

# Binding of ethidium to DNA measured using a 2D diffusion-modulated gradient COSY NMR experiment

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**Abstract** The binding of ethidium bromide to a DNA hairpin (dU<sub>5</sub>-hairpin) was investigated using a novel 2D diffusion-modulated gradient correlation spectroscopy (DMG-COSY) experiment to evaluate the applicability of this technique for studying the binding of drugs to DNA. The DMG-COSY experiment includes a preparation period during which coherent magnetization is attenuated due to molecular self-diffusion. Magnetization then evolves due to scalar coupling during an evolution delay, and is detected using gradient pulses for coherence selection. The time-domain data are processed in an analogous manner as for gradient-selected COSY experiments. The diffusion coefficient for uridine in DMSO solution was determined from the H5–H6 crosspeak intensities for a series of 2D DMG-COSY experiments that differed in the magnitude of the gradient pulses applied during the preparation period of the DMG-COSY experiment. The diffusion coefficient for uridine calculated from the DMG-COSY experiments was identical (within experimental error) to that determined from 1D diffusion experiments ( $5.24 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C). The diffusion coefficients for ethidium bromide and for the dU<sub>5</sub>-hairpin were first measured separately using the DMG-COSY experiment, and then measured in the putative complex. The diffusion coefficient for free ethidium bromide ( $4.15 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C) was considerably larger than for the dU<sub>5</sub>-hairpin ( $1.60 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C), as expected for the smaller molecule. The diffusion coefficient for ethidium was markedly decreased upon addition of the dU<sub>5</sub>-hairpin, consistent with complex formation ( $1.22 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C). Complex formation of 1:1 stoichiometry between ethidium and the stem of the dU<sub>5</sub>-hairpin was verified independently by fluorescence spectroscopy. These results demonstrate the utility of the DMG-COSY experiment for investigating the binding of drugs to DNA in aqueous solution.

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**Key words:** Diffusion; Two-dimensional nuclear magnetic resonance; Drug–DNA interaction; Bipolar pulse pair–longitudinal encode/decode

## 1. Introduction.

NMR (nuclear magnetic resonance) spectroscopy has proven to be a valuable tool for determining the three-dimensional structures of nucleic acid fragments and proteins in solution. In particular, NMR spectroscopy has played an im-

portant role in elucidating the structural basis for the recognition of duplex DNA by ligands that bind with sequence selectivity to duplex DNA [1]. Although the determination of molecular structures remains one of the most important objectives in biomolecular NMR spectroscopy, solution NMR studies of macromolecules have increasingly focused on understanding molecular dynamics and measuring molecular self-diffusion [2,3]. Information concerning molecular dynamics from NMR relaxation measurements and information concerning molecular shape from NMR measurements of self-diffusion complement the structural information derived predominantly from NOE (nuclear Overhauser effect) experiments to provide a comprehensive understanding of molecular structure and dynamics in solution [4]. In the present article we describe a novel 2D NMR experiment, DMG-COSY (diffusion-modulated gradient correlation spectroscopy) that utilizes the familiar COSY-type presentation of crosspeaks arising from scalar coupled <sup>1</sup>H resonances to determine the self-diffusion of molecules in solution.

The process of drug discovery has, in recent years, evolved from rational modification of naturally occurring substances that have demonstrable (albeit low) affinities for target molecules, to one of natural selection from large pools of molecules that display vast structural diversity (combinatorial chemistry). The role of NMR spectroscopy in drug discovery has adapted to accommodate this evolution in the drug discovery process. For example, Fesik and co-workers have developed the procedure of ‘SAR (structure–activity relationships) by NMR’ to identify ligands that bind weakly to proteins, and to identify the protein residues implicated in binding [5,6]. Other NMR technologies have also been developed to support the increasing emphasis on combinatorial approaches to drug design and development. These new methods include the acquisition of NMR spectra with minute quantities of sample, and the use of ‘flow probes’ to acquire NMR data on samples without requiring sample transfer to a sample tube [7,8].

