Why is SAXS a powerfull tool in GENE THERAPY?

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SAXS Workshop

1 October 2014
ALBA Synchrotron

Outline

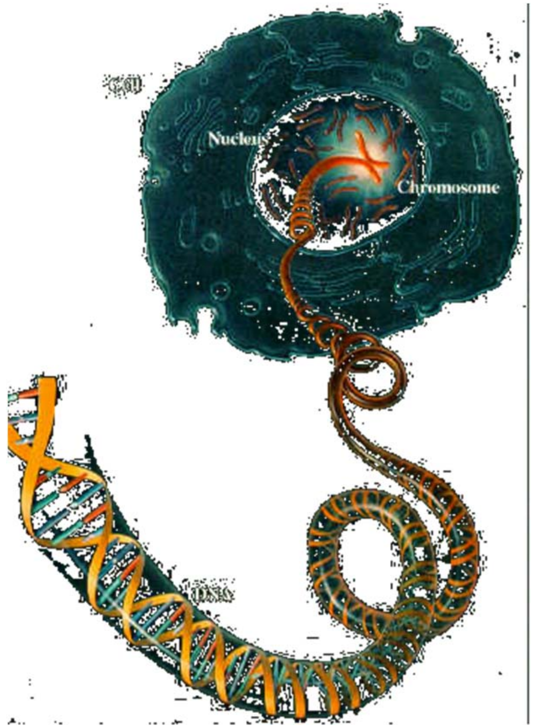
- Introduction: Gene therapy
- Application of SAXS in gene therapy
- Examples
  - ✓ Sample preparation
  - ✓ Results analyze

Introduction

- ***Genetic*** → quistic fibrosis, drepanocytic anaemia, haemophilia,

hypercholesterolemia

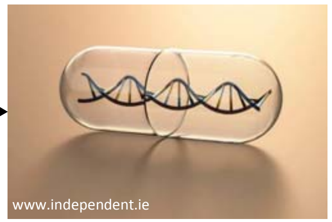
- ***Neurological*** → Alzheimer, Parkinson
- ***Cardiovasculars*** → arteriosclerosis, thrombosis, ischaemia, etc
- ***Infectious*** → AIDS, hepatitis B, herpes, etc.
- ***Tumors & cancers***
- ***Others*** → asthma, arthritis, diabetes, osteoporosis , etc



Genetic disorders

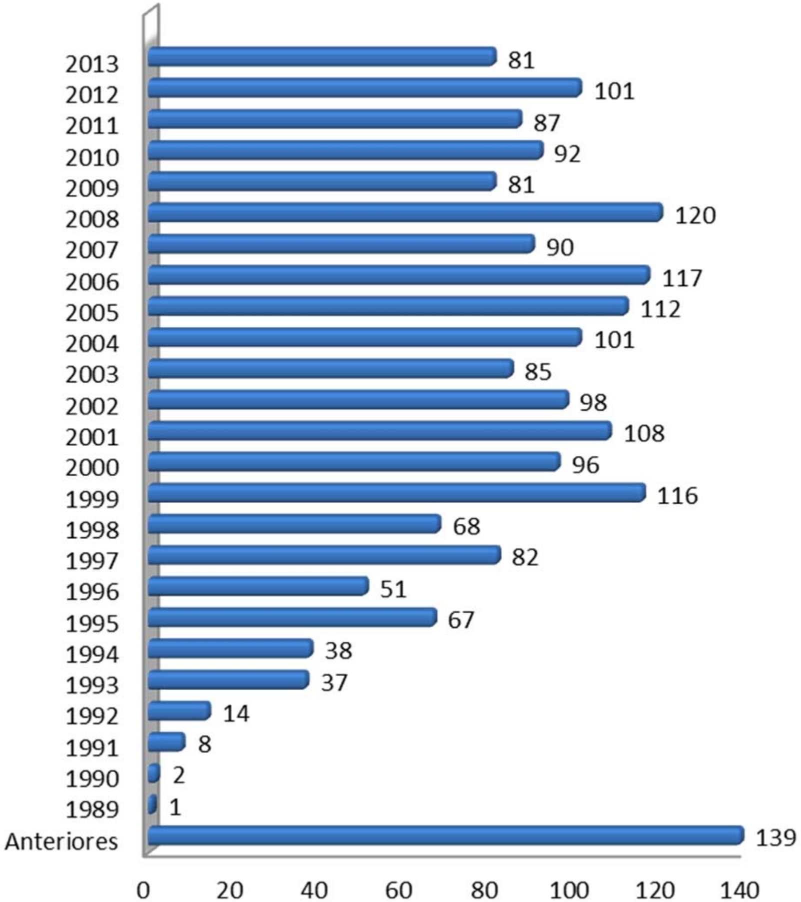
↓

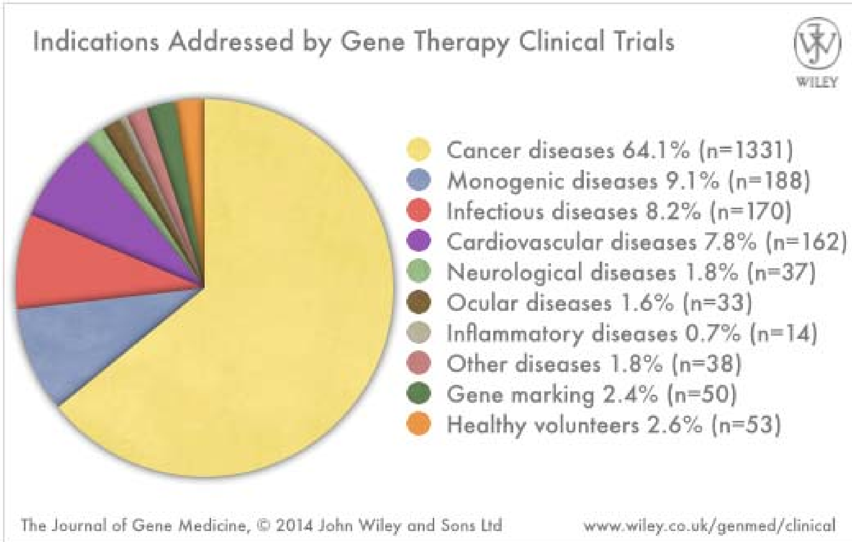
Gene Therapy



Introduction

Gene therapy clinical trials approved worldwide and indication addressed

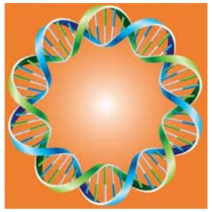


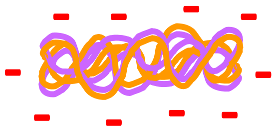


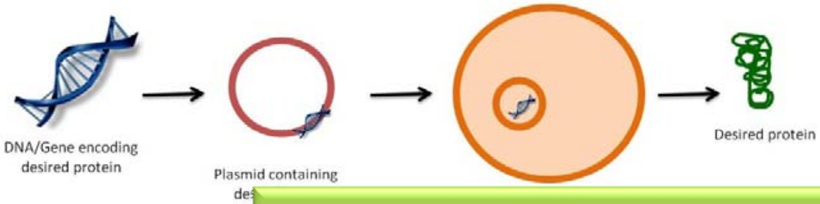
Introduction

Therapeutic agents based on different types of nucleic acids:

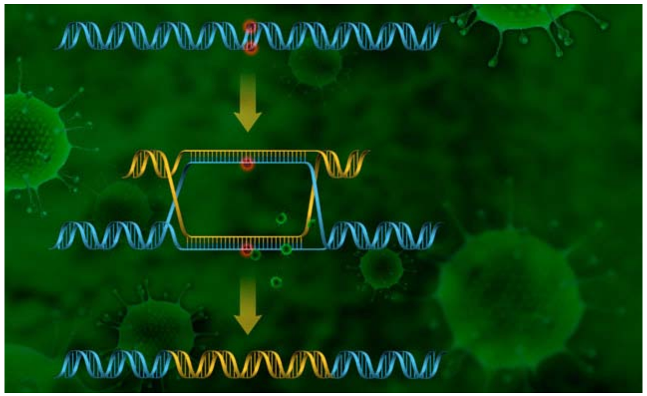
Plasmid DNA



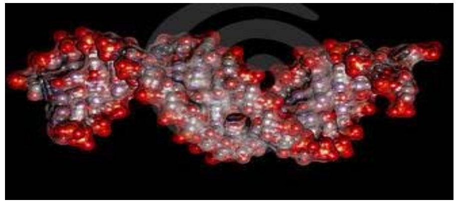


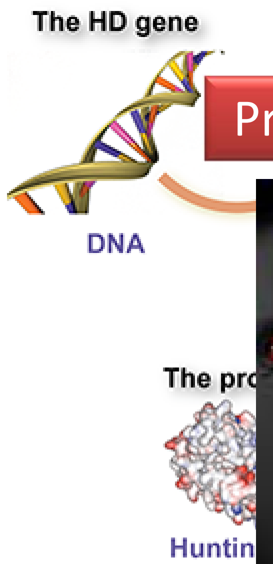


Produce a specific protein



siRNA

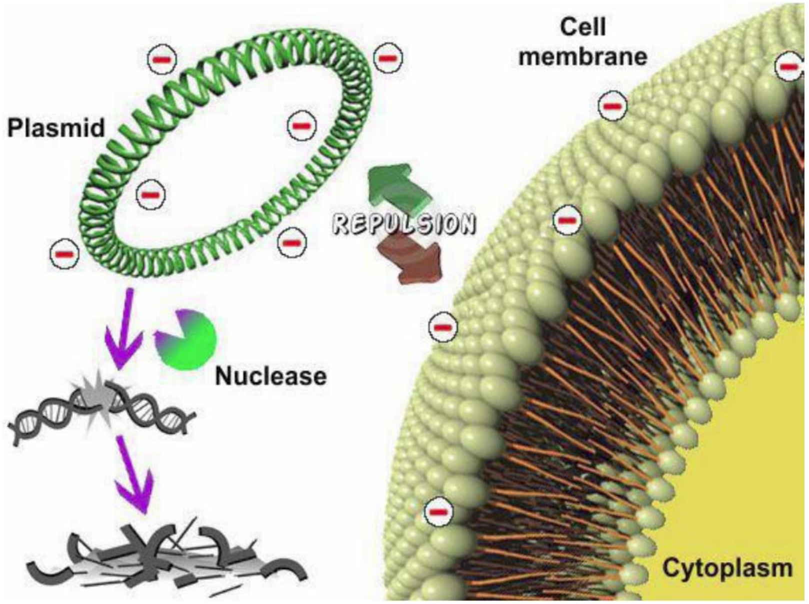




Protein knockdown

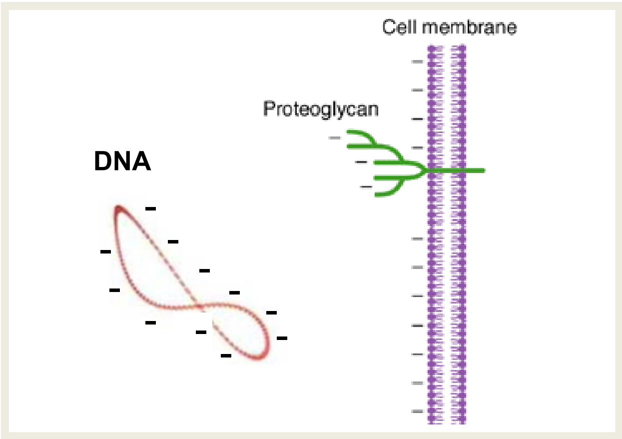
Introduction

Pharmacokinetic limitations of nucleic acid based therapeutic agents



The naked nucleic acid present low Transfection levels

- ✓ Nucleic acid are easily degraded by nucleases
- ✓ Membrane crossing abilities are limited by charge



Vector assisted techniques

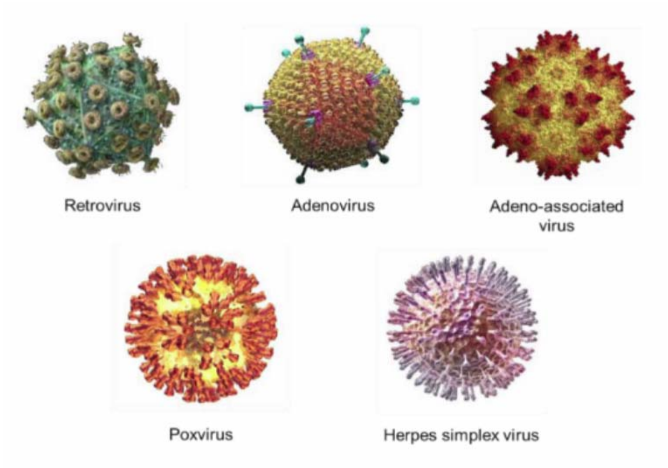
Gene carrier systems (vectors) should:

- ✓ Provide the nucleic acid protection
- ✓ Enable cellular uptake and delivery in a cellular target
- ✓ Promote it´s biological action

Introduction

GENE VECTORS

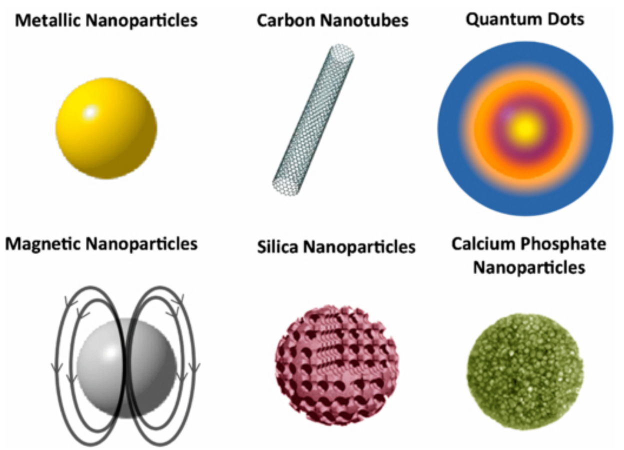
Viral vectors



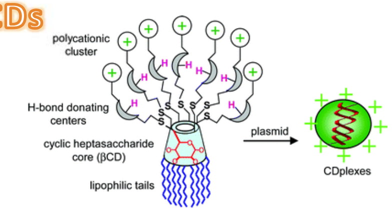
- ✓ Effectiveness
- ✓ Immunogenicity,
- ✓ Oncogenicity
- ✓ Potential virus recombination
- ✓ Costly production

Non-viral vectors

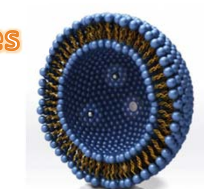
NPs



CDs



Liposomes

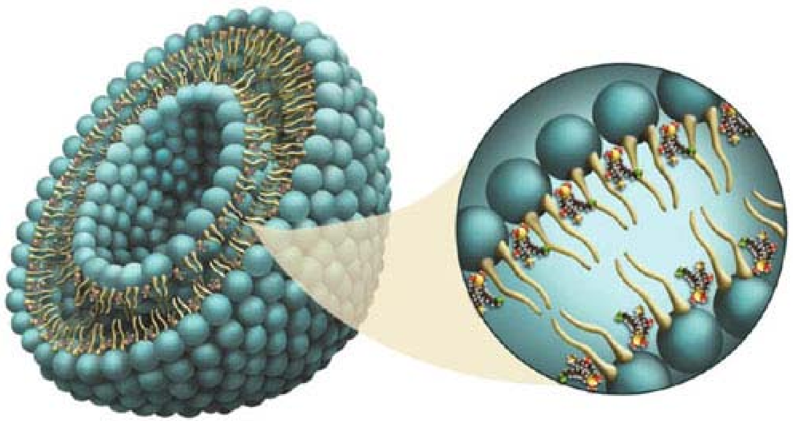


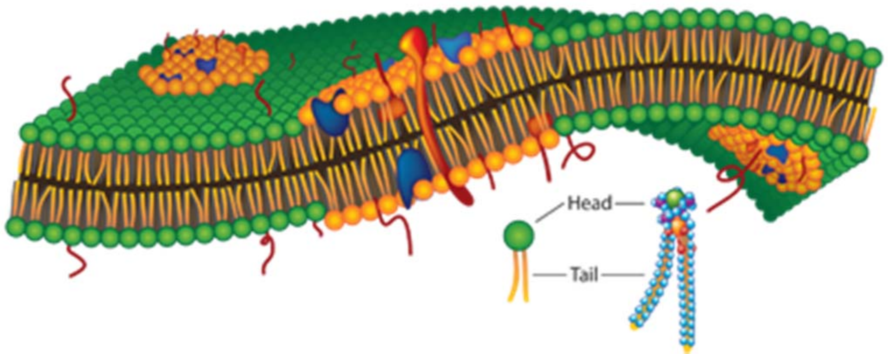
http://www.izon.com/

- ✓ Nula immune response
- ✓ Easy production
- ✓ Transport large size nucleic acids
- ✓ Show high toxicity levels
- ✓ Low Transfection levels

Liposomes

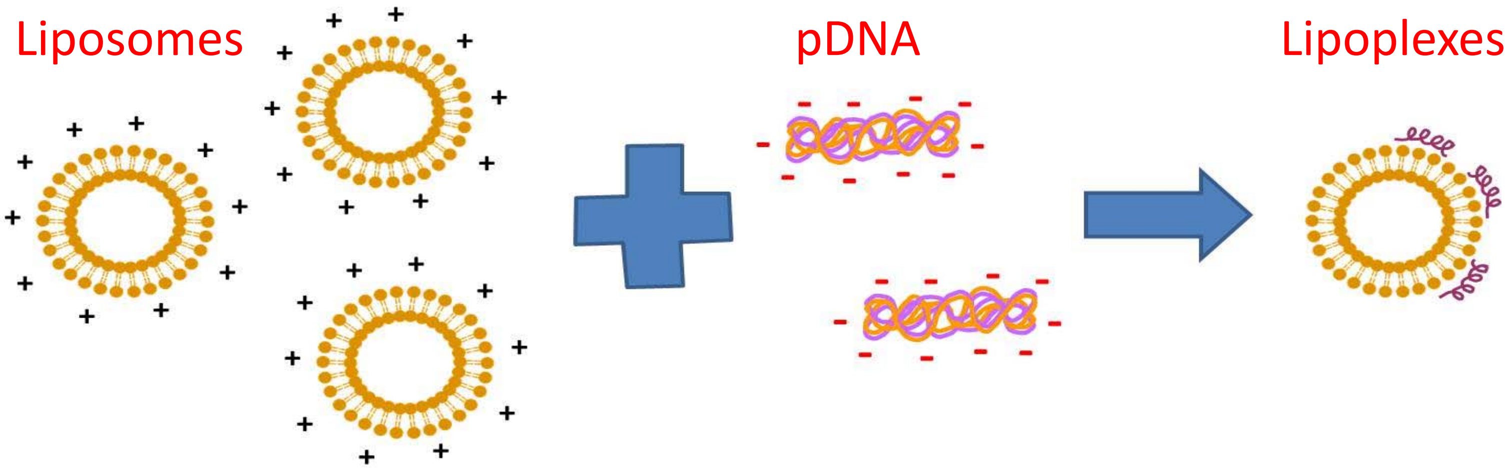
- Great similarity with the cell membrane
- Cationic liposomes are able to compact the DNA
- Cheaper production

