Gene Delivery and Gene Editing II

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Predicting polyspermines for therapeutic pulmonary RNA delivery by artificial intelligence

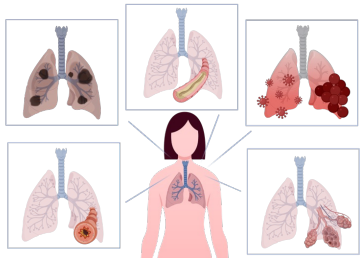
CONTROLLED RELEASE SOCIETY
CRS 2024 Annual Meeting AND Exposition
JULY 8-12, 2024 • BOLOGNA, ITALY

INTEGRATING
Delivery Science
ACROSS DISCIPLINES

Disclosure Statement

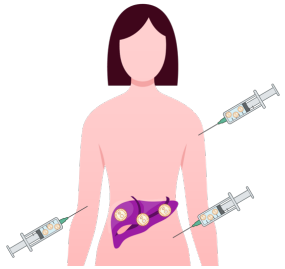
Olivia Merkel is a Co-Founder of RNhale, SAB member of Corden Pharma, AMW, and Coriolis Pharma, and a Consultant for AbbVie, PARI Pharma, and Boehringer-Ingelheim.

Unmet medical needs



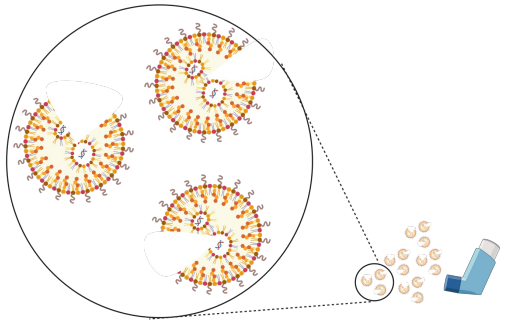
1) Lung Diseases Are Amongst the Leading Causes of Death Globally (WHO)

Solution: RNA Medicines to “Drug the Undruggable”



2) Approved RNA-Nano-Formulations Accumulate in the Liver

Solution: Local Administration Routes



3) Approved RNA-Nano-Formulations Are Not Suited for Inhalation Delivery

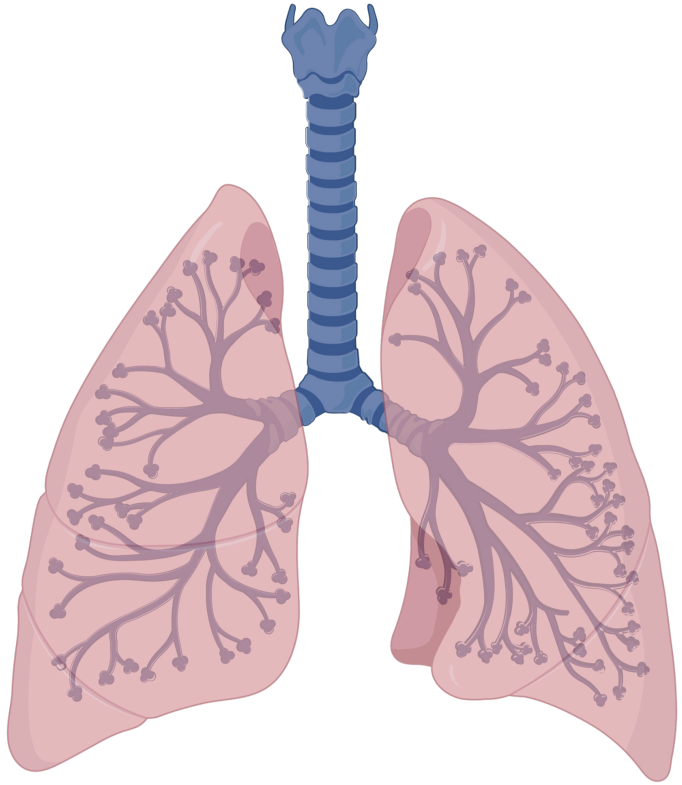
Solution: Biodegradable Polymer Nanoparticles

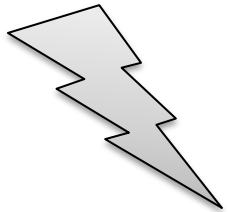
4) Nanocarrier Materials Are Commonly Optimized by Trial-And-Error Methodology

Solution: Rational and Computational Predictions

Pulmonary drug delivery

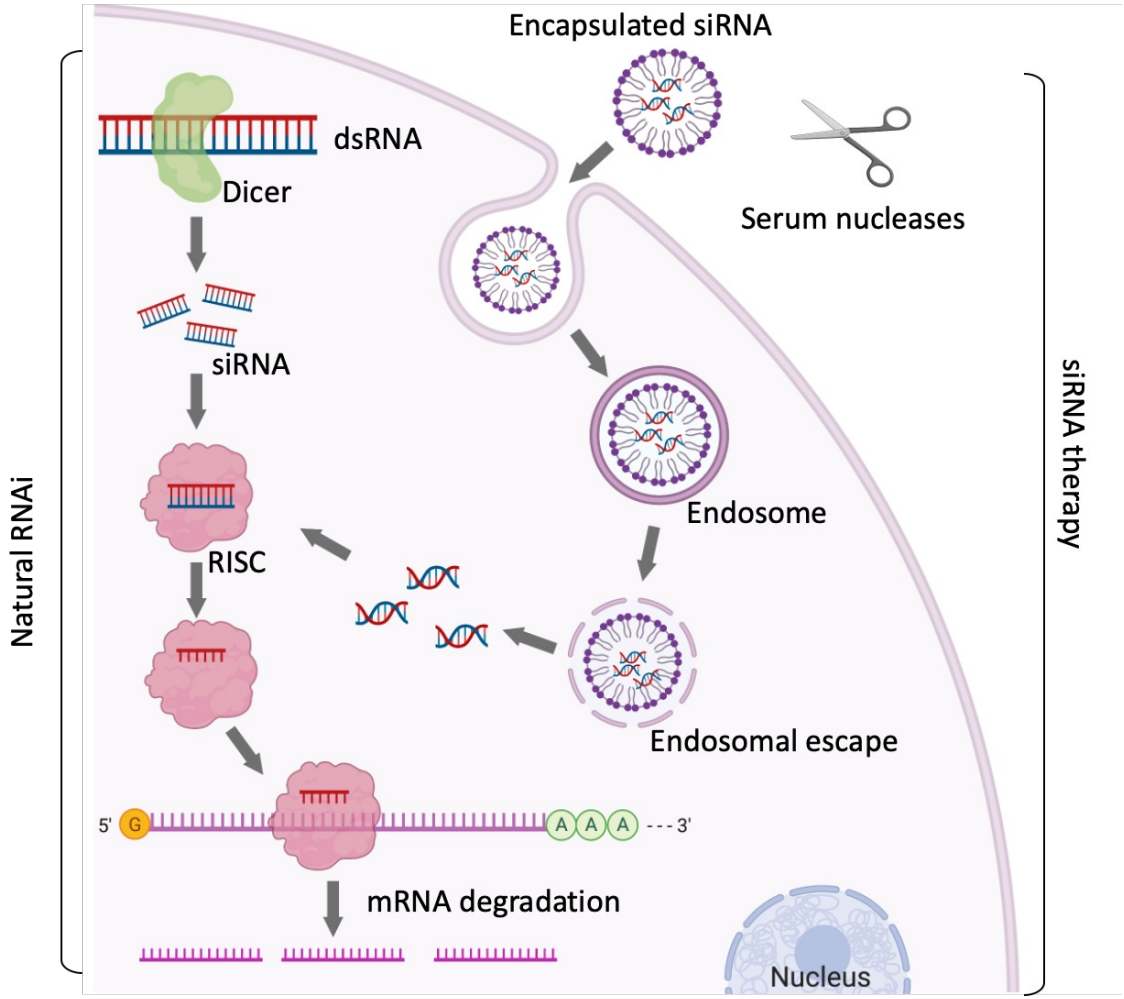
- ✓ Non invasive
- ✓ <u>Local delivery</u>
  - FPE ⇩
  - Systemic side effects ⇩
- ✓ Lung retention ⇧
- ✓ <u>Physiology</u>
  - Large surface area
  - High vascularity
  - No serum proteins
  - Thin epithelium





