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A reagentless and disposable electronic genosensor: from multiplexed analysis to molecular logic gates†

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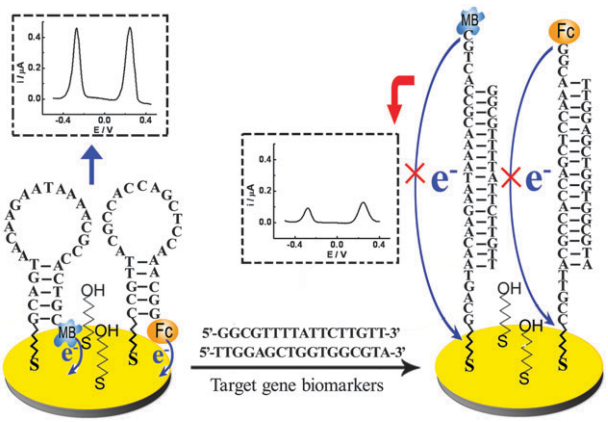
Reagentless, sensitive and multiplexed analysis of *gyrB* and *K-ras* gene biomarkers is achieved based on the proximity changes of two different redox-tags to the electrode surface upon DNA hybridizations, and the presence of the two gene biomarkers also acts as inputs and activates the logic gate.

The completion of the human genome project has generated a large number of sequence data. Although significant progress has been made in analyzing single component sequences, one-spot multiplexed analysis of DNA sequences remains a challenge because of the requirement to eliminate cross-activity, design optimal probes, and minimize non-specific bindings.[1] On the other hand, due to the important functions of DNA multiplexing in gene expression profiling, drug discovery, pathogen detection, and clinic diagnosis,[2] recent research activities have been focused on developing assays for simultaneous analysis of multiple DNA sequences. Various strategies for multiplexed analysis of DNA sequences based on distinct signal readouts upon DNA hybridization from quantum dots,[3] stripped metallic nanorods,[4] fluorescent dyes,[5] and DNA bio-barcode[1] thus have been demonstrated. These methods, however, encountered the limitations of extra labeling steps, long assay time, and high cost.

Indeed, the pioneering work by Plaxco's and Lai's groups on the fabrication of E-DNA sensors has greatly facilitated the detection of DNA.[6] Their approaches rely on the assembly of a redox-tag modified DNA probe on the electrode surface. Hybridization of the target DNA with the probe changes the distance and electron transfer efficiency between the redox-tag and the electrode surface and causes variations in electrochemical (EC) signal from the redox-tag, which leads to convenient detection of DNA target.

Inspired by these advances in DNA analysis, we report herein for the first time a reagentless, disposable, sensitive and multiplexed genosensor for one-spot simultaneous monitoring of two gene biomarkers from the *salmonella typhimurium* pathogen (*gyrB* gene) and the colorectal tumor (*K-ras* gene), respectively. This multiplexed genosensor is based on changes in current intensities from two distinct redox-tags (methylene blue, MB and ferrocene, Fc) conjugated to stem-loop probes on a disposable screen printed carbon electrode (SPCE) upon hybridization with the corresponding target DNAs. The redox-tags exhibit two well-resolved peaks, whose positions (MB: −0.28 V and Fc: +0.25 V) and sizes reflect the identities and concentrations of the target sequences. Moreover, molecular logic gates have received increasing attention owing to its important roles in developing molecular computations and "autonomously regulated" chemical systems.[7] Most of the molecular logic gates reported to date, however, are mainly based on optical outputs, which suffer the limitations of cumbersomely interfacing the optical outputs with non-molecular-based technologies.[7g] In response, based on our multiplexed DNA sensing strategy, we show that a robust "AND" molecular logic gate operation can be designed by employing the *gyrB* and *K-ras* genes as inputs and the distinct current suppressions from the redox-tags as the electronic outputs, respectively.

The new reagentless and multiplexed sensing protocol, as illustrated in Scheme 1, involves the co-assembly of MB and Fc modified stem-loop SH-DNA probes for *gyrB* and *K-ras* genes on a gold nanoparticle-deposited disposable SPCE,



Scheme 1 Illustration of the reagentless and multiplexed sensing platform for two target gene biomarkers based on proximity changes of two different redox-tags to the electrode surface upon DNA hybridizations.

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followed by surface blocking with 6-mercapto-1-hexanol, addition of the target gene sequences, and EC monitoring of the redox-tags. The SH-DNA probes were designed in such a way that each probe has five complementary bases at its 5′ and 3′ termini, which forms the double-stranded stem and brings the redox-tags to proximity of the electrode surface.[6e,8] The loop regions, meanwhile, specifically target the *gyrB* and *K-ras* genes, respectively. Hybridizations of the surface immobilized probes with the target analyte sequences unfold the stem-loop structures, force the redox-tags away from the electrode, and consequently lead to decreased current intensities. The distinct EC signatures of MB and Fc at resolved potentials with different degree of current suppressions thus correspond to the identities and concentrations of the target sequences.

