ITC XV - Nucleic Acid-Small Molecule interactions

Arabzadeh A., Bathaie S. Z., Farsam H., Amanlou M., Saboury A. A., Shockravi A., and Moosavi-Movahedi A. A. (2002) Studies on mechanism of 8-methoxypsoralen-DNA interaction in the dark. *Int J Pharm* **237,** 47-55.

Abstract: The interaction of 8-methoxypsoralen (8-MOP) with calf thymus DNA was studied in darkness at 25 degrees C and pH 7.4. The enthalpy curve for 8-MOP-DNA interaction was obtained by isothermal titration calorimetry and showed a two-step process for the interaction. According to the spectrophotometric data, it was suggested that some compaction may occur in the DNA structure at higher [8-MOP](t)/[DNA] ratio. Using the fluorescence quenching data, the Scatchard analysis was performed for 8-MOP-DNA interaction at the extended ranges of drug concentration. The results indicated that the first set of binding sites was occupied by 1 mol of drug bound per near eight base pairs of DNA. Also 8-MOP caused the quenching of the fluorescence emission of DNA-ethidium bromide complex. The Scatchard analysis of these data indicated the non-competitive manner for quenching. A non-displacement based quenching mechanism has been suggested for this behavior. The circular dichroism spectra also confirmed the non-intercalative binding of 8-MOP at higher concentrations accompanied by some conformational changes in DNA structure. It has been suggested that at low drug load, 8-MOP binds to DNA as an intercalator, which is an endothermic process, whereas at higher ratios of [8-MOP](t)/[DNA], it binds to the outside of DNA, probably in the minor groove and causes some compaction in DNA, which is the exothermic process.

Arora A., Balasubramanian C., Kumar N., Agrawal S., Ojha R. P. and Maiti S. (2008) Binding of berberine to human telomeric quadruplex - spectroscopic, calorimetric and molecular modeling studies. *FEBS J* **275,** 3971-3983.

Abstract: This study examines the characteristics of binding of berberine to the human telomeric d[AG(3)(T(2)AG(3))(3)] quadruplex. By employing UV-visible spectroscopy, fluorescence spectroscopy and isothermal titration calorimetry, we found that the binding affinity of berberine to the human telomeric quadruplex is 10(6). The complete thermodynamic profile for berberine binding to the quadruplex, at 25 degrees C, shows a small negative enthalpy (DeltaH) of -1.7 kcal.mol(-1), an entropy change with TDeltaS of +6.5 kcal.mol(-1), and an overall favorable free energy (DeltaG) of -8.2 kcal.mol(-1) .Through the temperature dependence of DeltaH, we obtained a heat capacity (DeltaC(p)) of -94 (+/- 5) cal.mol(-1).K(-1). The osmotic stress method revealed that there is an uptake of 13 water molecules in the complex relative to the free reactants. Furthermore, the molecular modeling studies on different quadruplex-berberine complexes show that berberine stacking at the external G-quartet is mainly aided by the pi-pi interaction and the stabilization of the high negative charge density of O6 of guanines by the positively charged N7 of berberine. The theoretical heat capacity (DeltaC(p)) values for quadruplex-berberine models are -89 and -156 cal.mol(-1).K(-1)

Arora A. and Maiti S. (2008) Effect of loop orientation on quadruplex-TMPyP4 interaction. *J Phys. Chem B* **112,** 8151-8159.

Abstract: G-quadruplexes are believed to be potential targets for therapeutic intervention and this has resulted in designing of various quadruplex interacting ligands. Moreover, reports about existence of quadruplex forming sequences across the genome have propelled greater interest in understanding their interaction with small molecules. An intramolecular quadruplex sequence can adopt different conformations, owing to different orientation of loops in the structure. The differences in the loop orientation can affect their molecular recognition. Herein, we have studied the interaction of 5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP4), a well-known G quadruplex binding ligand with three DNA quadruplexes differing in loop orientations. Results obtained from UV, ITC, and SPR studies have coherently revealed that the TMPyP4 molecule shows preferential binding to parallel G-quadruplex (c-myc and c-kit) over its antiparallel counterpart (human telomeric). The binding affinity for parallel quadruplex was (10(7)) 1 order of magnitude higher than that for antiparallel DNA quadruplex (10). The study shows two binding modes, stronger binding (10(7)) of TMPyP4 involving end stacking and a weaker external binding (10), while TMPyP4 shows only one binding mode with duplex with a binding affinity of the order of 10(6). Overall, the study emphasizes that differences in the loop orientation give rise

to different conformations of quadruplex, which in turn govern its binding to small molecules, and thereby play a pivotal role in molecular recognition

Arya D. P., Coffee R. L., Jr., and Xue L. (2004) From triplex to B-form duplex stabilization: reversal of target selectivity by aminoglycoside dimers. *Bioorg Med Chem Lett* **14,** 4643-4646.
Abstract: Aminoglycosides have been shown to target A-form nucleic acids. Our work has previously shown that neomycin (and other aminoglycosides) bind and stabilize DNA/RNA triplexes and other A-form nucleic acids. We report herein the unexpected B-form duplex stabilization shown by aminoglycoside dimers (neomycin-neomycin and neomycin-tobramycin). The dimers are highly selective for AT rich duplexes and show high affinity (K_a approximately $10^8 M^{-1}$) as determined by isothermal titration calorimetry.

Arya D. P., Micovic L., Charles I., Coffee R. L., Jr., Willis B., and Xue L. (2003) Neomycin binding to Watson-Hoogsteen (W-H) DNA triplex groove: a model. *J Am Chem Soc* **125,** 3733-3744.
Abstract: Neomycin is the most effective aminoglycoside (groove binder) in stabilizing a DNA triple helix. It stabilizes TAT, as well as mixed base DNA triplexes, better than known DNA minor groove binders (which usually destabilize the triplex) and polyamines. Neomycin selectively stabilizes the triplex (in the presence of salt), without any effect on the DNA duplex. (1) Triplex stabilization by neomycin is salt dependent (increased KCl and MgCl(2) concentrations decrease neomycin's effectiveness, at a fixed drug concentration). (2) Triplex stabilization by neomycin is pH dependent (increased pH decreases neomycin's effectiveness, at a fixed drug concentration). (3) CD binding studies indicate approximately 5-7 base triplets/drug apparent binding site, depending upon the structure/sequence of the triplex. (4) Neomycin shows nonintercalative groove binding to the DNA triplex, as evident from viscometric studies. (5) Neomycin shows a preference for stabilization of TAT triplets but can also accommodate CGC(+) triplets. (6) Isothermal titration calorimetry (ITC) studies reveal an association constant of approximately 2 x 10^5 M^{-1} between neomycin and an intramolecular triplex and a higher Ka for polydA.2polydT. (7) Binding/modeling studies show a marked preference for neomycin binding to the larger W-H groove. Ring I/II amino groups and ring IV amines are proposed to be involved in the recognition process. (8) The novel selectivity of neomycin is suggested to be a function of its charge and shape complementarity to the triplex W-H groove, making neomycin the first molecule that selectively recognizes a triplex groove over a duplex groove.

Babaian I., Mnzini G., and Quadrifoglio F. (1988) [Interaction of ethidium bromide with synthetic double-stranded polyribonucleotides]. *Mol Biol (Mosk)* **22,** 898-910.
Abstract: The interaction of ethidium bromide (EtBr) with double helical synthetic polyribonucleotides poly(G).poly(C), poly(A).poly(U) and poly(I).poly(C) has been investigated by the method of isothermal microcalorimetry and according to the character of changes on the spectra of circular dichroism, absorption and fluorescence at binding. The calculations showed that at binding of EtBr with poly(A).poly(U) the saturation stechiometry was one EtBr molecule per 2 base pairs with binding constant (2.5 +/- 0.5).10^6 M^{-1} at 30 degrees C and 0.1 M. NaCl. In the case of binding of EtBr with poly(G).poly(C) and poly(I).poly(C) the saturation stechiometry was not less than 0.2 EtBr molecule per 1 base pair with binding constant (4 +/- 1).10^3 M^{-1} and (1.5 +/- 0.3).10^4 M^{-1} respectively, at 25 degrees C and 0.1 M NaCl. The binding enthalpies of EtBr with poly(A).poly(U) and poly(G).poly(C) have been determined to be (-7.5 +/- 0.5) Kcal per 1 mol of bound EtBr in average for both polymers. It has been shown that the observed strong selectivity of EtBr binding with polyribonucleotides is of entropic origin.

Babaian I. and Manzini G. (1990) [Interaction of the antineoplastic agent mitoxantrone with double-stranded nucleic acids]. *Mol Biol (Mosk)* **24,** 1084-1094.
Abstract: The binding of mitoxantrone with double-helical nucleic acids was investigated by the methods of isothermal microcalorimetry, circular dichroism and absorption at the ionic strength mu = 0.11 and 0.011 M NaCl at temperature region of 30 divided by 60 degrees C. The investigation shows, that at mu = 0.11 M NaCl mitoxantrone interacts with double-helical nucleic acids in one way only. For such conditions using spectrophotometric titration data Scatchard plots for the binding of mitoxantrone with double-helical nucleic acids were constructed. The calculations show that the saturation stoichiometry is one mitoxantrone molecule per 2 divided by 3 base pairs DNA and 6 divided by 8 base pairs RNA. The dependence of binding constant from GC-content is observed. It is shown that the binding enthalpy of mitoxantrone with

DNA and RNA increases linearly and reaches -(3.0 +/- 0.5) kkal per 1 mol mitoxantrone. It is shown that a binding mitoxantrone with double-helical nucleic acids, besides the intercalation of rings, a determinate contribution in the binding is given also by electrostatic interaction of side chains mitoxantrone with nucleic acids.

Babayan Y., Manzini G., Xodo L. E., and Quadrifoglio F. (1987) Base specificity in the interaction of ethidium with synthetic polyribonucleotides. *Nucleic Acids Res* **15,** 5803-5812.
Abstract: Base specificity in the interaction of ethidium with double stranded synthetic RNA homopolymers has been studied by means of spectroscopic (UV-visible absorption and fluorescence), microcalorimetric and dilatometric techniques. The results show a strong base specificity in this interaction, the association constant with poly A:poly U being more than three order of magnitude higher than with poly O:poly C. The interaction is mainly enthalpy driven, the differences in affinity being essentially entropic in origin. These evidences along with the dilatometric data suggest that the observed base specificity may arise from the different extent of water release upon intercalation.

Baidya N. and Uhlenbeck O. C. (1997) A kinetic and thermodynamic analysis of cleavage site mutations in the hammerhead ribozyme. *Biochemistry* **36,** 1108-1114.
Abstract: Two kinetically well-characterized hammerheads with different arm lengths were used to reinvestigate the cleavage properties of substrates with the four natural nucleotides at position 17, the residue 5' to the cleavage site. From experiments measuring substrate binding affinity, cleavage rates, and the internal equilibrium, free energy profiles of the reaction of all four substrates were constructed. Each nucleotide at the cleavage site affects the energy profile quite differently. Whereas C and U have the same ground state energy, U destabilizes the transition state by 1 kcal/mol. A destabilizes both the ground and transition states by 1 kcal/mol, and G stabilizes the ground state by 2 kcal/mol and destabilizes the transition state by 4 kcal/mol. These data, along with experiments with the C3U mutant hammerhead, indicate that although an N3-N17 pair can form, the contribution to the binding energy for the wild-type (C3-C17) hammerhead is quite small. Thus, the energetic cost of disrupting the C3-C17 pair is not great, consistent with several proposals that this occurs during cleavage. The data also suggest that the structure in the transition state involves different stabilizing interactions with nucleotide 17 than those that are observed in the ground state. Finally, the A17 hammerhead may cleave by a slightly different reaction pathway.

Barbieri C. M., Li T. K., Guo S., Wang G., Shallop A. J., Pan W., Yang G., Gaffney B. L., Jones R. A., and Pilch D. S. (2003) Aminoglycoside complexation with a DNA.RNA hybrid duplex: the thermodynamics of recognition and inhibition of RNA processing enzymes. *J Am Chem Soc* **125,** 6469-6477.
Abstract: Spectroscopic and calorimetric techniques were employed to characterize and contrast the binding of the aminoglycoside paromomycin to three octamer nucleic acid duplexes of identical sequence but different strand composition (a DNA.RNA hybrid duplex and the corresponding DNA.DNA and RNA.RNA duplexes). In addition, the impact of paromomycin binding on both RNase H- and RNase A-mediated cleavage of the RNA strand in the DNA.RNA duplex was also determined. Our results reveal the following significant features: (i) Paromomycin binding enhances the thermal stabilities of the RNA.RNA and DNA.RNA duplexes to similar extents, with this thermal enhancement being substantially greater in magnitude than that of the DNA.DNA duplex. (ii) Paromomycin binding to the DNA.RNA hybrid duplex induces CD changes consistent with a shift from an A-like to a more canonical A-conformation. (iii) Paromomycin binding to all three octamer duplexes is linked to the uptake of a similar number of protons, with the magnitude of this number being dependent on pH. (iv) The affinity of paromomycin for the three host duplexes follows the hierarchy, RNA.RNA > DNA.RNA >> DNA.DNA. (v) The observed affinity of paromomycin for the RNA.RNA and DNA.RNA duplexes decreases with increasing pH. (vi) The binding of paromomycin to the DNA.RNA hybrid duplex inhibits both RNase H- and RNase A-mediated cleavage of the RNA strand. We discuss the implications of our combined results with regard to the specific targeting of DNA.RNA hybrid duplex domains and potential antiretroviral applications.

Barbieri C. M., Srinivasan A. R., and Pilch D. S. (2004) Deciphering the origins of observed heat capacity changes for aminoglycoside binding to prokaryotic and eukaryotic ribosomal RNA a-sites: a calorimetric, computational, and osmotic stress study. *J Am Chem Soc* **126,** 14380-14388.
Abstract: Isothermal titration calorimetry (ITC), computational, and osmotic stress techniques have been used to characterize the changes in heat capacity, solvent-accessible surface, and hydration that accompany

the binding of the aminoglycoside paromomycin to both prokaryotic and eukaryotic rRNA A-site model oligonucleotides. Regarded as a whole, the results of these studies suggest that the intrinsic heat capacity change (ΔC_p) for the binding of paromomycin to each rRNA A-site is near zero, with the negative ΔC_p observed for the binding of the drug to the prokaryotic rRNA A-site being dictated by the coupled destacking of the adenine residues at positions 1492 and 1493. In this connection, ΔC_p provides a useful calorimetric signature for assessing the relative impacts of novel and existing A-site targeting ligands on rRNA conformation, which, in turn, should provide a useful analytical tool for facilitating the drug design process, since aminoglycoside-induced destacking of A1492 and A1493 is thought to be a determining factor in the mistranslational and antimicrobial activities of the drugs.

Barbieri C. M. and Pilch D. S. (2006) Complete Thermodynamic Characterization of the Multiple Protonation Equilibria of the Aminoglycoside Antibiotic Paromomycin: A Calorimetric and Natural Abundance 15N NMR Study. *Biophys J* **90,** 1338-1349.
Abstract: The binding of aminoglycoside antibiotics to a broad range of macromolecular targets is coupled to protonation of one or more of the amino groups that typify this class of drugs. Determining how and to what extent this linkage influences the energetics of the aminoglycoside-macromolecule binding reaction requires a detailed understanding of the thermodynamics associated with the protonation equilibria of the aminoglycoside amino groups. In recognition of this need, a calorimetric- and NMR-based approach for obtaining the requisite thermodynamic information is presented using paromomycin as the model aminoglycoside. Temperature- and pH-dependent (15)N NMR studies provide pKa values for the five paromomycin amino groups, as well as the temperature dependence of these pKa values. These studies also indicate that the observed pKa values associated with the free base form of paromomycin are lower in magnitude than the corresponding values associated with the sulfate salt form of the drug. This difference in pKa is due to drug interactions with the sulfate counterions at the high drug concentrations (>/=812 mM) used in the (15)N NMR studies. ITC studies conducted at drug concentrations </=45 microM reveal that the extent of paromomycin protonation linked to the binding of the drug to its pharmacologically relevant target, the 16 S rRNA A-site, is consistent with the pKa values of the free base and not the sulfate salt form of the drug. Temperature- and pH-dependent isothermal titration calorimetry studies yield exothermic enthalpy changes (DeltaH) for protonation of the five paromomycin amino groups, as well as positive heat capacity changes (DeltaCp) for three of the five amino groups. Regarded as a whole, the results presented here represent an important first step toward establishing a thermodynamic database that can be used to predict how aminoglycoside-macromolecule binding energetics will be influenced by conditions such as temperature, pH, and ionic strength. Such a predictive capability is a critical component of any drug design strategy.

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)2]. 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)2] 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 $3.6 \times 10^5\ M^{-1}$ at 20 degrees C, in a solution containing 18 mM Na(+). The DNA-drug

interaction was enthalpy driven, with a ΔH(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 (ΔCp) 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 ΔG_{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. and Portugal J. (2004) Elsamicin A binding to DNA. A comparative thermodynamic characterization. *FEBS Lett* **576,** 68-72.
Abstract: The antitumor drug elsamicin A contains a coumarin-related chartarin chromophore that intercalates into DNA. It differs from other related molecules in its disaccharide moiety, which bears an amino sugar. Its binding to DNA was analyzed using isothermal titration calorimetry and UV thermal denaturation, and characterized thermodynamically. For the association of elsamicin A with DNA we found $\Delta G° = -8.6$ kcal mol^{-1}, $\Delta H = -10.4$ kcal mol^{-1}, $\Delta S = -6.1$ cal mol^{-1} K^{-1}, and $K_{obs} = 2.8(+/- 0.2) \times 10^{6}$ M^{-1} at 20 degrees C in 18 mM Na+. The contributions to the free energy of binding that lead to the DNA-elsamicin complex are compared with the binding to DNA of chartreusin, another chartarin-containing drug. The results are discussed in terms of the contributions of the disaccharide moieties into the strength 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(obs) = 1.2 (+/-0.3) x 10(5) M(-1)) is tighter than that of MSK (2.9 (+/-1.0) x 10(4) 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.

Bathaie S. Z., Moosavi-Movahedi A. A., and Saboury A. A. (1999) Energetic and binding properties of DNA upon interaction with dodecyl trimethylammonium bromide. *Nucleic Acids Res* **27,** 1001-1005.
Abstract: The interaction of dodecyl trimethylammonium bromide (DTAB), a cationic surfactant, with calf thymus DNA has been studied by various methods, including potentiometric technique using DTAB-selective plastic membrane electrode at 27 and 37 degrees C, isothermal titration microcalorimetry and UV spectrophotometry at 27 degrees C using 0.05 M Tris buffer and 0.01 M NaCl at pH 7.4. The free energy is calculated from binding isotherms on the basis of Wyman binding potential theory and the enthalpy of binding according to van't Hoff relation. The enthalpy of unfolding has been determined by subtraction of the enthalpy of binding from the microcalorimetric enthalpy. The results show that, after the interaction of first DTAB molecule to DNA (base molarity) through the electrostatic interaction, the second DTAB molecule also binds to DNA through electrostatic interaction. At this stage, the predom-inant DNA conformational change occurs. Afterwards up to 20 DTAB molecules, below the critical micelle concentration of DTAB, bind through hydrophobic interactions.

Bernacchi S., Freisz S., Maechling C., Spiess B., Marquet R., Dumas P. and Ennifar E. (2007) Aminoglycoside binding to the HIV-1 RNA dimerization initiation site: thermodynamics and effect on the kissing-loop to duplex conversion. *Nucleic Acids Res* **35,** 7128-7139.
Abstract: Owing to a striking, and most likely fortuitous, structural and sequence similarity with the bacterial 16 S ribosomal A site, the RNA kissing-loop complex formed by the HIV-1 genomic RNA dimerization initiation site (DIS) specifically binds 4,5-disubstituted 2-deoxystreptamine (2-DOS)

aminoglycoside antibiotics. We used chemical probing, molecular modeling, isothermal titration calorimetry (ITC) and UV melting to investigate aminoglycoside binding to the DIS loop-loop complex. We showed that apramycin, an aminoglycoside containing a bicyclic moiety, also binds the DIS, but in a different way than 4,5-disubstituted 2-DOS aminoglycosides. The determination of thermodynamic parameters for various aminoglycosides revealed the role of the different rings in the drug-RNA interaction. Surprisingly, we found that the affinity of lividomycin and neomycin for the DIS (K(d) approximately 30 nM) is significantly higher than that obtained in the same experimental conditions for their natural target, the bacterial A site (K(d) approximately 1.6 microM). In good agreement with their respective affinity, aminoglycoside increase the melting temperature of the loop-loop interaction and also block the conversion from kissing-loop complex to extended duplex. Taken together, our data might be useful for selecting new molecules with improved specificity and affinity toward the HIV-1 DIS RNA.

Bhadra K., Maiti M. and Kumar G. S. (2008) Berberine-DNA complexation: new insights into the cooperative binding and energetic aspects. *Biochim Biophys Acta* **1780,** 1054-1061.
Abstract: The equilibrium binding of the cytotoxic plant alkaloid berberine to various DNAs and energetics of the interaction have been studied. At low ratios of bound alkaloid to base pair, the binding exhibited cooperativity to natural DNAs having almost equal proportions of AT and GC sequences. In contrast, the binding was non-cooperative to DNAs with predominantly high AT or GC sequences. Among the synthetic DNAs, cooperative binding was observed with poly(dA).poly(dT) and poly(dG).poly(dC) while non-cooperative binding was seen with poly(dA-dT).poly(dA-dT) and poly(dG-dC).poly(dG-dC). Both cooperative and non-cooperative bindings were remarkably dependent on the salt concentration of the media. Linear plots of ln K(a) versus [Na(+)] for poly(dA).poly(dT) and poly(dA-dT).poly(dA-dT) showed the release of 0.56 and 0.75 sodium ions respectively per bound alkaloid. Isothermal titration calorimetry results revealed the binding to be exothermic and favoured by both enthalpy and entropy changes in all DNAs except the two AT polymers and AT rich DNA, where the same was predominantly entropy driven. Heat capacity values (DeltaCp(o)) of berberine binding to poly(dA).poly(dT), poly(dA-dT).poly(dA-dT), Clostridium perfringens and calf thymus DNA were -98, -140, -120 and -110 cal/mol K respectively. This study presents new insights into the binding dependent base pair heterogeneity in DNA conformation and the first complete thermodynamic profile of berberine binding to DNAs

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 x 10(4), 2.84 x 10(4), and 3.5 x 10(6) M(1) at 20 degrees C in buffer of 20 mM [Na(+)]. 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 (DeltaC(p)(o)) 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

Bhadra K., Maiti M. and Kumar G. S. (2008) Interaction of isoquinoline alkaloid palmatine with deoxyribonucleic acids: binding heterogeneity, and conformational and thermodynamic aspects. *Chem Biodivers.* **5,** 575-590.

Abstract: The binding heterogeneity, conformational aspects, and energetics of the interaction of the cytotoxic plant alkaloid palmatine have been studied with various natural and synthetic DNAs. The alkaloid binds to calf thymus and Escherichia coli DNA that have mixed AT and GC sequences in almost equal proportions with positive cooperativity, while, with Clostridium perfringens and Micrococcus lysodeikticus DNA with predominantly high AT and GC sequences, respectively, noncooperative binding was observed. On further investigation with synthetic DNAs, the binding was observed to be cooperative with polymers like poly(dA).poly(dT) and poly(dG).poly(dC) having poly(purine)poly(pyrimidine) sequences, while with polymers poly(dA-dT).poly(dA-dT), poly(dA-dC).poly(dG-dT) and poly(dG-dC).poly(dG-dC), which have alternating purine-pyrimidine sequences, a non-cooperative binding phenomenon was observed. This suggests the binding heterogeneity of palmatine to the two types of sequences of base pairs. Circular dichroism (CD) studies revealed that the binding induced conformational changes in all the DNAs, but more importantly, the bound alkaloid molecules acquired induced optical activity, and the extent was dependent on the AT content and showed AT base-pair specificity. Energetics of the interaction of the alkaloid studied by highly sensitive isothermal titration calorimetry revealed that the binding was in most cases exothermic and favored by both enthalpy and entropy changes, while, in the case of the homo and hetero AT polymers, the same was predominantly entropy-driven. This study defines base-pair-dependent heterogeneity, conformational aspects, and energetics of palmatine binding to DNA

Bhadra K., Maiti M. and Kumar G. S. (2007) Molecular recognition of DNA by small molecules: AT base pair specific intercalative binding of cytotoxic plant alkaloid palmatine. *Biochim Biophys Acta* **1770,** 1071-1080.
Abstract: The base dependent binding of the cytotoxic alkaloid palmatine to four synthetic polynucleotides, poly(dA).poly(dT), poly(dA-dT).poly(dA-dT), poly(dG).poly(dC) and poly(dG-dC).poly(dG-dC) was examined by competition dialysis, spectrophotometric, spectrofluorimetric, thermal melting, circular dichroic, viscometric and isothermal titration calorimetric (ITC) studies. Binding of the alkaloid to various polynucleotides was dependent upon sequences of base pairs. Binding data obtained from absorbance measurements according to neighbour exclusion model indicated that the intrinsic binding constants decreased in the order poly(dA).poly(dT)>poly(dA-dT).poly(dA-dT)>poly(dG-dC).poly(dG-dC)>poly(dG) .poly(dC). This affinity was also revealed by the competition dialysis, increase of steady state fluorescence intensity, increase in fluorescence quantum yield, stabilization against thermal denaturation and perturbations in circular dichroic spectrum. Among the polynucleotides, poly(dA).poly(dT) showed positive cooperativity at binding values lower than r=0.05. Viscosity studies revealed that in the strong binding region, the increase of contour length of DNA depended strongly on the sequence of base pairs being higher for AT polymers and induction of unwinding-rewinding process of covalently closed superhelical DNA. Isothermal titration calorimetric data showed a single entropy driven binding event in the AT homo polymer while that with the hetero polymer involved two binding modes, an entropy driven strong binding followed by an enthalpy driven weak binding. These results unequivocally established that the alkaloid palmatine binds strongly to AT homo and hetero polymers by mechanism of intercalation.

