

DSC VII - Vaccine and virus studies

Aranda F. J., Teruel J. A., and Ortiz A. (2003) Interaction of a synthetic peptide corresponding to the N terminus of canine distemper virus fusion protein with phospholipid vesicles: a biophysical study. *Biochim Biophys Acta* **1618**, 51-58.

Abstract: The F protein of canine distemper virus (CDV) is a classic type I glycoprotein formed by two polypeptides, F1 and F2. The N-terminal regions of the F1 polypeptides of CDV, measles virus and other paramyxoviruses present moderate to high homology, supporting the existence of a high conservation within these structures, which emphasises its role in viral-host cell membrane fusion. This N-terminal polypeptide is usually termed the fusion peptide. The fusion peptides of most viral fusion-mediating glycoproteins contain a high proportion of hydrophobic amino acids, which facilitates its insertion into target membranes during fusion. In this work we report on the interaction of a 31-residue synthetic peptide (FP31) corresponding to the N terminus of CDV F1 protein with phospholipid membranes composed of various phospholipids, as studied by means of various biophysical techniques. FTIR investigation of FP31 secondary structure in aqueous medium and in membranes resulted in a major proportion of alpha-helical structure which increased upon membrane insertion. Differential scanning calorimetry (DSC) showed that the presence of concentrations of FP31 as low as 0.1 mol%, in mixtures with L-alpha-dimyristoylphosphatidylcholine (DMPC), L-alpha-dipalmitoylphosphatidylcholine (DPPC) and L-alpha-distearoylphosphatidylcholine (DSPC), already affected the thermotropic properties of the gel to liquid-crystalline phase transition. In mixtures with the three lipids, increasing the concentration of peptide made the pretransition to disappear, and lowered and broadened the main transition. This effect was slightly stronger as the acyl chain length of the phospholipid grew larger. In the corresponding partial phase diagrams, no immiscibilities or critical points were observed. FTIR showed that incorporation of 1 mol% of peptide in DPPC shifted the antisymmetric and symmetric CH(2) stretching bands to higher values, indicating the existence of an additional disordering of the acyl chain region of the fluid bilayer. FTIR studies of the Cz.dbnd6;O stretching band indicated that incorporation of FP31 into phosphatidylcholine membranes produced a strong dehydration of the polar part of the bilayer. In mixtures with L-alpha-dielaidoylphosphatidylethanolamine (DEPE), increasing FP31 concentrations broadened and shifted to lower temperatures the lamellar to hexagonal-H(II) phase transition, indicating that this peptide destabilized the bilayer and promoted formation of the hexagonal-H(II) phase. The results are discussed in terms of lipid-peptide hydrophobic mismatch and its influence on the role of the N-terminal polypeptide of CDV F1 protein in viral membrane fusion.

Ausar S. F., Foubert T. R., Hudson M. H., Vedvick T. S., and Middaugh C. R. (2006) Conformational stability and disassembly of Norwalk virus-like particles. Effect of pH and temperature. *J Biol Chem* **281**, 19478-19488.

Abstract: Greater than 99% of the Norwalk virus (NV) capsid consists of 180 copies of a single 58-kDa protein. Recombinantly expressed monomers self-assemble into virus-like particles (VLPs) with a well defined icosahedral structure. NV-VLPs are an appropriate vaccine antigen since the antigenic determinants of the parent virion are preserved. They also constitute very simple models to study the mechanisms of assembly and disassembly of viral capsids. This work examines the inherent stability of NV-VLPs over a range of pH and temperature values and provides detailed insight into structural perturbations that accompany disassembly. The NV-VLP structure was monitored using a variety of biophysical techniques including intrinsic and extrinsic fluorescence, high resolution second-derivative UV absorption spectroscopy, circular dichroism (CD), dynamic light scattering, differential scanning calorimetry, and direct observation employing transmission electron microscopy. The data demonstrate that NV-VLPs are highly stable over a pH range of 3-7 and up to 55 degrees C. At pH 8, however, reversible capsid dissociation was correlated with increased solvent exposure of tyrosine residues and subtle changes in secondary structure. Above 60 degrees C NV-VLPs undergo distinct phase transitions arising from secondary-, tertiary-, and quaternary-level protein structural perturbations. By combining the spectroscopic data employing a multidimensional eigenvector phase space approach, an empirical phase diagram for NV-VLP was constructed. This strategy of visualization provides a comprehensive description of the physical stability of NV-VLP over a broad range of pH and temperature. Complementary, differential scanning calorimetric analyses suggest that the two domains of VP1 unfold independently in a pH-dependent manner.

Barrera F. N., Hurtado-Gomez E., Lidon-Moya M. C., and Neira J. L. (2006) Binding of the C-terminal domain of the HIV-1 capsid protein to lipid membranes: a biophysical characterization. *Biochem J* **394**, 345-353.

Abstract: The capsid protein, CA, of HIV-1 forms a capsid that surrounds the viral genome. However, recent studies have shown that an important proportion of the CA molecule does not form part of this capsid, and its location and function are still unknown. In the present work we show, by using fluorescence, differential scanning calorimetry and Fourier-transform infrared spectroscopy, that the C-terminal region of CA, CA-C, is able to bind lipid vesicles in vitro in a peripheral fashion. CA-C had a greater affinity for negatively charged lipids (phosphatidic acid and phosphatidylserine) than for zwitterionic lipids [PC/Cho/SM (equimolar mixture of phosphatidylcholine, cholesterol and sphingomyelin) and phosphatidylcholine]. The interaction of CA-C with lipid membranes was supported by theoretical studies, which predicted that different regions, occurring close in the three-dimensional CA-C structure, were responsible for the binding. These results show the flexibility of CA-C to undergo conformational rearrangements in the presence of different binding partners. We hypothesize that the CA molecules that do not form part of the mature capsid might be involved in lipid-binding interactions in the inner leaflet of the virion envelope.

Bertocco A., Formaggio F., Toniolo C., Broxterman Q. B., Epand R. F., and Epand R. M. (2003) Design and function of a conformationally restricted analog of the influenza virus fusion peptide. *J Pept Res* **62**, 19-26.

Abstract: A conformationally restricted analog of the N-terminal 12-residue peptide segment of the HA2 subunit of the PPV/34, PR/8/34, and Jap/57 strains of influenza virus hemagglutinin was synthesized containing three residues of α -methyl-valine. This peptide has a higher content of helical structure in the presence of 50% trifluoroethanol or when interacting with liposomes of egg phosphatidylcholine compared with the conformationally more flexible control peptide with the native sequence. The control and analog peptides had opposite effects on membrane curvature as measured by shifts in the bilayer-to-hexagonal phase transition temperature of a synthetic phosphatidylethanolamine derivative. The control peptide promoted more negative curvature, particularly at acidic pH and was also more potent than the analog in promoting lipid mixing. The results indicate that the ability of the peptide to sample a broader range of conformations is required for the influenza fusion peptide to destabilize membranes and that a rigid helical structure is less fusogenic. The difference between the two peptides and between pH 7.4 and pH 5.0 show a correlation between the ability to promote negative curvature and to accelerate lipid mixing.

Bong D. T., Janshoff A., Steinem C., and Ghadiri M. R. (2000) Membrane partitioning of the cleavage peptide in flock house virus. *Biophys J* **78**, 839-845.

Abstract: Membrane translocation of the ssRNA genome of nodaviruses has been proposed to be mediated by direct lipid-protein interactions between a postassembly autocatalytic cleavage product from the capsomere and the target membrane. We have recently shown that the 21-residue Met \rightarrow Nle variant of the N-terminal helical domain (denoted $\gamma(1)$) of the cleavage peptide in flock house nodavirus increases membrane permeability to hydrophilic solutes and can alter both membrane structure and function, suggesting the possibility of peptide-triggered disruption of the endosomal membrane as a prelude to viral uncoating in the host cytoplasm. Elucidation of partitioning energetics would allow an assessment of the likelihood of this mechanism. We report herein complete thermodynamic characterization of the partitioning of $\gamma(1)$ to phospholipids by lipid-peptide titrations following changes in ellipticity, fluorescence signature, or calorimetric response. These experiments revealed a partitioning energy comparable to natural membrane-active peptide toxins, suggesting that the proposed mechanism may be possible. Additionally, a novel switch in the balance of partitioning forces was found: when the lipid headgroup was changed from zwitterionic to negatively charged, membrane association of the peptide became completely entropy-driven.

Briers Y., Lavigne R., Plessers P., Hertveldt K., Hanssens I., Engelborghs Y., and Volckaert G. (2006) Stability analysis of the bacteriophage phiKMV lysin gp36C and its putative role during infection. *Cell Mol Life Sci* **63**, 1899-1905.

Abstract: The kinetic, thermodynamic and structural stability of gp36C, the virion-associated peptidoglycan hydrolase domain of bacteriophage phiKMV, is analyzed. Recombinant gp36C is highly thermoresistant ($k = 0.595 \text{ h}^{-1}$ at 95 degrees C), but not thermostable ($T(m) = 50.2 \text{ degrees C}$, $\Delta H(\text{cal})$

= 6.86×10^4 cal mol⁻¹). However, aggregation influences kinetic stability in an unusual manner since aggregation is more pronounced at 55 degrees C than at higher temperatures. Furthermore, gp36C reversibly unfolds in a two-state endothermic transition, and circular dichroism analysis shows that gp36C almost completely refolds after a 3-h heat treatment at 85 degrees C. These properties are in agreement with gp36C being part of the extensible tail which is ejected in an unfolded state during phage infection.

Carmo M., Faria T. Q., Falk H., Coroadinha A. S., Teixeira M., Merten O. W., Geny-Fiamma C., Alves P. M., Danos O., Panet A., Carrondo M. J., and Cruz P. E. (2006) Relationship between retroviral vector membrane and vector stability. *J Gen Virol* **87**, 1349-1356.

Abstract: The present work studies the physico-chemical properties of retroviral vector membrane, in order to provide some explanation for the inactivation kinetics of these vectors and to devise new ways of improving transduction efficiency. For this purpose, vectors with an amphotropic envelope produced by TE Fly A7 cells at two culture temperatures (37 and 32 degrees C) were characterized by different techniques. Electron paramagnetic resonance (EPR) results showed that vectors produced at 32 degrees C are more rigid than those produced at 37 degrees C. Further characterization of vector membrane composition allowed us to conclude that the vector inactivation rate increases with elevated cholesterol to phospholipid ratio. Differential scanning calorimetry (DSC) showed that production temperature also affects the conformation of the membrane proteins. Transduction studies using HCT116 cells and tri-dimensional organ cultures of mouse skin showed that vectors produced at 37 degrees C have higher stability and thus higher transduction efficiency in gene therapy relevant cells as compared with vectors produced at 32 degrees C. Overall, vectors produced at 37 degrees C show an increased stability at temperatures below 4 degrees C. Since vector membrane physico-chemical properties are affected in response to changes in culture temperature, such changes, along with alterations in medium composition, can be used prospectively to improve the stability and the transduction efficiency of retroviral vectors for therapeutic purposes.

Carreira A., Menendez M., Reguera J., Almendral J. M., and Mateu M. G. (2004) In vitro disassembly of a parvovirus capsid and effect on capsid stability of heterologous peptide insertions in surface loops. *J Biol Chem* **279**, 6517-6525.

Abstract: We have analyzed the in vitro disassembly of the capsid of the minute virus of mice, and the stability of capsid chimeras carrying heterologous epitope insertions. Upon heating in a physiological buffer, empty capsids formed by 60 copies of protein VP2 underwent first a reversible conformational change with a small enthalpy change detected by fluorescence. This change was associated with, but not limited to, externalization of the VP2 N terminus. Irreversible capsid dissociation as detected by changes in fluorescence, hemagglutination activity, and electrophoretic mobility occurred at much higher temperatures. Differential scanning calorimetry in the same conditions indicated that the dissociation/denaturation transition involved a high enthalpy change and proceeded through one or more intermediates. In contrast, in the presence of 1.5 M guanidinium chloride, heat-induced disassembly fitted a two-state irreversible process. Both thermally and chemically induced dissociation/denaturation yielded a form that had lost a part of the tertiary structure, but still retained the native secondary structure. Data from chemical dissociation indicates this form may correspond to a molten globule-like monomeric state of the capsid protein. All five antigenic peptide insertions attempted in exposed loops, despite being perhaps among the least disruptive, led to defects in folding/assembly of the capsid and, in most cases, to reduced capsid stability against thermal dissociation. The results with one of the simplest viral capsids reveal a complex pathway for disassembly, and a reduction in capsid assembly and stability upon insertion of peptides, even within the most exposed capsid loops.

Chen C. H., Wu R., Roth L. G., Guillot S., and Crainic R. (1997) Elucidating mechanisms of thermostabilization of poliovirus by D2O and MgCl₂. *Arch Biochem Biophys* **342**, 108-116.

Abstract: To understand a significant reduction in the loss of poliovirus infectivity by D2O and a combination of D2O and MgCl₂ at 37-45 degrees C, this paper attempts to elucidate the mechanisms underlying the thermostabilization of poliovirus. Three serotypes of Sabin oral poliovirus vaccine strains were investigated. Temperature-dependent fluorescence emission intensity studies showed that the effects of D2O and MgCl₂ on the stability and conformation of poliovirus are correlated with those of the infectivity of poliovirus. Fluorescence steady-state polarization revealed that the conformation of poliovirus capsid is sensitive to D2O medium and MgCl₂ salt, and that the rigidity of poliovirus conformation is

increased in their presence. The exposure of poliovirus tryptophan residues to water is modified by D2O and MgCl₂, as evidenced by changes in fluorescence emission intensity excited at 295 nm. The involvement of hydrogen bonding in the D2O effect was demonstrated by the greatly increased value of relative fluorescence intensity. Conformational alteration was also shown by changes in the positive band (193-230 nm) of circular dichroism spectra. D2O and MgCl₂ were also found to reduce the interaction of virus with water as examined by differential scanning microcalorimetry, leading to a decline in the extent of water penetration into the poliovirus capsid. All these observations were found to be more profound in a combination of D2O with MgCl₂ than D2O or MgCl₂ alone. By inducing a conformation favorable to maintaining the poliovirus assembly and by reducing virus-water interaction to decrease water penetration into the poliovirus capsid, D2O, MgCl₂, or D2O-MgCl₂ is able to exert its thermostabilization effect. Thus, to maintain the virus assembly and conformation of the virus and to reduce the swelling of the virus capsid are key factors in increasing the thermostability of poliovirus. These two factors are mutually complementary. The latter can provide a favorable environment for the formers and the formers, in turn, lead to the latter.

