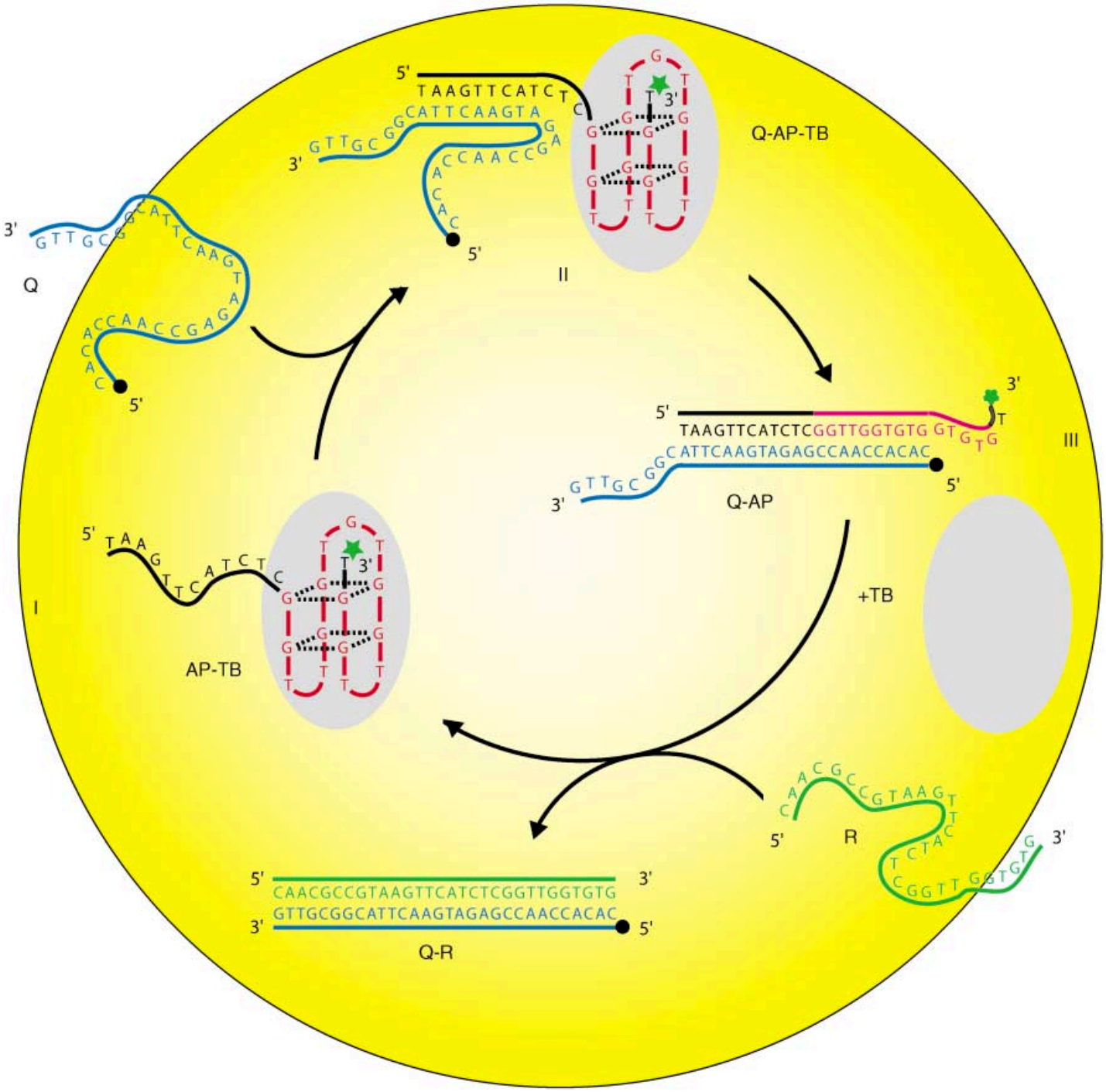
# Communications




In the Communication on the following pages F. C. Simmel and co-workers describe a molecular device based on a DNA aptamer which can be instructed to repeatedly bind and release the protein thrombin.


