

ITC XII - Protein-Lipid interactions

Abraham T., Lewis R. N., Hodges R. S., and McElhane R. N. (2005) Isothermal titration calorimetry studies of the binding of a rationally designed analogue of the antimicrobial peptide gramicidin S to phospholipid bilayer membranes. *Biochemistry* **44**, 2103-2112.

Abstract: The binding of the positively charged antimicrobial peptide cyclo[VKLDKVDYPLKVKLDYP] (GS14dK4) to various lipid bilayer model membranes was investigated using isothermal titration calorimetry. GS14dK4 is a diastereomeric lysine ring-size analogue of the naturally occurring antimicrobial peptide gramicidin S which exhibits enhanced antimicrobial and markedly reduced hemolytic activities compared with GS itself. Large unilamellar vesicles composed of various zwitterionic (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine [POPC]) and anionic phospholipids {1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(glycerol)] [POPG] and 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphoserine] [POPS]}, with or without cholesterol, were used as model membrane systems. Dynamic light scattering results indicate the absence of any peptide-induced major alteration in vesicle size or vesicle fusion under our experimental conditions. The binding of GS14dK4 is significantly influenced by the surface charge density of the phospholipid bilayer and by the presence of cholesterol. Specifically, a significant reduction in the degree of binding occurs when three-fourths of the anionic lipid molecules are replaced with zwitterionic POPC molecules. No measurable binding occurs to cholesterol-containing zwitterionic vesicles, and a dramatic drop in binding is observed in the cholesterol-containing anionic POPG and POPS membranes, indicating that the presence of cholesterol markedly reduces the affinity of this peptide for phospholipid bilayers. The binding isotherms can be described quantitatively by a one-site binding model. The measured endothermic binding enthalpy (ΔH) varies dramatically (+6.3 to +26.5 kcal/mol) and appears to be inversely related to the order of the phospholipid bilayer system. However, the negative free energy (ΔG) of binding remains relatively constant (-8.5 to -11.5 kcal/mol) for all lipid membranes examined. The relatively small variation of negative free energy of peptide binding together with a pronounced variation of positive enthalpy produces an equally strong variation of $T\Delta S$ (+16.2 to +35.0 kcal/mol), indicating that GS14dK4 binding to phospholipid bilayers is primarily entropy driven.

Abraham T., Lewis R. N., Hodges R. S., and McElhane R. N. (2005) Isothermal titration calorimetry studies of the binding of the antimicrobial peptide gramicidin S to phospholipid bilayer membranes. *Biochemistry* **44**, 11279-11285.

Abstract: The binding of the amphiphilic, positively charged, cyclic beta-sheet antimicrobial decapeptide gramicidin S (GS) to various lipid bilayer model membrane systems was studied by isothermal titration calorimetry. Large unilamellar vesicles composed of the zwitterionic phospholipid 1-palmitoyl-2-oleoylphosphatidylcholine or the anionic phospholipid 1-palmitoyl-2-oleoylphosphatidylglycerol, or a binary mixture of the two, with or without cholesterol, were used to mimic the lipid compositions of the outer monolayers of the lipid bilayers of mammalian and bacterial membranes, respectively. Dynamic light scattering results suggest the absence of major alterations in vesicle size or appreciable vesicle fusion upon the binding of GS to the lipid vesicles under our experimental conditions. The binding isotherms can be reasonably well described by a one-site binding model. GS is found to bind with higher affinity to anionic phosphatidylglycerol than to zwitterionic phosphatidylcholine vesicles, indicating that electrostatic interactions in the former system facilitate peptide binding. However, the presence of cholesterol reduced binding only slightly, indicating that the binding of GS is not highly sensitive to the order of the phospholipid bilayer system. Similarly, the measured positive endothermic binding enthalpy (ΔH) varies only modestly (2.6 to 4.4 kcal/mol), and the negative free energy of binding (ΔG) also remains relatively constant (-10.9 to -12.1 kcal/mol). The relatively large but invariant positive binding entropy, reflected in relatively large $T\Delta S$ values (13.4 to 16.4 kcal/mol), indicates that GS binding to phospholipid bilayers is primarily entropy driven. Finally, the relative binding affinities of GS for various phospholipid vesicles correlate relatively well with the relative lipid specificity for GS interactions with bacterial and erythrocyte membranes observed in vivo.

Adao R., Seixas R., Gomes P., Pessoa J. C. and Bastos M. (2008) Membrane structure and interactions of a short Lycotoxin I analogue. *J Pept. Sci* **14**, 528-534.

Abstract: Lycotoxin I and Lycotoxin II are natural anti-microbial peptides that were identified in the venom of the Wolf Spider *Lycosa carolinensis*. These peptides were found to be potent growth inhibitors

for bacteria (*Escherichia coli*) and yeast (*Candida glabrata*) at micromolar concentrations. Recently, shortened analogues of LycoI and LycoII have been reported to have decreased haemolytic effects. A shorter Lyco-I analogue studied, LycoI 1-15 (H-IWLTALKFLGKHAAK-NH₂), was active only above 10 microM, but was also the least haemolytic. On the basis of these findings, we became interested in obtaining a deeper insight into the membrane activity of LycoI 1-15, as this peptide may represent the first major step for the future development of selective, i.e. non-haemolytic, Lycotoxin-based antibiotics. The interaction of this peptide with liposomes of different composition was studied by microcalorimetry [differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC)] and CD. The results obtained from the calorimetric and spectroscopic techniques were jointly discussed in an attempt to further understand the interaction of this peptide with model membranes

Agnati L. F., Guidolin D., Genedani S., Ferre S., Bigiani A., Woods A. S., and Fuxe K. (2005) How proteins come together in the plasma membrane and function in macromolecular assemblies: focus on receptor mosaics. *J Mol Neurosci* **26**, 133-154.

Abstract: Some theoretical aspects on structure and function of proteins have been discussed previously. Proteins form multimeric complexes, as they have the capability of binding other proteins (Lego property) resulting in multimeric complexes capable of emergent functions. Multimeric proteins might have either a genomic or a postgenomic origin. Proteins spanning the plasma membrane have been analyzed by considering the effects of the microenvironment in which the protein is embedded. In particular, the different effects of the hydrophilic (extracellular and intracellular) versus the lipophilic (intramembrane) environment have been considered. These aspects have been discussed in the framework of membrane microdomains, in particular, the so-called rafts. In alpha-helix proteins the individual peptide dipoles align to produce a macrodipole crossing the entire membrane. This macrodipole has its positive (extracellular) pole at the N-terminal end of the helix and its negative (intracellular) pole at the C-terminal end. This arrangement has been analyzed in the framework of the counter-ion atmosphere, that is, the formation of a cloud of small ions bearing an opposite charge. Excitable cells reverse their resting potential during the all-or-none action potentials. Hence, the extracellular side of the plasma membrane becomes negative with respect to the intracellular side. This change of polarization affects also the direction and magnitude of the alpha-helix dipole in view of the fact that there is a displacement of the counter ions. The oscillation in the intensity of the dipole caused by the action potentials opens the possibility of an interaction among dipoles by electromagnetic waves.

Ananthanarayanan B., Stahelin R. V., Digman M. A., and Cho W. (2003) Activation mechanisms of conventional protein kinase C isoforms are determined by the ligand affinity and conformational flexibility of their C1 domains. *J Biol Chem* **278**, 46886-46894.

Abstract: The regulatory domains of conventional and novel protein kinases C (PKC) have two C1 domains (C1A and C1B) that have been identified as the interaction site for diacylglycerol (DAG) and phorbol ester. It has been reported that C1A and C1B domains of individual PKC isoforms play different roles in their membrane binding and activation; however, DAG affinity of individual C1 domains has not been quantitatively determined. In this study, we measured the affinity of isolated C1A and C1B domains of two conventional PKCs, PKC α and PKC γ , for soluble and membrane-incorporated DAG and phorbol ester by isothermal calorimetry and surface plasmon resonance. The C1A and C1B domains of PKC α have opposite affinities for DAG and phorbol ester; i.e. the C1A domain with high affinity for DAG and the C1B domain with high affinity for phorbol ester. In contrast, the C1A and C1B domains of PKC γ have comparably high affinities for both DAG and phorbol ester. Consistent with these results, mutational studies of full-length proteins showed that the C1A domain is critical for the DAG-induced activation of PKC α , whereas both C1A and C1B domains are involved in the DAG-induced activation of PKC γ . Further mutational studies in conjunction with in vitro activity assay and monolayer penetration analysis indicated that, unlike the C1A domain of PKC α , neither the C1A nor the C1B domain of PKC γ is conformationally restricted. Cell studies with enhanced green fluorescent protein-tagged PKCs showed that PKC α did not translocate to the plasma membrane in response to DAG at a basal intracellular calcium concentration due to the inaccessibility of its C1A domain, whereas PKC γ rapidly translocated to the plasma membrane under the same conditions. These data suggest that differential activation mechanisms of PKC isoforms are determined by the DAG affinity and conformational flexibility of their C1 domains.

Andra J., Lamata M., Martinez d. T., Bartels R., Koch M. H., and Brandenburg K. (2004) Cyclic antimicrobial peptides based on Limulus anti-lipopolysaccharide factor for neutralization of lipopolysaccharide. *Biochem Pharmacol* **68**, 1297-1307.

Abstract: Bacterial endotoxin (lipopolysaccharide, LPS) is responsible for the septic shock syndrome. As potential therapeutic agents cyclic cationic antimicrobial peptides of different length, based on the Limulus anti-lipopolysaccharide factor (LALF), were synthesized, and their interaction with LPS was characterized physico-chemically and related to results in biological assays. All peptides inhibited the LPS-induced cytokine production in human mononuclear cells and the Limulus amoebocyte lysate in a concentration-dependent way, with the peptide comprising the complete LPS-binding loop of the LALF (cLALF22) being the most effective. The peptides were neither cytotoxic nor hemolytic, except a slight effect of cLALF22. The peptides were able to displace Ca(2+) cations from a LPS monolayer, with cLALF22 being again most effective in accordance with results from isothermal titration calorimetry, in which saturation of binding was observed at an equimolar [cLALF22]:[LPS] ratio, and at a ratio 2-2.5 for the other peptides. For cLALF22, zeta (ξ) potential experiments exhibited a complete compensation of the negative charges of LPS, whereas for the other peptides a residual negative potential of -20 to -40mV was found. X-ray diffraction experiments showed that the mixed unilamellar/cubic inverted aggregate structure of the lipid A part of LPS was converted into a multilamellar one. The gel to liquid crystalline phase transition of the acyl chains of LPS was changed upon cLALF22 binding, leading to a clear fluidization, which was not observed or only to a lesser degree for the other peptides. The affinity of the peptides for LPS led to a reduced binding of lipopolysaccharide-binding protein (LBP) to target membranes and hence to an inhibition of cytokine induction in human mononuclear cells.

Andra J., Garidel P., Majerle A., Jerala R., Ridge R., Paus E., Novitsky T., Koch M. H., and Brandenburg K. (2004) Biophysical characterization of the interaction of Limulus polyphemus endotoxin neutralizing protein with lipopolysaccharide. *Eur J Biochem* **271**, 2037-2046.

Abstract: Endotoxin-neutralizing protein (ENP) of the horseshoe crab is one of the most potent neutralizers of endotoxins [bacterial lipopolysaccharide (LPS)]. Here, we report on the interaction of LPS with recombinant ENP using a variety of physical and biological techniques. In biological assays (Limulus amoebocyte lysate and tumour necrosis factor- α induction in human mononuclear cells), ENP causes a strong reduction of the immunostimulatory ability of LPS in a dose-dependent manner. Concomitantly, the accessible negative surface charges of LPS and lipid A (zeta potential) are neutralized and even converted into positive values. The gel to liquid crystalline phase transitions of LPS and lipid A shift to higher temperatures indicative of a rigidification of the acyl chains, however, the only slight enhancement of the transition enthalpy indicates that the hydrophobic moiety is not strongly disturbed. The aggregate structure of lipid A is converted from a cubic into a multilamellar phase upon ENP binding, whereas the secondary structure of ENP does not change due to the interaction with LPS. ENP contains a hydrophobic binding site to which the dye 1-anilino-8-sulfonic acid binds at a K_d of 19 μ M, which is displaced by LPS. Because lipopolysaccharide-binding protein (LBP) is not able to bind to LPS when ENP and LPS are preincubated, tight binding of ENP to LPS can be deduced with a K_d in the low nanomolar range. Importantly, ENP is able to incorporate by itself into target phospholipid liposomes, and is also able to mediate the intercalation of LPS into the liposomes thus acting as a transport protein in a manner similar to LBP. Thus, LPS-ENP complexes might enter target membranes of immunocompetent cells, but are not able to activate due to the ability of ENP to change LPS aggregates from an active into an inactive form.

Augusto L. A., Decottignies P., Synguelakis M., Nicaise M., Le Marechal P., and Chaby R. (2003) Histones: a novel class of lipopolysaccharide-binding molecules. *Biochemistry* **42**, 3929-3938.

Abstract: Unlike soluble and membrane forms of lipopolysaccharide (LPS)-binding proteins, intracellular LPS-binding molecules are poorly documented. We looked for such molecules in a murine lung epithelial cell line. Two proteins with LPS-binding activity were isolated and unambiguously identified as histones H2A.1 and H4 by mass spectrometry. Synthetic peptides representing partial structures indicated that the LPS binding site is located in the C-terminal moiety of the histones. Extending the study, we found that histones H1, H2A, H2B, H3, and H4 from calf thymus are all able to bind LPS. Bindings were specific, and affinities, determined by isothermal titration calorimetry, were (except for H4) higher than that of the LPS-binding antibiotic polymyxin B. In the presence of H2A the binding of LPS to the macrophage cell line RAW 264.7, and the LPS-induced production of TNF- α and nitric oxide by these cells, were markedly reduced. Histones may thus represent a new class of intracellular and extracellular LPS sensors.

Arnulphi C., Jin L., Tricerri M. A., and Jonas A. (2004) Enthalpy-driven apolipoprotein A-I and lipid bilayer interaction indicating protein penetration upon lipid binding. *Biochemistry* **43**, 12258-12264.

Abstract: The interaction of lipid-free apolipoprotein A-I (apoA-I) with small unilamellar vesicles (SUVs) of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) with and without free cholesterol (FC) was studied by isothermal titration calorimetry and circular dichroism spectroscopy. Parameters reported are the affinity constant (K_a), the number of protein molecules bound per vesicle (n), enthalpy change (ΔH°), entropy change (ΔS°), and the heat capacity change (ΔC_p°). The binding process of apoA-I to SUVs of POPC plus 0-20% (mole) FC was exothermic between 15 and 37 degrees C studied, accompanied by a small negative entropy change, making enthalpy the main driving force of the interaction. The presence of cholesterol in the vesicles increased the binding affinity and the alpha-helix content of apoA-I but lowered the number of apoA-I bound per vesicle and the enthalpy and entropy changes per bound apoA-I. Binding affinity and stoichiometry were essentially invariant of temperature for binding to SUVs of POPC/FC at a molar ratio of 6/1 at $(2.8-4) \times 10^6 \text{ M}^{-1}$ and 2.4 apoA-I molecules bound per vesicle or 1.4×10^2 phospholipids per bound apoA-I. A plot of ΔH degrees against temperature displayed a linear behavior, from which the ΔC_p° per mole of bound apoA-I was calculated to be $-2.73 \text{ kcal}/(\text{mol} \times \text{K})$. These results suggested that binding of apoA-I to POPC vesicles is characterized by nonclassical hydrophobic interactions, with alpha-helix formation as the main driving force for the binding to cholesterol-containing vesicles. In addition, comparison to literature data on peptides suggested a cooperativity of the helices in apoA-I in lipid interaction.

Arnulphi C., Sanchez S.A., Tricerri M.A., Gratton E., Jonas A. (2005) Interaction of human apolipoprotein A-I with model membranes exhibiting lipid domains. *Biophys J.* **89**, 285-95.

Abstract: Several mechanisms for cell cholesterol efflux have been proposed, including membrane microsolubilization, suggesting that the existence of specific domains could enhance the transfer of lipids to apolipoproteins. In this work isothermal titration calorimetry, circular dichroism spectroscopy, and two-photon microscopy are used to study the interaction of lipid-free apolipoprotein A-I (apoA-I) with small unilamellar vesicles (SUVs) of 1-palmitoyl, 2-oleoyl phosphatidylcholine (POPC) and sphingomyelin (SM), with and without cholesterol. Below 30 degrees C the calorimetric results show that apoA-I interaction with POPC/SM SUVs produces an exothermic reaction, characterized as nonclassical hydrophobic binding. The heat capacity change (ΔC_p degrees) is small and positive, whereas it was larger and negative for pure POPC bilayers, in the absence of SM. Inclusion of cholesterol in the membranes induces changes in the observed thermodynamic pattern of binding and counteracts the formation of alpha-helices in the protein. Above 30 degrees C the reactions are endothermic. Giant unilamellar vesicles (GUVs) of identical composition to the SUVs, and two-photon fluorescence microscopy techniques, were utilized to further characterize the interaction. Fluorescence imaging of the GUVs indicates coexistence of lipid domains under 30 degrees C. Binding experiments and Laurdan generalized-polarization measurements suggest that there is no preferential binding of the labeled apoA-I to any particular domain. Changes in the content of alpha-helix, binding, and fluidity data are discussed in the framework of the thermodynamic parameters.

Baier L. J., Sacchettini J. C., Knowler W. C., Eads J., Paolisso G., Tataranni P. A., Mochizuki H., Bennett P. H., Bogardus C., and Prochazka M. (1995) An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest* **95**, 1281-1287.

Abstract: The intestinal fatty acid binding protein locus (FABP2) was investigated as a possible genetic factor in determining insulin action in the Pima Indian population. A polymorphism at codon 54 of FABP2 was identified that results in an alanine-encoding allele (frequency 0.71) and a threonine-encoding allele (frequency 0.29). Pimas who were homozygous or heterozygous for the threonine-encoding allele were found to have a higher mean fasting plasma insulin concentration, a lower mean insulin-stimulated glucose uptake rate, a higher mean insulin response to oral glucose and a mixed meal, and a higher mean fat oxidation rate compared with Pimas who were homozygous for the alanine-encoding allele. Since the FABP2 threonine-encoding allele was found to be associated with insulin resistance and increased fat oxidation in vivo, we further analyzed the FABP2 gene products for potential functional differences. Titration microcalorimetry studies with purified recombinant protein showed that the threonine-containing protein had a twofold greater affinity for long-chain fatty acids than the alanine-containing protein. We

conclude that the threonine-containing protein may increase absorption and/or processing of dietary fatty acids by the intestine and thereby increase fat oxidation, which has been shown to reduce insulin action.

Balendiran G. K., Schnutgen F., Scapin G., Borchers T., Xhong N., Lim K., Godbout R., Spener F., and Sacchettini J. C. (2000) Crystal structure and thermodynamic analysis of human brain fatty acid-binding protein. *J Biol Chem* **275**, 27045-27054.

Abstract: Expression of brain fatty acid-binding protein (B-FABP) is spatially and temporally correlated with neuronal differentiation during brain development. Isothermal titration calorimetry demonstrates that recombinant human B-FABP clearly exhibits high affinity for the polyunsaturated n-3 fatty acids alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and for monounsaturated n-9 oleic acid (Kd from 28 to 53 nM) over polyunsaturated n-6 fatty acids, linoleic acid, and arachidonic acid Kd from 115 to 206 nM). B-FABP has low binding affinity for saturated long chain fatty acids. The three-dimensional structure of recombinant human B-FABP in complex with oleic acid shows that the oleic acid hydrocarbon tail assumes a "U-shaped" conformation, whereas in the complex with docosahexaenoic acid the hydrocarbon tail adopts a helical conformation. A comparison of the three-dimensional structures and binding properties of human B-FABP with other homologous FABPs, indicates that the binding specificity is in part the result of nonconserved amino acid Phe(104), which interacts with double bonds present in the lipid hydrocarbon tail. In this context, analysis of the primary and tertiary structures of human B-FABP provides a rationale for its high affinity and specificity for polyunsaturated fatty acids. The expression of B-FABP in glial cells and its high affinity for docosahexaenoic acid, which is known to be an important component of neuronal membranes, points toward a role for B-FABP in supplying brain abundant fatty acids to the developing neuron.

Baraldi E., Carugo K. D., Hyvonen M., Surdo P. L., Riley A. M., Potter B. V., O'Brien R., Ladbury J. E., and Saraste M. (1999) Structure of the PH domain from Bruton's tyrosine kinase in complex with inositol 1,3,4,5-tetrakisphosphate. *Structure Fold Des* **7**, 449-460.

Abstract: BACKGROUND: The activity of Bruton's tyrosine kinase (Btk) is important for the maturation of B cells. A variety of point mutations in this enzyme result in a severe human immunodeficiency known as X-linked agammaglobulinemia (XLA). Btk contains a pleckstrin-homology (PH) domain that specifically binds phosphatidylinositol 3,4,5-trisphosphate and, hence, responds to signalling via phosphatidylinositol 3-kinase. Point mutations in the PH domain might abolish membrane binding, preventing signalling via Btk. RESULTS: We have determined the crystal structures of the wild-type PH domain and a gain-of-function mutant E41K in complex with D-myo-inositol 1,3,4,5-tetra-kisphosphate (Ins (1,3,4,5)P₄). The inositol Ins (1,3,4,5)P₄ binds to a site that is similar to the inositol 1,4,5-trisphosphate binding site in the PH domain of phospholipase C- Δ . A second Ins (1,3,4,5)P₄ molecule is associated with the domain of the E41K mutant, suggesting a mechanism for its constitutive interaction with membrane. The affinities of Ins (1,3,4,5)P₄ to the wild type (K_d = 40 nM), and several XLA-causing mutants have been measured using isothermal titration calorimetry. CONCLUSIONS: Our data provide an explanation for the specificity and high affinity of the interaction with phosphatidylinositol 3,4,5-trisphosphate and lead to a classification of the XLA mutations that reside in the Btk PH domain. Mis-sense mutations that do not simply destabilize the PH fold either directly affect the interaction with the phosphates of the lipid head group or change electrostatic properties of the lipid-binding site. One point mutation (Q127H) cannot be explained by these facts, suggesting that the PH domain of Btk carries an additional function such as interaction with a G α protein.

Bastos M., Bai G., Gomes P., Andreu D., Goormaghtigh E. and Prieto M. (2008) Energetics and partition of two cecropin-melittin hybrid peptides to model membranes of different composition. *Biophys J* **94**, 2128-2141.

Abstract: The energetics and partition of two hybrid peptides of cecropin A and melittin (CA(1-8)M(1-18) and CA(1-7)M(2-9)) with liposomes of different composition were studied by time-resolved fluorescence spectroscopy, isothermal titration calorimetry, and surface plasmon resonance. The study was carried out with large unilamellar vesicles of three different lipid compositions: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DMPG), and a 3:1 binary mixture of DMPC/DMPG in a wide range of peptide/lipid ratios. The results are compatible with a model involving a strong electrostatic surface interaction between the peptides and the negatively charged liposomes, giving rise to aggregation and precipitation. A correlation is observed in the calorimetric

experiments between the observed events and charge neutralization for negatively charged and mixed membranes. In the case of zwitterionic membranes, a very interesting case study was obtained with the smaller peptide, CA(1-7)M(2-9). The calorimetric results obtained for this peptide in a large range of peptide/lipid ratios can be interpreted on the basis of an initial and progressive surface coverage until a threshold concentration, where the orientation changes from parallel to perpendicular to the membrane, followed by pore formation and eventually membrane disruption. The importance of negatively charged lipids on the discrimination between bacterial and eukaryotic membranes is emphasized

Benjwal S., Jayaraman S., and Gursky O. (2005) Electrostatic effects on the stability of discoidal high-density lipoproteins. *Biochemistry* **44**, 10218-10226.

Abstract: High-density lipoproteins (HDL) remove cholesterol from peripheral tissues and thereby help to prevent atherosclerosis. Nascent HDL are discoidal complexes composed of a phospholipid bilayer surrounded by protein α -helices that are thought to form extensive stabilizing interhelical salt bridges. Earlier we showed that HDL stability, which is necessary for HDL functions, is modulated by kinetic barriers. Here we test the role of electrostatic interactions in the kinetic stability by analyzing the effects of salt, pH, and point mutations on model discoidal HDL reconstituted from human apolipoprotein C-1 (apoC-1) and dimyristoyl phosphatidylcholine (DMPC). Circular dichroism, Trp fluorescence, and light scattering data show that molar concentrations of NaCl or Na₂SO₄ increase the apparent melting temperature of apoC-1:DMPC complexes by up to 20 degrees C and decelerate protein unfolding. Arrhenius analysis shows that 1 M NaCl stabilizes the disks by $\Delta\Delta G^*$ approximately equal 3.5 kcal/mol at 37 degrees C and increases the activation energy of their denaturation and fusion by $\Delta E(a)$ approximately equal $\Delta\Delta H^*$ approximately equal 13 kcal/mol, indicating that the salt-induced stabilization is enthalpy-driven. Denaturation studies in various solvent conditions (pH 5.7-8.2, 0-40% sucrose, 0-2 M trimethylamine N-oxide) suggest that the salt-induced disk stabilization results from ionic screening of unfavorable short-range Coulombic interactions. Thus, the dominant electrostatic interactions in apoC-1:DMPC disks are destabilizing. Comparison of the salt effects on the protein:lipid complexes of various composition reveals an inverse correlation between the lipoprotein stability and the salt-induced stabilization and suggests that short-range electrostatic interactions significantly contribute to lipoprotein stability: the better-optimized these interactions are, the more stable the complex is.

Berg O. G., Yu B. Z., Apatz-Castro R. J., and Jain M. K. (2004) Phosphatidylinositol-specific phospholipase C forms different complexes with monodisperse and micellar phosphatidylcholine. *Biochemistry* **43**, 2080-2090.

Abstract: Phosphatidylinositol-specific phospholipase C (PI-PLC) from *Bacillus cereus* forms a pre-micellar complex E(#₁) with monodisperse diheptanoylphosphatidylcholine (DC(7)PC) that is distinguishable from the E complex formed with micelles. Results are interpreted with the assumption that in both cases amphiphiles bind to the interfacial binding surface (i-face) of PI-PLC but not to the active site. Isothermal calorimetry and fluorescence titration results for the binding of monodisperse DC(7)PC give an apparent dissociation constant of $K(2) = 0.2$ mM with Hill coefficient of 2. The gel-permeation, spectroscopic, and probe partitioning behaviors of E(#₁) are distinct from those of the E complex. The aggregation and partitioning behaviors suggest that the acyl chains in E(#₁) but not in E remain exposed to the aqueous phase. The free (E) and complexed (E(#₁) and E) forms of PI-PLC, each with distinct spectroscopic signatures, readily equilibrate with changing DC(7)PC concentration. The underlying equilibria are modeled and their significance for the states of the PI-PLC under monomer kinetic conditions is discussed to suggest that the Michaelis-Menten complex formed with monodisperse DC(7)PC is likely to be E(#₁)S or its aggregate rather than the classical monodisperse ES complex.

Beschiaschvili G. and Seelig J. (1992) Peptide binding to lipid bilayers. Nonclassical hydrophobic effect and membrane-induced pK shifts. *Biochemistry* **31**, 10044-10053.

Abstract: The binding of the cyclic peptide (+)-D-Phe1-Cys2-Phe3-D-Trp4-(+)-Lys5-Thr6-Cys7-Thr(ol)8, a somatostatin analogue (SMS 201-995), and the potential-sensitive dye 2-(p-toluidinyl)naphthalene-6-sulfonate (TNS) to lipid membranes was investigated with high-sensitivity titration calorimetry. The binding enthalpy of the peptide was found to vary dramatically with the vesicle size. For highly curved vesicles with a diameter of d congruent to 30 nm, the binding reaction was enthalpy-driven with ΔH congruent to -7.0 ± 0.3 kcal/mol; for large vesicles with more tightly packed lipids, the binding reaction became endothermic with ΔH congruent to $+1.0 \pm 0.3$ kcal/mol and was

entropy-driven. In contrast, the free energy of binding was almost independent of the vesicle size. The thermodynamic analysis suggests that the observed enthalpy-entropy compensation of about 8 kcal/mol can be related to a change in the internal tension of the bilayer and is brought about by an entropy increase of the lipid matrix. The "entropy potential" of the membrane may have its molecular origin in the excitation of the hydrocarbon chains to a more disordered configuration and may play a more important role in membrane partition equilibria than the classical hydrophobic effect. The binding of the peptide to the membrane surface induced a pK shift of the peptide terminal amino group. Neutral membranes were found to destabilize the NH₃⁺ group, leading to a decrease in pK; negatively charged membranes, generated an apparent increase in pK due to the increase in proton concentration near the membrane surface. No pK shifts were seen for TNS. Titration calorimetry combined with the Gouy-Chapman theory can be used to determine both the reaction enthalpy and the binding constant of the membrane-binding equilibrium.

Bhunja A., Domadia P. N., Mohanram H. and Bhattacharjya S. (2008) NMR structural studies of the Ste11 SAM domain in the dodecyl phosphocholine micelle. *Proteins (epublication)*.

Abstract: The sterile alpha-motif (SAM), a relatively small (approximately 70 amino acids) interaction domain, is found in a variety of proteins involved in cell signaling, transcription regulation, and scaffolding. The Ste11 protein kinase from the mitogen activated protein kinase (MAPK) signaling cascades of the budding yeast is regulated by a SAM domain located at the N-terminus of full-length protein. In solution, the Ste11 SAM domain exists as a well-folded dimeric structure that is involved in interaction with the cognate SAM domain from an adaptor protein Ste50. In this work, we show that the Ste11 SAM domain has an intrinsic affinity towards the lipid membranes. The solution conformation of the Ste11 SAM determined in perdeuterated DPC micelle, using NMR spectroscopy, is defined by five helices of different lengths connected by a number of loops. In the micelle bound state, the non-polar and aromatic residues of the Ste11 SAM lack a native-like packing and are presumably engaged in interactions with the micelle. Using two different paramagnetic doxyl-lipids; we have mapped out localization of Ste11 SAM residues at the micelle surface. Most of the residues appear to localize at the interfacial region of the micelle. However, a number of non-polar residues from the central region of the domain are found to be located inside the core of the micelle including residues from the helix 4 and a loop between helix 2 and helix 3. Isothermal titration calorimetry studies demonstrate that a facile insertion of the Ste11 SAM into the DPC micelle is primarily driven by a large change in enthalpy, -50 kcal/mol with an apparent equilibrium association constant (K(a)) of $7.86 \times 10^6 \text{ M}^{-1}$. Interestingly, an interfacial mutant L60R of the Ste11 SAM lacking the dimeric structure does not show detectable interactions with the lipid micelle. The micelle-bound structure of the Ste11 SAM domain described in this work may have potential implications in the regulation of MAPK signaling whereby positioning of the Ste11 protein in close proximity to the membrane may facilitate efficient phosphorylation of the Ste11 kinase by the membrane attached upstream Ste20/pak kinase. *Proteins* 2008. (c) 2008 Wiley-Liss, Inc

Binder H. and Lindblom G. (2003) Charge-dependent translocation of the Trojan peptide penetratin across lipid membranes. *Biophys J* **85**, 982-995.

