

ITC XVI - Nucleic Acid-Nucleic Acid interactions

Archer E. A. and Krische M. J. (2002) Duplex oligomers defined via covalent casting of a one-dimensional hydrogen-bonding motif. *J Am Chem Soc* **124**, 5074-5083.

Abstract: Hydrogen-bonded tapes comprised of monomeric molecular precursors are used to define structural parameters for the design of related oligomers encoded with predetermined modes of assembly. Application of this "covalent casting" strategy vis-a-vis the one-dimensional H-bonding motif expressed by 2-amino-4,6-dichlorotriazine has enabled the design of high-affinity duplex molecular strands. Dimeric, trimeric, and tetrameric duplex oligomers are prepared through an iterative synthetic protocol involving sequential homologation of the oligo(aminotriazine). The mode of assembly and interstrand affinity of homologous oligomers are established in solution by ^1H NMR dilution experiments, isothermal titration calorimetry (ITC), vapor pressure osmometry (VPO), cross-hybridization experiments involving the analysis of dye-labeled strands via thin-layer chromatography (TLC), and in the solid state by X-ray crystallographic analysis. Binding free energy per unimer ($-\Delta G$ degrees/n) increases significantly upon extension from monomer to dimer to trimer, signifying a strong positive cooperative effect. Nanomolar binding affinity ($K(d) = 1.44 \pm 0.50$ nM) was determined for the duplex trimer by ITC in 1,2-dichloroethane at 20 degrees C. In-register duplex formation is not observed for the tetramer, which appears to adopt an alternative binding mode. These data give insight into the structural and interactional features of the oligomers required for high-affinity, high-specificity binding and define a platform for the design of second-generation systems and related duplex strands for use in nanoscale assembly.

Asensio J. L., Dosanjh H. S., Jenkins T. C., and Lane A. N. (1998) Thermodynamic, kinetic, and conformational properties of a parallel intermolecular DNA triplex containing 5' and 3' junctions. *Biochemistry* **37**, 15188-15198.

Abstract: The interaction of the 11-mer oligodeoxypyrimidine d(TCTTCTUTCCT) with the 17 bp duplex d(CGCTAGAAGAAAGGACG).d(CGTCCUTTCTTCTAGCG) in forming an intermolecular DNA triplex has been examined in solution by surface plasmon resonance (SPR), UV thermal denaturation, circular dichroism (CD), and NMR methods. Thermodynamic data were also acquired for the shorter 15 bp target duplex d(CGCTAGAAGAAAGGA). d(TCCUTTCTTCTAGCG), which forms a 3' flush-ended parallel triplex. CD titrations at pH 5 gave a triplex \rightarrow (duplex + strand) dissociation constant K_d of 0.5 μM at 15 degrees C and approximately 2 μM at 25 degrees C for both the 11-15.15 and 11-17.17 systems, in agreement with analysis of the UV melting data and a direct calorimetric measurement. In contrast, the "apparent" K_d value determined by SPR was 10-20-fold smaller. The rate constant for dissociation (k_d) of the third strand from the triplex was found to be approximately 0.0002 s^{-1} at 25 degrees C by SPR. The rate constant for exchange between the triplex and duplex states determined by NMR was approximately 2 s^{-1} at 40 degrees C. The dissociation kinetics measured by SPR are considerably underestimated, which largely accounts for the poor estimation of K_d using this technique. Extensive ^1H NMR assignments were obtained for both the 17 bp DNA duplex and the triplex. Large changes in chemical shifts were observed in the purine strand of the host duplex, but only small shift changes were induced in the complementary pyrimidine strand. Dramatic differences in shifts were observed for the G and A residues, especially in the minor groove, consistent with only small, localized conformational changes in the underlying duplex. The magnitude of the shift changes decreased to baseline within one base of the 3' triplex-duplex junction and over two to three bases at the 5' junction. Chemical shift changes at the 5' junction suggest small conformational anomalies at this site. COSY and NOESY spectra indicate that the nucleotides are in the "S" domain in both the triplex and duplex states. These data rule out major conformation changes at the triplex-duplex boundaries. NOEs between pyrimidines in the third strand and those in the duplex showed proximity for these bases in the major groove, which could be ascribed to buckling of the Hoogsteen bases out of the plane of the Watson-Crick base pairs.

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 (K_b), the enthalpies (ΔH_0), the free energies (ΔG_0), the entropies (ΔS_0) 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.

Chin T. M., Tseng M. H., Chung K. Y., Hung F. S., Lin S. B., and Kan L. S. (2001) Formation of DNA triple helix containing N(4)-(6-aminopyridin-2-yl)-2'-deoxycytidine. *J Biomol Struct Dyn* **19**, 543-553.
Abstract: A cytidinyl derivative, N(4)-(6-aminopyridin-2-yl)- 2'-deoxycytidine ((p)C), could interact with a CG base pair to support the triple-helix (triplex) formation of oligodeoxyribonucleotides. Characteristics of (p)C in the formation of both intramolecular triplex, i.e., a "paper clip type" triplex ((P)CT) and intermolecular triplex, i.e., a "linear type" triplex (LT) was monitored by optical methods and isothermal titration calorimetric measurements. Experimental results revealed that the LT with (p)C*CG internally was independent of the solution pH. Only single substitution of (p)C, situated internally but not terminally, facilitated the (P)CT formation by the UV thermal melting study at the neutral pH. However, the best stabilization of the PCT in acidic conditions occurred when (p)C at the end of the triplex rather than internally. In addition, an LT, but not a (P)CT, containing an alternating (p)CT(p)CT(p)C sequence, could be formed in the conditions of 20 mM MgCl(2) and/or 5 mM spermine. Thus, the presence of several nucleotides of (p)C in proximity along the Hoogsteen strand may lead to structural distortion such that the more flexible LT with multiple substitutions is formed in favor of the more rigid PCT.

Choosakoonkriang S., Lobo B. A., Koe G. S., Koe J. G., and Middaugh C. R. (2003) Biophysical characterization of PEI/DNA complexes. *J Pharm Sci* **92**, 1710-1722.

Abstract: The main goal of this study was to determine the effects of polyethylenimine (PEI) molecular weight and structure (750 kDa, 25 kDa, 2 kDa branched, and 25 kDa linear PEI) and the nitrogen/phosphate (N/P) molar ratio on the physical properties and transfection efficiencies of PEI/DNA complexes. Fourier transform infrared spectroscopy revealed that DNA remained in the B conformation when complexed to all PEIs. Unique alterations in the circular dichroism spectra of DNA were observed in the presence of each PEI, whereas differential scanning calorimetry measurements showed that all PEIs examined destabilized supercoiled DNA at N/P < 3/1, but not at higher ratios. Isothermal titration calorimetry revealed the existence of protonation changes at low ionic strength due to possible shifts in pK(a) of the ionizable groups of PEI during complex formation. Twenty-five kilodalton branched and 25 kDa linear PEI complexes showed the highest transfection efficiencies at an N/P ratio of 6:1 in COS-7 and CHO-K1 cells, respectively. These investigations have detected alterations in the physical and colloidal properties of the complexes that were sensitive to polymer structure, molecular weight, and polymer/DNA ratio, but these properties did not directly correlate with their transfection efficiencies. To further probe any possible relationship between these parameters and activity, a more refined biophysical analysis of any subpopulations in these samples that may differ in transfection activity is suggested, although the existence of such species remains unknown.

