Synthesis of Platinum Cluster Chains on DNA Templates: Conditions for a Template-Controlled Cluster Growth

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We investigate the conditions which should be fulfilled to grow chains of nanosized noble metal clusters on DNA templates according to a selectively heterogeneous, template-controlled mechanism. A long incubation of double-stranded DNA molecules with Pt(II) complexes is necessary to obtain a template-directed formation of thin and uniform cluster chains after chemical reduction of the DNA/salt solution. Without this "activation" step, DNA acts as a nonspecific capping agent for the formed clusters and does not hinder the formation of random cluster aggregates. The effect of binding Pt(II) complexes to the DNA is investigated by UV−vis spectroscopy, electrophoresis experiments, and scanning force microscopy, revealing that the base stacking along the DNA molecule is significantly distorted but the double-stranded DNA configuration is retained. Citrate ions can be used as additional stabilizers for the heterogeneously grown metal clusters, leading to significantly more regular metal cluster chains. After a systematic variation of the absolute concentration of the reactants (Pt salt, DNA, reducing agent), we can conclude that there is an optimum concentration value for the fabrication of cluster chains and that small variations around the optimum value do not have noticeable effects on the quality of the metallization products. The described metallization procedure can be resolved into a series of simple and efficient steps, which is essential for a biomimetic fabrication of nanostructures in a reproducible way.

I. Introduction

Metallic wires fabricated via the selective growth of metal on DNA templates are very promising candidates for use in microelectronic devices of future generation.[1−13] The aim of this approach is to create novel devices where quantum size effects and the large number of surface atoms influence their chemical, electronic, magnetic, and optical behavior.[14,15] Since DNA molecules have a diameter of only two nanometers and can be arbitrarily long, very thin (below 10 nm) and regular wire-like metallic structures can be efficiently produced.[6] Moreover, both the ends of DNA molecules and the surface of solid substrates can be functionalized through a variety of methods to create specific links between DNA and substrate, allowing the integration of DNA molecules into specific, predefined sites of microstructured electronic circuits.[1,13,16,17] Furthermore, by exploiting the specific recognition of complementary nucleotide sequences, complex structures made of DNA can be fabricated without much effort[9,16,13,18] and metallized in a later stage.[9]

Despite of the progress which has been made in the past years, the current techniques of biopolymer metallization for the production of nanoscopic conductive elements are still at an early stage of development. The major drawbacks of the today's biomimetic metallization techniques are the difficulties associated with the reproducible assembly of individual structures into working devices and with the control of their desired physical properties such as, e.g., electric conductivity. Moreover, it is not clear at the present stage whether the recently developed techniques for the metallization of biomolecules can be efficiently employed in large scale applications. To be suitable for industrial processing, the methods for the fabrication of wires (and other metallic structures) should be both (i) simple and (ii) robust. Namely, (i) the overall process should be composed by a few, clearly defined elementary steps which can be performed in sequence with high degree of automatizm, and (ii) the method should be as little as possible sensitive to small fluctuations of the set of parameters which define the optimum conditions for the fabrication of the products. In this work, our goal is to define the conditions which permit a selectively heterogeneous metallization of DNA molecules to be achieved in a way which is simple, robust, and reproducible.

The assembling of metallic nanoparticles along DNA templates can be performed in several ways.[19] For instance, chains of separated particles can be prepared by binding positively charged colloids to the negatively charged backbone of DNA via electrostatic interactions.[11] Moreover, particles can be grown directly on the DNA template after the previous formation of nucleation sites along the DNA followed by chemical reduction of dissolved metal salts. Through this technique, coarse palladium wires with a diameter of about 50 nm have been obtained,[2] which possess peculiar electric conduction properties both at room and at low temperatures.[3,8]

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More recently, by accurate tuning of the metallization conditions, continuous chains of platinum clusters no thicker than a few nanometers and extended over several micrometers have been realized by the present authors.[6] The key feature of the employed technique is the long incubation of DNA with an aged solution of K_2PtCl_4 to allow the binding of Pt(II) complexes to the biomolecule. The bound Pt(II) complexes act in the subsequent reduction step as preferential nucleation centers for a selectively heterogeneous cluster growth.[5,6] In fact, through a series of first principles investigations, we demonstrated that the presence of the nucleotide ligands both enhance the electronic affinity of the complexes and stabilize the formation of Pt−Pt bonds during the first reduction stages.[6,20] Since cluster growth is an autocatalytic process,[21] the first formed nuclei quickly develop to larger clusters and consume the dissolved Pt(II) complexes.[6,22] In this way, the homogeneous nucleation process is hindered. Therefore, the achievement of a selective DNA metallization without homogeneous formation of clusters in solution is the result of a delicate balance between the kinetics of reduction in the homogeneous and heterogeneous case.

Here we show how this balance is shifted by changing the absolute and relative concentrations of reactants, and the type of metal complexes. This can have a dramatic effect on the final products, leading in most cases to the formation of random cluster agglomerates or to a very coarse DNA metallization. However, we find that there is an optimum interval of parameters where thin and uniform cluster chains form selectively on DNA molecules. Moreover, small variations around the optimum set of parameters have only little or no influence on the quality of the metallization products. This makes our metallization technique robust, in the sense that once an optimum set of parameters is found for a given metal complex uncontrolled small fluctuations of the reducing conditions are very unlikely to lead to faulty products. We stress that, despite the fact that the actual molecular mechanisms leading to cluster formation are rather complicated, the overall process can be resolved into a series of very simple steps. In particular, uncomfortable processes such as dialysis, ion exchange, or changing of the solvent are avoided, which would be very expensive and inefficient at a level of massive production.

Defining the conditions which are necessary for a template-controlled cluster growth on DNA requires the detailed knowledge of all steps of the metallization process. This begins with the dissolution of metal salt in water and with the reaction of the dissolved metal complexes with the DNA bases and ends with the formation of the metallized products after the addition of a reducing agent to the solution in order to achieve the formation of metal clusters. Although the essential steps of the process have been presented in ref 6, a number of questions remain to be answered in more detail. In particular, we investigate here (i) the effect of the hydrolysis degree of dissolved the Pt(II) complexes on the kinetics of binding to the DNA bases during the activation step; (ii) whether the addition of citrate ions to stabilize the formed metal clusters improve the quality of metallization; (iii) to which extent variations of the concentrations of reactants influence the formation of regular and thin metal wires; (iv) the importance of the double-stranded DNA configuration with respect to the single-stranded DNA configuration for the formation of the reduction products; and (v) how the balance between homogeneous and heterogeneous nucleation can be shifted by changing the overall reduction kinetics using Pd(II) complexes instead of Pt(II) ones. Furthermore, we briefly discuss the results of previous investigations into the influence of the DNA composition on the overall reaction kinetics.[16] Finally, we give a summary of problems and open questions to stimulate future work in the field of DNA metallization.

II. Experimental Section

A. Metallization of DNA Molecules. If not otherwise stated, the reduction experiments were performed by aging a 10 mM solution of K_2PtCl_4 or K_2PdCl_4 (both purchased from Fluka) for at least 24 h, diluting the aged solution to 1 mM, and mixing it with 5 μg/mL of λ-DNA (New England Biolabs) or DNA from salmon testes (SIGMA). Both types of DNA exhibit statistically random sequences and GC base pairs contents of 49.9% and 41.2%, respectively. In this way we can exclude such effects of DNA metallization, which arise from the specific composition of DNA sequence. The concentration of DNA was varied between 0 and 100 μg/mL in the investigations described in section III.B. The kinetics of DNA activation was investigated by diluting four stock solutions of K_2PtCl_4 at different initial concentrations which were previously aged for at least 24 h to a final concentration of 1 mM and mixing them with 5 μg/mL of λ-DNA. The reduction of the dissolved complexes to form metal cluster was started by heating the solution to 37 °C and adding 100 μL of a 10 mM solution of dimethylaminoborane (DMAB, Fluka) to 1 mL of the DNA/Pt solution. In some cases, sodium citrate was added to the DNA solution in the concentration of 0.5 mM before starting the reduction.

The kinetics of both the activation process and the cluster formation process were studied using a Cary 50 UV/VIS photospectrometer (Varian), recording the absorbance spectra at wavelengths above 240 nm and above 550 nm, respectively, during the reaction course.

B. Microscopic Investigations. Samples for scanning force microscopy (SFM) and transmission electron microscopy (TEM) were prepared 30 min after starting the reduction. SFM samples were prepared as described by Bezanilla et al.[23] by diluting the DNA/cluster suspension with HEPES/Mg buffer (40mM HEPES, 5mM$MgCl_2$, pH 7.6) to a final DNA concentration of 1 μg/mL and placing a droplet onto freshly cleaved mica for 2 min. Subsequently, the sample was rinsed with water and blown dry with nitrogen. Imaging was performed with a Nanoscope IIIa (Digital Instruments) in tapping mode in air. SFM samples from activated DNA were prepared following the same protocol. TEM samples were prepared by placing a droplet of the DNA/cluster suspension onto a carbon-coated copper grid for 5 min. Afterward, the sample was rinsed with water and excess water was removed with filter paper. The TEM investigations were carried out using a Philips CM 200 with tungsten cathode at 160 kV for low-resolution imaging, whereas a Philips CM 200 microscope with a field emission cathode was employed for the high resolution work.

