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# DNA Analysis by Application of Pt Nanoparticle Electrochemical Amplification with Single Label Response

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Supporting Information

**ABSTRACT:** This study demonstrates a highly sensitive sensing scheme for the detection of low concentrations of DNA, in principle down to the single biomolecule level. The previously developed technique of electrochemical current amplification for detection of single nanoparticle (NP) collisions at an ultramicroelectrode (UME) has been employed to determine DNA. The Pt NP/Au UME/hydrazine oxidation reaction was employed, and individual NP collision events were monitored. The Pt NP was modified with a 20-base oligonucleotide with a C6 spacer thiol (detection probe), and the Au UME was modified with a 16-base oligonucleotide with a C6 spacer thiol (capture probe). The presence of a target oligonucleotide (31 base) that hybridized with both capture and detection probes brought a Pt NP on the electrode surface, where the resulting electrochemical oxidation of hydrazine resulted in a current response.

Metal nanoparticles (NPs) have been widely used in many fields because of their large surface-to-volume ratio and size-dependent optical properties.[1,2] NPs are often employed in biosensors, as labels for signal amplification,[3−6] or as supports to provide large surface areas.[7] For example, DNA sensors have been reported that use the color changes when DNA-modified NPs aggregate by DNA hybridization.[8,9] Wang's group developed a DNA sensor using anodic stripping of NPs,[10−12] and Yang's group employed the electrocatalytic properties of NPs as a signal amplification method.[13,14] These NP labels improve the sensitivity of the biosensor beyond that of enzyme[15,16] or optically detected quantum dot[17,18] labels. Thus, DNA can be detected at attomolar to picomolar concentration levels using various methods,[19,20] including redox cycling.[21] However, the challenge is to detect biomolecules at even lower levels, i.e., zeptomolar or even just a few molecules, since the ability to determine such small amounts, e.g., in the bloodstream, could be of diagnostic value. Previously, individual single-stranded DNA was studied with a nanopore in a membrane,[22] although this is mainly used for sequencing information. Scanning probe microscopy[23] can be used to image DNA but is not really a useful analytical approach for practical detection. In this study, we demonstrate a proof-of-concept study on the simple individual DNA sensing scheme using an ultramicroelectrode (UME).

Our recently developed electrocatalytic amplification (EA) method,[24−26] which demonstrates detection of single particle events, suggests the possibility of a highly sensitive biosensing scheme using NPs as the labels in the biosensor. The single NP contact event between an electrocatalytic NP and an inert UME produces a large current amplification and can be monitored as a current step or transient (blip). We describe here its use as a sensing scheme for biomolecule (DNA) detection, showing the real-time response of a single target molecule. We should note, however, that the total concentration of target molecules in this study is sufficiently high that responses by diffusion occurred in a reasonable time in a fairly large volume (10 mL). Success with samples at the zM or single molecule level will require dealing with the system fluidics and surface equilibria. Therefore, in this study, we mainly focus on whether this technique is adaptable for the biosensing scheme using a moderate sample concentration and detection time, and we show that the electrocatalytic amplification of signal is still obtainable even after the NP and UME have been modified with DNA.

A key issue in applying the EA method for a biosensor is verification of the electrocatalytic activity of the NPs after modification with DNA and demonstration of electrochemically effective contact between the DNA-modified NP and the DNA-modified UME. In previous related studies,[27] the single NP collision signal was very sensitive to many variables, such as a self-assembled monolayer (SAM) on the UME and the nature and amount of capping agent on the NP, in addition to the usual EA variables, like the nature of the electrolyte, electrolyte concentration, and pH. For example, the NP collision signal disappeared in the presence of a strongly binding capping agent such as poly(vinylpyrrolidone) (PVP) or long-chain alkanethiols. This may be related to the binding properties of NPs onto the electrode and electron-transfer kinetics through the capping agent layer. Citrate ion appears to be a good capping agent for NPs for observation of NP collision signals. A sandwich-type DNA sensor with a Au UME was prepared as shown in Scheme 1 (see the Supporting Information for experimental details).

