Synthesis of versatile fluorescent sensors based on Click chemistry: detection of unsaturated fatty acids by their pyrene-emission switching

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Supplementary Information

General Methods and Materials. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. MALDI-TOF mass spectra were measured with 3-hydroxypicolinic acid as a matrix. High-resolution mass spectra were obtained by ESI-TOF method. Reagents were purchased from commercial sources and used without further purification. The pyrene-modified ODNs **1´** and **2´**,[1] azide derivatives of cyclodextrins **3**[2] and **4**[2], bisadamantyl guest molecule **5**[3], and DMT-protected ethynyl β-D-deoxyribofuranoside **7**[4] (Scheme S1) were synthesised according to their published procedures.

Phosphoramidite 6. To a CH_2Cl_2 (10 mL) solution of **7**[4] (600 mg, 1.35 mmol) were added $NEt(^{i}Pr)_2$ (942 μL, 5.40 mmol) and *i*-$Pr_2NPClO(CH_2)_2CN$ (753 μL, 3.37 mmol) at room temperature under Ar atmosphere (Scheme S1). The reaction mixture was stirred for 20 min at that temperature, quenched by the addition of a few pieces of ice, and poured into $CHCl_3$. The $CHCl_3$ solution was washed with 2×$NaHCO_3$ aq and 1×brine. After removal of the solvent with a rotary evaporator, the residue was purified by reverse phase HPLC on a PREP-ODS column (20 × 250 mm; SHIMADZU TECHNO-RESEARCH, INC., eluent, CH_3CN) to give **6** as yellow oil (721 mg, 83%): $[\alpha]^{27}_{D}$ +21.2 (*c* 1.0, $CHCl_3$); IR (NaCl) 3288, 2967, 1608, 1509, 1251, 1179, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ1.07−1.18 (m, 12 H), 2.23−2.27 (m, 2 H), 2.43 (t, *J* = 13.2 Hz, 1 H), 2.49 (t, *J* = 4.4 Hz, 1 H), 2.57 (t, *J* = 13.2 Hz, 1 H),

3.12−3.24 (m, 2 H), 3.50−3.86 (m, 10 H), 4.07−4.13 (m, 1 H), 4.44−4.50 (m, 1 H), 4.75−4.80 (m, 1 H), 6.80−6.83 (m, 4 H), 7.17−7.21 (m, 1 H), 7.26−7.30 (m, 2 H), 7.34−7.38 (m, 4 H), 7.46−7.49 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.0, 20.1, 20.2, 20.3, 24.3, 24.37, 24.45, 24.6, 41.3, 43.0, 43.1, 43.2, 55.0, 55.2, 58.1, 58.2, 58.3, 63.9, 64.0, 67.4, 67.5, 67.67, 67.71, 73.2, 73.5, 75.4, 75.6, 82.6, 82.7, 85.6, 86.0, 112.9, 113.1, 117.38, 117.44, 126.5, 126.8, 127.5, 127.8, 128.1, 128.3, 130.0, 130.2, 135.9, 136.0, 144.8, 158.3; HRMS (ESI) *m/z* calcd for $C_{37}H_{45}N_2NaO_6P$ ($[M+Na]^+$) 667.2913, found 667.2934.

Precursor 1. The phosphoramidite **6** was used to introduce an alkynylnucleoside residue into ODN. The single-stranded 8-mer ODN **1** including the alkynylnucleoside residue (5′-end) and C3-alkylamino linker (3′-end) was prepared by using an Applied Biosystems 392 DNA/RNA synthesizer. The released ODN from the support was modified with the pyrene-based succinimidyl ester[5] at the 3′-end amino group of the C3-alkylamino linker according to a method[5] previously reported to give the crude **1**.

Precursor 2. The counterpart **2** was synthesised on Universal support II by the DNA synthesiser using two phosphoramidites, **6** and the pyrene-based one[5].

