

## DSC XV: Nucleic Acid –Small Molecule Interactions

Barcelo F., Ortiz-Lombardia M., and Portugal J. (2001) Heterogeneous DNA binding modes of berenil. *Biochim Biophys Acta* **1519**, 175-184.

**Abstract:** Isothermal titration calorimetry (ITC) profiles of berenil bound to different DNAs show that, despite the strong preference of berenil for AT-rich regions in DNA, it can bind to other DNA sequences significantly. The ITC results were used to quantify the binding of berenil, and the thermodynamic profiles were obtained using natural DNAs as well as synthetic polynucleotides. ITC binding isotherms cannot be simply described when a single set of identical binding sites is considered, except for poly[d(A-T)<sub>2</sub>]. Ultraviolet melting of DNA and differential scanning calorimetry were also used to quantify several aspects of the binding of berenil to salmon testes DNA. We present evidence for secondary binding sites for berenil in DNA, corresponding to G+C rich sites. Berenil binding to poly[d(G-C)<sub>2</sub>] is also observed. Circular dichroism experiments showed that binding to GC-rich sites involves drug intercalation. Using a molecular modeling approach we demonstrate that intercalation of berenil into CpG steps is sterically feasible.

Barcelo F., Capo D., and Portugal J. (2002) Thermodynamic characterization of the multivalent binding of chartreusin to DNA. *Nucleic Acids Res* **30**, 4567-4573.

**Abstract:** Characterization of the thermodynamics of DNA- drug interactions is a very useful part in rational drug design. Isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and UV melting experiments have been used to analyze the multivalent (intercalation plus minor groove) binding of the antitumor antibiotic chartreusin to DNA. Using DNA UV melting studies in the presence of the ligand and the binding enthalpy determined by ITC, we determined that the binding constant for the interaction was  $3.6 \times 10^5 \text{ M}^{-1}$  at 20 degrees C, in a solution containing 18 mM Na(+). The DNA-drug interaction was enthalpy driven, with a  $\Delta H(b)$  of -7.07 kcal/mol at 20 degrees C. Binding enthalpies were determined by ITC in the 20-35 degrees C range and used to calculate a binding-induced change in heat capacity ( $\Delta C_p$ ) of -391 cal/mol K. We have obtained a detailed thermodynamic profile for the interaction of this multivalent drug, which makes possible a dissection of  $\Delta G(\text{obs})$  into the component free energy terms. The hydrophobic transfer of the chartreusin chromophore from the solution to the DNA intercalating site is the main contributor to the free energy of binding.

Barcelo F., Scotta C., Ortiz-Lombardia M., Mendez C., Salas J. A. and Portugal J. (2007) Entropically-driven binding of mithramycin in the minor groove of C/G-rich DNA sequences. *Nucleic Acids Res* **35**, 2215-2226.

**Abstract:** The antitumour antibiotic mithramycin A (MTA) is a DNA minor-groove binding ligand. It binds to C/G-rich tracts as a dimer that forms in the presence of divalent cations such as Mg(2+). Differential scanning calorimetry, UV thermal denaturation, isothermal titration calorimetry and competition dialysis were used, together with computations of the hydrophobic free energy of binding, to determine the thermodynamic profile of MTA binding to DNA. The results were compared to those obtained in parallel using the structurally related mithramycin SK (MSK). The binding of MTA to salmon testes DNA determined by UV melting studies ( $K(\text{obs}) = 1.2 (+/-0.3) \times 10(5) \text{ M}(-1)$ ) is tighter than that of MSK ( $2.9 (+/-1.0) \times 10(4) \text{ M}(-1)$ ) at 25 degrees C. Competition dialysis studies showed a tighter MTA binding to both salmon testes DNA (42% C + G) and *Micrococcus lysodeikticus* DNA (72% C + G). The thermodynamic analysis of binding data at 25 degrees C shows that the binding of MTA and MSK to DNA is entropically driven, dominated by the hydrophobic transfer of the antibiotics from solution to the DNA-binding site. Direct molecular recognition between MTA or MSK and DNA through hydrogen bonding and van der Waals contacts may also contribute significantly to complex formation.

Bear S. and Remers W. A. (1996) Computer simulation of the binding of amonafide and azonafide to DNA. *J Comput Aided Mol Des* **10**, 165-175.

**Abstract:** Intercalative binding of the antitumor drugs amonafide and azonafide to the oligonucleotide duplex d(GGCCGGCCGG).d(CCGGCCGGCC) was compared using molecular dynamics in vacuum with the AMBER force field. A number of reasonable possible binding conformations were obtained, with the azonafide complexes favored over the amonafide complexes in net binding enthalpy. In comparison with amonafide, the larger chromophore of azonafide permits greater DNA distortion and wider side-chain swings, without falling out of the intercalation site. The best model obtained was used for further dynamics

on amonafide and azonafide with solvent and counterions present, and again the azonafide complex had a more favorable enthalpy. Furthermore, the enthalpy change on going from solvent into the intercalation site was less unfavorable for azonafide. These results are consistent with the stronger DNA binding of azonafide compared to amonafide, as observed in relative melting transition temperature increases and tumor inhibition in cell cultures.

Berezniak E. G., Kruglova E. B., Khrebtova A. S., Dukhopel'nikov E. V. and Zinchenko A. V. (2007) [Interaction of actinocin derivative with different poly(rC) structures]. *Biofizika* **52**, 812-821.

**Abstract:** The interaction of actinocin derivative Act III with single- and double-stranded poly(rC) has been investigated by the methods of differential scanning microcalorimetry and UV-vis absorption spectroscopy. It was shown that, after the addition of the ligand, the temperature, enthalpy and entropy of poly (rC) melting decrease. The analysis of poly(rC)-ActIII absorption spectra indicated that the conformation of polynucleotide differs from that of free poly (rC) in the presence of ActIII at pH 4.46 and pH 6.0. Using the DALSMOD optimization program, the parameters of interaction of Act III with poly (rC) were calculated. It was found that the binding constant of ActIII with double-stranded poly (rC) is essentially higher than that with the single-stranded one upon monomeric binding. On the basis of these data, we conclude that the conformation changes of the matrix are the main cause of the decrease in melting temperature and enthalpy observed by calorimetry. Possible mechanisms of interaction of actinocin derivative with poly (rC) are discussed.

Bhadra K., Maiti M. and Kumar G. S. (2008) DNA-Binding Cytotoxic Alkaloids: Comparative Study of the Energetics of Binding of Berberine, Palmatine, and Coralyne. *DNA Cell Biol (epublication)*.

**Abstract:** Deoxyribonucleic acid, the genetic material of living cells, is the site of storage and retrieval of information through interaction with proteins and other small molecules. In the present study, the interaction of two natural cytotoxic protoberberine plant alkaloids, berberine and palmatine, and a synthetic derivative, coralyne, with mammalian herring testis DNA was investigated using a combination of isothermal titration calorimetry, differential scanning calorimetry, and optical melting experiments to characterize the energetics of their binding. The binding constants of these alkaloids to DNA under identical conditions were evaluated from the UV melting data, and the enthalpy of binding was elucidated from isothermal titration studies. Under identical conditions, the binding constants of berberine, palmatine, and coralyne to DNA were found to be  $1.15 \times 10^4$ ,  $2.84 \times 10^4$ , and  $3.5 \times 10^6$  M<sup>-1</sup> at 20 degrees C in buffer of 20 mM [Na<sup>+</sup>]. Parsing of the free energy change of the interaction observed into polyelectrolytic and nonpolyelectrolytic components suggested that although these alkaloids are charged, the major contributor of about 75% of the binding free energy arises from the nonpolyelectrolytic forces. The binding in case of palmatine and coralyne was predominantly enthalpy driven with favoring smaller entropy terms, while that of berberine was favored by both negative enthalpy and positive entropy changes. Temperature dependence of the binding enthalpies determined from ITC studies in the range 20-40 degrees C was used to calculate the binding-induced change in heat capacity ( $\Delta C_p$ ) values as -117, -135, and -157 cal/mol K, respectively, for berberine, palmatine, and coralyne. Taken together, the results suggest that the DNA binding of the planar synthetic coralyne is stronger and thermodynamically more favored compared to the buckled natural berberine and palmatine

Bischoff G., Gromann U., Lindau S., Meister W. V., and Hoffmann S. (1999) A spectroscopic and thermodynamic study of Taxol nucleic acid complexes. *Nucleosides Nucleotides* **18**, 2201-2217.

**Abstract:** The interactions of natural and synthetic polynucleotide double strands with the antitumor agent paclitaxel and the oncological product "Taxol for Injection Concentrate" (abbreviated as taxol) were examined in diluted aqueous solutions by thermal denaturation profiles ( $T_m$ ), CD spectra and UV-absorption measurements. Furthermore, DNA-paclitaxel and -taxol complexes in condensed nucleic acid solutions were studied by differential scanning calorimetry. As polynucleotides alternating and homologous poly[d(AT)] and poly[d(GC)] and calf thymus DNA were used. The results point to stabilizing interactions of paclitaxel to AT nucleotides, whereas in the presence of GC base pairings no interaction took place. Thereby the interaction to homologous (dA).(dT)-tracts seems to be preferred.

Bogdanov A. A., Ivanov I., Kas'ianenko N. A., Potekhin S. A., Surzhik M. A., Timkovskii A. L., Feofanov S. A., Khusainova R. S. and Iakovlev K. I. (2008) [Destabilization and stabilization of poly(A).poly(U) structure by platinum(II) compounds]. *Biofizika* **53**, 740-743.

**Abstract:** On the basis of molecular biophysics, a methodology for the analysis of intramolecular structural order of the polynucleotide duplex poly(A).poly(U) has been developed. It was shown that the combination of circular dichroism spectroscopy with differential scanning calorimetry is an optimal approach, which ensures the screening of a wide set of substances and interaction conditions and the choice of compound(s) that can stabilize the structure and increase the biological activity of this duplex. The study is aimed at obtaining a new and highly active antiviral remedy

Booth V. K., Roberts J. C., Warters R. L., Wilmore B. H., and Lepock J. R. (2000) Radioprotective thiolamines WR-1065 and WR-33278 selectively denature nonhistone nuclear proteins. *Radiat Res* **153**, 813-822.

