J|A|C|S
ARTICLES
Published on Web 03/10/2004

Fluorescent Polymeric Transducer for the Rapid, Simple, and Specific Detection of Nucleic Acids at the Zeptomole Level

Kim Doré,[†,‡] Sébastien Dubus,[†] Hoang-Anh Ho,[†] Isabelle Lévesque,[†] Maryse Brunette,[†,‡] Geneviève Corbeil,[†] Maurice Boissinot,[§] Guy Boivin,[§] Michel G. Bergeron,[§] Denis Boudreau,*[,‡] and Mario Leclerc*[,†]

Contribution from the Centre de Recherche en Sciences et Ingénierie des Macromolécules and Centre d'Optique, Photonique et Laser, Département de Chimie, Université Laval, Quebec City, Quebec, Canada G1K 7P4, and Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec, Pavillon CHUL, Université Laval, Quebec City, Quebec, Canada G1V 4G2

Received October 6, 2003; E-mail: mario.leclerc@chm.ulaval.ca; denis.boudreau@chm.ulaval.ca

Abstract: We report the specific detection of a few hundred molecules of genetic material using a fluorescent polythiophene biosensor. Such recognition is based on simple electrostatic interactions between a cationic polymeric optical transducer and the negatively charged nucleic acid target and can be done in less than 1 h, simply and affordably, and without any chemical reaction. This simple system is versatile enough to detect nucleic acids of various lengths, including a segment from the RNA genome of the *Influenza* virus.

Introduction

The rapid, sensitive, and specific detection of nucleic acids is of central importance for the diagnosis of infections, identification of genetic mutations, and forensic analyses. In particular, the efficient and affordable detection of infectious disease agents is envisioned by the World Health Organization as the most critical biotechnological development for improving health in developing countries.[1] The recognition capabilities of DNA through hybridization reactions are well-established, but adequate transducing elements are needed to generate a physically measurable signal from the recognition event. In this regard, many interesting DNA hybridization transducers have been proposed, including molecular beacons,[2] DNA-derivatized nanoparticles,[3−6] redox-active nucleic acids,[7−9] and engineered allosteric enzyme.[10] Electrochemical and optical DNA biosensors based on conjugated polymers have also been reported,[11−19] in which, in most cases, the detection relies on a modification of the electrical and/or optical properties of an oligonucleotide-functionalized conjugated polymer through hybridization with the complementary nucleic acid strand. However, up to now, none of these otherwise very impressive methods offer the simultaneous advantages of rapidity, simplicity, specificity, and detection sensitivity such that a chemical tagging of the DNA probe or target and/or a prior polymerase chain reaction (PCR) amplification step, which increases the assay's turnaround time and may limit quantitative analysis, is not required. Interestingly, conjugated polymers offer an amplification of the signal as compared to the response of individual monomers,[20−23] an effect that could, in principle, lead to a genomic detection technique at ultralow concentrations. Along these lines, we report here a rapid and simple electrostatic approach based on a cationic water-soluble polythiophene which enables the direct detection and specific identification of a few hundred molecules of the negatively charged complementary DNA or RNA target without any chemical reaction on the probes or targets.

[†] Centre de Recherche en Sciences et Ingénierie des Macromolécules, Département de Chimie.
[‡] Centre d'Optique, Photonique et Laser, Département de Chimie.
[§] Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec.

