Nanomedicine and Nanoscale Delivery

Heinrich Haas

CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

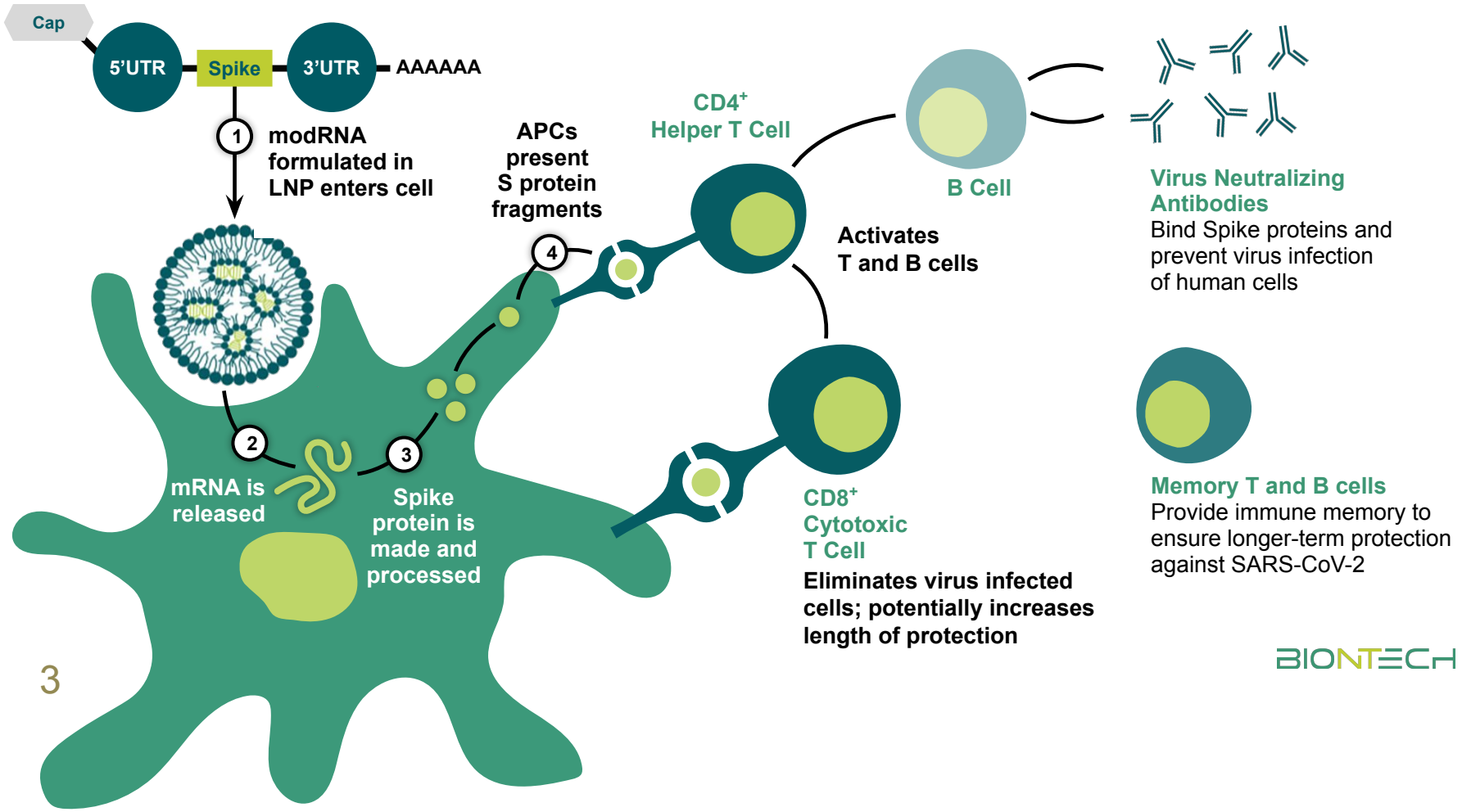
Nanoscale Coherencies in RNA and Drug Delivery Vehicles

Heinrich Haas

Vice President Formulation & Drug Delivery

BIONTECH

How mRNA vaccines work – training the immune system for a real infection



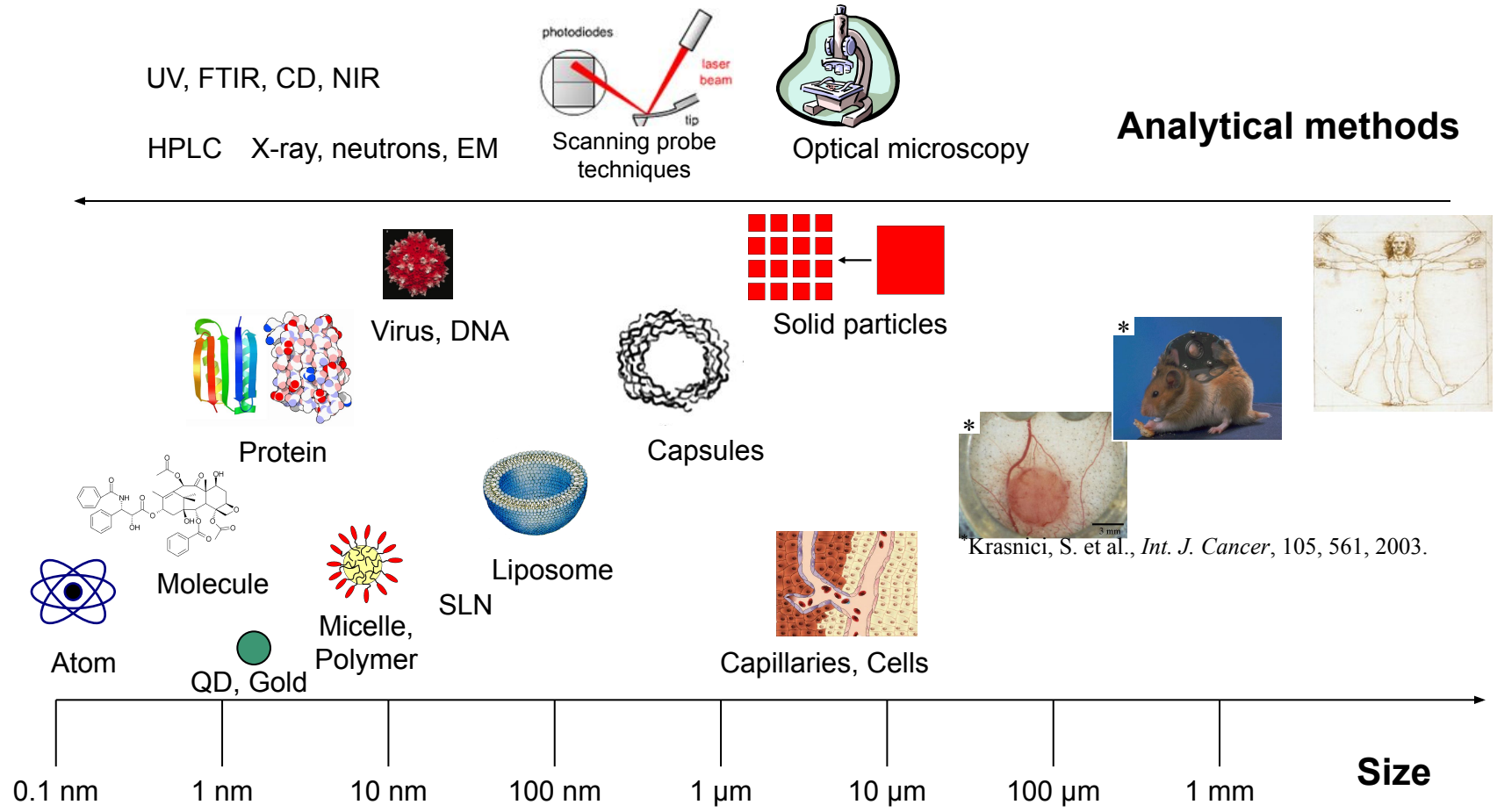
RNA Therapeutics at BioNTech

enger RNA:
acologically optimized protein-coding RNA for targeted *in*
v ery

BioNTech is applying its therapeutic platforms to different medical indication fields

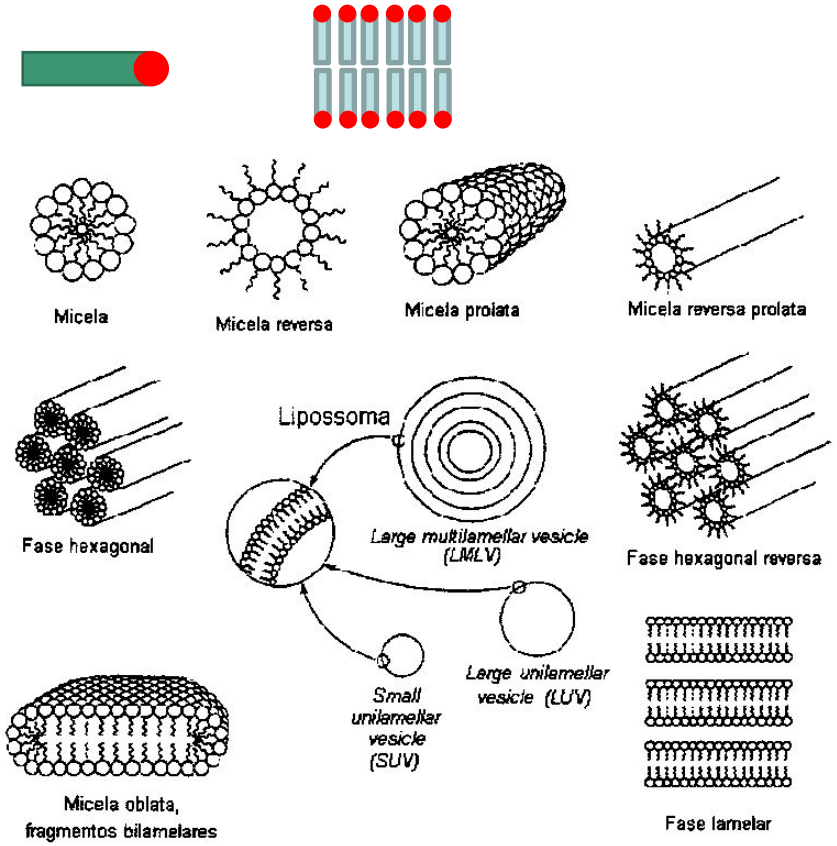
- **Cancer Immunotherapies**
  - FixVAC
  - RNA WAREHOUSE
  - IVAC® MUTANOME
  - RiboMABs
- **Infectious Diseases**
  - Nucleoside modified RNA
  - Self amplifying RNA
  - RiboMABs
  - Broadly neutralizing Abs
- **Other Indications**
  - Protein replacement
  - Antibodies
  - Other

What are Nanoparticulate Agents?

