Supplementary Material (ESI) for New Journal of Chemistry

Supporting information

Electrochemical detection of nucleic acids using pentaferrocenyl phosphoramidate α-oligonucleotides

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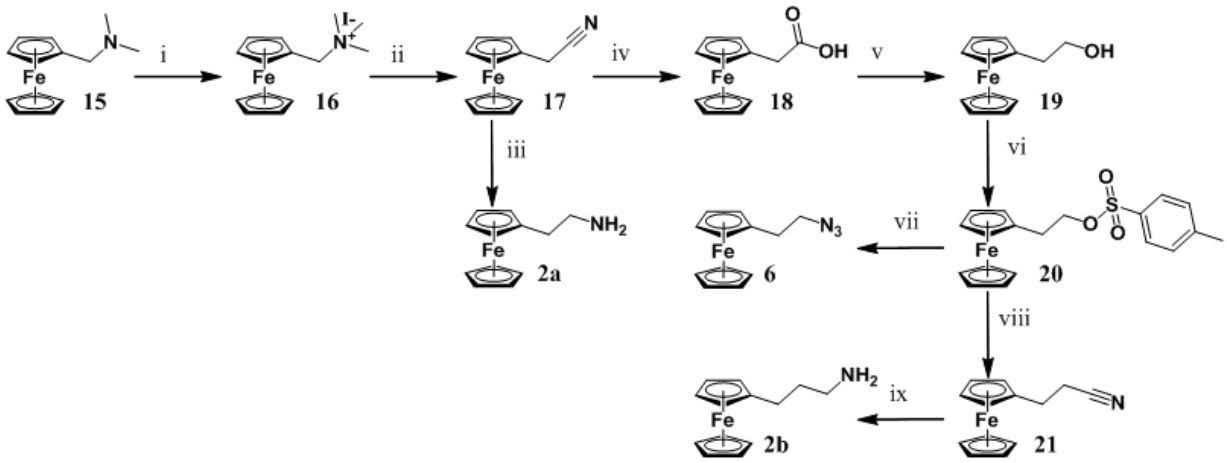
Synthetic route for the preparation of the ferrocenyl monomers 2a, b and 6:



Scheme S1 Synthetic route for the preparation of the ferrocenyl monomers **2a, b** and **6**. i) MeI, MeOH, rt, ii) NaCN, H_2O, reflux, iii) $LiAlH_4$, Et_2O, reflux, iv) KOH, EtOH, rt, v) $LiAlH_4$, THF, reflux, vi) *p*-TsCl, Py, CH_2Cl_2, 0°C, vii) NaN_3, H_2O, MeCN, reflux, viii) NaCN, H_2O, MeCN, reflux, ix) $LiAlH_4$, Et_2O, reflux.

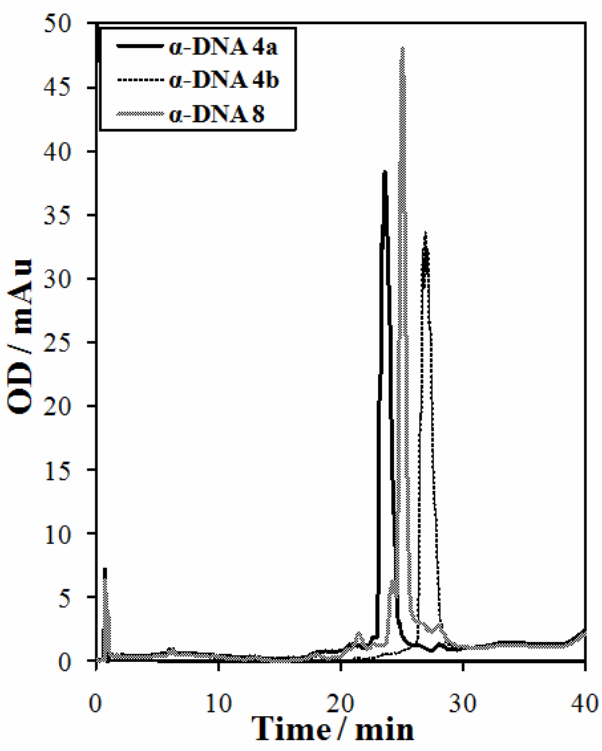


Fig. S1 HPLC spectra of Fc_5-α-DNAs **4a** (solid line), **4b** (dashed line) and **8** (hashed line) after purification. Analyses were achieved on a X-terra MS C18 2.5 µm column at 60°C using a linear gradient of acetonitrile from 12% to 64% in 30 min, then 64 % for 5 min and finally 64% to 80% in 5 min at 1 mL/min in TEAAc 50 mM pH 7.

