DSC VI - Antibiotic, antibacterial, antifungal and antimicrobial studies

Abbassi F., Galanth C., Amiche M., Saito K., Piesse C., Zargarian L., Hani K., Nicolas P., Lequin O. and Ladram A. (2008) Solution structure and model membrane interactions of temporins-SH, antimicrobial peptides from amphibian skin. A NMR spectroscopy and differential scanning calorimetry study. *Biochemistry* **47,** 10513-10525.
Abstract: Temporin-SHa and temporin-SHc are 13 residue long antimicrobial peptides from frog skin that have similar sequences but differ markedly in their membrane-damaging properties. Temporin-SHa contains a single basic lysine residue and has a unique antimicrobial spectrum of action among temporins, being very potent against Gram-positive and Gram-negative bacteria, yeasts, fungi, and protozoa. Temporin-SHc, which contains a single basic histidine residue, is inactive against Gram-negative bacteria, has a reduced efficacy against Gram-positive bacteria, but is still active against yeasts and fungi. Temporin-SHb, with no basic residue, has no antimicrobial activity. The three-dimensional structures of the peptides bound to SDS micelles were analyzed by CD and NMR spectroscopy combined with restrained molecular dynamics calculations. The peptides adopt well-defined amphipathic alpha-helical structures extending from residue 3 to residue 12, when bound to SDS micelles. The structures are stabilized by extensive interactions between aliphatic and aromatic side chains on the nonpolar face. Relaxation enhancements caused by paramagnetic probes showed that the peptides adopt nearly parallel orientations to the micelle surface and do not deeply penetrate into the micelle. The interaction of the peptides with model membranes was investigated by differential scanning calorimetry on anionic and zwitterionic multilamellar vesicles and membrane-permeabilization assays on calcein-loaded large unilamellar vesicles. Calorimetric data indicated that both temporin-SHa and -SHc reside at the hydrocarbon core-water interface of the anionic lipid bilayer but interact with anionic bilayers in a very different manner. This suggests that the charge-induced activity of temporins-SH for bacterial cells is due to changes in the membrane-disturbing mechanism of the bound peptides

Abraham T., Marwaha S., Kobewka D. M., Lewis R. N., Prenner E. J., Hodges S. and McElhaney R. N. (2007) The relationship between the binding to and permeabilization of phospholipid bilayer membranes by GS14dK(4), a designed analog of the antimicrobial peptide gramicidin S. *Biochim Biophys Acta*.
Abstract: The cationic beta-sheet cyclic tetradecapeptide cyclo[VKLdKVdYPLKVKLdYP] (GS14dK(4)) is a diastereomeric lysine ring-size analog of the potent naturally occurring antimicrobial peptide gramicidin S (GS) which exhibits enhanced antimicrobial but markedly reduced hemolytic activity compared to GS itself. We have previously studied the binding of GS14dK(4) to various phospholipid bilayer model membranes using isothermal titration calorimetry [Abraham, T. et al. (2005) Biochemistry 44, 2103-2112]. In the present study, we compare the ability of GS14dK(4) to bind to and disrupt these same phospholipid model membranes by employing a fluorescent dye leakage assay to determine the ability of this peptide to permeabilize large unilamellar vesicles. We find that in general, the ability of GS14dK(4) to bind to and to permeabilize phospholipid bilayers of different compositions are not well correlated. In particular, the binding affinity of GS14dK(4) varies markedly with the charge and to some extent with the polar headgroup structure of the phospholipid and with the cholesterol content of the model membrane. Specifically, this peptide binds much more tightly to anionic than to zwitterionic phospholipids and much less tightly to cholesterol-containing than to cholesterol-free model membranes. In addition, the maximum extent of binding of GS14dK(4) can also vary considerably with phospholipid composition in a parallel fashion. In contrast, the ability of this peptide to permeabilize phospholipid vesicles is only weakly dependent on phospholipid charge, polar headgroup structure or cholesterol content. We provide tentative explanations for the observed lack of a correlation between the affinity and extent of GS14dK(4) binding to, and degree of disruption of the structure and integrity of, phospholipid bilayers membranes. We also present evidence that the lack of correlation between these two parameters may be a general phenomenon among antimicrobial peptides. Finally, we demonstrate that the affinity of binding of GS14dK4 to various phospholipid bilayer membranes is much more strongly correlated with the antimicrobial and hemolytic activities of this peptide than with its effect on the rate and extent of dye leakage in these model membrane systems.

Abuja P. M., Zenz A., Trabi M., Craik D. J., and Lohner K. (2004) The cyclic antimicrobial peptide RTD-1 induces stabilized lipid-peptide domains more efficiently than its open-chain analogue. *FEBS Lett* **566,**

301-306.
Abstract: The effects of a mammalian cyclic antimicrobial peptide, rhesus theta defensin 1 (RTD-1) and its open chain analogue (oRTD-1), on the phase behaviour and structure of model membrane systems (dipalmitoyl phosphatidylcholine, DPPC and dipalmitoyl phosphatidylglycerol, DPPG) were studied. The increased selectivity of RTD-1 for anionic DPPG over zwitterionic DPPC was shown by differential scanning calorimetry. RTD-1, at a molar peptide-lipid ratio of 1:100, induced considerable changes in the phase behaviour of DPPG, but not of DPPC. The main transition temperature, Tm, was unchanged, but additional phase transitions appeared above Tm. oRTD-1 induced similar effects. However, the effects were not observable below a peptide:lipid molar ratio of 1:50, which correlates with the weaker biological activity of oRTD-1. Small- and wide-angle X-ray scattering revealed for DPPG the appearance of additional structural features induced by RTD-1 above Tm, which were interpreted as correlated lamellar structures, with increased order of the fatty acyl side chains of the lipid. It is proposed that after initial electrostatic interaction of the cationic rim of the peptide with the anionic DPPG headgroups, leading to stabilized lipid-peptide clusters, the hydrophobic face of the peptide assists in its interaction with the fatty acyl side chains eventually leading to membrane disruption.

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

Alves I. D., Correia I., Jiao C. Y., Sachon E., Sagan S., Lavielle S., Tollin G. and Chassaing G. (2008) The interaction of cell-penetrating peptides with lipid model systems and subsequent lipid reorganization: thermodynamic and structural characterization. *J Pept. Sci*. (epublication)
Abstract: Cell-penetrating peptides (CPPs) are cationic peptides that are able to induce cellular uptake and delivery of large and hydrophilic molecules, that otherwise do not cross the plasma membrane of eukaryotic cells. Despite their potential use for gene transfer and drug delivery, the mode of action of CPPs is still mysterious. Nonetheless, the interaction with phospholipid bilayers constitutes the first step in the process. The interaction of two CPPs with distinct charge distribution, penetratin (nonamphipathic) and RL16 (a secondary amphipathic peptide with antimicrobial properties) with lipid membranes was investigated. For this purpose, we employed three independent techniques, comprising (31)P-nuclear magnetic resonance, differential scanning calorimetry (DSC), and plasmon waveguide resonance (PWR) spectroscopy. In view of the cationic nature of the peptides, their interaction and affinity for zwitterionic versus anionic lipids was investigated. Although a strong affinity was observed when negative charged lipids were present, the peptides' thermodynamic behavior on binding to zwitterionic versus anionic lipids and the induced supramolecular structure organization in those lipids was quite different. The study suggests that the amphipathic profile and charge distribution of CPPs strongly influences the perturbation mechanism of the peptide on the bilayer establishing the frontier between a pure CPP and a CPP with antimicrobial properties. Copyright (c) 2008 European Peptide Society and John Wiley & Sons, Ltd

Alves I. D., Goasdoue N., Correia I., Aubry S., Galanth C., Sagan S., Lavielle S. and Chassaing G. (2008) Membrane interaction and perturbation mechanisms induced by two cationic cell penetrating peptides with distinct charge distribution. *Biochim Biophys Acta* **1780,** 948-959.
Abstract: Independently from the cell penetrating peptide uptake mechanism (endocytic or not), the interaction of the peptide with the lipid bilayer remains a common issue that needs further investigation. The cell penetrating or antimicrobial properties of exogenous peptides require probably different

preliminary interactions with the plasma membrane. Herein, we have employed (31)P NMR, differential scanning calorimetry and CD to study the membrane interaction and perturbation mechanisms of two basic peptides with similar length but distinct charge distribution, penetratin (non-amphipathic) and RL16, a secondary amphipathic peptide. The peptide effects on the thermotropic phase behavior of large multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG) and dipalmitoleoyl phosphatidylethanolamine (DiPoPE) were investigated. We have found that, even though both peptides are cationic, their interaction with zwitterionic versus anionic lipids is markedly distinct. Penetratin greatly affects the temperature, enthalpy and cooperativity of DMPG main phase transition but does not affect those of DMPC while RL16 presents opposite effects. Additionally, it was found that penetratin induces a negative curvature whereas RL16 induces a positive one, since a decrease in the fluid lamellar to inverted hexagonal phase transition temperature of DiPoPE (T(H)) was observed for penetratin and an increase for RL16. Contrary to penetratin, (31)P NMR of samples containing DMPC MLVs and RL16 shows an isotropic signal indicative of the formation of small vesicles, concomitant with a great decrease in sample turbidity both below and at the phase transition temperature. Opposite effects were also observed on DMPG where both peptides provoke strong aggregation and precipitation. Both CPPs adopt helical structures when contacting with anionic lipids, and possess a dual behavior by either presenting their cationic or hydrophobic domains towards the phospholipid face, depending on the lipid nature (anionic vs zwitterionic, respectively). Surprisingly, the increase of electrostatic interactions at the water membrane interface prevents the insertion of RL16 hydrophobic region in the bilayer, but is essential for the interaction of penetratin. Modulation of amphipathic profiles and charge distribution of CPPs can alter the balance of hydrophobic and electrostatic membrane interaction leading to translocation or and membrane permeabilisation. Penetratin has a relative pure CPP behavior whereas RL16 presents mixed CPP/AMP properties. A better understanding of those processes is essential to unveil their cell translocation mechanism

Andrushchenko V. V., Vogel H. J. and Prenner E. J. (2007) Interactions of tryptophan-rich cathelicidin antimicrobial peptides with model membranes studied by differential scanning calorimetry. *Biochim Biophys Acta* **1768,** 2447-2458.
Abstract: The 13-residue cathelicidins indolicidin and tritrpticin are part of a group of relatively short tryptophan-rich antimicrobial peptides that hold potential as future substitutes for antibiotics. Differential scanning calorimetry (DSC) has been applied here to study the effect of indolicidin and tritrpticin as well as five tritrpticin analogs on the phase transition behaviour of model membranes made up of zwitterionic dimyristoylphosphatidylcholine (DMPC, DMPC/cholesterol) and anionic dimyristoylphosphatidyl glycerol (DMPG) phospholipids. Most of the peptides studied significantly modified the phase transition profile, suggesting the importance of hydrophobic forces for the peptide interactions with the lipid bilayers and their insertion into the bilayer. Indolicidin and tritrpticin are both known to be flexible in aqueous solution, but they adopt turn-turn structures when they bind to and insert in a membrane surface. Pro-to-Ala substitutions in tritrpticin, which result in the formation of a stable alpha-helix in this peptide, lead to a substantial increase in the peptide interactions with both zwitterionic and anionic phospholipid vesicles. In contrast, the substitution of the three Trp residues by Tyr or Phe resulted in a significant decrease of the peptide's interaction with anionic vesicles and virtually eliminated binding of these peptides to the zwitterionic vesicles. An increase of the cationic charge of the peptide induced much smaller changes to the peptide interaction with all lipid systems than substitution of particular amino acids or modification of the peptide conformation. The presence of multiple lipid domains with a non-uniform peptide distribution was noticed. Slow equilibration of the lipid-peptide systems due to peptide redistribution was observed in some cases. Generally good agreement between the present DSC data and peptide antimicrobial activity data was obtained.

Barcelo F., Capo D., and Portugal J. (2002) Thermodynamic characterization of the multivalent binding of chartreusin to DNA. *Nucleic Acids Res* **30,** 4567-4573.
Abstract: Characterization of the thermodynamics of DNA- drug interactions is a very useful part in rational drug design. Isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and UV melting experiments have been used to analyze the multivalent (intercalation plus minor groove) binding of the antitumor antibiotic chartreusin to DNA. Using DNA UV melting studies in the presence of the ligand and the binding enthalpy determined by ITC, we determined that the binding constant for the interaction was 3.6×10^{5} M^{-1} at 20 degrees C, in a solution containing 18 mM Na(+). The DNA-drug

interaction was enthalpy driven, with a ΔH(b) of -7.07 kcal/mol at 20 degrees C. Binding enthalpies were determined by ITC in the 20-35 degrees C range and used to calculate a binding-induced change in heat capacity (ΔCp) of -391 cal/mol K. We have obtained a detailed thermodynamic profile for the interaction of this multivalent drug, which makes possible a dissection of ΔG(obs) into the component free energy terms. The hydrophobic transfer of the chartreusin chromophore from the solution to the DNA intercalating site is the main contributor to the free energy of binding.

Barcelo F., Scotta C., Ortiz-Lombardia M., Mendez C., Salas J. A. and Portugal J. (2007) Entropically-driven binding of mithramycin in the minor groove of C/G-rich DNA sequences. *Nucleic Acids Res* **35,** 2215-2226.
Abstract: The antitumour antibiotic mithramycin A (MTA) is a DNA minor-groove binding ligand. It binds to C/G-rich tracts as a dimer that forms in the presence of divalent cations such as Mg(2+). Differential scanning calorimetry, UV thermal denaturation, isothermal titration calorimetry and competition dialysis were used, together with computations of the hydrophobic free energy of binding, to determine the thermodynamic profile of MTA binding to DNA. The results were compared to those obtained in parallel using the structurally related mithramycin SK (MSK). The binding of MTA to salmon testes DNA determined by UV melting studies (K(obs) = 1.2 (+/-0.3) x 10(5) M(-1)) is tighter than that of MSK (2.9 (+/-1.0) x 10(4) M(-1)) at 25 degrees C. Competition dialysis studies showed a tighter MTA binding to both salmon testes DNA (42% C + G) and Micrococcus lysodeikticus DNA (72% C + G). The thermodynamic analysis of binding data at 25 degrees C shows that the binding of MTA and MSK to DNA is entropically driven, dominated by the hydrophobic transfer of the antibiotics from solution to the DNA-binding site. Direct molecular recognition between MTA or MSK and DNA through hydrogen bonding and van der Waals contacts may also contribute significantly to complex formation.

Berezniak E. G., Kruglova E. B., Khrebtova A. S., Dukhopel'nikov E. V. and Zinchenko A. V. (2007) [Interaction of actinocin derivative with different poly(rC) structures]. *Biofizika* **52,** 812-821.
Abstract: The interaction of actinocin derivative Act III with single- and double-stranded poly(rC) has been investigated by the methods of differential scanning microcalorimetry and UV-vis absorption spectroscopy. It was shown that, after the addition of the ligand, the temperature, enthalpy and entropy of poly (rC) melting decrease. The analysis of poly(rC)-ActIII absorption spectra indicated that the conformation of polynucleotide differs from that of free poly (rC) in the presence of ActHI at pH 4.46 and pH 6.0. Using the DALSMOD optimization program, the parameters of interaction of Act III with poly (rC) were calculated. It was found that the binding constant of ActHI with double-stranded poly (rC) is essentially higher than that with the single-stranded one upon monomeric binding. On the basis of these data, we conclude that the conformation changes of the matrix are the main cause of the decrease in melting temperature and enthalpy observed by calorimetry. Possible mechanisms of interaction of actinocin derivative with poly (rC) are discussed.

Bonhivers M., Desmadril M., Moeck G. S., Boulanger P., Colomer-Pallas A., and Letellier L. (2001) Stability studies of FhuA, a two-domain outer membrane protein from Escherichia coli. *Biochemistry* **40,** 2606-2613.
Abstract: FhuA (MM 78.9 kDa) is an Escherichia coli outer membrane protein that transports iron coupled to ferrichrome and is the receptor for a number of bacteriophages and protein antibiotics. Its three-dimensional structure consists of a 22-stranded beta-barrel lodged in the membrane, extracellular hydrophilic loops, and a globular domain (the "cork") located within the beta-barrel and occluding it. This unexpected structure raises questions about the connectivity of the different domains and their respective roles in the different functions of the protein. To address these questions, we have compared the properties of the wild-type receptor to those of a mutated FhuA (FhuA Δ) missing a large part of the cork. Differential scanning calorimetry experiments on wild-type FhuA indicated that the cork and the beta-barrel behave as autonomous domains that unfold at 65 and 75 degrees C, respectively. Ferrichrome had a strong stabilizing effect on the loops and cork since it shifted the first transition to 71.4 degrees C. Removal of the cork destabilized the protein since a unique transition at 61.6 degrees C was observed even in the presence of ferrichrome. FhuA Δ showed an increased sensitivity to proteolysis and to denaturant agents and an impairment in phage T5 and ferrichrome binding.

Brandenburg K., Jurgens G., Muller M., Fukuoka S., and Koch M. H. (2001) Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. *Biol Chem* **382,** 1215-1225.
Abstract: The interaction of bacterial endotoxins (LPS Re and lipid A, the 'endotoxic principle' of LPS) with the endogenous antibiotic lactoferrin (LF) was investigated using various physical techniques and biological assays. By applying Fourier-transform infrared (FTIR) spectroscopy, we find that LF binds to the phosphate group within the lipid A part and induces a rigidification of the acyl chains of LPS. The secondary structure of the protein - as monitored by the amide I band - is, however, not changed. Concomitant with the IR data, scanning calorimetric data indicate a sharpening of the acyl chain phase transition. From titration calorimetric and zeta potential data, saturation of LF binding to LPS was found to lie at a [LF]:[LPS] ratio of 1:3 to 1:5 M from the former and 1:10 M from the latter technique. X-ray scattering data indicate a change of the lipid A aggregate structure from inverted cubic to multilamellar, and with fluorescence (FRET) spectroscopy, LF is shown to intercalate by itself into phospholipid liposomes and may also block the lipopolysaccharide-binding protein (LBP)-induced intercalation of LPS. The LPS-induced cytokine production of human mononuclear cells exhibits a decrease due to LF binding, whereas the coagulation of amebocyte lysate in the Limulus test exhibited concentration-dependent changes. Based on these results, a model for the mechanisms of endotoxin inactivation by LF is proposed.