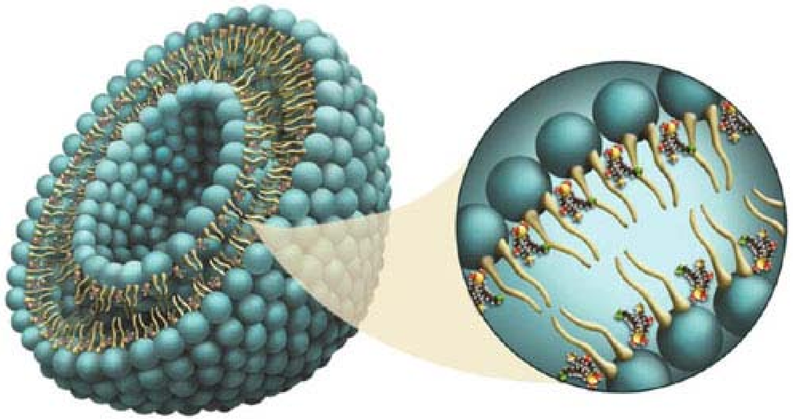




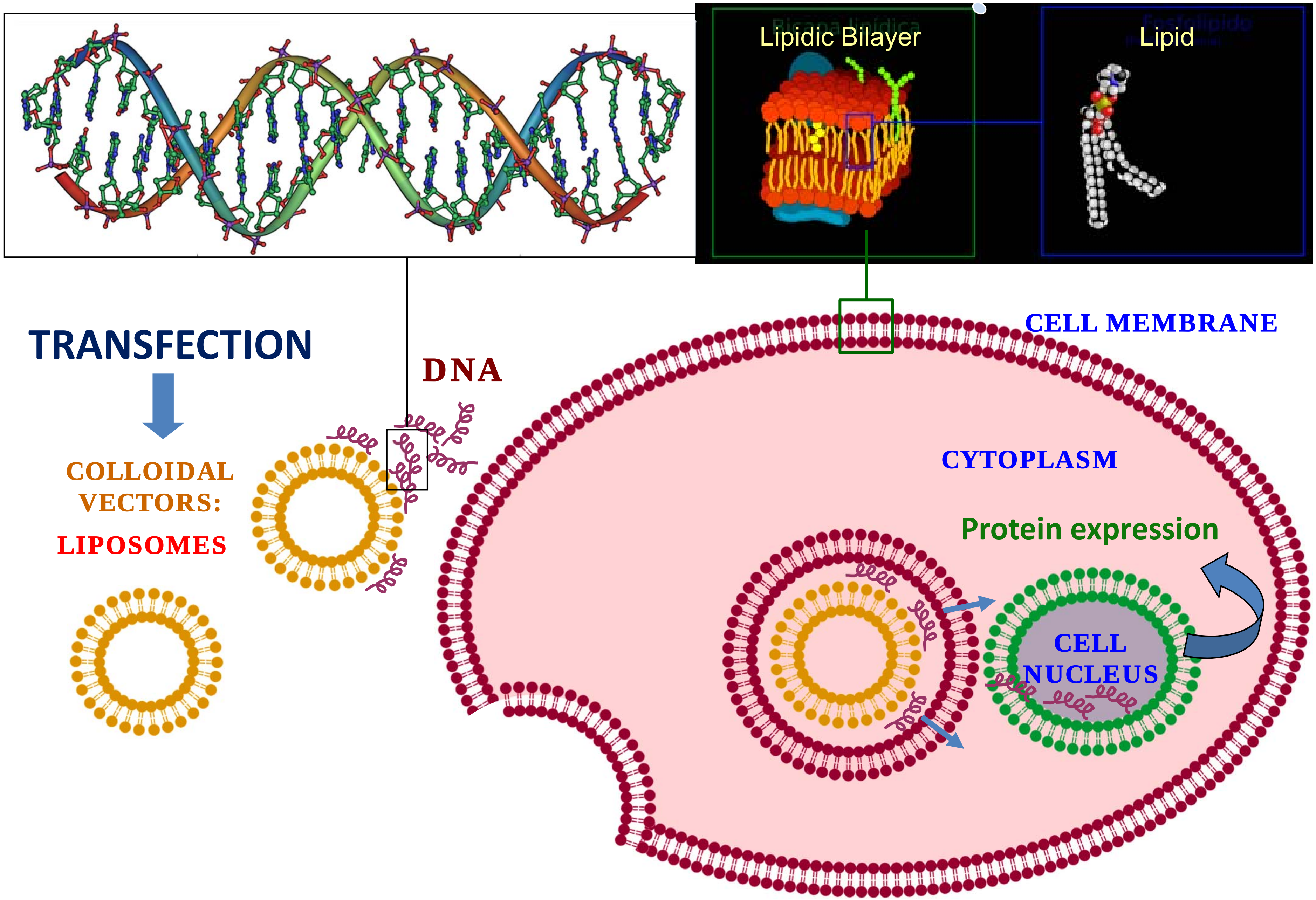
Introduction

Liposomes

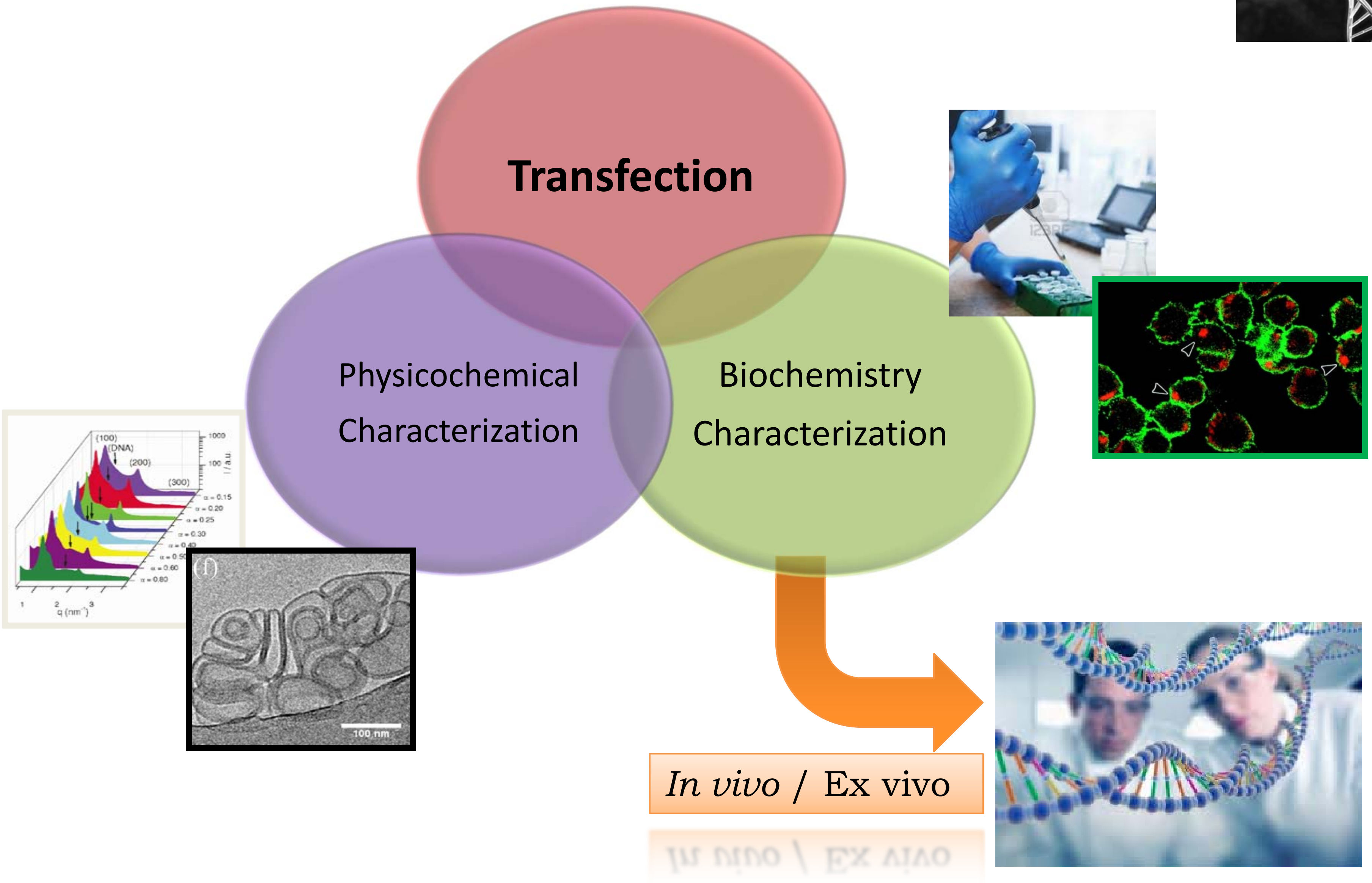




Introduction

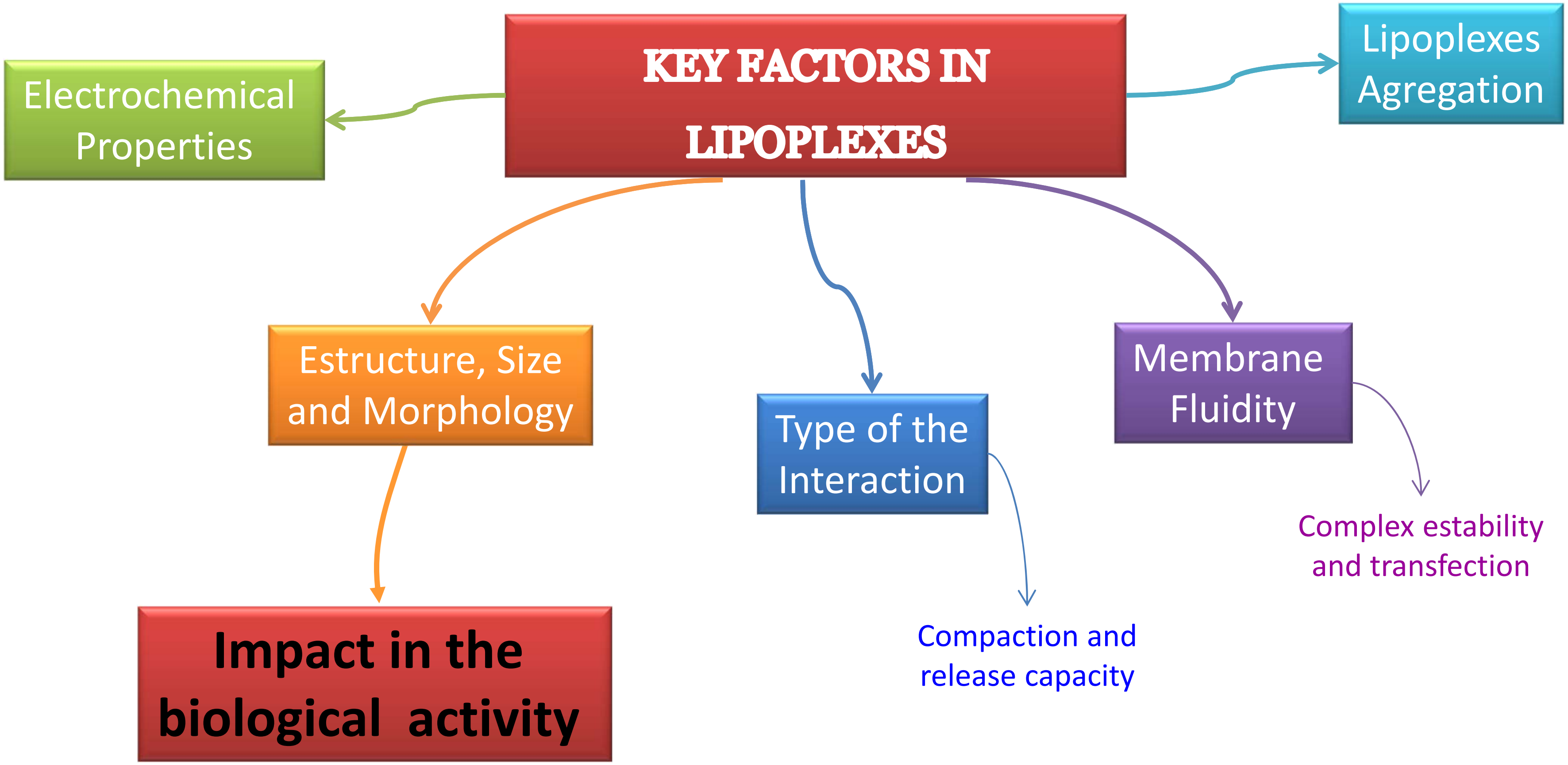


Introduction



Introduction

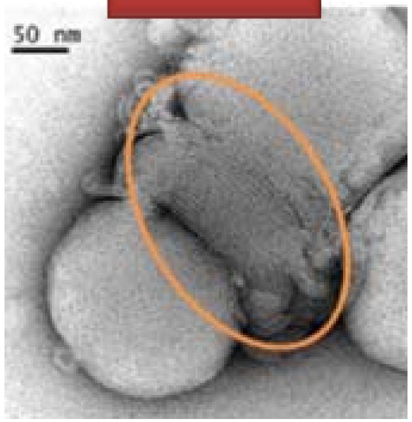
Physicochemical Characterization



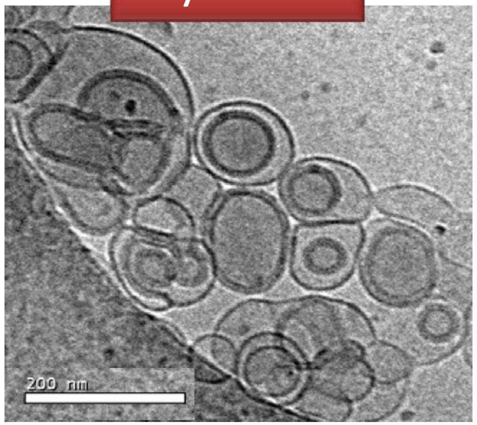
Introduction

Different techniques to determine:
Size, morphology and structure

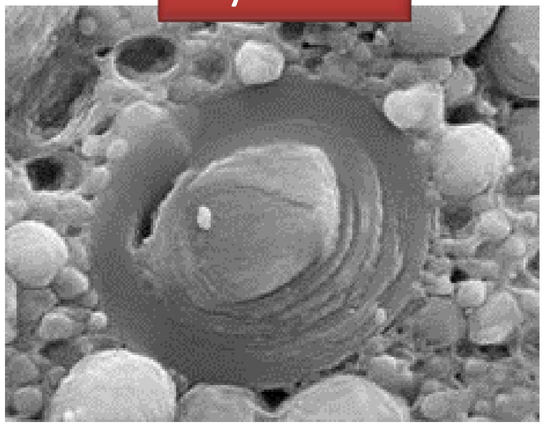
NS-TEM



Cryo-TEM

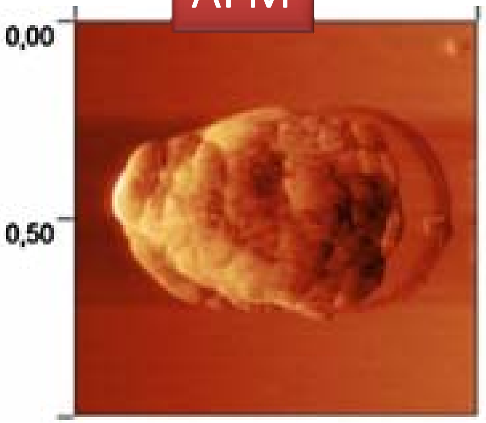


Cryo-SEM

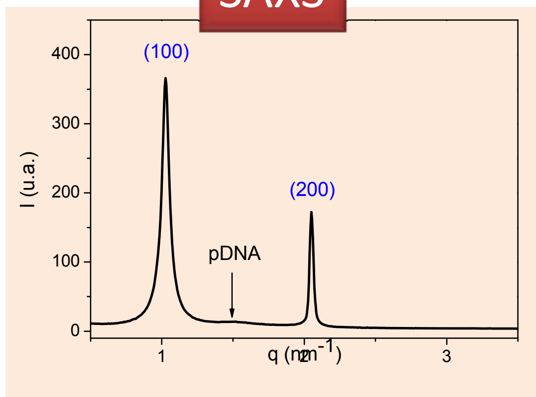


Freeze-Fracture

AFM

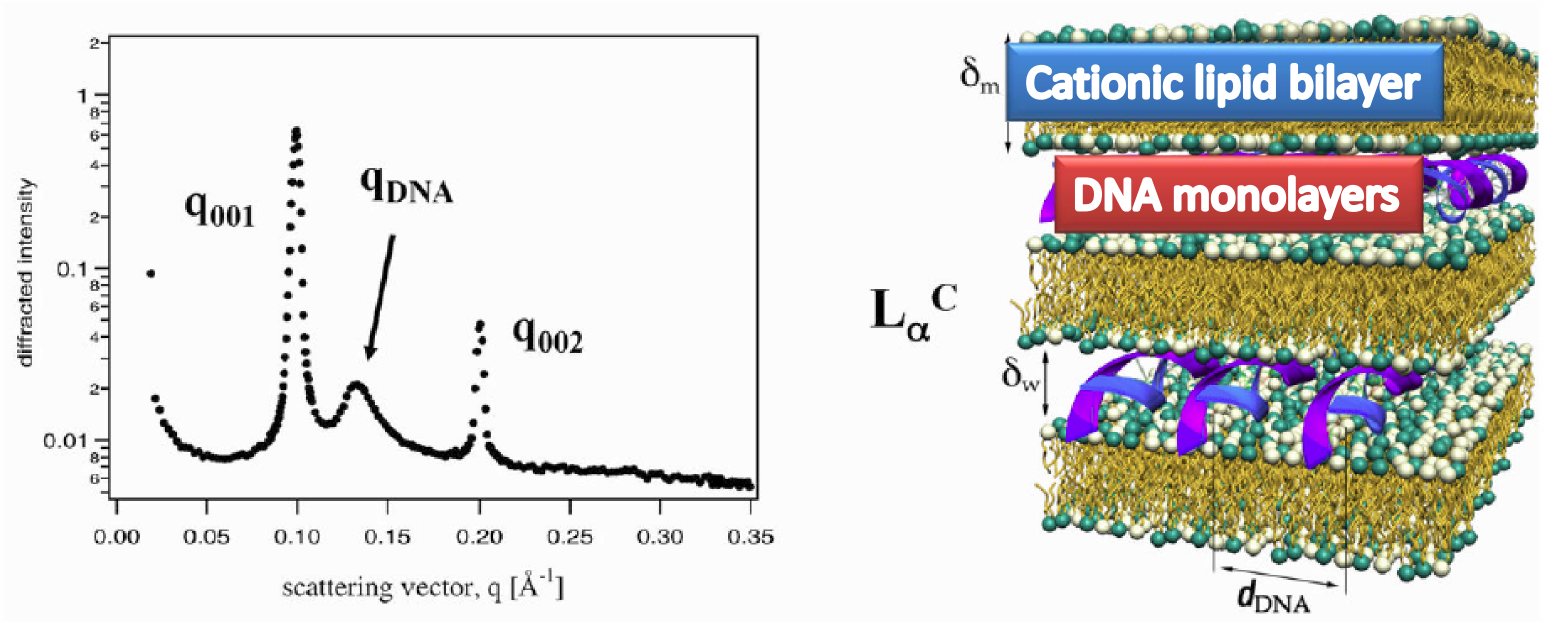


SAXS



Introduction

Self-assembly structure of CL-DNA complexes: Multilamellar structure L_α

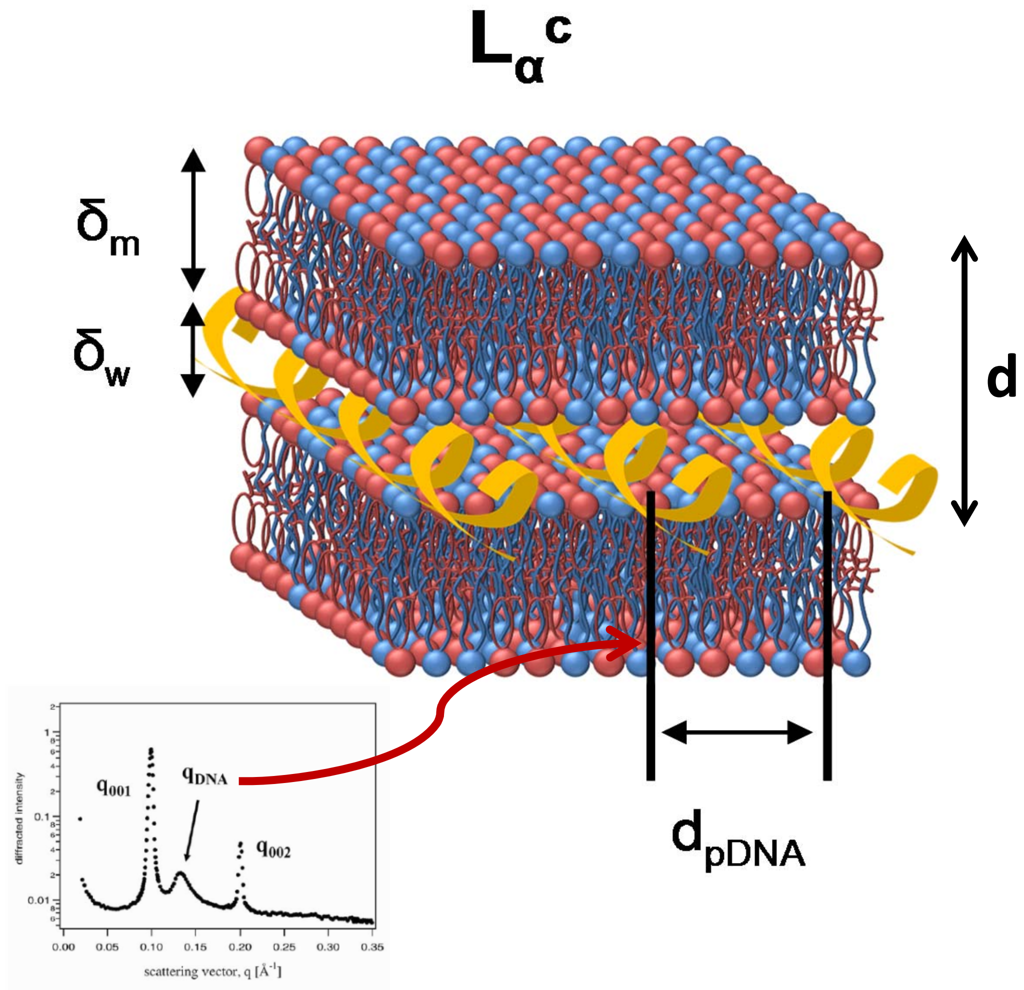


Where the cationic lipid liposomes consisted of mixtures of neutral (so called "helper lipid") DOPC and cationic DOTAP and λ-phage DNA α =0.5