As a first step of our goal towards the dual-target gene sequence multiplexing, we evaluated the single gene sequence detection assay performance. As expected, the presence of *gyrB* gene increases the distance between the MB tag on the stem-loop probe and the electrode surface, resulting in a current suppression. Fig. 1A depicts the current suppression dependence of the MB tag upon concentration of *gyrB* gene (curve a). We can see that the degree of current suppression is enhanced (from 10–1000 nM) and levels off thereafter as the concentration of *gyrB* gene increases. The detection limit for *gyrB* gene is estimated to be 8.3 nM. On the other hand, in the presence of various amounts of *gyrB* gene, no significant current suppression of Fc (corresponding to the *K-ras* gene) is observed in Fig. 1A (curve b), which indicates minimal cross activity of the two target genes because the presence of one target gene only causes current suppression of the corresponding tag. Similarly, the current suppression of Fc also shows concentration dependence upon the *K-ras* gene (Fig. 1B) with a detection limit of 10 nM. It should be noted that the current suppression of either MB or Fc in the presence of the corresponding target gene biomarkers levels off at about 80% and further increase of the target concentrations causes no significant current suppression. This is presumably due to two reasons. First, despite that hybridizations between the targets and probes unfold the hairpin structures, force the redox-tags away from the electrode surface and lower the electron transfer efficiency between the redox-tags and the electrode, the lowered electron transfer efficiency still generates residual current responses. Second, the inaccessibility of some hairpins by the targets because of their steric hindrances maintains these hairpins unfolded and results in residual current responses. The combination of these possible effects causes the current suppressions less than 80% even with higher concentrations of target genes in the sample.

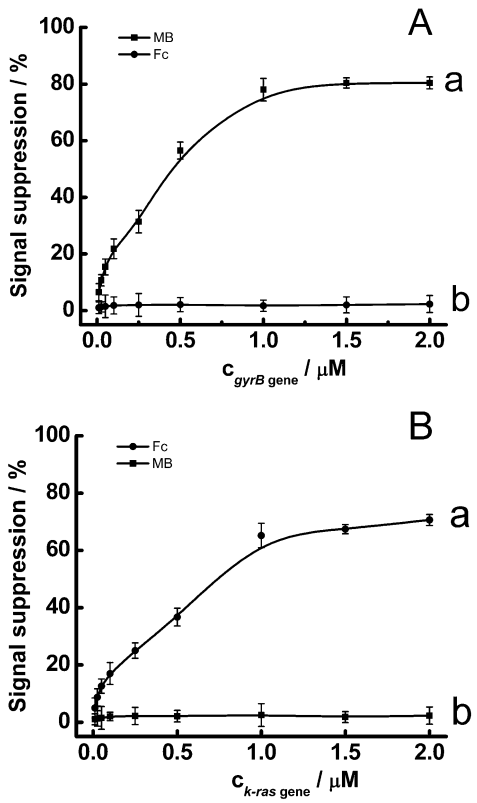

Fig. 1 Calibration curve for *gyrB* gene (A) and *K-ras* gene (B) detection in the range of 10 nM–2 μM using the proposed genosensor. Square wave voltammetric measurements were carried out in 20 mM Tris-HCl buffer (140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl and 1 mM $CaCl_2$, pH 7.0) by scanning the potential from −0.50 V to +0.45 V with a step potential of 4 mV, a frequency of 25 Hz and an amplitude of 25 mV. The error bars represent the standard deviations of three parallel tests.

Moving to the dual-target gene biomarker detection scenario, the current suppression of both MB and Fc is expected. Due to the signal suppressions in the presence of the target DNAs, the current intensities exhibit inverse dependence upon the target concentrations. As displayed in Fig. 2A, the current intensities of MB (*gyrB* gene) and Fc (*K-ras* gene) decrease accordingly as the concentrations of the target sequences increase from 25 to 1000 nM, suggesting the feasibility of our genosensor for simultaneous monitoring of multiple target sequences.

The selectivity of the multiplexed genosensor was investigated, respectively, against a random DNA sequence and a mixture of control sequences of the *gyrB* gene from *Shigella sonnei* pathogen and three-base mismatched *K-ras* gene. From Fig. 2B, we can see that despite the existence of an excess (50-fold, 2.5 μM) of the non-target sequences, no apparent signal suppression is observed with respect to the blank test (0 nM target analytes). However, the presence of 500 nM *gyrB* and *K-ras* genes leads to significant signal drops, indicating a good selectivity of our sensor.

