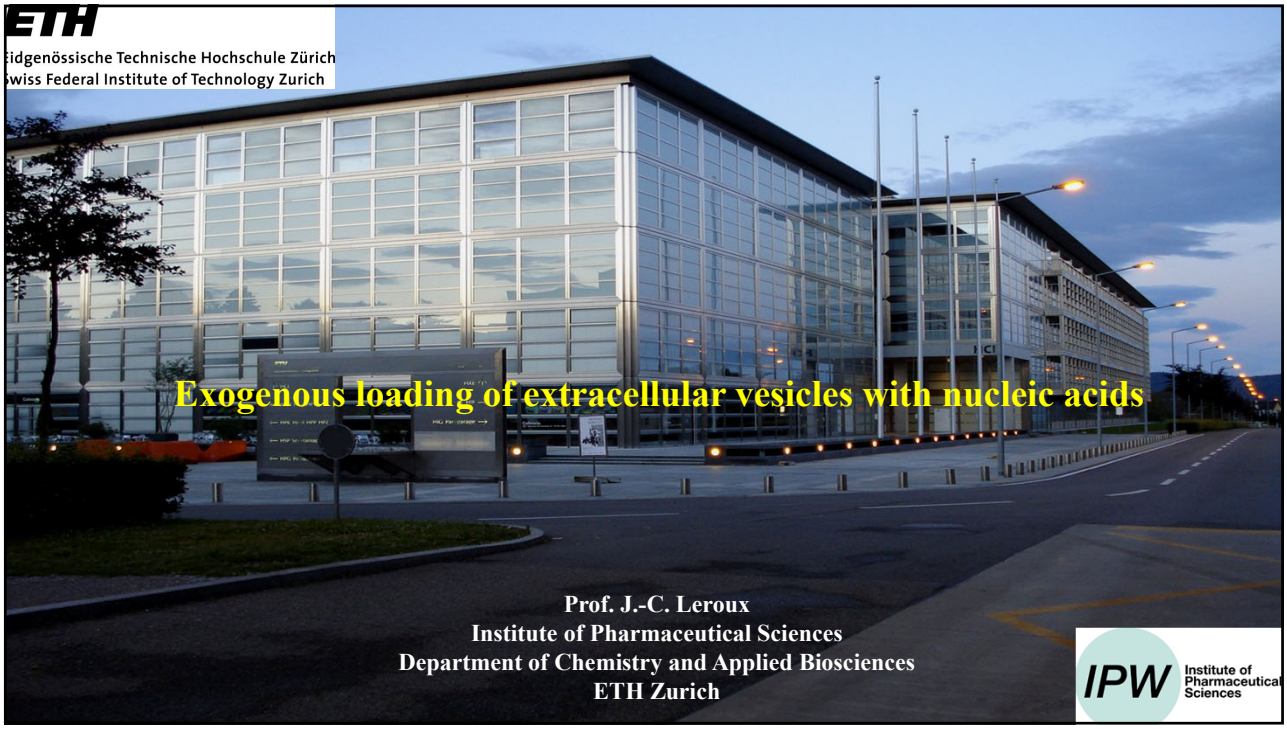
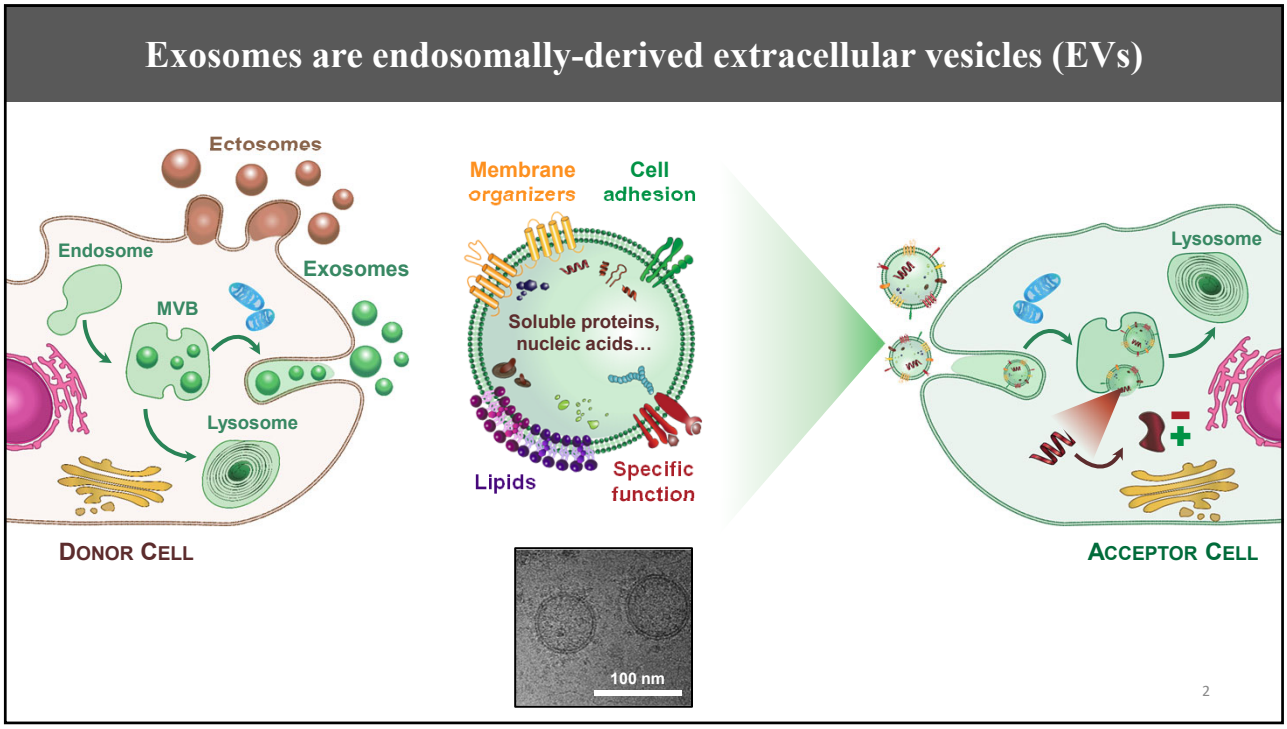