(1) Daar, A. S.; Thorsteinsdottir, H.; Martin, D. K.; Smith, A. C.; Nast, S.; Singer, A. C. *Nat. Genet.* **2002**, *32*, 229.
(2) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303.
(3) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
(4) Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1503.
(5) Dubertret, B.; Calame, M.; Libchaber, A. J. *Nat. Biotechnol.* **2001**, *19*, 365.
(6) Wang, J.; Polsky, R.; Merkoci, A.; Turner, K. L. *Langmuir* **2003**, *19*, 989.
(7) Ihara, T.; Nakayama, M.; Murata, M.; Nakano, K.; Maeda, M. *Chem. Commun.* **1997**, 1609.
(8) Patolsky, F.; Lichtenstein, A.; Willner, I. *Nat. Biotechnol.* **2001**, *19*, 253.
(9) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096.
(10) Saghatelian, A.; Guckian, K. M.; Thayer, D. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 344.
(11) Livache, T.; Roget, A.; Dejean, E.; Barthet, C.; Bidan, G.; Teoule, R. *Nucleic Acids Res.* **1994**, *22*, 2915.
(12) Youssoufi, H. K.; Garnier, F.; Srivastava, P.; Godillot, P.; Yassar, A. *J. Am. Chem. Soc.* **1997**, *119*, 7388.
(13) Bauerle, P.; Emge, A. *Adv. Mater.* **1998**, *10*, 324.
(14) Youssoufi, H. K.; Yassar, Y. *Biomacromolecules* **2001**, *2*, 58.
(15) Ho, H. A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Doré, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1548.
(16) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954.
(17) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 896.
(18) Liu, B.; Gaylord, B. S.; Wang, S.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 6705.
(19) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419.
(20) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201.
(21) Faïd, K.; Leclerc, M. *J. Am. Chem. Soc.* **1998**, *120*, 5274.
(22) Chen, L.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wuld, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287.
(23) Harrison, B. S.; Ramey, M. B.; Reynolds, J. R.; Schanze, K. S. *J. Am. Chem. Soc.* **2000**, *122*, 8561.

 10.1021/ja038900d CCC: $27.50

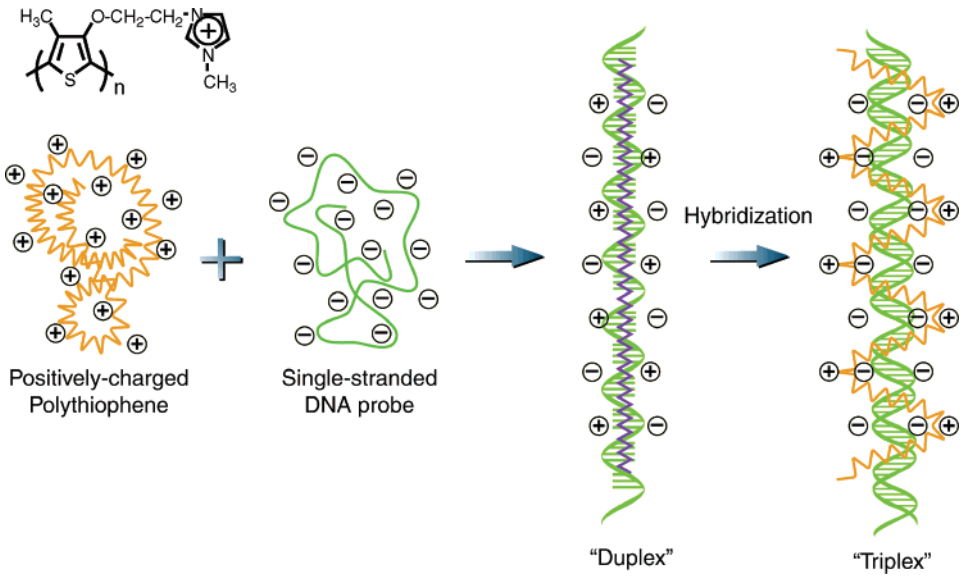

Figure 1. Schematic description of the formation of the polythiophene/single-stranded nucleic acid duplex and the polythiophene/hybridized nucleic acid triplex.

Results and Discussion

As with any polyelectrolytes, the cationic water-soluble polythiophene can form stoichiometric polyelectrolyte complexes (coacervates) with anionic oligonucleotide probes (duplex; see Figure 1). These stoichiometric complexes tend to form aggregates in aqueous solutions.[24] Depending upon the concentration of these coacervates, precipitation can occur after several hours. As a result of aggregation, the intrinsic fluorescence of the polymer alone (quantum yield of 0.03 in its random coil conformation) is red-shifted and quenched, due to close proximity and stiffening of the polythiophene chains.[15,19] However, fluorescence intensity increases again through the hybridization process with the perfectly matched complementary nucleic acid strand. Spectroscopic data suggest that the blue shift and increase in fluorescence is due to a helical structure of the polythiophene backbone that wraps with greater affinity around the anionic phosphate backbone of double-stranded nucleic acids (as compared to its affinity for single-stranded nucleic acids)[15] combined with a better solubilization of the resulting nonstoichiometric polyelectrolyte complex (triplex) which limits interchain quenching.[15,25]

