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DNA as a template for synthesis of fluorescent gold nanoclusters

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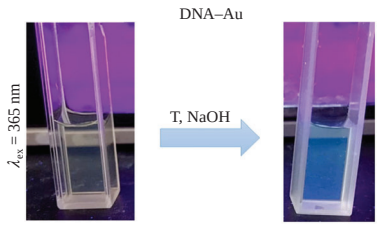
Water-soluble blue-emitting gold nanoclusters have been synthesized using dsDNA as a template without any additional reducing agent. The features of the formed nanoclusters have been revealed by fluorescence and electronic absorption spectroscopy as well as transmission electron microscopy. The prepared gold nanoclusters have been highly stable at physiological pH without any further modification.



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Fluorescent gold nanoclusters (AuNCs) smaller than 2 nm and consisting of less than 200 atoms are peculiar gold-based nanomaterials. Unlike larger gold nanoparticles, AuNCs do not exhibit surface plasmon resonance visible-range absorption but are prone to fluorescence in the visible to near-infrared spectral range.[1,2] Owing to photostability, biocompatibility, and non-toxicity, AuNCs have been used in various biomedical and sensing applications.[3,4] Fluorescent AuNCs have been synthesized in an aqueous medium *via* reduction of gold precursors and stabilized by various templates including dendrimers,[1] proteins,[5–7] peptides,[8] and single-stranded DNA.[9–12]

We have recently shown that gold nanoparticles smaller than 2 nm are formed in the presence of DNA and $NaBH_4$.[13,14] Herein we report a simple synthetic route to obtain fluorescent gold nanoclusters in the presence of DNA without any additional reducing agent.†

The interaction of DNA macromolecules with $HAuCl_4$ was accompanied by pronounced changes in the electronic absorption spectra; the corresponding data for the mixtures with different molar ratios of the components (DNA : $HAuCl_4$ of 1 : 1–20 : 1) are presented in Figure 1.

The addition of $HAuCl_4$ led to the strengthening of the DNA absorption band and the shift of its maximum from 260 to 268 nm [Figure 1(*a*)]. The effect became more pronounced with the increase in the fraction of $HAuCl_4$ in the mixture. The changes could be due to coordination of the aurate ions at the DNA, the nucleobases acting as donor sites. Indeed, it has been reported that coordination compounds including chelate complexes can be formed between gold(III) ions and the ring and amino nitrogen atoms of the nucleic acid bases.[16,17]

Upon overnight incubation with $HAuCl_4$, the initially colorless DNA solutions turned yellow, and a broad absorption band at 350–450 nm appeared; no localized surface plasmon resonance band was observed [Figure 1(*b*)]. The intensity of the appeared absorption band was increased with increasing $HAuCl_4$ fraction in the mixture.

Similar effects (yellow coloration and appearance of broad absorption band about 350–450 nm) have been attributed to the conversion of the gold(III) precursor into gold(0) atoms[18] or fluorescent gold nanoclusters.[19,20] It should be noted that the appearance of gold atoms in the double-stranded DNA matrix has been also observed elsewhere[21] upon incubation of DNA–Au complexes (Au : DNA of ~0.4) for a day. As shown in [Figure 2(*b*)], the DNA–Au complexes prepared *via* DNA

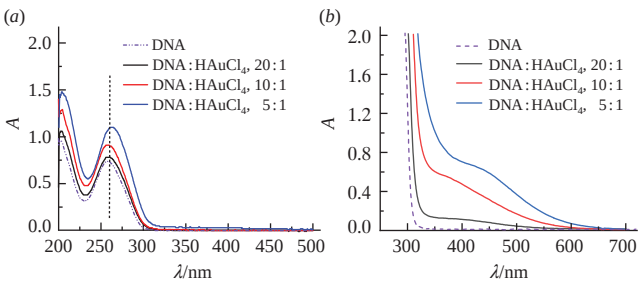

Figure 1 Electronic absorption spectra of native DNA and its mixtures with $HAuCl_4$ upon overnight incubation; pH 3.4, *c*(DNA) = 0.5 mM, and optical paths of (*a*) 2 mm and (*b*) 10 mm.

