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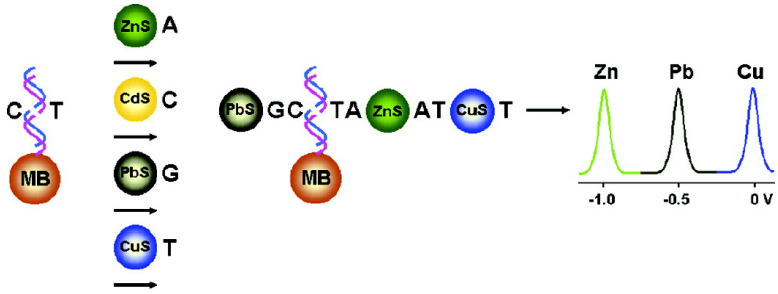
J. Am. Chem. Soc., **2005**, 127 (1), 38-39• DOI: 10.1021/ja043780a • Publication Date (Web): 15 December 2004



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Published on Web 12/15/2004

Nanocrystal-Based Bioelectronic Coding of Single Nucleotide Polymorphisms

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Single nucleotide polymorphisms (SNPs) are point mutations that constitute the most common genetic variation. Reliable methods for rapid screening of SNPs are highly desired for studying and identifying disease-causing genes.[1,2] Current methods for screening human SNPs rely on a wide variety of probes (e.g., molecular beacons,[3] peptide nucleic acids,[4] gold-particle labeled oligonucleotides[5]) and nucleic-acid protocols (such as ligation,[6] primer extension,[7] or endonuclease digestion[8]) in connection with different detection platforms (e.g., fluorescence,[9] gel electrophoresis,[10] mass spectroscopy,[11] electrochemistry,[12] and microgravimetry[13]). However, these protocols are not yet widely used in routine clinical settings. Additional efforts are thus needed to create more broadly applicable methods that would allow accurate, sensitive, rapid, and low-cost SNP identification. Herein, we demonstrate how inorganic nanocrystal tags can lead to distinct bioelectronic signatures for unknown individual SNPs as well as for known two-base mutations in a single DNA target.

The high performance of electrochemical devices, their inherent miniaturization, and their low cost and power requirements make them excellent candidates for decentralized DNA diagnostics.[14−16] Brazill et al.[17] and Di Giusto et al.[18] used redox-labeled nucleotide terminators, while Willner[13] used enzyme labels in connection to the single-base extension (SBE) technique for detecting known point mutations. When the mutation site corresponded to the labeled chain terminator, the captured extension product led to an electrical signal. Kerman et al.[19] used monobase-modified gold nanoparticles for electrochemical measurements of SNPs. A drawback of these systems is the need to perform four voltammetric measurements for a given unknown mutation. The above electrical schemes rely on a single signal (potential) and are not suitable for creating distinct fingerprints for individual SNPs.

In the present study we demonstrate that the electrodiverse population of inorganic nanocrystal tags[20] yields distinct four-potential voltammetric signatures for all eight unknown SNPs. For this purpose, ZnS, CdS, PbS, and CuS have been linked (using phosphoramidite chemistry through a cysteamine linker) to adenosine, cytidine, guanosine, and thymidine mononucleotides, respectively. Sequential introduction of the monobase-conjugated nanocrystals to the hybrid-coated magnetic-bead solution leads to their specific binding, via base-pairing, to different complementary mismatched sites, as well as to previously linked conjugates (Figure 1). Each mutation thus captures different nanocrystal−mononucleotide conjugates. Taking, for example, the T−G mutation (bottom of Figure 1), where the T and G capture A−ZnS and C−CdS, respectively, followed by base pairing of T−CuS and G−PbS to the captured A−ZnS and C−CdS, respectively. This results in a characteristic four-potential voltammogram, whose peak potentials reflect the identity of the mismatch. The other seven mutations are expected to yield different voltammetric signatures (based on capturing different nanocrystal−monobase conjugates), thus enabling bioelectronic coding of each of eight possible single-base

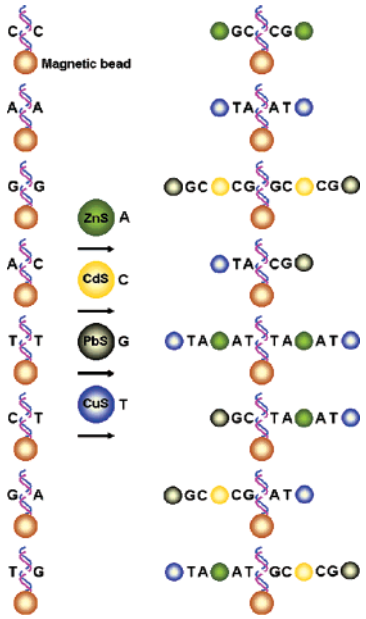

Figure 1. Electrochemical coding of all eight possible one-base mismatches using inorganic nanocrystal tracers. Use of mismatch-containing hybrids (captured on magnetic beads) followed by sequential additions of ZnS-linked adenosine-5′ monophosphate, CdS-linked cytidine-5′ monophosphate, PbS-linked guanosine-5′ monophosphate, and CuS-linked thymidine-5′ monophosphate. Also shown (right) are the corresponding assemblies of nanocrystal-linked DNA/magnetic beads. (Relative size of the magnetic beads and nanocrystal tags is not in scale.)

mismatches in a single voltammetric run. The somewhat unexpected stable binding of single nucleotide modified nanoparticles to mismatched sites and among the modified nanocrystals themselves is attributed to the unique binding properties of nanoparticle-labeled nucleotide[5] and/or to multiple binding sites on each nanoparticle.[19]

