Translational models to study lipid based gene delivery systems

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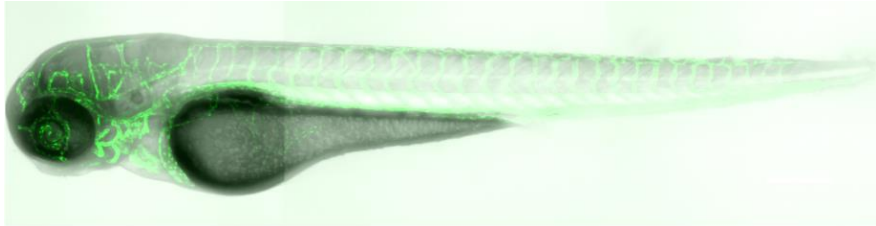
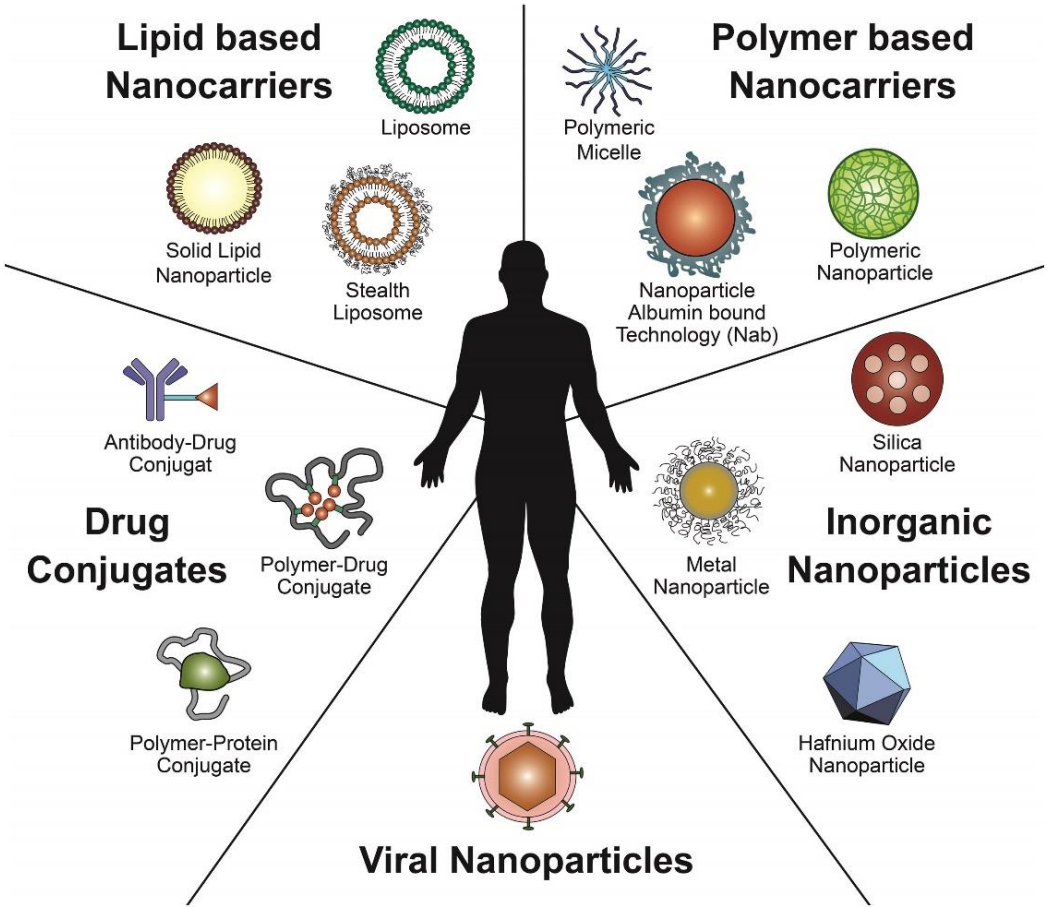


University of Basel

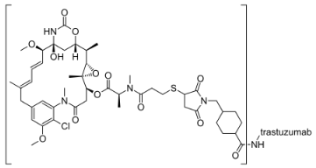
7th International Symposium on Phospholipids in Pharmaceutical Sciences

Particulate drug carriers as therapeutics



Moderna/Pfizer mRNA-LNP vaccines

Aerosil: Fumed silica
(thixotropic; anti-caking)



Trastuzumab emtansine

Abraxane (cancer):
nanoparticle albumin–bound paclitaxel

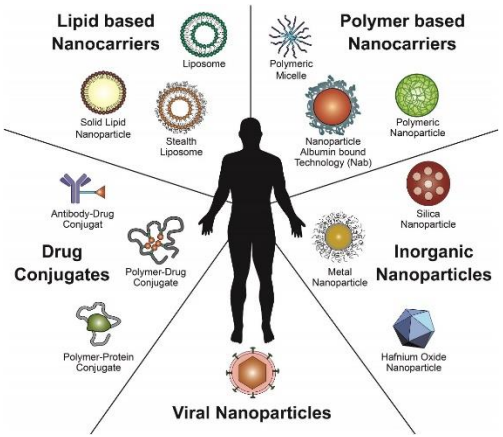
Doxil: pegylated liposomes
(cancer; doxorubicin)