Christensen D., Foged C., Rosenkrands I., Nielsen H. M., Andersen P. and Agger E. M. (2007) Trehalose preserves DDA/TDB liposomes and their adjuvant effect during freeze-drying. *Biochim Biophys Acta* **1768**, 2120-2129.

Abstract: Disaccharides are well-known reagents to protect biostructures like proteins and phospholipid-based liposomes during freezing and drying. We have investigated the ability of the two disaccharides trehalose and sucrose to stabilize a novel, non-phospholipid-based liposomal adjuvant composed of the cationic dimethyldioctadecylammonium (DDA) and trehalose 6,6'-dibehenate (TDB) upon freeze-drying. The liposomes were freeze-dried using a human dose concentration containing 2.5 mg/ml DDA and 0.5 mg/ml TDB with varying concentrations of the two sugars. The influence on particle size upon rehydration was investigated using photon correlation spectroscopy (PCS) and the gel to fluid phase transition was examined by differential scanning calorimetry (DSC). Data revealed that concentrations above 211 mM trehalose protected and preserved DDA/TDB during freeze-drying, and the liposomes were readily rehydrated. Sucrose was less efficient as a stabilizer and had to be used in concentrations above 396 mM in order to obtain the same effect. Immunization of mice with the tuberculosis vaccine candidate Ag85B-ESAT-6 in combination with the trehalose stabilized adjuvant showed that freeze-dried DDA/TDB liposomes retained their ability to stimulate both a strong cell-mediated immune response and an antibody response. These findings show that trehalose at isotonic concentrations protects cationic DDA/TDB-liposomes during freeze-drying. Since this is not the case for liposomes based on DDA solely, we suggest that the protection is facilitated via direct interaction with the headgroup of TDB and a kosmotropic effect, whereas direct interaction with DDA plays a minor role.

Christensen D., Alleso M., Rosenkrands I., Rantanen J., Foged C., Agger E. M., Andersen P. and Nielsen H. M. (2008) NIR transmission spectroscopy for rapid determination of lipid and lyoprotector content in liposomal vaccine adjuvant system CAF01. *Eur J Pharm. Biopharm (epublication)*.

Abstract: It is of crucial importance to determine the concentration of the different components in the formulation accurately, during production. In this respect, near-infrared (NIR) spectroscopy represents an intriguing alternative that offers rapid, non-invasive and non-destructive sample analysis. This method, combined with multivariate data analysis was successfully applied to quantify the total concentration of lipids in the liposomal CAF01 adjuvant, composed of the cationic surfactant dimethyldioctadecylammonium bromide (DDA) and the immunomodulator alpha, alpha'-trehalose 6,6'-dibehenate (TDB). The near-infrared (NIR) detection method was compared to a validated high-performance liquid chromatography (HPLC) method and a differential scanning calorimetry (DSC) analysis, and a blinded study with three different sample concentrations was performed, showing that there was no significant difference in the accuracy of the three methods. However, the NIR and DSC methods were more precise than the HPLC method. Also, with the NIR method it was possible to differentiate between various concentrations of trehalose added as cryo-/lyoprotector. These studies therefore suggest that NIR can be used for real-time process control analysis in the production of CAF01 liposomes

Contreras L. M., Aranda F. J., Gavilanes F., Gonzalez-Ros J. M., and Villalain J. (2001) Structure and interaction with membrane model systems of a peptide derived from the major epitope region of HIV protein gp41: implications on viral fusion mechanism. *Biochemistry* **40**, 3196-3207.

Abstract: The HIV-1 gp41 envelope protein mediates entry of the virus into the target cell by promoting membrane fusion. With a view toward possible new insights into viral fusion mechanisms, we have investigated by infrared, fluorescence, and nuclear magnetic resonance spectroscopies and calorimetry a fragment of 19 amino acids corresponding to the immunodominant region of the gp41 ectodomain, a highly conserved sequence and major epitope. Information on the structure of the peptide both in solution and in the presence of model membranes, its incorporation and location in the phospholipid bilayer, and the modulation of the phase behavior of the membrane has been gathered. Here we demonstrate that the peptide binds and interacts with negatively charged phospholipids, changes its conformation in the presence of a membraneous medium, and induces leakage of vesicle contents as well as a new phospholipid phase. These characteristics might be important for the formation of the fusion-active gp41 core structure, promoting the close apposition of the two viral and target-cell membranes and therefore provoking fusion.

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

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

Darboe N., Kenjale R., Picking W. L., Picking W. D., and Middaugh C. R. (2006) Physical characterization of MxiH and PrgI, the needle component of the type III secretion apparatus from Shigella and Salmonella. *Protein Sci* **15**, 543-552.

Abstract: Shigella and Salmonella use similar type III secretion systems for delivering effector proteins into host cells. This secretion system consists of a base anchored in both bacterial membranes and an extracellular "needle" that forms a rod-like structure exposed on the pathogen surface. The needle is composed of multiple subunits of a single protein and makes direct contact with host cells to facilitate protein delivery. The proteins that make up the needle of Shigella and Salmonella are MxiH and PrgI, respectively. These proteins are attractive vaccine candidates because of their essential role in virulence and surface exposure. We therefore isolated, purified, and characterized the monomeric forms of MxiH and PrgI. Their far-UV circular dichroism spectra show structural similarities with hints of subtle differences in their secondary structure. Both proteins are highly helical and thermally unstable, with PrgI having a midpoint of thermal unfolding (T_m) near 37 degrees C and MxiH having a value near 42 degrees C. The two proteins also have comparable intrinsic stabilities as measured by chemically induced (urea) unfolding. MxiH, however, with a free energy of unfolding (ΔG_{un}) of 1.6 kcal/mol, is slightly more stable than PrgI (1.2 kcal/mol). The relatively low m -values obtained for the urea-induced unfolding of the proteins suggest that they undergo only a small change in solvent-accessible surface area. This argues that when MxiH and PrgI are incorporated into the needle complex, they obtain a more stable structural state through the introduction of protein-protein interactions.

D'Aquino J. A., Tetenbaum-Novatt J., White A., Berkovitch F., and Ringe D. (2005) Mechanism of metal ion activation of the diphtheria toxin repressor DtxR. *Proc Natl Acad Sci U S A*, **102**, 18408-13.

Abstract: The diphtheria toxin repressor (DtxR) is a metal ion-activated transcriptional regulator that has

been linked to the virulence of *Corynebacterium diphtheriae*. Structure determination has shown that there are two metal ion binding sites per repressor monomer, and site-directed mutagenesis has demonstrated that binding site 2 (primary) is essential for recognition of the target DNA repressor, leaving the role of binding site 1 (ancillary) unclear. Calorimetric techniques have demonstrated that although binding site 1 (ancillary) has high affinity for metal ion with a binding constant of 2×10^{-7} , binding site 2 (primary) is a low-affinity binding site with a binding constant of 6.3×10^{-4} . These two binding sites act in an independent fashion, and their contribution can be easily dissected by traditional mutational analysis. Our results clearly demonstrate that binding site 1 (ancillary) is the first one to be occupied during metal ion activation, playing a critical role in stabilization of the repressor. In addition, structural data obtained for the mutants Ni-DtxR(H79A,C102D), reported here, and the previously reported DtxR(H79A) have allowed us to propose a mechanism of metal activation for DtxR.

Davies S. M., Epanand R. F., Bradshaw J. P., and Epanand R. M. (1998) Modulation of lipid polymorphism by the feline leukemia virus fusion peptide: implications for the fusion mechanism. *Biochemistry* **37**, 5720-5729.

Abstract: The structural effects of the fusion peptide of feline leukemia virus (FeLV) on lipid polymorphism were studied, using differential scanning calorimetry (DSC), ³¹P nuclear magnetic resonance (NMR), and time-resolved X-ray diffraction. This peptide lowers the transition temperature, T_H , of dipalmitoleoylphosphatidylethanolamine (DiPoPE) at peptide mole fractions of up to 1.5×10^{-3} at pH 5.0 and at pH 7.4. The temperature at which isotropic ³¹P NMR signals for monomethyldioleoylphosphatidylethanolamine (MeDOPE) first occurred is lowered by the FeLV peptide. The amount of isotropic signal seen at 40 degrees C is directly correlated to the peptide:lipid molar ratio. In the peptide-containing samples, more lipid remains in the isotropic state over the whole recorded temperature range. Isotropic ³¹P NMR signals were observed for DiPoPE in the presence of the FeLV peptide for the entire recorded temperature range of 35-50 degrees C, while pure DiPoPE showed no significant amount of isotropic signal. X-ray studies of DiPoPE show the formation of a new lipid phase with peptide, which is not seen in the pure lipid samples. Disordering of the α phase is evidenced by broadening of the diffraction peaks, and the hexagonal cell parameter is decreased with peptide present. Our results suggest that the FeLV peptide is increasing the negative curvature of the lipid system, which is thought to be crucial to the formation of highly bent, high-energy structural fusion intermediates, such as the "stalk" model. Fusion activity for this putative fusogenic peptide was also demonstrated, using a resonance energy transfer (RET) lipid mixing assay. To our knowledge, this work provides the first published experimental evidence of both fusogenic activity and effects on lipid polymorphism for the FeLV fusion peptide.

Epanand R. F., Moroder L., Lutz J., Flanagan T. D., Nir S., and Epanand R. M. (1997) Lipogastrins as potent inhibitors of viral fusion. *Biochim Biophys Acta* **1327**, 259-268.

Abstract: The rate and extent of membrane fusion is markedly sensitive to membrane interfacial properties. Lipopeptides with hydrophilic peptide moieties will insert into membranes, leaving the peptide portion at the membrane-water interface. In this work, we have used a lipopeptide composed of the peptide [Nle15]-gastrin-(2-17)-amide covalently linked to 1,2-diacyl-3-mercaptoplycerol-N(α)-maleoyl-beta-alanine to give DM-gastrin or DP-gastrin having 14 or 16 carbon atom acyl chains, respectively. The fluorescence emission from the two Trp residues of these lipopeptides exhibited little or no blue shift upon addition of liposomes of egg-phosphatidylethanolamine containing 5 mol% G(D1a). Iodide quenching of DP-gastrin fluorescence was also independent of lipid. These results indicate that the peptide moiety is exposed to the aqueous environment even though the lipopeptide is firmly anchored to the membrane. Both DM and DP-gastrin markedly raise the bilayer to hexagonal phase transition temperature of dipalmitoleoyl phosphatidylethanolamine. However, DM-E5 lowers this phase transition temperature. These lipopeptides have effects on the overall fusion of Sendai virus to liposomes in accord with their opposite effects on lipid curvature. The lipogastrins are potent inhibitors of viral fusion, while DM-E5 slightly promotes this process. Truncated forms of DM-gastrin are also inhibitory to viral fusion, but are less inhibitory than the full lipopeptide. Analysis of the fusion kinetics shows that DP-gastrin causes a reduction in the final extent of fusion and a marked lowering of the fusion rate constant. Binding of Sendai virus to the ganglioside receptor-containing liposomes was not affected. Consideration of the various contributions to the mechanism of inhibition of viral fusion suggests that effects of lipogastrin on membrane intrinsic monolayer curvature is of primary importance.

Epanand R. F. and Epanand R. M. (2003) Irreversible unfolding of the neutral pH form of influenza hemagglutinin demonstrates that it is not in a metastable state. *Biochemistry* **42**, 5052-5057.

Abstract: The thermal denaturation of the proteins of influenza virus has been measured by differential scanning calorimetry in the presence and absence of lipids as a function of scan rate. We have applied theories of irreversible thermodynamics to obtain the activation energy. In the presence of liposomes of dioleoylphosphatidylcholine with the ganglioside, GD(1a), the denaturation temperature of the hemagglutinin protein is lowered. This lowering of thermal stability is also reflected in the temperature dependence of the circular dichroism spectra. Quasi-elastic light scattering confirms that liposomes containing GD(1a) interact with the virus and inhibit the growth in the size of the particle as a function of temperature. Although the virus can fuse with the liposomes at higher temperatures, the enthalpy change for this process is not detectable. Our results also demonstrate that the compact folded structure of the influenza hemagglutinin protein is not a kinetically trapped metastable high-energy form.

Epanand R. F. and Epanand R. M. (2003) Irreversible unfolding of the neutral pH form of influenza hemagglutinin demonstrates that it is not in a metastable state. *Biochemistry* **42**, 5052-5057.

Abstract: The thermal denaturation of the proteins of influenza virus has been measured by differential scanning calorimetry in the presence and absence of lipids as a function of scan rate. We have applied theories of irreversible thermodynamics to obtain the activation energy. In the presence of liposomes of dioleoylphosphatidylcholine with the ganglioside, GD(1a), the denaturation temperature of the hemagglutinin protein is lowered. This lowering of thermal stability is also reflected in the temperature dependence of the circular dichroism spectra. Quasi-elastic light scattering confirms that liposomes containing GD(1a) interact with the virus and inhibit the growth in the size of the particle as a function of temperature. Although the virus can fuse with the liposomes at higher temperatures, the enthalpy change for this process is not detectable. Our results also demonstrate that the compact folded structure of the influenza hemagglutinin protein is not a kinetically trapped metastable high-energy form.

Epanand R. M. and Epanand R. F. (2002) Thermal denaturation of influenza virus and its relationship to membrane fusion. *Biochem J* **365**, 841-848.

