UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

CHOLESTERYL OLEATE-LOADED SOLID LIPID NANOPARTICLES FOR THE VECTORIZATION OF NUCLEIC ACIDS

MARC SUÑÉ POU BARCELONA, 2019

FDA approves $2M medicine, most expensive ever

By Associated Press

May 24, 2019 | 4:40pm | Updated

The one-time gene therapy developed by Novartis, Zolgensma, will cost $2.125 million.

Manufacturing Challenges Limit Gene Therapy Development

Moving forward with gene therapy development requires a “quantum leap” in manufacturing capabilities.

Nov 16, 2018 By Jill Wechsler

The biggest hurdle for advancing gene therapy from treating rare diseases to addressing more common conditions is difficulties in achieving efficient scale-up of production processes, says Peter Marks, director of FDA’s Center for Biologics Evaluation and Research (CBER). Marks noted that there are now

TYPES OF VECTORS

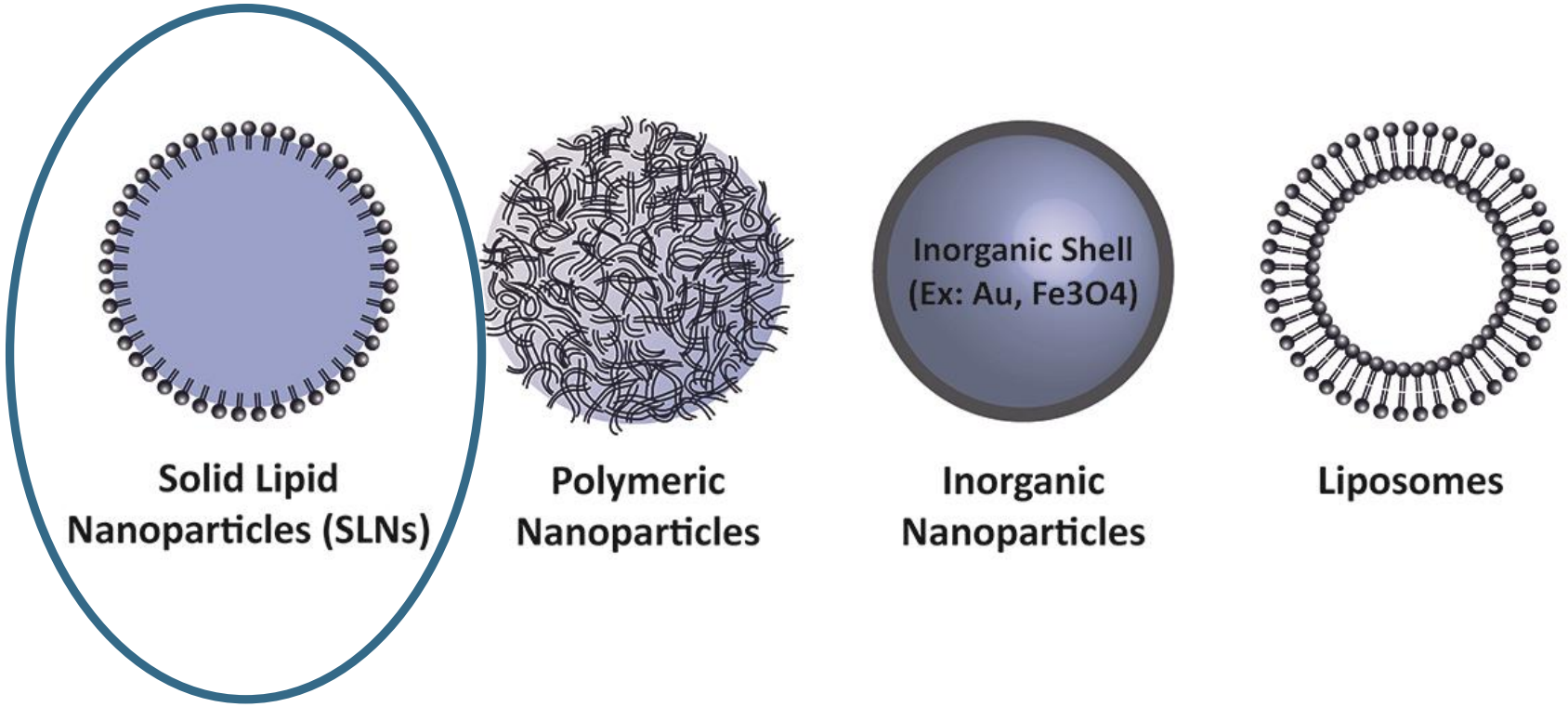
VIRAL VECTORS

- ✓ High transfection efficiency
- ✗ Expensive
- ✗ Possibility of immunogicity

NON VIRAL VECTORS

- ✓ Easy to manipulate and cheaper
- ✓ Low immunogicity
- ✗ Low transfection efficiency

NON VIRAL VECTORS. NANOPARTICLES

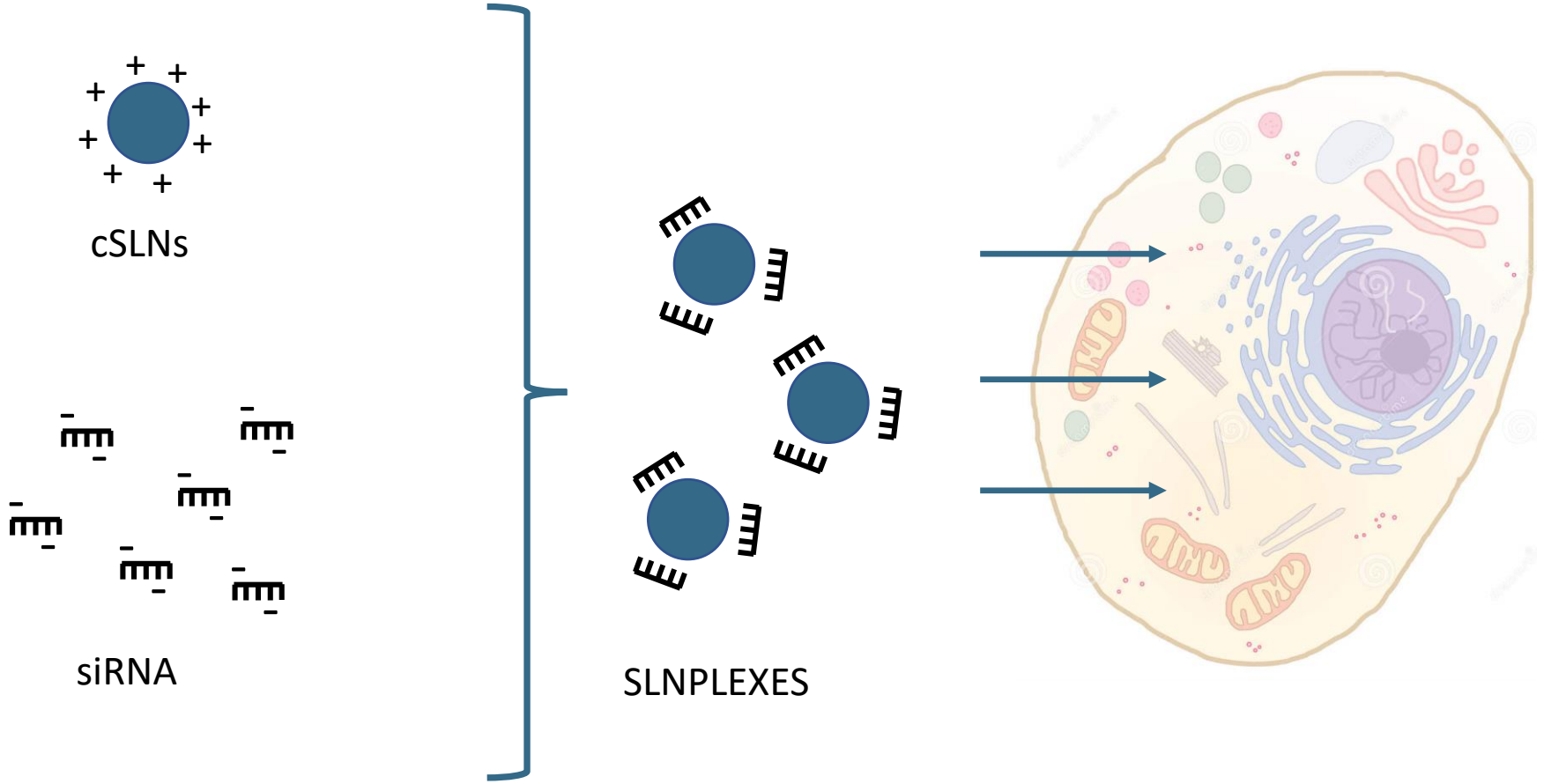


(Suñé-Pou et al, 2017)

SOLID LIPID NANOPARTICLES (SLN)

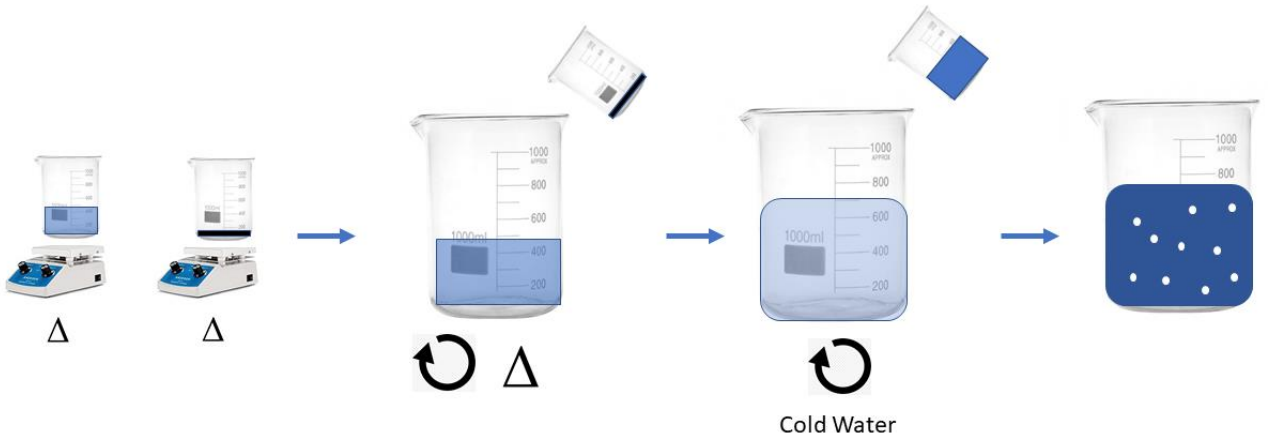
- Nanoparticles based on biocompatible lipids → Low toxicity.
- Easy manufacture process.
- Can be manufactured avoiding organic solvents.
- Can incorporate drugs inside its structure or outside, on the surface.
- Protect the drugs from external agents.
- SLN can be functionalized.

