Universitat Autònoma de Barcelona

Development of a purification process for HIV-1 VLPs: from supernatant to lyophilization

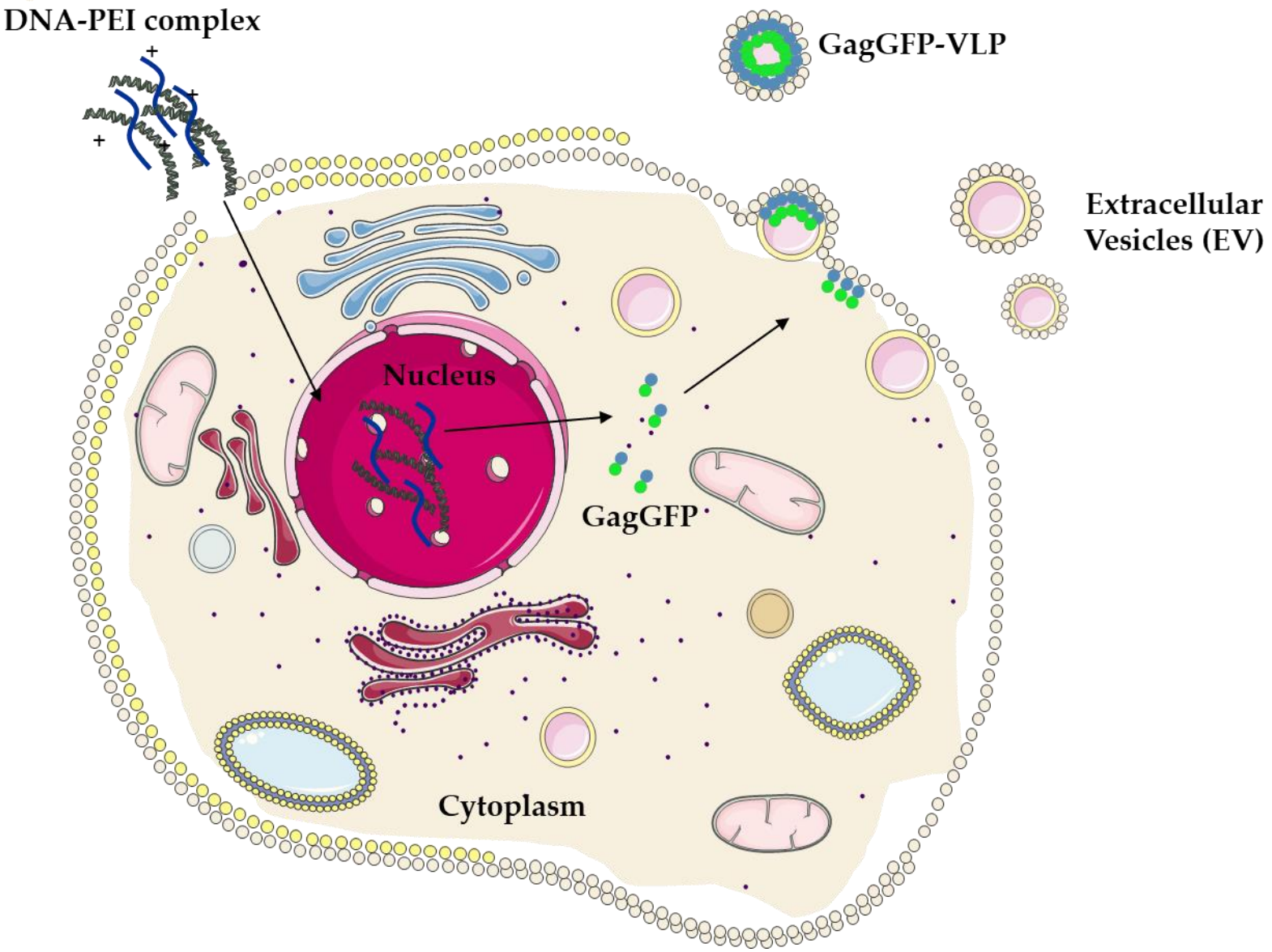
ELIANET LORENZO; Laia Miranda; Irene González-Domínguez; Laura Cervera; Francesc Gòdia

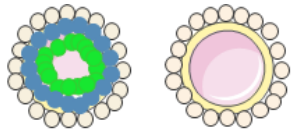
Department of Chemical, Biological and Environmental Engineering

CELL ENGINEERING AND BIOPROCESSES GROUP

ECI *Vaccine Technology VIII*

VLP Production





100nm -200nm

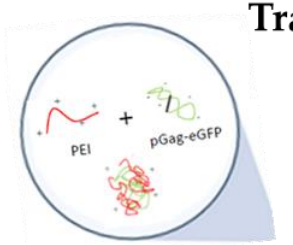
EVs and VLPs bud from the host cell membrane

Similar morphological and biophysical properties

Difficult the separation of both particles

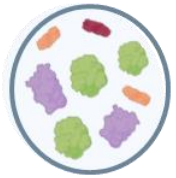
Vaccine candidate process

Transient Transfection



HEK293 cell line

Production of VLPs



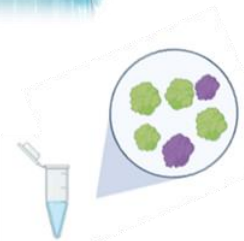
EV naturally co-produced

VLPs's purification process

Chromatography and membrane

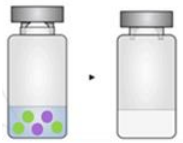
Purified VLPs

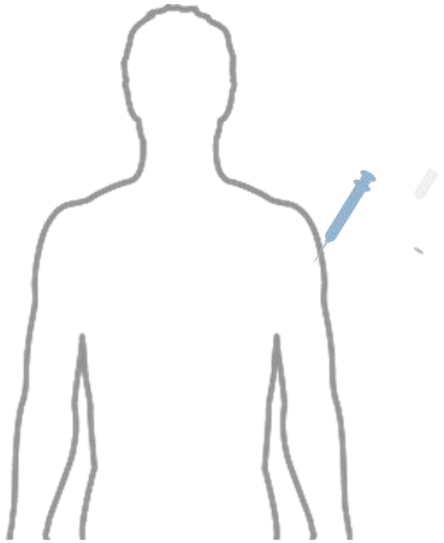
- *One dose of 1x10⁹ VLPs*
- *10 ng dsDNA/dose*
- *100 ppm of HCP/dose*
- *15 to 60 ug of antigen/dose*



Final Formulation

Lyophilization





Vaccine candidate process

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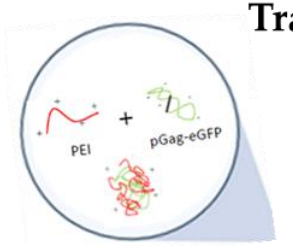

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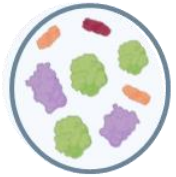

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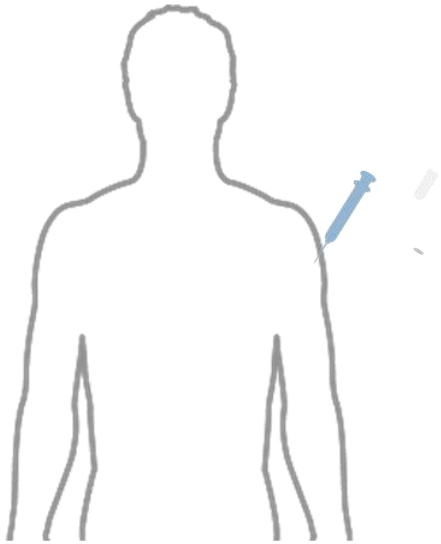

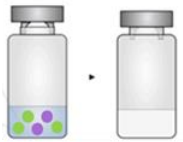

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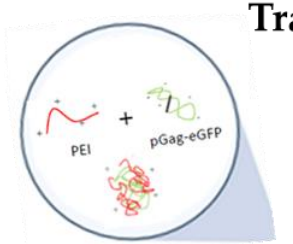

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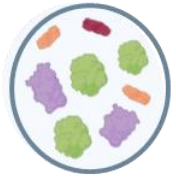

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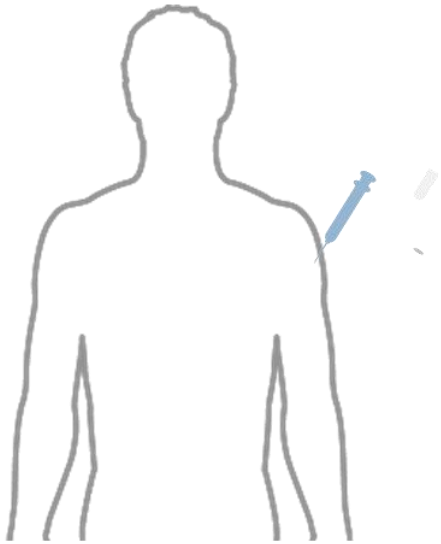

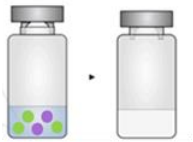

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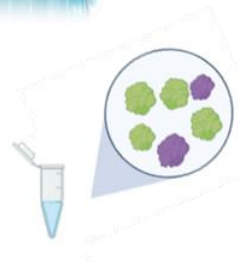