Abstract: The X-31 strain of influenza virus was studied by differential scanning calorimetry (DSC), CD and SDS/PAGE analysis as a function of both temperature and pH. A bromelain-treated virus was also studied by these methods. The major transition observed in the intact virus was a result of the denaturation of the haemagglutinin (HA) protein. At pH 7.4, this transition was similar in the intact virus and the isolated HA, but was absent in the bromelain-treated virus. However, at pH 5 the denaturation temperature and enthalpy were both higher for HA in the virus than in the isolated protein, indicating that HA interacts with other molecular components in the intact virus. The transition observed by DSC occurs at a higher temperature than does the thermal transition observed by CD. The temperature of the CD transition coincides with the temperature at which the fusogenicity of the virus increases, and probably corresponds to the formation of an extended coiled-coil conformation. Analysis by SDS/PAGE at neutral pH under non-reducing conditions demonstrates a selective loss of the HA protein trimer, resulting in the formation of aggregates in the range of temperatures of 55 to 70 degrees C. In contrast, at acidic pH, the HA protein is largely in the monomeric form at 25 degrees C, and there is little change with temperature. There is thus a weakening of the quaternary structure of HA at acidic pH prior to heating. At the temperature at which the virus exhibits an increased fusogenicity at neutral pH, there is a loss of secondary structure and a beginning of a destabilization of the trimeric form of HA. This temperature is lower than that required for the major endothermic peak observed in DSC experiments. The results demonstrate that there is no kinetically trapped high-energy form of HA at neutral pH.

Feinberg H., Guo Y., Mitchell D. A., Drickamer K., and Weis W. I. (2005) Extended neck regions stabilize tetramers of the receptors DC-SIGN and DC-SIGNR. *J Biol Chem* **280**, 1327-1335. **Abstract:** The human cell surface receptors DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin) and DC-SIGNR (DC-SIGN-related) bind to oligosaccharide ligands found on human tissues as well as on pathogens including viruses, bacteria, and parasites. The extracellular portion of each receptor contains a membrane-distal carbohydrate-recognition domain (CRD) and forms tetramers stabilized by an extended neck region consisting of 23 amino acid repeats. Cross-linking analysis of full-length receptors expressed in fibroblasts confirms the tetrameric state of the intact receptors. Hydrodynamic studies on truncated receptors demonstrate that the portion of the neck of each protein adjacent to the CRD is

sufficient to mediate the formation of dimers, whereas regions near the N terminus are needed to stabilize the tetramers. Some of the intervening repeats are missing from polymorphic forms of DC-SIGNR. Two different crystal forms of truncated DC-SIGNR comprising two neck repeats and the CRD reveal that the CRDs are flexibly linked to the neck, which contains alpha-helical segments interspersed with non-helical regions. Differential scanning calorimetry measurements indicate that the neck and CRDs are independently folded domains. Based on the crystal structures and hydrodynamic data, models for the full extracellular domains of the receptors have been generated. The observed flexibility of the CRDs in the tetramer, combined with previous data on the specificity of these receptors, suggests an important role for oligomerization in the recognition of endogenous glycans, in particular those present on the surfaces of enveloped viruses recognized by these proteins.

Ihnat P. M., Vellekamp G., Obenauer-Kutner L. J., Duan J., Han M. A., Witchev-Lakshmanan L. C., and Grace M. J. (2005) Comparative thermal stabilities of recombinant adenoviruses and hexon protein. *Biochim Biophys Acta* **1726**, 138-151.

Abstract: Differential scanning calorimetry was used to identify the thermal stability profile of the replication deficient and protein IX deleted recombinant adenovirus type 5 that contains the p53 transgene (rAd/p53) in phosphate buffered saline (vPBS) or 10% glycerol (TRIS/phosphate buffer). The wildtype adenovirus (Ad/WT) and purified hexon protein (major capsid protein) were also evaluated in 10% glycerol (TRIS/phosphate buffer) as controls. The thermal profile of rAd/p53 revealed three endothermic transitions (T1, T2 and T3) occurring between 25 degrees C and 90 degrees C. T1, which occurred at 46.7 degrees C in vPBS and 49.4 degrees C in TRIS/PO4 10% glycerol buffer, was irreversible following repeated scanning and attributed to the degradation of the intact vector. The latter two endothermic transitions, T2 and T3, occurring at 69 degrees C and 78 degrees C, respectively, corresponded with the two transitions of purified hexon in temperature and amount of heat absorbed. The thermal profile of Ad/WT revealed four endothermic transitions at 51.5 degrees C (T1), 70.5 degrees C (T2A), 73.6 degrees C (T2B), and 77.4 degrees C (T3). The higher temperature of degradation as well as additional transition was attributed to the presence of protein IX associated with the hexon. The positions and excess molar heat capacities of the intact rAds were found to be affected by pH, glycerol, vector concentration and the presence or absence of protein IX in the capsid. Irreversibility of T1 implied that the degradation of the intact virus may follow first-order kinetics. The thermal scan rate dependence of T1 further confirmed that degradation of the intact virus may be first-order. The apparent activation energies for the degradation of the intact vectors were determined from the scan rate dependence of T1 and shown to be affected by protein IX in the capsid and solution conditions. Analysis of rAd samples incubated at 45 degrees C by Field Emission Electron Microscopy (FESEM) confirmed that loss of single particles was first-order. Although aggregates were observed in the samples, degradation appeared to be the dominant reaction leading to disappearance of single virions from the aqueous matrix. Based on thermal and FESEM analysis, an empirical model was proposed that accounted for the disappearance of single rAd particles. At or near T1, degradation of rAd particles followed a unidirectional, pseudo-first order reaction. However, at lower temperatures, disappearance of single virions resulted from competing irreversible degradation and aggregation reactions.

Jelesarov I. and Lu M. (2001) Thermodynamics of trimer-of-hairpins formation by the SIV gp41 envelope protein. *J Mol Biol* **307**, 637-656.

Abstract: The gp41 envelope protein mediates the entry of primate immunodeficiency viruses into target cells by promoting the fusion of viral and cellular membranes. The structure of the gp41 ectodomain core represents a trimer of identical helical hairpins in which a central trimeric coiled-coil made up of three amino-terminal helices is wrapped in an outer layer of three antiparallel carboxyl-terminal helices. Triggering formation of this fusion-active gp41 conformation appears to cause close membrane apposition and thus overcome the activation energy barrier for lipid bilayer fusion. We present a detailed description of the folding thermodynamics of the simian immunodeficiency virus (SIV) gp41 core by using a recombinant trimeric N34(L6)C28 model. Differential scanning calorimetry and spectroscopic experiments on denaturant-induced and thermal unfolding indicate that the free energy of association of three 68 residue N34(L6)C28 peptides to a trimer-of-hairpins is 76 kJ mol⁻¹ at pH 7.0 and 25 degrees C in physiological buffer. The associated enthalpy change, $\Delta H(\text{unf})$, is 177 kJ mol⁻¹, while the entropy of unfolding, $\Delta S(\text{unf})$, is 0.32 kJ K⁻¹ mol⁻¹. The temperature of maximal stability is close to 20 degrees C. The unfolding heat capacity increment is approximately 9 kJ K⁻¹ mol⁻¹ (approximately 45 J K⁻¹ mol residue⁻¹), which is lower than expected for unfolding of the trimer to an extended and fully hydrated polypeptide chain. Replacement

by isoleucine of the polar residues Thr582 or Thr586 buried in the N-terminal trimeric coiled-coil interface leads to very strong stabilization of the trimer-of-hairpins, 30-35 kJ mol⁻¹. Single-point mutations in the central coiled-coil thus strongly stabilize the gp41 core structure. These thermodynamic characteristics may be important for the refolding of the gp41 envelope protein into its fusion-active conformation during membrane fusion.

Jones L. S., Peek L. J., Power J., Markham A., Yazzie B., and Middaugh C. R. (2005) Effects of adsorption to aluminum salt adjuvants on the structure and stability of model protein antigens. *J Biol Chem* **280**, 13406-13414.

Abstract: The effect of adsorption onto aluminum salt adjuvants on the structure and stability of three model protein antigens was studied using fluorescence and Fourier transform infrared spectroscopies, as well as isothermal titration and differential scanning calorimetric techniques. Lysozyme was preferentially adsorbed to aluminum phosphate (Adju-Phos), whereas ovalbumin and bovine serum albumin were better adsorbed to aluminum hydroxide (Alhydrogel). A linearized Langmuir adsorption isotherm was used to obtain information regarding the binding interactions between proteins and adjuvants. Binding energetics and stoichiometry data obtained from isothermal titration calorimetry measurements were complex. Based on the spectroscopic and differential scanning calorimetry studies, the structure of all three proteins, when adsorbed to the surface of an aluminum salt, was altered in such a way as to render the proteins less thermally stable. Besides the pharmaceutical significance of this destabilization, we consider the possibility that this phenomenon may facilitate the presentation of antigens and thus contribute to the adjuvant activity of the aluminum salts.

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

Abstract: The human immunodeficiency virus type 1 nucleocapsid protein (NC) is a nucleic acid chaperone that catalyzes the rearrangement of nucleic acids into their thermodynamically most stable structures. In the present study, a combination of optical and thermodynamic techniques were used to characterize the influence of NC on the secondary structure, thermal stability and energetics of monomolecular DNA quadruplexes formed by the sequence d(GGTTGGTGTGGTTGG) in the presence of K⁺ or Sr²⁺. Circular dichroism studies demonstrate that NC effectively unfolds the quadruplexes. Studies carried out with NC variants suggest that destabilization is mediated by the zinc fingers of NC. Calorimetric studies reveal that NC destabilization is enthalpic in origin, probably owing to unstacking of the G-quartets upon protein binding. In contrast, parallel studies performed on a related DNA duplex reveal that under conditions where NC readily destabilizes and unfolds the quadruplexes, its effect on the DNA duplex is much less pronounced. The differences in NC's ability to destabilize quadruplex versus duplex is in accordance with the higher DeltaG of melting for the latter, and with the inverse correlation between nucleic acid stability and the destabilizing activity of NC.

Kegel W. K. and van der S. P. (2006) Physical regulation of the self-assembly of tobacco mosaic virus coat protein. *Biophys J* **91**, 1501-1512.

Abstract: We present a statistical mechanical model based on the principle of mass action that explains the main features of the in vitro aggregation behavior of the coat protein of tobacco mosaic virus (TMV). By comparing our model to experimentally obtained stability diagrams, titration experiments, and calorimetric data, we pin down three competing factors that regulate the transitions between the different kinds of aggregated state of the coat protein. These are hydrophobic interactions, electrostatic interactions, and the formation of so-called "Caspar" carboxylate pairs. We suggest that these factors could be universal and relevant to a large class of virus coat proteins.

Khan A. R., Baker B. M., Ghosh P., Biddison W. E., and Wiley D. C. (2000) The structure and stability of an HLA-A*0201/octameric tax peptide complex with an empty conserved peptide-N-terminal binding site. *J Immunol* **164**, 6398-6405.

Abstract: The crystal structure of the human class I MHC molecule HLA-A2 complexed with of an octameric peptide, Tax8 (LFGYPVYV), from human T cell lymphotropic virus-1 (HTLV-1) has been determined. This structure is compared with a newly refined, higher resolution (1.8 Å) structure of HLA-A2 complexed with the nonameric Tax9 peptide (LLFGYPVYV) with one more N-terminal residue. Despite the absence of a peptide residue (P1) bound in the conserved N-terminal peptide-binding pocket of

the Tax8/HLA-A2 complex, the structures of the two complexes are essentially identical. Water molecules in the Tax8 complex replace the terminal amino group of the Tax9 peptide and mediate a network of hydrogen bonds among the secondary structural elements at that end of the peptide-binding groove. Thermal denaturation measurements indicate that the Tax8 complex is much less stable, $\Delta T_m = 16$ degrees C, than the Tax9 complex, but both can sensitize target cells for lysis by some Tax-specific CTL from HTLV-1 infected individuals. The absence of a P1 peptide residue is thus not enough to prevent formation of a "closed conformation" of the peptide-binding site. TCR affinity measurements and cytotoxic T cell assays indicate that the Tax8/HLA-A2 complex does not functionally cross-react with the A6-TCR-bearing T cell clone specific for Tax9/HLA-A2 complexes.

Kingston R. L., Gay L. S., Baase W. S. and Matthews B. W. (2008) Structure of the nucleocapsid-binding domain from the mumps virus polymerase; an example of protein folding induced by crystallization. *J Mol Biol* **379**, 719-731.

Abstract: The human pathogen mumps virus, like all paramyxoviruses, encodes a polymerase responsible for virally directed RNA synthesis. The template for the polymerase is the nucleocapsid, a filamentous protein-RNA complex harboring the viral genome. Interaction of the polymerase and the nucleocapsid is mediated by a small domain tethered to the end of the phosphoprotein (P), one of the polymerase subunits. We report the X-ray crystal structure of this region of mumps virus P (the nucleocapsid-binding domain, or NBD, amino acids 343-391). The mumps P NBD forms a compact bundle of three alpha-helices within the crystal, a fold apparently conserved across the Paramyxovirinae. In solution, however, the domain exists in the molten globule state. This is demonstrated through application of differential scanning calorimetry, circular dichroism spectroscopy, NMR spectroscopy, and dynamic light scattering. While the mumps P NBD is compact and has persistent secondary structure, it lacks a well-defined tertiary structure under normal solution conditions. It can, however, be induced to fold by addition of a stabilizing methylamine cosolute. The domain provides a rare example of a molten globule that can be crystallized. The structure that is stabilized in the crystal represents the fully folded state of the domain, which must be transiently realized during binding to the viral nucleocapsid. While the intermolecular forces that govern the polymerase-nucleocapsid interaction appear to be different in measles, mumps, and Sendai viruses, for each of these viruses, polymerase translocation involves the coupled binding and folding of protein domains. In all cases, we suggest that this will result in a weak-affinity protein complex with a short lifetime, which allows the polymerase to take rapid steps forward

Kirkitadze M. D., Barlow P. N., Price N. C., Kelly S. M., Boutell C. J., Rixon F. J., and McClelland D. A. (1998) The herpes simplex virus triplex protein, VP23, exists as a molten globule. *J Virol* **72**, 10066-10072.

