

## ITC XVII – Lipid-Small Molecule interactions (including drugs, detergent/surfactants)

Alakoskela J. M., Covey D. F. and Kinnunen P. K. (2007) Lack of enantiomeric specificity in the effects of anesthetic steroids on lipid bilayers. *Biochim Biophys Acta* **1768**, 131-145.

**Abstract:** The most important target protein for many anesthetics, including volatile and steroid anesthetics, appears to be the type A gamma-amino butyric acid receptor (GABA(A)R), yet direct binding remains to be demonstrated. Hypotheses of lipid-mediated anesthesia suggest that lipid bilayer properties are changed by anesthetics and that this in turn affects the functions of proteins. While other data could equally well support direct or lipid-mediated action, enantiomeric specificity displayed by some anesthetics is not reflected in their interactions with lipids. In the present study, we studied the effects of two pairs of anesthetic steroid enantiomers on bilayers of several compositions, measuring potentially relevant physical properties. For one of the pairs, allopregnanolone and ent-allopregnanolone, the natural enantiomer is 300% more efficacious as an anesthetic, while for the other, pregnanolone and ent-pregnanolone, there is little difference in anesthetic potency. For each enantiomer pair, we could find no differences. This strongly favors the view that the effects of these anesthetics on lipid bilayers are not relevant for the main features of anesthesia. These steroids also provide tools to distinguish in general the direct binding of steroids to proteins from lipid-mediated effects.

Anderson T. G., Tan A., Ganz P., and Seelig J. (2004) Calorimetric measurement of phospholipid interaction with methyl-beta-cyclodextrin. *Biochemistry* **43**, 2251-2261.

**Abstract:** Cyclodextrins are able to bind hydrophobic molecules in their interior cavity and as such have received a great deal of attention as carriers of cholesterol, lipophilic drugs, and other sparingly soluble compounds. Despite the importance of these biochemical applications, relatively little is known about the interactions of cyclodextrins with phospholipid membranes. Here we characterize the binding of randomly methylated beta-cyclodextrin (m beta CD) to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) using right-angle light scattering and isothermal titration calorimetry. Existing models of lipophile-membrane interactions are inadequate to describe the observed binding; we introduce a modified chemical reaction model in which the chemical activity of the phospholipid is independent of its concentration. We find that an average of four m beta CD molecules bind to each POPC molecule with an enthalpy of reaction of 46 kJ mol<sup>-1</sup> and an equilibrium constant of 90 M<sup>-3</sup>. These results are consistent with earlier qualitative observations and suggest that disruption of phospholipid membranes may be minimized if the concentration of m beta CD is kept below about 15 mM.

Andrushchenko V. V., Aarabi M. H., Nguyen L. T., Prenner E. J. and Vogel H. J. (2008) Thermodynamics of the interactions of tryptophan-rich cathelicidin antimicrobial peptides with model and natural membranes. *Biochim Biophys Acta* **1778**, 1004-1014.

**Abstract:** Tritrpticin and indolicidin are short 13-residue tryptophan-rich antimicrobial peptides that hold potential as future alternatives for antibiotics. Isothermal titration calorimetry (ITC) has been applied as the main tool in this study to investigate the thermodynamics of the interaction of these two cathelicidin peptides as well as five tritrpticin analogs with large unilamellar vesicles (LUVs), representing model and natural anionic membranes. The anionic LUVs were composed of (a) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine/1-palmitoyl-2-oleoyl -sn-glycero-3-phosphoglycerol (POPE/POPG) (7:3) and (b) natural E. coli polar lipid extract. 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was used to make model zwitterionic membranes. Binding isotherms were obtained to characterize the antimicrobial peptide binding to the LUVs, which then allowed for calculation of the thermodynamic parameters of the interaction. All peptides exhibited substantially stronger binding to anionic POPE/POPG and E. coli membrane systems than to the zwitterionic POPC system due to strong electrostatic attractions between the highly positively charged peptides and the negatively charged membrane surface, and results with tritrpticin derivatives further revealed the effects of various amino acid substitutions on membrane binding. No significant improvement was observed upon increasing the Tritrp peptide charge from +4 to +5. Replacement of Arg residues with Lys did not substantially change peptide binding to anionic vesicles but moderately decreased the binding to zwitterionic LUVs. Pro to Ala substitutions in tritrpticin, allowing the peptide to adopt an alpha-helical structure, resulted in a significant increase of the binding to both anionic and zwitterionic vesicles and therefore reduced the selectivity for bacterial and mammalian membranes. In

contrast, substitution of Trp with other aromatic amino acids significantly decreased the peptide's ability to bind to anionic LUVs and essentially eliminated binding to zwitterionic LUVs. The ITC results were consistent with the outcome of fluorescence spectroscopy membrane binding and perturbation studies. Overall, our work showed that a natural *E. coli* polar lipid extract as a bacterial membrane model was advantageous compared to the simpler and more widely used POPE/POPG lipid system

Arseneault M. and Laflleur M. (2006) Isothermal titration calorimetric study of calcium association to lipid bilayers: influence of the vesicle preparation and composition. *Chem Phys Lipids* **142**, 84-93.

**Abstract:** The association of Ca<sup>2+</sup> ions with phospholipid bilayers was investigated using isothermal titration calorimetry. The study reveals that the binding enthalpy of these cations to bilayers formed with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) depends strongly on the method of preparation of the unilamellar vesicles. Extruded vesicles lead to an exothermic association, whereas sonicated ones lead to an endothermic association. In the later case, the calorimetric signal is sensitive to the length of the sonication period. It is proposed that a reorganization of the lipid bilayers under stress, obtained with sonicated small unilamellar vesicles, contributes to the calorimetric signal upon the titration with Ca<sup>2+</sup>. The analysis of the titrations indicates that, as expected, the nature of the association of Ca<sup>2+</sup> with negatively charged phospholipid bilayers is essentially of electrostatic nature. Using a Scatchard approach, it is found that bilayers become saturated in Ca<sup>2+</sup> approximately when the electroneutrality of the bilayer interface is reached. Moreover, the affinity constant was reduced by the increase of the ionic strength of the aqueous buffer. It was found that the intrinsic binding constant of Ca<sup>2+</sup> to membranes containing 30 and 50 mol% of POPG was about 11 mM<sup>-1</sup>, in a MES buffer containing 10 mM NaCl, at pH 5.6.

Austin R. P., Barton P., Davis A. M., Fessey R. E., and Wenlock M. C. (2005) The thermodynamics of the partitioning of ionizing molecules between aqueous buffers and phospholipid membranes. *Pharm Res* **22**, 1649-1657.

**Abstract:** PURPOSE: To study the thermodynamics of partitioning of eight ionising dual D2-receptor beta2-adrenoceptor agonists between vesicles of L-alpha-dimyristoylphosphatidylcholine (DMPC) and aqueous buffers. METHODS: The thermodynamics of partitioning have been studied by isothermal titration calorimetry (ITC). RESULTS: Compounds which are predominantly cationic at pH 7.4 (designated as class 1 compounds) have a more exothermic partitioning than those which are predominantly in the electronically neutral form (designated as class 2 compounds) at pH 7.4, and less positive standard entropies of partitioning. Under acidic conditions (pH 4.0), class compounds 2 (predominantly electronically neutral at pH 7.4) are almost completely cationic and accordingly have a more exothermic partitioning than at pH 7.4. The standard entropies of partitioning also depend on the pH. When the compounds are predominantly cationic, the standard entropy change is less positive (less favourable) than under conditions where the compounds are predominantly electronically neutral. CONCLUSIONS: The observations are consistent with the notion of there being a favourable electrostatic interaction (enthalpically) between the positively charged amino-group of predominantly cationic compounds and the negatively charged phosphate group of the vesicle.

Barrow D. J., Jr., Chandrasekaran S., Heerklotz H. H., Henary M. M., Michniak B. B., Nguyen P. M., Song Y., Smith J. C., and Strekowski L. (2005) Mechanistic studies on percutaneous penetration enhancement by N-(4-halobenzoyl)-S,S-dimethyliminosulfuranes. *J Lipid Res* **46**, 2192-2201.

**Abstract:** Halogen-substituted iminosulfuranes are transdermal penetration enhancers (TPEs) in permeation studies using hairless mouse or human cadaver skin. The interaction of N-(4-R-benzoyl)-S,S-dimethyliminosulfuranes 1-4, where R=H, Cl, Br, and I, with 1-alpha-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) has been studied using differential scanning calorimetry, isothermal titration calorimetry, nuclear Overhauser effect spectroscopy (NOESY), and NMR spectroscopy, and by calculation of the iminosulfurane polarizabilities in order to elucidate the molecular basis of the TPE activity. The active compounds reduce the melting temperature of the gel-to-liquid-crystal phase transition and induce multiple components in the transition excess heat capacity profile. The partitioning of the bromo derivative 3, the most active compound, into DMPC is unique in that 3 may be trapped in the bilayer, affording an enhanced residence time and a reason for its high TPE activity. The entropy decrease associated with the transfer of 3 to the bilayer is much lower than that for the other compounds, indicating that 3 occupies or induces sites that afford it considerable local motional freedom. Correlations between the iminosulfurane

TPE activities, the partition coefficients, and NOESY crosspeak volume were observed. Molecular polarizabilities are not consistent with a TPE mode of action involving interaction of these agents with protein side chains.

Bauerle H. D. and Seelig J. (1991) Interaction of charged and uncharged calcium channel antagonists with phospholipid membranes. Binding equilibrium, binding enthalpy, and membrane location. *Biochemistry* **30**, 7203-7211.

**Abstract:** The membrane location and the binding mechanism of two Ca<sup>2+</sup> channel antagonists, amlodipine and nimodipine, in pure lipid membranes were investigated with deuterium and phosphorus-31 nuclear magnetic resonance, with thermodynamic methods such as high-sensitivity titration calorimetry, and by measuring the membrane surface charge via the zeta-potential. The two drugs exhibit quite different physical-chemical properties. The noncharged nimodipine is strongly hydrophobic, and selective deuteration of the lipid membrane reveals a homogeneous distribution of nimodipine across the whole hydrocarbon layer, but no interaction at the lipid headgroup level. The membrane behavior of the amphiphilic amlodipine (electric charge  $z = +1$ ) is distinctly more complex. Deuterium magnetic resonance demonstrates that amlodipine adopts a well-defined position in the bilayer membrane. In particular, the charged ethanolamine side group of amlodipine is located near the water-lipid interface, interacting with the dipoles of the headgroup region according to a nonspecific, electrostatic mechanism and inducing a reorientation of the phosphocholine dipoles toward the water phase. At the level of the hydrocarbon segment, the nonpolar ring system of amlodipine interacts specifically with the cis double bond of the membrane lipid, forming a weak association complex. With increasing amlodipine concentration the deuterium signal of the cis double bond gradually loses intensity, a phenomenon previously observed only in related studies on protein-lipid interactions. The binding equilibrium of amlodipine to phosphatidylcholine membranes was studied by measuring the electrophoretic mobility of lipid vesicles and with a centrifugation assay. Hydrophobic interactions of the nonpolar ring systems and electrostatic repulsions at the membrane surface contribute to the binding energy. (ABSTRACT TRUNCATED AT 250 WORDS).

Beck A., Tsamaloukas A. D., Jurcevic P. and Heerklotz H. (2008) Additive action of two or more solutes on lipid membranes. *Langmuir* **24**, 8833-8840.

**Abstract:** A wide variety of biological processes, pharmaceutical applications, and technical procedures is based on the combined action of two or more soluble compounds to perturb, permeabilize, or lyse biological membranes. Here we present a general model describing the additive action of solutes on the properties of membranes or micelles. The onset and completion of membrane solubilization induced by two surfactants (lauryl maltoside, with nonyl maltoside, octyl glucoside, or CHAPS, respectively) are very well described by our model on the basis of their individual partition coefficients, cmc's, and critical mole ratios  $R_{e\text{ sat}}$  and  $R_{e\text{ sol}}$  as detected by isothermal titration calorimetry. This suggests that the thermodynamic phase transition is governed by a single parameter (e.g., spontaneous curvature) in spite of the complexity of structural changes. Such surfactant mixtures show unique features such as nonlinear solubilization boundaries and concentration-dependent effective partition coefficients. Other phenomena such as membrane leakage are predicted to obey additive action if the solutes act via the same mechanism (e.g., toroidal pore formation) but deviate from the model in the case of independent, synergistic, or antagonistic action

Beni S., Budai M., Noszal B., and Grof P. (2006) Molecular interactions in imatinib-DPPC liposomes. *Eur J Pharm Sci* **27**, 205-211.

**Abstract:** Imatinib (Gleevec((R))) is a novel chemotherapeutic agent against Bcr-Abl protein tyrosine kinase, playing a crucial role in the therapy of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST). Our study aimed at designing a liposomal imatinib formulation and investigating molecular interactions between lipid and imatinib, within the liposomal membrane. Multilamellar (MLV) and small unilamellar (SUV) vesicles were prepared from alpha-l-dipalmitoyl-phosphatidylcholine (DPPC). The effect of imatinib on the DPPC membrane was studied by electron paramagnetic resonance (EPR) spectroscopy and differential scanning calorimetry (DSC), at pH 5.2 and 9.0, where imatinib is in monocationic and neutral form, respectively. Our results indicate that imatinib interacts mainly with the DPPC head groups, leading to a slight increase in the mobility of the polar headgroups in case of MLVs. Contrary to that, imatinib causes a significant decrease in the fluidity of SUVs, which can be the result of a

pH-dependent fusion/fission effect. The size distribution and morphology of liposomes were checked by dynamic light scattering and freeze-fracture electron microscopy. Our results direct attention to investigate the interactions of imatinib with artificial/biological membranes.

Binder H., Kohlstrunk B., and Heerklotz H. H. (1999) Hydration and Lyotropic Melting of Amphiphilic Molecules: A Thermodynamic Study Using Humidity Titration Calorimetry. *J Colloid Interface Sci* **220**, 235-249.

