

DSC XIV: Protein-Nucleic Acid Studies

Blaszczyk U. and Wasylewski Z. (2003) Interaction of cAMP receptor protein from *Escherichia coli* with cAMP and DNA studied by differential scanning calorimetry. *J Protein Chem* **22**, 285-293.

Abstract: The cyclic AMP receptor protein (CRP) regulates the expression of many genes in *Escherichia coli*. The protein is a homodimer, and each monomer is folded into two distinct structural domains. In this study, we have used differential scanning calorimetry (DSC) and circular dichroism (CD) to measure the enthalpy change and melting temperature of the apo-CRP and CRP complexes with cAMP or DNA sequences lac, gal, and palindromic ICAP. DSC and CD measurements showed irreversible thermal denaturation process of CRP. Enthalpy of dissociation of the protein-DNA complex, as measured by DSC, depends on the DNA sequence. The thermal transition of the protein in CRP-DNA complexes, measured by CD, indicates that the protein stability in the complex is also DNA sequence-dependent.

Bonincontro A., Cinelli S., Mengoni M., Onori G., Risuleo G., and Santucci A. (1998) Differential stability of *E. coli* ribosomal particles and free RNA towards thermal degradation studied by microcalorimetry. *Biophys Chem* **75**, 97-103.

Abstract: We investigated the thermal degradation of *E. coli* ribosomes by differential scanning microcalorimetry. The 70S particles show two distinctive and irreversible peaks upon thermal degradation. Free rRNA in solution produces, on the contrary, an unstructured denaturation profile. The thermal analysis of 50S particles shows a profile substantially identical to that observed in 70S, while 30S particles produce an unstructured denaturation pattern. Therefore the thermal behavior of the 70S particle is essentially attributable to the denaturation of the 50S subunit. Our data validate previous observations that the 50S has a more rigid structure as compared to 30S, which behaves as a 'floppy' particle. In addition our data suggest that protein/RNA interactions play a significant role to stabilize three-dimensional structures of the ribosome.

Bonincontro A., Cinelli S., Onori G., and Risuleo G. (2000) Instability of three-dimensional structures in ribosomal cores evidenced by microcalorimetric studies. *Z Naturforsch [C]* **55**, 410-412.

Abstract: In this paper we show a microcalorimetric investigation carried out on the so-called cores, i.e. ribosomes deprived of select proteins by LiCl treatment. Thermal degradation of native ribosomes gives rise to two thermal transitions occurring at different temperatures. In the cores the high temperature peak persists even after treatment at very high ion strength (2 M LiCl). This strongly suggests the existence of a very stable structure that was previously observed also in particles treated with agents that hydrolyze the RNA moiety. The low temperature peak gradually but dramatically decreases even though it never disappears completely. This indicates that the treatment to obtain ribosomal cores does not cause complete unfolding of the particle but only the destabilization of a structural three-dimensional domain present in native ribosomes. These data are discussed in the light of previous results obtained by dielectric spectroscopy and microcalorimetric studies on ribosomal particles.

Bonincontro A., Nierhaus K. H., Onori G., and Risuleo G. (2001) Intrinsic structural differences between "tight couples" and Kaltschmidt-Wittmann ribosomes evidenced by dielectric spectroscopy and scanning microcalorimetry. *FEBS Lett* **490**, 93-96.

Abstract: Measurements of dielectric spectroscopy (DS) and microcalorimetry (differential scanning calorimetry (DSC)) of *Escherichia coli* 70S, 50S and 30S were performed on particles prepared according either to the "classical" twice NH₄Cl-washed ribosomes, also known as loose couples (LC), or to the "tight couples" preparative protocol (TC). Results show that 70S particles prepared according to the two different protocols exhibit different structural properties. Two subsequent relaxation processes occur in both samples as measured by DS. However, in LC ribosomes the first one is shifted towards a lower frequency with a higher dielectric increment. This is suggestive of a more extensive exposure of RNA to the solvent and of an overall more relaxed structure. The smaller LC subunit exhibits only one relaxation while the TC 30S shows two dielectric dispersions as well as 70S. No substantial differences were evidenced in either 50S species. Two typical melting peaks were observed by DSC both in LC and TC 70S as well as in 50S. Thermograms obtained from the TC 30S show a single well structured peak while LC particles produce a large unstructured curve. On the basis of these results we conclude that TC 70S particles are more compact than LC ribosomes and that in the former ones the rRNA is less exposed to the

solvent phase. Furthermore 30S particles obtained from TC show a more stable structure with respect to LC 30S. We conclude that the 30S subunit gives a major contribution to the compact character of the whole TC 70S. These differences might be related to the intrinsic and well documented functional difference between the two ribosome species.

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

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

Bracken C., Carr P. A., Cavanagh J., and Palmer A. G., III (1999) Temperature dependence of intramolecular dynamics of the basic leucine zipper of GCN4: implications for the entropy of association with DNA. *J Mol Biol* **285**, 2133-2146.

Abstract: The basic leucine zipper domain of the yeast transcription factor GCN4 consists of a C-terminal leucine zipper and an N-terminal basic DNA-binding region that achieves a stable structure only after association with DNA. Backbone dynamics of a peptide encompassing the basic and leucine zipper bZip domain (residues 226-281) are described using NMR spectroscopy. The ¹⁵N longitudinal relaxation rates, ¹⁵N transverse relaxation rates, and inverted question mark ¹H inverted question mark-¹⁵N nuclear Overhauser effects were measured for the backbone amide nitrogen atoms at 290 K, 300 K, and 310 K. The relaxation data were interpreted using reduced spectral density mapping to determine values of the spectral density function, J(ω), at the frequencies 0, ω_N , and $0.87\omega_H$ to characterize overall and intramolecular motions on picosecond-nanosecond timescales. To account for the temperature dependence of overall rotational diffusion, the J(0) values were normalized using Stoke's Law. At 310 K, the ¹³C α and ¹³C O chemical shifts in conjunction with the spectral density values indicate that the leucine zipper sequence forms a highly ordered α -helix, while the basic region populates an ensemble of highly dynamic transient structures with substantial helical character. The normalized values of J(0) and the values of J($0.87\omega_H$) for residues in the leucine zipper dimerization domain are independent of temperature. In contrast, residues in the basic region exhibit pronounced increases in the normalized J(0) and decreases in J($0.87\omega_H$) as temperature is decreased. A strong correlation exists between the temperature dependence of ¹³C O chemical shifts and of J($0.87\omega_H$). These results suggest that, for the basic region, lowering the temperature increases the population of transient helical conformations, and concomitantly reduces the amplitude or timescale of conformational fluctuations on picosecond-nanosecond timescales. Changes in the conformational dynamics of the peptide backbone of the basic region that accompany DNA binding contribute to the overall thermodynamics of complex formation. The change in backbone conformational entropy derived from NMR spin-relaxation data agrees well with the result calculated from calorimetric measurements. Restriction of the conformational space accessible to the basic region may significantly reduce the entropic cost associated with formation of the basic region helices consequent to DNA binding.

