Horizons 2000– Aspects of Colloid and
Interface Science at the Turn of the Millenium

PROGRESS IN COLLOID & POLYMER SCIENCE

Editors: F. Kremer (Leipzig) and G. Lagaly (Kiel)

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The issue is dedicated to Professor Dr. Dr. h. c. Milan Schwuger on the occasion of his 60th birthday. A central topic of his scientific studies concerns the interaction of surface active agents with solid materials. Though most of the papers collected in this issue are related to this central theme, they comprise an impressing overview of the actual research in colloid science and reveal expected trends of this strongly developing field at the turn of the millennium.

G. Lagaly

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W. von Rybinski

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On the occasion of his 60th birthday Professor Schwuger is honored as a scientist with an extraordinary career, which not only includes an unusually broad field of colloid and interface science, but also both basic science and application.

Starting as a student of Physical Chemistry in 1957 his diploma thesis "Comparison of BET-surface areas for different adsorbents" led into the field of interface science. His PhD thesis continued this topic with a successful defense at the University of Aachen in 1966. In the same year he joined the chemical company Henkel in Düsseldorf, Germany. In collaboration with Professor Hermann Lange he extended his scientific work into another field of interface science with a specific focus on applied research. During this time his first remarkable papers on the structure of association colloids were published. This early scientific success resulted in him becoming group leader within Corporate Research at Henkel in 1968. His main interest was the study of the interfacial properties of association and dispersion colloids in aqueous phases based on specific application problems. This period of research led to the replacement of phosphates in detergents with zeolite A.

Based on fundamental studies of the mode of action of complexing agents Professor Schwuger proposed the replacement of phosphates not by one, but by a mixture of at least two different components. Instead of choosing a water-soluble complexing agent, the water-insoluble zeolite A in combination with a carrier fulfilled the performance profile of phosphates in detergents and became the new technical standard for builders in detergents.

From 1972 to 1977 Professor Schwuger was the leader of a project team, which would eventually establish the scientific and technical basis and the ecological evaluation of this invention. All aspects ranging from technical problems to the environmental compatibility were covered in this project. Additionally, physico-chemical studies of ion exchange, especially of heavy-metal ions, were conducted.

After the successful finalization of the project and the introduction of the first test detergent containing zeolite A, Professor Schwuger was appointed the head of Physical Chemical Research at Henkel. In recognition for his research in the replacement of phosphates in detergents he was honored by the Theodor–Steinkopff award of the German Colloid Society. Nowadays about 1.5 million tons of zeolite A and 5 million tons of detergents are produced according to the patents of Professor Schwuger. This invention is displayed in the Deutsche

Museum in Bonn, Germany, where the one hundred most important German inventions since 1945 are shown.

As head of Physical Chemical Research at Henkel from 1977 to 1989 Professor Schwuger succeeded in solving many problems of the worldwide activities of the company regarding colloid and interface science. In addition to his job at Henkel he became a lecturer at the University of Düsseldorf in 1982. In 1989 Professor Schwuger was appointed as the director of the Institute for Applied Physical Chemistry in Jülich, Germany, retaining his position as professor in Düsseldorf. Since then the physical chemistry of interfaces in the environment became his major field of research. He was able to build up a unique branch of research which deals with the physico-chemical interaction of surfactants in the aquatic and terrestrial environment, especially in multicomponent systems containing contaminants. The results regarding the kinetics and equilibrium during the adsorption of surfac-

tant-contaminant mixtures onto clay minerals and biodegradable microemulsions for soil decontamination received awards from the American Oil Chemist's Society and the company Nordac. In 1989 Professor Schwuger also became director of the Environmental Specimen Bank of Germany, which belongs to the Institute of Applied Physical Chemistry in Jülich. In 1991 Professor Schwuger was elected as chairman of the German Colloid Society. He is a Dr Honoris Causa from Clarkson University in Potsdam, New York.

During a period of more than 30 years Professor Schwuger's scientific research impact has been tremendous. He has published about 130 original scientific papers in international journals and 15 contributions to books. In addition to this, there are more than 70 patent applications which show a unique combination of basic science and application in the research of Professor Schwuger.

I have had the priviledge to work together with Professor Schwuger at

Henkel for seven years, when I joined Henkel in 1980. This time he convinced me that the study of the fundamentals in colloid and interface chemistry is of tremendous importance in many fields of applications. There was, and still is, a huge demand for further the understanding of technical processes regarding colloid and interface chemistry. Professor Schwuger is a brilliant scholar who in addition to this always had an open ear for his collegues and co-workers.

Looking at such a successful life's work, what remains for the future? Additional scientific achievements are a goal which Professor Schwuger will pursue and achieve of course, but one should not forget that there is also a private person in Professor Schwuger whose great passion is travelling, especially on the American continent. So, I would like to wish him and his wife, who has always given him strong support, enough spare time for travels.

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Lipid monolayers to understand and control properties of fluid interfaces

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Abstract Langmuir monolayers are
probably the best defined interfacial
systems since they enable variation of
many parameters independently and
since many techniques have been
established in recent years to charac-
terize them at the molecular level. In
the first part of the paper the progress
in the physical understanding of the
polymorphism is given. Ordered
mesophases are encountered, dis-
tinguished by aliphatic chain tilt,
azimuth and the freedom of chain
rotation about the long axis. The
orientation of polar molecules at the
interface in addition is responsible for
long range electrostatic interactions
causing peculiar domain shapes and
superlattices. Having largely
understood the polymorphism one

can use structural studies to
understand interactions of lipids with
adjacent molecules. Samples pre-
sented are phospholipid molecules
electrostatically interacting with
polyelectrolytes, enzymatically
attacked by phospholipases and
partly solubilized by hydrocarbons. It
is shown that these interactions
specifically affect the monolayer
structure, changing chain tilt and
partly increasing the order. In
addition it is shown that enzymatic
reactions at interfaces are varied via
structure and composition of the
monolayer.