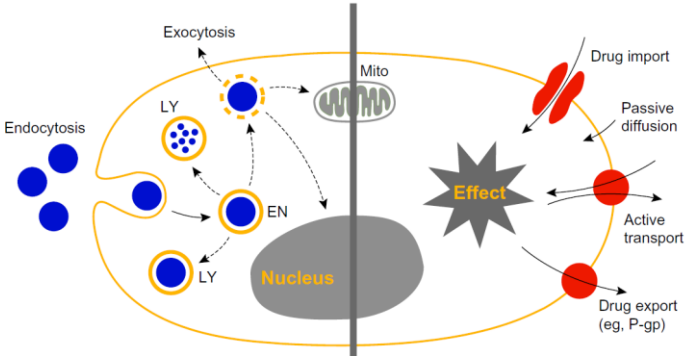
Wicki et al., Journal of Controlled Release, 2015

Unique properties and complex biological interactions of nanomaterials

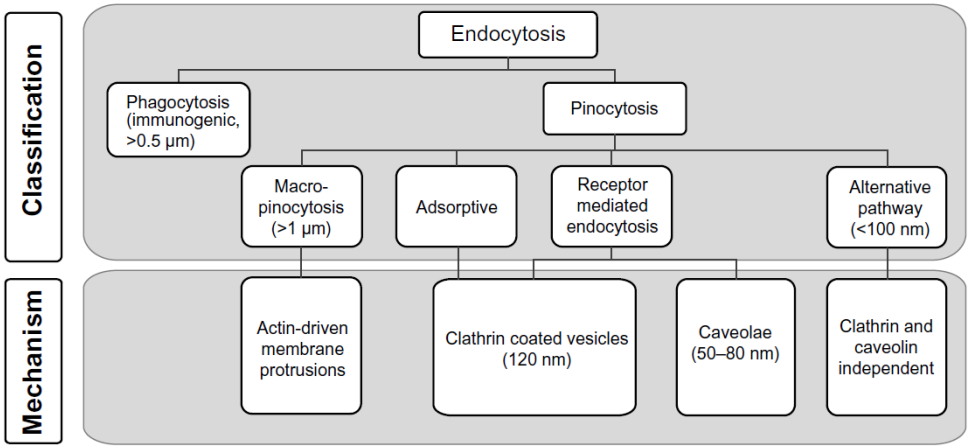
Unique properties of Engineered Nanomaterials:

- Chemical properties
  - huge specific surface (e.g. several 100 m^2/g for Aerosil)
- Physico-chemical properties
  - size, geometry, PDI, ζ-potential, colloidal stability
- Biological interactions
  - opsonization, receptor-mediated cellular uptake
  - immune reactions
- Regulatory challenges
  - no harmonization of existing safety and testing guidelines





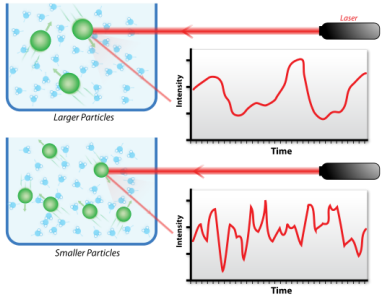
Appearance	Suspension	Solution
Molecular weight	High (>1,000 Da)	Low (<1,000 Da)
Transport	Active	Passive and active
Adverse effects	Delayed, cumulative	Often acute

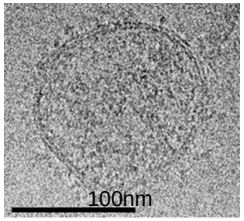


Kettiger et al., Int. J. Nanomedicine, 2013

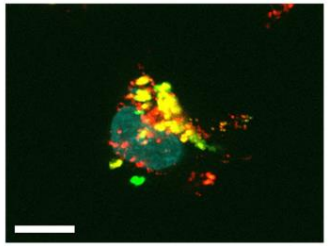
The challenge:
In vivo screening, development of therapeutics

in vitro



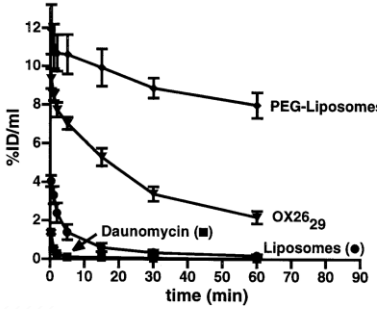


Physico-chemical characterization
e.g. DLS, TEM, cryo-TEM…



***In vitro* cellular uptake, intracellular processing, cytotoxicity**
e.g. confocal microscopy, FACS…

in vivo



??