Abstract: Two proteins, VP19C (50,260 Da) and VP23 (34,268 Da), make up the triplexes which connect adjacent hexons and pentons in the herpes simplex virus type 1 capsid. VP23 was expressed in *Escherichia coli* and purified to homogeneity by Ni-agarose affinity chromatography. In vitro capsid assembly experiments demonstrated that the purified protein was functionally active. Its physical status was examined by differential scanning calorimetry, ultracentrifugation, size exclusion chromatography, circular dichroism, fluorescence spectroscopy, and 8-anilino-1-naphthalene sulfonate binding studies. These studies established that the bacterially expressed VP23 exhibits properties consistent with its being in a partially folded, molten globule state. We propose that the molten globule represents a functionally relevant intermediate which is necessary to allow VP23 to undergo interaction with VP19C in the process of capsid assembly.

Kirkitadze M. D., Henderson C., Price N. C., Kelly S. M., Mullin N. P., Parkinson J., Dryden D. T., and Barlow P. N. (1999) Central modules of the vaccinia virus complement control protein are not in extensive contact. *Biochem J* **344 Pt 1**, 167-175.

Abstract: The 28.6 kDa vaccinia virus complement control protein (VCP) is an inhibitor of the complement system and has therapeutic potential. It is composed of four domains or modules and is a homologue of complement receptor 1 (CR1) and other mammalian regulators of complement activation. A key aspect to structure-function relationships in these proteins is the extent of intramolecular module-module interactions, since these dictate the overall shape and flexibility of the molecules. A protein fragment (VCP approximately 2,3) encompassing modules 2 and 3 of VCP was over-expressed in *Pichia pastoris*. Ultracentrifugation showed that VCP approximately 2,3 is highly asymmetric with an axial ratio of 5.3:1, which is consistent with an end-to-end arrangement of the two modules. NMR spectroscopy,

differential scanning calorimetry, CD and intrinsic tryptophan fluorescence were used to monitor unfolding of VCP approximately 2,3. Experiments performed over a range of temperatures and concentrations of guanidinium chloride revealed that module 2 unfolds under milder conditions than, and independently of, module 3. Unfolding of module 2 is not associated with extensive changes in amide (15)N and (1)H chemical shifts of module 3, implying that the modules do not form an extensive intermodular interface. Results obtained in this work for VCP approximately 2,3 are compared with those obtained in a study of CR1 modules 15-17 [Kirkitadze, Krych, Uhrin, Dryden, Smith, Cooper, Wang, Hauhart, Atkinson and Barlow (1999) *Biochemistry* 38, 7019-7031].

Kirkitadze M. D., Henderson C., Price N. C., Kelly S. M., Mullin N. P., Parkinson J., Dryden D. T., and Barlow P. N. (1999) Central modules of the vaccinia virus complement control protein are not in extensive contact. *Biochem J* **344 Pt 1**, 167-175.

Abstract: The 28.6 kDa vaccinia virus complement control protein (VCP) is an inhibitor of the complement system and has therapeutic potential. It is composed of four domains or modules and is a homologue of complement receptor 1 (CR1) and other mammalian regulators of complement activation. A key aspect to structure-function relationships in these proteins is the extent of intramolecular module-module interactions, since these dictate the overall shape and flexibility of the molecules. A protein fragment (VCP approximately 2,3) encompassing modules 2 and 3 of VCP was over-expressed in *Pichia pastoris*. Ultracentrifugation showed that VCP approximately 2,3 is highly asymmetric with an axial ratio of 5.3:1, which is consistent with an end-to-end arrangement of the two modules. NMR spectroscopy, differential scanning calorimetry, CD and intrinsic tryptophan fluorescence were used to monitor unfolding of VCP approximately 2,3. Experiments performed over a range of temperatures and concentrations of guanidinium chloride revealed that module 2 unfolds under milder conditions than, and independently of, module 3. Unfolding of module 2 is not associated with extensive changes in amide (15)N and (1)H chemical shifts of module 3, implying that the modules do not form an extensive intermodular interface. Results obtained in this work for VCP approximately 2,3 are compared with those obtained in a study of CR1 modules 15-17 [Kirkitadze, Krych, Uhrin, Dryden, Smith, Cooper, Wang, Hauhart, Atkinson and Barlow (1999) *Biochemistry* 38, 7019-7031].

Krell T., Greco F., Engel O., Dubayle J., Dubayle J., Kennel A., Charlotheaux B., Brasseur R., Chevalier M., Sodoyer R., and El Habib R. (2004) HIV-1 gp41 and gp160 are hyperthermostable proteins in a mesophilic environment. Characterization of gp41 mutants. *Eur J Biochem* **271**, 1566-1579.

Abstract: HIV gp41(24-157) unfolds cooperatively over the pH range of 1.0-4.0 with T_m values of > 100 degrees C. At pH 2.8, protein unfolding was 80% reversible and the $\Delta H_{vH}/\Delta H_{cal}$ ratio of 3.7 is indicative of gp41 being trimeric. No evidence for a monomer-trimer equilibrium in the concentration range of 0.3-36 micro m was obtained by DSC and tryptophan fluorescence. Glycosylation of gp41 was found to have only a marginal impact on the thermal stability. Reduction of the disulfide bond or mutation of both cysteine residues had only a marginal impact on protein stability. There was no cooperative unfolding event in the DSC thermogram of gp160 in NaCl/P_i, pH 7.4, over a temperature range of 8-129 degrees C. When the pH was lowered to 5.5-3.4, a single unfolding event at around 120 degrees C was noted, and three unfolding events at 93.3, 106.4 and 111.8 degrees C were observed at pH 2.8. Differences between gp41 and gp160, and hyperthermostable proteins from thermophile organisms are discussed. A series of gp41 mutants containing single, double, triple or quadruple point mutations were analysed by DSC and CD. The impact of mutations on the protein structure, in the context of generating a gp41 based vaccine antigen that resembles a fusion intermediate state, is discussed. A gp41 mutant, in which three hydrophobic amino acids in the gp41 loop were replaced with charged residues, showed an increased solubility at neutral pH.

Kissmann J., Ausar S. F., Foubert T. R., Brock J., Switzer M. H., Detzi E. J., Vedvick T. S. and Middaugh C. R. (2008) Physical stabilization of Norwalk virus-like particles. *J Pharm. Sci* **97**, 4208-4218.

Abstract: Virus-like particles (VLPs) used as vaccine antigens often elicit strong immune responses due to their intrinsic repetitive, high-density display of epitopes, and the fact that the mammalian immune system is highly attuned to recognizing particles in the size range of viruses (20-150 nm). To retain these immunogenic qualities, vaccines that utilize virus-like particle (VLP) antigens should be formulated to stabilize both native conformational epitopes and the overall particulate nature of the VLP. This work describes a systematic approach for identifying potential stabilizers for formulation of Norwalk VLPs (NV-VLPs) in aqueous suspension. A number of excipients were screened for their ability to inhibit aggregation

of NV-VLPs under conditions known to induce aggregation. Those compounds shown to inhibit aggregation were further evaluated under conditions of thermal stress and the NV-VLP structure was monitored using biophysical techniques such as CD, ANS fluorescence, and DSC to provide insight into the mechanisms by which stability was conferred. Increased thermal stability in the presence of chitosan glutamate, sucrose, and trehalose was correlated with stabilization of secondary and tertiary structural elements of NV-VLPs. These excipients may be useful for formulation of a stable NV-VLP vaccine

Krell T., Manin C., Nicolai M. C., Pierre-Justin C., Berard Y., Brass O., Gerentes L., Leung-Tack P., and Chevalier M. (2005) Characterization of different strains of poliovirus and influenza virus by differential scanning calorimetry. *Biotechnol Appl Biochem* **41**, 241-246.

Abstract: Vaccines against poliomyelitis and influenza contain inactivated forms of poliovirus and influenza virus. These antigens are generated on an industrial scale from the purified active viruses that have been analysed in this study by DSC (differential scanning calorimetry). Multiple unfolding transitions are seen for influenza virus A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2) and B/Shangdong/7/97. These data, combined with previously reported data on other influenza viruses, indicates that each influenza virus strain has a characteristic unfolding behaviour. Only minor changes were seen in the thermogram of betaPL (beta-propiolactone)-inactivated influenza virus, which is consistent with the proposition that betaPL reacts mainly with the nucleotide fraction of the virus. We demonstrate that a peak annotation of the thermogram of the native virus is possible using bromelain-treated virus and virosomes. At pH 1.5-2.5, poliovirus of type I unfolds in a single unfolding event with respective T(m) (midpoint of protein unfolding transition) values between 34 and 45 degrees C. At pH 2, polioviruses of type II unfold equally in a single event, but, compared with the type I virus, with a T(m) value increased by 3.7 degrees C. At neutral pH, the DSC thermogram of type I poliovirus was very 'noisy'. Data obtained offer the possibility of precisely characterizing and identifying

Krell T., Greco F., Nicolai M. C., Dubayle J., Renaud-Mongenie G., Poisson N., and Bernard I. (2003) The use of microcalorimetry to characterize tetanus neurotoxin, pertussis toxin and filamentous haemagglutinin. *Biotechnol Appl Biochem* **38**, 241-251.

Abstract: Tetanus neurotoxin (TeNT), pertussis toxin (PT) and pertussis filamentous haemagglutinin (FHA) are major virulence factors of *Clostridium tetani* and *Bordetella pertussis*, which are the causative agents of tetanus and whooping cough respectively. Inactivated forms of these virulence factors are the protein components of vaccines against these diseases. Here we report microcalorimetric studies to characterize these proteins. The microcalorimetric titration curves of TeNT with micelles of gangliosides G(D1b), G(T1b) and G(Q1b) were biphasic. For these gangliosides a high-affinity binding site (K(D) 45-277 nM) can be distinguished from a lower-affinity binding event (K(D) 666-1190 nM). This is direct evidence for multiple binding sites for gangliosides of the (1b) series at TeNT as proposed by Emsley et al. [Emsley, Fotinou, Black, Fairweather, Charles, Watts, Hewitt and Isaacs (2000) *J. Biol. Chem.* 275, 8889-8894]. In agreement with previous reports, no binding was observed for gangliosides G(M1), G(M2), G(M3) and G(D2). The thermal denaturation of TeNT was characterized by two unfolding transitions centred around 57.4 and 62.4 degrees C. The conversion of TeNT into the toxoid form by formaldehyde treatment was accompanied by a large increase in T(m) (the midpoint of protein unfolding transition, that is, the temperature at which half the protein is denatured and the other half is still present in its native form). Fetuin and asialofetuin bound to PT with similar affinities (K(D) 420 and 335 nM respectively). Binding was largely enthalpy-driven and counterbalanced by an unfavourable entropy change, indicating a loss of conformational flexibility. The latter could account for the observed inhibition of ATP binding after binding to fetuin. Furthermore, the molecular limits of mature PT subunit S5 were defined by MS and N-terminal peptide sequencing. The differential-scanning-calorimetry thermogram of FHA shows four well-resolved unfolding transitions, a finding consistent with the sequential denaturation of four structural domains.

Kueltzo L. A., Normand N., O'Hare P., and Middaugh C. R. (2000) Conformational lability of herpesvirus protein VP22. *J Biol Chem* **275**, 33213-33221.

Abstract: The herpesvirus protein VP22 traffics between cells, being exported from expressing cells in a non-Golgi-dependent manner and localizing in the nuclei of surrounding cells. This transport is retained in certain VP22 fusion proteins, making VP22 a candidate for use in macromolecular drug delivery. In an effort to understand the physical basis for this activity, we have initiated structural studies of VP22.C1, the

C-terminal half of VP22, which possesses the full transport activity of the native protein. CD and Fourier transform infrared analyses indicate a secondary structure consisting of approximately 30% alpha-helix, 17% beta-sheet, and 51% disordered and turn structure. Unfolding studies conducted by CD, differential scanning calorimetry, and fluorescence reveal a series of discrete structural transitions in the range of 20-60 degrees C. CD and fluorescence studies of interactions between VP22.C1 and divalent cations and model polyanions indicate that Mg(2+), Zn(2+), oligonucleotides, and heparin interact with the protein, causing changes in secondary structure and thermal stability. Additionally, the interaction of VP22.C1 with model lipids was examined. Fluorescence titrations of the protein with trans-parinaric acid at various temperatures suggest the binding of one to two molecules of parinaric acid to VP22.C1 at temperatures >40 degrees C, suggesting the possibility of conformation dependent membrane interaction under physiological conditions.

Kumar S., McDonnell P. C., Lehr R., Tierney L., Tzimas M. N., Griswold D. E., Capper E. A., Tal-Singer R., Wells G. I., Doyle M. L., and Young P. R. (2000) Identification and initial characterization of four novel members of the interleukin-1 family. *J Biol Chem* **275**, 10308-10314.

Abstract: Interleukin-1 (IL-1), fibroblast growth factors (FGFs), and their homologues are secreted factors that share a common beta-barrel structure and act on target cells by binding to cell surface receptors with immunoglobulin-like folds in their extracellular domain. While numerous members of the FGF family have been discovered, the IL-1 family has remained small and outnumbered by IL-1 receptor homologues. From expressed sequence tag data base searches, we have now identified four additional IL-1 homologues, IL-1H1, IL-1H2, IL-1H3, and IL-1H4. Like most other IL-1/FGFs, these proteins do not contain a hydrophobic leader sequence. IL-1H4 has a propeptide sequence, while IL-1H1, IL-1H2, and IL-1H3 encode only the mature protein. Circular dichroism spectra and thermal stability analysis suggest that IL-1H1 folds similarly to IL-1ra. The novel homologues are not widely expressed in mammals. IL-1H1 is constitutively expressed only in placenta and the squamous epithelium of the esophagus. However, IL-1H1 could be induced in vitro in keratinocytes by interferon-gamma and tumor necrosis factor-alpha and in vivo via a contact hypersensitivity reaction or herpes simplex virus infection. This suggests that IL-1H1 may be involved in pathogenesis of immune mediated disease processes. The addition of four novel IL-1 homologues suggests that the IL-1 family is significantly larger than previously thought.

