Nanoparticle Synthesis

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Poly(thymine)-Templated Selective Formation of Fluorescent Copper Nanoparticles**

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By virtue of a unique nanosized linear geometric structure, excellent molecule recognition properties, and high affinity for some metal ions, DNA has been used as a template for metallic nanostructures through the binding of metal ions on the DNA and subsequent chemical reduction of the DNA-complexed metal ions.[1] This approach has been used for fabricating metal nanoclusters with tunable fluorescence emission and high photostability that are suitabable for biological diagnostic techniques,[2,3] as well as conductive DNA nanowires by deposition of metal atoms on linear DNA. For example, DNA-templated nanowires of silver,[4] palladium,[5] platinum,[6] copper,[4c,7] and gold[8] have been obtained. However, the controlled reduction of DNA-complexed metal ions on pre-selected sections of DNA is still rarely reported and is confined to double-stranded DNA (dsDNA). The first example was reported by Braun and co-workers.[4b] They employed RecA proteins as the resist to protect a portion of the dsDNA. The unprotected dsDNA was then metallized by sequential reduction of Ag^+ and Au^{3+} ions, converting the unprotected regions to conductive metal wires. But this was limited to constructing rather large microstructures ($>1\,\mu m$) because RecA-directed homologous recombination is efficient only with long strands of DNA. Another example of the controlled reduction of DNA-complexed metal ions on pre-selected sections of dsDNA was recently reported by Mokhir and co-workers.[9] Selective formation of copper nanoparticles (CuNPs) on short dsDNA was realized by connecting two metallized dsDNAs with a non-metallized linker. The CuNPs formed in the dsDNA-templated metallization were fluorescent and proved efficient for biochemical sensing in further studies.[10] In addition, through site-specific hybridization of immobilized probe DNA with signal DNA to form dsDNA, another interesting technique for site-specific synthesis of AgNCs on a triangular DNA origami scaffold was achieved by Liu and co-workers.[11]

A single-stranded DNA (ssDNA) molecule that maintains its linear state without hybridization of a second strand would be an excellent programmable building block for nanotechnology. Therefore, the exact spatial positioning of metal deposition on ssDNAs is crucial for the future development of complex nanodevices such as integrated DNA-templated electronics.

Herein, we developed a method for the controlled reduction of DNA-complexed metal ions on specified sections of ssDNA using copper(II) (Cu^{2+}) ions (Figure 1). Five kinds of ssDNA, including random ssDNA, poly(adenine) (poly A), poly(thymine) (poly T), poly(cytosine) (poly C), and poly(guanine) (poly G), were investigated as templates for Cu^{2+} reduction and CuNPs formation. The fluorescence emission intensity of the CuNPs formed was used to determine the efficiency of CuNP formation on ssDNA templates. After the specific ssDNA with the capability to serve as a template for CuNP formation (Cu-ssDNA) was identified, we tried to regulate the size of the CuNPs by altering the length of the Cu-ssDNA. Simultaneously, other ssDNAs without the ability to serve as a template for Cu^{2+} reduction and CuNPs formation (NCu-ssDNA) were tested as rigid linkers or "smart glues" to create complex nanostructures with alternating metallized and non-metallized parts.

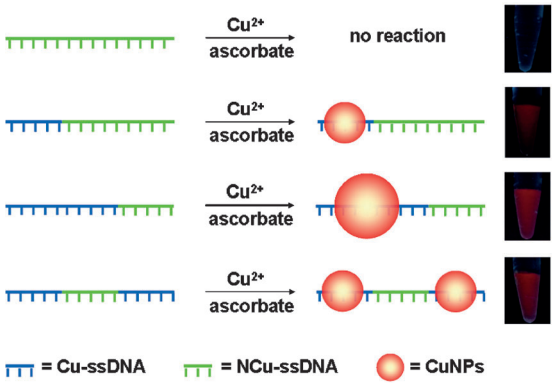

Figure 1. Controlled reduction of DNA-complexed Cu^{2+} ions on pre-selected sections of ssDNA to form nanoparticles. Cu-ssDNA (blue) represents ssDNA that can serve as a template for CuNP formation. The size of the CuNPs formed is dependent on the length of the Cu-ssDNA. NCu-ssDNA (green) represents ssDNA without the capability of forming CuNPs, which can be used to link nanoparticles together for the assembly of complex nanostructures.