Diamond J. M., Turner D. H., and Mathews D. H. (2001) Thermodynamics of three-way multibranch loops in RNA. *Biochemistry* **40**, 6971-6981.

Abstract: RNA multibranch loops (junctions) are loops from which three or more helices exit. They are nearly ubiquitous in RNA secondary structures determined by comparative sequence analysis. In this study, systems in which two strands combine to form three-way junctions were used to measure the stabilities of RNA multibranch loops by UV optical melting and isothermal titration calorimetry (ITC). These data were used to calculate the free energy increment for initiation of a three-way junction on the basis of a nearest neighbor model for secondary structure stability. Imino proton NMR spectra were also measured for two systems and are consistent with the hypothesized helical structures. Incorporation of the experimental data into the mfold and RNA structure computer programs has contributed to an improvement in prediction of RNA secondary structure from sequence.

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.

Feig A. L. (2007) Applications of isothermal titration calorimetry in RNA biochemistry and biophysics. *Biopolymers* **87**, 293-301.

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

Goobes R. and Minsky A. (2001) Contextual equilibrium effects in DNA molecules. *J Biol Chem* **276**, 16155-16160.

Abstract: The thermodynamic parameters of DNA triplex formation between oligonucleotides and double-stranded DNA segments containing adenine runs (A-tracts) were investigated to explore equilibrium structural effects exerted by flanking segments upon the A-tracts. Results obtained from isothermal titration calorimetry, temperature-dependent circular dichroism (CD), and UV melting experiments indicate that A-tracts, considered as a uniquely robust and inflexible DNA motif, can be structurally perturbed by neighboring sequences in a way that significantly affects the propensity of this motif to interact with triplex-forming oligonucleotides. These contextual equilibrium effects, which depend upon the composition and location of the flanking sequences, are likely to apply not only to the interaction of A-tracts with single-stranded DNA molecules but also to interactions with drugs and proteins. As such, the current results refine the guidelines for the design of triplex-forming oligonucleotides used for antigene strategies. More generally, they substantiate the notion that significant data might be encoded by structural DNA parameters.

Goobes R. and Minsky A. (2001) Thermodynamic aspects of triplex DNA formation in crowded environments. *J Am Chem Soc* **123**, 12692-12693.

Goobes R., Kahana N., Cohen O., and Minsky A. (2003) Metabolic buffering exerted by macromolecular crowding on DNA-DNA interactions: origin and physiological significance. *Biochemistry* **42**, 2431-2440.

Abstract: Crowding, which characterizes the interior of all living cells, has been shown to dramatically affect biochemical processes, leading to stabilization of compact morphologies, enhanced macromolecular associations, and altered reaction rates. Due to the crowding-mediated shift in binding equilibria toward association, crowding agents were proposed to act as a metabolic buffer, significantly extending the range of intracellular conditions under which interactions occur. Crowding may, however, impose a liability because, by greatly and generally enhancing macromolecular association, it can lead to irreversible

interactions. To better understand the physical determinants and physiological consequences of crowding-mediated buffering, we studied the effects of crowding, or excluded volume, on DNA structures. Results obtained from isothermal titration calorimetry (ITC) and UV melting experiments indicate that crowding-induced effects are marginal under conditions that a priori favor association of DNA strands but become progressively larger when conditions deteriorate. As such, crowding exerts "genuine" buffering activity. Unexpectedly, crowding-mediated effects are found to include enthalpy terms that favorably contribute to association processes. We propose that these enthalpy terms and preferential stabilization derive from a reconfiguration of DNA hydration that occurs in dense DNA-rich phases obtained in crowded environments.

Heilman-Miller S. L., Wu T., and Levin J. G. (2004) Alteration of nucleic acid structure and stability modulates the efficiency of minus-strand transfer mediated by the HIV-1 nucleocapsid protein. *J Biol Chem* **279**, 44154-44165.

Abstract: During human immunodeficiency virus type 1 minus-strand transfer, the nucleocapsid protein (NC) facilitates annealing of the complementary repeat regions at the 3'-ends of acceptor RNA and minus-strand strong-stop DNA ((-) SSDNA). In addition, NC destabilizes the highly structured complementary trans-activation response element (TAR) stem-loop (TAR DNA) at the 3'-end of (-) SSDNA and inhibits TAR-induced self-priming, a dead-end reaction that competes with minus-strand transfer. To investigate the relationship between nucleic acid secondary structure and NC function, a series of truncated (-) SSDNA and acceptor RNA constructs were used to assay minus-strand transfer and self-priming in vitro. The results were correlated with extensive enzymatic probing and mFold analysis. As the length of (-) SSDNA was decreased, self-priming increased and was highest when the DNA contained little more than TAR DNA, even if NC and acceptor were both present; in contrast, truncations within TAR DNA led to a striking reduction or elimination of self-priming. However, destabilization of TAR DNA was not sufficient for successful strand transfer: the stability of acceptor RNA was also crucial, and little or no strand transfer occurred if the RNA was highly stable. Significantly, NC may not be required for in vitro strand transfer if (-) SSDNA and acceptor RNA are small, relatively unstructured molecules with low thermodynamic stabilities. Collectively, these findings demonstrate that for efficient NC-mediated minus-strand transfer, a delicate thermodynamic balance between the RNA and DNA reactants must be maintained.

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 $-73 \pm 2 \text{ kcal (mol of duplex)}^{-1}$. 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 $-1.3 \pm 0.1 \text{ kcal K}^{-1} \text{ (mol of duplex)}^{-1}$. From DSC denaturation studies in 120 mM Na⁺, we obtain an enthalpy of duplex formation of $-120 \pm 5 \text{ kcal (mol of duplex)}^{-1}$ and an estimate of the corresponding heat capacity change of $-0.8 \pm 0.4 \text{ kcal K}^{-1} \text{ (mol of duplex)}^{-1}$ at the T_m 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 $-5.5 \pm 0.5 \text{ kcal}$ 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 $-64 \text{ kcal (mol of duplex)}^{-1}$ as compared to $-210 \text{ kcal (mol of duplex)}^{-1}$ calculated for duplex formation from completely unstructured single strands and to the experimental ITC value of $-73 \text{ kcal (mol of duplex)}^{-1}$. The intrinsic

heat capacity change for association of single helical strands to form the duplex is found to be small and positive [approximately $0.1 \text{ kcal K}^{-1} (\text{mol of duplex})^{-1}$], in agreement with the result of a surface area analysis, which also predicts an undetectably small heat capacity change for single helix formation.

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 $200(+/-40) \text{ JK}(-1) (\text{mol bp})(-1)$. 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 $-33(+/-2) \text{ kJ} (\text{mol bp})(-1)$, $-93(+/-4) \text{ J K}(-1) (\text{mol bp})(-1)$ and $-5.0(+/-0.5) \text{ kJ} (\text{mol bp})(-1)$, respectively.