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich
Exogenous loading of extracellular vesicles with nucleic acids
Prof. J.-C. Leroux
Institute of Pharmaceutical Sciences
Department of Chemistry and Applied Biosciences
ETH Zurich
IPW
Institute of Pharmaceutical Sciences

1


Exosomes are endosomally-derived extracellular vesicles (EVs)
Ectosomes
Endosome
Exosomes
MVB
Lysosome
DONOR CELL
Membrane organizers
Cell adhesion
Soluble proteins, nucleic acids…
Lipids
Specific function
100 nm
Lysosome
ACCEPTOR CELL
2

2

EVs are key drivers for bioeffects of mesenchymal stem cells

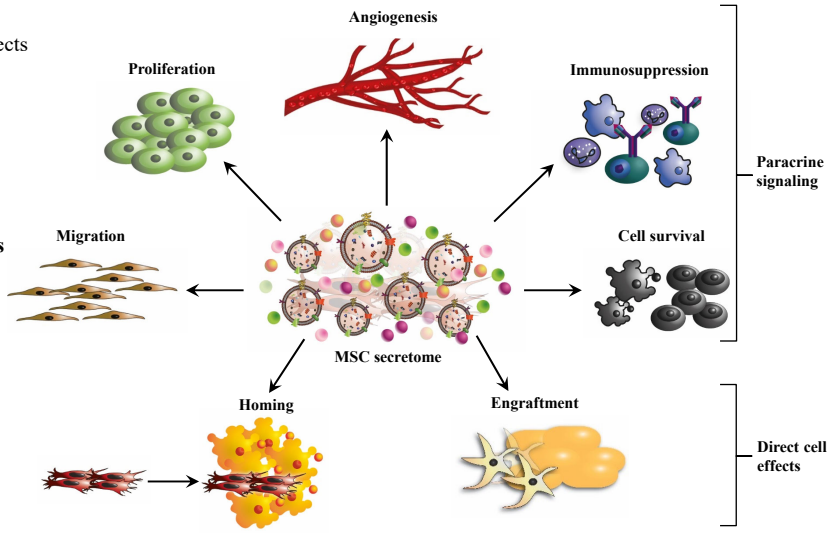
Mesenchymal (MSC) stem cells

Multipotent cells with various biological effects

Mechanism of action

- Direct cell effects
- Paracrine signaling

→ **Effects mediated partially *via* EVs**

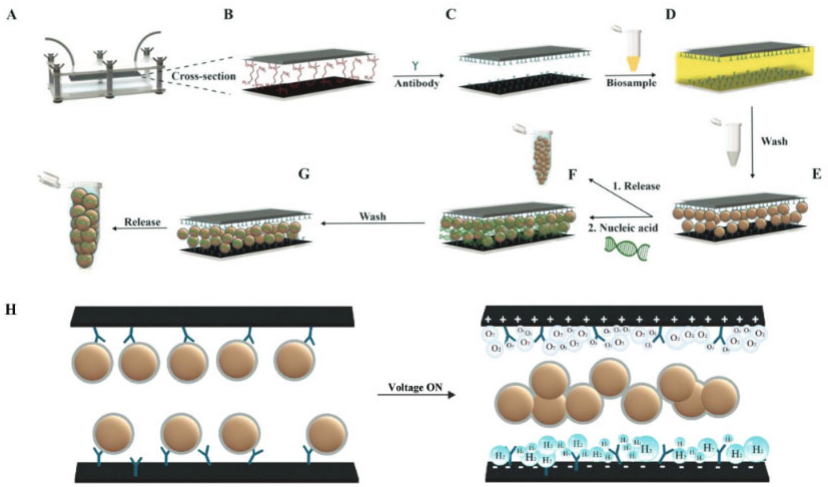


Roefs, M. T., *Trends Cell Biol.* 2020.
Rani, S., *Adv Mater.* 2016.

