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Optical Detection of DNA and Proteins with Cationic Polythiophenes

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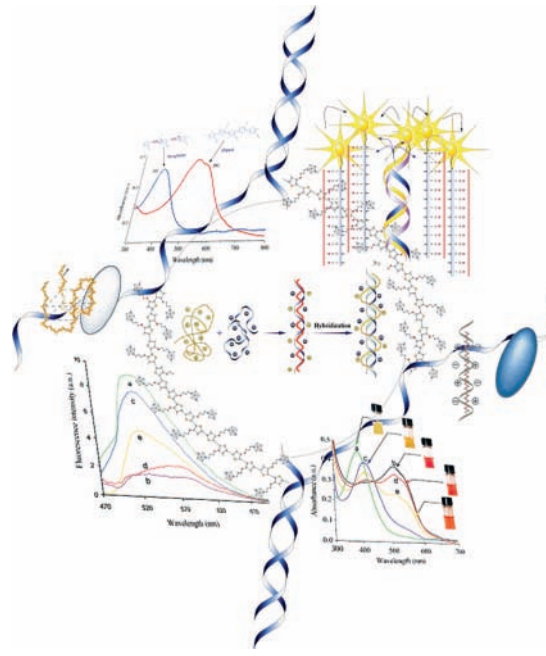
CONSPECTUS

In recent years, intense research has been carried out worldwide with the goal of developing simple, sensitive, and specific detection tools for biomedical applications. Along these lines, we reported in 2002 on cationic polythiophene derivatives able to provide ultrasensitive detection levels and the capability to distinguish perfect matches from oligonucleotides having as little as a single base mismatch. It was shown that the intrinsic fluorescence of the random-coil polymers quenches as a result of the planar, highly conjugated conformation adopted by the polymers when complexed with a single-strand DNA (ssDNA) capture probe but increases again after hybridization with the perfectly matched complementary strand. This change in fluorescence intensity is mainly due to a modification in the delocalization of π electrons along the carbon chain backbone that occurs when switching between the two conformations. Thus, by monitoring, via the change in fluorescence intensity, the hybridization of the complementary ssDNA target with the "duplex", one could detect as little as 220 complementary target molecules in a 150 μL sample volume (0.36 zmol) in less than 1 hour. Building on this initial concept, we then reported that tagging the DNA probe with a suitable fluorophore dramatically increases the detection sensitivity. This novel molecular system involves the self-assembly of aggregates of duplexes in solution, prior to the introduction of the target, which allows a highly efficient resonance energy transfer (RET) between a "donor" (being the complex formed of the DNA double helix and the polymer chain wrapped around it) and a large number of neighboring "acceptors" (the fluorophores attached to the DNA probes). The massive intrinsic signal amplification (fluorescence chain reaction or FCR) provided by this novel integrated molecular system allows the specific detection of as little as five dsDNA copies in a 3 mL sample volume in only 5 minutes, without the need for prior amplification of the target. Clearly, direct and reliable detection of DNA hybridization without prior PCR amplification or chemical tagging of the genetic target is now possible with this methodology. We have also shown that proteins can be detected following a similar strategy. Impressive results have also been reported by direct and specific staining of targeted proteins. All these features have recently allowed the development of responsive polymeric supports for the detection of DNA and proteins. All these assays that do not require any chemical manipulation of the biological targets or sophisticated experimental procedures should soon lead to major advances in genomics and proteomics.



Introduction

The fast-growing research fields related to genomics and proteomics have drawn much interest in the development of novel tools for the rapid, simple, and specific electrical or optical detection of biomolecules. In parallel, sensors based on conjugated polymers have been shown to be very sensitive to very minor perturbations due to amplification by a collective system response and therefore offer a remarkable advantage over small-molecule-based sensors.[1−3] It was then

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thought that by combining both types of macromolecules (the biological ones with synthetic conjugated polymers), new hybrid biosensing elements could emerge clearly out of the traditional toolbox of biochemists, molecular biologists, and physicians. Colorimetric detection of proteins with the use of ligand-functionalized polydiacetylenes was pioneered by Charych,[4] whereas first attempts for DNA detection were carried out by Garnier[5] by covalently attaching a single-stranded (ss) DNA probe onto an electroactive polypyrrole backbone. In both cases, the binding of the target molecules implied a change of the conformation of the conjugated polymer, which resulted in a modification of its optical or electrochemical properties. These results were promising, but it was clear that the utilization of water-soluble conjugated polymers would be more useful for *in situ* detection. We therefore tried to bind various biological ligands onto ionic water-soluble conjugated polymers but with limited success in terms of detection.[6–8] Then, we realized that DNA is itself an ionic polymer and could therefore serve as the template for the attachment of the conjugated polymer. Moreover, a careful analysis of the general behavior of the polyelectrolytes taught us that any anionic polyelectrolytes (e.g., DNA) can form strong electrostatic complexes with a cationic polyelectrolyte, neutral complexes (coacervates) being known to form stable aggregates.[9] On the basis of this knowledge, we decided to develop new cationic conjugated polymers. Our first choice was devoted to polythiophenes since some polythiophenes are known to exhibit interesting chromic features (change of color induced by a conformational change of the conjugated backbone, see Figure 1) in presence of different stimuli.[10] In many cases, such chromic effects also have a strong influence on the fluorescence properties of these conjugated polymers. As shown in Scheme 1, some chromic, cationic, water-soluble polythiophenes are now available.[11,12] By mixing ssDNA with such cationic polythiophenes, it was believed that this polymeric assembly would combine an optical (chromic) transducer together with a DNA probe. This Account will therefore focus on the recent and quite impressive progress of the use of such hybrid ssDNA/cationic polythiophene complexes as biosensors. This research area was certainly not among the first ones to be anticipated for conjugated polymers but seems to be now one of their most promising fields of applications.

