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# Labeling-free fluorescent detection of DNA hybridization through FRET from pyrene excimer to DNA intercalator SYBR green I

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ABSTRACT

A novel labeling-free fluorescence complex probe has been developed for DNA hybridization detection based on fluorescence resonance energy transfer (FRET) mechanism from pyrene excimer of pyrene-functionalized poly [2-(*N*, *N*-dimethylamino) ethyl methacrylate] (PFP) to SYBR Green I (SG, a specific intercalator of double-stranded DNA) in a cost-effective, rapid and simple manner. The complex probe consists of the positively charged PFP, SG and negatively charged single-stranded DNA (ssDNA). Upon adding a complementary strand to the complex probe solution, double-stranded DNA (dsDNA) was formed, followed by the intercalation of SG into dsDNA. The pyrene excimer emission was overlapped with the absorption of SG very well and the electrostatic interactions between PFP and dsDNA kept them in close proximity, enabling efficient FRET from pyrene excimer to SG. The fluorescence of SG in the duplex DNA resulting from FRET can be successfully applied to detect DNA hybridization with high sensitivity for a very low detection limit of 10 nM and excellent selectivity for detection of single base pair mismatch.



## 1. Introduction

During the past decades, the sequence-specific DNA detection has received great attention because of its application in many areas, such as clinical diagnosis, gene expression analysis, biomedical studies (Bi et al., 2010; Rosi et al., 2006; Sefah et al., 2009; Zheng et al., 2013b; Zhu et al., 2010). Various techniques for DNA detection have been developed, such as electrochemical (Ahangar and Mehrgardi, 2012; Ganguly et al., 2009; Qiu et al., 2011; Cheng et al., 2014) and fluorescent (Kang et al., 2009, 2010; Lee et al., 2014; Xiang et al., 2012; Zhang and Zhou, 2012) methods. Homogeneous DNA hybridization assays based on fluorescence resonance energy transfer (FRET) between energy-transfer chromophore pairs are attractive because of their simplicity of operation and use of standard optical equipment (Wang et al., 2011). Recently, the FRET strategy employing water-soluble cationic conjugated polymers (CCP) with a large number of chromophoric repeat units, which enable the transfer of excitation energy along the whole backbone of the CCP to the reported chromophore via FRET, has been paid more attention for DNA detection with high sensitivity and selectivity (Feng et al., 2011; Jiang et al., 2009; Liu et al., 2014; Xu et al., 2005, 2010). Bazan and co-workers designed a three-color DNA detection assay with CCP and a fluorophore-labeled peptide nucleic acid strand through efficient FRET from CCP to the fluorophore (Liu and Bazan, 2004). Wang and co-workers demonstrated a simple method using CCP, EB and fluorescein-labeled ssDNA for DNA detection based on two FRET process from CCP to fluorescein and then to EB with enhanced detection efficiency (Feng et al., 2008; Tian et al., 2007). In these assays, the fluorophore moieties as acceptors were covalently linked to the DNA or PNA probes and the negatively charged phosphate groups of the DNA interacted with CCP to form the complexes by electrostatic interactions, which could detect DNA hybridization through FRET from CCP to the fluorophore labeled at the end of nucleic acids. However, labeling process with chromophores is high-cost, complex and time-consuming, which may limit its applications in detection of nucleic acids (Ma et al., 2013; Pu and Liu, 2009; Wang et al., 2011). Thus, to overcome the aforementioned limitations, development of novel and simple labeling-free FRET probes for DNA detection is highly desirable.

The labeling-free assays using DNA intercalators can exhibit high sensitivity and selectivity with low cost and convenient operation (Aied et al., 2012; Lin et al., 2015; Pu and Liu, 2009; Wang et al., 2011, 2014; Xu et al., 2010). SYBR Green I (SG), a DNA intercalating dye, is an asymmetrical cyanine dye and has a significantly enhanced fluorescence in complex with dsDNA due to a dampening of its intra-molecular motions (Dragan et al., 2012), which can be potentially utilized to develop labeling-free sensors for DNA hybridization detection. In addition, SG has a high quantum yield of 0.80 that is 100 times larger than that of the other

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commonly used intercalating dye EB, which is also much less mutagenic than EB (Lee et al., 2008). Thus, SG has been widely used for the selective detection of metal ions ($K^+$, $Hg^{2+}$ etc.) (Liu, 2008; Xu et al., 2010) and biological molecules (DNA, proteins etc.) (Li et al., 2013; Zheng et al., 2013a) with low cost in a "mix-and-detect" manner. For example, Zheng and co-workers developed a labeling-free signal amplification assay for DNA detection based on exonuclease III and SG, which showed excellent detection selectivity for single-base discrimination.

Recently, we designed a labeling-free fluorescent complex probe composed of pyrene-functionalized water-soluble cationic polyelectrolytes and ssDNA for DNA hybridization detection based on a decreased fluorescence resulted from the intercalation of pyrene into the duplex DNA (Yang et al., 2012; Zhang et al., 2013; Zhao et al., 2013), while the fluorescence turn-off assay for hybridization detection might limit the sensitivity and selectivity. Herein, to improve the detection sensitivity and selectivity, we develop a novel labeling-free DNA complex probe composed of a water-soluble positively charged pyrene-functionalized PDMAEMA (PFP), DNA intercalator SG, and negatively charged ssDNA for detection of DNA hybridization through efficient FRET from pyrene excimer of PFP to SG. The novel probe shows high sensitivity with a 10 nM detection limit of the target DNA and excellent selectivity for single base mismatch detection, which provides great potential in DNA hybridization detection. This proposed strategy exhibits several advantages over existing techniques such as simple and low-cost in design, fast in operation and high specific as compared to the previously reported methods using fluorescent labeling DNA probe, which might provide a promising sensing platform for bioanalysis, clinical molecular diagnostics and environmental monitoring.