Kurganov B. I., Rafikova E. R., and Dobrov E. N. (2002) Kinetics of thermal aggregation of tobacco mosaic virus coat protein. *Biochemistry (Mosc)* **67**, 525-533.

Abstract: The kinetics of thermal aggregation of coat protein (CP) of tobacco mosaic virus (TMV) have been studied at 42 and 52 degrees C in a wide range of protein concentrations, [P]₀. The kinetics of aggregation were followed by monitoring the increase in the apparent absorbance (A) at 320 nm. At 52 degrees C the kinetic curves may be approximated by the exponential law in the range of TMV CP concentrations from 0.02 to 0.30 mg/ml, the first order rate constant being linearly proportional to [P]₀ (50 mM phosphate buffer, pH 8.0). The analogous picture was observed at 42 degrees C in the range of TMV CP concentrations from 0.01 to 0.04 mg/ml (100 mM phosphate buffer, pH 8.0). At higher TMV CP concentrations the time of half-conversion approaches a limiting value with increasing [P]₀ and at sufficiently high protein concentrations the kinetic curves fall on a common curve in the coordinates [A/A(lim); t] (t is time and A(lim) is the limiting value of A at t → infinity). According to a mechanism of aggregation of TMV CP proposed by the authors at rather low protein concentrations the rate of aggregation is limited by the stage of growth of aggregate, which proceeds as a reaction of the pseudo-first order, whereas at rather high protein concentrations the rate-limiting stage is the stage of protein molecule unfolding.

Lai Y. H. and D'Souza M. J. (2007) Formulation and evaluation of an oral melanoma vaccine. *J Microencapsul.* **24**, 235-252.

Abstract: The purpose of this study was to formulate and evaluate the physicochemical properties and efficacy of an oral melanoma vaccine. Blood, feces and vaginal wash were collected weekly and analysed by ELISA. The mortality and diameter of the tumors were determined using a vernier caliper. The oral melanoma vaccine microparticles demonstrated desirable particle size, product yield, and zeta potentials. In addition, FT-IR and DSC studies revealed that there was no significant degradation in microencapsulated extra-cellular antigen (ECA). The oral vaccine group showed 25% greater survival rate compared to the control in the efficacy and challenge studies.

Larios C., Casas J., Alsina M. A., Mestres C., Gomara M. J., and Haro I. (2005) Characterization of a putative fusogenic sequence in the E2 hepatitis G virus protein. *Arch Biochem Biophys* **442**, 149-159.

Abstract: With the aim of better understanding the fusion process mediated by the envelope proteins of the hepatitis G virus (HGV/GBV-C), we have investigated the interaction with model membranes of two overlapping peptides [(267-284) and (279-298)] belonging to the E2 structural protein. The peptides were compared for their ability to perturb lipid bilayers by means of different techniques such as differential scanning calorimetry and fluorescence spectroscopy. Furthermore, the conformational behaviour of the peptides in different membrane environments was studied by Fourier-transform infrared spectroscopy and circular dichroism. The results showed that only the E2(279-298) peptide sequence was able to bind with high affinity to negatively charged membranes, to permeabilize efficiently negative lipid bilayers, to induce haemolysis, and to promote inter-vesicle fusion. This fusogenic activity could be related to the induced peptide conformation upon interaction with the target membrane.

Li X., Lopez-Guisa J. M., Ninan N., Weiner E. J., Rauscher F. J., III, and Marmorstein R. (1997) Overexpression, purification, characterization, and crystallization of the BTB/POZ domain from the PLZF oncoprotein. *J Biol Chem* **272**, 27324-27329.

Abstract: The BTB/POZ domain defines a conserved region of about 120 residues and has been found in over 40 proteins to date. It is located predominantly at the N terminus of Zn-finger DNA-binding proteins, where it may function as a repression domain, and less frequently in actin-binding and poxvirus-encoded proteins, where it may function as a protein-protein interaction interface. A prototypic human BTB/POZ protein, PLZF (promyelocytic leukemia zinc finger) is fused to RARalpha (retinoic acid receptor alpha) in a subset of acute promyelocytic leukemias (APLs), where it acts as a potent oncogene. The exact role of the BTB/POZ domain in protein-protein interactions and/or transcriptional regulation is unknown. We have overexpressed, purified, characterized, and crystallized the BTB/POZ domain from PLZF (PLZF-BTB/POZ). Gel filtration, dynamic light scattering, and equilibrium sedimentation experiments show that PLZF-BTB/POZ forms a homodimer with a K_d below 200 nM. Differential scanning calorimetry and equilibrium denaturation experiments are consistent with the PLZF-BTB/POZ dimer undergoing a two-state unfolding transition with a T_m of 70.4 degrees C, and a ΔG of 12.8 +/- 0.4 kcal/mol. Circular dichroism shows that the PLZF-BTB/POZ dimer has significant secondary structure including about 45% helix and 20% beta-sheet. We have prepared crystals of the PLZF-BTB/POZ that are suitable for a high resolution structure determination using x-ray crystallography. The crystals form in the space group I222 or I212121 with $a = 38.8$, $b = 77.7$, and $c = 85.3$ A and contain 1 protein subunit per asymmetric unit with approximately 40% solvent. Our data support the hypothesis that the BTB/POZ domain mediates a functionally relevant dimerization function in vivo. The crystal structure of the PLZF-BTB/POZ domain will provide a paradigm for understanding the structural basis underlying BTB/POZ domain function.

Lidon-Moya M.C., Barrera F.N., Bueno M., Perez-Jimenez R., Sancho J., Mateu M.G., and Neira J.L. (2005) An extensive thermodynamic characterization of the dimerization domain of the HIV-1 capsid protein. *Protein Sci.* **14**, 2387-404.

Abstract: The type 1 human immunodeficiency virus presents a conical capsid formed by several hundred units of the capsid protein, CA. Homodimerization of CA occurs via its C-terminal domain, CA-C. This self-association process, which is thought to be pH-dependent, seems to constitute a key step in virus assembly. CA-C isolated in solution is able to dimerize. An extensive thermodynamic characterization of the dimeric and monomeric species of CA-C at different pHs has been carried out by using fluorescence, circular dichroism (CD), absorbance, nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), and size-exclusion chromatography (SEC). Thermal and chemical denaturation allowed the determination of the thermodynamic parameters describing the unfolding of both CA-C species. Three reversible thermal transitions were observed, depending on the technique employed. The first one was protein concentration-dependent; it was observed by FTIR and NMR, and consisted of a broad transition occurring between 290 and 315 K; this transition involves dimer dissociation. The second transition (T_m approximately 325 K) was observed by ANS-binding experiments, fluorescence anisotropy, and near-UV CD; it involves partial unfolding of the monomeric species. Finally, absorbance, far-UV CD, and NMR revealed a third transition occurring at T_m approximately 333 K, which involves global unfolding of the monomeric species. Thus, dimer dissociation and monomer unfolding were not coupled. At low pH, CA-C underwent a conformational transition, leading to a species displaying ANS binding, a low CD signal, a red-shifted fluorescence spectrum, and a change in compactness. These features are characteristic of molten

globule-like conformations, and they resemble the properties of the second species observed in thermal unfolding.

Lommer B. S. and Luo M. (2002) Structural plasticity in influenza virus protein NS2 (NEP). *J Biol Chem* **277**, 7108-7117.

Abstract: The cellular nuclear transport machinery relies on the assembly of specialized transport complexes between soluble transport receptors, transport substrates, and additional accessory proteins. This study focuses on the structural characteristics of influenza virus protein NS2 (NEP), which interacts with the nuclear export machinery during viral replication, and has been proposed to act as an adapter molecule between the nuclear export machinery and the viral ribonucleoprotein complex. For this purpose, we have purified recombinant NS2 under nondenaturing conditions, and have investigated its structure and aggregation state using optical spectroscopy, differential scanning calorimetry, as well as hydrodynamic techniques. Our results indicate that isolated NS2 exists as a monomer in solution, and adopts a compact, but very flexible conformation, which shows characteristics of the molten globule state under near physiological conditions. Proteolytic sensitivity suggests that, despite its overall plasticity, the structure of NS2 is heterogeneous. While the C terminus of the protein adopts a relatively rigid conformation, its N terminus, which is recognized by the nuclear export machinery, exists in a highly mobile and exposed state. It is proposed that the flexibility observed in the nuclear export domain of NS2 is an important element in the recognition of substrate proteins by the nuclear export machinery.

Maestro B., and Sanz J.M. (2005) Accumulation of partly folded states in the equilibrium unfolding of the pneumococcal choline-binding module C-LytA. *Biochem J* **387**, 479-88.

Abstract: Choline-binding modules are present in some virulence factors and many other proteins of *Streptococcus pneumoniae* (Pneumococcus). The most extensively studied choline-binding module is C-LytA, the C-terminal moiety of the pneumococcal cell-wall amidase LytA. The three-dimensional structure of C-LytA is built up from six loop-hairpin structures forming a left-handed beta-solenoid with four choline-binding sites. The affinity of C-LytA for choline and other structural analogues allows its use as an efficient fusion tag for single-step purification of hybrid proteins. In the present study, we characterize the folding and stability of C-LytA by chemical and thermal equilibrium denaturation experiments. Unfolding experiments using guanidinium chloride at pH 7.0 and 20 degrees C suggest the existence of two partly folded states (I1 and I2) in the following model: N (native) \rightleftharpoons I1 \rightleftharpoons I2. The N \rightarrow I1 transition is non-cooperative and irreversible, and is significant even in the absence of a denaturant. In contrast, the I1 \rightleftharpoons I2 transition is co-operative and reversible, with an associated freeenergy change ($\Delta G(0)$) of 30.9 \pm 0.8 kJ x mol⁻¹. The residual structure in the I2 state is unusually stable even in 7.4 M guanidinium chloride. Binding of choline stabilizes the structure of the native state, induces its dimerization and prevents the accumulation of the I1 species ($[N]_2 \rightleftharpoons [I2]_2$, $\Delta G(0) = 50.1 \pm 0.8$ kJ x mol⁻¹). Fluorescence and CD measurements, gel-filtration chromatography and limited proteolysis suggest that I1 differs from N in the local unfolding of the N-terminal beta-hairpins, and that I2 has a residual structure in the C-terminal region. Thermal denaturation of C-LytA suggests the accumulation of at least the I1 species. These results might pave the way for an effective improvement of its biotechnological applications by protein engineering.

Manfrinato M. C., Bellini T., Masserini M., Tomasi M., and Dallochio F. (2001) Thermal stability of the hemagglutinin-neuraminidase from Sendai virus evidences two folding domains. *FEBS Lett* **495**, 48-51.

Abstract: The domain structure of hemagglutinin-neuraminidase from Sendai virus (cHN) was investigated by studying the thermal stability in the 20-100 degrees C range. Differential scanning calorimetry evidences two conformational transitions. The first transition is apparently a reversible two-state process, with T_m 48.3 degrees C, and is shifted to 50.1 degrees C in the presence of the substrate analogue 2,3-dehydro-2-deoxy-N-acetyl neuraminic acid, meaning that the substrate binding domain is involved in the transition. The second transition, with apparent T_m 53.2 degrees C, is accompanied by irreversible loss of enzymatic activity of the protein, and the presence of the substrate analogue does not affect the T_m. The data indicate that cHN is composed of two independent folding domains, and that only one domain is involved in the binding of the substrate. Our results suggest that the paramyxovirus neuraminidases have the folding properties of a two-domain protein.

Martin I., Epand R. M., and Ruyschaert J. M. (1998) Structural properties of the putative fusion peptide of fertilin, a protein active in sperm-egg fusion, upon interaction with the lipid bilayer. *Biochemistry* **37**, 17030-17039.

Abstract: We recently demonstrated that a peptide representing the putative fusion domain of fertilin, a surface membrane protein of sperm involved in sperm-egg fusion, induces fusion of large unilamellar vesicles containing negatively charged lipids [Martin, I., and Ruyschaert, J. M. (1997) *FEBS Lett.* 405, 351-355]. In the present work, we demonstrate that increasing the concentration in negatively charged lipids strongly enhances the binding of the fertilin fusion peptide to the membrane, suggesting that electrostatic attractions play a crucial role in the binding process. While no significant change of the secondary structure content is observed by increasing the amounts of negatively charged lipids in the bilayer, the orientation of the alpha-helix changes from a parallel to an oblique orientation in the membrane. This topological change is confirmed by amide II hydrogen/deuterium exchange measurements that monitor the accessibility of the peptide to the water medium. Differential scanning calorimetry data also suggest that the fertilin fusion peptide lowers the bilayer to hexagonal phase transition temperature of model membranes composed of mixtures of dipalmitoleoylphosphatidylethanolamine and 1-palmitoyl-2-oleoylphosphatidylserine and therefore promotes negative curvature in lipid vesicles. A comparison of the biophysical properties and the membrane-perturbing activities of fertilin and of viral fusion peptides is discussed in terms of sperm-egg fusion and virus cell fusion.

Meher A. K., Bal N. C., Chary K. V., and Arora A. (2006) Mycobacterium tuberculosis H37Rv ESAT-6-CFP-10 complex formation confers thermodynamic and biochemical stability. *FEBS J* **273**, 1445-1462.

Abstract: The 6-kDa early secretory antigenic target (ESAT-6) and culture filtrate protein-10 (CFP-10), expressed from the region of deletion-1 (RD1) of Mycobacterium tuberculosis H37Rv, are known to play a key role in virulence. In this study, we explored the thermodynamic and biochemical changes associated with the formation of the 1 : 1 heterodimeric complex between ESAT-6 and CFP-10. Using isothermal titration calorimetry (ITC), we precisely determined the association constant and free energy change for formation of the complex to be 2×10^7 M⁻¹ and -9.95 kcal.mol⁻¹, respectively. Strikingly, the thermal unfolding of the ESAT-6-CFP-10 heterodimeric complex was completely reversible, with a T(m) of 53.4 degrees C and DeltaH of 69 kcal.mol⁻¹. Mixing of ESAT-6 and CFP-10 at any temperature below the T(m) of the complex led to induction of helical conformation, suggesting molecular recognition between specific segments of unfolded ESAT-6 and CFP-10. Enhanced biochemical stability of the complex was indicated by protection of ESAT-6 and an N-terminal fragment of CFP-10 from proteolysis with trypsin. However, the flexible C-terminal of CFP-10 in the complex, which has been shown to be responsible for binding to macrophages and monocytes, was cleaved by trypsin. In the presence of phospholipid membranes, ESAT-6, but not CFP-10 and the complex, showed an increase in alpha-helical content and enhanced thermal stability. Overall, complex formation resulted in structural changes, enhanced thermodynamic and biochemical stability, and loss of binding to phospholipid membranes. These features of complex formation probably determine the physiological role of ESAT-6, CFP-10 and/or the complex in vivo. The ITC and thermal unfolding approach described in this study can readily be applied to characterization of the 11 other pairs of ESAT-6 family proteins and for screening ESAT-6 and CFP-10 mutants.