Abstract: We studied the interaction of the cell-penetrating peptide penetratin with mixed dioleoylphosphatidylcholine/dioleoylphosphatidylglycerol (DOPC/DOPG) unilamellar vesicles as a function of the molar fraction of anionic lipid, X(PG), by means of isothermal titration calorimetry. The work was aimed at getting a better understanding of factors that affect the peptide binding to lipid membranes and its permeation through the bilayer. The binding was well described by a surface partitioning equilibrium using an effective charge of the peptide of $z(P)$ approximately 5.1 ± 0.5 . The peptide first binds to the outer surface of the vesicles, the effective binding capacity of which increases with X(PG). At X(PG) approximately 0.5 and a molar ratio of bound peptide-to-lipid of approximately 1/20 the membranes become permeable and penetratin binds also to the inner monolayer after internalization. The results were rationalized in terms of an "electroporation-like" mechanism, according to which the asymmetrical distribution of the peptide between the outer and inner surfaces of the charged bilayer causes a transmembrane electrical field, which alters the lateral and the curvature stress acting within the membrane. At a threshold value these effects induce internalization of penetratin presumably via inversely curved transient structures.

Black S. L., Stanley W. A., Filipp F. V., Bhairo M., Verma A., Wichmann O., Sattler M., Wilmanns M. and Schultz C. (2008) Probing lipid- and drug-binding domains with fluorescent dyes. *Bioorg. Med. Chem* **16**, 1162-1173.

Abstract: A series of 2- and 3-OH Nile red dyes was prepared in order to generate water-soluble probes that could be used to probe lipid binding to proteins. Various substitutions in positions 2-/3-, 6-, and 7-shifted wavelengths while maintaining the environmental sensitivity of Nile red. In order to increase the solubility of the dyes in aqueous solutions, we attached butyric acid groups to the 2- or 3-OH position. In addition, phenothiazine dyes, which exhibited particularly long excitation properties, were synthesized and tested for the first time. All dyes showed Stoke's shifts of 70-100 nm and changes in excitation and emission of over 100 nm, depending on the hydrophobicity of the environment. Binding studies with bovine serum albumin and the non-specific lipid transfer protein SCP2 revealed emission changes of more than 30 nm upon binding to the protein and a five-fold increase in emission intensity. Titration of the dye-loaded proteins with various lipids or drugs replaced the dye and thereby reversed the shift in wavelength intensity. This allowed us to estimate the lipid binding affinity of the investigated proteins. For SCP2, isothermal calorimetry (ITC) data verified the titration experiments. NMR titration experiments of SCP2 with Nile red 2-O-butyrac acid (1a) revealed that the dye is bound within the lipid binding pocket and competes with lipid ligands for this binding site. These results give valuable insight into lipid and drug transport by proteins outside and inside cells

Boguslavsky V., Hruba V. J., O'Brien D. F., Misicka A., and Lipkowski A. W. (2003) Effect of peptide conformation on membrane permeability. *J Pept Res* **61**, 287-297.

Abstract: The effect of peptide conformational constraint on the peptide permeation across the model membranes was examined by determining the permeability of pairs of cyclic and acyclic peptides related to c[d-Pen2, d-Pen5] enkephalin (DPDPE). The peptides were cyclized by formation of an intramolecular disulfide bridge between the second and fifth residues composed of either d-penicillamine or cysteine. In each case the acyclic peptide was three to seven times more permeable than corresponding cyclic peptide. The possibility that the differences in permeability of cyclic and acyclic peptides is based on the greater conformational freedom of the acyclic peptides in the presence of membrane was examined in more detail by isothermal titration calorimetric studies of Trp6-DPDPE and its acyclic analog. The membrane binding of the acyclic peptide is a more exothermic process than binding of its cyclic Trp6-DPDPE. The transfer of acyclic peptide from water to membrane is an enthalpy driven process, whereas the transfer of the cyclic peptide is driven by entropy.

Boots J. P., Chupin V., Killian J. A., Demel R. A., and de Kruijff B. (2001) The specificity of monoglyceride-protein interactions and mechanism of the protein induced L(beta) to coagel phase transition. *Biochim Biophys Acta* **1510**, 401-413.

Abstract: This study aims at gaining insight into the specificity and molecular mechanism of monoglyceride-protein interactions. We used beta-lactoglobulin (beta-LG) and lysozyme as model proteins and both monostearoylglycerol and monopalmitoylglycerol as defined gel phase monoglycerides. The monoglycerides were used in different combinations with the two negatively charged amphiphiles dicetylphosphate and distearylphosphate. The interactions were characterized using the monolayer technique, isothermal titration calorimetry, (2)H-nuclear magnetic resonance (NMR) using deuterium labelled monoglycerides and freeze fracture electron microscopy (EM). Our results show that lysozyme inserts efficiently into all monolayers tested, including pure monoglyceride layers. The insertion of beta-LG depends on the lipid composition of the monolayer and is promoted when the acylchains of the negatively charged amphiphile are shorter than that of the monoglyceride. The binding parameters found for the interaction of beta-LG and lysozyme with monoglyceride bilayers were generally similar. Moreover, in all cases a large exothermic binding enthalpy was observed which was found to depend on the nature of the monoglycerides but not of the proteins. (2)H-NMR and freeze fracture EM showed that this large enthalpy results from a protein mediated catalysis of the monoglyceride L(beta) to coagel phase transition. The mechanism of this phase transition consists of two steps, an initial protein mediated vesicle aggregation step which is followed by stacking and probably fusion of the bilayers.

Boots J. W., Chupin V., Killian J. A., Demel R. A., and de Kruijff B. (1999) Interaction mode specific reorganization of gel phase monoglyceride bilayers by beta-lactoglobulin. *Biochim Biophys Acta* **1420**, 241-251.

Abstract: The interaction between beta-lactoglobulin and sonicated aqueous dispersions of the gel phase forming monoglyceride monostearoylglycerol were studied using isothermal titration calorimetry, direct binding experiments, differential scanning calorimetry, leakage of a fluorescent dye and solid-state (31)P- and (2)H-NMR. In the absence of a charged amphiphile, monostearoylglycerol forms a precipitate. Under these conditions, no interaction with beta-lactoglobulin was observed. In the presence of the negatively charged amphiphile dicetylphosphate, the gel phase monostearoylglycerol formed stable and closed, probably unilamellar, vesicles with an average diameter of 465 nm. beta-Lactoglobulin interacts with these bilayer structures at pH 4, where the protein is positively charged, as well as at pH 7 where the protein is negatively charged. Under both conditions of pH, the binding affinity of beta-lactoglobulin is in the micromolar range as observed with ITC and the direct binding assay. At pH 4, two binding modes were found, one of which is determined with ITC while the direct binding assay determines the net result of both. The first binding mode is observed with ITC and is characterized by a large binding enthalpy, a decreased enthalpy of the MSG L(beta) to L(alpha) phase transition and leakage of a fluorescent dye. These characteristics are explained by a beta-lactoglobulin induced partial L(beta) to coagel phase transition that results from a specific electrostatic interaction between the protein and the charged amphiphile. This explanation is confirmed by solid-state (2)H-NMR using 1-monostearoylglycerol with a fully deuterated acyl chain. Upon interaction with beta-lactoglobulin, the isotropic signal in the (2)H-NMR spectrum of the monostearoylglycerol-dicetylphosphate mixture partially transforms into a broad anisotropic signal which could be assigned to coagel formation. The second binding mode probably results from an aspecific electrostatic attraction between the negatively charged bilayer and the positively charged protein and causes the precipitation of the dispersion. At pH 7, only the first binding mode is observed.

Bose H. S., Whittal R. M., Ran Y., Bose M., Baker B. Y. and Miller W. L. (2008) StAR-like activity and molten globule behavior of StARD6, a male germ-line protein. *Biochemistry* **47**, 2277-2288.

Abstract: The steroidogenic acute regulatory protein (StAR) belongs to a family of 15 StAR-related lipid transfer (START) domain proteins termed StARD1-StARD15. StAR (StARD1) induces adrenal and gonadal steroidogenesis by moving cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane by an unclear process that involves conformational changes that have been characterized as a molten globule transition. We expressed, purified, and assessed the activity and cholesterol-binding behavior of StARD1 and StARD3-D7, showing that StARD6 had activity equal to StARD1, whereas StARD4, D5, and D7 had little or no activity with adrenal mitochondria in vitro. Partial proteolysis examined by mass spectrometry suggests that StARD6 has a protease-sensitive C-terminus, similar to but smaller than that of StARD1. Experiments using urea denaturation, stopped-flow kinetics and measurements of mitochondrial membrane association suggests that StARD1 and StARD6 both unfold and refold slowly with similar kinetic patterns. Isothermal titration calorimetry suggests that StARD6 interacts with mitochondrial membranes as well as or better than StARD1. Computational modeling of StARD6 suggests that it has a similar fold to StARD1, with a hydrophobic sterol-binding pocket and a unique C-terminal extension. StARD6, which is expressed only in male germ-line cells, thus exhibits biological and biophysical properties that imply a role in steroidogenesis

Brandenburg K., Koch M. H., and Seydel U. (1998) Biophysical characterisation of lysozyme binding to LPS Re and lipid A. *Eur J Biochem* **258**, 686-695.

Abstract: The binding of lysozyme to bacterial deep rough mutant lipopolysaccharide (LPS) Re and to its lipid moiety lipid A, the 'endotoxic principle' of LPS, was investigated using biophysical techniques. The beta-<-->alpha gel to liquid crystalline phase transition, the nature of the functional groups of the endotoxins, the secondary structure of lysozyme, and competition with polymyxin B were studied by Fourier-transform infrared spectroscopy (FTIR); the supramolecular aggregate structure of the endotoxins was determined with synchrotron radiation X-ray diffraction and the binding stoichiometry with microcalorimetry. The results were compared with those found with zwitterionic and negatively charged phospholipids. It can clearly be shown that lysozyme binds electrostatically to charged groups of the endotoxin molecules with the consequence of acyl-chain rigidification and an initiation of a transition from inverted cubic to multilamellar structures. The binding stoichiometry of endotoxin and lysozyme is a 3:1 molar ratio for both LPS Re and lipid A, indicating a dominant binding of lysozyme to the lipid A-phosphates. This could be confirmed by the analysis of a phosphate vibration and by the use of a dephospho LPS. Parallel to lysozyme binding to endotoxin, a conformational change of the secondary structure in the protein from mainly alpha helix to more unordered structures takes place, while the residual

beta-sheet substructure does not exhibit a clear concentration dependence. Binding is found to be specific for the endotoxins since, for the zwitterionic phosphatidylcholine, no binding is observed and, for the negatively charged phosphatidylglycerol, only very weak binding is found. The results are discussed in the context of the ability of lysozyme to reduce endotoxicity.

Bringezu F., Wen S., Dante S., Hauss T., Majerowicz M. and Waring A. (2007) The insertion of the antimicrobial peptide dicynthaurin monomer in model membranes: thermodynamics and structural characterization. *Biochemistry* **46**, 5678-5686.

Abstract: This paper is focused on the thermodynamics and the structural investigation of the interaction of the antimicrobial peptide dicynthaurin monomer with model lipid membranes composed of mixtures of 1-palmitoyl-2-oleyl-glycerophosphocholine and -glycerophosphoglycerol. The thermodynamic binding parameters as obtained by isothermal titration calorimetry reveal strong binding toward the lipid model system dominated by large chemical binding constants which exceeds the electrostatic binding effects and thus suggests insertion of the amphipathic alpha-helical peptide into the hydrophobic membrane core. Circular dichroism study shows that the peptide exhibits trans-membrane alpha-helix secondary structure. Neutron diffraction measurements using partially deuterated sequences were successfully applied to determine the orientation of the peptide thus proving insertion into the hydrophobic membrane core. This insertion and the formation of higher order porelike aggregates is assumed to be the most relevant event in microbial membrane perturbation that in vivo finally leads to bacterial cell death on a fast time scale.

Carneiro F. A., Bianconi M. L., Weissmuller G., Stauffer F., and Da Poian A. T. (2002) Membrane recognition by vesicular stomatitis virus involves enthalpy-driven protein-lipid interactions. *J Virol* **76**, 3756-3764.

Abstract: Vesicular stomatitis virus (VSV) infection depends on the fusion of viral and cellular membranes, which is mediated by virus spike glycoprotein G at the acidic environment of the endosomal compartment. VSV G protein does not contain a hydrophobic amino acid sequence similar to the fusion peptides found among other viral glycoproteins, suggesting that membrane recognition occurs through an alternative mechanism. Here we studied the interaction between VSV G protein and liposomes of different phospholipid composition by force spectroscopy, isothermal titration calorimetry (ITC), and fluorescence spectroscopy. Force spectroscopy experiments revealed the requirement for negatively charged phospholipids for VSV binding to membranes, suggesting that this interaction is electrostatic in nature. In addition, ITC experiments showed that VSV binding to liposomes is an enthalpically driven process. Fluorescence data also showed the lack of VSV interaction with the vesicles as well as inhibition of VSV-induced membrane fusion at high ionic strength. Intrinsic fluorescence measurements showed that the extent of G protein conformational changes depends on the presence of phosphatidylserine (PS) on the target membrane. Although the increase in PS content did not change the binding profile, the rate of the fusion reaction was remarkably increased when the PS content was increased from 25 to 75%. On the basis of these data, we suggest that G protein binding to the target membrane essentially depends on electrostatic interactions, probably between positive charges on the protein surface and negatively charged phospholipids in the cellular membrane. In addition, the fusion is exothermic, indicating no entropic constraints to this process.

Carneiro F. A., Lapido-Loureiro P. A., Cordo S. M., Stauffer F., Weissmuller G., Bianconi M. L., Juliano M. A., Juliano L., Bisch P. M., and Poian A. T. (2006) Probing the interaction between vesicular stomatitis virus and phosphatidylserine. *Eur Biophys J* **35**, 145-154.

Abstract: The entry of enveloped animal viruses into their host cells always depends on membrane fusion triggered by conformational changes in viral envelope glycoproteins. Vesicular stomatitis virus (VSV) infection is mediated by virus spike glycoprotein G, which induces membrane fusion between the viral envelope and the endosomal membrane at the acidic environment of this compartment. In this work, we evaluated VSV interactions with membranes of different phospholipid compositions, at neutral and acidic pH, using atomic force microscopy (AFM) operating in the force spectroscopy mode, isothermal calorimetry (ITC) and molecular dynamics simulation. We found that the binding forces differed dramatically depending on the membrane phospholipid composition, revealing a high specificity of G protein binding to membranes containing phosphatidylserine (PS). In a previous work, we showed that the sequence corresponding amino acid 164 of VSV G protein was as efficient as the virus in catalyzing membrane fusion at pH 6.0. Here, we used this sequence to explore VSV-PS interaction using ITC. We

found that peptide binding to membranes was exothermic, suggesting the participation of electrostatic interactions. Peptide-membrane interaction at pH 7.5 was shown to be specific to PS and dependent on the presence of His residues in the fusion peptide. The application of the simplified continuum Gouy-Chapman theory to our system predicted a pH of 5.0 at membrane surface, suggesting that the His residues should be protonated when located close to the membrane. Molecular dynamics simulations suggested that the peptide interacts with the lipid bilayer through its N-terminal residues, especially Val(145) and His(148).

Coulon A., Mosbah A., Lopez A., Sautereau A. M., Schaller G., Urech K., Rouge P., and Darbon H. (2003) Comparative membrane interaction study of viscotoxins A3, A2 and B from mistletoe (*Viscum album*) and connections with their structures. *Biochem J* **374**, 71-78.

Abstract: Viscotoxins A2 (VA2) and B (VB) are, together with viscotxin A3 (VA3), among the most abundant viscotxin isoforms that occur in mistletoe-derived medicines used in anti-cancer therapy. Although these isoforms have a high degree of amino-acid-sequence similarity, they are strikingly different from each other in their in vitro cytotoxic potency towards tumour cells. First, as VA3 is the only viscotxin whose three-dimensional (3D) structure has been solved to date, we report the NMR determination of the 3D structures of VA2 and VB. Secondly, to account for the in vitro cytotoxicity discrepancy, we carried out a comparative study of the interaction of the three viscotoxins with model membranes. Although the overall 3D structure is highly conserved among the three isoforms, some discrete structural features and associated surface properties readily account for the different affinity and perturbation of model membranes. VA3 and VA2 interact in a similar way, but the weaker hydrophobic character of VA2 is thought to be mainly responsible for the apparent different affinity towards membranes. VB is much less active than the other two viscotoxins and does not insert into model membranes. This could be related to the occurrence of a single residue (Arg25) protruding outside the hydrophobic plane formed by the two amphipathic alpha-helices, through which viscotoxins are supposed to interact with plasma membranes.

Da Poian A.T., Carneiro F.A., and Stauffer F. (2005) Viral membrane fusion: is glycoprotein G of rhabdoviruses a representative of a new class of viral fusion proteins? *Braz J Med Biol Res.* **38**, 813-23.
Abstract: Enveloped viruses always gain entry into the cytoplasm by fusion of their lipid envelope with a cell membrane. Some enveloped viruses fuse directly with the host cell plasma membrane after virus binding to the cell receptor. Other enveloped viruses enter the cells by the endocytic pathway, and fusion depends on the acidification of the endosomal compartment. In both cases, virus-induced membrane fusion is triggered by conformational changes in viral envelope glycoproteins. Two different classes of viral fusion proteins have been described on the basis of their molecular architecture. Several structural data permitted the elucidation of the mechanisms of membrane fusion mediated by class I and class II fusion proteins. In this article, we review a number of results obtained by our laboratory and by others that suggest that the mechanisms involved in rhabdovirus fusion are different from those used by the two well-studied classes of viral glycoproteins. We focus our discussion on the electrostatic nature of virus binding and interaction with membranes, especially through phosphatidylserine, and on the reversibility of the conformational changes of the rhabdovirus glycoprotein involved in fusion. Taken together, these data suggest the existence of a third class of fusion proteins and support the idea that new insights should emerge from studies of membrane fusion mediated by the G protein of rhabdoviruses. In particular, the elucidation of the three-dimensional structure of the G protein or even of the fusion peptide at different pH's might provide valuable information for understanding the fusion mechanism of this new class of fusion proteins.

Dathe M., Nikolenko H., Klose J., and Bienert M. (2004) Cyclization increases the antimicrobial activity and selectivity of arginine- and tryptophan-containing hexapeptides. *Biochemistry* **43**, 9140-9150.

Abstract: Arginine- and tryptophan-rich motifs have been identified in antimicrobial peptides with various secondary structures. We synthesized a set of linear hexapeptides derived from the sequence AcRRWRF-NH₂ by substitution of tryptophan (W) by tyrosine (Y) or naphthylalanine (Nal) and by replacement of arginine (R) by lysine (K) to investigate the role of cationic charge and aromatic residues in membrane activity and selectivity. A second set of corresponding head-to-tail cyclic analogues was prepared to analyze the role of conformational constraints. The biological activity of the linear peptides followed the order Nal- >> W- > Y-containing compounds and slightly decreased upon R-K substitution. A pronounced activity-improving and bacterial selectivity-enhancing effect was found upon cyclization of the R- and W-

bearing parent peptide, whereas the activity-modifying effect of cyclization of Y- and Nal-containing peptides was low. The analysis of the driving forces of peptide interaction with model membranes showed that the activities correlated with the partition coefficients and the depths of peptide insertion into neutral and negatively charged lipid bilayers. Spectroscopic studies, RP-HPLC, and titration calorimetry implied that the combination of cationic and aromatic amino acid composition and conformational rigidity afforded a membrane-active, amphipathic structure with a highly charged face opposed by a cluster of aromatic side chains. However, threshold values of low and high hydrophobicity seemed to exist beyond which the activity-enhancing effect of cyclization was negligible. The results suggest that cyclization of small peptides of an appropriate amino acid composition may serve as a promising strategy in the design of antimicrobial peptides.

de Araujo P. S., Rosseneu M. Y., Kremer J. M., van Zoelen E. J., and de Haas G. H. (1979) Structure and thermodynamic properties of the complexes between phospholipase A2 and lipid micelles. *Biochemistry* **18**, 580-586.

Abstract: The interaction between porcine pancreatic phospholipase A2 and a homogeneous population of micelles of the substrate analogue n-hexadecylphosphorylcholine containing 155 lipid monomers was studied by light scattering, equilibrium gel filtration, and isothermal calorimetry. From the detergent/protein molar ratio and the equivalent "molecular weight" of the resulting complex it is concluded that insertion of the enzyme into the detergent micelle results in a protein--detergent complex containing two phospholipase A2 molecules and 80 lipid monomers at 25 degrees C. The affinity constants and complex composition have been determined at different temperatures, allowing calculation of the thermodynamic parameters of the binding process. It is concluded that the interaction of phospholipase A2 with micellar lipids is predominantly hydrophobic.

Derksen A., Gantz D., and Small D. M. (1996) Calorimetry of apolipoprotein-A1 binding to phosphatidylcholine-triolein-cholesterol emulsions. *Biophys J* **70**, 330-338.

Abstract: The thermotropic properties of triolein-rich, low-cholesterol dipalmitoyl phosphatidylcholine (DPPC) emulsion particles with well-defined chemical compositions (approximately 88% triolein, 1% cholesterol, 11% diacyl phosphatidylcholine) and particle size distributions (mean diameter, approximately 1000-1100 Å) were studied in the absence and presence of apolipoprotein-A1 by a combination of differential scanning and titration calorimetry. The results are compared to egg yolk PC emulsions of similar composition and size. Isothermal titration calorimetry at 30 degrees C was used to saturate the emulsion surface with apo-A1 and rapidly quantitate the binding constants (affinity $K_a = 11.1 \pm 3.5 \times 10^6 \text{ M}^{-1}$ and capacity $N = 1.0 \pm 0.09$ apo-A1 per 1000 DPPC) and heats of binding (enthalpy $H = -940 \pm 35 \text{ kcal mol}^{-1}$ apo-A1 or $-0.92 \pm 0.12 \text{ kcal mol}^{-1}$ DPPC). The entropy of association is $-3070 \text{ cal deg}^{-1} \text{ mol}^{-1}$ protein or $-3 \text{ cal deg}^{-1} \text{ mol}^{-1}$ DPPC. Without protein on the surface, the differential scanning calorimetry heating curve of the emulsion showed three endothermic transitions at 24.3 degrees C, 33.0 degrees C, and 40.0 degrees C with a combined enthalpy of $1.53 \pm 0.2 \text{ kcal mol}^{-1}$ DPPC. With apo-A1 on the surface, the heating curve showed the three transitions more clearly, in particular, the second transition became more prominent by significant increases in both the calorimetric and Van't Hoff enthalpies. The combined enthalpy was $2.70 \pm 0.12 \text{ kcal mol}^{-1}$ DPPC and remained constant upon repeated heating and cooling. Indicating that the newly formed DPPC emulsion-Apo-A1 complex is thermally reversible during calorimetry. Thus there is an increase in ΔH of $1.17 \text{ kcal mol}^{-1}$ DPPC after apo-A1 is bound, which is roughly balanced by the heat released during binding (-0.92 kcal) of apo-A1. The melting entropy increase, $+3.8 \text{ cal deg}^{-1} \text{ mol}^{-1}$ DPPC of the three transitions after apo-A1 binds, also roughly balances the entropy ($-3 \text{ cal deg}^{-1} \text{ mol}^{-1}$ DPPC) of association of apo-A1. These changes indicate that apo-A1 increases the amount of ordered gel-like phase on the surface of DPPC emulsions when added at 30 degrees C. From the stoichiometry of the emulsions we calculate that the mean area of DPPC at the triolein/DPPC interface is 54.5 Å^2 at 41 degrees C and 54.2 Å^2 at 30 degrees C. The binding of apo-A1 at 30 degrees C to the emulsion reduces the surface area per DPPC molecule from 54.2 Å^2 to 50.8 Å^2 . At 30 degrees apo-A1 binds with high affinity and low capacity to the surface of DPPC emulsions and increases the packing density of the lipid domain to which it binds. Apo-A1 was also titrated onto DPPC emulsions at 45 degrees C. This temperature is above the gel liquid crystal transition. No heat was released or adsorbed. Furthermore, egg yolk phosphatidylcholine emulsions of nearly identical composition were also titrated at 30 degrees C with apo-A1 and were eutermic. Association constants were previously measured using a classical centrifugation assay and were used to calculate the entropy of apo-A1 binding ($+28 \text{ cal deg}^{-1} \text{ mol}^{-1}$

apo-A1). This value indicates that apo-A1 binding to a fluid surface like egg yolk phosphatidylcholine or probably DPPC at 45 degrees C is hydrophobic and is consistent with hydrocarbon lipid or protein moieties coming together and excluding water. Thus the binding of apo-A1 to partly crystalline surfaces is entropically negative and increases the order of the already partly ordered phases, whereas binding to liquid surfaces is mainly an entropically driven hydrophobic process.

Dimitrova M. N., Matsumura H., Dimitrova A., and Neitchev V. Z. (2000) Interaction of albumins from different species with phospholipid liposomes. Multiple binding sites system. *Int J Biol Macromol* **27**, 187-194.

Abstract: The interactions of three serum albumin species (rat, human, and bovine) with liposomes containing dimyristoylphosphatidylcholine, distearoylphosphatidylcholine or mixtures of both under different membrane fluidity conditions have been investigated using isothermal titration calorimetry and steady-state fluorescence anisotropy. Calorimetric titration studies of the binding of liposomes to the albumin species indicate in all cases exothermic processes with multiple sites of binding in the albumin molecules. Distinct saturation of the protein-lipid binding processes was observed at low or high molar lipid/protein ratio depending on the particular system. The thermodynamic parameters, including the association enthalpy and entropy, and the optimal values for the binding constants were thoroughly varied as a function of the number of identical binding sites, defining the best value of the parameter. Our experimental results, obtained using complementary biophysical techniques, provide experimental evidence for a significant difference in the association of the three protein species to phospholipid membranes. These observations also suggest a close relation between the binding parameters of the protein/lipid association and the lipid state of the phospholipid membranes.

Douliez J. P., Jegou S., Pato C., Molle D., Tran V., and Marion D. (2001) Binding of two mono-acylated lipid monomers by the barley lipid transfer protein, LTP1, as viewed by fluorescence, isothermal titration calorimetry and molecular modelling. *Eur J Biochem* **268**, 384-388.

Abstract: The binding of two mono-acylated lipid monomers by plant lipid transfer proteins (LTP1s) presents an attractive field of research that could help our understanding of the functional role of this protein family. This task has been investigated in the case of barley LTP1 because it is known to exhibit a small cavity in its free state. The titration with lipids could not be followed by fluorescence with the native protein. Indeed, this LTP1 possesses a tyrosine residue on its C-terminus, Tyr91, which is not sensitive to lipid binding but mainly contributes to the fluorescence signal intensity. However, the binding of 1-myristoylglycerophosphatidylcholine (MyrGro-PCho) could be monitored by fluorescence after removal of Tyr91 by a carboxypeptidase. These experiments returned a dissociation constant of about 1 μ M and showed that the protein can indeed bind two monomers. This result was corroborated by molecular modelling where the structure of the complex between barley LTP1 and MyrGro-PCho was derived from that determined in the case of wheat [Charvolin, D., Douliez, J.P., Marion, D., Cohen-addad, C. & Pebay-Peyroula, E. (1999) *Eur. J. Biochem.* 264, 562-568.]. Results from isothermal titration calorimetry experiments indicated non-classic titration behaviour but also suggested that two lipids could be bound by the protein. Finally, barley LTP1 binds two omega-hydroxypalmitic acid, a compound found in the family of cutin monomers. The fact that the binding of two lipids could be related to the physiological role of this protein family is discussed.

egre-Cebollada J., Cunietti M., Herrero-Galan E., Gavilanes J. G. and Martinez-del-Pozo A. (2008) Calorimetric scrutiny of lipid binding by sticholysin II toxin mutants. *J Mol Biol* **382**, 920-930.

Abstract: The mechanisms by which pore-forming toxins are able to insert into lipid membranes are a subject of the highest interest in the field of lipid-protein interaction. Eight mutants affecting different regions of sticholysin II, a member of the pore-forming actinoporin family, have been produced, and their hemolytic and lipid-binding properties were compared to those of the wild-type protein. A thermodynamic approach to the mechanism of pore formation is also presented. Isothermal titration calorimetry experiments show that pore formation by sticholysin II is an enthalpy-driven process that occurs with a high affinity constant (1.7×10^8 M⁻¹). Results suggest that conformational flexibility at the N-terminus of the protein does not provide higher affinity for the membrane, although it is necessary for correct pore formation. Membrane binding is achieved through two separate mechanisms, that is, recognition of the lipid-water interface by a cluster of aromatic residues and additional specific interactions that include a

phosphocholine-binding site. Thermodynamic parameters derived from titration experiments are discussed in terms of a putative model for pore formation

Ek B. A., Cistola D. P., Hamilton J. A., Kaduce T. L., and Spector A. A. (1997) Fatty acid binding proteins reduce 15-lipoxygenase-induced oxygenation of linoleic acid and arachidonic acid. *Biochim Biophys Acta* **1346**, 75-85.

Abstract: Free fatty acids in plasma and cells are mainly bound to membranes and proteins such as albumin and fatty acid binding proteins (FABP), which can regulate their biological activities and metabolic transformations. We have investigated the effect of FABP and albumin on the peroxidation of linoleic acid (18:2) and arachidonic acid (20:4) by 15-lipoxygenase (15-LO). Rabbit reticulocyte 15-LO produced a rapid conversion of [1-¹⁴C]18:2 to 13-hydroxyoctadecadienoic acid (13-HODE) and [3H]20:4 to 15-hydroxyicosatetraenoic acid (15-HETE). 13-HODE formation was reduced when intestinal FABP (I-FABP), liver FABP (L-FABP) or albumin was added. The relative ability of these proteins to reduce 15-LO induced formation of 13-HODE and 15-HETE was BSA > L-FABP > I-FABP. Smaller reductions in activity were observed with 20:4 as compared to 18:2. The IC₅₀-values of I-FABP and L-FABP, using either 18:2 (3.4 μM) or 20:4 (3.4 μM), were 4.6 +/- 0.6 and 1.9 +/- 0.2 μM, respectively, for reduction of 13-HODE and 6.8 +/- 0.3 and 3.1 +/- 0.2 μM, respectively, for reduction of 15-HETE formation. The smaller 15-HETE reduction correlated with decreased binding of 20:4 to the FABP. Titration calorimetry also showed that the I-FABP IC₅₀ for 18:2, 0.25 μM, was lower than for 20:4, 0.6 μM. Thus the reduction in fatty acid lipid peroxidation relates to the binding capacity of each FABP. We also demonstrated that 18:2 rapidly diffuses (flip-flops) across the phospholipid bilayer of small unilamellar vesicles (SUV) and measured partitioning of 18:2 between proteins and SUV by the pyranin fluorescence method [Kamp, F. and Hamilton, J.A. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89, 11367-11370]. Addition of proteins to SUV in buffer resulted in a complete desorption of 18:2 from SUV with a relative effect of BSA > L-FABP > I-FABP. This suggests that the relative effects of these proteins on 18:2 peroxidation will not be altered by the presence of membranes. Our results indicate that FABPs protect intracellular polyunsaturated fatty acids against peroxidation and, through differential binding of 18:2 and 20:4, they may modulate the availability of these polyunsaturated fatty acids to intracellular oxidative pathways.

