ITC XXIV – Bacteriophage studies

Alley S. C., Trakselis M. A., Mayer M. U., Ishmael F. T., Jones A. D., and Benkovic S. J. (2001) Building a replisome solution structure by elucidation of protein-protein interactions in the bacteriophage T4 DNA polymerase holoenzyme. *J Biol Chem* **276,** 39340-39349.
Abstract: Assembly of DNA replication systems requires the coordinated actions of many proteins. The multiprotein complexes formed as intermediates on the pathway to the final DNA polymerase holoenzyme have been shown to have distinct structures relative to the ground-state structures of the individual proteins. By using a variety of solution-phase techniques, we have elucidated additional information about the solution structure of the bacteriophage T4 holoenzyme. Photocross-linking and mass spectrometry were used to demonstrate interactions between I107C of the sliding clamp and the DNA polymerase. Fluorescence resonance energy transfer, analytical ultracentrifugation, and isothermal titration calorimetry measurements were used to demonstrate that the C terminus of the DNA polymerase can interact at two distinct locations on the sliding clamp. Both of these binding modes may be used during holoenzyme assembly, but only one of these binding modes is found in the final holoenzyme. Present and previous solution interaction data were used to build a model of the holoenzyme that is consistent with these data.

Baxa U., Cooper A., Weintraub A., Pfeil W., and Seckler R. (2001) Enthalpic barriers to the hydrophobic binding of oligosaccharides to phage P22 tailspike protein. *Biochemistry* **40,** 5144-5150.
Abstract: The structural thermodynamics of the recognition of complex carbohydrates by proteins are not well understood. The recognition of O-antigen polysaccharide by phage P22 tailspike protein is a highly suitable model for advancing knowledge in this field. The binding to octa- and dodecasaccharides derived from Salmonella enteritidis O-antigen was studied by isothermal titration calorimetry and stopped-flow spectrofluorimetry. At room temperature, the binding reaction is enthalpically driven with an unfavorable change in entropy. A large change of -1.8 +/- 0.2 kJ mol^{-1} K^{-1} in heat capacity suggests that the hydrophobic effect and water reorganization contribute substantially to complex formation. As expected from the large heat-capacity change, we found enthalpy-entropy compensation. The calorimetrically measured binding enthalpies were identical within error to van't Hoff enthalpies determined from fluorescence titrations. Binding kinetics were determined at temperatures ranging from 10 to 30 degrees C. The second-order association rate constant varied from 1 x 10^5 M^{-1} s^{-1} for dodecasaccharide at 10 degrees C to 7 x 10^5 M^{-1} s^{-1} for octasaccharide at 30 degrees C. The first-order dissociation rate constants ranged from 0.2 to 3.8 s^{-1}. The Arrhenius activation energies were close to 50 and 100 kJ mol^{-1} for the association and dissociation reactions, respectively, indicating mainly enthalpic barriers. Despite the fact that this system is quite complex due to the flexibility of the saccharide, both the thermodynamic and kinetic data are compatible with a simple one-step binding model.

Bonin I., Muhlberger R., Bourenkov G. P., Huber R., Bacher A., Richter G., and Wahl M. C. (2004) Structural basis for the interaction of Escherichia coli NusA with protein N of phage lambda. *Proc Natl Acad Sci U S A* **101,** 13762-13767.
Abstract: The C terminus of transcription factor NusA from Escherichia coli comprises two repeat units, which bind during antitermination to protein N from phage lambda. To delineate the structural basis of the NusA-lambdaN interaction, we attempted to crystallize the NusA C-terminal repeats in complex with a lambdaN peptide (residues 34-47). The two NusA domains became proteolytically separated during crystallization, and crystals contained two copies of the first repeat unit in contact with a single lambdaN fragment. The NusA modules employ identical regions to contact the peptide but approach the ligand from opposite sides. In contrast to the alpha-helical conformation of the lambdaN N terminus in complex with boxB RNA, residues 34-40 of lambdaN remain extended upon interaction with NusA. Mutational analyses indicated that only one of the observed NusA-lambdaN interaction modes is biologically significant, supporting an equimolar ratio of NusA and lambdaN in antitermination complexes. Solution studies indicated that additional interactions are fostered by the second NusA repeat unit, consistent with known compensatory mutations in NusA and lambdaN. Contrary to the RNA polymerase alpha subunit, lambdaN binding does not stimulate RNA interaction of NusA. The results demonstrate that lambdaN serves as a scaffold to closely oppose NusA and the mRNA in antitermination complexes.

