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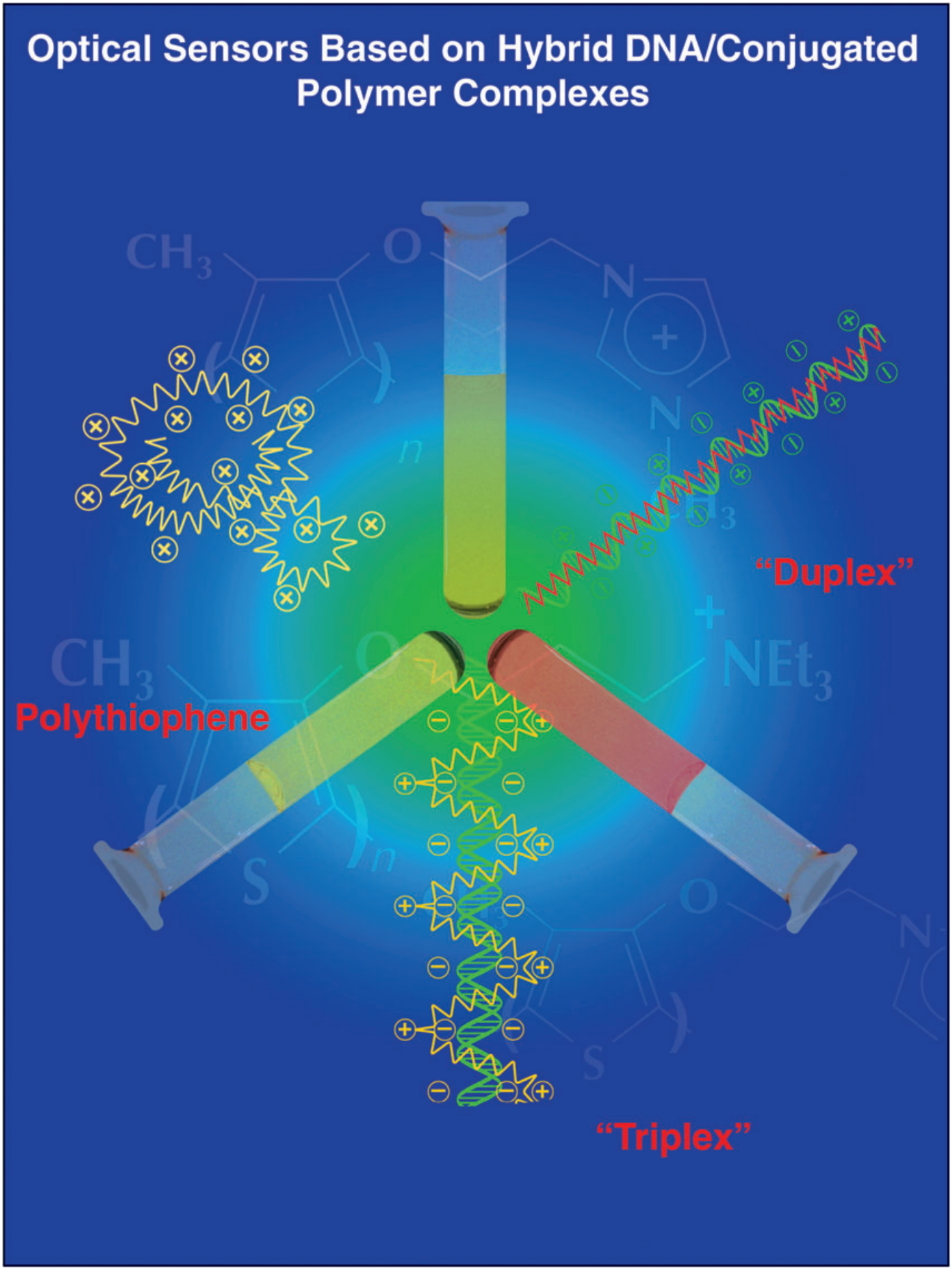

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Optical Sensors Based on Hybrid DNA/Conjugated Polymer Complexes

Hoang-Anh Ho, Maïté Béra-Abérem, and Mario Leclerc*[a]

Abstract: Single-stranded DNA (ss-DNA) can specifically bind to various targets, including a complementary ss-DNA, ions, proteins, drugs, and so forth. When binding takes place, the oligonucleotide probe often undergoes a conformational transition. This conformational change of the negatively charged ss-DNA can be detected by using a water-soluble, cationic polythiophene derivative, which transduces the complex formation into an optical (colorimetric or fluorometric) signal without any labeling of the probe or the target. This simple and rapid methodology has enabled the specific and sensitive detection of nucleic acids and human thrombin. This new biophotonic tool can easily be applied to the detection of various other biomolecules and is also useful in the high-throughput screening of new drugs.

