Supporting Information

Lysine-based amino-functionalized lipids for gene transfection: the influence of chain composition on 3D phase behaviour and transfection performance

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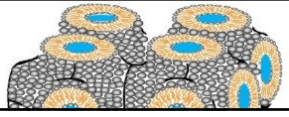
1. Cubic Mesophases

1.1 Im3m lattice

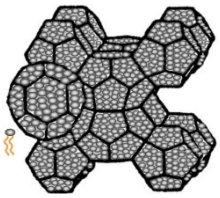
Table S1: The indexed reflexes for the bicontinuous cubic Im3m lattice ($Q^{229}\alpha$) and their Miller Indices.

index reflections $(h^2+k^2+l^2)^{0.5}$	Miller Indices	Im3m lattice (Q_α^{229})
√2	(110)	
√4	(200)	
√6	(211)	

√8	(220)
√10	(310)
√12	(222)
√14	(321)
√16	(400)
√18	(411) or (330)
√20	(420)
√22	(332)

1.2 Pm3n lattice

Table S2: The indexed reflexes of the micellar cubic Pm3n lattice ($Q^{223}\alpha$) and their Miller Indices.

index reflections $(h^2+k^2+l^2)^{0.5}$	**Miller Indices**	**Pm3n lattice (Q_α^{223})**
√2	(110)	
√4	(200)	
√5	(210)	
√6	(211)	
√8	(220)	
√10	(310)	
√12	(222)	

2. Different dispersions of lipids at 25 °C:

Table S3: Repeating distance *d* and hexagonal lattice parameter *a* of different lipid dispersion at 25 °C.

			10 wt%		**20 wt%**		
		25 °C	**bromid buffer (2 mM)**		**citrate buffer (5 mM)**	**water**	**carbonate buffer (5 mM)**
mesophase	**[Å]**		**pH 3**	**pH 10**	**pH 4**	**~pH 5.8**	**pH 10**
multi-lamellar	repeating distance *d*	**TH10**	52.4	61.1	60.7	57.8	63.2
		TT10	-	-	63.7	55.8	50.9
		OH10	53.9	54.0	60.0	74.2	64.2
		OT10	-	-	60.6	61.2	52.8
hexagonal	lattice parameter *a*	**OO10**	57.8	57.9	56.5	56.5	56.6
		DOPE	74.4	-	72.2	72.0	72.9

3. 20 wt% lipid dispersions in Citrate buffer (5 mM, pH 4):

The citrate buffer ($C_8H_7NaO_7$ – sodium citrate monohydrate) had a constant concentration of 5 mM and pH 4 was adjusted with NaOH solution (c = 1 M).

3.1. SAXS and WAXS of **TH10**, **TT10**, **OH10**, **OT10** and **OO10** at 25 °C:

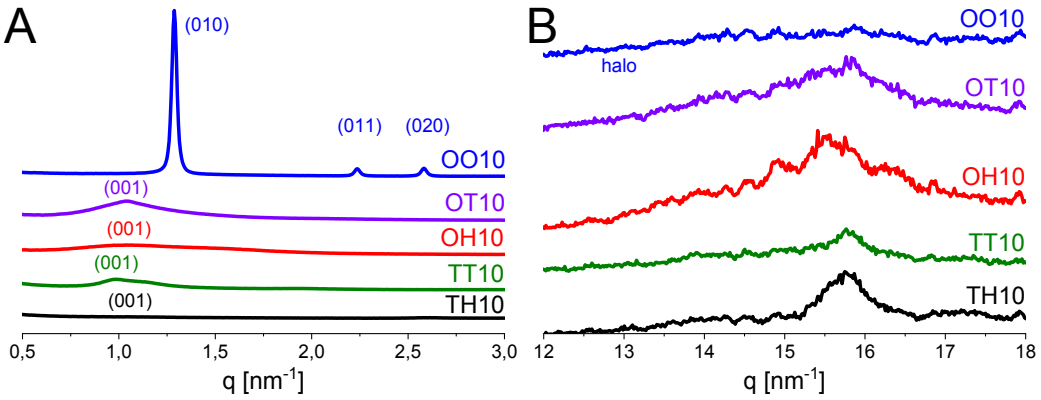


Figure S1: A) SAXS pattern and **B)** corresponding WAXS pattern of 20 wt% lipid dispersions in citrate buffer pH 4 at 25 °C. **TH10** (black line), **TT10** (green line), **OH10** (red line), **OT10** (violet line) and **OO10** (blue line).