The utility of the ‘SAR by NMR’ approach for identifying ligands that bind to nucleic acids, rather than proteins, with high affinity and selectivity is hampered by a lack of routine synthetic and biosynthetic approaches for preparing <sup>15</sup>N- and <sup>13</sup>C-labeled nucleic acids [9,10]. Ligand binding frequently does not induce substantial changes in <sup>1</sup>H chemical shifts, while accessing the wider dispersion of either <sup>13</sup>C or <sup>15</sup>N nuclei requires either isotopic enrichment or long acquisition times that are incompatible with rapid screening. For these reasons, NMR methods that detect changes in the self-diffusion of a putative ligand upon binding to a nucleic acid frag-

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ment may be preferred to NMR methods that detect chemical shift differences that occur upon complex formation for identifying ligands with high affinity for the nucleic acid target. Measurements of the self-diffusion of molecules using PFG-NMR (pulsed field gradient NMR) have become increasingly common with the relatively routine inclusion of pulsed field gradient amplifiers and probes with newer NMR systems [11–14]. These studies frequently utilize a single echo concept in which self-diffusion during the time interval between field gradient pulses results in attenuation of coherent magnetization [15]. Modifications have been introduced to this basic echo concept that reduce attenuation of coherent magnetization due to transverse relaxation and field inhomogeneities [16].

The characterization of ligand binding to nucleic acids using one-dimensional PFG-NMR techniques, such as BPP-LED (bipolar pulse pair–longitudinal encode/decode), is frequently limited by spectral overlap. Many of the ligands that bind to nucleic acids are comprised of one or more aromatic rings that intercalate between the DNA bases [1]. The  $^1\text{H}$  resonances from the aromatic rings of these intercalating moieties frequently are not well-resolved from the  $^1\text{H}$  resonances of the aromatic bases of DNA. The resolution of distinct  $^1\text{H}$  resonances that overlap in one-dimensional NMR spectra is frequently made possible via scalar coupling interactions to non-overlapped partners using 2D COSY experiments. The COSY-type display of 2D NMR spectra is familiar to most individuals using NMR spectroscopy in drug development research. Thus, a 2D NMR experiment with a COSY-type display for measurement of diffusion coefficients would provide a practical and familiar tool for researchers interested in studying ligand binding to nucleic acids using NMR spectroscopy. We have developed such a 2D NMR experiment, the DMG-COSY (Fig. 1), in which the intensity of the crosspeaks in the 2D COSY-type spectrum is attenuated by molecular self-diffusion in an identical manner to that accomplished in a 1D BPP-LED experiment. In the present article, we demonstrate the utility of this technique by investigating the binding of ethidium bromide to a DNA hairpin.

## 2. Materials and methods

### 2.1. Sample preparation

The oligodeoxynucleotide (ODN) 5' dGCGCUUUUUGCGC (dU<sub>5</sub>-hairpin) was synthesized by the Molecular Biology core facility at the UNMC/Eppley cancer center. The dU<sub>5</sub>-hairpin was HPLC purified and desalted by column chromatography. The concentration of ODN solutions was determined at 260 nm and 90°C using the extinction coefficient of  $116\text{ mM}^{-1}\text{cm}^{-1}$ . These values were calculated by extrapolation of the tabulated values of the dimers and monomer bases at 25°C to high temperatures [17]. Ethidium bromide was purchased from Sigma and used without further purification. Concentrations of ethidium were determined at 480 nm and room temperature using the extinction coefficient of  $5850\text{ M}^{-1}\text{cm}^{-1}$ . All experiments were done in 10 mM sodium phosphate buffer, adjusted to pH 7. For NMR experiments, the samples were lyophilized from this buffer and re-dissolved in 100% D<sub>2</sub>O.

### 2.2. Temperature-dependent UV spectroscopy

Absorbance versus temperature profiles (melting curves) in appropriate solution conditions were measured at 260 nm with a thermoelectrically controlled AVIV 14-DS spectrophotometer. The temperature was scanned at a constant heating rate of 0.5°C/min. Melting curves were carried out as a function of strand concentration. The analysis of the melting curves, using standard procedures, allowed measurement of transition temperatures,  $T_M$ , and van't Hoff enthalpies,  $\Delta H_{\text{vH}}$  [18].

### 2.3. Differential scanning calorimetry

Excess heat capacities as a function of temperature were measured using a Microcal MC-2 differential scanning calorimeter [19,20]. Two cells, the sample cell containing 1.5 ml of ODN solution, and the reference cell filled with buffer solution, were heated from 10 to 100°C at a rate of 0.75°C/min. Analysis of the resulting thermograms allowed calculation of thermodynamic profiles ( $\Delta H$ ,  $\Delta S$  and  $\Delta G$ ), and model-dependent enthalpies,  $\Delta H_{\text{vH}}$ , of the helix–coil transition of the DNA hairpin [18].

