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# An aggregated perylene-based broad-spectrum, efficient and label-free quencher for multiplexed fluorescent bioassays

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ABSTRACT

Fluorescent sensing systems based on the quenching of fluorophores have found wide applications in bioassays. An efficient quencher will endow the sensing system a high sensitivity. The frequently used quenchers are based on organic molecules or nanomaterials, which usually need tedious synthesizing and modifying steps, and exhibit different quenching efficiencies to different fluorophores. In this work, we for the first time report that aggregated perylene derivative can serve as a broad-spectrum and label-free quencher that is able to efficiently quench a variety of fluorophores, such as green, red and far red dyes labeled on DNA. By choosing nucleases as model biomolecules, such a broad-spectrum quencher was then employed to construct a multiplexed bioassay platform through a label-free manner. Due to the high quenching efficiency of the aggregated perylene, the proposed platform could detect nuclease with high sensitivity, with a detection limit of 0.03 U/mL for EcoRV, and 0.05 U/mL for EcoRI. The perylene quencher does not affect the activity of nuclease, which makes it possible to design post-addition type bioassay platform. Moreover, the proposed platform allows simultaneous and multicolor analysis of nucleases in homogeneous solution, demonstrating its value of potential application in rapid screening of multiple bio-targets.



## 1. Introduction

Fluorescence methods exhibit unique advantages such as high sensitivity, rapid analysis with spatial resolution, and little proclivity to sample or cell damage (Martinez-Manez, and Sancenon, 2003; Borisov and Wolfbeis2008; Liu et al., 2009; Gale, 2010). Subsequently, past decade has seen considerable interest in development of fluorescent sensing systems for biological molecules. Most of these sensing systems are based on the quenching of a fluorophore by a non-fluorescent quencher. To date, the most frequently used commercial quenchers are small organic molecules. However, they usually need to be conjugated on the probe through tedious modifying steps, and suffer from low quenching efficiency or variant quenching efficiency for fluorophores that emit at different wavelengths (Tyagi et al., 1998; Dubertret et al., 2001).

In recent years, a variety of nanomaterials, including carbon nanomaterials and gold nanoparticles (AuNPs), have been developed which can quench various fluorophores with high quenching efficiency (Yang et al., 2008; Pautler et al., 2013; Song et al., 2009; Zhao et al., 2011). For example, AuNPs are reported to be able to quench FAM with quenching efficiency 100-fold higher than that of organic quenchers (Song et al., 2010). Nevertheless, these AuNP-based methods generally possess poor salt stability and thermal stability, thus limiting their practical applications (Song et al., 2010; Elghanian et al., 1997). It has also been proven that the fluorescence of different kinds of dyes could be efficiently quenched by carbon nanotubes (CNTs) or graphene. However, proteins and other biomolecules are prone to nonspecifically adsorb on CNTs or graphene, which might inhibit the activity of these biomolecules to some extent (Mann et al., 2013). Chen et al. reported a broad-spectrum nanoquencher by incorporating a series of dark quenchers into mesoporous silica nanoparticles (MSNs) (Huang et al., 2012), which can efficiently quench a broad range of visible and near infrared (NIR) fluorophores. To obtain such a nanoquencher, several tedious preparing steps are necessary. The development of a broad-spectrum fluorescent quencher which is easy to synthesize, exhibits high quenching efficiency with high biocompatibility and stability, is therefore highly desirable.

Recently, aggregated cationic perylene diimide derivative (compound **1**, Fig. 1) was reported to be able to act as a superquencher to quench the fluorescence of the adjacent oligonucleotide-labeled

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FAM (Wang et al., 2011; Fu et al., 2013). Such a superquencher can be formed "in situ" in a label-free manner, which make it an attractive, universal and convenient quencher for constructing FAM-based fluorescence biosensors. In this work, we for the first time report that cationic aggregated perylene **1** can act as a broad-spectrum and label-free quencher which is able to efficiently quench a variety of anionic oligonucleotide-labeled fluorophores that emit at a wide wavelength range from 520 to 670 nm via the strong electrostatic interactions. In comparison with classic small organic molecule-based quenchers, compound **1** is label-free quencher that can efficiently quench a variety of adjacent anionic oligonucleotide-labeled fluorophores that emit at a wide wavelength range from the visible to NIR region via strong electrostatic interactions. It avoids to be conjugated on the probe through tedious modifying steps. By choosing nucleases as model biomolecules, such a broad-spectrum quencher was then employed to construct a multiplexed bioassay platform through a label-free and post-addition manner with satisfactory results (Fig. 1).

