

DSC XXIII - Bacteriophage studies

Ali S. A., Iwabuchi N., Matsui T., Hirota K., Kidokoro S., Arai M., Kuwajima K., Schuck P., and Arisaka F. (2003) Reversible and fast association equilibria of a molecular chaperone, gp57A, of bacteriophage T4. *Biophys J* **85**, 2606-2618.

Abstract: The association of a molecular chaperone, gp57A, of bacteriophage T4, which facilitates formation of the long and short tail fibers, was investigated by analytical ultracentrifugation, differential scanning microcalorimetry, and stopped-flow circular dichroism (CD) to establish the association scheme of the protein. Gp57A is an oligomeric alpha-helix protein with 79 amino acids. Analysis of the sedimentation velocity data by direct boundary modeling with Lamm equation solutions together with a more detailed boundary analysis incorporating association schemes led us to conclude that at least three oligomeric species of gp57A are in reversible and fast association equilibria and that a 3(mer)-6(mer)-12(mer) model described the data best. On the other hand, differential scanning microcalorimetry revealed a highly reversible two-step transition of dissociation/denaturation, both of which accompanied decrease in CD at 222 nm. The melting curve analysis revealed that it is consistent with a 6(mer)-3(mer)-1(mer) model. The refolding/association kinetics of gp57A measured by stopped-flow CD was consistent with the interpretation that the bimolecular reaction from trimer to hexamer was preceded by a fast alpha-helix formation in the dead-time. Trimer or hexamer is likely the functional oligomeric state of gp57A.

Betts S. and King J. (1999) There's a right way and a wrong way: in vivo and in vitro folding, misfolding and subunit assembly of the P22 tailspike. *Structure Fold Des* **7**, R131-R139.

Abstract: The in vivo and in vitro folding, assembly and misfolding of an elongated protein, the thermostable tailspike adhesin of phage P22, reveals important aspects of the sequence control of chain folding as well as its failure mode, inclusion body formation.

Boudko S. P., Londer Y. Y., Letarov A. V., Sernova N. V., Engel J., and Mesyanzhinov V. V. (2002) Domain organization, folding and stability of bacteriophage T4 fibrin, a segmented coiled-coil protein. *Eur J Biochem* **269**, 833-841.

Abstract: Fibrin is a segmented coiled-coil homotrimer of the 486-residue product of phage T4 gene wac. This protein attaches to a phage particle by the N-terminal region and forms fibrous whiskers of 530 Å, which perform a chaperone function during virus assembly. The short C-terminal region has a beta-annulus-like structure. We engineered a set of fibrin deletion mutants sequentially truncated from the N-termini, and the mutants were studied by differential scanning calorimetry (DSC) and CD measurements. The analysis of DSC curves indicates that full-length fibrin exhibits three thermal-heat-absorption peaks centred at 321 K ($\Delta H=1390 \text{ kJ} \times \text{mol trimer}^{-1}$), at 336 K ($\Delta H=7600 \text{ kJ} \times \text{mol trimer}^{-1}$), and at 345 K ($\Delta H=515 \text{ kJ} \times \text{mol trimer}^{-1}$). These transitions were assigned to the N-terminal, segmented coiled-coil, and C-terminal functional domains, respectively. The coiled-coil region, containing 13 segments, melts co-operatively as a single domain with a mean enthalpy $\Delta H_{\text{res}}=21 \text{ kJ} \times \text{mol residue}^{-1}$. The ratio of $\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$ for the coiled-coil part of the 120-, 182-, 258- and 281-residue per monomer mutants, truncated from the N-termini, and for full-length fibrin are 0.91, 0.88, 0.42, 0.39, and 0.13, respectively. This gives an indication of the decrease of the 'all-or-none' character of the transition with increasing protein size. The deletion of the 12-residue-long loop in the 120-residue fibrin increases the thermal stability of the coiled-coil region. According to CD data, full-length fibrin and all the mutants truncated from the N-termini refold properly after heat denaturation. In contrast, fibrin XN, which is deleted for the C-terminal domain, forms aggregates inside the cell. The XN protein can be partially refolded by dilution from urea and does not refold after heat denaturation. These results confirm that the C-terminal domain is essential for correct fibrin assembly both in vivo and in vitro and acts as a foldon.

Boudko S. P., Strelkov S. V., Engel J., and Stetefeld J. (2004) Design and crystal structure of bacteriophage T4 mini-fibrin NCCF. *J Mol Biol* **339**, 927-935.

Abstract: Fibrin is a fibrous protein that forms "whiskers" attached to the neck of bacteriophage T4. Whiskers interact with the long tail fibers regulating the assembly and infectivity of the virus. The fibrin trimer includes the N-terminal domain responsible for attachment to the phage particle and for the collar formation, the central domain forming a 500 Å long segmented coiled-coil structure, and the C-terminal "foldon" domain. We have designed a "mini" fibrin with most of the coiled-coil domain deleted, and

solved its crystal structure. The non-helical N-terminal part represents a new protein fold that tightly interacts with the coiled-coil segment forming a single domain, as revealed by calorimetry. The analysis of the crystal structure and earlier electron microscopy data on the collar-whisker complex suggests the necessity of other proteins to participate in the collar formation. Crystal structure determination of the N-terminal domain of fibrinin is the first step towards elucidating the detailed structure and assembly mechanism of the collar-whisker complex.

Briers Y., Lavigne R., Hertveldt K., Hanssens I., Engelborghs Y. and Volckaert G. (2007) Stability of phiKMV lysin gp36c reflects its role during bacteriophage infection. *Commun Agric. Appl. Biol Sci* **72**, 115-118. (no abstract)

Capen C. M. and Teschke C. M. (2000) Folding defects caused by single amino acid substitutions in a subunit are not alleviated by assembly. *Biochemistry* **39**, 1142-1151.