Epanand R. F., Lehrer R. I., Waring A., Wang W., Maget-Dana R., Lelievre D., and Epanand R. M. (2003) Direct comparison of membrane interactions of model peptides composed of only Leu and Lys residues. *Biopolymers* **71**, 2-16.

Abstract: We compared the properties of two peptides of identical size and amino acid composition, Ac-(LKKL)(5)-NH₂ and Ac-(KL)(10)-NH₂. Both are amphipathic, but only Ac-(LKKL)(5)-NH₂ is a potent promoter of negative curvature. CD studies performed in the presence of lipids confirmed that under these conditions Ac-(LKKL)(5)-NH₂ forms an alpha-helix, and Ac-(KL)(10)-NH₂ adopts a beta structure. We studied their binding affinity by centrifugation and isothermal titration calorimetry techniques. The Ac-(LKKL)(5)-NH₂ bound to zwitterionic and anionic liposomes, while Ac-(KL)(10)-NH₂ interacted mainly with anionic liposomes. Ac-(LKKL)(5)-NH₂ was more lytic than Ac-(KL)(10)-NH₂ for zwitterionic palmitoylcholine phosphatidylcholine (POPC) liposomes, and for liposomes composed of lipids extracted from either sheep or human erythrocytes (RBC). Both peptides had similar lytic and lipid mixing activities for liposomes containing anionic lipids. Both peptides were highly hemolytic, with Ac-(LKKL)(5)-NH₂ active against sheep RBC and Ac-(KL)(10)-NH₂ more active against human RBC. From their respective minimal effective concentrations (MECs) as antimicrobial agents, we judged Ac-(KL)(10)-NH₂ to be 2 to 5-fold more potent than Ac-(LKKL)(5)-NH₂ in media that contained physiological concentrations of NaCl. Notwithstanding, both peptides had MECs <1 μg/mL for *Escherichia coli* and *Pseudomonas aeruginosa* and <4 μg/mL for *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*. Although selectivity of antimicrobial peptides for bacterial membranes may result, in part, from the preferential display of anionic residues in these membranes, inability to interact with or bind to zwitterionic phospholipids offers no guarantee that the peptide will lack appreciable cytotoxicity for host cells.

Epanand R. M., Segrest J. P., and Anantharamaiah G. M. (1990) Thermodynamics of the binding of human apolipoprotein A-I to dimyristoylphosphatidylglycerol. *J Biol Chem* **265**, 20829-20832.

Abstract: The interaction of human serum apolipoprotein A-I with dimyristoylphosphatidylglycerol was analyzed by isothermal titration calorimetry. Binding of the apolipoprotein A-I to large unilamellar vesicles of dimyristoylphosphatidylglycerol, a negatively charged phospholipid, is characterized by thermodynamic

parameters which are invariant over the 30-40 degrees C temperature range. The enthalpy change resulting from the first additions of lipid are positive and decline in magnitude with subsequent additions of lipid. After several additions of lipid, the sign of the enthalpy changes to negative and then reaches a constant value/injection. This exothermic process is larger and opposite in sign to the heat of dilution. Similar behavior is also observed when the lipid is in the form of a dispersion in distilled water. Only a non-saturable exothermic process is observed at 30 degrees C with large unilamellar vesicles of the zwitterionic lipid, dimyristoylphosphatidylcholine. The beginning of an exothermic process can also be observed prior to the larger endotherm in the first injections of large unilamellar vesicles of dimyristoylphosphatidylglycerol into the protein. We analyze the enthalpy changes for the reaction of dimyristoylphosphatidylglycerol with the protein as arising from two distinct processes, one endothermic and the other exothermic. The binding isotherms for the high affinity binding of the apolipoprotein A-I to large unilamellar vesicles of dimyristoylphosphatidylglycerol, over the temperature range 30-40 degrees C, gave an enthalpy change of 1.43 +/- 0.07 kcal/mol of protein and a free energy change of -5.91 +/- 0.04 kcal/mol of protein for the binding of the protein to a cluster of 25 +/- 2 lipid molecules. Thus this reaction is entropically driven.

Fang Y., Tong G. C., and Means G. E. (2006) Structural changes accompanying human serum albumin's binding of fatty acids are concerted. *Biochim Biophys Acta* **1764**, 285-291.

Abstract: Long chain fatty acids (LCFAs), a major source of cellular energy, are solubilized and transported in the blood by binding to serum albumin. Changes in human serum albumin's (HSA's) UV absorption and characteristic reactivity with pyridoxal-5'-phosphate appear to reflect a concerted change in its structure upon binding five equivalents of myristate. Isothermal titrations with myristate and other LCFA anions are also consistent with the presence of five strong, interacting, binding sites. Although HSA is usually thought to have many independent LCFA anion binding sites, just five interacting sites appear to account for the changes in structure that accompany its binding of myristate.

Franklin C. L., Li H., and Martin S. F. (2003) Design, synthesis, and evaluation of water-soluble phospholipid analogues as inhibitors of phospholipase C from *Bacillus cereus*. *J Org Chem* **68**, 7298-7307.

Abstract: The rate of hydrolysis of natural phospholipids by the phosphatidylcholine-preferring phospholipase C from *Bacillus cereus* (PLC(Bc)) follows the order phosphatidylcholine > phosphatidylethanolamine >> phosphatidyl-l-serine. To probe the structural basis for this substrate specificity, a series of water-soluble, nonhydrolyzable substrate analogues were needed so their complexes with the enzyme could be studied via X-ray crystallography and isothermal titration calorimetry (ITC). Accordingly the water-soluble dithiophospholipids 2-10 having choline, ethanolamine, and l-serine headgroups were synthesized, and the inhibitory activity of each was determined in an assay using 1,2-dihexanoyl-sn-glycero-3-phosphocholine (C6PC) as the monomeric substrate. The 1,2-dibutanoyl dithiophosphocholine 2 was a weak inhibitor, whereas the related 1,2-dipentanoyl dithiophosphocholine 3 and the ethylene glycol dithiophosphocholines 4 and 5 were moderate inhibitors. The 1,2-omega-hydroxydiacyl dithiophosphocholines 6 and 7 were potent inhibitors, while the related compound 8, which had shorter acyl side chains, was a weak inhibitor. The dithiophosphoethanolamine 9 was a modest inhibitor, whereas the dithiophospho-l-serine 10 was a somewhat weaker inhibitor. Overall, the phospholipid analogues had increasing K(i) values according to the order 2 << 10 < 3 < 4 approximately 5 approximately 8 < 9 << 6 << 7 and increasing solubility according to the sequence 5 approximately 7 < 4 approximately 6 approximately 9 < 3 < 10 < 8 < 2.

Gazzara J. A., Phillips M. C., Lund-Katz S., Palgunachari M. N., Segrest J. P., Anantharamaiah G. M., Rodriguez W. V., and Snow J. W. (1997) Effect of vesicle size on their interaction with class A amphipathic helical peptides. *J Lipid Res* **38**, 2147-2154.

Abstract: Throughout the life span of a lipoprotein particle, the type and number of exchangeable apolipoproteins on its surface varies with particle size, suggesting a role of surface curvature on the lipid-binding properties of these proteins. Peptides 18A, Ac-18A-NH₂, Ac-18R-NH₂, 37pA, and 37aA have been designed to investigate the lipid-binding properties of the amphipathic alpha-helix structural motif that appears to modulate the lipid-binding properties of the exchangeable plasma apolipoproteins. We report here the results of a quantitative thermodynamic characterization of the effects of modifying helix length and of varying both the location of charged residues about the polar face of the peptides and vesicle size on the lipid affinity and depth of bilayer penetration for model amphipathic alpha-helices. Partition

coefficients, K_p , were determined by fluorescence spectroscopy, and binding enthalpies, ΔH , by titration calorimetry. The results indicate that K_p values are on the order of 10^5 , with similar ΔG_0 values for the interactions of the peptides with vesicles of various sizes. It appears that a class A motif and increased alpha-helical content optimize binding for 18-residue peptides. The interactions of the model peptides with 20 nm SUV are enthalpically driven with small, negative entropy changes; however, interactions for larger vesicles are entropically driven, likely due to disordering of bilayer hydrocarbon chains. Thermodynamic data indicate that 37pA and 37aA induce greater disordering of bilayer hydrocarbon chains than Ac-18A-NH2. The results of this study suggest that the type of interaction, i.e., enthalpically or entropically driven, may be modulated by the lateral compressibility of the bilayer membrane.

Gazzara J. A., Phillips M. C., Lund-Katz S., Palgunachari M. N., Segrest J. P., Anantharamaiah G. M., and Snow J. W. (1997) Interaction of class A amphipathic helical peptides with phospholipid unilamellar vesicles. *J Lipid Res* **38**, 2134-2146.

Abstract: The exchangeable apolipoproteins are important in determining the structure/function properties of lipoproteins. These proteins typically contain varying amounts of amphipathic helices. Five model peptides, 18A, Ac-18A-NH2, Ac-18R-NH2, 37pA, and 37aA, have been designed to investigate variations of the amphipathic alpha-helix structural motif on their lipid-binding properties. These include the 18-residue peptides, 18A and Ac-18A-NH2, examples of class A helices, and Ac-18R-NH2, which has the positions of acidic and basic residues interchanged relative to 18A. Three larger peptides were also studied: 36A, a dimer of 18A, 37pA and 37aA, dimers of 18A coupled by Pro (18A-Pro-18A) and Ala (18A-Ala-18A), respectively. We report here the results of a thermodynamic characterization of the binding properties of these peptides to small unilamellar vesicles of POPC. Partition coefficients, K_p , were determined by fluorescence spectroscopy and binding enthalpies, ΔH , by titration calorimetry. These parameters were used to obtain the free energies, ΔG_0 , and entropies, ΔS_0 , of binding. The results of this study indicate K_p values on the order of 10^5 , with interactions being enthalpically but not entropically favored in all cases. The presence of positively charged residues at the interface (18A and Ac-18A-NH2) enhances binding but has little effect on the extent of bilayer penetration. The presence of tandem repeats decreases lipid affinities for these small, highly curved bilayers. Our results are consistent with the idea that interaction appears to be confined largely to the surface, with some degree of penetration of the hydrophobic face of the helix into the interior of the bilayer.

Golovko M. Y., Faergeman N. J., Cole N. B., Castagnet P. I., Nussbaum R. L., and Murphy E. J. (2005) alpha-Synuclein Gene Deletion Decreases Brain Palmitate Uptake and Alters the Palmitate Metabolism in the Absence of alpha-Synuclein Palmitate Binding. *Biochemistry* **44**, 8251-8259.

Abstract: alpha-Synuclein is an abundant protein in the central nervous system that is associated with a number of neurodegenerative disorders, including Parkinson's disease. Its physiological function is poorly understood, although recently it was proposed to function as a fatty acid binding protein. To better define a role for alpha-synuclein in brain fatty acid uptake and metabolism, we infused awake, wild-type, or alpha-synuclein gene-ablated mice with [1-(14)C]palmitic acid (16:0) and assessed fatty acid uptake and turnover kinetics in brain phospholipids. alpha-Synuclein deficiency decreased brain 16:0 uptake 35% and reduced its targeting to the organic fraction. The incorporation coefficient for 16:0 entering the brain acyl-CoA pool was significantly decreased 36% in alpha-synuclein gene-ablated mice. Because incorporation coefficients alone are not predictive of fatty acid turnover in individual phospholipid classes, we calculated kinetic values for 16:0 entering brain phospholipid pools. alpha-Synuclein deficiency decreased the incorporation rate and fractional turnover of 16:0 in a number of phospholipid classes, but also increased the incorporation rate and fractional turnover of 16:0 in the choline glycerophospholipids. No differences in incorporation rate or turnover were observed in liver phospholipids, confirming that these changes in lipid metabolism were brain specific. Using titration microcalorimetry, we observed no binding of 16:0 or oleic acid to alpha-synuclein in vitro. Thus, alpha-synuclein has effects on 16:0 uptake and metabolism similar to those of an FABP, but unlike FABP, it does not directly bind 16:0; hence, the mechanism underlying these effects is different from that of a classical FABP.

Goncalves E., Kitas E., and Seelig J. (2005) Binding of oligoarginine to membrane lipids and heparan sulfate: structural and thermodynamic characterization of a cell-penetrating peptide. *Biochemistry* **44**, 2692-2702.

Abstract: Cell-penetrating peptides (CPPs) comprise a group of arginine-rich oligopeptides that are able to

deliver exogenous cargo into cells. A first step in the internalization of CPPs is their binding to the cell surface, a reaction likely to involve membrane phospholipids and/or heparan sulfate proteoglycans (HSPGs). The present work characterizes the interaction of R(9), one of the most efficient CPPs, with either heparan sulfate (HS) or lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylglycerol (POPG). Isothermal titration calorimetry shows that R(9) binds to HS with high affinity. Assuming that HS has n independent and equivalent binding sites for R(9), we find an association constant of $3.1 \times 10^6 \text{ M}^{-1}$ at 28 degrees C. At this temperature, the reaction enthalpy is $\Delta H(\text{degrees})_{\text{pep}} = -5.5 \text{ kcal/mol}$ and approximately 7 R(9) molecules bind per HS chain, which is equivalent to approximately 0.95 cationic/anionic charge ratio. ΔH decreases in magnitude upon an increase in temperature, and the reaction becomes entropy-driven at higher temperatures (≥ 37 degrees C). The positive heat-capacity change entailed by this reaction ($\Delta C(\text{degrees})_{\text{P}} = +167 \text{ cal mol}^{-1} \text{ K}^{-1}$) indicates the loss of polar residues on R(9)-HS binding, suggesting that hydrophobic forces play no major role on binding. Calorimetric analysis of the interaction of R(9) with POPC/POPG (75:25) vesicles reveals an association constant of $8.2 \times 10^4 \text{ M}^{-1}$ at 28 degrees C. Using a surface partition equilibrium model to correct for electrostatic effects, we find an intrinsic partition constant of approximately 900 M^{-1} , a value that is also confirmed by electrophoretic mobility measurements. This corresponds to an electrostatic contribution of approximately 33% to the total free energy of binding. Deuterium nuclear magnetic resonance (NMR) shows no change in the headgroup conformation of POPC and POPG, suggesting that binding takes place at some distance from the plane of the polar groups. ^{31}P NMR indicates that the lipid bilayer remains intact upon R(9) binding. The fact that R(9) binds with greater affinity to HS than to anionic lipid vesicles makes the former molecule a more likely target in binding this CPP to the cell surface.

Guerrero-Valero M., Marin-Vicente C., Gomez-Fernandez J. C. and Corbalan-Garcia S. (2007) The C2 domains of classical PKCs are specific PtdIns(4,5)P₂-sensing domains with different affinities for membrane binding. *J Mol Biol* **371**, 608-621.

Abstract: C2 domains are conserved protein modules in many eukaryotic signaling proteins, including the protein kinase (PKCs). The C2 domains of classical PKCs bind to membranes in a Ca²⁺-dependent manner and thereby act as cellular Ca²⁺ effectors. Recent findings suggest that the C2 domain of PKC α interacts specifically with phosphatidylinositols 4,5-bisphosphate (PtdIns(4,5)P₂) through its lysine rich cluster, for which it shows higher affinity than for POPS. In this work, we compared the three C2 domains of classical PKCs. Isothermal titration calorimetry revealed that the C2 domains of PKC α and β display a greater capacity to bind to PtdIns(4,5)P₂-containing vesicles than the C2 domain of PKC γ . Comparative studies using lipid vesicles containing both POPS and PtdIns(4,5)P₂ as ligands revealed that the domains behave as PtdIns(4,5)P₂-binding modules rather than as POPS-binding modules, suggesting that the presence of the phosphoinositide in membranes increases the affinity of each domain. When the magnitude of PtdIns(4,5)P₂ binding was compared with that of other polyphosphate phosphatidylinositols, it was seen to be greater in both PKC β - and PKC γ -C2 domains. The concentration of Ca²⁺ required to bind to membranes was seen to be lower in the presence of PtdIns(4,5)P₂ for all C2 domains, especially PKC α . In vivo experiments using differentiated PC12 cells transfected with each C2 domain fused to ECFP and stimulated with ATP demonstrated that, at limiting intracellular concentration of Ca²⁺, the three C2 domains translocate to the plasma membrane at very similar rates. However, the plasma membrane dissociation event differed in each case, PKC α persisting for the longest time in the plasma membrane, followed by PKC γ and, finally, PKC β , which probably reflects the different levels of Ca²⁺ needed by each domain and their different affinities for PtdIns(4,5)P₂.

Han X. and Tamm L. K. (2000) A host-guest system to study structure-function relationships of membrane fusion peptides. *Proc Natl Acad Sci U S A* **97**, 13097-13102.

Abstract: We designed a host-guest fusion peptide system, which is completely soluble in water and has a high affinity for biological and lipid model membranes. The guest sequences are those of the fusion peptides of influenza hemagglutinin, which are solubilized by a highly charged unstructured C-terminal host sequence. These peptides partition to the surface of negatively charged liposomes or erythrocytes and elicit membrane fusion or hemolysis. They undergo a conformational change from random coil to an obliquely inserted (approximately 33 degrees from the surface) α -helix on binding to model membranes. Partition coefficients for membrane insertion were measured for influenza fusion peptides of

increasing lengths (n = 8, 13, 16, and 20). The hydrophobic contribution to the free energy of binding of the 20-residue fusion peptide at pH 5.0 is -7.6 kcal/mol (1 cal = 4.18 J). This energy is sufficient to stabilize a "stalk" intermediate if a typical number of fusion peptides assemble at the site of membrane fusion. The fusion activity of the fusion peptides increases with each increment in length, and this increase strictly correlates with the hydrophobic binding energy and the angle of insertion.

Hanakam F., Gerisch G., Lotz S., Alt T., and Seelig A. (1996) Binding of hisactophilin I and II to lipid membranes is controlled by a pH-dependent myristoyl-histidine switch. *Biochemistry* **35**, 11036-11044.
Abstract: The interaction of the two N-terminally myristoylated isoforms of Dictyostelium hisactophilin with lipid model membranes was investigated by means of the monolayer expansion method and high-sensitivity titration calorimetry. The two isoforms, hisactophilin I and hisactophilin II, were found to insert with their N-terminal myristoyl residue into an electrically neutral POPC monolayer corresponding in its lateral packing density to that of a lipid bilayer. The partition coefficient for this insertion process was $K_p = (1.1 \pm 0.2) \times 10^4 \text{ M}^{-1}$. The area requirement of the protein in the lipid membrane was estimated as $44 \pm 6 \text{ \AA}^2$ which corresponds to the cross sectional area of the myristoyl moiety with an additional small contribution from amino acid side chains. The interaction of hisactophilin I (hisactophilin II) with negatively charged membrane surfaces is modulated in a pH-dependent manner by charged amino acid residues clustered around the myristoyl moiety. The electrostatic binding site consists of three lysine (one arginine and two lysine), seven (nine) histidine, and four (four) glutamic acid residues and has an isoelectric point of 6.9 (7.1). For small unilamellar POPC/POPG (75/25 mole/mole) vesicles, an apparent binding constant, $K_{app} = (8 \pm 1) \times 10^5 \text{ M}^{-1}$, was measured at pH 6.0 by means of high-sensitivity titration calorimetry. Electrostatic interactions hence increase the binding constant by about 2 orders of magnitude compared to hydrophobic binding alone. With increasing pH, the electrostatic attraction decreases and turns into an electrostatic repulsion at $\text{pH} > 7.0 \pm 0.1$. The area occupied by the cluster of charged residues constituting the membrane binding region was $280 \pm 20 \text{ \AA}^2$ as derived from monolayer measurements in close agreement with molecular modeling data derived from the NMR structure of hisactophilin I [Habazettl et al. (1992) *Nature* 359, 855-858].

Hanhoff T., Lucke C., and Spener F. (2002) Insights into binding of fatty acids by fatty acid binding proteins. *Mol Cell Biochem* **239**, 45-54.

Abstract: Members of the phylogenetically related intracellular lipid binding protein (iLBP) are characterized by a highly conserved tertiary structure, but reveal distinct binding preferences with regard to ligand structure and conformation, when binding is assessed by the Lipidex method (removal of unbound ligand by hydrophobic polymer) or by isothermal titration calorimetry, a true equilibrium method. Subfamily proteins bind retinoids, subfamily II proteins bind bulky ligands, examples are intestinal bile acid binding protein (I-BABP) and liver fatty acid binding protein (L-FABP) which binds 2 ligand molecules, preferably monounsaturated and n-3 fatty acids. Subfamily III intestinal fatty acid binding protein (I-FABP) binds fatty acid in a bent conformation. The fatty acid bound by subfamily IV FABPs has a U-shaped conformation; here heart (H-) FABP preferably binds n-6, brain (B-) FABP n-3 fatty acids. The ADIFAB-method is a fluorescent test for fatty acid in equilibrium with iLBP and reveals some correlation of binding affinity to fatty acid solubility in the aqueous phase; these data are often at variance with those obtained by the other methods. Thus, in this review published binding data are critically discussed, taking into account on the one hand binding increments calculated for fatty acid double bonds on the basis of the 'solubility' hypothesis, on the other hand the interpretation of calorimetric data on the basis of crystallographic and solution structures of iLBPs.

Heerklotz H. (2004) The microcalorimetry of lipid membranes. *J. Phys.: Condens. Matter* **16** R441-R467.

Abstract: Insight into the forces governing a system is essential for understanding its behaviour and function. Calorimetric investigations provide a wealth of information that is not, or is hardly, available by other methods. This paper reviews calorimetric approaches and assays for the study of lipid vesicles (liposomes) and biological membranes. With respect to the instrumentation, differential scanning calorimetry (DSC), pressure perturbation calorimetry (PPC), isothermal titration calorimetry (ITC) and water sorption calorimetry are considered. Applications of these techniques to lipid systems include the measurement of thermodynamic parameters and a detailed characterization of the thermotropic, barotropic, and lyotropic phase behaviour. The membrane binding or partitioning of solutes (proteins, peptides, drugs,

surfactants, ions, etc) can also be quantified. Many calorimetric assays are available for studying the effect of proteins and other additives on membranes, characterizing non-ideal mixing, domain formation, stability, curvature strain, permeability, solubilization, and fusion. Studies of membrane proteins in lipid environments elucidate lipid-protein interactions in membranes. The systems are described in terms of enthalpic and entropic forces, equilibrium constants, heat capacities, partial volume changes etc, shedding light also on the stability of structures and the molecular origin and mechanism of structural changes.

Heerklotz H. and Seelig J. (2007) Leakage and lysis of lipid membranes induced by the lipopeptide surfactin. *Eur Biophys J* **36**, 305-314.

Abstract: Surfactin is a lipopeptide produced by *Bacillus subtilis* which possesses antimicrobial activity. We have studied the leakage and lysis of POPC vesicles induced by surfactin using calcein fluorescence de-quenching, isothermal titration calorimetry and ^{31}P solid state NMR. Membrane leakage starts at a surfactin-to-lipid ratio in the membrane, $R(b)$ approximately 0.05, and an aqueous surfactin concentration of $C(S)(w)$ approximately 2 μM . The transient, graded nature of leakage and the apparent coupling with surfactin translocation to the inner leaflet of the vesicles, suggests that this low-concentration effect is due to a bilayer-couple mechanism. Different permeabilization behaviour is found at $R(b)$ approximately 0.15 and attributed to surfactin-rich clusters, which can induce leaks and stabilize them by covering their hydrophobic edges. Membrane lysis or solubilization to micellar structures starts at $R(b)(\text{sat}) = 0.22$ and $C(S)(w) = 9 \mu\text{M}$ and is completed at $R(m)(\text{sol}) = 0.43$ and $C(S)(w) = 11 \mu\text{M}$. The membrane-water partition coefficient of surfactin is obtained as $K = 2 \times 10^4 \text{ M}^{-1}$. These data resolve inconsistencies in the literature and shed light on the variety of effects often referred to as detergent-like effects of antibiotic peptides on membranes. The results are compared with published parameters characterizing the hemolytic and antibacterial activity.

Hu N. J., Yusof A. M., Winter A., Osman A., Reeve A. K. and Hofmann A. (2008) The crystal structure of calcium-bound annexin Gh1 from *Gossypium hirsutum* and its implications for membrane binding mechanisms of plant annexins. *J Biol Chem* **283**, 18314-18322.

Abstract: Plant annexins show distinct differences in comparison with their animal orthologues. In particular, the endonexin sequence, which is responsible for coordination of calcium ions in type II binding sites, is only partially conserved in plant annexins. The crystal structure of calcium-bound cotton annexin Gh1 was solved at 2.5 Å resolution and shows three metal ions coordinated in the first and fourth repeat in types II and III binding sites. Although the protein has no detectable affinity for calcium in solution, in the presence of phospholipid vesicles, we determined a stoichiometry of four calcium ions per protein molecule using isothermal titration calorimetry. Further analysis of the crystal structure showed that binding of a fourth calcium ion is structurally possible in the DE loop of the first repeat. Data from this study are in agreement with the canonical membrane binding of annexins, which is facilitated by the convex surface associating with the phospholipid bilayer by a calcium bridging mechanism. In annexin Gh1, this membrane-binding state is characterized by four calcium bridges in the I/IV module of the protein and by direct interactions of several surface-exposed basic and hydrophobic residues with the phospholipid membrane. Analysis of the protein fold stability revealed that the presence of calcium lowers the thermal stability of plant annexins. Furthermore, an additional unfolding step was detected at lower temperatures, which can be explained by the anchoring of the N-terminal domain to the C-terminal core by two conserved hydrogen bonds

Hunter H. N., Jing W., Schibli D. J., Trinh T., Park I. Y., Kim S. C., and Vogel H. J. (2005) The interactions of antimicrobial peptides derived from lysozyme with model membrane systems. *Biochim Biophys Acta* **1668**, 175-189.

Abstract: Two peptides, RAWVAWR-NH₂ and IVSDGNGMNAWVAWR-NH₂, derived from human and chicken lysozyme, respectively, exhibit antimicrobial activity. A comparison between the L-RAWVAWR, D-RAWVAWR, and the longer peptide has been carried out in membrane mimetic conditions to better understand how their interaction with lipid and detergent systems relates to the reported higher activity for the all L-peptide. Using CD and 2D ^1H NMR spectroscopy, the structures were studied with DPC and SDS micelles. Fluorescence spectroscopy was used to study peptide interactions with POPC and POPG vesicles and DOPC, DOPE, and DOPG mixed vesicle systems. Membrane-peptide interactions were also probed by ITC and DSC. The ability of fluorescein-labeled RAWVAWR to rapidly enter both E.

coli and Staphylococcus aureus was visualized using confocal microscopy. Reflecting the bactericidal activity, the long peptide interacted very weakly with the lipids. The RAWVAWR-NH₂ peptides preferred lipids with negatively charged headgroups and interacted predominantly in the solvent-lipid interface, causing significant perturbation of membrane mimetics containing PG headgroups. Peptide structures determined by ¹H NMR indicated a well-ordered coiled structure for the short peptides and the C-terminus of the longer peptide. Using each technique, the two enantiomers of RAWVAWR-NH₂ interacted in an identical fashion with the lipids, indicating that any difference in activity in vivo is limited to interactions not involving the membrane lipids.

Iram S. H. and Cronan J. E. (2005) Unexpected functional diversity among FadR fatty acid transcriptional regulatory proteins. *J Biol Chem* **280**, 32148-32156.

Abstract: The FadR protein of Escherichia coli has been shown to play a dual role in transcription of the genes of bacterial fatty acid metabolism. The protein acts as a repressor of beta-oxidation and an activator of unsaturated fatty acid synthesis. FadR DNA binding is antagonized by long chain acyl-CoAs, and thus FadR acts as a sensor of fatty acid availability in the environment. When viewed from a genomic viewpoint, FadR proteins are unusual in that the DNA binding domain is very highly conserved among FadR-containing bacteria, whereas the C-terminal acyl-CoA binding domain shows only weak conservation. To further our understanding of the role of FadR in bacterial lipid metabolism we have examined the in vivo and in vitro properties of a diverse set of FadR proteins expressed in E. coli. In addition to E. coli FadR the proteins examined were those of Salmonella enterica, Vibrio cholerae, Pasteurella multocida, and Haemophilus influenzae. These FadR proteins were found to differ markedly in their effects on repression and induction of beta-oxidation in E. coli and in their acyl-CoA binding abilities as measured by isothermal titration calorimetry. The E. coli and S. enterica proteins were the most similar, although they differed in their effects on utilization of oleic acid and acyl-CoA binding affinities, whereas the P. multocida and H. influenzae proteins showed only weak repression and poor acyl-CoA binding affinities. The V. cholerae FadR was strikingly superior to the other proteins in the amplitude of its regulatory response, and it bound long chain acyl-CoAs appreciably more strongly than the E. coli and S. enterica proteins. The significance of these findings is discussed in view of the protein sequences and the physiological niches occupied by these organisms.

Isenberg G., Doerhoefer S., Hoekstra D., and Goldmann W. H. (2002) Membrane fusion induced by the major lipid-binding domain of the cytoskeletal protein talin. *Biochem Biophys Res Commun* **295**, 636-643.

Abstract: Secondary structure predictions have led to the identification of a major membrane-anchoring domain of the cytoskeletal protein talin spanning from amino acid 385 to 406. Using a synthetically derived peptide of this region, researchers have shown that it inserts into POPC/POPG phospholipid membranes with a partition coefficient of $K_{app}=1.1\pm 0.2 \times 10^5 M^{-1}$ and has an average molar reaction enthalpy of $\Delta H=-2.5$ kcal/mol, as determined by monolayer expansion technique and isothermic titration calorimetry [J. Biol. Chem. 275, 17954]. We applied resonance energy transfer (RET) assays to analyze the fusogenic properties of this peptide by lipid mixing and used liposomes containing carboxyfluorescein to measure the contents leakage. We directly visualized talin peptide-induced vesicle membrane fusion using cryo-electron microscopy. This is the first example of a cytoskeletal protein domain that can trigger membrane fusion that might be of importance for understanding membrane targeting and motile events at the leading edge of the cell.

Jakoby M. G., Miller K. R., Toner J. J., Bauman A., Cheng L., Li E., and Cistola D. P. (1993) Ligand-protein electrostatic interactions govern the specificity of retinol- and fatty acid-binding proteins. *Biochemistry* **32**, 872-878.