Crowther L. J., Yamagata A., Craig L., Tainer J. A., and Donnenberg M. S. (2005) The ATPase Activity of BfpD Is Greatly Enhanced by Zinc and Allosteric Interactions with Other Bfp Proteins. *J Biol Chem* **280,** 24839-24848.
Abstract: Type IV pilus biogenesis, protein secretion, DNA transfer, and filamentous phage morphogenesis systems are thought to possess similar architectures and mechanisms. These multiprotein complexes include members of the PulE superfamily of putative NTPases that have extensive sequence similarity and probably similar functions as the energizers of macromolecular transport. We purified the PulE homologue BfpD of the enteropathogenic Escherichia coli bundle-forming pilus (BFP) biogenesis machine and characterized its ATPase activity, providing new insights into its mode of action. Numerous techniques revealed that BfpD forms hexamers in the presence of nucleotide. Hexameric BfpD displayed weak ATPase activity. We previously demonstrated that the N termini of membrane proteins BfpC and BfpE recruit BfpD to the cytoplasmic membrane. Here, we identified two BfpD-binding sites, BfpE(39-76) and BfpE(77-114), in the N terminus of BfpE using a yeast two-hybrid system. Isothermal titration calorimetry and protease sensitivity assays showed that hexameric BfpD-ATPgammaS binds to BfpE(77-114), whereas hexameric BfpD-ADP binds to BfpE(39-76). Interestingly, the N terminus of BfpC and BfpE(77-114) together increased the ATPase activity of hexameric BfpD over 1200-fold to a V(max) of 75.3 mumol of P(i) min(-1) mg(-1), which exceeds by over 1200-fold the activity of other PulE family members. This augmented activity occurred only in the presence of Zn(2+). We conclude that allosteric interactions between BfpD and BfpC and BfpE dramatically stimulate its ATPase activity. The differential nucleotide-dependent binding of hexameric BfpD to BfpE(39-76) and BfpE(77-114) suggests a model for the mechanism by which BfpD transduces mechanical energy to the biogenesis machine.

Datta A. B., Roy S., and Parrack P. (2005) Role of C-terminal residues in oligomerization and stability of lambda CII: implications for lysis-lysogeny decision of the phage. *J Mol Biol* **345,** 315-324.
Abstract: A crucial element in the lysis-lysogeny decision of the temperate coliphage lambda is the phage protein CII, which has several interesting properties. It promotes lysogeny through activation of three phage promoters p(E), p(I) and p(aQ), recognizing a direct repeat sequence TTGCN6TTGC at each. The three-dimensional structure of CII, a homo-tetramer of 97 residue subunits, is unknown. It is an unstable protein in vivo, being rapidly degraded by the host protease HflB (FtsH). This instability is essential for the function of CII in the lysis-lysogeny switch. From NMR and limited proteolysis we show that about 15 C-terminal residues of CII are highly flexible, and may act as a target for proteolysis in vivo. From in vitro transcription, isothermal calorimetry and gel chromatography of CII (1-97) and its truncated fragments CIIA (4-81/82) and CIIB (4-69), we find that residues 70-81/82 are essential for (a) tetramer formation, (b) operator binding and (c) transcription activation. Presumably, tetramerization is necessary for the latter functions. Based on these results, we propose a model for CII structure, in which protein-protein contacts for dimer and tetramer formation are different. The implications of tetrameric organization, essential for CII activity, on the recognition of the direct repeat sequence is discussed.

