Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Role of counter-ion and helper lipid content in the design and properties of nanocarrier systems: a biophysical study in 2D and 3D lipid assemblies

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siRNA complexation, serum stability and silencing efficiency of DODAX:MO systems.

Depending on the counter-ion DODAX:MO (2:1) systems have shown differences in the % of siRNA complexation; in the serum stability of lipoplexes and differences in transfection efficiency (Fig. S1).

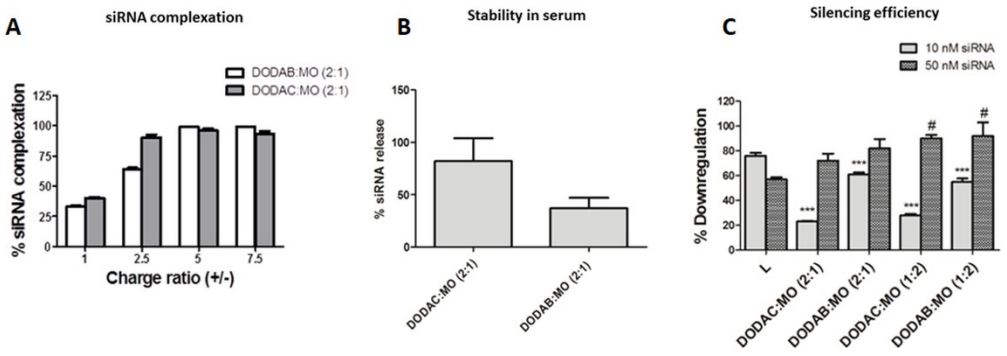

Fig. S1. Influence of counter-ion and MO content on siRNA complexation of DODAX:MO (2:1) liposomes (A), on the capacity of DODAX:MO (2:1) based lipoplexes to retain siRNA content in human serum (B) and on the transfection efficiency of DODAX:MO based lipoplexes. The percentage of RNA complexation after addition of DODAB:MO and DODAC:MO (2:1) liposomes to a RNA solution, was evaluated with the RNA intercalating probe Ribogreen. The stability in serum and silencing efficiency were adapted from[26].

To illustrate the impact of the counter-ion on the lipoplex formation, siRNA complexation was studied at different charge ratios C.R. (+/-) by the RiboGreen fluorimetric assay, according to the manufacturer specifications. Briefly, lipoplexes were incubated with RiboGreen solution in the dark (5 min) and then the fluorescence was measured in a Fluoroskan ACEN FL Microplate Fluorometer and Luminometer (Thermo Scientific, Waltham, MA), using the excitation/emission filter pair of 485/538 nm. A calibration curve was made in order to assure the linearity between the RiboGreen fluorescence and RNA concentration. The percentage of free RNA was attained from a control, of the same quantity of RNA used in the preparation of the lipoplexes. DODAC:MO (2:1) liposomes reached total siRNA complexation at lower C.R.

(+/-) = 2.5 comparing to DODAB:MO (2:1) liposomes that needed higher C.R. (+/-) = 5 to reach total siRNA complexation (Fig. S1 A). The colloidal stability of DODAX:MO (2:1) in human serum has been previously studied and has also shown to be dependent on the counter-ion (Fig. S1 B). [26] DODAB:MO (2:1) based lipoplexes are more stable in serum than DODAC:MO (2:1) based lipoplexes. Finally both the MO content and the counter-ion have influence in silencing efficiency, as stated in our previous study. [26] Lipoplexes based on DODAB systems with low MO content (DODAB:MO (2:1)) are more efficient in gene silencing than DODAC:MO (2:1) based lipoplexes (Fig. S1 C). At higher MO contents (1:2), the counter-ion influence is very much reduced and gene silencing is similar for lipoplexes based on DODAB:MO or DODAC:MO.