Kamiya M., Shimizume R., Shindo H., Torigoe H., and Sarai A. (1995) Mechanism of the formation of DNA triplex and effect of chemical modifications on its stability as studied by isothermal titration calorimetry. *Nucleic Acids Symp Ser* 57-58.

Abstract: The thermodynamic properties of DNA triplex formation with various single-stranded oligo-DNAs as well as mismatched sequences were investigated by isothermal titration calorimetry (ITC). For the triplex formation of perfectly matched sequences, enthalpy changes and dissociation constants were measured and their temperature-dependences suggest that conformational change of pyrimidine single-strand and the intermediate state(s) are involved in the triplex formation. Effects of mismatches and chemical modifications on stability and specificity of the triplex formation will also be discussed.

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, ΔC_p , 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

Ladbury J. E., Sturtevant J. M., and Leontis N. B. (1994) The thermodynamics of formation of a three-strand, DNA three-way junction complex. *Biochemistry* **33**, 6828-6833.

Abstract: Isothermal titration calorimetry (ITC) is used to study the thermodynamics of assembly of the three DNA oligonucleotides S1 (5'-GCCTGCCACCGC), S2 (5'-GCGGTGCGTCCG), and S3AA (5'-CGGACGAAGCAGGC) to form a three-way junction (TWJ) complex consisting of three double-helical arms radiating from a junction region having two unpaired adenosines in one strand (S3AA). The thermodynamics of assembly were measured for three different orders of addition of the component oligonucleotides at four temperatures between 10 and 25 degrees C. At each temperature studied, the overall values of ΔH , ΔS degrees, and ΔG degrees for assembly of the complex from the component single strands were found to be independent of the order of addition. The enthalpy of binding, ΔH , was found to be linearly dependent on temperature. From the temperature dependence of ΔH , the change in heat capacity ΔC_p , for the overall assembly of three strands to form the junction complex was calculated and found to be $-1.6 \text{ kcal mol}^{-1} \text{ K}^{-1}$. This work represents the first attempt to evaluate the thermodynamics of DNA TWJ formation by ITC.

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, ΔG 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 $-1.42 \pm 0.09 \text{ kJ mol}^{-1} \text{ K}^{-1}$ for DNA/DNA and $-0.87 \pm 0.05 \text{ kJ mol}^{-1} \text{ K}^{-1}$ for DNA/RNA. Values of ΔG 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 ΔG 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., 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

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 $5.28 \times 10^7 \text{M}^{-1}$ 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 $8.06 \times 10^{-3} \text{s}^{-1}$ 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.

Lu M., Guo Q., Marky L. A., Seeman N. C., and Kallenbach N. R. (1992) Thermodynamics of DNA branching. *J Mol Biol* **223**, 781-789.

Abstract: Branched DNA molecules arise transiently as intermediates in genetic recombination or on extrusion of cruciforms from covalent circular DNA duplexes that contain palindromic sequences. The free energy of these structures relative to normal DNA duplexes is of interest both physically and biologically. Oligonucleotide complexes that can form stable branched structures, DNA junctions, have made it possible to model normally unstable branched states of DNA such as Holliday recombinational intermediates. We present here an evaluation of the free energy of creating four-arm branch points in duplex DNA, using a system of two complementary junctions and four DNA duplexes formed from different combinations of the same set of eight 16-mer strands. The thermodynamics of formation of each branched structure from the matching pair of intact duplexes have been estimated in two experiments. In the first, labeled strands are allowed to partition between duplexes and junctions in a competition assay on polyacrylamide gels. In the second, the heats of forming branched or linear molecules from the component strands have been determined by titration microcalorimetry at several temperatures. Taken together these measurements allow us to determine the standard thermodynamic parameters for the process of creating a branch in an otherwise normal DNA duplex. The free energy for reacting two 16-mer duplexes to yield a four-arm junction in which the branch site is incapable of migrating is $+1.1 (+/- 0.4) \text{kcal mol}^{-1}$ (at 18 degrees C, 10 mM-Mg2+). Analysis of the distribution of duplex and tetramer products by electrophoresis confirms that the free energy difference between the four duplexes and two junctions is small at this temperature. The associated enthalpy change at 18 degrees C is $+27.1 (+/- 1.3) \text{kcal mol}^{-1}$, while the entropy is $+89 (+/- 30) \text{cal K}^{-1} \text{mol}^{-1}$. The free energy for branching is temperature dependent, with a large unfavorable enthalpy change compensated by a favorable entropy term. Since forming one four-stranded complex from two duplexes should be an entropically unfavorable process, branch formation is likely to be accompanied by significant changes in hydration and ion binding. A significant apparent ΔC_p is also observed for the formation of one mole of junction, $+0.97 (+/- 0.05) \text{kcal deg}^{-1} \text{mol}^{-1}$.

Lu M., Guo Q., and Kallenbach N. R. (1993) Thermodynamics of G-tetraplex formation by telomeric DNAs. *Biochemistry* **32**, 598-601.

Abstract: Telomeres are structures at the ends of eukaryotic chromosomes, the DNA of which contains stretches of tandemly repeated sequences with G clusters along one strand. Model telomeric G-rich DNAs can form different tetraplex structures, stabilized by cyclic hydrogen bonding of four guanines in the presence of metal ions such as Na+ or K+. Oligonucleotides with a single copy of the Oxytricha sequence dT4G4 form a tetramer, with a parallel-stranded, right-handed helical structure. Additional copies favor folded-back structures that associate to form an antiparallel dimer. The parallel-stranded tetramer has all G's in the anti configuration, while the folded-back dimer has alternating syn and anti nucleotide

conformations along each strand. Here we have constructed two G-tetraplex structures, containing identical G-tetrad base pairs, from oligonucleotides. One has the truncated telomeric sequence from *Oxytricha*, dG4T4G4, which forms an antiparallel G-quartet structure; the second is constrained to form a parallel G-strand arrangement by insertion of a 5'-p-5' linkage between two dT2G4 sequences. Each oligomer forms a defined G-tetraplex dimeric structure in the presence of Na⁺. The standard-state enthalpies, entropies, and free energy for formation of these tetraplexes have been determined. The parallel strand structure is thermodynamically more stable than the antiparallel one, primarily because of both greater enthalpy and entropy of formation. In addition, the two molecules differ in their interaction with sodium ions, reflecting a difference in ion binding and therefore in structure between the two forms.

Majhi P. R., Qi J., Tang C. F. and Shafer R. H. (2008) Heat capacity changes associated with guanine quadruplex formation: an isothermal titration calorimetry study. *Biopolymers* **89**, 302-309.

