

Extracellular vesicle-inspired carriers for therapeutic RNA delivery

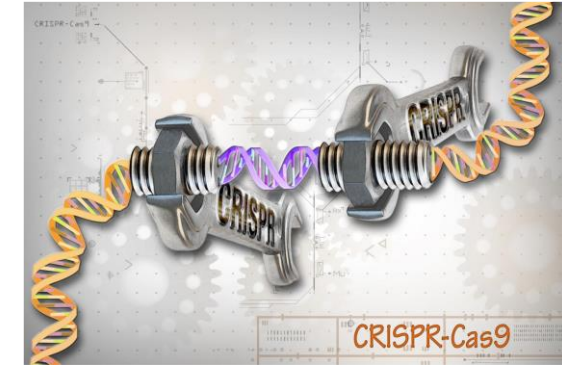
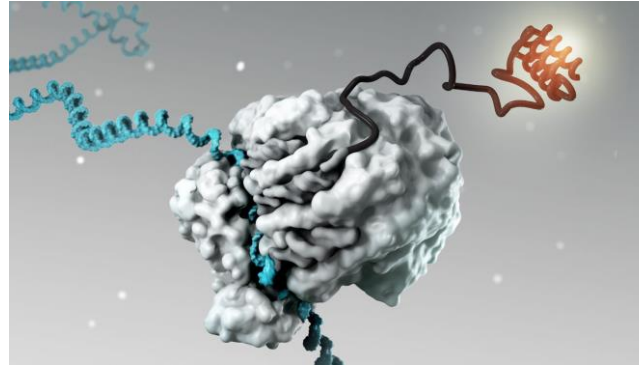
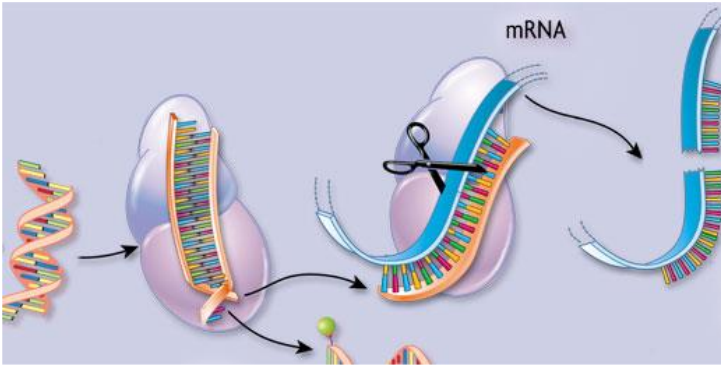
Dr Pieter Vader

pvader@umcutrecht.nl



PRC Symposium, Heidelberg, 2024

RNA-based therapeutics

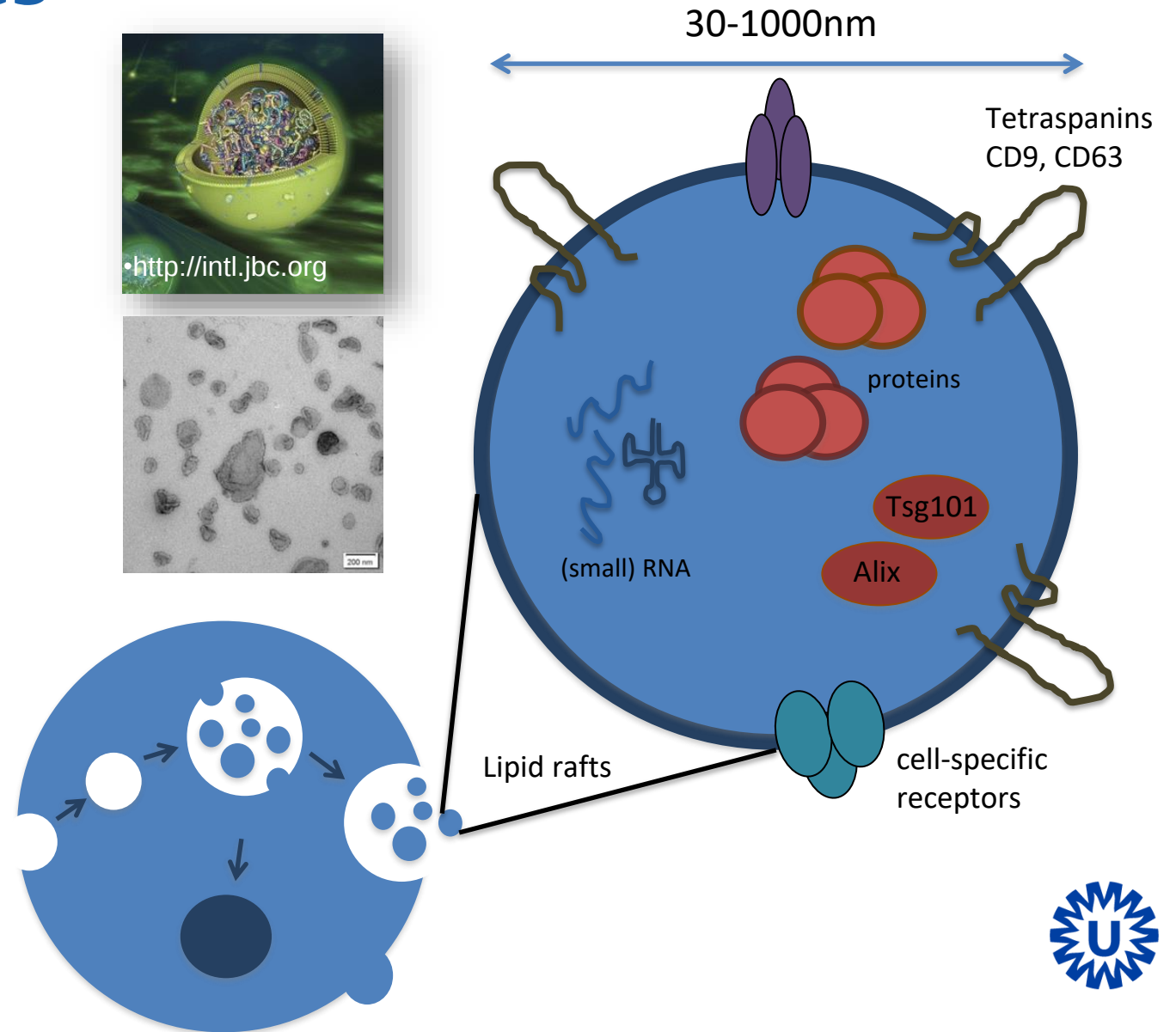
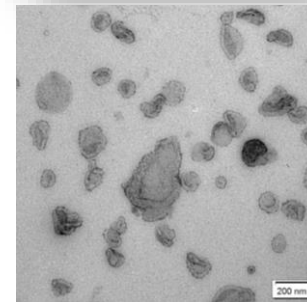
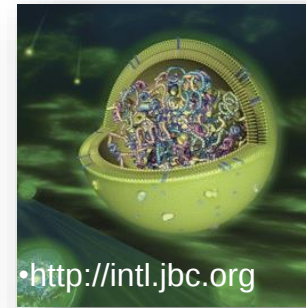


- Typically require delivery to site of action
- Delivery systems:
 - Immunogenic, dose-limiting toxicity
 - Inefficient uptake/endosomal escape



