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Electronic Supplementary Information

for

Ratiometric DNA Sensing with a Host-Guest FRET Pair

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Contents

1. Materials and Methods

CB7 and CB7-OPr were synthesized according to literature procedures.[1] DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride) and Type III salmon sperm DNA (ca. 2000 base pair)[2] were commercial samples (Sigma Aldrich) and used as received without further purification. 6-FAM-azide was purchased from Baseclick and used as received. Reagents for synthesis were from Fluka, Carl Roth, and Sigma-Aldrich. TLC was performed on SIL G/UV254 (Macherey-Nagel). Buffers and salts were of the highest purity available from Fluka and Sigma-Aldrich and used as received.

UV-Vis absorption measurements were performed with a Varian Cary 4000 spectrophotometer and the fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer. All measurements were performed at ambient temperature, except for the fluorescence-based DNA melting experiment, which was recorded in a temperature range from 20 to 90 °C with rectangular quartz cuvettes with 1-cm optical path length. The fluorescence lifetimes were measured by time-correlated single-photon counting (FLS920, Edinburgh Instruments Ltd.). For the lifetime measurements, DAPI and CB7-CF were excited at 373 nm by using a diode laser (PicoQuant, LDH-P-C 375, fwhm ca. 50 ps) and the fluorescence was followed at 470 nm. ^{1}H NMR spectra were recorded on a Jeol ECS400 MHz and chemical shifts (δ) are reported in ppm relative to TMS (δ = 0 ppm). Mass spectra were recorded on a Bruker MALDI TOF spectrometer; HCCA (α-cyano-4-hydroxycinnamic acid) was used as a matrix.

2. Synthesis and Characterization of CB7-CF

8.7 mg (7.2 μmol) CB7-OPr was dissolved in 0.7 mL anhydrous DMSO and 6 mg (13 μmol) 6-FAM-azide was added. Then, 10 mg (50 μmol) sodium L-ascorbate was added into 2.8 mL 55% DMSO aqueous solution containing 4.47 mg (28 μmol) $CuSO_4$ and 14.86 mg (28 μmol) tris(benzyltriazolylmethyl)amine (TBTA). These two solutions were mixed and stirred at room temperature for 24 h. 50 mL diethyl ether was added, and the resulting precipitate was washed three times with 25 mL MeOH. Drying under high vacuum afforded a dark solid. The crude product containing unreacted CB7-OPr, 6-FAM-Azide, and CB7-CF, was purified by column chromatography. In detail, the mixture was dissolved in 600 μL H_2O/HCOOH 1:1 and loaded onto silica gel 60 (0.04-0.063 mm) and the column was eluted with H_2O/AcOH/HCOOH 10:10:1.5. The eluent was collected in fractions of 2 mL (50 fractions) and the fractions containing pure CB7-CF were combined. Evaporation of the solvent gave 4 mg (2.4 μmol, 33% yield) CB7-CF as a brown solid. The product identity was confirmed by mass spectrometry

(Figure S2) and NMR (Figure S3). ^{1}H NMR (400 MHz, D_2O with excess *p*-xylene diamine[3]), δ (ppm) = 8.55 (H1), 8.44 (H2), 7.96 (H7), 7.75 (H6), 7.48 (H4), 7.46 (H3), 7.37 (H5, H10, Hb_{free}), 6.58 (Hb_{bound}), 5.73 (Hd), 5.52 (He), 4.79 (H11, HOD), 4.24 (H14, Hc), 4.00 (H13), 3.92 (Ha_{free}), 3.49 (Ha_{bound}), 1.90 (H12). MALDI-TOF MS calculated for [CB7-CF] 1675.43, found 1675.67; calculated for [CB7-CF–H^++Na^+] 1697.41, found 1697.63.

