Supporting Information

Superquencher Formation via Nucleic Acid Induced Noncovalent Perylene Probe Self-Assembly

Bin Wang, Huping Jiao, Wenying Li, Dongli Liao, Fangyuan Wang, and Cong Yu*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China

Fax: (+86) 431-8526-2710; E-mail: congyu@ciac.jl.cn

1. General experimental details.

Chemicals

Oligonucleotides used in this study were synthesized and purified with PAGE by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides are given in Table S1. Nuclease S1 was bought from Sangon Biotechnology Co., Ltd., and nicking endonuclease Nt.BbvCI was obtained from New England BioLabs, Inc. (MA, USA). Thrombin was purchased from Sigma (Missouri, USA). Hemoglobin was obtained from Worthington Biochemical Corporation (New Jersey, USA). Bovine serum albumin (BSA) was bought from Bio Basic Inc (Toronto, Canada). Lysozyme, trypsin, and collagenase I were obtained from Dingguo Biotechnology Development Co., Ltd (Beijing, China). Compound **1** was synthesized and purified as previously described.[s1-s4] All other chemicals were of analytical grade. The aptamer and protein solutions were stored at 4 ºC before use. All stock and buffer solutions were prepared using water purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA).

Fluorescence Measurements

Emission spectra were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). An excitation wavelength of 490 nm was normally used, and an excitation wavelength of 465 nm was used in order to get the full emission spectrum of the FAM-apt (Table S1) in Figure S5. Excitation and emission bandwidths were both of 2 nm [when 100 nM FAM labeled oligonucleotide (FAM-apt)

was used], or 5 nm (when 1 nM FAM-apt was used). Quartz cuvettes with 10-mm path length and 2-mm window width were used for UV-vis and emission spectra measurements. The fluorescence intensity changes at 517 nm (FAM maximum emission) was used to evaluate the performance of the proposed assay strategy. Unless otherwise specified, all spectra were taken at 25 ºC in 20 mM MOPS at pH 8.0. Under the optimized conditions, the limit of detection of the FAM labeled oligonucleotide (FAM-apt) by the spectrofluorometer was estimated to be 5-10 pM.

Assay procedures

Nuclease S1 digestion assay: The enzymatic digestion of FAM-apt was carried out by the addition of nuclease S1. Briefly, 2.5 μL of 10 μM FAM-apt was mixed with 21.5 μL of 20 mM HAc-NaAc buffer [pH 4.6, containing 1 mM $Zn(Ac)_2$], 1 μL of 10 U/μL nuclease S1, and incubated at 37 ºC for 30 min. 215 μL of 20 mM MOPS (pH 8.0) and 10 μL of compound **1** were then added, and the emission spectra were recorded.

Quantification of PDGF

Firstly, 2.5 μL of 100 nM FAM-apt and 2.5 μL of 100 nM apt-2 were added into a 500 μL centrifuge tube containing 43 μL NEBuffer 4 [50 mM KAc, 20 mM Tris-HAc, 10 mM $Mg(Ac)_2$, 1 mM DTT, pH 7.9]. The mixture was incubated at 72 ºC for 10 min, and gradually cooled to 37 ºC. 1 μL of PDGF-BB of various concentrations and 1 μL (2 U) of nicking endonuclease (Nt.BbvCI) were then added and the assay mixture was

incubated at 46 ºC for 7 h. Thereafter, 200 μL of 20 mM MOPS (pH 8.0) and 1 μL of compound **1** were added and the emission spectra were recorded.

