DSC XIII- Nucleic Acid Folding and Stability Studies

Amrane S. and Mergny J. L. (2006) Length and pH-dependent energetics of (CCG)n and (CGG)n trinucleotide repeats. *Biochimie* **88,** 1125-1134.
Abstract: Trinucleotide repeats are involved in a number of debilitating diseases such as fragile-X syndrome and myotonic dystrophy. Eighteen to 75 base-long (CCG)(n) and (CGG)(n) oligodeoxynucleotides were analysed using a combination of biophysical (UV-absorbance, differential scanning calorimetry) and biochemical methods (non-denaturing gel electrophoresis, enzymatic footprinting). All oligomers formed stable intramolecular structures under near physiological conditions with a melting temperature which was only weakly dependent on oligomer length. Thermodynamic analysis of the denaturation process by UV-melting and calorimetric experiments revealed a length-dependent discrepancy between the enthalpy values deduced from model-dependent (UV-melting) and model-independent experiments (calorimetry), as recently shown for CTG and CAG trinucleotides (Nucleic Acids Res. 33 (2005) 4065). Evidence for non-zero molar heat capacity changes was also derived from the analysis of the Arrhenius plots. Such behaviour is analysed in the framework of an intramolecular "branched" or "broken" hairpin model, in which long oligomers do not fold into a simple long hairpin-stem intramolecular structure, but allow the formation of several independent folding units of unequal stability. These results suggest that this observation may be extended to various trinucleotide repeats-containing sequences.

Amosova O., George J., and Fresco J. R. (1997) Effect of the 1-(2'-deoxy-beta-D-ribofuranosyl)-3-nitropyrrole residue on the stability of DNA duplexes and triplexes. *Nucleic Acids Res* **25,** 1930-1934.
Abstract: 3-Nitropyrrole (M) was introduced as a non-discriminating 'universal' base in nucleic acid duplexes by virtue of small size and a presumed tendency to stack but not hydrogen bond with canonical bases. However, the absence of thermally-induced hyperchromic changes by single-stranded deoxyoligomers in which M alternates with A or C residues shows that M does not stack strongly with A or C nearest neighbors. Yet, the insertion of a centrally located M opposite any canonical base in a duplex is sometimes even less destabilizing than that of some mismatches, and the variation in duplex stability is small. In triplexes, on the other hand, an M residue centrally located in the third strand reduces triplex stability drastically even when the X.Y target base pair is A.T or G. C in a homopurine. homopyrimidine segment. But, when the target duplex opposition is M-T and the third strand residue is T, the presence of M in the test triplet has little effect on triplex stability. Therefore, a lack of hydrogen bonding in an otherwise helix-compatible test triplet cannot be responsible for triplex destabilization when M is the third strand residue. Thus, M is non-discriminating and none-too-destabilizing in a duplex, but in a triplex it is extremely destabilizing when in the third strand.

Amrane S., Sacca B., Mills M., Chauhan M., Klump H. H., and Mergny J. L. (2005) Length-dependent energetics of (CTG)n and (CAG)n trinucleotide repeats. *Nucleic Acids Res* **33,** 4065-4077.
Abstract: Trinucleotide repeats are involved in a number of debilitating diseases such as myotonic dystrophy. Twelve to seventy-five base-long (CTG)n oligodeoxynucleotides were analysed using a combination of biophysical [UV-absorbance, circular dichroism and differential scanning calorimetry (DSC)] and biochemical methods (non-denaturing gel electrophoresis and enzymatic footprinting). All oligomers formed stable intramolecular structures under near physiological conditions with a melting temperature that was only weakly dependent on oligomer length. Thermodynamic analysis of the denaturation process by UV-melting and calorimetric experiments revealed an unprecedented length-dependent discrepancy between the enthalpy values deduced from model-dependent (UV-melting) and model-independent (calorimetry) experiments. Evidence for non-zero molar heat capacity changes was also derived from the analysis of the Arrhenius plots and DSC profiles. Such behaviour is analysed in the framework of an intramolecular 'branched-hairpin' model, in which long CTG oligomers do not fold into a simple long hairpin-stem intramolecular structure, but allow the formation of several independent folding units of unequal stability. We demonstrate that, for sequences ranging from 12 to 25 CTG repeats, an intramolecular structure with two loops is formed which we will call 'bis-hairpin'. Similar results were also found for CAG oligomers, suggesting that this observation may be extended to various trinucleotide repeats-containing sequences.

Antonacci C., Chaires J. B. and Sheardy R. D. (2007) Biophysical characterization of the human telomeric (TTAGGG)4 repeat in a potassium solution. *Biochemistry* **46,** 4654-4660.
Abstract: Quadruplex structures arise from four coplanar G bases arranged in a Hoogsteen base pairing motif to create a central pore that can coordinate cations. The termini of eukaryotic chromosomes contain structures, known as telomeres, which are capable of forming quadruplex structures. Quadruplexes have been implicated in a variety of disease states, including cancer. The literature seems to agree that the human telomeric repeat containing four stretches of three guanines displays conformational states that are different in the presence of Na+ and K+ and an unknown number of species involved in the quadruplex to single strand transition. Using circular dichroism spectroscopy, differential scanning calorimetry, and singular-value decomposition, the number of species present in the dissociation process is assessed. The results indicate that three species exist in equilibria during the melting process. We present a model for the heat-induced denaturation from the folded to the unfolded state, whereby the hybrid parallel-antiparallel quadruplex undergoes a transition to an unknown intramolecular intermediate followed by a transition to a single strand.

Babaian I., Karapetian L. G., Kazarian R. S., and Khudaverdian N. V. (1997) [Features of melting of DNA complexes with mitoxantrone at low concentrations]. *Biofizika* **42,** 367-371.
Abstract: The melting of DNA complexes with the anticancer mitoxantrone preparation at the mu = 0.11 and mu = 0.011 NaCl ionic strengths was investigated by the methods of microcalorimetry and spectrophotometry. It was shown, that at the 0.011 M NaCl the dependence of the melting temperature (Tm) upon the mitoxantrone concentration passed at minimum. The decrease of Tm was observed in the region, where one mitoxantrone molecule fell approximately on 100 base pairs DNA. There was observed a deeper minimum for DNA sarcoma 45. In the region of the mitoxantrone concentration the enthalpy of melting of the complexes increases linearly with the increase of the mitoxantrone concentration. The mentioned regularities were not observed at the mu = 0.11 M NaCl. The observed phenomenon is explained qualitatively by the entropy increase of the coiled state of DNA ligand complex on account of additional freedom of ligand's revolving.

Balbi C., Sanna P., Barboro P., Alberti I., I, Barbesino M., and Patrone E. (1999) Chromatin condensation is confined to the loop and involves an all-or-none structural change. *Biophys J* **77,** 2725-2735.
Abstract: Using differential scanning calorimetry in combination with pulsed field gel electrophoresis, we relate here the changes in the thermal profile of rat liver nuclei induced by very mild digestion of chromatin by endogenous nuclease with the chain length distribution of the DNA fragments. The enthalpy of the endotherm at 106 degrees C, which reflects the denaturation of the heterochromatic domains, decreases dramatically after the induction of a very small number of double-strand breaks per chromosome; the thermal transition disappears when the loops have undergone on average one DNA chain scission event. Quantitative analysis of the experimental data shows that the loop behaves like a topologically isolated domain. Also discussed is the process of heterochromatin formation, which occurs according to an all-or-none mechanism. In the presence of spermine, a strong condensation agent, only the loops that have undergone one break are able to refold, in confirmation of the extremely cooperative nature of the transition. Furthermore, our results suggest a relationship between the states that give rise to the endotherms at 90 degrees C and 106 degrees C and the morphologies referred to as class II and class III in a previous physicochemical study of the folding of chromatin fragments (. J. Mol. Biol. 190:411-424) and support the view that the overall process of condensation follows a sequential (two-step) pathway.

Barcelo F. and Portugal J. (1994) Calorimetric and spectroscopic studies on the poly[d(GA).d(CT)] structural polymorphism induced by zinc. *J Biomol Struct Dyn* **12,** 203-216.
Abstract: The interaction of zinc (II) with poly[d(GA).d(CT)] and salmon testes DNA has been investigated by Differential Scanning Calorimetry (DSC) and Circular Dichroism (CD). We have detected and energetically characterized the existence of two different structural forms in poly[d(GA).d(CT)] which behave differently during a DSC experiment. The overall melting of DNA shows two calorimetric transitions at different temperatures. Moreover, the presence of zinc, at an input ratio of ion to nucleotide (r) above two, renders a complex DSC profile which is characterized by a negative enthalpy transition. Besides, the low-temperature transition observed in the presence of zinc is practically reversible after re-cooling/re-heating cycles. Nevertheless, the high-temperature transition characterized by a negative Δ H degree cal does not appear in re-heating experiments, and remains stable below 100 degrees C. A

calorimetric negative enthalpy transition is also found using salmon DNA in the presence of zinc ions. It seems that the combination of a temperature effect and zinc binding might induce the production of a stable metal-DNA complex, which can also be detected by changes in some bands in the CD profiles. The experimental results show that the presence of DNA structures and binding processes involving a negative calorimetric enthalpy contribution might be more widespread than previously reckoned.

Barciszewski J., Bratek-Wiewiorowska M. D., Gornicki P., Naskret-Barciszewska M., Wiewiorowski M., Zielenkiewicz A., and Zielenkiewicz W. (1988) Comparative calorimetric studies on the dynamic conformation of plant 5S rRNA. I. Thermal unfolding pattern of lupin seeds and wheat germ 5S rRNAs, also in the presence of magnesium and sperminium cations. *Nucleic Acids Res* **16,** 685-701.
Abstract: An attempt has been made to correlate differential scanning calorimetry melting profiles of 5S rRNAs from lupin seeds (L.s.) and wheat germ (W.g.) with their structure. It is suggested that the observed differences in thermal unfolding are due to differences in RNA nucleotide sequence and as a consequence in higher order structures. Interesting effects induced by magnesium cation, perprotonated and permethylated sperminium tetracations are discussed. It is suggested that the difference in the stabilizing effect of the three cations results from different mode of their interactions with RNA. "Pure" electrostatic interactions expected for permethylated tetracations are rather weak due to the steric hindrance around each positively charged nitrogen atom. Electrostatic interactions of the other two cations are significantly enhanced by coordination bonding for magnesium and by hydrogen bonding for protonated sperminium cation.

Bastos M., Castro V., Mrevlishvili G., and Teixeira J. (2004) Hydration of ds-DNA and ss-DNA by neutron quasielastic scattering. *Biophys J* **86,** 3822-3827.
Abstract: Quasielastic neutron scattering measurements were performed in hydrated samples of ds-DNA and ss-DNA. The samples were hydrated in a high relative humidity atmosphere, and their final water content was 0.559 g H_2O/g ds-DNA and 0.434 g H_2O /g ss-DNA. The measurements were performed at 8 and 5.2 A for the ds-DNA sample, and at 5.2 A for the ss-DNA sample. The temperature was in both cases 298 K. Analysis of the obtained data indicates that in the ds-DNA sample we can distinguish two types of protons-those belonging to water molecules strongly attached to the ds-DNA surface and another fraction belonging to water that diffuses isotropically in a sphere of radius 2.8 A, with a local diffusion coefficient of 2.2×10^{-5} cm^2 s^{-1}. For ss-DNA, on the other hand, no indication was found of motionally restricted or confined water. Further, the fraction of protons strongly attached to the ds-DNA surface corresponds to 0.16 g H_2O /g ds-DNA, which equals the amount of water that is released by ds-DNA upon thermal denaturation, as studied by one of us (G.M.) by differential scanning calorimetry. This value also equals the difference between the critical hydration values of ds-DNA and ss-DNA, also determined by DSC. These results represent, thus, a completely independent measurement of water characteristics and behavior in ds- and ss-DNA at critical hydration values, and therefore substantiate the previous suggestions/conclusions of the results obtained by calorimetry.

Bergstrom D. E., Zhang P., and Johnson W. T. (1997) Comparison of the base pairing properties of a series of nitroazole nucleobase analogs in the oligodeoxyribonucleotide sequence 5'-d(CGCXAATTYGCG)-3'. *Nucleic Acids Res* **25,** 1935-1942.
Abstract: The nucleoside analogs 1-(2'-deoxy-beta-D-ribofuranosyl)- 3-nitropyrrole (9), 1-(2'-deoxy-beta-D-ribofuranosyl)-4-nitropyrazole (10), 1-(2'-deoxy-beta-D-ribofuranosyl)-4-nitroimidazole (11) and 1-(2'-deoxy-beta-D-ribofuranosyl)-5-nitroindole (21) were incorporated into the oligonucleotide 5'-d(CGCXAATTYGCG)-3'in the fourth position from the 5'-end. Procedures for synthesis of two of the nitroazole nucleosides, 10 and 11, were developed for this study. Each of the nitroazoles was converted into a 3'-phosphoramidite for oligonucleotide synthesis by conventional automated protocols. Four oligonucleotides were synthesized for each modified nucleoside in order to obtain duplexes in which each of the four natural bases was placed opposite (position 9) the nitroazole. In order to assess the role of the nitro group on base stacking interaction, sequences were also synthesized in which the fourth base was 1-(2'-deoxy-beta-D-ribofuranosyl)pyrazole. Corresponding sequences containing an abasic site, as well as sequences containing inosine, were synthesized for comparison. Thermal melting studies yielded T m values and thermodynamic parameters. Each nucleoside analog displayed a unique pattern of base pairing preferences. The least discriminating analog was 3-nitropyrrole, for which T m values differed by 5 degrees C and Δ G 25 degrees C ranged from -6.1 to -6.5 kcal/mol. 5-Nitroindole gave duplexes with significantly

higher thermal stability, with Tm values varying from 35.0 to 46.5 degrees C and -Δ G 25 degrees C ranging from 7.7 to 8.5 kcal/mol. Deoxyinosine (22), a natural analog which has found extensive use as a universal nucleoside, is far less non-discriminating than any of the nitroazole derivatives. Tm values ranged from 35.4 degrees C when paired with G to 62.3 degrees C when paired with C. The significance of the nitro substituent was determined by comparison of the base pairing properties of a simple azole nucleoside, 1-(2'-deoxy-beta-D-ribofuranosyl)pyrazole (12). The pyrazole-containing sequences melt at 10-20 degrees C lower than the corresponding nitropyrazole-containing sequences. On average, the pyrazole-containing sequences were equivalent in stability (average Δ G = -4.8 kcal/mol) to the sequences containing an abasic site (average Δ G = -4.7 kcal/mol).

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.

Blasi M., Bonincontro A., Cinelli S., Onori G., and Risuleo G. (2000) Structural stability of ribosomes subjected to RNase treatment evidenced by dielectric spectroscopy and differential scanning microcalorimetry. *Biophys Chem* **83,** 73-78.
Abstract: Previous studies from our laboratory demonstrated the existence of at least two levels of structural complexity in E. coli 70S ribosomes. Ribosomal RNA seems to be principally involved in the overall stability of these structures. In this paper we present an investigation of ribosomes subjected to treatment with RNase. The study is based on both differential scanning microcalorimetry and dielectric spectroscopy. In the thermograms obtained on treated ribosomes only the low temperature peak of the two typical denaturation events observed in native ribosomes, is promptly eliminated by the enzyme treatment. Dielectric spectroscopy measurements carried out on the same samples indicate an alteration of the dielectric behavior previously shown to consist of two subsequent relaxation processes. In fact, only the low frequency relaxation is affected by the treatment. The second one, observed at higher frequency, remains unaltered. The same effect on the dielectric parameters is observed if the ribosome particles are heated and then cooled prior to measurement. These results are consistent with the idea that two different structures are present within the ribosome. One is very stable and withstands both temperature and RNase treatment while the second is promptly abolished by both treatments. Data presented here strongly suggest that the RNA domains exposed to the solvent play a fundamental role in the stability of the 3-D structure of the ribosome particle.

Bourdelat-Parks B. N. and Wartell R. M. (2004) Thermodynamic stability of DNA tandem mismatches. *Biochemistry* **43,** 9918-9925.
Abstract: The thermodynamics of nine hairpin DNAs were evaluated using UV-monitored melting curves and differential scanning calorimetry (DSC). Each DNA has the same five-base loop and a stem with 8-10 base pairs. Five of the DNAs have a tandem mismatch in the stem, while four have all base pairs. The

tandem mismatches examined (ga/ga, aa/gc, ca/gc, ta/ac, and tc/tc) spanned the range of stability observed for this motif in a previous study of 28 tandem mismatches. UV-monitored melting curves were obtained in 1.0 M Na(+), 0.1 M Na(+), and 0.1 M Na(+) with 5 mM Mg(2+). DSC studies were conducted in 0.1 M Na(+). Transition T_m values were unchanged over a 50-fold range of strand concentration. Model-independent enthalpy changes (ΔH °) evaluated by DSC were in good agreement (+/-8%) with enthalpy values determined by van't Hoff analyses of the melting curves in 0.1 M Na(+). The average heat capacity change (ΔC_p) associated with the hairpin to single strands transitions was estimated from plots of ΔH ° and ΔS ° with T_m and ln T_m, respectively, and from profiles of DSC curves. The average ΔC_p values (113 +/- 9 and 42 +/- 27 cal x K^{-1} x mol^{-1} of bp), were in the range of values reported in previous studies. Consideration of ΔC_p produced large changes in ΔH ° and ΔS ° extrapolated from the transition region to 37 degrees C and smaller but significant changes to free energies. The loop free energy of the five tandem mismatches at 37 degrees C varied over a range of approximately 4 kcal x mol^{-1} for each solvent.

Bozza M., Sheardy R. D., Dilone E., Scypinski S., and Galazka M. (2006) Characterization of the secondary structure and stability of an RNA aptamer that binds vascular endothelial growth factor. *Biochemistry* **45,** 7639-7643.
Abstract: Thermal denaturation studies and spectroscopic studies were employed to investigate the secondary structure and stability of an RNA-PEG conjugate commercially called Macugen. The RNA aptamer is conjugated to a pegylated moiety, and the majority of its 2'-hydroxyl groups are methylated or otherwise modified. UV optical melting studies and differential scanning calorimetry (DSC) were carried out under different conditions to evaluate the effects of Na+ and oligomer concentrations on the stability of the secondary structure of the RNA oligomer. The results of these studies indicated that the T(m) of the RNA is independent of oligomer concentration but dependent on the salt concentration, in a predictable fashion. Further, the DSC melting profiles obtained under all conditions were highly reversible. Circular dichroism (CD) studies were determined under different salt concentrations, various RNA concentrations, and temperatures as well. Together, the thermal denaturation and CD studies provide evidence that the secondary structure of the RNA oligonucleotide is a stable hairpin at 25 degrees C and that the thermally induced hairpin to single strand transition is highly reversible.

Brabec V., Stehlikova K., Malina J., Vojtiiskova M., and Kasparkova J. (2006) Thermodynamic properties of damaged DNA and its recognition by xeroderma pigmentosum group A protein and replication protein A. *Arch Biochem Biophys* **446,** 1-10.
Abstract: The effects of the lesions induced by single, site-specific 1,2-GG or 1,3-GTG intrastrand adducts of cis-diamminedichloroplatinum(II) formed in oligodeoxyribonucleotide duplexes on energetics of DNA were examined by means of differential scanning calorimetry. These effects were correlated with affinity of these duplexes for damaged-DNA binding-proteins XPA and RPA; this affinity was examined by gel electrophoresis. The results confirm that rigid DNA bending is the specific determinant responsible for high-affinity interactions of XPA with damaged DNA, but that an additional important factor, which affects affinity of XPA to damaged DNA, is a change of thermodynamic stability of DNA induced by the damage. In addition, the results also confirm that RPA preferentially binds to DNA distorted so that hydrogen bonds between complementary bases are interrupted. RPA also binds to non-denaturational distortions in double-helical DNA, but affinity of RPA to these distortions is insensitive to alterations of thermodynamic stability of damaged DNA.

Brewood G. P., Rangineni Y., Fish D. J., Bhandiwad A. S., Evans D. R., Solanki R. and Benight A. S. (2008) Electrical detection of the temperature induced melting transition of a DNA hairpin covalently attached to gold interdigitated microelectrodes. *Nucleic Acids Res* **36,** e98.
Abstract: The temperature induced melting transition of a self-complementary DNA strand covalently attached at the 5' end to the surface of a gold interdigitated microelectrode (GIME) was monitored in a novel, label-free, manner. The structural state of the hairpin was assessed by measuring four different electronic properties of the GIME (capacitance, impedance, dissipation factor and phase angle) as a function of temperature from 25 degrees C to 80 degrees C. Consistent changes in all four electronic properties of the GIME were observed over this temperature range, and attributed to the transition of the attached single-stranded DNA (ssDNA) from an intramolecular, folded hairpin structure to a melted ssDNA. The melting curve of the self-complementary single strand was also measured in solution using

differential scanning calorimetry (DSC) and UV absorbance spectroscopy. Temperature dependent electronic measurements on the surface and absorbance versus temperature values measured in solution experiments were analyzed assuming a two-state process. The model analysis provided estimates of the thermodynamic transition parameters of the hairpin on the surface. Two-state analyses of optical melting data and DSC measurements provided evaluations of the thermodynamic transition parameters of the hairpin in solution. Comparison of surface and solution measurements provided quantitative evaluation of the effect of the surface on the thermodynamics of the melting transition of the DNA hairpin

Cao W. and Lai L. (1999) A thermodynamic study on the formation and stability of DNA duplex at transcription site for DNA binding proteins GCN4. *Biophys Chem* **80,** 217-226.
Abstract: Using isothermal titration calorimetry (ITC), we studied the thermodynamic parameters of the 15-mer duplex dsDNA [d(GAGATGACTCATCTC)].[d(GAGATGAGTCATCTC)] formation from its two complementary single strands (S1 and S2) over a range of temperatures. The two complementary single strands d(GAGATGACTCATCTC) (herein called S1) and d(GAGATGAGTCATCTC) (herein called S2) containing palindromic sequences may assume ordered structures at low temperatures, which made the duplex dsDNA formation rather complicated. The thermodynamic parameters for the duplex formation, such as the binding constants (Kb), the enthalpies (Δ H0), the free energies (Δ G0), the entropies (Δ S0) are strongly temperature-dependent. The thermally-induced disruptions of the duplex and its two complementary single strands, S1 and S2, were measured using differential scanning calorimetry (DSC) and CD spectroscopy, the results demonstrate that the DNA duplex is very stable, and its component single strands have an ordered structure at low temperature. This 15-mer specific sequence DNA may act as recognition site for DNA binding proteins GCN4 and plays a key role in transcription regulation of gene expression. Our analyses of the thermodynamic data suggest that the duplex formation is a coupled process between conformational transitions in the two single strands and their binding to form duplex dsDNA.

Cardellini E., Cinelli S., Gianfranceschi G. L., Onori G., Santucci A., and Urbanelli L. (2000) Differential scanning calorimetry of chromatin at different levels of condensation. *Mol Biol Rep* **27,** 175-180.
Abstract: The thermal denaturation of calf thymus total chromatin and of fractions enriched in heterochromatin or euchromatin, has been investigated by differential scanning calorimetry and compared to that of calf thymus DNA and DNA-histone complexes. In our experimental conditions, chromatin melts in three thermal transitions: the main one, assigned to separation of the DNA double helix, occurs at 83 degrees C, while the other two occur at 63 degrees C and 74 degrees C. The data show that: (a) the transition enthalpy for denaturation of DNA in the total chromatin and in DNA-histone complexes is nearly the same as that of DNA in solution; (b) the transition at 63 degrees C is present in the thermogram of the heterocromatin enriched fraction, while it is completely absent in that of the euchromatin enriched one. The results suggest that this transition can be attributed to the higher order structures of heterochromatin.

Cervantes-Cervantes M. P., Calderon-Salinas J. V., Albores A., and Munoz-Sanchez J. L. (2005) Copper increases the damage to DNA and proteins caused by reactive oxygen species. *Biol Trace Elem Res* **103,** 229-248.
Abstract: Copper [Cu(II)] is an ubiquitous transition and trace element in living organisms. It increases reactive oxygen species (ROS) and free-radical generation that might damage biomolecules like DNA, proteins, and lipids. Furthermore, ability of Cu(II) greatly increases in the presence of oxidants. ROS, like hydroxyl (.OH) and superoxide (.O(2)) radicals, alter both the structure of the DNA double helix and the nitrogen bases, resulting in mutations like the AT-->GC and GC-->AT transitions. Proteins, on the other hand, suffer irreversible oxidations and loss in their biological role. Thus, the aim of this investigation is to characterize, in vitro, the structural effects caused by ROS and Cu(II) on bacteriophage lambda DNA or proteins using either hydrogen peroxide (H(2)O(2)) or ascorbic acid with or without Cu(II). Exposure of DNA to ROS-generating mixtures results in electrophoretic (DNA breaks), spectrophotometric (band broadening, hypochromic, hyperchromic, and bathochromic effects), and calorimetric (denaturation temperature [T(d)], denaturation enthalpy [DeltaH], and heat capacity [C(p)] values) changes. As for proteins, ROS increased their thermal stability. However, the extent of the observed changes in DNA and proteins were distinct, depending on the efficiency of the systems assayed to generate ROS. The resulting effects were most evident when Cu(II) was present. In summary, these results show that the ROS, .O2 and .OH radicals, generated by the Cu(II) systems assayed deeply altered the chemical structure of both DNA and proteins. The physiological relevance of these structural effects should be further investigated.

Chakrabarti M. C. and Schwarz F. P. (1999) Thermal stability of PNA/DNA and DNA/DNA duplexes by differential scanning calorimetry. *Nucleic Acids Res* **27,** 4801-4806.
Abstract: Thermodynamics of the thermal dissociation transitions of 10 bp PNA/DNA duplexes and their corresponding DNA/DNA duplexes in 10 mM sodium phosphate buffer (pH 7.0) were determined from differential scanning calorimetry (DSC) measurements. The PNA/DNA transition temperatures ranged from 329 to 343 K and the calorimetric transition enthalpies ranged from 209 +/- 6 to 283 +/- 37 kJ mol^{-1}. The corresponding DNA/DNA transition temperatures were 7-20 K lower and the transition enthalpies ranged from 72 +/- 29 to 236 +/- 24 kJ mol^{-1}. Agreement between the DSC and UV monitored melting (UVM) determined transition enthalpies validated analyzing the UVM transitions in terms of a two-state transition model. The transitions exhibited reversibility and were analyzed in terms of an AB = A + B two-state transition model which yielded van't Hoff enthalpies in agreement with the transition enthalpies. Extrapolation of the transition enthalpies and free energy changes to ambient temperatures yielded more negative values than those determined directly from isothermal titration calorimetry measurements on formation of the duplexes. This discrepancy was attributed to thermodynamic differences in the single-strand structures at ambient and at the transition temperatures, as indicated by UVM measurements on single DNA and PNA strands.

Chalikian T. V., Volker J., Plum G. E., and Breslauer K. J. (1999) A more unified picture for the thermodynamics of nucleic acid duplex melting: a characterization by calorimetric and volumetric techniques. *Proc Natl Acad Sci U S A* **96,** 7853-7858.
Abstract: We use a combination of calorimetric and volumetric techniques to detect and to characterize the thermodynamic changes that accompany helix-to-coil transitions for five polymeric nucleic acid duplexes. Our calorimetric measurements reveal that melting of the duplexes is accompanied by positive changes in heat capacity (ΔCP) of similar magnitude, with an average ΔCP value of 64.6 +/- 21.4 cal deg-1 mol-1. When this heat capacity value is used to compare significantly different transition enthalpies (ΔHo) at a common reference temperature, Tref, we find ΔHTref for duplex melting to be far less dependent on duplex type, base composition, or base sequence than previously believed on the basis of the conventional assumption of a near-zero value for ΔCP. Similarly, our densimetric and acoustic measurements reveal that, at a given temperature, all the AT- and AU-containing duplexes studied here melt with nearly the same volume and compressibility changes. In the aggregate, our results, in conjunction with literature data, suggest a more unified picture for the thermodynamics of nucleic acid duplex melting. Specifically, when compared at a common temperature, the apparent large differences present in the literature for the transition enthalpies of different duplexes become much more compressed, and the melting of all-AT- and all-AU-containing duplexes exhibits similar volume and compressibility changes despite differences in sequence and conformation. Thus, insofar as thermodynamic properties are concerned, when comparing duplexes, the temperature under consideration is as important as, if not more important than, the duplex type, the base composition, or the base sequence. This general behavior has significant implications for our basic understanding of the forces that stabilize nucleic acid duplexes. This behavior also is of practical significance in connection with the use of thermodynamic databases for designing probes and for assessing the affinity and specificity associated with hybridization-based protocols used in a wide range of sequencing, diagnostic, and therapeutic applications.

Chinyengetere F. and Jamieson E. R. (2008) Impact of the oxidized guanine lesion spiroiminodihydantoin on the conformation and thermodynamic stability of a 15-mer DNA duplex. *Biochemistry* **47,** 2584-2591.
Abstract: Spiroiminodihydantoin (Sp) is a hyperoxidized guanine base produced from oxidation of the mutagenic DNA lesion 7,8-dihydro-8-oxo-2'-deoxguanosine (8-oxoG) by a variety of species including peroxynitrite, singlet oxygen, and the high-valent metals Ir(IV) and Cr(V). In this study, the conformation and thermodynamic stability of a 15-mer DNA duplex containing an Sp lesion are examined using spectroscopic techniques and differential scanning calorimetry (DSC). The Sp lesion does not alter the global B-form conformation of the DNA duplex as determined by circular dichroism spectroscopy. Thermal denaturation experiments find that Sp significantly lowers the thermal stability of the duplex by approximately 20 degrees C. The enthalpies, entropies, and free energies of duplex formation for 15-mers containing guanine, 8-oxoG, and Sp were determined by performing DSC experiments as well as van't Hoff analysis of UV melting spectroscopic data. The thermodynamic stability of the Sp duplex is significantly reduced compared to that of both the 8-oxoG and parent G duplexes, with the thermodynamic destabilization being enthalpic in origin. The thermodynamic impact of the Sp lesion is compared to what is

found for other types of DNA base damage and discussed in relation to how the presence of this lesion could affect cellular processes, in particular the recognition and repair of these adducts by the base excision repair enzymes

Cooper R. L. and Lee S. A. (2004) Differential scanning calorimetric study of the binding of the water of hydration to deoxyadenosine. *J Biomol Struct Dyn* **22,** 375-380.
Abstract: Differential scanning calorimetry was used to study the water of hydration in powder samples of deoxyadenosine (dA), a naturally occurring nucleoside. Though water of crystallization is present in samples which have not undergone heat treatment previously, dA was found to rehydrate at room temperature only at relative humidities (RHs) of 88% and higher. Rehydrated samples exhibited a single endothermic peak. At 95% RH, its activation energy was 1.61 +/- 0.06 eV and had an enthalpy change of 190 +/- 30 J/g. Experiments between 88 and 95% RH revealed that the energies are independent of RH, to within experimental error. This range of humidity corresponds to secondary hydration in DNA.

D'Onofrio J., Petraccone L., Erra E., Martino L., Fabio G. D., Napoli L. D., Giancola C. and Montesarchio D. (2007) 5'-Modified G-quadruplex forming oligonucleotides endowed with anti-HIV activity: synthesis and biophysical properties. *Bioconjug. Chem* **18,** 1194-1204.
Abstract: Oligodeoxyribonucleotides of sequence d(5'TGGGAG3') carrying bulky aromatic groups at the 5' end were found to exhibit potent anti-HIV activity [Hotoda, H., et al. (1998) J. Med. Chem. 41, 3655-3663 and references therein]. Structure-activity relationship investigations indicated that G-quadruplex formation, as well as the presence of large aromatic substituents at the 5'-end, were both essential for their antiviral activity. In this work, we synthesized some representative examples of the anti-HIV active Hotoda's 6-mers and analyzed the resulting G-quadruplexes by CD, DSC, and molecular modeling studies, in comparison with the unmodified oligonucleotide. In the case of the sequence carrying the 3,4-dibenzyloxybenzyl (DBB) group, identified as the best candidate for further drug optimization, we developed an alternative protocol to synthesize the 5'-DBB-thymidine phosphoramidite building block in higher yields. The thermodynamic and kinetic parameters for the association/dissociation processes of the 5'-conjugated quadruplexes, determined with respect to the unmodified one, were discussed in light of the molecular modeling studies. The aromatic groups at the 5' position of d(5'TGGGAG3') dramatically enhance both the equilibrium and the rate of formation of the quadruplex complexes. The overall stability of the investigated quadruplexes was found to correlate with the reported IC50 values, thus furnishing quantitative evidence for the hypothesis that the G-quadruplex structures are the ultimate active species, effectively responsible for the biological activity.

Del Vecchio P., Esposito D., Ricchi L., and Barone G. (1999) The effects of polyols on the thermal stability of calf thymus DNA. *Int J Biol Macromol* **24,** 361-369.
Abstract: The effects on thermal denaturation of calf thymus DNA (ct-DNA) and its conformational changes induced by the presence in solution of different polyols, namely glycerol, i-erytritol, L(--) and D(+) arabitol, D-mannitol, D-sorbitol and myo-inositol, have been investigated by means of differential scanning calorimetry (DSC) and circular dichroism (CD). By increasing the concentration of these additives a decrease in both the denaturation enthalpy (ΔdH) and temperature of the maximum of the denaturation peak (Tmax) of DNA is observed. The values of these thermodynamic parameters depend on both the nature and concentration of the solute. The overall destabilization of DNA molecule has been related to the different capability of polyhydric alcohols to interact with the polynucleotide solvation sites replacing water and to the modification of the electrostatic interactions between the polynucleotide and its surrounding atmosphere of counterions. The particular behaviour of L(--) arabitol, which showed a much greater destabilizing ability compared to the other polyols, was further investigated and attributed to a direct more effective interaction with the double helix of DNA. CD spectra showed only a slight alteration of DNA-B structure in the presence of all the molecules here studied, except for L(--) arabitol where the DNA molecule seems to undergo a meaningful conformational change. The salt concentration dependence of DNA thermal stability in the presence of L(--) arabitol indicates a conformational change of polynucleotide towards a more extended conformation.

Delrow J. J., Heath P. J., and Schurr J. M. (1997) On the origin of the temperature dependence of the supercoiling free energy. *Biophys J* **73,** 2688-2701.
Abstract: Monte Carlo simulations using temperature-invariant torsional and bending rigidities fail to

predict the rather steep decline of the experimental supercoiling free energy with increasing temperature, and consequently fail to predict the correct sign and magnitude of the supercoiling entropy. To illustrate this problem, values of the twist energy parameter (E(T)), which governs the supercoiling free energy, were simulated using temperature-invariant torsion and bending potentials and compared to experimental data on pBR322 over a range of temperatures. The slope, -dE(T)/dT, of the simulated values is also compared to the slope derived from previous calorimetric data. The possibility that the discrepancies arise from some hitherto undetected temperature dependence of the torsional rigidity was investigated. The torsion elastic constant of an 1876-bp restriction fragment of pBR322 was measured by time-resolved fluorescence polarization anisotropy of intercalated ethidium over the range 278-323 K, and found to decline substantially over that interval. Simulations of a 4349-bp model DNA were performed using these measured temperature-dependent torsional rigidities. The slope, -dE(T)/dT, of the simulated data agrees satisfactorily with the slope derived from previous calorimetric measurements, but still lies substantially below that of Duguet's data. Models that involve an equilibrium between different secondary structure states with different intrinsic twists and torsion constants provide the most likely explanation for the variation of the torsion constant with T and other pertinent observations.

