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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

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Electric Field Assisted Surface Plasmon-Coupled Directional Emission: An Active Strategy on Enhancing Sensitivity for DNA Sensing and Efficient Discrimination of Single Base Mutation

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Supporting Information

ABSTRACT: We have demonstrated the proof-of-principle of electric field assisted surface plasmon-coupled directional emission (E-SPCDE). The combination of SPCDE and electric field control produced a significant synergistic effect to amplify the right signal and suppress the wrong signal intelligently in an active strategy. A novel hairpin structured DNA biosensor based on the quenching and enhancing of fluorescence in SPCDE has been designed. With modulation of the fluorescence coupling efficiency, a high discrimination ratio up to more than 20-fold has been achieved by enhancing the signal of match and suppressing that of mismatch. E-SPCDE has shown a successful application in DNA sensing, eliminating false positives and false negatives in the detection. E-SPCDE should provide an opportunity to create a new generation of miniaturized high-performance sensing platforms especially in chip-based microarrays and to make the manipulation of the nanometer-scale processes more accessible and detectable.

In 2004, Lakowicz[1] put forward the concept and made the first systematic demonstration of surface plasmon-coupled directional emission (SPCDE) in which surface plasmons present in thin noble metal films, typically silver or gold, couple with an excited fluorophore located 20−250 nm from the metal surface and radiate through adhered prism in a narrow angular range. SPCDE was identified as having the advantages of increasing detection sensitivity by 20- to 1000-fold with high collection efficiency and background suppression capability. Since then, efforts to exploit this advance and demonstrate its potential for bioanalytical applications[2] have been slow-coming. Our previous work on the observation of highly polarized and directional SPCDE based on a conformational-switching signaling aptamer were also devoted to it.[3] We suggest that this advance opens the investigation of DNA molecular events at interfaces and the design of high-performance surface-based biosensors, overcoming the limitation of both surface plasmon resonance (SPR) and traditional fluorescence technology in a new vision for meeting the increasing requirements in bioanalysis, medical diagnosis, and drug discovery.[4] Application of an external electric field is a good way to control the conformation of DNA and facilitate the recognition of mutations.[5−8] We demonstrate the use of electric field assisted (E-) SPCDE as an active means to enhance the sensitivity of biosensors and provide a reliable method to detect single base mutation. The unique property of SPCDE dependence on interfacial conditions affected by electric potential makes the approach successful in DNA analysis, as described below.

The use of dye-labeled molecular beacon immobilized to gold has been proven to be a useful method for "target label-free" detection of DNA.[9] With the conformational reorganization of molecular beacon responding to targets, the dye−gold distance changes, leading to quenching and recovery of fluorescence. Transplanting this sensing strategy to SPCDE, the signal is much more susceptive to distance change. At distance very close to metal surface, the quenching effect is dominant. With increasing distance, the plasmon coupling enhancement efficiency increases sensitively and become dominant at suitable increasing distance from surface.[10] So the fluorescence is enhanced, not just recovered, after the hybridization to warrant a much higher sensitivity of measurement compared to the traditional detection.

With the assistance of an external electric field, we are able to control nanobiointerfaces in an active way and further improve the performance of the sensor in SPCDE. In the normal sensor design, the conditions to form DNA layers are passive and spontaneous. On the one hand, the hybridized DNA duplex anchored to the surface may form a slanted or random orientation,[5,11] leading to the labeled fluorophores close to the surface, which limits the efficiency of enhancement and results in potential signal deviation with the unpredictable orientation. On the other hand, a duplex formation involving a single base mismatch will also obviously cause an enhanced fluorescence signal in SPCDE, resulting in false positive (Figure 1, top). To overcome these problems, a negative potential is actively applied to modulate the conformation and hybridization of natively negative charged DNA layers. In the presence of matched target, efficient hybridization will occur as long as the target is inclined to hybridize with the probe rather than departing from the metal surface under the repulsive potential.[6] After hybridization, the hairpin stem is opened and the duplex stands straight up in a uniform way under negative potential, thus, ensuring all the labeled fluorophores locate in the enhancement zone to the largest extent. On the other hand, because the mismatched duplex is less stable, the repulsive potential will hinder the reaction and most fluorophores will be very close to the surface and not be plasmon coupled. So the low coupling effect results in only weak signal when mismatches are encountered (Figure 1, bottom). The synergistic effect on enhancing the fluorescence coupling efficiency of matched hybrids