**Abstract:** Differential scanning calorimetry was used to study the interactions of nuclei isolated from Chinese hamster V79 cells with the radioprotector WR-1065, other thiol compounds, and polyamines. Differential scanning calorimetry monitors denaturation of macromolecules and resolves the major nuclear components (e.g. constrained and relaxed DNA, nucleosome core, and nuclear matrix) of intact nuclei on the basis of thermal stability. WR-1065 treatment (0.5-10 mM) of isolated nuclei led to the irreversible denaturation of nuclear proteins, a fraction of which are nuclear matrix proteins. Denaturation of 50% of the total nonhistone nuclear protein content of isolated nuclei occurred after exposure to 4.7 mM WR-1065 for 20 min at 23 degrees C. In addition, a 22% increase in the insoluble protein content of nuclei isolated from V79 cells that had been treated with 4 mM WR-1065 for 30 min at 37 degrees C was observed, indicating that WR-1065-induced protein denaturation occurs not only in isolated nuclei but also in the nuclei of intact cells. From the extent of the increase in insoluble protein in the nucleus, protein denaturation by WR-1065 is expected to contribute to drug toxicity at concentrations greater than approximately 4 mM. WR-33278, the disulfide form of WR-1065, was approximately twice as effective as the free thiol at denaturing nuclear proteins. The proposed mechanism for nucleoprotein denaturation is through direct interactions with protein cysteine groups with the formation of destabilizing protein-WR-1065 disulfides. In comparison to its effect on nuclear proteins in isolated nuclei, WR-1065 had only a very small effect on non-nuclear proteins of whole cells, isolated nuclear matrix, or the thiol-rich Ca(2+)ATPase of sarcoplasmic reticulum, indicating that WR-1065 can effectively denature protein only inside an intact nucleus, probably due to the increased concentration of the positively charged drug in the vicinity of DNA.

Bursova V., Kasparikova J., Hofr C., and Brabec V. (2005) Effects of monofunctional adducts of platinum(II) complexes on thermodynamic stability and energetics of DNA duplexes. *Biophys J* **88**, 1207-1214.

**Abstract:** Effects of adducts of [PtCl(NH<sub>3</sub>)<sub>3</sub>]Cl or chlorodiethylenetriamineplatinum(II) on DNA stability were studied with emphasis on thermodynamic origins of that stability. Oligodeoxyribonucleotide duplexes (15-bp) containing the single, site-specific monofunctional adduct at G-residues of the central sequences TGT/ACA or 5'-AGT/5'-ACT were prepared and analyzed by differential scanning calorimetry, temperature-dependent ultraviolet absorption and circular dichroism. The unfolding of the platinated duplexes was accompanied by relatively small unfavorable free energy terms. This destabilization was enthalpic in origin. On the other hand, a relatively large reduction of melting temperature (T(m)) was observed as a consequence of the monofunctional adduct in the TGT sequence, whereas T(m) due to the adduct in the AGT sequence was reduced only slightly. We also examined the efficiency of the mammalian nucleotide excision repair system to remove from DNA the monofunctional adducts and found that these lesions were not recognized by this repair system. Thus, rather thermodynamic than thermal characterization of DNA adducts of monofunctional platinum compounds is a property implicated in the modulation of downstream effects such as protein recognition and repair.

Castelli F., De Guidi G., Giuffrida S., Miano P., and Sortino S. (1999) Molecular mechanisms of photosensitization XIII: a combined differential scanning calorimetry and DNA photosensitization study in non steroidal antiinflammatory drugs-DNA interaction. *Int J Pharm* **184**, 21-33.

**Abstract:** A combined differential scanning calorimetry (DSC) and photosensitization study has been carried out on the interaction of several NSAID on DNA, both from calf thymus and pBR 322 plasmid. The investigated compounds were both non-steroidal anti-inflammatory drugs as well as compounds related to NSAIDs for structural similar properties, to find evidence for their ability to interact with DNA as a function of steric hindrance and polarity of the chemical structures. The considered NSAIDs were diflunisal (DFN, a salicylic derivative), naproxen (NAP), ketoprofen (KPF), suprofen (SPF) and tiaprofenic acid

(TIA, arylpropionic acids). The structural criterion used was related to three different aromatic groups, biphenyl, naphthalene and benzophenone (BZP). In fact drug-DNA interaction can be revealed by variations of the enthalpies and temperatures of unfolding of DNA obtained by comparison of calorimetric peaks, where a decrease of the enthalpy is associated with the drug-DNA interaction, by engaging electrostatic bonds. Testing their ability in inducing DNA cleavage when UVA irradiated can evidence the photosensitizing properties of the drug. A good correlation was found between calorimetric and photosensitization studies. From the results obtained it can be reasonably supposed that the photocleavage depends only on the drug molecules bound to DNA. Copyright.

Chaires J. B. (1997) Energetics of drug-DNA interactions. *Biopolymers* **44**, 201-215.

**Abstract:** Understanding the thermodynamics of drug binding to DNA is of both practical and fundamental interest. The practical interest lies in the contribution that thermodynamics can make to the rational design process for the development of new DNA targeted drugs. Thermodynamics offer key insights into the molecular forces that drive complex formation that cannot be obtained by structural or computational studies alone. The fundamental interest in these interactions lies in what they can reveal about the general problems of parsing and predicting ligand binding free energies. For these problems, drug-DNA interactions offer several distinct advantages, among them being that the structures of many drug-DNA complexes are known at high resolution and that such structures reveal that in many cases the drug acts as a rigid body, with little conformational change upon binding. Complete thermodynamic profiles ( $\Delta G$ ,  $\Delta H$ ,  $\Delta S$ ,  $\Delta C_p$ ) for numerous drug-DNA interactions have been obtained, with the help of high-sensitivity microcalorimetry. The purpose of this article is to offer a perspective on the interpretation of these thermodynamics parameters, and in particular how they might be correlated with known structural features. Obligatory conformational changes in the DNA to accommodate intercalators and the loss of translational and rotational freedom upon complex formation both present unfavorable free energy barriers for binding. Such barriers must be overcome by favorable free energy contributions from the hydrophobic transfer of ligand from solution into the binding site, polyelectrolyte contributions from coupled ion release, and molecular interactions (hydrogen and ionic bonds, van der Waals interactions) that form within the binding site. Theoretical and semiempirical tools that allow estimates of these contributions to be made will be discussed, and their use in dissecting experimental data illustrated. This process, even at the current level of approximation, can shed considerable light on the drug-DNA binding process.

Chaires J. B., Leng F., Przewloka T., Fokt I., Ling Y. H., Perez-Soler R., and Priebe W. (1997) Structure-based design of a new bisintercalating anthracycline antibiotic. *J Med Chem* **40**, 261-266.

**Abstract:** A new bisintercalating anthracycline antibiotic, WP631, has been designed and synthesized. The rational design of the new compound was based upon the geometry of monomeric anthracyclines bound to DNA oligonucleotides observed in high-resolution crystal structures. Monomeric units of daunorubicin have been linked through their reactive 3' NH<sub>2</sub> substituents on the daunosamine moieties to form the new bisanthracycline WP631. Viscosity studies confirmed that WP631 binds to DNA by bisintercalation. Differential scanning calorimetry and UV melting experiments were used to measure the ultratight binding of WP631 to DNA. The binding constant for the interaction of WP631 with herring sperm DNA was determined to be  $2.7 \times 10^{11} \text{ M}^{-1}$  at 20 degrees C. The large, favorable binding free energy of  $-15.3 \text{ kcal mol}^{-1}$  was found to result from a large, negative enthalpic contribution of  $-30.2 \text{ kcal mol}^{-1}$ . A molecular model was generated that shows the favorable stereochemical fit of the linker in the DNA minor groove. The cytotoxicity of WP631 was compared to that of doxorubicin using MCF-7-sensitive and MCF-7/VP-16 MRP-mediated multidrug-resistant cell lines. These initial studies showed that while WP631 is slightly less cytotoxic than doxorubicin in the sensitive cell line, it appears to overcome MRP-mediated multidrug resistance and was much more cytotoxic against the MCF-7/VP-16 cell line than was doxorubicin. The design of new potential anticancer agents based on known structural principles was found to produce a compound with significantly increased DNA binding affinity and with interesting biological activity.

Chaires J. B. (1998) Drug--DNA interactions. *Curr Opin Struct Biol* **8**, 314-320.

**Abstract:** Significant progress has been made over the past few years in studies of drug-DNA interactions. Structure-based design strategies have yielded new DNA-binding agents with clinical promise. The hairpin polyamides represent the result of a design strategy with outstanding potential. One specific molecule of this class has now been proven to inhibit the expression of a specific gene in vivo. A new bisintercalating anthracycline antibiotic binds with high affinity to DNA, and appears to overcome a specific form of

multidrug resistance. Progress in fundamental studies of drug binding to DNA continues, with detailed thermodynamic studies providing new insights into the forces that drive complex formation. New tools have been developed in order to characterize both the binding mode and the sequence specificity of drug binding to DNA, tools that will enable the fundamental aspects of these biologically important reactions to be understood in more detail.

Costa D., Burrows H. D., and da Graca M. M. (2005) Changes in hydration of lanthanide ions on binding to DNA in aqueous solution. *Langmuir* **21**, 10492-10496.

**Abstract:** The interaction of the trivalent lanthanides Ce(III), Eu(III), and Tb(III) with sodium deoxyribonucleic acid (DNA) in aqueous solution has been studied using their luminescence spectra and decays. Complexation with DNA is indicated by changes in luminescence intensity. In the system terbium(III)-DNA, changes in luminescence with pH are suggested to be due to the protonation of phosphate groups. The degree of hydration of Tb(III) on binding to DNA is followed by luminescence lifetime measurements in water and deuterium oxide solutions, and it is found that the lanthanide ion loses at least one hydration water on binding to long double stranded DNA at pH 4.7 and pH 7. Rather different behavior is observed on binding to long or short single stranded DNA, where six water molecules are lost, independent of pH. It is suggested that in this case the lanthanide probably binds to the bases of the DNA backbone. The DNA conformation seems to be an important factor in the binding. In addition, the isotopic effect on terbium luminescence lifetime may provide a useful method to distinguish between single and double stranded DNA. DSC results are consistent with cleavage of the double helix of DNA at pH 9 in the presence of terbium.

Draper D. E., Xing Y., and Laing L. G. (1995) Thermodynamics of RNA unfolding: stabilization of a ribosomal RNA tertiary structure by thiostrepton and ammonium ion. *J Mol Biol* **249**, 231-238.

**Abstract:** RNAs with interesting secondary and tertiary structures tend to melt in several broad and overlapping transitions over a wide temperature range, and it has been consequently difficult to resolve the thermodynamics of individual unfolding steps. In the case that a ligand selectively binds a single folded state of the RNA, it is possible to obtain reliable thermodynamic parameters for both RNA unfolding and RNA-ligand binding simply from the hyperchromicity of RNA denaturation. The analysis procedure involves fitting a three-dimensional surface to absorbance data collected as a function of both temperature and ligand concentration. Analysis of the unfolding of a fragment of the large subunit ribosomal RNA (*Escherichia coli* sequence 1051 to 1109) is presented; both an antibiotic (thiostrepton) and ammonium ion specifically stabilize a tertiary structure within this RNA. A consistent set of thermodynamic parameters ( $\Delta H$  and  $t_m$ ) for the first two sequentially linked unfolding transitions is obtained from the experiments, and the binding constants obtained for the two ligands are consistent with other independent measurements. The approach is applicable to a variety of RNAs that specifically bind proteins, antibiotics, ions or other ligands.

Draper D. E. and Gluick T. C. (1995) Melting studies of RNA unfolding and RNA-ligand interactions. *Methods Enzymol* **259**, 281-305.

Freyer M. W., Buscaglia R., Kaplan K., Cashman D., Hurley L. H. and Lewis E. A. (2007) Biophysical studies of the c-MYC NHE III1 promoter: model quadruplex interactions with a cationic porphyrin. *Biophys J* **92**, 2007-2015.