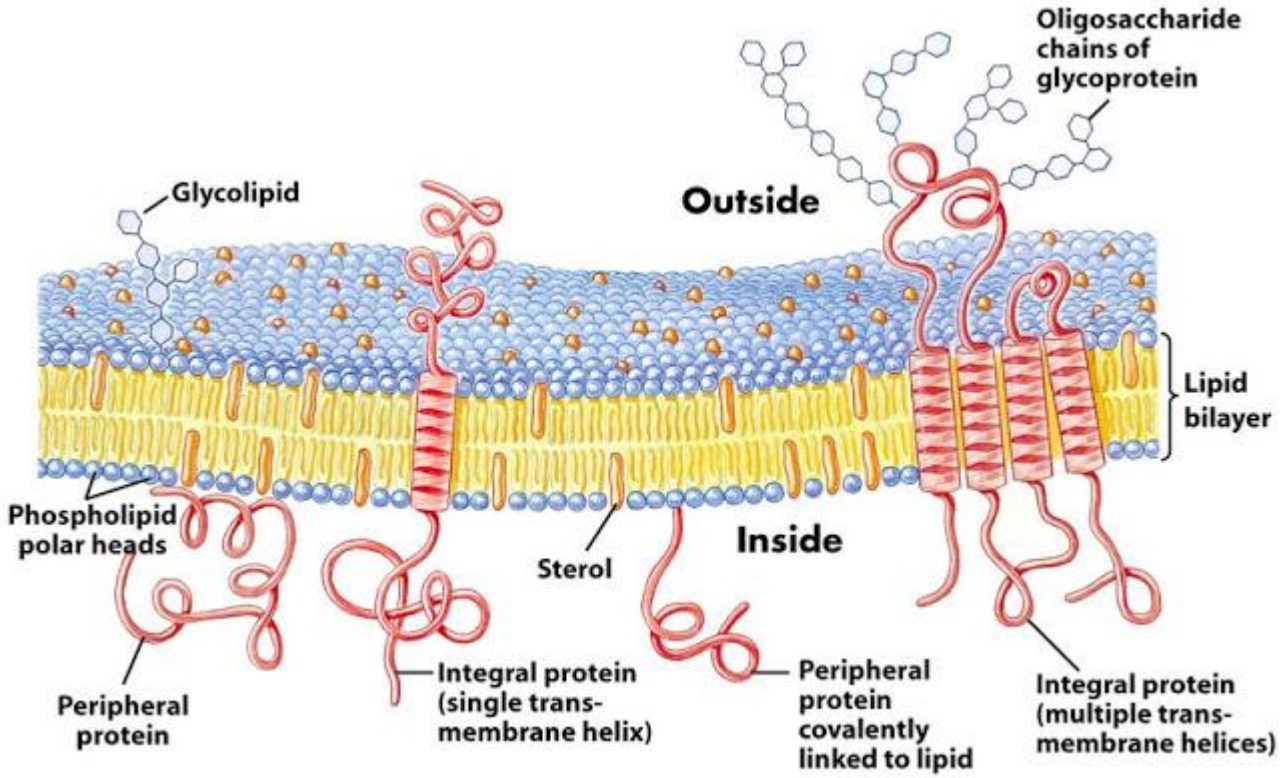


*Krasnici, S. et al., *Int. J. Cancer*, 105, 561, 2003.

Liposomes and Lipid Membranes



Fluid-mosaic model of cell membrane



Singer, Garth L. Nicolson: *The Fluid Mosaic Model of the Structure of Cell Membranes*. In: *Science*. Vol. 175, Nr. 4023, 1972, S. 720–731

Similarities between liposome and cell membrane structure

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Timeline of liposome / nanoparticle advancement

ACS NANO
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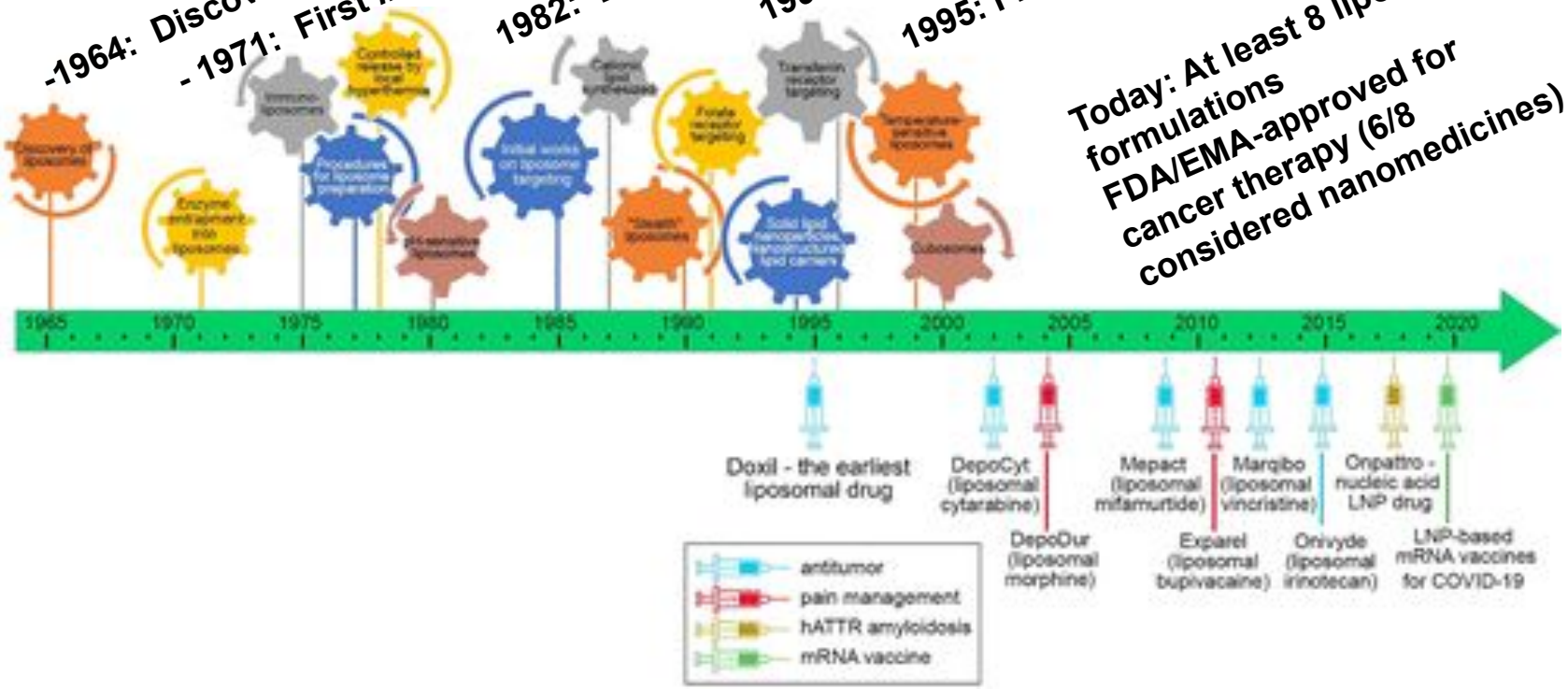
Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement

Rumiana Tenchov, Robert Bird, Allison E. Curtze, and Qiongqiong Zhou*

Cite This: ACS Nano 2021, 15, 16982–17015 Read Online

-1964: Discovery of liposomes – Alec Bangham
- 1971: First *in vivo* applications of liposomes for drug delivery
1982: Liposomes to reduce cardiac toxicity of doxorubicin (Dox)
1991-92: 1st tests of Peg-Lip-Dox in humans
1995: FDA approval of Peg-Lip-Dox
Today: At least 8 liposome formulations FDA/EMA-approved for cancer therapy (6/8 considered nanomedicines)



Liposomes are one leading technology behind developments in the field of Nanomedicines

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Timeline of liposome / nanoparticle advancement

-1964: Discovery of liposomes – Alec Bangham
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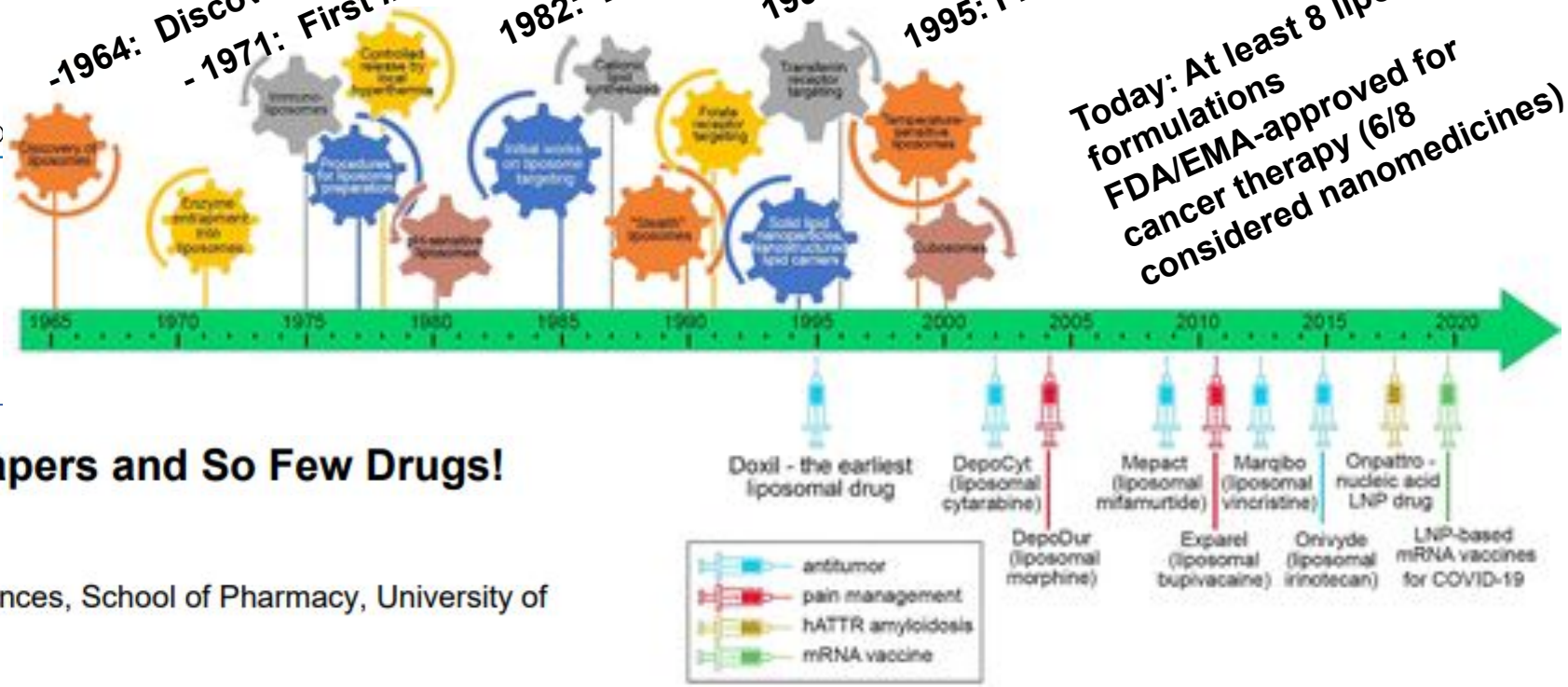

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Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement

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Cite This: *ACS Nano* 2021, 15, 16982–17015 Read Online

Cancer Nanomedicines: So Many Papers and So Few Drugs!