Bishop G. R., Ren J., Polander B. C., Jeanfreau B. D., Trent J. O., and Chaires J. B. (2007) Energetic basis of molecular recognition in a DNA aptamer. *Biophys Chem* 126, 165-75.
Abstract: The thermal stability and ligand binding properties of the l-argininamide-binding DNA aptamer (5'-GATCGAAACGTAGCGCCTTCGATC-3') were studied by spectroscopic and calorimetric methods. Differential calorimetric studies showed that the uncomplexed aptamer melted in a two-state reaction with a melting temperature T(m)=50.2+/-0.2 degrees C and a folding enthalpy DeltaH degrees (fold)=-49.0+/-2.1 kcal mol(-1). These values agree with values of T(m)=49.6 degrees C and DeltaH degrees (fold)=-51.2 kcal mol(-1) predicted for a simple hairpin structure. Melting of the uncomplexed aptamer was dependent upon salt concentration, but independent of strand concentration. The T(m) of aptamer melting was found to increase as l-argininamide concentrations increased. Analysis of circular dichroism titration data using a single-site binding model resulted in the determination of a binding free energy DeltaG degrees (bind)=-5.1 kcal mol(-1). Isothermal titration calorimetry studies revealed an exothermic binding reaction with DeltaH degrees (bind)=-8.7 kcal mol(-1). Combination of enthalpy and free energy produce an unfavorable entropy of -TDeltaS degrees =+3.6 kcal mol(-1). A molar heat capacity change of -116 cal mol(-1) K(-1) was determined from calorimetric measurements at four temperatures over the range of 15-40 degrees C. Molecular dynamics simulations were used to explore the structures of the unligated and ligated aptamer

structures. From the calculated changes in solvent accessible surface areas of these structures a molar heat capacity change of -125 cal mol(-1) K(-1) was calculated, a value in excellent agreement with the experimental value. The thermodynamic signature, along with the coupled CD spectral changes, suggest that the binding of l-argininamide to its DNA aptamer is an induced-fit process in which the binding of the ligand is thermodynamically coupled to a conformational ordering of the nucleic acid.

Breslauer K. J., Remeta D. P., Chou W. Y., Ferrante R., Curry J., Zaunczkowski D., Snyder J. G., and Marky L. A. (1987) Enthalpy-entropy compensations in drug-DNA binding studies. *Proc Natl Acad Sci U S A* **84,** 8922-8926.
Abstract: We present a comparative study of calorimetrically derived thermodynamic profiles for the binding of a series of drugs with selected DNA host duplexes. We use these data to demonstrate that comparisons between complete thermodynamic profiles (ΔG zero, ΔH zero, ΔS zero, ΔCp) are required before drug binding can be used as a probe of DNA conformation, since enthalpy-entropy compensations can cause two drug-DNA binding events to exhibit similar binding free energies (Δ G zero) despite being driven by entirely different thermodynamic forces (ΔH zero, ΔS zero). In this work, we employ a combination of spectroscopic and calorimetric techniques to characterize thermodynamically the DNA binding of netropsin and distamycin (two minor groove-directed ligands), ethidium (an intercalator), and daunomycin (a combined intercalator/groove binder). Our free energy data (ΔG zero) show that each drug exhibits similar binding affinities at 25 degrees C for the alternating copolymer duplex poly[d(A-T)].poly[d(A-T)] and for the homopolymer duplex poly(dA).poly(dT). However, our calorimetric measurements reveal that the nature of the thermodynamic forces (Δ H zero, Δ S zero) that drive drug binding to these two host duplexes at 25 degrees C are entirely different, despite similar binding free energies (Δ G zero) and similar salt dependencies (lnK/ln[Na+]). Specifically, the 25 degrees C binding of all four drugs to the alternating copolymer poly[d(A-T)].poly[d(A-T)] is overwhelmingly enthalpy driven, whereas the corresponding binding of each drug to the homopolymer duplex poly(dA).poly(dT) is overwhelmingly entropy driven. Thus, the similar binding free energies (Δ G zero) we measure for complexation of each drug with poly[d(A-T)].poly[d(A-T)] and poly(dA).poly(dT) result from compensating changes in the enthalpy and entropy terms. Comparison with the thermodynamic profiles for the complexation of these drug molecules to other DNA host duplexes at 25 degrees C reveals that the binding of each is strongly enthalpy driven, except when the poly(dA).poly(dT) homopolymer serves as the host duplex. This comparison allows us to conclude that poly[d(A-T)].poly[d(A-T)] behaves thermodynamically as the more "normal" host duplex toward drug binding, whereas the entropy-driven binding to the poly(dA).poly(dT) duplex represents "aberrant" behavior. Furthermore, since each of the four drugs exhibits different modes of DNA binding, we conclude that the observed entropy-driven behavior for binding to poly(dA).poly(dT) reflects an intrinsic property of the homopolymer duplex that is perturbed in a common manner upon ligation rather than a common property of all four binding ligands. To rationalize the large positive entropy changes that drive drug complexation with poly(dA).poly(dT) duplex, we propose a model that emphasizes binding-induced perturbations of the more highly hydrated, altered B conformation of the homopolymer. Our results suggest that an aberrant thermodynamic binding profile may reflect an unusual DNA conformation in the host duplex. However, before such a conclusion can be reached, complete thermodynamic binding profiles must be examined, since enthalpy-entropy compensations can cause two binding events to exhibit similar binding constants even when they are driven by very different thermodynamic forces.

Buchmueller K. L., Bailey S. L., Matthews D. A., Taherbhai Z. T., Register J. K., Davis Z. S., Bruce C. D., O'hare C., Hartley J. A., and Lee M. (2006) Physical and Structural Basis for the Strong Interactions of the -ImPy- Central Pairing Motif in the Polyamide f-ImPyIm. *Biochemistry* **45,** 13551-13565.
Abstract: The polyamide f-ImPyIm has a higher affinity for its cognate DNA than either the parent analogue, distamycin A (10-fold), or the structural isomer, f-PyImIm (250-fold), has for its respective cognate DNA sequence. These findings have led to the formulation of a two-letter polyamide "language" in which the -ImPy- central pairings associate more strongly with Watson-Crick DNA than -PyPy-, -PyIm-, and -ImIm-. Herein, we further characterize f-ImPyIm and f-PyImIm, and we report thermodynamic and structural differences between -ImPy- (f-ImPyIm) and -PyIm- (f-PyImIm) central pairings. DNase I footprinting studies confirmed that f-ImPyIm is a stronger binder than distamycin A and f-PyImIm and that f-ImPyIm preferentially binds CGCG over multiple competing sequences. The difference in the binding of f-ImPyIm and f-PyImIm to their cognate sequences was supported by the Na(+)-dependent nature of DNA

melting studies, in which significantly higher Na(+) concentrations were needed to match the ability of f-ImPyIm to stabilize CGCG with that of f-PyImIm stabilizing CCGG. The selectivity of f-ImPyIm beyond the four-base CGCG recognition site was tested by circular dichroism and isothermal titration microcalorimetry, which shows that f-ImPyIm has marginal selectivity for (A.T)CGCG(A.T) over (G.C)CGCG(G.C). In addition, changes adjacent to this 6 bp binding site do not affect f-ImPyIm affinity. Calorimetric studies revealed that binding of f-ImPyIm, f-PyImIm, and distamycin A to their respective hairpin cognate sequences is exothermic; however, changes in enthalpy, entropy, and heat capacity (DeltaC(p)()) contribute differently to formation of the 2:1 complexes for each triamide. Experimental and theoretical determinations of DeltaC(p)() for binding of f-ImPyIm to CGCG were in good agreement (-142 and -177 cal mol(-)(1) K(-)(1), respectively). (1)H NMR of f-ImPyIm and f-PyImIm complexed with their respective cognate DNAs confirmed positively cooperative formation of distinct 2:1 complexes. The NMR results also showed that these triamides bind in the DNA minor groove and that the oligonucleotide retains the B-form conformation. Using minimal distance restraints from the NMR experiments, molecular modeling and dynamics were used to illustrate the structural complementarity between f-ImPyIm and CGCG. Collectively, the NMR and ITC experiments show that formation of the 2:1 f-ImPyIm-CGCG complex achieves a structure more ordered and more thermodynamically favored than the structure of the 2:1 f-PyImIm-CCGG complex.

Buurma N. J. and Haq I. (2007) Advances in the analysis of isothermal titration calorimetry data for ligand-DNA interactions. *Methods* **42,** 162-172.
Abstract: Isothermal titration calorimetry (ITC) is a well established technique for the study of biological interactions. The strength of ITC is that it directly measures enthalpy changes associated with interactions. Experiments can also yield binding isotherms allowing quantification of equilibrium binding constants, hence an almost complete thermodynamic profile can be established. Principles and application of ITC have been well documented over recent years, experimentally the technique is simple to use and in ideal scenarios data analysis is trivial. However, ITC experiments can be designed such that previously inaccessible parameters can be evaluated. We outline some of these advances, including (1) exploiting different experimental conditions; (2) low affinity systems; (3) high affinity systems and displacement assays. In addition we ask the question: What if data cannot be fit using the fitting functions incorporated in the data-analysis software that came with your ITC? Examples where such data might be generated include systems following non 1:n binding patterns and systems where binding is coupled to other events such as ligand dissociation. Models dealing with such data are now appearing in literature and we summarise examples relevant for the study of ligand-DNA interactions.

Buurma N. J. and Haq I. (2008) Calorimetric and spectroscopic studies of Hoechst 33258: self-association and binding to non-cognate DNA. *J Mol Biol* **381,** 607-621.
Abstract: Sequence and structure-specific molecular recognition of DNA by small molecules is an important goal in biophysical chemistry and drug discovery. Many candidate ligands possess flat aromatic surfaces and other molecular features that allow them to self-associate. In addition, non-specific binding to the target is a complicating feature of these interactions. Therefore, multiple equilibria are present and need to be accounted for in data analysis in order to obtain meaningful thermodynamic parameters. In order to address these issues we have systematically examined the bis-benzimidazole dye Hoechst 33258 (H33258) in terms of self-aggregation and binding to DNA oligonucleotides lacking any cognate minor groove A.T sites. This model system has been interrogated using isothermal titration calorimetry (ITC), circular dichroism (CD), fluorescence spectroscopy and pulsed gradient spin echo NMR. Three distinct binding events and ligand self-aggregation have been identified and, where possible, quantified. H33258 self-aggregation involves a step-wise aggregation mechanism, driven by stacking interactions. The DNA binding process includes two specific binding modes and non-specific DNA-templated H33258 stacking. We have written novel ITC data-fitting software (IC-ITC; freely available to the biophysics community), which simultaneously fits ligand aggregation and ligand-DNA binding. Here, this numerical analysis, which uses simulated annealing of complex calorimetric data representing multiple coupled equilibria, is described

Cai P., Huang Q., Jiang D., Rong X., and Liang W. (2006) Microcalorimetric studies on the adsorption of DNA by soil colloidal particles. *Colloids Surf B Biointerfaces* **49,** 49-54.
Abstract: This study applied TAM air isothermal calorimeter to measure the adsorption enthalpies of DNA

on eight colloidal fractions from permanent-charge and variable-charge soils. The adsorption of DNA on soil colloids was also examined by equilibrium adsorption analysis. The data evaluated from isotherms fitted by Langmuirean model revealed that the affinity of DNA for variable-charge soil colloids was higher than that for permanent-charge soil colloids. More tightly bound DNA molecules were observed on coarse clays and inorganic clays than on fine clays and organic clays, respectively. The adsorption enthalpies of DNA on permanent-charge soil colloids were higher than those on variable-charge soil colloids. DNA adsorption on organic clays is endothermic, whereas that on inorganic clays is exothermic. Dehydration and electrostatic repulsion were considered to cause the higher adsorption enthalpies of DNA with organic clays, while hydrogen bonding, ligand exchange and electrostatic attraction result in the lower DNA adsorption enthalpies on inorganic clays. The thermodynamic parameters presented in this study have important implication for providing further insight into mechanisms of the adsorption of DNA on soil particles.

Cao M., Deng M., Wang X. L. and Wang Y. (2008) Decompaction of cationic gemini surfactant-induced DNA condensates by beta-cyclodextrin or anionic surfactant. *J Phys. Chem B* **112,** 13648-13654.
Abstract: Compaction of DNA by cationic gemini surfactant hexamethylene-1,6-bis-(dodecyldimethylammoniumbromide) (C12C6C12Br2) and the subsequent decompaction of the DNA-C12C6C12Br2 complexes by beta-cyclodextrin (beta-CD) or sodium dodecyl sulfate (SDS) have been studied by using zeta potential and particle size measurements, atomic force microscopy (AFM), isothermal titration microcalorimetry (ITC), and circular dichroism. The results show that C12C6C12Br2 can induce the collapse of DNA into densely packed bead-like structures with smaller size in an all-or-none manner, accompanied by the increase of zeta potential from highly negative values to highly positive values. In the decompaction of the DNA-C12C6C12Br2 complexes, beta-CD and SDS exhibit different behaviors. For beta-CD, the experimental results suggest that it can remove the outlayer hydrophobically bound C12C6C12Br2 molecules from the DNA-C12C6C12Br2 complexes by inclusion interaction, and the excess beta-CD may attach on the complexes by forming inclusion complexes with the hydrocarbon chains of the electrostatically bound C12C6C12Br2 that cannot be removed. The increase of steric hindrance due to the attachment of beta-CD molecules results in the decompaction of the DNA condensates though the true release of DNA cannot be attained. However, for SDS, the experimental results suggest that it can realize the decompaction and release of DNA from its complexes with C12C6C12Br2 due to both ion-pairing and hydrophobic interaction between SDS and C12C6C12Br2

Carrasco C., Vezin H., Wilson W. D., Ren J., Chaires J. B., and Bailly C. (2001) DNA binding properties of the indolocarbazole antitumor drug NB-506. *Anticancer Drug Des* **16,** 99-107.
Abstract: Indolocarbazoles derived from the antibiotic rebeccamycin represent an important group of antitumor agents. Several indolocarbazoles are currently undergoing clinical trials. These compounds inhibit topoisomerase 1 to produce DNA breaks that are responsible for cell death. Unlike classical topoisomerase I poisons like camptothecin, glycosyl indolocarbazoles can form stable complexes with DNA even in the absence of topoisomerase I. At least in part, their mode of action is reminiscent of that of the anthracyclines, which also bind to nucleic acids and interfere with topoisomerase II. The lead synthetic compound in the series is the uncharged drug NB-506, which bears a glucose residue attached to the indolocarbazole chromophore substituted with two hydroxyl groups at positions 1 and 11. Here we report a detailed biophysical study aimed at characterizing the DNA binding properties of NB-506. Molecular modeling was used to compare the conformation and electronic properties of NB-506 and its analogue ED-571 bearing the two hydroxyl groups at positions 2 and 10. Surface plasmon resonance experiments, performed with DNA hairpin oligomers, indicate that NB-506 binds almost equally well to both AT and GC base pairs, and the binding affinity ($K = 10^5\ M^{-1}$) is similar to that of certain classical intercalators such as amsacrine and bisantrene. Isothermal titration calorimetry experiments show that the binding of NB-506 is enthalpy-driven (ΔH = -7.2 kcal/mol). The binding enthalpy measured for NB-506 is similar to that obtained with doxorubicin but the DNA interaction processes for the two drugs differ markedly in terms of entropy and ΔG. The free energy of NB-506 binding to DNA is considerably less favorable than that of doxorubicin. These biophysical data help us to understand further how rebeccamycin-type anticancer drugs interact with DNA.

Chaires J. B. (2006) A thermodynamic signature for drug-DNA binding mode. *Arch Biochem Biophys* **453,** 26-31.

Abstract: A number of small molecules bind directly and selectively to DNA, acting as chemotherapeutic agents by inhibiting replication, transcription or topoisomerase activity. Two common binding modes for these small molecules are intercalation or groove-binding. Intercalation results from insertion of a planar aromatic substituent between DNA base pairs, with concomitant unwinding and lengthening of the DNA helix. Groove binding, in contrast, does not perturb the duplex structure to any great extent. Groove-binders are typically crescent-shaped, and fit snugly into the minor groove with little distortion of the DNA structure. Recent calorimetric studies have determined the enthalpic and entropic contributions to the DNA binding of representative DNA binding compounds. Analysis of such thermodynamic data culled from the literature reveals distinctive thermodynamic signatures for groove-binding and intercalating compounds. Plots of the binding enthalpy (DeltaH) against binding entropy (-TDeltaS) for 26 drug-DNA interactions reveal that groove-binding interactions are clustered in a region of the graph with favorable entropy contributions to the free energy, while intercalators are clustered in a region with unfavorable entropy but favorable enthalpy contributions. Groove-binding is predominantly entropically driven, while intercalation in enthalpically driven. The molecular basis of the contrasting thermodynamic signatures for the two binding modes is by no means clear, but the pattern should be of use in categorizing new DNA binding agents.

Chaires J. B., Satyanarayana S., Suh D., Fokt I., Przewloka T., and Priebe W. (1996) Parsing the free energy of anthracycline antibiotic binding to DNA. *Biochemistry* **35,** 2047-2053.
Abstract: The DNA binding free energy of eight anthracycline antibiotics was determined as a function of NaCl concentration. Compounds were chosen for study that differed from the parent compounds, doxorubicin or daunorubicin, at a single chemical substituent. Determination of the salt concentration dependence of the binding constant allowed us to dissect the DNA binding free energy of each compound into its component nonelectrostatic and polyelectrolyte contributions. Comparison of the nonelectrostatic free energy contribution allowed us to evaluate the net energetic contribution of specific functional groups to DNA binding. These quantitative data revealed a surprisingly large and favorable energetic contribution (2 kcal (mol^{-1})) of the groove-binding daunosamine moiety and a substantial energetic penalty for alteration of its stereochemistry. The energetic cost of removal of hydroxyl groups at the C-9 and C-14 positions (which structural studies indicate may participate in hydrogen-bonding interactions with the DNA) was approximately 1 kcal mol^{-1}. Replacement of the 3'-amino group with a hydroxyl group led to a loss of 0.7 kcal mol^{-1} in binding free energy, above and beyond the energetic penalty resulting from the removal of its positive charge from the antibiotic. The results and analysis presented here provide a rigorous and detailed description of structure-DNA affinity relationships among anthracycline antibiotics. The results are of general interest in understanding how total ligand binding free energies are partitioned among substituents and will be useful in the formulation of rules for the rational design of novel DNA binding agents.

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 (ΔG, ΔH, ΔS, ΔCp) 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. (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.

Chaires J. B. (2001) Analysis and interpretation of ligand-DNA binding isotherms. *Methods Enzymol* **340,** 3-22.
Abstract: Binding studies provide information of fundamental and central importance for the complete understanding of ligand-DNA interactions. Studies of ligand binding to long natural DNA samples, to synthetic deoxypolynucleotides of simple repeating sequence, and to oligonucleotides of defined sequence are all needed to begin to understand the interaction in detail. Binding studies provide entry into the thermodynamics of the DNA interactions, which in turn provides great insight into the molecular forces that drive the binding process. This chapter summarizes both model-dependent and -independent approaches for the analysis and interpretation of binding isotherms, and should serve as a concise guide for handling experimental data.

Charles I., Xi H. and Arya D. P. (2007) Sequence-specific targeting of RNA with an oligonucleotide-neomycin conjugate. *Bioconjug. Chem* **18,** 160-169.
Abstract: The synthesis of neomycin covalently attached at the C5-position of 2'-deoxyuridine is reported. The synthesis outlined allows for incorporation of an aminoglycoside (neomycin) at any given site in an oligonucleotide (ODN) where a thymidine (or uridine) is present. Incorporation of this modified base into an oligonucleotide, which is complementary to a seven-bases-long alpha-sarcin loop RNA sequence, leads to enhanced duplex hybridization. The increase in Tm for this duplex (DeltaTm = 6 degrees C) suggests a favorable interaction of neomycin within the duplex groove. CD spectroscopy shows that the modified duplex adopts an A-type confirmation. ITC measurements indicate the additive effects of ODN and neomycin binding to the RNA target (Ka = 4.5 x 107 M-1). The enhanced stability of the hybrid duplex from this neomycin-ODN conjugate originates primarily from the enthalpic contribution of neomycin {DeltaDeltaHobs = -7.21 kcal/mol (DeltaHneomycin conjugated - DeltaH nonconjugated)} binding to the hybrid duplex. The short linker length allows for selective stabilization of the hybrid duplex over the hybrid triplex. The results described here open up new avenues in the design and synthesis of nucleo-aminoglycoside-conjugates (N-Ag-C) where the inclusion of any number of aminoglycoside (neomycin) molecules per oligonucleotide can be accomplished.

Chatterjee A., Moulik S. P., Majhi P. R., and Sanyal S. K. (2002) Studies on surfactant-biopolymer interaction. I. Microcalorimetric investigation on the interaction of cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulfate (SDS) with gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA). *Biophys Chem* **98,** 313-327.
Abstract: The interaction of the surfactants cetyltrimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) with the biopolymers gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA) was studied by isothermal titration microcalorimetry at varied biopolymer concentration, pH and temperature. The nature of interaction of the surfactants with the biopolymers was assessed from the observed enthalpy-[surfactant] profiles. The biopolymer-induced aggregation of the surfactants was observed. The enthalpies of aggregation of amphiphiles, binding of aggregates with macromolecules, organisational change of bound aggregates, and threshold concentrations for micelle formation of surfactants in the presence of biopolymers were estimated. The results collected on the three biopolymers were analysed and compared.

Chou W. Y., Marky L. A., Zaunczkowski D., and Breslauer K. J. (1987) The thermodynamics of drug-DNA interactions: ethidium bromide and propidium iodide. *J Biomol Struct Dyn* **5,** 345-359.
Abstract: We report the first calorimetrically-derived characterization of the thermodynamics of ethidium bromide (EB) and propidium iodide (PI) binding to a series of nucleic acid host duplexes. Our spectroscopic and calorimetric measurements yield the following results: 1) At low salt (16mM Na^+) and 25 degrees C. PI binds more strongly than EB to a given host duplex. The magnitude of this PI preference depends only marginally on base sequence, with AT base pairs showing a greater PI preference than GC base pairs. 2) The enhanced binding of PI relative to EB at low salt and 25 degrees C reflects a more favorable entropic driving force for PI binding. 3) The PI binding preference diminishes at higher salt concentrations (216mM). In other words, the binding preference is electrostatic in origin. 4) The salt dependence of the binding constants ($\Delta \ln K_b / \Delta \ln[Na^+]$) reveal that PI binds as a dication while EB binds as a monocation. 5) PI and EB both exhibit impressive enthalpy-entropy compensations when they bind to the deoxy homopolymers poly dA.poly dT and poly dA.poly dU. We have observed a similar enthalpy-entropy compensation for netropsin binding to the poly dA.poly dT homopolymer duplex. We therefore conclude that the compensation phenomenon is an intrinsic property of the host duplex rather than reflecting a property of the binding ligand. 6) When either PI or EB bind to the corresponding ribo homopolymer (poly rA.poy rU) we do not observe the enthalpy-entropy compensation that characterizes the binding to the deoxy homopolymer. 7) EB and PI both bind more strongly to poly d(AT).poly d(AT) than to poly d(AU).poly d(AU). Specifically, the absence of the thymine methyl group in poly d(AU).poly d(AU) reduces the binding constant of both drugs by a factor of four. This reduction in binding is due to a less favorable entropy change. In this paper we present and discuss possible molecular origins for our observed thermodynamic and extra-thermodynamic data. In particular, we evoke solvent effects involving both the drugs and the host duplexes when we propose molecular interpretations which are consistent with our thermodynamic data.

Coles D. J., Yang S., Minchin R. F. and Toth I. (2008) The characterization of a novel dendritic system for gene delivery by isothermal titration calorimetry. *Biopolymers* **90,** 651-654.
Abstract: Understanding the nature of binding of polycationic dendrimers to DNA provides useful information on their role in gene delivery. In the present study, we have characterized the interaction of several peptide-based polycationic dendrimers with salmon sperm DNA using isothermal titration calorimetry. The dendrimers consisted of the cell penetrating peptide TAT, a nuclear localization signal peptide and dendritic polylysine. The binding affinity and thermodynamic parameters were found to increase as the number of positive charges on the dendrimer increased, indicating that ionic interactions were the major binding forces between the two molecules. The effect of acidic pH (3.2) compared to a more neutral pH (7.2) was also examined. The binding affinity was stronger at the lower pH but precipitation of the complex was more prominent at pH 7.2 which was shown by large enthalpies. The results indicate that our dendrimers are forming stable complexes with DNA

Cong X. and Nilsen-Hamilton M. (2005) Allosteric aptamers: targeted reversibly attenuated probes. *Biochemistry* **44,** 7945-7954.
Abstract: Aptamers are unique nucleic acids with regulatory potentials that differ markedly from those of proteins. A significant feature of aptamers not possessed by proteins is their ability to participate in at least two different types of three-dimensional structure: a single-stranded folded structure that makes multiple contacts with the aptamer target and a double-helical structure with a complementary nucleic acid sequence. We have made use of this structural flexibility to develop an aptamer-based biosensor (a targeted reversibly attenuated probe, TRAP) in which hybridization of a cis-complementary regulatory nucleic acid (attenuator) controls the ability of the aptamer to bind to its target molecule. The central portion of the TRAP, between the aptamer and the attenuator, is complementary to a target nucleic acid, such as an mRNA, which is referred to as a regulatory nucleic acid (regNA) because it regulates the activity of the aptamer in the TRAP by hybridization with the central (intervening) sequence. The studies reported here of the ATP-DNA TRAP suggest that, as well as inhibiting the aptamer, the attenuator also acts as a structural guide, much like a chaperone, to promote proper folding of the TRAP such that it can be fully activated by the regDNA. We also show that activation of the aptamer in the TRAP by the complementary nucleic acid at physiological temperatures is sensitive to single-base mismatches. Aptamers that can be regulated by a specific nucleic sequence such as in an mRNA have potential for many in vivo applications including regulating a particular enzyme or signal transduction pathway or imaging gene expression in vivo.

Cowan J. A., Ohyama T., Wang D., and Natarajan K. (2000) Recognition of a cognate RNA aptamer by neomycin B: quantitative evaluation of hydrogen bonding and electrostatic interactions. *Nucleic Acids Res* **28,** 2935-2942.
Abstract: Aminoglycosides are an important class of antibiotic that selectively target RNA structural motifs. Recently we have demonstrated copper derivatives of amino-glycosides to be efficient cleavage agents for cognate RNA motifs. To fully develop their potential as pharmaceutical agents it is necessary to understand both the structural mechanisms used by aminoglycosides to target RNA, and the relative contributions of hydrogen bonding and electrostatic interactions to recognition selectivity. Herein we report results from a calorimetric analysis of a stem-loop 23mer RNA aptamer complexed to the aminoglycoside neomycin B. Key thermodynamic parameters for complex formation have been determined by isothermal titration calorimetry, and from the metal-ion dependence of these binding parameters the relative contributions of electrostatics and hydrogen bonding toward binding affinity have been assessed. The principal mechanism for recognition and binding of neomycin B to the RNA major groove is mediated by hydrogen bonding.

