DNA Nanofunctional Units

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Selective dsDNA-Templated Formation of Copper Nanoparticles in Solution**

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DNA sequences can hybridize with each other in a predictable and a programmable manner to form linear and branched double-stranded (ds) helical structures. This ability makes DNA an excellent building block for preparation of nanostructures of defined shapes and sizes. For example, surface patterns and complex 2D and even 3D objects have been obtained by self-assembly of DNA strands.[1] To make dsDNA conductive, it has been coated with metals, metal oxides, or metal sulfides. For example, a number of methods for the complete coverage of DNA with Au^0, Pd^0, Pt^0, Ag^0, Cu^0, and CdS have been reported.[2,3] However, less is known about the controlled modification of pre-selected sections of DNA. The first example of the selective coating of DNA with metal was reported by Braun and co-workers.[4] In particular, they protected a portion of λ DNA with a RecA protein/ssDNA complex. This step was followed by metallization of the unprotected DNA by sequential reduction of Ag^+ and Au^{3+}. Finally, the RecA protein was degraded, exposing the protected region of λ DNA for further manipulations. This method provided two stretches of conducting DNA wires that are interrupted by a circa 1 μm-long stretch of nonconductive DNA. However, as RecA-induced homologous recombination is efficient only with long DNA sections, this approach is limited to construction of rather large molecular objects (>1 μm).

Chemical synthesis of dsDNA containing metal ions between coordinating base pairs has been reported.[5] By the variation of the number of such base pairs, the length of the metal-containing stretches within the DNA can be varied.[5] It still remains to be experimentally confirmed that the metal ion/DNA complexes obtained are conductive and, therefore, applicable as conducting wires.

Herein we describe a method for selective metallization of ds regions of DNA with copper(0) (Figure 1). ssDNA overhangs present in the duplexes could potentially be used as addressable anchors for preparation of functional devices based on metallized dsDNA. We prepared a simple device of this type containing two metallized dsDNA connected by a non-metallized rigid linker.

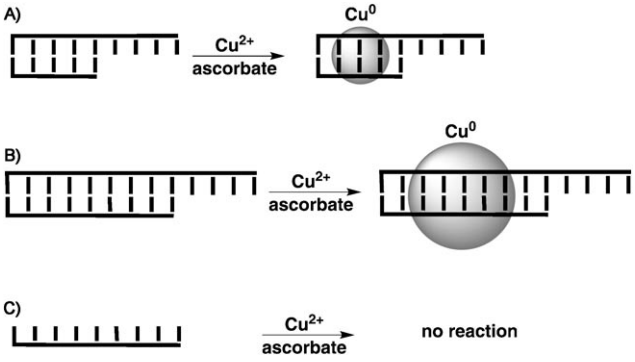

Figure 1. CuNPs formed in solutions of Cu^{2+} and ascorbate in the presence of a DNA duplex (reactions A and B); single stranded DNA do not promote this reaction (C). The size of the nanoparticles is dependent on the number of base pairs in the double stranded DNA template; the single-stranded part of the metallized duplex can be used as a handle to organize CuNPs into more complex structures.

The group of Woolley has reported that λ DNA attached to a silicon surface can be metallized with copper under rather harsh conditions, such as 0.1–1.0 M $Cu(NO_3)_2$ and 0.1 M ascorbate.[3] The high concentration of the reagents leads to unspecific formation of copper(0), even in the absence of the template. The latter problem could be alleviated by the pretreatment of a dsDNA-modified SiO_2 surface with alkali metal ions.[3] However, under these conditions, DNA templates are degraded by hydroxy radicals ($HO^{\bullet}$), which are generated in the concentrated Cu^{2+}/ascorbate mixture.[3,6]

In our experiments, we used substantially smaller (< 1000 times) concentrations of a copper(II) salt. According to HPLC analysis, both ssDNA and dsDNA are stable under these conditions (Supporting Information, Figure S1). Sequences of DNA tested in this study are given in Table 1.

By using atomic force microscopy (AFM), we were able to observe that ssDNA does not support nanoparticle formation at a low concentration of $CuSO_4$ (Figure 2 A), whereas dsDNA acts as an efficient template (Figure 2 B,C). Interestingly, the size of the nanoparticles formed is proportional to the number of base pairs in the dsDNA template (Figure 2; Supporting Information, Figure S4).

Copper nanoparticles (CuNPs) formed in the dsDNA-templated metallization are fluorescent (λ_{em} = 587–600 nm,

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Table 1: Sequences and a labeling scheme of DNA used in this study.

