Extracellular vesicles subpopulations yield different DNA encapsulation efficiencies

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Poster #252

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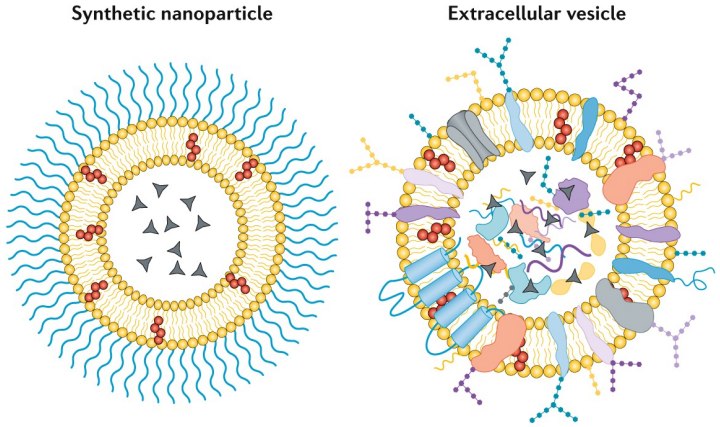
INTEGRATING
Delivery Science
ACROSS DISCIPLINES

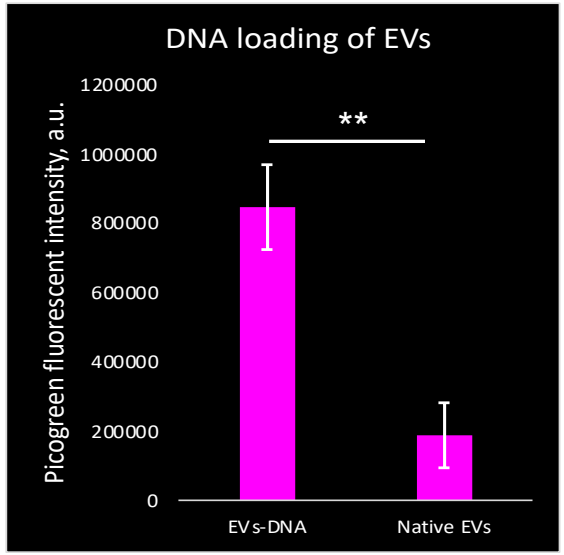
Extracellular vesicles (EVs) for gene delivery

- EVs are “body’s liposomes”
- Natural nucleic acid carriers
- Very promising for gene delivery
- Poor control over DNA loading in EVs

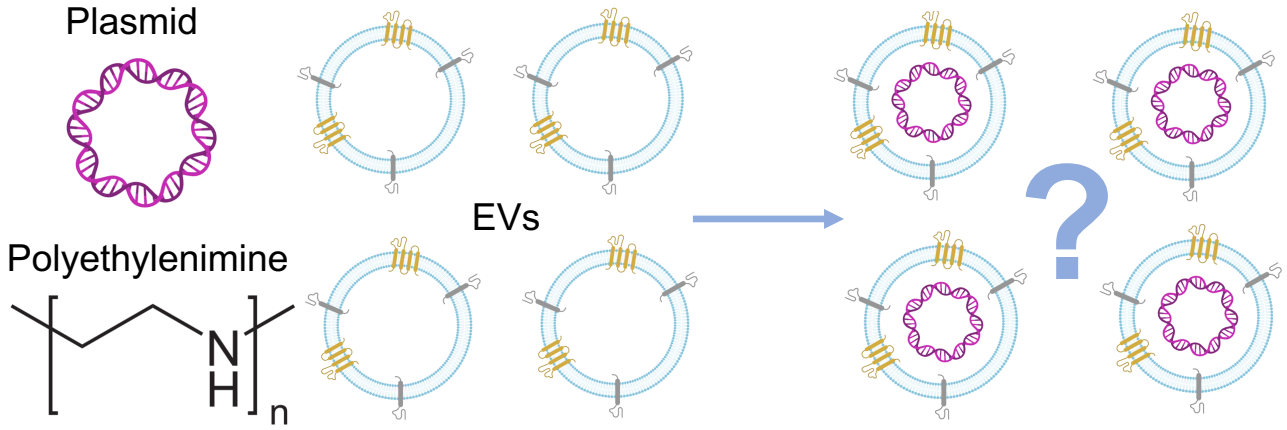


Complexity	++	+++
Variability	++	+++
Manufacturing expertise	++	+
Safety	++	++
Circulation time	+++	++
Multifunctionality	++	+++

Witwer, K. W., & Wolfram, J. (2021). Extracellular vesicles versus synthetic nanoparticles for drug delivery. *Nature Reviews Materials*, *6*(2), 103-106.



