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A novel pyrene-guanidiniocarbonyl-pyrrole cation efficiently differentiates between ds-DNA and ds-RNA by two independent, sensitive spectroscopic methods

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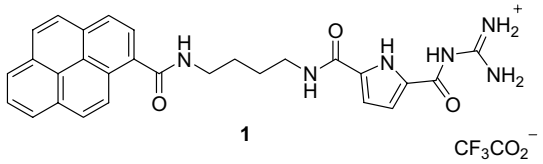
Abstract—At micromolar concentrations and equimolar conditions in respect to basepairs, a novel pyrene-guanidiniocarbonyl-pyrrole cation **1** exhibited a strong ICD signal at about $\lambda = 300$ nm specifically upon the interaction with ds-DNA, while under the same conditions a new fluorescence maximum at $\lambda = 480$ nm appeared exclusively upon the addition of ds-RNA.

Up till now only few small molecules are known which are able to specifically differentiate between simple double stranded (ds-) DNA (B-helix) and ds-RNA (A-helix), independently of their basepair sequence. Such a sensing is often based on the specific binding of molecules exclusively in the minor groove of ds-DNA.[1] Consequently, to the best of our knowledge, there is no small molecule known so far which gives different spectroscopic signals for ds-RNA and ds-DNA, respectively. We report here a new type of a molecular probe by combining a pyrene moiety with a guanidiniocarbonyl pyrrole cation. Pyrene is a well-known polarity-sensitive fluorescence probe often employed for probing microheterogeneous systems,[2a,b] especially DNA.[2c–f] In compound **1** a pyrene moiety was covalently linked via a flexible alkyl chain to a guanidiniocarbonyl pyrrole cation, known to be a highly efficient oxoanion binding site even in aqueous solutions.[3] The combination of an planar aromatic fluorescent probe with an efficient oxoanion binding site should allow for multiple non-covalent interactions with DNA/RNA such as H-bonds, electrostatic interactions and aromatic stacking. For example, guanidinium cations just recently have been used to increase the affinity of the antibiotic neomycin to RNA.[4] The linker between the pyrene and the guanidiniocarbonyl pyrrole cation should be long enough to allow for both intercalation of the pyrene into the nucleic acid double strand and ion pairing of the guanidiniocarbonyl pyrrole cation with the sugar phosphate backbone in the groves of either DNA or RNA, respectively. In preliminary experiments, smaller aromatics than pyrene (benzene or naphthalene) proved not to be efficient for intercalation. The linker length of four carbons was based on the hope to provide enough flexibility without loosing too much binding energy due to an unfavourable entropy. The linker was not yet optimized based on modelling studies for example and leaves way for further improvement of the compound (Scheme 1).

Due to the significant differences between the grooves of ds-DNA and ds-RNA, different combinations of non-covalent interactions of **1** with either nucleic acid type



Scheme 1. Structure of the studied compound **1**.

Keywords: Guanidinium; Pyrene; Pyrrole; ds-DNA; ds-RNA.
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was expected. This could allow for the occurrence of different spectroscopic signals upon complex formation with either ds-DNA or RNA. Furthermore, the binding features of the guanidinocarbonyl pyrrole cation are easily tunable by pH since oxoanion binding requires the protonation of the guanidinium cation. The pK_a-value of the acyl guanidium group in **1** is around 6 so in aqueous solution **1** can either be protonated or deprotonated. The synthesis of **1** is described in the Supporting Information.

The interactions of **1** with either ds-DNA or ds-RNA were therefore studied at pH 7 and pH 5, respectively. At pH 7 compound **1** is mainly present in the deprotonated form whereas at pH 5 it is mainly present as the protonated cation. As only the protonated cation is an oxoanion binding site, different interactions with the anionic nucleic acids was expected at both pHs. And indeed, the addition of **1** at pH 7 did not influence the thermal denaturation of either calf thymus (ct-) DNA or polyA–polyU (ds-RNA) even close to equimolar conditions ($r_{[1]/[polynucleotide]} = 0.3$). In contrast, at pH 5 compound **1** stabilized ct-DNA by $\Delta T_m = +7.3$ °C but weakly destabilized poly A–poly U ($\Delta T_m = -1.5$ °C). These results not only confirm that the protonation state of **1** controls its interactions with nucleic acids but also revealed different modes of interaction of **1** with either ds-DNA or ds-RNA, respectively. Unfortunately, the changes in the UV/Vis spectra of **1** upon the addition of ct-DNA were rather small in the region of $\lambda > 300$ nm, thus hampering the use of UV/Vis titrations for further studies. However, the changes of the fluorescence spectrum of **1** are strongly dependent on both the type of polynucleotide added and the pH of the solution. At pH 7, titration of **1** with DNA (ct-DNA, poly dA–poly dT) or RNA (polyA–polyU, polyG–polyC) resulted in an efficient quenching of its fluorescence independent of the type of nucleic acid used. The excellent fit of the titration data to Scatchard equation[6] suggested that only one dominant complex is formed. The calculated values for the binding constants show a rather high affinity of **1** for all types of ds-DNA and RNA ($\log K_s = 5.1 - 6.0$). However, the high Scatchard ratios $n_{[bound\ 1]/[polynucleotide]} = 0.6-3.6$ do not support an intercalative binding mode. This is in agreement also with the lack of any thermal stabilization at this pH.[12] These results suggest an unspecific hydrophobic-driven agglomeration of **1** which is neutral at pH 7 with either DNA or RNA, perhaps additionally stabilized by some intermolecular π-stacking of aromatic moieties in the nucleic acid and **1**.