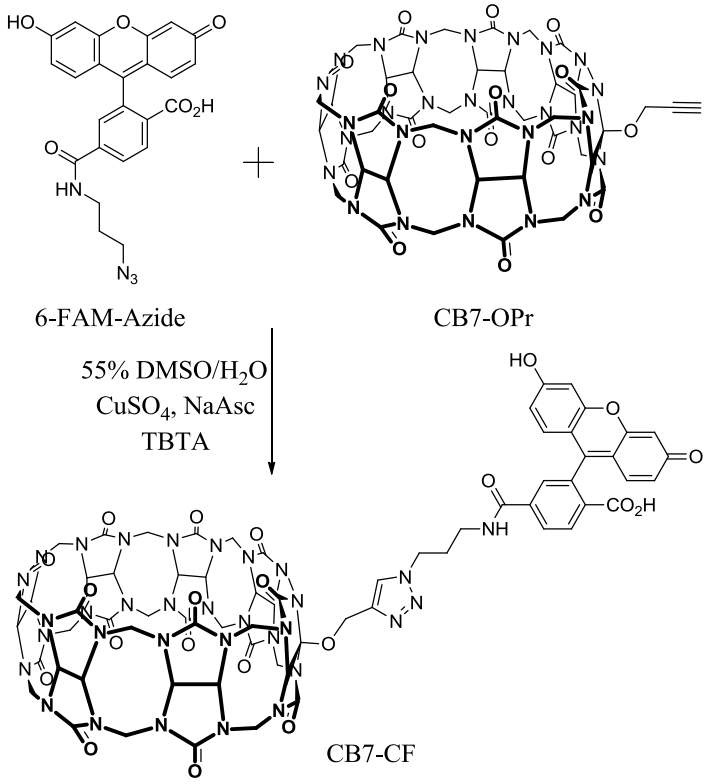

Fig. S1 Synthesis of CB7-CF.

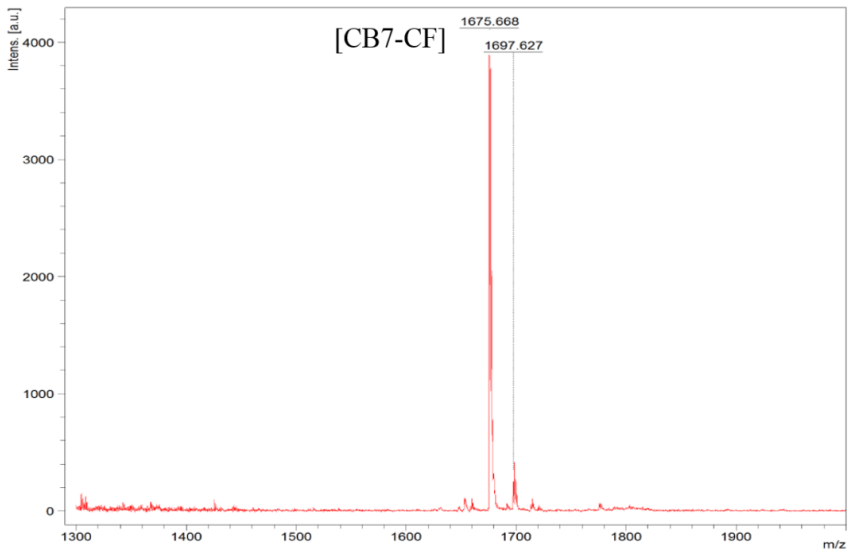

Fig. S2 MALDI-TOF mass spectra of CB7-CF (HCCA matrix, positive mode).