**Abstract:** Regulation of the structural equilibrium of G-quadruplex-forming sequences located in the promoter regions of oncogenes by the binding of small molecules has shown potential as a new avenue for cancer chemotherapy. In this study, microcalorimetry (isothermal titration calorimetry and differential scanning calorimetry), electronic spectroscopy (ultraviolet-visible and circular dichroism), and molecular modeling were used to probe the complex interactions between a cationic porphyrin mesotetra (N-methyl-4-pyridyl) porphine (TMPyP4) and the c-MYC PU 27-mer quadruplex. The stoichiometry at saturation is 4:1 mol of TMPyP4/c-MYC PU 27-mer G-quadruplex as determined by isothermal titration calorimetry, circular dichroism, and ultraviolet-visible spectroscopy. The four independent TMPyP4 binding sites fall into one of two modes. The two binding modes are different with respect to affinity, enthalpy change, and entropy change for formation of the 1:1 and 2:1, or 3:1 and 4:1 complexes. Binding of TMPyP4, at or near physiologic ionic strength ( $[K(+)] = 0.13$  M), is described by a "two-independent-sites model." The two highest-affinity sites exhibit a  $K(1)$  of  $1.6 \times 10^7$  M<sup>-1</sup> and the two lowest-affinity sites exhibit a  $K(2)$  of

$4.2 \times 10^5 \text{ M}^{-1}$ ). Dissection of the free-energy change into the enthalpy- and entropy-change contributions for the two modes is consistent with both "intercalative" and "exterior" binding mechanisms. An additional complexity is that there may be as many as six possible conformational quadruplex isomers based on the sequence. Differential scanning calorimetry experiments demonstrated two distinct melting events ( $T(m)1 = 74.7 \text{ degrees C}$  and  $T(m)2 = 91.2 \text{ degrees C}$ ) resulting from a mixture of at least two conformers for the c-MYC PU 27-mer in solution.

Freyer M. W., Buscaglia R., Nguyen B., Wilson W. D., and Lewis E. A. (2006) Binding of netropsin and 4,6-diamidino-2-phenylindole to an A2T2 DNA hairpin: a comparison of biophysical techniques. *Anal Biochem* **355**, 259-266.

**Abstract:** Isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), and biosensor-surface plasmon resonance (SPR) are evaluated for their accuracy in determining equilibrium constants, ease of use, and range of application. Systems chosen for comparison of the three techniques were the formation of complexes between two minor groove binding compounds, netropsin and 4,6-diamidino-2-phenylindole (DAPI), and a DNA hairpin having the sequence 5'-d(CGAATTCGTCTCCGAATTCG)-3'. These systems were chosen for their structural differences, simplicity (1:1 binding), and binding affinity in the range of interest ( $K$  approximately  $10^8 \text{ M}^{-1}$ ). The binding affinities determined from all three techniques were in excellent agreement; for example, netropsin/DNA formation constants were determined to be  $K = 1.7 \times 10^8 \text{ M}^{-1}$  (ITC),  $K = 2.4 \times 10^8 \text{ M}^{-1}$  (DSC), and  $K = 2.9 \times 10^8 \text{ M}^{-1}$  (SPR). DSC and SPR techniques have an advantage over ITC in studies of ligands that bind with affinities greater than  $10^8 \text{ M}^{-1}$ . The ITC technique has the advantage of determining a full set of thermodynamic parameters, including  $\Delta H$ ,  $\Delta S$ , and  $\Delta C(p)$  in addition to  $\Delta G$  (or  $K$ ). The ITC data revealed complex binding behavior in these minor groove binding systems not detected in the other methods. All three techniques provide accurate estimates of binding affinity, and each has unique benefits for drug binding studies.

Giri P. and Kumar G. S. (2007) Specific binding and self-structure induction to poly(A) by the cytotoxic plant alkaloid sanguinarine. *Biochim Biophys Acta* **1770**, 1419-1426.

**Abstract:** The cytotoxic plant alkaloid sanguinarine was found to bind preferentially and strongly to single stranded poly(A) with an association constant ( $K(a)$ ) in the range  $3.6\text{-}4.6 \times 10^6 \text{ M}^{-1}$  in comparison to several nucleic acids. The binding induced unique self-structure formation in poly(A) that showed cooperative melting transition in circular dichroism, absorbance, and differential scanning calorimetry studies. The alkaloid binding was characterized to be intercalation as revealed from fluorescence quenching experiments and was predominantly enthalpy driven as revealed from isothermal titration calorimetry. Sanguinarine is the first and only natural product so far known to induce a self-structure formation in poly(A).

Giri P. and Kumar G. S. (2008) Self-structure induction in single stranded poly(A) by small molecules: Studies on DNA intercalators, partial intercalators and groove binding molecules. *Arch Biochem Biophys* **474**, 183-192.

**Abstract:** Self-structure induction in single stranded poly(A) has been one typical example of the various ways that could be used to modulate nucleic acid structural aspects through binding of small molecules. For the first time, the interaction between a series of small molecules and poly(A) has been investigated to understand the nature of the structural features in DNA binding small molecules that could be responsible for the formation of self-structure in single stranded poly(A) molecules. Classical intercalators like ethidium, coralyne, quinacrine and proflavine, partial intercalators like berberine and palmatine and classical minor groove binders like hoechst 33258 and DAPI have been chosen for this study. The binding of each of these molecules to poly(A) has been characterized by absorption spectral titration, job plot and isothermal titration calorimetry. Self-structure formation was monitored from circular dichroic melting, optical melting and differential scanning calorimetry. The results revealed that while all the intercalators studied induced self-structure formation, partial intercalators did not induce the same in poly(A). Of the two classical DNA minor groove binding molecules investigated, hoechst was effective in inducing self-structure while DAPI was ineffective. Self-structure induction in poly(A) was observed to be directly linked to the cooperative binding of the molecules to poly(A) in that all the molecules that bound cooperatively induced self-structure in poly(A). Structural and thermodynamic aspects of the interaction leading to self-structure formation are described

Giri P. and Kumar G. S. (2008) Binding of protoberberine alkaloid coralyne with double stranded poly(A): a biophysical study. *Mol Biosyst.* **4**, 341-348.

**Abstract:** Recognition of double stranded ribonucleic acid is a critical event in many biological pathways such as trafficking, editing and maturation of mRNA, interferon antiviral response and RNA interference. In the context of probing double stranded RNA binding small molecules, the interaction of the antitumor protoberberine alkaloid coralyne with double stranded poly(A) has been studied by various biophysical techniques. Typical hypochromic and bathochromic shifts in the absorption spectrum and appreciable quenching of the intrinsic fluorescence of coralyne indicated the strong affinity of coralyne to poly(A). The corresponding intrinsic binding constant evaluated from Scatchard analysis was in the order of  $10^5 \text{ M}^{-1}$ . The strong binding was further characterized by significant polarization of the alkaloid fluorescence and stabilization of poly(A) helix against thermal strand separation. The binding process was manifested by remarkable perturbation of the intrinsic circular dichroic spectrum of poly(A) with concomitant generation of optical activity in the bound alkaloid molecules that are otherwise achiral. Job plot analysis showed the binding stoichiometry of the interaction process to be two base pairs per alkaloid molecule. The energetics of the strong interaction was studied by isothermal titration and differential scanning calorimetric techniques that suggested the binding to be exothermic and favoured by both negative enthalpy and positive entropy changes. All these results, together with the Stern-Volmer quenching experiment in fluorescence, revealed the molecular details of the intercalation of coralyne into poly(A) duplex leading to its potential use as an agent in gene regulation in eukaryotic cells

Gmeiner W. H., Cui W., Sharma S., Soto A. M., Marky L. A., and Lown J. W. (2000) Shape-selective binding of geometrically-constrained bis-distamycins to a DNA duplex and a model Okazaki fragment of identical sequence. *Nucleosides Nucleotides Nucleic Acids* **19**, 1365-1379.

**Abstract:** The binding of ligands to nucleic acids is of great interest for the control of gene expression and other nucleic acid mediated processes. We have evaluated the binding of several geometrically-constrained bis-distamycins to a model Okazaki fragment [OKA], or a DNA duplex having identical base sequence [DD], using gel-shift assays, optical spectroscopy and differential scanning calorimetry. In the case of covalent attachment of two distamycins to a central benzene ring, a similar binding profile was observed for [DD] as was observed for [OKA] (para binds [ $K(\text{app}) > 10^6 \text{ M}^{-1}$ ], meta binds only weakly). For a central pyridyl ring, however, clear distinction between the binding to [DD] and binding to [OKA] was observed. While none of the three meta isomers having a central pyridyl ring bound [OKA], two of them (MT-17 and MT-12) bound [DD] [ $K(\text{app}) > 10^6 \text{ M}^{-1}$ ]. These results demonstrate subtle differences in lexitropsin shape and placement of electronegative atoms may result in selective binding to a nucleic acid duplex based both on base sequence and chemical composition. Selective binding to DNA duplexes may be useful for designing ligands that regulate transcription, but do not interfere in other nucleic acid mediated processes.

Guthrie K. M., Parenty A. D., Smith L. V., Cronin L. and Cooper A. (2007) Microcalorimetry of interaction of dihydro-imidazo-phenanthridinium (DIP)-based compounds with duplex DNA. *Biophys Chem* **126**, 117-123.

**Abstract:** Isothermal titration (ITC) and differential scanning calorimetry (DSC) have been used to screen the binding thermodynamics of a family of DNA intercalators based on the dihydro-imidazo-phenanthridinium (DIP) framework. All members of this DIP-based ligand family bind to both genomic (calf thymus and/or salmon testes) and a synthetic dodecamer d(CGCGAATTCGCG) duplex DNA with broadly similar affinities regardless of side chain size or functionality. Viscosity measurements confirm that binding satisfies standard criteria for intercalation. Binding is exothermic but with an additional favourable positive entropy contribution in most cases at 25 degrees C, although a significant negative heat capacity effect ( $\Delta C_p$ ) means that both  $\Delta H(0)$  and  $\Delta S(0)$  decrease with increasing temperature. DIP-ligand binding to DNA also shows significant entropy-enthalpy compensation effects that are now almost standard in such situations, probably reflecting the conformational flexibility of macromolecular systems involving a multiplicity of weak non-covalent interactions. This ability to vary side chain functionality without compromising DNA binding suggests that the DIP framework should be a promising basis for more adventurous chemistry at the DNA level.

Hofr C. and Brabec V. (2005) Thermal stability and energetics of 15-mer DNA duplex interstrand crosslinked by trans-diamminedichloroplatinum(II). *Biopolymers* **77**, 222-229.

