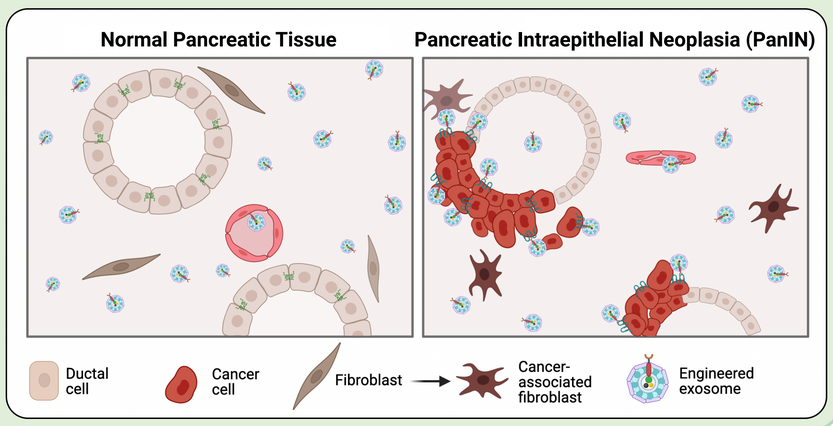
An innovative solution for early detection of pancreatic cancer

Introduction

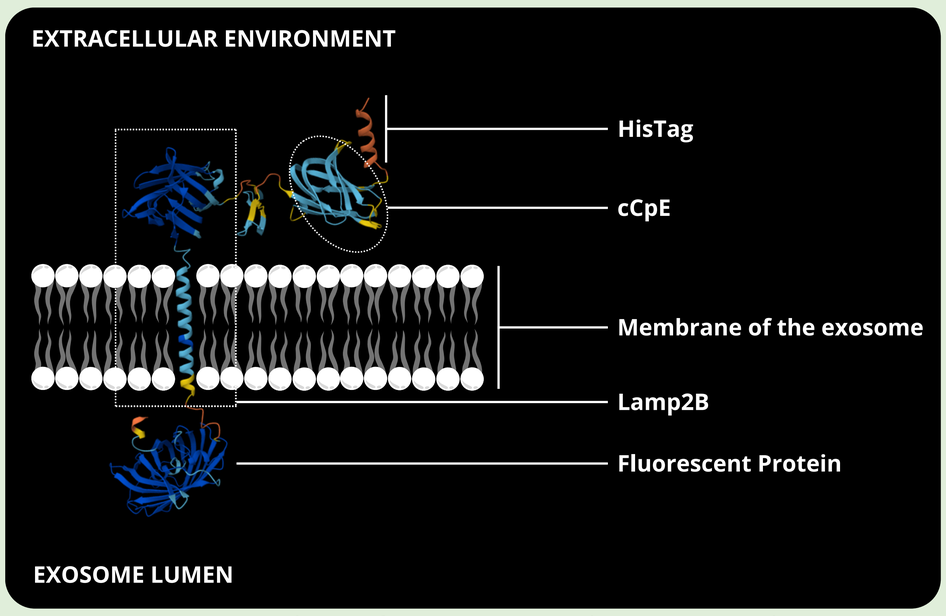
iGEM (International Genetically Engineered Machine) is an international synthetic biology competition launched by MIT in 2003. It brings together teams of students (from high school to undergraduate and master's levels) to design innovative projects addressing major societal challenges in fields such as health, environment, agriculture, energy, and more.
Beyond laboratory experimentation, teams are expected to build a wiki, collaborate with experts, engage with the public, and thoroughly document their work.

The strategy

The project, ExoSPY, aims to develop an exosome-based diagnostic and therapeutic tool for early detection of pancreatic cancer, which currently has a poor prognosis and a 5-year survival rate after diagnosis of ~10%. We engineer exosomes to display a ligand incorporing a fragment of the *Clostridium perfringens* enterotoxin known to bind the Claudin-4, a protein overexpressed and exposed on tumor cells. These exosomes are produced in HEK293T cells, purified, and characterized. Their targeting ability will be assessed *in vitro* on pancreatic cancer models.



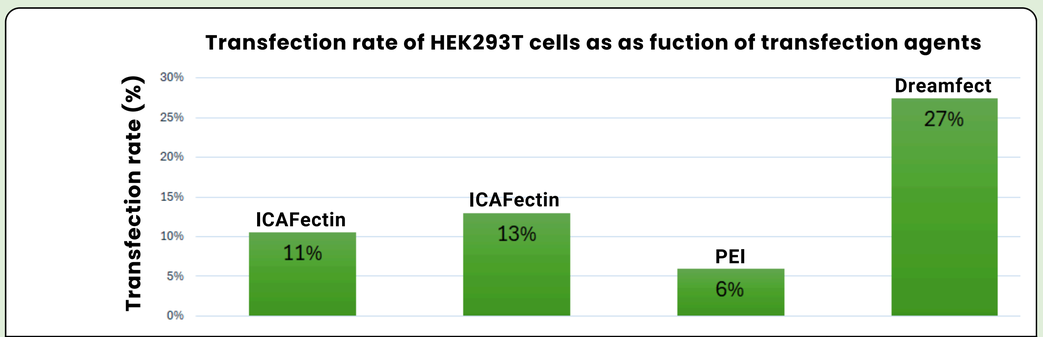
Modelisation



AlphaFold3-based modeling of the recombinant protein, anchored to the exosome membrane.