Destoumieux-Garzon D., Duquesne S., Peduzzi J., Goulard C., Desmadril M., Letellier L., Rebuffat S., and Boulanger P. (2005) The iron-siderophore transporter FhuA is the receptor for microcin J25. Role of the microcin Val11-Pro16 beta-hairpin region in the recognition mechanism. *Biochem J* **389,** 869-876.
Abstract: The role of the outer membrane iron-transporter FhuA as a potential receptor for microcin J25 (MccJ25) was studied through a series of in vivo and in vitro experiments. The requirement for both FhuA and the inner membrane TonB/ExbB/ExbD complex was evidenced by antibacterial assays using complementation of a fhuA - strain, and by using isogenic strains mutated in the complex encoding genes, respectively. In addition, MccJ25 was shown to block phage T5 infection of Escherichia coli, in vivo, by inhibiting phage adhesion, which suggested that MccJ25 prevents the interaction between the phage and its receptor FhuA. This in vivo activity was confirmed in vitro as MccJ25 inhibited phage T5 DNA ejection triggered by purified FhuA. Direct interaction of MccJ25 with FhuA was demonstrated for the first time by size-exclusion chromatography and isothermal titration calorimetry. MccJ25 bound to FhuA with a 2:1 stoichiometry and a K d of 1.2 microM. Altogether, our results demonstrate that FhuA is the receptor for MccJ25 and that the ligand-receptor interaction may occur in the absence of other components of the bacterial membrane. Finally, both differential scanning calorimetry and antimicrobial assays showed that MccJ25 binding involves FhuA external loops. Contrary to native MccJ25, a thermolysin-cleaved MccJ25 variant was unable to bind to FhuA and failed to prevent phage T5 infection of E. coli. Therefore, the

MccJ25 Val11-Pro16 beta-hairpin region, which is disrupted upon thermolysin cleavage, is required for microcin recognition.

Freeman A. D., Declais A. C., and Lilley D. M. (2003) Metal ion binding in the active site of the junction-resolving enzyme T7 endonuclease I in the presence and in the absence of DNA. *J Mol Biol* **333,** 59-73.
Abstract: Endonuclease I of bacteriophage T7 is a DNA junction-resolving enzyme. We have previously used crystallography to demonstrate the binding of two manganese ions into the active site that is formed by three carboxylate (Glu 20, Asp 55 and Glu 65) and a lysine residue (Lys 67). Endonuclease I is active in the presence of magnesium, manganese, iron (II) and cobalt (II) ions, weakly active in the presence of nickel, copper (II) and zinc ions, and completely inactive in the presence of calcium ions. However, using calorimetry, we have observed the binding of two calcium ions to the free enzyme in a manner very similar to the binding of manganese ions. In the presence of iron (II) ions, we have obtained a cleavage of the continuous strands of a junction bound by endonuclease I, at sites close to (but not identical with) enzyme-induced hydrolysis. The results suggest that this arises from attack by locally generated hydroxyl radicals, arising from iron (II) ions bound into the active site. This therefore provides an indirect way of examining metal ion binding in the enzyme-junction complex. Ion binding in free protein (by calorimetry) and the enzyme-junction complex (iron-induced cleavage) have been studied in series of active-site mutants. Both confirm the importance of the three carboxylate ligands, and the lack of a requirement for Lys67 for the ion binding. Calorimetry points to particularly critical role of Asp55, as mutation completely abolishes all binding of both manganese and calcium ions.

Gokce I., Raggett E. M., Hong Q., Virden R., Cooper A., and Lakey J. H. (2000) The TolA-recognition site of colicin N. ITC, SPR and stopped-flow fluorescence define a crucial 27-residue segment. *J Mol Biol* **304,** 621-632.
Abstract: Colicins translocate across the Escherichia coli outer membrane and periplasm by interacting with several receptors. After first binding to the outer membrane surface receptors via their central region, they interact with TolA or TonB proteins via their N-terminal region. Colicin N residues critical to TolA binding have been discovered, but the full extent of any colicin TolA site is unknown. We present, for the first time, a fully mapped TolA binding site for a colicin. It was determined through the use of alanine-scanning mutants, glutathione S-transferase fusion peptides and Biacore/fluorescence binding studies. The minimal TolA binding region is 27 residues and of similar size to the TolA binding region of bacteriophage g3p-D1 protein. Stopped-flow kinetic studies show that the binding to TolA follows slow association kinetics. The role of other E. coli Tol proteins in colicin translocation was also investigated. Isothermal titration microcalorimetry (ITC) and in vivo studies conclusively show that colicin N translocation does not require the presence of TolB. ITC also demonstrated colicin A interaction with TolB, and that colicin A in its native state does not interact with TolAII-III. Colicin N does not bind TolR-II. The TolA protein is shown to be unsuitable for direct immobilisation in Biacore analysis.