SOLID LIPID NANOPARTICLES (SLN)



**cSLNs → cationic Solid Lipid Nanoparticles

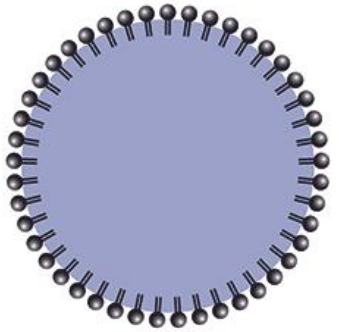
METHODS. HOT MICROEMULSIFICATION METHOD



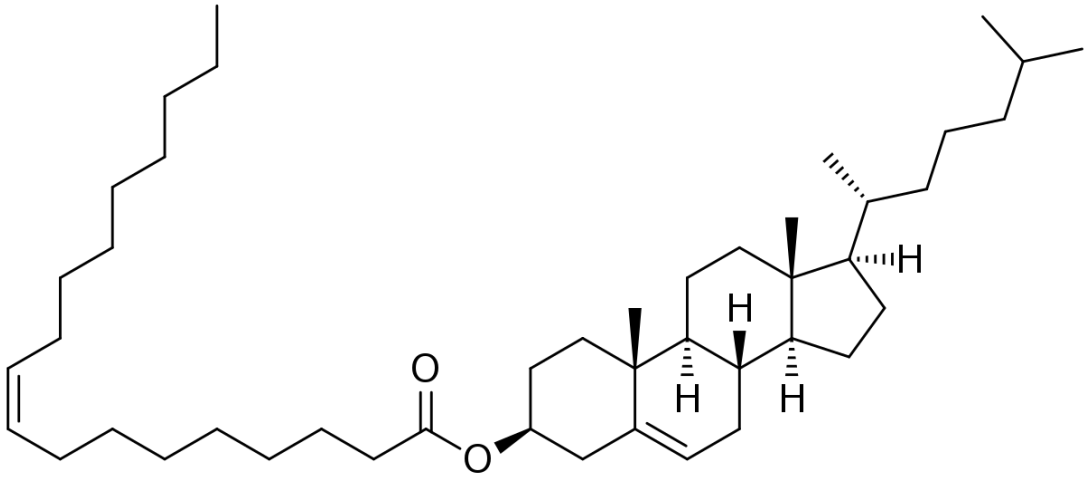
1. CENTRIFUGATION
2. FILTRATION
3. LIOFILIZATION

PREVIOUS FORMULATION

Matricial Lipids	Stearic Acid	500 mg
Cationic Lipid	Octadecylamine	600 mg
Surfactant	Poloxamer 188	100 mg



Solid Lipid Nanoparticles (SLNs)



COMPARISON OF FORMULATIONS WITH CHOLESTERYL OLEATE (CO)

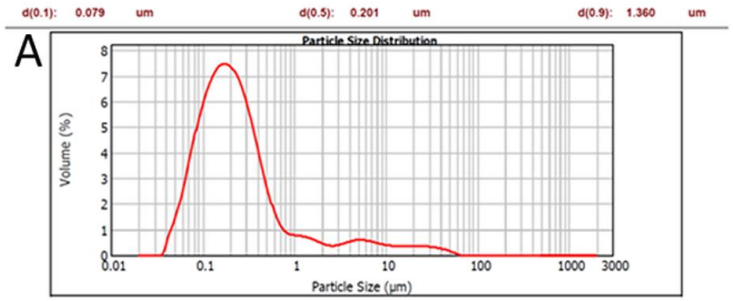
DEVELOPED AND TESTED FORMULATIONS

+ Cholesteryl Oleate (CO)
- Stearic Acid

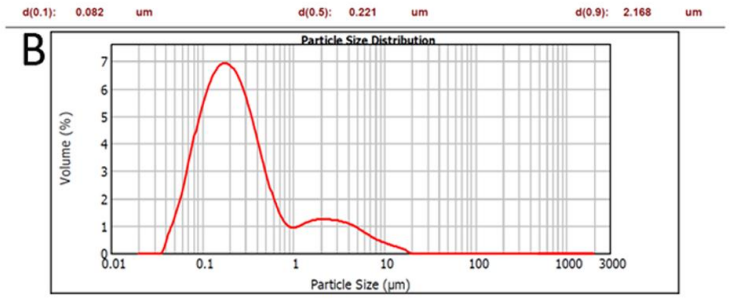
		Ref. 12	Ref. 13	Ref.14	Ref. 15	Ref. 16
Matricial Lipids	Stearic Acid	400 mg	300 mg	200 mg	100 mg	0 mg
	Cholesteryl Oleate	100 mg	200 mg	300 mg	400 mg	500 mg
Cationic Lipid	Octadecylamine	600 mg	600 mg	600 mg	600 mg	600 mg
Surfactant	Poloxamer 188	100 mg	100 mg	100 mg	100 mg	100 mg

PARTICLE SIZE DISTRIBUTION

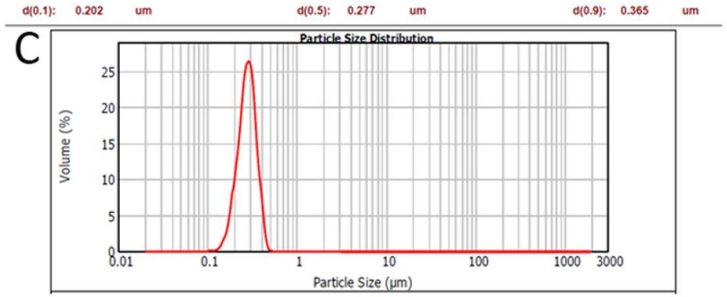
A



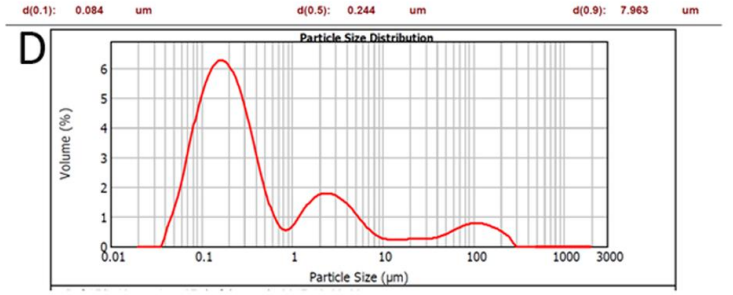
B



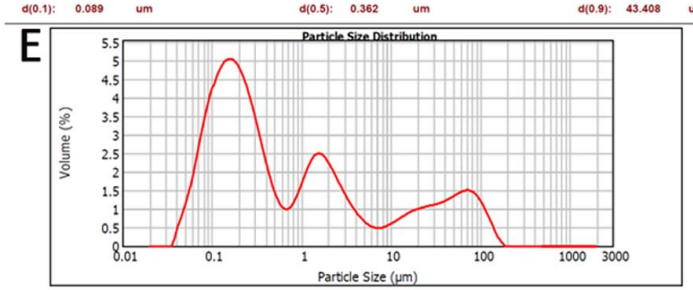
C



D

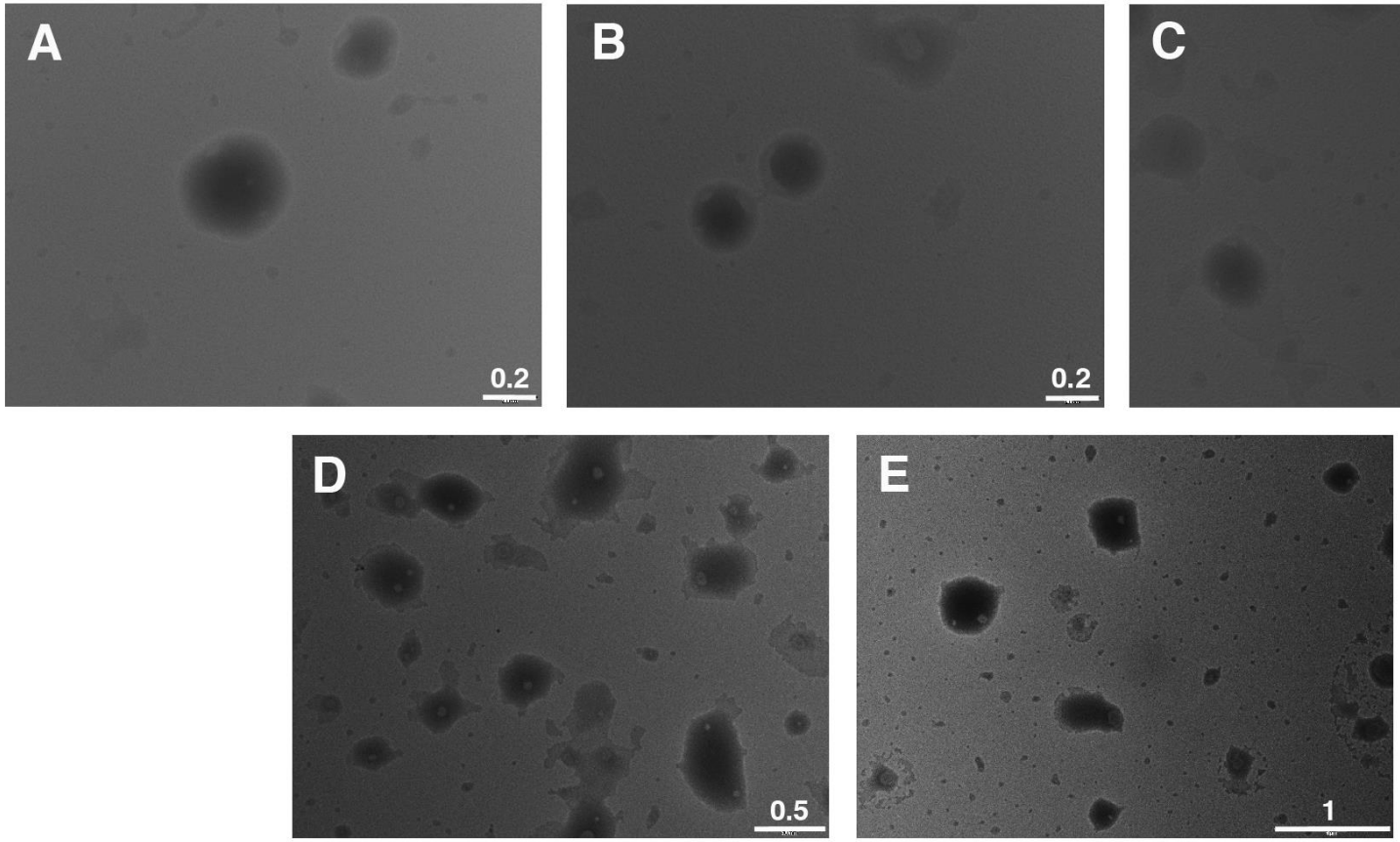


E



A → Reference 12
B → Reference 13
C → Reference 14
D → Reference 15
E → Reference 16