Vincent J. Venditto[1] and **Francis C. Szoka Jr.**[1,*]

[1]Departments of Bioengineering and Therapeutic Sciences, School of Pharmacy, University of California, San Francisco, California 94143-0912

Liposomes are one leading technology behind developments in the field of Nanomedicines

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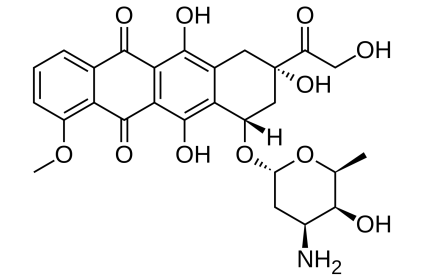
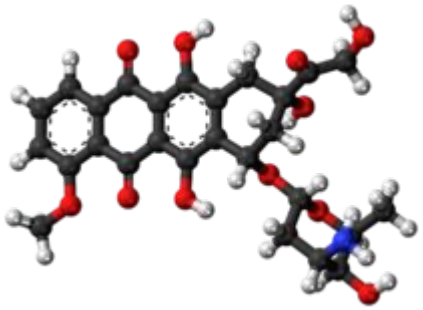
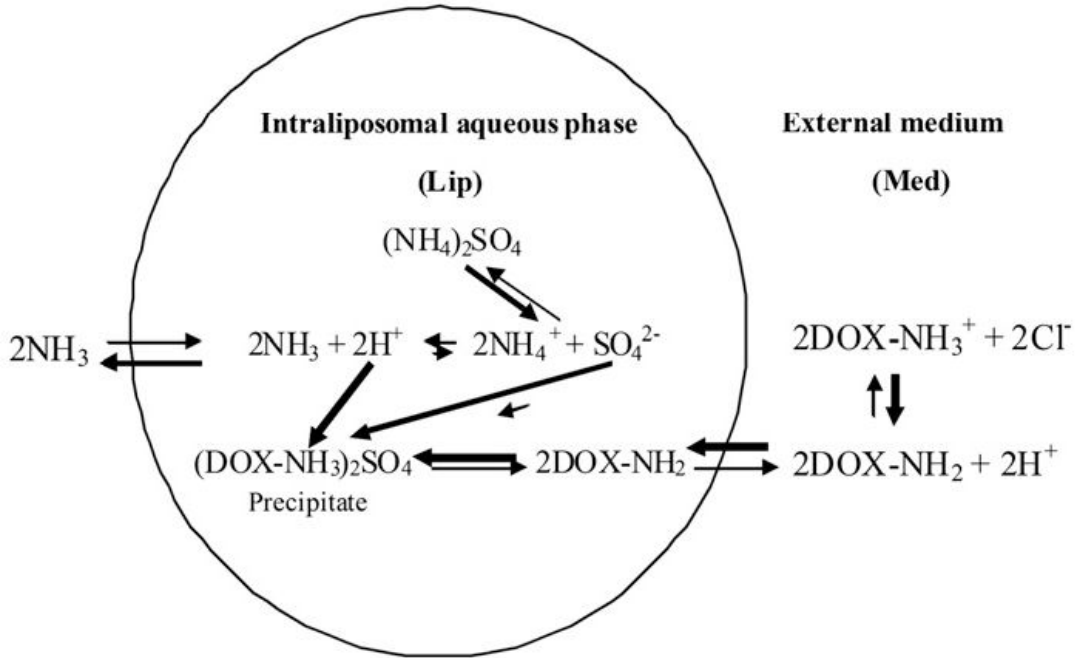
Doxil

Remote loading of water-soluble doxorubicin into liposomes by pH gradient

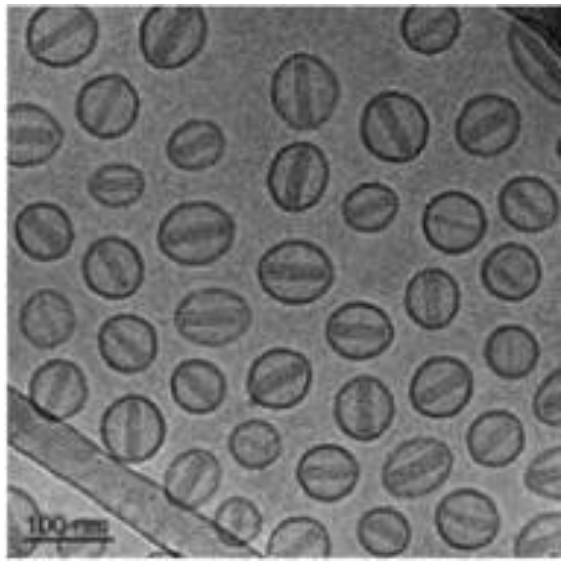




https://en.wikipedia.org/wiki/Doxorubicin



https://www.semanticscholar.org/paper/Doxil%C2%AE--the-first-FDA-approved-nano-drug%3A-lessons-Barenholz/3ce7c0a98376475fd26396b369ef6d64ce133b8b/figure/1



https://commons.wikimedia.org/wiki/File:Doxil_Cryo-TEM.jpg

The first FDA-approved nano-drug comprising the anti-cancer agent doxorubicin

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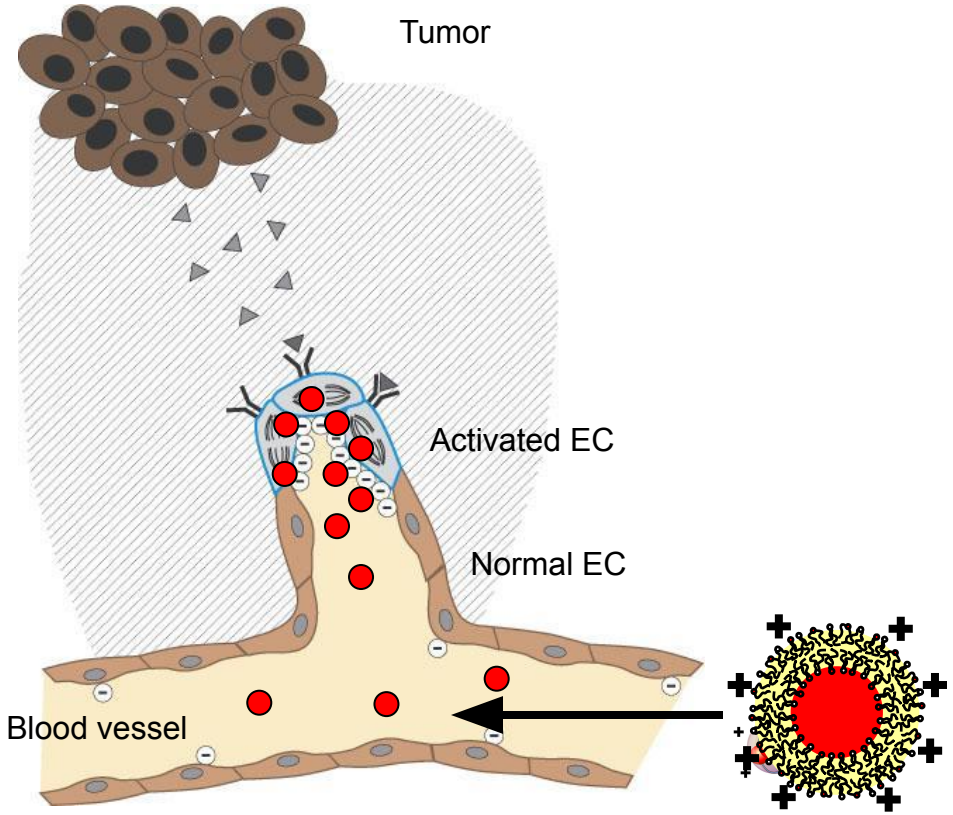
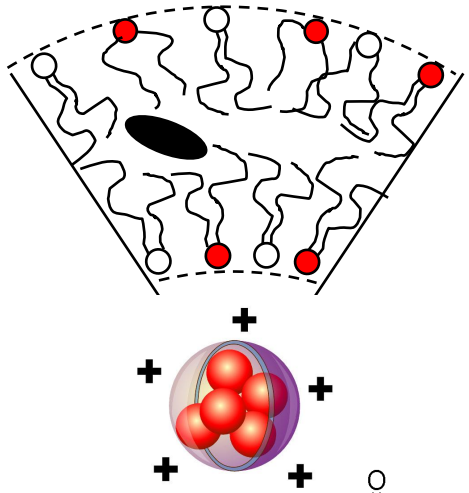
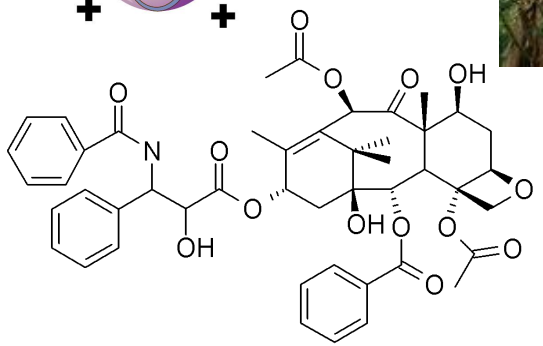
EndoTAG

Loading of the lipophilic anti-cancer drug paclitaxel to the lipid membrane







*G. Thurston et al, J. Clin Invest. 1998, 101(7), 1401-1413

Cationic liposomes for vascular delivery of anti-cancer drug

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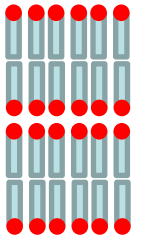
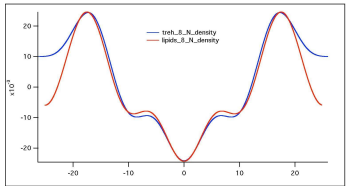
SAXS/WAXS to investigate lipid-based delivery systems

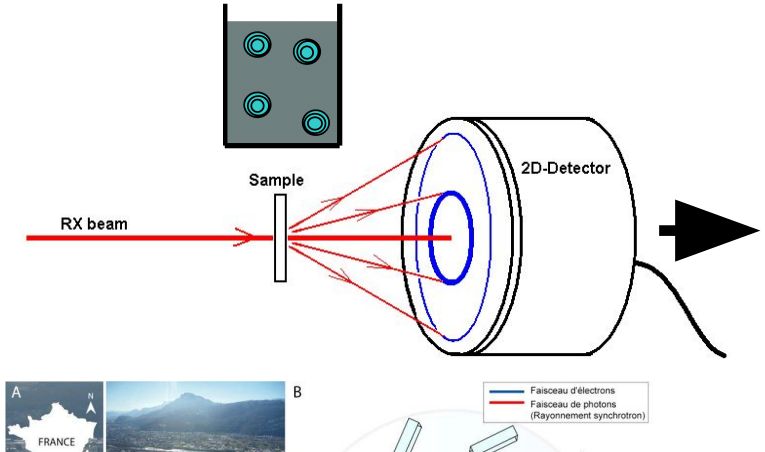
- Small angle X-ray scattering (SAXS) enables determination of subtle structural changes in liposome preparation in situ
- In multilamellar liposome preparations changes of Bragg peak positions (d-spacing) can be accurately determined

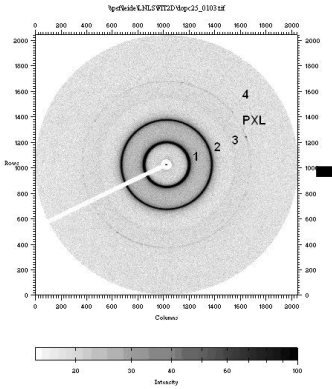


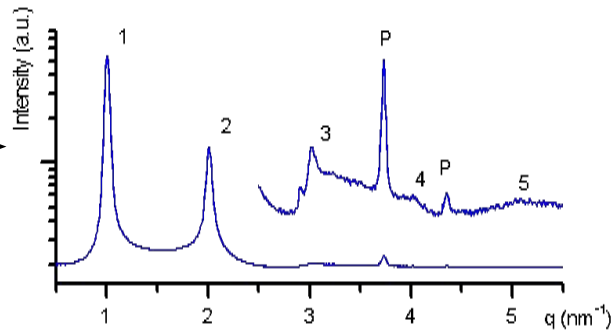
d spacing, lamellarity

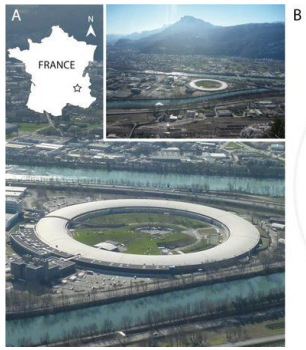


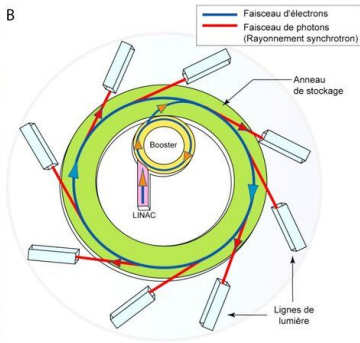
Membrane density profile

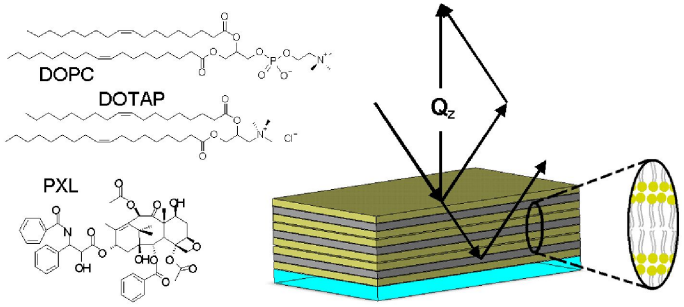


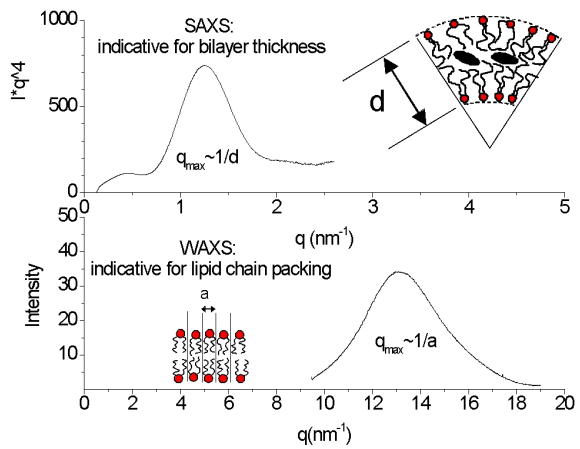












ESRF, DESY,..