Key words Lipid monolayers – model
membranes – interface structure

Introduction

Horizons 2000 – this must be the area in colloid and
interfacial science between what we just faintly observe
and what we may soon observe. It may also be the area
that we greatly desire to see, and there may be different
reasons for this desire: new applications and new demands,
e.g. in environmental science, biotechnology or informa-
tion technology; new principles, e.g. in general physics,
biophysics or chemistry. These different motivations are
not conflicting and one may ask: Where has been the most
progress, what can one expect from it and how will one
make use of it? Obviously answers on this will be highly
subjective, and we will try to argue from our backgrounds

in physics, chemistry and biology. In condensed matter
physics much is known about macroscopic systems ($>\mu$m)
and atoms and molecules, the "hot" areas being inter-
faces, clusters, soft matter and glasses. In chemistry the
field of supramolecular chemistry is exploding where re-
petitive synthetic routes and self-organization principles
are used to prepare functional macromolecules. In bio-
logy besides understanding the genetical code and the
interplay between functional entities there are large efforts
to resolve structure and dynamics of "small" units like
proteins. The dimensions of these systems are between
nm and μm, i.e. the colloidal domain, and it is therefore
not surprising that colloid science is part of these larger
areas and is thus per se also interdisciplinary. Interfacial
science on the other hand is also essential to understand

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colloids, because the behaviour of colloidal systems largely depends on their interfaces. Thus colloids and interfaces are merged, e.g. in most relevant journals or in names of institutes like ours. In addition it has a virtue on its own because via suitable control of interfaces interactions can be controlled and understood and also processes like wetting, adsorption and detergency can be manipulated.

This work considers a very well-defined fluid interface, a Langmuir monolayer of amphiphiles at the air/water and oil/water interface. It enables an independent variation of parameters like molecular density, pressure or ionic conditions. The systems have been known for a century [1] and are of no direct practical use. Yet, they have recently achieved much attention because many techniques had been developed in the last 15 years to resolve the film structure at the molecular level [2]. Hence, these systems can now be used as models to understand interfacial interactions. Consequently, this paper is organized as follows: following the description of experiments we will describe general features of Langmuir monolayers which when first observed were unexpected or controversial. These features result from the fact that polar molecules are partially oriented at interfaces thus leading to long range electrostatic interactions and that aliphatic tails can assume many different configurations causing a rich polymorphism. Then we will give examples of the use of Langmuir layers to understand interactions of relevance in colloid and biosciences.

Experimental

Materials

The double-chain phospholipids 1,2-dipalmitoylphosphatidylcholine (DPPC), 1,2-dipalmitoylphosphatidylethanolamine (DPPE) and 1,2-dipalmitoylphosphatidic acid (DPPA) are commercially available and were spread from a 10^{-3} M solution in chloroform onto an ultrapure water subphase. The purification of the water in a Millipore desktop filtering system leads to a specific resistance of 18.2 MΩ cm.

As an example the chemical structures of a polymer coupled lipid (DPPA) [3] are shown in Fig. 1. The polyelectrolyte poly (diallyldimethylammonium chloride) (PDADMAC) was added to the water subphase in a concentration of 10^{-3} M (referring to the molecular weight of one monomer unit) before spreading the monolayer solution. After spreading the lipid monolayer, the polymer was given about 30 min to adsorp. Longer adsorption times up to 8 h did not change the isotherm.
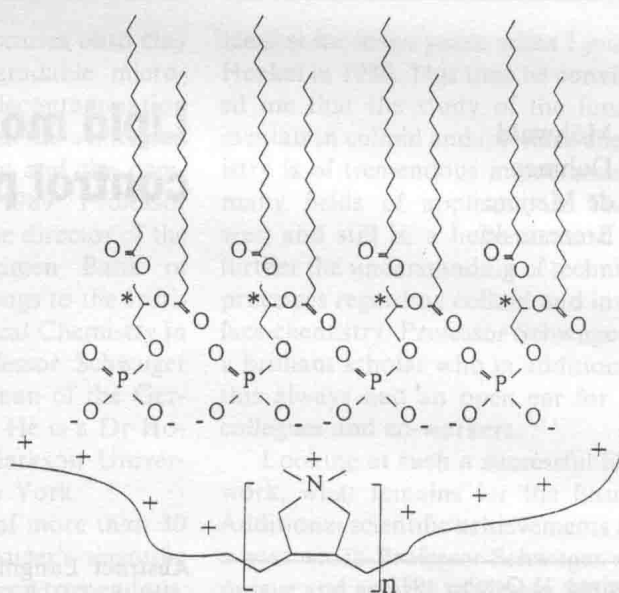
Fig. 1 Chemical structures of 1,2-dipalmitoylphosphatidic acid (*top*) and poly(diallyldimethylammonium chloride) (*bottom*). The asterisk marks the asymmetric carbon atom (chiral center)

Film balance and fluorescence microscopy

The pressure/area isotherms were measured on a thermostated film balance equiped with a Wilhelmy system to continuously record the surface pressure during the compression of the monolayer.