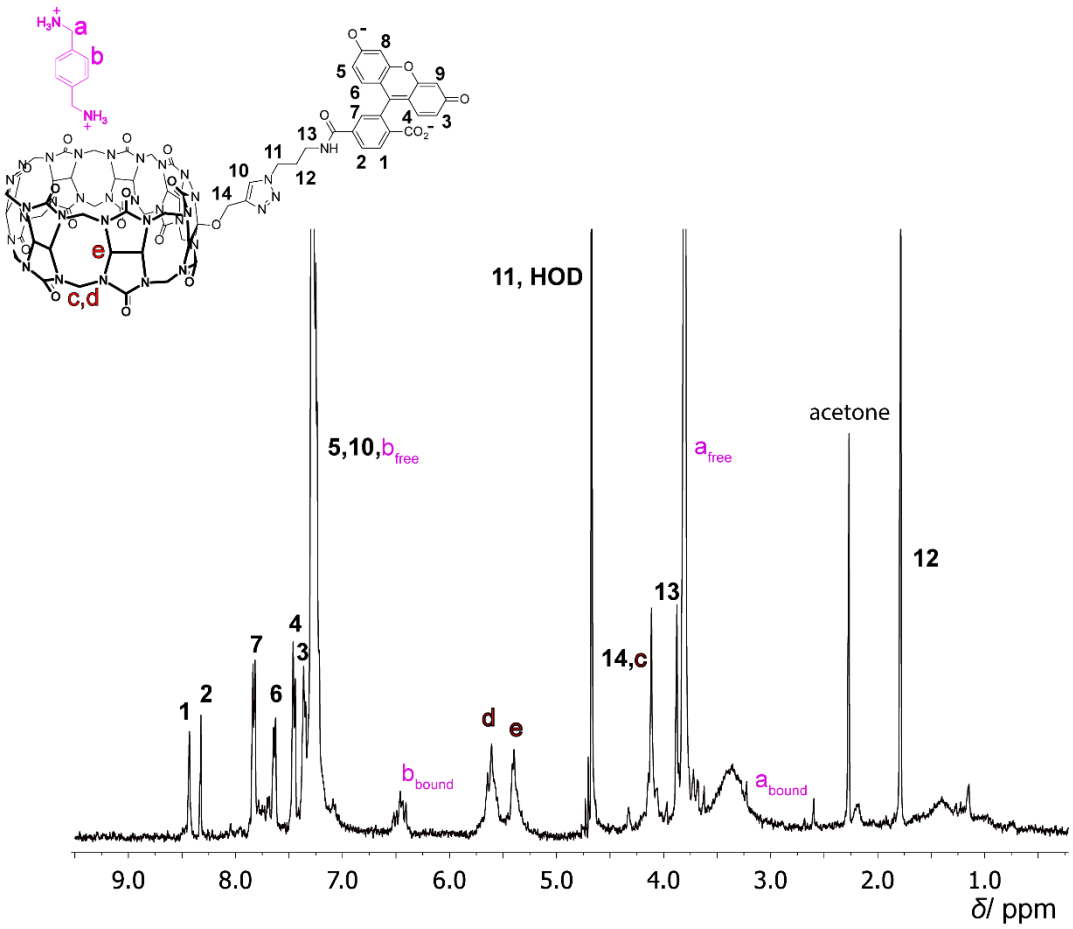

Fig. S3 ^{1}H NMR spectra of CB7-CF in D_2O with excess *p*-xylene diamine (see ref. 2) to aid in solubilization (magenta peak assignments).

