Technical Session I
Manufacturing Science and Technology

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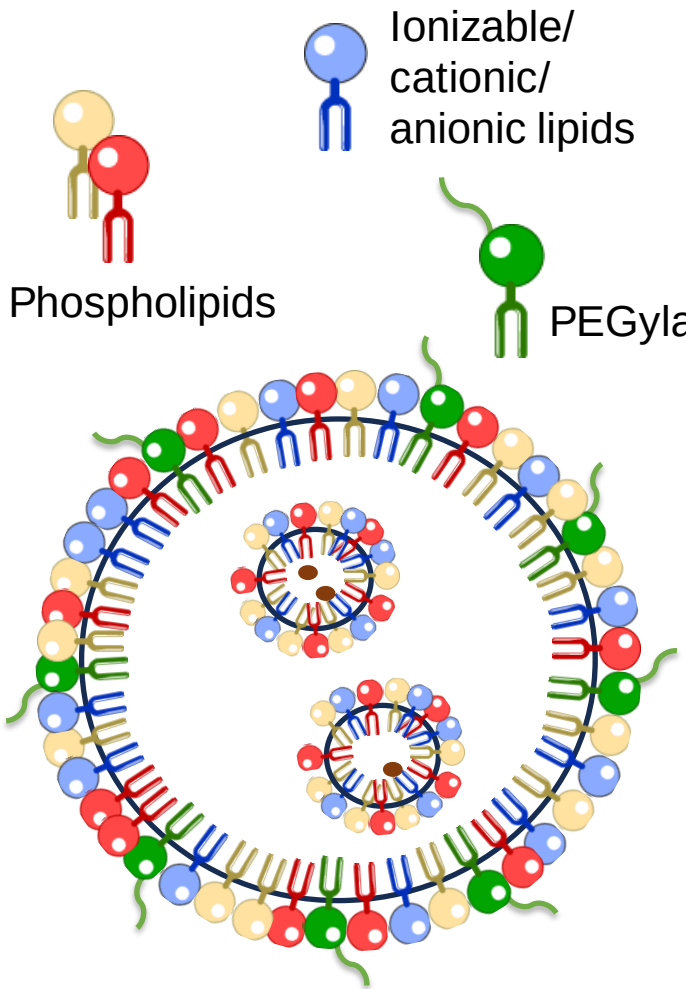
Human Leukocyte Antigen-free Biomimetic Proteolipid Vesicles as a donor-independent delivery platform for overcoming alloreactivity

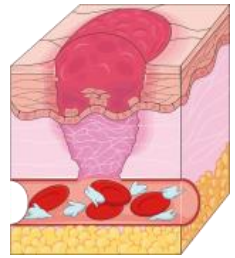
CONTROLLED RELEASE SOCIETY
CRS 2024 Annual Meeting AND Exposition
JULY 8-12, 2024 • BOLOGNA, ITALY

INTEGRATING
Delivery Science
ACROSS DISCIPLINES

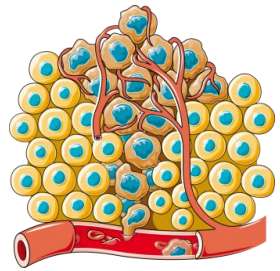
Lipid nano-particles (NPs): application

- Multiple benefits over conventional drug delivery systems like increased plasma half-life and improved biodistribution.
- Continuous, direct, and controlled drug release to malignant cells.
- Vast field for biomedical applications, such as gene delivery, site-specific targeted drug delivery system, or to design and develop new imaging methods.

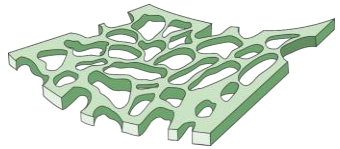




Wound healing



Cancer



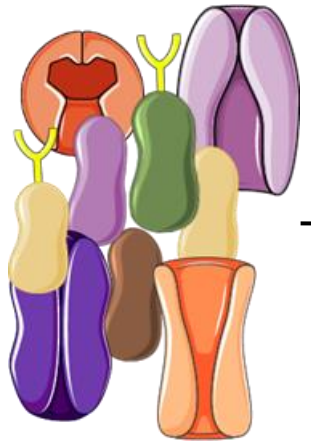
Tissue engineering

Drug delivery

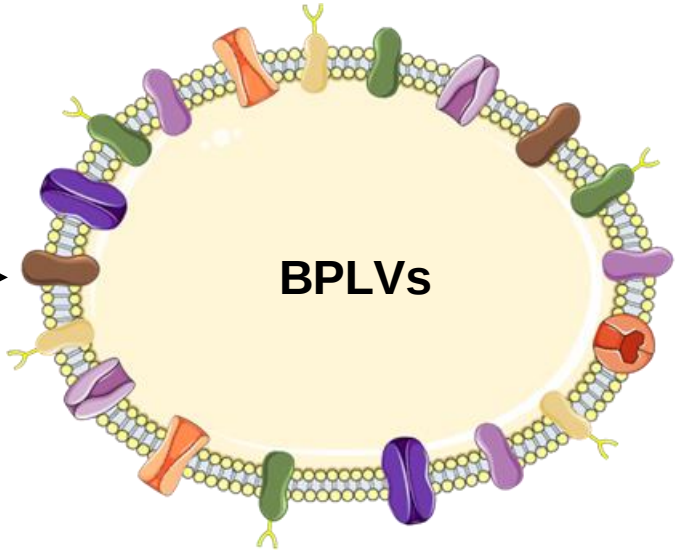
Biomimetic proteolipid vesicles (BPLVs)