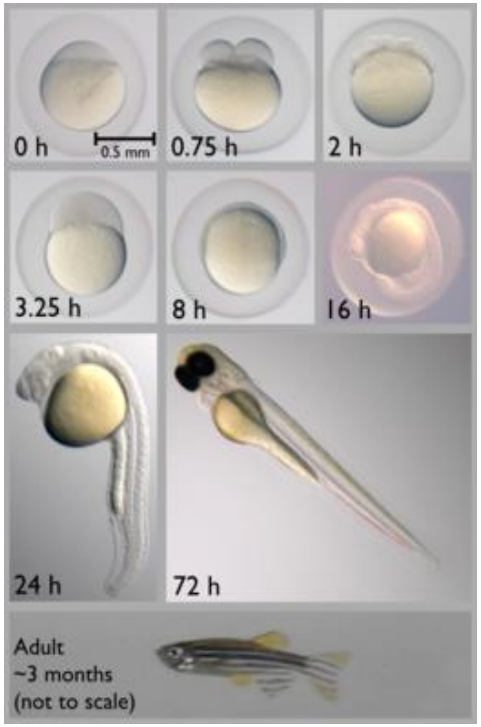
Screening?

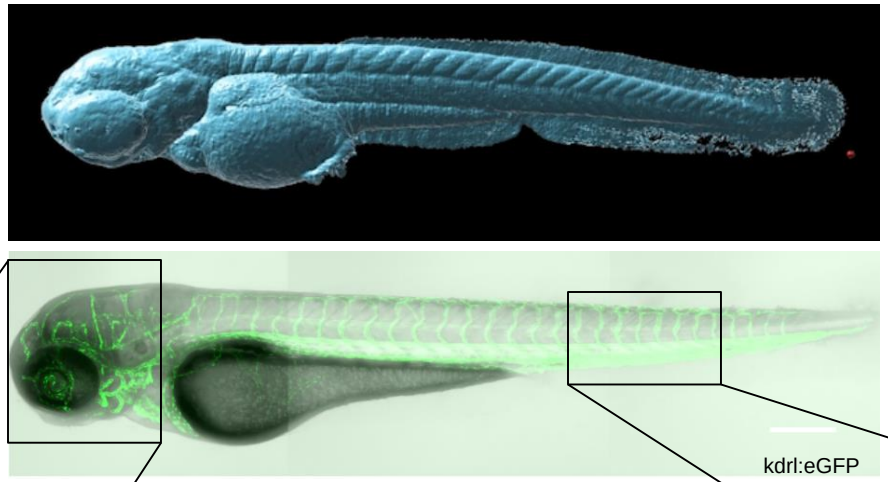
Extrapolation?

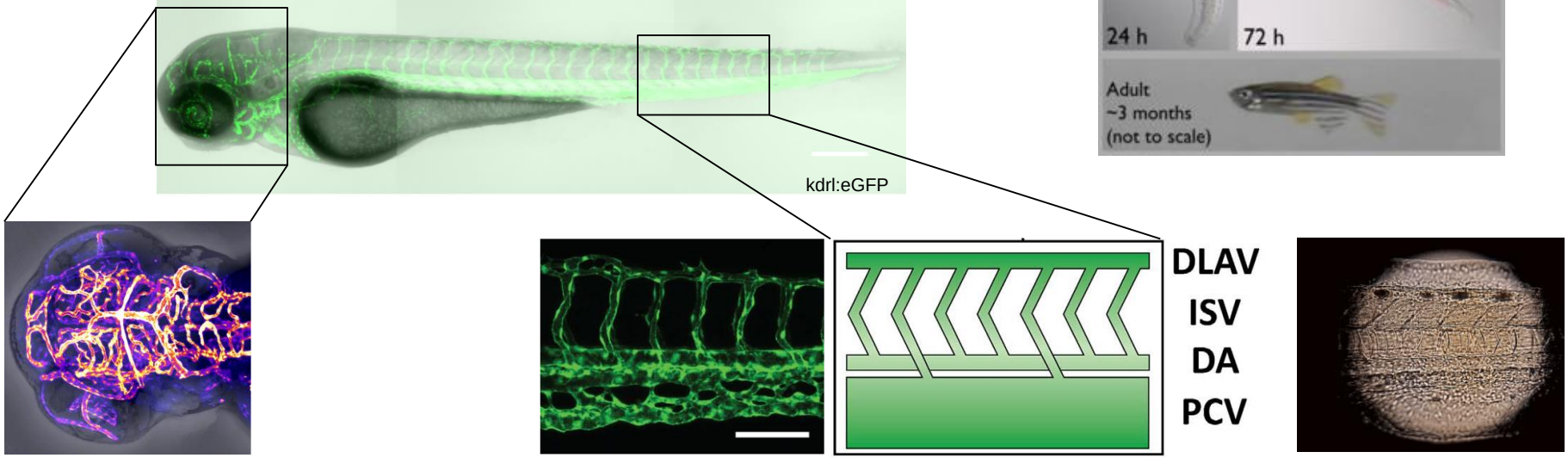
***In vivo* evaluation**
e.g. PK, PD, Tox, clinical trials, regulatory…