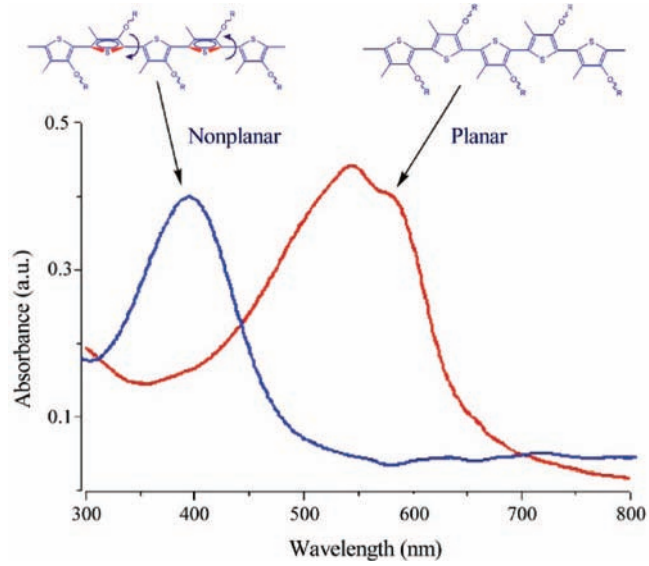
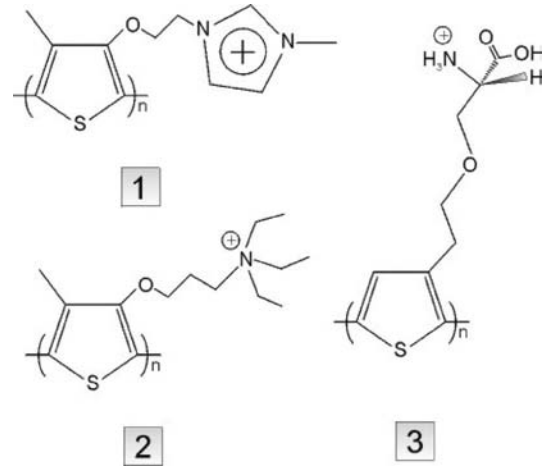


FIGURE 1. Conformations and corresponding UV–visible absorption spectra of a chromic polythiophene.

SCHEME 1. Chemical Structure of Some Cationic Chromic Polythiophenes



Detection of DNA

All living organisms contain DNA, with the exception of some viruses, which have RNA genomes. Thus, it could be of first importance to develop a simple and quite general method of sequence-specific detection of DNA for genotyping or for diagnosing infections. Moreover, an assay that does not require any chemical manipulation of nucleic acids or complex reaction mixtures would be greatly advantageous. To reach this goal and as mentioned above, we designed an approach based on the utilization of cationic, chromic polythiophene transducers.[11] For instance, at 55 °C, an aqueous solution of the cationic polymer **1** is yellow with an absorption wavelength near 400 nm (Figure 2a). This absorption maximum at a short wavelength is related to a random-coil conformation of the polythiophene derivative, any twist of the conjugated backbone leading to a decrease in the effective conjugation length. Upon addition of 1.0 equiv (on a monomer unit basis) of capture probe **X1** (5′-CATGATTGAACCATCCACCA-3′), the mixture becomes red ($\lambda_{max} = 527$ nm) (Figure 2b), because of the formation of a so-called duplex between the poly-

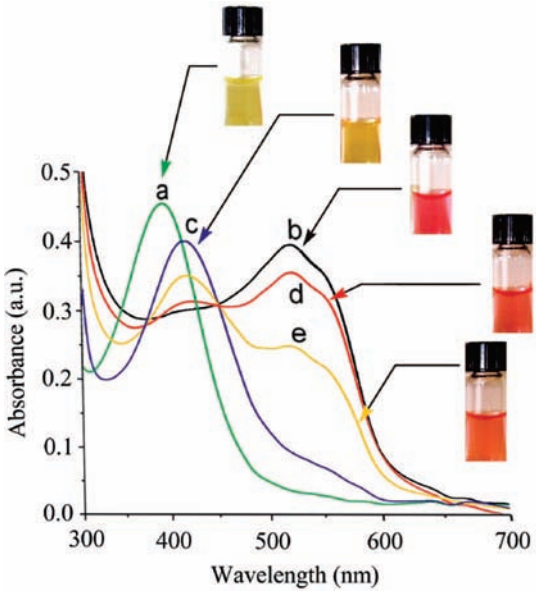


FIGURE 2. UV–vis absorption spectrum of a solution (7.9×10^{-5} M, on a monomeric unit basis) of (a) polymer **1**, (b) polymer **1**/**X1** duplex, (c) polymer **1**/**X1**/**Y1** perfect match triplex, (d) polymer **1**/**X1**/**Y2** mixture with two mismatches, and (e) polymer **1**/**X1**/**Y3** mixture with one mismatch after 5 min of mixing at 55 °C in 0.1 M NaCl.