PBMCs

Membrane protein extraction

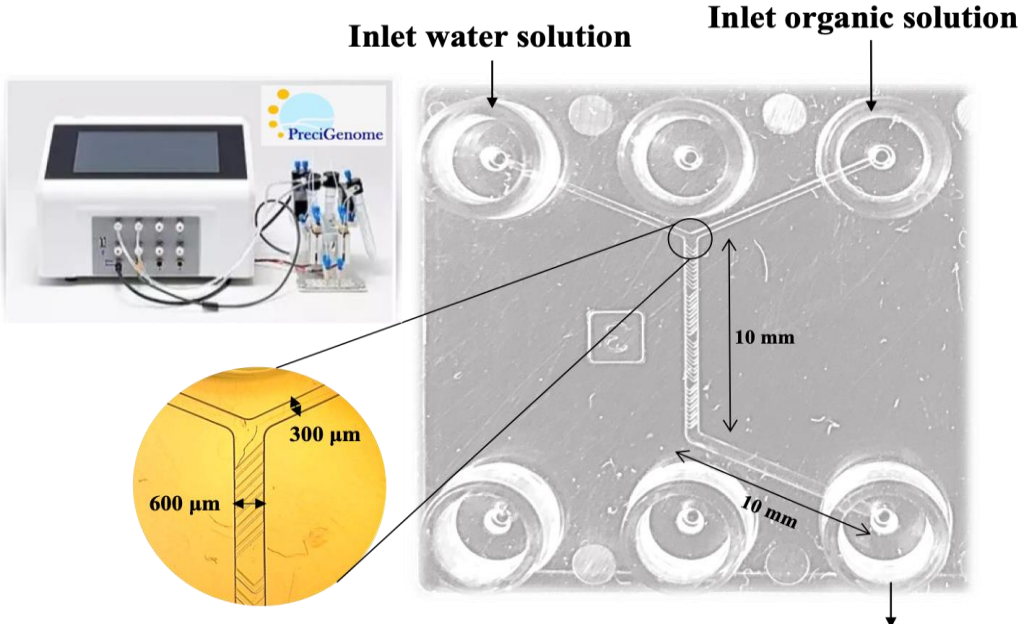


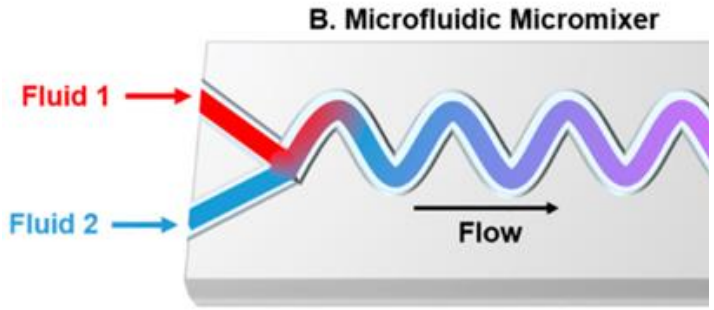
Membrane proteins

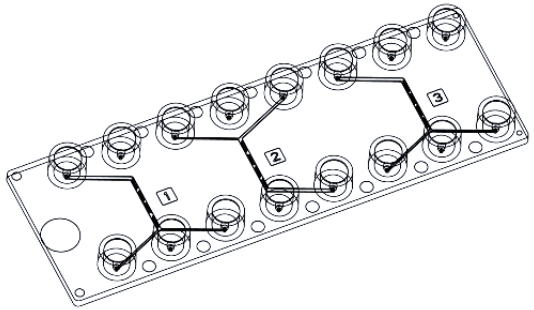


- ✓ A new potential protein-delivery system with high biocompatibility and tolerability.
- ✓ Proteins are incorporated within vesicles and correctly positioned.
- ✓ Loaded proteins are uptake by targeted cells and exposed on cell surface to revert specific protein deficiencies without modifying cell genome.

Microfluidic technologies



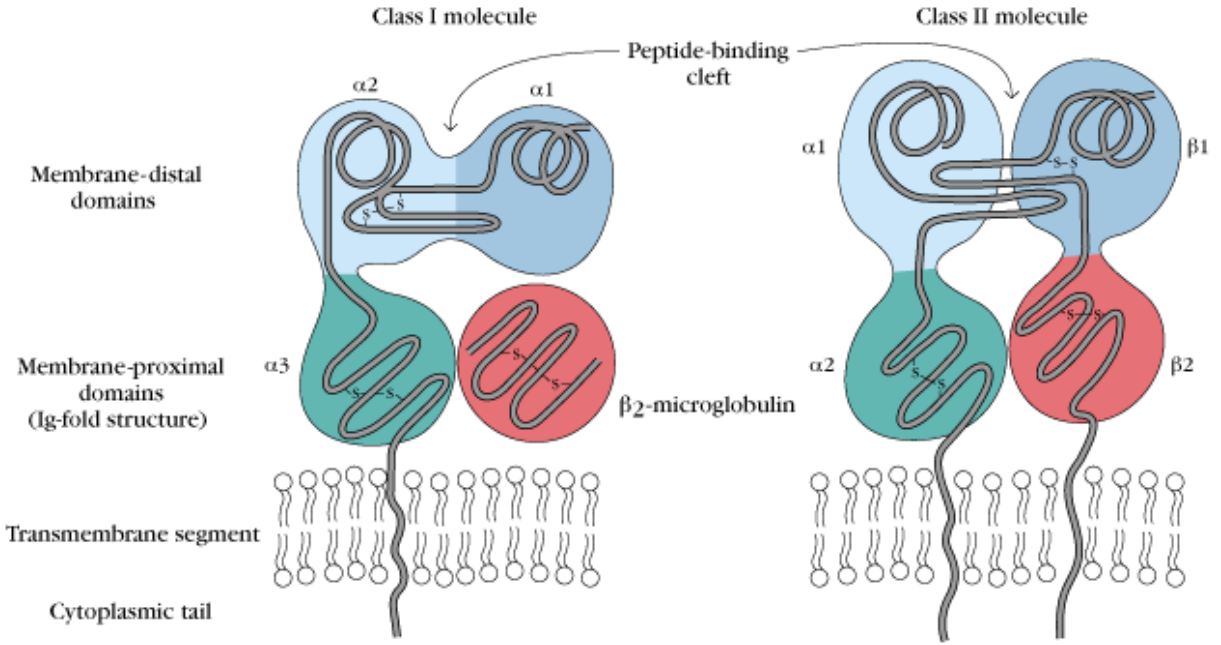




- 3D structure reactors consisting of micron-size (10–1000 µm) channel networks with different geometries.
  - ✓ ***Laminar flow***
  - ✓ ***Lower critical Reynolds number for transition***
  - ✓ ***Mixing by diffusion***
  - ✓ ***Fast heat and mass transfer***
  - ✓ ***Precise control***
  - ✓ ***Low reagent consumption***
  - ✓ ***Narrow residence time distributions***

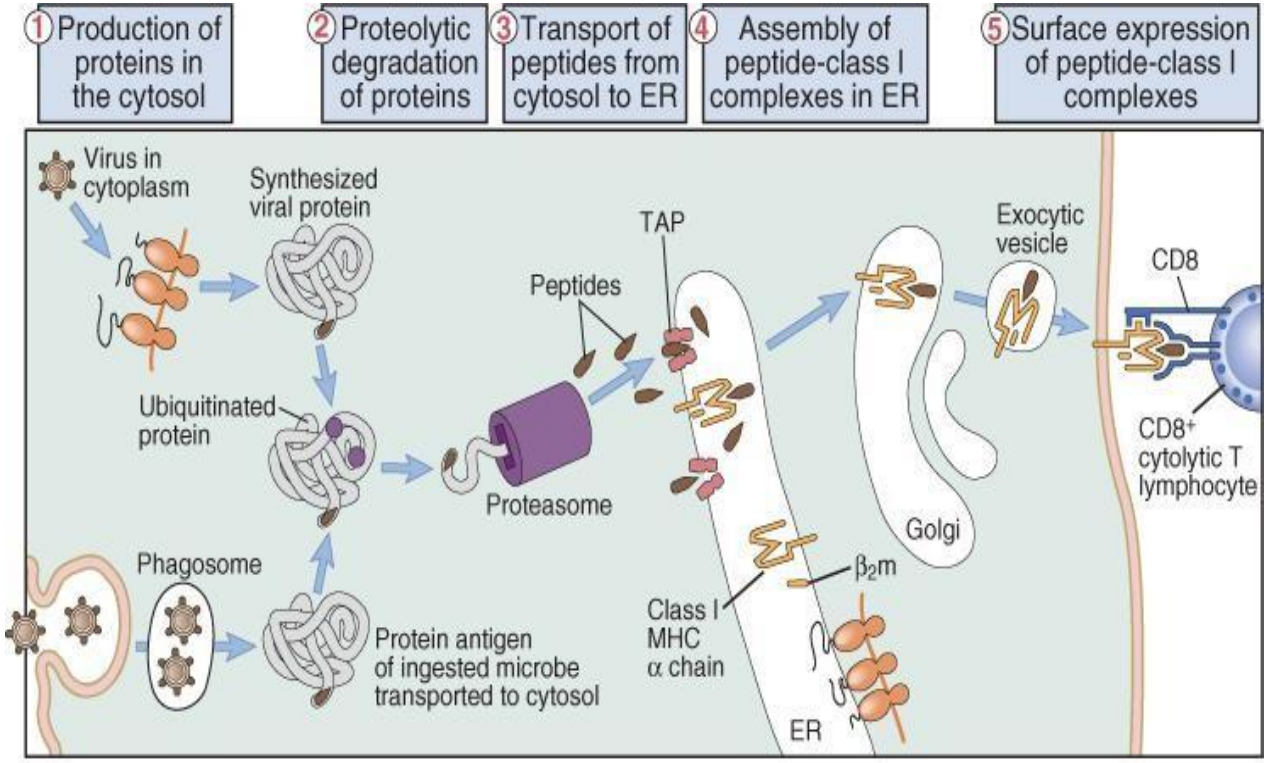
HLA complexity

- HLA (Human Leukocyte Antigen) discriminates between self- and non-self and is involved in immune responses. On chromosome 6p, divided in three regions:
- **HLA-class I, including A,B,C**
  - Expressed on all nucleated cells.
  - Can present antigens (non-self) to cytotoxic T cells.
- **HLA-class II, including DR, DP, DQ**
  - Expressed on macrophages, B lymphocytes, dendritic cells.
  - Present antigens to T helper cells.
- **HLA-class III, including C2, C4, TNF**
  - Secreted proteins with immunological functions.
  - Complement sistem
  - Prof-inflammatory cytokines.

