In vivo loading of recombinant proteins into EVs for the treatment of lysosomal storage diseases

Diego Baranda-Martínez-Abascal[1,2]

Marc Moltó-Abad[1,2]

Joaquin Seras-Franzoso[1,2]

Diana Rafael[1,2]

Fernanda Andrade[1,2]

José Luis Corchero[2,3]

Joan Sayós[4]

Águeda Martínez[4]

Simó Schwartz[1,2]

Ibane Abasolo[1,2]

1. Drug Delivery & Targeting (DDT), Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.

2. Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.

3. Institut de Biotecnologia i de Biomedicina (IBB) and Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain

4. Institut de Recerca Biomèdica de Lleida Fundació Dr. Pifarré (IRBLleida), Lleida, Spain.

Contact details presenting authors:
diego.baranda@vhir.org
marc.molto@vhir.org

Introduction

Extracellular vesicles (EVs) are increasingly being studied as drug delivery systems (DDSs) due to their high blood stability and low immunogenicity. Moreover, EVs can be naturally loaded with therapeutic proteins through the genetic modification of producing cells[1], and their cell internalization capability can be enhanced with Cell Penetrating Peptides (CPPs) such as developed-in-house F7 peptide. Remarkably, vehiculization of lysosomal enzymes in EVs has proved to increase their stability and catalytic activity, making EVs a promising drug nanocarrier for the development of the enzyme replacement therapy in lysosomal storage diseases (LSDs) [2,3,4].

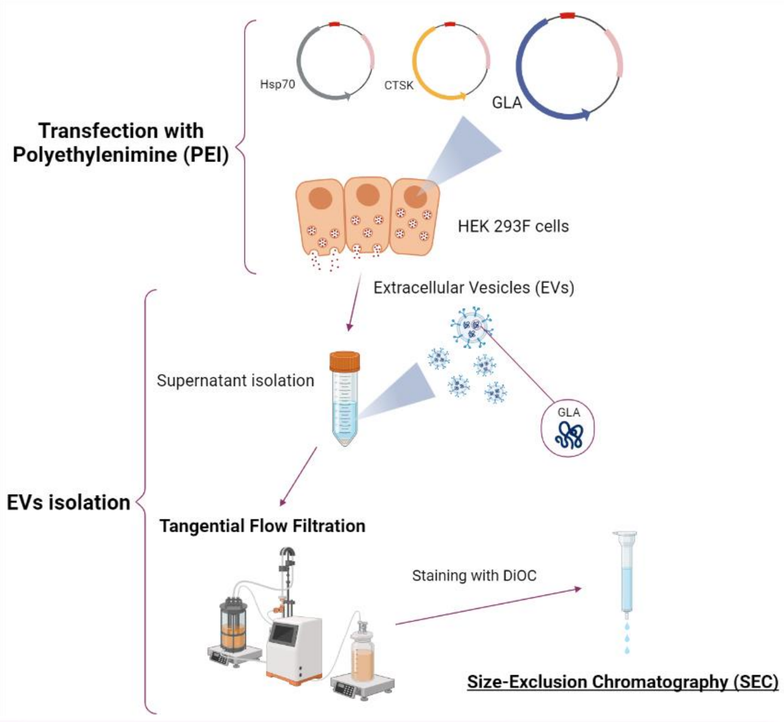
Objective

Production, isolation, functionalization and testing of HEK293F and CHO DG44-derived EVs as DDSs for three model LSDs, namely Fabry (EV-GLA), pycnodysostosis (EV-CTSK) and Niemann Pick (EV-Hsp70) diseases.