**Abstract:** The hydration of the lipid 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and of the cationic detergent dodecyltrimethylammonium bromide (DTAB) has been studied by means of isothermal titration calorimetry (ITC), gravimetry, and infrared (IR) spectroscopy. During the experiments films of the amphiphiles are perfused by an inert gas of variable relative humidity. The measurement of adsorption heats using ITC represents a new adaptation of adsorption calorimetry which has been called the humidity titration technique. This method yields the partial molar enthalpy of water upon adsorption. It is found to be endothermic with respect to the molar enthalpy of water on condensation for the water molecules which interact directly with the headgroups of POPC and DTAB. Consequently, the spontaneous hydration of the amphiphiles is entropy driven in an aqueous environment. IR spectroscopy shows that hydration is accompanied by the increase in the conformational and/or motional freedom of the amphiphilic molecules upon water binding. In particular, a lyotropic chain melting transition is induced at a certain characteristic relative humidity. This event is paralleled by the adsorption of water. The corresponding exothermic adsorption heat is consumed completely (POPC) or partially (DTAB) by the hydrocarbon chains upon melting. Differential scanning calorimetry was used as an independent method to determine transition enthalpies of the amphiphiles at a definite hydration degree. Water binding onto the headgroups is discussed in terms of hydrogen bonding and polar interactions. The adsorption isotherms yield a number of approximately 2.6 tightly bound water molecules per POPC and DTAB molecule. Copyright 1999 Academic Press.

Blume A. and Tuchtenhagen J. (1992) Thermodynamics of ion binding to phosphatidic acid bilayers. Titration calorimetry of the heat of dissociation of DMPA. *Biochemistry* **31**, 4636-4642.

**Abstract:** The heat of dissociation of the second proton of 1,2-dimyristoylphosphatidic acid (DMPA) was studied as a function of temperature using titration calorimetry. The dissociation of the second proton of DMPA was induced by addition of NaOH. From the calorimetric titration experiment, the intrinsic pK<sub>0</sub> for the dissociation reaction could be determined by applying the Gouy-Chapman theory. pK<sub>0</sub> decreases with temperature from ca. 6.2 at 11 degrees C to 5.4 at 54 degrees C. From the total heat of reaction, the dissociation enthalpy,  $\Delta H_{\text{diss}}$ , was determined by subtracting the heat of neutralization of water and the heat of dilution of NaOH. In the temperature range between 2 and 23 degrees C,  $\Delta H_{\text{diss}}$  is endothermic with an average value of ca. 2.5 kcal.mol<sup>-1</sup> and shows no clear-cut temperature dependence. In the temperature range between 23 and 52 degrees C,  $\Delta H_{\text{diss}}$  calculated after subtraction of the heat of neutralization and dilution is not the true dissociation enthalpy but includes contributions from the phase transition enthalpy,  $\Delta H_{\text{trans}}$ , as the pH jump induces a transition from the gel to the liquid-crystalline phase. The  $\Delta C_p$  for the reaction enthalpy observed in this temperature range is positive. Above 53 degrees C, the pH jump induces again only the dissociation of the second proton, and the bilayers stay in the liquid-crystalline phase. In this temperature range,  $\Delta H_{\text{diss}}$  seems to decrease with temperature. The thermodynamic data from titration calorimetry and differential scanning calorimetry as a function of pH can be combined to construct a complete enthalpy-temperature diagram of DMPA in its two ionization states.

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 amoebocyte 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.

Fan Y., Han Y. and Wang Y. (2007) Solubilization of phosphatidylcholine vesicles by hydrophobically modified poly(acrylamide)-co-(acrylic acid): effects of acrylic acid fraction and polymer concentration. *J Phys. Chem B* **111**, 10123-10129.

**Abstract:** The interaction of hydrophobically modified copolymers of acrylamide and acrylic acid, designated as PAM-C12-AA (X%) (X% indicates the percentage of acrylic acid unit and X = 5, 10, 20), with dimyristoylphosphatidylcholine (DMPC) vesicles has been studied. Complementary techniques including isothermal titration microcalorimetry (ITC), differential scanning calorimetry (DSC), turbidity measurement, calcein leakage measurement, dynamic light scattering (DLS), and transmission electron microscopy (TEM) were used to get comprehensive information. The results show that PAM-C12-AA leads to solubilization of DMPC vesicles. There is a critical concentration (C(s)) for PAM-C12-AA to induce obvious vesicle disruption. This concentration is very close to the critical aggregation concentration (CAC) for the polymer self-aggregation. The C<sub>s</sub> values are found to be similar for the three polymers. However, the disruption of DMPC vesicles induced by the polymers increases to a greater degree at higher AA fraction, owing to the increasing strength of interaction between the polymer and the lipid bilayer.

Fournier I., Barwicz J., and Tancrede P. (1998) The structuring effects of amphotericin B on pure and ergosterol- or cholesterol-containing dipalmitoylphosphatidylcholine bilayers: a differential scanning calorimetry study. *Biochim Biophys Acta* **1373**, 76-86.

**Abstract:** Amphotericin B (AmB) is the most widely used polyene antibiotic to treat systemic fungal infections which affect an increasing number of immunocompromised patients. It is generally thought that AmB forms pores within the fungi membranes by interacting with ergosterol, the main sterol of fungi. However, it also interacts with the cholesterol contained in mammalian cells, hence its toxicity. In order to have a better understanding of the interactions prevailing between AmB and sterols, differential scanning calorimetry was used to study various mixtures incorporating from 6.5 to 25 mol% of AmB in pure dipalmitoylphosphatidylcholine (DPPC) vesicles and in ergosterol- or cholesterol-containing DPPC vesicles. The sterol concentration was kept constant at 12.5 mol% with respect to the phospholipid. Our results show that three phases co-exist when AmB is dispersed in the pure phospholipid. One corresponds to the phospholipid phase alone. The two others are characterised by a broad transition at temperatures higher than the main transition temperature of the pure phospholipid, corresponding to the drug in interaction with the aliphatic chains of the lipid. The fact that the transition temperatures of these additional components are higher than that of the pure phospholipid suggests that AmB interacts strongly with the aliphatic chains of the lipid, consistent with the idea prevailing in the literature that AmB by itself may form pores in a lipid matrix. When AmB interacts with cholesterol-containing bilayers the thermograms also present three components. Upon increasing the concentration of AmB, though, an important broadening of these components is observed which is explained in terms of destabilisation of the organisation of the aliphatic chains. The situation is strikingly different if ergosterol is present in the lipid matrix. The thermograms remain unmodified as the concentration of AmB is increased and a broad transition, now involving only two components when the thermograms are decomposed, is observed. An analysis of the results shows that various interacting units, e.g. AmB+DPPC and (AmB+ergosterol)+DPPC, are present within the membrane. These units involve the phospholipid and hence contribute to its structuration. The important differences between the thermograms obtained with the ergosterol- as compared to the cholesterol-containing bilayers, in spite of the structural similarity of these two sterols, provides strong evidence for the selectivity of interaction of AmB with ergosterol as compared to cholesterol. It is thus clear that the action of AmB on cholesterol- as compared to ergosterol-containing membranes results from different mechanisms. Finally, UV-visible spectra of AmB in pure as well as sterol-containing DPPC vesicles show the presence of absorption bands that give support to the interpretation derived from the calorimetric data.

Frangopol P. T., Mihăbreve, and Ilescu D. (2001) Interactions of some local anesthetics and alcohols with membranes. *Colloids Surf B Biointerfaces* **22**, 3-22.

**Abstract:** A review of the results obtained by our group in the last decade regarding the interactions of procaine, lidocaine, dibucaine and tetracaine with membranes is presented in the context of the literature data. The action upon membranes, in first approximation monomolecular film of stearic acid spread at the air/water interface used as a membrane model, the modification of biomembrane structure and function using diffraction methods, lipid phase transition, fluidity of lipids and proteins, membrane expansion and platelet aggregation were studied. The thermodynamic knowledge of membrane-alcohol interactions improved by using highly sensitive calorimetric techniques are briefly reported. One of the main conclusions is that the physical state of a monolayer model membrane was the result of competitive interactions between film-film and film-substrate interactions. It was taken into account that local anesthetics, such as lidocaine, carbisocaine, mesocaine, showed changes in the bilayer structure, reflected in macroscopic mechanical properties. This restructuring of the lipid bilayer has a significant influence on the operation of functional subunits, e.g. ionic channels formed by gramicidin. The results support the concept of non-specific interactions of local anesthetics with lipid bilayers. The theoretical modeling of the interactions of local anesthetics is closely compared with experimental data. Our new theory of relaxation for these interactions is using a non-archimedean formalism based on a process resulting from superpositions of different component processes which take place at different scales of time.

Funari S. S., Nuscher B., Rapp G., and Beyer K. (2001) Detergent-phospholipid mixed micelles with a crystalline phospholipid core. *Proc Natl Acad Sci U S A* **98**, 8938-8943.

**Abstract:** An unusual micelle was discovered in mixtures of the nonionic detergent octaethyleneglycol-mono-n-dodecylether with disaturated phospholipids such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine or 1,2-dipalmitoyl-sn-glycero-3-phosphocholine in water. These mixtures undergo a structural transition upon cooling through the chain-melting temperatures of the respective phospholipids, resulting in the formation of mixed micelles. Structural features of the micellar particles were studied here by synchrotron x-ray scattering. The translucent micellar solutions showed characteristic wide-angle reflections that were attributed to ordered hydrocarbon chains, whereas the absence of small-angle x-ray reflections indicated that there is no long-range order in these mixtures. The presence of ordered phospholipid acyl chains was confirmed by differential scanning calorimetry and isothermal titration calorimetry. The endothermic differential scanning calorimetry signals observed in the up-scan mode were tentatively ascribed to chain melting and mixing of the components. Isothermal titration of the mixed-micellar solutions into an excess of the detergent octaethyleneglycol-mono-n-dodecylether resulted in sudden uptake of the latent heat by the gel-state phospholipids. The heat uptake per mol of phospholipid decreased with increasing detergent/phospholipid molar ratio. A simple geometric model is presented assuming that the dominating particle species in the mixtures is a discoidal phospholipid aggregate with ordered acyl chains, surrounded by a toroidal detergent hoop. The model implies that the fraction of ordered phospholipid chains decreases with increasing detergent/phospholipid molar ratio, in agreement with the calorimetric results and high-resolution NMR spectroscopy.

Garidel P., Hildebrand A., Knauf K. and Blume A. (2007) Membranolytic activity of bile salts: influence of biological membrane properties and composition. *Molecules*. **12**, 2292-2326.

**Abstract:** The two main steps of the membranolytic activity of detergents: 1) the partitioning of detergent molecules in the membrane and 2) the solubilisation of the membrane are systematically investigated. The interactions of two bile salt molecules, sodium cholate (NaC) and sodium deoxycholate (NaDC) with biological phospholipid model membranes are considered. The membranolytic activity is analysed as a function of the hydrophobicity of the bile salt, ionic strength, temperature, membrane phase properties, membrane surface charge and composition of the acyl chains of the lipids. The results are derived from calorimetric measurements (ITC, isothermal titration calorimetry). A thermodynamic model is described, taking into consideration electrostatic interactions, which is used for the calculation of the partition coefficient as well as to derive the complete thermodynamic parameters describing the interaction of detergents with biological membranes (change in enthalpy, change in free energy, change in entropy etc). The solubilisation properties are described in a so-called vesicle-to-micelle phase transition diagram. The obtained results are supplemented and confirmed by data obtained from other biophysical techniques (DSC differential scanning calorimetry, DLS dynamic light scattering, SANS small angle neutron scattering).

Gerebtzoff G., Li-Blatter X., Fischer H., Frentzel A., and Seelig A. (2004) Halogenation of drugs enhances membrane binding and permeation. *Chembiochem* **5**, 676-684.

**Abstract:** Halogenation of drugs is commonly used to enhance membrane binding and permeation. We quantify the effect of replacing a hydrogen residue by a chlorine or a trifluoromethyl residue in position C-2 of promazine, perazine, and perphenazine analogues. Moreover, we investigate the influence of the position (C-6 and C-7) of residue CF(3) in benzopyranols. The twelve drugs are characterized by surface activity measurements, which yield the cross-sectional area, the air-water partition coefficient, and the critical micelle concentration. By using the first two parameters ( $A_D$  and  $K_{aw}$ ) and the appropriate membrane packing density, the lipid-water partition coefficients, are calculated in excellent agreement with the lipid-water partition coefficients measured by means of isothermal titration calorimetry for small unilamellar vesicles of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. Replacement of a hydrogen residue by a chlorine and a trifluoromethyl residue enhances the free energy of partitioning into the lipid membrane, on average by  $\Delta G_{lw}$  approximately  $-1.3$  or  $-4.5$   $\text{kJ mol}^{-1}$ , respectively, and the permeability coefficient by a factor of approximately 2 or approximately 9, respectively. Despite exhibiting practically identical hydrophobicities, the two benzopyranol analogues differ in their permeability coefficients by almost an order of magnitude; this is due to their different cross-sectional areas at the air-water and lipid-water interfaces.

Gortzi O., Lala S., Chinou I. and Tsaknis J. (2007) Evaluation of the antimicrobial and antioxidant activities of *Origanum dictamnus* extracts before and after encapsulation in liposomes. *Molecules*. **12**, 932-945.

**Abstract:** The antioxidant and antimicrobial activity of methanol or dichloromethane extracts of *O. dictamnus*, produced from wild and organic cultivated specimens, were determined. The Rancimat and malondialdehyde (MDA) by HPLC methods were used to measure the antioxidant action, in comparison with that of the common commercial antioxidants butylated hydroxytoluene (BHT) and alpha-tocopherol. The extracts that presented high antioxidant activity were encapsulated in liposomes and their antioxidant action was again estimated using differential scanning calorimetry (DSC). Thermaloxidative decomposition of the samples (pure liposomes and encapsulated extracts) and the modification of the main transition temperature for the lipid mixture and the splitting of the calorimetric peak in the presence of the antioxidants were also studied by the DSC method. All extracts showed antioxidant and antimicrobial activities. Their action proved superior to alpha-tocopherol. The methanol extract of organic cultivated *O. dictamnus* (240 ppm) showed higher activity than butylated hydroxytoluene. After encapsulation in liposomes the antioxidant as well as antimicrobial activities proved to be higher than those of the same extracts in pure form.

Heerklotz H. and Seelig J. (2000) Correlation of membrane/water partition coefficients of detergents with the critical micelle concentration. *Biophys J* **78**, 2435-2440.

