Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Interstrand H-aggregation of Cationic Dyes for Narrowing the Absorption Spectra and Stabilizing the Duplex

Hiromu Kashida[a]; Hiroyuki Asanuma[b]; Makoto Komiyama[a]
[a] Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan [b] PRESTO, Japan Science and Technology Agency (JST), Japan

To cite this Article Kashida, Hiromu , Asanuma, Hiroyuki and Komiyama, Makoto(2004) 'Interstrand H-aggregation of Cationic Dyes for Narrowing the Absorption Spectra and Stabilizing the Duplex', Supramolecular Chemistry, 16: 6, 459 — 464
To link to this Article: DOI: 10.1080/10610270410001722358
URL: http://dx.doi.org/10.1080/10610270410001722358

Supramolecular Chemistry, September 2004, Vol. 16 (6), pp. 459–464

Taylor & Francis
Taylor & Francis Group

Interstrand H-aggregation of Cationic Dyes for Narrowing the Absorption Spectra and Stabilizing the Duplex

HIROMU KASHIDA[a], HIROYUKI ASANUMA[a,b,*] and MAKOTO KOMIYAMA[a]

[a]*Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8904, Japan;*
[b]*PRESTO, Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan*

Received (in Austin, USA) 22 April 2004; Accepted 6 May 2004

Interstrand H-aggregates of cationic dyes have been prepared by hybridization of two DNA–dye conjugates involving a Naphthyl Red moiety and spacer alternately in the middle of the sequence. At pH 5.0 where Naphthyl Red is positively charged, a single-stranded DNA–Naphthyl Red conjugate involving three dye groups and three spacer residues exhibited a broad absorption spectrum with λ_{max} at around 530 nm. However, hybridization of two DNA–Naphthyl Red conjugates that were complementary provided different spectra: a much narrower absorption band appeared at 507 nm, that is 23 nm shorter than the single-stranded conjugates. This spectroscopic behavior indicates that the dyes in the duplex are H-aggregated and excitons are strongly coupled in the aggregate. In addition to the appearance of the narrow H-band, the melting temperature dramatically increased by the H-aggregation of the stacked cationic dyes compared with that of the natural duplex without dye and spacer residues. Thus, the positive charge on the stacked dyes did not interfere with the duplex formation by electrostatic repulsion, but in fact promoted the hybridization.

Keywords: Azo compounds; DNA; Cationic dye; H-aggregation

INTRODUCTION

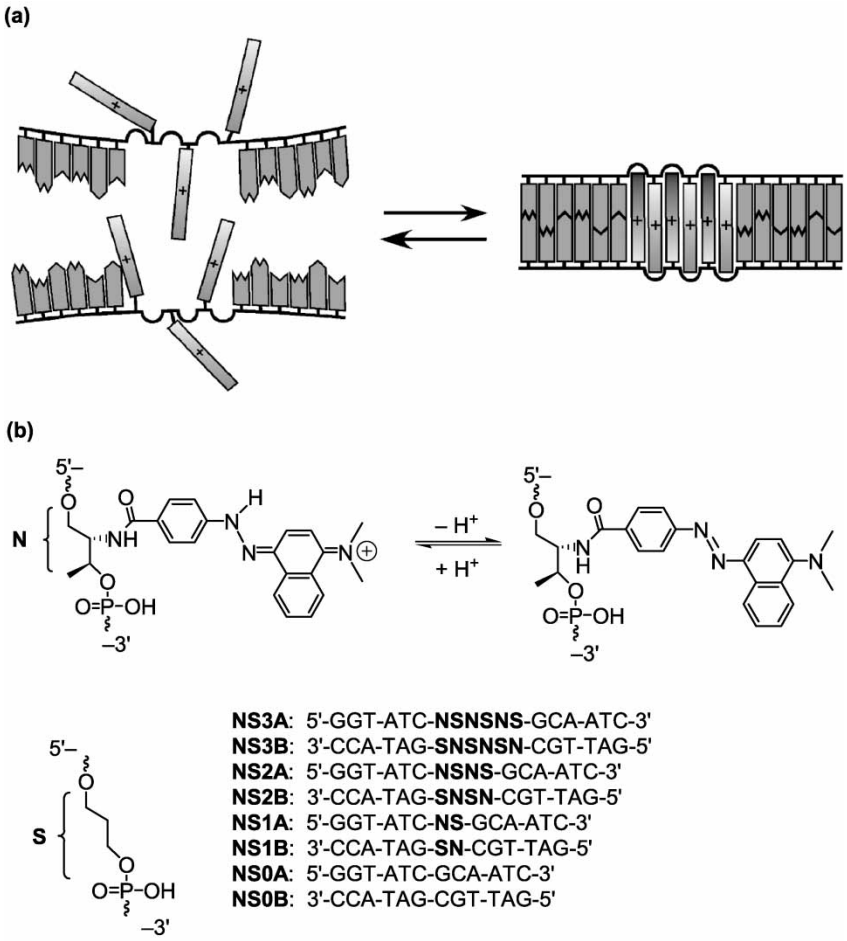
Oligodeoxyribonucleotide (DNA), a natural supramolecule composed of four nucleotides, forms a stable double helix spontaneously with its complementary strand through hydrogen bonding and hydrophobic stacking interaction. After the pioneering work of Kool and colleagues [1–5], various artificial nucleobases were synthesized for the expansion of the genetic alphabet [6–9] and the design of DNA-based supramolecules [10–12]. For these new nucleobases, hydrogen bonding is no longer essential for connecting two strands.