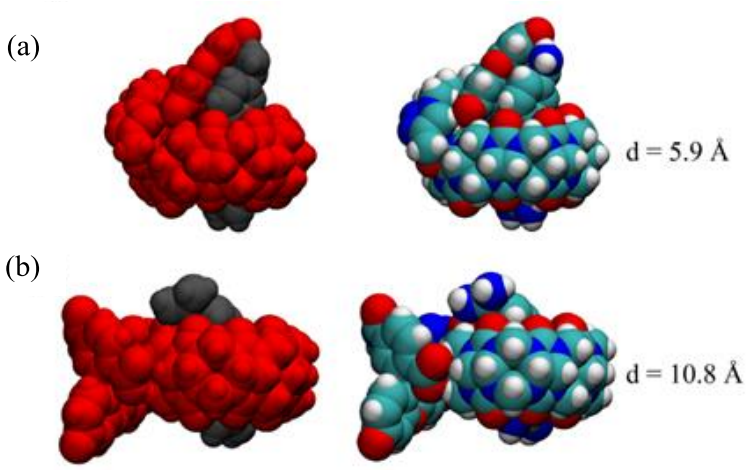

Fig. S4 DFT-optimized structures (B3LYP/3-21G*) of different possible co-conformations for the CB7-CF/DAPI complex (in gas phase). The distance (d) between the center of mass of the CF and DAPI is given in Å. The structure shown in (a) was found to be more stable than that in (b) by 4.9 kcal/mol.

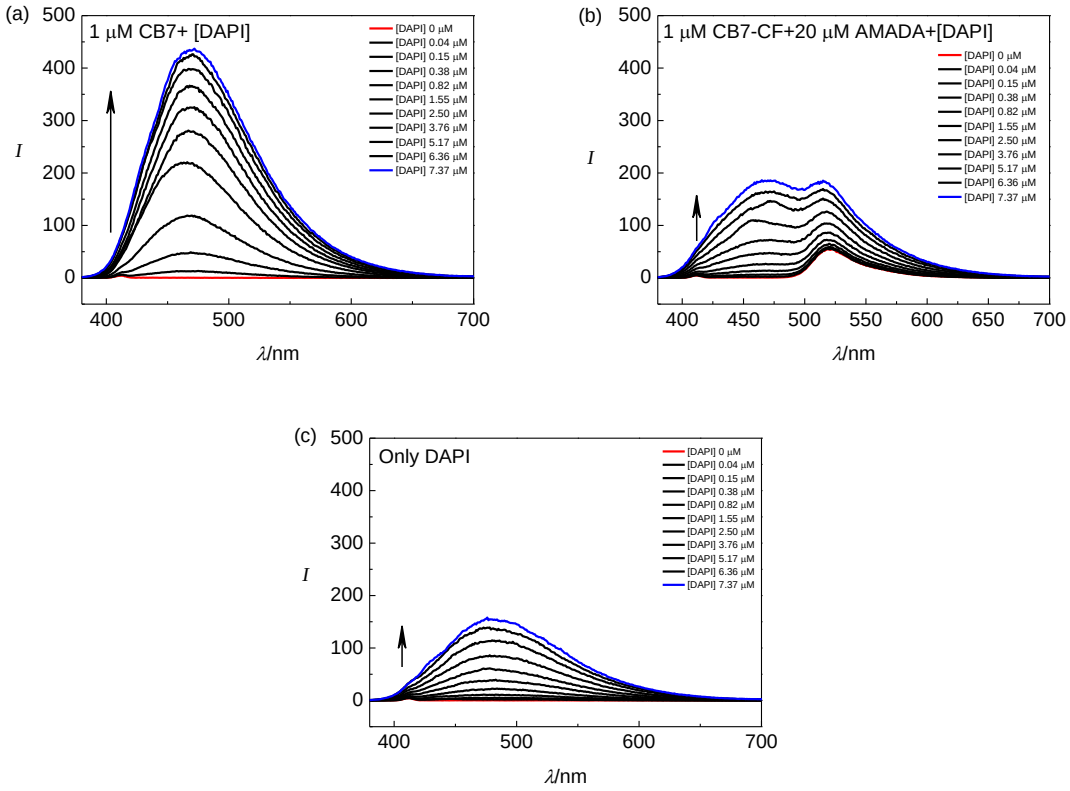

Fig. S5 Fluorescence titrations (λ_{ex} = 360 nm) with increasing amounts of DAPI (0 to 7.4 µM) in the presence of (a) 1 µM CB7, (b) 1 µM CB7-CF as well as 20 µM AMADA, and (c) only DAPI. All experiments were performed in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