Abstract: This study addresses the temperature dependence of the enthalpy of formation for several unimolecular quadruplexes in the presence of excess monovalent salt. We examined a series of biologically significant guanine-rich DNA sequences: thrombin binding aptamer (TBA) (d(G(2)T(2)G(2)TGTG(2)T(2)G(2))), PS2.M, a catalytically active aptamer (d(GTG(3)TAG(3)CG(3)T(2)G(2))), and the human telomere repeat (HT) (d(AG(3)(T(2)AG(3))(3))). Using CD spectra and UV melting, we confirmed the presence of quadruplex structures and established the temperature range in which quadruplex conformation is stable. We then performed ITC experiments, adding DNA to a solution containing excess NaCl or KCl. In this approach, only several additions are made, and only the enthalpy of quadruplex formation is measured. This measurement was repeated at different temperatures to determine the temperature dependence of the enthalpy change accompanying quadruplex formation. To control for the effect of nonspecific salt interactions during DNA folding, we repeated the experiment by replacing the quadruplex-forming sequences with a similar but nonfolding sequence. Dilution enthalpies were also subtracted to obtain the final enthalpy value involving only the quadruplex folding process. For all sequences studied, quadruplex formation was exothermic but with an increasing magnitude with increasing temperature. These results are discussed in terms of the change in heat capacity associated with quadruplex formation

Mikulecky P. J. and Feig A. L. (2004) Heat capacity changes in RNA folding: application of perturbation theory to hammerhead ribozyme cold denaturation. *Nucleic Acids Res* **32**, 3967-3976.

Abstract: In proteins, empirical correlations have shown that changes in heat capacity (ΔC_p) scale linearly with the hydrophobic surface area buried upon folding. The influence of ΔC_p on RNA folding has been widely overlooked and is poorly understood. In addition to considerations of solvent reorganization, electrostatic effects might contribute to ΔC_p s of folding in polyanionic species such as RNAs. Here, we employ a perturbation method based on electrostatic theory to probe the hot and cold denaturation behavior of the hammerhead ribozyme. This treatment avoids much of the error associated with imposing two-state folding models on non-two-state systems. Ribozyme stability is perturbed across a matrix of solvent conditions by varying the concentration of NaCl and methanol co-solvent. Temperature-dependent unfolding is then monitored by circular dichroism spectroscopy. The resulting array of unfolding transitions can be used to calculate a ΔC_p of folding that accurately predicts the observed cold denaturation temperature. We confirm the accuracy of the calculated ΔC_p by using isothermal titration calorimetry, and also demonstrate a methanol-dependence of the ΔC_p . We weigh the strengths and limitations of this method for determining ΔC_p values. Finally, we discuss the data in light of the physical origins of the ΔC_p s for RNA folding and consider their impact on biological function.

Mikulecky P. J., Takach J. C., and Feig A. L. (2004) Entropy-driven folding of an RNA helical junction: an isothermal titration calorimetric analysis of the hammerhead ribozyme. *Biochemistry* **43**, 5870-5881.

Abstract: Helical junctions are extremely common motifs in naturally occurring RNAs, but little is known about the thermodynamics that drive their folding. Studies of junction folding face several challenges: non-two-state folding behavior, superposition of secondary and tertiary structural energetics, and drastically opposing enthalpic and entropic contributions to folding. Here we describe a thermodynamic dissection of the folding of the hammerhead ribozyme, a three-way RNA helical junction, by using isothermal titration calorimetry of bimolecular RNA constructs. By using this method, we show that tertiary folding of the hammerhead core occurs with a highly unfavorable enthalpy change, and is therefore entropically driven.

Furthermore, the enthalpies and heat capacities of core folding are the same whether supported by monovalent or divalent ions. These properties appear to be general to the core sequence of bimolecular hammerhead constructs. We present a model for the ion-induced folding of the hammerhead core that is similar to those advanced for the folding of much larger RNAs, involving ion-induced collapse to a structured, non-native state accompanied by rearrangement of core residues to produce the native fold. In agreement with previous enzymological and structural studies, our thermodynamic data suggest that the hammerhead structure is stabilized in vitro predominantly by diffusely bound ions. Our approach addresses several significant challenges that accompany the study of junction folding, and should prove useful in defining the thermodynamic determinants of stability in these important RNA motifs.

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 (ΔC_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 ΔC_p due to the release of waters solvating aromatic surfaces of nucleotide bases. In previous work, we showed that the ΔC_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 ΔC_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 ΔC_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 ΔC_p from coupled equilibria and conclude that observed ΔC_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 (ΔC_p s) 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 ΔC_p s associated with nucleic acid folding transitions. This work helps to gather the molecular insights that can be gleaned from analysis of ΔC_p s and points toward the challenges that will need to be overcome if the energetic contribution of ΔC_p terms are to be put to use in improving free energy calculations for nucleic acid structure prediction.

Miyoshi D., Matsumura S., Nakano S., and Sugimoto N. (2004) Duplex dissociation of telomere DNAs induced by molecular crowding. *J Am Chem Soc* **126**, 165-169.

Abstract: Because of the importance of telomere DNAs, the structures of these DNAs in vivo are currently of great research interest in the medical, pharmaceutical, chemical, and industrial fields. To understand the structure of biomolecules in vivo, their properties studied in vitro are extrapolated to the in vivo condition, while the condition in a living cell is inherently molecularly crowded and a nonideal solution contains various biomolecules. We investigated the effect of molecular crowding, which is one of the most important cellular environmental conditions, on the structure and stability of the telomere and G-rich and C-rich DNAs using circular dichroism (CD) spectra, CD melting curves, and isothermal titration calorimetry (ITC). The CD spectra and CD melting curves of G-rich DNA, C-rich DNA, and the 1:1 mixture of G-rich and C-rich DNAs showed that each G-rich DNA, C-rich DNA, and the 1:1 mixture form the antiparallel G-quadruplex, I-motif, and duplex, respectively, in the noncrowding condition as previously considered. On the contrary, the G-rich and C-rich DNAs individually form the parallel G-quadruplex and I-motif, respectively, in the molecular crowding condition, and the 1:1 mixture folds into the parallel G-quadruplex and I-motif but does not form a duplex. The ITC measurements indicated that the thermodynamic stability (ΔG_{20}) of the duplex formation between the G-rich and C-rich DNAs in the noncrowding condition was $-10.2 \text{ kcal mol}^{-1}$, while only a small heat change was observed in the ITC

measurements in the molecular crowding condition. These ITC results also demonstrated that the molecular crowding condition prevents any duplex formation between G-rich and C-rich DNAs. These results indicate that a structural polymorphism of the telomere DNAs is induced by molecular crowding in vivo.

Ratilainen T., Holmen A., Tuite E., Haaime G., Christensen L., Nielsen P. E., and Norden B. (1998) Hybridization of peptide nucleic acid. *Biochemistry* **37**, 12331-12342.