Abstract: Significant stabilization of a protein often occurs when it is assembled into an oligomer. Bacteriophage P22 contains 420 monomers of coat protein that are stabilized by the assembly and maturation processes. The effects of eight single amino acid substitutions in coat protein that each cause a temperature-sensitive-folding defect were investigated to determine if the conformational differences previously observed in the monomers could be alleviated by assembly or maturation. Several techniques including differential scanning calorimetry, heat-induced expansion, urea denaturation, and sensitivity to protease digestion were used to explore the effects of the amino acid substitutions on the conformation of coat protein, once assembled. Each of the amino acid substitutions caused a change in the conformation as compared to wild-type coat protein, observed by at least one of the probes used. Thus, neither assembly nor expansion entirely corrected the conformational defects in the monomeric subunits of the folding mutants.

Cervantes-Cervantes M. P., Calderon-Salinas J. V., Albores A., and Munoz-Sanchez J. L. (2005) Copper increases the damage to DNA and proteins caused by reactive oxygen species. *Biol Trace Elem Res* **103**, 229-248.

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

Conway J. F., Cheng N., Ross P. D., Hendrix R. W., Duda R. L. and Steven A. C. (2007) A thermally induced phase transition in a viral capsid transforms the hexamers, leaving the pentamers unchanged. *J Struct. Biol* **158**, 224-232.

Abstract: Scanning calorimetry combined with cryo-electron microscopy affords a powerful approach to investigating hierarchical interactions in multi-protein complexes. Calorimetry can detect the temperatures at which certain interactions are disrupted and cryo-EM can reveal the accompanying structural changes. The procapsid of bacteriophage HK97 (Prohead I) is a 450A-diameter shell composed of 60 hexamers and 12 pentamers of gp5, organized with icosahedral symmetry. Gp5 consists of the N-terminal Delta-domain (11kDa) and gp5* (31 kDa): gp5* forms the contiguous shell from which clusters of Delta-domains extend inwards. At neutral pH, Prohead I exhibits an endothermic transition at 53 degrees C with an enthalpy change of 14 kcal/mole (of gp5 monomer). We show that this transition is reversible. To capture its structural expression, we incubated Prohead I at 60 degrees C followed by rapid freezing and, by cryo-EM,

observed a capsid species 10% larger than Prohead I. At 11 Å resolution, visible changes are confined to the gp5 hexamers. Their Delta-domain clusters have disappeared and are presumably disordered, either by unfolding or dispersal. The gp5* hexamer rings are thinned and flattened as they assume the conformation observed in Expansion Intermediate I, a transition state of the normal, proteolysis-induced, maturation pathway. We infer that, at ambient temperatures, the hexamer Delta-domains restrain their gp5* rings from switching to a lower free energy, EI-I-like, state; above 53 degrees, this restraint is overcome. Pentamers, on the other hand, are more stably anchored and resist this thermal perturbation.

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 μM . 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.

Duewel H. S., Daub E., and Honek J. F. (1995) Investigations of the interactions of saccharides with the lysozyme from bacteriophage lambda. *Biochim Biophys Acta* **1247**, 149-158.

Abstract: The bacteriophage lambda R gene has been isolated into an Escherichia coli expression system and the R gene product, a lysozyme, has been overexpressed and purified to homogeneity using an efficient purification procedure. A turbidimetric assay utilizing chloroform-treated E. coli cells has been optimized to assess the bacteriolytic activity of the purified enzyme. Using this assay, oligomers of beta (1 --> 4) N-acetyl-D-glucosamine at high concentrations were shown to inhibit lysozyme but were not cleaved by the enzyme. Differential scanning calorimetry revealed that the thermal denaturation of lysozyme was found to increase in the presence of (GlcNAc)₃ and (GlcNAc)₅. The lysozyme was also expressed in an E. coli strain auxotrophic for methionine, allowing for the incorporation of [methyl-¹³C]methionine into the enzyme. An alteration of the [1H-¹³C]HMQC NMR spectra of the labelled enzyme was observed in the presence of (GlcNAc)₅. Commercially available nitrophenyl glycosides did not act as substrates for lambda lysozyme. The results indicate that lambda lysozyme has specific interactions with oligosaccharides of N-acetylglucosamine, but is incapable of hydrolyzing these sugars. The relevance of the structure of peptidoglycan to the activity of lambda lysozyme is discussed.

Efimov V. P., Nepluev I. V., Sobolev B. N., Zurabishvili T. G., Schulthess T., Lustig A., Engel J., Haener M., Aebi U., Venyaminov S. Y., and . (1994) Fibrin encoded by bacteriophage T4 gene wac has a parallel triple-stranded alpha-helical coiled-coil structure. *J Mol Biol* **242**, 470-486.