Efficient nucleic acid detection by this polymeric sensor is greatly aided by the relatively large Stokes shift between the optimal excitation wavelength (at ca. 425 nm) and the wavelength for peak fluorescence emission (ca. 525 nm) of the triplex formed between polymer, oligonucleotide capture probe, and perfectly matched complementary nucleic acid strand (see Figure 2). This significant spectral separation allows the efficient rejection of background signals such as those generated by Rayleigh and Raman scattering using simple interference filters. In the present work, a tailored detection platform was developed specifically for the polythiophene sensor using such interference filters and a light-emitting diode (LED). LEDs are well-known for their very stable output intensity and have been used as light

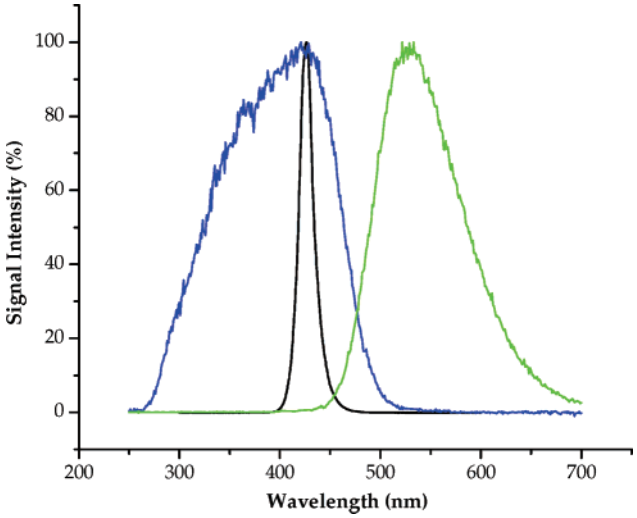

Figure 2. Excitation−emission spectrum for the polymer−DNA triplex, measured with a commercial spectrofluorimeter. The blue trace is the excitation spectral profile, and the green trace is the emission spectral profile. The black trace shows the spectral bandwidth of the LED excitation source.

sources for molecular absorption spectrometry,[26] for fluorimetry,[27] and even for optical detection in capillary electrophoresis.[28] In the latter case, the exceptional stability of a battery-powered LED source resulted in a relative standard deviation of the background signal of less than 0.0004%, despite the poor spatial beam quality of the LED and the resulting deficient coupling of the excitation beam with the small-diameter capillary. The simple detection platform developed herein and used in all the experiments mentioned in this paper combines a powerful and stable LED excitation source, a high-efficiency photon-counting detector, and the high light throughput of a nondispersive, filter-based optical design. This combination results in low light-measuring capabilities with a typical relative noise-to-signal of 0.004%.

(24) Petrak, K. In *Polyelectrolytes: Science and Technology*; Hara, M., Ed.; Dekker: New York, 1993; pp 265−297.
(25) Nilsson, K. P. R.; Andersson, M. R.; Inganäs, O. *J. Phys. Condens. Matter* **2002**, *14*, 10011.
(26) Imasaka, T.; Kamibuto, T.; Kawabata, Y.; Ishibishi, N. *Anal. Chim. Acta* **1983**, *153*, 261.
(27) Smith, B. W.; Jones, B. T.; Winefordner, J. D. *Appl. Spectrosc.* **1988**, *42*, 1469.
(28) Tong, W.; Yeung, E. S. *J. Chromatogr., A* **1995**, *718*, 177.

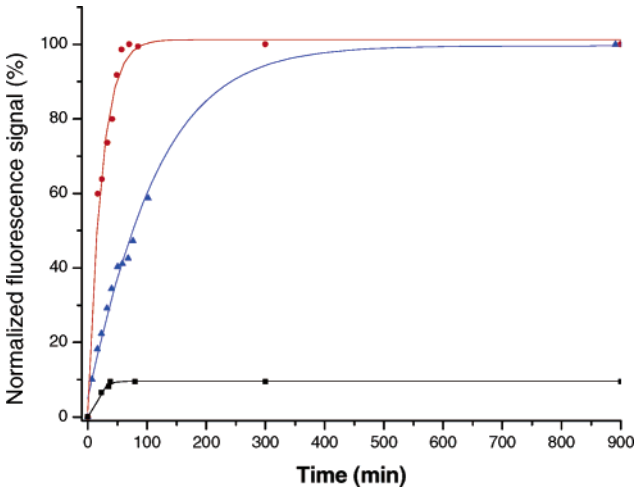

Figure 3. Hybridization kinetics of the perfectly matched target analyte (red curve), a two-mismatch sequence (black curve), and a single-mismatch sequence (blue curve).

