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Quantum Dots Enhanced Ultrasensitive Detection of DNA Adducts

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Here we demonstrate that quantum dots (QD) can greatly improve the ultrasensitive capillary electrophoresis-laser induced fluorescence immunoassay of trace *anti*-benzo(*a*)pyrene diol epoxide (BPDE)−DNA adducts from sensitivity to separation. We for the first time show that the target QD−antibody−DNA complex is not only effectively separated but also effectively focused by capillary electrophoresis. With the online laser-induced fluorescence detection coupled, the low limits of detection of 6.6 $\times$ 10^{-21} mol in mass and 120 fM in concentration are achieved for BPDE−DNA adducts. The achieved ultrasensitivity allows for human exposure biomonitoring and shows promising applications of QD in various DNA analyses, including DNA damage.

Ultrasensitive analysis is a combat of detecting weak signal against the noises resulting from nonspecific adsorption, fluorescence impurities, and Raman scattering. It is hard for the traditional organic dyes to overcome these restrictions. Because of the exceptional optical properties and well-controlled nanosurface, semiconductor quantum dots (QD) hold much promise for ultrasensitive analysis.[1−4] However, it also raises a great challenge because of the difficulty to separate the QD bound biomolecules of interest from the unbound QD, resulting from their similarly large size and high density.[5] The success in separation is essential for achieving desirable ultrasensitivity and specificity. By taking advantage of the potentials of QD and combining the advantages of highly efficient separation and ultrasensitivity of capillary electrophoresis coupled laser-induced fluorescence (CE-LIF), we demonstrate the quantification of representative DNA adducts, *anti*-benzo(*a*)pyrene diol epoxide (BPDE)−DNA adducts, with a low detection limit of several zeptomoles in mass and femtomoles in concentration using nanograms of DNA. The formation of carcinogenic BPDE−DNA adducts in organisms mainly results from the exposure to a ubiquitous environmental pollutant benzo(*a*)pyrene (B(a)P).[6−8] When armed with an ultrasensitive method for detection of trace DNA adducts resulting from low levels of exposure relevant to environmental settings, DNA adducts as carcinogen biomarkers can provide essential and quantitative information for a realistic and meaningful assessment of environmental exposure and cancer risk and prevention.[9−11]

EXPERIMENTAL SECTION

Caution. B(a)P and *anti*-BPDE are carcinogenic and should be handled with extreme caution.

Chemicals and Reagents. Benzo(a)pyrene (B(a)P) and *anti*-benzo(*a*)pyrene-trans-7,8-dihydrodiol-9,10-epoxide (*anti*-BPDE) were supplied by the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Kansas, MO). Mouse monoclonal *anti*-BPDE-dG antibody (mAb 8E11) was from Trevigen (Gaithersburg, MD). Qdot 625 goat F(ab')$_2$ antimouse IgG conjugate (H + L) and bovine serum albumin (BSA) were from Invitrogen (Carlsbad, CA). Deionized water was obtained from a Purelab Ultra Bioscience water purification system (ELGA, U.K.). Uncoated fused-silica square capillary (50 μm square i.d., 373 μm o.d.) was purchased from Polymicro Technologies (Phoenix, AZ). All other reagents and solvents were of analytical or HPLC grade.

Cell Culture and Treatment. To simulate the generation of BPDE−DNA adducts at environmental settings, human lung carcinoma A549 cells were incubated with *anti*-BPDE, B(a)P, and cigarette-smoke condensate as low as 0.1 nM, 1 nM, and from less than one cigarette, respectively. The A549 cells were grown in 6 cm plates and maintained in 4.0 mL/plate RPMI 1640 medium (Hyclone, South Logan, Australian) supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD), 100 U/mL penicillin G (Hyclone), and 100 U/mL streptomycin sulfate

