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Cationic Conjugated Polymers for Optical Detection of DNA Methylation, Lesions, and Single Nucleotide Polymorphisms

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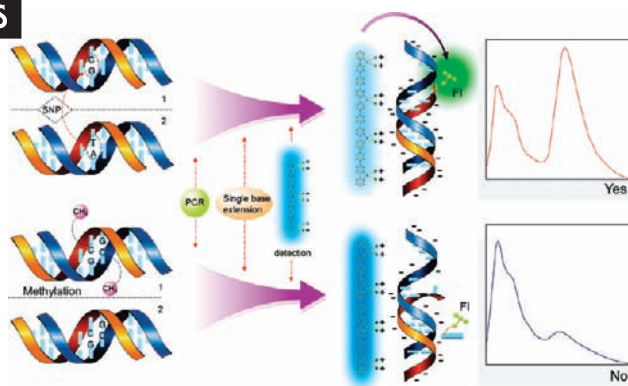
CONSPECTUS



Simple, rapid, and sensitive technologies to detect nucleic acid modifications have important applications in genetic analysis, clinical diagnosis, and molecular biology. Because genetic modifications such as single nucleotide polymorphisms (SNP), DNA methylation, and other lesions can serve as hallmarks of human disease, interest in such methods has increased in recent years. This Account describes a new strategy for the optical detection of these DNA targets using cationic conjugated polymers (CCPs).

Because of their unique signal amplification properties, researchers have extensively investigated conjugated polymers as optical transducers in highly sensitive biosensors. Recently, we have shown that cationic polyfluorene can detect SNPs within the DNA of clinical samples. When we incorporated deoxyguanosine triphosphate (dGTP-Fl) into the DNA chain at an SNP site where the target/probe pair is complementary, we observed higher fluorescence resonance energy transfer (FRET) efficiency between cationic polyfluorene and fluorescein label on the dGTP. By monitoring the change in emission intensity of cationic polyfluorene or fluorescein, we identified the homozygous or heterozygous SNP. The high sensitivity of this assay results from the 10-fold enhancement of fluorescein emission intensity by the FRET from polyfluorene. This method can detect allele frequencies (the proportion of all copies of a gene that is made up of a particular gene variant) as low as 2%. Using this novel method, we clearly discriminated among the SNP genotypes of 76 individuals of Chinese ancestry.

Improving on this initial system, we designed a method for multicolor and one-tube SNP genotyping assays based on cationic polyfluorene using fluorescein-labeled deoxyuridine triphosphate (dUTP-Fl) and Cy3-labeled deoxycytidine triphosphate (dCTP-Cy3) in extension reactions. We also developed a one-step method for direct detection of SNP genotypes from genomic DNA by combining allele-specific PCR with CCPs. In 2008, we developed a new method for DNA methylation detection based on single base extension reaction and CCPs. Treatment of DNA with bisulfite followed by PCR amplification converts unmethylated DNA into a C/T polymorphism, which allows us to characterize the methylation status of the target DNA. Furthermore, we used CCPs to detect DNA lesions caused by ultraviolet light irradiation for the first time. By monitoring the color change of cationic polythiophene before and after DNA cleavage, we also detected oxidative damage to DNA by hydroxyl radical.

These CCP-based new assays avoid primer labeling, cumbersome workups, and sophisticated instruments, leading to simpler procedures and improved sensitivity. We expect that these features could lead to major advances in human disease diagnostics and genomic study in the near future.

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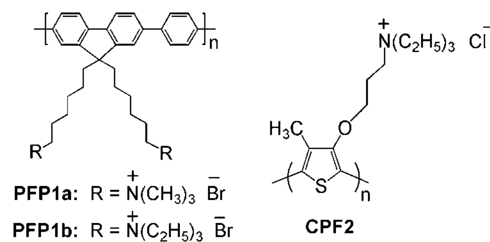
Introduction

In recent years, simple, rapid, and sensitive technologies for the detection of human disorder-related nucleic acids are receiving more and more interest due to their important applications in genetic analysis, clinical diagnosis, and molecular biology.[1] Single nucleotide polymorphism (SNP) is the most common sequence variation in the human genome. SNPs can be used as genetic markers for mapping genes, defining population structure, and performing gene association studies, as well as a fundamental tool in drug discovery and identification of genetic and inherited diseases.[2−5] Not only genetic but also epigenetic changes might be responsible for cancer initiation and progression.[6] DNA methylation is an important component of epigenetic regulation that can be used as a biomarker for early cancer diagnosis and determination of specific cancer types.[6−10] DNA damage has received much attention due to its involvement in mutagenesis, carcinogenesis, and aging.[11] Simple, sensitive, and rapid detection methods for DNA methylation, lesions, and SNPs are increasingly demanded.

Conjugated polymers (CPs) featured a delocalized electronic structure. Excitation energy along the polymer backbone can transfer to an acceptor by electron transfer or fluorescence resonance energy transfer (FRET), resulting in the superquenching of CPs or the amplification of the fluorescence signal of the acceptor.[12−14] CPs have been utilized in sensitive detection of biomacromolecules, such as nucleic acids and proteins.[15−27] Water-soluble conjugated polymers typically have charged functionalities on the side chain.[13,18] Cationic conjugated polymers (CCPs) provide for a convenient tool to interface with negatively charged DNA probes.[14,15] Of the various water-soluble CPs reported to date for biosensors, cationic poly(fluorene)[14,19,28] and polythiophene derivatives[15,29] initiated, respectively, by the Bazan group and the Leclerc group have been widely used in DNA sensing (see their typical chemical structures in Scheme 1). It is noted that the fluorescent properties of PFP1a and PFP1b have no noticeable differences. The unique properties of cationic poly(fluorene)s include facile substitution at the fluorene C9 position, good chemical and thermal stability, and high fluorescence quantum yields in water.[30] Aqueous solutions of cationic poly(fluorene) derivatives typically display absorption maxima at approximately 380 nm and emission maxima at 420 nm with fluorescence quantum efficiencies in the range of 20% to 40%.[14,19] Cationic polyfluorenes can transduce the hybridization event of single-stranded probe and target DNA to fluorescent signal by FRET with high sensitivity.[14,16] Binding of

