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A Novel Conformationally Constrained Parallel G Quadruplex

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Guanine-rich DNA sequences are known to form highly ordered structures called G quadruplexes.[1] These structures play an important role in many relevant biological processes, such as telomere stabilization,[2] oncogene activation,[3] and the regulation of the immunoglobulin switch region.[4] The G-quadruplex motif is based on the association of planar G quartets of four guanine residues that are held together by eight Hoogsteen-type hydrogen bonds (Figure 1 A). The G-quadruplex motif requires monovalent cations, such as Na^+ and K^+, for stabilization. A wide variety of topologies can be adopted depending on the number of strands involved in the structure, the strand direction, as well as variations in loop size and sequence (Figure 1).[5] The structure of parallel-stranded as well as antiparallel-stranded quadruplexes have been extensively studied by using different methods, such as NMR spectroscopy,[6] X-ray diffraction[7] and circular dichroism,[8] but the exact conformation present in vivo is still under discussion. The design of small molecules that can bind to G quadruplexes has thus received attention because these nucleic acid motifs represent valuable pharmaceutical targets. For this purpose, a large number of small molecules has been evaluated for their binding with these particular DNA structures.[9] However, as mentioned, the G quadruplex can adopt different topologies that can confuse the study of recognition phenomena. The design of a system that is able to mimic a well-defined conformation of G quadruplex is thus of great interest to precisely study the molecular interactions that can occur with small organic molecules.

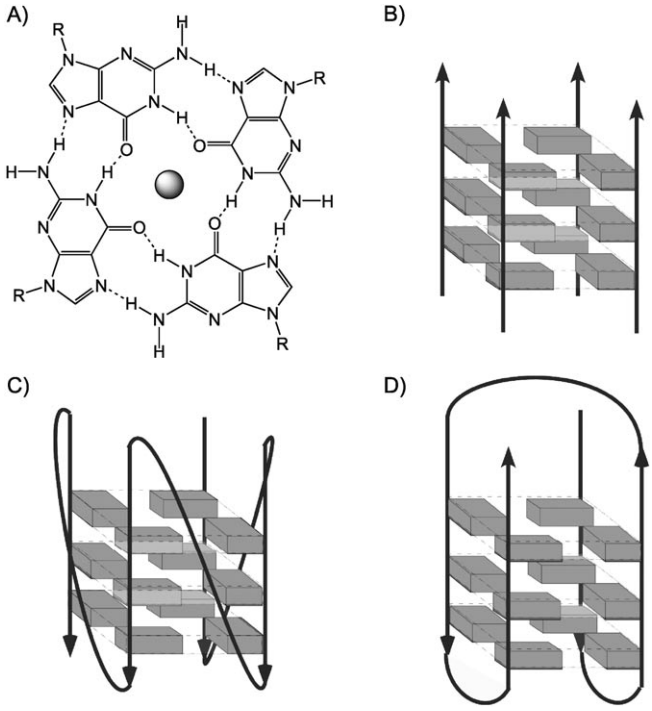

Figure 1. G-quartet motif and possible folded structures of the G quadruplex. A) G quartet; B) intermolecular parallel form; C) intramolecular parallel form; D) intramolecular antiparallel form.

In 1985, Mutter proposed the TASP concept (template-assembled synthetic proteins) for the design of folded proteins.[10] These pioneering works described the use of a cyclodecapeptide that allows the preparation of artificial proteins with a predetermined three-dimensional structure. Despite a large number of examples that use this template, to our knowledge, it has not been applied for the design of a specific folded structure of nucleic acid.[11]

With this in mind, we investigated the use of a peptidic scaffold as a topological template that directs the intramolecular assembly of covalently attached oligonucleotides into a single characteristic folding topology of G quadruplex. We anticipated that the scaffold should permit the preorganization of the DNA strands and the stabilization of the quadruplex structure. We report herein the synthesis and characterization of the novel water-soluble peptidic scaffold–oligonucleotide conjugate **1** that mimics the parallel-stranded conformation of G quadruplex (Scheme 1). We demonstrate that the use of the scaffold allows the precise control of the conformation of the quadruplex and dramatically increases the stability of the motif—all the more so as the formation of the quadruplex motif is possible even without the addition of any monovalent cations, such as K^+. We also show that mimic **1** can be used for surface functionalization, and this permits the study of the molecular interaction with G-quadruplex ligands by using surface plasmon resonance (SPR).