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(Hyclone) at 95% humidity and 5% CO_2 for 2−3 days prior to use. The cultured A549 cells (about 5×10^6 cells per plate) were treated for 2 h with racemic *anti*-BPDE of different doses (0−10 nM). The *anti*-BPDE was freshly dissolved in tetrahydrofuran/triethylamine solution (THF/TEA, 19:1, v/v) and 2.0 μL solution was added to each plate. For the control, only 2.0 μL of THF/TEA solution was added into each plate. For the B(a)P exposure, the cells were incubated for 16 h with B(a)P, dissolved in dimethylsulfoxide (DMSO) of different doses (0−50 nM). The control cells were treated only by DMSO (2.0 μL/4 mL medium). After treatments, the cells were subsequently washed with phosphate buffered saline prior to DNA extraction. The genomic DNA was extracted from the cultured A549 cells using Wizard genomic DNA purification kits (Promega, Madison, WI), according to the protocol of the manufacturer. The DNA concentration was estimated at UV 260 nm.

The commercial cigarettes used in the experiment were purchased from a local retail store. The smoke generated from one and five cigarettes were bubbled through 500 μL of ethanol at room temperature by using a designed smoke-aspirating apparatus (Supporting Information, Figure S1A) to extract B(a)P.[12] One tenth of the extract solution (50 μL) was added into one plate with confluent A549 cells for each dose. In the control dish, 50 μL of ethanol was added. The cell treatment was duplicated for each dose. After incubation for 16 h, the genomic DNA was extracted as described above.

CE-LIF Immunoassay of *anti*-BPDE−DNA Adducts. CE-LIF analysis was performed on a laboratory-built CE-LIF system as described previously.[13] A 488 nm solid-state laser (30 mW, Melles Griot, Irvine, CA) was used for excitation, and the emitted fluorescence was detected at 625 nm. Genomic DNA extracted from cell cultures was denatured by heating at 95 °C for 5 min followed by chilling in ice. The denatured DNA was mixed with 1.5 nM (0.25 μg/mL) primary antibody mAb 8E11, 5 nM secondary antibody QD-2°Ab, and 100 μg/mL BSA in a Tris-acetate buffer (TA, 20 mM Tris and 4 mM acetic acid, pH = 7.8). Addition of nonspecific proteins, such as BSA, can enhance the formation and stability of the ternary complex between the antibodies and the DNA adduct and improve the reproducibility of the analysis.[14] The samples were incubated at room temperature for at least 30 min prior to CE-LIF analysis. Uncoated fused-silica square capillaries of 50 μm square i.d. were covalently coated with a monolayer of linear polyacrylamide (PAA) as described previously.[13] The capillary was 26 cm long with an effective length of 20 cm from the inlet to the detection window. A negative voltage of 20 kV was applied to the injection end of the capillary, and the outlet end (near detection window) was grounded. Samples were electrokinetically injected into the capillary by applying an injection voltage of −20 kV for 10 s. All the separations were carried out at room temperature. The separation buffer was 1× Tris-glycine buffer (TG, 30 mM Tris and 160 mM glycine, pH = 8.5). All buffers were filtered through a 0.22 μm membrane filter and degassed using ultrasonication before use. After each analysis, the capillary was washed by 50% (v/v) methanol/water solution and running buffer for 5 min in sequence.