Fluorescence microscopic images were obtained with a Zeiss Axiotron microsocpe using a long distance objective ($d = 8$ mm). One mol% of the fluorescence dye L-α-phosphatidylcholine-β-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminohexanoyl]-γ-palmitoyl (Sigma, Taufkirchen, Germany) was added to the spreading solution. Its emission is excited by a 59-W Hg high-pressure lamp via a dichroic mirror and detected by means of a SIT camera (Hamamatsu C2400).

Grazing incidence X-ray diffraction (GIXD)

Synchrotron X-ray experiments at grazing incidence (GID) were carried out on the liquid surface diffractometer at the undulator beamline BW1 at HASYLAB, DESY (Hamburg, Germany) [4–7]. The Synchrotron beam was made monochromatic by Bragg reflection by a beryllium (0 0 2) crystal and was adjusted to strike the monolayer on the water surface at an angle of incidence $\alpha_i = 0.85\alpha_c$, where α_c is the critical angle of total external reflection. The intensity of the diffracted radiation is detected by a position sensitive detector (PSD) (OED-100-M, Braun,

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Garching, Germany). The resolution of the horizontal scattering angle $2\Theta_{hor}$ is given by a Soller collimator located in front of the PSD. The scattering vector Q has an in-plane component $Q_{xy} = (4\pi/\lambda)\sin(2\Theta_{hor}/2)$ and an out-of-plane component $Q_z = (2\pi/\lambda)\sin\alpha_f$, where λ is the X-ray wave length and α_f the vertical scattering angle. The accumulated position-resolved scans were corrected for polarization, footprint-area and powder-averaging (Lorentz factor). The intensities of the diffraction peaks were least-squares fitted to model peaks as products of a Lorentzian parallel and a Gaussian normal to the water surface. The lattice spacings are obtained from the in-plane diffraction. The lattice parameters can be calculated from the lattice spacings and the unit cell area A_{xy} is calculated from the lattice parameters.

If two diffraction maxima occur, the monolayer forms a centred rectangular lattice where the tilt azimuth can be in one of the two symmetry directions, towards the nearest neighbour (NN) or towards the next nearest neighbour (NNN). Three non-degenerate diffraction maxima at $Q_z > 0\,\text{Å}^{-1}$ are measured if the unit cell has an oblique symmetry with three different lattice spacings.

Polarization-modulated infrared reflection absorption spectroscopy (IRRAS)

The goal of the polarization modulated FTIR technique used is to obtain a difference spectrum $(R_p - R_s)/(R_p + R_s)$ with R_p and R_s, the p- and s-reflectivity. Only anisotropic absorptions contribute to a difference spectrum, isotropic absorptions (from water vapor, etc) are not detected. Our setup consists of a Bruker IFS66 (Bruker, Karlsruhe, Germany) spectrometer, and an external reflection unit. At the exit of the Michelson interferometer, the IR-beam is directed into the external reflection unit, where it passes a polarizer in p- and a photoelastic modulator (ZnSe). Then the IR-beam is focused onto the water surface with an angle of incidence of 73°. The reflected intensity is focused onto a liquid-nitrogen-cooled MCS detector. The electronical setup used (two bandpass filters and a lock-in amplifier) is designed to obtain two signals $(R_p - R_s)$ and $(R_p + R_s)$. The signals are multiplexed and connected with the standard electronics of the spectrometer. Here the resulting interferogram is amplified, digitized (16 bit AD-board) and Fourier transformed (OPUS-software).

The IR spectra were collected using 200 scans at $4\,\text{cm}^{-1}$ resolution with a scanning time of about 5 min.

General features

Figure 2 shows typical pressure/area isotherms as they are observed for phospholipids, fatty acids, fatty alcohols and
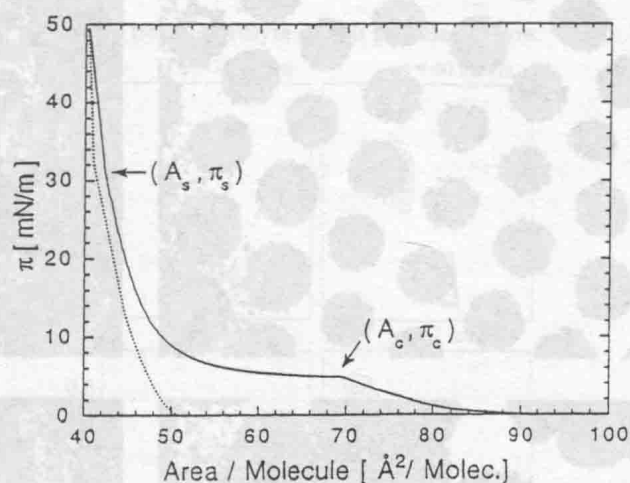
Fig. 2 Pressure–area isotherms of an 1,2-dimyristoylphosphatidyl-ethanolamine (DMPE) monolayer at $T = 20\,°C$ (*solid line*) and an 1,2-dipalmitoylphosphatidylethanolamine (DPPE) mono-layer at $T = 25\,°C$ (*dotted line*)

many other compounds. For sufficiently high temperature or small attraction one observes a so-called liquid expanded (LE) state with no order and diffusivity like in a fluid oil. On compression one enters an ordered state and the many different types of order will be discussed below. Reducing the temperature or increasing the lateral interaction one may suppress the LE phase and by compression the system can be sublimed from a gaseous to an ordered state.

