Supporting Information
Dual-labeled oligonucleotide probe for sensing adenosine *via* FRET: A novel alternative to genotyping SNPs

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General Experimental:

Oligonucleotide Synthesis and Characterisation: All the reagents and the Fluorophore **FAM** [5'-Fluorescein Phosphoramidite (6-FAM)] for DNA synthesis were purchased from Glen Research. The BDF nucleosides, ^{Py}**U** [A. Okamoto, K. Kanatani, I. Saito, *J. Am. Chem. Soc.* **2004**, *126*, 4820] and $^{2\text{-Ant}}$**U** [Y. Saito, K. Motegi, S. S. Bag, I. Saito, *Bioorg. Med. Chem.* **2006**, (Article in press)] were synthesized and characterized according to our previously published literature [ODNs were synthesized by a conventional phosphoramidite method by using an Applied Biosystems 392 DNA/RNA synthesizer]. ODNs were purified by reverse phase HPLC on a 5-ODS-H column (10 x 150 mm, elution with 50 mM ammonium formate buffer (AF), pH 7.0, linear gradient over 50 min from 0% to 50% acetonitrile at a flow rate 3.0 ml/min). ODNs containing modified nucleotides were fully digested with calf intestine alkaline phosphatase (50 U/mL), snake venom phosphodiesterase (0.15 U/mL), and P1 nuclease (50 U/mL) at 37 ^{o}C for 3 h. Digested solutions were analysed by HPLC on a CHEMCO-BOND 5-ODS-H column (4.6 x 150 mm), elution with a solvent mixture of 0.1 M TEAA, pH 7.0, flow rate 1.0 mL/min). The concentration of each ODNs were determined by comparing peak areas with standard solution containing dA, dC, dG, and dT at a concentration of 0.1 mM. Mass spectra of ODNs purified by HPLC were determined with a MALDI-TOF mass spectroscopy, Shimadzu, AXIMA-LNR.

Table S1: MALDI-TOF Mass Spectral data for the ODNs

ODNs	Sequences	MALDI-TOF Mass cald. for	MALDI-TOF Mass found
3 [ODN (**F2-^{Py}U**)]	5'-d(**F**-AT^{Py}**U**TAACGCACACG)-3'	5037.61	5038.70
3a [ODN (**2-^{Py}U**)]	5'-d(AT^{Py}**U**TAACGCACACG)-3'	4499.14	4500.34
4 [ODN (**F3-^{Py}U**)]	5'-d(**F**-AAT^{Py}**U**TAACGCACACG)-3'	5350.82	5351.11
4a [ODN (**3-^{Py}U**)]	5'-d(AAT^{Py}**U**TAACGCACACG)-3'	4812.35	4813.34
5 [ODN (**F4-^{Py}U**)]	5'-d(**F**-AAAT^{Py}**U**TAACGCACACG)-3'	5664.03	5664.74
5a [ODN (**4-^{Py}U**)]	5'-d(AAAT^{Py}**U**TAACGCACACG)-3'	5125.56	5126.48
5c [ODN (**F4-$^{2\text{-Ant}}$U**)]	5'-d(**F**-AAAT$^{2\text{-Ant}}$**U**TAACGCACACG)-3'	5642.03	5643.36
5d [ODN (**4-$^{2\text{-Ant}}$U**)]	5'-d(AAAT$^{2\text{-Ant}}$**U**TAACGCACACG)-3'	5103.55	5104.79
6 [ODN (**F5-^{Py}U**)]	5'-d(**F**-AAAAT^{Py}**U**TAACGCACACG)-3'	5977.25	5978.41
6a [ODN (**5-^{Py}U**)]	5'-d(AAAAT^{Py}**U**TAACGCACACG)-3'	5438.77	5438.37
7 [ODN (**F8-^{Py}U**)]	5'-d(**F**-AAAAAAAT^{Py}**U**TAACGCACACG)-3'	5037.61	5038.70
7a [ODN (**8-^{Py}U**)]	5'-d(AAAAAAAT^{Py}**U**TAACGCACACG)-3'	4499.14	4500.34

Melting temperature (T_m) measurements: All T_ms of the ODNs (2.5 mM, final duplex concentration) were taken in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance vs temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 ^{o}C to 90 ^{o}C with a heating rate of 1 ^{o}C/min. From these profiles, first derivatives were calculated to determine T_m values.