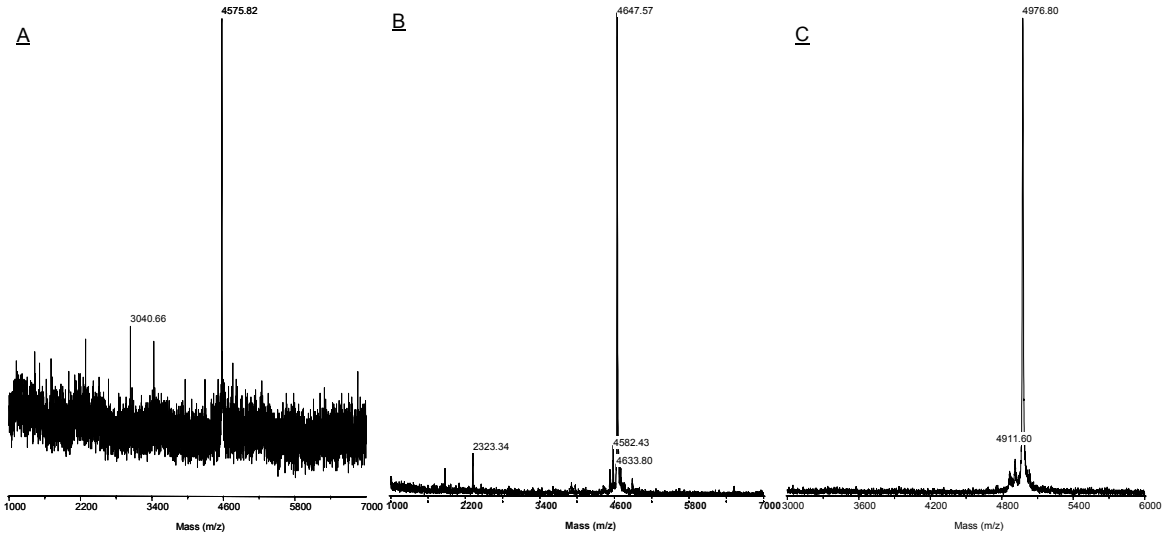


Fig. S2 MALDI TOF MS of the purified Fc_5-α-DNAs **4a** (A), **4b** (B) and **8** (C) (2,4,6-trihydroxyacetophenone (THAP) as matrix).

Table S1 Non modified DNA sequences used for the thermodynamic and electrochemical experiments

DNA	Anomeric configuration	Sequence 5'→3'	Linkage
9	*α*	TTCTCCTTCTTT	PO
10	*β*	AAGAGGAAGAAA	PO
11	*β*	TTTCTTCCTCTT	PO
12	*β*	ATTGAGATTCCCGAGATTGA	PO

Thermodynamic parameter determination

Thermodynamic parameter calculation (*Tm*, $\Delta S°$, $\Delta H°$ and $\Delta G°_{298}$): Dissociation constants ($K(T)$) were calculated for Fc_5-α-DNA/target duplexes as described by Marky et al.[1] and Durand et al.[2]. For this analysis, it is first necessary to cast raw optical data into the fraction of the single strand and duplex state at each temperature $\alpha(T)$, for Fc_5-α-ON/target binding experiments. To accomplish this, upper and lower absorption baselines (A_U and A_L) were determined by calculating the absorbance derivative (dA/dT). A_U and A_L values corresponded to both dA/dT minimums of the curve. Next, the bound fraction parameter $\alpha(T)$ was computed for each temperature (T) according to equation **1**:

$$\alpha(T)= (A_U-A(T))/(A_U-A_L) \quad \textbf{(1)}$$

where $A(T)$ is the absorbance at the temperature T.

