On the Amyloid-β transcytosis across the blood-brain barrier

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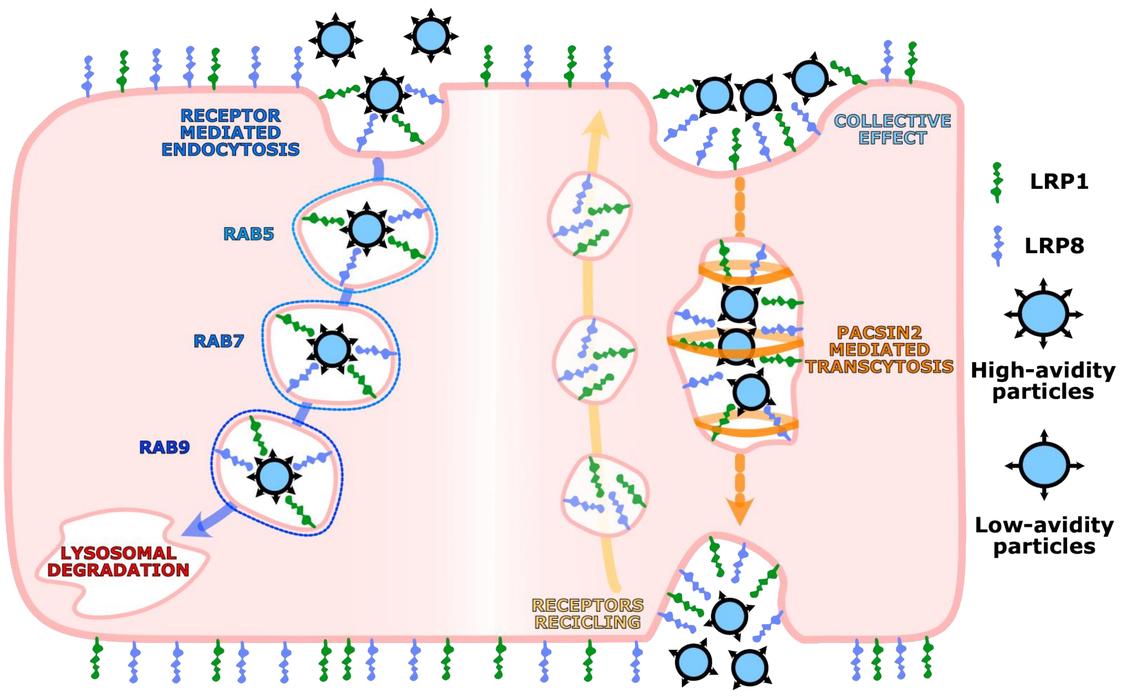
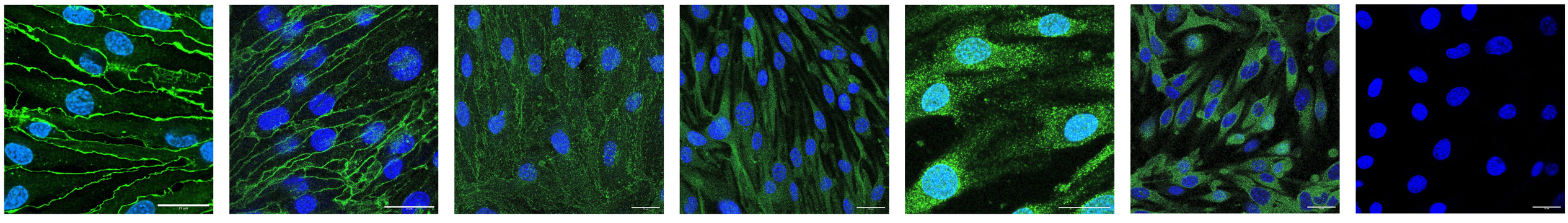
BACKGROUND



The **blood-brain barrier (BBB),** among its multiple metabolic functions, is also responsible for regulating the transport of misfolded proteins to and from the Central Nervous System (CNS). One of the key players in such a process is low-density lipoprotein receptor-related proteins, including **LRP1, LRP2, and LRP8**. LRP1 is known to control the shuttling of several misfolded proteins, including **amyloid-β (Aβ).** Our research has revealed that the BBB controls the trafficking of large molecules as a function of their avidity towards LRP1. **High-avidity molecules** can be retained within the **endocytic pathway**, while **mid-avidity molecules** are **transported via tubular vesicles** that are stabilised by the BAR domain protein, **PACSIN2**. We are currently investigating the role of other LRP receptors and developing multivalent polymeric nanoparticles (NPs) that can modulate and enhance this process to expedite the removal of Aβ.