5′→3′		
DNA 1	GAA CGT ATG	9-mer
DNA 2	TAC TCC ATA CGT TCT GTA C	19-mer
DNA 3	GTT CAT CAC G	10-mer
DNA 4a	GTT TAT CAC G	10-mer
DNA 4b	GTT CTT CAC G	10-mer
DNA 5	CGT GAT GAA CGT ATG AGC GTA T	22-mer
DNA 6	ATG AAC GTA TGA GC	14-mer
DNA 7	TAC TCG CTC ATA CGT TCA TTG TAC	24-mer
DNA 8	CGT GAT GAA CGT ATG AGC GTA T	22-mer
DNA 9	TACTCATACGCTCATACGTTCATCACGACTAC	32-mer
DNA 10	GTAGTCGTGATGAACGTATGAGCGTATGAGTA	32-mer

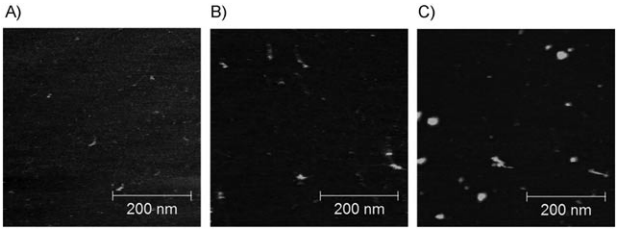


Figure 2. AFM images of CuNPs formed on a A) ssDNA template (DNA 7), B) a short dsDNA template (DNA 6/DNA 7), and C) a long dsDNA template (DNA 8/DNA 9). Concentration of DNA 9 nM, $CuSO_4$ 10 μM, ascorbic acid 2 mM, MOPS buffer pH 7.5:10 mM, Mg^{2+} 12.5 mM.

$\lambda_{ex} = 340$ nm; Figure 3 A). Metallic copper[7] and 12 nm and 30 nm CuNPs stabilized with poly-(*N*-vinylpyrrolidone) in ethanol[8] emit in the same spectral region (600 and 610 nm, respectively, $\lambda_{ex} = 337$ nm). Reduction of copper(II) by ascorbate in the presence of the dsDNA template is completed within several minutes after the reaction beginning (Figure 3 B, Figure 4). In the absence of dsDNA or in the presence of ssDNA templates, the nanoparticles are practically not formed (Figure 3). In particular, the ratio $F_{dsDNA}/F_{ssDNA} = 96$, where F_{dsDNA} is the fluorescence intensity of the copper(II)/ascorbate, DNA 8/DNA 9 mixture and F_{ssDNA} is the fluorescence intensity of the Cu^{2+}/ascorbate, DNA 8 mixture (Figure 3 A). The fluorescent signal resulting from the metallization is saturated at $[Cu^{2+}] \geq 100$ μM (Supporting Information, Figure S2). This metal ion concentration was used in all further experiments.

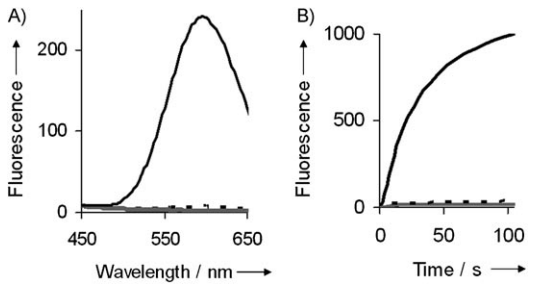


Figure 3. A) Fluorescence spectra ($\lambda_{ex} = 340$ nm) obtained 30 min after addition of $CuSO_4$ (100 μM) to mixtures containing MOPS (pH 7.5, 10 mM), NaCl (150 mM), sodium ascorbate (1 mM) and either DNA 8 (——), DNA 9 (-----), or DNA 8/DNA 9 duplex (——); DNA concentration was 200 nM. B) Time dependence of fluorescence intensity at $\lambda_{em} = 600$ nm after addition of $CuSO_4$ to buffered solutions of DNA. Trace labeling and concentration of the reagents are the same as those given in (A).

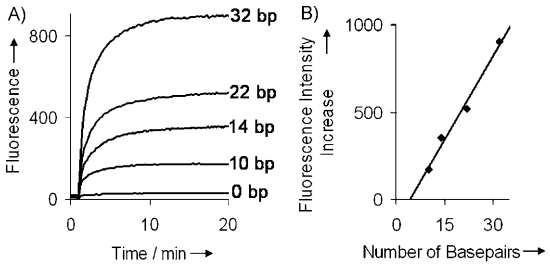


Figure 4. A) Time dependence of the fluorescence intensity at $\lambda_{em} = 600$ nm ($\lambda_{ex} = 340$ nm) after addition of $CuSO_4$ (100 μM) to buffered solutions (MOPS, pH 7.5, 10 mM; NaCl 150 mM, sodium ascorbate 1 mM) of DNA (100 nM): 32 bp, DNA 9/DNA 10; 22 bp, DNA 8/DNA 9; 14 bp, DNA 6/DNA 7; 10 bp, DNA 3/DNA 5; 0 bp, DNA 9. DNA 1/DNA 2 (9-mer dsDNA) did not act as a template for nanoparticle formation (data not shown). B) Increase of fluorescence intensity at $\lambda_{em} = 600$ nm ($\lambda_{ex} = 340$ nm) after addition of $CuSO_4$ (100 μM) to buffered solutions of dsDNA of different lengths.