Brescia C. C., Mikulecky P. J., Feig A. L., and Sledjeski D. D. (2003) Identification of the Hfq-binding site on DsrA RNA: Hfq binds without altering DsrA secondary structure. *RNA* **9**, 33-43.

Abstract: DsrA RNA regulates the translation of two global regulatory proteins in *Escherichia coli*. DsrA activates the translation of RpoS while repressing the translation of H-NS. The RNA-binding protein Hfq is necessary for DsrA to function in vivo. Although Hfq binds to DsrA in vitro, the role of Hfq in DsrA-mediated regulation is not known. One hypothesis was that Hfq acts as an RNA chaperone by unfolding DsrA, thereby facilitating interactions with target RNAs. To test this hypothesis, we have examined the structure of DsrA bound to Hfq in vitro. Comparison of free DsrA to DsrA bound to Hfq by RNase footprinting, circular dichroism, and thermal melt profiles shows that Hfq does not alter DsrA secondary structures, but might affect its tertiary conformation. We identify the site on DsrA where Hfq binds, which is a structural element in the middle of DsrA. In addition, we show that although long poly(U) RNAs compete with DsrA for binding to Hfq, a short poly(U) stretch present in DsrA is not necessary for Hfq binding. Finally, unlike other RNAs, DsrA binding to Hfq is not competed with by poly(A) RNA. In fact, DsrA:poly(A):Hfq may form a stable ternary complex, raising the possibility that Hfq has multiple RNA-binding sites.

Carra J. H. and Privalov P. L. (1997) Energetics of folding and DNA binding of the MAT alpha 2 homeodomain. *Biochemistry* **36**, 526-535.

Abstract: Homeodomains are a class of DNA-binding protein domains which play an important role in genetic regulation in eukaryotes. We have characterized the thermodynamics of folding and sequence-specific association with DNA of the MAT alpha 2 homeodomain of yeast. Using differential scanning and isothermal titration calorimetry, we measured the enthalpy, heat capacity, and Gibbs free energy changes of these processes. The protein-DNA interaction is enthalpically driven at physiological temperatures. DSC data on the process of melting the protein-DNA complex at different salt concentrations were dissected into its endothermic components, yielding the enthalpy change and dissociation constant of binding. A comparison of the circular dichroism spectra of the free and DNA-bound protein species revealed the formation of additional alpha-helical structure upon binding to DNA. We propose that the latter half of helix 3, the recognition helix, is substantially unfolded in the free protein under the conditions used, as has been observed with other homeodomains [Tsao, D. H. H., et al. (1994) *Biochemistry* **33**, 15053-15060; Cox, M., et al. (1995) *J. Biomol. NMR* **5**, 23-32]. Formation of protein structure is induced by DNA binding, and the energies measured for association therefore include a component due to folding.

Chen S. H., Suzuki C. K. and Wu S. H. (2008) Thermodynamic characterization of specific interactions between the human Lon protease and G-quartet DNA. *Nucleic Acids Res* **36**, 1273-1287.

Abstract: Lon is an ATP-powered protease that binds DNA. However, the function of DNA binding by Lon remains elusive. Studies suggest that human Lon (hLon) binds preferentially to a G-rich single-stranded DNA (ssDNA) sequence overlapping the light strand promoter of mitochondrial DNA. This sequence is contained within a 24-base oligonucleotide referred to as LSPas. Here, we use biochemical and biophysical approaches to elucidate the structural properties of ssDNAs bound by hLon, as well as the thermodynamics of DNA binding by hLon. Electrophoretic mobility shift assay and circular dichroism show that ssDNAs with a propensity for forming parallel G-quartets are specifically bound by hLon. Isothermal titration calorimetry demonstrates that hLon binding to LSPas is primarily driven by enthalpy change associated with a significant reduction in heat capacity. Differential scanning calorimetry pinpoints an excess heat capacity upon hLon binding to LSPas. By contrast, hLon binding to an 8-base G-rich core sequence is entropically driven with a relatively negligible change in heat capacity. A considerable enhancement of thermal stability accompanies hLon binding to LSPas as compared to the G-rich core. Taken together, these data support the notion that hLon binds G-quartets through rigid-body binding and that binding to LSPas is coupled with structural adaptation

Chivers P. T. and Sauer R. T. (1999) NikR is a ribbon-helix-helix DNA-binding protein. *Protein Sci* **8**, 2494-2500.

Abstract: *Escherichia coli* NikR, a repressor with homologs in other bacteria and archaea, was identified as a potential new member of the ribbon-helix-helix (beta-alpha-alpha) family of transcription factors in profile based sequence searches and in structure prediction experiments. Biophysical and biochemical characterization of the N-terminal domain of NikR show that it has many features expected of a beta-alpha-alpha protein including alpha-helical content, dimeric solution form, concentration dependent thermal

stability, and ability to bind DNA in sequence-specific manner. Mutation of a residue predicted to be important for DNA-binding reduces operator affinity but does not affect the secondary structure or stability of the protein.

Cooper A., McAlpine A., and Stockley P. G. (1994) Calorimetric studies of the energetics of protein-DNA interactions in the *E. coli* methionine repressor (MetJ) system. *FEBS Lett* **348**, 41-45.

Abstract: Calorimetric measurements of binding of a specific DNA fragment and S-adenosyl methionine (SAM) co-repressor molecules to the *E. coli* methionine repressor (MetJ) show significant differences in the energetics of binary and ternary protein-DNA complexes. Formation of the MetJ:SAM:DNA ternary complex is significantly more exothermic (ΔH congruent to -99 kJ.mol^{-1}) than either MetJ:DNA or MetJ:SAM binary complexes alone (ΔH congruent to -10 kJ.mol^{-1} each). The protein is also significantly more stable to unfolding (ΔT_m congruent to 5.4 degrees C) when bound to DNA. These observations suggest that binding of SAM to the protein-DNA complex leads to a significant reduction in dynamic flexibility of the ternary complex, with considerable entropy-enthalpy compensation, not necessarily involving any overall conformational change.