Zebrafish (*Danio rerio*) larvae as a vertebrate screening model

Fast development of zebrafish (egg -> larvae in 3 days)
Development outside of its mother
Optically transparent
Not an autonomous animal until 120 hpf
Transgenic/wt/mutant fish lines (> 30’000 at EZRC)

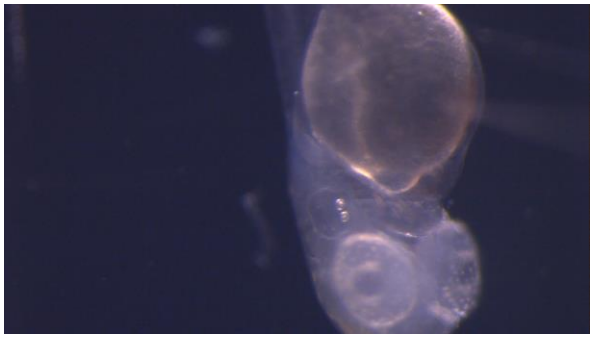




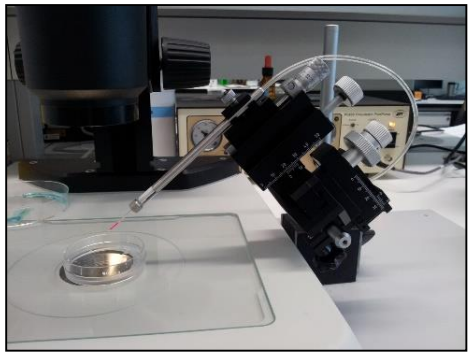


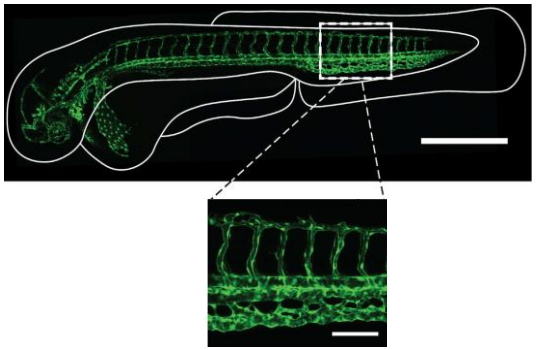
Synchrotron X-ray tomography @ PSI: Cörek et al., Small, 2020 (http://zebrafish.pharma-te.ch)

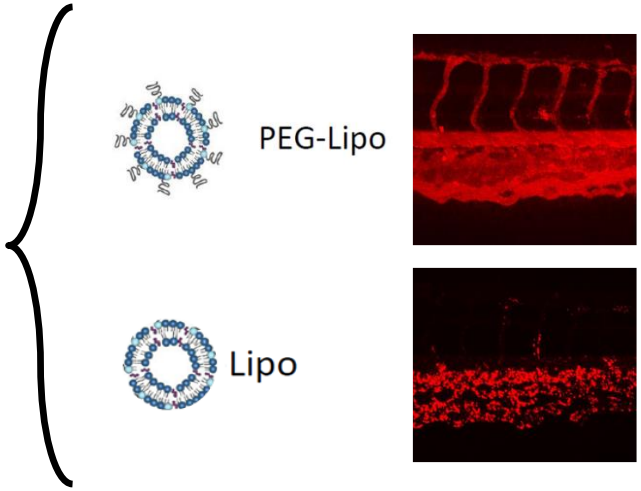
ZFL: intravenous injection



***i.v.* injection**
1-5 nl
Duct of Cuvier







Aim: Establish zebrafish as a vertebrate screening tool to study the circulation and tissue distribution of nanoparticles

J Control Release. 2017; 264:180-191
Nat Commun. 2018; 9(1):1127
ACS Nano. 2018; 12(3):2138-2150

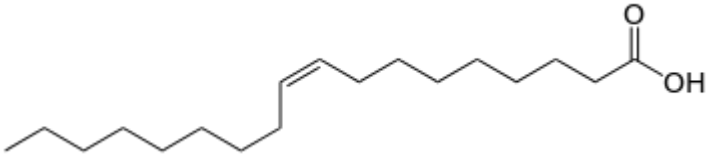
Presented projects

Application of the model:

- **Optimization of liposomal formulations**
- **Gene delivery**

Lipid based drug carriers: Optimization of PK properties

Goal:
Optimization of liposomal formulations to **reduce plasma clearance** and to **enhance tissue accumulation**