3.2. Temperature dependence of **TH10**, **TT10**, **OH10**, **OT10** and **OO10**:

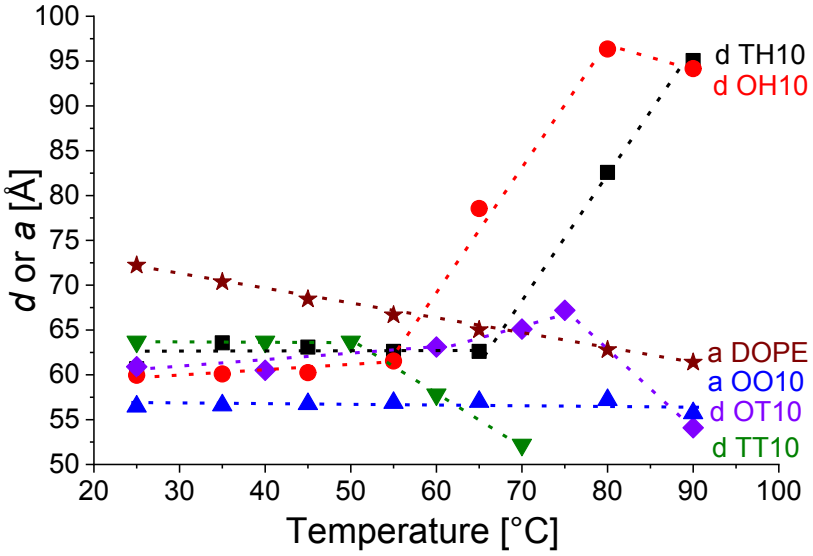


Figure S2: The repeating distance *d* of multi-lamellar bilayers of **TH10** (black squares), **TT10** (green triangles down), **OH10** (red dots) and **OT10** (violet diamonds) as well as the hexagonal lattice parameter *a* of **OO10** (blue triangles up) and **DOPE** (wine star) as 20 wt% dispersions in citrate buffer pH 4 as a function of temperature. Dotted lines are for guiding the eyes only.

