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Electropolymerization of intercalator-grafted conducting polymer for direct and amplified DNA detection†

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Efficient DNA intercalators comprising naphthalene diimide grafted with ethylenedioxythiophene monomer were successfully designed, synthesized and applied to mediate/promote conducting polymer growth electrochemically on biosensor electrodes for direct and amplified DNA detection.

Intercalating organic/inorganic compounds that bind selectively and reversibly to double-stranded DNA (dsDNA) have demonstrated applications for antitumor drugs, DNA probes and gene delivery vectors.[1] Redox-active and fluorescent intercalators are employed as indicators for DNA hybridization to avoid labeling of the target DNA.[2] Complementary DNA targets are detected by measuring optical/electrochemical signals of these intercalators. However, most of these methods are limited by low signal intensity and poor signal/noise ratio. Recently, several reports have demonstrated sensitive DNA detection with a greatly amplified signal generated by nanoparticle-mediated[3] or enzyme-catalyzed[4] material growth on sandwich DNA assay (capture probe/target DNA/detection probe). Herein we present a unique molecular approach for intercalator-mediated polymer growth by combining this signal amplification method with the advantageous use of intercalators for DNA detection. This method is based on a designed dual functional molecule with both intercalator and conductive monomer building blocks that facilitates the electrochemical growth of conducting polymer on an electrode with hybridized DNA.

Naphthalenediimide (ND) intercalators[5] conjugated to redox-active moieties have been applied for electrochemical DNA detection.[6] On the other hand, conducting polymers derived from EDOT are among the most promising conductive materials due to their high conductivity, long-term stability and structure versatility.[7,8] We synthesized two ND-derivatized molecules with EDOT building block symmetrically (EDOT–ND–EDOT) and unsymmetrically (EDOT–ND–Os) as shown in Scheme 1.

Starting from hydroxymethyl-functionalized EDOT,[9] we prepared a key EDOT intermediate containing amino functional group with oligoethylene glycol linkers (EDOT–EG*n*–NH_2; n = 3 or 4).[10] These linkers were introduced to improve the solubility of the compounds in aqueous medium. Condensation of excess EDOT–EG4–NH_2 with 1,4,5,8-naphthalene tetracarboxylic dianhydride yielded the symmetrical EDOT–ND–EDOT.[11] For the synthesis of asymmetric EDOT–ND–Os, the ND precursor was first reacted with 1 equivalent of 1-(3-aminopropyl)imidazole to form mono-imide.[12] Subsequently, the intermediate was reacted with EDOT–EG3–NH_2 to yield ND with asymmetrical imidazole and EDOT grafts (EDOT–ND–Im).[13] Complexation between this compound and $Os(bpy)_2Cl_2$ (bpy = bipyridine) was conducted in refluxing ethylene glycol.

In addition to ^1H and ^{13}C NMR and HR-MS, the target compounds were characterized by UV-Vis spectroscopy (Fig. 1A) and cyclic voltammetry (Fig. 1B). EDOT–ND–Os exhibited absorption bands from $Os(bpy)_2^{2+}$ (λ_{max} = 296 nm) and ND (λ_{max} = 359 and 379 nm), whereas EDOT–ND–EDOT only displayed absorption bands arising from ND. EDOT–ND–Os displayed redox wave from Os^{2+}/Os^{3+} ($E_{1/2}$ = 0.17 V) upon oxidation, and redox waves from the π-conjugated system of ND ($E_{1/2}$ = −1.04 and −0.63 V) upon reduction.[14]

In intercalation, insertion of the planar aromatic ring between dsDNA base pairs would result in hypochromism and red shifts in UV-Vis absorption spectra. As shown in Fig. 1C, addition of salmon sperm DNA to a solution of EDOT–ND–Os at a DNA base pair/EDOT–ND–Os ratio of 3.0 resulted in 40% decrease in ND absorption bands at 362 and 383 nm. These bands were also red-shifted by ~3 nm, consistent with the previous reports on ND with aliphatic tertiary amine side-chains[5,15] or symmetrical $Os(bpy)_2^{2+}$ complex.[6] In contrast, limited hypochromism and red-shifts were observed for EDOT–ND–EDOT (Fig. 1D), presumably due to limited binding capacity arising from the lack of positive charges. In most DNA detection, the concentration of capture probe–target DNA hybrid is so low that intercalation is unlikely to occur by pure diffusion. For positively charged EDOT–ND–Os, favorable binding occurred between the polyanionic DNA and the cationic intercalator due to the additional electrostatic interactions, leading to stronger intercalation. Intercalative properties of EDOT–ND–Os were further studied by a competitive displacement assay with thiazole orange dye. The binding constant was estimated as 8×10^5 M^{-1} with dsDNA.