Consequently, a plot of $\alpha(T)$ versus temperature was constructed and used to calculate the *Tm* value by interpolating α=0.5. The transition equilibrium $K(T)$ was calculated at various temperatures by using the equation **2**:

$$K(T)= \alpha(T)/([C_T/n]^{n-1}\,[1-\alpha(T)]^n) \quad \textbf{(2)}$$

where n is the number of strands involved in the thermodynamic equilibrium and C_T, the sum of their concentrations. In the case of the Fc_5-α-DNA/target solution, n=2. This equation is only available for non self-complementary duplexes. A van't Hoff plot of $\ln(K(T))$ versus $1/T$ was generated for duplex formation. The slope and intercept of the calculated line yielded to the enthalpy ($\Delta H°$) and the entropy ($\Delta S°$) respectively, according to the equation **3**:

$$\ln(K(T))= -(\Delta H°/R)\cdot 1/T + \Delta S°/R \quad \textbf{(3)}$$

where R is the gas constant (1.987 Cal K^{-1} mol^{-1}) and T the temperature in Kelvin. The Gibbs free energy at 298°K ($\Delta G°_{298}$) was calculated according to the equation **4**:

$$\Delta G°_{298}= \Delta H°-298\cdot\Delta S° \quad \textbf{(4)}$$

The obtained data represent at least the average of minimum five independent experiments on duplex formations. The errors addressed on the thermodynamic data result from the standard deviation of the three experiments. Standard deviation calculated for *T*m values are within ±0.5°C and those of $\Delta H°$, $\Delta S°$ and $\Delta G°_{298}$ are within ±7%. Values of $\Delta S°$ and $\Delta H°$ used for the calculation of $\Delta G°_{298}$ were gathered in Table S2.

Table S2 Thermodynamic parameters of Fc_5-α-DNAs **4a**, **4b** and **8** with complementary sequence **10** in comparison with PO α-DNA **9** and β-DNA **11**.

Duplex	*Tm* (°C)	Δ*Tm*	Δ*Tm*/Fc	ΔG_{298} (kCal mol^{-1})	Δ*H*° (kCal Mol^{-1})	Δ*S*° (Cal Mol^{-1} T^{-1})
9/**10**	46.6 ± 0.4	---	---	-13.9 ± 0.2	-78.7 (± 3.0)	-217 (± 9)
4a/**10**	48.4 ± 0.4	+1.8	+0.4	-12.6 ± 0.3	-55.8 (± 3.2)	-145 (± 10)
4b/**10**	47.1 ± 0.2	+0.5	+0.1	-12.7 ± 0.2	-60.1 (± 2.4)	-159 (± 7)
8/**10**	45.0 ± 0.5	-1.6	-0.3	-12.8 ± 0.6	-62.2 (± 3.3)	-166 (± 13)
11/**10**	43.1 ± 0.2	-3.5*		-13.2 ± 0.1	-80.1 (± 2.7)	-224 (± 9)

* Δ*Tm* corresponds to 12 modifications of α to β nucleoside. Experiments were carried out at 1μM concentration for each strand in 25mM sodium phosphate buffer, 250mM NaCl pH 6.4. Data are the mean values of minimum five experiments.

Electrochemical characterization

Electrochemical characterization and hybridization kinetics: Standard characterization of the Fc_5-α-DNAs electrochemical response has been achieved on a convention electrode device as described previously,[3] using an external reference electrode of Ag/AgCl, a gold working electrode (1.6 mm diameter) and a counter electrode of Pt, at a scan rate of 100 mV s^{-1}. 50 µM solutions of Fc_5-α-DNAs were used in a 25 mM sodium phosphate buffer, 250 mM $NaClO_4$, pH 6.4 (Table S3).

Table S3 Electrochemical characterization of the Fc_5-α-DNAs (50 µM) on a conventional electrode device; reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}.

Fc_5-α-DNA	$E_{1/2}$ ± 10 (mV)	*Epa* (mV)	*Epc* (mV)	Δ*Ep* (mV)
4a	226	228	223	5
4b	200	222	178	44
8	253	256	249	7

Hybridization experiments:

Hybridization tests were carried out by introducing the target in the droplet. Electrochemical response was followed by CV before and after the target addition (scan rate: 100 mV s^{-1}; potential range: -300 mV/+300 mV). [Fc_5-α-DNA]=10 µM and [target]=55 µM (5.5 eq).

