Deciphering Aromatic Oligoamide Foldamer–DNA Interactions**

Laurence Delaurière, Zeyuan Dong, Katta Laxmi-Reddy, Frédéric Godde, Jean-Jacques Toulmé,* and Ivan Huc*

Foldamers are artificial folded molecular architectures inspired by the structures of biopolymers.[1] Some synthetic foldamers feature properties that make them promising candidates for the selective recognition of protein and nucleic acid surfaces:[2] they adopt well-defined and predictable conformations that can be decorated with proteinogenic side chains; they are medium sized (0.5–5 kDa); they are often resistant to proteolytic degradation,[3] and some show good cell penetration ability.[4] However, despite these favorable properties, identifying a foldamer that binds selectively to a given protein or nucleic acid target remains an enormous challenge and there is a strong need for progress in this area. Some successful examples of structure-based designs have been reported, such as α-helix mimetics,[5] nucleic acid analogues,[6] DNA minor-groove binders,[7] and some G-quadruplex DNA ligands.[8] Other successes were based on combinatorial approaches and systematic screening to generate α/β + α chimeric peptide inhibitors of the interaction between Bcl-xL and its proapoptotic partner,[9] and α-helix mimetic inhibitors of the MDM2/p53 interaction.[10]

In the case of quinoline-based helical aromatic amide foldamers,[11,12] the fact that they do not relate closely to known biological structures that they could mimic makes the challenge to design ligands for proteins or nucleic acids all the more difficult. Encouraged by our finding that short—trimeric or tetrameric—oligoamides of quinoline $\mathbf{Q}^+$ (Figure 1a) could bind to DNA having a G-quadruplex struc-

[*] Dr. Z. Dong,[+] Dr. K. Laxmi-Reddy, Dr. F. Godde, Dr. I. Huc
Univ. Bordeaux—IPB—CNRS UMR5248
Institut Européen de Chimie et Biologie
2 rue Robert Escarpit, 33607 Pessac (France)
E-mail: i.huc@iecb.u-bordeaux.fr

Dr. L. Delaurière,[+] Dr. J.-J. Toulmé
Univ. Bordeaux, IECB, laboratoire ARNA
2 rue Robert Escarpit, 33607 Pessac (France)
E-mail: jean-jacques.toulme@inserm.fr

Dr. L. Delaurière,[+] Dr. J.-J. Toulmé
INSERM, U869, laboratoire ARNA
146 Rue Léo Saignat, 33076 Bordeaux (France)

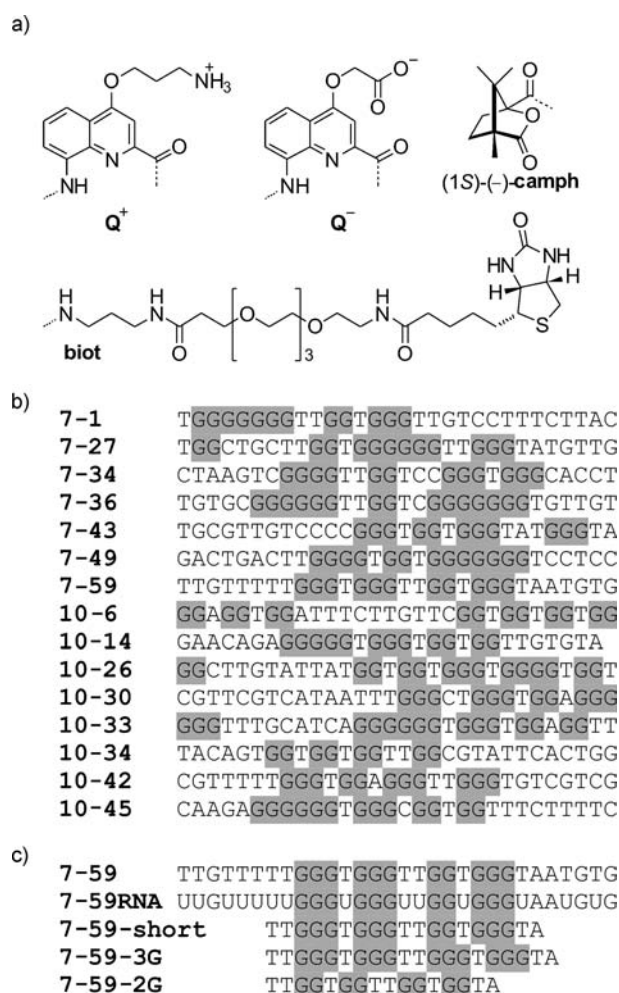[+] These authors contributed equally to this work.