3

3

Production and isolation

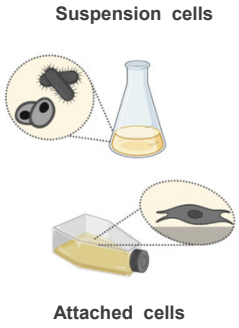


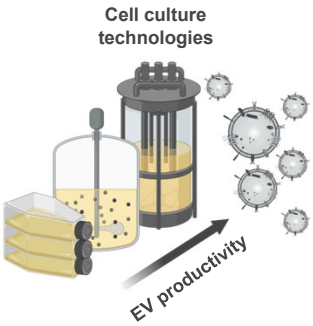
Krivitsky V *et al.* **35** *Adv Mater* (2023) 2212000.

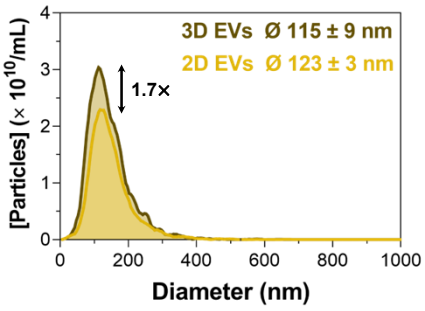
4

4

Production and isolation







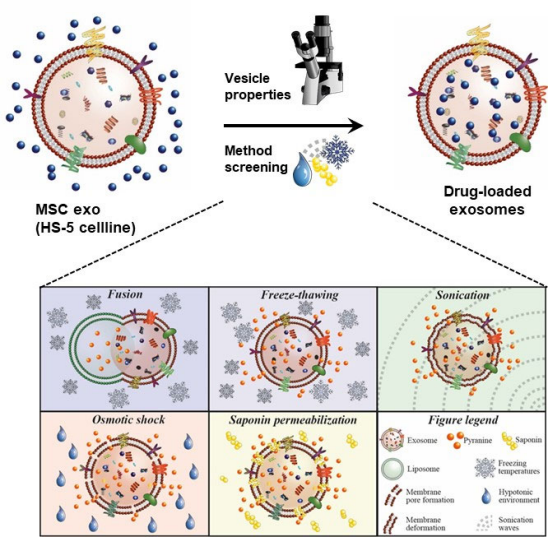
Bader J et al. *Eur J Pharm Biopharm* **182** (2023) 103-114.

5

5

Loading experiments

- Screening of loading techniques for hydrophilic compounds
- Exploring effects of the loading techniques on structural properties and bioactivity of EVs

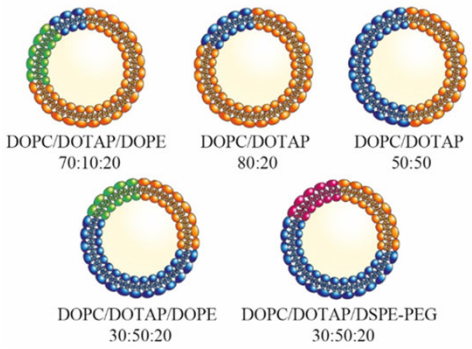


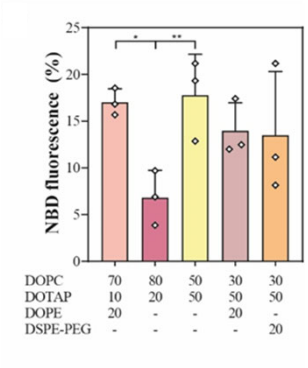
Hettich B *et al.* **11** *Adv Healthc Mater* (2021) 2100047.

6

6

hMSC EVs fuse with cationic liposomes



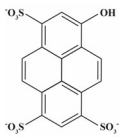


DOPE improved the fusion of positively charged liposomes with EVs independent of the DOTAP content

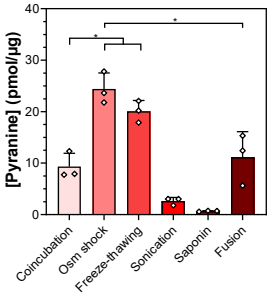
7

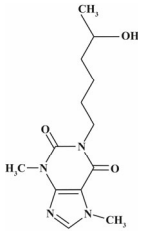
7

Hydrophilic probes can be efficiently loaded in EVs

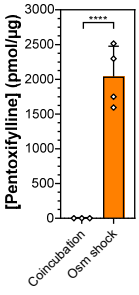


Pyranine
(524.4 g/mol)





Pentoxifylline
(278.3 g/mol)