Dima R. I., Hyeon C., and Thirumalai D. (2005) Extracting stacking interaction parameters for RNA from the data set of native structures. *J Mol Biol* **347,** 53-69.
Abstract: A crucial step in the determination of the three-dimensional native structures of RNA is the prediction of their secondary structures, which are stable independent of the tertiary fold. Accurate prediction of the secondary structure requires context-dependent estimates of the interaction parameters. We have exploited the growing database of natively folded RNA structures in the Protein Data Bank (PDB) to obtain stacking interaction parameters using a knowledge-based approach. Remarkably, the calculated values of the resulting statistical potentials (SPs) are in excellent agreement with the parameters determined using measurements in small oligonucleotides. We validate the SPs by predicting 74% of the base-pairs in a dataset of structures using the ViennaRNA package. Interestingly, this number is similar to that obtained using the measured thermodynamic parameters. We also tested the efficacy of the SP in predicting secondary structure by using gapless threading, which we advocate as an alternative method for rapidly predicting RNA structures. For RNA molecules with less than 700 nucleotides, about 70% of the native base-pairs are correctly predicted. As a further validation of the SPs we calculated Z-scores, which measure the relative stability of the native state with respect to a manifold of higher free energy states. The computed Z-scores agree with estimates made using calorimetric measurements for a few RNA molecules. Structural analysis was used to rationalize the success and failures of SP and experimentally determined parameters. First, from the near perfect linear relationship between the number of native base-pairs and sequence length, we show that nearly 46% of nucleotides are not in stacks. Second, by analyzing the suboptimal structures that are generated in gapless threading we show that the SPs and experimentally determined parameters are most successful in predicting stacks that end in hairpins. These results show that further improvement in secondary structure prediction requires reliable estimates of interaction parameters for loops, bulges, and stacks that do not end in hairpins.

Draper D. E., Xing Y., and Laing L. G. (1995) Thermodynamics of RNA unfolding: stabilization of a ribosomal RNA tertiary structure by thiostrepton and ammonium ion. *J Mol Biol* **249,** 231-238.
Abstract: RNAs with interesting secondary and tertiary structures tend to melt in several broad and overlapping transitions over a wide temperature range, and it has been consequently difficult to resolve the thermodynamics of individual unfolding steps. In the case that a ligand selectively binds a single folded state of the RNA, it is possible to obtain reliable thermodynamic parameters for both RNA unfolding and RNA-ligand binding simply from the hyperchromicity of RNA denaturation. The analysis procedure involves fitting a three-dimensional surface to absorbance data collected as a function of both temperature and ligand concentration. Analysis of the unfolding of a fragment of the large subunit ribosomal RNA (Escherichia coli sequence 1051 to 1109) is presented; both an antibiotic (thiostrepton) and ammonium ion specifically stabilize a tertiary structure within this RNA. A consistent set of thermodynamic parameters (Δ H and tm) for the first two sequentially linked unfolding transitions is obtained from the experiments, and the binding constants obtained for the two ligands are consistent with other independent measurements. The approach is applicable to a variety of RNAs that specifically bind proteins, antibiotics, ions or other ligands.

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

Duguid J. G. and Bloomfield V. A. (1995) Aggregation of melted DNA by divalent metal ion-mediated cross-linking. *Biophys J* **69,** 2642-2648.
Abstract: In an accompanying paper we reported the use of differential scanning calorimetry and optical densitometry to characterize the melting and aggregation of 160 bp fragments of calf thymus DNA during heating in the presence of divalent metal cations. Aggregation is observed as thermal denaturation begins and becomes more extensive with increasing temperature until the melting temperature Tm is reached, after which the aggregates dissolve extensively. The order of effectiveness of the metals in inducing aggregation is generally consistent with their ability to induce melting: Cd > Ni > Co > Mn approximately Ca > Mg. Under our experimental conditions (50 mg/ml DNA, 100 mM MCl2, [metal]/[DNA phosphate] approximately 0.6), no measurable aggregates were observed for BaDNA or SrDNA. In this paper we show that the Shibata-Schurr theory of aggregation in the thermal denaturation region provides a good model for our observations. Free energies of cross-linking, induced by the divalent cations, are estimated to be between 34% and 38% of the free energies of base stacking. The ability of a divalent metal cation to induce DNA aggregation can be attributed to its ability to disrupt DNA base pairing and simultaneously to link two different DNA sites.

Duguid J. G., Bloomfield V. A., Benevides J. M., and Thomas G. J., Jr. (1995) Raman spectroscopy of DNA-metal complexes. II. The thermal denaturation of DNA in the presence of Sr2+, Ba2+, Mg2+, Ca2+, Mn2+, Co2+, Ni2+, and Cd2+. *Biophys J* **69,** 2623-2641.
Abstract: Differential scanning calorimetry, laser Raman spectroscopy, optical densitometry, and pH potentiometry have been used to investigate DNA melting profiles in the presence of the chloride salts of Ba2+, Sr2+, Mg2+, Ca2+, Mn2+, Co2+, Ni2+, and Cd2+. Metal-DNA interactions have been observed for the molar ratio [M2+]/[PO2-] = 0.6 in aqueous solutions containing 5% by weight of 160 bp mononucleosomal calf thymus DNA. All of the alkaline earth metals, plus Mn2+, elevate the melting temperature of DNA (Tm > 75.5 degrees C), whereas the transition metals Co2+, Ni2+, and Cd2+ lower Tm. Calorimetric (Δ Hcal) and van't Hoff (Δ HVH) enthalpies of melting range from 6.2-8.7 kcal/mol bp and 75.6-188.6 kcal/mol cooperative unit, respectively, and entropies from 17.5 to 24.7 cal/K mol bp. The average number of base pairs in a cooperative melting unit (<nmelt>) varied from 11.3 to 28.1. No dichotomy was observed between alkaline earth and transition DNA-metal complexes for any of the thermodynamic parameters other than their effects on Tm. These results complement Raman difference spectra, which reveal decreases in backbone order, base unstacking, distortion of glycosyl torsion angles, and rupture of hydrogen bonds, which occur after thermal denaturation. Raman difference spectroscopy shows that transition metals interact with the N7 atom of guanine in duplex DNA. A broader range of interaction sites with single-stranded DNA includes ionic phosphates, the N1 and N7 atoms of purines, and the N3 atom of pyrimidines. For alkaline earth metals, very little interaction was observed with duplex DNA, whereas spectra of single-stranded complexes are very similar to those of melted DNA without metal. However, difference spectra reveal some metal-specific perturbations at 1092 cm-1 (nPO2-), 1258 cm-1 (dC, dA), and 1668 cm-1 (nC==O, dNH2 dT, dG, dC). Increased spectral intensity could also be observed near 1335 cm-1 (dA, dG) for CaDNA. Optical densitometry, employed to detect DNA aggregation, reveals increased turbidity during the melting transition for all divalent DNA-metal complexes, except SrDNA and BaDNA. Turbidity was not observed for DNA in the absence of metal. A correlation was made between DNA melting, aggregation, and the ratio of Raman intensities I1335/I1374. At room temperature, DNA-metal interactions result in a pH drop of 1.2-2.2 units for alkaline earths and more than 2.5 units for transition metals. Sr2+, Ba2+, and Mg2+ cause protonated sites on the DNA to become thermally labile. These results lead to a model that describes DNA aggregation and denaturation during heating in the presence of divalent metal cations; 1) The cations initially interact with the DNA at phosphate and/or base sites, resulting in proton displacement. 2) A combination of metal-base interactions and heating disrupts the base pairing within the DNA duplex. This allows divalent metals and protons to bind to additional sites on the DNA bases during the aggregation/melting process. 3) Strands whose bases have swung open upon disruption are linked to neighboring strands by metal ion bridges. 4) Near the midpoint of the melting transition, thermal energy breaks up the aggregate. We have no evidence to indicate whether metal ion cross-bridges or direct base-base interactions rupture first. 5) Finally, all cross-links break, resulting in single-stranded DNA complexed with metal ions.

Duguid J. G., Bloomfield V. A., Benevides J. M., and Thomas G. J., Jr. (1996) DNA melting investigated by differential scanning calorimetry and Raman spectroscopy. *Biophys J* **71,** 3350-3360.
Abstract: Thermal denaturation of the B form of double-stranded DNA has been probed by differential scanning calorimetry (DSC) and Raman spectroscopy of 160 base pair (bp) fragments of calf thymus DNA. The DSC results indicate a median melting temperature Tm = 75.5 degrees C with calorimetric enthalpy change Δ Hcal = 6.7 kcal/mol (bp), van't Hoff enthalpy change Δ HVH = 50.4 kcal/mol (cooperative unit), and calorimetric entropy change Δ Scal = 19.3 cal/deg.mol (bp), at the experimental conditions of 55 mg DNA/ml in 5 mM sodium cacodylate at pH 6.4. The average cooperative melting unit (nmelt) comprises 7.5 bp. The Raman signature of 160 bp DNA is highly sensitive to temperature. Analyses of several conformation-sensitive Raman bands indicate the following ranges for thermodynamic parameters of melting: 43 < Δ HVH < 61 kcal/mol (cooperative unit), 75 < Tm < 80 degrees C and 6 < (nmelt) < 9 bp, consistent with the DSC results. The changes observed in specific Raman band frequencies and intensities as a function of temperature reveal that thermal denaturation is accompanied by disruption of Watson-Crick base pairs, unstacking of the bases and disordering of the B form backbone. These three types of structural change are highly correlated throughout the investigated temperature range of 20 to 93 degrees C. Raman bands diagnostic of purine and pyrimidine unstacking, conformational rearrangements in the deoxyribose-phosphate moieties, and changes in environment of phosphate groups have been identified. Among these, bands at 834 cm-1 (due to a localized vibration of the phosphodiester group), 1240 cm-1 (thymine ring) and 1668 cm-1 (carbonyl groups of dT, dG and dC), are shown by comparison with DSC results to be the most reliable quantitative indicators of DNA melting. Conversely, the intensities of Raman marker bands at 786 cm-1 (cytosine ring), 1014 cm-1 (deoxyribose ring) and 1092 cm-1 (phosphate group) are largely invariant to melting and are proposed as appropriate standards for intensity normalizations.

Duguid J. G. and Bloomfield V. A. (1996) Electrostatic effects on the stability of condensed DNA in the presence of divalent cations. *Biophys J* **70,** 2838-2846.
Abstract: Cylindrical cell model Poisson-Boltzmann (P-B) calculations are used to evaluate the electrostatic contributions to the relative stability of various DNA conformations (A, B, C, Z, and single-stranded (ss) with charge spacings of 3.38 and 4.2 A) as a function of interhelix distance in a concentrated solution of divalent cations. The divalent ion concentration was set at 100 mM, to compare with our earlier reports of spectroscopic and calorimetric experiments, which demonstrate substantial disruption of B-DNA geometry. Monovalent cations neutralize the DNA phosphates in two ways, corresponding to different experimental situations: 1) There is no significant contribution to the ionic strength from the neutralizing cations, corresponding to DNA condensation from dilute solution and to osmotic stress experiments in which DNA segments are brought into close proximity to each other in the presence of a large excess of buffer. 2) The solution is uniformly concentrated in DNA, so that the neutralizing cations add significantly to those in the buffer at close DNA packing. In case 1), conformations with lower charge density (Z and ssDNA) have markedly lower electrostatic free energies than B-DNA as the DNA molecules approach closely, due largely to ionic entropy. If the divalent cations bind preferentially to single-stranded DNA or a distorted form of B-DNA, as is the case with transition metals, the base pairing and stacking free energies that stabilize the double helix against electrostatic denaturation may be overcome. Strong binding to the bases is favored by the high concentration of divalent cations at the DNA surface arising from the large negative surface potential; the surface concentration increases sharply as the interhelical distance decreases. In case 2), the concentration of neutralizing monovalent cations becomes very large and the electrostatic free energy difference between secondary structures becomes small as the interhelical spacing decreases. Such high ionic concentrations will be expected to modify the stability of DNA by changing water activity as well as by screening electrostatic interactions. This may be the root of the decreased thermal stability of DNA in the presence of high concentrations of magnesium ions.

Evertsz E. M., Rippe K., and Jovin T. M. (1994) Parallel-stranded duplex DNA containing blocks of trans purine-purine and purine-pyrimidine base pairs. *Nucleic Acids Res* **22,** 3293-3303.
Abstract: A 30 base pair parallel-stranded (ps) duplex ps-L1.L2 composed of two adjoined purine-purine and purine-pyrimidine sequence blocks has been characterized thermodynamically and spectroscopically. The 5'-terminal 15 residues in both strands ('left-half') consisted of the alternating d(GA)7G sequence that forms a ps homoduplex secondary structure stabilized by d(G.G) and d(A.A) base pairs. The 3'-terminal 15 positions of the sequence ('right-half') were combinations of A and T with complementary reverse Watson-Crick d(A.T) base pairing between the two strands. The characteristics of the full length duplex were

compared to those of the constituent left and right halves in order to determine the compatibility of the two ps helical forms. The thermal denaturation curves and hyperchromicity profiles of all three duplexes determined by UV absorption spectroscopy were characteristic of ps-DNA, in accordance with previous studies. The thermodynamic properties of the 30 bp duplex corresponded within experimental error to the linear combination of the two 15-mers. Thus, the Tm and Δ HvH of ps-L1.L2 in 10 mM MgCl2, derived from analyses according to a statistical mechanical formulation for the helix-coil transition, were 43 degrees C and 569 kJ mol-1, compared to 21 degrees C, 315 kJ mol-1 (ps-F5.F6) and 22 degrees C, 236 kJ mol-1 (ps-GA15). The UV absorption and CD spectra of ps-L1.L2 and the individual 15-mer ps motifs were also compared quantitatively. The sums of the two constituent native spectra (left+right halves) accurately matched that of the 30 bp duplex, with only small deviations in the 195-215 nm (CD) and 220-240 nm (absorption) regions. Based on analysis by native gel electrophoresis, the sequences studied formed duplex structures exclusively; there were no indications of higher order species. Chemical modification with diethyl pyrocarbonate showed no hyperreactivity of the junctional bases, indicating a smooth transition between the two parallel-stranded conformations. We conclude that under given salt conditions, oligonucleotides with normal primary chemical structures can readily form a parallel-stranded double helix based on blocks of very disparate non-canonical purine-purine and purine-pyrimidine base pairs and without perceptible destabilization at the junction. There are biological implications of these findings in relation to genetic structure and expression.

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.

Fish D. J., Horne M. T., Brewood G. P., Goodarzi J. P., Alemayehu S., Bhandiwad A., Searles R. P. and Benight A. S. (2007) DNA multiplex hybridization on microarrays and thermodynamic stability in solution: a direct comparison. *Nucleic Acids Res* **35,** 7197-7208.
Abstract: Hybridization intensities of 30 distinct short duplex DNAs measured on spotted microarrays, were directly compared with thermodynamic stabilities measured in solution. DNA sequences were designed to promote formation of perfect match, or hybrid duplexes containing tandem mismatches. Thermodynamic parameters DeltaH degrees , DeltaS degrees and DeltaG degrees of melting transitions in solution were evaluated directly using differential scanning calorimetry. Quantitative comparison with results from 63 multiplex microarray hybridization experiments provided a linear relationship for perfect match and most mismatch duplexes. Examination of outliers suggests that both duplex length and relative position of tandem mismatches could be important factors contributing to observed deviations from linearity. A detailed comparison of measured thermodynamic parameters with those calculated using the nearest-neighbor model was performed. Analysis revealed the nearest-neighbor model generally predicts

mismatch duplexes to be less stable than experimentally observed. Results also show the relative stability of a tandem mismatch is highly dependent on the identity of the flanking Watson-Crick (w/c) base pairs. Thus, specifying the stability contribution of a tandem mismatch requires consideration of the sequence identity of at least four base pair units (tandem mismatch and flanking w/c base pairs). These observations underscore the need for rigorous evaluation of thermodynamic parameters describing tandem mismatch stability.

Franzen J. S., Zhang M., Chay T. R., and Peebles C. L. (1994) Thermal activation of a group II intron ribozyme reveals multiple conformational states. *Biochemistry* **33,** 11315-11326.
Abstract: Conformational changes often accompany biological catalysis. Group II introns promote a variety of reactions in vitro that show an unusually sharp temperature dependence. This suggests that the chemical steps are accompanied by the conversion of a folded-but-inactive form to a differently folded active state. We report here the kinetic analysis of 5'-splice-junction hydrolysis (SJH) by E1:12345, a transcript containing the 5'-exon plus the first five of six intron secondary structure domains. The pseudo-first-order SJH reaction shows (1) activation by added KCl to 1.5 M; (2) cooperative activation by added MgCl2, nHill = 4.1-4.3, and [MgCl2]vmax/2 approximately 0.040 M; and (3) a rather high apparent activation energy, Ea approximately 50 kcal mol-l. In contrast, the 5'-terminal phosphodiester bond of a domain 5 transcript (GGD5) was hydrolyzed with Ea approximately 30 kcal mol-1 under SJH conditions; the 5'-GG leader dinucleotide presumably lacks secondary structure constraints. The effect of adding the chaotropic salt tetraethylammonium chloride (TEA) was also investigated. TEA reduced the melting temperatures of GGD5 and E1:12345. TEA also shifted the profile of rate versus temperature for SJH by E1:12345 toward lower temperatures without affecting the maximum rate. TEA had little effect on the rate of hydrolysis of the 5'-phosphodiester bond of GGD5. The high apparent activation enthalpy and entropy for SJH along with the effect of TEA on these parameters imply that conversion of an inactive form of E1:12345 to an active conformation accompanies enhanced occupation of the transition state as the temperature is raised to that for maximum SJH. Analytical modeling indicates that either a two-state model (open and disordered, with open being active) or a three-state model (compact, open, and disordered) could account for the temperature dependence of kSJH. However, the three-state model is clearly preferable, since it does not require that the activation parameters for phosphodiester bond hydrolysis exhibit exceptional values or that the rates for the chemical steps of SJH respond directly to TEA addition.

Fujiwara T., Sugiyama H., and Saito I. (1997) Structure of DNA containing aristeromycin analog. *Nucleic Acids Symp Ser* 51-52.
Abstract: The melting temperatures and thermodynamic properties of several oligodeoxynucleotide duplexes containing one modified deoxyadenosine residue (carbocyclic deoxyadenosine, dAr) were investigated. It was found that the introduction of carbocyclic deoxyadenosine slightly destabilized oligonucleotide duplexes mainly due to an entropic contribution.

Ganguly M., Wang F., Kaushik M., Stone M. P., Marky L. A. and Gold B. (2007) A study of 7-deaza-2'-deoxyguanosine 2'-deoxycytidine base pairing in DNA. *Nucleic Acids Res* **35,** 6181-6195.
Abstract: The incorporation of 7-deazaguanine modifications into DNA is frequently used to probe protein recognition of H-bonding information in the major groove of DNA. While it is generally assumed that 7-deazaguanine forms a normal Watson-Crick base pair with cytosine, detailed thermodynamic and structural analyses of this modification have not been reported. The replacement of the 7-N atom on guanine with a C-H, alters the electronic properties of the heterocycle and eliminates a major groove cation-binding site that could affect the organization of salts and water in the major groove. We report herein the characterization of synthetic DNA oligomers containing 7-deazaguanine using a variety of complementary approaches: UV thermal melting, differential scanning calorimetry (DSC), circular dichroism (CD), chemical probing and NMR. The results indicate that the incorporation of a 7-deazaguanine modification has a significant effect on the dynamic structure of the DNA at the flanking residue. This appears to be mediated by changes in hydration and cation organization.

Gelfand C. A., Plum G. E., Grollman A. P., Johnson F., and Breslauer K. J. (1998) The impact of an exocyclic cytosine adduct on DNA duplex properties: significant thermodynamic consequences despite modest lesion-induced structural alterations. *Biochemistry* **37,** 12507-12512.
Abstract: The exocyclic base adduct 3,N4-deoxyethenocytosine (epsilonC) is a common DNA lesion that

can arise from carcinogen exposure and/or as a biproduct of cellular processes. We have examined the thermal and thermodynamic impact of this lesion on DNA duplex properties, as well as the structural alterations imparted by the lesion. For these studies, we used calorimetric and spectroscopic techniques to investigate a family of 13-mer DNA duplexes of the form (5'CGCATGNGTACGC3')x(3'GCGTACNCATGCG5'), where the central NxN base pair represents the four standard Watson-Crick base pairs (corresponding to four control duplexes), and where either one of the N bases has been replaced by epsilonC, yielding eight test duplexes. Studies on these 12 duplexes permit us to assess the impact of the epsilonC lesion as a function of sequence context. Our spectroscopic and calorimetric data allow us to reach the following conclusions: (i) The epsilonC lesion imparts a large penalty on duplex stability, with sequence context only modestly modulating the extent of this lesion-induced destabilization. This result contrasts with our recent studies of duplexes with abasic sites, where sequence context was found to be the predominant determinant of thermodynamic damage. (ii) For the epsilonC-containing duplexes, sequence context effects are most often observed in the enthalpic contribution to lesion-induced duplex destabilization. However, due to compensating entropies, the free energy changes associated with this lesion-induced duplex destablization are nearly independent of sequence context. (iii) Despite significant lesion-induced changes in duplex energetics, our spectroscopic probes detect only modest lesion-induced changes in duplex structure. In fact, the overall duplex maintains a global B-form conformation, in agreement with NMR structural data. We discuss possible interpretations of the apparent disparity between the severe thermodynamic and relatively mild structural impacts of the epsilonC lesion on duplex properties. We also note and discuss the implications of empirical correlations between biophysical and biological properties of lesion-containing duplexes.

Gelfand C. A., Plum G. E., Grollman A. P., Johnson F., and Breslauer K. J. (1998) Thermodynamic consequences of an abasic lesion in duplex DNA are strongly dependent on base sequence. *Biochemistry* **37,** 7321-7327.
Abstract: The abasic site in DNA may arise spontaneously, as a result of nucleotide base damage, or as an intermediate in glycosylase-mediated DNA-repair pathways. It is the most common damage found in DNA. We have examined the consequences of this lesion and its sequence context on DNA duplex structure, as well as the thermal and thermodynamic stability of the duplex, including the energetic origins of that stability. To this end, we incorporated a tetrahydrofuran abasic site analogue into a family of 13-mer DNA duplexes, wherein the base opposite the lesion (A, C, G, or T) and the base pairs neighboring the lesion (C.G or G.C) were systematically varied and characterized by a combination of spectroscopic and calorimetric techniques. The resulting data allowed us to reach the following conclusions: (i) the presence of the lesion in all sequence contexts studied does not alter the global B-form conformation characteristic of the parent undamaged duplex; (ii) the presence of the lesion induces a significant enthalpic destabilization of the duplex, with the magnitude of this effect being dependent on the sequence context; (iii) the thermodynamic impact of the lesion is dominated by the identity of the neighboring base pairs, with the cross strand partner base exerting only a secondary thermodynamic effect on duplex properties. In the aggregate, our data reveal that even in the absence of lesion-induced alterations in global structure, the abasic lesion can significantly alter the thermodynamic properties of the host duplex, with the magnitude of this impact being strongly dependent on sequence context.

Giancola C., Petraccone L., Pieri M., and Barone G. (2001) Thermodynamic and computational studies of DNA triple helices containing a nucleotide or a non-nucleotide linker in the third strand. *Biophys Chem* **94,** 23-31.
Abstract: In this paper we report a thermodynamic characterisation of stability and melting behaviour of four different triple helices at pH 6.0. The target duplex consists of 16 base pairs in alternate sequence of the type 5'-(purine)(m)(pyrimidine)(m)-3'. The four triplexes are formed by targeting the 16-mer duplex with an all pyrimidine 16-mer or 15-mer or 14-mer third strand. The 16-mer oligonucleotide contains a 3'-3' phosphodiester junction and corresponding triplex was named 16-mer P. The 14-mer oligonucleotide contains a non-nucleotide linker, the 1,2,3 propanetriol residue and the corresponding triplex was named 14-mer PT. For the 15-mer oligonucleotide both junctions were alternatively used and the relative triplexes were named 15-mer P and 15-mer PT, respectively. These linkers introduce the appropriate polarity inversion and let the third strand switch from one oligopurine strand of the duplex to the other. Thermal denaturation profiles indicate the initial loss of the third strand followed by the dissociation of the target duplex. Transition enthalpies, entropies and free energies were derived from differential scanning

calorimetric measurements. The comparison of Gibbs energies reveals that a more stable triplex is obtained when in the third strand there is the lack of one nucleotide in the junction region and a propanetriol residue as linker was used. The thermodynamic data were discussed in light of molecular mechanics and dynamics calculations.

Giancola C., Petraccone L., Pieri M., De Napoli L., Montesarchio D., Piccialli G., and Barone G. (2001) Physico-chemical studies on DNA triplexes containing an alternate third strand with a non-nucleotide linker. *Int J Biol Macromol* **28,** 387-394.
Abstract: Differential scanning calorimetric (DSC), circular dichroism (CD) and molecular mechanics studies have been performed on two triple helices of DNA. The target duplex consists of 16 base pairs in alternate sequence of the type 5'-(purine)m(pyrimidine)m-3'. In both the triplexes, the third oligopyrimidine strand crosses the major groove at the purine-pyrimidine junction, with a simultaneous binding of the adjacent purine tracts on alternate strands of the Watson-Crick duplex. The switch is ensured by a non-nucleotide linker, the 1,2,3 propanetriol residue, that joins two 3'-3' phosphodiester ends. The third strands differ from each other for a nucleotide in the junction region. The resulting triple helices were termed 14-mer-PXP and 15-mer-PXP (where P = phosphate and X = 1,2,3-propanetriol residue) according to the number of nucleotides that compose the third strand. DSC data show two independent processes: the first corresponding to the dissociation of the third strand from the target duplex, the second to the dissociation of the double helix in two single strands. The two triple helices show the same stability at pH 6.6. At pH 6.0, the 15-mer-PXP triplex is thermodynamically more stable than the 14-mer-PXP triplex. Thermodynamic data are discussed in relation to structural models. The results are useful when considering the design of oligonucleotides that can bind in an antigene approach to the DNA for therapeutic purposes.

Gluick T. C. and Draper D. E. (1994) Thermodynamics of folding a pseudoknotted mRNA fragment. *J Mol Biol* **241,** 246-262.
Abstract: A sequence in the leader and first gene of the Escherichia coli alpha mRNA folds into a complex pseudoknot structure that is required for binding of a translational repressor. The thermal denaturation of a 112 nt RNA containing this structure has been followed by calorimetry and UV hyperchromicity. To determine the partially folded intermediates in unfolding, the denaturation of 13 mutants and of several fragments with successive deletions of helices were investigated as well. An unfolding pathway with seven states is proposed as the simplest mechanism that accounts for the data, and has several implications. (1) The lowest temperature transition appears only in the presence of moderate concentrations of Mg2+ or high concentrations of K+ (Δ H approximately 45 kcal/mol), and is the unfolding of tertiary structures, rather than secondary structure. Under some conditions it is destabilized by increasing salt concentration. (2) Two of the intermediates unfolding at higher temperature must have non-canonical or tertiary interactions in addition to the known secondary structure. (3) Two alternative structures compete for formation of the complete pseudoknot, and form as the pseudoknot unfolds. Thus structures not present in the completely folded pseudoknot affect the overall thermodynamics, and probably the kinetics, of unfolding. (4) Approximately 16 kcal/mol of free energy is required to completely expose the coding region to ribosomes at 37 degrees C, though approximately 6.5 kcal/mol is regained by refolding of upstream regions after the pseudoknot is unfolded. The substantial energy needed to unfold the pseudoknot may affect the rate of translation from this ribosome binding site. A simple model of RNA folding in which an optimum secondary structure forms first, followed by tertiary interactions that further stabilize the secondary structure, does not hold in this RNA.

Gluick T. C., Wills N. M., Gesteland R. F., and Draper D. E. (1997) Folding of an mRNA pseudoknot required for stop codon readthrough: effects of mono- and divalent ions on stability. *Biochemistry* **36,** 16173-16186.
Abstract: Unfolding of an mRNA pseudoknot that induces ribosome suppression of the gag gene stop codon in Moloney murine leukemia virus has been studied by UV hyperchromicity and calorimetry. The pseudoknot melts in two steps, corresponding to its two helical stems. The total enthalpy of denaturation is approximately 170 kcal/mol, approximately the value expected for the secondary structure. At low salt concentrations (<50 mM KCl) the unfolding transitions are not two-state, but they approach two-state behavior at higher salt concentrations. The structure is preferentially stabilized by smaller alkali metal ions (Li+ > Na+ > K+ > Rb+ > Cs+) and by NH4+; the same preferences are exhibited by one of the stems in the context of a hairpin. Divalent metal ions are not required to fold the pseudoknot but do stabilize it

further. To examine divalent ion effects over a wide concentration range, urea was used to lower the RNA unfolding temperature and was shown not to affect characteristics of the pseudoknot unfolding in other respects. The pseudoknot binds divalent ions somewhat more tightly than a hairpin but shows only weak selectivity for different size ions. It is suggested that a region of "intermediate" divalent ion binding affinity, in between highly ligated specific sites and purely delocalized ion binding in character, is created by the pseudoknot fold but that nonspecific, delocalized ion binding contributes at least half the free energy of pseudoknot stabilization by Mg2+.

Gu C. and Wang Y. (2005) Thermodynamic and in vitro replication studies of an intrastrand G[8-5]C cross-link lesion. *Biochemistry* **44,** 8883-8889.
Abstract: We recently identified, from the gamma-irradiation mixture of duplex DNA, a new intrastrand G[8-5]C cross-link lesion, in which the C8 atom of guanine and the C5 atom of its 3' neighboring cytosine are covalently bonded, and carried out in vitro replication studies for the lesion-bearing substrate with a translesion synthesis polymerase, yeast polymerase eta. Here we extended the in vitro replication studies to two replicative polymerases, exonuclease-deficient bacteriophage T7 DNA polymerase (T7(-)) and HIV reverse transcriptase (HIV-RT). Primer extension assays showed that both polymerases stopped synthesis after incorporating a nucleotide opposite the 3'-cytosine in the G[8-5]C lesion. Steady-state kinetic measurements for nucleotide incorporation opposite the 3'-cytosine of the lesion showed that both T7(-) and HIV-RT preferentially incorporated the correct nucleotide, dGMP. We also examined the thermal stabilities and base pairing properties of G[8-5]C in d(ATGGCG[8-5]CGCTAT). The G[8-5]C lesion destabilizes the duplex form by approximately 4 kcal/mol in free energy at 25 degrees C relative to the undamaged parent duplex, and the thermally most stable duplex has natural bases opposite the lesion.

Guo Q., Lu M., and Kallenbach N. R. (1995) Effect of hemimethylation and methylation of adenine on the structure and stability of model DNA duplexes. *Biochemistry* **34,** 16359-16364.
Abstract: Enzymatic methylation of adenine underlies a variety of biological regulatory mechanisms in Escherichia coli. We present here structural and thermodynamic characterization of a non-self-complementary DNA decamer duplex containing the dam sequence 5'-GATC in the unmethylated, hemimethylated (both forms), and methylated states. Differential scanning calorimetry measurements show that the free energies for adenine methylation of the decamer duplex are +1.1 and +2.0 kcal/mol for hemimethylation, respectively, and +3.3 kcal/mol for full methylation. In all cases, a large unfavorable enthalpy change is partially compensated by a favorable entropy term. CD spectroscopy indicates an overall conformational difference between the unmethylated decamer duplex and its methylated analogs. Reaction with diethyl pyrocarbonate (DEPC), a purine-specific probe sensitive to conformation, is enhanced in the vicinity of the methylation site of the duplex, consistent with loosening of base pairing at this site. Comparison of the scission patterns of these decamer duplexes by the reactive probes methidiumpropyl-EDTA.FeII [MPE.FeII] and CuI(o-phenanthroline)2 [(OP)2CuI] indicates that the methylation site of the decamer duplex represents a site of enhanced reactivity for these agents. On the basis of these thermodynamics and structural features, we suggest that the methylated base pair exists in two different helical states, which require local transient opening of the duplex for interconversion.

Haku T., Hibino T., Fukada H., Mishima Y., Yamashita I., and Kato M. (2006) DNA secondary structure forming at minisatellite repeat unit sequences. *Nucleic Acids Symp Ser (Oxf)* 229-230.
Abstract: The lengths of simple repeat sequences are generally unstable or polymorphic; that is, they are highly variable with respect to the numbers of tandem repeats. To determine the structural features that cause such variability, we examined a minisatellite DNA isolated from the yellow fin sea bream Acanthopagrus latus. Electrophoresis, CD spectra, and calorimetric analyses of oligodeoxyribonucleotides comprised of the minisatellite repeat unit suggest a stable secondary structure is formed within the repeat unit.

Harvey S. C. (1997) Slipped structures in DNA triplet repeat sequences: entropic contributions to genetic instabilities. *Biochemistry* **36,** 3047-3049.
Abstract: Slipped DNA structures can occur in sequences with direct repeats. DNA triplet repeats, particularly (CTG)n, (CGC)n, and (GAA)n, are known to be associated with several neurological diseases. Slippage is probably the cause of expansion of the number of repeats, a process called dynamic mutation, which is known to be the cause of the diseased state. Here it is shown that the conformational entropy

associated with slippage is more destabilizing for long direct repeats (300-1000 base pairs) than shorter runs (10-30 base pairs), by about 2 kcal/mol. This contributes to the greater instability of longer sequences. Entropic considerations also favor the formation of simple bulges, rather than hairpin structures. A model is presented for dynamic mutations, and experimentally testable predictions are made that will allow the model to be tested.

Hatters D. M., Wilson L., Atcliffe B. W., Mulhern T. D., Guzzo-Pernell N., and Howlett G. J. (2001) Sedimentation analysis of novel DNA structures formed by homo-oligonucleotides. *Biophys J* **81,** 371-381.
Abstract: Sedimentation velocity analysis has been used to examine the base-specific structural conformations and unusual hydrogen bonding patterns of model oligonucleotides. Homo-oligonucleotides composed of 8-28 residues of dA, dT, or dC nucleotides in 100 mM sodium phosphate, pH 7.4, at 20 degrees C behave as extended monomers. Comparison of experimentally determined sedimentation coefficients with theoretical values calculated for assumed helical structures show that dT and dC oligonucleotides are more compact than dA oligonucleotides. For dA oligonucleotides, the average width (1.7 nm), assuming a cylindrical model, is smaller than for control duplex DNA whereas the average rise per base (0.34 nm) is similar to that of B-DNA. For dC and dT oligonucleotides, there is an increase in the average widths (1.8 nm and 2.1 nm, respectively) whereas the average rise per base is smaller (0.28 nm and 0.23 nm, respectively). A significant shape change is observed for oligo dC(28) at lower temperatures (10 degrees C), corresponding to a fourfold decrease in axial ratio. Optical density, circular dichroism, and differential scanning calorimetry data confirm this shape change, attributable from nuclear magnetic resonance analysis to i-motif formation. Sedimentation equilibrium studies of oligo dG(8) and dG(16) reveal extensive self-association and the formation of G-quadruplexes. Continuous distribution analysis of sedimentation velocity data for oligo dG(16) identifies the presence of discrete dimers, tetramers, and dodecamers. These studies distinguish the conformational and colligative properties of the individual bases in DNA and their inherent capacity to promote specific folding pathways.

He J., Becher G., Budow S., and Seela F. (2003) Pyrazolo[3,4-d]pyrimidine nucleic acids: adjustment of the dA-dT to the dG-dC base pair stability. *Nucleosides Nucleotides Nucleic Acids* **22,** 573-576.
Abstract: The pyrazolo[3,4-d]pyrimidine-4,6-diamine nucleosides 2b-d stabilize the dA-dT base pair significantly when the dA-residue is replaced. Oligonucleotide duplexes incorporating 2b-d show a 4-6 degrees C Tm increase per modification. The 7-bromo compound 2b harmonizes the stability of the dA-dT vs. the dG-dC pair. According to this the stability of such duplexes depends no longer on the base pair composition of a DNA molecule.

He Y., Scaria P. V., and Shafer R. H. (1997) Studies on formation and stability of the d[G(AG)5]* d[G(AG)5]. d[C(TC)5] and d[G(TG)5]* d[G(AG)5]. d[C(TC)5] triple helices. *Biopolymers* **41,** 431-441.
Abstract: We have targeted the d[G(AG)5]. d[C(TC)5] duplex for triplex formation at neutral pH with either d[G(AG)5] or d[G(TG)5]. Using a combination of gel electrophoresis, uv and CD spectra, mixing and melting curves, along with DNase I digestion studies, we have investigated the stability of the 2:1 pur*pur.pyr triplex, d[G(AG)5]*d[G(AG)5].d[C(TC)5], in the presence of MgCl2. This triplex melts in a monophasic fashion at the same temperature as the underlying duplex. Although the uv spectrum changes little upon binding of the second purine strand, the CD spectrum shows significant changes in the wavelength range 200-230 nm and about a 7 nm shift in the positive band near 270 nm. In contrast, the 1:1:1 pur/pyr*pur.pyr triplex, d[G(TG)5]*d[G(AG)5].d[C(TC)5], is considerably less stable thermally, melting at a much lower temperature than the underlying duplex, and possesses a CD spectrum that is entirely negative from 200 to 300 nm. Ethidium bromide undergoes a strong fluorescence enhancement upon binding to each of these triplexes, and significantly stabilizes the pur/pyr*pur.pyr triplex. The uv melting and differential scanning calorimetry analysis of the alternating sequence duplex and pur*pur.pyr triplex shows that they are lower in thermodynamic stability than the corresponding 10-mer d(G3A4G3). d(C3T4C3) duplex and its pur*pur.pyr triplex under identical solution conditions.