UV Absorption Measurements: ODN solutions were prepared as described in Tm measurement experiment. Absorption spectra were obtained using a Shimadzu UV-

2550 spectrophotometer at room temperature using 1 cm path length cell.

Fluorescence measurements: ODN solutions were prepared as described in T_m measurement experiment. Fluorescence spectra were obtained using a Shimadzu RF-5300PC spectrophotometer at 25°C using 1cm path length cell. The excitation bandwidth was 1.5 nm. The emission bandwidth was 1.5 nm.

Table S2: Melting temperatures of the duplexes:

Duplexes	T_m (°C)	Duplexes	T_m (°C)	Duplexes	T_m (°C)
ODN 3/3b	54.74 (N = A) 53.46 (N = T) 51.52 (N = G) 49.94 (N = C)	**ODN 5/5b**	56.48 (N = A) 53.10 (N = T) 53.27 (N = G) 53.66 (N = C)	**ODN 6/6b**	59.50 (N = A) 55.62 (N = T) 54.31 (N = G) 54.45 (N = C)
ODN 3a/3b	59.50 (N = A) 59.84 (N = T) 57.30 (N = G) 58.47 (N = C)	**ODN 5a/5b**	57.46 (N = A) 55.57 (N = T) 52.44 (N = G) 54.88 (N = C)	**ODN 6a/6b**	59.96 (N = A) 54.37 (N = T) 53.54 (N = G) 55.27 (N = C)
ODN 4/4b	54.76 (N = A) 42.64 (N = T) 53.74 (N = G) 54.36 (N = C)	**ODN 5c/5b**	61.52 (N = A) 51.23 (N = T) 50.63 (N = G) 52.71 (N = C)	**ODN 7/bb**	62.34 (N = A) 59.04 (N = T) 59.63 (N = G) 56.85 (N = C)
ODN 4a/4b	59.65 (N = A) 56.74 (N = T) 59.65 (N = G) 59.44 (N = C)	**ODN 5d/5b**	57.54 (N = A) 50.45 (N = T) 52.54 (N = G) 55.24 (N = C)	**ODN 7a/7b**	61.86 (N = A) 59.47 (N = T) 57.54 (N = G) 57.86 (N = C)

Fluorescence decay measurements: Fluorescence decay (sample solution in sodium phosphate buffer, pH 7.0, was degassed by N_2 bubbling for 5 minutes) was measured by a two-dimensional photon-counting method with the picosecond fluorescence lifetime measurement system (C4334, Hamamatsu). A SHG (second hermonic generation) of titanium sapphire laser (Tsunami, Spectra Physics, 355 nm) was used as the excitation light source. The fluorescence emission was collected at 420 nm.

Calculation of Förster Radius (R_0): Förster distance R_0 was estimated from a plot of r/R_0 *vs.* number of inserted A/T base pairs utilising the following equation **1**,

$$\frac{1}{\tau_{rise}} \cong k_{FRET} = \frac{1}{\tau_D^0}\left[\frac{R_0}{r}\right]^6 \qquad \mathbf{1}$$

where, τ_D^0 is lifetime of donor (τ_2) as was shown in **Table 2** in the text, R_0 is Förster distance, and r is the interaction distance between donor and acceptor, respectively. Using the above equation **1**, a plot of r/R_0 against the number of inserted A/T base pairs (2, 3, 4, 5, and 8) gives rise to a straight line with a positive slope (**Fig. S1**). When R_0 is equal to r, the number of A/T base pairs obtained from the graph was 11.4 base pairs. Based on the average contour length and the total number of base pairs as was measured from scanning force microscope image, Rivetti *et al.* have reported the mean inter-nucleotide distance of double-stranded DNA is 2.9 Å. Thus, if we assume

the distance of one A/T base pair as 2.9 Å, we would obtain the Förster distance (R_0) as about 33 Å [**Ref.** (a) M. Masuko, S. Ohuchi, K. Sode, H. Ohtani and A. Shimadzu, *Nucl. Acids Res.,* 2000 **28**, e34; (b) C. Rivetti, C. Walker and C. Bustamante, *J. Mol. Biol.,* 1998, **280**, 41].