Keywords: conjugated polymers · DNA recognition · electrostatic interactions · proteins · sensors

Introduction

Intense research is being carried out worldwide with the goal of developing rapid, simple, specific, and sensitive detection tools for medical diagnostics and biomedical research applications.[1–9] In this regard, many interesting optical and electrochemical transducers have been recently proposed, including molecular beacons,[2] derivatized nanoparticles,[3] redox-active nucleic acids,[4] and so forth.[5–9] However, most of the available methods have the disadvantage of requiring the chemical coupling of a photoactive, electroactive, or radioactive tagging agent onto the target or the probe prior to detection. Along these lines, we recently developed a new concept that utilizes a water-soluble, cationic conjugated polymer as a "polymeric stain" that can specifically transduce the binding of an appropriate single-stranded DNA probe (ss-DNA or oligonucleotides) to its target into a clear optical (colorimetric or fluorometric) signal. This simple, rapid, sensitive, and selective methodology is based on conformationally flexible polyelectrolytes and complementary electrostatic interactions and does not require any chemical modification on the probes or targets. This new electrostatic methodology has already enabled the specific detection of molecules of biological interest at the zeptomole level.

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Nucleic Acid Detection

The sequence-specific detection of DNA is of central importance for genetic analysis to diagnose infections and various genetic diseases. For this purpose, and on the basis of previous studies on thermochromic, solvatochromic, and affinitychromic (changing color upon binding) poly(3-alkoxy-4-methylthiophene)s,[10] we designed and developed the following water-soluble, cationic polymeric transducer, poly(1*H*-imidazolium-1-methyl-3-{2-[(4-methyl-3-thienyl)-oxy]ethyl} chloride)[11,12] (Scheme 1). As expected, the resulting cationic polythiophene derivative is soluble in aqueous solutions with a maximum absorption at 397 nm. This absorption maximum, which is at a relatively short wavelength, should be related to a random-coil (nonplanar or nonconjugated) conformation of the polythiophene derivative, as any twisting of the conjugated backbone leads to a decrease of the effective conjugation length. In contrast, in the solid state, the maximum absorption wavelength is located at 540 nm that is attributed to an aggregated (planar or conjugated) form (Figure 1).

As with any water-soluble cationic polyelectrolytes, this polythiophene derivative can form strong electrostatic complexes with negatively charged oligomers and polymers. As a proof-of-concept for the electrostatic sequence-specific detection of oligonucleotides, we utilized four different negatively charged oligonucleotides: a capture probe sequence

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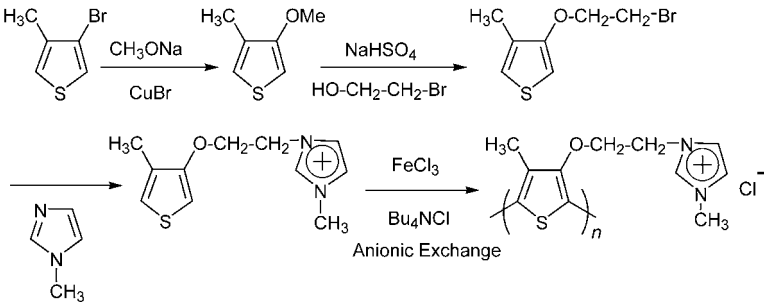


Scheme 1. Synthesis of a cationic poly(3-alkoxy-4-methylthiophene).

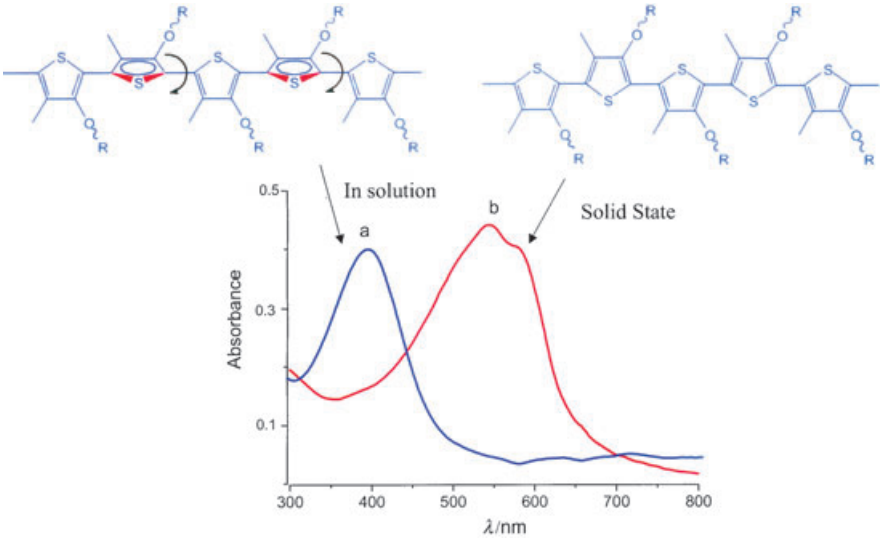


Figure 1. Conformational changes and their corresponding UV-visible absorption spectra of cationic polythiophene: a) in an aqueous solution; b) in the solid state.