Previously, we have synthesized H-aggregates of Methyl Reds, in which the dyes are axially stacked in an antiparallel manner along the helix axis, from DNA–dye conjugates [13,14]. For the design of these aggregates, the dye moiety on the threoninol linker was used in place of the natural nucleobase and a 1,3-propanediol unit was used as a counterpart, and these artificial scaffolds were introduced alternately into the middle of the DNA (see Scheme 1a). On hybridization of these two complementary strands, H-aggregates of the dyes were successfully prepared: both hypsochromic shifts and induced circular dichroism (ICD), which are characteristic of H-aggregation, were observed. Interestingly, although they involved some non-natural scaffolds, small but distinct increases in the melting temperature (T_m), compared with the natural duplex without these scaffolds, were also observed. This indicated that the aggregated dyes could sufficiently stabilize the duplex only by interstrand stacking interaction in DNA [15].

Our later interests shifted to the effect of positive charge on the H-aggregation for the following two reasons: the first is to narrow the absorption band due to the strong exciton

*Corresponding author. E-mail: asanuma@mkomi.rcast.u-tokyo.ac.jp

ISSN 1061-0278 print/ISSN 1029-0478 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10610270410001722358



SCHEME 1 Schematic illustration of (a) the strategy for the preparation of H-aggregates of the dyes and (b) DNA–Naphthyl Red conjugates synthesized in this study.

coupling [16], as distinct narrowing of the absorption band is often observed for cationic dyes such as cyanine [17,18].* Narrowing of the band makes the absorption coefficient at λ_{max} greater and raises the efficiency of photoinduced electron transfer at specific wavelength regions [19]. Furthermore, strong exciton coupling affects some interesting optical features such as enhancement of nonlinear optical properties, which are significant for optical materials. The other reason is to stabilize the duplex: if positive charges are provided to the dyes of these aggregates as illustrated in Scheme 1a, is the duplex stabilized or not? Estimation of charge effects on the stability of the duplex should contribute to the design of even more effective non-natural bases and artificial supramolecules. Here, we report the interstrand assembly of cationic dyes to estimate the effect of positive charge on the H-aggregation. Both narrowing of the band and significant stabilization of the DNA duplex by the stacked dye cations are attained.

RESULTS AND DISCUSSION

Interstrand H-aggregation of Naphthyl Reds

In this study, Naphthyl Red was used because the charge on this dye is controllable by the pH of solution (see Scheme 1b): neutral Naphthyl Red gives λ_{max} at 450 nm, which shifts towards around 530 nm when it is protonated, as shown in Fig. 1. Its pK_a was determined as 6.5 from this spectral change [20]. The *ab initio* calculation of this dye revealed that positively charged and neutral forms had similar planar structures as shown in Fig. 2. Therefore, structural differences between the charged and non-charged forms are minimized and the effect of positive charge on the melting temperature (T_m) of the duplex can be examined

*This suggests that a positive charge on the dye might facilitate the exciton coupling, although the effect of charge on the exciton coupling (narrowing of the band) has not yet been examined with an identical dye.

