# Interactions of Thionin with DNA Strands: Intercalation versus External Stacking†

**Christoph Hecht,‡ Josef Friedrich,*,‡ and Ta-Chau Chang§**

*Physik-Department, Lehrstuhl für Physik E14 Weihenstephan, TU München, 85350 Freising, Germany, and Institute of Atomic and Molecular Sciences, Academia Sinica, P.O. Box 23-166, Taipei 10764, Taiwan*

*Received: October 31, 2003; In Final Form: January 27, 2004*

We have investigated thionin in a glycerol/water glass bound to DNA quadruplexes and DNA duplexes with pressure-tuning hole-burning spectroscopy at 2 K. For free thionin as a reference we could identify the two tautomeric structures (imino and amino form) in the inhomogeneous absorption bands. The measurement of local compressibilities from the spectral hole shifts under pressure allowed the assignment of binding modes of thionin to the DNA strands. Thionin binds via external stacking to the dimeric hairpin quadruplex and binds to the monomeric quadruplex only by Coulombic interaction, whereas it intercalates into a linear GC-duplex structure. However, differences in the local compressibilities revealed that only the amino form is able to intercalate into the DNA duplex.

## Introduction

The interaction of aromatic dye molecules with DNA strands concerns an interesting topic, which touches various aspects. For instance, in antitumor therapy people focus on the anticancer drug aspect of such dyes because a proper interaction, for example, intercalation into the strands, may prohibit the correct information retrieval from the DNA.[1−5] On the other hand, some aromatic compounds have been examined for stabilizing quadruplex structures of DNA in the application of telomerase inhibitor,[6−11] because telomerase was found in most cancer cells, but not in somatic cells.[12] As to physics and chemistry, the binding modes of the chromophore to various DNA structures, including intercalation, groove binding, and external stacking, are of great interest.[13,14] Different binding modes may be identified by their characteristic changes in the spectral shift or in the inhomogeneous line broadening. A rich scenario of interactions may play a role in this binding, such as hydrogen bonding, electrostatic, dispersive, and van der Waals interactions as well as structural changes.[15] However, not only the spectral properties may change with the type of binding. For instance, the local elastic properties around the probe may be different as well for various binding types. If the probe binds to the DNA, it will be partially shielded from the solvent and, hence, will be less sensitive to a solvent compression relative to the pure solvent case. Such a situation would change the local compressibility. The scale of this change depends on the mode of binding (external stacking, intercalation, etc.)

Wu et al.[16] have applied circular dichroism (CD) spectroscopy to investigate binding of thionin to DNA strands at room temperature. They concluded that thionin undergoes a noncovalent external stacking to the end surfaces of DNA-quadruplex structures. However, this binding was not uniform but showed characteristic variations in the CD spectra depending on the specific quadruplex structures. Quite in contrast to the quadruplexes, Tuite and Kelly[17] concluded that thionin intercalates in linear duplex structures. Figure 1a shows the proposed

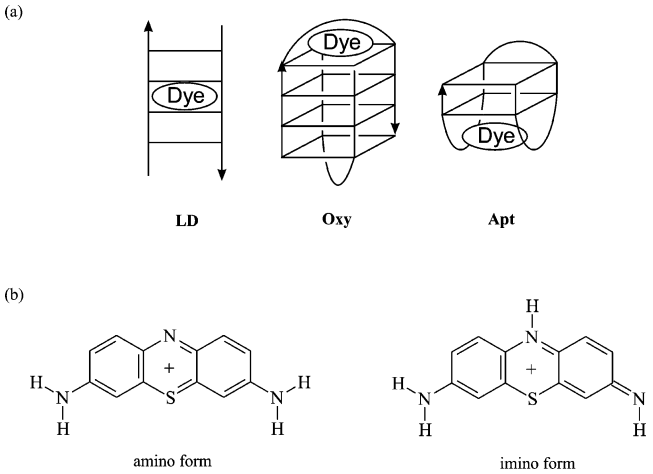


**Figure 1.** (a) Sketches of the investigated DNA strands including the proposed binding sites for thionin. (b) The two tautomeric structures of thionin.