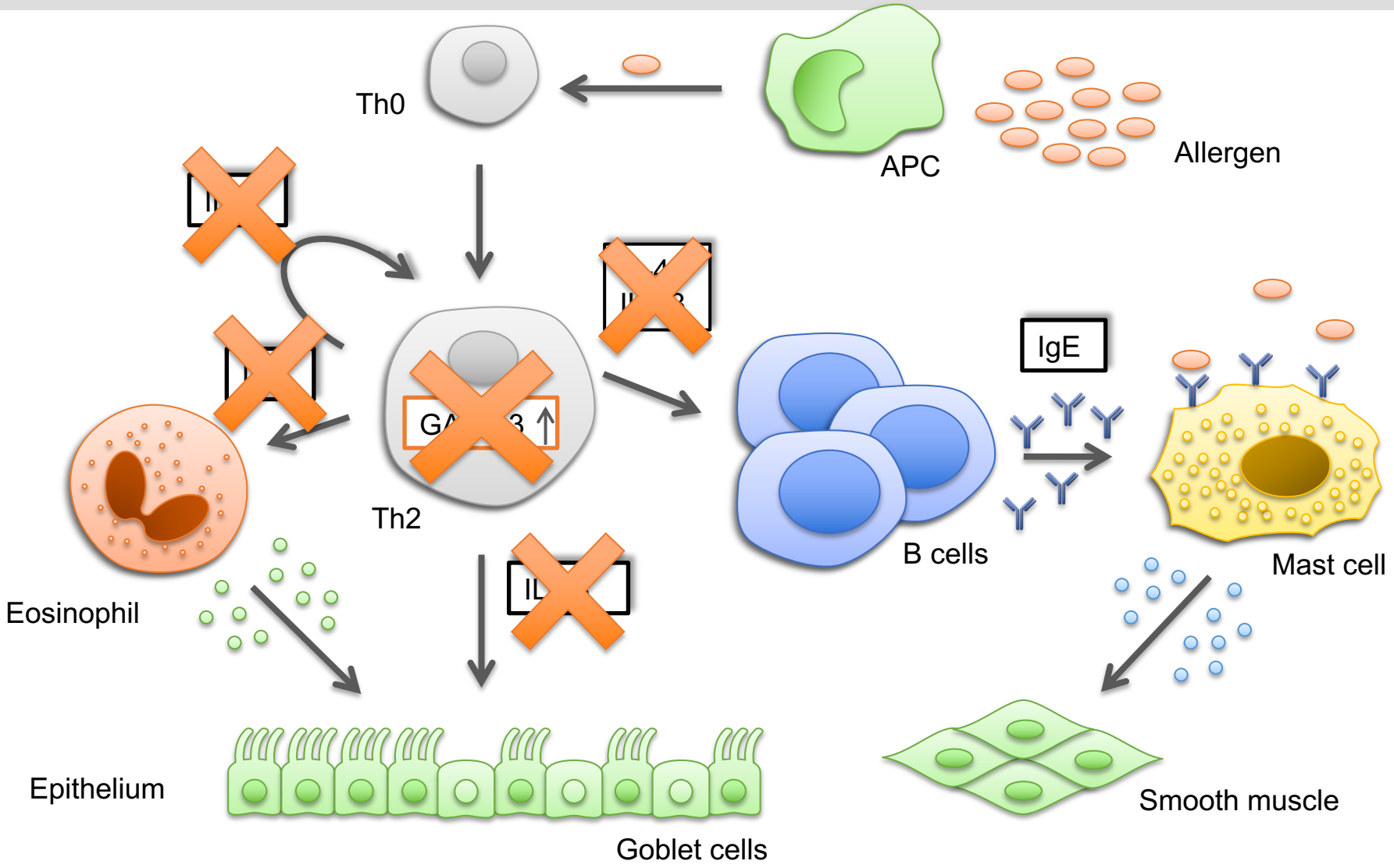
- ➢ Mucus / Surfactant
- ➢ Size requirements

The delivery challenge



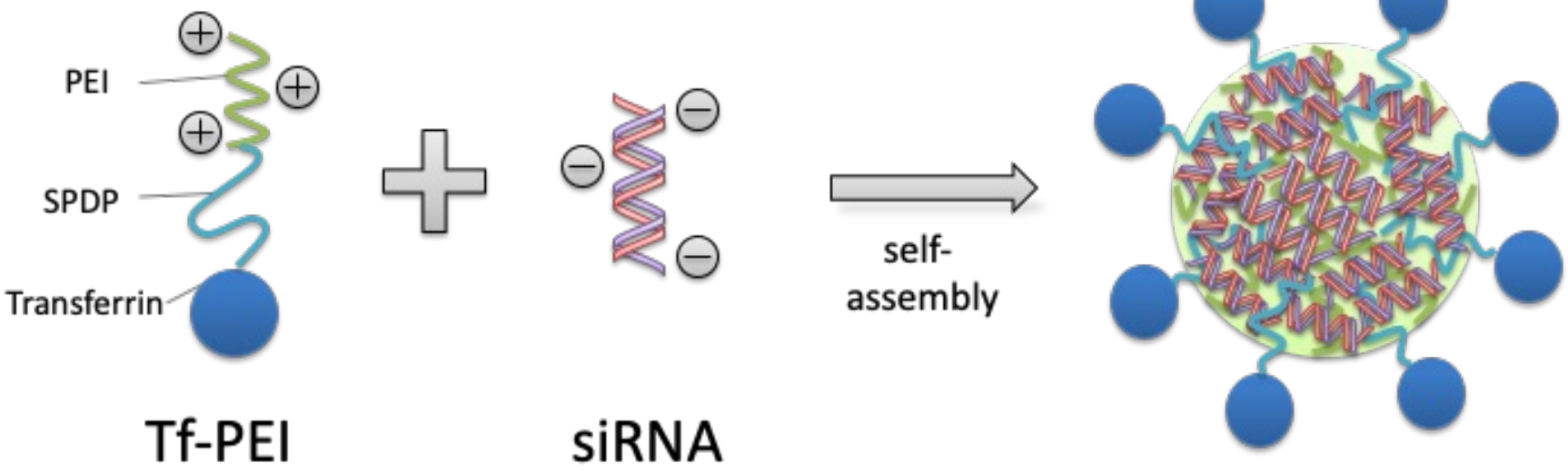
Mehta, Michler, Merkel, Adv Healthc Mater, 2021