**Abstract:** The membrane/water partition coefficients,  $K$ , of 15 electrically neutral (non-charged or zwitterionic) detergents were measured with phospholipid vesicles by using isothermal titration calorimetry, and were compared to the corresponding critical micellar concentrations, cmc. The detergents measured were oligo(ethylene oxide) alkyl ethers (C(m)EO(n) with  $m = 10/n = 3, 7$  and  $m = 12/n = 3.8$ ); alkylglucosides (octyl, decyl); alkylmaltosides (octyl, decyl, dodecyl); diheptanoylphosphatidylcholine; Tritons (X-100, X-114) and CHAPS. A linear relation between the free energies of partitioning into the membrane and micelle formation was found such that  $K \cdot \text{CMC}$  approximately 1. The identity  $K \cdot \text{CMC} = 1$  was used to classify detergents with respect to their membrane disruption potency. "Strong" detergents are characterized by  $K \cdot \text{CMC} < 1$  and solubilize lipid membranes at detergent-to-lipid ratios  $X(b) < 1$  (alkylmaltosides, tritons, heptaethylene glycol alkyl ethers). "Weak" detergents are characterized by  $K \cdot \text{CMC} > 1$  and accumulate in the membrane- to detergent-to-lipid ratios  $X(b) > 1$  before the bilayer disintegrates (alkylglucosides, pentaethylene glycol dodecyl ether).

Heerklotz H. and Seelig J. (2000) Titration calorimetry of surfactant-membrane partitioning and membrane solubilization. *Biochim Biophys Acta* **1508**, 69-85.

**Abstract:** The interaction of surfactants with membranes has been difficult to monitor since most detergents are small organic molecules without spectroscopic markers. The development of high sensitivity isothermal titration calorimetry (ITC) has changed this situation distinctly. The insertion of a detergent into the bilayer membrane is generally accompanied by a consumption or release of heat which can be

measured fast and reliably with modern titration calorimeters. It is possible to determine the full set of thermodynamic parameters, i.e., the partitioning enthalpy, the partitioning isotherm, the partition coefficient, the free energy, and the entropy of transfer. The application of ITC to the following problems is described: (i) measurement of the critical micellar concentration (CMC) of pure detergent solutions; (ii) analysis of surfactant-membrane partitioning equilibria, including asymmetric insertion; and (iii) membrane-surfactant phase diagrams. Finally, the thermodynamic parameters derived for non-ionic detergents are discussed and the affinity for micelle formation is compared with membrane incorporation.

Heerklotz H. (2001) Membrane stress and permeabilization induced by asymmetric incorporation of compounds. *Biophys J* **81**, 184-195.

**Abstract:** The area balance or imbalance between the inner and outer monolayer of biological membranes is a key parameter for driving shape changes (including exo and endocytosis) and controlling the bilayer curvature stress. The asymmetric incorporation of a drug or biological agent interferes with these processes, and the subsequent stress may lead to a membrane permeation or permeabilization. A main goal of this study is to introduce new methods to characterize such phenomena using isothermal titration calorimetry. POPC unilamellar vesicles and a series of alkyl maltosides are used as model systems; the unilamellarity was checked by NMR with the shift reagent Pr(3+). The free energy, enthalpy, and entropy associated with the asymmetry stress are estimated by comparing partitioning data of uptake versus release assays. The asymmetry stress is of enthalpic nature and somewhat reduced by entropic effects. Stimulated membrane permeation occurs at a mean maltoside-to-lipid ratio of approximately 0.2, which corresponds to an apparent area asymmetry of approximately 30% and a limiting free energy of the order of 2 kJ/mol of maltoside. Membrane solubilization to coexisting micelles proceeds at mole ratios of approximately 0.73, 0.81, and 0.88 (C(12)-, C(13)-, and C(14)-maltoside, respectively). Experiments with vesicles pre-loaded with surfactant in both monolayers provide evidence that the translocation threshold is controlled by the asymmetrically incorporated surfactant, whereas the onset of solubilization depends on the total surfactant content in the membrane. Free copies of the uptake and release fitting script including instructions are available upon request to heerklotz@gmx.net.

Heerklotz H. and Seelig J. (2001) Detergent-like action of the antibiotic peptide surfactin on lipid membranes. *Biophys J* **81**, 1547-1554.

**Abstract:** Surfactin is a bacterial lipopeptide with powerful surfactant-like properties. High-sensitivity isothermal titration calorimetry was used to study the self association and membrane partitioning of surfactin. The critical micellar concentration (CMC), was 7.5  $\mu\text{M}$ , the heat of micellization was endothermic with  $\Delta H(w \rightarrow m)(\text{Su}) = +4.0$  kcal/mol, and the free energy of micellization  $\Delta G(O, w \rightarrow m)(\text{Su}) = -9.3$  kcal/mol (25 degrees C; 100 mM NaCl; 10 mM TRIS, 1 mM EDTA; pH 8.5). The specific heat capacity of micellization was deduced from temperature dependence of  $\Delta H(w \rightarrow m)(\text{Su})$  as  $\Delta C(w \rightarrow m)(P) = -250 \pm 10$  cal/(mol.K). The data can be explained by combining the hydrophobicity of the fatty acyl chain with that of the hydrophobic amino acids. The membrane partition equilibrium was studied using small (30 nm) and large (100 nm) unilamellar POPC vesicles. At 25 degrees C, the partition coefficient,  $K$ , was  $(2.2 \pm 0.2) \times 10^4 \text{ M}^{-1}$  for large vesicles leading to a free energy of  $\Delta G(O, w \rightarrow b)(\text{Su}) = -8.3$  kcal/mol. The partition enthalpy was again endothermic, with  $\Delta H(w \rightarrow b)(\text{Su}) = 9 \pm 1$  kcal/mol. The strong preference of surfactin for micelle formation over membrane insertion explains the high membrane-destabilizing activity of the peptide. For surfactin and a variety of non-ionic detergents, the surfactant-to-lipid ratio, inducing membrane solubilization,  $R(\text{sat})(b)$ , can be predicted by the simple relationship  $R(\text{sat})(b)$  approximately  $K \cdot \text{CMC}$ .

Heerklotz H. (2002) Triton promotes domain formation in lipid raft mixtures. *Biophys J* **83**, 2693-2701.

**Abstract:** Biological membranes are supposed to contain functional domains (lipid rafts) made up in particular of sphingomyelin and cholesterol, glycolipids, and certain proteins. It is often assumed that the application of the detergent Triton at 4 degrees C allows the isolation of these rafts as a detergent-resistant membrane fraction. The current study aims to clarify whether and how Triton changes the domain properties. To this end, temperature-dependent transitions in vesicles of an equimolar mixture of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, egg sphingomyelin, and cholesterol were monitored at different Triton concentrations by differential scanning calorimetry and pressure perturbation calorimetry. Transitions initiated by the addition of Triton to the lipid mixture were studied by isothermal titration calorimetry, and the structure was investigated by  $(31)\text{P-NMR}$ . The results are discussed in terms of liquid-

disordered (ld) and -ordered (lo) bilayer and micellar (mic) phases, and the typical sequence encountered with increasing Triton content or decreasing temperature is ld, ld + lo, ld + lo + mic, and lo + mic. That means that addition of Triton may create ordered domains in a homogeneous fluid membrane, which are, in turn, Triton resistant upon subsequent membrane solubilization. Hence, detergent-resistant membranes should not be assumed to resemble biological rafts in size, structure, composition, or even existence. Functional rafts may not be steady phenomena; they might form, grow, cluster or break up, shrink, and vanish according to functional requirements, regulated by rather subtle changes in the activity of membrane disordering or ordering compounds.

Heerklotz H., Szadkowska H., Anderson T., and Seelig J. (2003) The sensitivity of lipid domains to small perturbations demonstrated by the effect of Triton. *J Mol Biol* **329**, 793-799.

**Abstract:** The hypothesis of lipid rafts describes functional domains in biological membranes. It is often assumed that rafts form by spontaneous de-mixing of certain lipids and that they can be isolated as detergent-resistant membrane particles (DRMs) using the detergent Triton X-100 (TX). Here, we present a model that describes the process of domain formation in membranes in the presence and in the absence of TX. We measure the interactions between TX and an equimolar mixture of sphingomyelin (SM), cholesterol (Cho), and 1-palmitoyl-2-oleoyl-3-sn-glycero-phosphatidylcholine (POPC) (1:1:1, mol) by means of isothermal titration calorimetry. Comparison with pure POPC membranes reveals a very unfavorable interaction between TX and SM/Cho, which causes a substantial tendency to segregate these molecules into separate, DRM-like (SM-rich) and fluid (TX-rich), domains. If rafts are indeed formed by spontaneous de-mixing of PC and SM/Cho, they must be very sensitive, and perturbations caused by techniques used to study rafts could lead to misleading results. If, however, rafts are much more stable than PC-SM-Cho domains, there must be an unknown raft stabilizer. Subtle changes of such a promoter could serve to modulate raft function.

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

**Abstract:** Insight into the forces governing a system is essential for understanding its behaviour and function. Calorimetric investigations provide a wealth of information that is not, or is hardly, available by other methods. This paper reviews calorimetric approaches and assays for the study of lipid vesicles (liposomes) and biological membranes. With respect to the instrumentation, differential scanning calorimetry (DSC), pressure perturbation calorimetry (PPC), isothermal titration calorimetry (ITC) and water sorption calorimetry are considered. Applications of these techniques to lipid systems include the measurement of thermodynamic parameters and a detailed characterization of the thermotropic, barotropic, and lyotropic phase behaviour. The membrane binding or partitioning of solutes (proteins, peptides, drugs, surfactants, ions, etc) can also be quantified. Many calorimetric assays are available for studying the effect of proteins and other additives on membranes, characterizing non-ideal mixing, domain formation, stability, curvature strain, permeability, solubilization, and fusion. Studies of membrane proteins in lipid environments elucidate lipid-protein interactions in membranes. The systems are described in terms of enthalpic and entropic forces, equilibrium constants, heat capacities, partial volume changes etc, shedding light also on the stability of structures and the molecular origin and mechanism of structural changes.

Heerklotz H. H., Binder H., and Epand R. M. (1999) A "release" protocol for isothermal titration calorimetry. *Biophys J* **76**, 2606-2613.

**Abstract:** Isothermal titration calorimetry (ITC) has become a standard method for investigating the binding of ligands to receptor molecules or the partitioning of solutes between water and lipid vesicles. Accordingly, solutes are mixed with membranes (or ligands with receptors), and the subsequent heats of incorporation (or binding) are measured. In this paper we derive a general formula for modeling ITC titration heats in both binding and partitioning systems that allows for the modeling of the classic incorporation or binding protocols, as well as of new protocols assessing the release of solute from previously solute-loaded vesicles (or the dissociation of ligand/receptor complexes) upon dilution. One major advantage of a simultaneous application of the incorporation/binding and release protocols is that it allows for the determination of whether a ligand is able to access the vesicle interior within the time scale of the ITC experiment. This information cannot be obtained from a classical partitioning experiment, but it must be known to determine the partition coefficient (or binding constant and stoichiometry) and the transfer enthalpy. The approach is presented using the partitioning of the nonionic detergent C12EO7 to

palmitoyloleoylphosphatidylcholine vesicles. The release protocol could also be advantageous in the case of receptors that are more stable in the ligand-saturated rather than the ligand-depleted state.

Hendrich A. B., Stanczak K., Komorowska M., Motohashi N., Kawase M., and Michalak K. (2006) A study on the perturbation of model lipid membranes by phenoxazines. *Bioorg Med Chem* **14**, 5948-5954.

**Abstract:** The interactions of six newly synthesized phenoxazine derivatives with lipid bilayers were studied by means of calorimetry, fluorescence spectroscopic methods and electron spin resonance. Depending on their structure studied compounds decreased membrane fluidity and increased lipid order in liquid-crystalline bilayers to different degrees. These studies showed also that phenoxazine molecules are located close to the polar/apolar interface of bilayer. The results allow to conclude that phenoxazines rather weakly interact with lipid bilayers.

Hildebrand A., Beyer K., Neubert R., Garidel P., and Blume A. (2004) Solubilization of negatively charged DPPC/DPPG liposomes by bile salts. *J Colloid Interface Sci* **279**, 559-571.

**Abstract:** The interactions of the bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC) in 0.1 M NaCl (pH 7.4) with membranes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol (DPPG) and mixtures of DPPC and DPPG at molar ratios of 3:1 and 1:1 were studied by means of high-sensitivity isothermal titration calorimetry (ITC), dynamic light scattering (DLS), and differential scanning calorimetry (DSC). The partition coefficients and the transfer enthalpies for the incorporation of bile salt molecules into the phospholipid membranes were determined by ITC. The vesicle-to-micelle transition was investigated by ITC, DLS, and DSC. The phase boundaries for the saturation of the vesicles and their complete solubilization established by ITC were in general agreement with DLS data, but systematic differences could be seen due to the difference in detected physical quantities. Electrostatic repulsion effects between the negatively charged bile salt molecules and the negatively charged membrane surfaces are not limiting factors for the vesicle-to-micelle transition. The membrane packing constraints of the phospholipid molecules and the associated spontaneous curvature of the vesicles play the dominant role. DPPG vesicles are transformed by the bile salts into mixed micelles more easily or similarly compared to DPPC vesicles. The saturation of mixed DPPC/DPPG vesicles requires less bile salt, but to induce the solubilization of the liposomes, significantly higher amounts of bile salt are needed compared to the concentrations required for the solubilization of the pure phospholipid systems. The different solubilization behavior of DPPC/DPPG liposomes compared to the pure liposomes could be due to a specific "extraction" of DPPG into the mixed micelles in the coexistence region.

Hung W. C., Chen F. Y., Lee C. C., Sun Y., Lee M. T. and Huang H. W. (2008) Membrane-thinning effect of curcumin. *Biophys J* **94**, 4331-4338.

**Abstract:** Interaction of curcumin with lipid bilayers is not well understood. A recent experiment showed that curcumin significantly affected the single-channel lifetime of gramicidin in a 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer without affecting its single-channel conductance. We performed two experiments to understand this result. By isothermal titration calorimetry, we measured the partition coefficient of curcumin binding to DOPC bilayers. By x-ray lamellar diffraction, we measured the thickness change of DOPC bilayers as a function of the curcumin/lipid ratio. A nonlinear membrane-thinning effect by curcumin was discovered. The gramicidin data were qualitatively interpreted by the combination of isothermal titration calorimetry and x-ray results. We show that not only does curcumin thin the lipid bilayer, it might also weaken its elasticity moduli. The result implies that curcumin may affect the function of membrane proteins by modifying the properties of the host membrane

Ishitsuka R., Yamaji-Hasegawa A., Makino A., Hirabayashi Y., and Kobayashi T. (2004) A lipid-specific toxin reveals heterogeneity of sphingomyelin-containing membranes. *Biophys J* **86**, 296-307.