→ Osmotic shock and freeze-thawing: highest pyranine loading

→ Osmotic shock encapsulates pentoxifylline

8

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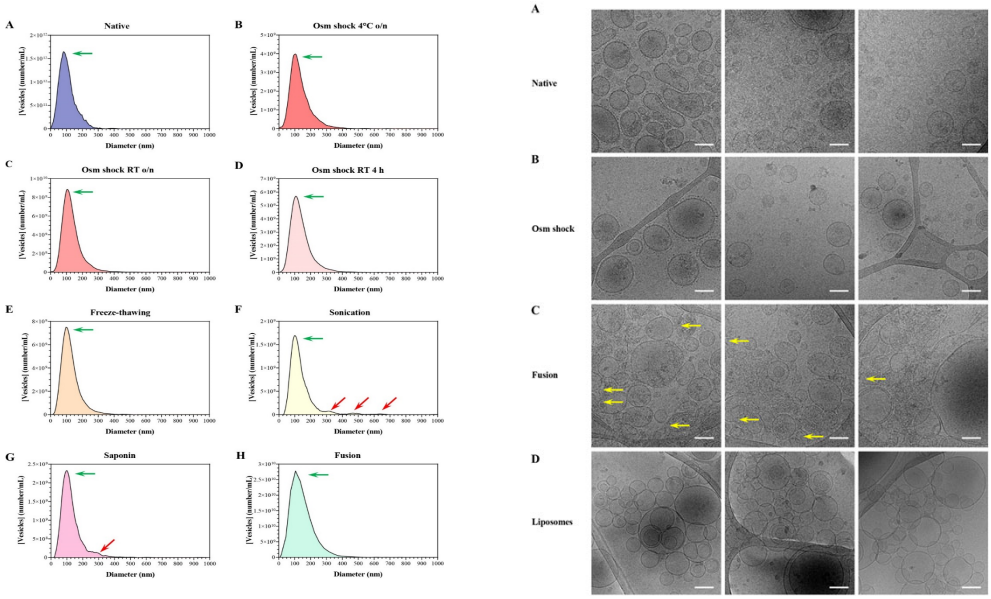
Size distribution



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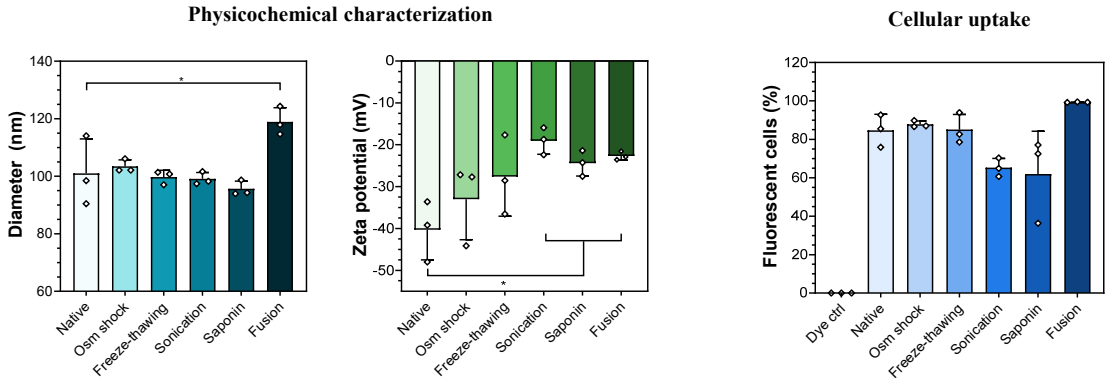
No apparent major effects on EVs characteristics



→ Physicochemical properties unaltered after osmotic shock and freeze-thawing

→ Cellular uptake of EVs shows no clear effects

10

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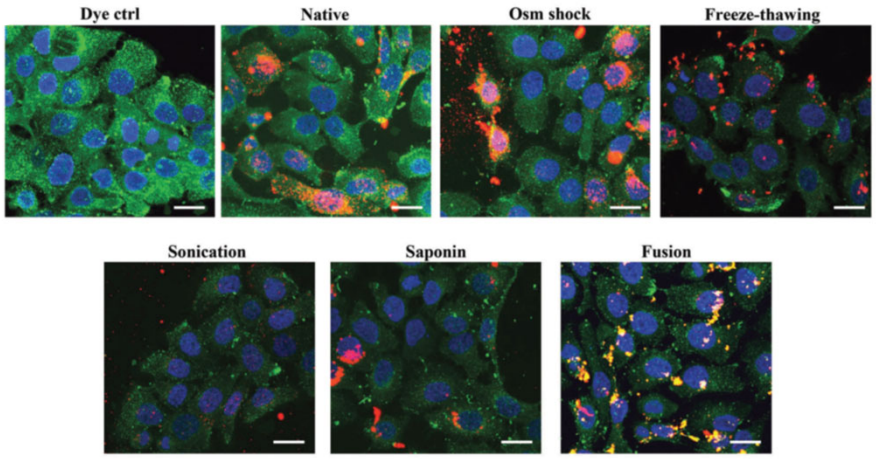
Cellular uptake