The scaffold used for the synthesis of mimic **1** is a cyclic decapeptide with two independently functionalizable faces, which are due to the orientation of the lysine side-chains. On one side, the four oligonucleotides derived from the human telomeric sequence d(^{5}TTAGGGT^3) were anchored by using oxime bond formation, and a biotin residue was incorporated on the other side for attachment to streptavidin-immobilized surfaces. Earlier work from our laboratory has demonstrated that the oxime coupling strategy allows the efficient preparation of peptide–oligonucleotide conjugates.[12]

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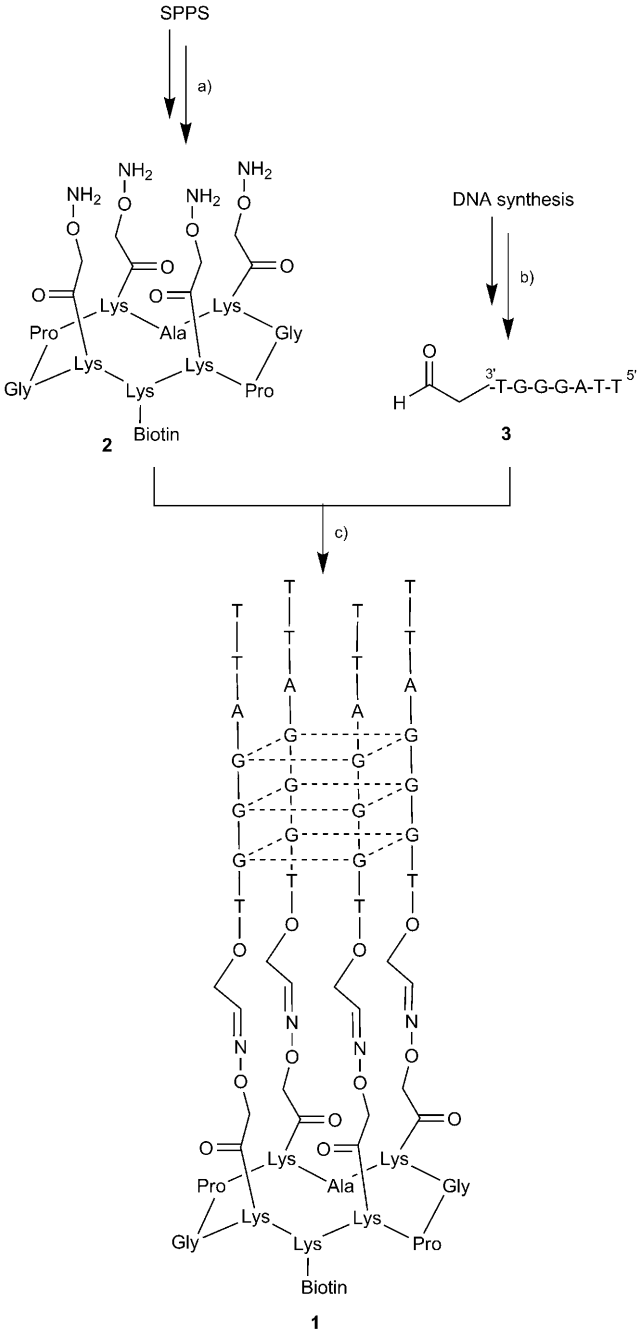

Scheme 1. Synthesis of the G quadruplex mimic **1**. a) 1) SPPS by using a Fmoc/*t*Bu strategy, then head-to-tail cyclization in solution, 2) cleavage of the Boc group under acidic conditions, 3) functionalization by an oxyamino linker, then acidic deprotection; b) automated DNA synthesis on a glyceryl resin then $NaIO_4$ oxidation; c) coupling reaction in 10 mM ammonium acetate buffer (see the Supporting Information).