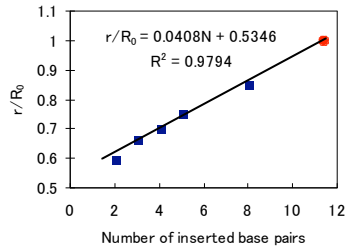
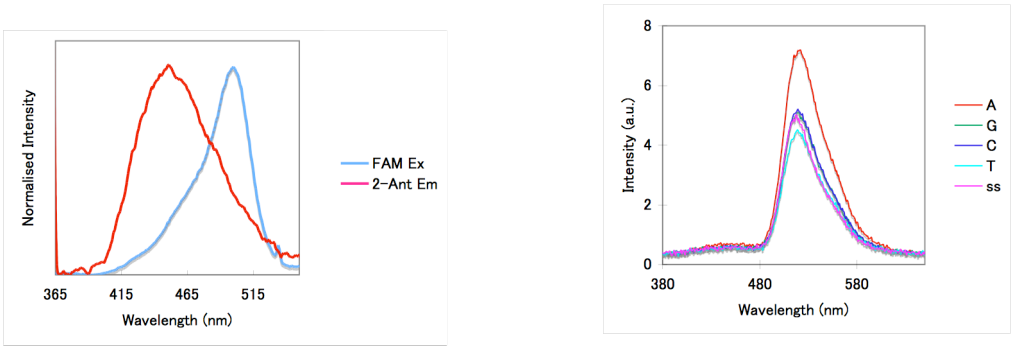
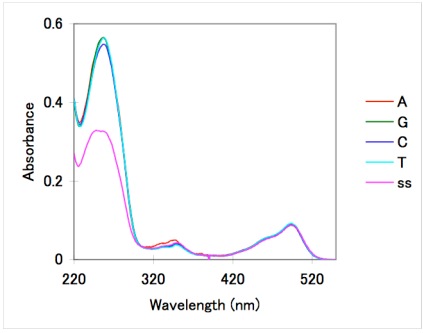


Figure S1: A plot r/R_0 *vs.* number of inserted A/T base pairs for ODNs containing ^{py}**U** as the donor and fluorescein as the acceptor.

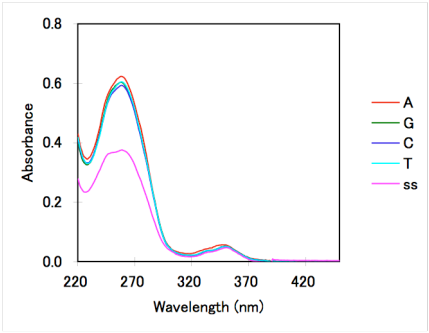


a **b**

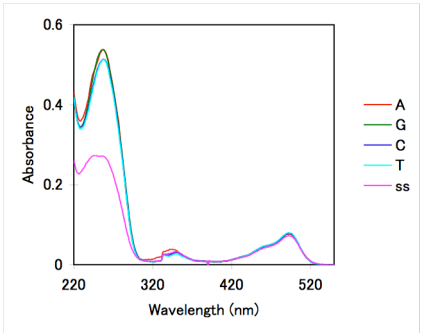
Figure S2: (a) Overlap of the emission spectrum of ODN **5d** [ODN (**4**-$^{2\text{-Ant}}$**U**)] (red) and the excitation spectrum of FAM (blue). (b) Fluorescence spectra of single stranded ODN **5c** [ODN (**F**4-$^{2\text{-Ant}}$**U**)] (2.5 μm) and the different duplexes formed by hybridization with ODN **5b** {[ODN (**4**-**N**) (**N** = A, C, G, T)]} (2.5 μm, 50 mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT). Excitation wavelength was 350 nm.