There has been a long discussion as to whether the break in the slope of the isotherm corresponds to a first order phase transition and the clearest proof of this would be to observe two-phase coexistence. This has been achieved by staining the LE phase by a fluorescing dye and recording fluorescence micrographs of the monolayer upon compression. As an example Fig. 3 shows dark (ordered phase) domains in a continuous LE phase (bright) environment. These results on phase coexistence were later confirmed with techniques not relying on probes like Brewster angle microscopy or imaging ellipsometry [8–10]. In addition these showed that the ordered state is distinguished by a uniform tilt of aliphatic tails. Figure 2 also shows that there may be peculiar domain shapes and domain superlattices. These shapes are stable over observation times of hours, and this leads to a specific feature of the interfacial system. For a three-dimensional system one would expect that following a nucleation period large domains would have to grow on expense of small ones in order to minimize the boundary energy corresponding to the phases. For the interfacial system, however, the molecules carrying dipole moments are partially aligned at the interface and this leads to a long range

Fig. 3 Fluorescence micrographs of lipid monolayers in the LE LC phase coexistence region. *Upper left*: 1,2-dimyristoylphosphatidic acid (**1**) at pH 5 and in the presence of monovalent ions. *Upper right*: compound (**1**) containing 1 mol% cholesterol at pH 11. *Lower left*: compound (**1**) at pH 5, no other ions added. *Lower right*: a diacetylenic lipid in the unpolymerized state
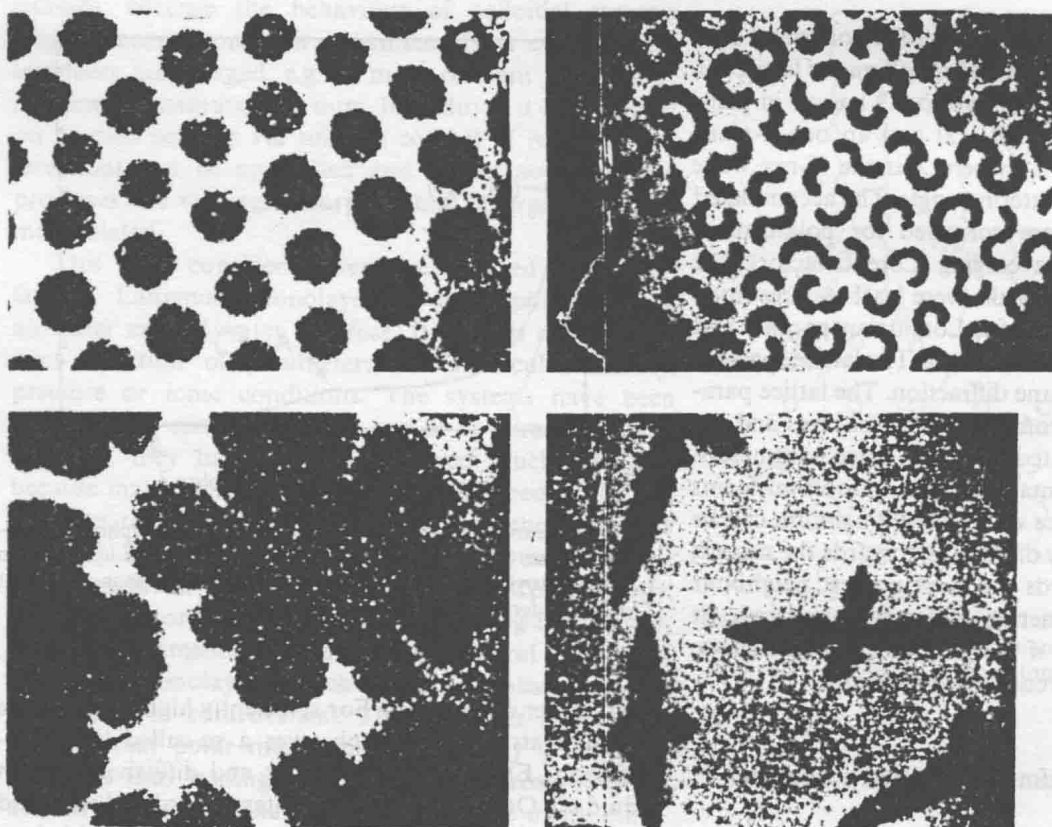
repulsion. For a two-dimensional system this energy increases with domain size faster than the boundary energy [11]. Hence there is a critical size R_c above which electrostatic repulsion dominates. This force limits the domain size and causes domain repulsion leading to a superlattice. If the boundary energy is anisotropic, its interplay with electrostatics can cause lamellar domains and if there is additionally a chiral interaction, spiral shapes can be formed. In order to quantify the ideas elegant theories have been formulated [11] and the electrostatic energy can be measured via the surface potential difference of coexisting phases [12]. Controversies exist concerning the boundary energies which may be strongly system dependent. They may vary from zero near a critical point to $\sim 10^{-4}$ mN/m [13]. Although there are undoubtedly systems where equilibrium between the two types of energies exists there are other systems where equilibrium may not be established at all. In fact it has been shown that establishment of chemical equilibrium may require days due to the very small chemical potential gradient [14].