Brouns S.J., Wu H., Akerboom J., Turnbull A.P., de Vos W.M., and van der Oost J. (2005) Engineering a selectable marker for hyperthermophiles. *J Biol Chem.* **280**, 11422-31.
Abstract: Limited thermostability of antibiotic resistance markers has restricted genetic research in the field of extremely thermophilic Archaea and bacteria. In this study, we used directed evolution and selection in the thermophilic bacterium Thermus thermophilus HB27 to find thermostable variants of a bleomycin-binding protein from the mesophilic bacterium Streptoalloteichus hindustanus. In a single selection round, we identified eight clones bearing five types of double mutated genes that provided T. thermophilus transformants with bleomycin resistance at 77 degrees C, while the wild-type gene could only do so up to 65 degrees C. Only six different amino acid positions were altered, three of which were glycine residues. All variant proteins were produced in Escherichia coli and analyzed biochemically for thermal stability and functionality at high temperature. A synthetic mutant resistance gene with low GC content was designed that combined four substitutions. The encoded protein showed up to 17 degrees C increased thermostability and unfolded at 85 degrees C in the absence of bleomycin, whereas in its presence the protein unfolded at 100 degrees C. Despite these highly thermophilic properties, this mutant was still able to function normally at mesophilic temperatures in vivo. The mutant protein was co-crystallized with bleomycin, and the structure of the binary complex was determined to a resolution of 1.5 A. Detailed structural analysis revealed possible molecular mechanisms of thermostabilization and enhanced antibiotic binding, which included the introduction of an intersubunit hydrogen bond network, improved hydrophobic packing of surface indentations, reduction of loop flexibility, and alpha-helix stabilization. The potential applicability of the thermostable selection marker is discussed.

Carver T.E., Bordeau B., Cummings M.D., Petrella E.C., Pucci M.J., Zawadzke L.E., Dougherty B.A., Tredup J.A., Bryson J.W., Yanchunas J. Jr, Doyle M.L., Witmer M.R., Nelen M.I., DesJarlais R.L., Jaeger E.P., Devine H., Asel E.D., Springer B.A., Bone R., Salemme F.R., and Todd M.J. (2005) Decrypting the biochemical function of an essential gene from Streptococcus pneumoniae using ThermoFluor technology. *J Biol Chem.* **280**, 11704-12.
Abstract: The protein product of an essential gene of unknown function from Streptococcus pneumoniae was expressed and purified for screening in the ThermoFluor affinity screening assay. This assay can detect ligand binding to proteins of unknown function. The recombinant protein was found to be in a dimeric, native-like folded state and to unfold cooperatively. ThermoFluor was used to screen the protein against a library of 3000 compounds that were specifically selected to provide information about possible biological functions. The results of this screen identified pyridoxal phosphate and pyridoxamine phosphate as equilibrium binding ligands (K(d) approximately 50 pM, K(d) approximately 2.5 microM, respectively), consistent with an enzymatic cofactor function. Several nucleotides and nucleotide sugars were also identified as ligands of this protein. Sequence comparison with two enzymes of known structure but relatively low overall sequence homology established that several key residues directly involved in pyridoxal phosphate binding were strictly conserved. Screening a collection of generic drugs and natural products identified the antifungal compound canescin A as an irreversible covalent modifier of the enzyme. Our investigation of this protein indicates that its probable biological role is that of a nucleoside diphospho-

keto-sugar aminotransferase, although the preferred keto-sugar substrate remains unknown. These experiments demonstrate the utility of a generic affinity-based ligand binding technology in decrypting possible biological functions of a protein, an approach that is both independent of and complementary to existing genomic and proteomic technologies.

Caturla N., Vera-Samper E., Villalain J., Mateo C. R., and Micol V. (2003) The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Radic Biol Med* **34,** 648-662.
Abstract: The effects of four catechins, (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG), on the physical properties of phospholipid model membranes and the correlation to their antioxidant and antibacterial capacities have been studied by using differential scanning calorimetry (DSC), fluorescence spectroscopy, infrared spectroscopy (IR), AAPH-induced oxidation, and leakage experiments. DSC data revealed that galloylated catechins, especially ECG, affected the physical properties of both the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) bilayers dramatically. Galloylated catechins showed higher phospholipid/water partition coefficients than their homologues and were immersed in the phospholipid palisade intercalating within the hydrocarbon chains, ECG being at the deepest position. In contrast, nongalloylated catechins presented a shallow location close to the phospholipid/water interface. ECG also exhibited the highest antioxidant capacity against lipid peroxidation, which correlated with its strong effect on DPH fluorescence anisotropy (as observed by the increase of the lipid order of fluid PC bilayers) and with the presence of highly cooperative transitions as seen by DSC. We propose that the high antioxidant capacity of some galloylated catechins such as ECG could be partially due to the formation of membrane structures showing resistance to detergent solubilization and in which the phospholipids have tightly packed acyl chains and highly hydrated phosphate groups. Significantly, PE was found to be essential to the promotion of carboxyfluorescein leakage from bacterial model membranes by galloylated catechins, indicating that their bactericidal activity, at least at the membrane level, could be due to the specific effect of these catechins on PE.

Caturla N., Perez-Fons L., Estepa A., and Micol V. (2005) Differential effects of oleuropein, a biophenol from Olea europaea, on anionic and zwiterionic phospholipid model membranes. *Chem Phys Lipids* **137,** 2-17.
Abstract: Oleuropein (Ole) is the major phenolic constituent of the olive leaf (Olea europaea) and it is also present in olive oil and fruit. In the last years several compounds from olive tree, oleuropein among them, have shown a variety of biological activities such as antimicrobial or antioxidant. A phospholipid model membrane system was used to study whether the Ole biological effects could be membrane related. Ole showed a significant partition level in phospholipid membranes, i.e. 80%, at lipid-saturating conditions. Moreover, fluorescence quenching experiments indicated a shallow location for Ole in membranes. Ole promoted weak effects on zwiterionic phospholipids such as phosphatidylcholine or phosphatidylethanolamine. In contrast, differential scanning microcalorimetry, light scattering and fluorescence anisotropy pH titration studies revealed strong effects on anionic phospholipids such as phosphatidylglycerol at physiological pH and salt conditions. These effects consisted on perturbations at the phospholipid membrane surface, which might involve specific molecular interactions between Ole and the negatively charged phosphate group and therefore modify the phospholipid/water interface properties. It is proposed that Ole induces lipid structures similar to the gel-fluid intermediate phase (IP) described for PG membranes, in a similar way than low ionic strength does. These effects on phosphatidylglycerol may account for the antimicrobial activity of Ole.

Chaires J. B., Leng F., Przewloka T., Fokt I., Ling Y. H., Perez-Soler R., and Priebe W. (1997) Structure-based design of a new bisintercalating anthracycline antibiotic. *J Med Chem* **40,** 261-266.
Abstract: A new bisintercalating anthracycline antibiotic, WP631, has been designed and synthesized. The rational design of the new compound was based upon the geometry of monomeric anthracyclines bound to DNA oligonucleotides observed in high-resolution crystal structures. Monomeric units of daunorubicin have been linked through their reactive 3' NH_2 substituents on the daunosamine moieties to form the new bisanthracycline WP631. Viscosity studies confirmed that WP631 binds to DNA by bisintercalation. Differential scanning calorimetry and UV melting experiments were used to measure the ultratight binding

of WP631 to DNA. The binding constant for the interaction of WP631 with herring sperm DNA was determined to be 2.7 x 10^{11} M^{-1} at 20 degrees C. The large, favorable binding free energy of -15.3 kcal mol^{-1} was found to result from a large, negative enthalpic contribution of -30.2 kcal mol^{-1}. A molecular model was generated that shows the favorable stereochemical fit of the linker in the DNA minor groove. The cytotoxicity of WP631 was compared to that of doxorubicin using MCF-7-sensitive and MCF-7/VP-16 MRP-mediated multidrug-resistant cell lines. These initial studies showed that while WP631 is slightly less cytotoxic than doxorubicin in the sensitive cell line, it appears to overcome MRP-mediated multidrug resistance and was much more cytotoxic against the MCF-7/VP-16 cell line than was doxorubicin. The design of new potential anticancer agents based on known structural principles was found to produce a compound with significantly increased DNA binding affinity and with interesting biological activity.

Chaires J. B. (1998) Drug--DNA interactions. *Curr Opin Struct Biol* **8,** 314-320.
Abstract: Significant progress has been made over the past few years in studies of drug-DNA interactions. Structure-based design strategies have yielded new DNA-binding agents with clinical promise. The hairpin polyamides represent the result of a design strategy with outstanding potential. One specific molecule of this class has now been proven to inhibit the expression of a specific gene in vivo. A new bisintercalating anthracycline antibiotic binds with high affinity to DNA, and appears to overcome a specific form of multidrug resistance. Progress in fundamental studies of drug binding to DNA continues, with detailed thermodynamic studies providing new insights into the forces that drive complex formation. New tools have been developed in order to characterize both the binding mode and the sequence specificity of drug binding to DNA, tools that will enable the fundamental aspects of these biologically important reactions to be understood in more detail.

Chen H. M., Leung K. W., Thakur N. N., Tan A., and Jack R. W. (2003) Distinguishing between different pathways of bilayer disruption by the related antimicrobial peptides cecropin B, B1 and B3. *Eur J Biochem* **270,** 911-920.
Abstract: Different pathways of bilayer disruption by the structurally related antimicrobial peptides cecropin B, B1 and B3, revealed by surface plasma resonance analysis of immobilized liposomes, differential scanning calorimetry of peptide-large unilamellar vesicle interactions, and light microscopic analysis of peptide-treated giant unilamellar vesicles, have been identified in this study. Natural cecropin B (CB) has one amphipathic and one hydrophobic alpha-helix, whereas cecropins B1 (CB1) and B3 (CB3), which are custom-designed, chimaeric analogues of CB, possess either two amphipathic or two hydrophobic alpha-helices, respectively. Surface plasma resonance analysis of unilamellar vesicles immobilized through a biotin-avidin interaction showed that both CB and CB1 bind to the lipid bilayers at high concentration (>10 μM); in contrast, CB3 induces disintegration of the vesicles at all concentrations tested. Differential scanning calorimetry showed the concentration-dependent effect of bilayer disruption, based on the different thermotrophic phase behaviours and the shapes of the thermal phase-transition curves obtained. The kinetics of the lysis of giant unilamellar vesicles observed by microscopy demonstrated that both CB and CB1 effect a continuous process involving loss of integrity followed by coalescence and resolution into smaller vesicles, whereas CB3 induces rapid formation of irregular-shaped, nonlamellar structures which rapidly disintegrate into twisted, microtubule-containing debris before being completely destroyed. On the basis of these observations, models by which CB, CB1 and CB3 induce lysis of lipid bilayers are discussed.

Cristani M., D'Arrigo M., Mandalari G., Castelli F., Sarpietro M. G., Micieli D., Venuti V., Bisignano G., Saija A. and Trombetta D. (2007) Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *J Agric. Food Chem* **55,** 6300-6308.
Abstract: The present article reports the antimicrobial efficacy of four monoterpenes (thymol, carvacrol, p-cymene, and gamma-terpinene) against the Gram-positive bacterium Staphylococcus aureus and the Gram-negative bacterium Escherichia coli. For a better understanding of their mechanism of action, the damage caused by these four monoterpenes on biomembranes was evaluated by monitoring the release, following exposure to the compounds under study, of the water-soluble fluorescent marker carboxyfluorescein (CF) from large unilamellar vesicles (LUVs) with different lipidic composition (phosphatidylcholine, PC, phosphatidylcholine/phosphatidylserine, PC/PS, 9:1; phosphatidylcholine/stearylamine, PC/SA, 9:1). Furthermore, the interaction of these terpenes with dimyristoylphosphatidylcholine multilamellar vesicles as model membranes was monitored by means of differential scanning calorimetry (DSC) technique.

Finally, the results were related also with the relative lipophilicity and water solubility of the compounds examined. We observed that thymol is considerably more toxic against S. aureus than the other three terpenes, while carvacrol and p-cymene are the most inhibitory against E. coli. Thymol and carvacrol, but not gamma-terpinene and p-cymene, caused a concentration-dependent CF leakage from all kinds of LUVs employed; in particular, thymol was more effective on PC and PC/SA LUVS than on PC/PS vesicles, while carvacrol challenge evoked a CF leakage from PC/PS LUVs similar to that induced from PC/SA LUVs, and lower than that measured with PC vesicles. Concerning DSC experiments, these four terpenes caused a decrease in Tm and (especially carvacrol and p-cymene) DeltaH values, very likely acting as substitutional impurities. Taken together, our findings lead us to speculate that the antimicrobial effect of thymol, carvacrol, p-cymene, and gamma-terpinene may result, partially at least, from a gross perturbation of the lipidic fraction of the plasmic membrane of the microorganism. In addition to being related to the physicochemical characteristics of the compounds (such as lipophilicity and water solubility), this effect seems to be dependent on the lipidic composition and net surface charge of the microbic membranes. Furthermore, the compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity.

Dave P. C., Billington E., Pan Y. L., and Straus S. K. (2005) Interaction of alamethicin with ether-linked phospholipid bilayers: oriented circular dichroism, 31P solid-state NMR, and differential scanning calorimetry studies. *Biophys J* **89,** 2434-2442.
Abstract: The arrangement of the antimicrobial peptide alamethicin was studied by oriented circular dichroism, 31P solid-state NMR, and differential scanning calorimetry in ether-linked phospholipid bilayers composed of 1,2-O-dihexadecyl-sn-glycero-3-phosphocholine (DHPC). The measurements were performed as a function of alamethicin concentration relative to the lipid concentration, and results were compared to those reported in the literature for ester-linked phospholipid bilayers. At ambient temperature, alamethicin incorporates into the hydrophobic core of DHPC bilayers but results in more lipid disorder than observed for ester-linked 1-palmitoyl, 2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) lipid bilayers. This orientational disorder appears to depend on lipid properties such as bilayer thickness. Moreover, the results suggest that alamethicin inserts into the hydrophobic core of the bilayers (at high peptide concentration) for both ether- and ester-linked lipids but using a different mechanism, namely toroidal for DHPC and barrel-stave for POPC.

Deleu M., Paquot M. and Nylander T. (2008) Effect of fengycin, a lipopeptide produced by Bacillus subtilis, on model biomembranes. *Biophys J* **94,** 2667-2679.
Abstract: Fengycin is a biologically active lipopeptide produced by several Bacillus subtilis strains. The lipopeptide is known to develop antifungal activity against filamentous fungi and to have hemolytic activity 40-fold lower than that of surfactin, another lipopeptide produced by B. subtilis. The aim of this work is to use complementary biophysical techniques to reveal the mechanism of membrane perturbation by fengycin. These include: 1), the Langmuir trough technique in combination with Brewster angle microscopy to study the lipopeptide penetration into monolayers; 2), ellipsometry to investigate the adsorption of fengycin onto supported lipid bilayers; 3), differential scanning calorimetry to determine the thermotropic properties of lipid bilayers in the presence of fengycin; and 4), cryogenic transmission electron microscopy, which provides information on the structural organization of the lipid/lipopeptide system. From these experiments, the mechanism of fengycin action appears to be based on a two-state transition controlled by the lipopeptide concentration. One state is the monomeric, not deeply anchored and nonperturbing lipopeptide, and the other state is a buried, aggregated form, which is responsible for membrane leakage and bioactivity. The mechanism, thus, appears to be driven mainly by the physicochemical properties of the lipopeptide, i.e., its amphiphilic character and affinity for lipid bilayers

Draper D. E., Xing Y., and Laing L. G. (1995) Thermodynamics of RNA unfolding: stabilization of a ribosomal RNA tertiary structure by thiostrepton and ammonium ion. *J Mol Biol* **249,** 231-238.
Abstract: RNAs with interesting secondary and tertiary structures tend to melt in several broad and overlapping transitions over a wide temperature range, and it has been consequently difficult to resolve the thermodynamics of individual unfolding steps. In the case that a ligand selectively binds a single folded state of the RNA, it is possible to obtain reliable thermodynamic parameters for both RNA unfolding and RNA-ligand binding simply from the hyperchromicity of RNA denaturation. The analysis procedure involves fitting a three-dimensional surface to absorbance data collected as a function of both temperature

and ligand concentration. Analysis of the unfolding of a fragment of the large subunit ribosomal RNA (Escherichia coli sequence 1051 to 1109) is presented; both an antibiotic (thiostrepton) and ammonium ion specifically stabilize a tertiary structure within this RNA. A consistent set of thermodynamic parameters (Δ H and tm) for the first two sequentially linked unfolding transitions is obtained from the experiments, and the binding constants obtained for the two ligands are consistent with other independent measurements. The approach is applicable to a variety of RNAs that specifically bind proteins, antibiotics, ions or other ligands.

Epand R. F., Raguse T. L., Gellman S. H., and Epand R. M. (2004) Antimicrobial 14-helical beta-peptides: potent bilayer disrupting agents. *Biochemistry* **43,** 9527-9535.
Abstract: The interactions of two amphiphilic and cationic, nine-residue beta-peptides with liposomal membranes were studied. These beta-peptides are shown to form 14-helices in the presence of bilayers. Membrane binding and membrane permeabilization occur preferentially in the presence of anionic lipids. The beta-peptides have the ability to cause tranbilayer diffusion of phospholipids, form pores, and promote lipid mixing between liposomes. These beta-peptides have previously been shown to display antimicrobial activity comparable to that of a longer beta-peptide, beta-17, which adopts a different type of helical conformation (12-helix), and to the 23 amino acid (Ala(8,13,18))-magainin-II-amide, which adopts an alpha-helical conformation. In addition, these 14-helical beta-peptides show relatively low hemolytic activity. The biological potency and microbial specificity of the 14-helical beta-peptides, despite their relatively short length, suggests that 14-helices can be particularly disruptive to microbial membranes.

Epand R. M. and Epand R. F. (2003) Liposomes as models for antimicrobial peptides. *Methods Enzymol* **372,** 124-133.