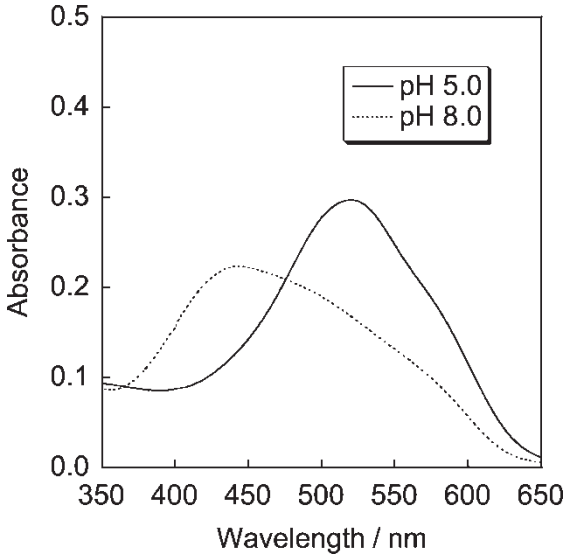


FIGURE 1 Effect of pH on the UV–Vis spectra of the single-stranded NS3B at 0°C in the presence of 0.1 M NaCl.

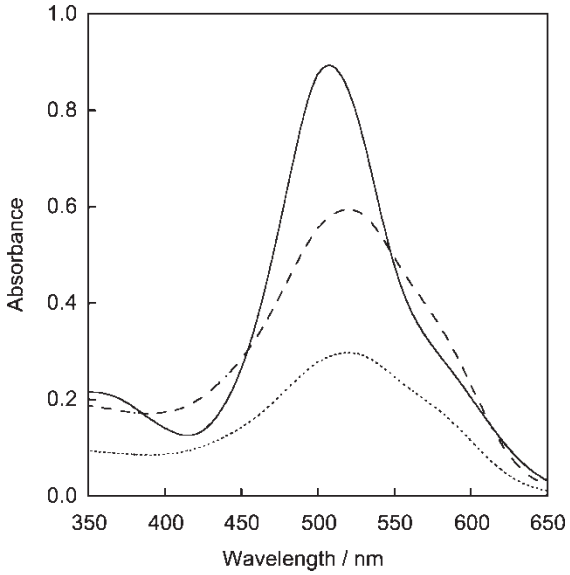


FIGURE 3 UV–Vis spectra of single-stranded NS3B (dotted line) and NS3A/NS3B duplex (solid line) at 0°C in the presence of 0.1 M NaCl at pH 5.0, under which conditions the Naphthyl Reds are protonated. The broken line represents the simple sum of the spectra of single-stranded NS3A and NS3B. The melting temperature of this duplex is 55.2°C under these conditions.

with the identical dye by changing only the pH of the solution.

Naphthyl Red was introduced into the DNA on D-threoninol instead of the natural deoxyribose framework (N residue in Scheme 1b) [20], and 1,3-propanediol (S residue in Scheme 1b) was used as a counterpart. The UV–Vis spectrum of single-stranded NS3B involving three N and three S residues at pH 5.0, where Naphthyl Red is positively charged, is shown as a dotted line in Fig. 3. Although NS3B is chiral and optically pure, circular dichroism (CD) was almost nonexistent (data not shown), indicating that the Naphthyl Reds were essentially isolated due to the inserted S residue. The absorption spectrum of NS3A was almost the same as that of NS3B, and a simple sum of these two spectra is shown as the broken line in Fig. 3. However, hybridization of these two complementary strands provided a different spectrum: a much narrower absorption band appeared at 507 nm, which is 23 nm shorter than the single-stranded NS3B. The half-line-width of the NS3A/NS3B duplex at 0°C was 3110 cm^{-1}, whereas that of NS3B was 4320 cm^{-1}. Strong induced CD (ICD), which is characteristic of the aggregated dyes, was also observed (solid line in Fig. 4a). These spectroscopic behaviors demonstrate that H-aggregates with strong exciton coupling among the stacked dyes were formed in the duplex. Thus, the narrowing is attained on hybridization of two complementary DNAs involving cationic Naphthyl Reds and spacers alternately. Similar narrowing was also observed for NS1A/NS1B and NS2A/NS2B duplexes at pH 5 (Fig. 5). In addition to the narrowing, a small but distinct hypsochromic shift was induced by increasing the number of aggregated dyes: λ_{max} shifted from 520 nm (NS1A/NS1B) to 507 nm (NS3A/NS3B) with accumulation of the dyes. This shift is also consistent with the property of aggregation predicted by McRae and Kasha [21]. As expected, broadening of the sharp H-band of NS3A/NS3B and a bathochromic shift occurred