Safinya et al. Nature 1997

Introduction

Self-assembly structure of CL-DNA complexes:
Multilamellar structure L_α



$$d = \frac{2\pi}{q_{100}}$$

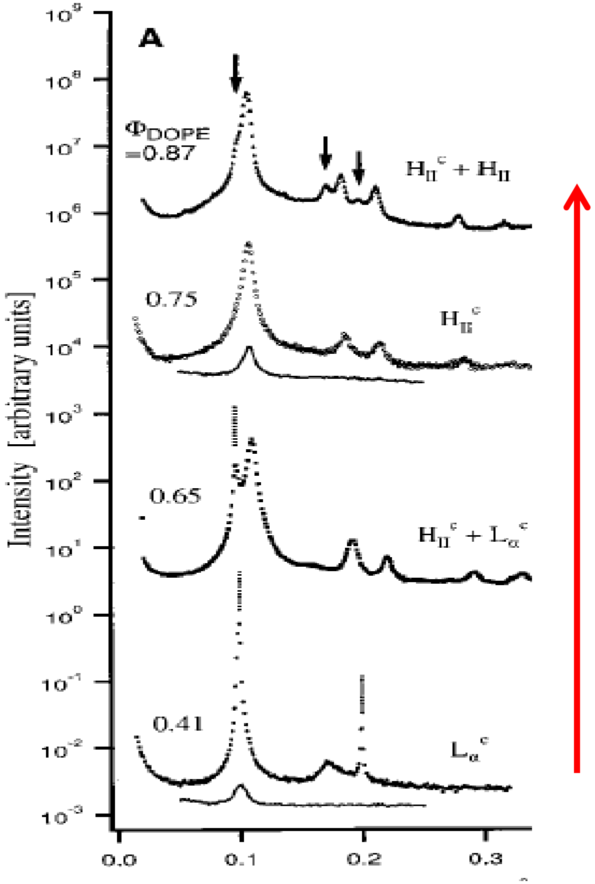
$$d = \delta_m + \delta_w$$

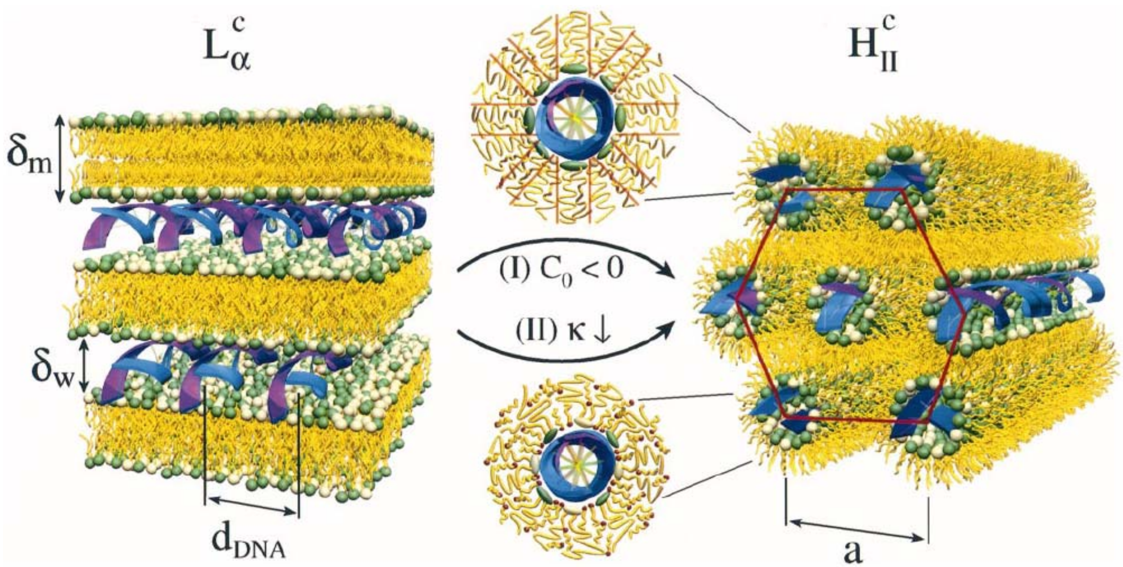
$$d_{pDNA} = \frac{2\pi}{q_{pDNA}}$$

Introduction

Self-assembly structure of CL-DNA complexes: Hexagonal structure H_{II}

DOPE/DOTAP-DNA

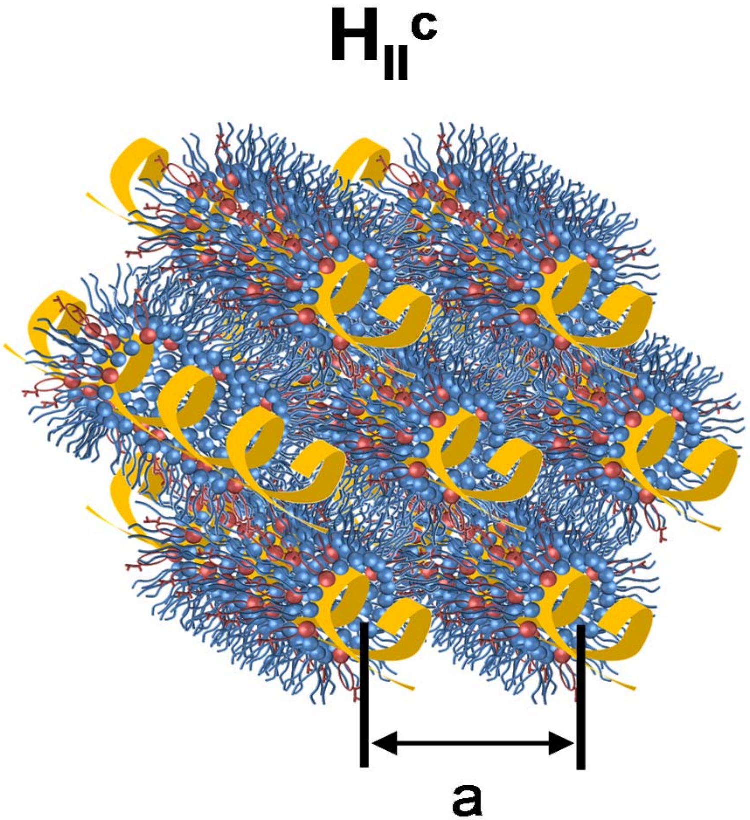




Safinya et al. Nature 1998

Introduction

Self-assembly structure of CL-DNA complexes: Hexagonal structure H_{II}



$$a = d_{DNA} = \frac{4\pi}{3^{(1/2)} q_{10}}$$

Introduction

Effect of lipoplexes structure in the transfection efficiency

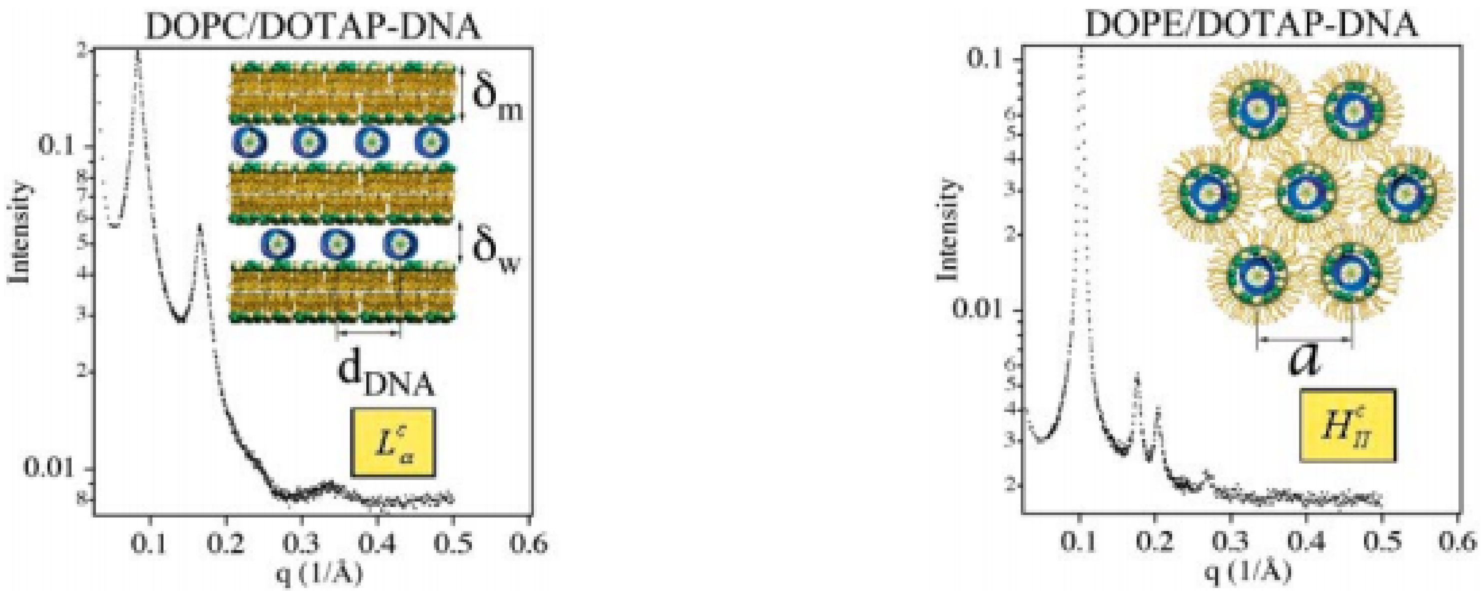


FIGURE 2 Comparison of structure and transfection efficiency (TE). Left (mole fraction $\Phi_{DOPC} = 0.67$) shows a typical XRD scan of lamellar (*inset*) L_{α}^{C} complexes. Right (mole fraction $\Phi_{DOPE} = 0.69$) shows a typical XRD scan of inverted hexagonal (*inset*) H_{II}^{C} complexes. Middle displays the corresponding TE, as measured by luciferase enzyme assays of transfected mouse L-cells.

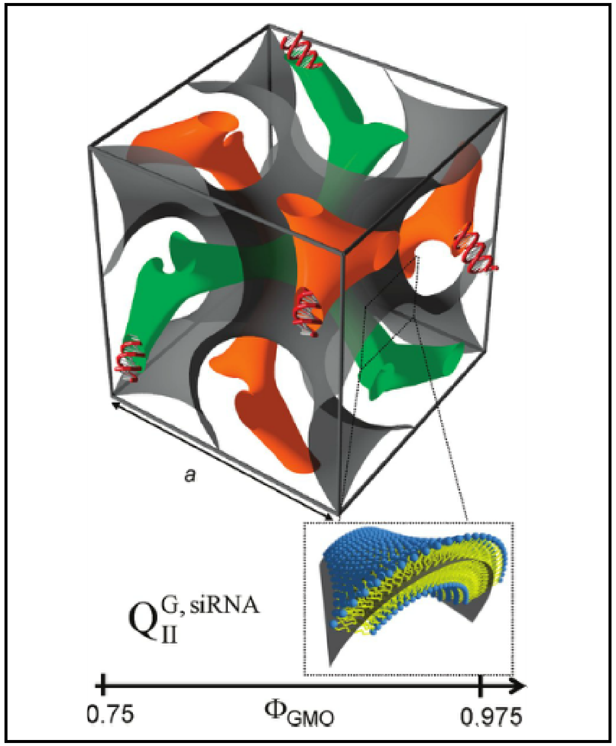
Safinya et al., Biophysical Journal, 2003

Introduction

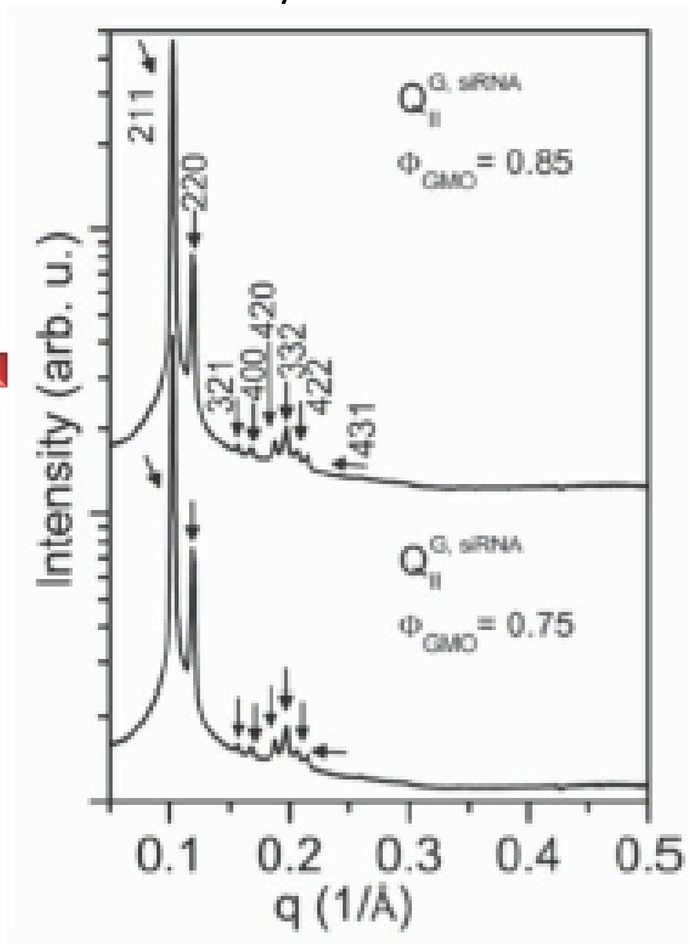
Self-assembly structure of CL-siRNA complexes:

Cubic structure Q_{II}

GMO/DOTAP



GMO/DOTAP-siRNA



Safinya et al. JACS 2010

$$a = \frac{2\pi}{q}\sqrt{h^2 + k^2 + l^2}$$

Introduction

Why use synchrotron SAXS?

- Syncrotron light sourses
- High intentity
- High collimation (small divergence)
- Wide wavelength tunability

Parameter	SAXS	Synchrotron SAXS
Resolution	Low	High
Time of measurement	Minute to hours	5-30 s
Vacuum	Yes	Not

Our work

Our group is involved in the physicochemical and biochemical characterization of non-viral gene vectors, in order to find better gene carries systems.

- Electrochemical properties
- Fluidity bilayers properties
- Influences of the nanostructure &
- Biological methods for the evaluation of transfection efficiency

Examples

Cationic mixed liposomes + DNA

- ✓ DC-Chol/DOPE-ctDNA
- ✓ $C_6(LL)_2$/DOPE-pDNA
- ✓ $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA
- ✓ $(C_{16}Im)_2(C_2O)_n$/DOPE-pDNA

Anionic mixed liposomes +Ca^{2+}+DNA

- ✓ DOPG/DOPE-Ca^{2+}-pDNA
- ✓ DOPS/DOPE-Ca^{2+}-pDNA

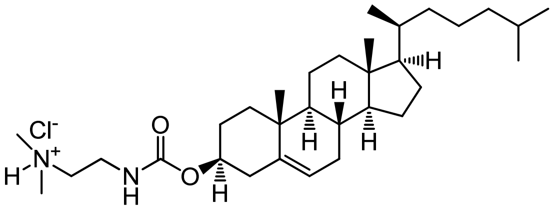
Cationic mixed liposomes + siRNA

- ✓ $(C_{16}Im)_2(C_2O)_n$/DOPE-siRNA

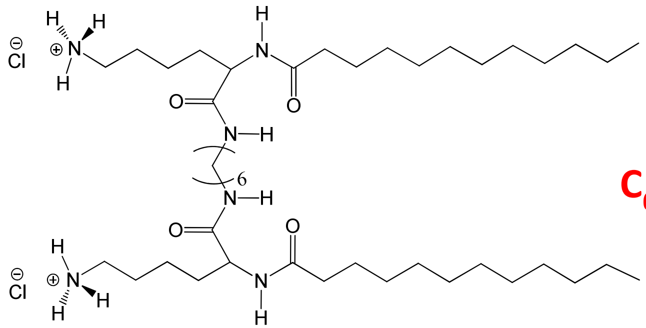
Materials

CATIONIC LIPIDS

DC-Chol



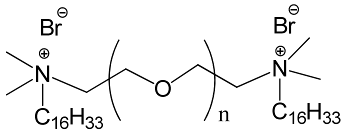
Gemini Lipids



$C_6(LL)_2$

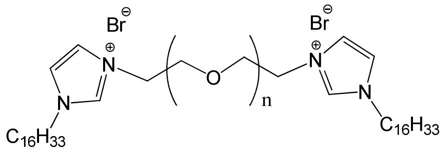
$(C_{16}Am)_2(C_2O)_n$

n= 1, 2 y 3



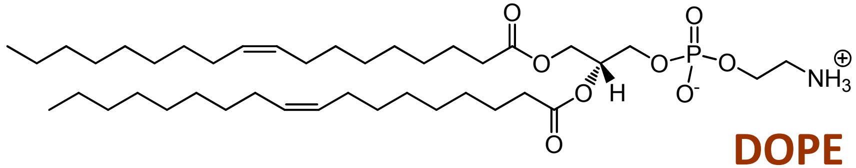
$(C_{16}Im)_2(C_2O)_n$

n= 1, 2 y 3



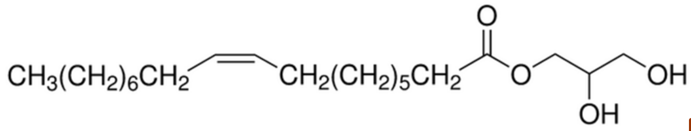
ZWITTERIONIC LIPID

(null charge at pH=7.4)



DOPE

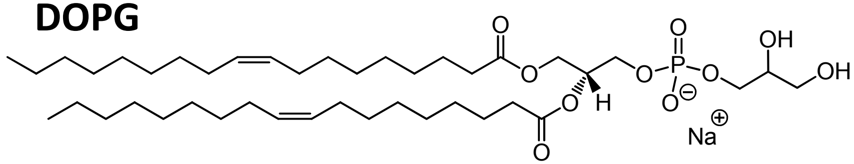
NEUTRAL LIPID



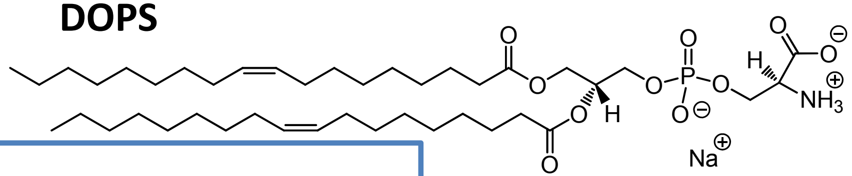
MOG

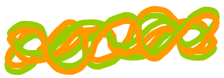
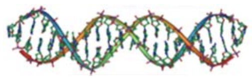
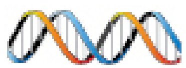
ANIONIC LIPIDS

