Pre-formation loading of extracellular vesicles with exogenous molecules by photoporation

J. Ramon[1], C. Pinheiro[2], C. Vandendriessche[3,4], H. De Keersmaecker[1,6], D. Punj[1], J.C. Fraire[1,7], E. Geeurickx[2], R.E. Vandenbroucke[3,4], A. Hendrix[2], S. Stremersch[1], S.C. De Smedt[1], K. Raemdonck[1], K. Braeckmans[1]

[1]Labgratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium ;
[2]Laboratory of Experimental Cancer Research, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium
[3]VIB Center for Inflammation Research, 9052 Ghent, Belgium
[4]Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium;
[5]Department of Biomolecular Health Sciences, Faculty of Veterinary Sciences, Utrecht University, 3584 CL Utrecht, The Netherlands;
[6]Ghent Light Microscopy Core Facility, Ghent University, 9000 Ghent, Belgium
[7]Institute of Bioengineering of Catalonia (IBEC), Smart Nano-Bio-Devices Group, 08028 Barcelona, Spain

Introduction

- EVs are important players in **cell-to-cell communication**.
- EVs need to be labeled to **study their cell tropism**.
- The labeling method **cannot interfere with the surface properties** of EVs.
- **Photoporation** is a potential **pre-formation EV loading method** that delivers molecules straight into the cell's cytosol.
- **Passive** EV loading with fluorescently labeled dextrans (AF10)
  Active EV targeting with fluorescently labeled anti-EGFP nanobodies (NBAF)

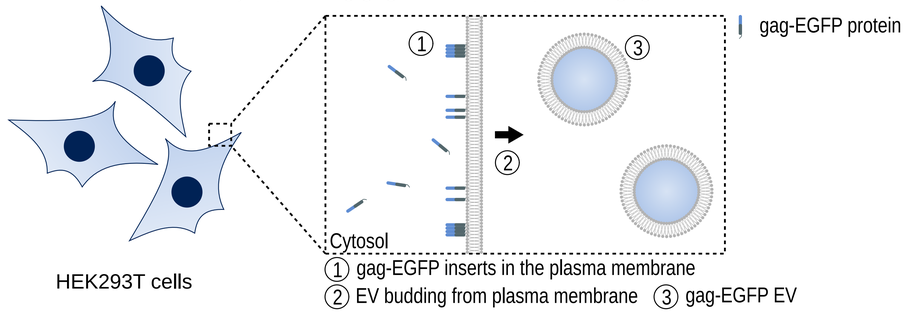
Intracellular delivery
Fluorescent labels in cytosol

EV loading
Encapsulation from cytosol

Different cargo types
Passive vs. active targeting

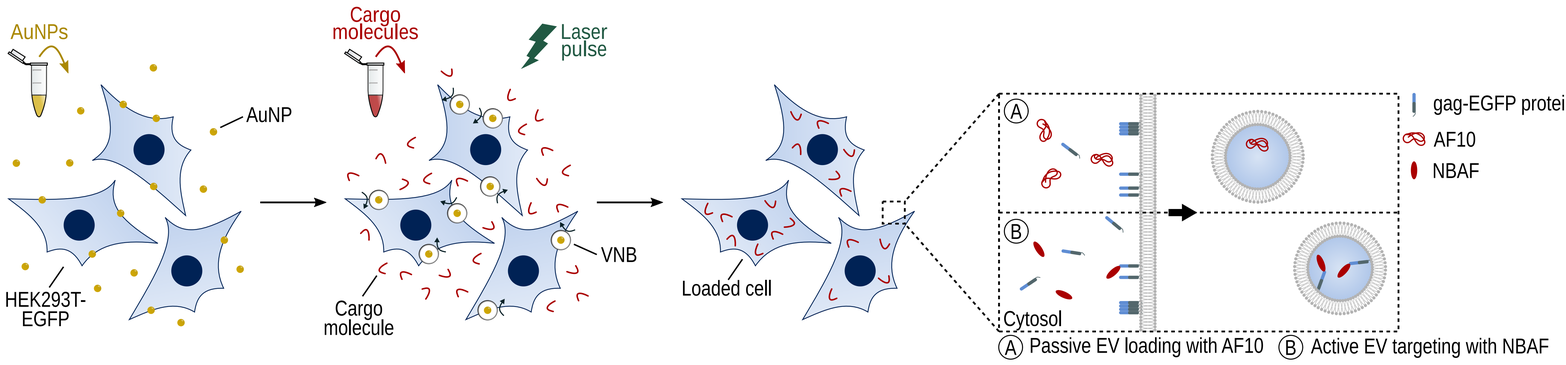
EV producing cell line: HEK293T gag-EGFP

HEK293T cells transiently expressing **gag-EGFP**, which produce **gag-EGFP EVs**.