**DOI: 10.1002/anie.200353537**

DNA Structures

# A DNA-Based Machine That Can Cyclically Bind and Release Thrombin**

*Wendy U. Dittmer, Andreas Reuter, and Friedrich C. Simmel**

Molecular machines can be used to produce controlled nanoscale movements.[1] In particular, the unique molecular recognition properties of DNA have been used to devise a number of nanomechanical devices that can be reversibly switched between several distinct conformations.[2] Although these devices display a variety of motions including rotational and stretching-type movements,[3,4] they do not perform any particular function. DNA structures with functionality can be found in DNA aptamers, which are short stretches of single-stranded DNA selected from a random pool of DNA sequences for their binding affinities to proteins or small molecules.[5] We report here on the construction of a protein-bearing molecular machine based on a DNA aptamer, which can be instructed to grab or release the human blood-clotting factor, α-thrombin, depending on an operator DNA sequence addressing it. In the operation of this DNA nanomachine, the thrombin-binding DNA aptamer is mechanically switched between a binding and a nonbinding form. The functioning of this "molecular hand" is demonstrated by gel electrophoresis, fluorescence resonance energy transfer (FRET), and fluorescence anisotropy measurements.

The machine is based on the 15-base DNA sequence 5′-GGTTGGTGTGGTTGG-3′, which has been found to bind