Extracellular vesicles

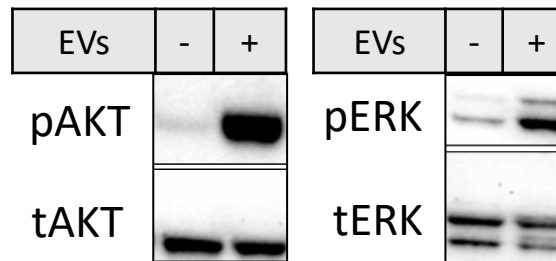
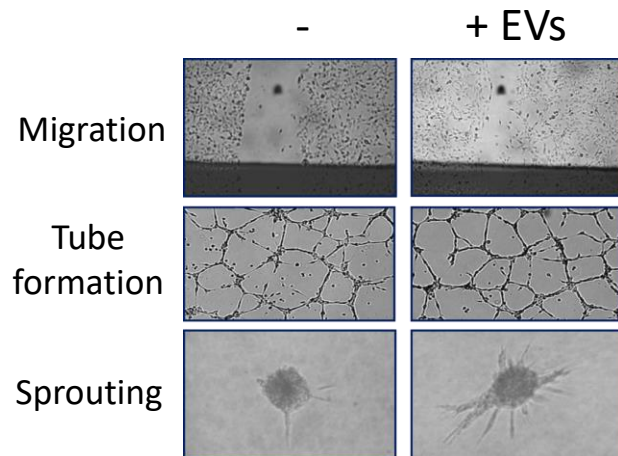
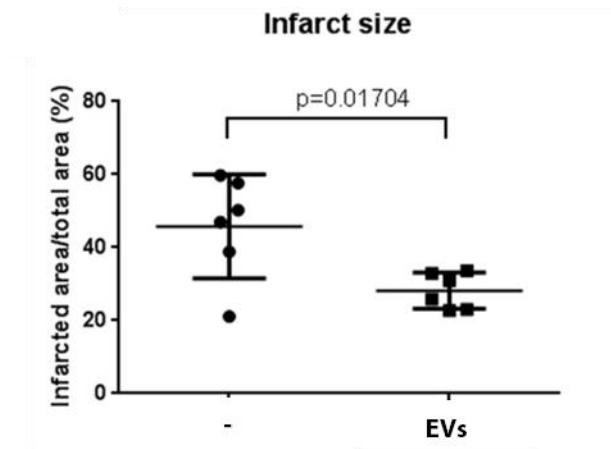
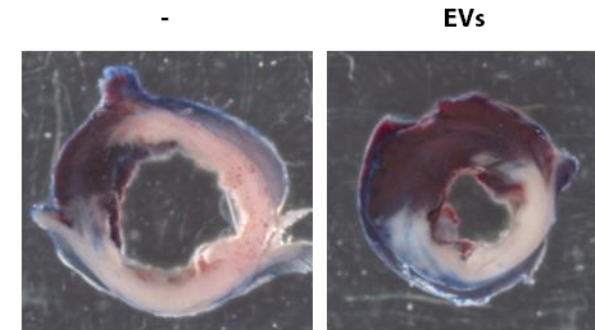
- EVs: membrane surrounded structures released by cells
- Natural carriers of biomolecules
- Capable of functionally transferring cargo to recipient cells



Extracellular vesicles for RNA delivery

Potential advantages over synthetic systems:

- No/low immunogenicity
- Stable
- Utilize native mechanisms for targeting, uptake and intracellular trafficking
- Intrinsic therapeutic efficacy



Vrijssen et al., *Adv Healthc Mat*, 2016;
Mol et al., *Nanomedicine NBM*, 2017;
Maring et al., *J Cardiovasc Transl Res*, 2019

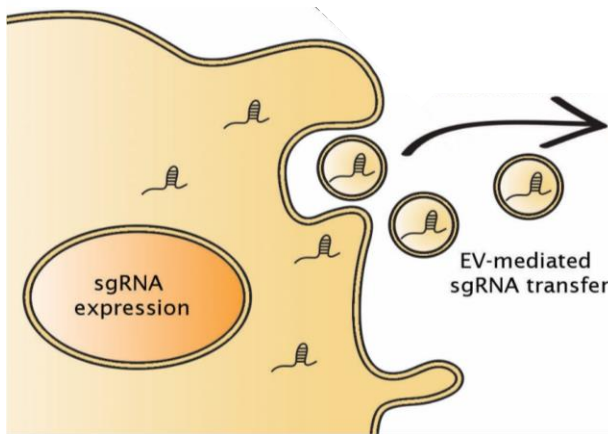


Research questions

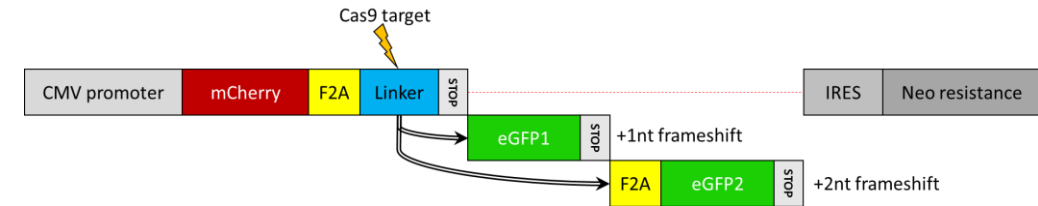
- Can we measure/quantify EV-mediated RNA delivery?
- How efficient is EV-mediated RNA delivery?
- How can we apply EVs for therapeutic RNA delivery?



EV-mediated RNA delivery



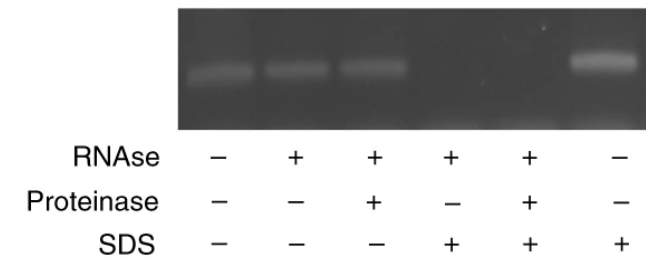
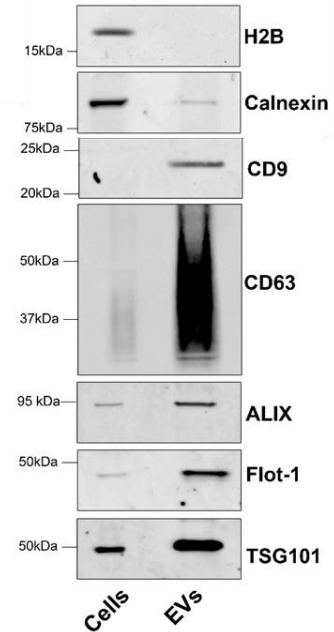
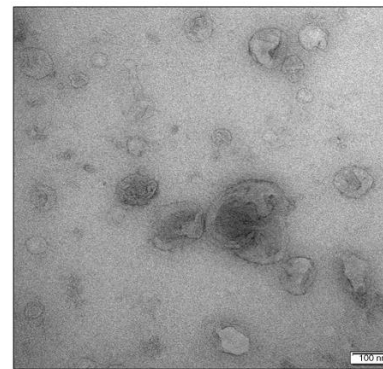
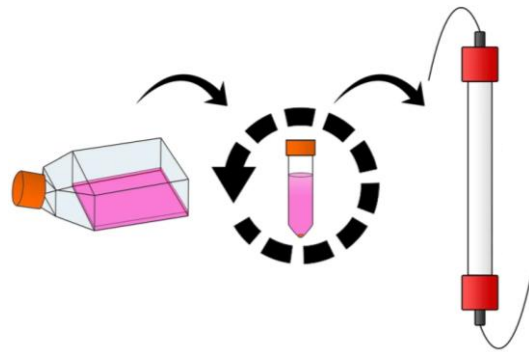
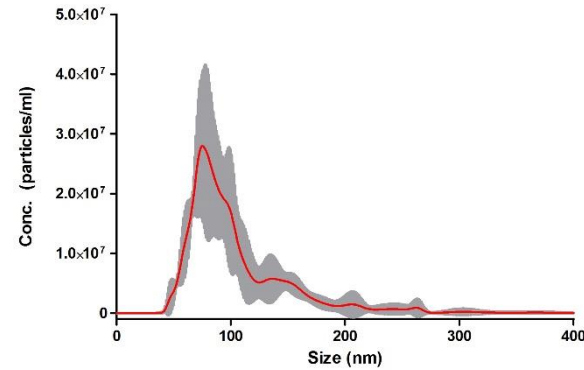
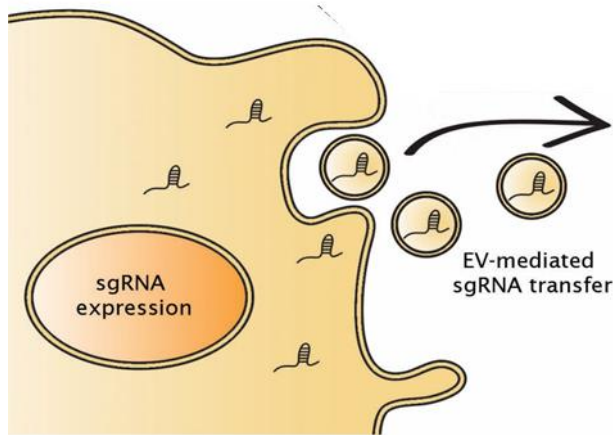
~1 sgRNA per 10^4 - 10^6 EVs