Michels B., Leimkuhler M., Lechner M. D., Adrian M., Lorber B., and Witz J. (1999) Polymorphism of turnip yellow mosaic virus empty shells and evidence for conformational changes occurring after release of the viral RNA. A differential scanning calorimetric study. *Eur J Biochem* **264**, 965-972.

Abstract: Turnip yellow mosaic virus (TYMV) is a small isometric plant virus which decapsidates by releasing its RNA through a hole in the capsid, leaving behind an empty shell [R. E. F. Matthews and J. Witz, (1985) *Virology* 144, 318-327]. Similar empty shells (artificial top component, ATC) can be obtained by submitting the virions to various treatments in vitro. We have used differential scanning calorimetry, analytical sedimentation, and electron microscopy to investigate the thermodenaturation of natural empty shells (NTC, natural top component) present in purified virus suspensions, and of several types of ATCs. ATCs divided in two major classes. Those obtained by alkaline titration, by the action of urea or butanol behaved as NTC: their thermograms contained only one peak corresponding to the irreversible dissociation of the shells and the denaturation of the coat protein. The temperature of this unique transition varied significantly with pH, from 71 degrees C at pH 4.5 to 84 degrees C at pH 8.5. The thermograms of ATCs obtained by freezing and thawing, or by the action of high pressure, contained two peaks: shells dissociated

first into smaller protein aggregates at 57 degrees C (at pH 5.0) to 61 degrees C (at pH 8.5), which denatured at the temperature of the unique transition of NTC. Shells obtained by heating virions to 55 degrees C at pH 7.6, changed conformation after the release of the viral RNA, as upon continuous heating to 95 degrees C, their thermograms were similar to those of the shells obtained by freezing and thawing, whereas after purification they behaved like NTC. Structural implications of these observations are discussed.

Moreno M. R., Perez-Berna A. J., Guillen J. and Villalain J. (2008) Biophysical characterization and membrane interaction of the most membranotropic region of the HIV-1 gp41 endodomain. *Biochim Biophys Acta* **1778**, 1298-1307.

Abstract: The membrane fusion protein of HIV-1 is the envelope transmembrane gp41 glycoprotein, which is the responsible of the membrane fusion between the virus and the target cell. Gp41 has an unusual cytoplasmic tail, the endodomain, containing highly helicoidal segments with large hydrophobic moments, the so called lentivirus lytic peptides or LLPs. According to our previous work, one of the most membranotropic regions along the whole gp41 glycoprotein was located in the LLP3 region of the gp41. In order to get new insights into the viral membrane fusion mechanism, a peptide pertaining to the LLP3 domain has been studied by infrared, fluorescence and calorimetry regarding its structure, its ability to induce membrane rupture and aggregation, as well as its affinity towards specific phospholipids. Our results demonstrate that this peptide interacts with phospholipid-containing model membranes, affects the phase-behavior of membrane phospholipids and induces leakage and aggregation of liposomes. The membrane-perturbing properties of LLP3, together with the possibility that the Kennedy sequence could be part of an external loop, open the possibility that these domains might function in modulating viral membrane fusion or budding, synergistically with other membranotropic regions of the gp41 glycoprotein

Nemykh M. A., Novikov V. K., Arutiunian A. M., Kalmykov P. V., Drachev V. A. and Dobrov E. N. (2007) [Comparative study of structural stability of potato virus X coat protein molecules in solution and in the virus particles]. *Mol Biol (Mosk)* **41**, 697-705.

Abstract: With help of several optical methods and differential scanning calorimetry we studied the structure and stability of molecules of coat protein (CP) of filamentous of potato virus X (PVX) in free state and in the virions. According to the results of all these methods, at room temperature (25 degrees C) free PVX CP subunits possess some fixed tertiary structure but this structure is highly unstable and is completely disrupted at temperatures as low as 35 degrees C. The free PVX CP tertiary structure was also disrupted by very low sodium dodecylsulfate and cetyltrimethylammonium bromide concentrations: 3 to 5 molecules of the surfactants per the CP molecule were sufficient to induce its total disruption. At the same time, these treatments did not result in any changes in the PVX CP secondary structure. Incorporation of the CP subunits into the PVX virions resulted in a strong increase in their stability to effects of increased temperatures and surfactants. This combination of highly labile tertiary structure and rather stable secondary structure of free PVX CP subunits may represent a structural basis for recently observed capacity of the PVX CP molecules to assume two different functional states in the virion.

Nemykh M. A., Efimov A. V., Novikov V. K., Orlov V. N., Arutyunyan A. M., Drachev V. A., Lukashina E. V., Baratova L. A. and Dobrov E. N. (2008) One more probable structural transition in potato virus X virions and a revised model of the virus coat protein structure. *Virology* **373**, 61-71.

Abstract: We found that a 2-h incubation of potato virus X (PVX) virions in 10 mM Tris-HCl buffer pH 7.5 at -20 degrees C results in a strong but reversible drop in virion stability. Under these conditions, the PVX virions are completely disrupted by low (starting from 50 mM) concentrations of LiCl and CaCl₂ but not of NaCl. Incubation of PVX samples with 0.05-2 M LiCl at +4 degrees C did not result in virion disassembly and the virions were not disrupted upon incubation at -20 degrees C in 10 mM Tris-HCl buffer pH 7.5 without LiCl. We suggest that a 2-h incubation of the PVX virions at -20 degrees C in 10 mM Tris-HCl pH 7.5 results in a structural transition in the virus particles. A revised model of the three-dimensional organization of coat protein subunits in the PVX virions is proposed. This two-domain model explains better the high plasticity of the PVX CP structure

Nunez E., Fernandez A. M., Estepa A., Gonzalez-Ros J. M., Gavilanes F., and Coll J. M. (1998) Phospholipid interactions of a peptide from the fusion-related domain of the glycoprotein of VHSV, a fish rhabdovirus. *Virology* **243**, 322-330.

Abstract: Previous studies mapped a p2 domain (aa 82-109) which binds phosphatidylserine (PS) (Estepa and Coll, 1996a) and contains three contiguous hydrophobic amino acid heptad repeats followed by a positively charged stretch (Coll, 1995b) in the glycoprotein G of the viral hemorrhagic septicemia virus (VHSV), a fish rhabdovirus. Anti-p2 antibodies inhibited low-pH VHSV-induced fusion (Estepa and Coll, 1997) and low-pH PS binding to VHSV (Estepa and Coll, 1996a). We report here further studies on the interaction of the synthetic peptide p2 with phospholipid vesicles. The synthetic p2 peptide was able to mediate aggregation, lipid mixing, and leakage of contents only with negatively charged phospholipid vesicles and in a concentration-dependent manner. As shown by its effect on lipid phase transitions deduced from data with fluorescence polarization and differential scanning calorimetry, the p2 peptide becomes inserted into the hydrophobic negatively charged phospholipid vesicle bilayers. In addition, data based on circular dichroism showed that the p2 peptide folds as a structure with a high content of beta-sheets stabilized by interaction with anionic phospholipids. These studies are potentially relevant to viral fusion in VHSV.

Orlov V. N., Kust S. V., Kalmykov P. V., Krivosheev V. P., Dobrov E. N., and Drachev V. A. (1998) A comparative differential scanning calorimetric study of tobacco mosaic virus and of its coat protein ts mutant. *FEBS Lett* **433**, 307-311.

Abstract: The differential scanning calorimetry (DSC) 'melting curves' for virions and coat proteins (CP) of wild-type tobacco mosaic virus (strain U1) and for its CP ts mutant ts21-66 were measured. Strain U1 and ts21-66 mutant (two amino acid substitutions in CP: 121 --> T and D66 --> G) differ in the type of symptoms they induce on some host plants. It was observed that CP subunits of both U1 and ts21-66 at pH 8.0, in the form of small (3-4S) aggregates, possess much lower thermal stability than in the virions. Assembly into the virus particles resulted in a DSC melting temperature increase from 41 to 72 degrees C for U1 and from 38 to 72 degrees C for ts21-66 CP. In the RNA-free helical virus-like protein assemblies U1 and ts21-66 CP subunits had a thermal stability intermediate between those in 3-4S aggregates and in the virions. ts21-66 helical protein displayed a somewhat lower thermal stability than U1.

Orlov V. N., Arutyunyan A. M., Kust S. V., Litmanovich E. A., Drachev V. A., and Dobrov E. N. (2001) Macroscopic aggregation of tobacco mosaic virus coat protein. *Biochemistry (Mosc)* **66**, 154-162.

Abstract: The relationship between processes of thermal denaturation and heat-induced aggregation of tobacco mosaic virus (TMV) coat protein (CP) was studied. Judging from differential scanning calorimetry "melting" curves, TMV CP in the form of a trimer-pentamer mixture ("4S-protein") has very low thermal stability, with a transition temperature at about 40 degrees C. Thermally denatured TMV CP displayed high propensity for large (macroscopic) aggregate formation. TMV CP macroscopic aggregation was strongly dependent on the protein concentration and solution ionic strength. By varying phosphate buffer molarity, it was possible to merge or to separate the denaturation and aggregation processes. Using far-UV CD spectroscopy, it was found that on thermal denaturation TMV CP subunits are converted into an intermediate that retains about half of its initial alpha-helix content and possesses high heat stability. We suppose that this stable thermal denaturation intermediate is directly responsible for the formation of TMV CP macroscopic aggregates.

Panyukov Y. V., Nemykh M. A., Dobrov E. N. and Drachev V. A. (2007) Surfactant-Induced Amorphous Aggregation of Tobacco Mosaic Virus Coat Protein: A Physical Methods Approach. *Macromol. Biosci* **8**, 199-209.

Abstract: The interactions of non-ionic surfactant Triton X-100 and the coat protein of tobacco mosaic virus, which is an established model for both ordered and non-ordered protein aggregation, were studied using turbidimetry, differential scanning calorimetry, isothermal titration calorimetry, and dynamic light scattering. It was found that at the critical aggregation concentration (equal to critical micelle concentration) of 138×10^{-6} M, Triton X-100 induces partial denaturation of tobacco mosaic virus coat protein molecules followed by protein amorphous aggregation. Protein aggregation has profound ionic strength dependence and proceeds due to hydrophobic sticking of surfactant-protein complexes (start aggregates) with initial radii of 46 nm. It has been suggested that the anionic surfactant sodium dodecyl sulfate forms mixed micelles with Triton X-100 and therefore reverses protein amorphous aggregation with

release of protein molecules from the amorphous aggregates. A stoichiometric ratio of 5 was found for Triton X-100-sodium dodecyl sulfate interactions.

Peek L. J., Martin T. T., Elk N. C., Pegram S. A. and Middaugh C. R. (2007) Effects of stabilizers on the destabilization of proteins upon adsorption to aluminum salt adjuvants. *J Pharm. Sci* **96**, 547-557.

Abstract: Excipients for protein-based vaccines are currently identified by evaluating the stability of the protein in solution. In most cases, however, the protein is adsorbed to the surface of an aluminum salt adjuvant in the final vaccine formulation. Previous studies showed that model protein antigens may be structurally altered and less thermally stable upon adsorption to aluminum salt adjuvants [Jones LS, Peek LJ, Power J, Markham A, Yazzie B, Middaugh CR, 2005, *J Biol Chem* 280:13406-13414]. The work presented herein provides evidence that compounds that stabilize the protein in solution also stabilize the adsorbed protein; however, the stability of the adsorbed protein in the presence of the stabilizer remains lower than that of the protein in solution. Potential implications of the reduced stability on the approach used to select excipients during formulation development are discussed.

Peisajovich S. G., Blank L., Epand R. F., Epand R. M., and Shai Y. (2003) On the interaction between gp41 and membranes: the immunodominant loop stabilizes gp41 helical hairpin conformation. *J Mol Biol* **326**, 1489-1501.

Abstract: gp41 is the protein responsible for the process of membrane fusion that allows primate lentiviruses (HIV and SIV) to enter into their host cells. gp41 ectodomain contains an N-terminal and a C-terminal heptad repeat region (NHR and CHR) connected by an immunodominant loop. In the absence of membranes, the NHR and CHR segments fold into a protease-resistant core with a trimeric helical hairpin structure. However, when the immunodominant loop is not present (either in a complex formed by HIV-1 gp41-derived NHR and CHR peptides or by mild treatment with protease of recombinant constructs of HIV-1 gp41 ectodomain, which also lack the N-terminal fusion peptide and the C-terminal Trp-rich region) membrane binding induces a conformational change in the gp41 core structure. Here, we further investigated whether covalently linking the NHR and CHR segments by the immunodominant loop affects this conformational change. Specifically, we analyzed a construct corresponding to a fragment of SIVmac239 gp41 ectodomain (residues 27-149, named e-gp41) by means of surface plasmon resonance, Trp and rhodamine fluorescence, ATR-FTIR spectroscopy, and differential scanning calorimetry. Our results suggest that the presence of the loop stabilizes the trimeric helical hairpin both when e-gp41 is in aqueous solution and when it is bound to the membrane surface. Bearing in mind possible differences between HIV-1 and SIV gp41, and considering that the gp41 ectodomain constructs analyzed to date lack the N-terminal fusion peptide and the C-terminal Trp-rich region, we discuss our observations in relation to the mechanism of virus-induced membrane fusion.