Selectivity study

The selectivity study of our assay method was carried out according to the above-mentioned procedures, except PDGF-BB was replaced with other interference proteins (thrombin, lysozyme, hemoglobin, BSA, collagenase, and trypsin). The applicability of the proposed method for the selective detection of PDGF in a complex sample solution (sera) was also studied. 2.5 μL of 100 nM FAM-apt and 2.5 μL of 100 nM apt-2 were added into a 500 μL centrifuge tube containing 38 μL NEBuffer 4 [50 mM KAc, 20 mM Tris-HAc, 10 mM $Mg(Ac)_2$, 1 mM DTT, pH 7.9]. The mixture was incubated at 72 ºC for 10 min, and gradually cooled to 37 ºC. 1 μL of 250 nM of PDGF-BB, 1 μL (2 U) of nicking endonuclease (Nt.BbvCI), and 5 μL 1% sera were added and incubated at 46 ºC for 7 h. Thereafter, 200 μL of 20 mM MOPS (pH 8.0) and 1 μL of compound **1** were added and the emission spectra were recorded, and the recovery of PDGF was determined.

Assay optimization

The aptamer sequence and the experimental conditions were optimized for accurate and sensitive quantification of the target protein. Since the recognition site of the nicking enzyme is at the end of a duplex structure, binding and DNA nicking reaction of the enzyme may be affected. We have investigated this aspect by elongating the

complementary sequence of the aptamer by 2, 4, and 6 bases (apt-2, apt-3, and apt-4, respectively, Table S1). Figure S6 shows that apt-2 gave the best performance. The results suggest that when the complementary sequence was elongated by 2 bases, the FAM labeled DNA strand was cleaved more efficiently, and increased emission intensity was obtained. The following assay optimization experiments were all conducted with apt-2.

The optimal assay temperature was determined. The assay temperature is a very important factor, since the modified PDGF binding aptamer (FAM-apt + apt-2, Table S1) has a ten base pair complementary end sequences, if the assay temperature was low, a duplex structure would form at the end of the aptamer without the addition of PDGF. And the subsequent binding of the nicking enzyme would cause cleavage of the FAM fluorophore and increase of the emission intensity. Figure S7-A shows that the FAM fluorophore was cleaved even in the absence of PDGF at lower assay temperatures. As a result, the background emission was very high. As the assay temperature was increased to 46 ºC, much reduced background emission was obtained. At this temperature, dehybridization of the FAM labeled aptamer end duplex structure occurred, which gave low background emission. However, at 46 ºC and in the presence of the target protein, binding of the target protein caused aptamer end duplex structure formation (Scheme 2), the enzymatic action of the nicking enzyme effectively cleaved the labeled FAM fluorophore, and a turn-on fluorescence signal was observed. At temperatures higher than 46 ºC, a small decrease of the background

emission was observed, but the intensity of the turn-on fluorescence signal also decreased. The ratio of signal to background reached to its maximum value at 46 ºC.

Figure S7-B shows the changes of the FAM emission intensity with the increase of the reaction time. The results show that the FAM emission intensity increased gradually, and the maximum emission was obtained after 7 hours of reaction, after which no further increase of the FAM emission was obtained, indicating that all the labeled FAM fluorophore molecules were cleaved. The optimized assay conditions are the following: an assay temperature of 46 ºC and an enzymatic reaction time of 7 hours.

Quenching of FAM fluorescence

FRET is a distance-dependent transfer of energy from the excited donor fluorophore to a suitable acceptor fluorophore or quencher. The efficiency of energy transfer depends largely on the distance between the donor and the acceptor, and the degree of the spectral overlap. Figure S5 shows the Uv-vis absorption spectra of compound **1** monomer (at 90 ºC) and aggregate (at 10 ºC) and the emission spectrum of FAM-apt.[s1] The results clearly show that the absorption spectrum of the compound **1** aggregate overlaps well with the emission spectrum of FAM-apt. Efficient FRET could take place between the FAM fluorophore and the compound **1** aggregate when they are close enough. And since compound **1** aggregate is not fluorescent, quenching of the FAM fluorescence was observed. On the contrary, the UV-vis spectrum of compound **1** free monomer also has a good spectral overlap with the emission

spectrum of FAM-apt, but since they were well separated in solution, no efficient energy transfer was observed, and therefore the emission spectra of both were observed (Figure 1, curve e). It should also be pointed out that it is possible that at least a portion of the FAM fluorophore molecules were quenched via c or static/contact quenching mechanisms.[1a]

References

[s1] B. Wang, C. Yu, *Angew. Chem. Int. Ed.* **2010**, *49*, 1485–1488.

