

ITC III- Protein-Nucleic Acid interactions (RNA, DNA, oligos)

Allen, M.D., Grummitt, C.G., Hilcenko, C., Min, S.Y., Tonkin, L.M., Johnson, C.M., Freund, S.M., Bycroft, M., and Warren, A.J. (2006) Solution structure of the nonmethyl-CpG-binding CXXC domain of the leukaemia-associated MLL histone methyltransferase. *EMBO J* **25**, 4503–4512.

Abstract: Methylation of CpG dinucleotides is the major epigenetic modification of mammalian genomes, critical for regulating chromatin structure and gene activity. The mixed-lineage leukaemia (MLL) CXXC domain selectively binds nonmethyl-CpG DNA, and is required for transformation by MLL fusion proteins that commonly arise from recurrent chromosomal translocations in infant and secondary treatment-related acute leukaemias. To elucidate the molecular basis of nonmethyl-CpG DNA recognition, we determined the structure of the human MLL CXXC domain by multidimensional NMR spectroscopy. The CXXC domain has a novel fold in which two zinc ions are each coordinated tetrahedrally by four conserved cysteine ligands provided by two CGXCXXC motifs and two distal cysteine residues. We have identified the CXXC domain DNA binding interface by means of chemical shift perturbation analysis, cross-saturation transfer and site-directed mutagenesis. In particular, we have shown that residues in an extended surface loop are in close contact with the DNA. These data provide a template for the design of specifically targeted therapeutics for poor prognosis MLL-associated leukaemias.

Amarasinghe G. K., De Guzman R. N., Turner R. B., Chancellor K. J., Wu Z. R., and Summers M. F. (2000) NMR structure of the HIV-1 nucleocapsid protein bound to stem-loop SL2 of the psi-RNA packaging signal. Implications for genome recognition. *J Mol Biol* **301**, 491-511.

Abstract: The RNA genome of the human immunodeficiency virus type-1 (HIV-1) contains a approximately 120 nucleotide Psi-packaging signal that is recognized by the nucleocapsid (NC) domain of the Gag polyprotein during virus assembly. The Psi-site contains four stem-loops (SL1-SL4) that possess overlapping and possibly redundant functions. The present studies demonstrate that the 19 residue SL2 stem-loop binds NC with affinity ($K_d=110(+/-50)$ nM) similar to that observed for NC binding to SL3 ($K(d)=170(+/-65)$ nM) and tighter than expected on the basis of earlier work, suggesting that NC-SL2 interactions probably play a direct role in the specific recognition and packaging of the full-length, unspliced genome. The structure of the NC-SL2 complex was determined by heteronuclear NMR methods using $(15)N,(13)C$ -isotopically labeled NC protein and SL2 RNA. The N and C-terminal "zinc knuckles" (Cys-X(2)-Cys-X(4)-His-X(4)-Cys; X=variable amino acid) of HIV-1 NC bind to exposed guanosine bases G9 and G11, respectively, of the G8-G9-U10-G11 tetraloop, and residues Lys3-Lys11 of the N-terminal tail forms a 3(10) helix that packs against the proximal zinc knuckle and interacts with the RNA stem. These structural features are similar to those observed previously in the NMR structure of NC bound to SL3. Other features of the complex are substantially different. In particular, the N-terminal zinc knuckle interacts with an A-U-A base triple platform in the minor groove of the SL2 RNA stem, but binds to the major groove of SL3. In addition, the relative orientations of the N and C-terminal zinc knuckles differ in the NC-SL2 and NC-SL3 complexes, and the side-chain of Phe6 makes minor groove hydrophobic contacts with G11 in the NC-SL2 complex but does not interact with RNA in the NC-SL3 complex. Finally, the N-terminal helix of NC interacts with the phosphodiester backbone of the SL2 RNA stem mainly via electrostatic interactions, but does not bind in the major groove or make specific H-bonding contacts as observed in the NC-SL3 structure. These findings demonstrate that NC binds in an adaptive manner to SL2 and SL3 via different subsets of inter and intra-molecular interactions, and support a genome recognition/packaging mechanism that involves interactions of two or more NC domains of assembling HIV-1 Gag molecules with multiple Psi-site stem-loop packaging elements during the early stages of retrovirus assembly.

Amarasinghe G. K., Zhou J., Miskimon M., Chancellor K. J., McDonald J. A., Matthews A. G., Miller R. R., Rouse M. D., and Summers M. F. (2001) Stem-loop SL4 of the HIV-1 psi RNA packaging signal exhibits weak affinity for the nucleocapsid protein. structural studies and implications for genome recognition. *J Mol Biol* **314**, 961-970.

Abstract: Encapsidation of the genome of the human immunodeficiency virus type-1 (HIV-1) during retrovirus assembly is mediated by interactions between the nucleocapsid (NC) domains of assembling Gag polyproteins and a approximately 110 nucleotide segment of the genome known as the Psi-site. The HIV-1

Psi-site contains four stem-loops (SL1 through SL4), all of which are important for genome packaging. Recent isothermal titration calorimetry (ITC) studies have demonstrated that SL2 and SL3 are capable of binding NC with high affinity (K_d approximately 140 nM), consistent with proposals for protein-interactive functions during packaging. To determine if SL4 may have a similar function, NC-interactive studies were conducted by NMR and gel-shift methods. In contrast to previous reports, we find that SL4 binds weakly to NC ($K_d = (+/-) 14 \mu\text{M}$), suggesting an alternative function. NMR studies indicate that the GAGA tetraloop of SL4 adopts a classical GNRA-type fold (R=purine, N=G, C, A or U), a motif that stabilizes RNA tertiary structures in other systems. In combination with previously reported gel mobility studies of Psi-site deletion mutants, these findings suggest that SL4 functions in genome recognition not by binding to Gag, but by stabilizing the structure of the Psi-site. Differences in the affinities of NC for SL2, SL3 and SL4 stem-loops can now be rationalized in terms of the different structural properties of stem loops that contain GGNG (SL2 and SL3) and GNRA (SL4) sequences.

Armengaud J., Urbonavicius J., Fernandez B., Chaussinand G., Bujnicki J. M., and Grosjean H. (2004) N2-methylation of guanosine at position 10 in tRNA is catalyzed by a THUMP domain-containing, S-adenosylmethionine-dependent methyltransferase, conserved in Archaea and Eukaryota. *J Biol Chem* **279**, 37142-37152.

Abstract: In sequenced genomes, genes belonging to the cluster of orthologous group COG1041 are exclusively, and almost ubiquitously, found in Eukaryota and Archaea but never in Bacteria. The corresponding gene products exhibit a characteristic Rossmann fold, S-adenosylmethionine-dependent methyltransferase domain in the C terminus and a predicted RNA-binding THUMP (thiouridine synthases, RNA methyltransferases, and pseudouridine synthases) domain in the N terminus. Recombinant PAB1283 protein from the archaeon *Pyrococcus abyssi* GE5, a member of COG1041, was purified and shown to behave as a monomeric 39-kDa entity. This protein (EC 2.1.1.32), now renamed (Pab)Trm-G10, which is extremely thermostable, forms a 1:1 complex with tRNA and catalyzes the adenosylmethionine-dependent methylation of the exocyclic amino group (N(2)) of guanosine located at position 10. Depending on the experimental conditions used, as well as the tRNA substrate tested, the enzymatic reaction leads to the formation of either N(2)-monomethyl (m(2)G) or N(2)-dimethylguanosine (m(2)(2)G). Interestingly, (Pab)Trm-G10 exhibits different domain organization and different catalytic site architecture from another, earlier characterized, tRNA-dimethyltransferase from *Pyrococcus furiosus* ((Pfu)Trm-G26, also known as (Pfu)Trm1, a member of COG1867) that catalyzes an identical two-step dimethylation of guanosine but at position 26 in tRNAs and is also conserved among all sequenced Eukaryota and Archaea. The co-occurrence of these two guanosine dimethyltransferases in both Archaea and Eukaryota but not in Bacteria is a hallmark of distinct tRNAs maturation strategies between these domains of life.

Berger C., Jelesarov I., and Bosshard H. R. (1996) Coupled folding and site-specific binding of the GCN4-bZIP transcription factor to the AP-1 and ATF/CREB DNA sites studied by microcalorimetry. *Biochemistry* **35**, 14984-14991.

Abstract: The site-specific interaction of the basic leucine zipper protein C62GCN4, which corresponds to the C-terminal sequence 220-281 of the yeast transcription factor GCN4, with the AP-1 and ATF/CREB DNA recognition sites was analyzed by isothermal titration microcalorimetry. Free C62GCN4 is a dimer composed of a C-terminal leucine zipper and a basic, mainly unstructured DNA binding domain. Upon association with the target DNA, C62GCN4 folds to a fully alpha-helical dimer [Ellenberger et al. (1992) *Cell* 71, 1223-1237; König and Richmond (1993) *J. Mol. Biol.* 233, 139-154]. The protein-bound AP-1 site is straight, and the protein-bound ATF/CREB site is bent by 20 degrees toward the leucine zipper domain. The coupling between protein folding and DNA association resulting in two conformationally different complexes with C62GCN4 poses interesting thermodynamic problems. The association was strongly exothermic for both DNA target sites. The free energies of binding were indistinguishable in buffers of low salt concentration, and no change of the protonation state of C62GCN4 and/or the DNA target site occurred on formation of the complexes. Both complexes exhibited large and negative heat capacity changes. The empirical correlation between buried nonpolar and polar surfaces and the reduction in heat capacity concomitant to complexation did hold for the reaction with the AP-1 site at low salt concentration. However, in the case of the ATF/CREB site, the change in heat capacity was larger than could be accounted for by the burial of solvent-accessible surface. Potential sources of the extra decrement in the heat capacity could be restrictions in the vibrational modes of polar groups and of bound water molecules at the protein-DNA interface, thought to result from the bending of the ATF/CREB site. In the presence of

high concentrations of glutamate and NaCl, the complex with the ATF/CREB site was significantly weaker than the complex with the AP-1 site.

Bergqvist S., O'Brien R., and Ladbury J. E. (2001) Site-specific cation binding mediates TATA binding protein-DNA interaction from a hyperthermophilic archaeon. *Biochemistry* **40**, 2419-2425.

Abstract: *Pyrococcus woesei* (Pw) is a hyperthermophilic archaeal organism that exists under conditions of high salt and elevated temperature. In a previous study [O'Brien, R., DeDecker, B., Fleming, K., Sigler, P. B., and Ladbury, J. E., (1998) *J. Mol. Biol.* 279, 117-125], we showed that, despite the similarity of primary and secondary structure, the TATA box binding protein (TBP) from Pw binds thermodynamically in a fundamentally different way to its mesophilic counterparts. The affinity of the interaction increases as the salt concentration is increased. The formation of the protein-DNA complex involves the release of water and the uptake of ions, which were hypothesized to be cations. Here we test this hypothesis by selecting potential cation binding sites at negatively charged, acidic residues in the complex interface. These were substituted using site-directed mutagenesis of specific residues. Changes in the thermodynamic parameters on formation of the mutant protein-DNA complex were determined using isothermal titration calorimetry and compared to the wild type interaction. Removal of a glutamate residue from the binding site resulted in the uptake of one less cation on formation of the complex. This glutamate (E12) is directly involved in the binding of cations in the complex interface. Substitution of another acidic residue proximal to the DNA binding site (D101) had no effect on cation uptake, suggesting that the location of the amino acid on the protein surface is important in dictating the potential to coordinate cations. Removal of the cation binding site provided a more favorable entropy of binding; however, this effect is significantly reduced at higher salt concentrations. The removal of the cation binding site led to an increase in affinity with respect to the wild-type TBP at low salt concentrations.

Bergqvist S., Williams M. A., O'Brien R., and Ladbury J. E. (2002) Reversal of halophilicity in a protein-DNA interaction by limited mutation strategy. *Structure (Camb)* **10**, 629-637.

Abstract: Comparison of the genes of functionally homologous proteins in organisms existing in different environments shows that adaptation is most often accomplished by mutation of an existing protein. However, from such comparisons, the significance of individual residues to the particular environmental adaptation is not generally discernable among the mass of changes that occur over evolutionary time. This can be exemplified by the general transcription factor found in eukaryotes and archaea, the TATA binding protein (TBP). TBP from *Pyrococcus woesei* is adapted for optimal binding to DNA at high salt and high temperature, with 34% of the amino acids altered in comparison to its nearest known mesophilic counterpart. We demonstrate that the halophilic nature of this protein can be attributed to only three mutations, revealing that the important phenotype of halophilicity could be rapidly acquired in evolutionary time.

Bergqvist S., Williams M. A., O'Brien R., and Ladbury J. E. (2003) Halophilic adaptation of protein-DNA interactions. *Biochem Soc Trans* **31**, 677-680.

Abstract: *Pyrococcus woesei* (Pw) is an archaeal organism adapted to living in conditions of elevated salt and temperature. Thermodynamic data reveal that the interaction between the TATA-box-binding protein (TBP) from this organism and DNA has an entirely different character to the same interaction in mesophilic counterparts. In the case of the Pw TBP, the affinity of its interaction with DNA increases with increasing salt concentration. The opposite effect is observed in all known mesophilic protein-DNA interactions. The halophilic behaviour can be attributed to sequestration of cations into the protein-DNA complex. By mutating residues in the Pw TBP DNA-binding site, potential sites of cation interaction can be removed. These mutations have a significant effect on the binding characteristics, and the halophilic nature of the Pw TBP-DNA interaction can be reversed, and made to resemble that of a mesophile, in just three mutations. The genes of functionally homologous proteins in organisms existing in different environments show that adaptation is most often accompanied by mutation of an existing protein. However, the importance of any individual residue to a phenotypic characteristic is usually difficult to assess amongst the multitude of changes that occur over evolutionary time. Since the halophilic nature of this protein can be attributed to only three mutations, this reveals that the important phenotype of halophilicity could be rapidly acquired in evolutionary time.

Beuth B., Pennell S., Arnvig K. B., Martin S. R., and Taylor I. A. (2005) Structure of a Mycobacterium tuberculosis NusA-RNA complex. *EMBO J* **24**, 3576-3587.

Abstract: NusA is a key regulator of bacterial transcriptional elongation, pausing, termination and antitermination, yet relatively little is known about the molecular basis of its activity in these fundamental processes. In Mycobacterium tuberculosis, NusA has been shown to bind with high affinity and specificity to BoxB-BoxA-BoxC antitermination sequences within the leader region of the single ribosomal RNA (rRNA) operon. We have determined high-resolution X-ray structures of a complex of NusA with two short oligo-ribonucleotides derived from the BoxC stem-loop motif and have characterised the interaction of NusA with a variety of RNAs derived from the antitermination region. These structures reveal the RNA bound in an extended conformation to a large interacting surface on both KH domains. Combining structural data with observed spectral and calorimetric changes, we now show that NusA binding destabilises secondary structure within rRNA antitermination sequences and propose a model where NusA functions as a chaperone for nascently forming RNA structures.

Bubunenko M., Korepanov A., Court D.L., Jagannathan I., Dickinson D., Chaudhuri B.R., Garber M.B., and Culver G.M. (2006) 30S ribosomal subunits can be assembled in vivo without primary binding ribosomal protein S15. *RNA*. **12**, 1229-39.

Abstract: Assembly of 30S ribosomal subunits from Escherichia coli has been dissected in detail using an in vitro system. Such studies have allowed characterization of the role for ribosomal protein S15 in the hierarchical assembly of 30S subunits; S15 is a primary binding protein that orchestrates the assembly of ribosomal proteins S6, S11, S18, and S21 with the central domain of 16S ribosomal RNA to form the platform of the 30S subunit. In vitro S15 is the sole primary binding protein in this cascade, performing a critical role during assembly of these four proteins. To investigate the role of S15 in vivo, the essential nature of rpsO, the gene encoding S15, was examined. Surprisingly, E. coli with an in-frame deletion of rpsO are viable, although at 37 degrees C this DeltarpsO strain has an exaggerated doubling time compared to its parental strain. In the absence of S15, the remaining four platform proteins are assembled into ribosomes in vivo, and the overall architecture of the 30S subunits formed in the DeltarpsO strain at 37 degrees C is not altered. Nonetheless, 30S subunits lacking S15 appear to be somewhat defective in subunit association in vivo and in vitro. In addition, this strain is cold sensitive, displaying a marked ribosome biogenesis defect at low temperature, suggesting that under nonideal conditions S15 is critical for assembly. The viability of this strain indicates that in vivo functional populations of 70S ribosomes must form in the absence of S15 and that 30S subunit assembly has a plasticity that has not previously been revealed or characterized.

Buczek P., Orr R.S., Pyper S.R., Shum M., Kimmel E., Ota I., Gerum S.E., and Horvath M.P. (2005) Binding linkage in a telomere DNA-protein complex at the ends of Oxytricha nova chromosomes. *J Mol Biol*. **350**, 938-52.

Abstract: Alpha and beta protein subunits of the telomere end binding protein from Oxytricha nova (OnTEBP) combine with telomere single strand DNA to form a protective cap at the ends of chromosomes. We tested how protein-protein interactions seen in the co-crystal structure relate to DNA binding through use of fusion proteins engineered as different combinations of domains and subunits derived from OnTEBP. Joining alpha and beta resulted in a protein that bound single strand telomere DNA with high affinity ($K(D-DNA)=1.4$ nM). Another fusion protein, constructed without the C-terminal protein-protein interaction domain of alpha, bound DNA with 200-fold diminished affinity ($K(D-DNA)=290$ nM) even though the DNA-binding domains of alpha and beta were joined through a peptide linker. Adding back the alpha C-terminal domain as a separate protein restored high-affinity DNA binding. The binding behaviors of these fusion proteins and the native protein subunits are consistent with cooperative linkage between protein-association and DNA-binding equilibria. Linking DNA-protein stability to protein-protein contacts at a remote site may provide a trigger point for DNA-protein disassembly during telomere replication when the single strand telomere DNA must exchange between a very stable OnTEBP complex and telomerase.

Buczek P. and Horvath M. P. (2006) Thermodynamic Characterization of Binding Oxytricha nova Single Strand Telomere DNA with the Alpha Protein N-terminal Domain. *J Mol Biol* **359**, 1217-1234.

Abstract: The Oxytricha nova telomere binding protein alpha subunit binds single strand DNA and participates in a nucleoprotein complex that protects the very ends of chromosomes. To understand how the

N-terminal, DNA binding domain of alpha interacts with DNA we measured the stoichiometry, enthalpy (ΔH), entropy (ΔS), and dissociation constant ($K(D-DNA)$) for binding telomere DNA fragments at different temperatures and salt concentrations using native gel electrophoresis and isothermal titration calorimetry (ITC). About 85% of the total free energy of binding corresponded with non-electrostatic interactions for all DNAs. Telomere DNA fragments d(T(2)G(4)), d(T(4)G(4)), d(G(3)T(4)G(4)), and d(G(4)T(4)G(4)) each formed monovalent protein complexes. In the case of d(T(4)G(4)T(4)G(4)), which has two tandemly repeated d(TTTTGGGG) telomere motifs, two binding sites were observed. The high-affinity "A site" has a dissociation constant, $K(D-DNA(A))=13(+/-4)$ nM, while the low-affinity "B site" is characterized by $K(D-DNA(B))=5600(+/-600)$ nM at 25 degrees C. Nucleotide substitution variants verified that the A site corresponds principally with the 3'-terminal portion of d(T(4)G(4)T(4)G(4)). The relative contributions of entropy (ΔS) and enthalpy (ΔH) for binding reactions were DNA length-dependent as was heat capacity (ΔC_p). These trends with respect to DNA length likely reflect structural transitions in the DNA molecule that are coupled with DNA-protein association. Results presented here are important for understanding early intermediates and subsequent stages in the assembly of the full telomere nucleoprotein complex and how binding events can prepare the telomere DNA for extension by telomerase, a critical event in telomere biology.

Buczek P. and Horvath M. P. (2006) Structural reorganization and the cooperative binding of single-stranded telomere DNA in *Sterkiella nova*. *J Biol Chem* **281**, 40124-40134.

Abstract: In *Sterkiella nova*, alpha and beta telomere proteins bind cooperatively with single-stranded DNA to form a ternary alpha.beta.DNA complex. Association of telomere protein subunits is DNA-dependent, and alpha-beta association enhances DNA affinity. To further understand the molecular basis for binding cooperativity, we characterized several possible stepwise assembly pathways using isothermal titration calorimetry. In one path, alpha and DNA first form a stable alpha.DNA complex followed by the addition of beta in a second step. Binding energy accumulates with nearly equal free energy of association for each of these steps. Heat capacity is nonetheless dramatically different, with $\Delta C_p = -305 \pm 3$ cal mol⁻¹ K⁻¹ for alpha binding with DNA and $\Delta C_p = -2010 \pm 20$ cal mol⁻¹ K⁻¹ for the addition of beta to complete the alpha.beta.DNA complex. By examining alternate routes including titration of single-stranded DNA with a preformed alpha.beta complex, a significant portion of binding energy and heat capacity could be assigned to structural reorganization involving protein-protein interactions and repositioning of the DNA. Structural reorganization probably affords a mechanism to regulate high affinity binding of telomere single-stranded DNA with important implications for telomere biology. Regulation of telomere complex dissociation is thought to involve post-translational modifications in the lysine-rich C-terminal portion of beta. We observed no difference in binding energetics or crystal structure when comparing complexes prepared with full-length beta or a C-terminally truncated form, supporting interesting parallels between the intrinsically disordered regions of histones and this portion of beta.

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.

Chan E., Amon M., Marano R. J., Wimmer N., Kearns P. S., Manolios N., Rakoczy P. E. and Toth I. (2007) Novel cationic lipophilic peptides for oligodeoxynucleotide delivery. *Bioorg. Med. Chem* **15**, 4091-4097.

Abstract: In search of new oligodeoxynucleotide (ODN) delivery agents, we evaluated novel peptides derived from core peptide H-GLRILLKLV-OH (CP). CP is a fragment designed from the T-cell antigen receptor (TCR) alpha-chain transmembrane sequence. CP was able to enter cells including T-cells and inhibited interleukin-2 (IL-2) production. To examine the effect of increased lipophilicity on cellular uptake and activity of CP, a lipophilic amino acid (2-aminododecanoic acid) was incorporated into peptide CP resulting in 2-aminododecanoyl-CP (LP). The toxicity of CP and LP was assessed by measuring the haemolytic activity. Neither compound caused any haemolysis of red blood cells. We have also compared the biological activities of the CP and LP. Using a T-cell antigen presentation assay, the more lipophilic LP caused greater inhibition of IL-2 production than the parent CP in the antigen stimulated T-cells. The LP also showed increased permeability than CP in the Caco-2 cell assay. We utilised the enhanced cell permeability property of LP in oligodeoxynucleotide ODN1 delivery. Isothermal titration calorimetry (ITC) suggested that CP and LP complex with ODN1 in a 12:1 (CP:ODN1) and 15:1 (LP:ODN1) ratio. These complexes were then transfected into human retinal pigment epithelial cells. The level of transfection was measured by the decreased production of the protein human vascular endothelial growth factor (hVEGF). The results revealed greater transfection efficiency for both CP and LP (47%, 55% more inhibition) compared to commercially available transfection agent cytofectin GSV. These results suggested that the CP and particularly its lipophilic analogue LP have the potential to be used as oligodeoxynucleotide delivery systems.

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

Cheng A., Wong S. M. and Yuan Y. A. (2008) Structural basis for dsRNA recognition by NS1 protein of influenza A virus. *Cell Res (epublication)*.

Abstract: Influenza A viruses are important human pathogens causing periodic pandemic threats. Nonstructural protein 1 (NS1) protein of influenza A virus (NS1A) shields the virus against host defense. Here, we report the crystal structure of NS1A RNA-binding domain (RBD) bound to a double-stranded RNA (dsRNA) at 1.7Å. NS1A RBD forms a homodimer to recognize the major groove of A-form dsRNA in a length-independent mode by its conserved concave surface formed by dimeric anti-parallel alpha-helices. dsRNA is anchored by a pair of invariable arginines (Arg38) from both monomers by extensive hydrogen bonds. In accordance with the structural observation, isothermal titration calorimetry assay shows that the unique Arg38-Arg38 pair and two Arg35-Arg46 pairs are crucial for dsRNA binding, and that Ser42 and Thr49 are also important for dsRNA binding. Agrobacterium co-infiltration assay further supports that the unique Arg38 pair plays important roles in dsRNA binding in vivo. Cell Research advance online publication 23 September 2008; doi: 10.1038/cr.2008.288

Cicero D. O., Nadra A. D., Eliseo T., Dellarole M., Paci M., and Prat-Gay G. (2006) Structural and thermodynamic basis for the enhanced transcriptional control by the human papillomavirus strain-16 E2 protein. *Biochemistry* **45**, 6551-6560.

Abstract: Strain 16 of the human papillomavirus is responsible for the largest number of cases of cervical cancers linked to this virus, and the E2 protein is the transcriptional regulator of all viral genes. We present the first structure for the DNA binding domain of HPV16 E2 bound to DNA, and in particular, to a natural

cognate sequence. The NMR structure of the protein backbone reveals that the overall conformation remains virtually unchanged, and chemical shift analysis of the protein bound to a shorter DNA duplex uncovered a contact out of the minimal E2 DNA binding site, made by lysine 349. This contact was confirmed by titration calorimetry and mutagenesis, with a contribution of 1.0 kcal mol⁻¹ to binding energy. HPV16 E2 has the highest DNA binding affinity and exerts a strict transcriptional control, translated into the repression of the E6 and E7 oncogenes. These novel features provide the structural and thermodynamic basis for this tight transcriptional control, the loss of which correlates with carcinogenesis.

Colegrove-Otero L.J., Minshall N., and Standart N. (2005) RNA-binding proteins in early development. *Crit Rev Biochem Mol Biol.* **40**, 21-73.

Abstract: RNA-binding proteins play a major part in the control of gene expression during early development. At this stage, the majority of regulation occurs at the levels of translation and RNA localization. These processes are, in general, mediated by RNA-binding proteins interacting with specific sequence motifs in the 3'-untranslated regions of their target RNAs. Although initial work concentrated on the analysis of these sequences and their trans-acting factors, we are now beginning to gain an understanding of the mechanisms by which some of these proteins function. In this review, we will describe a number of different families of RNA-binding proteins, grouping them together on the basis of common regulatory strategies, and emphasizing the recurrent themes that occur, both across different species and as a response to different biological problems.

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⁻¹each). 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.

Croy J. E., Fast J. L., Grimm N. E. and Wuttke D. S. (2008) Deciphering the mechanism of thermodynamic accommodation of telomeric oligonucleotide sequences by the Schizosaccharomyces pombe protection of telomeres 1 (Pot1pN) protein. *Biochemistry* **47**, 4345-4358.

Abstract: Linear chromosomes terminate in specialized nucleoprotein structures called telomeres, which are required for genomic stability and cellular proliferation. Telomeres end in an unusual 3' single-strand overhang that requires a special capping mechanism to prevent inappropriate recognition by the DNA damage machinery. In Schizosaccharomyces pombe, this protective function is mediated by the Pot1 protein, which binds specifically and with high affinity to telomeric ssDNA. We have characterized the thermodynamics and accommodation of both cognate and noncognate telomeric single-stranded DNA (ssDNA) sequences by Pot1pN, an autonomous ssDNA-binding domain (residues 1-187) found in full-length S. pombe Pot1. Direct calorimetric measurements of cognate telomeric ssDNA binding to Pot1pN show favorable enthalpy, unfavorable entropy, and a negative heat-capacity change. Thermodynamic analysis of the binding of noncognate telomeric ssDNA to Pot1pN resulted in unexpected changes in free energy, enthalpy, and entropy. Chemical-shift perturbation and structural analysis of these bound noncognate sequences show that these thermodynamic changes result from the structural rearrangement of both Pot1pN and the bound oligonucleotide. These data suggest that the ssDNA-binding interface is highly dynamic and, in addition to the conformation observed in the crystal structure of the Pot1pN/d(GGTTAC) complex, capable of adopting alternative thermodynamically equivalent conformations

Cui T., Wei S., Brew K., and Leng F. (2005) Energetics of binding the mammalian high mobility group protein HMGA2 to poly(dA-dT)₂ and poly(dA)-poly(dT). *J Mol Biol* **352**, 629-645.

Abstract: The mammalian high mobility group protein A2 (HMGA2) is a chromosomal architectural transcription factor involved in oncogenesis and cell transformation. It has three "AT-hook" DNA binding domains, which specifically bind to the minor groove of AT DNAs. The interaction of HMGA2 with

poly(dA-dT)₂ and poly(dA)poly(dT) has been investigated using the ethidium displacement assay, isothermal titration calorimetry, and UV melting studies. Each AT hook DNA binding domain was found to bind to 5 bp and each HMGA2 molecule binds to 15 bp. Although an individual AT hook DNA binding domain binds to AT DNAs with moderate affinity, HMGA2 binds with very high affinity to both DNAs in solutions containing 20 mM Na⁺ at 25 degrees C. The K_a and binding enthalpy for poly(dA-dT)₂ were determined to be, respectively, 1.9x10¹⁴M⁽⁻¹⁾ and -29.1(+/-0.5)kcal/mol. The binding reaction is enthalpy-driven with a favorable free energy of -19.5 kcal/mol and unfavorable entropy of -32.5 cal/mol K (-TΔS=+9.7kcal/mol) at a 1M reference state. Interestingly, although HMGA2 binds to poly(dA)poly(dT) with a binding constant of 9.6x10¹² M⁽⁻¹⁾, the binding reaction is entropy-driven with an unfavorable enthalpy of +0.6 kcal/mol, a free energy of -17.7 kcal/mol and an entropy of +61.4 cal/mol K (-TΔS=-18.3 kcal/mol) at the 1 M state. The enthalpy-entropy compensation is similar to that of several minor groove-binding drugs such as netropesin, distamycin A and Hoechst33258 and may be a reflection of dehydration difference of different ligand-DNA complexes. The salt-dependence of the binding constant of HMGA2 with both DNAs showed that electrostatic interaction is a dominant force for the binding reactions. The temperature dependence of binding enthalpy for poly(dA-dT)₂ indicates a large heat capacity of binding of -705(+/-113) cal/molK, consistent with an important role of solvent displacement in the linked folding/binding processes in this system.

D'Souza V., Melamed J., Habib D., Pullen K., Wallace K., and Summers M. F. (2001) Identification of a high affinity nucleocapsid protein binding element within the Moloney murine leukemia virus Psi-RNA packaging signal: implications for genome recognition. *J Mol Biol* **314**, 217-232.

Abstract: Murine leukemia virus (MLV) is currently the most widely used gene delivery system in gene therapy trials. The simple retrovirus packages two copies of its RNA genome by a mechanism that involves interactions between the nucleocapsid (NC) domain of a virally-encoded Gag polyprotein and a segment of the RNA genome located just upstream of the Gag initiation codon, known as the Psi-site. Previous studies indicated that the MLV Psi-site contains three stem loops (SLB-SLD), and that stem loops SLC and SLD play prominent roles in packaging. We have developed a method for the preparation and purification of large quantities of recombinant Moloney MLV NC protein, and have studied its interactions with a series of oligoribonucleotides that contain one or more of the Psi-RNA stem loops. At RNA concentrations above approximately 0.3 mM, isolated stem loop SLB forms a duplex and stem loops SL-C and SL-D form kissing complexes, as expected from previous studies. However, neither the monomeric nor the dimeric forms of these isolated stem loops binds NC with significant affinity. Longer constructs containing two stem loops (SL-BC and SL-CD) also exhibit low affinities for NC. However, NC binds with high affinity and stoichiometrically to both the monomeric and dimeric forms of an RNA construct that contains all three stem loops (SL-BCD; K_d=132(+/-55) nM). Titration of SL-BCD with NC also shifts monomer-dimer equilibrium toward the dimer. Mutagenesis experiments demonstrate that the conserved GACG tetraloops of stem loops C and D do not influence the monomer-dimer equilibrium of SL-BCD, that the tetraloop of stem loop B does not participate directly in NC binding, and that the tetraloops of stem loops C and D probably also do not bind to NC. These surprising results differ considerably from those observed for HIV-1, where NC binds to individual stem loops with high affinity via interactions with exposed residues of the tetraloops. The present results indicate that MLV NC binds to a pocket or surface that only exists in the presence of all three stem loops.

Da G., Lenkart J., Zhao K., Shiekhattar R., Cairns B.R., and Marmorstein R. (2006) Structure and function of the SWIRM domain, a conserved protein module found in chromatin regulatory complexes. *Proc Natl Acad Sci U S A*. **103**, 2057-62.