SCHEME 1. Chemical Structures of Cationic Polyfluorene and Polythiophene Derivatives

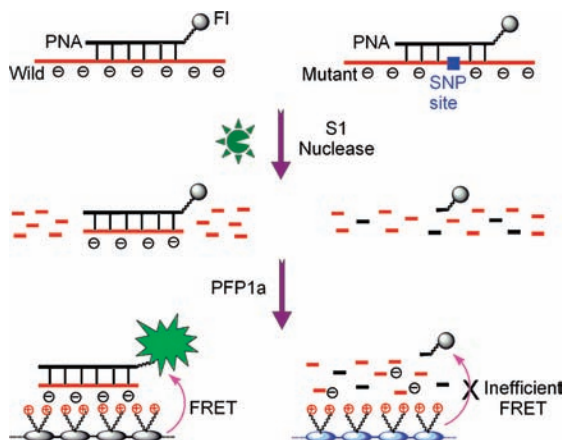


cationic polythiophene derivatives to DNA tunes their conformations, which in turn significantly changes optical properties of polymers. They have been successfully utilized for DNA detection with very high sensitivity.[15,29] We discovered that CCPs can be readily used as optical transducers for simple, rapid, sensitive, and homogeneous detections of SNP genotyping,[31−34] DNA methylation,[35,36] and DNA damage.[37,38] In this Account, the results of our recent studies aimed at the development of optical detection for human disorder-related nucleic acids using CCPs are discussed in detail.

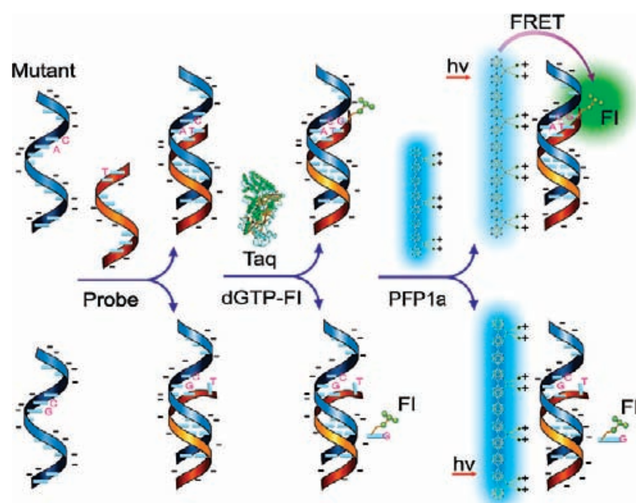
CCP-Based SNP Detection

In 2003, the Whitten group developed a microsphere-based DNA hybridization by using water-soluble poly(phenylene ethynylene) and a peptide nucleic acid (PNA) probe.[39] In 2005, the Fan group developed magnetically assisted DNA assays using a cationic polyfluorene derivative and a DNA probe.[40] These methods showed good potential for SNP detection. In 2005, the Bazan group developed a new fluorescence method for SNP detection by using cationic polyfluorene (PFP1a), fluorescein-labeled peptide nucleic acid (PNA-Fl), and S1 nuclease.[41] The fluorescein label in PNA, with absorption maximum at 480 nm, was chosen since its absorption spectra overlaps with the emission spectra of PFP1a. Irradiation at 380 nm selectively excites PFP1a, and fluorescence resonance energy transfer (FRET) from PFP1a to fluorescein is favored.[42,43] The hybridization between PNA probe and wild-type target is more stable than PNA probe and mutant-type target. After subsequent treatment with S1 nuclease, PFP1a electrostatically associates with the remaining PNA/DNA duplex, leading to sensitized emission of the fluorescein via FRET from PFP1a. The FRET signaling is achieved only for the wild-type DNA but not for mutant sequence containing the SNP site (Scheme 2). The addition of nonionic surfactant to the PFP1a/PNA system can enhance the sensitivity due to the increase of PFP1a quantum yield.[44] In these hybridization-based methods, the robustness of allelic discrimination

SCHEME 2. Schematic Representation of SNP Detection Based on CCP, PNA, and S1 Nuclease



SCHEME 3. Schematic Representation of SNP Genotyping Based on CCP and Allelic-Specific Primer Extension



depends on complex probe design, and expensive PNA probes are required, which will restrict the application of these methods.

In 2007, we initiated a new CCP-based SNP detection method, in which allelic specific primer extension is chosen as the allelic discrimination strategy and fluorophore-labeled nucleotide is incorporated into DNA during the extension reaction.[31] Primer extension is a very robust allelic discrimination strategy,[45] in which an allelic-specific DNA probe can be extended by DNA polymerase only when the 3′-end of the probe is perfectly complementary to the target at the polymorphism site. As shown in Scheme 3, target DNA fragment as part of p53 exon8 contains a polymorphic site, in which the nucleotide G in a 32-mer wild target (5′-CCTCTGTGCGCC*G*GTCTCTCCCAGGACAGGCA-3′) is replaced by A in a 32-mer mutant target (5′-CCTCTGTGCGCC*A*GTCTCTCCCAGGACAGGCA-3′). We design the 3′-end of the 20-mer mutant probe (5′-TGCCTGTCCTGGGAGAGAC*T*-3′) with a T that is complementary to A in the mutant-type target and is not complementary to G in the wild-type target. Taq DNA polymerase and fluorescein-labeled dGTP (dGTP-Fl) are used for extension reactions. For mutant-type target, the fluorescein-labeled base G is incorporated into the probe by extension reaction in the presence of Taq DNA polymerase. Upon addition of cationic polyfluorene (PFP1b) to the solution of the extension reaction, strong electrostatic interactions between PFP1b and extended probe will bring fluorescein close to PFP1b and induce the occurrence of efficient FRET from PFP1b to fluorescein. For wild-type target, the 3′-end of the probe is not complementary, thus the base extension reaction is blocked. In this case, upon addition of the PFP1b, FRET from PFP1b to fluorescein is inefficient. By monitoring the change in emission intensity of PFP1b or fluorescein, one can identify the SNP genotypes. As shown in Figure 1a, upon addition of PFP1b, fluorescence emission intensity of fluorescein for the perfect complementary target/probe pair (mutant target and probe) is about 4 times higher than that for single mismatched wild target/probe pair. Furthermore, the emission intensity of fluorescein using 380 nm excitation is approximately 10 times larger than that obtained by direct excitation at the fluorescein absorption maximum (480 nm) due to the optical amplification by the conjugated polymers. The enhanced emission intensity of fluorescein via FRET imparts the assay high sensitivity. Allele frequency is the proportion of all copies of a gene that is made up of a particular gene variant. Estimation of SNP allele frequency in a pooled DNA sample is an efficient method for investigating the association between SNPs and diseases.[46,47]

