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**Title:** Engineering Hybrid Extracellular Vesicles for mRNA delivery

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**Introduction:** Despite the potential of extracellular vesicles (EVs) as nucleic acid delivery vectors, achieving efficient mRNA loading in EVs and cytoplasmic release of EV-loaded cargo remain critical challenges. Here, the properties of lipid nanoparticles (LNPs) were explored to enhance mRNA loading and endosomal escape in human-induced pluripotent stem cell EVs (hiPSC-EVs) by generating EV-LNP hybrids (HEVs).

**Methods:** HEVs were formed through temperature and pH-controlled fusion of hiPSC-EVs and LNPs. To optimize HEV formation, various LNP lipid compositions were systematically screened. Hybrid formation efficiency was assessed at the single-particle level through nanoflow cytometry and Single Particle Automated Raman Analysis (SPARTA), while morphology was evaluated by Cryo-TEM. *In vitro* efficacy was tested using Cy5-labelled eGFP mRNA in a mCherry-Galectin 9 reporter cell line.

**Results:** Single-particle characterization revealed that up to 73.3% HEVs were formed with optimized LNPs, with diverse hybrid structures co-existing. Based on specific Raman spectral signatures, HEVs’ fingerprint was characterized by higher intensities in four Raman bands corresponding to cholesterol (605 cm⁻¹, 698 cm⁻¹), proteins (954 cm⁻¹), and nucleic acids (1371 cm⁻¹). *In vitro*, HEVs effectively delivered mRNA, achieving 100% transfection efficiency at a particle concentration eight times lower than that required for standard Dlin-MC3-DMA LNPs (6.25×108 particles.mL-1 vs 5×109 particles.mL-1).

**Summary/Conclusions:** We have demonstrated that HEVs are an effective strategy to functionalize EVs, significantly improving mRNA loading and providing EVs with the capability to escape endosomal degradation. Due to their hybrid nature, HEVs can potentially overcome safety issues associated with LNPs, which makes them a promising tool for therapeutic mRNA delivery.