Purification of 1 and 2. Both of the single-stranded building blocks were purified by reverse phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm; Chemco SCIENTIFIC CO., LTD., elution with 5.0 mM ammonium formate and a 5–15% acetonitrile linear gradient (0–40 min) at a flow rate of 3.0 mL/min). **1**: MS (MALDI-TOF) *m/z* Calcd for $C_{97}H_{117}N_{26}O_{50}P_8$ ($[M+H]^+$) 2693.53, Found 2693.23. **2**: MS (MALDI-TOF) *m/z* Calcd for $C_{94}H_{111}N_{28}O_{47}P_8$ ($[M+H]^+$) 2631.51, Found 2631.40.

Cyclodextrin-linked fluorescent ODNs. To a solution of the precursor **1** (10 nmol) in water were added a solution of **3** (1.0 μmol) in water and a solution of CuBr (0.40 μmol) and *tris*-(benzyltriazolylmethyl)amine[6] (TBTA, 0.40 μmol) in a mixed solvent (H_2O:DMSO:*t*-BuOH = 4:3:1). The reaction mixture was stirred at room temperature for 1 h. The crude ODN was purified by reverse phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm; Chemco SCIENTIFIC, LTD., elution with 5.0 mM ammonium formate and a 5–15% acetonitrile linear gradient (0–40 min) at a flow rate of 3.0 mL/min) to give **1α**. Other ODNs **2α**, **1β**, and, **2β** were prepared in the same procedure. **1α**: MS (MALDI-TOF) *m/z* Calcd for $C_{133}H_{176}N_{29}O_{79}P_8$ ($[M+H]^+$) 3690.85, Found 3690.73. **2α**: MS

(MALDI-TOF) *m/z* Calcd for $C_{130}H_{170}N_{31}O_{76}P_8$ ($[M+H]^+$) 3628.83, Found 3628.95. **1β**: MS (MALDI-TOF) *m/z* Calcd for $C_{139}H_{186}N_{29}O_{84}P_8$ ($[M+H]^+$) 3852.91, Found 3853.02. **2β**: MS (MALDI-TOF) *m/z* Calcd for $C_{136}H_{180}N_{31}O_{81}P_8$ ($[M+H]^+$) 3790.88, Found 3790.86.

Fluorescence titration of the sensor with 5. To a 1.0 μM (duplex concentration) solution of **1β**/**2β** in a 20 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl was added **5** (0–1.0 equiv. to the duplex) at 40 °C. Each mixture was stirred at 40 °C until no more change of the fluorescence spectrum occurred (ca. 15 min). The excitation wavelength was 345 nm, and the fluorescence spectra were recorded from 320 to 720 nm at every addition of **5**. The binding constant for the complex of **1β**/**2β** with **5** was calculated by a curve-fitting method on the basis of the titration experiment where **1β**/**2β** (200 nM as a duplex) was titrated with **5** (0–5.0 equiv. to the duplex).

Fluorescence Titration of the sensors with fatty acids. To a 200 nM (duplex concentration) solution of **1β**/**2β** in a 50 mM Gly-NaOH buffer (pH 10.0) containing 700 mM NaCl was added fatty acids (0–100 equiv. to the duplex) at 30 °C. Each mixture was stirred at 30 °C until no more change of the fluorescence spectrum occurred (ca. 15 min). In the case of **1α**/**2α**, fatty acids were added to a 2.0 μM (duplex concentration) solution at 45 °C. The excitation wavelength was 345 nm, and the fluorescence spectra were recorded from 320 to 720 nm at every addition of fatty acids.

Determination of T_m. Melting curves were measured at 260 nm at the temperature ranging from 5 to 65 °C (1.0 °C/min) by UV/vis spectroscopy. The solution for the measurement contained **1β/2β** (1.0 μM as a duplex), 20 mM Tris-HCl (pH 8.0), and 100 mM NaCl in the presence or absence of **5** (1.0 μM). In the case of **1α/2α** (2.0 μM as a duplex) in a 50 mM Gly-NaOH buffer (pH 10.0) containing 700 mM NaCl, oleic acid (15 μM) was added to the solution.