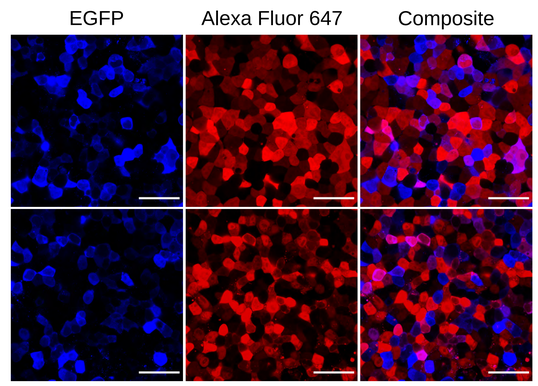
Cytosolic delivery of fluorescent molecules by photoporation

1 Gold nanoparticle (AuNP) membrane adsorption.
2 Pulsed laser irradiation and VNB formation.
3 Pore formation and cytosolic delivery.
4 Encapsulation in Extracellular Vesicles (EVs).



Alexa Fluor 647 Dextran 10 kDa

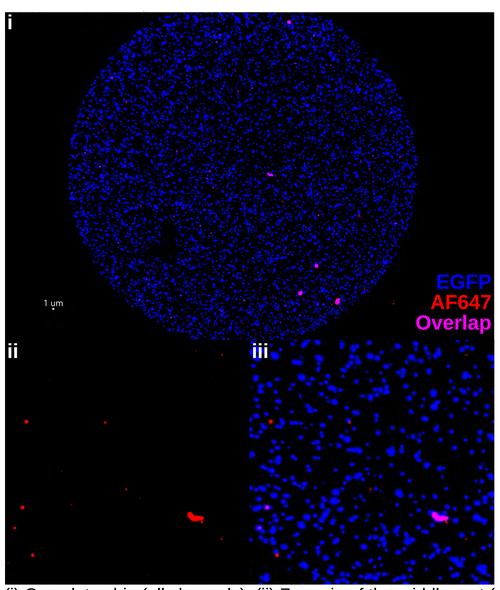
Alexa Fluor 647 anti-EGFP nanobody



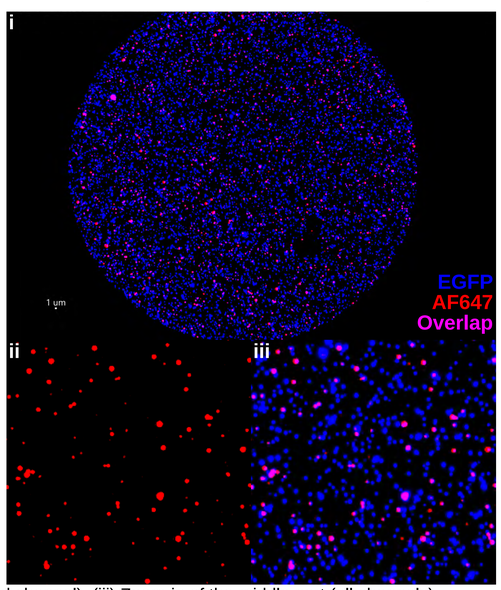
Encapsulation of fluorescent molecules in extracellular vesicles

Labeled EVs captured onto CD63 ExoView chips

Alexa Fluor 647 Dextran 10 kDa

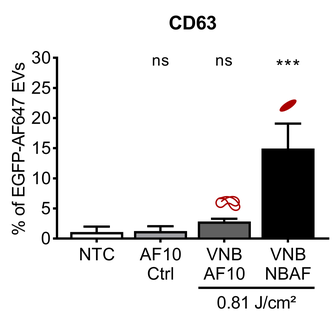


Alexa Fluor 647 anti-EGFP nanobody

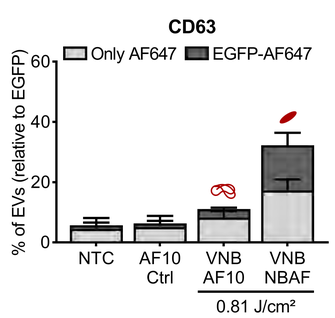


(i) Complete chip (all channels), (ii) Zoom-in of the middle part (red channel), (iii) Zoom-in of the middle part (all channels).

% of EGFP-AF647 EVs



% of EVs (relative to EGFP)



Higher EV loading with NBAF.

Differences in encapuslation efficiency inherent to type of molecule?

Higher non-EGFP loading with NBAF compared to AF10 .

EV characteristics

EV concentration =
% Fluorescent EVs =
#EVs/cell =

Size around 150 nm
Size =

Negative charge
No clear differences

No impact on distribution of CD63, CD81 and CD9

Conclusions

Successful loading of HEK293T gag-EGFP cells with AF10 and NBAF using photoporation.

Improved loading of gag-EGFP EVs with nanobodies.

No impact on EV characteristics by photoporation.

Dr. Jana Ramon
Jana.Ramon@UGent.be