De Jong et al., Nature Commun, 2020



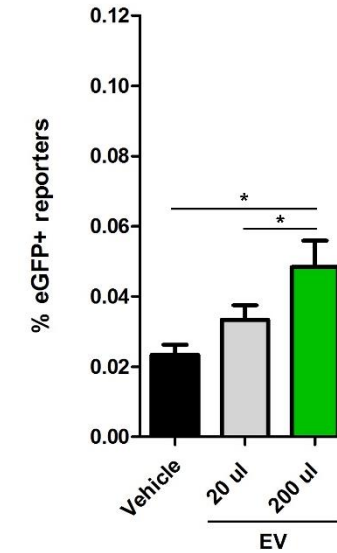
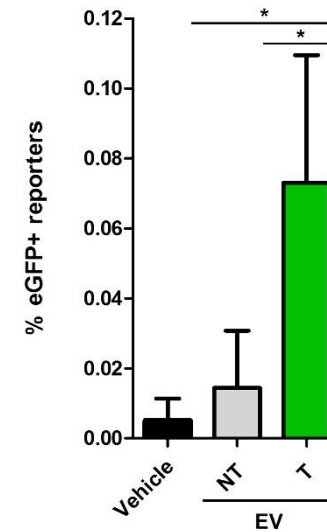
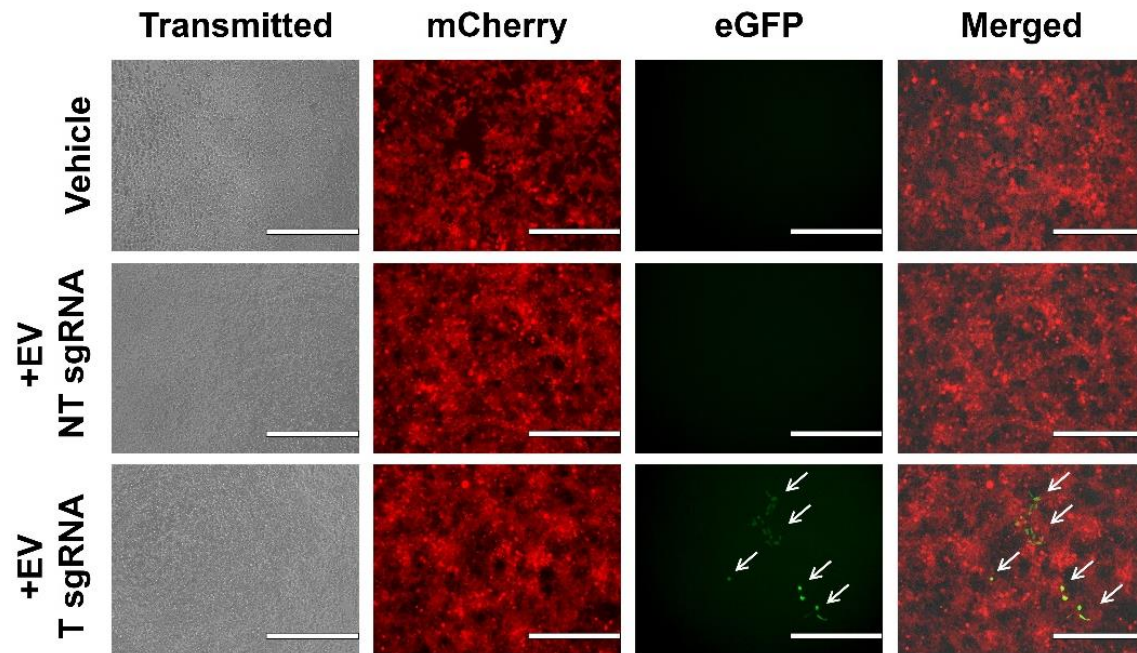
Isolation of sgRNA-carrying EVs



De Jong et al., Nature Commun, 2020
Murphy et al., Nano Letters, 2021



EV-mediated sgRNA transfer

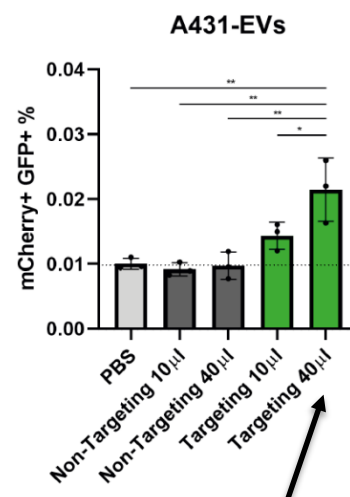
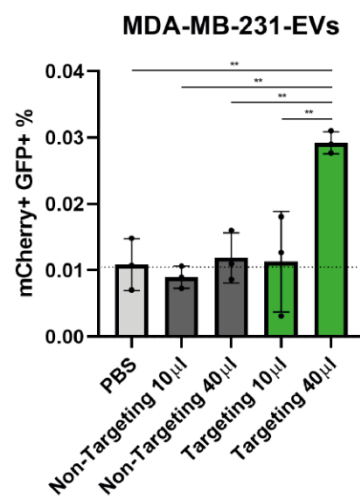
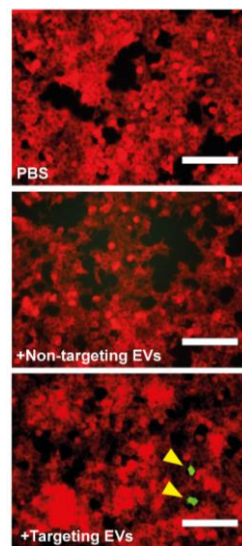
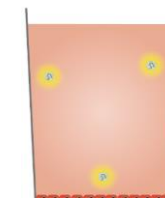
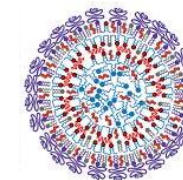
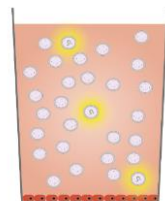
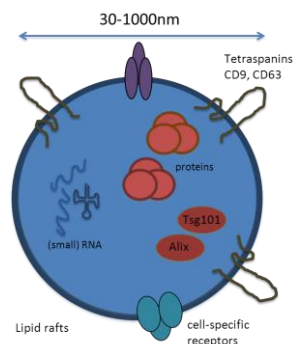