**Abstract:** Little is known about the heterogenous organization of lipids in biological membranes. Sphingomyelin (SM) is a major plasma membrane lipid that forms lipid domains together with cholesterol and glycolipids. Using SM-specific toxin, lysenin, we showed that in cultured epithelial cells the accessibility of the toxin to SM is different between apical and basolateral membranes. Apical membranes are highly enriched with glycolipids. The inhibitory role of glycolipids in the binding of lysenin to SM was confirmed by comparing the glycolipid-deficient mutant melanoma cell line with its parent cell. Model membrane experiments indicated that glycolipid altered the local density of SM so that the affinity of the lipid for lysenin was decreased. Our results indicate that lysenin recognizes the heterogenous organization

of SM in biomembranes and that the organization of SM differs between different cell types and between different membrane domains within the same cell. Isothermal titration calorimetry suggests that lysenin binding to SM is presumably the result of a SM-lysenin complex formation of specific stoichiometry, thus supporting the idea of the existence of small condensed lipid complexes consisting of just a few lipid molecules in living cells.

Johansson E., Engvall C., Arfvidsson M., Lundahl P., and Edwards K. (2005) Development and initial evaluation of PEG-stabilized bilayer disks as novel model membranes. *Biophys Chem* **113**, 183-192.

**Abstract:** We show in this study that stable dispersions dominated by flat bilayer disks may be prepared from a carefully optimized mixture of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylenegly col)-5000] [PEG-DSPE(5000)]. By varying the content of the latter component, the average diameter of the disks can be changed in the interval from about 15 to 60 nm. The disks show excellent long-term stability, and their size and structure remain unaltered in the temperature range between 25 and 37 degrees C. The utility of the disks as artificial model membranes was confirmed and compared to uni- and multilamellar liposomes in a series of drug partition studies. Data obtained by isothermal titration calorimetry and drug partition chromatography (also referred to as immobilized liposome chromatography) indicate that the bilayer disks may serve as an attractive and sometimes superior alternative to liposomes in studies aiming at the investigation of drug-membrane interactions. The disks may, in addition, hold great potential for structure/function studies of membrane-bound proteins. Furthermore, we suggest that the sterically stabilized bilayer disks may prove interesting as carriers for in vivo delivery of protein/peptide, as well as conventional amphiphilic and/or hydrophobic, drugs.

Keller S., Heerklotz H., Jahnke N., and Blume A. (2006) Thermodynamics of lipid membrane solubilization by sodium dodecyl sulfate. *Biophys J* **90**, 4509-4521.

**Abstract:** We provide a comprehensive thermodynamic description of lipid membrane dissolution by a charged detergent. To this end, we have studied the interactions between the anionic detergent sodium dodecyl sulfate (SDS) and the zwitterionic phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in dilute aqueous solution (10 mM phosphate buffer, 154 mM NaCl, pH 7.4). Thermodynamic parameters of vesicle solubilization and reconstitution, membrane partitioning, and micelle formation were assessed by right-angle light scattering and isothermal titration calorimetry. Membrane translocation and dissolution proceed very slowly at 25 degrees C but are considerably accelerated at 65 degrees C. At this temperature, a simple SDS/POPC phase diagram (comprising vesicular, coexistence, and micellar ranges) and a complete set of partition coefficients and transfer enthalpies were obtained. Electrostatic repulsion effects at the membrane surface were implemented by combining Gouy-Chapman theory with a Langmuir adsorption isotherm to account for Na<sup>+</sup> binding to membrane-incorporated DS-. This approach offered a quantitative understanding of solubilization and reconstitution processes, which were interpreted in terms of partition equilibria between and ideal mixing in all phases. More than any other property, the transbilayer flip-flop rate under given experimental conditions hence appears to dictate a detergent's suitability for thermodynamically controlled lipid membrane solubilization and reconstitution.

Keller S., Heerklotz H., and Blume A. (2006) Monitoring lipid membrane translocation of sodium dodecyl sulfate by isothermal titration calorimetry. *J Am Chem Soc* **128**, 1279-1286.

**Abstract:** We establish high-sensitivity isothermal titration calorimetry (ITC) as a fast, reliable, and versatile tool for assessing membrane translocation of charged compounds. A combination of ITC uptake and release titrations can discriminate between the two extreme cases of half-sided binding and complete transbilayer equilibration on the experimental time scale. To this end, we derive a general fit function for both assays that allows for incorporation of different membrane partitioning models. Electrostatic effects are taken into account with the aid of Gouy-Chapman theory, thus rendering uptake and release experiments amenable to the investigation of charged solutes. This is exemplified for the flip-flop of the anionic detergent sodium dodecyl sulfate (SDS) across the membranes of 100-nm-diameter unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in aqueous solution (10 mM phosphate buffer, 154 mM NaCl, pH 7.4). If repulsive electrostatic forces are accounted for adequately, SDS binding to POPC membranes can be evaluated on the basis of ideal mixing in all phases. At 25 degrees C, the intrinsic partition coefficient between the interfacial aqueous phase and the membrane amounts to  $3.5 \times 10^6$ ; however, detergent flip-flop is negligibly slow under these conditions. Raising the

temperature to 65 degrees C lowers the intrinsic partition coefficient to  $1.4 \times 10^6$  but enables rapid transbilayer distribution of the detergent and, therefore, binding to or desorption from both membrane leaflets. Thus, combining a surface partition equilibrium with simple electrostatic theory appears highly useful in monitoring transmembrane movement of ionic compounds by ITC, thereby eliminating the need for specific reporter groups.

Koch P. J., Frank J., Schuler J., Kahle C., and Bradaczek H. (1999) Thermodynamics and Structural Studies of the Interaction of Polymyxin B with Deep Rough Mutant Lipopolysaccharides. *J Colloid Interface Sci* **213**, 557-564.

**Abstract:** Deep rough mutant lipopolysaccharide (ReLPS) dissolved in aqueous solution spontaneously forms supramolecular structures which mainly consist of vesicles. Addition of Polymyxin B (PmB) to these ReLPS vesicles influence the shape of these structures as demonstrated here by electronmicroscopy and dynamic light scattering techniques. The main phase transition of the ReLPS is found at  $21.3 \pm 0.1$  degrees C for ReLPS from *Escherichia coli* and at  $24.0 \pm 0.5$  degrees C for ReLPS from *Salmonella minnesota* by differential scanning calorimetry (DSC). Using isothermal differential titration calorimetry (ITC), the thermodynamic behavior of the interaction of PmB with ReLPS vesicles has been studied. The stoichiometric ratio for the binding of PmB to ReLPS was found to lie between 0.6 and 1, as determined from ITC and monolayer experiments. No phase transition was observed for ReLPS monolayers saturated with PmB. The results indicate specific interaction of PmB with ReLPS. We propose a two-step mechanism for this interaction, which involves electrostatic attraction between charged parts of the molecules and, in the second step, hydrophobic interactions between the nonpolar parts of both compounds. Copyright 1999 Academic Press.

Lasch J. and Hildebrand A. (2002) Isothermic titration calorimetry to study CMCs of neutral surfactants and of the liposome-forming bolaamphiphile dequalinium. *J Liposome Res* **12**, 51-56.

**Abstract:** Isothermic titration calorimetry was used to measure the heat of micelle formation (molar enthalpy of transfer of surfactants monomers from water into micellar aggregates). The problems associated with the estimation of the CMC and the whole thermodynamic profile of micellization of surfactants via Gibbs-Helmholtz-Equation are discussed. CMC's of octylthioglucoside and the peculiar bolaamphiphile dequalinium which concentrates in mitochondria are measured. In contrast to earlier reports, no CMC of dequalinium could be found inspite of extensive systematic measurements.

Lehrmann R. and Seelig J. (1994) Adsorption of  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  to bilayer membranes: measurement of the adsorption enthalpy and binding constant with titration calorimetry. *Biochim Biophys Acta* **1189**, 89-95.

**Abstract:** The adsorption of  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  ions to the surface of lipid bilayer membranes was studied with high sensitivity titration calorimetry.  $\text{Ca}^{2+}$  adsorbs to mixed phosphatidylcholine/phosphatidylglycerol membranes with a reaction enthalpy of  $\Delta H$  approximately 0.1-0.2 kcal/mol.  $\text{La}^{3+}$  binds to sonified phosphatidylcholine vesicles with a reaction enthalpy of  $\Delta H$  approximately +1.8 kcal/mol. Adsorption of  $\text{La}^{3+}$  to phosphatidylcholine bilayers imparts a net positive charge to the membrane surface which makes the binding of further  $\text{La}^{3+}$  increasingly more difficult. From the decreasing amplitudes in the calorimetric titration experiment a  $\text{La}^{3+}$  adsorption constant of  $K$  approximately  $(4.1 \pm 1.1) \times 10^3 \text{ M}^{-1}$  was evaluated. Electrostatic effects were corrected for by means of the Gouy-Chapman theory. The adsorption constant of  $\text{Ca}^{2+}$  was determined previously as  $K$  approximately  $10^{20} \text{ M}^{-1}$  using the same binding model. Since the reaction enthalpies of  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  adsorption are endothermic, the adsorption of both metal ions to the membrane surface is driven by a distinct change in entropy.

Marcotte L., Barbeau J., and Lafleur M. (2005) Permeability and thermodynamics study of quaternary ammonium surfactants-phosphocholine vesicle system. *J Colloid Interface Sci* **292**, 219-227.

**Abstract:** Quaternary ammonium compounds (QACs) are recognized as membrane active agents widely used as biocides. The main purpose of this work was to investigate the influence of the QAC head group and acyl chain length on their permeability-perturbing power and on their affinity for lipidic membranes. Permeability perturbations were assessed by measuring the release of calcein entrapped inside vesicles. The affinity of QACs for bilayers was investigated by isothermal titration calorimetry (ITC). QACs bearing C(16) chain were found to be more efficient to decrease the membrane permeability than their C(12) analogues. On the other hand, the chemical nature of the ammonium head group has practically no

influence on the permeability perturbations caused by QACs bearing C(16) chains. It was difficult to assess the partitioning of the QACs between the aqueous and lipid phases since the ITC signals could also be associated to morphological changes such as vesicle aggregation. For the systems for which reliable thermodynamic parameters could be obtained, the Gibbs energy of transfer was similar to that for the micellization. The entropy variation represented the main contribution to the Gibbs energy, indicating that the insertion of QACs inside lipidic bilayers is driven by hydrophobic interactions.

Matos C., Lima J. L., Reis S., Lopes A., and Bastos M. (2004) Interaction of antiinflammatory drugs with EPC liposomes: calorimetric study in a broad concentration range. *Biophys J* **86**, 946-954.

**Abstract:** Isothermal titration calorimetry was used to characterize and quantify the partition of indomethacin and acetaminophen between the bulk aqueous phase and the membrane of egg phosphatidylcholine vesicles. Significant electrostatic effects were observed due to binding of the charged drugs to the membrane, which implied the use of the Gouy-Chapman theory to calculate the interfacial concentrations. The binding/partition phenomenon was quantified in terms of the partition coefficient ( $K_p$ ), and/or the equilibrium constant ( $K_b$ ). Mathematical expressions were developed, either to encompass the electrostatic effects in the partition model, or to numerically relate partition coefficients and binding constants. Calorimetric titrations conducted under a lipid/drug ratio >100:1 lead to a constant heat release and were used to directly calculate the enthalpy of the process,  $\Delta H$ , and indirectly,  $\Delta G$  and  $\Delta S$ . As the lipid/drug ratio decreased, the constancy of reaction enthalpy was tested in the fitting process. Under low lipid/drug ratio conditions simple partition was no longer valid and the interaction phenomenon was interpreted in terms of binding isotherms. A mathematical expression was deduced for quantification of the binding constants and the number of lipid molecules associated with one drug molecule. The broad range of concentrations used stressed the biphasic nature of the interaction under study. As the lipid/drug ratio was varied, the results showed that the interaction of both drugs does not present a unique behavior in all studied regimes: the extent of the interaction, as well as the binding stoichiometry, is affected by the lipid/drug ratio. The change in these parameters reflects the biphasic behavior of the interaction-possibly the consequence of a modification of the membrane's physical properties as it becomes saturated with the drug.

Mavromoustakos T., Zoumpoulakis P., Kyriakou I., Zoga A., Siapi E., Zervou M., Daliani I., Dimitriou D., Pitsas A., Kamoutsis C., and Laggner P. (2004) Efforts to understand the molecular basis of hypertension through drug:membrane interactions. *Curr Top Med Chem* **4**, 445-459.

**Abstract:** Biological membranes play an essential role in the drug action. They constitute the first barrier for drugs to exert their biological action. AT1 antagonists are amphiphilic molecules and are hypothesized to act on AT1 receptor through incorporation (first step) and lateral diffusion through membrane bilayers (second step). Various biophysical methods along with Molecular Modelling were applied in order to explore the plausible two step proposed mechanism of action for this class of antihypertensive drugs.

Meier M., Blatter X. L., Seelig A., and Seelig J. (2006) Interaction of verapamil with lipid membranes and P-glycoprotein: connecting thermodynamics and membrane structure with functional activity. *Biophys J* **91**, 2943-2955.

**Abstract:** Verapamil and amlodipine are calcium ion influx inhibitors of wide clinical use. They are partially charged at neutral pH and exhibit amphiphilic properties. The noncharged species can easily cross the lipid membrane. We have measured with solid-state NMR the structural changes induced by verapamil upon incorporation into phospholipid bilayers and have compared them with earlier data on amlodipine and nimodipine. Verapamil and amlodipine produce a rotation of the phosphocholine headgroup away from the membrane surface and a disordering of the fatty acid chains. We have determined the thermodynamics of verapamil partitioning into neutral and negatively charged membranes with isothermal titration calorimetry. Verapamil undergoes a pK-shift of  $\Delta pK(a) = 1.2$  units in neutral lipid membranes and the percentage of the noncharged species increases from 5% to 45%. Verapamil partitioning is increased for negatively charged membranes and the binding isotherms are strongly affected by the salt concentration. The electrostatic screening can be explained with the Gouy-Chapman theory. Using a functional phosphate assay we have measured the affinity of verapamil, amlodipine, and nimodipine for P-glycoprotein, and have calculated the free energy of drug binding from the aqueous phase to the active center of P-glycoprotein in the lipid phase. By combining the latter results with the lipid partitioning data it was

possible, for the first time, to determine the true affinity of the three drugs for the P-glycoprotein active center if the reaction takes place exclusively in the lipid matrix.

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 =  $4.2 \pm 0.5$ , 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 <sup>2</sup>H-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).

Nebel S., Ganz P., and Seelig J. (1997) Heat changes in lipid membranes under sudden osmotic stress. *Biochemistry* **36**, 2853-2859.