DyLight 680/ CellMask Green/ Hoechst 33342

11

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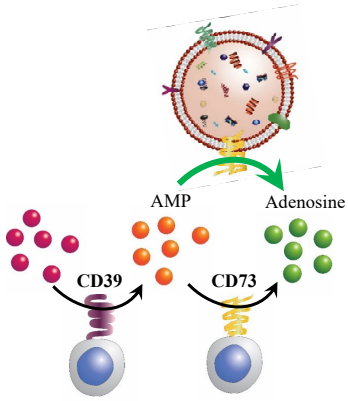
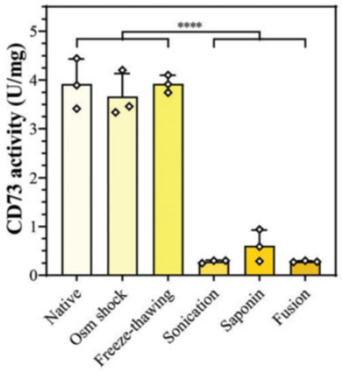
Biofunctions and membrane integrity of exosomes post-loading

Loading process can impair the EVs' inherent bioactivity





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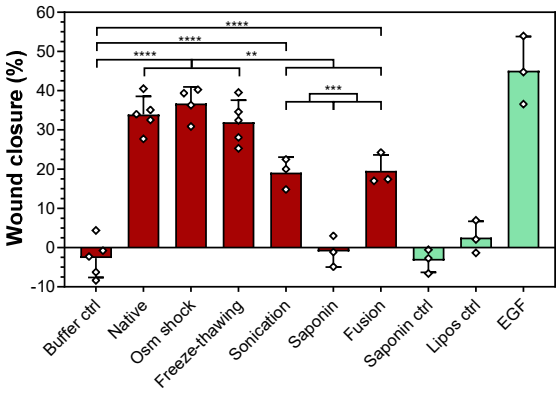
Biofunctions of exosomes post-loading

Wound closure *in vitro* (keratinocytes)



Osmotic shock preserves does not impair wound closure *in vitro*

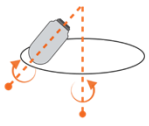
13

13

Loading of nucleic acids

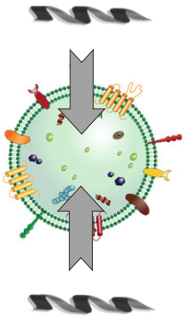
Sonication

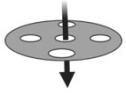
Lamichhane et al. *Cell Mol Bioeng.* 9, 315-325 (2016)



Dual asymmetric centrifugation

Roerig et al. *Small Methods* 6, e2201001 (2022)





Extrusion

Grossen et al. *Eur J Pharm Biopharm* 158, 198-1210 (2021)

Electroporation

Alvarez-Erviti et al. *Nat. Biotechnol.* 29, 341-345 (2011)

14

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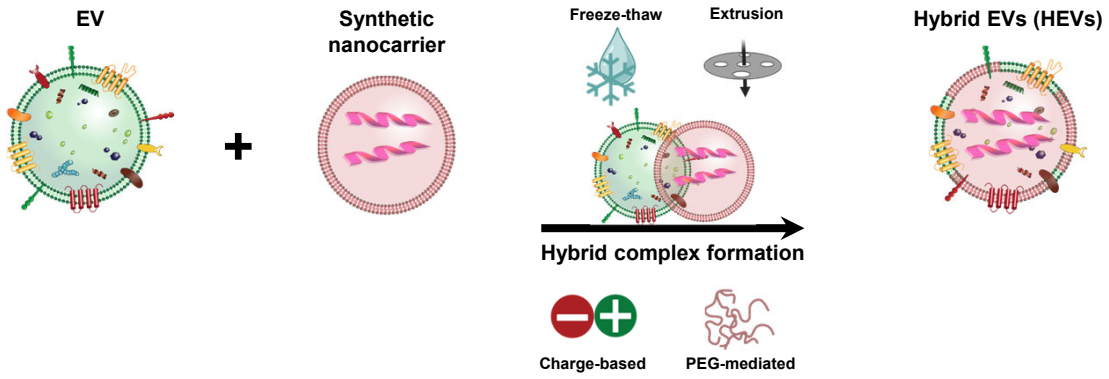
Fusion of EVs with preloaded lipid nanoparticles



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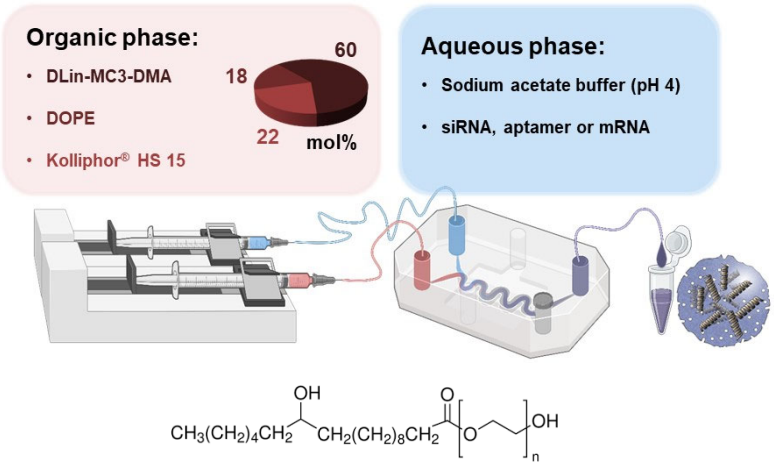
Manufacturing of lipid nanoparticles



Bader J *et al. bioRxiv* 2024; DOI: 10.1101/2024.04.10.588678.