For the last ten years it has been possible to characterize the ordered state, previously called liquid condensed (LC) by X-ray scattering studies [15, 16]. The fact that one observes Bragg diffraction reveals that there is positional order in the plane. However, often the peaks are broad indicating order only over some 10 nm, typical for a liquid crystal. Measuring the diffraction intensity along the plane normal one can also derive the tilt angle t of the aliphatic chains and the tilt direction. Generally, t decreases upon compression and from a systematic measurement and analysis one deduces a phase diagram as given in Fig. 4 for behenic acid [17, 18]. One realizes that there are at least eight different ordered phases. Two phases existing at low temperatures are crystalline and exhibit very narrow diffraction peaks, the others are mesophases with low positional order. They are distinguished by a tilt to a nearest neighbour (NN), a next nearest neighbour (NNN) or no tilt and by a free rotation or a hindered rotation about the long molecular axes. The latter modes are also found for n-alkanes [19]. This polymorphism is not unique as it is
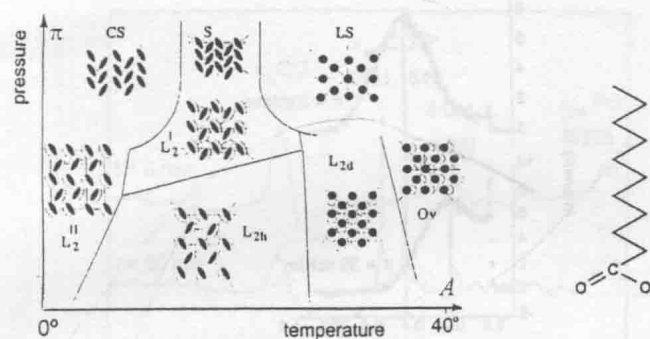
Fig. 4 Temperature-pressure phase diagram for a fatty acid mono-layer displaying hexatic phases (LS, L_{2d} and Ov), hindered rotator phases (S, L_2' and L_{2h}) and crystalline phases (CS and L_2''). The temperature axis is roughly correct for a behenic acid and would have to be shifted for chain length variations. The chemical structure of a typical fatty acid molecule is shown on the right side

also found, although at other pressures and temperatures, for amphiphiles with other chain lengths and head groups [20]. Mesophases can be suppressed for amphiphiles with strong and specific lateral interactions. Phospholipids as double-chain surfactants with an extended head group also exhibit mesophases, but crystalline monolayers have not yet been observed [21]. Probably the incommensurability of head and tail inhibits crystalline ordering. On the other hand it was possible to find head group chirality expressed in a chiral structure of the tail lattices [22, 23]. This among others indicates orientational order of the heads. This order is in addition reflected in the domain shapes. For example, the spirals are only observed for the pure enantiomer of the lipid, whereas the racemate exhibits linearly elongated domains [24].

Langmuir monolayers to model interactions

Polyelectrolyte/surfactant interactions

For various applications e.g. in separation and filtration technology stable lamellar structures existing of sheets of hydrophobic and hydrophilic regions are desired. One approach to this may be self-organized amphiphilic polymers or blockcopolymers, another approach, the biomimetic one, would be to design a lipid bilayer coupling to a polyelectrolyte [25]. In the latter case an important question concerns the influence of the polymer binding on the membrane structure. In the specific example the membrane exists of a negatively charged phospholipid, to which a positively charged polyelectrolyte is electrostatically bound. The question then arises: since binding reduces the electrostatic repulsion between the phospholipid heads does the membrane condense or does the disorder
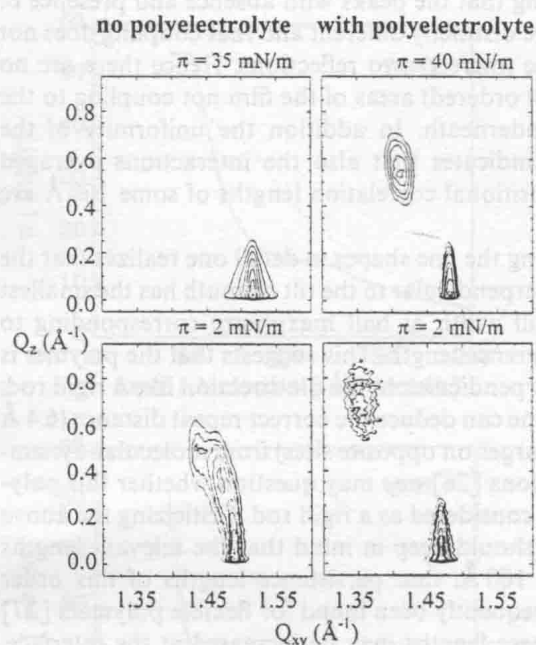

Fig. 5 Contour plots of the corrected X-ray intensities as a function of the in-plane component Q_{xy} and the out-of-plane component Q_z of the scattering vector Q of DPPA monolayers on water (left) and on a PDADMAC subphase (right) at different surface pressures (indicated)

induced by the flexible polymer dominate and lead to a lateral expansion of the membrane?