binding modes of thionin to various DNA structures: a linear duplex (LD) of $(d(GC)_6)_2$, a dimeric quadruplex of $(d(G_4T_4G_4))_2$ (Oxy), and a monomeric quadruplex of $d(G_2T_2G_2TGTG_2T_2G_2)$ (Apt). The dye probe we are dealing with in this paper has interesting properties as well. Marek et al.[18] predicted that there are two possible tautomers, the amino and the imino form, as shown in Figure 1b. This view was supported by Weng et al.[19] who found two sets of vibrational modes at a temperature of 6 K in the satellite hole spectra as a function of burning wavelength. They assigned them to these two tautomeric structures.

In this paper we report on pressure-tuning hole-burning experiments using thionin as a probe and various short-stranded DNA chains as complex-forming partners. Pressure-tuning spectroscopy in combination with spectral hole burning is one of the most sensitive spectroscopic techniques for research on probe−solvent interactions.[20−22] Our aim is to characterize the various binding modes of thionin to the DNA molecules via the pressure-tuning response using the free thionin in solution as a reference standard. The investigation of the binding modes may stress the existence of the two tautomeric structures and

† Part of the special issue "Gerald Small Festschrift".

* Corresponding author. E-mail: j.friedrich@lrz.tu-muenchen.de. Fax: ++49 8161 71 4517. Phone: ++49 8161 71 3293.

‡ TU München.

§ Academia Sinica.

10.1021/jp0372993 CCC: $27.50 
Published on Web 03/20/2004

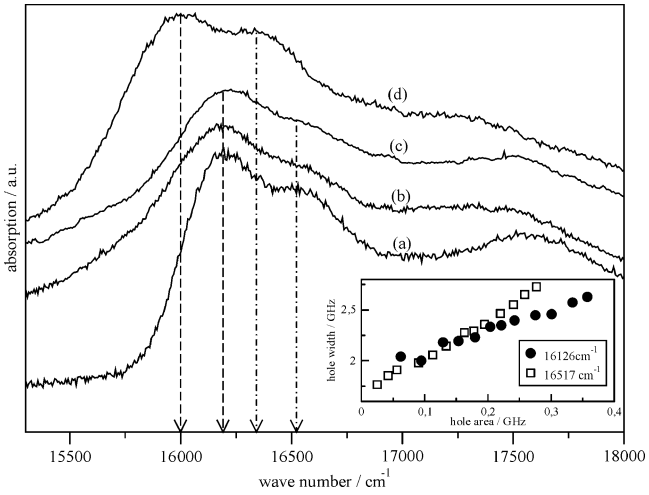


**Figure 2.** Absorption spectra of free thionin in a glycerol/water glass (a), thionin bound to the dimeric hairpin quadruplex (b), thionin bound to the monomeric quadruplex (c), and thionin bound to the linear duplex of GC (d). The spectra are set off for reasons of clarity. Inset: Hole widths as a function of hole area for the free thionin sample at two spectral positions in the two long wavelength peaks.

reveal their specific features with respect to quadruplex and duplex DNA structures.

## Experimental Section

**Materials.** Thionin was purchased from Sigma and used after purification with HPLC. The different samples of DNA were purified by HPLC, dried, and dissolved in 0.01 M Tris/HCl buffer at pH 7.5. To retain their quadruplex structures, 0.15 M NaCl was added to Oxy and Apt. All samples were subsequently mixed with glycerol to obtain a final volume ratio of 4:5 (v/v) $H_2O$/Gly.