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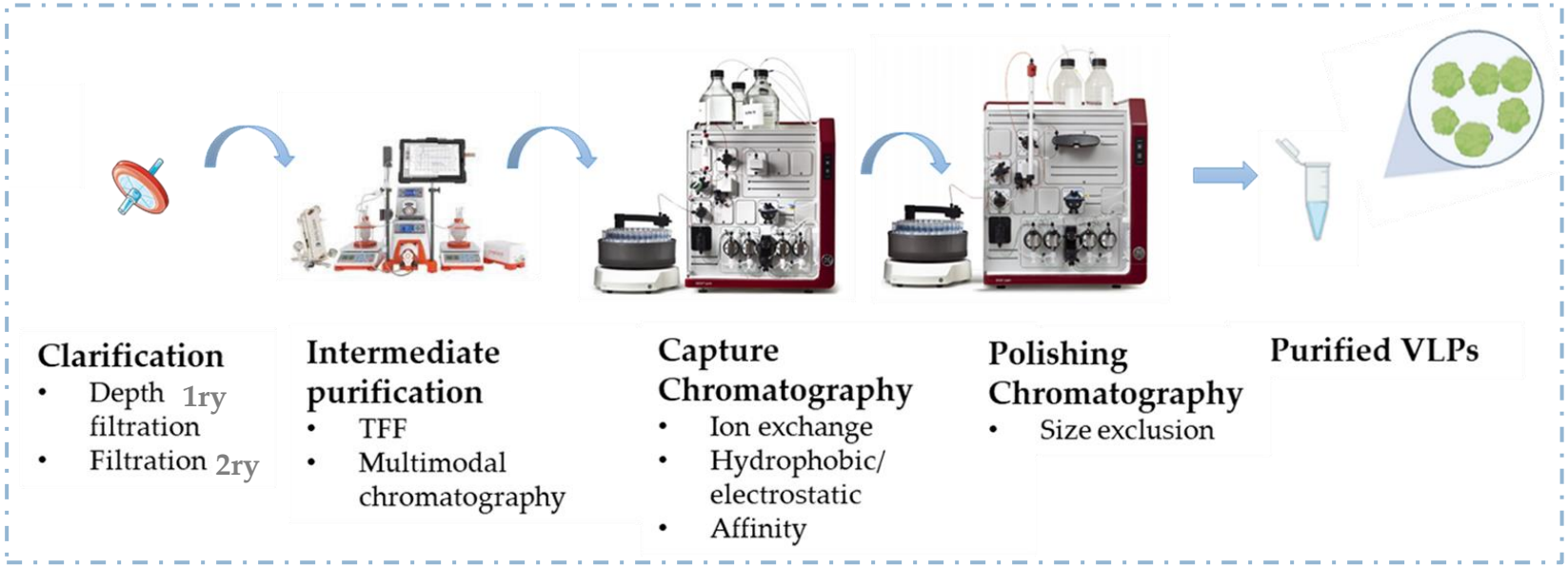
Experimental approach



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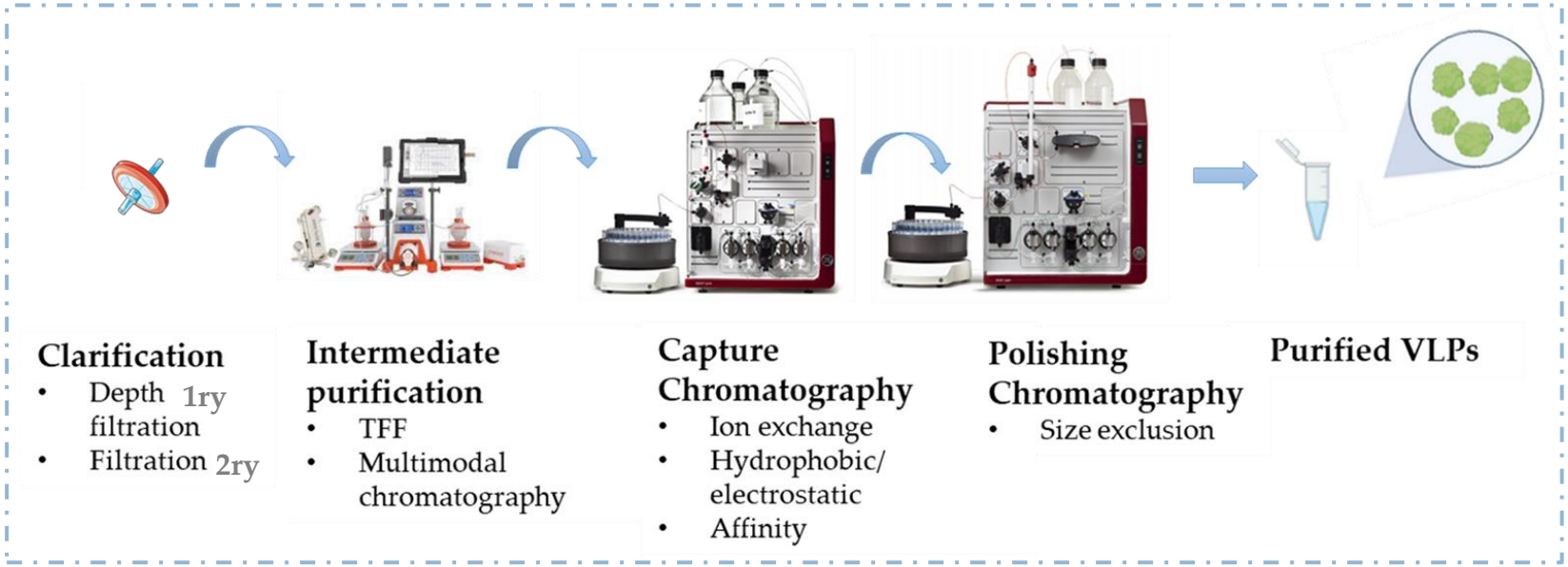
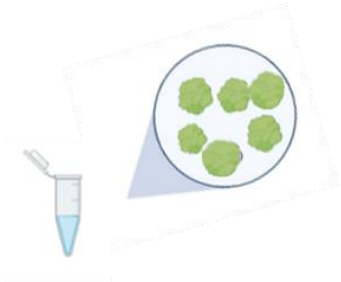




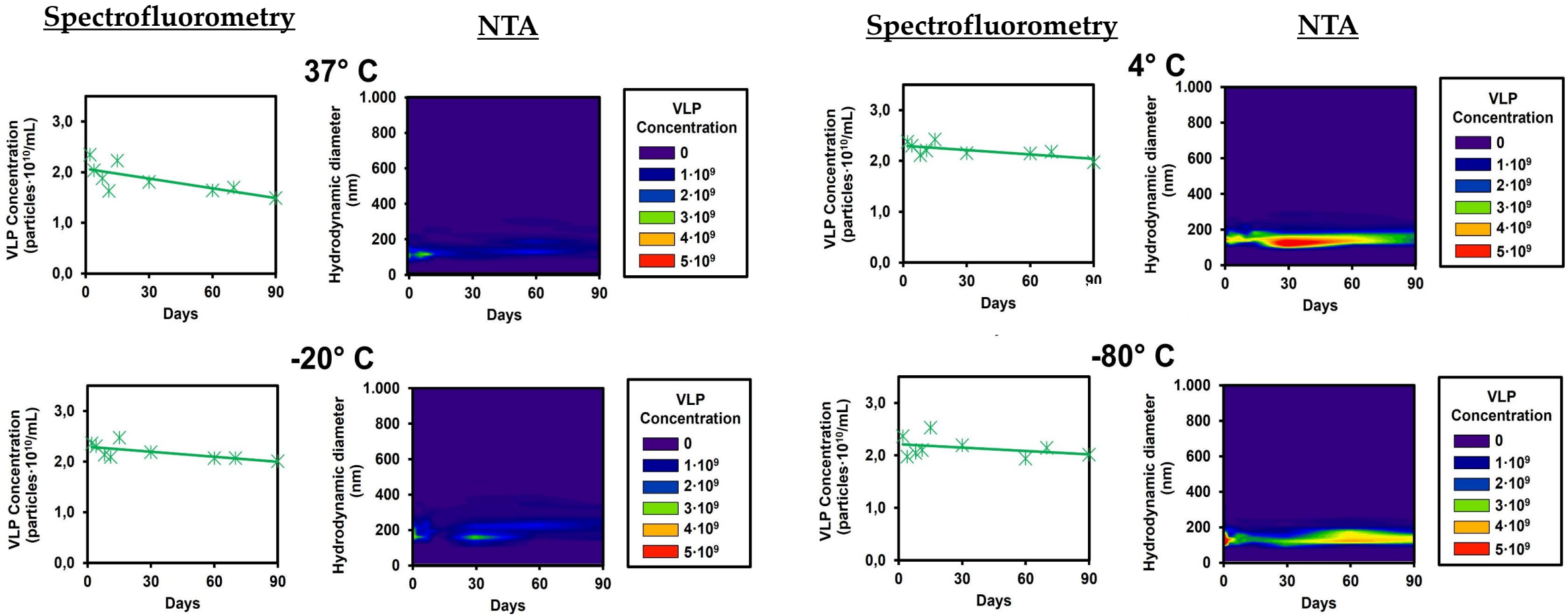
Purified VLPs

Characteristics

- **Recovery and enrichment of VLPs** → **NTA-Flow virometry**
- **Aggregation and size distribution** → **DLS**
- **Content of contaminants** → **Picogreen-BCA**
- **Morphology** → **CryoTEM**
- **GagGFP protein characterization** → **SDSPAGE-Western blot**

Analytics

Stability of Gag VLPs



A Four-Step Purification Process for Gag VLPs: From Culture Supernatant to High-Purity Lyophilized Particles
Irene González-Domínguez, Elianet Lorenzo , Alice Bernier , Laura Cervera , Francesc Gòdia and Amine Kamen . Vaccines, 2021

Gag-GFP VLPs showed to be **stable up to three months at 4 and -80 °C**, whereas it is not recommended to store them at 37 or -20 °C since a clear particle disruption is observed under these conditions

Primary clarification

Separation of the HIV-1 Gag-eGFP VLPs of the supernatant from the cells, directly out of the shake flask harvest.