Cranz S., Berger C., Baici A., Jelesarov I., and Bosshard H. R. (2004) Monomeric and dimeric bZIP transcription factor GCN4 bind at the same rate to their target DNA site. *Biochemistry* **43**, 718-727.

Abstract: Basic leucine zipper (bZIP) transcription factors are dimeric proteins that recognize dyadic and mostly palindromic DNA sites. Dimerization of bZIP transcription factor GCN4 is linked to the folding of its C-terminal leucine zipper domain. However, monomeric GCN4, lacking a folded leucine zipper, also recognizes the DNA site with dimerization taking place on the DNA. Here we report the kinetics of DNA recognition by unfolded monomeric and folded dimeric derivatives of GCN4 using a 19 bp double-stranded DNA containing a palindromic CRE site. The rate of DNA binding of both monomeric and dimeric GCN4 has a bimolecular rate constant of $3\text{-}5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, which is near the diffusion limit. Because the rate of dimerization of GCN4 is slower ($1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) than the rate of DNA association, the formation of the dimeric GCN4-DNA complex through consecutive binding of two monomers (monomer pathway) is faster when starting from free monomers. Thus, the results presented here support facilitated and rapid target recognition by the monomeric transcription factor. However, DNA binding of preformed folded dimeric GCN4 is as rapid as complex formation through the monomer pathway. Therefore, the monomer and dimer pathways are kinetically equivalent if monomeric and dimeric GCN4 are at equilibrium. Hence, the dimer pathway may also have a role under in vivo conditions. The lower affinity of GCN4 in which two DNA contacting residues have been mutated is due exclusively to the faster dissociation of the mutant protein-DNA complex and not to slower complex formation.

Crowther R. L., Remeta D. P., Minetti C. A., Das D., Montano S. P., and Georgiadis M. M. (2004) Structural and energetic characterization of nucleic acid-binding to the fingers domain of Moloney murine leukemia virus reverse transcriptase. *Proteins* **57**, 15-26.

Abstract: Reverse transcriptase is an essential retroviral enzyme that replicates the single-stranded RNA genome of the retrovirus producing a double-stranded DNA copy, which is subsequently integrated into the host's genome. We have previously reported that processive DNA synthesis of Moloney murine leukemia virus reverse transcriptase (MMLV RT) is severely compromised by substitution of an Ala for the fingers domain residue Arg 116. In order to further investigate the role of Arg 116 in interactions of MMLV RT with nucleic acids, we have determined the crystal structure of the R116A N-terminal fragment and characterized the binding of two self-complementary DNA duplexes [d(CATGCATG)₂ and d(CGCGCGCG)₂] to both the wild-type and R116A fragments by isothermal titration calorimetry. The resultant thermodynamic profiles extrapolated to 25 degrees C reveal that binding of the wild-type N-terminal fragment to both DNA duplexes is enthalpy-driven and characterized by an unfavorable entropy. Although the temperature dependence of the respective protein-DNA binding enthalpies is markedly different reflecting distinct heat capacity changes, the binding free energies are nearly identical and relatively invariant to temperature (ΔG approximately $-6.0 \text{ kcal} \times \text{mol}^{-1}$). In contrast to the wild-type fragment, the R116A fragment exhibits no measurable affinity for either DNA duplex, yet its crystal structure reveals no significant changes when compared to the wild-type structures. We suggest that hydrogen-bonding interactions involving the fingers domain residue Arg 116 are critical for DNA binding as well as processive DNA synthesis by MMLV RT.

Davis K. G., Plyte S. E., Robertson S. R., Cooper A., and Kneale G. G. (1995) Comparison of Pfl and Fd gene 5 proteins and their single-stranded DNA complexes by NMR spectroscopy and differential scanning calorimetry. *Biochemistry* **34**, 148-154.

Abstract: The Pfl gene 5 protein forms a large helical nucleoprotein complex ($M_r = 3.1 \times 10^7$) with single-stranded viral DNA, from which a 32 amino acid sequence rich in alanine, proline, and glutamine residues can be removed from the C-terminus by limited proteolysis. Sharp resonances in the 1H NMR spectrum of the Pfl nucleoprotein complex indicate that the C-terminal region of the protein subunits enjoys remarkable conformational flexibility in the complex. In contrast, the globular N-terminal domain of the protein subunits is rigidly held and does not contribute to the spectrum. The Fd gene 5 protein lacks this C-terminal flexible domain, and no distinct resonances can be observed in the 1H NMR spectrum when this protein is complexed to single-stranded viral DNA. Differential scanning calorimetry shows that the thermal stability of both the Pfl and Fd gene 5 protein is increased by 8 degrees C in the complex with DNA, and the transition is highly cooperative. Removal of the C-terminal domain of the Pfl gene 5 protein subunits has no appreciable effect either on the T_m of the DNA-protein complex or on the cooperative nature of the thermal transition. It is suggested that the C-terminal domain of the Pfl gene 5 protein acts as a dynamic clamp which kinetically stabilizes the nucleoprotein complex.

Dragan A. I., Frank L., Liu Y., Makeyeva E. N., Crane-Robinson C., and Privalov P. L. (2004) Thermodynamic signature of GCN4-bZIP binding to DNA indicates the role of water in discriminating between the AP-1 and ATF/CREB sites. *J Mol Biol* **343**, 865-878.