Abstract: The thermodynamics of hybridization and the conformations of decameric mixed purine-pyrimidine sequence PNA/PNA, PNA/DNA, and DNA/DNA duplexes have been studied using fluorescence energy transfer (FET), absorption hypochromicity (ABS), isothermal titration calorimetry (ITC), and circular dichroism (CD) techniques. The interchromophoric distances determined in the FET experiments on fluorescein- and rhodamine-labeled duplexes indicate that the solution structures of the duplexes are extended helices in agreement with available NMR (PNA/DNA) and crystal X-ray data (PNA/PNA). The melting thermodynamics of the duplexes was studied with both FET and ABS. The thermodynamic parameters obtained with ABS are in good agreement with the parameters from calorimetric measurements while FET detection of duplex melting gives in most cases more favorable free energies of hybridization. This discrepancy between FET and ABS detection is ascribed to the conjugated dyes which affect the stability of the duplexes substantially. Especially, the dianionic fluorescein attached via a flexible linker either to PNA or to DNA seems to be involved in an attractive interaction with the opposite dicationic lysine when hybridized to a PNA strand. This interaction leads to an increased thermal stability as manifested as a 3-4 degreesC increase of the melting temperature. For the PNA/DNA duplex where fluorescein is attached to the PNA strand, a large destabilization ($\Delta T_m = -12$ degreesC) occurs relative to the unlabeled duplex, probably originating from electrostatic repulsion between the fluorescein and the negatively charged DNA backbone. In the case of the PNA/PNA duplex, the sense of helicity of the duplex is reversed upon conjugation of fluorescein via a flexible linker arm, but not when the fluorescein is attached without a linker to the PNA.

Ratilainen T., Holmen A., Tuite E., Nielsen P. E., and Norden B. (2000) Thermodynamics of sequence-specific binding of PNA to DNA. *Biochemistry* **39**, 7781-7791.

Abstract: For further characterization of the hybridization properties of peptide nucleic acids (PNAs), the thermodynamics of hybridization of mixed sequence PNA-DNA duplexes have been studied. We have characterized the binding of PNA to DNA in terms of binding affinity (perfectly matched duplexes) and sequence specificity of binding (singly mismatched duplexes) using mainly absorption hypochromicity melting curves and isothermal titration calorimetry. For perfectly sequence-matched duplexes of varying lengths (6-20 bp), the average free energy of binding (ΔG degrees) was determined to be -6.5 ± 0.3 kJ mol⁻¹bp⁻¹, corresponding to a microscopic binding constant of about 14 M⁻¹ bp⁻¹. A variety of single mismatches were introduced in 9- and 12-mer PNA-DNA duplexes. Melting temperatures (T_m) of 9- and 12-mer PNA-DNA duplexes with a single mismatch dropped typically 15-20 degrees C relative to that of the perfectly matched sequence with a corresponding free energy penalty of about 15 kJ mol⁻¹bp⁻¹. The average cost of a single mismatch is therefore estimated to be on the order of or larger than the gain of two matched base pairs, resulting in an apparent binding constant of only 0.02 M⁻¹ per mismatch. The impact of a mismatch was found to be dependent on the neighboring base pairs. To a first approximation, increasing the stability of the surrounding region, i.e., the distribution of A.T and G.C base pairs, decreases the effect of the introduced mismatch.

Ratilainen T. and Norden B. (2002) Thermodynamics of PNA interactions with DNA and RNA. *Methods Mol Biol* **208**, 59-88.

Recht M. I. and Williamson J. R. (2004) RNA tertiary structure and cooperative assembly of a large ribonucleoprotein complex. *J Mol Biol* **344**, 395-407.

Abstract: The mechanisms that govern the ordered assembly of multiprotein ribonucleoprotein complexes are not well understood. The in vitro reconstitution of the small subunit of the bacterial ribosome provides a tractable system for the detailed study of ordered assembly. We present a quantitative thermodynamic description of the hierarchical binding of ribosomal proteins to 16S rRNA during assembly of the platform of the 30S ribosomal subunit. The binding of S8, S11, S15, and the S6:S18 heterodimer to the central domain of 16S rRNA has been measured both individually and in combination using isothermal titration

calorimetry and gel mobility shift assays. Both enthalpy and free energy measurements demonstrate the cooperative binding of S15 and the S6:S18 heterodimer, but no cooperativity is observed for either S8 or S11. The results define a thermodynamic framework that describes cooperative platform assembly.

Rentzeperis D., Kupke D. W., and Marky L. A. (1992) Differential hydration of homopurine sequences relative to alternating purine/pyrimidine sequences. *Biopolymers* **32**, 1065-1075.

Abstract: The minor groove ligand distamycin A has been used to probe the relative hydration of the minor groove of eight synthetic polynucleotides of known sequence and composition. A combination of densimetric, calorimetric, and temperature-dependent spectroscopic techniques have been used to obtain complete thermodynamic profiles (ΔG_{zero} , ΔH_{zero} , ΔS_{zero} , and ΔV_{zero}) for the association of distamycin A to all polymer duplexes. In 10 mM phosphate buffer, pH 7, binding of the drug to each of the polymeric duplexes resulted in characteristic negative changes in both the volume and enthalpy. Although the binding constants were found to be identical for pairs of isomer polynucleotides having identical compositions but different sequences, the values of ΔH_{zero} , ΔS_{zero} , and ΔV_{zero} of each such pair were remarkably different. The entropy changes were found to roughly parallel the volume changes; no such trend was seen between ΔH_{zero} and ΔV_{zero} . The data support the hypothesis that the volume changes observed for these systems reflect the coulombic-hydration contribution to the entropy. The heteropolymer duplexes generated much larger exothermic contributions, less favorable entropies and larger volume contractions than did the corresponding homopolymer duplexes of identical composition, and strongly suggest that polynucleotides with homopurine sequences are more hydrated than polynucleotides with alternating purine/pyrimidine sequences. In addition, it was found that duplexes containing guanine sharply reduced the affinity for the drug, also lowering the exothermicity but raising the entropy. This may be explained by the presence of an amino group in the minor groove that prevents hydrogen bonding. Substitution of the guanine with inosine reversed this trend in the thermodynamic properties. Furthermore, substitution of poly(dA) for poly(rA) in a duplex produced a similar reduction in the affinity, while raising the exothermic contribution and greatly reducing the favorable entropy effect in agreement with an apparent increase in the hydration state.

Rentzeperis D., Kupke D. W., and Marky L. A. (1993) Volume changes correlate with entropies and enthalpies in the formation of nucleic acid homoduplexes: differential hydration of A and B conformations. *Biopolymers* **33**, 117-125.

Abstract: We have used a combination of densimetric, calorimetric, and uv absorption techniques to obtain a complete thermodynamic characterization for the formation of nucleic acid homoduplexes of known sequence and conformation. The volume change ΔV accompanying the formation of four duplexes was interpreted to reflect changes in hydration based on the electrostriction phenomenon. In 10 mM sodium phosphate buffer at pH 7, the magnitude of the measured ΔV 's ranged from -2.0 to +7.2 ml/mol base pair and followed the order of poly(rA).poly(dT) approximately poly(dA).poly(dT) < poly(rA).poly(dU) approximately poly(rA).poly(rU). Inclusion of 100 mM NaCl in the same buffer gave the range of -17.4 to -2.3 mL/mol base pair and the following order: poly(dA).poly(dT) < poly(rA).poly(dT) < poly(rA).poly(rU) approximately poly(rA).poly(rU). Standard thermodynamic profiles of forming these duplexes from their corresponding complementary single strands indicated similar free energies that resulted from the compensation of favorable enthalpies with unfavorable entropies along with a similar counterion uptake at both ionic strengths. The differences in these compensating effects of entropy and enthalpy correlated very well with the volume change measurements in a manner suggesting that the homoduplexes in the B conformation are more hydrated than are those in the A conformation. Moreover, the increased thermal stability of these homoduplexes resulted from an increase in the salt concentration corresponding to larger hydration levels as reflected by the ΔV results.