Holbrook J. A., Capp M. W., Saecker R. M., and Record M. T., Jr. (1999) Enthalpy and heat capacity changes for formation of an oligomeric DNA duplex: interpretation in terms of coupled processes of formation and association of single-stranded helices. *Biochemistry* **38,** 8409-8422.
Abstract: The thermodynamics of self-assembly of a 14 base pair DNA double helix from complementary

strands have been investigated by titration (ITC) and differential scanning (DSC) calorimetry, in conjunction with van't Hoff analysis of UV thermal scans of individual strands. These studies demonstrate that thermodynamic characterization of the temperature-dependent contributions of coupled conformational equilibria in the individual "denatured" strands and in the duplex is essential to understand the origins of duplex stability and to derive stability prediction schemes of general applicability. ITC studies of strand association at 293 K and 120 mM Na+ yield an enthalpy change of -73 +/- 2 kcal (mol of duplex)-1. ITC studies between 282 and 312 K at 20, 50, and 120 mM Na+ show that the enthalpy of duplex formation is only weakly salt concentration-dependent but is very strongly temperature-dependent, decreasing approximately linearly with increasing temperature with a heat capacity change (282-312 K) of -1.3 +/- 0.1 kcal K-1 (mol of duplex)-1. From DSC denaturation studies in 120 mM Na+, we obtain an enthalpy of duplex formation of -120 +/- 5 kcal (mol of duplex)-1 and an estimate of the corresponding heat capacity change of -0.8 +/- 0.4 kcal K-1 (mol of duplex)-1 at the Tm of 339 K. van't Hoff analysis of UV thermal scans on the individual strands indicates that single helix formation is noncooperative with a temperature-independent enthalpy change of -5.5 +/- 0.5 kcal at 120 mM Na+. From these observed enthalpy and heat capacity changes, we obtain the corresponding thermodynamic quantities for two fundamental processes: (i) formation of single helices from disordered strands, involving only intrastrand (vertical) interactions between neighboring bases; and (ii) formation of double helices by association (docking) of single helical strands, involving interstrand (horizontal and vertical) interactions. At 293 K and 120 mM Na+, we calculate that the enthalpy change for association of single helical strands is approximately -64 kcal (mol of duplex)-1 as compared to -210 kcal (mol of duplex)-1 calculated for duplex formation from completely unstructured single strands and to the experimental ITC value of -73 kcal (mol of duplex)-1. The intrinsic heat capacity change for association of single helical strands to form the duplex is found to be small and positive [approximately 0.1 kcal K-1 (mol of duplex)-1], in agreement with the result of a surface area analysis, which also predicts an undetectably small heat capacity change for single helix formation.

Horton T. E., Maderia M., and DeRose V. J. (2000) Impact of phosphorothioate substitutions on the thermodynamic stability of an RNA GAAA tetraloop: an unexpected stabilization. *Biochemistry* **39,** 8201-8207.
Abstract: This study analyzes the impact of phosphorothioate substitutions on the thermodynamic stability of a 12-nt RNA hairpin containing a (5')GAAA(3') tetraloop. The thermodynamic consequences of stereospecific phosphorothioate substitutions 5' to each adenosine in the loop region are measured using optical melting and calorimetry experiments. Surprisingly, a single stereospecific phosphorothioate substitution 5' to the second adenosine of the tetraloop, R(p)-A7, results in a stabilization corresponding to a $\Delta(\Delta G(37)(degrees)(C))$ of approximately -2.9 kcal mol^{-1} (0.1 M NaCl) when compared with that of an unmodified sample. Five other phosphorothioate-substituted samples did not show significant thermodynamic differences in comparison with the unsubstituted samples. Addition of Mg(2+) to all of the hairpins studied results in increased t(m's) that are fit with a general electrostatic model to a dissociation constant of K(d)(Mg(2+)) approximately 2-3 mM (0.1 M NaCl). The R(p)-A7 phosphorothioate-substituted hairpin showed an unusual decrease in Tm and apparent increase in enthalpy of unfolding upon addition of Cd(2+). These results may impact the interpretation of interference mapping experiments that use phosphorothioate substitutions to characterize RNAs in solution.

Hoshika S., Minakawa N., and Matsuda A. (2004) Synthesis and physical and physiological properties of 4'-thioRNA: application to post-modification of RNA aptamer toward NF-kappaB. *Nucleic Acids Res* **32,** 3815-3825.
Abstract: We report herein full details of the preparation of 4'-thiouridine, -cytidine, -adenosine and -guanosine phosphoramidites based on our synthetic protocol via the Pummerer reaction. Fully modified 4'-thioRNAs containing four kinds of 4'-thioribonucleoside units were prepared according to the standard RNA synthesis. The T_m values and thermodynamic parameters of a series of duplexes were determined by UV melting and differential scanning calorimetry (DSC) measurements. The resulting overall order of thermal stabilities for the duplexes was 4'-thioRNA:4'-thioRNA >> 4'-thioRNA:RNA > RNA:RNA > RNA:DNA > 4'-thioRNA:DNA. In addition, it was shown that the dominant factor in the stability of the duplexes consisting of 4'-thioRNA was enthalpic in character. The CD spectra of not only 4'-thioRNA:RNA and 4'-thioRNA:4'-thioRNA but also 4'-thioRNA:DNA were all similar to those of duplexes in the A-conformation. The stability of 4'-thioRNA in human serum was 600 times greater than that of natural RNA. Neither the RNA:RNA nor the 4'-thioRNA:4'-thioRNA duplexes were digested under the

same conditions. The first example of a post-modification of an RNA aptamer by 4'-thioribonucleoside units was demonstrated. Full modification of the aptamer thioRNA3 resulted in complete loss of binding activity. In contrast, modifications at positions other than the binding site were tolerated without loss of binding activity. The post-modified RNA aptamer thioRNA5 was thermally stabilized and resistant toward nuclease digestion. The results presented in this paper will, it is hoped, contribute to the development of 4'-thioRNA as a new generation of artificial RNA.

Howard F. B., Miles H. T., and Ross P. D. (1995) The poly(dT).2poly(dA) triple helix. *Biochemistry* **34,** 7135-7144.
Abstract: A new homopolynucleotide triple helix, (dT)n.2(dA)n, detected by circular dichroism mixing curves, is the product of an endothermic reaction of (dA)n.(dT)n with (dA)n at moderate temperatures and high salt concentrations ([NaCl] > or = 2.6 M): (dA)n.(dT)n + (dA)n<-->(dT)n.2(dA)n. At higher temperatures (dT)n.2(DA)n forms from the (dA)n.(dT)n duplex alone: 3[(dA)n.(dT)n]<-->(dT)n.2(dA)n + (dA)n.2(dT)n. Upon further heating, (dT)n.2(dA)n is converted to the triplex (dA)n.2(dT)n: 2[(dT)n.2(dA)n]<-->(dA)n.2(dT)n + 3(dA)n. (dT)n.2(dA)n forms owing to a favorable entropy change; Δ Hm is unfavorable, ranging from 1 to 2.5 kcal mol-1, depending upon [NaCl]. The formation reaction is associated with a negative change in heat capacity. (dT)n.2(dA)n is an extremely weak complex with a free energy of stabilization, Δ Go < or = 100 cal mol-1. Tm values of ultraviolet, circular dichroism, and differential scanning calorimetry transition curves for the formation of (dT)n.2(dA)n decrease with increasing [NaCl], reflecting, in part, the net uptake of cations. The values of (dTm/d ln a +/-)(Δ Hm/RTm2) can be accounted for in terms of the charge spacing and cylindrical dimensions of the polynucleotides. The ionic strength dependence of this quantity is consistent with interaction of anions with (dAn). High concentrations of the anions Cl-, Br-, and ClO4- decrease the stability of (dT)n.2(dA)n according to the lyotropic series. The highly polarizable anion, ClO4-, entirely prevents the formation of (dT)n.2(dA)n. Phase diagrams of the (dA)n,(dT)n system in solutions of NaCl, NaBr, and NaClO4 are presented. A bonding scheme for (dT)n.2(dA)n is proposed, and implications of this work for Py.Pu.Pu triple helices are discussed.

Hu X., Tierney M. T., and Grinstaff M. W. (2002) Synthesis and characterization of phenothiazine labeled oligodeoxynucleotides: novel 2'-deoxyadenosine and thymidine probes for labeling DNA. *Bioconjug Chem* **13,** 83-89.
Abstract: A facile procedure for the incorporation of phenothiazine at the terminus of oligodeoxynucleotides is reported. Phenothiazine is covalently linked to the 5'-position of 2'-deoxyadenosine and thymidine. Next, the corresponding phosphoramidites are prepared, and then the labeled nucleosides are incorporated in DNA using an automated DNA solid-phase synthesizer. Phenothiazine labeled oligodeoxynucleotides form stable B-form duplexes with similar melting temperatures, CD spectra, and DSC traces compared to unlabeled DNA duplexes. The favorable photophysical properties of phenothiazine are also retained after covalent attachment to the oligodeoxynucleotide.

Hughesman C. B., Turner R. F. and Haynes C. (2008) Stability and mismatch discrimination of DNA duplexes containing 2,6-diaminopurine and 2-thiothymidine locked nucleic acid bases. *Nucleic Acids Symp. Ser. (Oxf)* 245-246.
Abstract: Hybridization thermodynamics measured by differential scanning calorimetry (DSC) and UV spectroscopy (UVM) are reported for 8- and 14-mer oligonucleotides containing two new LNA bases: 2,6 diaminopurine (D) and 2-thiothymidine (2sT). Oligonucleotides containing D or 2sT bases are shown to have enhanced stability and improved discrimination for several of the possible mismatched base pairs

Husler P. L. and Klump H. H. (1994) Unfolding of a branched double-helical DNA three-way junction with triple-helical ends. *Arch Biochem Biophys* **313,** 29-38.
Abstract: We have designed three oligonucleotides (33 mers) which when mixed in a 1:1:1 ratio form double-helical DNA three-way junctions with triple helical ends in the pH interval pH 4 to 5.5. The triplex to coil transition is initiated by raising the temperature and was recorded by temperature gradient gel electrophoresis, uv melting, and differential scanning calorimetry. The transitions can be deconvoluted into three subtransitions representing the independent thermal denaturation of each of the arms. We have

proposed a model for the unfolding pathway and give the thermodynamic parameters for each step as calculated using the formalism outlined in the appendix.

Husler P. L. and Klump H. H. (1995) Prediction of pH-dependent properties of DNA triple helices. *Arch Biochem Biophys* **317,** 46-56.
Abstract: The thermodynamic properties of two triple helices were investigated by uv thermal denaturation, differential scanning calorimetry, and pH titrations. Starting from the grand partition function and using matrix methods we present a formalism that describes pH effects on the thermal stability of triple helices. The formalism can be used over a wide pH range and is not restricted to the limiting case where the pH is larger or smaller than the pK alpha of cytosine. Furthermore, it covers nearest neighbor electrostatic effects of closely spaced cytosines in the Hoogsteen strand which can shift the pK alpha of cytosine to lower pH values. A procedure is employed to predict enthalpy and entropy changes for triplex formation. These values are in accordance with the results obtained by differential scanning calorimetry.

Husler P. L. and Klump H. H. (1995) Thermodynamic characterization of a triple-helical three-way junction containing a Hoogsteen branch point. *Arch Biochem Biophys* **322,** 149-166.
Abstract: We have designed a Hoogsteen (HG) triple-helical three-way junction (ternary complex) constructed from three 33-mer oligonucleotides based on the same subset of sequences used for the Watson-Crick (WC) triple-helical three-way junction, characterized previously (P. L. Husler and H. H. Klump (1994) Arch. Biochem. Biophys., 313, 29-38). The junction differs primarily in the assembly of the branch point and the ends of the arms. The three oligonucleotides can each fold into a WC hairpin, linked by a four-member cytosine loop, each containing a homo-pyrimidine 10-mer single-strand extension. On lowering the pH (between 6 and 4), the extensions mutually associate to one of the other hairpins via Hoogsteen (HG) hydrogen bonding. Collectively, this process results in the formation of the branch point and the triple-helical arms. The HG triple-helical three-way junction is characterized by gel electrophoresis, circular dichroism, uv melting, and differential scanning calorimetry. The junction undergoes thermal unfolding in two distinct temperature regions. In the temperature range 15 to 50 degrees C loss of HG base pairing results in the dissociation of the three-way junction. Between 55 and 95 degrees C the resulting hairpins undergo further successive unfolding. The overall calorimetric unfolding enthalpy and entropy changes associated with the loss of HG base pairing are approximately equal to the sum of the enthalpy and entropy changes associated with the dissociation of the HG base pairing in the isolated arms (170.6 kcal.mol-1; 540.1 cal.mol-1.K-1). It is apparent from these results that in the proximity of the branch point the structure is not perturb or strain. This result is contrary to the results obtained for the WC triple-helical three-way and for three-way junctions constructed from canonical double-helical DNA. Complete folding of the junction requires either high Na+ (600 mM) ion concentrations or 40-60 mM Mg2+.

Jelesarov I., Crane-Robinson C., and Privalov P. L. (1999) The energetics of HMG box interactions with DNA: thermodynamic description of the target DNA duplexes. *J Mol Biol* **294,** 981-995.
Abstract: The thermal properties and energetics of formation of 10, 12 and 16 bp DNA duplexes, specifically interacting with the HMG box of Sox-5, have been studied by isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). DSC studies show that the partial heat capacity of these short duplexes increases considerably prior to the cooperative process of strand separation. Direct extrapolation of the pre and post-transition heat capacity functions into the cooperative transition zone suggests that unfolding/dissociation of strands results in no apparent heat capacity increment. In contrast, ITC measurements show that the negative enthalpy of complementary strand association increases in magnitude with temperature rise, implying that strand association proceeds with significant decrease of heat capacity. Furthermore, the ITC-measured enthalpy of strand association is significantly smaller in magnitude than the enthalpy of cooperative unfolding measured by DSC. To resolve this paradox, the heat effects upon heating and cooling of the separate DNA strands have been measured by DSC. This showed that cooling of the strands from 100 degrees C to -10 degrees C proceeds with significant heat release associated with the formation of intra and inter-molecular interactions. When the enthalpy of residual structure in the strands and the temperature dependence of the heat capacity of the duplexes and of their unfolded strands have been taken into account, the ITC and DSC results are brought into agreement. The analysis shows that the considerable increase in heat capacity of the duplexes with temperature rise is due to increasing fluctuations of their structure (e.g. end fraying and twisting) and this effect obscures the heat capacity increment resulting from the cooperative separation of strands, which in fact amounts to 200(+/-

40) JK^{-1} (mol bp)$^{-1}$. Using this heat capacity increment, the averaged standard enthalpy, entropy and Gibbs energy of formation of fully folded duplexes from fully unfolded strands have been determined at 25 degrees C as -33(+/-2) kJ (mol bp)$^{-1}$, -93(+/-4) J K^{-1} (mol bp)$^{-1}$ and -5.0(+/-0.5) kJ (mol bp)$^{-1}$, respectively.

Kankia B. I. and Marky L. A. (2001) Folding of the thrombin aptamer into a G-quadruplex with Sr(2+): stability, heat, and hydration. *J Am Chem Soc* **123,** 10799-10804.
Abstract: It has been shown that the DNA aptamer d(G(2)T(2)G(2)TGTG(2)T(2)G(2)) adopts an intramolecular G-quadruplex structure in the presence of K+. Its affinity for trombin has been associated with the inhibition of thrombin-catalyzed fibrin clot formation. In this work, we used a combination of spectroscopy, calorimetry, density, and ultrasound techniques to determine the spectral characteristics, thermodynamics, and hydration effects for the formation of G-quadruplexes with a variety of monovalent and divalent metal ions. The formation of cation-aptamer complexes is relatively fast and highly reproducible. The comparison of their CD spectra and melting profiles as a function of strand concentration shows that K+, Rb+, NH(4)+, Sr(2+), and Ba(2+) form intramolecular cation-aptamer complexes with transition temperatures above 25 degrees C. However, the cations Li+, Na+, Cs+, Mg(2+), and Ca(2+) form weaker complexes at very low temperatures. This is consistent with the observation that metal ions with ionic radii in the range 1.3-1.5 A fit well within the two G-quartets of the complex, while the other cations cannot. The comparison of thermodynamic unfolding profiles of the Sr(2+)-aptamer and K+ -aptamer complexes shows that the Sr(2+)-aptamer complex is more stable, by approximately 18 degrees C, and unfolds with a lower endothermic heat of 8.3 kcal/mol. This is in excellent agreement with the exothermic heats of -16.8 kcal/mol and -25.7 kcal/mol for the binding of Sr(2+) and K+ to the aptamer, respectively. Furthermore, volume and compressibility parameters of cation binding show hydration effects resulting mainly from two contributions: the dehydration of both cation and guanine atomic groups and water uptake upon the folding of a single-strand into a G- quadruplex structure.

Katayose S. and Kataoka K. (1997) Water-soluble polyion complex associates of DNA and poly(ethylene glycol)-poly(L-lysine) block copolymer. *Bioconjug Chem* **8,** 702-707.
Abstract: Complex formation of poly(ethylene glycol)-poly(L-lysine) (PEG-PLL) AB type block copolymer with salmon testes DNA or Col E1 plasmid DNA in aqueous milieu was studied. The PLL segment of PEG-PLL interacts with nucleic acid through an electrostatic force to form a water-soluble complex associate with a diameter of ca. 50 nm. PEG segments surrounding the core of the polyion complex prevented the complex from precipitation even under stoichiometric conditions, at which the unit ratio of L-lysine in PEG-PLL and phosphate in the DNA is equal. The profile of the thermal melting curve revealed a higher stabilization of DNA structure in PEG-PLL/DNA complexes compared to that in the complex made from DNA and PLL homopolymer with the same molecular weight as the PLL segment in PEG-PLL. This stabilizing effect on the DNA structure may be due to the compartmentalization of DNA into the microenvironment of PEG with low permittivity. The reversible nature of the PEG-PLL/DNA complex was further verified through the addition of polyanion [poly-(L-aspartic acid)]: Poly(L-aspartic acid) replaced DNA in the complex with PEG-PLL, resulting in the release of free DNA in the medium. Furthermore, the PEG-PLL/DNA complex showed high resistance against DNase I attack, suggesting DNA protection through the segregation into the core of the associate having PEG palisade.

Kato M., Haku T., Hibino T., Fukada H., Mishima Y., Yamashita I., Minoshima S., Nagayama K. and Shimizu N. (2007) Stable minihairpin structures forming at minisatellite DNA isolated from yellow fin sea bream Acanthopagrus latus. *Comp Biochem Physiol B Biochem Mol Biol* **146,** 427-437.
Abstract: The lengths of simple repeat sequences are generally unstable or polymorphic (highly variable with respect to the numbers of tandem repeats). Previously we have isolated a family of minisatellite DNA (GenBank accession AF422186) that appears specifically and abundantly in the genome of yellow fin sea bream Acanthopagrus latus but not in closely-related red sea bream Pagrus major, and found that the numbers of tandem arrays in the homologous loci are polymorphic. This means that the minisatellite sequence has appeared and propagated in A. latus genome after speciation. In order to understand what makes the minisatellite widespread within the A. latus genome and what causes the polymorphic nature of the number of tandem repeats, the structural features of single-stranded polynucleotides were analyzed by electrophoresis, chemical modification, circular dichroism (CD), differential scanning calorimetry (DSC) and electron microscopy. The results suggest that a portion of the repeat unit forms a stable minihairpin structure, and it can cause polymerase pausing within the minisatellite DNA.

Kaur H., Arora A., Wengel J., and Maiti S. (2006) Thermodynamic, counterion, and hydration effects for the incorporation of locked nucleic acid nucleotides into DNA duplexes. *Biochemistry* **45,** 7347-7355.
Abstract: A locked nucleic acid (LNA) monomer is a conformationally restricted nucleotide analogue with an extra 2'-O, 4'-C-methylene bridge added to the ribose ring. LNA-modified oligonucleotides are known to exhibit enhanced hybridization affinity toward complementary DNA and RNA. In this work, we have evaluated the hybridization thermodynamics of a series of LNA-substituted DNA octamers, modified to various extents by one to three LNA substitutions, introduced at either adenine (5'-AGCACCAG) or thymine (5'-TGCTCCTG) nucleotides. To understand the energetics, counterion effects, and the hydration contribution of the incorporation of LNA modification, a combination of spectroscopic and calorimetric techniques was used. The CD spectra of the corresponding duplexes showed that the modified duplexes adopt an A-type conformation. UV and DSC melting studies revealed that each type of duplex unfolds in a two-state transition. A complete thermodynamic profile at 5 degrees C indicated that the net effect of modification on thermodynamic parameters might be positional and that the neighboring bases flanking the modification might influence the favorable formation of the modified duplexes. Furthermore, relative to the formation of the unmodified reference duplexes, the formation of modified duplexes is accompanied by a higher uptake of counterions and a lower uptake of water molecules.

Kaur H., Wengel J. and Maiti S. (2008) Thermodynamics of DNA-RNA heteroduplex formation: effects of locked nucleic acid nucleotides incorporated into the DNA strand. *Biochemistry* **47,** 1218-1227.
Abstract: A locked nucleic acid (LNA) monomer is a conformationally restricted nucleotide analogue exhibiting enhanced hybridization efficiency toward complementary strand. The potential of LNA-based oligonucleotides has been sought to improve the selectivity and specificity of probe sets employed in detection and specific targeting of nucleic acids. We have evaluated the influence of "locked nucleic acid" residues on hybridization thermodynamics, counterions and hydration of DNA.RNA heteroduplex using spectroscopic and calorimetric techniques. One to three LNA substitutions have been introduced either at the adenine (5'-AGCACCAG) or thymine (5'-TGCTCCTG) residues of the DNA strand. A complete thermodynamic profile for heteroduplex formation suggested that LNA-induced stabilization results from a favorable increase in the enthalpy of hybridization that compensates for the unfavorable entropy change. Analysis of differential scanning calorimetry data indicated a nonzero heat capacity change, DeltaCp, accompanying the heteroduplex formation. Isothermal titration calorimetry measurements indicated an increase in binding affinity of the two strands as the LNA content of the heteroduplex is increased. Overall our result demonstrated that the effect of LNA-substitution at the thymine residue is more pronounced compared to the adenine residue. Furthermore, optical melting studies showed that, compared to an unmodified duplex, the formation of LNA-modified duplex is accompanied by a higher uptake of counterions and a lower uptake of water molecules. Our result, thus, presents a preliminary attempt toward the characterization of hybridization thermodynamics of the LNA-based probe-target sets, which will in turn aid in the selection of optimal conditions for hybridization experiments, and evaluation of the minimum probe-length required for hybridization and cloning experiments

Kaur H., Arora A., Wengel J. and Maiti S. (2008) Thermodynamic, counterion and hydration effects for the incorporation of locked nucleic acid (LNA) nucleotides in duplex. *Nucleic Acids Symp. Ser. (Oxf)* 425-426.
Abstract: A Locked Nucleic Acid (LNA) monomer is a conformationally restricted nucleotide analogue with an extra 2'-O, 4'-C-methylene bridge added to the ribose ring that is known to exhibit enhanced hybridization affinity towards complementary DNA and RNA, however the underlying thermodynamic basis for this observation are poorly understood. We have evaluated the influence of LNA residues on hybridization thermodynamics, counterions and hydration of DNA*DNA and DNA*RNA heteroduplex using spectroscopic and calorimetric techniques. Thermodynamic analysis for duplex formation using UV and differential scanning calorimetry suggested that LNA-induced stabilization results from a large, favorable increase in the enthalpy of hybridization that compensates for the unfavorable entropy change. The heat capacity change, Delta C(p), accompanying the duplex formation, obtained through DSC, has also been reported and has been used to furnish thermodynamic parameters at 37 degrees C. Furthermore, it was observed that relative to the formation of unmodified duplex, the formation of LNA-modified duplexes was accompanied by a higher uptake of counterions and a lower uptake of water molecules

Kaushik M., Suehl N. and Marky L. A. (2007) Calorimetric unfolding of the bimolecular and i-motif complexes of the human telomere complementary strand, d(C(3)TA(2))(4). *Biophys Chem* **126,** 154-164.

Abstract: A combination of spectroscopic and calorimetric techniques is used to determine the unfolding thermodynamics of the complexes formed by the complementary sequence of the human telomere, d(C(3)TA(2))(4), in the pH range of 4.2 to 6. Calorimetric melting curves show biphasic transitions; both transitions are shifted to higher temperatures as the pH is decreased, indicative of cytosine protonation, which favors the formation of C*C(+) base pairs. Furthermore, the transition temperature, T(M), of the lower transition depends on strand concentration, while the T(M) of the higher transition is independent of strand concentration, indicating the following sequential melting: bimolecular complex(s)-->intramolecular complex-->random coil. The thermodynamic profiles for the formation of each complex, bimolecular and i-motif reveals small favorable free energy terms resulting from favorable enthalpy-unfavorable entropy compensations, uptake of protons, marginal uptake of counterions (i-motif) and marginal release of water molecules (i-motif). Furthermore, an enthalpy of 3.2 kcal/mol (bimolecular complex) and 5.0 kcal/mol (i-motif) is estimated for a single C*C(+)/C*C(+) base-pair stack.

Kawashima T., Sasaki A., and Sasaki S. (2006) Transition of nanostructure in DNA-cationic surfactant complexes with the added salt. *Biomacromolecules* **7,** 1942-1950.
Abstract: Nanostructures of complexes of DNA with single-chain surfactant of octadecyltrimethylammonium (OTA) and double-chain surfactant of didodecyldimethylammonium (DDA) in aqueous NaCl solution at concentration, Cs, from 0 to 500 mM were studied using small-angle-scattering techniques (SAXS). SAXS profiles of the DNA-OTA complex show two SAXS peaks with a spacing ratio of 1:3(1/2) in the solution at Cs below 150 mM and three peaks with a spacing ratio of 1:3(1/2):4(1/2) at Cs above 250 mM. Contents of Na+ and Cl- ions in the complexes evaluated from the atomic absorbance for Na+ and the potentiometry for Cl- revealed charge molar ratios of OTA/DNA = 1 and DDA/DNA = 1.25. Contents of Na+ and Cl- ions per ionic unit of DNA molecule in the DNA-OTA complex equilibrating with the solution at Cs below 100 mM were much less than 0.1, while they increased with NaCl concentration at Cs above 200 mM. The DNA-OTA complex in the solution at Cs above 260 mM exhibited an endothermic peak in the DSC measurements, and the others did not. On the basis of the experimental results, the salt concentration dependent nanostructures are discussed.

Kornilova S. V., Iasem P., Grigor'ev D. N., Kapinos L. E., Totova I., and Blagoi I. (1997) [Calorimetric study of Ca2+ and Mn2+ ions' effect on DNA helix-coil transition]. *Biofizika* **42,** 599-606.
Abstract: Using the method of differential scanning calorimetry, the DNA helix-coil transition studied in solutions (10^{-3} M Na+, 10^{-3}M tris HCl, pH 7.0) containing divalent metal ions (Mn2+ and Ca2+) at relative metal ion concentrations (Me/PDNA) ranging from 0.2 to 20. Dependences of the melting temperature and enthalpy on the ion relative concentration were stated. The fine structure of melting curves in DNA+Me complexes was observed. The resolution degree of this structure was shown to increase in the range of moderate ion concentrations. The data obtained were compared with values determined by UV-spectroscopy. Association constants were defined for Mn2+ and Ca2+ binding to DNA by the ligand theory.

Kornilova S. V., Yasem P., Grigor'ev D. N., Kapinos L. E., Totova I., Blagoi I., and Galkin V. L. (1998) [Calorimetric study of the helix-coil transition in DNA during interaction with Cu2+ ions]. *Biofizika* **43,** 46-52.
Abstract: Using the method of differential scanning calorimetry, the DNA helix-coil transition was studied in solutions (10^{-3} M Na+, 10^{-3} M tris HCl, pH 7.0) containing divalent copper ions at relative metal ion concentrations (Mt2+/PDNA) ranging from 0.2 to 20. Dependences of the melting temperature and enthalpy on relative ion concentration were determined. An aggregation of Cu(2+) + DNA complexes in the range of average ion concentration was established. It is shown that the melting enthalpy of "units" increases with copper ion concentration. The data obtained were compared with values determined by UV-spectroscopy. Association constants for Cu2+ binding to DNA were defined by the ligand theory.

Korolev N., Lyubartsev A. P., and Nordenskiold L. (1998) Application of polyelectrolyte theories for analysis of DNA melting in the presence of Na+ and Mg2+ ions. *Biophys J* **75,** 3041-3056.
Abstract: Numerical calculations, using Poisson-Boltzmann (PB) and counterion condensation (CC) polyelectrolyte theories, of the electrostatic free energy difference, ΔGel, between single-stranded (coil) and double-helical DNA have been performed for solutions of NaDNA + NaCl with and without added MgCl2. Calculations have been made for conditions relevant to systems where experimental values of helix

coil transition temperature (Tm) and other thermodynamic quantities have been measured. Comparison with experimental data has been possible by invoking values of Tm for solutions containing NaCl salt only. Resulting theoretical values of enthalpy, entropy, and heat capacity (for NaCl salt-containing solutions) and of Tm as a function of NaCl concentration in NaCl + MgCl2 solutions have thus been obtained. Qualitative and, to a large extent, quantitative reproduction of the experimental Tm, ΔHm, ΔSm, and ΔCp values have been found from the results of polyelectrolyte theories. However, the quantitative resemblance of experimental data is considerably better for PB theory as compared to the CC model. Furthermore, some rather implausible qualitative conclusions are obtained within the CC results for DNA melting in NaCl + MgCl2 solutions. Our results argue in favor of the Poisson-Boltzmann theory, as compared to the counterion condensation theory.

Korolev N., Lyubartsev A. P., and Nordenskiold L. (2002) Application of the Poisson Boltzmann polyelectrolyte model for analysis of equilibria between single-, double-, and triple-stranded polynucleotides in the presence of K(+), Na(+), and Mg(2+) ions. *J Biomol Struct Dyn* **20,** 275-290.
Abstract: The Poisson Boltzmann (PB) cell model of polyelectrolyte solution has been used for numerical calculations of the change in electrostatic free energy, ΔG(el), for transformations between different structural forms (tri-, double-, and single-stranded) of the polyribonucleotides poly(rA).poly(rU)2, poly(rA).poly(rU), poly(rA), and poly(rU). In particular, the dependence on monovalent salt concentration, MCl (M = Na or K) in the absence and in the presence of MgCl(2) has been calculated. The results were obtained for conditions relevant to available experimental values of structural transition ("melting") temperatures (Tm) and other thermodynamic quantities. Using the experimental Tm values and theoretical electrostatic ΔG(el), ΔH(el), and ΔS(el) functions, non-electrostatic contributions to the corresponding thermodynamic parameters of the poly(rA)/poly(rU) melting transitions were determined in MCl solutions in the absence of Mg(2+). Qualitative and to a large extent quantitative reproduction of the experimental calorimetry enthalpy, entropy and heat capacity values was found from the results of the PB theory. Furthermore, dependencies of Tm on MCl concentration in the presence of MgCl(2) for the poly(rA)/poly(rU) transitions were also calculated. Compared to a model considering Mg(2+) as a fully-hydrated ion, much better agreement between experimental and PB theory was achieved by assuming the ion size of Mg(2+) to be given by that of a bare non-hydrated ion smaller than that of hydrated Na(+) or K(+). In agreement with data of experimental studies reported in literature, this result indicates that magnesium(II) can bind to RNA as a bare ion in a way that is different from that of DNA. Generally, we can conclude that the PB polyelectrolyte theory can provide an adequate description of thermally induced structural transitions in polydeoxy- and polyribonucleotides in different salt solutions in spite of the rather simplified model treating the solvent as dielectric continuum, the polyion as a uniformly charged cylinder, and the mobile ions as hard spheres in the absence of excluded volume effects.

Korolev N. I., Vlasov A. P., and Kuznetsov I. A. (1994) Thermal denaturation of Na- and Li-DNA in salt-free solutions. *Biopolymers* **34,** 1275-1290.
Abstract: Thermal denaturation of Na- and Li-DNA from chicken erythrocytes was studied by means of scanning microcalorimetry in salt-free solutions at DNA concentrations (Cp) from 4.5 x 10^{-2} to 1 x 10^{-3} moles of nucleotides/liter (M). Linear dependencies of DNA melting temperature (Tm) vs lgCp were obtained: [formula: see text] for Na- and Li-DNA, respectively. Microcalorimetry data were compared with the results of spectrophotometric studies at 260 nm of DNA thermal denaturation in Me-DNA + MeCl solutions at Cp congruent to (6-8) x 10^{-5} M and Cs = 0-40 mM (Me is Na or Li, Cs is salt concentration). It was found that Eqs. (1) and (2) are valid in DNA salt-free solutions over the Cp range 6 x 10^{-5}-4.5 x 10^{-2} M. Protonation of DNA bases due to the absorption of CO2 from air in Na-DNA + NaCl solutions affects DNA melting parameters at Cs < 4 mM. Linear dependence of Tm on lga+ is found in Na-DNA + NaCl at Cs > 0.4 mM in the absence of contact of solutions with CO2 from air (a+ is cation activity). A dependence of [dTm/dlga+] on Li+ activity was observed in Li-DNA + LiCl solutions at Cs < 10 mM: [dTm/dlga+] increases from 17 degrees-18 degrees at Cs > 10 mM to 28 degrees-30 degrees at Cs congruent to 0.2-0.4 mM. Spectrophotometric measurements at 282 nm show that this effect was caused by protonation of bases in fragments of denatured DNA in neutral solutions. The Poisson-Boltzmann (PB) equation was solved for salt-free DNA at the melting point. The linear dependence of Tm vs lgCp was interpreted in terms of Manning's condensation theory. PB and Manning's theories fit the experimental data if charge density parameter (xi) of denatured DNA is in the range 1.8-2.1 (assuming for native DNA xi = 4.2). Specificity of Li ions in interactions with DNA is discussed.

Kulinski T., Bratek-Wiewiorowska M. D., Wiewiorowski M., Zielenkiewicz A., Zolkiewski M., and Zielenkiewicz W. (1991) Comparative calorimetric studies on the dynamic conformation of plant 5S rRNA: II. Structural interpretation of the thermal unfolding patterns for lupin seeds and wheat germ. *Nucleic Acids Res* **19,** 2449-2455.
Abstract: Thermal unfolding of 5S rRNA from wheat germ (WG) and lupin seeds (LS) was studied in solution. Experimental curves of differential scanning calorimetry (DSC) were resolved into particular components according to the thermodynamic model of two-state transitions. The DSC temperature profiles for WG and LS differ significantly in spite of very high similarities in the sequence of both molecules. Those results are interpreted according to a model of the secondary and tertiary molecular structure of 5S rRNA. A comparison of the 'nearest neighbour' model of interaction with the experimental thermodynamic results enables a complete interpretation of the process of the melting of its structures. In light of our observations, the crucial differences between both DSC melting profiles are mainly an outcome of different thermodynamic properties of the first helical fragment 'A' made up of 9 complementary base pairs. It contains 6 differences in the nucleotide sequence of both types of molecules, which still retain 9-meric double helixes. The temperature stability of his helix in WG is much lower than of the LS one. Moreover, the results supply evidence for a strong specific tertiary interaction between the two hairpin loops 'c' and 'e' in both 5S rRNA molecules, modulated by small differences in the thermodynamic properties of both 5S rRNA.