DOPG



DOPS



Plasmid (pDNA) pEGFP-C3	ctDNA	siRNA
		

Sample preparation

Key factors in lipoplexes preparation

Molar fraction composition, α

$$\alpha = \frac{L^{+/-} / M_{L^{+/-}}}{(L^{+/-} / M_{L^{+/-}}) + (L^{0} / M_{L^{0}})}$$

Charge Ratio, ρ

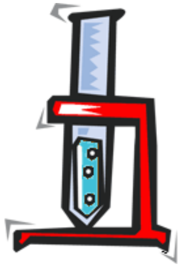
$$\rho = \frac{n^{+}}{n^{-}} = \frac{q^{+}_{L^{+}} L^{+} / M_{L^{+}}}{q^{-}_{DNA} D / \overline{M}_{bp}}$$

$\rho > 1$

Experiments were done at in HEPES buffer 40 mM at pH = 7.4

Sample preparation

$L^+ + L^0$ —Et-OH→

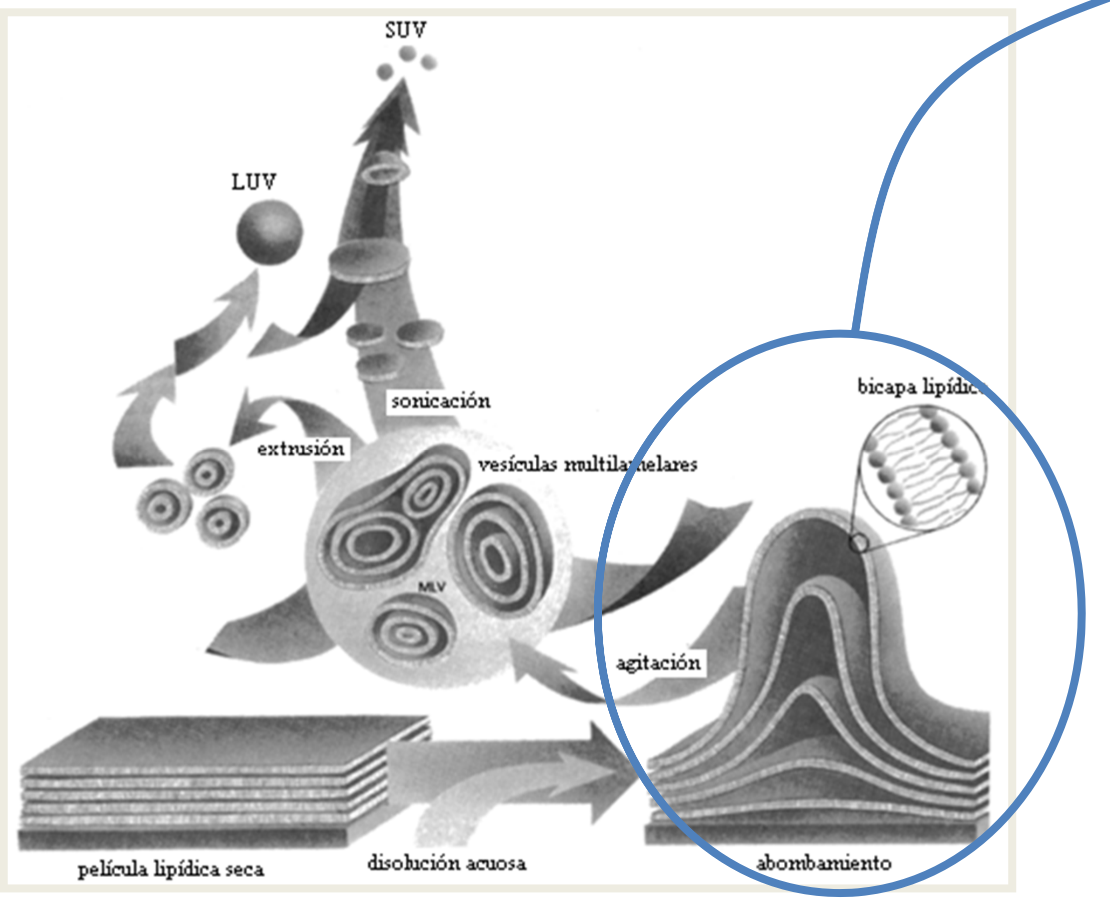


→ Et-OH → *Film*

HIDRATED → ***EXTRUSION***

- *Vortex*
- *Sonication*
- *Temperature*
- *HEPES 40mM*

- *35ºC*
- *400-200nm*

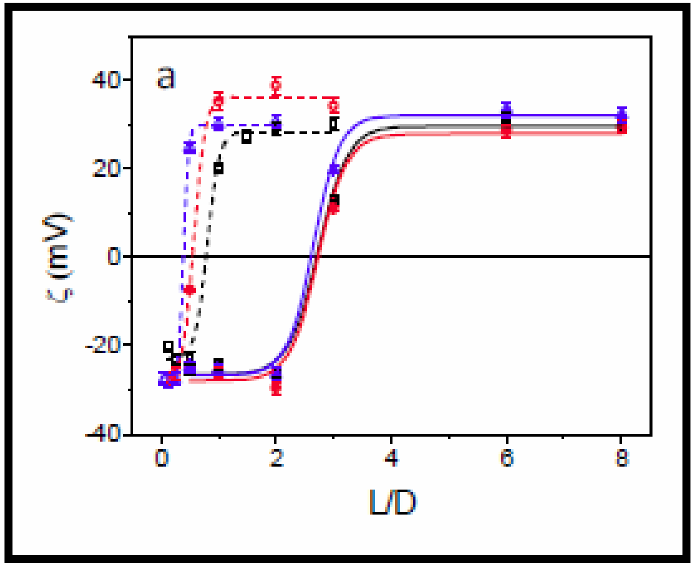


Mixed Liposome

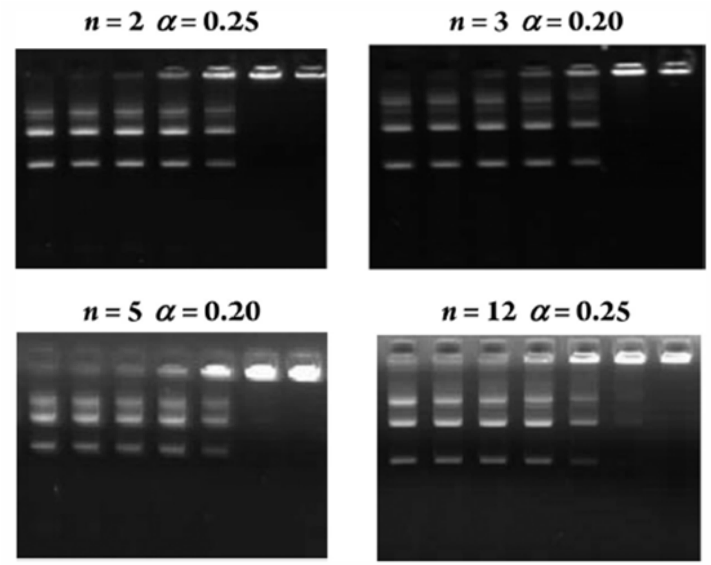
Sample preparation

Before the capillary sample preparation, complete electrochemical studies have been carried out in order to obtain the optimal condition for SAXS

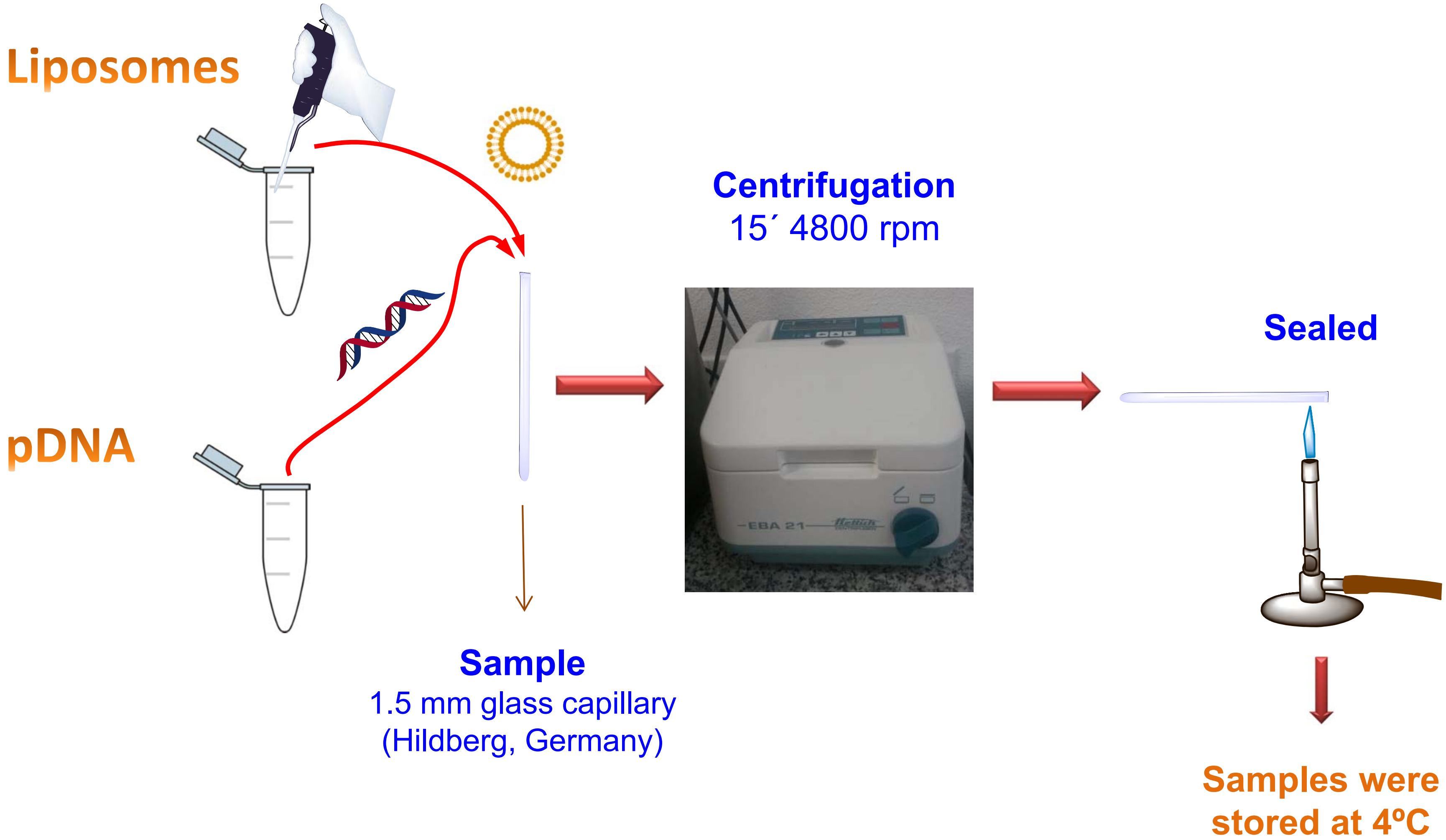
ζ Potential



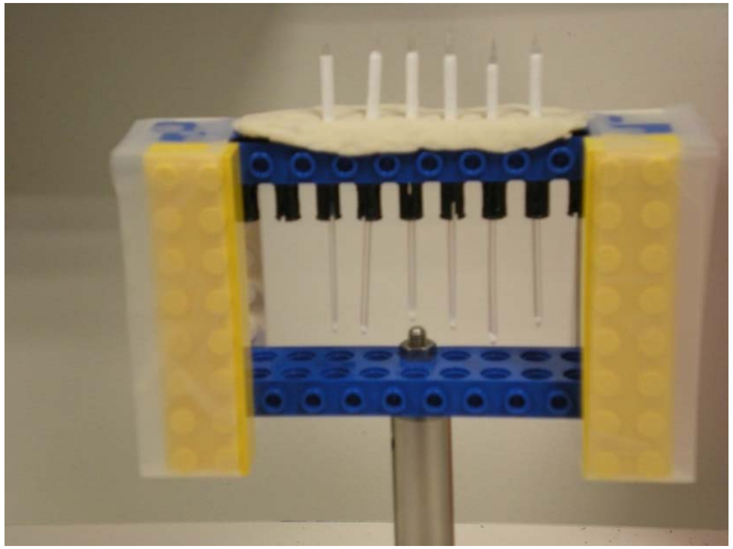
Gel Electrophoresis

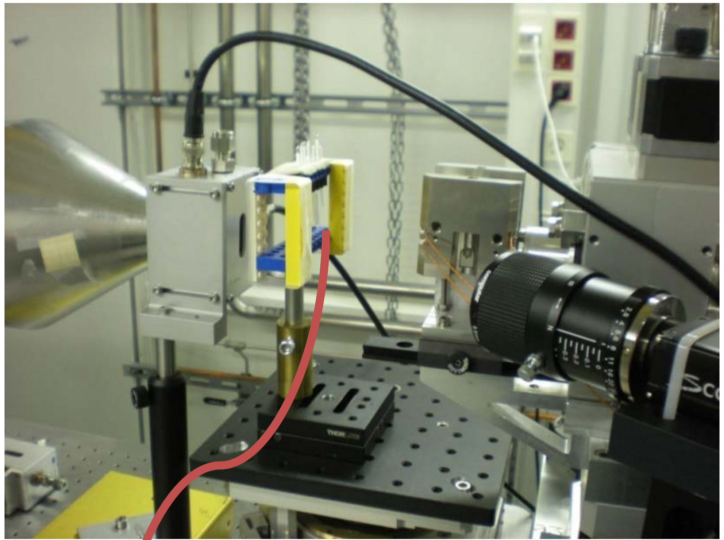


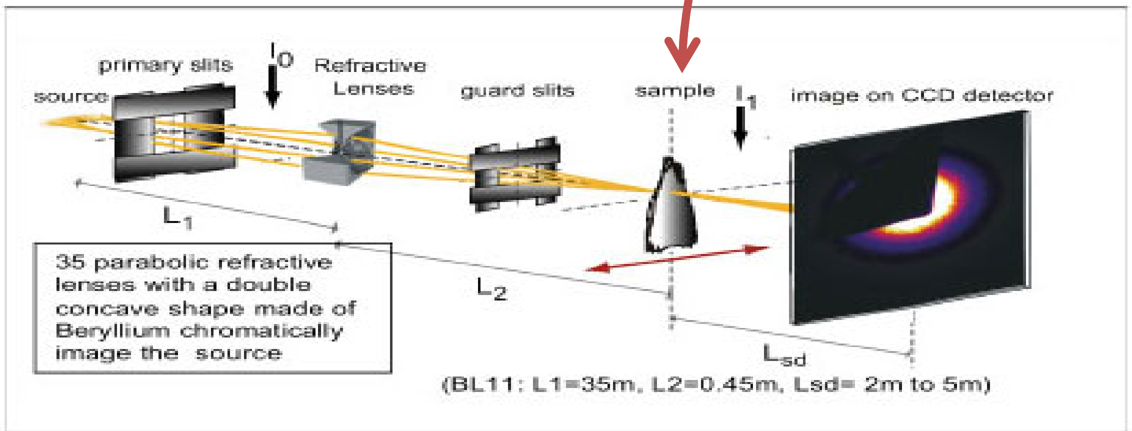
Sample preparation



In ALBA





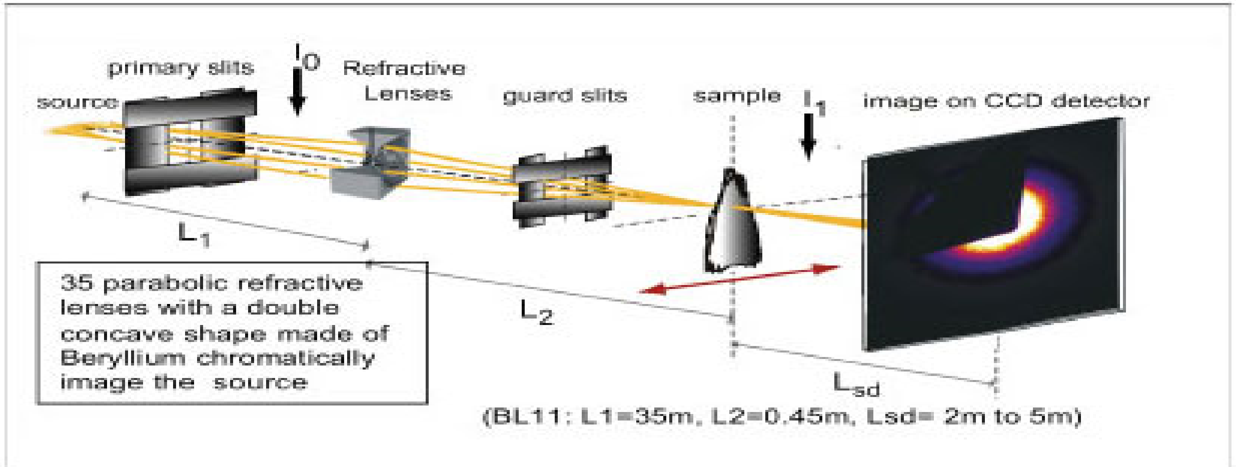

primary slits
I_0
Refractive Lenses
guard slits
sample
I_1
image on CCD detector
source
L_1
L_2
L_{sd}
35 parabolic refractive lenses with a double concave shape made of Beryllium chromatically image the source
(BL11: L1=35m, L2=0.45m, Lsd= 2m to 5m)

Measurement conditions

BL11-NCD beamline at the ALBA synchrotron

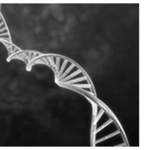
Beam characteristics

- ✓ **Energy of the incident**: 12.6 KeV (λ) 0.995 Å
- ✓ **Beam size:** 100 μm
- ✓ **Sample-to-detector distance:** 1.4 m
- ✓ **Detector:** Quantum 210r CCD



