Experimental Report ID02 (SC-3939 Taßler)

Proposal Title: Structure and thermotropic phase behavior of novel cationic lipid-mRNA complexes studied by SAXS, USAXS and WAXS.

1.) A short report

The gene delivery and the gene silencing are nucleic-acid based therapeutics for the treatment or prevention of viral infection (e. g. AIDS), cancer and monogenetic disorders (e. g. cystic fibrosis or sickle-cell anemia) – the so called gene therapy. Successful gene therapy requires an efficient delivery of genetic material (either DNA or RNA) into biological tissue or cells.

Synthetic cationic lipids are widely used for non-viral gene delivery (DNA) and gene silencing (RNA).

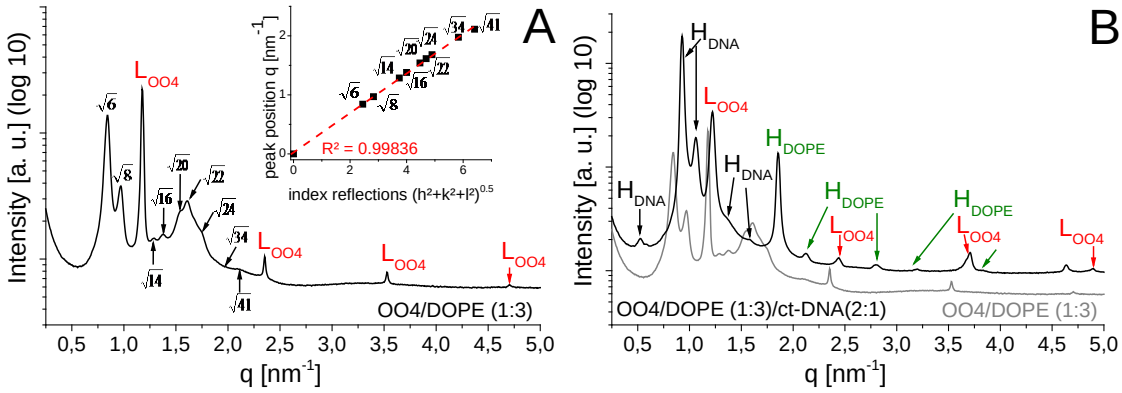
The structure and thermotropic phase behavior of novel cationic lipid-mRNA/lipid-ctDNA complexes have been studied by SAXS, USAXS and WAXS. Some of the achieved data from this beam time are already published, while some data are going to be published soon (e.g. the physical-chemical investigation of **OO4**).

OO4 is the most promising transfection lipid of the investigated cationic lipids. It has three primary amine groups, a branched tris(2-aminoethyl)amine spacer and two unsaturated oleyl-chains ($C_{18:1}$). Due to its malonic acid / lysine backbone, it has a peptide-mimic character and is biocompatible. **OO4** arranges in multilamellar bilayers (L_α) in HBr buffer pH 3 and pH 10 (d = 55.9 Å, 25 °C). Furthermore **OO4** was mixed with **DOPE** and **DPPE** as neutral helper lipids in order to change the liposome properties. **DOPE** has a conical molecular shape and tends to arrange in inverted hexagonal cylinders (H_{II}). At pH 3 and 25 °C, **DOPE** has a lattice parameter a equal 74.4 Å. **DPPE** tends to form multilamellar aggregations in bulk due to its cylinder-like molecule shape. At pH 3 and 25 °C, **DPPE** is in gel phase state and arranges in lamellar structures ($L_ß$) with a repeating distance d equal 54.9 Å.

Furthermore, the phase behavior in presence of calf thymus DNA has been investigated due to the fact that the lipoplex structure is crucial for the transfection process. The (N/P)-ratio of 2:1 was chosen, because it showed the best performance in the transfection experiments.

The SAXS and WAXS data for **OO4/DOPE** (1:3) and **OO4/DPPE** (1:3) liposomes as well as **OO4/DOPE/DNA** (lipids:DNA 2:1) and **OO4/DPPE/DNA** (lipids:DNA 2:1) lipoplexes were recorded at 25 °C and 37 °C. Considering that the preparation for the transfection experiments was performed at 25 °C and that 37 °C is the body temperature.