We observed that fluorescence intensity increase obtained in the result of template-directed CuNP formation correlates with the number of base pairs in dsDNA templates (Figure 4). El-Sayed and co-workers reported that the fluorescence quantum yield of CuNP increased with an increase in their size.[8] In accordance with these reports, our data indicate that longer dsDNA templates induce generation of larger CuNPs in the reaction studied. This conclusion is corroborated by the AFM measurements conducted for two dsDNA of different sizes: a 14-mer and a 32-mer (Figure 2, Supporting Information, Figure S4).

Even at very low concentrations of dsDNA, the CuNP formation reaction is quite efficient. In particular, the fluorescence intensity characteristics of the nanoparticles is increased upon addition of ascorbate and copper(II) ions to 3.5 nM 22-mer dsDNA template (Supporting Information, Figure S3).

The nanoparticle formation is highly sensitive to single nucleotide mismatches. For example, 10-mer dsDNA (DNA 4a/DNA 5 and DNA 4b/DNA 5), which contain single mismatched base pairs (C4→T4 mutations in DNA 4a and A5→T5 in DNA 4b) do not act as templates for CuNP formation at all (Figure 5). This result is not surprising, as at our reaction conditions these DNA exist in a single-stranded form as shown by the UV melting analysis: no melting was observed at ≥ 22 °C. In contrast, fully matched duplexes of the same size (DNA 3/DNA 5, $T_m = (40.5 \pm 0.7)$ °C) is an efficient template (Figure 5).

In all previous studies of templated metal precipitation, the DNA templates were first treated with a metal salt and then with a reducing agent.[2,5] This order of addition of the reagents has been used as it is believed that the initial coordination of DNA to copper(II) is a prerequisite for the selective precipitation of the metal on the nucleic acid. We observed however that this procedure leads to a very low

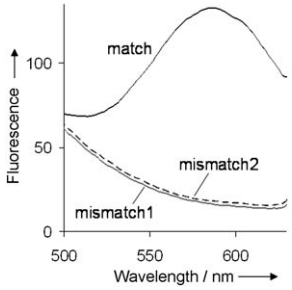
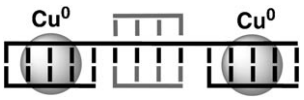


Figure 5. Fluorescence spectra ($\lambda_{ex} = 340$ nm) acquired 20 min after addition of $CuSO_4$ (100 μM) to buffered solutions of DNA (100 nM) labeled as match (DNA3/DNA5), mismatch1 (DNA4a/DNA5), and mismatch2 (DNA4b/DNA5). Buffer: MOPS (pH 7.5, 10 mM), NaCl (150 mM), sodium ascorbate (1 mM).

yield of CuNPs under our experimental conditions. In contrast, addition of $CuSO_4$ to a buffer already containing a reducing agent (ascorbate) results in efficient nanoparticle formation (Figure 3–5), which indicates that the initial binding of copper(II) to DNA is inhibiting rather than facilitating the reaction. The effect is not so surprising, as potential binding sites for copper(II) on DNA are either oxygen atoms of the phosphodiester groups or nitrogen and oxygen atoms of the nucleobases. These ligands can be classified as hard Lewis bases (oxygen atoms) or intermediate Lewis bases (nitrogen atoms). Ligands of this type are known to stabilize copper(II), which should therefore result in the inhibition of the $Cu^{2+} \rightarrow Cu^0$ transformation. These observations allow us to suggest that the first step in the reaction is reduction of copper(II) to copper(I), which is followed by the disproportionation of copper(I) into copper(II) and copper(0). The copper(0) that is formed is then clustered on dsDNA, thus producing stable nanoparticles. We also found that DNA triplexes do not promote the nanoparticle formation (Supporting Information, Figure S6). This observation indicates that the nanoparticles are accumulated in the major groove of the dsDNA, which is blocked in the triplex and which is absent in the ssDNA. The charge of the duplex also appears to be important, as less-charged PNA/DNA duplexes do not act as templates at all (Supporting Information, Figure S5).

As PNA/DNA duplexes and DNA triplexes do not act as templates for nanoparticle formation, they can be used as rigid linkers to create nanostructures with alternating metallized and non-metallized parts. We have confirmed this possibility by producing a (CuNP/duplex1)-triplex-(duplex2/CuNP) structure (Scheme 1; Supporting Information, Figure S9).

In summary, our method is the first example on the selective formation of metal nanoparticles on dsDNA in solution. It has several potential applications. In particular, it is a new method to control the size of copper nanoparticles by the use of a dsDNA template of a selected length. The metallization is highly selective for dsDNA compared to ssDNA, and as described here, it can be used for the detection of dsDNA by fluorescence. Furthermore, it is possible to detect single mismatches very efficiently. The method has the potential to be used for selective metallization of more complex DNA nanostructures. Complex patterns of DNA



Scheme 1. A structure of a nanodevice containing two metallized DNA duplexes linked together by a rigid triplex structure: (CuNP/duplex1)-triplex-(duplex2/CuNP).

nanostructures immobilized on surfaces may also be metallized by the same method. For example, DNA origami can be designed to contain single stranded domains, and we are currently studying the selective metallization of dsDNA in such structures immobilized on surfaces. Future studies will show whether the selectivity of this metallization procedure can be used for integrating metallized DNA-based structures in semiconductor-based electronic circuits.

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