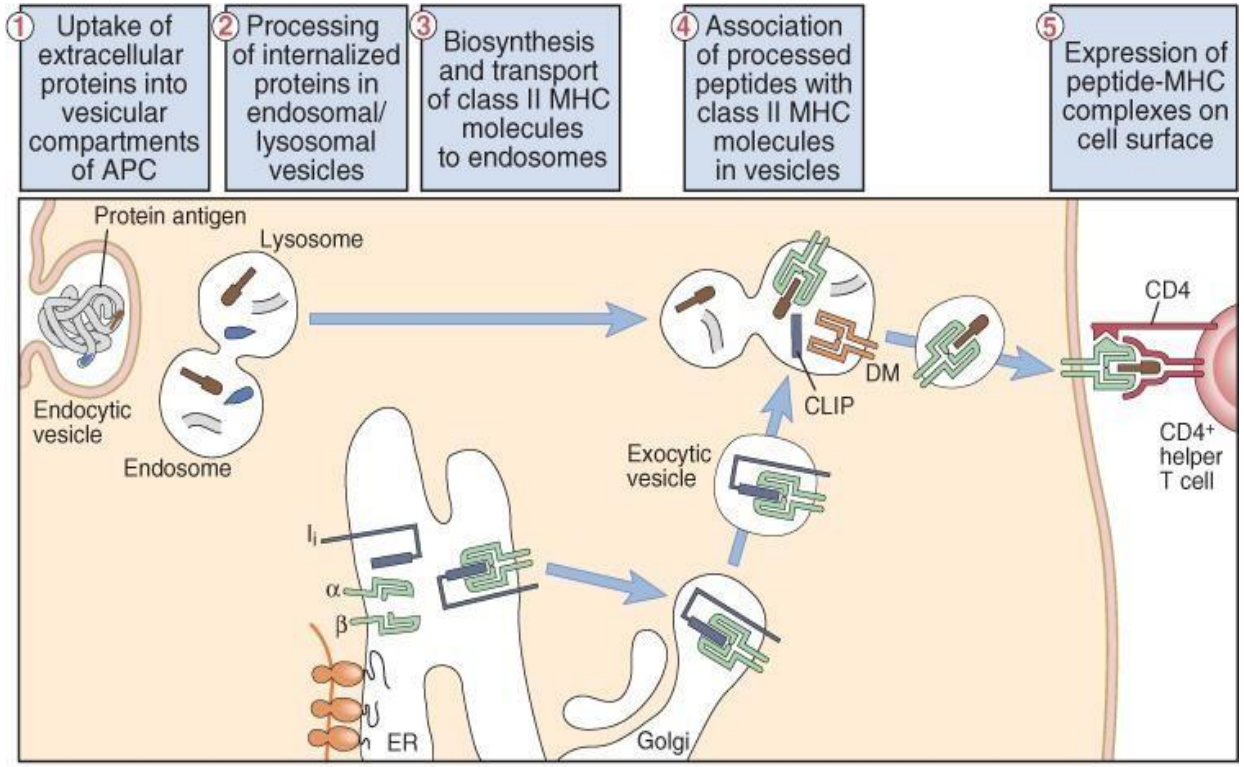


HLA-matching in immune responses

Class I



Class II



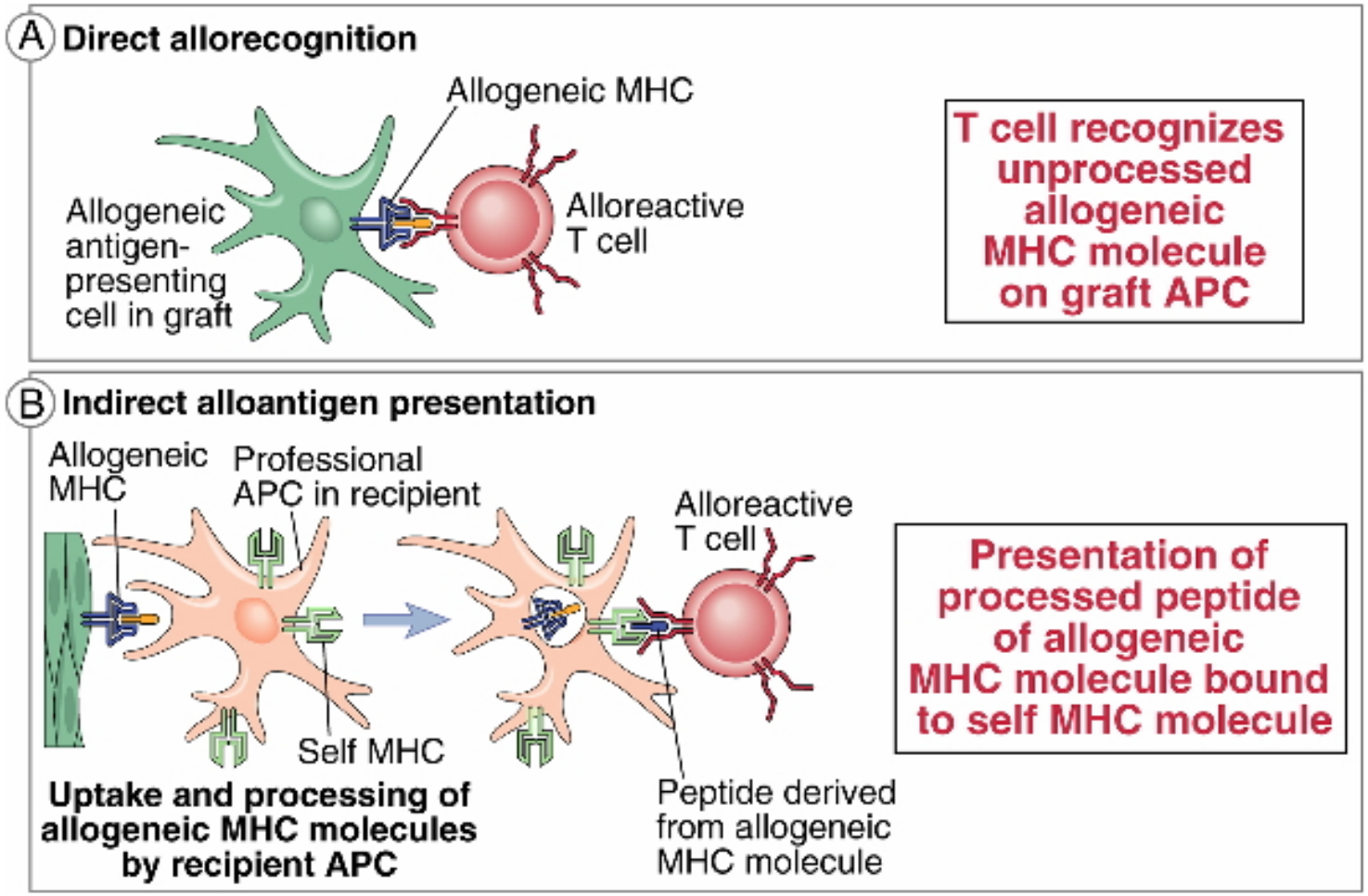
HLA-matching in tissue and organ transplantation

In organ and tissue transplantation, donor cells should not recognize as non-self recipient cells.
Otherwise, HLA-mismatch causes:

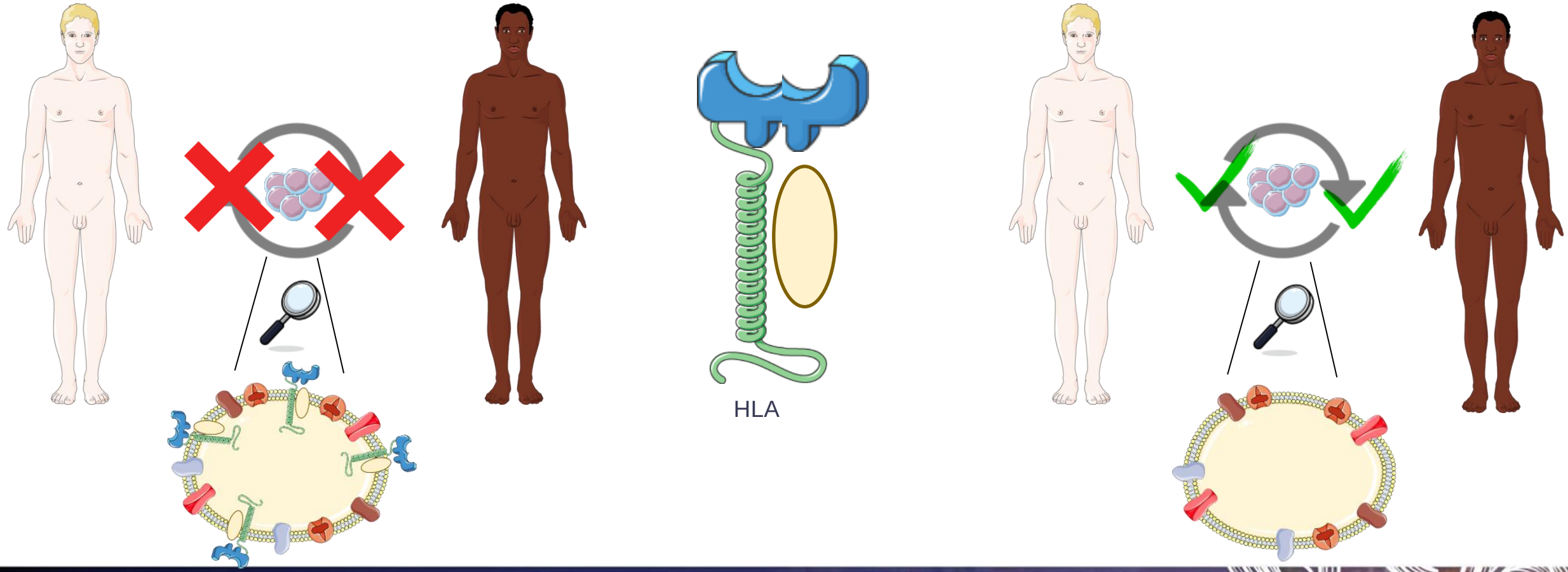
- **GRAFT FAILURE**
- **GRAFT VERSUS HOST DISEASE**

The only benefit in hematological malignancies:

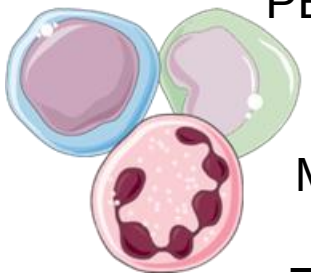
- **GRAFT VERSUS LEUKEMIA**



HLA-issue in allogenic transplantation: a troubleshoot

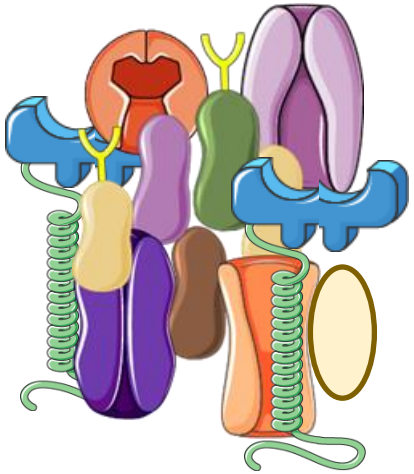


HLA immunoprecipitation



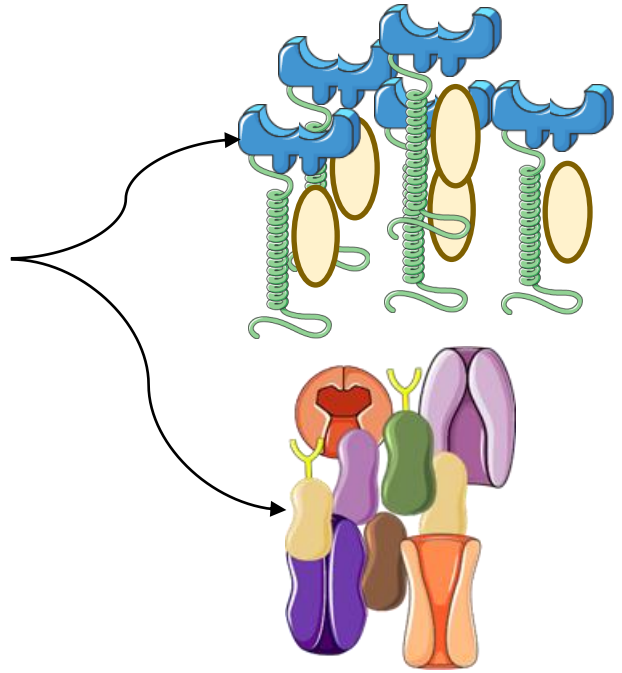
PBMCs

Membrane protein extraction



Membrane proteins

Immunoprecipitation using anti-HLA-A,B,C antibody



Total lysates from membrane proteins

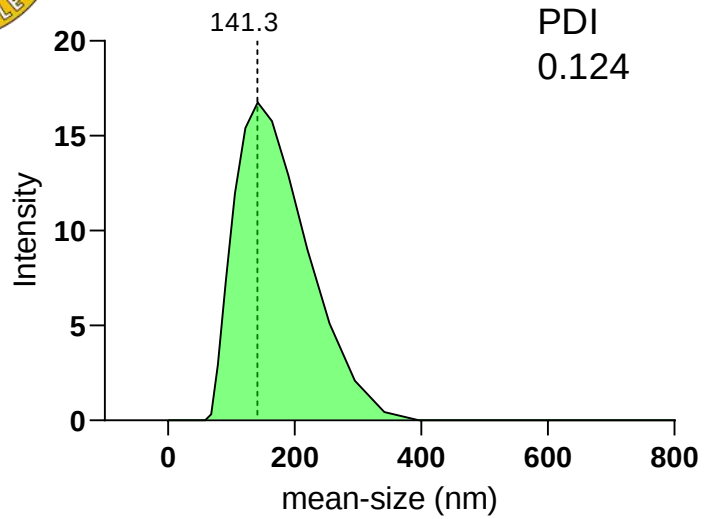
40 kDa

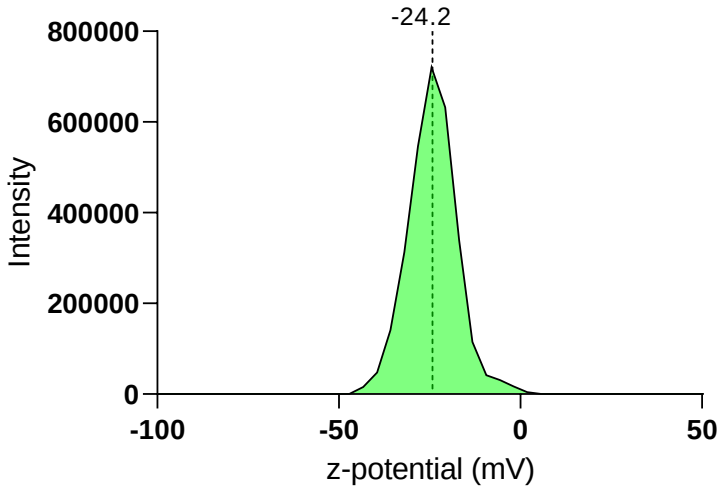