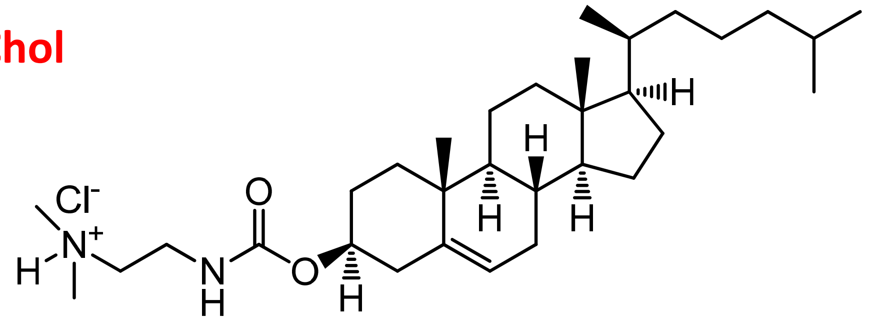
Results

Example 1: DC-Chol/DOPE

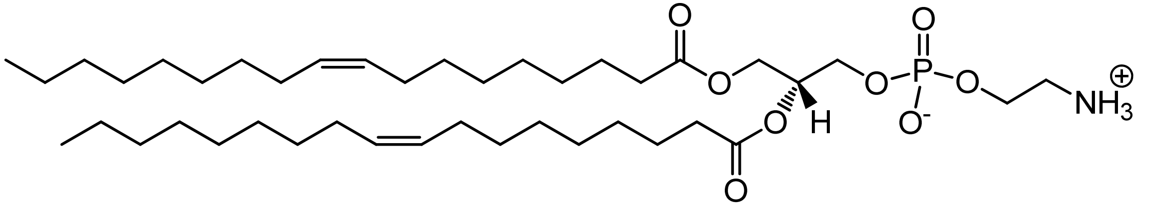


LIPOSOMES MIXED

DC-Chol

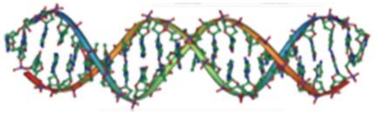


DOPE

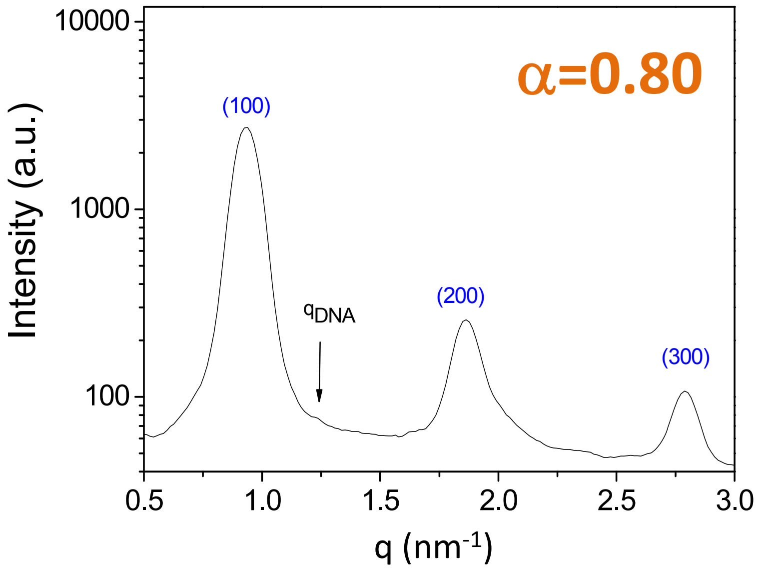


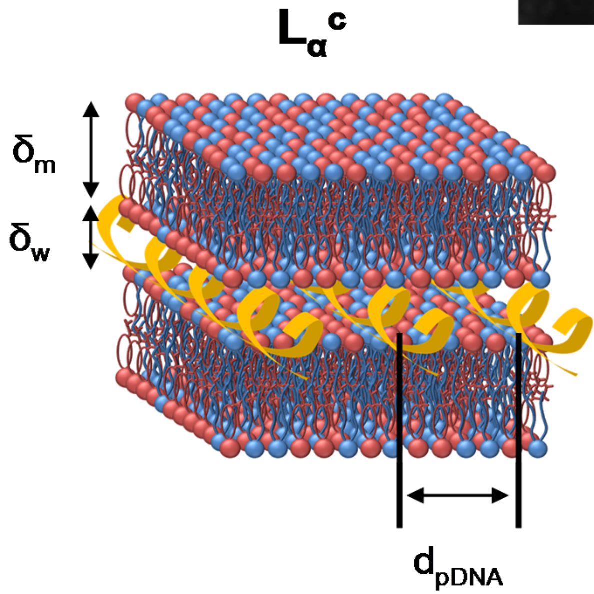
DNA

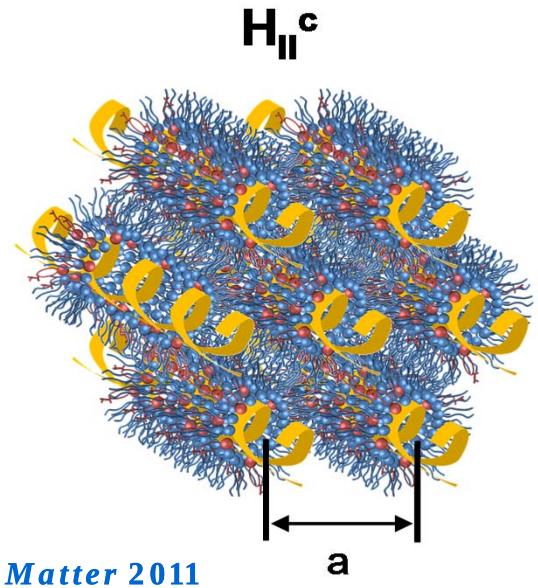
ctDNA

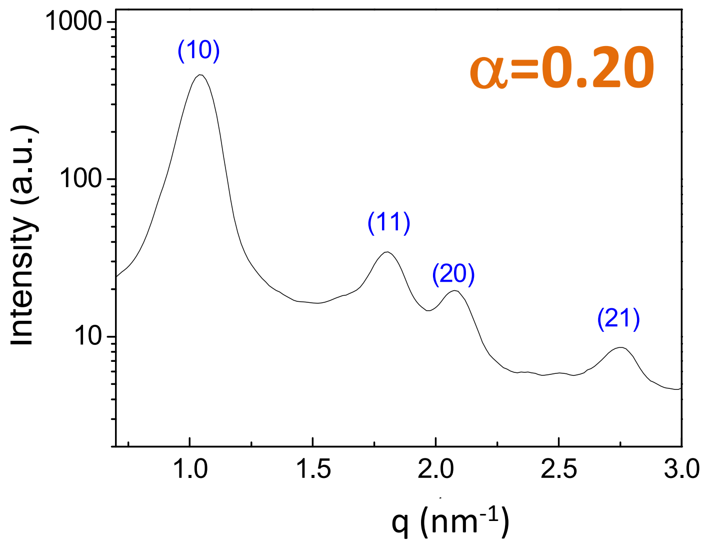


Results: DC-Chol/DOPE









Soft Matter 2011

MULTILAMELLAE FORMATION

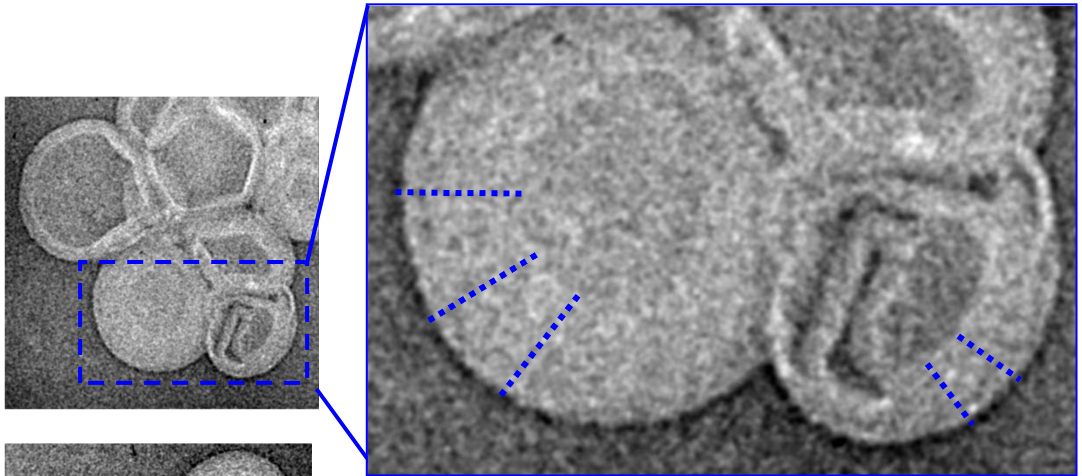
MULTILAMELLAE ANALYSIS → *EMAN Software*

