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# Template-directed excimer formation *via* specific non-covalent interactions between pyrene guanidinium derivatives and nucleic acids

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ABSTRACT

Structurally distinct guanidinium derivatives were evaluated for their ability to interact non-covalently with various nucleic acid sequences. Among the evaluated derivatives, 4-[(pyrene-1-ylmethyl)amino] butyl] guanidinium (**pbg**) was found to demonstrate strong excimer emission upon nucleic acid addition and high levels of discrimination between ds- and ss-DNA. The intensity of excimer emission proved to be dependent on the length of the linker probe as well as the oligonucleotide length and sequence. In particular, G-quadruplex prone structures were found to induce the highest excimer emissions among all nucleic acids tested.



Pyrene is undoubtedly one of the most useful proximity reporters in sensor design owing to its exceptional excimer forming ability. Typically, the monomer emission is characterized by well-defined peaks at ~370–380 nm which differs from the broad band at 450–500 nm observed when two pyrene rings are brought into close proximity. This feature has notably been used for sensing metal ions, phosphates[1] and biomolecules.[2–4] In particular, pyrene excimer fluorescence has proved to be extremely useful in the context of DNA sensing. In a large number of these studies the pyrene moiety is incorporated covalently into DNA sequences through the use of modified phosphoramidite units or post-synthetic modifications.[5–9] Non-covalent approaches have also been evaluated by exploiting electrostatic interactions between positively charged pyrene derivatives and negatively charged phosphodiester internucleosidic linkages of DNA or RNA.[10–16] Interestingly, it has been demonstrated by fluorescence studies that pyrene-ammonium, pyrene-imidazolium and pyrene-thiazolium derivatives induce large excimer/monomer intensity ratios in the presence of G-quadruplex structures.[10,11,14–16] Our interest in positively charged pyrene derivatives was originally stimulated by a desire to characterize non-covalently bound complexes of guanidinium receptors and single-stranded DNA by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

This study revealed the importance of hydrophobicity and π-π stacking interactions and highlighted the prevalence of the pyrene moiety to form very strong complexes with DNA.[17] These properties were eventually extended to the analysis of sulfated polysaccharides and sulfopeptides.[18,19] In light of these results we decided to evaluate the extent of excimer formation between guanidinium derivatives and ssDNA or ssRNA. This study will thus consolidate our ongoing work devoted to evaluate the Y-shape electrostatic interactions between guanidinium and phosphodiester internucleosidic linkages.[20] In order to identify the structural elements that favor excimer emission, 1-pyrenemethyl guanidinium (**pmg**), [4-[(pyrene-1-ylmethyl)amino]butyl] guanidinium (**pbg**), and [6-[(pyrene-1-ylmethyl)amino]hexyl] guanidinium (**phg**) were synthesized as previously reported[20] and evaluated in the presence of homo- and heteropolymeric DNA or RNA oligonucleotides of different lengths (Fig. 1).

The UV–Vis spectra of **pmg**, **pbg** and **phg** show that for a fixed concentration of each guanidinium derivative (50 and 100 mM), addition of an increasing amount of the heteropolymeric random sequence poly($dN_{25}$) (5′-AGCTCGTTTAGTGAACCGTCAGATC-3′) led to a decrease of the absorption bands of the pyrene units at 310, 330 and 340 nm, thus suggesting strong interactions between the oligonucleotide and the guanidinium moiety (Fig. 2). Based on these titrations curves the binding constants of **pmg**, **pbg** and **phg** were found to be 5.53 (±0.15) × $10^5$, 4.70 (±0.12) × $10^5$ and 3.95 (±0.10) × $10^5$ $M^{-1}$, respectively (ESI). These results indicate the preferential binding of **pmg** and **pbg** over the more flexible

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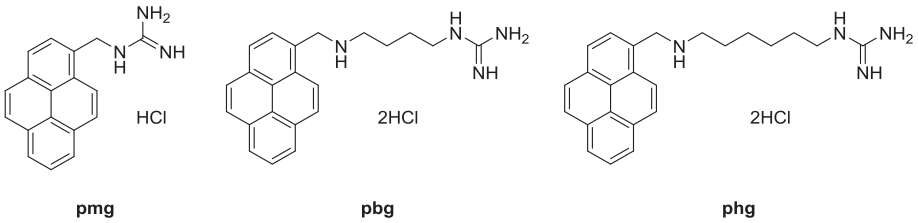