Abstract: The bacteriophage T4 late gene wac (whisker's antigen control) encodes a fibrous protein which forms a collar/whiskers complex. Whiskers function as a helper protein for the long tail fibres assembly and plays a role in regulating retraction of the long tail fibres in response to environmental conditions. In this work we show that expression of the cloned wac gene in Escherichia coli yields a protein oligomer of 53 nm length which we call fibrin, and which is able to complement gpwac T4 particles in vitro. CD spectroscopy of fibrin indicates a 90% alpha-helical content, and scanning calorimetry shows that the protein has several distinct domains. The analysis of the 486 amino acid sequence of fibrin reveals three structural components: a 408 amino acid region that contains 12 putative coiled-coil segments with a canonical heptad (a-b-c-d-e-f-g)_n substructure where the "a" and "d" positions are preferentially occupied

by apolar residues, and the N and C-terminal domains (47 and 29 amino acid residues, respectively) have no heptad substructure. The distribution of hydrophobic residues within heptads is more similar to a triple than to a double coiled-coil. The alpha-helical segments are separated by short "linker" regions, variable in length, that have a high proportion of glycine and proline residues. Each coiled-coil segment has, on the borders with linker regions, residues that are common to the N and C-terminal caps of the alpha-helices. Full-length and amino-terminally truncated fibrinins can be reassembled in vitro after temperature-induced denaturation. Co-assembly of full-length fibritin and the N-terminal deletion mutant, as well as analytical centrifugation, indicates that the protein is a parallel triple-standard alpha-helical coiled-coil. Deletions of various N-terminal portions of fibritin did not block trimerisation but the mutant trimers are unable to bind to T4 particles. The last 18 C-terminal residues of fibritin are required for correct trimerisation of gpwac monomers in vivo. We propose that fibritin might serve as a convenient model for the investigation of folding and assembly mechanisms of alpha-fibrous proteins.

Frank S., Kammerer R. A., Mechling D., Schulthess T., Landwehr R., Bann J., Guo Y., Lustig A., Bachinger H. P., and Engel J. (2001) Stabilization of short collagen-like triple helices by protein engineering. *J Mol Biol* **308**, 1081-1089.

Abstract: Recombinant expression of collagens and fragments of collagens is often difficult, as their biosynthesis requires specific post-translational enzymes, in particular prolyl 4-hydroxylase. Although the use of hydroxyproline-deficient variants offers one possibility to overcome this difficulty, these proteins usually differ markedly in stability when compared with the hydroxyproline-containing analogs. Here, we report a method to stabilize collagen-like peptides by fusing them to the N terminus of the bacteriophage T4 fibritin foldon domain. The isolated foldon domain and the chimeric protein (GlyProPro)(10)foldon were expressed in a soluble form in *Escherichia coli*. The recombinant proteins and the synthetic (ProProGly)(10) peptide were characterized by circular dichroism (CD) spectroscopy, differential scanning calorimetry, and analytical ultracentrifugation. We show that the foldon domain, which comprises only 27 amino acid residues, forms an obligatory trimer with a high degree of thermal stability. The CD thermal unfolding profiles recorded from foldon are monophasic and completely reversible upon cooling. Similar Van't Hoff and calorimetric enthalpy values of trimer formation indicated a cooperative all-or-none transition. As reported previously, (ProProGly)(10) peptides form collagen triple helices of only moderate stability. When fused to the foldon domain, however, triple helix formation of (GlyProPro)(10) is concentration independent, and the midpoint temperature of the triple helix unfolding is significantly increased. The stabilizing function of the trimeric foldon domain is explained by the close vicinity of its N termini, which induce a high local concentration in the range of 1 M for the C termini of the collagen-like-peptide. Collagen-foldon fusion proteins should be potentially useful to study receptor-collagen interactions.

Galisteo M. L., Gordon C. L., and King J. (1995) Stability of wild-type and temperature-sensitive protein subunits of the phage P22 capsid. *J Biol Chem* **270**, 16595-16601.

Abstract: Temperature-sensitive folding (tsf) mutants of the phage P22 coat protein prevent newly synthesized polypeptide chains from reaching the conformation competent for capsid assembly in cells, and can be rescued by the GroEL chaperone (Gordon, C., Sather, S., Casjens, S., and King, J. (1994) *J. Biol. Chem.* 269, 27941-27951). Here we investigate the stabilities of wild-type and four tsf mutant unpolymerized subunits. Wild-type coat protein subunits denatured at 40 degrees C, with a calorimetric enthalpy of approximately 600 kJ/mol. Comparison with coat protein denaturation within the shell lattice ($T_m = 87$ degrees C, ΔH approximately 1700 kJ/mol) (Galisteo, M.L., and King, J. (1993) *Biophys. J.* 65, 227-235) indicates that protein-protein interactions within the capsid provide enormous stabilization. The melting temperatures of the subunits carrying tsf substitutions were similar to wild-type. At low temperatures, the tsf mutants, but not the wild-type, formed non-covalent dimers, which were dissociated at temperatures above 30 degrees C. Spectroscopic and calorimetric studies indicated that the mutant proteins have reduced amounts of ordered structure at low temperature, as compared to the wild-type protein. Although complex, the in vitro phenotypes are consistent with the in vivo finding that the mutants are defective in folding, rather than subunit stability. These results suggest a role for incompletely folded subunits as precursors in viral capsid assembly, providing a mechanism of reaching multiple conformations in the polymerized form.

Holopainen J. M., Saily M., Caldentey J., and Kinnunen P. K. (2000) The assembly factor P17 from bacteriophage PRD1 interacts with positively charged lipid membranes. *Eur J Biochem* **267**, 6231-6238.

Abstract: The interactions of the assembly factor P17 of bacteriophage PRD1 with liposomes were investigated by static light scattering, fluorescence spectroscopy, and differential scanning calorimetry. Our data show that P17 binds to positively charged large unilamellar vesicles composed of the zwitterionic 1-palmitoyl-2-oleoyl-phosphatidylcholine and sphingosine, whereas only a weak interaction is evident for 1-palmitoyl-2-oleoyl-phosphatidylcholine vesicles. P17 does not bind to negatively charged membranes composed of 1-palmitoyl-2-oleoyl-phosphatidylglycerol and 1-palmitoyl-2-oleoyl-phosphatidylcholine. Our differential scanning calorimetry results reveal that P17 slightly perturbs the phase behaviour of neutral phosphatidylcholine and negatively charged multilamellar vesicles. In contrast, the phase transition temperature of positively charged dimyristoylphosphatidylcholine/sphingosine multilamellar vesicles (molar ratio 9 : 1, respectively) is increased by approximately 2.4 degrees C and the half width of the enthalpy peak broadened from 1.9 to 5.6 degrees C in the presence of P17 (protein : lipid molar ratio 1 : 47). Moreover, the enthalpy peak is asymmetrical, suggesting that lipid phase separation is induced by P17. Based on the far-UV CD spectra, the alpha-helicity of P17 increases upon binding to positively charged micelles composed of Triton X-100 and sphingosine. We propose that P17 can interact with positively charged lipid membranes and that this binding induces a structural change on P17 to a more tightly packed and ordered structure.