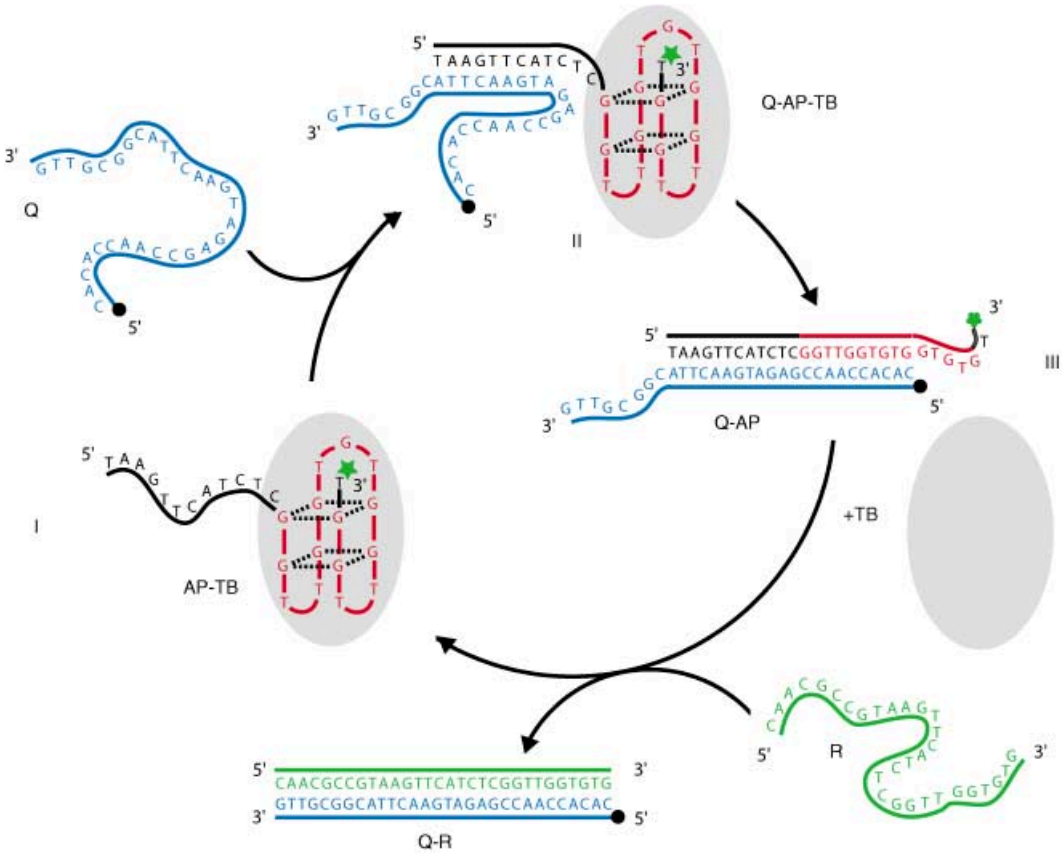



***Figure 1.*** Representation of the operation cycle of the aptamer-based molecular machine in the presence of thrombin (for an explanation see the text). Sequences are shown for the labeled strands A3, Q5, and R.

[*] Dr. W. U. Dittmer, A. Reuter, Dr. F. C. Simmel
Sektion Physik and Center for Nanoscience
LMU Munich
Geschwister Scholl Platz 1, 80539 Munich (Germany)
Fax: (+49) 89-2180-3182
E-mail: simmel@lmu.de

[**] We thank Prof. Dr. John S. McCaskill for helpful discussions and Prof. Dr. Jörg P. Kotthaus for continuous support. This work was funded by the Deutsche Forschungsgemeinschaft, the Bavarian State Ministry of Sciences, Research, and the Arts, and the Alexander von Humboldt Foundation.

strongly to α-thrombin[6] with a dissociation constant on the order of 3–450 nM.[7] In the presence of potassium ions, the aptamer assumes a conformation characterized by two stacked guanine quadruplex structures (see Figure 1). To construct the machine, we modified the aptamer at the 5′-end by adding a 12-base "toehold" section to address the device. The 5′-end was chosen in order not to interfere with thrombin binding sites.[8] We validated, using melting-curve experiments monitoring the absorbance, that in the presence of $K^+$ ions, the aptamer device (AP) indeed assumes its G quartet

  DOI: 10.1002/anie.200353537 

form.[9] The operation cycle of the machine is depicted in Figure 1. Upon the addition of thrombin (TB), strand AP binds to the protein (I in Figure 1). By the addition of an "opening" strand Q, the DNA device extends to a duplex structure AP-Q, which is not capable of binding to TB (III). Q is complementary to the toehold and the first ten bases of the aptamer sequence and additionally has an eight-base-long sequence at the 3′-end which serves as a point of attachment for the fully complementary "removal" strand R. This is the fundamental operating principle used to cycle DNA machines through their various conformations.[4] Q was not chosen to be completely complementary to AP, because R would otherwise also contain the full aptamer sequence and thus could bind to thrombin. Removal of Q by R returns the device to its original conformation (I), where it binds to TB again.

We observed the operation of the machine with an electrophoretic band-shift experiment (Figure 2). Binding of

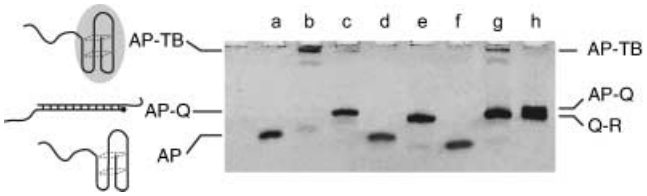



***Figure 2.*** Gel evidence for the operation of the DNA machine. Lanes contain: a) AP, b) AP + thrombin (TB), c) (AP + TB) + Q, d) Q, e) Q + R, f) R, g) ((AP + TB) + Q) + R, h) (((AP + TB) + Q) + R) + Q. Pairs in brackets were incubated for 1 h before the addition of the next component. In the hybridization reactions the oligonucleotides each have a concentration of 1 μM. Human α-thrombin (Fluka) was added in a protein:aptamer ratio of 10:1. Due to the higher fluorescence intensity of the gel stain when bound to DNA duplexes, the total amount of DNA loaded into wells c, e, g, and h was adjusted to obtain a uniform intensity for the bands without extensive broadening or smearing. In lane g only 60% of the amount of strand AP was added relative to that in lane b, which explains the reduced intensity for the AP–TB complex in this lane.