C. Electrophoresis Experiments. In the electrophoresis investigations, 5 μg of native λ-DNA and of λ-DNA digested with *Hin*d III (New England Biolabs) were activated for 20 h in 1 mL of a 1mM K_2PtCl_4 solution previously aged for 24 h. Single-stranded DNA (ss-DNA) was obtained by thermal denaturation of double-stranded DNA (ds-DNA) at 95 °C and subsequent cooling on ice. After activation, 20 μL of each sample were mixed with 1 μL of a 1 mM DMAB solution to cover the DNA with nucleation sites for the subsequent gold stain. Electrophoresis was performed in a 0.5% agarose gel for 1.5 h in 1× TAE-buffer at 4.2V/cm. Subsequently the gel was covered with a gold stain solution[7] until the DNA bands

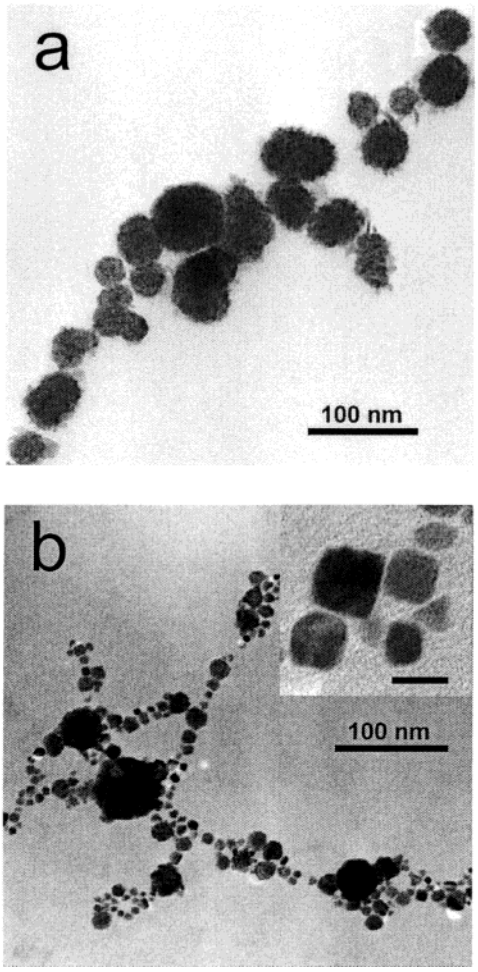

Figure 1. TEM micrographs of Pt particles formed after reduction of a 1 mM solution of $PtCl_4^{2-}$ with 1 mM DMAB. (a) In the absence of citrate; (b) in the presence of 0.5 mM citrate. The scale bar in the inset is 10 nm.

appeared. The reaction was stopped by rinsing the gel with abundant water.

III. Results

A. Pt Cluster Synthesis in the Absence of DNA. When K_2PtCl_4 salt is dissolved in water solution, it dissociates, and after the dissociation, the $PtCl_4^{2-}$ ions undergo the following solvolysis reactions:[24,25]

$$PtCl_4^{2-} + H_2O \rightleftharpoons PtCl_3(H_2O)^- + Cl^- \quad (1)$$

$$PtCl_3(H_2O)^- + H_2O \rightleftharpoons PtCl_2(H_2O)_2 + Cl^- \quad (2)$$

At the equilibrium, a 1 mM solution of K_2PtCl_4, which is usually considered as standard concentration to obtain suspensions of metal nanoparticles in reduction experiments,[26,27] consists of 5 $PtCl_4^{2-}$, 53% $PtCl_3(H_2O)^-$, and 42% $PtCl_2(H_2O)_2$.[28] At room temperature, the aging time necessary for reaching the equilibrium is about 1 day. The solution is then stable for some days. Within this time, the formation of higher-order solvolysis products can be neglected because the corresponding formation rates are 3 orders of magnitude lower.[25] After mixing an aged solution of K_2PtCl_4 with DMAB, the dissolved Pt(II) complexes are reduced to metallic platinum particles (Figure 1). Considering, for instance, doubly hydrolyzed complexes, an overall chemical reaction of this preparation can formally be written as follows (see, e.g., ref 29)

$$(CH_3)_2HNBH_3 + PtCl_2(H_2O)_2 + 2H_2O \rightarrow Pt\downarrow + (CH_3)_2HNH^+ + H_3BO_3 + 2HCl + OH^- + 2H_2 \quad (3)$$

In the absence of any capping agent, the formed metal clusters agglomerate into ~50 nm large, polycrystalline particles (Figure 1a). When a capping agent like sodium citrate is added to the solution prior to the reduction, small metal clusters are stabilized by a citrate ligand shell and are visible in the TEM images together with bigger agglomerates (Figure 1b).[30] The isolated particles are of polyhedral shape (tetrahedrons, cubes, and cuboctahedrons can be distinguished on the sample's surface), which is typical for Pt clusters stabilized by citrate.[31] Interestingly, the morphology of the formed cluster aggregates is in part chainlike. However, the typical length of these randomly formed chains is much shorter than the length of any heterogeneously grown chain of particles on DNA (see below and refs 5, 6, and 16).

Since the reduction is performed under air, the presence of dissolved oxygen is expected to lead to formation of PtO_x phases at the surface of the metallic particles.[26,33] Superficial platinum oxide phases have been in fact detected by time-of-flight secondary ion mass spectrometry (TOF−SIMS) in a previous analysis of the platinum powders obtained following a reduction protocol analogous to the one used in this work.[34] Furthermore, an XPS analysis of the same material confirmed the metallic character of the obtained particles, revealing the presence of oxygen and carbon adsorbates, together with chlorine and potassium ions.[35] Although no boron impurities could be detected in these analyses, the boron content in particles produced by reduction of Pt complexes with boron compounds (such as, e.g., DMAB) has been found to be about 0.05 wt % by other authors.[29,36]

B. Pt Cluster Synthesis in the Presence of Nonactivated DNA. We turn now to study the reduction of dissolved $PtCl_4^{2-}$ ions in the presence of native DNA. To this aim, an aged solution of K_2PtCl_4 is mixed with dissolved DNA, and DMAB is *immediately* added to the solution. Under these conditions, the characteristic reduction time is shorter than the characteristic binding time of Pt(II) complexes to the DNA bases,[37] so that only a few complexes have time to bind to the DNA before the reduction is fully completed. We refer to these conditions as reduction in the presence of "nonactivated" DNA.

The overall kinetics of the reduction process in the presence of DNA in different concentrations is monitored by means of UV−vis spectroscopy at the wavelength of 600 nm (Figure 2). At this wavelength, there is no absorbance due to either dissolved $[PtCl_4]^{2-}$ complexes or DNA. Far from the plasmon resonance frequency (which is at about 215 nm in the case of Pt colloids[26]), the absorption of light depends mostly on the concentration of the formed metal particles, and only to a minor extent on their shape. According to ref 26, the major contribution to the increase of absorption in this region of the spectrum is due to dipole−dipole interactions of small particles when they aggregate into bigger structures during the reduction reaction. Therefore, the measured curves represent the overall kinetics of the reduction process.

In the presence of DNA, the overall reduction kinetics is slowed (Figure 2). The maximum effect is reached at a DNA concentration of 100 μg/mL, and no further retardation is measured by increasing the DNA amount. The metallization products obtained at 5 μg/mL are small crystallites with a diameter of about 5 nm, as well as a few larger polycrystalline

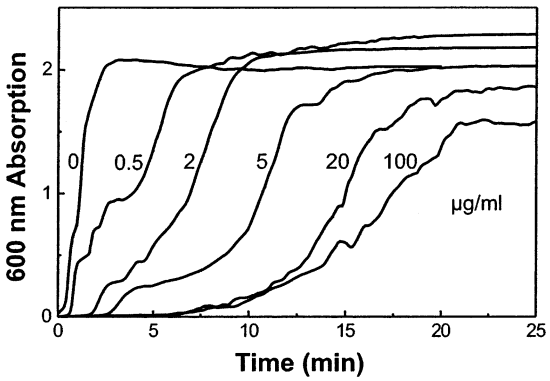

Figure 2. Reduction kinetics of a solution of $PtCl_4^{2-}$ in the presence of different amounts of nonactivated DNA, measured by UV−vis spectroscopy at 600 nm. The DNA concentration is given in μg/mL. The concentrations of $PtCl_4^{2-}$ (1mM) and DMAB (1 mM) are kept constant.