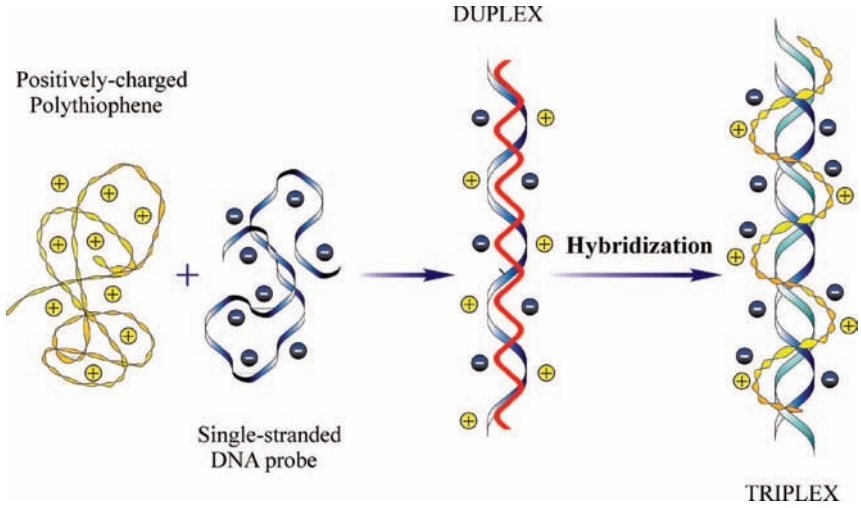
thiophene and the oligonucleotide probe (Scheme 2). It is now known that such duplexes are not isolated species but rather form aggregates.[13] Remarkably, after 5 min of mixing in the presence of 1.0 equiv of the complementary oligonucleotide **Y1** (3′-GTACTAACTTGGTAGGTGGT-5′), the solution becomes yellow ($\lambda_{max} = 420$ nm) (Figure 2c); presumably caused by the formation of a new complex termed a triplex (Scheme 2), obtained by complexation of the cationic polymer with the hybridized nucleic acids. On the basis of previous studies performed on chromic polythiophenes and as described in Scheme 2, it is believed that these colorimetric effects are made possible because of a different conformation of the conjugated polymer in the duplex form (planar and highly conjugated) compared with that observed in the triplex form (nonplanar, slightly conjugated). Interestingly, it seems that electrostatic interactions between the ssDNA probe and the polythiophene optical transducer do not interfere with the H-bonding of the two cDNA strands. As it was found later,[14] the cationic polythiophene is indeed favoring DNA hybrization by screening the electrostatic repulsion between the two complementary negatively charged ssDNAs.

In order to verify the specificity of this polymeric optical transducer, different oligonucleotides (20-mers differing by only 1 or 2 bases) were investigated. A very distinct and reproducible UV–vis absorption spectrum is observed in the case of oligonucleotide target with two mismatches (**Y2**, 3′-GTACTAACTTCGAAGGTGGT-5′) (Figure 2d) when compared with perfect hybridization (Figure 2c). It is also possible to discriminate the sequence with one mismatch (**Y3**, 3′-GTACTAACTTCGTAGGTGGT-5′) (Figure 2e). It is interesting to note that spectra of duplexes and triplexes show an isosbestic point, indicating the presence of only two distinct conformational structures for the polymeric transducer. The detection limit of this colorimetric method is about 1×10^{13} molecules of oligonucleotide (20-mers) in a total volume of 100 μL (which corresponds to a concentration of 2×10^{-7} M). Very similar results[11,12] have been obtained with chromic polythiophenes **2** and **3** and with various DNA sequences, indicating the generalization of this electrostatic method.

A fluorometric detection of oligonucleotide hybridization is also possible since the fluorescence of polythiophenes is quenched in the planar, aggregated form.[10] At 55 °C, the yellow form of polymer **1** is fluorescent (quantum yield of 0.03 with a maximum of emission at 530 nm) (Figure 3a). Upon addition of 1.0 equiv of a negatively charged oligonucleotide probe (**X1**), the fluorescence intensity decreases and the maximum of emission is slightly red-shifted (Figure 3b). When hybridization with the complementary strand **Y1** takes place, the formation of a polymeric triplex leads to about a 5-fold increase in fluorescence intensity (Figure 3c). Interestingly, upon addition of the oligonucleotide target having two mismatches (**Y2**), the fluorescence intensity is weak and not significantly modified (Figure 3d). It is even possible to distinguish oligonucleotides with one mismatch (Figure 3e). Once again, very similar results have been obtained with polythiophenes **2** and **3**.[11,12] With this method, it is possible to detect the presence of as few as 3×10^6 molecules of the perfect complementary oligonucleotide (20-mers) in a volume of 200 μL (this is a concentration of 2×10^{-14} M). Moreover, by using a custom-built fluorimeter based on a high-intensity blue diode (as the excitation source) and a nondispersive interference filter, a few hundred copies of either DNA or RNA can be specifically detected.[15] Interestingly, similar selectivity and sensitivity can be obtained when the fluorescent polymeric hybridization transducer is grafted on magnetic microbeads, allowing the possibility of performing both preconcentration and detection steps simultaneously.[16] Other studies have reported similar optical detection concepts based on electrostatic interactions between a cationic fluorescent polymeric transducer, chromophore-labeled probes, and negatively charged DNA targets.[17–19]

More recently, a combination of electrostatic interactions, chromism, and a Förster resonance energy transfer (FRET) mechanism has led to a novel fluorescence signal amplifica-

