Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Rapid and Enzyme-free Nucleic Acids Based on Exponential Hairpin Assembly in Complex Biological Fluids

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Experimental Section

Materials： Oligonucleotides involved in this study were designed by using NUPACK software (http://www.nupack.org/) and produced by Shanghai Bio-Engineering Company (Shanghai, China). All DNAs (sequences listed in Table S1-5) were purified by high-performance liquid chromatography (HPLC). RPMI 1640 cell medium including 10% fetal bovine serum (FBS) was purchased from Shanghai Bio-Engineering Company (Shanghai, China). All other reagents were of analytical grade and used as received.

Methods： The DNA stock solutions were prepared in sterilized hybridization buffer (20 mM Tris-HCl, 3 mM $MgCl_2$, pH 7.4), and were heated to 95°C for 5 min and then cooled to room temperature for use. Different concentrations of target DNA were added to a final volume 100 μL (10 mM PBS, 5 mM $MgCl_2$, pH 7.4) including each of four DNA hairpins in a final concentration of 5.0×10^{-7} M. The reaction system was incubated at room temperature for 15 min, followed by agarose gel electrophoresis or fluorescence detection by a Hitachi F-4500 spectrophotometer (Tokyo, Japan) equipped with a xenon lamp.

Supplementary Figures

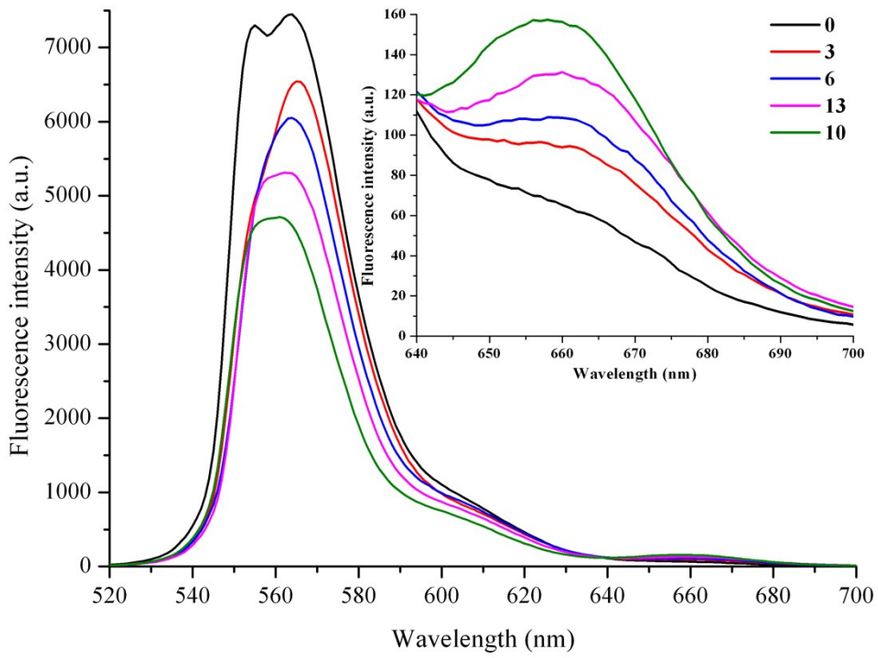

Figure S1. The FRET efficiency from Cy3 to Cy5 was optimized. The distance we optimized between Cy3 and Cy5 were 0 base, 3 bases, 6 bases, 10 bases and 13 bases. The sequences can be found in Table S1-5.

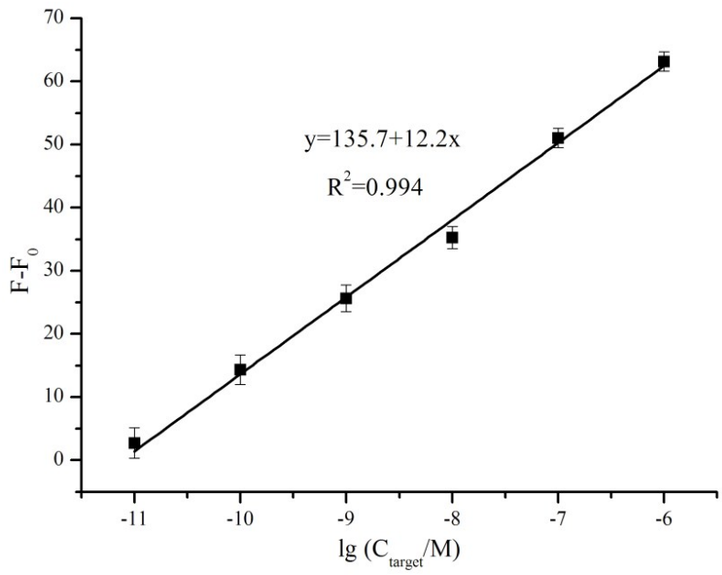

Figure S2. The relationship between the change in fluorescence signal of Cy5 and the logarithm of the target DNA concentration.