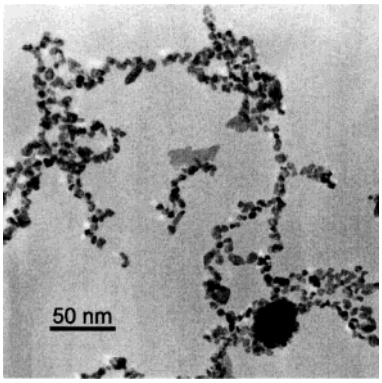

Figure 3. TEM micrograph showing the metallization products obtained after reduction of a 1 mM aged solution of $PtCl_4^{2-}$ with 1 mM DMAB in the presence of 5 μg/mL nonactivated DNA.

particle agglomerates (Figure 3). Note that TEM analysis alone does not permit the possibly present nonmetallized DNA molecules to be visualized. However, previously performed SFM studies[5,6] revealed that under similar conditions the large metal agglomerates are surrounded by free DNA molecules and that a few small crystallites appear to be located on the DNA strands. The addition of citrate to the Pt(II)/DNA solution prior to reduction led to similar aggregates (not shown) and was not effective in stabilizing long chains of particles aligned along the DNA molecules. From these results, we conclude that, to produce thin metallic wires, the reduction has to start necessarily after the completion of the binding reaction of Pt(II) complexes to the bases. The details of this process are investigated in the next section.

C. Activation of the DNA Template. The binding process of Pt(II) complexes to DNA is well investigated in the case of cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) which is widely used as an anticancer drug.[38] When DNA is incubated with Pt(II) complexes such as cisplatin, the Pt(II) atom binds to one or two stacked DNA bases forming monfunctional and bifunctional DNA·Pt(II) adducts, respectively. The most favorable binding site for cisplatin to the DNA is the N7 position of guanine, followed by the N7 position of adenine.[38] After these sites are occupied, the binding reaction proceeds more slowly and indiscriminately with other metal-binding sites of all bases.[37] It is known that the limiting step for the binding of $[Pt(NH_3)_2Cl_2]$ complexes to guanine or adenine is the hydrolysis of the complexes with loss of one or two chlorine ions, leading to the formation of $[Pt(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O)_2]^{2+}$ complexes, respectively.[39] In fact, the characteristic time for the binding of non-hydrolyzed complexes is about 2 h (which is comparable with the characteristic time of the chlorine/water substitution reaction), whereas 2-fold hydrolyzed complexes react with the DNA bases within a few minutes.[39] Although monofunctional adducts are formed fast, the formation of bifunctional adducts is much slower. In the case of cisplatin, the latter is completed within about 10 h.[39]

In the case of dissolved $[PtCl_4]^{2-}$ ions at high Pt(II)/base ratios, it is known that three Pt(II) complexes bind on average to each base pair.[37] This reaction is completed in about 10 h.[40] In analogy with the reaction mechanism proposed for hydrolyzed cisplatin complexes, the reaction of hydrolyzed Pt(II) complexes with DNA is expected to begin with the formation of a covalent bond between the N7 atom of a purine DNA base (A or G) and the Pt atom after loss of a water ligand. This reaction can be formally written as

$$G + PtCl_2(H_2O)_2 \rightarrow (G)PtCl_2(H_2O) + H_2O \qquad (4)$$

Then, after the loss of a second water ligand, bifunctional complexes like, e.g., $(AG)PtCl_2$ are expected to form, where a Pt(II) ion binds to two stacked bases. Similarly to the hydrolysis-limited binding process of cisplatin, we expect that the hydrolysis degree of the $[PtCl_4]^{2-}$ solution (see section III.A) will affect the kinetics of binding to DNA. To gain more information about the characteristic binding time of dissolved Pt(II) complexes under the conditions used in our metallization experiments, we measure the binding kinetics of $PtCl_4^{2-}$ to DNA at different hydrolysis degrees by means of UV−vis spectroscopy.

1. Kinetics of $[PtCl_4]^{2-}$ Binding to the DNA. DNA molecules have a maximum in the absorbance spectrum at ~260 nm, which is due to electronic excitations of the nitrogenous bases. The 260 nm absorbance increases passing from an ordered stack of bases to disordered conformations because of the diminished electronic interactions between the bases. This is evident, for instance, during the melting of DNA, which is the transition from the double-stranded to the single-stranded structure. Also the binding of dissolved $[PtCl_4]^{2-}$ ions to the DNA is known to lead to a significant distortion of the base stacking.[5,38] This allows us to investigate the kinetics of the binding process looking at the changes of the 260 nm absorbance peak during the incubation of DNA with $[PtCl_4]^{2-}$ (Figure 4). To separate the contribution of the DNA spectrum from that of the Pt(II) complexes, which also have a strong absorbance in the 260 nm region, we subtract from the spectrum of the Pt(II)/DNA solution the spectrum of a reference solution containing only $PtCl_4^{2-}$ ions at the same concentration (Figure 4a).

As expected, the binding of the Pt(II) complexes to DNA leads to an increase in 260 nm absorbance, i.e., to a strong distortion of the DNA base stacking, with increasing incubation time (Figure 4b). For each obtained DNA spectrum, we measure both the sample and the reference solution to account for the time-dependent changes of the $[PtCl_4]^{2-}$ spectrum due to ongoing hydrolysis processes. We start from a freshly prepared 1 mM solution and three aged stock solutions with different concentrations (100, 10, and 1.5 mM), which were diluted to a concentration of 1 mM immediately before mixing with the DNA solution. In the four samples, the absolute concentration of Pt(II) complexes is the same but the initial relative concentrations of non-hydrolyzed, 1-fold hydrolyzed, and 2-fold hydrolyzed complexes are different (Table 1). The measured kinetics curves are reported in Figure 4c. Initially, we observe a rapid increase of the absorbance, which slows down at long incubation times. However, the absorbance does not reach a saturation value

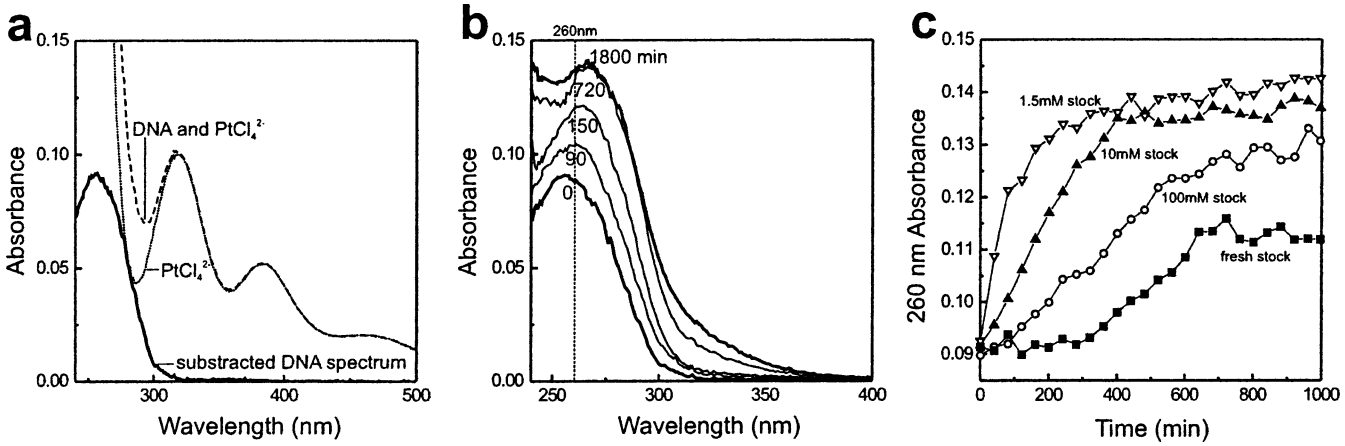

Figure 4. Distortion of the DNA base stacking due to the binding of $PtCl_4^{2-}$ complexes as a function of the incubation time. (a) Spectra of a 1 mM solution of $PtCl_4^{2-}$, and of a solution containing 1 mM $PtCl_4^{2-}$ and 5 μg/mL λ-DNA immediately after mixing (incubation time $t = 0$). Subtraction of the first spectrum form the second one provides a spectrum for the DNA only. The characteristic maximum at about 260 nm is visible. (b) Spectra of DNA incubated with $PtCl_4^{2-}$ obtained at increasing incubation times. Note the increase of the absorbance in the region around 260 nm, indicating the ongoing distortion of the DNA base stacking. (c) Distortion of the DNA base stacking recorded at 260 nm for differently hydrolyzed solutions of $PtCl_4^{2-}$, which were prepared by dilution of one fresh and three aged stock solutions with initial concentrations of 100, 10, and 1.5 mM to a final concentration of 1 mM. The percentages of the hydrolyzed species are reported in Table 1.

