Superquencher formation via nucleic acid induced noncovalent perylene probe self-assembly†

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A fluorophore labeled oligonucleotide could induce aggregation of a positively charged perylene probe. The perylene aggregate could very efficiently quench the fluorescence of the labeled fluorophore. Based on this observation, a new method for the highly sensitive and selective detection of a protein has been developed.

Sensitive and selective sensing of biomolecules is of fundamental importance in many areas of biochemical/biomedical research, disease treatment, and new drug development. Fluorescence based assay is a powerful tool because of its high sensitivity and ease of operation. Many fluorescence detection techniques rely on fluorescence resonance energy transfer (FRET) from a donor fluorophore to an acceptor fluorophore, or quenching of the donor’s fluorescence by a non-fluorescent quencher.1

To date, the most frequently used quenchers are small organic molecules. For example, a vast majority of molecular beacon probe designs employ an organic quencher.1a However, in practical applications, quenching of the fluorophore’s fluorescence is usually not very efficient. Therefore, great efforts have been made in recent years to reduce the background emission.2 In addition, advanced materials such as gold nanoparticle, single-walled carbon nanotube, and graphene have also been employed as efficient quenchers for the design of new biosensing assays in recent years.3

DNA-templated assembly of organic dye aggregates has been extensively studied in recent years.4 We have reported that a single-stranded nucleic acid could act as a polyanion to induce aggregation and self-assembly of various types of positively charged molecular probes, and strong UV-vis, emission, and CD spectral changes were observed.5 And as a variation of the well-documented fluorimetric displacement assay,6 we have showed that a positively charged perylene probe (compound 1, Scheme 1) displays strong fluorescence in an aqueous buffer solution. However, an oligonucleotide could induce strong aggregation of compound 1, and result in very efficient probe fluorescence quenching.7

In this work, we report that for the first time, the nucleic acid induced perylene probe noncovalent self-assembly was able to act as a quencher, and it could very efficiently quench the fluorescence of the donor fluorophore. This new class of universal quencher can be formed “in situ”, it differs substantially from the normally used organic quenchers, and the nano-material based quenchers as mentioned above, and therefore should find many applications in new biosensing technology developments. As a proof of principle demonstration, by using this new type of quencher, a nucleic acid aptamer based method for the highly sensitive and selective quantification of a target protein [PDGF-BB] has been developed.

Since a single-stranded DNA is a polyanion, it could induce aggregation of the positively charged compound 1 via electrostatic interactions, and the strong intermolecular hydrophobic and π-π stacking interactions among compound 1 molecules caused quenching of their fluorescence. Fig. 1 shows that both the FAM labeled oligonucleotide [FAM-apt, Table S1, ESI†] and compound 1 display strong fluorescence in 20 mM MOPS (pH 8.0) aqueous buffer solution. However, when FAM-apt was mixed with compound 1, the fluorescence of both disappeared. The results suggest that when FAM-apt was mixed with compound 1, the induced aggregation caused quenching of compound 1 fluorescence. In addition, because the FAM fluorophore was labeled on the oligonucleotide, it was in close contact with the nucleic acid induced perylene aggregate.
proximity to the oligonucleotide bound non-fluorescent compound 1 aggregate (the quencher), and the resonance energy transfer from the excited FAM fluorophore to the quencher compound 1 aggregate caused efficient quenching of the FAM fluorescence. Fig. S1 (ESI†) shows the gradual decrease of the FAM fluorescence with the increase of compound 1 concentration. We were able to obtain a quenching efficiency of up to 99.6%, which is comparable to the quenching performance of the 1.4 nm gold nanoparticle (98.4%–99.9%),3\textsuperscript{w} graphene (97%)3\textsuperscript{y} and single-wall carbon nanotube (98%)10\textsuperscript{y} based quenchers.