Figure 1. a) Positively and negatively charged monomers, biotin, and camphanyl terminal groups combined in this investigation. b) 30 nucleotide variable region of G-rich DNA sequences from SELEX rounds 7 and 10 against $O_2N–(\mathbf{Q}^+)_8–$**biot**; G stretches are in gray boxes. c) Variants of sequence **7–59**.

ture,[8] we set out to further investigate the scope of interactions between DNA and longer foldamer sequences. However, instead of undertaking a classical approach that would entail systematic variations of the foldamer structure and binding assays against a small range of DNA targets, we envisaged an alternative approach that consisted of screening a vast number of DNA sequences against a single foldamer. Specifically, we used SELEX (systematic evolution of ligands by exponential enrichment),[13] a method that has been successful against a wide range of targets,[14] to identify DNA aptamers that have a strong affinity for the octamer

O_2N–$(Q^+)_8$–OH,[15] which folds into a helix spanning over three turns in the solid state and in solution.[11] As described in the following, this unusual approach led to important results: 1) an independent and unbiased assay confirmed the prevalence of a specific interaction between multiturn $(Q^+)_n$ oligomers and G-quadruplex DNA, a motif of increasingly recognized biological relevance;[16] 2) this interaction can be made diastereoselective with a one-handed helix; 3) the first example of a DNA- vs. RNA-selective G-quadruplex synthetic ligand was identified; 4) a foldamer was found to selectively bind to one quadruplex sequence and not to others. Aptamers are thus useful tools to reveal the DNA-binding potential of foldamers.

A biotinylated octamer O_2N–$(Q^+)_8$–**biot** was prepared, immobilized on magnetic streptavidin beads, and subjected to ten rounds of selection from a library of DNA sequences having a 30 nucleotide central random region (see the Supporting Information). The initial library size was 1.8×10^{14} DNA molecules (300 pmol), consisting of mostly distinct sequences.[17] The ability of the library population to bind to O_2N–$(Q^+)_8$–**biot** was monitored after each round by surface plasmon resonance (SPR) with the biotinylated foldamer immobilized onto the sensorchip. The signal was hardly detectable up to the second SELEX round before rising sharply (Figure S1). Populations of rounds 7 and 10 gave strong SPR signals, from which a total of 62 candidates (25 from round 7 and 37 from round 10) were cloned and sequenced. Overall, the candidates were rich in thymine (T; 40.1%), poor in adenine (A; 9.2%), and contained 27.9% and 22.8% of guanine (G) and cytosine (C), respectively. Folding analysis with Mfold[18] revealed no Watson–Crick base pairing secondary structure. An apparent feature was a short conserved motif (TTCTT or GTTTT; Figure S2); most sequences were not investigated further. However, 15 sequences contained at least four GG or GGG repeats, a pattern known to potentially give rise to G-quadruplex structures (Figure 1b).[16] Incidentally, a Blast[19] analysis showed that 16-nucleotide windows including the oligo-G repeats all exist in the human genome.