Research questions

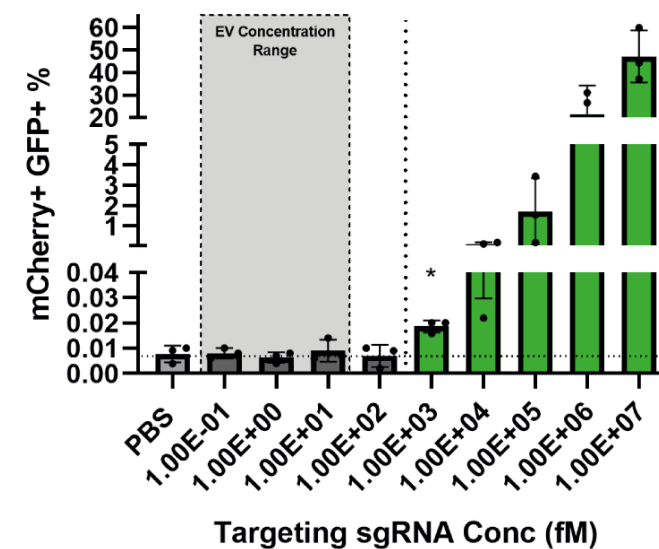
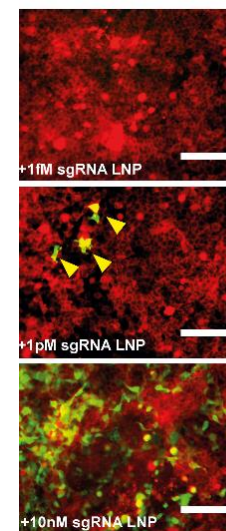
- Can we measure/quantify EV-mediated RNA delivery?
- How efficient is EV-mediated RNA delivery?
- How can we apply EVs for therapeutic RNA delivery?



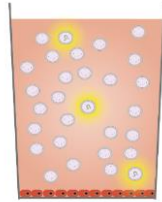
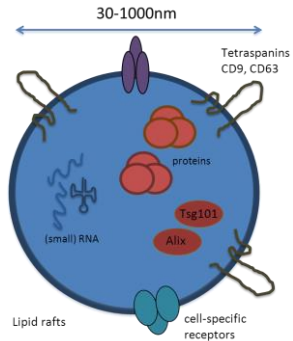
Functional comparison: EVs vs LNPs



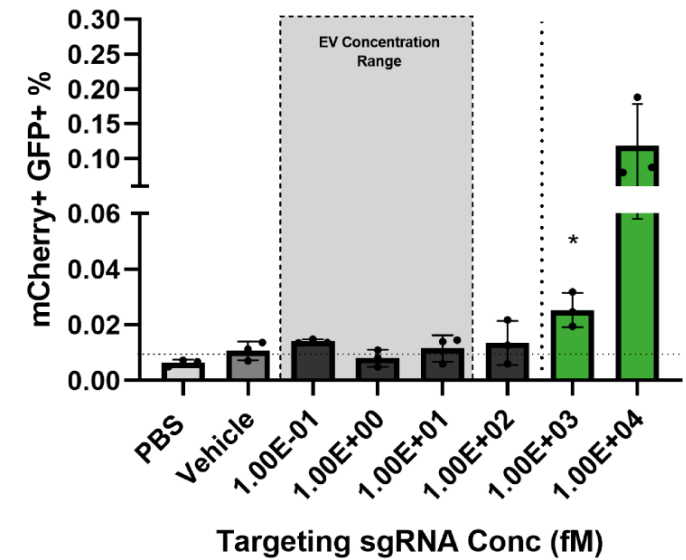
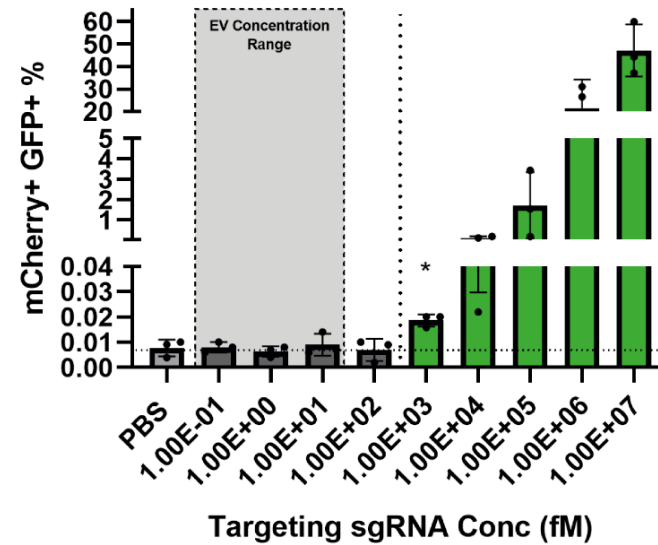
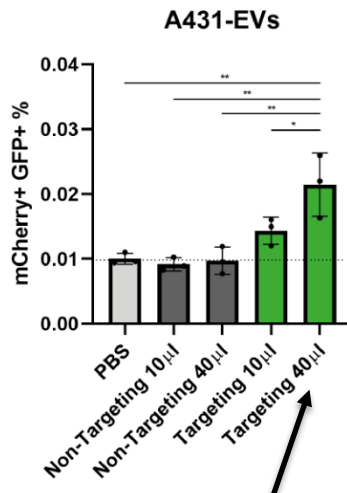
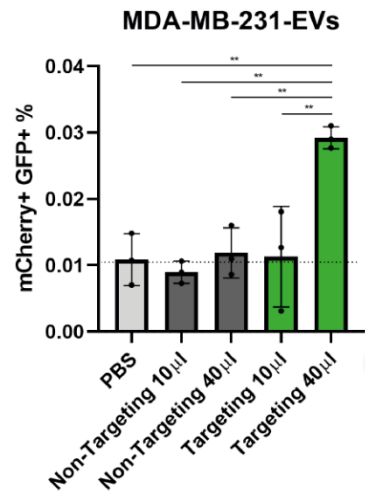
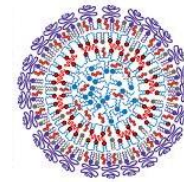
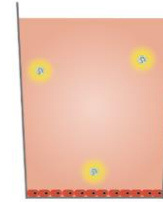
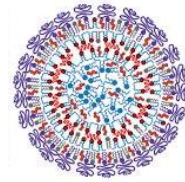
0.1-10 fM sgRNA