[s2] T. Deligeorgiev, D. Zaneva, I. Petkov, Il. Timcheva, R. Sabnis, *Dyes Pigm.* **1994**, *24*, 75–81.

[s3] Z. R. Liu, R. L. Rill, *Anal. Biochem.* **1996**, *236*, 139–145.

[s4] T. Ma, C. Li, G. Q. Shi, *Langmuir* **2008**, *24*, 43–48.

Table S1. Oligonucleotides used in the present study. End complementary sequences are marked with underline, the nicking endonuclease Nt.BbvCI binding site is given in red color, and the enzyme cleavage point is marked with "↓".

Oligonucleotide	Sequence
FAM-apt	5′-(FAM)-**CC↓T CAG C**AG GCT ACG GCA CGT AGA-3′
Apt-1	5′-GCA TCA CCA TGA TCC T**GC TGA GG**-3′
Apt-2	5′-GCA TCA CCA TGA TCC T**GC TGA GG**T A-3′
Apt-3	5′-GCA TCA CCA TGA TCC T**GC TGA GG**T ATC-3′
Apt-4	5′-GCA TCA CCA TGA TCC T**GC TGA GG**T ATC TA-3′

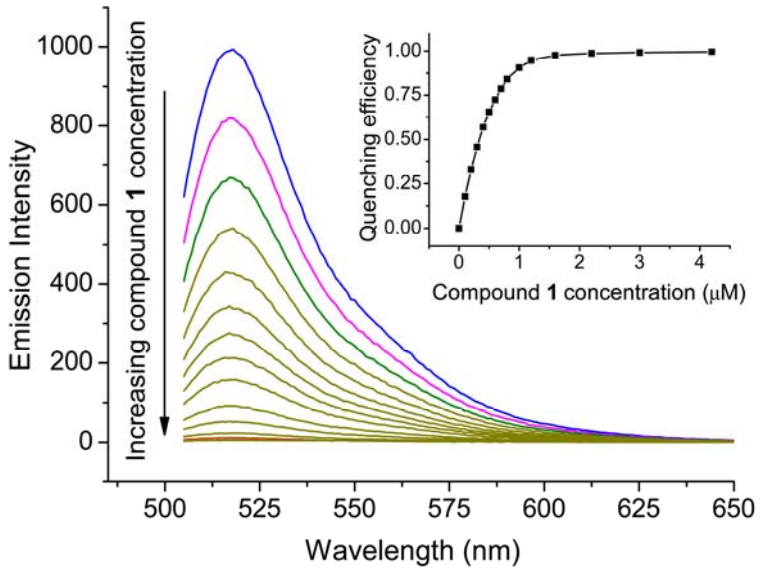

Figure S1. Emission spectral changes of 100 nM FAM-apt with the addition of increasing concentrations of compound **1**. Inset: Changes in quenching efficiency upon the addition of increasing concentrations of compound **1**.

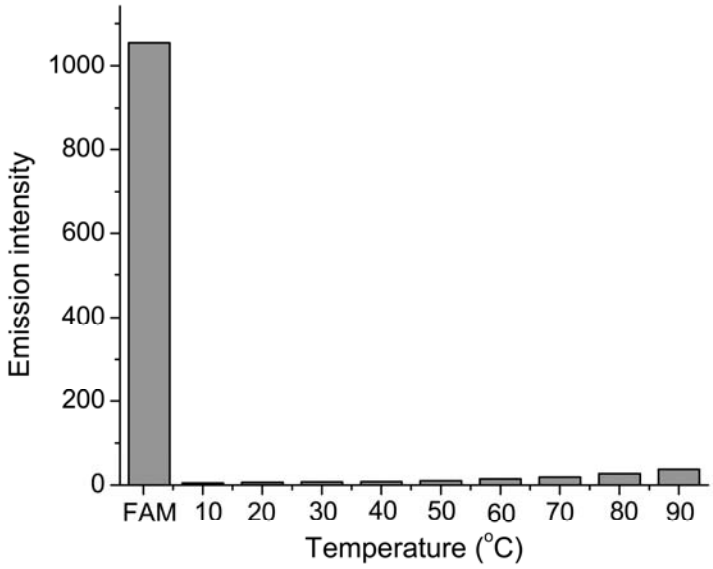

Figure S2. The effect of solution temperature on the emission intensity of the FAM fluorophore at 517 nm. FAM: FAM-apt only; the rest: 100 nM FAM-apt + 4.2 μM compound **1**.