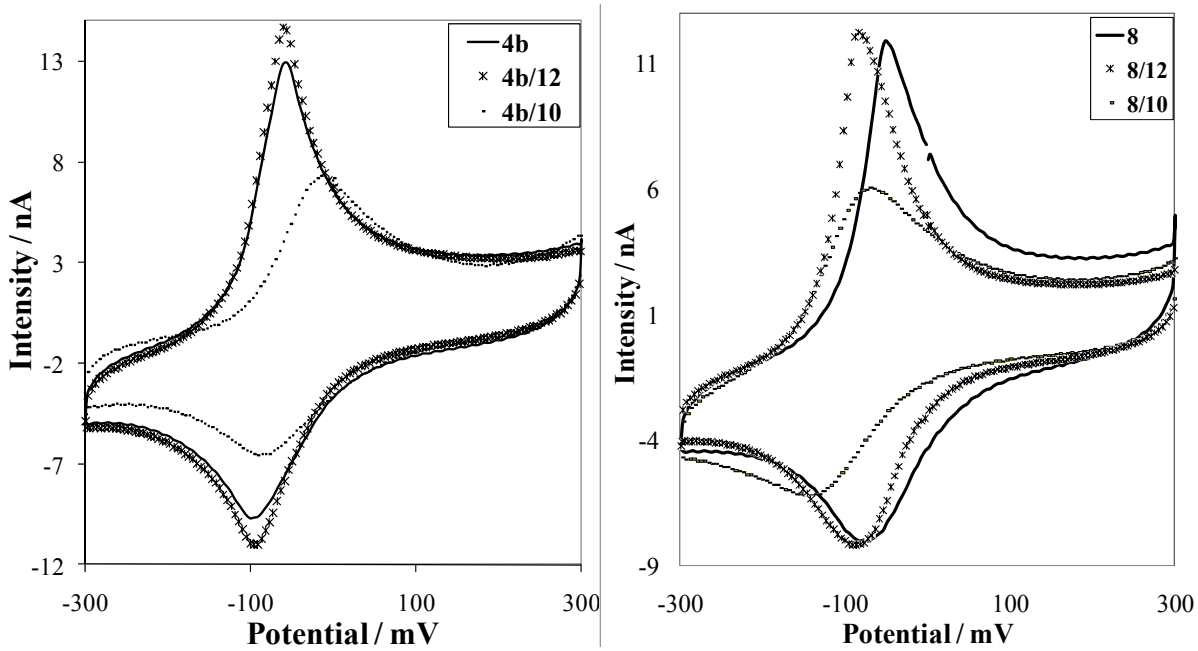


Fig. S3 CV of Fc_5-α-DNA **4b** or **8** alone (solid line), with non complementary **12** (∗) and complementary **10** (-) sequences. Experiments were carried out in 10 µL of 25 mM sodium phosphate buffer, 250 mM $NaClO_4$, pH 6.4 with a 10 µM concentration of Fc_5-α-DNA **4b** or **8**, and 5.5 eq of targets **10** or **12**. Scan rate: 100 mV s^{-1}.