(**X1**: 5′-CATGATTGAACCATCCACCA-3′), a perfect complementary target (**Y1**: 3′-GTACTAACTTGGTAGGTGGT-5′), a two-mismatch complementary target (**Y2**: 3′-GTACTAACTT*CG*A*A*GGTGGT-5′), and a one-mismatch complementary target (**Y3**: 3′-GTACTAACTT*C*GTAGGTGGT-5′). Upon addition of one equivalent (on a monomer unit basis) of capture oligonucleotide **X1**, the yellow polymeric solution (Figure 2A a and B a) becomes red ($\lambda_{max}=527$ nm; A b and B b), because of the formation of a so-called duplex between the polythiophene and the oligonucleotide probe (Scheme 2). Such stoichiometric (neutral) polyelectrolyte complexes (coacervates) tend to be insoluble in the medium in which they are formed. These red-violet aggregates (probably formed from planar polymer chains) have an absorption spectrum similar to that obtained in the solid state. After five minutes of mixing in the presence of one equivalent of the complementary oligonucleotide **Y1**, the solution again becomes yellow ($\lambda_{max}=421$ nm, Figure 2A c and B c); this change is presumably caused by the formation of a new more soluble complex (Scheme 2) between the cationic polymer and hybridized oligonucleotides. This new negatively charged complex, called a triplex, was characterized by circular dichroism measurements, which revealed a right-handed helical (twisted) orientation of the polythiophene backbone compatible with the binding of the polymer to the negatively charged phosphate backbone of double-stranded DNA.[11]

To verify the specificity of this polymeric optical transducer in the presence of imperfect or incomplete hybridizations, two different 20-mer oligonucleotides differing by only one or two nucleotides (but placed around the middle of the ssDNA) were investigated. A very distinct, stable, and reproducible UV-visible absorption spectrum is observed in the case of oligonucleotide target with two mismatches **Y2** (Figure 2B d) when compared to perfect hybridization (B c). In some cases, it is even possible to distinguish only one mismatch (Figure 2A e and B e).

Fluorometric detection of oligonucleotide hybridization is also possible, since the fluorescence of poly(3-alkoxy-4-methylthiophene)s is quenched in the planar, aggregated form.[10]

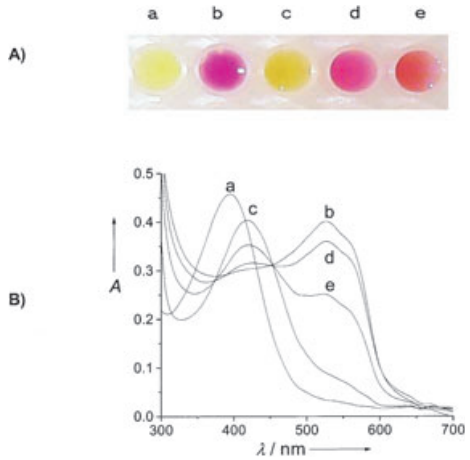
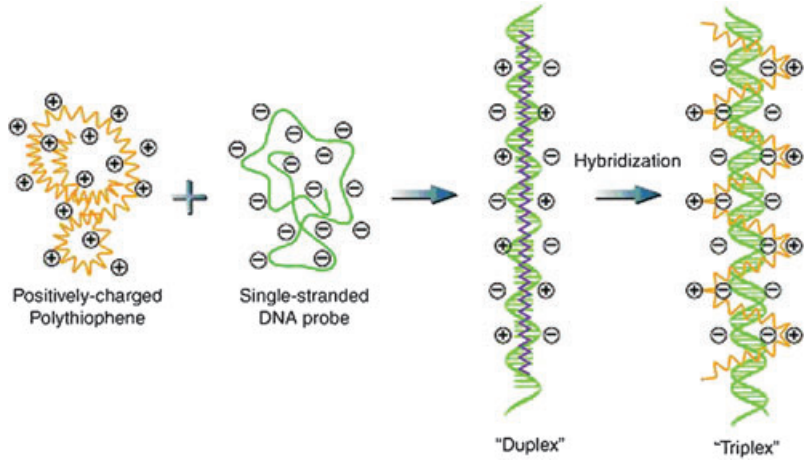


Figure 2. A) Photographs of 7.9×10^{-5} M (on a monomeric unit basis) solutions of a) cationic polymer alone, b) polymer/**X1** duplex, c) polymer/**X1**/**Y1** triplex, d) polymer/**X1**/**Y2** mixture, and e) polymer/**X1**/**Y3** mixture after five minutes of mixing at 55 °C in 0.1 M NaCl/H_2O. B) UV-visible absorption spectra corresponding to the different assays of photograph A. Reprinted with permission from reference [11], copyright (2002) Wiley-VCH.