Infrared reflection-absorption spectroscopy (IRRAS) to evaluate phase transition of DODAB, DODAC and MO monolayers

IRRAS is a powerful tool to obtain a widespread spectrum of information of lipid monolayers at the air–water interface and by the position of the CH_2 symmetric, $\nu_s(CH_2)$, and asymmetric, $\nu_{as}(CH_2)$, stretching bands the phase state of lipids can be determined. LE phases are characterized by asymmetric, $\nu_{as}(CH_2)$, stretching bands around 2925 cm^{-1}, respectively, while in the LC phase, these bands are shifted to lower wavenumbers (2920 cm^{-1}). Accordingly, Figure S2 shows the position of $\nu_{as}(CH_2)$ of DODAB, DODAC and MO at different pressures in a aqueous subphase and 20 ºC. The phase transition from LE to LC in DODAB monolayers is connected with a jump in the wavenumbers of the CH_2¬ stretching vibrations to lower values indicating a clear phase transition as observed in the *π-A* isotherms. In the case of DODAC the transition to the LC phase cannot be observed and the wavenumbers decrease continuously with increasing pressure. At the highest lateral pressure measured, the wavenumbers of DODAC are at least 1 cm^{-1} larger than those of DODAB indicating a less dense packing. MO, as expected, presented a LE phase with no transition.

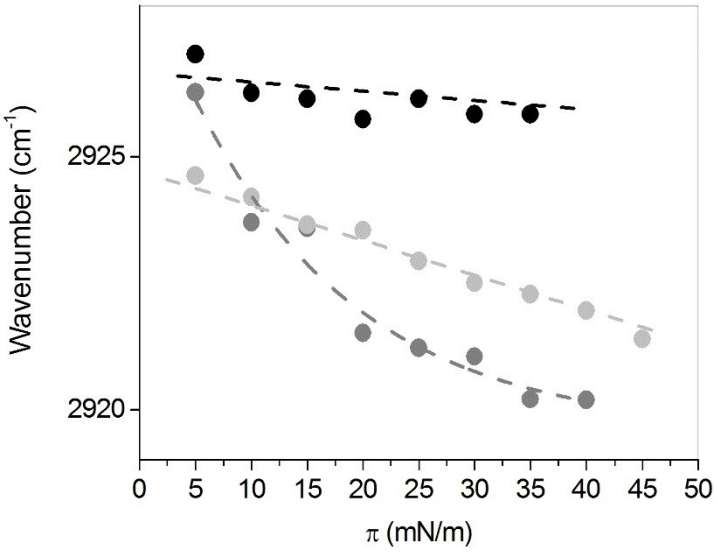

Fig. S2- Position of the asymmetric CH_2-stretching vibration of DODAB (dark grey dots), DODAC (light grey dots) and MO (black dots) on aqueous subphase at 20 °C in dependence of the surface pressure.

Langmuir isotherms of DODAB and DODAC on 10 mM NaBr or NaCl subphases

To illustrate the counter ion effect, DODAB and DODAC have been measured on 10 mM NaBr or NaCl solutions, respectively. In the case of DODAB, the phase transition region (coexistence of LE and LC) is seen as a perfect horizontal plateau at ~2 mN/m, whereas DODAC on the 10 mM NaCl solution does not exhibit any plateau but the area decreases continuously with increasing pressure. Above 20 mN/m, the molecular area of DODAC is smaller than that of DODAB indicating a less dense packing in DODAC.

