Structure and shape transitions in PEGylated paclitaxel-loaded cationic liposomes enhances delivery and cytotoxicity to human cancer cells

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Evolution of liposomes in drug/Nucleic Acid delivery (Bangham & Horne 1964)

Redrawn from CR Safinya & KK Ewert
Nature 2012, 489, 372-374 (News & Views article)

Stealth Liposomes
(1988-1990)

Cationic Liposomes:
(Felgner et al 1987: bead on string model)

+ DNA

CL-DNA Complex
(1997)

Fundamental Studies & Biomedical Applications: **Cationic Liposome (CL)-Carriers** used in ≈ 119 gene therapy clinical trials/2018 FDA approval of Onpattro (CL-siRNA) to silence mutated TTR gene

Outline:

(A) Surface-functionalized Cationic Liposome (CL)-DNA Nanoparticles (NPs): Imaging of intracellular pathways.

(B) Pegylated Cationic Liposome (PEG-CL) Vectors of Hydrophobic Paclitaxel (PTX) for Cancer Therapeutics.

Goal:

Mechanistic understanding of nucleic acid (DNA, RNA) and hydrophobic drug delivery including NP intracellular pathways and interactions of NPs with cell components (cell imaging + other techniques)

Cationic Liposomes (CLs): Nonviral Vectors for Nucleic Acids

INITIAL WORK:

Felgner, P. L. et al. *Proc. Nat. Acad. Sci. USA* **1987**, 84, 7413 (> 3500 citations)

(1987 PNAS) Electrostatic attachment to cells via surface sulfated proteoglycans

Early Clinical Trials:

G. Nabel, E. Nabel, L. Huang et al. *PNAS USA* **1993** (> 900 citations);
E. Hersh et al.; ..

Human Gene Therapy Clinical Trials:

<https://www.wiley.com/legacy/wileychi/genmed/clinical/> 2018
data ≈ 119 Trials (4.2%) use CL-vectors for targeting cancer, cystic fibrosis,... Total trials ≈ 2805

2018 FDA approval of Onpattro (CL-siRNA) to silence mutated TTR gene to treat rare genetic disease.

Q: How does non-viral nucleic acid delivery work?

Answer: Early stages of understanding of the pathways used by non-viral vectors in nucleic acid delivery applications

> 2800 Open gene therapy clinical trials worldwide

	Number of open clinical trials worldwide	Diseases treated
Viral Vectors		
Retrovirus	496 (17.7%)	Cancers, SCID, AIDS, hemophilia, others
Adenovirus	535 (19.1%)	Cancers, cystic fibrosis, peripheral artery disease, others
Pox virus	70 (2.5%)	Cancers
Adeno-associated virus	227 (8.1%)	Cystic fibrosis, hemophilia, prostate cancer
Herpes simplex virus	95 (3.4%)	Cancers
Nonviral Vectors		
Lipofection (lipids)	119 (4.2%)	Cancers, cystic fibrosis, coronary artery disease
Naked Plasmid DNA	446 (15.9%)	Cancers, artery diseases, others
RNA transfer	51 (1.8%)	Cancers
siRNA	5 (0.2%)	Cancers

Current information on clinical trials in the field of gene therapy can be found on the internet at <https://www.wiley.com/legacy/wileychi/genmed/clinical/>

Lipids

Cationic Lipids

DOTAP 1+

Multivalent
Cationic Lipids via
Custom synthesis
(Kai K. Ewert)

MVL5 5+

DOPC ($C_o = 0$)

Neutral Lipids

GMO
($C_1C_2 < 0$)

DOPE
($C_o < 0$)

LIPID SHAPE (C_o) → Distinct Self Assembled Structure

Cationic Liposomes (CLs): Carriers of DNA and si-RNA for Gene Delivery & Gene Silencing

CLs Drive 3D Organization of Nucleic Acids in Distinct Geometries

Structures of
Cationic Liposome-
Nucleic Acid
Complexes from
Synchrotron SAXS

J. Rädler *et al.*
Science **1997**,
275, 810-814

L_{α}^c
 $C_o = 0$

I. Koltover *et al.* *Science*
1998, 281,
78-81

H_{II}^c
 $C_o < 0$

K. Ewert *et al.* *J. Am.*
Chem. Soc. **2006**, 128,
3998-4006

H_I^c
 $C_o > 0$
(MVLBG2,
+16e)