Abstract: Cellular retinol-binding protein II (CRBP-II) and intestinal fatty acid-binding protein (I-FABP) are both expressed in small intestinal enterocytes and exhibit 31% sequence identity. I-FABP binds a single molecule of long-chain fatty acid and forms an ion-pair electrostatic interaction between the cationic side chain of arginine-106 and the anionic fatty acid carboxyl group. In contrast, CRBP-II binds all-trans-retinol or -retinal and contains a glutamine residue in the corresponding position, residue 109. We have characterized and compared the interactions of fatty acids and retinoids with I-FABP, CRBP-II, and two reciprocal mutant proteins. The mutants were designated CRBP-II(Q109R), where glutamine-109 was replaced by arginine, and I-FABP(R106Q), where arginine-106 was replaced by glutamine. As monitored by titration calorimetry and carbon-13 NMR spectroscopy, the fatty acid-binding properties of CRBP-

II(Q109R) were found to be essentially identical to those of wild-type I-FABP. Both proteins bound 1 molecule of fatty acid with identical affinities ($K_d = 0.2 \mu\text{M}$). The enthalpic contribution to the total free energy of binding was large for both proteins: 66% and 87%, respectively. In addition, the carboxyl groups of fatty acids bound to both proteins were solvent-inaccessible. There was little or no change in the ionization state of the bound fatty acid over a wide pH range, as monitored by the chemical shift of the fatty acid carboxyl ^{13}C resonance. Furthermore, the binding of fatty acid to both proteins was accompanied by a selective perturbation of the guanidino ^{13}C resonance of a single arginine residue.(ABSTRACT TRUNCATED AT 250 WORDS).

Jeppesen B., Smith C., Gibson D. F. and Tait J. F. (2008) Entropic and enthalpic contributions to annexin V-membrane binding: a comprehensive quantitative model. *J Biol Chem* **283**, 6126-6135.

Abstract: Annexin V binds to membranes with very high affinity, but the factors responsible remain to be quantitatively elucidated. Analysis by isothermal microcalorimetry and calcium titration under conditions of low membrane occupancy showed that there was a strongly positive entropy change upon binding. For vesicles containing 25% phosphatidylserine at 0.15 m ionic strength, the free energy of binding was -53 kcal/mol protein, whereas the enthalpy of binding was -38 kcal/mol. Addition of 4 m urea decreased the free energy of binding by about 30% without denaturing the protein, suggesting that hydrophobic forces make a significant contribution to binding affinity. This was confirmed by mutagenesis studies that showed that binding affinity was modulated by the hydrophobicity of surface residues that are likely to enter the interfacial region upon protein-membrane binding. The change in free energy was quantitatively consistent with predictions from the Wimley-White scale of interfacial hydrophobicity. In contrast, binding affinity was not increased by making the protein surface more positively charged, nor decreased by making it more negatively charged, ruling out general ionic interactions as major contributors to binding affinity. The affinity of annexin V was the same regardless of the head group present on the anionic phospholipids tested (phosphatidylserine, phosphatidylglycerol, phosphatidylmethanol, and cardiolipin), ruling out specific interactions between the protein and non-phosphate moieties of the head group as a significant contributor to binding affinity. Analysis by fluorescence resonance energy transfer showed that multimers did not form on phosphatidylserine membranes at low occupancy, indicating that annexin-annexin interactions did not contribute to binding affinity. In summary, binding of annexin V to membranes is driven by both enthalpic and entropic forces. Dehydration of hydrophobic regions of the protein surface as they enter the interfacial region makes an important contribution to overall binding affinity, supplementing the role of protein-calcium-phosphate chelates

Jing W., Hunter H. N., Hagel J., and Vogel H. J. (2003) The structure of the antimicrobial peptide Ac-RRWWRF-NH₂ bound to micelles and its interactions with phospholipid bilayers. *J Pept Res* **61**, 219-229.

Abstract: The hexapeptide Ac-RRWWRF-NH₂ has earlier been identified as a potent antimicrobial peptide by screening synthetic combinatorial hexapeptide libraries. In this study, it was found that this peptide had a large influence on the thermotropic phase behavior of model membranes containing the negatively charged headgroup phosphatidylglycerol, a major component of bacterial membranes. In contrast, differential scanning calorimetry showed that it had little effect on model membranes containing the zwitterionic phosphatidylcholine headgroup, the main component of erythrocyte membranes. This behavior is consistent with its biological activity and with its affinity to these membranes as determined by titration calorimetry, implying that peptide-lipid interactions play an important role in this process. The structure of this peptide bound to membrane-mimetic sodium dodecyl sulfate (SDS) and dodecylphosphocholine micelles has been determined using conventional two-dimensional nuclear magnetic resonance methods. It forms a marked amphipathic structure in SDS with its hydrophobic residues on one side of the structure and with the positively charged residues on the other side. This amphipathic structure may allow this peptide to penetrate deeper into the interfacial region of negatively charged membranes, leading to local membrane destabilization. Knowledge about the importance of electrostatic interactions of Arg and the role of Trp residues as a membrane interface anchor will provide insight into the future design of potent antimicrobial peptidomimetics.

Jing W., Demcoe A. R., and Vogel H. J. (2003) Conformation of a bactericidal domain of puroindoline a: structure and mechanism of action of a 13-residue antimicrobial peptide. *J Bacteriol* **185**, 4938-4947.

Abstract: Puroindoline a, a wheat endosperm-specific protein containing a tryptophan-rich domain, was reported to have antimicrobial activities. We found that a 13-residue fragment of puroindoline a

(FPVTWRWWKWWKG-NH(2)) (puroA) exhibits activity against both gram-positive and gram-negative bacteria. This suggests that puroA may be a bactericidal domain of puroindoline a. PuroA interacted strongly with negatively charged phospholipid vesicles and induced efficient dye release from these vesicles, suggesting that the microbicidal effect of puroA may be due to interactions with bacterial membranes. A variety of biophysical and biochemical methods, including fluorescence spectroscopy and microcalorimetry, were used to examine the mode of action of puroA. These studies showed that puroA is located at the membrane interface, probably due to its high content of Trp residues that have a high propensity to partition into the membrane interface. The penetration of these Trp residues in negatively charged phospholipid vesicles resembling bacterial membranes was more extensive than the penetration in neutral vesicles mimicking eukaryotic membranes. Peptide binding had a significant influence on the phase behavior of the former vesicles. The three-dimensional structure of micelle-bound puroA determined by two-dimensional nuclear magnetic resonance spectroscopy indicated that all the positively charged residues are oriented close to the face of Trp indole rings, forming energetically favorable cation- π interactions. This characteristic, along with its well-defined amphipathic structure upon binding to membrane mimetic systems, allows puroA to insert more deeply into bacterial membranes and disrupt the regular membrane bilayer structure.

Jurgens G., Muller M., Koch M. H., and Brandenburg K. (2001) Interaction of hemoglobin with enterobacterial lipopolysaccharide and lipid A. Physicochemical characterization and biological activity. *Eur J Biochem* **268**, 4233-4242.

Abstract: The interaction of hemoglobin (Hb) with endotoxins [i.e. with enterobacterial deep rough mutant lipopolysaccharide (LPS) Re and the "endotoxic principle" of LPS, lipid A] was investigated using a variety of physical techniques and with two biological assays, tumor necrosis factor (TNF)- α induction in human mononuclear cells and the Limulus amoebocyte lysate (LAL) assay. Fourier-transform IR-spectroscopic experiments indicate nonelectrostatic binding to the hydrophobic moiety with a slight rigidification of the lipid A acyl chains, and an increase in the inclination of the lipid A backbone with respect to the membrane surface from 35 degrees to more than 40 degrees due to Hb binding, but no change of the predominantly α -helical secondary structures of Hb due to LPS binding. From isothermal titration calorimetry, the molar [Hb] : [endotoxin] binding ratio lies between 1 : 3 and 1 : 5 molar. Synchrotron radiation X-ray diffraction measurements indicate a reorientation of the lipid A aggregates from one cubic structure to another, the final structure belonging to space group Q224. The LPS-induced TNF- α production of mononuclear cells is enhanced by Hb, whereas in the LAL assay an LPS concentration-dependent increase or decrease was observed. Although a detailed mechanism of action cannot be given, the enhancement of LPS bioactivity can be understood in the light of the previously presented conformational concept; Hb induces an increase in the conical shape of the lipid A moiety of LPS, higher cross-section of the hydrophobic than the hydrophilic part, and of the inclination angle of the diglucosamine backbone with respect to the direction of the acyl chains.

Jurgens G., Muller M., Garidel P., Koch M. H., Nakakubo H., Blume A., and Brandenburg K. (2002) Investigation into the interaction of recombinant human serum albumin with Re-lipopolysaccharide and lipid A. *J Endotoxin Res* **8**, 115-126.

Abstract: The interaction of bacterial endotoxins, deep rough mutant lipopolysaccharide LPS Re and the 'endotoxic principle' lipid A, with recombinant human serum albumin (rHSA) was investigated with a variety of physical techniques and biological assays. With Fourier-transform infrared spectroscopy and differential scanning calorimetry, the influence of albumin on the acyl chain melting behavior of the endotoxins was measured. Also, the effect on the functional groups of the endotoxins, in particular with respect to their orientation, was studied, including competition experiments with polymyxin B. Furthermore, the influence of endotoxin binding to rHSA on the protein's secondary structure was investigated. The results indicate a non-electrostatic binding with no change of the backbone orientation of LPS and only a slight change of the secondary structure of rHSA. Correspondingly, the amount of charge neutralization of the endotoxins due to rHSA measured by the electrophoretic mobility exhibited only a slight reduction of the surface potential. From these measurements and isothermal titration calorimetry, the lipid:protein binding stoichiometry was estimated to [LPS]:[rHSA], 10:1 molar. The determination of the aggregate structure of the endotoxins by X-ray small-angle scattering exhibited a complex change of a cubic into a non-lamellar structure. No influence of rHSA on endotoxin intercalation into phospholipid liposomes induced by lipopolysaccharide-binding protein could be detected by fluorescence resonance

energy transfer. Finally, the LPS-induced cytokine production of human mononuclear cells was only slightly increased at high molar rHSA excess, while the coagulation of amoebocyte lysate in the Limulus test yielded a complex change due to rHSA binding of LPS.

Kamp F. and Beyer K. (2006) Binding of alpha-synuclein affects the lipid packing in bilayers of small vesicles. *J Biol Chem* **281**, 9251-9259.

Abstract: The intracellular deposition of fibrillar aggregates of alpha-synuclein is a characteristic feature of Parkinson disease. Alternatively, as a result of its unusual conformational plasticity, alpha-synuclein may fold into an amphipathic helix upon contact with a lipid-water interface. Using spin label ESR and fluorescence spectroscopy, we show here that alpha-synuclein affects the lipid packing in small unilamellar vesicles. The ESR hyperfine splittings of spin-labeled phospholipid probes revealed that alpha-synuclein induces chain ordering at carbon 14 of the acyl chains below the chain melting phase transition temperature but not in the liquid crystalline state of electroneutral vesicle membranes. Binding of alpha-synuclein leads to an increase in the temperature and cooperativity of the phase transition according to the fluorescence anisotropy of the hydrophobic polyene 1,6-diphenylhexatriene and of the fluorescence emission maxima of the amphiphilic probe 6-dodecanoyl-2-dimethylaminonaphthalene. Binding parameters were obtained from the fluorescence anisotropy measurements in combination with our previous determinations by titration calorimetry (Nuscher, B., Kamp, F., Mehnert, T., Odoy, S., Haass, C., Kahle, P. J., and Beyer, K. (2004) *J. Biol. Chem.* 279, 21966-21975). We also show that alpha-synuclein interacts with vesicle membranes containing sphingomyelin and cholesterol. We propose that the protein is capable of annealing defects in curved vesicle membranes, which may prevent synaptic vesicles from premature fusion.

Kathir K. M., Ibrahim K., Rajalingam D., Prudovsky I., Yu C. and Kumar T. K. (2007) S100A13-lipid interactions-role in the non-classical release of the acidic fibroblast growth factor. *Biochim Biophys Acta* **1768**, 3080-3089.

Abstract: S100A13 is a 98-amino acid, calcium binding protein. It is known to participate in the non-classical secretion of signal peptide-less proteins, such as the acidic fibroblast growth factor. In this study, we investigate the lipid binding properties of S100A13 using a number of biophysical techniques, including multidimensional NMR spectroscopy. Isothermal titration calorimetry and steady state fluorescence experiments show that apoS100A13 exhibits preferential binding to small unilamellar vesicles of 1-phosphatidyl serine (pS). In comparison, Ca(2+)-bound S100A13 is observed to bind weakly to unilamellar vesicles (SUVs) of pS. Equilibrium thermal unfolding and limited trypsin digestion analysis reveal that apoS100A13 is significantly destabilized upon binding to SUVs of pS. Results of the far UV circular dichroism and ANS (8-anilino-1-naphthalene sulfonate) binding experiments indicate a subtle conformational change resulting in the increase in the solvent-accessible hydrophobic surface in the protein. Availability of the solvent-exposed hydrophobic surface(s) in apoS100A13 facilitates its interaction with the lipid vesicles. Our data suggest that Ca(2+) binding dictates the membrane binding affinity of S100A13. Based on the results of this study, a model describing the sequence of molecular events that possibly can occur during the non-classical secretion of FGF-1 is presented.

Kilby P. M., Primrose W. U., and Roberts G. C. (1995) Changes in the structure of bovine phospholipase A2 upon micelle binding. *Biochem J* **305 (Pt 3)**, 935-944.

Abstract: Phospholipase A2 (PLA2) is a calcium-dependent enzyme which hydrolyses the 2-acyl ester bond of phospholipids. The extracellular PLA2s are activated by as much as 10000-fold on binding to micelles or vesicles of substrate, possibly due to a conformational change induced in the enzyme. We have studied the complex of bovine pancreatic PLA2 with micelles of SDS by ultracentrifugation, equilibrium dialysis, microcalorimetry, fluorescence and n.m.r. spectroscopy. Ultracentrifugation and equilibrium dialysis measurements showed that on average 1.28 (+/- 0.17) PLA2 molecules and 26.4 (+/- 3.1) SDS molecules are involved in the complex and that there is a rapid equilibrium between micellar species containing one or more enzyme monomers. The estimated heat of formation of the complex, measured calorimetrically as the heat released when PLA2 was injected into excess 10 mM SDS, was 162.3 +/- 1.5) kJ/mol [38.8 (+/- 0.35) kcal/mol] of PLA2 added. The fluorescence of the single tryptophan at position 3 in the N-terminal helix of the protein increases when PLA2 binds to SDS micelles, indicating that this part of the protein is in a more hydrophobic environment in the complex. The structural changes in PLA2 on addition of [2H25]SDS were monitored using n.m.r. spectroscopy. The overall structure of the protein is unchanged, but changes in nuclear Overhauser effects (NOEs) were observed for residues in the N-terminal

helix, at the active site region and in a lysine-rich region near the C-terminus. The NOE changes at the N-terminus indicate that this portion of the protein molecule adopts a more ordered, helical conformation when bound to a micelle. We suggest that these conformational changes could be the mechanism by which the enzyme becomes activated in the presence of aggregated substrate.

Kurian E., Kirk W. R., and Prendergast F. G. (1996) Affinity of fatty acid for (r)rat intestinal fatty acid binding protein:further examination. *Biochemistry* **35**, 3865-3874.

Abstract: The enhancement of the fluorescence quantum yield of 1,8-anilinonaphthalenesulfonic acid (ANS) upon binding to intestinal fatty acid protein (I-FABP) was exploited to devise an assay for free I-FABP. With this assay, we monitored the competition for free I-FABP between ANS and fatty acids and thereby extracted values for the dissociation constants ($K(FA)$) of fatty acids for I-FABP. We obtained these constants for the I-FABP ligands oleic acid, arachidonic acid, and palmitic acid. In addition, we measured the dependence of $K(FA)$ for oleic acid upon temperature and at two pH values. From these data, we calculate the van't Hoff enthalpy of oleic acid binding. This enthalpy is compared with the enthalpies of binding obtained directly from titration calorimetry. Our experiments with the fluorescence-based assay generate values of $K(FA)$ which disagree with older values obtained from calorimetry and other methods. Our own calorimetric data were analyzed with a view to improving the technique involved in subtraction of a "reference" dilution of the ligand into solution in the absence of the protein. By this maneuver, we obtained "corrected" titrations which could be fitted to values of $K(FA)$ more in agreement with the values we determined via the fluorescence-based assay than were the older literature values. Our new values for $K(FA)$ also agree substantially with values derived using a complementary assay technique, one measuring the concentration of free fatty acid, that has recently been developed by Richiere et al [Richiere et al. (1995) *J. Biol. Chem.* 270, 15076-15084]. We compare the values of ΔH degrees, ΔS degrees, and ΔC_p degrees for fatty acid binding we have obtained in this work with those we found in earlier work with ANS binding to I-FABP [Kirk et al. (1996) *Biophys. J.* 70, 69-83]. Our interpretation of the origin of the thermodynamic changes for ANS binding in our earlier work is here substantiated and extended to include an evaluation in physical terms of the interaction of I-FABP with fatty acids.

Lai A.L., Park H., White J.M., and Tamm L.K. (2006) Fusion peptide of influenza hemagglutinin requires a fixed angle boomerang structure for activity. *J Biol Chem.* **281**, 5760-70.

Abstract: The fusion peptide of influenza hemagglutinin is crucial for cell entry of this virus. Previous studies showed that this peptide adopts a boomerang-shaped structure in lipid model membranes at the pH of membrane fusion. To examine the role of the boomerang in fusion, we changed several residues proposed to stabilize the kink in this structure and measured fusion. Among these, mutants E11A and W14A expressed hemagglutinins with hemifusion and no fusion activities, and F9A and N12A had no effect on fusion, respectively. Binding enthalpies and free energies of mutant peptides to model membranes and their ability to perturb lipid bilayer structures correlated well with the fusion activities of the parent full-length molecules. The structure of W14A determined by NMR and site-directed spin labeling features a flexible kink that points out of the membrane, in sharp contrast to the more ordered boomerang of the wild-type, which points into the membrane. A specific fixed angle boomerang structure is thus required to support membrane fusion.

LaLonde J. M., Levenson M. A., Roe J. J., Bernlohr D. A., and Banaszak L. J. (1994) Adipocyte lipid-binding protein complexed with arachidonic acid. Titration calorimetry and X-ray crystallographic studies. *J Biol Chem* **269**, 25339-25347.

Abstract: The association of the adipocyte lipid-binding protein (ALBP) with arachidonic acid (all cis, 20:4 Δ 5,8,11,14) and oleic acid (cis, 18:1 Δ 9) has been examined by titration calorimetry. In addition, the crystal structure of ALBP with bound arachidonic acid has also been obtained. Crystallographic analysis of the arachidonic acid-ALBP complex along with the previously reported oleic acid-ALBP structure (Xu, Z., Bernlohr, D. A., and Banaszak, L. J. (1993) *J. Biol. Chem.* 268, 7874-7884) provides a framework for the molecular examination of protein-lipid association. Isothermal titration calorimetry revealed high affinity association of both unsaturated fatty acids with the protein. The calorimetric data yielded the following thermodynamic parameters for arachidonic acid: $K_d = 4.4 \mu\text{M}$, $n = 0.8$, $\Delta G = -7370 \text{ cal/mol}$, $\Delta H = -6770 \text{ cal/mol}$, and $T \Delta S = +600 \text{ cal/mol}$. For oleic acid, the thermodynamic parameters were $K_d = 2.4 \mu\text{M}$, $n = 0.9$, $\Delta G = -7770 \text{ cal/mol}$, $\Delta H = -6050 \text{ cal/mol}$, and $T \Delta S = +1720 \text{ cal/mol}$.

The identification of thermodynamically dominating enthalpic factors for both fatty acids are consistent with the crystallographic studies demonstrating the interaction of the fatty acid carboxylate with a combination of Arg106, Arg126, and Tyr128. The crystallographic refinement of the protein-arachidonate complex was carried out to 1.6 Å with the resultant R factor of 0.19. Within the cavity of the crystalline binding protein, the arachidonate was found in a hairpin conformation. The conformation of the bound ligand is consistent with acceptable torsional angles and the four cis double bonds in arachidonate. These results demonstrate that arachidonate is a ligand for ALBP. They provide thermodynamic and structural data concerning the physical basis for protein-lipid interaction and suggest that intracellular lipid-binding proteins may mediate the biological effects of polyunsaturated fatty acids in vivo.

Lario P. I., Pfuetzner R. A., Frey E. A., Creagh L., Haynes C., Maurelli A. T., and Strynadka N. C. (2005) Structure and biochemical analysis of a secretin pilot protein. *EMBO J* **24**, 1111-1121.

Abstract: The ability to translocate virulence proteins into host cells through a type III secretion apparatus (TTSS) is a hallmark of several Gram-negative pathogens including Shigella, Salmonella, Yersinia, Pseudomonas, and enteropathogenic Escherichia coli. In common with other types of bacterial secretion apparatus, the assembly of the TTSS complex requires the preceding formation of its integral outer membrane secretin ring component. We have determined at 1.5 Å the structure of MxiM28-142, the Shigella pilot protein that is essential for the assembly and membrane association of the Shigella secretin, MxiD. This represents the first atomic structure of a secretin pilot protein from the several bacterial secretion systems containing an orthologous secretin component. A deep hydrophobic cavity is observed in the novel 'cracked barrel' structure of MxiM, providing a specific binding domain for the acyl chains of bacterial lipids, a proposal that is supported by our various lipid/MxiM complex structures. Isothermal titration analysis shows that the C-terminal domain of the secretin, MxiD525-570, hinders lipid binding to MxiM.

Lassiseraye D., Courtemanche L., Bergeron A., Manjunath P. and Lafleur M. (2008) Binding of bovine seminal plasma protein BSP-A1/-A2 to model membranes: lipid specificity and effect of the temperature. *Biochim Biophys Acta* **1778**, 502-513.

Abstract: Bovine seminal plasma (BSP) contains a family of phospholipid-binding proteins. The affinity of the protein BSP-A1/-A2 for lipid membranes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), and POPC containing 30% (mol/mol) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) or cholesterol, has been investigated by the isothermal titration calorimetry (ITC). This study confirms the association of these proteins to lipid bilayers, and provides a direct characterization of this exothermic process, at 37 degrees C. The measurements indicate that the protein affinity for lipid bilayers is modulated by the lipid composition, the lipid/protein ratio, and the temperature. The saturation lipid/protein ratio was increased in the presence of cholesterol and, to a lesser extent, of phosphatidylethanolamine, suggesting that it is modulated by the lipid acyl chain order. For all the investigated systems, the binding of BSP-A1/-A2 could not be modeled using a simple partitioning of the proteins between the aqueous and lipid phases. The existence of "binding sites", and lipid phase separations is discussed. The decrease of temperature, from 37 to 10 degrees C, converts the exothermic association of the proteins to the POPC bilayers to an endothermic process. A complementary 1-D and 2-D infrared spectroscopy study excludes the thermal denaturation of BSP-A1/-A2 as a contributor in the temperature dependence of the protein affinity for lipid bilayers. The reported findings suggest that changes in the affinity of BSP-A1/-A2 for lipid bilayers could be involved in modulating the association of these proteins to sperm membranes as a function of space and time; this would consequently modulate the extent of lipid extraction, including cholesterol, at a given place and given time

Letizia C., Andreozzi P., Scipioni A., La M. C., Bonincontro A. and Spigone E. (2007) Protein binding onto surfactant-based synthetic vesicles. *J Phys. Chem B* **111**, 898-908.

Abstract: Synthetic vesicles were prepared by mixing anionic and cationic surfactants, aqueous sodium dodecylsulfate with didodecyltrimethylammonium or cetyltrimethylammonium bromide. The overall surfactant content and the (anionic/cationic) mole ratios allow one to obtain negatively charged vesicles. In the phase diagram, the vesicular region is located between a solution phase, a lamellar liquid crystalline dispersion, and a precipitate area. Characterization of the vesicles was performed by electrophoretic mobility, NMR, TEM, and DLS and we determined their uni-lamellar character, size, stability, and charge

density. Negatively charged vesicular dispersions, made of sodium dodecylsulfate/didodecyltrimethylammonium bromide or sodium dodecylsulfate/cetyltrimethylammonium bromide, were mixed with lysozyme, to form lipoplexes. Depending on the protein/vesicle charge ratio, binding, surface saturation, and lipoplexes flocculation, or precipitation, occurs. The free protein in excess remains in solution, after binding saturation. The systems were investigated by thermodynamic (surface tension and solution calorimetry), DLS, CD, TEM, ¹H NMR, transport properties, electrophoretic mobility, and dielectric relaxation. The latter two methods give information on the vesicle charge neutralization by adsorbed protein. Binding is concomitant to modifications in the double layer thickness of vesicles and in the surface charge density of the resulting lipoplexes. This is also confirmed by developing the electrophoretic mobility results in terms of a Langmuir-like adsorption isotherm. Charges in excess with respect to the amount required to neutralize the vesicle surface promote lipoplexes clustering and/or flocculation. Protein-vesicle interactions were observed by DLS, indicating changes in particle size (and in their distribution functions) upon addition of LYSO. According to CD, the bound protein retains its native conformation, at least in the SDS/CTAB vesicular system. In fact, changes in the alpha-helix and beta-sheet conformations are moderate, if any. Calorimetric methods indicate that the maximum heat effect for LYSO binding occurs at charge neutralization. They also indicate that enthalpic are by far the dominant contributions to the system stability. Accordingly, energy effects associated with charge neutralization and double-layer contributions are much higher than counterion exchange and dehydration terms.

Li Y., Han X., and Tamm L. K. (2003) Thermodynamics of fusion peptide-membrane interactions. *Biochemistry* **42**, 7245-7251.

Abstract: The fusion peptides of viral membrane fusion proteins play a key role in the mechanism of viral spike glycoprotein mediated membrane fusion. These peptides insert into the lipid bilayers of cellular target membranes where they adopt mostly helical secondary structures. To better understand how membranes may be converted to high-energy intermediates during fusion, it is of interest to know how much energy, enthalpy and entropy, is provided by the insertion of fusion peptides into lipid bilayers. Here, we describe a detailed thermodynamic analysis of the binding of analogues of the influenza hemagglutinin fusion peptide of different lengths and amino acid compositions. In small unilamellar vesicles, the interaction of these peptides with lipid bilayers is driven by enthalpy (-16.5 kcal/mol) and opposed by entropy (-30 cal mol⁻¹ K⁻¹). Most of the driving force ($\Delta G = -7.6$ kcal/mol) comes from the enthalpy of peptide insertion deep into the lipid bilayer. Enthalpic gains and entropic losses of peptide folding in the lipid bilayer cancel to a large extent and account for only about 40% of the total binding free energy. The major folding event occurs in the N-terminal segment of the fusion peptide. The C-terminal segment mainly serves to drive the N-terminus deep into the membrane. The fusion-defective mutations G1S, which causes hemifusion, and particularly G1V, which blocks fusion, have major structural and thermodynamic consequences on the insertion of fusion peptides into lipid bilayers. The magnitudes of the enthalpies and entropies of binding of these mutant peptides are reduced, their helix contents are reduced, but their energies of self-association at the membrane surface are increased compared to the wild-type fusion peptide.

Li Y., Han X., Lai A.L., Bushweller J.H., Cafiso D.S., and Tamm L.K.. (2005) Membrane structures of the hemifusion-inducing fusion peptide mutant G1S and the fusion-blocking mutant G1V of influenza virus hemagglutinin suggest a mechanism for pore opening in membrane fusion. *J Virol.* **79**, 12065-76.

Abstract: Influenza virus hemagglutinin (HA)-mediated membrane fusion is initiated by a conformational change that releases a V-shaped hydrophobic fusion domain, the fusion peptide, into the lipid bilayer of the target membrane. The most N-terminal residue of this domain, a glycine, is highly conserved and is particularly critical for HA function; G1S and G1V mutant HAs cause hemifusion and abolish fusion, respectively. We have determined the atomic resolution structures of the G1S and G1V mutant fusion domains in membrane environments. G1S forms a V with a disrupted "glycine edge" on its N-terminal arm and G1V adopts a slightly tilted linear helical structure in membranes. Abolishment of the kink in G1V results in reduced hydrophobic penetration of the lipid bilayer and an increased propensity to form beta-structures at the membrane surface. These results underline the functional importance of the kink in the fusion peptide and suggest a structural role for the N-terminal glycine ridge in viral membrane fusion.

Lin M. S., Chiu H. M., Fan F. J., Tsai H. T., Wang S. S., Chang Y. and Chen W. Y. (2007) Kinetics and enthalpy measurements of interaction between beta-amyloid and liposomes by surface plasmon resonance and isothermal titration microcalorimetry. *Colloids Surf B Biointerfaces* **58**, 231-236.

Abstract: The objective of this research is to understand the interaction mechanism of beta-amyloid (Abeta) with cell and were basically divided into two parts. The first part focused on the time-dependent structural changes of Abeta (1-40) by circular dichroism (CD) spectroscopy, thioflavin T (ThT) fluorescence assay, and atomic force microscopy (AFM). The second part emphasized the kinetics and enthalpy of interaction between Abeta (1-40) and liposome by surface plasmon resonance (SPR) and isothermal titration microcalorimetry (ITC). Results obtained from CD, ThT and AFM confirmed the formation of 1 microm fibril after single day incubation. The driving force of kinetic interaction between Abeta and liposomes was revealed by SPR to be electrostatics. Further studies indicated that fresh Abeta has high GM1 affinity. Besides, addition of cholesterol to the liposome could alter membrane fluidity and affect the interactions of fresh Abeta with liposomes especially in the amount of Abeta absorbed and preserving the structure of liposome after adsorbing. Hydrophobicity was found to be the driving force leading to the interaction between Abeta fibrils and liposomes. These reactions are endothermic as supported by ITC measurements. When the composition of liposomes is zwitterionic lipids, the interaction of Abeta with liposomes is predominantly hydrophobic force. In contrast, the driving force of interaction of charged lipids with Abeta is electrostatic.

Maatman R. G., Degano M., Van Moerkerk H. T., Van Marrewijk W. J., Van der Horst D. J., Sacchettini J. C., and Veerkamp J. H. (1994) Primary structure and binding characteristics of locust and human muscle fatty-acid-binding proteins. *Eur J Biochem* **221**, 801-810.

Abstract: The conservation between muscle fatty-acid-binding proteins (M-FABP) of *Locusta migratoria* flight muscle and human skeletal muscle was investigated. The locust M-FABP cDNA (632 bp) was isolated by 5' and 3' rapid amplification of cDNA ends. The identities of the locust and human M-FABP on the cDNA and protein levels were 54% and 42%, respectively. The predicted amino acid sequence of locust M-FABP indicated a molecular mass of 14935 Da and isoelectric point 6.1. The locust M-FABP was expressed in *Escherichia coli*, purified by (NH₄)₂SO₄ precipitation, anion-exchange and gel-filtration chromatographies and compared with the recombinant human M-FABP with respect to immunological and binding properties. In spite of the high sequence similarity, the proteins did not show immunological cross-reactivity. The binding parameters of locust M-FABP were analyzed with radiolabeled oleic acid by the Lipidex assay and titration microcalorimetry. Both methods revealed a K_d for oleic acid of 0.5 μM and a binding stoichiometry of 1 mol fatty acid/mol FABP. The ΔH, ΔG and ΔS for oleic acid binding were -146 kJ.mol⁻¹ and -36 J.mol⁻¹ and -369 J.mol⁻¹.K⁻¹ respectively. All the information obtained from binding, fluorescence and displacement studies indicated that locust M-FABP has binding characteristics similar to human M-FABP. Finally the recombinant locust M-FABP was crystallized with and without oleic acid. All crystals were trigonal in the P3(1)21 space group. The unit cell dimensions were a = b = 5.89 nm and c = 14.42 nm.