Crenshaw J. M., Graves D. E., and Denny W. A. (1995) Interactions of acridine antitumor agents with DNA: binding energies and groove preferences. *Biochemistry* **34,** 13682-13687.
Abstract: Absorbance spectroscopy is used to examine the thermodynamic properties associated with the interaction of the experimental antitumor agents N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (AAC) and N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) with nucleic acids. Placement of the amino substituent at the C9 position on the acridine ring results in marked changes to the acridine chromophore's electronic properties, with the overall charge of AAC increasing to +2 in comparison to DACA's charge of +1 at neutral pH. In comparative DNA binding studies, we examine the influence that the electrostatic properties of these ligands have on the binding energies as well as their effects on enthalpy and entropy contributions. These studies show that placement of the amino moiety at C9 results in 6 times greater DNA binding affinity as compared the deamino analog (DACA). Comparisons of ionic strength dependence for these two analogs reveal a difference in the binding energies of the compounds which can be attributed to electrostatic effects. Further dissection of the enthalpy and entropy components of the binding energy reveals the enhanced electrostatic effects are related to an increased entropy contribution upon formation of the AAC-DNA complex. Groove selectivity of these acridine analogs was probed by examining the binding profiles to native and groove-modified DNAs which included glycosylated T4 DNA and the distamycin-DNA complex. These studies are indicative of minor groove interactions for both compounds with DNA.

Degtyareva N. N., Fresia M. J. and Petty J. T. (2007) DNA Conformational Effects on the Interaction of Netropsin with A-tract Sequences. *Biochemistry* **46,** 15136-15143.
Abstract: The influence of cosolutes and DNA sequence on the interaction of netropsin with three duplexes has been studied by isothermal titration calorimetry. In buffer, netropsin forms two complexes with a net stoichiometry of 1:1 in the minor groove of the oligonucleotide (GCGCGAATTCGCGC)2. One complex has a weaker affinity and is more enthalpically favored relative to the other one, consistent with previous studies [Freyer, M. W., et al. (2006) Biophys. Chem. 126, 186-196]. With the cosolutes betaine and 2-methyl-2,4-pentanediol, the enthalpy and heat capacity changes indicate that the complex with weaker affinity is disfavored relative to the complex with higher affinity. With (CGCGCAATTGCGCG)2, netropsin has one binding mode in buffer, and complex formation is not influenced by the cosolutes. The similarities of the enthalpy and heat capacity changes suggest that netropsin interacts similarly with these two oligonucleotides in the presence of cosolutes. The oligonucleotide (GCGCAAATTTGCGC)2 also forms two complexes with netropsin, and the complex with weaker affinity is again disfavored by the cosolutes. Thus, the interaction of netropsin with these A/T binding sites is influenced both by the bases adjacent to the binding site and by cosolutes. We suggest that these two factors influence the conformation of the minor-groove binding site of DNA.

Delehanty J.B., Stuart T.C., Knight D.A., Goldman E.R., Thach D.C., Bongard J.E., and Chang E.L. (2005) RNA hydrolysis and inhibition of translation by a Co(III)-cyclen complex. *RNA.* **11**, 831-6.
Abstract: Metal ion-chelator catalysts based on main-group, lanthanide, or transition metal complexes have been developed as nonenzymatic alternatives for the hydrolysis of the phosphodiester bonds in DNA and RNA. Cobalt (III), with its high-charge density, is known for its ability to hydrolyze phosphodiesters

with rate constants as high as 2 x 10(-4) s(-1). We have developed a kinetically inert Co(III)-cyclen-based complex, Co(III)-cycmmb that is very potent in inhibiting the translation of RNA into protein. Contact time as short as 10 min is sufficient to achieve the complete inhibition of the translation of a concentrated luciferase RNA solution into the enzyme in a cell-free translation system. The inhibition appears to proceed through two pathways. The first pathway involves the kinetic or substitutional inertness of Co(III) for the RNA template at short contact times. This interaction is mediated through the kinetic inertness of Co(III) for the phosphate groups of the nucleotides, as well as coordination of Co(III) to the nitrogenous bases. The second pathway occurs at longer contact times and is mediated by the hydrolysis of the phosphodiester backbone. This report represents the first demonstrated use of a metal-chelate complex to achieve the inhibition of the translation of RNA into protein. This Co(III) system can be useful in its present nonsequence-specific form as a novel viral decontamination agent. When functionalized to recognize specific nucleic acid sequences, such a system could potentially be used in gene-silencing applications as an alternative to standard antisense or RNAi technologies.

Dignam J. D., Qu X., Ren J. and Chaires J. B. (2007) Daunomycin binding to detergent micelles: a model system for evaluating the hydrophobic contribution to drug-DNA interactions. *J Phys. Chem B* **111,** 11576-11584.

Abstract: The interaction of daunomycin with sodium dodecyl sulfate and Triton X-100 micelles was investigated as a model for the hydrophobic contribution to the free energy of DNA intercalation reactions. Measurements of visible absorbance, fluorescence lifetime, steady-state fluorescence emission intensity, and fluorescence anisotropy indicate that the anthraquinone ring partitions into the hydrophobic micelle interior. Fluorescence quenching experiments using both steady-state and lifetime measurements demonstrate reduced accessibility of daunomycin in sodium dodecyl sulfate micelles to the anionic quencher iodide and to the neutral quencher acrylamide. Quenching of daunomycin fluorescence by iodide in Triton X-100 micelles was similar to that seen with free daunomycin. Studies of the energetics of the interaction of daunomycin with micelles by fluorescence and absorbance titration methods and by isothermal titration calorimetry in the presence of excess micelles revealed that association with sodium dodecyl sulfate and Triton X-100 micelles is driven by a large negative enthalpy. Association of the drug with both types of micelles also has a favorable entropic contribution, which is larger in magnitude for Triton X-100 micelles than for sodium dodecyl sulfate micelles. The thermodynamic profile for the interaction of daunomycin with both types of micelles is characteristic of the "nonclassical" hydrophobic effect. The enthalpy for the interaction of daunomycin with sodium dodecyl sulfate micelles increases nonlinearly with temperature, indicating a positive (and temperature dependent) heat capacity change. The binding isotherm for daunomycin association with sodium dodecyl sulfate micelles was cooperative, with a Hill coefficient of 1.6. The cooperative behavior and the positive heat capacity change suggest that the drug alters micelle size or imposes order on the hydrocarbon interior of the micelle.

Duff M. R., Tan W. B., Bhambhani A., Perrin B. S., Jr., Thota J., Rodger A., and Kumar C. V. (2006) Contributions of hydroxyethyl groups to the DNA binding affinities of anthracene probes. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110,** 20693-20701.

Abstract: Contributions of hydroxyethyl functions to the DNA binding affinities of substituted anthracenes are evaluated by calorimetry and spectroscopy. Isothermal titration calorimetry indicated that binding of the ligands to calf thymus DNA (5 mM Tris buffer, 50 mM NaCl, pH 7.2, 25 degrees C) is exothermic. The binding constants increased from 1.5 x 10(4) to 1.7 x 10(6) M(-1) as a function of increase in the number of hydroxyethyl functions (0-4). DNA binding was accompanied by red-shifted absorption (approximately 630 cm(-1)), strong hypochromism (>65%), positive induced-circular dichroism bands, and negative linear dichroism signals. DNA binding, in general, increased the helix stabilities to a significant extent (DeltaT(m) approximately 7 degrees C, DeltaDeltaH approximately 3 kcal/mol, DeltaDeltaS approximately 6-20 cal/K.mol). The binding constants showed a strong correlation with the number of hydroxyethyl groups present on the anthracene ring system. Analysis of the binding data using the hydrophobicity parameter (Log P) showed a poor correlation between the binding affinity and hydrophobicity. This observation was also supported by a comparison of the affinities of probes carrying N-ethyl (Kb = 0.8 x 10(5) M(-1)) versus N-hydroxyethyl side chains (Kb = 5.5 x 10(5) M(-1)). These are the very first examples of a strong quantitative correlation between the DNA binding affinity of a probe and the number of hydroxyethyl groups present on the probe. These quantitative findings are useful in the rational design of new ligands for high-affinity binding to DNA.

Feig A. L. (2007) Applications of isothermal titration calorimetry in RNA biochemistry and biophysics. *Biopolymers* **87,** 293-301.
Abstract: Isothermal titration calorimetry (ITC) has been applied to the study of proteins for many years. Its use in the biophysical analysis of RNAs has lagged significantly behind its use in protein biochemistry, however, in part because of the relatively large samples required. As the instrumentation has become more sensitive, the ability to obtain high quality data on RNA folding and RNA ligand interactions has improved dramatically. This review provides an overview of the ITC experiment and describes recent work on RNA systems that have taken advantage of its versatility for the study of small molecule binding, protein binding, and the analysis of RNA folding.

Ferreira J. M. and Sheardy R. D. (2006) Enthalpy of the B-to-Z conformational transition of a DNA oligonucleotide determined by isothermal titration calorimetry. *Biophys J* **91,** 3383-3389.
Abstract: The influence of high concentrations of Na(+) or [Co(NH(3))(6)](3+) on the conformation of two related DNA oligomers was investigated by circular dichroism spectropolarimetry (CD), isothermal titration calorimetry (ITC), and differential scanning calorimetry (DSC). As revealed by CD, DNA oligomers, (dC-dG)(4) and (dm(5)C-dG)(4), both form right-handed double helical structures (B-DNA) in standard phosphate buffer with 115 mM Na(+) at 25 degrees C. However, at 2.0 M Na(+) or 200 microM [Co(NH(3))(6)](3+), (dm(5)C-dG)(4) assumes a left-handed double helical structure (Z-DNA), whereas the unmethylated (dC-dG)(4) analog remains right-handed under those conditions. ITC was then used to determine the enthalpy change upon increasing the concentration of either Na(+) or [Co(NH(3))(6)](3+) for both DNA oligomers at 25 degrees C. The titration with Na(+) resulted in endothermic isotherms with (dm(5)C-dG)(4) being more endothermic than (dC-dG)(4) by 700 cal/mol basepair. In contrast, titration with [Co(NH(3))(6)](3+) resulted in exothermic isotherms with (dC-dG)(4) being more exothermic than (dm(5)C-dG)(4) by 720 cal/mol basepair. We attribute the enthalpy difference to the conformational transition from B-form DNA to Z-form DNA for (dm(5)C-dG)(4), a transition which does not occur for the unmethylated (dC-dG)(4). The value of approximately 700 cal/mol basepair for the enthalpy of the B-Z transition compares favorably with previously published results obtained by different techniques. DSC was used to monitor the duplex to single strand transitions for both oligomers under the different concentrations. These results indicated that methylation of the cytidine destabilizes (dm(5)C-dG)(4) relative to (dC-dG)(4). Coupling the DSC data with the ITC data allowed construction of a thermodynamic cycle which gives insight into the influence of both temperature and ionic strength on the heat content of the two DNA systems studied. Further, this study reveals the utility of using ITC for determinations of transition enthalpies with the appropriate choice of control.

Freyer M. W., Buscaglia R., Hollingsworth A., Ramos J., Blynn M., Pratt R., Wilson W. D. and Lewis E. A. (2007) Break in the heat capacity change at 303 K for complex binding of netropsin to AATT containing hairpin DNA constructs. *Biophys J* **92,** 2516-2522.
Abstract: Studies performed in our laboratory demonstrated the formation of two thermodynamically distinct complexes on binding of netropsin to a number of hairpin-forming DNA sequences containing AATT-binding regions. These two complexes were proposed to differ only by a bridging water molecule between the drug and the DNA in the lower affinity complex. A temperature-dependent isothermal titration calorimetry (ITC)-binding study was performed using one of these constructs (a 20-mer hairpin of sequence 5'-CGAATTCGTCTCCGAATTCG) and netropsin. This study demonstrated a break in the heat capacity change for the formation of the complex containing the bridging water molecule at approximately 303 K. In the plot of the binding enthalpy change versus temperature, the slope (DeltaCp) was -0.67 kcal mol-1 K-1 steeper after the break at 303 K. Because of the relatively low melting temperature of the 20-mer hairpin (341 K (68 degrees C)), the enthalpy change for complex formation might have included some energy of refolding of the partially denatured hairpin, giving the suggestion of a larger DeltaCp. Studies done on the binding of netropsin to similar constructs, a 24-mer and a 28-mer, with added GC basepairs in the hairpin stem to increase thermal stability, exhibit the same nonlinearity in DeltaCp over the temperature range of from 275 to 333 K. The slopes (DeltaCp) were -0.69 and -0.64 kcal mol-1 K-1 steeper after 303 K for the 24-mer and 28-mer, respectively. This observation strengthens the argument regarding the presence of a bridging water molecule in the lower affinity netropsin/DNA complex. The DeltaCp data seem to infer that because the break in the heat capacity change function for the lower affinity binding occurs at the isoequilibrium temperature for water, water may be included or trapped in the complex. The fact that this

break does not occur in the heat capacity change function for formation of the higher affinity complex can similarly be taken as evidence that water is not included in the higher affinity complex.

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 porphryin 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 x 10(7) M(-1) and the two lowest-affinity sites exhibit a K(2) of 4.2 x 10(5) 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 degrees C and T(m)2 = 91.2 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., Cashman D., Hyslop S., Wilson W. D., Chaires J. B., and Lewis E. A. (2007) Binding of netropsin to several DNA constructs: Evidence for at least two different 1:1 complexes formed from an -AATT-containing ds-DNA construct and a single minor groove binding ligand. *Biophys Chem.* **126,** 186-96.

Abstract: Isothermal titration calorimetry, ITC, has been used to determine the thermodynamics (DeltaG, DeltaH, and -TDeltaS) for binding netropsin to a number of DNA constructs. The DNA constructs included: six different 20-22mer hairpin forming sequences and an 8-mer DNA forming a duplex dimer. All DNA constructs had a single -AT-rich netropsin binding with one of the following sequences, (A(2)T(2))(2), (ATAT)(2), or (AAAA/TTTT). Binding energetics are less dependent on site sequence than on changes in the neighboring single stranded DNA (hairpin loop size and tail length). All of the 1:1 complexes exhibit an enthalpy change that is dependent on the fractional saturation of the binding site. Later binding ligands interact with a significantly more favorable enthalpy change (partial differentialDeltaH(1-2) from 2 to 6 kcal/mol) and a significantly less favorable entropy change (partial differential(-TDeltaS(1-2))) from -4 to -9 kcal/mol). The ITC data could only be fit within expected experimental error by use of a thermodynamic model that includes two independent binding processes with a combined stoichiometry of 1 mol of ligand per 1 mol of oligonucleotide. Based on the biophysical evidence reported here, including theoretical calculations for the energetics of "trapping" or structuring of a single water molecule and molecular docking computations, it is proposed that there are two modes by which flexible ligands can bind in the minor groove of duplex DNA. The higher affinity binding mode is for netropsin to lay along the floor of the minor groove in a bent conformation and exclude all water from the groove. The slightly weaker binding mode is for the netropsin molecule to have a slightly more linear conformation and for the required curvature to be the result of a water molecule that bridges between the floor of the minor groove and two of the amidino nitrogens located at one end of the bound netropsin molecule.

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) 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 = 1.7x10(8) M(-1) (ITC), K = 2.4x10(8) M(-1) (DSC), and K = 2.9x10(8) M(-1) (SPR). DSC and SPR techniques have an advantage over ITC in studies of ligands that bind with affinities greater than 10(8) M(-1). The ITC technique has the advantage of determining a full set of thermodynamic parameters, including deltaH, TdeltaS, and deltaC(p) in addition to deltaG (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.

Ghaderi M., Bathaie S. Z., Saboury A. A., Sharghi H. and Tangestaninejad S. (2007) Interaction of an Fe derivative of TMAP (Fe(TMAP)OAc) with DNA in comparison with free-base TMAP. *Int J Biol Macromol.* **41,** 173-179.
Abstract: We investigated the interaction of meso-tetrakis (N-para-methylanilium) porphyrin (TMAP) in its free base and Fe(II) form (Fe(TMAP)OAc) as a new derivative, with high molecular weight DNA at different ionic strengths, using various spectroscopic methods and microcalorimetry. The data obtained by spectrophotometery, circular dichroism (CD), fluorescence quenching and resonance light scattering (RLS) have demonstrated that TMAP association with DNA is via outside binding with self-stacking manner, which is accompanied with the "end-on" type complex formation in low ionic strength. However, in the case of Fe(TMAP)OAc, predominant mode of interaction is groove binding and after increasing in DNA concentration, unstable stacking-type aggregates are formed. In addition, isothermal titration calorimetric measurements have indicated the exothermic process of porphyrins binding to DNA, but the exothermisity in metal derivative of porphyrin is less than the free base. It confirmed the formation of a more organized aggregate of TMAP on DNA surface. Interactions of both porphyrins with DNA show high sensitivity to ionic strength. By addition of salt, the downfield CD signal of TMAP aggregates is shifted to a higher wavelength, which indicates some changes in the aggregates position. In the case of Fe(TMAP)OAc, addition of salt leads to changes in the mode of binding from groove binding to outside binding with self-stacking, which is accompanied with major changes in CD spectra, possibly indicating the formation of "face-on" type complex.

Giri P., Hossain M., and Kumar G. S. (2006) Molecular aspects on the specific interaction of cytotoxic plant alkaloid palmatine to poly(A). *Int J Biol Macromol* **39,** 210-221.
Abstract: The interaction of the protoberberine alkaloid palmatine with single and double stranded structures of poly(A) was studied by various biophysical techniques. Comparative binding studies were also performed with double stranded DNA, t-RNA, poly(C).poly(G), poly(U) and poly(C). The results of competition dialysis, fluorescence, and absorption spectral studies converge to reveal the molecular aspects of the strong and specific binding of palmatine to single stranded poly(A). The binding affinity of palmatine to natural DNA, t-RNA and double stranded poly(A) was weaker while no binding was apparent with single stranded poly(U), poly(C) and double stranded poly(C).poly(G). The strong affinity of the alkaloid to single stranded poly(A) in comparison to the double stranded structure was also revealed from circular dichroic and viscometric studies. The effect of [Na(+)] ion concentration on the binding process revealed the significant role of electrostatic forces in the complexation. The presence of bound alkaloid also remarkably affected denaturation-renaturation of stacked helical poly(A). The energetics of the strong binding to poly(A) was studied from thermodynamic estimation from van Hoff' analysis of the temperature dependent binding constants and ultra sensitive isothermal titration calorimertry, both suggesting the binding to be exothermic and enthalpy driven. This study provides detailed insight into the binding specificity of the natural alkaloid to single stranded poly(A) over several other single and double stranded nucleic acid structures suggesting its potential as a lead compound for RNA based drug targeting.

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 3.6-4.6 x 10(6) 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) 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

Gourishankar A., Shukla S., Ganesh K. N., and Sastry M. (2004) Isothermal titration calorimetry studies on the binding of DNA bases and PNA base monomers to gold nanoparticles. *J Am Chem Soc* **126,** 13186-13187.

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 (DeltaC(p)) means that both DeltaH(0) and DeltaS(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.

Hammann C., Cooper A., and Lilley D. M. (2001) Thermodynamics of ion-induced RNA folding in the hammerhead ribozyme: an isothermal titration calorimetric study. *Biochemistry* **40,** 1423-1429.
Abstract: The hammerhead ribozyme undergoes a well-defined two-stage conformational folding process, induced by the binding of magnesium ions. In this study, we have used isothermal titration calorimetry to analyze the thermodynamics of magnesium binding and magnesium ion-induced folding of the ribozyme. Binding to the natural sequence ribozyme is strongly exothermic and can be analyzed in terms of sequential interaction at two sites with association constants K(A) = 480 and 2840 M^{-1}. Sequence variants of the hammerhead RNA give very different isothermal titration curves. An A14G variant that cannot undergo ion-induced folding exhibits endothermic binding. By contrast, a deoxyribose G5 variant that can undergo only the first of the two folding transitions gives a complex titration curve. However, despite these differences the ITC data for all three species can be analyzed in terms of the sequential binding of magnesium ions at two sites. While the binding affinities are all in the region of 10^3 M^{-1}, corresponding to free energies of ΔG degrees = -3.5 to -4 kcal mol^{-1}, the enthalpic and entropic contributions show much greater variation. The ITC experiments are in good agreement with earlier conformational studies of the folding of the ion-induced folding of the hammerhead ribozyme.

Han F., Taulier N., and Chalikian T. V. (2005) Association of the minor groove binding drug Hoechst 33258 with d(CGCGAATTCGCG)2: volumetric, calorimetric, and spectroscopic characterizations. *Biochemistry* **44,** 9785-9794.
Abstract: We employed ultrasonic velocimetry, high-precision densimetry, circular dichroism and fluorescence spectroscopy, and isothermal titration calorimetry to characterize the binding of Hoechst 33258 to the d(CGCGAATTCGCG)(2) oligomeric duplex at 25 degrees C. We used this experimental combination to determine the full thermodynamic profile for the binding of Hoechst 33258 to the DNA. Specifically, we report changes in binding free energy, enthalpy, entropy, volume, and adiabatic compressibility accompanying the binding. We interpret our volumetric data in terms of hydration and evaluate the number of waters of hydration that become released to or taken up from the bulk. Our calorimetric data reveal that the drug-DNA binding event studied in this work is entropy-driven and proceeds with an unfavorable change in enthalpy. The favorable binding entropy predominantly results from hydration changes. In contrast to a large and positive change in hydrational entropy, the binding-induced change in configurational entropy is insignificant. The latter observation is consistent with the "lock-and-key" mode of minor groove binding.

Haq I., Ladbury J. E., Chowdhry B. Z., Jenkins T. C., and Chaires J. B. (1997) Specific binding of hoechst 33258 to the d(CGCAAATTTGCG)2 duplex: calorimetric and spectroscopic studies. *J Mol Biol* **271,** 244-257.
Abstract: Fluorescence spectroscopy and high-sensitivity isothermal titration calorimetry (ITC) techniques have been used to examine the binding characteristics of Hoechst 33258 with the extended AT-tract DNA duplex d(CGCAAATTTGCG)2 in aqueous solution. The method of continuous variation reveals a 1:1 binding stoichiometry. Fluorescence equilibrium studies carried out at three different, but fixed, ligand concentrations show that the binding isotherm shifts towards higher [DNA] as the concentration of ligand is increased. The data show tight binding with Kb=3.2(+/-0.6)x10^8 M$(duplex)^{-1}$ at 25 degrees C in solutions containing 200 mM Na+. Based on UV studies of duplex melting, which show that strand separation starts at approximately 35 degrees C and has a Tm at 54 degrees C in 300 mM NaCl, binding enthalpies were determined by ITC in the 10 to 30 degrees C range. Binding is endothermic at all temperatures examined,

with ΔH values ranging from +10.24(+/-0.18) to +4.2(+/-0.10) kcal mol(duplex)$^{-1}$ at 9.4 degrees C and 30.1 degrees C, indicating that the interaction is entropically driven. The temperature dependence of ΔH shows a binding-induced change in heat capacity (ΔCp) of -330(+/-50) cal mol^{-1} K^{-1}. This value is similar to that predicted from a consideration of the effects of hydrophobic and hydrophilic solvent-accessible surface burial on complexation. This result, almost entirely dictated by a removal from exposure of the non-polar reactant surfaces, represents the first demonstration of such behavior in a DNA-drug system. The salt dependence of the binding constant was examined using reverse-salt fluorescence titrations, with a value of 0.99 determined for the $\Delta \ln K/\Delta \ln[Na+]$ parameter. These data provide a detailed thermodynamic profile for the interaction that enables a dissection of ΔG_{obs} into the component free energy terms. Analysis of data obtained at 25 degrees C reveals that ΔG_{obs} is dominated by the free energy for hydrophobic transfer of ligand from solution to the DNA binding site. Molecular interactions, including H-bonding and van der Waals contacts, are found to play only a minor role in stabilizing the resulting complex, a somewhat surprising finding given the emphasis placed on such interactions from structural studies.

Haq I. and Ladbury J. (2000) Drug-DNA recognition: energetics and implications for design. *J Mol Recognit* **13,** 188-197.
Abstract: In this article we review thermodynamic studies designed to examine the interaction of low molecular weight ligands or drugs with DNA. Over the past 10 years there has been an increase in the number of rigorous biophysical studies of DNA-drug interactions and considerable insight has been gained into the energetics of these binding reactions. The advent of high-sensitivity calorimetric techniques has meant that the energetics of DNA-drug association reactions can be probed directly and enthalpic and entropic contributions to the binding free energy established. There are two principal consequences arising from this type of work, firstly three-dimensional structures of DNA-drug complexes from X-ray and NMR studies can be put into a thermodynamic context and the energetics responsible for stabilizing the observed structures can be more fully understood. Secondly, any rational approach to structure-based drug design requires a fundamental base of knowledge where structural detail and thermodynamic data on complex formation are intimately linked. Therefore these types of studies allow a set of general guidelines to be established, which can then be used to develop drug design algorithms. In this review we describe recent breakthroughs in duplex DNA-directed drug design and also discuss how similar principles are now being used to target higher-order DNA molecules, for example, triplex (three-stranded) and tetraplex (four-stranded) structures.

Haq I., Jenkins T. C., Chowdhry B. Z., Ren J., and Chaires J. B. (2000) Parsing free energies of drug-DNA interactions. *Methods Enzymol* **323,** 373-405.

Haq I., Chowdhry B. Z., and Jenkins T. C. (2001) Calorimetric techniques in the study of high-order DNA-drug interactions. *Methods Enzymol* **340,** 109-149.