Functional comparison: EVs vs LNPs

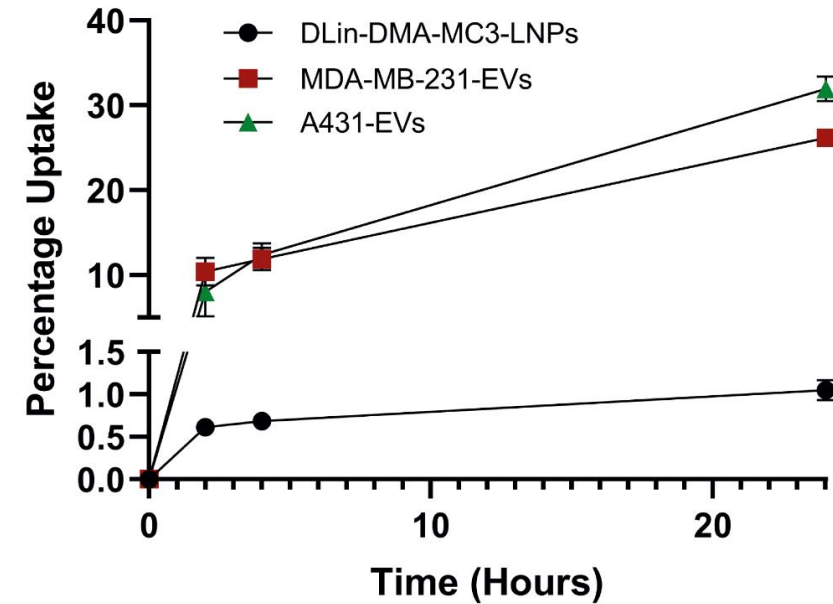
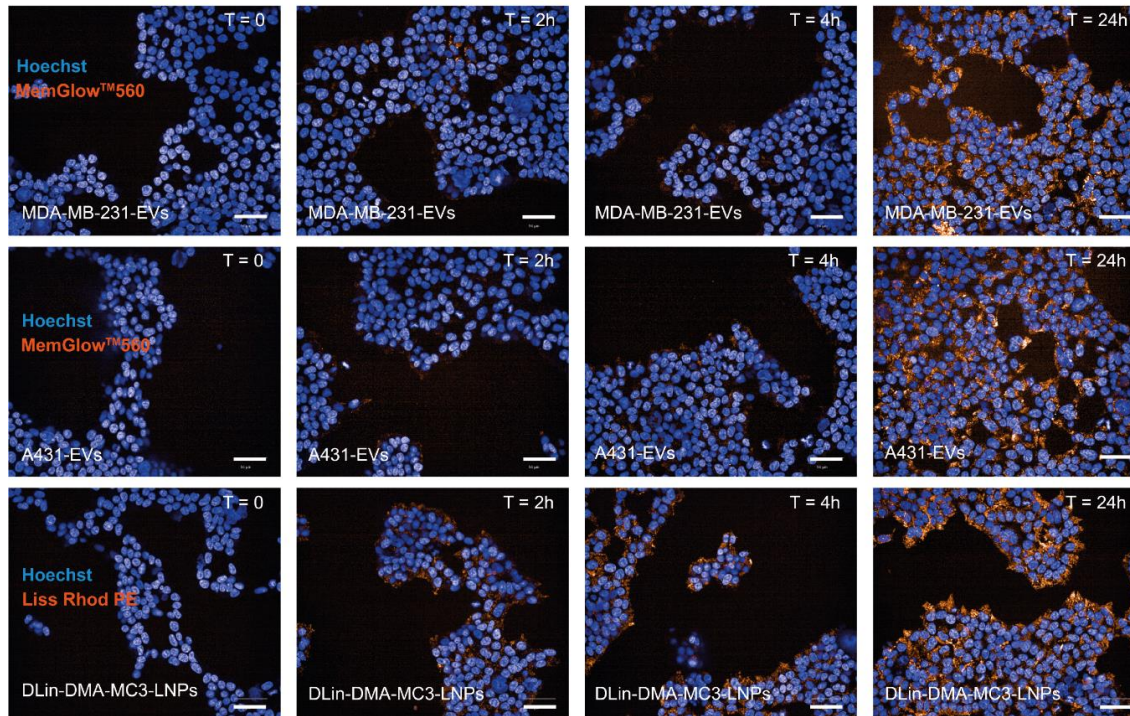