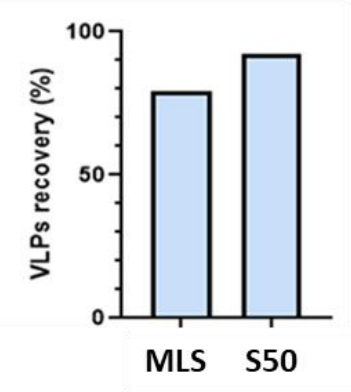
Depth filtration

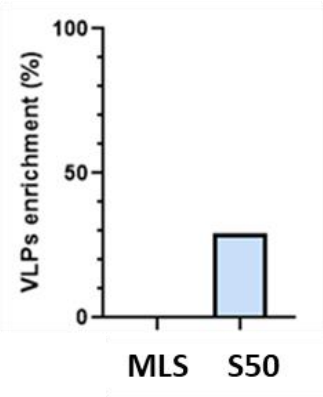
Millistak®+ D0HC µpod depth filter

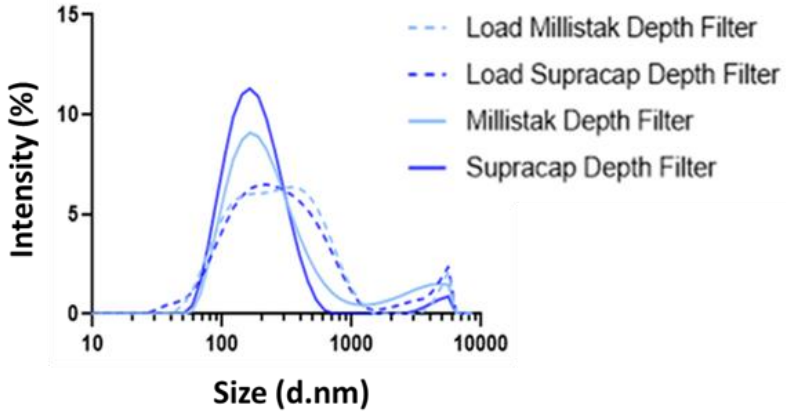
MLS

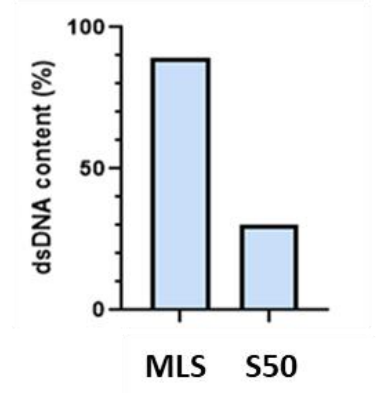
Supracap™ 50 depth filter capsule

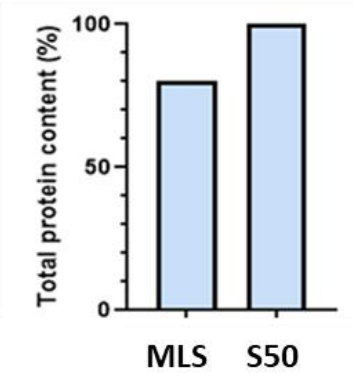
S50

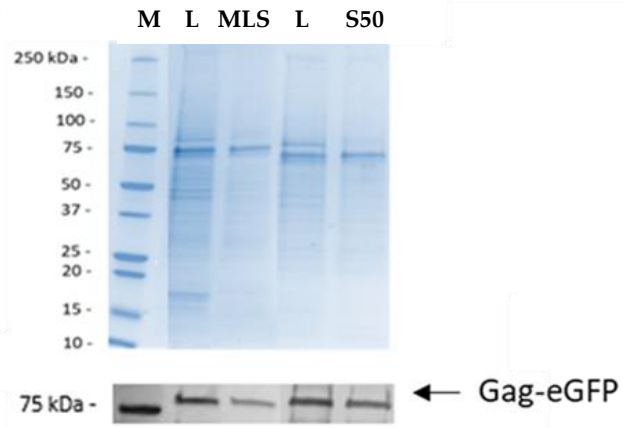












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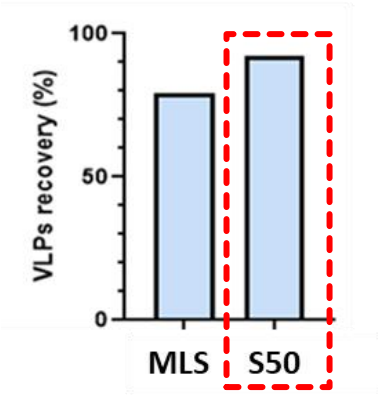
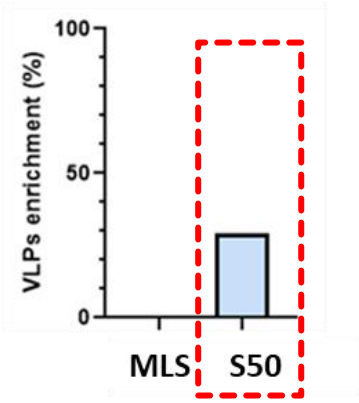
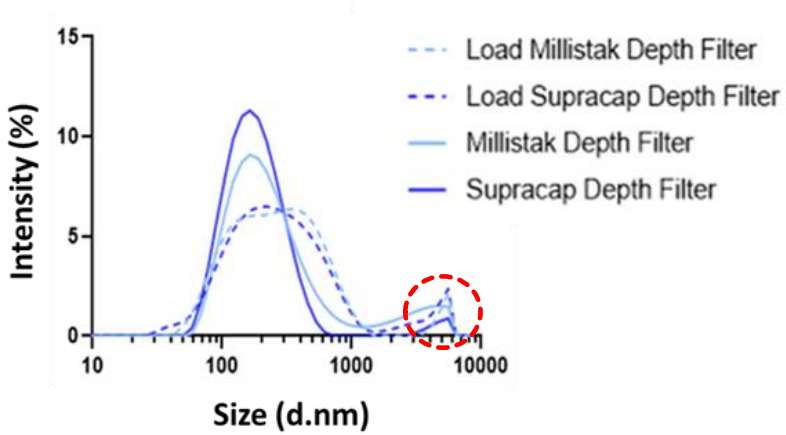
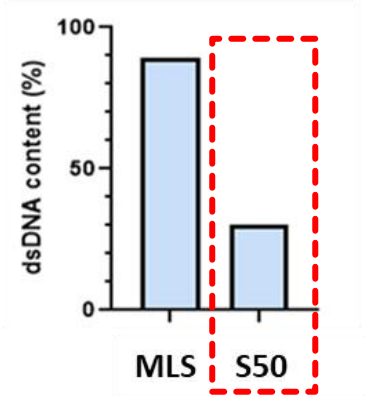
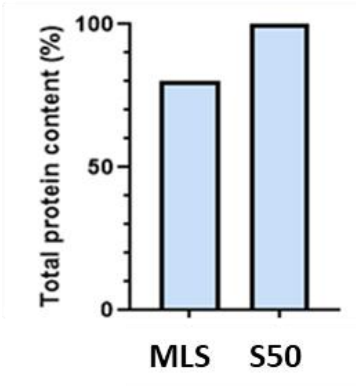
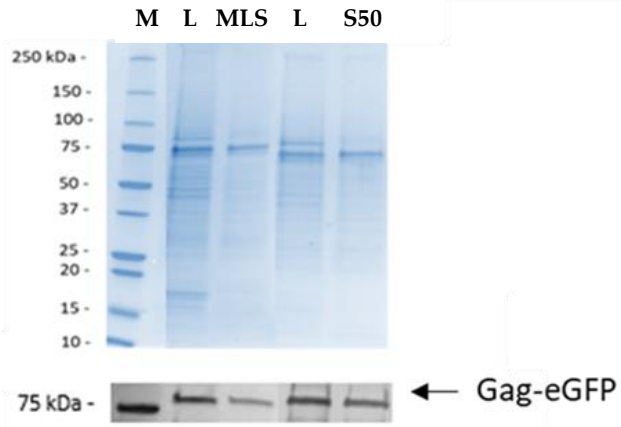












Higher recovery and purity were achieved with the **Supracap™ 50V100 depth filter**

Secondary clarification

Removal of precipitates formed when the clarified is freezed or stored several days in the fridge.

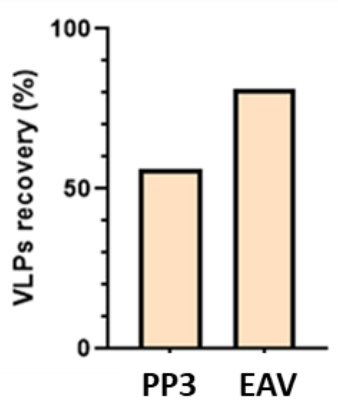
Filtration

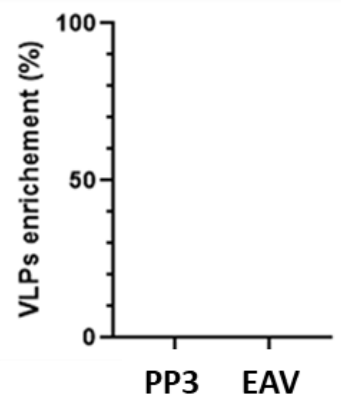
Sartopore® SartoScale 25 PP3 Filter

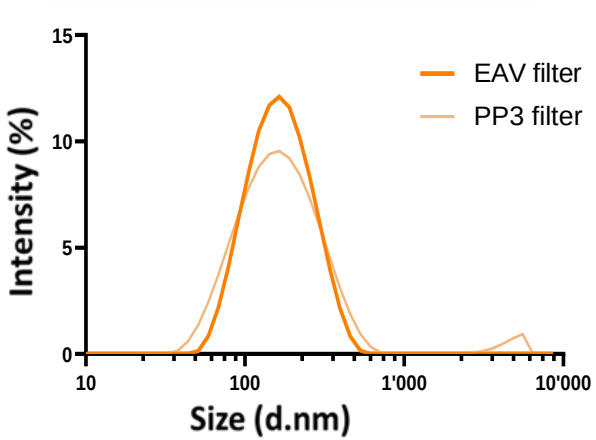
PP3

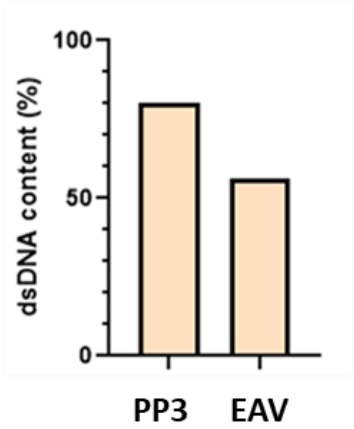
Supor® EAV - Mini Kleenpak™ 20 filter capsule