RESULTS AND DISCUSSION

The immunoassay of the BPDE−DNA adducts is illustrated in Figure 1a. Mouse monoclonal antibody (mAb) 8E11 was chosen for specific recognition of single *anti*-BPDE−N^2−dG adducts in genomic DNA because of its no observable cross-reaction against normal DNA and high affinity ($K_a = 2.5 \times 10^8$ M^{-1}).[15] QD 625 goat F(ab')$_2$ antimouse IgG conjugate (QD-2°Ab) was chosen for selectively labeling mAb 8E11 and its immunocomplex of DNA adducts. The QD-2°Ab was excited at 488 nm and detected at the maximum emission of 625 nm. By the excitation at 488 nm and the emission at 625 nm, the strong Raman scattering from water (distributed at 490−530 nm and 572−588 nm) can be bypassed (Supporting Information, Figure S2). In addition, agarose gel electrophoresis analysis shows that the QD-2°Ab migrated toward the anode (Supporting Information, Figure S3), suggesting that the QD-2°Ab has been sparsely modified by negative charge. This property will greatly reduce both the nonspecific adsorption of DNA on the nanosurface and the interaction of the QD-2°Ab with the inner capillary surface. Our agarose gel electrophoresis analysis of the mixture of QD-2°Ab and normal DNA confirms the former point (data not shown), and the symmetry CE peak of QD-2°Ab (peak 2, Figure 1b) confirms the latter point. In the immuno-reaction solutions, the QD-2°Ab, mAb 8E11, and BPDE adduct containing DNA can form the complexes of QD−antibody−DNA. However, these large complexes are mixed with QD-2°Ab and the complex of QD-2°Ab−mAb 8E11 (Figure 1a). The normal DNA because of a lack of target adducts will not be labeled by QD-2°Ab. Therefore, there is no need to separate the QD−antibody−DNA complexes from the QD unbound genomic DNA (>20 kb). However, it requires effective separation of the QD−antibody−DNA complexes from DNA unbound QD-2°Ab. By application of optimized neutral capillary electrophoresis, the large complex of QD−antibody−DNA (~1.5 min, ≤20 pM) can be completely separated from the 250 times more QD-2°Ab (2.8 min, 5 nM) (Figure 1b). The complexes of QD−antibody−DNA because of the excess negative charge of the bound DNA migrate faster toward the detection window (near the anode end) than the DNA unbound QD species (Figure 1a). Interestingly, even two major complexes of QD−antibody−DNA (1.50 and 1.53 min) are resolvable (Figure 1c). The observation of the two complexes is coincident with the bivalent property of the primary antibody IgG and consistent with previous work,[15] attributing to the immunocomplexes of different binding stoichiometry. Under the optimized conditions, the complex of QD-2°Ab−mAb 8E11 with BPDE adducts in a short 90mer DNA strand (≪20 kb genomic DNA) is also resolvable from the unbound QD-2°Ab (data not shown). All the separations were achieved on free solution CE and no sieving matrix was involved, demonstrating the separation potential of free solution CE in QD enhanced immunoassays. It is interesting that a DNA-driven focusing method described for enhancing protein−DNA binding assays[16] is also applicable to the focusing of the QD−antibody−