versus



0.1-10 fM sgRNA

EVs are internalized more rapidly

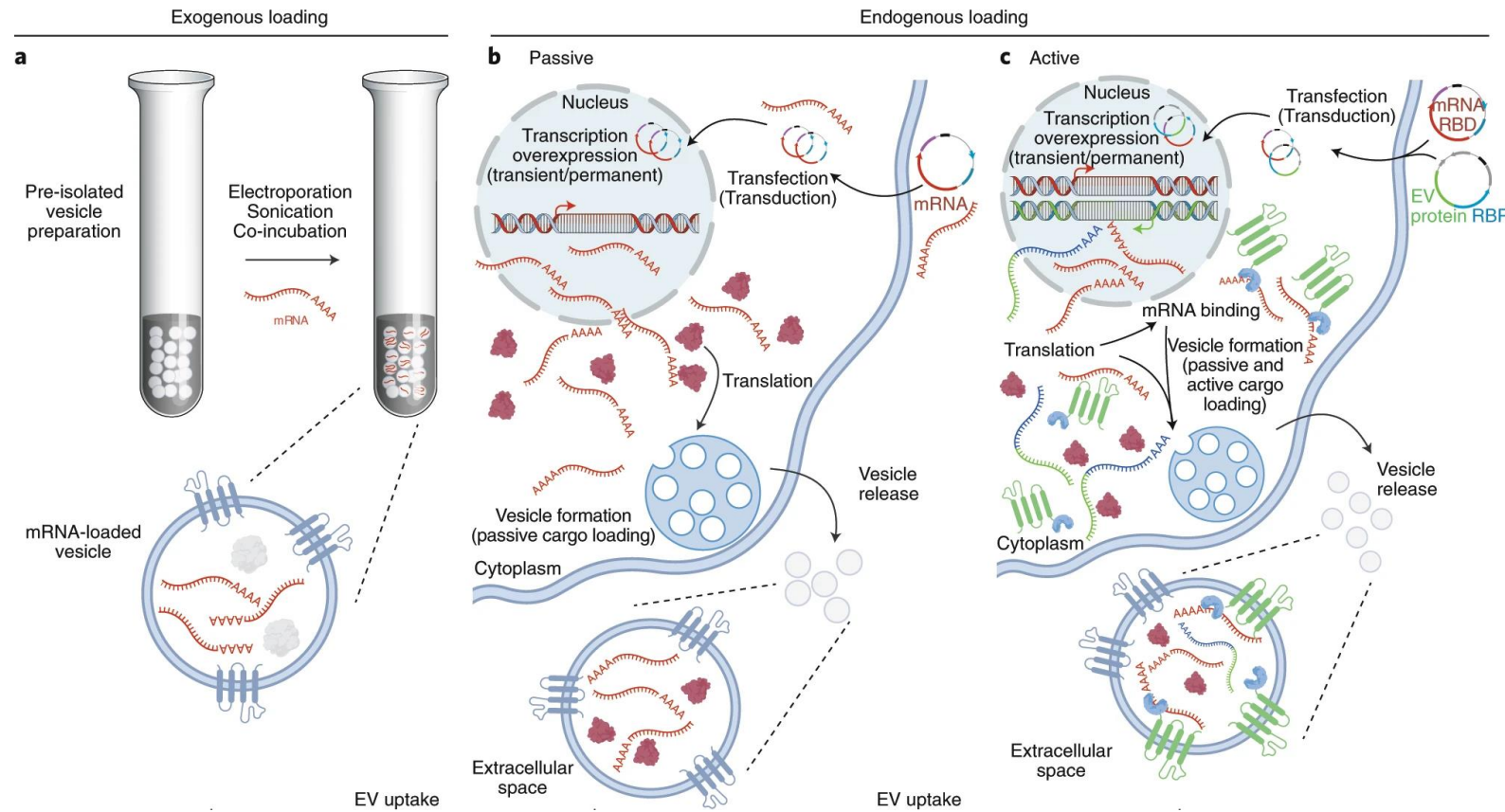


Research questions

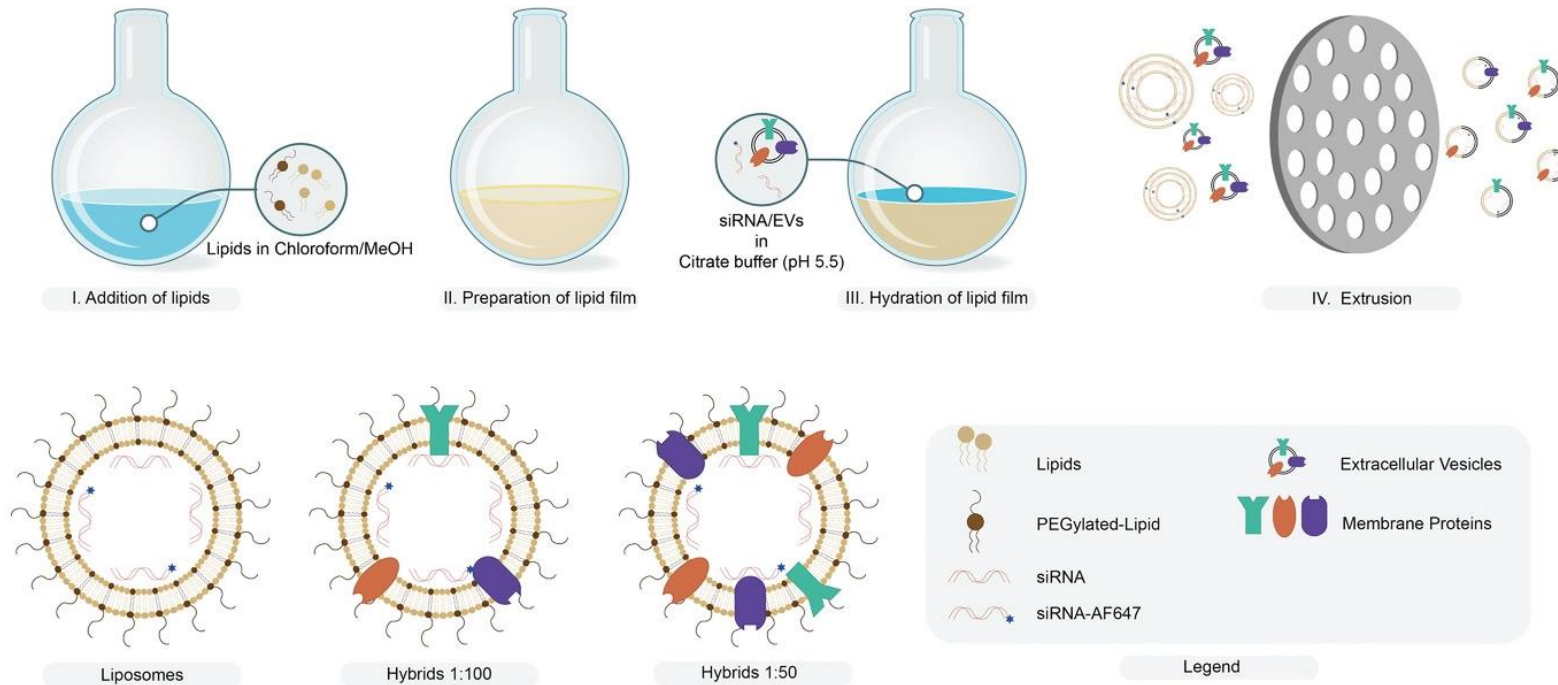
- Can we measure/quantify EV-mediated RNA delivery?
- How efficient is EV-mediated RNA delivery?
- How can we apply EVs for therapeutic RNA delivery?



EV-mediated RNA delivery: cargo loading



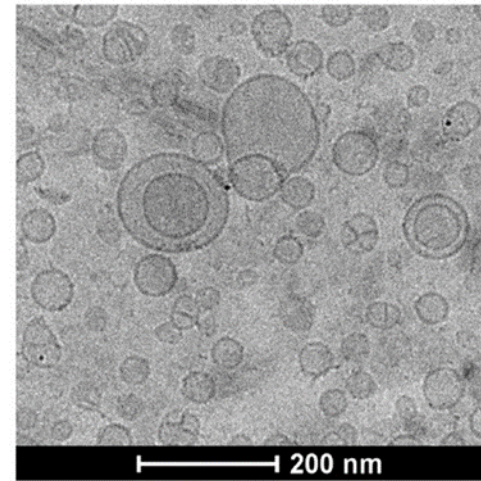
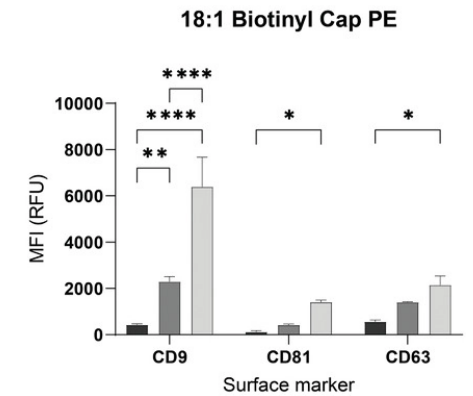
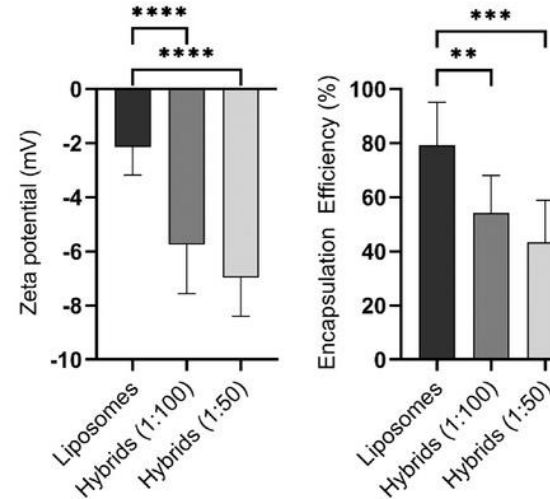
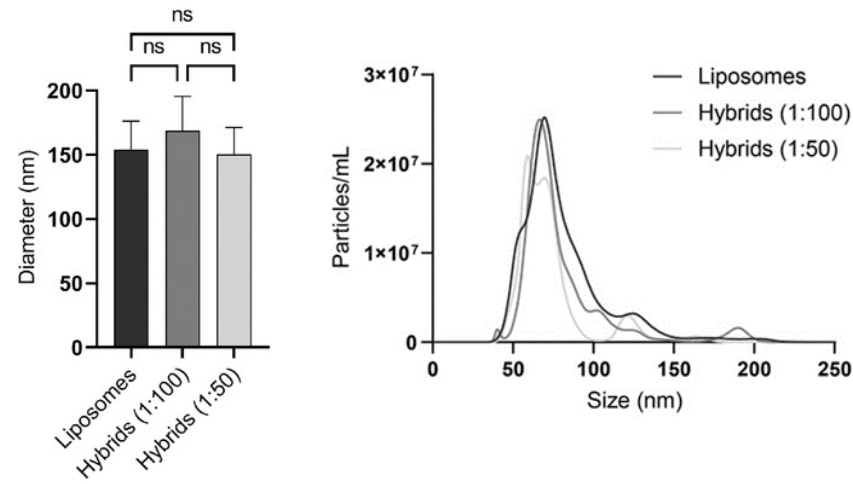
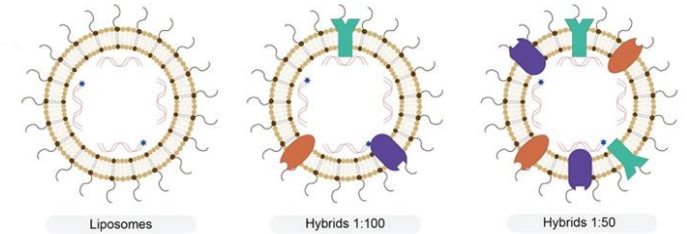
EV-liposome hybrids



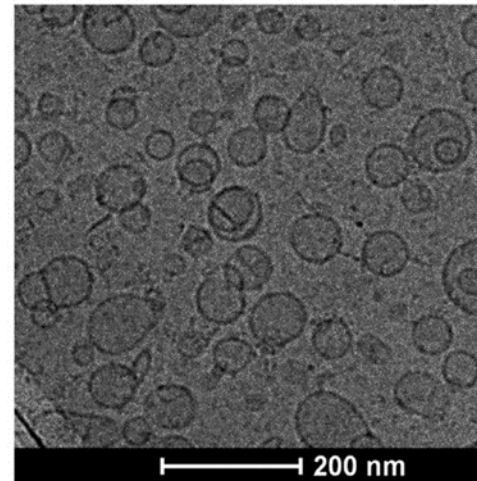
Can we combine biological (EV) and synthetic (liposome) properties into a hybrid structure?