Haq I. (2002) Thermodynamics of drug-DNA interactions. *Arch Biochem Biophys* **403,** 1-15.
Abstract: Many anticancer, antibiotic, and antiviral drugs exert their primary biological effects by reversibly interacting with nucleic acids. Therefore, these biomolecules represent a major target in drug development strategies designed to produce next generation therapeutics for diseases such as cancer. In order to improve the clinical efficacy of existing drugs and also to design new ones it is necessary to understand the molecular basis of drug-DNA interactions in structural, thermodynamic, and kinetic detail. The past decade has witnessed an increase in the number of rigorous biophysical studies of drug-DNA systems and considerable knowledge has been gained in the energetics of these binding reactions. This is, in part, due to the increased availability of high-sensitivity calorimetric techniques, which have allowed the thermodynamics of drug-DNA interactions to be probed directly and accurately. The focus of this article is to review thermodynamic approaches to examining drug-DNA recognition. Specifically, an overview of a recently developed method of analysis that dissects the binding free energy of these reactions into five component terms is presented. The results of applying this analysis to the DNA binding interactions of both minor groove drugs and intercalators are discussed. The solvent water plays a key role in nucleic acid structure and consequently in the binding of ligands to these biomolecules. Any rational approach to DNA-targeted drug design requires an understanding of how water participates in recognition and binding events. Recent studies examining hydration changes that accompany DNA binding by intercalators will be

reviewed. Finally some aspects of cooperativity in drug-DNA interactions are described and the importance of considering cooperative effects when examining these reactions is highlighted.

Henry J. A., Le N. M., Nguyen B., Howard C. M., Bailey S. L., Horick S. M., Buchmueller K. L., Kotecha M., Hochhauser D., Hartley J. A., Wilson W. D., and Lee M. (2004) Targeting the inverted CCAAT box 2 in the topoisomerase IIalpha promoter by JH-37, an imidazole-pyrrole polyamide hairpin: design, synthesis, molecular biology, and biophysical studies. *Biochemistry* **43,** 12249-12257.
Abstract: The topoisomerase IIalpha promoter is regulated through transcription factor interactions with five inverted CCAAT boxes (ICBs). In confluent cancer cells, binding of nuclear factor Y to ICB2 represses the expression of this gene, contributing to resistance to topoisomerase II poisons. The ICB sites within the topoisomerase IIalpha promoter are, therefore, potential targets for the design of anticancer drugs and gene control agents. The synthesis and DNA binding properties of a hairpin polyamide molecule (JH-37) that targets 5'-TTGGT-3' found in ICB2 and ICB3 sites are described. Gel shift and DNase I footprinting studies on the topoisomerase IIalpha promoter showed JH-37 to preferentially bind to ICB2,3 and ICB1 sites. The larger ΔT_M values for ICB2,3 (8-9 degrees C) over ICB1,4,5 (4-5 degrees C) indicated a preference of JH-37 for ICB2,3. CD titration studies confirmed the binding of JH-37 to the minor groove, with a 1:1 binding stoichiometry. Results from SPR studies showed JH-37 to bind most strongly to ICB2 ($K = 3 \times 10^7 M^{-1}$), followed by ICB1, the non-ICB sequence (TGCA), and finally the ICB mutant (ICB2m). The improved binding to ICB2 is largely due to a lower dissociation rate of the compound at the preferred site. To our knowledge, this is the first example on the use of SPR for studying the interactions of hairpin polyamides with DNA. Binding of JH-37 to ICB2 was corroborated by ITC studies, in which the $\Delta G°$ of binding is driven by both enthalpy and entropy. With knowledge of the fundamental thermodynamic and kinetic properties that govern the molecular recognition of polyamides with DNA, we are poised to systematically edit the structure of JH-37 in order to further enhance its binding affinity and selectivity for ICB2,3. Our strategy for designing molecules that control gene expression is to target shorter, but multiple, binding sites that are in close array within the promoter. Binding of JH-37 to multiple ICB sites in the topoisomerase IIalpha promoter is an ideal test for this strategy. This approach is in contrast to the traditional strategy of targeting 15-16 base pairs, which has not been successful in actual biological systems due to poor cell uptake and distribution.

Hernandez L. I., Zhong M., Courtney S. H., Marky L. A., and Kallenbach N. R. (1994) Equilibrium analysis of ethidium binding to DNA containing base mismatches and branches. *Biochemistry* **33,** 13140-13146.
Abstract: In the processes of DNA replication, recombination, and repair, duplex DNA can transiently form branched structures, such as Holliday junctions, as well as base pair mismatches and bulges. These stages have altered ligand and protein binding properties from normal double helical DNA. A variety of ligands have been reported to interact more tightly at branches and bulges than to normal duplex sites. The stoichiometry, structural basis, and thermodynamics of this effect have not been determined. We have investigated the binding of the intercalator, ethidium bromide, to several DNA constructs including base mismatches, bulges, and three- and four-arm branched structures, using chemical footprinting, titration calorimetry, and fluorescence lifetime measurements. Two classes of binding sites are detected in three- and four-arm junctions in our high ionic strength conditions: one class is characterized by a small number of ligands (2-4 per DNA), with high binding affinity ($K > 10^5$), and the second by a larger number of sites (10-12 per DNA) with lower affinity (K approximately 10^4). By use of appropriate control experiments, the former appear to be associated with sites at or near the branch point or mismatch, while the latter are consistent with binding to the normal duplex DNA region(s) of the molecule. Titration calorimetry indicates an enthalpy of -10 to -13 kcal/mol for binding of ethidium to a mismatch or three- and four-arm branch point. The tight binding class is associated with a fluorescence lifetime of 12-16 ns, distinct from that of free ethidium (ca. 2 ns) and the longer lifetime observed for ethidium intercalated in duplex DNA (22-26 ns).

Hopkins H. P., Jr., Ming Y., Wilson W. D., and Boykin D. W. (1991) Intercalation binding of 6-substituted naphthothiopheneamides to DNA: enthalpy and entropy components. *Biopolymers* **31,** 1105-1114.
Abstract: N-(3-dimethylaminopropyl)naphtho[2,1-b]thiophene-4-carboxamide and the 6-substituted methoxy, methyl, fluoro, chloro, bromo, trifluoromethyl, and cyano derivatives have been shown to bind to DNA via intercalation with binding constants in the 35-900 X 10^3 range at 25 degrees C, pH 7, and [Na+] =

0.019M. Both electron-donating and -withdrawing substituents enhance intercalation binding, but the binding affinity is most enhanced by the cyano substituent. Calorimetric titrations for calf thymus DNA differ dramatically from those reported for ethidium [Hopkins et al. (1990) Biopolymers Vol. 29, pp. 449-459]. Apparent enthalpy parameters (ΔHB) for intercalation are constant only at low coverage of sites and become much more positive as saturation is approached. In the plateau region, Δ HB values for the parent and the cyano-, fluoro-, chloro-, and bromo-substituted compounds are nearly the same (approximately -5.9 kcal/mol). For the methyl- (-6.8 kcal/mol) and methoxy- (-7.5 kcal/mol) substituted compounds, the Δ HB values are more exothermic than that for the unsubstituted compound, whereas Δ HB for the trifluoromethyl compound is approximately 1 kcal/mol less exothermic. The corresponding ΔSB values, corrected for mixing effects, are in the 7-15-cal/deg/mol range and are approximately linearly related to Δ HB if the cyano derivative is excluded.

Hopkins H. P., Jr. (1997) Calorimetric techniques for studying drug-DNA interactions. *Methods Mol Biol* **90,** 259-268.

Hormann A., Chaudhuri B., and Fretz H. (2001) DNA binding properties of the marine sponge pigment fascaplysin. *Bioorg Med Chem* **9,** 917-921.
Abstract: Association of fascaplysin with double-stranded calf thymus DNA was investigated by means of isothermal titration calorimetry, absorption spectroscopy, and circular dichroism. The UV spectroscopic data could be well interpreted in terms of a two-site model for the binding of fascaplysin to DNA revealing affinity constants of K1 = 2.5×10^{6} M^{-1} and K2 = 7.5×10^{4} M^{-1} (base pairs of DNA). Based on the typical change observed in the absorption and circular dichroism spectra, intercalation of fascaplysin is regarded as the major binding mode. The calorimetric titration curves showed an exothermic reaction which was exhausted at a 2:1 base pair/drug; ratio. This finding is in agreement with an intercalation model comprising nearest neighbor exclusion. In addition, significantly weaker non-intercalative DNA interactions can be observed at high drug concentration. By comparison of all these data with the binding behavior of known intercalating agents, it is concluded that fascaplysin intercalates into DNA.

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

Hutchins R. A., Crenshaw J. M., Graves D. E., and Denny W. A. (2003) Influence of substituent modifications on DNA binding energetics of acridine-based anticancer agents. *Biochemistry* **42,** 13754-13761.
Abstract: The DNA binding energetics of a series of analogues derived from the anticancer agent N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (AAC) are investigated. The effects of substituent modification at the C5 position of the acridine chromophore on the interaction of AAC with DNA are determined using spectrophotometry and isothermal titration calorimetry (ITC). The binding affinity and

binding free energy associated with the interaction of AAC with DNA are significantly enhanced upon substitution at the C5 position. Energetic profiles describing ligand-DNA complex formation obtained from ITC indicate that C5 substitution significantly enhances binding enthalpy relative to the parent AAC. In many cases, the enhanced binding enthalpies of the C5-substituted analogues correlate with anticancer activity. Because of the cationic character of AAC and its analogues, the DNA binding properties of these compounds are dependent on ionic strength. To quantitate the ionic contributions to complex formation, the observed binding free energy of each compound is parsed into its polyelectrolyte and nonelectrostatic components. Enhanced nonelectrostatic contributions to the overall binding free energies observed with C5-substituted analogues relative to the parent AAC suggest that C5 substituents play a critical role in directing both thermodynamic mechanisms associated with complex formation and molecular interactions between the ligand and its DNA binding site. These studies have demonstrated that substitution of AAC at the C5 position results in enhanced DNA binding affinity and energetics.

Ikonen M., Murtomaki L. and Kontturi K. (2008) Controlled complexation of plasmid DNA with cationic polymers: effect of surfactant on the complexation and stability of the complexes. *Colloids Surf B Biointerfaces* **66,** 77-83.
Abstract: The aggregation of the cationic polymer-plasmid DNA complexes of two commonly used polymers, polyethyleneimine (PEI) and poly-l-lysine (PLL) were systematically compared. The complexation was studied in 5% glucose solution at 25 degrees C using dynamic light scattering and isothermal titration calorimetry. The aggregation of the complexes was controlled by addition of the surfactant polyoxyethylene stearate (POES). The stability of the complexes was evaluated using dextran sulphate (DS) as relaxing agent. The relaxation of the complexes in the presence of DS was studied using agarose gel electrophoresis. This study elucidates the role of surfactant in controlling the size of the PEI/pDNA complex and reveals the differences of the two polymers as complexing agents

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.

Jin E., Katritch V., Olson W. K., Kharatisvili M., Abagyan R., and Pilch D. S. (2000) Aminoglycoside binding in the major groove of duplex RNA: the thermodynamic and electrostatic forces that govern recognition. *J Mol Biol* **298,** 95-110.
Abstract: We use a combination of spectroscopic, calorimetric, viscometric and computer modeling techniques to characterize the binding of the aminoglycoside antibiotic, tobramycin, to the polymeric RNA duplex, poly(rI).poly(rC), which exhibits the characteristic A-type conformation that is conserved among natural and synthetic double-helical RNA sequences. Our results reveal the following significant features: (i) CD-detected binding of tobramycin to poly(rI).poly(rC) reveals an apparent site size of four base-pairs per bound drug molecule; (ii) tobramycin binding enhances the thermal stability of the host poly(rI).poly(rC) duplex, the extent of which decreases upon increasing in Na(+) concentration and/or pH conditions; (iii) the enthalpy of tobramycin- poly(rI).poly(rC) complexation increases with increasing pH conditions, an observation consistent with binding-induced protonation of one or more drug amino groups; (iv) the affinity of tobramycin for poly(rI).poly(rC) is sensitive to both pH and Na(+) concentration, with

increases in pH and/or Na(+) concentration resulting in a concomitant reduction in binding affinity. The salt dependence of the tobramycin binding affinity reveals that the drug binds to the host RNA duplex as trication. (v) The thermodynamic driving force for tobramycin- poly(rI).poly(rC) complexation depends on pH conditions. Specifically, at pH< or =6.0, tobramycin binding is entropy driven, but is enthalpy driven at pH > 6.0. (vi) Viscometric data reveal non-intercalative binding properties when tobramycin complexes with poly(rI).poly(rC), consistent with a major groove-directed mode of binding. These data also are consistent with a binding-induced reduction in the apparent molecular length of the host RNA duplex. (vii) Computer modeling studies reveal a tobramycin-poly(rI). poly(rC) complex in which the drug fits snugly at the base of the RNA major groove and is stabilized, at least in part, by an array of hydrogen bonding interactions with both base and backbone atoms of the host RNA. These studies also demonstrate an inability of tobramycin to form a stable low-energy complex with the minor groove of the poly(rI).poly(rC) duplex. In the aggregate, our results suggest that tobramycin-RNA recognition is dictated and controlled by a broad range of factors that include electrostatic interactions, hydrogen bonding interactions, drug protonation reactions, and binding-induced alterations in the structure of the host RNA. These modulatory effects on tobramycin-RNA complexation are discussed in terms of their potential importance for the selective recognition of specific RNA structural motifs, such as asymmetric internal loops or hairpin loop-stem junctions, by aminoglycoside antibiotics and their derivatives.

Kankia B. I., Soto A. M., Burns N., Shikiya R., Tung C. S., and Marky L. A. (2002) DNA oligonucleotide duplexes containing intramolecular platinated cross-links: energetics, hydration, sequence, and ionic effects. *Biopolymers* **65,** 218-227.

Abstract: The anticancer activity of cisplatin arises from its ability to bind covalently to DNA, forming primarily intrastrand cross-links to adjacent purine residues; the most common adducts involve d(GpG) (65%) and d(ApG) (25%) intrastrand cross-links. The incorporation of these platinum adducts in a B-DNA helix induces local distortions, causing bending and unwinding of the DNA. In this work, we used temperature-dependent UV spectroscopy to investigate the unfolding thermodynamics, and associated ionic effects, of two sets of DNA decamer duplexes containing either cis-[Pt(NH(3))(2)[d(GpG]] or cis-[Pt(NH(3))(2) [d(ApG]] cross-links, and their corresponding unmodified duplexes. The platinated duplexes are less stable and unfold with lower T(M)s (and ΔG degrees s) in enthalpy-driven reactions, which indicates a loss of favorable base-pair stacking interactions. The folding thermodynamics and hydration effects for the first set of decamers containing the d(GpG) cross-link was investigated by a combination of titration calorimetry, density, and ultrasound techniques. The hydration parameters showed an uptake of structural water by the platinated duplex and a release of electrostricted water by the control duplex. Relative to the unmodified duplex, the folding of the platinated duplex at 20 degrees C yielded a positive Δ Δ G degrees term [and positive Δ Δ H-Δ(TΔS) compensation] and a negative differential volume change. The opposite signs of the Δ ΔG degrees and Δ ΔV terms confirmed its uptake of structural water. Further, solvent-accessible surface areas calculations for a similar pair of dodecamer duplexes indicated that the modified duplex has a 503 oeA(2) higher polar and nonpolar surface area that is exposed to the solvent. Therefore, the incorporation of a platinum adduct in duplex DNA disrupts favorable base-pair stacking interactions, yielding a greater exposure of aromatic bases to the solvent, which in turn immobilizes structural water. The overall results correlate nicely with the results reported in the available structural data of nuclear magnetic resonance solution studies.

Kankia B. I. (2003) Mg2+-induced triplex formation of an equimolar mixture of poly(rA) and poly(rU). *Nucleic Acids Res* **31,** 5101-5107.

Abstract: Magnesium ions strongly influence the structure and biochemical activity of RNA. The interaction of Mg2+ with an equimolar mixture of poly(rA) and poly(rU) has been investigated by UV spectroscopy, isothermal titration calorimetry, ultrasound velocimetry and densimetry. Measurements in dilute aqueous solutions at 20 degrees C revealed two differ ent processes: (i) Mg2+ binding to unfolded poly(rA)*poly(rU) up to [Mg2+]/[phosphate] = 0.25; and (ii) poly(rA)*2poly(rU) triplex formation at [Mg2+]/[phosphate] between 0.25 and 0.5. The enthalpies of these two different processes are favorable and similar to each other, approximately -1.6 kcal x mol^{-1} of base pairs. Volume and compressibility effects of the first process are positive, 8 cm3 x mol^{-1}and 24 x 10^{-4} cm3 x mol^{-1} x bar^{-1}, respectively, and correspond to the release of water molecules from the hydration shells of Mg2+ and the polynucleotides. The triplex formation is also accompanied by a positive change in compressibility, 14 x 10^{-4} cm3 x mol^{-1}x bar^{-1}, but only a small change in volume, 1 cm3 x mol^{-1}. A phase diagram has been constructed from the

melting experiments of poly(rA)*poly(rU) at a constant K+ concentration, 140 mM, and various amounts of Mg2+. Three discrete regions were observed, corresponding to single-, double- and triple-stranded complexes. The phase boundary corresponding to the transition between double and triple helical conformations lies near physiological salt concentrations and temperature.

Kaul M., Barbieri C. M., and Pilch D. S. (2005) Defining the basis for the specificity of aminoglycoside-rRNA recognition: a comparative study of drug binding to the A sites of Escherichia coli and human rRNA. *J Mol Biol* **346,** 119-134.
Abstract: 2-Deoxystreptamine (2-DOS) aminoglycoside antibiotics exert their antimicrobial activities by targeting the decoding region A site of the rRNA and inhibiting protein synthesis. A prokaryotic specificity of action is critical to therapeutic utility of 2-DOS aminoglycosides as antibiotics. Here, isothermal titration calorimetry (ITC) and fluorescence studies are presented that provide insight into the molecular basis for this prokaryotic specificity of action. Specifically, the rRNA binding properties of the 2-DOS aminoglycosides paromomycin and G418 (geneticin) are compared, using both human and Escherichia coli rRNA A site model oligonucleotides as drug targets. Paromomycin and G418 differ with respect to their specificities of action, with only paromomycin exhibiting a specificity for prokaryotic versus human ribosomes. G418 binds to both the human and E. coli rRNA A sites with a markedly lower affinity than paromomycin, with the affinities of both drugs for the human rRNA A site being lower than those they exhibit for the E. coli rRNA A site. Paromomycin induces the destacking of the base at position 1492 (by E. coli numbering) upon binding to the E. coli rRNA A site, but not the human rRNA A site. By contrast, the binding of G418 induces the destacking of base 1492 when either rRNA A site serves as the drug target. In the aggregate, these results suggest that binding-induced base destacking at the rRNA A site is a critical factor in determining the prokaryotic specificity of aminoglycoside action, with binding affinity for the A site being of secondary importance.

Kaul M., Barbieri C. M., and Pilch D. S. (2004) Fluorescence-based approach for detecting and characterizing antibiotic-induced conformational changes in ribosomal RNA: comparing aminoglycoside binding to prokaryotic and eukaryotic ribosomal RNA sequences. *J Am Chem Soc* **126,** 3447-3453.
Abstract: Aminoglycoside antibiotics bind specifically to a conserved sequence of the 16S ribosomal RNA (rRNA) A site and interfere with protein synthesis. One model for the mechanism underlying the deleterious effects of aminoglycosides on protein synthesis invokes a drug-induced conformational change in the rRNA that involves the destacking of two adenine residues (A1492 and A1493 in Escherichia coli) at the A site. We describe here a fluorescence-based approach for detecting and characterizing this drug-induced conformational change in the target rRNA. In this approach, we insert the fluorescent base analogue 2-aminopurine in place of A1492 in an E. coli 16S rRNA A-site model oligonucleotide (EcWT) as well as in a mutant form of this oligomer (A1408G) in which A1408 has been replaced with a guanine. The presence of guanine at 1408 instead of adenine represents one of the major sequence differences between prokaryotic and eukaryotic A sites, with the latter A sites being resistant to the deleterious effects of aminoglycosides. Binding of the aminoglycoside paromomycin to the 2AP-substituted forms of EcWT and A1408G induced changes in fluorescence quantum yield consistent with drug-induced base destacking in EcWT but not A1408G. Isothermal titration calorimetry studies reveal that paromomycin binds to the EcWT duplex with a 31-fold higher affinity than the A1408G duplex, with this differential affinity being enthalpic in origin. In the aggregate, these observations are consistent with both rRNA binding affinity and drug-induced base destacking being important determinants in the prokaryotic specificity of aminoglycosides. Combining fluorescence quantum yield and lifetime data allows for quantification of the extent of drug-induced base destacking, thereby providing a convenient tool for evaluating the relative impacts of both novel and existing A-site targeting ligands on rRNA conformation and potentially for predicting relative antibiotic activities and specificities.

Kaul M. and Pilch D. S. (2002) Thermodynamics of aminoglycoside-rRNA recognition: the binding of neomycin-class aminoglycosides to the A site of 16S rRNA. *Biochemistry* **41,** 7695-7706.
Abstract: We use spectroscopic and calorimetric techniques to characterize the binding of the aminoglycoside antibiotics neomycin, paromomycin, and ribostamycin to a RNA oligonucleotide that models the A-site of Escherichia coli 16S rRNA. Our results reveal the following significant features: (i) Aminoglycoside binding enhances the thermal stability of the A-site RNA duplex, with the extent of this thermal enhancement decreasing with increasing pH and/or Na(+) concentration. (ii) The RNA binding

enthalpies of the aminoglycosides become more exothermic (favorable) with increasing pH, an observation consistent with binding-linked protonation of one or more drug amino groups. (iii) Isothermal titration calorimetry (ITC) studies conducted as a function of buffer reveal that aminoglycoside binding to the host RNA is linked to the uptake of protons, with the number of linked protons being dependent on pH. Specifically, increasing the pH results in a corresponding increase in the number of linked protons. (iv) ITC studies conducted at 25 and 37 degrees C reveal that aminoglycoside-RNA complexation is associated with a negative heat capacity change (ΔCp), the magnitude of which becomes greater with increasing pH. (v) The observed RNA binding affinities of the aminoglycosides decrease with increasing pH and/or Na(+) concentration. In addition, the thermodynamic forces underlying these RNA binding affinities also change as a function of pH. Specifically, with increasing pH, the enthalpic contribution to the observed RNA binding affinity increases, while the corresponding entropic contribution to binding decreases. (vi) The affinities of the aminoglycosides for the host RNA follow the hierarchy neomycin > paromomycin > ribostamycin. The enhanced affinity of neomycin relative to either paromomycin or ribostamycin is primarily, if not entirely, enthalpic in origin. (vii) The salt dependencies of the RNA binding affinities of neomycin and paromomycin are consistent with at least three drug NH(3)(+) groups participating in electrostatic interactions with the host RNA. In the aggregate, our results reveal the impact of specific alterations in aminoglycoside structure on the thermodynamics of binding to an A-site model RNA oligonucleotide. Such systematic comparative studies are critical first steps toward establishing the thermodynamic database required for enhancing our understanding of the molecular forces that dictate and control aminoglycoside recognition of RNA.

Kaul M., Barbieri C. M., Kerrigan J. E., and Pilch D. S. (2003) Coupling of drug protonation to the specific binding of aminoglycosides to the A site of 16 S rRNA: elucidation of the number of drug amino groups involved and their identities. *J Mol Biol* **326,** 1373-1387.
Abstract: 2-Deoxystreptamine (2-DOS) aminoglycoside antibiotics bind specifically to the central region of the 16S rRNA A site and interfere with protein synthesis. Recently, we have shown that the binding of 2-DOS aminoglycosides to an A site model RNA oligonucleotide is linked to the protonation of drug amino groups. Here, we extend these studies to define the number of amino groups involved as well as their identities. Specifically, we use pH-dependent 15N NMR spectroscopy to determine the pKa values of the amino groups in neomycin B, paromomycin I, and lividomycin A sulfate, with the resulting pKa values ranging from 6.92 to 9.51. For each drug, the 3-amino group was associated with the lowest pKa, with this value being 6.92 in neomycin B, 7.07 in paromomycin I, and 7.24 in lividomycin A. In addition, we use buffer-dependent isothermal titration calorimetry (ITC) to determine the number of protons linked to the complexation of the three drugs with the A site model RNA oligomer at pH 5.5, 8.8, or 9.0. At pH 5.5, the binding of the three drugs to the host RNA is independent of drug protonation effects. By contrast, at pH 9.0, the RNA binding of paromomycin I and neomycin B is coupled to the uptake of 3.25 and 3.80 protons, respectively, with the RNA binding of lividomycin A at pH 8.8 being coupled to the uptake of 3.25 protons. A comparison of these values with the protonation states of the drugs predicted by our NMR-derived pKa values allows us to identify the specific drug amino groups whose protonation is linked to complexation with the host RNA. These determinations reveal that the binding of lividomycin A to the host RNA is coupled to the protonation of all five of its amino groups, with the RNA binding of paromomycin I and neomycin B being linked to the protonation of four and at least five amino groups, respectively. For paromomycin I, the protonation reactions involve the 1-, 3-, 2'-, and 2'''-amino groups, while, for neomycin B, the binding-linked protonation reactions involve at least the 1-, 3-, 2', 6'-, and 2'''-amino groups. Our results clearly identify drug protonation reactions as important thermodynamic participants in the specific binding of 2-DOS aminoglycosides to the A site of 16S rRNA.

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 Ki, i.e., the binding to an elongated DNA chain with a binding constant K1 and with K2 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 K1 > K2. For the DNA condensation, however, the relationships for K1 and K2 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.

Kiser J.R., Monk R.W., Smalls R.L., and Petty J.T. (2005) Hydration changes in the association of Hoechst 33258 with DNA. *Biochemistry*. **44**, 16988-97.
Abstract: The role of water in the interaction of Hoechst 33258 with the minor groove binding site of the (AATT)2 sequence was investigated using calorimetric and equilibrium constant measurements. Using isothermal titration calorimetry measurements, the heat capacity change for the reaction is -256 +/- 10 cal/(K mol of Hoechst). Comparison with the heat capacity changes based on area models supports the expulsion of water from the interface of the Hoechst-DNA complex. To further consider the role of water, the osmotic stress method was used to determine if the Hoechst association with DNA was coupled with hydration changes. Using four osmolytes with varying molecular weights and chemical properties, the Hoechst affinity for DNA decreases with increasing osmolyte concentration. From the dependence of the equilibrium constant on the solution osmolality, 60 +/- 13 waters are acquired in the complex relative to the reactants. It is proposed that the osmotic stress technique is measuring weakly bound waters that are not measured via the heat capacity changes.

