Gene therapy is one of the most promising concepts in order to heal hereditary but also acquired diseases. Apart from AIDS, cardiovascular disorders and neurological diseases like Parkinson and Alzheimer’s disease, cancer is the most addressed subject in this field. However, in most cases the therapeutic concepts still come to fail with the practical realization. The main difficulties lie in the finding of suitable gene carriers. Viral vectors show high transfection efficiencies but come along with the stimulation of the immune response, size limitations and complications in the production process. Other methods like transfection based upon the complexation of DNA with the help of cationic lipids (lipoplexes) are able to avoid these problems. The challenges in this case are low transfection rates and cell toxicity. Novel cationic lipids are devotedly synthesized and new formulations are persistently tested.

Apart from investigations of bulk systems, model experiments at the air-water interface are able to add valuable insights. For instance, it is possible to understand the mechanisms of binding and complexation of DNA at lipid monolayers in detail [1]. The correlation of monolayer results to the behavior of real lipoplexes is challenging. This work is focused on the physical-chemical behavior and the comparison of two new lipids in 2D systems at the air-water interface. Lipids 7 (N’-2-[(2,6-diamino-1-oxohexyl)amino]ethyl-2,N-(dihexadecyl)propane diamide) and 8 (N’-2-[(2,6-diamino-1-oxohexyl)amino]ethyl-2-hexadecyl-N-[(9Z)-octadec-9-enyl]propane diamide) were tested in a systematic study of 9 new structural similar lipids designed and synthesized for gene transfection. The 3D structures are reported in [2]. These lipids are characterized by the same head group structure but different chain patterns. Lipid 8 showed outstanding transfection results even in comparison with commercially available reagents like Lipofectamine®. In contrast, lipid 7 revealed almost no activity. In the present work, the phase behavior and monolayer structures of these two lipids on different subphases are investigated by IRRAS and GIXD. The coupling of DNA is quantified in a new approach using IRRAS.

The chain lattice structure of lipids in the ordered state (LC-phase) can be determined at the air-water interface by GIXD. In the case of lipid 7, two diffraction peaks, that can be assigned to the chain lattice, are found at pH 4 and at pH 8. Representative Bragg peaks and contour plots at 30 mNm⁻¹ are shown in Figure 3. The Bragg peaks at higher $Q_{xy}$ are located at $Q_z>0$ and are therefore indexed as the degenerate $(1,±1)$ reflections. The reflections located at smaller $Q_z$ are found at $Q_z=0$ and are consequently indexed as the non-degenerate $(0,2)$ peaks. From the peak patterns, an
orthorhombic lattice with only slightly tilted chains has been derived for both pH values. The lattices do almost not change upon compression. At pH 8, a typical herring bone arrangement is present while at pH 4 the cross-sectional area $A_0$ is larger due to stronger electrostatic repulsions between the charged head groups. $A_0$ at pH 8 is comparable with values observed by WAXS experiments in bulk systems of lipid 7 [2]. The correlation lengths $L_{xy}$ at pH 8 are significantly larger than at pH 4 indicating that the electrostatic repulsion leads to the formation of a chain lattice with more defects. An additional peak with low intensity at $Q_{xy}=1.32 \ \text{Å}^{-1}$ ($d=4.76 \ \text{Å}$) and $Q_z=0 \ \text{Å}^{-1}$ has been observed at both pH values. This peak is typical for hydrogen bonds between N–H•••O=C groups of β-sheets of proteins and peptides. The structure of lipid 7 provides many possibilities to build a hydrogen bond network. The decreasing intensity of this peak at pH 4 emphasizes that its origin are indeed hydrogen bonded head groups. The tight packing of the lipids at pH 8 allows the formation of hydrogen bonds while at pH 4 electrostatic repulsion increases the distance between head groups and reduces the possibility of hydrogen bond formation.

At pH 8, a fourth peak is found at $Q_{xy}=1.75 \ \text{Å}^{-1}$ with $Q_z=0 \ \text{Å}^{-1}$. This peak cannot be assigned to the chain lattice. It is likely that it is based on a second independently formed head group lattice. However, in the range of $Q_{xy}=1.2-2.6 \ \text{Å}^{-1}$ no other peaks are found. This very limited information does not allow the calculation of lattice parameters. A similar peak appears at pH 4 around $Q_{xy} \approx 1.70 \ \text{Å}^{-1}$ with a very low intensity indicating the limited possibilities for head group lattice formation due to increased repulsion.

The addition of DNA to the subphase leads to significant changes. Representative diffraction patterns at 30 mN m$^{-1}$ are given in Figure 3. The peaks corresponding to hydrogen bonding and to the head group lattice vanish completely at pH 4 and are strongly reduced in intensity at pH 8. At pH 4, no diffraction patterns can be found at low pressures. Obviously, the presence of DNA fluidizes the monolayer. At pressures ~20 mN m$^{-1}$, one broad Bragg peak occurs and is an indication for hexagonal packing of poorly correlated chains. This observation is in agreement with the obtained IRRAS data suggesting a very weak ordering of the chains (larger wavenumbers).

References