Rentzeperis D., Kupke D. W., and Marky L. A. (1994) Differential hydration of dA.dT base pairs in parallel-stranded DNA relative to antiparallel DNA. *Biochemistry* **33**, 9588-9591.

Abstract: Parallel-stranded DNA is a novel double-stranded helical form of DNA. Its secondary structure is established by reverse Watson-Crick base pairing between the bases of the complementary strands forming a double helix with equivalent grooves. We have used a combination of magnetic suspension densitometry and isothermal titration calorimetry to obtain complete thermodynamic profiles for the formation of two DNA 25 mer duplexes. The duplexes contain exclusively dA.dT base pairs in either parallel (ps-d1.D2) or antiparallel (aps-D1.D3) orientation. At 15 degrees C, the formation of each duplex

is accompanied by favorable free-energy terms resulting from the partial compensation of favorable exothermic enthalpies and unfavorable entropies and by an uptake of both counterions and water molecules. By taking into account the contribution of single-strand base-stacking interactions and using the formation of the aps-D1.D3 duplex as a reference state to establish a thermodynamic cycle in which the similar single strands cancel out, we obtained a $\Delta \Delta G$ zero term of $+10 \text{ kcal mol}^{-1}$ duplex formed that results from a partial differential enthalpy-entropy compensation of $+32 \text{ kcal mol}^{-1}$ and a $\Delta \Delta V$ of 257 mL mol^{-1} . The positive sign of this enthalpy-entropy compensation together with the marginal differential counterion uptake of $0.2 \text{ mol of Na}^+/\text{mol of duplex}$ is characteristic of processes driven by differential hydration and strongly suggests that the parallel duplex is much less hydrated than its antiparallel counterpart by approximately $4 \text{ mol of water/mol of base pair}$.

Salim N. N. and Feig A. L. (2008) Isothermal titration calorimetry of RNA. *Methods (epublication)*.

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

Schwarz F. P., Robinson S., and Butler J. M. (1999) Thermodynamic comparison of PNA/DNA and DNA/DNA hybridization reactions at ambient temperature. *Nucleic Acids Res* **27**, 4792-4800.

Abstract: The thermodynamics of 13 hybridization reactions between 10 base DNA sequences of design 5'-ATGCXYATGC-3' with X, Y = A, C, G, T and their complementary PNA and DNA sequences were determined from isothermal titration calorimetry (ITC) measurements at ambient temperature. For the PNA/DNA hybridization reactions, the binding constants range from $1.8 \times 10^6 \text{ M}^{-1}$ for PNA(TT)/DNA to $4.15 \times 10^7 \text{ M}^{-1}$ for PNA(GA)/DNA and the binding enthalpies range from -194 kJ mol^{-1} for PNA(CG)/DNA to -77 kJ mol^{-1} for PNA(GT)/DNA. For the corresponding DNA/DNA binding reactions, the binding constants range from $2.9 \times 10^5 \text{ M}^{-1}$ for DNA(GT)/DNA to $1.9 \times 10^7 \text{ M}^{-1}$ for DNA(CC)/DNA and the binding enthalpies range from -223 kJ mol^{-1} for DNA(CG)/DNA to -124 kJ mol^{-1} for DNA(TT)/DNA. Most of the PNA sequences exhibited tighter binding affinities than their corresponding DNA sequences resulting from smaller entropy changes in the PNA/DNA hybridization reactions. van't Hoff enthalpies and extrapolated ΔG values determined from UV melting studies on the duplexes exhibited closer agreement with the ITC binding enthalpies and ΔG values for the DNA/DNA duplexes than for the PNA/DNA duplexes.

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)

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 $\Delta\Delta H(\text{ITC})-\Delta(\Delta S)$ 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., 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.

Takach J. C., Mikulecky P. J., and Feig A. L. (2004) Salt-dependent heat capacity changes for RNA duplex formation. *J Am Chem Soc* **126**, 6530-6531.

Torigoe H., Shimizume R., Sarai A., and Shindo H. (1997) Effect of chemical modification of oligohomopyrimidine on triplex formation: thermodynamic and kinetic studies. *Nucleic Acids Symp Ser* 267-268.

Abstract: To investigate the effect of chemical modification of the third strand on the stability of triplex DNA, we have examined the thermodynamic properties of the triplex formation between a 23-mer double-stranded homopurine-homopyrimidine and each of five kinds of 15-mer chemically modified single-stranded homopyrimidines by isothermal titration calorimetry, and the kinetic properties by interaction analysis system. The modifications of the third strand included two base modifications, two sugar moiety modifications, and one phosphate backbone modification. The thermodynamic and kinetic parameters for the triplex formation were similar in magnitude among the two base-modified and two sugar-modified single strands. By contrast, the binding constant for the triplex formation with the single strand with phosphorothioate backbone was more than ten times as small as that for the other triplex formation. On the basis of the kinetic analyses, the single strand with phosphorothioate backbone was more difficult to associate with and easier to dissociate from the target double strand than the other single strands, which resulted in the much smaller binding constant.

Torigoe H., Ferdous A., Watanabe H., Akaike T., and Maruyama A. (1999) Poly(L-lysine)-graft-dextran copolymer promotes pyrimidine motif triplex DNA formation at physiological pH. Thermodynamic and kinetic studies. *J Biol Chem* **274**, 6161-6167.

Abstract: Extreme instability of pyrimidine motif triplex DNA at physiological pH severely limits its use for artificial control of gene expression in vivo. Stabilization of the pyrimidine motif triplex at physiological pH is therefore of great importance in improving its therapeutic potential. To this end, isothermal titration calorimetry interaction analysis system and electrophoretic mobility shift assay have been used to explore the thermodynamic and kinetic effects of our previously reported triplex stabilizer, poly(L-lysine)-graft-dextran (PLL-g-Dex) copolymer, on pyrimidine motif triplex formation at physiological pH. Both the thermodynamic and kinetic analyses have clearly indicated that in the presence of the PLL-g-Dex copolymer, the binding constant of the pyrimidine motif triplex formation at physiological pH was about 100 times higher than that observed without any triplex stabilizer. Of importance, the triplex-promoting efficiency of the copolymer was more than 20 times higher than that of physiological concentrations of spermine, a putative intracellular triplex stabilizer. Kinetic data have also demonstrated that the observed copolymer-mediated promotion of the triplex formation at physiological pH resulted from the considerable increase in the association rate constant rather than the decrease in the dissociation rate constant. Our results certainly support the idea that the PLL-g-Dex copolymer could be a key material and may eventually lead to progress in therapeutic applications of the antigene strategy in vivo.

