# Femtomolar Electrochemical Detection of DNA Targets Using Metal Sulfide Nanoparticles

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# Femtomolar Electrochemical Detection of DNA Targets Using Metal Sulfide Nanoparticles

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The development of highly sensitive and selective DNA sensors for diagnosis and forensic investigations is a field of ever increasing interest. Most detection methodologies rely on hybridization with probes providing an optical readout.[1] Within recent years, several inventive designs for DNA sensors based on an electrochemical readout have appeared.[2] Electrochemical detection assays have the advantage of being simple, reliable, cheap, sensitive, and selective for genetic detection. Electrochemical sensors based on impedance[3] or voltammetry[4] have been reported, and in recent years, metal nanoparticles have been applied for electrochemical DNA sensors with sensitivities in the pico- and femtomolar range.[5] Mirkin reported on gold nanoparticle-based electrochemical DNA chips.[6] Wang et al. reported on electrochemical assays based on quantum dot nanocrystals as tracers.[7,8] These quantum dots exhibit sharp and well resolved stripping voltammetry signals due to the well-defined oxidation potentials of the metal components. However, most of the electrochemical DNA sensors reported until now require the subsequent addition of a label or, with few exceptions,[9] suffer from low sensitivity.

Here we report on a new metal sulfide nanoparticle-based electrochemical detection method that provides detection capabilities down to 100 attomol of target DNA. The setup is constructed to give a signal-off response with a build-in control signal. The control signal eliminates the disadvantages commonly associated with signal-off sensors.

A highly selective assay for binding and detection of multiple metal sulfide nanoparticles on a solid substrate was first developed. This initial setup is related to the magnetic bead assay reported by Wang et al.[7] Semiconductor CdS, ZnS, and PbS nanoparticles were synthesized,[7] and TEM images of these particles reveal relatively monodisperse particles with an average diameter of 3 nm for CdS and 5 nm for PbS nanoparticles. The three different particles were each conjugated with 5′-thiolated DNA reporter sequences **r1**, **r2**, and **r3**. AFM analysis of the DNA-conjugated CdS and PbS nanoparticles on a mica surface revealed sizes of predominantly 5−10 and 10−15 nm, respectively (see Supporting Information). For binding of the nanoparticles to a solid substrate, a capture assay was prepared by immobilization of 5′-thiolated DNA capture sequences **c1**, **c2**, and **c3** on a gold substrate, which was subsequently treated with hexanethiol (Figure 1A).[10] The gold substrate was simply a flattened piece of gold wire with a surface area of approximately 0.5 $cm^2$. The capture sequences **c1**−**3** contain 15 bp regions complementary to the reporter sequences **r1**−**3**, respectively. In the experiment illustrated in Figure 1A, all three capture sequences are immobilized on the same gold substrate and subjected to a solution containing all three nanoparticle DNA conjugates. After a thorough washing procedure, the metal sulfide nanoparticles on the gold substrate were dissolved by adding 0.10 M $HNO_3$.

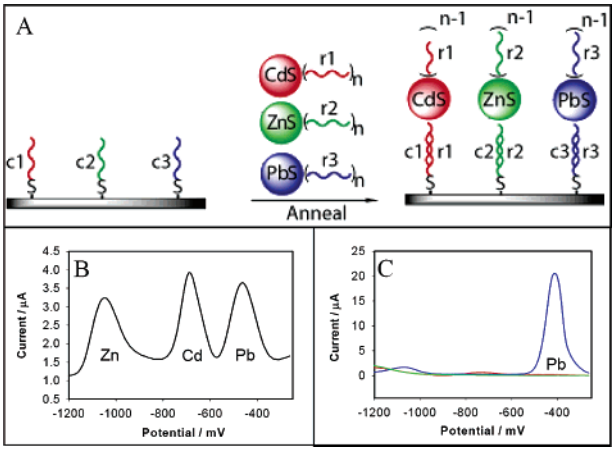


***Figure 1.*** Capturing of nanoparticle−DNA conjugates CdS−**r1**, ZnS−**r2**, and PbS−**r3** by hybridization to the three complementary sequences **c1**, **c2**, and **c3** immobilized on a gold surface (A). Anodic stripping voltammetry analysis of the nanoparticles captured at the surface after washing and dissolution of the metals (B). Anodic stripping voltammetry analysis of three surfaces containing only capture sequence **c3** after incubation with CdS−**r1** (red), ZnS−**r2** (green), and PbS−**r3** (blue), respectively (C).

Identification and quantification of the dissolved metals were performed by anodic stripping voltammetry (Figure 1B). This technique provides well-resolved signals for each of the three metal traces.

The sequence specificity and absence of nonspecific binding was demonstrated in a similar experiment with only one capture DNA sequence, **c3**, immobilized on gold substrates. Each of three substrates was treated with CdS−**r1**, ZnS−**r2**, and PbS−**r3** conjugates, and only Pb was detected in the subsequent stripping analyses (Figure 1C). Similar selectivity was observed for the other metals using the corresponding sequences of the capture probes.