Cavalcanti et al., X-ray diffraction from paclitaxel-loaded zwitterionic and cationic model membranes, Chemistry and Physics of Lipids, 2008

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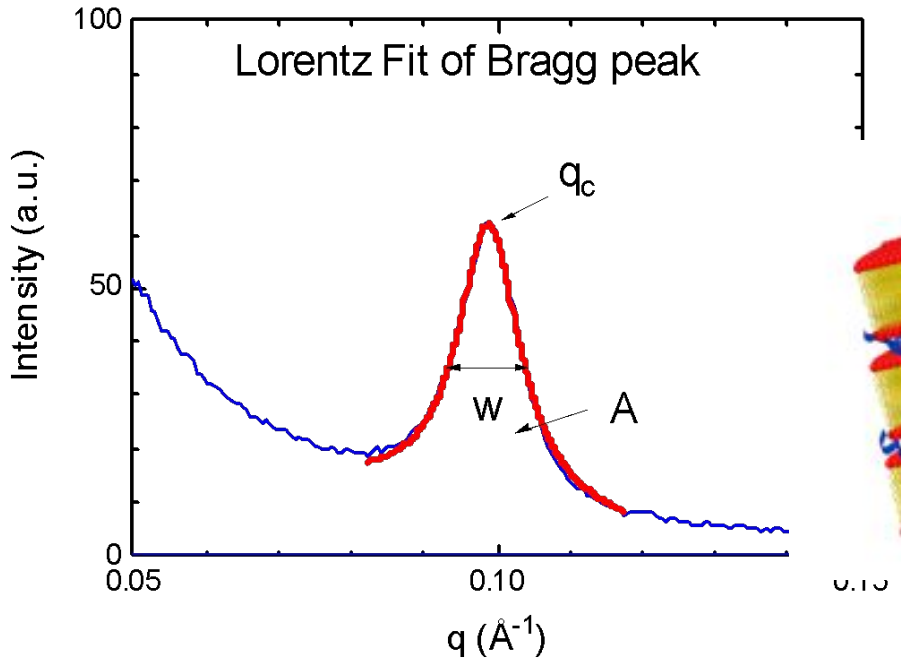
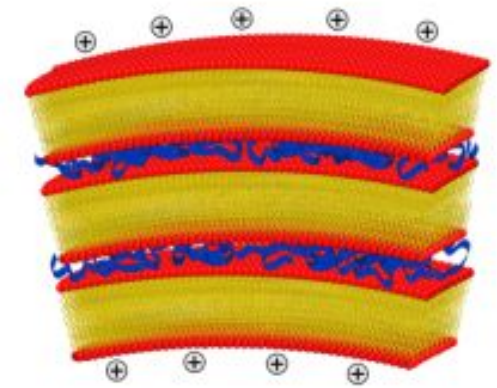
Peak Analysis

Lorentz fitting of Bragg peaks

$$y = y_0 + \frac{2 * A}{\pi} * \left(\frac{w}{(4 * (q - q_c)^2 + w^2)} \right)$$





$$d = \frac{2\pi}{q_c}$$

$$\xi_L = \frac{2}{w}$$

Information obtained on:

- **repeat distance**
- **size of ordered stacks**
- **Amount of ordered material**

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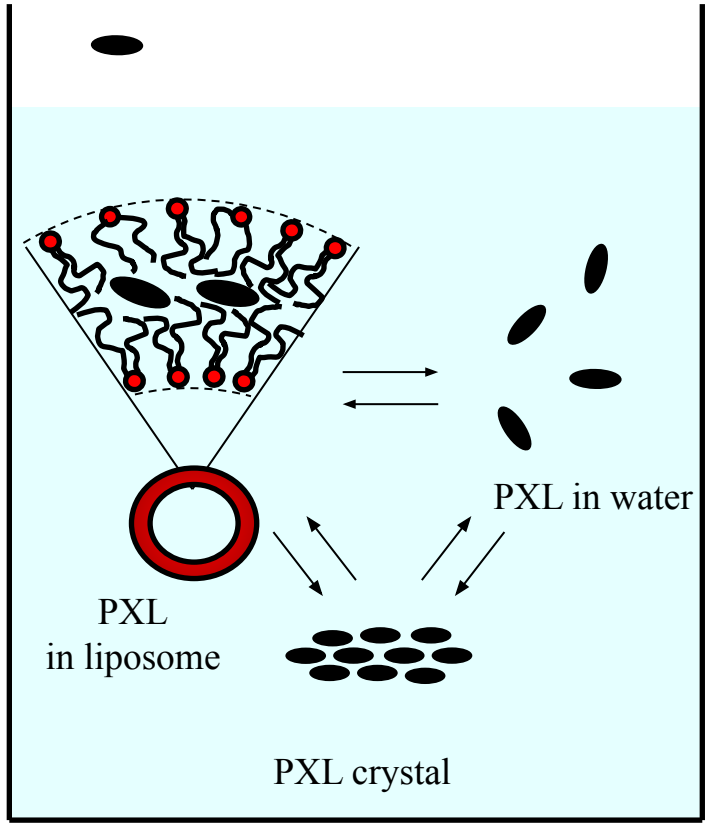
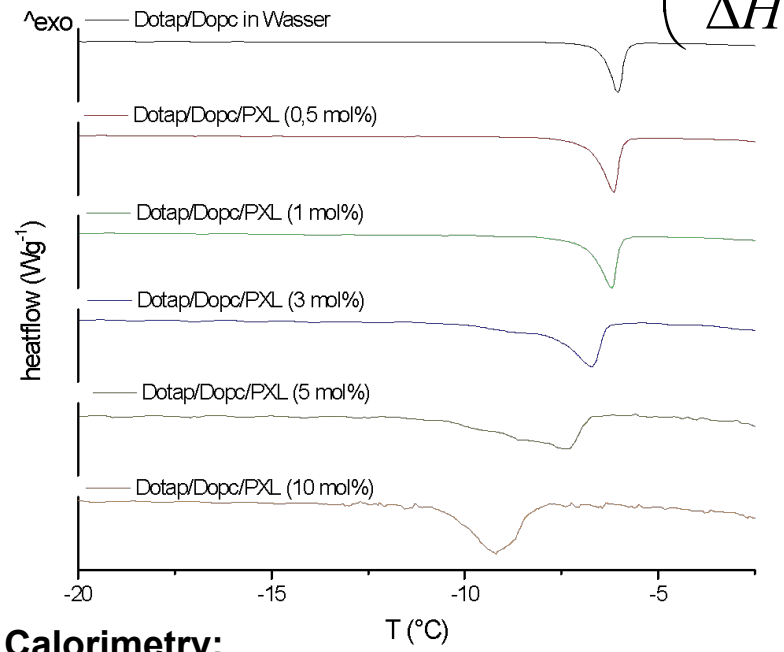
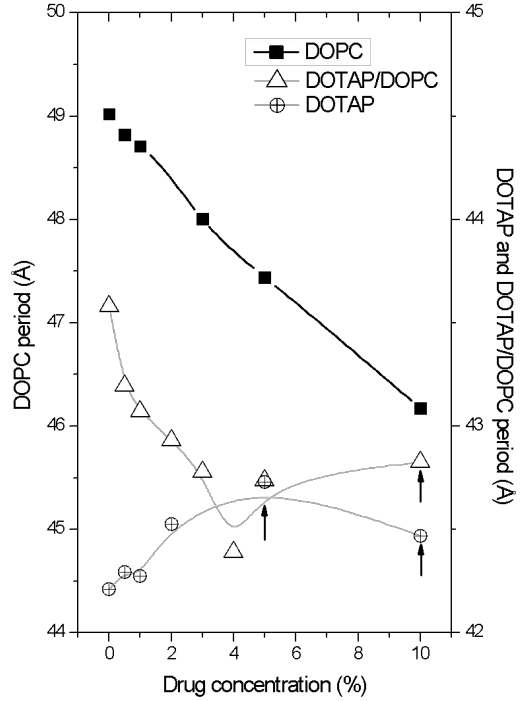
Partitioning of Paclitaxel to Liposome Membrane



$$\Delta T_m = -\left(\frac{RT_m^2}{\Delta H}\right) \cdot x_a$$



Calorimetry:
Change of phase transition temperature



X-ray scattering from model membranes:
Change of d-spacing

Cavalcanti et al., X-ray diffraction from paclitaxel-loaded zwitterionic and cationic model membranes, Chemistry and Physics of Lipids, 2008

Optimized loading to bilayer membrane determined by physicochemical methods

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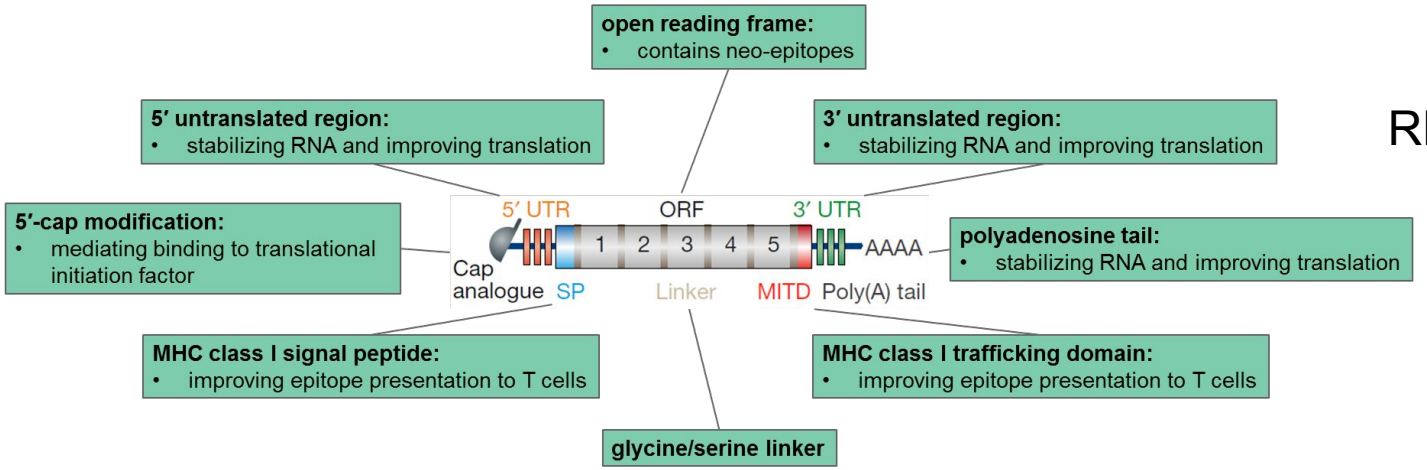
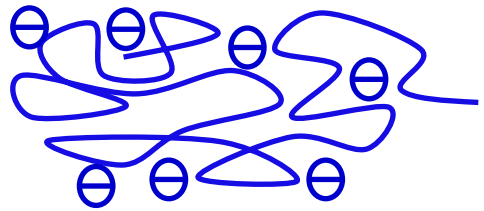
RNA as Therapeutic Molecule, RNA Biochemist‘s and Biophisicist‘s View



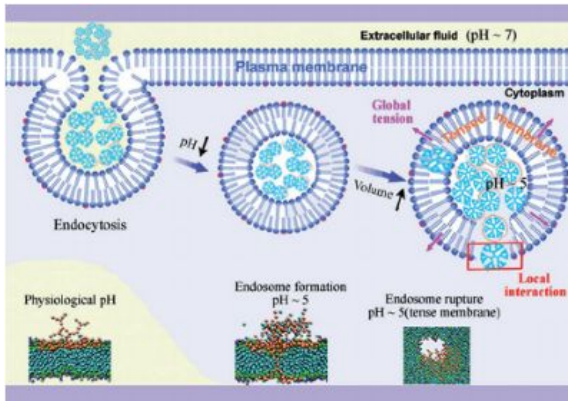
Kuhn et al., *Gene Therapy* 2010, *17*, 961.

