Supporting Information

DNA delivery systems based on peptide-mimicking cationic lipids – the effect of the co-lipid on the structure and DNA binding capacity

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1. Calculation of the theoretical mass for QCM-D

The measured data were compared with theoretically calculated values obtained by the assumption that a lipid bilayer consist of the monolayers, which means that two molecules in a bilayer (one above the other) require the same area such as one molecule in a monolayer. In this case, the molecular mass $M$ is twice the value for a monolayer. According to the lateral pressure obtained in biological membranes, the molecular area $A_M$ was taken from the $\pi$-$A$-isotherms at a surface pressure of 30 mN/m in order to estimate a theoretical mass deposition $m_{\text{theoretical}}$. Since the number of molecules at one cm$^2$ can be calculated from its molecular mass and the molecular area via the basic relations below:

$$c \cdot V = \frac{m}{M}$$

Equation S1

Equation S1 represents in principle the lipid solution spread on the Langmuir trough with $c$ being the concentration of the lipid solution [mM] and $V$ being the spreading volume [µL]. $M$ is the molecular mass [g/mol] and $m$ [mg] is the weighted sample, both were needed to prepare the lipid solution.

The theoretical mass deposition $m_{\text{theoretical}}$ [ng/cm$^2$] can be obtained from the mass of a single $m_M$ molecule and its required molecular area $A_M$ [$Å^2=10^{-16}$ cm$^2$] by using equation S2.

$$m_{\text{theoretical}} = \frac{m_M}{A_M}$$

Equation S2

The mass of a single molecule is defined by its molecular mass $M$ [g/mol = $10^9$ ng/mol] and the Avogadro constant ($N_A = 6.0221412927\cdot10^{23}$ mol$^{-1}$) as shown in equation S3.

$$m_M = \frac{M}{N_A}$$

Equation S3

Finally, the theoretical mass deposition $m_{\text{theoretical}}$ can be used to interpret the results gained by QCM. In literature, a frequency shift of 13 Hz indicates a lipid monolayer, consequently, but also proofed, a lipid bilayer causes a frequency shift of 26 Hz, and for vesicle adsorption a frequency shift of 90 Hz was reported. In this study the well-investigated phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was used as a reference for evaluation of obtained results.

The theoretical mass of calf thymus DNA was estimated assuming the DNA double-helix as a cylinder with the curved surface area $A_{cs}$ by using equation S4.

$$A_{cs} = \pi \cdot d_{DNA} \cdot h$$

Equation S4

Here $r$ is the radius of the cylinder and $h$ is the height. Calf thymus DNA is B-Form DNA with a diameter $d_{DNA} = 20$ Å and a distance of 3.4 Å between base pairs. Referring to the provider (Sigma-Aldrich, St. Louis (MO), United States of America) the used calf thymus DNA (Typ 1) consists of > 13 kb (thirteen thousand base pairs). Hence this approach was used for the height $h$. Since the cylindrical DNA only attaches the lipid bilayer with on side, while the other side faces the aqueous solution, just half of the value for $A_{cs}$ was taken into account for the calculation of the theoretical mass deposition of DNA (25.7 ng/cm$^2$).

According to Günter Sauerbrey, the weighting sensitivity of a quartz crystal with an electrode diameter of 4 mm in the center of the vibration region is about $10^{10}$ Hz/g. Taking an error bare for
the frequency measurement ($\Delta f = 1 \, Hz$) into account the weighting accuracy can be determined to 0.1 ng.

2. IRRA spectra of OO4 on bromide containing buffer and ct-DNA containing bromide buffer (CH$_2$ and PO$_2^-$ stretching vibration region)

Figure S1: OO4 at 30 mN·m$^{-1}$ on bromide containing buffer (straight lines) and ct-DNA containing bromide buffer (dashed lines) A) asymmetric and symmetric CH$_2$ stretching vibration region and B) asymmetric PO$_2^-$ stretching vibration region

3. QCM-D of DMPC

Figure S2: A) $\Delta f(t)$ and B) $\Delta D(t)$ of DMPC (1 mg/mL) in bromide containing buffer (2 mM, pH 3) at 20 °C. Supported DMPC bilayer is formed without observing a critical density of vesicles on the surface ($\Theta_c$). The 3$^{rd}$ overtone is shown.
4. Specular X-Ray Reflectivity curves of OO4 on bromide containing buffer

![Graph A: X-ray reflectivity](image1)

**Figure S3:** A) X-ray reflectivity of OO4 at room temperature on bromide containing buffer (2 mM) at 30 mN·m⁻¹ (BW1, HASYLAB at DESY in Hamburg, Germany) and B) electron density profile from a box-model fit of the reflectivity curve.