The FRET ratio (I_{535nm}/I_{424nm}) as a function of allele frequency was performed. As allele frequency increases, the FRET ratio increases, and as low as 2% allele frequency can be clearly detected (Figure 1b). Besides high sensitivity, our CCP-based new protocol also offers two significant features: (i) the assays are performed in homogeneous solution, and cumbersome workups are avoided to simplify operations and increase reproducibility; (ii) it does not require fluorescent labels on the primers, which should reduce the cost.

Obviously, the application of the current platform in real sample analysis is our final goal. After the above method is adapted to real sample analysis by the optimization of extension conditions and the involvement of shrimp alkaline phosphatase (SAP) treatment to hydrolyze unreacted dGTP-Fl, we have discriminated the SNP genotypes of 76 individuals of Chinese ancestry.[34] In these assays, target DNA fragments for subsequent SNP genotyping are obtained by PCR reaction from human genomic DNA. After an enzymatic treatment to

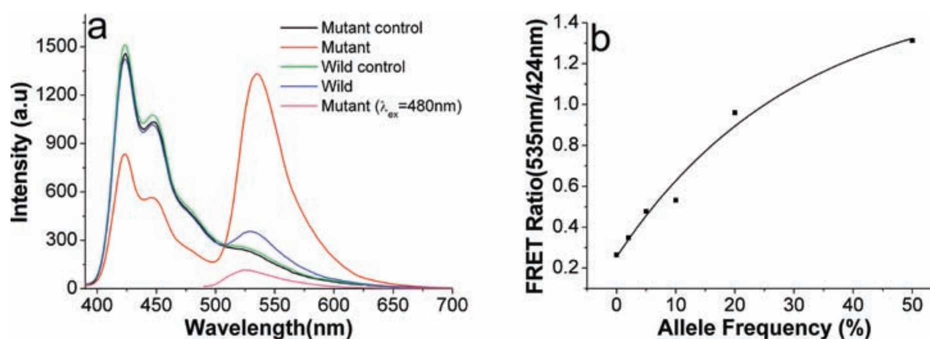


FIGURE 1. (a) Emission spectra from solutions containing PFP1b and extension products of mutant and wild target DNA and (b) FRET ratio (I_{535nm}/I_{424nm}) as a function of allele frequencies. Reproduced from ref 31. Copyright 2007 American Chemical Society.

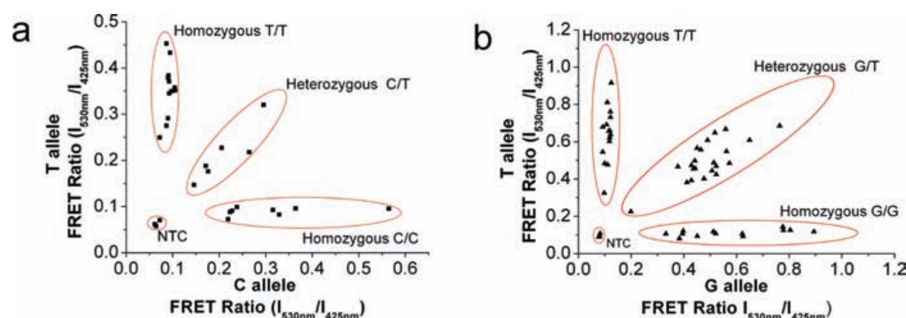


FIGURE 2. Genotyping results for SNP site rs1800469, C>T, of 26 individuals (a) and genotyping results for SNP site rs2241715, G>T, of 50 individuals (b) using CCP-based SNP genotyping method. FRET ratios of the allele-specific extension reactions are plotted as the *X* axis and *Y* axis, respectively. No-template control (NTC) is used as a blank. Reproduced from ref 34. Copyright 2009 Macmillan Publishers Ltd.

degrade redundant PCR primers, dNTPs (a mixture of dATP, dCTP, dTTP, dGTP), and pyrophosphate, the PCR product is used as a target in the extension reaction. Upon addition of polymer PFP1a to the extension solution, different FRET ratios (I_{530nm}/I_{425nm}) of extension solutions allow SNP genotypes to be determined. Most SNPs are biallele polymorphisms, namely, two possible alternative nucleotides at the SNP site. Generally speaking, a human being has two copies of each gene; genotype refers to the pair of alleles. Thus three kinds of genotypes at one SNP site are possible, two homozygous genotypes (same allele in two copies of each gene) and one heterozygous genotype (different allele in two copies of each gene). Each allele needs a specific primer for SNP genotyping. For homozygous genotypes, FRET will be detected with only one allele-specific primer, and for heterozygous genotypes, FRET will be detected with two different allele-specific primers. Genotypes of the SNP site rs1800469, C>T, of 26 individuals and genotypes of SNP site rs2241715, G>T, of 50 individuals are clearly distinguished (Figure 2).