RNA formats:

- mRNA
- **modRNA**
- saRNA
- ….



Biopolymer
Linear polyelectrolyte



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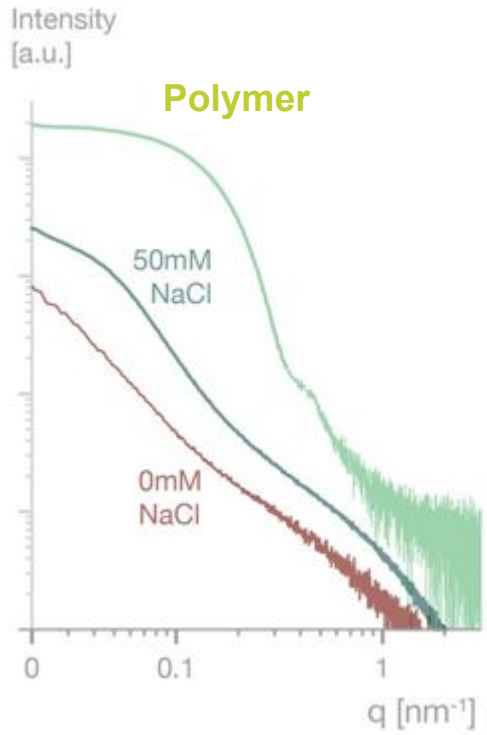
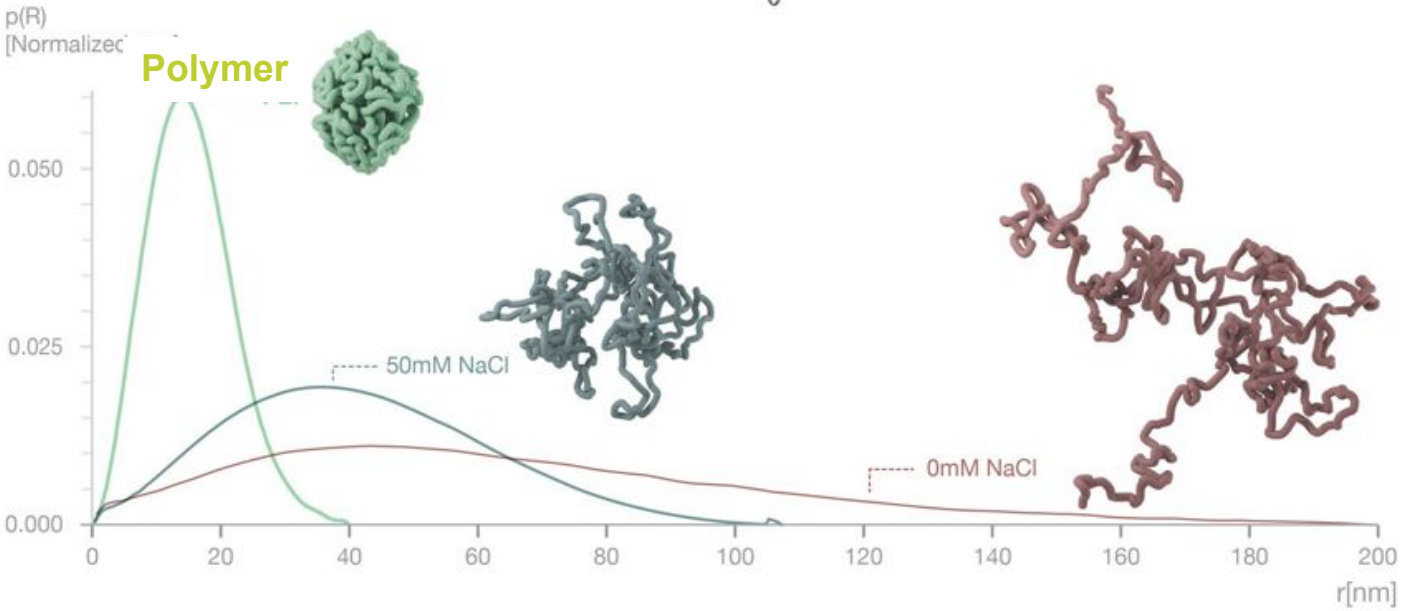
Organization of RNA together with Ions and Polymers

Comparison of different formulation approaches

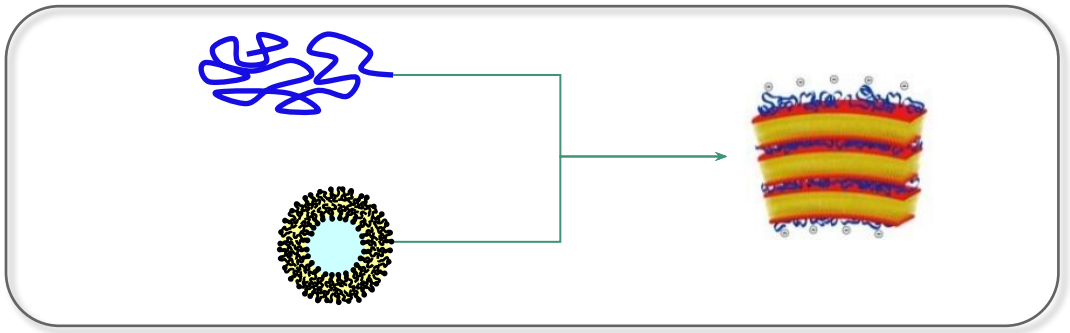


$$P(r) = \frac{r}{2\pi^2} \int_0^\infty I(q)\, q\, \sin(qr)\, dq$$



No internal repeat order, compaction by ions and polymers

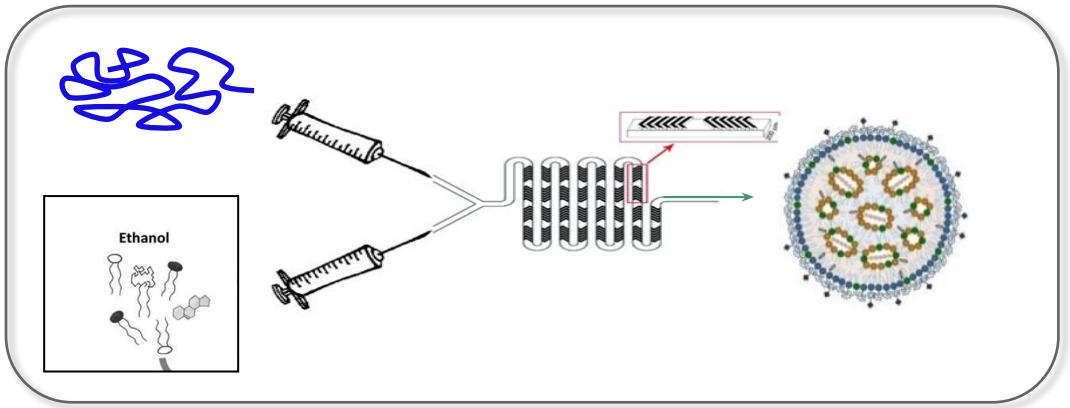
Key Assembly Protocols



Lipoplex Nanoparticles (LPX)

- formed from liposomes and RNA in aqueous buffers

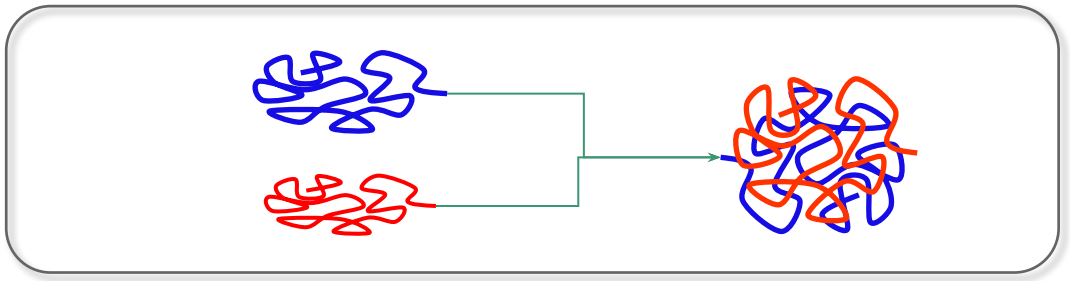
Kranz *et al.,* Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* **2016**, *534*
Grabbe, et al., Translating nanoparticulate-personalized cancer vaccines into clinical applications: case study with RNA-lipoplexes for the treatment of melanoma. *Nanomedicine* **2016**, *11*, 2723



Lipid Nanoparticles (LNPs)

- formed from lipids in ethanol and RNA in aqueous buffer

Nogueira et al., Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery,
ACS Appl. Nano Mater, 10.1021/acsanm.0c01834



Polyplex Nanoparticles (PLX)

- formed from polymers in and RNA in aqueous buffers

Vogel, et al.. Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. Mol Ther. 2018 Feb 7;26(2):446-455
Siewert et al. Investigation of charge ratio variation in mRNA - DEAE-dextran polyplex delivery systems. Biomaterials. 2019 Feb;192:612-620
Aldon et al., Immunogenicity of Stabilized HIV-1 Env Trimers Delivered by Self-Amplifying mRNA, Molecular Therapy Nucleic Acids, 2021, DOI:https://doi.org/10.1016/j.omtn.2021.06.008

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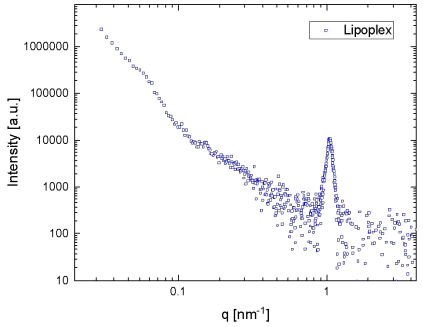
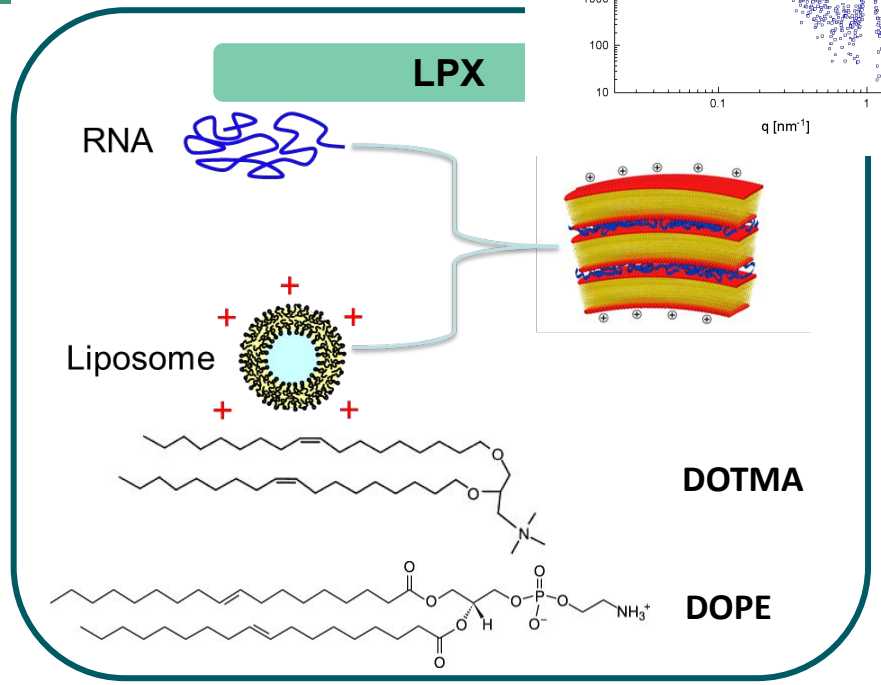
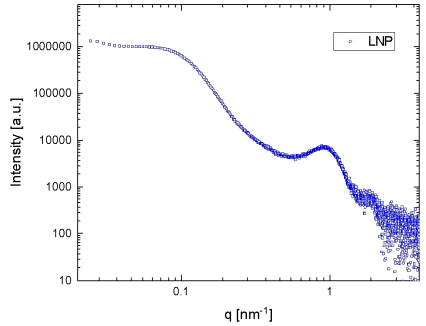
Lipoplexes and LNPs