References

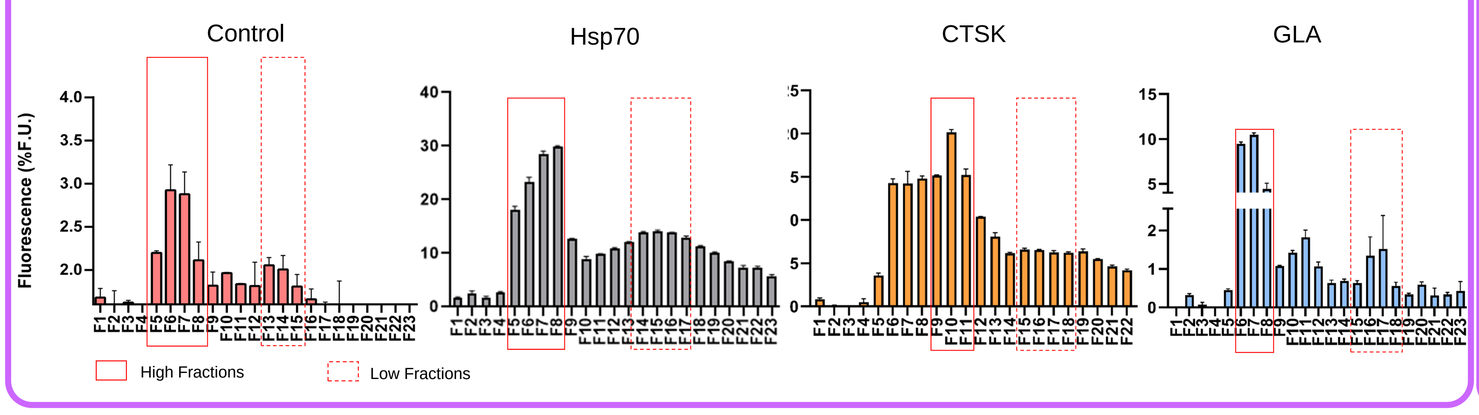
1. Sterzenbach U et al. Engineered Exosomes as Vehicles for Biologically Active Proteins. Molecular Therapy : The Journal of the American Society of Gene Therapy. 2017 Mar 30;25(6): 1269–1278. doi: 10.1016/J.YMTHE.2017.03.030. PMID: 28412169; PMCID: PMC5474961.
2. Gleason AM et al. The Role of Exosomes in Lysosomal Storage Disorders. Biomolecules. 2021 Apr 15;11(4):576. doi: 10.3390/biom11040576. PMID: 33920837; PMCID: PMC8071119.
3. Seras-Franzoso J et al. Extracellular vesicles from recombinant cell factories improve the activity and efficacy of enzymes defective in lysosomal storage disorders. J Extracell Vesicles. 2021 Mar;10(5):e12058. doi: 10.1002/jev2.12058. Epub 2021 Mar 12. PMID: 33738082; PMCID: PMC7953474.
4. Do MA et al. Targeted delivery of lysosomal enzymes to the endocytic compartment in human cells using engineered extracellular vesicles. Sci Rep. 2019 Nov 21;9(1):17274. doi: 10.1038/s41598-019-53844-5. PMID: 31754156; PMCID: PMC6872767.