Received: September 3, 2010
Published: January 25, 2011

ACS Publications © 2011 American Chemical Society

dx.doi.org/10.1021/ja107964s | J. Am. Chem. Soc. 2011, 133, 1787–1789

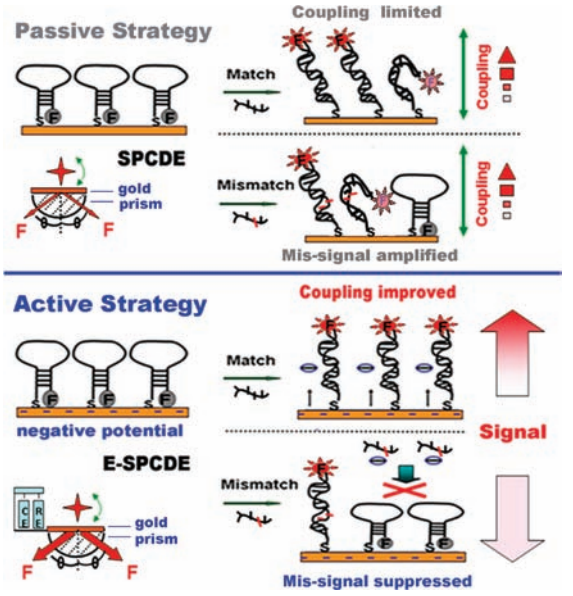

Figure 1. (Top) In SPCDE, fluorescence enhancement occurs. After hybridization, the duplexes orientate randomly, limiting the distance separation of the labeled fluorophore from the gold surface and thus the enhancement efficiency. The signal of mismatched duplexes will also be enhanced when duplex formation occurs. (Bottom) In E-SPCDE, a negative surface potential orientates the matched duplexes straight up with the fluorophore much better separated from the gold surface into the enhancement zone. Because the electrostatic repulsion restrains the hybridization of mismatched duplexes, their fluorophores are too close to surface to be efficiently plasmon coupled and little restoration of fluorescence is observed. Images were not drawn to scale.

with the extending of DNA duplex while lowing the coupling effect with the hindering of the hybridization of mismatched ones together in one assay provides great sensitivity and selectivity for DNA sensing.

Texas Red labeled DNA hairpin probes together with perfect matches (PM) and single base mismatches (MM) were chosen to demonstrate the feasibility of our proposed approach (Supporting Information for details). The SPCDE measurements were conducted in passive and electric field assisted conditions, respectively. A constant negative potential (−400 mV vs Ag/AgCl) avoiding electrochemical reactions was applied (Figures S6−S10). Figure 2 shows a typical result for a 36-base hairpin probe to PM. Increased emission was found to be sharply distributed at a direction (Figure 2a) and to be p-polarized (Figure 2b,c) through the prism with or without applying electric field after hybridization. The directional and highly polarized emission proves that there is coupling between the fluorophores and plasmons, because only a defined angle and p-polarized emission can satisfy the necessary match of plasmon wavevectors.[1] The intensity after hybridization increased more than 70-fold in SPCDE and 120-fold in E-SPCDE as compared to that before hybridization (Figure S3). Compared to the isotropic fluorescence of free space emission (FSE) collected in front of the gold surface, a significant fluorescence enhancement (more than 12-fold for E-SPCDE) was observed at the directional angle through the prism (Figure 2a inset). The amplified fluorescence signals under negative electric field are not due to an increase in hybridization efficiency, but to the conformational changes of DNA layers. It is worth noting that the increased ratio of E-SPCDE to SPCDE was much larger than that of E-FSE to

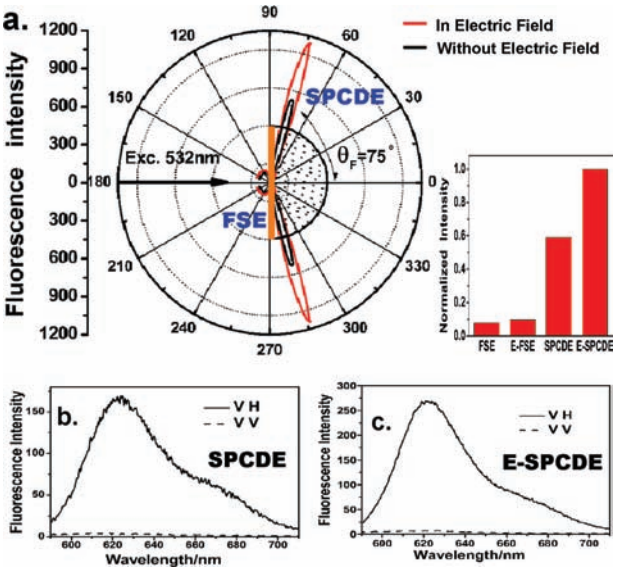

Figure 2. (a) Angular distribution of fluorescence with and without applying electric field after hybridization with 3.0 μM PM. Inset: normalized fluorescence intensity of FSE, E-FSE, SPCDE and E-SPCDE. (b and c) Polarized emission spectra with polarized excitation in SPCDE and E-SPCDE. V and H represent vertical and horizontal polarization in the order excitation−emission with polarizers. Reverse Kretschmann (RK) configuration with xenon light excitation.