- Image processing
- Noise elimination

Flattened lipoplexes stack to a template lipoplex

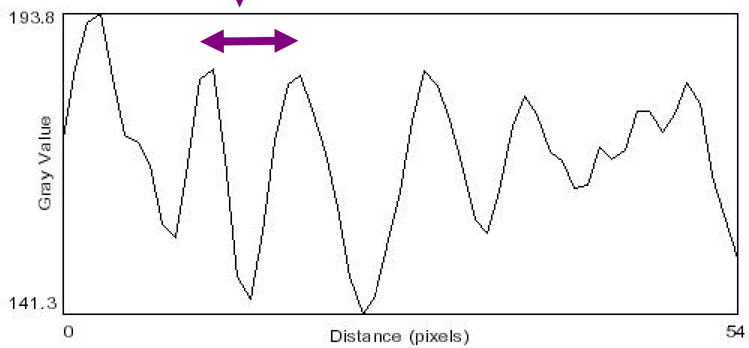
DC-Chol/DOPE-DNA

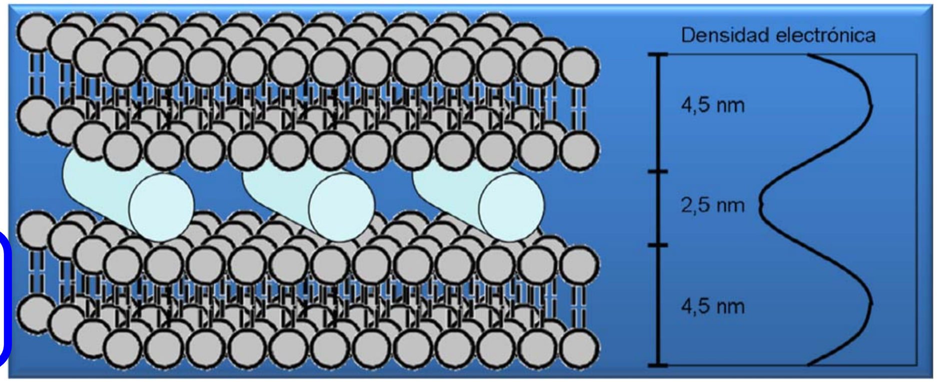
***J. Phys. Chem. B* 2009**



Periodicity **7 nm**

REPETITIVE BEHAVIOR



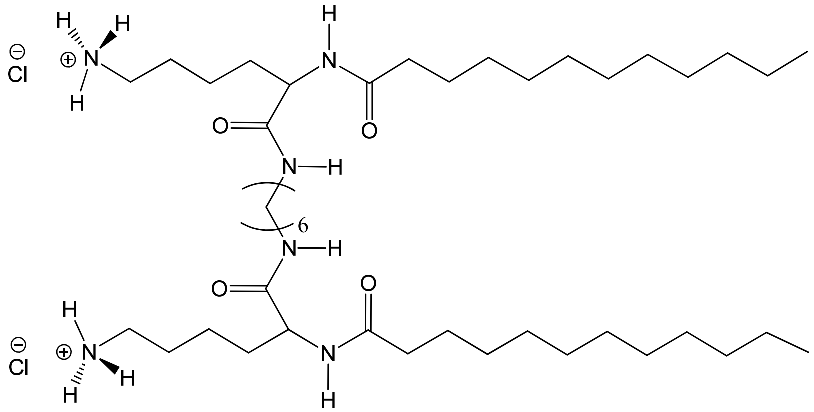


Periodicity **7 nm** = Lipidic bilayer **4.5 nm** + DNA **2.5 nm**

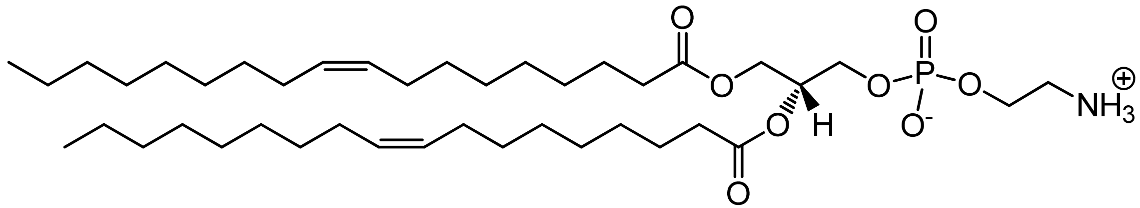
Example 2: $C_6(LL)_2$/DOPE

LIPOSOMES MIXED

$C_6(LL)_2$



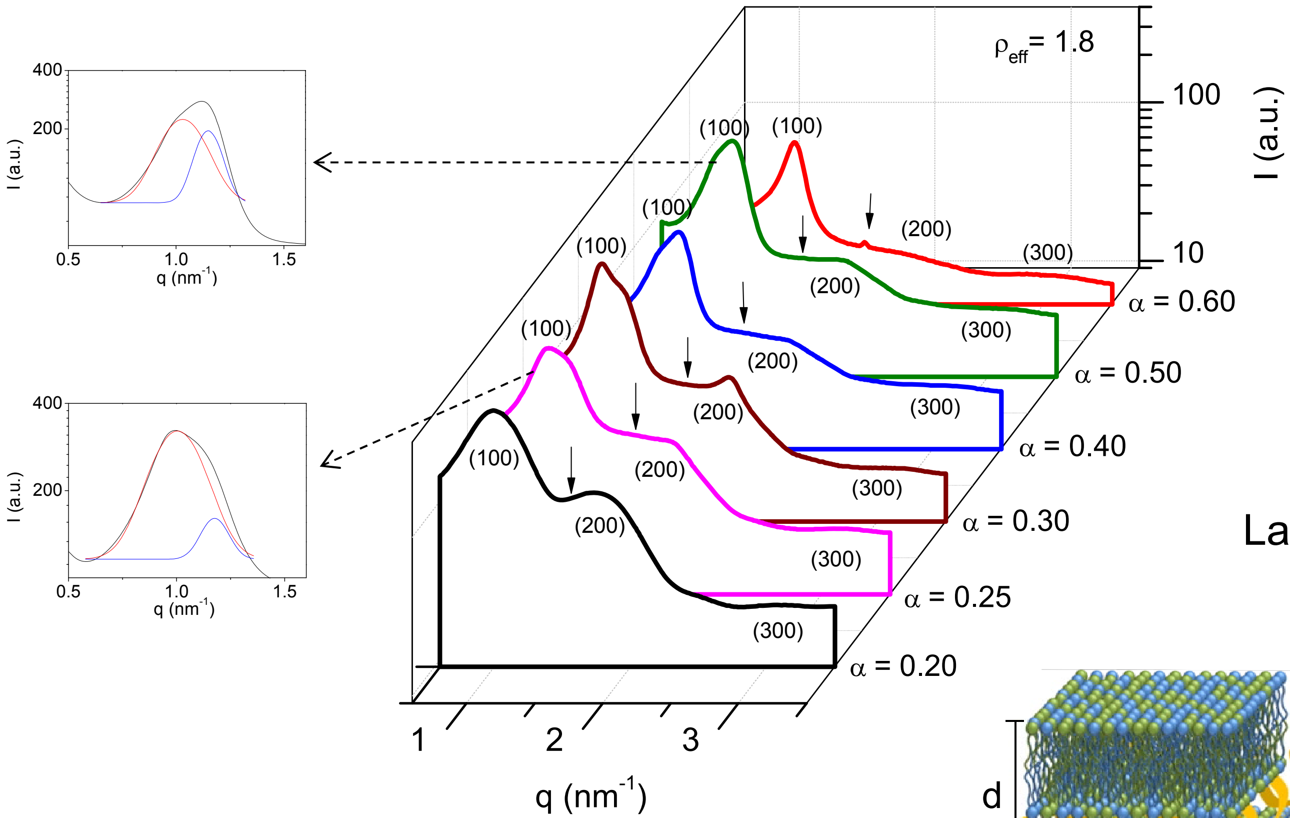
DOPE



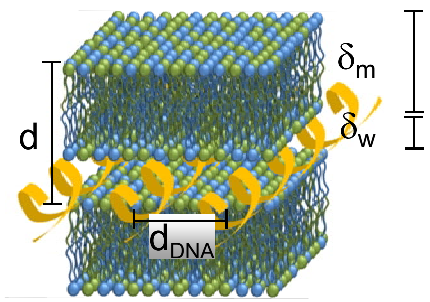
DNA

Plasmid (pDNA)
pEGFP-C3

Results: $C_6(LL)_2$/DOPE-pDNA



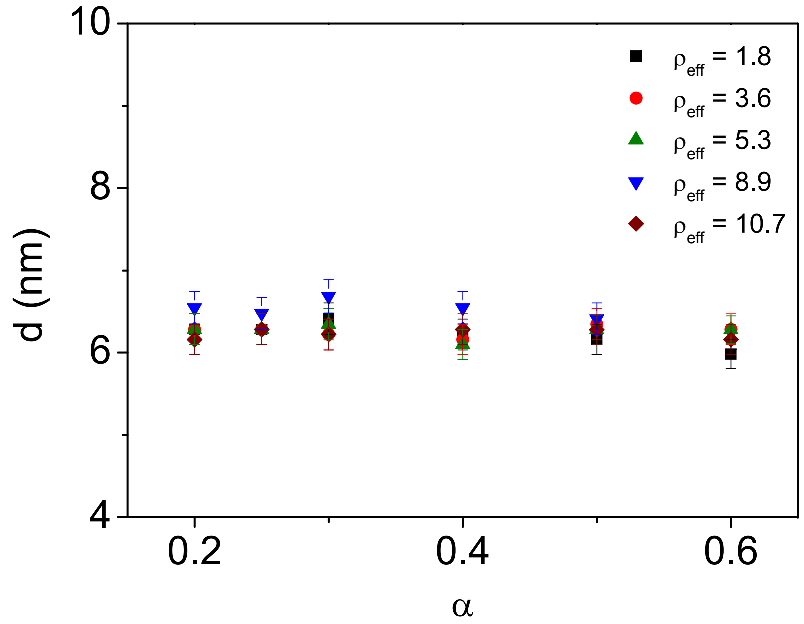
Lamellar L_α



$$d = \frac{2\pi}{q_{100}}$$

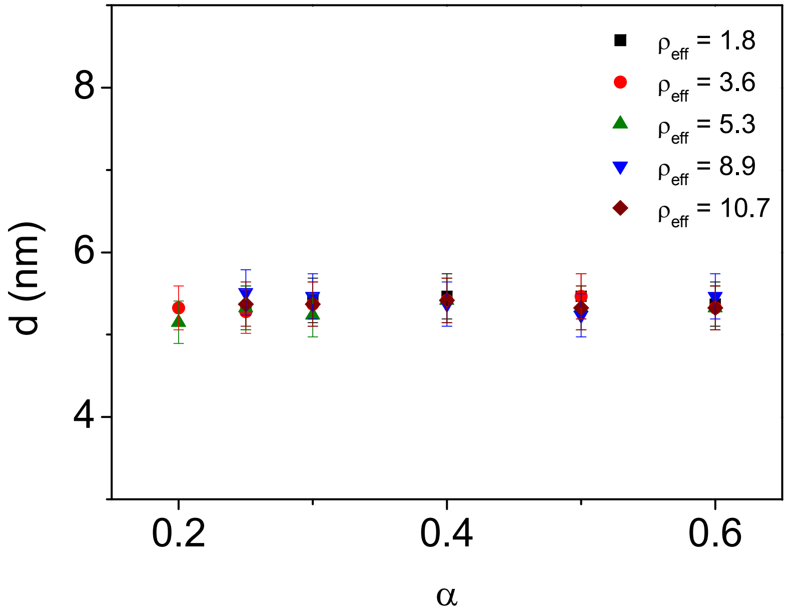
Soft Matter, 2012, **8**, 7368

Results: $C_6(LL)_2$/DOPE-pDNA



$d_1 = 6.3 \pm 0.2$ nm

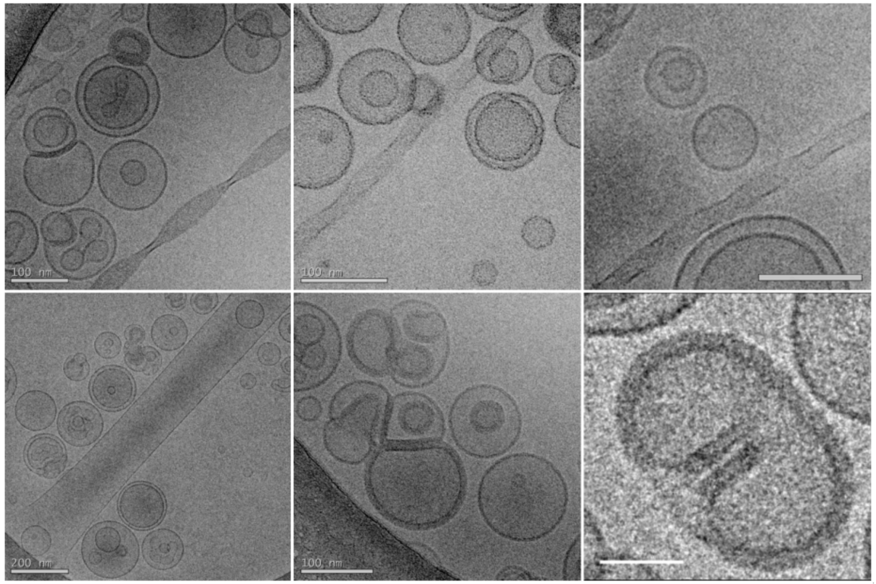
$d_2 = 5.4 \pm 0.2$ nm



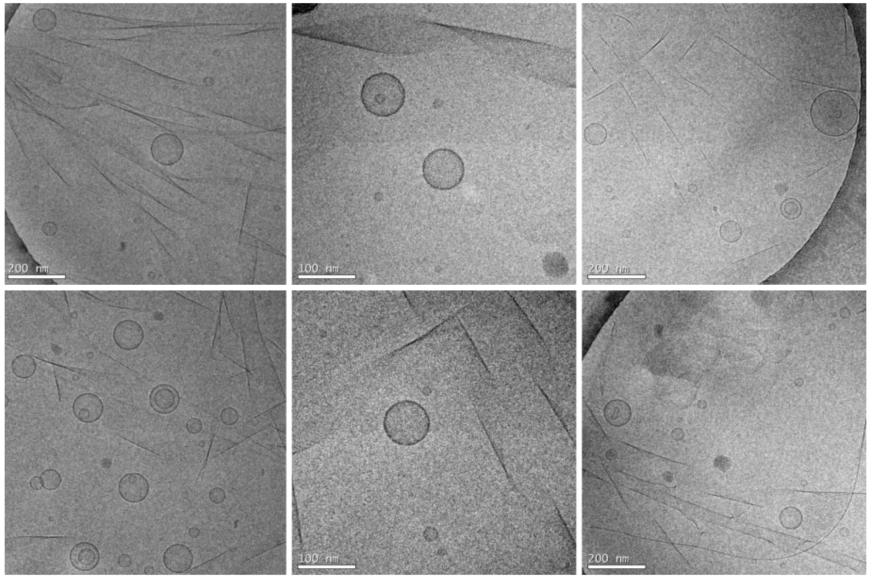
Results: $C_6(LL)_2$/DOPE-pDNA

Crio-TEM

α = 0.20



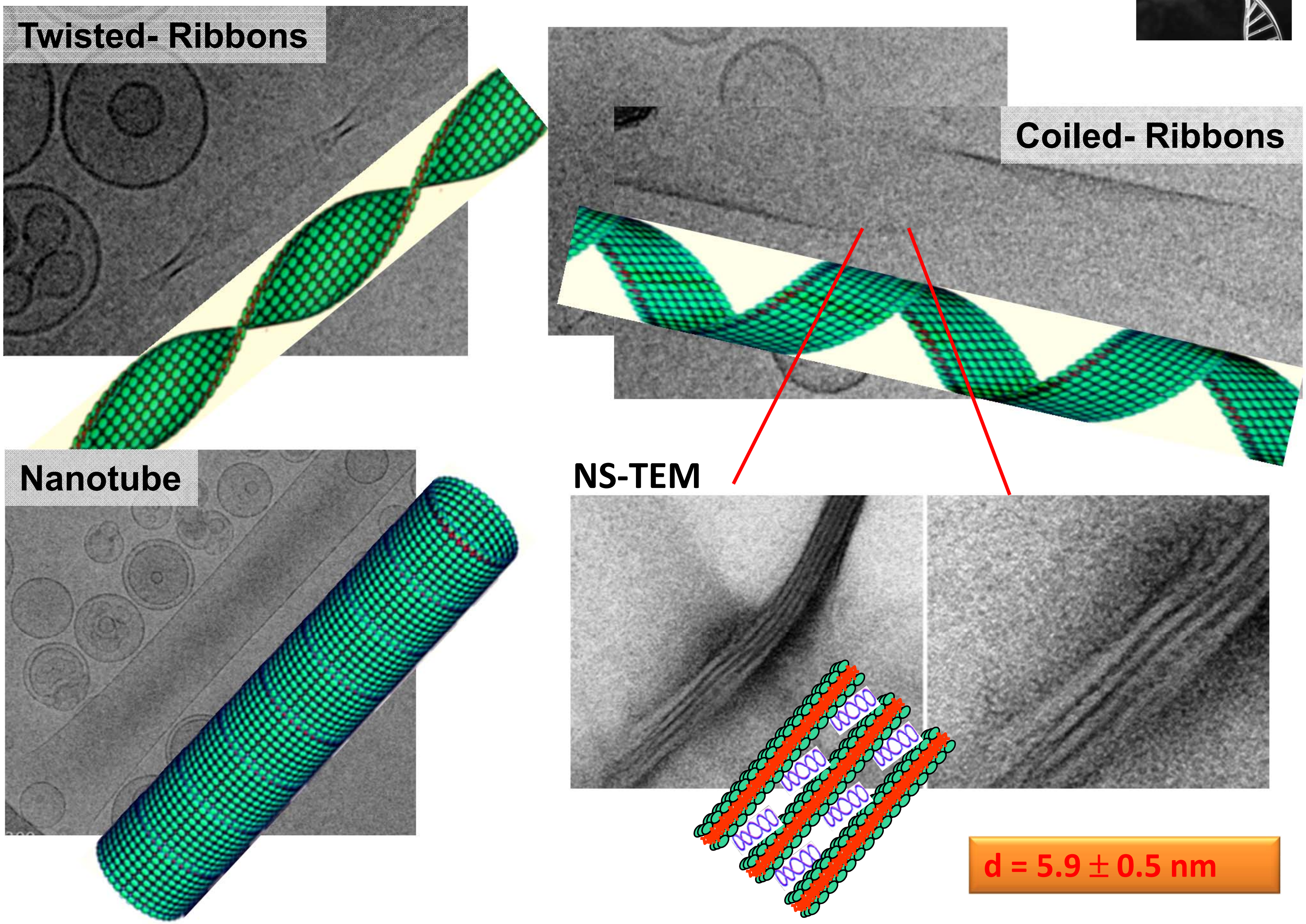
α = 0.50



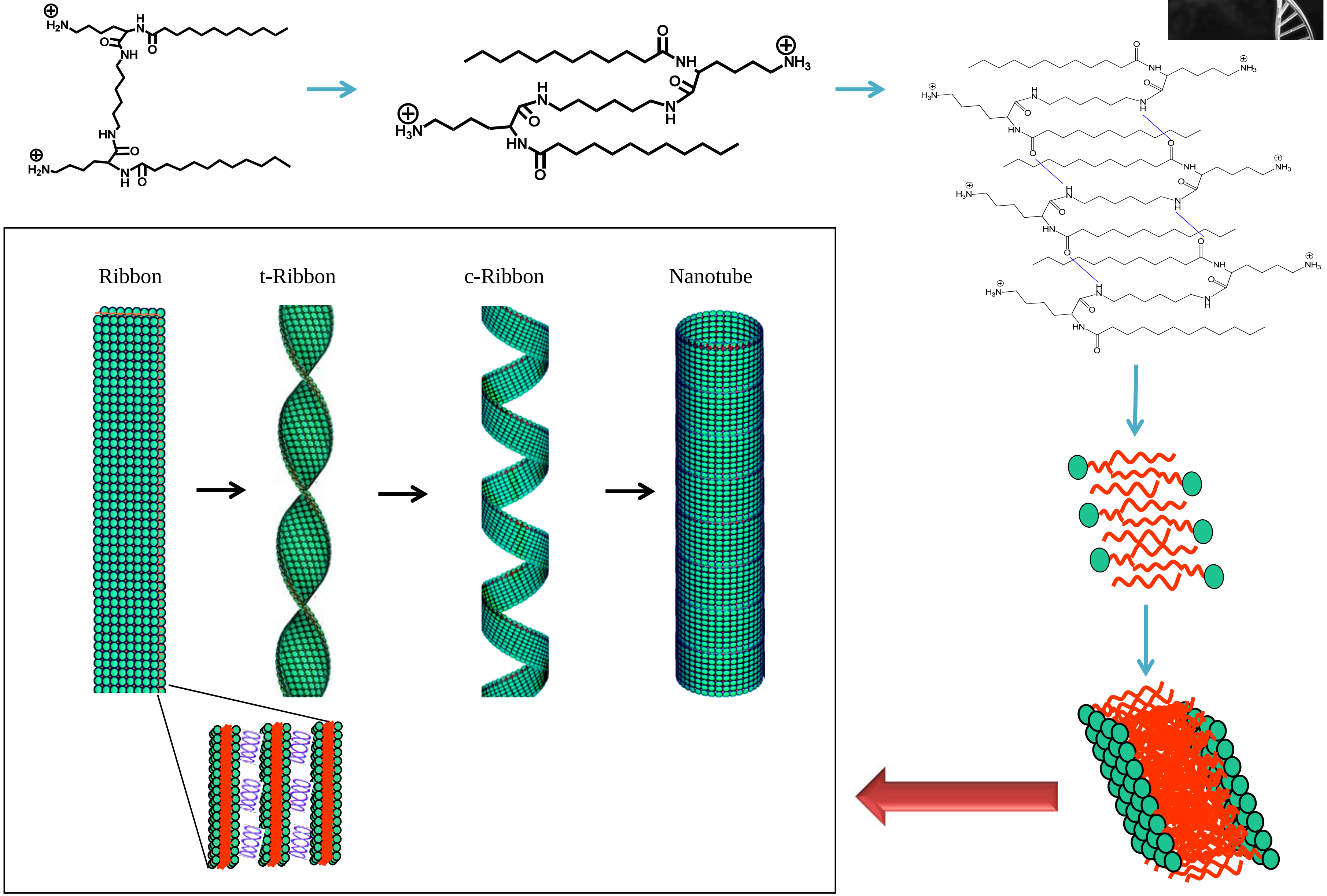
Ribbon-type

Cluster-type

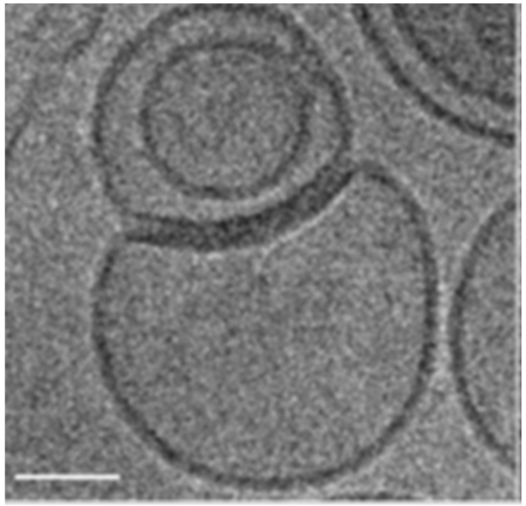
Results: Ribbon-type structures

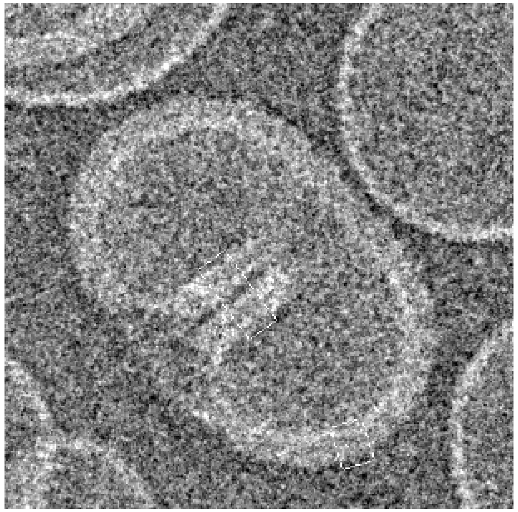


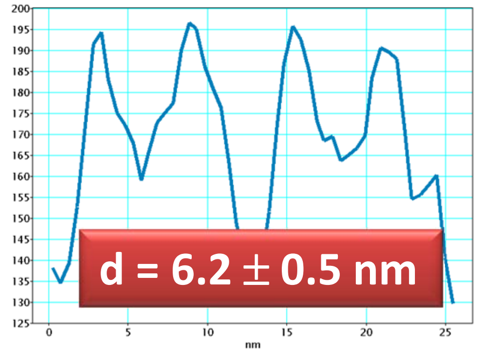
Results: $C_6(LL)_2$/DOPE-pDNA

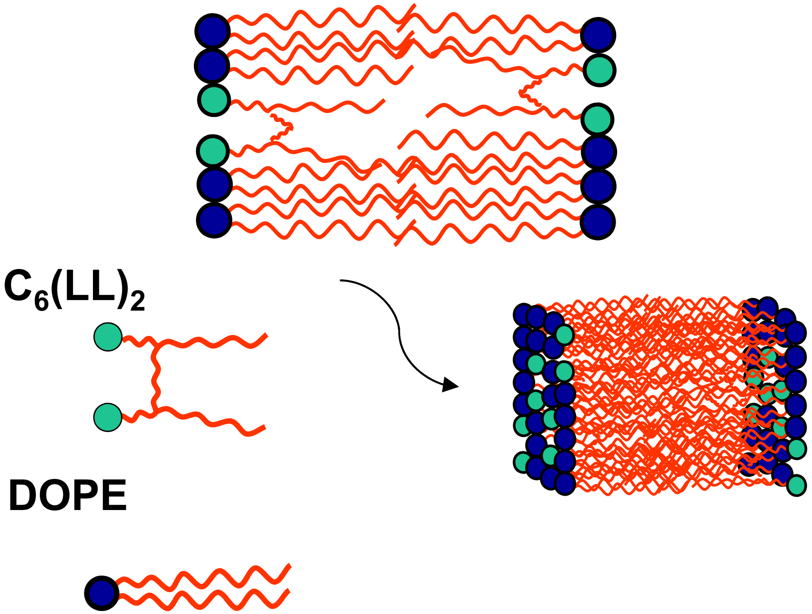


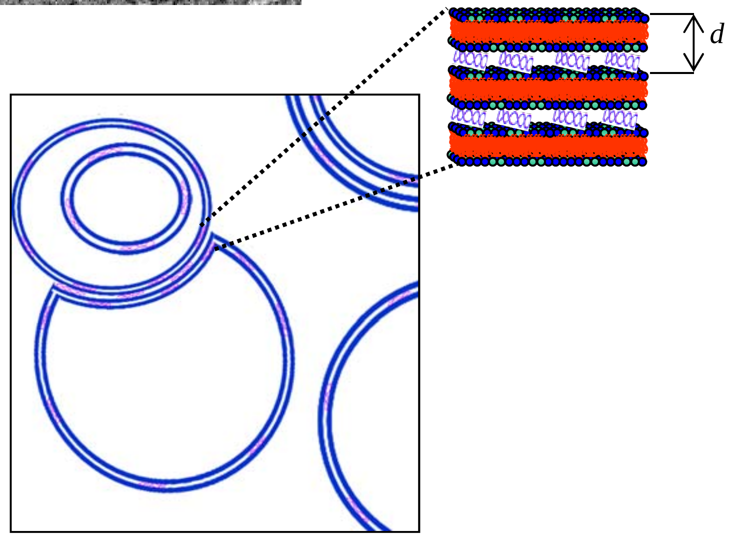
Results: Cluster-type structures



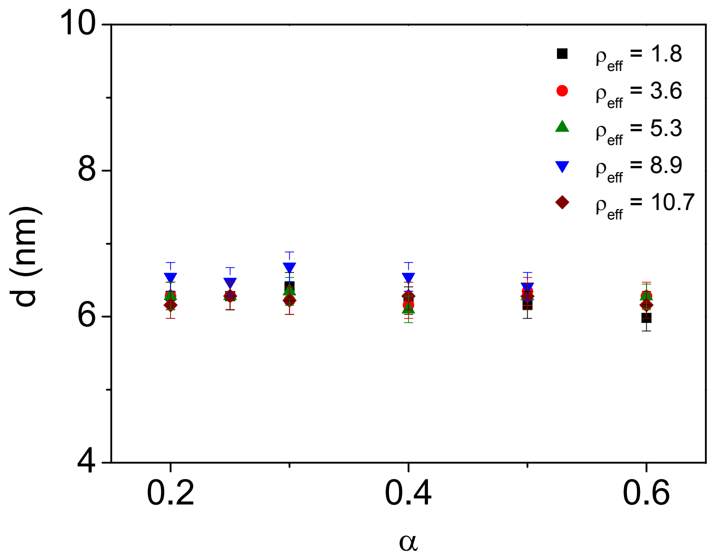


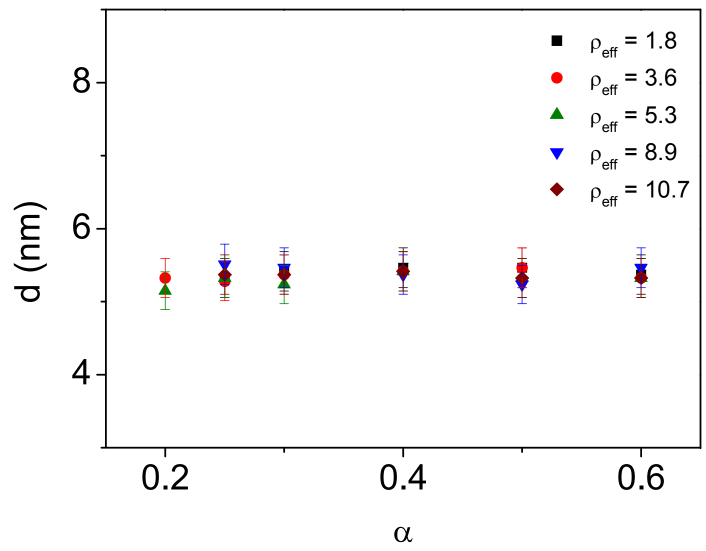






Results: $C_6(LL)_2$/DOPE-pDNA

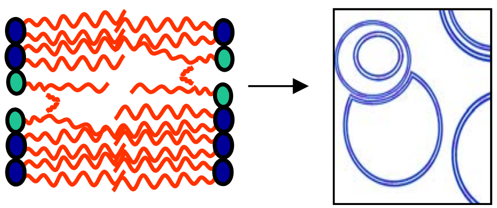


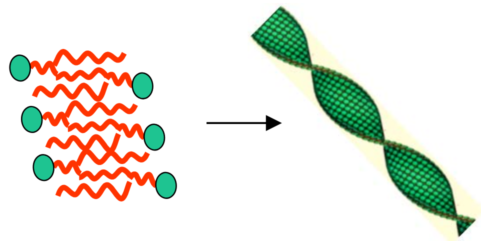


d = 6.3 ± 0.2 nm

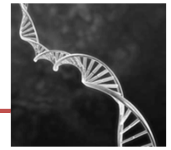
$$d = \frac{2\pi}{q_{100}}$$

d = 5.4 ± 0.2 nm





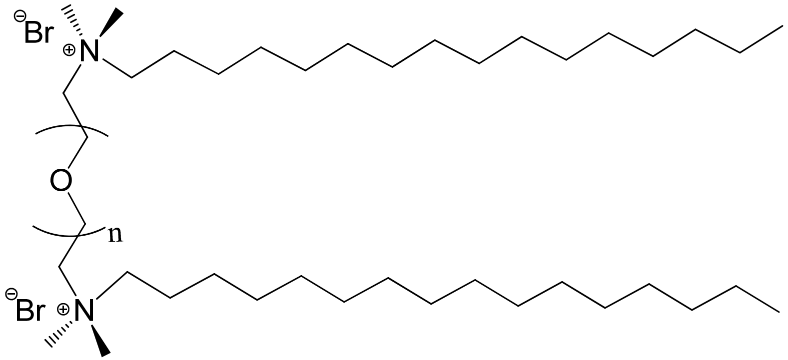
Example 3: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA



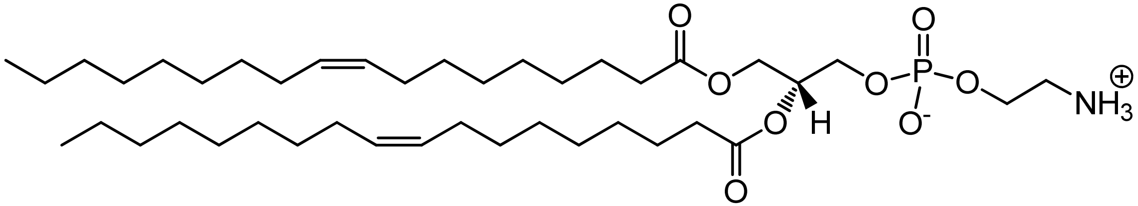
LIPOSOMES MIXED

$(C_{16}Am)_2(C_2O)_n$

n= 1, 2 y 3



DOPE



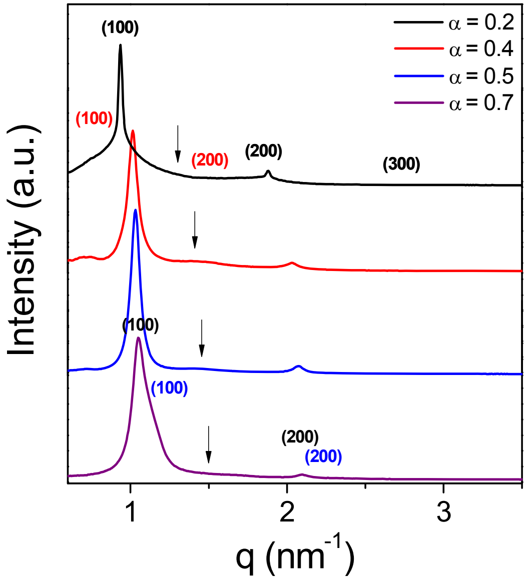
DNA

Plasmid (pDNA)

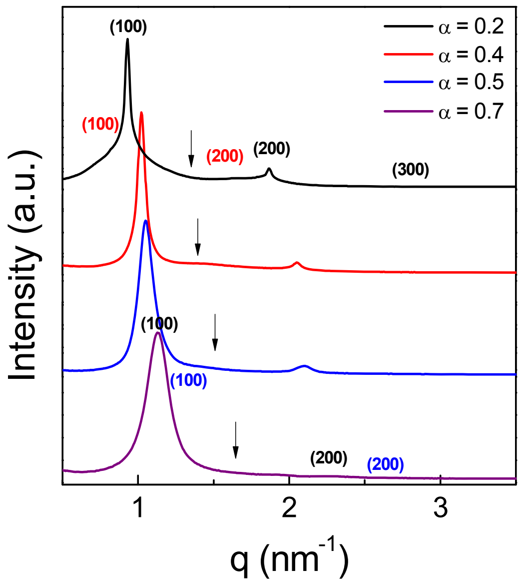
pEGFP-C3

Results: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA

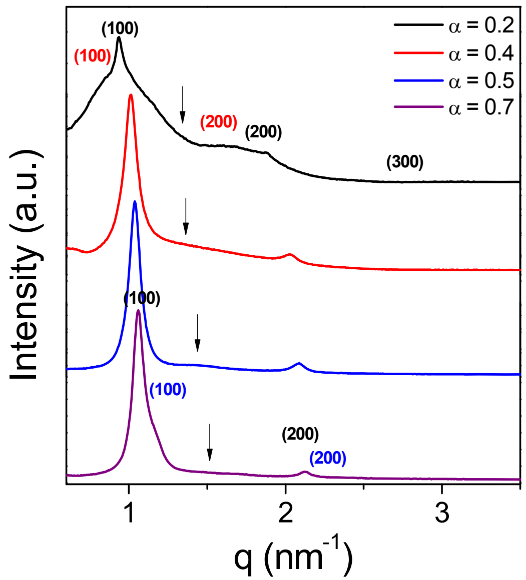
n = 1

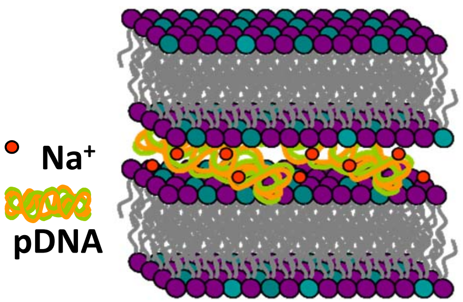


n = 2



n = 3



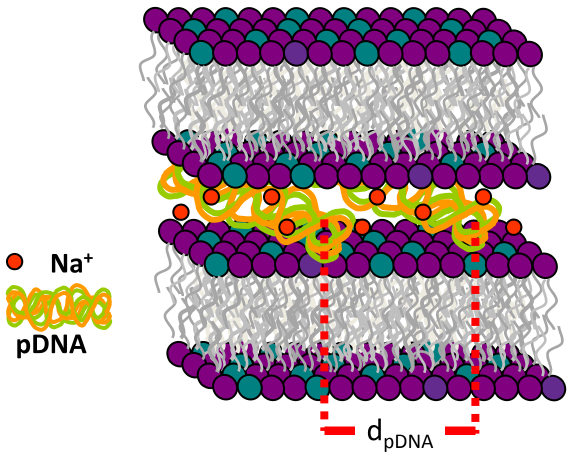


J. Mat. Chem. B, 2014,

- At all α compositions, diffractograms show a Bragg peaks corresponding to a lamellar structure (L_{α}).
- De-mixing phenomena is observed at α = 0.2 and 0.7
- Broad and smoothed peak correspond to DNA-DNA correlation

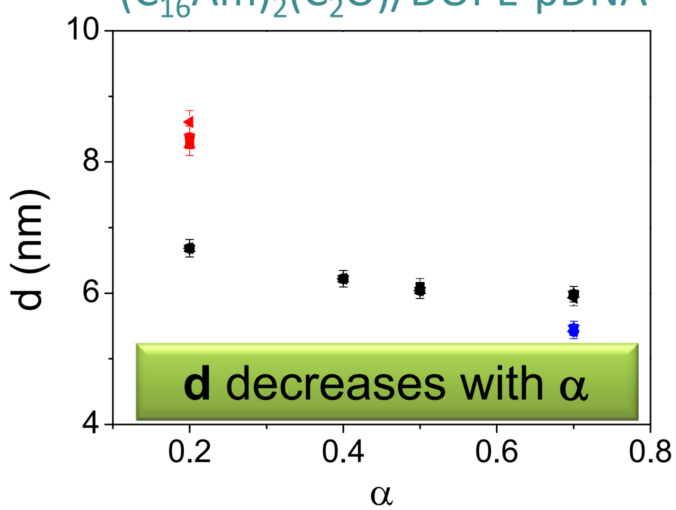
Results: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA

Interlamellar distance



$$d = \frac{2\pi}{q_{100}}$$

$$d_{pDNA} = \frac{2\pi}{q_{pDNA}}$$



d ≈ 7.0 - 6.0 nm

$$d = d_m + d_w$$

d_m≈ 4.5 - 4.0 nm

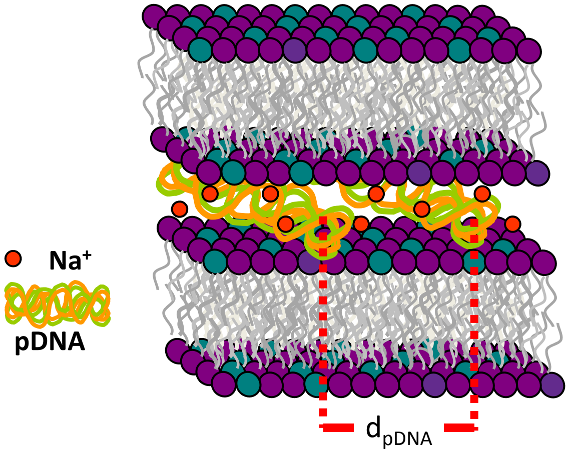
d_w≈ 2.5 - 2.0 nm

- A thinner bilayer (d_m) as α increases because the length of the GCL is slightly shorter than that of DOPE.

- The decrease of the monolayer (d_w) at higher α because the surface charge area in the liposomes is increased resulting in a higher compaction of pDNA.

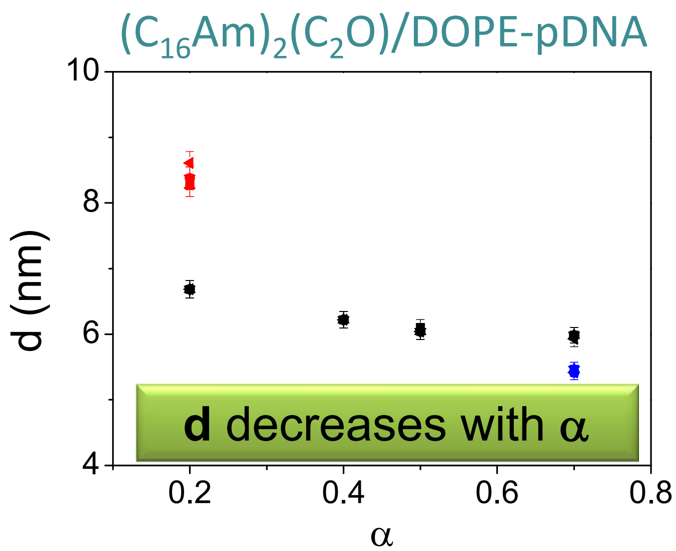
Results: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA

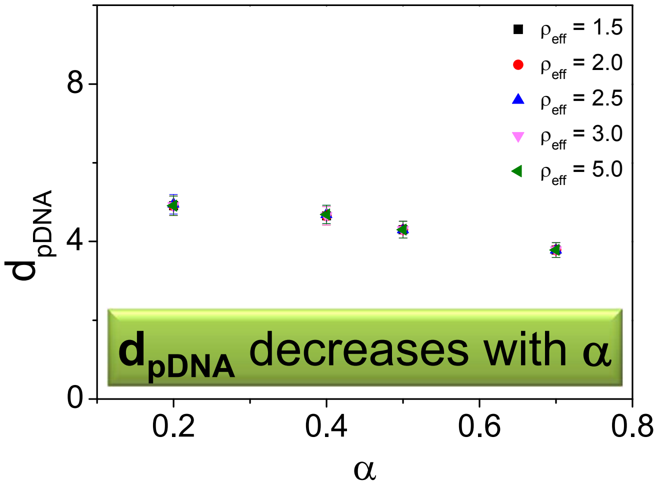
Interlamellar distance



$$d = \frac{2\pi}{q_{100}}$$

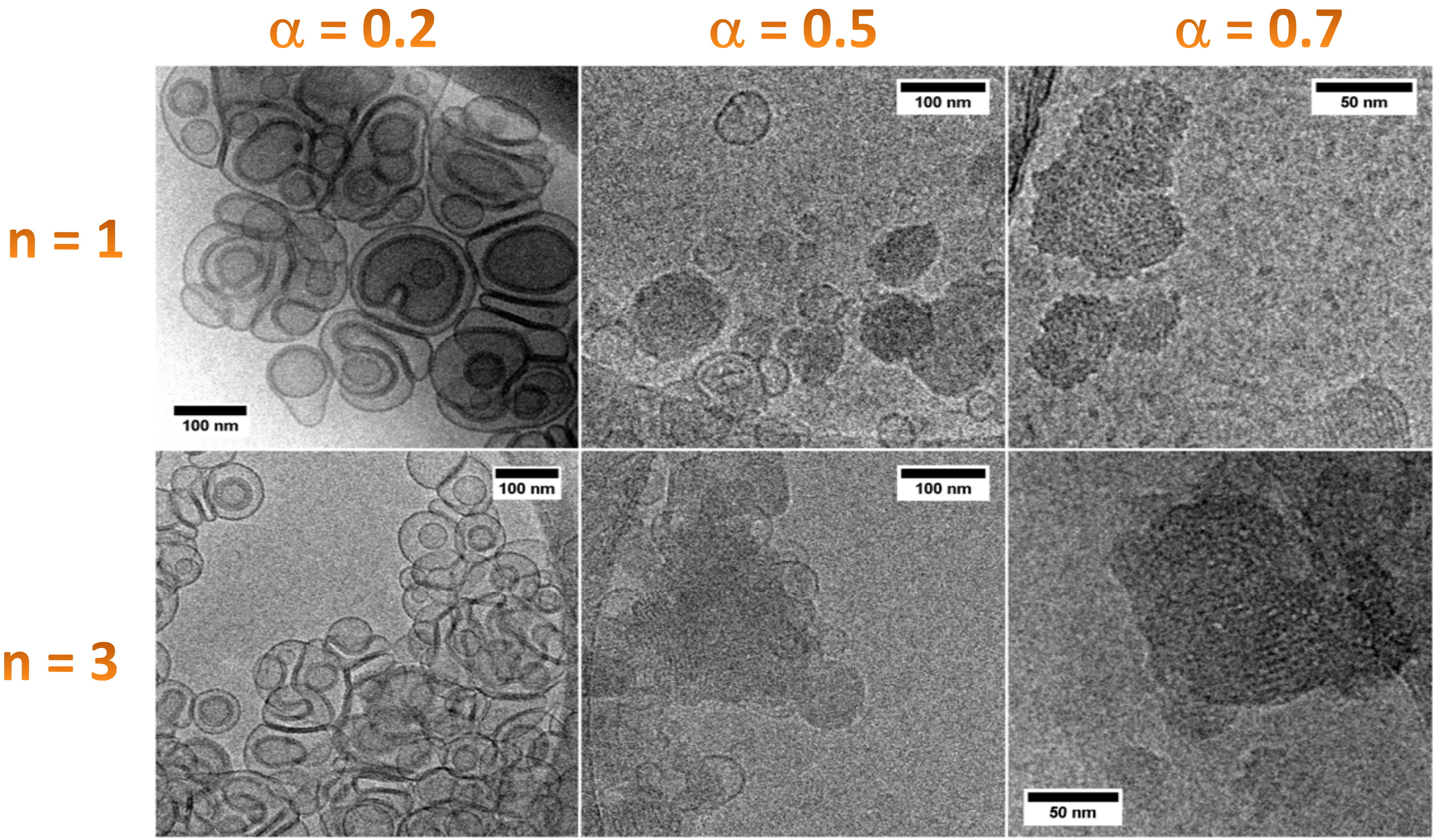
$$d_{pDNA} = \frac{2\pi}{q_{pDNA}}$$





DND-DNA distance are reduce because the increase on the surface charge area

Results: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA

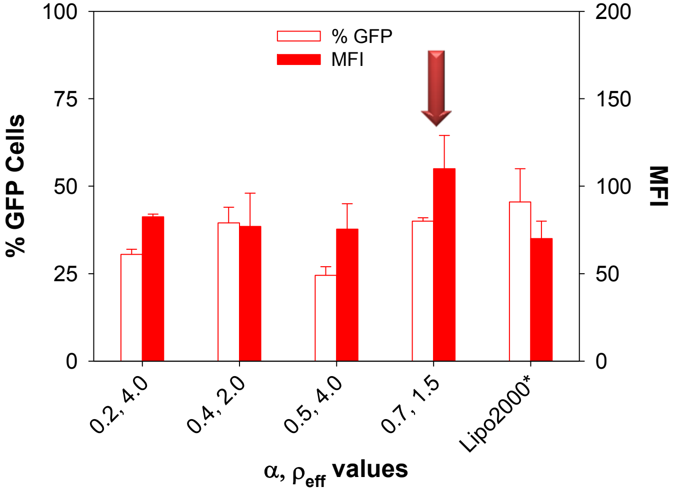


When α increase the structure become more compact

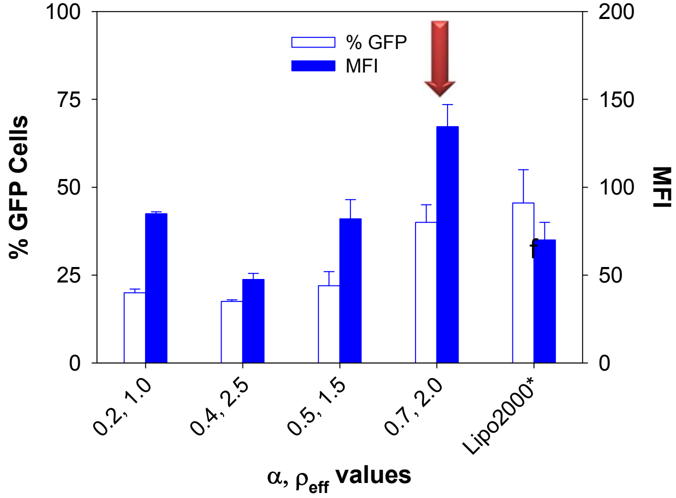
Results: $(C_{16}Am)_2(C_2O)_n$/DOPE-pDNA

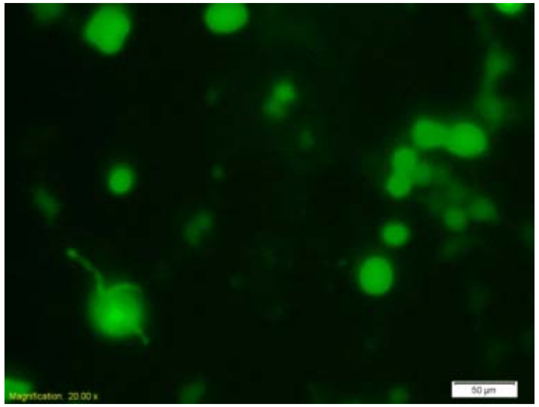
Transfection Results

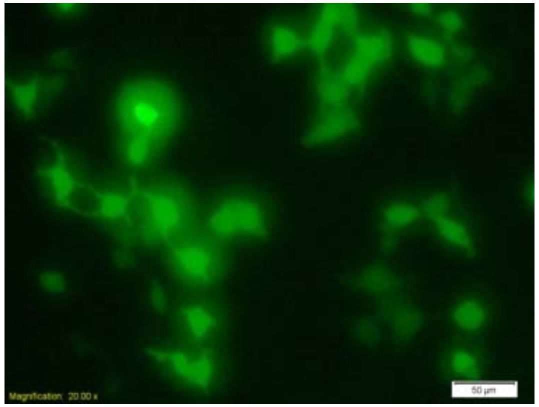
n = 1



n = 3

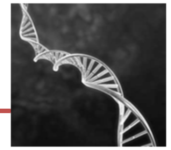






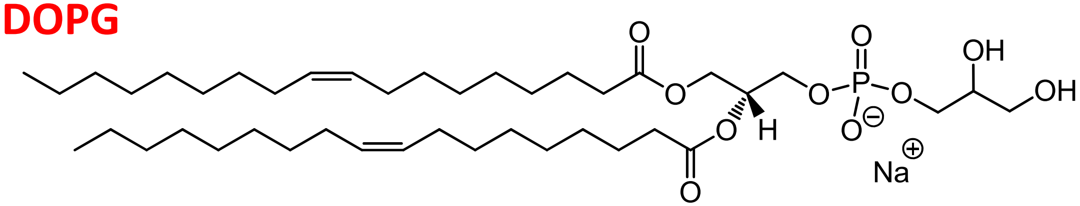
When the structure becomes more compact transfection better results are obtained

