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Bio-Barcodes Based on Oligonucleotide-Modified Nanoparticles

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Herein, we report a method that utilizes oligonucleotides as biochemical barcodes for detecting multiple protein structures in one solution (Figure 1). The approach takes advantage of protein recognition elements functionalized with oligonucleotide strands and the fact that hybridization events that result in the aggregation of gold nanoparticles can significantly alter their physical properties (e.g., optical, electrical, mechanical).[1−5] The general idea is that each protein recognition element can be encoded with a different oligonucleotide sequence with discrete and tailorable hybridization/dehybridization properties and a spectroscopic signature associated with the nanoparticles that changes upon melting to decode a series of analytes in a multi-analyte assay. The barcodes herein are different from the ones based on physical diagnostic markers such as nanorods,[6] fluorophore-labeled beads,[7] and quantum dots[8] in that the decoding information is in the form of chemical information stored in a predesigned oligonucleotide sequence.

An antibody, immunoglobulin E (IgE) or immunoglobulin G1 (IgG1), can be detected with oligonucleotide-modified nanoparticle probes prehybridized with oligonucleotide strands modified with the appropriate hapten (biotin in the case of IgG1 and dinitrophenyl (DNP) in the case of IgE; Figure 1A).[9,10] Oligonucleotide-modified 13 nm Au particles were prepared by literature methods (∼110 oligonucleotides/particle).[11,12] Hapten-modified oligonucleotides were prepared with a biotin-triethylene glycol phosphoramidite (Glen research) for **A1** and 2,4-dinitrophenyl-triethylene glycol phosphoramidite for **B1** using standard solid-phase DNA synthesis procedures.[13] IgE and IgG1 were purchased from Sigma (Milwaukee, WI) and dissolved in 0.3 M PBS buffer (0.3 M NaCl and 10 mM phosphate buffer, pH 7) with 0.05% Tween 20 and background proteins (10 μg/mL of lysozyme, 1% bovine serum albumin, and 5.3 μg/mL of anti-digoxin; 10 μL of each) prior to use. To prepare the probes, the oligonucleotide modified particles (13 nM, 300 μL) were hybridized with hapten-modified complementary oligonucleotides (10 μL of 10 μM) and biobarcode DNA (10 μL of 10 μM) at room temperature for 2−3 h, Figure 1A. The DNA sequences in the proof-of-concept assays presented herein were designed in a way that would ensure that the two different aggregates formed from the probe reactions with IgG1 and IgE would melt at different temperatures, Figure 1B. The probes for IgG1 have longer sequences and greater G,C base contents than those for IgE. Therefore, the former sequences, when hybridized to complementary strands, melt at a higher temperature than the latter ones. These sequence variations allow one to prepare probes with distinct melting signatures that can be used as codes to identify which targets have reacted with them to form nanoparticle aggregates. Three different systems have been studied: (1) two probes with one target antibody (IgG1 or IgE) present and background proteins, (2) two probes with the two different target antibodies and background proteins present, and (3) a control where no target antibodies are present.

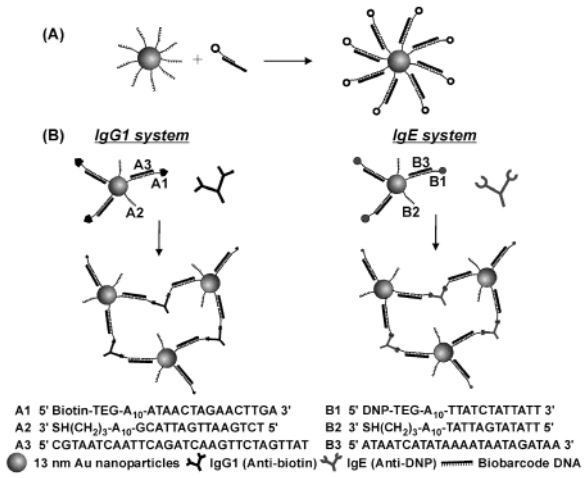

Figure 1. A DNA/Au nanoparticle-based protein detection scheme. (A) Preparation of hapten-modified nanoparticle probes. (B) Protein detection using protein binding probes. Notice that there are nine G,C pairs in sequence A, and there are only two G,C pairs in sequence B.

In a typical assay for IgE and/or IgG1, the target proteins (40 μL of 43 μg/mL for each) were added to the solution containing the probes (∼13 nM), and the mixture was incubated at 37 °C for 50 min to facilitate protein−hapten complexation. To ensure complete reaction among all the components, especially the complementary DNA strands, the solution was incubated to expedite hybridization at −15 °C for 20 min (Boekel Tropicooler Hot/Cold Block Incubator) and stored at 4 °C for ∼24 h. If the target protein is present, particle aggregation takes place effecting a shift in the gold nanoparticle plasmon band and a red-to-purple color change along with precipitation. The hybridized products were centrifuged (3000 rpm for 2 min), and the supernatant containing unreacted elements was decanted prior to analysis. To determine which proteins are present, a melting analysis which monitors the extinction at 260 nm as a function of temperature is carried out in the solution, Figure 2. When IgG1 is treated with the probes via the aforementioned protocol, the solution turns pinkish-blue, indicating the formation of nanoparticle aggregates. In a control experiment where no target but background proteins are present, there is no discernible precipitation. A melting analysis of the solution shows a sharp transition with a melting temperature (T_m) of 55 °C. This is the expected transition for the IgG1 target, Figure 2A (- - -). If IgE is added to a fresh solution of probes, the same color change is observed but the melting analysis provides a curve with a T_m of 36 °C, the expected transition for this target, Figure 2A (−). Significantly, when both protein targets are added to the solution of probes, the solution turns dark purple, and the melting analysis exhibits two distinct transitions. The first derivative of this