**Abstract:** The effect of the location of the interstrand cross-link formed by trans-diamminedichloroplatinum(II) (transplatin) on the thermal stability and energetics of 15-mer DNA duplex has been investigated. The duplex containing single, site-specific cross-link, thermodynamically equivalent model structures (hairpins) and nonmodified duplexes were characterized by differential scanning calorimetry, temperature-dependent uv absorption, and circular dichroism. The results demonstrate that the formation of the interstrand cross-link of transplatin does not affect pronouncedly thermodynamic stability of DNA: the cross-link induces no marked changes not only in enthalpy, but also in "reduced" (concentration independent) monomolecular transition entropy. These results are consistent with the previous observations that interstrand cross-links of transplatin structurally perturb DNA only to a relatively small extent. On the other hand, constraining the duplex with the interstrand cross-link of transplatin results in a significant increase in thermal stability that is primarily due to entropic effects: the cross-link reduces the molecularity of the oligomer system from bimolecular to monomolecular. Importantly, the position of the interstrand cross-link within the duplex modulates cooperativity of the melting transition of the duplex and consequently its thermal stability.

Hofr C., Farrell N., and Brabec V. (2001) Thermodynamic properties of duplex DNA containing a site-specific d(GpG) intrastrand crosslink formed by an antitumor dinuclear platinum complex. *Nucleic Acids Res* **29**, 2034-2040.

**Abstract:** Bifunctional polynuclear platinum compounds represent a novel class of metal-based antitumor drugs which are currently undergoing preclinical development. A typical agent is [(trans-PtCl(NH<sub>3</sub>)(2))(2)H(2)N(CH<sub>2</sub>)(4)NH(2)]Cl(2) (1,1/t,t), which coordinates to bases in DNA and forms various types of covalent crosslinks. It also forms a 1,2-d(GpG) intrastrand adduct, the equivalent of the major DNA lesion of 'classical' cisplatin. In the present study differential scanning calorimetry and spectroscopic techniques were employed to characterize the influence of this crosslink on the thermal stability and energetics of 20 bp DNA duplexes site-specifically modified by 1,1/t,t. Thermal denaturation data revealed that the crosslink of 1,1/t,t reduced thermal and thermodynamical stability of the duplex noticeably more than that of 'classical' cisplatin. The energetic consequences of the intrastrand crosslink at the d(GG) site are discussed in relation to the structural distortions induced by this adduct in DNA and to its recognition and binding by HMG domain proteins. It has been suggested that the results of the present work are consistent with different DNA binding modes of cisplatin and polynuclear bifunctional DNA-binding drugs, which might be relevant to their distinct biological effectiveness.

Hofr C. and Brabec V. (2001) Thermal and thermodynamic properties of duplex DNA containing site-specific interstrand cross-link of antitumor cisplatin or its clinically ineffective trans isomer. *J Biol Chem* **276**, 9655-9661.

**Abstract:** The effect of the single, site-specific interstrand cross-link formed by cisplatin or transplatin on the thermal stability and energetics of a 20-base pair DNA duplex is reported. The cross-linked or unplatinated 20-base pair duplexes were investigated with the aid of differential scanning calorimetry, temperature-dependent UV absorption, and circular dichroism. The cross-link of both platinum isomers increases the thermal stability of the modified duplexes by changing the molecularity of denaturation. The structural perturbation resulting from the interstrand cross-link of cisplatin increases entropy of the duplex and in this way entropically stabilizes the duplex. This entropic cross-link-induced stabilization of the duplex is partially but not completely compensated by the enthalpic destabilization of the duplex. The net result of these enthalpic and entropic effects is that the structural perturbation resulting from the formation of the interstrand cross-link by cisplatin induces a decrease in duplex thermodynamic stability, with this destabilization being enthalpic in origin. By contrast, the interstrand cross-link of transplatin is enthalpically almost neutral with the cross-link-induced destabilization entirely entropic in origin. These differences are consistent with distinct conformational distortions induced by the interstrand cross-links of the two isomers. Importantly, for the duplex cross-linked by cisplatin relative to that cross-linked by transplatin, the compensating enthalpic and entropic effects almost completely offset the difference in cross-link-induced energetic destabilization. It has been proposed that the results of the present work further support the view that the impact of the interstrand cross-links of cisplatin and transplatin on DNA is different for each and might also be associated with the distinctly different antitumor effects of these platinum compounds.

Hossain M., Giri P. and Kumar G. S. (2008) DNA intercalation by quinacrine and methylene blue: a comparative binding and thermodynamic characterization study. *DNA Cell Biol* **27**, 81-90.

**Abstract:** There is compelling evidence that cellular DNA is the target of many anticancer agents. Consequently, elucidation of the molecular nature governing the interaction of small molecules to DNA is paramount to the progression of rational drug design strategies. In this study, we have compared the binding and thermodynamic aspects of two known DNA-binding agents, quinacrine (QNA) and methylene blue (MB), with calf thymus (CT) DNA. The study revealed noncooperative binding phenomena for both the drugs to DNA with an affinity one order higher for QNA compared to MB as observed from diverse techniques, but both bindings obeyed neighbor exclusion principle. The data of the salt dependence of QNA and MB from the plot of  $\log K$  versus  $\log [Na^+]$  revealed a slope of 1.06 and 0.93 consistent with the values predicted by theories for the binding of monovalent cations, and have been analyzed for contributions from polyelectrolytic and nonpolyelectrolytic forces. The binding of both drugs was further characterized by strong stabilization of DNA against thermal strand separation in both optical melting and differential scanning calorimetry studies. The binding data analyzed from the thermal denaturation and from isothermal titration calorimetry (ITC) were in close proximity to those obtained from spectral titration data. ITC results revealed the binding to be exothermic and favored by both negative enthalpy and positive entropy changes. The heat capacity changes obtained from temperature dependence of enthalpy indicated -146 and -78 cal/(mol.K), respectively, for the binding of QNA and MB to CT DNA. Circular dichroism study further characterized the structural changes on DNA upon intercalation of these molecules. Molecular aspects of interaction of these molecules to DNA are discussed

Huang C. Y., Ma S. S., Lee S., Radhakrishnan R., Braun C. S., Choosakoonkriang S., Wiethoff C. M., Lobo B. A., and Middaugh C. R. (2002) Enhancements in gene expression by the choice of plasmid DNA formulations containing neutral polymeric excipients. *J Pharm Sci* **91**, 1371-1381.

**Abstract:** Formulations containing maltodextrin (2% w/v) were identified to facilitate intramuscular (im) delivery of plasmid DNA in mice using the reporter genes luciferase and chloramphenicol acetyltransferase (CAT) and the therapeutic gene of erythropoietin (EPO) as monitors of transfection efficiency. Even though considerable variability in gene expression was observed in animals, a 5-8-fold enhancement of reporter gene expression was observed with this excipient compared with saline formulations of DNA. In a therapeutically significant experiment, a single im injection of an EPO plasmid formulation containing 2% (w/v) maltodextrin resulted in a significant and prolonged elevation of the hematocrit levels of mice compared with control DNA in saline. Biophysical studies with Fourier transform infrared (FTIR) spectroscopy, isothermal titration, and differential scanning calorimetry (DSC) suggested a weak interaction between DNA and maltodextrin as well as a thermal stabilizing effect on the DNA. These in vivo and biophysical results with maltodextrin are comparable to those reported previously with other nonionic polymers, such as poly(vinyl pyrrolidone) and poloxamers, and indicate that maltodextrin is an additional nonionic excipient that displays the property of gene expression enhancement.

Islam M. M., Sinha R. and Kumar G. S. (2007) RNA binding small molecules: studies on t-RNA binding by cytotoxic plant alkaloids berberine, palmatine and the comparison to ethidium. *Biophys Chem* **125**, 508-520.

**Abstract:** The interaction of two natural protoberberine plant alkaloids berberine and palmatine with t-RNA(phe) was studied using various biophysical techniques and the data was compared with the binding of the classical DNA intercalator, ethidium. The results of optical thermal melting, differential scanning calorimetry and circular dichroism characterized the native cloverleaf structure of t-RNA under the conditions of the study. The strong binding of the alkaloids and ethidium to t-RNA was revealed from the absorption and fluorescence studies. The salt dependence of the binding constants enabled the dissection of the binding free energy to electrostatic and non-electrostatic contributions. This analysis revealed a surprisingly large favourable component of the non-electrostatic contribution to the binding of these charged alkaloids and ethidium to t-RNA. Isothermal titration calorimetric studies revealed that the binding of both the alkaloids is driven by a moderately favourable enthalpy decrease and a moderately favourable entropy increase while that of ethidium is driven by a large favourable enthalpy decrease. Taken together, the results suggest that the binding of these alkaloid molecules on the t-RNA structure appears to be mostly by partial intercalation while ethidium intercalates to the t-RNA. These results reveal the molecular aspects on the interaction of these alkaloids to t-RNA.

Jadhav V., Maiti S., Dasgupta A., Das P. K., Dias R. S., Miguel M. G. and Lindman B. (2008) Effect of the head-group geometry of amino acid-based cationic surfactants on interaction with plasmid DNA. *Biomacromolecules*. **9**, 1852-1859.

**Abstract:** The interaction between DNA and different types of amino acid-based cationic surfactants was investigated. Particular attention was directed to determine the extent of influence of surfactant head-group geometry toward tuning the interaction behavior of these surfactants with DNA. An overview is obtained by gel retardation assay, isothermal titration calorimetry, fluorescence spectroscopy, and circular dichroism at different mole ratios of surfactant/DNA; also, cell viability was assessed. The studies show that the surfactants with more complex/bulkier hydrophobic head group interact more strongly with DNA but exclude ethidium bromide less efficiently; thus, the accessibility of DNA to small molecules is preserved to a certain extent. The presence of more hydrophobic groups surrounding the positive amino charge also gave rise to a significantly lower cytotoxicity. The surfactant self-assembly pattern is quite different without and with DNA, illustrating the roles of electrostatic and steric effects in determining the effective shape of a surfactant molecule

Kawashima T., Sasaki A., and Sasaki S. (2006) Transition of nanostructure in DNA-cationic surfactant complexes with the added salt. *Biomacromolecules* **7**, 1942-1950.

**Abstract:** Nanostructures of complexes of DNA with single-chain surfactant of octadecyltrimethylammonium (OTA) and double-chain surfactant of didodecyltrimethylammonium (DDA) in aqueous NaCl solution at concentration,  $C_s$ , from 0 to 500 mM were studied using small-angle-scattering techniques (SAXS). SAXS profiles of the DNA-OTA complex show two SAXS peaks with a spacing ratio of 1:3(1/2) in the solution at  $C_s$  below 150 mM and three peaks with a spacing ratio of 1:3(1/2):4(1/2) at  $C_s$  above 250 mM. Contents of Na<sup>+</sup> and Cl<sup>-</sup> ions in the complexes evaluated from the atomic absorbance for Na<sup>+</sup> and the potentiometry for Cl<sup>-</sup> revealed charge molar ratios of OTA/DNA = 1 and DDA/DNA = 1.25. Contents of Na<sup>+</sup> and Cl<sup>-</sup> ions per ionic unit of DNA molecule in the DNA-OTA complex equilibrating with the solution at  $C_s$  below 100 mM were much less than 0.1, while they increased with NaCl concentration at  $C_s$  above 200 mM. The DNA-OTA complex in the solution at  $C_s$  above 260 mM exhibited an endothermic peak in the DSC measurements, and the others did not. On the basis of the experimental results, the salt concentration dependent nanostructures are discussed.

