Nucleic Acid Targeted Therapy: G4 Oligonucleotides Downregulate *HRAS* in Bladder Cancer Cells through a Decoy MechanismGiulia Miglietta,^{†,§} Alaa S. Gouda,^{‡,§,||} Susanna Cogoi,[†] Erik B. Pedersen,[‡] and Luigi E. Xodo^{*,†}[†]Department of Medical and Biological Sciences, University of Udine, 33100 Udine, Italy[‡]Nucleic Acid Center, Institute of Physics and Chemistry, University of Southern Denmark, DK-5230 Odense M, Denmark

Supporting Information

ABSTRACT: In a previous study we have demonstrated that two neighboring G-quadruplexes, *hras-1* and *hras-2*, located immediately upstream of the major transcription start site of *HRAS*, bind MAZ, a nuclear factor that activates transcription (Cogoi, S.; et al. *Nucl. Acid Res.* 2014, 42, 8379). For the present study we have designed G4 oligonucleotides with anthraquinone insertions and locked nucleic acids (LNA) modifications mimicking quadruplex *hras-1*. Luciferase, qRT-PCR, and Western blot data demonstrate that these constructs efficiently down regulate *HRAS* in T24 bladder cancer cells. The inhibitory efficiency of the G4 oligonucleotides correlates with their nuclease resistance in the cell environment. By chromatin immunoprecipitation we show that the association of MAZ to the *HRAS* promoter is strongly attenuated by the designed G4 oligonucleotides, thus suggesting that these constructs behave with a decoy mechanism.

KEYWORDS: *HRAS*, G4-oligonucleotides, anthraquinone insertions, T24 bladder cancer cells, decoy mechanism

Mutations in the *ras* genes have been associated with about one-third of all human cancers.¹ In particular, mutations in codon 12 of *HRAS* are found in more than 35% of bladder cancers.^{2,3} The *ras* genes encode for a GTP-binding protein activating downstream signal pathways that control several cell functions including proliferation.³ The involvement of mutant *HRAS* in the pathogenesis of bladder cancer is well established.^{4–6} Bladder cancer is commonly treated with cisplatin-based combination therapies, which, however, develop drug resistance. As this limits the efficacy of the therapy,⁷ innovative therapeutic strategies are desirable. Given the central role of mutant *HRAS* in the pathogenesis of bladder cancer, the downregulation of this oncogene should inhibit cell growth and/or sensitize cancer cells to chemotherapy.⁸ To address this issue, we focused on the *HRAS* region immediately upstream of the major transcription start site (TSS), including two neighboring G-rich quadruplex-forming sequences, namely, *hras-1* and *hras-2*, which play a critical role in transcription regulation (Figure 1).^{9,10} These two sequences overlap binding sites for MAZ and Sp1, two zinc-finger transcription factors that are essential for *HRAS* expression. By FRET experiments we demonstrated that MAZ upon binding to the promoter unfolds the G-quadruplexes.¹⁰ We proposed a regulatory mechanism according to which the two quadruplex-forming sequences behave as a molecular switch for the control of gene expression. Under normal conditions, *HRAS* transcription is blocked by the two G-quadruplexes. Transcription is then activated when MAZ binds to and unfolds the G-quadruplexes, thus favoring the formation of the activated transcription complex (Figure 1).^{10,11} In the light of this regulatory model we have

Figure 1. (A) Sequence of the *HRAS* promoter upstream of TSS. Two neighboring G4 sequences, *hras-1* and *hras-2*, form a molecular switch that control transcription. (B) Decoy strategy.

hypothesized a decoy strategy to inhibit oncogenic *HRAS* in human bladder cancer cells. According to this approach, when

Received: August 4, 2015

Accepted: October 18, 2015

Published: October 18, 2015

Table 1. Sequences of the Designed Anthraquinone G4 Decoys

G4-decoy	5' → 3' ^(a)	Mw calculated	Mw ^(b) Measured	ε (260 nm)	T _M ^(c) (°C)	Topology ^(d)
7213	TCGGGTT <u>HC</u> GGGCGCAGGG <u>CH</u> CGGGCGG	9044.78	9045.74	268.72	63.8	Antiparallel
7214	THGGGTTGC <u>GGG</u> C <u>GC</u> AGGGCACGGG <u>HGG</u>	9108.78	9109.64	278.62	56.8	mixed P/A
7215	THGGGTTGC <u>GGG</u> <u>CH</u> CAGGGCACGGGCGG	9040.82	9040.68	275.12	60.0	Antiparallel
7216	TCGGGTTGC <u>GGG</u> <u>CH</u> CAGGGCACGGG <u>HGG</u>	9040.82	9042.63	275.12	55.0	mixed P/A
7217	TCGGGTTGC <u>GGG</u> C <u>GC</u> AGGGCACGGGCGG	8852.52	8853.18	276.50	56.2	Antiparallel