Eight G-rich candidates were selected (**7–27**, **7–49**, **7–59**, **10–6**, **10–14**, **10–33**, **10–42**, **10–45**) and were shown to be G-quadruplexes. The affinity of these sequences for O_2N–$(Q^+)_8$–**biot** was measured by SPR (Figure 2a). In the selection buffer (140 mM K$^+$), all candidates, but also an unrelated DNA control sequence, were shown to bind to the foldamer. This non-selective binding was assigned to electrostatic interactions between negatively charged DNA and the positively charged foldamer. Indeed, under high salt conditions (1M K$^+$), the control sequence and aptamers containing TTCTT or GTTTT failed to bind, whereas all eight G-rich sequences injected at 1 μM bind, in particular **10–33**, **10–6**, **10–45,** and **7–59**. The sensorgrams could not be fitted to a 1:1 binding model, thus preventing the accurate determination of the affinity constants. This may arise from very slow complex dissociation, quadruplex aggregation (see below) as well as quadruplex conformational changes[20] and may not reflect complicated binding stoichiometries. It can be estimated that K_d values are submicromolar for most sequences. Variations of the maximal SPR signal of each sequence suggest that
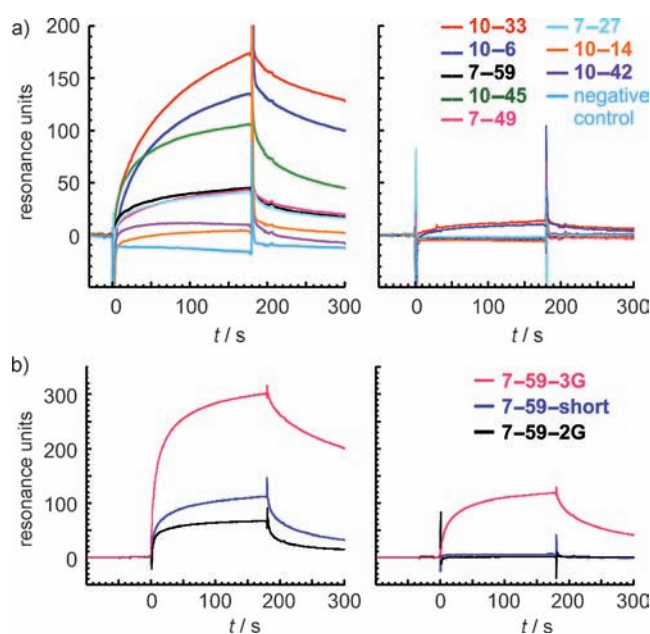

Figure 2. a) SPR sensorgrams of eight G-rich aptamers (1 μM in a 1 M salt buffer) and a negative control single-stranded DNA (ATCTTTATG-CAGTTCGCATCCCCTCGCATA) against O_2N–$(Q^+)_8$–**biot** (left) or O_2N–$(Q^+)_4(Q^-)_4$–**biot** (right) immobilized on the sensorchip. b) SPR sensorgrams of **7–59–short**, **7–59–2G**, and **7–59–3G** against O_2N–$(Q^+)_8$–**biot** (left) and O_2N–$(Q^+)_4(Q^-)_4$–**biot** (right).

affinities for O_2N–$(Q^+)_8$–**biot** do not differ by much more than one order of magnitude. Binding of DNA aptamers completely disappeared when the four C-terminal Q^+ residues of the foldamer were replaced by Q^- (O_2N–$(Q^+)_4(Q^-)_4$–**biot**), despite the high salt concentration which screens the effect of charges, hence suggesting intimate foldamer–DNA contacts at the C terminus. Further support for a selective interaction came from the observation that TG$_5$T, a tetramolecular DNA quadruplex without any loop, does not bind to O_2N–$(Q^+)_8$–**biot**.

