Synthesis of Hybrid Outer Membrane Vesicles for Drug Delivery through Cell Membranes

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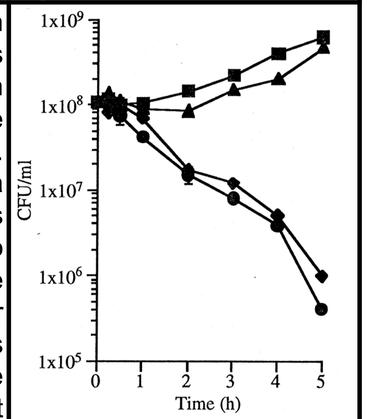
Motivation and Background

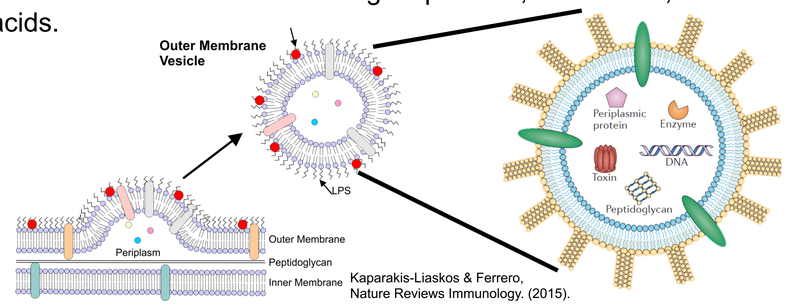
Bacteria evolve antibiotic resistance in several ways; relevant mechanisms include the modification of drug targets in the outer membrane and limiting uptake of antibiotic molecules into the periplasm. Using OMVs to deliver antibiotics via fusion to the cell membrane circumvents both mechanisms. We are interested to stabilize the OMVs at body temperature and prevent aggregation to improve their delivery capabilities; by extruding OMVs with synthetic liposomes (L-OMVs), we extend their lifespan and inhibit aggregation.



Kadurugamuwa, J. L. & Beveridge, T. J. J. Bacteriol. (1996).

Outer Membrane Vesicle (OMV) Biogenesis

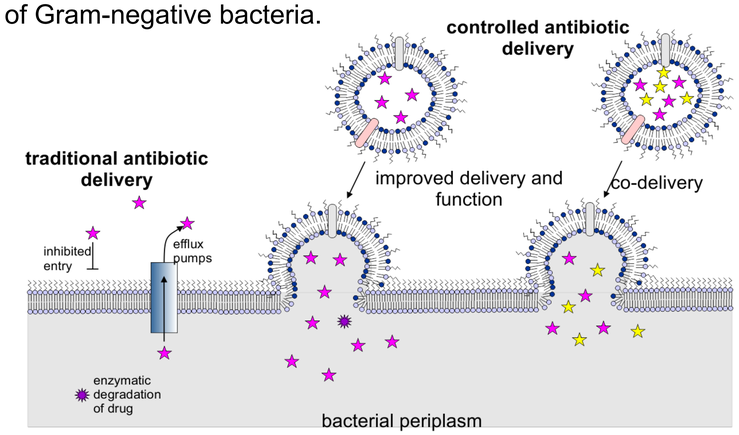
OMVs bleb from the membranes of Gram-negative bacteria and contain biomolecules such as signal proteins, endotoxins, and nucleic acids.



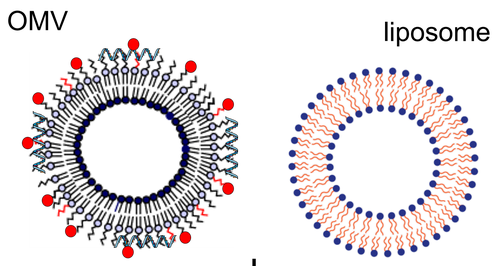
Kaparakis-Liaskos & Ferrero, Nature Reviews Immunology. (2015).

Delivery of Antibiotics through Cell Membranes

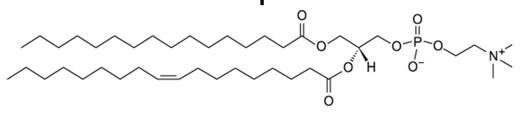
OMVs have been observed to deliver genes to Gram-negative bacterial cells, indicating their ability to transport biomolecules across the cell wall of Gram-negative bacteria.



