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Controlled Nucleation of DNA Metallization**

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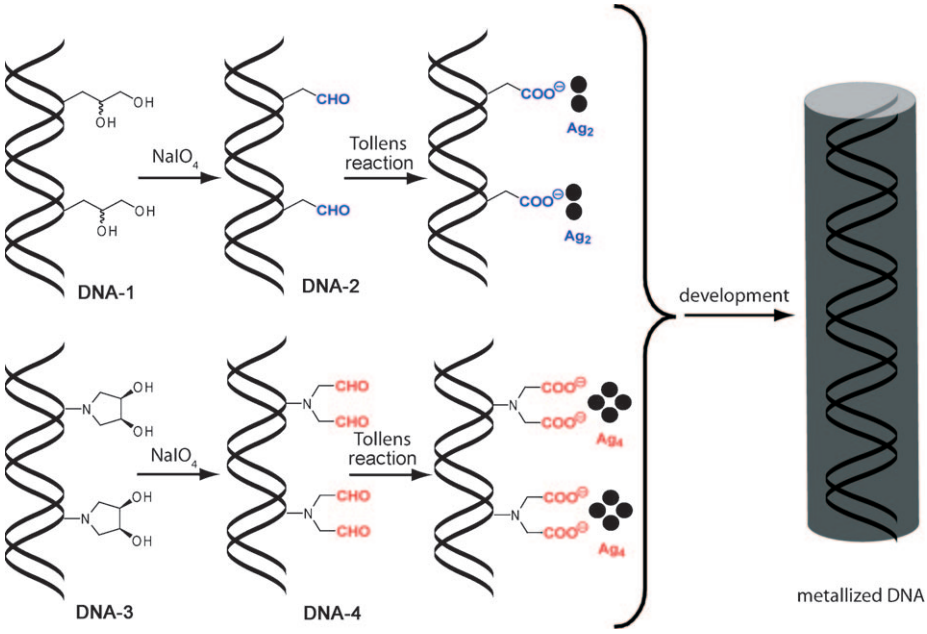
The outstanding self-recognition properties of DNA have been exploited in the use of this biomolecule as a template for the construction of nanoscale assemblies[1] and hybrid structures.[2,3] To further expand the utility of DNA for other applications, research is currently aimed at increasing its intrinsically low electric conductivity.[4] The selective coating of DNA with a thin layer of a conductive element, such as Ag,[5] Pd,[6] Pt,[7] Cu,[8] or Co,[9] has emerged as a promising avenue. Most coating procedures involve the reduction of electrostatically bound metal ions on the DNA by an exogeneous reductant to give small metal clusters attached to the DNA. In a second, development step, known from black-and-white photography, these metal clusters function as nucleation sites for the reductive deposition of metal atoms until a continuous conductive coating is formed. Novel procedures aimed at increasing the selectivity of the metallization process are the decoration of DNA with functional groups to control the spatial distribution of the nucleation sites,[10] the photochemical deposition of silver on DNA strands,[11] and the formation of DNA–Pt^{II} adducts as precursors for metal deposition on DNA.[12] The most critical step in the whole metallization process is the initial nucleation step. The uniformity and the distribution of the metal clusters define the homogeneity of the development step and hence the result of the metallization process. Unfortunately, nucleation in chemical reactions is still a poorly understood process and is therefore difficult to control.



Scheme 1. Transformation of diol-modified **DNA-1** and **DNA-3** into aldehyde- and dialdehyde-modified **DNA-2** and **DNA-4**, followed by metallization steps.

To coat DNA with silver, small silver clusters Ag_n ($n = 2, 4, 6\ldots$) that are able to undergo a development process need to be deposited on the DNA. These clusters can be formed in a redox reaction between Ag^+ in solution and aldehyde groups present on the DNA (Tollens reaction). Owing to the stoichiometry of the redox process, one aldehyde group can reduce two silver ions to form an Ag_2 cluster. Dialdehyde groups should be able to form an Ag_4 cluster (Scheme 1). It is suspected that these Ag_4 clusters, as a result of their electronic structure, are the smallest stable, developable (magic-size) silver clusters.[13] If this hypothesis is correct, the controlled formation of Ag_4 clusters on DNA should enable more reliable DNA metallization.