SCHEME 2. Schematic Description of the Formation Polythiophene/Single-Stranded Nucleic Acid Duplex and Polythiophene/Hybridized Nucleic Acid Triplex



tion detection. This technique was described as "superlighting" or "fluorescence chain reaction" (FCR)[14] mechanism (see Scheme 3) and consists of preparing stoichiometric (neutral) duplexes made from a chromic cationic polythiophene (e.g., polymer **1**, which serves as a potential donor) and oligonucleotide capture probes labeled with a fluorophore (acceptor). The choice of the acceptor (e.g., Alexa Fluor 546) was obviously driven by the need to get a good overlap between its excitation spectrum and the emission spectrum of the polymer donor together with an excellent fluorescence quantum yield for the acceptor. In the starting conditions, there is formation of nanoaggregates of duplexes[13] without any significant FRET mechanism between the quenched polymer donor and the acceptor. Upon hybridization with the perfect cDNA target, the cationic polythiophene becomes more fluorescent, which should lead to efficient energy transfer to the neighboring acceptor. In principle, since the fluorescence quantum yield of Alexa Fluor 546 is about 30 times higher than that of the cationic polythiophene, a similar increase of the sensitivity was expected. However, this new strategy has led to an improvement in detection sensitivity by a factor of 4000 and to the detection of as few as five copies of target DNA. It seems that the increase of sensitivity is made possible by efficient and direct and indirect (through homotransfers between aggregated Alexa Fluor chromophores) ultrafast energy transfers to many neighboring acceptors attached to the aggregated probes.[20] Interestingly, a large excess of duplex probes (i.e., 10^9 copies of aggregated capture probes for detecting one target molecule) can be utilized, allowing not only a large amplification of the optical detection but also increasing the hybridization speed. To verify the applicability of the method, various kinds of genetic materials were tested. For instance, it has been possible to work with the entire human genome and to specifically detect a few copies of a human gene.[14]

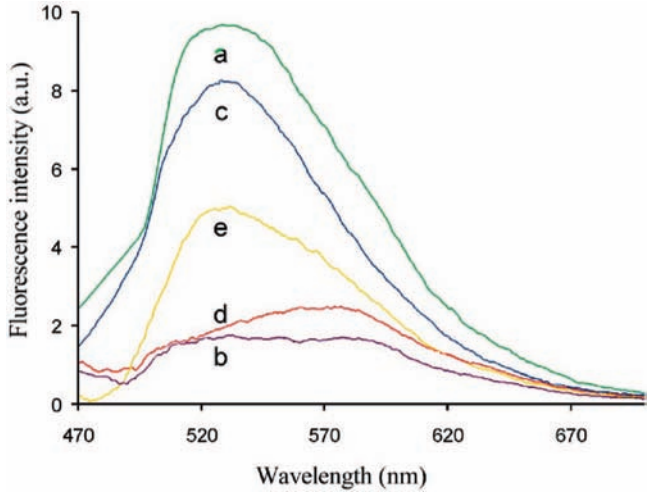
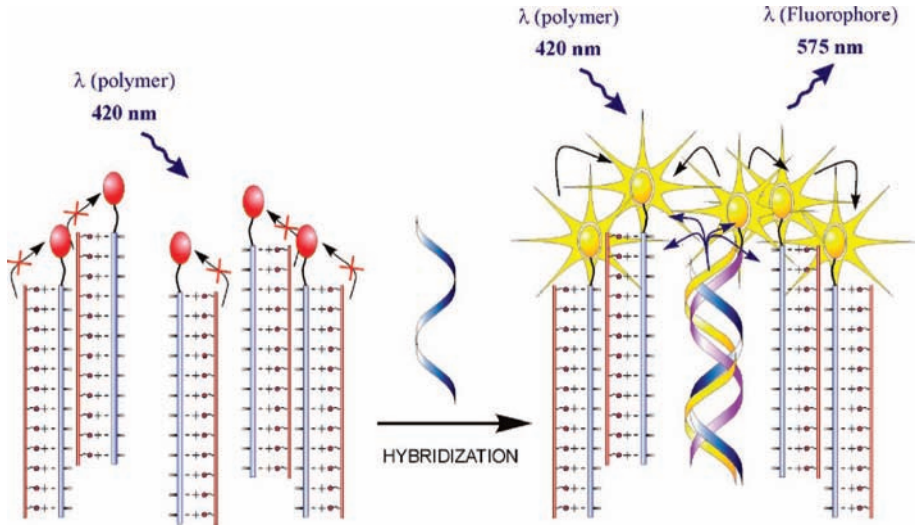


