Biocatalytically Synthesized Poly(3,4-ethylenedioxythiophene)

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ABSTRACT: Over the past decade, poly(3,4-ethylenedioxythiophene) (PEDOT) has been one of the widely investigated conjugated polymers due to its excellent electro-optical properties. The conventional synthesis of PEDOT/PSS involves oxidation of EDOT using strong oxidants in aqueous polystyrenesulfonate (SPS) solution. The low pH conditions and strong oxidants render this synthetic protocol unsuitable for use of PEDOT in applications such as biosensing. For the purpose of expanding the utility of PEDOT in these applications, it is important to develop a route that can provide the possibility of synthesizing PEDOT in the presence of the appropriate biological entities. Here we report the use of terthiophene as a radical mediator to synthesize PEDOT/PSS under milder pH conditions using soybean peroxidase (SBP). The oxidation potential of terthiophene is sufficiently low for initiation of the polymerization reaction catalyzed by SBP. The oxidized terthiophene helps the subsequent oxidation of EDOT, thus mediating the polymerization reaction. This novel approach involving the use of conjugated oligomers as redox mediators is generic and vastly expands the types of substrates (thiophenes, pyrroles) that can be polymerized using enzymatic methods and benign conditions.

Introduction

The synthesis of solution-processable inherently conducting polymers continues to be of interest due to their ease of processing for electronic and photonic applications. Poly(3,4-ethylenedioxythiophene) (PEDOT) is one of the successful and well-known π-conjugated polymers with good conducting and electro-optical properties.[1] Apart from being synthesized from a monomer 3,4-ethylenedioxothiophene (EDOT) of relatively low oxidation potential (as compared to other thiophene monomers), PEDOT possesses a unique combination of properties and reasonably good stability in the oxidized state.[2] PEDOT/PSS has good film-forming properties, exhibits conductivities[1] ranging between 10^{-3} and 10 S/cm, and is transparent in the visible wavelength region. PEDOT/PSS also exhibits electrochromic properties which have been demonstrated in devices.[3] The possibility of using this electrically conducting polymer for biosensing[4] (DNA sensors[5]) and drug delivery applications[6] has already been explored. PEDOT has also been found to be superior to poly(pyrrole) for sensing applications.[7] However, to date, most synthetic protocols used for the synthesis of PEDOT involves very low pH conditions and oxidants that are not compatible with biological systems. For the purpose of creating biosensors based on PEDOT, it is important to develop a synthetic route that can provide the possibility of synthesizing PEDOT in the presence of an appropriate biological entity. A biocatalytic approach could potentially pave the way to polymerizing EDOT in relatively benign conditions, rendering it suitable for a variety of biological applications.

During the initial stages of development, PEDOT that was synthesized using the conventional oxidative chemical/electrochemical method was insoluble in water. However, the solubility problem was overcome using a water-soluble polyelectrolyte, poly(styrenesulfonic acid) (PSS). PSS was also found to help in keeping the PEDOT segments dispersed in water. PSS also acts as the charge-balancing dopant for PEDOT, thus providing the desired electrical properties.[8] The most commonly used chemical method for the synthesis of PEDOT/PSS involves oxidation of EDOT by a strong oxidizing agent such as sodium persulfate in aqueous PSS solution.[9] The resulting PEDOT/PSS is not truly water-soluble but forms a dispersion in water which is stable and processable.

Experimental Section

Materials. The thiophene monomers, EDOT, and terthiophene were obtained from Sigma-Aldrich Co. and were used without further purification. Peroxidase from *Glycine max* (soybean) (activity 108 purpurogallin units/mg), poly(sodium 4-styrenesulfonate) [PSSNa] (M_w ca. 70 000), PEDOT/PSS (1.3 wt % dispersion in water), and hydrogen peroxide (30% solution) were also obtained from Sigma-Aldrich Co. The hydrogen peroxide was diluted to 0.3% (in deionized water), and this solution was used for polymerization.