To construct DNA with dialdehyde moieties, we functionalized DNA with *cis*-3,4-dihydroxypyrrolidine units, which

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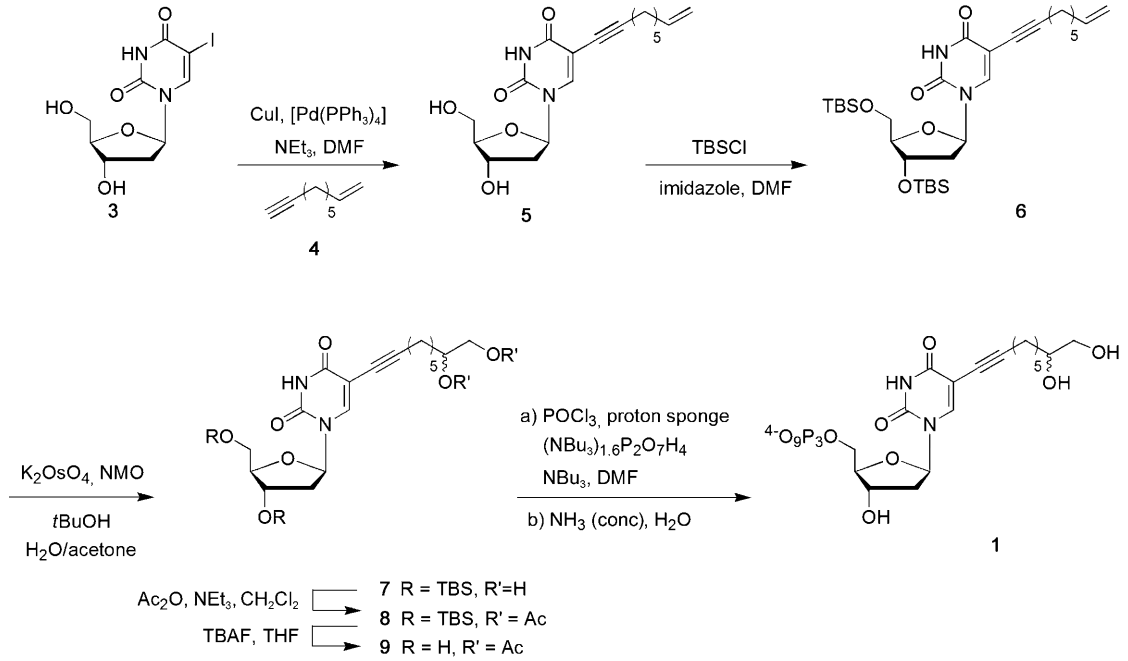
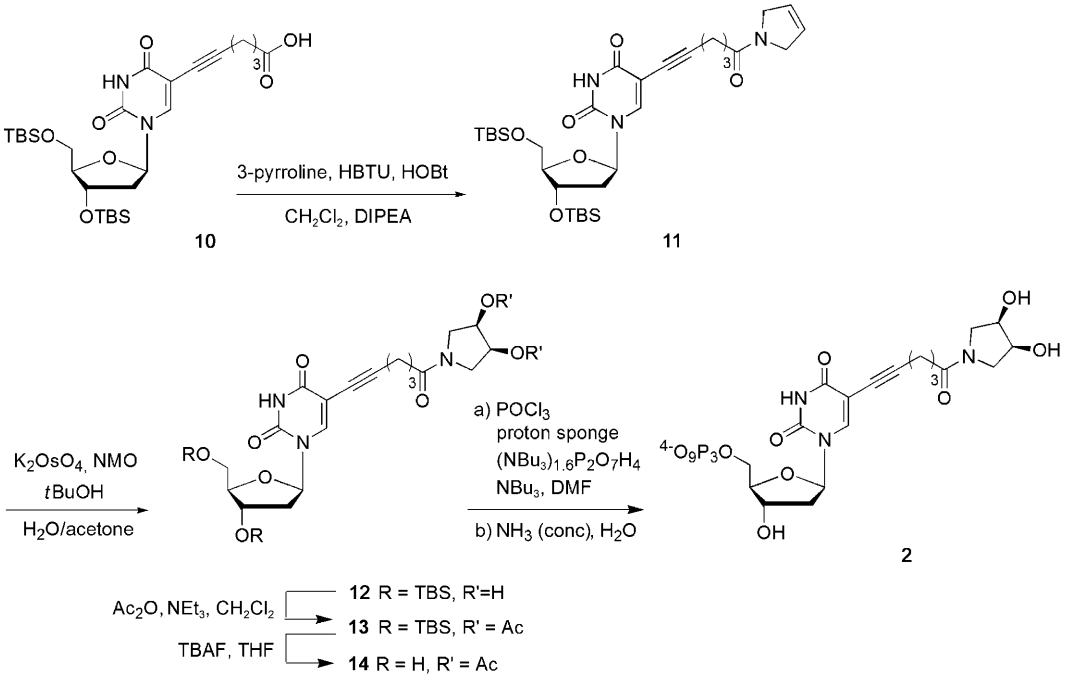
were cleaved directly on the DNA strand with sodium periodate[14] to give closely spaced dialdehyde moieties (Scheme 1). For comparison, we also prepared DNA containing open-chain 1,2-diol moieties, which were converted into monoaldehydes upon treatment with sodium periodate. For the preparation of sufficiently long DNA strands containing the desired modification, we installed the appropriate triphosphate building blocks enzymatically by PCR.[15] In this way, we prepared two types of DNA strand, **DNA-1** and **DNA-3**, which could be converted into monoaldehyde- and dialdehyde-modified **DNA-2** and **DNA-4**, respectively, upon treatment with sodium periodate.