Kitazawa D., Takeda S., Kageyama Y., Tomihara M., and Fukada H. (2005) Expression and Characterization of a Baseplate Protein for Bacteriophage Mu, gp44. *J Biochem (Tokyo)* **137**, 601-606.

Abstract: The gene product of gene 44 of Mu phage (gp44) is an essential protein for baseplate assembly and has been designated as gpP, a traditional genetic assignment. The function of gp44 during the assembly or infection process is not known. In the present study, we purified the recombinant gp44 and characterized it by analytical ultracentrifugation and differential scanning microcalorimetry. The results indicate that gp44 forms a trimer comprising a complex consisting of the 42 kDa and 40 kDa subunits that had been cleaved in the C-terminal region. Thermodynamic analysis also suggested that the C-terminal region forms a flexible domain.

Ladbury J. E., Wynn R., Thomson J. A., and Sturtevant J. M. (1995) Substitution of charged residues into the hydrophobic core of Escherichia coli thioredoxin results in a change in heat capacity of the native protein. *Biochemistry* **34**, 2148-2152.

Abstract: Two site-directed mutants of Escherichia coli thioredoxin (L78K and L78R) were designed to study the effect of placing a charged residue in the hydrophobic core of the protein. Both mutants retain catalytic activity in the assembly of phage M13. Thermal denaturation of both these mutant proteins at pH 7.0 shows a reduction of stability of approximately 4 kcal.mol⁻¹ with respect to the oxidized wild-type form. The thermal denaturation of the protein fits a dimeric state model. A significant reduction in the change in heat capacity (ΔC_p) on unfolding is observed compared to oxidized wild-type thioredoxin. We present data to indicate that this reduction in ΔC_p is attributable to structural perturbations resulting in localized unfolding of the native protein and exposure to solvent of residues that are buried in the wild-type protein.

Martin A. and Schmid F. X. (2003) Evolutionary stabilization of the gene-3-protein of phage fd reveals the principles that govern the thermodynamic stability of two-domain proteins. *J Mol Biol* **328**, 863-875.

Abstract: The gene-3-protein (G3P) of filamentous phage is essential for their propagation. It consists of three domains. The CT domain anchors G3P in the phage coat, the N2 domain binds to the F pilus of Escherichia coli and thus initiates infection, and the N1 domain continues by interacting with the TolA receptor. Phage are thus only infective when the three domains of G3P are tightly linked, and this requirement is exploited by Proside, an in vitro selection method for proteins with increased stability. In Proside, a repertoire of variants of the protein to be stabilized is inserted between the N2 and the CT domains of G3P. Stabilized variants can be selected because they resist cleavage by a protease and thus maintain the essential linkage between the domains. The method is limited by the proteolytic stability of G3P itself. We improved the stability of G3P by subjecting the phage without a guest protein to rounds of random in vivo mutagenesis and proteolytic Proside selections. Variants of G3P with one to four mutations were selected, and the temperature at which the corresponding phage became accessible for a protease increased in a stepwise manner from 40 degrees C to almost 60 degrees C. The N1-N2 fragments of wild-

type gene-3-protein and of the four selected variants were purified and their stabilities towards thermal and denaturant-induced unfolding were determined. In the biphasic transitions of these proteins domain dissociation and unfolding of N2 occur in a concerted reaction in the first step, followed by the independent unfolding of domain N1 in the second step. N2 is thus less stable than N1, and it unfolds when the interactions with N1 are broken. The strongest stabilizations were caused by mutations in domain N2, in particular in its hinge subdomain, which provides many stabilizing interactions between the N1 and N2 domains. These results reveal how the individual domains and their assembly contribute to the overall stability of two-domain proteins and how mutations are optimally placed to improve the stability of such proteins.

Mattos C., Cohen J. D., Green D. F., Tidor B., and Karplus M. (2004) X-ray structural and simulation analysis of a protein mutant: the value of a combined approach. *Proteins* **55**, 733-742.

Abstract: The effect of the mutation Arg 96 to His on the stability of bacteriophage T4 lysozyme has been previously studied by calorimetric experiments, X-ray crystallography, and free energy simulation techniques. The experimental and calculated values for the difference between the free energy of denaturation of the mutant and the wild type are in reasonable agreement. However, the two approaches led to different explanations for the loss in stability. To analyze the differences, a series of refinements based on the crystallographic data were performed, a number of aspects of the simulations were reexamined, and continuum electrostatic calculations were done to complement the latter. The results of those comparisons provide a better understanding of the origin of the free energy difference in this mutant. Furthermore, they show the importance of the combined use of simulations and crystallography for interpreting the effects of mutations on the energetics of the system.

Mdzinarashvili T., Khvedelidze M., Ivanova A., Mrevlishvili G., Kutateladze M., Balarjishvili N., Celia H., and Pattus F. (2006) Biophysics of T5, IRA phages, Escherichia coli outer membrane protein FhuA and T5-FhuA interaction. *Eur Biophys J* **35**, 231-238.