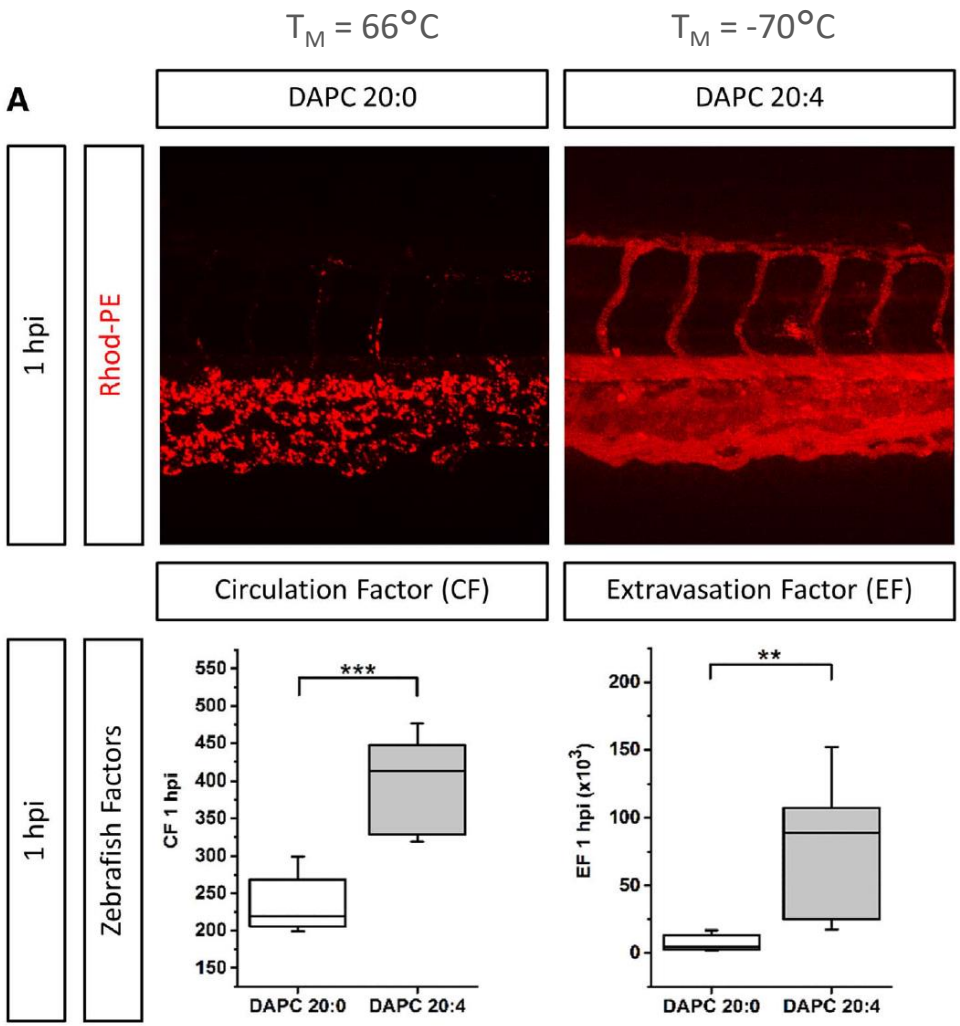


	Liposome	Lipid T_m (°C)	Size (nm)	PDI	Zeta potential (mV)
C20:4	DAPC 20:4	− 70	106.1 ± 3.4	0.096 ± 0.043	− 26.5 ± 5.8
C18:1	DOPC	− 22	116.8 ± 4.4	0.105 ± 0.031	− 25.4 ± 7.7
C14:0	DMPC	23	113.7 ± 3.2	0.075 ± 0.035	− 19.6 ± 9.7
C16:0	DPPC	41	105.9 ± 2.4	0.076 ± 0.015	− 16.9 ± 4.0
C18:0	DSPC	55	104.6 ± 2.3	0.062 ± 0.029	− 16.6 ± 5.6
C20:0	DAPC 20:0	66	110.7 ± 4.7	0.110 ± 0.037	− 17.1 ± 6.6

Preparation of liposomes (PC +/- cholesterol) by extrusion
Similar physico-chemical characteristics BUT
different lipid phase transition temperatures (Tm)

Journal of Controlled Release 264 (2017) 180–191 (Sieber…Witzigmann...)

Liposomes as drug carriers: Pharmacokinetics in ZFL



CF and EF calculated by image analysis:

$$EF = \frac{\overline{I_{Vasc}}}{A_{Vasc}} \frac{A_{Non\ Vasc}}{\overline{I_{Non\ Vasc}}}$$

$$CF = \frac{\overline{I_{Free}}}{A_{Vasc}}$$

PC lipids with a Tm < 28°C show increased circulation properties and extravasation.