The nucleoside triphosphate **1** with a terminal diol was prepared in a linear sequence of six steps from the known deoxyuridine derivative **3** (Scheme 2). The synthesis of triphosphate **2** with a terminal 3,4-dihydroxypyrrolidine unit is depicted in Scheme 3. Key steps in the preparation of **2** are the formation of an amide bond with 3-pyrroline and the



Scheme 2. Synthesis of the diol-modified deoxyuridine triphosphate **1**. DMF = *N*,*N*-dimethylformamide, NMO = *N*-methylmorpholine *N*-oxide, TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl.



Scheme 3. Synthesis of the cyclic-diol-modified deoxyuridine triphosphate **2**.

following selective OsO_4-mediated *cis* dihydroxylation of the pyrroline double bond. The triphosphate unit was synthesized by a one-pot procedure. The nonnatural nucleotide substrates **1** and **2** were incorporated efficiently into DNA by the polymerases Vent exo− and KOD XL in the PCR reaction. The desired diol-containing amplicons **DNA-1** and **DNA-3** were typically obtained in high yield and excellent purity (Figure 1).

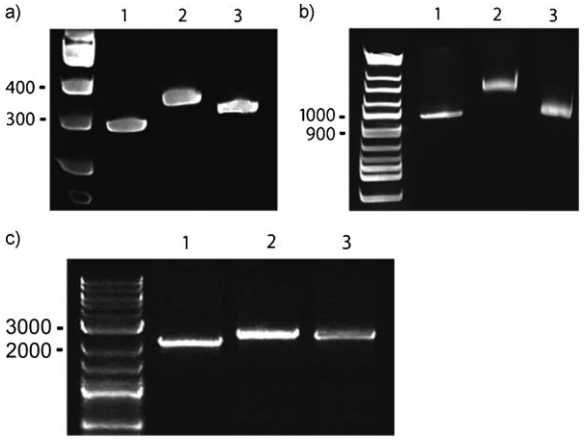


Figure 1. PCR synthesis of a) 300-mer DNA, b) 900-mer DNA, and c) 2000-mer DNA. Lane 1: native triphosphates: **DNA-N**; lane 2: triphosphate **2** substituted for 2′-deoxythymidine 5′-triphosphate (dTTP): **DNA-3**, lane 3: triphosphate **1** substituted for dTTP: **DNA-1**.

Diol-modified **DNA-1** and **DNA-3** were transformed subsequently into aldehyde-modified **DNA-2** and **DNA-4** by treatment with a 1 mM solution of $NaIO_4$ in acetate buffer (Scheme 1). To investigate the efficiency of the transformation on the long 300-mer DNA strands, we subjected the DNA molecules to enzymatic digestion before and after periodate treatment and analyzed the resulting nucleoside mixtures by HPLC–MS.[16] The quantitative conversion of the diol groups on both **DNA-1** and **DNA-3** into aldehyde groups was confirmed in this way. Importantly, we detected no by-products. It was possible that by-products might be generated by oxidation of the vulnerable bases dA and dG. Thus, the mild periodate cleavage conditions do not harm the canonical nucleobases.

To investigate the silver-deposition properties of the 300-mer DNA strands **300DNA-2** and **300DNA-4**, we treated both aldehyde-modified strands with Tollens solution. A rapid yellow coloration of the solutions could be observed by the naked eye in the case of dialdehyde-modified **DNA-4**, whereas hardly any coloration was detected with **DNA-2**, even when it was used at twice the concentration of **DNA-4**. We next monitored silver deposition during the Tollens reaction of **DNA-2** and **DNA-4** by UV/Vis spectroscopy. Plasmon absorption at 400–450 nm, which is characteristic of silver nanoparticles,[17] is clearly much more intense in the case of dialdehyde-modified **DNA-4** (Figure 2a,b). The metallization reaction proved to be selective and specific for aldehyde-modified DNA: No plasmon absorption was observed for unmodified DNA of the same sequence and length under the same conditions.[16] Concentration-dependent studies on the evolution of the plasmon peak showed that the initial concentration (measured by absorption) of monoaldehyde-modified **DNA-2** had to be four times as high as that of **DNA-4** for the same intensity of plasmon absorption to be reached (Figure 2c).