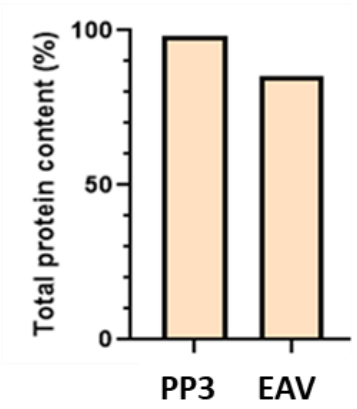
EAV

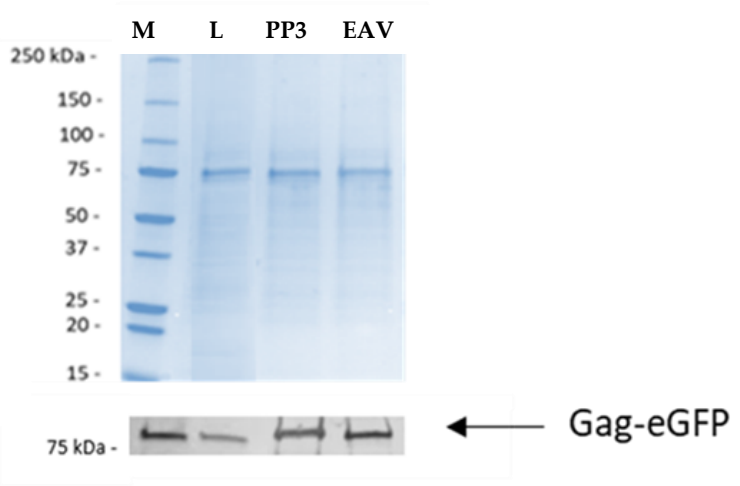












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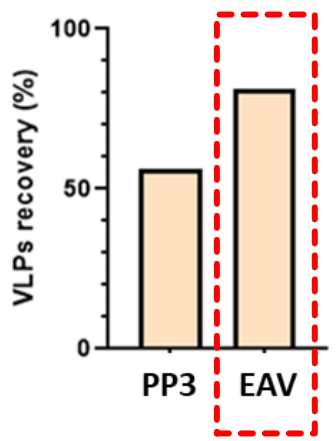

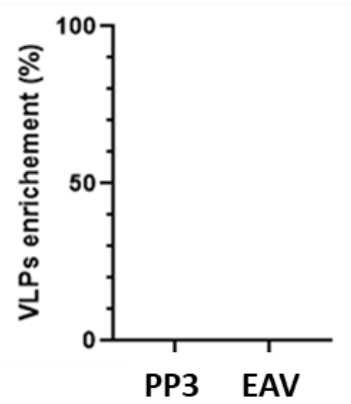

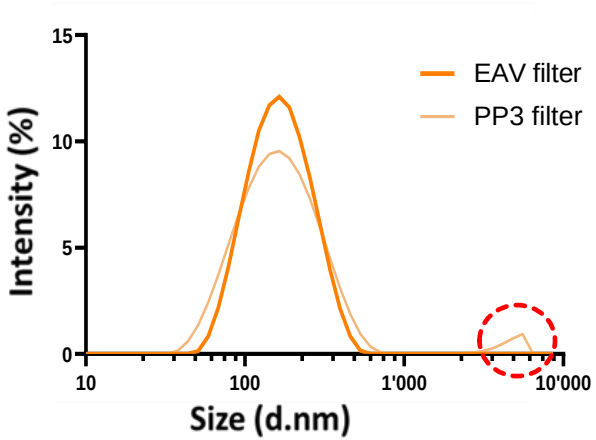

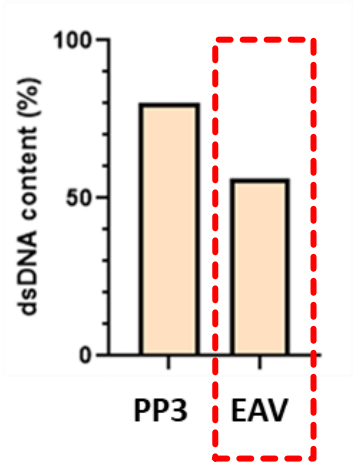

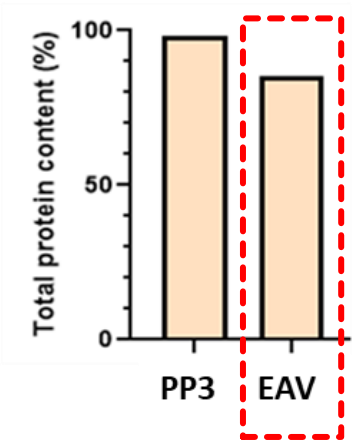

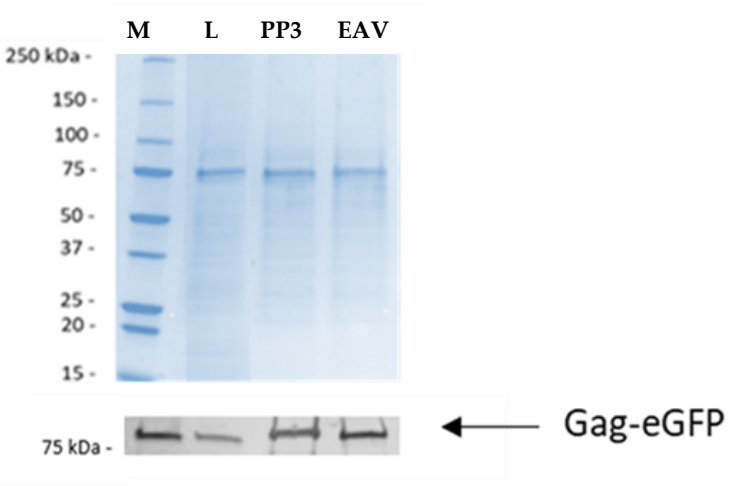

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Intermediate purification

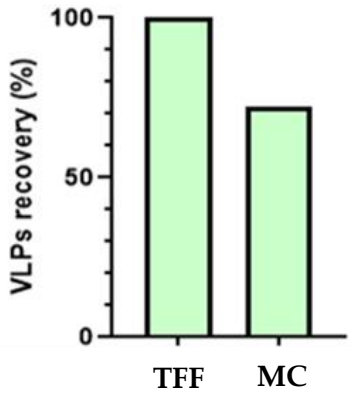
Intermediate purification

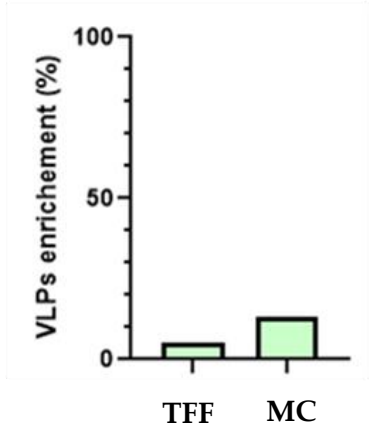
TFF: reduction of most part of the volume and concentration