† DNA from sturgeon testes (~300 base pairs) isolated from Derinat formulation was used as a template. Tetrachloroauric acid (Sigma-Aldrich) was used as received, as well as other chemicals purchased from local suppliers (NaCl, ethanol, NaOH, and HCl). The DNA–Au complexes were prepared as follows. DNA was dissolved in an aqueous saline (1 mM of NaCl, pH 5.6); the observed absorbance ratio of the prepared solution, A_{260}/A_{280} = 1.88, indicated that the DNA was not contaminated by proteins. The DNA concentration was determined from the absorbance at 260 nm using the molar extinction coefficient of 6500 $dm^3\ mol^{-1}\ cm^{-1}$.[15] An aqueous solution of $HAuCl_4$ was added to the obtained DNA solution (final pH 3.4), and the mixture was stirred for 24 h at room temperature. Upon the DNA–Au complex formation, the solution pH was adjusted to 11 by addition of NaOH, and the samples were heated at 90 °C for about 30 min. The electronic absorption and fluorescence spectra were recorded using Vernier Ocean Optics modular system equipped with a DH-2000 light source (absorption) or 365-nm diode (fluorescence) and a Maya Pro detector. The measurements were performed at room temperature in 2- or 10-mm quartz cells. All fluorescence measurements were carried out at room temperature. Transmission electron microscopy (TEM) images were obtained using a FEI Titan Themis G3 high-resolution microscope equipped with a Super-X EDX system, at 200 kV.

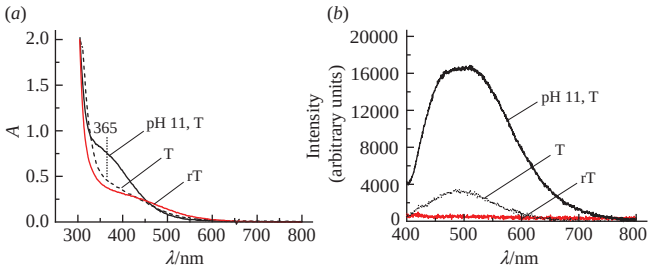

Figure 2 (*a*) Electronic absorption and (*b*) emission spectra of the DNA–Au complex upon preparation at room temperature and pH 3.4 (rT), upon heating at 90 °C for 30 min (T), and upon heating at 90 °C and pH 11 for 30 min (pH 11, T); $c(HAuCl_4)$ = 0.4 mM, DNA : Au of 10 : 1, and λ_{ex} = 365 nm.

incubation with $HAuCl_4$ initially showed almost no emission (excitation wavelength 365 nm), whereas noticeable fluorescence appeared upon heating at 90 °C. Note that only slight changes were observed in the absorption spectrum upon heating [Figure 2(*a*)].

Since macromolecules of native double-stranded DNA are rigid, aggregation of the gold atoms anchored at the DNA strand into clusters was only possible *via* migration of the gold atoms along the macromolecule; that process could be extremely slow at room temperature. When the temperature was increased, the mobility of the DNA chains was enhanced, and individual gold atoms could readily migrate along the macromolecule to afford the gold nanoclusters stabilized by the DNA molecules. If accelerated migration of the gold atoms bound to the DNA chain leading to their coalescence was the reason for the formation of AuNCs at 90 °C within 30 min, a similar effect of the mixture alkalinization could be expected (since rigid double-stranded DNA molecules are known to completely denature at pH 11). Flexible single-stranded DNA molecules (or their smaller fragments formed *via* partial chain scissoring) can efficiently stabilize the AuNCs. Therefore, we further explored the behavior of the DNA–Au complexes at pH 11.

To do so, we adjusted the DNA–Au complex solution to pH 11 with NaOH and incubated the samples at 90 °C for 30 min. The so-treated samples revealed significantly increased absorption at about 360 nm [Figure 2(*a*)] and blue fluorescence [Figure 2(*b*)], its intensity being much higher in comparison with the sample heated at pH 3.4. The observed strengthening of the absorption and emission bands was evidently associated with the formation of a large amount of fluorescent AuNCs. Overall, the obtained data confirmed that the mobility of DNA strands played a decisive role in the formation of AuNCs stabilized by DNA.

We further attempted characterization of the prepared samples by means of TEM. The DNA–Au complexes obtained upon the mixture incubation at room temperature and pH 3.4 revealed the gold atoms uniformly spread over the specimen area [Figure 3(*b*)]. However, ultrasmall (1–2 nm) gold particles could be clearly seen in the TEM images upon the sample heating at pH 11 [Figure 3(*c*),(*d*)] as bright spots in the dark-field TEM images, which were assigned to gold nanoclusters basing on the analysis of the EDX mapping. It should be noted that the DNA strands could be seen in the lower-magnification image of the sample prepared at room temperature [Figure 2(*a*)], whereas they were almost absent in the image for the sample heated at the alkaline pH. That observation suggested that the denaturation of the native DNA structure upon heating at pH 11 was irreversible.