**Spectroscopy.** The pressure-tuning experiments were performed at a temperature of 2 K. For hole burning the samples were sealed into plastic bags to ensure isotropic pressure distribution and subsequently fixed in a 2 mm cuvette. Irradiation was done with an argon ion laser pumped dye ring laser operated with sulforhodamine 101. The power density at the sample was around 1 $\mu$W/mm$^2$ for burning and 20 nW/mm$^2$ for reading. Under this condition, hole depths of around 10% burned into the red side gradually decreased to about 2% in the blue side of the inhomogeneous absorption of the free thionin. In the quadruplex structures of Oxy and Apt, holes burned into the blue wing of the inhomogeneous band were even less than 1% in depth. As a consequence an evaluation of the hole shape was impossible because of the poor signal-to-noise ratio. In the duplex structure the power density of the laser had to be increased by at least 2 orders of magnitude to ensure sufficient hole depths for hole burning. All holes were detected in transmission.

Pressure was transmitted via helium gas. The pressure-induced hole shift was measured for pressures up to 1.5 MPa. The pressure-induced broadening, that is, the width of the Gaussian pressure kernel, was not evaluated.

The holes in our experiments are narrow, as shown in the inset of Figure 2. As a consequence, the applied pressure for tuning the hole is quite small. At this pressure level the deformation in the materials investigated is elastic, and it seems rather safe to assume that pressure application only increases the already present probe−solvent interactions by shortening the distances between interacting species by solvent compression. Accordingly, the pressure shift is proportional to the solvent shift (pressure shift−solvent shift model[23]). Along these lines of reasoning, the spectral shift per pressure is given by

$$s_p/\Delta p = 2\kappa\,(\nu_L - \nu_{vac}) \qquad (1)$$

with the isothermal solvent compressibility $\kappa$ and the vacuum absorption wavenumber $\nu_{vac}$. The wavenumber $\nu_L$ characterizes the laser radiation and, hence, determines the wavenumber where hole burning is carried out within the inhomogeneous band. It is obvious from eq 1 that measuring the pressure shift as a function of $\nu_L$ enables the determination of the compressibility $\kappa$ and the vacuum absorption wavenumber $\nu_{vac}$. The factor 2 appears only if the probe−solvent interactions fall off with distance as $R^{-6}$. As to the compressibility, we want to stress two points: First, note that in optical pressure-tuning experiments $\kappa$ is a local quantity, because the pressure sensitive detector is a dye molecule whose interactions with the solvent are very short ranged. Second, $\kappa$ appears in eq 1 as a consequence of the simple pressure shift−solvent shift model.[23] In the present case we focus on relative changes. The absolute numbers are of minor importance.

## Results and Discussion

**Absorption Spectra.** Figure 2 shows the absorption spectra of the investigated systems at 4 K: Thionin in a glycerol/water glass (a), thionin bound to the dimeric hairpin quadruplex (Oxy, b), thionin bound to the monomeric quadruplex (Apt, c), and thionin bound to the linear duplex of GC (LD, d). The spectra albeit clearly different show characteristic common features: The long wavelength band displays a double-peak structure that is also present in the room temperature spectra. In the two quadruplexes this structure is significantly broadened but clearly discernible. The two peaks definitely correspond with two electronic origins as demonstrated by the narrow holes that could be burnt into both of them (cf. the inset). As the hole-burning time increases the area of the hole, that is, the number of burnt molecules, scales with the width of the hole in a linear fashion. Extrapolation to area zero yields twice the value of the homogeneous line width. The inset shows the widths for two holes in the two different bands compared to their areas. Accordingly, the possibility that the higher energy peak of the two may be a vibrational peak can safely be ruled out. The respective homogeneous hole widths are 950 and 700 MHz. The straightforward interpretation is that the two origins correspond to the two proposed tautomeric forms of thionin, the amino and the imino form as shown in Figure 1b. The relation between the two structures and the two peaks, however, is not quite clear, but we believe that the lower energy peak corresponds to the imino form whereas the higher one represents the amino form. This argument is based on the fact that the imino structure can be formed at two equivalent sites because of the respective mirror symmetry. Hence, its population is larger in agreement with the higher intensity. In addition, the conjugation of the $\pi$-electron system is slightly more extended in agreement with the lower absorption energy. Comparative measurements with thionin in PVB films support this notion.[19] In the thionin-quadruplex structures (Oxy and Apt) the two peaks are located at the same wavenumber as for thionin in solution. However, in the thionin-duplex structure a significant red shift of about 200 cm$^{-1}$ occurs. Recently it was suggested that the imino and amino forms correspond to educt and photoproduct. If so, the photoreaction mechanism would be a light-induced proton transfer involving the solvent.[19] Note that both tautomers are equally stable up to room temperature.