Abstract: In spite of the similarities in a structural organization of T5 and IRA phages their thermal and hydrodynamical peculiarities are completely different. One of the significant differences is observed in temperature value at which thermally induced DNA ejection starts. If in the case of physiological conditions this difference equals to 30 degrees capital ES, Cyrillic, then it decreases as ionic strength of the solvent decreases. Also, from our experimental results follows that in the opening of phage tail channel for T5 phage (at pH7) significant role-play electrostatic forces. In spite of that both of these phages grow on the same Escherichia coli strain, we have shown that these phages need different receptors to penetrate into the bacterial cell precisely FhuA serves as receptor only for T5 phage. The higher FhuA concentration in T5 phage suspension is, the more intensive DNA ejection in environment is. The minimal FhuA/T5 ratio, which is 300/1, correspondingly, necessary for effective DNA ejection from the phage head was experimentally determined. For the first time the ejection of T5 phage DNA induced by FhuA was observed in an incessant regime. The deconvolution of calorimetric curve of FhuA's denaturation has been shown that in a chosen condition there are four thermodynamically independent domains in the structure of FhuA.

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

Abstract: Knowledge of both the packaging of the linear, double-stranded (ds)DNA in bacteriophages and its subsequent release into the bacterial host is vital to our understanding of phage infection. There is now strong evidence that packaging requires a powerful rotary motor fuelled by ATP. From thermodynamic studies, however, it has been proposed that, at least for those viruses with a contractile tail, the dsDNA ejection from the phage head is a relatively simple physical process that does not require cellular energy and is facilitated by the difference in the conditions of the medium in the environments inside and outside the head. In this case, there should be no enthalpic effects associated with the dehiscence of the capsid and no destruction of it or the other structural elements of the phage. For the present study of temperature-induced phage dehiscence, we used a newly discovered phage with a contractile tail, named the Un (unknown) bacteriophage. Evidence is given of its characteristics in terms of ultrastructural morphology, serological parameters, host range and interaction with host cell. These show that, although it has

similarities with the T-even phages and, in particular, the DDVI phage, it appears to be a new type. Earlier viscometric studies with it had shown that the temperature-induced release of the capsid dsDNA was completed at 70 degrees C. In the present investigation, a concentrated suspension of purified phage was subjected to pycnometric analysis through the temperature range of 30 to 70 degrees C. This showed that a significant and abrupt increase in the phage partial volume takes place, which remarkably is in the order of threefold. Viscometric measurements over time at 72 degrees C gave a kinetic curve from which evidence it was suggested that the temperature-induced DNA release is similar to a second order phase transition. At the same time, data from differential scanning calorimetry over the same temperature range showed no enthalpic effect. Our results indicate that the ejection of DNA from the capsid tail is driven by an entropy change.

Merabet E. K., Burz D. S., and Ackers G. K. (1998) Thermal melting properties of C-terminal domain mutants of bacteriophage lambda cI repressor. *Methods Enzymol* **295**, 450-467.

Osumi-Davis P. A., Sreerama N., Volkin D. B., Middaugh C. R., Woody R. W., and Woody A. Y. (1994) Bacteriophage T7 RNA polymerase and its active-site mutants. Kinetic, spectroscopic and calorimetric characterization. *J Mol Biol* **237**, 5-19.

Abstract: It has been demonstrated that the amino acids Asp537, Asp812, Lys631, His811 and Tyr639 are involved in bacteriophage T7 RNA polymerase catalysis. In the present paper, we report kinetic, spectroscopic and calorimetric characterization of the wild-type and mutant T7 RNA polymerases generated at these five loci (D537N, E; K631M, R; Y639F, S, A, W; H811Q, A; D812N, E). The wild-type enzyme has a substantial amount of secondary structure as determined by CD analysis (alpha-helix, 43%; beta-sheet, 14%; beta-turn, 25%; unordered, 18%). The CD spectra of 12 mutants at five loci are very similar to that of the wild-type, except for the mutant Y639W. Within experimental error, the thermal transition temperatures measured by CD and DSC as well as the lambda max values of the fluorescence spectra were the same for the wild-type and all of the mutants. Therefore, the overall folding and stability of the mutant enzymes are very similar to those of the wild-type enzyme, although small local conformational changes cannot be excluded. For the synthesis of the pentamer pppGGACU, the mutants D537E and D812E showed an approximately two- to threefold decrease in (kcat)_{app} and an approximately two- to threefold increase in (K_m)_{app}, relative to the wild-type, in contrast to the mutants D537N and D812N which exhibited no detectable activity. The mutant K631R showed a sevenfold reduction in (kcat)_{app} and a two- to threefold increase in (K_m)_{app}, supporting our earlier observation with the mutant K631M that Lys631 may be involved in phosphodiester bond formation. The mutant Y639S can synthesize the trimer GGA with an approximately 50-fold decrease in (kcat)_{app} and a tenfold increase in (K_m)_{app}, relative to the wild-type, underlining the importance of the phenyl ring of Tyr639. The mutant H811A, in which the side-chain at position 811 is incapable of forming a hydrogen bond, can synthesize the trimer GGA with an approximately tenfold decrease in (kcat)_{app} and an approximately 35-fold increase in (K_m)_{app}. Thus, either the hydrogen-bonding capacity of this residue is non-essential or some other group can functionally substitute for the His811 side-chain. The wild-type enzyme showed significant effects of the base position in the sequence on the apparent binding constants for the NTPs. The kinetics of GpG-primed trimer, tetramer and pentamer synthesis on three 22 bp templates were investigated for the wild-type and mutant enzymes with measurable activity. (ABSTRACT TRUNCATED AT 400 WORDS).