Abstract: The SWIRM domain is a module found in the Swi3 and Rsc8 subunits of SWI/SNF-family chromatin remodeling complexes, and the Ada2 and BHC110/LSD1 subunits of chromatin modification complexes. Here we report the high-resolution crystal structure of the SWIRM domain from Swi3 and characterize the in vitro and in vivo function of the SWIRM domains from *Saccharomyces cerevisiae* Swi3 and Rsc8. The Swi3 SWIRM forms a four-helix bundle containing a pseudo 2-fold axis and a helix-turn-helix motif commonly found in DNA-binding proteins. We show that the Swi3 SWIRM binds free DNA and mononucleosomes with high and comparable affinity and that a subset of Swi3 substitution mutants that display growth defects in vivo also show impaired DNA-binding activity in vitro, consistent with a nucleosome targeting function of this domain. Genetic and biochemical studies also reveal that the Rsc8 and Swi3 SWIRM domains are essential for the proper assembly and in vivo functions of their respective

complexes. Together, these studies identify the SWIRM domain as an essential multifunctional module for the regulation of gene expression.

Datta K. and LiCata V. J. (2003) Thermodynamics of the binding of *Thermus aquaticus* DNA polymerase to primed-template DNA. *Nucleic Acids Res* **31**, 5590-5597.

Abstract: DNA binding of the Type 1 DNA polymerase from *Thermus aquaticus* (Taq polymerase) and its KlenTaq large fragment domain have been studied as a function of temperature. Equilibrium binding assays were performed from 5 to 70 degrees C using a fluorescence anisotropy assay and from 10 to 60 degrees C using isothermal titration calorimetry. In contrast to the usual behavior of thermophilic proteins at low temperatures, Taq and KlenTaq bind DNA with high affinity at temperatures down to 5 degrees C. The affinity is maximal at 40-50 degrees C. The ΔH and ΔS of binding are highly temperature dependent, and the ΔC_p of binding is -0.7 to -0.8 kcal/mol K, for both Taq and KlenTaq, with good agreement between van't Hoff and calorimetric values. Such a thermodynamic profile, however, is generally associated with sequence-specific DNA binding and not non-specific binding. Circular dichroism spectra show conformational rearrangements of both the DNA and the protein upon binding. The high ΔC_p of Taq/KlenTaq DNA binding may be correlated with structure-specific binding in analogy to sequence-specific binding, or may be a general characteristic of proteins that primarily bind non-specifically to DNA. The low temperature DNA binding of Taq/KlenTaq is suggested to be a general characteristic of thermophilic DNA binding proteins.

Datta K. and LiCata V. J. (2003) Salt dependence of DNA binding by *Thermus aquaticus* and *Escherichia coli* DNA polymerases. *J Biol Chem* **278**, 5694-5701.

Abstract: DNA binding properties of the Type 1 DNA polymerases from *Thermus aquaticus* (Taq, KlenTaq) and *Escherichia coli* (Klenow) have been examined as a function of [KCl] and [MgCl₂]. Full-length Taq and its KlenTaq "large fragment" behave similarly in all assays. The two different species of polymerases bind DNA with sub-micromolar affinities in very different salt concentration ranges. Consequently, at similar [KCl] the binding of Klenow is approximately 3 kcal/mol (150x) tighter than that of Taq/KlenTaq to the same DNA. Linkage analysis reveals a net release of 2-3 ions upon DNA binding of Taq/KlenTaq and 4-5 ions upon binding of Klenow. DNA binding of Taq at a higher temperature (60 degrees C) slightly decreases the ion release. Linkage analysis of binding versus [MgCl₂] reports the ultimate release of approximately 1 Mg²⁺ ion upon complex formation. However, the MgCl₂ dependence for Klenow, but not KlenTaq, shows two distinct phases. In 10 mM EDTA, both polymerase species still bind DNA, but their binding affinity is significantly diminished, Klenow more than KlenTaq. In summary, the two polymerase species, when binding to identical DNA, differ substantially in their sensitivity to the salt concentration range, bind with very different affinities when compared under similar conditions, release different numbers of ions upon binding, and differ in their interactions with divalent cations.

Datta K., Wowor A., J., Richard A., J., and LiCata V. J. (2006) Temperature dependence and thermodynamics of Klenow polymerase binding to primed-template DNA. *Biophys J.* **90**, 1739-51.

Abstract: DNA binding of Klenow polymerase has been characterized with respect to temperature to delineate the thermodynamic driving forces involved in the interaction of this polymerase with primed-template DNA. The temperature dependence of the binding affinity exhibits distinct curvature, with tightest binding at 25-30 degrees C. Nonlinear temperature dependence indicates Klenow binds different primed-template constructs with large heat capacity (ΔC_p) values (-870 to -1220 cal/mole K) and thus exhibits large temperature dependent changes in enthalpy and entropy. Binding is entropy driven at lower temperatures and enthalpy driven at physiological temperatures. Large negative ΔC_p values have been proposed to be a 'signature' of site-specific DNA binding, but type I DNA polymerases do not exhibit significant DNA sequence specificity. We suggest that the binding of Klenow to a specific DNA structure, the primed-template junction, results in a correlated thermodynamic profile that mirrors what is commonly seen for DNA sequence-specific binding proteins. Klenow joins a small number of other DNA-sequence independent DNA binding proteins which exhibit unexpectedly large negative ΔC_p values. Spectroscopic measurements show small conformational rearrangements of both the DNA and Klenow upon binding, and small angle x-ray scattering shows a global induced fit conformational compaction of the protein upon binding. Calculations from both crystal structure and solution structural data indicate that Klenow DNA binding is an exception to the often observed correlation between ΔC_p and changes in

accessible surface area. In the case of Klenow, surface area burial can account for only about half of the ΔC_p of binding.

DeDecker B. S., O'Brien R., Fleming P. J., Geiger J. H., Jackson S. P., and Sigler P. B. (1996) The crystal structure of a hyperthermophilic archaeal TATA-box binding protein. *J Mol Biol* **264**, 1072-1084.

Abstract: This study analyzes the three-dimensional structure of the TATA-box binding protein (TBP) from the hyperthermophilic archaea *Pyrococcus woesei*. The crystal structure of *P. woesei* TBP (PwTBP) was solved at 2.2 Å by X-ray diffraction and as expected from sequence homology (36% to 41% identical to eukaryotic TBPs) its overall structure is very similar to eukaryotic TBPs. The thermal unfolding transition temperature of this protein was measured by differential scanning calorimetry to be 101 degrees C, which is more than 40 degrees C higher than that of yeast TBP. Preliminary titration calorimetry data show that the affinity of PwTBP for its DNA target, unlike its eukaryotic counterparts, is enhanced by increasing the temperature and salt concentration. The structure reveals possible explanations for this thermostability and these unusual DNA binding properties. The crystal structure of this hyperthermostable protein was compared to its mesophilic homologs and analyzed for differences in the native structure that may contribute to thermostability. Differences found were: (1) a disulfide bond not found in mesophilic counterparts; (2) an increased number of surface electrostatic interactions; (3) more compact protein packing. The presumed DNA binding surface of PwTBP, like its eukaryotic counterparts, is hydrophobic but the electrostatic profile surrounding the protein is relatively neutral compared to the asymmetric positive potential that surrounds eukaryotic TBPs. The total reliance on a hydrophobic interface with DNA may explain the enhanced affinity of PwTBP for its DNA promoter at higher temperatures and increased salt concentration.

Del Vecchio. P., Carullo P., Barone G., Pagano B., Graziano G., Iannetti A., Acquaviva R., Leonardi A. and Formisano S. (2008) Conformational stability and DNA binding energetics of the rat thyroid transcription factor 1 homeodomain. *Proteins* **70**, 748-760.

Abstract: The conformational stability of the rat thyroid transcription factor 1 homeodomain, TTF-1HD, has been investigated by means of circular dichroism (CD) and differential scanning calorimetry (DSC) measurements at pH 5.0 as a function of KCl concentration. Thermal unfolding of TTF-1HD is a reversible two-state transition. The protein is not stable against temperature, showing a denaturation temperature of 32 degrees C in the absence of salt and 50 degrees C at 75 mM KCl. The binding energetics of TTF-1HD to its target DNA sequence has been characterized by means of isothermal titration calorimetry (ITC) measurements, complemented with CD data. At 25 degrees C, pH 5.0 and 75 mM KCl, the binding constant amounts to $1.5 \times 10^8 \text{ M}^{-1}$ and the binding enthalpy change amounts to -41 kJ mol^{-1} . The process is enthalpy driven, but also the entropy change is favorable to complex formation. To gain a molecular level understanding of the interactions determining the association of TTF-1HD to the target DNA sequence structural information would be requested, but it is not yet available. Therefore, structural models of two complexes, TTF-1HD with the target DNA sequence and TTF-1HD with a modified DNA sequence, have been constructed by using as a template the NMR structure of the complex between NK-2 HD and its target DNA, and by performing molecular dynamics simulations 3.5 ns long. Analysis of these models allows one to shed light on the origin of the DNA binding specificity characteristic of TTF-1HD. *Proteins* 2007. (c) 2007 Wiley-Liss, Inc.

Deleuw L., Tchernatynskaia A. V. and Lane A. N. (2008) Thermodynamics and specificity of the Mbp1-DNA interaction. *Biochemistry* **47**, 6378-6385.

Abstract: The DNA binding domain of the yeast transcription factor Mbp1 is a winged helix-turn-helix structure, with an extended DNA binding site involving C-terminal "tail" residues. The thermodynamics of the interaction of the DNA binding domain with its target DNA sequence have been determined using fluorescence anisotropy and calorimetry. The dissociation constant was determined as a function of pH and ionic strength in assessing the relative importance of specific and nonspecific ionic interactions. Mutational analysis of the residues in the binding site was used to determine their contributions to binding. The three tail histidine residues and His 63 in the recognition helix accounted for most of the pH dependence of the DNA binding. The tail histidine residues, along with two previously identified lysine residues, account for a major part of the polyelectrolyte contribution to binding and for the nonspecific affinity of Mbp1 for DNA. Gln67 was shown to be a very important residue, which interacts in the minor groove of the target

DNA. Systematic mutations of the DNA consensus binding sites showed that the CGCG core contributes most to recognition. Isothermal titration calorimetry revealed a strong temperature-dependent enthalpy change, with a ΔC_p of $-1.3 \text{ kJ mol}^{-1} \text{ K}^{-1}$, consistent with a specific binding mode and burial of surface area. Parsing the free energy contributions demonstrates that polyelectrolyte effects account for half of the total free energy at the physiological pH and salt concentration. We present a model for the origin of the sequence specificity and overall affinity of the protein that accounts for the observed thermodynamics

Dellarole M., Sanchez I. E., Freire E. and de Prat-Gay G. (2007) Increased stability and DNA site discrimination of "single chain" variants of the dimeric beta-barrel DNA binding domain of the human papillomavirus E2 transcriptional regulator. *Biochemistry* **46**, 12441-12450.

Abstract: Human papillomavirus infects millions of people worldwide and is a causal agent of cervical cancer in women. The HPV E2 protein controls the expression of all viral genes through binding of its dimeric C-terminal domain (E2C) to its target DNA site. We engineered monomeric versions of the HPV16 E2C, in order to probe the link of the dimeric beta-barrel fold to stability, dimerization, and DNA binding. Two single-chain variants, with 6 and 12 residue linkers (scE2C-6 and scE2C-12), were purified and characterized. Spectroscopy and crystallography show that the native structure is unperturbed in scE2C-12. The single chain variants are stabilized with respect to E2C, with effective concentrations of 0.6 to 6 mM. The early folding events of the E2C dimer and scE2C-12 are very similar and include formation of a compact species in the submillisecond time scale and a non-native monomeric intermediate with a half-life of 25 ms. However, monomerization changes the unfolding mechanism of the linked species from two-state to three-state, with a high-energy intermediate. Binding to the specific target site is up to 5-fold tighter in the single chain variants. Nonspecific DNA binding is up to 7-fold weaker in the single chain variants, leading to an overall 10-fold increased site discrimination capacity, the largest described so far for linked DNA binding domains. Titration calorimetric binding analysis, however, shows almost identical behavior for dimer and single-chain species, suggesting very subtle changes behind the increased specificity. Global analysis of the mechanisms probed suggests that the dynamics of the E2C domain, rather than the structure, are responsible for the differential properties. Thus, the plastic and dimeric nature of the domain did not evolve for a maximum affinity, specificity, and stability of the quaternary structure, likely because of regulatory reasons and for roles other than DNA binding played by partly folded dimeric or monomeric conformers.

Dhanasekaran M., Negi S., Imanishi M. and Sugiura Y. (2007) DNA-Binding ability of GAGA zinc finger depends on the nature of amino acids present in the beta-hairpin. *Biochemistry* **46**, 7506-7513.

Abstract: The GAGA factor of *Drosophila melanogaster* uses a single Cys2-His2-type zinc finger for specific DNA binding. Comparative sequence alignment of the GAGA zinc finger core with other structurally characterized zinc fingers reveals that the beta-hairpin of the GAGA zinc finger prefers amino acids with an aliphatic side-chain different from those of other zinc fingers. To probe the substitution effect of aromatic amino acids in the beta-hairpin on the DNA binding, three mutant peptides were designed by substituting consensus phenylalanine, an aromatic amino acid, at key positions in the beta-hairpin region. The metal-binding and the overall fold of the mutant peptides are very similar to those of the wild-type as shown by UV-vis absorption spectroscopy and circular dichroism spectroscopy. However, the gel mobility shift assay and isothermal calorimetric studies demonstrated that none of the mutants are able to bind the cognate DNA substrate, although the mutation is confined only to the beta-hairpin region. The present results suggest that the nature of the amino acids in the beta-hairpin plays an important role in the DNA-binding of the GAGA factor protein.

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 $1.45 \mu\text{M}$ 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 1.5nM and 6.4nM, corresponding to Gibbs energies of -49kJmol^{-1} and -46kJmol^{-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.

Dragan A. I., Li Z., Makeyeva E. N., Milgotina E. I., Liu Y., Crane-Robinson C., and Privalov P. L. (2006) Forces driving the binding of homeodomains to DNA. *Biochemistry* **45**, 141-151.

Abstract: Homeodomains are helix-turn-helix type DNA-binding domains that exhibit sequence-specific DNA binding by insertion of their "recognition" alpha helices into the major groove and a short N-terminal arm into the adjacent minor groove without inducing substantial distortion of the DNA. The stability and DNA binding of four representatives of this family, MATalpha2, engrailed, Antennapedia, and NK-2, and truncated forms of the last two lacking their N-terminal arms have been studied by a combination of optical and microcalorimetric methods at different temperatures and salt concentrations. It was found that the stability of the free homeodomains in solution is rather low and, surprisingly, is reduced by the presence of the N-terminal arm for the Antennapedia and NK-2 domains. Their stabilities depend significantly upon the presence of salt: strongly for NaCl but less so for NaF, demonstrating specific interactions with chloride ions. The enthalpies of association of the homeodomains with their cognate DNAs are negative, at 20 degrees C varying only between -12 and -26 kJ/mol for the intact homeodomains, and the entropies of association are positive; i.e., DNA binding is both enthalpy- and entropy-driven. Analysis of the salt dependence of the association constants showed that the electrostatic component of the Gibbs energy of association resulting from the entropy of mixing of released ions dominates the binding, being about twice the magnitude of the nonelectrostatic component that results from dehydration of the protein/DNA interface, van der Waals interactions, and hydrogen bonding. A comparison of the effects of NaCl/KCl with NaF showed that homeodomain binding results in a release not only of cations from the DNA phosphates but also of chloride ions specifically associated with the proteins. The binding of the basic N-terminal arms in the minor groove is entirely enthalpic with a negative heat capacity effect, i.e., is due to sequence-specific formation of hydrogen bonds and hydrophobic interactions rather than electrostatic contacts with the DNA phosphates.

Dragan A. I., Carrillo R., Gerasimova T. I. and Privalov P. L. (2008) Assembling the human IFN-beta enhanceosome in solution. *J Mol Biol* **384**, 335-348.

Abstract: Assembly of interferon-beta enhanceosome from its individual protein components and of enhancer DNA has been studied in solution using a combination of fluorescence anisotropy, microcalorimetry, and CD titration. It was shown that the enhancer binds only one full-length phosphomimetic IRF-3 dimer at the PRDIII-PRDI sites, and this binding does not exhibit cooperativity with binding of the ATF-2/c-Jun bZIP (leucine zipper dimer with basic DNA recognition segments) heterodimer at the PRDIV site. The orientation of the bZIP pair is, therefore, not determined by the

presence of the IRF-3 dimer, but is predetermined by the asymmetry of the PRDIV site. In contrast, bound IRF-3 dimer interacts strongly with the NF-kappaB (p50/p65) heterodimer bound at the neighboring PRDII site. The orientation of bound NF-kappaB is also predetermined by the asymmetry of the PRDII site and is the opposite of that found in the crystal structure. The HMG-I/Y protein, proposed as orchestrating enhanceosome assembly, interacts specifically with the PRDII site of the interferon-beta enhancer by inserting its DNA-binding segments (AT hooks) into the minor groove, resulting in a significant increase in NF-kappaB binding affinity for the major groove of this site

Duggin I. G., Matthews J. M., Dixon N. E., Wake R. G., and Mackay J. P. (2005) A complex mechanism determines polarity of DNA replication fork arrest by the replication terminator complex of *Bacillus subtilis*. *J Biol Chem* **280**, 13105-13113.

Abstract: Two dimers of the replication terminator protein (RTP) of *Bacillus subtilis* bind to a chromosomal DNA terminator site to effect polar replication fork arrest. Cooperative binding of the dimers to overlapping half-sites within the terminator is essential for arrest. It was suggested previously that polarity of fork arrest is the result of the RTP dimer at the blocking (proximal) side within the complex binding very tightly and the permissive-side RTP dimer binding relatively weakly. In order to investigate this "differential binding affinity" model, we have constructed a series of mutant terminators that contain half-sites of widely different RTP binding affinities in various combinations. Although there appeared to be a correlation between binding affinity at the proximal half-site and fork arrest efficiency *in vivo* for some terminators, several deviated significantly from this correlation. Some terminators exhibited greatly reduced binding cooperativity (and therefore have reduced affinity at each half-site) but were highly efficient in fork arrest, whereas one terminator had normal affinity over the proximal half-site, yet had low fork arrest efficiency. The results show clearly that there is no direct correlation between the RTP binding affinity (either within the full complex or at the proximal half-site within the full complex) and the efficiency of replication fork arrest *in vivo*. Thus, the differential binding affinity over the proximal and distal half-sites cannot be solely responsible for functional polarity of fork arrest. Furthermore, efficient fork arrest relies on features in addition to the tight binding of RTP to terminator DNA.

Edmondson S. P., Kahsai M. A., Gupta R., and Shriver J. W. (2004) Characterization of Sac10a, a hyperthermophile DNA-binding protein from *Sulfolobus acidocaldarius*. *Biochemistry* **43**, 13026-13036.

Abstract: Sac10a is a member of a group of basic DNA-binding proteins thought to be important in chromatin structure and regulation in the archaeon *Sulfolobus*. We describe here the isolation, gene identification, and biophysical characterization of native Sac10a. The protein exists as a 23.8 kDa homodimer at pH 7 and unfolds with a T degrees of 122 degrees C. Dissociation of the dimer into folded globular subunits is promoted by decreased pH and salt concentration. Thermal unfolding of the monomeric subunits occurred with two transitions, indicating two independent domains. The dimer demonstrated a high affinity for duplex poly(dAdT) with a K_D of 5×10^{-10} M and a site size of 17 bp (in 0.15 M KCl, pH 7), with only weak binding ($K_D > 5 \times 10^{-6}$ M) to poly(dA)-poly(dT), poly(dGdC), poly(dG)-poly(dC), and *Escherichia coli* DNA under similar conditions. Binding to poly(dAdT) resulted in distortions in the DNA duplex that were consistent with overwinding as indicated by inversion of the CD spectrum of the DNA. The monomeric subunits are predicted to adopt a winged helix DNA-binding motif which dimerizes through formation of a two-stranded coiled coil involving an extended C-terminal helix with more than four heptad repeats (about 45 Å in length). This is the first example of the conserved archaeal transcription regulator domain COG3432 to be characterized. Sequences for homologous proteins containing both COG3432 and predicted coiled coil domains occur in the genomes of both crenarchaeota (*Sulfolobus*, *Pyrobaculum*, *Aeropyrum*) and euryarchaeota (*Methanosarcina*, *Methanococcus*, *Archaeoglobus*, *Thermoplasma*), with multiple genes in some species. Sac10a shows no sequence similarity to the other *Sulfolobus* chromatin proteins Sac7d, Sac8, Sso10b2, and Alba.

Erra E., Petraccone L., Esposito V., Randazzo A., Mayol L., Ladbury J., Barone G., and Giancola C. (2005) Interaction of porphyrin with G-quadruplex structures. *Nucleosides Nucleotides Nucleic Acids* **24**, 753-756.

Abstract: Isothermal titration calorimetry (ITC) is a sensitive technique for probing bimolecular processes and can provide direct information about the binding affinity and stoichiometry and the key thermodynamic parameters involved. ITC has been used to investigate the interaction of the ligand H2TMPyP to the two DNA quadruplexes, [d(AGGGT)]4 and [d(TGGGGT)]. Analysis of the ITC data reveals that

porphyrin/quadruplex binding stoichiometry under saturating conditions is 1:2 for [d(AGGGT)]₄ and 2:1 for [d(TGGGGT)]₄, respectively.

Eulberg D., Buchner K., Maasch C., and Klussmann S. (2005) Development of an automated in vitro selection protocol to obtain RNA-based aptamers: identification of a biostable substance P antagonist. *Nucleic Acids Res* **33**, e45.

Abstract: We have developed an automated SELEX (Systematic Evolution of Ligands by EXponential Enrichment) process that allows the execution of in vitro selection cycles without any direct manual intervention steps. The automated selection protocol is designed to provide for high flexibility and versatility in terms of choice of buffers and reagents as well as stringency of selection conditions. Employing the automated SELEX process, we have identified RNA aptamers to the mirror-image configuration (d-peptide) of substance P. The peptide substance P belongs to the tachykinin family and exerts various biologically important functions, such as peripheral vasodilation, smooth muscle contraction and pain transmission. The aptamer that was identified most frequently was truncated to the 44mer SUP-A-004. The mirror-image configuration of SUP-A-004, the so-called Spiegelmer, has been shown to bind to naturally occurring l-substance P displaying a K(d) of 40 nM and to inhibit (IC₅₀ of 45 nM) l-substance P-mediated Ca²⁺ release in a cell culture assay.

Fisher R. J., Fivash M. J., Stephen A. G., Hagan N. A., Shenoy S. R., Medaglia M. V., Smith L. R., Worthy K. M., Simpson J. T., Shoemaker R., McNitt K. L., Johnson D. G., Hixson C. V., Gorelick R. J., Fabris D., Henderson L. E., and Rein A. (2006) Complex interactions of HIV-1 nucleocapsid protein with oligonucleotides. *Nucleic Acids Res* **34**, 472-484.

Abstract: The HIV-1 nucleocapsid (NC) protein is a small, basic protein containing two retroviral zinc fingers. It is a highly active nucleic acid chaperone; because of this activity, it plays a crucial role in virus replication as a cofactor during reverse transcription, and is probably important in other steps of the replication cycle as well. We previously reported that NC binds with high-affinity to the repeating sequence d(TG)_n. We have now analyzed the interaction between NC and d(TG)₄ in considerable detail, using surface plasmon resonance (SPR), tryptophan fluorescence quenching (TFQ), fluorescence anisotropy (FA), isothermal titration calorimetry (ITC) and electrospray ionization Fourier transform mass spectrometry (ESI-FTMS). Our results show that the interactions between these two molecules are surprisingly complex: while the K(d) for binding of a single d(TG)₄ molecule to NC is only approximately 5 nM in 150 mM NaCl, a single NC molecule is capable of interacting with more than one d(TG)₄ molecule, and conversely, more than one NC molecule can bind to a single d(TG)₄ molecule. The strengths of these additional binding reactions are quantitated. The implications of this multivalency for the functions of NC in virus replication are discussed.

Fodor E., Mack J. W., Maeng J. S., Ju J. H., Lee H. S., Gruschus J. M., Ferretti J. A., and Ginsburg A. (2005) Cardiac-specific Nkx2.5 homeodomain: conformational stability and specific DNA binding of Nkx2.5(C56S). *Biochemistry* **44**, 12480-12490.

Abstract: The cardiac-specific Nkx2.5 homeodomain has been expressed as a 79-residue protein with the oxidizable Cys(56) replaced with Ser. The Nkx2.5 or Nkx2.5(C56S) homeodomain is 73% identical in sequence to and has the same NMR structure as the vnd (ventral nervous system defective)/NK-2 homeodomain of *Drosophila* when bound to the same specific DNA. The thermal unfolding of Nkx2.5(C56S) at pH 6.0 or 7.4 is a reversible, two-state process with unit cooperativity, as measured by differential scanning calorimetry (DSC) and far-UV circular dichroism. Adding 100 mM NaCl to Nkx2.5(C56S) at pH 7.4 increases T(m) from 44 to 54 +/- 0.2 degrees C and DeltaH from 34 to 45 +/- 2 kcal/mol (giving a DeltaC(p) of approximately 1.2 kcal K(-)(1) mol(-)(1) for homeodomain unfolding). DSC profiles of Nkx2.5 indicate fluctuating natively-like structures at <37 degrees C. Titrations of specific 18 bp DNA with Nkx2.5(C56S) in buffer at pH 7.4 with 100 mM NaCl yield binding constants of 2-6 x 10(8) M(-)(1) from 10 to 37 degrees C and a stoichiometry of 1:1 for homeodomain binding DNA, using isothermal titration calorimetry. The DNA binding reaction of Nkx2.5 is enthalpically controlled, and the temperature dependence of DeltaH gives a DeltaC(p) of -0.18 +/- 0.01 kcal K(-)(1) mol(-)(1). This corresponds to 648 +/- 36 A(2) of buried apolar surface upon Nkx2.5(C56S) binding duplex B-DNA. Thermodynamic parameters differ for Nkx2.5 and vnd/NK-2 homeodomains binding specific DNA. Unbound NK-2 is more flexible than Nkx2.5.

Fodor E. and Ginsburg A. (2006) Specific DNA binding by the homeodomain Nkx2.5(C56S): detection of impaired DNA or unfolded protein by isothermal titration calorimetry. *Proteins* **64**, 13-18.

Abstract: Titrations of specific 18-bp duplex DNA with the cardiac-specific homeodomain Nkx2.5(C56S) have utilized an ultrasensitive isothermal titration calorimeter (ITC). As the free DNA nears depletion, we observe large apparent decreases in the binding enthalpy when the DNA is impaired or when the temperature is sufficiently high to produce some unfolding of the free protein. Either effect can be attributed to refolding of the biopolymer that occurs as a result of stabilization due to the large favorable change in free energy on the homeodomain binding to DNA (-49.4 kJ/mol at 298 K). In either case, thermodynamic parameters obtained in such ITC experiments are unreliable. By using a lower temperature (85 vs. 95 degrees C) during the annealing of complementary DNA strands, damage of the 18-bp duplex DNA ($T(m) = 72$ degrees C) is avoided, and titrations with the homeodomain are normal at temperatures from 10 to 40 degrees C when >95% of the protein is folded. Under the latter conditions, the heat capacity plot is linear with a $\Delta C(p)$ value of -0.80 ± 0.03 kJ K⁻¹ mol⁻¹, which is more negative than that calculated from the burial of solvent accessible surface areas (-0.64 ± 0.05 kJ K⁻¹ mol⁻¹), consistent with water structures being at the protein-DNA interfaces.

Gao Y. G., Suzuki H., Itou H., Zhou Y., Tanaka Y., Wachi M., Watanabe N., Tanaka I. and Yao M. (2008) Structural and functional characterization of the LldR from *Corynebacterium glutamicum*: a transcriptional repressor involved in L-lactate and sugar utilization. *Nucleic Acids Res.*(epublication)

Abstract: LldR (CGL2915) from *Corynebacterium glutamicum* is a transcription factor belonging to the GntR family, which is typically involved in the regulation of oxidized substrates associated with amino acid metabolism. In the present study, the crystal structure of LldR was determined at 2.05-Å resolution. The structure consists of N- and C-domains similar to those of FadR, but with distinct domain orientations. LldR and FadR dimers achieve similar structures by domain swapping, which was first observed in dimeric assembly of transcription factors. A structural feature of Zn(2+) binding in the regulatory domain was also observed, as a difference from the FadR subfamily. DNA microarray and DNase I footprint analyses suggested that LldR acts as a repressor regulating *cgl2917-lldD* and *cgl1934-fruK-ptsF* operons, which are indispensable for l-lactate and fructose/sucrose utilization, respectively. Furthermore, the stoichiometries and affinities of LldR and DNAs were determined by isothermal titration calorimetry measurements. The transcriptional start site and repression of LldR on the *cgl2917-lldD* operon were analysed by primer extension assay. Mutation experiments showed that residues Lys4, Arg32, Arg42 and Gly63 are crucial for DNA binding. The location of the putative ligand binding cavity and the regulatory mechanism of LldR on its affinity for DNA were proposed

Gilbert S. D., Love C. E., Edwards A. L. and Batey R. T. (2007) Mutational analysis of the purine riboswitch aptamer domain. *Biochemistry* **46**, 13297-13309.

Abstract: The purine riboswitch is one of a number of mRNA elements commonly found in the 5'-untranslated region capable of controlling expression in a cis-fashion via its ability to directly bind small-molecule metabolites. Extensive biochemical and structural analysis of the nucleobase-binding domain of the riboswitch, referred to as the aptamer domain, has revealed that the mRNA recognizes its cognate ligand using an intricately folded three-way junction motif that completely encapsulates the ligand. High-affinity binding of the purine nucleobase is facilitated by a distal loop-loop interaction that is conserved between both the adenine and guanine riboswitches. To understand the contribution of conserved nucleotides in both the three-way junction and the loop-loop interaction of this RNA, we performed a detailed mutagenic survey of these elements in the context of an adenine-responsive variant of the *xpt-pbuX* guanine riboswitch from *Bacillus subtilis*. The varying ability of these mutants to bind ligand as measured by isothermal titration calorimetry uncovered the conserved nucleotides whose identity is required for purine binding. Crystallographic analysis of the bound form of five mutants and chemical probing of their free state demonstrate that the identity of several universally conserved nucleotides is not essential for formation of the RNA-ligand complex but rather for maintaining a binding-competent form of the free RNA. These data show that conservation patterns in riboswitches arise from a combination of formation of the ligand-bound complex, promoting an open form of the free RNA, and participating in the secondary structural switch with the expression platform.

Gonzalez M., Weiler S., Ferretti J. A., and Ginsburg A. (2001) The vnd/NK-2 homeodomain: thermodynamics of reversible unfolding and DNA binding for wild-type and with residue replacements

H52R and H52R/T56W in helix III. *Biochemistry* **40**, 4923-4931.

Abstract: The conformational stabilities of the vnd (ventral nervous system defective)/NK-2 homeodomain [HD(wt); residues 1-80 that encompass the 60-residue homeodomain] and those harboring mutations in helix III of the DNA recognition site [HD(H52R) and HD(H52R/T56W)] have been investigated by differential scanning calorimetry (DSC) and ellipticity changes at 222 nm. Thermal unfolding reactions at pH 7.4 are reversible and repeatable in the presence of 50-500 mM NaCl with $\Delta C_p = 0.52 \pm 0.04 \text{ kcal K}^{-1} \text{ mol}^{-1}$. A substantial stabilization of HD(wt) is produced by 50 mM phosphate or by the addition of 100-500 mM NaCl to 50 mM Hepes, pH 7.4, buffer (from $T_m = 35.5$ degrees C to $T_m 43-51$ degrees C; $\Delta H(\text{vH})$ congruent with $47 \pm 5 \text{ kcal mol}^{-1}$). The order of stability is HD(H52R/T56W) > HD(H52R) > HD(wt), irrespective of the anions present. Progress curves for ellipticity changes at 222 nm as a function of increasing temperature are fitted well by a two-state unfolding model, and the cooperativity of secondary structure changes is greater for mutant homeodomains than for HD(wt) and also is increased by adding 100 mM NaCl to Hepes buffer. A 33% quench of the intrinsic tryptophanyl residue fluorescence of HD(wt) by phosphate binding ($K(D)^1 = 2.6 \pm 0.3 \text{ mM phosphate}$) is reversed approximately 60% by DNA binding. Thermodynamic parameters for vnd/NK-2 homeodomain proteins binding sequence-specific 18 bp DNA have been determined by isothermal titration calorimetry (10-30 degrees C). Values of ΔC_p are +0.25, -0.17, and $-0.10 \pm 0.04 \text{ kcal K}^{-1} \text{ mol}^{-1}$ for HD(wt), HD(H52R), and HD(H52R/T56W) binding duplex DNA, respectively. Interactions of homeodomains with DNA are enthalpically controlled at 298 K and pH 7.4 with corresponding ΔH values of -6.6 ± 0.5 , -10.8 ± 0.1 , and $-9.0 \pm 0.6 \text{ kcal mol}^{-1}$ and ΔG^1 values of -11.0 ± 0.1 , -11.0 ± 0.1 , and $-11.3 \pm 0.3 \text{ kcal mol}^{-1}$ with a binding stoichiometry of 1.0 ± 0.1 . Thermodynamic parameters for DNA binding are not predicted from homeodomain structural changes that occur upon complexing to DNA and must reflect also solvent and possibly DNA rearrangements.