TABLE 1: Relative Equilibrium Concentrations of the Hydrolyzed Pt(II) Species in One Fresh and Three Stock Solutions of K_2PtCl_4 Aged for 16 h[a]

	$PtCl_4^{2-}$	$Pt(H_2O)Cl_3^-$	$Pt(H_2O)_2Cl_2$	$t_{\Delta Abs/2}$ (min)
fresh $PtCl_4^{2-}$	1	0	0	440
aged 100mM $PtCl_4^{2-}$	0.700	0.292	0.008	350
aged 10mM $PtCl_4^{2-}$	0.350	0.585	0.065	190
aged 1.5mM $PtCl_4^{2-}$	0.170	0.562	0.268	70

[a] The data were obtained using the equilibrium constants from ref 25. The right column in the table gives the characteristic time for the distortion of the base stacking of DNA (see text and Figure 4) upon incubation with the four differently hydrolyzed stock solutions after their dilution to a final concentration of 1 mM.

within the whole duration of the experiment, indicating that the binding of Pt(II) to the DNA still continues after more than 16 h of incubation. The time after which half of the maximum absorbance is reached for each solution is reported in Table 1. The more the $[PtCl_4]^{2-}$ solution is hydrolyzed, the faster the helix-breakdown process is, indicating a faster binding of the complexes to the DNA. Like in the case of *cis*-$Pt(NH_3)_2Cl_2$, the binding process appears thus to be limited by the hydrolysis of the complexes.

2. Double-Stranded DNA Structure after $PtCl_4^{2-}$ Binding. From the kinetics measurements presented above, it is unclear whether the DNA still retains its double-stranded structure after the binding of Pt(II) complexes. Indeed, the binding of Pt(II) complexes could break the hydrogen bonds between the strands causing a transition from double-stranded to single-stranded DNA, which could as well lead to an increase of the absorbance peak at 260 nm. To rule out this possibility, we perform a series of gel electrophoresis experiments (Figure 5). Solutions of double strand λ-DNA, thermally denatured λ-DNA, λ-DNA digested with *Hin*d III, and thermally denatured λ-DNA digested with *Hin*d III are incubated with a 1 mM aged solution of K_2PtCl_4. After 20 h of incubation, the four solutions are treated with a highly diluted DMAB solution and let run in an agarose gel. Subsequent gold staining (see Experimental Section) reveals the appearance of clear bands in the case of both full length and digested λ-DNA (lanes 2 and 4 of Figure 5). However, no bands appear in the lanes of the previously thermally denaturated ss-DNA samples (lanes 1 and 3 of Figure 5). This clearly

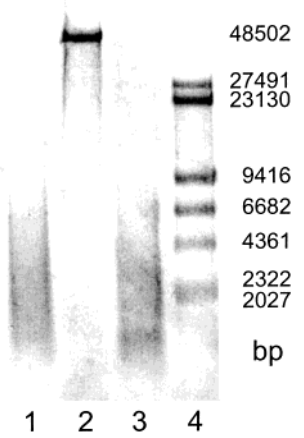

Figure 5. Electrophoresis experiments of single and double stranded DNA after 16 h of activation with $PtCl_4^{2-}$. Lane 1: single-stranded λ-DNA. Lane 2: double-stranded λ-DNA. Lane 3: single-stranded λ-DNA digested with *Hin*d III. Lane 4: double-stranded λ-DNA digested with *Hin*d III.

indicates that the process of Pt(II) binding to ds-DNA does not break the double strand configuration.[41]

3. DNA Morphology after Pt(II) Binding. The binding of Pt(II) complexes to ds-DNA is known to cause an overall shrinkage of the molecule length, as it was previously noticed in SFM experiments at low Pt/base ratios.[42] Here we investigate by means of SFM the structural changes which DNA undergoes upon $PtCl_4^{2-}$ binding at a Pt/base ratio of about 65:1 (Figure 6). Compared with native λ-DNA (Figure 6a), the incubation with hydrolyzed $PtCl_4^{2-}$ ions reveals the formation of kinks and coils in the DNA structure (Figure 6, parts b and c). This results in an overall shortening of the DNA molecules from a native length of about 16 μm to lengths in the range of 1.5−2.9 μm. The appearance of these short segments cannot be due to rupture of long molecules, as this would not result in the appearance of a single, sharp λ-DNA band in the electrophoresis experiments (see Figure 5, lane 2), which is indicative of a unique molecule weight.

D. Synthesis of Pt Cluster Chains in the Presence of Activated DNA. In section B we have shown that the reduction of metal salt in the presence of nonactivated DNA leads to the

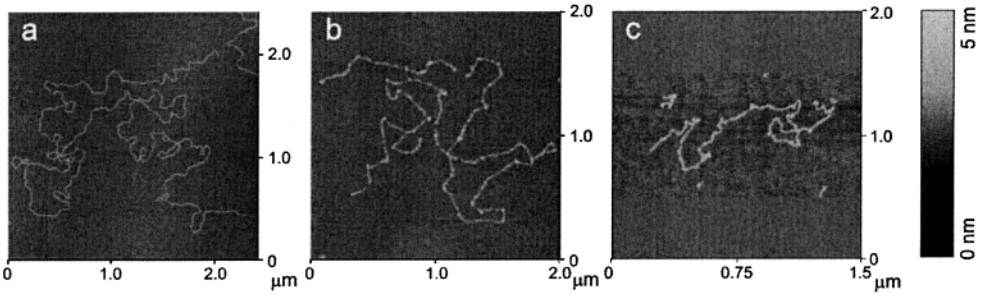

Figure 6. AFM images of DNA after incubation with $PtCl_4^{2-}$. (a) Native λ-DNA. (b) λ-DNA after 1 h of incubation. (c) λ-DNA after 16 h of incubation.

formation of particle aggregates. They are in part bound to the DNA[5,6] but also form bigger agglomerates of homogeneously nucleated particles. As demonstrated in ref 6, the formation of long cluster chains via a clean, purely heterogeneous cluster formation process necessarily requires the activation of the DNA for about 20 h prior to the reduction.[5,6,16] At fixed concentration of Pt(II) complexes (1 mM), analysis of metallization products at different concentrations of activated DNA (5, 20, and 100 μg/mL) revealed that continuous chains were obtained at a DNA concentration of 5 μg/mL. These concentrations correspond to a Pt(II)/base ratio of about 65:1, and will be considered as the "standard" concentrations in our metallization experiments. After long activation, about 1.5 complexes are expected to be bound to each base on average,[40] which means that less than 3% of the total complexes are bound to the DNA. Despite the large amount of free complexes in solution, no large agglomerates of homogeneously formed particles are present after reduction with DMAB (see Figure 7a and refs 5, 6, and 16). In other words, the balance between the homogeneous and heterogeneous nucleation processes is shifted from mixed homogeneous and heterogeneous nucleation in the case of nonactivated DNA to purely heterogeneous nucleation in the case of activated DNA.[6] A numbers of parameters which control this balance are investigated below.

The parameters which in principle can influence the metallization process are the absolute and relative concentrations of the reactants (metal complexes, DNA, reducing agent); the type of metal salt and its hydrolysis degree; the strength of the reducing agent (i.e., both the reduction potential between metal complexes and reducing agent, and the kinetics of electron transfer); the temperature and the pH of the reducing bath; the composition of the DNA sequence.[16] Finally, variations of the activation time influence both the number of complexes which bind to the DNA prior to reduction and, possibly, the hydrolysis degree of the bound complexes. Here we study how changing the absolute concentrations of reactants and the metal type influence the metallization process. Furthermore, we analyze the metallization products obtained with different DNA molecular structures (ds-DNA, ss-DNA, and single nucleotides) and in the presence of citrate as a capping agent for the formed clusters. The effect of the guanine content of the DNA template on the kinetics of metallization has been studied in ref 16 and will be discussed later in this paper. The strength of the reducing agent, the temperature, and the pH of the reducing bath can be considered parameters for a "fine-tuning" of the metallization procedure, and a systematic study of their effects on the metallization products will require further investigations.

1. Variation of the Absolute Concentration of the Reactants. Varying the absolute concentrations of the reactants (Pt salt, DNA, and reducing agent) can have a dramatic effect on the reaction products. Therefore, we perform metallization experiments (activation followed by reduction) using Pt(II) concentrations of 1/4, 1/2, 2, and 4 times the standard concentration, while keeping constant the concentration ratios of the reactants. A chain of clusters obtained under the standard conditions is shown as a reference in Figure 7a. The inset of Figure 7b shows a high resolution TEM image of the same preparation, which confirms the metallic character of the obtained particles (see the caption of Figure 7b). Remarkably, also when the absolute concentrations of the reactants are either half or twice the standard ones, we obtain very clean samples with purely heterogeneously formed cluster chains (Figure 7c). Instead, in the case of either one-fourth or four times the standard concentrations, only random cluster agglomerates are found on the sample's surface (not shown).