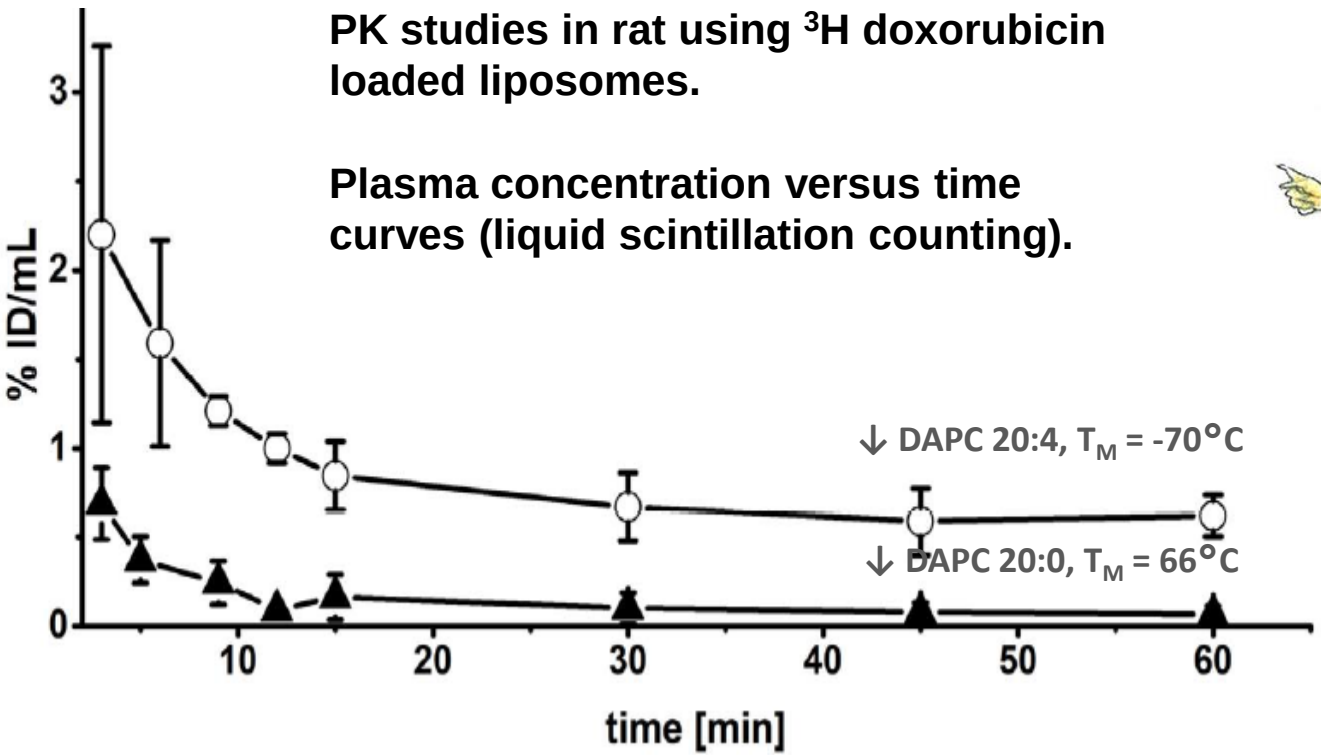
This effect is enhanced by cholesterol.

"Flexibility" of membrane favours extravasation and alters protein binding ?

Liposomes as drug carriers: Pharmacokinetics in the rat

PK studies in rat using ^{3}H doxorubicin loaded liposomes.

Plasma concentration versus time curves (liquid scintillation counting).



Liposome	$CF_{zebrafish}$	$EF_{zebrafish}$ ($\times 10^3$)	$t_{1/2}$ $rat_{terminal}$ (min)	AUC_0^∞ rat (% ID $*$ mL/min)
DAPC 20:0	233.8 ± 38.2	7.0 ± 6.1	12.5 ± 10.1	19.6 ± 6.1
DAPC 20:4	398.2 ± 61.6	77.9 ± 49.4	54.4 ± 21.5	120.4 ± 31.9

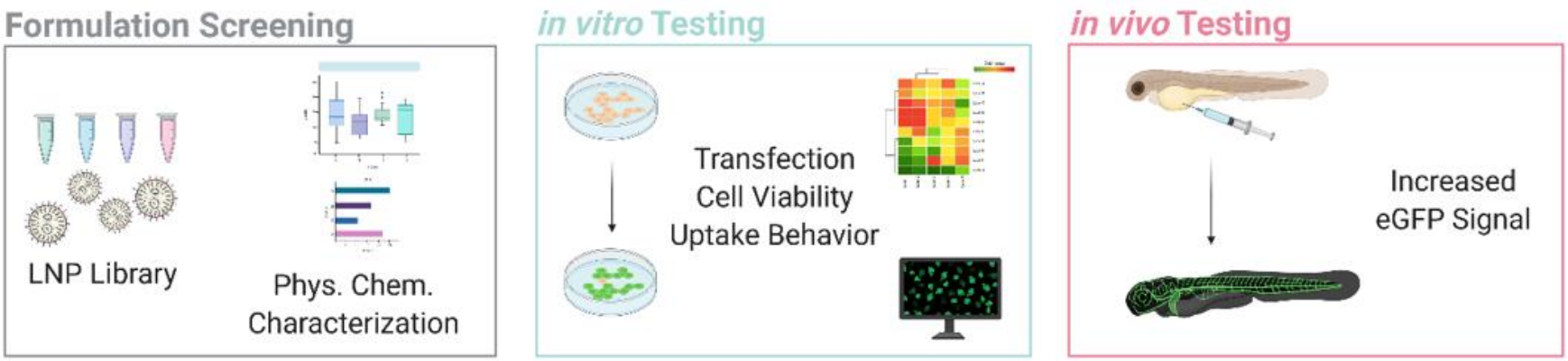
Results confirmed in rat (and mouse)