**Abstract:** Closed lipid vesicles act as osmometers increasing or decreasing their volume under the influence of osmotic gradients. The enthalpy changes accompanying membrane compression or expansion have not been measured yet, and first results obtained with high-sensitivity titration calorimetry are reported here. Phospholipid vesicles suspended in and in equilibrium with an electrolyte or nonelectrolyte with a defined initial concentration of  $c(i)$ , were injected into a solution with a final concentration of  $c(f)$ , and the heat changes were monitored with a titration microcalorimeter. Osmotic compression ( $\Delta c = c(f) - c(i) > 0$ ) produced an exothermic heat change with  $\Delta H$  approximately  $-500 \pm 100$  cal/mol and osmotic expansion ( $\Delta c < 0$ ) an endothermic heat change with  $\Delta H$  approximately  $1000 \pm 200$  cal/mol; both results normalized to a concentration gradient of inverted question mark  $\Delta c$  inverted question mark = 1 M NaCl. The heats of compression and expansion varied linearly with the lipid content and the size of the osmotic gradient but were independent of the vesicle size. The cubic thermal expansion coefficient  $\alpha(v)$  which equals  $(1/V)(\Delta V/\Delta T)_p$  could be derived and was found to be  $1.25 \times 10^{-3}$  and  $2.5 \times 10^{-3} \text{ K}^{-1}$  for the compressed and expanded bilayer vesicles, respectively. The entropy changes associated with compression and expansion could be estimated. Compression of the membrane led to a negative entropy change and increased the hydrocarbon chain order. Expansion of the membrane was accompanied by a positive entropy change which can be explained, in part, by more disordered hydrocarbon chains. Vesicle expansion and compression thus appear to be asymmetric as far as the thermodynamic driving force is concerned.

Ollila F. and Slotte J. P. (2002) Partitioning of Triton X-100, deoxycholate and C(10)EO(8) into bilayers composed of native and hydrogenated egg yolk sphingomyelin. *Biochim Biophys Acta* **1564**, 281-288.

**Abstract:** We have used isothermal titration calorimetry (ITC) to study the thermodynamics of Triton X-100 (T(X-100)), deoxycholate and decyl octaethylene glycol (C(10)EO(8)) penetration into bilayers composed of native (ESM) and hydrogenated egg yolk sphingomyelin (DHSM). Light scattering measurements were used to study the point of saturation ( $R(e,sat)$ ) and the onset of solubilization of membranes by the detergents. We found that DHSM bilayers at 25 degrees C were much more resistant to detergent partitioning (lower  $K$ ) and gave higher reaction enthalpies ( $\Delta H$ ) for all three detergents compared to the ESM bilayer system. Because DHSM lacks double bonds ( $\Delta(4trans)$ ) and some cis bonds as well), attractive acyl chain interactions are favored in membranes of this lipid class. The high stability and cohesion of DHSM in membranes could be a crucial functional property of this lipid as it is enriched in eye lens membranes.

Opatowski E., Lichtenberg D., and Kozlov M. M. (1997) The heat of transfer of lipid and surfactant from vesicles into micelles in mixtures of phospholipid and surfactant. *Biophys J* **73**, 1458-1467.

**Abstract:** We study the heat associated with the transformation of vesicles into micelles in mixtures of bilayer-forming phospholipids and micelle-forming surfactants. We subdivide the total heat evolution  $\Delta Q(\text{coex})$  within the range of coexistence of vesicles and micelles into three contributions related to the transition of  $dN(D)_{m-b}$  molecules of surfactant and  $dN(L)_{m-b}$  molecules of lipid from micelles to vesicles and to the extraction of  $dN(D)_{m-w}$  molecules of surfactant from micelles to the aqueous solution, so that  $\Delta Q(\text{coex}) = \Delta H(D)_{m-w} \times dN(D)_{m-w} + \Delta H(D)_{m-b} \times dN(D)_{m-b} + \Delta H(L)_{m-b} \times dN(L)_{m-b}$  where  $\Delta H(D)_{m-w}$ ,  $\Delta H(L)_{m-b}$ , and  $\Delta H(D)_{m-b}$  are the respective molar "transfer" enthalpies. We design a method for the evaluation of all three molar enthalpies, from isothermal calorimetric titrations conducted according to two different protocols of titration of lipid-surfactant mixtures. In the first protocol the mixture is titrated with an aqueous solution of pure lipid vesicles, and in the second the mixture is titrated with an aqueous solution of pure surfactant. Titration of the mixed systems by a buffer solution serves to verify the results obtained under these protocols. In addition to the values of molar enthalpies, our method yields the cmc value of the pure surfactant. We apply our method to investigating the heat evolution in mixtures of egg yolk phosphatidylcholine and the nonionic surfactant octylglucoside in a phosphate-buffered saline solution at 28 degrees C. These studies gave the following values:  $\Delta H(D)_{m-w} = -1732$  cal/mol,  $\Delta H(L)_{m-b} = -592$  cal/mol,  $\Delta H(D)_{m-b} = 645$  cal/mol, and  $\text{cmc} = 23.5$  mM. We discuss the possible physical insight of these values and the perspectives of applications of the proposed method.

Opatowski E., Kozlov M. M., and Lichtenberg D. (1997) Partitioning of octyl glucoside between octyl glucoside/phosphatidylcholine mixed aggregates and aqueous media as studied by isothermal titration calorimetry. *Biophys J* **73**, 1448-1457.

**Abstract:** Stepwise dilution of lipid-surfactant mixed micelles first results in extraction of surfactant from the mixed micelles into the aqueous medium. Subsequently mixed micelles transform into vesicles, within a range of compositions that corresponds to equilibrium coexistence between these two types of aggregates. Further dilution results in extraction of surfactant from the resultant mixed vesicles. In the present study, we have investigated the heat evolution of these processes, as they occur in mixed systems composed of egg phosphatidylcholine (PC) and the nonionic surfactant octylglucoside (OG). A combined use of isothermal titration calorimetry (ITC) and photon correlation spectroscopy (PCS), capable of monitoring phase transformations, revealed that 1) The sum of all of the studied processes (i.e., extraction of OG from mixed micelles and vesicles and the phase transformation) is isocaloric at approximately 40 degrees C throughout the whole dilution. At lower temperatures, all of the dilution steps are exothermic, whereas at higher temperatures all of them are endothermic. 2) At all temperatures, the absolute value of the heat associated with each dilution step within the range of coexistence of micelles and vesicles is almost constant and larger than in either the micellar or the vesicular range. We give an interpretation of these calorimetric data in terms of the relationship between the composition of the mixed aggregates  $R_e$  and the aqueous concentration of surfactant monomers  $D_w$ . Assuming that the main contribution to the heat evolution is due to extraction of surfactant from mixed aggregates to the aqueous solution, we deduce the relationship  $D_w(R_e)$  characterizing the system over the whole range of compositions. We find that, in accord with thermodynamic expectations,  $D_w$  is almost constant throughout the range of coexistence of mixed micelles and vesicles.

Panicker L. and Mishra K. P. (2006) Nuclear magnetic resonance and thermal studies on the interaction between salicylic acid and model membranes. *Biophys Chem* **120**, 15-23.

**Abstract:** DSC and ((1)H and (31)P) NMR measurements are used to investigate the perturbation caused by the keratolytic drug, salicylic acid (SA) on the physicochemical properties of the model membranes. Model membranes (in unilamellar vesicular (ULV) form) in the present studies are prepared with the phospholipids, dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidic acid (DPPA) and mixed lipid DPPC-DPPE (with weight ratio, 2.5:2.2). These lipids have the same acyl (dipalmitoyl) chains but differed in the headgroup. The molar ratio of the drug to lipid (lipid mixture), is in the range 0 to 0.4. The DSC and NMR results suggest that the lipid head groups have a pivotal role in controlling (i) the behavior of the membranes and (ii) their interactions with SA. In the presence of SA, the main phase transition temperature of (a) DPPE membrane decreases, (b) DPPA membrane increases and (c) DPPC and DPPC-DPPE membranes are not significantly changed. The drug increases the transition enthalpy (i.e., acyl chain order) in DPPC, DPPA and DPPC-DPPE membranes.

However, the presence of the drug in DPPC membrane formed using water (instead of buffer), shows a decrease in the transition temperature and enthalpy. In all the systems studied, the drug molecules seem to be located in the interfacial region neighboring the glycerol backbone or polar headgroup. However, in DPPC-water system, the drug seems to penetrate the acyl chain region also.

Rowe E. S., Zhang F., Leung T. W., Parr J. S., and Guy P. T. (1998) Thermodynamics of membrane partitioning for a series of n-alcohols determined by titration calorimetry: role of hydrophobic effects. *Biochemistry* **37**, 2430-2440.

**Abstract:** Recent studies have shown that the traditional paradigm relying on hydrophobic effects is not adequate to describe membrane partitioning of amphiphilic solutes. To elucidate the thermodynamics and determine the role of the hydrophobic effect in the partitioning of small amphiphilic molecules into lipid bilayers, we have used titration calorimetry to directly measure the enthalpy, partition coefficients, and heat capacity change for the partitioning of a series of n-alcohols into lipid bilayers of several lipid compositions. The incremental thermodynamic quantities have been compared with model compound data for partitioning into bulk hydrocarbon solvents. We have found that there is a large negative heat capacity change upon partitioning, indicating a major contribution from the dehydration of nonpolar solute moieties; however, these hydrophobic effects also involve changes in lipid interactions with water in the interfacial region of the bilayer. In addition, we have found that the enthalpy effects are large, indicating that the partitioning process is accompanied by significant changes in the intralipid interactions within the bilayer. Cholesterol has a large effect on partitioning thermodynamics, making both the enthalpy and entropy contributions significantly more positive, resulting in a relatively small net decrease in the negative free energy of partitioning. These results demonstrate that while hydrophobic interactions play a major role in partitioning, the process is considerably more complex than the partitioning of model compounds between water and bulk hydrocarbons, with major contributions coming from changes in the structure and thermodynamic state of the bilayer, including the interfacial region. The results are discussed in terms of both the thermodynamics of partitioning and the role of lipid properties in membrane function. Our results support a paradigm for membrane structure and function in which the thermodynamic state, which is a function of lipid composition, temperature, and dissolved solutes, is a critical membrane property.

Rozwarski D. A., Swami B. M., Brewer C. F., and Sacchettini J. C. (1998) Crystal structure of the lectin from *Dioclea grandiflora* complexed with core trimannoside of asparagine-linked carbohydrates. *J Biol Chem* **273**, 32818-32825.

**Abstract:** The seed lectin from *Dioclea grandiflora* (DGL) has recently been shown to possess high affinity for 3, 6-di-O-( $\alpha$ -D-mannopyranosyl)- $\alpha$ -D-mannopyranose, the core trimannoside of asparagine-linked carbohydrates, but lower affinity for biantennary complex carbohydrates. In the previous paper, the thermodynamics of DGL binding to deoxy analogs of the core trimannoside and to a biantennary complex carbohydrate were determined by isothermal titration microcalorimetry. The data suggest that DGL recognizes specific hydroxyl groups of the trimannoside similar to that of the jack bean lectin concanavalin A (ConA) (Gupta, D. Dam, T. K., Oscarson, S., and Brewer, C. F. (1997) *J. Biol. Chem.* **272**, 6388-6392). However, the thermodynamics of DGL binding to certain deoxy analogs and to the complex carbohydrate are different from that of ConA. In the present paper, the x-ray crystal structure of DGL complexed to the core trimannoside was determined to a resolution of 2.6 Å. The overall structure of the DGL complex is similar to the structure of the ConA-trimannoside complex (Naismith, J. H., and Field, R. A. (1996) *J. Biol. Chem.* **271**, 972-976). The location and conformation of the bound trimannoside as well as its hydrogen-bonding interactions in both complexes are nearly identical. However, differences exist in the location of two loops outside of the respective binding sites containing residues 114-125 and 222-227. The latter residues affect the location of a network of hydrogen-bonded water molecules that interact with the trisaccharide. Differences in the arrangement of ordered water molecules in the binding site and/or protein conformational differences outside of the binding site may account for the differences in the thermodynamics of binding of the two lectins to deoxy analogs of the trimannoside. Molecular modeling studies suggest how DGL discriminates against binding the biantennary complex carbohydrate relative to ConA.

Rozycka-Roszak B., Zylka R., and Sarapuk J. (2001) Micellization process--temperature influence on the counterion effect. *Z Naturforsch [C]* **56**, 154-157.

**Abstract:** The micellization process of dodecyltrimethylammonium chloride (DTAC) and bromide

(DTAB) was studied at 313 K. Nuclear magnetic resonance and calorimetric methods were used. The calorimetric titration curves permitted determination of the critical micelle concentration (CMC) and enthalpy of the micellization process ( $\Delta H_m$ ) of the compounds studied. The results obtained were compared to those obtained at 298 K. It was found that calorimetric curves obtained at 313 K for both compounds were similar to each other in contrast to 298 K. Especially a great difference in the shape of curves was observed for DTAC. NMR ( $^1H$  NMR and  $^{13}C$  NMR) spectra were taken below and above the CMC values and chemical shifts ( $\Delta$ ) analysed as a function of concentration of the compounds. Comparison of chemical shift-concentration plots with those obtained from measurements performed at lower temperature showed that chemical shifts are of very similar character in both cases for analyzed groups. However, there are some quantitative differences that indicate at smaller difference in hydration of DTAB and DTAC micelles at elevated temperature. This may be the reason of decrease of differences between micellization processes of DTAC and DTAB compounds. The smaller hydration may be, in turn, the result of diminishing differences in physicochemical properties of bromide and chloride ions with temperature.

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

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

Schote U. and Seelig J. (1998) Interaction of the neuronal marker dye FM1-43 with lipid membranes. Thermodynamics and lipid ordering. *Biochim Biophys Acta* **1415**, 135-146.