CD Measurements. CD spectra of **1β/2β** (1.0 μM as a duplex) with **5** (0–1.5 equiv. to the duplex) were measured in a 20 mM Tris-HCl buffer containing 100 mM NaCl (pH 8.0) at 40 °C. The similar titration experiment for **1α/2α** (2.0 μM as a duplex) was carried out with oleic acid (0–7.0 equiv. to the duplex) in a 50 mM Gly-NaOH buffer (pH 10.0) containing 700 mM NaCl at 45 °C.

References

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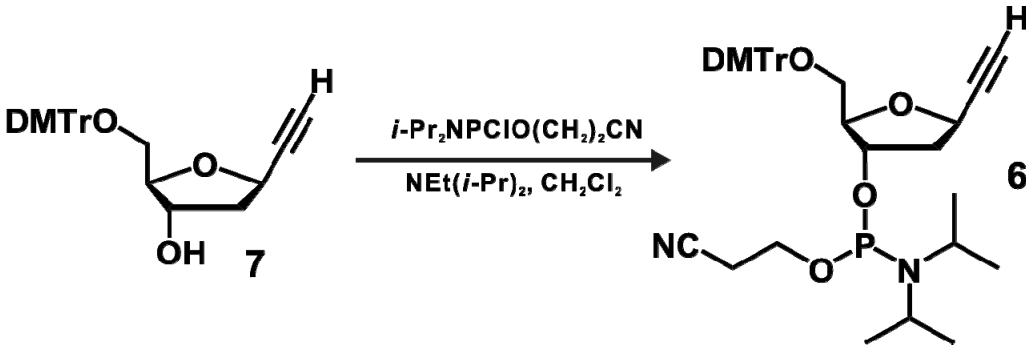
Scheme S1



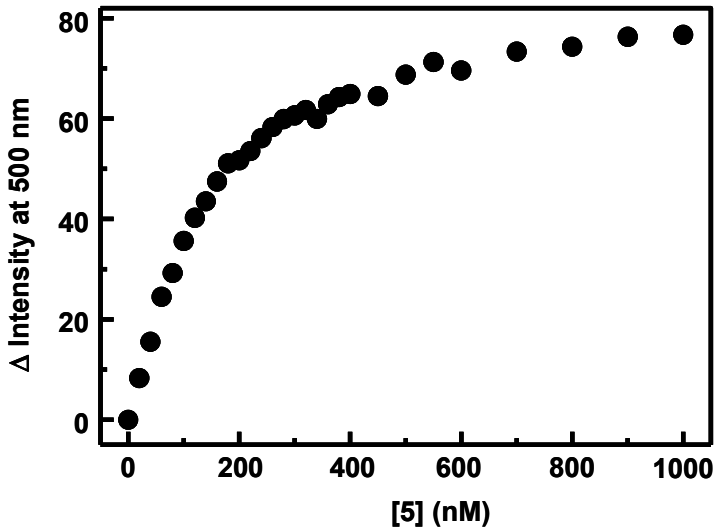


Fig. S1 Change for the fluorescence intensity at 500 nm upon the titration experiment of **1β**/**2β** (200 nM as a duplex) with **5** (0–1000 nM) in a 20 mM Tris-HCl buffer containing 100 mM NaCl (pH 8.0) at 40 °C, plotted against the concentration of **5**. The excitation wavelength was 345 nm.