Machaidze G., Ziegler A., and Seelig J. (2002) Specific binding of Ro 09-0198 (cinnamycin) to phosphatidylethanolamine: a thermodynamic analysis. *Biochemistry* **41**, 1965-1971.

Abstract: Ro 09-0198 (cinnamycin) is a tetracyclic peptide antibiotic that is used to monitor the transbilayer movement of phosphatidylethanolamine (PE) in biological membranes during cell division and apoptosis. The molecule is one of the very rare examples where a small peptide binds specifically to a particular lipid. In model membranes and biological membranes containing phosphatidylethanolamine, Ro 09-0198 forms a 1:1 complex with this lipid. We have measured the thermodynamic parameters of complex formation with high sensitivity isothermal titration calorimetry and have investigated the structural consequences with deuterium and phosphorus solid-state NMR. Complex formation is characterized by a large binding constant, K₀, of 10⁷ to 10⁸ M⁻¹, depending on the experimental conditions. The reaction enthalpy, ΔHdegrees, varies between zero at 10 degrees C to strongly exothermic -10 kcal/mol at 50 degrees C. For large vesicles with a diameter of approximately 100 nm, ΔHdegrees decreases linearly with temperature and the molar heat capacity of complex formation can be evaluated as = -245 cal/mol, indicating a hydrophobic binding mechanism. The free energy of binding is ΔGdegrees = -10.5 kcal/mol and shows only little temperature dependence. The constancy of ΔGdegrees together with the distinct temperature-dependence of ΔHdegrees provide evidence for an entropy-enthalpy compensation mechanism: at 10 degrees C, complex formation is completely entropy-driven, at 50 degrees C it is

enthalpy-driven. Varying the PE fatty acid chain-length between 6 and 18 carbon atoms produces similar binding constants and ΔH values. Addition of Ro 09-0198 to PE containing bilayers eliminates the typical bilayer structure and produces ²H- and ³¹P-NMR spectra characteristic of slow isotropic tumbling. This reorganization of the lipid matrix is not limited to PE but also includes other lipids.

Machaidze G. and Seelig J. (2003) Specific binding of cinnamycin (Ro 09-0198) to phosphatidylethanolamine. Comparison between micellar and membrane environments. *Biochemistry* **42**, 12570-12576.

Abstract: Cinnamycin (Ro 09-0198) is a tetracyclic peptide antibiotic that binds specifically to phosphatidylethanolamine (PE). Formation of a complex with phosphatidylethanolamine follows a 1:1 stoichiometry. Using high-sensitivity isothermal titration calorimetry (ITC), we have measured the thermodynamic parameters of complex formation for two different PE environments, namely, PE dissolved either in octyl glucoside (OG) micelles or in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer membrane. We have compared diacyl-PE with lyso-PE and have varied the carbon chain length from 6 to 18. Binding requires both a PE headgroup and at least one fatty acyl chain. The optimum chain length for complex formation (n) is eight. Longer chains do not enhance the binding affinity; for shorter chains, the interaction is weakened. The cinnamycin-PE complex has a binding constant K_0 of approximately 10^7 - 10^8 M⁻¹ in the POPC membrane and only approximately 10^6 M⁻¹ in the octyl glucoside micelle. The difference can be attributed to the nonspecific hydrophobic interaction of cinnamycin with the lipid membrane. Complex formation is enthalpy-driven in OG micelles, whereas enthalpy and entropy make equal contributions in bilayer membranes. However, for the optimum chain length (n) of eight, the binding reaction is also completely enthalpy-driven for the bilayer membrane.

Marynka K., Rotem S., Portnaya I., Cogan U. and Mor A. (2007) In vitro discriminative antipseudomonal properties resulting from acyl substitution of N-terminal sequence of dermaseptin s4 derivatives. *Chem Biol* **14**, 75-85.

Abstract: Truncation and acylation were combined to investigate the broad-spectrum bactericidal and hemolytic peptide S4(1-15). Substitution of up to seven residues with dodecanoic acid (C(12)) gradually led to specific antipseudomonal activity: out of 40 bacterial strains tested in vitro, C(12)-S4(8-15) displayed similar minimal inhibitory concentrations (MICs) as S4(1-15) against *Pseudomonas aeruginosa* sp. (identical MIC(90)) but was practically inactive against most other bacteria or erythrocytes. Surface plasmon resonance and isothermal titration calorimetry experiments revealed the binding properties of S4(1-15) to be consistent with its nonselective activities, while discriminative activities of C(12)-S4(8-15) correlated with high binding affinity to a membrane containing pseudomonal lipopolysaccharides and with lower affinities to membranes containing nonpseudomonal lipopolysaccharides or cholesterol. Various mechanistic studies failed to detect significant differences in secondary structure, bactericidal kinetics, or ability to perturb the cytoplasmic membrane, pointing to a similar mode of action.

Massolini G. and Calleri E. (2003) Survey of binding properties of fatty acid-binding proteins. Chromatographic methods. *J Chromatogr B Analyt Technol Biomed Life Sci* **797**, 255-268.

Abstract: Fatty acid-binding proteins (FABPs) are members of a super family of lipid-binding proteins, and occur intracellularly in vertebrates and invertebrates. This review briefly addresses the structural and molecular properties of the fatty acid binding proteins, together with their potential physiological role. Special attention is paid to the methods used to study the binding characteristics of FABPs. An overview of the conventional (Lipidex, the ADIFAB and ITC) and innovative separation-based techniques (chromatographic and electrophoretic methods) for the study of ligand-protein interactions is presented along with a discussion of their strengths, weak points and potential applications. The best conventional approaches with natural fatty acids have generally revealed only limited information about the interactions of fatty acid proteins. In contrast, high-performance affinity chromatography (HPAC) studies of several proteins provide full information on the binding characteristics. The review uses, as an example, the application of immobilized liver basic FABP as a probe for the study of ligand-protein binding by high-performance affinity chromatography. The FABP from chicken liver has been immobilized on aminopropyl silica and the developed stationary phase was used to examine the enantioselective properties of this protein and to study the binding of drugs to FABP. In order to clarify the retention mechanism, competitive displacement studies were also carried out by adding short chain fatty acids to the mobile phase as displacing agents and preliminary quantitative structure-retention relationship (QSRRs) correlations were

developed to describe the nature of the interactions between the chemical structures of the analytes and the observed chromatographic results. The results of these studies may shed light on the proposed roles of these proteins in biological systems and may find applications in medicine and medicinal chemistry. This knowledge will yield a deeper insight into the mechanism of fatty acid binding in order to indisputably show the central role played by FABPs in cellular FA transport and utilization for a proper lipid metabolism.

McIntosh T. J., Vidal A., and Simon S. A. (2001) The energetics of binding of a signal peptide to lipid bilayers: the role of bilayer properties. *Biochem Soc Trans* **29**, 594-598.

Abstract: The interactions between a signal peptide and a variety of lipid bilayers have been studied with direct binding assays, CD and isothermal titration calorimetry. We find that the binding of this peptide is influenced by charges and dipoles located in the bilayer interfacial region, as well as by the presence of cholesterol in the bilayer. These studies show that bilayer compositional and mechanical variations found in different biological membranes can affect the partitioning of peptides into the bilayer.

Meier M. and Seelig J. (2008) Length dependence of the coil \leftrightarrow beta-sheet transition in a membrane environment. *J Am. Chem Soc.* **130**, 1017-1024.

Abstract: The most abundant structural element in protein aggregates is the beta-sheet. Designed peptides that fold into a beta-sheet structure upon binding to lipid membranes are useful models to elucidate the thermodynamic characteristics of the random coil \leftrightarrow beta-structure transition. Here, we examine the effect of strand length on the random coil \leftrightarrow beta-sheet transition of the (KIGAKI)_n peptide with the total chain length varying between 7 and 30 amino acids. The beta-sheet content of the peptides in the presence and absence of membranes was measured with circular dichroism spectroscopy. The peptides were titrated with small unilamellar lipid vesicles, and the thermodynamic binding parameters were determined with isothermal titration calorimetry (ITC). Membrane binding includes at least two processes, namely (i) the transfer of the peptide from the aqueous phase to the lipid surface and (ii) the conformational change from a random coil conformation to a beta-sheet structure. CD spectroscopy and ITC analysis demonstrate that beta-sheet formation depends cooperatively on the peptide chain length with a distinct increase in beta-structure for $n > 10-12$. Binding to the lipid membrane is an entropy-driven process as the binding enthalpy is always endothermic. The contribution of the beta-sheet folding reaction to the overall process was determined with analogues of the KIGAKI repeat where two adjacent amino acids were replaced by their D-enantiomers. The folding reaction for peptides with $n \geq 12$ is characterized by a negative free folding energy of $\Delta G(\text{degree})_{\text{beta}}$ approximately equal -0.15 kcal/mol per amino acid residue. The folding step proper is exothermic with $\Delta H(\text{degree})_{\text{beta}}$ approximately equal -0.2 to -0.6 kcal/mol per residue and counteracted by a negative entropy term $T\Delta S(\text{degree})_{\text{beta}} = -0.1$ to -0.5 kcal/mol per residue, depending on the chain length ($18 \leq n \leq 30$). For a short chain with $n = 12$, beta-sheet formation is unfavorable with $\Delta G(\text{degree})_{\text{beta}}$ approximately $+0.08$ kcal/mol per residue. Small changes of environmental parameters like pH or temperature can thus be anticipated to have profound effects on aggregation reactions, leading to amyloid fibril formation

Miller K. R. and Cistola D. P. (1993) Titration calorimetry as a binding assay for lipid-binding proteins. *Mol Cell Biochem* **123**, 29-37.

Abstract: Titration calorimetry has been evaluated as a method for obtaining binding constants and thermodynamic parameters for the cytosolic fatty acid- and lipid-binding proteins. An important feature of this method was its ability to accurately determine binding constants in a non-perturbing manner. The equilibrium was not perturbed, since there was no requirement to separate bound and free ligand in order to obtain binding parameters. Also, the structure of the lipid-protein complex was not perturbed, since native ligands were used rather than non-native analogues. As illustrated for liver fatty acid-binding protein, the method distinguished affinity classes whose dissociation constants differed by an order of magnitude or less. It also distinguished endothermic from exothermic binding reactions, as illustrated for the binding of two closely related bile salts to ileal lipid-binding protein. The main limitations of the method were its relatively low sensitivity and the difficulty working with highly insoluble ligands, such as cholesterol or saturated long-chain fatty acids. However, the signal-to-noise ratio was improved by manipulating the buffer conditions, as illustrated for oleate binding to rat intestinal fatty acid binding protein. Binding parameters are reported for oleate interactions with several wild-type and mutant lipid-binding proteins

from intestine. Where possible, the binding parameters obtained from calorimetry were compared with results obtained from fluorescence and Lipidex binding assays of comparable systems.

Mishra V. K., Palgunachari M. N., Segrest J. P., and Anantharamaiah G. M. (1994) Interactions of synthetic peptide analogs of the class A amphipathic helix with lipids. Evidence for the snorkel hypothesis. *J Biol Chem* **269**, 7185-7191.

Abstract: Class A amphipathic helices present in exchangeable plasma apolipoproteins are characterized by the location of positively charged amino acid residues at the non-polar-polar interface and negatively charged amino acid residues at the center of the polar face. The objectives of the present study were: (i) to investigate the role of hydrocarbon side chain length of the interfacial positively charged amino acid residues in the lipid affinity of class A amphipathic helices, and (ii) to investigate the importance of the nature of interfacial charge in the lipid affinity of class A amphipathic helices. Toward this end, lipid interactions of the following two analogs of the class A amphipathic helix, Ac-18A-NH₂ (acetyl-Asp-Trp-Leu-Lys-Ala-Phe-Tyr- Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe-NH₂), and Ac-18A(Lys > Haa)-NH₂ (acetyl-Asp-Trp-Leu-Haa-Ala-Phe-Tyr-Asp-Haa-Val-Ala-Glu-Haa-Leu-Haa-Glu- Ala-Phe-NH₂) (Haa = homoaminoalanine), were studied. The side chain of Haa has two CH₂ groups less than that of lysine. The lipid affinities of these two peptide analogs were compared with that of Ac-18R-NH₂, an analog of Ac-18A-NH₂ with positions of the charged amino acid residues reversed. The techniques used in these studies were circular dichroism, fluorescence spectroscopy, right-angle light scattering measurements, and differential scanning calorimetry. The results of these studies indicated the following rank order of lipid affinity: Ac-18A-NH₂ > Ac-18A(Lys > Haa)-NH₂ > Ac-18R-NH₂. These results are in agreement with the "snorkel" model proposed earlier to explain the higher lipid affinity of class A amphipathic helices (Segrest, J. P., Loof, H. D., Dohlman, J. G., Brouillette, C. G., and Anantharamaiah, G. M. (1990) *Proteins Struct. Funct. Genetics* 8, 103-117). In addition, it was observed from the differential scanning calorimetry studies that Ac-18A-NH₂ and Ac-18A(Lys > Haa)-NH₂ interact more strongly than Ac-18R-NH₂ with negatively charged dimyristoyl phosphatidylglycerol. The weaker interaction of Ac-18R-NH₂ with dimyristoyl phosphatidylglycerol is suggested to be due to electrostatic repulsion between the negatively charged lipid and the interfacial negative charges of the peptide.

Mishra V. K., Palgunachari M. N., Lund-Katz S., Phillips M. C., Segrest J. P., and Anantharamaiah G. M. (1995) Effect of the arrangement of tandem repeating units of class A amphipathic alpha-helices on lipid interaction. *J Biol Chem* **270**, 1602-1611.

Abstract: Exchangeable apolipoproteins possess tandem repeating units of class A amphipathic helical segments and many of them are linked together by proline residues. To understand the optimal arrangement of the amphipathic helices for lipid association, we have studied the interactions of three model class A amphipathic helical peptides with lipids. The three peptides are: 37pA, a dimer of 18A (DWLKAFYDKVAEKLKEAF) linked together by a Pro (18A-Pro-18A); 37aA, a dimer of 18A linked together by an Ala (18A-Ala-18A); and 36A, a dimer of 18A without any linker residue (18A-18A). Circular dichroism (CD) spectra showed that the peptides are predominantly alpha-helical in aqueous and lipid environments. Temperature dependent CD studies indicated that in buffer helix stability decreases in the order 36A > 37aA > 37pA; however, in the presence of dimyristoyl phosphatidylcholine (DMPC), the above order is reversed. The retention times of the peptides on a C18 reversed-phase high performance liquid chromatography column decreased in the order 36A > 37aA > 37pA, consistent with the lengths of the nonpolar faces of the alpha-helices being in the same order; the retention time of the parent 18A was shorter than 37pA. While 37pA adsorbed to egg phosphatidylcholine monolayers most strongly, the degree and rate of association of 36A were significantly lower. Differential scanning calorimetry indicated that, while 37pA was most effective in reducing the enthalpy of the gel to liquid-crystalline phase transition of DMPC multilamellar vesicles, 36A was least effective; 36A was even less effective than 18A. Fluorescence quenching experiments with iodide and acrylamide indicated that, in the presence of DMPC, Trp residues in 36A are most exposed to the quenchers while in 37pA they are least exposed. In the presence of DMPC, shielding of Trp in 18A from the quenchers was more than that observed with Trp residues in 36A. The results of this study suggest that the arrangement of tandem repeating amphipathic helical units which results in the formation of a class A amphipathic helix with a nonpolar face longer than five or six turns reduces the ability of the helix to associate with phospholipid.

Mishra V. K. and Palgunachari M. N. (1996) Interaction of model class A1, class A2, and class Y amphipathic helical peptides with membranes. *Biochemistry* **35**, 11210-11220.

Abstract: To test the hypothesis that differences in the lipid affinity of exchangeable apolipoproteins are due to the presence of different classes of amphipathic alpha-helical motifs which differ primarily in the distribution of charged amino acid residues, we designed and synthesized model peptides mimicking class A1, class A2, and class Y amphipathic helices present in these apolipoproteins. Both class A1 and class A2 helices have positive residues at the polar-nonpolar interface and negative residues at the center of the polar face. However, clustering of positive and negative residues is less exact in class A1 compared to class A2 helices. The class Y helices have two negative residue clusters on the polar face separating the two arms and the base of the Y motif formed by three positive residue clusters. The lipid affinities of three 18 residue model peptides representing these classes, Ac-18A1-NH2 (Ac-ELLEKWA EKLAALKEALK-NH2), Ac-18A2-NH2 (Ac-ELLEKWKEALAA LAEKLK-NH2), and Ac-18Y-NH2 (Ac-ELLKAWKEALEALKEKLA-NH2), were determined by right-angle light scattering, circular dichroism spectroscopy, differential scanning calorimetry, and fluorescence spectroscopy. The observed rank order of lipid affinity of these three peptides is: Ac-18A2-NH2 > Ac-18Y-NH2 > Ac-18A1-NH2. This order is consistent with the known lipid affinity of exchangeable apolipoproteins containing class A1, class A2, and class Y helices (class A2 > class Y > class A1). Results of this study illustrate the important role of interfacial lysine residues in modulating the lipid affinity of amphipathic helices and suggest that the effect of interfacial lysine residues in increasing lipid affinity is additive. We propose that interfacial lysine residues, in addition to widening the hydrophobic face because of snorkeling, also help anchor the amphipathic helix in the lipid bilayer.

Montich G., Scarlata S., McLaughlin S., Lehrmann R., and Seelig J. (1993) Thermodynamic characterization of the association of small basic peptides with membranes containing acidic lipids. *Biochim Biophys Acta* **1146**, 17-24.

Abstract: We measured the binding of the peptide acetyl-Trp-Lys7-amide to membranes formed from mixtures of the zwitterionic lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC) and the acidic lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (PG). Direct filtration and equilibrium dialysis measurements demonstrate that binding increases sigmoidally with the mole fraction of PG as predicted from a simple Gouy-Chapman/mass action theoretical model. We used these measurements to calibrate two binding assays, one based on the increase in Trp fluorescence that occurs when the peptide binds to the membrane, the other on the quenching of Trp fluorescence that occurs when the peptide binds to membranes containing fluorescent lipids. Both fluorescence assays demonstrate that binding does not depend strongly on temperature, which suggests the enthalpy change, ΔH , is small. Calorimetric measurements demonstrate this directly for the analogous basic peptide Lys5: ΔH congruent to +1 kcal/mol for the binding of Lys5 to sonicated phospholipid vesicles and ΔH congruent to 0 kcal/mol for its binding to large unilamellar vesicles. Thus, the decrease in the free energy that occurs when these peptides bind to the membrane is due to a positive change in the entropy of the system. Fluorescence measurements demonstrate the binding of the Trp-containing peptide to 4:1 PC/PG membranes is independent of pressure up to 2 kbar, which suggests that binding occurs without a significant change in volume.

Mosbahi K., Walker D., James R., Moore G. R., and Kleanthous C. (2006) Global structural rearrangement of the cell penetrating ribonuclease colicin E3 on interaction with phospholipid membranes. *Protein Sci* **15**, 620-627.

Abstract: Nuclease type colicins and related bacteriocins possess the unprecedented ability to translocate an enzymatic polypeptide chain across the Gram-negative cell envelope. Here we use the rRNase domain of the cytotoxic ribonuclease colicin E3 to examine the structural changes on its interaction with the membrane. Using phospholipid vesicles as model membranes we show that anionic membranes destabilize the nuclease domain of the rRNase type colicin E3. Intrinsic tryptophan fluorescence and circular dichroism show that vesicles consisting of pure DOPA act as a powerful protein denaturant toward the rRNase domain, although this interaction can be entirely prevented by the addition of salt. Binding of E3 rRNase to DOPA vesicles is an endothermic process ($\Delta H=24$ kcal mol⁻¹), reflecting unfolding of the protein. Consistent with this, binding of a highly destabilized mutant of the E3 rRNase to DOPA vesicles is exothermic. With mixed vesicles containing anionic and neutral phospholipids at a ratio of 1:3, set to mimic the charge of the Escherichia coli inner membrane, destabilization of E3 rRNase is lessened, although the melting temperature of the protein at pH 7.0 is greatly reduced from 50 degrees C to 30

degrees C. The interaction of E3 rRNase with 1:3 DOPA:DOPC vesicles is also highly dependent on both ionic strength and temperature. We discuss these results in terms of the likely interaction of the E3 rRNase and the related E9 DNase domains with the E. coli inner membrane and their subsequent translocation to the cell cytoplasm.

Myers M., Mayorga O. L., Emtage J., and Freire E. (1987) Thermodynamic characterization of interactions between ornithine transcarbamylase leader peptide and phospholipid bilayer membranes. *Biochemistry* **26**, 4309-4315.

Abstract: The interactions of the targeting sequence of the mitochondrial enzyme ornithine transcarbamylase with phospholipid bilayers of different molecular compositions have been studied by high-sensitivity heating and cooling differential scanning calorimetry, high-sensitivity isothermal titration calorimetry, fluorescence spectroscopy, and electron microscopy. These studies indicate that the leader peptide interacts strongly with dipalmitoylphosphatidylcholine (DPPC) bilayer membranes containing small mole percents of the anionic phospholipids dipalmitoylphosphatidylglycerol (DPPG) or brain phosphatidylserine (brain PS) but not with pure phosphatidylcholines. For the first time, the energetics of the leader peptide-membrane interaction have been measured directly by using calorimetric techniques. At 20 degrees C, the association of the peptide with the membrane is exothermic and characterized by an association constant of $2.3 \times 10^6 \text{ M}^{-1}$ in the case of phosphatidylglycerol-containing and $0.35 \times 10^6 \text{ M}^{-1}$ in the case of phosphatidylserine-containing phospholipid bilayers. In both cases, the enthalpy of association is -60 kcal/mol of peptide. Additional experiments using fluorescence techniques suggest that the peptide does not penetrate deeply into the hydrophobic core of the membrane. The addition of the leader peptide to DPPC/DPPG (5:1) or DPPC/brain PS (5:1) small sonicated vesicles results in vesicle fusion. The fusion process is dependent on peptide concentration and is maximal at the phase transition temperature of the vesicles and minimal at temperatures below the phase transition.

Naderi S., Doyle K., and Melchior D. L. (1995) Preferential association of membrane phospholipids with the human erythrocyte hexose transporter. *Biochim Biophys Acta* **1236**, 10-14.

Abstract: This study reports the results of an investigation to determine to what extent the influence of membrane lipids on the human erythrocyte sugar transporter protein activity (Caruthers, A. and Melchior, D.L. (1988) *Annu. Rev. Physiol.* 50, 257-271) is related to lipid/protein associations in the membrane bilayer. Differential scanning calorimetry was carried out on the human erythrocyte transport protein reconstituted into artificial bilayers formed from preselected lipids. It was found that the transport protein displays a preferential and in some cases strongly preferential affinity for specific lipid types. This association is a function of lipid head group, backbone and hydrocarbon chain length. It appears that the affinity of the transport protein for various lipids can correlate with the lipid's ability to influence transporter activity. This study further suggests that certain lipids (in this case sphingomyelin) can induce an oligomeric association of HEST monomers in the bilayer.

Nomura K. and Corzo G. (2006) The effect of binding of spider-derived antimicrobial peptides, oxyopinins, on lipid membranes. *Biochim Biophys Acta* **1758**, 1475-1482.

Abstract: Oxyopinins (Oxki1 and Oxki2) are antimicrobial peptides isolated from the crude venom of the wolf spider *Oxyopes kitabensis*. The effect of oxyopinins on lipid bilayers was investigated using high-sensitivity titration calorimetry and (^{31}P) solid-state NMR spectroscopy. High-sensitivity titration calorimetry experiments showed that the binding of oxyopinins was exothermic, and the binding enthalpies (ΔH) to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) small unilamellar vesicles (SUVs) were -18.1 kcal/mol and -15.0 kcal/mol for Oxki1 and Oxki2, respectively, and peptide partition coefficient ($K(p)$) was found to be $3.9 \times 10(3) \text{ M}^{-1}$. (^{31}P) NMR spectra of 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (DEPE) membranes in the presence of oxyopinins indicated that they induced a positive curvature in lipid bilayers. The induced positive curvature was stronger in the presence of Oxki2 than in the presence of Oxki1. (^{31}P) NMR spectra of phosphatidylcholine (PC) membranes in the presence of Oxki2 showed that Oxki2 produced micellization of membranes at low peptide concentrations, but unsaturated PC membranes or acidic phospholipids prevented micellization from occurring. Furthermore, (^{31}P) NMR spectra using membrane lipids from E. coli suggested that Oxki1 was more disruptive to bacterial membranes than Oxki2. These results strongly correlate to the known biological activity of the oxyopinins.

Nuscher B., Kamp F., Mehnert T., Odoy S., Haass C., Kahle P. J., and Beyer K. (2004) Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study. *J Biol Chem* **279**, 21966-21975.

Abstract: A number of neurodegenerative disorders, including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy, are characterized by the intracellular deposition of fibrillar aggregates that contain a high proportion of alpha-synuclein (alphaS). The interaction with the membrane-water interface strongly modulates folding and aggregation of the protein. The present study investigates the lipid binding and the coil-helix transition of alphaS, using titration calorimetry, differential scanning calorimetry, and circular dichroism spectroscopy. Titration of the protein with small unilamellar vesicles composed of zwitterionic phospholipids below the chain melting temperature of the lipids yielded exceptionally large exothermic heat values. The sigmoidal titration curves were evaluated in terms of a simple model that assumes saturable binding sites at the vesicle surface. The cumulative heat release and the ellipticity were linearly correlated as a result of simultaneous binding and helix folding. There was no heat release and folding of alphaS in the presence of large unilamellar vesicles, indicating that a small radius of curvature is necessary for the alphaS-membrane interaction. The heat release and the negative heat capacity of the protein-vesicle interaction could not be attributed to the coil-helix transition of the protein alone. We speculate that binding and helix folding of alphaS depends on the presence of defect structures in the membrane-water interface, which in turn results in lipid ordering in the highly curved vesicular membranes. This will be discussed with regard to a possible role of the protein for the stabilization of synaptic vesicle membranes.

Oldenburg K. R., Epanand R. F., D'Orfani A., Vo K., Selick H., and Epanand R. M. (1996) Conformational studies on analogs of recombinant parathyroid hormone and their interactions with phospholipids. *J Biol Chem* **271**, 17582-17591.

Abstract: Through the use of oligonucleotide-directed mutagenesis we have generated variants of a recombinant human parathyroid (PTH) hormone-(1-34)-homoserine (RPTH) in which a positively charged residue (Arg or Lys), a negatively charged residue (Glu), or a neutral residue (Gly) has been substituted at every position throughout the peptide. These 106 PTH analogs have been tested for their ability to stimulate cAMP production in the rat osteosarcoma cell line, UMR106. Analysis of these peptides led to the construction of several analogs containing multiple substitutions at sites of potential structural importance. Several of these analogs were shown to have 3-5-fold enhanced activity and receptor affinity. Circular dichroism (CD) and lipid binding studies were then performed on these analogs. Circular dichroism demonstrates enhanced helical content in the presence of lipid vesicles, particularly anionic lipid. The [Arg15,19,22,Lys29]RPTH (+6RPTH) analog requires higher concentrations of trifluoroethanol to attain enhanced helicity. The intrinsic tryptophan fluorescence of the peptides are blue shifted more in the presence of the anionic lipid dimyristoyl phosphatidylglycerol (DMPG) than with the zwitterionic lipid dimyristoyl phosphatidylcholine (DMPC). Effects of the peptides on the phase transition behavior of DMPC shows that +6RPTH has less effect on the lipid than does RPTH. This difference in lipid interaction is also exhibited with isothermal titration calorimetry, in which RPTH reacts exothermally with DMPG, while +6RPTH shows little or no heat change. The pH dependence of binding of the hydrophobic probe 1,1'-bis(4-anilino)-naphthalene-5,5'-trisulfonic acid, also shows a difference in exposure of hydrophobic sites between RPTH and +6RPTH. The +6RPTH has about a 5-fold greater affinity for receptor binding. We suggest that this enhanced activity is a consequence of the altered lipid interaction of +6RPTH, combined with increased conformational flexibility, particularly in the carboxyl-terminal region of the molecule.

Olofsson A., Borowik T., Grobner G. and Sauer-Eriksson A. E. (2007) Negatively charged phospholipid membranes induce amyloid formation of medin via an alpha-helical intermediate. *J Mol Biol* **374**, 186-194.

Abstract: Medin, a recently discovered 5.5 kDa peptide, is associated with amyloid deposits in the medial layer of human arteries and the prevalence is nearly 100% within individuals above 50 years. Presently, not much is known about its biochemical and biophysical properties or its pathway from soluble peptide to insoluble amyloid. Here we have characterized the behavior of medin in the presence of lipid membranes, using circular dichroism, isothermal titration calorimetry, differential scanning calorimetry, size exclusion chromatography, and atomic force microscopy (AFM). Medin was shown to exist as a monomer in solution with a predominantly random-coil structure. It binds lipid vesicles that have either a neutral or a negative

surface potential. Upon association to membranes containing acidic lipids, it undergoes an electrostatically driven conformational change towards a mainly alpha-helical state. Prolonged incubation converts medin from an alpha-helical structure into an amyloid beta-sheet fibrillar state as confirmed by AFM. Based on these findings, we propose a mechanism of medin-amyloid formation where medin electrostatically associates in its monomeric form to biological interfaces displaying a negative potential. This process both increases the local peptide concentration and induces an aggregation-prone alpha-helical fold.

Patel D. R., Isas J. M., Ladokhin A. S., Jao C. C., Kim Y. E., Kirsch T., Langen R., and Haigler H. T. (2005) The conserved core domains of annexins A1, A2, A5, and B12 can be divided into two groups with different Ca²⁺-dependent membrane-binding properties. *Biochemistry* **44**, 2833-2844.

Abstract: The hallmark of the annexin super family of proteins is Ca²⁺-dependent binding to phospholipid bilayers, a property that resides in the conserved core domain of these proteins. Despite the structural similarity between the core domains, studies reported herein showed that annexins A1, A2, A5, and B12 could be divided into two groups with distinctively different Ca²⁺-dependent membrane-binding properties. The division correlates with the ability of the annexins to form Ca²⁺-dependent membrane-bound trimers. Site-directed spin-labeling and Forster resonance energy transfer experimental approaches confirmed the well-known ability of annexins A5 and B12 to form trimers, but neither method detected self-association of annexin A1 or A2 on bilayers. Studies of chimeras in which the N-terminal and core domains of annexins A2 and A5 were swapped showed that trimer formation was mediated by the core domain. The trimer-forming annexin A5 and B12 group had the following Ca²⁺-dependent membrane-binding properties: (1) high Ca²⁺ stoichiometry for membrane binding (approximately 12 mol of Ca²⁺/mol of protein); (2) binding to membranes was very exothermic (> -60 kcal/ mol of protein); and (3) binding to bilayers that were in the liquid-crystal phase but not to bilayers in the gel phase. In contrast, the nontrimer-forming annexin A1 and A2 group had the following Ca²⁺-dependent membrane-binding properties: (1) lower Ca²⁺ stoichiometry for membrane binding (<or=4 mol of Ca²⁺/mol of protein); (2) binding to membranes was relatively less exothermic (< -33 kcal/ mol of protein); and (3) binding to bilayers that were in either the liquid-crystal phase or gel phase. The biological implications of this subdivision are discussed.

