Supplementary Information

Flavylium dye as pH tunable fluorescent and CD probe for double stranded DNA and RNA

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Table S1. Groove widths and depths for used nucleic acid sequences [1,2].

Structure type	Groove width [Å]		Groove depth [Å]	
	major	minor	major	minor
[a] poly rA – poly rU	3.8	10.9	13.5	2.8
[b] poly dA – poly dT	11.4	3.3	7.5	7.9
[c] poly dGdC – poly dGdC	13.5	9.5	10.0	7.2
[c] poly dAdT – poly dAdT	11.2	6.3	8.5	7.5

[a] A-helical structure (e.g. A-DNA)

[b] C-helical structure (e.g. C-DNA)

[c] B- helical structure (e.g. B-DNA)

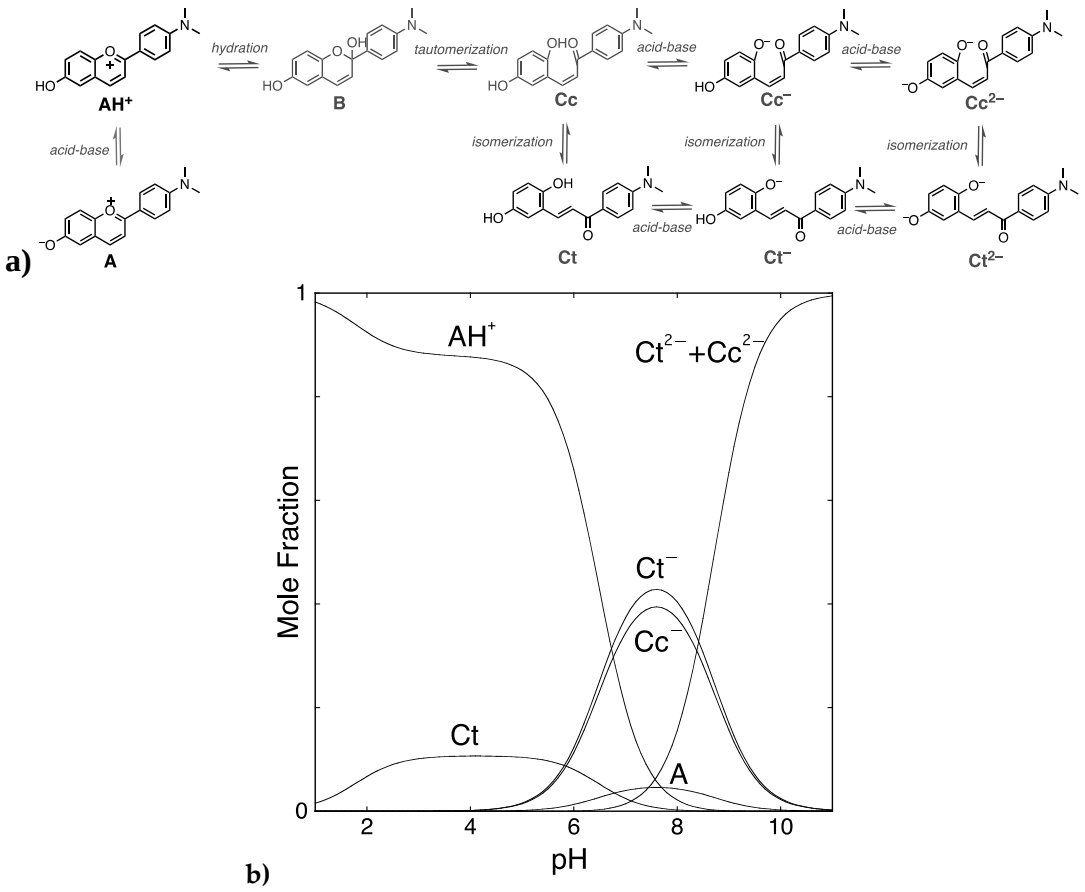

Figure S1. Chemical equilibria (**a**) and mole fraction distribution of species (**b**) for 4'-(*N*,*N*-dimethylamino)-6-hydroxyflavylium in aqueous solution; data from ref. 20 in the paper