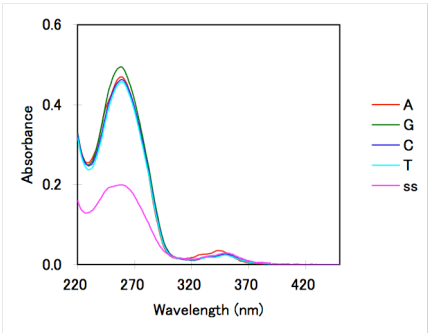
Probe ODN **3** and with its targets



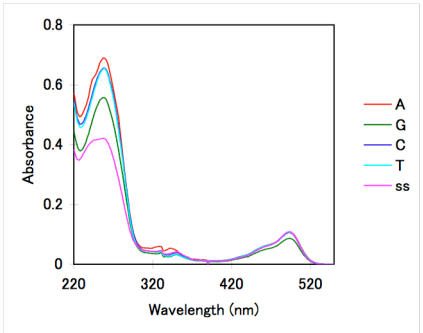
Probe ODN **3a** and with its targets



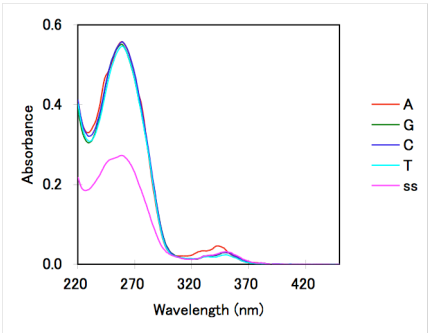
ODN **4** with or without target ODNs



ODN **4a** with or without target ODNs

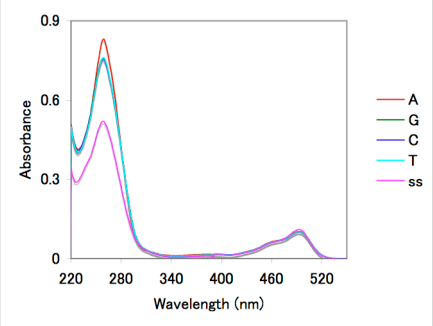


ODN **5** with or without target ODNs

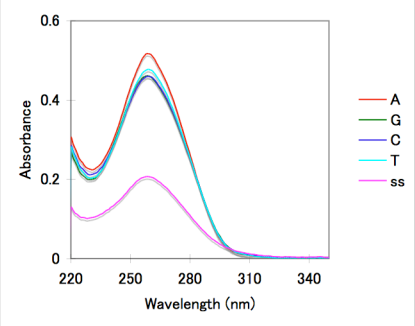


ODN **5a** with or without target ODNs

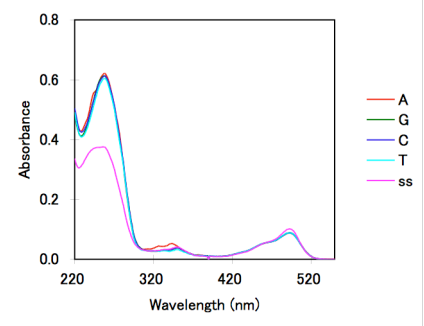
Figure S3: UV-visible spectra of probes alone (ss) and with their targets (A, G, C, T) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]