**Fig. 1.** Structures of the evaluated pyrene-guanidinium derivatives.

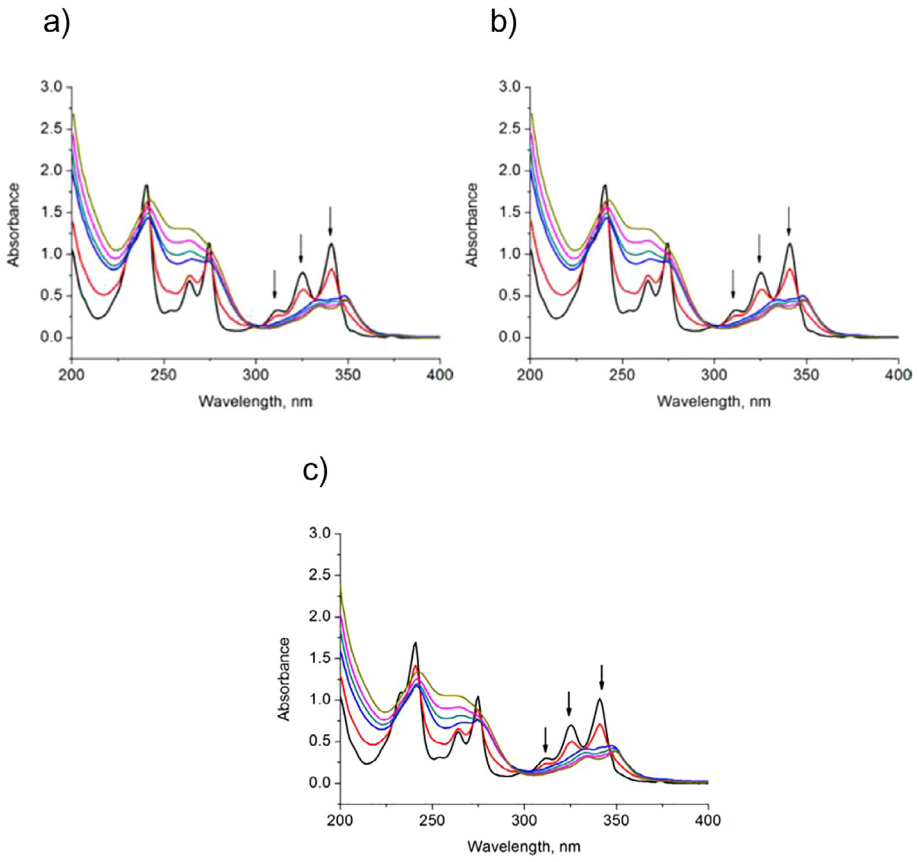


**Fig. 2.** UV–vis absorption spectra of a) **pmg**, b) **pbg** and c) **phg** titrated with poly(dN)$_{25}$. Downward arrows show a decrease in absorbance upon interaction with poly(dN)$_{25}$. Experiments were performed in MOPS buffer (1 mM MOPS and 1 mM NaCl) at pH = 7.5.

**phg** and the slight influence of the added positive charge carried by **pbg** and **phg**.

We then evaluated the fluorescence changes upon titration of **pmg**, **pbg** and **phg** (50 and 100 μM) with increasing amounts of homo- and heteropolymeric DNA oligonucleotides of different lengths. For all oligonucleotides the spectra showed a decrease of the monomer emission (at 375 and 395 nm) concomitant with the appearance of a red-shifted emission band at 470 nm characteristic of pyrene excimer emission. In all cases, as the concentration of DNA was augmented the excimer/monomer emission ($I_E/I_M$) increased up to a maximum point before it started to decrease.

As a general trend higher $I_E/I_M$ maximum values were obtained when the concentration of the guanidinium probe was fixed at 100 μM. Interestingly, the relative intensity of the pyrene excimer emission was highly dependent on the spacer length. As can be seen in Fig. 3, **pmg** which has the shortest alkyl linker (methyl), demonstrates low excimer emission compared to the aminobutyl and aminohexyl linkers. The lower flexibility for stacking interactions imposed by the methylene linker might explain this observation. Meanwhile, with longer linkers, charge repulsion does not seem to inhibit excimer formation. The $I_E/I_M$ maximum value was also strongly dependent on the DNA sequence especially for **pbg** and **phg**. Poly

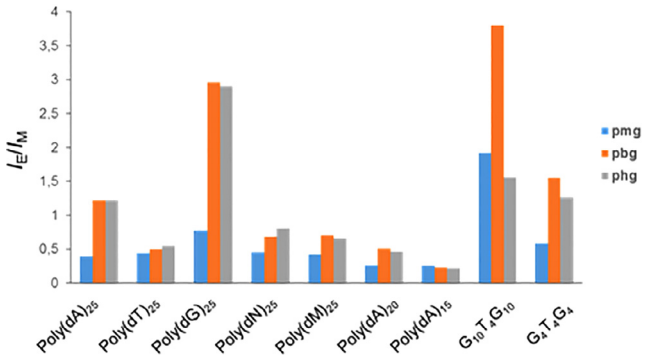


**Fig. 3.** $I_E/I_M$ maximum values obtained for **pmg**, **pbg** and **phg** (100 μM) mixed with various DNA sequences. Experiments were performed in MOPS buffer (1 mM MOPS and 1 mM NaCl) at pH = 7.5.