Example 4: Anionic Lipoplexes

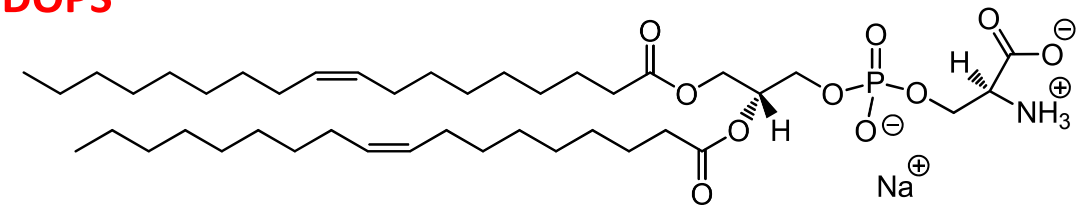


LIPOSOMES MIXED

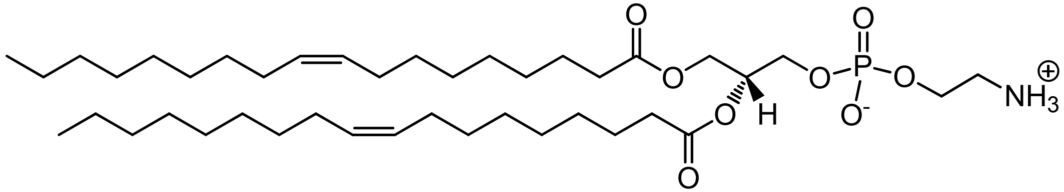
DOPG



DOPS



DOPE

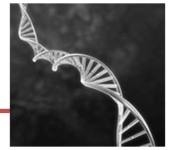


DNA

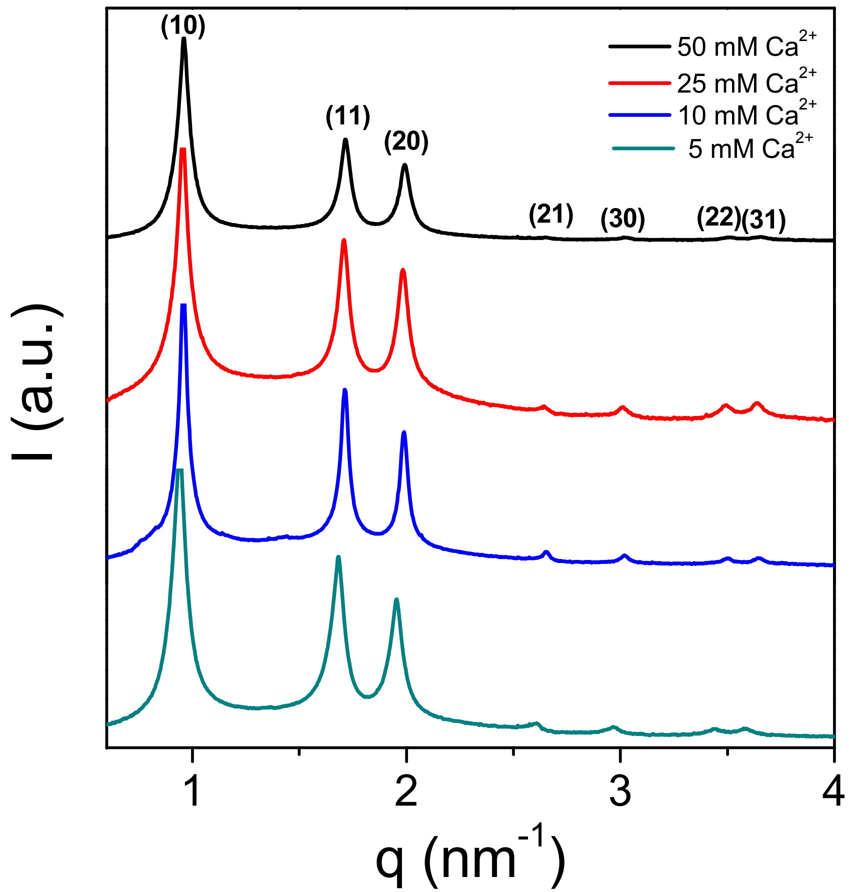
Plasmid (pDNA)
pEGFP-C3

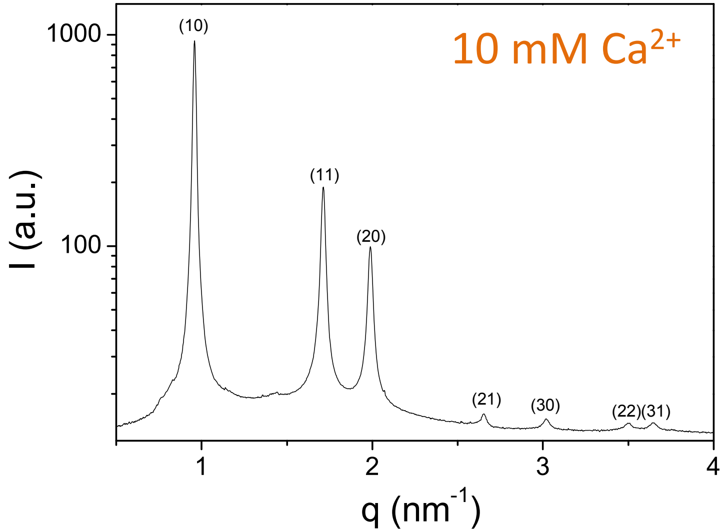
DIVALENT CATION: Ca^{2+}

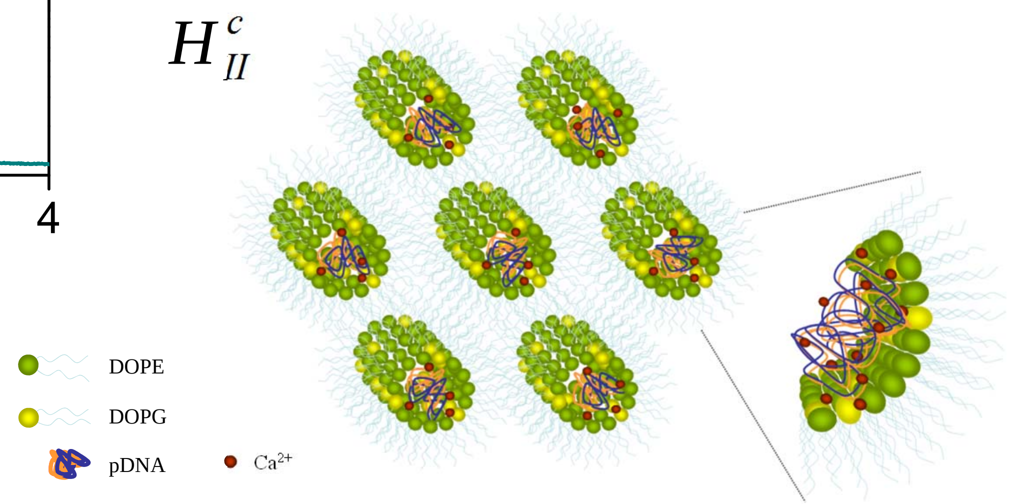
Results: DOPG/DOPE-Ca^{2+}-pDNA



α = 0.20

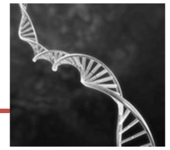




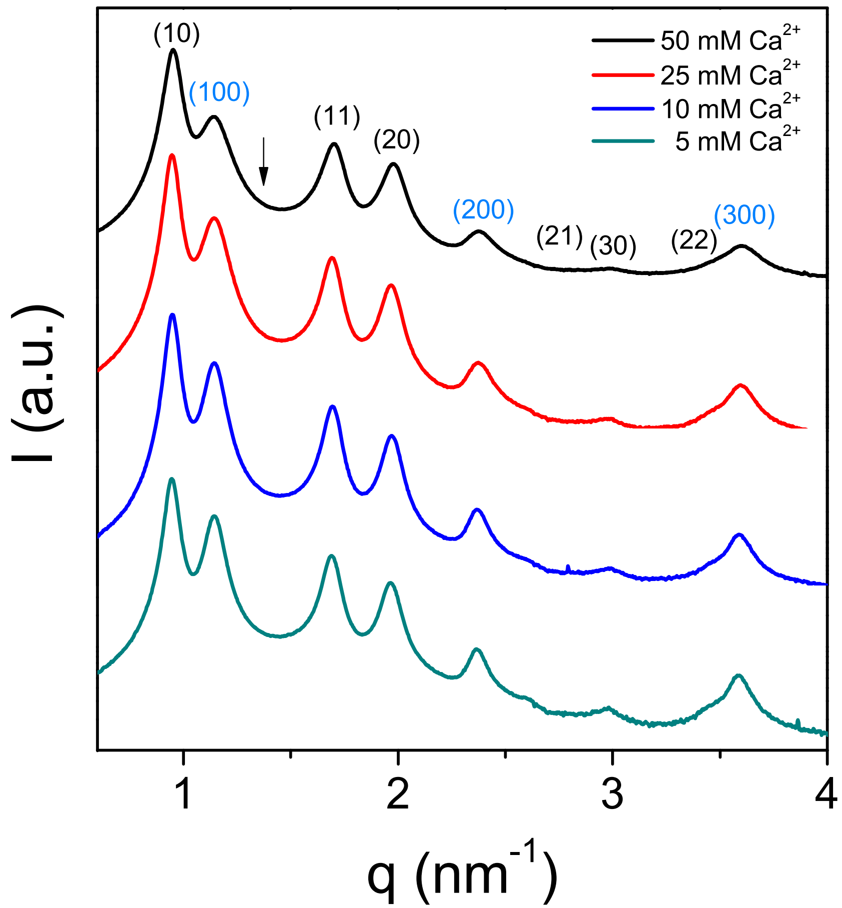


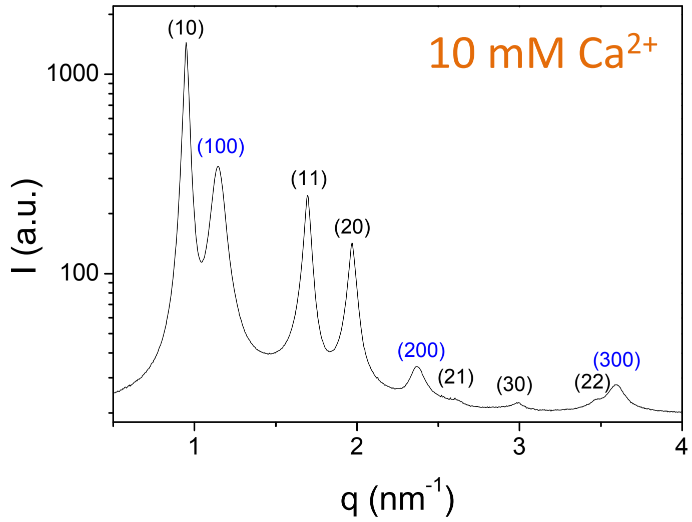
Langmuir, 2014

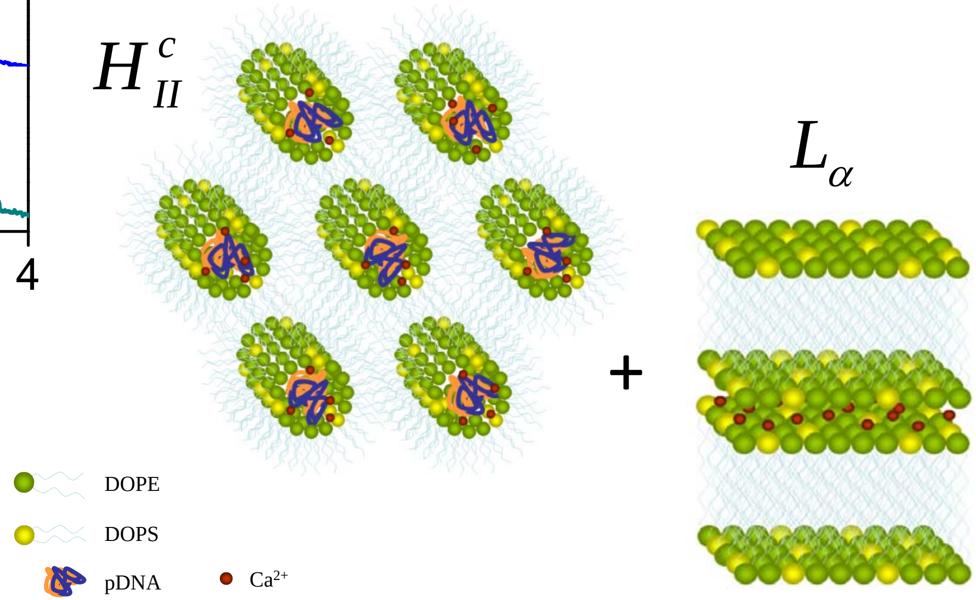
Results: DOPS/DOPE-Ca^{2+}-pDNA



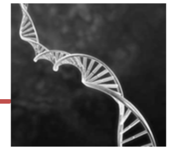
α = 0.20





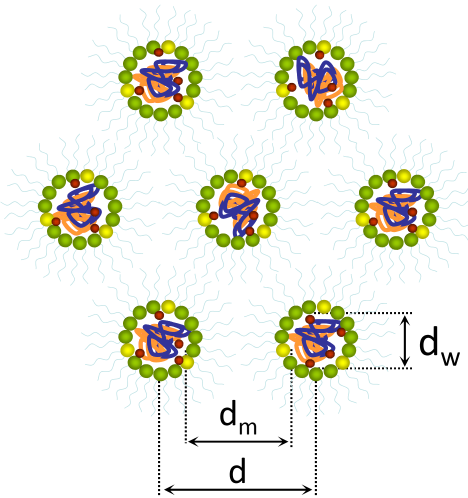


Results: AL/DOPE-Ca^{2+}-pDNA



Interlayer distance

H_{II}^{c} → DOPG and DOPS lipoplexes



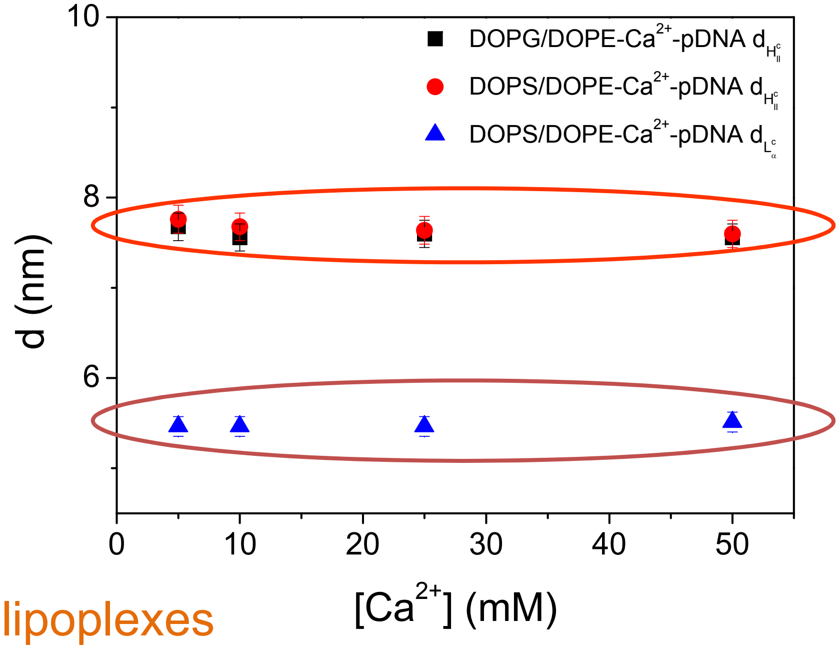
$$d = d_{pDNA}\frac{4\pi}{\sqrt{3}q_{10}}$$

$$d = d_m + d_w$$

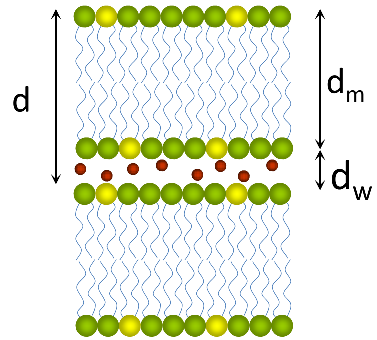
d ≈ 7.6 nm

d_m ≈ 4.5 nm

d_w ≈ 3.1 nm



L_{α} → DOPS lipoplexes



$$d = \frac{2\pi}{q_{100}}$$

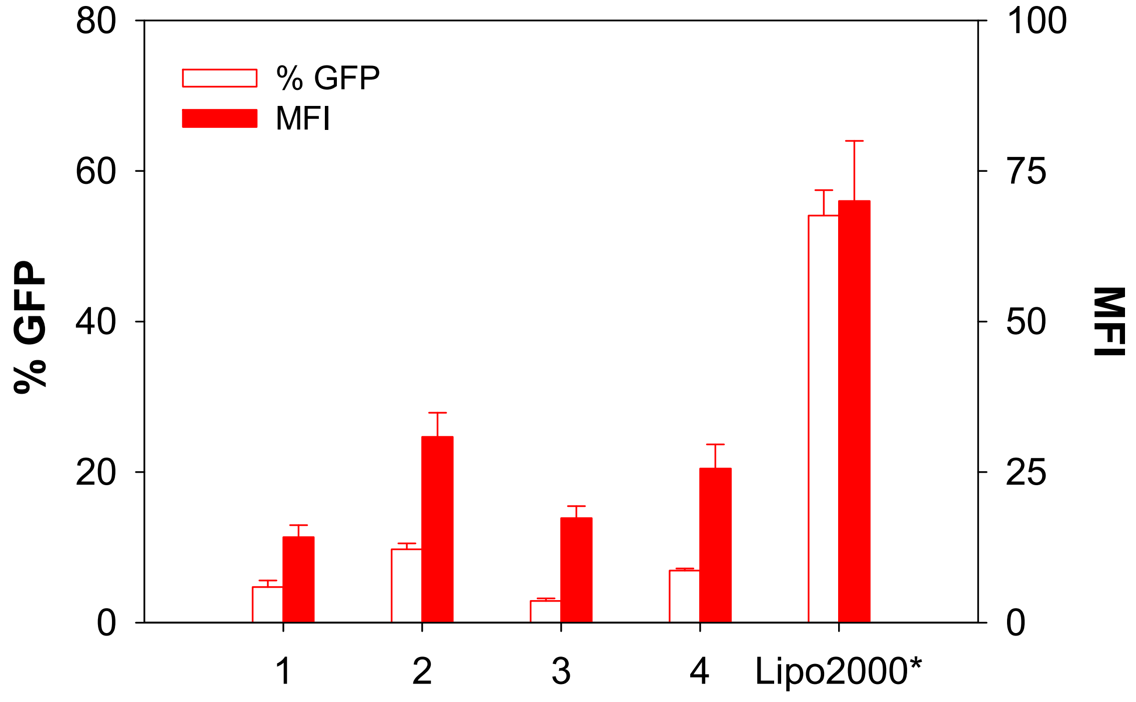
d ≈ 5.5 nm

d_m ≈ 4.5 nm

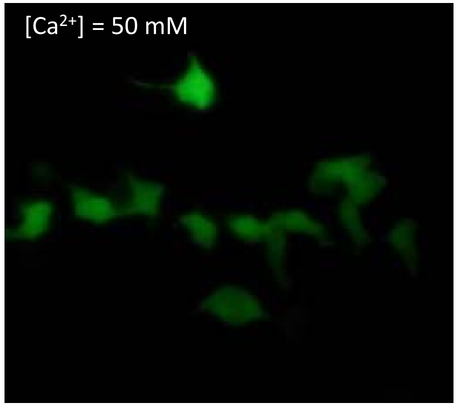
d_w ≈ 1.0 nm

Results: AL/DOPE-Ca^{2+}-pDNA

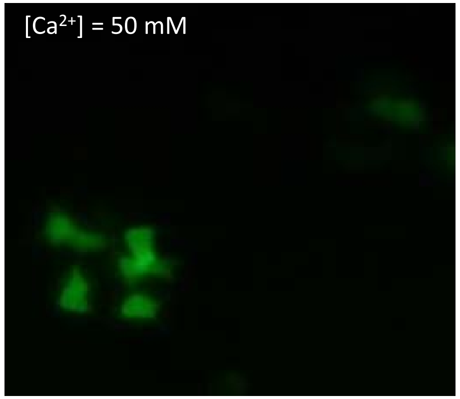
Transfection Efficiency (TE): HEK293T cells



DOPG/DOPE-Ca^{2+}-pDNA



DOPS/DOPE-Ca^{2+}-pDNA



AL lipoplexes mediated by Ca^{2+} are available to transfect the pEGFP-c3 plasmid in HEK293T cells.

Conclusions

The long term objective of the studies described of liquid crystalline liposome-nucleic acid complexes, is to develop a fundamental science base, which will lead to the design and synthesis of optimal carriers of DNA and siRNA for gene delivery, gene silencing, and disease control.

The structure-function data obtained from such studies should eventually allow one to begin the formidable task of a rational design of these self assemblies for enhanced nucleic acid delivery applications from the ground up beginning with chemical structure of the lipids and the correct compositions in mixtures including functional DNA and RNA.

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Thank you!

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