Synthesis of PEDOT/PSSNa. The reactions were carried out in 80/20 (v/v) mixtures of citrate buffer (pH 3.5–5.5) and dimethyl sulfoxide (DMSO). The polymerization of EDOT in the presence of PSSNa and terthiophene was carried out using SBP and hydrogen peroxide under ambient conditions. 20.6 mg (10 mM) of PSSNa was dissolved in 7.8 mL of citrate buffer. A 1 mM stock solution of terthiophene was prepared by dissolving 2.48 mg in 10 mL of DMSO. A stock solution of SBP (12.5 mg/mL) was also prepared in deionized water. A typical reaction mixture contained 7.8 mL of citrate buffer containing PSSNa, 1 mL of DMSO containing 10.6 μL (10 mM) of EDOT monomer, 1 mL of the terthiophene stock solution, and 200 μL of SBP solution. The polymerization was initiated by the addition of 25 μL aliquots of 0.3% H_2O_2 solution. A total of 20 aliquots of the H_2O_2 solution were added at 3 min intervals to prevent inhibition of the enzyme by the H_2O_2 solution. The reaction mixture was stirred gently for several hours. The water-soluble complex was then transferred to individual regenerated natural cellulose membrane bags (molecular weight cutoff 1000 Da) and was dialyzed against 5000 mL of acidified deionized water maintained at pH 4.3. Dialysis was carried out for 72 h with fresh acidified deionized water being added every 6 h to expedite the removal of oligomers and unreacted monomer. The dry polymer

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was obtained by evaporation of solvent and drying at 40 °C under vacuum for 72 h. The gravimetric yield was around 78%.

Characterization. Products were characterized using a Perkin-Elmer Lambda 9 UV–vis spectrometer. The FTIR measurements were carried out on films cast on a ZnSe disk by use of a Thermo Nicolet 370 FT-IR spectrometer. Thermogravimetric analysis (TGA) was performed using a TA Instruments Hi-Res 2950 thermogravimetric analyzer. The TGA of all samples were carried out in air. Conductivity measurements were carried out on PEDOT/PSS pressed pellet samples using a Cascade Microtech collinear four-point probe connected to a current source and electrometer. The conductivity values reported are the average of several readings at different regions and sides of the disk. Cyclic voltammetry (CV) experiments were performed under nitrogen at room temperature using lithium perchlorate as electrolyte in deionized water. Platinum wire was used as a counter electrode; Ag/AgCl was used as the reference electrode and glassy carbon as the working electrode. A scan rate of 100 mV/s was used and the potential of the working electrode was swept between −0.9 and 0.6 V vs Ag/AgCl. Under similar conditions, potassium ferrocyanide gave an oxidation peak at 0.384 V.

Results and Discussion

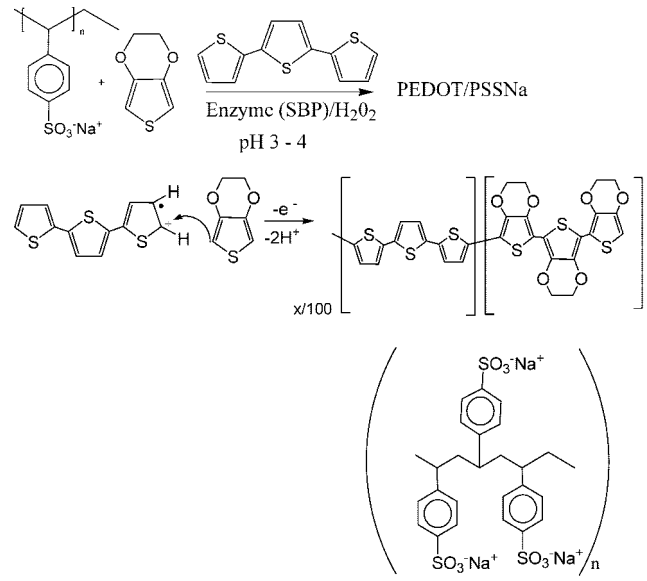
Enzymatic Polymerization. In the recent years we have shown that the synthesis of conducting polymers such as polyaniline can be carried out enzymatically in the presence of biological entities like genomic DNA.[10] Enzyme-catalyzed polymerizations are known to offer many advantages over traditional chemical approaches including environmentally friendly reaction conditions and ease of synthesis. Horseradish peroxidase (HRP) has been used for the oxidative polymerization of aniline in the presence of various charged macromolecular "templates" to yield water-soluble and conducting polyaniline complexes.[11]

However the catalytic use of HRP has been restricted to aniline-based monomers mainly due to the low redox potential [∼0.95 V with respect to standard hydrogen electrode (SHE) of the enzyme].[12] In EDOT, the substituents in the 3- and 4-positions cause a decrease in the oxidation potential of the monomer[13] to around 1.1 V, which is still significantly higher than the potential favorable for polymerization catalyzed by HRP. While there have been reports on the use of HRP for the polymerization of EDOT,[14] these reactions proceed well only at low pH conditions and temperatures.