T cell Targeting in Asthma

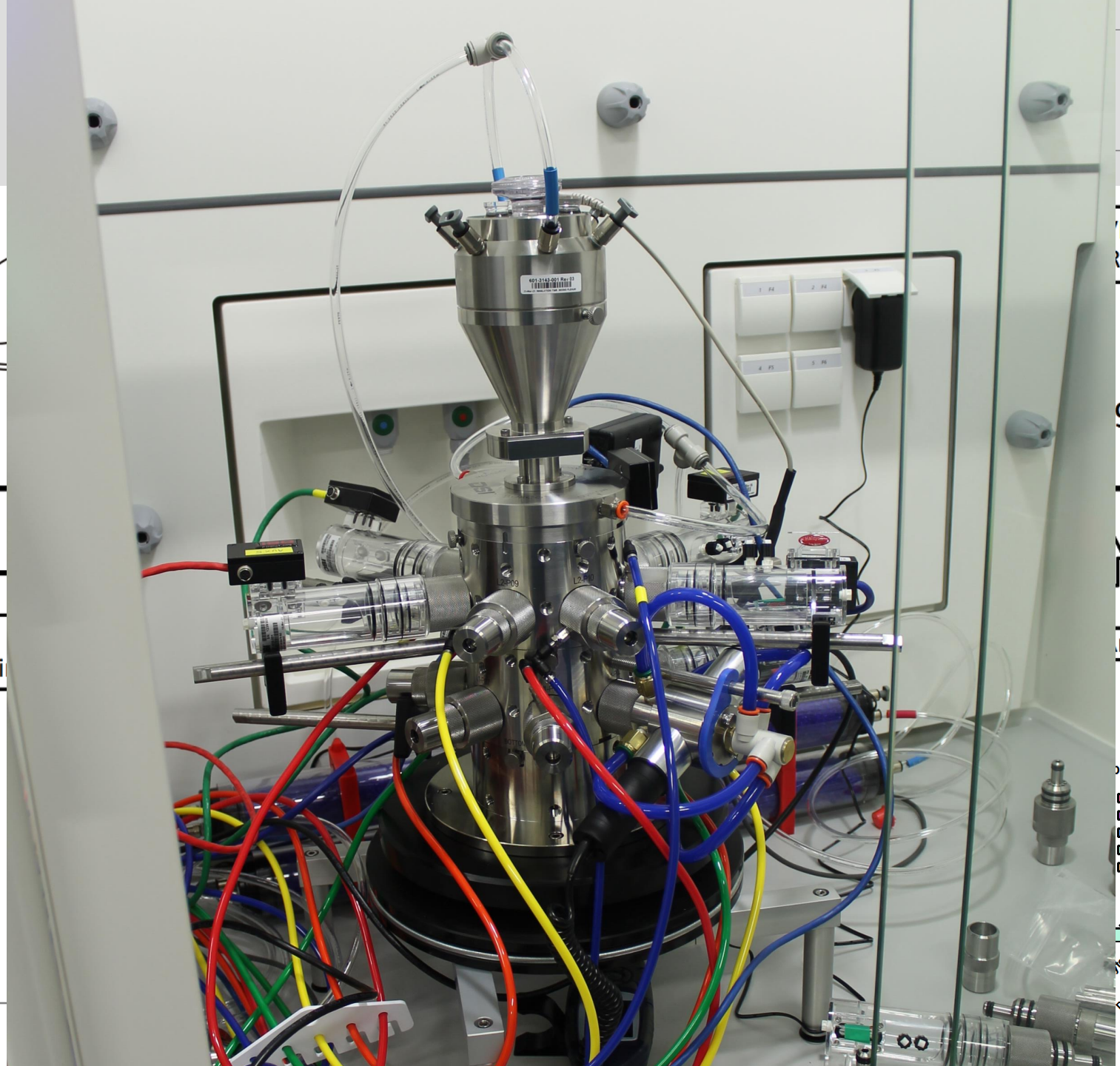


Adapted from Xie and Merkel, Archiv der Pharmazie 2015

T cell targeting



Xie et al, JCR 2016



Day 0

i.p. i

plexes
RNA

9 40

nalysis

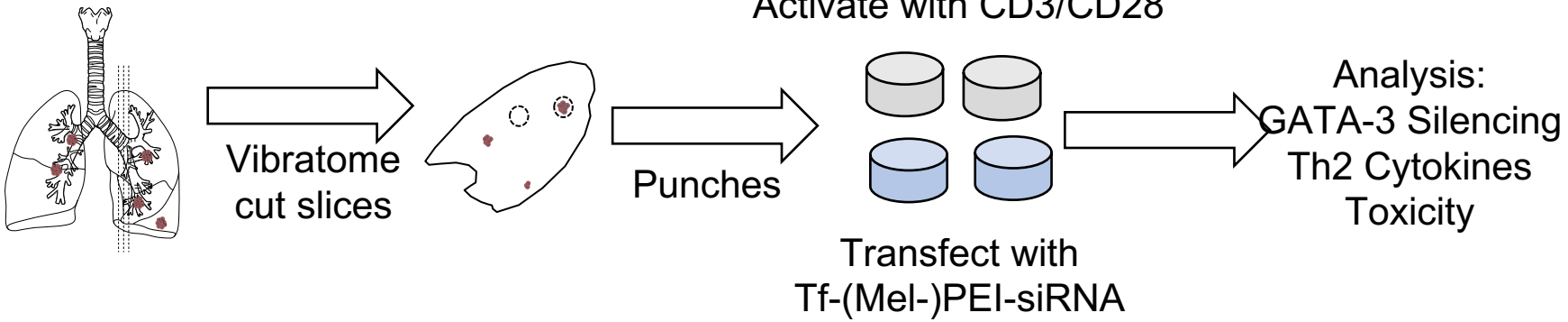
by Tf-PEI

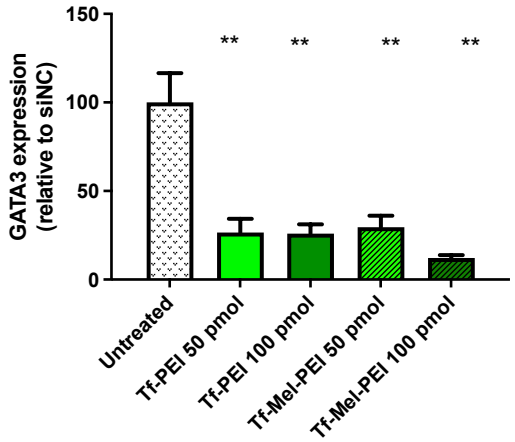
Macrophages
T cells
Eosinophils
Type II pneumocytes
Dendritic cells

Dendritic cells

Human ex vivo model

Precision Cut Lung Slices (PCLS)