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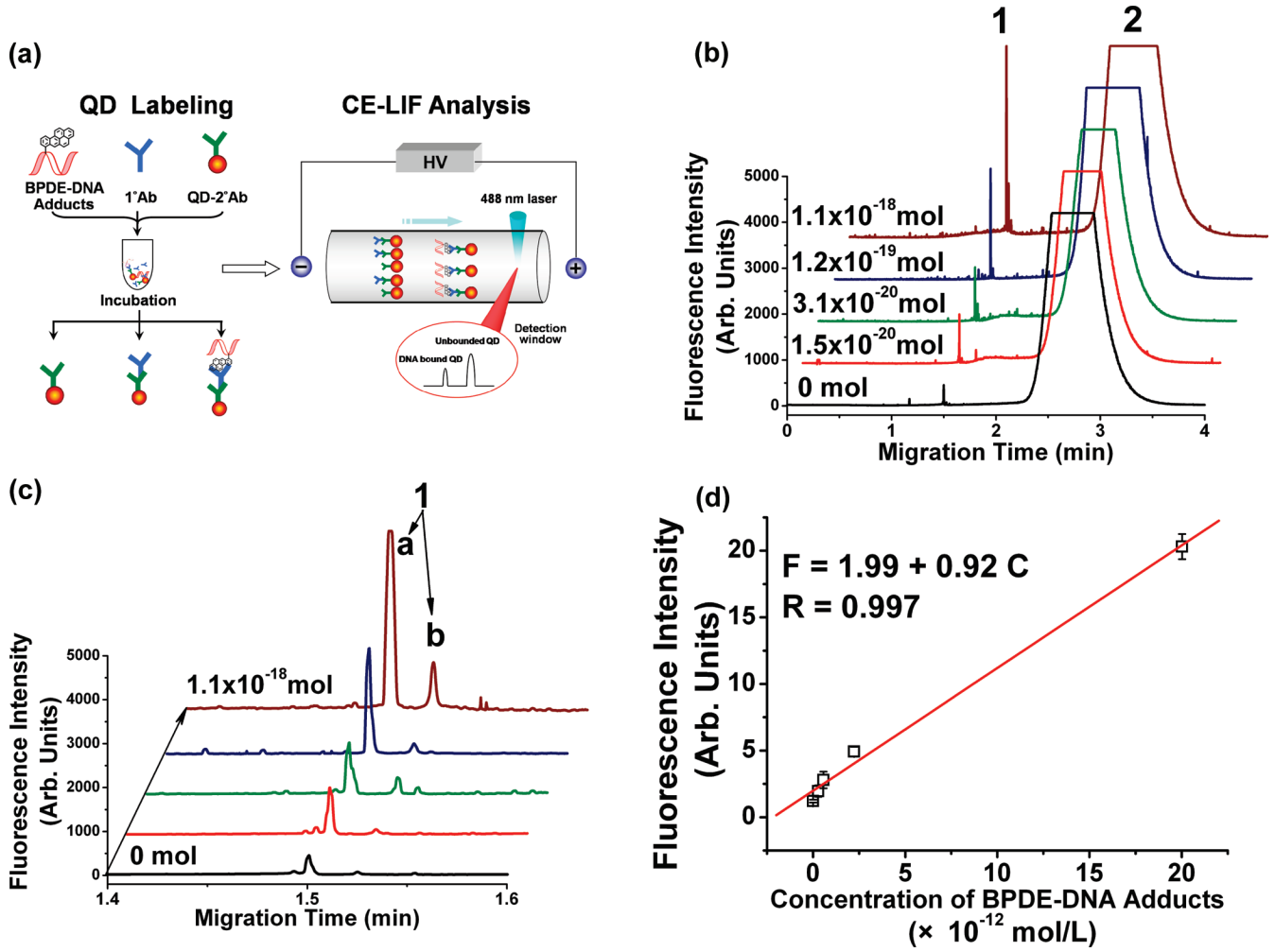

Figure 1. A QD based CE-LIF immunoassay for detection of BPDE−DNA adducts. (a) The BPDE−DNA adducts are first specifically bound to 1°Ab (mAb 8E11), then selectively labeled by QD-2°Ab, and followed by CE-LIF analysis. (b) Electropherograms from CE-LIF analysis of QD labeled BPDE−DNA adducts. The standard BPDE−DNA adducts are varied from 0 to 1.1 × 10^{-18} mol. (c) The electropherogram in part b from 1.4−1.6 min. Peaks 1a and 1b correspond to the two complexes of Qdot-2°Ab−mAb 8E11−DNA and peak 2 corresponds to the excess DNA unbound QD. (d) The linear plot of the QD−antibody−DNA complex against the concentration of BPDE−DNA adducts.

DNA complex. As shown in Figure 1b, the large complex of QD−antibody−DNA has a 2000 times higher separation efficiency (over 1.7 million theoretical plates per meter) than QD-2°Ab itself (800 theoretical plates per meter). The focusable property further enhances the detection sensitivity of QD−antibody−DNA.

A BPDE−DNA adduct standard in a genomic DNA size was prepared according to a recent work.[17] By ultraperformance liquid chromatography tandem mass spectrometry analysis and calibration with stable isotope dilution, it was found that the standard has a total adduct frequency of 5.98 BPDE per 10^5 nucleotides (Supporting Information, Figure S4). By using this well-characterized BPDE−DNA adduct standard, we tested the linearity and sensitivity of the method. As shown in Figure 1d, the complexes accounted in the sum of the peak areas of the two complexes increases proportionally to the concentration of the standard (from 2.8 × 10^{-13} to ~2.0 × 10^{-11} mol/L). The linear dynamic range is over 2 orders of magnitude with a correlation coefficient of $R = 0.997$ ($n = 5$). The lower limits of detection are about 1.2 × 10^{-13} mol/L in concentration and 6.6 × 10^{-21} mol in mass by estimation from the ratio of signal-to-noise (S/N) = 3 after the deduction of background signal. The achieved mass sensitivity is about 5400 times higher than that of nuclease P1 enhanced ^{32}P-postlabeling assays,[18,19] which is often used in the detection of DNA adducts for human biomonitoring although precaution and restriction for the use of radiation are required.