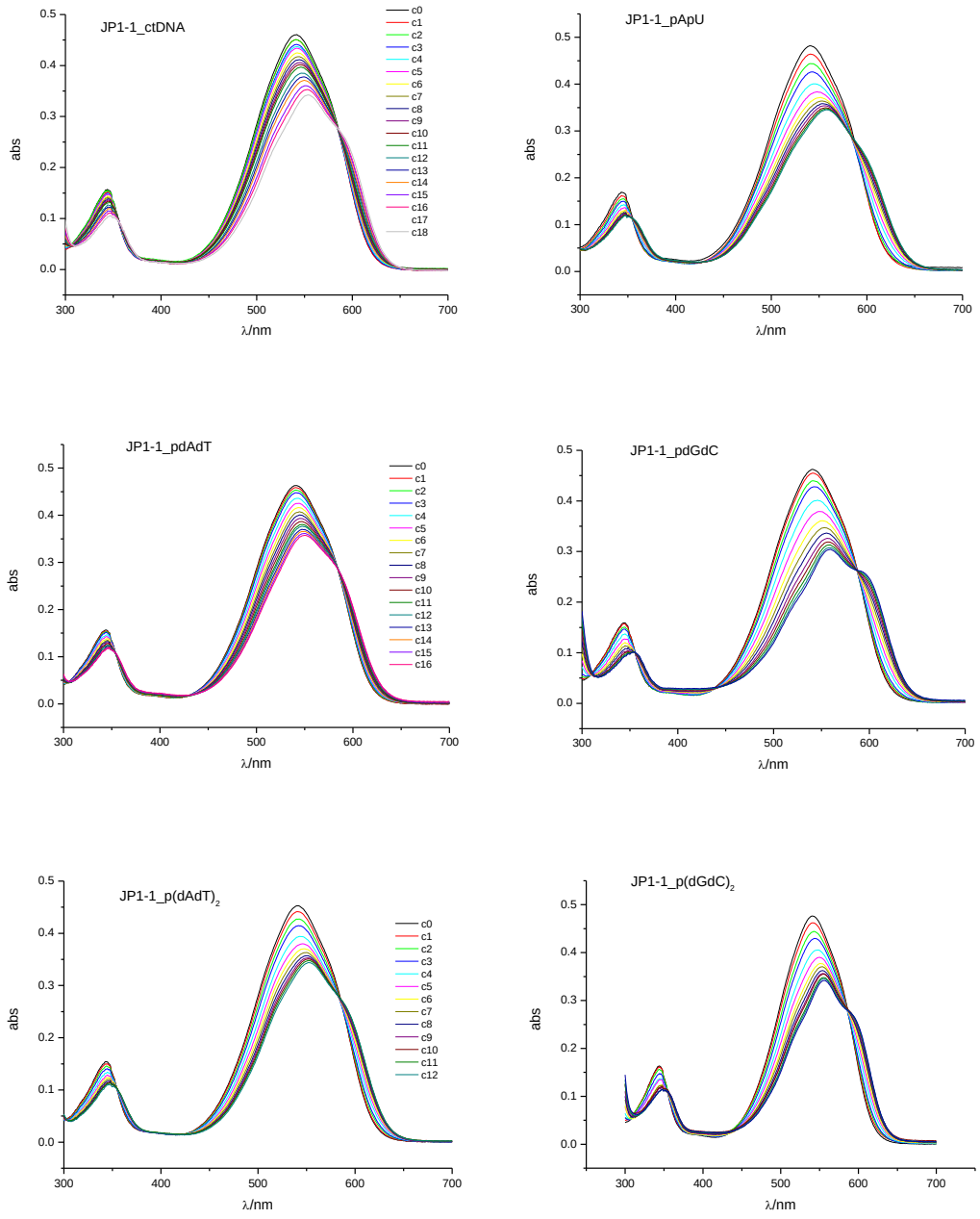

Figure S2. Changes in the UV/Vis spectra of **F1** (c = 1 × 10^{-5} mol dm^{-3}, sodium cacodylate buffer, pH = 5.0, I=0.05 mol dm^{-3}) upon addition of polynucleotides.