Gorfe A. A. and Jelesarov I. (2003) Energetics of sequence-specific protein-DNA association: computational analysis of integrase Tn916 binding to its target DNA. *Biochemistry* **42**, 11568-11576.

Abstract: The N-terminal domain of the bacterial integrase Tn916 specifically recognizes the 11 bp DNA target site by positioning the face of a three-stranded beta-sheet into the major groove. Binding is linked to structural adaptation. We have characterized INT-DBD binding to DNA in detail by calorimetry [Milev, S., Gorfe, A., Karshikoff, A., Clubb, R. T., Bosshard, H. R., and Jelesarov, I. (2003) *Biochemistry* **42**, 3481-3491]. Our thermodynamic analysis has indicated that the major driving force of association is the hydrophobic effect while polar interactions contribute less. To gain more comprehensive information about the binding process, we performed a computational analysis of the binding free energy and report here the results. A hybrid molecular mechanics/continuum approach was followed. The total binding free energy is predicted with reasonable accuracy. The calculations confirm that nonpolar effects stabilize the protein-DNA complex while electrostatics opposes binding. Structural changes optimizing surface complementarity are costly in terms of energy. The energetic consequences from the replacement of nine DNA-contacting residues by alanine were investigated. The calculations correctly predict the binding affinity decrease of eight mutations and the destabilizing effect of one wild-type residue. Bulky side chains stabilize the wild-type complex through packing interactions and favorable nonpolar dehydration, but the net nonpolar energy changes do not correlate with the relative affinity loss upon mutation. Discrete protein-DNA electrostatic interactions may be net stabilizing or net destabilizing depending on the local environment. In contrast to nonpolar energy changes, the magnitude of the electrostatic free energy ranks the mutations according to the experimentally measured $\Delta\Delta G$. Free energy decomposition analysis from a structural perspective leads to detailed information about the thermodynamic strategy used by INT-DBD for sequence-specific DNA binding.

Gu S. Q., Jockel J., Beinker P., Warnecke J., Semenov Y. P., Rodnina M. V., and Wintermeyer W. (2005) Conformation of 4.5S RNA in the signal recognition particle and on the 30S ribosomal subunit. *RNA* **11**, 1374-1384.

Abstract: The signal recognition particle (SRP) from *Escherichia coli* consists of 4.5S RNA and protein Ffh. It is essential for targeting ribosomes that are translating integral membrane proteins to the translocation pore in the plasma membrane. Independently of Ffh, 4.5S RNA also interacts with elongation factor G (EF-G) and the 30S ribosomal subunit. Here we use a cross-linking approach to probe the conformation of 4.5S RNA in SRP and in the complex with the 30S ribosomal subunit and to map the binding site. The UV-activatable cross-linker p-azidophenacyl bromide (AzP) was attached to positions 1, 21, and 54 of wild-type or modified 4.5S RNA. In SRP, cross-links to Ffh were formed from AzP in all

three positions in 4.5S RNA, indicating a strongly bent conformation in which the 5' end (position 1) and the tetraloop region (including position 54) of the molecule are close to one another and to Ffh. In ribosomal complexes of 4.5S RNA, AzP in both positions 1 and 54 formed cross-links to the 30S ribosomal subunit, independently of the presence of Ffh. The major cross-linking target on the ribosome was protein S7; minor cross-links were formed to S2, S18, and S21. There were no cross-links from 4.5S RNA to the 50S subunit, where the primary binding site of SRP is located close to the peptide exit. The functional role of 4.5S RNA binding to the 30S subunit is unclear, as the RNA had no effect on translation or tRNA translocation on the ribosome.

Guazzaroni M. E., Krell T., Felipe A., Ruiz R., Meng C., Zhang X., Gallegos M. T., and Ramos J. L. (2005) The multidrug efflux regulator TtgV recognizes a wide range of structurally different effectors in solution and complexed with target DNA: evidence from isothermal titration calorimetry. *J Biol Chem* **280**, 20887-20893.

Abstract: TtgV modulates the expression of the *ttgGHI* operon, which encodes an efflux pump that extrudes a wide variety of chemicals including mono- and binuclear aromatic hydrocarbons, aliphatic alcohols, and antibiotics of dissimilar chemical structure. Using a *'lacZ* fusion to the *ttgG* promoter, we show that the most efficient *in vivo* inducers were 1-naphthol, 2,3-dihydroxynaphthalene, 4-nitrotoluene, benzonitrile, and indole. The thermodynamic parameters for the binding of different effector molecules to purified TtgV were determined by isothermal titration calorimetry. For the majority of effectors, the interaction was enthalpy-driven and counterbalanced by unfavorable entropy changes. The TtgV-effector dissociation constants were found to vary between 2 and 890 μM . There was a relationship between TtgV affinity for the different effectors and their potential to induce gene expression *in vivo*, indicating that the effector binding constant is a major determinant for efficient efflux pump gene expression. Equilibrium dialysis and isothermal titration calorimetry studies indicated that a TtgV dimer binds one effector molecule. No evidence for the simultaneous binding of multiple effectors to TtgV was obtained. The binding of TtgV to a 63-bp DNA fragment containing its cognate operator was tight and entropy-driven ($K(D) = 2.4 \pm 0.35 \text{ nM}$, $\Delta H = 5.5 \pm 0.04 \text{ kcal/mol}$). The TtgV-DNA complex was shown to bind 1-naphthol with an affinity comparable with the free soluble TtgV protein, $K(D) = 4.8 \pm 0.19$ and $3.0 \pm 0.15 \mu\text{M}$, respectively. The biological relevance of this finding is discussed.

Guazzaroni M. E., Krell T., Gutierrez del A. P., Velez M., Jimenez M., Rivas G. and Ramos J. L. (2007) The transcriptional repressor TtgV recognizes a complex operator as a tetramer and induces convex DNA bending. *J Mol Biol* **369**, 927-939.

Abstract: The TtgV repressor belongs to the large but infrequently investigated IclR family of transcriptional regulators. Although members of this family usually exhibit high effector specificity, TtgV possesses multidrug binding properties. The TtgV protein regulates the expression of the *ttgGHI* operon encoding the main solvent extrusion pump of the extremophile *Pseudomonas putida* DOT-T1E strain. Here we used a multidisciplinary approach to study the functional oligomeric state of TtgV during repression and derepression events, as well as the molecular basis of TtgV-DNA operator interactions. Analytical ultracentrifugation studies (AUC) show that TtgV is a tetramer in solution and that this oligomeric state does not change in the presence of effectors. We also show that the binding of effectors leads to the dissociation of TtgV as a tetramer from the DNA-TtgV complex. Previous dimethyl sulfate and DNase I footprints revealed that TtgV protected a 42 bp region. Based on AUC, electrophoretic mobility shift assays and isothermal titration calorimetry analyses we show that TtgV recognition specificity is restricted within this operator to a 34-nucleotide stretch and that TtgV may interact with intercalated inverted repeats that share no significant DNA sequence similarities within this short 34-nucleotide segment. Binding stoichiometry is one TtgV tetramer per operator, and affinity for its target DNA is around 200 nM. Circular dichroism analysis reveals that TtgV binding causes DNA distortion and atomic force microscopy imaging of TtgV-DNA operator complexes shows that TtgV induces a 57 degrees convex bend in its operator DNA. We propose that the mechanism of TtgV repression is based on the steric occlusion of the RNA polymerase binding site reinforced by DNA-bending of the *ttgV-ttgG* promoter region.

Guazzaroni M. E., Gallegos M. T., Ramos J. L. and Krell T. (2007) Different modes of binding of mono- and biaromatic effectors to the transcriptional regulator TtgV: role in differential derepression from its cognate operator. *J Biol Chem* **282**, 16308-16316.

Abstract: Members of the IclR family of regulators exhibit a highly conserved effector recognition domain and interact with a limited number of effectors. In contrast with most IclR family members, TtgV, the transcriptional repressor of the TtgGHI efflux pump, exhibits multidrug recognition properties. A three-dimensional model of the effector domain of TtgV was generated based on the available three-dimensional structure of several IclR members, and a series of point mutants was created. Using isothermal titration calorimetry, we determined the binding parameters of the most efficient effectors for TtgV and its mutant variants. All mutants bound biaromatic compounds with higher affinity than the wild-type protein, whereas monoaromatic compounds were bound with lower affinity. This tendency was particularly pronounced for mutants F134A and H200A. TtgVF134A bound 4-nitrotoluene with an affinity 13-fold lower than that of TtgV (17.4±0.6 µM). This mutant bound 1-naphthol with an affinity of 5.7 µM, which is seven times as great as that of TtgV (40 µM). The TtgVV223A mutant bound to DNA with the same affinity as the wild-type TtgV protein, but it remained bound to the target operator in the presence of effectors, suggesting that Val-223 could be part of an intra-TtgV signal recognition pathway. Thermodynamic analyses of the binding of effectors to TtgV and to its mutants in complex with their target DNA revealed that the binding of biaromatic compounds resulted in a more efficient release of the repressor protein than the binding of monoaromatics. The physiological significance of these findings is discussed.

Hagan N. and Fabris D. (2003) Direct mass spectrometric determination of the stoichiometry and binding affinity of the complexes between nucleocapsid protein and RNA stem-loop hairpins of the HIV-1 Psi-recognition element. *Biochemistry* **42**, 10736-10745.

Abstract: The formation of noncovalent complexes between the HIV-1 nucleocapsid protein p7 (NC) and RNA hairpins SL2-SL4 of the Psi-recognition element was investigated by direct infusion electrospray ionization-Fourier transform mass spectrometry (ESI-FTMS). The high resolution afforded by this method provided the unambiguous characterization of the stoichiometry and composition of complexes formed by multiple equilibria in solution. For each hairpin, the formation of a 1:1 complex was found to be the primary binding mode in solutions of intermediate salt content (150 mM ammonium acetate). Binding of multiple units of NC was observed with lower affinity and a maximum stoichiometry matching the limit calculated from the number of nucleotides in the construct and the size of the footprint of NC onto single-stranded nucleic acids, thus implying the defolding of the hairpin three-dimensional (3D) structure. Dissociation constants of 62 ± 22 nM, 178 ± 64 nM, and 1.3 ± 0.5 µM were determined for SL2, SL3-2, and SL4, respectively, which are similar to values obtained by spectroscopic and calorimetric methods with the additional confidence offered by a direct, rather than inferred, knowledge of the binding stoichiometry. Competitive binding experiments carried out in solutions of intermediate ionic strength, which has the effect of weakening the electrostatic interactions in solution, provided a direct way of evaluating the stabilizing contributions of H-bonding and hydrophobic interactions that are more sensitive to the sequence and structural context of the different hairpins. The relative scale of binding affinity obtained in this environment reflects the combination of contributions provided by the different structures of both the tetraloop and the double-stranded stem. The importance of the stem 3D structure in modulating the binding activity was tested by a competitive binding experiment that included the SL3-2 RNA construct, a DNA analogue of SL3 (SL3(DNA)), and a DNA analogue in which all four loop bases were replaced with abasic nucleotides (SL3(abasic)). NC was found to bind the A-type double-stranded stem of SL3-2 RNA at least 30 times more tightly than the B-type helical structure of SL3(DNA). Eliminating the stabilization provided by the interactions with the tetraloop bases made the binding of SL3(abasic) approximately 50 times weaker than that of SL3(DNA).

Haq I., O'Brien R., Lagunavicius A., Siksnys V., and Ladbury J. E. (2001) Specific DNA recognition by the type II restriction endonuclease MunI: the effect of pH. *Biochemistry* **40**, 14960-14967.

Abstract: To investigate the effect of pH on sequence-specific binding, a thermodynamic characterization of the interaction of the protein MunI with a specific, and a nonspecific, oligonucleotide was performed. MunI is a type II restriction endonuclease which is able to bind specifically, but loses its enzymatic activity in the absence of magnesium ions. Comparison of the specific and nonspecific interactions at 10 and 25 degrees C shows that the latter is accompanied by a small change in enthalpy, and a negligible change in constant pressure heat capacity. On going through the pH range 5.75-9.0 at 25 degrees C, the affinity of specific complex formation is reduced by 20-fold. The interaction is accompanied by the protonation of groups assumed to be on the protein. Based on the simplest model that will fit the data, two distinct protonation events are observed. At low pH, two groups per protein molecule undergo protonation with a

pK(a) of 6.0 and 6.9 in the free and bound forms, respectively. At high pH, a further independent protonation occurs involving two groups with pK(a) values of 8.9 and approximately 10.7 in the free and bound forms, respectively. The change in heat capacity ranges from -2.7 to -1.7 kJ mol⁻¹ K⁻¹ in going from pH 6.5 to 8.5. This range of variation of change in heat capacity can be accounted for by the effects of protonation of the interacting molecules. The change in heat capacity, calculated from surface area burial using a previously established relationship (1.15 kJ mol⁻¹ K⁻¹), does not correlate well with the experimentally determined values.

Hargreaves V. V., Makeyeva E. N., Dragan A. I., and Privalov P. L. (2005) Stability and DNA binding ability of the DNA binding domains of interferon regulatory factors 1 and 3. *Biochemistry* **44**, 14202-14209.

Abstract: The thermodynamic properties and DNA binding ability of the N-terminal DNA binding domains of interferon regulatory factors IRF-1 (DBD1) and IRF-3 (DBD3) were studied using microcalorimetric and optical methods. DBD3 is significantly more stable than DBD1: at 20 degrees C the Gibbs energy of unfolding of DBD3 is -28.6 kJ/mol, which is 2 times larger than that of DBD1, -14.9 kJ/mol. Fluorescence anisotropy titration experiments showed that at this temperature the association constants with the PRDI binding site are $1.1 \times 10(6) \text{ M}(-)(1)$ for DBD1 and $3.6 \times 10(6) \text{ M}(-)(1)$ for DBD3, corresponding to Gibbs energies of association of -34 and -37 kJ/mol, respectively. However, the larger binding energy of DBD3 is due to its larger electrostatic component, while its nonelectrostatic component is smaller than that of DBD1. Therefore, DBD1 appears to have more sequence specificity than DBD3. Binding of DBD1 to target DNA is characterized by a substantially larger negative enthalpy than binding of DBD3, implying that the more flexible structure of DBD1 forms tighter contacts with DNA than the more rigid structure of DBD3. Thus, the strength of the DBDs' specific association with DNA is inversely related to the stability of the free DBDs.

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.

Herrera M. C. and Ramos J. L. (2007) Catabolism of phenylalanine by *Pseudomonas putida*: the NtrC-family PhhR regulator binds to two sites upstream from the phhA gene and stimulates transcription with sigma70. *J Mol Biol* **366**, 1374-1386.

Abstract: *Pseudomonas putida* uses L-phenylalanine as the sole nitrogen source for growth by converting L-phenylalanine to L-tyrosine, which acts as a donor of the amino group. This metabolic step requires the products of the phhA and phhB genes, which form an operon. Expression of the phhA promoter is mediated by the phhR gene product in the presence of L-phenylalanine or L-tyrosine. The PhhR protein belongs to the NtrC family of enhancers. In contrast with most members of this family of regulators, transcription from the promoter of the phhAB operon (P(phhA)) is mediated by RNA polymerase with sigma(70) rather than with sigma(54). The PhhR regulator binds two similar but non-identical upstream PhhR motifs (5'-TGTAATAATTATCGTTACG-3' and 5'-ACAAAACTGTGTTTCCG-3') that are located 39 and 97

nucleotides upstream of the proposed -35 hexamer for RNA polymerase, respectively. These motifs are called PhhR proximal and PhhR distal binding motifs because of their position with respect to the RNA polymerase binding site. Affinity of PhhR for its target sequences was determined by isothermal titration calorimetry and was found to be around 30 nM for the proximal site and 2 microM for the distal site, and the binding stoichiometry is of a dimer per binding site. Both target sequences are sine qua non requirements for transcription, since inactivation of either of them resulted in no transcription from the phhA promoter. An IHF binding site overlaps the proximal PhhR proximal motif, which is recognized by IHF with a $K(D)$ of around 1.2 microM. IHF may consequently compete with PhhR for binding and indeed inhibits PhhR-dependent phhAB operon expression.

Holbrook J. A., Tsodikov O. V., Saecker R. M., and Record M. T., Jr. (2001) Specific and non-specific interactions of integration host factor with DNA: thermodynamic evidence for disruption of multiple IHF surface salt-bridges coupled to DNA binding. *J Mol Biol* **310**, 379-401.

Abstract: Site-specific DNA binding of architectural protein integration host factor (IHF) is involved in formation of functional multiprotein-DNA assemblies in *Escherichia coli*, while non-specific binding of IHF and other histone-like proteins serves to structure the nucleoid. Here, we report an isothermal titration calorimetry study of the thermodynamics of binding IHF to a 34 bp fragment composed entirely of the specific H' site from lambda-phage DNA. At low to moderate $[K(+)]$ (60-100 mM), strong competition is observed between specific and non-specific binding as a result of a low specificity ratio (approximately 10(2)) and a very small non-specific site size. In this $[K(+)]$ range, both specific and non-specific binding are enthalpy-driven, with large negative enthalpy, entropy and heat capacity changes and binding constants that are insensitive to $[K(+)]$. Above 100 mM $K(+)$, only specific binding is observed, and both the binding constant and the magnitudes of enthalpy, entropy and heat capacity changes all decrease strongly with increasing $[K(+)]$. When interpreted in the context of the structure of the specific complex, the thermodynamics provide compelling evidence for a previously unrecognized design principle by which proteins that form extensive binding interfaces with nucleic acids control binding constants, binding site sizes and effects of temperature and ion concentrations on stability and specificity. We propose that up to 22 of the 23 IHF cationic side-chains that are located within 6 Å of DNA phosphate oxygen atoms in the complex, are masked in the absence of DNA by pairing with anionic carboxylate groups in intramolecular salt-bridges (dehydrated ion-pairs). These salt-bridges increase in stability with increasing temperature and decreasing $[K(+)]$. To explain the unusual thermodynamics of IHF-DNA interactions, we propose that both specific and non-specific binding at low $[K(+)]$ require disruption of salt-bridges (as many as 18 for specific binding) whereupon many of the unmasked charged groups hydrate and the cationic groups interact with DNA. From structural or thermodynamic parallels with IHF, we propose that large-scale coupling of disruption of protein salt-bridges to DNA binding is significant for other large-interface DNA wrapping proteins including the nucleosome, lac repressor core tetramer, RNA polymerase core protein, HU and SSB.

Houbaviy H. B. and Burley S. K. (2001) Thermodynamic analysis of the interaction between YY1 and the AAV P5 promoter initiator element. *Chem Biol* **8**, 179-187.

Abstract: BACKGROUND: We previously determined the co-crystal structure of the zinc finger region of transcription factor YY1 (YY1Δ) bound to the initiator element (Inr) of the adenoassociated virus (AAV) P5 gene promoter [Houbaviy, H.B. et al. (1996) Proc. Natl. Acad. Sci. USA 93, 13577-13582]. Our structure explained both binding specificity and the ability of YY1 to support specific, unidirectional transcription initiation. RESULTS: To further understand Inr recognition by YY1, we analyzed the YY1Δ-Inr interaction by isothermal titration calorimetry (ITC) and used limited proteolysis, DNase I footprinting and missing nucleoside experiments to show that YY1Δ and full-length YY1 (YY1WT) have indistinguishable DNA binding properties. CONCLUSIONS: YY1 binding occurs at an equilibrium dissociation constant (K_d) of about 1 μM, and exhibits a large negative heat capacity change (ΔC_p). We analyzed the thermodynamic behavior of YY1Δ in terms of buried solvent-accessible surface area resulting from interaction of two rigid bodies, which could not explain our measured value of ΔC_p . We must, therefore, postulate conformational changes in YY1 and/or the Inr or question the validity of current $\Delta C(p)$ analysis methods for protein-DNA interactions.

Huang D., Korolev N., Eom K. D., Tam J. P. and Nordenskiöld L. (2008) Design and biophysical characterization of novel polycationic epsilon-peptides for DNA compaction and delivery. *Biomacromolecules*. **9**, 321-330.

Abstract: Design and solid-phase synthesis of novel and chemically defined linear and branched -oligo(L-lysines) (denoted -K n, where n is the number of lysine residues) and their alpha-substituted homologues (epsilon-(R)K10, epsilon-(Y)K10, epsilon-(L)K10, epsilon-(YR)K10, and epsilon-(LYR)K10) for DNA compaction and delivery are reported. The ability to condense viral (T2 and T4) and plasmid DNA as well as the size of -peptide DNA complexes under different conditions was investigated with static and dynamic light scattering, isothermal titration calorimetry, and fluorescence microscopy. Nanoparticle diameters varied from 100 to 150 and 375 to 550 nm for plasmid and T4 DNA peptide complexes, respectively. Smaller sizes were observed for oligo(L-lysines) compared to alpha-poly(L-lysine). The linear -oligo-lysines are less toxic and epsilon-(LYR)K10 showed higher transfection efficiency in HeLa cells than corresponding controls. The results also demonstrate that with a branched design having pendent groups of short alpha-oligopeptides, improved transfection can be achieved. This study supports the hypothesis that available alpha-oligolysine derived systems would potentially have more favorable delivery properties if they are based instead on epsilon-oligo(L-lysines). The flexible design and unambiguous synthesis that enables variation of pendent groups holds promise for optimization of such -peptides to achieve improved DNA compaction and delivery

Hyre D. E. and Spicer L. D. (1995) Thermodynamic evaluation of binding interactions in the methionine repressor system of Escherichia coli using isothermal titration calorimetry. *Biochemistry* **34**, 3212-3221.

Abstract: The binding interactions of the methionine repressor protein, MetJ, from Escherichia coli with its cognate, metbox DNA sequence and corepressor S-adenosylmethionine were examined using calorimetric methods. A detailed thermodynamic characterization of this system which exhibits the recently reported (beta alpha alpha)₂ binding motif provides values for ΔG , ΔH , and ΔS for each step in the repressor binding cycle. These studies show that, in the presence of corepressor, MetJ binds to a single metbox operator site with $\Delta G = -7.7 \text{ kcal.mol}^{-1}$, whereas in the absence of corepressor, the free energy of interaction with a single site is $-5.8 \text{ kcal.mol}^{-1}$. Cooperative interactions between two repressor molecules bound to two adjacent sites contribute an additional free energy of $-1.3 \text{ kcal.mol}^{-1}$ to binding at the second site. Binding is enthalpically unfavorable in the absence of the corepressor with $\Delta H = +2.6 \text{ kcal.mol}^{-1}$ but becomes exothermic with $\Delta H = -4.6 \text{ kcal.mol}^{-1}$ when corepressor is present. The heat capacity for the system decreases significantly by $\Delta C_p = -290 \text{ cal.mol}^{-1}.\text{K}^{-1}$ on a per site basis when the protein binds to DNA, and interactions between repressor molecules bound to adjacent sites contribute a $\Delta C_p = -800 \text{ cal.mol}^{-1}.\text{K}^{-1}$, indicating that solvent exclusion plays a significant role in binding in this system. The corepressor binds to the unbound repressor protein with a free energy of $\Delta G = -6.0 \text{ kcal.mol}^{-1}$ and to the MetJ-operator complex with $\Delta G = -6.95 \text{ kcal.mol}^{-1}$. Repressor binding to random-sequence DNA was estimated to occur with a free energy of $-5.7 \text{ kcal.mol}^{-1}$ in the presence of corepressor. These data clearly indicate that MetJ repressor dimer binds specifically to the central region of its 8 bp cognate metbox operator but recognizes partial operator sequences as short as 6 bp. Cooperativity in binding of adjacent MetJ dimers to a double metbox sequence is demonstrated to be important in determining the energetics of the interaction. Finally, the corepressor S-adenosylmethionine enhances the affinity of MetJ for its recognition site DNA by a factor of 25 and contributes significantly to the net exothermicity of repressor binding.

Inomata K., Ohki I., Tochio H., Fujiwara K., Hiroaki H. and Shirakawa M. (2008) Kinetic and thermodynamic evidence for flipping of a methyl-CpG binding domain on methylated DNA. *Biochemistry* **47**, 3266-3271.

Abstract: The methyl-CpG binding domain (MBD) is a conserved domain in transcriptional factors that binds to methylated CpG dinucleotide DNA sequences in vertebrates. The complex is comprised of an asymmetric MBD monomer and a symmetric DNA duplex. Therefore, in the complex, each strand of the duplex DNA is in contact with the protein at a distinct surface and thus exhibits a different chemical shift in NMR spectra. Two-dimensional chemical exchange spectroscopy revealed the presence of a stochastic exchange of the two strands of the duplex DNA in the complex at a rate of 4 s^{-1} at 25 degrees C, which indicates the existence of a motion of the MBD such that the orientation of the MBD becomes reversed with respect to the DNA duplex. Kinetic and thermodynamic analyses using surface plasmon resonance, quartz crystal microbalance, and isothermal titration calorimetry suggest that the reversal of MBD with

respect to the DNA duplex takes place without its complete dissociation from DNA, indicating the presence of an intermediate protein-DNA binding state that allows the protein to undergo a flip motion upon DNA

Janin J. (1995) Elusive affinities. *Proteins* **21**, 30-39.

Abstract: The affinity of two molecules for each other and its temperature dependence are determined by the change in enthalpy, free enthalpy, entropy, and heat capacity upon dissociation. As we know the forces that stabilize protein-protein or protein-DNA association and the three-dimensional structures of the complex, we can in principle derive values for each one of these parameters. The calculation is done first in gas phase by molecular mechanics, then in solution with the help of hydration parameters calibrated on small molecules. However, estimates of enthalpy and entropy changes in gas phase have excessively large error bars even under the approximation that the components of the complex associate as rigid bodies. No reliable result can be expected at the end. The fit to experimental values derived from binding and calorimetric measurements is poor, except for the dissociation heat capacity. This parameter can be attributed mostly to the hydration step and it correlates with the size of the interface. Many protein-protein complexes have interface areas in the range 1200-2000 Å² and only small conformation changes, so the rigid body approximation applies. It is less generally valid in protein-DNA complexes, which have interfaces covering 2200-3100 Å², large dissociation heat capacities, and affect both the conformation and the dynamics of their components.

Jason W. C., Lucius A. L., and Lohman T. M. (2005) Energetics of DNA end binding by E.coli RecBC and RecBCD helicases indicate loop formation in the 3'-single-stranded DNA tail. *J Mol Biol* **352**, 765-782.

Abstract: We examined the equilibrium binding of Escherichia coli RecBC and RecBCD helicases to duplex DNA ends possessing pre-existing single-stranded (ss) DNA ((dT)(n)) tails varying in length (n=0 to 20 nucleotides) in order to determine the contributions of both the 3' and 5' single strands to the energetics of complex formation. Protein binding was monitored by the fluorescence enhancement of a reference DNA labeled at its end with a Cy3 fluorophore. Binding to unlabeled DNA was examined by competition titrations with the Cy3-labeled reference DNA. The affinities of both RecBC and RecBCD increase as the 3'-(dT)(n) tail length increases from zero to six nucleotides, but then decrease dramatically as the 3'-(dT)(n) tail length increases from six to 20 nucleotides. Isothermal titration calorimetry experiments with RecBC show that the binding enthalpy is negative and increases in magnitude with increasing 3'-(dT)(n) tail length up to n=6 nucleotides, but remains constant for n > or =6. Hence, the decrease in binding affinity for 3'-(dT)(n) tail lengths with n > or =6 is due to an unfavorable entropic contribution. RecBC binds optimally to duplex DNA with (dT)6 tails on both the 3' and 5'-ends while RecBCD prefers duplex DNA with 3'-(dT)6 and 5'-(dT)10 tails. These data suggest that both RecBC and RecBCD helicases can destabilize or "melt out" six base-pairs upon binding to a blunt DNA duplex end in the absence of ATP. These results also provide the first evidence that a loop in the 3'-ssDNA tail can form upon binding of RecBC or RecBCD with DNA duplexes containing a pre-formed 3'-ssDNA tail with n > or =6 nucleotides. Such loops may be representative of those hypothesized to form upon interaction of a Chi site contained within the unwound 3' ss-DNA tail with the RecC subunit during DNA unwinding.

Jeng W. Y., Ko T. P., Liu C. I., Guo R. T., Liu C. L., Shr H. L. and Wang A. H. (2008) Crystal structure of IcaR, a repressor of the TetR family implicated in biofilm formation in Staphylococcus epidermidis. *Nucleic Acids Res* **36**, 1567-1577.

Abstract: Expression of the gene cluster icaADBC is necessary for biofilm production in Staphylococcus epidermidis. The ica operon is negatively controlled by the repressor IcaR. Here, the crystal structure of IcaR was determined and the refined structure revealed a homodimer comprising entirely alpha-helices, typical of the tetracycline repressor protein family for gene regulations. The N-terminal domain contains a conserved helix-turn-helix DNA-binding motif with some conformational variations, indicating flexibility in this region. The C-terminal domain shows a complementary surface charge distribution about the dyad axis, ideal for efficient and specific dimer formation. The results of the electrophoretic mobility shift assay and isothermal titration calorimetry suggested that a 28 bp core segment of the ica operator is implicated in the cooperative binding of two IcaR dimers on opposite sides of the duplex DNA. Computer modeling based on the known DNA-complex structure of QacR and site-specific mutagenesis experiments showed that direct protein-DNA interactions are mostly conserved, but with slight variations for recognizing the different sequences. By interfering with the binding of IcaR to DNA, aminoglycoside gentamicin and other

antibiotics may activate the *icaADBC* genes and elicit biofilm production in *S. epidermidis*, and likely *S. aureus*, as a defense mechanism

Jenkins J. L., Shen H., Green M. R. and Kielkopf C. L. (2008) Solution Conformation and Thermodynamic Characteristics of RNA Binding by the Splicing Factor U2AF65. *J Biol Chem* **283**, 33641-33649.

Abstract: The U2 auxiliary factor large subunit (U2AF(65)) is an essential pre-mRNA splicing factor for the initial stages of spliceosome assembly. Tandem RNA recognition motifs (RRM)s of U2AF(65) recognize polypyrimidine tract signals adjacent to 3' splice sites. Despite the central importance of U2AF(65) for splice site recognition, the relative arrangement of the U2AF(65) RRM)s and the energetic forces driving polypyrimidine tract recognition remain unknown. Here, the solution conformation of the U2AF(65) RNA binding domain determined using small angle x-ray scattering reveals a bilobal shape without apparent interdomain contacts. The proximity of the N and C termini within the inter-RRM configuration is sufficient to explain the action of U2AF(65) on spliceosome components located both 5' and 3' to its binding site. Isothermal titration calorimetry further demonstrates that an unusually large enthalpy-entropy compensation underlies U2AF(65) recognition of an optimal polyuridine tract. Qualitative similarities were observed between the pairwise distance distribution functions of the U2AF(65) RNA binding domain and those either previously observed for N-terminal RRM)s of Py tract-binding protein that lack interdomain contacts or calculated from the high resolution coordinates of a U2AF(65) deletion variant bound to RNA. To further test this model, the shapes and RNA interactions of the wild-type U2AF(65) RNA binding domain were compared with those of U2AF(65) variants containing either Py tract-binding protein linker sequences or a deletion within the inter-RRM linker. Results of these studies suggest inter-RRM conformational plasticity as a possible means for U2AF(65) to universally identify diverse pre-mRNA splice sites

Jin L., Yang J., and Carey J. (1993) Thermodynamics of ligand binding to trp repressor. *Biochemistry* **32**, 7302-7309.

Abstract: The thermodynamics of L-tryptophan and operator DNA binding to the tryptophan repressor of *Escherichia coli* were analyzed by titration microcalorimetry and van't Hoff analysis of footprinting titrations, respectively. At 25 degrees C in 10 mM sodium phosphate, pH 7.6, and 0.1 M NaCl, the binding of L-tryptophan to the repressor is characterized by values of ΔG degree = -6.04, ΔH degree = -14.7, and ΔS degree = -8.67 kcal/mol. The temperature dependence of ΔH degree yields ΔC_p degree = -0.46 +/- 0.08 kcal/(mol.K) per dimer. The binding is noncooperative at all temperatures studied. At 23 degrees C in 2.5 mM sodium phosphate, pH 7.6, and 25 mM NaCl, the binding of operator DNA to the repressor is characterized by values of ΔG degree = -13.3 kcal/mol, ΔH degree = -1.55 kcal/mol, ΔS degree = 11.8 kcal/mol, and ΔC_p degree = -0.54 +/- 0.10 kcal/(mol.K). Changes in water-accessible surface areas upon binding of L-tryptophan or DNA were calculated from X-ray crystal structures. The experimentally observed ΔC_p degree values were compared with ΔC_p degree values calculated according to several methods based on various proposed relationships between surface area changes and heat capacity changes. Regardless of which method is used, we find poor agreement between the calorimetric results for L-tryptophan binding and the surface areas calculated from X-ray data; the direction of the discrepancy is that the X-ray data underestimate the value of ΔC_p degree. (ABSTRACT TRUNCATED AT 250 WORDS).