Here we report the use of terthiophene as a radical mediator to synthesize PEDOT/PSSNa under milder pH and at ambient conditions using the enzyme soybean peroxidase (SBP) and poly(sodium *p*-styrenesulfonate) (PSSNa). SBP belongs to the family of plant peroxidases that can oxidize a wide variety of organic and inorganic substrates using hydrogen peroxide. SBP has been reported to have a higher redox potential as compared to HRP,[15] but SBP cannot catalyze the polymerization of EDOT under standard conditions. However, we found that the polymerization of EDOT can be accomplished by introducing a small amount (<1%) of an oligomeric thiophene such as 2,5-di(2-thienyl)thiophene [terthiophene] in the reaction mixture. The terthiophene monomer with a slightly lower oxidation potential (∼1.02 V) acts as a redox mediator facilitating the polymerization of EDOT in the presence of PSS as shown in Scheme 1a.

It has been shown that the polymerization of EDOT does not proceed without the addition of terthiophene.[16] The polymerization of terthiophene under identical conditions also yielded only low molecular weight oligomeric products. Further the concentration of terthiophene used was 100 times lower than that of EDOT monomer. Hence, the amount of oligomeric species formed by the oxidation of terthiophene is expected to be significantly lower (∼1%). Nevertheless, even at such low

Scheme 1. (a) Polymerization of EDOT Catalyzed by SBP Using Terthiophene As a Redox Mediator; (b) Proposed Mechanism for the Reaction



concentrations, the low oxidation potential of terthiophene permits the formation of terthiophene radicals that can in turn initiate the polymerization of EDOT. Since the oxidation of the EDOT monomer to form the dimer is the most difficult step, the introduction of a small amount of terthiophene facilitates this process. This result agrees well with earlier reports[17] on enhancement of rate of electrochemical polymerization of thiophene by addition of terthiophene and/or bithiophene. Wei et al.[17] have also proposed a mechanism for this electrochemical polymerization. This mechanism can be extended to polymerization of EDOT as shown in Scheme 1b. Although the terthiophene is present only is small concentrations in the reaction mixture, we believe that the terthiophene is incorporated along with the PEDOT in the final polymer. The exact mechanism of mediation by terthiophene is under investigation, and at the current time it is still unclear whether the terthiophene is incorporated as blocks or in a random fashion in the polymer formed.

Polymer Characterization. In order to determine the effect of concentration of the redox mediator on the formation of PEDOT/PSSNa, a series of reactions were performed where the concentration of terthiophene was varied (from 0.025% to 1%). As shown in Figure 1a, it was found that at least 0.5% (by weight of monomer concentration) of the terthiophene is required to initiate the polymerization of EDOT using SBP. PEDOT/PSSNa was not formed when the concentration of terthiophene was 0.025%. The polymerization of EDOT was also carried out at various pH conditions, and the concentration of the final polymer was monitored spectroscopically as shown in Figure 1b. As seen in the figure, it is evident that the polymerization can be carried out even at a pH of 4.0 to yield doped PEDOT/PSSNa polymer as seen by the absorption beyond 700 nm. The synthesis of PEDOT in aqueous conditions can be accomplished using biomimetic catalysts such as pegylated hematin.[18] Copolymers of PEDOT and pyrrole can also be synthesized in the presence of PSS using pegylated hematin as the catalyst.[19] However, these reactions can only be carried out at very low pH conditions (pH ∼1).