In our initial method for SNP detection, two steps are required: the first one is the amplification of the target DNA fragment by PCR; the second one is the single base extension reaction followed by the detection of SNP genotypes using PFP1a. The genomic DNA could not be detected directly. Very recently, we have developed a homogeneous, sensitive, and economical method to detect SNP genotypes of genomic DNA by combining the allele-specific PCR technique with CCPs.[33] In this approach, the PCR product relies on the DNA polymerase that extends a primer only when its 3′-end is complementary to the target DNA. We use dGTP-Fl and dUTP-Fl to partly replace dGTP and dTTP in the dNTPs mixture for PCR. When PCR amplification occurs, dGTP-Fl and dUTP-Fl are incorporated into formed PCR amplicons. By detecting the existence of PCR product with the FRET signaling from PFP1a to fluorescein-labeled PCR amplicons, the SNP genotype can be identified (Figure 3). The genotyping of 50 ng of genomic DNA from a human lung cancer cell is easily detected using this method.

To identify three genotypes in one extension reaction, we designed an improved method for multicolor and one-tube SNP genotyping assays based on PFP1a/DNA assemblies using fluorescein-modified dUTP and Cy3-modified dCTP for extension reactions.[32] Fluorescein and Cy3 are chosen as acceptors for PFP1a. Fluorescein can also act as the donor for Cy3 because of their overlap integral as required by efficient FRET. In this assay, a sequence on exon8 of human p53 containing a polymorphic site is used as DNA target. For this SNP site, there are three possible genotypes: homozygous G/G, homozygous A/A, and heterozygous G/A. The primer (5′-TGCCTGTCCTGGGAGAGAC-3′) is complementary to upstream of the SNP site. Upon performance of the extension reaction

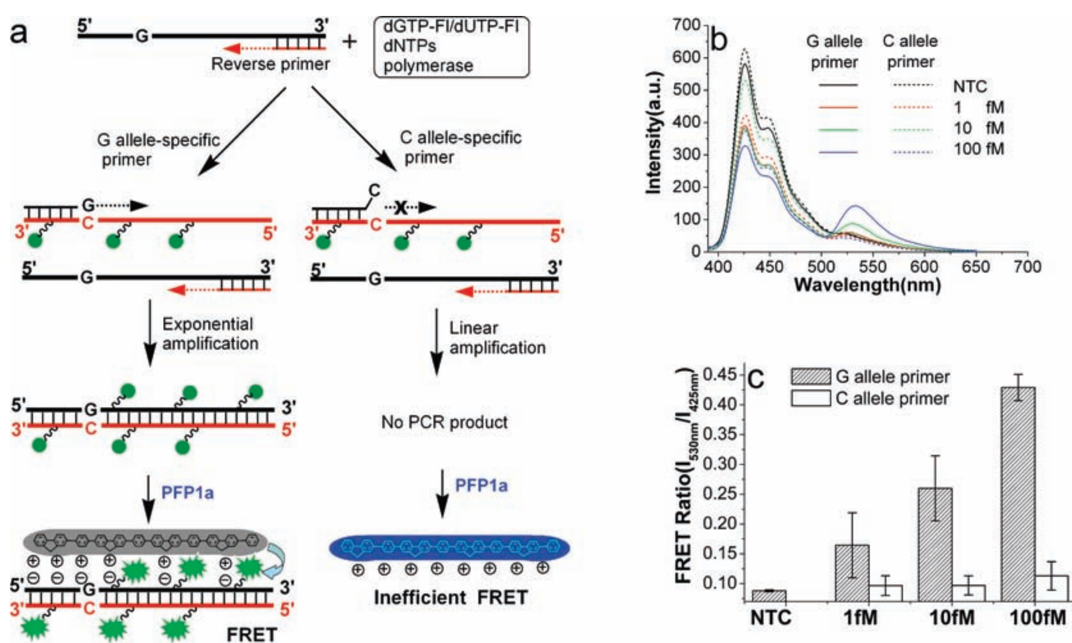
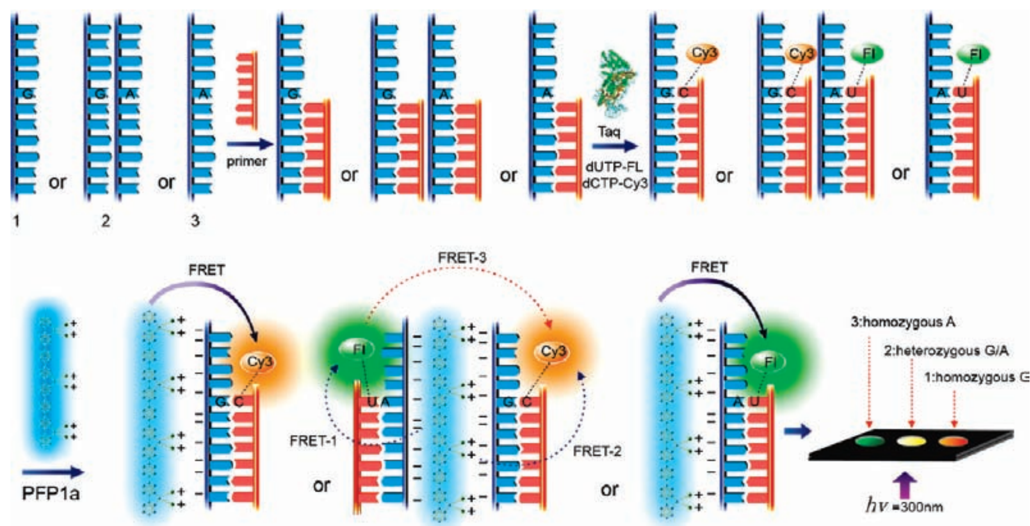


FIGURE 3. (a) Schematic representation of the SNP assay using CCP and allelic-specific PCR. Emission spectra (b) and FRET ratio (c) from solution containing PFP1a and G or C allele-specific primer PCR products from G allele template with varying concentrations. Reproduced with permission from ref 33. Copyright 2009 Elsevier.