Moreover, recent publications have reported the strong influence of surfactants on the luminescent properties of water-soluble conjugated polymers.[29−31] The detection of DNA in the presence of different nonionic surfactants was investigated. In highly dilute regimes, it was observed that the presence of 0.3 mM Triton X-100 improves the limit of detection by at least a factor of 10. This effect may be related to an increase of both the molar extinction coefficient and the luminescence quantum yield which can be related to the release of interfacial water molecules and the formation of more extended polymer chains.[29,31] The combined use of Triton X-100 with the dedicated fluorometer instrument allowed unprecedented simplicity, versatility, and analytical sensitivity.

As illustrated in Figures 3 and 4, it is now possible with this molecular system to specifically detect the hybridization of a few hundred molecules of complementary oligonucleotides within 60 min. A target DNA with two mismatches (black curve, Figure 3) can easily be discriminated from the perfectly matched analyte (red curve, Figure 3). Moreover, under kinetic monitoring, it is possible to discriminate perfect hybridization from a single defect in sequence matching (blue curve, Figure 3). As shown in Figure 4, there is a linear relationship between luminescence intensity and the concentration of the target analyte. In the case of 20-mer target oligonucleotides, one can extrapolate a limit of detection of ~310 molecules or 0.54×10^{-21} mol in an effective volume of 150 μL, or 3.6×10^{-18} M. In comparison with the perfect hybridization, the presence of sequences having one or two mismatches induces only a slight increase of the luminescence intensity, and the addition of a large excess (100 equiv) of the oligonucleotide with two mismatches only leads to a moderate increase of the luminescence.

Interestingly, the binding of 50-mer oligonucleotides onto a complex made of the polythiophene transducer and a 20-mer oligonucleotide capture probe produces an even higher luminescent signal (curve A, Figure 5) than that obtained when a

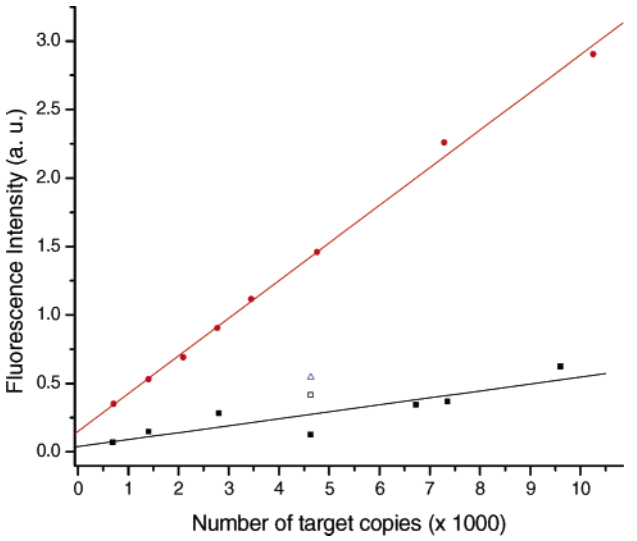

Figure 4. Calibration curves for 20-mer double-stranded DNA targets complexed with the polymer: calibration curve in red (filled circles) for the perfectly matched target analyte (polymer/X1/Y1) and in black (filled squares) for the two-mismatch sequence (polymer/X1/Y2). The open square symbol indicates the signal from a 100 equiv excess of the two-mismatch sequence (polymer/X1/100eqY2), and the open triangle symbol indicates the signal from 1 equiv of the single-mismatch sequence (polymer/X1/Y3). The limit of detection for the perfectly matched 20-mer target is 310 molecules, or 0.54 zmol, in a volume of 150 μL.