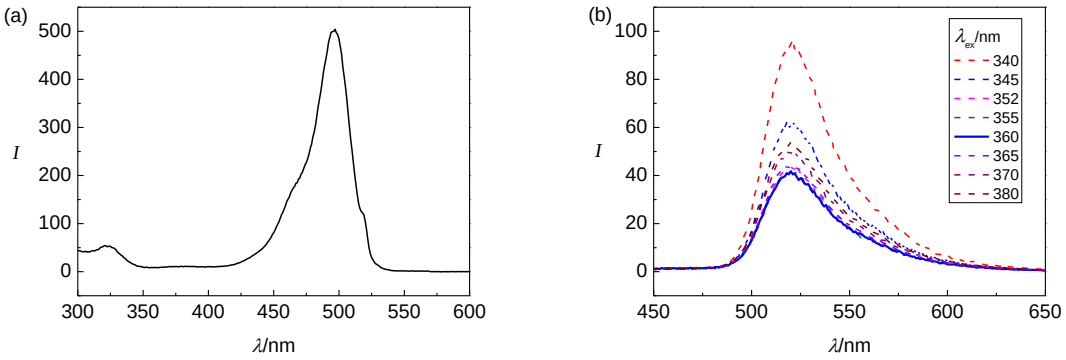

Fig. S6 (a) Excitation spectrum (λ_{em} = 520 nm) of 1 µM CB7-CF in 10 mM $(NH_4)_2HPO_4$, pH 7.2. (b) Emission spectra of 1 µM CB7-CF at varying excitation wavelengths.

3. Data Analysis

Binding constants were calculated from the fluorescence titrations (Fig. 3b, inset) by assuming a 1:1 complex stoichiometry and performing a nonlinear fitting according to eq. S5. [G] and

[HG] are the concentrations of the uncomplexed guest and the host-guest complex, and $[G]_0$ and $[H]_0$ are the total concentrations of guest and host. K_a is the association constant of the guest with the host. a and b are constants depending on instrumental settings of the fluorometer.

At a suitable low concentration, the fluorescence intensity of a fluorophore has a linear relationship with fluorophore concentration (eq. S1).

$$I = a[\mathrm{G}] + b[\mathrm{HG}] \tag{S1}$$

Conservation of mass requires that:

$$[\mathrm{G}]_0 = [\mathrm{G}] + [\mathrm{HG}] \tag{S2}$$

From eq. 1 and eq. S2 one obtains:

$$I = (a - b) \cdot [\mathrm{G}] + b \cdot [\mathrm{G}]_0 \tag{S3}$$

According to the law of mass action, the concentration of host-guest complex under equilibrium conditions is:

$$[\mathrm{G}] = \frac{[\mathrm{G}]_0 - [\mathrm{H}]_0 - \frac{1}{K_a}}{2} + \sqrt{\frac{\left([\mathrm{H}]_0 + [\mathrm{G}]_0 + \frac{1}{K_a}\right)^2}{4} - [\mathrm{H}]_0[\mathrm{G}]_0} \tag{S4}$$

Substitution of eq. S4 into eq. S3 affords eq. S5, which can be implemented into fitting programs and which was used in the fitting in Figure 3b, inset, in the main text.

$$I = (\mathrm{a} - \mathrm{b}) \cdot \frac{[\mathrm{G}]_0 - [\mathrm{H}]_0 - \frac{1}{K_a}}{2} + \sqrt{\frac{\left([\mathrm{H}]_0 + [\mathrm{G}]_0 + \frac{1}{K_a}\right)^2}{4} - [\mathrm{H}]_0[\mathrm{G}]_0} + \mathrm{b} \cdot [\mathrm{G}]_0 \tag{S5}$$