The preparation of mimic **1** involves the synthesis of the cyclodecapeptide **2** that possesses lysines on the upper face, each of which is functionalized on the side-chain by an oxyamino group, and a subsequent coupling reaction with an aldehyde that contains the desired sequence of oligonucleotides (Scheme 1, see also the Supporting Information). Briefly, the peptide was prepared by using standard Fmoc/*t*Bu solid-phase peptide synthesis on an acid-labile Sasrin® resin, and by head-to-tail cyclization in solution by adapting the previously described procedures.[13] After deprotection of the side-chains of the lysine residues under acidic conditions, the oxyamino groups were introduced by using 2-(1-ethoxyethylideneaminooxy)acetic acid.[14] Final acidic deprotection provided the key intermediate **2**. The oligonucleotide d($^{5'}$TTAGGGTX$^{3'}$) **3**, in which X represents the 3′-aldehyde linker, was synthesized as previously reported.[12b] The coupling reaction was carried out in ammonium acetate buffer (pH 4) by using a slight excess of the aldehyde-containing oligonucleotide **3** and was monitored by anion-exchange HPLC. The reaction proceeded to completion to yield conjugate **1** as the major product (see the Supporting Information). Purification was performed by anion-exchange chromatography; it was noted that RP-HPLC was unsuitable. Mimic **1** was obtained in satisfactory yields (50%) and characterized by MALDI-ToF MS analysis, which showed excellent agreement between the experimentally determined molecular weight and the calculated value.

Circular dichroism (CD) spectroscopy was used to determine the topology adopted by the four oligonucleotides in quadruplex mimic **1**. Previous studies have shown that different topologies of G quadruplex DNA can be associated with a specific CD signature.[15] CD spectra of mimic **1** display a characteristic positive peak at 263 nm and a negative peak at 240 nm (Figure 2A), both of which are expected when a parallel-stranded quadruplex structure is formed. More interestingly, the parallel-stranded quadruplex structure could still be observed even in the absence of cations (Na^+ or K^+).

The thermal stability of G quadruplex conformation was then evaluated by CD spectroscopic denaturation studies (Figure 2B). The samples were incubated at each temperature to achieve equilibrium before recording the CD signal. As shown in Table 1, a dramatic stabilization was observed for mimic **1** in comparison with the parallel-stranded quadruplex formed by the intermolecular association of four strands of sequence d($^{5'}$TTAGGGT$^{3'}$). In fact, a melting temperature (T_m) of 87 °C was measured in the presence of 100 mM of KCl for mimic **1**, whereas the intermolecular quadruplex (d($^{5'}$TTAGGGT$^{3'}$)×4) showed a T_m of 55 °C under the same conditions.[16] The use of the scaffold thus improved the thermal stability of the quadruplex significantly by maintaining the four oligonucleotides bonded together. We also checked whether mimic **1** forms an intra- or intermolecular quadruplex. As expected, the melting temperature of quadruplex mimic **1** was independent of the concentration; this demonstrates the formation of an intramolecular quadruplex (Supporting Information).

The thermodynamic parameters were calculated from the CD spectroscopic denaturation–renaturation studies by using the classical Van't Hoff relation and were compared to those obtained with sequence d($^{5'}$TTAGGG$^{3'}$)$_4$. This latter can fold to an intramolecular quadruplex and is usually considered to be a model of the human telomeric sequence. The calculated values are given in Table 1 (see also the Supporting Information). For both systems the quadruplex is the predominant form at physiological temperature whenever $\Delta G < 0$. The T_m was higher in KCl than in NaCl, which is a well-known property of G quadruplexes.[17] The peptidic scaffold, however, confers a