Methodology



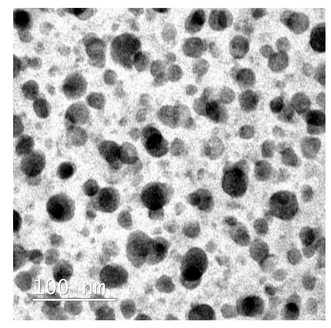
Results

EVs SEC fluorimetry

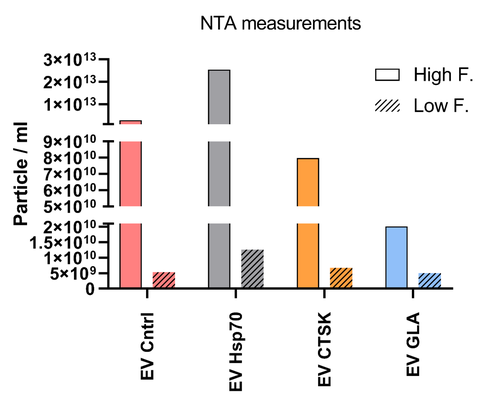


Transmission Electron Microscopy

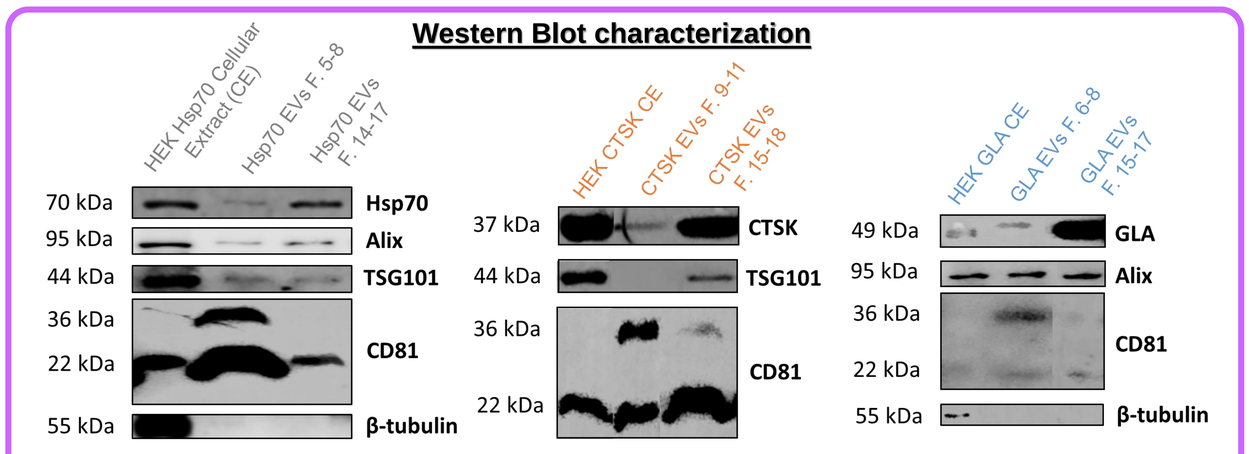
EVs Control High Fraction



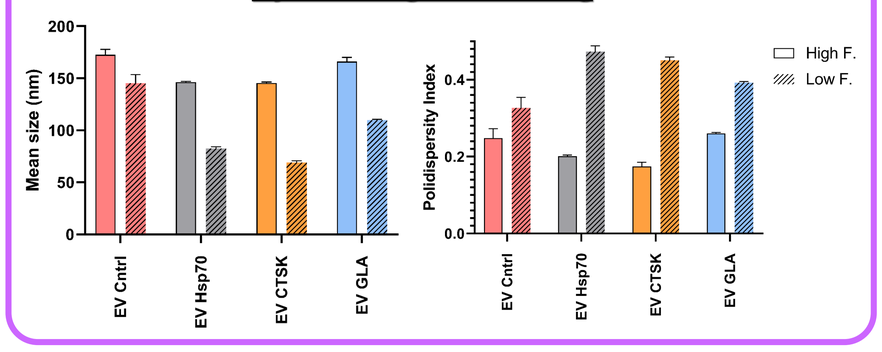
Nanoparticle Tracking Analysis



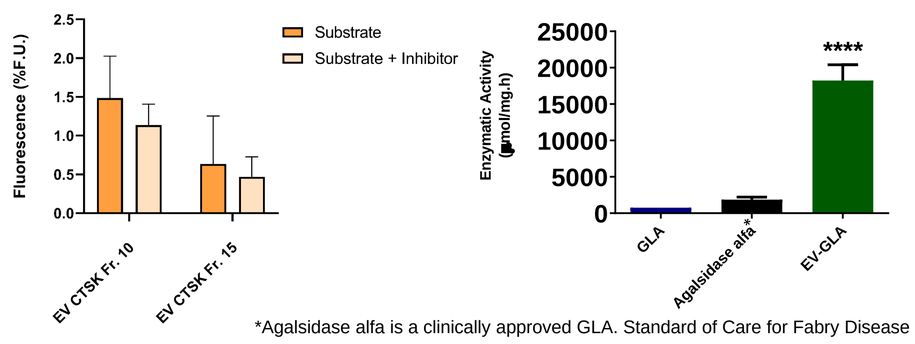
Western Blot characterization



Dynamic Light Scattering

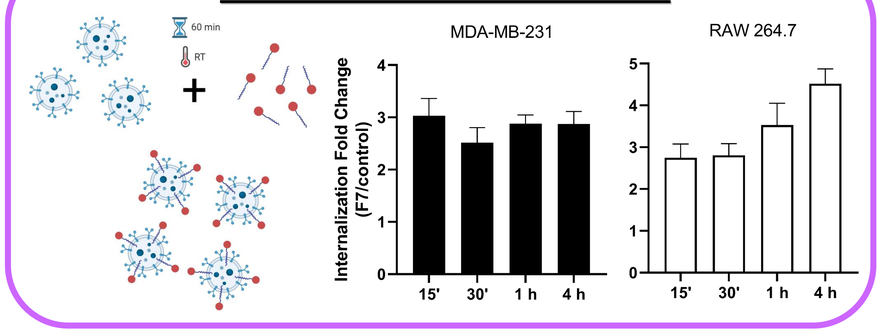


Activity of recombinant enzyme

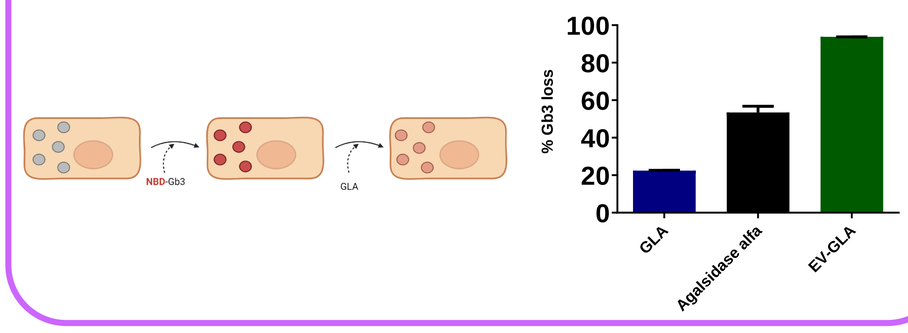


*Agalsidase alfa is a clinically approved GLA. Standard of Care for Fabry Disease

Surface modification with CPP F7



EV-GLA activity and efficacy in cell cultures



Conclusions

- ✓ Fractions obtained from SEC contain an **accurate size gradient** of vesicles at a **high concentration**
- ✓ Obtained EVs **contain significant amounts of recombinant proteins GLA, CTSK, Hsp70** as well as relevant EV markers (Alix, TSG101, CD81)
- ✓ Chemical conjugation of F7 penetrating peptide **enhances the EVs internalization rate** in two different cell lines
- ✓ **EV-CTSK and EV-GLA show enzymatic activity. EV-GLA** present an **improved therapeutic effect** when compared to Fabry disease Standard of Care

Acknowledgments:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964386. This work has been funded by the P21/00936 grant of the Instituto de Salud Carlos III and co-funded by the European Regional Development Fund (ERDF), the European Mimic-key project (GA 964386) and the ADVERT-Proyectos Valorización CIBER-BBN. DBM-A was supported by a FPU grant (FPU20/04210) from the Ministerio de Educación y Ciencia and MMA received a PERIS-FI grant (SLT/017/20/000181) from the Catalan Government.