Kvaratskhelia M., George S. J., Cooper A., and White M. F. (1999) Quantitation of metal ion and DNA junction binding to the Holliday junction endonuclease Cce1. *Biochemistry* **38,** 16613-16619.
Abstract: Cce1 is a magnesium-dependent Holliday junction endonuclease involved in the resolution of recombining mitochondrial DNA in Saccharomyces cerevisiae. Cce1 binds four-way DNA junctions as a dimer, opening the junction into an extended, 4-fold symmetric structure, and resolves junctions by the introduction of paired nicks in opposing strands at the point of strand exchange. In the present study, we have examined the interactions of wild-type Cce1 with a noncleavable four-way DNA junction and metal ions (Mg(2+) and Mn(2+)) using isothermal titration calorimetry, EPR, and gel electrophoresis techniques. Mg(2+) or Mn(2+) ions bind to Cce1 in the absence of DNA junctions with a stoichiometry of two metal ions per Cce1 monomer. Cce1 binds to four-way junctions with a stoichiometry of two Cce1 dimers per junction molecule in the presence of EDTA, and one dimer of Cce1 per junction in 15 mM magnesium. The presence of 15 mM Mg(2+) dramatically reduces the affinity of Cce1 for four-way DNA junctions, by about 900-fold. This allows an estimation of ΔG degrees for stacking of four-way DNA junction 7 of -4.1 kcal/mol, consistent with the estimate of -3.3 to -4.5 kcal/mol calculated from branch migration and NMR experiments [Overmars and Altona (1997) J. Mol. Biol. 273, 519-524; Panyutin et al. (1995) EMBO J. 14, 1819-1826]. The striking effect of magnesium ions on the affinity of Cce1 binding to the four-way junction is predicted to be a general one for proteins that unfold the stacked X-structure of the Holliday junction on binding.

Lacy E. R., Nguyen B., Le M., Cox K. K., OHare C., Hartley J. A., Lee M., and Wilson W. D. (2004) Energetic basis for selective recognition of T*G mismatched base pairs in DNA by imidazole-rich polyamides. *Nucleic Acids Res* **32,** 2000-2007.
Abstract: To complement available structure and binding results and to develop a detailed understanding of the basis for selective molecular recognition of T.G mismatches in DNA by imidazole containing polyamides, a full thermodynamic profile for formation of the T.G-polyamide complex has been determined. The amide-linked heterocycles f-ImImIm and f-PyImIm (where f is formamido group, Im is imidazole and Py is pyrrole) were studied by using biosensor-surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) with a T.G mismatch containing DNA hairpin duplex and a similar DNA with only Watson-Crick base pairs. Large negative binding enthalpies for all of the polyamide-DNA complexes indicate that the interactions are enthalpically driven. SPR results show slower complex formation and stronger binding of f-ImImIm to the T.G than to the match site. The thermodynamic analysis indicates that the enhanced binding to the T.G site is the result of better entropic contributions. Negative heat capacity changes for the complex are correlated with calculated solvent accessible surface area changes and indicate hydrophobic contributions to complex formation. DNase I footprinting analysis in a long DNA sequence provided supporting evidence that f-ImImIm binds selectively to T.G mismatch sites.

Lafontaine I. and Lavery R. (2000) Optimization of nucleic acid sequences. *Biophys J* **79,** 680-685.
Abstract: Base sequence influences the structure, mechanics, dynamics, and interactions of nucleic acids. However, studying all possible sequences for a given fragment leads to a number of base combinations that increases exponentially with length. We present here a novel methodology based on a multi-copy approach enabling us to determine which base sequence favors a given structural change or interaction via a single energy minimization. This methodology, termed ADAPT, has been implemented starting from the JUMNA molecular mechanics program by adding special nucleotides, "lexides," containing all four bases, whose contribution to the energy of the system is weighted by continuously variable coefficients. We illustrate the application of this approach in the case of double-stranded DNA by determining the optimal sequences satisfying structural (B-Z transition), mechanical (intrinsic curvature), and interaction (ligand-binding) properties.

Lah, J., Carl, N., Drobnak, I, Šumiga, B. and Vesnaver, G. (2006) Competition of Some Minor Groove Binders for a Single DNA Binding Site. *Acta Chim. Slov*. **53,** 284–291.
Abstract: We employed circular dichroism (CD) and isothermal titration calorimetry (ITC) to characterize binding of netropsin (NET) and distamycin A (DST) to the hairpin (D) formed from the 5′-CGAATTGACGTCTCCGTCAATTCG-3′ oligonucleotide. From fitting the appropriate model to the CD titration curves describing NET and DST binding to the D and CD and ITC titration curves describing the displacement of NET bound to D by the added DST and vice versa we were able to determine the corresponding binding and displacement constants at 25 °C. The displacement constant (K12) determined for 2DST + NET-D ↔ DST2-D + NET process is in good agreement with the corresponding value calculated from the individual binding events (NET + D ↔NET-D, k1 = 1.6 106 M-1; DST + D ↔DST-D, k1 = 1.0 107 M-1 and DST + DST-D ↔DST2-D, k2 = 0.9 104 M-1). ITC results reveal that the free energy of displacement ΔG12° = -29 kJ mol-1 in combination with the enthalpy of displacement ΔH12° = -74 kJ mol-1 results in the corresponding entropy contribution TΔS12° = -45 kJ mol-1. Evidently, the 2DST + NET-D ↔DST2-D + NET displacement is a strongly enthalpy driven process. What we find important with the displacement studies is the observation that NET entirely displaces DST from one half of its 1:1 complexes with the hairpin in spite of the fact that the 1:1 binding constant of NET is about one order of magnitude lower than the corresponding 1:1 constant of DST. We propose that the driving force of this displacement is strong binding of DST molecules that are displaced as a result of the overall NET-DST-D equilibrium to the available DST-hairpin 1:1 complexes.

Lah J. and Vesnaver G. (2004) Energetic diversity of DNA minor-groove recognition by small molecules displayed through some model ligand-DNA systems. *J Mol Biol* **342,** 73-89.
Abstract: Energetics of interactions occurring in the model ligand-DNA systems constituted from distamycin A (DST), netropsin (NET) and the oligomeric duplexes d(GCAAGTTGCGATATACG)d(CGTATATCGCAACTTGC)=D#1 and d(GCAAGTTGCGAAAAACG)d(CGTTTTTCGCAACTTGC)=D#2 was studied by spectropolarimetry, UV-absorption spectroscopy and isothermal titration calorimetry. Model analysis of the measured signals was applied to describe individual and competitive binding in terms of populations of various species in the solution. Our results reveal several unprecedented ligand-DNA binding features. DST binds to the neighboring 5'-AAGTT-3' and 5'-ATATA-3' sites of D#1 statistically in a 2:1 binding mode. By contrast, its association to D#2 appears to be a 2:1 binding event only at the DST/D#2 molar ratios between 0 and 2 while its further binding to D#2 may be considered as a step-by-step binding to the unoccupied 5'-AAAAA-3' sites resulting first in DST3D#2 and finally in DST4D#2 complex formation. Competition between DST and NET binding shows that for the most part DST displaces NET from its complexes with D#1 and D#2. In contrast to the obligatory 1:1 binding of DST to the ligand-free 5'-AAAAA-3' sites observed at DST/5'-AAAAA-3' <1 the displacement of NET bound to the 5'-AAAAA-3' sites by added DST occurs even at the smallest additions of DST in a 2:1 manner. NET can also displace DST molecules but only those bound monomerically to the 5'-AAAAA-3' sites of DST3D#2. Actually, only half of these molecules can be displaced due to the simultaneous rebinding of the displaced DST to the unreacted 5'-AAAAA-3' sites in DST3D#2. Binding of DST and NET to D#1 and D#2 is an enthalpy driven process accompanied by large unfavorable (DST), small (NET) or large favorable (NET binding to 5'-AAAAA-3') entropy contributions and negative ΔCP degrees that are reasonably close to ΔCP degrees predicted from

the calculated changes in solvent-accessible surface areas that accompany complex formation. Although various modes of DST and NET binding within D#1 and D#2 are characterized by significant energetic differences they seem to be governed by the same driving forces; the hydrophobic transfer of ligand from the solution into the duplex binding site and the accompanying specific non-covalent ligand-DNA and ligand-ligand interactions occurring within the DNA minor groove.

Lah J. and Vesnaver G. (2000) Binding of distamycin A and netropsin to the 12mer DNA duplexes containing mixed AT.GC sequences with at most five or three successive AT base pairs. *Biochemistry* **39,** 9317-9326.
Abstract: Circular dichroism (CD), isothermal calorimetric titrations (ITC), and temperature-dependent UV spectroscopy were used to investigate binding of the minor groove-directed ligands distamycin A (Dst) and netropsin (Net) to the following duplexes: d(GTTAGTATTTGG). d(CCAAATACTAAC), d(GTTAGTATATGG).d(CCATATACTAAC), d(GTTAGTACTTGG). d(CCAAGTACTAAC), and d(GTTAGTAGTTGG).d(CCAACTACTAAC). Our results reveal that Dst binds within the minor grooves of these dodecamers that contain five-AT and/or four-AT.GC binding sites exclusively in a dimeric high-affinity 2:1 binding mode (K approximately 10^{16} M^{-2}). By contrast, Net exhibits high-affinity binding only when it binds in a 1:1 mode (K(1) approximately 10^{9} M^{-1}) to the two duplexes that contain five-AT sites (5'-TATTT-3' and 5'-TATAT-3'). Its further binding to these two duplexes occurs in a low-affinity mode (K(2) approximately 10^{6} M^{-1}) and results in the formation of 2:1 Net-DNA complexes. To the other two duplexes that contain sequences with at most three AT consecutive base pairs Net binds in two distinctive low-affinity 1:1 binding modes (K(1) approximately 10^{7} M^{-1}, K(2) approximately 10^{6} M^{-1}). Competition experiments (CD and ITC titrations) reveal that Dst entirely displaces Net from its 1:1 and 2:1 complexes with any of the four duplexes. We discuss and interpret our optical and calorimetric results in the context of the available structural information about the complexes between DNA and the sequence-specific minor groove binders Dst and Net.

Lah J., Drobnak I., Dolinar M. and Vesnaver G. (2008) What drives the binding of minor groove-directed ligands to DNA hairpins? *Nucleic Acids Res* **36,** 897-904.
Abstract: Understanding the molecular basis of ligand-DNA-binding events, and its application to the rational design of novel drugs, requires knowledge of the structural features and forces that drive the corresponding recognition processes. Existing structural evidence on DNA complexation with classical minor groove-directed ligands and the corresponding studies of binding energetics have suggested that this type of binding can be described as a rigid-body association. In contrast, we show here that the binding-coupled conformational changes may be crucial for the interpretation of DNA (hairpin) association with a classical minor groove binder (netropsin). We found that, although the hairpin form is the only accessible state of ligand-free DNA, its association with the ligand may lead to its transition into a duplex conformation. It appears that formation of the fully ligated duplex from the ligand-free hairpin, occurring via two pathways, is enthalpically driven and accompanied by a significant contribution of the hydrophobic effect. Our thermodynamic and structure-based analysis, together with corresponding theoretical studies, shows that none of the predicted binding steps can be considered as a rigid-body association. In this light we anticipate our thermodynamic approach to be the basis of more sophisticated nucleic acid recognition mechanisms, which take into account the dynamic nature of both the nucleic acid and the ligand molecule

Li T. K., Barbieri C. M., Lin H. C., Rabson A. B., Yang G., Fan Y., Gaffney B. L., Jones R. A., and Pilch D. S. (2004) Drug targeting of HIV-1 RNA.DNA hybrid structures: thermodynamics of recognition and impact on reverse transcriptase-mediated ribonuclease H activity and viral replication. *Biochemistry* **43,** 9732-9742.
Abstract: RNA degradation via the ribonuclease H (RNase H) activity of human immunodeficiency virus type I (HIV-1) reverse transcriptase (RT) is a critical component of the reverse transcription process. In this connection, mutations of RT that inactivate RNase H activity result in noninfectious virus particles. Thus, interfering with the RNase H activity of RT represents a potential vehicle for the inhibition of HIV-1 replication. Here, we demonstrate an approach for inhibiting the RNase H activity of HIV-1 RT by targeting its RNA.DNA hybrid substrates. Specifically, we show that the binding of the 4,5-disubstituted 2-deoxystreptamine aminoglycosides, neomycin, paromomycin, and ribostamycin, to two different chimeric RNA-DNA duplexes, which mimic two distinct intermediates in the reverse transcription process, inhibits specific RT-mediated RNase H cleavage, with this inhibition being competitive in nature. UV melting and

isothermal titration calorimetry studies reveal a correlation between the relative binding affinities of the three drugs for each of the chimeric RNA-DNA host duplexes and the relative extents to which the drugs inhibit RT-mediated RNase H cleavage of the duplexes. Significantly, this correlation also extends to the relative efficacies with which the drugs inhibit HIV-1 replication. In the aggregate, our results highlight a potential strategy for AIDS chemotherapy that should not be compromised by the unusual genetic diversity of HIV-1.

Lin P. H., Yen S. L., Lin M. S., Chang Y., Louis S. R., Higuchi A. and Chen W. Y. (2008) Microcalorimetrics studies of the thermodynamics and binding mechanism between L-tyrosinamide and aptamer. *J Phys. Chem B* **112,** 6665-6673.
Abstract: In recent years, several high-resolution structures of aptamer complexes have shed light on the binding mode and recognition principles of aptamer complex interactions. In some cases, however, the aptamer complex binding behavior and mechanism are not clearly understood, especially with the absence of structural information. In this study, it was demonstrated that isothermal titration calorimetry (ITC) and circular dichroism (CD) were useful tools for studying the fundamental binding mechanism between a DNA aptamer and L-tyrosinamide (L-TyrNH2). To gain further insight into this behavior, thermodynamic and conformational measurements under different parameters such as salt concentration, temperature, pH value, analogue of L-TyrNH2, and metal ion were carried out. The thermodynamic signature along with the coupled CD spectral change suggest that this binding behavior is an enthalpy-driven process, and the aptamer has a conformational change from B-form to A-form. The results showed that the interaction is an induced fit binding, and the driving forces in this binding behavior may include electrostatic interactions, hydrophobic effects, hydrogen bonding, and the binding-linked protonation process. The amide group and phenolic hydroxyl group of the L-TyrNH2 play a vital role in this binding mechanism. In addition, it should be noted that Mg(2+) not only improves binding affinity but also helps change the structure of the DNA aptamer

Liu Y., Collar C. J., Kumar A., Stephens C. E., Boykin D. W. and Wilson W. D. (2008) Heterocyclic diamidine interactions at AT base pairs in the DNA minor groove: effects of heterocycle differences, DNA AT sequence and length. *J Phys. Chem B* **112,** 11809-11818.
Abstract: Given the increasing significance of diamidines as DNA-targeted therapeutics and biotechnology reagents, it is important to establish the variations in thermodynamic quantities that characterize the interactions of closely related compounds to different sequence AT binding sites. In this study, an array of methods including biosensor-surface plasmon resonance (SPR), isothermal titration microcalorimetry (ITC), circular dichroism (CD), thermal melting (Tm) and molecular modeling have been used to characterize the binding of dicationic diamidines related to DB75 (amidine-phenyl-furan-phenyl-amidine) with alternating and nonalternating AT sequences. Conversion of the central furan of DB75 to other similar groups, such as thiophene or selenophene, can yield compounds with increased affinity and sequence binding selectivity for the minor groove. Calorimetric measurements revealed that the thermodynamic parameters (Delta G, Delta H, Delta S) that drive diamidine binding to alternating and nonalternating oligomers can be quite different and depend on both DNA sequence and length. Small changes in a compound can have major effects on DNA interactions. By choosing an appropriate central group it is possible to "tune" the shape of the molecule to match DNA for enhanced affinity and sequence recognition

Mackay H., Brown T., Uthe P. B., Westrate L., Sielaff A., Jones J., Lajiness J. P., Kluza J., O'Hare C., Nguyen B., Davis Z., Bruce C., Wilson W. D., Hartley J. A. and Lee M. (2008) Sequence specific and high affinity recognition of 5'-ACGCGT-3' by rationally designed pyrrole-imidazole H-pin polyamides: thermodynamic and structural studies. *Bioorg. Med. Chem* **16,** 9145-9153.
Abstract: Imidazole (Im) and Pyrrole (Py)-containing polyamides that can form stacked dimers can be programmed to target specific sequences in the minor groove of DNA and control gene expression. Even though various designs of polyamides have been thoroughly investigated for DNA sequence recognition, the use of H-pin polyamides (covalently cross-linked polyamides) has not received as much attention. Therefore, experiments were designed to systematically investigate the DNA recognition properties of two symmetrical H-pin polyamides composed of PyImPyIm (5) or f-ImPyIm (3e, f=formamido) tethered with an ethylene glycol linker. These compounds were created to recognize the cognate 5'-ACGCGT-3' through an overlapped and staggered binding motif, respectively. Results from DNaseI footprinting, thermal denaturation, circular dichroism, surface plasmon resonance and isothermal titration microcalorimetry

studies demonstrated that both H-pin polyamides bound with higher affinity than their respective monomers. The binding affinity of formamido-containing H-pin 3e was more than a hundred times greater than that for the tetraamide H-pin 5, demonstrating the importance of having a formamido group and the staggered motif in enhancing affinity. However, compared to H-pin 3e, tetraamide H-pin 5 demonstrated superior binding preference for the cognate sequence over its non-cognates, ACCGGT and AAATTT. Data from SPR experiments yielded binding constants of 1.6x10(8)M(-1) and 2.0x10(10)M(-1) for PyImPyIm H-pin 5 and f-ImPyIm H-pin 3e, respectively. Both H-pins bound with significantly higher affinity (ca. 100-fold) than their corresponding unlinked PyImPyIm 4 and f-ImPyIm 2 counterparts. ITC analyses revealed modest enthalpies of reactions at 298 K (DeltaH of -3.3 and -1.0 kcal mol(-1) for 5 and 3e, respectively), indicating these were entropic-driven interactions. The heat capacities (DeltaC(p)) were determined to be -116 and -499 cal mol(-1)K(-1), respectively. These results are in general agreement with DeltaC(p) values determined from changes in the solvent accessible surface areas using complexes of the H-pins bound to (5'-CCACGCGTGG)(2). According to the models, the H-pins fit snugly in the minor groove and the linker comfortably holds both polyamide portions in place, with the oxygen atoms pointing into the solvent. In summary, the H-pin polyamide provides an important molecular design motif for the discovery of future generations of programmable small molecules capable of binding to target DNA sequences with high affinity and selectivity

Mallena S., Lee M. P., Bailly C., Neidle S., Kumar A., Boykin D. W., and Wilson W. D. (2004) Thiophene-based diamidine forms a "super" at binding minor groove agent. *J Am Chem Soc* **126,** 13659-13669.
Abstract: The DNA minor groove is the interaction site for many enzymes and transcription control proteins and as a result, development of compounds that target the minor groove is an active research area. In an effort to develop biologically active minor groove agents, we are preparing and exploring the DNA interactions of a systematic set of diamidine derivatives with a powerful array of methods including DNase I footprinting, biosensor-SPR methods, and X-ray crystallography. Surprisingly, conversion of the parent phenyl-furan-phenyl diamidine to a phenyl-thiophene-benzimidazole derivative yields a compound with over 10-fold-increased affinity for the minor groove at AT sequences. Single conversion of the furan to a thiophene or a phenyl to benzimidazole does not cause a similar increase in affinity. X-ray results indicate a small bond angle difference between the C-S-C angle of thiophene and the C-O-C angle of furan that, when amplified out to the terminal amidines of the benzimidazole compounds, yields a very significant difference in the positions of the amidines and their DNA interaction strength.

Marky L. A. and Breslauer K. J. (1987) Origins of netropsin binding affinity and specificity: correlations of thermodynamic and structural data. *Proc Natl Acad Sci U S A* **84,** 4359-4363.
Abstract: We report complete thermodynamic profiles for netropsin binding to an oligomeric and to several polymeric DNA host duplexes. These data allow us to reach the following conclusions: netropsin binding by deep penetration into the minor groove is overwhelmingly enthalpy driven and exhibits a very high binding affinity (K approximately 10^9 at 25 degrees C); deep penetration into the minor groove is required to form those drug-DNA interactions responsible for the enthalpy-driven high binding affinity of netropsin; I-C base pairs form binding sites for netropsin that thermodynamically are equivalent to those formed by A-T base pairs; the positive binding entropies reflect entropic contributions from molecular events other than just water spine disruption; the thermodynamic binding data primarily reflect local netropsin-DNA interactions rather than long-range binding-induced conformational changes at regions distant from the binding site; the enhanced binding affinity associated with deep penetration of netropsin into the minor groove does not result from more favorable electrostatic interactions; the binding of netropsin to the central AATT core of the decamer duplex [d(GCGAATTCGC)]2 is thermodynamically modeled best by netropsin binding to the poly[d(AT)].poly[d(AT)] duplex rather than the poly(dA).poly(dT) duplex. We propose correlations between our thermodynamic data and specific molecular interactions defined by NMR and x-ray structural studies on similar and identical drug-DNA complexes.

Martino L., Virno A., Pagano B., Virgilio A., Micco S. D., Galeone A., Giancola C., Bifulco G., Mayol L. and Randazzo A. (2007) Structural and Thermodynamic Studies of the Interaction of Distamycin A with the Parallel Quadruplex Structure [d(TGGGGT)](4). *J Am. Chem Soc* **129,** 16048-16056.

Abstract: The complex between distamycin A and the parallel DNA quadruplex [d(TGGGGT)]4 has been studied by 1H NMR spectroscopy and isothermal titration calorimetry (ITC). To unambiguously assert that distamycin A interacts with the grooves of the quadruplex [d(TGGGGT)]4, we have analyzed the NMR titration profile of a modified quadruplex, namely [d(TGGMeGGT)]4, and we have applied the recently developed differential frequency-saturation transfer difference (DF-STD) method, for assessing the ligand-DNA binding mode. The three-dimensional structure of the 4:1 distamycin A/[d(TGGGGT)]4 complex has been determined by an in-depth NMR study followed by dynamics and mechanics calculations. All results unequivocally indicate that distamycin molecules interact with [d(TGGGGT)]4 in a 4:1 binding mode, with two antiparallel distamycin dimers that bind simultaneously two opposite grooves of the quadruplex. The affinity between distamycin A and [d(TGGGGT)]4 enhances (approximately 10-fold) when the ratio of distamycin A to the quadruplex is increased. In this paper we report the first three-dimensional structure of a groove-binder molecule complexed to a DNA quadruplex structure.

Matulis D., Rouzina I., and Bloomfield V. A. (2000) Thermodynamics of DNA binding and condensation: isothermal titration calorimetry and electrostatic mechanism. *J Mol Biol* **296,** 1053-1063.
Abstract: The thermodynamics of binding of the trivalent cations cobalt hexammine and spermidine to plasmid DNA was studied by isothermal titration calorimetry. Two stages were observed in the course of titration, the first attributed to cation binding and the second to DNA condensation. A standard calorimetric data analysis was extended by applying an electrostatic binding model, which accounted for most of the observed data. Both the binding and condensation reactions were entropically driven ($T\Delta S$ approximately +10 kcal/mol cation) and enthalpically opposed (ΔH approximately +1 kcal/mol cation). As predicted from their relative sizes, the binding constants of the cations were indistinguishable, but cobalt hexammine had a much greater DNA condensing capacity because it is more compact than spermidine. The dependence of both the free energy of cobalt hexammine binding and the critical cobalt hexammine concentration for DNA condensation on temperature and monovalent cation concentration followed the electrostatic model quite precisely. The heat capacity changes of both stages were positive, perhaps reflecting both the temperature dependence of the dielectric constant of water and the burial of polar surfaces. DNA condensation occurred when about 67 % of the DNA phosphate charge was neutralized by cobalt hexammine and 87 % by spermidine. During condensation, the remaining DNA charge was neutralized.

Mazur S., Tanious F. A., Ding D., Kumar A., Boykin D. W., Simpson I. J., Neidle S., and Wilson W. D. (2000) A thermodynamic and structural analysis of DNA minor-groove complex formation. *J Mol Biol* **300,** 321-337.
Abstract: As part of an effort to develop a better understanding of the structural and thermodynamic principles of DNA minor groove recognition, we have investigated complexes of three diphenylfuran dications with the d(CGCGAATTCGCG)(2) duplex. The parent compound, furamidine (DB75), has two amidine substituents while DB244 has cyclopentyl amidine substituents and DB226 has 3-pentyl amidines. The structure for the DB244-DNA complex is reported here and is compared to the structure of the DB75 complex. Crystals were not obtained with DB226 but information from the DB75 and DB244 structures as well as previous NMR results on DB226 indicate that all three compounds bind in the minor groove at the AATT site of the duplex. DB244 and DB75 penetrate to the floor of the groove and form hydrogen bonds with T8 on one strand and T20 on the opposite strand while DB226 forms a complex with fewer interactions. Binding studies by surface plasmon resonance (SPR) yield -ΔG degrees values in the order DB244>DB75>DB226 that are relatively constant with temperature. The equilibrium binding constants for DB244 are 10-20 times greater than that for DB226. Isothermal titration calorimetric (ITC) experiments indicate that, in contrast to ΔG degrees, ΔH degrees varies considerably with temperature to yield large negative ΔCp degrees values. The thermodynamic results, analyzed in terms of structures of the DNA complexes, provide an explanation of why DB244 binds more strongly to DNA than DB75, while DB266 binds more weakly. All three compounds have a major contribution to binding from hydrophobic interactions but the hydrophobic term is most favorable for DB244. DB244 also has strong contributions from molecular interactions in its DNA complex and all of these factors combine to give it the largest-Δ G degrees for binding. Although the factors that influence the energetics of minor groove interactions are varied and complex, results from the literature coupled with those on the furan derivatives indicate that there are some common characteristics for minor groove recognition by unfused heterocyclic cations that can be used in molecular design.