Patel D. R., Jao C. C., Mailliard W. S., Isas J. M., Langen R., and Haigler H. T. (2001) Calcium-dependent binding of annexin 12 to phospholipid bilayers: stoichiometry and implications. *Biochemistry* **40**, 7054-7060.

Abstract: Annexins (ANXs) are a superfamily of proteins whose functional hallmark is Ca²⁺-dependent binding to anionic phospholipids. Their core domains are usually composed of a 4-fold repeat of a conserved amino acid sequence, with each repeat containing a type II Ca²⁺ binding site that is generally thought to mediate Ca²⁺-dependent binding to the membrane. We now report that ANX12 binding to phospholipid vesicles is highly cooperative with respect to Ca²⁺ concentration (Hill constant approximately 7), thereby suggesting that more than the four well-characterized type II Ca²⁺ binding sites are involved in phospholipid binding. Two independent approaches, a novel 45Ca²⁺ copelleting assay and isothermal titration calorimetry, indicate a stoichiometry of approximately 12 mol of Ca²⁺/mol of ANX12 for binding to phospholipid vesicles. On the basis of the "low-affinity" Ca²⁺-binding sites in a number of ANX X-ray crystal structures, we propose a model for ANX12 bilayer binding that involves three types of Ca²⁺ sites in each of the four repeats. In this model, there is a complementarity between the spacing of the ANX12 Ca²⁺ binding sites and the spacing of the phospholipid headgroups in bilayers. We tested the implications of the model by manipulating the physical state of vesicles composed of phospholipids with saturated acyl chains with temperature and measuring its influence on ANX12 binding. ANX12 bound to vesicles in a Ca²⁺-dependent manner when the vesicles were in the liquid crystal phase but not when the phospholipid was in the gel phase. Furthermore, ANX12 bound initially to fluid bilayers remained bound when cooled to 4 degrees C, a temperature that should induce the gel phase transition. Overall, these studies suggest that ANX12 is well suited to being a Ca²⁺ sensor for rapid all-or-none intercellular membrane-related events.

Plager D. A. and Nelsestuen G. L. (1994) Direct enthalpy measurements of factor X and prothrombin association with small and large unilamellar vesicles. *Biochemistry* **33**, 7005-7013.

Abstract: Isothermal titration calorimetry was used to determine the enthalpy for the calcium-dependent protein conformation change and subsequent interaction of blood clotting factor X and prothrombin with

phospholipid vesicles. The effect of vesicle size was also determined. The protein conformation change was accompanied by -12 ± 1 and -7 to -15 kcal/mol for factor X and prothrombin, respectively. The range of values for prothrombin arose from use of different protein preparations and may be due to non-ideal behavior of this protein when calcium was added. The apparent enthalpy of association ($\Delta H(\text{assoc})$) of both factor X and prothrombin with phosphatidylserine (PS)/phosphatidylcholine (PC) large unilamellar vesicles (LUVs, 120 nm diameter) was shown to be near 0 kcal/mol. In comparison, $\Delta H(\text{assoc})$ for interaction with PS/PC small unilamellar vesicles (SUVs, 40 nm diameter) was -9 ± 3 and -7 ± 2 kcal/mol for factor X and prothrombin, respectively. This difference appeared complementary to $\Delta H(\text{assoc})$ for calcium binding to these vesicles. That is, the interaction of calcium was athermic with SUVs and exothermic with LUVs. While such properties might suggest a considerable difference in the manner of calcium binding to LUVs versus SUVs, little difference in the quantity of calcium bound to SUVs and LUVs was detected by equilibrium dialysis. In any event, the results indicate that protein binding to LUVs was primarily entropy driven whereas binding to SUVs was primarily enthalpy driven. The exothermic process for calcium-dependent factor X or prothrombin binding to SUVs may result from protein-induced changes in the phospholipid packing/calcium interaction, possibly related to changes in how calcium is bound to the phospholipid.

Rebolj K., Ulrich N. P., Macek P., and Sepcic K. (2006) Steroid structural requirements for interaction of ostreolysin, a lipid-raft binding cytolysin, with lipid monolayers and bilayers. *Biochim Biophys Acta* **1758**, 1662-1670.

Abstract: Ostreolysin, a cytolytic protein from the edible oyster mushroom (*Pleurotus ostreatus*), recognizes and binds specifically to membrane domains enriched in cholesterol and sphingomyelin (or saturated phosphatidylcholine). These events, leading to permeabilization of the membrane, suggest that a cholesterol-rich liquid-ordered membrane phase, which is characteristic of lipid rafts, could be its possible binding site. In this work, we present effects of ostreolysin on membranes containing various steroids. Binding and membrane permeabilizing activity of ostreolysin was studied using lipid mono- and bilayers composed of sphingomyelin combined, in a 1/1 molar ratio, with natural and synthetic steroids (cholesterol, ergosterol, beta-sitosterol, stigmaterol, lanosterol, 7-dehydrocholesterol, cholesteryl acetate, and 5-cholesten-3-one). Binding to membranes and lytic activity of the protein are both shown to be dependent on the intact sterol 3 β -OH group, and are decreased by introducing additional double bonds and methylation of the steroid skeleton or C17-isoctyl chain. The activity of ostreolysin mainly correlates with the ability of the steroids to promote formation of liquid-ordered membrane domains, and is the highest with cholesterol-containing membranes. Furthermore, increasing the cholesterol concentration enhanced ostreolysin binding in a highly cooperative manner, suggesting that the membrane lateral distribution and accessibility of the sterols are crucial for the activity of this new member of cholesterol-dependent cytolysins.

Rezansoff A. J., Hunter H. N., Jing W., Park I. Y., Kim S. C., and Vogel H. J. (2005) Interactions of the antimicrobial peptide Ac-FRWWHR-NH(2) with model membrane systems and bacterial cells. *J Pept Res* **65**, 491-501.

Abstract: The acetylated and amidated hexapeptide FRWWHR (combi-2), previously identified by combinatorial chemistry methods, shows strong antimicrobial activity. The binding of the peptide to 1-palmitoyl-2-oleoyl-sn-glycero-3-[(phospho-rac-(1-glycerol))] (POPG) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles was studied using fluorescence spectroscopy and isothermal titration calorimetry (ITC). Differential scanning calorimetry (DSC) with dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) multilamellar vesicles was performed to determine changes in the lipid phase behaviour upon binding the peptide. Two-dimensional proton nuclear magnetic resonance (NMR) spectroscopy, to solve the bound peptide structure, was performed in the presence of dodecylphosphatidylcholine (DPC) and sodium dodecyl sulphate (SDS) micelles. The fluorescence, ITC and DSC studies indicate that the peptide interacts preferentially with lipid vesicles containing negatively charged head groups. Conformational information determined using NMR indicate that the combi-2 peptide adopts a coiled amphipathic conformation when bound to SDS and DPC micelles. Leakage assays indicate that the peptide is not very efficient at causing leakage from calcein-filled large unilamellar vesicles comprised of POPG/POPC (1 : 1). The rapid passage of either the fluorescent-tagged peptides combi-2 or the previously studied peptide Ac-RRWRF-NH(2) (combi-1) into *Escherichia coli* and *Staphylococcus*

aureus suggests that instead of membrane disruption, the main bactericidal site of action of these peptides might be located inside bacteria.

Rourke A. M., Cha Y., and Collins D. (1996) Stabilization of granulocyte colony-stimulating factor and structurally analogous growth factors by anionic phospholipids. *Biochemistry* **35**, 11913-11917.

Abstract: Recombinant granulocyte colony-stimulating factor (rhG-CSF) interacts with liposomes composed of the anionic phospholipid dioleoylphosphatidylglycerol (DOPG), and this interaction enhances the stability of the protein [Collins, D., & Cha, Y. (1994) *Biochemistry* 33, 4521-4526]. In the present studies, we have examined the interaction of rhG-CSF with phospholipids other than DOPG. Fluorescence spectroscopy of rhG-CSF with a variety of lipid vesicles demonstrated that rhG-CSF inserts into bilayers of anionic, but not zwitterionic, phospholipids. Isothermal titration calorimetry of the interaction between DMPG and rhG-CSF indicates that the binding is saturable and involves 10 lipids/rhG-CSF. Studies of phosphatidylglycerols with varying alkyl chain lengths determined that the stabilization of rhG-CSF by anionic phospholipids required a certain alkyl chain length; no stabilization was observed with lipids of shorter chain length. Also investigated was the stabilization of other growth factors, which are structurally similar to rhG-CSF, by anionic phospholipids. These proteins include recombinant porcine somatotropin (rpSt), recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF), recombinant human interleukin 4 (rhIL-4), and recombinant human interleukin 2 (rhIL-2). The helical secondary structure of the proteins was recoverable after heating and cooling in the presence of anionic phospholipids as observed by circular dichroism; the presence of zwitterionic lipids did not induce this effect. Results of these investigations concluded that a group of structurally similar proteins interact preferentially with anionic phospholipids and that the complexation of the growth factors with vesicles composed of anionic phospholipids improves the stability of the proteins under conditions where they normally denature.

Saito H., Dhanasekaran P., Baldwin F., Weisgraber K. H., Lund-Katz S., and Phillips M. C. (2001) Lipid binding-induced conformational change in human apolipoprotein E. Evidence for two lipid-bound states on spherical particles. *J Biol Chem* **276**, 40949-40954.

Abstract: Apolipoprotein (apo) E contains two structural domains, a 22-kDa (amino acids 1-191) N-terminal domain and a 10-kDa (amino acids 223-299) C-terminal domain. To better understand apoE-lipid interactions on lipoprotein surfaces, we determined the thermodynamic parameters for binding of apoE4 and its 22- and 10-kDa fragments to triolein-egg phosphatidylcholine emulsions using a centrifugation assay and titration calorimetry. In both large (120 nm) and small (35 nm) emulsion particles, the binding affinities decreased in the order 10-kDa fragment approximately 34-kDa intact apoE4 > 22-kDa fragment, whereas the maximal binding capacity of intact apoE4 was much larger than those of the 22- and 10-kDa fragments. These results suggest that at maximal binding, the binding behavior of intact apoE4 is different from that of each fragment and that the N-terminal domain of intact apoE4 does not contact lipid. Isothermal titration calorimetry measurements showed that apoE binding to emulsions was an exothermic process. Binding to large particles is enthalpically driven, and binding to small particles is entropically driven. At a low surface concentration of protein, the binding enthalpy of intact apoE4 (-69 kcal/mol) was approximately equal to the sum of the enthalpies for the 22- and 10-kDa fragments, indicating that both the 22- and 10-kDa fragments interact with lipids. In a saturated condition, however, the binding enthalpy of intact apoE4 (-39 kcal/mol) was less exothermic and rather similar to that of each fragment, supporting the hypothesis that only the C-terminal domain of intact apoE4 binds to lipid. We conclude that the N-terminal four-helix bundle can adopt either open or closed conformations, depending upon the surface concentration of emulsion-bound apoE.

Saito H., Dhanasekaran P., Baldwin F., Weisgraber K. H., Phillips M. C., and Lund-Katz S. (2003) Effects of polymorphism on the lipid interaction of human apolipoprotein E. *J Biol Chem* **278**, 40723-40729.

Abstract: ApoE exists as three common isoforms, apoE2, apoE3, and apoE4; apoE2 and apoE3 preferentially bind to high density lipoproteins, whereas apoE4 prefers very low density lipoproteins (VLDL). To understand the molecular basis for the different lipoprotein distributions of these isoforms in human plasma, we examined the lipid-binding properties of the apoE isoforms and some mutants using lipid emulsions. With both large (120 nm) and small (35 nm) emulsion particles, the binding affinity of apoE4 was much higher than that of apoE2 and apoE3, whereas the maximal binding capacities were similar among the three isoforms. The 22-kDa N-terminal fragment of apoE4 displayed a much higher binding capacity than did apoE2 and apoE3. The apoE4(E255A) mutant, which has no electrostatic

interaction between Arg61 and Glu255, showed binding behavior similar to that of apoE3, indicating that N- and C-terminal domain interaction in apoE4 is responsible for its high affinity for lipid. In addition, the apoE3(P267A) mutant, which is postulated to contain a long alpha-helix in the C-terminal domain, had significantly decreased binding capacities for both sizes of emulsion particle, suggesting that the apoE4 preference for VLDL is not due to a stabilized long alpha-helical structure. Isothermal titration calorimetry measurements showed that there is no significant difference in thermodynamic parameters for emulsion binding among the apoE isoforms. However, fluorescence measurements of 8-anilino-1-naphthalenesulfonic acid binding to apoE indicated that apoE4 has more exposed hydrophobic surface compared with apoE3 mainly due to the different tertiary organization of the C-terminal domain. The less organized structure in the C-terminal domain of apoE4 leads to the higher affinity for lipid, contributing to its preferential association with VLDL. In fact, we found that apoE4 binds to VLDL with higher affinity compared with apoE3.\

Saito H., Dhanasekaran P., Nguyen D., Deridder E., Holvoet P., Lund-Katz S., and Phillips M. C. (2004) Alpha-helix formation is required for high affinity binding of human apolipoprotein A-I to lipids. *J Biol Chem* **279**, 20974-20981.

Abstract: Apolipoprotein (apo) A-I is thought to undergo a conformational change during lipid association that results in the transition of random coil to alpha-helix. Using a series of deletion mutants lacking different regions along the molecule, we examined the contribution of alpha-helix formation in apoA-I to the binding to egg phosphatidylcholine (PC) small unilamellar vesicles (SUV). Binding isotherms determined by gel filtration showed that apoA-I binds to SUV with high affinity and deletions in the C-terminal region markedly decrease the affinity. Circular dichroism measurements demonstrated that binding to SUV led to an increase in alpha-helix content, but the helix content was somewhat less than in reconstituted discoidal PC.apoA-I complexes for all apoA-I variants, suggesting that the helical structure of apoA-I on SUV is different from that in discs. Isothermal titration calorimetry showed that the binding of apoA-I to SUV is accompanied by a large exothermic heat and deletions in the C-terminal regions greatly decrease the heat. Analysis of the rate of release of heat on binding, as well as the kinetics of quenching of tryptophan fluorescence by brominated PC, indicated that the opening of the N-terminal helix bundle is a rate-limiting step in apoA-I binding to the SUV surface. Significantly, the correlation of thermodynamic parameters of binding with the increase in the number of helical residues revealed that the contribution of alpha-helix formation upon lipid binding to the enthalpy and the free energy of the binding of apoA-I is -1.1 and -0.04 kcal/mol per residue, respectively. These results indicate that alpha-helix formation, especially in the C-terminal regions, provides the energetic source for high affinity binding of apoA-I to lipids.

Sakai H., Hisamoto S., Fukutomi I., Sou K., Takeoka S., and Tsuchida E. (2004) Detection of lipopolysaccharide in hemoglobin-vesicles by Limulus amoebocyte lysate test with kinetic-turbidimetric gel clotting analysis and pretreatment of surfactant. *J Pharm Sci* **93**, 310-321.

Abstract: A method to quantitatively measure the bacterial endotoxin content (lipopolysaccharide, LPS) in phospholipid vesicles or liposomes is necessary because the conventional Limulus amoebocyte lysate (LAL) test does not provide an accurate measurement due to the hydrophobic interaction of LPS and vesicles that shields the activity of LPS to clot the LAL coagulant. This interference was evident from isothermal titration calorimetry results in our study that clearly demonstrated the insertion of the LPS molecule into the phospholipid bilayer membrane. Hemoglobin-vesicles (HbVs; particle diameter = 251 +/- 80 nm; [Hb] = 10 g/dL) are artificial oxygen carriers encapsulating a conc. Hb solution in phospholipid vesicles, and their oxygen transporting ability has been extensively studied. To accurately measure the LPS content in the HbV suspension, we tested the solubilization of HbV with deca(oxyethylene) dodecyl ether (C(12)E(10)), used to release the LPS entrapped in the vesicles, as a pretreatment for the succeeding LAL assay of the kinetic-turbidimetric gel clotting (detecting wavelength, 660 nm). The C(12)E(10) surfactant interferes with the gel clotting in a concentration-dependent manner, and the optimal condition was determined in terms of minimizing the dilution factor and C(12)E(10) concentration. We clarified the condition that allowed the measurement of LPS at >0.1 endotoxin units (EU)/mL in the HbV suspension. Moreover, the utilization of histidine-immobilized agarose gel effectively concentrated the trace amount of LPS from the C(12)E(10)-solubilized HbV solution and washed out C(12)E(10) as an inhibitory element. The LAL assay with the LPS-adsorbed gel resulted in the detection limit of 0.0025 EU/mL. Pretreatment

with C(12)E(10) would be applicable not only to HbVs but also to other drug delivery systems using phospholipid vesicles encapsulating or incorporating functional molecules.

Scott, D.L., Diez, G., and Goldmann, W.H.. (2006) Protein-lipid interactions: correlation of a predictive algorithm for lipid-binding sites with three-dimensional structural data. *Theor Biol Med Model* **3**, 17.
Abstract: BACKGROUND: Over the past decade our laboratory has focused on understanding how soluble cytoskeleton-associated proteins interact with membranes and other lipid aggregates. Many protein domains mediating specific cell membrane interactions appear by fluorescence microscopy and other precision techniques to be partially inserted into the lipid bilayer. It is unclear whether these protein-lipid interactions are dependent on shared protein motifs or unique regional physiochemistry, or are due to more global characteristics of the protein. RESULTS: We have developed a novel computational program that predicts a protein's lipid-binding site(s) from primary sequence data. Hydrophobic labeling, Fourier transform infrared spectroscopy (FTIR), film balance, T-jump, CD spectroscopy and calorimetry experiments confirm that the interfaces predicted for several key cytoskeletal proteins (alpha-actinin, Arp2, CapZ, talin and vinculin) partially insert into lipid aggregates. The validity of these predictions is supported by an analysis of the available three-dimensional structural data. The lipid interfaces predicted by our algorithm generally contain energetically favorable secondary structures (e.g., an amphipathic alpha-helix flanked by a flexible hinge or loop region), are solvent-exposed in the intact protein, and possess favorable local or global electrostatic properties. CONCLUSION: At present, there are few reliable methods to determine the region of a protein that mediates biologically important interactions with lipids or lipid aggregates. Our matrix-based algorithm predicts lipid interaction sites that are consistent with the available biochemical and structural data. To determine whether these sites are indeed correctly identified, and whether use of the algorithm can be safely extended to other classes of proteins, will require further mapping of these sites, including genetic manipulation and/or targeted crystallography.

Seelig A., Alt T., Lotz S., and Holzemann G. (1996) Binding of substance P agonists to lipid membranes and to the neurokinin-1 receptor. *Biochemistry* **35**, 4365-4374.
Abstract: Three new analogues of the neuropeptide substance P (SP) were synthesized. The C-terminal message segment was made more hydrophilic in (Arg9)SP or more hydrophobic in (Nle9)SP. In (AcPro2, Arg9)SP the charge at the N-terminal address segment was reduced, while that of the message segment was increased. The rationale underlying these substitutions was to correlate the physical-chemical properties of the SP-analogues, in particular their lipid-induced conformation and membrane-binding affinity, with receptor binding and functional activity. In solution, all three analogues exhibited random coil conformations as evidenced by circular dichroism spectroscopy. Addition of SDS micelles induced partially alpha-helical structures. The same structure was also produced by negatively charged lipid vesicles for (AcPro2, Arg9)SP and (Arg9)SP whereas both alpha-helix-like structures and beta-sheet structures were observed for SP and (Nle9)SP. The measurement of the Gibbs adsorption isotherms and monolayer expansion studies provided quantitative data on the surface area requirement and on the membrane penetration area of the SP analogues. The thermodynamic parameters for lipid binding were determined with monolayer expansion for measurements and high-sensitivity titration calorimetry. The apparent binding constants, K_{app} , for membranes containing 100% POPG were of the order of 10^3 - 10^5 M^{-1} . The binding was due to electrostatic attraction of the cationic peptides to the negatively charged membrane surface. The intrinsic (hydrophobic) binding constants, obtained after correcting for electrostatic effects, were much smaller with $K_p=10 \pm 1$ M^{-1} for (Arg9)SP, 9 ± 1 M^{-1} for (AcPro2, Arg9)SP, and 39 ± 3 M^{-1} for (Nle9)SP. The measurement of the binding affinities to the NK-1 receptor and of the in vitro activities showed that all three peptides behaved as agonists. Their binding affinity to the neurokinin-1 receptor decreased with the size of the side chains at position 9 of the amino acid sequence but was independent of the cationic charge of the peptides. The fact that even the highly charged (Arg9)SP has agonistic activity provides evidence that the binding epitope at the receptor is in a rather hydrophilic environment. This finding is in agreement with the low hydrophobic binding constants and the weak penetration of the three peptides into negatively charged membranes. It argues against a membrane mediated receptor mechanism and suggests that the agonist approaches the receptor binding site from the aqueous phase.

Seelig A., Blatter X. L., Frentzel A., and Isenberg G. (2000) Phospholipid binding of synthetic talin peptides provides evidence for an intrinsic membrane anchor of talin. *J Biol Chem* **275**, 17954-17961.

Abstract: Talin, an actin-binding protein, is assumed to anchor at the membrane via an intrinsic amino acid sequence. Three N-terminal talin fragments, 21-39 (S19), 287-304 (H18), and 385-406 (H17) have been proposed as potential membrane anchors. The interaction of the corresponding synthetic peptides with lipid model systems was investigated with CD spectroscopy, isothermal titration calorimetry, and monolayer expansion measurements. The membrane model systems were neutral or negatively charged small unilamellar vesicles or monolayers with a lateral packing density of bilayers (32 mN/m). S19 partitions into charged monolayers/bilayers with a penetration area $A(p) = 140 \pm 30 \text{ \AA}^2$ and a free energy of binding of $\Delta G(0) = -5.7 \text{ kcal/mol}$, thereby forming a partially alpha-helical structure. H18 does not interact with lipid monolayers or bilayers. H17 penetrates into neutral and charged monolayers/bilayers with $A(p) = 148 \pm 23 \text{ \AA}^2$ and $A(p) = 160 \pm 15 \text{ \AA}^2$, respectively, forming an alpha-helix in the membrane-bound state. Membrane partitioning is mainly entropy-driven. Under physiological conditions the free energy of binding to negatively charged membranes is $\Delta G(0) = -9.4 \text{ kcal/mol}$ with a hydrophobic contribution of $\Delta G(h) = -7.8 \text{ kcal/mol}$, comparable to that of post-translationally attached membrane anchors, and an electrostatic contribution of $\Delta G(h) = -1.6 \text{ kcal/mol}$. The latter becomes more negative with decreasing pH. We show that H17 provides the binding energy required for a membrane anchor.

Seelig J. (2004) Thermodynamics of lipid-peptide interactions. *Biochim Biophys Acta* **1666**, 40-50.

Abstract: This review is focused on peptide molecules which exhibit a limited solubility in the aqueous phase and bind to the lipid membrane from the aqueous medium. Surface adsorption, membrane insertion, and specific binding are usually accompanied by changes in the heat content of the system and can be measured conveniently with isothermal titration calorimetry, avoiding the necessity of peptide labeling. The driving forces for peptide adsorption and binding are hydrophobicity, electrostatics, and hydrogen bonding. An exclusively hydrophobic interaction is exemplified by the immunosuppressant drug cyclosporine A. Its insertion into the membrane can be described by a simple partition equilibrium $X_b = K_0 C_{eq}$. If peptide and membrane are both charged, electrostatic interactions are dominant leading to nonlinear binding curves. The concentration of the peptide near the membrane interface can then be much larger than its bulk concentration. Electrostatic effects must be accounted for by means of the Gouy-Chapman theory before conventional binding models can be applied. A small number of peptides and proteins bind with very high affinity to a specific lipid species only. This is illustrated for the lantibiotic cinnamycin (Ro 09-0198) which forms a 1:1 complex with phosphatidylethanolamine with a binding constant of 10^8 M^{-1} . Membrane adsorption and insertion can be accompanied by conformational transitions facilitated, in part, by hydrogen bonding mechanisms. The two membrane-induced conformational changes to be discussed are the random coil-to-alpha-helix transition of amphipathic peptides and the random coil-to-beta-structure transition of Alzheimer peptides.

Seelig J., Nebel S., Ganz P., and Bruns C. (1993) Electrostatic and nonpolar peptide-membrane interactions. Lipid binding and functional properties of somatostatin analogues of charge $z = +1$ to $z = +3$. *Biochemistry* **32**, 9714-9721.

Abstract: The interaction of four structurally related somatostatin analogues (effective electric charge $+0.4 < \text{or} = < \text{or} = +3$) with lipid membranes was studied with titration calorimetry and was compared with the functional activity of the peptides. Surface activity measurements provided average cross-sections of 70 or 135 \AA^2 , indicating that the cyclic molecules orient at the air-water interface with their ring system either parallel ($z = +3$) or perpendicular ($z = +1$) to the surface or switching between the two orientations according to the surface density ($z = +2$). The nonspecific binding of the peptides to sonified lipid vesicles was enthalpy-driven with a ΔH of -4 to -7.5 kcal/mol. A consistent quantitative analysis of the binding isotherms was achieved by combining electrostatic attractions, calculated via the Gouy-Chapman theory, with a nonspecific surface partition equilibrium for the nonpolar interactions. The electrostatic attraction of the cationic peptides varied strongly according to the peptide charge. Due to the flat ring structure of the cyclic peptides, their true physical charge was sensed at the membrane surface, and no "charge screening" was observed. Peptide binding to the negative charged membrane was accompanied by a proton-uptake of the N-terminal amino group of 0.23-0.38 H^+ /peptide. Deviations from the theoretical prediction of 0.39 H^+ /peptide can be explained by a preferential binding of the nonprotonated species. The nonpolar interactions, as described by the surface partition coefficients of the four peptides, fell into a narrow range of K congruent to 50-230 M^{-1} whereas the apparent overall binding constants were between 200 and 5000 M^{-1} . (ABSTRACT TRUNCATED AT 250 WORDS).

Seelig J., Lehrmann R., and Terzi E. (1995) Domain formation induced by lipid-ion and lipid-peptide interactions. *Mol Membr Biol* **12**, 51-57.

Abstract: High sensitivity titration calorimetry was performed for metal ions such as calcium and lanthanum and for different types of Alzheimer peptides. Ca²⁺ adsorbs to mixed phosphatidylcholine (PC)/phosphatidylglycerol (PG) membranes with an endothermic reaction enthalpy of ΔH approximately +0.1 kcal/mol. La³⁺ binds to sonified PC vesicles with a reaction enthalpy of ΔH approximately + 1.8 kcal/mol. The binding constants are of the order of 10 M⁻¹ for Ca²⁺ and 4 x 10³ M⁻¹ for La³⁺. The role of lipids in the random coil \leftrightarrow beta-sheet equilibrium of different types of Alzheimer model peptides was investigated with circular dichroism (CD) and high sensitivity titration calorimetry. Alzheimer peptide beta AP(1-40)OH and several fragments of this peptide undergo a concentration-dependent, co-operative random coil \leftrightarrow beta-sheet transition in solution which can be described by a linear association model with a nucleation parameter sigma approximately 0.2-0.01 and a growth parameter s approximately 10⁴ M⁻¹. Addition of sonified lipid vesicles containing negatively charged lipids shifts the equilibrium towards the beta-sheet conformation. This can be explained by an aggregation phenomenon at the lipid/water interphase. The cationic peptides are attracted to the negatively charged membrane surface causing a local increase in peptide concentration. The high peptide concentration, together with the ordering of the peptide molecules on the membrane surface, facilitates beta-sheet formation, constituting the first experimental evidence for the induction of beta-sheet formation via the membrane surface. The binding of Alzheimer peptide fragments to the lipid membrane is accompanied by an exothermic heat of reaction with ΔH in the range -2 - -8 kcal/mol.

Seelig J. (1997) Titration calorimetry of lipid-peptide interactions. *Biochim Biophys Acta* **1331**, 103-116.

Shalev D. E., Rotem S., Fish A., and Mor A. (2006) Consequences of N-acylation on structure and membrane binding properties of dermaseptin derivative K4-S4-(1-13). *J Biol Chem* **281**, 9432-9438.

Abstract: Acyl conjugation to antimicrobial peptides is known to enhance antimicrobial properties. Here, we investigated the consequences of aminolauryl (NC(12)) conjugation to the dermaseptin derivative K(4)-S4-(1-13) (P) on binding properties to bilayer models mimicking bacterial plasma membrane, which is often cited as the ultimate site of action. Isothermal titration calorimetry revealed that acylation was responsible for enhancing the binding affinity of NC(12)-P compared with P (K = 13 x 10⁵ and 1.5 x 10⁵ m⁻¹, respectively). Surface plasmon resonance measurements confirmed the isothermal titration calorimetry results (K_{app} = 12.6 x 10⁵ and 1.53 x 10⁵ m⁻¹, respectively) and further indicated that enhanced adhesion affinity (K_{adhesion} = 3 x 10⁵ and 1 x 10⁵ m⁻¹, respectively) was coupled to enhanced tendency to insert within the bilayer (K_{insertion} = 4.5 and 1.5, respectively). To gain insight into the molecular basis for these observations, we investigated the three-dimensional structures in the presence of dodecylphosphocholine using NMR. The ensemble of NMR-calculated structures (backbone root mean square deviation <0.6 Å) showed that the acyl moiety was responsible for a significant molecular reorganization, possibly affecting the electrostatic potential distribution in NC(12)-P relative to that of P. The combined data present compelling evidence in support of the hypothesis that N-acylation affects antimicrobial properties by modifying the secondary structure of the peptide in a manner that facilitates contact with the membrane and consequently increases its disruption.

Stahelin R. V., Digman M. A., Medkova M., Ananthanarayanan B., Melowic H. R., Rafter J. D., and Cho W. (2005) Diacylglycerol-induced membrane targeting and activation of protein kinase Cepsilon: mechanistic differences between protein kinases Cdelta and Cepsilon. *J Biol Chem* **280**, 19784-19793.

Abstract: Two novel protein kinases C (PKC), PKCdelta and PKCepsilon, have been reported to have opposing functions in some mammalian cells. To understand the basis of their distinct cellular functions and regulation, we investigated the mechanism of in vitro and cellular sn-1,2-diacylglycerol (DAG)-mediated membrane binding of PKCepsilon and compared it with that of PKCdelta. The regulatory domains of novel PKC contain a C2 domain and a tandem repeat of C1 domains (C1A and C1B), which have been identified as the interaction site for DAG and phorbol ester. Isothermal titration calorimetry and surface plasmon resonance measurements showed that isolated C1A and C1B domains of PKCepsilon have comparably high affinities for DAG and phorbol ester. Furthermore, in vitro activity and membrane binding analyses of PKCepsilon mutants showed that both the C1A and C1B domains play a role in the DAG-induced membrane binding and activation of PKCepsilon. The C1 domains of PKCepsilon are not conformationally restricted and readily accessible for DAG binding unlike those of PKCdelta.