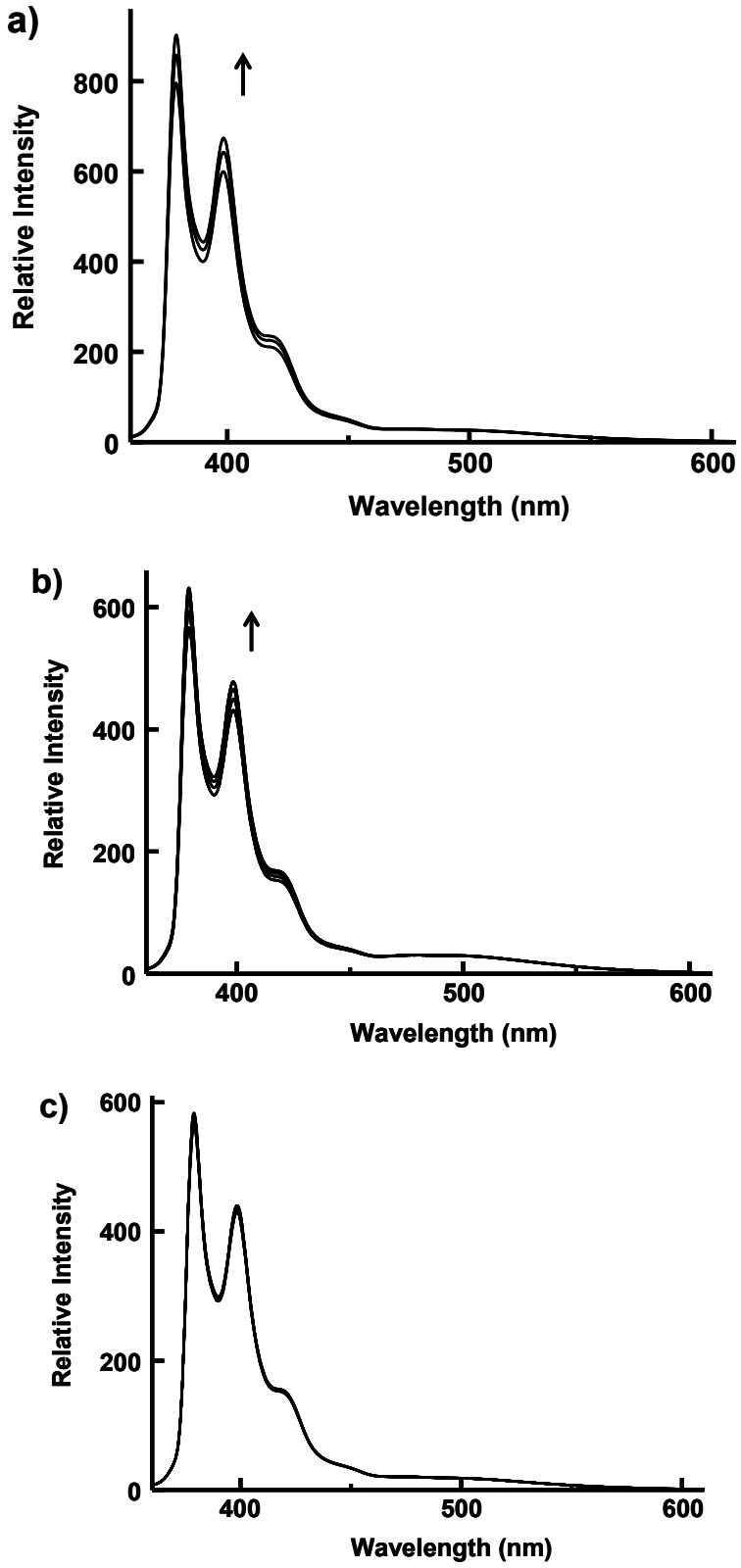


Fig. S2 Fluorescence titration spectra of the combination of a) **1′**/**2β**, b) **1β**/**2′**, and c) **1′**/**2′** ([**1β**] = [**2β**] = [**1′**] = [**2′**] = 1.0 μM) in a 20 mM Tris-HCl buffer containing 100 mM NaCl (pH 8.0) on titration with **5** (0–1.5 equiv. to the duplex) at 40 °C. The excitation wavelength was 345 nm.