McKnight R. E., Onogul B., Polasani S. R., Gannon M. K. and Detty M. R. (2008) Substituent control of DNA binding modes in a series of chalcogenoxanthylium photosensitizers as determined by isothermal titration calorimetry and topoisomerase I DNA unwinding assay. *Bioorg. Med. Chem* **16,** 10221-10227.
Abstract: The DNA binding efficacy and preferred mode of binding of a series of rhodamine-related chalcogenoxanthylium dyes was investigated by isothermal titration calorimetry (ITC) using ctDNA, [poly(dCdG)](2) and [poly(dAdT)](2), and by a topoisomerase I DNA unwinding (Topo I) assay. The dyes of this study showed tight binding to ctDNA with binding constants, K(b), on the order of 10(6)-10(7)M(-1). The ITC and Topo I assay studies suggested that the 9-substituent has a strong impact on binding modes ranging from an apparent preference for intercalation with a 9-2-thienyl substituent (similar binding to [poly(dCdG)](2) and [poly(dAdT)](2), re-supercoiling of DNA in the Topo I assay at <10(-5)M dye), to mixed binding modes with 9-phenyl derivatives (2- to 3-fold preference for binding to [poly(dAdT)](2), re-supercoiling of DNA in the Topo I assay at approximately 2 x 10(-5)M dye), to minor groove binding in a 9-(2-thienyl-5-diethylcarboxamide) derivative (strong preference for binding to [poly(dAdT)](2), did not show complete re-supercoiling in the Topo I assay). No binding to ctDNA was observed in one derivative with a 9-(3-thienyl-2-diethylcarboxamide) substituent, which cannot be co-planar with the xanthylium core. In series of dyes where the chalcogen atom was varied, the selenoxanthylium derivatives had 2- to 3-fold higher values of K(b) than the corresponding xanthylium, thioxanthylium, or telluroxanthylium derivatives, which all showed comparable values of K(b). The chalcogen atom appeared to have little influence on binding mode

Miao Y., Lee M. P., Parkinson G. N., Batista-Parra A., Ismail M. A., Neidle S., Boykin D. W., and Wilson W. D. (2005) Out-of-shape DNA minor groove binders: induced fit interactions of heterocyclic dications with the DNA minor groove. *Biochemistry* **44,** 14701-14708.
Abstract: DB921 and DB911 are benzimidazole-biphenyl isomers with terminal charged amidines. DB911 has a central meta-substituted phenyl that gives it a shape similar to those of known minor groove binding compounds. DB921 has a central para-substituted phenyl with a linear conformation that lacks the appropriate radius of curvature to match the groove shape. It is thus expected that DB911, but not DB921, should be an effective minor groove binder, but we find that DB921 not only binds in the groove but also has an unusually high binding constant in SPR experiments (2.9 x 10(8) M(-)(1), vs 2.1 x 10(7) M(-)(1) for DB911). ITC thermodynamic analysis with an AATT sequence shows that the stronger binding of DB921 is due to a more favorable binding enthalpy relative to that of DB911. CD results support minor groove binding for both compounds but do not provide an explanation for the binding of DB921. X-ray crystallographic analysis of DB921 bound to AATT shows that an induced fit structural change in DB921 reduces the twist of the biphenyl to complement the groove, and places the functional groups in position to interact with bases at the floor of the groove. The phenylamidine of DB921 forms indirect contacts with the bases through a bound water. The DB921-water pair forms a curved binding module that matches the shape of the minor groove and provides a number of strong interactions that are not possible with DB911. This result suggests that traditional views of compound curvature required for minor groove complex formation should be reevaluated.

Muller M., Weigand J. E., Weichenrieder O., and Suess B. (2006) Thermodynamic characterization of an engineered tetracycline-binding riboswitch. *Nucleic Acids Res* **34,** 2607-2617.
Abstract: Riboswitches reflect a novel concept in gene regulation that is particularly suited for technological adaptation. Therefore, we characterized thermodynamically the ligand binding properties of a synthetic, tetracycline (tc)-binding RNA aptamer, which regulates gene expression in a dose-dependent manner when inserted into the untranslated region of an mRNA. In vitro, one molecule of tc is bound by one molecule of partially pre-structured and conformationally homogeneous apo-RNA. The dissociation constant of 770 pM, as determined by fluorimetry, is the lowest reported so far for a small molecule-binding RNA aptamer. Additional calorimetric analysis of RNA point mutants and tc derivatives identifies functional groups crucial for the interaction and including their respective enthalpic and entropic contributions we can propose detailed structural and functional roles for certain groups. The conclusions are consistent with mutational analyses in vivo and support the hypothesis that tc-binding reinforces the structure of the RNA aptamer, preventing the scanning ribosome from melting it efficiently.

Munde M., Lee M., Neidle S., Arafa R., Boykin D. W., Liu Y., Bailly C. and Wilson W. D. (2007) Induced fit conformational changes of a "reversed amidine" heterocycle: optimized interactions in a DNA minor groove complex. *J Am. Chem Soc.* **129,** 5688-5698.

Abstract: To better understand the molecular basis for recognition of the DNA minor groove by heterocyclic cations, a series of "reversed amidine" substituted heterocycles has been prepared. Amidine derivatives for targeting the minor groove have the amidine carbon linked to a central heterocyclic system, whereas in the reverse orientation, an amidine nitrogen provides the link. The reverse system has a larger dihedral angle as well as a modified spatial relationship with the groove relative to amidines. Because of the large dihedral, the reversed amidines should have reduced binding to DNA relative to similar amidines. Such a reduction is observed in footprinting, circular dichroism (CD), biosensor-surface plasmon resonance (SPR), and isothermal titration calorimetric (ITC) experiments with DB613, which has a central phenyl-furan-phenyl heterocyclic system. The reduction is not seen when a pyrrole (DB884) is substituted for the furan. Analysis of a number of derivatives defines the pyrrole and a terminal phenyl substituent on the reversed amidine groups as critical components in the strong binding of DB884. ITC and SPR comparisons showed that the better binding of DB884 was due to a more favorable binding enthalpy and that it had exceptionally slow dissociation from DNA. Crystallographic analysis of DB884 bound to an AATT site shows that the compound was bound in the minor groove in a 1:1 complex as suggested by CD solution studies. Surprisingly, unlike the amidine derivative, the pyrrole -NH of DB884 formed an H-bond with a central T of the AATT site and this accounts for the enthalpy-driven strong binding. The structural results and molecular modeling studies provide an explanation for the differences in binding affinities for related amidine and reversed amidine analogues.

Munde M., Ismail M. A., Arafa R., Peixoto P., Collar C. J., Liu Y., Hu L., vid-Cordonnier M. H., Lansiaux A., Bailly C., Boykin D. W. and Wilson W. D. (2007) Design of DNA minor groove binding diamidines that recognize GC base pair sequences: a dimeric-hinge interaction motif. *J Am. Chem Soc.* **129,** 13732-13743.

Abstract: The classical model of DNA minor groove binding compounds is that they should have a crescent shape that closely fits the helical twist of the groove. Several compounds with relatively linear shape and large dihedral twist, however, have been found recently to bind strongly to the minor groove. These observations raise the question of how far the curvature requirement could be relaxed. As an initial step in experimental analysis of this question, a linear triphenyl diamidine, DB1111, and a series of nitrogen tricyclic analogues were prepared. The goal with the heterocycles is to design GC binding selectivity into heterocyclic compounds that can get into cells and exert biological effects. The compounds have a zero radius of curvature from amidine carbon to amidine carbon but a significant dihedral twist across the tricyclic and amidine-ring junctions. They would not be expected to bind well to the DNA minor groove by shape-matching criteria. Detailed DNase I footprinting studies of the sequence specificity of this set of diamidines indicated that a pyrimidine heterocyclic derivative, DB1242, binds specifically to a GC-rich sequence, -GCTCG-. It binds to the GC sequence more strongly than to the usual AT recognition sequences for curved minor groove agents. Other similar derivatives did not exhibit the GC specificity. Biosensor-surface plasmon resonance and isothermal titration calorimetry experiments indicate that DB1242 binds to the GC sequence as a highly cooperative stacked dimer. Circular dichroism results indicate that the compound binds in the minor groove. Molecular modeling studies support a minor groove complex and provide an inter-compound and compound-DNA hydrogen-bonding rational for the unusual GC binding specificity and the requirement for a pyrimidine heterocycle. This compound represents a new direction in the development of DNA sequence-specific agents, and it is the first non-polyamide, synthetic compound to specifically recognize a DNA sequence with a majority of GC base pairs.

Mundoma C. and Greenbaum N. L. (2002) Sequestering of Eu(III) by a GAAA RNA tetraloop. *J Am Chem Soc* **124,** 3525-3532.

Abstract: The site-specific binding of metal ions maintains an important role in the structure, thermal stability, and function of folded RNA structures. RNA tetraloops of the "GNRA" family (where N = any base and R = any purine), which owe their unusual stability to base stacking and an extensive hydrogen bonding network, have been observed to bind metal ions having different chemical and geometric properties. We have used laser-induced lanthanide luminescence and isothermal titration calorimetry (ITC) to examine the metal-binding properties of an RNA stem loop of the GNRA family. Previous research has shown that a single Eu(III) ion binds the stem loop fragment in a highly dehydrated site with a K(d) of

approximately 12 μM. Curve-fitting analysis of the broad luminescence excitation spectrum of Eu(III) upon complexation with the tetraloop fragment indicates the possibility of two microenvironments that do not differ in hydration number. Binding of Eu(III) to the loop was accompanied by positive enthalpic changes, consistent with energetic cost of removal of water molecules and suggesting that the binding is entropically driven. By comparison, binding of Mg(II) or Mn(II) to the RNA loop, or Eu(III) to the DNA analogue of the loop, was associated with exothermic changes, consistent with predominantly outer-sphere coordination. These results suggest specific binding, most probably involving ligands on the 5' side of the loop.

Mundoma C. and Greenbaum N. L. (2003) Binding of europium(III) ions to RNA stem loops: role of the primary hydration sphere in complex formation. *Biopolymers* **69,** 100-109.
Abstract: Understanding the process by which RNA molecules fold into stable structures includes study of the role of site-bound metal ions. Because the alkaline earth metal ions typically associated with RNA structure [most often Mg(II)] do not provide convenient spectroscopic signals, replacement with metal ions having spectroscopically useful properties has been a valuable approach. The luminescence properties of the lanthanide(III) series, in particular europium(III), have made them useful in the study of complexation with biomolecules. We review the physical, chemical, and spectroscopic characteristics of Eu(III) that contribute to its value as a probe of RNA-metal ion interactions, and examples of information obtained from studies of Eu(III) bound to small RNA stem loops. Although Eu(III) has similar site preference to Mg(II), luminescence and isothermal titration calorimetry measurements indicate that Ln(III) loses water molecules from the inner hydration sphere more readily than does Mg(II), resulting in more direct coordination between RNA and the metal ion and very different energetics of binding. In some cases, e.g., a GAAA tetraloop, binding appears to occur by a lock and key process; in the same base sequence containing certain deoxynucleoside substitutions that alter loop structure, binding appears to occur by an induced fit process.

Nakatani K., Sando S., Kumasawa H., Kikuchi J., and Saito I. (2001) Recognition of guanine-guanine mismatches by the dimeric form of 2-amino-1,8-naphthyridine. *J Am Chem Soc* **123,** 12650-12657.
Abstract: Dimeric 2-amino-1,8-naphthyridine selectively binds to a G-G mismatch with high affinity (Kd= 53 nM). We have investigated a binding mechanism of naphthyridine dimer 2 to a G-G mismatch by spectroscopic studies, thermodynamic analysis, and structure-activity studies for the thermal stabilization of the mismatch. 1H NMR spectra of a complex of 2 with 9-mer duplex d(CATCGGATG)2 containing a G-G mismatch showed that all hydrogens in two naphthyridine rings of 2 were observed upfield compared to those of 2 in a free state. The 2D-NOESY experiments showed that each naphthyridine of 2 binds to a guanine in the G-G mismatch within the pi-stack. In CD spectra, a large conformational change of the G-G mismatch-containing duplex was observed upon complex formation with 2. Isothermal calorimetry titration of 2 binding to the G-G mismatch showed that the stoichiometry for the binding is about 1:1 and that the binding is enthalpy-controlled. It is clarified by structure-activity studies that show (i) the linker connecting two naphthyridine rings was essential for the stabilization of the G-G mismatch, (ii) the binding efficiency was very sensitive to the linker structure, and (iii) the binding of two naphthyridines to each one of two Gs in the G-G mismatch is essential for a strong stabilization. These results strongly supported the intercalation of both naphthyridine rings of 2 into DNA base pairs and the formation of a hydrogen bonded complex with the G-G mismatch.

Nakatani K., Hagihara S., Goto Y., Kobori A., Hagihara M., Hayashi G., Kyo M., Nomura M., Mishima M., and Kojima C. (2005) Small-molecule ligand induces nucleotide flipping in (CAG)n trinucleotide repeats. *Nat Chem Biol.* **1**, 39-43.
Abstract: DNA trinucleotide repeats, particularly CXG, are common within the human genome. However, expansion of trinucleotide repeats is associated with a number of disorders, including Huntington disease, spinobulbar muscular atrophy and spinocerebellar ataxia. In these cases, the repeat length is known to correlate with decreased age of onset and disease severity. Repeat expansion of (CAG)n, (CTG)n and (CGG)n trinucleotides may be related to the increased stability of alternative DNA hairpin structures consisting of CXG-CXG triads with X-X mismatches. Small-molecule ligands that selectively bound to CAG repeats could provide an important probe for determining repeat length and an important tool for investigating the in vivo repeat extension mechanism. Here we report that napthyridine-azaquinolone (NA, 1) is a ligand for CAG repeats and can be used as a diagnostic tool for determining repeat length. We show

by NMR spectroscopy that binding of NA to CAG repeats induces the extrusion of a cytidine nucleotide from the DNA helix.

Nguyen B., Stanek J., and Wilson W. D. (2006) Binding-linked protonation of a DNA minor-groove agent. *Biophys J* **90,** 1319-1328.
Abstract: The energetics for binding of a diphenyl diamidine antitrypanosomal agent CGP 40215A to DNA have been studied by spectroscopy, isothermal titration calorimetry, and surface plasmon resonance biosensor methods. Both amidines are positively charged under experimental conditions, but the linking group for the two phenyl amidines has a pK(a) of 6.3 that is susceptible to a protonation process. Spectroscopic studies indicate an increase of 2.7 pK(a) units in the linking group when the compound binds to an A/T minor-groove site. Calorimetric titrations in different buffers and pH conditions support the proton-linkage process and are in a good agreement with spectroscopic titrations. The two methods established a proton-uptake profile as a function of pH. The exothermic enthalpy of complex formation varies with different pH conditions. The observed binding enthalpy increases as a function of temperature indicating a negative heat capacity change that is typical for DNA minor-groove binders. Solvent accessible surface area calculations suggest that surface burial accounts for about one-half of the observed intrinsic negative heat capacity change. Biosensor and calorimetric experiments indicate that the binding affinities vary with pH values and salt concentrations due to protonation and electrostatic interactions. The surface plasmon resonance binding studies indicate that the charge density per phosphate in DNA hairpins is smaller than that in polymers. Energetic contributions from different factors were also estimated for the ligand/DNA complex.

Nishimura T., Okobira T., Kelly A. M., Shimada N., Takeda Y. and Sakurai K. (2007) DNA binding of tilorone: 1H NMR and calorimetric studies of the intercalation. *Biochemistry* **46,** 8156-8163.
Abstract: The fluorene derivative tilorone has received great attention as a DNA intercalator and has been widely recognized as an inducer of interferon. The biological activity of tilorone is known to be related to its binding mode with DNA; however, few structural and thermodynamic studies have elaborated on this issue. This paper presents two-dimensional (2-D) NMR and isothermal titration calorimetry (ITC) for the tilorone/DNA complex, coupled with circular dichroism (CD) spectroscopy and viscosity measurements. NMR investigation suggests that tilorone binds to DNA through intercalation, showing greater affinity for insertion between AT base pairs than between CG pairs. CD spectral changes were observed for T/B (tilorone/DNA base pair molar ratio) ratios greater than the stoichiometric ratio generally expected for intercalators (i.e., T/B = 0.5, according to the neighbor-exclusion principle). However, there was a clear plateau in the CD intensity between T/B < 0.35 and T/B > 0.45. From comparison with NMR and other measurements, we postulate that CD changes below the plateau should be related to the intercalation and the latter to electrostatic interactions and nonspecific bindings. ITC data showed that DeltaH < -TDeltaS < 0, which indicated that tilorone/DNA binding is enthalpy controlled. The magnitude of Kb (the binding constant) was of the same order as that of ethidium bromide. The stoichiometric number, obtained from ITC, CD, and UV data, implied a relatively smaller value (0.28-0.35) than that of the neighbor-exclusion principle. This is because side chains located in the groove disrupt further intercalation to the adjacent sites.

Ou T. M., Lu Y. J., Tan J. H., Huang Z. S., Wong K. Y. and Gu L. Q. (2008) G-quadruplexes: targets in anticancer drug design. *ChemMedChem.* **3,** 690-713.
Abstract: G-quadruplexes are special secondary structures adopted in some guanine-rich DNA sequences. As guanine-rich sequences are present in important regions of the eukaryotic genome, such as telomeres and the regulatory regions of many genes, such structures may play important roles in the regulation of biological events in the body. G-quadruplexes have become valid targets for new anticancer drugs in the past few decades. Many leading compounds that target these structures have been reported, and a few of them have entered preclinical or clinical trials. Nonetheless, the selectivity of this kind of antitumor compound has yet to be improved in order to suppress the side effects caused by nonselective binding. As drug design targets, the topology and structural characteristics of quadruplexes, their possible biological roles, and the modes and sites of small-ligand binding to these structures should be understood clearly. Herein we provide a summary of published research that has set out to address the above problem to provide useful information on the design of small ligands that target G-quadruplexes. This review also covers research methodologies that have been developed to study the binding of ligands to G-quadruplexes

Pagano B., Mattia C. A., Virno A., Randazzo A., Mayol L. and Giancola C. (2007) Thermodynamic analysis of quadruplex DNA-drug interaction. *Nucleosides Nucleotides Nucleic Acids* **26,** 761-765.
Abstract: This work studies the binding properties of distamycin and its carbamoyl analog, containing four pyrrole units, with the [d(TGGGGT)](4) quadruplex by means of isothermal titration calorimetry (ITC). Analysis of the ITC data reveals that drug/quadruplex binding stoichiometry is 1:1 for both interactions and that distamycin analog gives approximately a 10-fold increase in the quadruplex affinity.

Pagano B., Virno A., Mattia C. A., Mayol L., Randazzo A. and Giancola C. (2008) Targeting DNA quadruplexes with distamycin A and its derivatives: an ITC and NMR study. *Biochimie* **90,** 1224-1232.
Abstract: The use of small molecules that bind and stabilize G-quadruplex structures is emerging as a promising way to inhibit telomerase activity in tumor cells. In this paper, isothermal titration calorimetry (ITC) and 1H NMR studies have been conducted to examine the binding of distamycin A and its two carbamoyl derivatives (compounds 1 and 2) to the target [d(TGGGGT)]4 and d[AG3(T2AG3)3] quadruplexes from the Tetrahymena and human telomeres, respectively. The interactions were examined using two different buffered solutions containing either K+ or Na+ at a fixed ionic strength, to evaluate any influence of the ions present in solution on the binding behaviour. Experiments reveal that distamycin A and compound 1 bind the investigated quadruplexes in both solution conditions; conversely, compound 2 appears to have a poor affinity in any case. Moreover, these studies indicate that the presence of different cations in solution affects the stoichiometry and thermodynamics of the interactions

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.

Patel M.M., Anchordoquy T.J. (2005) Contribution of hydrophobicity to thermodynamics of ligand-DNA binding and DNA collapse. *Biophys J.* **88,** 2089-2103.
Abstract: The importance of understanding the dynamics of DNA condensation is inherent in the biological significance of DNA packaging in cell nuclei, as well as for gene therapy applications. Specifically, the role of ligand hydrophobicity in DNA condensation has received little attention. Considering that only multivalent cations can induce true DNA condensation, previous studies exploring monovalent lipids have been unable to address this question. In this study we have elucidated the contribution of the hydrophobic effect to multivalent cation- and cationic lipid-DNA binding and DNA collapse by studying the thermodynamics of cobalt hexammine-, spermine-, and lipospermine-plasmid DNA binding at different temperatures. Comparable molar heat capacity changes (DeltaC(p)) associated with cobalt hexammine- and spermine-DNA binding (-23.39 cal/mol K and -17.98 cal/mol K, respectively) suggest that upon binding to DNA, there are insignificant changes in the hydration state of the methylene groups in spermine. In contrast, the acyl chain contribution to the DeltaC(p) of lipospermine-DNA binding

(DeltaC(p) = DeltaC(p lipospermine) - DeltaC(p spermine)) is significant (-220.94 cal/mol K). Although lipopermine induces DNA ordering into "tubular" suprastructures, such structures do not assume toroidal dimensions as observed for spermine-DNA complexes. We postulate that a steric barrier posed by the acyl chains in lipospermine precludes packaging of DNA into dimensions comparable to those found in nature.

Patel M. M. and Anchordoquy T. J. (2006) Ability of spermine to differentiate between DNA sequences-Preferential stabilization of A-tracts. *Biophys Chem* **122,** 5-15.
Abstract: The regulatory roles fulfilled by polyamines by governance of chromatin structure are made possible by their strong association with cellular DNA, and hence by their ability to modulate DNA structure and function. Towards this end, it is crucial to understand the manifestation of sequence-dependent polyamine binding at the secondary and tertiary structural levels of DNA. This study utilizes circular dichroism (CD) and isothermal titration calorimetry (ITC) to address this relationship by using 20bp oligonucleotides with sequences-poly(dA):poly(dT), poly(dAdT):poly(dAdT), poly(dG):poly(dC), poly(dGdC):poly(dGdC)-that yield physiologically relevant structures, and poly(dIdC):poly(dIdC). CD studies show that at physiological ionic strength (150mM NaCl), spermine preferentially stabilizes A-tracts, and increases flexibility of the G-tract oligomer; the latter is also suggested by the larger change in entropy (DeltaS) of spermine binding to G-tracts. Given the chromatin destabilizing property of these sequences, these findings suggest a role for spermine in stabilization of non-nucleosomal A-tracts, and a compensating mechanism for incorporation of G-tracts in the chromatin structure. Other implications of these findings in sequence dependent DNA packaging are discussed.

Phillips T., Haq I., Meijer A. J., Adams H., Soutar I., Swanson L., Sykes M. J., and Thomas J. A. (2004) DNA binding of an organic dppz-based intercalator. *Biochemistry* **43,** 13657-13665.
Abstract: An improved synthesis of a water-soluble derivative of dipyrido[3,2-a:2',3'-c]phenazine (dppz) is reported. The structures of both dppz and the cation ethylene-bipyridyldiylium-phenazine dinitrate [[1][(PF(6))(2)]] have been obtained via X-ray crystallography. Metal complex derivatives of dppz are very well studied. However, using the water soluble [1][(NO(3))(2)], the nature of the interaction of a simple dppz unit with duplex DNA has been investigated for the first time. In both organic solvents and water, 1 displays unstructured luminescence, assigned to an intramolecular charge transfer. The emission is quenched on binding to natural and synthetic duplex DNA, including poly(dA).poly(dT). A variety of techniques reveal that the cation binds to DNA with an affinity comparable to those of many metal dppz complexes, via an intercalative binding mode.

Pilch D. S., Yu C., Makhey D., LaVoie E. J., Srinivasan A. R., Olson W. K., Sauers R. R., Breslauer K. J., Geacintov N. E., and Liu L. F. (1997) Minor groove-directed and intercalative ligand-DNA interactions in the poisoning of human DNA topoisomerase I by protoberberine analogs. *Biochemistry* **36,** 12542-12553.
Abstract: Spectroscopic, calorimetric, DNA cleavage, electrophoretic, and computer modeling techniques have been employed to characterize the DNA binding and topoisomerase poisoning properties of three protoberberine analogs, 8-desmethylcoralyne (DMC), 5,6-dihydro-8-desmethylcoralyne (DHDMC), and palmatine, which differ in the chemical structures of their B- and/or D-rings. DNA topoisomerase-mediated cleavage assays revealed that these compounds were unable to poison mammalian type II topoisomerase. By contrast, the three protoberberine analogs poisoned human topoisomerase I according to the following hierarchy: DHDMC > DMC > palmatine. DNA binding by all three protoberberine analogs induced negative flow linear dichroism signals as well as unwinding of the host duplex. These two observations are consistent with an intercalative mode of protoberberine binding to duplex DNA. However, a comparison of the DNA binding properties for DMC and DHDMC, which differ only by the state of saturation at the 5,6 positions of the B-ring, revealed that the protoberberine analogs do not "behave" like classic DNA intercalators. Specifically, saturation of the 5-6 double bond in the B-ring of DMC, thereby converting it to the DHDMC molecule, was associated with enhanced DNA unwinding as well as a reversal of DNA binding preference from a DNA duplex with an inaccessible or occluded minor groove inverted question markpoly[d(G-C)]2 inverted question mark to DNA duplexes with accessible or unobstructed minor grooves inverted question markpoly[d(A-T)]2 and poly[d(I-C)]2 inverted question mark. In addition, a comparison of the DNA binding properties for DHDMC and palmatine revealed that transferring the 11-methoxy moiety on the D-ring of DHDMC to the 9 position, thereby converting it to palmatine, was associated with a reduction in binding affinity for both duplexes with unobstructed minor grooves as well

as for duplexes with occluded minor grooves. These DNA binding properties are consistent with a "mixed-mode" DNA binding model for protoberberines in which a portion of the ligand molecule intercalates into the double helix, while the nonintercalated portion of the ligand molecule protrudes into the minor groove of the host duplex, where it is thereby available for interactions with atoms lining the floor and/or walls of the minor groove. Furthermore, saturation at the 5,6 positions of the B-ring, which causes the A-ring to be tilted relative to the plane formed by rings C and D, appears to stabilize the interaction between the host duplex and the minor groove-directed portion of the protoberberine ligand. Computer modeling studies on the DHDMC-poly[d(A-T)]2 complex suggest that this interaction may involve van der Waals contacts between the ligand A-ring and backbone sugar atoms lining the minor groove of the host duplex. The hierarchy of topoisomerase I poisoning noted above suggests that this minor groove-directed interaction may play an important role in topoisomerase I poisoning by protoberberine analogs. In the aggregate, our results presented here, coupled with the recent demonstration of topoisomerase I poisoning by minor groove-binding terbenzimidazoles [Sun, Q., Gatto, B., Yu, C., Liu, A. , Liu, L. F., & LaVoie, E. J. (1995) J. Med. Chem. 38, 3638-3644], suggest that minor groove-directed ligand-DNA interactions may be of general importance in the poisoning of topoisomerase I.