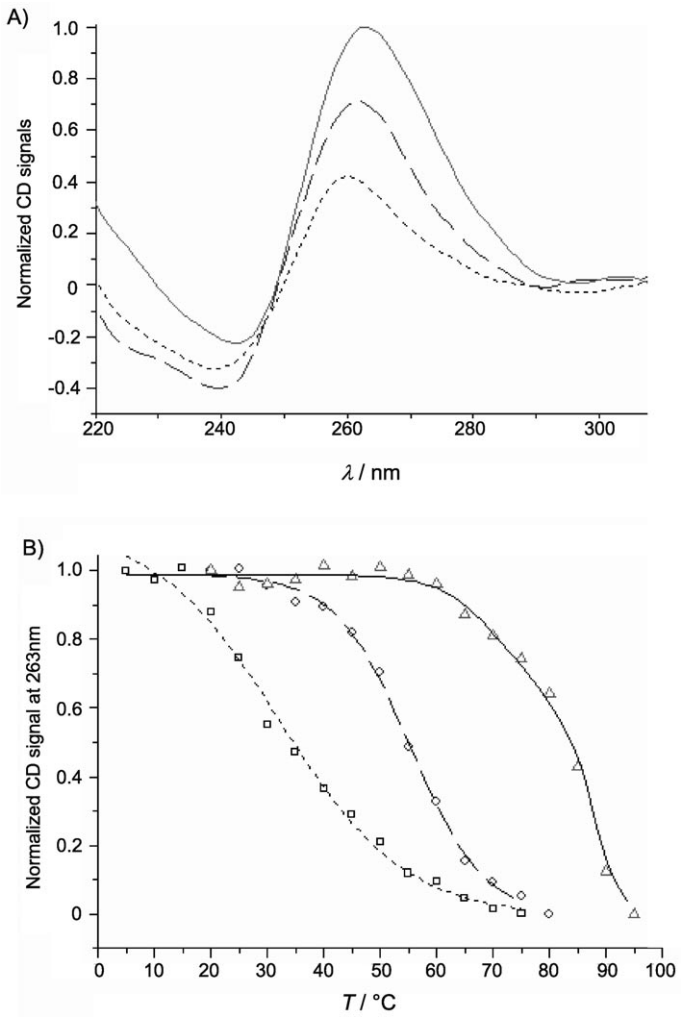

Figure 2. A) CD spectra of mimic **1**, spectra were obtained in 10 mM Tris·HCl buffer with 100 mM KCl (——), 100 mM NaCl (– – –), and without salt (-----) at pH 7.4 at 20 °C; B) thermal denaturation profiles of quadruplex mimic **1**; denaturation was performed in 10 mM Tris·HCl buffer with 100 mM KCl (△), 100 mM NaCl (○), and without addition of salt (□).

greater stability on the quadruplex and allows the formation of the quadruplex motif in the absence of cations to stabilize the structure. The entropy values of mimic **1** and the intramolecular G quadruplex d($^{5'}$TTAGGG$^{3'}$)$_4$ are quite similar; this demonstrates that the formation of quadruplex conformation in **1** is entropically driven due to the preorganization afforded by the scaffold.

Surface plasmon resonance (SPR) is a powerful method for investigating molecular interactions. A number of quadruplex sequences, including the human telomeric quadruplex, the Tetrahymena telomeric quadruplex, the c-MYC promoter quadruplex, the thrombin binding aptamer, and a G_2T_4 basket quadruplex have been immobilized on surfaces for SPR studies.[18] All these quadruplexes consist of an intramolecular folded single strand; this can complicate the interpretation of the results due to the propensity of quadruplex sequences for adopting different topologies. To our knowledge there is no published attempt to immobilize intermolecular quadruplexes on a SPR sensor. Indeed an obvious limitation is that denaturation can occur during the course of SPR experiments. In this way, we examined the ability of mimic **1** to interact with known G-quadruplex ligands. The use of mimic **1** should provide a good way to specifically study the interaction of small molecules with an intermolecular-like quadruplex constrained in a parallel structure. Furthermore, additional structures that could prevent unspecific interactions, such as a loop, are not present in our mimic.

We chose an easily accessible ligand (*N,N'*-bis-(2-(dimethylamino)ethyl)-3,4,9,10-perylenetetracarboxylic acid diimide) to test this concept.[19] Biotinylated mimic **1** was efficiently immobilized on streptavidin-coated SPR biochips. Optimized experimental conditions allowed clear sensorgrams to be collected and interpreted (Figure 3, see also the Supporting Information). We found that the perylene derivative ligand exhibits a high affinity for mimic **1**, and the affinity constant was estimated to be 10^9 M^{-1}.[20] Thus, mimic **1** represents an interesting tool to

Table 1. T_m values and thermodynamic parameters from CD denaturation studies.[a]

Quadruplex	Conditions	T_m [°C]	ΔG [kcal mol^{-1}]	ΔH° [kcal mol^{-1}]	ΔS° [cal mol^{-1} K^{-1}]
1	–	32	−0.7	−17	−56
	NaCl	56	−2.5	−29	−88
	KCl	87	−5.9	−37	−106
d($^{5'}$TTAGGG$^{3'}$)$_4$	NaCl	45	−2.2	−32	−99
	KCl	51	−3.3	−41	−128
d($^{5'}$TTAGGGT$^{3'}$)×4	NaCl	24[b]	n.d.	n.d.	n.d.
	KCl	55[b]	n.d.	n.d.	n.d.

[a] Conditions: quadruplexes were annealed by heating the oligonucleotides and mimic **1** at 90 °C for 10 min in Tris·HCl buffer (10 mM, pH 7.4) with 100 mM KCl or NaCl, or without cations, and cooled slowly to room temperature to favor the thermodynamically stable form. The samples were prepared at 2.5 μM according to UV measurements. All the parameters were determined at 298 K. [b] From ref. [16], n.d.: not determined. The melting transition was found to be reversible.