The UV–vis spectrum of the doped PEDOT/PSSNa closely resembles (Figure 2a) the PEDOT/PSS obtained from a commercial source (Aldrich). It has been reported that the oxidized (doped) and reduced (undoped) forms of PEDOT show different absorption characteristics.[1] Figure 2b,c shows the

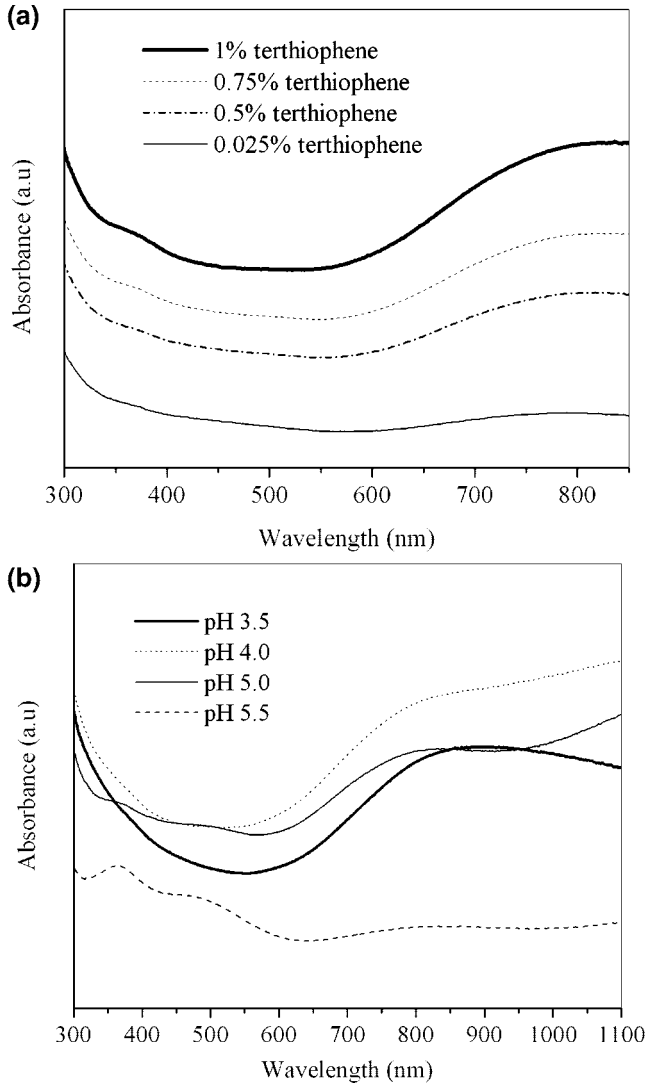


Figure 1. (a) UV–vis spectra for polymerization of EDOT at different terthiophene concentrations. (b) UV−vis spectra for polymerization of EDOT at different pH.

doping−dedoping studies on the enzymatically synthesized PEDOT/PSSNa. On increasing the pH of the solution from 2.7 to 11, an increase in the intensity of the band at 570 nm was observed (Figure 2b). At pH 11, the polymer is dedoped. The polymer can be redoped back as shown in Figure 2c. Upon doping, the absorbance at 570 nm assigned to a $\pi-\pi^*$ transition decreases continuously while giving rise to new absorptions beyond 700 nm. PEDOT/PSSNa is a molecular complex of PEDOT with PSSNa. The molecular weight/polydispersity of the PEDOT by itself could not be determined because the polymer remains soluble only when complexed with the polyelectrolyte (PSSNa). When the PSS is removed, the polymer was found to precipitate out of solution.

FT-IR Spectra. Figure 3 illustrates the FTIR spectrum of the commercially available PEDOT/PSS and the enzymatically synthesized PEDOT/PSSNa. The spectra are scaled individually for clearer comparison. The vibrations at 1195, 1139, and 1089 cm^{-1} are due to the C−O−C bond stretch in the ethylenedioxy group. The peak at 1521 cm^{-1} is due to the ring stretching of the thiophene ring. The weak vibration at 1062 cm^{-1} is possibly due to the C−O stretch. Peaks at 979, 937, and 840 cm^{-1} are assigned to thiophene C−S bond stretching. As seen in Figure 3, the PEDOT/PSSNa synthesized enzymatically shows similar features as that of the standard, and no major additional peaks are observed.