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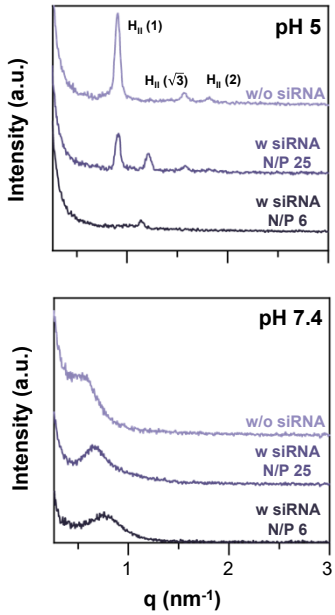
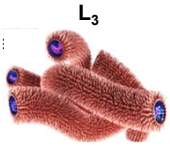
Structure of lipid nanoparticles

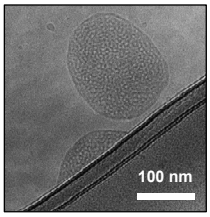


Inverted hexagonal H_{II}

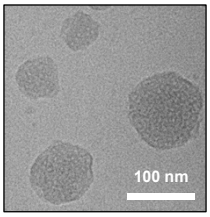
Sponge L_3



LCNPs (w siRNA) **pH 5**



LCNPs (w siRNA) **pH 7.4**



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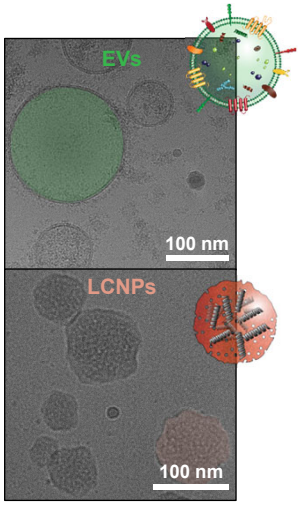
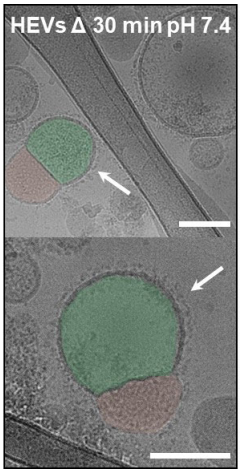
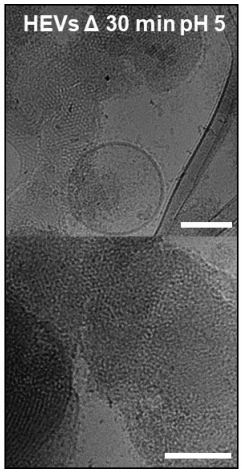
Characterization of hybridization process, formation of HEVs







LCNPs hybridize with EVs at pH 7.4 but aggregate at pH 5

18

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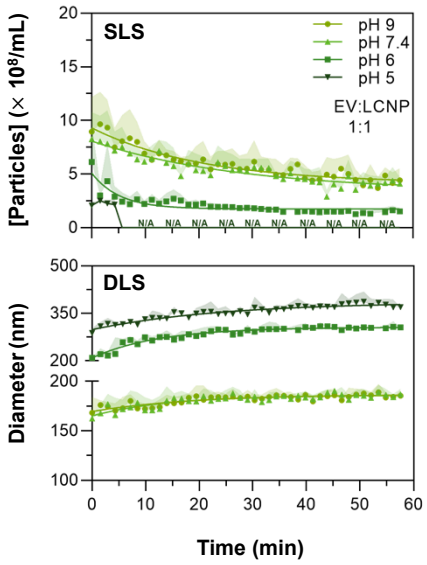
Hybridization kinetics



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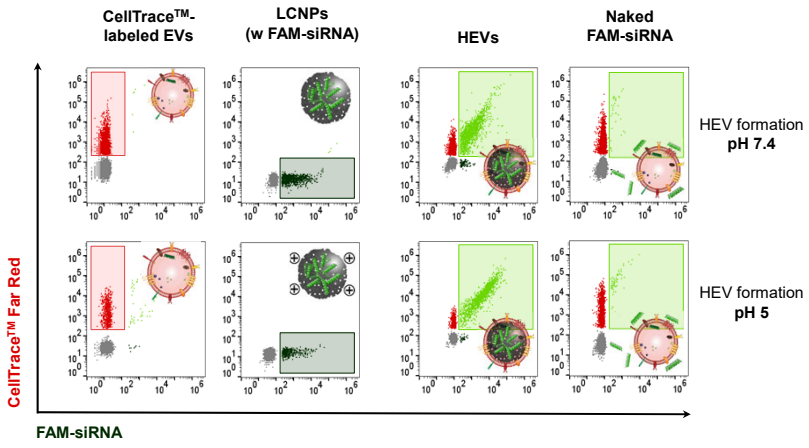
Characterization of hybridization process