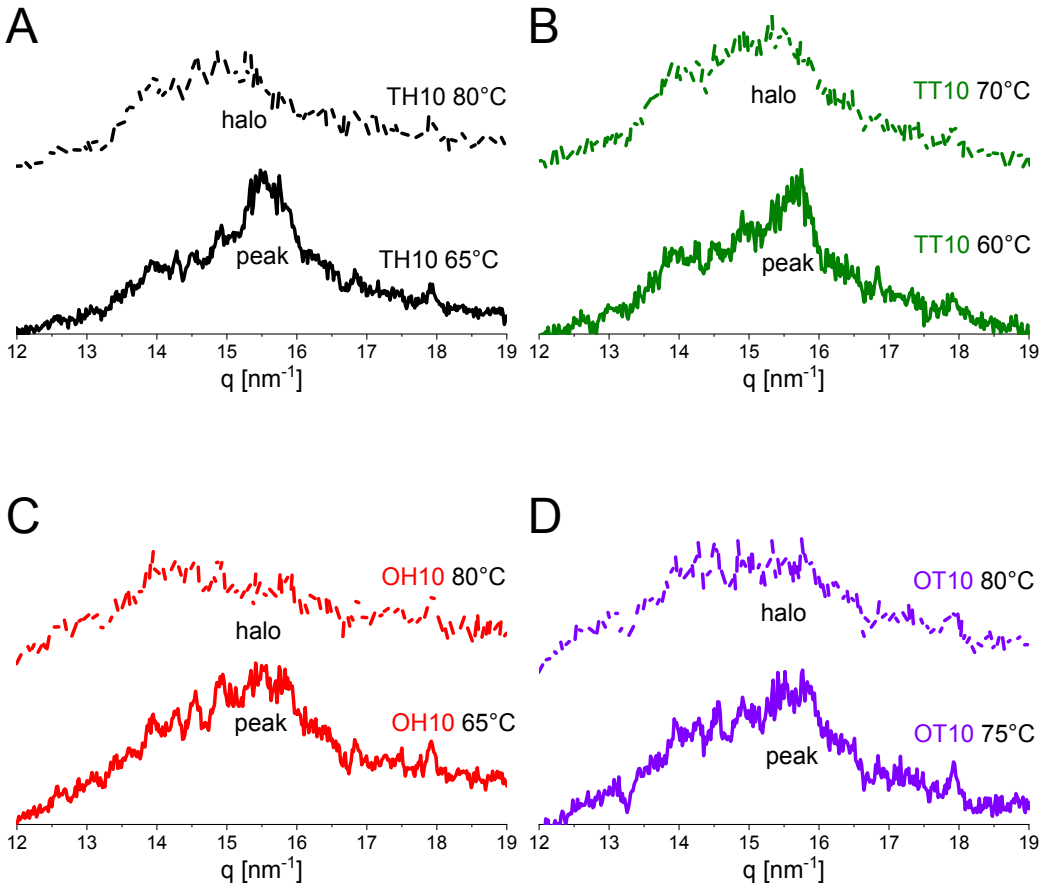


Figure S3: WAXS of 20 wt% lipid dispersions in citrate buffer pH 4 at different temperatures. **A) TH10** in gel state at 65 °C (black straight line) and in liquid-crystalline state at 80 °C (black dashed line), **B) TT10** in gel state at 60 °C (green straight line) and in liquid-crystalline state at 70 °C (green dashed line), **C) OH10** in gel state at 65 °C (red straight line) and in liquid-crystalline state at 80 °C (red dashed line) and **D) OT10** in gel state at 75 °C (violet straight line) and in liquid-crystalline state at 80 °C (violet dashed line).

4. Temperature dependence of 20 wt% lipid dispersions in Carbonate buffer (5 mM, pH 10):

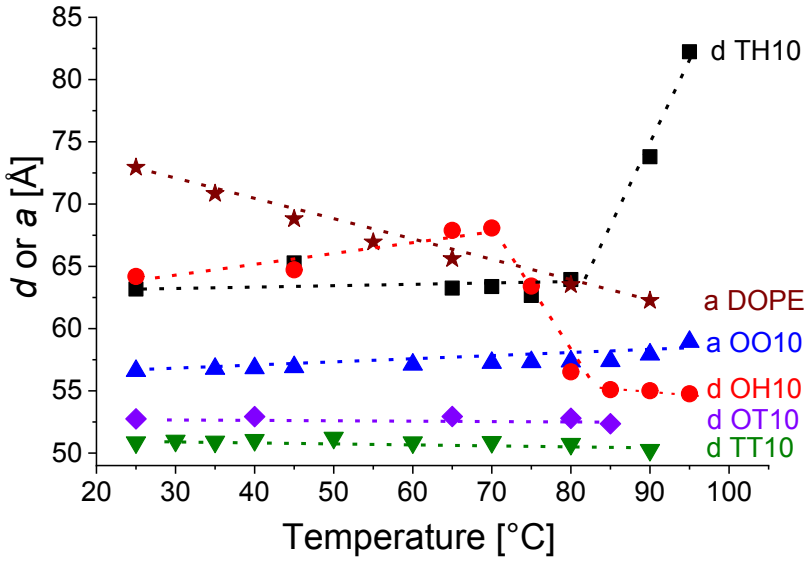


Figure S4: The repeating distance *d* of multi-lamellar bilayers of **TH10** (black squares), **TT10** (green triangles down), **OH10** (red dots) and **OT10** (violet diamonds) as well as the hexagonal lattice parameter *a* of **OO10**

(blue triangles up) and **DOPE** (wine star) as 20 wt% dispersions in carbonate buffer pH 10 as a function of temperature. Dotted lines are for guiding the eyes only.