Scheme 2. Schematic description of the formation polythiophene/single-stranded nucleic acid duplex and polythiophene/hybridized nucleic acid triplex.

For instance, at 55 °C, the yellow form of cationic polythiophene is fluorescent (quantum yield of 0.03 with a maximum of emission at 530 nm, see Figure 3 curve a), but upon addition of one equivalent of a negatively charged capture oligonucleotide probe **X1** the fluorescence intensity decreases

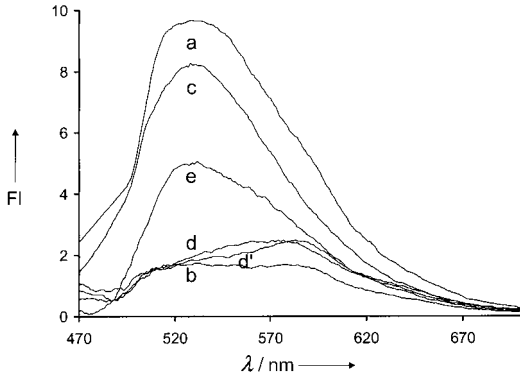


Figure 3. Fluorescence spectrum of a 2.0×10^{-7} M (on a monomeric unit basis) solution of a) cationic polymer alone, b) polymer/**X1** duplex, c) polymer/**X1**/**Y1** triplex, d) polymer/**X1**/**Y2** mixture, d′) polymer/**X1**/**Y2** (100 equivalents) mixture, and e) polymer/**X1**/**Y3** mixture at 55 °C, in 10 mM Tris buffer containing 0.1 M NaCl (pH 8). Reprinted with permission from reference [11], copyright (2002) Wiley-VCH.

and the maximum of emission is slightly red-shifted (curve b). When hybridization with the perfect complementary strand **Y1** takes place, the formation of the more soluble and helical polymeric triplex leads to a fivefold rise in fluorescence intensity (curve c). Interestingly, upon addition of one (Figure 3 curve d) or even 100 equivalents (curve d′) of the target oligonucleotide with two mismatches **Y2**, the fluorescence intensity is not significantly modified. Oligonucleotides with one mismatch can be discriminated (Figure 3 curve e). By measuring the fluorescence intensity at 530 nm (without recording the entire emission spectrum) with a dedicated fluorometer, a few hundred copies of either DNA or RNA were specifically detected.[12] The turn-on of the fluorescence intensity and the good stability of the electrostatic complexes should explain this sensitivity comparable to the best methods reported in the literature so far.[2–9] However, it must be pointed out that the electrostatic approach necessitates the presence of ssDNA targets only and will suffer from interferences coming from other polyelectrolytes or double-stranded (ds) DNA.

Ion Detection

To further investigate the potential of this new concept, we used artificial nucleic acid ligands (i.e., aptamers) that are known to exhibit high affinity and selectivity against a variety of targets, including ions, small organic molecules, amino acids, proteins, and so forth. RNA and DNA aptamers are generally selected and generated by the so-called SELEX procedure,[13,14] which involves repeated cycles of selection, recovery, and amplification. The monovalent potassium cation was our first target, because of its known fold-inducing properties for several classes of nucleic acids.[15] In agreement with our previous results, an aqueous solution of the polythiophene alone shows a maximum of absorption (λ_{max}) around 400 nm (illustrations a in both panels of Figure 4). A red color ($\lambda_{max}=527$ nm) was observed in the presence of LiCl (illustrations b), NaCl (illustrations c), RbCl (illustrations e), and ss-DNA (**X2**: 5′-

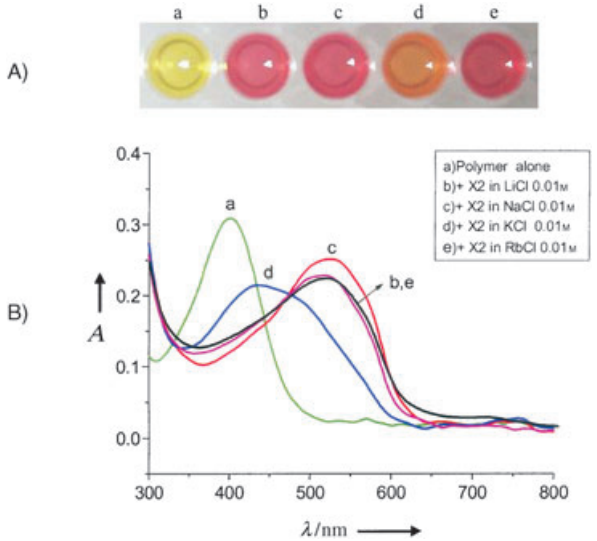
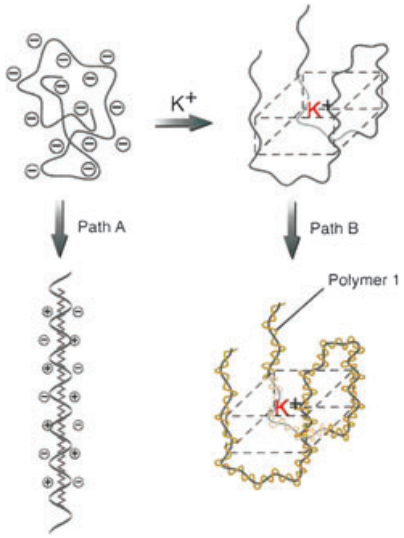