C. Leal *et al.* *J. Am.*
Chem. Soc. **2010**,
132, 16841-16847

$Q_{II}^{G,siRNA}$

$C_1 C_2 < 0$

3D Double
Gyroid Cubic
Lattice

Self-Assembly of DNA and Cationic Liposomes into the Lamellar L_{α}^C Phase

**Driving Force for Self-Assembly:
Entropy gain through release of counter-ions**

Rädler, J. O.; Koltover, I.; Salditt, T.; Safinya, C. R.: Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. *Science* **1997**, 275, 810-814

Multivalent Cationic Lipids

Ewert, K. K.; et al. *J. Med. Chem.* **2002**, *45*, 5023-5029

MVL2 2^+
(Maximum)

MVL3 3^+
(Maximum)

MVL4 4^+
(Maximum)

TMVL5 5^+
(Maximum)

MVL5 5^+
(Maximum)

Multivalent CL-DNA Complexes: Transfection Efficiency (TE) vs Membrane Charge Density (σ_M)

Plot
versus
 σ_M

L_{α}^C Complexes: Bell Curve for TE vs Membrane Charge Density (σ_M).
 σ_M is a Predictor of Transfection Efficiency for lamellar L_{α}^C complexes.

H_{II}^C complexes (DOPE) show no σ_M dependence (open circles)

TE is dependent on Structure of CL-NA complex: Non-lamellar complexes (H_{II} and H_I) independent of σ_M

Ahmad, A.; Evans, H. M.; et al. *J. Gene Med.* **2005**, 7, 739-748.

Zidovska, A.; et al. *J. Phys. Chem. B* **2009**, 113, 3694-3703

Surface-Functionalized PEGylated CL-DNA Nanoparticles (NPs)

PEGylation required for in vivo applications
(to suppress immune cell clearing of NPs)

Peptide Sequence	Spacer
GRGDSP (& cyclic RGD)	PEG 2000 (n = 46)
	Hydrolyzable PEG
(SV 40 NLS)	PEG 1000 (n = 23)

Specific Cell-Receptor attachment via RGD (R. Majzoub et al., *Biomaterials* 2014, 35, 4996.)

Endosomal escape via HPEG-L (C-L Chan et al. *Biomaterials* 2012, 33, 4928)

Nuclear Localization Sequence

Spatial-Temporal tracking of Nanoparticles by Co-localization with Rab-GFP

Rab GTPases: Coordinators of Vesicle Trafficking

Location and Function of Rab GTPases

(adapted
from H.
Stenmark
, *Nature
Reviews
MCB*
2011)

Rab 5 (Early
endosome
marker)

Rab5 cycles between
PM and EE:
mediates vesicle
budding (endocytosis),
trafficking and fusion to
Early Endosomes)

Rab 7, 9 (Late
endosome
markers)

Tagging of intracellular organelles with **Rab-GFP**

Co-localization of NPs and Mutant Rab5-Q79L

Mutant Rab5-Q79L inhibits endosome maturation into Late Endosome:
Formation of Giant Early Endosomes

(A,B) $\approx$ few PEG-CL-DNA NPs in endosomes (missing peptide ligand):

(C,D) RGD-PEG-CL-DNA NPs are seen in endosomes:

Visualization of NPs inside endosomes: New Technique to unravel mechanisms of endosomal escape.

Majzoub, R. N.; et al.: Fluorescence microscopy colocalization of lipid-nucleic acid nanoparticles with wildtype and mutant Rab5-GFP: A platform for investigating early endosomal events. *Biochim. Biophys. Acta – Biomembranes* **2015**, 1848, 1308-1318. DOI: [10.1016/j.bbamem.2015.03.001](https://doi.org/10.1016/j.bbamem.2015.03.001)

Cationic Liposome (CL) Vectors for Cancer Therapeutics

**Paclitaxel (PTX,
hydrophobic)**

breast, ovarian, lung, bladder,
prostate, melanoma, esophageal
cancers

(World Health Organization's
List of Essential Medicines)