No HLA fraction

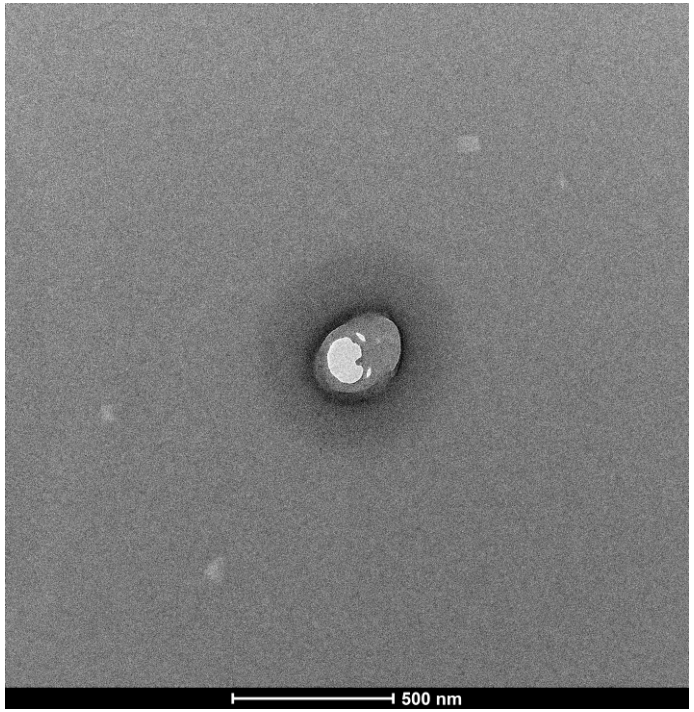
Eluate fraction

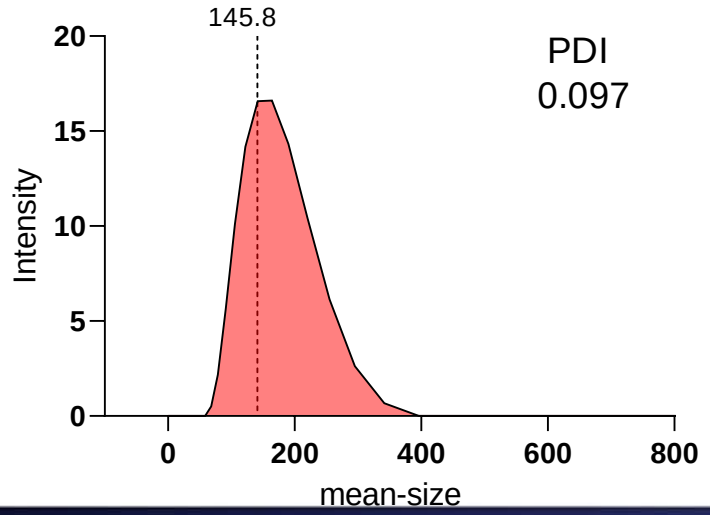
40 kDa

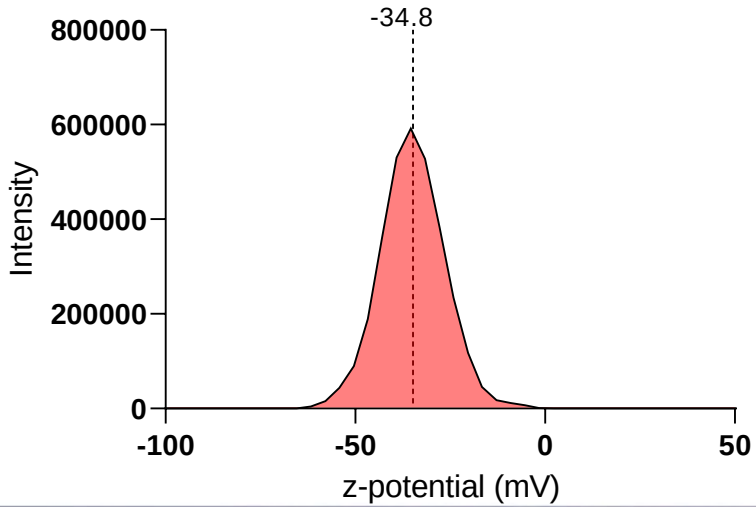
Morphological characterization



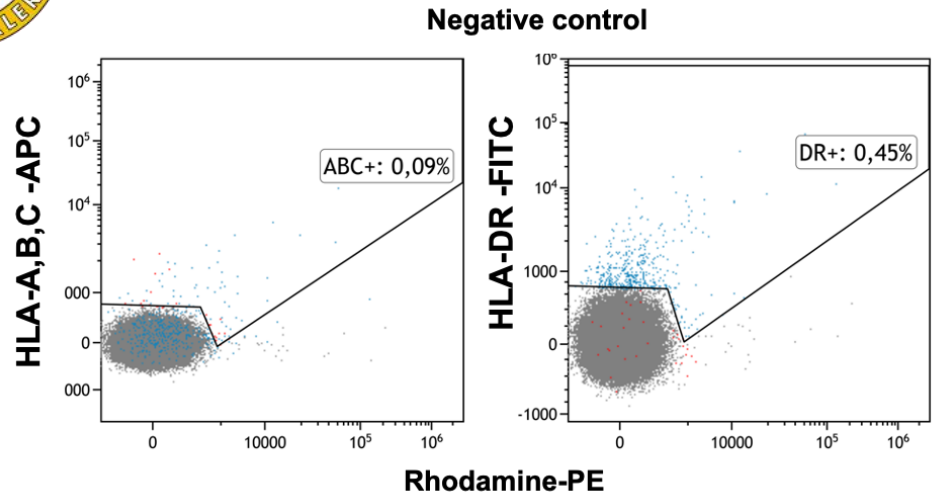


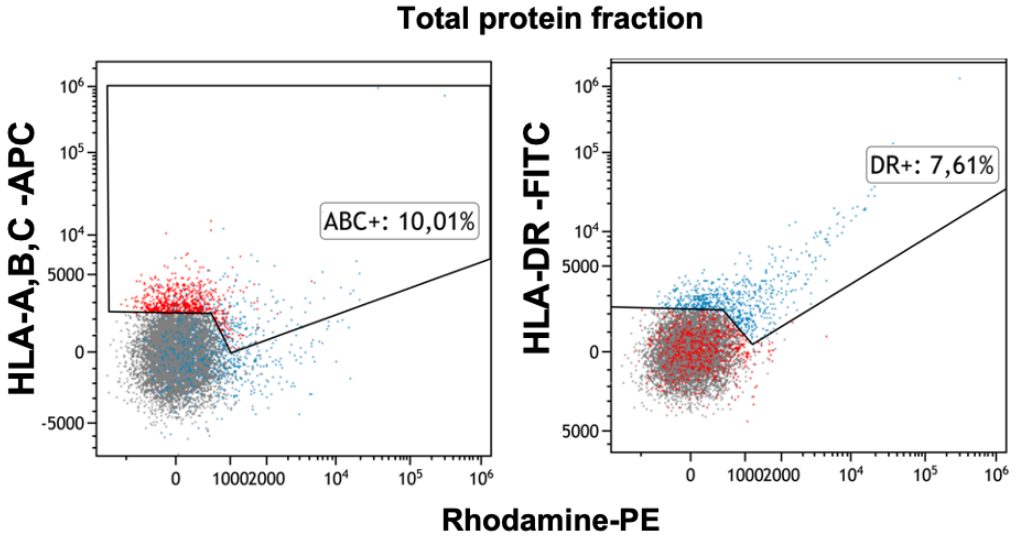


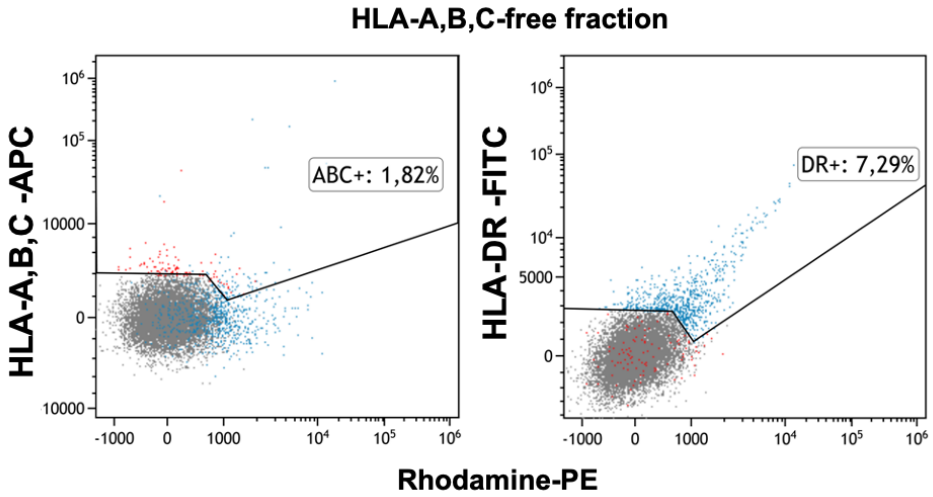


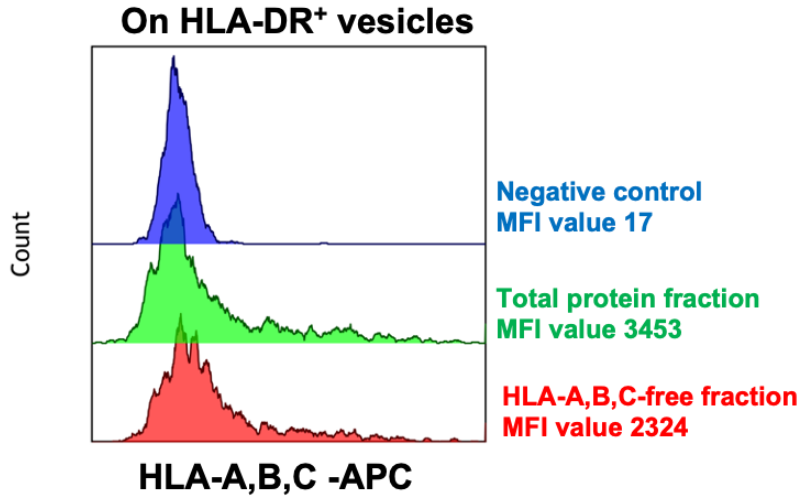


Protein characterization: Flow cytometry



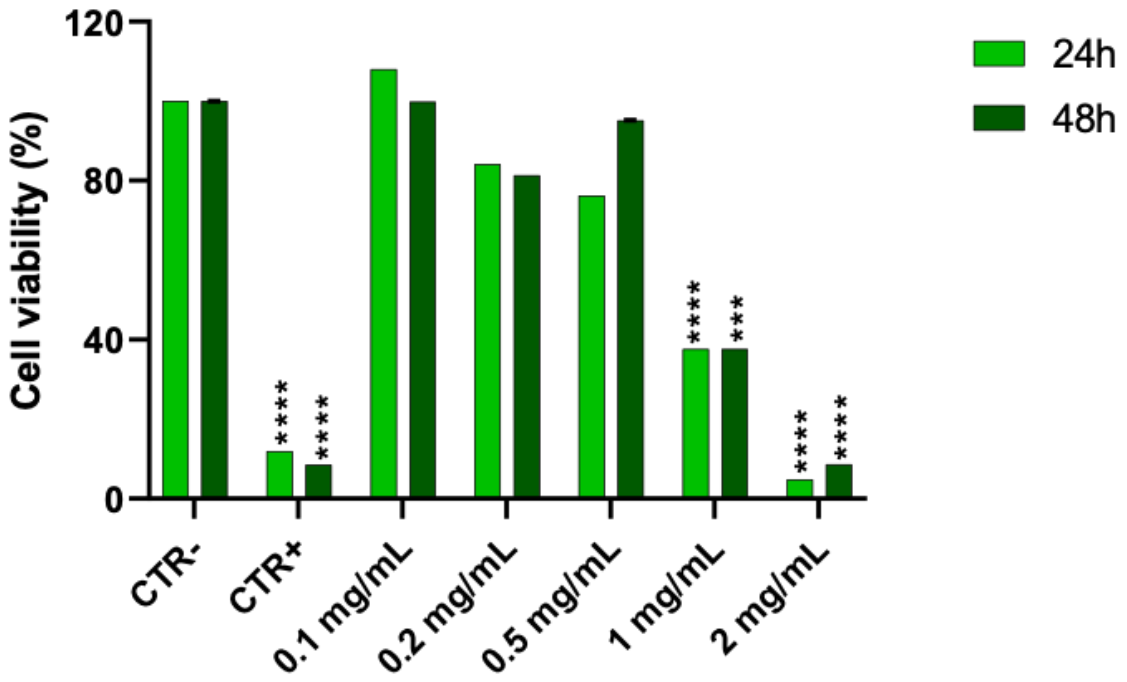