5. Small-angle and wide-angle X-ray scattering of **TH10** as aqueous dispersion with different amounts of **DOPE** in absence and presence of calf thymus DNA:

5.1. TH10/DOPE liposomes:

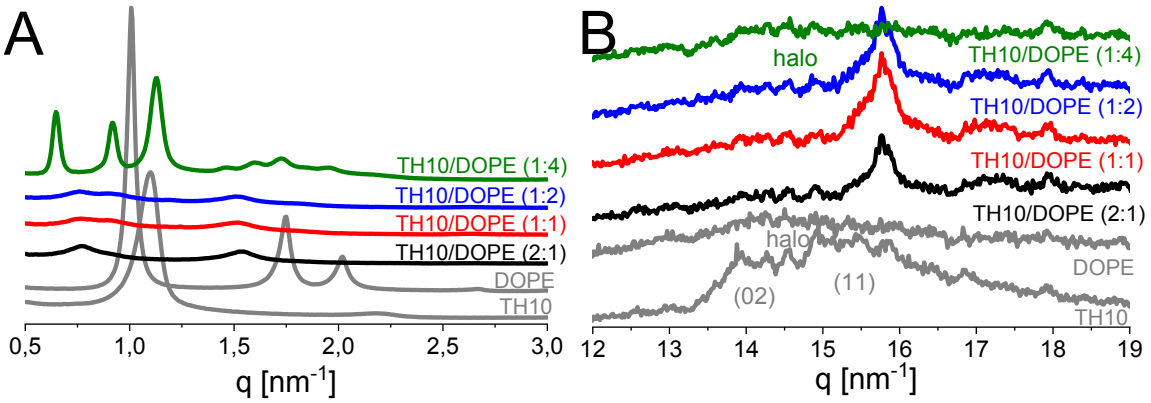


Figure S5: A) SAXS pattern and **B)** corresponding WAXS pattern of **TH10/DOPE (2:1)** black, **TH10/DOPE (1:1)** red, **TH10/DOPE (1:2)** blue and **TH10/DOPE (1:4)** olive green as 20 wt% dispersion in water at 25 °C, while the pure **TH10** and **DOPE** are given in grey.

Table S4: Phase state, peak position, repeating distance or lattice parameter and cross-sectional area obtained in SAXS and WAXS of 20 wt% dispersion of **TH10** and **DOPE** in different ratios in water at 25 °C.

	phase state	**d [Å]**	**q_{11} [Å^{-1}]**	**q_{02} [Å^{-1}]**	**A_0 [Å^2]**
TH10	$L_{\beta'}$	57.8	1.54	1.35	20.7
TH10/DOPE 2:1	$L_{\beta'}$	81.6	peak → gel state		
TH10/DOPE 1:1	$L_{\beta'}$	80.2	peak → gel state		
TH10/DOPE 1:2	$L_{\beta'}$	81.8	peak → gel state		
	phase state	**a [Å]**	**q_{11} [Å^{-1}]**	**q_{02} [Å^{-1}]**	**A_0 [Å^2]**
DOPE	H_α	72	halo → molten chains		
TH10/DOPE 1:4	Q_α[229]	138	halo → molten chains		

