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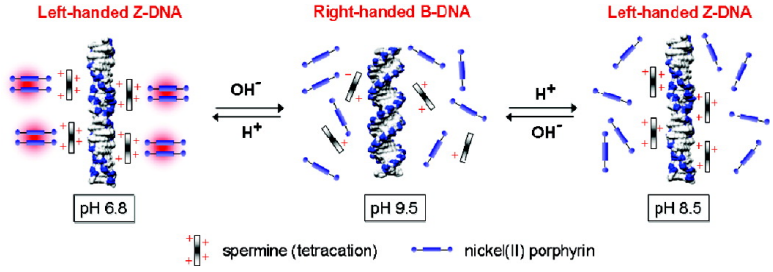

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Interactions of a Tetraanionic Porphyrin with DNA: from a Z-DNA Sensor to a Versatile Supramolecular Device

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Left-handed Z-DNA is a higher-energy conformation of double stranded DNA.[1] The biological relevance of Z-DNA has been recently demonstrated by the discovery of proteins that bind specifically to Z-DNA.[2] Detection and recognition of noncanonical Z-DNA represents an important but difficult challenge.[3−5] The Norden and Barton groups were the first to report recognition of B- and Z-DNA by using the chiral complexes of transition metals.[3] The Sugiyama group reported that chiral helicenes can discriminate between B- and Z-DNA.[5]

We have shown that cationic zinc(II) porphyrin (ZnT4) can selectively detect the left-handed Z-form of DNA.[6] Spectroscopic properties of metallo-porphyrins are very attractive for sensing Z-DNA: their absorption (∼400 nm) is far from the "crowded" UV region where both nucleic acids and proteins strongly absorb. This allows for detection of Z-DNA in complex mixtures. Interactions between porphyrins and B-DNA have been well characterized.[7] However only a few studies have been performed on porphyrins as probes to distinguish between various DNA forms.[6,8] We demonstrate here that a tetraanionic nickel(II) *meso*-tetrakis(4-sulfonatophenyl)porphyrin NiTPPS (**1**, inset Figure 1) is able to selectively sense the spermine induced Z-form of DNA. In addition, the resulting **1**-Z-DNA complex behaves as reversible information storage system and an AND logic gate.

We used poly(dG-dC)$_2$ as a tunable B-to-Z DNA scaffold and spermine, a tetra-amine, as a micromolar inducer of the Z-DNA conformation.[9] Spermine is the most efficient inducer of the Z-DNA and can be found in high concentrations in eukaryotic cells. Logically the effect of spermine on the B-Z DNA transition has received much attention.[10] In the presence of the right-handed B-form of poly(dG-dC)$_2$, no induced circular dichroism (ICD) signal is observed at ∼400 nm for **1** (Figure 1, blue curve), while in the presence of left-handed Z-DNA (Figure 1, red curve) an intense negative exciton coupled CD signal appears. This demonstrates that anionic nickel(II) porphyrin **1** is able (i) to selectively sense Z-DNA and (ii) to spectroscopically discriminate between the B-DNA and Z-DNA at micromolar concentrations. The intensity of the ICD signal in the presence of Z-DNA rises with increasing concentration of **1**. The binding curve has a sigmoidal profile (Supporting Information, Figure S2). The saturation of the ICD signal is observed at ∼9 μM of **1** with ICD of −16 mdeg. The mixing ratio, defined by the concentration of porphyrin unit per DNA base [**1**]/[DNA], then equals 0.18. Hence no more than two porphyrins per each DNA turn contribute to the ICD signal. The degree of cooperation $n = 2$ (Hill equation fit, Figure S2) indicates a minimum