Figure 2 displays typical voltammograms for four representative single-base mismatches (B−E) as well as for a control (complementary) sequence (A). As expected from the binding events of Figure 1, the C-C (B), A-C (C), C-T (D), and T-G (E) mutation sites capture the G−PbS, T−CuS/G−PbS, G−PbS/A−ZnS/T−CuS, and T−CuS/A−ZnS/C−CdS/G−PbS nanocrystal−nucleotide conjugates, respectively. The corresponding voltammetric signatures, displayed in Figure 2, thus contain one, two, three, or four characteristic well-resolved metal peaks, and reflect the identity of the mismatch. As expected from Figure 1, the intensity of the lead peak (corresponding to the C-C mutation (B)), is 2-fold higher than those of other mutations (note the different scales). The mismatch recognition events are being amplified by the metal accumulation feature of the stripping voltammetric transduction mode. As desired for a sensitive SNP detection, such amplification is coupled to the absence of signal for the complementary sequence

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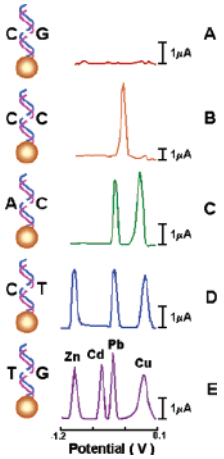

Figure 2. Typical stripping voltammograms for complementary DNA (A), and hybrids containing C-C (B), A-C (C), C-T (D), and T-G (E) mismatches using adenosine-5′ monophosphate/ZnS, cytidine-5′ monophosphate/CdS, guanosine-5′ monophosphate/PbS and thymidine-5′ monophosphate/CuS conjugates (based on the protocol of Figure 1). The measuring solution was a 0.1 M acetate buffer (pH 4.9) containing 10 μg mL^{-1} of Hg(II). Other conditions: in-situ plated mercury-coated glassy-carbon electrode, with 1-min pretreatment at +0.6 V, followed by 1-min accumulation at −1.4 V, a 10-s rest period (without stirring), and a square-wave voltammetric scan from −1.2 to +0.1 V.

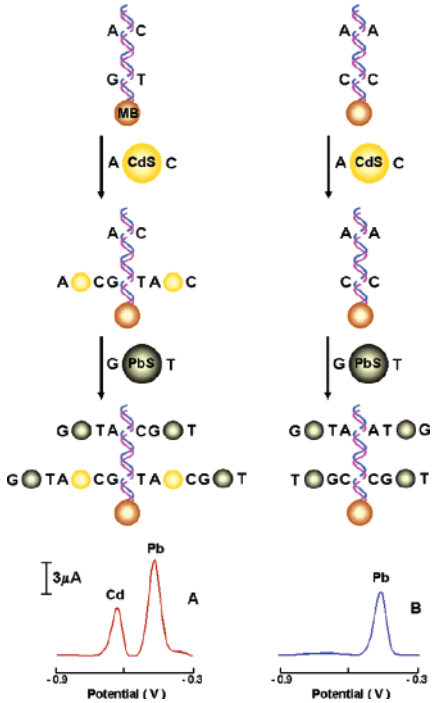

Figure 3. (Top) Electrochemical coding of known two-base mismatches using two monobase-modified inorganic nanocrystal tracers. Use of two-base mutated DNA captured on magnetic beads, followed by sequential additions of adenosine-5′ monophosphate/cytidine-5′ monophosphate-modified CdS conjugate and guanosine-5′ monophosphate/thymidine-5′ monophosphate-modified PbS conjugate, along with the resulting nanocrystal-linked DNA/magnetic-bead assemblies. (Bottom) Typical stripping voltammograms for known two-base mismatches, (A): A-C, G-T; (B) A-A, C-C. Accumulation potential, −1.1 V; scanning potential range: −0.9 to −0.3 V; other conditions, as Figure 1.

(A). Note that the four conjugates are added sequentially (in the given order) to the same vial, with a wash step between each addition, to avoid potential cross base pairing, and that only one electrochemical measurement is needed to identify the unknown SNP. This assay is characterized with reproducible signals, with relative standard deviations of less than 10%.

Next, we will demonstrate how the nanocrystal−nucleotide tags can be used for identifying two known mismatches within a single DNA target in one voltammetric run. Such protocol relies on the use of nanocrystals modified with two monobases (Figure 3) and is illustrated below using the A-C and G-T (left) and A-A and C-C (right) model mutations. In the first case, the A−CdS−C conjugate is initially incubated with magnetic beads functionalized with the DNA hybrid containing the two mutations, to link with the G-T mutation site. Subsequently, a G−PbS−T conjugate is introduced to bind with the A-C mismatch, as well as with the previously captured A−CdS−C. This results in a two-potential voltammogram, with a Cd/Pb peak intensity ratio of 1:2. In contrast, only a single lead peak is observed for the A−A and C-C mutated DNA (as expected from the capture of the G−PbS−T conjugate and the absence of A−CdS−C binding). Other mutation pairs would require sequential additions of different monobase−nanocrystal conjugates.

In summary, we have reported a new and effective nanocrystal-based bioelectronic strategy for coding of individual SNPs. Each mutation captures via base pairing different nanocrystal−mononucleotide conjugates to yield a distinct electronic fingerprint. In contrast to other electrical methods for detecting unknown SNPs,[13,17,19] our method relies on multiple signals and leads to distinct multipotential fingerprints for specific SNPs in a single voltammetric run. The new protocol should facilitate the simple, fast, cost-effective screening of important SNPs and could be readily scaled up for a high-throughput automated operation.

Acknowledgment. This work was supported by the National Science Foundation (Grant Numbers CHE 0209707 and OCE 332918)) and the NIH (Award Number R01A 1056047-01).

Supporting Information Available: Related instrumentation and procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA043780A