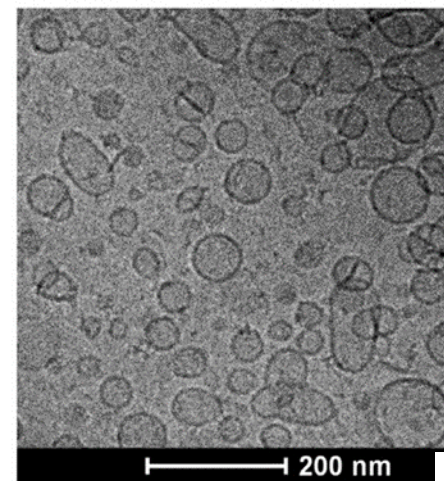
Characterization of hybrids



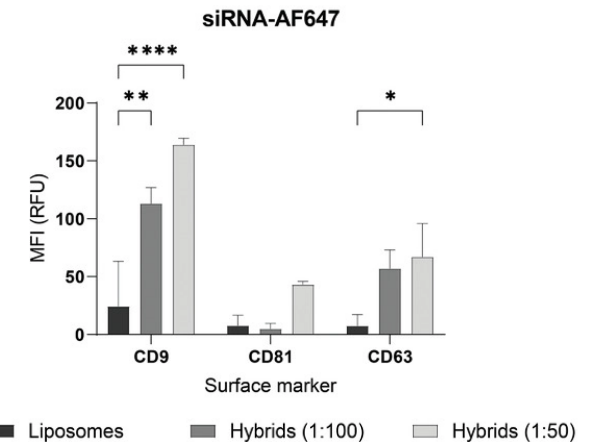
Liposomes



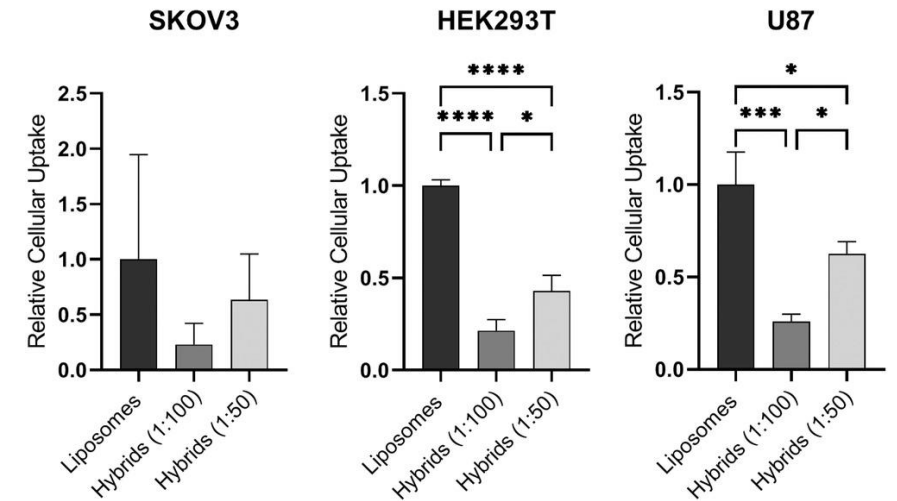
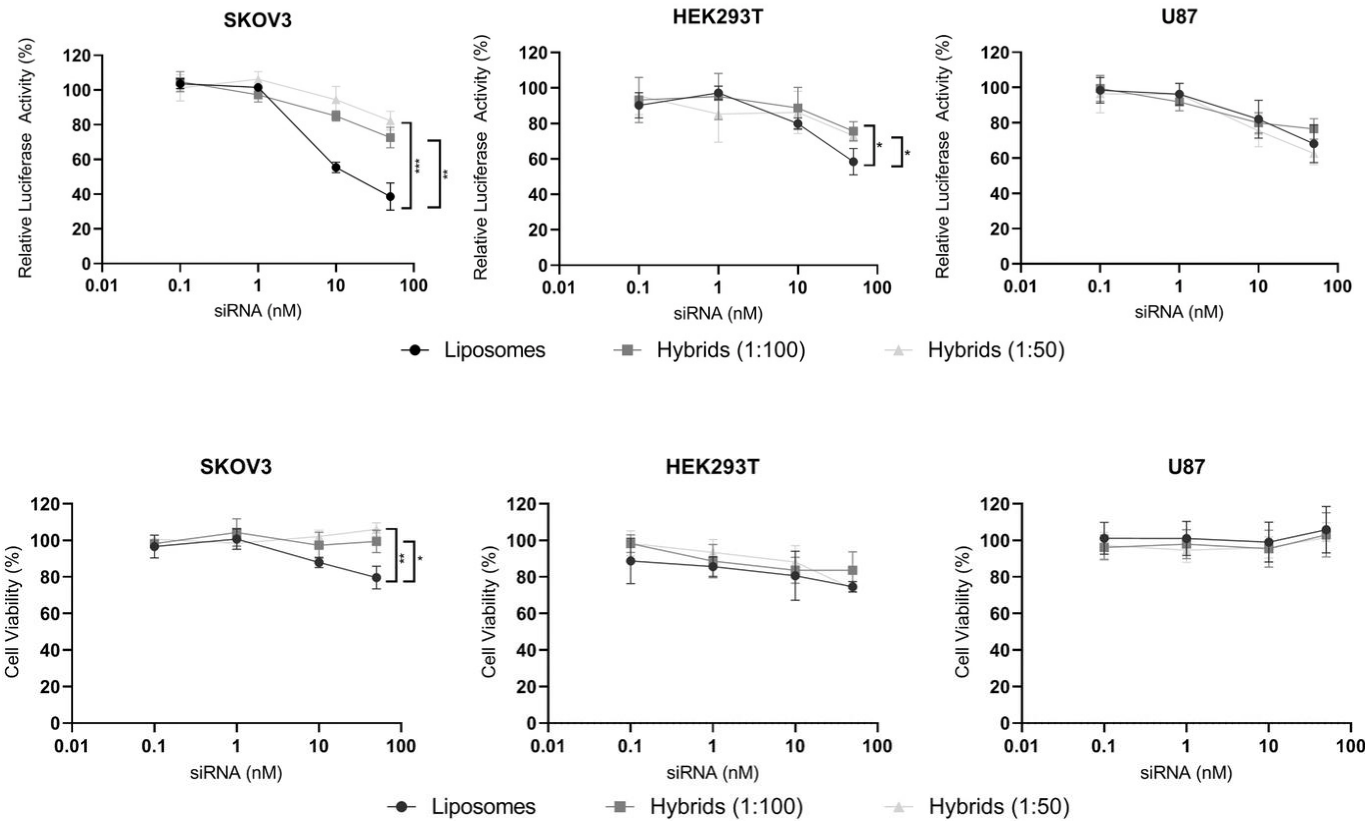
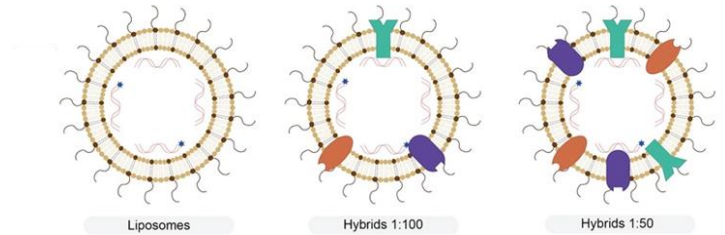
Hybrids (1:100)



Hybrids (1:50)