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One 20-mer random ssDNA (R20) and four 20-mer DNA homopolymers (A20 = poly A, T20 = poly T, C20 = poly C, G20 = poly G) were first tested as templates for Cu^{2+} reduction and CuNPs formation. Sequences of the ssDNAs tested in this study are provided in the Supporting Information, Table S1. The reaction was carried out using a solution of ssDNA (500 nM) and $CuSO_4$ (100 μM) in MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6), and sodium ascorbate (2 mM) was used as the reducing agent. Reaction solutions were studied by fluorescence spectroscopy, as shown in Figure 2 A.

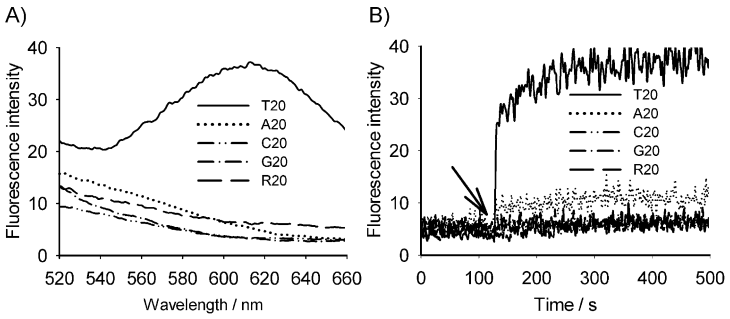

Figure 2. A) Fluorescence spectra and B) real-time fluorescence recorded at $\lambda_{em} = 615$ nm in the presence of Cu^{2+} ions and different kinds of 20-mer ssDNA. The arrow marks the addition of $CuSO_4$ to the buffered solutions of DNA.

We found that the solution containing T20 emitted fluorescence around 615 nm under excitation with 340 nm UV light. However, no fluorescence was observed when either R20, A20, C20, or G20 was used in the solutions. We also found that the fluorescence induced by poly T was complete within several minutes of the start of the reaction (Figure 2 B), therefore we obtained fluorescence spectra in all studies after five minutes reaction. It was reported that both poly(*N*-vinylpyrrolidone)-stabilized CuNPs[12] and dsDNA-templated CuNPs[9] emitted in a similar region (around 600 nm) upon excitation with 340 nm UV light. We found that T20 served as a template for Cu^{2+} reduction and CuNPs formation by studying the resulting structures using transmission electron microscopy (TEM; Figure 3 A). We also found that the size of the CuNPs was dependent on the length of the poly T strand. Herein, three lengths of poly T (T20, T30 and T40) were investigated. Under the same reaction conditions (10 mM MOPS, 150 mM NaCl, 500 nM poly T, 2 mM sodium ascorbate, 100 μM $CuSO_4$, pH 7.6), increasing the length of poly T caused in increase in the size of the CuNPs (Figure 3; Figure S1), which was consistent with previous findings of longer dsDNA templates generating larger CuNPs.[9]