FAM-apt induced compound 1 aggregation and FAM fluorescence quenching was a fairly rapid process, a simple “add and mix” procedure was sufficient to reach the equilibrium (compound 1 aggregation can be induced by either single-stranded or duplex DNA, and similar quenching efficiency was obtained, Fig. S8 (ESI†)). In addition, it was surprising to find out that although formed via only noncovalent interactions, the quencher (perylene self-assembly) is fairly thermally stable. With the increase of the solution temperature, very little emission intensity increase at 517 nm was observed, indicating that quenching of FAM fluorescence was still very efficient at elevated temperatures (Fig. S2, ESI†). We were able to obtain a quenching efficiency of 99.1% at 50 °C, and even at 90 °C, a quenching efficiency of 96.5% was still obtained. The quencher self-assembly was also found to be very resistant to high solution ionic strength (Fig. S3, ESI†). We were able to obtain a quenching efficiency of 99.1% with a buffer solution containing 100 mM NaCl, and a quenching efficiency of 97.6% was still obtained with a buffer solution containing 500 mM NaCl.

In addition, when the concentration of FAM-apt was reduced, compound 1 needed to induce efficient quenching was also substantially reduced. For example, when 2 nM of FAM-apt was used, only 20 nM of compound 1 was needed to reach a quenching efficiency of 97.6%. It should also be pointed out that since most of the compound 1 monomer molecules had aggregated in the vicinity of the oligonucleotide, little free dye monomer was left in the sample solution, as judged by the almost complete quenching of the dye monomer emission.

When FAM-apt was treated with nuclease S1, the emission intensity of FAM increased evidently (Fig. 1, curve d). Nuclease S1 is a single-stranded nucleic acid specific nuclease that can digest single-stranded DNA very efficiently. Nuclease S1 treatment effectively degrated the oligonucleotide, and therefore the fluorescence of the released FAM fluorophore was detected (Scheme 1). It is interesting to note that the fluorescence intensity of the released FAM is higher than the oligonucleotide bound FAM. Literature reports have shown that nucleic acid bases could act as quenchers to quench the nearby fluorophore’s fluorescence.8 Degradation of the oligonucleotide effectively separated the FAM fluorophore from the nucleic acid bases, as a result, increased FAM fluorescence was observed. In addition, since the degraded oligonucleotide was no longer a polyanion, no induced aggregation was observed when compound 1 was added to the assay mixture, and therefore compound 1 monomer emission was also observed (Fig. 1, curve e).

Based on the FAM-apt induced compound 1 aggregation and the quenching of the FAM fluorescence, we have designed a novel nucleic acid aptamer based method for the highly sensitive and selective sensing of a protein.9

Platelet-derived growth factor (PDGF), a family of growth factor proteins in human platelets, is directly related to human tumor growth.10 Literature reports related to the selective detection of PDGF include fluorescent,11 colorimetric,12 and electrochemical13 methods. PDGF-BB (an isoform of PDGF) was chosen as the model protein. A PDGF binding aptamer was selected.11–14 The aptamer binds to the target protein through a three-way helix folding conformation (Scheme 2). It has two complementary sequences (four base pair) at the ends (5'CAGG-aptamer-CCTG-3'), which could potentially form an end duplex structure.

The sensing strategy is as follows (Scheme 2): (A) The PDGF aptamer was split into two halves (FAM-apt + apt-2, Table S1 (ESI†)), and the ends of the aptamer were elongated by six base pairs so a recognition site for a restriction endonuclease (nicking enzyme Nt.BbvCI) was introduced. Nt.BbvCI can recognize a specific sequence of a double-stranded DNA and create a nick point at a specific position of one of the strands. The nicking enzyme does not bind to single-stranded DNA. (B) The DNA strand with the nicking site was end labeled with an FAM fluorophore (FAM-apt). In the absence of PDGF, the two halves of the aptamer were separated, no binding of the restriction endonuclease took place at an elevated temperature (46 °C). When compound 1 was added, induced aggregation of compound 1 took place, compound 1 monomer emission disappeared, and the FAM fluorescence was efficiently quenched by the compound 1.
aggregate as a quencher to quench the FAM fluorescence highly efficiently (the exact mechanism of quenching, including the possible formation of any ternary structures is currently under investigation). Thus our perylene aggregate represents a new class of highly efficient quencher, it is formed in situ via only noncovalent interactions, and is stable at higher temperature and ionic strength conditions. By using the perylene aggregate quencher, a novel fluorescence turn-on assay method for the highly sensitive and selective sensing of a target protein (PDGF) has been demonstrated. We envision that our perylene aggregate quencher could facilitate new biosensing method developments.

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Notes and references