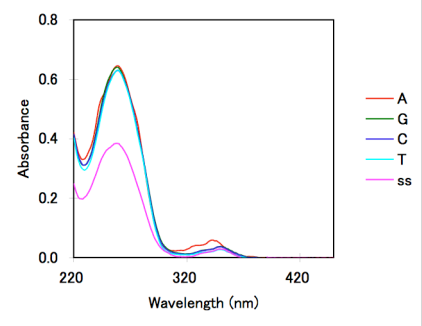
ODN **5c** with or without target ODNs



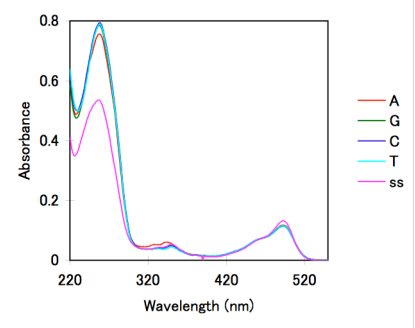
ODN **5d** with or without target ODNs



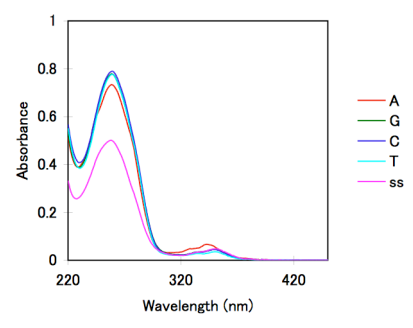
ODN **6** with or without target ODNs



ODN **6a** with or without target ODNs

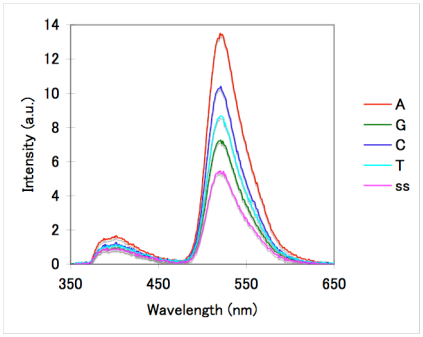


ODN **7** with or without target ODNs

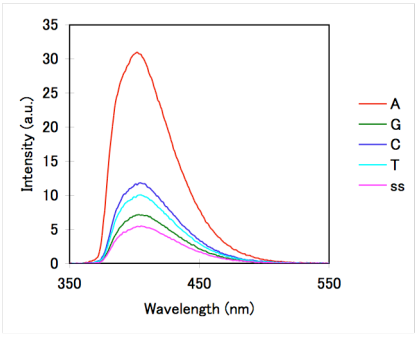


ODN **7a** with or without target ODNs

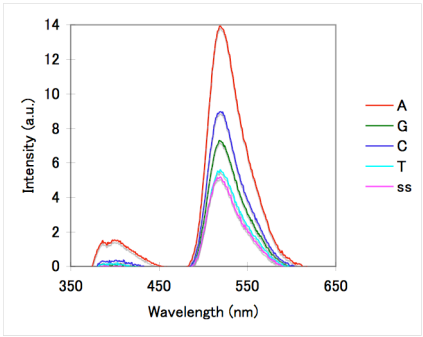
Figure S4: UV-visible spectra of probes alone (ss) and with their targets (A, G, C, T) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT].



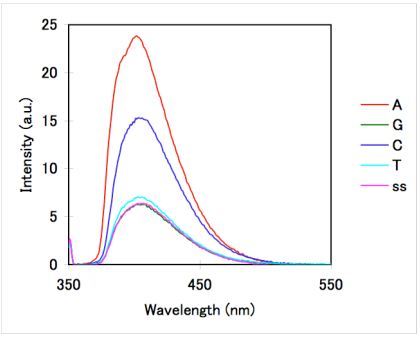
ODN **3** with or without target ODNs



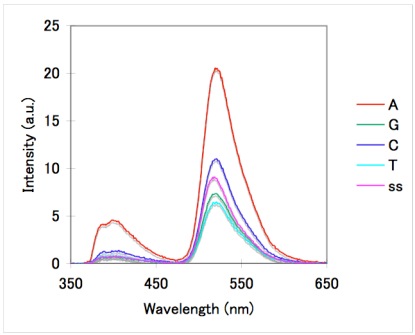
ODN **3a** with or without target ODNs



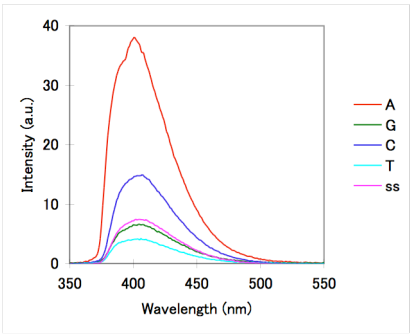
ODN **4** with or without target ODNs



ODN **4a** with or without target ODNs

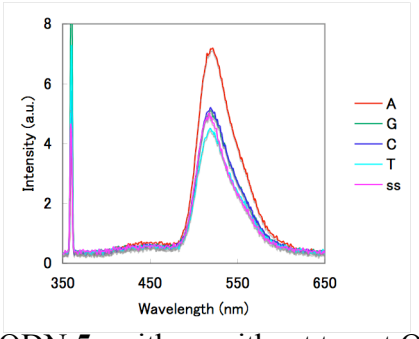


ODN **5** with or without target ODNs

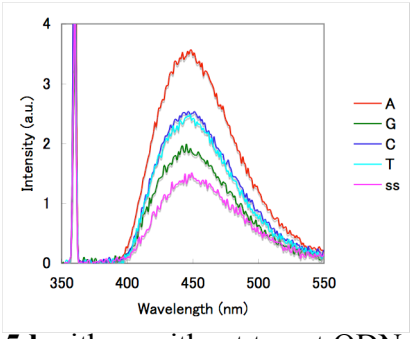


ODN **5a** with or without target ODNs

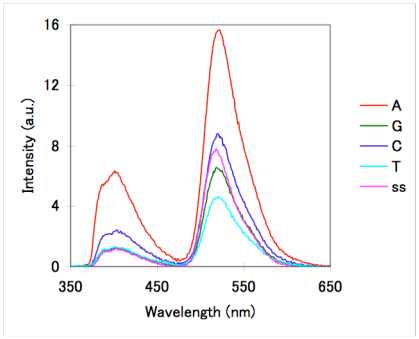
Figure S5: Fluorescence spectra of probes alone (ss) and with their targets (A, G, C, T) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]. Excitation wavelength was 340 nm. "ss"denotes single-stranded ODN.