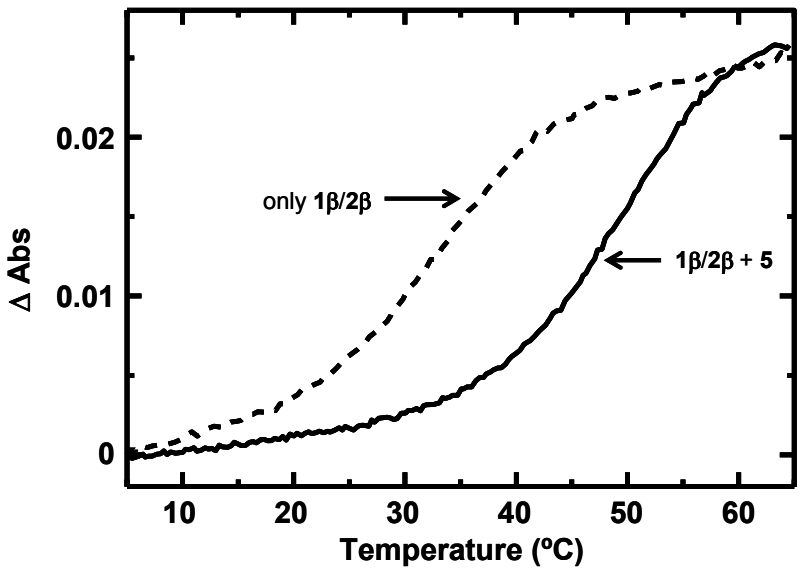


Fig. S3 Melting curves for **1β**/**2β** (dashed line) and **1β**/**2β** + **5** (line) at 260 nm by UV/vis spectroscopy. The solution for the measurements contained **1β**/**2β** (1.0 μM as a duplex), 20 mM Tris-HCl (pH 8.0), and 100 mM NaCl in the presence or absence of **5** (1.0 μM).