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 A 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.

McMahon S.A., Miller J.L., Lawton J.A., Kerkow D.E., Hodes A., Marti-Renom M.A., Doulatov S., Narayanan E., Sali A., Miller J.F., Ghosh P.(2005) The C-type lectin fold as an evolutionary solution for massive sequence variation. *Nat Struct Mol Biol.* **12,** 886-92.
Abstract: Only few instances are known of protein folds that tolerate massive sequence variation for the sake of binding diversity. The most extensively characterized is the immunoglobulin fold. We now add to this the C-type lectin (CLec) fold, as found in the major tropism determinant (Mtd), a retroelement-encoded receptor-binding protein of Bordetella bacteriophage. Variation in Mtd, with its approximately 10(13) possible sequences, enables phage adaptation to Bordetella spp. Mtd is an intertwined, pyramid-shaped trimer, with variable residues organized by its CLec fold into discrete receptor-binding sites. The CLec fold provides a highly static scaffold for combinatorial display of variable residues, probably reflecting a different evolutionary solution for balancing diversity against stability from that in the immunoglobulin fold. Mtd variants are biased toward the receptor pertactin, and there is evidence that the CLec fold is used broadly for sequence variation by related retroelements.

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).

Morton A., Baase W. A., and Matthews B. W. (1995) Energetic origins of specificity of ligand binding in an interior nonpolar cavity of T4 lysozyme. *Biochemistry* **34,** 8564-8575.

Abstract: To determine the constraints on interactions within the core of a folded protein, we have analyzed the binding of 91 different compounds to an internal cavity created in the interior of phage T4 lysozyme by site-directed mutagenesis [Eriksson et al. (1992a) Nature 355, 371-373]. The cavity is able to accommodate a variety of small, mainly nonpolar, ligands. Molecules which do not appear to bind include those that are very polar, those that are too large, and those that have appropriate volume and polarity but inappropriate shape. Calorimetric analysis of 16 of these ligands reveals that their free energies of binding are poorly correlated with their solvent-transfer free energies. In addition, their enthalpies of binding are much larger than expected on the basis of transfer of the ligands from an aqueous to a nonpolar liquid phase. The binding energetics were analyzed by dividing the reaction into three processes: desolvation, immobilization, and packing. This analysis indicates that all three processes contribute to binding specificity. For a subset of these ligands that are structurally related, however, packing interactions in the protein interior are well modeled by the interactions of the ligands with octanol.

Olia A. S., Bhardwaj A., Joss L., Casjens S. and Cingolani G. (2007) Role of gene 10 protein in the hierarchical assembly of the bacteriophage P22 portal vertex structure. *Biochemistry* **46,** 8776-8784.
Abstract: The portal vertex structure of the phage P22 is a 2.8 MDa molecular machine that mediates attachment and injection of the viral genome into the host Salmonella enterica serovar Typhimurium. Five proteins form this molecular machine: the portal protein, gp1; the tail-spike, gp9; the tail-needle, gp26, and the tail accessory factors, gp4 and gp10. In order to understand the assembly of the portal vertex structure, we have isolated the gene encoding tail accessory factor gp10 and defined its structural composition and assembly within the portal vertex structure. In solution, monomeric gp10 is a beta-sheet-rich protein with a stable conformational structure, which spontaneously assembles into hexamers, likely via a dimeric intermediate. This oligomerization enhances the structural stability of the protein, which then becomes competent for assembly to a preformed portal protein:gp4 complex, and acts as a structural adaptor bridging the nascent phage tail to gp26 and gp9. Notably, in vitro purified tail accessory factors gp4, gp10, and gp26 do not significantly interact with each other in solution, but their assembly takes place efficiently when these factors are added sequentially onto an immobilized portal protein. This suggests that the assembly of the P22 tail is a highly sequential and cooperative process, likely mediated by structural rearrangements in the assembly components. The assembled portal vertex structure represents both a membrane-binding and penetrating device as well as a plug that retains the pressurized phage DNA inside the capsid.