Epand R. F., Savage P. B. and Epand R. M. (2007) Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). *Biochim Biophys Acta* **1768,** 2500-2509.
Abstract: Ceragenins are cationic bile salt derivatives having antimicrobial activity. The interactions of several ceragenins with phospholipid bilayers were tested in different systems. The ceragenins are capable of forming specific associations with several phospholipid species that may be involved with their antimicrobial action. Their antimicrobial activity is lower in bacteria that have a high content of phosphatidylethanolamine. Gram negative bacteria with a high content of phosphatidylethanolamine exhibit sensitivity to different ceragenins that corresponds to the extent of interaction of these compounds with phospholipids, including the ability of different ceragenins to induce leakage of aqueous contents from phosphatidylethanolamine-rich liposomes. A second class of bacteria having cell membranes composed largely of anionic lipids and having a low content of phosphatidylethanolamine are very sensitive to the action of the ceragenins but they exhibit similar minimal inhibitory concentrations with most of the ceragenins and for different strains of bacteria. Although Gram negative bacteria generally have a high content of phosphatidylethanolamine, there are a few exceptions. In addition, a mutant strain of Escherichia coli has been made that is essentially devoid of phophatidylethanolamine, although 80% of the lipid of the wild-type strain is phosphatidylethanolamine. Furthermore, certain Gram positive bacteria are also exceptions in that they can have a high content of phosphatidylethanolamine. We find that the antimicrobial action of the ceragenins correlates better with the content of phosphatidylethanolamine in the bacterial membrane than whether or not the bacteria has an outer membrane. Thus, the bacterial lipid composition can be an important factor in determining the sensitivity of bacteria to antimicrobial agents.

Epand R. F., Schmitt M. A., Gellman S. H., and Epand R. M. (2006) Role of membrane lipids in the mechanism of bacterial species selective toxicity by two alpha/beta-antimicrobial peptides. *Biochim Biophys Acta* **1758,** 1343-1350.
Abstract: We have previously shown that two synthetic antimicrobial peptides with alternating alpha- and beta-amino acid residues, designated simply as alpha/beta-peptide I and alpha/beta-peptide II, had toxicity toward bacteria and affected the morphology of bacterial membranes in a manner that correlated with their effects on liposomes with lipid composition similar to those of the bacteria. In the present study we account for the weak effects of alpha/beta-peptide I on liposomes or bacteria whose membranes are enriched in phosphatidylethanolamine (PE) and why such membranes are particularly susceptible to damage by alpha/beta-peptide II. The alpha/beta-peptide II has marked effects on unilamellar vesicles enriched in PE causing vesicle aggregation and loss of their internal aqueous contents. The molecular basis of these effects

is the ability of alpha/beta-peptide II to induce phase segregation of anionic and zwitterionic lipids as shown by fluorescence and differential scanning calorimetry. This phase separation could result in the formation of defects through which polar materials could pass across the membrane as well as form a PE-rich membrane domain that would not be a stable bilayer. alpha/beta-Peptide II is more effective in this regard because, unlike alpha/beta-peptide I, it has a string of two or three adjacent cationic residues that can interact with anionic lipids. Although alpha/beta-peptide I can destroy membrane barriers by converting lamellar to non-lamellar structures, it does so only weakly with unilamellar vesicles or with bacteria because it is not as efficient in the aggregation of these membranes leading to the bilayer-bilayer contacts required for this phase conversion. This study provides further understanding of why alpha/beta-peptide II is more toxic to micro-organisms with a high PE content in their membrane as well as for the lack of toxicity of alpha/beta-peptide I with these cells, emphasizing the potential importance of the lipid composition of the cell surface in determining selective toxicity of anti-microbial agents.

Fa N., Ronkart S., Schanck A., Deleu M., Gaigneaux A., Goormaghtigh E., and Mingeot-Leclercq M. P. (2006) Effect of the antibiotic azithromycin on thermotropic behavior of DOPC or DPPC bilayers. *Chem Phys Lipids* **144,** 108-116.
Abstract: Azithromycin is a macrolide antibiotic known to bind to lipids and to affect endocytosis probably by interacting with lipid membranes [Tyteca, D., Schanck, A., Dufrene, Y.F., Deleu, M., Courtoy, P.J., Tulkens, P.M., Mingeot-Leclercq, M.P., 2003. The macrolide antibiotic azithromycin interacts with lipids and affects membrane organization and fluidity: studies on Langmuir-Blodgett monolayers, liposomes and J774 macrophages. J. Membr. Biol. 192, 203-215]. In this work, we investigate the effect of azithromycin on lipid model membranes made of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). Thermal transitions of both lipids in contact with azithromycin are studied by (31)P NMR and DSC on multilamellar vesicles. Concerning the DPPC, azithromycin induces a suppression of the pretransition whereas a phase separation between the DOPC and the antibiotic is observed. For both lipids, the enthalpy associated with the phase transition is strongly decreased with azithromycin. Such effects may be due to an increase of the available space between hydrophobic chains after insertion of azithromycin in lipids. The findings provide a molecular insight of the phase merging of DPPC gel in DOPC fluid matrix induced by azithromycin [Berquand, A., Mingeot-Leclercq, M.P., Dufrene, Y.F., 2004. Real-time imaging of drug-membrane interactions by atomic force microscopy. Biochim. Biophys. Acta 1664, 198-205] and could help to a better understanding of azithromycin-cell interaction.

Falck E., Hautala J. T., Karttunen M., Kinnunen P. K., Patra M., Saaren-Seppala H., Vattulainen I., Wiedmer S. K., and Holopainen J. M. (2006) Interaction of fusidic acid with lipid membranes: Implications to the mechanism of antibiotic activity. *Biophys J* **91,** 1787-1799.
Abstract: We have studied the effects of cholesterol and steroid-based antibiotic fusidic acid (FA) on the behavior of lipid bilayers using a variety of experimental techniques together with atomic-scale molecular dynamics simulations. Capillary electrophoretic measurements showed that FA was incorporated into fluid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine membranes. Differential scanning calorimetry in turn showed that FA only slightly altered the thermodynamic properties of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers, whereas cholesterol abolished all endotherms when the mole fraction of cholesterol (X(chol)) was >0.20. Fluorescence spectroscopy was then used to further characterize the influence of these two steroids on DPPC large unilamellar vesicles. In the case of FA, our result strongly suggested that FA was organized into lateral microdomains with increased water penetration into the membrane. For cholesterol/DPPC mixtures, fluorescence spectroscopy results were compatible with the formation of the liquid-ordered phase. A comparison of FA and cholesterol-induced effects on DPPC bilayers through atomistic molecular dynamics simulations showed that both FA and cholesterol tend to order neighboring lipid chains. However, the ordering effect of FA was slightly weaker than that of cholesterol, and especially for deprotonated FA the difference was significant. Summarizing, our results show that FA is readily incorporated into the lipid bilayer where it is likely to be enriched into lateral microdomains. These domains could facilitate the association of elongation factor-G into lipid rafts in living bacteria, enhancing markedly the antibiotic efficacy of FA.

Fournier I., Barwicz J., Auger M. and Tancrede P. (2008) The chain conformational order of ergosterol- or cholesterol-containing DPPC bilayers as modulated by Amphotericin B: a FTIR study. *Chem Phys. Lipids* **151,** 41-50.
Abstract: Amphotericin B (AmB) is the most widely used antibiotic to treat systemic fungal infections. However, the molecular mechanism of its activity is still not completely understood. In the present work we have used FTIR spectroscopy to investigate the conformational state of the aliphatic chains of DPPC liposomes using the 2850 cm(-1) band, associated with the methylene symmetric stretching mode. The liposomes were either binary mixtures of the lipid with AmB, cholesterol or ergosterol, or ternary systems of these constituents. The two sterols contribute to an ordering of the aliphatic chains of the lipid, this ordering being slightly more important with ergosterol. In the gel state, AmB does not change the conformational order of DPPC even at high concentration. In the fluid phase, however, the drug clearly structures its lipid environment. Our results show that AmB can initiate a redistribution of the ergosterol in the plane of the membrane, but not of the cholesterol molecules, which might constitute an additional mechanism to explain the activity of the antibiotic

Fukuoka S. and Karube I. (1994) Influence of cationic antibiotics on phase behavior of rough-form lipopolysaccharide. *Appl Biochem Biotechnol* **49,** 1-9.
Abstract: The rough-form lipopolysaccharide (LPS) interacted with cationic antibiotic polymyxin B and gramicidin S in solution, and showed altered thermotropic phase behavior and viscoelasticity. The phase behavior was measured by differential scanning calorimetry and quartz crystal microbalance (QCM). Addition of polymyxin B of up to 0.5 mg/mL to the 5.0 mg/mL LPS solution increased gel-to-liquid crystalline phase transition enthalpy (Δ H) and raised the transition temperature (tmax). The further addition of polymyxin B reduced the Δ H value. Gramicidin S produced a different effect, whereby a minor addition reduced tmax and Δ H value of the LPS. The LPS film on the platinum electrode of the QCM indicated a downward shift of resonant frequency and an upward shift of resonant resistance when in contact with the antibiotic solution. An interpretation of these variations is that the LPS on the QCM electrode changed not only film weight, but also viscoelasticity owing to contact with the antibiotic solution. The different effects between the antibiotics between polymyxin B and gramicidin S on the LPS are induced by the difference of the governing effect. Polymyxin B interacts with the LPS electrostatically, whereas gramicidin S interacts by hydrophobic moieties

Fukuoka S., Howe J., Andra J., Gutsmann T., Rossle M. and Brandenburg K. (2008) Physico-chemical and biophysical study of the interaction of hexa- and heptaacyl lipid A from Erwinia carotovora with magainin 2-derived antimicrobial peptides. *Biochim Biophys Acta* **1778,** 2051-2057.
Abstract: The neutralization of endotoxin structures such as the active 'endotoxic principle' lipid A by suitable compounds has been shown to be a key step in the treatment of infectious diseases, in particular in the case of Gram-negative bacteria which frequently may lead to the septic shock syndrome. An effective antimicrobial peptide, originally found in the skin of an African frog, is magainin 2. Here, the interaction of magainin 2-amide and a peptide derived thereof, M2V, with chemically defined and homogeneous hexaacyl and heptaacyl lipids A isolated from LPS of Erwinia carotovora, was investigated. By using Fourier-transform infrared spectroscopy, the gel to liquid crystalline phase transition of the acyl chains of lipid A and the conformation of their phosphate groups due to peptide binding was investigated. The former parameter was also determined by using differential scanning calorimetry. The electrophoretic mobility of lipid A aggregates under the influence of the peptides was studied to determine the Zeta potential, and small-angle X-ray scattering was applied for the elucidation of the types of aggregate structures in the absence and presence of the peptides. The lipid A-induced cytokine production in human mononuclear cells shows that the ability of the two peptides to inhibit a tumor necrosis factor-alpha production correlates with characteristic changes of the biophysical parameters. These are much stronger expressed for the peptide M2V than for magainin 2-amide, which apparently is connected with the higher number of positive as well as more hydrophobic amino acids, leading to a stronger amphiphilicity necessary to neutralize the amphiphilic lipid A aggregates

Grau A., Ortiz A., de Godos A., and Gomez-Fernandez J. C. (2000) A biophysical study of the interaction of the lipopeptide antibiotic iturin A with aqueous phospholipid bilayers. *Arch Biochem Biophys* **377,** 315-323.
Abstract: Iturin A is a lipopeptide extracted from the culture media of Bacillus subtilis which shows a

strong antifungal action. The interaction of iturin A with multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) induced structures which did not sediment during centrifugation. Electron microscopy after negative staining showed that, at 30 mol%, iturin A/DMPC vesicles were visible but smaller than those formed by pure DMPC. Thermograms of DMPC/iturinA obtained after differential scanning calorimetry, at low concentrations of iturin A, were interpreted as indicating the presence of two laterally separated phases, one formed by pure phospholipid and the other by lipopeptide-phospholipid complexes, these two separated phases being already detected even at low concentrations such as 2 mol%. Fluorescence quenching experiments showed that the D-Tyr residue of the lipopeptide was fully accessible to the aqueous medium, indicating that the polar part of iturin A is located outside of the membrane hydrophobic palisade. It was concluded that the membrane barrier properties are likely to be damaged in the area where the lipid complexes are accumulated, due to structural fluctuations, and this may be one of the bases of its biological activity. Iturin-A was also able to greatly destabilize dielaidoylphosphatidylethanolamine (DEPE) membranes in the fluid form, producing a new structure which had a poor correlation in X-ray diffraction, and in 31P NMR spectroscopy gave rise to a spectrum containing a double isotropic signal. Iturin A was shown to induce DEPE to adopt phases other than H(II) inverted hexagonal, underlining that this lipopeptide is capable of modifying the curvature of the membrane, which may also be important in explaining the tendency of iturin A to create small vesicles and which may be another of the bases of its biological activity.

Hallock K. J., Lee D. K., and Ramamoorthy A. (2003) MSI-78, an analogue of the magainin antimicrobial peptides, disrupts lipid bilayer structure via positive curvature strain. *Biophys J* **84,** 3052-3060.
Abstract: In this work, we present the first characterization of the cell lysing mechanism of MSI-78, an antimicrobial peptide. MSI-78 is an amphipathic alpha-helical peptide designed by Genaera Corporation as a synthetic analog to peptides from the magainin family. (31)P-NMR of mechanically aligned samples and differential scanning calorimetry (DSC) were used to study peptide-containing lipid bilayers. DSC showed that MSI-78 increased the fluid lamellar to inverted hexagonal phase transition temperature of 1,2-dipalmitoleoyl-phosphatidylethanolamine indicating the peptide induces positive curvature strain in lipid bilayers. (31)P-NMR of lipid bilayers composed of MSI-78 and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine demonstrated that the peptide inhibited the fluid lamellar to inverted hexagonal phase transition of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine, supporting the DSC results, and the peptide did not induce the formation of nonlamellar phases, even at very high peptide concentrations (15 mol %). (31)P-NMR of samples containing 1-palmitoyl-2-oleoyl-phosphatidylcholine and MSI-78 revealed that MSI-78 induces significant changes in the bilayer structure, particularly at high peptide concentrations. At lower concentrations (1-5%), the peptide altered the morphology of the bilayer in a way consistent with the formation of a toroidal pore. Higher concentrations of peptide (10-15%) led to the formation of a mixture of normal hexagonal phase and lamellar phase lipids. This work shows that MSI-78 induces significant changes in lipid bilayers via positive curvature strain and presents a model consistent with both the observed spectral changes and previously published work.

Haney E. F., Nazmi K., Lau F., Bolscher J. G. and Vogel H. J. (2008) Novel lactoferrampin antimicrobial peptides derived from human lactoferrin. *Biochimie*. (epublication)
Abstract: Human lactoferrampin is a novel antimicrobial peptide found in the cationic N-terminal lobe of the iron-binding human lactoferrin protein. The amino acid sequence that directly corresponds to the previously characterized bovine lactoferrin-derived lactoferrampin peptide is inactive on its own (WNLLRQAQEKFGKDKSP, residues 269-285). However, by increasing the net positive charge near the C-terminal end of human lactoferrampin, a significant increase in its antibacterial and Candidacidal activity was obtained. Conversely, the addition of an N-terminal helix cap (sequence DAI) did not have any appreciable effect on the antibacterial or antifungal activity of human lactoferrampin peptides, even though it markedly influenced that of bovine lactoferrampin. The solution structure of five human lactoferrampin variants was determined in SDS micelles and all of the structures display a well-defined amphipathic N-terminal helix and a flexible cationic C-terminus. Differential scanning calorimetry studies indicate that this peptide is capable of inserting into the hydrophobic core of a membrane, while fluorescence spectroscopy results suggest that a hydrophobic patch encompassing the single Trp and Phe residues as well as Leu, Ile and Ala side chains mediates the interaction between the peptide and the hydrophobic core of a phospholipid bilayer

Henzler-Wildman K. A., Martinez G. V., Brown M. F., and Ramamoorthy A. (2004) Perturbation of the hydrophobic core of lipid bilayers by the human antimicrobial peptide LL-37. *Biochemistry* **43,** 8459-8469.
Abstract: LL-37 is a cationic, amphipathic alpha-helical antimicrobial peptide found in humans that kills cells by disrupting the cell membrane. To disrupt membranes, antimicrobial peptides such as LL-37 must alter the hydrophobic core of the bilayer. Differential scanning calorimetry and deuterium (^{2}H) NMR experiments on acyl chain perdeuterated lipids demonstrate that LL-37 inserts into the hydrophobic region of the bilayer and alters the chain packing and cooperativity. The results show that hydrophobic interactions between LL-37 and the hydrophobic acyl chains are as important for the ability of this peptide to disrupt lipid bilayers as its electrostatic interactions with the polar headgroups. The ^{2}H NMR data are consistent with the previously determined surface orientation of LL-37 (Henzler Wildman, K. A., et al. (2003) Biochemistry 42, 6545) with an estimated 5-6 A depth of penetration of the hydrophobic face of the amphipathic helix into the hydrophobic interior of the bilayer. LL-37 also alters the material properties of lipid bilayers, including the area per lipid, hydrophobic thickness, and coefficient of thermal expansion in a manner that varies with lipid type and temperature. Comparison of the effect of LL-37 on 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC-d(31)) and 1,2-dimyristoyl-phosphatidylcholine (DMPC-d(54)) at different temperatures demonstrates the importance of bilayer order in determining the type and extent of disordering and disruption of the hydrophobic core by LL-37. One possible explanation, which accounts for both the ^{2}H NMR data presented here and the known surface orientation of LL-37 under identical conditions, is that bilayer order influences the depth of insertion of LL-37 into the hydrophobic/hydrophilic interface of the bilayer, altering the balance of electrostatic and hydrophobic interactions between the peptide and the lipids.