Quantum yield (QY) of fluorescence of the prepared AuNCs was determined using quinine sulfate reference (λ_{ex} = 365 nm, λ_{em} = 400–600 nm, and QY = 0.54[22]); it was found dependent on the initial ratios of the components, being of 1.1, 1.3, 0.7, and 0.3% at DNA : Au ratios of 20 : 1, 10 : 1, 5 : 1, and 2 : 1, respectively.

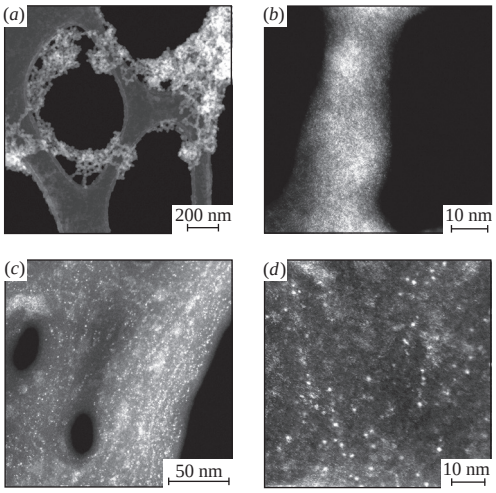

Figure 3 High-resolution TEM images of the DNA–Au complex prepared (*a*, *b*) at pH 3.4 [$c(HAuCl_4)$ = 0.4 mM, DNA : Au of 10 : 1] and (*c*, *d*) the AuNCs formed in the same sample upon heating at pH 11. The specimens for TEM examination were prepared by depositing the liquid sample on a carbon-coated copper grid by solution casting, followed by air drying.

It was reasonable to assume that at low fraction of DNA it was not sufficient to completely reduce gold(III).

The fluorescence intensity of the obtained AuNCs remained constant during keeping at daylight for more than 20 days. Thus, the prepared AuNCs could be stored at room temperature without isolation and without any additional stabilizer. Moreover, the absorption and fluorescence spectra of the samples were found independent of the NaCl concentration (up to 0.5 M at pH 7.4), which confirmed applicability of the synthesized AuNCs under physiological conditions. Moreover, the resistance against the action of quite a high concentration of the salt evidenced that stabilization of the AuNCs with DNA was non-electrostatic.

We also investigated the effect of the medium pH on the fluorescence properties of the AuNCs (Figure 4). To do so, the AuNCs were synthesized via heating at pH 11 as described earlier, and then the sample pH was adjusted with HCl. The fluorescence intensity as a function of pH followed a curve with maximum at pH 8–9, being significantly weakened in both acidic and alkaline media.

In summary, fluorescent gold nanoclusters could be obtained using DNA as a template and the reductant, without any additional reducing agent. The formation of gold nanoclusters was additionally confirmed by UV spectroscopy, high-resolution TEM and EDX spectroscopy. The obtained blue-emitting AuNCs were stable over wide range of pH (7–11) and at high ionic strength of the solution.

Using the obtained data, we suggested the following possible mechanism of the formation of the fluorescent AuNCs, involving several steps. The first step was the complex formation between

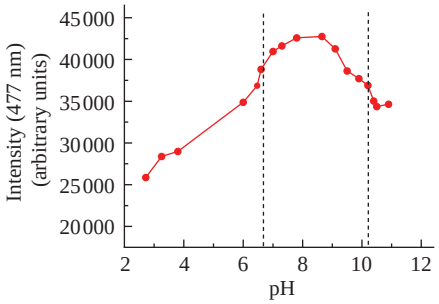

Figure 4 Fluorescence intensity (λ_{ex} = 365 nm, λ_{em} = 512 nm) of the AuNC sample as a function of pH. The AuNCs were prepared at $c(HAuCl_4)$ = 0.4 mM and DNA : Au of 10 : 1 *via* heating of the DNA–Au complex at 90 °C and pH 11 for 30 min.

Au^{III} and DNA macromolecules. The second step was reduction of Au^{III} to Au^{II}, Au^{I}, and Au^{0} with guanosine, forming the reduced Au species stabilized by DNA. Such reduction of Au^{III} to Au^{0} has been recently proposed by Kunoh *et al*.[23] Those steps occurred readily at room temperature and weakly acidic pH, but possibly could be accelerated by heating or alkalinization. The final step, which was initiated by a simultaneous action of temperature and NaOH, was the melting of DNA and the aggregation of Au^{0} into AuNCs. The DNA–Au complexes in the solution were not fluorescent, in contrast to the AuNCs obtained upon heating at pH 11, so we can speculate about aggregation-induced emission. A similar phenomenon has recently been observed in luminophore systems involving organic, organometallic, and polymeric luminogens.[24]

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