Kulinski T., Bratek-Wiewiorowska M. D., Zielenkiewicz A., and Zielenkiewicz W. (1997) Mg2+ dependence of the structure and thermodynamics of wheat germ and lupin seeds 5S rRNA. *J Biomol Struct Dyn* **14,** 495-507.
Abstract: The formation and stability of structural elements in two 5S rRNA molecules from wheat germ (WG) and lupin seeds (LS) as a function of Mg2+ concentration in solution was determined using the adiabatic differential scanning microcalorimetry (DSC). The experimentally determined thermodynamic parameters are compared with calculations using thermodynamic databases used for prediction of RNA structure. The 5S rRNA molecules which show minor differences in the nucleotide sequence display very different thermal unfolding profiles (DSC profiles). Numerical deconvolution of DSC profiles provided information about structural transformations that take place in both 5S rRNA molecules. A comparative analysis of DSC data and the theoretical thermodynamic models of the structure was used to establish a relationship between the constituting transitions found in the melting profiles and the unfolding of structural domains of the 5S rRNA and stability of its particular helical elements. Increased concentrations of Mg2+ ions induces additional internal interactions stabilising 5S rRNA structures found at low Na+ concentrations. Observed conformational transitions suggest a structural model in which the extension of helical region E dominates over the postulated tertiary interaction between hairpin loops. We propose that helix E is stabilised by a sequence of non-standard pairings extending this helix by the formation of tetra loop e and an almost total reduction of loop d between helices E and D. Two hairpin structures in both 5S rRNA molecules: the extended C-C' and the extended E-E'-E" hairpins appear as the most stable elements of the structure. The cooperativity of the unfolding of helixes in these 5S rRNA molecules changes already at 2 mM Mg2+.

Kumar N. and Maiti S. (2007) Role of locked nucleic acid modified complementary strand in quadruplex/Watson-Crick duplex equilibrium. *J Phys. Chem B* **111,** 12328-12337.
Abstract: In the human genome, the G-rich sequences that form quadruplexes are present along with their C-rich complementary strands; this suggests the existence of equilibrium between a quadruplex and a Watson-Crick duplex which allows the execution of their respective biological functions. We have investigated the sensitivity of this equilibrium to pharmacological agents by employing locked nucleic acid (LNA) modified complementary strands, and demonstrated successful invasion of the stable telomeric quadruplex d[(G(3)TTA)(3)G(3)]. Fluorescence, UV, ITC, and SPR studies were performed to understand the binding process involving the preformed quadruplex and LNA-modified complementary strands compared with that involving the unmodified complementary strand. Our data indicate that LNA modifications in the complementary strand shift the equilibrium toward the duplex state. These modifications confer increased thermodynamic stability to the duplex and increase the magnitude of relative free energy (DeltaDeltaG degrees) difference between duplex and quadruplex, thus favoring the predominance of duplex population over quadruplex. This superior ability of LNA-modified complementary strand can be exploited to pave an exploratory approach in which it hybridizes to a

telomeric quadruplex and drives duplex formation, and inhibits the recognition of 3' G-rich overhang by RNA template of telomerase which guides telomere extension.

Kumar N. and Maiti S. (2008) A thermodynamic overview of naturally occurring intramolecular DNA quadruplexes. *Nucleic Acids Res* **36,** 5610-5622.
Abstract: Loop length and its composition are important for the structural and functional versatility of quadruplexes. To date studies on the loops have mainly concerned model sequences compared with naturally occurring quadruplex sequences which have diverse loop lengths and compositions. Herein, we have characterized 36 quadruplex-forming sequences from the promoter regions of various proto-oncogenes using CD, UV and native gel electrophoresis. We examined folding topologies and determined the thermodynamic profile for quadruplexes varying in total loop length (5-18 bases) and composition. We found that naturally occurring quadruplexes have variable thermodynamic stabilities (DeltaG(37)) ranging from -1.7 to -15.6 kcal/mol. Overall, our results suggest that both loop length and its composition affect quadruplex structure and thermodynamics, thus making it difficult to draw generalized correlations between loop length and thermodynamic stability. Additionally, we compared the thermodynamic stability of quadruplexes and their respective duplexes to understand quadruplex-duplex competition. Our findings invoke a discussion on whether biological function is associated with quadruplexes with lower thermodynamic stability which undergo facile formation and disruption, or by quadruplexes with high thermodynamic stability

Kuo J. H., Lo Y. L., Shau M. D., and Cherng J. Y. (2002) A thermodynamic study of cationic polymer-plasmid DNA complexes by highly-sensitive differential scanning calorimetry. *J Control Release* **81,** 321-325.
Abstract: The characteristics of polymer-DNA complexes formed by positively-negatively charged interaction have a great influence on their transfection potential. Since the limit changes in thermal transitions which were hardly measured in conventional calorimetry, now in this study they have been successfully carried out by highly-sensitive differential scanning calorimetry for better understanding the pDMAEMA-plasmid DNA complexing process. Thermal behaviors of plasmid DNA, polymer and their formed complexes were recorded to give insights into their conformational changes when temperature was raised. In results, the supercoiled or open-circular plasmid DNA is not thermal reversible indicated by the decrease of denaturation peak and disappearance of DNA conformational transition related to its twist status at 50-70 degrees C. The cationic polymer is thermally stable by showing reversible transition peaks after two heating processes. For the cationic polymer-plasmid DNA complexes, electrostatic forces lead to a higher denaturation temperature of plasmid DNA and transition temperature of polymer. Also, heat can cause a topological change in plasmid DNA and then change their mutual complexation capacity.

Laing L. G. and Draper D. E. (1994) Thermodynamics of RNA folding in a conserved ribosomal RNA domain. *J Mol Biol* **237,** 560-576.
Abstract: A small, 58 nt domain of the large subunit ribosomal RNA (Escherichia coli sequence 1051 to 1108) is a highly conserved junction of three helices whose secondary structure has been established by phylogenetic comparisons. To detect any contributions of additional tertiary interactions, the thermal denaturation of the rRNA domain was followed by either UV hyperchromicity or calorimetry in buffers containing a wide range of Mg2+ concentrations. Several smaller fragments corresponding to two different hairpin stem-loop structures within the domain were also synthesized and melted for comparison with the larger molecule. A model of the secondary structure unfolding was devised, based on measured enthalpies and melting temperatures of the component hairpins and tabulated parameters of base-pair stacking and loop closure. The model closely simulates the observed melting data when three additional factors are included: two parameters to account for coaxial stackings within a junction of helices, and a set of undefined "tertiary" interactions that unfolds before the secondary structure and is preferentially stabilized by Mg2+. A critical feature of this model is a conserved pair, U1082/A1086, that is within the junction loop and hypothesized to stack with an adjacent helix. The model correctly predicts the effects of disrupting this pair in a U1086 sequence variant. Although the set of "tertiary" interactions contributes a significant fraction of the RNA unfolding enthalpy (Δ H approximately 25 kcal/mol, out of 180 kcal/mol total), its overall stability is marginal at 37 degrees C.

Lang B. E. and Schwarz F. P. (2007) Thermodynamic dependence of DNA/DNA and DNA/RNA hybridization reactions on temperature and ionic strength. *Biophys Chem* **131,** 96-104.
Abstract: The thermodynamics of 5'-ATGCTGATGC-3' binding to its complementary DNA and RNA strands was determined in sodium phosphate buffer under varying conditions of temperature and salt concentration from isothermal titration calorimetry (ITC). The Gibbs free energy change, DeltaG degrees of the DNA hybridization reactions increased by about 6 kJ mol(-1) from 20 degrees C to 37 degrees C and exhibited heat capacity changes of -1.42 +/- 0.09 kJ mol(-1) K(-1) for DNA/DNA and -0.87 +/- 0.05 kJ mol(-1) K(-1) for DNA/RNA. Values of DeltaG degrees decreased non-linearly by 3.5 kJ mol(-1) at 25 degrees C and 6.0 kJ mol(-1) at 37 degrees C with increase in the log of the sodium chloride concentration from 0.10 M to 1.0 M. A near-linear relationship was observed, however, between DeltaG degrees and the activity coefficient of the water component of the salt solutions. The thermodynamic parameters of the hybridization reaction along with the heat capacity changes were combined with thermodynamic contributions from the stacking to unstacking transitions of the single-stranded oligonucleotides from differential scanning calorimetry (DSC) measurements, resulting in good agreement with extrapolation of the free energy changes to 37 degrees C from the melting transition at 56 degrees C.

Lee H. T., Arciniegas S. and Marky L. A. (2008) Unfolding thermodynamics of DNA pyrimidine triplexes with different molecularities. *J Phys. Chem B* **112,** 4833-4840.
Abstract: Nucleic acid oligonucleotides (ODNs), as drugs, present an exquisite selectivity and affinity that can be used in antigene and antisense strategies for the control of gene expression. In this work we try to answer the following question: How does the molecularity of a DNA triplex affect its overall stability and melting behavior? To this end, we used a combination of temperature-dependent UV spectroscopy and calorimetric (differential scanning calorimetry) techniques to investigate the melting behavior of DNA triplexes with a similar helical stem, TC+TC+TC+T/AGAGAGA/TCTCTCT, but formed with different strand molecularity. We determined standard thermodynamic profiles and the differential binding of protons and counterions accompanying their unfolding. The formation of a triplex is accompanied by a favorable free energy term, resulting from the typical compensation of favorable enthalpy-unfavorable entropy contributions, i.e., the folding of a particular triplex is enthalpy driven. The magnitude of the favorable enthalpy contributions corresponds to the number and strength of the base-triplet stacks formed, which are helped by stacking contributions due to the incorporation of dangling ends or loops. Triplex stability is in the following order: monomolecular > bimolecular > trimolecular; this is explained in terms of additional stacking contributions due to the inclusion of loops. As expected, acidic pH stabilized all triplexes by allowing protonation of the cytosines in the third strand; however, the percentage of protonation increases as the molecularity decreases. The results help to choose adequate solution conditions for the study of triplexes containing different ratios of CGC+ and TAT base triplets and to aid in the design of oligonucleotide sequences as targeting reagents that could effectively react with mRNA sequences involved in human diseases, thereby increasing the feasibility of using the antisense strategy for therapeutic purposes

Lee H. T., Olsen C. M., Waters L., Sukup H. and Marky L. A. (2008) Thermodynamic contributions of the reactions of DNA intramolecular structures with their complementary strands. *Biochimie* **90,** 1052-1063.
Abstract: One focus of our research is to further our understanding of the physico-chemical properties of unusual DNA structures and their interaction with complementary oligonucleotides. We have investigated three types of reactions involving the interaction of intramolecular DNA complexes with their complementary single strands of varied length. Specifically, we have used a combination of isothermal titration (ITC) and differential scanning (DSC) calorimetry and spectroscopy techniques to determine standard thermodynamic profiles for the reaction of an i-motif, G-quadruplex, and triplex with their complementary strands. The enthalpies for each reaction are measured directly in ITC titrations and compared with those obtained indirectly from Hess cycles using DSC unfolding data. All reactions investigated yielded favorable free energy contributions, indicating that each single strand is able to invade and disrupt the corresponding intramolecular DNA complex. These favorable free energy terms are enthalpy driven, which result from a compensation of exothermic contributions, due to the formation of additional base-pair stacks (or base-triplet stacks) in the duplex product (or triplex product), immobilization of electrostricted water by the base-pair and base-triplet stacks, and the removal of structural water from the reactant single strands; and endothermic contributions from the disruption of base-base stacking interactions of the reactant single strands. This investigation of nucleic acid reactions has provided new

methodology, based on physico-chemical principles, to determine the molecular forces involved in the interactions between DNA nucleic acid structures. This methodology may be used in targeting reactions for the control of gene expression

Lhomme J., Constant J. F., and Demeunynck M. (1999) Abasic DNA structure, reactivity, and recognition. *Biopolymers* **52,** 65-83.
Abstract: Loss of a base in DNA, i.e., creation of an abasic site leaving a deoxyribose residue in the strand, is a frequent lesion that may occur spontaneously, or under the action of radiations and alkylating agents, or enzymatically as an intermediate in the repair of modified or abnormal bases. The abasic site lesion is mutagenic or lethal if not repaired. From a chemical point of view,the abasic site is an alkali-labile residue that leads to strand breakage through beta- and delta- elimination. Progress in the understanding of the chemistry and enzymology of abasic DNA largely relies upon the study of synthetic abasic duplexes. Several efficient synthetic methods have thus been developed to introduce the lesion (or a stable analogue) at defined position in the sequence. Physicochemical and spectroscopic examination of such duplexes, including calorimetry, melting temperature, high-field nmr and molecular modeling indicate that the lesion strongly destabilizes the duplex, although remaining in the canonical B-form with structural modifications strictly located at the site of the lesion. Probes have been developed to titrate the damage in DNA in vitro. Series of molecules have been devised to recognize specifically the abasic site, exhibiting a cleavage activity and mimicking the AP nucleases. Others have been prepared that bind strongly to the abasic site and show promise in potentiating the cytotoxic and antitumor activity of the clinically used nitrosourea (bis-chloroethylnitrosurea).

Li J. S., Shikiya R., Marky L. A., and Gold B. (2004) Triple helix forming TRIPside molecules that target mixed purine/pyrimidine DNA sequences. *Biochemistry* **43,** 1440-1448.
Abstract: A new strategy to form stable and sequence-specific triple helical DNA structures at mixed purine/pyrimidine sequences using a combination of four C-glycosides (TRIPsides) has been described [Li et al. (2003) J. Am. Chem. Soc. 125, 2084]. The partial realization of the approach is demonstrated by incorporating two of the four TRIPsides into oligomers that can potentially fold into intramolecular triplexes that contain one or two major groove crossovers of the purine Hoogsteen H-bond information. Using temperature-dependent electronic and fluorescence spectroscopy and differential scanning calorimetry, it is demonstrated that stable triplexes form at physiological conditions at non-homopurine targets. In addition, triplexes using the TRIPsides form in a highly sequence specific manner.

Li J. S., Fan Y. H., Zhang Y., Marky L. A., and Gold B. (2003) Design of triple helix forming C-glycoside molecules. *J Am Chem Soc* **125,** 2084-2093.
Abstract: The modeling, synthesis, and characterization of oligomers containing 2-aminoquinazolin-5-yl 2'-deoxynucleotide residues are reported. The 2-aminoquinazoline residues sequence specifically bind via Hoogsteen base pairing as a third strand in the center of the major groove at T:A base pair Watson-Crick duplex sequences. Evidence for the formation of a sequence specific three-stranded structure is based on thermal denaturation UV-vis and fluorescence studies. The novel 2-aminoquinazoline C-nucleotide is a component of a system designed to overcome the homopurine requirement for triple helix structures.

Li W., Wu P., Ohmichi T., and Sugimoto N. (2002) Characterization and thermodynamic properties of quadruplex/duplex competition. *FEBS Lett* **526,** 77-81.
Abstract: Structural characteristics and thermodynamic properties of dG(3)(T(2)AG(3))(3), d(C(3)TA(2))(3)C(3) and dG(3)(T(2)AG(3))(3)/d(C(3)TA(2))(3)C(3) were intensively investigated. It was indicated that metal ions greatly affected the conformation and stability of the G-quadruplex. A competition of a structure transition among the G-quadruplex, I-motif, and the duplex was confirmed to be dependent on both cation species and pH values. The structural competitive mechanism is discussed for the first time. This study shows an intriguing potential in modulating DNA structures in vivo, which is of great importance in drug design and cancer chemotherapy.

Li W., Miyoshi D., Nakano S., and Sugimoto N. (2003) Structural competition involving G-quadruplex DNA and its complement. *Biochemistry* **42,** 11736-11744.
Abstract: Structural competition between the G-quadruplex, the I-motif, and the Watson-Crick duplex has been implicated for repetitive DNA sequences, but the competitive mechanism of these multistranded

structures still needs to be elucidated. We investigated the effects of sequence context, cation species, and pH on duplex formation by the G-quadruplex of dG(3)(T(2)AG(3))(3) and its complement the I-motif of d(C(3)TA(2))(3)C(3), using ITC, DSC, PAGE, CD, UV, and CD stopped-flow kinetic techniques. ITC and PAGE experiments confirmed Watson-Crick duplex formation by the complementary strands. The binding constant of the two DNA strands in the presence of 10 mM Mg(2+) at pH 7.0 was shown to be 5.28 x 10^{7} M^{-1} at 20 degrees C, about 400 times larger than that in the presence of 100 mM Na(+) at pH 5.5. The dynamic transition traces of the duplex formation from the equimolar mixture of G-/C-rich complementary sequences were obtained at both pH 7.0 and pH 5.5. Fitting to a single-exponential function gave an observed rate of 8.06 x 10^{-3} s^{-1} at 20 degrees C in 10 mM Mg(2+) buffer at pH 7.0, which was about 10 times the observed rate at pH 5.5 under the same conditions. Both of the observed rates increased as temperature rose, implying that the dissociation of the single-stranded structured DNAs is the rate-limiting step for the WC duplex formation. The difference between the apparent activation energy at pH 7.0 and that at pH 5.5 reflects the fact that pH significantly influences the structural competition between the G-quadruplex, the I-motif, and the Watson-Crick duplex, which also implies a possible biological role for I-motifs in biological regulation.

Liang F. and Cho B. P. (2007) Probing the thermodynamics of aminofluorene-induced translesion DNA synthesis by differential scanning calorimetry. *J Am. Chem Soc.* **129,** 12108-12109

Maderia M., Shenoy S., Van Q. N., Marquez V. E. and Barchi J. J., Jr. (2007) Biophysical studies of DNA modified with conformationally constrained nucleotides: comparison of 2'-exo (north) and 3'-exo (south) 'locked' templates. *Nucleic Acids Res* **35,** 1978-1991.
Abstract: The biophysical properties of oligodeoxyribonucleotides (ODNs) selectively modified with conformationally 'locked' bicyclo[3.1.0]hexane pseudosugars (Maier,M.A., Choi,Y., Gaus,H., Barchi,J.J. Jr, Marquez,V.E., Manoharan,M. (2004) Synthesis and characterization of oligonucleotides containing conformationally constrained bicyclo[3.1.0]hexane pseudosugar analogs Nucleic Acids Res., 32, 3642-3650) have been studied by various techniques. Six separate synthetic ODNs based on the Dickerson Drew dodecamer sequence (CGCGAAT*T*CGCG) were examined where each one (or both) of the thymidines (T*) were substituted with a bicyclic pseudosugar locked in either a North (2'-exo) or South (3'-exo) ring pucker. Circular dichroism spectroscopy, differential scanning calorimetry and (1)H NMR spectroscopy were used to examine the duplex stability and conformational properties of the ODNs. Replacement of one or both thymidines with North-locked sugars (RNA-like) into the dodecamer did not greatly affect duplex formation or melt temperatures but distinct differences in thermodynamic parameters were observed. In contrast, incorporation of South-locked sugar derivatives that were predicted to stabilize this standard B-DNA, had the unexpected effect of causing a conformational equilibrium between different duplex forms at specific strand and salt concentrations. Our data and those of others suggest that although DNA can tolerate modifications with RNA-like (North) nucleotides, a more complicated spectrum of changes emerges with modifications restricted to South (DNA-like) puckers.

Makube N., Klump H., Pikkemaat J., and Altona C. (1999) Thermodynamic properties of an intramolecular DNA four-way junction. *Arch Biochem Biophys* **364,** 53-60.
Abstract: We have investigated the thermodynamic properties of two homologous DNA four-way junctions, J4 and J4M, based on 46-mer linear DNA molecules. J4 and J4M have the same base sequence with the only difference that the latter contains an uncharged methylene-acetal linkage, -O3'-CH2-O5', instead of the phosphodiester linkage, -O3'-PO2-O5'-, between the residues T18 and C19. The comparison of the thermal unfolding of the J4 junction and J4M junction serves to investigate the effect of the uncharged methylene-acetal linkage on the stability of the junction. Our analysis is based on CD, UV absorbance spectroscopy, DSC, and chemical footprinting. The aim is to characterize in detail the structure and stability of the junctions. As demonstrated before by NMR, in the presence of 5 mM MgCl2 +/- 50 mM NaCl, both J4 and J4M form a complete four-way junction. This is now evidenced by protection from OsO4 cleavage (chemical footprinting). We can assume that full base pairing occurs throughout the arms even at the center of the junction. CD spectra suggest that the helices within the junctions adopt the regular B-DNA conformation. Almost identical melting temperatures and unfolding enthalpies are obtained for J4 and J4M both by UV and DSC. Furthermore, the Van't Hoff enthalpy (ΔHVH) derived from UV melting equals the calorimetric enthalpy (ΔHcal), which means that the melting process of the structures proceeds in a two-state manner. All results taken together support the conclusion that there are no major

conformational and energetic differences between J4 and J4M. The inclusion of the uncharged methylene-acetal group into the junction has no effect on its stability.

Makube N. and Klump H. H. (2000) A four-way junction with triple-helical arms: design, characterization, and stability. *Arch Biochem Biophys* **377,** 31-42.
Abstract: The formation of the four-way junction containing four triple-helical arms has been demonstrated using chemical methods (polyacrylamide gel electrophoresis and chemical footprinting using OsO(4) as a probe) and physical methods (UV absorbance melting and DSC). The junction J(T1T3) was assembled from two 20-mer purine strands and two 44-mer pyrimidine strands. To determine the contribution of the different arms to the stability of the complete structure of J(T1T3), the junction was compared to two simplified substructures, J(T1) and J(T3), respectively. Common to these complexes is the underlying double-helical four-way junction Js. Addition of Na(+) had a profound effect on stabilizing and subsequently folding the junctions into the stacked X-structures. The following results support the structure present: (i) The native polyacrylamide electrophoresis exhibits only a single band(s) corresponding to one species present when all four single strands are mixed in equal amounts. (ii) OsO(4) modifications were investigated at pH 5.0 and in the presence of 10 mM Mg(2+) and 100 mM Na(+). There is no cleavage of thymine residues at the branch point and throughout the structure. (iii) The thermal unfolding of J(T1) and J(T3) illustrates that the triple-helical arms are more stable than the double-helical arms which are contained in these junctions and that J(T1T3) with four triple-helical arms is slightly more stable than J(T1) and J(T3). (iv) The calorimetric transition enthalpies determined for the arms of J(T1T3) are comparable to those associated with the unfolding of its corresponding arms in J(T1) and J(T3). The results also illustrate that the formation of the junctions is not restricted by the pH, [Na(+)], sequence composition of the arms, and/or the loop position.

Makube N. and Klump H. H. (2001) Impact of the third-strand orientation on the thermodynamic stability of the four-way DNA junction. *Arch Biochem Biophys* **393,** 1-13.
Abstract: The physical properties of a triple-helical DNA four-way junction J(T2T4) have been characterized by means of UV spectroscopy, CD spectroscopy, and differential scanning calorimetry (DSC). J(T2T4) is another four-way junction that was designed in addition to J(T1T3) (N. Makube and H. H. Klump (2000) Arch. Biochem. Biophys. 377, 31-42) to study the effects of third strands on the stability of the four-way junction with triple-helical arms. The pH titration curves illustrate the sequential folding of single strands to double-helical four-way junctions and finally the binding of third strands to their respective W-C duplexes. CD measurements confirm triplex formation under appropriate pH and ionic strength conditions. The CD spectra also suggest different melting patterns for the triple-helical arms of J(T2T4). The melting temperature as a function of pH or ionic strength characterizes the effect of the third strands on the structural stability. Increased sodium concentration and low pH conditions enhances and stabilizes the overall structure of the junction. The results also indicate that all triplexes in J(T2T4) are formed in the absence of salt and at low pH; however, the junction may, under these conditions, assume a conformation different from the one assumed in the presence of salt. Through the deconvolution of DSC data, the calorimetric enthalpies associated with melting of arms of the junctions were determined. The loops are designed to have the same enthalpic effect on the different arms. The stabilizing effect of the loops is more pronounced when those loops are shifted from arms 1 and 3 in J(T1T3) to arms 2 and 4 in J(T2T4) without changing any of the sequences. Overall, J(T2T4) is slightly more stable than J(T1T3). The differences can be attributed to sequence effects rather than structural effects. All the results illustrate that binding of the third strand in either of the two orientations 5'5'3' (J(T2T4)) or 5'3'3' (J(T1T3)) stabilizes the underlying double-helical four-way junction and its triple-helical arms.

Mandell K. E., Vallone P. M., Owczarzy R., Riccelli P. V., and Benight A. S. (2006) Studies of DNA dumbbells VIII. Melting analysis of DNA dumbbells with dinucleotide repeat stem sequences. *Biopolymers* **82,** 199-221.
Abstract: Melting curves and circular dichroism spectra were measured for a number of DNA dumbbell and linear molecules containing dinucleotide repeat sequences of different lengths. To study effects of different sequences on the melting and spectroscopic properties, six DNA dumbbells whose stems contain the central sequences (AA)(10), (AC)(10), (AG)(10), (AT)(10), (GC)(10), and (GG)(10) were prepared. These represent the minimal set of 10 possible dinucleotide repeats. To study effects of dinucleotide repeat length, dumbbells with the central sequences (AG)(n), n = 5 and 20, were prepared. Control molecules,

dumbbells with a random central sequence, (RN)(n), n = 5, 10, and 20, were also prepared. The central sequence of each dumbbell was flanked on both sides by the same 12 base pairs and T(4) end-loops. Melting curves were measured by optical absorbance and differential scanning calorimetry in solvents containing 25, 55, 85, and 115 mM Na(+). CD spectra were collected from 20 to 45 degrees C and [Na(+)] from 25 to 115 mM. The spectral database did not reveal any apparent temperature dependence in the pretransition region. Analysis of the melting thermodynamics evaluated as a function of Na(+) provided a means for quantitatively estimating the counterion release with melting for the different sequences. Results show a very definite sequence dependence, indicating the salt-dependent properties of duplex DNA are also sequence dependent. Linear DNA molecules containing the (AG)(n) and (RN)(n), sequences, n = 5, 10, 20, and 30, were also prepared and studied. The linear DNA molecules had the exact sequences of the dumbbell stems. That is, the central repeat sequence in each linear duplex was flanked on both sides by the same 12-bp sequence. Melting and CD studies were also performed on the linear DNA molecules. Comparison of results obtained for the same sequences in dumbbell and linear molecular environments reveals several interesting features of the interplay between sequence-dependent structural variability, sequence length, and the unconstrained (linear) or constrained (dumbbell) molecular environments.

Marchetti S., Onori G., and Cametti C. (2006) Calorimetric and dynamic light-scattering investigation of cationic surfactant--DNA complexes. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110,** 24761-24765.
Abstract: By means of combined calorimetric and dynamic light-scattering measurements, we have investigated the conformational behavior of DNA chains after thermal melting in the presence of a cationic surfactant at different concentrations, up to a surfactant-to-phosphate group molar ratio close to unity. Both the specific heat capacity, C(ex)(p) and the hydrodynamic radius R of the DNA chains provide support for the existence of two structural arrangements with different thermal stabilities, coexisting in the bulk solution. Although a component remains an elongated unfolded DNA chain originated in the thermal denaturation, the second component, consisting of DNA-surfactant complexes, assumes a compact structure with an average size of about 80 nm, whose thermal denaturation occurs at temperatures higher than 100 degrees C.

Marky L. A. and Kupke D. W. (2000) Enthalpy-entropy compensations in nucleic acids: contribution of electrostriction and structural hydration. *Methods Enzymol* **323,** 419-441.

Marlowe R. L., Lukan A. M., Lee S. A., Anthony L., Chandrasekaran R., and Rupprecht A. (1996) Differential scanning calorimetric and X-ray study of the binding of the water of primary hydration to calf-thymus DNA. *J Biomol Struct Dyn* **14,** 373-379.
Abstract: Differential scanning calorimetry has been used to study the thermal properties of hydrated films of calf-thymus Na-, K- and CsDNA between 20 and 320 degrees C. A broad endothermic transition near 75 degrees C and a sharp exothermic transition near 240 degrees C are observed. The broad transition is due to the dehydration of the DNA, while the exothermic transition is due to pyrolysis of the sample. the peak temperatures of both transitions increase as the scan rate is increased. Based on a Kissinger analysis, the net activation energy for the desorption of the primary water of hydration is about 0.6 eV while that for the pyrolysis is about 1.9 eV. X-ray diffraction patterns suggest that heating the DNA films to 180 degrees C once does not, but thrice does, destroy their structural ordering.

Mathis G., Schutz R., and Hunziker J. (2003) Towards a DNA-like duplex without hydrogen bonds. *Nucleosides Nucleotides Nucleic Acids* **22,** 1183-1185.
Abstract: The inverse quadrupolar moments of the phenyl and pentafluorophenyl residues in the base pair P-F5 promotes strong intramolecular stacking interactions in DNA duplexes. The more natural base pairs are replaced by this novel pair the higher the thermodynamic stability of the resulting duplex if they are arranged in an alternating fashion.

Mdzinarashvili T. J., Mrevlishvili G. M., Khvedelidze M. M., Ivanova A. T., Janelidze N., Kiziria E. L., Tushishvili D. G., Tediashvili M. I., and Kemp R. B. (2006) Pycnometric, viscometric and calorimetric studies of the process to release the double-stranded DNA from the Un bacteriophage. *Biophys Chem* **124,** 43-51.

Abstract: Knowledge of both the packaging of the linear, double-stranded (ds)DNA in bacteriophages and its subsequent release into the bacterial host is vital to our understanding of phage infection. There is now strong evidence that packaging requires a powerful rotary motor fuelled by ATP. From thermodynamic studies, however, it has been proposed that, at least for those viruses with a contractile tail, the dsDNA ejection from the phage head is a relatively simple physical process that does not require cellular energy and is facilitated by the difference in the conditions of the medium in the environments inside and outside the head. In this case, there should be no enthalpic effects associated with the dehiscence of the capsid and no destruction of it or the other structural elements of the phage. For the present study of temperature-induced phage dehiscence, we used a newly discovered phage with a contractile tail, named the Un (unknown) bacteriophage. Evidence is given of its characteristics in terms of ultrastructural morphology, serological parameters, host range and interaction with host cell. These show that, although it has similarities with the T-even phages and, in particular, the DDVI phage, it appears to be a new type. Earlier viscometric studies with it had shown that the temperature-induced release of the capsid dsDNA was completed at 70 degrees C. In the present investigation, a concentrated suspension of purified phage was subjected to pycnometric analysis through the temperature range of 30 to 70 degrees C. This showed that a significant and abrupt increase in the phage partial volume takes place, which remarkably is in the order of threefold. Viscometric measurements over time at 72 degrees C gave a kinetic curve from which evidence it was suggested that the temperature-induced DNA release is similar to a second order phase transition. At the same time, data from differential scanning calorimetry over the same temperature range showed no enthalpic effect. Our results indicate that the ejection of DNA from the capsid tail is driven by an entropy change.

Mergny J. L., De Cian A., Amrane S., and Webba d. S. (2006) Kinetics of double-chain reversals bridging contiguous quartets in tetramolecular quadruplexes. *Nucleic Acids Res* **34,** 2386-2397.
Abstract: Repetitive 5'GGXGG DNA segments abound in, or near, regulatory regions of the genome and may form unusual structures called G-quadruplexes. Using NMR spectroscopy, we demonstrate that a family of 5'GCGGXGGY sequences adopts a folding topology containing double-chain reversals. The topology is composed of two bistranded quadruplex monomeric units linked by formation of G:C:G:C tetrads. We provide a complete thermodynamic and kinetic analysis of 13 different sequences using absorbance spectroscopy and DSC, and compare their kinetics with a canonical tetrameric parallel-stranded quadruplex formed by TG4T. We demonstrate large differences (up to 10(5)-fold) in the association constants of these quadruplexes depending on primary sequence; the fastest samples exhibiting association rate equal or higher than the canonical TG4T quadruplex. In contrast, all sequences studied here unfold at a lower temperature than this quadruplex. Some sequences have thermodynamic stability comparable to the canonical TG4T tetramolecular quadruplex, but with faster association and dissociation. Sequence effects on the dissociation processes are discussed in light of structural data.

Mikulecky P. J. and Feig A. L. (2006) Heat capacity changes associated with DNA duplex formation: salt- and sequence-dependent effects. *Biochemistry* **45,** 604-616.
Abstract: Duplexes are the most fundamental elements of nucleic acid folding. Although it has become increasingly clear that duplex formation can be associated with a significant change in heat capacity (deltaC(p)), this parameter is typically overlooked in thermodynamic studies of nucleic acid folding. Analogy to protein folding suggests that base stacking events coupled to duplex formation should give rise to a deltaC(p) due to the release of waters solvating aromatic surfaces of nucleotide bases. In previous work, we showed that the deltaC(p) observed by isothermal titration calorimetry (ITC) for RNA duplex formation depended on salt and sequence [Takach, J. C., Mikulecky, P. J., and Feig, A. L. (2004) J. Am. Chem. Soc. 126, 6530-6531]. In the present work, we apply calorimetric and spectroscopic techniques to a series of designed DNA duplexes to demonstrate that both the salt dependence and sequence dependence of deltaC(p)s observed by ITC reflect perturbations to the same fundamental phenomenon: stacking in the single-stranded state. By measuring the thermodynamics of single strand melting, one can accurately predict the deltaC(p)s observed for duplex formation by ITC at high and low ionic strength. We discuss our results in light of the larger issue of contributions to deltaC(p) from coupled equilibria and conclude that observed deltaC(p)s can be useful indicators of intermediate states in nucleic acid folding phenomena.

Mikulecky P. J. and Feig A. L. (2006) Heat capacity changes associated with nucleic acid folding. *Biopolymers* **82,** 38-58.

Abstract: Whereas heat capacity changes (DeltaCPs) associated with folding transitions are commonplace in the literature of protein folding, they have long been considered a minor energetic contributor in nucleic acid folding. Recent advances in the understanding of nucleic acid folding and improved technology for measuring the energetics of folding transitions have allowed a greater experimental window for measuring these effects. We present in this review a survey of current literature that confronts the issue of DeltaCPs associated with nucleic acid folding transitions. This work helps to gather the molecular insights that can be gleaned from analysis of DeltaCPs and points toward the challenges that will need to be overcome if the energetic contribution of DeltaCP terms are to be put to use in improving free energy calculations for nucleic acid structure prediction.

Milev S., Gorfe A. A., Karshikoff A., Clubb R. T., Bosshard H. R., and Jelesarov I. (2003) Energetics of sequence-specific protein-DNA association: conformational stability of the DNA binding domain of integrase Tn916 and its cognate DNA duplex. *Biochemistry* **42,** 3492-3502.
Abstract: Sequence-specific DNA recognition by bacterial integrase Tn916 involves structural rearrangements of both the protein and the DNA duplex. Energetic contributions from changes of conformation, thermal motions and soft vibrational modi of the protein, the DNA, and the complex significantly influence the energetic profile of protein-DNA association. Understanding the energetics of such a complicated system requires not only a detailed calorimetric investigation of the association reaction but also of the components in isolation. Here we report on the conformational stability of the integrase Tn916 DNA binding domain and its cognate 13 base pair target DNA duplex. Using a combination of temperature and denaturant induced unfolding experiments, we find that the 74-residue DNA binding domain is compact and unfolds cooperatively with only small deviation from two-state behavior. Scanning calorimetry reveals an increase of the heat capacity of the native protein attributable to increased thermal fluctuations. From the combined calorimetric and spectroscopic experiments, the parameters of protein unfolding are Tm = 43.8 +/- 0.3 degrees C, ΔH(m) = 255 +/- 18 kJ mol^{-1}, ΔS(m) = 0.80 +/- 0.06 kJ mol^{-1}, and ΔCp = 5.0 +/- 0.8 kJ K^{-1} mol^{-1}. The DNA target duplex displays a thermodynamic signature typical of short oligonucleotide duplexes: significant heat absorption due to end fraying and twisting precedes cooperative unfolding and dissociation. The parameters for DNA unfolding and dissociation are ΔH(m) = 335 +/- 4 kJ mol^{-1} and ΔCp = 2.7 +/- 0.9 kJ K^{-1} mol^{-1}. The results reported here have been instrumental in interpreting the thermodynamic features of the association reaction of the integrase with its 13 base pair target DNA duplex reported in the accompanying paper [Milev et al. (2003) Biochemistry 42, 3481-3491].