Kim W., Yamasaki Y., and Kataoka K. (2006) Development of a fitting model suitable for the isothermal titration calorimetric curve of DNA with cationic ligands. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 10919-10925.

**Abstract:** A novel curve fitting model was developed for the isothermal titration calorimetry (ITC) of a cationic ligand binding to DNA. The ligand binding often generates a DNA conformational change from an elongated random coil into a compact collapsed form that is referred to as "DNA condensation". The ligand binding can be classified into two regimes having different binding constants  $K_i$ , i.e., the binding to an elongated DNA chain with a binding constant  $K_1$  and with  $K_2$  that occurred during the conformational transition. The two-variable curve fitting models are usually bound by a strict regulation on the difference in the values of the binding constants  $K_1 > K_2$ . For the DNA condensation, however, the relationships for  $K_1$  and  $K_2$  are still unclear. The novel curve fitting model developed in this study takes into account this uncertainty on the relationship of the binding constants and is highly flexible for the two-variable binding constant system.

Law S. M., Eritja R., Goodman M. F., and Breslauer K. J. (1996) Spectroscopic and calorimetric characterizations of DNA duplexes containing 2-aminopurine. *Biochemistry* **35**, 12329-12337.

**Abstract:** The base analog 2-aminopurine (AP) strongly promotes A.T to G.C and G.C to A.T transitions in bacteria and bacteriophage. During DNA replication, the primary mutagenic event involves formation of a heteroduplex with an AP.C site at a much higher frequency than formation of the corresponding heteroduplex with an A.C site. It is not known if AP-induced mutagenesis correlates with differences in the thermodynamic properties of an AP.C versus an A.C site, or whether interactions involving DNA polymerases are controlling. To address this specific question, and more generally to characterize AP-containing duplexes, we have used a combination of spectroscopic and calorimetric techniques to determine the thermodynamic properties of six 11-mer duplexes. The sequences of these duplexes are identical except for the identity of the variable central base pair which can be either A.T, A.C, AP.T, AP.C, AP.A, or AP.G, and which we use to designate each duplex. Analyses and interpretation of the optically

and calorimetrically derived thermal and thermodynamic data on these six duplexes reveal the relative stabilizing influence of the central base pairs to be A.T > AP.T > AP.C > AP.A > AP.G > A.C, with the AP.C-containing duplex being significantly more stable than the A.C-containing duplex. In the aggregate, our results suggest that during incorporation, base pair discrimination by DNA polymerases is influenced, in part, by differences in the thermodynamic stabilities of the newly formed base pairs.

Leng F., Priebe W., and Chaires J. B. (1998) Ultratight DNA binding of a new bisintercalating anthracycline antibiotic. *Biochemistry* **37**, 1743-1753.

**Abstract:** Differential scanning calorimetry and absorption spectroscopy were used to characterize the interaction of the new bisintercalating anthracycline antibiotic, WP631, with DNA. The method of continuous variations revealed five distinct binding modes for WP631, corresponding to 6, 3, 1.3, 0.5, and 0.25 mol of base pairs (bp) per mole of ligand. The binding of one drug to 6 bp corresponds to the bisintercalative binding mode determined previously, and was the mode studied in detail. UV melting experiments and differential scanning calorimetry were used to measure the ultratight binding of WP631 to DNA. The binding constant for the interaction of WP631 with herring sperm DNA was determined to be  $3.1 (+/- 0.2) \times 10^{11} \text{ M}^{-1}$  at 20 degrees C. The large, favorable binding free energy of  $-15.3 \text{ kcal mol}^{-1}$  was found to result from a large, negative enthalpic contribution of  $-30.2 \text{ kcal mol}^{-1}$ . DNA melting curves at different concentrations of WP631 were fitted to McGhee's model of DNA melting in the presence of ligands, yielding an independent estimate of DNA binding parameters. The salt dependence of the WP631 binding constant was examined, yielding a slope  $SK = \Delta(\log K)/\Delta(\log[\text{Na}^+]) = 1.63$ . The observed salt dependence of the equilibrium constant, interpreted according to polyelectrolyte theory, indicates that there is a significant nonpolyelectrolyte contribution to the binding free energy. DNA melting studies using a homogeneous 214 bp DNA fragment showed that WP631 binds preferentially to the GC-rich region of the DNA.

Leng F., Chaires J. B., and Waring M. J. (2003) Energetics of echinomycin binding to DNA. *Nucleic Acids Res* **31**, 6191-6197.

**Abstract:** Differential scanning calorimetry and UV thermal denaturation have been used to determine a complete thermodynamic profile for the bis-intercalative interaction of the peptide antibiotic echinomycin with DNA. The new calorimetric data are consistent with all previously published binding data, and afford the most rigorous and direct determination of the binding enthalpy possible. For the association of echinomycin with DNA, we found  $\Delta G$  degrees =  $-7.6 \text{ kcal mol}^{-1}$ ,  $\Delta H = +3.8 \text{ kcal mol}^{-1}$  and  $\Delta S = +38.9 \text{ cal mol}^{-1} \text{ K}^{-1}$  at 20 degrees C. The binding reaction is clearly entropically driven, a hallmark of a process that is predominantly stabilized by hydrophobic interactions, though a deeper analysis of the free energy contributions suggests that direct molecular recognition between echinomycin and DNA, mediated by hydrogen bonding and van der Waals contacts, also plays an important role in stabilizing the complex.

Li T. K., Bathory E., LaVoie E. J., Srinivasan A. R., Olson W. K., Sauers R. R., Liu L. F., and Pilch D. S. (2000) Human topoisomerase I poisoning by protoberberines: potential roles for both drug-DNA and drug-enzyme interactions. *Biochemistry* **39**, 7107-7116.

**Abstract:** Protoberberines represent a structural class of organic cations that induce topoisomerase I-mediated DNA cleavage, a behavior termed topoisomerase I poisoning. We have employed a broad range of biophysical, biochemical, and computer modeling techniques to characterize and cross-correlate the DNA-binding and topoisomerase poisoning properties of four protoberberine analogues that differ with respect to the substituents on their A- and/or D-rings. Our data reveal the following significant features: (i) The binding of the four protoberberines unwinds duplex DNA by approximately 11 degrees, an observation consistent with an intercalative mode of interaction. (ii) Enthalpically favorable interactions, such as stacking interactions between the intercalated ligand and the neighboring base pairs, provide <50% of the thermodynamic driving force for the complexation of the protoberberines to duplex DNA. Computer modeling studies on protoberberine-DNA complexes suggest that only rings C and D intercalate into the host DNA helix, while rings A and B protrude out of the helix interior into the minor groove. (iii) All four protoberberine analogues are topoisomerase I-specific poisons, exhibiting little or no topoisomerase II poisoning activity. (iv) Modifications of the D-ring influence both DNA binding and topoisomerase I poisoning properties. Specifically, transference of a methoxy substituent from the 11- to the 9-position diminishes both DNA binding affinity and topoisomerase I poisoning activity, an observation suggesting that DNA binding is important in the poisoning of topoisomerase I by protoberberines. (v) Modifications of

the A-ring have a negligible impact on DNA binding affinity, while exerting a profound influence on topoisomerase I poisoning activity. Specifically, protoberberine analogues containing either 2,3-dimethoxy; 3,4-dimethoxy; or 3, 4-methylenedioxy substituents all bind DNA with a similar affinity. By contrast, these analogues exhibit markedly different topoisomerase I poisoning activities, with these activities following the hierarchy: 3,4-methylenedioxy > 2,3-dimethoxy >> 3, 4-dimethoxy. These differences in topoisomerase I poisoning activity may reflect the differing abilities of the analogues to interact with specific functionalities on the enzyme, thereby stabilizing the enzyme in its cleavable state. In the aggregate, our results are consistent with a mechanistic model in which both ligand-DNA and ligand-enzyme interactions are important for the poisoning of topoisomerase I by protoberberines, with the DNA-directed interactions involving ring D and the enzyme-directed interactions involving ring A. It is reasonable to suggest that the poisoning of topoisomerase I by a broad range of other naturally occurring and synthetic ligands may entail a similar mechanism.

Maeda Y. and Ohtsubo E. (1988) Differential scanning calorimetry of antitumor antibiotics-plasmid DNA interaction. *Nucleic Acids Symp Ser* 101-104.

**Abstract:** Differential scanning calorimetry (DSC) can detect stepwise melting of plasmid DNA along the molecular chain with high resolution. This method was applied to study interaction of some antitumor antibiotics with the plasmid pJL3-TB5 DNA (5277 base-pairs in length). Analysis of DSC curves of the plasmid DNA in the presence of, for example, adriamycin, an antitumor antibiotics of anthracycline group, together with theoretical analysis of the DNA melting curves obtained by calculation from the entire base sequence, led to the conclusion that adriamycin bound preferentially to the four particular regions with high G + C content. The DSC method would thus be useful for the study of properties of drugs which bind to DNA.

Maeda Y., Nunomura K., and Ohtsubo E. (1990) Differential scanning calorimetric study of the effect of intercalators and other kinds of DNA-binding drugs on the stepwise melting of plasmid DNA. *J Mol Biol* **215**, 321-329.

**Abstract:** The effect of intercalating drugs (the anthracycline group of antibiotics, ethidium bromide, actinomycin D) on stepwise melting of DNA was studied by differential scanning calorimetry (DSC). The DSC DNA melting profile of plasmid pJL3-TB5 DNA (5277 base-pairs in length) consists of seven peaks, and all the intercalators caused shifting of these peaks, particularly those formed at the high temperature ranges, to the higher temperature ranges in a characteristic manner depending upon the binding strength of the drug. The analysis of the anthracycline group of antibiotics, such as aclacinomycin A, daunomycin, adriamycin and pyrrubicin, indicates that the difference in binding is due to the sugar moiety at position O-7 of the chromophore in these antibiotics. Analysis on the basis of the helix-coil transition theory suggests that the anthracycline group of antibiotics interact preferentially with the 5'-CG-3' sequences. The effect of various DNA-binding drugs other than intercalators on stepwise melting of DNA was then studied by DSC. The representative drugs examined were distamycin A, peplomycin, cis-dichlorodiamine-platinum(II) (cis-DDP or cis-Platin) and mitomycin C, which differ in their mode of interaction with DNA; namely, minor groove binding, strand cleavage and intrastrand or interstrand cross-linking. Distamycin A caused shifting of the DSC peaks at the low temperature ranges to a higher temperature range, whereas peplomycin and cis-DDP caused shifting of all the DSC peaks to form a broad peak at a lower temperature range, suggesting that the DSC DNA melting profiles are affected in a characteristic manner depending upon the interaction mode of the drug.

Malina J., Hofr C., Maresca L., Natile G., and Brabec V. (2000) DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands. *Biophys J* **78**, 2008-2021.