AP (lane a) to thrombin results in a strong band shift (lane b). By the addition of Q, thrombin is released from AP and a band appears for the duplex AP–Q (lane c). Upon addition of R, AP is released and can bind to thrombin once again (lane g). This can be judged from the reappearance of the AP–TB band and a second band from the "waste" product Q–R. An additional, faint band is observed in lanes b and g just below that corresponding to AP–TB. It is found that the intensity of this band varies from one thrombin sample to another. Furthermore, it is more intense with older thrombin samples than with fresh ones. We therefore attribute this additional band to an impurity in our thrombin solution, possibly a thrombin decay product to which the aptamer can also bind. In lane h, further addition of Q releases thrombin again and two closely spaced bands appear for the duplex AP–Q and the waste product from the previous cycle. The gel experiments thus show unambiguously that the aptamer device can be switched repeatedly between its two conformations and cyclically binds and releases thrombin.

To follow the functioning of the aptamer device in real time, FRET experiments were performed. FRET is the nonradiative transfer of excitation energy from a "donor" fluorophore to an "acceptor" chromophore whose absorption spectrum overlaps with the emission spectrum of the donor. The efficiency of this process is strongly dependent on the distance between the two dyes and can be used to determine the proximity of labeled molecules.[10] Several FRET sensors based on aptamers have been constructed.[7,11] Here, FRET is used to follow the conformational changes of the DNA device accompanying the binding and release of thrombin. In Figure 3 the results of FRET experiments are shown in

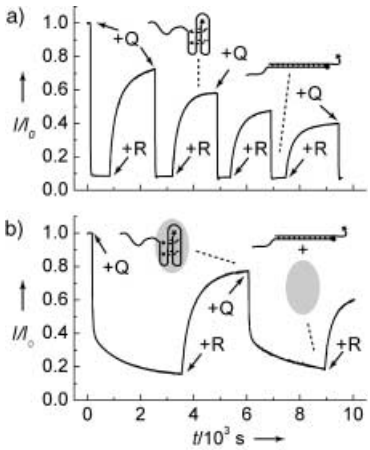



***Figure 3.*** Normalized fluorescence intensity $I/I_0$ collected during the operation of the DNA device, a) without thrombin, b) in the presence of thrombin. The conformations corresponding to the different fluorescence levels are indicated by the icons.

which the strands AP3, Q5 (labeled at the 3′- and 5′-ends, respectively, see Figure 1), and R are used in the absence (Figure 3a) and presence (Figure 3b) of thrombin. Upon binding of Q5 to AP3, the fluorophore on AP3 is quenched by the FRET acceptor on Q5. AP3 is already partly quenched when Q5 attaches to the toehold (cf. II in Figure 1); full quenching occurs after complete binding of Q5 to AP3. Figure 3 indicates that the device can be switched from the G quartet to the duplex form much more rapidly in the absence of thrombin. In contrast, the removal step does not involve thrombin and displays second order kinetics with roughly the same time constants for both situations ($\tau = 220$–$240$ s without thrombin, $\tau = 290$–$350$ s with thrombin).

The operation kinetics of the machine is influenced to a great extent by the presence of the toehold section attached to the aptamer sequence. To elucidate this effect, a shorter opening strand, Q5-S, was constructed to be complementary to the original 15-base aptamer sequence but not to contain the complement of the toehold. In the absence of thrombin, the device rapidly undergoes a transition to the duplex form upon addition of either Q5 or Q5-S. The final fluorescence level for the AP3–Q5-S structure is slightly lower, since the fluorophores are closer together here than in the AP3–Q5 complex. In both cases the half-lives for the transitions are on the order of $\tau = 5$ s. The situation is very different in the presence of thrombin. The fluorescence decay induced by the opening strand Q5 is well modeled by the superposition of a

second order and a first order process. The half-life for the second order step is $\tau = 24$ s. The half-life for the first order step is $\tau = 960$ s. The fast component is likely due to reaction of Q5 with thrombin-free AP strand and also due to its binding to and release from the toehold section (II in Figure 1). The slow first order part corresponds to the release of protein from the Q–AP–TB complex. When the short opening strand Q5-S, which does not attach to the toehold section, is used, a much slower first order step with half-life $\tau = 1740$ s is observed. The fits indicate that the toehold section increases the rate of protein release approximately twofold (Figure 4).