Jung K. C., Rhee H. S., Park C. H., and Yang C. H. (2005) Determination of the dissociation constants for recombinant c-Myc, Max, and DNA complexes: the inhibitory effect of linoleic acid on the DNA-binding step. *Biochem Biophys Res Commun* **334**, 269-275.

Abstract: c-Myc, the protein product of protooncogene *c-myc*, functions in cell proliferation, differentiation, and neoplastic disease. In this study, recombinant c-Myc and Max proteins, encompassing DNA binding (basic region) and dimerization (helix-loop-helix/leucine zipper) domain of human origin, were expressed in bacteria as Myc87 and Max85. Myc87 was purified under denatured conditions and was renatured again. The dissociation constant for the protein dimers and for dimer/DNA complexes were not detectable by isothermal titration calorimetry because of the low degree of solubility of Myc87 and Max85. Therefore, we set up equations which were used to determine the dissociation constants from the proportion of protein-DNA complexes. The dimer dissociation constants in TBS were $5.90(+/-0.54) \times 10^{-7}$ M for Max85/Max85 homodimer, $6.85(+/-0.25) \times 10^{-3}$ M for Myc87/Myc87 homodimer, and $2.55(+/-0.29) \times 10^{-8}$ M for Myc87/Max85 heterodimer, and the DNA-binding dissociation constants in TBS were $1.33(+/-0.21) \times 10^{-9}$ M for Max85/Max85/DNA, $2.27(+/-0.08) \times 10^{-12}$ M for Myc87/Myc87/DNA, and $4.43(+/-$

0.37) $\times 10^{-10}$ M for Myc87/Max85/DNA. In addition, we revealed that linoleic acid which is known as an inhibitor for the formation of Max/Max/DNA complex reduced the affinity of Max homodimer for DNA. This result indicates that linoleic acid may bind to the DNA-binding region of Max homodimer.

Ke A., Mathias J. R., Vershon A. K., and Wolberger C. (2002) Structural and thermodynamic characterization of the DNA binding properties of a triple alanine mutant of MATalpha2. *Structure (Camb)* **10**, 961-971.

Abstract: Triply mutated MATalpha2 protein, alpha2-3A, in which all three major groove-contacting residues are mutated to alanine, is defective in binding DNA alone or in complex with Mcm1 yet binds with MATa1 with near wild-type affinity and specificity. To gain insight into this unexpected behavior, we determined the crystal structure of the a1/alpha2-3A/DNA complex. The structure shows that the triple mutation causes a collapse of the alpha2-3A/DNA interface that results in a reorganized set of alpha2-3A/DNA contacts, thereby enabling the mutant protein to recognize the wild-type DNA sequence. Isothermal titration calorimetry measurements reveal that a much more favorable entropic component stabilizes the a1/alpha2-3A/DNA complex than the alpha2-3A/DNA complex. The combined structural and thermodynamic studies provide an explanation of how partner proteins influence the sequence specificity of a DNA binding protein.

Keller M., Tagawa T., Preuss M., and Miller A. D. (2002) Biophysical characterization of the DNA binding and condensing properties of adenoviral core peptide mu. *Biochemistry* **41**, 652-659.

Abstract: Cationic peptides containing Lys and Arg residues interact with DNA via charge-charge interactions and are known to play an important role in DNA charge neutralization and condensation processes. In this paper, we describe investigations of the interaction of the cationic adenovirus core complex peptide mu with a dodecameric ODN (12 bp) and pDNA (7528 bp) using a combination of fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, and photon correlation spectroscopy. Comparisons are made with protamine, a cationic peptide well-known for DNA charge neutralization and condensation. Equilibrium dissociation constants are derived independently by both CD and ITC methods for the interaction between protamine or mu with pDNA ($K_d = 0.6\text{-}1 \mu\text{M}$). Thermodynamic data are also obtained by ITC, indicating strong charge-charge interactions. The interaction of protamine with pDNA takes place with decreasing entropy ($-28.7 \text{ cal mol}^{-1} \text{ K}^{-1}$); unusually, the interaction of mu with pDNA takes place with increasing entropy ($\Delta S \text{ degrees (bind)} = 11.3 \text{ cal mol}^{-1} \text{ K}^{-1}$). Although protamine and mu appear to destabilize pDNA double helix character to similar extents, according to CD thermal titration analyses, PCS studies show that interactions between mu and pDNA result in the formation of significantly more size-stable condensed particles than protamine. The enhanced flexibility and size stability of mu-DNA (MD) particles (80-110 nm) compared to protamine counterparts suggest that MD particles are ideal for use as a part of new nonviral gene delivery vectors.

Kielkopf C. L., Rodionova N. A., Green M. R., and Burley S. K. (2001) A novel peptide recognition mode revealed by the X-ray structure of a core U2AF35/U2AF65 heterodimer. *Cell* **106**, 595-605.

Abstract: U2 auxiliary factor (U2AF) is an essential splicing factor that recognizes the 3' splice site and recruits the U2 snRNP to the branch point. The X-ray structure of the human core U2AF heterodimer, consisting of the U2AF35 central domain and a proline-rich region of U2AF65, has been determined at 2.2 Å resolution. The structure reveals a novel protein-protein recognition strategy, in which an atypical RNA recognition motif (RRM) of U2AF35 and the U2AF65 polyproline segment interact via reciprocal "tongue-in-groove" tryptophan residues. Complementary biochemical experiments demonstrate that the core U2AF heterodimer binds RNA, and that the interacting tryptophan side chains are essential for U2AF dimerization. Atypical RRMs in other splicing factors may serve as protein-protein interaction motifs elsewhere during spliceosome assembly.

Kim I., Liu C. W., and Puglisi J. D. (2006) Specific recognition of HIV TAR RNA by the dsRNA binding domains (dsRBD1-dsRBD2) of PKR. *J Mol Biol* **358**, 430-442.

Abstract: PKR (double-stranded RNA-dependent protein kinase) is an important component of host defense to virus infection. Binding of dsRNA to two dsRBDs (double-stranded RNA binding domains) of PKR modulates its own kinase activation. How structural features of natural target RNAs, such as bulges and loops, have an effect on the binding to two dsRBDs of PKR still remains unclear. By using ITC and NMR, we show here that both the bulge and loop of TAR RNA are necessary for the high affinity binding

to dsRBD1-dsRBD2 of PKR with 1:1 stoichiometry. The binding site for the dsRBD1-dsRBD2 spans from upper bulge to lower stem of the TAR RNA, based on chemical shift mapping. The backbone resonances in the 40 kDa TAR.dsRBD1-dsRBD2 were assigned. NMR chemical shift perturbation data suggest that the beta1-beta2 loop of the dsRBD1 interacts with the TAR RNA, whereas that of the dsRBD2 is less involved in the TAR RNA recognition. In addition, the residues of the interdomain linker between the dsRBD1 and the dsRBD2 also show large chemical perturbations indicating that the linker is involved in the recognition of TAR RNA. The results presented here provide the biophysical and spectroscopic basis for high-resolution structural studies, and show how local RNA structural features modulate recognition by dsRBDs.

Kozlov A. G. and Lohman T. M. (1998) Calorimetric studies of E. coli SSB protein-single-stranded DNA interactions. Effects of monovalent salts on binding enthalpy. *J Mol Biol* **278**, 999-1014.

Abstract: Isothermal titration calorimetry (ITC) was used to examine the effects of monovalent salts (NaCl, NaBr, NaF and ChCl) on the binding enthalpy (ΔH_{obs}) for E. coli SSB tetramer binding to the single-stranded oligodeoxythymidylates, dT(pT)69 and dT(pT)34 over a wide range of salt concentrations from 10 mM to 2.0 M (25 degrees C, pH 8.1), and when possible, the binding free energy and entropy (ΔG degrees obs, ΔS degrees obs). At low monovalent salt concentrations (<0.1 M), the total ΔH_{obs} for saturating all sites on the SSB tetramer with ssDNA shows little dependence on salt concentration, but is extremely large and exothermic ($\Delta H_{obs} = -150(\pm 5)$ kcal/mol). This is much larger than any ΔH_{obs} previously reported for a protein-nucleic acid interaction. However, at salt concentrations above 0.1 M, ΔH_{obs} is quite sensitive to NaCl and NaBr concentration, becoming less negative with increasing salt concentration ($\Delta H_{obs} = -70(\pm 1)$ -kcal/mol in 2 M NaBr). These salt effects on ΔH_{obs} were mainly a function of anion type and concentration, with the largest effects observed in NaBr, and then NaCl, with little effect of [NaF]. These large effects of salt on ΔH_{obs} appear to be coupled to a net release of weakly bound anions (Br⁻ and Cl⁻) from the SSB protein upon DNA binding. However, at lower salt concentrations (≤ 0.1 M), specific cation effects on ΔH_{obs} also are observed. Under conditions where we can determine ΔG degrees obs, ΔS degrees obs, and ΔH_{obs} (25 degrees C, pH 8.1, 0.17 to 2 M NaBr), SSB binding to dT(pT)69 is enthalpically driven with a large unfavorable entropic contribution, both of which are dependent upon [NaBr]. These studies show that weak anion binding to a protein can result in large effects of salt concentration on ΔH_{obs} (as well as ΔG degrees obs and ΔS degrees obs) for a protein-ssDNA interaction. The possibility of such effects needs to be considered in any interpretation of the thermodynamics of this and other protein-nucleic acid interactions.

Koh J., Saecker R. M. and Record M. T., Jr. (2008) DNA Binding Mode Transitions of Escherichia coli HU(alpha-beta): Evidence for Formation of a Bent. *J Mol Biol* (epublication).

Abstract: Escherichia coli HU(alpha-beta), a major nucleoid-associated protein, organizes chromosomal DNA and facilitates numerous DNA transactions. Using isothermal titration calorimetry, fluorescence resonance energy transfer and a series of DNA lengths (8 bp, 15 bp, 34 bp, 38 bp and 160 bp) we established that HU(alpha-beta) interacts with duplex DNA using three different nonspecific binding modes. Both the HU to DNA molar ratio ([HU]/[DNA]) and DNA length dictate the dominant HU binding mode. On sufficiently long DNA (≥ 34 bp), at low [HU]/[DNA], HU populates a noncooperative 34 bp binding mode with a binding constant of $2.1 \pm 0.4 \times 10^6$ M⁻¹, and a binding enthalpy of $+7.7 \pm 0.6$ kcal/mol at 15 degrees C and 0.15 M Na⁺. With increasing [HU]/[DNA], HU bound in the noncooperative 34 bp mode progressively converts to two cooperative (omega approximately 20) modes with site sizes of 10 bp and 6 bp. These latter modes exhibit smaller binding constants ($1.1 \pm 0.2 \times 10^5$ M⁻¹ for the 10 bp mode, $3.5 \pm 1.4 \times 10^4$ M⁻¹ for the 6 bp mode) and binding enthalpies (4.2 ± 0.3 kcal/mol for the 10 bp mode, -1.6 ± 0.3 kcal/mol for the 6 bp mode). As DNA length increases to 34 bp or more at low [HU]/[DNA], the small modes are replaced by the 34 bp binding mode. Fluorescence resonance energy transfer data demonstrate that the 34 bp mode bends DNA by 143 ± 6 degrees whereas the 6 bp and 10 bp modes do not. The model proposed in this study provides a novel quantitative and comprehensive framework for reconciling previous structural and solution studies of HU, including single molecule (force extension measurement), fluorescence, and electrophoretic gel mobility-shift assays. In particular, it explains how HU condenses or extends DNA depending on the relative concentrations of HU and DNA

Kozlov A. G. and Lohman T. M. (1999) Adenine base unstacking dominates the observed enthalpy and heat capacity changes for the Escherichia coli SSB tetramer binding to single-stranded oligoadenylates. *Biochemistry* **38**, 7388-7397.

Abstract: Isothermal titration calorimetry (ITC) was used to test the hypothesis that the relatively small enthalpy change (ΔH_{obs}) and large negative heat capacity change ($\Delta C_{p,\text{obs}}$) observed for the binding of the Escherichia coli SSB protein to single-stranded (ss) oligodeoxyadenylates result from the temperature-dependent adenine base unstacking equilibrium that is thermodynamically coupled to binding. We have determined $\Delta H_{1,\text{obs}}$ for the binding of 1 mole of each of dT(pT)₃₄, dC(pC)₃₄, and dA(pA)₃₄ to the SSB tetramer (20 mM NaCl at pH 8.1). For dT(pT)₃₄ and dC(pC)₃₄, we found large, negative values for $\Delta H_{1,\text{obs}}$ of -75 ± 1 and -85 ± 2 kcal/mol at 25 degrees C, with $\Delta C_{p,\text{obs}}$ values of -540 ± 20 and -570 ± 30 cal mol⁻¹ K⁻¹ (7-50 degrees C), respectively. However, for SSB-dA(pA)₃₄ binding, $\Delta H_{1,\text{obs}}$ is considerably less negative (-14 ± 1 kcal/mol at 25 degrees C), even becoming positive at temperatures below 13 degrees C, and $\Delta C_{p,\text{obs}}$ is nearly twice as large in magnitude (-1180 ± 40 cal mol⁻¹ K⁻¹). These very different thermodynamic properties for SSB-dA(pA)₃₄ binding appear to result from the fact that the bases in dA(pA)₃₄ are more stacked at any temperature than are the bases in dC(pC)₃₄ or dT(pT)₃₄ and that the bases become unstacked within the SSB-ssDNA complexes. Therefore, the $\Delta C_{p,\text{obs}}$ for SSB-ssDNA binding has multiple contributions, a major one being the coupling to binding of a temperature-dependent conformational change in the ssDNA, although SSB binding to unstacked ssDNA still has an "intrinsic" negative $\Delta C_{p,0}$. In general, such temperature-dependent changes in the conformational "end states" of interacting macromolecules can contribute significantly to both $\Delta C_{p,\text{obs}}$ and ΔH_{obs} .

Kozlov A. G. and Lohman T. M. (2000) Large contributions of coupled protonation equilibria to the observed enthalpy and heat capacity changes for ssDNA binding to Escherichia coli SSB protein. *Proteins Suppl 4*, 8-22.

Abstract: Many macromolecular interactions, including protein-nucleic acid interactions, are accompanied by a substantial negative heat capacity change, the molecular origins of which have generated substantial interest. We have shown previously that temperature-dependent unstacking of the bases within oligo(dA) upon binding to the Escherichia coli SSB tetramer dominates the binding enthalpy, $\Delta H(\text{obs})$, and accounts for as much as a half of the observed heat capacity change, ΔC_p . However, there is still a substantial ΔC_p associated with SSB binding to ssDNA, such as oligo(dT), that does not undergo substantial base stacking. In an attempt to determine the origins of this heat capacity change, we have examined by isothermal titration calorimetry (ITC) the equilibrium binding of dT(pT)₃₄ to SSB over a broad pH range (pH 5.0-10.0) at 0.02 M, 0.2 M NaCl and 1 M NaCl (25 degrees C), and as a function of temperature at pH 8.1. A net protonation of the SSB protein occurs upon dT(pT)₃₄ binding over this entire pH range, with contributions from at least three sets of protonation sites ($pK(a_1) = 5.9-6.6$, $pK(a_2) = 8.2-8.4$, and $pK(a_3) = 10.2-10.3$) and these protonation equilibria contribute substantially to the observed ΔH and ΔC_p for the SSB-dT(pT)₃₄ interaction. The contribution of this coupled protonation (approximately -260 to -320 cal mol⁻¹ K⁻¹) accounts for as much as half of the total ΔC_p . The values of the "intrinsic" $\Delta C_{p,0}$ range from -210 ± 33 cal mol⁻¹ degrees K⁻¹ to -237 ± 36 cal mol⁻¹ K⁻¹, independent of [NaCl]. These results indicate that the coupling of a temperature-dependent protonation equilibria to a macromolecular interaction can result in a large negative ΔC_p , and this finding needs to be considered in interpretations of the molecular origins of heat capacity changes associated with ligand-macromolecular interactions, as well as protein folding.

Kozlov A. G. and Lohman T. M. (2006) Effects of monovalent anions on a temperature-dependent heat capacity change for Escherichia coli SSB tetramer binding to single-stranded DNA. *Biochemistry* **45**, 5190-5205.

Abstract: We have previously shown that the linkage of temperature-dependent protonation and DNA base unstacking equilibria contribute significantly to both the negative enthalpy change ($\Delta H(\text{obs})$) and the negative heat capacity change ($\Delta C_{p,\text{obs}}$) for Escherichia coli SSB homotetramer binding to single-stranded (ss) DNA. Using isothermal titration calorimetry we have now examined $\Delta H(\text{obs})$ over a much wider temperature range (5-60 degrees C) and as a function of monovalent salt concentration and type for SSB binding to (dT)₇₀ under solution conditions that favor the fully wrapped (SSB)₆₅ complex (monovalent salt concentration ≥ 0.20 M). Over this wider temperature range we observe a strongly temperature-dependent $\Delta C_{p,\text{obs}}$. The $\Delta H(\text{obs})$ decreases as temperature increases from 5 to 35 degrees C ($\Delta C_{p,\text{obs}} < 0$) but then increases at higher temperatures up to 60 degrees C ($\Delta C_{p,\text{obs}} > 0$). Both salt concentration and anion type have large effects on $\Delta H(\text{obs})$ and $\Delta C_{p,\text{obs}}$. These observations can be explained by a model in which SSB protein can undergo a temperature- and salt-dependent conformational transition (below 35 degrees C), the midpoint of which shifts to higher temperature (above 35 degrees C) for SSB bound to ssDNA. Anions bind weakly to free SSB, with the

preference $\text{Br}(-) > \text{Cl}(-) > \text{F}(-)$, and these anions are then released upon binding ssDNA, affecting both $\Delta H(\text{obs})$ and $\Delta C(\text{p,obs})$. We conclude that the experimentally measured values of $\Delta C(\text{p,obs})$ for SSB binding to ssDNA cannot be explained solely on the basis of changes in accessible surface area (ASA) upon complex formation but rather result from a series of temperature-dependent equilibria (ion binding, protonation, and protein conformational changes) that are coupled to the SSB-ssDNA binding equilibrium. This is also likely true for many other protein-nucleic acid interactions.

Kozlov A. G. and Lohman T. M. (2006) Effects of monovalent anions on a temperature-dependent heat capacity change for Escherichia coli SSB tetramer binding to single-stranded DNA. *Biochemistry* **45**, 5190-5205.

Abstract: We have previously shown that the linkage of temperature-dependent protonation and DNA base unstacking equilibria contribute significantly to both the negative enthalpy change ($\Delta H(\text{obs})$) and the negative heat capacity change ($\Delta C(\text{p,obs})$) for Escherichia coli SSB homotetramer binding to single-stranded (ss) DNA. Using isothermal titration calorimetry we have now examined $\Delta H(\text{obs})$ over a much wider temperature range (5-60 degrees C) and as a function of monovalent salt concentration and type for SSB binding to (dT)(70) under solution conditions that favor the fully wrapped (SSB)(65) complex (monovalent salt concentration ≥ 0.20 M). Over this wider temperature range we observe a strongly temperature-dependent $\Delta C(\text{p,obs})$. The $\Delta H(\text{obs})$ decreases as temperature increases from 5 to 35 degrees C ($\Delta C(\text{p,obs}) < 0$) but then increases at higher temperatures up to 60 degrees C ($\Delta C(\text{p,obs}) > 0$). Both salt concentration and anion type have large effects on $\Delta H(\text{obs})$ and $\Delta C(\text{p,obs})$. These observations can be explained by a model in which SSB protein can undergo a temperature- and salt-dependent conformational transition (below 35 degrees C), the midpoint of which shifts to higher temperature (above 35 degrees C) for SSB bound to ssDNA. Anions bind weakly to free SSB, with the preference $\text{Br}(-) > \text{Cl}(-) > \text{F}(-)$, and these anions are then released upon binding ssDNA, affecting both $\Delta H(\text{obs})$ and $\Delta C(\text{p,obs})$. We conclude that the experimentally measured values of $\Delta C(\text{p,obs})$ for SSB binding to ssDNA cannot be explained solely on the basis of changes in accessible surface area (ASA) upon complex formation but rather result from a series of temperature-dependent equilibria (ion binding, protonation, and protein conformational changes) that are coupled to the SSB-ssDNA binding equilibrium. This is also likely true for many other protein-nucleic acid interactions.

Krell T., Teran W., Mayorga O. L., Rivas G., Jimenez M., Daniels C., Molina-Henares A. J., Martinez-Bueno M., Gallegos M. T. and Ramos J. L. (2007) Optimization of the palindromic order of the TtgR operator enhances binding cooperativity. *J Mol Biol* **369**, 1188-1199.

Abstract: TtgR is the specific transcriptional repressor of the TtgABC efflux pump. TtgR and the TtgB efflux pump proteins possess multidrug-binding capacity, and their concerted action is responsible for the multidrug resistance phenotype of Pseudomonas putida DOT-T1E. TtgR binds to a pseudo-palindromic site that overlaps the ttgR/ttgA promoters. Dimethylsulfate footprint assays reveal a close interaction between TtgR and the central region of this operator. The results of analytical ultracentrifugation demonstrate that TtgR forms stable dimers in solution, and that two dimers bind to the operator. Microcalorimetric analysis of the binding of the two TtgR dimers to the cognate operator showed biphasic behavior, and an interaction model was developed for the cooperative binding of two TtgR dimers to their target operators. The binding of the two TtgR dimers to the operator was characterized by a Hill coefficient of 1.63 ± 0.13 ($k(D) = 18.2 (\pm 6.3)$ μM , $k(D)' = 0.91 (\pm 0.49)$ μM), indicating positive cooperativity. These data are in close agreement with the results of sedimentation equilibrium studies of TtgR-DNA complexes. A series of oligonucleotides were generated in which the imperfect palindrome of the TtgR operator was empirically optimized. Optimization of the palindrome did not significantly alter the binding of the initial TtgR dimer to the operator, but increased the cooperativity of binding and consequently the overall affinity. The minimal fragment for TtgR binding was a 30-mer DNA duplex, and analysis of its sequence revealed two partially overlapping inverted repeats co-existing within the large pseudo-palindrome operator. Based on the architecture of the operator, the thermodynamics of the process, and the TtgR-operator interactions we propose a model for the binding of TtgR to its target sequence.

Kumaran S., Kozlov A. G., and Lohman T. M. (2006) Saccharomyces cerevisiae replication protein A binds to single-stranded DNA in multiple salt-dependent modes. *Biochemistry* **45**, 11958-11973.

Abstract: We have examined the single-stranded DNA (ssDNA) binding properties of the Saccharomyces cerevisiae replication protein A (scRPA) using fluorescence titrations, isothermal titration calorimetry, and

sedimentation equilibrium to determine whether scRPA can bind to ssDNA in multiple binding modes. We measured the occluded site size for scRPA binding poly(dT), as well as the stoichiometry, equilibrium binding constants, and binding enthalpy of scRPA-(dT)L complexes as a function of the oligodeoxynucleotide length, L. Sedimentation equilibrium studies show that scRPA is a stable heterotrimer over the range of [NaCl] examined (0.02-1.5 M). However, the occluded site size, n, undergoes a salt-dependent transition between values of n = 18-20 nucleotides at low [NaCl] and values of n = 26-28 nucleotides at high [NaCl], with a transition midpoint near 0.36 M NaCl (25.0 degrees C, pH 8.1). Measurements of the stoichiometry of scRPA-(dT)L complexes also show a [NaCl]-dependent change in stoichiometry consistent with the observed change in the occluded site size. Measurements of the ΔH (obsd) for scRPA binding to (dT)L at 1.5 M NaCl yield a contact site size of 28 nucleotides, similar to the occluded site size determined at this [NaCl]. Altogether, these data support a model in which scRPA can bind to ssDNA in at least two binding modes, a low site size mode (n = 18 +/- 1 nucleotides), stabilized at low [NaCl], in which only three of its oligonucleotide/oligosaccharide binding folds (OB-folds) are used, and a higher site size mode (n = 27 +/- 1 nucleotides), stabilized at higher [NaCl], which uses four of its OB-folds. No evidence for highly cooperative binding of scRPA to ssDNA was found under any conditions examined. Thus, scRPA shows some behavior similar to that of the E. coli SSB homotetramer, which also shows binding mode transitions, but some significant differences also exist.

K-unne A. G., Sieber M., Meierhans D., and Allemann R. K. (1998) Thermodynamics of the DNA binding reaction of transcription factor MASH-1. *Biochemistry* **37**, 4217-4223.

Abstract: MASH-1, a member of the basic helix-loop-helix (BHLH) family of transcription factors, promotes the differentiation of committed neuronal precursor cells. We have determined the thermodynamic parameters of the DNA binding reaction of the BHLH domain of MASH-1 (MASH-BHLH) by isothermal titration calorimetry and found that the specificity of the binding reaction was rather low. At 27 degrees C, the association constant for binding was $5.13 (+/-0.51) \times 10^8 \text{ M}^{-1}$ for an E-box containing oligonucleotide, while for a heterologous DNA sequence it was $5.14 (+/-1.93) \times 10^7 \text{ M}^{-1}$. The reaction enthalpy and the reaction entropy were strongly dependent on the temperature, but the reaction free energy was almost independent of temperature. The association reaction was enthalpically driven throughout the physiological temperature range and characterized by a large negative heat capacity change. No change in the protonation state of the protein and/or the DNA was observed at pH 6. Within experimental error, the reaction was independent of pH between pH 6 and 8. Dissection of the entropy change of the binding reaction indicated that binding was coupled to local protein folding of approximately 25 amino acids per protein subunit. The circular dichroism spectra of free and DNA-bound MASH-BHLH revealed the formation of additional alpha-helical structure comprising approximately 25 amino acids upon complex formation. Therefore, while the basic region was in an alpha-helical conformation in the DNA complex, in free MASH-BHLH it was substantially unfolded even at concentrations where the protein is mainly dimeric. The association between MASH-1 and DNA is therefore an example of "induced fit".

Kvaratskhelia M., Wardleworth B. N., Bond C. S., Fogg J. M., Lilley D. M., and White M. F. (2002) Holliday junction resolution is modulated by archaeal chromatin components in vitro. *J Biol Chem* **277**, 2992-2996.

Abstract: The Holliday junction-resolving enzyme Hjc is conserved in the archaea and probably plays a role analogous to that of Escherichia coli RuvC in the pathway of homologous recombination. Hjc specifically recognizes four-way DNA junctions, cleaving them without sequence preference to generate recombinant DNA duplex products. Hjc imposes an X-shaped global conformation on the bound DNA junction and distorts base stacking around the point of cleavage, three nucleotides 3' of the junction center. We show that Hjc is autoinhibitory under single turnover assay conditions and that this can be relieved by the addition of either competitor duplex DNA or the architectural double-stranded DNA-binding protein Sso7d (i.e. by approximating in vivo conditions more closely). Using a combination of isothermal titration calorimetry and fluorescent resonance energy transfer, we demonstrate that multiple Hjc dimers can bind to each synthetic four-way junction and provide evidence for significant distortion of the junction structure at high protein:DNA ratios. Analysis of crystal packing interactions in the crystal structure of Hjc suggests a molecular basis for this autoinhibition. The wider implications of these findings for the quantitative study of DNA-protein interactions is discussed.

Lacal J., Guazzaroni M. E., Busch A., Krell T. and Ramos J. L. (2008) Hierarchical binding of the TodT response regulator to its multiple recognition sites at the *tod* pathway operon promoter. *J Mol Biol* **376**, 325-337.

Abstract: The TodS and TodT proteins form a highly specific two-component regulatory system that controls the expression of genes involved in the degradation of toluene, benzene, and ethylbenzene via the toluene dioxygenase pathway. The catabolic genes of the toluene dioxygenase pathway are transcribed from a single promoter called P(*todX*) once the response regulator TodT is phosphorylated by the TodS sensor kinase in response to pathway substrates. We show here that TodT is a monomer in solution and that it binds to three specific sites in the P(*todX*) promoter, centered at -57, -85, and -106 with respect to the transcription start site. The -85 and -106 sites are pseudopalindromic, whereas the -57 site is half a palindrome. TodT binding to its target sites is sequential, as shown by electrophoresis mobility gel shift assays and footprinting. The binding affinity values of TodT, as determined by isothermal titration calorimetry, are 1.8 ± 0.2 , 5 ± 0.4 , and 6.3 ± 0.8 μM for the -106, -85, and -57 sites, respectively, and the binding stoichiometry is one monomer per half-palindromic element. Mutational analysis revealed that all three sites contribute to P(*todX*) strength, although the most relevant site is the distal one with respect to the -10 extended element of the downstream promoter element. The C-TodT [C-terminal TodT fragment (amino acids 154-206)], a truncated variant of TodT that contains the C-terminal half of the protein bearing the DNA binding domain, binds in vitro to all three sites with affinity similar to that of the full-length protein. However, C-TodT, in contrast to the full-length regulator, does not activate in vitro transcription from P(*todX*). We discuss the consequences of the organization of the binding sites on transcriptional control and propose that the N-terminal domain of TodT is necessary for appropriate interactions with other transcriptional elements

Ladbury J. E., Wright J. G., Sturtevant J. M., and Sigler P. B. (1994) A thermodynamic study of the *trp* repressor-operator interaction. *J Mol Biol* **238**, 669-681.

Abstract: We have measured the heats of formation of the *trp* repressor/operator complex by direct titration calorimetry over the temperature range 10 degrees C to 40 degrees C. A primary strong mode of binding displays the characteristic large negative heat capacity change observed by other methods in the formation of specific protein/DNA complexes. Unlike most such reactions, however, the formation of the *trp* repressor/operator complex is enthalpically driven throughout the physiological temperature range. After saturation of this principal mode, we also detected a secondary weaker binding mode, which we ascribe to a now well documented interaction called "half-site" binding. Although weak, this mode also exhibits an unusually large negative heat capacity change. Since the interface of the proposed secondary half-site binding mode has the same complementary stereochemistry as the primary one (due to internal symmetry), we correlate the negative heat capacity change with the formation of a stereospecific interface and not with high affinity. As in similar cases, the empirical correlation between buried non-polar surfaces and reduction of heat capacity does not account for the large negative ΔC_p , nor do crystal structures reveal any further reduction in solvent excluded surfaces within the reactants upon complex formation. We attribute the "unaccounted for" decrement in the heat capacity of the complex to the stereospecific restriction of the hydrated polar elements that form the specific interface. We suggest that the "tightening of soft internal modes" at and near the polar interface of the complex is more important than previously recognized because previous considerations did not take into account the highly hydrated nature of these polar elements and the concomitant reduction in the degrees of freedom of the water structure.

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.

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.

Lamers, M.H., Roxana E. Georgescu, R.E., Lee, S.G., O'Donnell, M., and Kuriyan, J. (2006) Crystal Structure of the Catalytic α Subunit of E. coli Replicative DNA Polymerase III. *Cell* **126**, 881-892.

Abstract: Bacterial replicative DNA polymerases such as Polymerase III (Pol III) share no sequence similarity with other polymerases. The crystal structure, determined at 2.3 Å resolution, of a large fragment of Pol III (residues 1–917), reveals a unique chain fold with localized similarity in the catalytic domain to DNA polymerase β and related nucleotidyltransferases. The structure of Pol III is strikingly different from those of members of the canonical DNA polymerase families, which include eukaryotic replicative polymerases, suggesting that the DNA replication machinery in bacteria arose independently. A structural element near the active site in Pol III that is not present in nucleotidyltransferases but which resembles an element at the active sites of some canonical DNA polymerases suggests that, at a more distant level, all DNA polymerases may share a common ancestor. The structure also suggests a model for interaction of Pol III with the sliding clamp and DNA.

Lebbink J. H., Georgijevic D., Natrajan G., Fish A., Winterwerp H. H., Sixma T. K., and de Wind N. (2006) Dual role of MutS glutamate 38 in DNA mismatch discrimination and in the authorization of repair. *EMBO J* **25**, 409-419.

Abstract: MutS plays a critical role in DNA mismatch repair in Escherichia coli by binding to mismatches and initiating repair in an ATP-dependent manner. Mutational analysis of a highly conserved glutamate, Glu38, has revealed its role in mismatch recognition by enabling MutS to discriminate between homoduplex and mismatched DNA. Crystal structures of MutS have shown that Glu38 forms a hydrogen bond to one of the mismatched bases. In this study, we have analyzed the crystal structures, DNA binding and the response to ATP binding of three Glu38 mutants. While confirming the role of the negative charge in initial discrimination, we show that in vivo mismatch repair can proceed even when discrimination is low. We demonstrate that the formation of a hydrogen bond by residue 38 to the mismatched base authorizes repair by inducing intramolecular signaling, which results in the inhibition of rapid hydrolysis of distally bound ATP. This allows formation of the stable MutS-ATP-DNA clamp, a key intermediate in triggering downstream repair events.