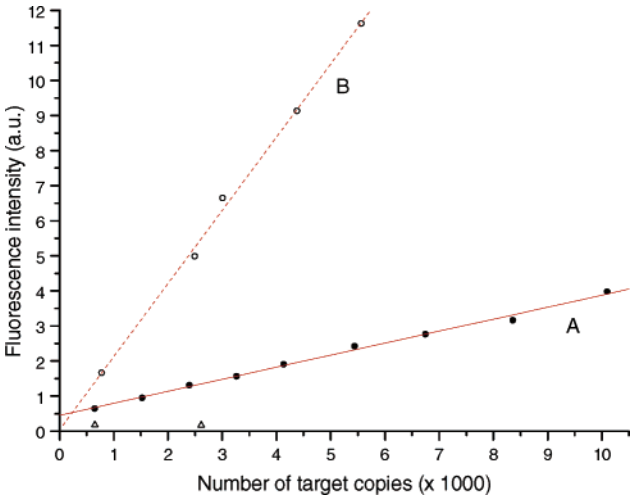

Figure 5. (A) Calibration curve for a 50-mer oligonucleotide target analyte (perfectly matched over a 20 nucleotide sequence of the capture probe) as detected by the polymer + 20-mer capture probe complex (polymer/Y2/X2-L). The triangle symbols are for the single-mismatch sequence (polymer/Y3/X2-L). The limit of detection for the 50-mer perfectly matched target is 0.36 zmol or 220 molecules in a volume of 150 μL. (B) Calibration curve for *Influenza A* viral RNA as detected by the polymer + 32-mer probe complex.

20-mer target is hybridized with the capture probe. This could be related to the formation of more soluble and more extended oligomeric moieties which limits intrachain and interchain quenching. In this case, the complex (triplex) formed between polymer, oligonucleotide capture probe, and perfectly matched complementary nucleic acid strand contains significantly more negative charges. These effects lead to a lower limit of detection of 220 molecules or 0.36 zeptomole (zmol) in 150 μL, (i.e. 2.4×10^{-18} M). We have also observed that hybridization with 50-mer targets is faster than with 20-mers. The hybridization of 50-mer target oligonucleotides presenting one and two mis-

(29) Chen, L.; Xu, S.; McBranch, D.; Whitten, D. *J. Am. Chem. Soc.* **2000**, *122*, 9302.
(30) Gaylord, B. S.; Wang, S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2001**, *123*, 6417.
(31) Lavigne, J. J.; Broughton, D. L.; Wilson, J. N.; Erdogan, B.; Bunz, U. H. F. *Macromolecules* **2003**, *36*, 7409.

matches (in the region complementary to the sequence of the 20-mer capture probe) was also tested. Results with one mismatch show that the system is still specific with analyte targets that are longer than the capture probe (Figure 5). This versatility of the fluorescent polymer in transducing the hybridization of oligonucleotides of varying lengths is a great asset in the design of molecular diagnostic tools for diverse genetic material. These results highlight again the stronger affinity of the cationic polythiophene toward double-stranded nucleic acids. However, it is worth noting that the optical detection of very low concentrations of nucleic acid targets is only possible when the polythiophene-capture probe complexes are hybridized at those low concentrations with the complementary strand of the analyte. Dilution of similar complexes of hybridized nucleic acids with the cationic polythiophene transducer made at higher concentrations (e.g. 10^{-12} M) leads to lower luminescence intensities, probably due to differences in the aggregation behavior of the stoichiometric complexes (i.e. duplexes) and structure of the resulting triplexes generated in diluted versus concentrated regimes. This property is indeed partly responsible for the high sensitivity and selectivity provided by the system and also enables the design of detection devices requiring only minimal amounts of reagents.

To test the validity of our system, a 32-mer oligonucleotide designed for the specific recognition of all *Influenza A* strains[32] was complexed with the polythiophene and hybridized with crude lysates from viral particules. In parallel, a control experiment was carried out with a nonspecific capture probe, and the fluorescent signal obtained from this control was subtracted from the signal generated by the *Influenza A*-specific probe. In less than 30 min, it was possible to detect nucleic acids corresponding to as few as 750 genome copies of the virus (curve B, Figure 5). Interestingly, this system based on a hybrid 32-mer oligonucleotide/polythiophene probe gives an enhanced fluorescence signal which can be related to an amplification of the detection coming from at least 32 repeat units of the fluorescent polymeric transducer together with a better solubilization of the resulting negatively charged complexes.