### 2.4. Isothermal titration calorimetry

The heat of interaction of ethidium bromide with the dU<sub>5</sub>-hairpin was measured directly by titration calorimetry using an Omega calorimeter from Microcal Inc. The ethidium solution used was 50-fold higher in concentration than the dU<sub>5</sub>-hairpin solution in the reaction cell. Complete mixing of reagents was accomplished by stirring of the syringe paddle at 400 rpm. The reference cell of the calorimeter was filled with water, and the instrument was calibrated by means of a known standard electrical pulse. Twenty-three injections of 5  $\mu\text{l}$  each were performed in a single titration. The heat measured by this procedure was corrected for the dilution heat of the ligand, and normalized by the concentration of added titrant, to obtain the binding enthalpy. The precision of the resulting heat of each injection was less than 0.5  $\mu\text{cal}$ . Analysis of the resulting binding isotherm allowed calculation of the binding enthalpy, binding affinities, and the stoichiometry of the resulting complexes [19,20].

### 2.5. Fluorometric job plots

The stoichiometry of the ethidium–dU<sub>5</sub> DNA complex(es) was determined initially by fluorescence, using an AVIV ATF-105 spectrofluorometer. Solutions of the complex were excited at 512 nm using a slit of 2 nm for both excitation and emission. Emission spectra were taken between 520 and 700 nm, and stoichiometries were determined using the method of continuous variations in which the dU<sub>5</sub>-hairpin solution was titrated with ethidium at constant total concentration of dU<sub>5</sub>-hairpin and ethidium. Complex formation was determined from the change in emission at 600 nm as a function of molar fraction of ethidium. The intersection of the two lines of intensity versus molar fraction of ethidium represents molar fraction values that correspond to the stoichiometry of the complex [19–21].

### 2.6. NMR spectroscopy

PFG-NMR self-diffusion measurements were made using a Varian Unity 500 spectrometer equipped with a 3 mm ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ )  $z$ -gradient, triple-resonance probe. The depth of the samples for PFG-NMR analysis was maintained at 1 cm using D<sub>2</sub>O-matched susceptibility plugs (Doty Scientific), to ensure that the entire sample experienced the same gradient in magnetic field strength. Diffusion measurements were collected at 26°C. The BPP-LED experiment was used to minimize the effects of eddy current recovery time during 1D PFG-NMR measurements of diffusion [16]. For each BPP-LED NMR experiment, 4000 data points were collected over an 8 kHz spectral window [22]. Gradient field strengths (0.5–15 Gauss/cm for  $^1\text{H}$ ) were calibrated by fitting the integrated  $^1\text{H}$  signal intensities from residual HDO in each sample to Eq. 1, and back-calculating  $g$ . Evolution times ( $\Delta$ ) of 0.05–0.10 s were used for the  $^1\text{H}$ -observed experiments. 2D DMG-COSY experiments were acquired using identical parameters for the BPP-LED element as were used in the 1D NMR experiments. Spectral windows of 8 kHz were used in both the direct and the indirect dimensions of the DMG-COSY experiment with 2000 points collected in  $t_2$  and 128 points collected in  $t_1$ . Data were zero-filled to 1000 in the  $t_1$  dimension and were apodized in both dimensions using squared sinebells.

## 3. Results and discussion

### 3.1. BPP-LED measurement of uridine self-diffusion

The BPP-LED experiment is among the pulse sequences most frequently used for the measurement of molecular self-diffusion. The pulse sequence for the BPP-LED experiment is shown in Fig. 1. The experiment encodes the longitudinal position of each molecule in the sample with two  $z$ -gradient pulses of opposite phase that are applied to transverse mag-