Lee M., Shea R. G., Hartley J. A., Lown J. W., Kissinger K., Dabrowiak J. C., Vesnaver G., Breslauer K. J., and Pon R. T. (1989) Molecular recognition between oligopeptides and nucleic acids. Sequence specific binding of (4S)-(+)- and (4R)-(-)-dihydrokikumycin B to DNA deduced from 1H NMR, footprinting studies and thermodynamic data. *J Mol Recognit* **2**, 6-17.

Abstract: The sequence specific binding of the antibiotic (4S)-(+)-dihydrokikumycin B and its (4R)-(-) enantiomer, [(S)-1 and (R)-1, respectively] to DNA were characterized by DNase I and MPE footprinting, calorimetry, UV spectroscopy, circular dichroism, and 1H NMR studies. Footprinting analyses showed that both enantiomers [(S)-1 and (R)-1] bind to AT-rich regions of DNA. 1H NMR studies (ligand induced chemical shift changes and NOE differences) of the dihydrokikumycins with d-[CGCAATTGCG]₂ show unambiguously that the N to C termini of the ligands are bound to 5'-A5T6T7-3' reading from left to right. From quantitative 1D-NOE studies, the AH2(5)-ligand H7 distance of complex A [(S)-1 plus decamer (which is bound more strongly)] and complex B [(R)-1 and decamer] are estimated to be 3.8 +/- 0.3 Å and 4.9 +/- 0.4 Å, respectively. This difference in binding properties is reflected in the thermodynamic profiles of the two enantiomeric ligands determined by a combination of spectroscopic and calorimetric techniques. The binding free energies (ΔG degrees) of (S)-1 and (R)-1 to poly d(AT).poly d(AT) at 25 degrees C are -

31.8 and -29.3 kJ mol⁻¹, respectively while the corresponding binding enthalpies (ΔH degrees) are -11.3 and -0.8 kJ mol⁻¹. These data permit the construction of models for the binding of the enantiomeric dihydrokikumycins to DNA and account for the more efficient binding of the natural (S) isomer to DNA.

Li L., Uversky V. N., Dunker A. K. and Meroueh S. O. (2007) A computational investigation of allostery in the catabolite activator protein. *J Am. Chem Soc.* **129**, 15668-15676.

Abstract: The catabolite activator protein is a dimer that consists of two cAMP-binding subunits, each containing a C-terminus DNA-binding module and a N-terminus ligand binding domain. The system is well-known to exhibit negative cooperativity, whereby the binding of one cAMP molecule reduces the binding affinity of the other cAMP molecule by 2 orders of magnitude, despite the large separation between the cAMP binding pockets. Here we use extensive explicit-solvent molecular dynamics simulations (135 ns) to investigate the allosteric mechanism of CAP. Six trajectories were carried out for apo, singly liganded, and doubly liganded CAP, both in the presence and absence of DNA. Thorough analyses of the dynamics through the construction of dynamical cross-correlated maps, as well as essential dynamics analyses, indicated that the system experienced a switch in motion as a result of cAMP binding, in accordance with recent NMR experiments carried out on a truncated form of the protein. Analyses of conformer structures collected from the simulations revealed a remarkable event: the DNA-binding module was found to dissociate from the N-terminus ligand binding domain. An interesting aspect of this structural change is that it only occurred in unoccupied subunits, suggesting that the binding of cAMP provides additional stability to the system, consistent with the increase in entropy that was observed in our calculations and from isothermal titration calorimetry. Analysis of the distribution of intrinsic disorder propensities in CAP amino acid sequence using PONDR VLXT and VSL1 predictors revealed that the region connecting ligand-binding and DNA-binding domains of CAP have the potential to exhibit increased flexibility. We complemented these trajectories with free energy calculations following the MM-PBSA approach on more than 2000 snapshots that included 880 normal mode analysis. The resulting free energy differences between the singly liganded and doubly liganded states were in excellent agreement with isothermal titration calorimetry data. When the free energy calculations were carried out in the presence of DNA, we discovered that a switch in cooperativity occurred, so that the binding of the first cAMP promoted the binding of the other cAMP. The components of the free energy reveal that this effect is mainly entropic in nature, whereby the DNA reduces the degree of tightening that is observed in its absence, thereby promoting binding of the second cAMP. This finding prompted us to propose a new mechanism by which CAP triggers the transcription activation that is based on an order to disorder transition mediated by cAMP binding as well as DNA.

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

Abstract: Protoberberines represent a structural class of organic cations that induce topoisomerase I-mediated DNA cleavage, a behavior termed topoisomerase I poisoning. We have employed a broad range of biophysical, biochemical, and computer modeling techniques to characterize and cross-correlate the DNA-binding and topoisomerase poisoning properties of four protoberberine analogues that differ with respect to the substituents on their A- and/or D-rings. Our data reveal the following significant features: (i) The binding of the four protoberberines unwinds duplex DNA by approximately 11 degrees, an observation consistent with an intercalative mode of interaction. (ii) Enthalpically favorable interactions, such as stacking interactions between the intercalated ligand and the neighboring base pairs, provide <50% of the thermodynamic driving force for the complexation of the protoberberines to duplex DNA. Computer modeling studies on protoberberine-DNA complexes suggest that only rings C and D intercalate into the host DNA helix, while rings A and B protrude out of the helix interior into the minor groove. (iii) All four protoberberine analogues are topoisomerase I-specific poisons, exhibiting little or no topoisomerase II poisoning activity. (iv) Modifications of the D-ring influence both DNA binding and topoisomerase I poisoning properties. Specifically, transference of a methoxy substituent from the 11- to the 9-position diminishes both DNA binding affinity and topoisomerase I poisoning activity, an observation suggesting that DNA binding is important in the poisoning of topoisomerase I by protoberberines. (v) Modifications of the A-ring have a negligible impact on DNA binding affinity, while exerting a profound influence on topoisomerase I poisoning activity. Specifically, protoberberine analogues containing either 2,3-dimethoxy; 3,4-dimethoxy; or 3, 4-methylenedioxy substituents all bind DNA with a similar affinity. By contrast, these

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

Liang Y., Du F., Zhou B. R., Zhou H., Zou G. L., Wang C. X., and Qu S. S. (2002) Thermodynamics and kinetics of the cleavage of DNA catalyzed by bleomycin A5. *Eur J Biochem* **269**, 2851-2859.

Abstract: Microcalorimetry and UV-vis spectroscopy were used to conduct thermodynamic and kinetic investigations of the scission of calf thymus DNA catalyzed by bleomycin A5 (BLM-A5) in the presence of ferrous ion and oxygen. The molar reaction enthalpy for the cleavage, the Michaelis-Menten constant for calf thymus DNA and the turnover number of BLM-A5 were calculated by a novel thermokinetic method for an enzyme-catalyzed reaction to be $-577 \pm 19 \text{ kJ.mol}^{-1}$, $20.4 \pm 3.8 \mu\text{M}$ and $2.28 \pm 0.49 \times 10^{-2} \text{ s}^{-1}$, respectively, at 37.0 degrees C. This DNA cleavage was a largely exothermic reaction. The catalytic efficiency of BLM-A5 is of the same order of magnitude as that of lysozyme but several orders of magnitude lower than those of TaqI restriction endonuclease, NaeI endonuclease and BamHI endonuclease. By comparing the molar enthalpy change for the cleavage of calf thymus DNA induced by BLM-A5 with those for the scission of calf thymus DNA mediated by adriamycin and by (1,10-phenanthroline)-copper, it was found that BLM-A5 possessed the highest DNA cleavage efficiency among these DNA-damaging agents. These results suggest that BLM-A5 is not as efficient as a DNA-cleaving enzyme although the cleavage of DNA by BLM-A5 follows Michaelis-Menten kinetics. Binding of BLM-A5 to calf thymus DNA is driven by a favorable entropy increase with a less favorable enthalpy decrease, in line with a partial intercalation mode involved in BLM-catalyzed breakage of DNA.

Liew, C.W., Rand, K.D., Simpson, R.J.Y., Yung, W.W., Mansfield, R.E., Crossley, M., Proetorius-Ibba, M., Nerlov, C., Poulsen, F.M., and Mackay, J.P. (2006) Molecular Analysis of the Interaction between the Hematopoietic Master Transcription Factors GATA-1 and PU.1. *J. Biol. Chem* **281**, 28296-28306.

Abstract: GATA-1 and PU.1 are transcription factors that control erythroid and myeloid development, respectively. The two proteins have been shown to function in an antagonistic fashion, with GATA-1 repressing PU.1 activity during erythropoiesis and PU.1 repressing GATA-1 function during myelopoiesis. It has also become clear that this functional antagonism involves direct interactions between the two proteins. However, the molecular basis for these interactions is not known, and a number of inconsistencies exist in the literature. We have used a range of biophysical methods to define the molecular details of the GATA-1-PU.1 interaction. A combination of NMR titration data and extensive mutagenesis revealed that the PU.1-Ets domain and the GATA-1 C-terminal zinc finger (CF) form a low affinity interaction in which specific regions of each protein are implicated. Surprisingly, the interaction cannot be disrupted by single alanine substitution mutations, suggesting that binding is distributed over an extended interface. The C-terminal basic tail region of CF appears to be sufficient to mediate an interaction with PU.1-Ets, and neither acetylation nor phosphorylation of a peptide corresponding to this region disrupts binding, indicating that the interaction is not dominated by electrostatic interactions. The CF basic tail shares significant sequence homology with the PU.1 interacting motif from c-Jun, suggesting that GATA-1 and c-Jun might compete to bind PU.1. Taken together, our data provide a molecular perspective on the GATA-1-PU.1 interaction, resolving several issues in the existing data and providing insight into the mechanisms through which these two proteins combine to regulate blood development.

Liew C. K., Simpson R. J. Y., Kwan A. H. Y., Crofts L. A., Loughlin F. E., Matthews J. M., Crossley M., and Mackay J. P. (2005) Zinc fingers as protein recognition motifs: Structural basis for the GATA-1/Friend of GATA interaction. *Proc Natl Acad Sci U S A* **102**, 583-588.

Abstract: GATA-1 and friend of GATA (FOG) are zinc-finger transcription factors that physically interact to play essential roles in erythroid and megakaryocytic development. Several naturally occurring mutations in the *GATA-1* gene that alter the FOG-binding domain have been reported. The mutations are associated with familial anemias and thrombocytopenias of differing severity. To elucidate the molecular basis for the

GATA-1/FOG interaction, we have determined the three-dimensional structure of a complex comprising the interaction domains of these proteins. The structure reveals how zinc fingers can act as protein recognition motifs. Details of the architecture of the contact domains and their physical properties provide a molecular explanation for how the *GATA-1* mutations contribute to distinct but related genetic diseases.

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 (zf1-3) 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 zf1-3 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 ΔH_{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 zf1-3/DNA complex (approximately $1900 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$) cannot be balanced by the reduction in translational/rotational and configurational freedom of the protein to the level of the observed entropy of binding ($38 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$). It is suggested that the additional negative entropy contribution comes from a damping of torsional motions in the DNA duplex.

Lohman T. M., Overman L. B., Ferrari M. E., and Kozlov A. G. (1996) A highly salt-dependent enthalpy change for *Escherichia coli* SSB protein-nucleic acid binding due to ion-protein interactions. *Biochemistry* **35**, 5272-5279.

Abstract: We have examined the linkage between salt concentration and temperature for the equilibrium binding of the tetrameric *Escherichia coli* single-stranded binding (SSB) protein to three single-stranded nucleic acids, poly(U), dA(pA)69, and dT(pT)69, by van't Hoff analysis and isothermal titration calorimetry (ITC). For SSB binding to poly(U) in its (SSB)65 mode, the equilibrium association constant, K_{obs} , decreases with increasing salt concentration at all temperatures examined, and binding is enthalpy-driven; however, the value of $[\text{symbol see text}] \log K_{\text{obs}} / [\text{symbol see text}] \log [\text{NaCl}]$ is highly temperature-dependent, varying from -9.3 ± 0.3 at 10 degrees C to -5.1 ± 0.4 at 37 degrees C. This indicates that ΔH_{obs} for SSB-poly(U) binding is strongly dependent on $[\text{NaCl}]$; based on van't Hoff analyses, ΔH_{obs} varies from $-57 \pm 3 \text{ kcal/mol}$ at 0.18 M NaCl to $-34 \pm 3 \text{ kcal/mol}$ at 0.42 M NaCl ($[\text{symbol see text}] \Delta H_{\text{obs}} / [\text{symbol see text}] \log [\text{NaCl}] = 60 \pm 5 \text{ kcal/mol}$). However, $[\text{symbol see text}] \Delta H_{\text{obs}} / [\text{symbol see text}] \log [\text{NaF}]$ is independent of temperature (25-37 degrees C), indicating that the effect of $[\text{NaCl}]$ on ΔH_{obs} is due primarily to Cl^- . Similar effects were also observed for SSB binding to dA(pA)69. We also measured ΔH_{obs} and its dependence on $[\text{NaCl}]$ for SSB binding dT(pT)69 by ITC and find $\Delta H_{\text{obs}} = -144 \pm 4 \text{ kcal/mol}$ (0.175 M NaCl, pH 8.1, 25 degrees C) and $[\text{symbol see text}] \Delta H_{\text{obs}} / [\text{symbol see text}] \log [\text{NaCl}] = 46 \pm 2 \text{ kcal/mol}$ (0.175-2.0 M NaCl). These large effects of $[\text{NaCl}]$ on ΔH_{obs} appear to result, at least partly, from the release of preferentially bound Cl^- from SSB protein upon binding nucleic acid, with the release of Cl^- being linked to a process with $\Delta H > 0$. Effects of salt concentration on ΔH_{obs} are not observed for processes in which only monovalent cations are released from the nucleic acid, presumably since Na^+ or K^+ are bound to linear nucleic acids as delocalized, fully hydrated cations. Such salt effects on ΔH_{obs} may serve as a signature for differential ion-protein binding. These results underscore the need to examine the linkage of $[\text{salt}]$ to ΔH_{obs} , as well as ΔH_{obs} degrees and ΔS_{obs} degrees, in order to understand the bases for stability and specificity of protein-nucleic acid interactions.

Lopez M. M., Yutani K., and Makhatadze G. I. (1999) Interactions of the major cold shock protein of *Bacillus subtilis* CspB with single-stranded DNA templates of different base composition. *J Biol Chem* **274**, 33601-33608.

Abstract: CspB is a small acidic protein of *Bacillus subtilis*, the induction of which is increased dramatically in response to cold shock. Although the exact functional role of CspB is unknown, it has been demonstrated that this protein binds single-stranded deoxynucleic acids (ssDNA). We addressed the question of the effect of base composition on the CspB binding to ssDNA by analyzing the thermodynamics of CspB interactions with model oligodeoxynucleotides. Combinations of four different techniques, fluorescence spectroscopy, gel shift mobility assays, isothermal titration calorimetry, and analytical ultracentrifugation, allowed us to show that: 1) CspB can preferentially bind poly-pyrimidine but not poly-purine ssDNA templates; 2) binding to T-based ssDNA template occurs with high affinity ($K(d(25 \text{ degrees C}))$ approximately 42 nM) and is salt-independent, whereas binding of CspB to C-based ssDNA template is strongly salt-dependent (no binding is observed at 1 M NaCl), indicating large electrostatic component involved in the interactions; 3) upon binding each CspB covers a stretch of 6-7 thymine bases on T-based ssDNA; and 4) the binding of CspB to T-based ssDNA template is enthalpically driven, indicating the possible involvement of interactions between aromatic side chains on the protein with the thymine bases. The significance of these results with respect to the functional role of CspB in the bacterial cold shock response is discussed.

Lopez M. M. and Makhatadze G. I. (2000) Major cold shock proteins, CspA from *Escherichia coli* and CspB from *Bacillus subtilis*, interact differently with single-stranded DNA templates. *Biochim Biophys Acta* **1479**, 196-202.

Abstract: The family of bacterial major cold shock proteins is characterized by a conserved sequence of 65-75 amino acid residues long which form a three-dimensional structure consisting of five beta-sheets arranged into a beta-barrel topology. CspA from *Escherichia coli* and CspB from *Bacillus subtilis* are typical representative members of this class of proteins. The exact biological role of these proteins is still unclear; however, they have been implicated to possess ssDNA-binding activity. In this paper, we report the results of a comparative quantitative analysis of ssDNA-binding activity of CspA and CspB. We show that in spite of high homology on the level of primary structure and very similar three-dimensional structures, CspA and CspB have different ssDNA-binding properties. Both proteins preferentially bind polypyrimidine ssDNA templates, but CspB binds to the T-based templates with one order of magnitude higher affinity than to U- or C-based ssDNA, whereas CspA binds T-, U- or C-based ssDNA with comparable affinity. They also show similarities and differences in their binding to ssDNA at high ionic strength. The results of these findings are related to the chemical structure of DNA bases.

Lopez M. M., Yutani K., and Makhatadze G. I. (2001) Interactions of the cold shock protein CspB from *Bacillus subtilis* with single-stranded DNA. Importance of the T base content and position within the template. *J Biol Chem* **276**, 15511-15518.

Abstract: The cold shock protein CspB from *Bacillus subtilis* binds T-based single-stranded DNA (ssDNA) with high affinity (Lopez, M. M., Yutani, K., and Makhatadze, G. I. (1999) *J. Biol. Chem.* **274**, 33601-33608). In this paper we report the results of CspB interactions with non-homogeneous ssDNA templates containing continuous and non-continuous stretches of T bases. The analysis of CspB-ssDNA interactions was performed using fluorescence spectroscopy, analytical centrifugation and isothermal titration calorimetry. We show that (i) there is a strong correlation between the CspB affinity and stoichiometry and the T content in the oligonucleotide that is independent of which other bases are incorporated into the sequence of ssDNA; (ii) the binding properties of CspB to ssDNA templates with continuous or non-continuous stretches of T bases with similar T content is very similar, and (iii) the mechanism of interaction between CspB and the T-based non-homogeneous ssDNA is mainly through the bases (a stretch of three T bases located in the middle of the ssDNA templates makes the binding independent of the ionic strength). The biological relevance of these results to the role of CspB as an RNA chaperone is discussed.

Loregian A., Sinigaglia E., Mercorelli B., Palu G. and Coen D. M. (2007) Binding parameters and thermodynamics of the interaction of the human cytomegalovirus DNA polymerase accessory protein, UL44, with DNA: implications for the processivity mechanism. *Nucleic Acids Res* **35**, 4779-4791.

Abstract: The mechanisms of processivity factors of herpesvirus DNA polymerases remain poorly understood. The proposed processivity factor for human cytomegalovirus DNA polymerase is a DNA-binding protein, UL44. Previous findings, including the crystal structure of UL44, have led to the hypothesis that UL44 binds DNA as a dimer via lysine residues. To understand how UL44 interacts with

DNA, we used filter-binding and electrophoretic mobility shift assays and isothermal titration calorimetry (ITC) analysis of binding to oligonucleotides. UL44 bound directly to double-stranded DNA as short as 12 bp, with apparent dissociation constants in the nanomolar range for DNAs >18 bp, suggesting a minimum DNA length for UL44 interaction. UL44 also bound single-stranded DNA, albeit with lower affinity, and for either single- or double-stranded DNA, there was no apparent sequence specificity. ITC analysis revealed that UL44 binds to duplex DNA as a dimer. Binding was endothermic, indicating an entropically driven process, likely due to release of bound ions. Consistent with this hypothesis, analysis of the relationship between binding and ionic strength indicated that, on average, 4 +/- 1 monovalent ions are released in the interaction of each monomer of UL44 with DNA. The results taken together reveal interesting implications for how UL44 may mediate processivity.

Lu, P., Li, Y., Gorman, A., and Chi, Y.I. (2006) Crystallization of Hepatocyte Nuclear Factor 1 β in Complex with DNA. *Acta Crystallograph Sect F Struct Biol Cryst Commun.* **62**, 525–529.

Abstract: Hepatocyte nuclear factor 1 β (HNF1 β) is a member of the POU transcriptionfactor family and binds the target DNA as a dimer with nanomolar affinity. The HNF1 β -DNA complex has been prepared and crystallized by hanging-drop vapor diffusion in 6%(v/v) PEG 300, 5%(w/v) PEG 8000, 8%(v/v) glycerol and 0.1 M Tris pH 8.0. The crystals diffracted to 3.2 \AA (93.9% completeness) using a synchrotron-radiation source under cryogenic (100 K) conditions and belong to space group R3, with unit-cell parameters $a = b = 172.69$, $c = 72.43\text{\AA}$. A molecular-replacement solution has been obtained and structure refinement is in progress. This structure will shed light on the molecular mechanism of promoter recognition by HNF1 β and the molecular basis of the disease-causing mutations found in it.

Lundback T. and Hard T. (1996) Sequence-specific DNA-binding dominated by dehydration. *Proc Natl Acad Sci U S A* **93**, 4754-4759.

Abstract: Fluorescence spectroscopy and isothermal titration calorimetry were used to study the thermodynamics of binding of the glucocorticoid receptor DNA-binding domain to four different, but similar, DNA-binding sites. The binding sites are two naturally occurring sites that differ in the composition of one base pair, i.e., an A-T to G-C mutation, and two sites containing chemical intermediates of these base pairs. The calorimetrically determined heat capacity change ($\Delta C(p)_{o}(obs)$) for glucocorticoid receptor DNA-binding domain binding agrees with that calculated for dehydration of solvent-accessible surface areas. A dominating effect of dehydration or solvent reorganization on the thermodynamics is also consistent with an observed linear relationship between observed enthalpy change ($\Delta H_{o}(obs)$) and observed entropy change ($\Delta S_{o}(obs)$) with a slope close to the experimental temperature. Comparisons with structural data allow us to rationalize individual differences between $\Delta H_{o}(obs)$ (and $\Delta S_{o}(obs)$) for the four complexes. For instance, we find that the removal of a methyl group at the DNA-protein interface is enthalpically favorable but entropically unfavorable, which is consistent with a replacement by an ordered water molecule.

Lundback T., Hansson H., Knapp S., Ladenstein R., and Hard T. (1998) Thermodynamic characterization of non-sequence-specific DNA-binding by the Sso7d protein from *Sulfolobus solfataricus*. *J Mol Biol* **276**, 775-786.

Abstract: We used isothermal titration calorimetry and fluorescence spectroscopy to investigate the thermodynamics of non-sequence-specific DNA-binding by the Sso7d protein from the archaeon *Sulfolobus solfataricus*. We report the Sso7d-poly(dGdC) binding thermodynamics as a function of buffer composition (Tris-HCl or phosphate), temperature (15 to 45 degrees C), pH (7.1 to 8.0), osmotic stress and solvent (H₂O/2H₂O), and compare it to poly (dAdT) binding; and we have previously also reported the salt concentration dependence. Binding isotherms can be represented by the McGhee-von Hippel model for non-cooperative binding, with a binding site size of four to five DNA base-pairs and binding free energies in the range ΔG degrees approximately -7 to ΔG degrees approximately -10 kcal mol⁻¹, depending on experimental conditions. The non-specific nature of the binding is reflected in similar thermodynamics for binding to poly(dAdT) and poly(dGdC). The native lysine methylation of Sso7d has only minor effects on the binding thermodynamics. Sso7d binding to poly(dGdC) is endothermic at 25 degrees C with a binding enthalpy ΔH degrees approximately 10 kcal mol⁻¹ in both phosphate and Tris-HCl buffers at pH 7.6, indicating that ΔH degrees does not include large contributions from coupled buffer ionization equilibria at this pH. The binding enthalpy is temperature dependent with a measured heat capacity change ΔC_p degrees=-0.25(+/-0.01) kcal mol⁻¹ K⁻¹ and extrapolations of thermodynamic data indicate that the complex is

heat stable with exothermic binding close to the growth temperature (75 to 80 degrees C) of *S. solfataricus*. Addition of neutral solutes (osmotic stress) has minor effects on ΔG degrees and the exchange of H₂O for 2H₂O has only a small effect on ΔH degrees, consistent with the inference that complex formation is not accompanied by net changes in surface hydration. Thus, other mechanisms for the heat capacity change must be found. The observed thermodynamics is discussed in relation to the nature of non-sequence-specific DNA-binding by proteins.

Lundback T., Chang J. F., Phillips K., Luisi B., and Ladbury J. E. (2000) Characterization of sequence-specific DNA binding by the transcription factor Oct-1. *Biochemistry* **39**, 7570-7579.

Abstract: The DNA-binding domain of the Oct-1 transcription factor, POU, recognizes a defined DNA sequence known as the octamer element to regulate the expression of both general and cell-type-specific genes. The two-part DNA-binding domain partially encircles the DNA to recognize the eight base pairs of the octamer element. We have characterized the binding of Oct-1/POU to an octamer element using isothermal titration calorimetry. As found for other cognate protein/DNA complexes, the formation of the Oct-1 POU/DNA complex is associated with a large negative heat capacity change, $\Delta C(p)$ (, obs). However, the observed change is much greater than expected by empirical relationships with buried surface area. Supported by data from proteolysis studies on the free and DNA-bound protein, we propose that the discrepancy in heat capacity arises principally from the partial folding of the Oct-1 POU protein upon complex formation. Formation of the Oct-1 POU/DNA complex is strongly dependent on ionic strength, and the detailed quantification of this relationship suggests that six charged contacts are made between the protein and the phosphate groups of the DNA. This agrees with observations from the crystal structure of an Oct-1 POU/DNA complex.

Lundback T., van Den B. S., and Hard T. (2000) Sequence-specific DNA binding by the glucocorticoid receptor DNA-binding domain is linked to a salt-dependent histidine protonation. *Biochemistry* **39**, 8909-8916.

Abstract: We used isothermal titration calorimetry in the temperature range 21-25 degrees C to investigate the effect of pH on the calorimetric enthalpy ($\Delta H(\text{cal})$) for sequence specific DNA-binding of the glucocorticoid receptor DNA-binding domain (GR DBD). Titrations were carried out in solutions containing 100 mM NaCl, 1 mM dithiothreitol, 5% glycerol by volume, and 20 mM Tris, Hepes, Mops, or sodium phosphate buffers at pH 7.5. A strong dependence of $\Delta H(\text{cal})$ on the buffer ionization enthalpy is observed, demonstrating that the DNA binding of the GR DBD is linked to proton uptake at these conditions. The apparent increase in the $pK(a)$ for an amino acid side chain upon DNA binding is supported by the results of complementary titrations, where $\Delta H(\text{cal})$ shows a characteristic dependence on the solution pH. $\Delta H(\text{cal})$ is also a function of the NaCl concentration, with opposite dependencies in Tris and Hepes buffers, respectively, such that a similar $\Delta H(\text{cal})$ value is approached at 300 mM NaCl. This behavior shows that the DNA-binding induced protonation is inhibited by increased concentrations of NaCl. A comparison with structural data suggests that the protonation involves a histidine (His451) in the GR DBD, because in the complex this residue is located close to a DNA phosphate at an orientation that is consistent with a charged-charged hydrogen bond in the protonated state. NMR spectra show that His451 is not protonated in the unbound protein at pH 7.5. The pH dependence in $\Delta H(\text{cal})$ can be quantitatively described by a shift of the $pK(a)$ of His451 from approximately 6 in the unbound state to close to 8 when bound to DNA at low salt concentration conditions. A simple model involving a binding competition between a proton and a Na(+) counterion to the GR DBD-DNA complex reproduces the qualitative features of the salt dependence.

Lynch S. R. and Puglisi J. D. (2001) Structural origins of aminoglycoside specificity for prokaryotic ribosomes. *J Mol Biol* **306**, 1037-1058.

Abstract: Aminoglycoside antibiotics, including paromomycin, neomycin and gentamicin, target a region of highly conserved nucleotides in the decoding region aminoacyl-tRNA site (A site) of 16 S rRNA on the 30 S subunit. Change of a single nucleotide, A1408 to G, reduces the affinity of many aminoglycosides for the ribosome; G1408 distinguishes between prokaryotic and eukaryotic ribosomes. The structures of a prokaryotic decoding region A-site oligonucleotide free in solution and bound to the aminoglycosides paromomycin and gentamicin C1a were determined previously. Here, the structure of a eukaryotic decoding region A-site oligonucleotide bound to paromomycin has been determined using NMR spectroscopy and compared to the prokaryotic A-site-paromomycin structure. A conformational change in

three adenosine residues of an internal loop, critical for high-affinity antibiotic binding, was observed in the prokaryotic RNA-paromomycin complex in comparison to its free form. This conformational change is not observed in the eukaryotic RNA-paromomycin complex, disrupting the binding pocket for ring I of the antibiotic. The lack of the conformational change supports footprinting and titration calorimetry data that demonstrate approximately 25-50-fold weaker binding of paromomycin to the eukaryotic decoding-site oligonucleotide. Neomycin, which is much less active against *Escherichia coli* ribosomes with an A1408G mutation, binds non-specifically to the oligonucleotide. These results suggest that eukaryotic ribosomal RNA has a shallow binding pocket for aminoglycosides, which accommodates only certain antibiotics.

Madl T., Van Melderen L., Mine N., Respondek M., Oberer M., Keller W., Khatai L., and Zangger K. (2006) Structural basis for nucleic acid and toxin recognition of the bacterial antitoxin CcdA. *J Mol Biol* **364**, 170-185.

Abstract: Toxin-antitoxin systems are highly abundant in plasmids and bacterial chromosomes. They ensure plasmid maintenance by killing bacteria that have lost the plasmid. Their expression is autoregulated at the level of transcription. Here, we present the solution structure of CcdA, the antitoxin of the *ccd* system, as a free protein (16.7 kDa) and in complex with its cognate DNA (25.3 kDa). CcdA is composed of two distinct and independent domains: the N-terminal domain, responsible for DNA binding, which establishes a new family of the ribbon-helix-helix fold and the C-terminal region, which is responsible for the interaction with the toxin CcdB. The C-terminal domain is intrinsically unstructured and forms a tight complex with the toxin. We show that CcdA specifically recognizes a 6 bp palindromic DNA sequence within the operator-promoter (OP) region of the *ccd* operon and binds to DNA by insertion of the positively charged N-terminal beta-sheet into the major groove. The binding of up to three CcdA dimers to a 33mer DNA of its operator-promoter region was studied by NMR spectroscopy, isothermal titration calorimetry and single point mutation. The highly flexible C-terminal region of free CcdA explains its susceptibility to proteolysis by the Lon ATP-dependent protease.

Maiti A., Morgan M. T., Pozharski E. and Drohat A. C. (2008) Crystal structure of human thymine DNA glycosylase bound to DNA elucidates sequence-specific mismatch recognition. *Proc. Natl. Acad. Sci U. S. A* **105**, 8890-8895.

Abstract: Cytosine methylation at CpG dinucleotides produces m(5)CpG, an epigenetic modification that is important for transcriptional regulation and genomic stability in vertebrate cells. However, m(5)C deamination yields mutagenic G.T mismatches, which are implicated in genetic disease, cancer, and aging. Human thymine DNA glycosylase (hTDG) removes T from G.T mismatches, producing an abasic (or AP) site, and follow-on base excision repair proteins restore the G.C pair. hTDG is inactive against normal A.T pairs, and is most effective for G.T mismatches and other damage located in a CpG context. The molecular basis of these important catalytic properties has remained unknown. Here, we report a crystal structure of hTDG (catalytic domain, hTDG(cat)) in complex with abasic DNA, at 2.8 Å resolution. Surprisingly, the enzyme crystallized in a 2:1 complex with DNA, one subunit bound at the abasic site, as anticipated, and the other at an undamaged (nonspecific) site. Isothermal titration calorimetry and electrophoretic mobility-shift experiments indicate that hTDG and hTDG(cat) can bind abasic DNA with 1:1 or 2:1 stoichiometry. Kinetics experiments show that the 1:1 complex is sufficient for full catalytic (base excision) activity, suggesting that the 2:1 complex, if adopted *in vivo*, might be important for some other activity of hTDG, perhaps binding interactions with other proteins. Our structure reveals interactions that promote the stringent specificity for guanine versus adenine as the pairing partner of the target base and interactions that likely confer CpG sequence specificity. We find striking differences between hTDG and its prokaryotic ortholog (MUG), despite the relatively high (32%) sequence identity.

McElroy C. A., Manfreda A., Gollnick P., and Foster M. P. (2006) Thermodynamics of Tryptophan-Mediated Activation of the *trp* RNA-Binding Attenuation Protein. *Biochemistry* **45**, 7844-7853.