Consequently, phosphatidylserine-dependent unleashing of C1 domains seen with PKCdelta was not necessary for PKCepsilon. Cell studies with fluorescent protein-tagged PKCs showed that, due to the lack of lipid headgroup selectivity, PKCepsilon translocated to both the plasma membrane and the nuclear membrane, whereas PKCdelta migrates specifically to the plasma membrane under the conditions in which DAG is evenly distributed among intracellular membranes of HEK293 cells. Also, PKCepsilon translocated much faster than PKCdelta due to conformational flexibility of its C1 domains. Collectively, these results provide new insight into the differential activation mechanisms of PKCdelta and PKCepsilon based on different structural and functional properties of their C1 domains.

Stahelin R. V., Digman M. A., Medkova M., Ananthanarayanan B., Rafter J. D., Melowic H. R., and Cho W. (2004) Mechanism of diacylglycerol-induced membrane targeting and activation of protein kinase Cdelta. *J Biol Chem* **279**, 29501-29512.

Abstract: The regulatory domains of novel protein kinases C (PKC) contain two C1 domains (C1A and C1B), which have been identified as the interaction site for sn-1,2-diacylglycerol (DAG) and phorbol ester, and a C2 domain that may be involved in interaction with lipids and/or proteins. Although recent reports have indicated that C1A and C1B domains of conventional PKCs play different roles in their DAG-mediated membrane binding and activation, the individual roles of C1A and C1B domains in the DAG-mediated activation of novel PKCs have not been fully understood. In this study, we determined the roles of C1A and C1B domains of PKCdelta by means of in vitro lipid binding analyses and cellular protein translocation measurements. Isothermal titration calorimetry and surface plasmon resonance measurements showed that isolated C1A and C1B domains of PKCdelta have opposite affinities for DAG and phorbol ester; i.e. the C1A domain with high affinity for DAG and the C1B domain with high affinity for phorbol ester. Furthermore, in vitro activity and membrane binding analyses of PKCdelta mutants showed that the C1A domain is critical for the DAG-induced membrane binding and activation of PKCdelta. The studies also indicated that an anionic residue, Glu(177), in the C1A domain plays a key role in controlling the DAG accessibility of the conformationally restricted C1A domain in a phosphatidylserine-dependent manner. Cell studies with enhanced green fluorescent protein-tagged PKCdelta and mutants showed that because of its phosphatidylserine specificity PKCdelta preferentially translocated to the plasma membrane under the conditions in which DAG is randomly distributed among intracellular membranes of HEK293 cells. Collectively, these results provide new insight into the differential roles of C1 domains in the DAG-induced membrane activation of PKCdelta and the origin of its specific subcellular localization in response to DAG.

Stolt P. C., Chen Y., Liu P., Bock H. H., Blacklow S. C., and Herz J. (2005) Phosphoinositide Binding by the Disabled-1 PTB Domain Is Necessary for Membrane Localization and Reelin Signal Transduction. *J. Biol. Chem* **280**, 9671-9677.

Abstract: Disabled-1 (Dab1) is an essential adaptor protein that functions in the Reelin signaling pathway and is required for the regulation of neuronal migration during embryonic development. Dab1 interacts with NPXY motifs in the cytoplasmic tails of the lipoprotein receptors ApoER2 and very low density lipoprotein receptor through an amino-terminal phosphotyrosine binding (PTB) domain. Binding of Reelin to these receptors leads to tyrosine phosphorylation of Dab1 and the initiation of a signaling cascade that results in remodeling of the cytoskeleton. Structural and biochemical studies of the Dab1 PTB domain have demonstrated that this domain binds to both the NPXY peptide motif in the lipoprotein receptor tails as well as to the head group of phosphoinositide 4,5-P₂ through energetically independent mechanisms. Here we have investigated how phosphoinositide binding by the Dab1 PTB domain influences Reelin signal transduction. Our findings in cultured primary neurons that have been transduced with lentiviral constructs expressing mutant *Dab1* forms reveal that phosphoinositide binding by the Dab1 PTB domain is necessary for proper membrane localization of Dab1 and for effective transduction of a Reelin signal.

Suurkuusk M. and Singh S. K. (2000) Formation of HDL-like complexes from apolipoprotein A-I(M) and DMPC. *Int J Pharm* **194**, 21-38.

Abstract: Conditions for the preparation of reconstituted high density lipoproteins (HDLs) by incubation of the synthetic lipid dimyristoylphosphatidylcholine (DMPC) and recombinant apolipoprotein A-I(M) have been investigated as a function of ratio of incubation lipid to protein, incubation temperature and the lipid form (multilamellar (MLV) or small unilamellar (SUV) vesicles). The size distributions of the resultant lipid-protein complex particles from various incubations have been evaluated by native gel

electrophoresis. Structural changes of the protein after incorporation into these complex particles have been estimated by CD. Thermal characteristics of the particles has been examined by DSC and correlated with CD results. Titration calorimetry has been used to obtain interaction parameters based on a simplified binding model. It is hypothesized that the major enthalpic step in the production of rHDLs is the primary association step between protein and lipid vesicles. It has been shown that by raising the temperature and incubation ratio, the formation of rHDL particles can be directed towards smaller size and a narrower size distribution. The results have been described on the basis of a model where formation of discoidal particles requires prior saturation of vesicle surface area by adsorbed protein, thus explaining differences between particles formed from MLVs and SUVs.

Tanaka M., Saito H., Dhanasekaran P., Wehrli S., Handa T., Lund-Katz S., and Phillips M. C. (2005) Effects of the core lipid on the energetics of binding of ApoA-I to model lipoprotein particles of different sizes. *Biochemistry* **44**, 10689-10695.

Abstract: Interaction of apolipoproteins (apo) with lipid surfaces plays crucial roles in lipoprotein metabolism and cholesterol homeostasis. To elucidate the thermodynamics of binding of apoA-I to lipid, we used lipid emulsions composed of triolein (TO) and egg phosphatidylcholine (PC) as lipoprotein models. Determination of the level of binding of wild-type (WT) apoA-I and some deletion mutants to large (120 nm diameter; LEM) and small (35 nm diameter; SEM) emulsions indicated that N-terminal (residues 44-65) and C-terminal (residues 190-243 and 223-243) deletions have large effects on lipid interaction, whereas deletion of the central region (residues 123-166) has little effect. Substitution of amino acids at either L230 or L230, L233, and Y236 with proline residues also decreases the level of binding, indicating that an alpha-helix conformation in this C-terminal region is required for efficient lipid binding. Calorimetry showed that binding of WT apoA-I to SEM generates endothermic heat (ΔH approximately 30 kcal/mol) in contrast to the exothermic heat (ca. -85 kcal/mol) generated upon binding to LEM and egg PC small unilamellar vesicles (SUV). This exothermic heat arises from an approximately 25% increase in alpha-helix content, and it drives the binding of apoA-I to LEM and SUV. There is a similar increase in alpha-helix content of apoA-I upon binding to either SEM or SUV, but the binding of apoA-I to SEM is an entropy-driven process. These results suggest that the presence of a core triglyceride modifies the highly curved SEM surface packing and thereby the thermodynamics of apoA-I binding in a manner that compensates for the exothermic heat generated by alpha-helix formation.

Tanaka M., Dhanasekaran P., Nguyen D., Ohta S., Lund-Katz S., Phillips M. C., and Saito H. (2006) Contributions of the N- and C-terminal helical segments to the lipid-free structure and lipid interaction of apolipoprotein A-I. *Biochemistry* **45**, 10351-10358.

Abstract: The tertiary structure of lipid-free apolipoprotein (apo) A-I in the monomeric state comprises two domains: a N-terminal alpha-helix bundle and a less organized C-terminal domain. This study examined how the N- and C-terminal segments of apoA-I (residues 1-43 and 223-243), which contain the most hydrophobic regions in the molecule and are located in opposite structural domains, contribute to the lipid-free conformation and lipid interaction. Measurements of circular dichroism in conjunction with tryptophan and 8-anilino-1-naphthalenesulfonic acid fluorescence data demonstrated that single (L230P) or triple (L230P/L233P/Y236P) proline insertions into the C-terminal alpha helix disrupted the organization of the C-terminal domain without affecting the stability of the N-terminal helix bundle. In contrast, proline insertion into the N terminus (Y18P) disrupted the bundle structure in the N-terminal domain, indicating that the alpha-helical segment in this region is part of the helix bundle. Calorimetric and gel-filtration measurements showed that disruption of the C-terminal alpha helix significantly reduced the enthalpy and free energy of binding of apoA-I to lipids, whereas disruption of the N-terminal alpha helix had only a small effect on lipid binding. Significantly, the presence of the Y18P mutation offset the negative effects of disruption/removal of the C-terminal helical domain on lipid binding, suggesting that the alpha helix around Y18 concealed a potential lipid-binding region in the N-terminal domain, which was exposed by the disruption of the helix-bundle structure. When these results are taken together, they indicate that the alpha-helical segment in the N terminus of apoA-I modulates the lipid-free structure and lipid interaction in concert with the C-terminal domain.

Tempone A. J., Bianconi M. L., and Rumjanek F. D. (1997) The interaction of human LDL with the tegument of adult *Schistosoma mansoni*. *Mol Cell Biochem* **177**, 139-144.

Abstract: The occurrence of a receptor for human LDL was investigated in the tegument of adult

Schistosoma mansoni employing several approaches. Binding of LDL to SDS-PAGE fractionated tegument proteins was measured directly on nitro-cellulose membranes and visualised by an anti-human LDL antibody. Proteins with an Mr of 60, 35 and 14 kDa were evidenced. Affinity chromatography of 125I-labelled tegument proteins on a LDL-Sepharose column, revealed the same pattern of proteins observed in the immunoblot experiments. Finally, the binding of human LDL to the intact tegument was measured by microcalorimetry. Binding was shown to be an exothermic reaction, releasing approximately 2500 kcal/mol, it was saturable, and reproducibly displayed a biphasic curve suggesting that binding of LDL to S. mansoni might occur through a two step process, initiated by a nonspecific hydrophobic interaction followed by a specific high affinity ligand-receptor reaction. Pre-treatment of the tegument with trypsin reduced the binding of LDL to the tegument. Furthermore, albumin, which is an abundant lipid carrier protein in the serum and thus a potential ligand, failed to release any measurable heat when incubated with the tegument.

Terzi E., Holzemann G., and Seelig J. (1994) Alzheimer beta-amyloid peptide 25-35: electrostatic interactions with phospholipid membranes. *Biochemistry* **33**, 7434-7441.

Abstract: The role of lipids in the aggregation of three Alzheimer model peptides was investigated with circular dichroism spectroscopy and high-sensitivity titration calorimetry under conditions of low ionic strength. In solution, the peptides beta AP(25-35)OH and beta AP(25-35Nle)NH₂ exhibit a reversible random-coil \leftrightarrow beta-sheet (or beta-structured aggregate) transition. Addition of lipid vesicles containing negatively charged lipids shifts the random-coil \leftrightarrow beta-sheet equilibrium almost completely toward beta-sheet structure, which can be explained by the specific conditions created at the membrane surface: the cationic peptides are attracted to the negatively charged membrane, and the increase in peptide concentration together with the partial alignment of the peptide molecules then facilitates beta-sheet formation. The third peptide, beta AP-(25-35)NH₂, also binds to the lipid membrane but was found to adopt an essentially random-coil structure, both with and without lipids. A quantitative characterization of the binding equilibrium was possible with high-sensitivity titration calorimetry. All three peptides exhibited exothermic binding enthalpies which varied between ΔH approximately -2 kcal/mol for beta AP(25-35)OH and -8 kcal/mol for beta AP(25-35)NH₂. The apparent binding constants, calculated with bulk concentrations, were large and varied between 500 and $5 \times 10^4 \text{ M}^{-1}$, depending on the experimental conditions. However, after correction for electrostatic charge effects using the Gouy-Chapman theory, the intrinsic binding constants were found to be constant and much smaller with K approximately $2-10 \text{ M}^{-1}$. (ABSTRACT TRUNCATED AT 250 WORDS).

Terzi E., Holzemann G., and Seelig J. (1995) Self-association of beta-amyloid peptide (1-40) in solution and binding to lipid membranes. *J Mol Biol* **252**, 633-642.

Abstract: The beta-amyloid peptide (beta AP), a 39 to 43 residue peptide, is the major component of Alzheimer plaques. Using circular dichroism spectroscopy, titration calorimetry, and analytical ultracentrifugation we have analyzed the self-association of beta AP(1-40) in aqueous solution and the binding of beta AP(1-40) to negatively charged lipid vesicles. The CD spectra of both aggregation and membrane binding are characterized by an isodichroic point at 212 nm, indicating a simple two-state equilibrium for both cases. In aqueous solution beta AP(1-40) exhibits a reversible, concentration-dependent random coil \leftrightarrow beta-structure transition which can be described by a cooperative aggregation model with an association constant of $s = 1.05 \times 10^4 \text{ M}^{-1}$ and a nucleation parameter of $\sigma = 0.012$. A similar conformational change is observed upon addition of lipid. At a given peptide concentration, the addition of negatively charged, small unilamellar vesicles also induces a conformational change from a random coil conformation to a conformation with 40 to 60% beta-structure. The binding isotherm can be measured with high sensitivity titration calorimetry. It is approximately linear in the initial binding phase and exhibits an apparent saturation behaviour. The apparent binding constant decreases with concentration from K_{app} approximately 2100 M^{-1} at low concentration to 700 M^{-1} at the highest concentration measured. Peptide penetration into the lipid membrane and peptide aggregation at the membrane surface are proposed as possible mechanisms to explain the lipid-induced random coil \leftrightarrow beta-structure transition.

Thennarasu S., Lee D. K., Tan A., Prasad K. U., and Ramamoorthy A. (2005) Antimicrobial activity and membrane selective interactions of a synthetic lipopeptide MSI-843. *Biochim Biophys Acta* **1711**, 49-58.

Abstract: Lipopeptide MSI-843 consisting of the nonstandard amino acid ornithine (Oct-OOLLOOLOOL-NH₂) was designed with an objective towards generating non-lytic short antimicrobial peptides, which can

have significant pharmaceutical applications. Octanoic acid was coupled to the N-terminus of the peptide to increase the overall hydrophobicity of the peptide. MSI-843 shows activity against bacteria and fungi at micromolar concentrations. It permeabilizes the outer membrane of Gram-negative bacterium and a model membrane mimicking bacterial inner membrane. Circular dichroism investigations demonstrate that the peptide adopts alpha-helical conformation upon binding to lipid membranes. Isothermal titration calorimetry studies suggest that the peptide binding to membranes results in exothermic heat of reaction, which arises from helix formation and membrane insertion of the peptide. ²H NMR of deuterated-POPC multilamellar vesicles shows the peptide-induced disorder in the hydrophobic core of bilayers. ³¹P NMR data indicate changes in the lipid head group orientation of POPC, POPG and Escherichia coli total lipid bilayers upon peptide binding. Results from ³¹P NMR and dye leakage experiments suggest that the peptide selectively interacts with anionic bilayers at low concentrations (up to 5 mol%). Differential scanning calorimetry experiments on DiPOPE bilayers and ³¹P NMR data from E. coli total lipid multilamellar vesicles indicate that MSI-843 increases the fluid lamellar to inverted hexagonal phase transition temperature of bilayers by inducing positive curvature strain. Combination of all these data suggests the formation of a lipid-peptide complex resulting in a transient pore as a plausible mechanism for the membrane permeabilization and antimicrobial activity of the lipopeptide MSI-843.

Torreillas A., Laynez J., Menendez M., Corbalan-Garcia S., and Gomez-Fernandez J. C. (2004) Calorimetric study of the interaction of the C2 domains of classical protein kinase C isoenzymes with Ca²⁺ and phospholipids. *Biochemistry* **43**, 11727-11739.

Abstract: The affinities of Ca²⁺ and anionic lipid vesicles from the C2 domains of classical protein kinase C subfamily (alpha, betaII, and gamma) were studied using isothermal titration calorimetry (ITC). In addition, the thermal stability of these C2 domains in the presence of different ligand concentrations was analyzed using differential scanning calorimetry (DSC). These three closely related C2 domains bind Ca²⁺ in a similar way, demonstrating the presence of two sets of sites. The first set of sites binds one Ca²⁺ ion exothermically with similar high affinity for the three proteins (K_d around 1 μM), while the second set of sites binds endothermically approximately two Ca²⁺ ions with lower affinity, which varies for each C2 domain: 22.2 μM for the PKCalpha-C2 domain, 17.2 μM for the PKCbetaII-C2 domain, and 4.3 μM for the PKCgamma-C2 domain. In the absence of Ca²⁺ the three C2 domains showed a weak interaction with vesicles containing anionic phospholipids. However, in the presence of a saturating Ca²⁺ concentration, the C2 domains increased their affinities for the anionic lipid vesicles. In all cases, the C2 domains bound the vesicles exothermically and with similar affinities. A DSC thermal stability study of the C2 domains in the presence of Ca²⁺ and anionic lipids provided further information about this protein-ligand interaction. The presence of increasing Ca²⁺ concentrations was matched by an increase in the T_m in all cases, which was even greater in the presence of anionic lipid vesicles. The extent of the change in T_m differed for each C2 domain, reflecting the differing effect of the ligands bound during the protein stabilization. Denaturation of the C2 domains was irreversible both in the absence and in the presence of ligands, although the thermograms were not kinetically controlled. The dependence of the T_m on the Ca²⁺ concentration indicates that the protein stabilization observed by DSC primarily reflects the saturation by the cation of the low-affinity set of sites.

Tricerri M. A., Sanchez S. A., Arnulphi C., Durbin D. M., Gratton E., and Jonas A. (2002) Interaction of apolipoprotein A-I in three different conformations with palmitoyl oleoyl phosphatidylcholine vesicles. *J Lipid Res* **43**, 187-197.

Abstract: Interactions of apolipoprotein A-I (apoA-I) with cell membranes appear to be important in the initial steps of reverse cholesterol transport. The objective of this work was to examine the effect of three distinct conformations of apoA-I (lipid-free and in 78 A or 96 A reconstituted high density lipoproteins, rHDL) on its ability to bind to, and abstract lipids from, palmitoyl oleoyl phosphatidylcholine membrane vesicles (small unilamellar vesicles, SUV, and giant unilamellar vesicles, GUV). The molecular interactions were observed by two-photon fluorescence microscopy, and the binding parameters were quantified by gel-permeation chromatography or isothermal titration microcalorimetry. Rearrangement of apoA-I-containing particles after exposure to SUVs was examined by native gel electrophoresis. The results indicate that lipid-free apoA-I binds reversibly, with high affinity, to the vesicles but does not abstract a significant amount of lipid nor perturb the vesicle structure. The 96 A rHDL, where all the amphipathic helices of apoA-I are saturated with lipid within the particles, do not bind to vesicles or perturb their structure. In contrast, the 78 A rHDL have a region of apoA-I, corresponding to a few

amphipathic helical segments, which is available for external or internal phospholipid binding. These particles bind to vesicles with measurable affinity (lower than lipid-free apoA-I), abstract lipids from the membranes, and form particles of larger diameters, including 96 Å rHDL. We conclude that the conformation of apoA-I regulates its binding affinity for phospholipid membranes and its ability to abstract lipids from the membranes.

Turner D. C., Straume M., Kasimova M. R., and Gaber B. P. (1995) Thermodynamics of interaction of the fusion-inhibiting peptide Z-D-Phe-L-Phe-Gly with dioleoylphosphatidylcholine vesicles: direct calorimetric determination. *Biochemistry* **34**, 9517-9525.

Abstract: The binding of the fusion-inhibiting peptide Z-D-Phe-L-Phe-Gly to unilamellar lipid vesicles of dioleoylphosphatidylcholine (DOPC) was investigated by isothermal titration calorimetry (ITC). The peptide Z-D-Phe-L-Phe-Gly is known to inhibit fusion of myxo- and paramyxoviruses with cells as well as cell-cell and vesicle-vesicle fusion in model systems. Calorimetric titrations conducted over a range of temperatures permitted characterization of the thermodynamics of the interaction of Z-D-Phe-L-Phe-Gly with model DOPC lipid membranes. Simultaneous global analysis of 15 ITC binding curves acquired at four different temperatures allowed determination of the equilibrium site association constant (K), stoichiometry of binding (n), binding enthalpy change (ΔH), and heat capacity change of binding (ΔC_p) in a single set of experiments. The binding affinity and enthalpy change per mole of DOPC bound at 25 degrees C was $\log K = 2.463 \pm 0.075$ and $\Delta H = -1.07 \pm 0.12$ kcal/mol DOPC while the binding heat capacity change per mole of DOPC bound was $\Delta C_p = -20.3 \pm 2.8$ cal/(K.mol DOPC) with a temperature dependence (from 10-45 degrees C) of $d(\Delta C_p)/dT = 0.37 \pm 0.18$ cal/(K².mol DOPC). A temperature-independent binding stoichiometry was determined to be $n = 5.56 \pm 0.33$ DOPC molecules per Z-D-Phe-L-Phe-Gly. A comparison of these results with previous peptide-lipid binding studies is discussed as is their relevance to a current model of the interaction of fusion-inhibiting peptides with phospholipid membranes.

Varadi B., Kolev K., Tenekedjiev K., Meszaros G., Kovalszky I., Longstaff C., and Machovich R. (2004) Phospholipid barrier to fibrinolysis: role for the anionic polar head charge and the gel phase crystalline structure. *J Biol Chem* **279**, 39863-39871.

Abstract: The massive presence of phospholipids is demonstrated in frozen sections of human arterial thrombi. Purified platelet phospholipids and synthetic phospholipids retard in vitro tissue-type plasminogen activator (tPA)-induced fibrinolysis through effects on plasminogen activation and plasmin function. The inhibition of plasminogen activation on the surface of fibrin correlates with the fraction of anionic phospholipid. The phospholipids decrease the amount of tPA penetrating into the clot by 75% and the depth of the reactive surface layer occupied by the activator by up to 30%, whereas for plasmin both of these parameters decrease by approximately 50%. The phospholipids are not only a diffusion barrier, they also bind the components of the fibrinolytic system. Isothermal titration calorimetry shows binding characterized with dissociation constants in the range 0.35-7.64 μ M for plasmin and tPA (lower values with more negative phospholipids). The interactions are endothermic and thermodynamically driven by an increase in entropy, probably caused by the rearrangements in the ordered gel structure of the phospholipids (in line with the stronger inhibition at gel phase temperatures compared with liquid crystalline phase temperatures). These findings show a phospholipid barrier, which should be overcome during lysis of arterial thrombi.

Velkov T., Chuang S., Pranker R., Sakellaris H., Porter C. J., and Scanlon M. J. (2005) An improved method for the purification of rat liver-type fatty acid binding protein from Escherichia coli. *Protein Expr Purif* **44**, 23-31.

Abstract: Rat liver fatty acid binding protein (L-FABP) was efficiently expressed in Escherichia coli and purified to homogeneity. The cDNA encoding L-FABP was ligated into the pTrc99A expression vector and expressed by induction with isopropyl-beta-D-thiogalactopyranoside under the control of the P(trc) promoter. Following an 18h induction period, L-FABP constituted approximately 3% of the cytosolic protein. The protein could be purified to electrophoretic homogeneity (silver-stained polyacrylamide gel detection) by ammonium sulfate fractionation (65% saturation) of the soluble bacterial lysate followed by the chromatographic sequence of anion-exchange-->hydrophobic interaction-->anion-exchange chromatography. The recombinant protein displayed an isoelectric point of 7.0 and cross-reactivity with rabbit anti-(human L-FABP) polyclonal antibody. The ligand binding properties of the delipidated L-FABP were examined by titration with the fluorescent probe 1-anilino-8-naphthalene sulfonic acid and isothermal

titration calorimetric analysis of oleic acid binding. The purified rat L-FABP displayed a binding stoichiometry of 2:1 (ANS:L-FABP) with dissociation constants ($K(d)$) of 1.7 and 15.5 μM for the high and low affinity binding sites, respectively. The $K(d)$ values determined by ITC for oleic acid binding were 0.155 and 4.04 μM with a binding stoichiometry of approximately 2 mol of fatty acid/mol of protein. These physicochemical and binding properties are in agreement with those of L-FABP isolated from rat liver tissue.

Verly R. M., Rodrigues M. A., Daghasanli K. R., Denadai A. M., Cuccovia I. M., Bloch C., Jr., Frezard F., Santoro M. M., Pilo-Veloso D. and Bemquerer M. P. (2007) Effect of cholesterol on the interaction of the amphibian antimicrobial peptide DD K with liposomes. *Peptides* **29**, 15-24.

Abstract: DD K is an antimicrobial peptide previously isolated from the skin of the amphibian *Phyllomedusa distincta*. The effect of cholesterol on synthetic DD K binding to egg lecithin liposomes was investigated by intrinsic fluorescence of tryptophan residue, measurements of kinetics of 5(6)-carboxyfluorescein (CF) leakage, dynamic light scattering and isothermal titration microcalorimetry. An 8nm blue shift of tryptophan maximum emission fluorescence was observed when DD K was in the presence of lecithin liposomes compared to the value observed for liposomes containing 43mol% cholesterol. The rate and the extent of CF release were also significantly reduced by the presence of cholesterol. Dynamic light scattering showed that lecithin liposome size increase from 115 to 140nm when titrated with DD K but addition of cholesterol reduces the liposome size increments. Isothermal titration microcalorimetry studies showed that DD K binding both to liposomes containing cholesterol as to liposomes devoid of it is more entropically than enthalpically favored. Nevertheless, the peptide concentration necessary to furnish an adjustable titration curve is much higher for liposomes containing cholesterol at 43mol% (2mmolL⁻¹) than in its absence (93 μmolL^{-1}). Apparent binding constant values were 2160 and 10,000Lmol⁻¹, respectively. The whole data indicate that DD K binding to phosphatidylcholine liposomes is significantly affected by cholesterol, which contributes to explain the low hemolytic activity of the peptide.

Wei S. Y., Wu J. M., Kuo Y. Y., Chen H. L., Yip B. S., Tzeng S. R., and Cheng J. W. (2006) Solution structure of a novel tryptophan-rich peptide with bidirectional antimicrobial activity. *J Bacteriol* **188**, 328-334.

Abstract: Trp-rich antimicrobial peptides play important roles in the host innate defense mechanisms of many plants, insects, and mammals. A new type of Trp-rich peptide, Ac-KWRRWVRWI-NH(2), designated Pac-525, was found to possess improved activity against both gram-positive and -negative bacteria. We have determined that the solution structures of Pac-525 bound to membrane-mimetic sodium dodecyl sulfate (SDS) micelles. The SDS micelle-bound structure of Pac-525 adopts an alpha-helical segment at residues Trp2, Arg3, and Arg4. The positively charged residues are clustered together to form a hydrophilic patch. The three hydrophobic residues Trp2, Val6, and Ile9 form a hydrophobic core. The surface electrostatic potential map indicates the three tryptophan indole rings are packed against the peptide backbone and form an amphipathic structure. Moreover, the reverse sequence of Pac-525, Ac-IWRVWRRWK-NH(2), designated Pac-525(rev), also demonstrates similar antimicrobial activity and structure in membrane-mimetic micelles and vesicles. A variety of biophysical and biochemical methods, including circular dichroism, fluorescence spectroscopy, and microcalorimetry, were used to show that Pac-525 interacted strongly with negatively charged phospholipid vesicles and induced efficient dye release from these vesicles, suggesting that the antimicrobial activity of Pac-525 may be due to interactions with bacterial membranes.

Wen S., Majerowicz M., Waring A. and Bringezu F. (2007) Dicynthaurin (ala) monomer interaction with phospholipid bilayers studied by fluorescence leakage and isothermal titration calorimetry. *J Phys. Chem B* **111**, 6280-6287.

Abstract: The interaction of the antimicrobial peptide dicynthaurin (ala) monomer with model membranes of zwitterionic and negatively charged lipids and mixtures thereof was studied by means of isothermal titration calorimetry (ITC), fluorescent leakage, and dynamic light scattering (DLS) measurements. For the ITC analysis, we have applied the surface partitioning equilibrium model which shows that the interaction is predominately driven by hydrophobic effects (K_b between 2×10^4 and 1×10^5 M⁻¹). Under low salt conditions, the enhanced electrostatic interaction leads to larger peptide concentrations immediately above the vesicle surface, which initiates the insertion of the peptide into the bilayer more effectively.

Fluorescent leakage measurements have shown a fast leakage of the fluorescent dye within seconds after peptide addition. The analysis of the leakage kinetics was performed in terms of an initial pore formation model (up to $t = 1000$ s) that takes the reversible surface aggregation of bound peptide monomers into account. From this analysis, a minimum aggregation number of $n = 7 \pm 2$ per pore is obtained.

Wenk M. R. and Seelig J. (1998) Magainin 2 amide interaction with lipid membranes: calorimetric detection of peptide binding and pore formation. *Biochemistry* **37**, 3909-3916.

Abstract: The interaction of the antibiotic magainin 2 amide (M2a) with lipid bilayers was investigated with high-sensitivity titration calorimetry. The enthalpy of transfer of the cationic M2a to negatively charged small unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) (75:25, mol/mol) was measured as $\Delta H = -17.0 \pm 1$ kcal/mol of peptide. The adsorption isotherm was determined by injecting lipid vesicles into peptide solutions at low peptide concentrations ($c_{Po} < 7 \mu\text{M}$). The apparent partition coefficient was K_{app} approximately $1.2 \times 10^4 \text{ M}^{-1}$ at a peptide equilibrium concentration of $1 \mu\text{M}$ but decreased with increasing peptide concentration. The hydrophobic partitioning of M2a into the lipid membrane is modulated by electrostatic effects that arise from the attraction of the positively charged peptide to the negatively charged membrane. Using the Gouy-Chapman theory to correct for electrostatic attraction, the experimental binding isotherms can be explained with an intrinsic (hydrophobic) partition coefficient of $K = 55 \pm 5 \text{ M}^{-1}$ and an effective peptide charge of $z = 3.7-3.8$. The free energy of binding is $\Delta G = -4.8$ kcal/mol. At peptide concentrations $c_{Po} > \text{approximately } 7 \mu\text{M}$, a second effect comes into play, and the titration enthalpies can no longer be explained exclusively by peptide partitioning. The first few injections produce enthalpies of reaction which are distinctly smaller than expected from a pure partition equilibrium, followed by a series of injections with reaction heats larger than expected. After subtracting the enthalpic contribution due to partitioning, the residual enthalpies are endothermic for the first few injections, and exothermic for the consecutive steps. Furthermore, the endothermic excess heat is compensated exactly by the exothermic excess heat; i.e., the excess heat consumed in the first part of the titration experiment is returned during the second part. Endothermic excess enthalpies are observed for total molar peptide-to-lipid ratios of $P/L > \text{approximately } 3.0\%$, whereas exothermic excess heats were seen for $0.7\% < P/L < 3.0\%$. Below $P/L < \text{approximately } 0.7\%$, the binding follows the partition equilibrium. Based on earlier spectroscopic evidence, it is suggested that magainin 2 amide binds to the lipid membrane and forms pores at high peptide-to-lipid ratio, this process being characterized by an endothermic reaction enthalpy. Pore formation is reversed with increasing lipid concentration, and the peptide pores disintegrate. The limiting peptide-to-lipid ratio deduced from titration calorimetry for M2a pore formation is in excellent agreement with spectroscopic methods. The enthalpy of pore formation amounts to $\Delta H = +6.2 \pm 1.6$ kcal/mol peptide or ΔH approximately 25-45 kcal/mol pore if the pore is comprised of 4-7 peptide molecules.