The achieved ultrasensitivity enables us to determine the BPDE−DNA adducts in cultured human lung carcinoma A549 cells exposed to *anti*-BPDE, a reactive metabolite of B(a)P, as low as 0.1 nM. The lowest dose is at least 100 times lower than that of previous works.[20−22] Human lung cells were hence chosen because of being a major target of B(a)P. The BPDE−DNA

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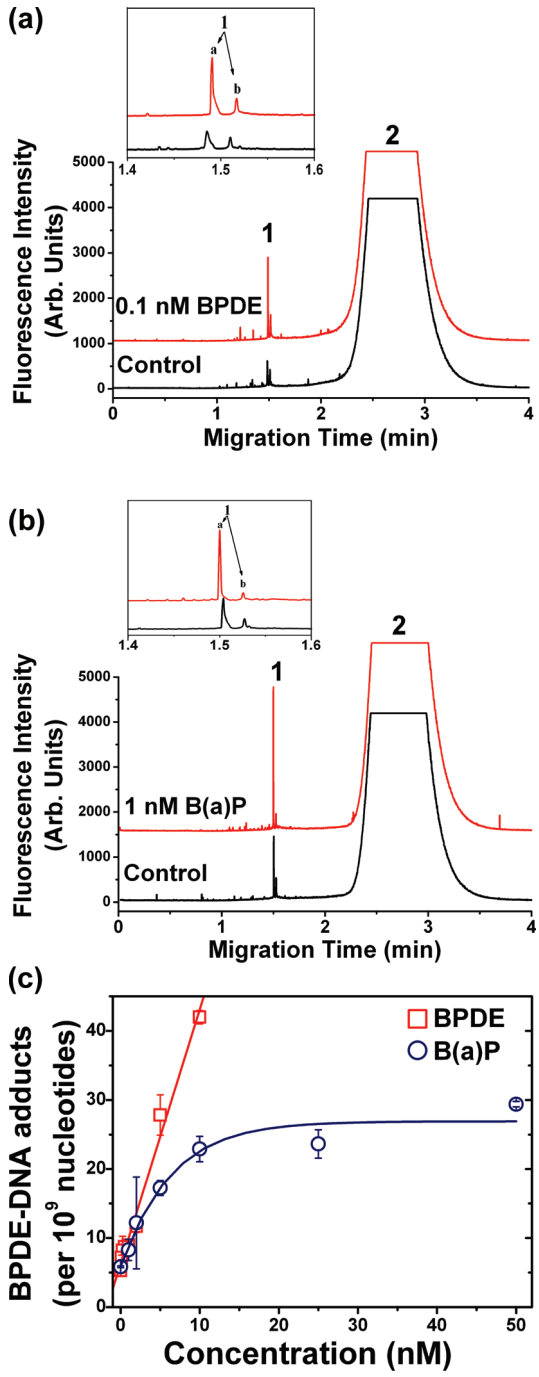

Figure 2. Electropherograms from QD based CE-LIF immunoassay of BPDE−DNA adducts in human lung carcinoma A549 cells by 2 h exposure to 0.1 nM anti-BPDE (a) or 16 h exposure to 1 nM B(a)P (b). (c) The dose−response curves for BPDE−DNA adducts in A549 cells treated with B(a)P (1−50 nM) and its reactive metabolite, anti-BPDE (0.1−10 nM). Genomic DNA of 5 ng was injected for each analysis.

adducts formed in confluent A549 cells by 2 h exposure to 0.1 nM *anti*-BPDE and 16 h exposure to 1 nM B(a)P (top traces, Figure 2a and 2b) can be well distinguished from the control (bottom traces, parts a and b of Figure 2) and corresponding to about 7.8 (±0.8) and 8.3 (±1.5) adducts per 10^9 nucleotides by standard calibration ($n = 3$). In contrast, the measured adduct frequency for the control is only about 5.2 (±0.5) adducts per 10^9 nucleotides. Interestingly, clear dose−response relationships were observed for both exposures even at such never examined low levels. The exposure to *anti*-BPDE (0.1−10 nM, 2 h) exhibits linear dose−response with a correlation coefficient of $R = 0.99$ ($n = 8$) (Figure 2c). However, the exposure to bioactivation required B(a)P (1−50 nM, 16 h) generates a plateau curve at high doses (Figure 2c), indicating the probable inhibition or saturation of bioactivation of B(a)P at high doses.