Lipid based gene delivery

Goals:

- Design of long circulating lipid-nanoparticles
- Condensation of nucleic acids
- (Targeted) gene delivery in the ZFL

DODMA



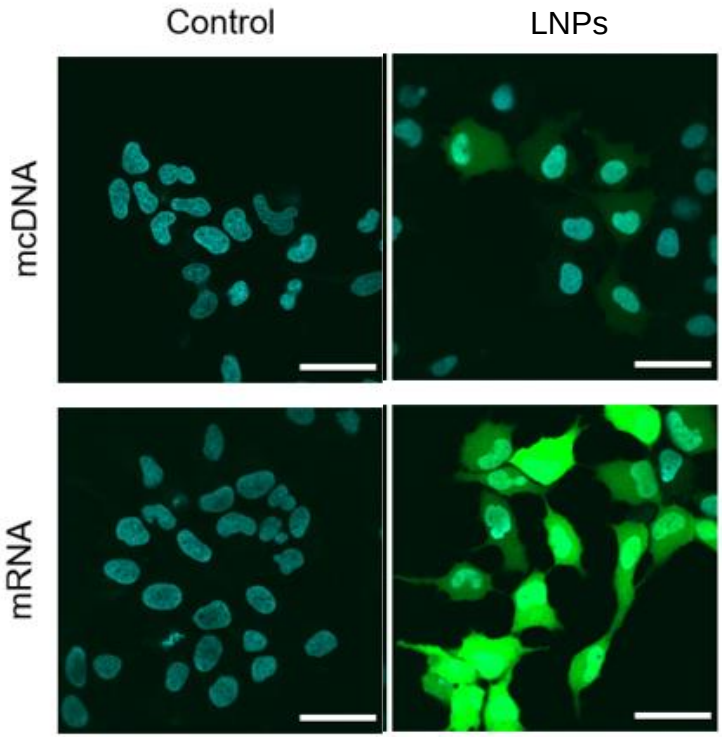
Design of LNPs:

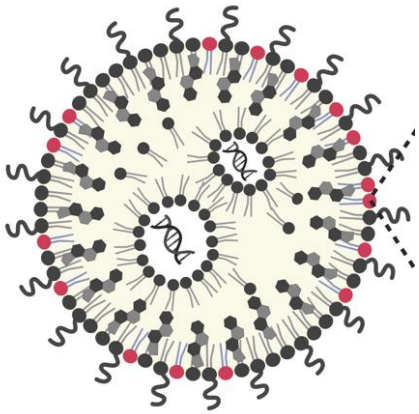
- Phospholipids and DODMA as an ionizable nucleic acid condensation agent
- mRNA or nanovectors* coding for eGFP

* ACS Omega 2020, 5, 24724−24732

Gene delivery (*in vitro*)