Torigoe H., Shimizume R., Sarai A., and Shindo H. (1999) Triplex formation of chemically modified homopyrimidine oligonucleotides: thermodynamic and kinetic studies. *Biochemistry* **38**, 14653-14659.

Abstract: We have investigated effects of chemical modifications of a third strand on the thermodynamic and kinetic properties of the triplex formation between a 23-bp duplex and each of four kinds of 15-mer chemically modified third strands using isothermal titration calorimetry and interaction analysis system. The chemical modifications of the third strand included one base modification, with replacement of thymine by uracil; two sugar moiety modifications, RNA and 2'-O-methyl-RNA; and one phosphate backbone modification, with replacement of phosphodiester by phosphorothioate backbone. The thermodynamic and kinetic parameters obtained were similar in magnitude at room temperature for the triplex formation with the base-modified and the sugar-modified third strands. By contrast, binding constant for the triplex formation with the third strand containing phosphorothioate backbone was much

smaller by a factor of 10 than that for the other triplex formations. Kinetic analyses have also demonstrated that the third strand containing phosphorothioate backbone was much slower in the association step and much faster in the dissociation step than the other third strands, which resulted in the much smaller binding constant. The reason for the instability of the triplex with the third strand containing phosphorothioate backbone will be discussed. We conclude that, at least in the triplex formation with the chemically modified third strands studied in the present work, the modification of phosphate backbone of the third strand produces more significant effect on the triplex formation than the modifications of base and sugar moiety.

Torigoe H., Akaike T., and Maruyama A. (1999) Promotion mechanism of triplex DNA formation by comb-type polycations: thermodynamic analyses of sequence specificity and ionic strength dependence. *Nucleic Acids Symp Ser* 137-138.

Abstract: We have previously reported that in the presence of poly (L-lysine)-graft-Dextran (PLL-g-Dex) copolymer, the binding constant of the pyrimidine-motif triplex formation at neutral pH is about 100-times higher than that observed without any triplex stabilizer. Here, to explore the mechanism of the promotion effect of the PLL-g-Dex copolymer at neutral pH, the sequence specificity and the ionic strength dependence of the pyrimidine-motif triplex formation was examined in the absence or presence of the copolymer. The sequence specificity of the pyrimidine-motif triplex formation at neutral pH in the presence of copolymer was almost similar to that at acidic pH without the copolymer. As the concentration of magnesium cation increased, the binding constant of the pyrimidine-motif triplex formation without the copolymer increased. On the other hand, the binding constant of the pyrimidine-motif triplex formation in the presence of the copolymer decreased upon the increase in the concentration of magnesium cation. Considering these results in light of counterion condensation (CC) theory, we conclude that the copolymer does not hinder the sequence specificity of the triplex formation, and isolates the triplex formation from the CC effect, which may lead to a net increase in entropy change upon the triplex formation, providing a favorable component to binding constant of the triplex formation.

Torigoe H. and Shimizume R. (2000) Thermodynamic analyses of triplex formation with homopurine oligonucleotide. *Nucleic Acids Symp Ser* 61-62.

Abstract: We analyzed the thermodynamics of purine motif triplex formation by isothermal titration calorimetry. The signs of calorimetric enthalpy change, ΔH_{cal} , and entropy change, ΔS , of the triplex formation were negative in the temperature range between 15 and 35 degrees C. Since an observed negative ΔS was unfavorable for the triplex formation, the triplex formation was driven by a large negative ΔH_{cal} . ΔH_{cal} decreased with increasing temperature, yielding a negative heat capacity change, ΔC_p , of approximately $-1.2 \text{ kcal mol}^{-1} \text{ K}^{-1}$. We found that the binding constant, K_a , increased with increasing temperature, leading to an apparent positive van't Hoff enthalpy change, ΔH_{vh} , which was in sharp contrast with the large negative ΔH_{cal} . The analyses of the observed temperature dependence of K_a and ΔH_{cal} and the negative ΔC_p suggest that the purine motif triplex formation near room temperature is not a simple two-state binding process but exhibits multiple states, which was previously observed for the pyrimidine motif triplex formation near room temperature.

Torigoe H. (2001) Thermodynamic and kinetic effects of N3'-->P5' phosphoramidate modification on pyrimidine motif triplex DNA formation. *Biochemistry* **40**, 1063-1069.

Abstract: I have investigated the thermodynamic and kinetic effects of N3'-->P5' phosphoramidate (PN) backbone modification of triplex-forming oligonucleotide (TFO) on the pyrimidine motif triplex formation between a 23-bp target duplex and a 15-mer TFO using electrophoretic mobility shift assay, UV melting, isothermal titration calorimetry, and interaction analysis system. The thermodynamic and kinetic analyses have clearly indicated that the PN modification of TFO not only significantly increased the thermal stability of the pyrimidine motif triplex at neutral pH but also increased the binding constant of the pyrimidine motif triplex formation at room temperature and neutral pH by nearly 2 orders of magnitude. The consideration of the observed thermodynamic parameters has suggested that the more rigidity of the PN TFO in the free state relative to the unmodified TFO may enable the significant increase in the binding constant of the pyrimidine motif triplex formation at neutral pH. Kinetic data have also demonstrated that the observed PN modification-mediated promotion of pyrimidine motif triplex formation at neutral pH resulted from the considerable decrease in the dissociation rate constant rather than the increase in the association rate constant. This information will present an effective approach for designing chemically modified TFO with

higher binding affinity in the triplex formation under physiological conditions, which may eventually lead to progress in therapeutic applications of the antigene strategy in vivo.

Torigoe H., Katayama T., and Umegaki Y. (2003) Thermodynamic analyses of purine motif triplex DNA formation. *Nucleic Acids Res Suppl* 159-160.

Abstract: We analyzed the thermodynamics of purine motif triplex formations involving single mismatches of four different base triplets (A x A:T, T x A:T, A x T:A and T x T:A) by isothermal titration calorimetry. The T x A:T and A x A:T base triplets are the most stable and the A x T:A base triplet is the least stable among the four base triplets. The magnitude of the Gibbs free energy change varies within 1.0 kcal mol⁻¹ for the single mismatches of the base triplet. On the other hand, the magnitude of the enthalpy change exhibits very large sequence dependence with up to 14 kcal mol⁻¹ variation per the single mismatches among the four base triplets, which is 14 times larger than the sequence-dependent variation of the magnitude of the Gibbs free energy change. Thus, the single mismatches in the purine motif triplex do not have strong effects on the magnitude of the Gibbs free energy change, but the magnitude of the enthalpy change exhibits much larger sequence-dependent variations. The present results support the previously proposed nucleation-elongation mechanism of the triplex formation and offer some hints to design novel triplex-forming oligonucleotides with enhanced sequence specificity.

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

Wang F., Li F., Ganguly M., Marky L. A., Gold B., Egli M. and Stone M. P. (2008) A bridging water anchors the tethered 5-(3-aminopropyl)-2'-deoxyuridine amine in the DNA major groove proximate to the N+2 C.G base pair: implications for formation of interstrand 5'-GNC-3' cross-links by nitrogen mustards. *Biochemistry* **47**, 7147-7157.