SCHEME 4. Schematic Representation of Multicolor and One-Tube SNP Genotyping Assays Using CCPs



using Taq DNA polymerase, for homozygous A/A, only dUTP-Fl is incorporated into the primer; for homozygous G/G, only dCTP-Cy3 is incorporated into the primer; for heterozygous G/A, both dCTP-Cy3 and dUTP-Fl are incorporated into the primer (Scheme 4). After the addition of PFP1a to the extension solution, different FRET processes regulate the fluorescence intensities of PFP1a, fluorescein, and Cy3, leading to different pattern of fluorescence emission spectra (Figure 4a). By triggering the emission colors or the change of emission intensities of fluorescein and Cy3, one can assay the three kinds of SNP genotypes in one extension reaction. A limit of detection (LOD) as low as 5.3 fmol (3σ) is achieved (Figure 4b), which implies high sensitivity of this SNP genotyping assay.

Label-free SNP genotyping can also be realized using water-soluble polythiophene derivatives. In 2003, the Inganäs group designed polythiophene bearing amino acid moieties (PT) for DNA hybridization and SNP detections.[48] In the presence of complementary strands, the PT/ssDNA duplex is changed to a PT/dsDNA complex, with an alteration of PT conformation from a rod-shaped to a nonplanar form followed by a 3-fold enhancement of PT emission intensity. By fluoromet-

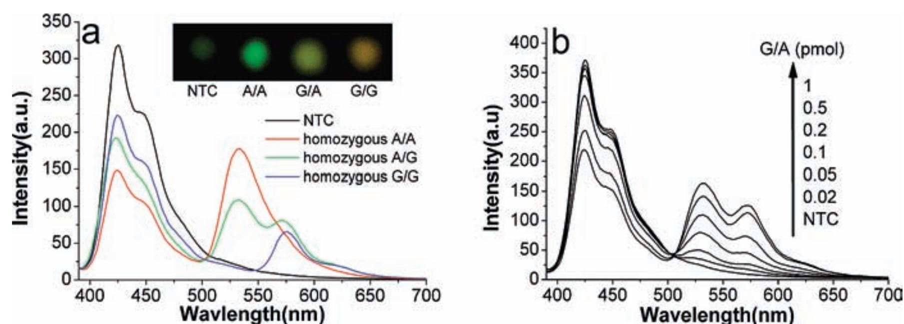


FIGURE 4. (a) Emission spectra from solutions containing PFP1a and extension products of homozygous A/A, heterozygous G/A, and homozygous G/G. A no-template control (NTC) was used as the blank. (b) Emission spectra from solutions containing PFP1a and extension products in the presence of varying concentrations of heterozygous G/A. A no-template control (NTC) was used as the blank. Reproduced from ref 32. Copyright 2008 The Royal Society of Chemistry.

ric means, mismatches can be distinguished at room temperature because they affect the intra- and interchain processes in the polymer, which enables the PT to detect DNA hybridization and SNPs without a denaturation step. In 2005, the Leclerc group developed an integrated PCR-free fluorescence DNA sensor using cationic polythiophene.[49] The sensor is based on the different conformations adopted by cationic polythiophene when it electrostatically binds to ssDNA or dsDNA, and on the energy transfer from the resulting fluorescent polythiophene/dsDNA complex to neighboring fluorophore labeled in ssDNA probe. This sensing system can directly detect SNPs from clinical samples in only a few minutes. Very recently, a label-free SNP genotyping method has also been developed for DNA and RNA targets combining a conformational change of cationic polythiophene derivative (CPT2) and primer extension reactions.[50] Because CPT2 forms different interpolyelectrolyte complexes with extended dsDNA and nonextended ssDNA, the SNP genotypes can be identified by triggering the color change of the extension solution. Furthermore, through the specific cleavage of RNA strands in the RNA/DNA hybrids by RNase H, SNP genotyping for RNA templates is also achieved.