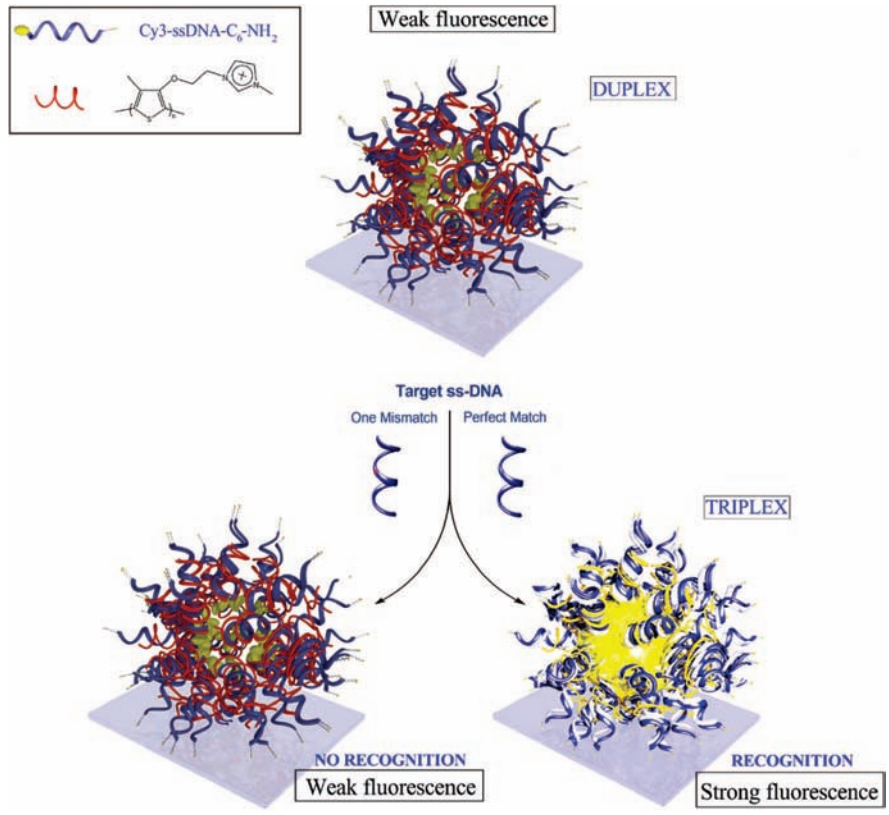
FIGURE 3. Fluorescence spectrum of a solution (2.0×10^{-7} M, on a monomeric unit basis) of (a) polymer **1**, (b) polymer **1**/**X1** duplex, (c) polymer **1**/**X1**/**Y1** perfect match triplex, (d) polymer **1**/**X1**/**Y2** mixture with two mismatches, and (e) polymer **1**/**X1**/**Y3** mixture with one mismatch, at 55 °C in 0.1 M NaCl.

On the other hand, future devices will certainly involve multiparametric analyses and probably, the utilization of microarrays on various solid supports. Indeed, biochips[21,22] are certainly one of the most promising approaches for medical applications since many diagnostic tests can be performed simultaneously from the same analyte. However, most of the present techniques rely on a fluorescent tagging of the analytes to make the detection. In this regard, some recent work on conjugated polymers using a fluorometric platform has revealed interesting and promising features.[12,23,24] For instance, it has been possible to put the luminescent poly-

SCHEME 3. Schematic Description of the Proposed Signal Amplification Detection Mechanism Based on the Conformation Change of Cationic Polythiophene and Energy Transfers for Ultrasensitive, Selective, and Rapid DNA Detection



SCHEME 4. Schematic Description of the Specific Recognition of Target ssDNA by Duplex Aggregates onto Glass Slides with Visualization of Signal Amplification Detection Mechanism Based on the Conformational Change of Cationic Polythiophene and Fast Energy Transfers within the Micelle



mer **3** on a hydrogel surface to study the hybridization of nucleic acids.[12]

The limit of the detection can be improved significantly by adapting the FCR mechanism for detection onto solid supports.[25] The novel responsive polymeric biochips were designed as described in Scheme 4. First, stoichiometric complexes (duplexes) were prepared by mixing a cationic water-soluble polythiophene transducer with a 3′-Cy3-labeled ssDNA capture probe. As previously mentioned, these stoichiometric complexes tend to form aggregates in aqueous solutions. In order to allow the covalent binding of these aggregates onto surface-treated glass slides, an amine group was also inserted at the 5′-end of the ssDNA cap-

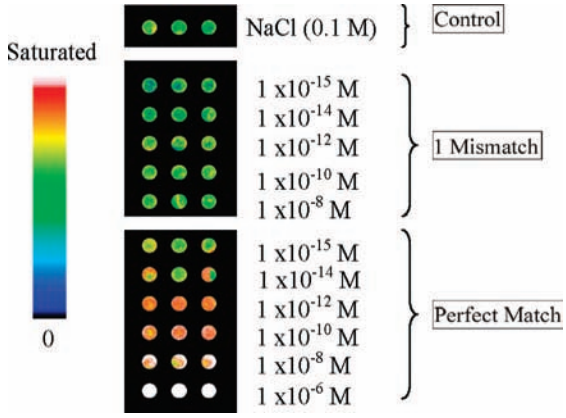


FIGURE 4. Fluorometric detection of hybridization of 20-mer DNA oligomers on arrays. Fluorescence intensity was measured at 570 nm with excitation at 408 nm.