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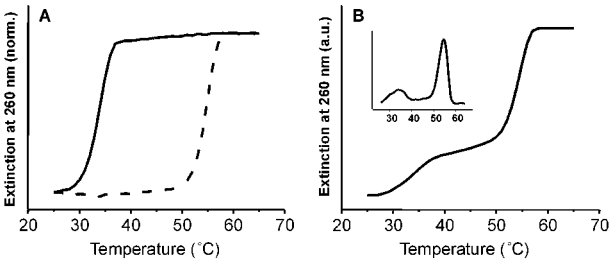
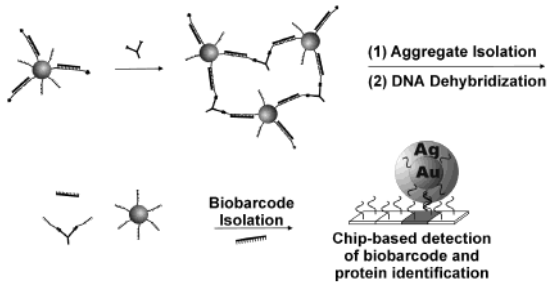


Figure 2. Thermal denaturation profiles for Au nanoparticle aggregates linked by DNA and proteins. Extinction at 260 nm was monitored as a function of increasing temperature (1 °C/min, 1 min holding time). Each UV−Vis spectrum was measured under constant stirring to suspend the aggregates. All the aggregates were suspended in 1 mL of 0.3 M PBS prior to performing the melting analyses. (A) Two probes with one target antibody present (IgE (−), IgG1 (- - -)). (B) Two probes with both target antibodies present. Inset: first derivative of the thermal denaturation curve.

Scheme 1



curve shows two peaks centered at 36 and 55 °C, respectively, Figure 2B. This demonstrates that two distinct assemblies form and their melting properties, which derive from the oligonucleotide barcodes, can be used to distinguish two protein targets.

A variation of this strategy can be used to increase the sensitivity of the aforementioned system and to increase the number of targets that can be interrogated in one solution (Scheme 1). With this strategy, the protein targets can be detected indirectly via the DNA biobarcodes. A 12-mer oligonucleotide has 4^{12} different sequences, many of which can be used to prepare a barcode for a polyvalent protein of interest via Figure 1A. In this variation of the assay, the melting properties of the aggregates that form are not measured in solution, but rather the DNA biobarcodes within the aggregates are separated via centrifugation (3000 rpm for 2 min) from the unreacted probes and target molecules. The aggregates are then denatured by adding water to the solution, freeing the complexed DNA. The particles and proteins can be separated from the DNA barcodes with a centrifugal filter device (Millipore Microcon YM-100, 3500 rpm for 20 min). Once the DNA barcodes are isolated, they can be captured on an oligonucleotide array and can be identified using one of many DNA detection assays (Scheme 1). For the examples described herein involving IgG1 and IgE, the barcodes are captured on a microscope slide that has been functionalized with oligonucleotides that are complementary to one-half of the barcode of interest (**A3** and **B3** in Figure 1). If the barcode is captured by the oligonucleotide array, a DNA-modified particle that is complementary to the remaining portion of the barcode can be hybridized to the array. When developed via the standard scanometric approach[4] (which involves treatment with photographic developing solution), a flat bed scanner can be used to quantify the results, Figure 3. If IgG1 is present, only the spot designed for IgG1 shows measurable signal. Similarly if IgE is the only protein present, the spot designed for it only exhibits signal. Finally, if both proteins are present, both spots exhibit intense signals.

This work is important because it provides two strategies for

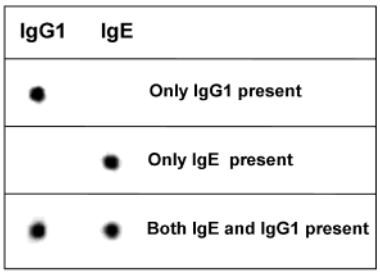


Figure 3. Scanometric DNA array detection of the DNA biobarcodes. Left column is for the detection of the biobarcode associated with IgG1 and the right column is for the biobarcode associated with IgE. The capture oligonucleotides are 5′-thiol-modified ATA ACT AGA ACT TGA for the IgG1 system and 5′-thiol-modified TTA TCT ATT ATT for the IgE system. Each spot is approximately 250 μm in diameter and read via gray scale with an Epson Expression 1640XL flatbed scanner (Epson America, Longbeach, California). These assays have been studied and work comparably well over the 20 nM to 700 nM target concentration range.

using gold nanoparticle probes, heavily functionalized with oligonucleotides, to detect single or multiple polyvalent proteins in one solution. Indeed, the detection of multiple proteins in one sample is not trivial and often requires time-consuming, expensive assay protocols. In this regard, others have recently used fluorophore-labeled peptidonucleic acids and DNA microarrays to recognize multiple protein targets in one solution.[14] However, this method relies on the binding of the proteins labeled with oligonucleotides to a microarray surface. The final step of the method described herein is based solely on the surface hybridization chemistry of ordinary DNA. Therefore, it can incorporate many of the high sensitivity aspects of state-of-the-art nanoparticle DNA detection methods,[2,4] but allows one to detect proteins rather than DNA without having the proteins present during the detection event. For surface assays, proteins are typically more difficult to work with than short oligonucleotides because they tend to exhibit greater nonspecific binding to solid supports, which often leads to higher background signals. Finally, for the homogeneous assay, the unusually sharp melting profiles[11] associated with these nanoparticle structures will allow one to design more biobarcodes than what would be possible with probes that exhibit normal and broad DNA melting behavior.

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