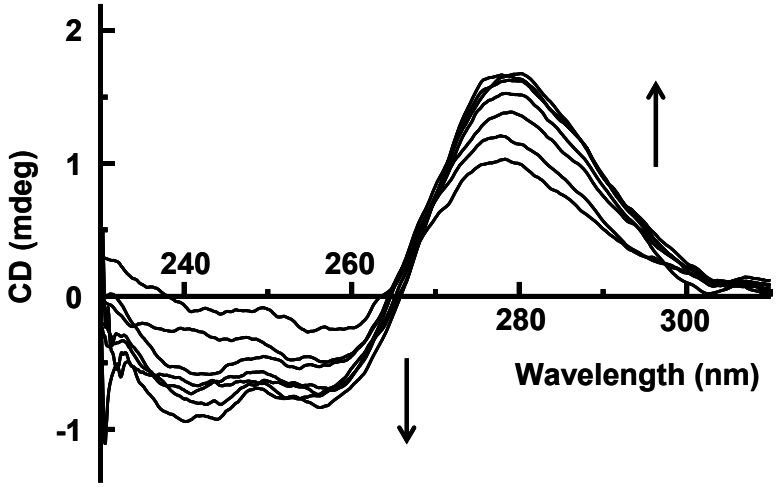
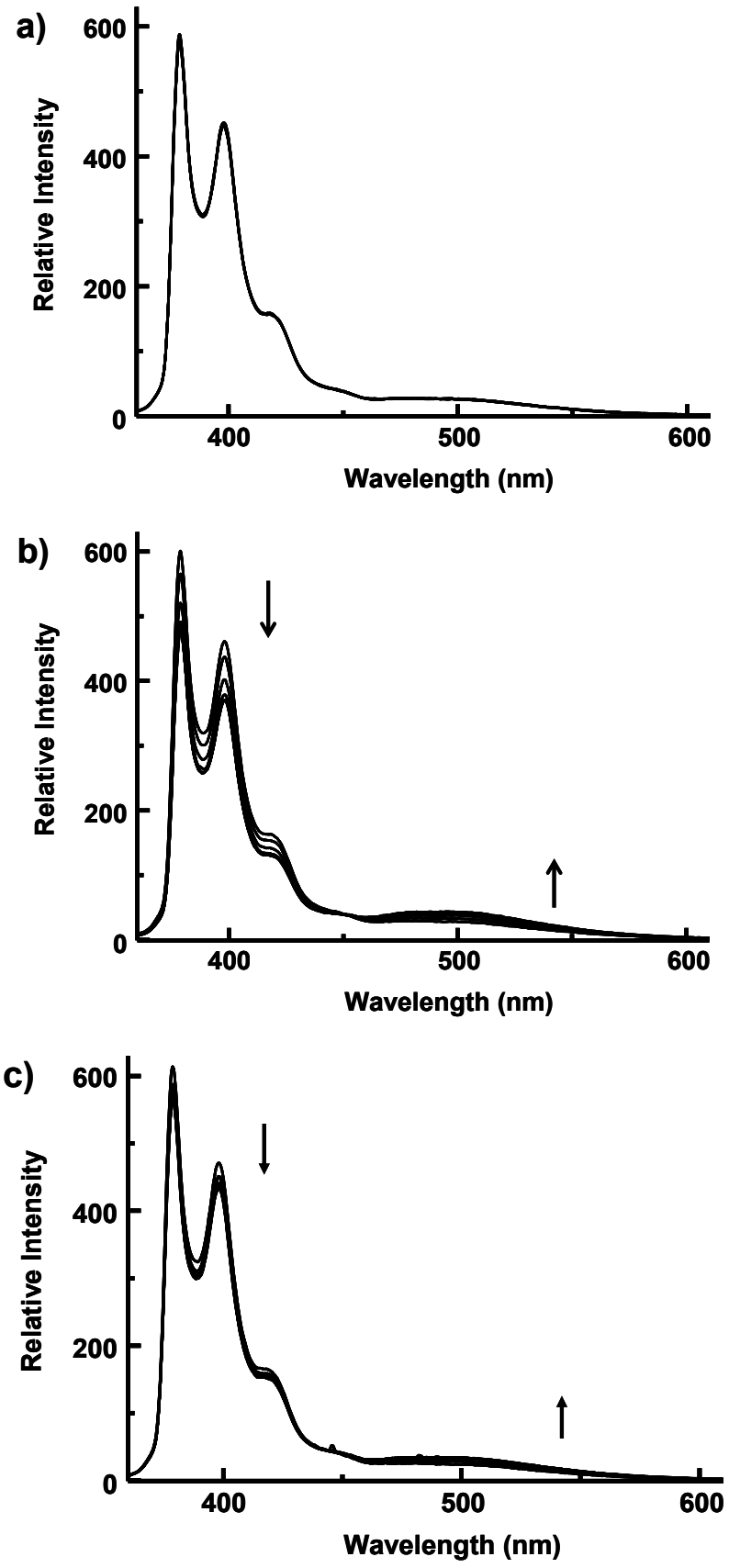


Fig. S4 CD titration spectra of **1β**/**2β** (1.0 μM as a duplex) in a 20 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl on titration with **5** (0–1.5 equiv. to the duplex) at 40 ºC.


a)
Relative Intensity
600
400
200
0
400
500
600
Wavelength (nm)
b)
Relative Intensity
600
400
200
0
400
500
600
Wavelength (nm)
c)
Relative Intensity
600
400
200
0
400
500
600
Wavelength (nm)