Circular dichroism (CD) spectra of the G-rich aptamers all show a characteristic G-quadruplex signature (Figure 3a). According to the position and amplitude of CD bands (a minimum of ellipticity at 264 nm and a maximum at 295 nm),[21] an antiparallel quadruplex structure prevails in **10–6**, whereas all other sequences seem to be predominantly parallel G-quadruplexes. Adding O_2N–$(Q^+)_8$–OH to the parallel quadruplexes does not significantly change the CD spectra (a minor decrease in intensity was observed). On the contrary, the spectrum of **10–6** undergoes a major reversal consistent with an anti-parallel to parallel transition (Figure 3b).[22] All DNA sequences seem to be in a parallel conformation when complexed to the cationic foldamer. The titration of **10–6** with O_2N–$(Q^+)_8$–OH suggests a binding stoichiometry in the 1:1 to 2:1 range.

Consistent with CD data, UV/Vis absorption properties of the G-rich aptamers were also characteristic of G-quadruplexes. The difference between absorption spectra at high and low temperatures that induce unfolded and folded states of the oligonucleotide, respectively (thermal difference spectra, TDS) have an isosbestic point at 285 nm, positive peaks at 243
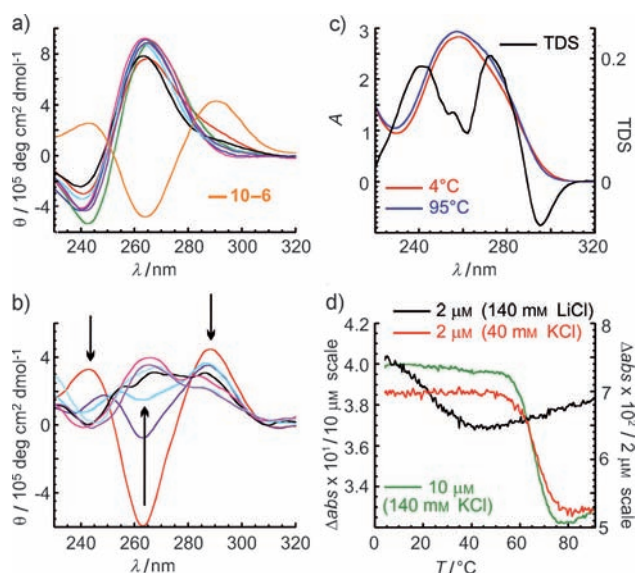
Figure 3. a) CD spectra of the eight sequences of Figure 2a (2 μM in a 140 mM KCl buffer); b) CD titration of **10–6** (1.5 μM in a 140 mM KCl buffer) by $O_2N–(Q^+)_8–OH$ showing the conformational change of the G-quadruplex. Successive curves following the arrows are for 0, 0.5, 1, 1.5, 2, 3, and 4 equiv of added foldamer. The mixture was allowed to equilibrate for 2 h after each foldamer addition before recording a spectrum. c) Representative example of a TDS spectrum for aptamer **7–59** at 10 μM in cacodylate buffer. The red and blue curves are the absorption spectra at 4°C and 95°C (left scale) and the black curve is the difference spectrum (right scale). d) Representative example of the melting curves monitored at 295 nm of aptamer **7–59** at different concentrations in KCl or LiCl.

Table 1: UV-monitored melting experiments and electrophoretic behavior of some G-rich aptamers against $O_2N–(Q^+)_8–$**biot**.

Entry	T_m [a] at 2 μM[b]	at 2 μM[c]	at 10 μM[c]	n[d]
7–27	21 ± 2.1	46.5 ± 0	49.5 ± 0	3
7–49	–[e]	71 ± 0.7	71.5 ± 0	3
7–59	20 ± 0	66.5 ± 0.7	66 ± 0	1
10–6	31.5 ± 0	38 ± 0	40 ± 0	1
10–14	35.7 ± 1.8	57.5 ± 0.7	67.5 ± 0	3
10–33	34 ± 4.2	64.5 ± 1.1	72.5 ± 0.2	3
10–42	31.5 ± 2.8	67 ± 0	67.5 ± 0	1
10–45	–[e]	61 ± 1	71 ± 0.2	2

[a] Melting temperatures in °C. [b] In 140 mM LiCl. [c] In 140 mM KCl. [d] Number of bands on a nondenaturing gel. [e] No observed transition.

multimolecular structures, consistent with their hysteretic melting behavior (not shown).