Hickel A., nner-Pongratz S., Amenitsch H., Degovics G., Rappolt M., Lohner K. and Pabst G. (2008) Influence of antimicrobial peptides on the formation of nonlamellar lipid mesophases. *Biochim Biophys Acta* **1778,** 2325-2333.
Abstract: We have studied the influence of four antimicrobial peptides of different secondary and ternary structure--melittin (Mel), protegrin-1 (PG-1), peptidyl-glycylleucine-carboxyamide (PGLa), and gramicidin S (GS)--on the lamellar-to-nonlamellar transition of palmitoyloleoyl phosphatidylethanolamine (POPE) applying differential scanning calorimetry and small-angle X-ray diffraction. None of the peptides studied led to the formation of an inverted hexagonal phase observed for pure POPE at high temperatures. Instead either cubic or lamellar phases were stabilized to different degrees. GS was most effective in inducing a cubic phase, whereas Mel fully stabilized the lamellar phase. The behavior of POPE in the presence of PG-1 and PGLa was intermediate to GS and Mel. In addition to the known role of membrane elasticity we propose two mechanisms, which cause stabilization of the lamellar phase: electrostatic repulsion and lipid/peptide pore formation. Both mechanisms prevent transmembrane contact required to form either an inverted hexagonal phase or fusion pores, as precursors of the cubic phase

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.

Jelokhani-Niaraki M., Hodges R. S., Meissner J. E., Hassenstein U. E. and Wheaton L. (2008) Interaction of gramicidin S and its aromatic amino-acid analog with phospholipid membranes. *Biophys J* **95,** 3306-3321.
Abstract: To investigate the mechanism of interaction of gramicidin S-like antimicrobial peptides with biological membranes, a series of five decameric cyclic cationic beta-sheet-beta-turn peptides with all possible combinations of aromatic D-amino acids, Cyclo(Val-Lys-Leu-D-Ar1-Pro-Val-Lys-Leu-D-Ar2-Pro) (Ar identical with Phe, Tyr, Trp), were synthesized. Conformations of these cyclic peptides were comparable in aqueous solutions and lipid vesicles. Isothermal titration calorimetry measurements revealed entropy-driven binding of cyclic peptides to POPC and POPE/POPG lipid vesicles. Binding of peptides to both vesicle systems was endothermic-exceptions were peptides containing the Trp-Trp and Tyr-Trp pairs with exothermic binding to POPC vesicles. Application of one- and two-site binding (partitioning) models to binding isotherms of exothermic and endothermic binding processes, respectively, resulted in determination of peptide-lipid membrane binding constants (K(b)). The K(b1) and K(b2) values for endothermic two-step binding processes corresponded to high and low binding affinities (K(b1) >or= 100 K(b2)). Conformational change of cyclic peptides in transferring from buffer to lipid bilayer surfaces was estimated using fluorescence resonance energy transfer between the Tyr-Trp pair in one of the peptide constructs. The cyclic peptide conformation expands upon adsorption on lipid bilayer surface and interacts more deeply with the outer monolayer causing bilayer deformation, which may lead to formation of nonspecific transient peptide-lipid porelike zones causing membrane lysis

Jing W., Prenner E. J., Vogel H. J., Waring A. J., Lehrer R. I., and Lohner K. (2005) Headgroup structure and fatty acid chain length of the acidic phospholipids modulate the interaction of membrane mimetic vesicles with the antimicrobial peptide protegrin-1. *J Pept Sci* **11,** 735-743.
Abstract: The interaction of protegrin-1 (PG-1), a small beta-sheet antimicrobial peptide with acidic phospholipid model membranes was investigated by differential scanning calorimetry. We found that PG-1 can distinguish between liposomes of the anionic phospholipids DPPG, DPPS and DPPA, eventhough the headgroups of these phospholipids all have the same net charge and they carry the same hydrocarbon chains. Specifically, PG-1 had only a minor effect on the thermotropic phase behavior of DPPA liposomes, while it interacted preferentially with the fluid phase of DPPS. Furthermore, PG-1 could induce a phase separation in DPPG liposomes resulting in the formation of peptide-rich domains even at low concentrations of the peptide. However, this peptide-rich domain was not evident when the fatty acyl chains were longer or shorter by two carbon atoms. In addition, PG-1 can also form peptide-rich domains in DPPS vesicles but only at high concentrations of the peptide. These results suggest that in addition to an overall negative charge, the structural features of the phospholipid headgroups, lipid packing and thus membrane fluidity will influence the interaction with PG-1, thereby modulating its biological activity.

Jung D., Powers J. P., Straus S. K. and Hancock R. E. (2008) Lipid-specific binding of the calcium-dependent antibiotic daptomycin leads to changes in lipid polymorphism of model membranes. *Chem Phys. Lipids* **154,** 120-128.
Abstract: Daptomycin is a cyclic anionic lipopeptide with an antibiotic activity that is completely dependent on the presence of calcium (as Ca2+). In a previous study [Jung et al., 2004. Chem. Biol. 11, 949-957], it was concluded that daptomycin underwent two Ca2+-dependent structural transitions, whereby the first transition was solely dependent on Ca2+, while the second transition was dependent on both Ca2+ and the presence of negatively charged lipids that allowed daptomycin to insert into and perturb bilayer membranes with acidic character. Differences in the interaction of daptomycin with acidic and neutral membranes were further investigated by spectroscopic means. The lack of quenching of intrinsic fluorescence by the water-soluble quencher, KI, confirmed the insertion of the daptomycin Trp residue into the membrane bilayer, while the kynurenine residue was inaccessible even in an aqueous environment. Differential scanning calorimetry (DSC) indicated that the binding of daptomycin to neutral bilayers occurred through a combination of electrostatic and hydrophobic interactions, while the binding of daptomycin to bilayers containing acidic lipids primarily involved electrostatic interactions. The binding of daptomycin to acidic membranes led to the induction of non-lamellar lipid phases and membrane fusion

Kedracka-Krok S. and Wasylewski Z. (2003) A differential scanning calorimetry study of tetracycline repressor. *Eur J Biochem* **270,** 4564-4573.
Abstract: Tetracycline repressor (TetR), which constitutes the most common mechanism of bacterial

resistance to an antibiotic, is a homodimeric protein composed of two identical subunits, each of which contains a domain possessing a helix-turn-helix motif and a domain responsible for binding tetracycline. Binding of tetracycline in the protein pocket is accompanied by conformational changes in TetR, which abolish the specific interaction between the protein and DNA. Differential scanning calorimetry (DSC) and CD measurements, performed at pH 8.0, were used to observe the thermal denaturation of TetR in the absence and presence of tetracycline. The DSC results show that, in the absence of tetracycline, the thermally induced transitions of TetR can be described as an irreversible process, strongly dependent on scan rate and indicating that the protein denaturation is under kinetic control described by the simple kinetic scheme: N(2)--->D(2), where k is a first-order kinetic constant, N is the native state, and D is the denatured state. On the other hand, analysis of the scan rate effect on the transitions of TetR in the presence of tetracycline shows that thermal unfolding of the protein can be described by the two-state model: N(2)<--->U(2)--->D. In the proposed model, TetR in the presence of tetracycline undergoes co-operative unfolding, characterized by an enthalpy change (ΔH(cal) = 1067 kJ x mol^{-1}) and an entropy change (ΔS = 3.1 kJ x mol(-1)).

Kiricsi M., Prenner E. J., Jelokhani-Niaraki M., Lewis R. N., Hodges R. S., and McElhaney R. N. (2002) The effects of ring-size analogs of the antimicrobial peptide gramicidin S on phospholipid bilayer model membranes and on the growth of Acholeplasma laidlawii B. *Eur J Biochem* **269,** 5911-5920.
Abstract: We have examined the effects of three ring-size analogs of the cyclic beta-sheet antimicrobial peptide gramicidin S (GS) on the thermotropic phase behavior and permeability of phospholipid model membranes and on the growth of the cell wall-less Gram-positive bacteria Acholeplasma laidlawii B. These three analogs have ring sizes of 10 (GS10), 12 (GS12) or 14 (GS14) amino acids, respectively. Our high-sensitivity differential scanning calorimetric studies indicate that all three of these GS analogs perturb the gel/liquid-crystalline phase transition of zwitterionic phosphatidylcholine (PtdCho) vesicles to a greater extent than of zwitterionic phosphatidylethanolamine (PtdEtn) or of anionic phosphatidylglycerol (PtdGro) vesicles, in contrast to GS itself, which interacts more strongly with PtdGro than with PtdCho and PtdEtn bilayers. However, the relative potency of the perturbation of phospholipid phase behavior varies markedly between the three peptides, generally decreasing in the order GS14 > GS10 > GS12. Similarly, these three GS ring-size analogs also differ considerably in their ability to cause fluorescence dye leakage from phospholipid vesicles, with the potency of permeabilization also generally decreasing in the order GS14 > GS10 > GS12. Finally, these GS ring-size analogs also differentially inhibit the growth of A. laidlawii with growth inhibition also decreasing in the order GS14 > GS10 > GS12. These results indicate that the relative potencies of GS and its ring-size analogs in perturbing the organization and increasing the permeability of phospholipid bilayer model membranes, and of inhibiting the growth of A. laidlawii B cells, are at least qualitatively correlated, and provide further support for the hypothesis that the primary target of these antimicrobial peptides is the lipid bilayer of the bacterial membrane. The very high antimicrobial activity of GS14 against the cell wall-less bacteria A. laidlawii as compared to various conventional bacteria confirms our earlier suggestion that the avid binding of this peptide to the bacterial cell wall is primarily responsible for its reduced antimicrobial activity against such organisms. The relative magnitude of the effects of GS itself, and of the three ring-size GS analogs, on phospholipid bilayer organization and cell growth correlate relatively well with the effective hydrophobicities and amphiphilicities of these peptides but less well with their relative charge density, intrinsic hydrophobicities or conformational flexibilities. Nevertheless, all of these parameters, as well as others, may influence the antimicrobial potency and hemolytic activity of GS analogs.

Klajnert B., Janiszewska J., Urbanczyk-Lipkowska Z., Bryszewska M., and Epand R. M. (2006) DSC studies on interactions between low molecular mass peptide dendrimers and model lipid membranes. *Int J Pharm* **327,** 145-152.
Abstract: It has recently been shown that a newly synthesized peptide dendrimers possess antimicrobial activity against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria as well as against fungal pathogens (Candida albicans) [Klajnert, B., Janiszewska, J., Urbanczyk-Lipkowska, Z., Bryszewska, M., Shcharbin, D., Labieniec, M., 2006. Biological properties of low molecular mass peptide dendrimers. Int. J. Pharm. 309, 208-217]. To extend our knowledge about their impact on biological systems, interactions between a group of low molecular mass lysine based dendrimers and model lipid bilayers were examined by differential scanning calorimetry (DSC). Conformational stability of dendrimers in 5-85 degrees C temperature range was confirmed by circular dichroism measurements (CD).

The dendrimer structure has been shown to play an important role in interactions with the membranes. A two-step mechanism of dendrimer-bilayer interactions was proposed. The first step involves electrostatic attractions between dendrimers and polar lipid heads, while the second one is a result of hydrophobic interactions between acyl chains and arms of dendrimers. While one dendrimer did not interact with the membrane, another with long hydrophobic arms significantly perturbed the membrane. Nevertheless, for all tested dendrimers the main transition in DSC scans was retained that indicates that these compounds at the tested concentrations did not cause the loss of membrane integrity.

Lacadena J., Martinez d. P., Gasset M., Patino B., Campos-Olivas R., Vazquez C., Martinez-Ruiz A., Mancheno J. M., Onaderra M., and Gavilanes J. G. (1995) Characterization of the antifungal protein secreted by the mould Aspergillus giganteus. *Arch Biochem Biophys* **324,** 273-281.
Abstract: An antifungal polypeptide (AFP) of 51 amino acid residues, secreted by the mould Aspergillus giganteus, has been purified to homogeneity and characterized. The inhibitory effect of this protein on the growth of different microorganisms has been studied. Whereas the growth of many of the filamentous fungi assayed is inhibited, no effect has been observed against yeasts or bacteria. The minimal concentration for total inhibition of the growth is in the range 6 to 25 μM. The antifungal polypeptide does not produce any effect on the growth of the producing mould. The polypeptide promotes aggregation of acidic phospholipid vesicles. A remarkable resistance to proteolysis and a low hydrogen x deuterium exchange have been observed for this protein. The protein does not show any thermal transition up to 80 degrees C when studied by differential scanning calorimetry and infrared spectroscopy. The uv absorbance, fluorescence emission, and circular dichroism (CD) characteristics of this protein have been studied. The protein exhibits a strong positive band at 230 nm as a prominent feature of the CD spectrum in the far uv region. All the spectroscopical properties of the antifungal protein are highly influenced by the abundance of tyrosine residues. These can be grouped in two different populations, buried and exposed, based on the results of pH-titration experiments. Fourier-transform infrared spectroscopy reveals a high content of beta-structure in AFP. Reduction and carboxy-amidomethylation produces a rather unstructured polypeptide as deduced from its spectroscopical properties.

Lafitte D., Lamour V., Tsvetkov P. O., Makarov A. A., Klich M., Deprez P., Moras D., Briand C., and Gilli R. (2002) DNA gyrase interaction with coumarin-based inhibitors: the role of the hydroxybenzoate isopentenyl moiety and the 5'-methyl group of the noviose. *Biochemistry* **41,** 7217-7223.
Abstract: DNA gyrase is a major bacterial protein that is involved in replication and transcription and catalyzes the negative supercoiling of bacterial circular DNA. DNA gyrase is a known target for antibacterial agents since its blocking induces bacterial death. Quinolones, coumarins, and cyclothialidines have been designed to inhibit gyrase. Significant improvements can still be envisioned for a better coumarin-gyrase interaction. In this work, we obtained the crystal costructures of the natural coumarin clorobiocin and a synthetic analogue with the 24 kDa gyrase fragment. We used isothermal titration microcalorimetry and differential scanning calorimetry to obtain the thermodynamic parameters representative of the molecular interactions occurring during the binding process between coumarins and the 24 kDa gyrase fragment. We provide the first experimental evidence that clorobiocin binds gyrase with a stronger affinity than novobiocin. We also demonstrate the crucial role of both the hydroxybenzoate isopentenyl moiety and the 5'-alkyl group on the noviose of the coumarins in the binding affinity for gyrase.

Langdon G. M., Bruix M., Galvez A., Valdivia E., Maqueda M., and Rico M. (1998) Sequence-specific 1H assignment and secondary structure of the bacteriocin AS-48 cyclic peptide. *J Biomol NMR* **12,** 173-175.
Abstract: The bacteriocin AS-48 is a cationic peptide (7149 Da) having a broad antimicrobial spectrum, encoded by the 68 kb conjugative plasmid pMB2 from Enterococcus faecalis S-48. It is a unique peptide since it has a cyclic structure, which is achieved by the formation of a tail-head peptide bond after ribosomal synthesis (Galvez et al., 1989; Martinez-Bueno et al., 1994; Samyn et al., 1994). Preliminary CD and calorimetric studies (data not shown) pointed towards a highly helical and very stable three dimensional structure. All the information gathered until now indicates that the target of AS-48 is the cytoplasmic membrane in which it opens channels or pores, leading to dissipation of the proton motive force and cell death, which in some cases is also followed by bacterial lysis (Galvez et al., 1991). This peptide is a suitable tool for studying protein-membrane interactions, and it also offers promising perspectives for biotechnological applications. Knowledge of the 3D structure of AS-48 is a first step in the

conduct of further structure-function studies. Here we report the complete 1H NMR assignment of its proton resonances together with the resulting secondary structure pattern as prerequisites for the determination of a high-resolution 3D solution structure.

Laporta O., Funes L., Garzon M. T., Villalain J. and Micol V. (2007) Role of membranes on the antibacterial and anti-inflammatory activities of the bioactive compounds from Hypoxis rooperi corm extract. *Arch Biochem Biophys* **467,** 119-131.
Abstract: Hypoxis rooperi corm extract ('African potato') is known for its traditional and ethnomedical uses in the treatment of a large variety of diseases. Its main bioactive compound hypoxoside (HYP) and its aglycone derivative rooperol (RO) were isolated and the interaction of these compounds with several types of model membranes was studied in order to contribute to the understanding of their molecular mechanism. The results show that RO abolishes the main transition phase and perturb the van der Waals interactions between phospholipid acyl chains in a stronger way than HYP in dimiristoylphosphatidylcholine (DMPC), dielaidoylphosphatidylethanolamine (DEPE) and dimiristoylphosphatidylglycerol membranes (DMPG), probably indicating that this molecule inserts into the bilayer. This effect decreases as the acyl chain length of the phospholipid increases. RO also promoted the formation of hexagonal H(II) phases at lower temperatures compared to pure DEPE. On the contrary, HYP showed a shallow interaction with phospholipids. This compound promoted the formation of gel-fluid like intermediate structures with isotropic motion in phosphatidylglycerol membranes at physiological pH, and affected the phospholipid/water interface probably through the variation of the surface charge of the phospholipid phosphate groups. Moreover, RO inhibited Staphylococcus aureus in a stronger manner than Escherichia coli and promoted a higher leakage level in E. coli, PG and PE-containing synthetic membranes. Furthermore, RO showed a significant degree of inhibition of cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1) evidencing an approximate COX-2/COX-1 IC50 ratio of 1.9, therefore this compound may be responsible for the anti-inflammatory activity of H. rooperi corm extract. These results may contribute to understand the molecular mechanism of the antibacterial and/or anti-inflammatory properties of the bioactive compounds deriving from the African potato corm extract.

Latal A., Degovics G., Epand R. F., Epand R. M., and Lohner K. (1997) Structural aspects of the interaction of peptidyl-glycylleucine-carboxyamide, a highly potent antimicrobial peptide from frog skin, with lipids. *Eur J Biochem* **248,** 938-946.
Abstract: The interaction of PGLa (peptidyl-glycylleucine-carboxyamide), a 21-amino-acid residue cationic peptide, isolated from the skin of the South African clawed frog, Xenopus laevis, with model membrane systems was investigated. Our studies focussed on the importance of the difference in the phospholipid composition of bacterial and erythrocyte membranes. This is of particular interest to gain information on the specificity of membranolysis exhibited by this peptide against bacteria but not against erythrocytes. In phosphate buffer at physiological pH, as well as in the presence of the zwitterionic phosphatidylcholine and sphingomyelin. the peptide had a random structure but it adopted an alpha-helical conformation in the presence of negatively charged lipids. Furthermore, calorimetric experiments showed that PGLa had no effects on the thermotropic phase behavior of liposomes composed of the choline phosphatides, while separation of a distinct peptide-rich domain was observed for phosphatidylglycerol liposomes. In addition to the main transition of pure 1,2-dipalmitoylglycerophosphoglycerol at 40 degrees C a second transition owing to the peptide-perturbed lipid domains was found at 41 degrees C. This conclusion is supported by X-ray diffraction experiments which indicated that PGLa penetrates into the hydrophobic core of the bilayer inducing an untilting of the hydrocarbon chains as observed in the gel phase of the pure lipid. These results demonstrate that this antibacterial peptide specifically interacts with negatively charged lipid membranes, which are characteristic of bacterial membranes. This can be explained based on the structural features of PGLa.