OMV and Liposome Characteristics

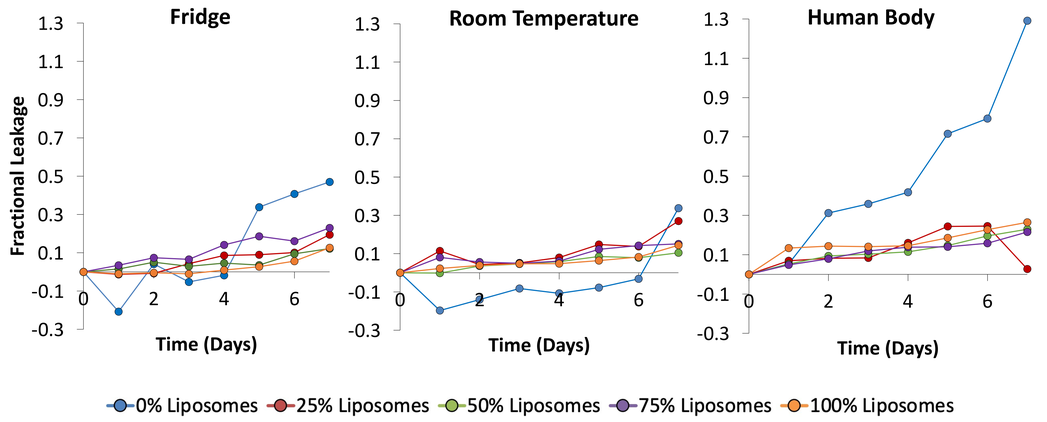


Pros	• Propensity for fusion to bacterial cells	• Blank canvas • Tunable properties • No aggregation
Cons	• Laden with periplasmic content • Unstable at body temperature • Aggregatory tendencies	• Synthetic; low rate of fusion

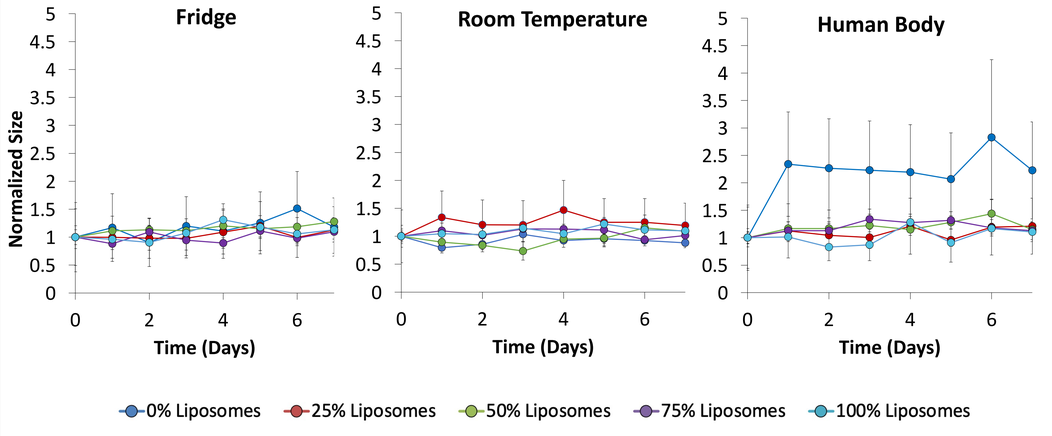


1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)

Temperature-Dependent Stability of L-OMVs



Results of Dynamic Light Scattering (DLS) Experiment



Conclusions

- **L-OMVs are stable at body temperature over timespans that destabilize antibiotic-laden OMVs.**
- **L-OMVs do not aggregate, which inhibits delivery of antibiotics via OMVs.**

Future Work

- Fine-tune liposome properties (protein composition, surface charge, fluidity) to enhance fusion capabilities of L-OMVs
- Monitor delivery of antibiotics to pathogenic *Pseudomonas aeruginosa* cells using fluorescent dye

Acknowledgements

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