The figure illustrates the design of the recombinant protein for targeting pancreatic cancer using exosomes. The protein is anchored to the exosome membrane through Lamp2B and presents at an extraexosomal level a His-tag and a cCPE domain for purification and Claudin-4 recognition, respectively. An internal fluorescent protein (GFP or meOsFast1) allows us to track the exosomes.

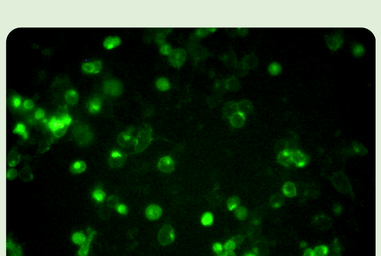
Transfection



Transfection reagent (µL)	1,5	1,5	1,5	1,5
DNA (µg)	0,5	1	0,5	0,5
Ratio [transfection reagent :DNA]	[3:1]	[3:2]	[3:1]	[3:1]

We will produce modified exosomes with HEK293T cells, transfected in 24-well plate. We compared different [DNA:transfection agent] ratios and assessed efficacy via expression of GFP encoded by the pcDNA3.1 plasmid. Among the conditions tested, Dreamfect® proved to be the most efficient transfection agent, achieving a transfection rate of 27.4%.

ICAFectin®441 showed intermediate efficacy (up to 13%), while PEI produced only limited results. Additional tests showed that culture volume and antibiotics had little impact on transfection rates.



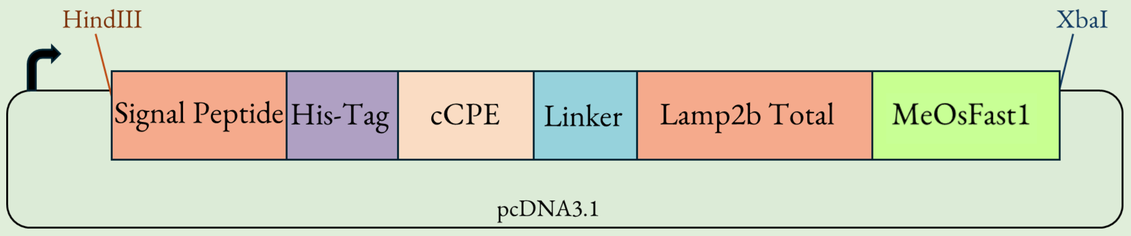
Dreamfect®transfection of HEK293T with 0,5µg DNA ratio [1:3]

Recombinant protein

We used HindIII and XbaI to clone the insert including the His-tag, cCPE, Lamp2B and a linker.
Several constructs were designed to assess whether the length of some domains (Lamp2B and the Linker) within the recombinant protein could influence the exosome's ability to bind the Claudin-4.

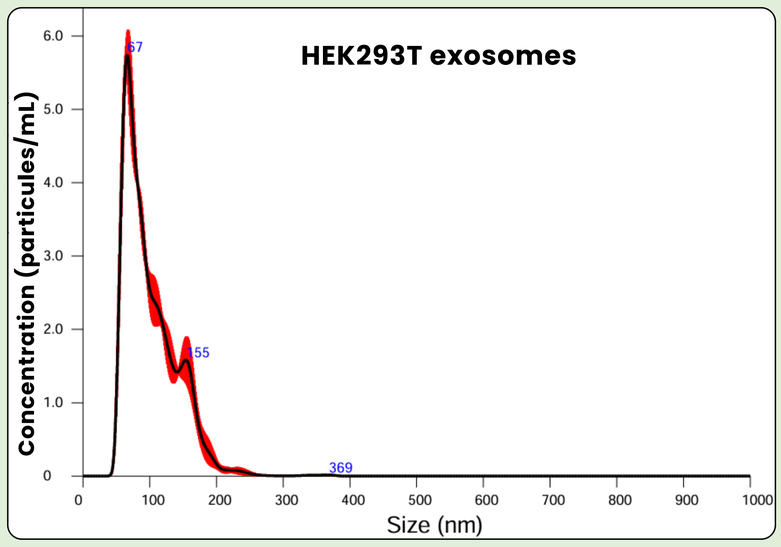
We will use a Golden Gate with Type IIS enzyme (Esp31) cloning to insert different fluorescent protein :

- GFP
- MeOsFast1, in order to later perform PALM microscopy



In the project, we will transfect HEK293T cells with this plasmid and the protein will be in the membrane of exosomes to catch the cancerous pancreatic cells.

Exosomes Characterisation



D10	59.4 nm
D50	88.5 nm
D90	156.1 nm

Size distribution of exosomes performed using NTA analysis with the NanoSight NS300 system. Sample diluted 1:500

From 100 mL of HEK293T cell culture, a concentration of 3.40×10^{11} particles/mL was obtained, with the most frequent value being 65.9 nm.
Among the extracellular vesicles collected, 10% were smaller than 59.4 nm, 50% were smaller than 88.6 nm, and 90% were smaller than 155.4 nm.

Perspectives

- Compare exosome populations from transfected versus non-transfected HEK293T and assess transfection efficiency via flow cytometry.
- Use electron microscopy to visualize the fusion protein on exosome surfaces and characterize modified vesicles.

Sponsored by:

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