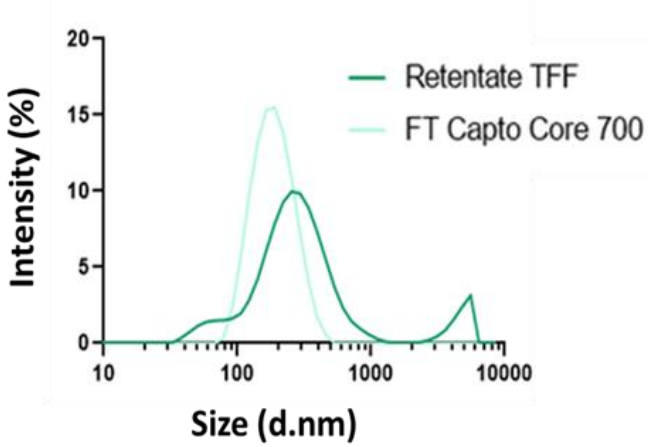
MC: removal of bulk impurities

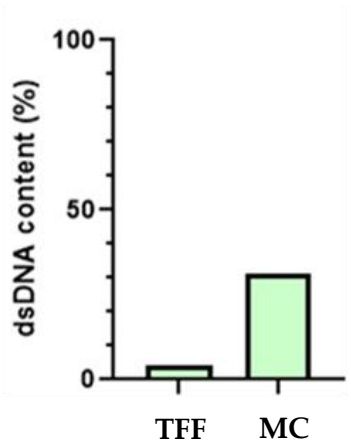
PES membrane TFF

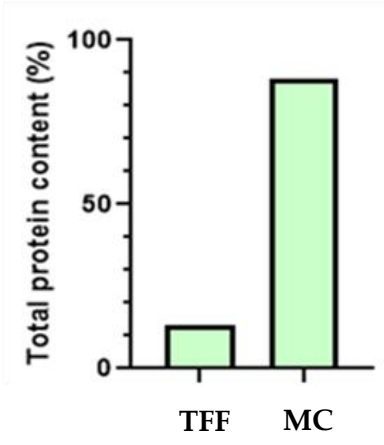
HiScreen™ Capto™ Core 700 column

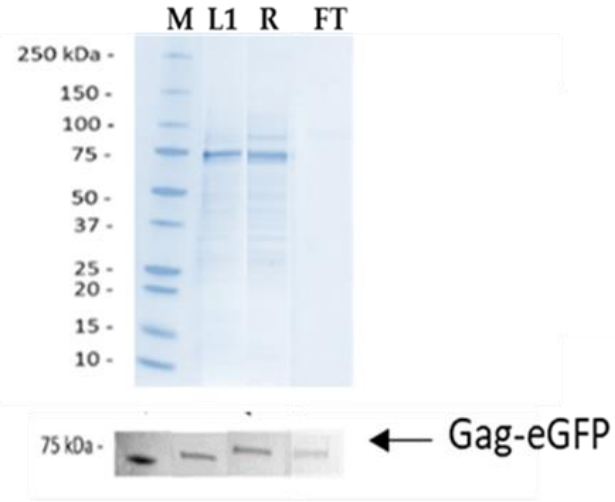












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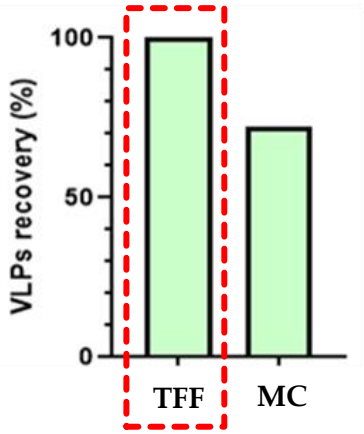
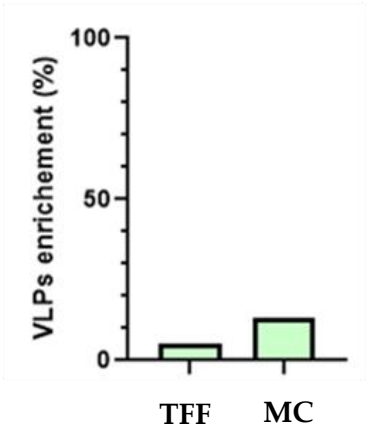
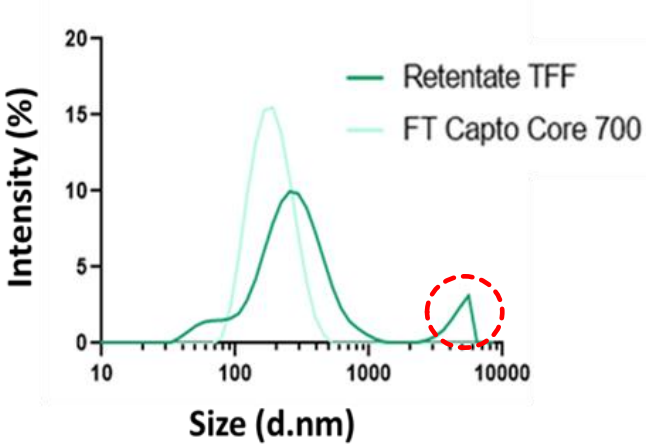
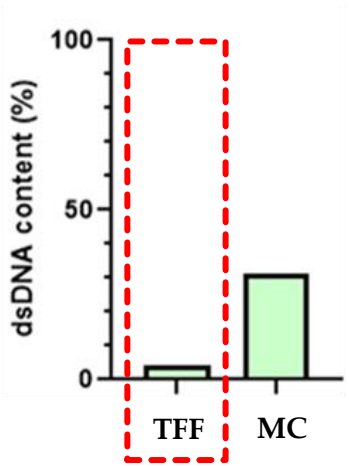
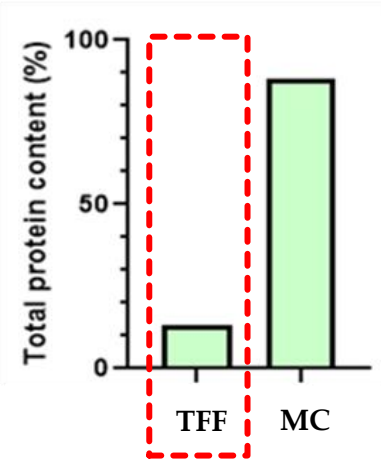
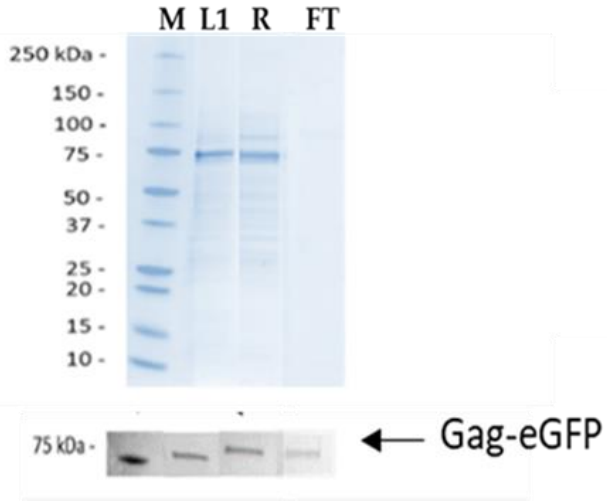












In the **TFF** all Gag-GFP VLPs were recovered, and concentrated in a volume ten times smaller, with presence of some aggregates. A considerable decrease in contaminating proteins and dsDNA were shown