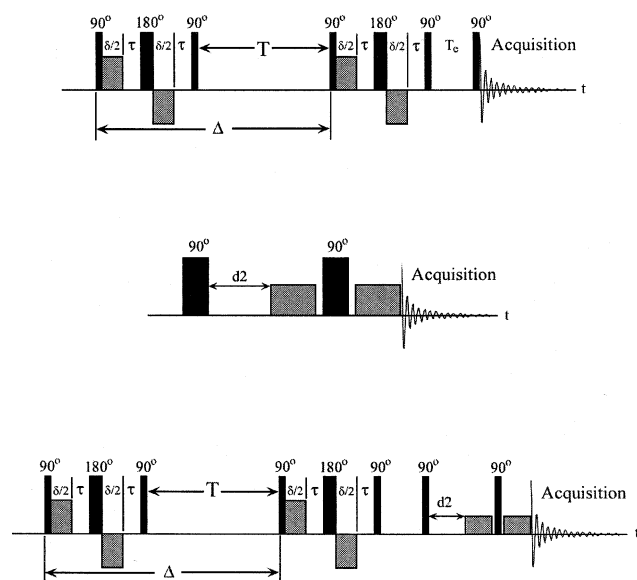


Fig. 1. Pulse sequences for the BPP-LED one-dimensional NMR experiment for measuring diffusion coefficients (top) [16] and the gCOSY two-dimensional NMR experiment for identifying  $^1\text{H}$  pairs connected via scalar coupling (center). In the bottom of the figure is the pulse sequence for the DMG-COSY described in this article. Crosspeaks are apparent in DMG-COSY spectra for  $^1\text{H}$  pairs connected via scalar coupling as in the gCOSY experiment, but the intensity of the crosspeak is modulated by molecular self-diffusion during the BPP-LED preparation period of the DMG-COSY experiment.

netization either prior to, or subsequent to, a  $180^\circ$  pulse. The resulting spatially encoded coherent magnetization is then stored along the  $z$ -axis during a time period for diffusion ( $T$  in Fig. 1) by application of a  $90^\circ$  pulse. Following the diffusion period, an identical procedure to that used to spatially encode the magnetization is applied to the sample to homogenize the phase of the remaining coherent magnetization. Following a final time period allowing eddy currents to dissipate ( $T_e$  in Fig. 1), transverse magnetization is generated by a final  $90^\circ$  pulse, and a 1D NMR spectrum is acquired and processed in the usual manner. Using this pulse sequence, the amplitude of the observed PFG-NMR signal can be expressed as:

$$A = A_0 \exp[-\gamma^2 g^2 \delta^2 D (\Delta - \delta/3 - \tau/2)] \quad (1)$$

where  $A_0$  is the signal in the absence of gradient pulses,  $\gamma$  is the gyromagnetic ratio ( $^1\text{H} = 2.675197 \times 10^4 \text{ G}^{-1}\text{s}^{-1}$ ),  $g$  and  $\delta$  are the magnitude and duration of the PFG pulses, and  $\Delta$  is the total evolution time. The self-diffusion coefficient for a 15 mM sample of uridine in DMSO was determined from a series of 1D BPP-LED experiments. The signal intensity for the H6  $^1\text{H}$  resonance of uridine as a function of increasing gradient strength is shown in Fig. 2. The ratio of the integral values of the H6 resonance in the presence and absence of field gradients was fit by least squares linear regression according to Eq. 1, to obtain the diffusion coefficient. The self-diffusion coefficient for uridine measured using the data from the BPP-LED experiments was  $5.24 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $26^\circ\text{C}$ .

### 3.2. DMG-COSY measurement of uridine self-diffusion

The self-diffusion of uridine was also measured using a 2D DMG-COSY experiment. The pulse sequence for the DMG-

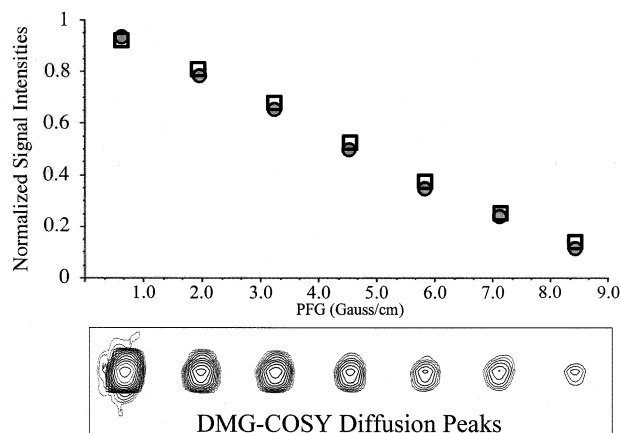


Fig. 2. The normalized signal intensities for the H6 resonance of uridine measured using BPP-LED experiments (filled circles) and for the H5–H6 crosspeak of uridine measured using DMG-COSY experiments (open squares). Gradient pulses of the indicated values were used in both experiments. The H5–H6 crosspeaks (5.72–7.96 ppm) from uridine from each of the DMG-COSY experiments are shown underneath the value of the gradient pulse used in the preparation period of the DMG-COSY experiment.