**Table S1:** structural data obtained from fits of the specular X-ray reflectivity curves of OO4 on bromide containing (2 mM) buffer pH 3 and pH 10, 20 °C, 30 mN·m⁻¹.

|       | chain | head group |
|-------|-------|------------|
|       | z [Å] | ρ [e-/Å⁻³] | e⁻/Å² | theoretical number of electrons | z [Å] | ρ [e-/Å⁻³] | e⁻/Å² | theoretical number of electrons | measured numbers of electrons |
| pH 3  | 13.492| 1.24       | 3.421 | 286                           | 11.267| 1.332      | 5.029 | 194                           | 413                            |
| pH 10 | 14.606| 1.207      | 3.412 | 286                           | 10.872| 1.27       | 4.617 | 194                           | 294                            |

10 e⁻ for H₂O; 36 e⁻ for Br⁻
5. Cubic Mesophases:

Fm3m phase

Table S2: The indexed reflexes of the micellar cubic Fm3m lattice ($Q^{225}_a$) and their Miller indices.

| index reflections $(h^2+k^2+l^2)^{0.5}$ | Miller Indices | Fm3m lattice ($Q^{225}_a$) |
|----------------------------------------|----------------|---------------------------|
| $\sqrt{3}$                            | (111)          |                           |
| $\sqrt{4}$                            | (200)          |                           |
| $\sqrt{8}$                            | (220)          |                           |
| $\sqrt{11}$                           | (311)          |                           |
| $\sqrt{12}$                           | (222)          |                           |
| $\sqrt{16}$                           | (400)          |                           |
| $\sqrt{19}$                           | (331)          |                           |
| $\sqrt{20}$                           | (420)          |                           |
| $\sqrt{24}$                           | (422)          |                           |
| $\sqrt{27}$                           | (333)          |                           |

Ia3d phase

Table S3: The indexed reflexes of the bicontinuous cubic Ia3d lattice ($Q^{230}_a$) and their Miller indices.

| index reflections $(h^2+k^2+l^2)^{0.5}$ | Miller Indices | Ia3d lattice ($Q^{230}_a$) |
|----------------------------------------|----------------|---------------------------|
| $\sqrt{6}$                            | (221)          |                           |
| $\sqrt{8}$                            | (220)          |                           |
| $\sqrt{14}$                           | (321)          |                           |
| $\sqrt{16}$                           | (400)          |                           |
| $\sqrt{20}$                           | (420)          |                           |
| $\sqrt{22}$                           | (332)          |                           |
| $\sqrt{24}$                           | (422)          |                           |
| $\sqrt{34}$                           | (433)          |                           |
| $\sqrt{41}$                           | (443)          |                           |

Pm3n phase

Table S4: The indexed reflexes of the micellar cubic Pm3n lattice ($Q^{223}_a$) and their Miller Indices.

| index reflections $(h^2+k^2+l^2)^{0.5}$ | Miller Indices | Pm3n lattice ($Q^{223}_a$) |
|----------------------------------------|----------------|---------------------------|
| $\sqrt{2}$                            | (110)          |                           |
| $\sqrt{4}$                            | (200)          |                           |
| $\sqrt{5}$                            | (210)          |                           |
| $\sqrt{6}$                            | (211)          |                           |
| $\sqrt{8}$                            | (220)          |                           |
| $\sqrt{10}$                           | (310)          |                           |
| $\sqrt{12}$                           | (222)          |                           |
6. SAXS/WAXS of DOPE and DPPE in bromide containing buffer

**Figure S4:** A) SAXS and B) WAXS of DOPE and DPPE as 10 wt% lipid dispersion in bromide containing buffer (2 mM, pH 3) at 25 °C.
7. Additional SAXS/WAXS data of OO4, DOPE and DPPE in MES buffer at 20 °C and 37 °C

Figure S5: A) SAXS and B) WAXS of OO4 (black line), DOPE (green line) and DPPE (red line) in MES buffer (pH 6.5) at 20 °C and 37 °C.

Figure S6: A) SAXS and B) WAXS of OO4/DOPE (black line) and OO4/DOPE/DNA (red line) in MES buffer (pH 6.5) at 20 °C and 37 °C.

Figure S7: A) SAXS and B) WAXS of OO4/DPPE (black line) and OO4/DPPE/DNA (red line) in MES buffer (pH 6.5) at 20 °C and 37 °C.
8. TEM image

Figure S8: TEM images of negatively stained samples prepared from an aqueous **OO4/DOPE 1:3 (n:n)** lipoplex dispersion at N/P5 (c = 0.05 mg/mL in MES buffer pH 6.5).

The lipoplex dispersions were diluted with MES to a concentration of 0.05 mg/mL. The negatively stained samples were prepared by spreading the dispersion (5 μL) onto a Copper grid coated with a Formvar film (Plano, Wetzlar, Germany). After 1 min, excess liquid was removed by blotting with filter paper and 1% aqueous uranyl acetate (5 μL) was placed onto the grid and drained off after 1 min. The dried specimens were examined using an EM 900 transmission electron microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Micrographs were acquired using a SSCCD SM-1k-120 camera (TRS, Moorenweis, Germany).
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