Wieprecht T., Dathe M., Epanand R. M., Beyermann M., Krause E., Maloy W. L., MacDonald D. L., and Bienert M. (1997) Influence of the angle subtended by the positively charged helix face on the membrane activity of amphipathic, antibacterial peptides. *Biochemistry* **36**, 12869-12880.

Abstract: To investigate the influence of the angle subtended by the positively charged helix face on membrane activity, six amphipathic alpha-helical peptides with angles between 80 degrees and 180 degrees, but with retained hydrophobicity, hydrophobic moment, and positive overall charge, were designed starting from the sequence of the antibacterial peptide magainin 2. CD investigations revealed that all analogs are in an alpha-helical conformation in vesicle suspension. The ability of the peptides to induce dye release from negatively charged phosphatidylglycerol (PG) vesicles decreased with increasing angle. However, peptides with a large angle of positively charged residues (140-180 degrees) exhibited a considerably higher permeabilizing activity at zwitterionic phosphatidylcholine (PC) and mixed PC/PG (3:1) vesicles than analogs with a small angle (80-120 degrees). In addition, analogs with large angles were more active in antibacterial and hemolytic assays. The antibacterial specificity of these analogs was decreased. Binding investigations showed that peptide binding is favored by a large angle and a high content of negatively charged phospholipid. In contrast, a small angle and a low negative membrane charge enhanced the membrane-permeabilizing efficiency of the bound peptide fraction. All analogs stabilized the bilayer phase of phosphatidylethanolamine over the inverted hexagonal phase. Therefore, a class L mechanism of permeabilization can be excluded. Furthermore, the analogs do not act by the induction of positive curvature strain or by a "carpet-like" mechanism. Our results are in accordance with a pore mechanism: The membrane-permeabilizing efficiency of analogs with enhanced angle of positively

charged residues is reduced due to electrostatic repulsion between adjacent helices within the pore, thus resulting in a decreased pore-forming probability and/or pore destabilization.

Wieprecht T., Apostolov O., Beyermann M., and Seelig J. (1999) Thermodynamics of the alpha-helix-coil transition of amphipathic peptides in a membrane environment: implications for the peptide-membrane binding equilibrium. *J Mol Biol* **294**, 785-794.

Abstract: Amphipathic alpha-helices are the membrane binding motif in many proteins. The corresponding peptides are often random coil in solution but are folded into an alpha-helix upon interaction with the membrane. The energetics of this ubiquitous folding process are still a matter of conjecture. Here, we present a new method to quantitatively analyze the thermodynamics of peptide folding at the membrane interface. We have systematically varied the helix content of a given amphipathic peptide when bound to the membrane and have correlated the thermodynamic binding parameters determined by isothermal titration calorimetry with the alpha-helix content obtained by circular dichroism spectroscopy. The peptides investigated were the antibiotic magainin 2 amide and three analogs in which two adjacent amino acid residues were substituted by their d-enantiomers. The thermodynamic parameters controlling the alpha-helix formation were found to be linearly related to the helicity of the membrane-bound peptides. Helix formation at the membrane surface is characterized by an enthalpy change of $\Delta H(\text{helix})$ approximately -0.7 kcal/mol per residue, an entropy change of $\Delta S(\text{helix})$ approximately -1.9 cal/molK residue and a free energy change of $\Delta G(\text{helix}) = -0.14$ kcal/mol residue. Helix formation is a strong driving force of peptide insertion into the membrane and accounts for about 50 % of the free energy of binding. An increase in temperature entails an unfolding of the membrane-bound helix. The temperature dependence can be described with the Zimm-Bragg theory and the enthalpy of unfolding agrees with that deduced from isothermal titration calorimetry.

Wieprecht T., Beyermann M., and Seelig J. (1999) Binding of antibacterial magainin peptides to electrically neutral membranes: thermodynamics and structure. *Biochemistry* **38**, 10377-10387.

Abstract: Magainins are positively charged amphipathic peptides which permeabilize cell membranes and display antimicrobial activity. They are usually thought to bind specifically to anionic lipids, and binding studies have been performed almost exclusively with negatively charged membranes. Here we demonstrate that binding of magainins to neutral membranes, a reaction which is difficult to assess with spectroscopic means, can be followed with high accuracy using isothermal titration calorimetry. The binding mechanism can be described by a surface partition equilibrium after correcting for electrostatic repulsion by means of the Gouy-Chapman theory. Unusual thermodynamic parameters are observed for the binding process. (i) The three magainin analogues that were investigated bind to neutral membranes with large exothermic reaction enthalpies ΔH of -15 to -18 kcal/mol (at 30 degrees C). (ii) The reaction enthalpies increase with increasing temperature, leading to a large positive heat capacity ΔC_p of approximately 130 cal mol⁻¹K⁻¹ (at 25 degrees C). (iii) The Gibbs free energies of binding ΔG are between -6.4 and -8.6 kcal/mol, resulting in a large negative binding entropy ΔS . The binding of magainin to small unilamellar vesicles is hence an enthalpy-driven reaction. The negative ΔH and ΔS and the large positive ΔC_p contradict the conventional understanding of the hydrophobic effect. CD experiments reveal that the membrane-bound fraction of magainin is approximately 80% helical at 8 degrees C, decreasing to approximately 60% at 45 degrees C. Since the random coil --> alpha-helix transition in aqueous solution is known to be an exothermic process, the same process occurring at the membrane surface is shown to account for up to 65% of the measured reaction enthalpy. In addition to membrane-facilitated helix formation, the second main driving force for membrane binding is the insertion of the nonpolar amino acid side chains into the lipid bilayer. It also contributes a negative ΔH and follows the pattern for the nonclassical hydrophobic effect. Addition of cholesterol drastically reduces the extent of peptide binding and reveals an enthalpy-entropy compensation mechanism. Membrane permeability was measured with a dye assay and correlated with the extent of peptide binding. The level of dye efflux is linearly related to the amount of surface-bound peptide and can be traced back to a membrane perturbation effect.

Wieprecht T., Apostolov O., and Seelig J. (2000) Binding of the antibacterial peptide magainin 2 amide to small and large unilamellar vesicles. *Biophys Chem* **85**, 187-198.

Abstract: The thermodynamics of binding of the antibacterial peptide magainin 2 amide (M2a) to negatively charged small (SUVs) and large (LUVs) unilamellar vesicles has been studied with isothermal titration calorimetry (ITC) and CD spectroscopy at 45 degrees C. The binding isotherms as well as the

ability of the peptide to permeabilize membranes were found to be qualitatively and quantitatively similar for both model membranes. The binding isotherms could be described with a surface partition equilibrium where the surface concentration of the peptide immediately above the plane of binding was calculated with the Gouy-Chapman theory. The standard free energy of binding was ΔG_0 approximately -22 kJ/mol and was almost identical for LUVs and SUVs. However, the standard enthalpy and entropy of binding were distinctly higher for LUVs ($\Delta H_0 = -15.1$ kJ/mol, $\Delta S_0 = 24.7$ J/molK) than for SUVs ($\Delta H_0 = -38.5$ kJ/mol, $\Delta S_0 = -55.3$ J/molK). This enthalpy-entropy compensation mechanism is explained by differences in the lipid packing. The cohesive forces between lipid molecules are larger in well-packed LUVs and incorporation of M2a leads to a stronger disruption of cohesive forces and to a larger increase in the lipid flexibility than peptide incorporation into the more disordered SUVs. At 45 degrees C the peptide easily translocates from the outer to the inner monolayer as judged from the simulation of the ITC curves.

Wieprecht T., Apostolov O., Beyermann M., and Seelig J. (2000) Interaction of a mitochondrial presequence with lipid membranes: role of helix formation for membrane binding and perturbation. *Biochemistry* **39**, 15297-15305.

Abstract: The binding of a peptide to a biological membrane is often accompanied by a transition from a random coil structure to an amphipathic α -helix. Recently, we have presented a new approach which allows the determination of the thermodynamic parameters of membrane-induced helix formation [Wieprecht et al. (1999) *J. Mol Biol.* 294, 785]. It involves a systematic variation of the helix content of a given peptide by double D-substitution and a correlation of the binding parameters with the helicity. Here we have used this method to study membrane-induced helix formation for the presequence of rat mitochondrial rhodanese (RHD). The thermodynamic parameters of binding of the peptide RHD and of four of its double D-isomers were determined for 30 nm (SUVs) and 100 nm (LUVs) unilamellar vesicles composed of phosphatidylcholine/phosphatidylglycerol (3:1) using circular dichroism spectroscopy, fluorescence spectroscopy, and isothermal titration calorimetry. The incremental changes of the thermodynamic parameters of helix formation were found to be very similar for SUVs and LUVs. Membrane-induced helix formation of RHD entailed a negative enthalpy of $\Delta H(\text{helix}) = -0.5$ to -0.6 kcal/mol/residue and was opposed by an entropy of about $\Delta S(\text{helix}) = -1$ to -1.4 cal/mol K/residue. The free energy of helix formation, $\Delta G(\text{helix})$, was about -0.2 kcal/mol, and helix formation accounted for 50-60% of the total free energy of membrane binding. Dye-release experiments were used to assess the role of helix formation for the membrane perturbation potential of the peptides. While helix formation plays a major role for membrane binding, it appears to have little importance for inducing membrane leakiness.

Wieprecht T., Apostolov O., Beyermann M., and Seelig J. (2000) Membrane binding and pore formation of the antibacterial peptide PGLa: thermodynamic and mechanistic aspects. *Biochemistry* **39**, 442-452.

Abstract: The antibacterial peptide PGLa exerts its activity by permeabilizing bacterial membranes whereas eukaryotic membranes are not affected. To provide insight into the selectivity and the permeabilization mechanism, the binding of PGLa to neutral and negatively charged model membranes was studied with high-sensitivity isothermal titration calorimetry (ITC), circular dichroism (CD), and solid-state deuterium nuclear magnetic resonance ($(2)\text{H}$ NMR). The binding of PGLa to negatively charged phosphatidylcholine (PC)/phosphatidylglycerol (PG) (3:1) vesicles was by a factor of approximately 50 larger than that to neutral PC vesicles. The negatively charged membrane accumulates the cationic peptide at the lipid-water interface, thus facilitating the binding to the membrane. However, if bulk concentrations are replaced by surface concentrations, very similar binding constants are obtained for neutral and charged membranes (K approximately 800 - 1500 M^{-1}). Membrane selectivity is thus caused almost exclusively by electrostatic attraction to the membrane surface and not by hydrophobic insertion. Membrane insertion is driven by an exothermic enthalpy (ΔH approximately -11 to -15 kcal/mol) but opposed by entropy. An important contribution to the binding process is the membrane-induced random coil \rightarrow α -helix transition of PGLa. The peptide is random coil in solution but adopts an approximately 80% α -helical conformation when bound to the membrane. Helix formation is an exothermic process, contributing approximately 70% to the binding enthalpy and approximately 30% to the free energy of binding. The $(2)\text{H}$ NMR measurements with selectively deuterated lipids revealed small structural changes in the lipid headgroups and in the hydrocarbon interior upon peptide binding which were continuous over the whole concentration range. In contrast, isothermal titration calorimetry of PGLa solutions with PC/PG(3:1) vesicles gave rise to two processes: (i) an exothermic binding of PGLa to the membrane followed by (ii) a slower endothermic process. The latter is only detected at peptide-to-lipid ratios >17 mmol/mol and is

paralleled by the induction of membrane leakiness. Dye efflux measurements are consistent with the critical limit derived from ITC measurements. The endothermic process is assigned to peptide pore formation and/or lipid perturbation. The enthalpy of pore formation is 9.7 kcal/mol of peptide. If the same excess enthalpy is assigned to the lipid phase, the lipid perturbation enthalpy is 180 cal/mol of lipid. The functional synergism between PGLa and magainin 2 amide could also be followed by ITC and dye release experiments and is traced back to an enhanced pore formation activity of a peptide mixture.

Wieprecht T., Beyermann M., and Seelig J. (2002) Thermodynamics of the coil-alpha-helix transition of amphipathic peptides in a membrane environment: the role of vesicle curvature. *Biophys Chem* **96**, 191-201.

Abstract: The binding of peptides or proteins to a bilayer membrane is often coupled with a random coil->alpha-helix transition. Knowledge of the energetics of this membrane-induced folding event is essential for the understanding of the mechanism of membrane activity. In a recent study [Wieprecht et al., *J. Mol. Biol.* 294 (1999) 785-794], we have developed an approach which allows an analysis of the energetics of membrane-induced folding. We have systematically varied the helix content of the amphipathic peptide magainin-2-amide by synthesizing analogs where two adjacent amino acid residues were substituted by their corresponding D-enantiomers and have measured their binding to small unilamellar vesicles (SUVs). Correlation of the binding parameters with the helicities allowed the evaluation of the thermodynamic parameters of helix formation. Since SUVs (30 nm in diameter) are characterized by a non-ideal lipid packing due to their high membrane curvature, we have now extended our studies to large unilamellar vesicles (LUVs) (100 nm in diameter) with a lipid packing close to planar membranes. While the free energy of binding was similar for SUVs and LUVs, the binding enthalpies and entropies were distinctly different for the two membrane systems. The thermodynamic parameters of the coil-helix transition were nevertheless not affected by the vesicle size. Helix formation at the membrane surface of LUVs (SUVs) was characterized by an enthalpy change of -0.8 (-0.7) kcal/mol per residue, an entropy change of -2.3 (-1.9) cal/mol K per residue, and a free energy change of -0.12 (-0.14) kcal/mol per residue. Helix formation accounted for approximately 50% of the free energy of binding underlining its major role as a driving force for membrane-binding.

Wimley W. C. and White S. H. (2004) Reversible unfolding of beta-sheets in membranes: a calorimetric study. *J Mol Biol* **342**, 703-711.

Abstract: The hexapeptide acetyl-Trp-Leu(5) (AcWL(5)) has the remarkable ability to assemble reversibly and spontaneously into beta-sheets on lipid membranes as a result of monomer partitioning followed by cooperative assembly. This system provides a unique opportunity to study the thermodynamics of protein folding in membranes, which we have done using isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). The results, which may represent the first example of reversible thermal unfolding of peptides in membranes, help to define the contribution of hydrogen bonding to the extreme thermal stability of membrane proteins. ITC revealed that the enthalpy change for partitioning of monomeric, unstructured AcWL(5) from water into membranes was zero within experimental error over the temperature range of 5 degrees C to 75 degrees C. DSC showed that the beta-sheet aggregates underwent a reversible, endothermic, and very asymmetric thermal transition with a concentration-dependent transition temperature (T_m) in the range of 60 degrees C to 80 degrees C. A numerical model of nucleation and growth-dependent assembly of oligomeric beta-sheets, proposed earlier to describe beta-sheet formation in membranes, recreated remarkably well the unusual shape and concentration-dependence of the transition peaks. The enthalpy for thermal unfolding of AcWL(5) beta-sheets in the membrane was found to be about 8(+/-1)kcal mol⁻¹, or about 1.3(+/-0.2)kcal mol⁻¹ per residue.

Wolfrum C., Borchers T., Sacchettini J. C., and Spener F. (2000) Binding of fatty acids and peroxisome proliferators to orthologous fatty acid binding proteins from human, murine, and bovine liver. *Biochemistry* **39**, 1469-1474.

Abstract: Liver-type fatty acid binding protein (L-FABP) has been proposed to be involved in the transport of fatty acids and peroxisome proliferators from the cytosol into the nucleus for interaction with the peroxisome proliferator-activated receptors (PPARs). On the basis of this premise, we investigated by isothermal titration calorimetry the binding of myristic, stearic, oleic, and docosahexaenoic acids to three orthologous L-FABPs and compared these results to those obtained for several xenobiotics [Wy14,643, bezafibrate, 5,8,11,14-eicosatetraynoic acid (ETYA), and BRL48,482] known for their peroxisome

proliferating activity in rodents. Recombinant human, murine, and bovine L-FABPs were analyzed and the thermodynamic data were obtained. Our studies showed that fatty acids bound with a stoichiometry of 2:1, fatty acid to protein, with dissociation constants for the first binding site in the nanomolar range. With dissociation constants above 1 μM the drug peroxisome proliferators showed weaker binding, with the exception of arachidonate analogue ETYA, which bound with a similar affinity as the natural fatty acid. Some of the thermodynamic data obtained for fatty acid binding could be explained by differences in protein structure. Moreover, our results revealed that binding affinities were not determined by ligand solubility in the aqueous phase.

Yang Q., Alemany R., Casas J., Kitajka K., Lanier S.M., and Escriba P.V. (2005) Influence of the membrane lipid structure on signal processing via G protein-coupled receptors. *Mol Pharmacol.* **68**, 210-7. **Abstract:** We have recently reported that lipid structure regulates the interaction with membranes, recruitment to membranes, and distribution to membrane domains of heterotrimeric Galphabeta gamma proteins, Galpha subunits, and Gbetagamma dimers (J Biol Chem 279:36540-36545, 2004). Here, we demonstrate that modulation of the membrane structure not only determines G protein localization but also regulates the function of G proteins and related signaling proteins. In this context, the antitumor drug daunorubicin (daunomycin) and oleic acid changed the membrane structure and inhibited G protein activity in biological membranes. They also induced marked changes in the activity of the alpha(2A/D)-adrenergic receptor and adenylyl cyclase. In contrast, elaidic and stearic acid did not change the activity of the above-mentioned proteins. These fatty acids are chemical but not structural analogs of oleic acid, supporting the structural basis of the modulation of membrane lipid organization and subsequent regulation of G protein-coupled receptor signaling. In addition, oleic acid (and also daunorubicin) did not alter G protein activity in a membrane-free system, further demonstrating the involvement of membrane structure in this signal modulation. The present work also unravels in part the molecular bases involved in the antihypertensive (Hypertension 43:249-254, 2004) and anticancer (Mol Pharmacol 67:531-540, 2005) activities of synthetic oleic acid derivatives (e.g., 2-hydroxyoleic acid) as well as the molecular bases of the effects of diet fats on human health.

Yin N., Marshall R. L., Matheson S., and Savage P. B. (2003) Synthesis of lipid A derivatives and their interactions with polymyxin B and polymyxin B nonapeptide. *J Am Chem Soc* **125**, 2426-2435. **Abstract:** Lipid A is the causative agent of Gram-negative sepsis, a leading cause of mortality among hospitalized patients. Compounds that bind lipid A can limit its detrimental effects. Polymyxin B, a cationic peptide antibiotic, is one of the simplest molecules capable of selectively binding lipid A and may serve as a model for further development of lipid A binding agents. However, association of polymyxin B with lipid A is not fully understood, primarily due to the low solubility of lipid A in water and inhomogeneity of lipid A preparations. To better understand lipid A-polymyxin B interaction, pure lipid A derivatives were prepared with incrementally varied lipid chain lengths. These compounds proved to be more soluble in water than lipid A, with higher aggregation concentrations. Isothermal titration calorimetric studies of these lipid A derivatives with polymyxin B and polymyxin B nonapeptide indicate that binding stoichiometries (peptide to lipid A derivative) are less than 1 and that affinities of these binding partners correlate with the aggregation states of the lipid A derivatives. These studies also suggest that cooperative ionic interactions dominate association of polymyxin B and polymyxin B nonapeptide with lipid A.

Yu B. Z., Polenova T., Jain M. K., and Berg O. G. (2005) Premicellar complexes of sphingomyelinase mediate enzyme exchange for the stationary phase turnover. *Biochim Biophys Acta* **1712**, 137-151. **Abstract:** During the steady state reaction progress in the scooting mode with highly processive turnover, Bacillus cereus sphingomyelinase (SMase) remains tightly bound to sphingomyelin (SM) vesicles (Yu et al., Biochim. Biophys. Acta 1583, 121-131, 2002). In this paper, we analyze the kinetics of SMase-catalyzed hydrolysis of SM dispersed in diheptanoylphosphatidyl-choline (DC7PC) micelles. Results show that the resulting decrease in the turnover processivity induces the stationary phase in the reaction progress. The exchange of the bound enzyme (E^*) between the vesicle during such reaction progress is mediated via the premicellar complexes ($E(i)\#$) of SMase with DC7PC. Biophysical studies indicate that in $E(i)\#$ monodisperse DC7PC is bound to the interface binding surface (i-face) of SMase that is also involved in its binding to micelles or vesicles. In the presence of magnesium, required for the catalytic turnover, three different complexes of SMase with monodisperse DC7PC ($E(i)\#$ with $i=1, 2, 3$) are sequentially formed

with Hill coefficients of 3, 4 and 8, respectively. As a result, during the stationary phase reaction progress, the initial rate is linear for an extended period and all the substrate in the reaction mixture is hydrolyzed at the end of the reaction progress. At low mole fraction (X) of total added SM, exchange is rapid and the processive turnover is limited by the steps of the interfacial turnover cycle without becoming microscopically limited by local substrate depletion or enzyme exchange. At high X, less DC7PC will be monodisperse, E(i)# does not form and the turnover becomes limited by slow enzyme exchange. Transferred NOESY enhancement results show that monomeric DC7PC in solution is in a rapid exchange with that bound to E(i)# at a rate comparable to that in micelles. Significance of the exchange and equilibrium properties of the E(i)# complexes for the interpretation of the stationary phase reaction progress is discussed.

Zhang F. and Rowe E. S. (1994) Calorimetric studies of the interactions of cytochrome c with dioleoylphosphatidylglycerol extruded vesicles: ionic strength effects. *Biochim Biophys Acta* **1193**, 219-225.

Abstract: Cytochrome c has been studied as an example of a peripheral membrane protein which interacts with the lipids as well as the proteins of the inner mitochondrial membrane. In order to elucidate the thermodynamic properties of these interactions, isothermal titration calorimetry and differential scanning calorimetry (DSC) were used to study the binding of cytochrome c to negatively charged dioleoylphosphatidylglycerol (DOPG) extruded vesicles as a function of ionic strength. The binding constant and enthalpy of association decrease with increasing ionic strength, with no binding detected above 0.5 M NaCl. The enthalpy of the binding of cytochrome c to DOPG-extruded vesicles was 15 kcal/mol, and the binding constant was $6 \times 10^6 \text{ M}^{-1}$ at the lowest ionic strengths. The minimum size of the lipid cluster to which the protein bound was found to be approx. 9 lipid molecules in the titration calorimetry measurements and as low as 5 lipid molecules in the DSC measurements. The stability of the bound cytochrome c was found to be reduced; the thermal denaturation temperature was lowered from 83 to 50 degrees when bound to DOPG. The results of this study support previous suggestions that cytochrome c may undergo a conformational change when it binds to charged lipids such as DOPG. The results also support the suggestion that the protein penetrates partially into the lipid bilayer.

Zhang W., Krishnan N., and Becker D. F. (2006) Kinetic and thermodynamic analysis of Bradyrhizobium japonicum PutA-membrane associations. *Arch Biochem Biophys* **445**, 174-183.

Abstract: In Escherichia coli, proline induces tight membrane binding of the PutA flavoenzyme and transforms PutA from a transcriptional repressor to a membrane-associated proline catabolic enzyme. In other gram-negative bacteria such as Bradyrhizobium japonicum, PutA lacks DNA binding activity and functions only as a proline catabolic enzyme. Here, we characterize the membrane binding properties of PutA from B. japonicum (BjPutA) to address whether proline regulates BjPutA-lipid binding similar to Escherichia coli PutA (EcPutA). Surface plasmon resonance (SPR) kinetic measurements of BjPutA-lipid binding show BjPutA forms a complex with lipids in the absence and presence of proline with similar dissociation constant (K(D)) values of 2.5 and 1.7nM, respectively. SPR experiments using differently charged lipid bilayers indicate BjPutA selectively binds negatively charged lipids, which contrasts with the charge independent membrane binding of EcPutA. Analysis of BjPutA-lipid binding by isothermal titration calorimetry at 25 degrees C revealed an endothermic binding reaction that is entropically driven. This work shows that BjPutA-membrane associations vary significantly from EcPutA.

Zhang X., Qi X. R., and Zhang Q. (2002) [Interaction between insulin with liposome]. *Yao Xue Xue Bao* **37**, 370-373.

Abstract: AIM: To study the characteristics of the interaction between insulin and liposome. METHODS: The interaction between insulin and liposome was studied by fluorescence spectra and microcalorimetry methods. The sample of insulin liposome interaction after separation by super-centrifugalization or gel filtration was determined by fluorescence and HPLC. RESULTS: The results indicate that there was only little increase in fluorescence intensity and no blue shift of peak in fluorescence spectrum. Fluorescence quenching experiments with NaI and acrylamide as quenchers showed that the KSVs (the slope of Strm-Volmer equation) of insulin were more similar to that with added liposome, indicating low interaction between insulin with liposome. The microcalorimetric results indicate that the heat released during the mixture of liposome with insulin, was $1.98 \text{ kcal.mol}^{-1}$, suggesting that the reaction belongs to weak reaction. The quantity of insulin in the insulin-liposome mixture sample after separation by ultracentrifuge

or by Sephadex G-75 determined by HPLC, the combination percent was only 0.2%, indicating low interaction between insulin and liposome. CONCLUSION: The interaction between insulin and liposome was weak.

Zhu P. P., Szczepanowski R. H., Nosworthy N. J., Ginsburg A., and Peterkofsky A. (1999) Reconstitution studies using the helical and carboxy-terminal domains of enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system. *Biochemistry* **38**, 15470-15479.

Abstract: Enzyme I of the bacterial phosphoenolpyruvate:sugar phosphotransferase system can be phosphorylated by PEP on an active-site histidine residue, localized to a cleft between an alpha-helical domain and an alpha/beta domain on the amino terminal half of the protein. The phosphoryl group on the active-site histidine can be passed to an active-site histidine residue of HPr. It has been proposed that the major interaction between enzyme I and HPr occurs via the alpha-helical domain of enzyme I. The isolated recombinant alpha-helical domain (residues 25-145) with approximately 80% alpha-helices as well as enzyme I deficient in that domain [EI(Δ HD)] with approximately 50% alpha-helix content from *M. capricolum* were used to further elucidate the nature of the enzyme I-HPr complex. Isothermal titration calorimetry demonstrated that HPr binds to the alpha-helical domain and intact enzyme I with $K_d = 5 \times 10^4$ and $1.4 \times 10^5 \text{ M}^{-1}$ at pH 7.5 and 25 degrees C, respectively, but not to EI(Δ HD), which contains the active-site histidine of enzyme I and can be autophosphorylated by PEP. In vitro reconstitution experiments with proteins from both *M. capricolum* and *E. coli* showed that EI(Δ HD) can donate its bound phosphoryl group to HPr in the presence of the isolated alpha-helical domain. Furthermore, *M. capricolum* recombinant C-terminal domain of enzyme I (EIC) was shown to reconstitute phosphotransfer activity with recombinant N-terminal domain (EIN) approximately 5% as efficiently as the HD-EI(Δ HD) pair. Recombinant EIC strongly self-associates (approximately 10^{10} M^{-1}) in comparison to dimerization constants of 10^5 - 10^7 M^{-1} measured for EI and EI(Δ HD).

Ziegler A., Blatter X. L., Seelig A., and Seelig J. (2003) Protein transduction domains of HIV-1 and SIV TAT interact with charged lipid vesicles. Binding mechanism and thermodynamic analysis. *Biochemistry* **42**, 9185-9194.

Abstract: Cell-penetrating peptides (CPPs) traverse cell membranes of cultured cells very efficiently by a mechanism not yet identified. Recent theories for the translocation suggest either the binding of the CPPs to extracellular glycosaminoglycans or the formation of inverted micelles with negatively charged lipids. In the present study, the binding of the protein transduction domains (PTD) of human (HIV-1) and simian immunodeficiency virus (SIV) TAT peptide (amino acid residues 47-57, electric charge $z(p) = +8$) to membranes containing various proportions of negatively charged lipid (POPG) is characterized. Monolayer expansion measurements demonstrate that TAT-PTD insertion between lipids requires loosely packed monolayer films. For densely packed monolayers ($\pi > 29 \text{ mN/m}$) and lipid bilayers, no insertion is possible, and binding occurs via electrostatic adsorption to the membrane surface. Light scattering experiments show an aggregation of anionic lipid vesicles when the electric surface charge is neutralized by TAT-PTD, the observed stoichiometry being close to the theoretical value of 1:8. Membrane binding was quantitated with isothermal titration calorimetry and three further methods. The reaction enthalpy is ΔH degrees approximately equal to -1.5 kcal/mol peptide and is almost temperature-independent with $\Delta C(p)$ degrees approximately 0 kcal/(mol K) , indicating equal contributions of polar and hydrophobic interactions to the reaction heat capacity. The binding of TAT-PTD to the anionic membrane is described by an electrostatic attraction/chemical partition model. The electrostatic attraction energy, calculated with the Gouy-Chapman theory, accounts for approximately 80% of the binding energy. The overall binding constant, $K(\text{app})$, is approximately 10^3 - 10^4 M^{-1} . The intrinsic binding constant (K_p), corrected for electrostatic effects and describing the partitioning of the peptide between the lipid-water interface and the membrane, is small and is K_p approximately 1 - 10 M^{-1} . Deuterium and phosphorus-31 nuclear magnetic resonance demonstrate that the lipid bilayer remains intact upon TAT-PTD binding. The NMR data provide no evidence for nonbilayer structures and also not for domain formation. This is further supported by the absence of dye efflux from single-walled lipid vesicles. The electrostatic interaction between TAT-PTD and anionic phosphatidylglycerol is strong enough to induce a change in the headgroup conformation of the anionic lipid, indicating a short-lived but distinct correlation between the TAT-PTD and the anionic lipids on the membrane outside. TAT-PTD has a much lower affinity for lipid membranes than for glycosaminoglycans, making the latter interaction a more probable pathway for CPP binding to biological membranes.

Zuckermann M. J. and Heimburg T. (2001) Insertion and pore formation driven by adsorption of proteins onto lipid bilayer membrane-water interfaces. *Biophys J* **81**, 2458-2472.

Abstract: We describe the binding of proteins to lipid bilayers in the case for which binding can occur either by adsorption to the lipid bilayer membrane-water interface or by direct insertion into the bilayer itself. We examine in particular the case when the insertion and pore formation are driven by the adsorption process using scaled particle theory. The adsorbed proteins form a two-dimensional "surface gas" at the lipid bilayer membrane-water interface that exerts a lateral pressure on the lipid bilayer membrane. Under conditions of strong intrinsic binding and a high degree of interfacial converge, this pressure can become high enough to overcome the energy barrier for protein insertion. Under these conditions, a subtle equilibrium exists between the adsorbed and inserted proteins. We propose that this provides a control mechanism for reversible insertion and pore formation of proteins such as melittin and magainin. Next, we discuss experimental data for the binding isotherms of cytochrome c to charged lipid membranes in the light of our theory and predict that cytochrome c inserts into charged lipid bilayers at low ionic strength. This prediction is supported by titration calorimetry results that are reported here. We were furthermore able to describe the observed binding isotherms of the pore-forming peptides endotoxin (alpha 5-helix) and of pardaxin to zwitterionic vesicles from our theory by assuming adsorption/insertion equilibrium.