IMMUNOCYTOCHEMISTRY

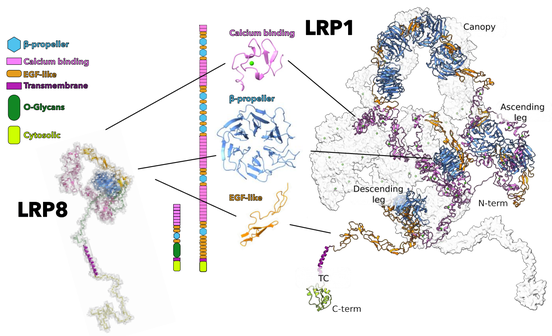
ZO-1 | CLAUDIN5 | OCCLUDIN | PACSIN2 | LRP1 | LRP8 | N.C.



PROXIMITY LIGATION ASSAY

AIM

We want to study the **colocalisation** between **LRP1 and LRP8** receptors and intracellular protein as **PACSIN2**.

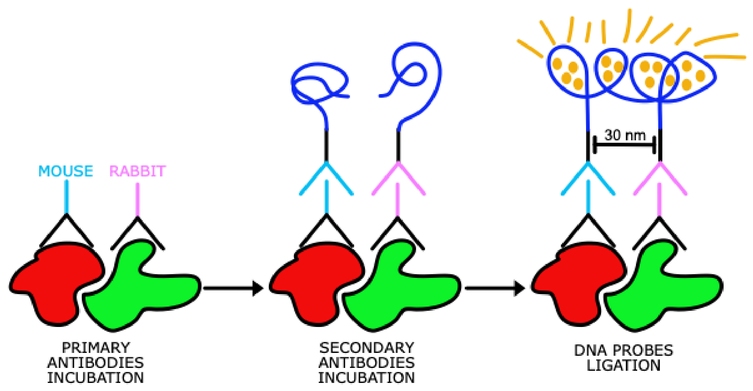


We are particularly interested in understanding their behaviours when cells are treated with **Aß peptides** as oligomers of various diameters and **fibrils**.

HOW IT WORKS

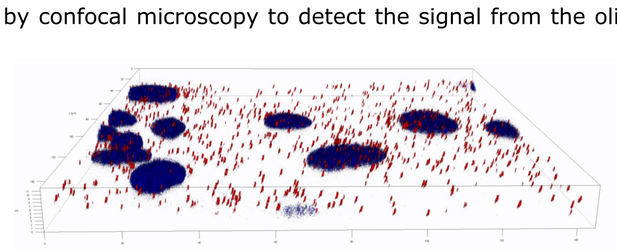
The Proximity Ligation Assay (PLA) is an immunocytochemistry-based technique that allows the identification of **protein-protein colocalisation within a 30 nm range**.

In such a technique, the **two secondary antibodies have DNA-based oligos that hybridise.** Then, the ligation and amplification processes ensure signal amplification from labelled oligos.

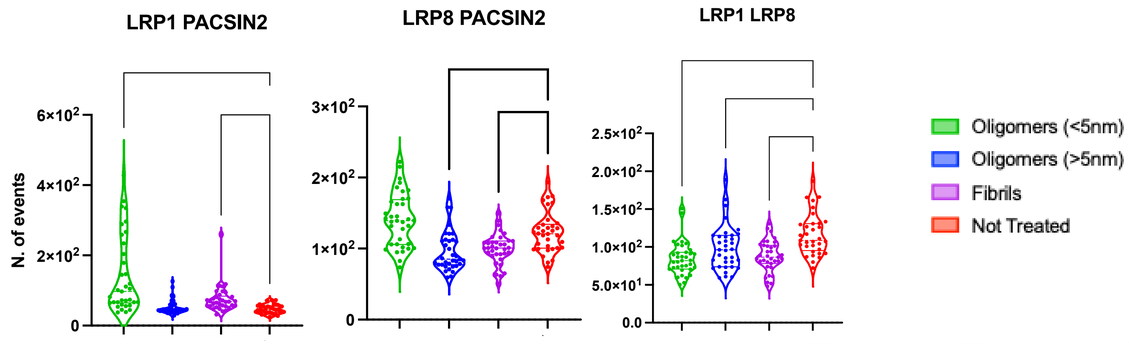


HOW WE ANALYSE IT

The samples are then imaged by confocal microscopy to detect the signal from the oligos, shown in red on the image.