**Abstract:** The fluorescent dye FM1-43 labels nerve terminals in an activity-dependent fashion and has been found increasingly useful in exploring the exo- and endocytosis of synaptic vesicles and other cells by fluorescence methods. The dye distributes between the aqueous phase and the lipid membrane but the physical-chemical parameters characterizing the adsorption/partition equilibrium have not yet been determined. Fluorescence spectroscopy alone is not sufficient for a detailed elucidation of the adsorption mechanism since the method can be applied only in a rather narrow low-concentration window. In addition to fluorescence spectroscopy, we have therefore employed high sensitivity isothermal titration calorimetry (ITC) and deuterium magnetic resonance ( $^2H$ -NMR). ITC allows the measurement of the adsorption isotherm up to 100  $\mu$ M dye concentration whereas  $^2H$ -NMR provides information on the location of the dye with respect to the plane of the membrane. Dye adsorption/partition isotherms were measured for neutral and negatively-charged phospholipid vesicles. A non-linear dependence between the extent of adsorption and the free dye concentration was observed. Though the adsorption was mainly driven by the insertion of the non-polar part of the dye into the hydrophobic membrane interior, the adsorption equilibrium was further modulated by an electrostatic attraction/repulsion interaction of the cationic dye ( $z=+2$ ) with the membrane surface. The Gouy-Chapman theory was employed to separate electrostatic and hydrophobic effects. After correcting for electrostatic effects, the dye-membrane interaction could be described by a simple partition equilibrium ( $X_b = K_{cdye}$ ) with a partition constant of  $10^3$ - $10^4$   $M^{-1}$ , a

partition enthalpy of  $\Delta H = -2.0$  kcal/mol and a free energy of binding of  $\Delta G = -7.8$  kcal/mol. The insertion of FM1-43 into lipid membranes at room temperature is thus an entropy-driven reaction following the classical hydrophobic effect. Deuterium nuclear magnetic resonance provided insight into the structural changes of the lipid bilayer induced by the insertion of FM1-43. The dye disturbed the packing of the fatty acyl chains and decreased the fatty acyl chain order. FM1-43 also induced a conformational change in the phosphocholine headgroup. The -P-N<sup>+</sup> dipole was parallel to the membrane surface in the absence of dye and was rotated with its positive end towards the water phase upon dye insertion. The extent of rotation was, however, much smaller than that induced by other cationic molecules of similar charge, suggesting an alignment of FM1-43 such that the POPC phosphate group is sandwiched by the two quaternary FM1-43 ammonium groups. In such an arrangement the two cationic charges counteract each other in a rotation of the -P-N<sup>+</sup> dipole.

Schote U., Ganz P., Fahr A., and Seelig J. (2002) Interactions of cyclosporines with lipid membranes as studied by solid-state nuclear magnetic resonance spectroscopy and high-sensitivity titration calorimetry. *J Pharm Sci* **91**, 856-867.

**Abstract:** Cyclosporin A (CyA) interacts with lipid membranes. Binding reaction and membrane location of CyA and analogs were examined with <sup>2</sup>H-NMR, high-sensitivity isothermal titration calorimetry (ITC), and CD spectroscopy. Effects of CyA and charged analogs on the phosphocholine head group and on the membrane interior were investigated using selectively deuterated phospholipids. Incorporation of cyclosporin generated small disordering of the lipid acyl chains. Binding of CyA and neutral and positively charged analogs to lipid membranes showed endothermic heats of reaction between + 5.9 and + 11.3 kcal/mol, whereas enthalpy of binding was close to zero for the negatively charged derivative. Binding constants of cyclosporines to liposomal membranes were in the range of  $K(P) = 1650-5560 \text{ M}^{-1}$  depending on the cholesterol content. (<sup>2</sup>H)-NMR provides evidence that CyA is essentially located in the interior of the bilayer membrane. For the charged analogs an additional interaction occurs at the head group level, placing the polar groups of these CyA analogs in the vicinity of the phosphocholine dipoles. The association of CyA and its analogs is accompanied by a positive enthalpy change, which is overcompensated by positive entropy changes. Binding of CyA to lipid membranes thus follows the classical hydrophobic effect, which is in contrast to many other peptide-lipid binding reactions.

Schwarz G., Damian L. and Winterhalter M. (2007) Model-free analysis of binding at lipid membranes employing micro-calorimetric measurements. *Eur Biophys J* **36**, 571-579.

**Abstract:** Based on universal thermodynamic principles (Schwarz in *Biophys Chem* 86:119-129, 2000) it is shown how measured enthalpy changes can be utilized to determine the relevant binding isotherm as well as the variation of the molar enthalpy change. This is carried out in a novel way involving multiple titration experiments whose evaluation requires no beforehand assumptions or models whatever. An appropriate specific model mechanism may be discussed afterwards and developed in view of the given experimental results. The pertinent procedure is demonstrated using micro-calorimetric data obtained in the case of the local anesthetic dibucaine as it associates with POPC liposomes. Mutual interactions of the bound ligand molecules could be described in terms of repulsive enthalpic and entropic activity coefficients. Apparently these are induced by electrostatic forces and by the finite size of binding sites, respectively.

Seelig J. and Ganz P. (1991) Nonclassical hydrophobic effect in membrane binding equilibria. *Biochemistry* **30**, 9354-9359.

**Abstract:** The enthalpy of transfer of four different amphiphilic molecules from the aqueous phase to the lipid membrane was determined by titration calorimetry. The four molecules investigated were the potential-sensitive dye 2-(p-toluidinyl)naphthalene-6-sulfonate (TNS), the membrane conductivity inducing anion tetraphenylborate (TPB), the Ca<sup>2+</sup> channel blocker amlodipine [Bauerle, H. D., & Seelig, J. (1991) *Biochemistry* 30, 7203-7211], and the positively charged local anesthetic dibucaine. All four amphiphiles penetrate into the hydrophobic part of the membrane, and their binding constants, after correcting for electrostatic effects, range between  $600 \text{ M}^{-1}$  for dibucaine and  $60,000 \text{ M}^{-1}$  for tetraphenylborate. The corresponding changes in free energy were about -6 to -9 kcal/mol. Binding of the amphiphiles to membrane vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine was accompanied by exothermic heats of reaction for all four molecules. For TNS, TPB, and amlodipine, the enthalpies of transfer were almost identical and corresponded to  $\Delta H$  approximately -9 kcal/mol, essentially accounting for the total free energy change. Thus, the binding of these charged amphiphiles to the hydrophobic

membrane was driven by enthalpy. This is in contrast to the classical hydrophobic effect, where the transfer is considered to be entropy driven. For dibucaine, the enthalpy of transfer was smaller with  $\Delta H$  approximately -2 kcal/mol but was still about one-third of the total free energy change. All enthalpies of transfer exhibited a distinct temperature dependence with molar heat capacities  $\Delta C_p$  of -30 to -100 cal mol<sup>-1</sup> K<sup>-1</sup> for the transfer from water to the membrane.(ABSTRACT TRUNCATED AT 250 WORDS).

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

**Abstract:** High sensitivity titration calorimetry was performed for metal ions such as calcium and lanthanum and for different types of Alzheimer peptides. Ca<sup>2+</sup> adsorbs to mixed phosphatidylcholine (PC)/phosphatidylglycerol (PG) membranes with an endothermic reaction enthalpy of  $\Delta H$  approximately +0.1 kcal/mol. La<sup>3+</sup> binds to sonified PC vesicles with a reaction enthalpy of  $\Delta H$  approximately + 1.8 kcal/mol. The binding constants are of the order of 10 M<sup>-1</sup> for Ca<sup>2+</sup> and 4 x 10<sup>3</sup> M<sup>-1</sup> for La<sup>3+</sup>. The role of lipids in the random coil $\leftrightarrow$ beta-sheet equilibrium of different types of Alzheimer model peptides was investigated with circular dichroism (CD) and high sensitivity titration calorimetry. Alzheimer peptide beta AP(1-40)OH and several fragments of this peptide undergo a concentration-dependent, co-operative random coil $\leftrightarrow$ beta-sheet transition in solution which can be described by a linear association model with a nucleation parameter sigma approximately 0.2-0.01 and a growth parameter s approximately 10<sup>4</sup> M<sup>-1</sup>. Addition of sonified lipid vesicles containing negatively charged lipids shifts the equilibrium towards the beta-sheet conformation. This can be explained by an aggregation phenomenon at the lipid/water interphase. The cationic peptides are attracted to the negatively charged membrane surface causing a local increase in peptide concentration. The high peptide concentration, together with the ordering of the peptide molecules on the membrane surface, facilitates beta-sheet formation, constituting the first experimental evidence for the induction of beta-sheet formation via the membrane surface. The binding of Alzheimer peptide fragments to the lipid membrane is accompanied by an exothermic heat of reaction with  $\Delta H$  in the range -2 - -8 kcal/mol.

Shimada H., Grutzner J. B., Kozlowski J. F., and McLaughlin J. L. (1998) Membrane conformations and their relation to cytotoxicity of asimicin and its analogues. *Biochemistry* **37**, 854-866.

**Abstract:** Certain plant species belonging to the family Annonaceae produce Annonaceous acetogenins, which are a unique class of long-chain fatty acid derivatives with potent cytotoxicity. Putative protein targets of the acetogenins are membrane-associated proteins, including complex I. Asimicin and its analogues constitute a class of Annonaceous acetogenins containing two tetrahydrofuran (THF) rings with hydrocarbon chains tethered to each ring; an alpha,beta-unsaturated gamma-lactone ring is terminal to one of the alkyl chains. The compounds examined in this study differ in the length of the alkyl chain between the THF rings and the lactone ring. The positions of both the THF and the lactone rings within liposomal membranes were determined by proton (1H) nuclear magnetic resonance spectroscopy. The depth of membrane penetration of acetogenins, coupled to membrane diffusion, controls the conformation of acetogenins as they diffuse to an active site. Based on 1H intermolecular nuclear Overhauser effects (NOEs), the THF rings of all acetogenins studied reside near the polar interfacial head group region of the DMPC. This was corroborated by 1H two-dimensional NOE spectroscopy and differential scanning calorimetry studies. The 1H difference NOE spectra indicated that the lactone rings of asimicin and parviflorin, the latter of which has two fewer carbons in its alkyl chain, are located below the glycerol backbone in the membrane. In contrast with asimicin and parviflorin, the lactone ring of longimicin B, an asimicin analogue with an alkyl chain four carbons shorter, resides close to the midplane in the membrane. This was corroborated by manganese-induced broadening studies. Since the THF rings are located near the center of the acetogenin molecules and the lactone ring is terminal to a long alkyl chain, these observations indicate that an asimicin-type acetogenin can be in either sickle-shaped or U-shaped conformations, depending on the length of the alkyl chain between the THF rings and the lactone ring. Interestingly, longimicin B does not exhibit significant cytotoxicity, but parviflorin is as cytotoxic as asimicin. The cytotoxicity of the asimicin-type of acetogenins would seem to be strongly related to the membrane conformation. This is the first report elucidating the conformation of Annonaceous acetogenins in membranes.

Simon S. A., Disalvo E. A., Gawrisch K., Borovyagin V., Toone E., Schiffman S. S., Needham D., and McIntosh T. J. (1994) Increased adhesion between neutral lipid bilayers: interbilayer bridges formed by tannic acid. *Biophys J* **66**, 1943-1958.

**Abstract:** Tannic acid (TA) is a naturally occurring polyphenolic compound that aggregates membranes and neutral phospholipid vesicles and precipitates many proteins. This study analyzes TA binding to lipid membranes and the ensuing aggregation. The optical density of dispersions of phosphatidylcholine (PC) vesicles increased upon the addition of TA and electron micrographs showed that TA caused the vesicles to aggregate and form stacks of tightly packed disks. Solution calorimetry showed that TA bound to PC bilayers with a molar binding enthalpy of -8.3 kcal/mol and zeta potential measurements revealed that TA imparted a small negative charge to PC vesicles. Monolayer studies showed that TA bound to PC with a dissociation constant of 1.5  $\mu$ M and reduced the dipole potential by up to 250 mV. Both the increase in optical density and decrease in dipole potential produced by TA could be reversed by the addition of polyvinylpyrrolidone, a compound that chelates TA by providing H-bond acceptor groups. NMR, micropipette aspiration, and x-ray diffraction experiments showed that TA incorporated into liquid crystalline PC membranes, increasing the area per lipid molecule and decreasing the bilayer thickness by 2 to 4%. <sup>2</sup>H-NMR quadrupole splitting measurements also showed that TA associated with a PC molecule for times much less than 10(-4) s. In gel phase bilayers, TA caused the hydrocarbon chains from apposing monolayers to fully interdigitate. X-ray diffraction measurements of both gel and liquid crystalline dispersions showed that TA, at a critical concentration of about 1 mM, reduced the fluid spacing between adjacent bilayers by 8-10 Å. These data place severe constraints on how TA can pack between adjacent bilayers and cause vesicles to adhere. We conclude that TA promotes vesicle aggregation by reducing the fluid spacing between bilayers by the formation of transient interbilayer bridges by inserting its digallic acid residues into the interfacial regions of adjacent bilayers and spanning the interbilayer space.

Soderlund T., Lehtonen J. Y., and Kinnunen P. K. (1999) Interactions of cyclosporin A with phospholipid membranes: effect of cholesterol. *Mol Pharmacol* **55**, 32-38.

**Abstract:** Cyclosporin A (CsA) is a highly hydrophobic drug used to prevent graft rejection after organ transplantation. Interactions of CsA with phosphatidylcholine as well as with binary mixtures containing phosphatidylcholine and cholesterol were investigated by measuring the penetration of CsA into lipid monolayers at an air/water interface, by differential scanning calorimetry, and by imaging with fluorescence microscopy the effects of CsA on the lateral distribution of a fluorescent probe, 1-palmitoyl-2-(N-4-nitrobenz-2-oxa-1, 3-diazol)aminocaproyl-phosphocholine, in monolayers. Film penetration studies revealed the association of CsA with lipids to be a biphasic process. Cholesterol diminished the intercalation of CsA into the monolayer at surface pressures of >19 mN/m. CsA broadened the main transition of dimyristoylphosphatidylcholine (DMPC)/beta-cholesterol (10:1, mol/mol) multilamellar vesicles. The behavior of the transition enthalpy was more complex; the behavior of DMPC/beta-cholesterol multilamellar vesicles in the XCsA of 0 to 0.1 showed at most ratios a increase, but several well distinct dips were observed. The results are interpreted in terms of regular structures in tertiary alloy. Influence of CsA on lateral organization could be verified for lipid domains observed by fluorescence microscopy of lipid monolayers. More specifically, CsA altered the distribution of 1-palmitoyl-2-(N-4-nitrobenz-2-oxa-1, 3-diazol)aminocaproyl-phosphocholine in a dipalmitoylphosphatidylcholine film and in DPPC/beta-cholesterol (88:10, mol/mol) mixtures in a manner that suggests that CsA partitions into the boundaries between fluid and gel domains. To our knowledge, this constitutes the first demonstration of a change in lipid domain morphology to be induced by a drug molecule.

Soderlund T., Jutila A., and Kinnunen P. K. (1999) Binding of adriamycin to liposomes as a probe for membrane lateral organization. *Biophys J* **76**, 896-907.