MORPHOLOGY (TEM)



A → Reference 12
B → Reference 13
C → Reference 14
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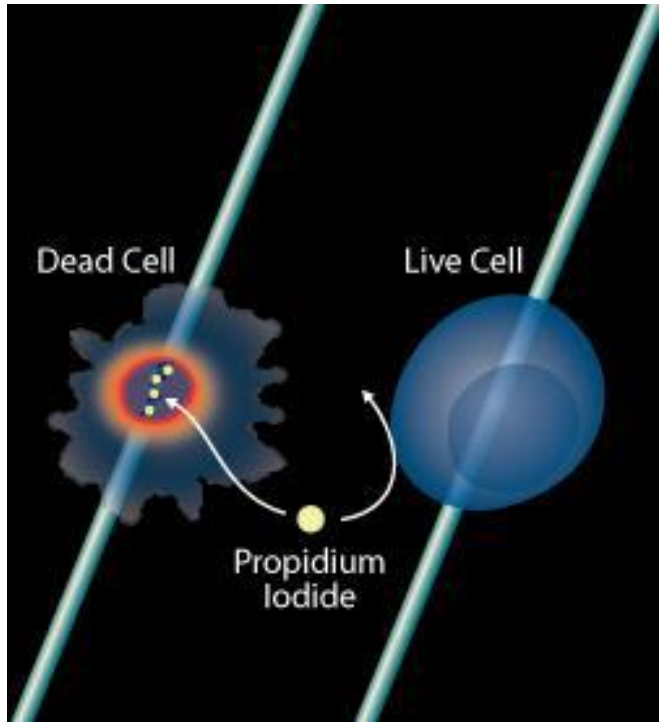
E → Reference 16

ZETA POTENTIAL

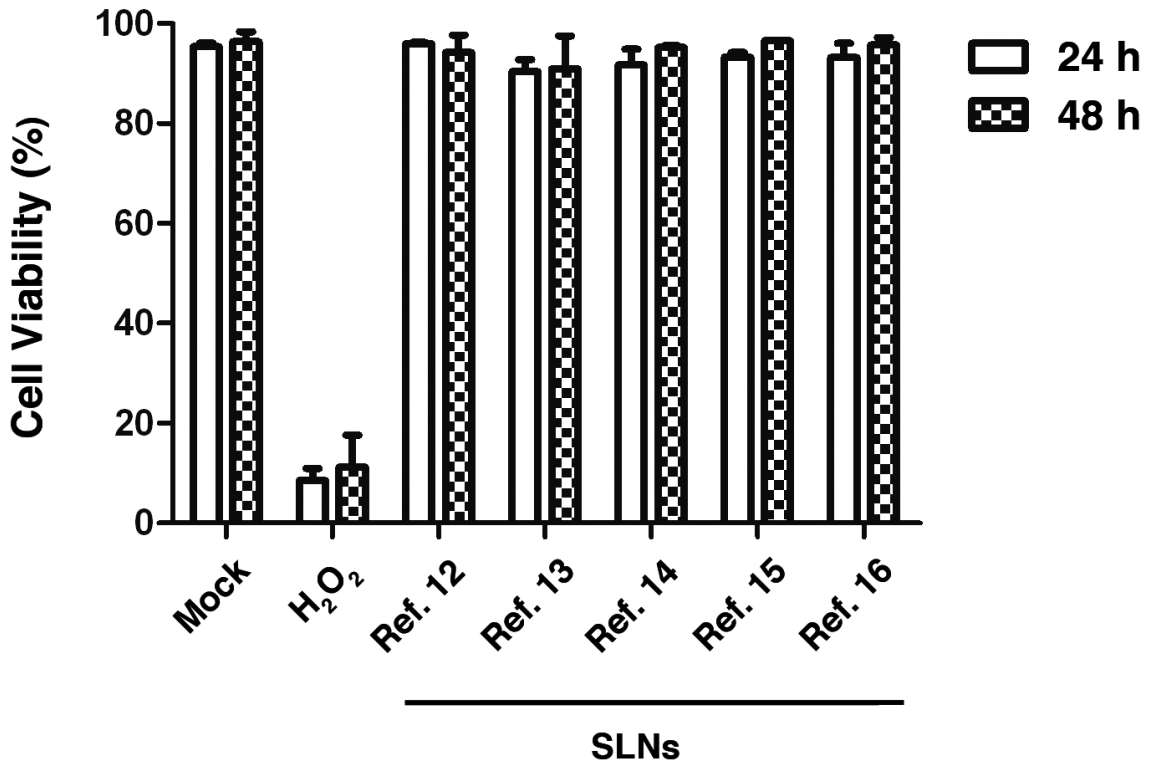
SLNs	Zeta potential (mV)
Ref. 12	38.3
Ref. 13	35.3
Ref. 14	35.7
Ref. 15	29.5
Ref. 16	41.9

CELL VIABILITY (PROPIDIUM IODIDE ASSAY)

- Propidium Iodide is a fluorescent intercalating agent that can be used to stain cells.
- Propidium iodide is used as a DNA stain in flow cytometry to evaluate cell viability.
- Propidium Iodide is not membrane-permeable making it useful to differentiate healthy cells based on membrane integrity.



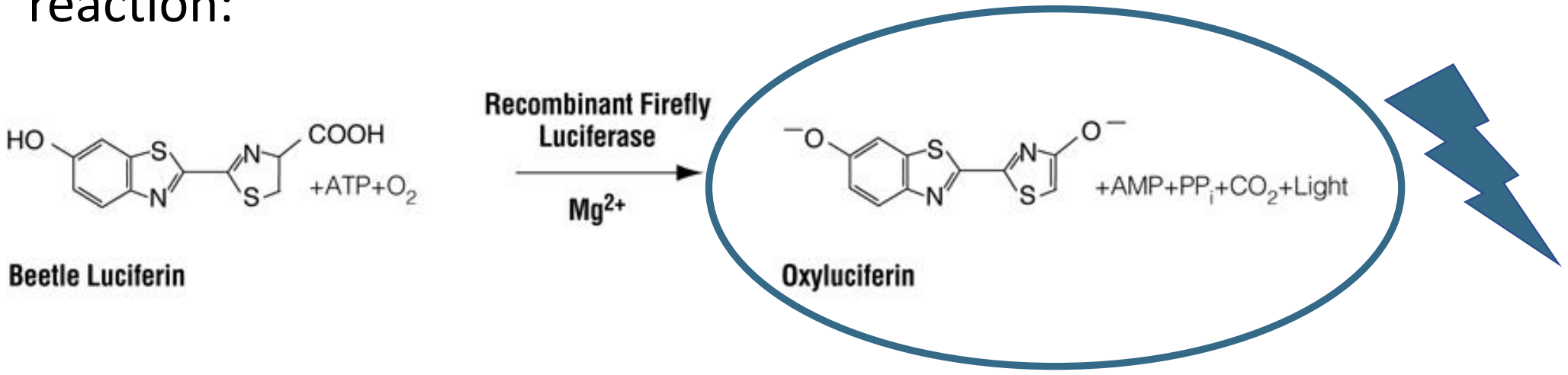
CELL VIABILITY (PROPIDIUM IODIDE ASSAY)



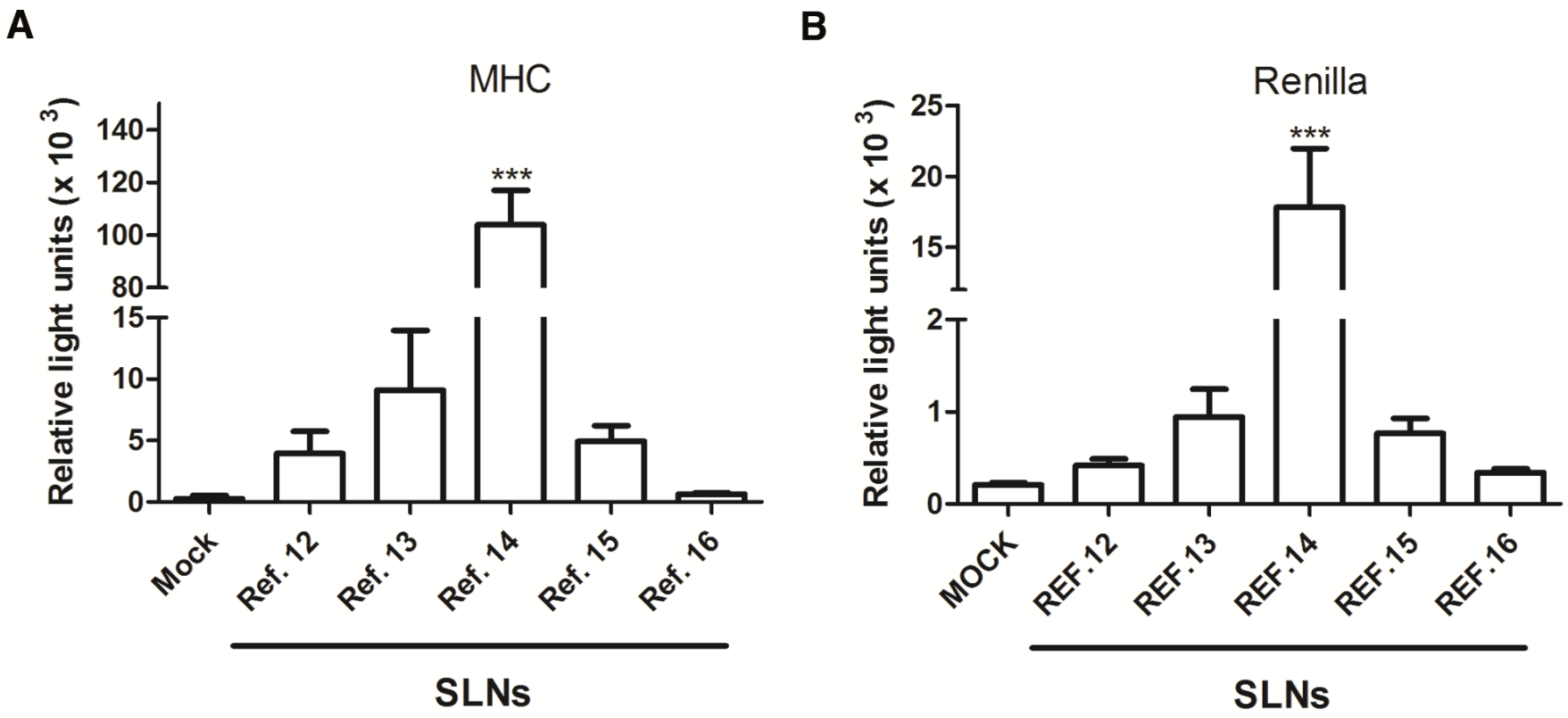
- All the formulations do not cause cellular death at the concentrations studied
- CO-SLNs are biocompatible in all the concentrations tested