Figure 4. A) Photographs and B) UV-visible absorption spectra of the cationic polymer (2.9×10^{-9} mol on a monomer unit basis) in the presence of **X2** (1.9×10^{-10} mol of the 15-mer) and different salts in 100 μL of water, at 25 °C. Reprinted with permission from reference [16], copyright (2004) American Chemical Society.

GGTTGGTGTGGTTGG-3′). This red shift is related to a stoichiometric complexation between unfolded anionic ss-DNA and the cationic polythiophene derivative (Scheme 3, path A). However, the optical properties (illustrations d in both panels of Figure 4) are different when potassium ions are present. The formation of a quadruplex state of oligonucleotide **X2** stabilized by K^+ allows polythiophene to wrap this folded structure (Scheme 3, path B) through electrostatic interactions. Moreover, similar results were observed when the chloride counterion was replaced by a bromide or an iodide anion, indicating the specificity of the detection toward potassium cations.[16]



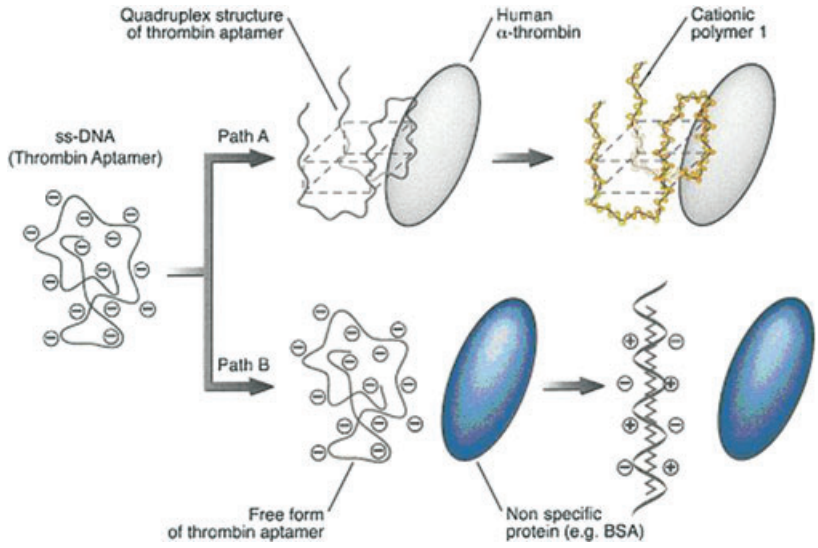
Scheme 3. Principle for the specific detection of potassium ions.

Protein Detection

Detection of the human α-thrombin is also possible using the same strategy, since oligonucleotide **X2** (5′-GGTTGGTGTGGTTGG-3′) is also known to be a specific binding sequence (i.e., an aptamer) of this protein.[17] A conformational change occurs when the aptamer binds to the thrombin molecule. Both NMR[18] and X-ray diffraction studies[19] have revealed that the aptamer adopts a compact unimolecular quadruplex structure with two G-quartets. Therefore, as shown in Scheme 4, the specific detection of human α-thrombin could be realized due to the formation of a quadruplex structure of the aptamer (**X2**).

The 1:1:1 complex formed between cationic polymer, aptamer **X2**, and thrombin has the same orange color and UV-visible absorption spectrum (Figure 5 curve b) as that induced by K^+. The thrombin promotes the formation of quadruplex form of the thrombin aptamer, and the cationic polythiophene wraps around this quadruplex structure; this wrapping seems to partially hinder the aggregation and planarization of the positively charged polymer in the presence of ssDNA **X2** (Scheme 4, path A). It is worth noting that only the stoichiometry of the aptamer (in terms of negative charges) and of the polymeric transducer (in terms of positive charges) has to be balanced, whereas an excess of thrombin does not influence its detection. In order to verify the specificity of the detection, two control experiments with a nonbinding sequence (**X3**: 5′-GGTGGTGGTTGTGGT-3′; Figure 5 curve c) and BSA (bovine serum albumin; curve d) were carried out under identical conditions. In both cases, an important red shift towards the lower energy ($\lambda_{max} = 505$ nm) was observed, and the color of these solutions was red-violet, a typical color of the planar and highly conjugated structure of the polythiophene backbone when mixed with unfolded ssDNA (Scheme 4, path B).