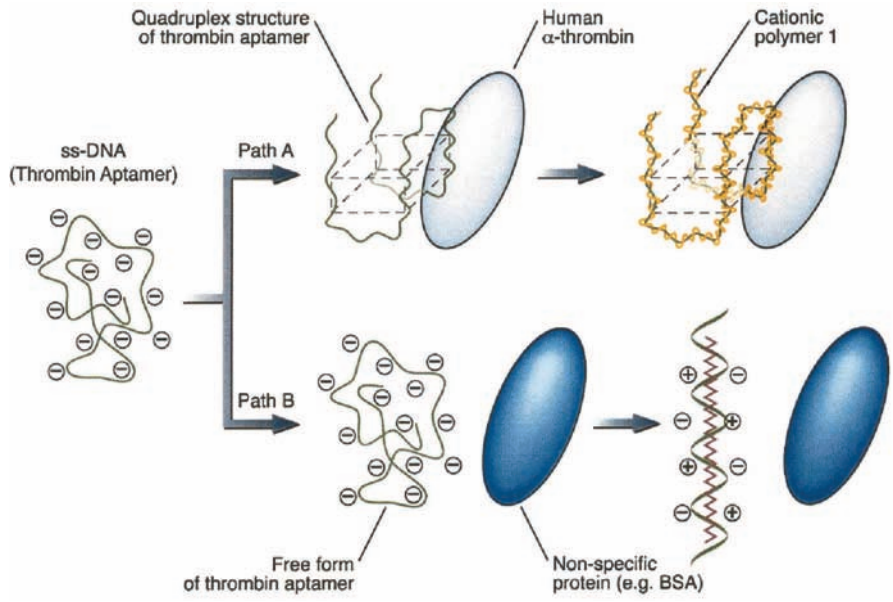
ture probes. Upon spotting, these micelles made of hybrid polythiophene/ssDNA (5′-NH_2-C_6-CATGATTGAACCATCCACCA-Cy3-3′) complexes were therefore bound onto glass slides (Scheme 4). The average aggregate diameter is around 200−250 nm, while the height is around 20−30 nm. The diameter of the spots is about 1.5−1.7 mm, each including about 1×10^{12} probes. Figure 4 exhibits the fluorescence intensity of the duplex after hybridization (formation of triplex) with the perfect complementary target oligonucleotide (3′-GTACTAACTTGGTAGGTGGT-5′) and a target having one mismatch (3′-GTACTAACTTCGTAGGTGGT-5′) for concentrations ranging from 1×10^{-6} to 1×10^{-15} M. The fluorescence signal, measured at 570 nm, shows a clear contrast between perfect complementary targets and those having one mismatch at concentrations greater than 10^{-14} M (Figure 4). Upon specific hybridization, it seems that the polythiophene undergoes a conformational change that makes it fluorescent upon excitation at 408 nm. The emission spectrum of the polythiophene derivative overlaps well with the excitation spectrum of the Cy3 fluorophore, allowing efficient FRET within the aggregates. Measurements at very low concentrations permit us to calculate a limit of detection around 5×10^{-16} M for a perfect complementary target oligonucleotide in a volume of 400 nL (corresponding to ca. 300 copies). The detection sensitivity obtained with the system described above is improved by a factor of ca. 1000 over that obtained using an unlabeled duplex (5′-NH_2-C_6-CATGATTGAACCATCCACCA-3′ with cationic polymer). Given the fact that the fluorescence quantum yield of the Cy3 fluorophore is 5 times higher than the polythiophene transducer, this seems to imply that, in addition to the FRET phenomenon, these molecular structures exhibit a significant amplification of the detection through a fast and efficient energy transfer (FCR mechanism) to many neighboring chromophores within the micelles.

Detection of Proteins

It is important to know that ssDNA not only binds to complementary ssDNA but can be a molecular recognition element for ions, small molecules, or even proteins. Indeed, artificial nucleic acid ligands (aptamers) have recently attracted much interest due to their capability to bind various molecules with high affinity and specificity.[26−32] Aptamers are usually isolated from combinatorial libraries of synthetic nucleic acids by an iterative process of adsorption, recovery, and amplification (coined SELEX for *systematic evolution of ligands by exponential procedure*). However, in most cases, the binding of a protein to its aptamer has been detected by tagging the target or the probe. It is worth noting that labeling with various functional groups may even compromise the binding properties of the aptamers. To solve this problem, we utilized a water-soluble cationic polythiophene as a "polymeric stain" that can specifically transduce the binding of an aptamer to its target into a clear optical (colorimetric or fluorometric) signal. This simple, rapid, sensitive, and selective methodology does not require any chemical modification on the probes or the analytes and is, once again, based on different electrostatic interactions and conformational structures between a cationic poly(3-alkoxy-4-methylthiophene) derivative and anionic single-stranded oligonucleotides as specific aptamer for a given protein. For instance, the detection of human α-thrombin is made possible by combining polymer **1** and aptamer **X2** (5′-GGTTGGTGTGGTTGG-3′), which is known to be a specific binding sequence of this protein.[33] A conformational change occurs when the aptamer binds to the thrombin molecule. Both NMR[34] and X-ray diffraction studies[35] have revealed that the aptamer adopts a compact unimolecular quadruplex structure with two G-quartets. Therefore, as shown in Scheme 5, 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 between cationic polymer **1**, aptamer **X2**, and thrombin has an orange color.[36] The thrombin promotes the formation of the quadruplex form of thrombin aptamer, and the cationic polythiophene wraps this quadruplex structure, which seems to partially hinder the aggregation and planarization (which would lead to a red-violet color) of the positively charged polymer in the presence of ssDNA **X2** (Scheme 5, 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 detec-

SCHEME 5. Schematic Description of the Specific Recognition of Human α-Thrombin Using ssDNA Thrombin Aptamer and Cationic Polythiophene