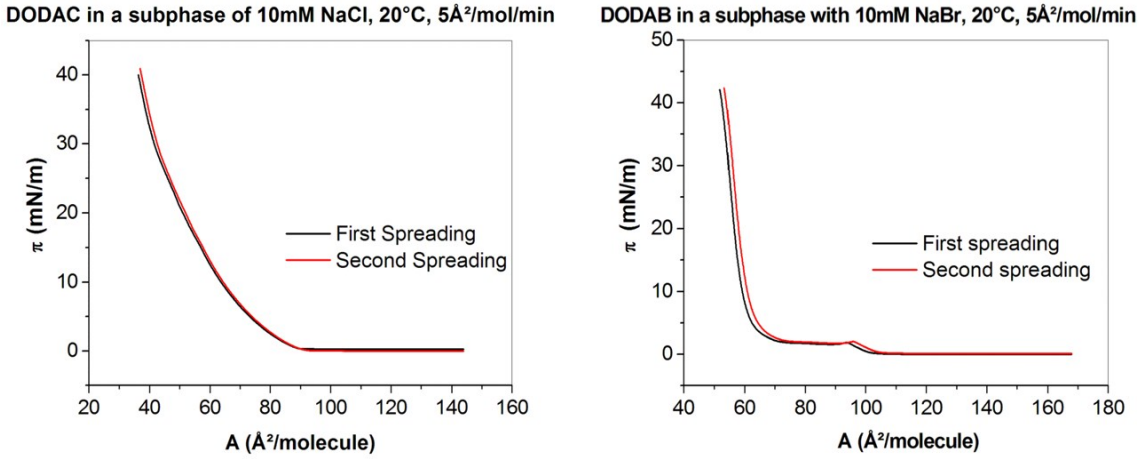

Fig. S3- Surface pressure-molecular area (π-A) isotherms (20 °C, aqueous subphase containing 10 mM of NaCl or NaBr) of DODAC (left) and DODAB (right) monolayers.

Brewster Angle Microscopy (BAM) of DODAB:MO and DODAC:MO (2:1)

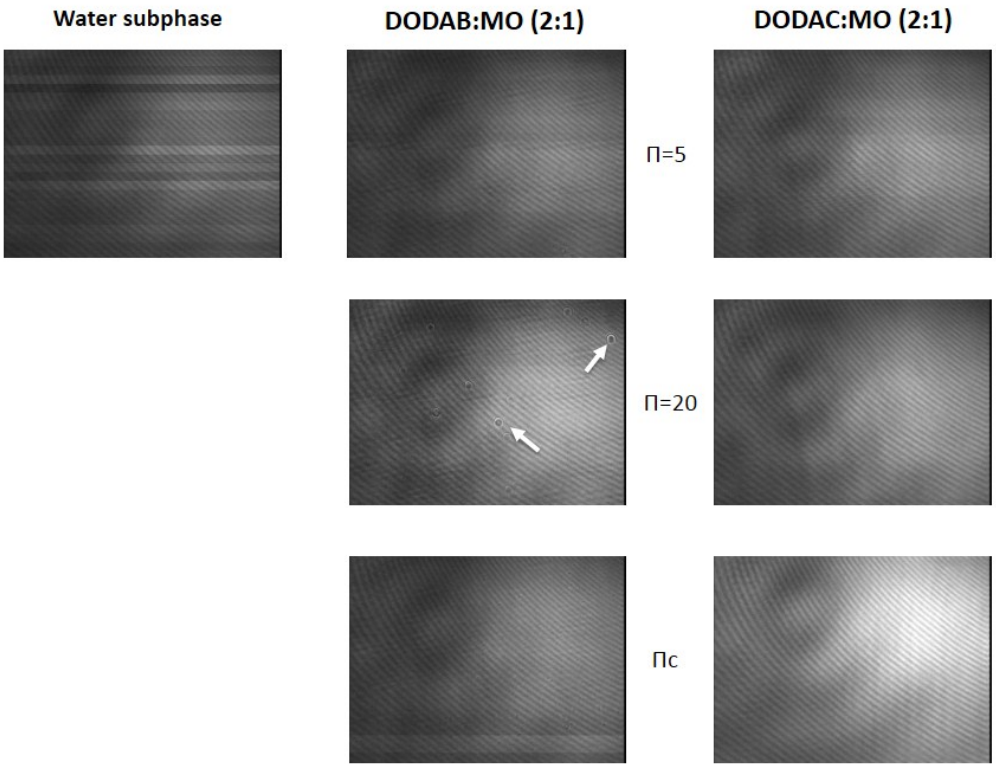

Fig. S4- BAM images of DODAB:MO and DODAC:MO (2:1) monolayer on an aqueous subphase at different pressures. Arrows indicate the presence of presence of small domains showing 'bubble-like' features in DODAB mixed monolayers that do not appear in DODAC mixed monolayers.