This question can be answered studying a phospholipid monolayer with and without polyelectrolyte in the subphase [3]. Figure 5 shows contour plots of X-ray diffraction intensity of a monolayer of enantiomeric dipalmitoylphosphatidic acid (DPPA) in absence (left) and presence (right) of poly(diallyldimethylammonium chloride) (PDADMAC) at low and high surface pressures. For the monolayer without polyelectrolyte coupling one observes the typical behaviour of a phospholipid with small head: at low lateral pressure there are three diffraction spots indicative of an oblique lattice and the fact that all peaks are above the horizon ($Q_z > 0 \, Å^{-1}$) proves that there is a chain tilt. On compression the peak maxima shift towards $Q_z = 0 \, Å^{-1}$, and at high pressure there is no chain tilt. All reflections degenerate indicating a hexagonal lattice. The situation is qualitatively different for the polymer coupled lipid monolayer. At all pressures the lattice remains rectangular and the chains tilted. The positions of the two reflections change only slightly upon compression and differ from those in the absence of polymer. Therefore, the unit cell is larger compared to the uncoupled system. This means that the expansion effected by the polymer dominates the reduction of the Coulombic repulsion which would lead to a shrinking of the unit cell. It is also

worth noting that the peaks with absence and presence of coupling are distinctly different and that coupling does not broaden the (merely) two reflections. Hence there are no (at least not ordered) areas of the film not coupling to the polymer underneath. In addition the uniformity of the reflections indicates that also the interactions averaged over the positional correlation lengths of some 100 Å are uniform.

Analyzing the line shapes in detail one realizes that the reflection perpendicular to the tilt azimuth has the smallest FWHM (full width at half maximum) corresponding to a large coherence length. This suggests that the polymer is oriented perpendicular to the tilt direction like a rigid rod. Although one can deduce the correct repeat distance (6.4 Å between charges on opposite sites) from molecular dynamics calculations [26] one may question whether this polymer can be considered as a rigid rod. Criticizing the above model one should keep in mind that the relevant lengths are merely 100 Å, that persistence lengths of this order have also frequently been found for flexible polymers [27] and that these lengths may be increased at the interface. Experiments systematically varying both the polymer and lipid charge density as well as polymer flexibility should help to conclude on this matter.

Enzymatic interfacial reactions

Many lipolytic enzyme reactions occur at interfaces and in one type of reaction, with much biophysical significance, phospholipids are cleaved by phospholipases. In the case considered here, the reactions with phospholipase A_2, the products are a fatty acid, presumably remaining in the membrane, and a lysolipid which is partially water soluble [28]. The reaction depends on the phase structure and the chemical structure of the phospholipid. Studies with well-defined monolayers should help to conclude on this in a precise way. The reaction is also enantio-selective, i.e. only the L-enantiomer is cleaved. However, using fluorescence microscopy one can show that the enzyme also binds to the monolayer of the D-enantiomer [29]. Hence the structural influence of the two steps, binding and reaction, can be separated.

The binding can again be studied via measurement of X-ray diffraction of a D-dipalmitoylphosphatidylcholine (D-DPPC) monolayer with phospholipase A_2 homogeneously distributed in the subphase (Fig. 6). In this case one realizes that all reflections can be shifted towards $Q_z = 0$ Å^{-1}, i.e. the tilt decreases near zero. This is surprising because for the pure DPPC monolayer the tilt angle cannot be reduced below 25°. This is generally ascribed to the large hydrated choline head group and its orientation nearly parallel to the surface [30]. Hence we conclude that
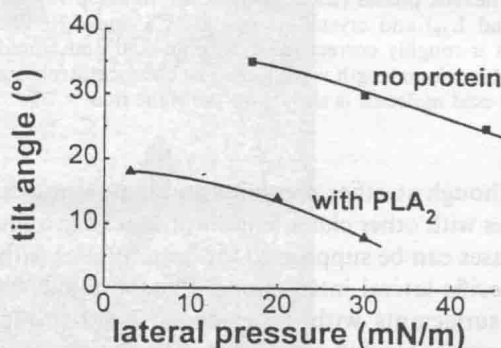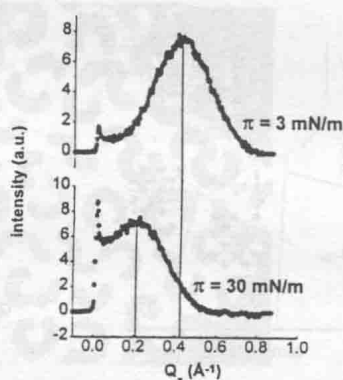

Fig. 6 *Top*: Diffraction intensity (Bragg rod) as a function of out-of-plane scattering vector component Q_z integrated over the Q_{xy} interval of the twofold degenerate peak ($\pi = 3$ mN/m: 1.435 Å$^{-1}\langle Q_{xy}\langle$ 1.465 Å^{-1}, $\pi = 30$ mN/m: 1.475 Å$^{-1}\langle Q_{xy}\langle$1.495 Å^{-1}) of a centered rectangular lattice of D-DPPC after adsorption of PLA$_2$. *Bottom*: Tilt angle versus lateral pressure for a D-DPPC monolayer on a buffer solution and after adsorption of PLA$_2$
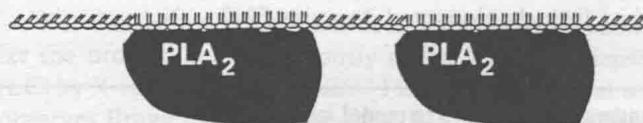
Fig. 7 Schematic diagram of the structural change induced upon PLA$_2$ adsorption

protein binding enforces a dehydration and reorientation of the head group. It is remarkable, that the protein binding does not only affect a single head which might be pulled into a binding pocket, but induces a cooperative change involving at least a hundred head groups (see Fig. 7). In fact from the line widths one can deduce the area over which positional order extends. It amounts to 180×80 Å^2 which, incidentally, is close to be projected area of the enzyme [29].