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.

Ravoo B. J., Weringa W. D., and Engberts J. B. (2000) Fusion of Sendai virus with vesicles of oligomerizable lipids: a microcalorimetric analysis of membrane fusion. *Cell Biol Int* **24**, 787-797.

Abstract: Sendai virus fuses efficiently with small and large unilamellar vesicles of the lipid 1,2-di-n-hexadecyloxypropyl-4- (beta-nitrostyryl) phosphate (DHPBNS) at pH 7.4 and 37 degrees C, as shown by lipid mixing assays and electron microscopy. However, fusion is strongly inhibited by oligomerization of the head groups of DHPBNS in the bilayer vesicles. The enthalpy associated with fusion of Sendai virus with DHPBNS vesicles was measured by isothermal titration microcalorimetry, comparing titrations of Sendai virus into (i) solutions of DHPBNS vesicles (which fuse with the virus) and (ii) oligomerized DHPBNS vesicles (which do not fuse with the virus), respectively. The observed heat effect of fusion of Sendai virus with DHPBNS vesicles is strongly dependent on the buffer medium, reflecting a partial charge neutralization of the Sendai F and HN proteins upon insertion into the negatively-charged vesicle membrane. No buffer effect was observed for the titration of Sendai virus into oligomerized DHPBNS vesicles, indicating that inhibition of fusion is a result of inhibition of insertion of the fusion protein into the target membrane. Fusion of Sendai virus with DHPBNS vesicles is endothermic and entropy-driven. The positive enthalpy term is dominated by heat effects resulting from merging of the protein-rich viral envelope with the lipid vesicle bilayers rather than by the fusion of the viral with the vesicle bilayers per se.

Remeta D. P., Krumbiegel M., Minetti C. A., Puri A., Ginsburg A., and Blumenthal R. (2002) Acid-induced changes in thermal stability and fusion activity of influenza hemagglutinin. *Biochemistry* **41**, 2044-2054.

Abstract: The conformational and thermal stability of full-length hemagglutinin (HA) of influenza virus (strain X31) has been investigated using a combination of differential scanning calorimetry (DSC), analytical ultracentrifugation, fluorescence, and circular dichroism (CD) spectroscopy as a function of pH. HA sediments as a rosette comprised of 5-6 trimers (31-35 S) over the pH range of 7.4-5.4. The DSC profile of HA in the native state at pH 7.4 is characterized by a single cooperative endotherm with a transition temperature (T_m) of 66 degrees C and unfolding enthalpy ($\Delta H(\text{cal})$) of 800 kcal x (mol of trimer)⁻¹. Upon acidification to pH 5.4, there is a significant decrease in the transition temperature (from 66 to 45 degrees C), unfolding enthalpy [from 800 to 260 kcal x (mol of trimer)⁻¹], and $\Delta H(\text{cal})/\Delta H(\text{vH})$ ratio (from 3.0 to approximately 1.3). Whereas the far- and near-UV ellipticities are maintained over this pH range, there is an acid-induced increase in surface hydrophobicity and decrease in intrinsic tryptophanyl fluorescence. The major contribution to the DSC endotherm arises from unfolding HA1 domains. The relationship between acid-induced changes in thermal stability and the fusion activity of HA has been examined by evaluating the kinetics and extent of fusion of influenza virus with erythrocytes over the temperature and pH range of the DSC measurements. Surprisingly, X31 influenza virus retains its fusion activity at acidic pH and temperatures significantly below the unfolding transition of HA. This finding is consistent with the notion that the fusion activity of influenza virus may involve structural changes of only a small fraction of HA molecules.

Rexroad J., Wiethoff C. M., Green A. P., Kierstead T. D., Scott M. O., and Middaugh C. R. (2003) Structural stability of adenovirus type 5. *J Pharm Sci* **92**, 665-678.

Abstract: Thermally induced structural changes in adenovirus type 5 (Ad) in the presence of either 2 or 10% sucrose were investigated using a variety of biophysical techniques. In solutions containing 2% sucrose, a highly cooperative transition in the structure of the virus was observed at 45 degrees C as detected by tryptophan fluorescence, derivative UV absorption spectroscopy, circular dichroism (CD), and dynamic and static light scattering. This transition resulted in (at least partial) disassembly of the virus and a concomitant increase in the accessibility of the viral DNA to the fluorescent dye, TOTO-1. Capsid disassembly was verified by transmission electron microscopy, which showed ruptured icosahedral vertices near 45 degrees C followed by complete capsid disassembly at higher temperatures. SDS-PAGE of thermally treated Ad suggests that the penton base (protein III) and protein IIIa (located in the peripentonal region) are significantly more labile than other capsid proteins and may be the initial instigators in capsid disassembly. Additional discrete structural transitions were observed in viral proteins using the aforementioned spectroscopic techniques. Thermally induced rearrangements of the condensed DNA at higher temperatures were also detected by the appearance of "psi"-like features in the CD spectra as well as a dramatic decrease in accessibility of DNA to TOTO-1. These transitions corresponded to discrete endothermic events that are also detected by differential scanning calorimetry. By increasing the

concentration of sucrose to 10%, secondary and tertiary structural features of adenoviral proteins were significantly stabilized, although loss of quaternary structure at 45 degrees C was still observed.

Rodriguez J. A., Valentine J. S., Eggers D. K., Roe J. A., Tiwari A., Brown R. H., Jr., and Hayward L. J. (2002) Familial amyotrophic lateral sclerosis-associated mutations decrease the thermal stability of distinctly metallated species of human copper/zinc superoxide dismutase. *J Biol Chem* **277**, 15932-15937.
Abstract: We report the thermal stability of wild type (WT) and 14 different variants of human copper/zinc superoxide dismutase (SOD1) associated with familial amyotrophic lateral sclerosis (FALS). Multiple endothermic unfolding transitions were observed by differential scanning calorimetry for partially metallated SOD1 enzymes isolated from a baculovirus system. We correlated the metal ion contents of SOD1 variants with the occurrence of distinct melting transitions. Altered thermal stability upon reduction of copper with dithionite identified transitions resulting from the unfolding of copper-containing SOD1 species. We demonstrated that copper or zinc binding to a subset of "WT-like" FALS mutants (A4V, L38V, G41S, G72S, D76Y, D90A, G93A, and E133Δ) conferred a similar degree of incremental stabilization as did metal ion binding to WT SOD1. However, these mutants were all destabilized by approximately 1-6 degrees C compared with the corresponding WT SOD1 species. Most of the "metal binding region" FALS mutants (H46R, G85R, D124V, D125H, and S134N) exhibited transitions that probably resulted from unfolding of metal-free species at approximately 4-12 degrees C below the observed melting of the least stable WT species. We conclude that decreased conformational stability shared by all of these mutant SOD1s may contribute to SOD1 toxicity in FALS.

Rojo N., Gomara M. J., Alsina M. A., and Haro I. (2003) Lipophilic derivatization of synthetic peptides belonging to NS3 and E2 proteins of GB virus-C (hepatitis G virus) and its effect on the interaction with model lipid membranes. *J Pept Res* **61**, 318-330.
Abstract: The synthesis by solid-phase methodologies of peptides belonging to structural and non-structural proteins of GB virus C as well as its N-alpha-acylation with myristate and palmitate fatty acids is described. To explore the peptide-lipid interactions we have used liposomes composed of dipalmitoylphosphatidylcholine as model membranes and complementary spectroscopic and calorimetric techniques. Our results show that structural and more clearly the structural lipophilic peptide sequences incorporated into lipid bilayers perturb the packing of lipids and affect their thermotropic properties, more than the non-structural selected sequence. However, the binding of the synthetic sequences to lipid membranes occurred without any restructuring of the peptides.

Rozak D. A., Orban J., and Bryan P. N. (2005) G148-GA3: a streptococcal virulence module with atypical thermodynamics of folding optimally binds human serum albumin at physiological temperatures. *Biochim Biophys Acta* **1753**, 226-233.
Abstract: The third albumin binding domain of streptococcal protein G strain 148 (G148-GA3) belongs to a novel class of prokaryotic albumin binding modules that is thought to support virulence in several bacterial species. Here, we characterize G148-GA3 folding and albumin binding by using differential scanning calorimetry and isothermal titration calorimetry to obtain the most complete set of thermodynamic state functions for any member of this medically significant module. When buffered at pH 7.0 the 46-amino acid alpha-helical domain melts at 72 degrees C and exhibits marginal stability (15 kJ/mol) at 37 degrees C. G148-GA3 unfolding is characterized by small contributions to entropy from non-hydrophobic forces and a low DeltaCp (1.1 kJ/(deg mol)). Isothermal titration calorimetry reveals that the domain has evolved to optimally bind human serum albumin near 37 degrees C with a binding constant of 1.4×10^7 M⁻¹. Analysis of G148-GA3 thermodynamics suggests that the domain experiences atypically small per residue changes in structural dynamics and heat capacity while transiting between folded and unfolded states.

Rozzelle J. E., Dauber D. S., Todd S., Kelley R., and Craik C. S. (2000) Macromolecular inhibitors of HIV-1 protease. Characterization of designed heterodimers. *J Biol Chem* **275**, 7080-7086.
Abstract: Defective variants of human immunodeficiency virus type 1 (HIV-1) protease (HIV PR) have been engineered to inhibit wild-type (wt) HIV PR activity. These variants were designed to promote the formation of heterodimers and to destabilize the formation of inactive variant homodimers of HIV-1 protease through substitutions at Asp-25, Ile-49, and Gly-50 (Babe, L. M., Rose, J., and Craik, C. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10069-10073; McPhee, F., Good, A. C., Kuntz, I. D., and Craik, C. S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11477-11481). The mechanism of action of these dominant-

negative inhibitors was established using recombinantly expressed defective monomers. The defective monomers were refolded *in vitro* in the presence of wt HIV PR and showed dose-dependent inhibition of proteolytic activity. This inhibition was shown to result from the formation of inactive heterodimers between defective and wt HIV PR monomers. Heterodimer formation was detected by (i) isolating refolded, inactive heterodimers using histidine-tagged defective monomers and (ii) isolating heterodimers from bacteria coexpressing both wt and defective variants of HIV PR. Single-chain variants of HIV PR, in which the C terminus of the wt HIV PR monomer was covalently tethered to the N terminus of the defective monomer, were also expressed and analyzed. Thermal denaturation of these single-chain heterodimers using differential scanning calorimetry revealed a 1.5-7.2 degrees C greater thermal stability than single-chain wt HIV PR. The thermodynamic trend shown by these three variants mirrors their relative inhibition in provirus transfection assays. These data support the model that the effects seen both in tissue culture and *in vitro* arise from an increase in stability conferred on these heterodimers by interface mutations and identifies heterodimer formation as their mechanism of inhibition.

Shank-Retzlaff M.L., Zhao Q., Anderson C., Hamm M., High H., Nguyen M., Wang F., Wang N., Wang B., Wang Y., Washabaugh M., Sitrin R., and Shi L. (2006) Evaluation of the Thermal Stability of Gardasil. *Human Vaccines* **2**, 147-154.

Abstract: The thermostability of GARDASIL® (Merck & Co., Inc, Whitehouse Station, NJ, USA), a developmental vaccine against human papillomavirus (HPV), was evaluated using an enzyme immunoassay, referred to as the *in vitro* relative potency (IVRP) assay and differential scanning calorimetry (DSC). Gardasil samples were stored at temperatures ranging from 4 to 42 °C and tested for IVRP at various time points. Extrapolation of the IVRP results indicates GARDASIL is extremely stable. The half-life of the vaccine is estimated to be 130 months or longer at temperatures up to 25 °C. At 37 °C, the half-life is predicted to be 18 months and at 42 °C, the half-life is predicted to be approximately 3 months. Differential scanning calorimetry (DSC) analysis was used to evaluate the process of protein denaturation during a rapid temperature increase (as opposed to longterm storage at a specific temperature). Differences were seen among the DSC profiles of the four HPV types tested. This indicates that small differences in the amino acid structure can have a significant effect on the intermolecular contacts that stabilize the L1 proteins and the VLP assembly. For the Gardasil samples evaluated here, DSC results demonstrated the relative overall structural stability of the VLPs, but were not predictive of the excellent long-term stability observed with the IVRP assay.

Shnyrov V. L., Zhadan G. G., Cobaleda C., Sagraera A., Munoz-Barroso I., and Villar E. (1997) A differential scanning calorimetric study of Newcastle disease virus: identification of proteins involved in thermal transitions. *Arch Biochem Biophys* **341**, 89-97.

Abstract: The irreversible thermal denaturation of Newcastle disease virus was investigated using different techniques including high-sensitivity differential scanning calorimetry, thermal gel analysis intrinsic fluorescence, and neuraminidase activity assays. Application of a successive annealing procedure to the scanning calorimetric endotherm of Newcastle disease virus furnished four elementary thermal transitions below the overall endotherm; these were further identified as coming from the denaturation of each viral protein. The shape of these transitions, as well as their scanrate dependence, was explained by assuming that thermal denaturation takes place according to the kinetic scheme $N \xrightarrow{k} D$, where k is a first-order kinetic constant that changes with temperature, as given by the Arrhenius equation; N is the native state; and D is the denatured state. On the basis of this model, activation energy values were calculated. The data obtained with the other methods used in this work support the proposed two-state kinetic model.

Smith S. A., Krishnasamy G., Murthy K. H., Cooper A., Bromek K., Barlow P. N., and Kotwal G. J. (2002) Vaccinia virus complement control protein is monomeric, and retains structural and functional integrity after exposure to adverse conditions. *Biochim Biophys Acta* **1598**, 55-64.

Abstract: Vaccinia virus complement control protein (VCP) possesses the ability to inhibit both classical and alternative pathways of complement activation, as well as bind to heparin or heparan sulfate proteoglycans, making it a unique multifunctional protein with therapeutic potential. Recently, the structure of the complete molecule of VCP was determined by X-ray crystallography. Two or three VCP molecules were packed within the unit cells of both crystal forms. Using gel filtration, VCP has now been shown to exist as a monomer in solution. To test the stability of this molecule, VCP was studied by nuclear magnetic resonance (NMR) over a range of temperatures and by differential scanning calorimetry (DSC). It was also

subjected to adverse physical conditions, including, freeze-thawing, changes in pH, changes in temperature, and storage at room temperature. VCP melts fully reversibly, and it maintained its 3-D structure and the ability to inhibit serum-induced hemolysis of sheep red blood cells after exposure to many extreme conditions. The robustness of VCP may be rationalized in terms of its architecture.