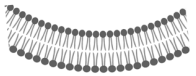
- DNA loading is usually measured at the macro scale
- What happens at the nano scale?



Materials & Methods

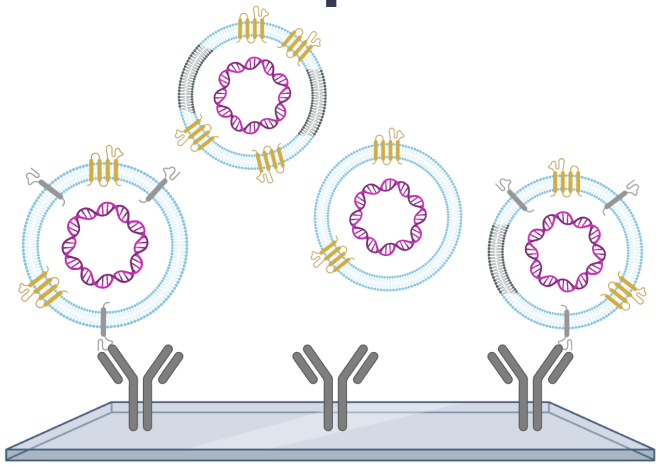
GYPA, canonical marker of the EVs' parent cells

CD9, protein commonly found on EVs

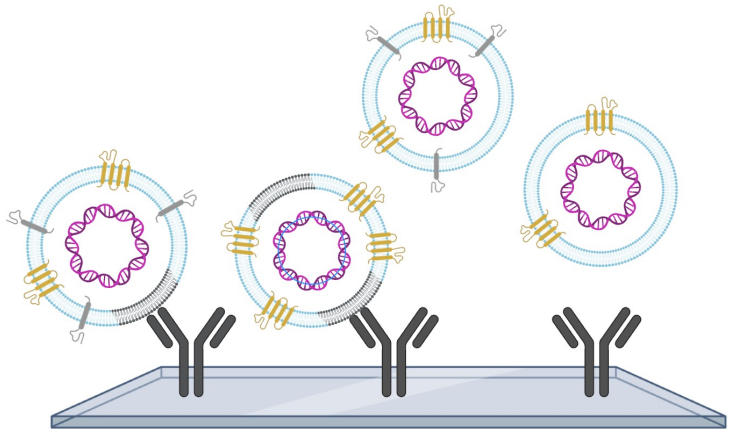


Phosphatidylserine (PS), lipid commonly found on EVs

1. Capture