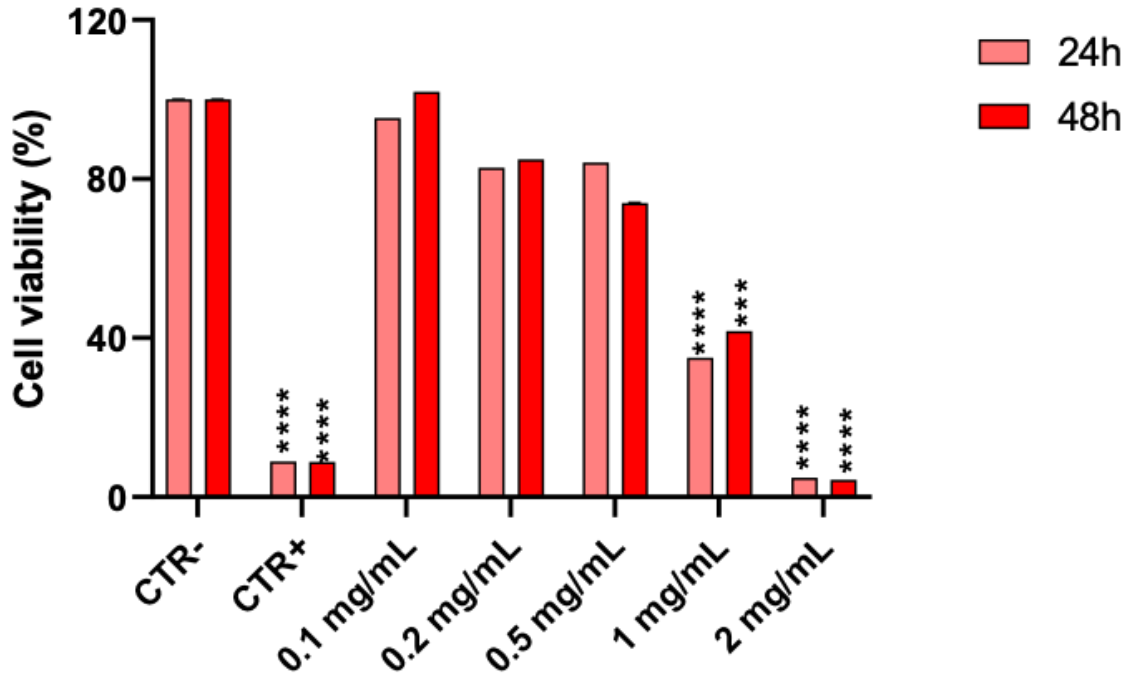


Biocompatibility: cell viability

BPLVs + total membrane proteins



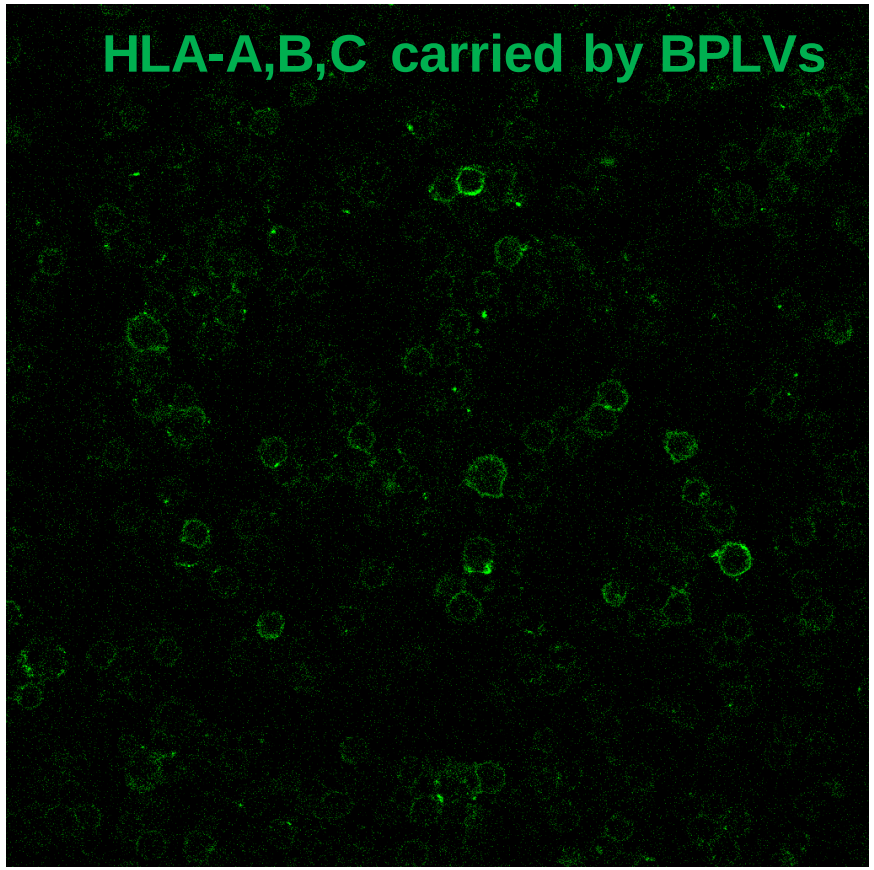
BPLVs-HLA-A,B,C free



Extremely high biocompatibility

Cellular uptake

BPLVs + total membrane proteins



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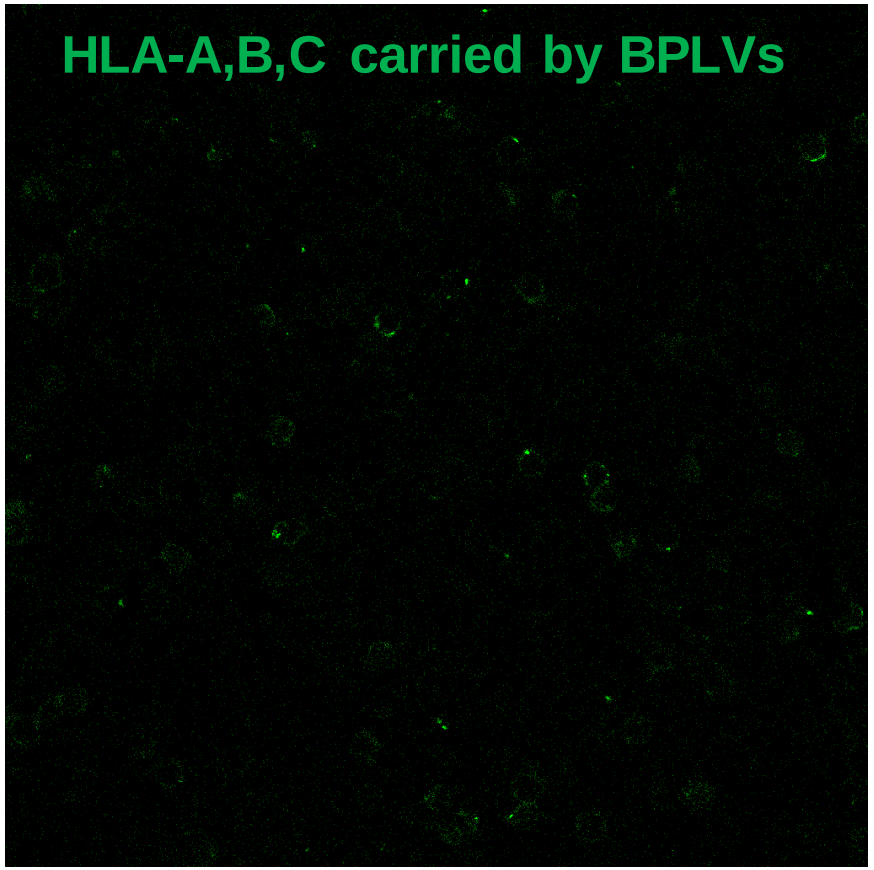

Cellular uptake

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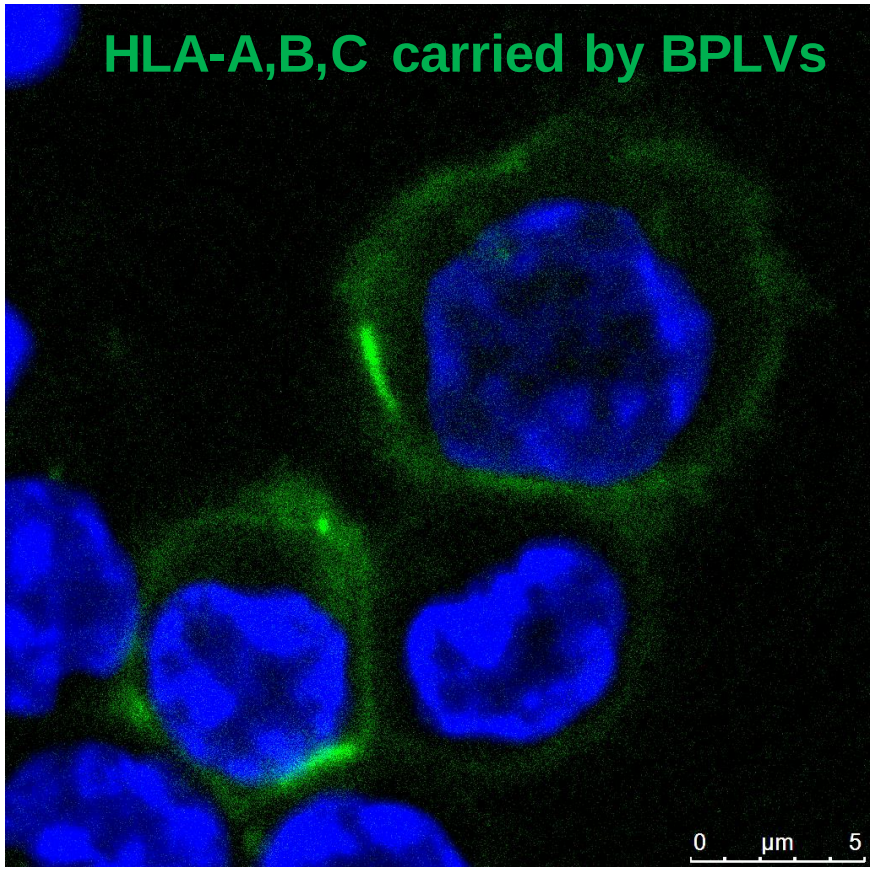