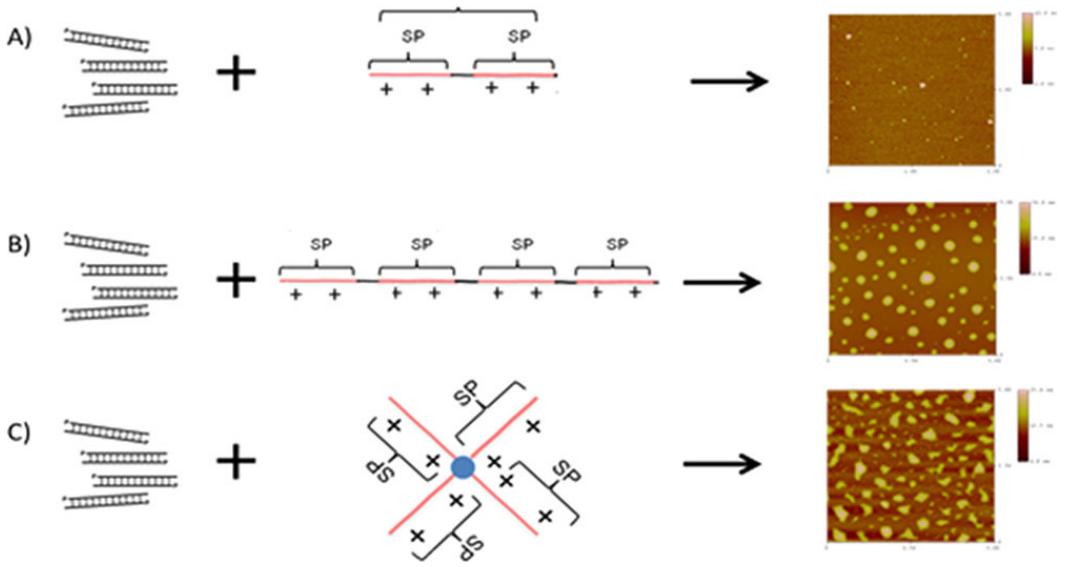


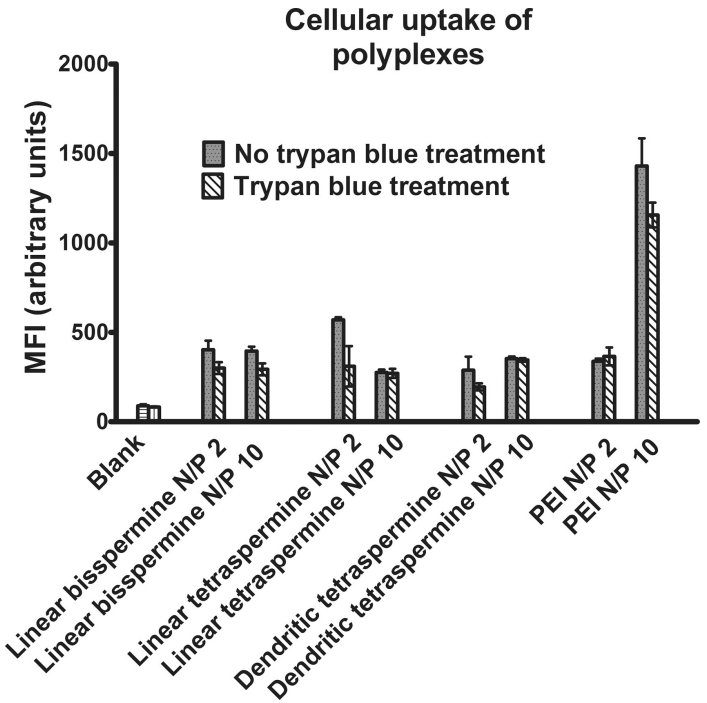


Kandil et al, JCR 2023

Oligospermines

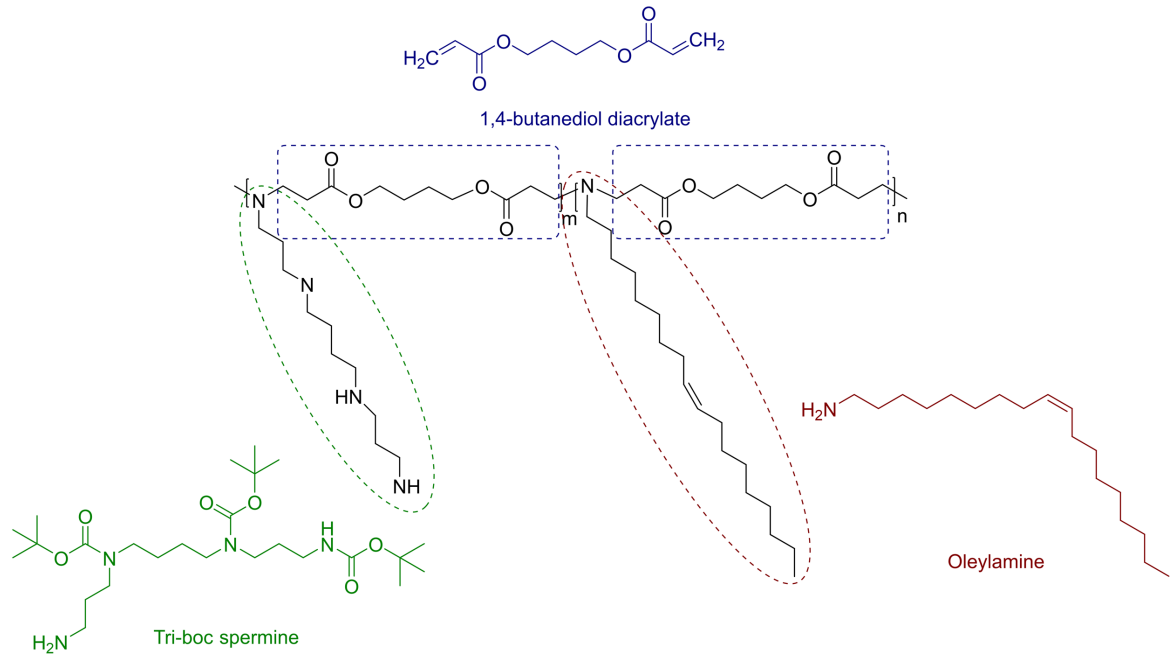
- Spermine Oligomers with DCC- and EDTA-based linkers
- Biodegradable and biocompatible