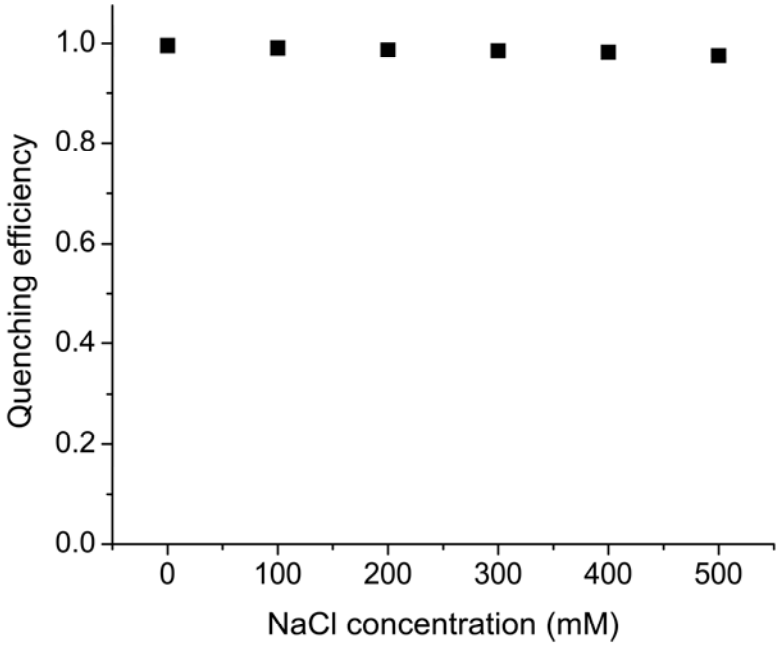

Figure S3. The effect of solution NaCl concentration on the FAM fluorophore quenching efficiency. Final concentration: FAM-apt, 100 nM; compound **1** needed, 4.2, 4.5, 6.2, 13.2, 37.0, 74.4 μM, respectively (with 0 → 500 mM increase in NaCl concentration). Buffer: 10 mM MOPS, pH 8.0.

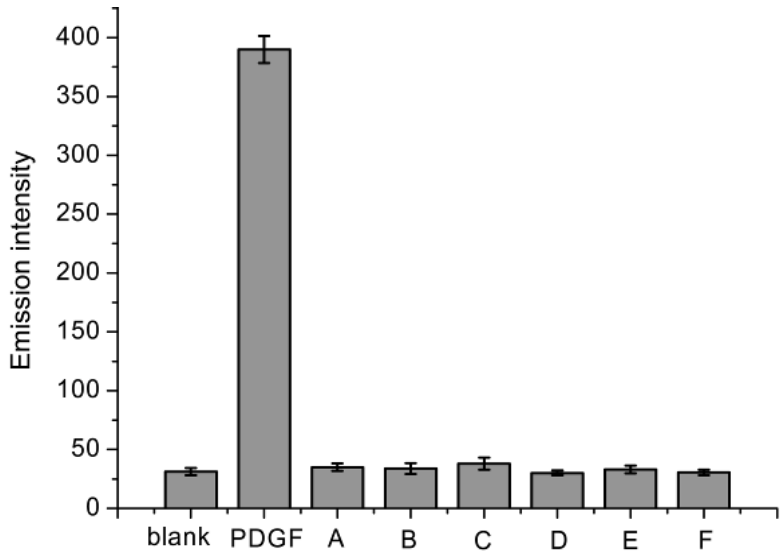

Figure S4. Selectivity of the present assay. Proteins were thrombin (A), BSA (B), hemoglobin (C), lysozyme (D), collagenase (E), and trypsin (F). Protein concentration: 20 nM.

