Peptide-Based Surface Engineering of Biomimetic Liposomes for Biomedical Applications

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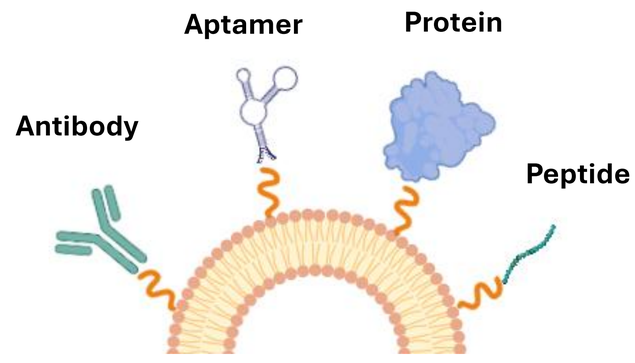
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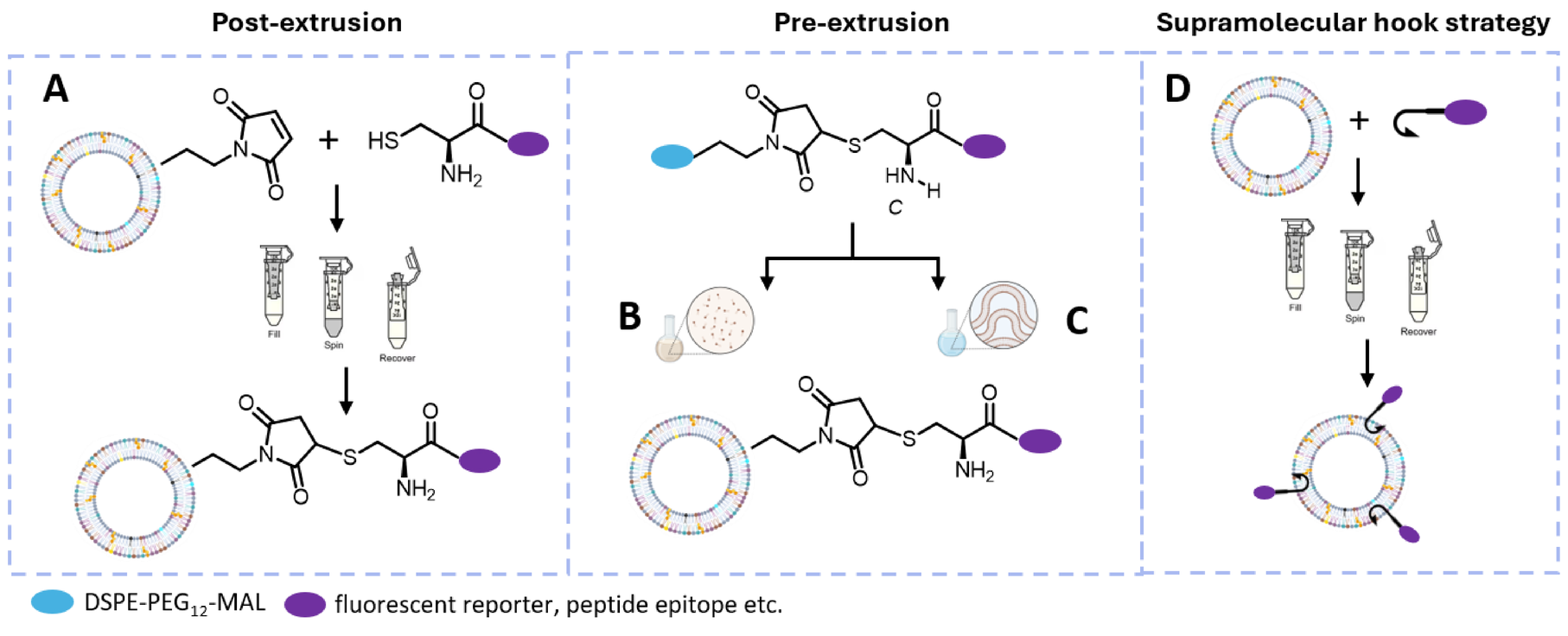
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Peptides for liposome surface functionalization

Efficient and selective targeting remains a major challenge in liposome-based therapies. Surface functionalization with peptides offers a versatile strategy to enhance receptor-mediated uptake, combining small size, low steric hindrance, chemical versatility, and precise control over sequence and surface density.