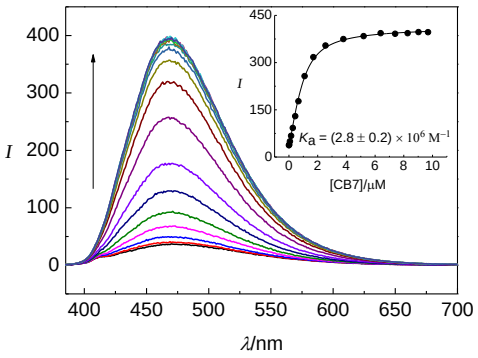

Fig. S7 Variation of the fluorescence spectrum (λ_{ex} = 360 nm) of 1 μM DAPI in 10 mM $(NH_4)_2HPO_4$, pH 7.2, upon addition of CB7. Inset: CB7 concentration dependence of the fluorescence intensity at 470 nm, R^2 = 0.998. The curve was fitted by 1:1 binding stoichiometry.

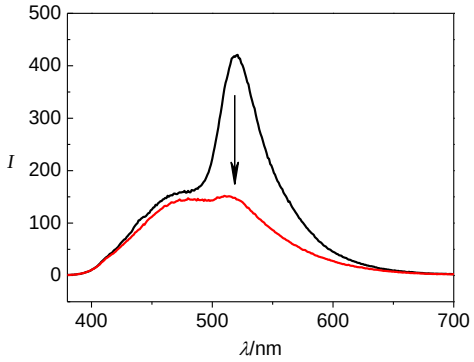

Fig. S8 Fluorescence spectra (λ_{ex} = 360 nm) of 1 μM CB7-CF and 7.4 μM DAPI without (black line) and with (red line) 32 μM AMADA in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The addition of AMADA leads to a strong fluorescence decrease of CB7-CF.

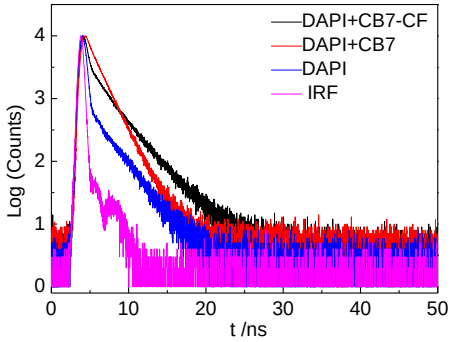

Fig. S9 Time-resolved fluorescence decay traces of 5 μM DAPI alone (blue) and in the presence of 10 μM CB7-CF (black) and CB7 (red); instrument response function is shown in magenta. λ_{exc} = 373 nm, λ_{obs} = 450 nm.

Table S1. Fitting results of lifetime decays of DAPI with and without CB7 or CB7-CF in 10 mM $(NH_4)_2HPO_4$, pH 7.2 with 0.5% DMSO.

Sample	**τ/ns**	***B***	**Rel%**	χ^2
DAPI[a]	τ_1 = 0.29	0.056	81.1	2.24
	τ_2 = 2.77	0.001	18.9	
DAPI	τ_1 = 0.31	0.054	79.2	2.66
	τ_2 = 2.74	0.002	20.8	
DAPI+CB7	τ_1 = 1.59	0.026	100	2.40
DAPI+CB7-CF	τ_1 = 0.65	0.021	30.2	1.94
	τ_2 = 3.05	0.010	69.8	

[a] Without DMSO.

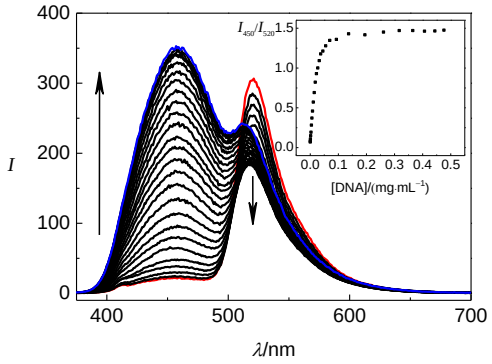

Fig. S10 Fluorescence spectra (λ_{ex} = 360 nm) of 0.5 μM DAPI and 1 μM CB7-CF with increasing concentration of salmon sperm DNA (0 to 0.5 mg/mL). The inset shows the corresponding change in the fluorescence intensity ratio at 450 nm and 520 nm.