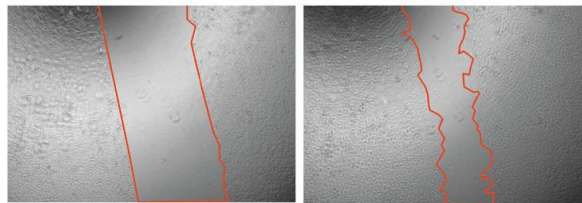
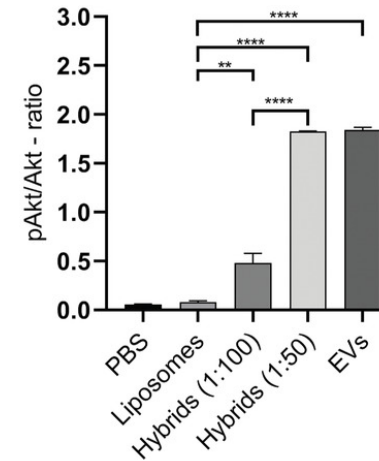
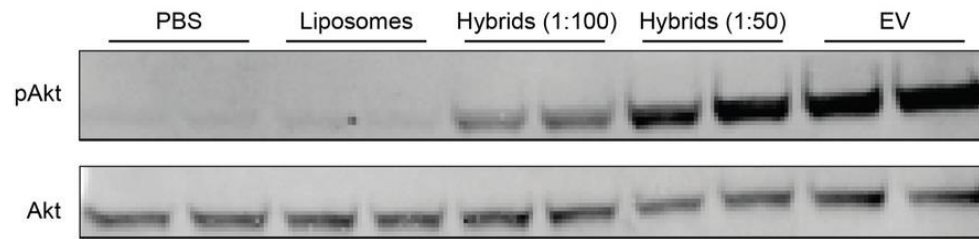
Functional evaluation of hybrids



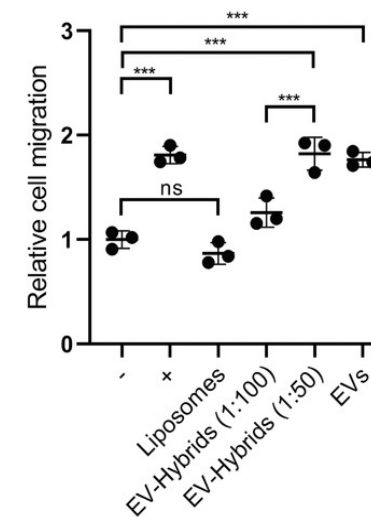
Hybrid targeting/uptake is mediated by EV components



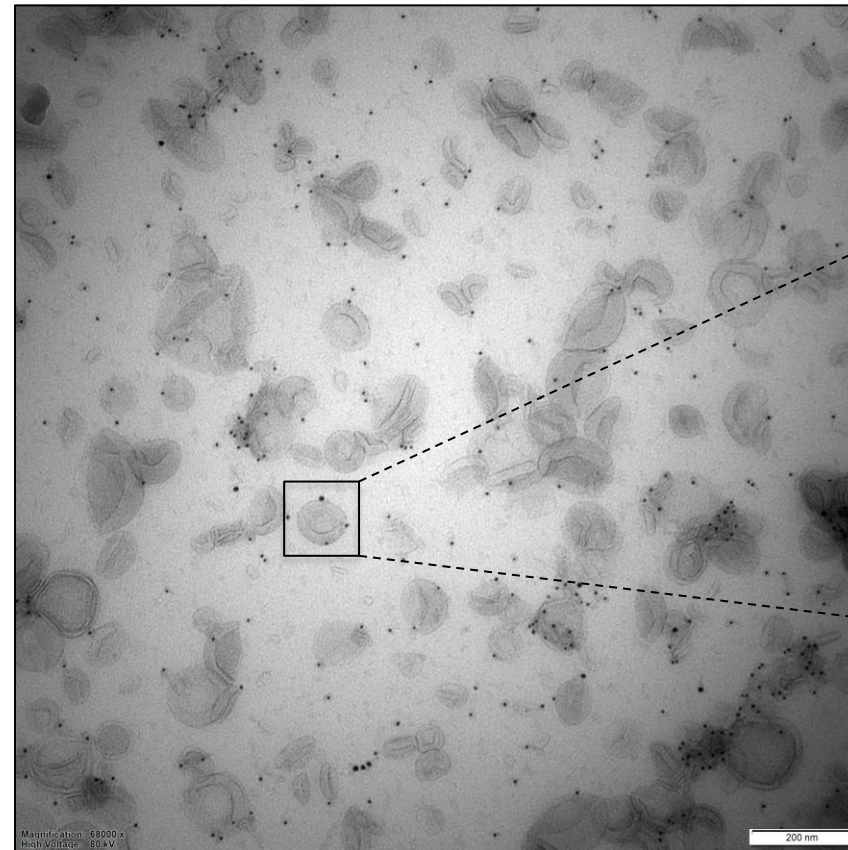
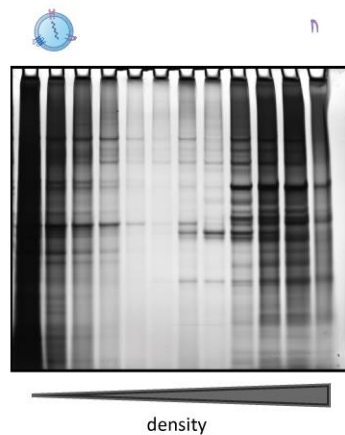
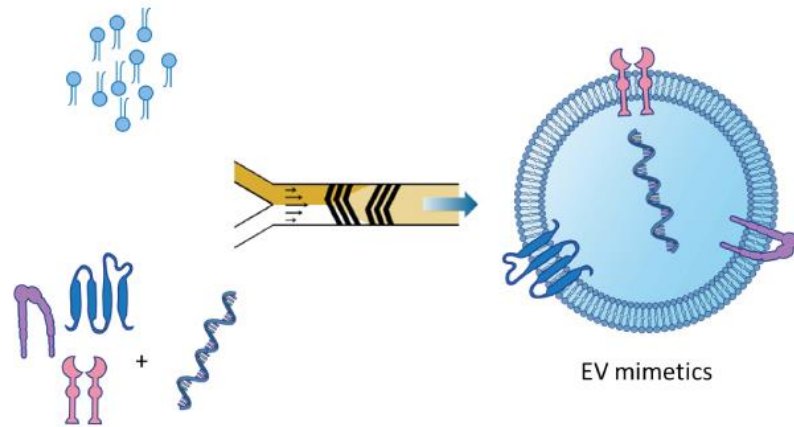
Hybrids retain regenerative properties



Endothelial cell migration



Towards scalable hybrid production....



Scale bar: 200 nm
5 nm: Alexa-NHS
10 nm: Biotin



Conclusions/perspectives

- EVs can outperform synthetic carriers in terms of delivery efficiency
 - EV-based hybrids or mimetics may be employed to delivery therapeutic RNA and/or CRISPR/Cas RNPs
-
- Improve therapeutic RNA loading of EVs
 - Improve synthetic delivery systems through EV-inspired engineering



Acknowledgements

UMC Utrecht, The Netherlands

- **Omnia Elsharkasy**
- **Dr. Olivier de Jong**
- **Dr. Dan Murphy**
- **Dr. Martijn Evers**
- Dr. Maria Laura Tognoli
- Naomie Zhou
- Jerney Gitz-Francois
- Cor Seinen
- Willemijn de Voogt
- Luuk van de Schepop
- Diego Aguilar Rodriquez
- Dr. Sander Kooijmans
- Dr. Tom Driedonks
- Dr. Marieke Roefs
- Dr. Simon van de Wakker
- Prof. Raymond Schiffelers
- Prof. Joost Sluijter

Université de Paris, INSERM, France

- Dr. Grégory Lavieu

Paracelsus Medical University, Austria

- Prof. Dirk Strunk
- Dr. Martin Wolf

Karolinska Institute, Sweden

- Prof. Samir El Andaloussi

University of Oxford, UK

- Prof. Matthew Wood



25-26 November Utrecht

Leeuwenbergh
Servaasbolwerk 1a
3512 NK Utrecht

Abstract submission: 1st Sept – 11th Oct

Registration: 1st Sept – 24th Oct

NLSEV 2024

Netherlands Society for Extracellular Vesicles

Keynote speakers



Janis Müller

Institute of Virology
Marburg, Germany



Maria Yáñez-Mó

Universidad Autónoma
de Madrid, Spain



Raymond Schiffelers

Central Diagnostic Laboratory,
UMC Utrecht

More info: nlsev.nl
loc@nlsev.nl