2. Variation of the DNA Molecular Structure. To investigate the influence of the DNA structure on the morphology of the metallization products, we perform reduction experiments in the presence of ss-DNA. The persistence length of ss-DNA is about 1 nm, i.e., much smaller than the persistence length of ds-DNA which is ~100 nm in solutions with ionic strength of about 1 mM.[43] Thermally denaturated λ-DNA is activated for 16 h, and subsequently, DMAB is added to the solution. The metallization products are shown in Figure 7d. In contrast with the case of ds-DNA, only very short cluster chains and random aggregates of small clusters are obtained. The small clusters appear to be stabilized to a certain extent by the ss-DNA, which act as a capping agent in a similar way as nonactivated ds-DNA (cf. Figure 3).

To study the capping action of DNA for the formed clusters in more detail, we perform reduction experiments in the presence of a mixture of single nucleotides, dATP, dTTP, dCTP, and dGTP. The relative ratios of the different nucleotides are chosen to match the base composition of λ-DNA. The nucleotide mixture is incubated for 16 h with an aged solution of K_2PtCl_4 at the standard reaction concentration. Subsequently, DMAB is added to the solution, and the metallization products are imaged with TEM. The formed clusters are shown in Figure 7e. The surface of the TEM grid is covered by separated small polyhedral crystallites surrounded by a capping layer, indicating that single nucleotides efficiently stabilize the formed clusters. A few bigger agglomerates are also present, which could presumably be avoided by more carefully tuning the concentrations of the reactants (here they are kept equal to those of the standard DNA metallization protocol).

3. Effect of Citrate. Citrate ions are known to be good capping agents for metal clusters[31] (cf. Figure 1b). With the aim of improving the quality of the obtained cluster chains, we add citrate to a solution of activated DNA immediately before starting the reduction.[44] In the presence of citrate, the metallization kinetics is slightly slowed, as expected in the presence of capping agents.[31] The obtained products are extremely regular

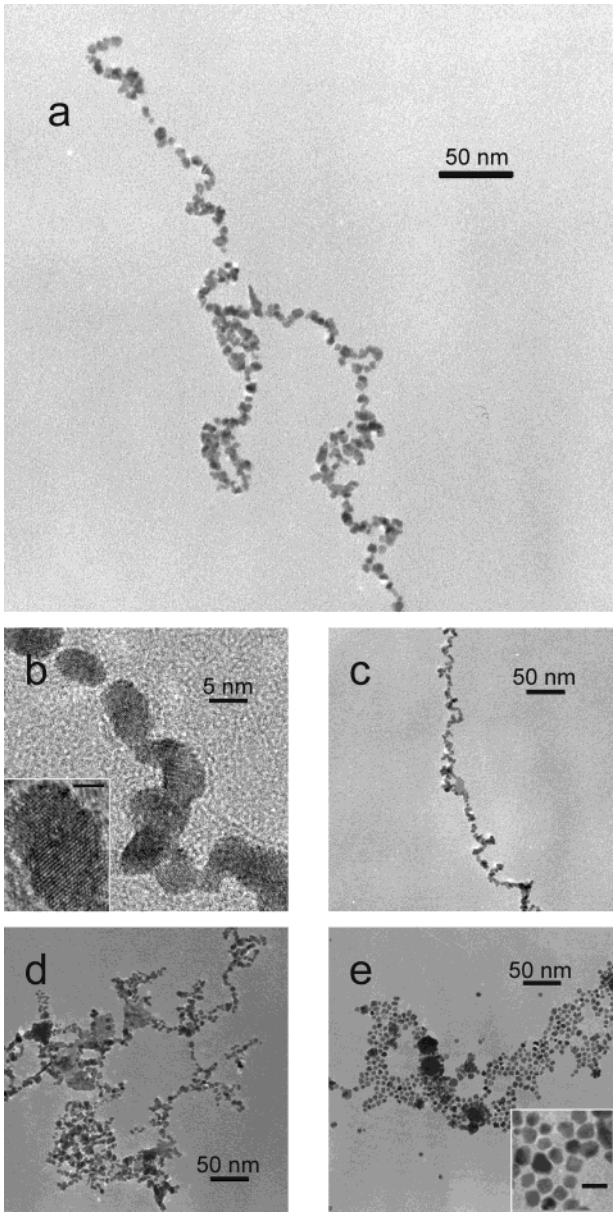

Figure 7. TEM micrographs of the metallization products formed after reduction of a $PtCl_4^{2-}$ solution with DMAB at different concentrations in the presence of ds-DNA, ss-DNA, and dissolved nucleotides. (a) 5 μg/mL λ-DNA, 1 mM $PtCl_4^{2-}$, and 1 mM DMAB. (b) as in (a), at higher resolution. Power spectrum analysis of multiple particles revealed interlayer distances correspondent to the [111], [200], and [202] crystallographic planes of bulk Pt, thus confirming the metallic character of the obtained particles. (c) 10 μg/mL λ-DNA, 2 mM $PtCl_4^{2-}$, and 2 mM DMAB. (d) 5 μg/mL single-stranded λ-DNA, 1 mM $PtCl_4^{2-}$, and 1 mM DMAB. (e) 5 μg/mL of nucleotides at a composition corresponding to that of λ-DNA, 1 mM $PtCl_4^{2-}$, and 1 mM DMAB.

chains of clusters of approximately the same size (~5 nm), which uniformly cover the DNA template over its whole length (Figure 8). Neither bigger agglomerates of homogeneously formed clusters nor random cluster aggregates are present in any of the investigated samples, whose backgrounds appear to be consistently clean of any spurious product of homogeneous metallization processes. In comparison with the chains obtained in the absence of citrate, the chains obtained here are significantly less curled (compare, for instance, Figure 8 with Figure 7a). Most of the crystallites are of regular shape, which is a direct influence of the capping action of citrate. Whereas without citrate the single clusters composing the chains tend to grow

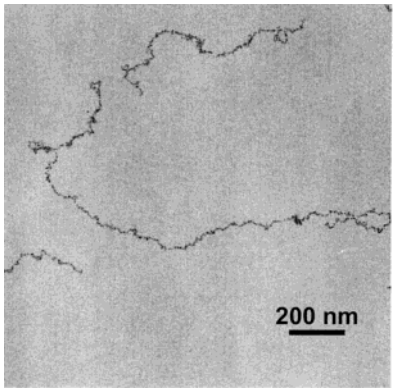

Figure 8. TEM micrograph of a chain of Pt clusters grown on λ-DNA in the presence of citrate (see text).

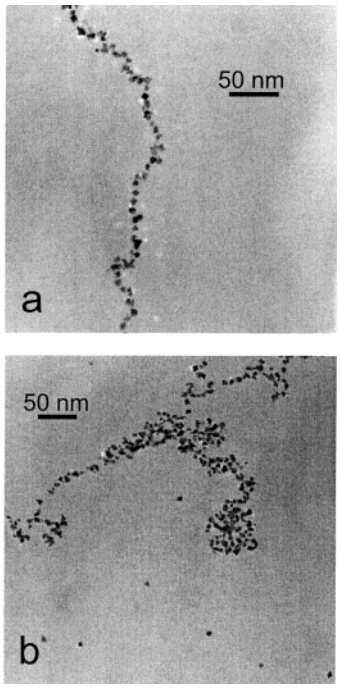

Figure 9. TEM micrographs of chains of (a) Pt and (b) Pd clusters grown on λ-DNA in the presence of citrate at our standard concentrations (see text). Note the presence of homogeneously nucleated particles in the case of palladium.

together and form continuous wires with a typical length of 50−100 nm (cf. Figure 7b), in the presence of citrate, they are clearly separated by a ligand shell (see also Figure 9a).

4. Comparison Pd vs Pt. As demonstrated in ref 6, increasing the activation time leads to an acceleration of the overall kinetics of metallization. At the same time, the nucleation behavior changes from mixed heterogeneous and homogeneous nucleation to purely heterogeneous nucleation (see ref 6). However, since only less than 3% of the Pt(II) complexes are bound to the DNA, this can only happen if the heterogeneous nucleation channel is considerably faster than the homogeneous nucleation channel. If, on the other hand, the homogeneous process itself is too fast, then the formation of homogeneously nucleated particles remains highly probable. This would lead to undesired agglomeration processes and formation of thicker metallic wires and/or cluster agglomerates. We investigate this issue using K_2PdCl_4 instead of K_2PtCl_4 as the salt precursor for both the activation and the subsequent metallization. Indeed, the reduction of Pd(II) complexes is known to be faster than the reduction of the corresponding Pt(II) complexes.[45]

In reduction experiments in the absence of DNA, the characteristic time for the reaction of an aged 1 mM solution of $[PdCl_4]^{2-}$ ions with DMAB lies below one second, and only large cluster agglomerates could be found in TEM investigations (not shown). The addition of citrate has a similar effect as shown in the case of Pt(II) complexes, leading to separate, crystalline clusters, as well as a number of bigger agglomerates (cf. Figure 1b). When Pd(II) ions are reduced in the presence of activated DNA and citrate, the characteristic time for metal formation is about 30 s. In comparison, the metallization starting from $PtCl_4^{2-}$ in the presence of citrate is significantly slower, being completed after about 8 min. TEM images of the obtained metallization products are shown in Figure 9. In the case of Pt, all obtained clusters appear to be grown specifically onto the DNA template (Figure 9a) forming long and ordered chains over the whole DNA length. Instead, in the case of Pd, a significant number of isolated clusters are present. These are clearly the result of homogeneous nucleation and growth processes (Figure 9b). Chains of Pd clusters along the DNA template are still obtained, but the metallization is coarser than in the case of Pt due to the fact that homogeneously formed clusters form aggregates on the heterogeneously formed chains (see also ref 2).