In this study, we investigated different peptide conjugation strategies for our biomimetic **Lipo-EV platform**, comparing **covalent maleimide–thiol coupling** and a non-covalent **"hook peptide" approach** enabling spontaneous membrane insertion. Different peptide-to-lipid ratios were evaluated in terms of **conjugation efficiency, stability, and peptide accessibility** using complementary analytical techniques. Peptide epitope exposure and functionality were further assessed by **peptide microarray immunoassays** and **fluorescence-based nanoparticle tracking analysis (NTA)**.



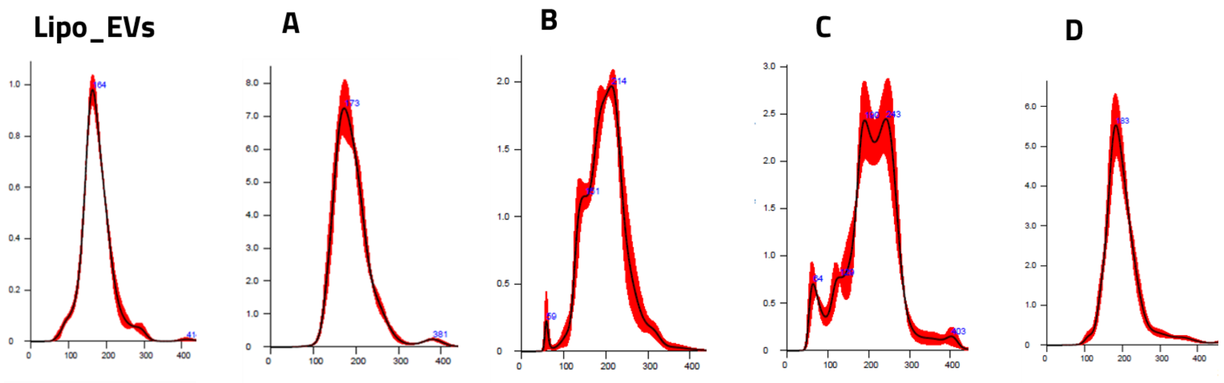


(A) Covalent peptide conjugation to maleimide-functionalized liposomes, followed by purification. **(B–C)** Peptide–lipid incorporation during liposome preparation by hydration and extrusion.

(D) Post-assembly insertion of hook peptides into preformed liposomes, followed by purification, enabling stable peptide insertion into the lipid bilayer.

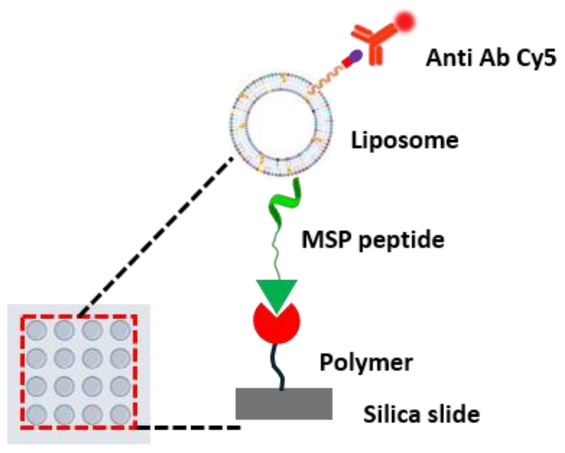
Assessing functionalization

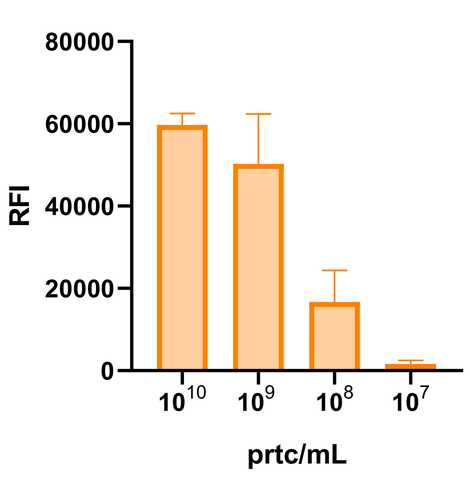
NTA



NTA analysis showed comparable particle size distributions for most functionalization strategies, while **Strategy C** displayed increased polydispersity, likely due to peptide–lipid micelle formation during hydration.