Once again, fluorometric detection of the human α-thrombin is possible, since the fluorescence of poly(3-alkoxy-4-methylthiophene) is quenched in the planar, aggregated form. The yellow, random-coil form of polymer is fluorescent (Figure 6 curve a) with an emission maximum at 525 nm. With use of non-specific thrombin aptamer (**X3**) (curve c) or the absence of human thrombin (curve d), the red-violet, highly conjugated form has a much lower fluorescence intensity and the maximum of emission is red-shifted ($\lambda_{em} = 590$ nm). However, when the 1:1:1 complex (human



Scheme 4. Schematic description of the specific detection of human α-thrombin using ss-DNA thrombin aptamer and cationic polythiophene.

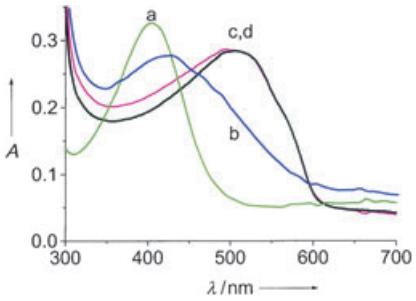


Figure 5. UV-visible absorption spectra corresponding to the different assays recorded at 5 °C: a) Polymer alone in water; b) Complex(1/1/1): human thrombin/specific thrombin DNA aptamer (**X2**)/polymer; c) mixture: human thrombin/nonspecific thrombin DNA aptamer (**X3**)/polymer; and d) mixture: BSA/specific thrombin DNA aptamer (**X2**)/polymer. Reprinted with permission from reference [16], copyright (2004) American Chemical Society.

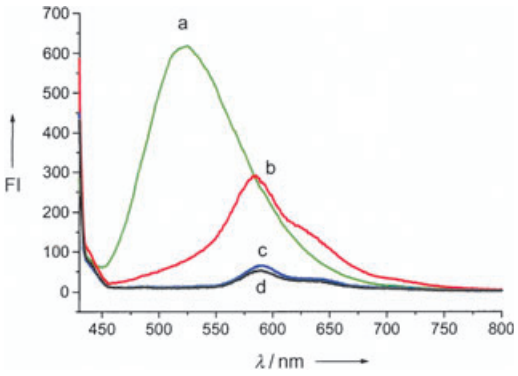


Figure 6. Fluorescence spectra of a) polymer, b) human thrombin/**X2**/polymer complex, c) human thrombin/**X3**/polymer mixture, and d) **X2**/polymer complex in water, measured at 5 °C. Reprinted with permission from reference [16], copyright (2004) American Chemical Society.

thrombin/specific thrombin DNA aptamer **X2**/polymer) is formed (Figure 6 curve b), the resulting orange intermediate form is less fluorescent than the yellow form, but more fluorescent (ca. sixfold increase) than the red-violet form. This higher intensity (turn on) of emission could be related to a partially planar conformation of the polythiophene chain, but with less aggregation of the chains. By using a standard spectrofluorimeter, a detection limit of 2×10^{-15} mol (this is a concentration of 1×10^{-11} M in 200 μL) of the human α-thrombin was obtained.[16]

Enantiomer Detection

Finally, as a fourth example, we describe here an easy and rapid methodology for the enantiomeric resolution of D- and L-adenosine. This approach is based on the fact that two stacked G-quartets are formed by mixing D-adenosine to the aptamer **X4** (5′-ATTATACCTGGGGGAGTATTGCGGAGGAAGGTATAAT-3′), which is not the case with L-adenosine.[20]

As usual, the cationic polythiophene gives a yellow color in an aqueous solution with a maximum absorption at 397 nm (Figure 7 curve a). Then, since L-adenosine is not

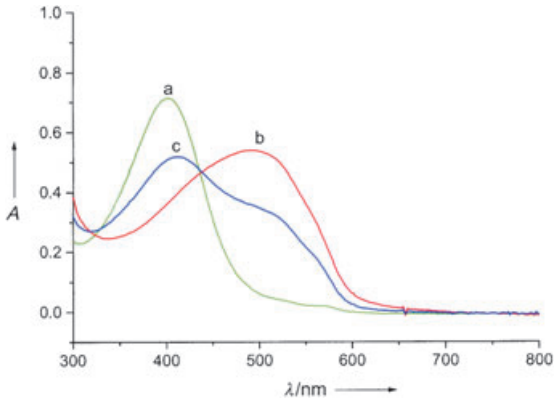


Figure 7. UV-visible absorption spectra of a) the cationic polymer (1.08×10^{-7} mol) in water (200 μL) at 5 °C; b) a mixture of L-adenosine (2.9×10^{-9} mol), DNA D-adenosine aptamer **X4** (1.08×10^{-7} mol based on monomeric negative charge or 2.9×10^{-9} mol of 37-mer) and polymer (1.08×10^{-7} mol based on charge unit) in water (200 μL) at 5 °C; and c) a mixture of D-adenosine (2.9×10^{-9} mol), DNA D-adenosine aptamer **X4** (1.08×10^{-7} mol based on monomeric negative charge or 2.9×10^{-9} mol of 37-mer) and polymer (1.08×10^{-7} mol based on charge unit) in water (200 μL) at 5 °C.