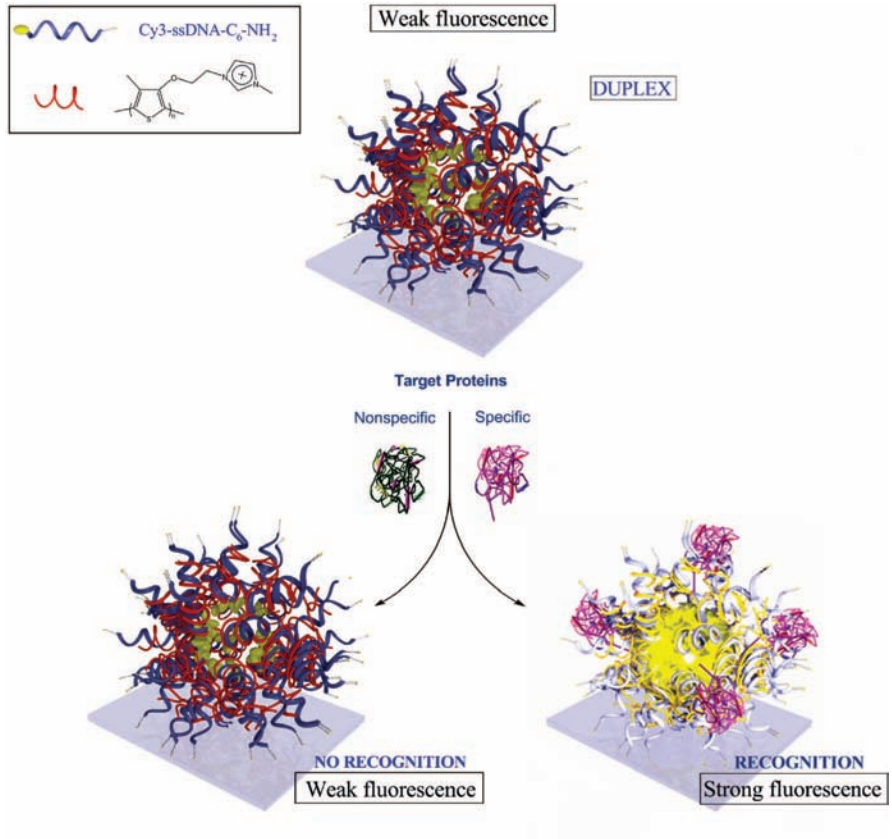
tion, two control experiments using a nonbinding sequence (**X3**, 5′-GGTGGTGGTTGTGGT-3′) and bovine serum albumin (BSA) were carried out under identical conditions. In both cases, an important red shift toward lower energy (maximum of absorption at 505 nm) was observed, and the color of these solutions was red-violet, a typical color of the planar and highly conjugated structure of the polythiophene backbone when mixed with unfolded ssDNA (Scheme 5, path B).

Interestingly, the adaptation of the FCR approach can also be applied for detecting proteins on solid supports.[37] Stoichiometric complexes (duplexes) were therefore prepared by mixing the polythiophene optical transducer with a 3′-Cy3-labeled ssDNA aptamer. In order to allow the covalent binding of these aggregated duplexes onto surface-treated glass slides, an amine group was also inserted at the 5′-end of the ssDNA capture probes. Upon spotting, these aggregates made of hybrid polythiophene/ssDNA complexes were therefore bound onto glass slides (Scheme 6). Then, for the specific detection of human thrombin, the following strategy was designed: first, P_1 sequence (5′-NH_2-C_6-GGTTGGTGTGGTTGG-Cy3-3′) or P_2 (5′-NH_2-C_6-GGTGGTGGTTGTGGT-Cy3-3′) was mixed with cationic polythiophene in order to form stoichiometric duplexes. P_1 sequence is specific to thrombin whereas P_2 is a labeled sequence that does not bind to thrombin.[33] It has been observed that in presence of the thrombin, the spots having the hybrid labeled aptamer P_1/polythiophene complexes (binding sequence) show a significant increase of the fluorescence. Three control experiments were done to verify the specificity of the detection. Two proteins, BSA and immunoglobulin E (IgE), were used in the same conditions, and fluorescence intensities remained quite low with both proteins.[37] This reveals an excellent specificity of the detection with respect to the target. The use of a nonbinding sequence (P_2) for human thrombin confirms also the specificity of the detection with respect to the probe.

Experiments probing the fluorescence intensity as a function of concentration of the proteins in the presence of polythiophene/P1 duplexes revealed a limit of detection of about 1.5×10^7 molecules for human thrombin (i.e., 6.2×10^{-11} M in 0.4 μL) together with a very good specificity.[37] The amplification of the detection through the FCR scheme was verified by the use of a unlabeled probe under the same conditions. The sensitivity is about 1000 times lower in the case of unlabeled probes when compared with labeled probes. These results are in agreement with our previous results on DNA for which the amplification of the fluorescence signal was assumed to result not only from FRET but also from FCR. It therefore seems that the resulting fluorescent folded polythiophene rapidly transfers[20] its energy to nearby Cy3 moieties, which seems to be combined with ultrafast and efficient homotransfers within the aggregated Cy3 fluorophores.

It is also worth noting that cationic fluorescent polythiophenes can directly report conformational changes occurring in proteins or different forms of the same protein.[38–42] This is a very important topic since many diseases are attributed to conformational changes in proteins. As an example, polymer **3** has been utilized to optically detect calcium-induced conformational changes in calmodulin and calmodulin–

SCHEME 6. Description of the Specific Detection of Target Proteins by Polythiophene/DNA Aptamer Duplex Aggregates on Glass Slides.