**Abstract:** Modifications of natural DNA and synthetic oligodeoxyribonucleotide duplexes in a cell-free medium by analogs of antitumor cisplatin containing enantiomeric amine ligands, such as cis-[PtCl<sub>2</sub>(RR-DAB)] and cis-[PtCl<sub>2</sub>(SS-DAB)] (DAB = 2,3-diaminobutane), were studied by various methods of molecular biophysics and biophysical chemistry. These methods include DNA binding studies by pulse polarography and atomic absorption spectrophotometry, mapping of DNA adducts using transcription assay, interstrand cross-linking assay using gel electrophoresis under denaturing conditions, differential scanning calorimetry, chemical probing, and bending and unwinding studies of the duplexes containing single, site-specific cross-link. The major differences resulting from the modification of DNA by the two enantiomers are the thermodynamical destabilization and conformational distortions induced in DNA by

the 1,2-d(GpG) intrastrand cross-link. It has been suggested that these differences are associated with a different biological activity of the two enantiomers observed previously. In addition, the results of the present work are also consistent with the view that formation of hydrogen bonds between the carbonyl oxygen of the guanine residues and the "quasi equatorial" hydrogen of the cis amine in the 1, 2-d(GpG) intrastrand cross-link plays an important role in determining the character of the distortion induced in DNA by this lesion.

Marky L. A. and Macgregor R. B., Jr. (1990) Hydration of dA.dT polymers: role of water in the thermodynamics of ethidium and propidium intercalation. *Biochemistry* **29**, 4805-4811.

**Abstract:** We report differences in the interaction of two structurally similar phenanthroline intercalators, ethidium and propidium, with poly(dA).poly(dT) and poly[d(A-T)] as a function of ionic strength based on titration microcalorimetry, fluorescence titration, and hydrostatic pressure measurements. Both ethidium and propidium bind more strongly to poly[d(A-T)].poly[d(A-T)] than to poly(dA).poly(dT). Ethidium intercalation into the latter polymer displays titrations with positive cooperativity; this is not found with propidium. The enthalpy of intercalation ( $\Delta H$  degrees) is exothermic for both dyes with poly[d(A-T)].poly[d(A-T)]; however, the value of this parameter is nearly zero in the case of poly(dA).poly(dT). The molar volume change ( $\Delta V$  degrees) accompanying dye intercalation is negative under all conditions for poly[d(A-T)].poly[d(A-T)] whereas it is positive for poly(dA).poly(dT). The changes observed in  $\Delta V$  degrees correlate well with the entropy changes derived from the titration and calorimetric data for this reaction. The results, interpreted in terms of the relative hydration of these two polymers, are consistent with a higher extent of hydration of poly(dA).poly(dT) relative to poly[d(A-T)].poly[d(A-T)].

Novakova O., Kasparkova J., Bursova V., Hofr C., Vojtkova M., Chen H., Sadler P. J., and Brabec V. (2005) Conformation of DNA modified by monofunctional Ru(II) arene complexes: recognition by DNA binding proteins and repair. Relationship to cytotoxicity. *Chem Biol* **12**, 121-129.

**Abstract:** We analyzed DNA duplexes modified at central guanine residues by monofunctional Ru(II) arene complexes [(eta(6)-arene)Ru(II)(en)(Cl)](+) (arene = tetrahydroanthracene or p-cymene, Ru-THA or Ru-CYM, respectively). These two complexes were chosen as representatives of two different classes of Ru(II) arene compounds for which initial studies revealed different binding modes: one that may involve DNA intercalation (tricyclic-ring Ru-THA) and the other (mono-ring Ru-CYM) that may not. Ru-THA is approximately 20 times more toxic to cancer cells than Ru-CYM. The adducts of Ru-THA and Ru-CYM have contrasting effects on the conformation, thermodynamic stability, and polymerization of DNA in vitro. In addition, the adducts of Ru-CYM are removed from DNA more efficiently than those of Ru-THA. Interestingly, the mammalian nucleotide excision repair system has low efficiency for excision of ruthenium adducts compared to cisplatin intrastrand crosslinks.

Park Y. W. and Breslauer K. J. (1992) Drug binding to higher ordered DNA structures: netropsin complexation with a nucleic acid triple helix. *Proc Natl Acad Sci U S A* **89**, 6653-6657.

**Abstract:** We have used a combination of spectroscopic and calorimetric techniques to characterize how netropsin, a ligand that binds in the minor groove of DNA, influences the properties of a DNA triple helix. Specifically, our data allow us to reach the following conclusions: (i) netropsin binds to the triplex without displacing the major-groove-bound third strand; (ii) netropsin binding to the triplex exhibits a lower saturation binding density (7.0 base triplets per netropsin bound) than netropsin binding to the corresponding duplex (5.5 base pairs per netropsin bound); (iii) the netropsin-free and the netropsin-bound triplexes each melt in two well-resolved transitions, initial conversion of the triplex to the duplex state followed by duplex melting to the component single-stranded states; (iv) netropsin remains bound to DNA as the triplex melts to the duplex state; (v) netropsin binding thermally destabilizes the triplex in equilibrium with duplex equilibrium dramatically, while thermally stabilizing the duplex to single-strand equilibrium; (vi) netropsin binding to the triplex is enthalpically 4 times more favorable (more exothermic) than netropsin binding to the corresponding duplex; (vii) netropsin binding to the triplex decreases the cooperativity of the triplex----duplex melting event. These results demonstrate that occupancy of the minor groove of a triplex by a ligand such as netropsin can exert a profound impact on the properties of the host triplex, particularly with regard to the equilibrium in which the third strand is expelled from the major groove. Thus, our results reveal considerable major groove/minor groove crosstalk. Such knowledge may prove of practical importance by providing an approach for modulating the affinity and specificity of major-groove-binding third strands in triplex-forming protocols designed to target specific duplex domains.

Fundamentally, our results provide insights into the crosstalk that can result when ligands bind to the two major receptor sites of duplex DNA--namely, the major and minor grooves.

Pilch D. S., Kirolos M. A., and Breslauer K. J. (1995) Berenil binding to higher ordered nucleic acid structures: complexation with a DNA and RNA triple helix. *Biochemistry* **34**, 16107-16124.

**Abstract:** Berenil is an antitrypanosomal agent that binds to nucleic acid duplexes. Recently, we reported that this drug can bind to both DNA and RNA duplexes, while exhibiting properties characteristic of both intercalation and groove binding [Pilch, D. S., Kirolos, M. A., Liu, X., Plum, G. E., & Breslauer, K. J. (1995) *Biochemistry* 34, 9962-9976]. In this work, we use spectroscopic, calorimetric, and hydrodynamic techniques to demonstrate that berenil also can bind to DNA and RNA triplexes. Our results reveal the following significant features: (i) Berenil binds to the poly(dA).2poly(dT) DNA triplex and to the poly(rA).2poly(rU) RNA triplex without displacing the major groove-bound third strands. (ii) Both berenil-bound triplexes melt via two distinct transitions: initial conversion of the triplex to the duplex state, with the berenil remaining bound, followed by denaturation of the duplex to its component single strands. (iii) The magnitude and even the direction of the impact of berenil binding on the thermal stability of the DNA triplex depends on both the Na<sup>+</sup> concentration and the drug binding density (the [base triplet]/[total berenil] ratio). Specifically, at Na<sup>+</sup> concentrations  $\leq 0.08$  M, the DNA triplex to duplex transition is thermally stabilized, while at Na<sup>+</sup> concentrations  $\geq 0.125$  M it is thermally destabilized. Between these two salt concentrations, berenil binding either enhances or diminishes the thermal stability of the DNA triplex in a manner that depends on the [base triplet]/[total berenil] ratio. (iv) The effect of berenil binding on the thermal stability of the RNA triplex to duplex equilibrium also depends on the [base triplet]/[total berenil] ratio, having a weakly destabilizing effect on this equilibrium at [base triplet]/[total berenil] ratios  $> 5$ , while thermally stabilizing this equilibrium at [base triplet]/[total berenil] ratios  $< 5$ . (v) The apparent "site sizes" associated with berenil binding to the triplexes range from approximately 1 to 12 base triplets per bound berenil and depend, in part, on the host triplex. One of the site sizes common to both triplexes is consistent with berenil binding to the minor groove. (vi) Berenil exhibits a higher apparent binding affinity for the DNA triplex relative to the RNA triplex. (vii) Viscometric data reveal nonintercalative binding properties when berenil complexes with both triplexes, consistent with a minor groove binding mode. (viii) Berenil binding to either the DNA or the RNA triplex is enthalpically more favorable than berenil binding to the corresponding duplex. (ix) Berenil binding to both triplexes decreases the cooperativity of the triplex to duplex melting event.(ABSTRACT TRUNCATED AT 400 WORDS).

Pilch D. S., Kirolos M. A., Liu X., Plum G. E., and Breslauer K. J. (1995) Berenil [1,3-bis(4'-amidino-phenyl)triazene] binding to DNA duplexes and to a RNA duplex: evidence for both intercalative and minor groove binding properties. *Biochemistry* **34**, 9962-9976.

**Abstract:** Berenil is an antitrypanosomal agent that binds to nucleic acid duplexes. The generally accepted mode of berenil binding is via complexation into the minor groove of AT-rich domains of DNA double helices. We find that berenil can bind to RNA as well as DNA duplexes, while exhibiting properties characteristic of both intercalation as well as minor groove binding. More specifically, we use spectroscopic, calorimetric, and hydrodynamic techniques to characterize berenil binding to four DNA duplexes and to one RNA duplex. Our results reveal the following features: (i) Berenil binding to the poly[d(A-T)]<sub>2</sub>, poly(dA).poly(dT), poly[d(I-C)]<sub>2</sub>, poly[d(G-C)]<sub>2</sub>, and poly(rA).poly(rU) duplexes exhibits intercalative as well as minor groove binding characteristics. (ii) The apparent "site sizes" associated with berenil binding to these five duplexes range from 1 to 13 base pairs per bound berenil and depend, in part, on the host duplex. One of the site sizes common to all five duplexes is consistent with berenil binding to the minor groove. (iii) The apparent berenil binding affinity follows the hierarchy: poly(dA).poly(dT)  $>$  poly-[d(A-T)]<sub>2</sub> approximately poly[d(I-C)]<sub>2</sub>  $\gg$  poly(rA).poly(rU)  $>$  poly[d(G-C)]<sub>2</sub>. (iv) Viscometric data reveal properties characteristic of a significant contribution from an intercalative mode of binding when berenil interacts with the poly[d(A-T)]<sub>2</sub>, poly[d(I-C)]<sub>2</sub>, poly[d(G-C)]<sub>2</sub>, and poly(rA).poly(rU) duplexes, while revealing an apparent nonintercalative mode when the drug binds to the poly(dA).poly(dT) duplex. (v) Berenil binding unwinds negative supercoils in the pBR322 plasmid, an observation consistent with an intercalative mode of binding to duplex DNA. (vi) Salt-dependent melting data suggest that both positively charged amidino groups of berenil participate in the complexation of the drug to the poly[d(I-C)]<sub>2</sub>, poly[d(A-T)]<sub>2</sub>, poly(dA).poly(dT), and poly(rA).poly(rU) duplexes, while also suggesting that the binding event is site-specific. In the aggregate, our results suggest that, in contrast to the conventional wisdom, berenil can exhibit intercalative as well as minor groove binding properties when it binds to both DNA and

RNA duplexes, while also exhibiting a preference for DNA duplexes with unobstructed minor grooves. We comment on the potential correlation between drugs, such as berenil, that exhibit "mixed" binding motifs and those that express anticancer activity via inhibition of topoisomerase I activity.