Because poly T was found to be a specific template for CuNPs formation, we tested what length of poly T segments within the ssDNA supported Cu^{2+} reduction and subsequent CuNPs formation. A number of 40-mer ssDNAs containing different types of T-rich domains, including $(T\text{-}C)_{20}$, $(T5\text{-}C5)_4$, $(T10\text{-}C10)_2$, T15-C10-T5-C10 and T20-C20 (for sequences of these ssDNAs, see Table S1), were investigated for CuNP formation. Among the ssDNAs tested, the length of the DNA and the number of T bases were uniform. However, we found that only the ssDNA T20-C20 induced bright fluorescence around 615 nm (Figure 4 A, Figure S2), and the fluorescence intensity was almost equivalent to that with T20. Even though T15-C10-T5-C10 has a section of 15 consecutive T bases, it still did not induce fluorescence. This study indicated that the degree of T polymerization plays an important role in the formation of templated CuNPs. In addition, we observed that the fluorescence intensity of templated CuNPs increased as a function of the length poly T segment within the ssDNA when the number of consecutive T bases was more than 20 (Figure 4 B; Figure S3). As Mokhir, El-Sayed, and co-workers have reported, the fluorescence quantum yield of CuNPs increases with increasing size.[9,12] Consistent with these reports, we found a positive correlation between the fluorescence intensity of the templated CuNPs and the length of poly T, wherein longer poly T segments induced formation of larger CuNPs following the reduction of Cu^{2+} ions. Our TEM images of the three kinds of poly T (T20, T30, and T40) also supported this conclusion (Figure 3).

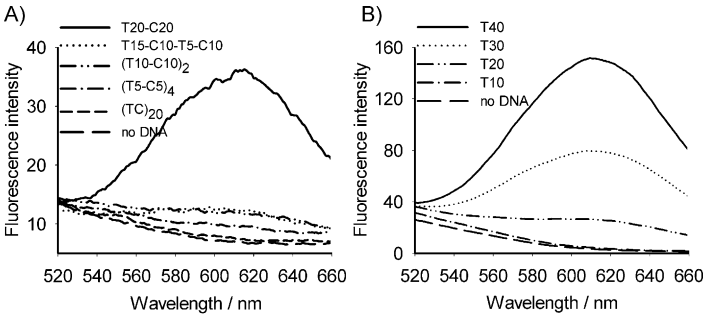

Figure 4. Fluorescence spectra of solutions containing Cu^{2+} ions and A) 40-mer ssDNA with different types of T-rich domains or B) poly T of different lengths.

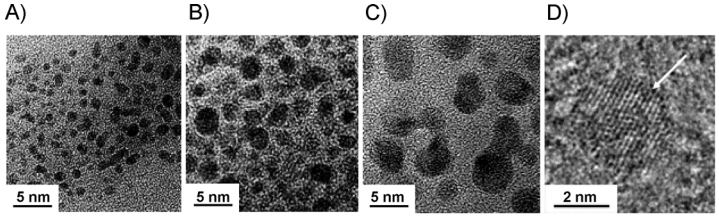

Figure 3. TEM images of CuNPs templated by A) T20, B) T30, and C) T40. D) High-resolution TEM image of the crystal lattice (arrow) structure of the CuNPs formed.

Thus, we concluded that poly T segments of more than 20 bases could selectively template the formation of fluorescent CuNPs, and the size of the nanoparticles was dependent on the length of poly T. As previously reported, nucleotides have high affinity for some metal cations, and these localized metal ions can be reduced

to form metallic nanomaterials in the shape of the DNA scaffold.[1–8] We reasoned that the formation of CuNPs templated by poly T was due to binding interactions between thymine and Cu^{2+} ions, and the thymine-complexed Cu^{2+} ions were reduced to Cu^0 by ascorbic acid along the contour of the poly T template.

The influences of Cu^{2+} and poly T concentration on CuNP formation were further investigated. We selected T30 as a model template for CuNP formation, and we first fixed the T30 concentration at 500 nM and varied the Cu^{2+} concentration from 0 μM to 150 μM. The fluorescence emission intensity increased with increasing Cu^{2+} concentration and gradually reached a plateau at 100 μM (Figure S4). As reported by Mokhir and co-workers,[9] at such low Cu^{2+} concentrations, DNA templates are stable and not degraded by the hydroxyl radicals (HO•) generated from the concentrated Cu^{2+}/ascorbate mixture. We next fixed the Cu^{2+} concentration at 100 μM and varied the T30 concentration. Using fluorescence spectroscopy, we found that poly T templated CuNP formation was quite efficient. CuNP fluorescence was detected from 10 nM T30, and the higher the T30 concentration, the stronger the fluorescence (Figure S5). The stability of CuNPs as a function of time was also investigated. We found that the CuNPs maintained over 80% of their initial fluorescence after two hours (Figure S6).