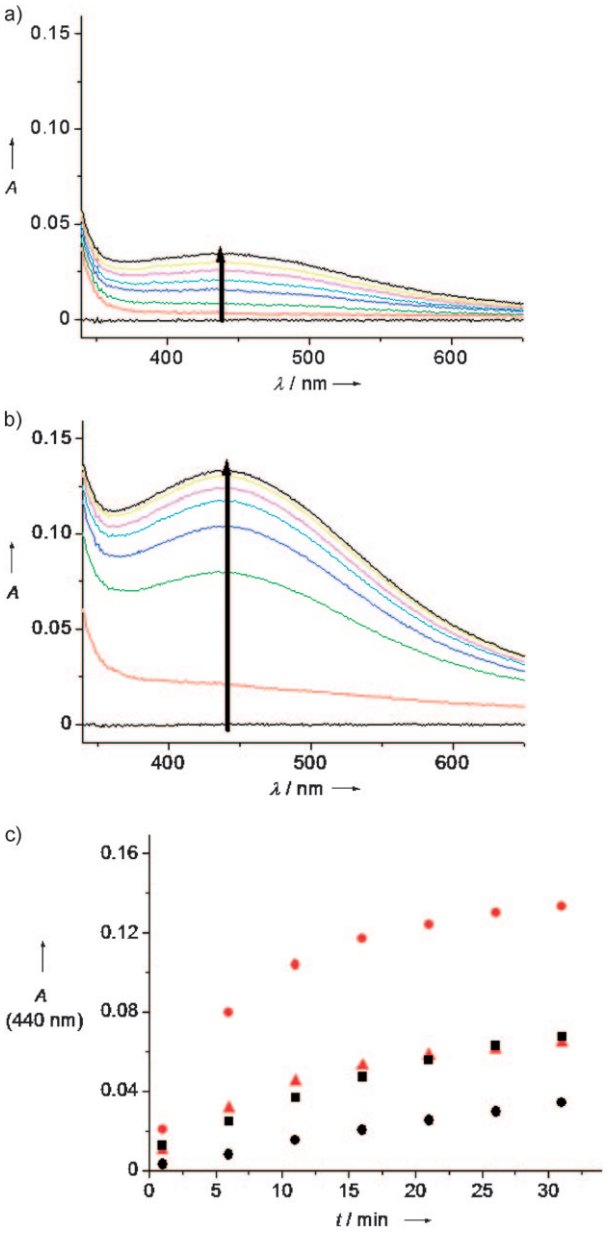


Figure 2. UV/Vis spectroscopic monitoring of the Tollens reaction of aldehyde-modified DNA: a) **300DNA-2**; b) **300DNA-4**. The optical density (OD) of DNA is 0.25. Bottom black line: before the addition of Tollens solution; colored lines: measurements at 5 min intervals after the addition of Tollens solution. c) Comparison of the development of the plasmon peak at different concentrations of **300DNA-2** (black) and **300DNA-4** (red). Triangles: OD 0.125, circles: OD 0.25, squares: OD 0.5.

We performed silver-staining experiments on nylon blotting membranes to assess whether the enhanced formation of silver nanoparticles leads to an improved detection limit. To this end, the aldehyde-modified DNA strands **300DNA-2** and **300DNA-4** and native DNA were spotted onto a blotting

membrane and treated first with the Tollens reagent to induce silver-seed formation and then with a gold enhancement solution[18] to develop the silver seeds formed (Figure 3).[16] Native DNA did not stain. A comparison of **300DNA-2** and **300DNA-4** revealed a dramatic improvement of the detection limit for the dialdehyde-modified DNA **300DNA-4**, which could be detected down to a limit of less than 0.4 ng μL^{-1} (40 pg in the example shown).[16]

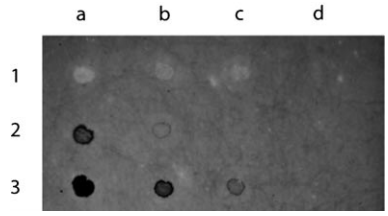


Figure 3. Membrane-staining experiment with 1) **300DNA-N**, 2) **300DNA-2**, 3) **300DNA-4**. Concentration: a) 40, b) 4, c) 0.4, d) 0.04 ng μL^{-1}.