Both EDOT–ND–EDOT and EDOT–ND–Os electropolymerized to form homopolymer or copolymers in either organic or aqueous microemulsion electrolyte solutions. Cyclic voltammograms of copolymer films of EDOT–ND–Os/EDOT–OH clearly displayed reversible peaks ($E_{1/2}$ = 0.2 V) from Os^{2+}/Os^{3+} redox centers. The peaks were superimposed on the broad wave of redox activities from the PEDOT backbone, confirming the formation of the copolymer.

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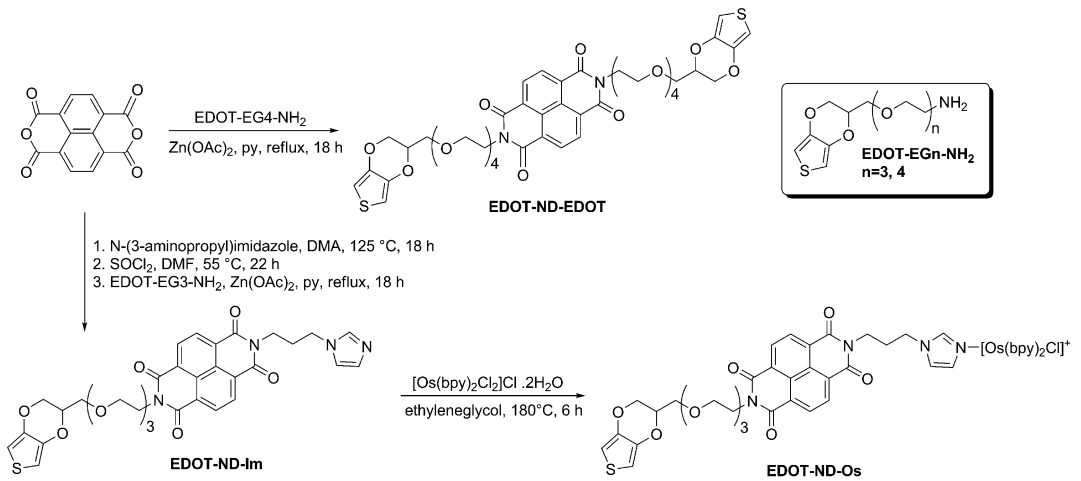

Scheme 1 Synthesis of EDOT-grafted naphthalenediimide intercalators.

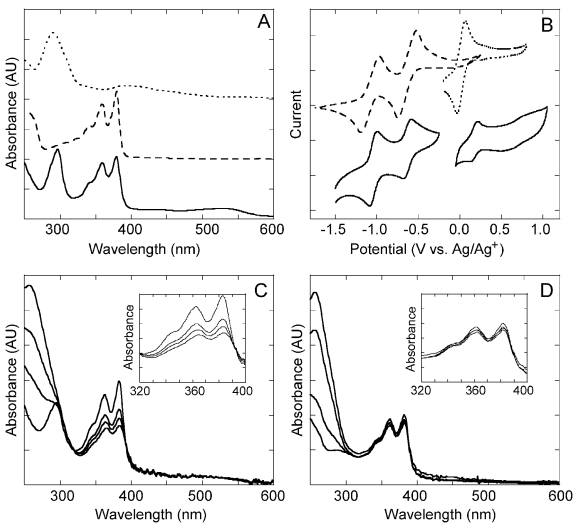

Fig. 1 (A) UV-Vis absorption spectra and (B) cyclic voltammograms of EDOT–ND–Os (—), EDOT–ND–EDOT (- - -), and $Os(bpy)_2Cl_2$ (···). The UV-Vis spectra were obtained in ethanol solution. Cyclic voltammograms were measured in 0.1 M of $n Bu_4NPF_6/CH_3CN$ (for EDOT–ND–Os and EDOT–ND–EDOT) and phosphate buffered saline (PBS) (for $Os(bpy)_2Cl_2$) at a scan rate of 100 mV s^{-1}. UV-Vis absorption spectra of 25 μM of (C) EDOT–ND–Os and (D) EDOT–ND–EDOT in PBS buffer solution containing 0, 25, 50, and 75 μM (from top to bottom) of salmon sperm DNA. The concentration of DNA is based on base pairs.