Elsayed et al, Biomacromolecules 2014

Poly-beta-aminoesters

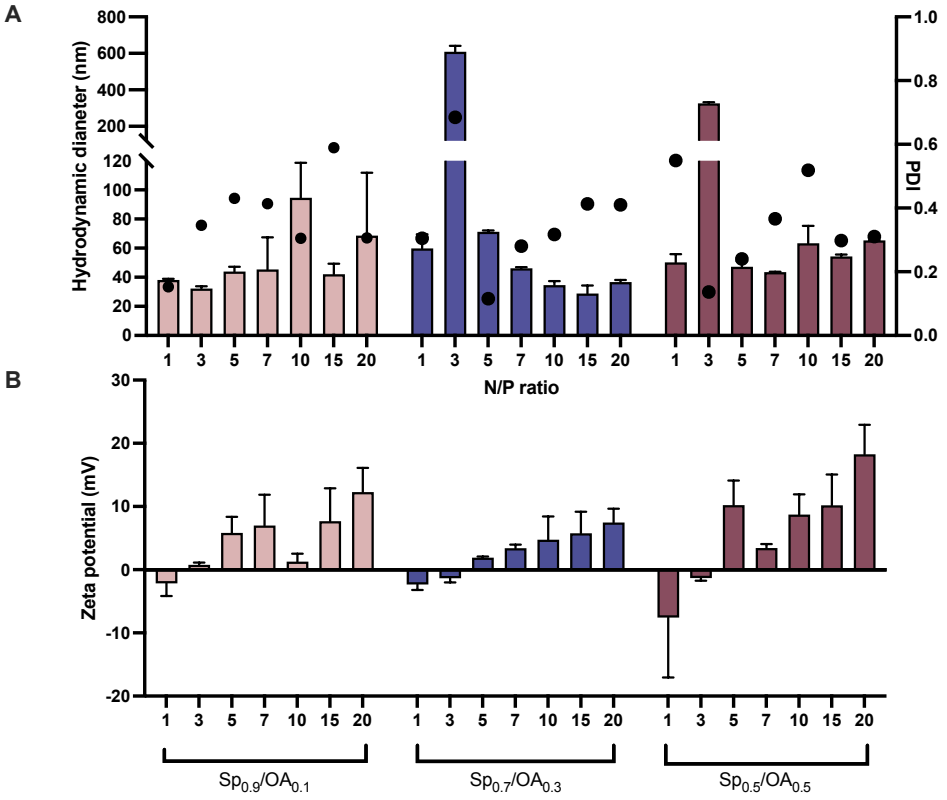


10% oleylamine/90% spermine	30% oleylamine/70% spermine	50% oleylamine/50% spermine
2 kDa	2 kDa	2 kDa
6 kDa	6 kDa	6 kDa
10 kDa	10 kDa	10 kDa

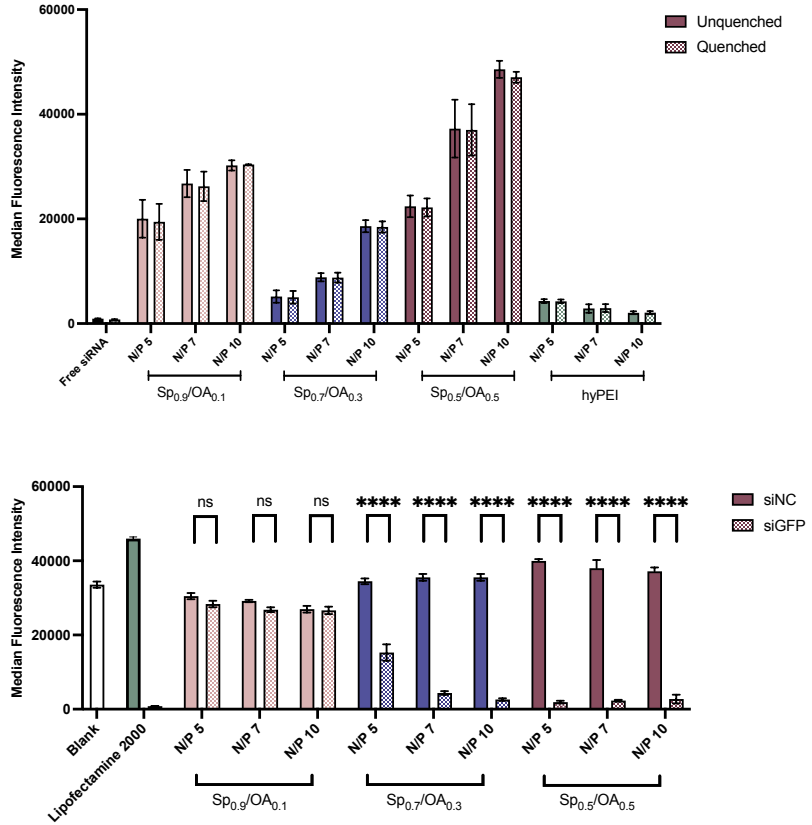
Jin et al, Biomacromolecules 2024

In vitro characterization

Physicochemical characteristics

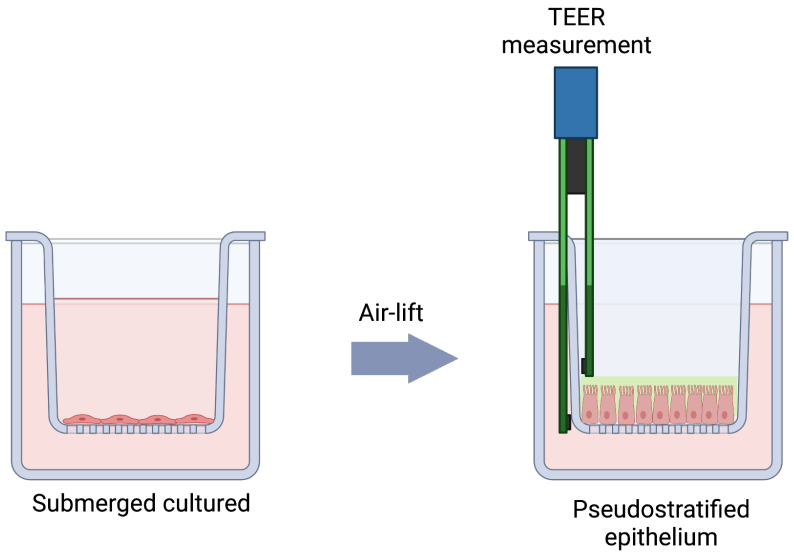


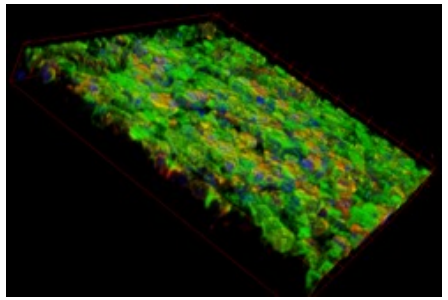
In vitro performance



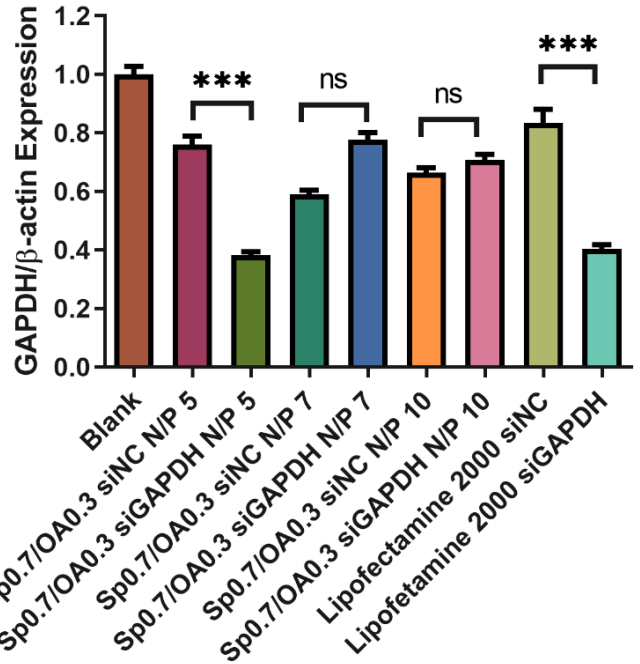
Jin et al, Biomacromolecules 2024

Air-liquid interface model





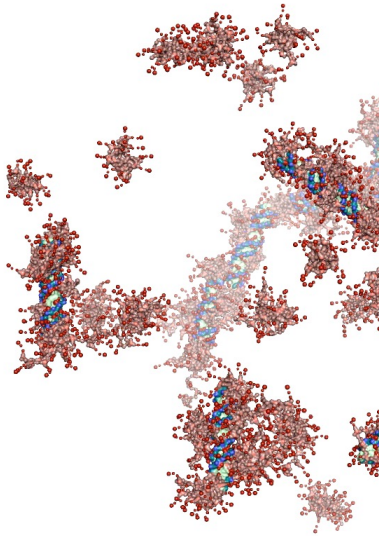
Calu-3 cells in ALI culture, mucus stained in green, actin red, nuclei blue