^aH = 1,4-anthraquinone insertion; underlined bases are LNA modified. ^bMolecular weight measured by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). ^cT_Ms from UV-melting profiles of compounds in 100 mM KCl and 20 mM phosphate buffer, pH 7.4. ^dTopology of the G4 decoys determined by circular dichroism.

short DNA fragments mimicking one of the *HRAS* G-quadruplexes are delivered to the cells, they should compete with the binding of the transcription factors MAZ and Sp1 to the promoter, with the result that transcription will be inhibited.

This nucleic-acid target strategy was first applied to block the binding of NF-κB and STAT3 to the corresponding *cis*-elements.^{12–17} Recently, we employed decoy oligonucleotides against *KRAS* in Panc-1 cells obtaining very promising results both in vitro and in vivo.¹⁸ To suppress *HRAS* in cancer cells by a decoy strategy, we designed quadruplex-forming (G4) oligonucleotides with locked nucleic acids (LNA) modifications and anthraquinone insertions.

The primary structure of these compounds is reported in Table 1. The LNA modifications should increase the resistance to nucleases,¹⁹ whereas the anthraquinone insertions, by promoting π -stacking interactions, should increase the stability of the folded decoy oligonucleotides. The anthraquinone chromophore is a well-known intercalator that has been used in the development of G4 conjugates.²⁰ To insert the anthraquinone moiety into specific positions of the decoy oligonucleotides, we used a 1,4-substituted anthraquinone phosphoramidite²¹ and synthesized the decoy oligonucleotides in solid phase (Supporting Information, S1). The anthraquinone-modified G4 oligonucleotides have been purified by HPLC and their molecular weights verified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis (Table 1). The calculated and measured molecular weights were in excellent agreement, with a difference of <0.03%. The G4 decoys have been designed with the sequence of the promoter *HRAS* segment called *hras-1* (Figure 1), which is known to assume an antiparallel G-quadruplex in the presence of KCl (S2).^{9,10} All the compounds were engineered with two or three LNA modifications and two anthraquinone insertions, each replacing one nucleotide, in positions H2/H4 (7213), H1/H5 (7214), H1/H3 (7215), and H3/H5 (7216) (Figure 2A,B). Compound 7217 was instead designed with only LNA modifications. By CD we examined if the insertions modified the conformation of the G-quadruplex formed by *hras-1*. Figure 2C shows the CD spectra of the designed compounds in 100 mM KCl, 20 mM phosphate buffer, pH 7.4. It can be seen that 7213, 7215, and 7217 maintain the antiparallel conformation with positive and negative ellipticities at 290 and 260 nm,¹⁷ whereas compounds 7214 and 7216 adopt a mixed parallel/antiparallel (P/A) form characterized by two positive ellipticities at 290 and 260 nm.²² Thus, the positions of the anthraquinone insertions influence

Figure 2. (A) Putative structure of the designed decoy oligonucleotides reported in Table 1 with LNA modifications and anthraquinone insertions H2/H4 (7213), H1/H5 (7214), H1/H3 (7215), H3/H5 (7216). (B) Structure of the anthraquinone insertion.²¹ (C) CD spectra of the designed decoy oligonucleotides in 20 mM phosphate buffer, pH 7.4, 100 mM KCl. (D) putative structure of the decoy oligonucleotides, H1–H5 = anthraquinone insertions.

the folding of the resulting G4 oligonucleotides. The change in G4 topology modified the electrophoretic mobility of the compounds. Figure 3A shows that the two oligonucleotides with a mixed P/A conformation migrate a little more slowly than the sequences in the antiparallel form. Moreover, by UV-melting we found that the compounds with a mixed P/A conformation, 7214 and 7216, have a thermal stability, 56.8 and 55.0 °C, respectively, similar to that of 7217 (56.2 °C), which lacks the anthraquinone insertions (Table 1). This suggests that in the oligonucleotide with a mixed P/A conformation, the two anthraquinone insertions, which are placed at opposite ends of the quadruplex, should not promote stabilizing π -interactions neither with the external G-quartets