supposed to induce a conformational change of the aptamer **X4**, the cationic polythiophene should bind to the aptamer and lead to the formation of a duplex. As expected, when the polymer is put in the presence of both the aptamer **X4** and L-adenosine, the aqueous solution becomes red with a maximum of absorption at 500 nm (Figure 7 curve b). In contrast, a maximum of absorption at 410 nm (with still a shoulder near 500 nm) is observed when the cationic polythiophene is added to a solution containing D-adenosine and its aptamer **X4** (curve c).

Since fluorescence spectroscopy is more sensitive than UV-visible absorption spectroscopy, the emission properties of the cationic polymer can also be used in this case to detect small quantities of D-adenosine. The yellow aqueous solution of the cationic polythiophene is fluorescent with a maximum of emission at 525 nm (Figure 8 curve a). When L-adenosine is put in presence of aptamer **X4**, the fluorescence of the optical transducer is red-shifted and quenched (curve b). In the case of the complexation between D-adenosine and the aptamer **X4**, a partial recovery of the fluorescence of the cationic polymer is observed (Figure 8 curve c). The limit of detection obtained by using a standard spectrofluorimeter is about 2×10^{-14} mole of D-adenosine in a total volume of approximately 200 μL.

Conclusion

These four examples of specific detections with hybrid DNA/polythiophene complexes have clearly shown the

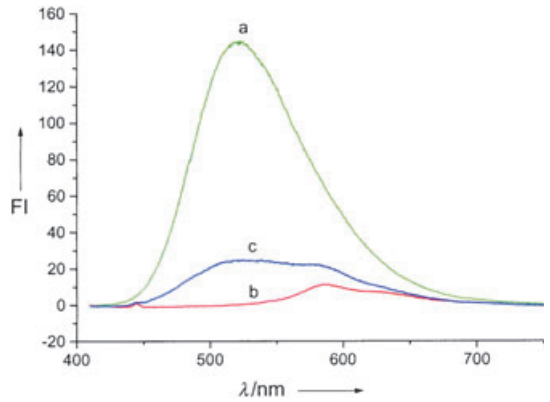


Figure 8. Fluorescence spectra of a) the cationic polymer (1.08×10^{-7} mol) in water (200 μL) at 5 °C; b) a mixture of L-adenosine (2.9×10^{-9} mol), DNA D-adenosine aptamer **X4** (1.08×10^{-7} mol based on monomeric negative charge or 2.9×10^{-9} mol of 37-mer), and polymer (1.08×10^{-7} mol based on charge unit) in water (200 μL) at 5 °C; and c) a mixture of D-adenosine (2.9×10^{-9} mol), DNA D-adenosine aptamer **X4** (1.08×10^{-7} mol based on monomeric negative charge or 2.9×10^{-9} mol of 37-mer), and polymer (1.08×10^{-7} mol based on charge unit) in water (200 μL) at 5 °C.

great potential of this new, rapid, specific, sensitive, and versatile electrostatic approach. This methodology does not require any chemical modification of the probes or the analytes and is based on conformational modifications of the conjugated backbone of a cationic poly(3-alkoxy-4-methylthiophene), when mixed with single-stranded DNA and an appropriate target. This electrostatic concept could therefore be adapted[11,12,16,21] to provide various inexpensive means for the rapid detection and identification of target nucleic acids, proteins, or other biological molecules as well as interesting tools for high-throughput screening for drug discovery.