Capture step

Capture and purification

CIMmultus™ QA Monolithic Column

MQA

Mustang® Q XT Acrodisc® Column

MQ

HiScreen™ Capto™ Q ImpRes Column

CQ

CIMmultus™ OH- Monolithic Column

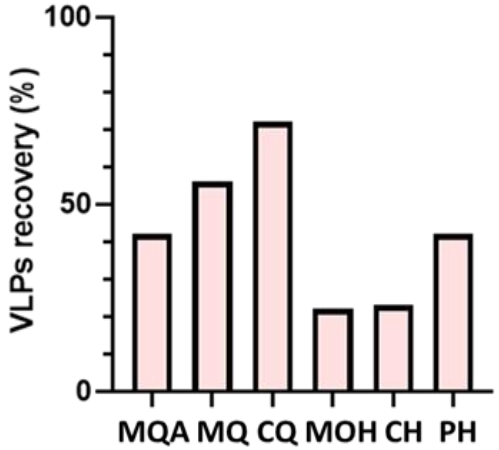
MOH

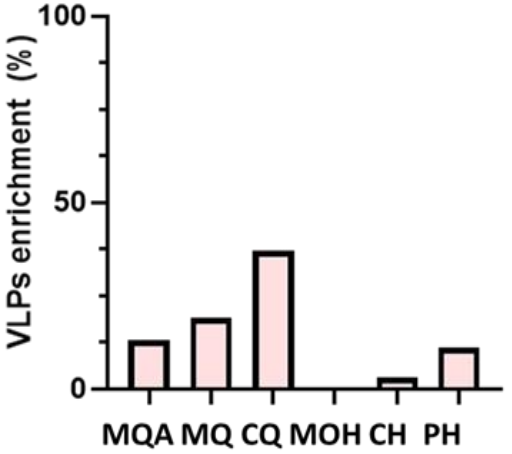
HiTrap Capto Heparin column

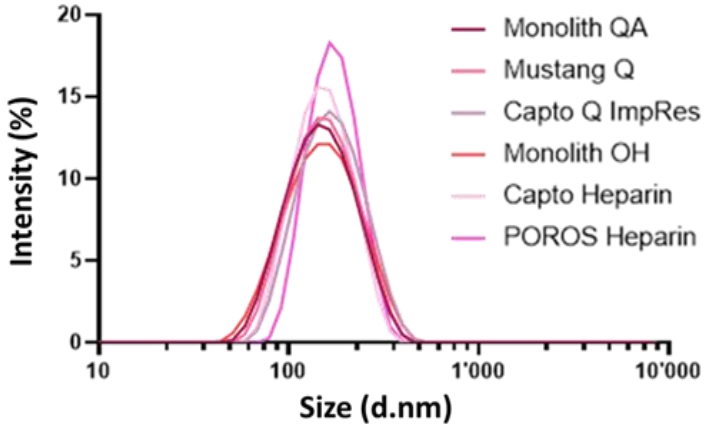
CH

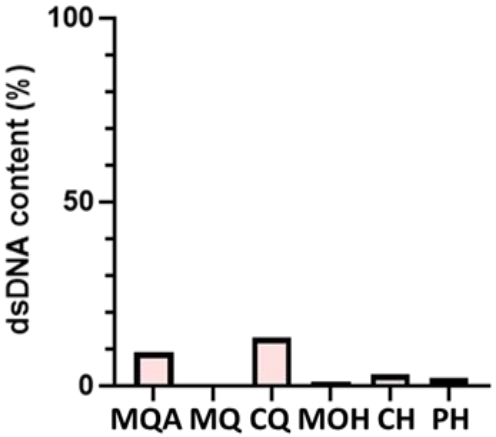
XK 16/20 POROS Heparin

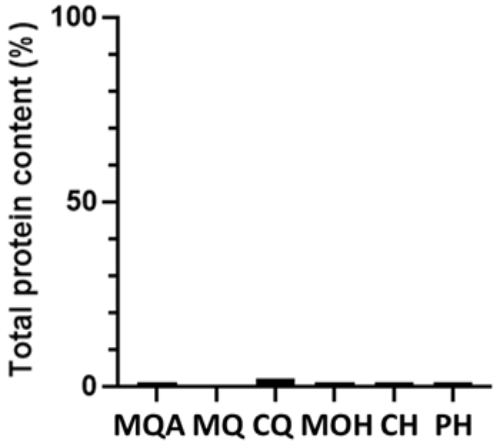
PH

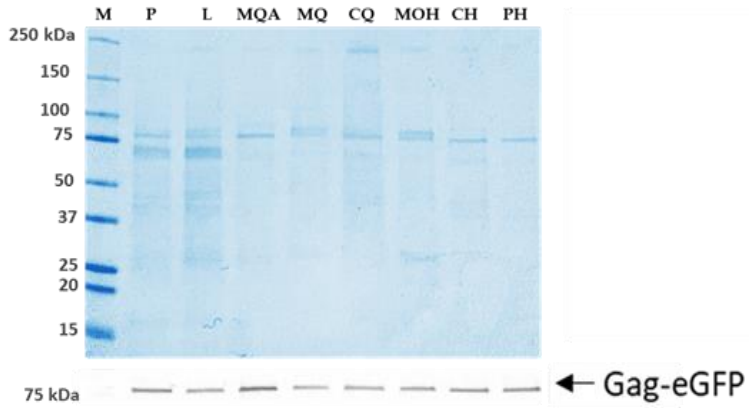












Capture step

Capture and purification

CIMmultus™ QA Monolithic Column

MQA

Mustang® Q XT Acrodisc® Column

MQ

HiScreen™ Capto™ Q ImpRes Column

CQ

CIMmultus™ OH- Monolithic Column

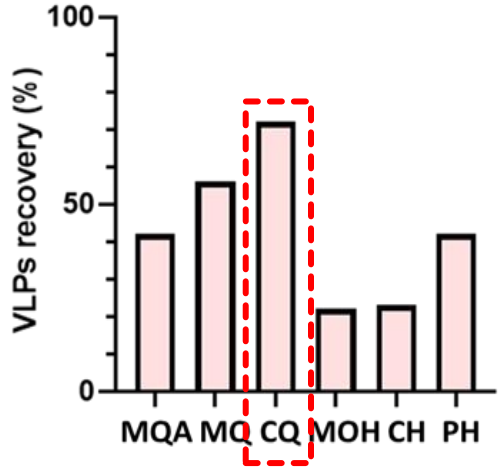
MOH

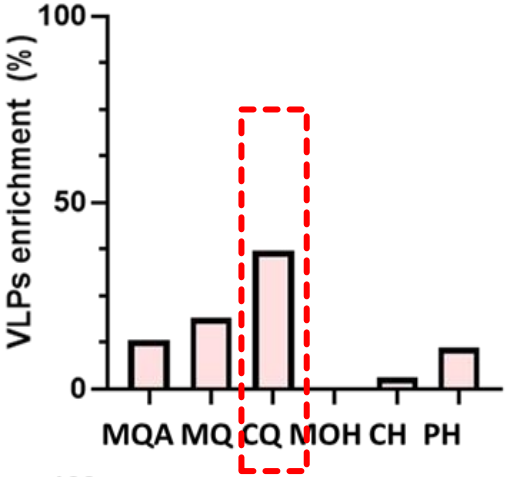
HiTrap Capto Heparin column

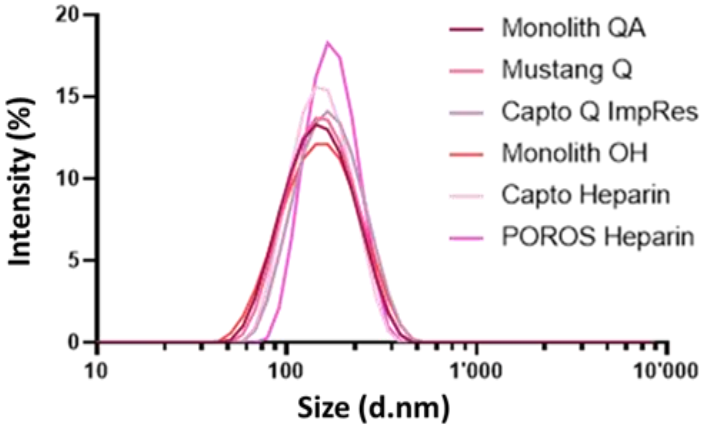
CH

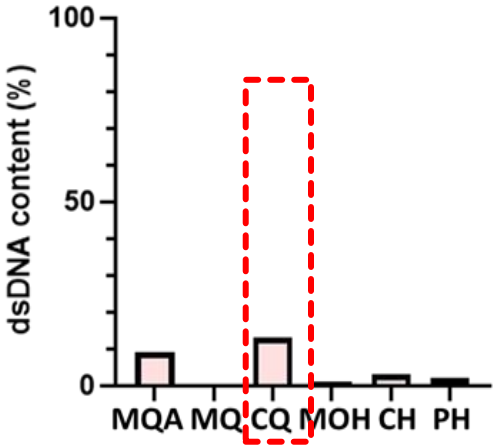
XK 16/20 POROS Heparin

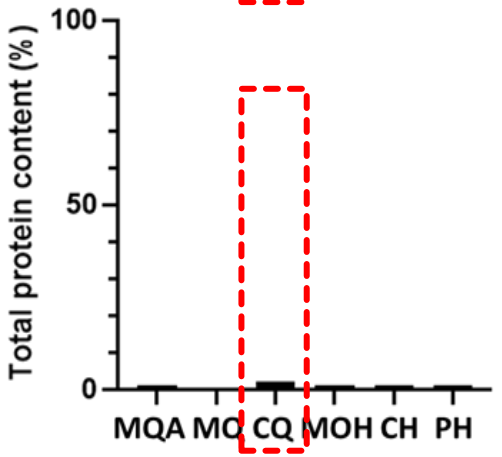
PH

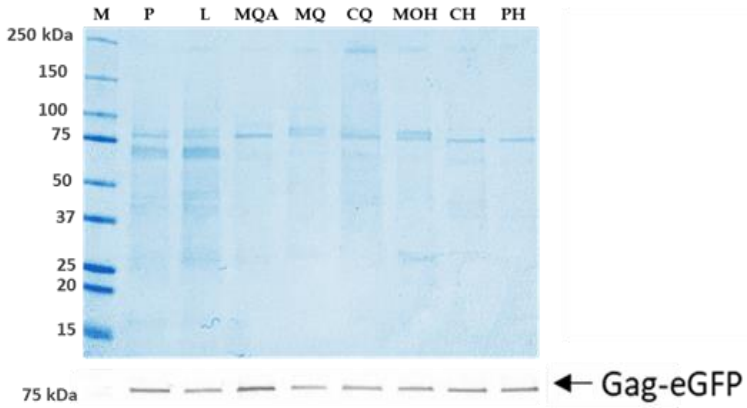












Capto Q ImpRes offers a better recovery of VLPs, enriching its presence with respect to the rest of the particles, and at the same time maintaining low amounts of contaminants

Polishing step

Removal of bulk impurities, final level of purity achievement, desalting and buffer exchange.