Figure 3. (A) Native PAGE of the G4 decoys and of 15-, 20-, 24-, 30-, and 36-mer reference oligonucleotides, *m* = mixed P/A, *A* = antiparallel. (B) Fluorescence spectra of the decoy oligonucleotides in 50 mM Tris-HCl pH 7.4, 100 mM KCl; Ex = 444 nm, Em = 470–750 nm. (C) Dual luciferase assay with pHRAS-luc, pRL-CMV, and decoy oligonucleotides. The ordinate reports % luciferase, i.e., $T/C \times 100$, where *T* = firefly/*Renilla* in oligonucleotide-treated cells and *C* = firefly/*Renilla* in oligonucleotide-untreated cells. **P* < 0.05.

nor with the neighboring bases. In contrast, compound **7213** with insertions H_2 and H_4 in the two lateral loops lying at the same side of the quadruplex (Figure 2A,D), shows a T_M 8 °C higher than that of **7217**. In this oligonucleotide the two anthraquinones are in close proximity and probably stacked on each other. To support this interpretation we performed fluorescence experiments, as the polycyclic anthraquinone chromophore may change its fluorescence quantum yield when it is involved in π -interactions.²³ When it is excited at 444 nm, it emits at 560 nm (Figure 3B). By fixing the emission of **7214** to 1, all the other compounds showed a higher emission: **7213**, 3.75-fold more intense, **7215**, 1.75-fold, and **7216**, 1.3-fold. Since the T_M of **7214** (56.8 °C), with two anthraquinone at the 5' and 3' ends, is similar to the T_M of **7217** (56.2 °C), we jumped to the conclusion that in **7214** the two chromophores are oriented outside the structure and do not promote π -interactions with the adjacent G-quartets. In contrast, compound **7213** shows a dramatic increase of fluorescence, as the two anthraquinones, inserted in neighboring lateral loops, promote reciprocal π -interactions. In this case the quadruplex is stabilized by 8 °C compared to **7217**.

In keeping with this interpretation, compound **7215** shows an increase of both T_M (4 °C) and fluorescence (1.75 fold), as the two anthraquinones lying at the same side of the quadruplex can promote π -interactions, to some extent. Instead, **7216** shows T_M similar to that of **7217**, as the anthraquinones are at opposing ends of the quadruplex (Figure 2D). In a parallel work, we inserted two anthraquinones in the neighboring loops of the thrombin aptamer, which is known to adopt an antiparallel structure.²⁴ This modified aptamer showed a CD similar to that of **7213** (S3). Molecular modeling studies showed that the two anthraquinones are indeed stacked in a face-to-face manner, as we have hypothesized for the parental **7213** compound (S4).

Next, we interrogated if the designed oligonucleotides behave as decoys and are able to downregulate *HRAS* in human T24 bladder cancer cells. To address this issue, we first carried out a dual luciferase assay with pHRAS-luc (an

expressing vector where the whole *HRAS* promoter drives the synthesis of firefly luciferase¹⁰) and pRL-CMV (a vector in which *Renilla* luciferase is driven by the CMV promoter). T24 cancer cells were treated with increasing amounts (50–400 nM) of G4 oligonucleotides, control oligonucleotides unable to form a G-quadruplex [CTRL-1, 5'-GAGGGAGC-GGCTGC-AGCGCTGGGAG; CTRL-2, 5'-GAAGGAGTG-AGTGAGG-GAGCGGCTGCAG; CTRL-3, 5'-TGCAGCC-GCTCCC-TCACTCACTCCTTCCCT], pHRAS-luc, and pRL-CMV. Figure 3C shows that all the designed G4 oligonucleotides cause the inhibition of firefly luciferase compared to *Renilla* luciferase in a dose-response manner, whereas the three control sequences that did not assume any folded structure, were not effective, even at their highest concentration (400 nM). The result of this reporter assay showed that both types of G4 oligonucleotides, antiparallel and mixed P/A, disrupt the mechanism leading to gene expression, seemingly by sequestering the proteins interacting with *hras*-1. To provide further support for the activity of the designed G4 oligonucleotides, we focused on genomic *HRAS*. We treated T24 cancer cells with 800 nM G4 or control oligonucleotides. Twelve hours after treatment, *HRAS* mRNA in both untreated and oligonucleotide-treated cells was determined by qRT-PCR. The qRT-PCR data showed that all G4 decoys, but not the control oligonucleotides, reduced *HRAS* mRNA compared to those of β 2-microglobulin and HPRT to ~50% of the control (untreated cells) (S5). We also examined the effect of the G4 decoys on *HRAS* expression by Western blots (Figure 4). In