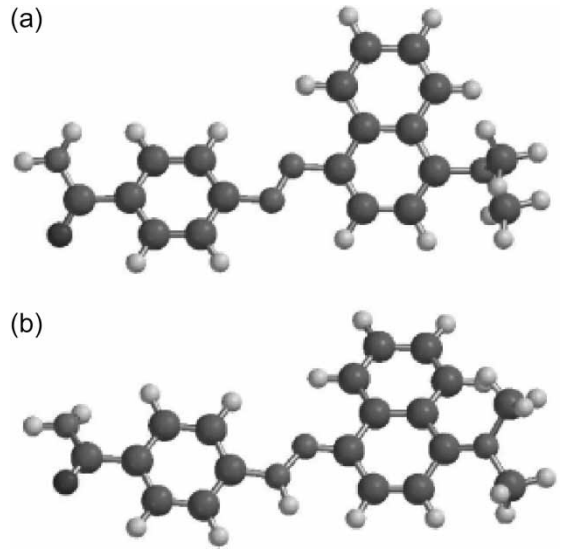


FIGURE 2 Energy-minimized structures of Naphthyl Reds in the (a) neutral and (b) the protonated forms determined from the *ab initio* calculation (basis set HF/6-31G*).

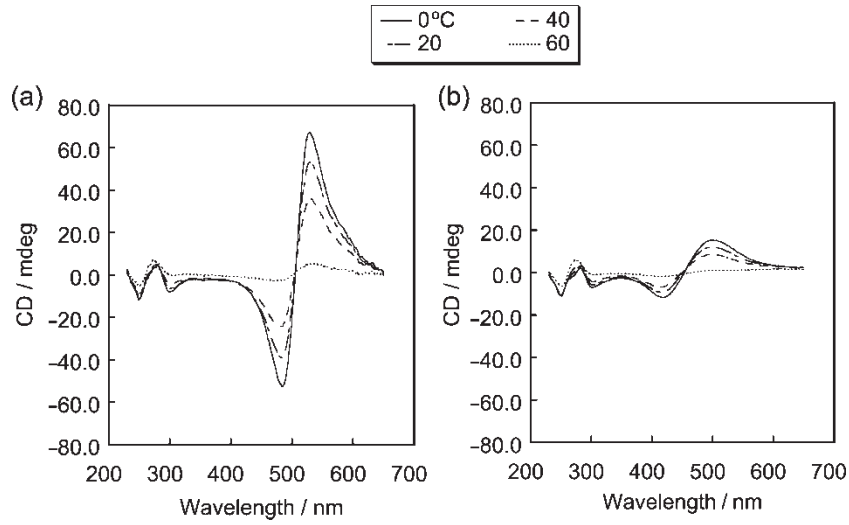


FIGURE 4 Effect of the temperature on the CD spectra of the NS3A/NS3B duplex at (a) pH 5.0 and (b) 8.0 in the presence of 0.1 M NaCl.

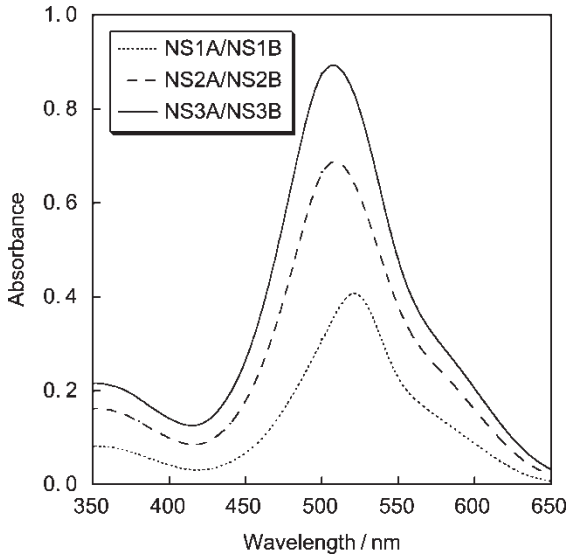


FIGURE 5 UV–Vis spectra of the NS1A/NS1B (dotted line), NS2A/NS2B (broken line) and NS3A/NS3B (solid line) duplexes at 0°C in the presence of 0.1 M NaCl at pH 5.0.

on elevating the temperature above T_m due to the dissociation of the duplex (Fig. 6a). Concurrently, strong ICD at around 500 nm derived from the stacked dyes disappeared (compare the solid line with the dotted line in Fig. 4a). Thus, H-aggregation of the Naphthyl Reds could be reversibly regulated by the temperature.