Parker M. H., Brouillette C. G., and Prevelige P. E., Jr. (2001) Kinetic and calorimetric evidence for two distinct scaffolding protein binding populations within the bacteriophage P22 procapsid. *Biochemistry* **40,** 8962-8970.
Abstract: A wide variety of viruses require the transient presence of scaffolding proteins to direct capsid assembly. In the case of bacteriophage P22, a model in which the scaffolding protein selectively stabilizes on-pathway growing intermediates has been proposed. The stoichiometry and thermodynamics of binding of the bacteriophage P22 scaffolding protein within the procapsid were analyzed by light scattering and isothermal titration calorimetry. Calorimetric experiments carried out between 10 and 37 degrees C were consistent with the presence of at least two distinct populations of binding sites, in agreement with kinetic evidence obtained by a light scattering assay. Binding to the high-affinity sites occurred at 20 degrees C with a stoichiometry of approximately 60 scaffolding molecules per procapsid and an apparent Kd of approximately 100-300 nM and was almost completely enthalpy-driven. For the second binding population, precise fitting of the data was impossible due to small heats of binding, but the thermodynamics of binding were clearly distinct from the high-affinity phase. The heat capacity change ($\Delta Cp()$) of binding was large for the high-affinity sites and negative for both sets of sites. Addition of sodium chloride (1 M) greatly reduced the magnitude of the apparent ΔH, in agreement with previous evidence that electrostatic interactions play a major role in binding. A mutant scaffolding protein that forms covalent dimers (R74C/L177I) bound only to the high-affinity sites. These data comprise the first quantitative measurements of the energetics of the coat protein/scaffolding protein interaction.

Piszczek G., Rozycki J., Singh S. K., Ginsburg A., and Maurizi M. R. (2005) The molecular chaperone, ClpA, has a single high affinity peptide binding site per hexamer. *J Biol Chem* **280,** 12221-12230.
Abstract: Substrate recognition by Clp chaperones is dependent on interactions with motifs composed of specific peptide sequences. We studied the binding of short motif-bearing peptides to ClpA, the chaperone

component of the ATP-dependent ClpAP protease of Escherichia coli in the presence of ATPgammaS and Mg2+ at pH 7.5. Binding was measured by isothermal titration calorimetry (ITC) using the peptide, AANDENYALAA, which corresponds to the SsrA degradation motif found at the C terminus of abnormal nascent polypeptides in vivo. One SsrA peptide was bound per hexamer of ClpA with an association constant (K(A)) of 5 x 10(6) m(-1). Binding was also assayed by changes in fluorescence of an N-terminal dansylated SsrA peptide, which bound with the same stoichiometry of one per ClpA hexamer (K(A) approximately 1 x 10(7) m(-1)). Similar results were obtained when ATP was substituted for ATPgammaS at 6 degrees C. Two additional peptides, derived from the phage P1 RepA protein and the E. coli HemA protein, which bear different substrate motifs, were competitive inhibitors of SsrA binding and bound to ClpA hexamers with K(A)' > 3 x 10(7) m(-1). DNS-SsrA bound with only slightly reduced affinity to deletion mutants of ClpA missing either the N-terminal domain or the C-terminal nucleotide-binding domain, indicating that the binding site for SsrA lies within the N-terminal nucleotide-binding domain. Because only one protein at a time can be unfolded and translocated by ClpA hexamers, restricting the number of peptides initially bound should avoid nonproductive binding of substrates and aggregation of partially processed proteins.