High-resolution scanning transmission electron microscopy (HR STEM) was employed for the microstructural analysis of the complex DNA–silver-cluster nanostructures. For these experiments we used the 900-mer DNA amplicons **900DNA-N** (unmodified DNA), **900DNA-2**, and **900DNA-4**. A tiny drop of each of the solutions obtained upon Tollens treatment (incubation for 20 min) of the DNA strands was placed on conventional, amorphous-carbon-coated copper TEM grids. The samples were dried in air and examined immediately afterwards with the microscope (Figure 4). A TEM foil with Tollens solution only (without DNA) was prepared as a control. In this control experiment, a small number of randomly distributed silver nanoparticles with a mean diameter of (1.1 ± 0.4) nm[19] were detected (Figure 4a).

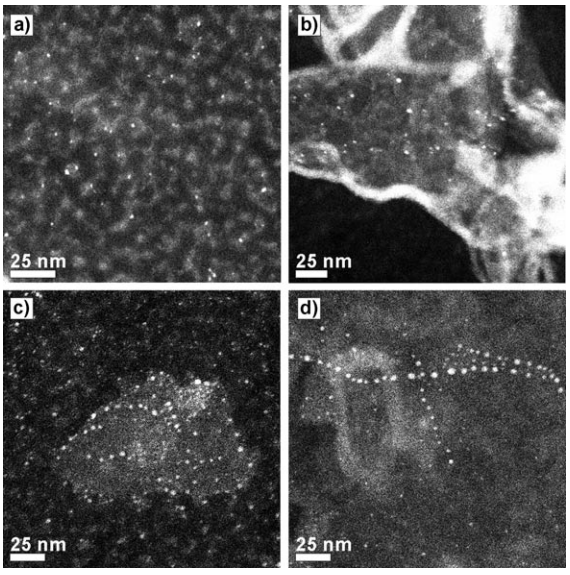


Figure 4. HR STEM micrographs: a) Tollens solution without DNA; b) Tollens solution incubated with native DNA; c) Tollens solution incubated with **900DNA-2**; d) Tollens solution incubated with **900DNA-4**. The incubation time was 20 min in each case.

For unmodified DNA treated with Tollens solution, the HR STEM image shows few nanoparticles, which are nearly exclusively present in large clouds of organic material (Figure 4b). The silver nanoparticles within the organic cloud were found to be slightly larger ((1.8 ± 0.7) nm) than those generated with the Tollens solution alone.[19] This difference can be explained in terms of the observation by Wei et al. that DNA can function as a template for silver-particle formation.[20] The HR STEM micrograph of monoaldehyde-modified **900DNA-2** after incubation with the Tollens solution revealed the presence of large amounts of small silver particles ((1.2 ± 0.5) nm; Figure 4c).[19] Thorough analysis of different areas of the TEM foil confirmed that these small particles were the main product of the reaction. Chainlike arrangements of larger silver nanoparticles were also detected, but these arrangements were rare, and the particles constituting the chains were distributed rather irregularly, which indicates rather inefficient silver-cluster formation.

The HR STEM results for the specimen with **900DNA-4**, which contains closely spaced dialdehyde groups, were prominently different. A much larger number of chainlike formations of silver particles were found. Figure 4d shows a representative section of the micrograph. These silver particles are larger ((2.6 ± 0.9) nm) than those formed with **900DNA-2**. Although the DNA itself cannot be resolved in the STEM images, the length of the cluster chains and the regularity of the distribution of the particles along the chains support the hypothesis that the particles are attached to a DNA strand. Interestingly, the distance between the clusters is much greater than the distance between pairs of aldehyde groups on the DNA template. We currently believe that the initial Ag_4 clusters are able to diffuse along the DNA to form larger clusters, as also suggested by Wei et al.[20] The driving force for the process may be a decrease in the free surface energy of the metal clusters. If the clusters diffuse with the same efficiency in both directions, and if the growing silver clusters consume all available silver, then a roughly equidistant distribution of silver clusters along the modified DNA should occur, as observed. This process needs further investigation.

Our results show that the initial nucleation event in the silver-deposition process is of key importance for metallization. This step can be controlled chemically by functionalizing DNA with dialdehyde groups, which seem to produce particularly stable developable Ag nanoclusters. Importantly, the triphosphate building blocks required are readily available and can be inserted into DNA with unprecedented efficiency by PCR.

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