PLASMID TRANSFECTION. LUCIFERASE ASSAY

- The **Luciferase Assay** is an extremely sensitive and rapid reagent for quantitation of firefly **luciferase**.
- Experiments tot transfect plasmid p3xMHC-LUC with the SLNs as non viral vector were performed.
- The more transfection, the more levels of Firefly Luciferase will be observed,.
- Firefly Luciferase is an enzyme that catalazises the following reaction:

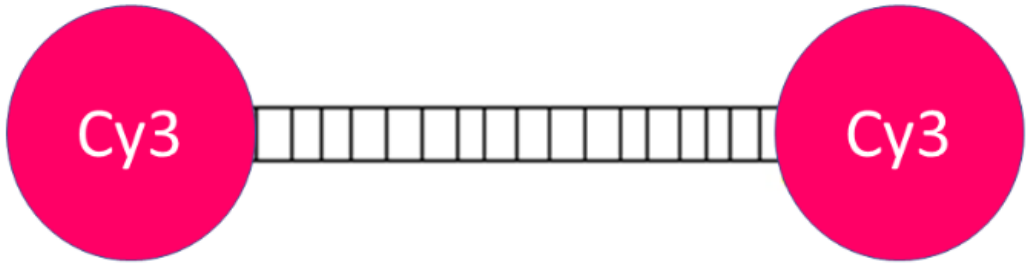


PLASMID TRANSFECTION. LUCIFERASE ASSAY

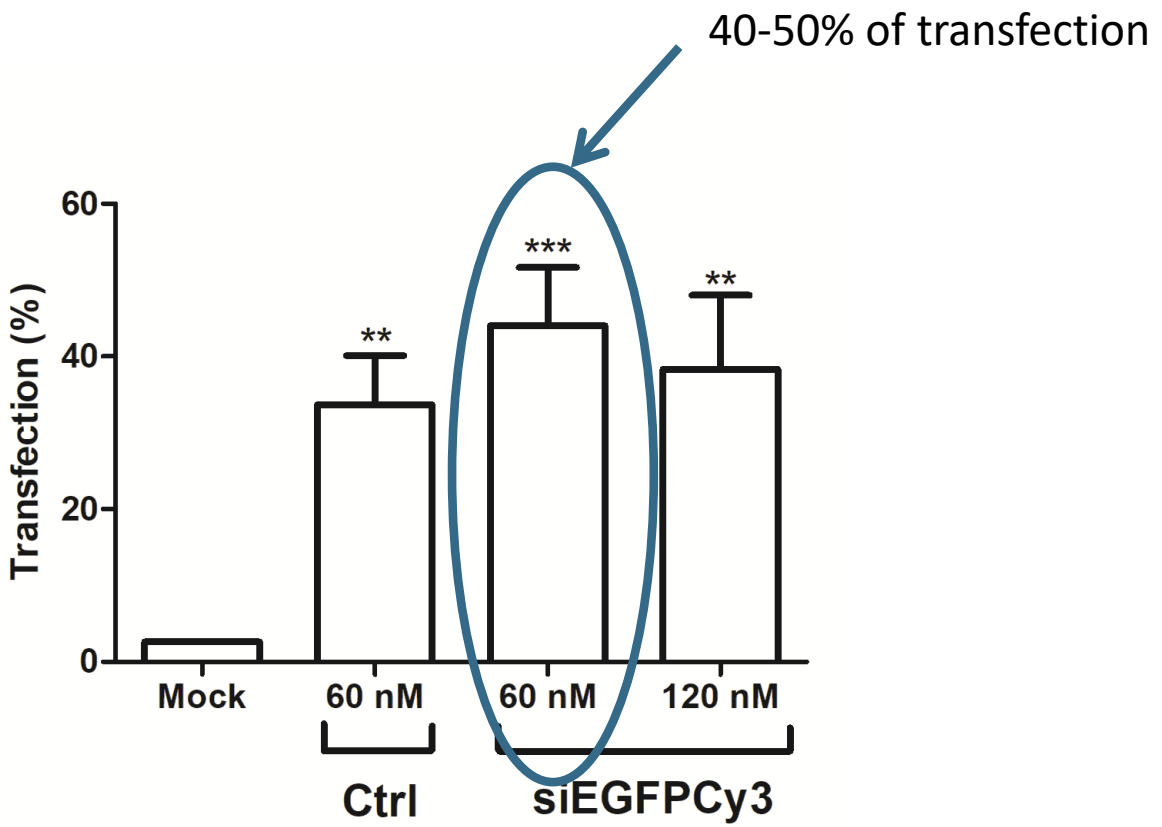


siRNA TRANSFECTION. siEGFPCy3

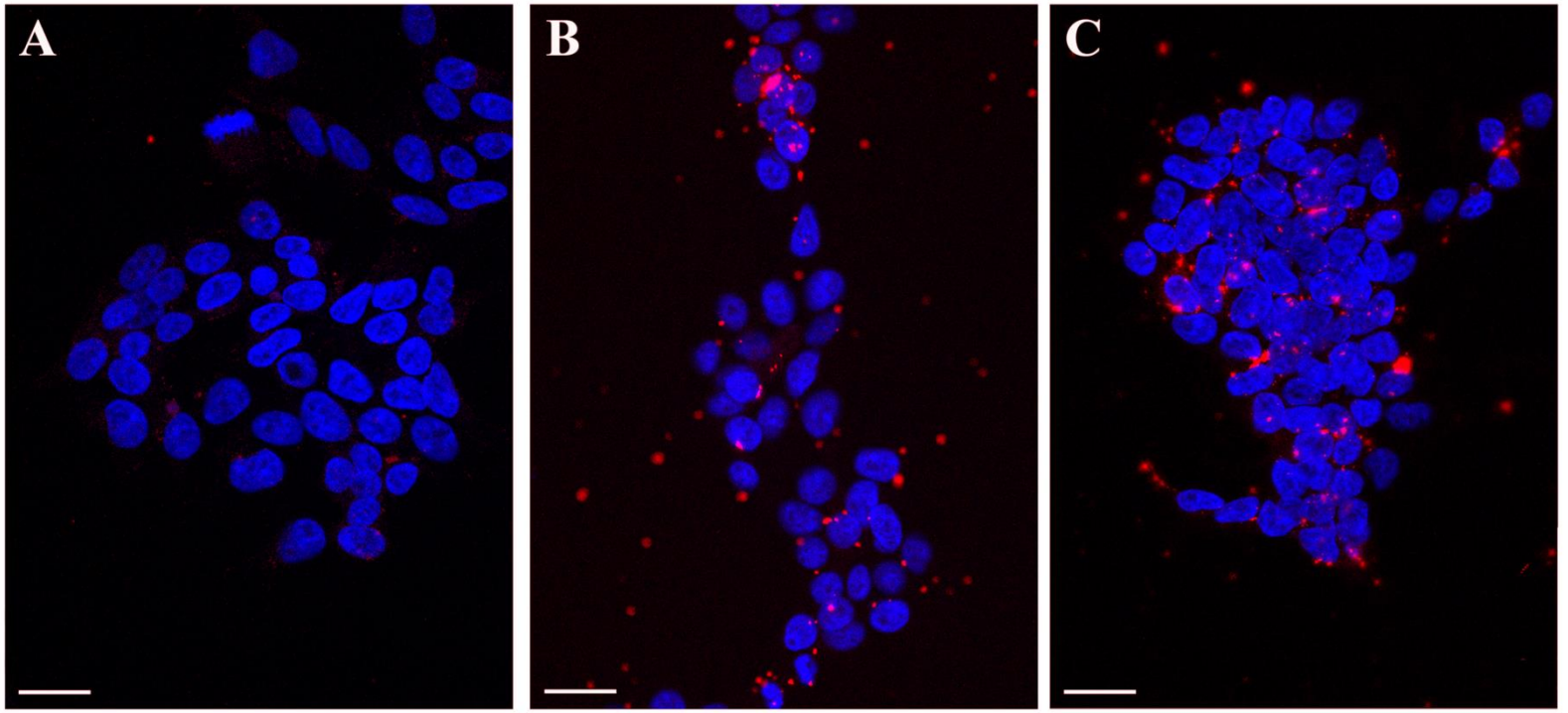
- Cy3 is a cyanine, therefore it can be used as a fluorescent dye.
- Cy3 is excited with λ = 570 nm-1
- siRNA can be marked with Cy3, so you can know the % of cells transfected (flow citometry) and track the transfection in cells with images (confocal microscopy)