5.2. TH10/DOPE/ct-DNA lipoplexes:

5.2.1. SAXS and WAXS:

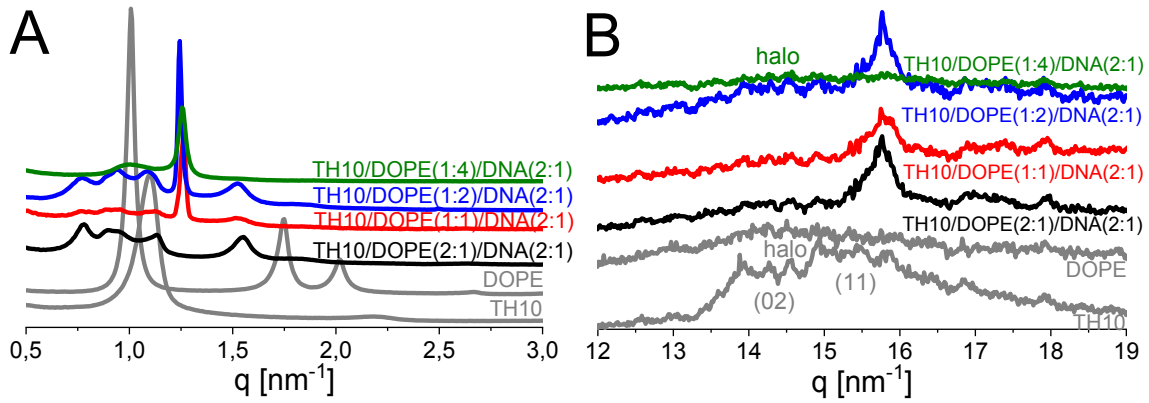


Figure S6: A) SAXS pattern and **B)** corresponding WAXS pattern of **TH10/DOPE (2:1) / DNA (2:1)** black, **TH10/DOPE (1:1) / DNA (2:1)** red, **TH10/DOPE (1:2) / DNA (2:1)** blue and **TH10/DOPE (1:4) / DNA (2:1)** olive green as 20 wt% dispersion in water at 25 °C, while the pure **TH10** and **DOPE** are given in grey.

5.2.2. Transfection efficiency and cell viability in A549 cells:

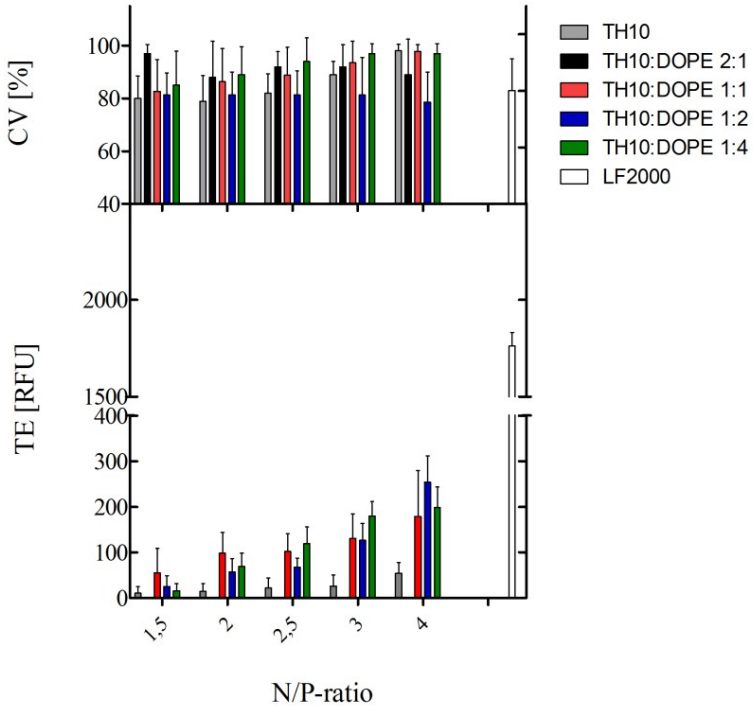


Figure S7: Transfection efficiency of **TH10** and **DOPE** in different ratios measured by detecting EGFP fluorescence and the corresponding cell viability values for different lipid dispersions complexed with pEGFP-C2-DNA in comparison with the standard Lipofectamine 2000® in A549 (human lung cancer) cells.