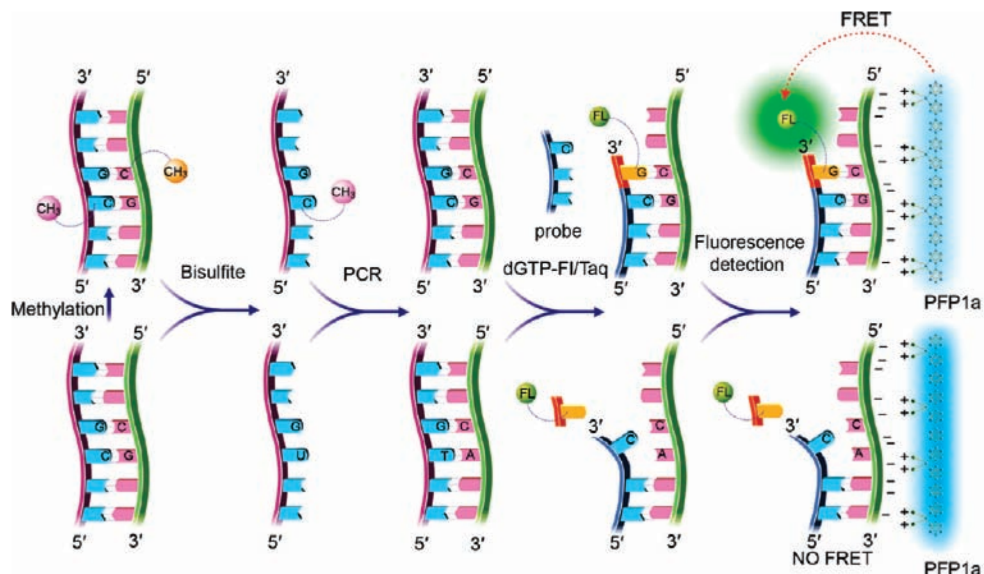
CCP-Based Detection of DNA Methylation

Epigenetic regulation that involves DNA methylation and histone modification leads to heritable change of genes expression without alteration of their coding sequence. Epigenetic abnormalities can cause several major pathologies, including cancer, syndromes involving chromosomal instabilities, and mental retardation.[51,52] DNA methylation in mammals occurs by the addition of a methyl group to the 5′ position of cytosine almost exclusively within a CpG dinucleotide.[7] Hypermethylation of the CpG island in the promoter region of gene has been regarded as a hallmarker of various diseases, cancer in particular.[8] Cancer-linked DNA hypermethylation leads to functional silencing of some tumor suppressor genes due to chromatin compaction, which is responsible for tumor formation and progression.[6] Thus, the detection of DNA methylation could provide a powerful tool for early cancer diagnosis and cancer type identification.[9,10]

In 2007, we developed a novel CCP-based method for detecting DNA methylation based on the cleavage of DNA by restriction endonuclease.[35] The cleavage of methylated DNA by restriction endonuclease is blocked by the methylation at specific sites in duplex DNA. The fluorescein-labeled target model DNA is first treated with *Eco*RI methyltransferase to methylate the adenine residue at the sequence 5′-GAATTC-3′ in the presence of a methyl group donor, S-adenosyl-L-methionine (SAM). The methylated DNA is then treated with its specific *Eco*RI nuclease that can specifically cleave unmethylated DNA, but not methylated DNA. Upon addition of PFP1b, efficient FRET from PFP1b to fluorescein occurs for methylated DNA while less efficient FRET signaling is observed for unmethylated DNA.

To attain the goal in real sample analysis, very recently we have initiated a convenient, sensitive and label-free method to determine the DNA methylation status of CpG sites of plasmid and human colon cancer cells.[36] The system relies on a highly selective single base extension reaction and significant optical amplification of conjugated polymers. The assay strategy is illustrated in Scheme 5. Bisulfite treatment changes nonmethylated cytosine into uracil but does not influence the methylated cytosine, and the resulting uracil is substituted by thymine after PCR amplification.[53] Thus, the DNA methylation status can convert into a C/T polymorphism. After single nucleotide base extension with Taq polymerase in the presence of dGTP-Fl and methylation-specific probe, the dGTP-Fl is incorporated into the probe for the methylated DNA, but not for nonmethylated DNA. Upon addition of PFP1a, strong electrostatic interactions between DNA and PFP1a bring the fluo-

SCHEME 5. Schematic Representation of DNA Methylation Detection



rescein close to PFP1a, and efficient FRET from PFP1a to fluorescein occurs for the methylated DNA but not for nonmethylated DNA. The methylation status of a specific CpG site can be monitored by fluorescence spectra in view of the observed PFP1a or fluorescein emission changes in aqueous solutions. A pUC57 plasmid containing a 283-bp DNA fragment from the promoter region of a tumor-suppressor gene (p16)[54] is first used for establishing the method. This DNA fragment contains three CpG sites. The full methylated plasmid is obtained by a methylase (M.SssI) treatment to the nonmethylated plasmid. As shown in Figure 5a−c, upon addition of PFP1a, the comparison of the resulting fluorescence from fluorescein obtained by excitation at 380 nm shows approximately 2−4 times higher signal for the complementary target/probe pair (three methylation-specific probes for sites 1−3 of methylated plasmid were used, respectively), relative to the