**Pressure-Tuning Experiments.** Figure 3 shows the behavior of the holes under increasing pressure up to 1.5 MPa. As an

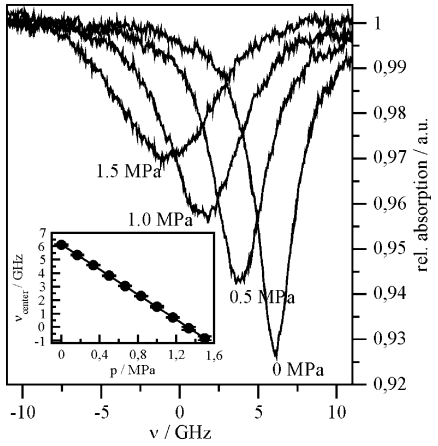


**Figure 3.** Spectral hole in the free thionin sample at 2 K. As pressure increases the hole broadens and shifts to the red side. Inset: The shift of the hole center scales linearly with increasing pressure.

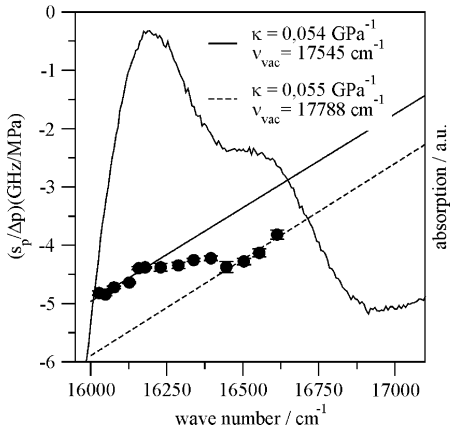


**Figure 4.** Shift of the hole center per pressure as a function of wavenumber for free thionin in a glycerol/water glass at 2 K. Two spectrally overlapping species with similar $\kappa$ are discernible, separated by an intermediate range of compensating pressure shifts. In the background the absorption spectrum of thionin is shown.

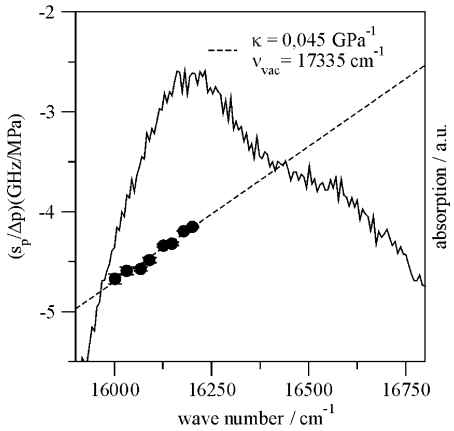


**Figure 5.** Shift of the hole center per pressure as a function of wavenumber for thionin bound to a dimeric hairpin quadruplex (Oxy) at 2 K. Holes of sufficient quality could only be burnt in the red band. Because of the low optical density, the broad band absorption spectrum is rather noisy.

example we present the initial hole as well as three holes under pressure burnt into thionin in a glycerol/water matrix. The inset in Figure 3 demonstrates that the shift of the hole center is perfectly linear with pressure in agreement with eq 1.