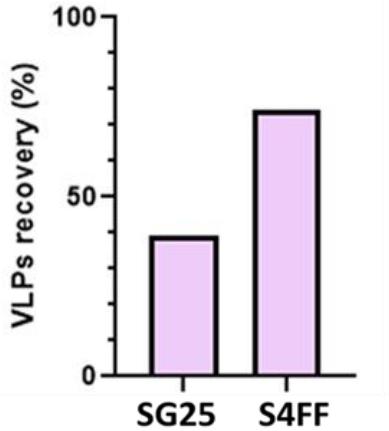
Size exclusion chromatography

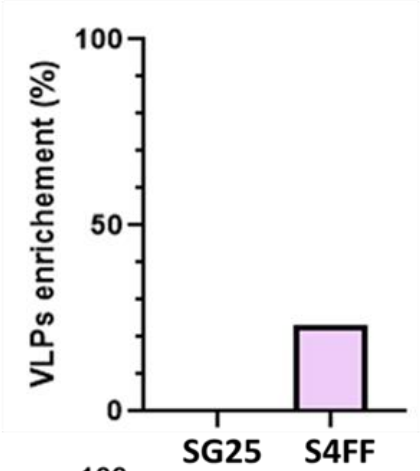
HiTrap®

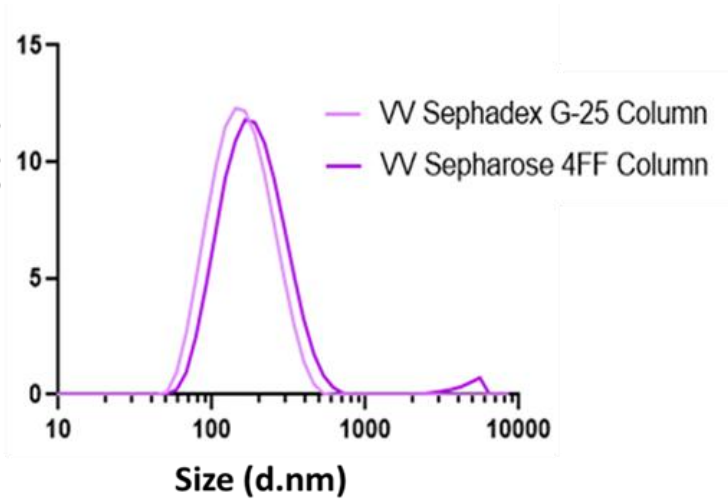
SG25

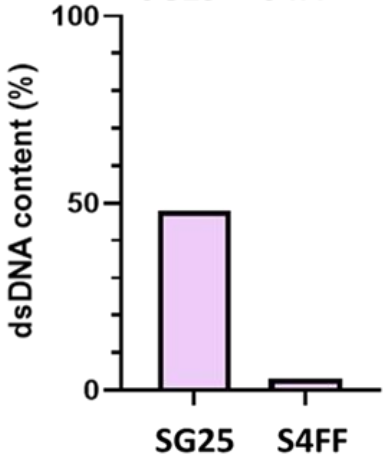
XK 16/40

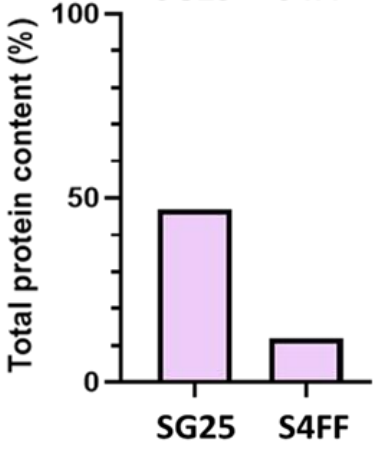
S4FF

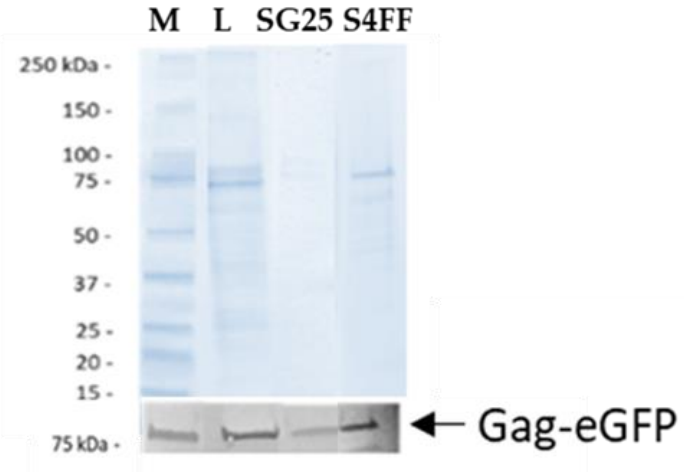












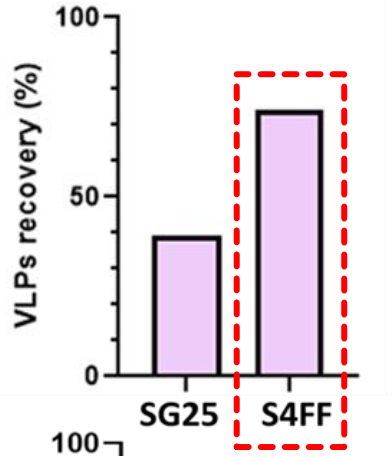
Polishing step

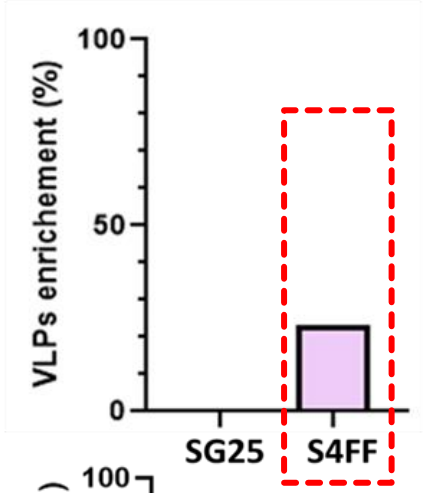
Removal of bulk impurities, final level of purity achievement, desalting and buffer exchange.

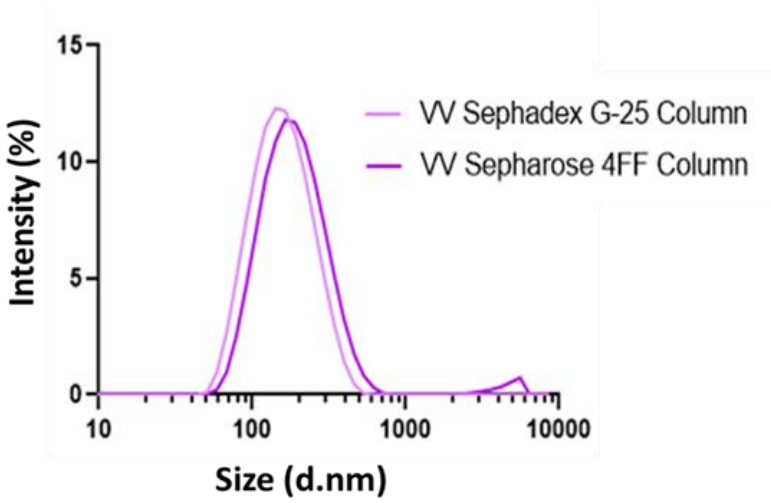
Size exclusion chromatography

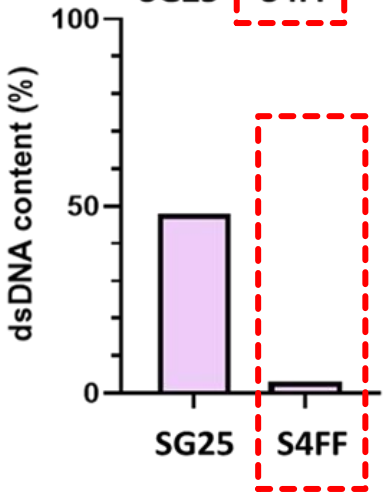
SG25

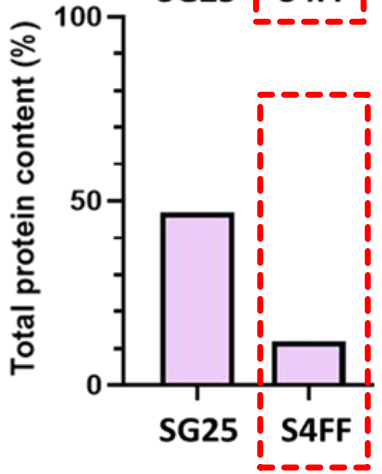
S4FF

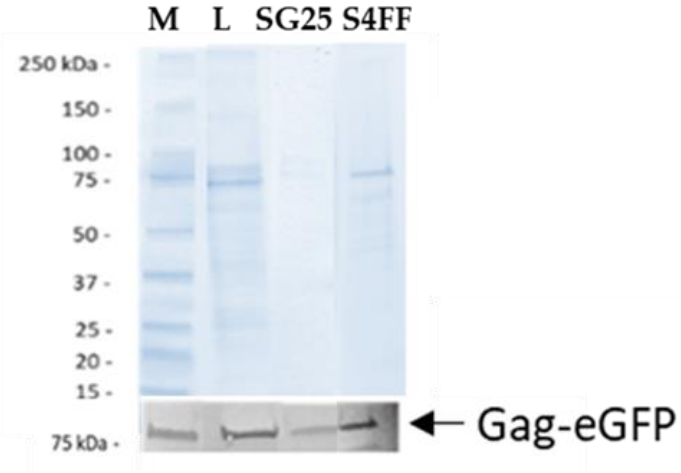






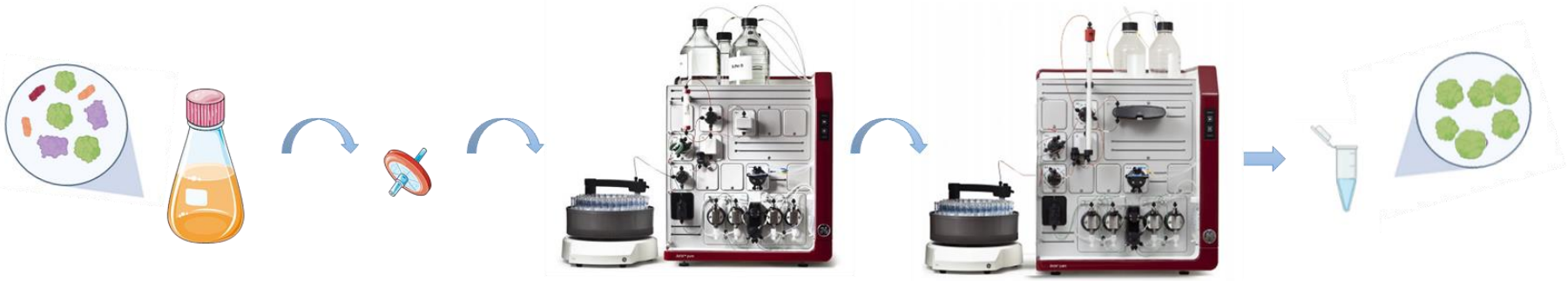






Higher recovery , purity and contaminants reduction were achieved with **S4FF**

Final integrated DSP



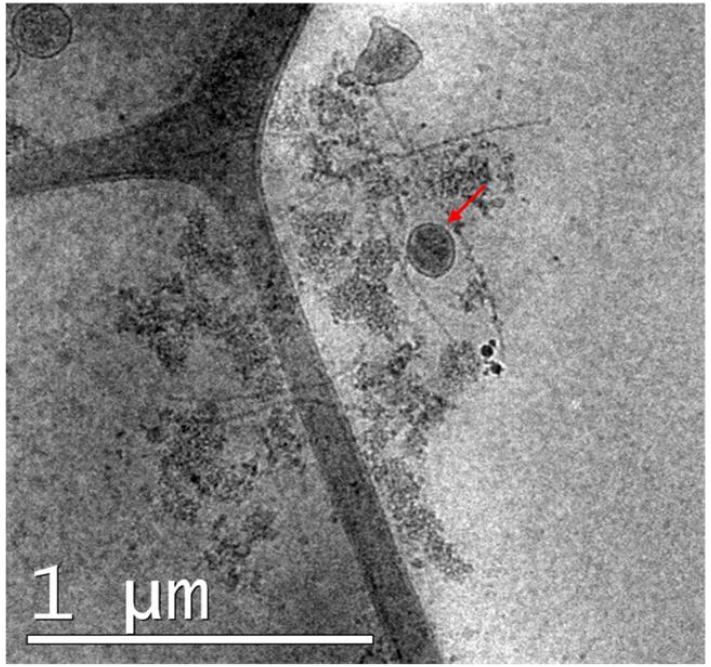
	Vol (mL)	VLPs's Recovery (%)	VLPs / Total Particles (%)	Total Protein Reduction (%)	dsDNA Reduction (%)	Gag-eGFP/Total protein (%)
Production	100	100	38	-	-	3
Clarified	100	90	43	2	65	2
E2 fraction CaptoQ ImpRes	2	76	60	98	96	32
VV fraction S4FF	6	55	64	83	96	70
Overall	**6**	**38**	**64**	**100**	**100**	**70**