IV. Discussion

A. Reduction in the Presence of Nonactivated DNA. In the presence of nonactivated DNA, the overall kinetics of the process of metal cluster formation is slowed (see Figure 2). This effect is associated with the hindering of the aggregation of small metal crystallites into bigger agglomerates (cf. Figure 1a with Figure 3), strongly suggesting that nonactivated DNA acts like a capping agent for the metal clusters. Indeed, the reduction kinetics during the formation of metal particles is known to be retarded with increasing concentration of capping agents (see, e.g., ref 31). The capping action of DNA is mostly evident for the reduction of $PtCl_4^{2-}$ in the presence of single nucleotides. In these experiments, the Pt/nucleotide ratio is chosen to be about 65:1. Thus, only very few complexes are bound to nucleotides before the chemical reduction begins. Despite this, the obtained metal particles appear to be perfectly stabilized and form small crystallites surrounded by a ligand shell (see Figure 7e). This clearly proves the great affinity of nucleotides to the metal surface, to which they are expected to bind mainly via covalent bonds between surface metal atoms and deprotonated nitrogen atoms of the bases.

Although the aggregation of small clusters is hindered by the presence of DNA, the processes of nucleation and growth of the small crystallites are expected to be influenced only to a minor extent. The reason for this is that the metal reduction is relatively fast. It is completed within a few minutes (Figure 2), whereas the characteristic time for binding Pt(II) to the DNA bases varies from 1 to 10 h (Figure 4), depending on the hydrolysis degree of Pt(II). Therefore, homogeneous metal reduction is expected to take place before Pt(II) complexes will form adducts with DNA.[46] In fact, if the metal salt is reduced immediately after addition of dissolved DNA, particles grow according to a prevalently homogeneous nucleation channel, as it was observed in previous SFM experiments.[5,6] The chainlike aggregate structures visible in Figure 3 are most likely due to aggregation processes after the formation of small clusters, similarly to the case of absence of DNA but presence of citrate (see Figure 1b). These results show that uncontrolled adsorption of homogeneously formed clusters to the DNA does not lead to the formation of long, ordered cluster chains. This means that the binding of Pt(II) complexes to DNA bases prior to chemical reduction of the Pt(II)/DNA solution is *necessary* to promote a uniform, template-controlled growth of clusters along the DNA templates.[6] We call this step 'activation' of the DNA template.

B. Activation of the DNA Template. From the spectroscopic experiments reported in section III.C, it is clear that the binding kinetics of dissolved $[PtCl_4]^{2-}$ ions to λ-DNA depends on the hydrolysis degree of the Pt(II) complexes. This is in agreement with the data for cisplatin at low Pt/base ratios.[39] At the Pt(II) concentration used in our standard metallization protocol (10 mM stock solution diluted to 1mM prior to reduction), the absorbance reaches half of its maximum value after a characteristic time of about 3 h (Table 1), and still increases after more than 16 h. This behavior is in general consistent with the results of the metallization experiments reported in refs 6 and 16. However, in those works, the reduction rate (which is mainly related with the number of Pt(II) complexes bound to the DNA) was found to increase even after more than 30 h of activation. This result indicates that the DNA metallization kinetics is also influenced by processes which take place after the number of Pt(II) complexes bound to DNA has reached the saturation value of 1.5 complexes per base.[40] Processes characterized by extremely slow kinetics are, for instance, the formation of bifunctional complexes and the further hydrolysis of bound complexes.[39] Evidence that they can influence the kinetics of DNA metallization comes from previous theoretical work, where it was found that the hydrolysis of DNA·Pt(II) complexes have a great influence on the stability of the first-formed Pt nuclei.[20] In the same work, bifunctional (and fully hydrolyzed) complexes were found to have higher electron affinity with respect to monofunctional (and less hydrolyzed) complexes.

C. Formation of Cluster Chains. The results of our metallization experiments show that the Pt(II) complexes which are bound to the DNA act as very efficient nucleation centers for the growth of small crystalline particles. The mechanisms of the initial nucleation process of noble metal clusters in reducing baths have been extensively studied by means of first principles molecular dynamics simulation, both in the homogeneous case[47,22] and in the heterogeneous case.[6,20] In these works, it was found that the initial formation of Pt dimers between *unreduced* Pt(II) complexes and singly reduced Pt(I) complexes is the limiting step of the whole cluster formation process, especially at high concentration of metal salt and in the presence of mild reducing agents.[47] After the initial nucleation steps, the nucleus (which is not necessarily completely reduced to the zerovalent state) continues to grow via steps of addition of unreduced Pt(II) complexes[22] and reduction of the whole cluster, according to an autocatalytic mechanism.[21]

In the presence of activated DNA, the initial reduction reaction can involve either dissolved Pt(II) complexes or DNA·Pt(II) complexes. On one hand, our previously performed theoretical work[6,20] suggests an enhancement of the electronic affinity of Pt(II) complexes after binding to the DNA (see, in particular, ref 20 for a thorough discussion about possible DNA·Pt(II) species involved in the reduction reaction). This is due to the presence of the heterocyclic DNA bases as ligands for Pt(II), which can "accommodate" the additional electrons in a more favorable way. On the other hand, reduction in solution cannot be excluded a priori because of the large number of dissolved complexes. However, our first principles molecular dynamics studies led to the conclusion that the heterogeneous formation of a Pt(I)−Pt(II) dimer at DNA is considerably favored over the analogous homogeneous reaction in solution

irrespective whether the initial reduction involve a free or a bound Pt(II) complex.[6,20] In other words, the presence of DNA bases as ligands for Pt(II) complexes has both the effect of enhancing the electron affinity of the metal complexes and increasing the bond energy of the first-formed Pt−Pt bonds during the reduction process. This means that the formation of heterogeneous nucleation centers (the DNA·Pt(II) adducts formed during the activation) leads to a *locally* enhanced nucleation kinetics along the DNA. Since the clusters grow in an autocatalytic, nucleation-limited way, this leads in turn to an *overall* enhancement of the metallization kinetics, as observed in the experiments reported in ref 6. Therefore, enhancement of the stability of the first-formed nuclei due to the complexation of Pt(II) by heterocyclic systems such as the DNA bases leads to a purely heterogeneous growth of particles on the DNA under the right reaction conditions. In particular, the concentration of metal complexes and the strength of the reducing agents should be carefully tuned to ensure that the autocatalytic growth started from the heterogeneous nuclei quickly consume the feedstock of free metal complexes before homogeneous processes lead to the formation of free clusters in the solution. If this condition is fulfilled, then the metallization occurs via a selectively heterogeneous, DNA-controlled mechanism.

1. Effect of the Kinetics of Metal Reduction. Consistently with the discussion above, the balance between heterogeneous and homogeneous nucleation appears to be directly influenced by the kinetics of the reduction process. Indeed, in the metallization experiments performed with Pd(II) complexes, which are reduced considerably faster than Pt(II) complexes, both the heterogeneous formation of cluster chains on the DNA and the homogeneous formation of metal particles in solution are simultaneously observed (see Figure 9). Especially in the absence of additional stabilizers such as citrate, these homogeneously formed particles can form random aggregates. This generally results in a much coarser and irregular metallization, as reported in refs 2 and 3, where the obtained wires have a diameter of about 50 nm and the formation of bigger, polycrystalline agglomerates of clusters is evident.[48]

2. Effect of Changing the Concentration of Reactants. In our investigations, we have identified a standard set of concentrations which results in a purely heterogeneous growth of platinum on DNA templates. In addition, we have shown that small deviations from the standard conditions (from 1/2 up to 2 times the standard concentration) have no effect on the quality of the metallization products. However, larger deviations (1/4 and 4 times the standard concentration) have a dramatic effect on the metallization products, and in both cases random agglomerates of particles are obtained. On one hand, when the concentration of Pt and DNA is too high, the formation of agglomerates is expected to happen both as a result of aggregation of the formed metallic chains, and, possibly, of the formation of links between different DNA strands by Pt(II) complexes during the activation step. On the other hand, when the concentration of Pt and DNA is too low, the formation of metal clusters is likely to occur through the classical mechanism of full reduction of the metal salt to the zerovalent state, followed by agglomeration of Pt(0) complexes. Namely, as discussed in ref 47, at very low concentration of Pt salt unreduced complexes are expected to have little effect on the mechanism of cluster formation. However, to observe a catalytic activity of DNA in promoting heterogeneous metal formation, the nucleation process necessarily has to involved *unreduced* complexes. This means that at too low concentration of metal salt the balance between homogeneous and heterogeneous nucleation is shifted toward the *homogeneous* nucleation channel, resulting in the formation of random aggregates of homogeneously nucleated particles.