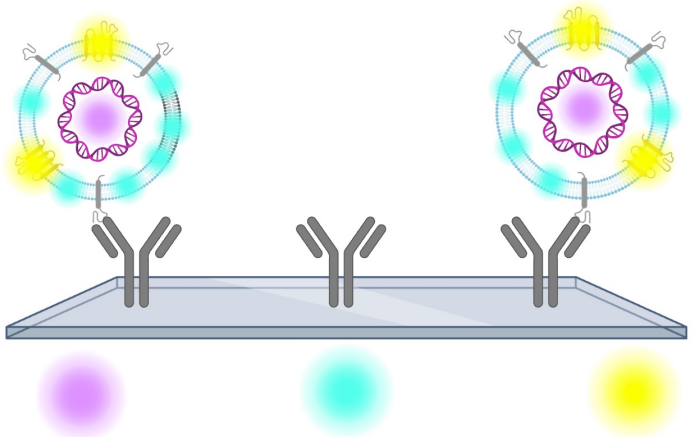


GYPA+ EV capture

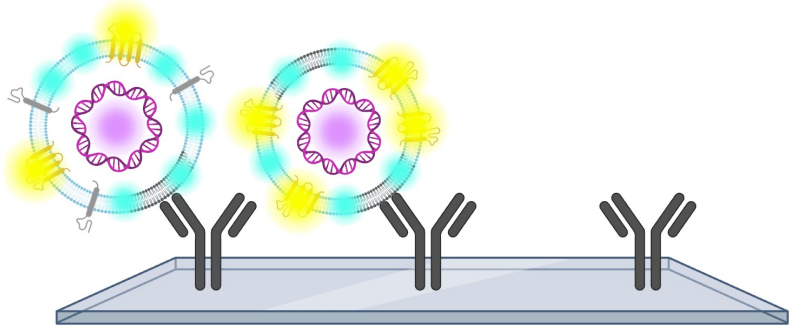


PS+ EV capture

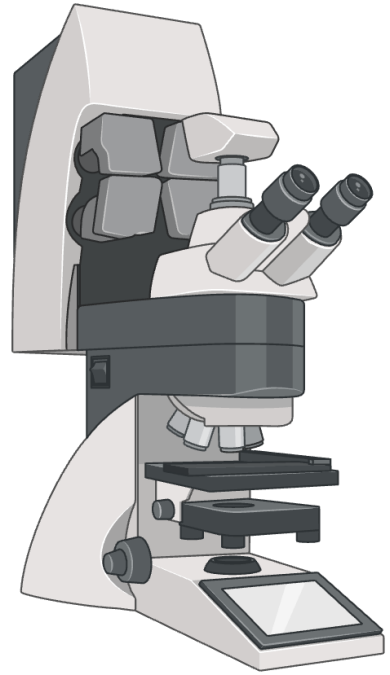
2. Staining



Cy5 plasmid Cy3 membrane CF488 CD9



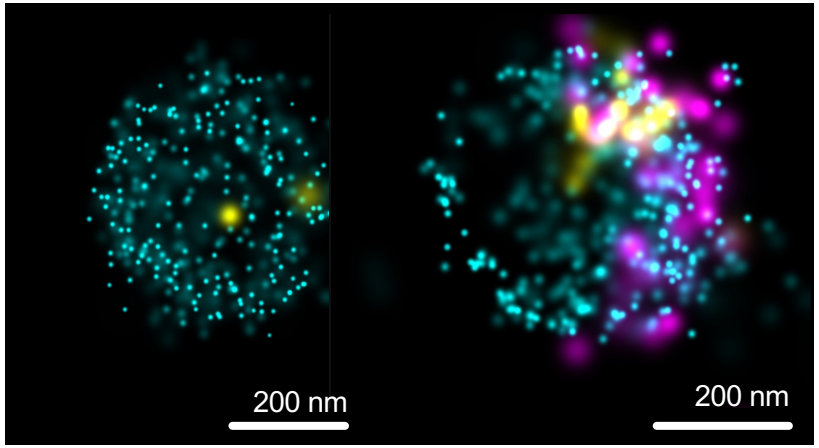
3. dSTORM Imaging



20 nm resolution

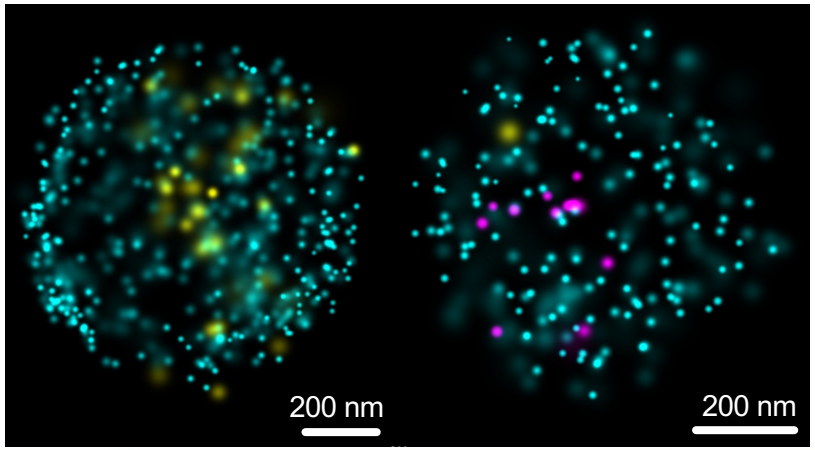
Observation 1: Inter- and intra-group heterogeneity