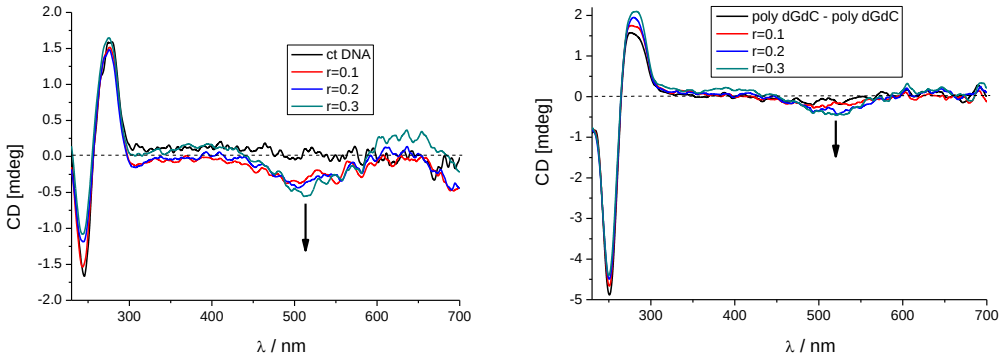

Figure S3. Changes in CD spectra of ds-polynucleotides (c = 2 × 10^{-5} mol dm^{-3}) upon addition of **F1** at different ratios r = [**F1**] / [ds-polynucleotide] (sodium cacodylate buffer, pH = 5.0, I=0.05 mol dm^{-3}).

Fitting titration data for calculation of binding constants

Large errors with Scatchard analysis[3]are often encountered (please see I. R. Klotz, Ligand-Receptor Energetics, John Wiley & Sons, Inc. New York, 1997). Since the concentration of observable species can determine the number of binding sites reflected in the isotherm, the apparent stoichiometry can change based upon the concentration of the observable species. In addition, the errors associated with assigning spectral properties of the 100% "free" versus the 100% "bound" become amplified in all the data points, since the fraction bound at each data point is calculated from these two extremes. The data points for the 100% free and the 100% bound states are, therefore, "weighed" much more heavily than the points in the middle of the titration.

On the other hand, non-linear analysis of binding data can help reduce the errors associated with quantifying the spectral properties of these "extreme" (and often inaccurate) data points. Non-linear analysis typically weighs all data points equally and fits all the points to a theoretical curve. However, it is advisable to carefully choose experimental conditions to assure that all dye molecules bind to dominant binding sites – this is done by preliminary experiment for rough estimation of binding affinity and then repeating more detailed titration at conditions of an excess of DNA/RNA binding sites over c(dye), which allows each dye molecule to find its dominant binding site according to J.D. Mc Ghee, P.H. von Hippel formalism for non-cooperative binding[4]. More detailed considerations how to organize titration experiment and analysis are nicely summarised in J. Lah and G. Vesnaver, J Mol Biol, 2004, 342, 73 (pp 80).

Scatchard equation adapted for non-linear fitting procedure:

Parameter Names: K, EpsKompleks, EpsLigand, n

Independent variables: cDNA,cLigand

Dependent variable: A

A = EpsKompleks*cK + EpsLigand*(cLigand-cK)+ EpsDNA*(n*cDNA-cK);

cK=(n*cDNA+cLigand+1/K-((n*cDNA+cLigand+1/K)^2-

4*n*cDNA*cLigand)^0.5)/2;

Whereby K is binding constant; EpsKompleks is fluorescence intensity of dye/ polynucleotide complex divided by c(dye); EpsLigand is fluorescence intensity of dye divided by c(dye); n =[bound dye] / [polynucleotide]; cDNA is c(polynucleotide); cLigand is c(dye).

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1 Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag, New York, 1983.

2 Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry* **1980**, *3*, 1109-1181.

3 Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* **1949**;*51*,660-672.

4 McGhee JD, von Hippel PH. Theoretical aspects of DNA-protein interactions, Co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. *J Mol Biol* **1976**;*103*,679-684.