**Abstract:** A stopped-flow spectrofluorometer equipped with a rapid scanning emission monochromator was utilized to monitor the binding of adriamycin to phospholipid liposomes. The latter process is evident as a decrease in fluorescence emission from a trace amount of a pyrene-labeled phospholipid analog (PPDPG, 1-palmitoyl-2-[(6-pyren-1-yl)]decanoyl-sn-glycero-3-phospho-rac-++ +glycerol) used as a donor for resonance energy transfer to adriamycin. For zwitterionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposomes, fluorescence decay was slow, with a half-time  $t_{1/2}$  of approximately 2 s. When the mole fraction of the acidic phospholipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-rac-glycerol (POPG), was increased to XPG  $\geq$  0.04, the decay of fluorescence became double exponential, and an additional, significantly faster process with  $t_{1/2}$  in the range between 2 and 4 ms was observed.

Subsequently, as XPG was increased further, the amplitude of the fast process increased, whereas the slower process was attenuated, its  $t_{1/2}$  increasing to 20 s. Increasing [NaCl] above 50 mM or [CaCl<sub>2</sub>] above 150  $\mu$ M abolished the fast component, thus confirming this interaction to be electrostatic. The critical dependence of the fast component on XPG allows the use of this process to probe the organization of acidic phospholipids in liposomes. This was demonstrated with 1, 2-palmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes incorporating PPDPG (XPPDPG = 0.03), i.e., conditions where XPG in fluid bilayers is below the required threshold yielding the fast component. In keeping with the presence of clusters of PPDPG, the fast component was observed for gel-state liposomes. At approximately 34 degreesC (i.e., 6 degrees below  $T_m$ ), the slower fluorescence decay also appeared, and it was seen throughout the main phase transition region as well as in the liquid-crystalline state. The fluorescence decay behavior at temperatures below, above, and at the main phase transition temperature is interpreted in terms of thermal density fluctuations and an intermediate state between gel and liquid-crystalline states being involved in the phospholipid main phase transition. This is the first observation of a cluster constituted by acidic phospholipids controlling the membrane association of a drug.

Sou K., Endo T., Takeoka S., and Tsuchida E. (2000) Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly(ethylene glycol)-lipid into the vesicles. *Bioconjug Chem* **11**, 372-379.

**Abstract:** The critical micelle concentrations of 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[monomethoxy poly(ethylene glycol) (5000)] (PEG-DPPE) and its distearoyl analogue (PEG-DSPE) were 70 and 9  $\mu$ M, respectively, in buffer solutions ([Tris] = 20 mM, [NaCl] = 140 mM, pH 7.4) at 37 degrees C. When these PEG-lipid micelle dispersions were mixed with the dispersions of phospholipid vesicles comprised of a C16 membrane, of which the carbon number is 16, or a C18 membrane, the PEG-lipid micelles were dissociated into monomers and then spontaneously incorporated into the surface of the preformed vesicles. The incorporation rates and the enthalpy changes during incorporation were measured with an isothermal titration microcalorimeter. The incorporation rate of PEG-DPPE was faster than that of PEG-DSPE, because the dissociation rate of the PEG-DPPE micelles was faster than that of PEG-DSPE micelles. The incorporation equilibrium constant of PEG-DSPE was larger than that of PEG-DPPE due to its slow dissociation rate from the membrane, caused by the stronger hydrophobic interaction. The combination of PEG-DSPE and the C18 membrane was the most thermodynamically stabilized pair. Furthermore, the dispersion stability of the surface-modified vesicles prepared by this spontaneous incorporation was analyzed by using the critical molecular weight of the polymer for the aggregation of vesicles. The aggregation of the vesicles was successfully suppressed with an increase in the molecular weight of the PEG in the PEG-lipid and its incorporation ratio.

Srimal S., Surolia N., Balasubramanian S., and Surolia A. (1996) Titration calorimetric studies to elucidate the specificity of the interactions of polymyxin B with lipopolysaccharides and lipid A. *Biochem J* **315** ( Pt 2), 679-686.

**Abstract:** Lipopolysaccharide (LPS), the major cell wall constituent of Gram-negative bacteria, evokes a multitude of biological effects in mammals including pyrogenicity and toxic shock syndrome. Polymyxin B (PmB), a polycationic cyclic peptide, is known to neutralize most of its activities. The nature of the interaction of PmB with LPS and lipid A was investigated by isothermal titration calorimetry. PmB binds to LPS as well as lipid A stoichiometrically and non-co-operatively with  $\mu$ molar affinity. These interactions are driven primarily by a favourable change in entropy ( $\Delta S$ ) and are endothermic in nature. These positive changes in enthalpies decrease with increasing temperature, yielding a heat capacity change,  $\Delta C_p$ , of -2385 J.mol<sup>-1</sup>.degree<sup>-1</sup> for PmB-LPS interactions while the binding of PmB to lipid A displays a  $\Delta C_p$  of -2259 J.mol<sup>-1</sup>.degree<sup>-1</sup>. The negative heat capacity changes provide strong evidence for the role of hydrophobic interactions as the driving force for the association of PmB with LPS and lipid A. A correlation of the energetics of these interactions with analyses of the molecular models of PmB suggests that a cluster of solvent-exposed non-polar amino acid side-chains that line one surface of the molecule, together with a ring of positively charged residues on its other surface, are responsible for its strong and stoichiometric binding to LPS.

Tan A., Ziegler A., Steinbauer B., and Seelig J. (2002) Thermodynamics of sodium dodecyl sulfate partitioning into lipid membranes. *Biophys J* **83**, 1547-1556.

**Abstract:** The partition equilibria of sodium dodecyl sulfate (SDS) and lithium dodecyl sulfate between water and bilayer membranes were investigated with isothermal titration calorimetry and spectroscopic methods (light scattering,  $^{31}\text{P}$ -nuclear magnetic resonance) in the temperature range of 28 degrees C to 56 degrees C. The partitioning of the dodecyl sulfate anion ( $\text{DS}(-)$ ) into the bilayer membrane is energetically favored by an exothermic partition enthalpy of  $\Delta H(\text{O})(\text{D}) = -6.0 \text{ kcal/mol}$  at 28 degrees C. This is in contrast to nonionic detergents where  $\Delta H(\text{O})(\text{D})$  is usually positive. The partition enthalpy decreases linearly with increasing temperature and the molar heat capacity is  $\Delta C(\text{O})(\text{P}) = -50 \pm 3 \text{ cal mol}^{-1} \text{ K}^{-1}$ . The partition isotherm is nonlinear if the bound detergent is plotted versus the free detergent concentration in bulk solution. This is caused by the electrostatic repulsion between the  $\text{DS}(-)$  ions inserted into the membrane and those free in solution near the membrane surface. The surface concentration of  $\text{DS}(-)$  immediately above the plane of binding was hence calculated with the Gouy-Chapman theory, and a strictly linear relationship was obtained between the surface concentration and the extent of  $\text{DS}(-)$  partitioning. The surface partition constant  $K$  describes the chemical equilibrium in the absence of electrostatic effects. For the SDS-membrane equilibrium  $K$  was found to be  $1.2 \times 10^4 \text{ M}^{-1}$  to  $6 \times 10^4 \text{ M}^{-1}$  for the various systems and conditions investigated, very similar to data available for nonionic detergents of the same chain length. The membrane-micelle phase diagram was also studied. Complete membrane solubilization requires a ratio of 2.2 mol SDS bound per mole of total lipid at 56 degrees C. The corresponding equilibrium concentration of SDS free in solution is  $C(\text{sat})(\text{D},\text{F})$  approximately 1.7 mM and is slightly below the critical micelles concentration ( $\text{CMC}$ ) = 2.1 mM (at 56 degrees C and 0.11 M buffer). Membrane saturation occurs at approximately 0.3 mol SDS per mol lipid and the equilibrium SDS concentration is  $C(\text{sat})(\text{D},\text{F})$  approximately equal 2.2 mM  $\pm$  0.6 mM. SDS translocation across the bilayer is slow at ambient temperature but increases at high temperatures.

Thomas P. G. and Seelig J. (1993) Binding of the calcium antagonist flunarizine to phosphatidylcholine bilayers: charge effects and thermodynamics. *Biochem J* **291** ( Pt 2), 397-402.

**Abstract:** We have examined the partitioning/transfer of the  $\text{Ca}^{2+}$  antagonist flunarizine from the aqueous phase into phospholipid bilayers. We show that the binding of the cationic amphiphilic drug flunarizine to phospholipid bilayers displays traditional linear concentration-dependent characteristics once unmasked of electrostatic effects. The coefficient for the binding/partitioning of flunarizine to phosphatidylcholine was found to be  $28700 \text{ M}^{-1}$ , supporting the notion that this drug may be particularly membrane-active. The thermodynamics of the partitioning/transfer process have also been studied using high-sensitivity titration calorimetry. Binding was found to be predominantly enthalpy-driven with only a small entropic contribution;  $\Delta H = -22.1 \text{ kJ}\cdot\text{mol}^{-1}$  ( $-5.3 \text{ kcal}\cdot\text{mol}^{-1}$ ) at 27 degrees C. This is in conflict with established ideas of entropy-driven partitioning of drugs into phospholipid membranes as a result of the 'hydrophobic effect'. The strong enthalpic nature of binding is interpreted as being indicative of strong lipophilic interactions between the drug and the phospholipid phase.

Trandum C., Westh P., Jorgensen K., and Mouritsen O. G. (1999) Association of ethanol with lipid membranes containing cholesterol, sphingomyelin and ganglioside: a titration calorimetry study. *Biochim Biophys Acta* **1420**, 179-188.

**Abstract:** The association of ethanol at physiologically relevant concentrations with lipid bilayers of different lipid composition has been investigated by use of isothermal titration calorimetry (ITC). The liposomes examined were composed of combinations of lipids commonly found in neural cell membranes: dimyristoyl phosphatidylcholine (DMPC), ganglioside (GM(1)), sphingomyelin and cholesterol. The calorimetric results show that the interaction of ethanol with fluid lipid bilayers is endothermic and strongly dependent on the lipid composition of the liposomes. The data have been used to estimate partitioning coefficients for ethanol into the fluid lipid bilayer phase and the results are discussed in terms of the thermodynamics of partitioning. The presence of 10 mol% sphingomyelin or ganglioside in DMPC liposomes enhances the partitioning coefficient by a factor of 3. Correspondingly, cholesterol (30 mol%) reduces the partitioning coefficient by a factor of 3. This connection between lipid composition and partitioning coefficient correlates with in vivo observations. Comparison of the data with the molecular structure of the lipid molecules suggests that ethanol partitioning is highly sensitive to changes in the lipid backbone (glycerol or ceramide) while it appears much less sensitive to the nature of the head group.

Trandum C., Westh P., Jorgensen K., and Mouritsen O. G. (2000) A thermodynamic study of the effects of cholesterol on the interaction between liposomes and ethanol. *Biophys J* **78**, 2486-2492.

**Abstract:** The association of ethanol with unilamellar dimyristoyl phosphatidylcholine (DMPC) liposomes of varying cholesterol content has been investigated by isothermal titration calorimetry over a wide temperature range (8-45 degrees C). The calorimetric data show that the interaction of ethanol with the lipid membranes is endothermic and strongly dependent on the phase behavior of the mixed lipid bilayer, specifically whether the lipid bilayer is in the solid ordered (so), liquid disordered (ld), or liquid ordered (lo) phase. In the low concentration regime (<10 mol%), cholesterol enhances the affinity of ethanol for the lipid bilayer compared to pure DMPC bilayers, whereas higher levels of cholesterol (>10 mol%) reduce affinity of ethanol for the lipid bilayer. Moreover, the experimental data reveal that the affinity of ethanol for the DMPC bilayers containing small amounts of cholesterol is enhanced in the region around the main phase transition. The results suggest the existence of a close relationship between the physical structure of the lipid bilayer and the association of ethanol with the bilayer. In particular, the existence of dynamically coexisting domains of gel and fluid lipids in the transition temperature region may play an important role for association of ethanol with the lipid bilayers. Finally, the relation between cholesterol content and the affinity of ethanol for the lipid bilayer provides some support for the in vivo observation that cholesterol acts as a natural antagonist against alcohol intoxication.

Tsamaloukas A. D., Szadkowska H., Slotte P. J., and Heerklotz H. H. (2005) Interactions of cholesterol with lipid membranes and cyclodextrin characterized by calorimetry. *Biophys J* **89**, 1109-1119.

**Abstract:** Interactions of cholesterol (cho) with different lipids are commonly believed to play a key role in the formation of functional domains in membranes. We introduce a novel approach to characterize cholesterol-lipid interactions by isothermal titration calorimetry. Cho is solubilized in the aqueous phase by reversible complexation with methyl-beta-cyclodextrin (cyd). Uptake of cho into the membrane is measured upon a series of injections of lipid vesicles into a cyd/cho solution. As an independent assay, cho release from membranes is measured upon titrating lipid/cho mixed vesicles into a cyd solution. The most consistent fit to the data is obtained with a mole fraction (rather than mole ratio) partition coefficient and considering a cho:cyd stoichiometry of 1:2. The results are discussed in terms of contributions from (i) the transfer of cho from cyd into a hypothetical, ideally mixed membrane and (ii) from non-ideal interactions with POPC. The latter are exothermic but opposed by a strong loss in entropy, in agreement with cho-induced acyl chain ordering and membrane condensation. They are accompanied by a positive heat capacity change which cannot be interpreted in terms of the hydrophobic effect, suggesting that additive-induced chain ordering itself increases the heat capacity. The new assays have a great potential for a better understanding of sterol-lipid interactions and yield suggestions how to optimize cho extraction from membranes.

Tsamaloukas A. D., Keller S. and Heerklotz H. (2007) Uptake and release protocol for assessing membrane binding and permeation by way of isothermal titration calorimetry. *Nat. Protoc.* **2**, 695-704.

**Abstract:** The activity of many biomolecules and drugs crucially depends on whether they bind to biological membranes and whether they translocate to the opposite lipid leaflet and trans aqueous compartment. A general strategy to measure membrane binding and permeation is the uptake and release assay, which compares two apparent equilibrium situations established either by the addition or by the extraction of the solute of interest. Only solutes that permeate the membrane sufficiently fast do not show any dependence on the history of sample preparation. This strategy can be pursued for virtually all membrane-binding solutes, using any method suitable for detecting binding. Here, we present in detail one example that is particularly well developed, namely the nonspecific membrane partitioning and flip-flop of small, nonionic solutes as characterized by isothermal titration calorimetry. A complete set of experiments, including all sample preparation procedures, can typically be accomplished within 2 days. Analogous protocols for studying charged solutes, virtually water-insoluble, hydrophobic compounds or specific ligands are also considered.