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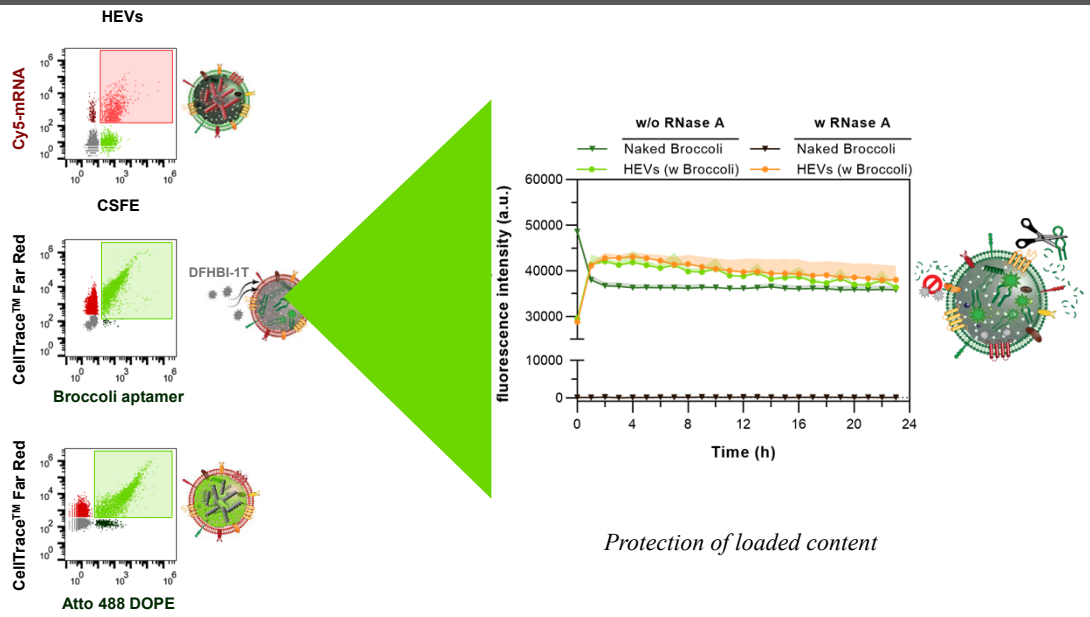
Characterization of hybridization process



Protection of loaded content

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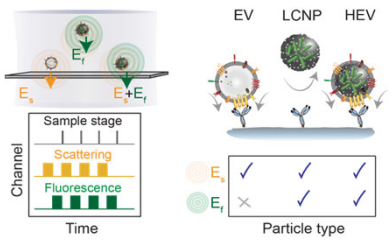
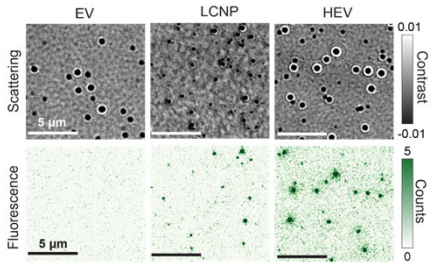
Characterization of hybridization process





Dr. J. Ortega Arroyo &
P. Rüedi
ETH Zurich
Quidant Group

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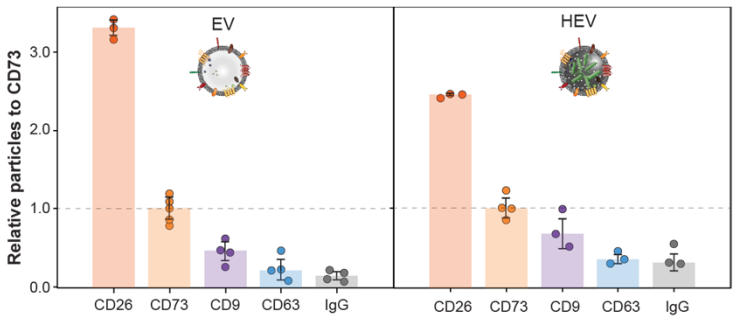
Characterization of hybridization process



EV membrane proteins are located on HEV surfaces

23

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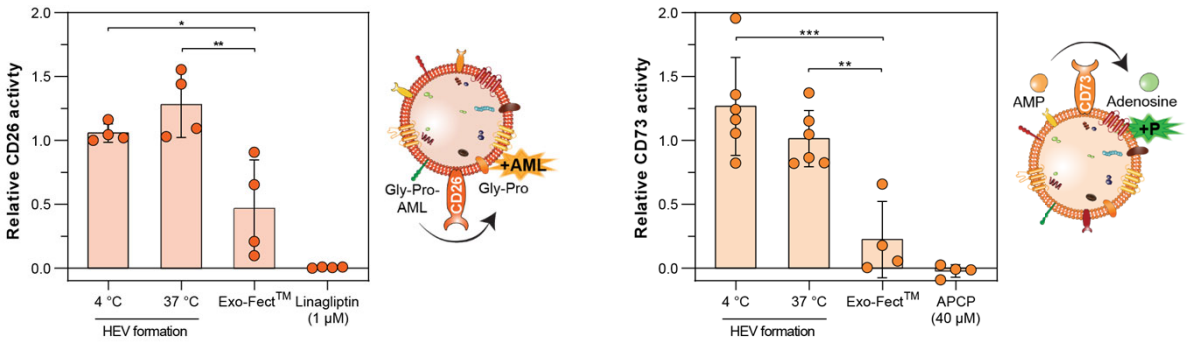
Characterization of hybridization process