That G-quadruplexes emerge as the only well-folded aptamers from a SELEX experiment most likely reflects strong and specific interactions with the foldamer target that other DNA folds do not provide. To decipher these interactions, the well-behaved unimolecular parallel G-quadruplex **7–59** was studied further. Its sequence contains one GG and three GGG blocks interspersed by one or two Ts. Several variants of this aptamer were investigated (Figure 1c): a sequence shortened at each extremity yet still containing the four guanosine blocks (**7–59–short**); a shortened sequence in which one G has been inserted resulting in four GGG blocks (**7–59–3G**); and a shortened sequence in which three Gs have been removed resulting in four GG blocks (**7–59–2G**). CD, TDS, and T_m measurements show that these three variants also exhibit a parallel G-quadruplex signature. However, **7–59–2G** is much less stable than **7–59–3G** and **7–59–short** ($T_m =$ 33, 60 and 63°C, respectively) as could be expected for a quadruplex having a maximum of two G-quartets. SPR analysis indicates that **7–59–short** and **7–59** bind similarly to $O_2N–(Q^+)_8–$**biot** (Figure 2b). The binding affinity is slightly reduced for **7–59–2G** and, quite surprisingly, it is strongly enhanced for **7–59–3G**. However, this rise in affinity is accompanied by a loss in selectivity: contrary to other **7–59** variants and to other G-rich sequences, **7–59–3G** binds to $O_2N–(Q^+)_4(Q^-)_4–$**biot**. This serendipitous discovery demonstrates that changing side-chains makes it possible to find ligands that are highly selective to some G-quadruplex DNA sequences, a desirable property that has proven difficult to achieve:[26] under the buffer and concentration conditions used, $O_2N–(Q^+)_4(Q^-)_4–$**biot** binds exclusively to **7–59–3G** and no other G-quadruplex sequences investigated herein.

An important feature of the foldamer used as a target for SELEX ($O_2N–(Q^+)_8–$**biot**) is that it exists as a 1:1 mixture of *P* and *M* helices.[11] Aptamers were thus exposed to the two conformers and may have been selected for by one or the other, or both. In order to assess whether foldamer/aptamer interactions are diastereoselective, a chiral camphanyl group was introduced instead of the N-terminal nitro group of the foldamer to control handedness: as shown in an earlier study,[27] (1S)-(−)-**camph**–$(Q^+)_8–$**biot** is exclusively *P* helical whilst (1R)-(+)-**camph**-$(Q^+)_8–$**biot** is *M* helical (Figure S7). Unfortunately, for almost all aptamers, the bulky camphanyl

and 273 nm and a negative peak at 295 nm, which is typical of G-quadruplex structures (Figure 3c).[23] In UV-monitored melting experiments, unfolding of G-quadruplexes results in a reversible cooperative absorbance decrease at 295 nm.[24] Aptamers **7–59** (Figure 3d), **10–42**, and **10–6** displayed the expected reversible sigmoidal profile. Sequences **7–27**, **7–49**, **10–14**, **10–33**, and **10–45** have a hysteretic, imperfectly reversible transition, suggesting that their structural transition is slow relative to the temperature ramp. This result might indicate the formation of multimolecular structures.[25] In support of this hypothesis, four out of these five oligonucleotides were found to have significantly higher T_m values at 10 μM compared to 2 μM (Table 1). Further evidence that the G-rich aptamers are G-quadruplexes came from T_m measurements performed in the presence of LiCl instead of KCl. In all cases, T_m values underwent a dramatic decrease (Table 1); **7–49** and **10–45** do not even exhibit a transition in the presence of LiCl.