COSY experiment is shown in Fig. 1. The DMG-COSY experiment uses the same strategy as the BPP-LED experiment for phase encoding and decoding the coherent magnetization according to the longitudinal position of molecules in the sample. Rather than acquiring a 1D NMR spectrum following the final  $90^\circ$  pulse of the BPP-LED element however, the magnetization in the DMG-COSY experiment is allowed to evolve further according to the Hamiltonian for scalar coupling during a variable evolution period  $d_2$  (Fig. 1). At the end of the COSY evolution period, a gradient pulse is applied to encode the antiphase magnetization of interest that had de-

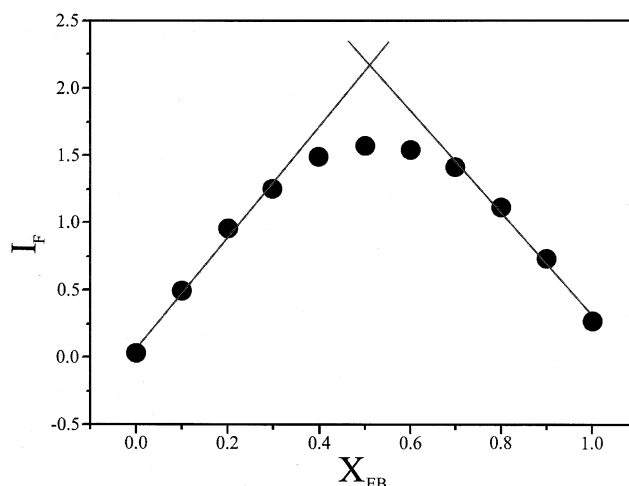


Fig. 3. Fluorometric job plot of the binding of ethidium to dU<sub>5</sub>-hairpin DNA. Complex formation was determined from the change in emission at 600 nm as a function of molar fraction of ethidium. The intersection of the two lines at a molar fraction of 0.5 is consistent with formation of a complex of one ethidium to each dU<sub>5</sub>-hairpin. Thermodynamic parameters derived from isothermal titration calorimetry show that this tight binding of 1:1 stoichiometry occurs to the stem region of the dU<sub>5</sub>-hairpin. Weaker binding of ethidium to the loop region of the dU<sub>5</sub>-hairpin also occurs with 2:1 stoichiometry.