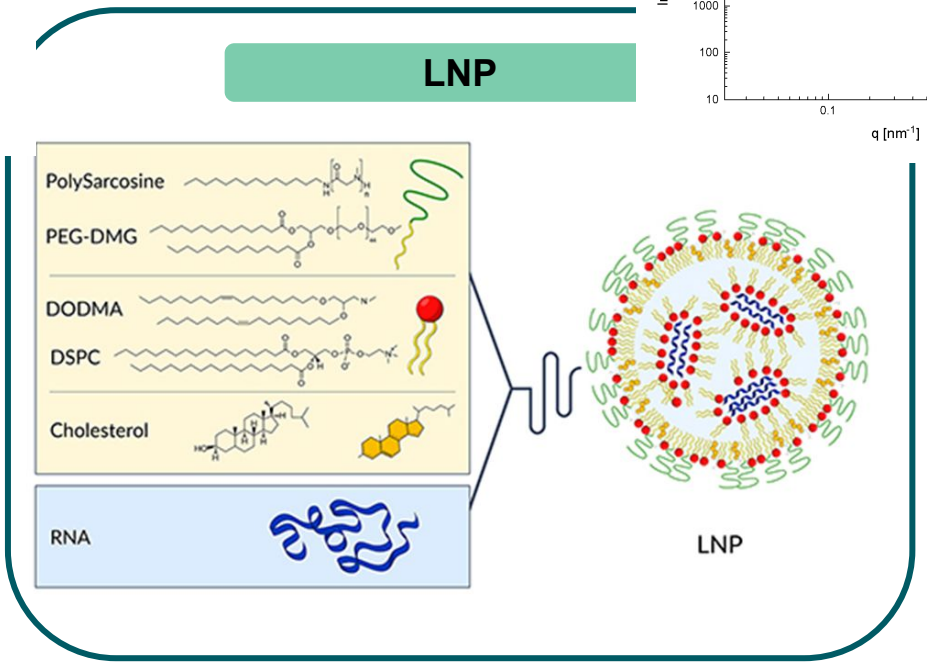




- **Lipids (liposomes) or polymer in water with RNA in water**

Kranz *et al.,* Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* **2016**, *534*
Grabbe, et al., Translating nanoparticulate-personalized cancer vaccines into clinical applications: case study with RNA-lipoplexes for the treatment of melanoma. *Nanomedicine* **2016**, *11*, 2723
Rosigkeit et al., Monitoring Translation Activity of mRNA-Loaded Nanoparticles in Mice, Mol. Pharmaceutics 2018, 15, 9, 3909





- **Lipids in ethanol with RNA in water**

Nogueira et al., Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery, ACS Appl. Nano Mater, 10.1021/acsanm.0c01834

LNPs and LPX are both representations of lipid-based RNA nanoparticles

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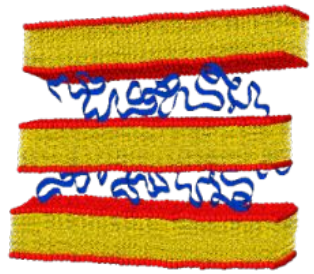
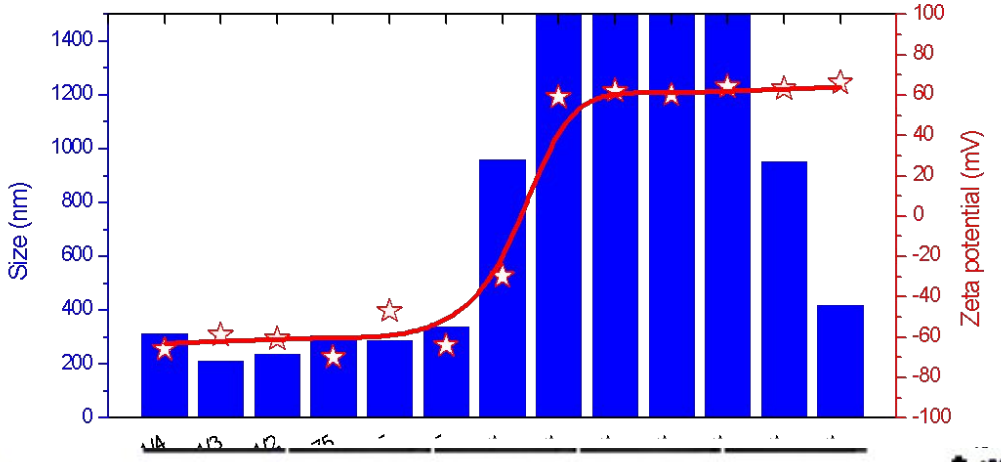
Lipid N/P Variation

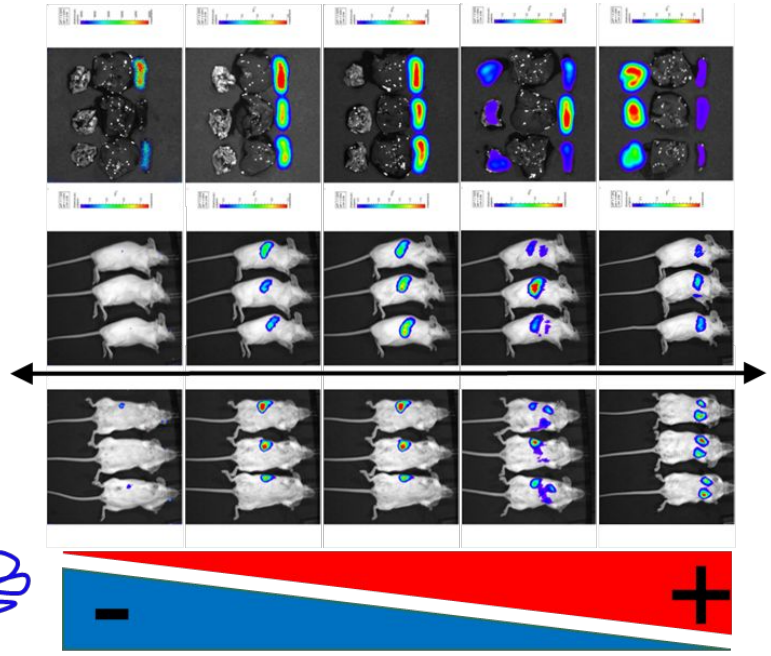
Variation of RNA/lipid charge ratio



Size and zeta potential for RNA lipoplexes at different +/- ratios

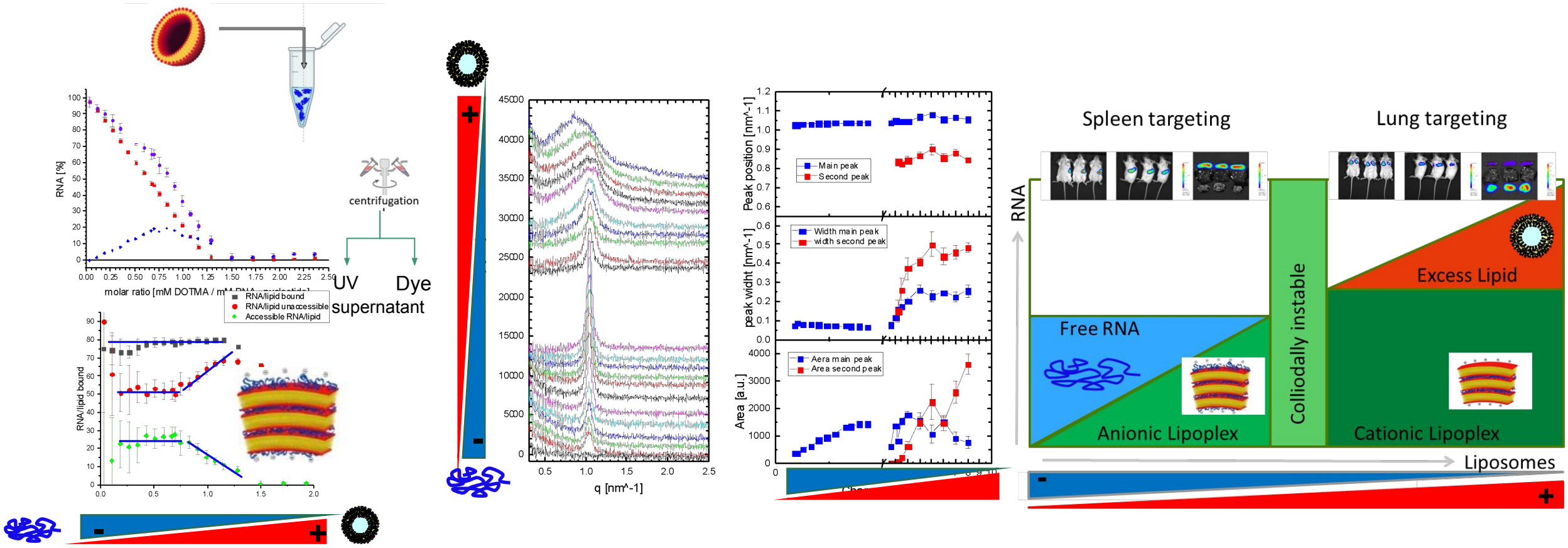


In vivo luciferase expression



Charge ratio drives targeting selectivity

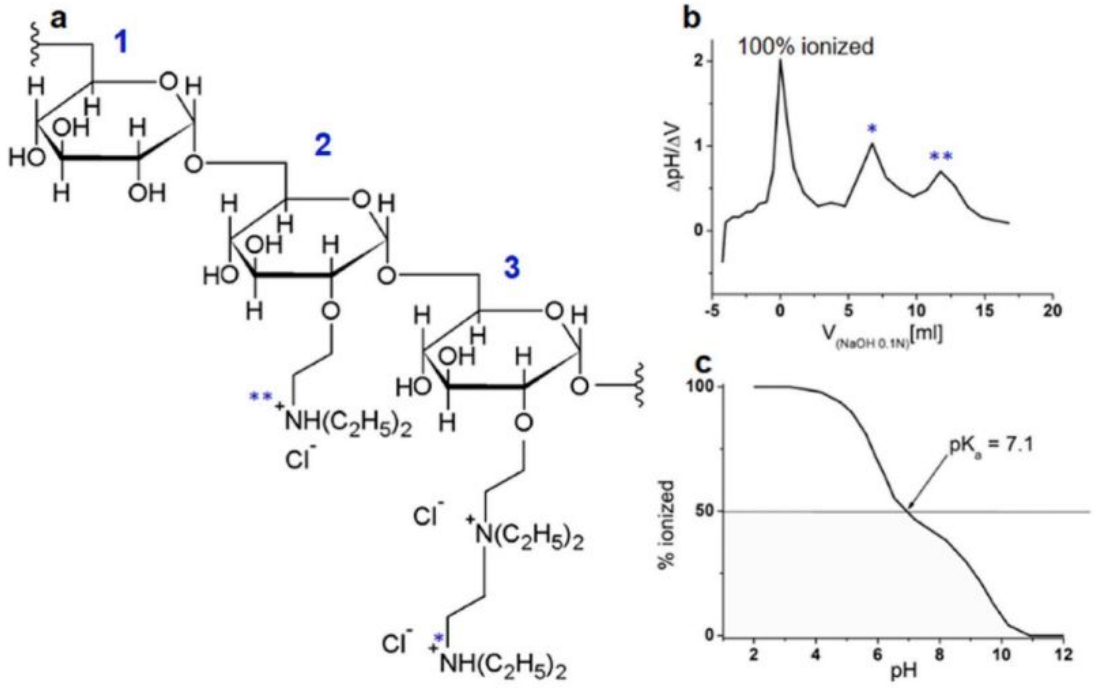
Advanced Characterization of Lipoplex Nanoparticle Formulations