3. Metallization of ss-DNA. In section III. D.2, we have shown that regular chains of particles cannot be grown in solution on single stranded DNA with our method.[49] Compared with double stranded DNA, the extent of random aggregation is increased, because the more flexible structure is not able to separate efficiently the heterogeneously formed clusters. Indeed, this can only be achieved if the persistence length of the molecular template is larger than the cluster diameter. Therefore, it is necessary to use ds-DNA as metallization template to obtain the formation of regular wire-like metallic structures in solution.[50]

4. Effect of Citrate. Citrate is known to act as an efficient capping agent for noble metal clusters (see ref 31 and Figure 1b). Therefore, we have added citrate prior to the reduction of platinum in the presence of DNA to fully suppress the local agglomeration of heterogeneously nucleated particles. Indeed, the addition of citrate ions leads to less curled and, therefore, longer cluster chains than those obtained in the absence of citrate (see Figure 8). However, the clusters obtained in this way are no longer in metallic contact. They are surrounded by a ligand shell, so that the obtained cluster necklace is not expected to be conductive. A thermal treatment after formation of the structure may be possibly sufficient to decompose the citrate layer restoring the metallic continuity.

On the other hand, when citrate is not added as a stabilizer, the produced wires appear to be continuous over distances of about 50 to 100 nm (see Figure 7b). However, small gaps are still present, which are expected to interrupt the metallic conductivity over longer distances. We expect that a further selective reduction of metal complexes on the cluster chains (similarly to the protocol used to produce "core−shell" nanoparticles[51]) will fill the gaps without increasing the thickness of the wire of more than a few nm (thus keeping the wire diameter below 10 nm). Investigations of this possibility, as well as measurements of the I/V characteristics of the obtained structures are currently under investigation in our laboratory.

5. Purity of the Pt Nanoparticles. Pt powders produced by reduction of Pt(II) complexes with DMAB under air predominantly reveal metallic character. PtO_x phases are present but are limited to the outermost atomic layers of the particles (see section III.A and refs 26, 34, and 35). Boron impurities are expected to be present in the bulk of the particles at a concentration of about 0.05 wt %.[29,36] We assume that the presence of DNA has little or no influence on the amount of impurities in the particle chains. In fact, HR-TEM investigations (see Figure 7b) confirm the metallic character of the synthesized clusters. Moreover, evidence of the predominantly metallic character of structures obtained using a similar protocol comes from previous work on 50 nm thick Pd wires grown on DNA templates after the reduction of Pd complexes with DMAB.[3,8] Conductivity measurements showed that these wires present ohmic behavior, and that their specific resistivity is only 10 times larger than that of bulk palladium.[3,8] Electron scattering at the grain boundaries of the clusters was identified as the dominant scattering mechanism.[3] Whether this effect is also relevant for the conductivity of the ultrathin cluster chains composed by series of single clusters remains to be investigated. Certainly, the present impurities will affect the conductivity of the particle chains in some way. To reduce the amount of impurities to the minimum, the cluster chains could be produced under exclusion of oxygen, or using hydrogen instead of DMAB as reducing agent.

6. Effects of the DNA Composition. All of the investigations presented here are carried out using DNA molecules with a random sequence and a content of GC base-pair between 40 and 50%. Thus, the influence of sequence and composition of the DNA template is excluded from the present study. However, we briefly discuss in this section results of previous investigations showing a peculiar influence of the content of GC vs AT base pairs on the kinetics of the reduction reaction in the presence of DNA.[16]

Since for short interaction times Pt(II) complexes are known to bind primarily to the guanine bases,[37] one would expect that the reaction kinetics becomes increasingly accelerated with increasing content of GC base pairs in the DNA used. This issue was investigated performing metallization experiments using three different types of DNA, containing 27%, 42%, and 72% of GC pairs.[16] The metallization rate was found to be independent of the DNA content when no complexes are bound to the DNA bases (no activation), or after very long activation times, where all metal-binding sites along the DNA are indiscriminately occupied by Pt(II) complexes. However, for a window of activation time ranging from 30 to 300 min, the reduction process was found to be faster for higher G contents. Indeed, in this time window the kinetics of Pt(II) binding to guanine is substantially faster than the binding to other bases.[37] Thus, in this time window, the number of complexes bound to the DNA depends directly on the GC content of the DNA. This confirms the active role of the formed Pt(II)·DNA adducts in promoting an acceleration of the reduction reaction. In particular, these experiments suggest that the DNA sequence could be designed and the metallization performed in such a way that only G-rich DNA sequences become fully metallized, while A-rich parts of the template remain in their native form or are decorated by a few, isolated clusters.

V. Future Perspectives

In conclusion, we have described in detail a procedure for the selective growth of metal on DNA templates according to a heterogeneous nucleation and growth mechanism which is controlled by the template itself. The growth of metal clusters on other organic polymers, in particular on proteins, can be achieved according to similar procedures.[52−56] The described metallization method is both simple and robust and thus in principle suitable to be extended to larger scale processing. Namely, although the molecular mechanisms of the processes of heterogeneous cluster formation on DNA templates are rather complicated, the sequence of steps of the metallization procedure which permit the fabrication of uniform and thin cluster chains appears to be astonishingly simple. Moreover, we stress that small variation of the reactant concentrations are extremely unlikely to affect the quality of the metallization products, once the "standard" concentrations have been carefully chosen. Therefore, we expect that the metallization procedure presented here will be a good starting point toward the development of large scale biopolymer metallization techniques. The overcoming of the difficulties that will invariably be associated with the scaling-up of the DNA metallization is a fundamental issue to be addressed in future work.

Various mechanistic aspects of the single steps composing the metallization protocol has been addressed and discussed also taking into account previously performed first principles atomistic simulations. While the global metallization mechanisms are now sufficiently clear, a number of more specific questions still remain to be answered, which are expected to stimulate future basic research work in the field of DNA metallization. The reaction conditions described in this work are tuned to fabricate chains of metal clusters in the bulk solution. However, application in electronic circuits or sensors will require the in-situ metallization of molecules bound to the surface of a solid substrate. The optimal reaction conditions for the metallization of molecules on a solid surface should be the subject of further investigations. Moreover, although continuous metal wires are obtained over relatively short distances, long chains of clusters invariably present small gaps that are expected to limit their conductivity. Additional work, including systematic measurements of the electric characteristic of the cluster chains and the continuous metal wires, should be devoted to address the problem of closing these gaps. If a thermal annealing of the metallized DNA is not sufficient, then the selective deposition of a thin metal layer on the formed clusters should lead to continuous wires with a diameter below 10 nm. Moreover, the influence of the impurities present in the bulk and on the surface of the obtained metallic structures on their conductivity remains to be investigated.

Finally, previously performed metallization experiments using DNA with different GC contents revealed that the metallization kinetics can be remarkably influenced by the nucleotide composition of the DNA sequence under particular conditions.[16] We stress that the metallization procedure presented in this paper is not designed to be affected by the DNA sequence and composition. However, we expect that the present metallization protocol can be modified by carefully acting on the kinetics of Pt(II) binding and reduction to develop a procedure for a sequence-specific metallization of DNA, where only guanine-rich sequences become fully metallized. This would enable a direct control of the metal growth by the DNA template at an unprecedented level.

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References and Notes

(1) Braun, E.; Eichen, Y.; Sivan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775−778.

(2) Richter, J.; Seidel, R.; Kirsch, R.; Mertig, M.; Pompe, W.; Plaschke, J.; Schackert, H. K. *Adv. Mater.* **2000**, *12*, 507−510.

(3) Richter, J.; Mertig, M.; Pompe, W.; Mönch, I.; Schackert, H. K. *Appl. Phys. Lett.* **2001**, *78*, 536−538.

(4) Harnack, O.; Ford, W. E.; Yasuda, A.; Wessels, J. M. *Nano Lett.* **2002**, *2*, 919−923. Ford, W. E.; Harnack, O.; Yasuda, A.; Wessels, J. M. *Adv. Mater.* **2001**, *13*, 1793−1797.

(5) Seidel, R.; Mertig, M.; Pompe, W. *Surf. Interface Anal.* **2002**, *33*, 151−154.

(6) Mertig, M.; Colombi Ciacchi, L.; Seidel, R.; Pompe, W.; De Vita, A. *Nano Lett.* **2002**, *2*, 841−844.