GYPA+ EV, no DNA

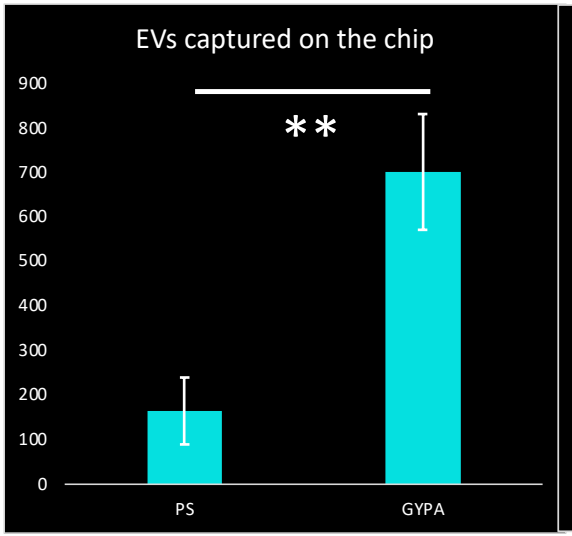


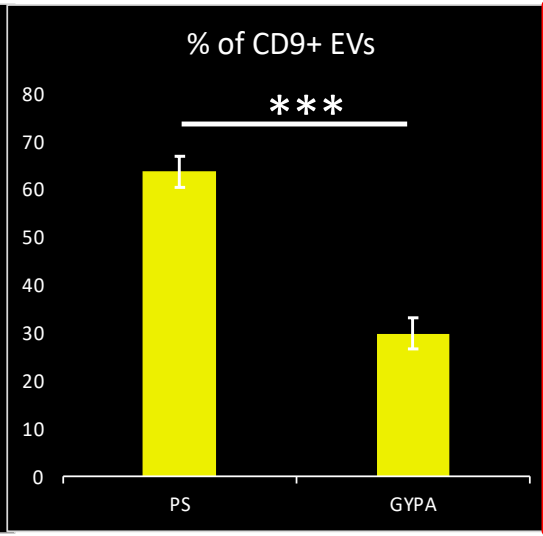
GYPA+ EV, DNA

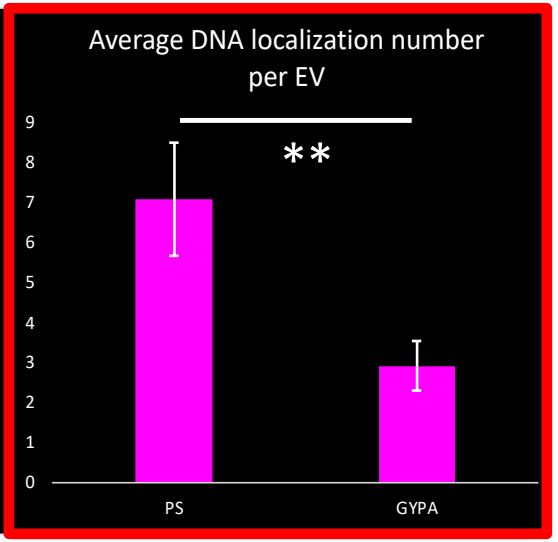
PS+ EV, no DNA



PS+ EV, DNA



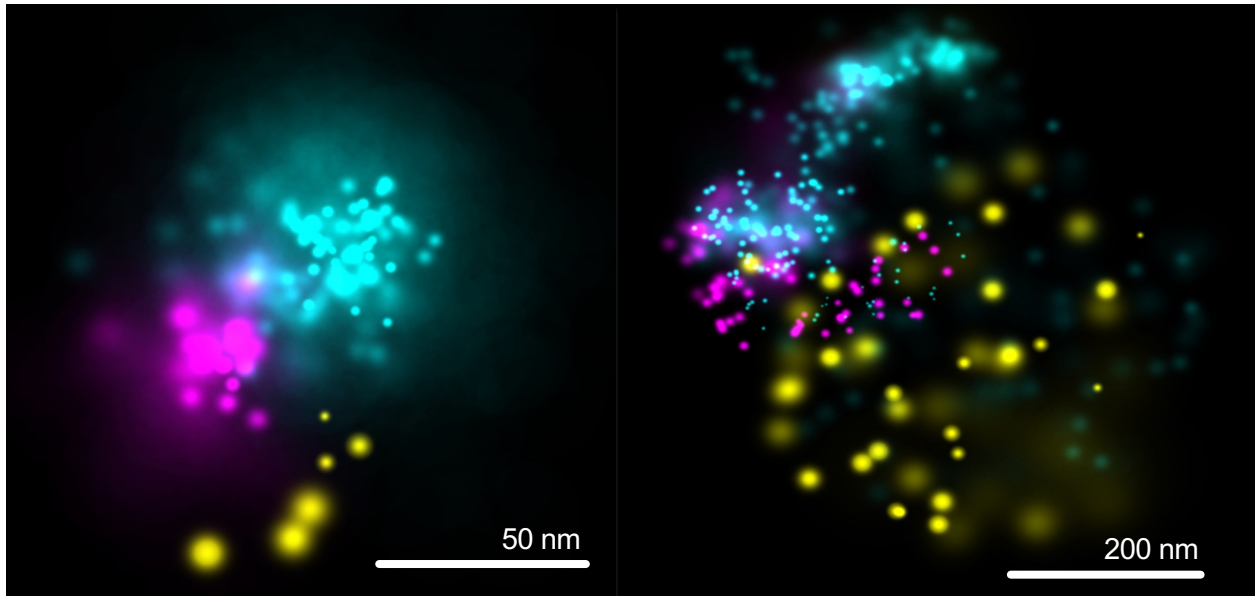


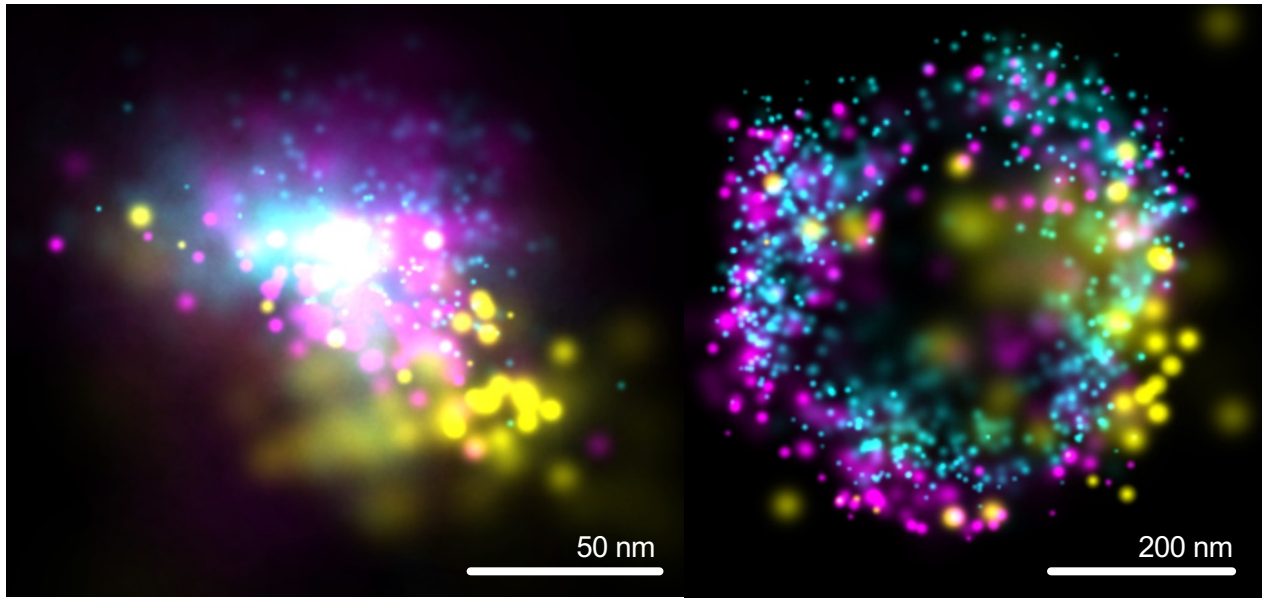


- Only half of EVs are loaded with DNA
- PS+ EVs are less abundant than GYPA+ EVs
- PS+ EVs exhibit CD9 twice as frequently as GYPA+ EVs
- **PS+ EVs have 2.4 times more DNA localizations than GYPA+ EVs**

… But why?

Observation 2: two EV-DNA interaction modes





- ”Sticky mode”, local patch of DNA on the EV surface
- “Mixed mode”, DNA localizations are found all over the EV, potentially inside

Conclusion

- Only half of EVs interact with DNA upon loading
- The PS+ EV subset is scarcer but displays enhanced DNA loading
- Two EV-DNA interaction modes were identified

- As a genetic lysosomal storage disorder, Gaucher disease could be an excellent application for PS+EV – mediated gene delivery

Acknowledgements

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Thank you! Grazie!
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