuNp-DNA conjugates was done as described above to achieve the final concentration of 300 mM NaCl. The conjugates were centrifuged and washed 2-3 times with PBS buffer containing 300 mM NaCl. The conjugates were finally reconstituted in PBS buffer for further analysis.

Stability of dithiocarbamate-capped AuNp-DNA conjugates in solutions of different pH.

Table S1

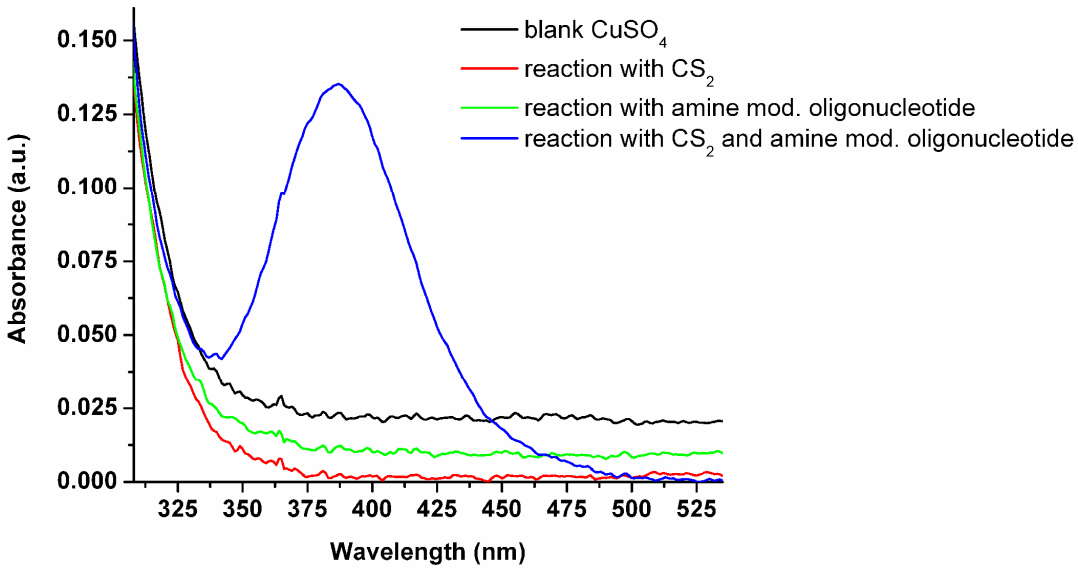
Solution pH	2	4	6	8	10
Stability of AuNp-DNA conjugates	2 hrs	5 hrs	week	months	months

Quantification of monothiolated DNA and DTC-DNA on gold nanoparticles. AuNp-DNA conjugates were prepared by mixing both monothiolated DNA and DTC-DNA (prepared by mixing amine-modified DNA with CS_2 as outlined above) with 13 nm gold nanoparticles in a molar ratio of 1000:1 (oligonucleotide:nanoparticle) and the mixture was stirred for 12 hrs. The conjugates were then aged to a final concentration of 0.3 M NaCl to enhance the surface coverage of AuNps with respective oligonucleotides. AuNps were quantified by measuring the UV-Vis absorption of AuNp-DNA conjugates prepared both by monothiolated DNA and DTC-DNA at the absorption maximum of ~520 nm. The conjugates were then centrifuged and the supernatant was quantified for DNA oligonucleotides by measuring the UV-Vis absorption at the absorption maximum of ~260 nm. The quantity of DNA adsorbed on AuNps was calculated by

subtracting the DNA concentration of the supernatant from the initial concentration of the DNA used for conjugation. The surface coverage of the gold nanoparticles was quantified to be ~33 pmol/cm^2 for monothiolated DNA and ~ 31 pmol/cm^2 for DTC-DNA.

Confirmation of in situ generation of dithiocarbamate ligands. Dithiocarbamates were generated in situ by mixing amine-modified oligonucleotides with an equimolar concentration of saturated aqueous solution of CS_2 in borate buffer, pH 9 for one hour. The resulting reaction mixture was desalted by G-25 columns to remove the salts. The solution thus obtained was acidified with 10 mM HCl and treated with excess solution of 1 mM $CuSO_4$. UV-Vis absorbance spectrum of the resulting yellow solution was taken (supplemental figure S1).

Figure S1. Confirmation of in situ generation of dithiocarbamate ligands by complexing with $CuSO_4$



References:

[1] K. C. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.* **1995**, *67*, 735-743.