OO4 mixtures with **DOPE** and calf thymus DNA:



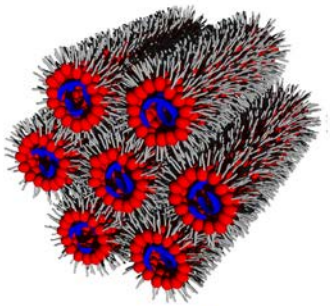


Figure 1: A SAXS pattern of **OO4/DOPE** in black at 25 °C and in HBr buffer pH 3, **B** SAXS pattern of **OO4/DOPE/DNA** in black at 25 °C and in HBr buffer pH 3, while **OO4/DOPE** is gray and **C** inverted hexagonal lipoplex – DNA is blue.

The mixing of **OO4** and **DOPE** in the ratio 1:3 results in phase separation (**Figure 1A**). The lamellar phase with the repeating distance of 53.4 Å corresponds most likely to phase separated **OO4**, since pure **OO4** arranges in multilamellar bilayers with similar *d*-values. Additionally there are Bragg peaks in the SAXS pattern in the ratio of √6:√8:√14:√16:√20:√22:√24:√34:√41 indicating an inverted cubic Ia3d phase ($Q^{230}\alpha$, so-called Gyroid minimal surface). The adding of DNA to the system changes the SAXS pattern drastically (**Figure 1B**). The cubic phase disappears, while the lamellar phase remains and two hexagonal phases appear. The *d*-value of the lamellar phase is with 51.5 Å slightly smaller than for the mixture without DNA. Therefore, the DNA cannot be incorporated in the lamellar phase. Most probably, the lamellar phase consists of phase separated **OO4**. Subsequently the DNA has to be incorporated in one of the hexagonal phases. The lattice parameter of H_{DNA} is equal to 138.1 Å, while the lattice parameter of H_{DOPE} is with a = 68.2 Å much smaller. Since the lattice parameter of H_{DOPE} is similar to the lattice parameter of pure **DOPE** (a = 74.4 Å), it seems like H_{DOPE} contains most likely phase separated **DOPE**. It is reasonable to assume that the DNA is completely incorporated in H_{DNA} (**Figure 1C**). In the SAXS pattern is no trace of free DNA. The double helix diameter of DNA is 20 Å, which means that the incorporation of DNA should result in an increase of the lattice parameter about 20 Å or less (partial penetration) per DNA layer.

OO4 mixtures with **DPPE** and calf thymus DNA:

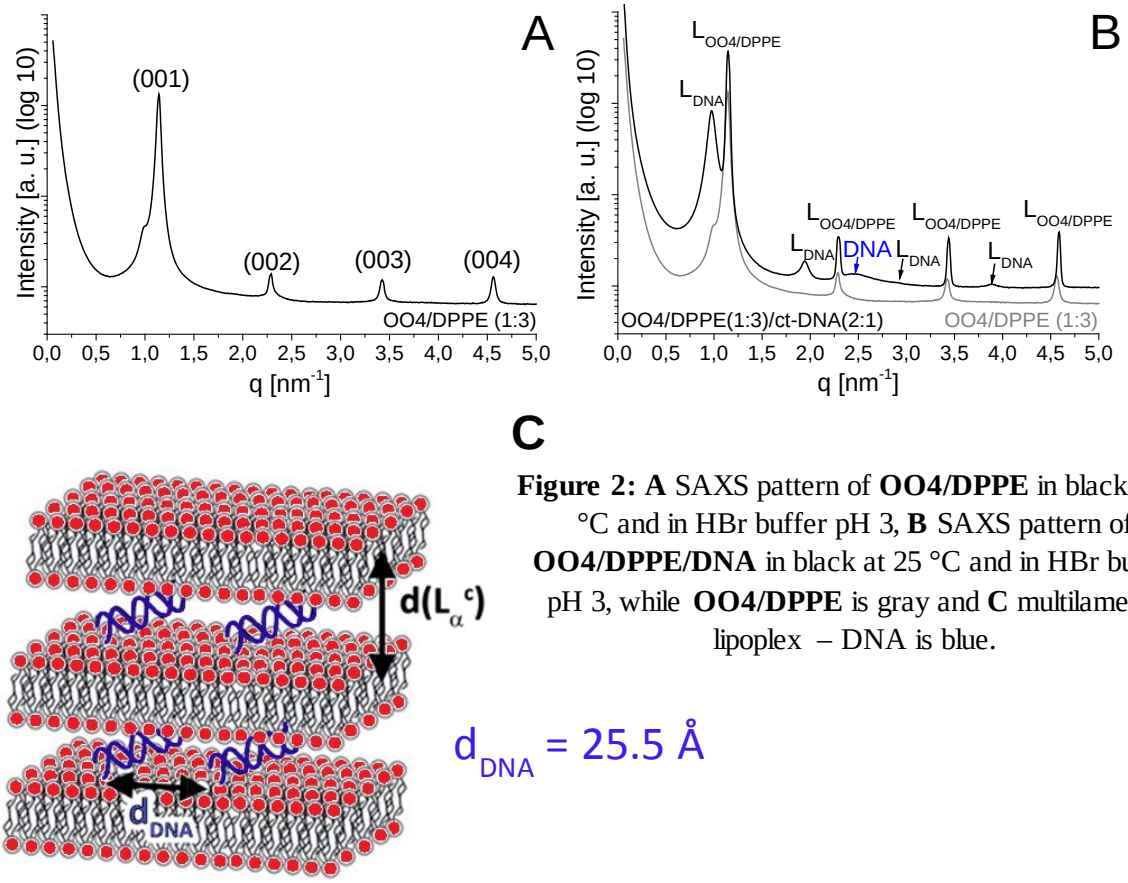


Figure 2: A SAXS pattern of **OO4/DPPE** in black at 25 °C and in HBr buffer pH 3, **B** SAXS pattern of **OO4/DPPE/DNA** in black at 25 °C and in HBr buffer pH 3, while **OO4/DPPE** is gray and **C** multilamellar lipoplex – DNA is blue.

The 1:3 mixture of **DPPE** and the cylinder-like lipid **OO4** also arranges in multilamellar bilayers. The sharp Bragg peaks in the SAXS (**Figure 2A**) are visible up to the 4th order in the observed q range, which indicates a long correlation length between the bilayers and characterizes an arrangement without significant defects. The adding of DNA causes a phase separation, but no change in the aggregation structure as shown in **Figure 2B**. Both phases are lamellar and at least one phase has tilted chains in gel state, because there are two peaks in the WAXS region. L_{DNA} (d = 64.6 Å) incorporates most probably the DNA, because to repeating distance of L_{DNA} is larger than for $L_{OO4/DPPE}$ (d = 54.8 Å). Further the d-value of $L_{OO4/DPPE}$ is similar to those of the **OO4/DPPE** mixture and pure **DPPE**. The broad peak at q = 2.47 nm^{-1} corresponds to a DNA-DNA in-plane distance of 25.5 Å. In the lamellar lipoplex the DNA is complexed sandwich-like between the lipid bilayers. Here the DNA rods align in a 1D lattice with a repeating distance of 25.5 Å (**Figure 2C**). This is a very tight packing.

2.) A complete list of publications from this beam time

R. Tanasescu, M. Lanz, D. Mueller, S. Tassler, T. Ishikawa, R. Reiter, G. Brezesinski, A. Zumbuehl (2016). "Vesicle Origami and the Influence of Cholesterol on Lipid Packing", Langmuir **32**(19): 4896-4903.

C. Janich, S. Taßler, A. Meister, G. Hause, J. Schäfer, U. Bakowsky, G. Brezesinski, C. Wölk (2016). "Structures of malonic acid diamide/phospholipid composites and of their lipoplexes",Soft Matter (accepted manuscript)

3) A complete list of all conference contributions from this beam time

Oral presentation:

S. Taßler and G. Brezesinski
"Physical-chemical Investigation of newly synthesised DNA transfection lipids"
6th Iberian Meeting on Colloids and Interfaces 2015 - Guimarães, Portugal

4.) A complete list of all eventual student thesis from this beam time

Doctoral dissertation:

S. Taßler (2015). "Physical-Chemical Investigation of newly synthesised Lysine-Based Amino-Functionalised Lipids for gene transfection in 2D and 3D model systems." Physical Chemistry, University of Potsdam: 142 pages.