For DNA detection, thiol-functionalized peptide nucleic acid (PNA) capture probes (5′-TTTGAGTCTGTTGCTTGG) and mercaptoundecanol were self-assembled on gold electrode surface.[16] PNA probes were used in order to reduce the non-specific binding of cationic EDOT–ND–Os and enhance the hybridization efficiency by eliminating the anionic repulsion between DNA targets and DNA probes. The probe sequence was designed to target the N1 gene of avian flu virus. After hybridization with the complementary target (5′-CCAAGCAA-CAGACTCAAA), EDOT–ND–Os intercalation and washing, redox activity of Os^{2+}/Os^{3+} was observed electrochemically. Using the more sensitive square-wave voltammetric method, a higher peak current was obtained for 20 pM of complementary target as compared to that of a blank experiment without target and for controls of 100 pM of non-complementary target (5′-GGTTCGTTGTCTGAGTTT) and 2-base mismatched target (5′-CCAAGGAACAGAGTCAAA). However, the signal differences were limited, even after background correction. Detection of 20 pM target using this method is not satisfactory. Even at 100 pM target concentration, the current signal of the complementary target was only ~4 fold of the signal from the non-complementary target (Fig. 2A).

One advantageous feature of these new intercalators was their capability to electropolymerize to form PEDOT films as described earlier. As shown in Fig. 2A, direct electrochemical detection of electroactive intercalators is not sensitive enough and could only lead to a detection limit of around 100 pM and a very low current output (~10 nA) even with a more sensitive square wave voltammetric method. We would like to amplify the electrochemical signal output using the greater electroactivity of PEDOT films, which could lead to easier and cheaper instrumentation. The biosensor electrode was first placed in aqueous electrolyte solution (0.1 M of $LiClO_4$), and subjected to cyclic potential between −0.2 and 1.0 V. For electrodes hybridized with complementary target, a greater

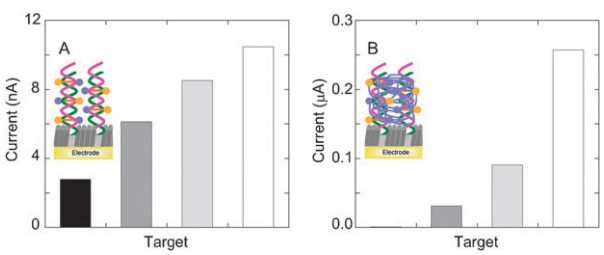

Fig. 2 (A) Peak current from square-wave voltammograms of EDOT–ND–Os bound to DNA biosensor electrodes at 0.14 V. (B) Voltammetric current from cyclic voltammograms of PEDOTs formed on assembled biosensor electrodes at 0.3 V (oxidation). The electrodes were hybridized with 100 pM of complementary target (white), 20 pM of complementary target (light grey), 20 pM of two-base mismatched target (dark grey) and 100 pM of non-complementary target (black). Base line signal (no target) was subtracted from the experimental data.

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increase of current was obtained between the initial and subsequent cycles due to the formation of oligomers/polymers from the surface-bound EDOT–ND–Os. We found a greater difference between the electrochemical signals of the experimental and control biosensor electrodes. The oligomers/polymers formed at this stage would serve as 'nuclei' for subsequent growth of PEDOT.

To further enhance the signal difference, the electrodes were placed in an aqueous electrolyte solution containing 5 mM of EDOT–OH. Previous reports on electrochemical growth of conducting polymers have shown that the polymer would form at a much lower potential after the initial layer of polymer has been deposited.[9] This was likely due to the lower over-potential required from improved electron transfer and alternative stepwise polymer propagation steps besides the original radical–radical coupling.[17] After fine-tuning the electrochemical polymer growth condition, it was found that amperometric polymer growth at 0.9 V for 120 s was optimal. At this controlled condition, polymer growth is severely limited in the absence of any nucleating sites. This results in a lower background, and thus potentially lower detection limit. This improved detection method is summarized in Scheme S1 (ESI†). As shown in Fig. 2B, greater signal difference was observed between complementary and non-complementary targets as compared to the electrochemical signal previously observed from Os^{2+}/Os^{3+} even with a less sensitive cyclic voltammetric method (Fig. S1, ESI†). Applying higher potentials or prolonging the polymerization time would result in a significant increase of polymer growth on the control experiments, effectively reducing the contrast. A much higher contrast of current outputs was obtained between biosensors with complementary and non-complementary DNA targets. This enhanced contrast was >100-fold improvement compared to the contrast obtained from the square-wave method applied in Fig. 2A, and detection of 20 pM target can be satisfactorily achieved. Using the same method with various concentrations of complementary target, a calibration plot was obtained as presented in Fig. S3 (ESI†).

In conclusion, a bi-functional molecule containing both an efficient dsDNA intercalator (ND) and a monomer unit of thiophene-based conducting polymer (EDOT) has been designed and synthesized. This molecule provides a unique approach to promote electrochemical growth of conducting polymer on dsDNA-immobilized electrode for sensitive DNA detection. Combining with cheaper and portable electrical instruments, the low detection limit and large current output would be potentially useful for point-of-care diagnostics.

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