Additionally, pyrene can also act as a spatially sensitive fluorescent dye and have been introduced to nucleic acids detection in complex biological fluids.[1] We have labeled hairpins H2 and H3 at two ends with pyrene moieties, and the result was shown in Figure S3. According to the literature,[2-3] the formation of the pyrene excimer of aromatic hydrocarbons is restricted to a parallel, aspectant configuration, while two pyrene molecule were brought into close enough by a head- to- head way in our detection system. Therefore, the result was not satisfactory.

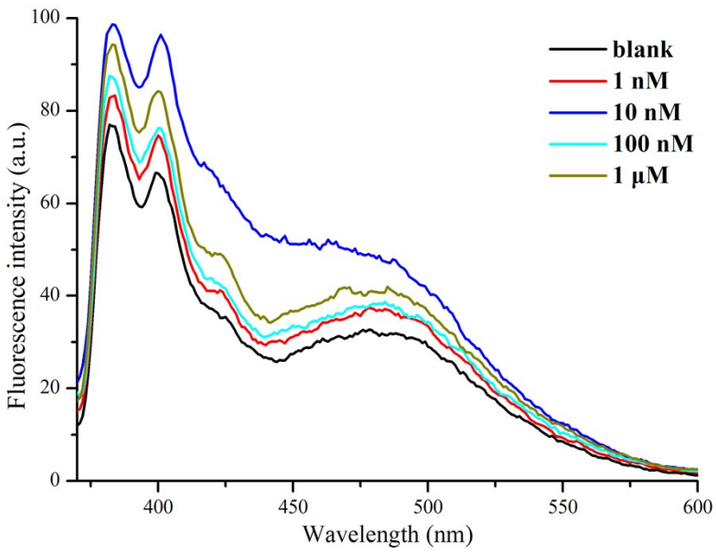

Figure S3. Fluorescence spectra in the presence of different concentrations of target DNA under λ_{ex} = 340 nm. The DNA hairpins H2 and H3 were dual-labeled with pyrene moieties.

Supplementary Tables

Supplementary Table S1: Sequences used in this work.

Name (domain)	Sequence (from 5′ to 3′)
H1(5-2'-3'-4-3-2-1)	CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC-***CAGAAC*-**GTTGGCTCTAACGGTTTC-GGTTGT-**GGATTG
H2(4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC-***CAATCC-ACAACC*-**GAAACCGTTAGAGCCAAC**
H3(2-5'-6-4-5)	GGTTGT-**CTCGCAGT**(Cy3)**TCACTCACAG-***AGGAGT-CAGAAC*-**CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC*-**CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT-Cy5
T (matched)(1'-2'-3')	CAATCC-ACAACC-GAAACCGTTAGAGCCAAC
T(mismatched)(1'-2'-3')	CAATCC-ACAAC**T**-GAAACCGTTAGAGCCAAC
T(inserted)(1'-2'-3')	CAATCC-ACAACC-**A**GAAACCGTTAGAGCCAAC
T(deleted)(1'-2'-3')	CAATCC-ACAACC-**_**AAACCGTTAGAGCCAAC

All sequences used were annotated with domain names, each of which represented a short oligonucleotide fragment. The numbered domain was complementary with the corresponding marked domain by a symbol ('). The boldface and italic portions in hairpins were stems and loops, respectively. The mismatched, deleted, and inserted bases of target DNA were shown by boxes.

Supplementary Table S2: All Sequences used for the Cy3-Cy5 distance with 0 base.