This type of nanoparticle assay is applicable for DNA sequence detection in a competition setup, as shown in Figure 2A. CdS−**r1** and PbS−**r3** conjugates were immobilized on the gold substrate by hybridization with **c1** and **c3** as described above. The presence of both nanoparticle species at the surface was verified by stripping analysis (Figure 2B). Addition of a competing nucleotide target **t3** (50 fmol) consisting of a 20 bp sequence complementary to the **r3** sequence and stirring for 5−6 h led to dissociation of PbS from the surface. This was verified by the absence of the Pb signal in the anodic stripping voltammetry recorded after washing the substrate and dissolving the nanoparticles at the surface (Figure 2C). The **c3**−**r3** 15 bp duplex is apparently ousted by the stronger interaction between the 20 bp **t3**−**r3** duplex, while CdS−**r1** remains immobilized at the surface. We also assume that hybridization of the single stranded **r3** sequences on the immobilized PbS nano-

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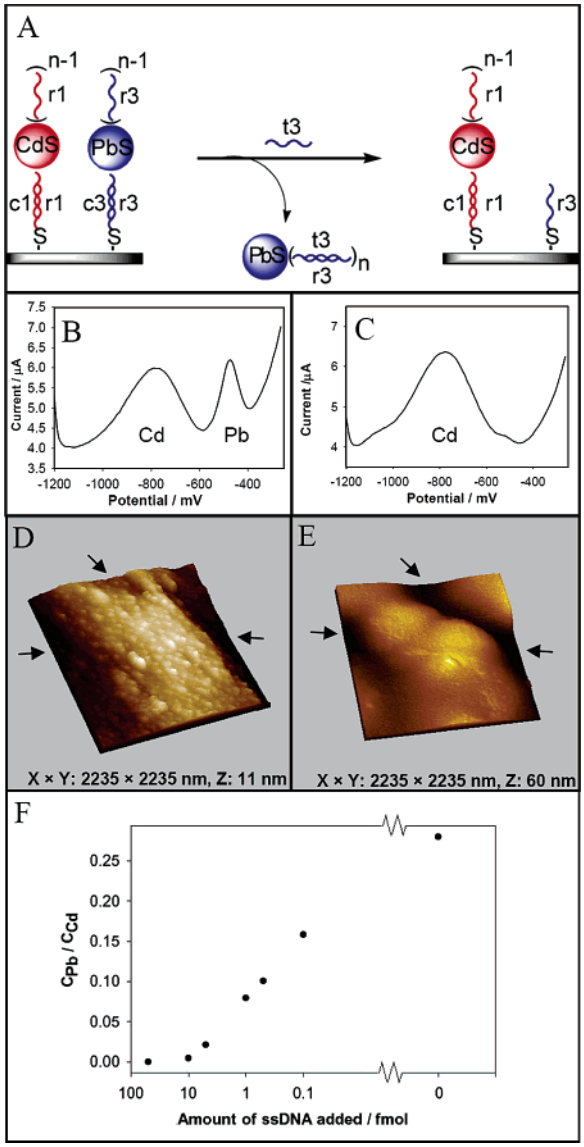


***Figure 2.*** Competition assay for detection of DNA target **t3** (50 fmol in 3 mL) (A) and anodic stripping voltammetry analysis of the particles remaining at the surface before (B) and after (C) addition of **t3**. AFM images of an area covered with the PbS−**r3** nanoparticle complexed to the immobilized **c3** sequence (D), and the same area after addition of the competing DNA target **t3** (E). That the imaging was carried out on the same area is evident from the depressions observed in the areas indicated with dotted arrow marks. Ratio between the areas (charge) of the Pb and Cd peaks obtained by stripping voltammograms performed after addition of 50 to 0.1 fmol of **t3** and in the absence of **t3** (F).

particle with the target **t3** causes increased steric and charge repulsion favoring dissociation of the nanoparticle.

Addition of target **t1** to CdS−**r1** and PbS−**r3** captured on a gold substrate led to dissociation of the CdS nanoparticles from the surface (see Supporting Information).

A gold substrate containing PbS−**r3** immobilized via hybridization with **c3** was imaged by AFM before and after the addition of target **t3** (Figure 2D and 2E). The AFM imaging was performed at room temperature (23 ± 1 °C) in buffered condition. Before addition of **t3**, the surface is covered with the PbS nanoparticles, whereas the same area is observed to be almost devoid of the nanoparticles after treatment with the competing DNA target **t3**.

Decreasing the amount of target DNA (**t3**) from 50 to 0.1 fmol in a series of experiments revealed very high sensitivity of the method (Figure 2F). This sensor is capable of efficiently detecting down to 0.1 fmol (33 fM, 3 mL) of the target **t3**.

In summary, we have developed a new method for the electrochemical detection of DNA targets. Compared to previously reported sandwiched assays, in which a labeled sequence is added after capture of the target, the competition assay reported here is, in principle, label-free and in this regard a major advancement.[5−8] The sensor provides a "signal-off" response, but the presence of a second type of metal sulfide nanoparticle at the surface attached by another DNA sequence (e.g., CdS in Figure 2A−C) constitutes a built-in control which confirms that the disappearance of the target signal is due to a specific interaction. It is therefore straightforward to rule out nonspecific binding, which presents an advantage compared to "signal-on" sensors.[5−8] The method offers high sensitivity of 0.1 fmol of target DNA. Since the signals from multiple metal sulfide nanoparticles can be resolved by anodic stripping voltammetry,[7] the method can possibly be extended to detect a multitude of hybridization events in a single experiment. In future studies, we will also develop this assay for the detection of biologically interesting DNA targets with even higher sensitivity.

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**Supporting Information Available:** DNA sequences, experimental procedures, stripping voltammograms relating to Figure 2F, and AFM images for characterization of nanoparticle−DNA conjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

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