Abstract: The *trp* RNA-binding attenuation protein (TRAP) functions in many bacilli to control the expression of the tryptophan biosynthesis genes. Transcription of the *trp* operon is controlled by TRAP through an attenuation mechanism, in which competition between two alternative secondary-structural elements in the 5' leader sequence of the nascent mRNA is influenced by tryptophan-dependent binding of TRAP to the RNA. Previously, NMR studies of the undecamer (11-mer) suggested that tryptophan-dependent control of RNA binding by TRAP is accomplished through ligand-induced changes in protein dynamics. We now present further insights into this ligand-coupled event from hydrogen/deuterium (H/D)

exchange analysis, differential scanning calorimetry (DSC), and isothermal titration calorimetry (ITC). Scanning calorimetry showed tryptophan dissociation to be independent of global protein unfolding, while analysis of the temperature dependence of the binding enthalpy by ITC revealed a negative heat capacity change larger than expected from surface burial, a hallmark of binding-coupled processes. Analysis of this excess heat capacity change using parameters derived from protein folding studies corresponds to the ordering of 17-24 residues per monomer of TRAP upon tryptophan binding. This result is in agreement with qualitative analysis of residue-specific broadening observed in TROSY NMR spectra of the 91 kDa oligomer. Implications for the mechanism of ligand-mediated TRAP activation through a shift in a preexisting conformational equilibrium and an induced-fit conformational change are discussed.

McKenna S. A., Kim I., Liu C. W., and Puglisi J. D. (2006) Uncoupling of RNA binding and PKR kinase activation by viral inhibitor RNAs. *J Mol Biol* **358**, 1270-1285.

Abstract: Protein kinase RNA-activated (PKR) is a serine/threonine kinase that contains an N-terminal RNA-binding domain and a C-terminal kinase domain. Upon binding double-stranded RNA (dsRNA), PKR can become activated and phosphorylate cellular targets, such as eukaryotic translation initiation factor 2alpha (eIF-2alpha). Phosphorylation of eIF-2alpha results in attenuation of protein translation by the ribosome in either a general or an mRNA-specific manner. Therefore, the interaction between PKR and dsRNAs represents a crucial host cell defense mechanism against viral infection. Viruses can circumvent PKR function by transcription of virus-encoded dsRNA inhibitors that bind to and inactivate PKR. We present here a biophysical characterization of the interactions between human PKR and two viral inhibitor RNAs, EBER(I) (from Epstein-Barr virus) and VA(I) (from human adenovirus). Autophosphorylation assays confirmed that both EBER(I) and VA(I) are inhibitors of PKR activation, and profiled the kinetics of the inhibition. Binding affinities of dsRNAs to PKR double-stranded RNA-binding domains (dsRBDs) were determined by isothermal titration calorimetry and gel electrophoresis. A single stem-loop domain from each inhibitory RNA mediates the interaction with both dsRBDs of PKR. The binding sites on inhibitor RNAs and the dsRBDs of PKR have been mapped by NMR chemical shift perturbation experiments, which indicate that inhibitors of PKR employ similar surfaces of interaction as activators. Finally, we show that dsRNA binding and inactivation are non-equivalent; regions other than the dsRBD stem-loops of inhibitory RNA are required for inhibition.

McKenna S. A., Lindhout D. A., Shimoike T., Aitken C. E. and Puglisi J. D. (2007) Viral dsRNA inhibitors prevent self-association and autophosphorylation of PKR. *J Mol Biol* **372**, 103-113.

Abstract: Host response to viral RNA genomes and replication products represents an effective strategy to combat viral invasion. PKR is a Ser/Thr protein kinase that binds to double-stranded (ds)RNA, autophosphorylates its kinase domain, and subsequently phosphorylates eukaryotic initiation factor 2alpha (eIF2alpha). This results in attenuation of protein translation, preventing synthesis of necessary viral proteins. In certain DNA viruses, PKR function can be evaded by transcription of highly structured virus-encoded dsRNA inhibitors that bind to and inactivate PKR. We probe here the mechanism of PKR inhibition by two viral inhibitor RNAs, EBER(I) (from Epstein-Barr) and VA(I) (from human adenovirus). Native gel shift mobility assays and isothermal titration calorimetry experiments confirmed that the RNA-binding domains of PKR are sufficient and necessary for the interaction with dsRNA inhibitors. Both EBER(I) and VA(I) are effective inhibitors of PKR activation by preventing trans-autophosphorylation between two PKR molecules. The RNA inhibitors prevent self-association of PKR molecules, providing a mechanistic basis for kinase inhibition. A variety of approaches indicated that dsRNA inhibitors remain associated with PKR under activating conditions, as opposed to activator dsRNA molecules that dissociate due to reduced affinity for the phosphorylated form of PKR. Finally, we show using a HeLa cell extract system that inhibitors of PKR result in translational recovery by the protein synthesis machinery. These data indicate that inhibitory dsRNAs bind preferentially to the latent, dephosphorylated form of PKR and prevent dimerization that is required for trans-autophosphorylation.

McKnight R. E., Ye M., Ohulchanskyy T. Y., Sahabi S., Wetzel B. R., Wagner S. J., Skripchenko A. and Detty M. R. (2007) Synthesis of analogues of a flexible thiopyrylium photosensitizer for purging blood-borne pathogens and binding mode and affinity studies of their complexes with DNA. *Bioorg. Med. Chem* **15**, 4406-4418.

Abstract: A series of thio- and selenopyrylium analogues of 2,4-di(4-dimethylaminophen-yl)-6-methylthiopyrylium iodide were prepared in five steps from 4-dimethylaminophenyl-propargyl aldehyde

and the corresponding lithium acetylde. When bound to DNA, all of the dyes absorb at wavelengths >600nm, which avoids the hemoglobin band I maximum at 575nm. The binding of the series of dyes to double-stranded DNA was examined spectrophotometrically and by isothermal titration calorimetry to determine binding constants, by a topoisomerase I DNA unwinding assay, by competition dialysis with [poly(dGdC)](2) and [poly(dAdT)](2), and by ethidium bromide displacement studies to examine propensities for intercalation, and by circular dichroism studies. The dyes were found to show mixed binding modes.

McKnight R. E., Gleason A. B., Keyes J. A. and Sahabi S. (2007) Binding mode and affinity studies of DNA-binding agents using topoisomerase I DNA unwinding assay. *Bioorg. Med. Chem Lett* **17**, 1013-1017.

Abstract: A topoisomerase I DNA unwinding assay has been used to determine the relative DNA-binding affinities of a model pair of homologous naphthalene diimides. Binding affinity data were corroborated using calorimetric (ITC) and spectrophotometric (titration and T(m)) studies, with substituent size playing a significant role in binding. The assay was also used to investigate the mode of binding adopted by several known DNA-binding agents, including SYBR Green and PicoGreen. Some of the compounds exhibited unexpected binding modes.

Meier-Andrejszki L., Bjelic S., Naud J. F., Lavigne P. and Jelesarov I. (2007) Thermodynamics of b-HLH-LZ protein binding to DNA: the energetic importance of protein-DNA contacts in site-specific E-box recognition by the complete gene product of the Max p21 transcription factor. *Biochemistry* **46**, 12427-12440.

Abstract: The Myc/Mad/Max network of dimeric basic region-helix-loop-helix-leucine zipper (b-HLH-LZ) transcription factors bind to enhancer box sequences (E-box) in the promoters of a large set of genes that control cell metabolism, proliferation, and differentiation. Max (Myc-associated factor X) is the obligate heterodimerization partner of Myc and Mad proteins. On the other hand, Max is the only member of the family capable of forming a stable homodimer. As part of the transcriptional regulation mechanism, Myc/Max and Mad/Max heterodimers and Max homodimers are thought to compete for binding to the E-box target sequences. E-box recognition is structurally supported by the b-HLH-LZ structural motif, which also promotes dimerization. However, the actual dimerization and heterodimerization constants of the complete gene products and their affinities for E-box sequences are not known. Also, the detailed thermodynamic characterization of DNA binding by these transcription factors has not been done yet. Such knowledge is necessary for complete understanding of the transcriptional regulation carried out by the Myc/Mad/Max network. Here, we report the first in-depth thermodynamic characterization of the stability and specific DNA binding of a full length gene product of the Myc/Mad/Max family, namely, Max protein isoform p21 (Max p21). Using calorimetric methods (DSC and ITC) we have determined the dimerization constant of Max p21 in the low micromolar range, and the Max p21/E-box complex dissociation constant in the low nanomolar range at 37 degrees C. The association is driven by a large exothermic effect, which is partly compensated by entropic factors. The energetic contribution to binding affinity of seven highly conserved residues that contact the DNA was probed by X-to-Ala mutagenesis. The results demonstrate that high binding affinity critically relies on the side chain of Arg 26. Furthermore, the mutational analysis points to the important role of the persistent helical turn that comprises this residue at the junction of the basic region and helix H1. Altogether, the study supports the idea that Max p21 can bind E-box sequences in vivo and likely participates directly in the regulation of transcription as homodimer.

Melkko S., Dumelin C. E., Scheuermann J., and Neri D. (2006) On the magnitude of the chelate effect for the recognition of proteins by pharmacophores scaffolded by self-assembling oligonucleotides. *Chem Biol* **13**, 225-231.

Abstract: The simultaneous interaction of the binding moieties of a bidentate ligand on adjacent epitopes of a target protein represents an attractive avenue for the discovery of specific, high-affinity binders. We used short DNA fragments in heteroduplex format to scaffold pairs of binding molecules with defined spatial arrangements. Iminobiotin derivatives were coupled either via bifunctional linkers or by using various oligonucleotides, thus allowing monovalent or bivalent binding to streptavidin. We determined the binding affinities of the synthesized constructs in solution. We also investigated the efficiency of recovery of superior bidentate ligands in affinity capture experiments, by using both radioactive counts and DNA microarrays as readouts. This analysis confirmed the suitability of the DNA heteroduplex as a scaffold for

the identification of synergistic pairs of binding moieties, capable of a high-affinity interaction with protein targets by virtue of the chelate effect.

Merabet E. and Ackers G. K. (1995) Calorimetric analysis of lambda cI repressor binding to DNA operator sites. *Biochemistry* **34**, 8554-8563.

Abstract: Enthalpies and heat capacities were determined by isothermal titration calorimetry for bacteriophage lambda cI repressor binding to DNA containing various combinations of the three operator sites OR1, OR2, and OR3 (each comprising a consensus half-site and a specific nonconsensus half-site). Differential scanning calorimetry was employed to evaluate the effects of specific DNA binding on thermal melting of the N-terminal and C-terminal repressor domains. Principal findings of this study are as follows: (1) Binding of repressor to each of the DNA operators is dominated by a large negative enthalpy, in agreement with earlier van't Hoff analyses of quantitative footprint titration data [Koblan & Ackers (1992) *Biochemistry* 31, 57-65]. The calorimetric data also revealed negative heat capacities for cI binding that are of comparable magnitude with many other systems [Spolar & Record (1994) *Science* 263, 777-784]. However, this feature in combination with the large negative values of binding enthalpies leads to an enthalpic dominance throughout the physiological temperature range. The resulting thermodynamic profile is opposite to the entropically dominated binding observed for many systems, including lambda cro repressor which binds to the same sites as cI and also employs a helix-turn-helix binding domain [Takeda et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89, 8180-8184]. It is suggested that these thermodynamic differences may arise from interactions of the cI repressor's N-terminal "arm" with the DNA. (2) Repressor monomers do not bind significantly to DNA containing either a consensus half-site or a nonconsensus half-site. Binding affinity to the double-consensus operator is much weaker than to any of the natural full-site operators. The same was found with other combinations of half-sites. A mutant repressor (PT158) which is severely defective in dimerization [Burz et al. (1994) *Biochemistry* 33, 8399-8405] was also found to bind only full-site operators and showed dimeric stoichiometry. (3) The thermal melting unit for N-terminal domains in the absence of DNA was found to reach values of 6-8 (monomer units) at concentrations where high-order oligomers of wild-type protein are formed [Senear et al. (1993) *Biochemistry* 32, 6179-6189]. However, in the presence of DNA operator sites, the cooperative unit for thermal unfolding was reduced to precisely two monomers, indicating that the N-terminal domain binds strictly as a dimer. (4) Significant nonadditivity was observed for the repressor binding enthalpies and heat capacities determined with multiple combinations of full-site operators. (ABSTRACT TRUNCATED AT 400 WORDS).

Milev S., Bosshard H. R., and Jelesarov I. (2005) Enthalpic and entropic effects of salt and polyol osmolytes on site-specific protein-DNA association: the integrase Tn916-DNA complex. *Biochemistry* **44**, 285-293.

Abstract: The effect of low molecular-weight compounds on the equilibrium constant $K(A)$ can be used to explore the energetics and molecular mechanism of protein-DNA interactions. Here we use the complex composed of the integrase Tn916 DNA-binding domain and its target DNA duplex to investigate the effects of salt and the nonionic osmolytes glycerol and sorbitol on sequence-specific protein-DNA association. Increasing Na^+ concentration from 0.12 to 0.32 M weakens the binding affinity by a factor of 20. The decrease of affinity is dominated by a large loss of binding enthalpy but only a small loss of binding entropy. This contrasts the concept that the salt-induced weakening of protein-DNA binding is mainly entropic. The large enthalpy loss is discussed in the light of recent views about the nature of the general salt effect. Addition of up to 2.5 M sorbitol and up to 3.3 M glycerol causes a slight increase of the binding affinity. However, both osmolytes lead to a large enthalpy gain and a similarly large entropy loss. This intriguing enthalpy-entropy compensation can be explained in part by an enthalpic chelate effect: The osmolyte tightens the structure of the protein-DNA complex whereby the formation of enthalpically favorable noncovalent interactions is promoted at the entropic cost of a more rigid complex. The results were obtained by isothermal titration calorimetry. They are supported by kinetic experiments showing that the rate of formation of the complex is reduced by salt, but the rate of complex dissociation is not. Glycerol and sorbitol reduce both rates in line with an only small effect on complex stability. This work clarifies the thermodynamic and kinetic response of a novel protein-DNA complex to increased salt and the presence of two common, nonionic osmolytes.

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 $-1.4 \text{ kJ K}^{-1} \text{ mol}^{-1}$ at 4 degrees C to $-2.9 \text{ kJ K}^{-1} \text{ mol}^{-1}$ 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 $-1.8 \text{ kJ K}^{-1} \text{ mol}^{-1}$ and temperature-independent. However, this value is still higher than ΔC_p of $-1.2 \text{ kJ K}^{-1} \text{ mol}^{-1}$ 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].

Minetti C. A., Remeta D. P., Zharkov D. O., Plum G. E., Johnson F., Grollman A. P., and Breslauer K. J. (2003) Energetics of lesion recognition by a DNA repair protein: thermodynamic characterization of formamidopyrimidine-glycosylase (Fpg) interactions with damaged DNA duplexes. *J Mol Biol* **328**, 1047-1060.

Abstract: As part of an overall effort to map the energetic landscape of the base excision repair pathway, we report the first thermodynamic characterization of repair enzyme binding to lesion-containing duplexes. Isothermal titration calorimetry (ITC) in conjunction with spectroscopic measurements and protease protection assays have been employed to characterize the binding of *Escherichia coli* formamidopyrimidine-glycosylase (Fpg), a bifunctional repair enzyme, to a series of 13-mer DNA duplexes. To resolve energetically the binding and the catalytic events, several of these duplexes are constructed with non-hydrolyzable lesion analogs that mimic the natural 8-oxo-dG substrate and the abasic-like intermediates. Specifically, one of the duplexes contains a central, non-hydrolyzable, tetrahydrofuran (THF) abasic site analog, while another duplex contains a central, carbocyclic substrate analog (carba-8-oxo-dG). ITC-binding studies conducted between 5.0 degrees C and 15.0 degrees C reveal that Fpg association with the THF-containing duplex is characterized by binding free energies that are relatively invariant to temperature (ΔG approximately $-9.5 \text{ kcalmol}^{-1}$), in contrast to both the reaction enthalpy and entropy that are strongly temperature-dependent. Complex formation between Fpg and the THF-containing duplex at 15 degrees C exhibits an unfavorable association enthalpy ($\Delta H=+7.5 \text{ kcalmol}^{-1}$) that is compensated by a favorable association entropy ($T\Delta S=+17.0 \text{ kcalmol}^{-1}$). The entropic nature of the binding interaction, coupled with the large negative heat capacity ($\Delta C_p=-0.67 \text{ kcaldeg}^{-1}\text{mol}^{-1}$), is consistent with Fpg complexation to the THF-containing duplex involving significant burial of non-polar surface areas. By contrast, under the high ionic strength buffer conditions employed herein (200 mM NaCl), no appreciable Fpg affinity for the carba-8-oxo-dG substrate analog is detected. Our results suggest that initial Fpg recognition of a damaged DNA site is predominantly electrostatic in nature, and does not involve large contact interfaces. Subsequent base excision presumably facilitates accommodation of the resulting lesion site into the binding pocket, as the enzyme interaction with the THF-containing duplex is characterized by high affinity and a large negative heat capacity change. Our data are consistent with a pathway in which Fpg glycosylase activity renders the base excision product a preferred ligand relative to the natural substrate, thereby ensuring the fidelity of removing highly reactive and potentially mutagenic abasic-like intermediates through catalytic elimination reactions.

Mishra S. H., Shelley C. M., Barrow D. J., Jr., Darby M. K., and Germann M. W. (2006) Solution structures and characterization of human immunodeficiency virus Rev responsive element IIB RNA targeting zinc finger proteins. *Biopolymers* **83**, 352-364.

Abstract: The Rev responsive element (RRE), a part of unspliced human immunodeficiency virus (HIV)

RNA, serves a crucial role in the production of infectious HIV virions. The viral protein Rev binds to RRE and facilitates transport of mRNA to the cytoplasm. Inhibition of the Rev-RRE interaction disrupts the viral life cycle. Using a phage display protocol, dual zinc finger proteins (ZNFs) were generated that bind specifically to RREIIB at the high affinity Rev binding site. These proteins were further shortened and simplified, and they still retained their RNA binding affinity. The solution structures of ZNF29 and a mutant, ZNF29G29R, have been determined by nuclear magnetic resonance (NMR) spectroscopy. Both proteins form C(2)H(2)-type zinc fingers with essentially identical structures. RNA protein interactions were evaluated quantitatively by isothermal titration calorimetry, which revealed dissociation constants ($K(d)$'s) in the nanomolar range. The interaction with the RNA is dependent upon the zinc finger structure; in the presence of EDTA, RNA binding is abolished. For both proteins, RNA binding is mediated by the alpha-helical portion of the zinc fingers and target the bulge region of RREIIB-TR. However, ZNF29G29R exhibits significantly stronger binding to the RNA target than ZNF29; this illustrates that the binding of the zinc finger scaffold is amenable to further improvements.

Morgan H. P., Estibeiro P., Wear M. A., Max K. E., Heinemann U., Cubeddu L., Gallagher M. P., Sadler P. J. and Walkinshaw M. D. (2007) Sequence specificity of single-stranded DNA-binding proteins: a novel DNA microarray approach. *Nucleic Acids Res* **35**, e75.

Abstract: We have developed a novel DNA microarray-based approach for identification of the sequence-specificity of single-stranded nucleic-acid-binding proteins (SNABPs). For verification, we have shown that the major cold shock protein (CspB) from *Bacillus subtilis* binds with high affinity to pyrimidine-rich sequences, with a binding preference for the consensus sequence, 5'-GTCTTTG/T-3'. The sequence was modelled onto the known structure of CspB and a cytosine-binding pocket was identified, which explains the strong preference for a cytosine base at position 3. This microarray method offers a rapid high-throughput approach for determining the specificity and strength of ss DNA-protein interactions. Further screening of this newly emerging family of transcription factors will help provide an insight into their cellular function.

Muller S., Bianchi M. E., and Knapp S. (2001) Thermodynamics of HMGB1 interaction with duplex DNA. *Biochemistry* **40**, 10254-10261.

Abstract: The high mobility group protein HMGB1 is a small, highly abundant protein that binds to DNA in a non-sequence-specific manner. HMGB1 consists of 2 DNA binding domains, the HMG boxes A and B, followed by a short basic region and a continuous stretch of 30 glutamate or aspartate residues. Isothermal titration calorimetry was used to characterize the binding of HMGB1 to the double-stranded model DNAs poly(dAdT).(dTdA) and poly(dGdC).(dCdG). To elucidate the contribution of the different structural motifs to DNA binding, calorimetric measurements were performed comparing the single boxes A and B, the two boxes plus or minus the basic sequence stretch (AB(bt) and AB), and the full-length HMGB1 protein. Thermodynamically, binding of HMGB1 and all truncated constructs to duplex DNA was characterized by a positive enthalpy change at 15 degrees C. From the slopes of the temperature dependence of the binding enthalpies, heat capacity changes of -0.129 ± 0.02 and -0.105 ± 0.05 kcal $\text{mol}^{-1} \text{K}^{-1}$ were determined for box A and full-length HMGB1, respectively. Significant differences in the binding characteristics were observed using full-length HMGB1, suggesting an important role for the acid tail in modulating DNA binding. Moreover, full-length HMGB1 binds differently these two DNA templates: binding to poly(dAdT).(dTdA) was cooperative, had a larger apparent binding site size, and proceeded with a much larger unfavorable binding enthalpy than binding to poly(dGdC).(dCdG).

Mishra, S.H., Shelley, C.M., Barrow Jr, D.J., Darby, M.K., and Germann, M.W. (2006) Solution structures and characterization of human immunodeficiency virus Rev responsive element IIB RNA targeting zinc finger proteins. *Biopolymers* **83**, 352 – 364.

Abstract: The Rev responsive element (RRE), a part of unspliced human immunodeficiency virus (HIV) RNA, serves a crucial role in the production of infectious HIV virions. The viral protein Rev binds to RRE and facilitates transport of mRNA to the cytoplasm. Inhibition of the Rev-RRE interaction disrupts the viral life cycle. Using a phage display protocol, dual zinc finger proteins (ZNFs) were generated that bind specifically to RREIIB at the high affinity Rev binding site. These proteins were further shortened and simplified, and they still retained their RNA binding affinity. The solution structures of ZNF29 and a mutant, ZNF29G29R, have been determined by nuclear magnetic resonance (NMR) spectroscopy. Both proteins form C2H2-type zinc fingers with essentially identical structures. RNA protein interactions were

evaluated quantitatively by isothermal titration calorimetry, which revealed dissociation constants (K_d 's) in the nanomolar range. The interaction with the RNA is dependent upon the zinc finger structure; in the presence of EDTA, RNA binding is abolished. For both proteins, RNA binding is mediated by the α -helical portion of the zinc fingers and target the bulge region of RREIIB-TR. However, ZNF29G29R exhibits significantly stronger binding to the RNA target than ZNF29; this illustrates that the binding of the zinc finger scaffold is amenable to further improvements.

O'Brien R., DeDecker B., Fleming K. G., Sigler P. B., and Ladbury J. E. (1998) The effects of salt on the TATA binding protein-DNA interaction from a hyperthermophilic archaeon. *J Mol Biol* **279**, 117-125.
Abstract: This study investigates the thermodynamics of the interaction of the TATA box binding protein (TBP) from *Pyrococcus woesei* (Pw) with an oligonucleotide containing a specific binding site. Pw is a hyperthermophilic archeal organism which exists under conditions of high salt and high temperature. A measurable protein-DNA interaction only occurs at high salt concentrations. Isothermal titration calorimetric binding studies were performed under a range of salts (potassium chloride, potassium phosphate, potassium acetate and sodium acetate) at varying concentrations (0.8 to 1.6 M). At the high salt concentrations used the observed equilibrium binding constant increases with increasing salt concentration. This is very different to the effect reported for all other protein-DNA interactions which have been studied at lower salt concentrations. Thermodynamic data suggest that the protein-DNA interaction at high salt concentration is accompanied by the removal of large numbers of water molecules from the buried hydrophobic surface area. In addition, the involvement of ions appears to influence the binding which can be explained by binding of cations in the interface between the electrostatically negative lateral lobes on the protein and the negatively charged DNA.

Oda M., Furukawa K., Ogata K., Sarai A., and Nakamura H. (1998) Thermodynamics of specific and non-specific DNA binding by the c-Myb DNA-binding domain. *J Mol Biol* **276**, 571-590.
Abstract: The thermodynamics of the c-Myb DNA-binding domain (R2R3) interaction with its target DNA have been analyzed using isothermal titration calorimetry and amino acid mutagenesis. The enthalpy of association between the standard R2R3, the Cys130 mutant substituted with Ile, and the cognate DNA is -12.5 (± 0.1) kcal mol⁻¹ at pH 7.5 and at 20 degrees C, and this interaction is enthalpically driven throughout the physiological temperature range. In order to understand the DNA recognition mechanism, several pairs of interactions were investigated using single and multiple-base alterations with single and multiple-amino acid substituted mutants. The interactions between the standard R2R3 and many non-cognate DNAs were accompanied by binding enthalpy changes and heat capacity changes, although their affinities were reduced. The roles of the electrostatic interactions in binding to the cognate and the non-cognate DNAs were also analyzed from the dependency of the thermodynamic parameters on the salt concentration. The heat capacity change was found to be significantly dependent upon the salt concentration. Several mutant proteins bound to the multiple-base altered DNA with very small enthalpy changes, although they bound to the cognate and the single-base altered DNAs with detectable enthalpy and heat capacity changes. From the thermodynamic cycles derived from the DNA binding of the amino acid substituted R2R3 to the base substituted DNA duplexes, the individual thermodynamic mechanisms of the specific DNA recognition of R2R3 were dissected. The local folding mechanism was highlighted by the substitution of Pro with either Gly or Ala at the linker between R2 and R3. The characteristic thermodynamic features of specific and non-specific DNA binding are discussed.

Oda M., Furukawa K., Sarai A., and Nakamura H. (1999) Construction of an artificial tandem protein of the c-Myb DNA-binding domain and analysis of its DNA binding specificity. *Biochem Biophys Res Commun* **262**, 94-97.

Abstract: An artificial tandem protein was generated using the third repeat of the c-Myb DNA-binding domain, and its DNA binding affinity and specificity were analyzed by a filter binding assay, isothermal titration calorimetry, and surface plasmon resonance. Although this artificial protein had the proper secondary structure, which is similar to the third repeat by itself, it could not bind to the expected base sequences specifically. Compared with the successful results of the zinc finger fusion proteins with novel sequence specificities, the cooperativity between the adjacent repeats, observed in the c-Myb-DNA complex, should also be required for the DNA recognition by the artificial tandem protein. Using the previous analyses of the DNA binding specificities by Myb homologous proteins, the differences in the DNA recognition mechanisms between the animal and plant Myb domains are also discussed.

Oda M. and Nakamura H. (2000) Thermodynamic and kinetic analyses for understanding sequence-specific DNA recognition. *Genes Cells* **5**, 319-326.

Abstract: Thermodynamic and kinetic analyses of biomolecular interactions reveal details of the energetic and dynamic features of molecular recognition processes, and complement structural analyses of the free and complexed conformations. The recent improvements in both isothermal titration calorimetry and surface plasmon resonance sensing provide powerful tools for analysing biomolecular interactions in thermodynamic and kinetic approaches. The thermodynamic and kinetic parameters obtained for binding between protein and DNA indicate the mechanism of specific DNA recognition, in the high-resolution structures of the protein-DNA complexes. The effects of temperature and ionic strength reflect the conformational changes of the protein and DNA molecules upon complex formation, including important contributions of water and solutes. When combined with mutational studies, the interactions can be reduced to several energetic contributions from individual contacts. These studies should be useful to determine general features of protein functions in genetic regulation.

Oddo C., Freire E., Frappier L., and Prat-Gay G. (2006) Mechanism of DNA recognition at a viral replication origin. *J Biol Chem* **281**, 26893-26903.

Abstract: Recognition of the DNA origin by the Epstein-Barr nuclear antigen 1 (EBNA1) protein is the primary event in latent phase genome replication of the Epstein-Barr virus, a model for replication initiation in eukaryotes. We carried out an extensive thermodynamic and kinetic characterization of the binding mechanism of the DNA binding domain of EBNA1, EBNA1452-641, to a DNA fragment containing a single specific origin site. The interaction displays a binding energy of 12.7 kcal mol⁻¹, with 11.9 kcal mol⁻¹ coming from the enthalpic change with a minimal entropic contribution. Formation of the EBNA1452-641.DNA complex is accompanied by a heat capacity change of -1.22 kcal mol⁻¹ K⁻¹, a very large value considering the surface area buried, which we assign to an unusually apolar protein-DNA interface. Kinetic dissociation experiments, including fluorescence anisotropy and a continuous native electrophoretic mobility shift assay, confirmed that two EBNA1.DNA complex conformers are in slow equilibrium; one dissociates slowly (t_{1/2} approximately 41 min) through an undissociated intermediate species and the other corresponds to a fast twostep dissociation route (t_{1/2} approximately 0.8 min). In line with this, at least two parallel association events from two populations of protein conformers are observed, with on-rates of 0.25-1.6x10⁸ m⁻¹ s⁻¹, which occur differentially either in excess protein or DNA molecules. Both parallel complexes undergo subsequent first order rearrangements of approximately 2.0 s⁻¹ to yield two consolidated complexes. These parallel association and dissociation routes likely allow additional flexible regulatory events for site recognition depending on site availability according to nucleus environmental conditions, which may lock a final recognition event, dissociate and re-bind, or slide along the DNA.

Oddone A., Lorentzen E., Basquin J., Gasch A., Rybin V., Conti E., and Sattler M. (2007) Structural and biochemical characterization of the yeast exosome component Rrp40. *EMBO Rep* **8**, 63-69.

Abstract: The exosome is a protein complex that is important in both degradation and 3'-processing of eukaryotic RNAs. We present the crystal structure of the Rrp40 exosome subunit from *Saccharomyces cerevisiae* at a resolution of 2.2 Å. The structure comprises an S1 domain and an unusual KH (K homology) domain. Close packing of the S1 and KH domains is stabilized by a GxNG sequence, which is uniquely conserved in exosome KH domains. Nuclear magnetic resonance data reveal the presence of a manganese-binding site at the interface of the two domains. Isothermal titration calorimetry shows that Rrp40 and archaeal Rrp4 alone have very low intrinsic affinity for RNA. The affinity of an archaeal core exosome for RNA is significantly increased in the presence of the S1-KH subunit Rrp4, indicating that multiple subunits might contribute to cooperative binding of RNA substrates by the exosome.

Ono S., Goldberg M.D., Olsson T., Esposito D., Hinton J.C., and Ladbury J.E. (2005) H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. *Biochem J* **391**, 203-13.

Abstract: Temperature is a primary environmental stress to which micro-organisms must be able to adapt and respond rapidly. Whereas some bacteria are restricted to specific niches and have limited abilities to survive changes in their environment, others, such as members of the Enterobacteriaceae, can withstand wide fluctuations in temperature. In addition to regulating cellular physiology, pathogenic bacteria use temperature as a cue for activating virulence gene expression. This work confirms that the nucleoid-associated protein H-NS (histone-like nucleoid structuring protein) is an essential component in thermoregulation of *Salmonella*. On increasing the temperature from 25 to 37 degrees C, more than 200

genes from *Salmonella enterica* serovar Typhimurium showed H-NS-dependent up-regulation. The thermal activation of gene expression is extremely rapid and change in temperature affects the DNA-binding properties of H-NS. The reduction in gene repression brought about by the increase in temperature is concomitant with a conformational change in the protein, resulting in the decrease in size of high-order oligomers and the appearance of increasing concentrations of discrete dimers of H-NS. The present study addresses one of the key complex mechanisms by which H-NS regulates gene expression.

Osawa M., Tong K. I., Lilliehook C., Wasco W., Buxbaum J. D., Cheng H. Y., Penninger J. M., Ikura M., and Ames J. B. (2001) Calcium-regulated DNA binding and oligomerization of the neuronal calcium-sensing protein, calsenilin/DREAM/KChIP3. *J Biol Chem* **276**, 41005-41013.

Abstract: Calsenilin/DREAM/KChIP3, a member of the recoverin branch of the EF-hand superfamily, interacts with presenilins, serves as a calcium-regulated transcriptional repressor, and interacts with A-type potassium channels. Here we report physicochemical characterization of calcium binding, oligomerization, and DNA binding of human calsenilin/DREAM/KChIP3. Equilibrium $\text{Ca}(2+)$ binding measurements indicate that the protein binds 3 $\text{Ca}(2+)$ with a dissociation constant of 14 μM and a Hill coefficient of 0.7. Dynamic light scattering and size exclusion chromatography show that the $\text{Ca}(2+)$ -bound protein exists as a dimer at protein concentrations lower than 150 μM and forms a tetramer at concentrations above 200 μM . The $\text{Ca}(2+)$ -free protein is a tetramer in the concentration range 20-450 μM . Isothermal titration calorimetry and dynamic light scattering indicate that the $\text{Ca}(2+)$ -free protein tetramer binds endothermically ($\Delta H = +25$ kcal/mol) to four molecules of DNA derived from the downstream regulatory element (DRE) of either the prodynorphin or c-fos genes. One DRE molecule binds tightly to the protein with a dissociation constant (K_d) of 75 nM, and the other three bind more weakly ($K_d = 640$ nM). No significant DNA binding was observed for the $\text{Ca}(2+)$ -bound protein. The N-terminal protein fragment (residues 1-70) binds nonspecifically to DRE in a $\text{Ca}(2+)$ -independent manner, whereas a C-terminal fragment containing the four EF-hands (residues 65-256) binds DRE ($K(d) = 200$ nM) in a $\text{Ca}(2+)$ -regulated and sequence-specific fashion. The C-terminal fragment is a tetramer in the $\text{Ca}(2+)$ -free state and dissociates into dimers at saturating $\text{Ca}(2+)$ levels.