Miyoshi D., Matsumura S., Li W., and Sugimoto N. (2003) Structural polymorphism of telomeric DNA regulated by pH and divalent cation. *Nucleosides Nucleotides Nucleic Acids* **22,** 203-221.
Abstract: DNA oligonucleotides can form multi-stranded structures such as a duplex, triplex, and quadruplex, while the double helical structure is generally considered as the canonical structure of DNA oligonucleotides. Guanine-rich or cytosine-rich oligonucleotides, which are observed in telomere, centromere, and other biologically important sequences in vivo, can form four-stranded G-quadruplex and I-motif structures in vitro. In this study, we have investigated the effects of pH and cation on the structures and their stabilities of d(G4T4G4) and d(C4A4C4). The CD spectra and thermal melting curves of DNAs at various pHs demonstrated that acidic conditions induced a stable I-motif structure of d(C4A4C4), while the pH value did not affect the G-quadruplex structure and stability of d(G4T4G4). The CD spectra of the 1:1 mixture of d(G4T4G4) and d(C4A4C4) indicated that the acidic conditions inhibit the duplex formation between d(G4T4G4) and d(C4A4C4). Isothermal titration calorimetry measurements of the duplex formation at various pHs also quantitatively indicated that the acidic conditions inhibit the duplex formation. On the other hand, the CD spectra and thermal melting curves of DNAs in the absence and presence of Ca2+ indicated that Ca2+ induces a parallel G-quadruplex structure of d(G4T4G4) and then inhibits the duplex formation. These results lead to the conclusion that both the pH and coexisting cation can induce and regulate the structural polymorphisms the oligonucleotides in which they form the G-quadruplex, I-motif, and duplex depending on the conditions. Thus, the results reported here indicate pivotal roles of pH and coexisting cations in biological processes by regulating the conformational switching between the duplex and quadruplexes structures of the guanine-rich or cytosine-rich oligonucleotides in vivo.

Monaselidze J., Majagaladze G., Barbakadze S., Khachidze D., Gorgoshidze M., Kalandadze Y., Haroutiunian S., Dalyan Y. and Vardanyan V. (2008) Microcalorimetric investigation of DNA,

poly(dA)poly(dT) and poly[d(A-C)]poly[d(G-T)] melting in the presence of water soluble (meso tetra (4 N oxyethylpyridyl) porphyrin) and its Zn complex. *J Biomol. Struct. Dyn.* **25,** 419-424.
Abstract: Thermodynamic parameters of melting process (DeltaHm, Tm, DeltaTm) of calf thymus DNA, poly(dA)poly(dT) and poly(d(A-C)).poly(d(G-T)) were determined in the presence of various concentrations of TOEPyP(4) and its Zn complex. The investigated porphyrins caused serious stabilization of calf thymus DNA and poly poly(dA)poly(dT), but not poly(d(A-C))poly(d(G-T)). It was shown that TOEpyp(4) revealed GC specificity, it increased Tm of satellite fraction by 24 degrees C, but ZnTOEpyp(4), on the contrary, predominantly bound with AT-rich sites and increased DNA main stage Tm by 18 degrees C, and Tm of poly(dA)poly(dT) increased by 40 degrees C, in comparison with the same polymers without porphyrin. ZnTOEpyp(4) binds with DNA and poly(dA)poly(dT) in two modes--strong and weak ones. In the range of r from 0.005 to 0.08 both modes were fulfilled, and in the range of r from 0.165 to 0.25 only one mode--strong binding--took place. The weak binding is characterized with shifting of Tm by some grades, and for the strong binding Tm shifts by approximately 30-40 degrees C. Invariability of DeltaHm of DNA and poly(dA)poly(dT), and sharp increase of Tm in the range of r from 0.08 to 0.25 for thymus DNA and 0.01-0.2 for poly(dA)poly(dT) we interpret as entropic character of these complexes melting. It was suggested that this entropic character of melting is connected with forcing out of H2O molecules from AT sites by ZnTOEpyp(4) and with formation of outside stacking at the sites of binding. Four-fold decrease of calf thymus DNA melting range width DeltaTm caused by increase of added ZnTOEpyp(4) concentration is explained by rapprochement of AT and GC pairs thermal stability, and it is in agreement with a well-known dependence, according to which DeltaT approximately TGC-TAT for DNA obtained from higher organisms (L. V. Berestetskaya, M. D. Frank-Kamenetskii, and Yu. S. Lazurkin. Biopolymers 13, 193-205 (1974)). Poly (d(A-C))poly(d(G-T)) in the presence of ZnTOEpyp(4) gives only one mode of weak binding. The conclusion is that binding of ZnTOEpyp(4) with DNA depends on its nucleotide sequence

Montesarchio D. (2008) G-quadruplex forming oligonucleotides as finely tunable aptamers: towards better DNA mimics. *Nucleic Acids Symp. Ser. (Oxf)* 9-10.
Abstract: The intense search for oligonucleotides (ODNs) endowed with pharmacological activities has led, in the past decade, to the identification of tens of candidate drugs, now being evaluated in preclinical or clinical trials. Based on G-rich DNA sequences, several aptamers, adopting G-quadruplex structures with different topologies, have been selected as potent in vitro antiviral and/or antitumoral agents. In order to develop novel therapeutically relevant G-quadruplex-based aptamers, we have investigated - as a model compound - the (5')d(TGGGAG)(3') sequence, known to be anti-HIV-1 active if 5'-modified with bulky aromatic residues. A set of 5'-conjugated analogues has been analyzed by integrated CD, DSC and molecular modelling studies, allowing a detailed biophysical characterization of the resulting G-quadruplexes. Following the assumption that the kinetically and thermodynamically favoured formation of the quadruplex complexes is a pre-requisite for their efficient antiviral activity, novel hybrid ODNs, carrying diverse terminal modifications, were prepared via a fully automated, on-line phosphoramidite-based strategy and evaluated for anti-HIV activity

Mukerji I. and Williams A. P. (2002) UV resonance Raman and circular dichroism studies of a DNA duplex containing an A(3)T(3) tract: evidence for a premelting transition and three-centered H-bonds. *Biochemistry* **41,** 69-77.
Abstract: The presence of A(n) and A(n)T(n) tracts in double-helical sequences perturbs the structural properties of DNA molecules, resulting in the formation of an alternate conformation to standard B-DNA known as B'-DNA. Evidence for a transition occurring prior to duplex melting in molecules containing A(n) tracts was previously detected by circular dichroism (CD) and calorimetric studies. This premelting transition was attributed to a conformational change from B'- to B-DNA. Structural features of A(n) and A(n)T(n) tracts revealed by X-ray crystallography include a large degree of propeller twisting of adenine bases, narrowed minor grooves, and the formation of three-centered H-bonds between dA and dT bases. We report UV resonance Raman (UVRR) and CD spectroscopic studies of two related DNA dodecamer duplexes, d(CGCAAATTTGCG)(2) (A(3)T(3)) and d(CGCATATATGCG)(2) [(AT)(3)]. These studies address the presence of three-centered H-bonds in the B' conformation and gauge the impact of these putative H-bonds on the structural and thermodynamic properties of the A(3)T(3) duplex. UVRR and CD spectra reveal that the premelting transition is only observed for the A(3)T(3) duplex, is primarily localized to the dA and dT bases, and is associated with base stacking interactions. Spectroscopic changes associated

with the premelting transition are not readily detectable for the sugar-phosphate backbone or the cytosine and guanosine bases. The temperature-dependent concerted frequency shifts of dA exocyclic NH(2) and dT C4=O vibrational modes suggest that the A(3)T(3) duplex forms three-centered hydrogen bonds at low temperatures, while the (AT)(3) duplex does not. The enthalpy of this H-bond, estimated from the thermally induced frequency shift of the dT C4=O vibrational mode, is approximately 1.9 kJ/mol or 0.46 kcal/mol.

Nicolini C., Carrara S., and Mascetti G. (1997) High order DNA structure as inferred by optical fluorimetry and scanning calorimetry. *Mol Biol Rep* **24,** 235-246.
Abstract: New quantitative insights on the native high order chromatin-DNA structure existing within interphase nuclei are obtained by monitoring the effects of two common well-characterized fixatives, glutaraldehyde and ethanol/acetic acid mixture, at the level of the intranuclear DNA distribution and structures. Reproducible distinct levels of DNA fluorescence intensity and their intranuclear distribution are apparent in unfixed and fixed thymocytes by using DAPI and quantitative optical microscopy based on a charge coupled device. The fluorescent histograms correlated with the calorimetric thermograms on the very same thymocytes fixed and unfixed, establish an unequivocal baseline for the different levels of structural organization of the chromatin within the intact nucleus; namely their number, DNA packing ratio and fiber diameter. A systematic comparison among all the numerous models, being so far proposed for the quinternary and quaternary levels of DNA folding, to identifies the rope or ribbon-like and the chromonema as the ones that best fit with the in situ distribution.

Nixon P. L. and Giedroc D. P. (2000) Energetics of a strongly pH dependent RNA tertiary structure in a frameshifting pseudoknot. *J Mol Biol* **296,** 659-671.
Abstract: Retroviruses employ -1 translational frameshifting to regulate the relative concentrations of structural and non-structural proteins critical to the viral life cycle. The 1.6 A crystal structure of the -1 frameshifting pseudoknot from beet western yellows virus reveals, in addition to Watson-Crick base-pairing, many loop-stem RNA tertiary structural interactions and a bound Na(+). Investigation of the thermodynamics of unfolding of the beet western yellows virus pseudoknot reveals strongly pH-dependent loop-stem tertiary structural interactions which stabilize the molecule, contributing a net of ΔH approximately -30 kcal mol^{-1} and ΔG degrees (37) of -3.3 kcal mol^{-1} to a total ΔH and ΔG degrees (37) of -121 and -16 kcal mol^{-1}, respectively, at pH 6.0, 0.5 M K(+) by DSC. Characterization of mutant RNAs supports the presence of a C8(+).G12-C26 loop 1-stem 2 base-triple (pK(a)=6.8), protonation of which contributes nearly -3.5 kcal mol^{-1} in net stability in the presence of a wild-type loop 2. Substitution of the nucleotides in loop 2 with uridine bases, which would eliminate the minor groove triplex, destroys pseudoknot formation. An examination of the dependence of the monovalent ion and type on melting profiles suggests that tertiary structure unfolding occurs in a manner quantitatively consistent with previous studies on the stabilizing effects of K(+), NH(4)(+) and Na(+) on other simple duplex and pseudoknotted RNAs.

Nixon P. L., Cornish P. V., Suram S. V., and Giedroc D. P. (2002) Thermodynamic analysis of conserved loop-stem interactions in P1-P2 frameshifting RNA pseudoknots from plant Luteoviridae. *Biochemistry* **41,** 10665-10674.
Abstract: The RNA genomes of plant luteovirids beet western yellows virus (BWYV), potato leaf roll virus (PLRV), and pea enation mosaic virus (PEMV RNA1; PEMV-1) contain a short mRNA pseudoknotted motif overlapping the P1 and P2 open reading frames required for programmed -1 mRNA ribosomal frameshifting. The relationship between structure, stability, and function is poorly understood in these RNA systems. A m(5)-C(8)-substituted BWYV RNA is employed to establish that the BWYV P1-P2 pseudoknot is protonated at cytidine 8 in loop L1 (delta(N(3)H)+ = 12.98 ppm), which stabilizes a C(+.)(G-C) major groove base triple by Δ(ΔG(37))(protonation) = 3.1 (+/-0.4) kcal mol^{-1}. The stabilities of both the PLRV and PEMV-1 P1-P2 pseudoknots are also strongly pH-dependent, with Δ(ΔG(37))(protonation) = 2.1 (+/-0.2) kcal mol^{-1} for the PEMV-1 pseudoknot despite a distinct structural context. As previously found for the BWYV pseudoknot [Nixon and Giedroc (2000) J. Mol. Biol. 296, 659], both the PLRV and PEMV-1 RNAs are stabilized by ΔH > or = 30 kcal mol(-)(1) in excess of secondary structure predictions, attributed to loop L2-stem S1 minor groove triplex interactions. BWYV RNAs containing single 2'-deoxy or A --> G substitutions that disrupt L2-S1 hydrogen bonding are strongly destabilized with Δ(ΔG(37))(folding) (pH = 7.0) ranging from approximately 1.8 (+/-0.3) to > or =4.0 kcal mol^{-1}, relative to

the wild-type BWYV RNA. These findings suggest that each member of this family of pseudoknots adopts a tightly folded structure that maximizes the cooperativity and complementarity of L1-S2 and L2-S1 loop-stem interactions required in part to offset the low intrinsic stability of the short three base pair pseudoknot stem S2.

Nystrom B. and Nilsson L. (2007) Molecular dynamics study of intrinsic stability in six RNA terminal loop motifs. *J Biomol. Struct. Dyn.* **24,** 525-536.
Abstract: Single stranded RNA molecules can assume a wide range of tertiary structures beyond the canonical A-form double helix. Certain sequences, termed motifs, are more common than a random distribution would suggest. The existence of such motifs can be rationalized in structural terms. In this study, we have investigated the intrinsic structural stability of RNA terminal loop motifs using multiple MD simulations in explicit water. Representative loops were chosen from the major tetraloop motifs, including also the U-turn motif. Not all loops retain their folded starting structure, but lowering the temperature to 277 K, or adding adjacent base pairs from the stem to which the motif is attached, helps stabilizing the folded loop structure.

Olsen C. M., Gmeiner W. H., and Marky L. A. (2006) Unfolding of G-quadruplexes: energetic, and ion and water contributions of G-quartet stacking. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110,** 6962-6969.
Abstract: It has been shown that DNA oligonucleotides composed, in part, of G repeat sequences can adopt G-quadruplex structures in the presence of specific metal ions. In this work, we use a combination of spectroscopic and calorimetric techniques to determine the spectral and thermodynamic characteristics of two DNA aptamers, d(G2T2G2TGTG2T2G2), G2, and d(G3T2G3TGTG3T2G3), G3; a sequence in the promoter region of the c-MYC oncogene, d(TG4AG3TG4AG3TG4A2G2), NHE-III; and the human telomere sequence d(AG3T2AG3T2AG3T2AG3), 22GG. The circular dichroism spectra of these oligonucleotides in the presence of K+ indicate that all form G-quadruplexes with G-quartets in an antiparallel arrangement (G2), in a parallel arrangement (NHE-III and 22GG), or in a mixed parallel and antiparallel G-quartet arrangement (G3). Melting profiles show transition temperatures, TM, above 45 degrees C that are independent of strand concentration, consistent with the formation of very stable intramolecular G-quadruplexes. We used differential scanning calorimetry to obtain complete thermodynamic profiles for the unfolding of each quadruplex. Subtracting the thermodynamic folding profiles of G2 from those of G3 yielded the following thermodynamic profile for the formation of a G-quartet stack: DeltaG degrees 20 = -2.2 kcal/mol, DeltaHcal = -14.6 kcal/mol, TDeltaScal = -12.4 kcal/mol, DeltanK+ = -0.3 mol of K+/mol, and DeltanW = 13 mol of H2O/mol. Furthermore, we used this profile to estimate the thermodynamic contributions of the loops and/or extra base sequences of each oligonucleotide in the G-quadruplex state. The average free energy contributions of the latter indicate that the incorporation of loops and base overhangs stabilizes quadruplex structures. This stabilization is enthalpy-driven and is due to base-stacking contributions.

Olsen C. M., Lee H. T. and Marky L. A. (2008) Unfolding Thermodynamics of Intramolecular G-Quadruplexes: Base Sequence Contributions of the Loops. *J Phys. Chem B*. (epublication)
Abstract: G-quadruplexes are a highly studied DNA motif with a potential role in a variety of cellular processes and more recently are considered novel targets for drug therapy in aging and anticancer research. In this work, we have investigated the thermodynamic contributions of the loops on the stable formation of G-quadruplexes. Specifically, we use a combination of UV, circular dichroism (CD) and fluorescence spectroscopies, and differential scanning calorimetry (DSC) to determine thermodynamic profiles, including the differential binding of ions and water, for the unfolding of the thrombin aptamer: d(GG T 2 GG TGTGG T 2 GG) that is referred to as G2. The sequences in italics, TGT and T 2 , are known to form loops. Other sequences examined contained base substitutions in the TGT loop (TAT, TCT, TTT, TAPT, and UUU), in the T 2 loops (T 4, U 2), or in both loops (UGU and U 2, UUU and U 2). The CD spectra of all molecules show a positive band centered at 292 nm, which corresponds to the "chair" conformation. The UV and DSC melting curves of each G-quadruplex show monophasic transitions with transition temperatures (T Ms) that remained constant with increasing strand concentration, confirming their intramolecular formation. These G-quadruplexes unfold with T Ms in the range from 43.2 to 56.5 degrees C and endothermic enthalpies from 22.9 to 37.2 kcal/mol. Subtracting the contribution of a G-quartet stack from each experimental profile indicated that the presence of the loops stabilize each G-quadruplex by

favorable enthalpy contributions, larger differential binding of K (+) ions (0.1-0.6 mol K (+)/mol), and a variable uptake/release of water molecules (-6 to 8 mol H 2O/mol). The thermodynamic contributions for these specific base substitutions are discussed in terms of loop stacking (base-base stacking within the loops) and their hydration effects

Owczarzy R. (2005) Melting temperatures of nucleic acids: Discrepancies in analysis. *Biophys Chem*.
Abstract: Melting temperature, T(m), is an important property of nucleic acid duplexes. It is typically determined from spectroscopic or calorimetric melting experiments. More than one analytical method has been used to extract T(m) values from experimental melting data. Unfortunately, different methods do not give the same results; the same melting data can be assigned different T(m) values depending upon which method is used to process that data. Inconsistencies or systematic errors between T(m)s reported in published data sets can be significant and add confusion to the field. Errors introduced from analysis can be greater than experimental errors, ranging from a fraction of degree to several degrees. Of the various methods, the most consistent and meaningful approach defines melting temperature as the temperature at the transition midpoint where half of the base pairs are melted and standard free energy is zero. Assuming a two-state melting behavior, we present here a set of general equations that can be used to reconcile these analytical T(m) differences and convert results to the correct melting temperatures at the transition midpoint. Melting temperatures collected from published sources, which were analyzed using different methods, can now be corrected for these discrepancies and compared on equal footing. The similar corrections apply to T(m) differences between calorimetric and spectroscopic melting curves. New algorithm for selection of linear sloping baselines, 2nd derivative method, is suggested, which can be used to automate melting curve analysis.

Owczarzy R., You Y., Moreira B. G., Manthey J. A., Huang L., Behlke M. A., and Walder J. A. (2004) Effects of sodium ions on DNA duplex oligomers: improved predictions of melting temperatures. *Biochemistry* **43,** 3537-3554.
Abstract: Melting temperatures, T_m, were systematically studied for a set of 92 DNA duplex oligomers in a variety of sodium ion concentrations ranging from 69 mM to 1.02 M. The relationship between T_m and ln $[Na^+]$ was nonlinear over this range of sodium ion concentrations, and the observed melting temperatures were poorly predicted by existing algorithms. A new empirical relationship was derived from UV melting data that employs a quadratic function, which better models the melting temperatures of DNA duplex oligomers as sodium ion concentration is varied. Statistical analysis shows that this improved salt correction is significantly more accurate than previously suggested algorithms and predicts salt-corrected melting temperatures with an average error of only 1.6 degrees C when tested against an independent validation set of T_m measurements obtained from the literature. Differential scanning calorimetry studies demonstrate that this T_m salt correction is insensitive to DNA concentration. The T_m salt correction function was found to be sequence-dependent and varied with the fraction of G.C base pairs, in agreement with previous studies of genomic and polymeric DNAs. The salt correction function is independent of oligomer length, suggesting that end-fraying and other end effects have little influence on the amount of sodium counterions released during duplex melting. The results are discussed in the context of counterion condensation theory.

Owczarzy R., Vallone P. M., Goldstein R. F., and Benight A. S. (1999) Studies of DNA dumbbells VII: evaluation of the next-nearest-neighbor sequence-dependent interactions in duplex DNA. *Biopolymers* **52,** 29-56.
Abstract: Melting experiments were conducted on 22 DNA dumbbells as a function of solvent ionic strength from 25-115 mM Na(+). The dumbbell molecules have short duplex regions comprised of 16-20 base pairs linked on both ends by T(4) single-strand loops. Only the 4-8 central base pairs of the dumbbell stems differ for different molecules, and the six base pairs on both sides of the central sequence and adjoining loops on both ends are the same in every molecule. Results of melting analysis on the 22 new DNA dumbbells are combined with our previous results on 17 other DNA dumbbells, with stem lengths containing from 14-18 base pairs, reported in the first article of this series (Doktycz, Goldstein, Paner, Gallo, and Benight, Biopoly 32, 1992, 849-864). The combination of results comprises a database of optical melting parameters for 39 DNA dumbbells in ionic strengths from 25-115 mM Na(+). This database is employed to evaluate the thermodynamics of singlet, doublet, and triplet sequence-dependent interactions in duplex DNA. Analysis of the 25 mM Na(+) data reveals the existence of significant

sequence-dependent triplet or next-nearest-neighbor interactions. The enthalpy of these interactions is evaluated for all possible triplets. Some of the triplet enthalpy values are less than the uncertainty in their evaluation, indicating no measurable interaction for that particular sequence. This finding suggests that the thermodynamic stability of duplex DNA depends on solvent ionic strength in a sequence-dependent manner. As a part of the analysis, the nearest-neighbor (base pair doublet) interactions in 55, 85, and 115 mM Na(+) are also reevaluated from the larger database.

Paiva A. M. and Sheardy R. D. (2004) Influence of sequence context and length on the structure and stability of triplet repeat DNA oligomers. *Biochemistry* **43,** 14218-14227.
Abstract: Genetic expansion diseases have been linked to the properties of triplet repeat DNA sequences during replication. The most common triplet repeats associated with such diseases are CAG, CCG, CGG, and CTG. It has been suggested that gene expansion occurs as a result of hairpin formation of long stretches of these sequences on the leading daughter strand synthesized during DNA replication [Gellibolian, R., Bacolla, A., and Wells, R. D. (1997) J. Biol. Chem. 272, 16793-7]. To test the biophysical basis for this model, oligonucleotides of general sequence $(CNG)_n$, where N = A, C, G, or T and n = 4, 5, 10, 15, or 25, were synthesized and characterized by circular dichroism (CD) spectropolarimetry, optical melting studies, and differential scanning calorimetry (DSC). The goal of these studies was to evaluate the influence of sequence context and oligomer length on their secondary structures and stabilities. The results indicate that all single oligomers, even those as short as 12 nucleotides, form stable hairpin structures at 25 degrees C. Such hairpins are characterized by the presence of N:N mismatched base pairs sandwiched between G:C base pairs in the stems and loops of three to four unpaired bases. Thermodynamic analysis of these structures reveals that their stabilities are influenced by both the sequence of the particular oligomer and its length. Specifically, the stability order of CGG > CTG > CAG > CCG was observed. In addition, longer oligomers were found to be more stable than shorter oligomers of the same sequence. However, a stability plateau above 45 nucleotides suggests that the length dependence reaches a maximum value where the stability of the G:C base pairs can no longer compensate the instability of the N:N mismatches in the stems of the hairpins. The results are discussed in terms of the above model proposed for gene expansion.

Pagano B., Martino L., Randazzo A. and Giancola C. (2008) Stability and binding properties of a modified thrombin binding aptamer. *Biophys J* **94,** 562-569.
Abstract: Aptamer-based drugs represent an attractive approach in pharmacological therapy. The most studied aptamer, thrombin binding aptamer (TBA), folds into a well-defined quadruplex structure and binds to its target with good specificity and affinity. Modified aptamers with improved biophysical properties could constitute a new class of therapeutic aptamers. In this study we show that the modified thrombin binding aptamer (mTBA), (3')GGT(5')-(5')TGGTGTGGTTGG(3'), which also folds into a quadruplex structure, is more stable than its unmodified counterpart and shows a higher thrombin affinity. The stability of the modified aptamer was investigated using differential scanning calorimetry, and the energetics of mTBA and TBA binding to thrombin was characterized by means of isothermal titration calorimetry (ITC). ITC data revealed that TBA/thrombin and mTBA/thrombin binding stoichiometry is 1:2 for both interactions. Structural models of the two complexes of thrombin with TBA and with mTBA were also obtained and subjected to molecular dynamics simulations in explicit water. Analysis of the models led to an improvement of the understanding of the aptamer-thrombin recognition at a molecular level

Petraccone L., Pagano B., Esposito V., Randazzo A., Piccialli G., Barone G., Mattia C. A., and Giancola C. (2005) Thermodynamics and kinetics of PNA-DNA quadruplex-forming chimeras. *J Am Chem Soc* **127,** 16215-16223.
Abstract: PNA-DNA chimeras present the interesting properties of PNA, such as the high binding affinity to complementary single-strand (DNA or RNA), and the resistance to nuclease and protease degradation. At the same time, the limitations of an oligomer containing all PNA residues, such as low water solubility, self-aggregation, and low cellular uptake, are effectively overcome. Further, PNA-DNA chimeras possess interesting biological properties as antisense agents. We have explored the ability of PNA-DNA chimeric strands to assemble in quadruplex structures. The rate constant for association of the quadruplexes and their thermodynamic properties have been determined by CD spectroscopy and differential scanning calorimetry (DSC). Thermal denaturation experiments indicated higher thermal and thermodynamic stabilities for chimeric quadruplexes in comparison with the corresponding unmodified DNA quadruplex. Singular value decomposition analysis (SVD) suggests the presence of kinetically stable intermediate species in the

quadruplex formation process. The experimental results have been discussed on the basis of molecular dynamic simulations. The ability of PNA-DNA chimeras to form stable quadruplex structures expands their potential utility as therapeutic agents.

Petraccone L., Erra E., Messere A., Montesarchio D., Piccialli G., De Napoli L., Barone G., and Giancola C. (2004) Targeting duplex DNA with DNA-PNA chimeras? Physico-chemical characterization of a triplex DNA-PNA/DNA/DNA. *Biopolymers* **73,** 434-442.
Abstract: Targeting double-stranded DNA with homopyrimidine PNAs results in strand displacement complexes PNA/DNA/PNA rather than PNA/DNA/DNA triplex structures. Not much is known about the binding properties of DNA-PNA chimeras. A 16-mer 5'-DNA-3'-p-(N)PNA(C) has been investigated for its ability to hybridize a complementary duplex DNA by DSC, CD, and molecular modeling studies. The obtained results showed the formation of a triplex structure having similar, if not slightly higher, stability compared to the same all-DNA complex.

Petraccone L., Erra E., Esposito V., Randazzo A., Mayol L., Nasti L., Barone G., and Giancola C. (2004) Stability and structure of telomeric DNA sequences forming quadruplexes containing four G-tetrads with different topological arrangements. *Biochemistry* **43,** 4877-4884.
Abstract: Telomeres are DNA-protein structures at the ends of eukaryotic chromosomes, the DNA of which comprise noncoding repeats of guanine-rich sequences. Telomeric DNA plays a fundamental role in protecting the cell from recombination and degradation. Telomeric sequences can form quadruplex structures stabilized by guanine quartets. These structures can be constructed from one, two, or four oligonucleotidic strands. Here, we report the thermodynamic characterization of the stability, analyzed by differential scanning calorimetry, of three DNA quadruplexes of different molecularity, all containing four G-tetrads. The conformational properties of these quadruple helices were studied by circular dichroism. The investigated oligomers form well-defined G-quadruplex structures in the presence of sodium ions. Two have the truncated telomeric sequence from Oxytricha, d(TGGGGT) and d(GGGGTTTTGGGG), which form a tetramolecular and bimolecular quadruplex, respectively. The third sequence, d(GGGGTTGGGGTGTGGGGTTGGGG) was designed to form a unimolecular quadruplex. The thermodynamic parameters of these quadruplexes have been determined. The tetramolecular structure is thermodynamically more stable than the bimolecular one, which, in turn, is more stable than the unimolecular one. The experimental data were discussed in light of the molecular-modeling study.

Petraccone L., Erra E., Mattia C. A., Fedullo V., Barone G., and Giancola C. (2004) Linkage of proton binding to the thermal dissociation of triple helix complex. *Biophys Chem* **110,** 73-81.
Abstract: The effects of cytosine protonation on the thermodynamic properties of parallel pyrimidine motif DNA triplex were investigated and characterized by different techniques, such as circular dichroism (CD), ultraviolet spectroscopy (UV) and differential scanning calorimetry (DSC). A thermodynamic model was developed which, by linking the cytosine ionization equilibrium to the dissociation process of the triplex, is able to rationalize the experimental data and to reproduce the pH dependence of the free energy, enthalpy and entropy changes associated with the triplex formation. The results are useful to systematically introduce the effect of pH in a more general model able to predict the stability of DNA triplexes on the basis of the sequence alone.

Petraccone L., Erra E., Messere A., Montesarchio D., Piccialli G., Barone G., and Giancola C. (2003) Physico-chemical studies of a DNA triplex containing a new ferrocenemethyl-thymidine residue in the third strand. *Biophys Chem* **104,** 259-270.
Abstract: The stability of a 16-mer DNA triple helix containing a 3-N(ferrocenemethyl)-thymidine residue in the third strand has been investigated in comparison with the unmodified triplex of the same sequence. A complete physico-chemical characterization of the two triple helices on changing the pH by means of calorimetry, circular dichroism and molecular modeling is therefore reported. The thermodynamic parameters were obtained in the pH range 5.5-7.2 by differential scanning calorimetry (DSC). For both triplexes the Tm and Δ H degrees (Tm) values increase on decreasing the pH. In the pH range 7.2-6.0 the triplex containing the ferrocenemethyl nucleoside is less stable than the unmodified one, whereas the modified triplex becomes more stable at pH 5.5. Such difference in stability at each pH value is overwhelmingly enthalpic in origin. CD spectra show conformational changes on decreasing the pH for both the triplexes. By spectroscopic pH titration the apparent pK(a) values of the cytosines in the two

triplexes could be estimated, with the cytosines in the TFO containing the ferrocenemethyl residue having lower apparent pK(a) values. These results are consistent with the calorimetric data, showing a decrease of the thermodynamic parameters in the pH range 7.2-6.0 and an increase at pH 5.5 for the ferrocenylated triplex with respect to the unmodified one. The thermodynamic and spectroscopic data are also discussed in relation to molecular models.

Petraccone L., Erra E., Nasti L., Galeone A., Randazzo A., Mayol L., Barone G., and Giancola C. (2003) Effect of a modified thymine on the structure and stability of [d(TGGGT)]4 quadruplex. *Int J Biol Macromol* **31,** 131-137.
Abstract: Telomeric guanine-rich sequence can adopt quadruplex structures that are important for their biological role in chromosomal stabilisation. G quartets are characterised by the cyclic hydrogen bonding of four guanine bases in a coplanar arrangement and their stability is ion-dependent. In this work we compare the stability of [d(TGGGT)](4) and [d(T*GGGT)](4) quadruplexes. The last one contains a modified thymine, where the hydroxyl group substitutes one hydrogen atom of the methyl group of the thymine in the [d(TGGGT)](4) sequence. We used a combination of spectroscopic, calorimetric and computational techniques to characterise the G-quadruplex formation. NMR and CD spectra of [d(T*GGGT)](4) were characteristic of parallel-stranded, tetramolecular quadruplex. CD and DSC melting experiments reveal that [d(T*GGGT)](4) is less stable that unmodified quadruplex. Molecular models suggest possible explanation for the observed behaviour.

Petraccone L., Duro I., Erra E., Randazzo A., Virno A. and Giancola C. (2007) Effect of the incorporation of 2'-deoxy-8-(hydroxyl)adenosine on the stability of quadruplexes formed by modified human telomeric DNA. *Nucleosides Nucleotides Nucleic Acids* **26,** 675-679.
Abstract: Differential scanning calorimetry (DSC) and circular dichroism (CD) techniques were used to investigate the physico-chemical properties of the quadruplexes formed by the two different truncations of human telomeric sequence d(TAGGGT) and d(AGGGT), where the adenines were substituted by 2'-deoxy-8-(hydroxyl)adenosine (A --> A(OH)). CD spectra show that the modified sequences are able to form parallel-stranded quadruplex structure. Analysis of the thermodynamic parameters reveals that the introduction of the modified adenine affects in different way the thermal stability of the [d(TAGGGT)](4) and [d(AGGGT)](4) quadruplexes.

Plum G. E., Park Y. W., Singleton S. F., Dervan P. B., and Breslauer K. J. (1990) Thermodynamic characterization of the stability and the melting behavior of a DNA triplex: a spectroscopic and calorimetric study. *Proc Natl Acad Sci U S A* **87,** 9436-9440.
Abstract: We report a complete thermodynamic characterization of the stability and the melting behavior of an oligomeric DNA triplex. The triplex chosen for study forms by way of major-groove Hoogsteen association of an all-pyrimidine 15-mer single strand (termed y15) with a Watson-Crick 21-mer duplex composed of one purine-rich strand (termed u21) and one pyrimidine-rich strand (termed y21). We find that the near-UV CD spectrum of the triplex can be duplicated by the addition of the B-like CD spectrum of the isolated 21-mer duplex and the CD spectrum of the 15-mer single strand. Spectroscopic and calorimetric measurements show that the triplex (y15.u21.y21) melts by two well-resolved sequential transitions. The first transition (melting temperature, Tm, approximately 30 degrees C) is pH-dependent and involves the thermal expulsion of the 15-mer strand to form the free duplex u21.y21 and the free single strand y15. The second transition (Tm approximately 65 degrees C) is pH-independent between pH 6 and 7 and reflects the thermal disruption of the u21.y21 Watson-Crick duplex to form the component single strands. The thermal stability of the y15.u21.y21 triplex increases with increasing Na+ concentration but is nearly independent of DNA strand concentration. Differential scanning calorimetric measurements at pH 6.5 show the triplex to be enthalpically stabilized by only 2.0 +/- 0.1 kcal/mol of base triplets (1 cal = 4.184 J), whereas the duplex is stabilized by 6.3 +/- 0.3 kcal/mol of base pairs. From the calorimetric data, we calculate that at 25 degrees C the y15.u21.y21 triplex is stabilized by a free energy of only 1.3 +/- 0.1 kcal/mol relative to its component u21.y21 duplex and y15 single strand, whereas the 21-mer duplex is stabilized by a free energy of 17.2 +/- 1.2 kcal/mol relative to its component single strands. The y15 single strand modified by methylation of cytosine at the C-5 position forms a triplex with the u21.y21 duplex, which exhibits enhanced thermal stability. The spectroscopic and calorimetric data reported here provide a quantitative measure of the influence of salt, temperature, pH, strand concentration, and base modification on the stability and the melting behavior of a DNA triplex. Such information should prove useful in

designing third-strand oligonucleotides and in defining solution conditions for the effective use of triplex structure formation as a tool for modulating biochemical events.

Plum G. E., Grollman A. P., Johnson F., and Breslauer K. J. (1995) Influence of the oxidatively damaged adduct 8-oxodeoxyguanosine on the conformation, energetics, and thermodynamic stability of a DNA duplex. *Biochemistry* **34,** 16148-16160.
Abstract: As part of an overall program to characterize the impact of mutagenic lesions on the physiochemical properties of DNA, we report here the results of a comparative spectroscopic and calorimetric study on a family of DNA duplexes both with and without the oxidative lesion 2'-deoxy-7-hydro-8-oxoguanosine (8-oxodG). Specifically, we have studied a family of eight 13-mer duplexes of the form [5'-GCGTAC[G* or G]CATGCG-3'].[3'-CGCATG[C, A, T, or G]GTACGC-5'] in which G* is the 8-oxodG lesion. These eight duplexes, which we designate by the identity of the variable central base pair (e.g., G*C), reflect two subsets: four duplexes in which the modified guanine base is positioned opposite each of the four possible canonical residues (G*C, G*A, G*G, G*T) and the corresponding four "control" duplexes in which the guanine is not modified (GC, GA, GG, GT). The data derived from our spectroscopic and calorimetric measurements on these eight duplexes allow us to evaluate the influence of the 8-oxodG lesion, as well as the base opposite the lesion, on the conformation, the thermal and thermodynamic stability, and the melting thermodynamics of the host DNA duplex. We find that modification of dG to 8-oxodG (G*) does not change the global DNA duplex conformation as judged by circular dichroism spectra. Despite this structural similarity, our data reveal that the dG to dG* modification does influence duplex thermal and thermodynamic properties, some of which depend on the base opposite the lesion. Thus, apparent structural identity does not mean that two duplexes necessarily will exhibit equivalent thermal and/or thermodynamic properties. In general, we find that the thermodynamic effects induced by the lesion (e.g., GC vs G*C) or by mismatched base pairs (e.g., GC vs GG) can result in relatively large changes in enthalpy which are partially or wholly compensated entropically to produce relatively modest changes in free energy. Our data also suggest that the biologically observed differential recognition of 8-oxodG duplexes and the preferential nucleotide insertion opposite 8-oxodG residues cannot be rationalized simply in terms of large thermodynamic differences.