Pilch D. S., Poklar N., Baird E. E., Dervan P. B., and Breslauer K. J. (1999) The thermodynamics of polyamide-DNA recognition: hairpin polyamide binding in the minor groove of duplex DNA. *Biochemistry* **38,** 2143-2151.
Abstract: Crescent-shaped synthetic ligands containing aromatic amino acids have been designed for specific recognition of predetermined DNA sequences in the minor groove of DNA. Simple rules have been developed that relate the side-by-side pairings of Imidazole (Im) and Pyrrole (Py) amino acids to their predicted target DNA sequences. We report here thermodynamic characterization of the DNA-binding properties of the six-ring hairpin polyamide, ImImPy-gamma-PyPyPy-beta-Dp (where gamma = gamma-aminobutyric acid, beta = beta-alanine, and Dp = dimethylaminopropylamide). Our data reveal that, at 20 degrees C, this ligand binds with a relatively modest 1.8-fold preference for the designated match site, 5'-TGGTA-3', over the single base pair mismatch site, 5'-TGTTA-3'. By contrast, we find that the ligand exhibits a 102-fold greater affinity for its designated match site relative to the double base pair mismatch site, 5'-TATTA-3'. These results demonstrate that the energetic cost of binding to a double mismatch site is not necessarily equal to twice the energetic cost of binding to a single mismatch site. Our calorimetrically measured binding enthalpies and calculated entropy data at 20 degrees C reveal the ligand sequence specificity to be enthalpic in origin. We have compared the DNA-binding properties of ImImPy-gamma-PyPyPy-beta-Dp with the hairpin polyamide, ImPyPy-gamma-PyPyPy-beta-Dp (an Im --> Py "mutant"). Our data reveal that both ligands exhibit high affinities for their designated match sites, consistent with the Dervan pairing rules. Our data also reveal that, relative to their corresponding single mismatch sites, ImImPy-gamma-PyPyPy-beta-Dp is less selective than ImPyPy-gamma-PyPyPy-beta-Dp for its designated match site. This result suggests, at least in this case, that enhanced binding affinity can be accompanied by some loss in sequence specificity. Such systematic comparative studies allow us to begin to establish the thermodynamic database required for the rational design of synthetic polyamides with predictable DNA-binding affinities and specificities.

Pilch D. S., Kaul M., Barbieri C. M., and Kerrigan J. E. (2003) Thermodynamics of aminoglycoside-rRNA recognition. *Biopolymers* **70,** 58-79.
Abstract: 2-Deoxystreptamine (2-DOS) aminoglycosides are a family of structurally related broad-spectrum antibiotics that are used widely in the treatment of infections caused by aerobic Gram-negative bacilli. Their antibiotic activities are ascribed to their abilities to bind a highly conserved A site in the 16 S rRNA of the 30 S ribosomal subunit and interfere with protein synthesis. The abilities of the 2-DOS aminoglycosides to recognize a specific subdomain of a large RNA molecule make these compounds archetypical models for RNA-targeting drugs. This article presents a series of calorimetric, spectroscopic, osmotic stress, and computational studies designed to evaluate the thermodynamics (ΔG, ΔH, ΔS, ΔCp) of aminoglycoside-rRNA interactions, as well as the hydration changes that accompany these interactions. In conjunction with the current structural database, the results of these studies provide important insights into the molecular forces that dictate and control the rRNA binding affinities and specificities of the aminoglycosides. Significantly, identification of these molecular driving forces [which include binding-linked drug protonation reactions, polyelectrolyte contributions from counterion release, conformational changes, hydration effects, and molecular interactions (e.g., hydrogen bonds and van der Waals

interactions)], as well as the relative magnitudes of their contributions to the binding free energy, could not be achieved by consideration of structural data alone, highlighting the importance of acquiring both thermodynamic and structural information for developing a complete understanding of the drug-RNA binding process. The results presented here begin to establish a database that can be used to predict, over a range of conditions, the relative affinity of a given aminoglycoside or aminoglycoside mimetic for a targeted RNA site vs binding to potential competing secondary sites. This type of predictive capability is essential for establishment of a rational design approach to the development of new RNA-targeted drugs.

Pilch D.S., Kaul M., and Barbieri C. M. (2005) Ribosomal RNA Recognition by Aminoglycoside Antibiotics. *Top Curr Chem* **253,** 179-204.
Abstract: 2-Deoxystreptamine (2-DOS) aminoglycosides are a family of structurally related broad-spectrum antibiotics that are used widely in the clinic. Their antibiotic activities are ascribed to their abilities to bind a highly conserved sequence (termed the A site) in the 16S rRNA of the 30S ribosomal subunit and interfere with protein synthesis. The aminoglycosides represent a paradigm for both drug–RNA and drug–ribosome interactions, and information gleaned from their study has relevance with regard to other RNA- and ribosome-directed drugs of acute clinical importance. This contribution provides an integrated overview of structural and thermodynamic studies of the rRNA binding of aminoglycosides. The results of these studies have enhanced our understanding of the molecular forces that govern aminoglycoside recognition of the rRNA A site, and underlie the mechanism and specificity of action of these drugs. Such knowledge provides the type of predictive capabilities that are essential for the devlopment of a rational basis for future drug design strategies

Prevette L. E., Lynch M. L., Kizjakina K. and Reineke T. M. (2008) Correlation of amine number and pDNA binding mechanism for trehalose-based polycations. *Langmuir* **24,** 8090-8101.
Abstract: Glycopolymers with repeat units comprised of the disaccharide trehalose and an oligoamine of increasing amine have been previously synthesized by our group and shown to efficiently deliver pDNA (plasmid DNA) to HeLa cells while remaining relatively nontoxic. Complexes formed between the most amine-dense of these polycations and pDNA were also found to be relatively stable in serum and have low aggregation, which is desirable for in vivo gene delivery. To lend insight into these interesting results, this study was aimed at investigating the binding strength and mechanism of interaction between these macromolecules, via isothermal titration calorimetry (ITC) and ethidium bromide exclusion assays. The size of these pDNA-polymer complexes, or polyplexes, at various states of formation was determined through light scattering and zeta-potential measurements. Varying degrees of pDNA secondary structure change occurred upon interaction with the polymers, as evidenced by circular dichroism spectra through increasing molar ratios of polymer amine to DNA phosphate, and Fourier transform infrared (FT-IR) results demonstrated stronger electrostatic binding with the phosphate backbone with the least amine-dense of the series. It was concluded that, depending on the number of secondary amines in the repeat unit, these polymers interact with pDNA via different mechanisms with varying extents of electrostatic interaction and hydrogen bonding. These differing mechanisms may affect the ability of trehalose to serve as a deterrent against aggregation in serum conditions and lend insight into the roles of polymer-pDNA binding during the complex transfection process

Qu X., Ren J., Riccelli P. V., Benight A. S., and Chaires J. B. (2003) Enthalpy/entropy compensation: influence of DNA flanking sequence on the binding of 7-amino actinomycin D to its primary binding site in short DNA duplexes. *Biochemistry* **42,** 11960-11967.
Abstract: The effect of the context of the flanking sequence on ligand binding to DNA oligonucleotides that contain consensus binding sites was investigated for the binding of the intercalator 7-amino actinomycin D. Seven self-complementary DNA oligomers each containing a centrally located primary binding site, 5'-A-G-C-T-3', flanked on either side by the sequences (AT)(n) or (AA)(n) (with n = 2, 3, 4) and AA(AT)(2), were studied. For different flanking sequences, (AA)(n)-series or (AT)(n)-series, differential fluorescence enhancements of the ligand due to binding were observed. Thermodynamic studies indicated that the flanking sequences not only affected DNA stability and secondary structure but also modulated ligand binding to the primary binding site. The magnitude of the ligand binding affinity to the primary site was inversely related to the sequence dependent stability. The enthalpy of ligand binding was directly measured by isothermal titration calorimetry, and this made it possible to parse the binding free energy into its energetic and entropic terms. Our results reveal a pronounced enthalpy-entropy

compensation for 7-amino actinomycin D binding to this family of oligonucleotides and suggest that the DNA sequences flanking the primary binding site can strongly influence ligand recognition of specific sites on target DNA molecules.

Rajput C., Rutkaite R., Swanson L., Haq I., and Thomas J. A. (2006) Dinuclear monointercalating RuII complexes that display high affinity binding to duplex and quadruplex DNA. *Chemistry* **12,** 4611-4619.
Abstract: The DNA duplex binding properties of previously reported dinuclear Ru(II) complexes based on the ditopic ligands tetrapyrido[3,2-a:2',3'-c:3",2"-h:2",3"-j]phenazine (tppz) and tetraazatetrapyrido[3,2-a:2'3'-c:3",2"-l:2'",3'"-n]pentacene (tatpp) are reported. Photophysical and biophysical studies indicate that, even at high ionic strengths, these complexes bind to duplex DNA, through intercalation, with affinities that are higher than any other monointercalating complex and are only equalled by DNA-threaded bisintercalating complexes. Additional studies at high ionic strengths using the 22-mer d(AG(3)[T(2)AG(3)](3)) [G3] human telomeric sequence reveal that the dinuclear tppz-based systems also bind with high affinity to quadruplex DNA. Furthermore, for these complexes, quadruplex binding is accompanied by a distinctive blue-shifted "light-switch" effect, characterized by higher emission enhancements than those observed in the analogous duplex effect. Calorimetry studies reveal that the thermodynamics of duplex and quadruplex binding is distinctly different, with the former being entirely entropically driven and the latter being both enthalpically and entropically favored.

Remeta D. P., Mudd C. P., Berger R. L., and Breslauer K. J. (1993) Thermodynamic characterization of daunomycin-DNA interactions: comparison of complete binding profiles for a series of DNA host duplexes. *Biochemistry* **32,** 5064-5073.
Abstract: Using a combination of spectroscopic and calorimetric techniques, we have determined complete thermodynamic binding profiles (Δ G degree, Δ H degree, and Δ S degree) for the complexation of daunomycin to a series of 10 polymeric DNA duplexes. We find the resulting drug binding data to be sensitive to the base composition and sequence of the host duplex, with the binding free energies ranging from -7.5 to -10.8 kcal/mol of bound drug and the binding enthalpies ranging from +4.11 to -10.76 kcal/mol of bound drug at 25 degrees C. The smaller range in the free energy term reflects the impact of large enthalpy-entropy compensations. We observe that the three synthetic duplexes which exhibit the highest daunomycin binding affinities all contain GC (or IC) base pairs as part of alternating purine/pyrimidine sequence motifs, with these high binding affinities being strongly enthalpy driven at 25 degrees C. Specific comparisons between the binding profiles for daunomycin complexation with select pairs of host duplexes lead to the following observations: (1) The presence or absence of a major-groove methyl group does not alter daunomycin binding thermodynamics. (2) The presence or absence of a minor-groove amino group does alter daunomycin binding thermodynamics. (3) Duplexes with different base compositions but identical minor-groove functionality exhibit similar daunomycin binding thermodynamics. (4) Homopolymeric duplexes composed of either AT or AU base pairs, but not GC base pairs, exhibit large enthalpy-entropy compensations in their daunomycin binding profiles. We propose interpretations of these and other features of our thermodynamic data in terms of specific daunomycin-DNA interactions deduced from available structural data.

Ren J., Jenkins T. C., and Chaires J. B. (2000) Energetics of DNA intercalation reactions. *Biochemistry* **39,** 8439-8447.
Abstract: Isothermal titration calorimetry has been used to determine the binding enthalpy and heat capacity change (ΔCp()) for a series of DNA intercalators, including ethidium, propidium, daunorubicin, and adriamycin. Temperature-dependent binding enthalpies were measured directly for the ligands, from which ΔCp() values of -140 to -160 cal mol^{-1}K^{-1}were calculated. Published van't Hoff plots were reanalyzed to obtain ΔCp() values of -337 to -423 cal mol^{-1} $^{-1}$ or the binding of actinomycin D to several DNA oligonucleotide duplexes with defined sequences. Heat capacity changes for DNA intercalation were found to correlate with the alterations in solvent-accessible surface area calculated from available high-resolution structural data. Multiple linear regression was used to derive the relationship ΔCp() = 0. 382(+/-0.026)ΔA(np) - 0.121(+/-0.077)ΔA(p) cal mol^{-1} K^{-1}, where ΔA(np) and ΔA(p) are the binding-induced changes in nonpolar and polar solvent-accessible surface areas (in square angstroms), respectively. The ΔCp() terms were used to estimate the hydrophobic contribution to intercalative binding free energies, yielding values that ranged from -11.2 (ethidium) to -30 kcal mol^{-1} (actinomycin D). An attempt was made to parse the observed binding free energies of ethidium and propidium into five underlying contributions.

Such analysis showed that the DNA binding behavior of these simple intercalators is driven almost equally by hydrophobic effects and van der Waals contacts within the intercalation site.

Rentzeperis D., Marky L. A., Dwyer T. J., Geierstanger B. H., Pelton J. G., and Wemmer D. E. (1995) Interaction of minor groove ligands to an AAATT/AATTT site: correlation of thermodynamic characterization and solution structure. *Biochemistry* **34,** 2937-2945.
Abstract: A combination of circular dichroism spectroscopy, titration calorimetry, and optical melting has been used to investigate the association of the minor groove ligands netropsin and distamycin to the central A3T2 binding site of the DNA duplex d(CGCAAATTGGC).d(GCCAATTTGCG). For the complex with netropsin at 20 degrees C, a ligand/duplex stoichiometry of 1:1 was obtained with Kb approximately 4.3 x 10^7 M^{-1}, Δ Hb approximately -7.5 kcal mol^{-1}, Δ Sb approximately 9.3 cal K^{-1} mol^{-1}, and ΔCp approximately 0. Previous NMR studies characterized the distamycin complex with A3T2 at saturation as a dimeric side-by-side complex. Consistent with this result, we found a ligand/duplex stoichiometry of 2:1. In the current study, the relative thermodynamic contributions of the two distamycin ligands in the formation of this side-by-side complex (2:1 Dst.A3T2) were evaluated and compared with the thermodynamic characteristics of netropsin binding. The association of the first distamycin molecule of the 2:1 Dst.A3T2 complex yielded the following thermodynamic profile: Kb approximately 3.1 x 10^7) M^{-1}, Δ Hb = -12.3 kcal mol^{-1}, Δ Sb = -8 cal K^{-1} mol^{-1}, and Δ Cp = -42 cal K^{-1} mol^{-1}. The binding of the second distamycin molecule occurs with a lower Kb of approximately 3.3 x 10^6 M^{-1}, a more favorable Δ Hb of -18.8 kcal mol^{-1}, a more unfavorable Δ Sb of -34 cal K^{-1} mol^{-1}, and a higher Δ Cp of -196 cal K^{-1} mol^{-1}. The latter term indicates an ordering of electrostricted and structural water molecules by the complexes. These results correlate well with the NMR titrations and are discussed in context of the solution structure of the 2:1 Dst.A3T2 complex.

Rentzeperis D., Medero M., and Marky L. A. (1995) Thermodynamic investigation of the association of ethidium, propidium and bis-ethidium to DNA hairpins. *Bioorg Med Chem* **3,** 751-759.
Abstract: We have used a combination of calorimetric and spectroscopic techniques to investigate the association of the bis-intercalator ethidium homodimer (bis-ethidium) to short DNA hairpins with sequences: d(GCGCT5GCGC) and d(CGCGT5CGCG). The helix-coil transition of each hairpin, investigated by UV and calorimetric melting protocol, takes place in monomolecular two-state transitions with characteristic enthalpies of approximately 37 kcal mol^{-1} for disrupting the four dG-dC base pairs of the hairpin stems. Deconvolution of the bis-ethidium-hairpin calorimetric titration curves indicate that each hairpin contains two distinct binding sites for the ligand: a high affinity site in the stem (Kb approximately 10^7) that accommodates one bis-ethidium molecule and a lower affinity site (Kb approximately 10^6) located probably at the loop that accommodates two bis-ethidium molecules. The overall stoichiometries of three ligands per hairpin are in agreement with those obtained in continuous variation experiments using visible spectroscopy. The interaction of bis-ethidium for each type of sites results in enthalpy driven reactions, with average binding enthalpies, Δ Hb, of -13.1 and -12.1 kcal mol^{-1} for the stem and loop sites, respectively. Comparison to the thermodynamic profiles of ethidium and propidium binding reveals that the bis-ethidium binding to the stem site of each hairpin has a more favorable free energy term of -1.4 kcal mol-1 and more favorable enthalpy of -4.2 kcal mol^{-1}. These suggest that only one phenanthridine ring of bis-ethidium intercalates in the stem, while the second planar ring is exposed to solvent or weakly associated to the surface of DNA.

Roche C. J., Thomson J. A., and Crothers D. M. (1994) Site selectivity of daunomycin. *Biochemistry* **33,** 926-935.
Abstract: We have reexamined the binding properties of the antitumor drug daunomycin using double-helical oligonucleotides 16 base pairs long that were designed to contain preferred binding sites for the drug. The preferred sites are contained in a six base pair core which is flanked on the 5' and 3' ends by tracts of adenines. The flanking sequences, which augment helix stability and reduce and effects, were chosen because daunomycin is known to bind poorly to poly(dA).poly(dT). Four major sequences were examined in the six base pair core: CGTACG, TAGCTG, TCATCC, and (TA)3 and compared with calf thymus DNA. A randomly generated 16 bp sequence containing no A tracts and a sequence containing only tracts of As and Ts were also used. Fluorometric, absorption, calorimetric, and stopped-flow techniques were used to examine the binding. The affinity of the drug for oligomers containing known binding sites was comparable to or enhanced relative to that for calf thymus bulk DNA. Association constants ranged from 1.0 x 10^8 to 3.0 x 10^7 M^{-1}. The strongest core binding site found was CGTACG, but its affinity is only

2-fold larger than that of other core sequences. Appreciable binding to the flanking A tracts was observed. An oligonucleotide which incorporates the CGTACG sequence in a short hairpin helix binds an order of magnitude more weakly. Complex lifetimes measured by stopped flow generally increase with equilibrium stability; the kinetics confirm the existence of a set of weaker sites. The exothermic binding enthalpy for daunomycin with the CGTACG core sequence is more than twice as large as for the TATATA sequence. Binding to dA20.dT20 is endothermic, and a less exothermic component can be detected in the calorimetric binding curve of the oligomers containing flanking A tracts.

Salim N. N. and Feig A. L. (2008) Isothermal titration calorimetry of RNA. *Methods (epublication)*.
Abstract: Isothermal titration calorimetry (ITC) is a fast and robust method to study the physical basis of molecular interactions. A single well-designed experiment can provide complete thermodynamic characterization of a binding reaction, including K(a), DeltaG, DeltaH, DeltaS and reaction stoichiometry (n). Repeating the experiment at different temperatures allows determination of the heat capacity change (DeltaC(P)) of the interaction. Modern calorimeters are sensitive enough to probe even weak biological interactions making ITC a very popular method among biochemists. Although ITC has been applied to protein studies for many years, it is becoming widely applicable in RNA biochemistry as well, especially in studies which involve RNA folding and RNA interactions with small molecules, proteins and with other RNAs. This review focuses on best practices for planning, designing and executing effective ITC experiments when one or more of the reactants is an RNA

Sasaki S., Shibata T., Torigoe H., Shibata Y., and Maeda M. (2001) Novel class of DNA binding motifs based on bistetrahydrofuran and bisfuran skeleton with long alkyl chains. *Nucleosides Nucleotides Nucleic Acids* **20,** 551-558.
Abstract: Small molecules with DNA-binding affinity within the minor groove have become of great interest. In this paper, new DNA binding molecules; diamino-bistetrahydrofuran (bisTHF) and diamino-bisfuran are reported. The bisTHF ligand with RR configuration at the amino groups and C8 alkyl chains (RR8) stabilized GC-rich duplex. In contrast, bisfuran compounds stabilized AT-rich duplex. The binding affinity of RR8 with 12 mer duplex DNA was determined by isothermal titration calorimetry to be 3.3 x 10^8 M^{-1}.

Sato Y., Seino T., Nishizawa S., and Teramae N. (2006) Thermodynamic characterization of the binding of naphthyridines to the AP site-containing DNA duplexes. *Nucleic Acids Symp Ser (Oxf)* 219-220.
Abstract: We here report on the thermodynamics of the hydrogen bond-mediated binding of 2-amino-7-methyl-1,8-naphthyridine (AMND) to a cytosine base opposite an abasic site (AP site) in a 21-meric DNA duplex (5'-GCA GCT CCC GXG GTC TCC TCG-3'/3'-CGT CGA GGG CCC CAG AGG AGC-5', X= AP site, C = target). The examination by fluorescence titration experiments shows a 1:1 binding constant of 2.7x10(6) M(-1) at 20 degrees C in solutions containing 110 mM Na(+) (pH 7.0). From the analysis of salt dependence of binding constants, polyelectrolyte (DeltaG(pe)) and non-polyelectrolyte (DeltaG(t)) contributions are calculated as -1.7 kcal/mol and -6.9 kcal/mol, respectively, at 110 mM Na(+) concentration. The binding enthalpy determined by isothermal titration calorimetry (ITC) is -18.5 kcal/mol in 110 mM Na(+) at 20 degrees C. We discuss these results with a view towards further development of our ligand-based fluorescence assay for SNPs (single nucleotide polymorphisms) typing.

Sha F. and Chen F. M. (2000) Actinomycin D binds strongly to d(CGACGACG) and d(CGTCGTCG). *Biophys J* **79,** 2095-2104.
Abstract: Earlier calorimetric studies had indicated that despite the absence of a GpC sequence, the self-complementary octamer d(CGTCGACG) binds strongly to actinomycin D (ACTD) with high cooperativity and a 2:1 drug/duplex ratio. A subsequent optical spectral study with related oligomers led us to suggest that ACTD may likely stack at the G. C basepairs of the duplex termini. New findings are reported herein to indicate that despite the lack of complete self-complementarity, oligomers of d(CGXCGXCG) [X = A or T] motif exhibit unusually strong ACTD affinities with binding constants of roughly 2 x 10^7 $M^{-1}$and binding densities of 1 drug molecule per strand. The ACTD binding affinity for the corresponding heteroduplex obtained by annealing these two oligomers is, however, considerably reduced. Although spectroscopic results with related oligomers obtained by removing, replacing, or appending bases at the termini appear to be consistent with the end-stacking model, capillary electrophoretic (CE) evidence provides additional insights into the binding mode. CE experiments with the self-complementary oligomers

d(CGAGCTCG) and d(CGTCGACG) revealed contrasting migration patterns in the presence of ACTD, with mobility retardation and acceleration exhibited by the GpC- and non-GpC-containing octamers, respectively, whereas the X/X-mismatched d(CGXCGXCG) experienced retardation. These results, along with those of related oligomers, suggest that ACTD may in fact stack at the duplex stem end of a monomeric hairpin or at the 3'-end of dG as a single strand. The seemingly cooperative ACTD binding and the curved Scatchard plot for the self-complementary d(CGTCGACG) may thus be attributed to the drug-induced duplex denaturation resulting from strong binding to single strands of d(CGXCGYCG) motif. Detailed structural information on the ACTD-DNA complexes, however, must await further NMR investigations.

Shah D., KELLY J., Zhang Y., Dande P., Martinez J., Ortiz G., Fronza G., Tran H., Soto A. M., Marky L., and Gold B. (2001) Evidence in Escherichia coli that N3-methyladenine lesions induced by a minor groove binding methyl sulfonate ester can be processed by both base and nucleotide excision repair. *Biochemistry* **40,** 1796-1803.
Abstract: It has been previously reported that a neutral DNA equilibrium binding agent based on an N-methylpyrrolecarboxamide dipeptide (lex) and modified with an O-methyl sulfonate ester functionality (MeOSO(2)-lex) selectively affords N3-methyladenine lesions. To study the interaction of the neutral lex dipeptide with calf thymus DNA, we have prepared stable, nonmethylating sulfone analogues of MeOSO(2)-lex that are neutral and cationic. Thermodynamic studies show that both the neutral and monocationic sulfone compounds bind to DNA with Kb's of 10^5 in primarily entropy-driven reactions. To determine how the cytotoxic N3-methyladenine adduct generated from MeOSO(2)-lex is repaired in E. coli, MeOSO(2)-lex was tested for toxicity in wild-type E. coli and in mutant strains defective in base excision repair (tag and/or alkA glycosylases or apn endonuclease), nucleotide excision repair (uvrA), and both base and nucleotide excision repair (tag/alkA/uvrA). The results clearly demonstrate the cellular toxicity of the N3-methyladenine lesion, and the protective role of base excision glycosylase proteins. A novel finding is that in the absence of functional base excision glycosylases, nucleotide excision repair can also protect cells from this cytotoxic minor groove lesion. Interaction between base and nucleotide excision repair systems is also seen in the protection of cells treated with cis-diamminedichloroplatinum(II) but not with anti-(+/-)-r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyren e.

Sielaff A., Mackay H., Brown T. and Lee M. (2008) 2-Aminopurine/cytosine base pair containing oligonucleotides: fluorescence spectroscopy studies on DNA-polyamide binding. *Biochem Biophys Res Commun* **369,** 630-634.
Abstract: Studies on the binding of a triamide f-IPI (1) to its cognate sequence labeled with a 2-aminopurine (2AP or G(*)) group are described. ITC studies showed that f-IPI (1) bound to the cognate site (ACG(*)CGT) with only 3.5-fold lower affinity than binding to the unlabeled DNA (ACGCGT) (K(eq)=2 x 10(7) and 7 x 10(7)M(-1), respectively). Titration of f-IPI (1) to both sequences gave strong induced bands at 330 nm via circular dichroism studies. The compound also gave comparable DeltaT(m) values of 5.0 and 7.8 degrees C, respectively. These techniques also proved that the sequence selectivity of f-IPI (1) was uncompromised, as only limited binding to the non-cognate sequence ACCG(*)GT was observed. Fluorescence studies demonstrated a 2:1 ligand:DNA binding motif as anticipated, and indicated that the limit of detection for this technique was 20muM DNA concentration. The results demonstrate that 2-aminopurine is a sufficient substitute for guanine in a G.C base pair useful in DNA binding studies

Sinha R., Hossain M. and Kumar G. S. (2007) RNA targeting by DNA binding drugs: structural, conformational and energetic aspects of the binding of quinacrine and DAPI to A-form and H(L)-form of poly(rC).poly(rG). *Biochim Biophys Acta* **1770,** 1636-1650.
Abstract: A key step in the rational design of new RNA binding small molecules necessitates a complete elucidation of the molecular aspects of the binding of existing molecules to RNA structures. This work focuses towards the understanding of the interaction of a DNA intercalator, quinacrine and a minor groove binder 4',6-diamidino-2-phenylindole (DAPI) with the right handed Watson-Crick base paired A-form and the left-handed Hoogsteen base paired H(L)-form of poly(rC).poly(rG) evaluated by multifaceted spectroscopic and viscometric techniques. The energetics of their interaction has also been elucidated by isothermal titration calorimetry. Results of this study converge to suggest that (i) quinacrine intercalates to both A-form and H(L)-form of poly(rC).poly(rG); (ii) DAPI shows both intercalative and groove-binding modes to the A-form of the RNA but binds by intercalative mode to the H(L)-form. Isothermal calorimetric

patterns of quinacrine binding to both the forms of RNA and of DAPI binding to the H(L)-form are indicative of single binding while the binding of DAPI to the A-form reveals two kinds of binding. The binding of both the drugs to both conformations of RNA is exothermic; while the binding of quinacrine to both conformations and DAPI to the A-form (first site) is entropy driven, the binding of DAPI to the second site of A-form and H(L)-conformation is enthalpy driven. Temperature dependence of the binding enthalpy revealed that the RNA-ligand interaction reactions are accompanied by small heat capacity changes that are nonetheless significant. We conclude that the binding affinity characteristics and energetics of interaction of these DNA binding molecules to the RNA conformations are significantly different and may serve as data for the development of effective structure selective RNA-based antiviral drugs.