The amount of DNA that was applied to the NP as a detection probe has to be controlled. This was accomplished to produce an average of 10 DNAs per NP by incubating the Pt NP in a solution containing a 10 times higher concentration of DNA than NPs for 48 h. Similarly, a dense SAM or adsorbed

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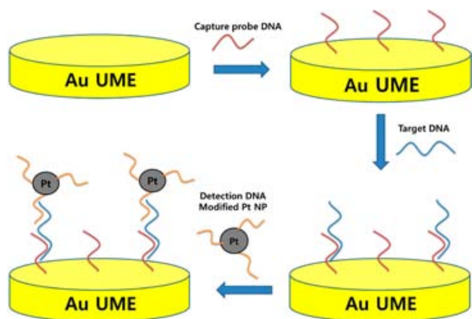
**Scheme 1. Illustration of Sandwich-Type DNA Sensor on the Au UME (Radius 5 $\mu$m)**




layer on the UME may block electron transfer between the tethered NP and electrode. Thus, in preparation of the UME with capture probe, no back-filling step with a short-chain alkanethiol, which is generally used in a preparation of DNA-mixed SAMs,[28] was carried out. To test the strategy in Scheme 1 first with a larger number of molecules, the Au UME (radius 5 $\mu$m) was cleaned with piranha solution ($H_2O_2$:$H_2SO_4$ (v/v) = 1:2). A synthesized thiol-functionalized DNA oligomer (the capture probe) was introduced by immersing the clean Au UME in the probe-containing solution for 12 h. After being rinsed with buffer (RB), the UME was incubated in a complementary DNA (target DNA) solution for 30 min. After being rinsed with RB, the electrode was incubated in a detection probe DNA-modified Pt NP solution for 30 min to form a layer of particles on the surface of the UME. After the electrode was rinsed, cyclic voltammetry (CV) and chronoamperometry (CA) were used to demonstrate the presence of labeled Pt NPs on the Au electrode surface. Figure 1 shows

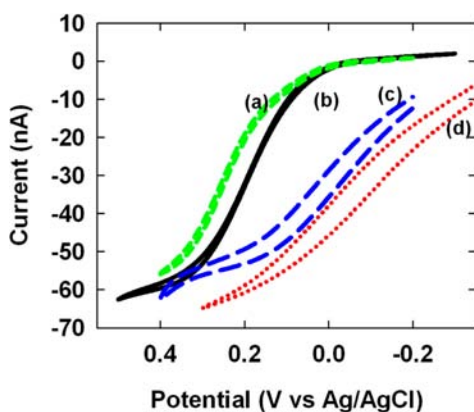



**Figure 1.** Cyclic voltammograms of hydrazine oxidation at (a) capture probe DNA-modified Au UME (green), (b) bare Au UME (black), (c) Au UME tethered Pt NP by DNA hybridization (1 $\mu$M of target DNA) (blue), or (d) bare Pt UME (red) (in all cases, UME radius is 5 $\mu$m) in 50 mM pH 7 phosphate buffer ( PB) containing 10 mM hydrazine. Scan rate is 50 mV/s.

hydrazine oxidation at variously prepared electrodes. A Au UME modified with capture probe (Figure 1a) showed a larger overpotential compared to a bare Au UME (Figure 1b), consistent with some blocking of active sites for hydrazine oxidation on the Au surface. However, the peak shifted to less positive potentials after the Pt NPs were placed on the surface using sandwich-type DNA hybridization (Figure 1c, i.e., the last step of Scheme 1.) This shifted peak was close to the peak at a bare Pt UME (Figure 1d). This indicated the Pt NP-modified (by DNA hybridization) Au UME with the tethered Pt NPs showed electrocatalytic activity, even with the DNA SAM on the electrode. We also investigated the peak shift from Au- to Pt-like behavior in the presence of different concentrations of target DNA (Figure 2). The peak shift increased with an