Plum G. E. and Breslauer K. J. (1995) Thermodynamics of an intramolecular DNA triple helix: a calorimetric and spectroscopic study of the pH and salt dependence of thermally induced structural transitions. *J Mol Biol* **248,** 679-695.
Abstract: We have characterized thermodynamically the melting transitions of a DNA 31-mer oligonucleotide (5'-GAAGAGGTTTTTCCTCTTCTTTTTCTTCTCC-3') which is designed to fold into an intramolecular triple helix. The first 19 residues fold back on themselves to form an antiparallel Watson-Crick hairpin duplex with a T5 loop. The 3'-terminal seven residues, which are connected to the Watson-Crick hairpin duplex by a second T5 loop, form Hoogsteen interactions in the major groove of the Watson-Crick hairpin. From ultraviolet (UV) melting studies we find that the 31-mer exhibits either one or two transitions, depending on solution conditions. We use pH- and temperature-dependent circular dichroism (CD) to assign the initial and final states associated with each transition. We find that the disruption of the Hoogsteen hairpin is accompanied by a release of protons and an uptake of sodium ions while the disruption of the Watson-Crick hairpin is accompanied by a release of sodium ions with no change in protonation state. From these data, we construct a phase diagram for this intramolecular DNA triple helix as a function of pH, sodium ion concentration, and temperature. We characterize the energetics of each transition using a van't Hoff analysis and differential scanning calorimetry (DSC). Significantly, the DSC data provide a model-independent thermodynamic characterization of the thermally induced transitions of this triplex. By combining the spectroscopic and calorimetric data, we develop a semi-empirical model which describes the state of the 31-mer as a function of pH, sodium ion concentration, and temperature. With this model we successfully predict characteristics of the 31-mer, which are beyond the data which are used in establishing the model (for example, the salt dependence of the apparent pKa of the Hoogsteen strand). This semi-empirical model may serve as a prototype for developing a method to predict the phase diagrams of intramolecular triple helix systems.

Plum G. E., Pilch D. S., Singleton S. F., and Breslauer K. J. (1995) Nucleic acid hybridization: triplex stability and energetics. *Annu Rev Biophys Biomol Struct* **24,** 319-350.
Abstract: In this chapter, we review the current state of the thermodynamic database for triple helical

oligonucleotide hybridization reactions and present a critical assessment of the methods used to obtain the relevant data. The thermodynamic stability of triple-helix oligonucleotide constructs is discussed in terms of its dependence on temperature, chain length, pH, salt, base sequence, base and backbone modifications, and ligand binding. In particular, we examine the coupling of hybridization equilibria to proton, cation, and drug-binding equilibria. Throughout the chapter, we emphasize that a detailed understanding of the endogenous and exogenous variables that control triplex stability is required for the rational design of oligonucleotides for specific therapeutic, diagnostic, and/or biotechnological applications, as well as for elucidating the potential cellular roles of these higher-order nucleic acid complexes.

Plum G. E., Gelfand C. A., and Breslauer K. J. (1999) Effects of 3,N4-ethenodeoxycytidine on duplex stability and energetics. *IARC Sci Publ* 169-177.
Abstract: The exocyclic cytosine adduct 3,N4-ethenocytosine is highly mutagenic in mammalian cells. We describe the impact of this adduct on DNA duplex stability. The adduct does not disrupt the overall B-form DNA structure; however, structural accommodation of the adduct is necessary at the lesion site. Despite the relatively small structural perturbation imparted by the adduct, there is a large adduct-induced destabilization of the DNA duplex. This destabilization is observed to be independent of the cross-strand partner base and neighbouring base pairs. The thermodynamic origins of the destabilization are, however, strongly dependent on the cross-strand partner base and neighbouring base pairs. Comparisons are made between the impact of the 3,N4-ethenocytosine adduct and other lesions on DNA thermodynamics. The lesions are similar in that all result in destabilization of the DNA duplex. The magnitudes and the thermodynamic origins of that destabilization vary widely, the 3,N4-ethenocytosine adduct being dramatically more destabilizing than other lesions. The impact of damaged sites on the stability of the DNA helix suggests that energetic differences between damaged and normal DNA may contribute to the recognition of damage by the cellular DNA repair machinery.

Pope L. H., Davies M. C., Laughton C. A., Roberts C. J., Tendler S. J., and Williams P. M. (2001) Force-induced melting of a short DNA double helix. *Eur Biophys J* **30,** 53-62.
Abstract: The dynamic behaviour of DNA is of fundamental importance to many cellular processes. One principal characteristic, central to transcription and replication, is the ability of the duplex to "melt". It has recently been shown that dynamic force spectroscopy provides information about the energetics of biomolecular dissociation. We have employed this technique to investigate the unbinding of single dodecanucleotide molecules. To separate the duplex to single-stranded DNA, forces ranging from 17 to 40 pN were required over a range of loading rates. Interpretation of the dependence of melting force on loading rate revealed that the energy barrier to rupture is between 9 and 13 kcal mol-1 in height and situated 0.58 nm from an intermediate structural state. Thermal melting studies show that, prior to dissociation, the oligonucleotide underwent a transition which required between 7 and 11 kcal mol^{-1} in energy. Through combined dynamic force spectroscopy and thermal melting studies we show the derivation of an energy landscape to dissociate a 12-mer duplex. Until very recently, this type of information was only accessible by computational analysis. Additionally, the force spectroscopy data allow an estimation of the kinetics of duplex formation and melting.

Prislan I., Lah J. and Vesnaver G. (2008) Diverse polymorphism of G-quadruplexes as a kinetic phenomenon. *J Am. Chem Soc.* **130,** 14161-14169.
Abstract: Knowledge of forces that drive conformational transitions of G-quadruplexes is crucial for understanding the molecular basis of several key cellular processes. It can only be acquired by combining structural, thermodynamic and kinetic information. Existing biophysical and structural evidences on polymorphism of intermolecular G-quadruplexes have shown that the formation of a number of these structures is a kinetically controlled process. Reported kinetic models that have been used to describe the association of single strands into quadruplex structures seem to be inappropriate since the corresponding model-predicted activation energies turn out to be negative. By contrast, we propose here a novel kinetic model that successfully describes experimentally monitored folding/unfolding transitions of G-quadruplexes and gives positive activation energies for all elementary steps, including those describing association of two single strands into bimolecular quadruplex structures. It is based on a combined thermodynamic and kinetic investigation of polymorphic behavior of bimolecular G-quadruplexes formed from d(G4T4G4) and d(G4T4G3) strands in the presence of Na(+) ions, monitored by spectroscopic (UV, CD) and calorimetric (DSC) techniques. According to our experiment and model analysis the topology of

the measured G-quadruplexes is clearly flexible with the conformational forms that respond to the rate of temperature change at which global unfolding/folding transitions occur

Ramprakash J. and Schwarz F. P. (2008) Energetic contributions to the initiation of transcription in E. coli. *Biophys Chem* **138,** 91-98.
Abstract: The thermodynamics of RNA polymerase (RNAP) binding to a 108 base pair (bp) synthetic promoter with consensus sequences at the -35 and -10 bp binding regions upstream from the transcription start point were determined using isothermal titration calorimetry (ITC). The binding constant at 25 degrees C is 2.37+/-0.18x10(7) M(-1), which is reduced to 0.17+/-0.06x10(7) M(-1) with mutations in the -10 bp region but remained the same with mutations in the -35 binding region. The binding reactions were enthalpically-driven with exothermic binding enthalpies ranging from -57+/-6 kJ mol(-1) at 15 degrees C to -271+/-20 kJ mol(-1) at 35 degrees C yielding a large binding heat capacity change of -10.7+/-1.9 kJ mol(-1) K(-1), indicating a conformational change upon binding to the RNAP. Differential scanning calorimetry (DSC) scans of the thermal unfolding of RNAP and the promoter-RNAP complex exhibited an unfolding transition at 55.5+/-0.6 degrees C and at 58.9+/-0.5 degrees C for the RNAP but only one transition at 60.5+/-1.1 degrees C for the complex with van't Hoff enthalpy to transition enthalpy ratios of, resp., 3.2+/-0.3 and 4.3+/-0.5. The single transition of the complex results from a shift to 60.5 degrees C of the low temperature transition upon promoter binding to the structural unit unfolding at the lower temperature in RNAP. The large transition enthalpy ratios indicate that the sigma, alpha, alpha, beta, and beta' subunits unfold as almost independent entities. The dissociation thermodynamics of short transcription "bubble" duplexes of 7 promoters sequenced from -1 to -12 bp were determined from ITC and DSC measurements. The free energy change of the promoter binding to the RNAP and the free energy requirement for formation of the transcription bubble at the low promoter concentrations in the cell are sufficient to drive the initiation of transcription through the isomerization of the closed to the open form step of the RNAP-promoter complex

Ramprakash J., Lang B. and Schwarz F. P. (2008) Thermodynamics of single strand DNA base stacking. *Biopolymers* **89,** 969-979.
Abstract: The thermodynamics of the stacking to unstacking transitions of 24 single-stranded DNA sequences (ssDNA), 10-12 bases in length, in sodium phosphate buffer were determined from 10 to 95 degrees C, using differential scanning calorimetry (DSC). An additional 22 ssDNA sequences did not exhibit an S<=>U transition in this temperature range. The transition properties of the ssDNA sequences with <or=60% self-complementarily in the reverse direction were independent of concentration with transition temperatures ranging from 15 to 70 degrees C, van't Hoff transition enthalpies from 92 to 201 kJ mol(-1) and transition enthalpies from 5 to 75% of the corresponding van't Hoff transition enthalpies. Since all the 16 doublets and 60 of the 64 triplets are present in both the transition and the non-transition ssDNA sequences, it is unlikely that the nucleation subset initiating stacking of the sequence is a specific doublet or triplet subset. Of the 141 quadruplet subsets of the 46 sequences, each transition ssDNA sequence contained at least one or more quadruplets not found in the non-transition ssDNA sequences. It could be concluded that the thermal stability of the stacked conformation was dependent on the presence of a possible nucleation quadruplet and the length of the ssDNA sequence and not on the G or C content of the ssDNA sequence, nor on the number of purine bases in the sequence

Rauch J., Wolf D., Hausmann M., and Cremer C. (2000) The influence of formamide on thermal denaturation profiles of DNA and metaphase chromosomes in suspension. *Z Naturforsch [C]* **55,** 737-746.
Abstract: Systematic photometric studies are presented to analyze the thermal denaturation behaviour with and without formamide of metaphase chromosome suspensions in comparison to DNA solutions. Temperature dependent hyperchromicity measurements at 256 nm and 313 nm were performed using an appropriately designed computer-controlled photometer device. Due to an upright optical axis, this allowed absorbance measurements with negligible sedimentation effects not only for solutions of pure DNA, but also for particle suspensions of isolated metaphase chromosomes. This device has a temperature resolution of +/- 0.5 degrees C and an optical sensitivity of $10^{-3}$to 10^{-4} optical density. For calf thymus DNA the reduction of the melting point with the increase of formamide in the solution was measured at pH 7.0 and pH 3.2. The good correlation of the theoretical approximation to experimental data indicated the suitability of the apparatus to quantitatively describe DNA conformation changes induced by thermal denaturation. For metaphase chromosome preparations of Chinese hamster culture cells, absorbance changes were

measured between 20 degrees C and 95 degrees C with a temperature gradient of 1 degrees C/min. These measurements were performed at pH 7.0 and at pH 3.2. The denaturation profiles (= first derivative of the absorbance curve) resulted in a highly variable peak pattern at 256 nm and 313 nm indicating complex conformation changes. A statistical evaluation of the temperature values of the peak maxima resulted in temperature ranges typical for chromosomal conformation changes during thermal treatment. Especially the range of highest temperature values was independent from pH modifications. For pH 3.2 the influence of formamide on the denaturation behaviour of metaphase chromosome preparations was analyzed. In contrast to pure DNA solutions, a reduction of the "melting point" (i.e. the maximum temperature at which a conformation change takes place) was not found. However, the denaturation behaviour depended on the duration of formamide treatment before the measurement.

Ren J., Qu X., Chaires J. B., Trempe J. P., Dignam S. S., and Dignam J. D. (1999) Spectral and physical characterization of the inverted terminal repeat DNA structure from adenoassociated virus 2. *Nucleic Acids Res* **27,** 1985-1990.
Abstract: An oligodeoxynucleotide (ODN) that includes elements found in secondary structures at the 5'- and 3'- ends of adenoassociated virus 2 virion DNA was synthesized by ligation of three overlapping ODNs. The most stable secondary structure was calculated to be branched, with a 61 bp duplex stem, terminating in a three-way junction with 9 bp arms. The electrophoretic mobility of the ODN is slower than expected for normal duplex DNA of the same size, suggesting a bent or branched conformation. CD spectra indicate that the ITR structure is largely B form DNA, although there is a slight blue shift compared to the spectra of the isolated stem and loop elements. Thermal melting experiments indicate that the hairpin is significantly more stable than the isolated stem and loop elements. Singular value decomposition of UV spectra obtained as a function of temperature indicates that four species contribute to changes in the spectra upon denaturation, indicating that the melting is not a simple two-state process. Characterization of the branched ODN by differential scanning calorimetry permits estimation of the enthalpy of melting by a model-free analysis, yielding ΔHcal= 614 kcal mol^{-1}. This value agrees with the enthalpy computed for the most stable secondary structure.

Ren J., Qu X., Trent J. O., and Chaires J. B. (2002) Tiny telomere DNA. *Nucleic Acids Res* **30,** 2307-2315.
Abstract: We describe the design, synthesis and biophysical characterization of a novel DNA construct in which a folded quadruplex structure is joined to a standard double helix. Circular dichroism, gel electrophoresis, three-dimensional UV melting and differential scanning calorimetry were all used to characterize the structure. Rigorous molecular dynamics simulations were used to build a plausible atomic-level structural model of the DNA construct. This novel DNA construct provides a model for the duplex-quadruplex junction region at the end of chromosomal DNA and offers a system for the study of structure-selective ligand binding.

Riccelli P. V., Hilario J., Gallo F. J., Young A. P., and Benight A. S. (1996) DNA and RNA oligomer sequences from the 3' noncoding region of the chicken glutamine synthetase gene from intramolecular hairpins. *Biochemistry* **35,** 15364-15372.
Abstract: The DNA sequence of the chicken glutamine synthetase gene contains an A.T-rich stretch of approximately 1500 base pairs in the 3' noncoding regions of exon 7 [Pu, H., & Young, A. P. (1989) Gene 18, 169-175]. Within this region several palindromic sequences occur that could conceivably form intramolecular structures. One such perfect inverted repeat sequence resides between positions 2605 and 2623. To investigate the hairpin-forming potential for this sequence, optical and calorimetric melting and gel electrophoresis studies have been performed on the following synthetically prepared DNA and RNA oligomer subsequences: DNA, 5'd-T-T-T-T-T-T-A-A-T-A-A-T-T-A-A-A-A-A-A-3'; and RNA, 5'r-U-U-U-U-U-U-A-A-U-A-A-U-U-A-A-A-A-A-A-3'. The DNA strand corresponds to the coding strand sequence while the RNA strand represents the transcribed mRNA. Results of melting analysis of these 19-base, partially self-complementary strands performed in 115 mM Na+ yielded evaluations of their thermodynamic transition parameters. These values are consistent with the melting of unimolecular structures, presumably hairpins. Thermodynamic parameters evaluated by analysis of the optical melting transitions assuming a two-state model and measured directly by differential scanning calorimetry agreed within experimental error. Therefore, melting behavior of the hairpins is all-or-none like. The DNA hairpin is slightly more stable than the RNA hairpin with melting enthalpy Δ H0 = 41.2 +/- 3.8 kcal/mol and entropy Δ S0 = 133 +/- 11 cal/K.mol (eu) compared to Δ H0 = 32.0 +/- 6.0 kcal/mol and entropy Δ S0 =

105 +/- 20 eu for the RNA. Gel electrophoretic analysis of these oligomers alone and in various mixtures with their DNA and RNA complementary strands was also performed. Consistent with interpretations of melting results, these experiments revealed both strands alone preferentially form intramolecular hairpin structures. In mixtures in which their complementary strands are in vast molar excess (stoichiometric ratios > 10:1), the intramolecular structures are converted to intermolecular duplexes. For the DNA and RNA strands examined, the conversion is not complete until over a 1000-fold excess of the complementary strand is added. Semiquantitative analysis of gel electrophoretograms enabled evaluations of the relative free energies of the hairpin and duplex states as a function of complementary strand concentration. With the finding that these sequences preferentially form hairpins, potential roles these structures could play in regulatory activities are considered.

Riccelli P. V., Vallone P. M., Kashin I., Faldasz B. D., Lane M. J., and Benight A. S. (1999) Thermodynamic, spectroscopic, and equilibrium binding studies of DNA sequence context effects in six 22-base pair deoxyoligonucleotides. *Biochemistry* **38,** 11197-11208.
Abstract: Effects of different end sequences on stability, circular dichroism spectra (CD), and enzyme binding properties were investigated for six 22-base pair, non-self-complementary duplex DNA oligomers. The center sequences of these deoxyoligonucleotides have 8-14 base pairs in common and are flanked on both sides by sequences differing in context and A-T content. Temperature-induced melting transitions monitored by differential scanning calorimetry (DSC) and ultraviolet absorbance were measured for the six duplexes in buffered 115 mM Na(+) solutions. Values of the melting transition enthalpy, ΔH(cal), and entropy, ΔS(cal), were obtained directly from DSC experiments. Melting transition parameters, ΔH(vH) and ΔS(vH), were also estimated from van't Hoff analysis of optical melting curves collected as a function of DNA concentration, assuming a two-state melting transition. Melting free energies (20 degrees C) of the six DNAs evaluated from DSC experiments ranged from -18.7 to -32.7 kcal/mol. van't Hoff estimates of the free energies ranged from -18.5 to -48.0 kcal/mol. With either method, the trends in free energy as a function of sequence were identical. Equilibrium binding by BamHI restriction endonuclease to the 22-base pair DNAs was also investigated. The central eight base pairs of all six molecules, 5'-A-GGATCC-A-3', contained a BamHI recognition sequence bounded by A-T base pairs. Magnesium free binding assays were performed by titering BamHI against a constant concentration of each of the deoxyoligonucleotide substrates and analyzing reaction products by gel retardation. Binding isotherms of the total amount of bound DNA versus protein concentration were constructed which provided semiquantitative estimates of the equilibrium dissociation constants for dissociation of BamHI from the six DNA oligomers. Dissociation constants ranged from 0.5 x 10^{-9} to 12.0 x 10^{-9} M with corresponding binding free energies of -12.5 to -10.6 (+/-0. 1) kcal/mol. An inverse relationship is found when binding and stability are compared.

Riccelli P. V., Mandell K. E., and Benight A. S. (2002) Melting studies of dangling-ended DNA hairpins: effects of end length, loop sequence and biotinylation of loop bases. *Nucleic Acids Res* **30,** 4088-4093.
Abstract: The effects of 3' single-strand dangling-ends of different lengths, sequence identity of hairpin loop, and hairpin loop biotinylation at different loop residues on DNA hairpin thermodynamic stability were investigated. Hairpins contained 16 bp stem regions and five base loops formed from the sequence, 5'-TAGTCGACGTGGTCC-N5-GGACCACGTCGACTAG-E(n)-3'. The length of the 3' dangling-ends (E(n)) was n = 13 or 22 bases. The identities of loop bases at positions 2 and 4 were varied. Biotinylation was varied at loop base positions 2, 3 or 4. Melting buffers contained 25 or 115 mM Na+. Average Tm values for all molecules were 73.5 and 84.0 degrees C in 25 and 115 mM Na+, respectively. Average two-state parameters evaluated from van't Hoff analysis of the melting curve shapes in 25 mM Na+ were ΔH(vH) = 84.8 +/- 15.5 kcal/mol, ΔS(vH) = 244.8 +/- 45.0 cal/K.mol and ΔG(vH) = 11.9 +/- 2.1 kcal/mol. In 115 mM Na+, two-state parameters were not very different at ΔH(vH) = 80.42 +/- 12.74 kcal/mol, ΔS(vH) = 225.24 +/- 35.88 cal/K.mol and ΔG(vH) = 13.3 +/- 2.0 kcal/mol. Differential scanning calorimetry (DSC) was performed to test the validity of the two-state assumption and evaluated van't Hoff parameters. Thermodynamic parameters from DSC measurements (within experimental error) agreed with van't Hoff parameters, consistent with a two-state process. Overall, dangling-end DNA hairpin stabilities are not affected by dangling-end length, loop biotinylation or sequence and vary uniformly with [Na+]. Consider able freedom is afforded when designing DNA hairpins as probes in nucleic acid based detection assays, such as microarrays.

Riccelli P. V., Hall T. S., Pancoska P., Mandell K. E., and Benight A. S. (2003) DNA sequence context and multiplex hybridization reactions: melting studies of heteromorphic duplex DNA complexes. *J Am Chem Soc* **125,** 141-150.
Abstract: Heteromorphic hybrid duplex DNA complexes are duplex states, other than perfectly matched duplexes, that can form when single strands comprising several different perfectly matched duplexes are simultaneously present in solution. Such cross-hybridization "side reactions" are of particular nuisance in multiplex reaction schemes, where many strands are designed to hybridize in parallel fashion with only their corresponding perfect complement strand. Relative to the perfect match duplexes, the sequence dependent features of these heteromorphic duplex states and their thermodynamic stability are an important consideration for multiplex hybridization reaction design. We have measured absorbance versus temperature melting curves and performed differential scanning calorimetry measurements on various mixtures of eight different 24 base single strands. When perfect complementary pairs of strands are mixed in single reactions, four perfectly matched duplexes form. When mixtures of strands that are not perfectly matched are prepared and analyzed, melting transitions for cross-hybridization are observed along with significant hyperchromicity changes. This is indicative of a melting hybrid, heteromorphic duplex states formed from two nonperfectly matched strands. In addition, when both the perfectly matched and noncomplementary strands are mixed together (in multiplex hybridization reactions) at molar ratios of 1:1, 3:1, and 1:3, evidence of perfect duplex and heteromorphic duplex complexes is found in all cases. A new analytical tool for considering heterogeneous, duplex complexes in multiplex hybridization mixtures is presented and employed to interpret the acquired melting data.

Roberts R. W. and Crothers D. M. (1996) Prediction of the stability of DNA triplexes. *Proc Natl Acad Sci U S A* **93,** 4320-4325.
Abstract: We present rules that allow one to predict the stability of DNA pyrimidine.purine.pyrimidine (Y.R.Y) triple helices on the basis of the sequence. The rules were derived from van't Hoff analysis of 23 oligonucleotide triplexes tested at a variety of pH values. To predict the enthalpy of triplex formation (Δ H degrees), a simple nearest-neighbor model was found to be sufficient. However, to accurately predict the free energy of the triplex (Δ G degrees), a combination model consisting of five parameters was needed. These parameters were (i) the Δ G degrees for helix initiation, (ii) the Δ G degrees for adding a T-A.T triple, (iii) the Δ G degrees for adding a C(+)-G.C triple, (iv) the penalty for adjacent C bases, and (v) the pH dependence of the C(+)-G.C triple's stability. The fitted parameters are highly consistent with thermodynamic data from the basis set, generally predicting both Δ H degrees and Δ G degrees to within the experimental error. Examination of the parameters points out several interesting features. The combination model predicts that C(+) -G.C. triples are much more stabilizing than T-A.T triples below pH 7.0 and that the stability of the former increases approximately equal to 1 kcal/mol per pH unit as the pH is decreased. Surprisingly though, the most stable sequence is predicted to be a CT repeat, as adjacent C bases partially cancel the stability of one another. The parameters successfully predict tm values from other laboratories, with some interesting exceptions.

Ross P. D. and Howard F. B. (2003) The thermodynamic contribution of the 5-methyl group of thymine in the two- and three-stranded complexes formed by poly(dU) and poly(dT) with poly(dA). *Biopolymers* **68,** 210-222.
Abstract: To assess the thermodynamic contribution of the 5-methyl group of thymine, we have studied the two-stranded helical complexes poly(dA).poly(dU) and poly(dA).poly(dT) and the three-stranded complexes--poly(dA).2poly(dU), poly(dA).poly(dT).poly(dU) and poly(dA).2poly(dT)--by differential scanning calorimetry, and uv optical melting experiments. The thermodynamic quantities associated with the 3 --> 2, 2 --> 1, and 3 --> 1 melting transitions are found to vary with salt concentration and temperature in a more complex manner than commonly believed. The transition temperatures, Tm, are generally not linear in the logarithm of concentration or activity of NaCl. The change in enthalpy and in entropy upon melting varies with salt concentration and temperature, and a change in heat capacity accompanies each transition. The poly(dA).2poly(dU) triple helix is markedly different from poly(dA).2poly(dT) in both its CD spectrum and thermodynamic behavior, while the poly(dA).poly(dT).poly(dU) triple helix resembles poly(dA).2poly(dT) in these properties. In comparing poly(dA).2poly(dT) with either the poly(dA).poly(dT).poly(dU) or the poly(dA).2poly(dU) triplexes, the substitution of thymine for uracil in the third strand results in an enhancement of stability against the 3 --> 2 dissociation of $\Delta\Delta$G degrees = -135 +/- 85 cal (mol A)$^{-1}$ at 37 degrees C. This represents a doubling of

the absolute stability toward dissociation compared to the triplexes with poly(dU) as the third strand. The poly (dA).poly (dT) duplex is more stable than poly(dA).poly(dU) by ΔΔG degrees = -350 +/- 60 cal (mol base pair) $^{-1}$ at 37 degrees C. Poly(dA).poly(dT) has 50% greater stability than poly(dA).poly(dU) as a result of the dT for dU substitution in the duplex.

Rouzina I. and Bloomfield V. A. (1999) Heat capacity effects on the melting of DNA. 1. General aspects. *Biophys J* **77,** 3242-3251.
Abstract: In this paper we analyze published data on ΔH and ΔS values for the DNA melting transition under various conditions. We show that there is a significant heat capacity increase ΔCp associated with DNA melting, in the range of 40-100 cal/mol K per base pair. This is larger than the transition entropy per base pair, ΔS(0) approximately 25 cal/mol K. The ratio of ΔCp/ΔS(0) determines the importance of heat capacity effects on melting. For DNA this ratio is 2-4, larger than for many proteins. We discuss how ΔCp values can be extracted from experimental data on the dependence of ΔH and ΔS on the melting temperature Tm. We consider studies of DNA melting as a function of ionic strength and show that while polyelectrolyte theory provides a good description of the dependence of Tm on salt, electrostatics alone cannot explain the accompanying strong variation of ΔH and ΔS. While Tm is only weakly affected by ΔCp, its dependence on one parameter (e.g., salt) as a function of another (e.g., DNA composition) is determined by ΔCp. We show how this accounts for the stronger stabilization of AT relative to GC base pairs with increasing ionic strength. We analyze the source of discrepancies in ΔH as determined by calorimetry and van't Hoff analysis and discuss ways of analyzing data that yield valid van't Hoff ΔH. Finally, we define a standard state for DNA melting, the temperature at which thermal contributions to ΔH and ΔS vanish, by analyzing experimental data over a broad range of stabilities.

Rouzina I. and Bloomfield V. A. (2001) Force-induced melting of the DNA double helix. 2. Effect of solution conditions. *Biophys J* **80,** 894-900.
Abstract: In this paper, we consider the implications of the general theory developed in the accompanying paper, to interpret experiments on DNA overstretching that involve variables such as solution temperature, pH, and ionic strength. We find the DNA helix-coil phase boundary in the force-temperature space. At temperatures significantly below the regular (zero force) DNA melting temperature, the overstretching force, f(ov)(T), is predicted to decrease nearly linearly with temperature. We calculate the slope of this dependence as a function of entropy and heat-capacity changes upon DNA melting. Fitting of the experimental f(ov)(T) dependence allows determination of both of these quantities in very good agreement with their calorimetric values. At temperatures slightly above the regular DNA melting temperature, we predict stabilization of dsDNA by moderate forces, and destabilization by higher forces. Thus the DNA stretching curves, f(b), should exhibit two rather than one overstretching transitions: from single stranded (ss) to double stranded (ds) and then back at the higher force. We also predict that any change in DNA solution conditions that affects its melting temperature should have a similar effect on DNA overstretching force. This result is used to calculate the dependence of DNA overstretching force on solution pH, f(ov)(pH), from the known dependence of DNA melting temperature on pH. The calculated f(ov)(pH) is in excellent agreement with its experimental determination (M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, Biophys. J., accepted for publication). Finally, we quantitatively explain the measured dependence of DNA overstretching force on solution ionic strength for crosslinked and noncrosslinked DNA. The much stronger salt dependence of f(ov) in noncrosslinked DNA results from its lower linear charge density in the melted state, compared to crosslinked or double-stranded overstretched S-DNA.

Rumora A. E., Kolodziejczak K. M., Malhowski W. A. and Nunez M. E. (2008) Thymine Dimer-Induced Structural Changes to the DNA Duplex Examined with Reactive Probes. *Biochemistry (epublication).*
Abstract: Despite significant progress in the past decade, questions still remain about the complete structural, dynamic, and thermodynamic effect of the cis-syn cyclobutane pyrimidine dimer lesion (hereafter called the thymine dimer) on double-stranded genomic DNA. We examined a 19-mer oligodeoxynucleotide duplex containing a thymine dimer lesion using several small, base-selective reactive chemical probes. These molecules probe whether the presence of the dimer causes the base pairs to be more accessible to the solution, either globally or adjacent to the dimer. Though all of the probes confirm that the overall structure of the dimer-containing duplex is conserved compared to that of the undamaged parent duplex, reactions with both diethyl pyrocarbonate and Rh(bpy) 2(chrysi) (3+) indicate that the duplex is locally destabilized near the lesion. Reactions with potassium permanganate and DEPC hint that the dimer-

containing duplex may also be globally more accessible to the solution through a subtle shift in the double-stranded DNA <--> single-stranded DNA equilibrium. To begin to distinguish between kinetic and thermodynamic effects, we determined the helix melting thermodynamic parameters for the dimer-containing and undamaged parent duplexes by microcalorimetry and UV melting. The presence of the thymine dimer causes this DNA duplex to be slightly less stable enthalpically but slightly less unstable entropically at 298 K, causing the overall free energy of duplex melting to remain unchanged by the dimer lesion within the error of the experiment. Here we consider these results in the context of what has been learned about the thymine dimer lesion from NMR, X-ray crystallographic, and molecular biological methods

Sacca B., Lacroix L., and Mergny J. L. (2005) The effect of chemical modifications on the thermal stability of different G-quadruplex-forming oligonucleotides. *Nucleic Acids Res* **33,** 1182-1192.
Abstract: A systematic study of the thermal and conformational properties of chemically modified G-quadruplexes of different molecularities is reported. The effect of backbone charge and atom size, thymine/uracyl substitution as well as the effect of modification at the ribose 2'-position was analyzed by UV spectroscopy. Additional calorimetric studies were performed on different modified forms of the human telomeric sequence. Determination of the differential spectra allowed more insights into the conformational properties of the oligonucleotides. Lack of negative charge at the phosphate backbone yielded to a general destabilization of the G-quadruplex structure. On the other hand, substitution of thymine with uracyl resulted in a moderate or strong stabilization of the structure. Additional modification at the sugar 2'-position gave rise to different effects depending on the molecularity of the quadruplex. In particular, loss of hydrogen bond capacity at the 2'-position strongly affected the conformation of the G-quadruplex. Altogether, these results demonstrate that the effect of some modifications depends on the sequence context, thus providing helpful information for the use of chemically modified quadruplexes as therapeutic agents or as structural elements of supramolecular complexes.

SantaLucia J., Jr. and Turner D. H. (1997) Measuring the thermodynamics of RNA secondary structure formation. *Biopolymers* **44,** 309-319.
Abstract: The thermodynamics of RNA secondary structure formation in small model systems provides a database for predicting RNA structure from sequence. Methods for making these measurements are reviewed with emphasis on optical methods and treatment of experimental errors. Analysis of experimental results in terms of simple nearest-neighbor models is presented. Some measured sequence dependences of non-Watson-Crick motifs are discussed.

Scaria P. V. and Shafer R. H. (1996) Calorimetric analysis of triple helices targeted to the d(G3A4G3).d(C3T4C3) duplex. *Biochemistry* **35,** 10985-10994.
Abstract: We present a thermodynamic analysis based on differential scanning calorimetry (DSC) of three short intermolecular DNA triplexes targeted to the same DNA duplex: d(C+3T4C+3)*d-(G3A4G3).d(C3T4C3) (PYR), d(G3A4G3)*d(G3A4G3).d(C3T4C3) (PUR), and d(G3T4G3)*d(G3A4G3).d(C3T4C3) (PUR/PYR). Enthalpies, ΔH, and entropies, ΔS, are measured by model-free integration of the DSC curves and are compared to the same quantities determined by van't Hoff analysis of the DSC curves and, in the case of the PYR and PUR/PYR triplexes, UV melting curves as well. In the case of the PUR triplex, which exhibits monophasic melting behavior, the calorimetric ΔH and the calorimetrically determined van't Hoff ΔH are in excellent agreement, indicating an all-or-none transition for this triplex. For the PYR and PUR/PYR triplexes, which melt in a biphasic manner, the calorimetrically determined van't Hoff ΔH values are somewhat larger than the model-independent calorimetric ΔH values. In those cases, however, good agreement is found between the calorimetric ΔH values and the spectrophotometrically determined van't Hoff ΔH values. The calorimetrically determined ΔH values, expressed per mole of triplet, for the three triplexes are 4.5, 3.8, and 2.4 kcal/mol for the PUR, PYR, and PUR/PYR triplexes, respectively. The same order of stability is observed in terms of ΔG and Tm values. The high stability of the PUR triplex at neutral pH indicates that purine oligonucleotides may be the most effective at targeting duplex regions for triple helix formation in vivo.

Schoppe A., Hinz H. J., Rosemeyer H., and Seela F. (1996) Xylose-DNA: comparison of the thermodynamic stability of oligo(2'-deoxyxylonucleotide) and oligo(2'-deoxyribonucleotide) duplexes. *Eur J Biochem* **239,** 33-41.

Abstract: Measurements of differential scanning calorimetry, ultraviolet absorption and circular dichroism have been performed on two synthetic oligo(2'-deoxyxylonucleotides): (A) d[xA)3-(xT)3-(xA)3-(xT)3-T] and (B) d[(xA-xT)6-T], and on the oligo(2'-deoxyribonucleotide) (C) d[(A)3-(T)3-(A)3-(T)3]. Oligonucleotides having 2'-deoxyxylose instead of 2'-deoxyribose exhibit unusual thermodynamic, optical and structural features. At identical concentrations the transition temperatures of the oligo(2'-deoxyxylooligomers) are higher than those of the oligo(2'-deoxyribooligomers) indicating higher stability. The calorimetric transition enthalpy of (C) is 270 +/- 15 kJ . mol^{-1}, the corresponding van't Hoff value is 280 +/- 15 kJ . (mol of cooperative unit) $^{-1}$. The ratio of Δ HvH/Δ Hcal = 1.04 suggests all-or-none behaviour for the transition of the 2'-deoxyribose oligonucleotide. The analogous parameters of (A) are: Δ Hcal = 310 +/- 30 kJ . mol^{-1}, Δ HvH = 220 +/- 30 kJ.(mol of cooperative unit) $^{-1}$. The ratio of 0.71 indicates multistate melting for this compound. The sequence dependence of the thermodynamic quantities becomes apparent when the parameters of the alternating oligo(2'-deoxyxylonucleotide) d[(xA-xT)6-T] are compared to those of d[(xA)3-(xT)3-(xA)3-(xT)3-T). The values are Δ Hcal = 330 +/- 30 kJ.mol^{-1}; Δ HvH = 180 +/- 15 kJ.(mol of cooperative unit) $^{-1}$ Δ HvH/ Δ Hcal = 0.55. The transition enthalpy of the alternating oligo(2'-deoxyxylonucleotide) (B) is the highest but the cooperativity of transition is the lowest of the oligonucleotides studied. The circular dichroic spectra of the two oligo(2'-deoxyxylonucleotides) show unusual features in that d[(xA)3-(xT)3-(XA)3-(xT)3-T] exhibits a spectrum that is suggestive of a left-handed double helix, while the spectrum of the alternating oligo(2'-deoxyxylonucleotide) (B) resembles neither that of (C) nor that of (A).