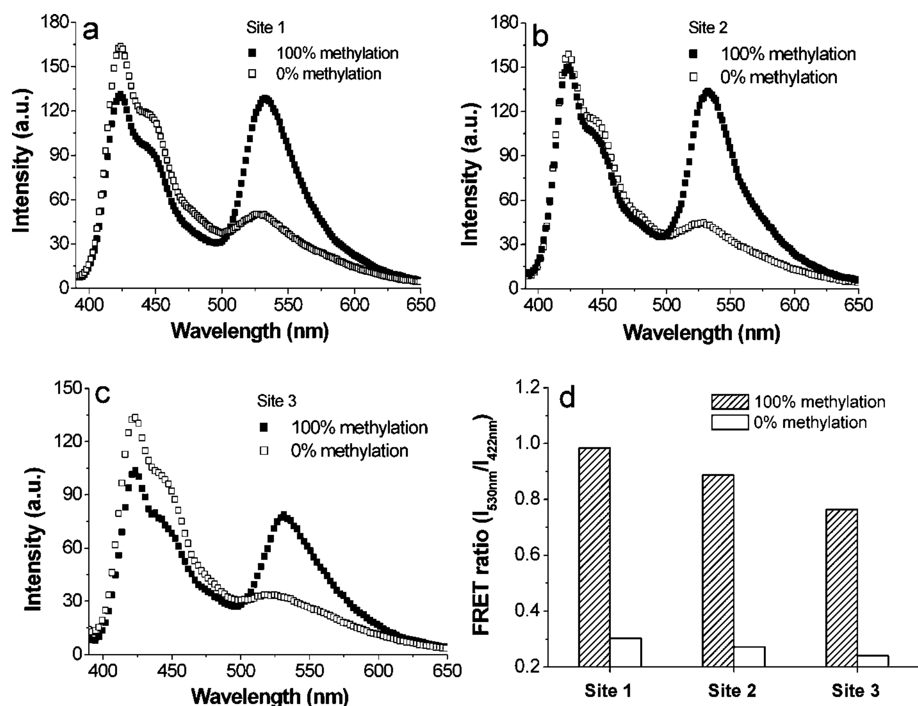


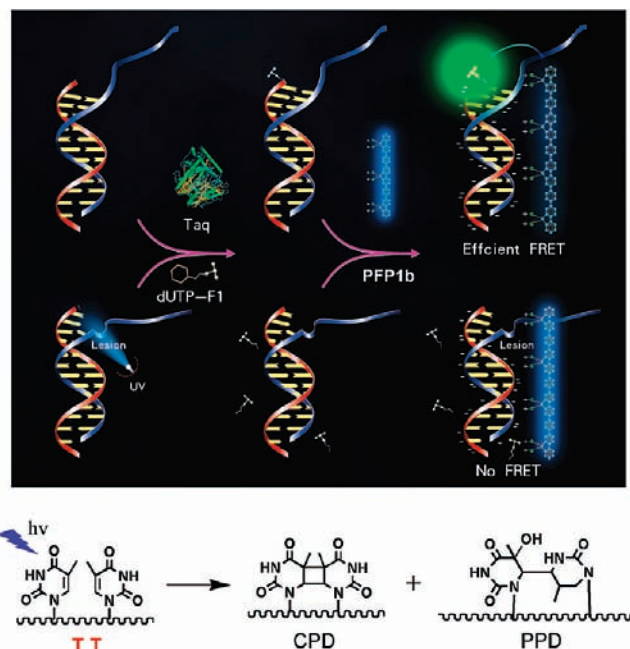
FIGURE 5. Fluorescence spectra (a−c) from solutions containing PFP1a and extension products of three CpG sites of methylated plasmid and nonmethylated plasmid using methylation-specific probes. Fluorescence spectra (c) and FRET ratio (I_{530nm}/I_{422nm}) (d) of the extension product with varying proportions of methylated DNA in the presence of PFP1a. Reproduced from ref 36. Copyright 2008 American Chemical Society.

mismatched target/probe pair (nonmethylation-specific probes were used). The FRET ratios (I_{530nm}/I_{422nm}) for specific extension are 3−5 times higher than that of the nonspecific extension (Figure 5d), which demonstrates the good selectivity of this assay method for the detection of the methylation status of specific CpG sites. The improved fluorescein emission by FRET shows the optical amplification by the PFP1a, and analyte DNA at the picomolar level can be easily detected. Based on the detection model, methylation status of three CpG sites in the *p16* promoter region of the human colon cancer cell line HT29 has been detected. The genomic DNA is extracted from HT29 followed by bisulfite treatment and PCR amplification. For each site, two extension reactions are carried out by using methylation-specific probe and nonmethylation-specific probe, respectively. At three CpG sites, significant FRET signaling for the methylation-specific probe implies that hypermethylation occurs in the *p16* promoter region of HT29 cells. This new technique has several important features. First, the method is continuous and homogeneous and does not need radioactive handling. The isolation and washing workups are avoided to simplify operations and increase reproducibility. Second, the method is applicable for analyzing trace amounts of DNA, and only microgram orders of DNA or less from human cells or plasmid are required. Third, this method does not require designing dye-labeled DNA probes, which should significantly reduce the cost. These advantages will make the system promising in the future for early cancer diagnosis.

CCP-Based Detection of DNA Damage

Ultraviolet (UV) damage to DNA produces a variety of lesions in cellular DNA, which may cause lethal mutagenic and carcinogenic risks. The most abundant lesions are cyclobutane pyrimidine dimer (CPD) and [6−4] photoproduct PPD (pyrimidine−pyrimidone dimer) that occur between adjacent thymine bases.[55] It has been reported that CPD and PPD can quantitatively inhibit DNA polymerization.[56] On the basis of this knowledge, we developed a new platform for DNA lesion detection using PFP1b (Scheme 6).[37] The target DNA has two adjacent thymine bases (-TT-). The primer with two adjacent adenines (-AA-) at the 3′-terminus is perfectly complementary to the target sequence. Taq DNA polymerase and dUTP-Fl are used for primer extension reactions. After the extension reaction, dUTP-Fl is incorporated into the primer. Upon addition of PFP1b to reaction solution, efficient FRET from PFP1b to fluorescein occurs. In contrast, when CPD and PPD lesions are formed due to UV irradiation, dUTP-Fl cannot be incorporated into the primer by the extension reaction, leading to inefficient FRET from PFP1b to fluorescein. As shown in Figure 6a, the initial assay solution shows intense fluorescein emission at 528 nm resulting from the efficient FRET from PFP1b. As UV light irradiation time extends from 0 to 180 min, the emission intensity of PFP1b at 424 nm gradually increases and that of fluorescein at 528 nm gradually decreases. Owing to the significant optical amplification of fluorescein by CCP, low concentrations of damaged DNA, below nanomolar levels, could be detected with a standard commercial fluorometer (Hitachi F-4500) equipped with a xenon lamp excitation source and a photomultiplier tube. Furthermore, as demonstrated in Figure 6b, the CPD and PPD lesions in DNA can be quantitatively analyzed by FRET means through the assistance of HPLC assay. The new CCP-based method offers a convenient approach for homogeneous, ratiometric, and rapid detections of DNA lesions at specific portions of a genome in aqueous solutions.

SCHEME 6. Schematic Representation of the CCP-Based DNA Lesion Detection



Oxidative damage of DNA by reactive oxygen species (ROS) generates characteristic mutagenic base lesions that are involved in mutagenesis, carcinogenesis, and aging.[11] In 2005, we developed a label-free, convenient detection method for DNA damage by hydroxyl radicals (•OH) using cationic polythiophene (Scheme 7).[38] CPT2 can form an interpolyelectrolyte complex with 25-mer ssDNA (5′-GAGTTAGCACCCGCATAGTCAAGAT-3′) through electrostatic interactions. In this case, CPT2 takes a highly planar conformation and thus exhibits a relatively red-shifted absorption wavelength (path A). When the ssDNA is damaged by •OH into small fragments, the complex cannot form and CPT2 takes a