Dose of 1 x 10^9 VLPs:

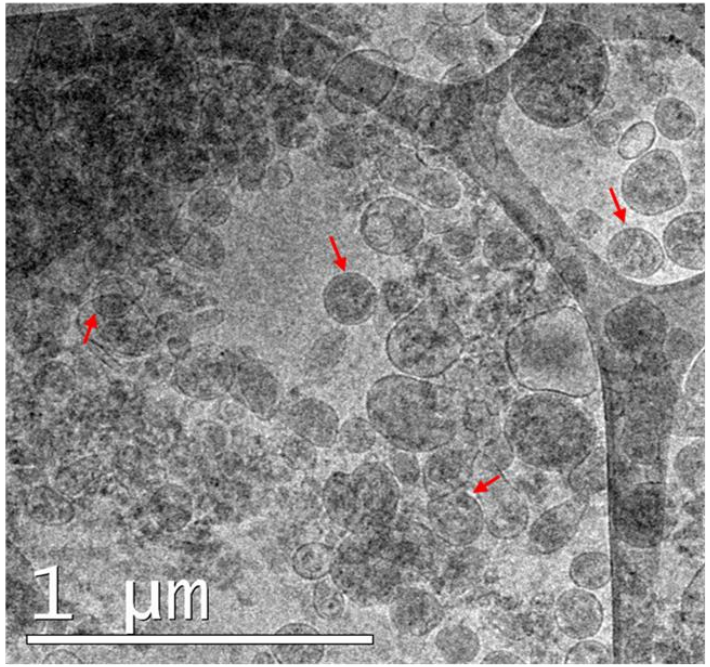
- ✓ **84 vaccination doses**
- ✓ **0,12 ng dsDNA/dose**
- ✓ **21µg of Gag-eGFP polyprotein**

Characterization of purified Gag-eGFP VLPs

Supernatant



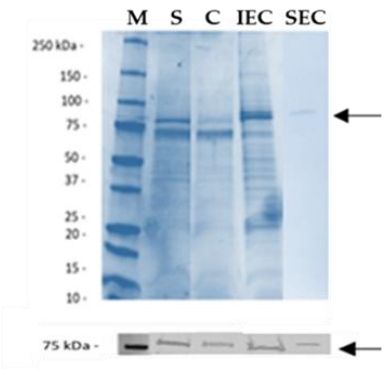
Purified Gag-eGFP VLPs after SEC



→ Gag-eGFP VLPs

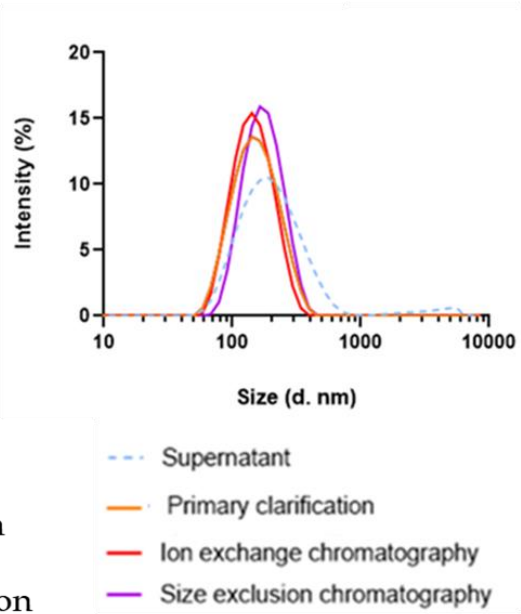
→ Gag-eGFP protein

SDS-PAGE and Western blot



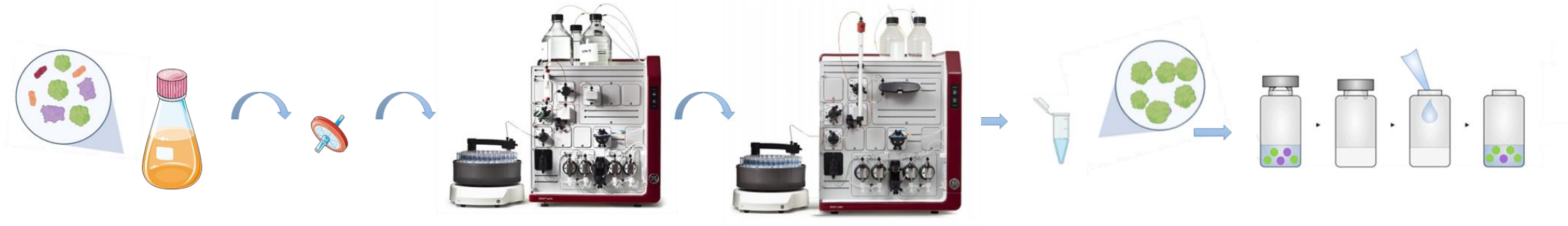
M: Marker
S: Supernatant
C: Clarified
IEC: Elution peak 2 from Capto Q ImpRes
SEC: Void volume fraction from Sepharose 4 fast flow

DLS profile

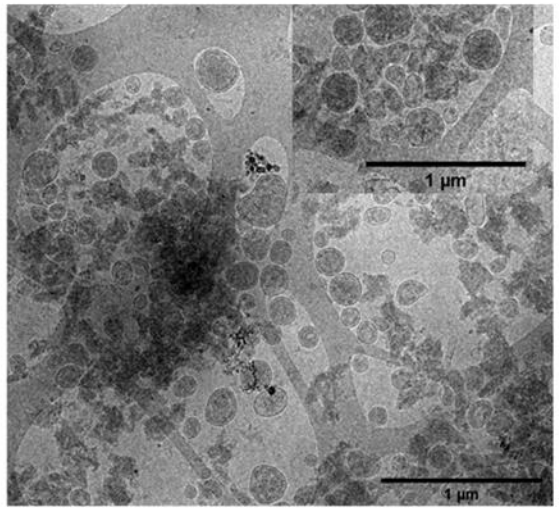


Final concentrated and purified Gag-eGFP VLPs are clearly observed in cryo-TEM micrographs compared to the initial clarified material

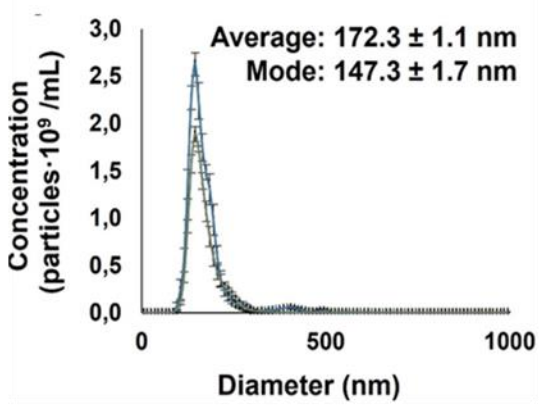
Lyophilization of VLPs



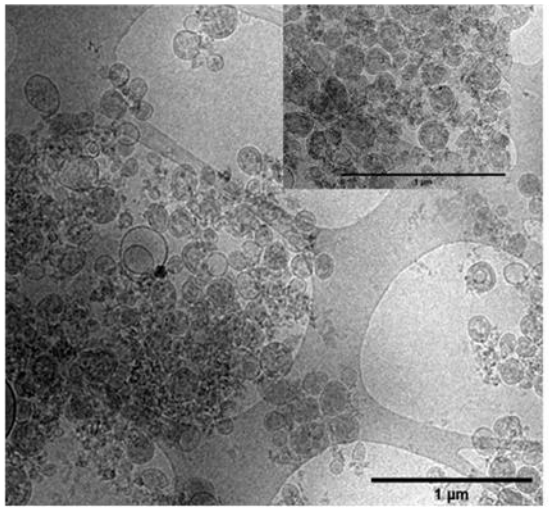
Frozen GagGFP VLPs



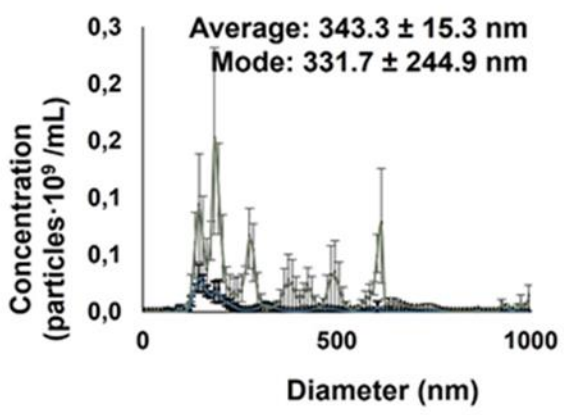
NTA



Lyophilized GagVLPs



NTA



Despite the aggregation, the presence of EVs was below LOD in lyophilized samples according cryo-TEM micrographs

Concluding Remarks

- ✓ This work has resulted in the establishment of a defined procedure of DSP for HIV-1 Gag-eGFP VLPs suitable for scale-up.
- ✓ Also, the present strategy meets the required standard quality and offers great promise for the development of novel vaccine candidates using this platform.
- ✓ The proposed process can also be applied to the purification of other VLPs and related products.

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Claudio Prieto
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Thank You!!

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