**Color Effects and Compressibilities.** Figures 4−7 show pressure-induced hole shifts as a function of burning wave-

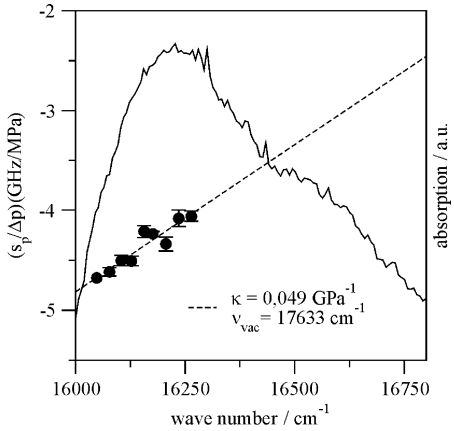


**Figure 6.** Shift of the hole center per pressure as a function of wavenumber for thionin bound to a monomeric quadruplex (Apt) at 2 K. Holes of sufficient quality could only be burnt in the red band. Because of the low optical density, the broad band absorption spectrum is rather noisy.

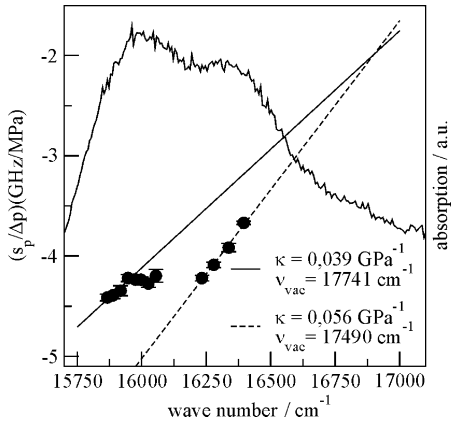


**Figure 7.** Shift of the hole center per pressure as a function of wavenumber for thionin bound to a GC duplex (LD) at 2 K. Two spectrally overlapping species with different $\kappa$ are discernible, separated by an intermediate range of compensating pressure shifts. In the background the absorption spectrum of LD is shown.

number $\nu_L$ within the respective inhomogeneous bands. The data of thionin in a glycerol/water glass appear in Figure 4 together with the inhomogeneous absorption band. The data are typical for two different spectrally overlapping molecular species in a common solvent environment.[24] There are two clearly discernible ranges with a linear color effect. The respective slopes are identical within the error limit yielding a solvent compressibility of $0.055 \pm 0.010$ GPa$^{-1}$ in the short wavelength band and a compressibility of $0.054 \pm 0.008$ GPa$^{-1}$ in the long wavelength band. Within the overlap range of the bands, holes are simultaneously burnt into either of the two forms. Therefore, the pressure-induced shift in this range does not depend on the wavenumber, because the individual shifts along the two straight lines just compensate. The two species have vacuum absorption wavenumbers which differ by about 240 cm$^{-1}$. The absolute solvent shifts $\nu_0 - \nu_{vac}$, with $\nu_0$ being the wavenumber of the band maximum as deduced from the pressure shift−solvent shift model, are huge and total around 1350 cm$^{-1}$.

The data for the quadruplexes (Oxy and Apt) are shown in Figures 5 and 6. Holes with sufficient quality could be burnt in the red band only. The main result from these experiments is that, at least for the imino form, in both cases the data can be compared to the respective ones of thionin in solution. Compared to the free thionin, the compressibilities of $0.045 \pm 0.003$ GPa$^{-1}$ for Oxy and $0.049 \pm 0.07$ GPa$^{-1}$ for Apt are somewhat smaller

by about 10% to 20%. This means that the interaction of the thionin probe with the solvent is partially shielded by the quadruplexes as a result of stacking to the end surface of the quartets. Considering the influence of the diagonal loop and the edge loop in the two quadruplexes, these results are also in agreement with the proposed stacking interaction, as shown in Figure 1a. That the compressibilities show a tendency toward smaller values obviously just reflects the fact that a larger, rather incompressible molecular entity is close to the probe. In addition, from the significant changes in the inhomogeneous widths it seems that the stacking binding of thionin to the two quadruplexes leaves quite a lot of structural freedom. This is probably due to the relative size between the G-quartet and thionin. Quite an interesting observation concerns the fact that despite the clear appearance of thionin binding to the DNA strands there is no shift of the bands. The conclusion is that the binding energy must be the same in the ground and the excited state of the probe. This is the case if the binding originated solely from pure Coulombic interaction. Charges are not affected by exciting an ionic probe, whereas dipole moments, dispersive forces, and hydrogen-bonding strengths are. Thus, pure Coulombic interactions do not contribute to the solvent shift but they may contribute to the dispersion of the solvent shift, that is, the inhomogeneous bandwidth via the ion−dipole interaction. Because thionin as well as the DNA quadruplexes are charged, this must be taken into consideration.