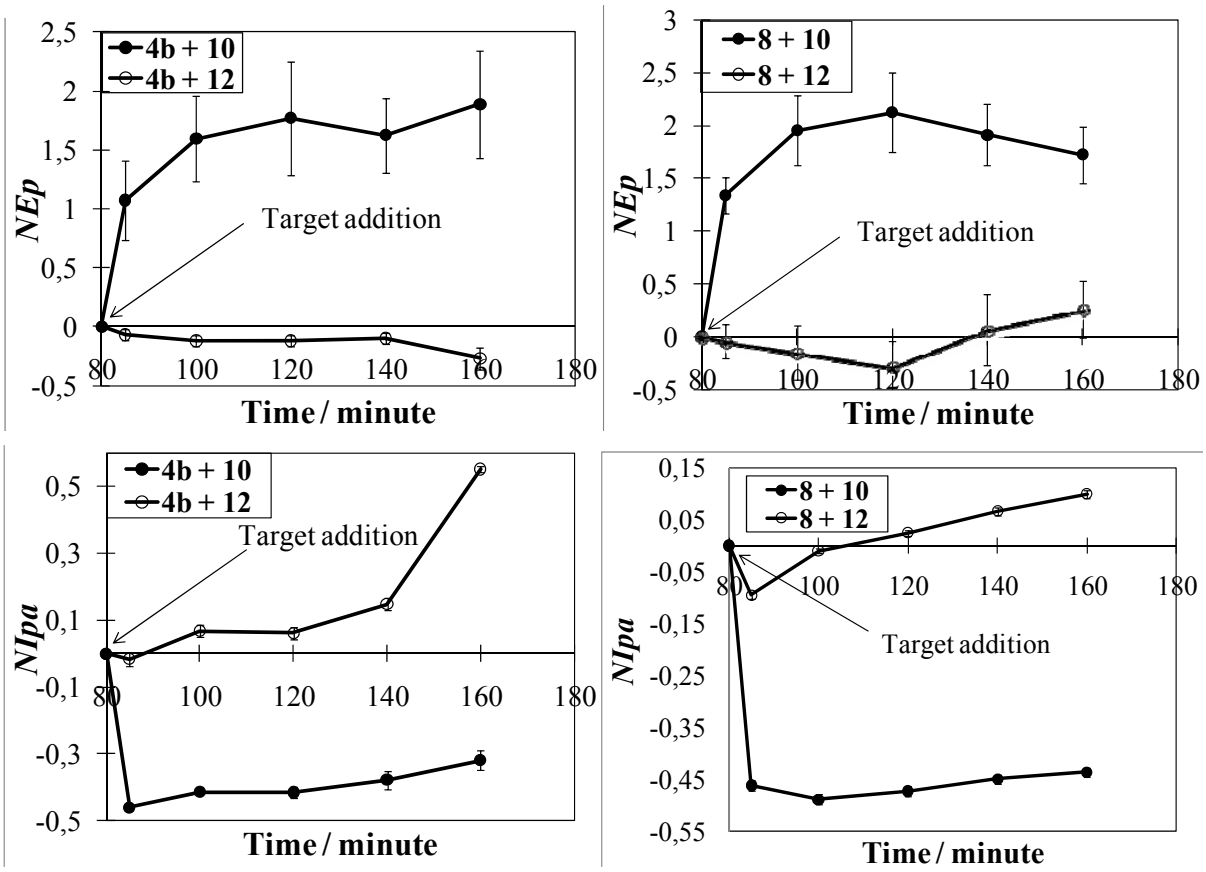


Fig. S4 Evolution of the *NEp* and the *NIpa* of **4b** and **8** by CV after the addition of the complementary **10** or the non complementary **12** in the solution. Experiments were carried out in 10 µL of 25 mM sodium phosphate buffer, 250 mM $NaClO_4$, pH 6.4 with a 10 µM concentration of Fc_5-α-DNA **4b** or **8** and 5.5 eq of **10** or **12**. Scan rate: 100 mV s^{-1}.

It is worth noting that the addition of the non complementary target slightly decreased *NEp* and increase *NIpa* of **4b** and **8** responses, probably due to an electrostatic effect of the negatively charged target on probes adsorbed on the electrode, after introduction in the medium. This phenomenon was previously reported with ferrocenyl-stem-loop oligonucleotides, in the same conditions.[4]

Progressive addition of the target 10:

To determine the sensitivity range of our system, a hybridization experiment was carried out with an increasing concentration of **10,** from 0 to 88 µM, at a constant probe concentration of 10 µM. This experiment was achieved to see how the Δ*Ep* parameter evolved with a variation of the complementary target concentration in the solution (Fig. S5).

10 µL droplet of the probe in sodium phosphate buffer (25 mM)/$NaClO_4$ (250 mM)/pH 6.4 were deposited on the biochip's working area. The system was left for stabilization during 80 min. 0.3 µL of target **10** solutions at different concentrations was successively added in the solution. CVs were recorded at 5 min, 20 min, and 40 min after the target addition. The values at 40 min that corresponded to a stable response of the system were represented on Fig. S5 for Fc_5-α-DNAs **4a**, **4b** and **8**.