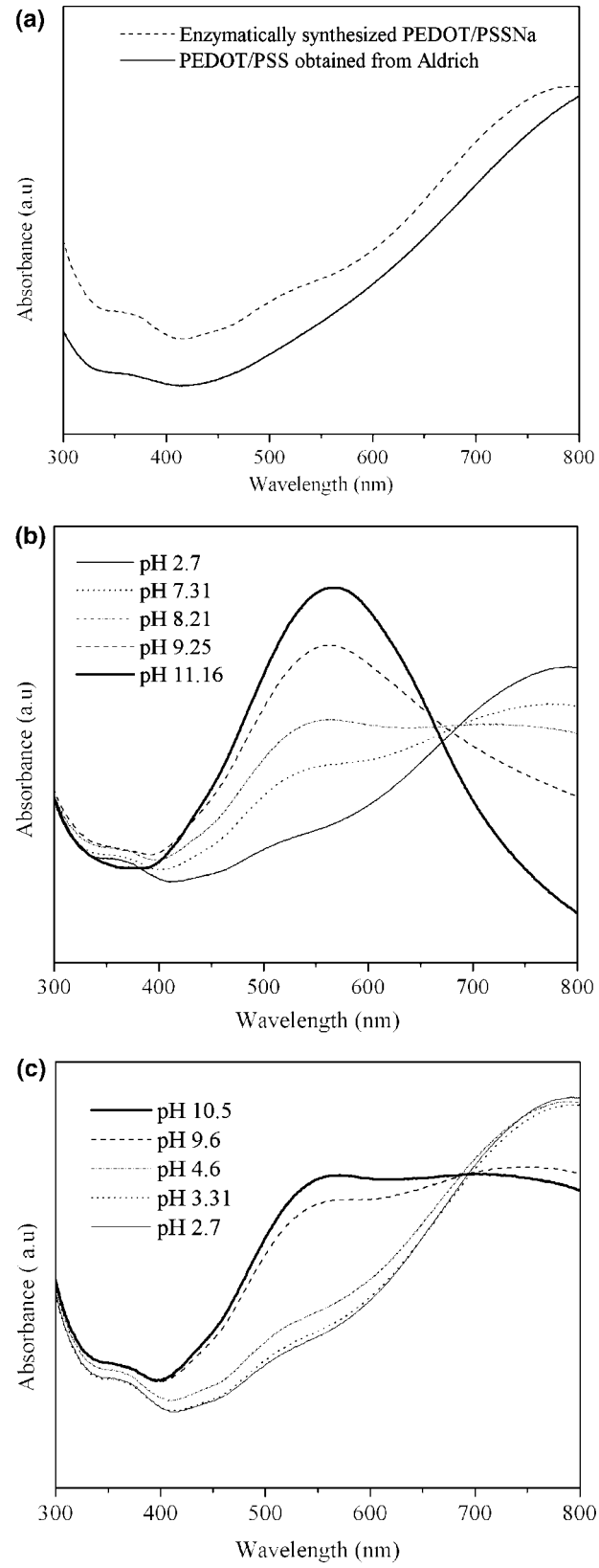


Figure 2. (a) UV–vis spectra of the PEDOT/PSS at pH 2.5. (b) Dedoping studies of PEDOT/PSSNa (pH 2.7−11). (c) Redoping of PEDOT/PSSNa (pH 10.5−2.7).

TGA. Thermal gravimetric analysis (TGA) done on the PEDOT/PSSNa synthesized enzymatically indicates an initial 8% weight loss until 150 °C which is attributed to the loss of water. The enzymatically synthesized polymer is fairly stable up to 200 °C and then starts to decompose (Figure 4). It is also

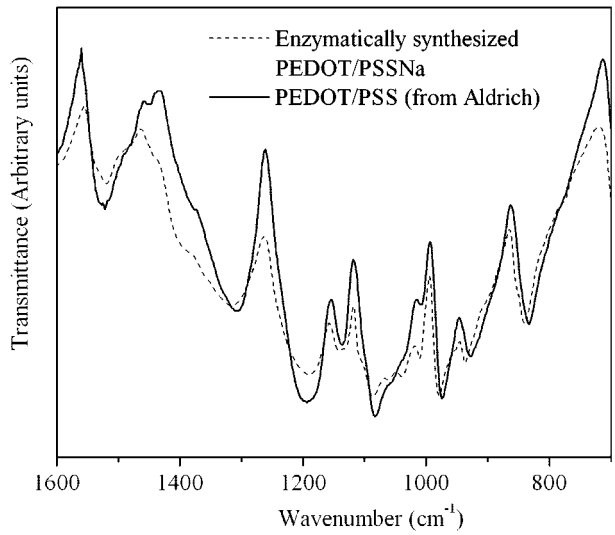


Figure 3. IR spectra of commercial PEDOT/PSS and enzymatically synthesized PEDOT/PSSNa.