Oste-Triantafyllou A., Wehtje E., Adlercreutz P., and Mattiasson B. (1996) Calorimetric studies on solid alpha-chymotrypsin preparations in air and in organic solvents. *Biochim Biophys Acta* **1295**, 110-118.

Abstract: Differential scanning calorimetry was the method to investigate the thermostability of chymotrypsin. The transition temperature decreased by approx. 30 degrees C when the dry enzyme became highly hydrated. High degree of hydration corresponded to extensive conformational changes during protein denaturation, reflected by large enthalpy values. Sorbitol, lyophilized together with the enzyme, caused the destabilization of the complex within the whole range of water activities. When the enzyme was equilibrated through the apolar solvent, iso-octane, stabilization of chymotrypsin was observed at high water activities, compared to equilibration in air. The presence of iso-octane resulted in a remarkable stabilization of the chymotrypsin-sorbitol complex. A sorbitol concentration of 5 mmol/g of protein was prerequisite to induce stabilization when equilibrated through iso-octane at high water activities. The transition enthalpy increased with increasing amounts of sorbitol. Different hydration isotherms were obtained for the air-equilibrated and solvent-equilibrated enzyme preparations. Increasing amounts of buffer salts within the chymotrypsin preparation caused the enhancement of both the temperature and the enthalpy of the transition at a water activity 0.97. Variations on the hydration of the preparations both offered the explanation to the thermal stability results and supported the evidence obtained from enzyme activity studies. Generally, the catalyst whose hydration was suppressed due to its environment exhibited low enzymatic activity.

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.

Palma E., Klapper D. G., and Cho M. J. (2005) Antibodies as drug carriers III: design of oligonucleotides with enhanced binding affinity for immunoglobulin G. *Pharm Res* **22**, 122-127.

Abstract: PURPOSE: To understand the structural requirements in designing epitope-bearing oligonucleotides with high antibody-binding affinity. METHODS: Binding affinity (K_A) and stoichiometry (n) of dinitrophenyl (DNP)-derivatized model 27-mer oligonucleotides (ODNs), GGG(AAA)7GGG, to monoclonal anti-trinitrophenyl (TNP) antibodies were determined using isothermal titration calorimetry (ITC). Structural variations were made in the ODNs to assess the effects of antigenic valence, epitope density, inter-epitope linker length, and linker flexibility. Binding isotherms were fitted with a single binding-site model to obtain $K(A)$ and n , from which changes in Gibbs free energy ($\Delta G(0)$), entropy ($\Delta S(0)$), and enthalpy ($\Delta H(0)$) were derived. RESULTS: As expected, ligands displaying increased epitope density showed increases in $K(A)$: for example, $K(A)$ for (DNP)2-Cys is 3.3-fold greater than that for DNP-Lys. Introduction of multiple DNP groups via long and flexible linkers to one end of the 27-mer ODN resulted in a bivalent behavior with n value of 1. A bivalent ligand, derivatized at both ends with a long and flexible linker, failed to form an immune complex when hybridized to its antisense strand, presumably due to intercalation of the DNP moiety to the double strand. ODNs derivatized with flexible linkers exhibited a higher $K(A)$ than those with a rigid linker. Ligands with flexible inter-epitope linkers measuring distances of 110, 60, and 40 angstroms yielded 13-, 30-, and 13-fold increases in $K(A)$, respectively. The combination of these factors; namely, bivalence, flexible inter-epitope linkers, and optimal inter-epitope distance, resulted in an overall 66-fold increase in $K(A)$. Thermodynamic analysis of binding indicates that the formation of high-affinity ODN-IgG complexes was a spontaneous and exothermic event, characterized by large negative ΔS degrees, ΔH degrees, and ΔG degrees values. CONCLUSIONS: All four strategies tested during this investigation, namely bivalence, epitope density, inter-epitope linker flexibility, and optimal inter-epitope distance, proved to be useful in improving the binding affinity of DNP-labeled ODNs to anti-TNP IgG. The final ODN design incorporating these strategies will be used in testing the systemic pharmacokinetic advantage gained from complexing such ODNs to IgG.

Park C., Schultz L. W., and Raines R. T. (2001) Contribution of the active site histidine residues of ribonuclease A to nucleic acid binding. *Biochemistry* **40**, 4949-4956.

Abstract: His12 and His119 are critical for catalysis of RNA cleavage by ribonuclease A (RNase A). Substitution of either residue with an alanine decreases the value of $k(\text{cat})/K(M)$ by more than 10(4)-fold. His12 and His119 are proximal to the scissile phosphoryl group of an RNA substrate in enzyme-substrate complexes. Here, the role of these active site histidines in RNA binding was investigated by monitoring the effect of mutagenesis and pH on the stability of enzyme-nucleic acid complexes. X-ray diffraction analysis of the H12A and H119A variants at a resolution of 1.7 and 1.8 Å, respectively, shows that the amino acid substitutions do not perturb the overall structure of the variants. Isothermal titration calorimetric studies on the complexation of wild-type RNase A and the variants with 3'-UMP at pH 6.0 show that His12 and His119 contribute 1.4 and 1.1 kcal/mol to complex stability, respectively. Determination of the stability of the complex of wild-type RNase A and 6-carboxyfluorescein approximately d(AUAA) at varying pHs by fluorescence anisotropy shows that the stability increases by 2.4 kcal/mol as the pH decreases from 8.0 to 4.0. At pH 4.0, replacing His12 with an alanine residue decreases the stability of the complex with 6-carboxyfluorescein approximately d(AUAA) by 2.3 kcal/mol. Together, these structural and thermodynamic data provide the first thorough analysis of the contribution of histidine residues to nucleic acid binding.

Patel M. M. and Anchordoquy T. J. (2006) Ability of spermine to differentiate between DNA sequences--preferential stabilization of A-tracts. *Biophys Chem* **122**, 5-15.

Abstract: The regulatory roles fulfilled by polyamines by governance of chromatin structure are made

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

Patston P. A., Church F. C., and Olson S. T. (2004) Serpin-ligand interactions. *Methods* **32**, 93-109.

Abstract: One of the more common features of serpins is the ability to bind various ligands. Ligand binding can occur so that the inhibitory properties of the serpin are regulated, so that the serpin can be localized, or to produce or modulate some other biological function of the serpin. Ligands known to affect serpin biologic activity include glycosaminoglycans such as heparin, heparan sulfate and dermatan sulfate, DNA, extracellular matrix proteins such as vitronectin and collagen, and small organic molecule hormones. Many different biochemical and biophysical techniques in conjunction with molecular biology and cell biology approaches have been used to study the binding of various ligands to serpins and to assess the influence of this binding on activity and structure. We summarize here the different approaches that have been used to identify serpin ligands and the many methods that have been used to characterize the interactions of these ligands with their cognate serpins.

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 ($K_a = 3.5(+/-1.6) \times 10^6 M^{-1}$) 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+/-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 ($\Delta C(p)$) 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 $\Delta C(p)$ 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.

Pommer A. J., Cal S., Keeble A. H., Walker D., Evans S. J., Kuhlmann U. C., Cooper A., Connolly B. A., Hemmings A. M., Moore G. R., James R., and Kleanthous C. (2001) Mechanism and cleavage specificity of the H-N-H endonuclease colicin E9. *J Mol Biol* **314**, 735-749.

Abstract: Colicin endonucleases and the H-N-H family of homing enzymes share a common active site structural motif that has similarities to the active sites of a variety of other nucleases such as the non-specific endonuclease from *Serratia* and the sequence-specific His-Cys box homing enzyme I-PpoI. In contrast to these latter enzymes, however, it remains unclear how H-N-H enzymes cleave nucleic acid substrates. Here, we show that the H-N-H enzyme from colicin E9 (the E9 DNase) shares many of the same basic enzymological characteristics as sequence-specific H-N-H enzymes including a dependence for high concentrations of Mg^{2+} or Ca^{2+} with double-stranded substrates, a high pH optimum (pH 8-9) and inhibition by monovalent cations. We also show that this seemingly non-specific enzyme preferentially nicks double-stranded DNA at thymine bases producing 3'-hydroxy and 5'-phosphate termini, and that the enzyme does not cleave small substrates, such as dinucleotides or nucleotide analogues, which has implications for its mode of inhibition in bacteria by immunity proteins. The E9 DNase will also bind single-stranded DNA above a certain length and in a sequence-independent manner, with transition metals such as Ni^{2+} optimal for cleavage but Mg^{2+} a poor cofactor. Ironically, the H-N-H motif of the E9 DNase although resembling the zinc binding site of a metalloenzyme does not support zinc-mediated hydrolysis of any DNA substrate. Finally, we demonstrate that the E9 DNase also degrades RNA in the absence of metal ions. In the context of current structural information, our data show that the H-N-H motif is an adaptable catalytic centre able to hydrolyse nucleic acid by different mechanisms depending on the substrate and metal ion regime.

Potty A. S., Kourentzi K., Fang H., Jackson G. W., Zhang X., Legge G. B. and Willson R. C. (2008) Biophysical characterization of DNA aptamer interactions with vascular endothelial growth factor. *Biopolymers* **91**, 145-156.

Abstract: The binding of a DNA aptamer (5'-CCGTCTTCCAGACAAGAGTGCAGGG-3') to recombinant human vascular endothelial growth factor (VEGF(165)) was characterized using surface plasmon resonance (SPR), fluorescence anisotropy and isothermal titration calorimetry (ITC). Results from both fluorescence anisotropy and ITC indicated that a single aptamer molecule binds to each VEGF homodimer, unlike other VEGF inhibitors that exhibit 2(ligand):1(VEGF homodimer) stoichiometry. In addition, ITC revealed that the association of the aptamer to VEGF at 20 degrees C is enthalpically driven, with an unfavorable entropy contribution. SPR kinetic studies, with careful control of possible mass transfer effects, demonstrated that the aptamer binds to VEGF with an association rate constant $k(on) = 4.79 \pm 0.03 \times 10(4) M^{-1} s^{-1}$ and a dissociation rate constant $k(off) = 5.21 \pm 0.02 \times 10(-4) s^{-1}$ at 25 degrees C. Key recognition hot-spots were determined by a combination of aptamer sequence substitutions, truncations, and extensions. Most single-nucleotide substitutions, particularly within an mfold-predicted stem, suppress binding, whereas those within a predicted loop have a minimal effect. The 5'-end of the aptamer plays a key role in VEGF recognition, as a single-nucleotide truncation abolished VEGF binding. Conversely, an 11-fold increase in the association rate (and affinity) is observed with a single cytosine nucleotide extension, due to pairing of the 3'-GGG with 5'-CCC in the extended aptamer. Our approach effectively maps the secondary structural elements in the free aptamer, which present the unpaired interface for high affinity VEGF recognition. These data demonstrate that a directed binding analysis can be used in concert with library screening to characterize and improve aptamer/ligand recognition. (c) 2008 Wiley Periodicals, Inc. *Biopolymers* 91: 145-156, 2009. This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of the preprint by emailing the *Biopolymers* editorial office at biopolymers@wiley.com

Poujol N., Margeat E., Baud S., and Royer C. A. (2003) RAR antagonists diminish the level of DNA binding by the RAR/RXR heterodimer. *Biochemistry* **42**, 4918-4925.

Abstract: A purified RAR/RXR- Δ AB heterodimer was obtained by production of His-tagged RAR and untagged RXR in *Escherichia coli*, followed by combined purification on a Ni(2+) affinity column using excess RXR extract, and finally a gel filtration chromatography step to isolate a pure heterodimer. The purified heterodimer preparation bound 9-cisRA at a level of 0.85-0.95 mol of binding sites per mole of protein monomer. Titration of a 26 kDa fluorescent labeled fragment of the SRC-1 coactivator protein with the purified heterodimer in the presence of the agonist 9-cisRA yielded a binding affinity near 300 nM, whereas no binding was observed in the absence of agonist. Binding of the purified heterodimer to a DR5 target was identical in the absence of ligand and in the presence of 9-cisRA. Competition by unlabeled specific and nonspecific DNA allowed us to demonstrate that the binding curve was bimodal. The first phase of binding was highly specific and of high affinity. This phase also exhibited a high degree of cooperativity in the binding profile. Nonspecific DNA efficiently competes for the second phase. Thus, the first phase of binding likely corresponds to the formation of the specific heterodimer complex in which heterodimerization is energetically coupled to DNA binding. While agonist binding had no effect on the apparent affinity of the heterodimer for DR5, a series of antagonists significantly destabilized the heterodimer-DR5 complex, either through a direct decrease in the affinity of the protein for the DNA or through destabilization of the heterodimer itself. Impeding the interaction between the heterodimer and DNA appears as an additional mechanism of antagonist action of varying efficiency, depending upon the chemical structure of the antagonist.

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 $4 \times 10^7 \text{ M}^{-1}$, 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 $-4 \text{ kJ K}^{-1} \text{ mol}^{-1}$, 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 $-2 \text{ kJ K}^{-1} \text{ mol}^{-1}$, 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 predenaturational 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.

Prongidi-Fix L., Sugawara M., Bertani P., Raya J., Leborgne C., Kichler A. and Bechinger B. (2007) Self-promoted cellular uptake of peptide/DNA transfection complexes. *Biochemistry* **46**, 11253-11262.

Abstract: The designed alpha-helical amphipathic peptide LAH4 assembles several properties, which makes it an interesting candidate as a gene-delivery vehicle. Besides being short and soluble in aqueous solutions, LAH4 presents cationic residues, which allow for efficient complexation of DNA. In addition, this peptide is poorly hemolytic at neutral pH, while it is able to destabilize biological membranes in acidic conditions. In this study, the structure of the peptide/DNA transfection complex was examined by circular dichroism and solid-state nuclear magnetic resonance spectroscopies and the thermodynamics of its formation and disassembly was monitored in a quantitative manner as a function of pH by isothermal titration calorimetry. Notably, the number of peptides within the complex considerably decreases upon acidification of the medium. This observation has direct and important consequences for the mechanism of action because the acidification of the endosome results in high local concentrations of free peptide in this organelle. Thus, these peptides become available to interact with the endosomal membranes and thereby responsible for the delivery of the transfection complex to the cytoplasm. When these data are taken together, they indicate a dual role of the peptide during the transfection process, namely, DNA complexation and membrane permeabilization.

Rajan, R., Wisler, J.W., and Bell, C.E. (2006) Probing the DNA sequence specificity of Escherichia coli RECA protein. *Nucleic Acids Res* **34**, 2463-2471.

Abstract: Escherichia coli RecA protein catalyzes the central DNA strand-exchange step of homologous recombination, which is essential for the repair of double-stranded DNA breaks. In this reaction, RecA first polymerizes on single-stranded DNA (ssDNA) to form a right-handed helical filament with one monomer per 3 nt of ssDNA. RecA generally binds to any sequence of ssDNA but has a preference for GT-rich sequences, as found in the recombination hot spot Chi (5'-GCTGGTGG-3'). When this sequence is located within an oligonucleotide, binding of RecA is phased relative to it, with a periodicity of three nucleotides. This implies that there are three separate nucleotide-binding sites within a RecA monomer that may exhibit preferences for the four different nucleotides. Here we have used a RecA coprotease assay to further probe the ssDNA sequence specificity of E.coli RecA protein. The extent of self-cleavage of a λ repressor fragment in the presence of RecA, ADP-AlF₄ and 64 different trinucleotide-repeating 15mer oligonucleotides was determined. The coprotease activity of RecA is strongly dependent on the ssDNA sequence, with TGG-repeating sequences giving by far the highest coprotease activity, and GC and AT-rich sequences the lowest. For selected trinucleotide-repeating sequences, the DNA-dependent ATPase and DNA-binding activities of RecA were also determined. The DNA-binding and coprotease activities of RecA have the same sequence dependence, which is essentially opposite to that of the ATPase activity of RecA. The implications with regard to the biological mechanism of RecA are discussed.

Ramprakesh J. and Schwarz F. P. (2005) Energetic differences between the specific binding of a 40bp DNA duplex and the lac promoter to lac repressor protein. *Arch Biochem Biophys* **438**, 162-173.

Abstract: The energetics of LRP binding to a 104bp lac promoter determined from ITC measurements were compared to the energetics of binding to a shorter 40bp DNA duplex with the 21bp promoter binding site sequence. The promoter binding affinity of $2.47 \pm 0.01 \times 10^7 \text{ M}^{-1}$ was higher than the DNA binding affinity of $1.81 \pm 0.67 \times 10^7 \text{ M}^{-1}$ while the binding enthalpy of $-804 \pm 41 \text{ kJ mol}^{-1}$ was lower than that of the DNA binding enthalpy of $-145 \pm 16 \text{ kJ mol}^{-1}$ at 298.15K. Both the promoter and DNA binding reactions were exothermic in phosphate buffer but endothermic in Tris buffer that showed the transfer of four protons to LRP in the former reaction but only two in the latter. A more complicated dependence of these parameters on temperature was observed for promoter binding. These energetic differences are attributable to additional LRP-promoter interactions from wrapping of the promoter around the LRP.

Randell J.C., Komazin G., Jiang C., Hwang C.B., and Coen DM. (2005) Effects of substitutions of arginine residues on the basic surface of herpes simplex virus UL42 support a role for DNA binding in processive DNA synthesis. *J Virol.* **79**, 12025-34.

Abstract: The way that UL42, the processivity subunit of the herpes simplex virus DNA polymerase, interacts with DNA and promotes processivity remains unclear. A positively charged face of UL42 has

been proposed to participate in electrostatic interactions with DNA that would tether the polymerase to a template without preventing its translocation via DNA sliding. An alternative model proposes that DNA binding by UL42 is not important for processivity. To investigate these issues, we substituted alanine for each of four conserved arginine residues on the positively charged surface. Each single substitution decreased the DNA binding affinity of UL42, with 14- to 30-fold increases in apparent dissociation constants. The mutant proteins exhibited no meaningful change in affinity for binding to the C terminus of the catalytic subunit of the polymerase, indicating that the substitutions exert a specific effect on DNA binding. The substitutions decreased UL42-mediated long-chain DNA synthesis by the polymerase in the same rank order in which they affected DNA binding, consistent with a role for DNA binding in polymerase processivity. Combining these substitutions decreased DNA binding further and impaired the complementation of a UL42 null virus in transfected cells. Additionally, using a revised mathematical model to analyze rates of dissociation of UL42 from DNAs of various lengths, we found that dissociation from internal sites, which would be the most important for tethering the polymerase, was relatively slow, even at ionic strengths that permit processive DNA synthesis by the holoenzyme. These data provide evidence that the basic surface of UL42 interacts with DNA and support a model in which DNA binding by UL42 is important for processive DNA synthesis.

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

Read M. A., Wood A. A., Harrison J. R., Gowan S. M., Kelland L. R., Dosanjh H. S., and Neidle S. (1999) Molecular modeling studies on G-quadruplex complexes of telomerase inhibitors: structure-activity relationships. *J Med Chem* **42**, 4538-4546.

Abstract: Inhibition of the ability of the enzyme telomerase to add telomeric repeats to the end of chromosomes is a novel target for potential anticancer therapy. This paper examines the hypothesis that compounds possessing a planar aromatic chromophore inhibit telomerase via stabilization of, and binding to, a folded guanine quadruplex structure. Two series of telomerase inhibitors have been designed based on the 2,6-disubstituted amidoanthracene-9,10-dione and 3,6-disubstituted acridine chromophores in order to investigate structure-activity relationships between biological activity and substituent group size. The relative binding energies between these compounds and the folded human telomere DNA quadruplex were determined using molecular simulation methods, involving explicitly solvated structures. The results obtained are in excellent agreement with the biological activity as measured in vitro using a modified TRAP assay and in general agreement with the ranking order of binding enthalpies found in isothermal titration calorimetry studies. This broad agreement provides strong support for the hypothesis that guanine quadruplexes are the primary target for telomerase inhibitors with extended planar chromophores.

Recht M. I. and Williamson J. R. (2001) Central domain assembly: thermodynamics and kinetics of S6 and S18 binding to an S15-RNA complex. *J Mol Biol* **313**, 35-48.

Abstract: The 30 S ribosomal subunit assembles in vitro through the hierarchical binding of 21 ribosomal proteins to 16 S rRNA. The central domain of 16 S rRNA becomes the platform of the 30 S subunit upon binding of ribosomal proteins S6, S8, S11, S15, S18 and S21. The assembly of the platform is nucleated by binding of S15 to 16 S rRNA, followed by the cooperative binding of S6 and S18. The prior binding of S6 and S18 is required for binding of S11 and S21. We have studied the mechanism of the cooperative binding of S6 and S18 to the S15-rRNA complex by isothermal titration calorimetry and gel mobility shift assays with rRNA and proteins from the hyperthermophilic bacterium *Aquifex aeolicus*. S6 and S18 form a stable heterodimer in solution with an apparent dissociation constant of 8.7 nM at 40 degrees C. The S6:S18 heterodimer binds to the S15-rRNA complex with an equilibrium dissociation constant of 2.7 nM at 40 degrees C. Consistent with previous studies using rRNA and proteins from *Escherichia coli*, we observed no binding of S6 or S18 in the absence of the other protein or S15. The presence of S15 increases the affinity of S6:S18 for the RNA by at least four orders of magnitude. The kinetics of S6:S18 binding to the S15-rRNA complex are slow, with an apparent bimolecular rate constant of $8.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and an apparent unimolecular dissociation rate of $1.6 \times 10^{-4} \text{ s}^{-1}$. These results, which are consistent with a model in which S6 and S18 bind as a heterodimer to the S15-rRNA complex, provide a mechanistic framework to describe the previously observed S15-mediated cooperative binding of S6 and S18 in the ordered assembly of a multi-protein ribonucleoprotein complex.

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

Schuermann J. P., Henzl M. T., Deutscher S. L., and Tanner J. J. (2004) Structure of an anti-DNA fab complexed with a non-DNA ligand provides insights into cross-reactivity and molecular mimicry. *Proteins* **57**, 269-278.

Abstract: Antibodies that recognize DNA (anti-DNA) are part of the autoimmune response underlying systemic lupus erythematosus. To better understand molecular recognition by anti-DNA antibodies, crystallographic studies have been performed using an anti-ssDNA antigen-binding fragment (Fab) known as DNA-1. The previously determined structure of a DNA-1/dT5 complex revealed that thymine bases insert into a narrow groove, and that ligand recognition primarily involves the bases of DNA. We now report the 1.75-Å resolution structure of DNA-1 complexed with the biological buffer HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid). All three light chain complementarity-determining regions (CDRs) and HCDR3 contribute to binding. The HEPES sulfonate hydrogen bonds to His L91, Asn L50, and to the backbone of Tyr H100 and Tyr H100A. The Tyr side-chains of L32, L92, H100, and H100A form nonpolar contacts with the HEPES ethylene and piperazine groups. Comparison to the DNA-1/dT5 structure reveals that the dual recognition of dT5 and HEPES requires a 13-Å movement of HCDR3. This dramatic structural change converts the combining site from a narrow groove, appropriate for the edge-on insertion of thymine bases, to one sufficiently wide to accommodate the HEPES sulfonate and piperazine. Isothermal titration calorimetry verified the association of HEPES with DNA-1 under conditions similar those used for crystallization (2 M ammonium sulfate). Interestingly, the presence of 2 M ammonium sulfate increases the affinities of DNA-1 for both HEPES and dT5, suggesting that non-polar Fab-ligand interactions are important for molecular recognition in highly ionic solvent conditions. The structural and thermodynamic data suggest a molecular mimicry mechanism based on structural plasticity and hydrophobic interactions.

Seldeen K. L., McDonald C. B., Deegan B. J. and Farooq A. (2008) Coupling of folding and DNA-binding in the bZIP domains of Jun-Fos heterodimeric transcription factor. *Arch Biochem Biophys* **473**, 48-60.

Abstract: In response to mitogenic stimuli, the heterodimeric transcription factor Jun-Fos binds to the promoters of a diverse array of genes involved in critical cellular responses such as cell growth and proliferation, cell cycle regulation, embryogenic development and cancer. In so doing, Jun-Fos heterodimer regulates gene expression central to physiology and pathology of the cell in a specific and timely manner. Here, using the technique of isothermal titration calorimetry (ITC), we report detailed thermodynamics of the bZIP domains of Jun-Fos heterodimer to synthetic dsDNA oligos containing the TRE and CRE consensus promoter elements. Our data suggest that binding of the bZIP domains to both TRE and CRE is under enthalpic control and accompanied by entropic penalty at physiological temperatures. Although the bZIP domains bind to both TRE and CRE with very similar affinities, the enthalpic contributions to the free energy of binding to CRE are more favorable than TRE, while the entropic penalty to the free energy of binding to TRE is smaller than CRE. Despite such differences in their thermodynamic signatures, enthalpy and entropy of binding of the bZIP domains to both TRE and CRE are highly temperature-dependent and largely compensate each other resulting in negligible effect of temperature on the free energy of binding. From the plot of enthalpy change versus temperature, the magnitude of heat capacity change determined is much larger than that expected from the direct association of bZIP domains with DNA. This observation is interpreted to suggest that the basic regions in the bZIP domains are largely unstructured in the absence of DNA and only become structured upon interaction with DNA in a coupled folding and binding manner. Our new findings are rationalized in the context of 3D structural models of bZIP domains of Jun-Fos heterodimer in complex with dsDNA oligos containing the TRE and CRE consensus sequences. Taken

together, our study demonstrates that enthalpy is the major driving force for a key protein-DNA interaction pertinent to cellular signaling and that protein-DNA interactions with similar binding affinities may be accompanied by differential thermodynamic signatures. Our data corroborate the notion that the DNA-induced protein structural changes are a general feature of the bZIP family of transcription factors

Seldeen K. L., McDonald C. B., Deegan B. J. and Farooq A. (2008) Evidence that the bZIP domains of the Jun transcription factor bind to DNA as monomers prior to folding and homodimerization. *Arch Biochem Biophys* **480**, 75-84.

Abstract: The Jun oncoprotein belongs to the AP1 family of transcription factors that is collectively engaged in diverse cellular processes by virtue of their ability to bind to the promoters of a wide spectrum of genes in a DNA sequence-dependent manner. Here, using isothermal titration calorimetry, we report detailed thermodynamics of the binding of bZIP domain of Jun to synthetic dsDNA oligos containing the TRE and CRE consensus promoter elements. Our data suggest that binding of Jun to both sites occurs with indistinguishable affinities but with distinct thermodynamic signatures comprised of favorable enthalpic contributions accompanied by entropic penalty at physiological temperatures. Furthermore, anomalously large negative heat capacity changes observed provoke a model in which Jun loads onto DNA as unfolded monomers coupled with subsequent folding and homodimerization upon association. Taken together, our data provide novel insights into the energetics of a key protein-DNA interaction pertinent to cellular signaling and cancer. Our study underscores the notion that the folding and dimerization of transcription factors upon association with DNA may be a more general mechanism employed in protein-DNA interactions and that the conventional school of thought may need to be re-evaluated

Shi Y., Wang S., Krueger S., and Schwarz F. P. (1999) Effect of mutations at the monomer-monomer interface of cAMP receptor protein on specific DNA binding. *J Biol Chem* **274**, 6946-6956.

Abstract: To determine the thermodynamic role of binding of an operon to cAMP receptor protein (CRP) in the activation of transcription, isothermal titration calorimetry measurements were performed on the binding of three 40-base pair DNA sequences to the cyclic nucleoside complexes of CRP and its mutants at 296 K. The three 40-base pair sequences consisted of a consensus DNA (conDNA) duplex derived from the CRP-binding site sequences of the operons activated by CRP and two DNA sequences based on the CRP-binding site sequences of the lac operon (lacDNA) and of the gal operon (galDNA). The mutants of CRP consisted of a T127L mutant, a S128A mutant, and a mutant containing both mutations (CRP*) which not only alter the transcriptional activity of the CRP complexes but also are involved in the monomer-monomer interfacial interactions of the CRP dimer. The binding reactions of the DNA duplexes to the fully cNMP-ligated CRP-mutant complexes were endothermic with binding constants as high as $6.6 \pm 1.1 \times 10^6 \text{ M}^{-1}$ (conDNA.CRP(cAMP)₂). ConDNA binding to the unligated T127L and CRP* mutants was observed as well as conDNA and lacDNA binding to CRP with cAMP bound to only one monomer. The reduction of the binding constants with increase in KCl concentration indicated the formation of two ion pairs for the cAMP-ligated CRP and S128A complexes and four ion pairs for the cAMP-ligated T127L and CRP* complexes. Reduction of the DNA binding constants upon substitution of D₂O for H₂O in the buffer, the large heat capacity changes, and the enthalpy-entropy compensation exhibited by the binding reactions indicate the importance of dehydration in the binding reaction. Small angle neutron scattering measurements on the lacDNA.CRP(cAMP)₂ complex in D₂O/H₂O mixtures show that the DNA is bent around the cAMP-ligated protein in solution.

Shuttleworth G., Fogg M. J., Kurpiewski M. R., Jen-Jacobson L., and Connolly B. A. (2004) Recognition of the pro-mutagenic base uracil by family B DNA polymerases from archaea. *J Mol Biol* **337**, 621-634.

Abstract: Archaeal family B DNA polymerases contain a specialised pocket that binds tightly to template-strand uracil, causing the stalling of DNA replication. The mechanism of this unique "template-strand proof-reading" has been studied using equilibrium binding measurements, DNA footprinting, van't Hoff analysis and calorimetry. Binding assays have shown that the polymerase preferentially binds to uracil in single as opposed to double-stranded DNA. Tightest binding is observed using primer-templates that contain uracil four bases in front of the primer-template junction, corresponding to the observed stalling position. Ethylation interference analysis of primer-templates shows that the two phosphates, immediately flanking the uracil (NpUpN), are important for binding; contacts are also made to phosphates in the primer-strand. Microcalorimetry and van't Hoff analysis have given a fuller understanding of the thermodynamic parameters involved in uracil recognition. All the results are consistent with a "read-ahead" mechanism, in

which the replicating polymerase scans the template, ahead of the replication fork, for the presence of uracil and halts polymerisation on detecting this base. Post-stalling events, serving to eliminate uracil, await full elucidation.

Sieber M. and Allemann R. K. (2000) Thermodynamics of DNA binding of MM17, a 'single chain dimer' of transcription factor MASH-1. *Nucleic Acids Res* **28**, 2122-2127.

Abstract: MASH-1, a member of the basic helix-loop-helix (bHLH) family of transcriptional regulators, is a central factor for the regulation of the differentiation of committed neuronal precursor cells of the peripheral nervous system. We have previously produced MM17, a single chain version of this dimeric protein, by linking the C-terminal end of the first subunit to the N-terminal residue of the second subunit through a flexible peptide linker. We have now determined by isothermal titration calorimetry the thermodynamic parameters characterising the DNA binding reactions of MM17. The DNA binding specificity was relatively low and comparable to that observed for wild-type MASH bHLH. At 32 degrees C and pH 7, the concentration of MM17 at which 50% DNA binding occurred was determined as 22.8 and 152 nM for binding to MCK-S and the heterologous SP-1, respectively. Similarly to MASH bHLH the free energy of the association was only slightly temperature dependent, while both the entropy and the enthalpy change were strong functions of temperature. The free energy of DNA binding was independent of the pH for the pH range between 6 and 8. Dissection of the entropy change of the association reaction suggested that the two basic domains and the linker region between the subunits underwent a folding transition from a mainly unfolded to a predominantly ordered conformation. Therefore, like wild-type MASH bHLH, the DNA binding reaction of MM17 follows an induced fit mechanism.

Singh M., D'Silva L., and Holak T. A. (2006) DNA-binding properties of the recombinant high-mobility-group-like AT-hook-containing region from human BRG1 protein. *Biol Chem* **387**, 1469-1478.

Abstract: The hBRG1 protein, a central ATPase of the human switching/sucrose non-fermenting (SWI/SNF) remodeling complex, has a catalytic ATPase domain, an AT-hook motif and a bromodomain. Bromodomains, found in many chromatin-associated proteins, recognize N-acetyl-lysine in histones and other proteins. The AT-hook motif, first described in the high-mobility group of non-histone chromosomal proteins HMGA1/2, is a DNA-binding motif. The AT-hook binds to the AT-rich DNA sequences in the minor groove of B-DNA in a non-sequence specific manner. AT-hook motifs have been identified in many other DNA-binding proteins. In this study we cloned and purified a fragment of hBRG1 encompassing the AT-hook region and the bromodomain. Nuclear magnetic resonance (NMR) and circular dichroism (CD) analyses show that the recombinant domains are structured. The functionality of subdomains was checked by assessing their interactions with N-acetylated peptides from histones and with DNA. Isothermal titration calorimetric (ITC) analysis demonstrates that the primary micromolar interaction is through the AT-hook motif. The AT-hook region binds to linear DNA by unwinding it. These properties resemble the characteristics of the HMGA1/2 proteins and their interaction with DNA.