Abstract: The energetic basis of GCN4-bZIP complexes with the AP-1 and ATF/CREB sites was investigated by optical methods and scanning and isothermal titration microcalorimetry. The dissociation constant of the bZIP dimer was found to be significantly higher than that of its isolated leucine zipper domain: at 20 degrees C it is 1.45 μ M and increases with temperature. To avoid complications from dissociation of this dimer, DNA binding experiments were carried out using an SS crosslinked version of the bZIP. The thermodynamic characteristics of the bZIP/DNA association measured at different temperatures and salt concentrations were corrected for the contribution of refolding the basic segment upon binding, determined from the scanning calorimetric experiments. Fluorescence anisotropy titration experiments showed that the association constants of the bZIP at 20 degrees C with the AP-1 and ATF/CREB binding sites do not differ much, being 1.5 nM and 6.4 nM, corresponding to Gibbs energies of -49 kJ mol^{-1} and -46 kJ mol^{-1} , respectively. Almost half of the Gibbs energy is attributable to the electrostatic component, resulting from the entropic effect of counterion release upon DNA association with the bZIP and is identical for both sites. In contrast to the Gibbs energies, the enthalpies of association of the fully folded bZIP with the AP-1 and ATF/CREB sites, and correspondingly the entropies of association, are very different. bZIP binding to the AP-1 site is characterized by a substantially larger negative enthalpy and non-electrostatic entropy than to the ATF/CREB site, implying that the AP-1 complex incorporates significantly more water molecules than the ATF/CREB complex.

Dragan A. I., Read C. M., Makeyeva E. N., Milgotina E. I., Churchill M. E., Crane-Robinson C., and Privalov P. L. (2004) DNA binding and bending by HMG boxes: energetic determinants of specificity. *J Mol Biol* **343**, 371-393.

Abstract: To clarify the physical basis of DNA binding specificity, the thermodynamic properties and DNA binding and bending abilities of the DNA binding domains (DBDs) of sequence-specific (SS) and non-sequence-specific (NSS) HMG box proteins were studied with various DNA recognition sequences using micro-calorimetric and optical methods. Temperature-induced unfolding of the free DBDs showed that their structure does not represent a single cooperative unit but is subdivided into two (in the case of NSS DBDs) or three (in the case of SS DBDs) sub-domains, which differ in stability. Both types of HMG box, most particularly SS, are partially unfolded even at room temperature but association with DNA results in stabilization and cooperation of all the sub-domains. Binding and bending measurements using fluorescence spectroscopy over a range of ionic strengths, combined with calorimetric data, allowed separation of the electrostatic and non-electrostatic components of the Gibbs energies of DNA binding, yielding their enthalpic and entropic terms and an estimate of their contributions to DNA binding and bending. In all cases electrostatic interactions dominate non-electrostatic in the association of a DBD with DNA. The main difference between SS and NSS complexes is that SS are formed with an enthalpy close to zero and a negative heat capacity effect, while NSS are formed with a very positive enthalpy and a positive heat capacity effect. This indicates that formation of SS HMG box-DNA complexes is specified by

extensive van der Waals contacts between apolar groups, i.e. a more tightly packed interface forms than in NSS complexes. The other principal difference is that DNA bending by the NSS DBDs is driven almost entirely by the electrostatic component of the binding energy, while DNA bending by SS DBDs is driven mainly by the non-electrostatic component. The basic extensions of both categories of HMG box play a similar role in DNA binding and bending, making solely electrostatic interactions with the DNA.

Dragan A. I., Klass J., Read C., Churchill M. E., Crane-Robinson C., and Privalov P. L. (2003) DNA binding of a non-sequence-specific HMG-D protein is entropy driven with a substantial non-electrostatic contribution. *J Mol Biol* **331**, 795-813.

Abstract: The thermal properties of two forms of the *Drosophila melanogaster* HMG-D protein, with and without its highly basic 26 residue C-terminal tail (D100 and D74) and the thermodynamics of their non-sequence-specific interaction with linear DNA duplexes were studied using scanning and titration microcalorimetry, spectropolarimetry, fluorescence anisotropy and FRET techniques at different temperatures and salt concentrations. It was shown that the C-terminal tail of D100 is unfolded at all temperatures, whilst the state of the globular part depends on temperature in a rather complex way, being completely folded only at temperatures close to 0 degrees C and unfolding with significant heat absorption at temperatures below those of the gross denaturational changes. The association constant and thus Gibbs energy of binding for D100 is much greater than for D74 but the enthalpies of their association are similar and are large and positive, i.e. DNA binding is a completely entropy-driven process. The positive entropy of association is due to release of counterions and dehydration upon forming the protein/DNA complex. Ionic strength variation showed that electrostatic interactions play an important but not exclusive role in the DNA binding of the globular part of this non-sequence-specific protein, whilst binding of the positively charged C-terminal tail of D100 is almost completely electrostatic in origin. This interaction with the negative charges of the DNA phosphate groups significantly enhances the DNA bending. An important feature of the non-sequence-specific association of these HMG boxes with DNA is that the binding enthalpy is significantly more positive than for the sequence-specific association of the HMG box from Sox-5, despite the fact that these proteins bend the DNA duplex to a similar extent. This difference shows that the enthalpy of dehydration of apolar groups at the HMG-D/DNA interface is not fully compensated by the energy of van der Waals interactions between these groups, i.e. the packing density at the interface must be lower than for the sequence-specific Sox-5 HMG box.

Dragan A. I., Liggins J. R., Crane-Robinson C., and Privalov P. L. (2003) The energetics of specific binding of AT-hooks from HMGA1 to target DNA. *J Mol Biol* **327**, 393-411.

Abstract: The interaction of the second and third AT-hooks of HMGA1 (formerly HMGI/Y), which bind selectively in the minor groove of an AT-rich DNA sequence, was studied at different temperatures and ionic strengths by spectropolarimetry, spectrofluorimetry, isothermal titration calorimetry and differential scanning calorimetry. The data show that binding of the ten amino acid core element of the two AT-hooks, which penetrates deep into the minor groove, is entropically driven: both the entropy and enthalpy of association of the peptides to the target DNA are positive up to 50 degrees C. The seven amino acid extension of the core in the second AT-hook, which extends out from the minor groove and loops over the phosphodiester backbone, adds a substantial negative enthalpic component into the binding of the 17 residue DBD2 peptide to DNA that corresponds in magnitude to the enthalpy of formation of two hydrogen bonds. The ionic strength dependence of the association constant allowed an estimation of the electrostatic component of binding and, by subtraction, the contribution of the non-electrostatic component, which results from dehydration of the contacting surfaces and makes up almost 70% of the total energy of complex formation. The exceptionally large positive entropy and enthalpy of association of the core AT-hook peptides with target DNA suggest that the water, which is removed from the minor groove of DNA upon binding, is in a highly ordered state. Acetylation of the lysine residue in the second AT-hook, which corresponds to Lys65 of HMGA1, has little effect on the DNA binding; so it appears that repression of the hIFNbeta gene, which follows this modification, is not a direct result of the abrogation of DNA binding.