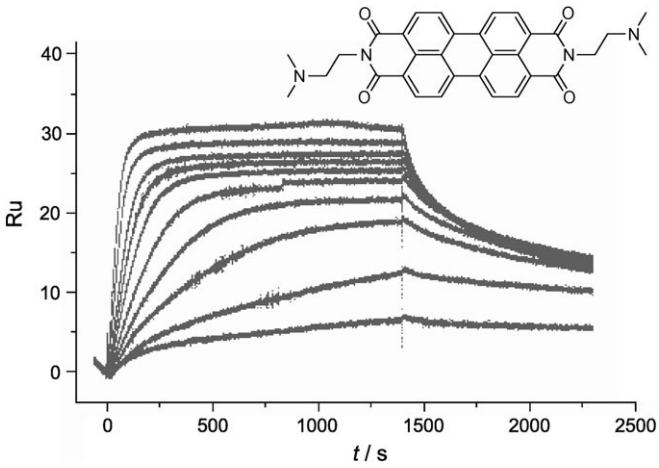

Figure 3. SPR sensorgrams for the interaction of ligand with mimic **1**, which was immobilized on a streptavidin-coated surface. The ligand concentration was varied from 1 nM (bottom curve) to 50 nM (top curve); inset: structure of perylene derivative ligand.

select ligands and to study their interactions with the parallel-stranded conformation of the G quadruplex.

Our studies represent the first template-assembled synthesis of the G quadruplex. We demonstrated that this template allows the formation of a very stable G-quadruplex motif in a unique conformation, in aqueous medium. NMR spectroscopic studies are in progress to determine the exact folding of the DNA strands. Mimic **1** was also anchored on surfaces for SPR studies. This technique should permit the analysis of molecular interactions of various ligands for a single conformation of G quadruplex. The possibility of extending the use of the scaffold to the formation of other specific conformations of DNA (antiparallel G quadruplex, i motifs) is also under investigation.

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Keywords: biosensors · DNA structures · G quadruplexes · surface plasmon resonance · template synthesis

[1] For general reviews on quadruplexes, see: a) J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698; b) *Quadruplex Nucleic Acids* (Eds.: S. Neidle, S. Balasubramanian), Royal Society of Chemistry, Cambridge, **2006**.

[2] a) A. M. Zahler, J. R. Williamson, T. R. Cech, D. M. Prescott, *Nature* **1991**, *350*, 718–720; b) T. M. Fletcher, D. Sun, M. Salazar, L. H. Hurley, *Biochemistry* **1998**, *37*, 5536–5541; c) A. J. Zaug, E. R. Podell, T. R. Cech, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10864–10869.

[3] a) A. Siddiqui-Jain, C. L. Grand, D. J. Bearss, L. H. Hurley, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11593–11598; b) M. Bejugam, S. Sewitz, P. S. Shirude, R. Rodriguez, R. Shahid, S. Balasubramanian, *J. Am. Chem. Soc.* **2007**, *129*, 12926–12927.

[4] M. L. Duquette, P. Pham, M. F. Goodman, N. Maizels, *Oncogene* **2005**, *24*, 5791–5798.

[5] M. A. Keniry, *Biopolymers* **2001**, *56*, 123–146.

[6] a) Y. Wang, D. J. Patel, *Biochemistry* **1992**, *31*, 8112–8119; b) Y. Wang, D. J. Patel, *Structure* **1993**, *1*, 263–282; c) G. N. Parkinson, M. P. H. Lee, S. Neidle, *Nature* **2002**, *417*, 876–880; d) Y. Wang, D. J. Patel, *Structure* **1994**, *2*, 1141–1156; e) A. T. Phan, Y. S. Modi, D. J. Patel, *J. Am. Chem. Soc.* **2004**, *126*, 8710–8716.

[7] a) K. Phillips, Z. Dauter, A. I. H. Murchie, D. M. J. Lilley, B. Luisi, *J. Mol. Biol.* **1997**, *273*, 171–182; b) S. Haider, G. N. Parkinson, S. Neidle, *J. Mol. Biol.* **2002**, *320*, 189–200.