Figure 4. Western blot showing that the G4 decoys (A) but not the control oligonucleotides (B) (800 nM) reduce protein p21^{HRAS} in T24 cancer cells. The % inhibition of p21^{HRAS} is shown in the histogram as $T/C \times 100$, where *T* and *C* are the p21^{HRAS}/ β -actin ratios in treated and untreated cells, respectively.

accord with the luciferase and qRT-PCR data, all the compounds decreased the level of protein *HRAS*, in particular **7215**, **7216**, and **7217** (to <30% of control), while the control oligonucleotides CTRL 1–3 did not.

Next, we examined the stability of the designed decoy oligonucleotides in a T24 cellular extract, up to 93 h of incubation (Figure 5A). The results showed that wild-type *hras*-1 oligonucleotide undergoes a nearly complete degradation in 93 h. In contrast, all the other compounds appeared more stable, and this provides a key to rationalize their activity. So, there is a correlation between the stability of the G4 oligonucleotides in the cell environment and their capacity to lower luciferase and *HRAS* expression.

Figure 5. (A) Denaturing PAGE showing the stability of the G4 decoys and wild-type *hras-1* in a T24 cellular extract up to 93 h. (B) ChIP showing that MAZ is associated with the *HRAS* promoter in the untreated (NT) or control oligonucleotide-treated T24 cancer cells (CTRL-2). In contrast, in 7214 and 7217 treated cells, the binding of MAZ to the promoter is attenuated. The product of ChIP-PCR is a 188 bp fragment (S1). The histogram shows levels of MAZ associated with *HRAS* promoter in untreated and control/decoy-treated cells.

As the designed G4 oligonucleotides suppress *HRAS*, we wondered if they really act through a decoy mechanism. To provide evidence for this, we examined by chromatin immunoprecipitation (ChIP) if the G4 decoys attenuate the occupancy of the *HRAS* promoter by proteins essential for transcription. We previously demonstrated that MAZ is a key transcription factor for *HRAS*;^{9,10} therefore, we focused our ChIP analysis on this protein, by using an antibody specific for MAZ. A typical ChIP analysis for 7214, 7217, and CTRL-2 is reported in Figure 5B. The histogram summarizes the results obtained with all the G4 decoys. The occupancy of the *HRAS* promoter by MAZ in the decoy-treated cells is about 1/3 of the occupancy observed with the untreated or control-treated cells. Note that *hras-1*, being located near to TSS, is significantly occupied by RNA Pol II too (positive control) but not by IgG (negative control).

Furthermore, by an EMSA competition assay we found that all the designed G4 decoys are able to compete with the binding of MAZ to the *hras-1* quadruplex (S6). So, both ChIP-PCR and EMSA proved that MAZ, under *in vivo* conditions, is associated with the critical G-rich elements of the *HRAS* promoter and that the binding of MAZ to the *HRAS* promoter is attenuated by the G4 decoy oligonucleotides.

As a final point we tested the efficacy of the G4 decoys to inhibit the proliferation of the *HRAS* mutant T24 bladder cancer cells. The cells were transfected with increasing amounts of decoy or control oligonucleotides and incubated for 72 h, before a resazurin assay was carried out. To various extents, all

compounds lowered the percentage of viable cells in a dose-response manner. The estimated IC_{50} values (nM) are ~600 for 7213, ~300 for 7214, >800 for 7215, ~800 for 7216, and >800 for 7217. In contrast, in noncancer 293 cells the compounds had a weaker effect on cell viability, with $IC_{50} \gg 800$ nM (S7).