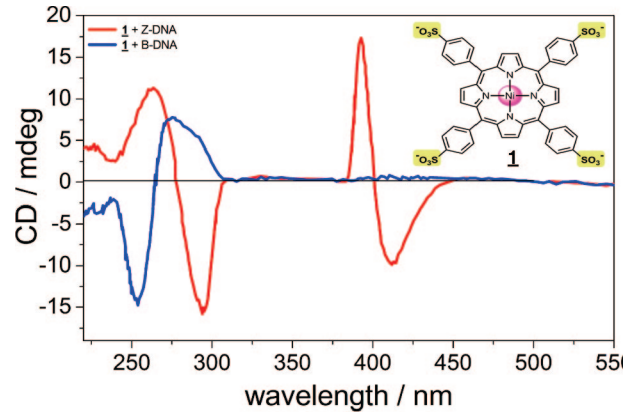

Figure 1. CD spectrum of NiTPPS **1** (4 μM) in the presence of poly(dG-dC)$_2$ (50 μM) having left-handed Z conformation (red curve) or right-handed B conformation (blue curve). Experimental conditions: spermine (14 μM), NaCl (10 mM), cacodylate buffer (1 mM), pH = 6.8, room temp.

of two binding events and is in agreement with the minimum number of porphyrins necessary to acquire an exciton couplet. Titration of **1** against the right-handed B-DNA also shows a sigmoidal curve with Hill coefficient $n = 5.5$ (Figure S2). However a long initial lag period with almost no ICD is observed for up to 4 μM of **1**. Only a small ICD increase is detected above 5 μM of **1** with saturation of the ICD signal at 9 μM (−2 mdeg).

The interactions between the anionic **1** and negatively charged DNA are mediated by structural differences between B- and Z-DNA as well as by protonated spermine, (four positive charges, the predominant species at pH 6.8).[11] On one hand, spermine shields the negative charge repulsions and stabilizes the **1**-Z-DNA complex.[10] On the other hand, the B to Z transition causes exposure of nitrogen N7 of guanine which, similarly to what we have proposed earlier,[6] provides additional stabilization through axial coordination with nickel in **1**. Resonance light scattering (RLS, Figure S3)[12] shows that **1** aggregates onto both Z- and B-DNA. Because of the loss of some vibrational freedom, the emission of **1** increases in both cases (Figure S4), but to a much larger extent in the presence of the Z-form. This suggests that the central Ni(II) binds to guanine N7, increasing the "immobilization" of **1**.[6]

We anticipated that it is possible to modulate both **1**−Z-DNA interactions and DNA helicity just by varying the degree of spermine protonation (i.e., the number of positive charges it carries) by varying the pH. Each state and pH (Figure 2) shows a characteristic chiroptical signature making the system a reversible information storing device, as seen in Figure 3. As the complex **1**−Z-DNA is assembled at pH 6.9, an intense ICD appears in the Soret region (Figure 3, black curve). Raising the pH to 8.2 the ICD disappears (Figure 3, red curve), indicating the disruption of the

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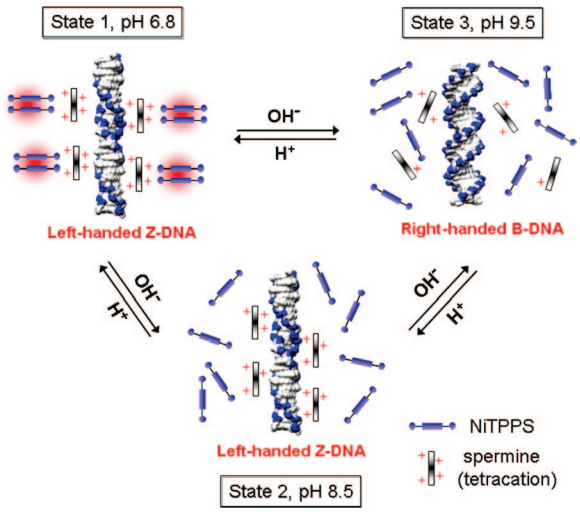

Figure 2. Modulation of **1**-poly(dG-dC)$_2$-spermine complex with pH.