Sengupta B., Uematsu T., Jacobsson P., and Swenson J. (2006) Exploring the antioxidant property of bioflavonoid quercetin in preventing DNA glycation: A calorimetric and spectroscopic study. *Biochem Biophys Res Commun* **339,** 355-361.
Abstract: Reducing sugars for example glucose, fructose, etc., and their phosphate derivatives non-enzymatically glycate biological macromolecules (e.g., proteins, DNA and lipids) and is related to the production of free radicals. Here we present a novel study, using differential scanning calorimetry (DSC) along with UV/Vis absorption and photon correlation spectroscopy (PCS), on normal and glycated human placenta DNA and have explored the antioxidant property of the naturally occurring polyhydroxy flavone quercetin (3,3',4',5,7-pentahydroxyflavone) in preventing the glycation. The decrease in the absorption intensity of DNA in presence of sugars clearly indicates the existence of sugar molecules between the two bases of a base pair in the duplex DNA molecule. Variations were perceptible in the PCS relaxation profiles of normal and glycated DNA. The melting temperature of placenta DNA was decreased when glycated suggesting a decrease in the structural stability of the double-stranded glycated DNA. Our DSC and PCS data showed, for the first time, that the dramatic changes in the structural properties of glycated DNA can be prevented to a significant extent by adding quercetin. This study provides valuable insights regarding the structure, function, and dynamics of normal and glycated DNA molecules, underlying the manifestation of free radical mediated diseases, and their prevention using therapeutically active naturally occurring flavonoid quercetin.

Shafer R. H. (1998) Stability and structure of model DNA triplexes and quadruplexes and their interactions with small ligands. *Prog Nucleic Acid Res Mol Biol* **59,** 55-94.
Abstract: This review focuses on the structural and thermodynamic characterization of model DNA triplex and quadruplex structures, taking into account effects of stoichiometry and sequence. Methods such as gel electrophoresis, UV melting, and scanning calorimetry, and the results thereof, are described for determination of the thermodynamic stability of such systems. Three classes of triplexes are considered based on the composition of the third strand, while quadruplex systems are limited to those based on the guanine quartet. X-ray crystallography and high resolution NMR studies are also described for these two classes of unusual structures. Ligand binding to triplexes and quadruplexes is also reviewed, with emphasis on specific molecular recognition. The availability of three-dimensional structures for triplex and quadruplex species sets the stage for structure-based development of ligands capable of binding to them specifically. To this end, we consider the application of DOCK, a program for the discovery of small molecules that can recognize macromolecular structures, to the problem of recognizing folded quadruplex structures. Such studies may ultimately lead to pharmaceutically active compounds.

Shaw N. N., Xi H. and Arya D. P. (2008) Molecular recognition of a DNA:RNA hybrid: sub-nanomolar binding by a neomycin-methidium conjugate. *Bioorg. Med. Chem Lett* **18,** 4142-4145.

Abstract: A novel neomycin-methidium conjugate was synthesized. The covalent linkage of the aminoglycoside to an intercalator, a derivative of ethidium bromide, results in a new conjugate capable of selective recognition of the DNA:RNA hybrid duplex. Spectroscopic methods: UV, CD, fluorescence, and calorimetric techniques: DSC and ITC were used to characterize the sub-nanomolar binding displayed by the conjugate for the DNA:RNA hybrid duplex, poly(dA):poly(rU)

Shiber M. C., Braswell E. H., Klump H., and Fresco J. R. (1996) Duplex-tetraplex equilibrium between a hairpin and two interacting hairpins of d(A-G)10 at neutral pH. *Nucleic Acids Res* **24,** 5004-5012.
Abstract: d(A-G)10 forms two helical structures at neutrality, at low ionic strength a single-hairpin duplex, and at higher ionic strength a double-hairpin tetraplex. An ionic strength-dependent equilibrium between these forms is indicated by native PAGE, which also reveals additional single-stranded species below 0.3 M Na+, probably corresponding to partially denatured states. The equilibrium also depends upon oligomer concentration: at very low concentrations, d(A-G)10 migrates faster than the random coil d(C-T)10, probably because it is a more compact single hairpin; at high concentrations, it co-migrates with the linear duplex d(A-G)10 x d(C-T)10, probably because it is a two-hairpin tetraplex. Molecular weights measured by equilibrium sedimentation in 0.1 M Na+, pH 7, reveal a mixture of monomer and dimer species at 1 degree C, but only a monomer at 40 degrees C; in 0.6 M Na+, pH 7, only a dimer species is observed at 4 degrees C. That the single- and double-stranded species are hairpin helices, is indicated by preferential S1 nuclease cleavage at the center of the oligomer(s), i.e., the loop of the hairpin(s). The UV melting transition below 0.3 M Na+ or K+, exhibits a dTm/dlog[Na+/K+] of 33 or 36 degrees C, respectively, consistent with conversion of a two-hairpin tetraplex to a single-hairpin duplex with extrahelical residues. When [Na+/K+] > or = 0.3 M, dTm/dlog [Na+/K+] is 19 or 17 degrees C, respectively, consistent with conversion of a two-hairpin tetraplex directly to single strands. A two-hairpin structure stabilized by G-tetrads is indicated by differential scanning calorimetry in 0.15 M Na+/5 mM Mg2+, with ΔH of formation per mole of the two-hairpin tetraplex of -116.9 kcal or -29.2 kcal/mol of G-tetrad.

Shikiya R., Li J. S., Gold B., and Marky L. A. (2005) Incorporation of cationic chains in the Dickerson-Drew dodecamer: correlation of energetics, structure, and ion and water binding. *Biochemistry* **44,** 12582-12588.
Abstract: We have investigated the unfolding thermodynamics for incorporating cationic side chains in the Dickerson-Drew dodecamer duplex. Incorporation of two 3-aminopropyl-2'-deoxyuridine residues (one on each self-complementary strand) lowers the stability of the duplex. This reduction is driven by unfavorable heat contributions due to the removal of electrostricted water and higher exposure of polar and nonpolar atomic groups that immobilize structural water. These cationic chains effectively remove counterions from the major groove, neutralizing some negatively charged phosphates. The overall results are consistent with the NMR solution of the modified duplex that showed a small bend at each modified site.

Smirnov I. and Shafer R. H. (2000) Effect of loop sequence and size on DNA aptamer stability. *Biochemistry* **39,** 1462-1468.
Abstract: The thrombin aptamer is a 15-mer oligodeoxyribonucleotide that folds into a unimolecular quadruplex consisting of a stack of two guanine quartets connected by two external loops and one central loop and possesses a high affinity for thrombin. We have undertaken a systematic examination, in KCl, of the thermodynamic stability of thrombin aptamer analogues containing sequence modifications in one or more of the loops, as well as in the number of quartets. UV melting studies have been carried out to obtain the relevant thermodynamic parameters for these aptamers. van't Hoff analysis of these data, with a two-state model for unimolecular denaturation, gave excellent fits to the experimental observations. Thermodynamic analysis indicates that the central loop sequence in the parent aptamer is optimal for stability. Modifications in this or other loops can effect either ΔH degrees, ΔS degrees, or both. Addition of a single G at the 5'-end decreases stability while addition of a G at the 3'-end increases stability. Differential scanning calorimetry experiments on the thrombin aptamer reveal that a heat capacity change, not detected by UV measurements, accompanies the unfolding of the aptamer.

Soto A. M., Kankia B. I., Dande P., Gold B., and Marky L. A. (2001) Incorporation of a cationic aminopropyl chain in DNA hairpins: thermodynamics and hydration. *Nucleic Acids Res* **29,** 3638-3645.
Abstract: We report on the physicochemical effects resulting from incorporating a 5-(3-aminopropyl) side

chain onto a 2'-deoxyuridine (dU) residue in a short DNA hairpin. A combination of spectroscopy, calorimetry, density and ultrasound techniques were used to investigate both the helix-coil transition of a set of hairpins with the following sequence: d(GCGACTTTTTGNCGC) [N = dU, deoxythymidine (dT) or 5-(3-aminopropyl)-2'-deoxyuridine (dU*)], and the interaction of each hairpin with Mg(2+). All three molecules undergo two-state transitions with melting temperatures (TM) independent of strand concentration that indicates their intramolecular hairpin formation. The unfolding of each hairpin takes place with similar TM values of 64-66 degrees C and similar thermodynamic profiles. The unfavorable unfolding free energies of 6.4-6.9 kcal/mol result from the typical compensation of unfavorable enthalpies, 36-39 kcal/mol, and favorable entropies of approximately 110 cal/mol. Furthermore, the stability of each hairpin increases as the salt concentration increases, the TM-dependence on salt yielded slopes of 2.3-2.9 degrees C, which correspond to counterion releases of 0.53 (dU and dT) and 0.44 (dU*) moles of Na(+) per mole of hairpin. Absolute volumetric and compressibility measurements reveal that all three hairpins have similar hydration levels. The electrostatic interaction of Mg(2+) with each hairpin yielded binding affinities in the order: dU > dT > dU*, and a similar release of 2-4 electrostricted water molecules. The main result is that the incorporation of the cationic 3-aminopropyl side chain in the major groove of the hairpin stem neutralizes some local negative charges yielding a hairpin molecule with lower charge density.

Soto A. M., Gmeiner W. H., and Marky L. A. (2002) Energetic and conformational contributions to the stability of Okazaki fragments. *Biochemistry* **41,** 6842-6849.
Abstract: A combination of spectroscopic and calorimetric techniques was used to determine complete thermodynamic profiles accompanying the folding of a model Okazaki fragment with sequence 5'-r(gagga)d(ATCTTTG)-3'/5'-d(CAAAGATTCCTC)-3' and control DNA (with and without thymidine substitutions for uridine), RNA, and hybrid duplexes. Circular dichroism spectroscopy indicated that all DNA duplexes are in the B conformation, the RNA and hybrid duplexes are in the A conformation, and the Okazaki fragment exhibits a spectrum between the A and B conformations. Ultraviolet and differential scanning calorimetry melting experiments reveal that all duplexes unfold in two-state transitions with thermal stabilities that follow the order RNA > OKA > DNA (with thymidines) > hybrids > DNA (with uridines). The dependence of the transition temperature on salt concentration yielded counterion releases in the following order: DNA (with thymidines) > RNA > DNA (with uridines) > OKA > hybrids. Thus, Okazaki fragments have a conformation and charge density between those of its components DNA and hybrid segments. However, the presence of the RNA-DNA/DNA junction confers on them higher stabilities than their component hybrid and DNA segments. The binding of intercalators to an Okazaki hairpin of sequence 5'-r(gc)d(GCU5GCGC)-3' and to its control DNA hairpin has also been studied. The results show that the binding of intercalators to Okazaki fragments is accompanied with higher heats and lower binding affinities, compared with DNA duplexes. This suggests that the presence of an RNA/DNA junction yields a larger surface contact to interact with the phenanthroline ring of the intercalators, which may lead to a larger disruption of the flexible flanking bases of the junction. The overall results suggest that the presence of this junction stabilizes Okazaki fragments and provides a structural feature that can be exploited in the design of drugs to specifically target these molecules.

Soto A. M., Kankia B. I., Dande P., Gold B., and Marky L. A. (2002) Thermodynamic and hydration effects for the incorporation of a cationic 3-aminopropyl chain into DNA. *Nucleic Acids Res* **30,** 3171-3180.
Abstract: The introduction of cationic 5-(omega-aminoalkyl)-2'-deoxypyrimidines into duplex DNA has been shown to induce DNA bending. In order to understand the energetic and hydration contributions for the incorporation of a cationic side chain in DNA a combination of spectroscopy, calorimetry and density techniques were used. Specifically, the temperature unfolding and isothermal formation was studied for a pair of duplexes with sequence d(CGTAGUCG TGC)/d(GCACGACTACG), where U represents 2'-deoxyuridine ('control') or 5-(3-aminopropyl)-2'-deoxyuridine ('modified'). Continuous variation experiments confirmed 1:1 stoichiometries for each duplex and the circular dichroism spectra show that both duplexes adopted the B conformation. UV and differential scanning calorimetry melting experiments reveal that each duplex unfolds in two-state transitions. In low salt buffer, the 'modified' duplex is more stable and unfolds with a lower endothermic heat and lower release of counterion and water. This electrostatic stabilization is entropy driven and disappears at higher salt concentrations. Complete thermodynamic profiles at 15 degrees C show that the favorable formation of each duplex results from the compensation of a favorable exothermic heat with an unfavorable entropy contribution. However, the

isothermal profiles yielded a differential enthalpy of 8.8 kcal/mol, which is 4.3 kcal/mol higher than the differential enthalpy observed in the unfolding profiles. This indicates that the presence of the aminopropyl chain induces an increase in base stacking interactions in the modified single strand and a decrease in base stacking interactions in the modified duplex. Furthermore, the formation of the 'control' duplex releases water while the 'modified' duplex takes up water. Relative to the control duplex, formation of the modified duplex at 15 degrees C yielded a marginal differential ΔG degrees term, positive $\Delta\Delta H(ITC)-\Delta(T\Delta S)$ compensation, negative $\Delta\Delta V$ and a net release of counterions. The opposite signs of the differential enthalpy-entropy compensation and differential volume change terms show a net uptake of structural water around polar and non-polar groups. This indicates that incorporation of the aminopropyl chain induces a higher exposure of aromatic bases to the solvent, which may be consistent with a small and local bend in the 'modified' duplex.

Soto A. M., Loo J., and Marky L. A. (2002) Energetic contributions for the formation of TAT/TAT, TAT/CGC(+), and CGC(+)/CGC(+) base triplet stacks. *J Am Chem Soc* **124,** 14355-14363.
Abstract: We used a combination of spectroscopic and calorimetric techniques to determine complete thermodynamic profiles accompanying the folding of a set of triple helices and control duplexes. Specifically, we studied the sequences: d(A(7)C(5)T(7)C(5)T(7)), d(A(6)C(5)T(6)C(5)T(6)), d(A(6)C(5)T(6)), d(AGAGAGAC(5)TCTCTCTC(5)TCTCTCT), d(AGAGAC(5)TCTCTC(5)TCTCT), d(AGAGAC(5)TCTCTC(2)), d(AAGGAC(5)TCCTTC(5)TTCCT), d(AGGAAC(5)TTCCTC(5)TCCTT), and d(GAAAGC(5)CTTTCC(5)CTTTC). Circular dichroism spectroscopy indicated that all triplexes and duplexes are in the "B" conformation. DSC melting experiments revealed that the formation of triplexes is accompanied by a favorable free energy change, which arises from the compensation of a large and favorable enthalpic contribution with an unfavorable entropic contribution. Comparison of the thermodynamic profiles of these triplexes yielded enthalpic contributions of -24 kcal/mol, -23 kcal/mol, and -22 kcal/mol for the formation of TAT/TAT, TAT/CGC(+), and CGC(+)/CGC(+) base triplet stacks, respectively. UV melts as a function of sodium concentration show sodium ions stabilize the triplexes that contain only TAT triplets but destabilize the triplexes that contain CGC(+) triplets. UV melts as a function of pH indicate that the protonation of the third strand and loop cytosines stabilizes the triplexes that contain CGC(+) and TAT triplets, respectively. Our overall results suggest that the triplex to duplex transition of triplexes that contain CGC(+) triplets is accompanied by a release of protons and an uptake of sodium, while their duplex to random coil transition is accompanied by a release of sodium ions. A consequence of this opposite sodium dependence is that their coupled transitions are nearly independent of sodium concentration but are dependent on the experimental pH.

Soto A. M. and Marky L. A. (2002) Thermodynamic contributions for the incorporation of GTA triplets within canonical TAT/TAT and C+GC/C+GC base-triplet stacks of DNA triplexes. *Biochemistry* **41,** 12475-12482.
Abstract: Nucleic acid triple helices may be used in the control of gene expression. One limitation of using triplex-forming oligonucleotides as therapeutic agents is that their target sequences are limited to homopurine tracts. To increase the repertoire of sequences that can be targeted, it has been postulated that a guanine can target a thymidine forming a stable GTA mismatch triplet. In this work, we have used a combination of optical and calorimetric techniques to determine thermodynamic unfolding profiles of two triplexes containing a single GTA triplet, d(A(3)TA(3)C(5)T(3)AT(3)C(5)T(3)GT(3)) (ATA) and d(AGTGAC(5)TCACTC(5)TCGCT) (GTG), and their control triplexes, d(A(7)C(5)T(7)C(5)T(7)) (TAT7) and d(AGAGAC(5)TCTCTC(5)TCTCT) (AG5T). In general, the presence of a GTA mismatch in DNA triplexes is destabilizing; however, this destabilization is greater when placed in a C(+)GC/C(+)GC base-triplet stack than between a TAT/TAT stack. These destabilizations are accompanied by a reduced unfolding enthalpy of approximately 10 kcal/mol, suggesting a decrease in the base stacking contributions surrounding the mismatch. Relative to their corresponding control triplexes, the folding of ATA is accompanied by a lower counterion uptake and a similar proton uptake, while GTG folding is accompanied by an increase in the counterion and proton uptakes. These effects are consistent with the observed decrease in stacking interactions. The overall results indicate that the main difficulty of targeting pyrimidine interruptions is that the decrease in stacking contributions, due to the incorporation of a GTA mismatch, affects the stability of the neighboring base triplets. This suggests that nucleotide analogues that increase the strength of these base-triplet stacks will result in a more effective targeting of pyrimidine interruptions.

Soto A. M., Rentzeperis D., Shikiya R., Alonso M., and Marky L. A. (2006) DNA intramolecular triplexes containing dT --> dU substitutions: unfolding energetics and ligand binding. *Biochemistry* **45,** 3051-3059.
Abstract: We used a combination of optical and calorimetric techniques to investigate the incorporation of deoxythymidine --> deoxyuridine (dT --> dU) substitutions in the duplex and third strand of the parallel intramolecular triplex d(A(7)C(5)T(7)C(5)T(7)) (ATT). UV and differential scanning calorimetry melting experiments show that the incorporation of two substitutions yielded triplexes with lower thermal stability and lower unfolding enthalpies. The enthalpies decrease with an increase in salt concentration, indirectly yielding a heat capacity effect, and the magnitude of this effect was lower for the substituted triplexes. The combined results indicate that the destabilizing effect is due to a decrease in the level of stacking interactions. Furthermore, the minor groove ligand netropsin binds to the minor groove and to the hydrophobic groove, created by the double chain of thymine methyl groups in the major groove of these triplexes. Binding of netropsin to the minor groove yielded thermodynamic profiles similar to that of a DNA duplex with a similar sequence. However, and relative to ATT, binding of netropsin to the hydrophobic groove has a decreased binding affinity and lower binding enthalpy. This shows that the presence of uridine bases disrupts the hydrophobic groove and lowers its cooperativity toward ligand binding. The overall results suggest that the stabilizing effect of methyl groups may arise from the combination of both hydrophobic and electronic effects.

Spadiliero B., Nicolini C., Mascetti G., Henriquez D., and Vergani L. (2002) Chromatin of Trypanosoma cruzi: in situ analysis revealed its unusual structure and nuclear organization. *J Cell Biochem* **85,** 798-808.
Abstract: Chromatin of Trypanosoma cruzi is known to be organized in classical nucleosomal filaments, but surprisingly, these filaments do not fold in visible chromosomes and the nuclear envelope is preserved during cell division. Our hypothesis about the role of chromatin structure in regulating gene expression and, more generally, cell functioning, pressed us to verify if chromatin organization is modulated during the parasite life-cycle. To this end, we analyzed in situ the fine structural organization of T. cruzi chromatin by means of an integrated biophysical approach, using differential scanning calorimetry and fluorescence microscopy. We observed that logarithmic forms exhibit a less condensed chromatin with respect to the stationary ones. Thermal analysis revealed that parasite chromatin is organized in three main levels of condensation, barring from the polynucleosomal filament till to superstructured fibers. Besides, the fluorescence images of nuclei showed a characteristic chromatin distribution, with defined domains localized near to the nuclear envelope. While in stationary parasites, these regions are highly condensed, in logarithmic forms they unfold by extending themselves toward the center of nucleus. These observations suggest that, in comparison with higher eukaryotes, in T. cruzi the nuclear envelope plays an unusual and pivotal role in interphase and in mitosis.

Spink C. H., Garbett N. and Chaires J. B. (2007) Enthalpies of DNA melting in the presence of osmolytes. *Biophys Chem* **126,** 176-185.
Abstract: The melting of DNA in the presence of osmolytes has been studied with the intention of obtaining information about how base pair stability is affected by changes in solution conditions. In previous investigations, the melting enthalpies were assumed to be constant as osmolalities change, but no systematic evaluation of whether this condition is true has been offered. This paper presents calorimetric data on the melting of two synthetic DNA samples in the presence of a number of common osmolytes. Poly(dAdT)*poly(dTdA) and poly(dGdC)*poly(dCdG) melting have been examined by differential scanning calorimetry in solutions containing ethylene glycol, glycerol, sucrose, urea, betaine, PEG 200 and PEG 1450 at increasing osmolalities. The results show small, but significant changes in the enthalpy of melting of the two polynucleotides that are different, depending on the structure of the cosolvent. The polyols, ethylene glycol, glycerol, PEG 200 and also urea all show decreases in melting enthalpy, while betaine and sucrose display increases with increasing concentration of cosolvent. The large stabilizing PEG 1450 shows no change within the experimental errors. Using concepts relating to preferential interactions of the cosolvents with the DNA base pairs, it is possible to interpret some of the observed changes in the thermodynamic properties of melting. The results indicate that there is strong entropy-enthalpy compensation upon melting base pairs, but entropy increases dominate to cause the decreases in stability with increased cosolvent concentration. Excess hydration parameters are evaluated and their magnitudes discussed in terms of changes in cosolvent interactions with the DNA base pairs.

Sugimoto N., Satoh N., and Yamamoto K. (1999) Comparison of thermodynamic stabilities between PNA (peptide nucleic acid)/DNA hybrid duplexes and DNA/DNA duplexes. *Nucleic Acids Symp Ser* 93-94.
Abstract: We have examined quantitatively stabilities of PNA/DNA hybrid duplexes with identical nearest-neighbor base pairs and compared stabilities between PNA/DNA and DNA/DNA. The average difference of stabilization energy of the short PNA/DNA was 0.9 kcal mol^{-1}, which suggests that the stability of the hybrids with identical nearest-neighbor base pairs can be predicted with the nearest-neighbor model as well as those of nucleic acid duplexes.

Sugimoto N., Wu P., Hara H., and Kawamoto Y. (2001) pH and cation effects on the properties of parallel pyrimidine motif DNA triplexes. *Biochemistry* **40,** 9396-9405.
Abstract: The effects of cytosine protonation and various cations on the properties of parallel pyrimidine motif DNA triplexes were intensively investigated and characterized by several different techniques, such as circular dichroism (CD) conformation, ultraviolet (UV) melting, differential scanning calorimetry (DSC) thermal denaturation, and surface plasmon resonance (SPR) real-time dynamics. The comparative CD spectra of the triplex and the corresponding homoduplexes showed that the negative peak at approximately 218 nm would be the eigenpeak of the Hoogsteen paired strand, and moreover, the formation pathway of a triplex was significantly pH-dependent and fell into three groups: under acidic conditions, the triplex is formed by a one-step docking, under near physiological conditions, the Watson-Crick duplex is first structured and then accepts the Hoogsteen third strand into its major groove, and under basic conditions, the triplex is not formed. The pH-dependent thermodynamics of the global triplex, the Watson-Crick antiparallel duplex, and the Crick-Hoogsteen parallel duplex were comparatively discussed for the first time. These data revealed that the thermodynamic stabilities of the Watson-Crick-Hoogsteen triplex and the Crick-Hoogsteen duplex would be strongly dependent on cytosine protonation, but a low-pH environment somewhat destabilized the Watson-Crick duplex. The binding energy of triplex formation would be different from the unfolding energy of triplex melting under acidic conditions due to the disparity in the pathway between the formation and unfolding of a triplex. Real-time dynamic measurements showed that the association and dissociation rate constants of a duplex-to-triplex formation are (1.98 +/- 0.24) x 10^{3} M^{-1} s^{-1} and (4.09 +/- 0.96) x 10^{-4} s^{-1} at 20 degrees C and pH 6.0, respectively. The formation energy of the duplex-to-triplex transition derived from SPR measurements was in agreement with the unfolding energy of the free Hoogsteen paired duplex derived from UV measurements. The calorimetric enthalpies of the triplex-to-duplex-to-single transition were 39.3 and 75.3 kcal/mol under near physiological conditions (pH 7.0), respectively, which were underestimated relative to the van't Hoff enthalpies. In addition, the effects of various cations, ionic strength, mixed-valent cations, and the position of the C(+)xG.C triplets on the thermodynamics of the triplexes were addressed under near physiological conditions. The interaction of metal ions with the triplexes clearly depended on the type and ionic strength of the cations, and the efficiency with which the cations stabilized the global triplex was in the order Mg(2+) > Mn(2+) > Ca(2+) > Ba(2+) >> Na(+). These observations would be useful for the design of triplex-forming oligonucleotides for antigene drugs and therapeutic purposes

Theimer C. A. and Giedroc D. P. (1999) Equilibrium unfolding pathway of an H-type RNA pseudoknot which promotes programmed -1 ribosomal frameshifting. *J Mol Biol* **289,** 1283-1299.
Abstract: The equilibrium unfolding pathway of a 41-nucleotide frameshifting RNA pseudoknot from the gag-pro junction of mouse intracisternal A-type particles (mIAP), an endogenous retrovirus, has been determined through analysis of dual optical wavelength, equilibrium thermal melting profiles and differential scanning calorimetry. The mIAP pseudoknot is an H-type pseudoknot proposed to have structural features in common with the gag-pro frameshifting pseudoknots from simian retrovirus-1 (SRV-1) and mouse mammary tumor virus (MMTV). In particular, the mIAP pseudoknot is proposed to contain an unpaired adenosine base at the junction of the two helical stems (A15), as well as one in the middle of stem 2 (A35). A mutational analysis of stem 1 hairpins and compensatory base-pair substitutions incorporated into helical stem 2 was used to assign optical melting transitions to molecular unfolding events. The optical melting profile of the wild-type RNA is most simply described by four sequential two-state unfolding transitions. Stem 2 melts first in two closely coupled low-enthalpy transitions at low tmin which the stem 3' to A35, unfolds first, followed by unfolding of the remainder of the helical stem. The third unfolding transition is associated with some type of stacking interactions in the stem 1 hairpin loop not present in the pseudoknot. The fourth transition is assigned to unfolding of stem 1. In all RNAs investigated, ΔHvH approximately ΔHcal, suggesting that ΔCpfor unfolding is small. A35 has the

thermodynamic properties expected for an extrahelical, unpaired nucleotide. Deletion of A15 destabilizes the stem 2 unfolding transition in the context of both the wild-type and DeltaA35 mutant RNAs only slightly, by ΔΔG degrees approximately 1 kcal mol^{-1} (at 37 degrees C). The DeltaA15 RNA is considerably more susceptible to thermal denaturation in the presence of moderate urea concentrations than is the wild-type RNA, further evidence of a detectable global destabilization of the molecule. Interestingly, substitution of the nine loop 2 nucleotides with uridine residues induces a more pronounced destabilization of the molecule (ΔΔG degrees approximately 2.0 kcal mol^{-1}), a long-range, non-nearest neighbor effect. These findings provide the thermodynamic basis with which to further refine the relationship between efficient ribosomal frameshifting and pseudoknot structure and stability.

Theimer C. A. and Giedroc D. P. (2000) Contribution of the intercalated adenosine at the helical junction to the stability of the gag-pro frameshifting pseudoknot from mouse mammary tumor virus. *RNA* **6,** 409-421.
Abstract: The mouse mammary tumor virus (MMTV) gag-pro frameshifting pseudoknot is an H-type RNA pseudoknot that contains an unpaired adenosine (A14) at the junction of the two helical stems required for efficient frameshifting activity. The thermodynamics of folding of the MMTV vpk pseudoknot have been compared with a structurally homologous mutant RNA containing a G x U to G-C substitution at the helical junction (U13C RNA), and an A14 deletion mutation in that context (U13CdeltaA14 RNA). Dual wavelength optical melting and differential scanning calorimetry reveal that the unpaired adenosine contributes 0.7 (+/-0.2) kcal mol^{-1} at low salt and 1.4 (+/-0.2) kcal mol^{-1} to the stability (ΔG(0)37) at 1 M NaCl. This stability increment derives from a favorable enthalpy contribution to the stability ΔΔH = 6.6 (+/-2.1) kcal mol^{-1} with ΔΔG(0)37 comparable to that predicted for the stacking of a dangling 3' unpaired adenosine on a G-C or G x U base pair. Group 1A monovalent ions, NH4+, Mg2+, and Co(NH3)6(3+) ions stabilize the A14 and deltaA14 pseudoknots to largely identical extents, revealing that the observed differences in stability in these molecules do not derive from a differential or specific accumulation of ions in the A14 versus deltaA14 pseudoknots. Knowledge of this free energy contribution may facilitate the prediction of RNA pseudoknot formation from primary nucleotide sequence (Gultyaev et al., 1999, RNA 5:609-617).

Tikhomirova A., Taulier N., and Chalikian T. V. (2004) Energetics of Nucleic Acid Stability: The Effect of ΔC_p. *J Am Chem Soc* **126,** 16387-16394.
Abstract: We report high-resolution differential scanning calorimetric data on the poly(dAdT)poly(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)poly(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) nucleic acid duplexes. We use these data to evaluate the melting temperatures, T_M, enthalpy changes, ΔH_M, and heat capacity changes, ΔC_p, accompanying helix-to-coil transitions of each polymeric duplex studied in this work at different NaCl concentrations. In agreement with previous reports, we have found that ΔC_p exhibits a positive, nonzero value, which, on average, equals 268 +/- 33 J mol^{-1} K^{-1}. With ΔC_p, we have calculated the transition free energies, ΔG, enthalpies, ΔH, and entropies, ΔS, for the duplexes as a function of temperature. Since, ΔG, ΔH, and ΔS all strongly depend on temperature, the thermodynamic comparison between DNA and/or RNA duplexes (that may differ from one another with respect to sequence, composition, conformation, etc.) is physically meaningful only if extrapolated to a common temperature. We have performed such comparative analyses to derive differential thermodynamic parameters of formation of GC versus AT, AU, and IC base pairs as well as B' versus A and B helix conformations. We have proposed some general microscopic interpretations for the observed sequence-specific and conformation-specific thermodynamic differences between the duplexes.

Tikhomirova A., Beletskaya I. V., and Chalikian T. V. (2006) Stability of DNA duplexes containing GG, CC, AA, and TT mismatches. *Biochemistry* **45,** 10563-10571.
Abstract: We employed salt-dependent differential scanning calorimetric measurements to characterize the stability of six oligomeric DNA duplexes (5'-GCCGGAXTGCCGG-3'/5'-CCGGCAYTCCGGC-3') that contain in the central XY position the GC, AT, GG, CC, AA, or TT base pair. The heat-induced helix-to-coil transitions of all the duplexes are associated with positive changes in heat capacity, DeltaC(p), ranging from 0.43 to 0.53 kcal/mol. Positive values of DeltaC(p) result in strong temperature dependences of changes in enthalpy, DeltaH degrees, and entropy, DeltaS degrees , accompanying duplex melting and cause melting free energies, DeltaG degrees, to exhibit characteristically curved shapes. These observations suggest that DeltaC(p) needs to be carefully taken into account when the parameters of duplex stability are

extrapolated to temperatures distant from the transition temperature, T(M). Comparison of the calorimetric and van't Hoff enthalpies revealed that none of the duplexes studied in this work exhibits two-state melting. Within the context of the central AXT/TYA triplet, the thermal and thermodynamic stabilities of the duplexes in question change in the following order: GC > AT > GG > AA approximately TT > CC. Our estimates revealed that the thermodynamic impact of the GG, AA, and TT mismatches is confined within the central triplet. In contrast, the thermodynamic impact of the CC mismatch propagates into the adjacent helix domains and may involve 7-9 bp. We discuss implications of our results for understanding the origins of initial recognition of mismatched DNA sites by enzymes of the DNA repair machinery.

Torigoe H., Kamiya M., Shindo H., and Sarai A. (1995) Structural polymorphism and thermal stability of telomere DNAs (T2G4)n and (T4G4)n. *Nucleic Acids Symp Ser* 199-200.
Abstract: The ends of eukaryotic chromosomes, termed telomeres, contain a single-stranded 3' overhang composed of tandemly repeated guanine-rich sequences, such as (T2G4)n and (T4G4)n, along one strand. The sequences can form defined folded tetraplex structures. Here we have systematically examined structural polymorphism and thermal stability of a series of oligonucleotide sequences, Tet n: (T2G4)n and Oxy n: (T4G4)n (n = 1, 2, 3, 4), using circular dichroism (CD) spectroscopy. The CD spectra of Tet 1 and Oxy 1 are consistent with those observed in tetraplex structures consisting of four parallel strands (type I conformation); whereas the spectra of Tet 2, Oxy 2, Oxy 3, and Oxy 4 correspond with those observed for tetraplex conformations where the strands are antiparallel (type II conformation). The spectra of Tet 3 and Tet 4 suggests that Tet 3 and Tet 4 can adopt both type I and type II conformations and they are structurally polymorphic. The melting temperatures of Tet n and Oxy n (n = 1, 2, 3, 4) measured by CD melting are consistent with the previously reported values obtained from differential scanning calorimetry (DSC). Furthermore, the CD melting of Tet 4 suggests that the type II conformation of Tet 4 changes into type I conformation between 55 degrees C and 70 degrees C.

Tumanova I., Boyer J., Ausar S. F., Burzynski J., Rosencrance D., White J., Scheidel J., Parkinson R., Maguire H., Middaugh C. R., Weiner D., and Green A. P. (2005) Analytical and biological characterization of supercoiled plasmids purified by various chromatographic techniques. *DNA Cell Biol* **24,** 819-831.
Abstract: Supercoiled plasmids are an important component of gene-based delivery vehicles. A number of production methods for clinical applications have been developed, each resulting in very high-quality product with low levels of residual contaminants. There is, however, no consensus on the optimal methods to characterize plasmid quality, and further, to determine if these methods are predictive of either product stability or biological activity. We have produced two plasmids using four production purification methodologies based on PolyFlo((R)) and hydrophobic interaction chromatography (HIC), either alone or in tandem processes. In each case, the product was analyzed using standard molecular biological methods. We also performed a number of biophysical analyses such as dynamic light scattering (DLS), circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). Minimal differences were detected among the preparations based on the more standard molecular biological methods. Some small differences were detected, however, using biophysical techniques, particularly FTIR and DSC, which may reflect small variations in plasmid tertiary structure and thermal stability. Stability after heat exposure at 60 degrees C, exposure to fetal bovine serum and long-term storage at 4 degrees C varied between plasmids. One plasmid showed no difference in stability depending on the production process, but the other showed significant differences. Evaluation in vivo in models for gene immunization and gene therapy showed significant differences in the response depending on the method of purification. Preparations using a tandem process of PolyFlo used in two separation modes provided higher biological activity compared to a tandem HIC/PolyFlo process or either resin used alone in a single column process. These data indicate that the process by which supercoiled plasmids are made can influence plasmid stability and biological activity and emphasize the need for more rigorous methods to evaluate supercoiled plasmids as gene-delivery vehicles.