Leng F., Priebe W., and Chaires J. B. (1998) Ultratight DNA binding of a new bisintercalating anthracycline antibiotic. *Biochemistry* **37,** 1743-1753.
Abstract: Differential scanning calorimetry and absorption spectroscopy were used to characterize the interaction of the new bisintercalating anthracycline antibiotic, WP631, with DNA. The method of continuous variations revealed five distinct binding modes for WP631, corresponding to 6, 3, 1.3, 0.5, and 0.25 mol of base pairs (bp) per mole of ligand. The binding of one drug to 6 bp corresponds to the bisintercalative binding mode determined previously, and was the mode studied in detail. UV melting

experiments and differential scanning calorimetry were used to measure the ultratight binding of WP631 to DNA. The binding constant for the interaction of WP631 with herring sperm DNA was determined to be 3.1 (+/- 0.2) x 10^{11} M^{-1} at 20 degrees C. The large, favorable binding free energy of -15.3 kcal mol^{-1} was found to result from a large, negative enthalpic contribution of -30.2 kcal mol^{-1}. DNA melting curves at different concentrations of WP631 were fitted to McGhee's model of DNA melting in the presence of ligands, yielding an independent estimate of DNA binding parameters. The salt dependence of the WP631 binding constant was examined, yielding a slope SK = Δ (log K)/Δ (log[Na+]) = 1.63. The observed salt dependence of the equilibrium constant, interpreted according to polyelectrolyte theory, indicates that there is a significant nonpolyelectrolyte contribution to the binding free energy. DNA melting studies using a homogeneous 214 bp DNA fragment showed that WP631 binds preferentially to the GC-rich region of the DNA.

Leng F., Chaires J. B., and Waring M. J. (2003) Energetics of echinomycin binding to DNA. *Nucleic Acids Res* **31,** 6191-6197.
Abstract: Differential scanning calorimetry and UV thermal denaturation have been used to determine a complete thermodynamic profile for the bis-intercalative interaction of the peptide antibiotic echinomycin with DNA. The new calorimetric data are consistent with all previously published binding data, and afford the most rigorous and direct determination of the binding enthalpy possible. For the association of echinomycin with DNA, we found ΔG degrees = -7.6 kcal mol^{-1}, ΔH = +3.8 kcal mol^{-1} and ΔS = +38.9 cal mol^{-1} K^{-1} at 20 degrees C. The binding reaction is clearly entropically driven, a hallmark of a process that is predominantly stabilized by hydrophobic interactions, though a deeper analysis of the free energy contributions suggests that direct molecular recognition between echinomycin and DNA, mediated by hydrogen bonding and van der Waals contacts, also plays an important role in stabilizing the complex.

Lisetski L. N., Vashchenko O. V., Tolmachev A. V., and Vodolazhskiy K. B. (2002) Effects of membranotropic agents on mono- and multilayer structures of dipalmitoylphosphatidylcholine. *Eur Biophys J* **31,** 554-558.
Abstract: We have studied the action of some membranotropic agents (MTAs) on the parameters of mono- and multilayers of dipalmitoylphosphatidylcholine (DPPC). The MTAs used included an antimicrobial drug, decamethoxinum, the model amphiphilic agent stearoyl-L-alpha-alanine, and cholesterol as a reference substance. Using differential scanning calorimetry and the Langmuir monolayer technique, we measured the temperature and enthalpy of the main phase transition of DPPC, the mean molecular area, the collapse pressure and the free energy of the mixed monolayers of DPPC and MTA. A good correlation has been obtained between the structure of the MTA used and changes in the parameters of both mono- and multilayers. Thus, for cholesterol, its well-known condensing effect in the L alpha phase correlates with its behavior in the mixed monolayers. The disturbing action of decamethoxinum (depression of the phase transition in DPPC multilayers and relatively high free energy of mixing in monolayers) is presumably connected with interaction of its charged ammonium moieties with polar phospholipid heads. At the same time, stearoyl-L-alpha- alpha-alanine condensed the lipid layers and increased the melting point of DPPC, owing to its interaction with both polar and non-polar lipid moieties. One can conclude that the three MTAs used can really be considered as representative examples of three different types of behavior in mono- and multilayers.

Liu J., Rosenberg E. Y., and Nikaido H. (1995) Fluidity of the lipid domain of cell wall from Mycobacterium chelonae. *Proc Natl Acad Sci U S A* **92,** 11254-11258.
Abstract: The mycobacterial cell wall contains large amounts of unusual lipids, including mycolic acids that are covalently linked to the underlying arabinogalactan-peptidoglycan complex. Hydrocarbon chains of much of these lipids have been shown to be packed in a direction perpendicular to the plane of the cell surface. In this study, we examined the dynamic properties of the organized lipid domains in the cell wall isolated from Mycobacterium chelonae grown at 30 degrees C. Differential scanning calorimetry showed that much of the lipids underwent major thermal transitions between 30 degree C and 65 degrees C, that is at temperatures above the growth temperature, a result suggesting that a significant portion of the lipids existed in a structure of extremely low fluidity in the growing cells. Spin-labeled fatty acid probes were successfully inserted into the more fluid part of the cell wall. Our model of the cell wall suggests that this domain corresponds to the outermost leaflet, a conclusion reinforced by the observation that labeling of intact cells produced electron spin resonance spectra similar to those of the isolated cell wall. Use of

stearate labeled at different positions showed that the fluidity within the outer leaflet increased only slightly as the nitroxide group was placed farther away from the surface. These results are consistent with the model of mycobacterial cell wall containing an asymmetric lipid bilayer, with an internal, less fluid mycolic acid leaflet and an external, more fluid leaflet composed of lipids containing shorter chain fatty acids. The presence of the low-fluidity layer will lower the permeability of the cell wall to lipophilic antibiotics and chemotherapeutic agents and may contribute to the well-known intrinsic resistance of mycobacteria to such compounds.

Liu J., Barry C. E., III, Besra G. S., and Nikaido H. (1996) Mycolic acid structure determines the fluidity of the mycobacterial cell wall. *J Biol Chem* **271,** 29545-29551.
Abstract: The low permeability of the mycobacterial cell wall is thought to contribute to the well known resistance of mycobacteria to antibiotics and chemotherapeutic agents. We have used differential scanning calorimetry to demonstrate that the high temperature phase transition observed in purified cell walls, usually in the 60-70 degrees C range, suggestive of a lipid environment of extremely low fluidity, can also be observed in whole organisms and in cell walls from which much of the free lipids was removed by extraction with Triton X-114. A survey of seven mycobacterial species demonstrated that this high temperature transition was a general property of these organisms. Cell walls isolated from two Corynebacterium species, which contain much shorter corynemycolic acids, displayed a much lower temperature transition, suggesting that the transition temperature was directly correlated to the length of mycolic acid. Methyl esters of mycolic acids were found to have a phase transition temperature that was linearly related to the amount of trans-mycolate. Both Mycobacterium avium and M. smegmatis responded to increasing growth temperature by increasing the proportion of trans-mycolate and displaying a correspondingly higher melting temperature. Whole cells of M. smegmatis grown at higher temperature allowed a less rapid influx of two lipophilic agents, norfloxacin and chenodeoxycholate. These results provide strong evidence that the nature of mycolic acid plays a crucial role in determining the fluidity and permeability of mycobacterial cell wall.

Lohner K., Latal A., Lehrer R. I., and Ganz T. (1997) Differential scanning microcalorimetry indicates that human defensin, HNP-2, interacts specifically with biomembrane mimetic systems. *Biochemistry* **36,** 1525-1531.
Abstract: alpha-Defensins are antimicrobial peptides with 29-35 amino acid residues and cysteine-stabilized amphiphilic, triple-stranded beta-sheet structures. We used high-precision differential scanning microcalorimetry to investigate the effects of a human neutrophil alpha-defensin, HNP-2, on the phase behavior of model membranes mimicking bacterial and erythrocyte cell membranes. In the presence of this positively charged peptide, the phase behavior of liposomes containing negatively charged phosphatidylglycerol was markedly altered even at a high lipid-to-peptide molar ratio of 500:1. Addition of HNP-2 to liposomes mimicking bacterial membranes (mixtures of dipalmitoylphosphatidylglycerol and -ethanolamine) resulted in phase separation owing to some domains being peptide-poor and others peptide-rich. The latter are characterized by an increase of the main transition temperature, most likely arising from electric shielding of the phospholipid headgroups by the peptide. On the other hand, HNP-2 did not affect the phase behavior of membranes mimicking erythrocyte membranes (equimolar mixtures of dipalmitoylphosphatidylcholine and sphingomyelin) as well as the pure single components. This is in contrast to melittin, which significantly affected the phase behavior of choline phospholipids in accordance with its unspecific lytic activity. These results support the hypothesis of preferential interaction of defensins with negatively charged membrane cell surfaces, a common feature of bacterial cell membranes, and demonstrate that HNP-2 discriminates between model membrane systems mimicking prokaryotic and eukaryotic cell membranes.

Lohner K. and Prenner E. J. (1999) Differential scanning calorimetry and X-ray diffraction studies of the specificity of the interaction of antimicrobial peptides with membrane-mimetic systems. *Biochim Biophys Acta* **1462,** 141-156.
Abstract: Interest in biophysical studies on the interaction of antimicrobial peptides and lipids has strongly increased because of the rapid emergence of antibiotic-resistant bacterial strains. An understanding of the molecular mechanism(s) of membrane perturbation by these peptides will allow a design of novel peptide antibiotics as an alternative to conventional antibiotics. Differential scanning calorimetry and X-ray diffraction studies have yielded a wealth of quantitative information on the effects of antimicrobial peptides

on membrane structure as well as on peptide location. These studies clearly demonstrated that antimicrobial peptides show preferential interaction with specific phospholipid classes. Furthermore, they revealed that in addition to charge-charge interactions, membrane curvature strain and hydrophobic mismatch between peptides and lipids are important parameters in determining the mechanism of membrane perturbation. Hence, depending on the molecular properties of both lipid and peptide, creation of bilayer defects such as phase separation or membrane thinning, pore formation, promotion of nonlamellar lipid structures or bilayer disruption by the carpet model or detergent-like action, may occur. Moreover, these studies suggest that these different processes may represent gradual steps of membrane perturbation. A better understanding of the mutual dependence of these parameters will help to elucidate the molecular mechanism of membrane damage by antimicrobial peptides and their target membrane specificity, keys for the rationale design of novel types of peptide antibiotics.

Ludtke S. J., He K., Heller W. T., Harroun T. A., Yang L., and Huang H. W. (1996) Membrane pores induced by magainin. *Biochemistry* **35,** 13723-13728.
Abstract: Magainin, found in the skin of Xenopus laevis, belongs to a broad class of antimicrobial peptides which kill bacteria by permeabilizing the cytoplasmic membrane but do not lyse eukaryotic cells. The 23-residue peptide has been shown to form an amphiphilic helix when associated with membranes. However, its molecular mechanism of action has been controversial. Oriented circular dichroism has detected helical magainin oriented perpendicular to the plane of the membrane at high peptide concentrations, but Raman, fluorescence, differential scanning calorimetry, and NMR all indicate that the peptide is associated with the head groups of the lipid bilayer. Here we show that neutron in-plane scattering detects pores formed by magainin 2 in membranes only when a substantial fraction of the peptide is oriented perpendicular to the membrane. The pores are almost twice as large as the alamethicin pores. On the basis of the in-plane scattering data, we propose a toroidal (or wormhole) model, which differs from the barrel-stave model of alamethicin in that the lipid bends back on itself like the inside of a torus. The bending requires a lateral expansion in the head group region of the bilayer. Magainin monomers play the role of fillers in the expansion region thereby stabilizing the pore. This molecular configuration is consistent with all published magainin data.

Maeda Y. and Ohtsubo E. (1988) Differential scanning calorimetry of antitumor antibiotics-plasmid DNA interaction. *Nucleic Acids Symp Ser* 101-104.
Abstract: Differential scanning calorimetry (DSC) can detect stepwise melting of plasmid DNA along the molecular chain with high resolution. This method was applied to study interaction of some antitumor antibiotics with the plasmid pJL3-TB5 DNA (5277 base-pairs in length). Analysis of DSC curves of the plasmid DNA in the presence of, for example, adriamycin, an antitumor antibiotics of anthracycline group, together with theoretical analysis of the DNA melting curves obtained by calculation from the entire base sequence, led to the conclusion that adriamycin bound preferentially to the four particular regions with high G + C content. The DSC method would thus be useful for the study of properties of drugs which bind to DNA.

Maeda Y., Nunomura K., and Ohtsubo E. (1990) Differential scanning calorimetric study of the effect of intercalators and other kinds of DNA-binding drugs on the stepwise melting of plasmid DNA. *J Mol Biol* **215,** 321-329.
Abstract: The effect of intercalating drugs (the anthracycline group of antibiotics, ethidium bromide, actinomycin D) on stepwise melting of DNA was studied by differential scanning calorimetry (DSC). The DSC DNA melting profile of plasmid pJL3-TB5 DNA (5277 base-pairs in length) consists of seven peaks, and all the intercalators caused shifting of these peaks, particularly those formed at the high temperature ranges, to the higher temperature ranges in a characteristic manner depending upon the binding strength of the drug. The analysis of the anthracycline group of antibiotics, such as aclacinomycin A, daunomycin, adriamycin and pyrarubicin, indicates that the difference in binding is due to the sugar moiety at position O-7 of the chromophore in these antibiotics. Analysis on the basis of the helix-coil transition theory suggests that the anthracycline group of antibiotics interact preferentially with the 5'-CG-3' sequences. The effect of various DNA-binding drugs other than intercalators on stepwise melting of DNA was then studied by DSC. The representative drugs examined were distamycin A, peplomycin, cis-dichlorodiamine-platinum(II) (cis-DDP or cis-Platin) and mitomycin C, which differ in their mode of interaction with DNA; namely, minor groove binding, strand cleavage and intrastrand or interstrand cross-linking. Distamycin A

caused shifting of the DSC peaks at the low temperature ranges to a higher temperature range, whereas peplomycin and cis-DDP caused shifting of all the DSC peaks to form a broad peak at a lower temperature range, suggesting that the DSC DNA melting profiles are affected in a characteristic manner depending upon the interaction mode of the drug.

Mavromoustakos T., Papadopoulos A., Theodoropoulou E., Dimitriou C., and Antoniadou-Vyza E. (1998) Thermal properties of adamantanol derivatives and their beta-cyclodextrin complexes in phosphatidylcholine bilayers. *Life Sci* **62,** 1901-1910.
Abstract: Differential Scanning Calorimetry (DSC) has been applied to study the thermal properties of the membrane perturbing antibacterial octyl- and dodecyl-bromide salts of quaternary dimethylamino adamantanol (ADM-8 and ADM-12 correspondingly) incorporated in free or complexed form with beta-cyclodextrin (beta-CD) into dipalmitoylphosphatidylcholine (DPPC) containing bilayers. The DSC results showed that the studied compounds exert pronounced thermotropic changes in DPPC bilayers when inserted as free molecules. These effects are reduced when are present in a complex form with beta-CD. Since the studied compounds exert destructive effects in membrane bilayers their insertion in membrane bilayers as complexes with cyclodextrin may result in differentiation of their activity. The obtained results suggest that their complexation with beta-CD may improve their biological profile. It also increases their aqueous solubility, a limited factor for their use as drugs.

Micol V., Mateo C. R., Shapiro S., Aranda F. J., and Villalain J. (2001) Effects of (+)-totarol, a diterpenoid antibacterial agent, on phospholipid model membranes. *Biochim Biophys Acta* **1511,** 281-290.
Abstract: (+)-Totarol, a highly hydrophobic diterpenoid isolated from Podocarpus spp., is inhibitory towards the growth of diverse bacterial species. (+)-Totarol decreased the onset temperature of the gel to liquid-crystalline phase transition of DMPC and DMPG membranes and was immiscible with these lipids in the fluid phase at concentrations greater than 5 mol%. Different (+)-totarol/phospholipid mixtures having different stoichiometries appear to coexist with the pure phospholipid in the fluid phase. At concentrations greater than 15 mol% (+)-totarol completely suppressed the gel to liquid-crystalline phase transition in both DMPC and DMPG vesicles. Incorporation of increasing amounts of (+)-totarol into DEPE vesicles induced the appearance of the H(II) hexagonal phase at low temperatures in accordance with NMR data. At (+)-totarol concentrations between 5 and 35 mol% complex thermograms were observed, with new immiscible phases appearing at temperatures below the main transition of DEPE. Steady-state fluorescence anisotropy measurements showed that (+)-totarol decreased and increased the structural order of the phospholipid bilayer below and above the main gel to liquid-crystalline phase transition of DMPC respectively. The changes that (+)-totarol promotes in the physical properties of model membranes, compromising the functional integrity of the cell membrane, could explain its antibacterial effects.