(7) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yoseph, G.; Sivan, U.; Braun, E. *Science* **2002**, *297*, 72−75.

(8) Richter, J.; Mertig, M.; Pompe, W.; Vinzelberg, H. *Appl. Phys. A* **2002**, *74*, 725−728.

(9) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882−1884.

(10) Deng, Z.; Mao, C. *Nano Lett.* **2003**, *3*, 1545−1548.

(11) Nakao, H.; Shiigi, H.; Yamamoto, Y.; Tokonami, S.; Nagaoka, T.; Sugiyama, S.; Ohtani, T. *Nano Lett.* **2003**, *3*, 1391−1394.

(12) Monson, C. F.; Woolley, A. T. *Nano Lett.* **2003**, *3*, 359−363.

(13) Mertig, M.; Pompe, W. in: *Nanobiotechnology − Concepts, Applications and Perspectives*; Niemeyer, C. M., Mirkin, C., Eds.; Wiley-VCH: Weinheim, Germany, 2004; pp 256−277.

(14) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609−611.

(15) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607−609.

(16) Mertig, M.; Seidel, R.; Colombi Ciacchi, L.; Pompe, W. *AIP Conf. Proc.* **2002**, *633*, 449−453.

(17) Zhang, Y.; Austin, R. H.; Kraeft, J.; Cox, E. C.; Ong, N. P. *Phys. Rev. Lett.* **2002**, *89*, 198102−1−4.

(18) Seeman, N. C. *Curr. Opin. Struct. Biol.* **1996**, *6*, 519−526.

(19) Richter, J. *Physica E* **2003**, *16*, 157−173.

(20) Colombi Ciacchi, L.; Mertig, M.; Seidel, R.; Pompe, W.; De Vita, A. *Nanotechnology* **2003**, *14*, 840−848.

(21) Watzky, M. A.; Finke, R. G. *J. Am. Chem. Soc.* **1997**, *119*, 10382−10400.

(22) Colombi Ciacchi, L.; Pompe, W.; De Vita, A. *J. Phys. Chem. B* **2003**, *107*, 1755−1764.

(23) Bezanilla, M.; Manne, S.; Laney, D. E.; Lyubchenko, Y. L.; Hansma, H. G. *Langmuir* **1995**, *11*, 655−659.

(24) Sanders, C. I.; Martin, Don, S., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 807−810.

(25) Elding, L. I. *Acta Chem. Scand.* **1970**, *24*, 1527−1540.

(26) Henglein, A.; Ershov, B. G.; Malow, M. *J. Phys. Chem.* **1995**, *99*, 14129−14136.

(27) Duff, D. G.; Edwards, P. P.; Johnson, B. F. G. *J. Phys. Chem.* **1995**, *99*, 15934−15944.

(28) Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochmann, M. *Advanced Inorganic Chemistry*, 6th ed.; John Wiley: New York, 1999.

(29) van Rheenen, P. R.; McKelvy, M. J.; Glaunsinger, W. S. *J. Solid State Chem.* **1987**, *67*, 151−169.

(30) In principle, it is possible to tune the concentrations of Pt(II) and capping agent and the strength of the reducing agent to obtain monodisperse colloidal solution of metal clusters.[29,31,32] The conditions applied here mainly serve as a reference for the later described experiments of DNA metallization, where the same concentrations of $PtCl_4^{2-}$ and DMAB are used.

(31) Henglein, A.; Giersig, M. *J. Phys. Chem. B* **2000**, *104*, 6767−6772.

(32) Henglein, A.; Giersig, M. *J. Phys. Chem. B* **1999**, *103*, 9533−9539.

(33) Henglein, A.; Lindig, B.; Westerhausen, J. *Radiat. Phys. Chem.* **1984**, *23*, 199.

(34) Schindler, A.; König, R.; Herrmannsdörfer, T.; Braun, H. F.; Eska, G.; Günther, D.; Meissner, M.; Mertig, M.; Wahl, R.; Pompe, W. *Europhys. Lett.* **2002**, *58*, 885−891.

(35) Rossbach, P.; Becker, J. S. private communication.

(36) Bönnemann, H.; Brijoux, W.; Brinkmann, R.; Fretzen, R.; Joussen, T.; Köppler, R.; Korall, B.; Neiteler, P.; Richter, J. *J. Mol. Catal.* **1994**, *86*, 129−177.

(37) Macquet, J. P.; Theophanides, T. *Biopolymers* **1975**, *14*, 781−799. Macquet, J. P.; Theophanides, T. *Inorg. Chim. Acta* **1976**, *18*, 189−194. Macquet, J. P.; Butour, J. L. *Eur. J. Biochem* **1978**, *83*, 375−387.

(38) *Cisplatin: chemistry and biochemistry of a leading anticancer drug*; Lippert, B., Ed.; Wiley-VCH: Weinheim, Germany, 1999.

(39) Bancroft, D. P.; Lepre, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 6860−6871.

(40) Sacharenko, E. T.; Moschkowski, Yu. S. *Biofizika* **1972**, *7*, 373−378, *in Russian*.

(41) The *Hin*d III digested double stranded λ-DNA consists of fragments composed by 23130, 2027, 2322, 9416, 564, 6682, 4361 bp, each terminated with 4 bp long single stranded ends (sticky ends) having sequences defined by the site of the enzymatic cut, which do not re-hybridize at room temperature. Only the extremities of the molecule, that is, the 5′ end of the 23130 bp fragment and the 3′ end of the 4361 bp fragment are terminated by 12 bp long sticky ends, which are known to hybridize at room temperature. In fact, in the gel a band correspondent to a fragment of about 27500 bp is present, which derives from hybridization of a number of 4361 bp fragments with 23130 bp fragments. These fragments are held together only by the hydrogen bonds between the complementary sticky ends, since the linking of the backbones requires specific enzymatic action. The fact that these long fragments are found after incubation with Pt(II) complexes means that the double stranded configuration is maintained over a length as short as 12 bp.

(42) Onoa, G. B.; Cervantes, G.; Moreno, V.; Prieto, M. J. *Nucleic Acids Res.* **1998**, *26*, 1473−1480.

(43) Baumann, C. G.; Smith, S. B.; Bloomfield, V. A.; Bustamante, C. *Proc. Natl. Acad. Sci.* **1997**, *94*, 6185−6190.

(44) Note that citrate is used in this work exclusively as a capping agent and not as a reducing agent. This would require much higher reaction temperatures and longer reaction times.

(45) Wu, M. L.; Chen, D. H.; Huang, T. C. *J. Colloid Interface Sci.* **2001**, *243*, 102−108.

(46) In fact, in an SFM analysis of metallization products in the presence of 20 μg/mL of DNA, we found the presence of isolated small clusters with a height of about 5 nm on the surface of the sample after 3 min of reduction (image not shown), that is, when the measured turbidity is still negligible.

(47) Colombi Ciacchi, L.; Pompe, W.; De Vita, A. *J. Am. Chem. Soc.* **2001**, *123*, 7371−7380.

(48) We note that the DNA metallization with Pd reported in ref 2 and 3 was performed at a much higher Pd:base ratio (about 2700:1) and with shorter activation time (2 h).

(49) Note, however, that chains could actually be grown on single stranded DNA when the molecules are stretched and fixed between two metal contacts, which would avoid the formation of tight random coils.

(50) A way to keep ss-DNA linear over long distances could be the attachment of its ends to a solid substrate prior to the activation and reduction steps. This, however, will certainly require adjustments of the reaction parameters and needs further investigations.

(51) Hodak, J. H.; Henglein, A.; Giersig, M.; Hartland, G. V. *J. Phys. Chem. B* **2000**, *104*, 11708−11718.

(52) Shenton, W.; Pum, D.; Sleytr, U. B.; Mann, S. *Nature* **1997**, *389*, 585−587.

(53) Mertig, M.; Kirsch, R.; Pompe, W.; Engelhardt, H. *Eur. Phys. J. D* **1999**, *9*, 45−48. Mertig, M.; Wahl, R.; Lehmann, M.; Simon, P.; Pompe, W. *Eur. Phys. J. D* **2001**, *16*, 317−320.

(54) Kirsch, R.; Mertig, M.; Pompe, W.; Wahl, R.; Sadowski, G.; Boehm, K. J.; Unger, E. *Thin Solid Films* **1997**, *305*, 248−253. Mertig, M.; Kirsch, R.; Pompe, W. *Appl. Phys. A* **1998**, *196*, S723−S727.

(55) Behrens, S.; Rahn, K.; Habicht, W.; Böhm, K.-J.; Rösner, H.; Dinjus, E.; Unger, E. *Adv. Mater.* **2002**, *14*, 1621−1625.

(56) Dujardin, E.; Peet, C.; Stubbs, G.; Culver, J. N.; Mann, S. *Nano Lett.* **2003**, *3*, 413−417.