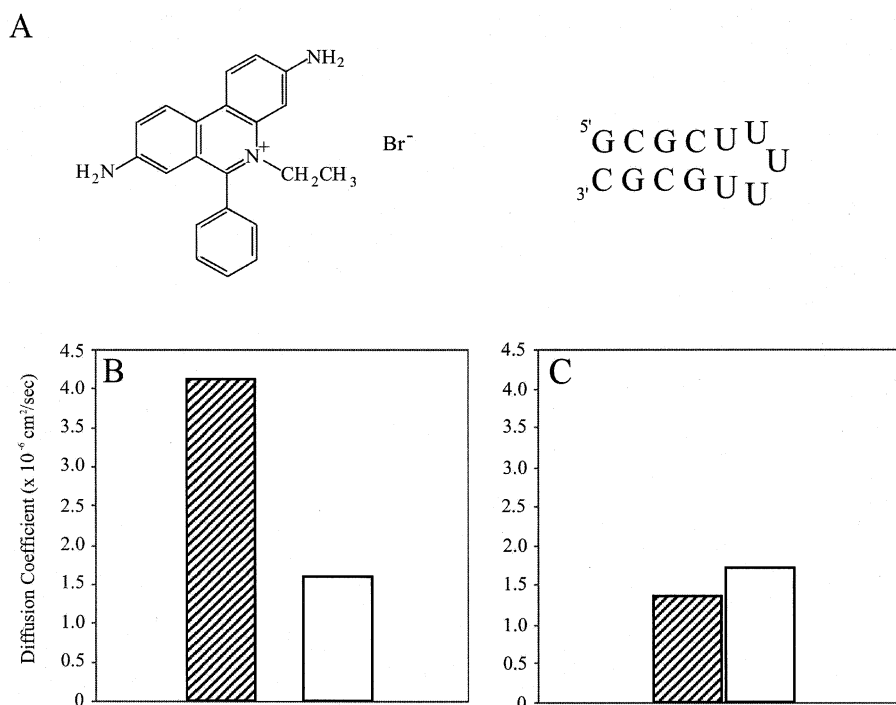


Fig. 4. A: The chemical structure of ethidium bromide and the secondary structure for the dU<sub>5</sub>-hairpin considered in the present study. B: Diffusion coefficients for free ethidium and for the dU<sub>5</sub>-hairpin calculated from fitting the intensities of crosspeaks in the DMG-COSY spectra. C: Diffusion coefficients for ethidium and for the dU<sub>5</sub>-hairpin in a sample of 1:1 stoichiometry. Diffusion coefficients were calculated from fitting the intensities of crosspeaks in DMG-COSY spectra.

veloped during the evolution period. The final 90° pulse of the DMG-COSY experiment interconverts the longitudinal and transverse components of the antiphase magnetization of interest, and this coherence is selected for by application of a final gradient pulse prior to data acquisition. The FID collected is then subjected to two successive Fourier transformations resulting in a spectrum with the appearance of a 2D COSY experiment. Crosspeaks in the DMG-COSY experiment differ from crosspeaks in a gradient-selected COSY experiment in that the intensity of the crosspeaks is not only a function of the magnitude of the scalar coupling between the two spins, but is also a function of the same variables that attenuate the signal intensity of 1D BPP-LED experiments, notably the self-diffusion coefficient. The results of applying the DMG-COSY to a 15 mM sample of uridine in DMSO solution are shown in Fig. 2. The intensity of the crosspeak for the H5–H6 interaction of uridine was modulated by the magnitude of the gradient pulses applied during the BPP-LED preparation period of the DMG-COSY experiment exactly as the 1D intensities varied in the BPP-LED experiment. The diffusion coefficient for uridine calculated from the DMG-COSY experiment is  $5.23 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C, within experimental error of the value calculated for uridine from the 1D BPP-LED experiment.

### 3.3. Helix–coil transition of dU<sub>5</sub> DNA

The exclusive formation of a monomolecular hairpin for the sequence 5' dGCGCUUUUGCGC (dU<sub>5</sub>-hairpin) was characterized by checking if the transition temperature,  $T_M$ , for the unfolding of this molecule, was independent of strand concentration. UV and calorimetric melting curves were per-

formed in 10 mM sodium phosphate buffer using strand concentrations of 6–600 μM. The melting of the dU<sub>5</sub>-hairpin occurred in broad monophasic transitions for both optical and calorimetric observables to yield a  $T_M$  of 67.3°C, independent of strand concentration. This result confirmed the formation of a single-stranded hairpin at low temperatures. Further, a calorimetric unfolding heat of 36.9 kcal/mol and van't Hoff enthalpies of 37 kcal/mol was obtained for the dU<sub>5</sub>-hairpin. A  $\Delta H_{vH}/\Delta H_{cal}$  value of 1.03 was obtained indicating that this hairpin melts in a two-state transition. These results are consistent with previous findings of similar DNA hairpins containing thymine loops [19].

### 3.4. Stoichiometry of the ethidium–hairpin complex

The stoichiometry of the ethidium–hairpin complex was determined initially by fluorescence spectroscopy using the continuous variation approach in which solutions of the hairpin and ethidium (Fig. 4A) were prepared at constant total concentration of ethidium and dU<sub>5</sub>-hairpin (equal to 7 μM). The intensity of ethidium emission was measured at 600 nm, with an excitation wavelength of 512 nm. The intersection of the two lines in the resulting job plot corresponds to molar fraction values that yield the stoichiometry of the complex(es) (Fig. 3). The two lines intercept at  $x=0.5$  consistent with complex formation having a stoichiometry of one ethidium molecule per hairpin molecule.

### 3.5. Thermodynamic parameters of ethidium binding to the hairpin

The interaction of ethidium with the dU<sub>5</sub>-hairpin was also investigated by isothermal titration calorimetry (ITC), using

concentrations of 10  $\mu\text{M}$  and 524  $\mu\text{M}$  for the dU<sub>5</sub>-hairpin and ethidium (titrant), respectively. Thermodynamic parameters were determined by fitting the resulting binding isotherm (total heat versus concentration of added ethidium). For the fitting procedure, a binding model of two sets of independent binding sites (stem and loop) was considered. At 25°C, the interaction of ethidium with the dU<sub>5</sub>-hairpin was characterized by exothermic heats of  $-9.4$  and  $-7.0$  kcal/mol, binding affinities of  $5.9 \times 10^6$  and  $1.1 \times 10^5$  M<sup>-1</sup>, and stoichiometries of one and two ligands per molecule, for the stem and loop, respectively. These results are in agreement with previous studies [19,20], and indicate that a single equivalent of ethidium binds preferentially to the stem of the dU<sub>5</sub>-hairpin with 1:1 stoichiometry and micromolar affinity.