siRNA TRANSFECTION. siEGFPCy3



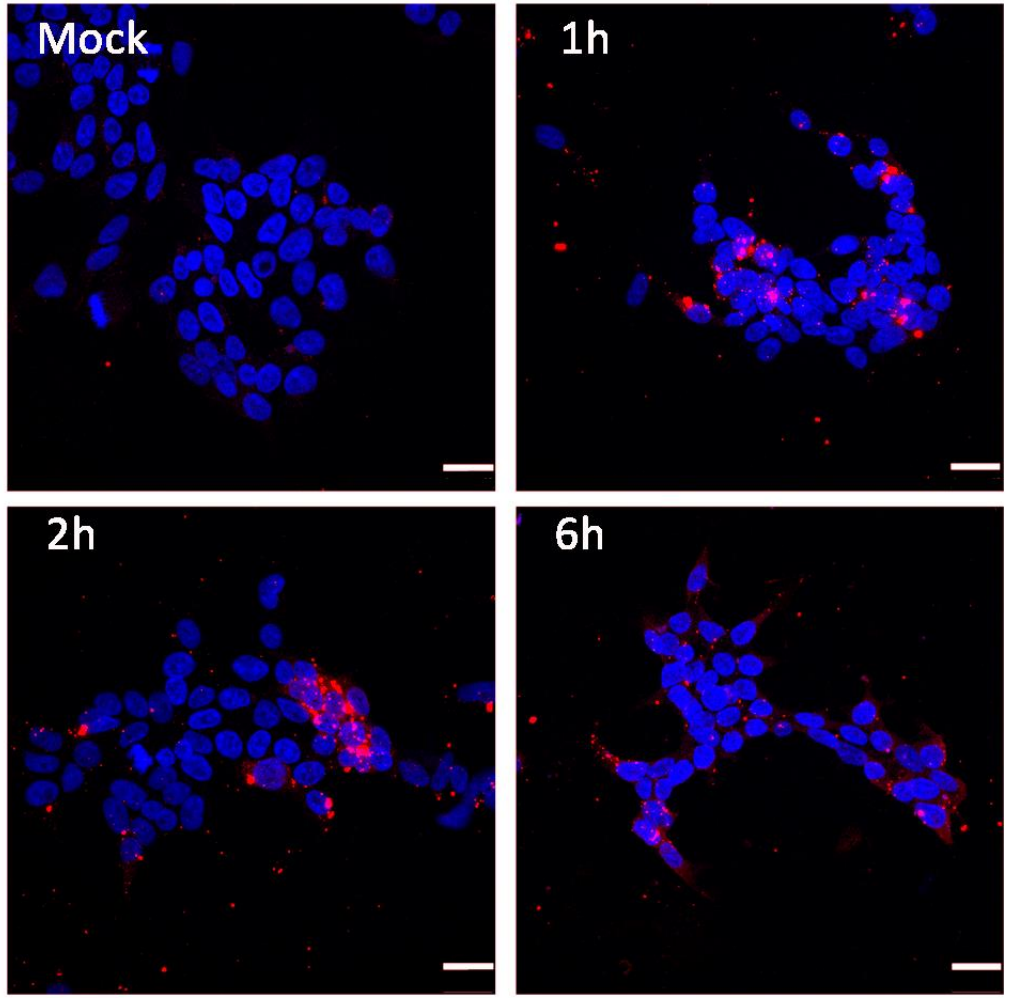
siRNA TRANSFECTION. siEGFPCy3

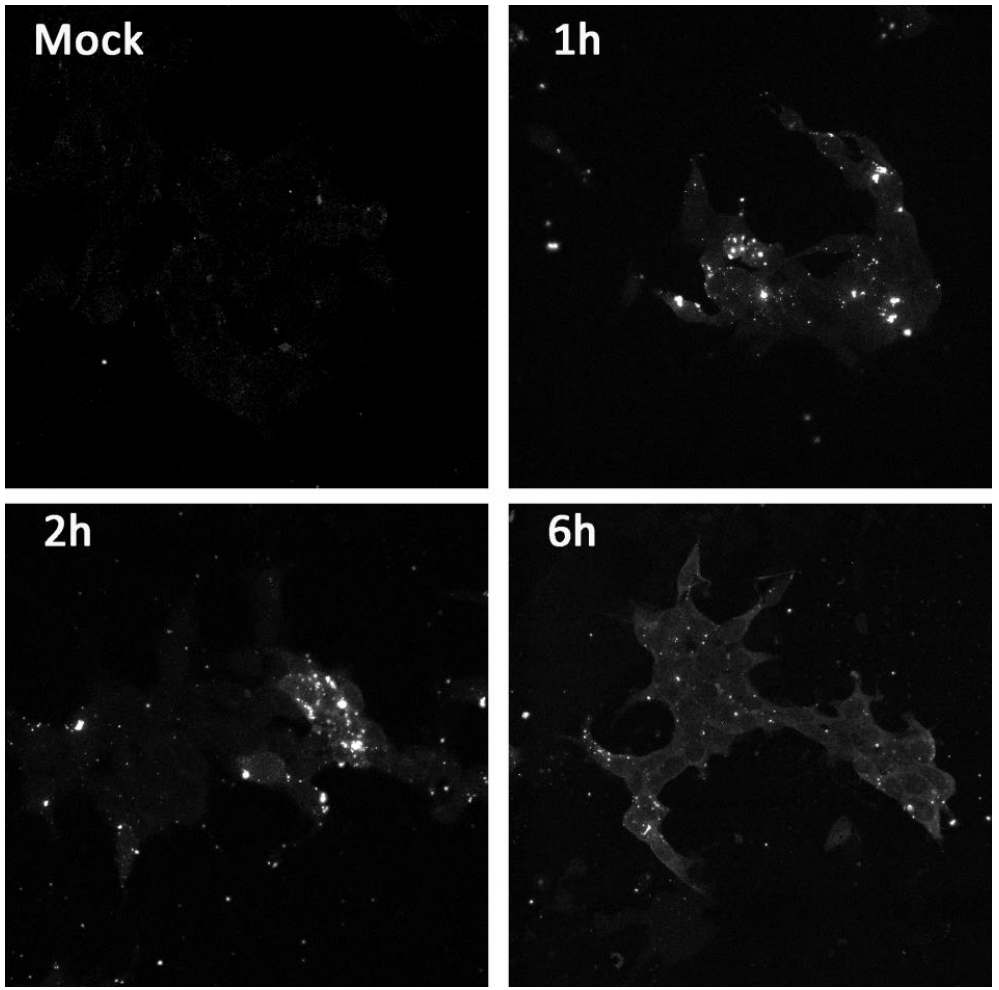


A → Negative Control
B → Positive Control
C → Reference 14

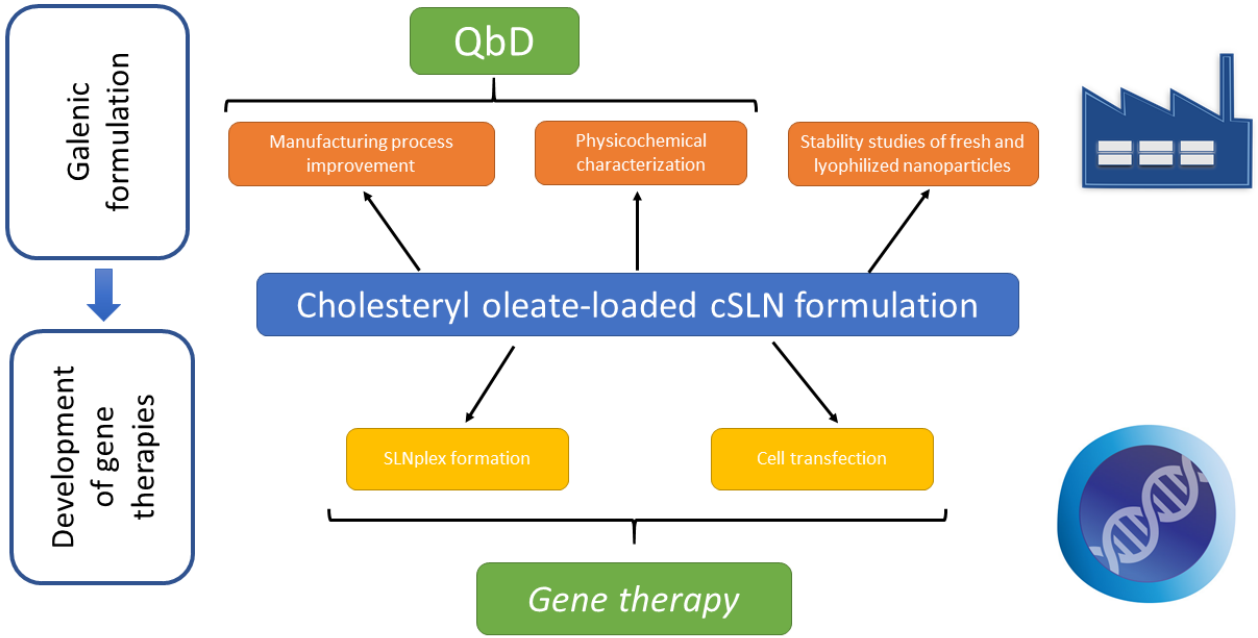
BLUE → NUCLEUS (DAPI)
RED → SLNPLEXES (siRNACy3)

siRNA TRANSFECTION. siEGFPCy3





BLUE → NUCLEUS (DAPI)
RED → SLNPLEXES (siRNACy3)



CHARACTERIZATION OF REF. 14

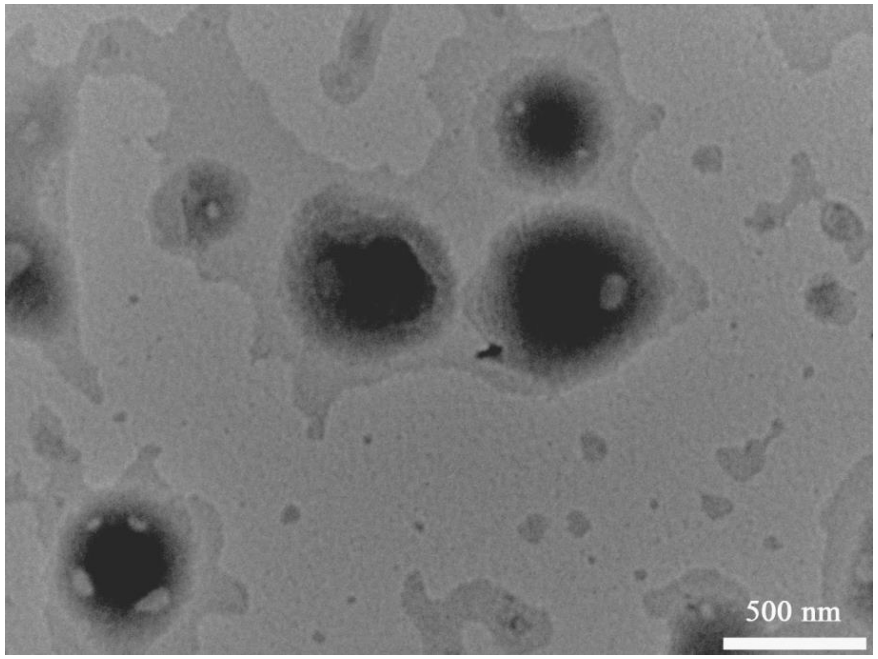
CHARACTERIZATION OF REF. 14

- All the galenic and biologic characterization was performed with the lyophilized resuspended.
- The assays performed were:

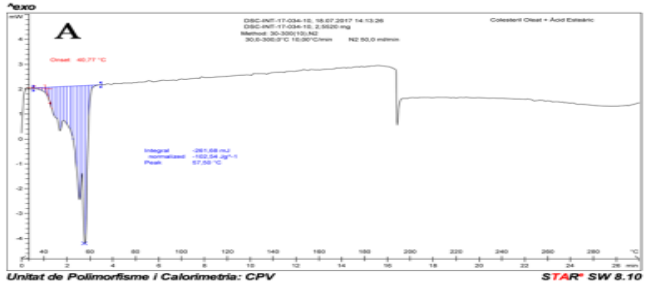
  - Size
  - Zeta Potential
  - Morphology and shape
  - Surface composition
  - Excipients Compatibility
  - Critical Collapse Temperature
  - Stability Studies
  - Cell viability (MTT)
  - Studies of SLNplexes formation
  - Cellular location of siRNA transfected

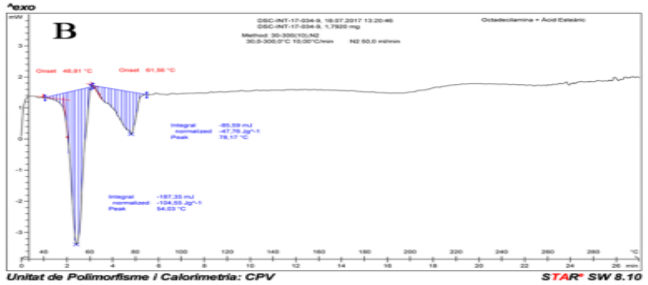
SIZE, ZETA POTENTIAL AND MORPHOLOGY (TEM)

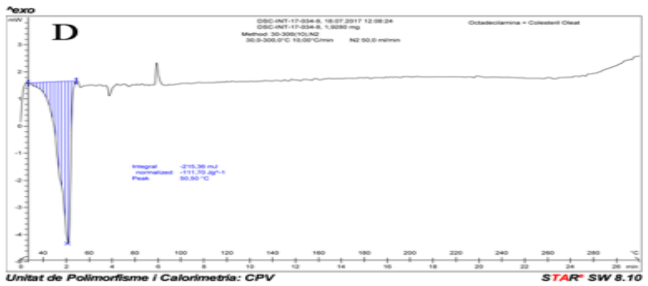
Experiments	Particle size (nm)	PdI	Zeta potential (mV)
1	172	0,210	39,8
2	224	0,209	34,0
3	255	0,224	30,0
4	173	0,204	33,1
5	210	0,226	36,9
Mean	207	0,215	34,76
Standard Deviation	35,06	0,0098	3,74

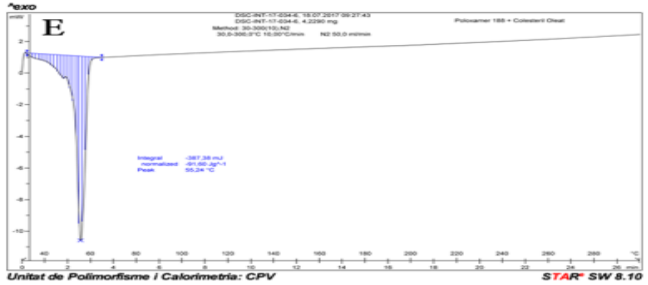


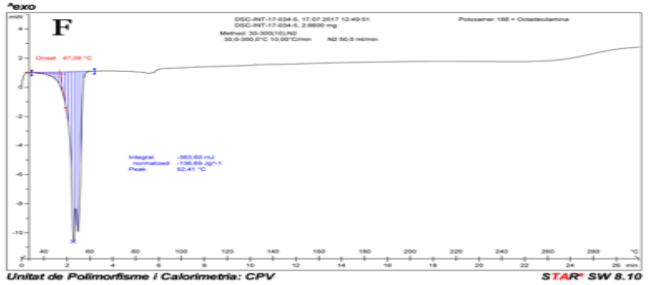
EXCIPIENTS COMPATIBILITY (DSC)











A: Stearic Acid + CO

B: Stearic Acid + Octadecylamine

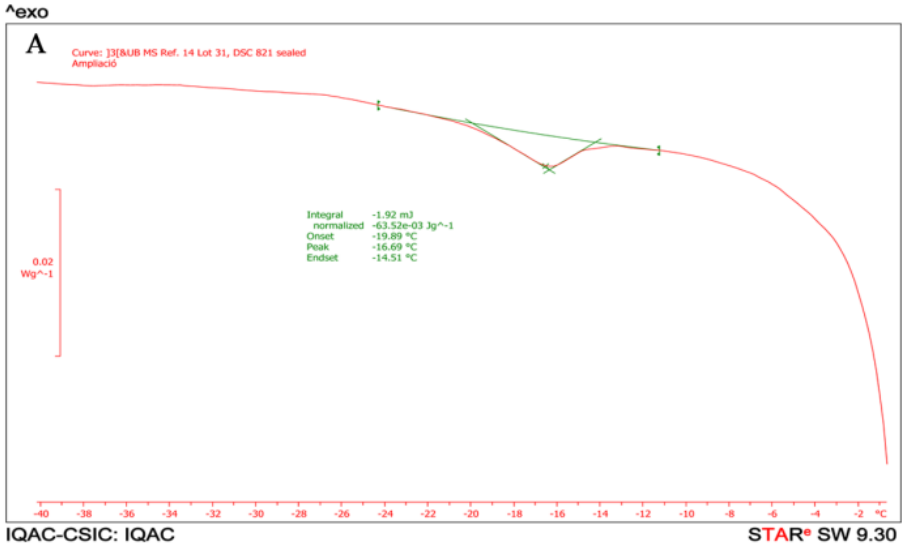
C: Stearic Acid + Poloxamer 188

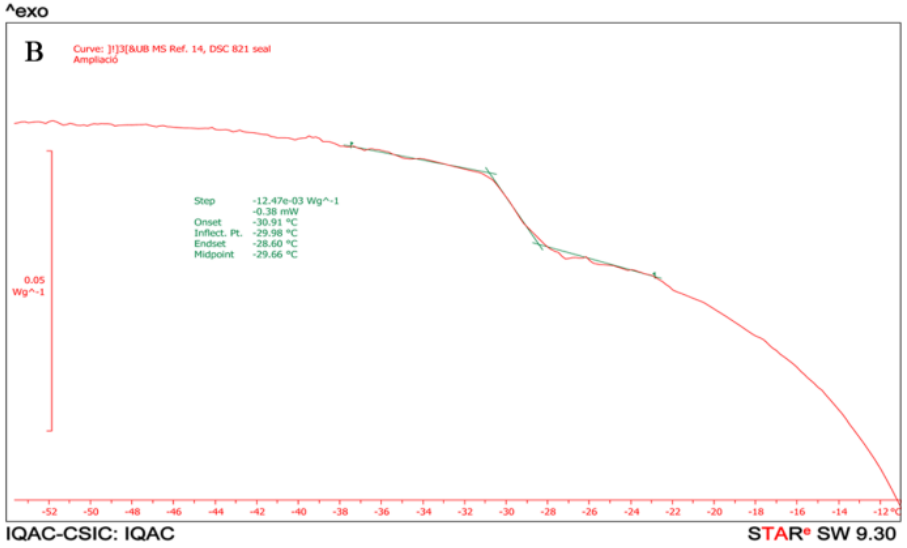
D: CO + Octadecylamine

E: CO + Poloxamer 188

F: Octadecylamine + Poloxamer 188

CRITICAL COLLAPSE TEMPERATURE (DSC)