Name (domain)	Sequence (from 5′ to 3′)
H1(5-2'-3'-4-3-2-1)	Cy3-CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC-***CAGAAC***-GTTGGCTCTAACGGTTTC-GGTTGT-**GGATTG
H2(4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC-***CAATCC-ACAACC***-GAAACCGTTAGAGCCAAC**
H3(2-5'-6-4-5)	GGTTGT-**CTCGCAGTTCACTCACAG-***AGGAGT-CAGAAC***-CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC***-CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT-Cy5
T(1'-2'-3')	CAATCC-ACAACC-GAAACCGTTAGAGCCAAC

All sequences used were annotated with domain names, each of which represented a short oligonucleotide fragment. The numbered domain was complementary with the corresponding marked domain by a symbol ('). The boldface and italic portions in hairpins were stems and loops, respectively.

Supplementary Table S3: All Sequences used for the Cy3-Cy5 distance with 3 bases.

Name (domain)	Sequence (from 5′ to 3′)
H1(5-2'-3'-4-3-2-1)	Cy3-CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC-***CAGAAC***-GTTGGCTCTAACGGTTTC-GGTTGT-**GGATTG
H2(4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC-***CAATCC-ACAACC***-GAAACCGTTAGAGCCAAC**
H3(2-5'-6-4-5)	GGTTGT-**CTCGCAGTTCACTCACAG-**TTT*AGGAGT-CAGAAC***-**CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC***-CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT-Cy5
T(1'-2'-3')	CAATCC-ACAACC-GAAACCGTTAGAGCCAAC

All sequences used were annotated with domain names, each of which represented a short oligonucleotide fragment. The numbered domain was complementary with the corresponding marked domain by a symbol ('). The boldface and italic portions in hairpins were stems and loops, respectively. Three poly-T inserted into domain 5′ and 6 of H3 were boxed.

Supplementary Table S4: All Sequences used for the Cy3-Cy5 distance with 6 bases.

Name (domain)	Sequence (from 5′ to 3′)
H1(5-2'-3'-4-3-2-1)	CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC-***CAGAAC*-**GTTGGCTCTAACGGTTTC-GGTTGT-**GGATTG
H2(4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC-***CAATCC-ACAACC*-**GAAACCGTTAGAGCCAAC**
H3(2-5'-6-4-5)	GGTTGT-**CTCGCAGTTCAC(Cy3)TCACAG-***AGGAGT-CAGAAC*-**CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC*-**CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT-Cy5
T(1'-2'-3')	CAATCC-ACAACC-GAAACCGTTAGAGCCAAC

All sequences used were annotated with domain names, each of which represented a short oligonucleotide fragment. The numbered domain was complementary with the corresponding marked domain by a symbol ('). The boldface and italic portions in hairpins were stems and loops, respectively.

Supplementary Table S5: All Sequences used for the Cy3-Cy5 distance with 13 bases.

Name (domain)	Sequence (from 5′ to 3′)
H1(5-2'-3'-4-3-2-1)	CTGTGAGTGAACTGCGAG-**ACAACC-GAAACCGTTAGAGCCAAC-***CAGAAC*-**GTTGGCTCTAACGGTTTC-GGTTGT-**GGATTG
H2(4'-3-1'-2'-3')	GTTCTG-**GTTGGCTCTAACGGTTTC-***CAATCC-ACAACC*-**GAAACCGTTAGAGCCAAC**
H3(2-5'-6-4-5)	GGTTGT-**CTCGC(Cy3)AGTTCACTCACAG-***AGGAGT-CAGAAC*-**CTGTGAGTGAACTGCGAG**
H4(3'-4-5-2'-5'-4'-6')	GAAACCGTTAGAGCCAAC-**CAGAAC-CTGTGAGTGAACTGCGAG-***ACAACC*-**CTCGCAGTTCACTCACAG-GTTCTG-**ACTCCT-Cy5
T(1'-2'-3')	CAATCC-ACAACC-GAAACCGTTAGAGCCAAC

All sequences used were annotated with domain names, each of which represented a short oligonucleotide fragment. The numbered domain was complementary with the corresponding marked domain by a symbol ('). The boldface and italic portions in hairpins were stems and loops, respectively.

Reference:

1 C. C. Wu, C. M. Wang, L. Yan and C. Y. J. Yang, *J. Biomed. Nanotechnol.* 2009, **5**, 1-10.

2 Z. H. Qing, X. X. He, J. Huang, K. M. Wang, Z. Zou, T. P. Qing, Z. G. Mao, H. Shi and D. G. He, *Anal. Chem.* 2014, **86**, 4934-4939.

3 M. Masuko, H. Ohtani, K. Ebata and A. Shimadzu, *Nucleic Acids Res.* 1998, **26**, 5409-5416.