Microarray immunoassay

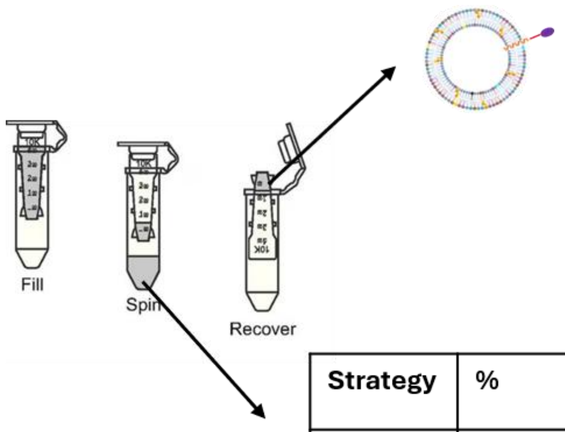




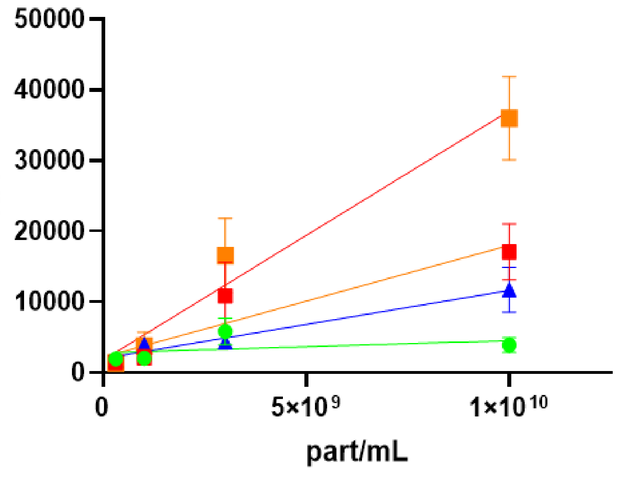
Epitope exposure was assessed using V5-tagged liposomes (5% peptide/lipid) captured on a silica MSP4 slides and detected by Cy5-labelled antibody. DiI Lipo_Evs were captured on MSP silica slide and detected through fluorescence to verify the capturing efficiency using this approach.

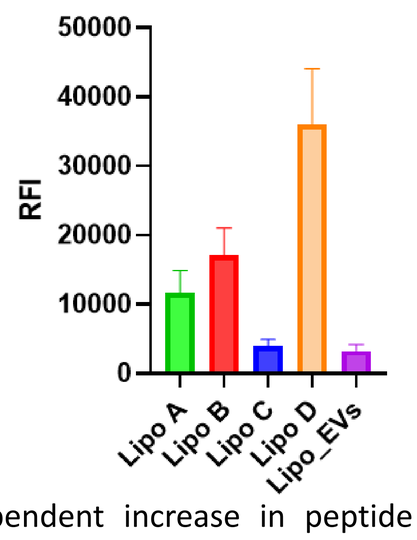
HPLC

HPLC was used to assess functionalization efficiency by monitoring peptide consumption. For Strategies A and B, Amicon wash fractions were analyzed, while for Strategies B and C, peptide–lipid conjugation was monitored directly.



Strategy	%
A	80%
B*	100%
C*	100%
D	65 %





Lipo A, B, and D showed a clear concentration-dependent increase in peptide functionalization, with Lipo D exhibiting the highest efficiency (~12-fold signal vs. unfunctionalized Lipo-EVs).

Conclusion

This study systematically compared different peptide functionalization strategies for EV-inspired liposomal nanoplatforms, highlighting the key role of functionalization approach in peptide display and accessibility. Strategy B emerged as the most effective conventional approach, providing efficient functionalization and favorable peptide exposure. Notably, Strategy D, based on a supramolecular hook peptide, offers greater versatility and modularity while avoiding extensive membrane chemical modification. Its simple, rapid, and adaptable design makes Strategy D particularly promising for future biomedical and clinical translation.

[1] Liu, Y., et al. (2021). **Nanoscale Horizons, 6**, 78–94. **[2]** Aronson, M. R., et al. (2021). **APL Bioengineering, 5**(1), 011501. **[3]** Sonju, J. N., et al. (2021). **Journal of Controlled Release, 329**, 624–644. **[4]** A. Gori, A. et al. **Journal of extracellular vesicles**, 2020, 9, 1751428.