Gavazzo P., Vergani L., Mascetti G. C., and Nicolini C. (1997) Effects of histone acetylation on chromatin structure. *J Cell Biochem* **64**, 466-475.

Abstract: The effect of histone acetylation was monitored on CHO chromatin structure, following the addition of 7 mM Na-butyrate to the cell culture medium. The properties of both control and hyperacetylated chromatins and nuclei were investigated by circular dichroism, ethidium bromide

intercalation, differential scanning calorimetry, and affinity chromatography. Our results are compatible with modest but significant alterations in the various levels of chromatin organization, as a result of the charge neutralization of some lysine residues within the N-terminal region of the histonic octamer. Namely, large statistically significant differences do exist in the heat capacity thermograms of native nuclei, where unfolding into single nucleofilament of the highly packed native chromatin superfiber appears associated with acetylation; at the same time CD, EB, and affinity chromatography point to modest but consistent differences in the compactness of isolated nucleosomes and polynucleosomes.

Grattarola M., Spaggiari S., Chessa L., Savio C., Nicolini C., and Vergani L. (2003) A structural characterization of in situ chromatin on cell lines isolated from patients affected by ataxia telangiectasia. *Int J Biol Macromol* **33**, 23-29.

Abstract: Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by numerous clinical and cellular features. The pleiotropic nature of the AT syndrome attests to the multiple roles of ATM, the protein codified by the gene altered in AT patients. We investigated if different mutations of ATM could reflect on different alterations of nuclear architecture and chromatin organization. We selected three lymphoblastoid cell lines isolated from AT patients affected by different mutations of ATM gene and one healthy control. We characterized the in situ chromatin structure of each cell line by a biophysical approach: (1) we evaluated the rearrangements of the chromatin domains at the level of single cell by quantitative fluorescence microscopy; (2) we analysed the changes of the average chromatin condensation by differential scanning calorimetry. The results show that the three different ATM mutations produce significant modifications of both nuclear architecture and chromatin condensation.

Kahsai M. A., Martin E., Edmondson S. P., and Shriver J. W. (2005) Stability and Flexibility in the Structure of the Hyperthermophile DNA-Binding Protein Sac7d. *Biochemistry* **44**, 13500-13509.

Abstract: Sac7d is a chromatin protein from the hyperthermophile *Sulfolobus acidocaldarius* that severely kinks duplex DNA with negligible change in protein structure. In previous work, the overall stability of Sac7d has been well-characterized with a global analysis of the linkage of folding, protonation, and anion binding. We extend that work here with NMR measurements of global stability as well as the distribution of stability and flexibility in the solution structure. Native state amide hydrogen exchange has been used to identify the most-protected core amide protons which exchange through global unfolding. The pH and temperature dependence of stability defined by native state exchange is in excellent agreement with the free energy surface determined by a linkage analysis of the dependence of folding on pH, salt, and temperature. These results confirm that the $\Delta C(P)$ obtained from a Kirchoff analysis of DSC data (i.e., ΔH vs $T(m)$) is incorrect, and an accurate description of the protein stability curve for Sac7d requires a measure of the thermodynamic contributions of protonation and anion binding. Amide hydrogen exchange, along with generalized order parameters determined by (^{15}N) relaxation data, demonstrates considerable variation in stability throughout the structure with some of the least stable regions occurring at the N- and C-termini. The most stable and inflexible region of the backbone occurs primarily in the DNA-binding beta-sheet which is responsible for bending DNA.

Kankia B. I., Barany G., and Musier-Forsyth K. (2005) Unfolding of DNA quadruplexes induced by HIV-1 nucleocapsid protein. *Nucleic Acids Res* **33**, 4395-4403.

Abstract: The human immunodeficiency virus type 1 nucleocapsid protein (NC) is a nucleic acid chaperone that catalyzes the rearrangement of nucleic acids into their thermodynamically most stable structures. In the present study, a combination of optical and thermodynamic techniques were used to characterize the influence of NC on the secondary structure, thermal stability and energetics of monomolecular DNA quadruplexes formed by the sequence d(GGTTGGTGTGGTTGG) in the presence of K^+ or Sr^{2+} . Circular dichroism studies demonstrate that NC effectively unfolds the quadruplexes. Studies carried out with NC variants suggest that destabilization is mediated by the zinc fingers of NC. Calorimetric studies reveal that NC destabilization is enthalpic in origin, probably owing to unstacking of the G-quartets upon protein binding. In contrast, parallel studies performed on a related DNA duplex reveal that under conditions where NC readily destabilizes and unfolds the quadruplexes, its effect on the DNA duplex is much less pronounced. The differences in NC's ability to destabilize quadruplex versus duplex is in accordance with the higher ΔG of melting for the latter, and with the inverse correlation between nucleic acid stability and the destabilizing activity of NC.

Ladbury J. E. (1995) Counting the calories to stay in the groove. *Structure* **3**, 635-639.

Abstract: High-sensitivity microcalorimetry is beginning to make an impact on the determination of thermodynamic parameters associated with protein-DNA interactions and the understanding of the relationship of these data to structural details of complex formation.

Lafitte D, Lamour V, Tsvetkov PO, Makarov AA, Klich M, Deprez P, Moras D, Briand C, Gilli R. (2002) DNA gyrase interaction with coumarin-based inhibitors: the role of the hydroxybenzoate isopentenyl moiety and the 5'-methyl group of the noviose. *Biochemistry*; **41**, 7217-7223.

Abstract: DNA gyrase is a major bacterial protein that is involved in replication and transcription and catalyzes the negative supercoiling of bacterial circular DNA. DNA gyrase is a known target for antibacterial agents since its blocking induces bacterial death. Quinolones, coumarins, and cyclothialidines have been designed to inhibit gyrase. Significant improvements can still be envisioned for a better coumarin-gyrase interaction. In this work, we obtained the crystal costructures of the natural coumarin clorobiocin and a synthetic analogue with the 24 kDa gyrase fragment. We used isothermal titration microcalorimetry and differential scanning calorimetry to obtain the thermodynamic parameters representative of the molecular interactions occurring during the binding process between coumarins and the 24 kDa gyrase fragment. We provide the first experimental evidence that clorobiocin binds gyrase with a stronger affinity than novobiocin. We also demonstrate the crucial role of both the hydroxybenzoate isopentenyl moiety and the 5'-alkyl group on the noviose of the coumarins in the binding affinity for gyrase.