Pilch D. S., Dunham S. U., Jamieson E. R., Lippard S. J., and Breslauer K. J. (2000) DNA sequence context modulates the impact of a cisplatin 1,2-d(GpG) intrastrand cross-link on the conformational and thermodynamic properties of duplex DNA. *J Mol Biol* **296**, 803-812.

**Abstract:** The anticancer activity of cisplatin derives from its ability to bind and cross-link DNA, with the major adduct being the 1,2-d(GpG) intrastrand cross-link. Here, the consequences of this adduct on the conformation, thermal stability, and energetics of duplex DNA are assessed, and the modulation of these parameters by the sequence context of the adduct is evaluated. The properties of a family of 15-mer DNA duplexes containing a single 1,2-d(GpG) cis- inverted question markPt(NH(3))(2) inverted question mark(2+) intrastrand cross-link are probed in different sequence contexts where the flanking base-pairs are systematically varied from T.A to C.G to A.T. By using a combination of spectroscopic and calorimetric techniques, the structural, thermal, and thermodynamic properties of each duplex, both with and without the cross-link, are characterized. Circular dichroism spectroscopic data reveal that the cross-link alters the structure of the host duplex in a manner consistent with a shift from a B-like to an A-like conformation. Thermal denaturation data reveal that the cross-link induces substantial thermal and thermodynamic destabilization of the host duplex. Significantly, the magnitudes of these cross-link-induced effects on duplex structure, thermal stability, and energetics are influenced by the bases that flank the adduct. The presence of flanking A.T base-pairs, relative to T.A or C.G base-pairs, enhances the extent of cross-link-induced alteration to an A-like conformation and dampens the extent of cross-link-induced duplex destabilization. These results are discussed in terms of available structural data, and in terms of the selective recognition of cisplatin-DNA adducts by HMG-domain proteins.

Poklar N., Pilch D. S., Lippard S. J., Redding E. A., Dunham S. U., and Breslauer K. J. (1996) Influence of cisplatin intrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex. *Proc Natl Acad Sci U S A* **93**, 7606-7611.

**Abstract:** cis-Diamminedichloroplatinum(II) (cisplatin) is a widely used anticancer drug that binds to and crosslinks DNA. The major DNA adduct of the drug results from coordination of two adjacent guanine bases to platinum to form the intrastrand crosslink cis-[Pt(NH<sub>3</sub>)<sub>2</sub>[d(GpG)-N7(1), -N7(2)]] (cis-Pt-GG). In the present study, spectroscopic and calorimetric techniques were employed to characterize the influence of this crosslink on the conformation, thermal stability, and energetics of a site-specifically platinated 20-mer DNA duplex. CD spectroscopic and thermal denaturation data revealed that the crosslink alters the structure of the host duplex, consistent with a shift from a B-like to an A-like conformation; lowers its thermal stability by approximately 9 degrees C; and reduces its thermodynamic stability by 6.3 kcal/mol at 25 degrees C, most of which is enthalpic in origin; but it does not alter the two-state melting behavior exhibited by the parent, unmodified duplex, despite the significant crosslink-induced changes noted above. The energetic consequences of the cis-Pt-GG crosslink are discussed in relation to the structural perturbations it induces in DNA and to how these crosslink-induced perturbations might modulate protein binding.

Rosa M., Dias R., da Graca M. M., and Lindman B. (2005) DNA-cationic surfactant interactions are different for double- and single-stranded DNA. *Biomacromolecules* **6**, 2164-2171.

**Abstract:** The stability of DNA in solution and the phase behavior in mixtures with dodecyltrimethylammonium bromide (DTAB) were investigated. By means of circular dichroism, UV absorption, and differential scanning calorimetry, we found that for dilute solutions of DNA with no addition of salt the DNA molecules are in the single-stranded conformation, whereas the addition of a small amount of NaBr, 1 mM, is sufficient to stabilize the DNA double-helix. Furthermore, at higher DNA concentrations, native DNA becomes the most stable structure, which is due to a self-screening effect. By phase diagram determinations of the DNA-surfactant system, we found that the effect of salt on phase behavior mainly relates to a difference in interaction of the amphiphile between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). The difference in association between ss and dsDNA with surfactants of different chain lengths can be interpreted in terms of an interplay between hydrophobic and electrostatic interactions, the latter being influenced by polymer flexibility. In this way, a nonmonotonic variation can be rationalized. A crossing of the phase separation lines with DNA concentration can be

rationalized in terms of a change in relative stability of ss and dsDNA. The fact that ssDNA phase separates earlier than dsDNA in association with DTAB, may serve as a basis for a method of easily separating dsDNA from ssDNA by the addition of surfactant; this is verified as monitored by circular dichroism measurements.

Schmitz H. U., Hubner W., and Ackermann T. (1995) Thermodynamic and spectroscopic characterization of the berenil and pentamidine complexes with the dodecanucleotide d(CGCGATATCGCG)<sub>2</sub>. *Z Naturforsch [C]* **50**, 263-274.

**Abstract:** The dodecanucleotide d(CGCGATATCGCG)<sub>2</sub> was characterized by thermodynamic and UV-spectrophotometric measurements. A van't Hoff enthalpy of  $\Delta H_{uv}$  approximately -190 kJ/mol was determined for the thermal transition using UV spectroscopy. This value was confirmed by differential scanning calorimetry (DSC). In addition we obtained the thermodynamic data  $\Delta \Delta HDSC = -405.1$  kJ/mol,  $\Delta SDSC = -1290$  J/mol.K and  $\Delta GDSC = -53.2$  kJ/mol for the helix to coil transition of the dodecanucleotide. The association of berenil and the oligonucleotide was accompanied with a stabilization of the host duplex (increase in  $T_m$ ) and an increase in the van't Hoff enthalpy. The berenil binding parameters ( $\Delta \Delta HDSC = -32.6$  kJ/mol,  $\Delta \Delta SDSC = -72$  J/mol.K and  $\Delta \Delta GDSC = -11.1$  kJ/mol) revealed significant differences compared to those of the pentamidine aggregation ( $\Delta \Delta HDSC = -23.7$  kJ/mol,  $\Delta \Delta SDSC = -53$  J/mol.K and  $\Delta \Delta GDSC = -7.8$  kJ/mol). The transition of the pure oligonucleotide was characterized by a substantial amount of intermediate states ( $\sigma DSC = 0.43$ ) which decreased significantly upon binding of the drugs ( $\sigma DSC$  approximately 0.80). The structural features of the complexes were analyzed by FT-IR spectroscopy. From these experiments we conclude that the configurations in the berenil and pentamidine complexes are different.

Sengupta B., Uematsu T., Jacobsson P., and Swenson J. (2006) Exploring the antioxidant property of bioflavonoid quercetin in preventing DNA glycation: a calorimetric and spectroscopic study. *Biochem Biophys Res Commun* **339**, 355-361.

**Abstract:** Reducing sugars for example glucose, fructose, etc., and their phosphate derivatives non-enzymatically glycate biological macromolecules (e.g., proteins, DNA and lipids) and is related to the production of free radicals. Here we present a novel study, using differential scanning calorimetry (DSC) along with UV/Vis absorption and photon correlation spectroscopy (PCS), on normal and glycated human placenta DNA and have explored the antioxidant property of the naturally occurring polyhydroxy flavone quercetin (3,3',4',5,7-pentahydroxyflavone) in preventing the glycation. The decrease in the absorption intensity of DNA in presence of sugars clearly indicates the existence of sugar molecules between the two bases of a base pair in the duplex DNA molecule. Variations were perceptible in the PCS relaxation profiles of normal and glycated DNA. The melting temperature of placenta DNA was decreased when glycated suggesting a decrease in the structural stability of the double-stranded glycated DNA. Our DSC and PCS data showed, for the first time, that the dramatic changes in the structural properties of glycated DNA can be prevented to a significant extent by adding quercetin. This study provides valuable insights regarding the structure, function, and dynamics of normal and glycated DNA molecules, underlying the manifestation of free radical mediated diseases, and their prevention using therapeutically active naturally occurring flavonoid quercetin.

Soto A. M., Rentzeperis D., Shikiya R., Alonso M., and Marky L. A. (2006) DNA intramolecular triplexes containing dT  $\rightarrow$  dU substitutions: unfolding energetics and ligand binding. *Biochemistry* **45**, 3051-3059.

**Abstract:** We used a combination of optical and calorimetric techniques to investigate the incorporation of deoxythymidine  $\rightarrow$  deoxyuridine (dT  $\rightarrow$  dU) substitutions in the duplex and third strand of the parallel intramolecular triplex d(A(7)C(5)T(7)C(5)T(7)) (ATT). UV and differential scanning calorimetry melting experiments show that the incorporation of two substitutions yielded triplexes with lower thermal stability and lower unfolding enthalpies. The enthalpies decrease with an increase in salt concentration, indirectly yielding a heat capacity effect, and the magnitude of this effect was lower for the substituted triplexes. The combined results indicate that the destabilizing effect is due to a decrease in the level of stacking interactions. Furthermore, the minor groove ligand netropsin binds to the minor groove and to the hydrophobic groove, created by the double chain of thymine methyl groups in the major groove of these triplexes. Binding of netropsin to the minor groove yielded thermodynamic profiles similar to that of a DNA duplex with a similar sequence. However, and relative to ATT, binding of netropsin to the hydrophobic groove has a decreased binding affinity and lower binding enthalpy. This shows that the

presence of uridine bases disrupts the hydrophobic groove and lowers its cooperativity toward ligand binding. The overall results suggest that the stabilizing effect of methyl groups may arise from the combination of both hydrophobic and electronic effects.

Spink C. H., Garbett N. and Chaires J. B. (2007) Enthalpies of DNA melting in the presence of osmolytes. *Biophys Chem* **126**, 176-185.

**Abstract:** The melting of DNA in the presence of osmolytes has been studied with the intention of obtaining information about how base pair stability is affected by changes in solution conditions. In previous investigations, the melting enthalpies were assumed to be constant as osmolalities change, but no systematic evaluation of whether this condition is true has been offered. This paper presents calorimetric data on the melting of two synthetic DNA samples in the presence of a number of common osmolytes. Poly(dAdT)\*poly(dTdA) and poly(dGdC)\*poly(dCdG) melting have been examined by differential scanning calorimetry in solutions containing ethylene glycol, glycerol, sucrose, urea, betaine, PEG 200 and PEG 1450 at increasing osmolalities. The results show small, but significant changes in the enthalpy of melting of the two polynucleotides that are different, depending on the structure of the cosolvent. The polyols, ethylene glycol, glycerol, PEG 200 and also urea all show decreases in melting enthalpy, while betaine and sucrose display increases with increasing concentration of cosolvent. The large stabilizing PEG 1450 shows no change within the experimental errors. Using concepts relating to preferential interactions of the cosolvents with the DNA base pairs, it is possible to interpret some of the observed changes in the thermodynamic properties of melting. The results indicate that there is strong entropy-enthalpy compensation upon melting base pairs, but entropy increases dominate to cause the decreases in stability with increased cosolvent concentration. Excess hydration parameters are evaluated and their magnitudes discussed in terms of changes in cosolvent interactions with the DNA base pairs.