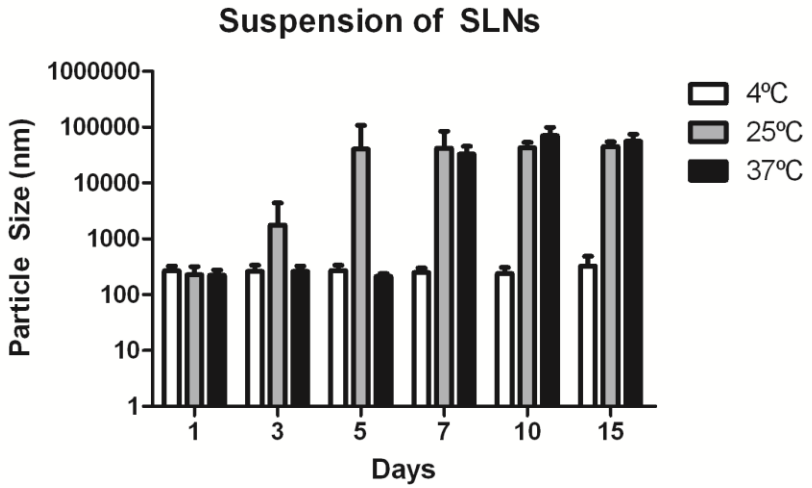


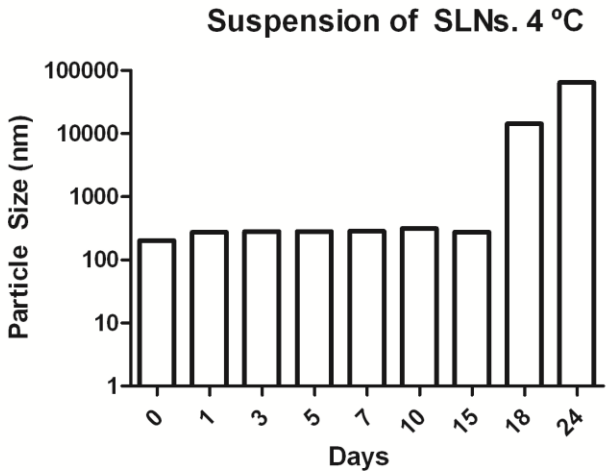


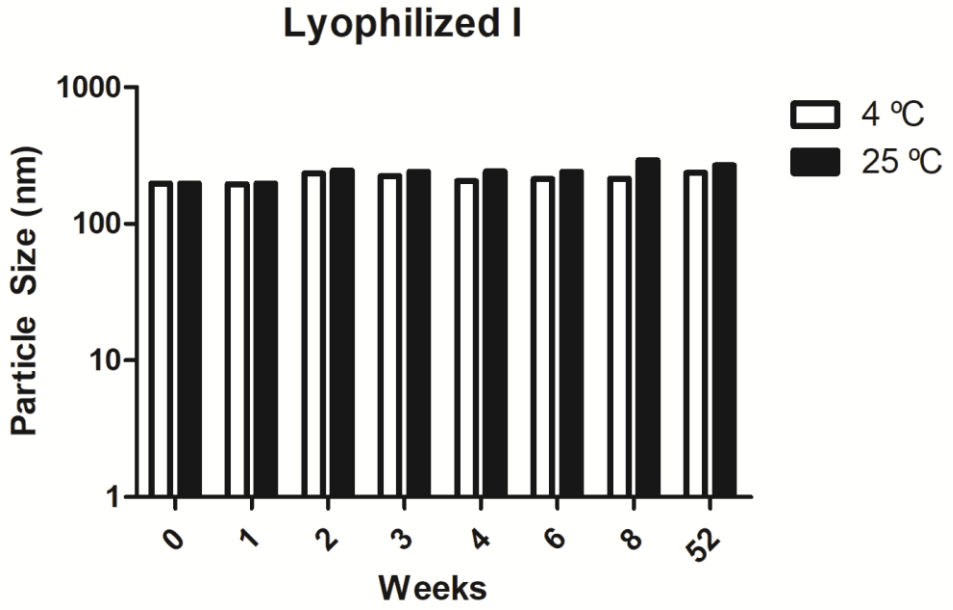
A: Critical collapse temperature (SLNs) → -18 ˚C

B: Critical collapse temperature (SLNs + Trehalose) → -28 ˚C

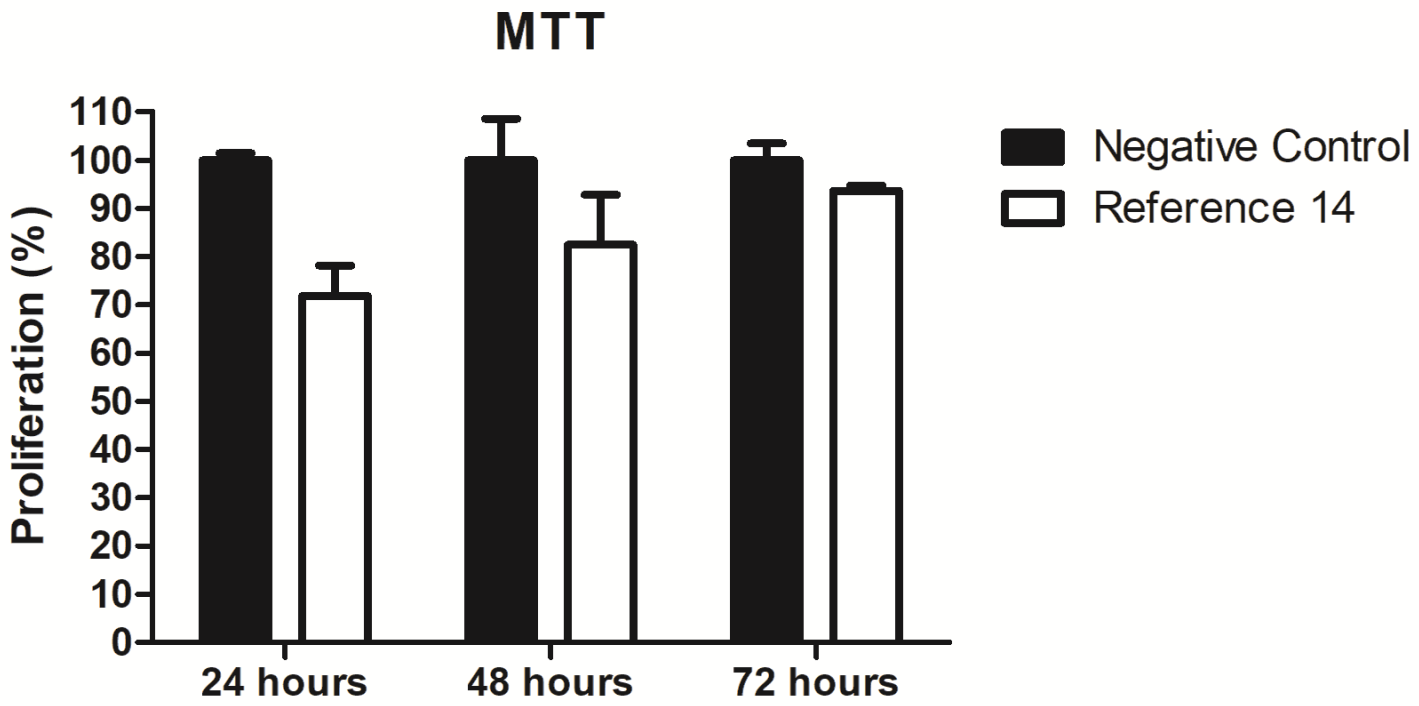
STABILITY STUDIES



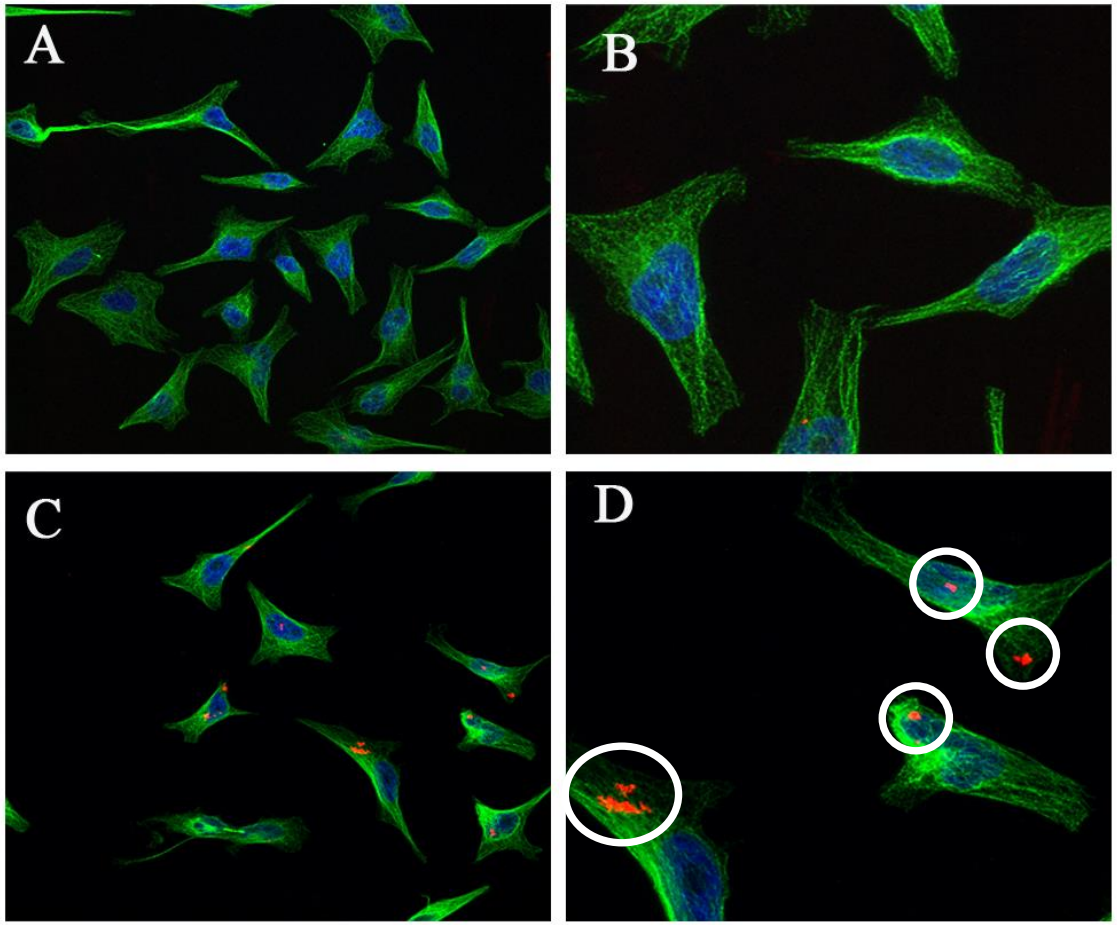




CELL VIABILITY (MTT ASSAY)