Lah J., Marianovsky I., Glaser G., Engelberg-Kulka H., Kinne J., Wyns L., and Loris R. (2003) Recognition of the intrinsically flexible addiction antidote MazE by a dromedary single domain antibody fragment. Structure, thermodynamics of binding, stability, and influence on interactions with DNA. *J Biol Chem* **278**, 14101-14111.

Abstract: The Escherichia coli mazEF operon defines a chromosomal addiction module that programs cell death under various stress conditions. It encodes the toxic and long-lived MazF and the labile antidote MazE. The denaturation of MazE is a two-state reversible dimer-monomer transition. At lower concentrations the denatured state is significantly populated. This leads to a new aspect of the regulation of MazE concentration, which may decide about the life and death of the cell. Interactions of MazE with a dromedary antibody domain, cAbMaz1 (previously used as a crystallization aid), as well as with promoter DNA were studied using microcalorimetric and spectroscopic techniques. Unique features of cAbMaz1 enable a specific enthalpy-driven recognition of MazE and, thus, a significant stabilization of its dimeric native conformation. The MazE dimer and the MazE dimer-cAbMaz1 complex show very similar binding characteristics with promoter DNA, i.e. three binding sites with apparent affinities in micromolar range and highly exothermic binding accompanied by large negative entropy contributions. A working model for the MazE-DNA assembly is proposed on the basis of the structural and binding data. Both binding and stability studies lead to a picture of MazE solution structure that is significantly more unfolded than the structure observed in a crystal of the MazE-cAbMaz1 complex.

Liggins J. R. and Privalov P. L. (2000) Energetics of the specific binding interaction of the first three zinc fingers of the transcription factor TFIIIA with its cognate DNA sequence. *Proteins Suppl* **4**, 50-62.

Abstract: The energetics of the specific interaction of a protein fragment (zfl-3) containing the three N-terminal zinc fingers of the Xenopus laevis transcription factor TFIIIA with its cognate DNA sequence, contained in a 15 bp DNA duplex were studied using isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and fluorescence titration. The use of both ITC and DSC is necessary to provide values for the thermodynamic parameters that have been corrected for thermal fluctuations of the interacting molecules. In the temperature range from 13 degrees C to 45 degrees C (where all the binding reaction components are folded), formation of the complex is enthalpically driven with a negative heat capacity effect (ΔC_p). In this respect, the binding reaction of zfl-3 is similar to those of other proteins that bind in the major groove of DNA. It is dissimilar to the association reactions of proteins, however, that bind in the minor groove of DNA and that are driven by a dominating entropy factor. Comparison of the experimental values of $\Delta H_{(ass)}$ and ΔC_p with expected values of these parameters, calculated from the burial of polar and nonpolar molecular surfaces, indicates that the polar groups at the protein/DNA interface are not completely dehydrated upon formation of the complex. It also seems that the expected large positive entropy of dehydration upon forming the zfl-3/DNA complex (approximately $1900 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$)

mol⁻¹) cannot be balanced by the reduction in translational/rotational and configurational freedom of the protein to the level of the observed entropy of binding (38 J * K⁻¹ * mol⁻¹). It is suggested that the additional negative entropy contribution comes from a damping of torsional motions in the DNA duplex.

Milev S., Gorfé A. A., Karshikoff A., Clubb R. T., Bosshard H. R., and Jelesarov I. (2003) Energetics of sequence-specific protein-DNA association: binding of integrase Tn916 to its target DNA. *Biochemistry* **42**, 3481-3491.

Abstract: The DNA binding domain of the transposon Tn916 integrase (INT-DBD) binds to its DNA target site by positioning the face of a three-stranded antiparallel beta-sheet within the major groove. Binding of INT-DBD to a 13 base pair duplex DNA target site was studied by isothermal titration calorimetry, differential scanning calorimetry, thermal melting followed by circular dichroism spectroscopy, and fluorescence spectroscopy. The observed heat capacity change accompanying the association reaction (ΔC_p) is temperature-dependent, decreasing from -1.4 kJ K⁻¹ mol⁻¹ at 4 degrees C to -2.9 kJ K⁻¹ mol⁻¹ at 30 degrees C. The reason is that the partial molar heat capacities of the free protein, the free DNA duplex, and the protein-DNA complex are not changing in parallel when the temperature increases and that thermal motions of the protein and the DNA are restricted in the complex. After correction for this effect, ΔC_p is -1.8 kJ K⁻¹ mol⁻¹ and temperature-independent. However, this value is still higher than ΔC_p of -1.2 kJ K⁻¹ mol⁻¹ estimated by semiempirical methods from dehydration of surface area buried at the complex interface. We propose that the discrepancy between the measured and the structure-based prediction of binding energetics is caused by incomplete dehydration of polar groups in the complex. In support, we identify cavities at the interface that are large enough to accommodate approximately 10 water molecules. Our results highlight the difficulties of structure-based prediction of ΔC_p (and other thermodynamic parameters) and emphasize how important it is to consider changes of thermal motions and soft vibrational modi in protein-DNA association reactions. This requires not only a detailed investigation of the energetics of the complex but also of the folding thermodynamics of the protein and the DNA alone, which are described in the accompanying paper [Milev et al. (2003) *Biochemistry* 42, 3492-3502].

Mudhivarthi V. K., Bhambhani A. and Kumar C. V. (2007) Novel enzyme/DNA/inorganic nanomaterials: a new generation of biocatalysts. *Dalton Trans.* 5483-5497.

Abstract: The design, synthesis and properties of a new class of enzyme/DNA/inorganic nanobiomaterials are described here. DNA has been used to stabilize the enzymes intercalated in the galleries of the inorganic solid, alpha-Zr(iv) phosphate (alpha-Zr(HPO₄)(2).H₂O, abbreviated as alpha-ZrP). Interestingly, the presence of DNA improved the activity and stability of the bound enzymes. Key studies leading to the current strategy are presented initially, and these are followed by more recent developments. Several enzymes and proteins, including horseradish peroxidase, lysozyme, glucose oxidase, chymotrypsin, bovine serum albumin, cytochrome c, met-hemoglobin and met-myoglobin are successfully intercalated in the galleries of alpha-ZrP, under benign ambient conditions (aqueous buffered solutions, at room temperature and neutral pH). These novel materials are characterized by XRD, SEM and TEM as well as by biochemical, calorimetric and spectroscopic methods. Spectroscopic studies (circular dichroism, CD), for example, indicated that co-intercalation of DNA improved the retention of bound enzyme structure. The activity was enhanced markedly (five-fold) when DNA is co-intercalated, when compared to the activity in the absence of DNA. Addition of DNA to the sample, after enzyme intercalation, did not make any improvements. Our hypothesis is that enzyme-DNA supramolecular complex binds to the solid and the unfavorable interactions between the enzyme and the solid are minimized. These novel nanobiocomposite materials provide a simple method for packaging DNA and aid in engineering more effective synthetic materials for gene/RNA-delivery and drug delivery applications.