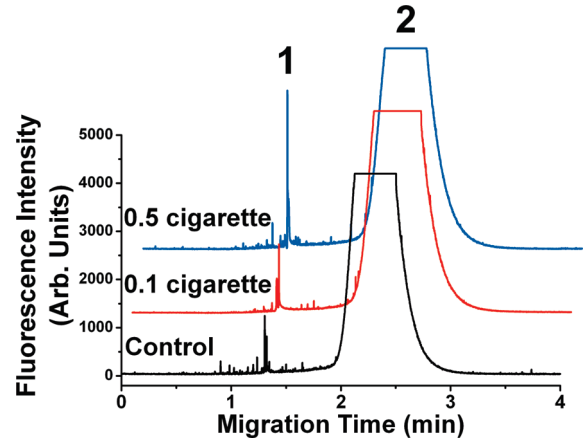

Figure 3. Electropherograms from QD based CE-LIF immunoassays for BPDE−DNA adducts in human A549 cells treated for 16 h with cigarette smoke condensate from 0, 0.1, and 0.5 cigarettes. Genomic DNA of 5 ng was injected for each analysis.

Cigarette smoke (CS) that is causally associated with lung cancer and affects about 1.3 billion smokers in the world[23] contains trace but carcinogenic B(a)P. We further determined the trace BPDE−DNA adducts formed in A549 cells treated with CS condensate from less than single cigarette smoke (Figure 3). The adduct frequencies in the A549 cells are 4.8 (±0.9), 7.9 (±0.5), and 21.9 (±2.9) adducts per 10^9 nucleotides for the respective treatment of the CS condensates from 0, 0.1, and 0.5 cigarette, showing a prominent dose-dependent response. The CS condensates induced significantly higher adduct frequency than control ($P < 0.007$). The amount of B(a)P in CS condensate from one cigarette's mainstream smoke is about 20 ng (Supporting Information, Figure S1B and Table S1). With the total nucleotides accounted for in the complete human genome (3.3×10^9 base pairs) and cell number (5.0×10^6 cells per plate), the estimated total BPDE−DNA adducts generated by 1 ng of B(a)P in CS condensate is 5.2×10^7 adducts, which is comparable with that in A549 cells treated by the same amount of B(a)P alone (8.2×10^7 adducts). The results suggest that the measurement of BPDE−DNA adducts by our method is not affected by the presence of other carcinogenic polycyclic aromatic hydrocarbons with similar amounts (Supporting Information, Table S1), showing the high specificity of the method.

In summary, we for the first time report a QD enhanced immunoassay for the detection of trace DNA adducts at environmental exposure levels. The detection mass sensitivity is about 5400 times higher than sensitive ^{32}P-postlabeling assays. Moreover, the method consumes only 1000 times less DNA (about 5 ng of genomic DNA) and does not require the labor-intensive and time-consuming DNA digestion and caution-taken radioac-

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tive labeling. With benefit from the ultrasensitivity, it enables us to quantify the trace BPDE−DNA adducts in A549 cells exposed to the smoke condensate from 0.1 cigarettes, 1 nM B(a)P, and 0.1 nM *anti*-BPDE. The ultrasensitive assay of BPDE−DNA adducts can be applied to DNA repair studies, human exposure biomonitoring, assessment of cancer risk and population susceptibility, and chemoprevention study. This method also shows promising applications of bright QD to various DNA analyses, including damage analysis. The achieved ultrasensitivity benefits from the exceptional optical properties and well-controlled nanosuface of QD and from the highly efficient separation and focusing of QDs by capillary electrophoresis.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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