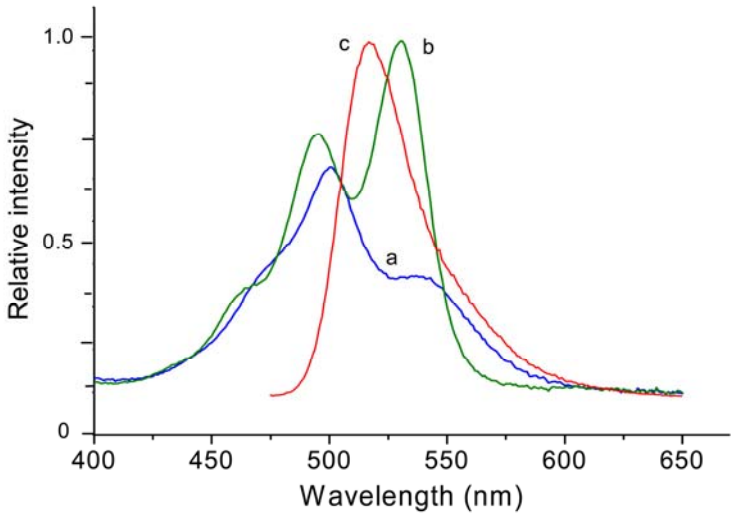

Figure S5. Uv-vis absorption spectra of 1 μM compound **1** at 10 ºC (a), at 95 ºC (b), and the emission spectrum of 100 nM FAM-apt (c).

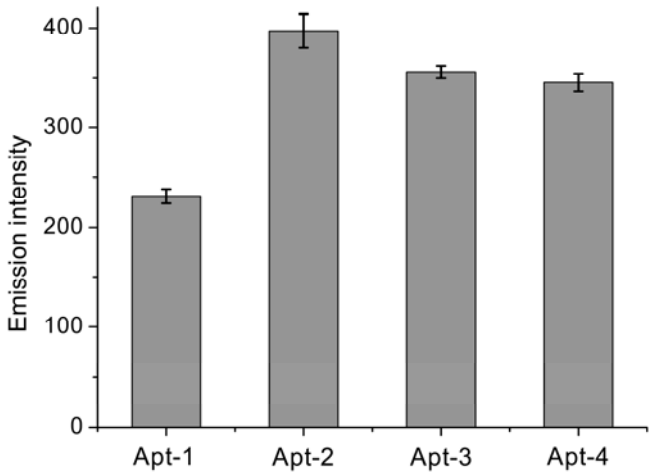

Figure S6. Aptamer sequence dependent sensing of PDGF. FAM-apt was separately mixed with apt-1, apt-2, apt-3, or apt-4. 20 nM PDGF and 2 U nicking enzyme were added, the assay mixtures (50 μL each) were incubated at 46 ºC for 7 hours. 200 μL of 20 mM MOPS (pH 8.0) and 1 μL of compound **1** were added, and the fluorescence spectra were recorded. Final concentration: FAM-apt, 1 nM; apt-1 (or apt-2, apt-3, and apt-4), 1 nM; compound **1**, 20 nM.