Conclusions

All these results show that the present polymeric biosensor can detect and specifically identify a few hundred copies of either DNA or RNA. This very sensitive optical detection system allows the direct detection and identification of the perfect match between a nucleic acid target and its complementary sequence and possibly of single nucleotide polymorphisms (SNPs). A large excess of an oligonucleotide with a sequence having two mismatches did not affect significantly the sensor response to the perfectly matched target, an observation of importance in cases where the concentration of the analyte is not known. This study also demonstrates that the direct detection of the flu virus is possible. These viruses harbor a ~10000 nucleotides (nt) genome composed of seven to eight single-stranded RNA segments with lengths varying from 890 nt to 2400 nt. The 32-mer probe we used here for *Influenza A* is complementary to the 1028 nt genome segment. It is thus possible with our polymer sensor to detect the presence of long RNA segments in complex mixtures of genetic material. The sensitivity and specificity of this fluorescence-based approach should lead to simple and cost-effective molecular diagnostic tools without any amplification steps. Moreover, the possibility to follow hybridization reactions in real time at such low concentrations can also find interesting applications for kinetics and thermodynamics studies of nucleic acids in various biological systems. In addition to its fluorescence, this polythiophene possesses electroactive properties which may serve for detection purposes or to generate structures such as conducting nanowires wrapped around nucleic acid scaffolds.

Experimental Section

General Procedure for the Chemical Polymerization of Monomer. Monomer 1H-imidazolium, 1-methyl-3-[2-[(4-methyl-3-thienyl)-oxy]ethyl]-, bromide was prepared as previously described.[15] The cationic, water-soluble, fluorescent polythiophene derivative, poly(1H-imidazolium, 1-methyl-3-[2-[(4-methyl-3-thienyl)oxy]ethyl]-, chloride), was synthesized from the chemical oxidation of this monomer using iron trichloride in chloroform. In 33 mL of chloroform, 0.650 g (2.16 mmol) of the monomer was dissolved followed by the dropwise addition of 22 mL of a 0.26 M iron trichloride chloroform solution, under nitrogen atmosphere. The mixture was left at room temperature for 48 h. The solvent was then removed under reduced pressure, and the crude black powder was washed with a minimum of cold methanol (10 mL). The polymer was then dissolved by adding a large volume of methanol (2500 mL), and a few drops of hydrazine were added. The solution is filtered and the solvent removed under reduced pressure. The resulting powder was washed using a Soxhlet apparatus and acetone as the solvent for 24 h. This procedure removed low-molecular-weight materials. The nonsoluble part (the resulting powder in the Soxhlet tube) was dried and washed again, this time using ethanol in the Soxhlet apparatus. The ethanol-soluble fraction of the polymer was recovered. The solvent was removed under reduced pressure. This procedure led to a dark red powder with a yield after purification of 55%. This polymer was soluble in water, forming a yellow solution. Ion chromatography measurements indicated that the counterions to the cationic polythiophene, Cl^- and Br^-, are present in a proportion of 88/12, which leads to a molecular weight of 262.83 per repeat unit.

Experimental Conditions for the Detection of Nucleic Acid Target Analytes. All hybridization experiments were carried out at 50 °C, in a solution comprising sterile Nanopure water, 0.1 M NaCl, and 0.3 mM hydrogenated Triton X-100. All dilutions and solution handling were performed in plasticware. First, the complex composed of the polymer + oligonucleotide probe was prepared. Stoichiometric quantities of the polymer and of the oligonucleotide capture probe were mixed to give a concentration of 4×10^{13} copies/μL of the resulting complex, which was then diluted to 1×10^4 copies/μL for the next experiments. The target DNA or RNA analyte was also diluted to the same concentration to form stoichiometric complexes. For the fluorescence measurements, 3 mL of hybridization solution was placed in a standard quartz cell (Starna). The polymer + probe complex was added, and the signal for this blank solution was measured. The target DNA or RNA analyte was then added, and the fluorescent signal was measured for a period of approximately 60 min allowed for hybridization and stabilization. The 50-mer DNA and RNA target analytes were denatured for 10 min at 100 °C prior to their addition into the sample quartz cell, to avoid secondary structures and the resulting signal artifacts. To acquire the data points presented in each calibration curve, additional amounts of polymer + probe complex and stoichiometric quantities of target analyte were added in the same quartz cell, and the measurements were taken as in the sequence described above. For all measurements, the blank signal measured for the probe + polymer complex was subtracted from the fluorescence signal measured for the polymer + probe + target analyte nucleic acid. This methodology was found to provide the best reproducibility.

(32) Klimov, A. I.; Rocha, E.; Hayden, F. G.; Shult, P. A.; Roumillat, L. F.; Cox, N. J. *J. Infect. Dis.* **1995**, *172*, 1352.