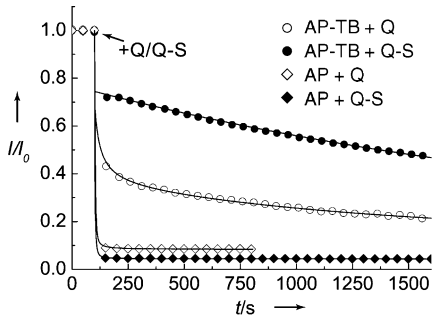



***Figure 4.*** Fluorescence decay after the addition of a quencher sequence Q to the device in the presence of TB (circles) or without TB (squares). Every 50th data point is displayed. The continuous lines are fits to the data (see text).

In order to directly observe the binding and release of thrombin by the machine, measurements of the fluorescence anisotropy $r$ were completed (see the Experimental Section).[12] In Figure 5 $r$ is displayed for several operation cycles of the device. For these experiments only the aptamer strand AP3 was labeled. Fluorescence anisotropy is sensitive to the environment of a fluorophore and the presence of the protein constrains the dye's rotational degree of freedom and thus enhances the anisotropy. The change in $r$ therefore corresponds to protein-binding or -release events. The opening step confirms the first order release kinetics with a half-life of roughly 600 s, the binding step is second order with a half-life

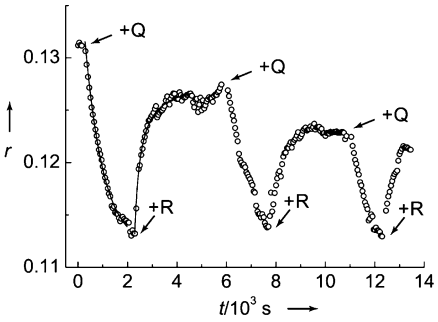



***Figure 5.*** Fluorescence anisotropy $r$ collected during the operation of the aptamer device in the presence of thrombin. The signal is only sensitive to the binding of the device to the protein and thus directly shows the binding and releasing of thrombin. The continuous lines are fits to the data.

$\tau = 230$ s. A thorough investigation of the kinetics of the device is in progress.

We have constructed a DNA molecular machine based on a DNA aptamer that can be instructed repeatedly to bind or release a protein. In essence, the device functions to precisely control the concentration of thrombin protein in solution between a depleted and an enriched state. The combination of the operating principles of DNA-based nanomechanical devices with the binding properties of DNA aptamers significantly opens up design possibilities for the construction of further functional DNA nanostructures. As aptamers can be selected to bind to a large variety of arbitrarily chosen compounds, DNA-based nanomachines useful for carrying, binding, and releasing molecules other than thrombin could be easily constructed from the device reported here.

## Experimental Section

The DNA sequences for AP3, Q5, and R are shown in Figure 1: The 3′-label of AP3 is Oregon Green 488 (Molecular Probes, Oregon). The 5′-label of Q5 is TAMRA (5(6)-carboxytetramethylrhodamine). Strand AP is like AP3 without the thymine spacer and the label at 3′. Unlabeled Q has the same sequence as Q5. Short Q5-S is 5′-TAMRA CCAACCACACCAACC-3′; R is 5′-CAACGCCGTAAGTT-CATCTCGGTTGGTGTG-3′. All oligonucleotides were synthesized by biomers.net, Ulm, Germany. Unless stated otherwise, chemicals were obtained from Sigma-Aldrich, Germany, and used without further purification. The buffer used for all experiments, unless otherwise indicated, was a modified physiological buffer consisting of 20 mM Tris HCl, 140 mM NaCl, 10 mM $MgCl_2$, and 10 mM KCl at pH 8.5.