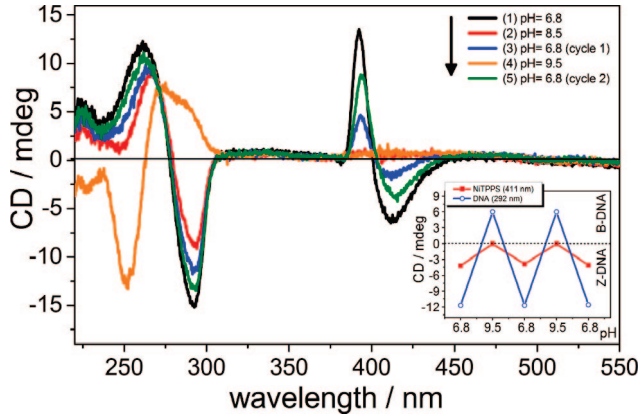

Figure 3. CD spectrum of porphyrin **1** (4 μM) in the presence of 50 μM poly(dG-dC)$_2$ at pH = 6.8 (black curve) and after the reported sequential pH variations. Experimental conditions: spermine (14 μM), NaCl (10 mM), cacodylate buffer (1 mM), room temp. *Inset*: Reversible CD signal changes at 48 °C in the **1**-poly(dG-dC)$_2$-spermine complex at different pH recorded at 411 nm (red line, **1**) and 292 nm (blue line, DNA).

1-Z-DNA complex by partial deprotonation of spermine (from four to three positive charges). CD features below 300 nm are not affected testifying preservation of Z conformation.[13]

Lowering the pH back to 6.9 is followed by the restoration of **1** ICD signal in less than 1 h, showing that the process is reversible (blue curve). Raising the pH up to 9.3 (from 6.9) causes the disappearance of Soret ICD and the Z-DNA to B-DNA transition (orange curve, CD below 300 nm). Lowering the pH back to 6.9 leads to the slow appearance of (i) the CD signal typical of the left-handed Z-DNA in the UV region of the spectrum (Figure 3, CD below 300 nm) and (ii) ICD signal of the **1** in the Soret region (Figure 3, green curve), verifying that it is possible to cycle reversibly among various states.[14] The simultaneous restoration of both left-handed Z-DNA helix and **1**-Z-DNA complex (directly from state 3 to state 1) proved to be slow (~16 h) and incomplete even at elevated temperatures although the B to Z transition is enthalpy driven.[14,15] Therefore we decided to go *via* a two-step process (pH 9.5 → 8.5 → 6.8, Figure 2) and to increase the temperature to 48 °C.[16] Under these conditions the **1**-Z-DNA-spermine complex was fully restored in less than 15 min (for two steps, Figures S5, S6). Several pH cycles were performed successfully at 48 °C (inset Figure 3).

Finally, using pH and temperature as input and ICD as output our system behaves as a reversible AND logic gate (Table 1).

Table 1. Truth Table: AND Logic Gate

inputs		output (ICD)
i_1 (pH)	i_2(T)	AND
1 (6.5)	**1** (25 °C)	**1** (ICD)
1 (6.5)	**0** (60 °C)	**0** (no ICD)
0 (9.5)	**1** (25 °C)	**0** (no ICD)
0 (9.5)	**0** (60 °C)	**0** (no ICD)

In conclusion, we showed that the sulfonated nickel(II) porphyrin **1** can spectroscopically discriminate between the right-handed B-DNA and left-handed Z-DNA. In the presence of left-handed Z-DNA **1** gives rise to a strong induced negative exciton coupled CD signal, in the presence of right-handed B-DNA no CD signal is observed in the Soret region. The porphyrin−porphyrin through space electronic interaction[6] is the origin of the observed induced exciton coupled CD signal. **1** interacts with the negatively charged Z form of DNA as a result of the shielding action of the protonated spermine. In the absence of spermine there are no interactions between **1** and DNA. We showed that the ternary **1**-DNA-spermine system can be modulated reversibly by pH variations. These characteristics make it a supramolecular reversible information storage system and an AND logic gate.

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Supporting Information Available: Temperature and pH modulated circular dichroic spectra, kinetic, fluorescence and RLS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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