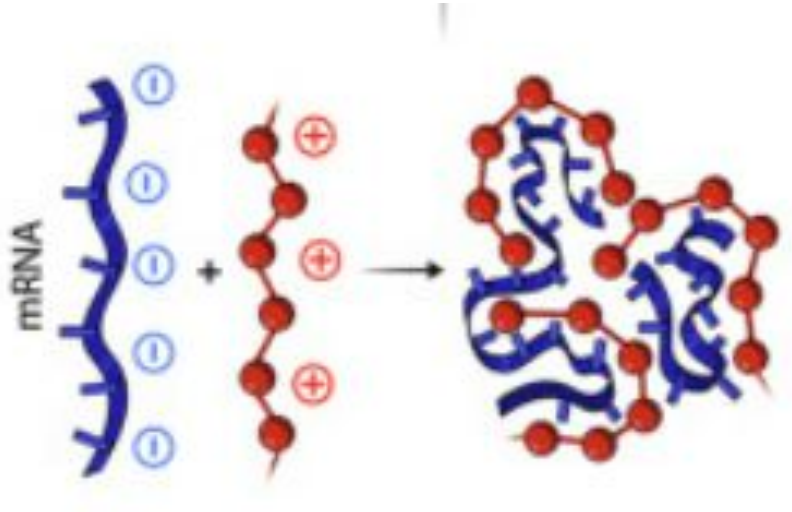


Understanding structural coherencies helps to define CQAs for a given product

Polymer N/P Variation

Charge ratio variation with DEAE Dextran as Model Polymer





Investigation of charge ratio variation in mRNA–DEAE-dextran polyplex delivery systems
C Siewert, H Haas, T Nawroth, A Ziller, SS Nogueira, MA Schroer, **Biomaterials 192, 612-620**

RNA Polyplex nanoparticles formed with DEAE dextran

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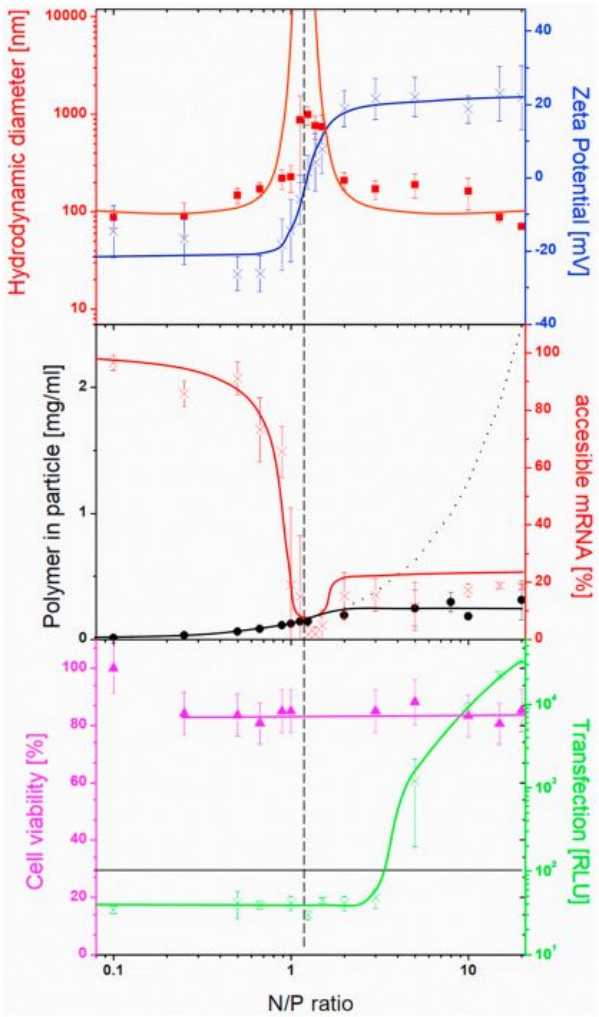
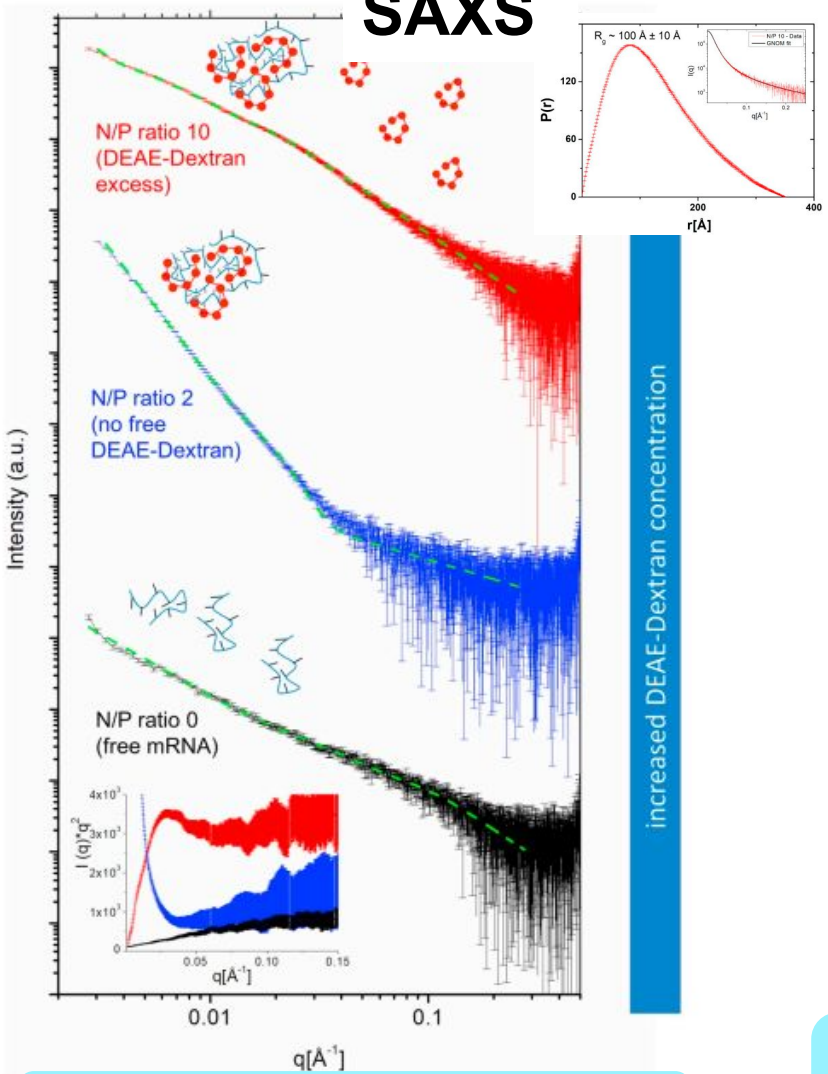
Molecular Organization and Activity for DEAE Dextran Polyplex NPs



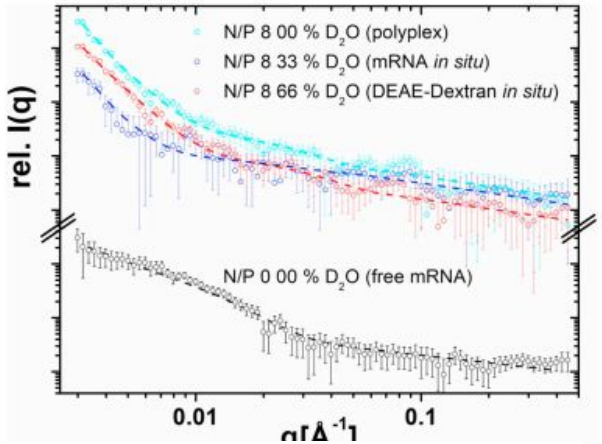
Activity only at N/P > 1

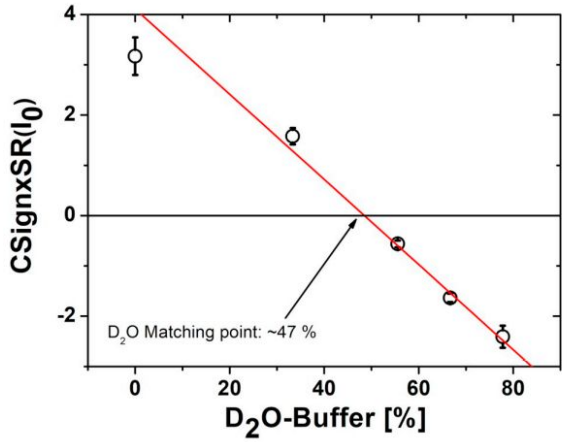
SAXS



Globular Particles (N/P 10)

SANS



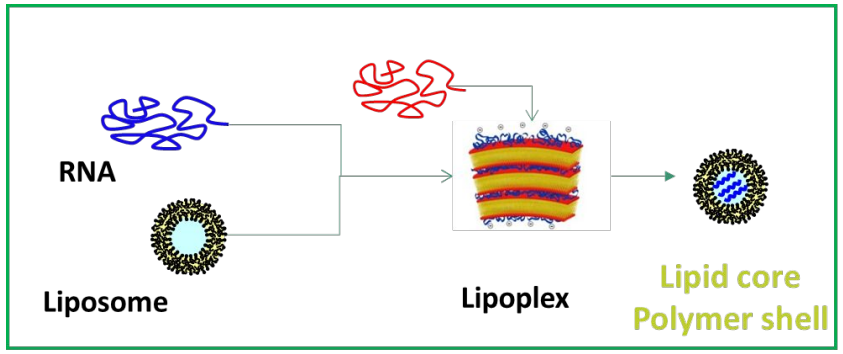


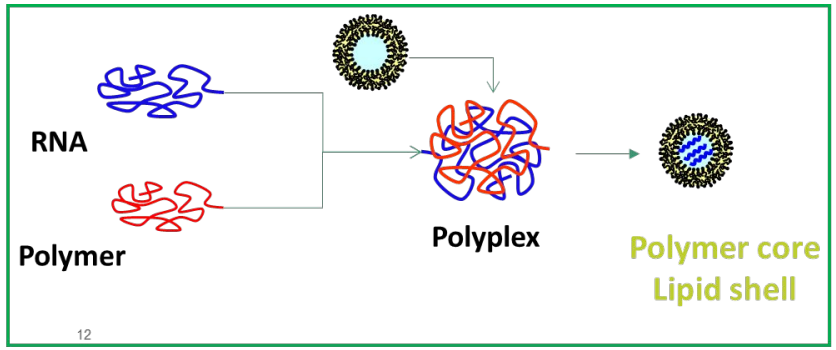
Matching point reveals particle N/P ~1
Coexistance with free dextrane?