In addition to selective and sensitive monitoring of multiple gene sequences, we also employed the multiplexed sensing platform as molecular logic gates by setting the two target gene sequences as inputs and the current intensity suppressions of MB and Fc as outputs (Fig. 3A). Consequently, we define the current intensity suppression (> 5%) of MB or Fc as the "OFF" or "0" output and the suppression of both as "ON" or "1" output. As can be seen from the truth table in Fig. 3B, only when both inputs are "1", a "1" output is obtained. If both inputs are "0" or only one of the inputs is "1", a "0" output is observed. The multiplexed sensing platform thus serves as an "AND" gate.

In summary, we have demonstrated a reagentless, disposable and multiplexed electronic DNA sensing platform and its application to the design of a molecular logic gate.

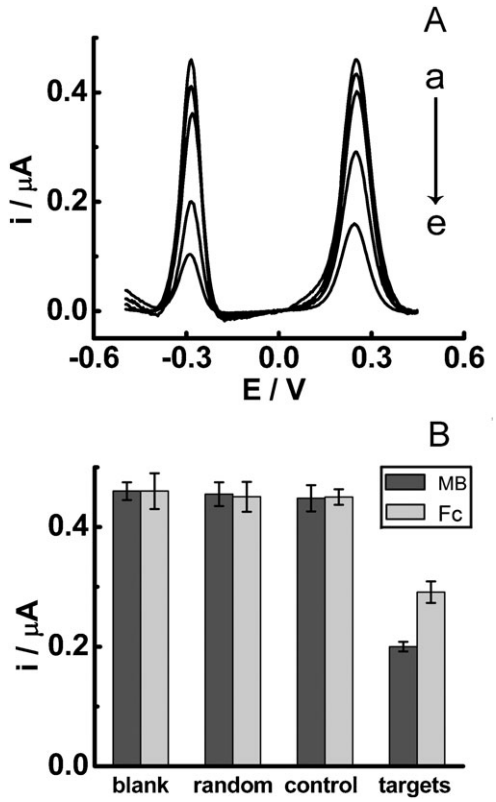

Fig. 2 (A) Square wave voltammograms for multiplexed analysis of mixtures of *gyrB* and *K-ras* genes at: (a) 0, (b), 25, (c) 100, (d) 500, (e) 1000 nM. (B) Specificity investigations against non-target molecules: mixture of target gene sequences (500 nM) *versus* random (2.5 μM) and control sequences (2.5 μM). Other conditions as in Fig. 1.

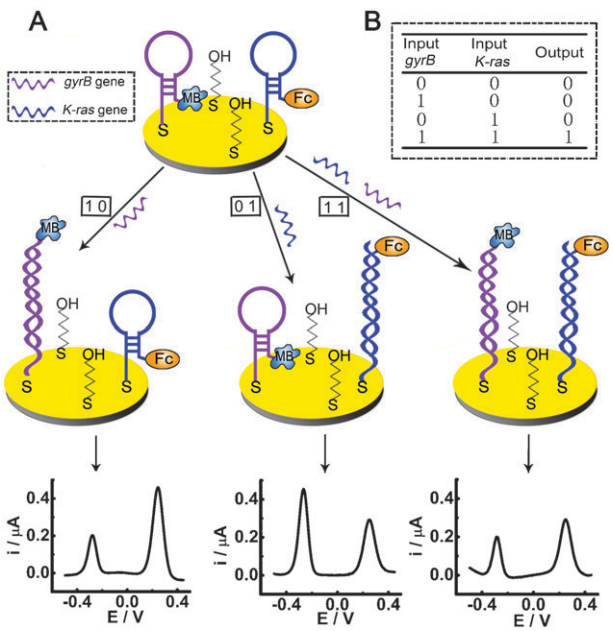

Fig. 3 (A) Schematic presentation of the "AND" logic gate system based on current intensity suppressions of MB and Fc at −0.28 V and +0.25 V by the *gyrB* and *K-ras* gene inputs: 500 nM *gyrB* gene (1, 0), 500 nM *K-ras* gene (0, 1), 500 nM *gyrB* and *K-ras* gene (1, 1). (B) The truth table for this two-input logic gate.

The sensing mechanism relies on the changes in surface proximity of two redox-tags at distinct potentials. Considering the possibility of a wider potential window for EC measurement, the multi-analyte sensing strategy and the logic gate operation can be readily expended by careful selection of more redox-tags with distinct potential positions (*e.g.* thionine at −0.17 V, anthraquinone at −0.45 V), which could potentially pave the way for the development of multiplexed point-of-care diagnostic devices.