Suh D., Oh Y. K., Hur M. W., Ahn B., and Chaires J. B. (2002) Daunomycin binding to deoxypolynucleotides with alternating sequences: complete thermodynamic profiles of heterogeneous binding sites. *Nucleosides Nucleotides Nucleic Acids* **21,** 637-649.
Abstract: Complete thermodynamic binding profiles for the interaction of the anticancer drug, daunomycin with natural DNA and synthetic deoxypolynucleotides were described. Fluorescence titration method was used to estimate the equilibrium binding constants. Binding isotherms were found to be surprisingly complex in some cases, presumably because there were heterogeneous sites even in simple deoxypolynucleotides of repeating sequence. Some polynucleotides consisting of alternating sequence contain at least two different binding sites for daunomycin. The binding affinity of the primary binding sites of alternating and non-alternating sequences was found to differ by two orders of magnitude. An isothermal microtitration calorimeter was used to directly measure the binding enthalpy at 25 degrees C with a high sensitivity. The binding enthalpy of poly[d(A-T)] was found to be -5.5 Kcal/mol, which was much lower than any other polynucleotides, while the binding constant of the high affinity sites, was similar. In this report, the complete thermodynamic profiles of daunomycin binding to deoxypolynucleotides were reliably shown for the first time.

Sun T. and Zhang Y. (2008) Pentamidine binds to tRNA through non-specific hydrophobic interactions and inhibits aminoacylation and translation. *Nucleic Acids Res* **36,** 1654-1664.
Abstract: The selective and potent inhibition of mitochondrial translation in Saccharomyces cerevisiae by pentamidine suggests a novel antimicrobial action for this drug. Electrophoresis mobility shift assay, T1 ribonuclease footprinting, hydroxyl radical footprinting and isothermal titration calorimetry collectively demonstrated that pentamidine non-specifically binds to two distinct classes of sites on tRNA. The binding was driven by favorable entropy changes indicative of a large hydrophobic interaction, suggesting that the aromatic rings of pentamidine are inserted into the stacked base pairs of tRNA helices. Pentamidine binding disrupts the tRNA secondary structure and masks the anticodon loop in the tertiary structure. Consistently, we showed that pentamidine specifically inhibits tRNA aminoacylation but not the cognate amino acid adenylation. Pentamidine inhibited protein translation in vitro with an EC(50) equivalent to that binds to tRNA and inhibits tRNA aminoacylation in vitro, but drastically higher than that inhibits translation in vivo, supporting the established notion that the antimicrobial activity of pentamidine is largely due to its selective accumulation by the pathogen rather than by the host cell. Therefore, interrupting tRNA aminoacylation by the entropy-driven non-specific binding is an important mechanism of pentamidine in inhibiting protein translation, providing new insights into the development of antimicrobial drugs

Tan J. F., Too H. P., Hatton T. A., and Tam K. C. (2006) Aggregation behavior and thermodynamics of binding between poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate) and plasmid DNA. *Langmuir* **22,** 3744-3750.
Abstract: The aggregation behavior and the thermodynamics of binding between poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate) (PEO-b-PDEAEMA) block copolymers and plasmid DNA were examined. Binding between the polymer and DNA were confirmed by gel electrophoresis. The high affinity between the polymer and DNA was demonstrated through the ethidium bromide (EtBr) displacement assay, and the binding was found to be related to the stoichiometric balance between the amine group of the polymer and the DNA nucleotide molar ratio (N/P molar ratio). The light scattering and TEM results showed that, at low polymer concentration, the hydrodynamic radii (R(h)) of the polymer/DNA complexes was around 90 nm; however, at sufficiently high polymer concentration, the

complexes condensed to around 35 nm induced by a structural rearrangement of the amphiphilic nature of the block copolymer. The isothermal titration calorimetric results showed that the binding between the polymer and DNA is driven by a large favorable enthalpy.

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 (CT DNA), poly(dA-dT), and poly(dG) x poly(dC) are reported. One contained bulky, cyclic cationic substituents at the 9 and 10 positions, and the other carried acylic, 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, 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 thermoneutral binding, smaller hypochromism, no bathochromism, very weak induced CD, and no change in the helix melting temperature with any of the DNA polymers. A sharp distinction between the binding properties of the two probes is indicated, and both have intrinsic binding constants of approximately 10(6) M(-1) for the three 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 the acyclic derivative corresponded with those of the cyclic derivative. The acyclic derivative showed large preference (10-fold) for poly(dG) x poly(dC) over poly(dA-dT), whereas 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.

Tanious F. A., Laine W., Peixoto P., Bailly C., Goodwin K. D., Lewis M. A., Long E. C., Georgiadis M. M., Tidwell R. R. and Wilson W. D. (2007) Unusually strong binding to the DNA minor groove by a highly twisted benzimidazole diphenylether: induced fit and bound water. *Biochemistry* **46,** 6944-6956.
Abstract: RT29 is a dicationic diamidine derivative that does not obey the classical "rules" for shape and functional group placement that are expected to result in strong binding and specific recognition of the DNA minor groove. The compound contains a benzimidazole diphenyl ether core that is flanked by the amidine cations. The diphenyl ether is highly twisted and gives the entire compound too much curvature to fit well to the shape of the minor groove. DNase I footprinting, fluorescence intercalator displacement studies, and circular dichroism spectra, however, indicate that the compound is an AT specific minor groove binding agent. Even more surprisingly, quantitative biosensor-surface plasmon resonance and isothermal titration calorimetric results indicate that the compound binds with exceptional strength to certain AT sequences in DNA with a large negative enthalpy of binding. Crystallographic results for the DNA complex of RT29 compared to calculated results for the free compound show that the compound undergoes significant conformational changes to enhance its minor groove interactions. In addition, a water molecule is incorporated directly into the complex to complete the compound-DNA interface, and it forms an essential link between the compound and base pair edges at the floor of the minor groove. The calculated DeltaCp value for complex formation is substantially less than the experimentally observed value, which supports the idea of water being an intrinsic part of the complex with a major contribution to the DeltaCp value. Both the induced fit conformational changes of the compound and the bound water are essential for strong binding to DNA by RT29.

Thomas J. R., Liu X., and Hergenrother P. J. (2006) Biochemical and thermodynamic characterization of compounds that bind to RNA hairpin loops: toward an understanding of selectivity. *Biochemistry* **45,** 10928-10938.
Abstract: Elucidation of the molecular forces governing small molecule-RNA binding is paramount to the progress of rational design strategies. The extensive characterization of the aminoglycoside-16S rRNA A-site interaction has deepened our understanding of how aminoglycosides bind to their target and exert their antimicrobial effects. However, to date no other RNA binding compounds have undergone such rigorous evaluation, and in general the origins of small molecule-RNA binding remain a mystery. We recently reported the identification of small molecules, dimers of 2-deoxystreptamine, which are able to bind selectively to RNA tetraloops and octaloops, respectively [Thomas, Liu, and Hergenrother (2005) J. Am. Chem. Soc. 127, 12434-12435]. Described herein is the biochemical and biophysical characterization of the RNA binding properties of the most selective compound, B-12, as well as closely related analogues. These

studies further substantiate that B-12 is indeed selective for RNA octaloop sequences and indicate that the origin of this selectivity may lie in B-12's unusual binding mode, in which entropic factors are major contributors to the overall binding energy. In fact, isothermal titration calorimetry (ITC) experiments indicate that the binding of B-12 and most of its analogues is associated with a strong entropic contribution to the total binding energy. This is in stark contrast to the aminoglycosides, for which favorable enthalpy typically provides the driving force for binding. These studies are the first to examine small molecule-RNA hairpin loop binding in detail and are a necessary step toward the design of compounds that are specific binders for a given RNA sequence.

Torigoe H., Kozasa T. and Ono A. (2007) The specific interaction between two C:C mismatch base pairs and silver (I) cation. *Nucleic Acids Symp. Ser. (Oxf)* 183-184.
Abstract: We have already found that a single silver (I) cation specifically binds to a single C:C mismatch base pair in heteroduplex, which increases the melting temperature of heteroduplex involving a single C:C mismatch base pair by about 4 degrees C. Here, to examine the thermodynamic properties involving two C:C mismatch base pairs, we analyzed the interaction between silver (I) cations and heteroduplex involving two C:C mismatch base pairs by isothermal titration calorimetry. The difference in the positions of the two C:C mismatch base pairs did not significantly affect the magnitudes of the stoichiometry and the thermodynamic parameters for the interaction between silver (I) cations and the two C:C mismatch base pairs. Two silver (I) cations bind with two C:C mismatch base pairs. The binding affinity for the second silver (I) cation was similar to that for the first silver (I) cation. Our results certainly support the idea that addition of the silver (I) cation is a promising strategy for the C:C mismatch base pair detection in the heteroduplex analysis and may eventually lead to progress in SNP genotyping.

Torigoe H., Miyakawa Y., Kozasa T. and Ono A. (2007) The specific interaction between two T:T mismatch base pairs and mercury (II) cation. *Nucleic Acids Symp. Ser. (Oxf)* 185-186.
Abstract: We have already found that a single mercury (II) cation specifically binds to a single T:T mismatch base pair in heteroduplex, which increases the melting temperature of heteroduplex involving a single T:T mismatch base pair by about 4 degrees C. Here, to examine the thermodynamic properties involving two T:T mismatch base pairs, we analyzed the interaction between mercury (II) cations and heteroduplex involving two T:T mismatch base pairs by isothermal titration calorimetry. The difference in the positions of the two T:T mismatch base pairs did not significantly affect the magnitudes of the stoichiometry and the thermodynamic parameters for the interaction between mercury (II) cations and the two T:T mismatch base pairs. Two mercury (II) cations bind with two T:T mismatch base pairs. The binding affinity for the second mercury (II) cation was significantly larger than that for the first mercury (II) cation. Our results certainly support the idea that addition of the mercury (II) cation is a promising strategy for the T:T mismatch base pair detection in the heteroduplex analysis and may eventually lead to progress in SNP genotyping.

Utsuno K., Kojima K., Maeda Y., and Tsuboi M. (1998) The average unwinding angle of DNA duplex produced by the binding of chromomycin A3. *Chem Pharm Bull (Tokyo)* **46,** 1667-1671.
Abstract: The effect of chromomycin A3 binding on the geometry of DNA duplex (plasmid pBR322) has been examined using topoisomerase I relaxation followed by gel electrophoresis. To determine the equilibrium constant of this drug-DNA binding-dissociation reaction in the same concentration range (ca. 10^{-5} M) in the same buffer as those for the topoisomerase reaction (at 37 degrees C), fluorescence measurements were made of the same plasmid-drug system, followed by a Scatchard plot and an analysis using McGhee-von Hippel's exclusion site model. The binding constant has been found to be 3.8 x 10^{5} M^{-1} in the particular buffer (buffer-T) at 37 degrees C, and the number of base pairs involved in the site of one chromomycin molecule on the duplex has been found to be 5. It has been concluded that one chromomycin molecule, bound to the duplex, unwinds it by 11.8 +/- 1.1 degrees. In addition, the enthalpy of binding was determined to be 31.81 kJ/mole using a titration calorimeter with a more concentrated (6.2 mM) solution.

Utsuno K., Maeda Y., and Tsuboi M. (1999) How and how much can Hoechst 33258 cause unwinding in a DNA duplex? *Chem Pharm Bull (Tokyo)* **47,** 1363-1368.
Abstract: The effect of Hoechst 33258 binding on the geometry of a DNA duplex (plasmid pBR322) has been examined using topoisomerase II relaxation followed by gel electrophoresis. Of this drug-DNA system, fluorescence, optical absorption, and calorimetric measurements were also made at various drug

and DNA concentrations and in the same buffer as that for the topoisomerase reaction. It has been confirmed that there are two modes of drug-DNA interaction. When the drug concentration is much lower than the DNA base pair concentration, the Hoechst 33258 molecule binds in the minor groove of the DNA duplex and occupies a site formed of five continuous base pair sequences that contain no G.C pair. Here, the equilibrium constant K1 is 1.8 x 10^{7} M^{-1} (at 37 degrees C), and the enthalpy of binding Δ H1 is -865 cal/mol. When the drug concentration is much higher, on the other hand, it shows another binding mode which is much weaker, so that K2 = 2.25 x 10^{4} M^{-1} and Δ H2 is -464 cal/mol, which gives fluorescence quenching, which has no base pair preference, and which causes an unwinding of the duplex by 1 degree.

Utsuno K. (2008) Thermodynamics of DNA condensation caused by mn2+ binding. *Chem Pharm. Bull. (Tokyo)* **56,** 247-249.
Abstract: Interaction between Mn2+ ion and the two forms of DNA duplex (supercoiled and linearized pUC119 DNA) in solution has been examined by isothermal titration calorimetry. Although DNA condensation reaction heat was observed at 323 K, this was not the case at 298 K. DNA condensation was entropically driven and supercoiled DNA was found to be more susceptive. The enthalpy of DNA condensation is estimated 0.42 kJ/mol for both DNA forms. Conversely, the entropy of DNA condensation was 0.13 kJ/mol K for supercoiled DNA, and 0.12 kJ/mol K for linearized DNA. The difference of entropy is attributable to their DNA conformation

Veselkov A. N., Maleev V. Y., Glibin E. N., Karawajew L., and Davies D. B. (2003) Structure-activity relation for synthetic phenoxazone drugs: evidence for a direct correlation between DNA and pro-apoptotic activity. *Eur J Biochem* **270,** 4200-4207.
Abstract: The structure-activity relations of a series of synthetic phenoxazone drugs with aminoalkyl side chains of variable length and different terminal groups were investigated by examining their biological activity and DNA complexation affinity. Biological activity was determined from their ability to induce apoptosis and cell cycle perturbations (activation of cell cycle checkpoints) using the human malignant MOLT-3 cell line. The thermodynamic parameters of drug-DNA complexation were determined by differential scanning calorimetry. By comparing the activities of compounds with different terminal groups (amino, dimethylamino and diethylamino), we found that the existence of a terminal dimethylamino group in the alkylamino side chain is an important factor for anti-tumour activity. Minor modifications in the dimethylaminoalkyl side chain (e.g. elongation by one methylene group) led to notable changes in both the anti-tumour activity and DNA-binding properties of the drug, providing unambiguous evidence of a marked structure-activity relation.

Wang L., Kumar A., Boykin D. W., Bailly C., and Wilson W. D. (2002) Comparative thermodynamics for monomer and dimer sequence-dependent binding of a heterocyclic dication in the DNA minor groove. *J Mol Biol* **317,** 361-374.
Abstract: Phenylamidine cationic groups linked by a furan ring (furamidine) and related symmetric diamidine compounds bind as monomers in the minor groove of AT sequences of DNA. DB293, an unsymmetric derivative with one of the phenyl rings of furamidine replaced with a benzimidazole, can bind to AT sequences as a monomer but binds more strongly to GC-containing minor-groove DNA sites as a stacked dimer. The dimer-binding mode has high affinity, is highly cooperative and sequence selective. In order to develop a better understanding of the correlation between structural and thermodynamic aspects of DNA molecular recognition, DB293 was used as a model to compare the binding of minor-groove agents with AT and mixed sequence DNA sites. Isothermal titration calorimetry and surface plasmon resonance results clearly show that the binding of DB293 and other related compounds into the minor groove of AT sequences is largely entropy-driven while the binding of DB293 as a dimer into the minor groove of GC-containing sequences is largely enthalpy-driven. At 25 degrees C, for example, the AT binding has ΔG degrees, ΔH degrees and TΔS degrees values of -9.6, -3.6 and 6.0 kcal/mol while the values for dimer binding to a GC-containing site are -9.0, -10.9 and -1.9 kcal/mol (per mol of bound compound), respectively. These results show that the thermodynamic components for binding of compounds of this type to DNA are very dependent on the structure, solvation and sequence of the DNA binding site.

Wettig S. D., Wood D. O., and Lee J. S. (2003) Thermodynamic investigation of M-DNA: a novel metal ion-DNA complex. *J Inorg Biochem* **94,** 94-99.
Abstract: The thermodynamics of formation of a novel divalent metal ion-DNA complex known as M-

DNA have been investigated using an ethidium bromide (EB) fluorescence assay, and with isothermal titration calorimetry. The process of M-DNA formation was observed from the EB assay to be strongly temperature-dependent. The binding of Zn(2+) to calf thymus (42% GC content) and Escherichia coli (50% GC content) DNA at pH 8.5 exhibited an endothermic cooperative binding process at Zn(2+) concentrations of approximately 0.1 mM, indicating an entropy driven process. This binding process is consistent with a site-specific binding interaction, similar in nature to Z-DNA formation; however, the interaction occurs at much lower metal ion concentrations. The enthalpy of M-DNA formation for calf thymus DNA was determined to be 10.5+/-0.7 and 9+/-2 kJ/mbp at DNA concentrations of 100 and 50 µg ml(-1), respectively. An enthalpy of 13+/-3 kJ/mbp was obtained for M-DNA formation for 50 µg ml(-1) E. coli DNA. No evidence of M-DNA formation was observed in either DNA at pH 7.5 with Zn(2+) or at either pH 7.5 or 8.5 with Mg(2+).

White E. W., Tanious F., Ismail M. A., Reszka A. P., Neidle S., Boykin D. W. and Wilson W. D. (2007) Structure-specific recognition of quadruplex DNA by organic cations: influence of shape, substituents and charge. *Biophys Chem* **126,** 140-153.
Abstract: Combining structure-specific recognition of nucleic acids with limited sequence reading is a promising method to reduce the size of the recognition unit required to achieve the necessary selectivity and binding affinity to control function. It has been demonstrated recently that G-quadruplex DNA structures can be targeted by organic cations in a structure-specific manner. Structural targets of quadruplexes include the planar end surfaces of the G-tetrad stacked columns and four grooves. These provide different geometries and functional groups relative to duplex DNA. We have used surface plasmon resonance and isothermal titration calorimetry to show that binding affinity and selectivity of a series of quadruplex end-stacking molecules to human telomeric DNA are sensitive to compound shape as well as substituent type and position. ITC results indicate that binding is largely enthalpy driven. Circular dichroism was also used to identify a group of structurally related compounds that selectively target quadruplex grooves.

Wu J., Du F., Zhang P., Khan I. A., Chen J., and Liang Y. (2005) Thermodynamics of the interaction of aluminum ions with DNA: implications for the biological function of aluminum. *J Inorg Biochem* **99,** 1145-1154.
Abstract: Aluminum is a known neurotoxic agent and its neurotoxic effects may be due to its binding to DNA. However, the mechanism for the interaction of aluminum ions with DNA is not well understood. Here, we report the application of isothermal titration calorimetry (ITC), fluorescence spectroscopy, and UV spectroscopy to investigate the thermodynamics of the binding of aluminum ions to calf thymus DNA (CT DNA) under various pH and temperature conditions. The binding reaction is driven entirely by a large favorable entropy increase but with an unfavorable enthalpy increase in the pH range of 3.5-5.5 and at all temperatures examined. Aluminum ions show a strong and pH-dependent binding affinity to CT DNA, and a large positive molar heat capacity change for the binding, 1.57 kcal mol(-1) K(-1), demonstrates the burial of the polar surface of CT DNA upon groove binding. The fluorescence of ethidium bromide bound to CT DNA is quenched by aluminum ions in a dynamic way. Both Stern-Volmer quenching constant and the binding constant increase with the increase of the pH values, reaching a maximum at pH 4.5, and decline with further increasing the pH to 5.5. At pH 6.0 and 7.0, aluminum ions precipitate CT DNA completely and no binding of aluminum ions to CT DNA is observed by ITC. Combining the results from these three methods, we conclude that aluminum ions bind to CT DNA with high affinity through groove binding under aluminum toxicity pH conditions and precipitate CT DNA under physiological conditions.

Xu H., Liang Y., Zhang P., Du F., Zhou B. R., Wu J., Liu J. H., Liu Z. G., and Ji L. N. (2005) Biophysical studies of a ruthenium(II) polypyridyl complex binding to DNA and RNA prove that nucleic acid structure has significant effects on binding behaviors. *J Biol Inorg Chem* **10,** 529-538.
Abstract: The interactions of a metal complex [Ru(phen)(2)PMIP](2+) {Ru=ruthenium, phen=1,10-phenanthroline, PMIP=2-(4-methylphenyl)imidazo[4,5-f]1,10-phenanthroline} with yeast tRNA and calf thymus DNA (CT DNA) have been investigated comparatively by UV-vis spectroscopy, fluorescence spectroscopy, viscosity measurements, isothermal titration calorimetry (ITC), as well as equilibrium dialysis and circular dichroism (CD). Spectroscopic studies together with ITC and viscosity measurements indicate that both binding modes of the Ru(II) polypyridyl complex to yeast tRNA and CT DNA are intercalation and yeast tRNA binding of the complex is stronger than CT DNA binding. ITC experiments

show that the interaction of the complex with yeast tRNA is driven by a moderately favorable enthalpy decrease in combination with a moderately favorable entropy increase, while the binding of the complex to CT DNA is driven by a large favorable enthalpy decrease with a less favorable entropy increase. The results from equilibrium dialysis and CD suggest that both interactions are enantioselective and the Delta enantiomer of the complex may bind more favorably to both yeast tRNA and CT DNA than the Lambda enantiomer does, and that the complex is a better candidate for an enantioselective binder to yeast tRNA than to CT DNA. Taken together, these results indicate that the structures of nucleic acids have significant effects on the binding behaviors of metal complexes.

Zhong M., Rashes M. S., Marky L. A., and Kallenbach N. R. (1992) T-T base mismatches enhance drug binding at the branch site in a four-arm DNA junction. *Biochemistry* **31,** 8064-8071.
Abstract: Base mismatches--non Watson-Crick pairing between bases--can arise in duplex DNA as a consequence of mutational events or by recombination. In a duplex, the sequence of the two bases involved, and those flanking the site of mismatch, determines the local structure and extent of destabilization of the helix. Base mismatches can arise also in recombination of nonhomologous strands, and their occurrence in Holliday recombination intermediates can influence the outcome of general or specialized recombination events. We have previously reported that the branch site in a DNA junction can interact selectively with a variety of ligands. Here we describe the thermodynamics of junctions containing T-T mismatches flanking the branch and show that these structures bind methidium and other intercalators with higher affinity than junctions lacking mismatches.

Zhong W., Yu J. S., and Liang Y. (2003) Chlorobenzylidine-herring sperm DNA interaction: binding mode and thermodynamic studies. *Spectrochim Acta A Mol Biomol Spectrosc* **59,** 1281-1288.
Abstract: The interaction of chlorobenzylidine with herring sperm DNA has been investigated by fluorescence, absorption, DNA melting experiment and differential scanning calorimetry (DSC). When bound to DNA, chlorobenzylidine shows hypochromism and red shift in absorption spectra, fluorescence quenching and polarization increasing in fluorescence spectra and increasing in DNA melting temperature. These spectral characteristics strongly support intercalation of chlorobenzylidine into herring sperm DNA. Scatchard plots constructed from fluorescence titration data give a binding constant of 3.2 x 10^4 $M^{-1}$and a binding site size of six base pairs per bound drug molecule. The intercalative interaction is exothermic with a van't Hoff enthalpy of -30.6 kJ mol^{-1}. This result is obtained from DSC experiment. In addition, ΔG degrees =-28.5 kJ mol^{-1}, and ΔS degrees =-7.1 J $mol^{-1}K^{-1}$. These results show that the binding of chlorobenzylidine to herring sperm DNA is exothermic.

Zhu D. M. and Evans R. K. (2006) Molecular mechanism and thermodynamics study of plasmid DNA and cationic surfactants interactions. *Langmuir* **22,** 3735-3743.
Abstract: The molecular mechanism and thermodynamics of the interactions between plasmid DNA and cationic surfactants were investigated by isothermal titration calorimetry (ITC), dynamic light scattering, surface tension measurements, and UV spectroscopy. The cationic surfactants studied include benzyldimethyldodecylammonium chloride, benzyldimethyltetradecylammonium chloride, cetylpyridinium chloride, and cetyltrimethylammonium chloride. The results indicate a critical aggregation concentration (cac) of a surfactant: above the cac the surfactant forms aggregates with plasmid DNA; below the cac, however, there is no detectable interaction between DNA and surfactant. Surfactants with longer hydrocarbon chains have smaller cac, indicating that hydrophobic interaction plays a key role in DNA-surfactant complexation. Moreover, an increase in ionic strength (I) increases the cac but decreases the critical micellization concentration (cmc). These opposite effects lead to a critical ionic strength (I(c)) at which cac = cmc; when I < I(c), cac < cmc; when I > I(c), DNA does not form complexes with surfactant micelles. In the interaction DNA exhibits a pseudophase property as the cac is a constant over a wide range of DNA concentrations. ITC data showed that the reaction is solely driven by entropy because both deltaH(o) (approximately 2-6 kJ mol(-1)) and deltaS(o) (approximately 70-110 J K(-1) mol(-1)) have positive values. In the complex, the molar ratio of DNA phosphate to surfactant is in the range of 0.63-1.05. The reaction forms sub-micrometer-sized primary particles; those aggregate at high surfactant concentrations. Taken together, the results led to an inference that there is no interaction between surfactant monomers and DNA molecules and demonstrated that DNA-cationic surfactant interactions are mediated by the hydrophobic interactions of surfactant molecules and counterion binding of DNA phosphates to the cationic surfactant aggregates.