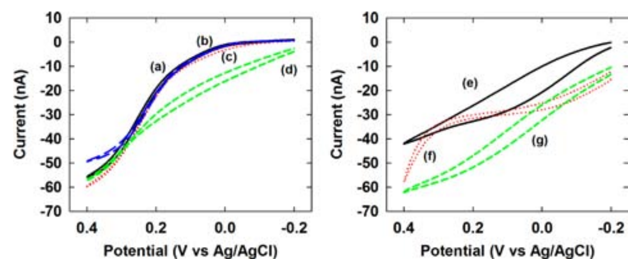



**Figure 2.** Cyclic voltammograms of hydrazine oxidation at prepared Au UME having DNA sensing layer. The UME was first incubated in a capture probe DNA and then incubated in (a) 10 nM (blue) noncomplementary DNA or (b) 0 M (black), (c) 10 pM (red), (d) 100 pM (green), (e) 1 nM (black), (f) 10 nM (red), or (g) 100 nM (green) complementary target DNA solution. The UMEs were then incubated in detection DNA-modified Pt NP solution. After the hybridization was finished, the electrode was rinsed and tested by CV in 50 mM pH 7 PB containing 10 mM hydrazine. Scan rate is 50 mV/s.

increase of target DNA concentration, i.e., only a very small shift with a 10 pM concentration but very close to that of a Pt UME with a 100 nM concentration. This peak shift, which is the result of ensemble properties of Pt NPs on the Au electrode, demonstrates an increase in the number of particles with higher target DNA concentrations. The use of an ensemble property of NPs as a sensor signal has been widely shown for biosensors employing NPs as labels.[8,10] Note that the overpotential shift caused by individual NPs is too small to be distinguished by CV at concentrations below about 100 pM.

However, by introducing the single NP EA method, we could obtain signals at lower target DNA concentrations and also show responses from a single NP. As shown in Figure 3, a current staircase was observed upon addition of target DNA-Pt NP samples to form a 10 pM solution. The single NP staircase increase of current represents the result of individual DNA-tethered Pt NPs colliding with and attaching to the UME and

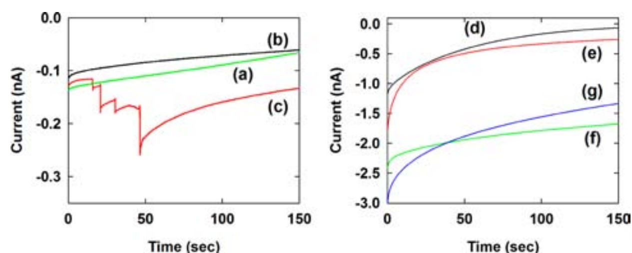



**Figure 3.** Chronoamperometric curves for single Pt NP collisions at the prepared DNA sensing Au UME. The UME was first incubated in a capture probe DNA and then incubated in (a) 10 nM (green) noncomplementary DNA or (b) 0 M (black), (c) 10 pM (red), (d) 100 pM (black), (e) 1 nM (red), (f) 10 nM (green), or (g) 100 nM (blue) complementary target DNA solution. The UMEs were then incubated in detection DNA-modified Pt NP solution. After the hybridization was finished, the electrode was rinsed and tested by CA in 50 mM pH 7 PB containing 10 mM hydrazine at 0 V vs Ag/AgCl.

existing at tunneling distance with the UME surface. The reason that a signal is obtained only with a low target concentration may be due to the background current level. At higher target DNA concentrations, the background current was sufficiently high that it was difficult to distinguish a step signal from the background level (see SI). Note that the absence of signals in the absence of target DNA (Figure 3, 0 M line) demonstrates the absence of nonspecific binding of modified Pt NPs.

We observed step increases of current under similar conditions in several experiments, but sometimes we did not observe them. Such variations in reproducibility have been observed in other EA experiments because of the extreme sensitivity of the method to small variations in experimental conditions. With the appropriate microfluidic flow conditions and using the EA of NP sensing scheme, we should be able to detect DNA in solutions with much lower concentrations of target DNA. With an optimization of conditions, samples containing single biomolecules should be analyzable, allowing, for example, detection of single protein molecules in an immunochemical assay.

We have demonstrated the observation of single NP collisions as a detection method in a sandwich-type DNA sensor. When we used typical ensemble properties of NPs as a detection signal, the detection limit for the target DNA was about 100 pM. However, target DNA at the 10 pM level was identified using EA with single NP collisions. This ability to detect individual biomolecules should be applicable to the development of even more sensitive biosensors.

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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