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Notes and references

1 S. I. Stoeva, J. S. Lee, C. S. Thaxton and C. A. Mirkin, *Angew. Chem.*, 2006, **118**, 3381.
2 (*a*) J. C. Miller, E. B. Butler, B. S. Teh and B. B. Haab, *Dis. Markers*, 2001, **17**, 225; (*b*) J. A. Ludwig and J. N. Weinstein, *Nat. Rev. Cancer*, 2005, **5**, 845; (*c*) M. Ferrari, *Nat. Rev. Cancer*, 2005, **5**, 161; (*d*) B. K. Kim, J. W. Lee, P. J. Park, Y. S. Shin, W. Y. Lee, K. A. Lee, S. Ye, H. Hyun, K. N. Kang, D. Yeo, Y. Kim, S. Y. Ohn, D. Y. Noh and C. W. Kim, *Breast Cancer Res.*, 2009, **11**, R22.
3 (*a*) J. Wang, G. Liu and A. Merkoci, *J. Am. Chem. Soc.*, 2003, **125**, 3214; (*b*) J. Li, X. W. Zhao, Y. J. Zhao and Z. Z. Gu, *Chem. Commun.*, 2009, 2329; (*c*) M. Han, X. Gao, J. Su and S. Nie, *Nat. Biotechnol.*, 2001, **19**, 631.
4 (*a*) G. Zheng, F. Patolsky, Y. Cui, W. U. Wang and C. M. Lieber, *Nat. Biotechnol.*, 2005, **23**, 1294; (*b*) J. B. H. Tok, F. Y. S. Chuang, M. C. Kao, K. A. Rose, S. S. Pannu, M. Y. Sha, G. Chakarova, S. G. Penn and G. M. Dougherty, *Angew. Chem.*, 2006, **118**, 7054; (*c*) W. M. Zheng and L. He, *J. Am. Chem. Soc.*, 2009, **131**, 3432.
5 (*a*) H. Fenniri, L. Ding, A. E. Ribbe and Y. Zyrianov, *J. Am. Chem. Soc.*, 2001, **123**, 8151; (*b*) Y. W. Cao, R. Jin and C. A. Mirkin, *Science*, 2002, **297**, 1536; (*c*) D. C. Pregibon, M. Toner and P. S. Doyle, *Science*, 2007, **315**, 1393; (*d*) Y. G. Li, Y. T. H. Cu and D. Luo, *Nat. Biotechnol.*, 2005, **23**, 885; (*e*) J. Hu, X. W. Zhao, Y. J. Zhao, J. Li, W. Y. Xu and Z. Y. Wen, *J. Mater. Chem.*, 2009, **19**, 5730; (*f*) S. O. Meade, M. Y. Chen, M. J. Sailor and G. M. Miskelly, *Anal. Chem.*, 2009, **81**, 2618.
6 (*a*) C. H. Fan, K. W. Plaxco and A. J. Heeger, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 9134; (*b*) X. L. Zuo, S. P. Song, J. Zhang, D. Pan, L. H. Wang and C. H. Fan, *J. Am. Chem. Soc.*, 2007, **129**, 1042; (*c*) F. Ricci and K. W. Plaxco, *Microchim. Acta*, 2008, **163**, 149; (*d*) S. J. P. Canete, W. W. Yang and R. Y. Lai, *Chem. Commun.*, 2009, 4835; (*e*) W. W. Yang, J. Y. Gerasimov and R. Y. Lai, *Chem. Commun.*, 2009, 2902; (*f*) S. J. P. Canete and R. Y. Lai, *Chem. Commun.*, 2010, **46**, 3941.
7 (*a*) Q. Liu, L. Wang, A. G. Frutos, A. E. Condon, R. M. Corn and L. M. Smith, *Nature*, 2000, **403**, 175; (*b*) M. N. Stojanovic and D. Stefanovic, *Nat. Biotechnol.*, 2003, **21**, 1069; (*c*) Y. Benenson, B. Gil, U. Ben-Dor, R. Adar and E. Shapiro, *Nature*, 2004, **429**, 423; (*d*) A. Okamoto, K. Tanaka and I. Saito, *J. Am. Chem. Soc.*, 2004, **126**, 9458; (*e*) G. Seelig, D. Soloveichik, D. Zhang and E. Winfree, *Science*, 2006, **314**, 1585; (*f*) J. Elbaz, M. Moshe and I. Willner, *Angew. Chem.*, 2009, **121**, 3892; (*g*) F. Xia, X. Zuo, R. Yang, R. J. White, Y. Xiao, D. Kang, X. Gong, A. A. Lubin, A. Vallée-Bélisle, J. D. Yuen, B. Y. B. Hsu and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 8557.
8 R. Y. Lai, E. T. Lagally, S. Lee, H. T. Soh, K. W. Plaxco and A. J. Heeger, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4017.