We have designed a decoy strategy to inhibit the expression of oncogene *HRAS* in bladder cancer cells. To strengthen the nuclease resistance and the folded conformation, the G4 decoys have been synthesized with LNA modifications and anthraquinone insertions. Luciferase, qRT-PCR, and Western blot assays showed that the decoy oligonucleotide repressed *HRAS* expression, while ChIP and EMSA provided evidence that the molecules actually behave through the postulated decoy mechanism. It might be argued that the decoy strategy lacks specificity because the sequestration of a transcription factor (as MAZ) could have impact on other genes as well. However, bladder cancer cells are addicted to oncogenic *HRAS*, just as pancreatic cancer cells are addicted to mutant *KRAS*.²⁵ This means that the metabolism of these transformed cells strongly depends on the expression of these oncogenes. Indeed, it has been reported that the *ras* oncogenes remodel the metabolic pathways of cancer cells to fuel their higher proliferation rate.²⁵ So, cancer cells are more responsive to *ras* gene expression than normal cells. In other words, the inhibition of *HRAS* in *HRAS* mutant bladder cancer cells is less tolerated than in noncancer 293 cells bearing wild-type *HRAS*. The concept of oncogene addiction for targeted anticancer therapy has been recently illustrated.²⁶

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00315.

Materials and Methods, oligonucleotides synthesis, CD spectra, qRT-PCR, EMSA, and cell viability assays (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: luigi.xodo@uniud.it. Fax: +39.043249430.

Present Address

^{||}Department of Chemistry, Faculty of Science, Benha University, Benha 13518, Egypt.

Author Contributions

[§]G.M. and A.S.G. have contributed equally to this work. The manuscript was written by L.X. All authors have given approval to the final version of the manuscript.

Funding

AIRC (Italian association for cancer research), IG2013, project Code 14301

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ChIP, chromatin immune precipitation; *HRAS*, Harvey *ras* gene; MAZ, myc associated zinc-finger protein; LNA, locked nucleic acid

■ REFERENCES

(1) Ding, L.; Getz, G.; Wheeler, D. A.; et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **2008**, *455*, 1069–1075.