G-based four-stranded structures may result from intra- or intermolecular tetrad formation. The molecularity of the quadruplexes formed by the selected sequences was analyzed by nondenaturing poly(acrylamide) gel electrophoresis (Table 1, Figure S6). Aptamers **7–59**, **10–6**, and **10–42** displayed a single low-molecular-weight fast moving band, likely corresponding to a unimolecular quadruplex. Aptamers **7–27**, **7–49**, **10–14**, **10–33**, and **10–45** also displayed a fast-moving band as well as one or two slower-moving bands assigned to

residue, whether 1*S* or 1*R*, resulted in a complete disruption of the aptamer/foldamer interaction, thus suggesting intimate contacts between each aptamer and the nitro-substituted region of the foldamer. The only exception was **7–59–2G**. This sequence was found to bind the *M* helix and not at all the *P* helix (Figure S4). Consistently, the enantiomeric L-DNA version of **7–59–2G** binds the *P* helix and not the *M* helix, and both DNA enantiomers show reduced (50%) binding to a 1:1 mixture of *P* and *M* helices. Thus, the three-turn aromatic amide helix (**Q**$^+$)$_8$ can recognize G-quadruplex DNA with full diastereoselectivity.[28] It might then be expected that the aptamers would induce a preferred handedness in the non-chiral O$_2$N–(**Q**$^+$)$_8$–OH. However, this was not observed because of the very high kinetic barrier between *P* and *M* helices under the conditions used.

Finally, the RNA vs. DNA selectivity was also assessed. Many compounds have been demonstrated to selectively recognize G-quadruplex DNA and not double- or triple-stranded DNA.[29] However, to the best of our knowledge, no synthetic ligand has been shown to discriminate between a DNA G-quadruplex and its corresponding RNA G-quadruplex sequence. The RNA sequence of **7–59** (**7–59RNA**) was shown to fold in a stable G-quadruplex ($T_m = 74\,^\circ$C),[30] but it binds to O$_2$N–(**Q**$^+$)$_8$–**biot** with a substantially lower affinity than **7–59** (Figure S4). This behavior occurs despite the fact that both **7–59RNA** and **7–59** have parallel structures and may not differ in major ways. Meanwhile, **7–59RNA** does not bind at all to O$_2$N–(**Q**$^+$)$_4$(**Q**$^-$)$_4$–**biot**.

[1] For a recent overview, see: G. Guichard, I. Huc, *Chem. Commun.* **2011**, *47*, 5933.

[2] T. A. Edwards, A. J. Wilson, *Amino Acids* **2011**, *41*, 743; J. Becerril, J. M. Rodriguez, P. N. Wyrembak, A. D. Hamilton in *Protein Surface Recognition* (Eds.: E. Giralt, M. W. Peczuh, X. Salvatella), Wiley, Chichester, **2011**, pp. 105–131; B. Baptiste, F. Godde, I. Huc, *ChemBioChem* **2009**, *10*, 1765; A. J. Wilson, *Chem. Soc. Rev.* **2009**, *38*, 3289.

[3] J. Frackenpohl, P. I. Arvidsson, J. V. Schreiber, D. Seebach, *ChemBioChem* **2001**, *2*, 445; J. V. Schreiber, J. Frackenpohl, F. Moser, T. Fleischmann, H. P. E. Kohler, D. Seebach, *ChemBioChem* **2002**, *3*, 424; S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr, W. H. Moos, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657; J. D. Sadowsky, J. K. Murray, Y. Tomita, S. H. Gellman, *ChemBioChem* **2007**, *8*, 903; V. V. Demidov, V. N. Potaman, M. D. Frank-Kamenetskii, M. Egholm, O. Buchard, S. H. Sönnichsen, P. E. Nielsen, *Biochem. Pharmacol.* **1994**, *48*, 1310; E. R. Gillies, F. Deiss, C. Staedel, J.-M. Schmitter, I. Huc, *Angew. Chem.* **2007**, *119*, 4159; *Angew. Chem. Int. Ed.* **2007**, *46*, 4081.