***In vitro* transfection (Huh-7 hepatocellular carcinoma)**

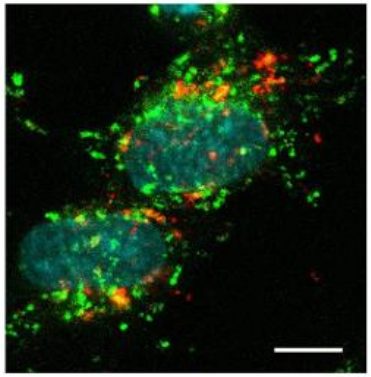




120 nm D_H

0.09 PDI

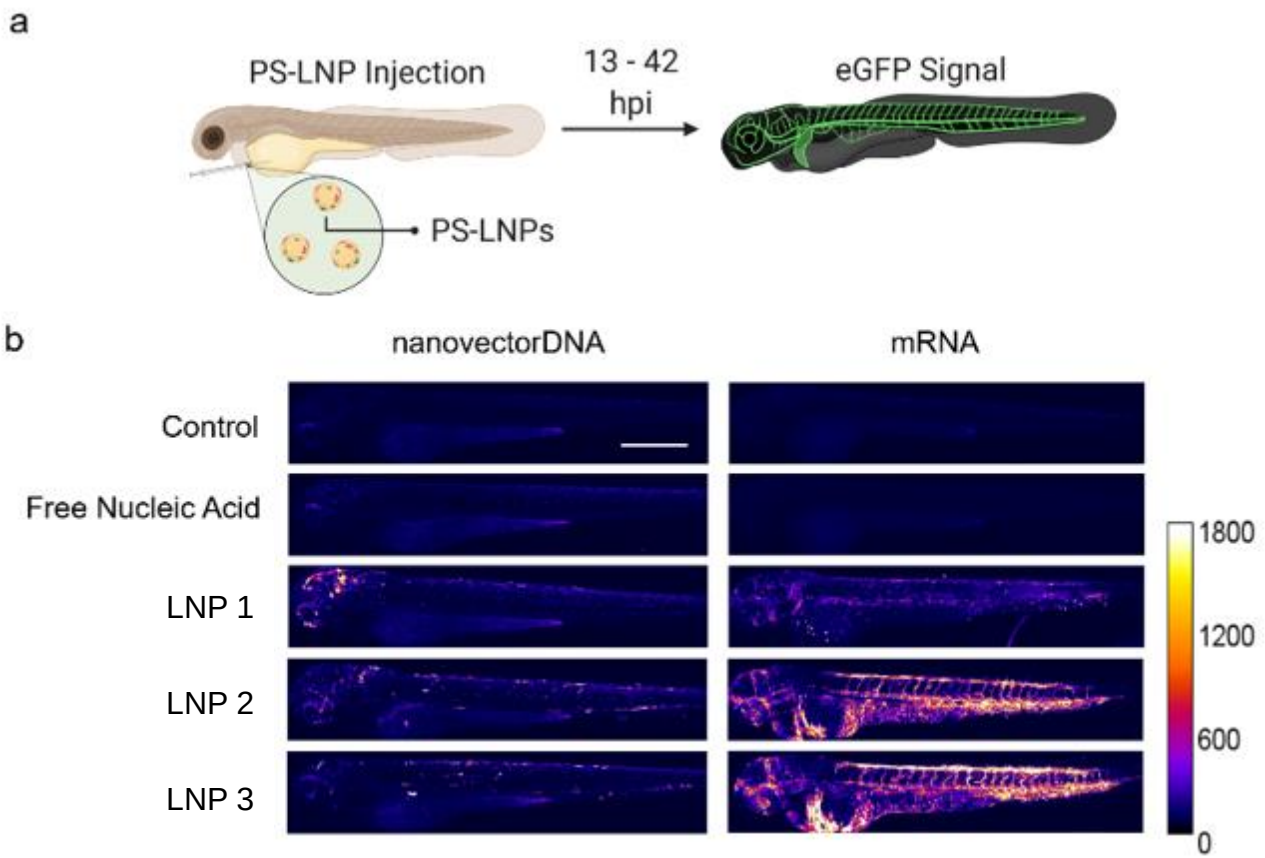
-5 mV ζ-potential



Red: DiI LNPs
Green: Lyso-Tracker
Nucleus: Hoechst stain

Cellular uptake: clathrin-mediated endocytosis
(inhibition by Chlorpromazine and Dynasore)
High transfection efficiency *in vitro* (75% for mcDNA, 100% for mRNA)
Strong eGFP signals *in vitro*

Zebrafish larvae to study gene delivery



In vivo **gene delivery (36 hpf ZFL) of mcDNA or mRNA using optimized LNPs**

Lipid composition as a critical factor

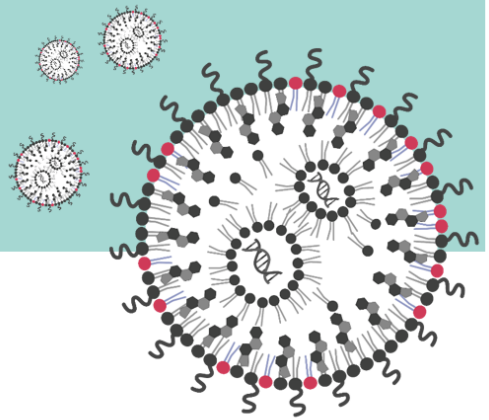
University of Basel

Incorporation of Phosphatidylserine Improves Efficiency of Lipid Based Gene Delivery Systems

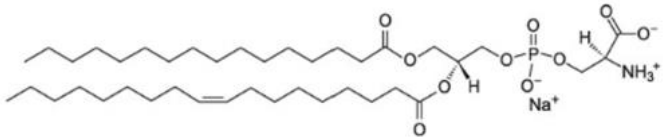
7th International Symposium on Phospholipids in Pharmaceutical Sciences
Poster Flash Presentation

Claudia Lotter, 13.09.2022

Poster Number 18



Phosphatidylserine (PS)



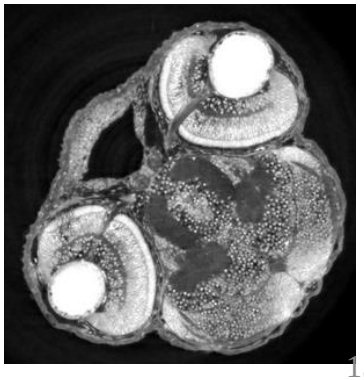
Summary

1. Complex interactions of engineered nanomaterials (ENMs) with biological systems

2. Use of zebrafish larvae as a predictive vertebrate screening model (3R principles!)

3. Applications of the ZFL model:
   - Screening & optimization of liposomal formulations
   - Optimization of gene delivery vectors

4. Outlook
   - Tissue distribution and gene expression (luciferase)
   - Intravital imaging
     (delivery, cellular uptake and processing, gene expression)



http://zebrafish.pharma-te.ch

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