Snyder S. W., Edalji R. P., Lindh F. G., Walter K. A., Solomon L., Pratt S., Steffy K., and Holzman T. F. (1996) Initial characterization of autoprocessing and active-center mutants of CMV proteinase. *J Protein Chem* **15**, 763-774.

Abstract: Human cytomegalovirus (CMV) encodes a unique serine proteinase that is required in the maturation of the viral capsid. The CMV proteinase can undergo autocatalytic activation and is subject to proteolytic self-inactivation. Mutant enzyme forms were prepared to eliminate the initial autoprocessing site and thus form an active single-chain protein for structure-function studies. Two mutants of CMV proteinase were cloned and expressed in *Escherichia coli*. The A143V mutant was a conservative substitution at the first internal cleavage site. The S132A mutant modified one of the triad of residues responsible for catalytic activity. Through the use of computer-controlled high-cell-density fermentations the mutant proteins were expressed in *E. coli* at approximately 170 mg/L as both soluble (approximately 40% of total) and inclusion-body forms (approximately 60% of total). The soluble enzyme was purified by standard methods; inclusion-body protein was isolated by standard methods after refolding and solubilization in guanidine or urea. Sedimentation equilibrium and sedimentation velocity analyses reveal that the enzyme undergoes concentration-dependent aggregation. It exhibits a monomer \rightleftharpoons dimer equilibrium ($K_d = 1 \mu\text{M}$) at low concentrations and remains dimeric at high concentrations (28 mg/ml). Differential scanning calorimetry data for protein thermal unfolding fit best to a non-two-state model with two components ($T_m = 52.3$ and 55.3 degrees C) which subsequently aggregate upon unfolding. Analysis of the short-UV circular dichroism spectra of protein forms resulting from expression as soluble molecules (not refolded) reveals that the two mutants have very similar secondary structures which comprise a mixed structural motif of 20% alpha-helix, 26% beta-sheet, and 53% random coil. Though soluble and active (A143V mutant only), CD analysis revealed that protein refolded from inclusion bodies did not exhibit spectra identical to that of protein expressed only in soluble form.

Steven A.C., Heymann J.B., Cheng N., Trus B.L., and Conway J.F. (2005) Virus maturation: dynamics and mechanism of a stabilizing structural transition that leads to infectivity. *Curr Opin Struct Biol.* **15**, 227-36.

Abstract: For many viruses, the final stage of assembly involves structural transitions that convert an innocuous precursor particle into an infectious agent. This process -- maturation -- is controlled by proteases that trigger large-scale conformational changes. In this context, protease inhibitor antiviral drugs act by blocking maturation. Recent work has succeeded in determining the folds of representative examples of the five major proteins -- major capsid protein, scaffolding protein, portal, protease and accessory protein -- that are typically involved in capsid assembly. These data provide a framework for detailed mechanistic investigations and elucidation of mutations that affect assembly in various ways. The nature of the conformational change has been elucidated: it entails rigid-body rotations and translations of the arrayed subunits that transfer the interactions between them to different molecular surfaces, accompanied by refolding and redeployment of local motifs. Moreover, it has been possible to visualize maturation at the submolecular level in movies based on time-resolved cryo-electron microscopy.

Szeltner Z. and Polgar L. (1996) Conformational stability and catalytic activity of HIV-1 protease are both enhanced at high salt concentration. *J Biol Chem* **271**, 5458-5463.

Abstract: The activity of human immunodeficiency virus protease is markedly increased at elevated salt concentration. The structural basis of this effect has been explored by several independent methods by using both the wild-type enzyme and its triple mutant (Q7K/L33I/L63I) (Mildner, A. M., Rothrock, D. J., Leone, J. W., Bannow, C. A., Lull, J. M., Reardon, I. M., Sarcich, J. L., Howe, W. J., Tomich, C.-S. C., Smith, C. W., Heinrikson, R. L., and Tomasselli, A. G. (1994) *Biochemistry* **33**, 9405-9413), designed to better resist autolysis. Monitoring the intrinsic fluorescence of the two enzymes during urea-mediated denaturation has shown that at high NaCl concentration, both the conformational stability (ΔG_0) and the transition midpoint ($D_{1/2}$) between the folded and unfolded states increase, indicating that the salt stabilizes the enzyme structure. These equilibrium data are supported by kinetic studies on the urea-mediated unfolding by measuring fluorescence change, red shifting in the maximum of the emission

spectrum, and far- and near-UV CD. The salt effects observed in urea-mediated unfolding reactions prevail upon heat denaturation. All these findings support the existence of a two-state equilibrium between the folded and unfolded proteins. The pH dependence of fluorescence intensity indicated that the conformation of human immunodeficiency virus type 1 protease should change in the catalytically competent pH region. It is concluded that preferential hydration stabilizes the protease structure in the presence of salt, providing entropic contribution to enhance the catalytic activity.

Upadhyay A., Williams C., Gill A. C., Philippe D. L., Davis K., Taylor L. A., Stevens M. P., Galyov E. E., and Bagby S. (2004) Biophysical characterization of the catalytic domain of guanine nucleotide exchange factor BopE from *Burkholderia pseudomallei*. *Biochim Biophys Acta* **1698**, 111-119.

Abstract: BopE is a type III secreted protein from *Burkholderia pseudomallei*, the aetiological agent of melioidosis. Like its *Salmonella* homologues SopE and SopE2, BopE is a guanine nucleotide exchange factor for Rho GTPases. It is thought that, in order to be secreted by the type III system, proteins must be unfolded or only partially folded. As part of a study of *B. pseudomallei* virulence proteins, we have expressed, purified and characterized the catalytic domain of BopE (amino acids 78-261). Analytical ultracentrifugation experiments in conjunction with analytical size exclusion chromatography show that BopE(78-261) is monomeric in aqueous solution. CD spectroscopy indicates that the protein is predominantly alpha-helical, with predicted secondary structure composition of 59% alpha-helix and 7% beta-strand. NMR spectroscopy confirms that BopE(78-261) adopts a single, stable conformation. In differential scanning calorimetry experiments, thermal denaturation of BopE(78-261) (T_m 52 degrees C) is reversible. Also, the secondary structure composition of BopE(78-261) changes little over a range of pH values from 3.5 to 10.5. BopE may therefore fold spontaneously to a functional form upon secretion into the host cell cytoplasm, and retains a native or native-like fold in varied environments. These properties are likely to be advantageous for a secreted bacterial effector protein.

Vangala A., Kirby D., Rosenkrands I., Agger E. M., Andersen P., and Perrie Y. (2006) A comparative study of cationic liposome and niosome-based adjuvant systems for protein subunit vaccines: characterisation, environmental scanning electron microscopy and immunisation studies in mice. *J Pharm Pharmacol* **58**, 787-799.

Abstract: Vesicular adjuvant systems composing dimethyldioctadecylammonium (DDA) can promote both cell-mediated and humoral immune responses to the tuberculosis vaccine fusion protein in mice. However, these DDA preparations were found to be physically unstable, forming aggregates under ambient storage conditions. Therefore there is a need to improve the stability of such systems without undermining their potent adjuvanticity. To this end, the effect of incorporating non-ionic surfactants, such as 1-monopalmitoyl glycerol (MP), in addition to cholesterol (Chol) and trehalose 6,6'-dibehenate (TDB), on the stability and efficacy of these vaccine delivery systems was investigated. Differential scanning calorimetry revealed a reduction in the phase transition temperature ($T(c)$) of DDA-based vesicles by approximately 12 degrees C when MP and cholesterol (1:1 molar ratio) were incorporated into the DDA system. Transmission electron microscopy (TEM) revealed the addition of MP to DDA vesicles resulted in the formation of multi-lamellar vesicles. Environmental scanning electron microscopy (ESEM) of MP-Chol-DDA-TDB (16:16:4:0.5 micromol) indicated that incorporation of antigen led to increased stability of the vesicles, perhaps as a result of the antigen embedding within the vesicle bilayers. At 4 degrees C DDA liposomes showed significant vesicle aggregation after 28 days, although addition of MP-Chol or TDB was shown to inhibit this instability. Alternatively, at 25 degrees C only the MP-based systems retained their original size. The presence of MP within the vesicle formulation was also shown to promote a sustained release of antigen in-vitro. The adjuvant activity of various systems was tested in mice against three subunit antigens, including mycobacterial fusion protein Ag85B-ESAT-6, and two malarial antigens (Merozoite surface protein 1, MSP1, and the glutamate rich protein, GLURP). The MP- and DDA-based systems induced antibody responses at comparable levels whereas the DDA-based systems induced more powerful cell-mediated immune responses.

Vessely C., Estey T., Randolph T. W., Henderson I., Cooper J., Nayar R., Braun L. J. and Carpenter J. F. (2008) Stability of a trivalent recombinant protein vaccine formulation against botulinum neurotoxin during storage in aqueous solution. *J Pharm. Sci.* (epublication)

Abstract: The adsorption of recombinant botulinum neurotoxin (BoNT) protein-derived vaccine antigens to aluminum salt adjuvants has been previously studied for the development of a trivalent vaccine against

the neurotoxins (Vessely et al., in press, J Pharm Sci). The current paper describes an investigation of the stability of recombinant BoNT antigens adsorbed to aluminum salt adjuvants during storage in aqueous solution. Both chemical and physical changes occurred during storage. Phosphate groups in the buffer exchanged with hydroxyl groups on the adjuvant surface. The resulting changes in solution pH and adjuvant surface chemistry promoted more favorable electrostatic interaction between the BoNT proteins and the surface, possibly increasing the affinity of the proteins for the surface during storage. Fluorescence and UV spectroscopy suggested changes to protein structure during storage, whereas differential scanning calorimetry showed changes to thermal processes related to protein conformation and/or surface adsorption. The consequence of the chemical and physical changes to the proteins was a decrease in the ability to desorb protein from the adjuvant surface during storage. Overall, the results of this study emphasize the utility of a thorough characterization of the interactions between protein antigens and aluminum salt adjuvants. (c) 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci

Welfle K., Misselwitz R., Hausdorf G., Hohne W., and Welfle H. (1999) Conformation, pH-induced conformational changes, and thermal unfolding of anti-p24 (HIV-1) monoclonal antibody CB4-1 and its Fab and Fc fragments. *Biochim Biophys Acta* **1431**, 120-131.

Abstract: Conformation, acid-induced conformational changes and stability of the murine monoclonal antibody CB4-1 directed against the human immunodeficiency virus type 1 capsid protein p24, and its Fab and Fc fragments, were analysed by circular dichroism (CD), fluorescence, and differential scanning calorimetry (DSC) measurements. CD spectra show the characteristics expected for beta-proteins. Lowering the pH to 3.5 reduces the stability, but does not change the conformation. Between pH 3.5 and 2.0 conformational changes and the formation of new structures are indicated. Deconvolution of the bimodal DSC curves of CB4-1 reveals five 'two-state' transitions at pH 7.5. At pH 5 and below, only four transitions are found. Half transition temperatures T_m and molar enthalpy changes ΔH_m gradually decrease at pH 4 and 3.4. At pH 2.1, two low-temperature ($T_m=36.9$ and 44.1 degrees C) and two high-temperature ($T_m=74.6$ and 76.8 degrees C) transitions are identified. The Fab and Fc fragments behave similarly. Deconvolution of their monophasic DSC curves yields two 'two-state' transitions for each fragment. T_m and ΔH_m values gradually decrease at pH 4.0 and 3.4; and at pH 2.1 and 2.8 for Fab and Fc, respectively, one of the transitions is found at high temperature ($T_m=67.2$ and 75.9 degrees C for Fab and Fc, respectively).

Wingfield P. T., Stahl S. J., Williams R. W., and Steven A. C. (1995) Hepatitis core antigen produced in *Escherichia coli*: subunit composition, conformational analysis, and in vitro capsid assembly. *Biochemistry* **34**, 4919-4932.

Abstract: The production and biochemical and physicochemical analysis are described of recombinant-produced hepatitis B virus core antigen (HBcAg capsid) and the corresponding particle produced by a deletion mutant missing the C-terminal 39 residues (HBeAg). Conditions for producing HBeAg from HBcAg capsids by in vitro proteolysis are also described. The morphology and masses of these capsids were determined by scanning transmission electron microscopy. Both HBcAg and HBeAg capsids comprise two size classes that correspond to icosahedral lattices with triangulation numbers (T) of 3 and 4, containing 180 and 240 subunits per capsid, respectively. This dimorphism was confirmed by sedimentation equilibrium and sedimentation velocity measurements on a Beckman Optima XL-A analytical ultracentrifuge. More than 60% of HBcAg capsids were T = 4, whereas only 15-20% of HBeAg capsids were of this size class: the remainder, in each case, were T = 3. Circular dichroism and Raman spectroscopy were used to determine the overall secondary structures of HBcAg and HBeAg capsids. Both have high alpha-helical contents, implying that this capsid protein does not conform to the canonical beta-barrel motif seen for all plant and animal icosahedral viral capsids solved to date. We suggest that the C-terminal domain of HBcAg has a random coil conformation. In vitro dissociation of HBeAg capsids under relatively mild conditions yielded stable dimers. The reassociation of HBeAg dimers into capsids appears to be driven by hydrophobic processes at neutral pH. Capsid assembly is accompanied by little change in subunit conformation as judged by circular dichroism and fluorescence spectroscopy. The thermal stability of HBcAg capsids was compared calorimetrically with that of in vitro assembled HBeAg capsids. Both have melting temperatures > 90 degrees C, implying that the C-terminal region makes little difference to the thermal stability of HBcAg; nevertheless, we discuss its possible role in facilitating disassembly and the release of viral nucleic acid.