Milhaud J., Lancelin J. M., Michels B., and Blume A. (1996) Association of polyene antibiotics with sterol-free lipid membranes: I. Hydrophobic binding of filipin to dimyristoylphosphatidylcholine bilayers. *Biochim Biophys Acta* **1278,** 223-232.
Abstract: The interaction of filipin III with multilamellar vesicles (MLV) of dimyristoylphosphatidylcholine (DMPC) was studied by four complementary methods leading to the following results: (1) The modifications of the filipin dichroic spectrum, by adding preformed fluid DMPC MLV, provide evidence of a saturable association with the stoichiometry DMPC/filipin = 4.2 +/- 0.5, constant between 24 and 35 degrees Celsius. (2) Thermograms obtained by differential scanning calorimetry (DSC) on mixtures where filipin is incorporated during the formation of MLV exhibit a high-temperature tail the more marked the higher the filipin content and some structures at temperatures which depend on this content. The corresponding evolution with the temperature of the CD spectra reveals that the characteristic bound filipin spectrum appears at the temperature at which a structure emerges. (3) Titration calorimetry measurements reveal that the association process is exothermic in the temperature range of the DSC endotherms in agreement with the filipin-induced ordering of the lipid chains, previously established by 2H-NMR in the same temperature range (Milhaud et al.(1989) Eur. Biophys. J. 17, 151-158). A discussion of the relevancy of this exothermicity to the hydrophobic effect is developed by referring to the paper by Wimley and White ((1993) Biochemistry 32, 6307-6312).

Milhaud J., Berrehar J., Lancelin J. M., Michels B., Raffard G., and Dufourc E. J. (1997) Association of polyene antibiotics with sterol-free lipid membranes. II. Hydrophobic binding of nystatin to

dilauroylphosphatidylcholine bilayers. *Biochim Biophys Acta* **1326,** 54-66.
Abstract: Interaction of nystatin A1 with multilamellar vesicles (MLV) of dilauroylphosphatidylcholine (DLPC), observed either by adding nystatin to preformed MLV (mixtures I) or by incorporating it during the formation of vesicles (mixtures II, inner lamellas of MLV in contact with nystatin) was investigated for 0.002 < or = nystatin/DLPC = R(A) < or = 0.20, by four complementary methods. The main results were: (i) Ultraviolet absorption and circular dichroism (CD) spectra of mixtures I revealed the occurrence of a saturable association with a stoichiometry (R(A) = 0.007 +/- 0.002) constant between 3 and 33 degrees C. (ii) By differential scanning calorimetry, thermograms of the two types of mixtures were similar only when water was in great excess. In the opposite (e.g., (H2O)/(DLPC) = R(W) < or = 300), mixture II thermograms displayed two features, upshifted by about 6.5 degrees C with respect to the sharp peak observed with mixture I, resembling those obtained for pure DLPC when the low-temperature phase was the subgel phase. For this R(W), the nystatin absolute concentrations were those for which nystatin form superaggregates as revealed by the nystatin CD spectra. It is proposed that these superaggregates are excluded from the interlamellar spacings of MLV and exert a pumping action on the interlamellar water. The subsequent dehydration of the inner lamellas is thought to convert them into the subgel state. (iii) 2H-NMR spectra of sn-2-perdeuterated DLPC MLV + nystatin mixtures II, confirmed such a temperature shift of the main transition. They showed, in addition, an ordering of the aliphatic chains immediately above the transition temperature, equivalent to a bilayer thickening of 2 A.

Milhaud J., Ponsinet V., Takashi M., and Michels B. (2002) Interactions of the drug amphotericin B with phospholipid membranes containing or not ergosterol: new insight into the role of ergosterol. *Biochim Biophys Acta* **1558,** 95-108.
Abstract: Amphotericin B (AmB) is an amphipathic polyene antibiotic which permeabilizes ergosterol-containing membranes, supposedly by formation of pores. In water, AmB forms chiral aggregates, modelled as stacks of planar dimers in which the joined polyene chains in each dimer turn round, from one dimer to the following in these stacks, by forming a helical array. Studies of the binding of AmB with L-dipalmitoylphosphatidylcholine (L-DPPC) and L-dilauroylphosphatidylcholine (L-DLPC) bilayers disclose the main following results. (1) An inversion of the helicity of the L-DPPC-bound AmB aggregates, when the L-DPPC bilayers are in the gel phase, is inferred from the evolution of the circular dichroism spectra of AmB+L-DPPC mixtures. (2) An AmB-induced gel-to-subgel transformation of L-DPPC bilayers, in the previous mixtures, is revealed by a differential scanning calorimetry study. (3) The role played by ergosterol in the location of phospholipid-bound AmB aggregates with respect to a phospholipid bilayer is directly demonstrated from atomic force microscopy observations of mica-supported AmB+L-DLPC mixtures, in the presence or absence of ergosterol. While in the absence of ergosterol AmB aggregates remained at the surface of the bilayer, in the presence of ergosterol they appeared embedded within this bilayer and became hollow-centered. As such an embedding in the hydrophobic core of a bilayer requires a rearrangement of the aggregates with respect to their architecture in water, this rearrangement is held responsible for the hollowing of aggregates. The hollow-centered sublayer-embedded AmB aggregates are thought to be the precursors of the formation of AmB pores.

Motohashi N., Kawase M., Molnar J., Ferenczy L., Wesolowska O., Hendrich A. B., Bobrowska-Hagerstrand M., Hagerstrand H., and Michalak K. (2003) Antimicrobial activity of N-acylphenothiazines and their influence on lipid model membranes and erythrocyte membranes. *Arzneimittelforschung* **53,** 590-599.
Abstract: The antibacterial activity and influence on lipid model membranes and erythrocyte membranes of 24 N-acylphenothiazines and trifluoperazine were studied. (1) Among 24 phenothiazines, the antimicrobial activity of amino maleates was the highest. (2) The influence of phenothiazines on model liposome and erythrocyte membranes was studied using N-phenyl-1-naphthylamine (NPN) as fluorescence probe. From the three types of phenothiazine substitution (H, Cl, CF3) at position 2, CF3-phenothiazines were the most effective in the interaction with liposomal membranes. (3) As measured by the polarization degree of 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence, the alteration of membrane fluidity induced by CF3-phenothiazines was the biggest. Surprisingly, phenothiazines induced stomatocytic shape alterations (invaginations) in erythrocytes and at higher concentrations, also hemolysis of erythrocytes was observed. (4) The microcalorimetic measurements of influence of phenothiazines on thermal behaviour of synthetic lipid systems confirmed the previously obtained results. The main transition temperature and enthalpy of transition of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) were significantly

modified by CF3-phenothiazines, suggesting their penetration of the lipid bilayer. Above results show that phenothiazine maleates were generally more effective than other phenothiazines used in this study.

Nomura K., Corzo G., Nakajima T., and Iwashita T. (2004) Orientation and pore-forming mechanism of a scorpion pore-forming peptide bound to magnetically oriented lipid bilayers. *Biophys J* **87,** 2497-2507.
Abstract: The orientation and pore-forming mechanisms of pandinin 2 (pin2), an antimicrobial peptide isolated from venom of the African scorpion Pandinus imperator, bound to magnetically oriented lipid bilayers were examined by 31P and 13C solid-state, and 15N liquid-state NMR spectroscopy. 31P NMR measurements at various temperatures, under neutral and acidic conditions, showed that membrane lysis occurred only under acidic conditions, and at temperatures below the liquid crystal-gel phase transition of the lipid bilayers, after incubation for two days in the magnet. Differential scanning calorimetry measurements showed that pin2 induced negative curvature strain in lipid bilayers. The 13C chemical shift values of synthetic pin2 labeled at Gly3, Gly8, Leu12, Phe17, or Ser18 under static or slow magic-angle spinning conditions, indicate that pin2 penetrates the membrane with its average helical axis perpendicular to the membrane surface. Furthermore, amide H-D exchange experiments of 15N-Ala4, Gly8, and Ala9 triply-labeled pin2 suggest that this peptide forms oligomers and confirms that the N-terminal region creates membrane pores.

Ogata N. (2007) Denaturation of protein by chlorine dioxide: oxidative modification of tryptophan and tyrosine residues. *Biochemistry* **46,** 4898-4911.
Abstract: Oxychlorine compounds, such as hypochlorous acid (HOCl) and chlorine dioxide (ClO2), have potent antimicrobial activity. Although the biochemical mechanism of the antimicrobial activity of HOCl has been extensively investigated, little is known about that of ClO2. Using bovine serum albumin and glucose-6-phosphate dehydrogenase of Saccharomyces cerevisiae as model proteins, here I demonstrate that the antimicrobial activity of ClO2 is attributable primarily to its protein-denaturing activity. By solubility analysis, circular dichroism spectroscopy, differential scanning calorimetry, and measurement of enzymatic activity, I demonstrate that protein is rapidly denatured by ClO2 with a concomitant decrease in the concentration of ClO2 in the reaction mixture. Circular dichroism spectra of the ClO2-treated proteins show a change in ellipticity at 220 nm, indicating a decrease in alpha-helical content. Differential scanning calorimetry shows that transition temperature and endothermic transition enthalpy of heat-induced unfolding decrease in the ClO2-treated protein. The enzymatic activity of glucose-6-phosphate dehydrogenase decreases to 10% within 15 s of treatment with 10 microM ClO2. Elemental analyses show that oxygen, but not chlorine, atoms are incorporated in the ClO2-treated protein, providing direct evidence that protein is oxidized by ClO2. Furthermore, mass spectrometry and nuclear magnetic resonance spectroscopy show that tryptophan residues become N-formylkynurenine and tyrosine residues become 3,4-dihydroxyphenylalanine (DOPA) or 2,4,5-trihydroxyphenylalanine (TOPA) in the ClO2-treated proteins. Taking these results together, I conclude that microbes are inactivated by ClO2 owing to denaturation of constituent proteins critical to their integrity and/or function, and that this denaturation is caused primarily by covalent oxidative modification of their tryptophan and tyrosine residues.

Oszlanczi A., Bota A. and Klumpp E. (2007) Layer formations in the bacteria membrane mimetic DPPE-DPPG/water system induced by sulfadiazine. *Biophys Chem* **125,** 334-340.
Abstract: The effect of the frequently used antibiotic sulfadiazine (SD) was studied on a bacteria membrane mimetic model system by using differential scanning calorimetric (DSC), small- and wide-angle X-ray scattering (SWAXS) and freeze-fracture methods. The membrane model system consisted of dipalmitoylphosphatidylethanolamine (DPPE, 0.8 molar ratio) and dipalmitoylphosphatidylglycerol (DPPG, 0.2 molar ratio). The SD molar ratio (relative to the lipids) was varied between 10(-3) and 1. In the presence of SD, two transitions between the gel and liquid crystalline phases appear at 60.5 degrees C and about at 65 degrees C. In the temperature domain of the gel phase, the subcell of the chain packing is strongly temperature dependent indicating the increased dominance of the hydration forces during the first transition and the location of SD molecules in the neighbourhood of the polar lipid head groups. The second transition is accompanied by the changes in the nanometer-scale layer arrangements observed by SAXS and in the mum-scale morphology observed by freeze-fracture. Above the temperature of the second transition, the SD-induced metastable structures undergo further formations to produce a more homogeneous state favoured by the geometrical packing of the cylindrical-shaped lipid molecules.

Pabst G., Grage S., nner-Pongratz S., Jing W., Ulrich A. S., Watts A., Lohner K. and Hickel A. (2008) Membrane Thickening by the Antimicrobial Peptide PGLa. *Biophys J*. (epublication)
Abstract: We have observed a hydrocarbon chain length dependent perturbation of saturated acyl chain phosphatidylglycerol bilayers by the antimicrobial peptide peptidyl-glycylleucine-carboxyamide (PGLa) using X-ray diffraction, solid-state (2)H-NMR, differential scanning calorimetry and dilatometry. In the gel phase, PGLa induces a quasi-interdigitated phase, previously reported also for other peptides, which is most pronounced for C18 phosphatidylglycerol. In the fluid phase we found an increase of the membrane thickness and NMR order parameter for C14 and C16 phosphatidylglycerol bilayers, though not for C18. The data is best understood in terms of a close hydrophobic match between the C18 bilayer core and the peptide length when PGLa is inserted with its helical axis normal to the bilayer surface. The C16 acyl chains appear to stretch in order to accommodate PGLa, whereas tilting within the bilayer seems to be energetically favorable for the peptide when inserted into bilayers of C14 phosphatidylglycerol. In contrast to the commonly accepted membrane thinning effect of antimicrobial peptides, the data demonstrate that pore formation does not necessarily relate to changes in the overall bilayer structure

Pashynskaya V. A., Kosevich M. V., Gomory A., Vashchenko O. V., and Lisetski L. N. (2002) Mechanistic investigation of the interaction between bisquaternary antimicrobial agents and phospholipids by liquid secondary ion mass spectrometry and differential scanning calorimetry. *Rapid Commun Mass Spectrom* **16,** 1706-1713.
Abstract: Mechanisms of interaction between the antimicrobial drugs decamethoxinum and aethonium, which are based on bisquaternary ammonium compounds, and a phospholipid component of biological membranes, dipalmitoylphosphatidylcholine, were studied by means of liquid secondary ion mass spectrometry (LSIMS) and differential scanning calorimetry (DSC). Supramolecular complexes of the drugs with this phospholipid were recorded under secondary ion mass spectrometric conditions. The dependence of the structures of these complexes on structural parameters of the dications of the bisquaternary ammonium compounds was demonstrated. Tandem mass spectrometric investigations of the metastable decay of doubly charged ions of decamethoxinum and aethonium complexes with dipalmitoylphosphatidylcholine allowed estimation of structural parameters of these complexes in the gas phase. Interactions of decamethoxinum and aethonium with model membrane assemblies built from hydrated dipalmitoylphosphatidylcholine were studied using DSC. It was shown that while both drugs can interact with model membranes, the mechanisms of such interactions for decamethoxinum and aethonium differ. The correlation between the nature of these interactions and structural and electronic parameters of the dications of the two bisquaternary agents is discussed. Interpretation of combined mass spectrometric and calorimetric experimental data led to proposals that the molecular mechanisms of antimicrobial action of bisquaternary ammonium compounds are related to their effect on the membrane phospholipid components of microbial cells.

Percec V., Dulcey A. E., Balagurusamy V. S., Miura Y., Smidrkal J., Peterca M., Nummelin S., Edlund U., Hudson S. D., Heiney P. A., Duan H., Magonov S. N., and Vinogradov S. A. (2004) Self-assembly of amphiphilic dendritic dipeptides into helical pores. *Nature* **430,** 764-768.
Abstract: Natural pore-forming proteins act as viral helical coats and transmembrane channels, exhibit antibacterial activity and are used in synthetic systems, such as for reversible encapsulation or stochastic sensing. These diverse functions are intimately linked to protein structure. The close link between protein structure and protein function makes the design of synthetic mimics a formidable challenge, given that structure formation needs to be carefully controlled on all hierarchy levels, in solution and in the bulk. In fact, with few exceptions, synthetic pore structures capable of assembling into periodically ordered assemblies that are stable in solution and in the solid state have not yet been realized. In the case of dendrimers, covalent and non-covalent coating and assembly of a range of different structures has only yielded closed columns. Here we describe a library of amphiphilic dendritic dipeptides that self-assemble in solution and in bulk through a complex recognition process into helical pores. We find that the molecular recognition and self-assembly process is sufficiently robust to tolerate a range of modifications to the amphiphile structure, while preliminary proton transport measurements establish that the pores are functional. We expect that this class of self-assembling dendrimers will allow the design of a variety of biologically inspired systems with functional properties arising from their porous structure.

Peters K. M., Schuman J. T., Skurray R. A., Brown M. H., Brennan R. G. and Schumacher M. A. (2008) QacR-cation recognition is mediated by a redundancy of residues capable of charge neutralization. *Biochemistry* **47,** 8122-8129.
Abstract: The Staphylococcus aureus multidrug binding protein QacR binds to a broad spectrum of structurally dissimilar cationic, lipophilic drugs. Our previous structural analyses suggested that five QacR glutamic acid residues are critical for charge neutralization and specification of certain drugs. For example, E57 and E58 interact with berberine and with one of the positively charged moieties of the bivalent drug dequalinium. Here we report the structural and biochemical effects of substituting E57 and E58 with alanine and glutamine. Unexpectedly, individual substitutions of these residues did not significantly affect QacR drug binding affinity. Structures of QacR(E57Q) and QacR(E58Q) bound to dequalinium indicated that E57 and E58 are redundant for charge neutralization. The most significant finding was that berberine was reoriented in the QacR multidrug binding pocket so that its positive charge was neutralized by side chain oxygen atoms and aromatic residues. Together, these data emphasize the remarkable versatility of the QacR multidrug binding pocket, illustrating that the capacity of QacR to bind myriad cationic drugs is largely governed by the presence in the pocket of a redundancy of polar, charged, and aromatic residues that are capable of electrostatic neutralization

Polikandritou L. M., Sheu E., Lin J. S., and Pereira H. A. (1997) Interaction of a synthetic peptide based on the neutrophil-derived antimicrobial protein CAP37 with dipalmitoyl-phosphatidylcholine membranes. *Biochim Biophys Acta* **1329,** 285-290.
Abstract: CAP37, a cationic antimicrobial protein of Mr 37 kDa is constitutively expressed in human neutrophils. A synthetic peptide, CAP37 P20-44, corresponding to amino acid residues 20 through 44 of the native CAP37 molecule has been shown to mimic the antimicrobial activity of the native protein. An analog of peptide CAP37 P20-44 was synthesized in which the cysteine residues at positions 26 and 42 were replaced with serine residues (CAP37 P20-44Ser). This resulted in a peptide that no longer exhibited bactericidal activity. The effect of different concentrations of the active CAP37 peptide, CAP37 P20-44, and its inactive analog, CAP37 P20-44Ser, on artificial lipid membranes composed of dipalmitoyl phosphatidylcholine (DPPC) was studied using small-angle X-ray scattering and differential scanning calorimetry. The results indicated that CAP37 P20-44 perturbs the periodicity of the lamellar structure as shown by small angle X-ray diffraction, while the effect of the inactive peptide is not as strong. Differential scanning calorimetry further confirms that CAP37 P20-44 interacts with lipid membranes as indicated by increased width of the transition and decreased peak height. Moreover, it completely abolishes the pretransition temperature of the DPPC membranes. The effect of the inactive peptide, CAP37 P20-44Ser on the thermotropic properties of DPPC was small. These studies suggest that CAP37 perturbs the lamellar structure of lipid bilayers and further suggests that the antibiotic action of the molecule may be through its interactions with the lipid components of the Gram negative bacterial membrane.