Tan W. B., Bhambhani A., Duff M. R., Rodger A., and Kumar C. V. (2006) Spectroscopic Identification of Binding Modes of Anthracene Probes and DNA Sequence Recognition. *Photochem Photobiol* **82**, 20-30.

**Abstract:** The binding properties of two anthracene derivatives with calf thymus DNA (CTDNA), poly(dA-dT), and poly(dG).poly(dC) are reported. One contained bulky, cyclic cationic substituents at the 9 and 10 positions while the other carried acyclic, branched, cationic substituents. Binding of the probes to the DNA was examined by calorimetry, spectroscopy, and helix melting studies. The cyclic derivative indicated exothermic binding, strong hypochromism, bathochromism, positive induced circular dichroism (CD, 300-400 nm), significant unwinding of the helix, facile energy transfer from the singlet excited states of the DNA to the probe, large increases in the helix melting temperature, strong but negative linear dichroism (LD, 300-400 nm), and considerable stabilization of the helix. In contrast, the acyclic analog indicated thermo-neutral binding, smaller hypochromism, no bathochromism, very weak induced CD, and no change in the helix melting temperature with any of the DNA polymers. However, when the ionic strength of the medium was lowered (10 mM NaCl), the absorption as well as CD spectral changes associated with the binding of acyclic derivative to CT DNA corresponded with those noted with the cyclic derivative. A sharp distinction between the binding properties of the two probes is indicated, and both had intrinsic binding constants of  $\sim 10(6) \text{ M}^{-1}$  for the three polymers. The acyclic derivative showed large preference (10-fold) for poly(dG).poly(dC) over poly(dA-dT), while the cyclic analog showed no preference. The characteristic spectroscopic signatures of the two distinct binding modes of these probes will be helpful in deciphering the interaction of other anthracene derivatives with DNA.

Terui Y., Ohnuma M., Hiraga K., Kawashima E., and Oshima T. (2005) Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*. *Biochem J* **388**, 427-433.

**Abstract:** Extreme thermophiles produce two types of unusual polyamine: long linear polyamines such as caldopentamine and caldohexamine, and branched polyamines such as quaternary ammonium compounds [e.g. tetrakis(3-aminopropyl)ammonium]. To clarify the physiological roles of long linear and branched polyamines in thermophiles, we synthesized them chemically and tested their effects on the stability of ds (double-stranded) and ss (single-stranded) DNAs and tRNA in response to thermal denaturation, as measured by differential scanning calorimetry. Linear polyamines stabilized dsDNA in proportion to the number of amino nitrogen atoms within their molecular structure. We used the empirical results to derive formulae that estimate the melting temperature of dsDNA in the presence of polyamines of a particular molecular composition. ssDNA and tRNA were stabilized more effectively by tetrakis(3-aminopropyl)ammonium than any of the other polyamines tested. We propose that long linear polyamines

are effective to stabilize DNA, and tetrakis(3-aminopropyl)ammonium plays important roles in stabilizing RNAs in thermophile cells.

Ueta H., Maeda Y., and Kawai Y. (2001) Features of distamycin preferential binding sites on natural DNA predicted using differential scanning calorimetry. *Biosci Biotechnol Biochem* **65**, 1261-1264.

**Abstract:** The interaction of distamycin with ColE1 DNA was examined by using differential scanning calorimetry (DSC) taking the helix-coil transition theory of DNA into consideration. Our results here strongly indicate that the affinity of distamycin to DNA, at a low distamycin concentration, depends highly on the DNA sequence, and preferential binding occurs to the sites of four to six successive A-T pairs having two or more successive G-C pairs on both their ends.

Willis B. and Arya D. P. (2006) Recognition of B-DNA by neomycin--Hoechst 33258 conjugates. *Biochemistry* **45**, 10217-10232.

**Abstract:** Recent developments have indicated that aminoglycoside binding is limited not to RNA but to nucleic acids that, like RNA, adopt conformations similar to the A-form. We have further sought to expand the utility of aminoglycoside binding to B-DNA structures by conjugating neomycin, an aminoglycoside antibiotic, with the B-DNA minor groove binding ligand Hoechst 33258. Described herein are novel neomycin-Hoechst 33258 conjugates developed for exploring B-DNA groove recognition. We have varied the two reported conjugates in linker length and composition in an effort to improve our understanding of the spatial differences that define B-DNA binding. Spectroscopic studies such as ultraviolet (UV) melting, isothermal fluorescence titrations, differential scanning calorimetry (DSC), and circular dichroism (CD) together illustrate the mode of binding by such conjugates. Both conjugates exhibit enhanced thermal stabilization of A.T rich duplexes when compared to Hoechst 33258.

Willis B. and Arya D. P. (2006) An expanding view of aminoglycoside-nucleic acid recognition. *Adv Carbohydr Chem Biochem* **60**, 251-302.

Yang M., Pang R., Jia X., Li Q., and Wang K. (2005) DNA interaction of dioxycyclobutenedione-(1,2-cyclohexanediamine) platinum(II) complex with potential anticancer activity. *J Inorg Biochem* **99**, 376-382.

**Abstract:** Dioxycyclobutenedione-(1,2-cyclohexanediamine)platinum(II), (R,R-DC-Pt) was found to have stronger cytotoxicity against six cancer cell lines than cisplatin and its DNA interactions was studied by calorimetric measurements, <sup>13</sup>C NMR. The binding specificity study of DNA base with R,R-DC-Pt was conducted by HPLC. To understand the molecular mechanism of R,R-DC-Pt with stronger cytotoxicity than that of cisplatin, we studied R,R-DC-Pt interaction with an oligonucleotide, d(ACCACGTGGT)(2), which contained c-H-ras gene encoding GGT by NMR spectroscopy. The oligomer DNA double helix was destroyed almost completely upon the R,R-DC-Pt binding. However under the same condition, the cisplatin binding with DNA was not so affected, and instead another conformation was formed, which suggests that larger damage to DNA can be induced by R,R-DC-Pt complex than that by cisplatin.

Xu Z., Pilch D. S., Srinivasan A. R., Olson W. K., Geacintov N. E., and Breslauer K. J. (1997) Modulation of nucleic acid structure by ligand binding: induction of a DNA.RNA.DNA hybrid triplex by DAPI intercalation. *Bioorg Med Chem* **5**, 1137-1147.

**Abstract:** The aromatic diamidine, DAPI (4',6-diamidino-2-phenylindole), is used as an important biological and cytological tool since it forms highly fluorescent complexes with nucleic acid duplexes via minor groove-directed/intercalative modes of interaction. In this study, we find that DAPI binding can induce the formation of an RNA-DNA hybrid triplex that would not otherwise form. More specifically, through application of a broad range of spectroscopic, viscometric, and molecular modeling techniques, we demonstrate that DAPI intercalation induces the formation of the poly(dT).poly(rA).poly(dT) hybrid triple helix, a structure which does not form in the absence of the ligand. Using UV mixing studies, we demonstrate that, in the presence of DAPI, the poly(rA).poly(dT) duplex and the poly(dT) single strand form a 1:1 complex (a triplex) that does not form in the absence of DAPI. Through temperature-dependent absorbance measurements, we show that the poly(dT).poly(rA).poly(dT) triplex melts via two distinct transitions: initial conversion of the triplex to the duplex state, with the DAPI remaining bound, followed by denaturation of the duplex-DAPI complex to its component single strands and free DAPI. Using optical melting profiles, we show that DAPI binding enhances the thermal stability of the

poly(dT).poly(rA).poly(dT) triplex, an observation consistent with the preferential binding of the ligand to the triplex versus the duplex and single-stranded states. Our differential scanning calorimetric measurements reveal melting of the DAPI-saturated poly(dT).poly(rA).poly(dT) triplex to be associated with a lower enthalpy but greater cooperativity than melting of the corresponding DAPI-saturated poly(rA).poly(dT) duplex. Our flow linear dichroism and viscometric data are consistent with an intercalative mode of binding when DAPI interacts with both the poly(dT).poly(rA).poly(dT) triplex and the poly(rA).poly(dT) duplex. Finally, computer modeling studies suggest that a combination of both stacking and electrostatic interactions between the intercalated ligand and the host nucleic acid play important roles in the DAPI-induced stabilization of the poly(dT).poly(rA).poly(dT) triplex. In the aggregate, our results demonstrate that ligand binding can be used to induce the formation of triplex structures that do not form in the absence of the ligand. This triplex-inducing capacity has potentially important implications in the design of novel antisense, antigene, antiviral, and diagnostic strategies.

Xu Z., Li T. K., Kim J. S., LaVoie E. J., Breslauer K. J., Liu L. F., and Pilch D. S. (1998) DNA minor groove binding-directed poisoning of human DNA topoisomerase I by terbenzimidazoles. *Biochemistry* **37**, 3558-3566.

**Abstract:** We have employed a broad range of spectroscopic, calorimetric, DNA cleavage, and DNA winding/unwinding measurements to characterize the DNA binding and topoisomerase I (TOP1) poisoning properties of three terbenzimidazole analogues, 5-phenylterbenzimidazole (5PTB), terbenzimidazole (TB), and 5-(naphthyl[2,3-d]imidazo-2-yl)bibenzenzimidazole (5NIBB), which differ with respect to the substitutions at their C5 and/or C6 positions. Our results reveal the following significant features. (i) The overall extent to which the three terbenzimidazole analogues poison human TOP1 follows the hierarchy 5PTB > TB >> 5NIBB. (ii) The impact of the three terbenzimidazole analogues on the superhelical state of plasmid DNA depends on the [total ligand] to [base pair] ratio (rbp), having no effect on DNA superhelicity at rbp ratios  $\leq 0.1$ , while weakly unwinding DNA at rbp ratios  $> 0.1$ . This weak DNA unwinding activity exhibited by the three terbenzimidazoles does not appear to be correlated with the abilities of these compounds to poison TOP1. (iii) Upon complexation with both poly(dA).poly(dT) and salmon testes DNA, the three terbenzimidazole analogues exhibit flow linear dichroism properties characteristic of a minor groove-directed mode of binding to these host DNA duplexes. (iv) The apparent minor groove binding affinities of the three terbenzimidazole analogues for the d(GA4T4C)<sub>2</sub> duplex follow a qualitatively similar hierarchy to that noted above for ligand-induced poisoning of human TOP1—namely, 5PTB > TB > 5NIBB. In the aggregate, our results suggest that DNA minor groove binding, but not DNA unwinding, is important in the poisoning of TOP1 by terbenzimidazoles.