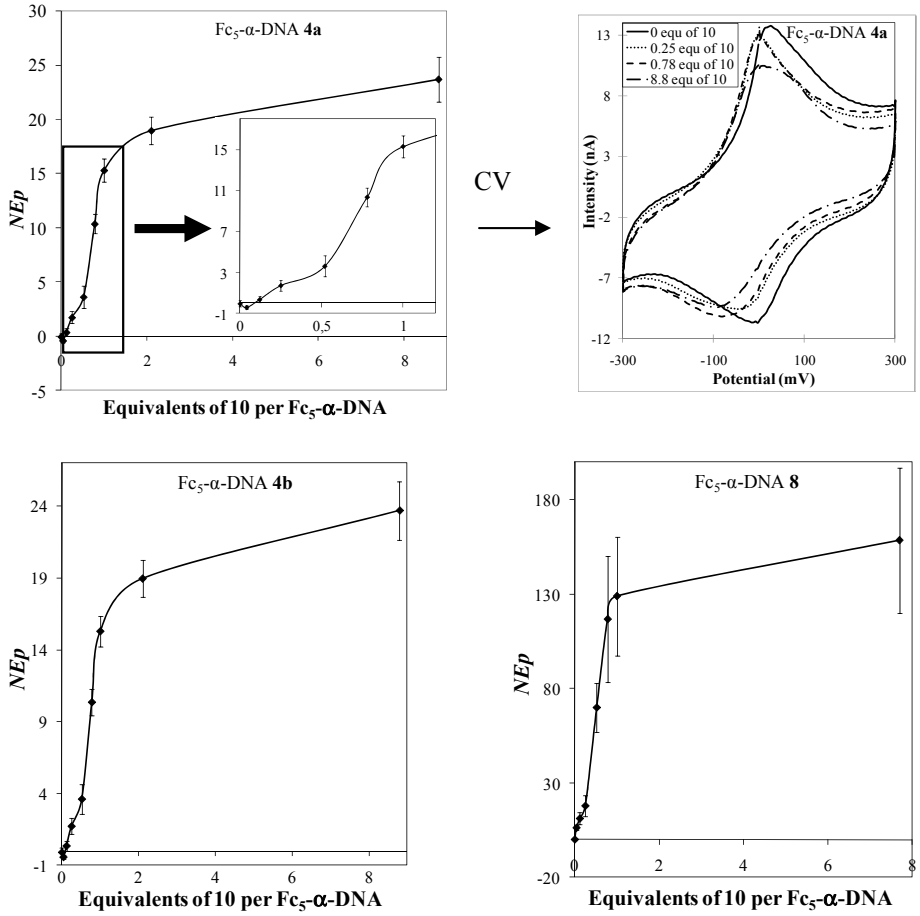


Fig. S5 Evolution of the *NEp* of Fc_5-α-DNAs **4a**, **4b** and **8** by CV after successive additions of target **10** from 0 to 8.8 equivalents. Experiments were carried out in 10 µL of sodium phosphate buffer (25 mM)/$NaClO_4$ (250 mM)/pH 6.4 with a 10 µM concentration of Fc_5-α-DNAs. Scan rate: 100 mV s^{-1}

The maximum variation was recorded after an addition of 2.1eq of **10**. Moreover, the response was considered to be significant for a Δ*Ep* variation higher than 10% of the value reached at the plateau. A minimum concentration of 2.5 µM (0.25 equivalents) of **10** was detected for Fc_5-α-DNA **4a** at 10 µM. The same detection threshold was obtained for Fc_5-α-DNAs **4b** and **8**. A quantitative determination of the **10** can be measured between 0.25 and 2.1 eq of the electrochemical probe. The presence of two species involved, binding and non-binding was not observed by CV. Only global oxidation and reduction waves were recorded during the experiment (see CV for **4a** on the top right).

1. L. A. Marky and K. J. Breslauer, *Biopolymers*, 1987, **26**, 1601-1620.
2. M. Durand, K. Chevrie, M. Chassignol, N. T. Thuong and J. C. Mauriziot, *Nucleic Acids Res.*, 1990, **18**, 6353-6359.
3. C. Farre, N. Spinelli, A. Bouchet, C. Marquette, B. Mandrand, F. Garnier and C. Chaix, *Synth. Met.*, 2007, **157**, 125-133.
4. G. Chatelain, H. Brisset and C. Chaix, *New J. Chem.*, 2009, **33**, 1139-1147.