Abstract: Site-specific insertion of 5-(3-aminopropyl)-2'-deoxyuridine (Z3dU) and 7-deaza-dG into the Dickerson-Drew dodecamers 5'-d(C (1)G (2)C (3)G (4)A (5)A (6)T (7)T (8)C (9) Z (10)C (11)G (12))-3'.5'-d(C (13)G (14)C (15)G (16)A (17)A (18)T (19)T (20)C (21) Z (22)C (23)G (24))-3' (named DDD (Z10)) and 5'-d(C (1)G (2)C (3)G (4)A (5)A (6)T (7) X (8)C (9) Z (10)C (11)G (12))-3'.5'-d(C (13)G (14)C (15)G (16)A (17)A (18)T (19) X (20)C (21) Z (22)C (23)G (24))-3' (named DDD (2+Z10)) (X = Z3dU; Z = 7-deaza-dG) suggests a mechanism underlying the formation of interstrand N+2 DNA cross-links by nitrogen mustards, e.g., melphalan and mechlorethamine. Analysis of the DDD (2+Z10) duplex reveals that the tethered cations at base pairs A (5).X (20) and X (8).A (17) extend within the major groove in the 3'-direction, toward conserved Mg (2+) binding sites located adjacent to N+2 base pairs C (3).Z (22) and Z (10).C (15). Bridging waters located between the tethered amines and either Z (10) or Z (22) O (6) stabilize the tethered cations and allow interactions with the N + 2 base pairs without DNA bending. Incorporation of 7-deaza-dG into the DDD (2+Z10) duplex weakens but does not eliminate electrostatic interactions between tethered amines and Z (10) O (6) and Z (22) O (6). The results suggest a mechanism by which tethered N7-dG aziridinium ions, the active species involved in formation of interstrand 5'-GNC-3' cross-

links by nitrogen mustards, modify the electrostatics of the major groove and position the aziridinium ions proximate to the major groove edge of the N+2 C.G base pair, facilitating interstrand cross-linking

Zahn A., Brotschi C., and Leumann C. J. (2005) pentafluorophenyl-phenyl interactions in biphenyl-DNA. *Chemistry* **11**, 2125-2129.

Abstract: We prepared and investigated oligonucleotide duplexes of the sequence d(GATGAC(X)(n)GCTAG)d(CTAGC(Y)(n)GTCATC), in which X and Y designate biphenyl- (bph) and pentafluorobiphenyl- ((5F)bph) C-nucleotides, respectively, and n varies from 0-4. These hydrophobic base substitutes are expected to adopt a zipperlike, interstrand stacking motif, in which not only bph/bph or (5F)bph/(5F)bph homo pairs, but also (5F)bph/bph mixed pairs can be formed. By performing UV-melting curve analysis we found that incorporation of a single (5F)bph/(5F)bph pair leads to a duplex that is essentially as stable as the unmodified duplex (n=0), and 2.4 K more stable than the duplex with the nonfluorinated bph/bph pair. The T(m) of the mixed bph/(5F)bph pair was in between the T(m) values of the respective homo pairs. Additional, unnatural aromatic pairs increased the T(m) by +3.0-4.4 K/couple, irrespective of the nature of the aromatic residue. A thermodynamic analysis using isothermal titration calorimetry (ITC) of a series of duplexes with n=3 revealed lower (less negative) duplex formation enthalpies (ΔH) in the (5F)bph/(5F)bph case than in the bph/bph case, and confirmed the higher thermodynamic stability (ΔG) of the fluorinated duplex, suggesting it to be of entropic origin. Our data are compatible with a model in which the stacking of (5F)bph versus bph is dominated by dehydration of the aromatic units upon duplex formation. They do not support a model in which van der Waals dispersive forces (induced dipoles) or electrostatic (quadrupole) interactions play a dominant role.

Zhong M., Marky L. A., Kallenbach N. R., and Kupke D. W. (1997) Thermodynamics of dT--dT base pair mismatching in linear DNA duplexes and three-arm DNA junctions. *Biochemistry* **36**, 2485-2491.

Abstract: We have used a combination of magnetic-suspension densimetry and calorimetry to derive complete thermodynamic profiles, including volume changes, for the formation of linear DNA duplexes and three-arm branched DNA junctions, from their component strands, with and without dT-dT mismatches. The formation of each type of complex at 20 degrees C is accompanied by a favorable free energy, with a favorable enthalpy term partially compensated by an unfavorable entropy. Formation is associated also with net uptake of water molecules. Using the formation of the fully-paired linear duplex or three-arm junction as reference states, we can establish a thermodynamic cycle in which the contribution of the single-strand species cancels. From this cycle, we determine that substitution of dA for dT has a differential free energy of $\Delta\Delta G$ degrees of +2.4 kcal mol⁻¹ for mismatched duplex and +2.0 kcal mol⁻¹ (on the average) for the mismatched junction. These unfavorable differential free energies result from an unfavorable enthalpy, partially compensated by a favorable entropy, and a negative $\Delta\Delta V$. The free energies in the two cases have signs opposed to those of $\Delta\Delta V$, a situation that implicates hydration changes in creating the mismatch. When the $\Delta\Delta V$ terms are normalized by the total number of base pairs involved, the immobilization of structural water molecules (and/or substitution of electrostricted for hydrophobic water molecules) is about 7 times greater for junctions than duplexes. This is consistent with more extensive hydrophobic hydration of branched DNA structures than of duplexes.

Zieba K., Chu T. M., Kupke D. W., and Marky L. A. (1991) Differential hydration of dA.dT base pairing and dA and dT bulges in deoxyoligonucleotides. *Biochemistry* **30**, 8018-8026.

Abstract: The role of water in the formation of stable duplexes of nucleic acids is being studied by determining the concurrent volume change, heats, and counterion uptake that accompany the duplexation process. The variability of the volume contraction that we have observed in the formation of a variety of homoduplexes suggests that sequence and conformation acutely affect the degree of hydration. We have used a combination of densimetric and calorimetric techniques to measure the change in volume and enthalpy resulting from the mixing of two complementary strands to form (a) fully paired duplexes with 10 or 11 base pairs and (b) bulged decameric duplexes with an extra dA or dT unmatched residue. We also monitored absorbance vs temperature profiles as a function of strand and salt concentration for all four duplexes. Relative to the decamer duplex, insertion of an extra dA.dT base pair to form an undecamer duplex results in a favorable enthalpy of -5.6 kcal/mol that is nearly compensated by an unfavorable entropy term of -5.1 kcal/mol. This enthalpy difference correlates with a differential uptake of water molecules, corresponding to an additional hydration of 16 mol of water molecules/mol of base pair. Relative to the fully paired duplexes, both bulged duplexes are 12-16 degrees C less stable and exhibit

marginally larger counterion uptake on forming the duplex. The enthalpy change is slightly lower for the T-bulge duplex and less still for the A-bulge duplex. The volume change results indicate that an unmatched residue increases the amount of coulombic and/or structural hydration. The combined results strongly suggest that the destabilizing forces in bulged duplexes are partially compensated by an increase in hydration levels.