[4] A. Unciti-Broceta, F. Diezmann, C. Y. Ou-Yang, M. A. Fara, M. Bradley, *Bioorg. Med. Chem.* **2009**, *17*, 959; P. A. Wender, J. B. Rothbard, T. C. Jessop, E. L. Kreider, B. L. Wylie, *J. Am. Chem. Soc.* **2002**, *124*, 13382; A. D. Bautista, J. S. Appelbaum, C. J. Craig, J. Michel, A. Schepartz, *J. Am. Chem. Soc.* **2010**, *132*,

2904; T. B. Potocky, J. Silvius, A. K. Menon, S. H. Gellman, *ChemBioChem* **2007**, *8*, 917; M. Okuyama, H. Laman, S. R. Kingsbury, C. Visintin, E. Leo, K. L. Eward, K. Stoeber, C. Boshoff, G. H. Williams, D. L. Selwood, *Nat. Methods* **2007**, *4*, 153; J. Iriondo-Alberdi, K. Laxmi-Reddy, B. Bouguerne, C. Staedel, I. Huc, *ChemBioChem* **2010**, *11*, 1679.

[5] I. Saraogi, J. A. Hebda, J. Becerril, L. A. Estroff, A. D. Miranker, A. D. Hamilton, *Angew. Chem.* **2010**, *122*, 748; *Angew. Chem. Int. Ed.* **2010**, *49*, 736; J. T. Ernst, J. Becerril, H. S. Park, H. Yin, A. D. Hamilton, *Angew. Chem.* **2003**, *115*, 553; *Angew. Chem. Int. Ed.* **2003**, *42*, 535; J. P. Plante, T. Burnley, B. Malkova, M. E. Webb, S. L. Warriner, T. A. Edwards, A. J. Wilson, *Chem. Commun.* **2009**, 5091.

[6] P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624.

[7] P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 2215.

[8] P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-ya, I. Huc, S. Balasubramanian, *J. Am. Chem. Soc.* **2007**, *129*, 11890.

[9] W. S. Horne, M. D. Boersma, M. A. Windsor, S. H. Gellman, *Angew. Chem.* **2008**, *120*, 2895; *Angew. Chem. Int. Ed.* **2008**, *47*, 2853.

[10] A. Shaginian, L. R. Whitby, S. Hong, I. Hwang, B. Farooqi, M. Searcey, J. Chen, P. K. Vogt, D. L. Boger, *J. Am. Chem. Soc.* **2009**, *131*, 5564.

[11] H. Jiang, J.-M. Léger, I. Huc, *J. Am. Chem. Soc.* **2003**, *125*, 3448; C. Dolain, A. Grélard, M. Laguerre, H. Jiang, V. Maurizot, I. Huc, *Chem. Eur. J.* **2005**, *11*, 6135.

[12] N. Delsuc, T. Kawanami, J. Lefeuvre, A. Shundo, H. Ihara, M. Takafuji, I. Huc, *ChemPhysChem* **2008**, *9*, 1882.

[13] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505; A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.

[14] For reviews, see: E. Dausse, S. Da Rocha Gomes, J.-J. Toulmé, *Curr. Opin. Pharmacol.* **2009**, *9*, 602; J.-J. Toulmé, F. Darfeuille, C. Di Primo, E. Dausse in *The aptamer handbook* (Eds.: S. Klussmann), Wiley-VCH, Weinheim, **2006**, pp. 167–187.

[15] In this notation, the N-terminal nitro group replaces the "NH" group of the structure shown in Figure 1.

[16] G. W. Collie, G. N. Parkinson, *Chem. Soc. Rev.* **2011**, *40*, 5867; D. Sen, W. Gilbert, *Nature* **1988**, *334*, 364; *Quadruplex Nucleic Acids* (Eds.: S. Neidle, S. Balasubramanian), RSC Biomolecular Sciences, Cambridge, **2006**, pp. 1–301; X. Ji, H. Sun, H. Zhou, J. Xiang, Y. Tang, C. Zhao, *Nucleic Acid Therapeutics* **2011**, *21*, 185.