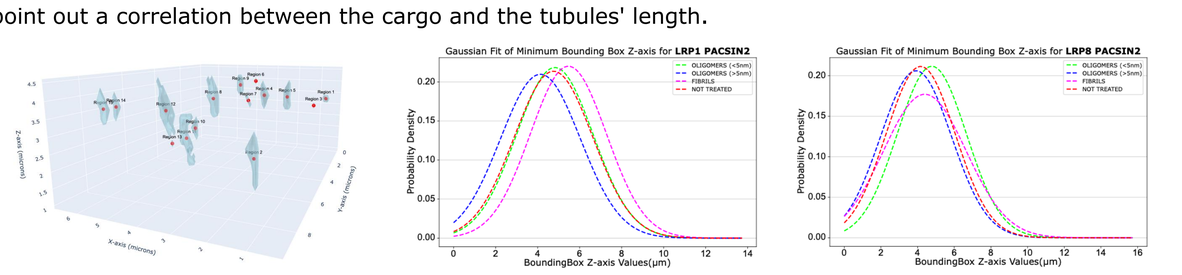


The first step consists in evaluating the 'number of events' happening for every colocalisation we are considering.



Data have been tested for Normality and Lognormality. A nonparametric test, the Kruskal-Wallis test, has been done. Pvalue: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), <0.0001 (****)

Then, we also analyse the dimensions of such tubular structures, particularly the height on the z-axis, to point out a correlation between the cargo and the tubules' length.



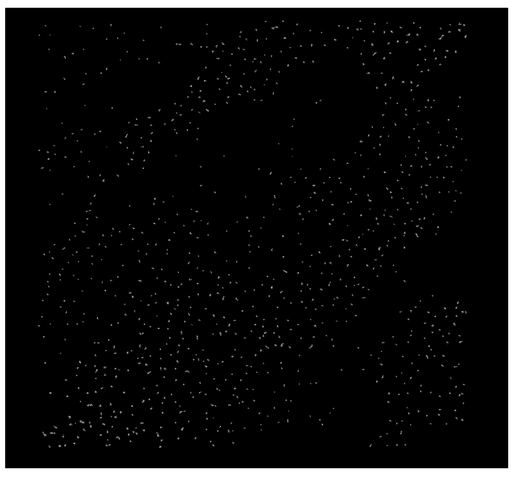
Data were tested using both ANOVA and t-tests. All the conditions are significantly different.

STORM

STORM (STochastic Optical Reconstruction Microscopy) is a super-resolution imaging technique that allows visualisation of structures at the nanoscale by sequentially activating and localising individual fluorescent molecules.

We aim to study the **clusterisation** and colocalisation **of LRP receptors** at the cellular membrane.

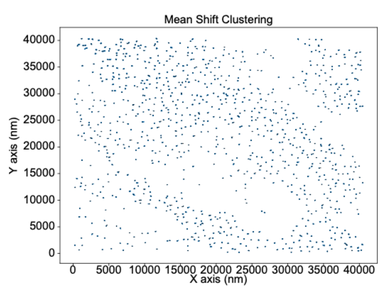
STORM image of LRP1 on the membrane of bEnd3

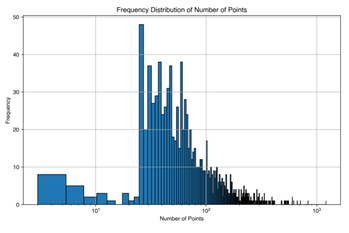


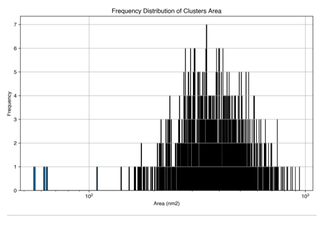
Primary antibody: rabbit anti-LRP1

Secondary antibody: anti-rabbit Alexa488

Mean Shift Clustering Analysis

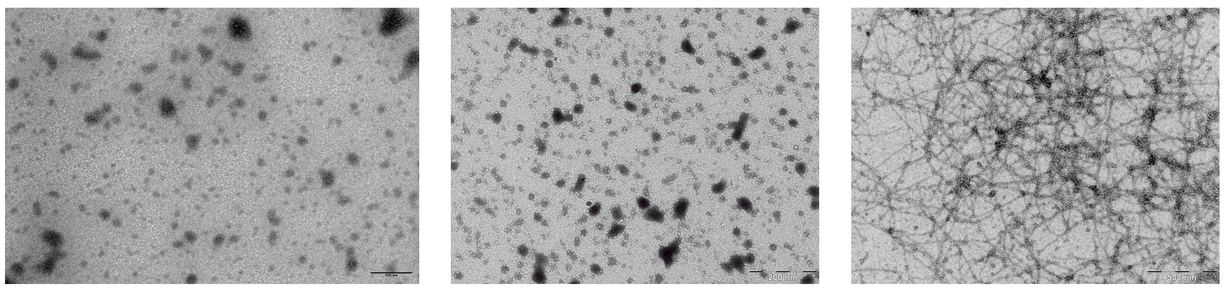






AMYLOID BETA

We also do Transmission Electron Microscopy (TEM) imaging to analyse the morphology of our **Aß-42** assemblies.



Oligomers (<5nm) | **Oligomers (>5nm)** | **Fibrils**

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