**Intercalation into the Linear DNA-Duplex Structure.** Figure 7 shows the data for the thionin-duplex structure. Hole burning was much more complicated in this case than it was for the other DNA structures. To get sufficiently deep holes the burning power had to be increased by 2 orders of magnitude. As reported in various studies,[25,26] the excited state of thionin is quenched very rapidly when binding near guanine bases occurs, so most of the excited molecules fall back to the ground state instead of undergoing a photoreaction. The influence of pressure is clearly different from the respective spectra obtained for free thionin. Two distinct slopes in the frequency dependence of the pressure shift can be observed, so two different local compressibilities can be assigned to the associated bands. One of the two compressibilities is the same as that in solution with a value of $0.056 \pm 0.004$ GPa$^{-1}$, but the other is significantly lower and yields a value of $0.039 \pm 0.009$ GPa$^{-1}$. The conclusion is that one of the two forms must be shielded to some extent from solvent compression. In line with the results in quadruplexes, this form obviously intercalates into the DNA strand, whereas the other form does not. The hole-burning results also show that the species which absorbs at longer wavelengths has a vacuum wavenumber farther to the blue as compared to the other, quite in contrast to the findings for thionin in solution.

One possibility for interpreting the spectral pattern is to assume that it is the blue-absorbing form of thionin which is able to intercalate into the DNA strand of the duplex. The subsequent red shift is so strong that the two forms "swap" their spectral position. This interpretation gains support through the observation of similar vacuum wavenumbers if the two bands of free thionin and the thionin-duplex structure are compared crosswise. Along these lines of reasoning, and according to our assignment above, the intercalating thionin would correspond to the amino form. If so, the imino form would just experience a rather slight red shift which leaves the overall solvent shift almost unchanged. The fact that despite this "swapping" the relative intensities of the two absorption peaks is not changed significantly could be due to a lower ground state energy of the redmost form, gaining intensity via the Boltzmann factor.

## Conclusions

We have investigated thionin in a glycerol/water glass attached to various oligonucleotides by pressure-tuning hole-burning spectroscopy to gain insights into the nature of DNA binding types. Two different tautomeric forms of thionin could clearly be identified by their narrow hole widths. Although the two forms in the pure solvent show the same local compressibility, deviations from this behavior could be found in the mixtures of thionin and DNA strands. Thionin bound to quadruplex structures such as a dimeric hairpin quadruplex (Oxy) and a monomeric quadruplex (Apt) is partially exposed to the surrounding solvents, which is reflected in a only 10% to 20% lowered local compressibility. This supports the notion that the binding mechanism of thionin to quadruplexes is external stacking. Furthermore, the binding seems to stem solely from Coulombic interaction, since the solvent shift does not change as compared to free thionin. However, a mixture of thionin with a linear duplex of GC features different local compressibilities for the two tautomeric forms of thionin. Although the amino form intercalates into the DNA strand and is therefore shielded from the solvent to a significant degree, the imino form is not intercalated and remains exposed to the solvent.

**Acknowledgment.** Support was granted through DFG (FOR353/2-1, A1), DAAD (D/0212772), and the Fonds der Chemischen Industrie, which is gratefully acknowledged. T.-C.C. acknowledges support from the NSC of the Republic of China.