Powers J. P., Tan A., Ramamoorthy A., and Hancock R. E. (2005) Solution structure and interaction of the antimicrobial polyphemusins with lipid membranes(,). *Biochemistry* **44,** 15504-15513.
Abstract: The horseshoe crab cationic antimicrobial peptide polyphemusin I is highly active in vitro but not protective in mouse models of bacterial and LPS challenge, while a synthetic polyphemusin variant, PV5, was previously shown to be protective in vivo. In this study, we investigated the interaction of these peptides with lipid membranes in an effort to propose a mechanism of interaction. The solution structure of PV5 was determined by proton NMR in the absence and presence of dodecylphosphocholine (DPC) micelles. Like polyphemusin I, PV5 is a beta-hairpin but appeared less amphipathic in solution. Upon association with DPC micelles, PV5 underwent side chain rearrangements which resulted in an increased amphipathic conformation. Using fluorescence spectroscopy, both peptides were found to have limited affinity for neutral vesicles composed of phosphatidylcholine (PC). Incorporation of 25 mol % cholesterol or phosphatidylethanolamine into PC vesicles produced little change in the partitioning of either peptide. Incorporation of 25 mol % phosphatidylglycerol (PG) into PC vesicles, a simple prokaryotic model, resulted in a large increase in the affinity for both peptides, but the partition coefficient for PV5 was almost twice that of polyphemusin I. Differential scanning calorimetry studies supported the partitioning data and demonstrated that neither peptide interacted readily with neutral PC vesicles. Both peptides showed affinity for negatively charged membranes incorporating PG. The affinity of PV5 was much greater as the pretransition peak was absent at low peptide to lipid ratios (1:400) and the reduction in enthalpy of the main transition was greater than that produced by polyphemusin I. Both peptides decreased the lamellar to

inverted hexagonal phase transition temperature of PE indicating the induction of negative curvature strain. These results, combined with previous findings that polyphemusin I promotes lipid flip-flop but does not induce significant vesicle leakage, ruled out the torroidal pore and carpet mechanisms of antimicrobial action for these polyphemusins.

Prenner E. J., Lewis R. N., Neuman K. C., Gruner S. M., Kondejewski L. H., Hodges R. S., and McElhaney R. N. (1997) Nonlamellar phases induced by the interaction of gramicidin S with lipid bilayers. A possible relationship to membrane-disrupting activity. *Biochemistry* **36,** 7906-7916.
Abstract: The interactions of the cyclic peptide gramicidin S (GS) with a variety of single-component lipid bilayers, and with membrane polar lipid extracts of Acholeplasma laidlawii B and Escherichia coli, were examined by differential scanning calorimetry (DSC), 31P-nuclear magnetic resonance (NMR) spectroscopy, and X-ray diffraction. The DSC data indicate that the effects of GS on the thermotropic phase behavior of phosphatidylcholine and phosphatidylethanolamine dispersions are compatible with those expected of peptides interacting primarily with the polar headgroup and/or the polar/apolar interfaces of lipid bilayers. These DSC studies also suggest that GS exhibits stronger interactions with the more fluid bilayers. For mixtures of GS with lipids such as phosphatidylcholine, phosphatidylserine, cardiolipin, and sphingomyelin, axially symmetric 31P-NMR powder patterns are observed throughout the entire temperature range examined (0-90 degrees C), and there is little evidence for significant destabilization of the lipid bilayer with respect to nonlamellar phases. With mixtures of GS with either phosphatidylethanolamine, phosphatidylglycerol, or a nonlamellar phase-forming phosphatidylcholine, axially symmetric 31P-NMR powder patterns are also observed at low temperatures. However, at high temperatures, an isotropic component is observed in their 31P-NMR spectra, and the relative intensity of this component increases significantly with temperature and with GS concentration. Once formed at high temperatures, this isotropic component exhibits a marked cooling hysteresis and in most cases disappears only when the sample is recooled to temperatures well below the lipid hydrocarbon chain-melting phase transition temperature. We also show that GS induces the formation of isotropic components in the 31P-NMR spectra of heterogeneous lipid mixtures such as occur in A. laidlawii B and E. coli membranes. These observations suggest that GS induces the formation of cubic or other three dimensionally ordered inverted nonlamellar phases when it interacts with some types of lipid bilayers, a suggestion strongly supported by our X-ray diffraction studies. Our results also suggest that the capacity of GS to induce the formation of such phases increases with the intrinsic nonlamellar phase-preferring tendencies of the lipids with which it interacts probably by producing localized increases in membrane monolayer curvature stress. The latter effect could be part of the mechanism through which this peptide exhibits its antimicrobial and hemolytic activities.

Prenner E. J., Lewis R. N., Kondejewski L. H., Hodges R. S., and McElhaney R. N. (1999) Differential scanning calorimetric study of the effect of the antimicrobial peptide gramicidin S on the thermotropic phase behavior of phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol lipid bilayer membranes. *Biochim Biophys Acta* **1417,** 211-223.
Abstract: We have studied the effects of the antimicrobial peptide gramicidin S (GS) on the thermotropic phase behavior of large multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylethanolamine (DMPE) and dimyristoyl phosphatidylglycerol (DMPG) by high-sensitivity differential scanning calorimetry. We find that the effect of GS on the lamellar gel to liquid-crystalline phase transition of these phospholipids varies markedly with the structure and charge of their polar headgroups. Specifically, the presence of even large quantities of GS has essentially no effect on the main phase transition of zwitterionic DMPE vesicles, even after repeating cycling through the phase transition, unless these vesicles are exposed to high temperatures, after which a small reduction in the temperature, enthalpy and cooperativity of the gel to liquid-crystalline phase transitions is observed. Similarly, even large amounts of GS produce similar modest decreases in the temperature, enthalpy and cooperativity of the main phase transition of DMPC vesicles, although the pretransition is abolished at low peptide concentrations. However, exposure to high temperatures is not required for these effects of GS on DMPC bilayers to be manifested. In contrast, GS has a much greater effect on the thermotropic phase behavior of anionic DMPG vesicles, substantially reducing the temperature, enthalpy and cooperativity of the main phase transition at higher peptide concentrations, and abolishing the pretransition at lower peptide concentrations as compared to DMPC. Moreover, the relatively larger effects of GS on the thermotropic phase behavior of DMPG vesicles are also manifest without cycling through the phase transition or

exposure to high temperatures. Furthermore, the addition of GS to DMPG vesicles protects the phospholipid molecules from the chemical hydrolysis induced by their repeated exposure to high temperatures. These results indicate that GS interacts more strongly with anionic than with zwitterionic phospholipid bilayers, probably because of the more favorable net attractive electrostatic interactions between the positively charged peptide and the negatively charged polar headgroup in such systems. Moreover, at comparable reduced temperatures, GS appears to interact more strongly with zwitterionic DMPC than with zwitterionic DMPE bilayers, probably because of the more fluid character of the former system. In addition, the general effects of GS on the thermotropic phase behavior of zwitterionic and anionic phospholipids suggest that it is located at the polar/apolar interface of liquid-crystalline bilayers, where it interacts primarily with the polar headgroup and glycerol-backbone regions of the phospholipid molecules and only secondarily with the lipid hydrocarbon chains. Finally, the considerable lipid specificity of GS interactions with phospholipid bilayers may prove useful in the design of peptide analogs with stronger interactions with microbial as opposed to eucaryotic membrane lipids.

Prenner E. J., Lewis R. N., and McElhaney R. N. (1999) The interaction of the antimicrobial peptide gramicidin S with lipid bilayer model and biological membranes. *Biochim Biophys Acta* **1462,** 201-221.
Abstract: Gramicidin S (GS) is a cyclic decapeptide of primary structure [cyclo-(Val-Orn-Leu-D-Phe-Pro)(2)] secreted by Bacillus brevis. It is a powerful antimicrobial agent with potent cidal action on a wide variety of Gram-negative and Gram-positive bacteria as well as on several pathogenic fungi. Unfortunately, however, GS is rather non-specific in its actions and also exhibits a high hemolytic activity, limiting its use as an antibiotic to topical applications. In a wide variety of environments, the GS molecule exists as a very stable amphiphilic antiparallel beta-sheet structure with a polar and a non-polar surface. Moreover, the large number of structure-activity studies of GS analogs which have been carried out indicate that this 'sidedness' structure is required for its antimicrobial action. In this review, we summarize both published and unpublished biophysical studies of the interactions of GS with lipid bilayer model and with biological membranes. In general, these studies show that GS partitions strongly into liquid-crystalline lipid bilayers in both model and biological membranes, and seems to be located primarily in the glycerol backbone region below the polar headgroups and above the hydrocarbon chains. The presence of GS appears to perturb lipid packing in liquid-crystalline bilayers and GS can induce the formation of inverted cubic phases at lower temperatures in lipids capable of forming such phases at higher temperature in the absence of peptide. The presence of GS at lower concentrations also increases the permeability of model and biological membranes and at higher concentrations causes membrane destabilization. There is good evidence from studies of the interaction of GS with bacterial cells that the destruction of the integrity of the lipid bilayer of the inner membrane is the primary mode of the antimicrobial action of this peptide. The considerable lipid specificity of GS for binding to and destabilization of lipid bilayer model membranes indicates that the design of GS analogs with an improved antimicrobial potency and a markedly decreased toxicity for eukaryotic cell plasma membranes should be possible.

Ramamoorthy A., Thennarasu S., Lee D. K., Tan A., and Maloy L. (2006) Solid-state NMR investigation of the membrane-disrupting mechanism of antimicrobial peptides MSI-78 and MSI-594 derived from magainin 2 and melittin. *Biophys J* **91,** 206-216.
Abstract: The mechanism of membrane interaction of two amphipathic antimicrobial peptides, MSI-78 and MSI-594, derived from magainin-2 and melittin, is presented. Both the peptides show excellent antimicrobial activity. The 8-anilinonaphthalene-1-sulfonic acid uptake experiment using Escherichia coli cells suggests that the outer membrane permeabilization is mainly due to electrostatic interactions. The interaction of MSI-78 and MSI-594 with lipid membranes was studied using 31P and 2H solid-state NMR, circular dichroism, and differential scanning calorimetry techniques. The binding of MSI-78 and MSI-594 to the lipid membrane is associated with a random coil to alpha-helix structural transition. MSI-78 and MSI-594 also induce the release of entrapped dye from POPC/POPG (3:1) vesicles. Measurement of the phase-transition temperature of peptide-DiPoPE dispersions shows that both MSI-78 and MSI-594 repress the lamellar-to-inverted hexagonal phase transition by inducing positive curvature strain. 15N NMR data suggest that both the peptides are oriented nearly perpendicular to the bilayer normal, which infers that the peptides most likely do not function via a barrel-stave mechanism of membrane-disruption. Data obtained from 31P NMR measurements using peptide-incorporated POPC and POPG oriented lamellar bilayers show a disorder in the orientation of lipids up to a peptide/lipid ratio of 1:20, and the formation of nonbilayer structures at peptide/lipid ratio>1:8. 2H-NMR experiments with selectively deuterated lipids

reveal peptide-induced disorder in the methylene units of the lipid acyl chains. These results are discussed in light of lipid-peptide interactions leading to the disruption of membrane via either a carpet or a toroidal-type mechanism.

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-RRWWRF-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.

Sedlak E., Zoldak G., Antalik M., and Sprinzl M. (2002) Thermodynamic properties of nucleotide-free EF-Tu from Thermus thermophilus in the presence of low-molecular weight effectors of its GTPase activity. *Biochim Biophys Acta* **1597,** 22-27.
Abstract: The thermal transition of elongation factor EF-Tu from Thermus thermophilus in the presence of low-molecular weight effectors was studied by differential scanning calorimetry. The effectors of GTPase activity used were the antibiotic kirromycin and the cations Li(+), Na(+), K(+) and NH(4)(+) in the chloride form. The temperature of thermal denaturation and the cooperativity of the transition of nucleotide-free EF-Tu (EF-Tu(f)) in the presence of kirromycin are comparable with those of the EF-Tu x guanosine-5'-[beta,gamma-imido]triphosphate (GppNHp) form, indicating similar conformational states. Increased concentrations of Na(+) and K(+) stabilized EF-Tu(f) in a manner similar to GppNHp. NH(4)(+) decreased the transition temperature of EF-Tu(f) and Li(+) decreased both the temperature and the calorimetric enthalpy of the thermal transition of EF-Tu(f). In the presence of salts, binding of kirromycin had a stabilizing effect on EF-Tu(f). Correlation between the GTPase activity and thermodynamic characteristics of EF-Tu(f) induced by kirromycin in the absence or presence of the cations is discussed.

Seto G. W., Marwaha S., Kobewka D. M., Lewis R. N., Separovic F. and McElhaney R. N. (2007) Interactions of the Australian tree frog antimicrobial peptides aurein 1.2, citropin 1.1 and maculatin 1.1 with lipid model membranes: Differential scanning calorimetric and Fourier transform infrared spectroscopic studies. *Biochim Biophys Acta* **1768,** 2787-2800.
Abstract: The interactions of the antimicrobial peptides aurein 1.2, citropin 1.1 and maculatin 1.1 with dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG) and dimyristoylphosphatidylethanolamine (DMPE) were studied by differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectroscopy. The effects of these peptides on the thermotropic phase behavior of DMPC and DMPG are qualitatively similar and manifested by the suppression of the pretransition, and by peptide concentration-dependent decreases in the temperature, cooperativity and enthalpy of the gel/liquid-crystalline phase transition. However, at all peptide concentrations, anionic DMPG bilayers are more strongly perturbed than zwitterionic DMPC bilayers, consistent with membrane surface charge being an important aspect of the interactions of these peptides with phospholipids. However, at all peptide concentrations, the perturbation of the thermotropic phase behavior of zwitterionic DMPE bilayers is weak and discernable only when samples are exposed to high temperatures. FTIR spectroscopy indicates that these peptides are unstructured in aqueous solution and that they fold into alpha-helices when

incorporated into lipid membranes. All three peptides undergo rapid and extensive H-D exchange when incorporated into D(2)O-hydrated phospholipid bilayers, suggesting that they are located in solvent-accessible environments, most probably in the polar/apolar interfacial regions of phospholipid bilayers. The perturbation of model lipid membranes by these peptides decreases in magnitude in the order maculatin 1.1>aurein 1.2>citropin 1.1, whereas the capacity to inhibit Acholeplasma laidlawii B growth decreases in the order maculatin 1.1>aurein 1.2 congruent withcitropin 1.1. The higher efficacy of maculatin 1.1 in disrupting model and biological membranes can be rationalized by its larger size and higher net charge. However, despite its smaller size and lower net charge, aurein 1.2 is more disruptive of model lipid membranes than citropin 1.1 and exhibits comparable antimicrobial activity, probably because aurein 1.2 has a higher propensity for partitioning into phospholipid membranes.

Tahir A., Grabielle-Madelmont C., Betrencourt C., Ollivon M., and Peretti P. (1999) A differential scanning calorimetry study of the interaction of lasalocid antibiotic with phospholipid bilayers. *Chem Phys Lipids* **103,** 57-65.
Abstract: Interaction of lasalocid sodium salt (Las-Na) with dipalmitoylphosphatidylcholine (DPPC) as a membrane model was investigated by highly-sensitive differential scanning calorimetry (DSC). The insertion properties of the antibiotic were studied both in multilamellar suspensions and unilamellar vesicles, for Las-Na/DPPC molar ratios (r) ranging from 0.005 to 0.1. The effect of the antibiotic on the lipid thermotropic behavior is concentration dependent and drastically changes at a critical r of 0.04 in both model membranes. Below this ratio, Las-Na molecules interact with DPPC bilayers without disrupting the global organization of the membrane. In the multilamellar systems only the transition cooperativity is affected whereas for the mixed vesicles, a decrease in the enthalpy change suggests a different mode of insertion. Above this ratio, implantation of the antibiotic give rise to lateral phase separation in multilamellar systems. These structural modifications have repercussions on the formation of mixed LAS-Na/DPPC vesicles which seems limited to an r value of 0.04.

Tanaka Y., Morikawa K., Ohki Y., Yao M., Tsumoto K., Watanabe N., Ohta T. and Tanaka I. (2007) Structural and mutational analyses of Drp35 from Staphylococcus aureus: a possible mechanism for its lactonase activity. *J Biol Chem* **282,** 5770-5780.
Abstract: Drp35 is a protein induced by cell wall-affecting antibiotics or detergents; it possesses calcium-dependent lactonase activity. To determine the molecular basis of the lactonase activity, we first solved the crystal structures of Drp35 with and without Ca(2+); these showed that the molecule has a six-bladed beta-propeller structure with two calcium ions bound at the center of the beta-propeller and surface region. Mutational analyses of evolutionarily conserved residues revealed that the central calcium-binding site is essential for the enzymatic activity of Drp35. Substitution of some other amino acid residues for the calcium-binding residues demonstrated the critical contributions of Glu(48), Asp(138), and Asp(236) to the enzymatic activity. Differential scanning calorimetric analysis revealed that the loss of activity of E48Q and D236N, but not D138N, was attributed to their inability to hold the calcium ion. Further structural analysis of the D138N mutant indicates that it lacks a water molecule bound to the calcium ion rather than the calcium ion itself. Based on these observations and structural information, a possible catalytic mechanism in which the calcium ion and its binding residues play direct roles was proposed for the lactonase activity of Drp35.

Taylor T. M., Davidson P. M., Bruce B. D., and Weiss J. (2005) Ultrasonic spectroscopy and differential scanning calorimetry of liposomal-encapsulated nisin. *J Agric Food Chem* **53,** 8722-8728.
Abstract: The thermal stability of phosphatidylcholine (PC) liposomes (colloidal dispersions of bilayer-forming polar lipids in aqueous solvents) in the presence and absence of the antimicrobial polypeptide nisin was evaluated using differential scanning calorimetry (DSC) and low-intensity ultrasonic spectroscopy (US). PC liposome mixtures with varying acyl chain lengths (C16:0 and C18:0) were formed in buffer with or without entrapped nisin. Gel-to-liquid crystalline phase transition temperatures (T(M)) of liposomes determined from DSC thermograms were in excellent agreement with those determined by ultrasonic velocity and attenuation coefficient measurements recorded at 5 MHz. The dipalmitoylphosphatidylcholine (DPPC) T(M) measured by DSC was approximately 41.3 and approximately 40.7 degrees C when measured by ultrasonic spectroscopy. The T(M) of distearoylphosphatidylcholine (DSPC) and DPPC/DSPC 1:1 liposomes was 54.3 and 54.9 degrees C and approximately 44.8 and approximately 47.3 degrees C when measured by DSC and US, respectively. The thermotropic stability generally increased

upon addition of nisin. Analysis of the stepwise decrease in ultrasonic velocity with temperature indicated an increased compressibility corresponding to a loss of structure upon heating.