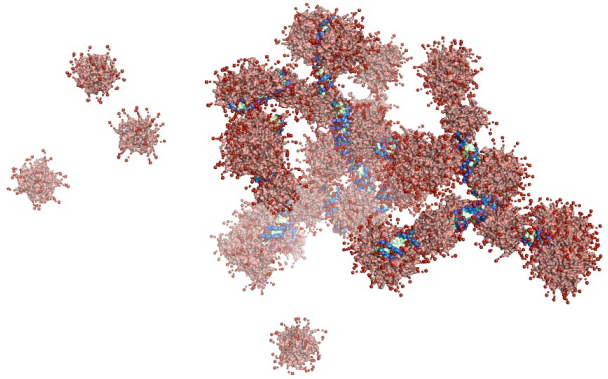


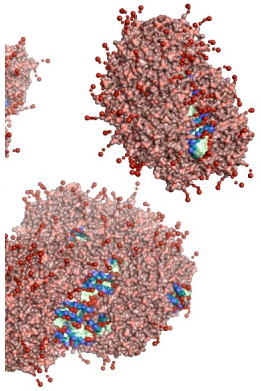
- 16HBE14o- cells
- PBAE polyplexes containing 50 pmol siRNA against GAPDH or scrambled control
- mRNA levels by qRT-PCR 48h post transfection

Jin et al, Biomacromolecules 2024

MD simulations



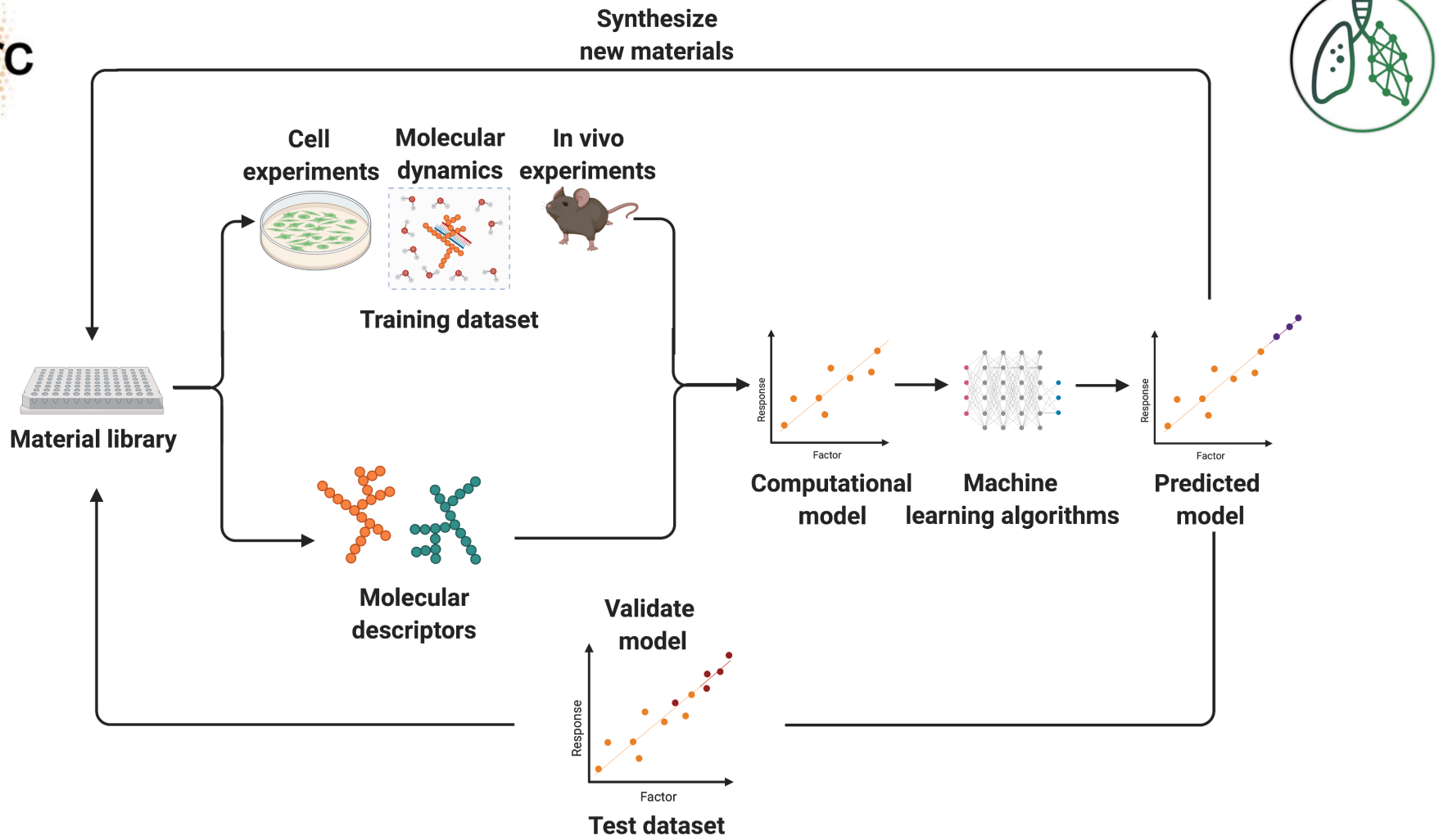




10% oleylamine/90% spermine	50% oleylamine/50% spermine	80% oleylamine/20% spermine