Padmanabhan S., Laurents D. V., Fernandez A. M., Elias-Arnanz M., Ruiz-Sanz J., Mateo P. L., Rico M., and Filimonov V. V. (1999) Thermodynamic analysis of the structural stability of phage 434 Cro protein. *Biochemistry* **38**, 15536-15547.

Abstract: Thermodynamic parameters describing the phage 434 Cro protein have been determined by calorimetry and, independently, by far-UV circular dichroism (CD) measurements of isothermal urea denaturations and thermal denaturations at fixed urea concentrations. These equilibrium unfolding transitions are adequately described by the two-state model. The far-UV CD denaturation data yield average temperature-independent values of $0.99 \pm 0.10 \text{ kcal mol}^{-1} \text{ M}^{-1}$ for m and $0.98 \pm 0.05 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for $\Delta C_p(U)$, the heat capacity change accompanying unfolding. Calorimetric data yield a temperature-independent $\Delta C_p(U)$ of $0.95 \pm 0.30 \text{ kcal mol}^{-1} \text{ K}^{-1}$ or a temperature-dependent value of $1.00 \pm 0.10 \text{ kcal mol}^{-1} \text{ K}^{-1}$ at 25 degrees C. $\Delta C_p(U)$ and m determined for 434 Cro are in accord with values predicted using known empirical correlations with structure. The free energy of unfolding is pH-dependent, and the protein is completely unfolded at pH 2.0 and 25 degrees C as

judged by calorimetry or CD. The stability of 434 Cro is lower than those observed for the structurally similar N-terminal domain of the repressor of phage 434 (R1-69) or of phage lambda (lambda(6)(-)(85)), but is close to the value reported for the putative monomeric lambda Cro. Since a protein's structural stability is important in determining its intracellular stability and turnover, the stability of Cro relative to the repressor could be a key component of the regulatory circuit controlling the levels and, consequently, the functions of the two proteins in vivo.

Plancon L., Janmot C., le Maire M., Desmadril M., Bonhivers M., Letellier L., and Boulanger P. (2002) Characterization of a high-affinity complex between the bacterial outer membrane protein FhuA and the phage T5 protein pb5. *J Mol Biol* **318**, 557-569.

Abstract: Binding of bacteriophage T5 to *Escherichia coli* cells is mediated by specific interactions between the receptor-binding protein pb5 (67.8 kDa) and the outer membrane iron-transporter FhuA. A histidine-tagged form of pb5 was overproduced and purified. Isolated pb5 is monomeric and organized mostly as beta-sheets (51%). pb5 functionality was attested in vivo by its ability to impair infection of *E. coli* cells by phage T5 and Phi80, and to prevent growth of bacteria on iron-ferrichrome as unique iron source. pb5 was functional in vitro, since addition of an equimolar concentration of pb5 to purified FhuA prevented DNA release from phage T5. However, pb5 alone was not sufficient for the conversion of FhuA into an open channel. Direct interaction of pb5 with FhuA was demonstrated by isolating a pb5/FhuA complex using size-exclusion chromatography. The stoichiometry, 1 mol of pb5/1 mol of FhuA, was deduced from its molecular mass, established by analytical ultracentrifugation after determination of the amount of bound detergent. SDS-PAGE and differential scanning calorimetry experiments highlighted the great stability of the complex: (i) it was not dissociated by 2% SDS even when the temperature was raised to 70 degrees C; (ii) thermal denaturation of the complex occurred at 85 degrees C, while pb5 and FhuA were denatured at 45 degrees C and 74 degrees C, respectively. The stability of the complex renders it suitable for high-resolution structural studies, allowing future analysis of conformational changes into both FhuA and pb5 upon adsorption of the virus to its host.

Protasevich I. I., Memelova L. V., Kochetkov S. N., and Makarov A. A. (1994) The studies of cooperative regions in T7 RNA polymerase. *FEBS Lett* **349**, 429-432.

Abstract: The heat denaturation of bacteriophage T7 RNA polymerase (T7RNAP) was studied by scanning microcalorimetry. The thermodynamic parameters of the denaturation were estimated within the pH range 6-9. The analysis of the denaturation curves showed the presence of two cooperative parts of the T7RNAP molecule melting according to the 'all-or-none' principle. The molecular masses of these parts were determined as 22 and 77 kDa. These values are close to the molecular masses of protein domains obtained from X-ray diffraction and limited trypsinolysis data. The smaller N-terminal domain was shown to increase the thermostability of the 'catalytic' C-terminal domain within the intact T7RNAP molecule.

Riechmann L. and Winter G. (2000) Novel folded protein domains generated by combinatorial shuffling of polypeptide segments. *Proc Natl Acad Sci U S A* **97**, 10068-10073.

Abstract: It has been proposed that the architecture of protein domains has evolved by the combinatorial assembly and/or exchange of smaller polypeptide segments. To investigate this proposal, we fused DNA encoding the N-terminal half of a beta-barrel domain (from cold shock protein CspA) with fragmented genomic *Escherichia coli* DNA and cloned the repertoire of chimeric polypeptides for display on filamentous bacteriophage. Phage displaying folded polypeptides were selected by proteolysis; in most cases the protease-resistant chimeric polypeptides comprised genomic segments in their natural reading frames. Although the genomic segments appeared to have no sequence homologies with CspA, one of the originating proteins had the same fold as CspA, but another had a different fold. Four of the chimeric proteins were expressed as soluble polypeptides; they formed monomers and exhibited cooperative unfolding. Indeed, one of the chimeric proteins contained a set of very slowly exchanging amides and proved more stable than CspA itself. These results indicate that native-like proteins can be generated directly by combinatorial segment assembly from nonhomologous proteins, with implications for theories of the evolution of new protein folds, as well as providing a means of creating novel domains and architectures in vitro.