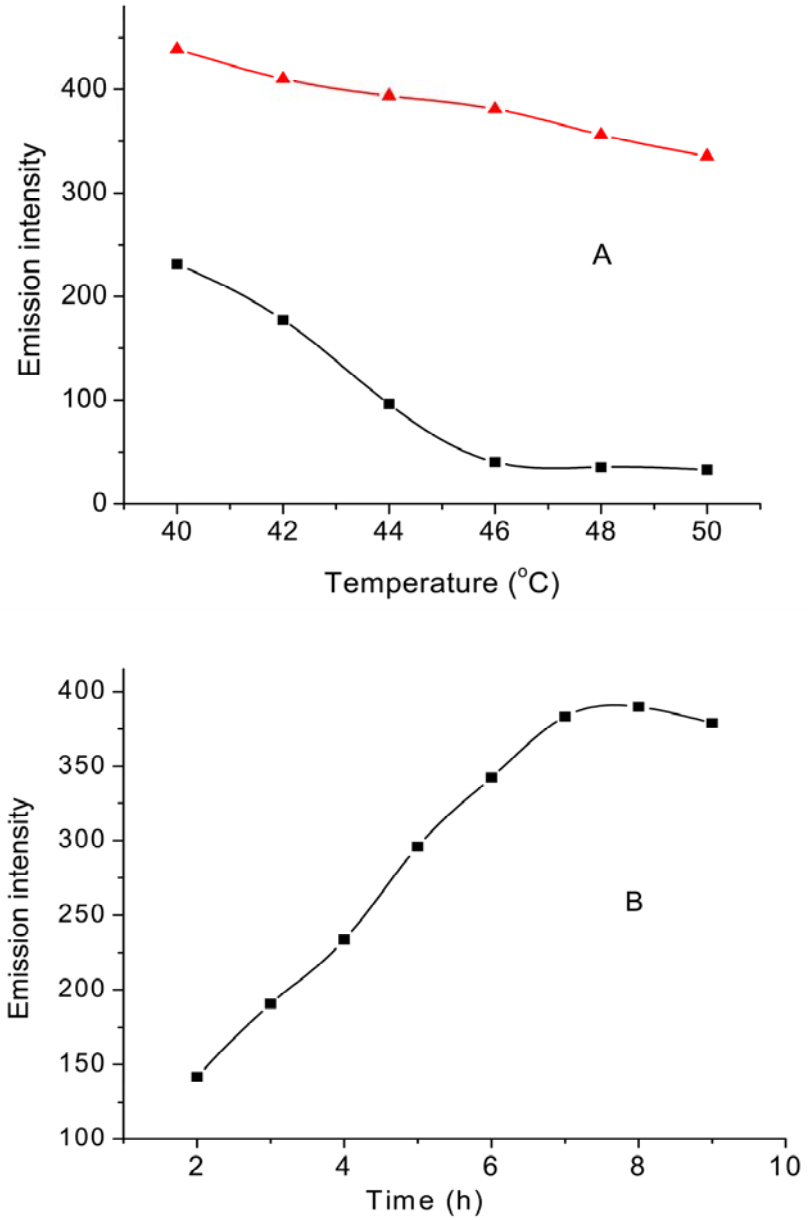

Figure S7. The effect of the assay temperature (A) and time (B) on the detection of PDGF. A: Fluorescence intensity changes with the assay temperature in the presence (▲) and absence (■) of 20 nM PDGF. Reaction time: 7 hours. B: Fluorescence intensity changes with reaction time. Conditions: 20 nM PDGF, and at a reaction temperature of 46 ºC. Final concentration: FAM-apt, 1 nM; apt-2, 1 nM; compound **1**, 20 nM.

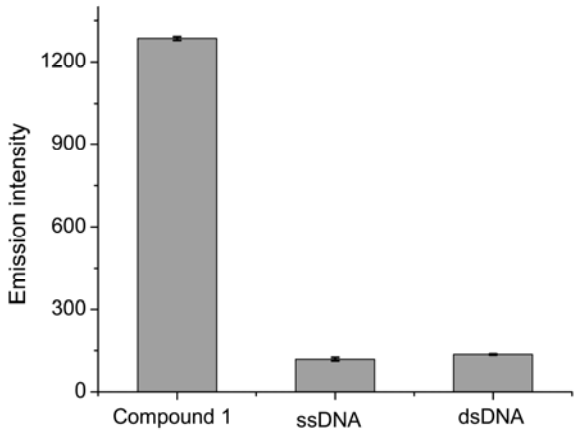

Figure S8. Comparison of the induced aggregation of compound **1** by single stranded DNA (oligo-dM: GAA CTC AGC ATC CAT GAT CGA CAG T) and duplex DNA (oligo-dM + oligo-dN; dN: ACT GTC GAT CAT GGA TGC TGA GTT C). Compound **1** concentration was 100 nM; oligo-dM only: 8 nM; duplex DNA: oligo-dM and oligo-dN each of 4 nM.