For the experiments with 20-mer oligonucleotides, the capture probe (X1) was 5′-CATGATTGAACCATCCACCA-3′ with perfect target (Y1) 3′-GTACTAACTTGGTAGGTGGT-5′. This corresponds to a conserved region of the *Candida albicans* yeast genome. The nonspecific target DNA was Y2: 3′-GTACTAACTTCGAAGGTTGGT-5′, which contains two mismatches from YI and corresponds to a sequence specific to *Candida dubliniensis*. Y3, 3′-GTACTAACTTCGTAGGTTGGT-5′, contains only one mismatch from Y1. With the 50-mer target, named X2-L, 5′-GGAATGGTGACAACATGATTGAAGCTTCCACCAACTGTCCATGGTACAAG-3′ (Trilink Biotechnologies Inc.), Y2 was used for the perfect match, Y1 for two mismatches, and Y3 for one mismatch. For the experiments with the *Influenza A* virus, the specific 32 capture probe used corresponds to probe M683 published by Klimov et al.,[32] while probe X1 was used for nonspecific control experiments.

***Influenza A* Virus Culture and Purification.** Confluent monolayers of Madin Darby canine kidney (MDCK) cells were infected with recombinant *Influenza* A/WSN/33 in an F75 flask. The infection medium was Eagle's minimum essential medium (EMEM) supplemented with 0.3% bovine serum albumin. After 4 days of incubation at 37 °C in a 5% CO_2 atmosphere, supernatants from infected cells were preclarified by low-speed centrifugation (6000 rpm for 5 min at 4 °C) and viruses were subsequently pelleted by ultracentrifugation (28 000 rpm for 2 h at 4 °C). The virus pellet was resuspended in 1 mL of phosphate buffered saline (PBS, pH 7.4) and stored at −80 °C.

Preparation of the RNA Samples from *Influenza* A Infected Cells. The purified viral particles were thawed on ice. A 100 μL aliquot of a stock solution of proteinase-K (Sigma) (20 μg/mL) was added to 1 mL of the viral suspension. The mixture was then vortexed for at least 10 s. After that, the resulting solution was incubated at 37 °C for 1 h. Finally, the preparation was heated at 95 °C for 10 min, and the sample was ready to be tested.

Description of the Fluorimetry Detection Platform. The excitation source is a prototype high-intensity blue LED (Lumileds Lighting, LLC) with an emission maximum at 425 nm and a spectral bandwidth (fwhm) of 16 nm. The spectral emission profile of the LED overlaps quite neatly with the excitation spectral profile of the polymer sensor (see Figure 2). The optical output power of the LED source was measured at 140 mW when operated at a current of 200 mA. The LED is powered by an ultralow-noise current source (ILX Lightwave LDX-3620) with a broadband current noise of only 850 nA rms, or less than 0.0004% at the nominal operating current of the LED. Detection of the fluorescence is made at 90° from the excitation axis by a photon counting detector (Hamamatsu H7421-40). Interference filters (CVI Laser Corp.) of appropriate central wavelength and bandwidth for the excitation (425 nm/15 nm) and emission (530 nm/30 nm) were used. The sample cell holder was kept at a constant temperature by a temperature-regulated water circulator (Fisher Scientific 1006 P). An effective sample volume of 150 μL was estimated from the volume probed by the excitation beam in the sample cell. For the purpose of comparing the analytical performances of this LED-based fluorimeter with a commercially available instrument, a Varian Cary Eclipse spectrofluorimeter was used in the following configuration: spectral band-pass of 20 nm, photomultiplier tube (PMT) voltage at 1 kV, in a standard 1 cm cuvette. The detection limits obtained with the LED-based detection platform for the perfectly matched hybridized complexes were about 2 orders of magnitude better than what is achievable with the dispersive spectrofluorometer.

Data Analysis. Each fluorescence data point in the accompanying figures is the average of at least 10 optical measurements. Each optical measurement is obtained by the integration of the fluorescence signal over a period of 10 s. The limit of detection is calculated as 3 times the standard deviation of the optical measurements for the blank signal, divided by the slope of the calibration curve.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Institutes of Health Research (CIHR), Infectio Diagnostic (IDI) Inc., and the Chemical, Biological, Radiological and Nuclear Research and Technology Initiative (CRTI). Lumileds Lighting, LLC, is gratefully acknowledged for the donation of LED prototypes. The authors acknowledge O. Desjardins for some optical measurements, Y. Abed for viral cultures, and J. P. Gayral and B. W. Smith for much useful advice.

JA038900D