Rogov V. V., Lucke C., Muresanu L., Wienk H., Kleinhaus I., Werner K., Lohr F., Pristovsek P., and Ruterjans H. (2003) Solution structure and stability of the full-length excisionase from bacteriophage

HK022. *Eur J Biochem* **270**, 4846-4858.

Abstract: Heteronuclear high-resolution NMR spectroscopy was employed to determine the solution structure of the excisionase protein (Xis) from the lambda-like bacteriophage HK022 and to study its sequence-specific DNA interaction. As wild-type Xis was previously characterized as a generally unstable protein, a biologically active HK022 Xis mutant with a single amino acid substitution Cys28-->Ser was used in this work. This substitution has been shown to diminish the irreversibility of Xis denaturation and subsequent degradation, but does not affect the structural or thermodynamic properties of the protein, as evidenced by NMR and differential scanning calorimetry. The solution structure of HK022 Xis forms a compact, highly ordered protein core with two well-defined alpha-helices (residues 5-11 and 18-27) and five beta-strands (residues 2-4, 30-31, 35-36, 41-44 and 48-49). These data correlate well with ¹H₂O-²H₂O exchange experiments and imply a different organization of the HK022 Xis secondary structure elements in comparison with the previously determined structure of the bacteriophage lambda excisionase. Superposition of both Xis structures indicates a better correspondence of the full-length HK022 Xis to the typical 'winged-helix' DNA-binding motif, as found, for example, in the DNA-binding domain of the Mu-phage repressor. Residues 51-72, which were not resolved in the lambda Xis, do not show any regular structure in HK022 Xis and thus appear to be completely disordered in solution. The resonance assignments have shown, however, that an unusual connectivity exists between residues Asn66 and Gly67 owing to asparagine-isoaspartyl isomerization. Such an isomerization has been previously observed and characterized only in eukaryotic proteins.

Ross P. D., Cheng N., Conway J. F., Firek B. A., Hendrix R. W., Duda R. L., and Steven A. C. (2005) Crosslinking renders bacteriophage HK97 capsid maturation irreversible and effects an essential stabilization. *EMBO J* **24**, 1352-1363.

Abstract: In HK97 capsid maturation, structural change ('expansion') is accompanied by formation of covalent crosslinks, connecting residue K169 in the 'E-loop' of each subunit with N356 on another subunit. We show by complementation experiments with the K169Y mutant, which cannot crosslink, that crosslinking is an essential function. The precursor Prohead-II passes through three expansion intermediate (EI) states en route to the end state, Head-II. We investigated the effects of expansion and crosslinking on stability by differential scanning calorimetry of wild-type and K169Y capsids. After expansion, the denaturation temperature (T_p) of K169Y capsids is slightly reduced, indicating that their thermal stability is not enhanced, but crosslinking effects a major stabilization (delta T_p, +11 degrees C). EI-II is the earliest capsid to form crosslinks. Cryo-electron microscopy shows that for both wild-type and K169Y EI-II, most E-loops are in the 'up' position, 30 Å from the nearest N356: thus, crosslinking in EI-II represents capture of mobile E-loops in 'down' positions. At pH 4, most K169Y capsids remain as EI-II, whereas wild-type capsids proceed to EI-III, suggesting that crosslink formation drives maturation by a Brownian ratchet mechanism.

Ross P. D., Conway J. F., Cheng N., Dierkes L., Firek B. A., Hendrix R. W., Steven A. C., and Duda R. L. (2006) A free energy cascade with locks drives assembly and maturation of bacteriophage HK97 capsid. *J Mol Biol* **364**, 512-525.

Abstract: We investigated the thermodynamic basis of HK97 assembly by scanning calorimetry and cryo-electron microscopy. This pathway involves self-assembly of hexamers and pentamers of the precursor capsid protein gp5 into procapsids; proteolysis of their N-terminal Delta-domains; expansion, a major conformational change; and covalent crosslinking. The thermal denaturation parameters convey the changes in stability at successive steps in assembly, and afford estimates of the corresponding changes in free energy. The procapsid represents a kinetically accessible local minimum of free energy. In maturation, it progresses to lower minima in a cascade punctuated by irreversible processes ("locks"), i.e. proteolysis and crosslinking, that lower kinetic barriers and prevent regression. We infer that Delta-domains not only guide assembly but also restrain the procapsid from premature expansion; their removal by proteolysis is conducive to initiating expansion and to its proceeding to completion. We also analyzed the mutant E219K, whose capsomers reassemble in vitro into procapsids with vacant vertices called "whiffleballs". E219K assemblies all have markedly reduced stability compared to wild-type gp5 (Delta T(p) approximately -7 degrees C to -10 degrees C; where T(p) is the denaturation temperature). As the mutated residue is buried in the core of gp5, we attribute the observed reduction in stability to steric and electrostatic perturbations of the packing of side-chains in the subunit interior. To explain the whiffleball phenotype, we suggest that

these effects propagate to the capsomer periphery in such a way as to differentially affect the stability or solubility of dissociated pentamers, leaving only hexamers to reassemble.

Ruiz-Sanz J., Simoncsits A., Toro I., Pongor S., Mateo P. L., and Filimonov V. V. (1999) A thermodynamic study of the 434-repressor N-terminal domain and of its covalently linked dimers. *Eur J Biochem* **263**, 246-253.