Polyacrylamide gel electrophoresis was performed on a 15% native gel in TB buffer (89 mM Tris HCl, 89 mM boric acid) with 10 mM KCl and run for 1.5 h with a field of 10 $V\,cm^{-1}$ at 20 °C. Gels were stained using the nucleic acid stain SYBR gold (Molecular Probes). Thrombin is not fluorescent itself and is not stained by the dye.

For FRET experiments, fluorescence was excited with an Argon ion laser ($\lambda = 488$ nm) and detected with a Si photodiode through a 10-nm bandpass filter centered at 514.5 nm using lock-in detection. The temperature was thermostated at 25 °C. The concentration of strand AP was 1 μM in all cases; other strands were added stoichiometrically. Thrombin was added at fivefold excess over AP.

In anisotropy experiments, fluorescence was excited through a Glan–Thompson (GT) polarizer. Parallel and perpendicular components $I_{\parallel}$, $I_{\perp}$ of the fluorescence intensity were collected through two GT analyzers in a T-format. Anisotropy is defined as $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$.

Received: December 15, 2003 [Z53537]
Published Online: April 28, 2004

**Keywords:** aptamers · DNA structures · DNA · molecular devices · nanostructures

[1] V. Balzani, A. Credi, F. M. Raymo, J. F. Stoddart, *Angew. Chem.* **2000**, *112*, 3484; *Angew. Chem. Int. Ed.* **2000**, *39*, 3349.
[2] C. M. Niemeyer, M. Adler, *Angew. Chem.* **2002**, *114*, 3933; *Angew. Chem. Int. Ed.* **2002**, *41*, 3779.
[3] C. D. Mao, W. Q. Sun, Z. Y. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144.
[4] a) B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605; b) F. C. Simmel, B. Yurke,

*Phys. Rev. E* **2001**, *6304*; c) F. C. Simmel, B. Yurke, *Appl. Phys. Lett.* **2002**, *80*, 883; d) H. Yan, X. P. Zhang, Z. Y. Shen, N. C. Seeman, *Nature* **2002**, *415*, 62; e) J. W. J. Li, W. H. Tan, *Nano Lett.* **2002**, *2*, 315; f) P. Alberti, J. L. Mergny, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1569; g) A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey, F. C. Simmel, *Phys. Rev. Lett.* **2003**, *90*; h) L. P. Feng, S. H. Park, J. H. Reif, H. Yan, *Angew. Chem.* **2003**, *115*, 4478; *Angew. Chem. Int. Ed.* **2003**, *42*, 4342.

[5] D. S. Wilson, J. W. Szostak, *Annu. Rev. Biochem.* **1999**, *68*, 611.

[6] L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564.

[7] J. W. J. Li, X. H. Fang, W. H. Tan, *Biochem. Biophys. Res. Commun.* **2002**, *292*, 31.

[8] K. Padmanabhan, K. P. Padmanabhan, J. D. Ferrara, J. E. Sadler, A. Tulinsky, *J. Biol. Chem.* **1993**, *268*, 17651.

[9] J. L. Mergny, A. T. Phan, L. Lacroix, *FEBS Lett.* **1998**, *435*, 74.

[10] a) T. Förster, *Ann. Phys.* **1948**, *2*, 55; b) L. Stryer, R. P. Haugland, *Proc. Natl. Acad. Sci. USA* **1967**, *58*, 719.

[11] a) R. Nutiu, Y. F. Li, *J. Am. Chem. Soc.* **2003**, *125*, 4771; b) M. Rajendran, A. D. Ellington, *Nucleic Acids Res.* **2003**, *31*, 5700.

[12] a) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd ed., Kluwer Academic/Plenum Publishers, New York, **1999**; b) R. A. Potyrailo, R. C. Conrad, A. D. Ellington, G. M. Hieftje, *Anal. Chem.* **1998**, *70*, 3419.