## 2. Materials and methods

### 2.1. Materials and apparatus

Phosphorous tribromide (98.5%) from Sinopharm Chemical Reagent Co. Ltd., 1-pyrenemethanol (99%), 2-(Dimethylamino) ethyl methacrylate (99%), ethyl 2-bromoisobutyrate (98%), copper bromide (98%), 1.1.4.7.10.10-hexamethyltriethylenetetramine (97%) from Aldrich and the conventional reagents were used as received. All atmosphere sensitive reactions were done under nitrogen. Deionized water was obtained from a Millipore water purification system. SG was purchased from Life Technologies. The HPLC-purified single stranded DNAs (ssDNAs) and phosphate buffer solution used in this work were obtained from the Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The sequences of the DNA oligonucleotides were as follows:

ssDNA$_1$: 5′-CAA GTA GAA TGT ATG TGC-3′
ssDNA2: 5′-GCA CAT ACA TTC TAC TTG-3′
ssDNA1nc: 5′-GCA CAT A**T**A TTC TAC TTG-3′
ssDNA2nc: 5′-GCA CA**G** ACA TTC **A**AC TTG-3′
ssDNA3nc: 5′-GCA **G**AT AC**C** TTC TA**A** TTG-3′
ssDNAa: 5′-AAA AAA AAA AAA AAA AAA-3′
ssDNAt: 5′-TTT TTT TTT TTT TTT TTT-3′
ssDNAc: 5′-CCC CCC CCC CCC CCC CCC-3′

Here ssDNA$_2$ is complementary with ssDNA$_1$; ssDNA$_{1nc}$, ssDNA$_{2nc}$ and ssDNA$_{3nc}$ are single-mismatched, double-mismatched and triple-mismatched DNAs with ssDNA$_1$, respectively; ssDNA$_a$, ssDNA$_c$ and ssDNA$_t$ are non-complementary ssDNAs.

$^1$H-NMR spectra were recorded from $CDCl_3$ solution on a Bruker AM 400 spectrometer. The molecular weight of the polymer was determined by gel permeation chromatography (GPC) (Waters 1515) with styragel columns relative to polystyrene standards using THF as eluent. UV–visible absorption spectra were taken using a Shimadzu UV-3100 UV–vis spectrophotometer. The fluorescence spectra were measured on a Hitachi F-4500 spectrofluorometer, in which all the measurements were done in 10 mM phosphate buffer solutions (pH=7.4).

### 2.2. Polymer synthesis

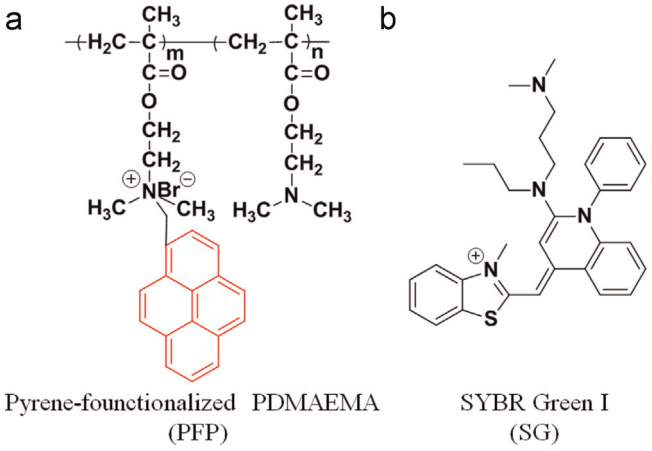
First, the polymer PDMAEMA was synthesized by atom transfer radical polymerization (ATRP). The number-average molecular weight (Mn) and polydispersity index (PDI) of the polymer were determined to be $2.32 \times 10^4$ and 1.09, respectively, by GPC. Next, 1-(Bromomethy) pyrene was synthesized and incorporated into PDMAEMA through quaternization with the dimethylaminoethyl unit to achieve the pyrene-functionalized PDMAEMA.

### 2.3. Sample preparation

The complex probes were prepared by dissolving a suitable amount of the polymer PFP in phosphate buffer solution (10 mM, pH=7.4), following addition of SG and ssDNA$_1$. Complementary or non-complementary ssDNAs with equal amount to ssDNA$_1$was mixed with the complex probe to investigate its fluorescence changes. All the samples containing DNA were annealed at 90 °C for 10 min and slowly cooled down to room temperature prior to measurement.

## 3. Results and discussion

The water soluble fluorescent pyrene-functionalized PDMAEMA was synthesized through the quaternization of the dimethylaminoethyl units of the polymer PDMAEMA with 1-(bromomethy) pyrene. The detailed procedure of the synthesis can be found in the Supporting Information. In this work, the polymer PDMAEMA was synthesized by atom transfer radical polymerization (ATRP). The number-average molecular weight (Mn) and polydispersity index (PDI) of the polymer were determined to be $2.32 \times 10^4$ and 1.09, respectively, by gel permeation chromatography (GPC). Here a pyrene-functionalized polymer PFP with a functionalization degree of 13.0% was used to form the complex probe. The fluorescence quantum yield ($\Phi_F$) of PFP was determined to be 0.081, using quinine sulfate in sulfuric acid (1.0 N) as a reference ($\Phi_R$=0.55). Scheme 1 shows the chemical structures of the




**Scheme 1.** Chemical structures of PFP (a) and SG (b).