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[1] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767.
[2] a) S. Tyagi, F. R. Kramer, *Nat. Biotechnol.* **1996**, *14*, 303; b) N. Hamaguchi, A. Ellington, M. Stanton, *Anal. Biochem.* **2001**, *294*, 126.
[3] a) T. A. Taton, C. A. Mirkin, R. L. Letsinger, *Science* **2000**, *289*, 1757; b) Y. C. Cao, R. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1503; c) B. Dubertret, M. Calame, A. J. Libchaber, *Nat. Biotechnol.* **2001**, *19*, 365; d) J. Wang, R. Polsky, A. Merkoci, K. L. Turner, *Langmuir* **2003**, *19*, 989; e) J.-M. Nam, C. S. Thaxton, C. A. Mirkin, *Science* **2003**, *301*, 1884; f) J.-M. Nam, S. I. Stoeva, C. A. Mirkin, *J. Am. Chem. Soc.* **2004**, *126*, 5932.
[4] a) T. Ihara, M. Nakayama, M. Murata, K. Nakano, M. Maeda, *Chem. Commun.* **1997**, 1609; b) F. Patolsky, A. Lichtenstein, I. Willner, *Nat. Biotechnol.* **2001**, *19*, 253; c) E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill, J. K. Barton, *Nat. Biotechnol.* **2000**, *18*, 1096.
[5] a) M. Leclerc, *Adv. Mater.* **1999**, *11*, 1491; b) D. T. McQuade, A. E. Pullen, T. M. Swager, *Chem. Rev.* **2000**, *100*, 2537.
[6] L. Chen, D. W. McBranch, H. L. Wang, R. Hegelson, F. Wudl, D. G. Whitten, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12287.
[7] T. Livache, A. Roget, E. Dejean, C. Barthet, G. Bidan, R. Teoule, *Nucleic Acids Res.* **1994**, *22*, 2915.
[8] M. Liss, B. Petersen, H. Wolf, E. Prohaska, *Anal. Chem.* **2002**, *74*, 4488.
[9] K. Enander, G. T. Dolphin, B. Liedberg, I. Lundström, L. Baltzer, *Chem. Eur. J.* **2004**, *10*, 2375.
[10] a) G. Daoust, M. Leclerc, *Macromolecules* **1991**, *24*, 455; b) C. Roux, M. Leclerc, *Macromolecules* **1992**, *25*, 2141; c) C. Roux, J. Y. Bergeron, M. Leclerc, *Makromol. Chem.* **1993**, *194*, 869; d) L. Robitaille, M. Leclerc, *Macromolecules* **1994**, *27*, 1847; e) I. Lévesque, M. Leclerc, *Chem. Mater.* **1996**, *8*, 2843; f) N. DiCesare, M. Belletête, G. Durocher, M. Leclerc, *Chem. Phys. Lett.* **1997**, *275*, 533; g) M. Leclerc, K. Faïd, *Adv. Mater.* **1997**, *9*, 1087; h) K. Faïd, M. Leclerc, *J. Am. Chem. Soc.* **1998**, *120*, 5274; i) S. Bernier, S. Garreau, M. Béra-Abérem, C. Gravel, M. Leclerc, *J. Am. Chem. Soc.* **2002**, *124*, 12463; j) H. A. Ho, M. Leclerc, *J. Am. Chem. Soc.* **2003**, *125*, 4412.
[11] H. A. Ho, M. Boissinot, M. G. Bergeron, G. Corbeil, K. Doré, D. Boudreau, M. Leclerc, *Angew. Chem.* **2002**, *114*, 1618; *Angew. Chem. Int. Ed.* **2002**, *41*, 1548.
[12] K. Doré, S. Dubus, H. A. Ho, I. Lévesque, M. Brunette, G. Corbeil, M. Boissinot, G. Boivin, M. G. Bergeron, D. Boudreau, M. Leclerc, *J. Am. Chem. Soc.* **2004**, *126*, 4240.
[13] C. Tuerk, L. Gold, *Science*, **1990**, *249*, 505.
[14] A. D. Ellington, J. Szostak, *Nature*, **1990**, *346*, 818.
[15] a) S. Basu, A. A. Szewczak, M. Cocco, S. A. Strobel, *J. Am. Chem. Soc.* **2000**, *122*, 3240; b) H. Ueyama, M. Takagi, S. Takenaka, *J. Am. Chem. Soc.* **2002**, *124*, 14286.
[16] H. A. Ho, M. Leclerc, *J. Am. Chem. Soc.* **2004**, *126*, 1384.
[17] L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564.
[18] K. Y. Wang, S. McCurdy, R. G. Shea, S. Swaminathan, P. H. Bolton, *Biochemistry* **1993**, *32*, 1899.
[19] K. Padmanabhan, K. P. Padmanablan, J. D. Ferrara, J. E. Sadler, A. J. Tulinsky, *J. Biol. Chem.* **1993**, *268*, 17651.
[20] a) D. A. Huizenga, J. W. Szostak, *Biochemistry*, **1995**, *34*, 656; b) M. Michaud, E. Jourdan, C. Ravelet, A. Villet, A. Ravel, C. Grosset, E. Peyrin, *Anal. Chem.* **2004**, *76*, 1015.
[21] a) B. S. Gaylord, A. J. Heeger, G. C. Bazan, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10954; b) B. S. Gaylord, A. J. Heeger, G. C. Bazan, *J. Am. Chem. Soc.* **2003**, *125*, 896; c) B. Liu, B. S. Gaylord, S. Wang, G. C. Bazan, *J. Am. Chem. Soc.* **2003**, *125*, 6705; d) K. P. R. Nilsson, O. Inganäs, *Nat. Mater.* **2003**, *2*, 419; e) S. Wang, G. C. Bazan, *Adv. Mater.* **2003**, *15*, 1425; f) K. P. R. Nilsson, J. Rydberg, L. Baltzer, O. Inganäs, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10170.

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