In contrast with the sharp spectra at pH 5.0, the absorption band was rather broad at pH 8.0 where the dye was non-charged: the UV–Vis spectra did not change at all even when the temperature was changed from 60 to 0°C (see Fig. 6b), despite the appearance of ICD at around 500 nm (see Fig. 4b).[†] Thus, positive charges in the aggregates enhanced the exciton coupling among the stacked dyes and made the UV–Vis spectra sharp.

Effect of Positive Charge on the Melting Temperature

Positive charges on the dye aggregates also significantly affected the stability of the duplex. At pH 8.0, T_m slightly increased with the number of aggregated dyes due to the stacking interaction as shown by the right column in Table I. Increase in the T_m by dye accumulation (NS3A/NS3B *vs.* NS0A/NS0B) was 4.9°C at pH 8.0 where dyes were neutral. Interestingly, T_m of all the H-aggregated duplexes increased when the pH was lowered from 8.0 to 5.0, whereas the natural NS0A/NS0B duplex was largely destabilized due to the protonation of cytidine. Under acidic conditions, the increase in the T_m was as large as 15.4°C. Thus, positive charges on the stacked dyes did not interfere with the duplex formation by electrostatic repulsion, but in fact promoted the hybridization. These facts demonstrate that anionic phosphodiesters on the backbone of DNA can sufficiently compensate for these positive charges and enable accumulation of positive charges as is observed in tetraplexed telomere DNA [22].

In conclusion, narrowing of the H-band is attained on hybridization of two complementary DNAs involving cationic Naphthyl Reds and spacers alternately. Accumulation of positive charge does not interfere with the hybridization, but significantly stabilizes the duplex. Thus, incorporation of cationic dyes into the DNA is effective for stabilization

[†]The appearance of induced CD indicates that the dyes are stacked although the UV–Vis spectra were unchanged.

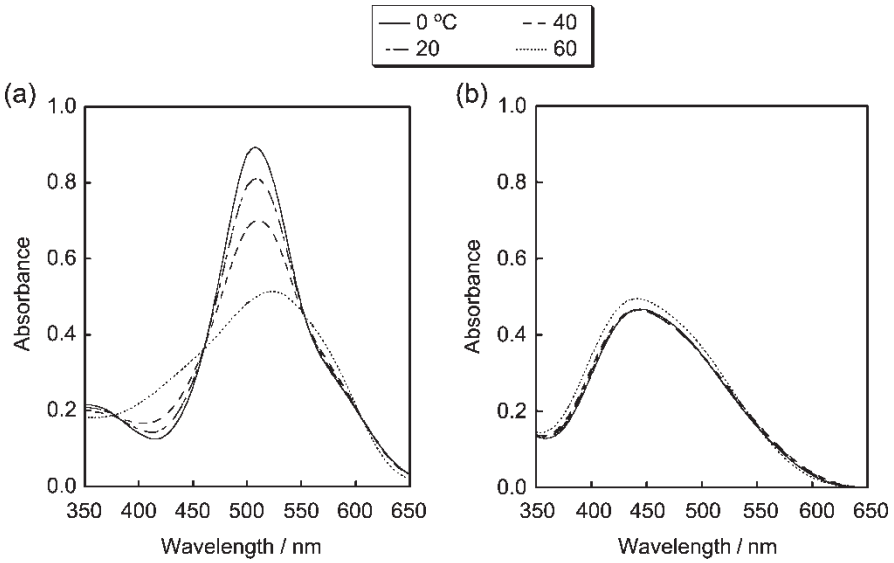


FIGURE 6 Effect of the temperature on the UV–Vis spectra of the NS3A/NS3B duplex (a) at pH 5.0 and (b) 8.0 in the presence of 0.1 M NaCl.

TABLE I Melting temperatures of Naphthyl Red conjugates at different pH values

Duplex	Number of dyes in the aggregate	T_m/°C*	
		pH 5.0	pH 8.0
NS0A/NS0B	0	39.8	46.1
NS1A/NS1B	2	49.5	48.6
NS2A/NS2B	4	52.2	49.7
NS3A/NS3B	6	55.2	51.0

*Melting temperatures were determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature.

of the duplex, especially at low pH where the natural duplex is destabilized. Narrowing of the absorption spectrum based on strong exciton coupling is also promising for the application to the DNA-based optical materials. These findings will contribute to the design of new non-natural nucleobases and supramolecules based on DNA.