### 3.6. DMG-COSY measurement of ethidium binding to DNA

The applicability of the DMG-COSY experiment for measuring the binding of drugs to DNA was assessed by monitoring the binding of ethidium bromide to the dU<sub>5</sub>-hairpin (Fig. 4A). The binding stoichiometry for this system was established using fluorescence spectroscopy as being tight binding of one molecule of ethidium bromide to the stem region of each molecule of the dU<sub>5</sub>-hairpin (Fig. 3). The self-diffusions of ethidium bromide and of the dU<sub>5</sub>-hairpin were first each investigated separately. The diffusion coefficients for free ethidium bromide and the dU<sub>5</sub>-hairpin calculated from the DMG-COSY experiments are shown in Fig. 4B. The values for self-diffusion of ethidium bromide were obtained from averaging the diffusion coefficients obtained from analysis of intensity data for two crosspeaks. The values determined from each crosspeak were within 10% of each other. For the dU<sub>5</sub>-hairpin, five H6–H5 crosspeaks were analyzed and the diffusion coefficients obtained were within 3% of each other. The greater uncertainty in the diffusion coefficient for ethidium bromide probably results from the proximity of both crosspeaks used in the analysis to the diagonal, while the H6–H5 crosspeaks analyzed for the dU<sub>5</sub>-hairpin were well-resolved from the diagonal. As expected, the relatively low molecular weight ethidium bromide molecule diffuses much more rapidly than the larger dU<sub>5</sub>-hairpin ( $4.15 \times 10^{-6}$  cm<sup>2</sup>/s vs.  $1.60 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C). Upon mixing the ethidium bromide and the dU<sub>5</sub>-hairpin together, the diffusion coefficient calculated from the DMG-COSY data for the ethidium bromide crosspeak is markedly reduced ( $1.22 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C), while that for the dU<sub>5</sub>-hairpin is very slightly increased ( $1.69 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C). The slight increase in the diffusion coefficient for the dU<sub>5</sub>-hairpin in the complex with ethidium, relative to the free hairpin, probably results from the DNA adopting a more compact and more rigid conformation in the complex. The slightly lower value for ethidium in the 1:1 complex, relative to the hairpin, probably results from the greater uncertainty in the self-diffusion coefficients calculated for ethidium due to the proximity of the two crosspeaks evaluated relative to the diagonal, although dynamics of complex dissociation may also be a factor. The values for the diffusion coefficients for ethidium complexed to the dU<sub>5</sub>-hairpin calculated from the DMG-COSY data are shown in Fig. 4C. These results are consistent with the fluorescence data that indicate that one molecule of ethidium bromide binds tightly to the stem region of the dU<sub>5</sub>-hairpin. These results demonstrate the utility of the DMG-COSY experiment for investigating the binding of drugs to DNA in solution.

## 4. Conclusions

A novel 2D DMG-COSY experiment was developed to measure diffusion rates from crosspeak intensities using a 2D COSY-type experiment rather than from a 1D BPP-LED experiment. The self-diffusion coefficient of uridine measured using the DMG-COSY experiment was identical within experimental error to that measured from 1D BPP-LED experiments ( $5.24 \times 10^{-6}$  cm<sup>2</sup>/s at 26°C). The applicability of the DMG-COSY experiment for investigating interactions between drugs and DNA was evaluated using the binding of ethidium bromide to dU<sub>5</sub>-hairpin DNA. Data from fluorescence spectroscopy showed that the stoichiometry of binding in this system was 1:1. DMG-COSY data demonstrated that the self-diffusion of ethidium bromide was markedly decreased upon addition of the dU<sub>5</sub>-hairpin DNA, and that both ethidium and the dU<sub>5</sub>-hairpin had nearly identical diffusion coefficients. These results are consistent with ethidium binding to dU<sub>5</sub>-hairpin DNA with 1:1 stoichiometry. These results demonstrate the applicability of the DMG-COSY system for investigating drug–DNA interactions.

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