We should also note that the extent of head group change strongly depends on the head group, e.g. it is much less pronounced if an ester linkage is replaced by an ether

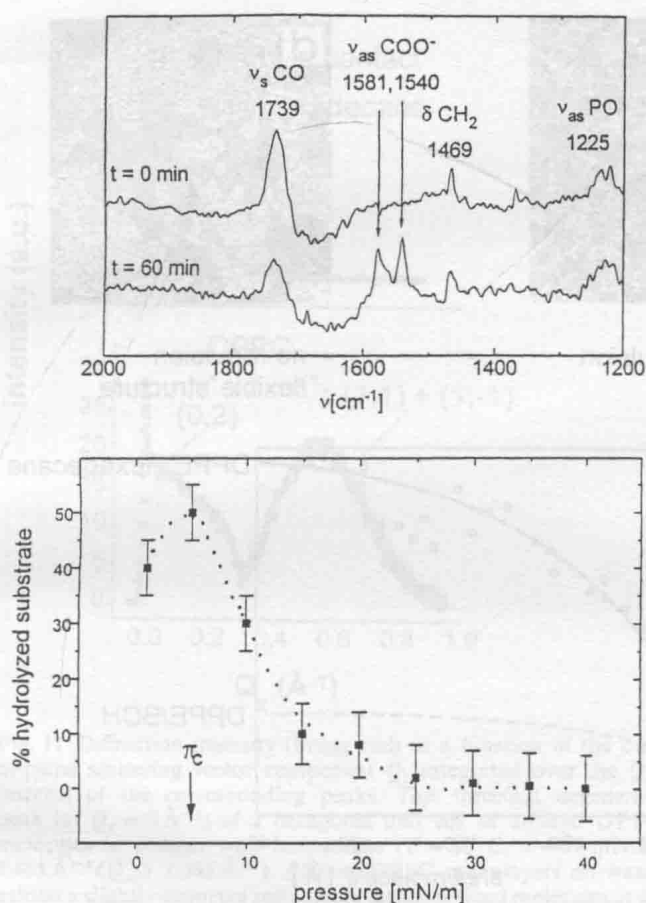Fig. 8 *Top*: Spectra of L-DPPC before (*t* = 0 min) and after 60 min of hydrolysis at 5 mN/m by PLA$_2$ (0.3 ng enzyme/ml subphase; PLA$_2$ from *Crotalus Atrox* venom); spectra measured at 40 mN/m. The asymmetric stretching vibrations of the carboxylate (fatty acid) have been assigned to free carboxylate (1540 cm^{-1}) and enzyme associated carboxylate (1581 cm^{-1}), the shoulder at 1562 cm^{-1} is due to Ca^{2+} associated carboxylate. *Bottom*: Percentage (%) hydrolyzed substrate after 60 min vs. lateral pressure applied during hydrolysis

Fig. 9 Pressure–area isotherms of DPPC (*top*) and DPPE (*bottom*) monolayers at bicyclohexyl (BCH)–water (*T* = 20 °C) (*dotted lines*) and hexadecane (C$_{16}$)–water(*T* = 25 °C) (*solid lines*) interfaces

linkage near the head. In this case the larger change correlates with larger enzyme activity. Also there were experiments where reflections of protein bound and protein free monolayers were observed simultaneously. This means that two types of areas may coexist if the monolayer is not completely covered by the enzyme.

In order to follow the enzyme activity FTIR spectroscopy has proven to be extremely valuable since product and educt are distinguished among others by vibrations in the carbonyl and the phosphate region. Because the interface causes partial molecular orientation, this technique can be interface specific by using polarization modulation and comparing the p-(in-plane polarization) and s-(out-of-plane polarization) IR reflectivities [31]. An example of polarization modulated IR-reflection spectra of
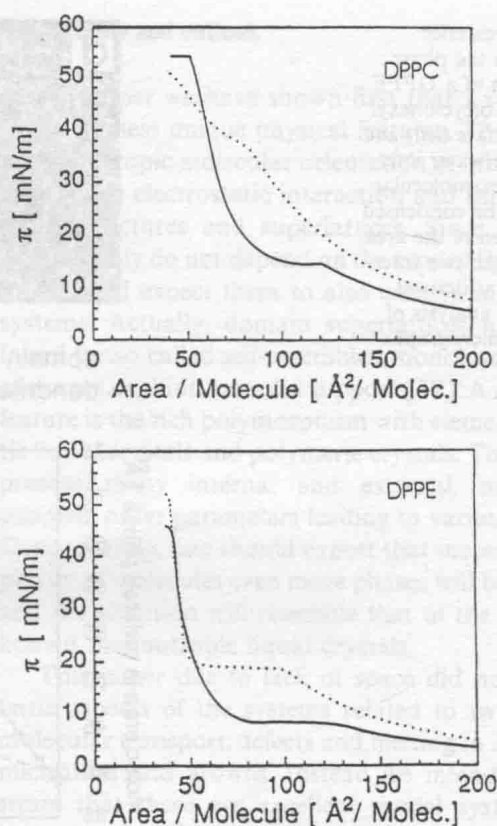
an L-DPPC monolayer before and after enzymatic reaction is given in Fig. 8. One clearly observes the appearance of peaks characteristic of fatty acid and the decrease of the ester vibrations. At this stage typical measurement times for spectra like in Fig. 8 are on the order of 5 min, and this determines the time resolution. From an integration of characteristic peaks one can determine reaction rates and it turns out that these are maximal for pressures corresponding to LE/LC phase coexistence [32]. This result agrees with fluorescence microscopic observations of maximum enzyme activity at the phase boundary [33].