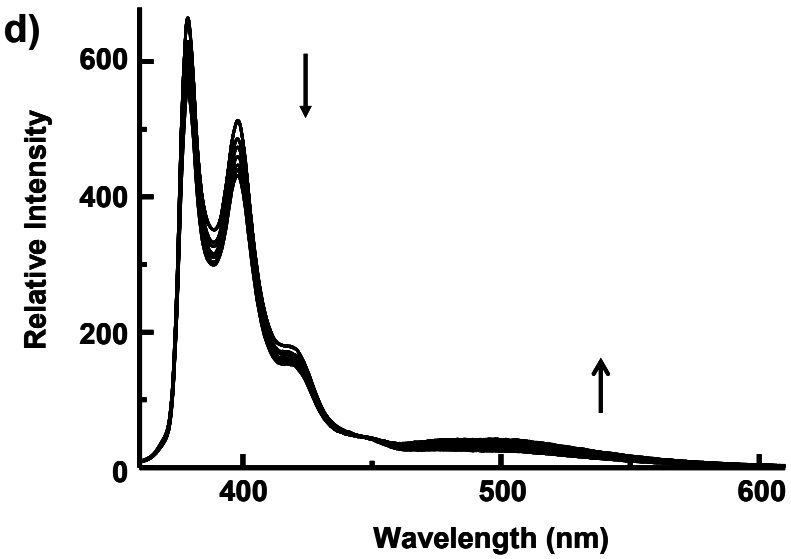


Fig. S5 Fluorescence titration spectra of **1**β/**2**β ([**1**β] = [**2**β] = 200 nM) in a 50 mM Gly-NaOH buffer containing 700 mM NaCl (pH 10.0) on titration with a) stearic acid (0–30 equiv. to the duplex), b) oleic acid (0–50 equiv. to the duplex), c) elaidic acid (0–50 equiv. to the duplex), and d) arachidonic acid (0–100 equiv. to the duplex) at 30 °C. The excitation wavelength was 345 nm.

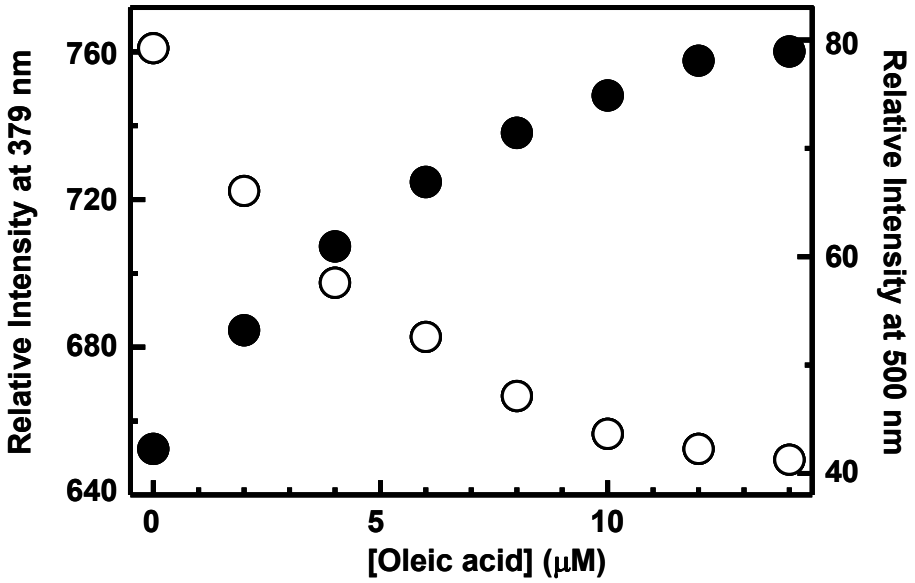


Fig. S6 Change for the fluorescence intensities at 379 nm (open circle) and 500 nm (closed circle) upon the titration experiment of **1**α/**2**α (2.0 μM as a duplex) with oleic acid (0–14 μM) in a 50 mM Gly-NaOH buffer containing 700 mM NaCl (pH 10.0) at 45 °C, plotted against the concentration of oleic acid. The excitation wavelength was 345 nm.