EV membrane proteins remain functional

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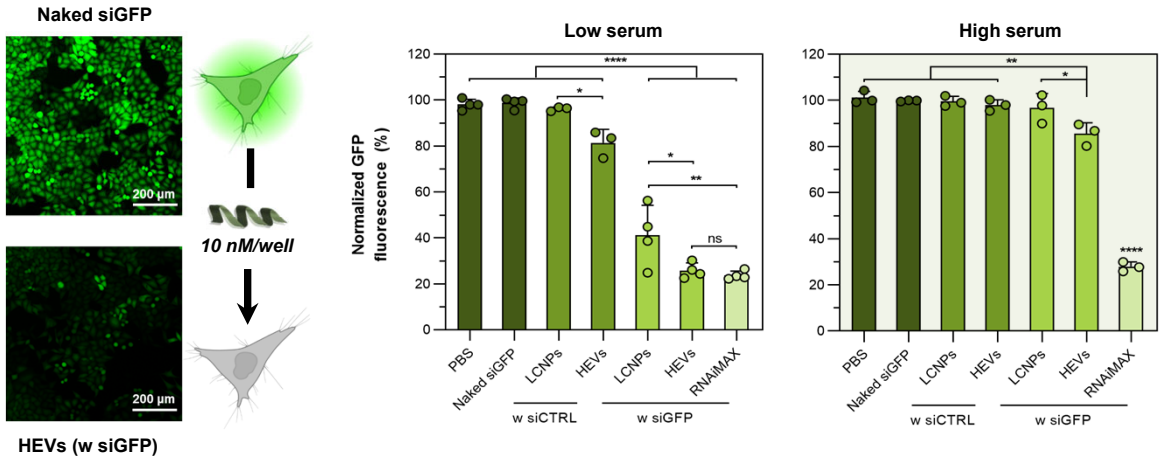
Transfection efficacy - siRNA



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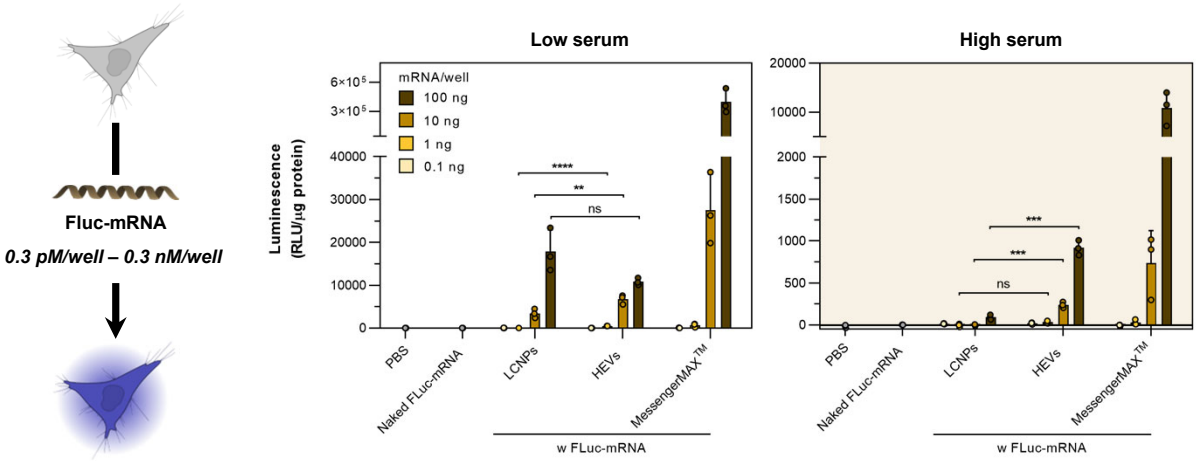
Transfection efficacy - mRNA



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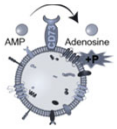
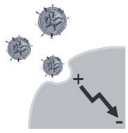
Conclusions

- Sponge-like LCNPs fuse with EVs and form HEVs
- EV membrane proteins are located on HEV surfaces and stay intact
- HEV formation enhance the transfection potency of LCNPs.





Open questions

→ Fusion and cell uptake mechanisms
→ *In vivo* biodistribution

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Acknowledgements

Collaborators
Prof. Dr. S. Werner (ETH)
Dr. M. Ben-Yehuda Greenwald
Dr. J. Ortega Arroyo
Prof. Dr. P. Rüedi
Prof. Dr. P. Arosio

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Phospholipid Research Center

ETH research grant

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