## References and Notes

(1) Georghiou, S. *Photochem. Photobiol.* **1977**, *26* (1), 59.
(2) Baguley, B. C.; Wakelin, L. P.; Jacintho, J. D.; Kovacic, P. *Curr. Med. Chem.* **2003**, *10* (24), 2643.
(3) Lisgarten, J. N.; Coll, M.; Portugal, J.; Wright, C. W.; Aymami, J. *Nat. Struct. Biol.* **2002**, *9* (1), 57.
(4) Jenkins, T. C. *Curr. Med. Chem.* **2000**, *7* (1), 99.
(5) Perry, P. J.; Jenkins, T. C. *Mini—Rev. Med. Chem.* **2001**, *2* (1), 31.
(6) Kerwin, S. M. *Curr. Pharm. Des.* **2000**, *6*, 441.
(7) Han, H.; Hurley, L. H. *Trends Pharmacol. Sci.* **2001**, *21*, 136.
(8) Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3253.
(9) Perry, P. J.; Read, M. A.; Davies, R. T.; Gowan, S. M.; Reszka, A. P.; Wood, A. A.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1999**, *42*, 2679.
(10) Koeppel, F.; Riou, J. F.; Laoui, A.; Mailliet, P.; Arimondo, P. B.; Labit, D.; Petitgenet, O.; Hélène, C.; Mergny, J. L. *Nucleic Acids Res.* **2001**, *29*, 1087.
(11) Chang, C. C.; Wu, J. Y.; Chien, C. W.; Wu, W. S.; Liu, H.; Kang, C. C.; Yu, L. J.; Chang, T.-C. *Anal. Chem.*, in press.
(12) Kim, N. W.; Piatyszek, M. A.; Prowas, K. R.; Harley, C. B.; West, M. D.; Ho, P. L.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. *Science* **1994**, *266*, 2011.
(13) Kellogg, G. E.; Searsdale J. N.; Fornari, F. A., Jr. *Nucleic Acids Res.* **1998**, *26* (20), 4721.
(14) Baguley, B. C. *Mol. Cell Biochem.* **1982**, *42* (3), 167.
(15) Tuite, E. M.; Kelly, J. M. *J. Photochem. Photobiol. B* **1993**, *21* (2−3), 103.
(16) Wu, J. Y.; Chang, C. C.; Yan, C. S.; Chen, K. Y.; Kuo, I. C.; Mou, C. Y.; Chang, T.-C. *J. Biomol. Struct. Dyn.* **2003**, *21* (1), 135.
(17) Tuite, E. M.; Kelly, J. M. *Biopolymers* **1995**, *35*, 419.
(18) Marek, N.; Kunsagi-Mate, S.; Szabo, K. *J. Mol. Struct. (THEOCHEM)* **1994**, *315*, 187.
(19) Weng, K. C.; Chiang, C. C.; Cheng, J. Y.; Cheng, S. Y.; Personov, R. I.; Chang, T.-C. *Chem. Phys. Lett.* **1999**, *302*, 347.
(20) Yang, Y. P.; Chang, T.-C.; Stübner, M.; Friedrich, J. *J. Phys. Chem. B* **2003**, *107*, 1458.
(21) Stübner, M.; Hecht, C.; Friedrich, J. *Biophys. J.* **2002**, *83*, 3553.
(22) Köhler, M.; Friedrich, J.; Fidy, J. *Biochim. Biophys. Acta—Protein Struct. Mol. Enzymol.* **1998**, *1386*, 255.
(23) Laird, B. B.; Skinner, J. L. *J. Chem. Phys.* **1989**, *90*, 3274.
(24) Friedrich, J.; Gafert, J.; Zollfrank, J.; Vanderkooi, J.; Fidy, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1029.
(25) Dohno, C.; Stemp, E. D.; Barton, J. K. *J. Am. Chem. Soc.* **2003**, *125* (32), 9586.
(26) Reid, G. D.; Whittaker, D. J.; Day, M. A.; Creely, C. M.; Tuite, E. M.; Kelly, J. M.; Beddard, G. S. *J. Am. Chem. Soc.* **2001**, *28*, 6953.