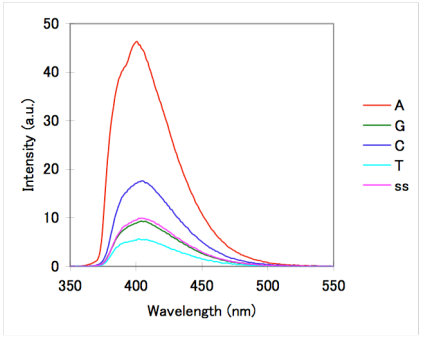
ODN **5c** with or without target ODNs



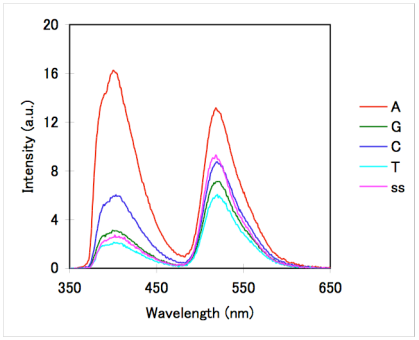
ODN **5d** with or without target ODNs



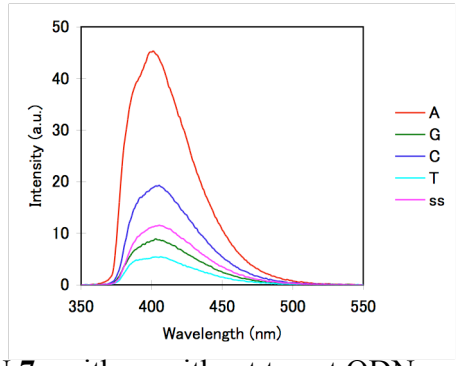
ODN **6** with or without target ODNs



ODN **6a** with or without target ODNs

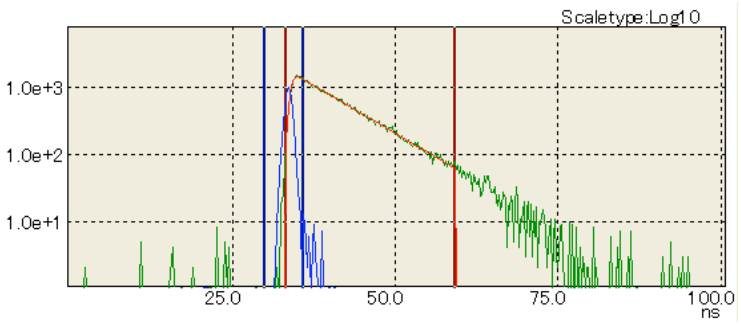


ODN **7** with or without target ODNs

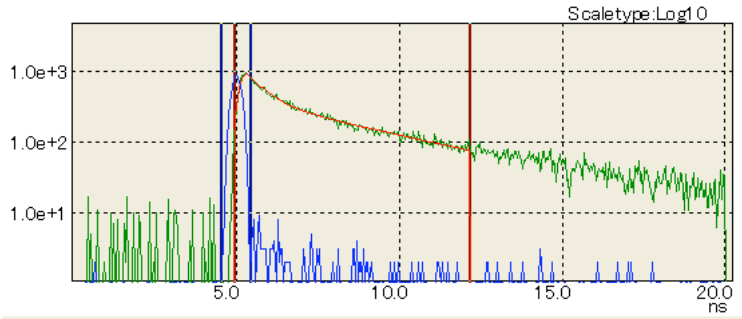


ODN **7a** with or without target ODNs

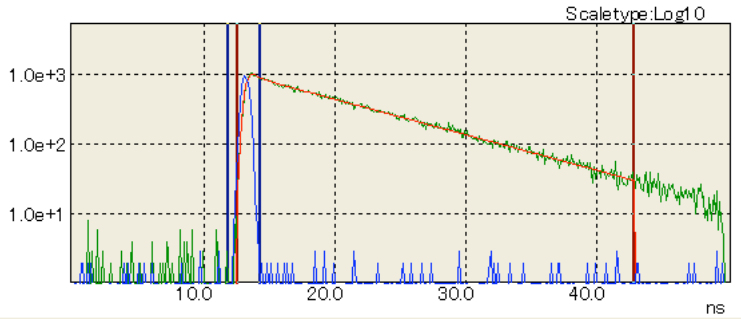
Figure S6: Fluorescence spectra of probes alone (ss) and with their targets (A, G, C, T) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]. Excitation wavelength was 340 nm. "ss"denotes single-stranded ODN.



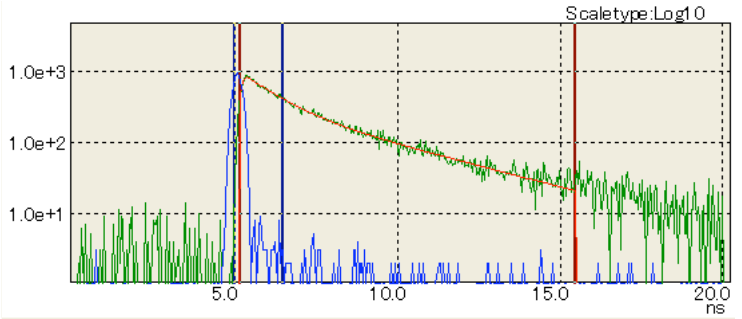