Ni X. and Cole R. D. (1994) Effects of various salts and pH on the stability of the nucleosome in chromatin fragments. *Biochemistry* **33**, 9276-9284.

Abstract: The stability of nucleosomes in long chromatin fragments was observed by differential scanning calorimetry over a wide range of solution conditions. The thermal denaturation of chromatin was characterized in general as three major transitions, although the process clearly is more complex. The three major transitions were (1) denaturation of the nucleosome, (2) base unstacking of DNA in the resulting denatured nucleoprotein, and (3) base unstacking of naked DNA. In very low salt concentrations (e.g., 2 mM sodium cacodylate), these three processes were essentially coincident (near 76 degrees C), but in medium salt concentrations (e.g., 100 mM NaCl) the nucleosome denaturation occurred first at about 69

degrees C and then base unstacking occurred at 85 degrees C. As [NaCl] was increased, all three processes were resolved with the observation of increasing amounts of naked DNA being melted, until at 2000 mM NaCl the calorimetric profile showed mainly the melting of DNA. The transition temperature for nucleosome denaturation decreased from 76 to 63 degrees C as the salt concentration increased from 1 to 600 mM. Destabilization of the nucleosome by increasing [NaCl] was also evident above 100 mM as a decrease in enthalpic change attributable to nucleosome denaturation. Similarly, as [NaCl] was increased above 100 mM, less and less denatured nucleoprotein was evident as more and more of the DNA melted as naked DNA. The fatty acid salts, sodium valerate and sodium caproate, destabilized the nucleosome but not the denatured nucleoprotein that resulted from the collapse of the nucleosome. In the series acetate, butyrate, valerate, caproate, it was clear that destabilization of the nucleosome increased as hydrophobicity (chain length) increased.(ABSTRACT TRUNCATED AT 250 WORDS).

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 has been investigated using differential scanning calorimetry (DSC), and the energetics of mTBA and TBA binding to thrombin has been characterized by means of isothermal titration calorimetry (ITC) measurements. 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.

Patrone E., Coradeghini R., Barboro P., D'Arrigo C., Mormino M., Parodi S., and Balbi C. (2006) SCN-binding to the charged lysines of histones end domains mimics acetylation and shows the major histone-DNA interactions involved in eu and heterochromatin stabilization. *J Cell Biochem* 97, 869-881.

Abstract: SCN⁻ binds to the charged amino group of lysines, inducing local changes in the electrostatic free energy of histones. We exploited this property to selectively perturb the histone-DNA interactions involved in the stabilization of eu and heterochromatin. Differential scanning calorimetry (DSC) was used as leading technique in combination with trypsin digestion that selectively cleaves the histone end domains. Euchromatin undergoes progressive destabilization with increasing KSCN concentration from 0 to 0.3 M. Trypsin digestion in the presence of 0.2 M KSCN show that the stability of the linker decreases as a consequence of the competitive binding of SCN⁻ to the amino groups located in the C and N-terminal domain of H1 and H3, respectively; likewise, the release of the N-terminal domain of H4 induces an appreciable depression in both the temperature and enthalpy of melting of core particle DNA. Unfolding of heterochromatin requires, in addition to further cleavage of H4, extensive digestion of H2A and H2B, strongly suggesting that these histones stabilize the higher order structure by forming a protein network which extends throughout the heterochromatin domain.

Peters W. B., Edmondson S. P., and Shriver J. W. (2004) Thermodynamics of DNA binding and distortion by the hyperthermophile chromatin protein Sac7d. *J Mol Biol* 343, 339-360.

Abstract: Sac7d is a hyperthermophile chromatin protein which binds non-specifically to the minor groove of duplex DNA and induces a sharp kink of 66 degrees with intercalation of valine and methionine side-chains. We have utilized the thermal stability of Sac7d and the lack of sequence specificity to define the thermodynamics of DNA binding over a wide temperature range. The binding affinity for poly(dGdC) was moderate at 25 degrees C ($K_a = 3.5(\pm 1.6) \times 10^6 \text{ M}^{-1}$) and increased by nearly an order of magnitude from 10 degrees C to 80 degrees C. The enthalpy of binding was unfavorable at 25 degrees C, and decreased linearly from 5 degrees C to 60 degrees C. A positive binding heat at 25 degrees C is attributed in part to the energy of distorting DNA, and ensures that the temperature of maximal binding affinity (75.1 \pm 5.6 degrees C) is near the growth temperature of *Sulfolobus acidocaldarius*. Truncation of the two intercalating residues to alanine led to a decreased ability to bend and unwind DNA at 25 degrees C with a small

decrease in binding affinity. The energy gained from intercalation is slightly greater than the free energy penalty of bending duplex DNA. Surprisingly, reduced distortion from the double alanine substitution did not lead to a significant decrease in the heat of binding at 25 degrees C. In addition, an anomalous positive ΔC_p of binding was observed for the double alanine mutant protein which could not be explained by the change in polar and apolar accessible surface areas. Both the larger than expected binding enthalpy and the positive heat capacity can be explained by a temperature dependent structural transition in the protein-DNA complex with a T_m of 15-20 degrees C and a ΔH of 15 kcal/mol. Data are discussed which indicate that the endothermic transition in the complex is consistent with DNA distortion.

Peters W. B., Edmondson S. P., and Shriver J. W. (2005) Effect of mutation of the Sac7d intercalating residues on the temperature dependence of DNA distortion and binding thermodynamics. *Biochemistry* **44**, 4794-4804.