Abstract: The isolated N-terminal 1-69 domain of the 434-phage repressor, R69, and its covalently linked (head-to-tail and tail-to-tail) dimers have been studied by differential scanning microcalorimetry (DSC) and CD. At neutral solvent conditions the R69 domain maintains its native structure, both in isolated form and within the dimers. The stability of the domain depends highly upon pH within the acidic range, thus at pH 2 and low ionic strength R69 is already partially unfolded at room temperature. The thermodynamic parameters of unfolding calculated from the DSC data are typical for small globular proteins. At neutral pH and moderate ionic strength, the domains of the dimers behave as two independent units with unfolding parameters similar to those of the isolated domain, which means that linking two R69 domains, either by a long peptide linker or by a designed C-terminal disulfide bridge, does not induce any cooperation between them.

Saiz J. L., Lopez-Zumel C., Monterroso B., Varea J., Arrondo J. L., Iloro I., Garcia J. L., Laynez J., and Menendez M. (2002) Characterization of Ejl, the cell-wall amidase coded by the pneumococcal bacteriophage Ejl-1. *Protein Sci* **11**, 1788-1799.

Abstract: The Ejl amidase is coded by Ejl-1, a temperate phage isolated from the atypical pneumococcus strain 101/87. Like all the pneumococcal cell-wall lysins, Ejl has a bimodular organization; the catalytic region is located in the N-terminal module, and the C-terminal module attaches the enzyme to the choline residues of the pneumococcal cell wall. The structural features of the Ejl amidase, its interaction with choline, and the structural changes accompanying the ligand binding have been characterized by CD and IR spectroscopies, differential scanning calorimetry, analytical ultracentrifugation, and FPLC. According to prediction and spectroscopic (CD and IR) results, Ejl would be composed of short beta-strands (ca. 36%) connected by long loops (ca. 17%), presenting only two well-predicted alpha-helices (ca. 12%) in the catalytic module. Its polypeptide chain folds into two cooperative domains, corresponding to the N- and C-terminal modules, and exhibits a monomer <--> dimer self-association equilibrium. Choline binding induces small rearrangements in Ejl secondary structure but enhances the amidase self-association by preferential binding to Ejl dimers and tetramers. Comparison of LytA, the major pneumococcal amidase, with Ejl shows that the sequence differences (15% divergence) strongly influence the amidase stability, the organization of the catalytic module in cooperative domains, and the self-association state induced by choline. Moreover, the ligand affinity for the choline-binding locus involved in regulation of the amidase dimerization is reduced by a factor of 10 in Ejl. Present results evidence that sequence differences resulting from the natural variability found in the cell wall amidases coded by pneumococcus and its bacteriophages may significantly alter the protein structure and its attachment to the cell wall.

Xie D. and Freire E. (1994) Structure based prediction of protein folding intermediates. *J Mol Biol* **242**, 62-80.

Abstract: The complete unfolding of a protein involves the disruption of non-covalent intramolecular interactions within the protein and the subsequent hydration of the backbone and amino acid side-chains. The magnitude of the thermodynamic parameters associated with this process is known accurately for a growing number of globular proteins for which high-resolution structures are also available. The existence of this database of structural and thermodynamic information has facilitated the development of statistical procedures aimed at quantifying the relationships existing between protein structure and the thermodynamic parameters of folding/unfolding. Under some conditions proteins do not unfold completely, giving rise to states (commonly known as molten globules) in which the molecule retains some secondary structure and remains in a compact configuration after denaturation. This phenomenon is reflected in the thermodynamics of the process. Depending on the nature of the residual structure that exists after denaturation, the observed enthalpy, entropy and heat capacity changes will deviate in a particular and predictable way from the values expected for complete unfolding. For several proteins, these deviations have been shown to exhibit similar characteristics, suggesting that their equilibrium folding intermediates exhibit some common structural features. Employing empirically derived structure-energetic relationships, it is possible to identify in the native structure of the protein those regions with the higher probability of

being structured in equilibrium partly folded states. In this work, a thermodynamic search algorithm aimed at identifying the structural determinants of the molten globule state has been applied to six globular proteins; alpha-lactalbumin, barnase, IIIgIc, interleukin-1 beta, phage T4 lysozyme and phage 434 repressor. Remarkably, the structural features of the predicted equilibrium intermediates coincide to a large extent with the known structural features of the corresponding intermediates determined by NMR hydrogen-exchange experiments.

Xing Y. and Draper D. E. (1995) Stabilization of a ribosomal RNA tertiary structure by ribosomal protein L11. *J Mol Biol* **249**, 319-331.

Abstract: Interactions between ribosomal protein L11 and a domain of large subunit rRNA have been highly conserved and are essential for efficient protein synthesis. To study the effects of L11 on rRNA folding, a homolog of the Escherichia coli L11 gene has been amplified from Bacillus stearothermophilus DNA and cloned into a phage T7 polymerase-based expression system. The expressed protein is 93% homologous to the L11 homolog from Bacillus subtilis, denatures at temperatures above 72 degrees C, and has nearly identical rRNA binding properties as the Escherichia coli L11 in terms of RNA affinity constants and their dependences on temperature, Mg²⁺ concentration, monovalent cation, and RNA mutations. Mg²⁺ and NH₄⁺ are specifically bound by the RNA-protein complex, with apparent ion-RNA affinities of 1.6 mM⁻¹ and 19 M⁻¹, respectively, at 0 degree C. The effect of the thermostable L11 on the unfolding of a 60 nucleotide rRNA fragment containing its binding domain has been examined in melting experiments. The lowest temperature RNA transition, which is attributed to tertiary structure unfolding, is stabilized by approximately 25 degrees C, and the interaction has an intrinsic enthalpy of approximately 13 kcal/mol. The thermal stability of the protein-RNA complex is enhanced by increasing Mg²⁺ concentration and by NH₄⁺ relative to Na⁺. Thus L11, NH₄⁺, and Mg²⁺ all bind and stabilize the same rRNA tertiary interactions, which are conserved and presumably important for ribosome function.