Thennarasu S., Lee D. K., Poon A., Kawulka K. E., Vederas J. C., and Ramamoorthy A. (2005) Membrane permeabilization, orientation, and antimicrobial mechanism of subtilosin A. *Chem Phys Lipids* **137,** 38-51.
Abstract: Subtilosin A is an antimicrobial peptide produced by the soil bacterium Bacillus subtilis that possesses bactericidal activity against a diverse range of bacteria, including Listeria monocytogenes. Recent structural studies have found that subtilosin A is posttranslationally modified in a unique way, placing it in a new class of bacteriocins. In this study, in order to understand the mechanism of membrane-disruption by subtilosin A, the interaction of the peptide with model phospholipid bilayers is characterized using fluorescence, solid-state NMR and differential scanning calorimetry (DSC) experiments. Our results in this study show that subtilosin A interacts with the lipid head group region of bilayer membranes in a concentration dependent manner. Fluorescence experiments reveal the interaction of subtilosin A with small unilamellar vesicles (SUVs) composed of POPC, POPG and E. coli total lipids, and that at least one edge of the molecule is buried in membrane bilayers. At high concentrations, it induces leakage from SUVs of POPC and POPE/POPG (7:3) mixture. (15)N solid-state NMR data suggests that the cyclic peptide is partially inserted into bilayers, which is in agreement with the fluorescence data. (31)P and (2)H NMR experiments and DSC data support the hypothesis that subtilosin A adopts a partially buried orientation in lipid bilayers, by showing that it induces a conformational change in the lipid headgroup and disordering in the hydrophobic region of bilayers. These results suggest that the lipid perturbation observed in this study may be one of the consequences of subtilosin A binding to lipid bilayers, which results in membrane permeabilization at high peptide concentrations.

Thomas A. M., Ginj C., Jelesarov I., Amrhein N., and Macheroux P. (2004) Role of K22 and R120 in the covalent binding of the antibiotic fosfomycin and the substrate-induced conformational change in UDP-N-acetylglucosamine enolpyruvyl transferase. *Eur J Biochem* **271,** 2682-2690.
Abstract: UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), catalyzes the first step in the biosynthesis of peptidoglycan, involving the transfer of the intact enolpyruvyl moiety from phosphoenolpyruvate to the 3'-hydroxyl group of UDP-N-acetylglucosamine (UDPNAG). The enzyme is irreversibly inhibited by the antibiotic fosfomycin. The inactivation is caused by alkylation of a highly conserved cysteine residue (C115) that participates in the binding of phosphoenolpyruvate. The three-dimensional structure of the enzyme suggests that two residues may play a decisive role in fosfomycin binding: K22 and R120. To investigate the role of these residues, we have generated the K22V, K22E, K22R and R120K single mutant proteins as well as the K22V/R120K and K22V/R120V double mutant proteins. We demonstrated that the K22R mutant protein behaves similarly to wild-type enzyme, whereas the K22E mutant protein failed to form the covalent adduct. On the other hand, the K22V mutant protein requires the presence of UDPNAG for the formation of the adduct indicating that UDPNAG plays a crucial role in the organization of productive interactions in the active site. This model receives strong support from heat capacity changes observed for the K22V/R120K and R120K mutant proteins: in both mutant proteins, the heat capacity changes are markedly reduced indicating that their ability to form a closed protein conformation is impeded due to the R120K exchange.

Tong J. and McIntosh T. J. (2004) Structure of supported bilayers composed of lipopolysaccharides and bacterial phospholipids: raft formation and implications for bacterial resistance. *Biophys J* **86,** 3759-3771.
Abstract: Lipopolysaccharide (LPS), the major lipid on the surface of Gram-negative bacteria, plays a key role in bacterial resistance to hydrophobic antibiotics and antimicrobial peptides. Using atomic force microscopy (AFM) we characterized supported bilayers composed of LPSs from two bacterial chemotypes with different sensitivities to such antibiotics and peptides. Rd LPS, from more sensitive "deep rough" mutants, contains only an inner saccharide core, whereas Ra LPS, from "rough" mutants, contains a longer polysaccharide region. A vesicle fusion technique was used to deposit LPS onto either freshly cleaved mica or polyethylenimine-coated mica substrates. The thickness of the supported bilayers measured with contact-mode AFM was 7 nm for Rd LPS and 9 nm for Ra LPS, consistent with previous x-ray diffraction measurements. In water the Ra LPS bilayer surface was more disordered than Rd LPS bilayers, likely due to the greater volume occupied by the longer Ra LPS polysaccharide region. Since deep rough mutants contain bacterial phospholipid (BPL) as well as LPS on their surfaces, we also investigated the organization of Rd LPS/BPL bilayers. Differential scanning calorimetry and x-ray diffraction indicated that

incorporation of BPL reduced the phase transition temperature, enthalpy, and average bilayer thickness of Rd LPS. For Rd LPS/BPL mixtures, AFM showed irregularly shaped regions thinner than Rd LPS bilayers by 2 nm (the difference in thickness between Rd LPS and BPL bilayers), whose area increased with increasing BPL concentration. We argue that the increased permeability of deep rough mutants is due to structural modifications caused by BPL to the LPS membrane, in LPS hydrocarbon chain packing and in the formation of BPL-enriched microdomains.

Trombetta D., Castelli F., Sarpietro M. G., Venuti V., Cristani M., Daniele C., Saija A., Mazzanti G., and Bisignano G. (2005) Mechanisms of antibacterial action of three monoterpenes. *Antimicrob Agents Chemother* **49,** 2474-2478.
Abstract: In the present paper, we report the antimicrobial efficacy of three monoterpenes [linalyl acetate, (+)menthol, and thymol] against the gram-positive bacterium Staphylococcus aureus and the gram-negative bacterium Escherichia coli. For a better understanding of their mechanisms of action, the capability of these three monoterpenes to damage biomembranes was evaluated by monitoring the release, following exposure to the compounds under study, of the water-soluble fluorescent marker carboxyfluorescein from unilamellar vesicles with different lipidic compositions (phosphatidylcholine, phosphatidylcholine/phosphatidylserine [9:1], phosphatidylcholine/stearylamine [9:1], and phosphatidylglycerol/cardiolipin [9:1]). Furthermore, the interaction of the terpenes tested with dimyristoylphosphatidylcholine multilamellar vesicles as model membranes was monitored by means of differential scanning calorimetry. Finally, the results were related to the relative lipophilicity and water solubility of the compounds examined. Taken together, our findings lead us to speculate that the antimicrobial effect of (+)menthol, thymol, and linalyl acetate may result, at least partially, from a perturbation of the lipid fraction of microorganism plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials. Besides being related to physicochemical characteristics of the drugs (such as lipophilicity and water solubility), this effect seems to be dependent on lipid composition and net surface charge of microbial membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity.

van Kan E. J., Ganchev D. N., Snel M. M., Chupin V., van der B. A., and de Kruijff B. (2003) The peptide antibiotic clavanin A interacts strongly and specifically with lipid bilayers. *Biochemistry* **42,** 11366-11372.
Abstract: In this study the interaction of the antimicrobial peptide clavanin A with phosphatidylcholine bilayers is investigated by DSC, NMR, and AFM techniques. It is shown that the peptide interacts strongly and specifically with the lipids, resulting in increased order-disorder phase transition temperatures, phase separation, altered acyl chain and headgroup packing, and a drastically changed surface morphology of the bilayer. These results are interpreted in terms of clavanin-specific interactions with lipids and are discussed in the light of the different mechanisms by which clavanin A can destroy the barrier function of biological membranes.

Vogel H. J., Schibli D. J., Jing W., Lohmeier-Vogel E. M., Epand R. F., and Epand R. M. (2002) Towards a structure-function analysis of bovine lactoferricin and related tryptophan- and arginine-containing peptides. *Biochem Cell Biol* **80,** 49-63.
Abstract: The iron-binding protein lactoferrin is a multifunctional protein that has antibacterial, antifungal, antiviral, antitumour, anti-inflammatory, and immunoregulatory properties. All of these additional properties appear to be related to its highly basic N-terminal region. This part of the protein can be released in the stomach by pepsin cleavage at acid pH. The 25-residue antimicrobial peptide that is released is called lactoferricin. In this work, we review our knowledge about the structure of the peptide and attempt to relate this to its many functions. Microcalorimetry and fluorescence spectroscopy data regarding the interaction of the peptide with model membranes show that binding to net negatively charged bacterial and cancer cell membranes is preferred over neutral eukaryotic membranes. Binding of the peptide destabilizes the regular membrane bilayer structure. Residues that are of particular importance for the activity of lactoferricin are tryptophan and arginine. These two amino acids are also prevalent in "penetratins", which are regions of proteins or synthetic peptides that can spontaneously cross membranes and in short hexapeptide antimicrobial peptides derived through combinatorial chemistry. While the antimicrobial, antifungal, antitumour, and antiviral properties of lactoferricin can be related to the Trp/Arg-rich portion of the peptide, we suggest that the anti-inflammatory and immunomodulating properties are more related to a

positively charged region of the molecule, which, like the alpha- and beta-defensins, may act as a chemokine. Few small peptides are involved in as wide a range of host defense functions as bovine and human lactoferricin.

Volke F., Waschipky R., Pampel A., Donnerstag A., Lantzsch G., Pfeiffer H., Richter W., Klose G., and Welzel P. (1997) Characterisation of antibiotic moenomycin A interaction with phospholipid model membranes. *Chem Phys Lipids* **85,** 115-123.
Abstract: Using a combination of physico-chemical techniques (MAS NMR, DSC, freeze-fracture electron microscopy, molecular modelling) the antibiotic moenomycin A was found to be anchored by its hydrophobic chain into multilamellar POPC membranes. The lamellar phase structure of the modified membrane is retained, while moenomycin A in water at different concentrations does not form any other but isotropic phase structures. The mobility of POPC molecule segments is reduced with increasing moenomycin A concentrations. Freeze-fracture electron microscopy images show ripple like structures for low moenomycin A concentrations, which are rare for high concentrations. A sugar-group network of the antibiotic seems to cover the whole membrane surface for molar ratios moenomycin A/POPC of 1:2, which is supported by 13C-MAS (Magic Angle Spinning) 31P-NMR, and molecular modelling.

Willis B. and Arya D. P. (2006) Recognition of B-DNA by neomycin--Hoechst 33258 conjugates. *Biochemistry* **45,** 10217-10232.
Abstract: Recent developments have indicated that aminoglycoside binding is limited not to RNA but to nucleic acids that, like RNA, adopt conformations similar to the A-form. We have further sought to expand the utility of aminoglycoside binding to B-DNA structures by conjugating neomycin, an aminoglycoside antibiotic, with the B-DNA minor groove binding ligand Hoechst 33258. Described herein are novel neomycin-Hoechst 33258 conjugates developed for exploring B-DNA groove recognition. We have varied the two reported conjugates in linker length and composition in an effort to improve our understanding of the spatial differences that define B-DNA binding. Spectroscopic studies such as ultraviolet (UV) melting, isothermal fluorescence titrations, differential scanning calorimetry (DSC), and circular dichroism (CD) together illustrate the mode of binding by such conjugates. Both conjugates exhibit enhanced thermal stabilization of A.T rich duplexes when compared to Hoechst 33258.

Willis B. and Arya D. P. (2006) An expanding view of aminoglycoside-nucleic acid recognition. *Adv Carbohydr Chem Biochem* **60,** 251-302.

Wu J. M., Wei S. Y., Chen H. L., Weng K. Y., Cheng H. T. and Cheng J. W. (2007) Solution structure of a novel D-naphthylalanine substituted peptide with potential antibacterial and antifungal activities. *Biopolymers* **88,** 738-745.
Abstract: A new type of Trp-rich peptide, Ac-KWRRWVRWI-NH2, designated as Pac-525, was found to possess improved activity against both gram-positive and negative bacteria. We have synthesized two Pac-525 analogues, D-Pac-525 containing all D-amino acids and D-Nal-Pac-525, the D-Pac-525 analogue with tryptophan replaced by D-beta-naphthylalanine. We have determined the solution structure of D-Nal-Pac-525 bound to membrane-mimetic DPC micelles by two-dimensional NMR methods. The DPC micelle-bound structure of D-Nal-Pac-525 adopts a left-hand alpha-helical segment and the positively charged residues are clustered together to form a hydrophilic patch. The surface electrostatic potential map indicates the three D-beta-naphthylalanines are packed against the peptide backbone and form an amphipathic structure. A variety of biophysical and biochemical experiments, including circular dichroism, fluorescence spectroscopy, and microcalorimetry, were used to show that D-Nal-Pac-525 interacted strongly with negatively charged phospholipid vesicles and induced efficient dye release from these vesicles, suggesting that the strong antimicrobial activity of D-Nal-Pac-525 may be due to interactions with bacterial and fungus membranes.

Zhang Y., Roy S., Jones L. S., Krishnan S., Kerwin B. A., Chang B. S., Manning M. C., Randolph T. W., and Carpenter J. F. (2004) Mechanism for benzyl alcohol-induced aggregation of recombinant human interleukin-1 receptor antagonist in aqueous solution. *J Pharm Sci* **93,** 3076-3089.
Abstract: Benzyl alcohol, an antimicrobial preservative, accelerates aggregation and precipitation of recombinant human interleukin-1 receptor antagonist (rhIL-1ra) in aqueous solution. The loss of native monomer during incubation at 37 degrees C was determined by analysis of sample aliquots with size

exclusion high performance liquid chromatography (SE-HPLC). Benzyl alcohol caused minor perturbation of the tertiary structure of the protein without changing its secondary structure, documenting that the preservative caused a minor shift in the protein molecular population toward partially unfolded species. Consistent with this conclusion, in the presence of benzyl alcohol the rate of H-D exchange was accelerated and the fluorescence of 1-anilinonaphthalene-8-sulfonic acid in the presence of rhIL1ra was increased. Benzyl alcohol did not alter the free energy of unfolding based on unfolding experiments in urea or guanidine HCl. With differential scanning calorimetry it was determined that benzyl alcohol reduced the apparent Tm of rhIL-1ra, but this effect occurred because the preservative lowered the temperature at which the protein aggregated during heating. Isothermal calorimetry documented that the interaction of benzyl alcohol with rhIL-1ra is relatively weak and hydrophobically driven. Thus, benzyl alcohol accelerates protein aggregation by binding to the protein and favoring an increase in the level of partially unfolded, aggregation-competent species. Sucrose partially inhibited benzyl alcohol-induced aggregation and tertiary structural change. Sucrose is preferentially excluded from the surface of the protein, favoring most compact native state species over expanded aggregation-prone forms.

Zhuang P., Eisenstein E., and Howell E. E. (1994) Equilibrium folding studies of tetrameric R67 dihydrofolate reductase. *Biochemistry* **33,** 4237-4244.
Abstract: R67 dihydrofolate reductase (DHFR) is an R-plasmid encoded enzyme that confers resistance to the antibacterial drug trimethoprim. This enzyme is not homologous in sequence or structure to chromosomal DHFRs. Equilibrium folding of tetrameric R67 DHFR was studied and found to be fully reversible. Formation of an inactive intermediate was assayed by loss of enzyme activity. Denaturation of the intermediate was monitored by concurrent changes in fluorescence and circular dichroism signals. Both transitions are protein concentration dependent. A simple model fitting these data is tetramer<==>2 dimers<==>4 unfolded monomers. No evidence for folded monomer was found. Global fitting of all the folding data yielded a Δ GH2O of -9.63 kcal/mol for the initial transition and a Δ GH2O of -12.35 kcal/mol for the second transition. In addition, thermal unfolding of tetrameric R67 DHFR was found to be reversible A folding intermediate also occurred during thermal unfolding as evidenced by the asymmetric endotherms and a Δ Hcalorimetric/Δ H(van't Hoff) ratio of 2.1.

Zweytick D., Pabst G., Abuja P. M., Jilek A., Blondelle S. E., Andra J., Jerala R., Monreal D., Martinez d. T., and Lohner K. (2006) Influence of N-acylation of a peptide derived from human lactoferricin on membrane selectivity. *Biochim Biophys Acta* **1758,** 1426-1435.
Abstract: Increasing numbers of bacterial strains being resistant to conventional antibiotics emphasize the urgent need for new antimicrobial agents. One strategy is based on host defence peptides that can be found in every organism including humans. We have studied the antimicrobial peptide LF11, derived from the pepsin cleavage product of human lactoferrin, known for its antimicrobial and lipid A-binding activity, and peptide C12LF11, the N-lauryl-derivative of LF11, which has owing to the attached hydrocarbon chain an additional hydrophobic segment. The influence of this hydrocarbon chain on membrane selectivity was studied using model membranes composed of dipalmitoylphosphatidylglycerol (DPPG), mimicking bacterial plasma membranes, and of dipalmitoylphosphatidylcholine (DPPC), a model system for mammalian membranes. A variety of biophysical techniques was applied. Thereby, we found that LF11 did not affect DPPC bilayers and showed only moderate effects on DPPG membranes in accordance with its non-hemolytic and weak antimicrobial activity. In contrast, the introduction of the N-lauryl group caused significant changes in the phase behaviour and lipid chain packing in both model membrane systems. These findings correlate with the in vitro tests on methicillin resistant S. aureus, E. coli, P. aeruginosa and human red blood cells, showing increased biological activity of C12LF11 towards these test organisms. This provides evidence that both electrostatic and hydrophobic interactions are crucial for biological activity of antimicrobial peptides, whereas a certain balance between the two components has to be kept, in order not to loose the specificity for bacterial membranes.