Vallone P. M., Paner T. M., Hilario J., Lane M. J., Faldasz B. D., and Benight A. S. (1999) Melting studies of short DNA hairpins: influence of loop sequence and adjoining base pair identity on hairpin thermodynamic stability. *Biopolymers* **50,** 425-442.
Abstract: Spectroscopic and calorimetric melting studies of 28 DNA hairpins were performed. These hairpins form by intramolecular folding of 16 base self-complementary DNA oligomer sequences. Sequence design dictated that the hairpin structures have a six base pair duplex linked by a four base loop

and that the first five base pairs in the stem are the same in every molecule. Only loop sequence and identity of the duplex base pair closing the loop vary for the set of hairpins. For these DNA samples, melting studies were carried out to investigate effects of the variables on hairpin stability. Stability of the 28 oligomers was ascertained from their temperature-induced melting transitions in buffered 115 mM Na(+) solvent, monitored by ultraviolet absorbance and differential scanning calorimetry (DSC). Experiments revealed the melting temperatures of these molecules range from 32.4 to 60.5 degrees C and are concentration independent over strand concentrations of 0.5 to 260 μM; thus, as expected for hairpins, the melting transitions are apparently unimolecular. Model independent thermodynamic transition parameters, ΔH(cal), ΔS(cal), and ΔG(cal), were determined from DSC measurements. Model dependent transition parameters, ΔH(vH), ΔS(vH), and ΔG(vH) were estimated from a van't Hoff (two-state) analysis of optical melting transitions. Results of these studies reveal a significant sequence dependence to DNA hairpin stability. Thermodynamic parameters evaluated by either procedure reveal the transition enthalpy, ΔH(cal) (ΔH(vH)) can differ by as much as 20 kcal/mol depending on sequence. Similarly, values of the transition entropy ΔS(cal) (ΔS(vH)) can differ by as much as 60 cal/Kmol (eu) for different molecules. Differences in free energies ΔG(cal) (ΔG(vH)) are as large as 4 kcal/mol for hairpins with different sequences. Comparisons between the model independent calorimetric values and the thermodynamic parameters evaluated assuming a two-state model reveal that 10 of the 28 hairpins display non-two-state melting behavior. The database of sequence-dependent melting free energies obtained for the hairpins was employed to extract a set of n-n (nearest-neighbor) sequence dependent loop parameters that were able to reproduce the input data within error (with only two exceptions). Surprisingly, this suggests that the thermodynamic stability of the DNA hairpins can in large part be reasonably represented in terms of sums of appropriate nearest-neighbor loop sequence parameters.

Vallone P. M. and Benight A. S. (1999) Melting studies of short DNA hairpins containing the universal base 5-nitroindole. *Nucleic Acids Res* **27,** 3589-3596.
Abstract: Effects of the universal base 5-nitroindole on the thermodynamic stability of DNA hairpins having a 6 bp stem and four base loops were investigated by optical absorbance and differential scanning calorimetry techniques. Melting studies were conducted in buffer containing 115 mM Na(+). Five different modified versions of DNA hairpins containing a 5-nitroindole base or bases substituted at different positions in the stem and loop regions were examined. Thermo-dynamic parameters of the melting transitions estimated from a two-state analysis of optical melting curves and measured directly by calorimetry revealed that the presence of 5-nitroindole bases in the duplex stem or loop regions of short DNA hairpins significantly affects both their enthalpic and entropic melting components in a compensating manner, while the transition free energy varies linearly with the transition temperature. The calorimetrically determined enthalpy and entropy values of the modified hairpins were considerably smaller (43-53%) than the two-state optical parameters, suggesting that solvent effects may be significant in the melting processes of these hairpins. Results of circular dichroism measurements also revealed slight differences between the modified hairpins and the control in both the duplex and melted states, suggesting subtle structural differences between the control and DNA hairpins containing a 5-nitroindole base or bases.

Vallone P. M. and Benight A. S. (2000) Thermodynamic, spectroscopic, and equilibrium binding studies of DNA sequence context effects in four 40 base pair deoxyoligonucleotides. *Biochemistry* **39,** 7835-7846.
Abstract: Effects of different end sequences on melting, circular dichroism spectra (CD), and enzyme binding properties were investigated for four 40 base pair, non-self-complementary duplex DNA oligomers. The center sequences of these oligoduplexes have either of two 22 base pair modules flanked on both sides by sequences differing in AT content. Temperature-induced melting transitions monitored by differential scanning calorimetry (DSC) and ultraviolet absorbance were measured for the six duplexes in buffered 115 mM Na(+) solutions. Values of the melting transition enthalpy, ΔH(cal), and entropy, ΔS(cal), were obtained directly from DSC experiments. Melting transition parameters, ΔH(vH) and ΔS(vH), were also estimated from a van't Hoff analysis of optical melting curves collected as a function of DNA concentration, assuming that the melting transition is two-state. Melting free energies (20 degrees C) evaluated from DSC melting experiments on the four duplex DNAs ranged from -52.2 to -77.5 kcal/mol. Free energies based on the van't Hoff analysis were -37.9 to -58.8 kcal/mol. Although the values are different, trends in the melting free energies of the four duplex DNAs as a function of sequence were identical in both DSC and optical analyses. Subject to several assumptions, values for the initiation free energy were estimated for each duplex, defined as ΔG(int) = ΔG(cal) - ΔG(pred), where ΔG(cal) is the

experimental free energy at 20 degrees C determined from the experimentially measured values of the transition enthalpy, ΔH(cal), and entropy, ΔS(cal). The predicted free energy of the sequence, ΔG(pred)(20 degrees C), is obtained using published nearest-neighbor sequence stability values. For three of the four duplexes, values of ΔG(int) are essentially nil. In contrast, the duplex with 81.8% GC has a considerably higher estimate of ΔG(int) = 7.1 kcal/mol. The CD spectra for the six duplexes collected over the wavelength range from 200 to 320 nm are also sequence-dependent. Factor analysis of the CD spectra by singular value decomposition revealed that the experimental CD spectra could be reconstructed from linear combinations of two minor and one major subspectra. Changes in the coefficients of the major subspectrum for different sequences reflect incremental sequence-dependent variations of the CD spectra. Equilibrium binding by BamHI restriction endonuclease to the 40 base pair DNAs whose central eight base pairs contain the recognition sequence for BamHI restriction enzyme bounded by A.T base pairs, 5'-A-GGATCC-A-3' was investigated. Binding assays were performed by titering BamHI against a constant concentration of each of the duplex DNA substrates, in the absence of Mg(2+), followed by analysis by gel retardation. Under the conditions employed, the enzyme binds but does not cleave the DNAs. Results of the assays revealed two binding modes with retarded gel mobilities. Binding isotherms for the fraction of bound DNA species versus enzyme concentration for each binding mode were constructed and analyzed with a simple two-step equilibrium binding model. This analysis provided semiquantitative estimates on the equilibrium binding constants for BamHI to the four DNAs. Values obtained for the binding constants varied only 7-fold and ranged from 6×10^{-8} to 42×10^{-8} M, with binding free energies from -8.6 to -9.7 (+/-0.2) kcal/mol depending on the sequence that flanks the enzyme binding site. Unlike what was found earlier in binding studies of the 22 base pair duplexes that constitute the core modules of the present 40-mers [Riccelli, P. V., Vallone, P. M., Kashin, I., Faldasz, B. D., Lane, M. J., and Benight, A. S. (1999) Biochemistry 38, 11197-11208], no obvious relationship between binding and stability was found for these longer DNAs. Apparently, effects of flanking sequence stability on restriction enzyme binding may only be measurable in very short duplex deoxyoligonucl.

Vander Meulen K. A., Davis J. H., Foster T. R., Record M. T., Jr. and Butcher S. E. (2008) Thermodynamics and folding pathway of tetraloop receptor-mediated RNA helical packing. *J Mol Biol* **384,** 702-717.
Abstract: Little is known about the thermodynamic forces that drive the folding pathways of higher-order RNA structure. In this study, we employ calorimetric [isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC)] and spectroscopic (NMR and UV) methods to characterize the thermodynamics of the GAAA tetraloop-receptor interaction, utilizing a previously described bivalent construct. ITC studies indicate that the bivalent interaction is enthalpy driven and highly stable, with a binding constant (K(obs)) of 5.5x10(6) M(-1) and enthalpy (DeltaH(obs)(o)) of -33.8 kcal/mol at 45 degrees C in 20 mM KCl and 2 mM MgCl(2). Thus, we derive the DeltaH(obs)(o) for a single tetraloop-receptor interaction to be -16.9 kcal/mol at these conditions. UV absorbance data indicate that an increase in base stacking quality contributes to the enthalpy of complex formation. These highly favorable thermodynamics are consistent with the known critical role for the tetraloop-receptor motif in the folding of large RNAs. Additionally, a significant heat capacity change (DeltaC(p,obs)(o)) of -0.24 kcal mol(-1) K(-1) was determined by ITC. DSC and UV-monitored thermal denaturation experiments indicate that the bivalent tetraloop-receptor construct follows a minimally five-state unfolding pathway and suggest the observed DeltaC(p,obs)(o) for the interaction results from a temperature-dependent unbound receptor RNA structure

Vives M., Tauler R., and Gargallo R. (2002) Study of the influence of metal ions on tRNA(Phe) thermal unfolding equilibria by UV spectroscopy and multivariate curve resolution. *J Inorg Biochem* **89,** 115-122.
Abstract: The influence of metal ions (Na(+), Mg(2+) and Cd(2+)) on the thermal unfolding of phenylalanine transfer ribonucleic acid (tRNA(Phe)) was studied by UV spectroscopy-monitored melting experiments. Absorbance data were obtained during the unfolding process in the range 220-340 nm and later analyzed by a multivariate curve resolution approach (MCR-ALS) based on factor analysis. This procedure determines the number of spectroscopically distinct conformations present during the unfolding process and reveals their concentration profiles and pure spectra, without any initial assumption having to be made about the number of steps in the unfolding pathway. From the concentration profiles and pure spectra, information such as Tm values can be recovered. The results were compared with those obtained

previously in spectroscopic and calorimetric unfolding experiments, showing that the multivariate approach recovers information that complements that obtained in traditional spectroscopic melting experiments.

Volker J., Osborne S. E., Glick G. D., and Breslauer K. J. (1997) Thermodynamic properties of a conformationally constrained intramolecular DNA triple helix. *Biochemistry* **36,** 756-767.
Abstract: We describe the thermodynamic properties of an intramolecular triple helix with two all-thymine linker loops in which the Hoogsteen strand is covalently crosslinked to the underlying Watson-Crick hairpin duplex by means of a disulfide bridge. We compare these properties to those of the corresponding intramolecular triplex without the disulfide crosslink. Optical and calorimetric measurements reveal that the uncrosslinked parent triplex melts in a biphasic manner above pH 6, with the initial triplex to duplex transition (Hoogsteen strand release) occurring at lower temperatures than subsequent melting of the hairpin helix. By contrast, crosslinking increases the thermal stability of the Hoogsteen transition such that the triplex and underlying hairpin duplex melt as a single transition under all conditions studied. Model independent thermodynamic data obtained by differential scanning calorimetry reveals the crosslink-induced increase in triplex thermal stability corresponds to a free energy stabilization of about 3 kcal/mol, with this stabilization being entirely entropic in origin. In other words, the crosslink is enthalpically neutral, but nevertheless, induces a triplex stabilization of 3 kcal/mol due to a reduction in the entropy change associated with triplex melting. In an effort to define the origin(s) of this entropic impact, we measured the pH and ionic strength dependence of the melting transitions. From a comparison of the melting transitions at different pH values and ionic strengths, we estimate that 0.4 more protons are associated with the crosslinked triplex state than with the uncrosslinked triplex, and 1.3 fewer counterions are released on melting the crosslinked triplex. We discuss how such crosslink-induced changes in proton binding and counterion release, in conjunction with potential changes in hydration and conformational freedom, could combine to give rise to the observed changes in entropy.

Volker J., Blake R. D., Delcourt S. G., and Breslauer K. J. (1999) High-resolution calorimetric and optical melting profiles of DNA plasmids: resolving contributions from intrinsic melting domains and specifically designed inserts. *Biopolymers* **50,** 303-318.
Abstract: We demonstrate that differential scanning calorimetry (DSC) can be used to yield high-resolution melting profiles for DNA plasmids that agree in all major features with the corresponding plasmid melting profiles derived using more traditional optical techniques. We further demonstrate that by combining information derived from both calorimetric and optical melting profiles one can glean insights that are unavailable from either melting curve alone. By using both optical and calorimetric observables, we show how one can resolve, identify, and measure the thermodynamic properties of particular sequences/domains of interest within a plasmid. We also show that complementary DSC and optical melting studies on plasmids with and without specifically designed inserts can provide fundamental advantages over the corresponding melting studies on other model system constructs for thermodynamically characterizing nucleic acid sequences/structures.

Volker J., Klump H. H., Manning G. S., and Breslauer K. J. (2001) Counterion association with native and denatured nucleic acids: an experimental approach. *J Mol Biol* **310,** 1011-1025.
Abstract: The melting temperature of the poly(dA) . poly(dT) double helix is exquisitely sensitive to salt concentration, and the helix-to-coil transition is sharp. Modern calorimetric instrumentation allows this transition to be detected and characterized with high precision at extremely low duplex concentrations. We have taken advantage of these properties to show that this duplex can be used as a sensitive probe to detect and to characterize the influence of other solutes on solution properties. We demonstrate how the temperature associated with poly(dA) . poly(dT) melting can be used to define the change in bulk solution cation concentration imparted by the presence of other duplex and triplex solutes, in both their native and denatured states. We use this information to critically evaluate features of counterion condensation theory, as well as to illustrate "crosstalk" between different, non-contacting solute molecules. Specifically, we probe the melting of a synthetic homopolymer, poly(dA) . poly(dT), in the presence of excess genomic salmon sperm DNA, or in the presence of one of two synthetic RNA polymers (the poly(rA) . poly(rU) duplex or the poly(rU) . poly(rA) . poly(rU) triplex). We find that these additions cause a shift in the melting temperature of poly(dA) . poly(dT), which is proportional to the concentration of the added polymer and dependent on its conformational state (B versus A, native versus denatured, and triplex versus duplex). To a first approximation, the magnitude of the observed tm shift does not depend significantly on

whether the added polymer is RNA or DNA, but it does depend on the number of strands making up the helix of the added polymer. We ascribe the observed changes in melting temperature of poly(dA) . poly(dT) to the increase in ionic strength of the bulk solution brought about by the presence of the added nucleic acid and its associated counterions. We refer to this communication between non-contacting biopolymers in solution as solvent-mediated crosstalk. By comparison with a known standard curve of tm versus log[Na+] for poly(dA) . poly(dT), we estimate the magnitude of the apparent change in ionic strength resulting from the presence of the bulk nucleic acid, and we compare these results with predictions from theory. We find that current theoretical considerations correctly predict the direction of the Tm shift (the melting temperature increases), while overestimating its magnitude. Specifically, we observe an apparent increase in ionic strength equal to 5% of the concentration of the added duplex DNA or RNA (in mol phosphate), and an additional apparent increase of about 9.5 % of the nucleic acid concentration (mol phosphate) upon denaturation of the added DNA or RNA, yielding a total apparent increase of 14.5 %. For the poly(rU) . poly(rA) . poly(rU) triplex, the total apparent increase in ionic strength corresponds to about 13.6% of the amount of added triplex (moles phosphate). The effect we observe is due to coupled equilibria between the solute molecules mediated by modulations in cation concentration induced by the presence and/or the transition of one of the solute molecules. We note that our results are general, so one can use a different solute probe sensitive to proton binding to characterize subtle changes in solution pH induced by the presence of another solute in solution. We discuss some of the broader implications of these measurements/results in terms of nucleic acid melting in multicomponent systems, in terms of probing counterion environments, and in terms of potential regulatory mechanisms.

Volker J., Makube N., Plum G. E., Klump H. H., and Breslauer K. J. (2002) Conformational energetics of stable and metastable states formed by DNA triplet repeat oligonucleotides: implications for triplet expansion diseases. *Proc Natl Acad Sci U S A* **99,** 14700-14705.
Abstract: We have embedded the hexameric triplet repeats (CAG)(6) and (CTG)(6) between two (GC)(3) domains to produce two 30-mer hairpins with the sequences d[(GC)(3)(CAG)(6)(GC)(3)] and d[(GC)(3)(CTG)(6)(GC)(3)]. This construct reduces the conformational space available to these repetitive DNA sequences. We find that the (CAG)(6) and (CTG)(6) repeats form stable, ordered, single-stranded structures. These structures are stabilized at 62 degrees C by an average enthalpy per base of 1.38 kcal.mol^{-1} for the CAG triplet and 2.87 kcal.mol^{-1} for the CTG triplet, while being entropically destabilized by 3.50 cal.K^{-1}.mol^{-1} for the CAG triplet and 7.6 cal.K^{-1}.mol^{-1} for the CTG triplet. Remarkably, these values correspond, respectively, to 1/3 (for CAG) and 2/3 (for CTG) of the enthalpy and entropy per base values associated with Watson-Crick base pairs. We show that the presence of the loop structure kinetically inhibits duplex formation from the two complementary 30-mer hairpins, even though the duplex is the thermodynamically more stable state. Duplex formation, however, does occur at elevated temperatures. We propose that this thermally induced formation of a more stable duplex results from thermal disruption of the single-stranded order, thereby allowing the complementary domains to associate (perhaps via "kissing hairpins"). Our melting profiles show that, once duplex formation has occurred, the hairpin intermediate state cannot be reformed, consistent with our interpretation of kinetically trapped hairpin structures. The duplex formed by the two complementary oligonucleotides does not have any unusual optical or thermodynamic properties. By contrast, the very stable structures formed by the individual single-stranded triplet repeat sequences are thermally and thermodynamically unusual. We discuss this stable, triplet repeat, single-stranded structure and its interconversion with duplex in terms of triplet expansion diseases.

Volker J., Klump H. H. and Breslauer K. J. (2007) The energetics of i-DNA tetraplex structures formed intermolecularly by d(TC5) and intramolecularly by d[(C5T3)3C5]. *Biopolymers* **86,** 136-147.
Abstract: Cytosine-rich DNA at low pH adopts an antiparallel tetraplex structure via the intercalation of two partially protonated, parallel stranded duplexes. This intriguing structural motif has been named i-DNA. We have used a combination of spectroscopic and calorimetric techniques to characterize the properties of an intermolecular i-DNA formed by d(TC(5)) and an intramolecular i-DNA formed by d[(C(5)T(3))(3)C(5)]. Our measurements reveal that both i-DNA complexes are enthalpically stabilized by 6.5-7.0 kcal/mol(base) and entropically destabilized by 20 cal/mol(base)/K. These values are about 50% larger than the corresponding enthalpy and entropy values per base for Watson and Crick duplexes and for Hoogsteen triplexes, while being similar to per base enthalpy and entropy values reported for G-quadruplexes. Our data also reveal a positive heat capacity change between 20 and 30 cal/mol(base)/K, values similar to that reported for polymeric Watson & Crick DNA duplexes. Solution-dependent studies

reveal the overall thermal and thermodynamic stability of i-DNA complexes to be dictated by an interplay between pH and ionic strength. Based on the thermodynamic data measured, we discuss the feasibility of i-DNA formation in the context of conventional DNA sequences, while commenting on potential roles for this structural motif in biological regulatory mechanisms.

Volker J., Klump H. H. and Breslauer K. J. (2007) DNA metastability and biological regulation: conformational dynamics of metastable omega-DNA bulge loops. *J Am. Chem Soc.* **129,** 5272-5280.
Abstract: Dynamic interchange between DNA conformations, including metastable states, can be of importance to biological function. In this study, we use a combination of spectroscopic and calorimetric techniques to detect and characterize kinetically trapped, metastable states in strand exchange and strand displacement reactions for bulge loop DNA conformations, here referred to as Omega-DNAs. We show that such metastable, Omega-DNA bulge loop states can stably coexist below 50 degrees C, while rearranging irreversibly at elevated temperatures to thermodynamically more stable states. Such dynamic interchange between metastable and globally stable DNA conformational states can be of importance in biological regulatory mechanisms.

Volker J., Klump H. H. and Breslauer K. J. (2008) DNA energy landscapes via calorimetric detection of microstate ensembles of metastable macrostates and triplet repeat diseases. *Proc. Natl. Acad. Sci U. S. A* **105,** 18326-18330.
Abstract: Biopolymers exhibit rough energy landscapes, thereby allowing biological processes to access a broad range of kinetic and thermodynamic states. In contrast to proteins, the energy landscapes of nucleic acids have been the subject of relatively few experimental investigations. In this study, we use calorimetric and spectroscopic observables to detect, resolve, and selectively enrich energetically discrete ensembles of microstates within metastable DNA structures. Our results are consistent with metastable, "native" DNA states being composed of an ensemble of discrete and kinetically stable microstates of differential stabilities, rather than exclusively being a single, discrete thermodynamic species. This conceptual construct is important for understanding the linkage between biopolymer conformational/configurational space and biological function, such as in protein folding, allosteric control of enzyme activity, RNA and DNA folding and function, DNA structure and biological regulation, etc. For the specific DNA sequences and structures studied here, the demonstration of discrete, kinetically stable microstates potentially has biological consequences for understanding the development and onset of DNA expansion and triplet repeat diseases

Wei Z., Tung C. H., Zhu T., Dickerhof W. A., Breslauer K. J., Georgopoulos D. E., Leibowitz M. J., and Stein S. (1996) Hybridization properties of oligodeoxynucleotide pairs bridged by polyarginine peptides. *Nucleic Acids Res* **24,** 655-661.
Abstract: The hybridization properties of a series of probes, based on two 9mer oligodeoxynucleotides (designated as I and II) having an appended oligoarginine chain (Rn) to produce peptide-oligonucleotide conjugates or peptide-bridged oligonucleotide pairs (e.g. Rn-I or II-Rn-I), were investigated. For the double-linked probes, we found that the peptide bridge induces the two 9mers to bind complementary single-stranded DNA or RNA targets with substantially enhanced thermal stability. The resulting hybrid with complementary DNA was found to assume a 1:1 complex in the B conformation as judged by UV mixing curves and CD spectroscopy. Complexes of single or double-linked probes with complementary RNA exhibited sensitivity to RNase H digestion. The influence of the identity and chirality of the repeating unit in the bridge, the length of the bridge, the gap size and the salt concentration on the hybridization properties of this new class of oligonucleotide probes was also studied. Our data reveal that these compounds exhibit properties that should prove useful in the development of antisense strategies.

Wheeler G., Miskovsky P., Jancura D., and Chinsky L. (1998) A study of metalloporphyrin-polynucleotide interactions by microcalorimetry and circular dichroism. *J Biomol Struct Dyn* **15,** 967-985.
Abstract: In this paper we examine the interactions of Calf Thymus DNA and the model polynucleotides poly(dA).poly(dT), poly(dAdT)2 and poly(dG.dC)2 with a group of metalloporphyrins derived from the freebase porphyrin tetrakis(4-N-methylpyridyl)porphine, H2(TMpy-P4), by means of ultraviolet absorption spectroscopy, circular dichroism spectroscopy and microcalorimetry. We have studied the interactions of the copper, cobalt, nickel and zinc derivatives of H2(TMpy-P4) in addition to the free base porphyrin itself. We have found strong evidence for an external self-stacking interaction of the Cu(TMpy-P4) and

Zn(TMpy-P4) derivatives with poly(dA).poly(dT) and poly(dAdT)2 even at low concentrations of porphyrin, and all of the porphyrin derivatives studied appear to display such a self-stacking in interaction with poly(dA.dT)2 at sufficiently high ratios of porphyrin to polynucleotide.

Williams M. C., Wenner J. R., Rouzina I., and Bloomfield V. A. (2001) Entropy and heat capacity of DNA melting from temperature dependence of single molecule stretching. *Biophys J* **80,** 1932-1939.
Abstract: When a single molecule of double-stranded DNA is stretched beyond its B-form contour length, the measured force shows a highly cooperative overstretching transition. We have measured the force at which this transition occurs as a function of temperature. To do this, single molecules of DNA were captured between two polystyrene beads in an optical tweezers apparatus. As the temperature of the solution surrounding a captured molecule was increased from 11 degrees C to 52 degrees C in 500 mM NaCl, the overstretching transition force decreased from 69 pN to 50 pN. This reduction is attributed to a decrease in the stability of the DNA double helix with increasing temperature. These results quantitatively agree with a model that asserts that DNA melting occurs during the overstretching transition. With this model, the data may be analyzed to obtain the change in the melting entropy ΔS of DNA with temperature. The observed nonlinear temperature dependence of ΔS is a result of the positive change in heat capacity of DNA upon melting, which we determine from our stretching measurements to be $\Delta Cp = 60 +/- 10$ cal/mol K bp, in agreement with calorimetric measurements.

Wu P. and Sugimoto N. (2000) New thermodynamic characterization and transition mechanism of DNA duplex formation. *Nucleic Acids Symp Ser* 15-16.
Abstract: A new transition mechanism of DNA duplex association was proposed and a segregated transition model (STM) was further derived. The experimental results in various molar ratios showed that the duplex association transition is imperfect and the thermodynamic properties and self-transition behavior of single strands exert a significant influence on DNA duplex formation.

Wu P. and Sugimoto N. (2000) Transition characteristics and thermodynamic analysis of DNA duplex formation: a quantitative consideration for the extent of duplex association. *Nucleic Acids Res* **28,** 4762-4768.
Abstract: Transition characteristics and thermodynamic properties of the single-stranded self-transition and the double-stranded association were investigated and analyzed for 9-, 15- and 21-bp non-self-complementary DNA sequences. The multiple transition processes for the single-stranded self-transition and the double-stranded association were further put forth. The experimental results confirmed that the double-stranded association transition was generally imperfect and the thermodynamic properties of the single-stranded self-transition would exert an influence on a duplex formation. Combining ultraviolet melting experiments in various molar ratios, the extent of duplex association was estimated for three double-stranded DNAs. In our experimental range, the extent of duplex association decreases with increasing the number of base pairs in DNA sequences, which suggest that the short oligonucleotides may proceed in a two-state transition while the long oligonucleotides may not. When the extent of duplex association was considered, the true transition enthalpies of a duplex formation derived from UV and differential scanning calorimetry measurements were in good agreement.

Wu P., Kawamoto Y., Hara H., and Sugimoto N. (2002) Effect of divalent cations and cytosine protonation on thermodynamic properties of intermolecular DNA double and triple helices. *J Inorg Biochem* **91,** 277-285.
Abstract: The contribution of divalent cations and cytosine protonation to conformation and stability of duplex and triplex formation were intensively investigated and characterized by ultraviolet (UV), circular dichroism (CD), differential scanning calorimetry (DSC), and electrophoresis mobility shift assay (EMSA). CD spectra showed that the divalent cations investigated would not significantly distort nucleotide geometry, while UV and DSC melting experiments revealed that the cation binding abilities to duplexes and triplexes were clearly dependent on the types of cations under near physiological conditions. The calorimetric enthalpies were generally underestimated relative to the corresponding van't Hoff enthalpies for Hoogsteen and Watson-Crick transitions, but free energy changes derived from the DSC measurements were in good agreement with those derived from the UV measurements. The adjacent placing of the C(+) x G.C triplets in triplexes lowered the stabilities of not only Hoogsteen base-pairing but also Watson-Crick base-pairing. The protonation contribution of the given cytosine residues might depend on the local and

global structure of the protonated cytosine complex. A rigid structural targeted-strand would favor the protonation of cytosine residues. The apparent pK(a) values for parallel duplex and triplex investigated were determined to be 6.4 and 7.6, respectively, which are considerably heightened by 2.1 and 3.3 pH unit as compared to the intrinsic pK(a) value of the free cytosine residues.

Wu P., Nakano S., and Sugimoto N. (2002) Temperature dependence of thermodynamic properties for DNA/DNA and RNA/DNA duplex formation. *Eur J Biochem* **269,** 2821-2830.
Abstract: A clear difference in the enthalpy changes derived from spectroscopic and calorimetric measurements has recently been shown. The exact interpretation of this deviation varied from study to study, but it was generally attributed to the non-two-state transition and heat capacity change. Although the temperature-dependent thermodynamics of the duplex formation was often implied, systemic and extensive studies have been lacking in universally assigning the appropriate thermodynamic parameter sets. In the present study, the 24 DNA/DNA and 41 RNA/DNA oligonucleotide duplexes, designed to avoid the formation of hairpin or slipped duplex structures and to limit the base pair length less than 12 bp, were selected to evaluate the heat capacity changes and temperature-dependent thermodynamic properties of duplex formation. Direct comparison reveals that the temperature-independent thermodynamic parameters could provide a reasonable approximation only when the temperature of interest has a small deviation from the mean melting temperature over the experimental range. The heat capacity changes depend on the base composition and sequences and are generally limited in the range of -160 to approximately -40 cal.mol^{-1}.K^{-1} per base pair. In contrast to the enthalpy and entropy changes, the free energy change and melting temperature are relatively insensitive to the heat capacity change. Finally, the 16 NN-model free energy parameters and one helix initiation at physiological temperature were extracted from the temperature-dependent thermodynamic data of the 41 RNA/DNA hybrids.

Xodo L. E., Alunni-Fabbroni M., and Manzini G. (1994) Effect of 5-methylcytosine on the structure and stability of DNA. Formation of triple-stranded concatenamers by overlapping oligonucleotides. *J Biomol Struct Dyn* **11,** 703-720.
Abstract: A triple helix can be formed upon binding of a pyrimidine oligonucleotide to the major groove of a homopurine-homopyrimidine (R.Y) double-stranded DNA target site. Here, we report that this reaction can be influenced by base methylation. The pyrimidine strand 5'-TmCTmCTmCTmCTTmCT (mY12), whose cytosine residues are methylated at C5, does not bind the duplex 5'-AGAGAGAGAAGA.3'-TCTCTCTCTTCT (R12.Y12) to yield a 12-triad triplex, as would be expected from these DNA sequences. Rather, a complex of overlapping oligonucleotides, which we define concatenamer, is formed. The concatenamer is clearly evidenced by polyacrylamide gel electrophoresis (PAGE) since it migrates with a smeared band of very low mobility. The stoichiometry of the concatenamer, determined by both UV mixing curves and electrophoresis, is surprisingly found to be (R12.2mY12)n, thus showing that the unmethylated Y12 strand is excluded from the complex. Denaturation experiments performed by ultraviolet absorbance (UV) and differential scanning calorimetry (DSC) show that the concatenamers melt with a single and highly cooperative transition whose Tm strongly depends on pH. Overall, the data point to the conclusion that the concatenamers are in triple helix, where the methylated mY12 strand is engaged in both Watson-Crick and Hoogsteen base pairings, thus displacing the Y12 strand from the R12.Y12 duplex. A possible mechanism of concatenamer formation is proposed. The results presented in this paper show that 5-methylcytosine brings about a strong stabilizing effect on both double and triple DNA helices, and that pyrimidine oligonucleotides containing 5-methylcytosine can displace from R.Y duplexes the analogous non-methylated strand. The advantage of using methylated oligonucleotides in antisense technology is discussed.

Xodo L. E. (1995) Characterization of the DNA triplex formed by d(TGGGTGGGTGGTTGGGTGGG) and a critical R.Y sequence located in the promoter of the murine Ki-ras proto-oncogene. *FEBS Lett* **370,** 153-157.
Abstract: The binding of the G-rich oligonucleotide d(TGGGTGGGTGGTTGGGTGGG) to a critical homopurine-homopyrimidine sequence located in the promoter of the murine Ki-ras proto-oncogene has been investigated. The duplex and the oligonucleotide form a triple helix as evidenced by band-shift electrophoresis, hydroxyapatite (HA) chromatography, UV-melting and circular dichroism (CD) experiments. Upon thermal denaturation in 50 mM Tris-acetate, pH 7.4, 50 mM NaCl, 10 mM MgCl2, 0.1 mM spermine the triplex exhibits two cooperative transitions: one of these is attributed to the triplex-to-

duplex transformation, the other to the duplex-to-coil transformation. The thermodynamic parameters of triplex formation have been determined by a van't Hoff analysis of the UV-melting curves which provided values of Δ H = 79 +/- 8 kcal/mol, Δ S = 224 +/- 22 e.u., delta G298 = 12.2 +/- 1.2 kcal/mol. These data are compared with those reported for the YRY triplex motif.

Zhong M., Rashes M. S., Leontis N. B., and Kallenbach N. R. (1994) Effects of unpaired bases on the conformation and stability of three-arm DNA junctions. *Biochemistry* **33,** 3660-3667.
Abstract: Three-arm DNA junctions, in which three double helices intersect at a branch, have unique structure and reactivity of bases at and near the branch. Their solution conformation is asymmetric in the presence of Mg2+, while bases at the branch are sensitive to single-strand-specific agents. Following the surprising report that unpaired bases at the branch stabilize three-arm junctions, we have investigated the geometry and thermodynamics of three-arm junctions containing pendant T and A bases. The results are consistent with additional structure formation in junctions containing up to four pendant bases at the branch: relative to the tight junction, the thermal stability of junctions with two T's or A's at the branch increases; bases near the branch become less reactive to single-strand-reactive probes; and the enthalpy of formation is more negative. The interaction of ethidium observed at the branch in three-arm junctions is enhanced in junctions with unpaired bases at the branch. The geometry of three-arm junctions is perturbed by the presence of pendant bases, as seen by measuring the electrophoretic mobility of junctions to which long duplex arms are appended pairwise.

Zhou J., Gregurick S. K., Krueger S., and Schwarz F. P. (2006) Conformational changes in single-strand DNA as a function of temperature by SANS. *Biophys J* **90,** 544-551.
Abstract: Small-angle neutron scattering (SANS) measurements were performed on a solution of single-strand DNA, 5'-ATGCTGATGC-3', in sodium phosphate buffer solution at 10 degrees C temperature increments from 25 degrees C to 80 degrees C. Cylindrical, helical, and random coil shape models were fitted to the SANS measurements at each temperature. All the shapes exhibited an expansion in the diameter direction causing a slightly shortened pitch from 25 degrees C to 43 degrees C, an expansion in the pitch direction with a slight decrease in the diameter from 43 degrees C to 53 degrees C, and finally a dramatic increase in the pitch and diameter from 53 degrees C to 80 degrees C. Differential scanning calorimeter scans of the sequence in solution exhibited a reversible two-state transition profile with a transition temperature of 47.5 +/- 0.5 degrees C, the midpoint of the conformational changes observed in the SANS measurements, and a calorimetric transition enthalpy of 60 +/- 3 kJ mol(-1) that indicates a broad transition as is observed in the SANS measurements. A transition temperature of 47 +/- 1 degrees C was also obtained from ultraviolet optical density measurements of strand melting scans of the single-strand DNA. This transition corresponds to unstacking of the bases of the sequence and is responsible for the thermodynamic discrepancy between its binding stability to its complementary sequence determined directly at ambient temperatures and determined from extrapolated values of the melting of the duplex at high temperature.

Zivkovic A. and Engels J. W. (2003) Synthesis of modified RNA-oligonucleotides for structural investigations. *Nucleosides Nucleotides Nucleic Acids* **22,** 1167-1170.
Abstract: RNA exhibits a higher structural diversity than DNA and is an important molecule in biology of life. It shows a number of secondary structures such as duplexes, hairpin loops, bulges, internal loops etc. However, in natural RNA, bases are limited to the four predominant structures U, C, A, and G and so the number of compounds that can be used for investigation of parameters of base stacking, base pairing and hydrogen bond, is limited. We synthesized different fluoromodifications of RNA building blocks: 1'-deoxy-1'-(2,4,6-trifluorophenyl)-beta-D-ribofuranose (F), 1'-deoxy-1'-(2,4,5-trifluorophenyl)-beta-D-ribofuranose (M) and 1'-deoxy-1'-(5-trifluoromethyl-1H-benzimidazol-1-yl)-beta-D-ribofuranose (D). Those amidites were incorporated and tested in a defined A, U-rich RNA sequence (12-mer, 5'-CUU UUC XUU CUU-3' paired with 3'-GAA AAG YAA GAA-5') (Schweitzer, B.A.; Kool, E.T. Aromatic nonpolar nucleosides as hydrophobic isosters of pyrimidine and purine nucleosides. J. Org. Chem. 1994, 59, 7238 pp.). Only one position was modified, marked as X and Y respectively. UV melting profiles of those oligonucleotides were measured.