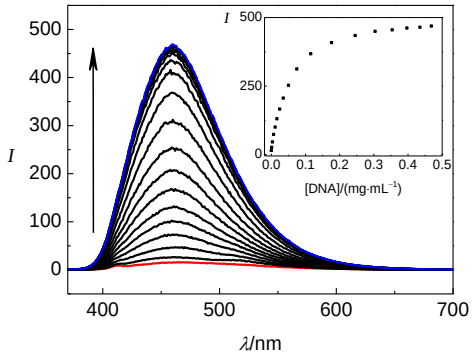

Fig. S11 Variation of the fluorescence spectrum (λ_{ex} = 360 nm) of 0.5 μM DAPI in 10 mM $(NH_4)_2HPO_4$, pH 7.2 upon addition of salmon sperm DNA. Inset: Dependence of the fluorescence intensity at 450 nm on DNA concentration.

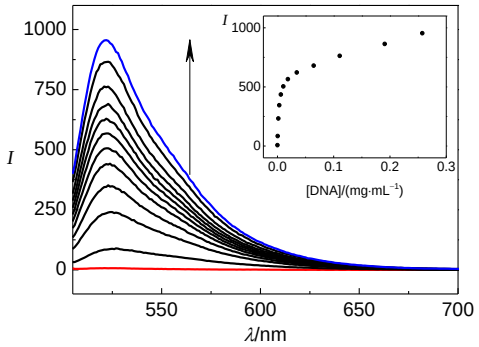

Fig. S12 Variation of the fluorescence spectrum (λ_{ex} = 497 nm) of 0.5 μM SYBR Green in 10 mM $(NH_4)_2HPO_4$, pH 7.2 upon addition of salmon sperm DNA. Inset: Dependence of the fluorescence intensity at 520 nm on DNA concentration.

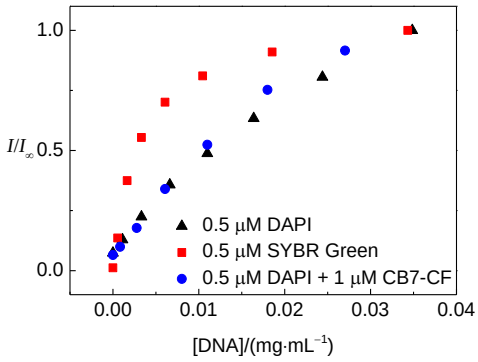

Fig. S13 Normalized fluorescence intensity of DAPI, SYBR-Green, and DAPI/CB7-CF in dependence on DNA concentration. Note that for DAPI/CB7-CF the ratiometric response is shown (compare Figures S10-S12).

4. CF Labelling Degree Calculation

As the FRET efficiency is >99%, the DAPI fluorescence in Fig. 3a should not increase upon addition of CB7-CF. However, there is a slight fluorescence increase by ca. 30% at 460 nm of DAPI, attributed to complexation by unlabelled CB7. For comparison, complexation of DAPI by unlabelled (parent) CB7 affords a fluorescence increase by a factor of 12 (Fig. S8, at 460 nm). The ratio of these two factors (1.3 divided by 12) is 11%, which represents the degree of unlabelled CB7 corresponding to ca. 90% labelling efficiency.

5. Limit of Detection

The limit of detection[4] (LOD) of the FRET probe was determined according to eq. S6 from the slope of the calibration curve, *b*, and the standard deviation of the y-intercept (inset in Fig. 4a) of the calibration curve, S_a (with $n \geq 7$). The molecular weight of salmon sperm DNA was taken from the literature as 1.3×10^6 g/mol.[2]

$$\mathrm{LOD} = 3S_a/b \qquad \text{(S6)}$$

6. References

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(4) A. Shrivastava and V. B. Gupta, *Chron. Young Sci.*, 2011, **2**, 21.