As suggested by the our results, random ssDNA, poly A, poly C, and poly G could not act as templates for CuNPs formation. Thus, they could act as a resist for creating nanostructures with alternating metallized and non-metallized parts. To test this, C10 was used as a linker and T25 was used as a template for CuNP formation. Different kinds of ssDNA assemblies with alternating T25 and C10 segments were investigated for CuNP formation under the same conditions used earlier (10 mM MOPS, 150 mM NaCl, 500 nM ssDNA, 2 mM sodium ascorbate, 100 μM $CuSO_4$, pH 7.6; sequences of these ssDNAs are listed in Table S1). The fluorescence intensity at 615 nm in the presence of different ssDNA assembles increased almost linearly with an increase in the number of T25 sections (Figure 5). Simultaneously, the fluorescence images show that the red fluorescence of CuNPs increased proportionally with an increasing number of T25 segments (Figure 5, inset). Also, no fluorescence signal was detected for the resulting solutions in the presence of C10 only. Thus, we can conclude that the C10 segments acted as a resist and that nanostructures with alternating CuNP/T25 and non-metallized C10 parts were produced. In addition, TEM images of the products templated by T40, T30-R20-T30, and T25-C10-T25-C10-T25 further confirmed that nanostructures with alternating metallized and non-metallized parts could be created. As shown in Figure S7, monodisperse CuNPs were templated by T40, while T30-R20-T30 produced "dimer" CuNPs and T25-C10-T25-C10-T25 gave "trimer" CuNPs with gaps as expected.

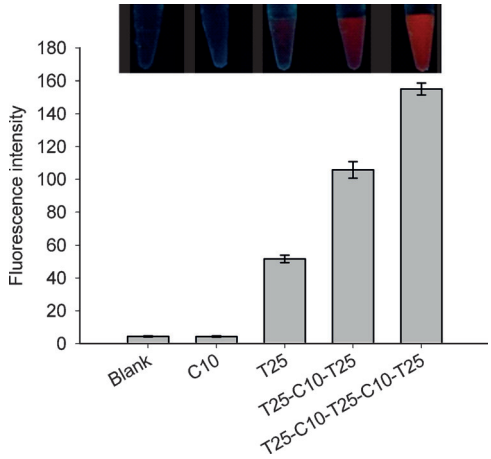

Figure 5. Fluorescence emission intensity at 615 nm of solutions containing Cu^{2+} ions and ssDNAs with different numbers of T25 segments. Inset: corresponding fluorescence emission image upon illumination with UV light.

In summary, our study was the first example of poly T being used as a single-stranded template for the formation of fluorescent CuNPs and the size of CuNPs could be simply regulated by altering the length of the poly T. Other ssDNAs, such as random ssDNA, poly A, poly C, and poly G failed to serve as templates for CuNPs under the same conditions. Using these properties, selective metallization of ssDNA gave nanostructures with alternating metallized and non-metallized parts using poly T as a template for CuNP formation and other ssDNAs as linkers. Because the non-metallized segments can be pre-designed, they can be conveniently connected with other objects or immobilized on surfaces. This method has the potential to create more complex DNA-metal nanostructures, which could be a significant step toward integrated DNA-templated electronics. In addition, because of the excellent fluorescence of the poly T templated CuNPs, poly T could be used as a probe for biochemical sensing without complicated modifications. Further studies of the conductivity of CuNPs on interfaces and applications for biochemical analysis are currently being undertaken in our lab.

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