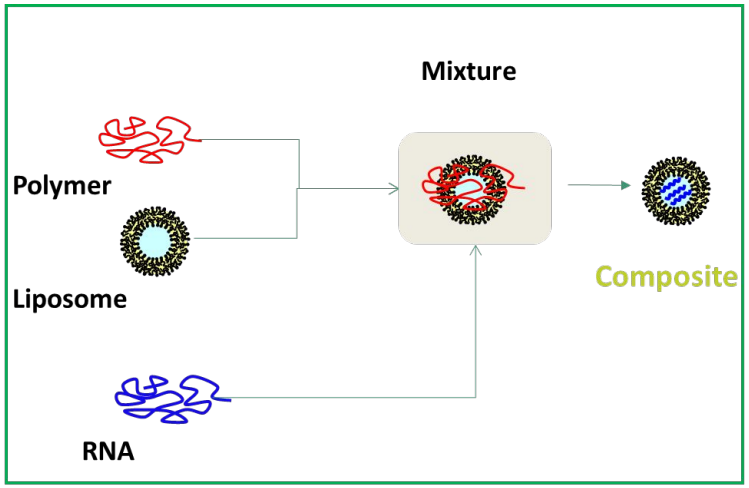
Lipid/Polymer Hybrid Nanoparticles

Hybrid Polymer-Lipid Nanoparticles

Comparison of different formulation approaches



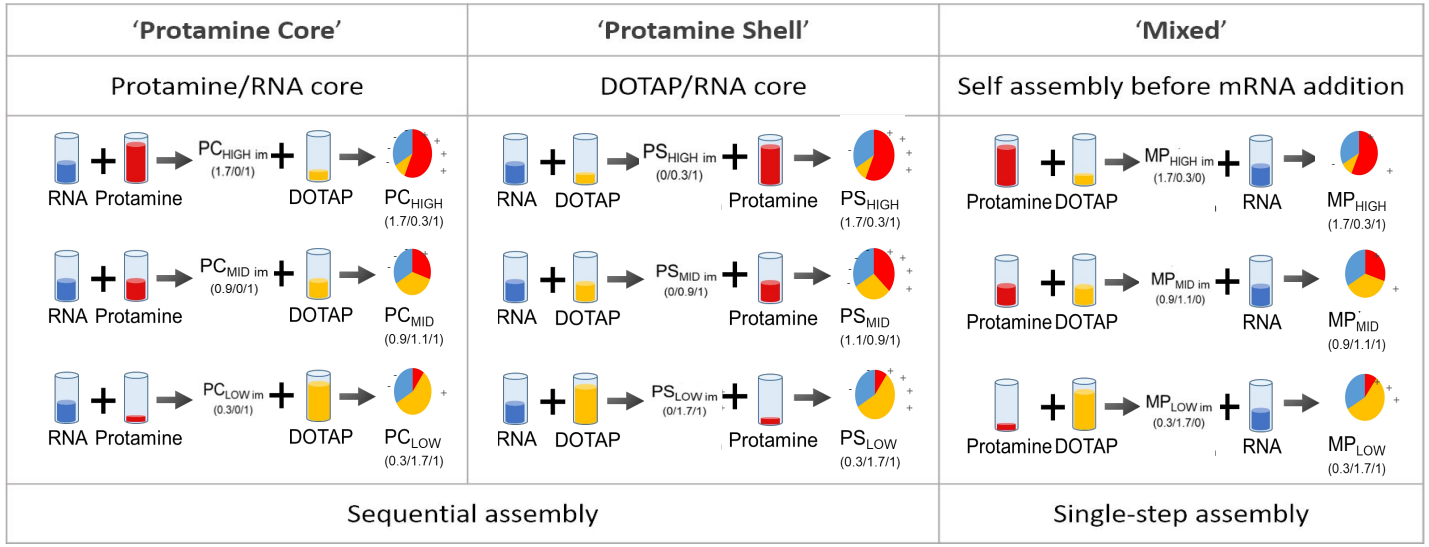




Complex internal structure to be obtained by hybrid and core-shell assembly strategies

Hybrid Polymer-Lipid Nanoparticles

Test of core-shell approaches for assembly of protamine and DOTAP nanoparticles



- Packing inside particles can be sensitively adjusted
- Allows tailoring of structure upon delivery pathway

Assembly of nanoparticles using different molar ratios and complexation sequence

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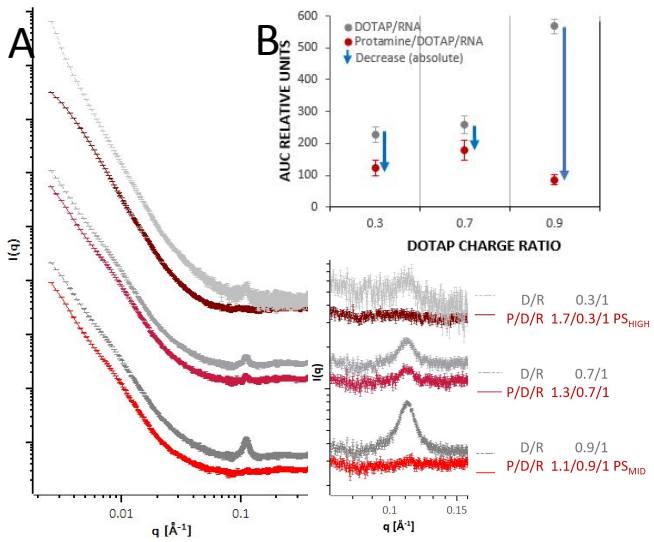
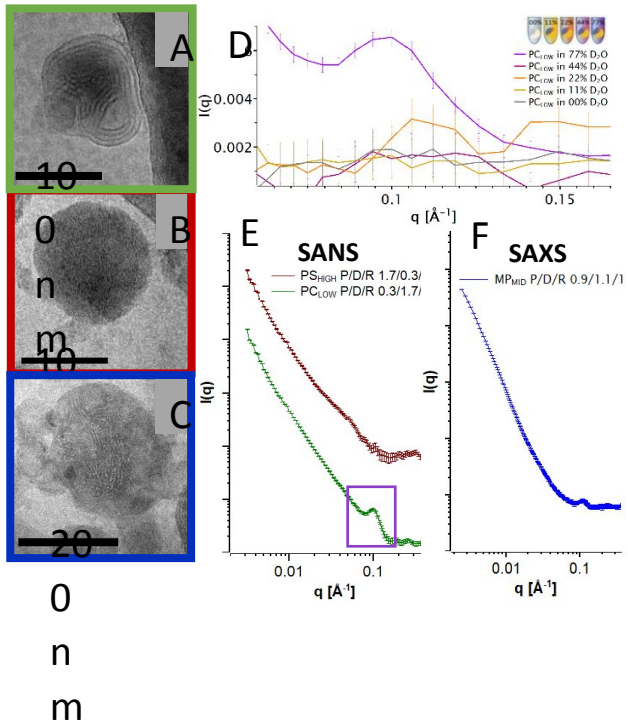
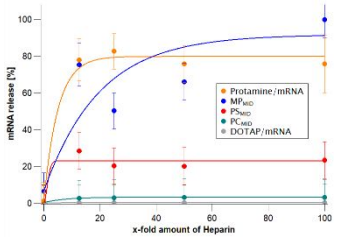
Hybrid Polymer-Lipid Nanoparticles

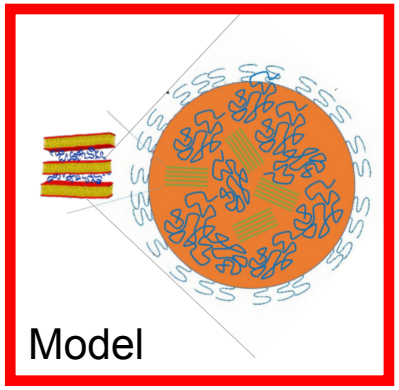
Core-shell approaches for assembly of protamine and DOTAP nanoparticles



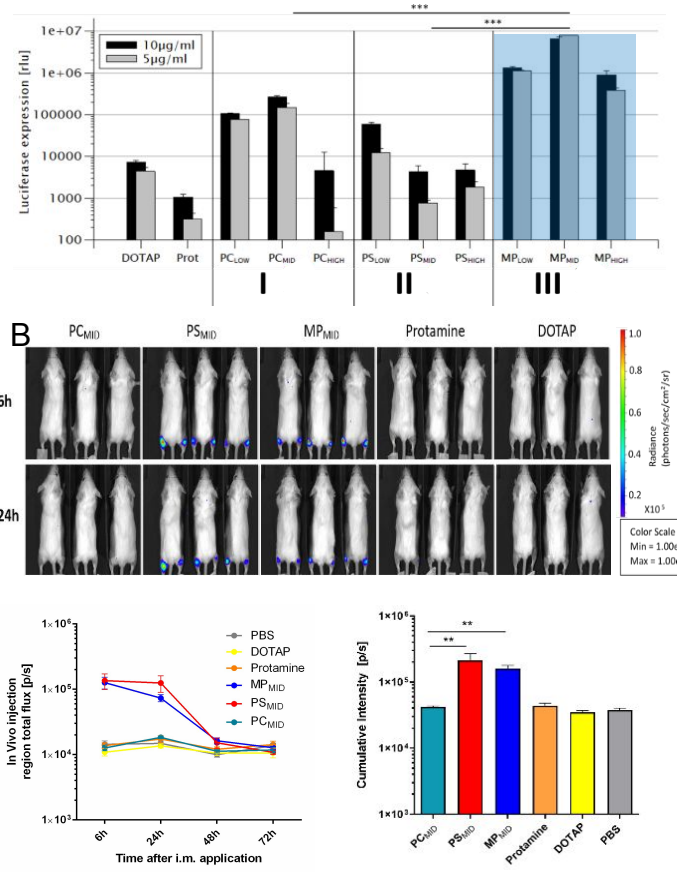




Release



Model



- **Coexistance or ordered and non-ordered structure correlated with improved activity**

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Conclusions

- **Nanoparticle technologies** are an important for development of pharmaceutical drug and RNA delivery products
- Control of **particle architecture at different length scales** can be relevant for product activity/quality
- Thorough **understanding** of the systems by combination of orthogonal methods for **advanced characterization** is helpful for rational formulation design
- **Tailored formulations** for a given therapeutic intervention need to be provided
- To be applied for n**ext generation** nanoparticle and RNA **therapeutics**

Acknowledgements

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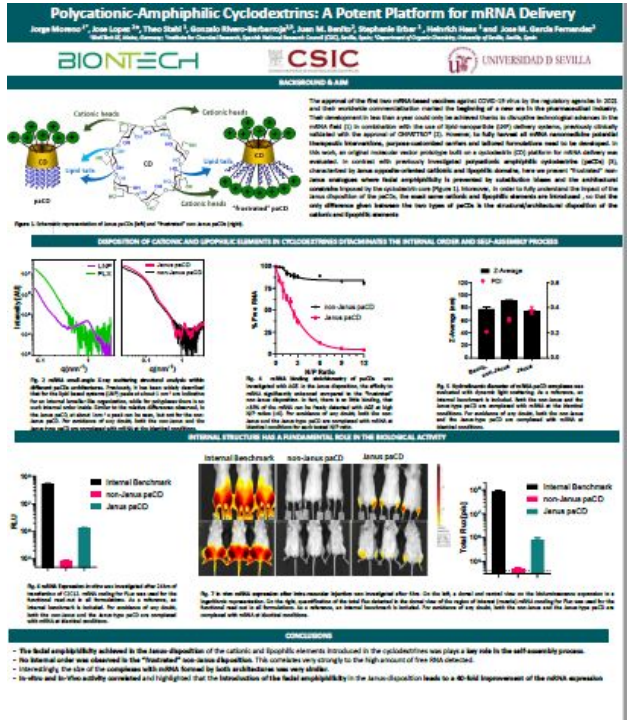
poster 286
Jorge Moreno



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An der Goldgrube 12
55131 Mainz
Germany

T: +49 6131 9084-0
F: +49 6131 9084-390
M: **info@biontech.de**