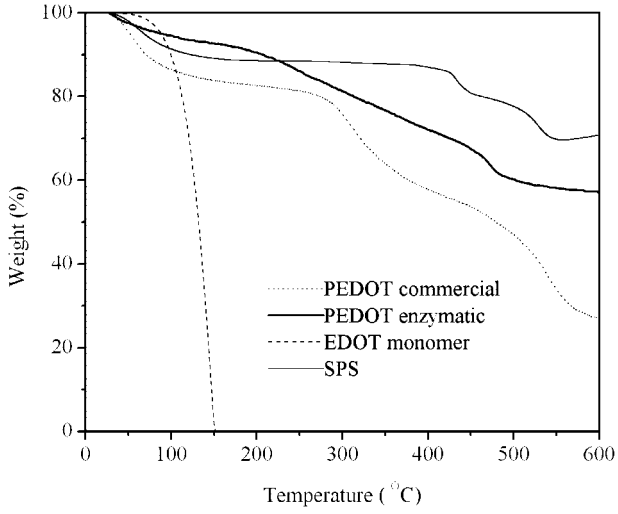


Figure 4. TGA curves of PEDOT/PSS (commercial), PEDOT/PSSNa, PSSNa, and EDOT.

more stable than PEDOT/PSS obtained from Aldrich. We believe the difference is due to greater amounts of template (PSSNa) in the enzymatically synthesized PEDOT.

Conductivity of pellets of PEDOT/PSSNa synthesized enzymatically was measured using a four-point probe and was compared with the PEDOT/PSS obtained from Aldrich. The enzymatically synthesized PEDOT/PSS was found to possess conductivities in the range of $10^{-3}-10^{-4}$ S cm^{-1}. In both cases pellets of the polymer were used, and the conductivities were of the same order of magnitude. Although the absolute value of the conductivities are slightly lower in the enzymatically synthesized PEDOT, they are comparable (of the same order of magnitude), and hence we can conclude that the PEDOT/PSS synthesized enzymatically is substantially similar to that synthesized chemically (obtained commercially).

Redox Studies. Cyclic voltammetric (CV) studies have been done on the enzymatically synthesized PEDOT/PSS. These results have been compared with PEDOT/PSS obtained from Aldrich. The redox behavior of the enzymatic PEDOT/PSS closely resembles that of the commercial polymer. The enzymatically synthesized PEDOT/PSS exhibits a reduction peak at −0.59V Vs Ag/AgCl and an oxidation peak at around −0.06 V. In comparison, commercially available PEDOT/PSS exhibits a reduction potential at around −0.5 V and a broad oxidation peak around −0.1 V.

Conclusions

In summary, the biocatalytic polymerization of EDOT has been accomplished using terthiophene as a redox mediator/initiator. The oxidation potential of terthiophene is sufficiently low to be oxidized by SBP in the initial stages of the reaction. The oxidized terthiophene helps in the oxidation of EDOT, thus mediating the polymerization reaction leading to the formation of PEDOT polymer. This approach also provides the ability to carry out the polymerization under milder (higher pH) reaction conditions. Furthermore, the choice of a thiophene-based material as the redox mediator ensures that any residual mediator that might be incorporated in the final polymer would still be composed of only conjugated thiophene segments. The unique combination of this redox-mediator approach with enzyme catalysis vastly expands the range of substrates (with higher oxidation potentials) that can potentially be polymerized using enzymatic/biomimetic methods. Further investigations are in progress to obtain a comprehensive understanding of the role of redox mediators in enzymatic synthesis of conducting polymers. Efforts are underway to explore the possibilities of using this biocatalytic synthesis for incorporating these polymers along with biological polyelectrolytes such as DNA for biosensing applications.

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