Tsogas I., Tsiourvas D., Paleos C. M., Giatrellis S., and Nounesis G. (2005) Interaction of L-arginine with dihexadecylphosphate unilamellar liposomes: the effect of the lipid phase organization. *Chem Phys Lipids* **134**, 59-68.

**Abstract:** The interaction of L-arginine with unilamellar liposomes of dihexadecylphosphate sodium salt (DHP-Na) has been investigated using calorimetric, light scattering, fluorescence spectroscopy and zeta-potential techniques. Heating from room temperature, the bilayer exhibits a phase transition from a subgel

(L(c)) to the gel (L(beta')) phase as well as a pre-transition (L(beta')-P(beta')), which is followed by the main lipid phase transition (P(beta')-L(alpha)). Direct studies of the interaction of L-arginine with the DHP-Na bilayers via isothermal titration calorimetry at 27 degrees C depict significant differences between samples in the L(c) and the L(beta') phases reflecting the effect of molecular organization of the lipids upon the interaction. While L-arginine has only a small impact upon the L(c) to L(beta') phase transition, it affects more significantly the transition temperature as well as the shape of the DSC peaks of the main lipid phase transition. Based on fluorescence and zeta-potential studies, the permeability of L-arginine through the liposomal membrane is higher within the temperature range of the main lipid phase transition. Encapsulated l-arginine obstructs the formation of the subgel phase.

Wenk M. R., Fahr A., Reszka R., and Seelig J. (1996) Paclitaxel partitioning into lipid bilayers. *J Pharm Sci* **85**, 228-231.

**Abstract:** Paclitaxel (taxol) is diterpenoid anticancer drug with a new mechanism of cytostatic action. It is under investigation in clinical trials for treatment of various types of human cancer. A major difficulty in developing paclitaxel as a chemotherapeutic agent is its poor water solubility. In order to improve the bioavailability of paclitaxel, novel vehicle systems such as mixed micelles or liposome-based formulations are being developed. In this study we determined the partition coefficient of paclitaxel partitioning into small unilamellar lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine using two different methods, namely high-sensitivity titration calorimetry and fluorescence spectrometry. We measured a partition coefficient of  $K_p$  approximately equal to  $9,500 \text{ M}^{-1}$ , a partition enthalpy of  $\Delta H = -25 \pm 3 \text{ kcal mol}^{-1}$  and a free energy of binding of  $\Delta G = -7.9 \text{ kcal mol}^{-1}$ . The binding reaction is enthalpy-driven, which can be explained by van der Waals interactions between the hydrophobic drug and the strong temperature dependence of the partition equilibrium. A temperature increase of 10 degrees C reduces the paclitaxel solubility in the lipid phase by a factor of 4.

Wenk M. R., Alt T., Seelig A., and Seelig J. (1997) Octyl-beta-D-glucopyranoside partitioning into lipid bilayers: thermodynamics of binding and structural changes of the bilayer. *Biophys J* **72**, 1719-1731.

**Abstract:** The interaction of the nonionic detergent octyl-beta-D-glucopyranoside (OG) with lipid bilayers was studied with high-sensitivity isothermal titration calorimetry (ITC) and solid-state  $^2\text{H-NMR}$  spectroscopy. The transfer of OG from the aqueous phase to lipid bilayers composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) can be investigated by employing detergent at concentrations below the critical micellar concentration; it can be defined by a surface partition equilibrium with a partition coefficient of  $K = 120 \pm 10 \text{ M}^{-1}$ , a molar binding enthalpy of  $\Delta H = 1.3 \pm 0.15 \text{ kcal/mol}$ , and a free energy of binding of  $\Delta G = -5.2 \text{ kcal/mol}$ . The heat of transfer is temperature dependent, with a molar heat capacity of  $\Delta C_p = -75 \text{ cal K}^{-1} \text{ mol}^{-1}$ . The large heat capacity and the near-zero  $\Delta H$  are typical for a hydrophobic binding equilibrium. The partition constant  $K$  decreased to approximately  $100 \text{ M}^{-1}$  for POPC membranes mixed with either negatively charged lipids or cholesterol, but was independent of membrane curvature. In contrast, a much larger variation was observed in the partition enthalpy.  $\Delta H$  increased by about 50% for large vesicles and by 75% for membranes containing 50 mol% cholesterol. Structural changes in the lipid bilayer were investigated with solid-state  $^2\text{H-NMR}$ . POPC was selectively deuterated at the headgroup segments and at different positions of the fatty acyl chains, and the measurement of the quadrupolar splittings provided information on the conformation and the order of the bilayer membrane. Addition of OG had almost no influence on the lipid headgroup region, even at concentrations close to bilayer disruption. In contrast, the fluctuations of fatty acyl chain segments located in the inner part of the bilayer increased strongly with increasing OG concentration. The  $^2\text{H-NMR}$  results demonstrate that the headgroup region is the most stable structural element of the lipid membrane, remaining intact until the disordering of the chains reaches a critical limit. The perturbing effect of OG is thus different from that of another nonionic detergent, octaethyleneglycol mono-n-dodecylether (C12E8), which produces a general disordering at all levels of the lipid bilayer. The OG-POPC interaction was also investigated with POPC monolayers, using a Langmuir trough. In the absence of lipid, the measurement of the Gibbs adsorption isotherm for pure OG solutions yielded an OG surface area of  $A_S = 51 \pm 3 \text{ \AA}^2$ . On the other hand, the insertion area  $A_I$  of OG in a POPC monolayer was determined by a monolayer expansion technique as  $A_I = 58 \pm 10 \text{ \AA}^2$ . The similar area requirements with  $A_S$  approximately  $A_I$  indicate an almost complete insertion of OG into the lipid monolayer. The OG

partition constant for a POPC monolayer at 32 mN/m was  $K_p$  approximately  $320 \text{ M}^{-1}$  and thus was larger than that for a POPC bilayer.

Wenk M. R. and Seelig J. (1997) Interaction of octyl-beta-thioglucopyranoside with lipid membranes. *Biophys J* **73**, 2565-2574.

**Abstract:** Octyl-beta-thioglucopyranoside (octyl thioglucoside, OTG) is a nonionic surfactant used for the purification, reconstitution, and crystallization of membrane proteins. The thermodynamic properties of the OTG-membrane partition equilibrium are not known and have been investigated here with high-sensitivity titration calorimetry. The critical concentration for inducing the bilayer  $\rightleftharpoons$  micelle transition was determined as  $cD^* = 7.3 \text{ mM}$  by 90 degree light scattering. All thermodynamic studies were performed well below this limit. Sonified, unilamellar lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with and without cholesterol were employed in the titration calorimetry experiments, and the temperature was varied between 28 degrees C and 45 degrees C. Depending on the surfactant concentration in the membrane, the partition enthalpy was found to be exothermic or endothermic, leading to unusual titration patterns. A quantitative interpretation of all titration curves was possible with the following model: 1) The partitioning of OTG into the membrane follows a simple partition law, i.e.,  $X_b = K_c(D, f)$ , where  $X_b$  denotes the molar amount of detergent bound per mole of lipid and  $c(D, f)$  is the detergent concentration in bulk solution. 2) The partition enthalpy for the transfer of OTG from the aqueous phase to the membrane depends linearly on the mole fraction,  $R$ , of detergent in the membrane. All calorimetric OTG titration curves can be characterized quantitatively by using a composition-dependent partition enthalpy of the form  $\Delta H(D, R) = -0.08 + 1.7 R$  (kcal/mol) (at 28 degrees C). At low OTG concentrations ( $R \leq 0.05$ ) the reaction enthalpy is exothermic; it becomes distinctly endothermic as more and more surfactant is incorporated into the membrane. OTG has a partition constant of  $240 \text{ M}^{-1}$  and is more hydrophobic than its oxygen-containing analog, octyl-beta-D-glucopyranoside (OG). Including a third nonionic amphiphile, octa(ethyleneoxide) dodecylether (C12EO8), an empirical relation can be established between the Gibbs energies of membrane partitioning,  $\Delta G_p$ , and micelle formation,  $\Delta G_{mic}$ , with  $\Delta G_p = 1.398 + 0.647 \Delta G_{mic}$  (kcal/mol). The partition constant of OTG is practically independent of temperature and of the cholesterol content of the membrane. In contrast, the partition enthalpy shows a strong temperature dependence. The molar specific heat capacity of the transfer of OTG from the aqueous phase to the membrane is  $\Delta C_p = -98 \text{ cal}/(\text{mol} \times \text{K})$ . The OTG partition enthalpy is also dependent on the cholesterol content of the membrane. It increases by approximately 1 kcal/mol at 50 mol% cholesterol. As the partition constant remains unchanged, the increase in enthalpy is compensated for by a corresponding increase in entropy, presumably caused by a restructuring of the membrane hydration layer.

Westh P. and Trandum C. (1999) Thermodynamics of alcohol-lipid bilayer interactions: application of a binding model. *Biochim Biophys Acta* **1421**, 261-272.

**Abstract:** Several recent reports have provided evidence that interactions of small alcohols with lipid bilayer membranes are dominated by adsorption to the membrane-water interface. This mode of interaction is better modeled by binding models than solution theories. In the present study, alcohol-membrane interactions are examined by applying the 'solvent exchange model' [J.A. Schellmann, *Biophys. Chem.* **37** (1990) 121] to calorimetric measurements. Binding constants (in mole fraction units) for small alcohols to unilamellar liposomes of dimyristoyl phosphatidylcholine were found to be close to unity, and in contrast to partitioning coefficients they decrease through the sequence ethanol, 1-propanol, 1-butanol. Thus, the direct (intrinsic) affinity of the bilayer for these alcohols is lower the longer the acyl chain. A distinction between binding and partitioning is discussed, and it is demonstrated that a high concentration of solute in the bilayer (large partitioning coefficients) can be obtained even in cases of weak binding. Other results from the model suggest that the number of binding sites on the lipid bilayer interface is 1-3 times the number of lipid molecules and that the binding is endothermic with an enthalpy change of 10-15 kJ/mol. Close to the main phase transition of the lipid bilayer the results suggest the presence of two distinct classes of binding sites: 'normal' sites similar to those observed at higher temperatures, and a lower number of high-affinity sites with binding constants larger by one or two orders of magnitude. The occurrence of high-affinity sites is discussed with respect to fluctuating gel and fluid domains in bilayer membranes close to the main phase transition.

Westh P. (2003) Unilamellar DMPC vesicles in aqueous glycerol: preferential interactions and thermochemistry. *Biophys J* **84**, 341-349.

**Abstract:** Glycerol is accumulated in response to environmental stresses in a diverse range of organisms. Understanding of favorable in vivo effects of this solute requires insight into its interactions with biological macromolecules, and one access to this information is the quantification of so-called preferential interactions in glycerol-biopolymer solutions. For model membrane systems, preferential interactions have been discussed, but not directly measured. Hence, we have applied a new differential vapor pressure equipment to quantify the isoosmotic preferential binding parameter,  $\Gamma(\mu)$ , for systems of unilamellar vesicles of DMPC in aqueous glycerol. It is found that  $\Gamma(\mu)$  decreases linearly with the glycerol concentration with a slope of  $-0.14 \pm 0.014$  per molal. This implies that glycerol is preferentially excluded from the membrane-solvent interface. Calorimetric investigations of the same systems showed that the glycerol-DMPC interactions are weakly endothermic, and the temperature of the main phase transition increases slightly ( $0.16$  degrees C per molal) with the glycerol concentration. The results are discussed with respect to a molecular picture which takes into account both the partitioning of glycerol into the membrane and the preferential exclusion from the hydration layer, and it is concluded that the latter effect contributes about four times stronger than the former to the net interaction.

Wood S. J., Miller K. A., and David S. A. (2004) Anti-endotoxin agents. 2. Pilot high-throughput screening for novel lipopolysaccharide-recognizing motifs in small molecules. *Comb Chem High Throughput Screen* **7**, 733-747.

**Abstract:** Lipopolysaccharides (LPS), otherwise termed 'endotoxins', are an integral part of the outer leaflet of the outer-membrane of Gram-negative bacteria. Lipopolysaccharides play a pivotal role in the pathogenesis of 'Septic Shock', a major cause of mortality in the critically ill patient, worldwide. The sequestration of circulatory endotoxin may be a viable therapeutic strategy for the prophylaxis and treatment of Gram-negative sepsis. We have earlier shown that the pharmacophore necessary for small molecules to bind LPS is simple, comprising of two protonatable cationic functions separated by about 15 Å, permitting the simultaneous interaction with the negatively charged phosphates on lipid A, the toxically active center of endotoxin. In this report, we employ high-throughput screening methods, using a novel fluorescent probe displacement method. Searches in three-dimensional structure databases yielded about approximately 4000 commercially available small molecules, each possessing two cationic functions spaced approximately 15 Å apart. Approximately 400 such compounds have been screened in an effort to validate the method by which high-affinity endotoxin binders can be identified. We show that the IC<sub>50</sub> values that are obtained from the fluorescence-based primary screen are correlated both to the enthalpy of binding, as measured by isothermal titration calorimetry, as well as to biological potency in vitro assays. By performing rapid toxicity screens in tandem with the bioassays, lead compounds of interest can be easily identified for further systematic structural modifications and SAR studies.

Zhang F. and Rowe E. S. (1992) Titration calorimetric and differential scanning calorimetric studies of the interactions of n-butanol with several phases of dipalmitoylphosphatidylcholine. *Biochemistry* **31**, 2005-2011.

**Abstract:** The interactions of n-butanol with dipalmitoylphosphatidylcholine (DPPC) were studied using titration calorimetry and differential scanning calorimetry (DSC). DSC results indicated that n-butanol induces the interdigitated phase in DPPC above 10 mg/mL butanol. A new application of titration calorimetry for measuring partition coefficients of nonsaturating solutes into lipids was developed. The partition coefficients and the heat of binding of n-butanol into DPPC were measured for the L beta', P beta', L alpha, and L beta I phases of DPPC. The partition coefficients were temperature dependent and ranged from 70 to 110 for the L beta I phase, from 170 to 183 for the L alpha phase, and similar to that for the L beta I phase in the P beta' phase. The binding to the L beta' phase could not be detected, giving an upper limit for this partition coefficient of 23. The enthalpies for binding to the L beta I and L alpha phases were 1.0 and 1.5 kcal/mol, respectively. The van't Hoff enthalpy was in good agreement with the calorimetric enthalpy for the partitioning into the L alpha phase; however, it was greater than the calorimetric enthalpy for the L beta I phase, suggesting that the interaction of n-butanol with this phase is cooperative in some way.