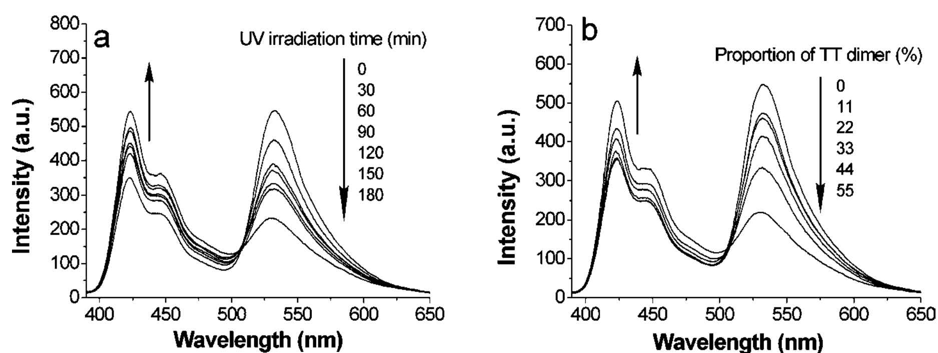
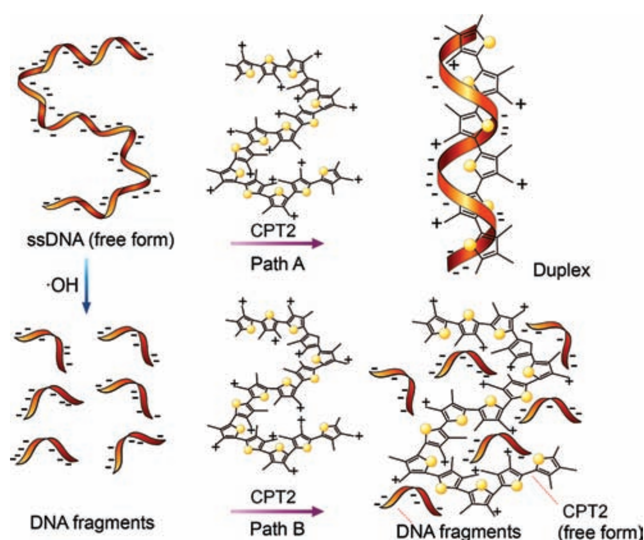


FIGURE 6. (a) Emission spectra of the assay solutions containing PFP1b and extension products as a function of UV light irradiation time (a) and DNA lesion content (b). Reproduced from ref 37. Copyright 2009 Wiley-VCH.

SCHEME 7. Schematic Representation of the Assay for DNA Damage by Hydroxyl Radicals



random-coil conformation and exhibits relatively short absorption wavelength (path B). The oxidative damage of DNA by •OH can be monitored by absorption spectra or color changes of CPT2. Figure 7a compares the absorption spectra of CPT2/ssDNA before and after treatment by •OH. Upon addition of ssDNA, the absorption maximum of CPT2 is red-shifted to 520 nm with a color change from yellow to pink-red. When ssDNA is damaged by •OH, the solution is still yellow and

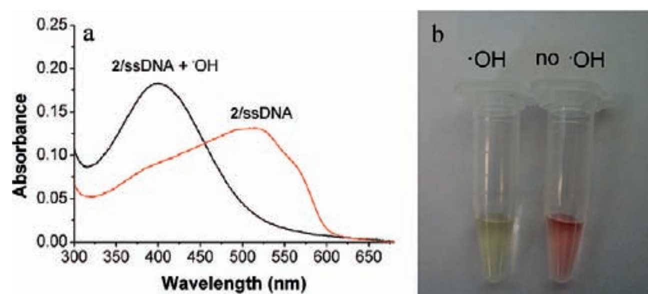


FIGURE 7. (a) UV−vis absorption spectra of CPT2/ssDNA in the presence and absence of hydroxyl radicals and (b) photographs of solutions corresponding to the absorption spectra. Reproduced from ref 38. Copyright 2006 American Chemical Society.

the absorption maximum of CPT2 appears at around 394 nm (Figure 7b). A relevant characteristic of the assay is its ability to relay information by direct visual inspection, which makes it more convenient than instrumentation-based methods.

Conclusion and Outlook

Nucleic acid detection techniques based on gel electrophoresis or solid support phases require cumbersome protocols or multiple washing steps. Most current homogeneous FRET methods (such as, TaqMan and molecular beacon) require doubly labeled DNA probes.[57,58] The use of CCP as a FRET donor has several advantages: (i) the assays are performed in homogeneous solution and cumbersome workups are avoided to simplify operations and increase reproducibility; (ii) the fluorescence detection could be performed using a common spectrofluorometer or UV viewing cabinet and does not require sophisticated instruments; (iii) the amplification of fluorescence signal from CPs can improve the detection sensitivity. The method requires only trace amounts of analyte DNA; (iv) the CCPs can form a complex with oppositely charged DNA through electrostatic interactions to avoid labeling the DNA by covalent linkage; also it does not require fluorescent labels on the primers, which should significantly reduce the cost. As reported in this Account, the CCP-based nucleic acid assay offers a very promising tool for the identification of susceptibility genes, early stage cancer diagnosis, mutagenesis, or aging assays. Although a great deal of progress has already been made in this field, major challenges remain before these sensors can be put into real-world applications. The main disadvantages of CCPs include insufficient biocompatibility, intractable self-quenching due to aggregation in water, and noticeable photobleaching. Novel designs for polymer chemical structures are desired to circumvent these limitations. Further advances and challenges will likely be

directed toward improving throughput of current methods to meet the requirement of large-scale research projects, such as genomic study. In this regard, recent advances of conjugated polymer-based microarrays and multiplexed target detection are very promising.[25,48,59−61] Furthermore, the association study between the diseases and susceptibility genes should be performed using CCP-based new methods. Despite these remaining challenges, it exhibits a very bright future of conjugated polymers for human disease diagnosis. There is good potential for CCP-based approaches to be incorporated into routine biological assay protocols in the near future.

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FOOTNOTES

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