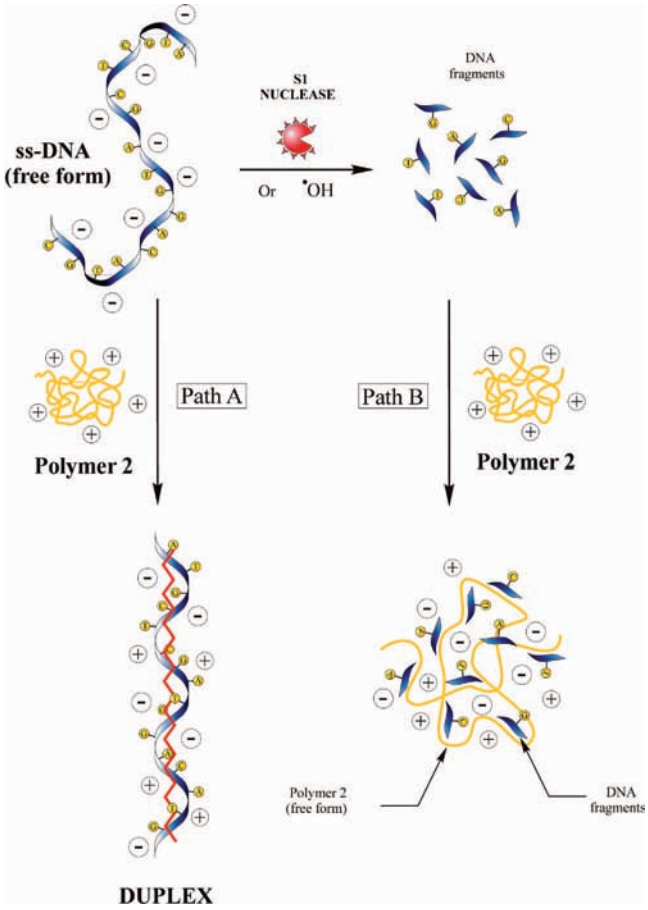
calcineurin interactions.[39] These novel conformation-sensitive optical methods have also been successfully applied to the detection of amyloid fibrils in chicken lysozyme.[40] Such characteristic fibrillar assemblies usually appear when the native form of a protein is destabilized by a high temperature or an acidic medium. Initial experiments were carried out *in vitro* but these chromic polyelectrolytes can also be used as an amyloid-specific probe in histological staining of tissue samples.[41,42]

Detection of Small Molecules of Biological Interest

Building on the same general concepts, an adenosine triphosphate (ATP) sensing method[43] was also recently described. Indeed, exposure of a cationic water-soluble polythiophene (similar to polymer **2**) to increasing concentrations of ATP in water led to a pronounced red shift in the absorbance spectra, changing the solution color from yellow to pink-red. The changes in the absorbance of the polythiophene upon exposure to ATP were due to formation of an electrostatic complex between the cationic polymer and anionic ATP, which led to increased planarity of the conjugated polymer backbone. This method was selective for ATP over other anions such as Cl^-, HPO_4^{2-}, and HCO_3^{2-}, adenosine diphosphate (ADP), adenosine monophosphate (AMP), or uridine triphosphate (UTP). In addition to red-shifted absorbance, the addition of ATP to an aqueous solution of the cationic polythiophene also resulted in fluorescence quenching. The fluorescence response was most sensitive to ATP over ADP or AMP. The detection limit for ATP, based upon fluorescence quenching, was reported to be approximately 10^{-8} M.

Moreover, a new method has been recently developed for the label-free, convenient, and real-time monitoring of the cleavage of single-stranded DNA by single-strand specific S1 nuclease and hydroxyl radical[44] based on polymer **2**. The cationic polymer **2** can form an interpolyelectrolyte complex with single-stranded DNA, called duplex, through electrostatic interaction in which polymer **2** adopts a highly conjugated or planar conformation and thus the positively charged polymer exhibits a relatively red-shifted absorption. In contrast, when ssDNA is digested by S1 nuclease or hydroxyl radical into small fragments, the polymer **2**/ssDNA duplex is not formed (Scheme 7). In this case, polymer **2** remains in a random-coil or nonconjugated conformation and shows a relatively short absorption wavelength. The nuclease digestion or oxidative damage by hydroxyl radical of DNA can be observed by UV–visible absorption spectroscopy or just visualized by naked eye.

SCHEME 7. Schematic Representation of the Assay for Nuclease



Conclusion and Outlook

Most of the current methods for the detection of DNA are based on an enzymatic amplification (polymerase chain reaction or PCR) of the targets combined with small-molecule optical reporters such as Taqman probes[45] and molecular beacons.[46] Other optical reporters are currently developed using fluorescent molecules such as fluorene,[47,48] fluorescein,[49] and oligothiophenes.[50–52] In most cases, specificity is excellent, but the sensitivity is not sufficient to avoid the PCR amplification step for DNA materials. Interestingly, as reported in this Account, by combining polythiophenes, biological ligands, and optical amplification tools (light harvesting, FRET, FCR, etc.), impressive biosensors have emerged. Indeed, all data presented here have clearly demonstrated the great potential of optical biosensors based on hybrid DNA/polythiophene electrostatic complexes, in particular for PCR-free detection of DNA. For instance, this simple and rapid methodology has enabled the specific and direct detection of a few copies of genomic materials. These remarkable results have been achieved by the use of a novel amplification mechanism (fluorescence chain reaction or FCR) that is based on the "turn-on" of a polythiophene donor and the resulting efficient and ultrafast energy transfer to many neighboring highly fluorescent acceptors attached to aggregated ssDNA probes. Impressive results have also been reported for proteomic applications by using ssDNA aptamer probes or by direct staining of the targeted protein. However, in most cases, specificity and sensitivity were observed with highly purified samples, and it will be important to further test the robustness and reproducibility of such devices with complex samples. In this regard, recent results obtained on microbeads or on arrays as well as demonstrations of specific staining inside tissue sections are extremely promising, and we therefore anticipate a very bright future for this relatively new field of applications for conjugated polymers.

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BIOGRAPHICAL INFORMATION

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FOOTNOTES

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