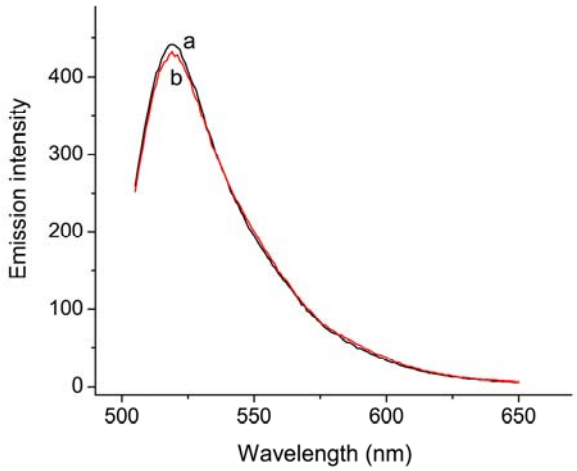

Figure S9. (a) Compound **1** was added after the addition of the nicking enzyme. (b) Compound **1** was added before the addition of the nicking enzyme. Conditions: the same as described in Figure 2.

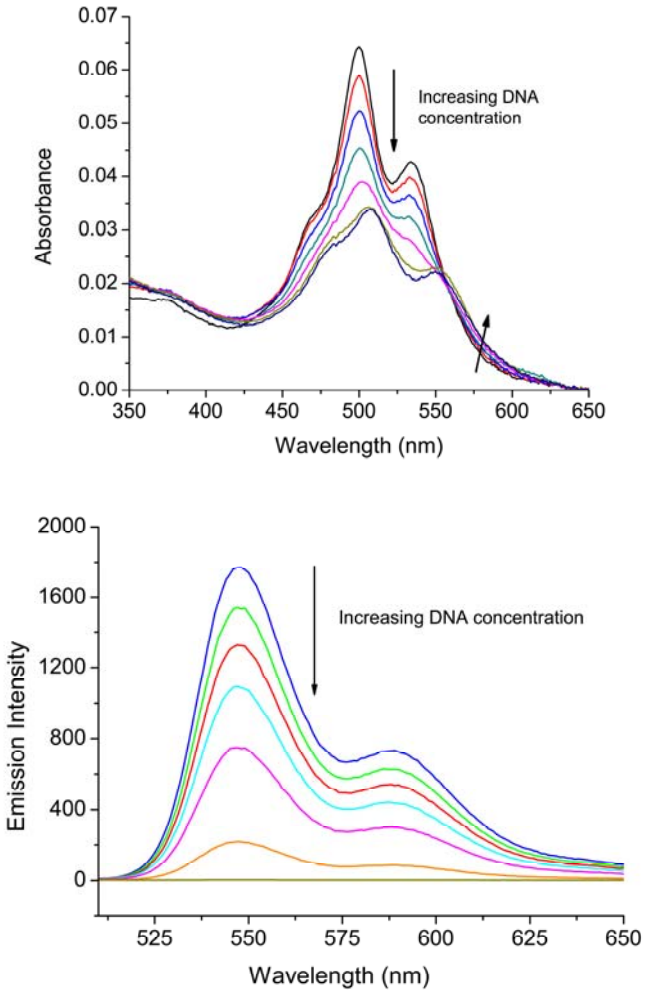

Figure S10. Uv-vis (top) and emission (bottom) spectral changes with the increase of the solution oligonucleotide concentration. Compound **1** concentration: 1 μM; oligo-dM: GAA CTC AGC ATC CAT GAT CGA CAG T; buffer: 10 mM MOPS pH 8.0. 30 nM of oligo-dM was needed for the complete quenching of compound **1** fluorescence.