Decay profile of ^{Py}**U** in Duplex ODN **3a/3b**
[τ_2 = 7.913 ns]



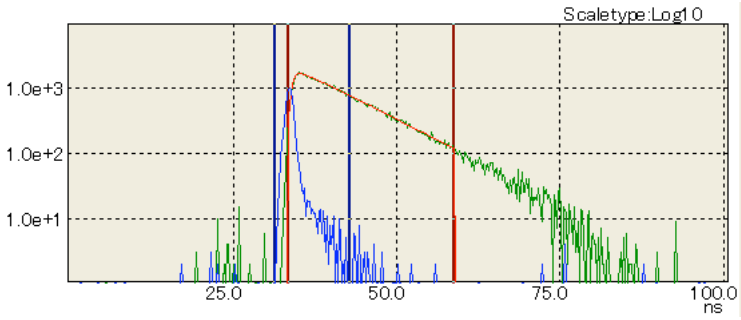
Decay profile of ^{Py}**U** in Duplex ODN **3/3b**
[(τ_1 = 0.599 (69%) ns and τ_2 = 4.363 (31%) ns)



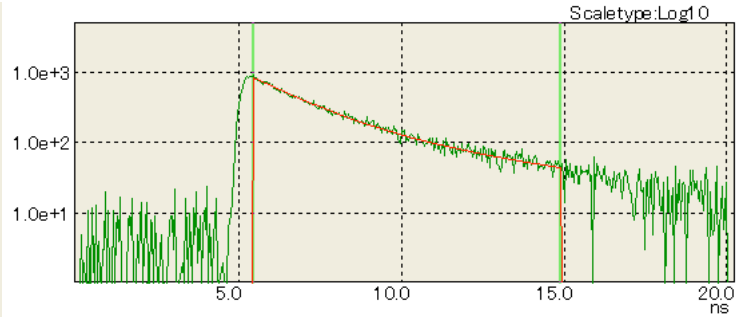
Decay profile of ^{Py}**U** in Duplex ODN **5a/5b**
[τ_2 = 8.458 ns]



Decay profile of ^{Py}**U** in Duplex ODN **5/5b**
[(τ_1 = 0.995 (66%) ns and τ_2 = 3.800 (34%) ns]



Decay profile of ^{Py}**U** in Duplex ODN **6a/6b**
[τ_2 = 8.788 ns]



Decay profile of ^{Py}**U** in Duplex ODN **6/6b**
[(τ_1 = 1.758 (88%) ns and τ_2 = 7.190 (12%) ns)

Figure S7: Fluorescence decay profiles of some selected duplexes in which the ODN contains both donor and acceptor and also with no acceptor.