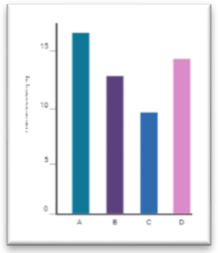
Steinegger et al, in preparation

AI in RNA formulation

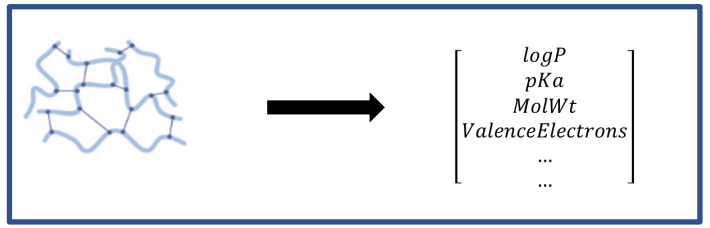


Sieber-Schäfer et al, unpublished

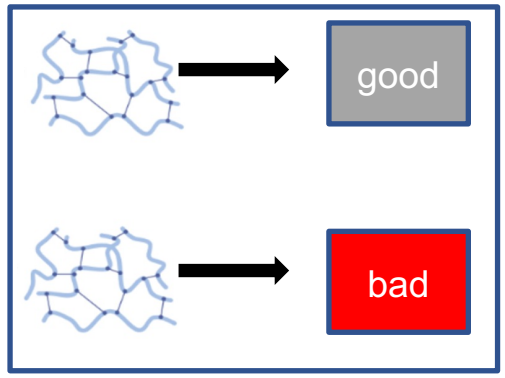
Machine Learning Algorithm



Literature Data

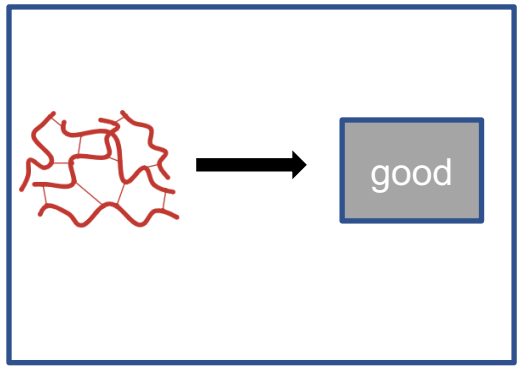


Encoding into Molecular Descriptors



Labeling with Performance Data

Train Machine Learning Model

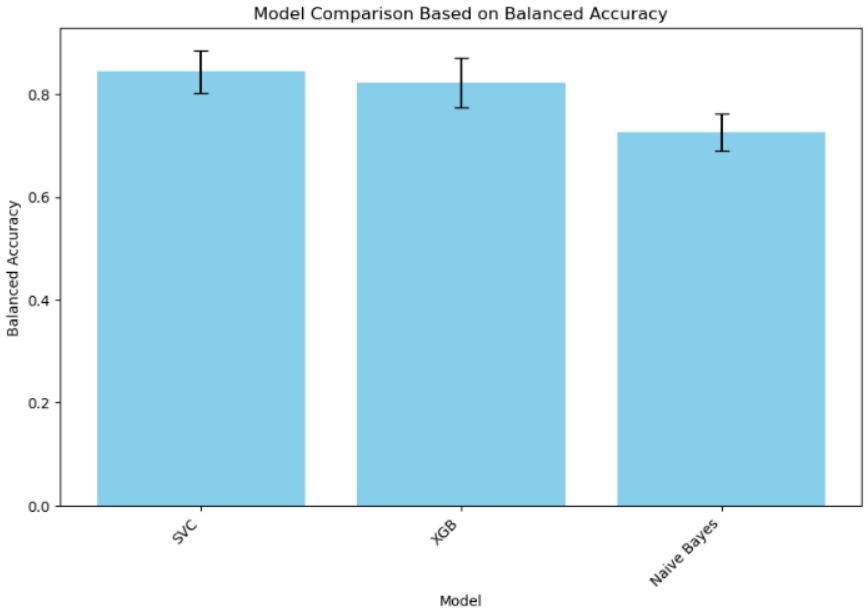


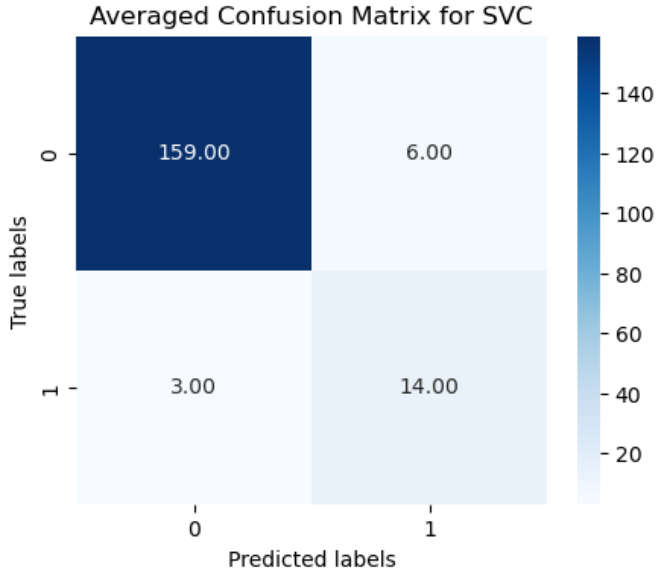
Prediction of New Candidate

Sieber-Schäfer et al, unpublished

Machine Learning Algorithm

Model Evaluation on Unseen Validation-Set

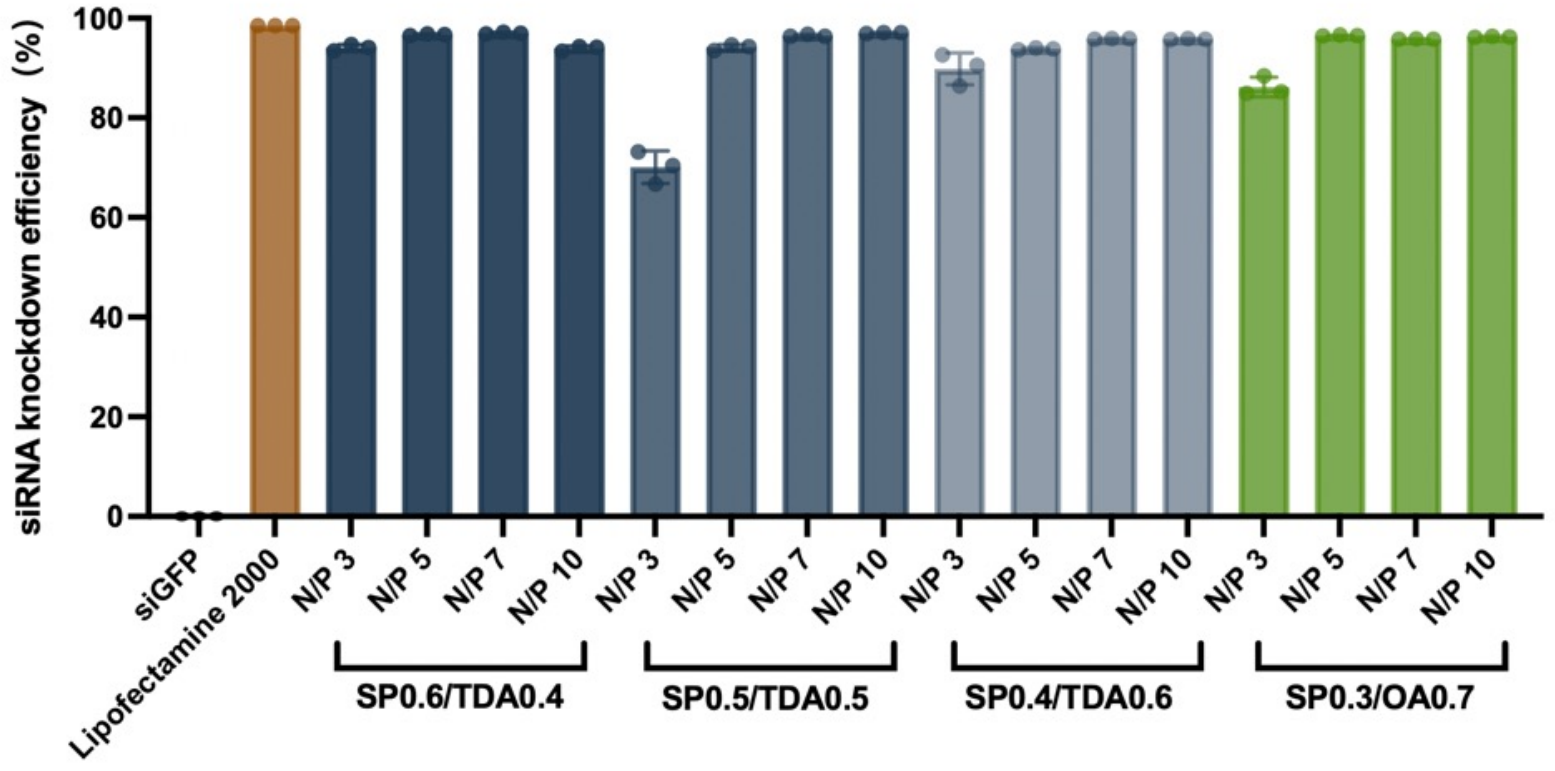




Sieber-Schäfer et al, unpublished

In vitro gene silencing

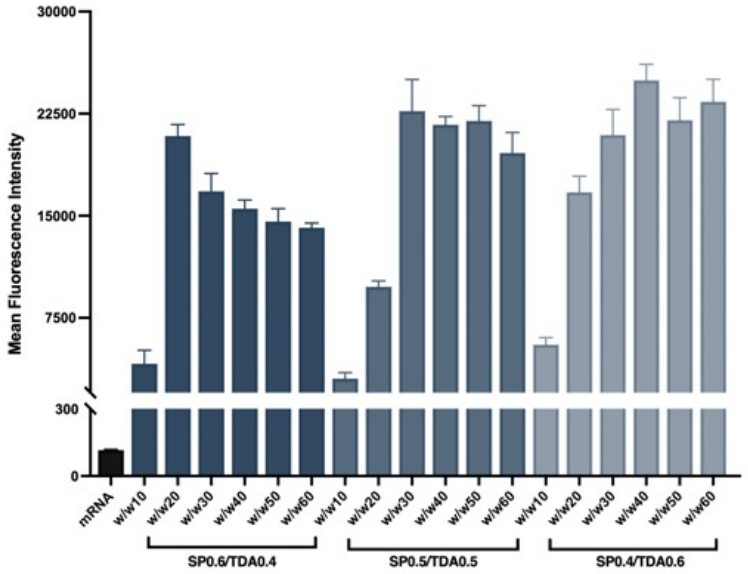
GFP silencing in H1299/GFP cells

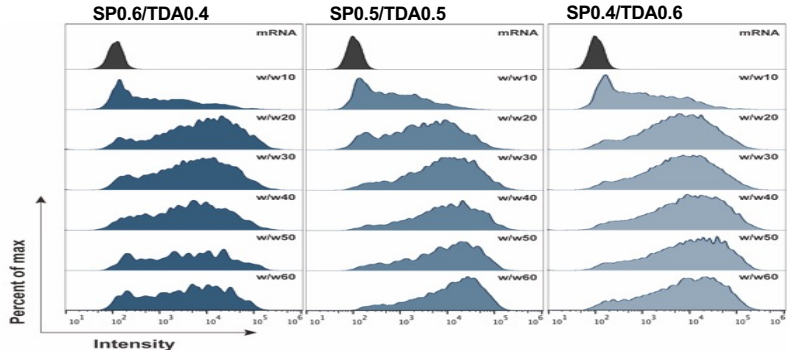


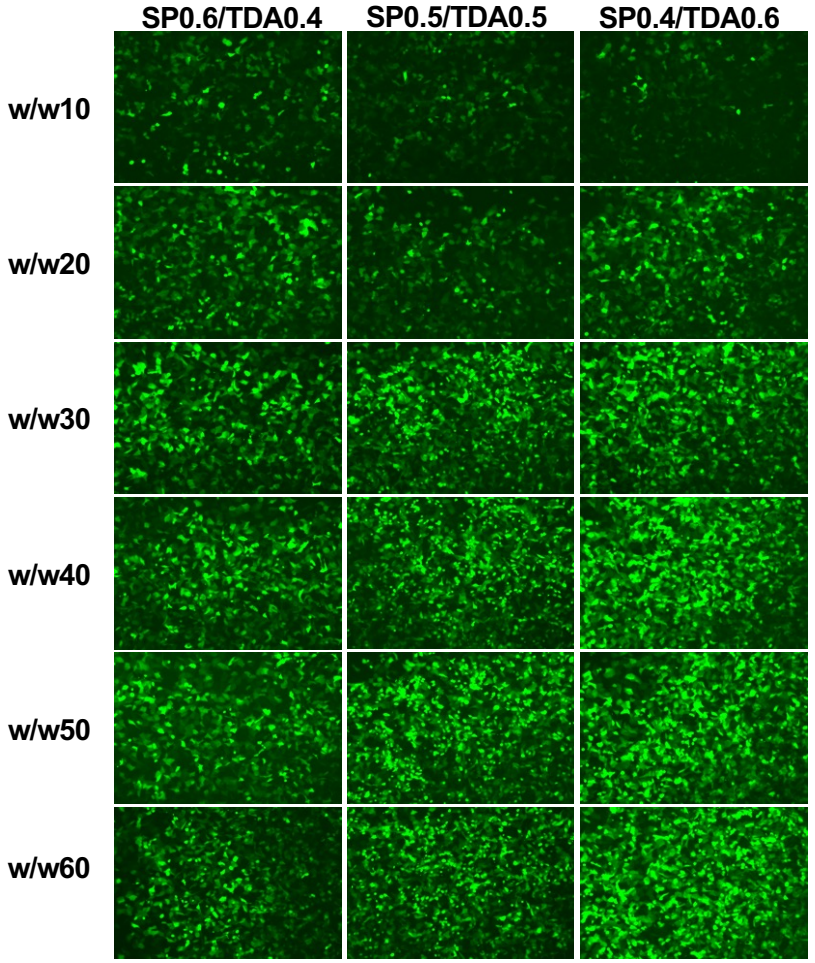
Sieber-Schäfer, Jiang et al, in preparation

mRNA delivery

GFP expression in H1299 cells







Sieber-Schäfer, Jiang et al, in preparation

Summary and Outlook

- Summary:
  - The potential of therapeutic RNA delivery for pulmonary diseases
  - Efficacy testing in *in vitro*, *ex vivo*, and *in vivo* models of disease
  - AI-based predictions for improved RNA nanocarriers

- Outlook for pulmonary RNA delivery
  - Synthesis of polyspermine-based bioconjugates
  - Assessment in disease models

- Aim: Development of nanoparticle based nucleic acid therapies

Thank you!

<u>Collaborators:</u>

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Walter Mier,
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Nora Urbanetz, *Daiichi-Sankyo*
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Ernst Wagner,
Knut Müller-Caspary,
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Achim Hartschuh, *LMU*

erc
Bundesministerium für Bildung und Forschung
Bundesministerium für Wirtschaft und Klimaschutz
DFG Deutsche Forschungsgemeinschaft
Bayerische Forschungsstiftung
VolkswagenStiftung
SPRIN-D BUNDESAGENTUR FÜR SPRUNGINNOVATIONEN
CNATM Cluster for Nucleic Acid Therapeutics Munich
Elite Network of Bavaria