Monolayers at the oil/water interface

Microemulsion stability is largely determined by the lowering of the oil/water interfacial energy by surfactant enrichment at the interface. Therefore understanding the surfactant layer at this interface is of utmost importance to control microemulsions. In this respect the planar monolayer is very promising because of its defined geometry and

Fig. 10 *Top*: fluorescence micrographs from the phase coexistence region of a DPPE monolayer at the bicyclohexyl (BCH)/water interface (*left*) and at the hexadecane/water interface (*right*). *Bottom*: molecular area per lipid in the condensed phase as a function of the area per molecule for the two interfaces from above as derived from quantitative analysis of the fluorescence micrographs

- domain repulsion
- dendrites

- no repulsion
- "flexible" structure
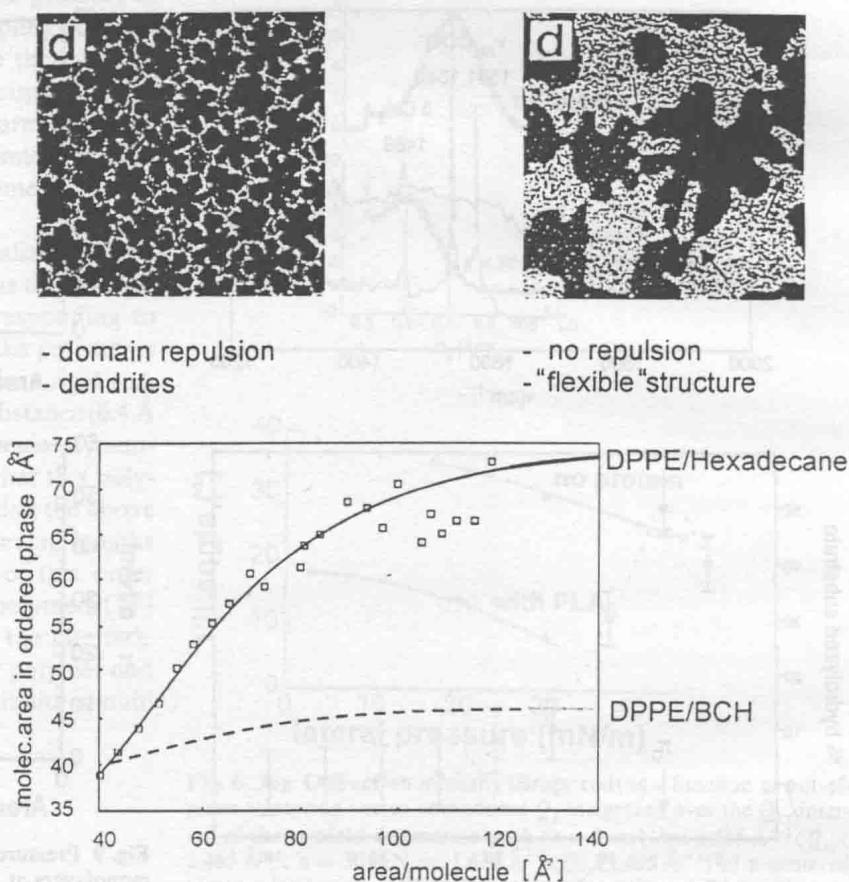
suitability for optical, X-ray and Neutron reflection techniques. Preparing the monolayer with phospholipids that are insoluble in the water as well as the oil phase one can also change the surfactant density and state in a well-defined way.

The pressure/area isotherms (Fig. 9) reveal that also in this case the pronounced break in the slope typical for a first order phase transition is observed [34]. The area differences of LE and LC phases are much larger compared to the air/water interface and this as well as the flat isotherm in the LE phase can be ascribed to the entropic repulsion by oil penetrating the monolayer [34]. The change of transition pressure and limiting molecular area at high pressures depend on the lipid [35] as well as the type of oil [34]. Concerning the latter it is also informative to analyse quantitatively the fluorescence micrographs observed for the phase coexistence range (Fig. 10, top). Comparing the bulky oil bicyclohexyl (BCH) and the (linear) n-alkane hexadecane (C_{16}) in the first case, contrary to the latter, one finds a dendritic structure and domain repulsion. Determining the LC phase area fraction as a function of molecular area one can also derive the area

per phospholipid A_p in the LC domains [36]. One derives values between 40 and 45 Å^2 in the case of BCH as expected for two nearly perpendicularly oriented tails of the phospholipid DPPE. In the case of C_{16}, however, A_p is well above 45 Å^2 and is decreased upon compression (Fig. 10, bottom). This indicates that commencing the transition the alkane partly remains in the ordered phase and is continuously squeezed out upon compression. To quantify this statement, since in the ordered state at low pressure the molecular area demand of the two tilted tails of the phospholipid is about 45 Å^2 and that of an ordered alkane similarly tilted is about 22 Å^2, one estimates 1–2 alkanes per phospholipid at the beginning of the transition.

The pressure/area isotherms are qualitatively much more expanded if a lipid like DPPC with a large head group is studied in contact with hexadecane [35]. In this region isotherms with oil contact and at the oil/water interface agree and there are good arguments that this also holds for the monolayer structure. The latter can again be studied by surface diffraction [37], and the analysis of the measurements in Fig. 11 shows that the tails can be aligned vertically. In this case, opposite to the situation of enzyme