## 2. Materials and methods

### 2.1. Reagents

DNA oligonucleotides used in this work were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences are shown in Table 1. Perylenetetracarboxylic dianhydride and N, N-Dimethyl-1, 3-propanediamine were purchased from Alfa Aesar. Methyl iodide and other compounds were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). All chemicals were of analytical grade and used without further purification. The restriction nucleases EcoRI, EcoRV and NEB buffer 3 were purchased from New England Biolabs. All solutions were prepared in Milli-Q water (resistance > 18 MΩ cm) from a Millipore system.

### 2.2. Sensing system preparation

#### 2.2.1. Quenching efficiency investigation

Different concentrations of compound **1** were added into solution containing 10 μLof 1 μM various fluorophores-labeled DNA (3-FAM-A, TA-A, TE-A, and Cy5-A). The experiments were performed in 100 μL reaction solution which contained 1 × NEB buffer 3 (50 mM Tris–HCl, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9 ). After incubating for

**Table 1**
Sequences of oligonucleotides used in this work.

| Name | Sequences (from 5′ to 3′) |
|---|---|
| 3-FAM-A | FAM-CTGAATTCTGGATGTAGTTAGTAGTC |
| 3-FAM-B | GACTACTAACTACATCCAGAATTCAG |
| 5-FAM-A | FAM-CACTGAATTCTGGATGTAGTTAGTAGTC |
| 5-FAM-B | GACTACTAACTACATCCAGAATTCAGTG |
| 7-FAM-A | FAM-CTCACTGAATTCTGGATGTAGTTAGTAGTC |
| 7-FAM-B | GACTACTAACTACATCCAGAATTCAGTGAG |
| TA-A | TAMRA-CAGATATCGTTAAGTAGGTGCATGTGTC |
| TA-B | GACACATGCACCTACTTAACGATATCTG |
| TE-A | Texas Red-ATGAAGGACGATGTATGCTTGAGGTC |
| Cy5-A | Cy5-CAGATATCGTTAAGTAGGTGCATGTGTC |

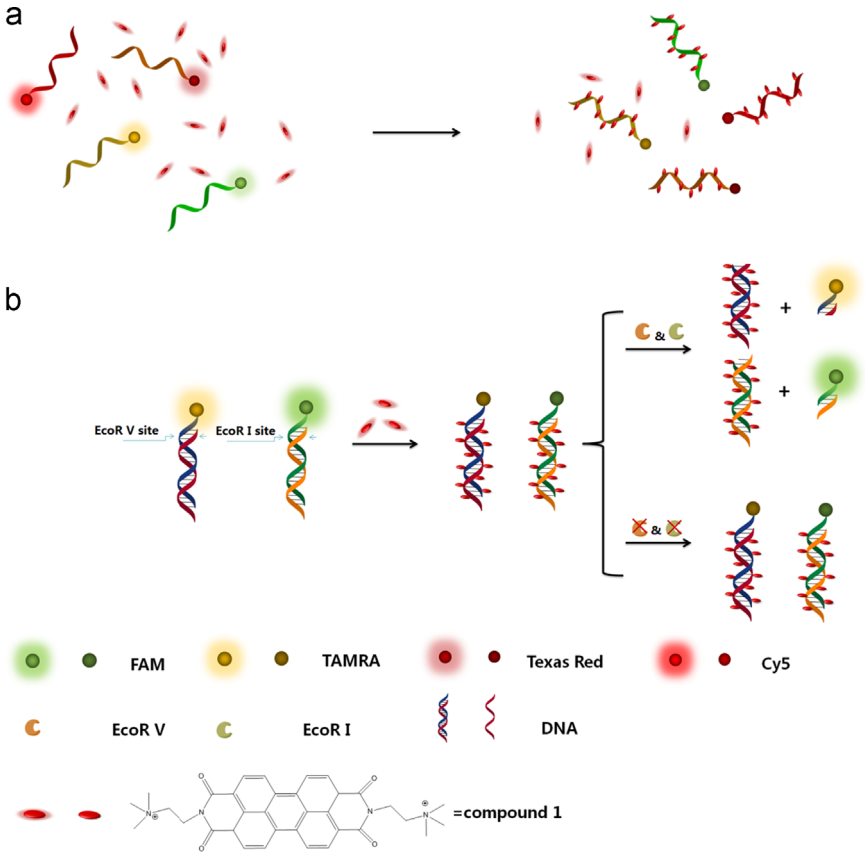


**Fig. 1.** (a) Schematic illustration of quenching properties of perylene derivative against various dyes. The fluorescence of the fluorophore was quenched by the compound **1**. (b) Schematic illustration of the multiplexed bioassay system for nucleases based on the broad-spectrum quencher of aggregated cationic perylene. Oligonucleotides labeled with FAM and TAMRA were designed as substrates for nuclease EcoRI and EcoRV, whose fluorescence was first quenched by compound **1**. The addition of EcoRI or EcoRV would induce the cleavage of the double strand DNA, releasing a short FAM-linked or TAMRA-linked oligonucleotide fragment, with their fluorescence signal being recovered.