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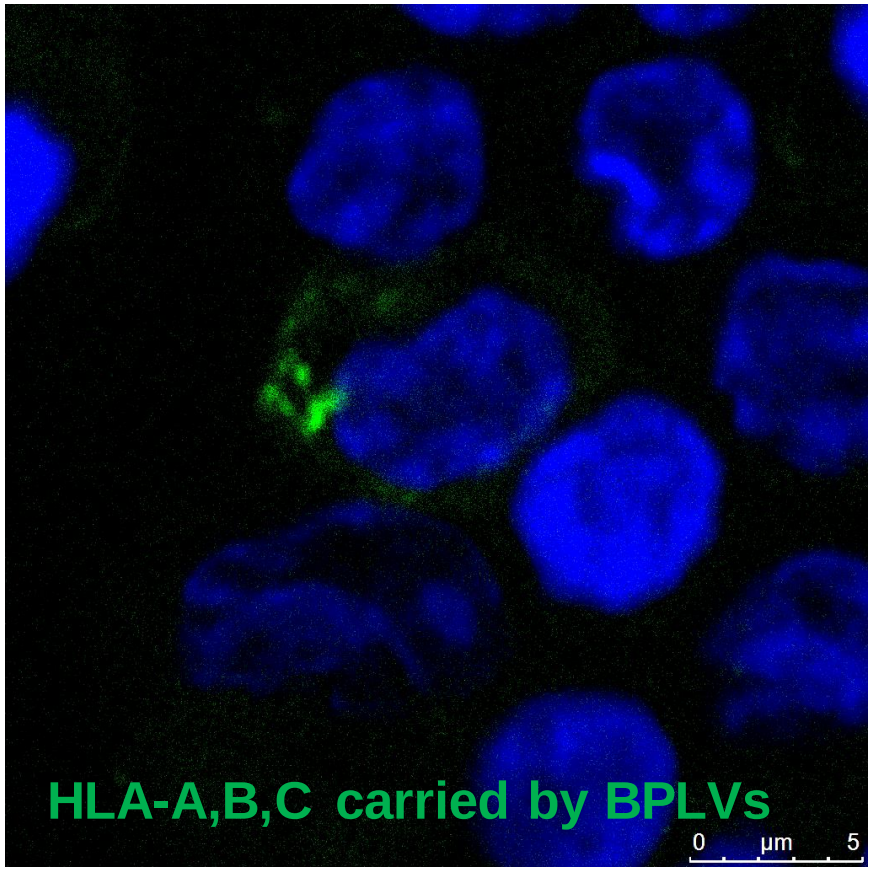
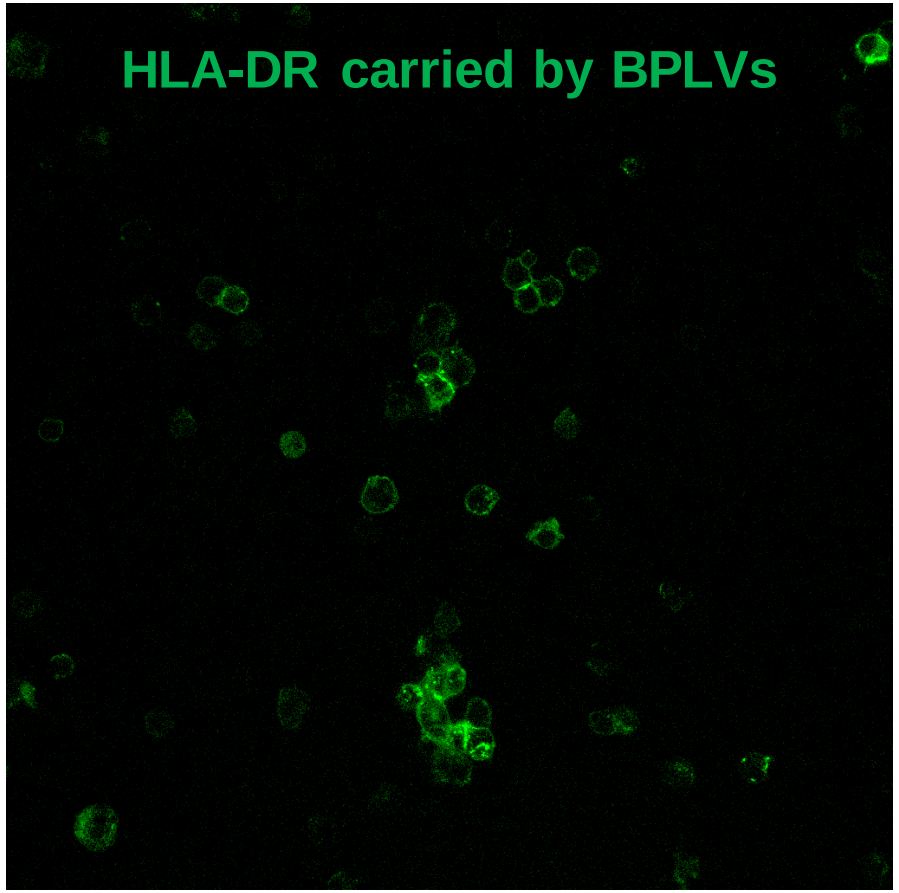
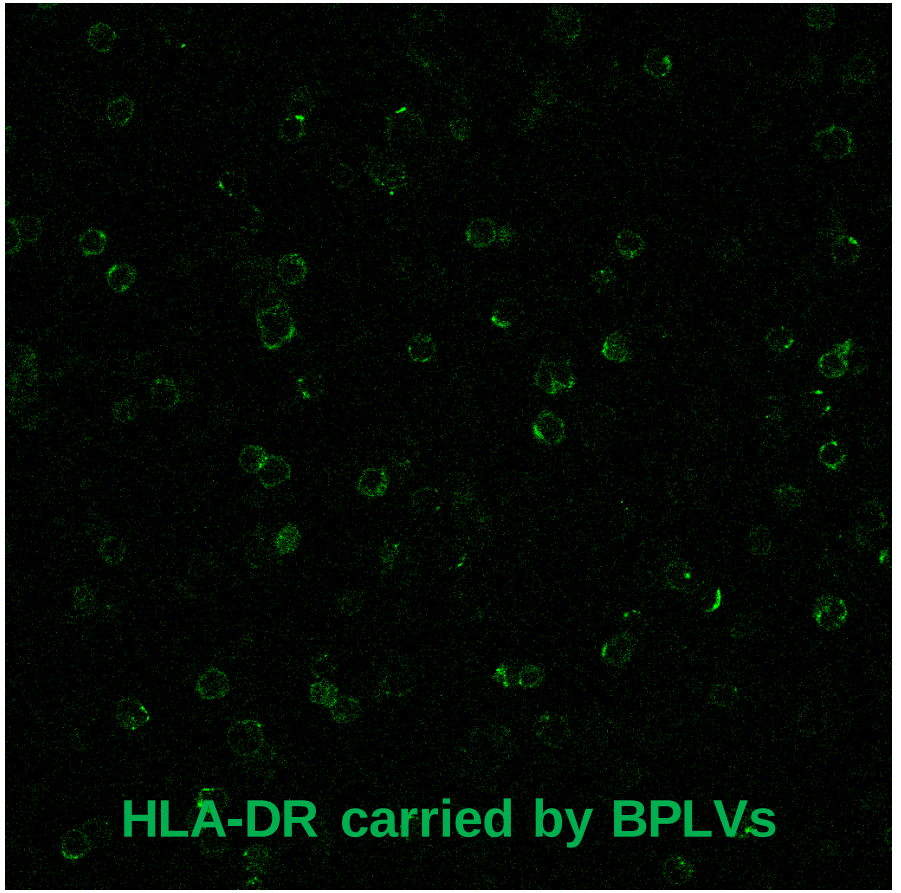


Cellular uptake

BPLVs + total membrane proteins



BPLVs-HLA-A,B,C free



Conclusions and further perspectives

- BPLVs can be efficiently and reproducibly produced by microfluidics approach and can be used to transfer proteins, drugs, or bioactive agents to target cells.
- BPLVs can be employed as ready-to-use protein delivery platform.
- Saving time, lowering cost, and making this therapeutic approach more accessible to patients.
- HLA matching limits the broad use of donor-derived BPLVs and can induce immune-mediated reactions.
- Using our approach, HLA-matching can be overcome and BPLVs can become an HLA-free protein delivery platforms.
- Future perfect continuous: increase the purity of HLA-A,B,C fraction and remove also HLA-DR,DP.DQ.
- Making BPLV uptake faster and easier.

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