Abstract: Sac7d is a small chromatin protein from the hyperthermophile *Sulfolobus acidocaldarius* which kinks duplex DNA by approximately 66 degrees at a single base pair step with intercalation of V26 and M29 side chains. Site-directed mutagenesis coupled with calorimetric and spectroscopic data has been used to characterize the influence of the intercalating side chains on the structure and thermodynamics of the DNA complex from 5 to 85 degrees C. Two single-alanine substitutions (V26A and M29A) and five double-glycine, -alanine, -leucine, -phenylalanine, and -tryptophan substitutions of the surface residues have been created. NMR and fluorescence titrations indicated that the substitutions had little effect on the structure of the protein or DNA binding site size. Each of the mutant proteins demonstrated a temperature-dependent binding enthalpy which was correlated with a similar temperature dependence in the structure of the complex reflected by changes in fluorescence and circular dichroism. A positive heat capacity change (ΔC_p) for DNA binding was observed for only those mutants which also demonstrated a thermotropic structural transition in the complex, and the temperature range for the positive ΔC_p coincided with that observed for the structural transition. The thermodynamic data are interpreted using a model in which binding is linked to an endothermic distortion of the DNA in the complex. The results support the proposal that the unfavorable enthalpy of binding of Sac7d at 25 degrees C is due in part to the distortion of DNA.

Privalov P. L., Jelesarov I., Read C. M., Dragan A. I., and Crane-Robinson C. (1999) The energetics of HMG box interactions with DNA: thermodynamics of the DNA binding of the HMG box from mouse sox-5. *J Mol Biol* **294**, 997-1013.

Abstract: The energetics of the Sox-5 HMG box interaction with DNA duplexes, containing the recognition sequence AACAAAT, were studied by fluorescence spectroscopy, isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). Fluorescence titration showed that the association constant of this HMG box with the duplexes is of the order $4 \times 10^7 M^{-1}$, increasing somewhat with temperature rise, i.e. the Gibbs energy is $-40 kJ mol^{-1}$ at 5 degrees C, decreasing to $-48 kJ mol^{-1}$ at 32 degrees C. ITC measurements of the enthalpy of association over this temperature range showed an endothermic effect below 17 degrees C and an exothermic effect above, suggesting a heat capacity change on binding of about $-4 kJ K^{-1} mol^{-1}$, a value twice larger than expected from structural considerations. A straightforward interpretation of ITC data in heat capacity terms assumes, however, that the heat capacities of all participants in the association reaction do not change over the considered temperature range. Our previous studies showed that over the temperature range of the ITC experiments the HMG box of Sox-5 starts to unfold, absorbing heat and the heat capacities of the DNA duplexes also increase significantly. These heat capacity effects differ from that of the DNA/Sox-5 complex. Correcting the ITC measured binding enthalpies for the heat capacity changes of the components and complex yielded the net enthalpies which exhibit a temperature dependence of about $-2 kJ K^{-1} mol^{-1}$, in good agreement with that predicted on the basis of dehydration of the protein-DNA interface. Using the derived heat capacity change and the enthalpy and Gibbs energy of association measured at 5 degrees C, the net enthalpy and entropy of association of the fully folded HMG box with the target DNA duplexes was determined over a broad temperature range. These functions were compared with those for other known cases of sequence specific DNA/protein association. It appears that the enthalpy and entropy of association of minor groove binding proteins are more positive than for proteins binding in the major groove. The observed thermodynamic characteristics of protein binding to the A+T-rich minor groove of DNA might result from dehydration of both polar and non-polar groups at the interface and release of counterions. The expected entropy of dehydration was calculated and found to be too large to be compensated by the negative entropy of

reduction of translational/rotational freedom. This implies that DNA/HMG box association proceeds with significant decrease of conformational entropy, i.e. reduction in conformational mobility.

Privalov P. L. and Dragan A. I. (2007) Microcalorimetry of biological macromolecules. *Biophys Chem* **126**, 16-24.

Abstract: The capabilities of contemporary differential scanning and isothermal titration microcalorimetry for studying the thermodynamics of protein unfolding/refolding and their association with partners, particularly target DNA duplexes, are considered. It is shown that the predenatural changes of proteins must not be ignored in studying the thermodynamics of formation of their native structure and their complexes with partners, particularly their cognate DNA duplexes.

Read C. M. and Jelesarov I. (2001) Calorimetry of protein-DNA complexes and their components. *Methods Mol Biol* **148**, 511-533.

Swinger K. K. and Rice P. A. (2004) IHF and HU: flexible architects of bent DNA. *Curr Opin Struct Biol* **14**, 28-35.

Abstract: The energetic cost of bending short segments of DNA is very high. This bending is critical for the packaging of DNA and is exploited to regulate many cellular processes. In prokaryotes, IHF and HU are key architectural proteins present at high concentrations. New protein-DNA co-crystal structures, and the adaptation of advanced biophysical and biochemical techniques have led to an improved understanding of how these proteins interact with DNA. These techniques include time-resolved synchrotron X-ray footprinting, differential scanning calorimetry, isothermal titration calorimetry and single-molecule experiments.

Tan W. B., Cheng W., Webber A., Bhambhani A., Duff M. R., Kumar C. V., and McLendon G. L. (2005) Endonuclease-like activity of heme proteins. *J Biol Inorg Chem* **10**, 790-799.

Abstract: Heme proteins, metmyoglobin, methemoglobin, and metcytochrome c showed unusual affinity for double-stranded DNA. Calorimetric studies show that binding of methemoglobin to calf thymus DNA (CTDNA) is weakly endothermic, and the binding constant is $4.9 \pm 0.7 \times 10^5 \text{ M}^{-1}$. The Soret absorption bands of the heme proteins remained unchanged, in the presence of excess CTDNA, but a new circular dichroic band appeared at 210 nm. Helix melting studies indicated that the protein-DNA mixture denatures at a lower temperature than the individual components. Thermograms obtained by differential scanning calorimetry of the mixture indicated two distinct transitions, which are comparable to the thermograms obtained for individual components, but there was a reduction in the excess heat capacity. Activation of heme proteins by hydrogen peroxide resulted in the formation of high valent Fe(IV) oxo intermediates, and CTDNA reacted rapidly under these conditions. The rate was first-order in DNA concentration, and this reactivity resulted in DNA strand cleavage. Upon activation with hydrogen peroxide, for example, the heme proteins converted the supercoiled pUC18 DNA into nicked circular and linear DNA. No reaction occurred in the absence of the heme protein, or hydrogen peroxide. These data clearly indicate a novel property of several heme proteins, and this is first report of the endonuclease-like activity of the heme proteins.