[17] The probability that a given sequence is found twice in the library is of the order of 10^{-8}.

[18] M. Zuker, *Nucleic Acids Res.* **2003**, *31*, 3406.

[19] http://blst.ncbi.nih.gov/Blast.cgi

[20] P. V. Jena, P. S. Shirude, B. Okumus, K. Laxmi-Reddy, F. Godde, I. Huc, S. Balasubramanian, T. Ha, *J. Am. Chem. Soc.* **2009**, *131*, 12522.

[21] D. M. Gray, R. L. Ratliff, M. R. Vaughan, *Methods Enzymol.* **1992**, *211*, 389; S. Paramasivan, I. Rujan, P. H. Bolton, *Methods* **2007**, *43*, 324; R. Rodriguez, G. D. Pantos, D. P. N. Gonçalves, J. K. M. Sanders, S. Balasubramanian, *Angew. Chem.* **2007**, *119*, 5501; *Angew. Chem. Int. Ed.* **2007**, *46*, 5405.

[22] The absence of a CD band above 400 nm indicates that there is no contribution of the foldamer to these CD spectra, allowing the monitoring of the fate of the quadruplex during the titration.

[23] J.-L. Mergny, J. Li, L. Lacroix, S. Amrane, J. Chaires, *Nucleic Acids Res.* **2005**, *33*, e138.

[24] P. V. Scaria, S. J. Shire, R. H. Shafer, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10336; J.-L. Mergny, A. T. Phan, L. Lacroix, *FEBS Lett.* **1998**, *435*, 74.

[25] J.-L. Mergny, L. Lacroix, *Curr. Protoc. Nucleic Acid Chem.* **2009**, chap. 17, unit 17.1.

[26] F. Hamon, E. Largy, A. Guédin-Beaurepaire, M. Rouchon-Dagois, A. Sidibe, D. Monchaud, J.-L. Mergny, J.-F. Riou, C.-H.

Nguyen, M.-P. Teulade-Fichou, Angew. Chem. **2011**, *123*, 8904; Angew. Chem. Int. Ed. **2011**, *50*, 8745.

[27] A. M. Kendhale, L. Poniman, Z. Dong, K. Laxmi-Reddy, B. Kauffmann, Y. Ferrand, I. Huc, *J. Org. Chem.* **2011**, *76*, 195.

[28] K.-i. Shinohara, Y. Sannohe, S. Kaieda, K.-i. Tanaka, H. Osuga, H. Tahara, Y. Xu, T. Kawase, T. Bando, H. Sugiyama, *J. Am. Chem. Soc.* **2010**, *132*, 3778; H. Yu, X. Wang, M. Fu, J. Ren, X. Qu, *Nucleic Acids Res.* **2008**, *36*, 5695.

[29] D. Monchaud, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627; S. N. Georgiades, N. H. Abd Karim, K. Suntharalingam,

R. Vilar, *Angew. Chem.* **2010**, *122*, 4114; *Angew. Chem. Int. Ed.* **2010**, *49*, 4020; T.-M. Ou, Y.-J. Lu, T. Jia-Heng, Z.-S. Zhang, K.-Y. Wong, L.-Q. Gu, *ChemMedChem* **2008**, *3*, 690; S. Neidle, *Curr. Opin. Struct. Biol.* **2009**, *19*, 239.

[30] For studies of RNA quadruplex stability and their propensity to adopt parallel structures, see: A. Y. Q. Zhang, A. Bugaut, S. Balasubramanian, *Biochemistry* **2011**, *50*, 7251; D. H. Zhang, G. Y. Zhi, *J. Biotechnol.* **2010**, *150*, 6.