CELLULAR LOCATION OF siRNA TRANSFECTED WITH REF. 14



HeLa Cells

BLUE → NUCLEUS (DAPI)
GREEN → CYTOSKELETON (α-TUBULIN)
RED → SLNPLEXES (siRNACy3)

utmb Health

Working together to work wonders.™

DENGUE INFECTION AFTER THE TRANSFECTION OF siP2.1 USING SLN AS A VECTOR

DENGUE VIRUS

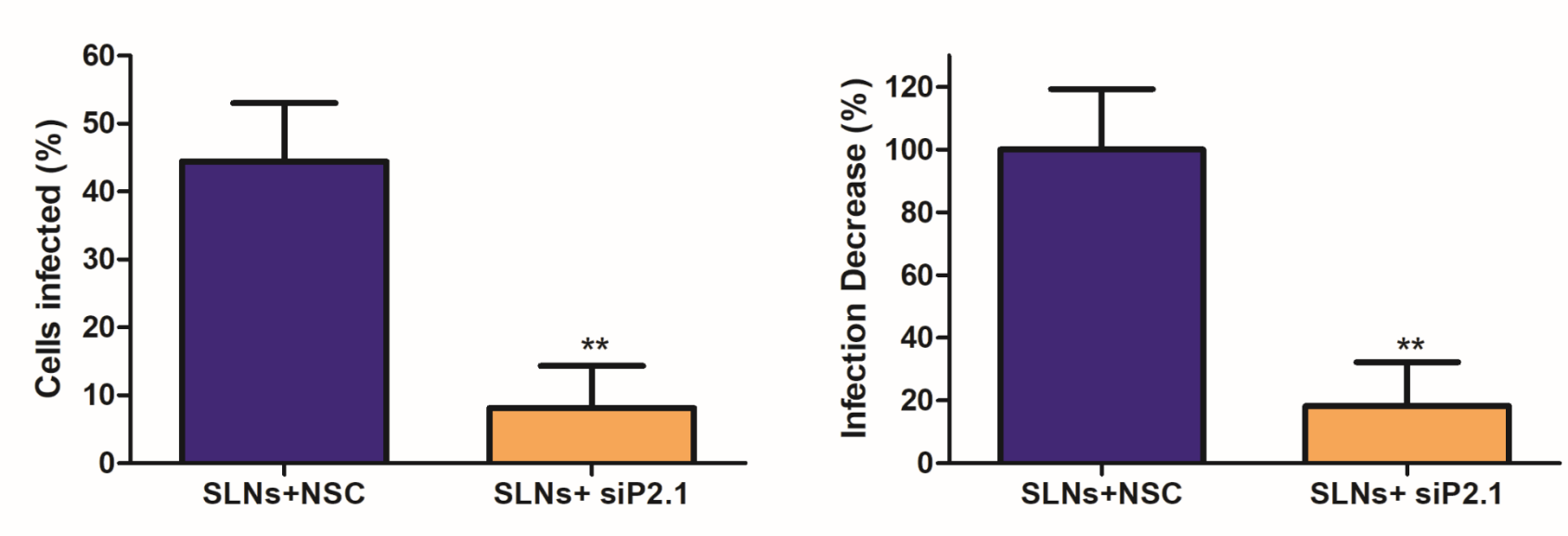
- Dengue Virus (DENV) is the cause of Dengue Feber.
- DENV is a Flavivirus, like Zika Virus, Yellow Feber Virus, etc.
- More than 100 million of cases around the world.
- 25.000 people die because of its infection every year.
- Currently, there is not any vaccine.

RPLP1/2

- RPLP1 and RPLP2 (RPLP 1/2) are essential for flaviviruses to infect cells, because they promote the early accumulation of viral proteins (Campos et al., J Vir, 2017)

- If RPLP1 and RPLP2 are knockdown, the DENV infection decreases extremely (Campos et al., J Vir, 2017)

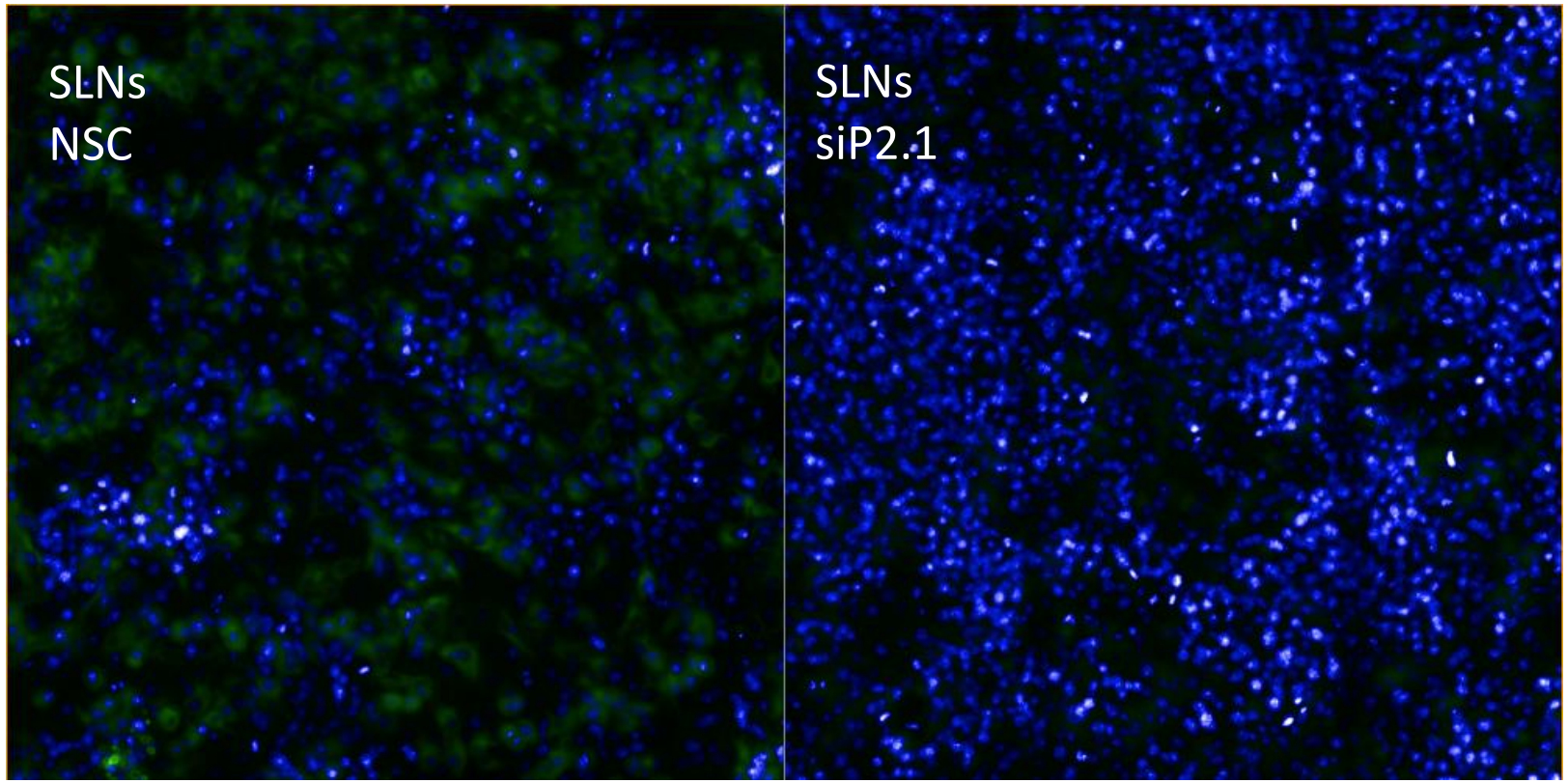
RESULTS



SLNs+NSC → Negative control

SLNs+siP2.1 → Treatment with the SLNs+siRNA

RESULTS

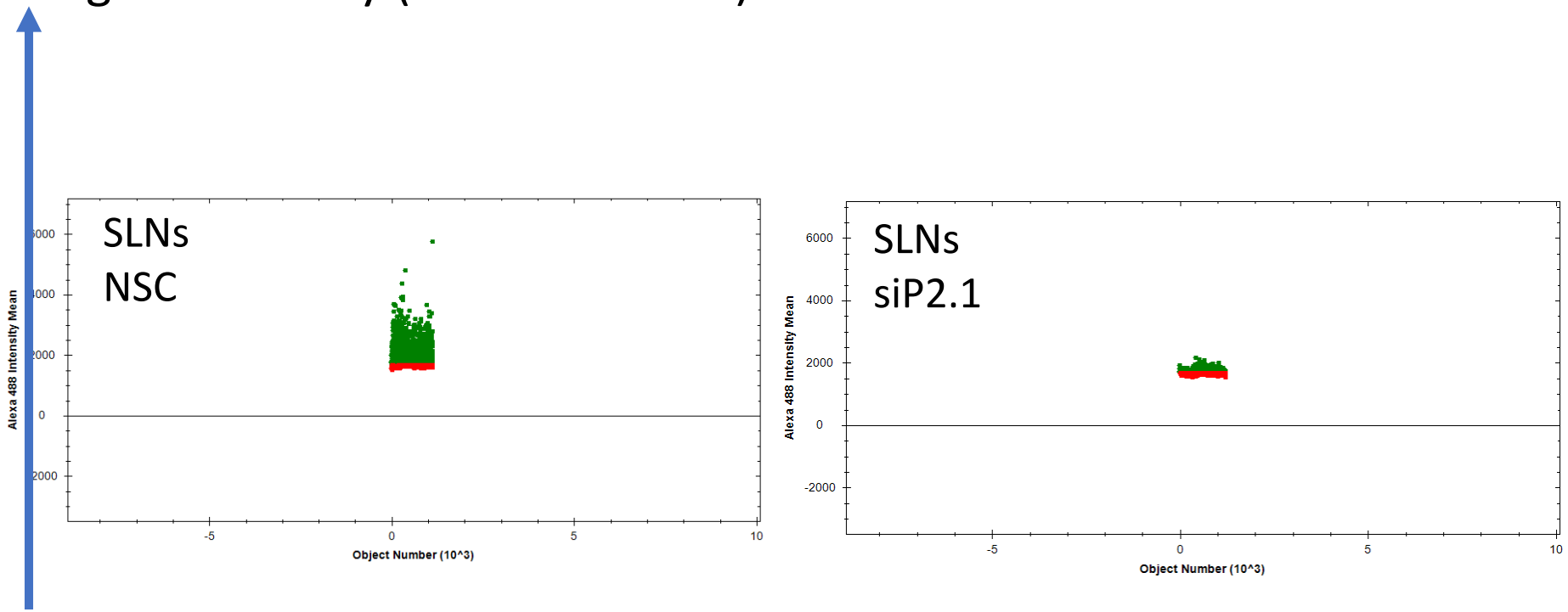


BLUE → NUCLEUS (DAPI)
GREEN → Virus Protein

SLNs+NSC → Negative Control
SLNs+siP2.1 → SLN:siRNA

RESULTS

+ Signal Intensity (more infection)



GREEN → Positive (infected cell)

RED → Negative (non-infected cell

NEXT STEPS

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Cristina Moreno-Castro
Younes El Yousfi
Sofía Boyero-Corral

Salvador Guerrero Fernández
Blanca Martínez

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JR Ticó-Grau, PhD, PI
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Débora Mercadé Frutos, PhD
Susana Fuentes Sánchez
Marta Toro
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Steven T. Powell
Jingru Fang


OUI
à
l'amour


JOHN SMITH