In contrast to the situation at pH 7, at pH 5 fluorescence titrations of **1** with all types of ds-DNA studied yielded two opposite tendencies of fluorescence changes (Fig. 1)[†], most likely due to the coexistence of at least two different types of complexes. For ct-DNA, the observed 'break point' between these two opposite spectroscopic changes around a ratio $r_{[1]/[ct\text{-}DNA]} = 0.14$ is consistent with an intercalative binding as such a behaviour is also seen for many other aromatic intercalators.[7,12] Namely, at excess of intercalator over intercalation binding sites the non-intercalated molecules of **1** tend to unspecifically stack on the polynucleotide causing a strong quenching of the fluorescence of **1**. However, during the titration the ratio changes to finally an excess of binding sites over **1**. The intercalative binding mode then becomes dominant. With increasing numbers of molecules of **1** being intercalated, one observes an increase in the fluorescence emission of the pyrene moiety of **1** at $\lambda = 398$ nm, which differs significantly from the fluorescence maximum of free **1** at $\lambda = 382$ nm. The excellent fit of the experimental data collected at an excess of ds-DNA (ct-DNA and poly dA-poly dT) over **1** ($r_{[1]/[ds\text{-}DNA]} < 0.2$) to the Scatchard equation[6] also supports the presence of only one dominant binding mode under these conditions. The resulting binding constants ($\log K_s = 5.9 - 6.8$)[†] and ratio $n_{[bound\ 1]/[ds\text{-}DNA]} = 0.01$–$0.09$, as well as the results of viscometry measurements ($\alpha(\mathbf{1}) = 0.75$)[†], are in excellent agreement with an intercalative binding mode.

In the fluorimetric titrations of **1** with ds-RNA (polyA–polyU, polyG–polyC) again two opposite tendencies of fluorescence changes are observed with a break point at about $r_{[1]/[ds\text{-}RNA]} = 0.3$ (Fig. 1)[†]. However, in sharp contrast to the titration of **1** with ds-DNA, a new fluorescence maximum at $\lambda = 480$ nm appeared specifically in titrations of **1** with ds-RNA (Fig. 1 bottom). It is important to stress that this new maximum at 480 nm is dominant at excess of **1** over ds-RNA ($r > 0.5$), decreasing with increased concentrations of ds-RNA. The presence of an isoemissive point (Fig. 1 bottom) in the range of $r_{[1]/[poly\ A\text{–}poly\ U]} = 0.32 - 0.09$ points again towards the co-existence of only two dominant species in this concentration range. It is known that pyrene exhibits a fluorescence maximum around $\lambda = 450$–480 nm either as a consequence of (a) photoinduced intermolecular excimers formed by two or more pyrenes;[8] or (b) by exciplex emission resulting from the strong association of the intercalated excited state of the pyrene with adjacent nucleobases.[5,9] Latter possibility can be excluded in this case because if the intercalation is responsible for this new maximum, its intensity should increase with the increasing concentration of the polynucleotide due to the increasing number of intercalated molecules of **1**. However, this maximum ($\lambda = 480$ nm) is associated with the dominant binding mode at high excess of **1** over ds-RNA intercalation binding sites and its intensity decreases with an increasing concentration of ds-RNA. This is exactly the opposite behaviour which is expected if intercalation was responsible for this emission maximum. Furthermore, also the destabilization of the ds-RNA upon the addition of **1**in the thermal denaturation experiments does not agree with an intercalative binding mode.[12]Thus, the fluorescence maximum at $\lambda = 480$ nm can not result from intercalation but must be due to a photoinduced intermolecular pyrene-excimer[8] formed by two or more molecules of **1** aggregated somehow within a complex formed with the ds-RNA.

[†] Electronic Supplementary Information (ESI) available: Synthesis and characterization of compound **1**, fluorimetric and CD titrations, details about modeling studies.