MATERIALS AND METHODS

Synthesis of the Modified DNA involving Naphthyl Red

All conventional phosphoramidite monomers, CPG columns, reagents for DNA synthesis and Poly-Pak cartridges were purchased from Glen Research Co. The modified DNAs carrying both N and S residues were synthesized on an ABI DNA/RNA Synthesizer Model 394 by typical phosphoramidite chemistry with the corresponding phosphoramidite monomers synthesized according to our previous paper [20]. All the modified DNAs listed in Scheme 1b were purified by reversed-phase HPLC and characterized by MALDI-TOFMS. MALDI-TOFMS for NS3A: obsd. 5463 (calcd. for [NS3A-H^+]: 5462), NS3B: obsd. 5464 (calcd. for [NS3B-H^+]: 5462). NS2A: obsd. 4857 (calcd. for [NS2A-H^+]: 4856). NS2B: obsd. 4856 (calcd. for [NS2B-H^+]: 4856). NS1A: obsd. 4250 (calcd. for [NS1A-H^+]: 4249). NS1B: obsd. 4250 (calcd. for [NS1B-H^+] 4249).

Spectroscopic Measurements

The UV–Vis spectra and CD spectra were measured on a JASCO model V-530 and a JASCO model J-725 with a 10 mm quartz cell, respectively. Both instruments were equipped with programmed temperature-controllers. Conditions of the sample solutions were as follows: [NaCl] = 0.1 M, pH 8.0 (10 mM Tris buffer) or pH 5.0 (10 mM MES buffer), [DNA] = 5 μM. The T_m value was determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature. The temperature ramp was 1.0°C min^{-1}.

Quantum Chemical Calculation

The energy-minimized structure of Naphthyl Red in either the neutral or the protonated form was determined from the *ab initio* calculation by use of Spartan '02 (Wavefunction, Inc.) on a personal computer (Windows). The HF/6-31G* basis set was applied to this calculation.

Acknowledgements

This work was supported by Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST). Partial support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by the Bio-oriented Technology Research Advancement Institution is also acknowledged.

References

[1] Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863.
[2] Moran, S.; Ren, R. X.-F.; Kool, E. T. *Proc. Natl Acad. Sci. USA* **1997**, *94*, 10506.
[3] Guckian, K. M.; Krugh, T. R.; Kool, E. T. *Nat. Struct. Biol.* **1998**, *5*, 950.
[4] Kool, E. T. *Biopolymers* **1998**, *48*, 3.
[5] Moralets, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **1999**, *121*, 2323.
[6] McMinn, D. L.; Ogawa, A. K.; Wu, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, *121*, 11585.
[7] Tae, E. L.; Wu, Y.; Xia, G.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2001**, *123*, 7439.
[8] Hirao, I.; Ohtsuki, T.; Fujiwara, T.; Mitsui, T.; Yokogawa, T.; Okuni, T.; Nakayama, H.; Takio, K.; Yabuki, T.; Kigawa, T.; Kodama, K.; Yokogawa, T.; Nishikawa, K.; Yokoyama, S. *Nat. Biotechnol.* **2002**, *20*, 177.
[9] Mitsui, T.; Kitamura, A.; Kimoto, M.; To, T.; Sato, A.; Hirao, I.; Yokoyama, S. *J. Am. Chem. Soc.* **2003**, *125*, 5298.
[10] Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shiro, M.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 12494.
[11] Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212.
[12] Mathis, G.; Hunziker, J. *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 3203.
[13] Asanuma, H.; Shirasuka, K.; Komiyama, M. *Chem. Lett.* **2002**, 490.
[14] Asanuma, H.; Shirasuka, K.; Takarada, T.; Kashida, H.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 2217.
[15] Brotschi, C.; Leumann, C. J. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 1655.
[16] Knapp, E. W. *Chem. Phys.* **1984**, *85*, 73.
[17] Wang, M.; Silvia, G. L.; Armitage, B. A. *J. Am. Chem. Soc.* **2000**, *122*, 9977.
[18] Asanuma, H.; Tani, T. *J. Phys. Chem. B* **1997**, *101*, 2149.
[19] Tani, T. In *Photographic Sensitivity*; Oxford, 1995; 118.
[20] Asanuma, H.; Kashida, H.; Liang, X.; Komiyama, M. *Chem. Commun.* **2003**, 1536.
[21] McRae, E. G.; Kasha, M. *J. Chem. Phys.* **1958**, *28*, 721.
[22] Several K^+ ions are accumulated and stabilized in the cavity of the guanine quartet: Hud, N. V.; Smith, F. W.; Anet, F. A. L.; Feigon, J. *Biochemistry* **1996**, *35*, 15383.