Pozharski E. and MacDonald R. C. (2003) Lipoplex Thermodynamics: Determination of DNA-Cationic Lipoid Interaction Energies. *Biophys J* **85,** 3969-3978.
Abstract: An experimental study of the cationic lipid-DNA binding affinity is presented. The binding free energy was determined by monitoring lipoplex dissociation under conditions of increasing salt concentration. The primary procedure was based on the extent of quenching by energy transfer of fluorophores on DNA molecules by fluorophore on a lipid as these molecules came into close association in the lipoplex. Titration calorimetry on the Dickerson dodecamer was also done, with results that were in agreement with the fluorescence data. Measurements on short oligonucleotides allowed estimation of the binding energy per nucleotide. The binding free energy is approximately 0.6 kcal/mole nucleotide for the Dickerson dodecamer and declines for longer oligonucleotides. The entropy gained upon complex formation is approximately 1 entropy unit per released counterion. The method was applied to long DNA molecules (herring and lambda-phage DNA) and revealed that complete dissociation occurs at 750 mM NaCl. Likely contributions of macromolecular desolvation and DNA flexibility to the binding energy are discussed.

Raggett E. M., Bainbridge G., Evans L. J., Cooper A., and Lakey J. H. (1998) Discovery of critical Tol A-binding residues in the bactericidal toxin colicin N: a biophysical approach. *Mol Microbiol* **28,** 1335-1343.
Abstract: Colicins translocate across the Escherichia coli outer membrane and periplasm by interacting with several receptors. After first binding to outer membrane surface receptors via their central region, they interact with TolA or TonB proteins via their N-terminal regions. Finally, the toxic C-terminal region is inserted into or across the cytoplasmic membrane. We have measured the binding of colicin N to TolA by isothermal titration microcalorimetry (ITC) and tryptophan fluorescence. The isolated N-terminal domain exhibits a higher affinity for TolA (Kd = 1 μM) than does the whole colicin (18 μM), and similar behaviour has been observed when the N-terminal domain of the g3p protein of the bacteriophage fd, which also binds TolA, is examined in isolation and in situ. This may indicate a similar mechanism in which a cryptic TolA binding site is revealed after primary receptor binding. The isolated colicin N N-terminal domain appears to be unstructured in circular dichroism and fluorescence studies. We have used mutagenesis and ITC to characterize the TolA binding site and have shown it to be of a different sequence and much further from the N-terminus than previously thought.

Valentine A. M., Ishmael F. T., Shier V. K., and Benkovic S. J. (2001) A zinc ribbon protein in DNA replication: primer synthesis and macromolecular interactions by the bacteriophage T4 primase. *Biochemistry* **40,** 15074-15085.
Abstract: The gene product 61 primase protein from bacteriophage T4 was expressed as an intein fusion and purified to homogeneity. The primase binds one zinc ion, which is coordinated by four cysteine residues to form a zinc ribbon motif. Factors that influence the rate of priming were investigated, and a physiologically relevant priming rate of approximately 1 primer per second per primosome was achieved. Primase binding to the single-stranded binding protein (1 primase:4 gp32 monomers; Kd approximately

860 nM) and to the helicase protein in the presence of DNA and ATP-gamma-S (1 primase:1 helicase monomer; Kd approximately 100 nM) was investigated by isothermal titration calorimetry (ITC). Because the helicase is hexameric, the inferred stoichiometry of primase binding as part of the primosome is helicase hexamer:primase in a ratio of 1:6, suggesting that the active primase, like the helicase, might have a ring-like structure. The primase is a monomer in solution but binds to single-stranded DNA (ssDNA) primarily as a trimer (Kd approximately 50-100 nM) as demonstrated by ITC and chemical cross-linking. Magnesium is required for primase-ssDNA binding. The minimum length of ssDNA required for stable binding is 22-24 bases, although cross-linking reveals transient interactions on oligonucleotides as short as 8 bases. The association is endothermic at physiologically relevant temperatures, which suggests an overall gain in entropy upon binding. Some possible sources of this gain in entropy are discussed.

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.