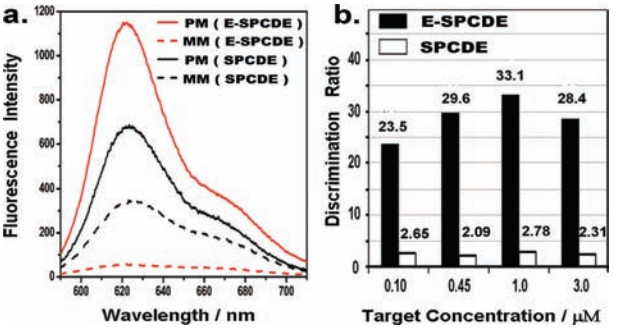

Figure 3. (a) SPCDE and E-SPCDE fluorescence spectra of DNA hairpin responding to 3.0 μM PM and MM. (b) Discrimination ratios with target concentrations ranging from 0.10 μM PM measured in SPCDE and E-SPCDE.

FSE, indicating SPCDE is more sensitive to conformational change than FSE. With DNA duplexes standing up under negative potential, the distance extension is susceptive enough to greatly improve the coupling effect, resulting in strong enhancement in E-SPCDE, while only small signal increase was observed in E-FSE. This ability to improve coupling efficiency of the sensor has been further approved by comparing different chain length probes. More obvious electric enhancement was observed in SPCDE comparing 48-base (16 nm chain length) to 36-base (12 nm chain length) hairpin probes (Figure S5). These results can be explained on the basis that coupling efficiency increases with the labeled dyes departing from surface as DNA duplexes orientated perpendicularly at negative potential. Thus, larger conformation changes with longer chain length produce higher signal responses in the distance-sensitive detection. In our present E-SPCDE measurement, the response was detectable to a target concentration as low as 100 pM for the 36-base hairpin (Figure S4). If

dx.doi.org/10.1021/ja107964s |J. Am. Chem. Soc. 2011, 133, 1787–1789

SPCDE Kretschmann (KR) configuration[12] and laser excitation are introduced together with the optimization of probe density and sequence, even higher sensitivity and lower detection limit can be expected. Instead of letting the sensor be passive, E-SPCDE offers a new approach to actively improve the coupling efficiency and thus the sensitivity in DNA detection. Moreover, it has the advantages of controlling and investigating interfacial molecular conformation for more effective detection of surface events.

To test the efficiency of detection of a single base mismatch, a series of concentrations ranging from 0.10 to 3.0 μM of both PM and MM to a 36-base hairpin were examined (Figure 3). In passive conditions, an obvious enhanced fluorescence generation was found after hybridization with MM in SPCDE, indicating that the enhancement amplifies the false signal and badly limits the selectivity of the biosensor (Figure S2). However, in E-SPCDE, with the synergistic effect of combining the unique property of SPCDE and the electric field control to DNA interface, greatly improved results were obtained. The SPCDE intensities of the chosen concentrations in passive and electric field assisted conditions were examined. The signal-to-background ratio, $S/B = (F_{hybrid} - F_{buffer})/(F_{probe} - F_{buffer})$, changing from PM to MM ($Ratio_{PM}/Ratio_{MM}$) was taken as the discrimination ratio to evaluate the specificity to PM compared to MM when both were present in equivalent concentrations, where F_{probe} and F_{hybrid} are the fluorescence of hairpin probe before and after hybridization, and F_{buffer} is the background intensity of buffer.[10,13] Excitingly, a greatly increased intensity gap between PM and MM in electric field assisted condition was found. As seen in Figure 3a, with coupling efficiency improved in electric field, the amplified matched signal was enhanced further, while the mismatched signal was very much suppressed in electric field. The weak MM signal can be attributed to the low fluorescence coupling effect due to the hindering of MM hybridization in electric field. Most labeled fluorophores are too close to the metal surface to be plasmon coupled, quenching the fluorescence signal. The discrimination ratio grew by more than 20-fold in E-SPCDE, approximately a 10-fold increase to that in SPCDE (Figure 3b). With increasing signal of PM to ensure sensitive sensing, and avoiding amplifying mismatched signal, both false positives and false negatives for DNA sensing can be eliminated.

In summary, we have demonstrated the proof-of-principle of E-SPCDE, which can be applied to a variety of distance-sensitive surface techniques. By actively modulating the conditions locally at interface with the synergistic effect of intelligently amplifying the right signal and suppressing the wrong signal, E-SPCDE has shown a successful application in sensitive DNA sensing with high discriminatory capacity for a single base mutation. E-SPCDE should provide an opportunity to create a new generation of miniaturized high-performance sensing platforms especially in chip-based microarray assays, with the active strategy to make the manipulation of the nanometer-scale processes more accessible and detectable.

■ ASSOCIATED CONTENT

Supporting Information. Detailed description of experimental procedures and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work is supported by funds from the NSFC (20975084, 20575055) and the 973 Program of China (2007CB935600). We thank Professor Richard N. Zare, Professor Chaoyong Yang and Professor G. M. Blackburn for critical reading of this manuscript.

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