- (2) Porter, A. C.; Vaillancourt, R. R. Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. *Oncogene* **1998**, *17*, 1343–1352.
- (3) Schubbert, S.; Shannon, K.; Bollag, G. Hyperactive Ras in developmental disorders and cancer. *Nat. Rev. Cancer* **2007**, *7*, 295–308.
- (4) Vageli, D.; Kiaris, H.; Delakas, D.; Anezinis, P.; Cranidis, A.; Spandidos, D. A. Transcriptional activation of H-ras, K-ras and N-ras proto-oncogenes in human bladder tumors. *Cancer Lett.* **1996**, *107*, 241–247.
- (5) Theodorescu, D.; Cornil, I.; Fernandez, B. J.; Kerbel, R. Overexpression of normal and mutated forms of HRAS induces orthotopic bladder invasion in a human transitional cell carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 9047–9051.
- (6) Mo, L.; Zheng, X.; Huang, H. Y.; Shapiro, E.; Lepor, H.; Cordon-Cardo, C.; Sun, T. T.; Wu, X. R. Hyperactivation of Ha-ras oncogene, but not Ink4a/Arf deficiency, triggers bladder tumorigenesis. *J. Clin. Invest.* **2007**, *117*, 314–325.
- (7) Yafi, F. A.; North, S.; Kassouf, W. First- and second-line therapy for metastatic urothelial carcinoma of the bladder. *Curr. Oncol.* **2011**, *18*, e25–e34.
- (8) Baines, A. T.; Depeng, X.; Der, C. J. Inhibition of Ras for cancer treatment: the search continue. *Future Med. Chem.* **2011**, *3*, 1787–1808.
- (9) Membrino, A.; Cogoi, S.; Pedersen, E. B.; Xodo, L. E. G4-DNA formation in the HRAS promoter and rational design of decoy oligonucleotides for cancer therapy. *PLoS One* **2011**, *6*, e24421.
- (10) Cogoi, S.; Shchekotikhin, A. E.; Xodo, L. E. HRAS is silenced by two neighbouring G-quadruplexes and activated by MAZ, a zinc-finger transcription factor with DNA unfolding property. *Nucleic Acids Res.* **2014**, *42*, 8379–8388.
- (11) Cogoi, S.; Zorzet, S.; Rapozzi, V.; Géci, I.; Pedersen, E. B.; Xodo, L. E. MAZ-binding G4-decoy with locked nucleic acid and twisted intercalating nucleic acid modifications suppresses KRAS in pancreatic cancer cells and delays tumor growth in mice. *Nucleic Acids Res.* **2013**, *41*, 4049–4064.
- (12) Sun, X.; Zhang, J. STAT3 Decoy ODN Therapy for Cancer. *Methods Mol. Biol.* **2015**, *1317*, 167–83.
- (13) Kim, K. H.; Lee, W. R.; Kang, Y. N.; Chang, Y. C.; Park, K. K. Inhibitory effect of nuclear factor- κ B decoy oligo-deoxynucleotide on liver fibrosis through regulation of the epithelial-mesenchymal transition. *Hum. Gene Ther.* **2014**, *25*, 721–729.
- (14) Chan, K. S.; Sano, S.; Kiguchi, K.; Anders, J.; Komazawa, N.; Takeda, J.; DiGiovanni, J. Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. *J. Clin. Invest.* **2004**, *114*, 720–728.
- (15) Sen, M.; Paul, K.; Freilino, M. L.; Li, H.; Li, C.; Johnson, D. E.; Wangm, L.; Eiseman, J.; Grandis, J. R. Systemic administration of a cyclic signal transducer and activator of transcription 3 (STAT3) decoy oligonucleotide inhibits tumor growth without inducing toxicological effects. *Mol. Med.* **2014**, *20*, 46–56.
- (16) Kim, K. H.; Park, J. H.; Lee, W. R.; Park, J. S.; Kim, H. C.; Park, K. K. The inhibitory effect of chimeric decoy oligodeoxynucleotide against NF- κ B and Sp1 in renal interstitial fibrosis. *J. Mol. Med. (Heidelberg, Ger.)* **2013**, *91*, 573–586.
- (17) De Rosa, G.; De Stefano, D.; Laguardia, V.; Arpicco, S.; Simeon, V.; Carnuccio, R.; Fattal, E. Novel cationic liposome formulation for the delivery of an oligonucleotide decoy to NF- κ B into activated macrophages. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 7–18.
- (18) Cogoi, S.; Zorzet, S.; Rapozzi, V.; Géci, I.; Pedersen, E. B.; Xodo, L. E. MAZ-binding G4-decoy with locked nucleic acid and twisted intercalating nucleic acid modifications suppresses KRAS in pancreatic cancer cells and delays tumor growth in mice. *Nucleic Acids Res.* **2013**, *41*, 4049–4064.
- (19) Campbell, M. A.; Wengel, J. Locked vs. unlocked nucleic acids (LNA vs. UNA): contrasting structures work towards common therapeutic goals. *Chem. Soc. Rev.* **2011**, *40*, 5680–5689.
- (20) Ndlebe, T.; Schuster, G. B. Long-distance radical cation transport in DNA: horizontal charge hopping in a dimeric quadruplex. *Org. Biomol. Chem.* **2006**, *4*, 4015–4021.
- (21) Gouda, A. S.; Amine, M. S.; Pedersen, E. B. Synthesis of new DNA G-quadruplex constructs with anthraquinone insertions and their anticoagulant activity. *Helv. Chim. Acta*, in press.
- (22) Randazzo, A.; Spada, G. P.; da Silva, M. W. Circular dichroism of quadruplex structures. *Top. Curr. Chem.* **2012**, *330*, 67–86.
- (23) Suzuki, I.; Nakayama, C.; Ui, M.; Hirose, K.; Yamauchi, A. Greater fluorescence from styrylpyridinium dye upon complexation with cyclodextrin derivatives through π - π interactions. *Anal. Sci.* **2007**, *3*, 249–251.
- (24) Mao, X. A.; Gmeiner, W. H. NMR study of the folding-unfolding mechanism for the thrombin-binding DNA aptamer d(GGTTGGTGTGGTTGG). *Biophys. Chem.* **2005**, *113*, 155–160.
- (25) Ying, H.; Kimmelman, A. C.; Lyssiotis, C. A.; et al. Oncogenic Kras maintains pancreatic tumours through regulation of anabolic glucose metabolism. *Cell* **2012**, *149*, 656–670.
- (26) Torti, D.; Truscolino, L. Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. *EMBO Mol. Med.* **2011**, *3*, 623–636.