**PTX stops cell division by stabilizing
microtubules: mitotic block and apoptosis**

Steffes, V. M.; et al. : Distinct solubility and cytotoxicity regimes of paclitaxel-loaded cationic liposomes at low and high drug content revealed by kinetic phase behavior and cancer cell viability studies. *Biomaterials*, **2017**, *145*, 242-255. DOI: [10.1016/j.biomaterials.2017.08.026](https://doi.org/10.1016/j.biomaterials.2017.08.026)

NPs for in vivo applications require PEG-polymer coat to delay nanoparticle clearance by immune cells

CL-DNA NPs: Gene delivery efficiency decreases with PEG

Majzoub, R. N.; Chan, C.-L.; et al.,
Biomaterials **2014**, 35, 4996-5005. DOI:
[10.1016/j.biomaterials.2014.03.007](https://doi.org/10.1016/j.biomaterials.2014.03.007)

DNA delivery by cationic liposomes hindered by PEGylation (Brush regime)

10% PEG2K (DOTAP, Low σ_M)

10% RGD PEG2K (DOTAP, Low σ_M)

lipid
DNA

A

B

Active targeting by RGD-PEG2k-lipid required for CL-DNA particle uptake

Majzoub, R. N.; Chan, C.-L.; et al.,
Biomaterials **2014**, 35, 4996-5005. DOI:
[10.1016/j.biomaterials.2014.03.007](https://doi.org/10.1016/j.biomaterials.2014.03.007)

PEGylated Cationic Liposomes: PTXL vs. Gene delivery

CL-PTXL NPs: Cell death efficacy **increases** with PEG

less efficient PTXL delivery, more cancer cells remain alive

UNEXPECTED result:
more efficient PTXL delivery in sterically stabilized brush regime

Steffes, V. M.; Zhang, Z.; et al: *ACS Appl. Mat. Interf.* **2020**, *12*, 151-162. DOI: [10.1021/acsami.9b16150](https://doi.org/10.1021/acsami.9b16150).

CL-DNA NPs: Gene delivery efficiency **decreases** with PEG

Majzoub, R. N.; Chan, C.-L.; et al., *Biomaterials* **2014**, *35*, 4996-5005. DOI: [10.1016/j.biomaterials.2014.03.007](https://doi.org/10.1016/j.biomaterials.2014.03.007)

Paclitaxel (PTXL) delivery by cationic liposomes enhanced by PEGylation

**PEGylated vesicles (in brush regime)
enter cells in high numbers**

Flow cytometry

Flow cytometry consistent with confocal microscopy, indicating higher PTX uptake from PEGylated liposomes

Cationic Liposomes as Vectors in Nucleic Acid and Hydrophobic Drug Delivery (gene therapy, gene silencing, cancer therapeutics)

Ongoing studies

In vitro and in vivo work with CL-PTX and CL-Nucleic Acid NPs (in vivo studies in collaboration with Tambet Teesalu group)

Steffes, V. M.; Zhang, Z.; et al: PEGylation of paclitaxel-loaded cationic liposomes drives steric stabilization of bicelles and vesicles thereby enhancing delivery and cytotoxicity to human cancer cells. *ACS Appl. Mat. Interf.* **2020**, *12*, 151-162. DOI: [10.1021/acsami.9b16150](https://doi.org/10.1021/acsami.9b16150).

Wonder, E. A.; Simón-Gracia, L. et al: Competition of Charge-Mediated and Specific Binding by Peptide-Tagged Cationic Liposome–DNA Nanoparticles *In Vitro* and *In Vivo*. *Biomaterials* **2018**, *166*, 52-63. DOI: [10.1016/j.biomaterials.2018.02.052](https://doi.org/10.1016/j.biomaterials.2018.02.052)

Steffes, V. M.; et al.: Distinct solubility and cytotoxicity regimes of paclitaxel-loaded cationic liposomes at low and high drug content revealed by kinetic phase behavior and cancer cell viability studies. *Biomaterials*, **2017**, *145*, 242-255. DOI: [10.1016/j.biomaterials.2017.08.026](https://doi.org/10.1016/j.biomaterials.2017.08.026); PMCID: [5610109](https://pubmed.ncbi.nlm.nih.gov/2610109/).

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Cationic liposome gene
delivery/ gene
silencing/hydrophobic drug
delivery

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