[8] a) M. Lu, Q. Guo, N. R. Kallenback, *Biochemistry* **1992**, *31*, 2455–2459; b) P. Balagurumoorthy, S. K. Brahmachari, D. Mohanty, M. Bansal, V. Sasisekharan, *Nucleic Acids Res.* **1992**, *20*, 4061–4067; c) P. Balagurumoorthy, S. K. Brahmachari, *J. Biol. Chem.* **1994**, *269*, 21858–21869; d) M. Lu, Q. Guo, N. R. Kallenbach, *Biochemistry* **1993**, *32*, 598–601.

[9] For recent reviews, see: a) A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J.-F. Riou, J.-L. Mergny, *Biochimie* **2008**, *90*, 131–155; b) D. Monchaud, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627–636.

[10] a) M. Mutter, *Angew. Chem.* **1985**, *97*, 639–654; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 639–653; b) M. Mutter, P. Dumy, P. Garrouste, C. Lehman, M. Mathieu, C. Peggion, S. Peluso, A. Razaname, G. Tuchscherer, *Angew. Chem.* **1996**, *108*, 1588–1591; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1482–1485.

[11] Use of other nonpeptidic scaffold: a) M. Nikan, J. C. Sherman, *Angew. Chem.* **2008**, *120*, 4978–4980; *Angew. Chem. Int. Ed.* **2008**, *47*, 4900–4902; b) G. Oliviero, J. Amato, N. Borbone, A. Galeone, L. Petraccone, M. Varra, G. Piccialli, L. Mayol, *Bioconjugate Chem.* **2006**, *17*, 889–898.

[12] a) D. Forget, D. Boturyn, E. Defrancq, J. Lhomme, P. Dumy, *Chem. Eur. J.* **2001**, *7*, 3976–3984; b) O. P. Edupuganti, Y. Singh, E. Defrancq, P. Dumy, *Chem. Eur. J.* **2004**, *10*, 5988–5995; c) Y. Singh, N. Spinelli, E. Defrancq, *Curr. Org. Chem.* **2008**, *12*, 263–290.

[13] E. Garanger, D. Boturyn, O. Renaudet, E. Defrancq, P. Dumy, *J. Org. Chem.* **2006**, *71*, 2402–2410.

[14] S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturyn, P. Dumy, *J. Org. Chem.* **2008**, *73*, 983–991.

[15] For some recent examples, see: a) D. Monchaud, P. Yang, L. Lacroix, M.-P. Teulade-Fichou, J.-L. Mergny, *Angew. Chem.* **2008**, *120*, 4936–4939; *Angew. Chem. Int. Ed.* **2008**, *47*, 4858–4861; b) V. Dapic, V. Abdomerovic, R. Marrington, J. Peberdy, A. Rodger, J. O. Trent, P. J. Bates, *Nucleic Acids Res.* **2003**, *31*, 2097–2107; c) R. Rodriguez, G. D. Pantos, D. P. N. Gonçalves, J. K. M. Sanders, S. Balasubramanian, *Angew. Chem.* **2007**, *119*, 5501–5503; *Angew. Chem. Int. Ed.* **2007**, *46*, 5405–5407; d) P. A. Rachwal, T. Brown, K. R. Fox, *Biochemistry* **2007**, *46*, 3036–3044.

[16] J.-L. Mergny, A. De Cian, A. Ghelab, B. Saccà, L. Lacroix, *Nucleic Acids Res.* **2005**, *33*, 81–94.

[17] J.-L. Mergny, A.-T. Phan, L. Lacroix, *FEBS Lett.* **1998**, *435*, 74–78.

[18] For general reviews on the use of SPR for the study of quadruplex–ligand interactions see: a) J. E. Redman, *Methods* **2007**, *43*, 302–312; b) M.-P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J.-L. Mergny, A. David, J.-M. Lehn, D. Wilson, *J. Am. Chem. Soc.* **2003**, *125*, 4732–4740; c) J. Seenisamy, S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shin-ya, E. White, W. D. Wilson, L. H. Hurley, *J. Am. Chem. Soc.* **2005**, *127*, 2944–2959.

[19] J. T. Kern, P. W. Thomas, S. M. Kerwin, *Biochemistry* **2002**, *41*, 11379–11389.

[20] Comparable affinities have been observed for similar perylene derivatives see: R. Samudrala, X. Zhang, R. M. Wadkins, D. L. Mattern, *Bioorg. Med. Chem.* **2007**, *15*, 186–193.

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