Spadaccini R., Reidt U., Dybkov O., Will C., Frank R., Stier G., Corsini L., Wahl M.C., Luhrmann R., and Sattler M. (2006) Biochemical and NMR analyses of an SF3b155-p14-U2AF-RNA interaction network involved in branch point definition during pre-mRNA splicing. *RNA*. **12**, 410-25.

Abstract: The p14 subunit of the essential splicing factor 3b (SF3b) can be cross-linked to the branch-point adenosine of pre-mRNA introns within the spliceosome. p14 stably interacts with the SF3b subunit SF3b155, which also binds the 65-kDa subunit of U2 auxiliary splicing factor (U2AF65). We combined biochemical and NMR techniques to study the conformation of p14 either alone or complexed with SF3b155 fragments, as well as an interaction network involving p14, SF3b155, U2AF65, and U2 snRNA/pre-mRNA. p14 comprises a canonical RNA recognition motif (RRM) with an additional C-terminal helix (alphaC) and a beta hairpin insertion. SF3b155 binds to the beta-sheet surface of p14, thereby occupying the canonical RNA-binding site of the p14 RRM. The minimal region of SF3b155 interacting with p14 (i.e., residues 381-424) consists of four alpha-helices, which are partially preformed in isolation. Helices alpha2 and alpha3 (residues 401-415) constitute the core p14-binding epitope. Regions of SF3b155 binding to p14 and U2AF65 are nonoverlapping. This allows for a simultaneous interaction of SF3b155 with both proteins, which may support the stable association of U2 snRNP with the pre-mRNA. p14-RNA interactions are modulated by SF3b155 and the RNA-binding site of the p14-SF3b155 complex involves the noncanonical beta hairpin insertion of the p14 RRM, consistent with the beta-sheet surface being occupied by the helical SF3b155 peptide and p14 helix alphaC. Our data suggest that p14 lacks

inherent specificity for recognizing the branch point, but that some specificity may be achieved by scaffolding interactions involving other components of SF3b.

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.

Takahashi M., Maraboeuf F., Morimatsu K., Selmane T., Fleury F., and Norden B. (2007) Calorimetric Analysis of Binding of two Consecutive DNA Strands to RecA Protein Illuminates Mechanism for Recognition Of Homology. *J Mol Biol* **365**, 603-611.

Abstract: RecA protein recognises two complementary DNA strands for homologous recombination. To gain insight into the molecular mechanism, the thermodynamic parameters of the DNA binding have been characterised by isothermal calorimetry. Specifically, conformational changes of protein and DNA were searched for by measuring variations in enthalpy change (ΔH) with temperature (heat capacity change, $\Delta C(p)$). In the presence of the ATP analogue ATP γ S, the ΔH for the binding of the first DNA strand depends upon temperature (large $\Delta C(p)$) and the type of buffer, in a way that is consistent with the organisation of disordered parts and the protonation of RecA upon complex formation. In contrast, the binding of the second DNA strand occurs without any pronounced $\Delta C(p)$, indicating the absence of further reorganisation of the RecA-DNA filament. In agreement with these findings, a significant change in the CD spectrum of RecA was observed only upon the binding of the first DNA strand. In the absence of nucleotide cofactor, the ΔH of DNA binding is almost independent of temperature, indicating a requirement for ATP in the reorganisation of RecA. When the second DNA strand is complementary to the first, the ΔH is larger than that for non-complementary DNA strand, but less than the ΔH of the annealing of the complementary DNA without RecA. This small ΔH could reflect a weak binding that may facilitate the dissociation of only partly complementary DNA and thus speed the search for complementary DNA. The ΔH of binding DNA sequences displaying strong base-base stacking is small for both the first and second binding DNA strand, suggesting that the second is also stretched upon interaction with RecA. These results support the proposal that the RecA protein restructures DNA, preparing it for the recognition of a complementary second DNA strand, and that the recognition is due mainly to direct base-base contacts between DNA strands.

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.

Terrier P., Tortajada J., Zin G. and Buchmann W. (2007) Noncovalent complexes between DNA and basic polypeptides or polyamines by MALDI-TOF. *J Am. Soc. Mass Spectrom.* **18**, 1977-1989.

Abstract: MALDI-MS was evaluated as a method for the study of noncovalent complexes involving DNA oligonucleotides and various polybasic compounds (basic polypeptides and polyamines). Complexes involving single-stranded DNA were successfully detected using DHAP matrix in the presence of an ammonium salt. Control experiments confirmed that the interactions involved basic sites of the polybasic compounds and that the complexes were not formed in the gas phase but were pre-existing in the matrix crystals. Moreover, the pre-existence in solution was probed by isothermal titration calorimetry at concentration and ionic strength similar to those used for mass spectrometry. Spectra showed no important difference between negative and positive ion modes. The influence of nature and size of DNA and polybasic compound on the relative intensities and stoichiometries of the complexes was investigated. Despite the fact that relative intensities can be affected by ionization yields and the gas-phase stabilities of the different species, numerous trends observed in the MALDI study were consistent with the expected in-solution behaviors. Experimental conditions related to sample preparation were investigated also. Complex abundance generally decreased when increasing the ammonium acetate concentration. It was dramatically decreased when using ATT instead of DHAP. Penta-L-arginine is an exception to these observations. Lastly, in the case of complexes involving DNA duplex, the ATT matrix was shown to favor the observation of specific DNA duplex but not that of its complex with polybasic compounds. Inversely, DHAP was appropriate for the conservation of DNA-polybasic compound interaction but not for the transfer of intact duplex.

Torigoe H., Dohmae N., Hanaoka F. and Furukawa A. (2007) Mutational analyses of a single-stranded telomeric DNA binding domain of fission yeast pot1: conflict with X-ray crystallographic structure. *Biosci. Biotechnol. Biochem* **71**, 481-490.

Abstract: To understand the telomere regulation mechanism in relation to cell aging and cancer, we examined the single-stranded telomeric DNA binding domain (ssDBD) of fission yeast telomere-binding protein Pot1 by constructing a series of deletion mutants. We found that Pot1(1-182) (amino acids 1-182) stably expressed in *Escherichia coli* without any degradation retained a stable folded structure and functional telomeric DNA binding activity, indicating that Pot1(1-182) corresponds to ssDBD. We investigated the amino acids of Pot1(1-182) involved in single-stranded telomeric DNA recognition by constructing a series of site-directed mutants. Although the previously reported X-ray crystallographic structure suggests that 12 amino acids contact the telomeric DNA, an electrophoretic mobility shift assay and isothermal titration calorimetry analyses of the binding ability of the site-directed mutants indicated that only five amino acids significantly contributed to telomeric DNA recognition. We conclude that the contribution to recognition is quite different in magnitude among the amino acids judged to contact the target by X-ray crystallographic structure.

Tsodikov O. V., Holbrook J. A., Shkel I. A., and Record M. T., Jr. (2001) Analytic binding isotherms describing competitive interactions of a protein ligand with specific and nonspecific sites on the same DNA oligomer. *Biophys J* **81**, 1960-1969.

Abstract: Many studies of specific protein-nucleic acid binding use short oligonucleotides or restriction fragments, in part to minimize the potential for nonspecific binding of the protein. However, when the specificity ratio is low, multiple nonspecifically bound proteins may occupy the region of DNA corresponding to one specific site; this situation was encountered in our recent calorimetric study of binding of integration host factor (IHF) protein to its specific 34-bp H' DNA site. Here, beginning from the analytical McGhee and von Hippel infinite-lattice nonspecific binding isotherm, we derive a novel analytic isotherm for nonspecific binding of a ligand to a finite lattice. This isotherm is an excellent approximation to the exact factorial-based Epstein finite lattice isotherm even for short lattices and therefore is of great practical significance for analysis of experimental data and for analytic theory. Using this isotherm, we develop an analytic treatment of the competition between specific and nonspecific binding of a large ligand to the same finite lattice (i.e., DNA oligomer) containing one specific and multiple overlapping nonspecific binding sites. Analysis of calorimetric data for IHF-H' DNA binding using this treatment yields enthalpies and binding constants for both specific and nonspecific binding and the nonspecific site size. This novel analysis demonstrates the potential contribution of nonspecific binding to the observed thermodynamics of specific binding, even with very short DNA oligomers, and the need for reverse (constant protein) titrations or titrations with nonspecific DNA to resolve specific and nonspecific contributions. The competition

treatment is useful in analyzing low-specificity systems, including those where specificity is weakened by mutations or the absence of specificity factors.

Vander Meulen K. A., Saecker R. M. and Record M. T., Jr. (2008) Formation of a wrapped DNA-protein interface: experimental characterization and analysis of the large contributions of ions and water to the thermodynamics of binding IHF to H' DNA. *J Mol Biol* **377**, 9-27.

Abstract: To characterize driving forces and driven processes in formation of a large-interface, wrapped protein-DNA complex analogous to the nucleosome, we have investigated the thermodynamics of binding the 34-base pair (bp) H' DNA sequence to the Escherichia coli DNA-remodeling protein integration host factor (IHF). Isothermal titration calorimetry and fluorescence resonance energy transfer are applied to determine effects of salt concentration [KCl, KF, K glutamate (KGlu)] and of the excluded solute glycine betaine (GB) on the binding thermodynamics at 20 degrees C. Both the binding constant $K(\text{obs})$ and enthalpy $\Delta H(\text{obs})$ depend strongly on [salt] and anion identity. Formation of the wrapped complex is enthalpy driven, especially at low [salt] (e.g., $\Delta H(\text{obs}) = -20.2 \text{ kcal} \times \text{mol}^{-1}$ in 0.04 M KCl). $\Delta H(\text{obs})$ increases linearly with [salt] with a slope ($d\Delta H(\text{obs})/d[\text{salt}]$), which is much larger in KCl ($38 \pm 3 \text{ kcal} \times \text{mol}^{-1} \text{ M}^{-1}$) than in KF or KGlu ($11 \pm 2 \text{ kcal} \times \text{mol}^{-1} \text{ M}^{-1}$). At 0.33 M [salt], $K(\text{obs})$ is approximately 30-fold larger in KGlu or KF than in KCl, and the [salt] derivative $\text{SK}(\text{obs}) = d\ln K(\text{obs})/d\ln[\text{salt}]$ is almost twice as large in magnitude in KCl (-8.8 ± 0.7) as in KF or KGlu (-4.7 ± 0.6). A novel analysis of the large effects of anion identity on $K(\text{obs})$, $\text{SK}(\text{obs})$ and on $\Delta H(\text{obs})$ dissects coulombic, Hofmeister, and osmotic contributions to these quantities. This analysis attributes anion-specific differences in $K(\text{obs})$, $\text{SK}(\text{obs})$, and $\Delta H(\text{obs})$ to (i) displacement of a large number of water molecules of hydration [estimated to be $1.0(\pm 0.2) \times 10^3$] from the 5340 Å² of IHF and H' DNA surface buried in complex formation, and (ii) significant local exclusion of F(-) and Glu(-) from this hydration water, relative to the situation with Cl(-), which we propose is randomly distributed. To quantify net water release from anionic surface (22% of the surface buried in complexation, mostly from DNA phosphates), we determined the stabilizing effect of GB on $K(\text{obs})$: $d\ln K(\text{obs})/d[\text{GB}] = 2.7 \pm 0.4$ at constant KCl activity, indicating the net release of ca. 150 H₂O molecules from anionic surface

Verdemato P. E., Brannigan J. A., Damblon C., Zuccotto F., Moody P. C., and Lian L. Y. (2000) DNA-binding mechanism of the Escherichia coli Ada O(6)-alkylguanine-DNA alkyltransferase. *Nucleic Acids Res* **28**, 3710-3718.

Abstract: The C-terminal domain of the Escherichia coli Ada protein (Ada-C) aids in the maintenance of genomic integrity by efficiently repairing pre-mutagenic O(6)-alkylguanine lesions in DNA. Structural and thermodynamic studies were carried out to obtain a model of the DNA-binding process. Nuclear magnetic resonance (NMR) studies map the DNA-binding site to helix 5, and a loop region (residues 151-160) which form the recognition helix and the 'wing' of a helix-turn-wing motif, respectively. The NMR data also suggest the absence of a large conformational change in the protein upon binding to DNA. Hence, an O(6)-methylguanine (O(6)meG) lesion would be inaccessible to active site nucleophile Cys146 if the modified base remained stacked within the DNA duplex. The experimentally determined DNA-binding face of Ada-C was used in combination with homology modelling, based on the catabolite activator protein, and the accepted base-flipping mechanism, to construct a model of how Ada-C binds to DNA in a productive manner. To complement the structural studies, thermodynamic data were obtained which demonstrate that binding to unmethylated DNA was entropically driven, whilst the demethylation reaction provoked an exothermic heat change. Methylation of Cys146 leads to a loss of structural integrity of the DNA-binding subdomain.

Volpon L., D'Orso I., Young C. R., Frasch A. C., and Gehring K. (2005) NMR structural study of TcUBP1, a single RRM domain protein from Trypanosoma cruzi: contribution of a beta hairpin to RNA binding. *Biochemistry* **44**, 3708-3717.

Abstract: TcUBP1 is a trypanosome cytoplasmic RNA-binding protein containing a single, conserved RNA-recognition motif (RRM) domain involved in selective destabilization of U-rich mRNAs such as the Trypanosoma cruzi small mucin gene family mRNA, TcSMUG. TcUBP1 binds specific transcripts in vivo and co-localizes in the perinuclear part of the cell with components of the mRNA-stability determinant pathway such as poly(A)-binding protein 1 (PABP1) and TcUBP2, a closely related RRM-containing protein. In TcUBP proteins, the RRM domain is flanked by N-terminal Gln-rich and C-terminal Gly-Gln-

rich extensions, which are involved in protein-protein interactions. In this work, we determined the solution structure of the TcUBP1 RRM domain by nuclear magnetic resonance (NMR) spectroscopy. The domain has a characteristic betaalphabetabetaalphabetabeta fold, consisting of a beta sheet composed of four antiparallel betastrands and two alpha helices packed against one face of the beta sheet. A unique aspect of TcUBP1 is the participation of a beta hairpin (beta4-beta5) in the beta sheet, resulting in an enlarged RNA-binding surface. Detailed analysis of the TcUBP1 interaction with a short single-stranded RNA derived from the 3' UTR of TcSMUG was carried out by titration experiments using both NMR spectroscopy and isothermal titration calorimetry. This analysis revealed that amino acids located within the beta hairpin (beta4-beta5) contribute to complex formation. This enlarged protein-RNA interface could compensate for the lack of additional RNA-binding domains in TcUBP1, as observed in many other RRM-containing proteins. The structure of TcUBP1 reveals new aspects of single RRM-RNA interactions and insight into how N- and C-terminal extensions can contribute to RNA binding.

Wahid A. M., Coventry V. K. and Conn G. L. (2008) Systematic deletion of the adenovirus-associated RNAI terminal stem reveals a surprisingly active RNA inhibitor of double-stranded RNA-activated protein kinase. *J Biol Chem* **283**, 17485-17493.

Abstract: Adenoviruses use the short noncoding RNA transcript virus-associated (VA) RNA(I) to counteract two critical elements of the host cell defense system, innate cellular immunity and RNA interference, mediated by the double-stranded RNA-activated protein kinase (PKR) and Dicer/RNA-induced silencing complex, respectively. We progressively shortened the VA RNA(I) terminal stem to examine its necessity for inhibition of PKR. Each deletion, up to 15 bp into the terminal stem, resulted in a cumulative decrease in PKR inhibitory activity. Remarkably, however, despite significant apparent destabilization of the RNA structure, the final RNA mutant that lacked the entire terminal stem (TSDelta21 RNA) efficiently bound PKR and exhibited wild-type inhibitory activity. TSDelta21 RNA stability was strongly influenced by solution pH, indicating the involvement of a protonated base within the VA RNA(I) central domain tertiary structure. Gel filtration chromatography and isothermal titration calorimetry analysis indicated that wild-type VA RNA(I) and TSDelta21 RNA form similar 1:1 complexes with PKR but that the latter lacks secondary binding site(s) that might be provided by the terminal stem. Although TSDelta21 RNA bound PKR with wild-type $K(d)$, and overall change in free energy (ΔG), the thermodynamics of binding (ΔH and ΔS) were significantly altered. These results demonstrate that the VA RNA(I) terminal stem is entirely dispensable for inhibition of PKR. Potentially, VA RNA(I) is therefore a truly bi-functional RNA; Dicer processing of the VA RNA(I) terminal stem saturates the RNA interference system while generating a "mini-VA RNA(I)" molecule that remains fully active against PKR

Wang E., Bauer M. C., Rogstam A., Linse S., Logan D. T. and von W. C. (2008) Structure and functional properties of the *Bacillus subtilis* transcriptional repressor Rex. *Mol Microbiol* **69**, 466-478.

Abstract: The transcription factor Rex has been implicated in regulation of the expression of genes important for fermentative growth and for growth under conditions of low oxygen tension in several Gram-positive bacteria. Rex senses the redox poise of the cell through changes in the NADH/NAD(+) ratio. The crystal structures of two essentially identical Rex proteins, from *Thermus aquaticus* and *T. thermophilus*, have previously been determined in complex with NADH. Here we present the crystal structure of the Rex protein from *Bacillus subtilis*, as well as extensive studies of its affinity for nucleotides and DNA, using surface plasmon resonance, isothermal titration calorimetry and electrophoretic mobility shift assays. We show that Rex has a very high affinity for NADH but that its affinity for NAD(+) is 20 000 times lower. However, the NAD(+) affinity is increased by a factor of 30 upon DNA binding, suggesting that there is a positive allosteric coupling between DNA binding and NAD(+) binding. The crystal structures of two pseudo-apo forms (from crystals soaked with NADH and cocrystallized with ATP) show a very different conformation from the previously determined Rex:NADH complexes, in which the N-terminal domains are splayed away from the dimer core. A mechanism is proposed whereby conformational changes in a C-terminal domain-swapped helix mediate the transition from a flexible DNA binding form to a locked NADH-bound form incapable of binding DNA

Wang X., Cao W., Cao A., and Lai L. (2003) Thermodynamic characterization of the folding coupled DNA binding by the monomeric transcription activator GCN4 peptide. *Biophys J* **84**, 1867-1875.

Abstract: Dimerization is a widely believed critical requirement for the yeast transcriptional activator GCN4 specifically recognizing its DNA target sites. Nonetheless, the binding of the monomeric GCN4 to

DNA target sites AP-1 and ATF/CREB was recently detected by kinetic studies. Here, for the first time, we present a detailed description of the thermodynamics of a monomeric peptide GCN4-br, the basic region (226-252) of GCN4, binding to AP-1, and ATF/CREB. GCN4 specifically binds to AP-1 and ATF/CREB in the monomeric form as shown by our circular dichroism thermal unfolding measurements. Isothermal titration calorimetry experiments indicate that the binding process of GCN4-br with DNA is enthalpically driven, accompanied by an unfavorable entropy change. The temperature dependence of $\Delta H(0)$ reveals negative changes in heat capacity ΔC_p $\Delta C_p = -0.92 \text{ kJ. mol}^{-1} \text{ K}^{-1}$ and $\Delta C_p = -0.95 \text{ kJ. mol}^{-1} \text{ K}^{-1}$ for GCN4-br binding to AP-1 and ATF/CREB, respectively, which is a striking manifestation of GCN4-br specifically recognizing DNA target sites. These thermodynamic characteristics may give new insight into the mechanism by which GCN4 protein binds to DNA target sites for its transcriptional regulation.

Wardleworth B. N., Russell R. J., Bell S. D., Taylor G. L., and White M. F. (2002) Structure of Alba: an archaeal chromatin protein modulated by acetylation. *EMBO J* **21**, 4654-4662.

Abstract: Eukaryotic DNA is packaged into nucleosomes that regulate the accessibility of the genome to replication, transcription and repair factors. Chromatin accessibility is controlled by histone modifications including acetylation and methylation. Archaea possess eukaryotic-like machineries for DNA replication, transcription and information processing. The conserved archaeal DNA binding protein Alba (formerly Sso10b) interacts with the silencing protein Sir2, which regulates Alba's DNA binding affinity by deacetylation of a lysine residue. We present the crystal structure of Alba from *Sulfolobus solfataricus* at 2.6 Å resolution (PDB code 1h0x). The fold is reminiscent of the N-terminal DNA binding domain of DNase I and the C-terminal domain of initiation factor IF3. The Alba dimer has two extended beta-hairpins flanking a central body containing the acetylated lysine, Lys16, suggesting three main points of contact with the DNA. Fluorescence, calorimetry and electrophoresis data suggest a final binding stoichiometry of approximately 5 bp DNA per Alba dimer. We present a model for the Alba-DNA interaction consistent with the available structural, biophysical and electron microscopy data.

Welfle K., Pratto F., Misselwitz R., Behlke J., Alonso J. C., and Welfle H. (2005) Role of the N-terminal region and of beta-sheet residue Thr29 on the activity of the omega2 global regulator from the broad-host range *Streptococcus pyogenes* plasmid pSM19035. *Biol Chem* **386**, 881-894.

Abstract: The dimeric regulatory protein wild-type omega (wt omega2) binds to arrays of 7-bp sequences (heptads) present in the operator DNA region of copy control and partition functions of plasmid pSM19035. Each omega2 protein probably binds with an antiparallel beta-sheet structure in the major groove of the 7-bp subsite of the operator DNA. Exchange of threonine at position 29 to alanine (T29A) drastically affects the activity of variant protein omega2T29A both in vivo and in vitro, and reduces the thermodynamic stability $\Delta G(0)$, but does not change the conformation. Likewise, the binding affinity to DNA is reduced and the association of the two monomeric subunits of the omega2T29A dimer is weakened, as manifested by an increase in the dissociation constant from 3.2 μM for wt omega2 to 6.3 μM for omega2T29A. Denatured dimers are formed upon thermal unfolding of wt omega2 and omega2T29A at ca. 45 μM ($D(n) \leftrightarrow D(u)$). Removal of 8 (omega2deltaN8), or even 18 (omega2deltaN18) N-terminal amino acids has no obvious effect either on the core structure or on the activity in comparison to wt omega2. The stability of variants omega2deltaN8 and omega2deltaN18 is similar to that of wt omega2, and their binding to operator DNA is not impaired.

Williams K. R., Sillerud L. O., Schafer D. E., and Konigsberg W. H. (1979) DNA binding properties of the T4 DNA helix-destabilizing protein. A calorimetric study. *J Biol Chem* **254**, 6426-6432.

Wittung P., Ellouze C., Maraboeuf F., Takahashi M., and Norden B. (1997) Thermochemical and kinetic evidence for nucleotide-sequence-dependent RecA-DNA interactions. *Eur J Biochem* **245**, 715-719.

Abstract: RecA catalyses homologous recombination in *Escherichia coli* by promoting pairing of homologous DNA molecules after formation of a helical nucleoprotein filament with single-stranded DNA. The primary reaction of RecA with DNA is generally assumed to be unspecific. We show here, by direct measurement of the interaction enthalpy by means of isothermal titration calorimetry, that the polymerisation of RecA on single-stranded DNA depends on the DNA sequence, with a high exothermic preference for thymine bases. This enthalpic sequence preference of thymine by RecA correlates with faster binding kinetics of RecA to thymine DNA. Furthermore, the enthalpy of interaction between the RecA x DNA filament and a second DNA strand is large only when the added DNA is complementary to

the bound DNA in RecA. This result suggests a possibility for a rapid search mechanism by RecA x DNA filaments for homologous DNA molecules.

Wong C. J. and Lohman T. M. (2008) Kinetic control of Mg²⁺-dependent melting of duplex DNA ends by *Escherichia coli* RecBC. *J Mol Biol* **378**, 761-777.

Abstract: *Escherichia coli* RecBCD is a highly processive DNA helicase involved in double-strand break repair and recombination that possesses two helicase/translocase subunits with opposite translocation directionality (RecB (3' to 5') and RecD (5' to 3')). RecBCD has been shown to melt out approximately 5-6 bp upon binding to a blunt-ended duplex DNA in a Mg²⁺-dependent, but ATP-independent reaction. Here, we examine the binding of *E. coli* RecBC helicase (minus RecD), also a processive helicase, to duplex DNA ends in the presence and in the absence of Mg²⁺ in order to determine if RecBC can also melt a duplex DNA end in the absence of ATP. Equilibrium binding of RecBC to DNA substrates with ends possessing pre-formed 3' and/or 5' single-stranded (ss)-(dT)(n) flanking regions (tails) (n ranging from zero to 20 nt) was examined by competition with a fluorescently labeled reference DNA and by isothermal titration calorimetry. The presence of Mg²⁺ enhances the affinity of RecBC for DNA ends possessing 3' or 5'-(dT)(n) ssDNA tails with n<6 nt, with the relative enhancement decreasing as n increases from zero to six nt. No effect of Mg²⁺ was observed for either the binding constant or the enthalpy of binding ($\Delta H(\text{obs})$) for RecBC binding to DNA with ssDNA tail lengths, n>=6 nucleotides. Upon RecBC binding to a blunt duplex DNA end in the presence of Mg²⁺, at least 4 bp at the duplex end become accessible to KMnO₄ attack, consistent with melting of the duplex end. Since Mg²⁺ has no effect on the affinity or binding enthalpy of RecBC for a DNA end that is fully pre-melted, this suggests that the role of Mg²⁺ is to overcome a kinetic barrier to melting of the DNA by RecBC and presumably also by RecBCD. These data also provide an accurate estimate ($\Delta H(\text{obs})=8\pm 1$ kcal/mol) for the average enthalpy change associated with the melting of a DNA base-pair by RecBC

Yang J., Xi J., Zhuang Z., and Benkovic S.J. (2005) The oligomeric T4 primase is the functional form during replication. *J Biol Chem.* **280**, 25416-23.

Abstract: Replisome DNA primases are responsible for the synthesis of short RNA primers required for the initiation of repetitive Okazaki fragment synthesis on the lagging strand during DNA replication. In bacteriophage T4, the primase (gp61) interacts with the helicase (gp41) to form the primosome complex, an interaction that greatly stimulates the priming activity of gp61. Because gp41 is hexameric, a question arises as to whether gp61 also forms a hexameric structure during replication. Several results from this study support such a structure. Titration of the primase/single-stranded DNA binding followed by fluorescence anisotropy implicated a 6:1 stoichiometry. The observed rate constant, k(cat), for priming was found to increase with the primase concentration, implicating an oligomeric form of the primase as the major functional species. The generation of hetero-oligomeric populations of the hexameric primase by controlled mixing of wild type and an inactive mutant primase confirmed the oligomeric nature of the most active primase form. Mutant primases defective in either the N- or C-terminal domains and catalytically inactive could be mixed to create oligomeric primases with restored catalytic activity suggesting an active site shared between subunits. Collectively, these results provide strong evidence for the functional oligomerization of gp61. The potential roles of gp61 oligomerization during lagging strand synthesis are discussed.

Zhang W., Ni H., Capp M. W., Anderson C. F., Lohman T. M., and Record M. T., Jr. (1999) The importance of coulombic end effects: experimental characterization of the effects of oligonucleotide flanking charges on the strength and salt dependence of oligocation (L8⁺) binding to single-stranded DNA oligomers. *Biophys J* **76**, 1008-1017.

Abstract: Binding constants K_{obs}, expressed per site and evaluated in the limit of zero binding density, are quantified as functions of salt (sodium acetate) concentration for the interactions of the oligopeptide ligand KWK6NH₂ (designated L8⁺, with ZL = 8 charges) with three single-stranded DNA oligomers (ss dT-mers, with |ZD| = 15, 39, and 69 charges). These results provide the first systematic experimental information about the effect of changing |ZD| on the strength and salt dependence of oligocation-oligonucleotide binding interactions. In a comparative study of L8⁺ binding to poly dT and to a short dT oligomer (|ZD| = 10), Proc. Natl. Acad. Sci. USA. 93:2511-2516) demonstrated the profound thermodynamic effects of phosphate charges that flank isolated nonspecific L8⁺ binding sites on DNA. Here we find that both K_{obs}

and the magnitude of its power dependence on salt activity ($|SaKobs|$) increase monotonically with increasing $|ZD|$. The dependences of $Kobs$ and $SaKobs$ on $|ZD|$ are interpreted by introducing a simple two-state thermodynamic model for Coulombic end effects, which accounts for our finding that when L8+ binds to sufficiently long dT-mers, both $\Delta G_{obs} = -RT \ln Kobs$ and $SaKobs$ approach the values characteristic of binding to poly-dT as linear functions of the reciprocal of the number of potential oligocation binding sites on the DNA lattice. Analysis of our L8+-dT-mer binding data in terms of this model indicates that the axial range of the Coulombic end effect for ss DNA extends over approximately 10 phosphate charges. We conclude that Coulombic interactions cause an oligocation (with $ZL < |ZD|$) to bind preferentially to interior rather than terminal binding sites on oligoanionic or polyanionic DNA, and we quantify the strong increase of this preference with decreasing salt concentration. Coulombic end effects must be considered when oligonucleotides are used as models for polyanionic DNA in thermodynamic studies of the binding of charged ligands, including proteins.

Zhou Y., Larson J. D., Bottoms C. A., Arturo E. C., Henzl M. T., Jenkins J. L., Nix J. C., Becker D. F. and Tanner J. J. (2008) Structural basis of the transcriptional regulation of the proline utilization regulon by multifunctional PutA. *J Mol Biol* **381**, 174-188.

Abstract: The multifunctional *Escherichia coli* proline utilization A (PutA) flavoprotein functions both as a membrane-associated proline catabolic enzyme and as a transcriptional repressor of the proline utilization genes *putA* and *putP*. To better understand the mechanism of transcriptional regulation by PutA, we have mapped the put-regulatory region, determined a crystal structure of the PutA ribbon-helix-helix domain (PutA52, a polypeptide corresponding to residues 1-52 of *E. coli* PutA) complexed with DNA, and examined the thermodynamics of DNA binding to PutA52. Five operator sites, each containing the sequence motif 5'-GTTGCA-3', were identified using gel-shift analysis. Three of the sites are shown to be critical for repression of *putA*, whereas the two other sites are important for repression of *putP*. The 2.25-Å-resolution crystal structure of PutA52 bound to one of the operators (operator 2; 21 bp) shows that the protein contacts a 9-bp fragment corresponding to the GTTGCA consensus motif plus three flanking base pairs. Since the operator sequences differ in flanking bases, the structure implies that PutA may have different affinities for the five operators. This hypothesis was explored using isothermal titration calorimetry. The binding of PutA52 to operator 2 is exothermic, with an enthalpy of -1.8 kcal/mol and a dissociation constant of 210 nM. Substitution of the flanking bases of operator 4 into operator 2 results in an unfavorable enthalpy of 0.2 kcal/mol and a 15-fold-lower affinity, showing that base pairs outside of the consensus motif impact binding. Structural and thermodynamic data suggest that hydrogen bonds between Lys9 and bases adjacent to the GTTGCA motif contribute to transcriptional regulation by fine-tuning the affinity of PutA for put control operators

Ziegler A. and Seelig J. (2007) High affinity of the cell-penetrating peptide HIV-1 Tat-PTD for DNA. *Biochemistry* **46**, 8138-8145.

Abstract: During cellular uptake of fluorescently labeled cell-penetrating peptides (CPPs), intense fluorescent signals are commonly observed in the nucleus of the cell, suggesting intracellular CPP relocation and potential binding to the genome of the host. We therefore investigated the interaction of the CPP HIV-1 Tat(47-57) with double-stranded DNA, and we also tested whether the fluorescence intensity of the labeled CPP allows for linear predictions of its intracellular concentration. Using isothermal titration calorimetry, we observe that the CPP has a high affinity for salmon sperm DNA as characterized by a microscopic dissociation constant of 126 nM. The binding is exothermic, with a reaction enthalpy of -4.63 kcal/mol CPP (28 degrees C). The dissociation constant and reaction enthalpy decrease further at higher temperatures. The affinity of the CPP for DNA is thus 1-2 magnitudes higher than for extracellular heparan sulfate, the likely mediator of the CPP uptake. Accordingly, the high affinity for DNA confers stability to extracellular transport complexes of CPP and DNA but potentially affects the regulation and molecular organization of the host's genome after nuclear uptake. Moreover, the CPP leads to the condensation of DNA as evidenced by the pronounced increase in light-scattering intensity. The fluorescence quantum yield of the FITC-labeled CPP decreases considerably at concentrations > 5 micromol/L, at $pH < 7$, and upon binding to DNA and glycosaminoglycans. This change in fluorescence quantum yield impedes the microscopic identification of uptake routes and the comparison of uptake efficiency of different CPPs, especially if the accumulation in subcellular compartments (self-quenching and pH difference) and transitory binding partners (quenching and condensation) is unknown