Modulating LRP1 and LRP8 in the blood-brain barrier using multivalent nanomedicines

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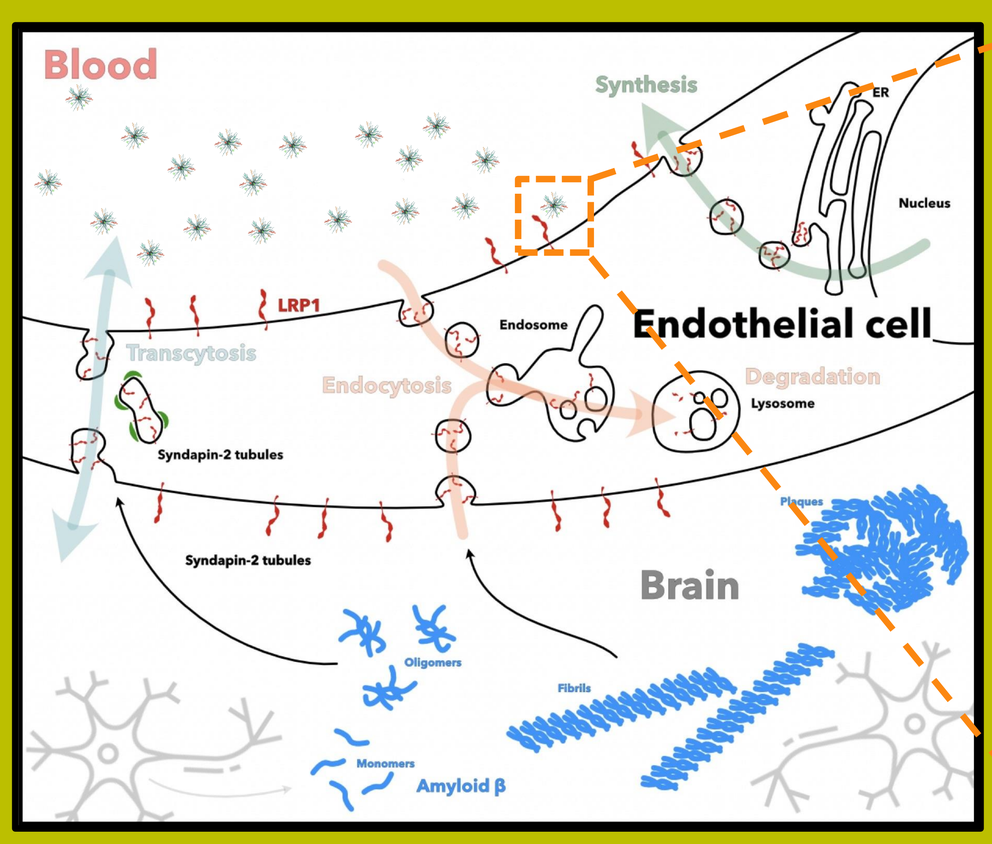
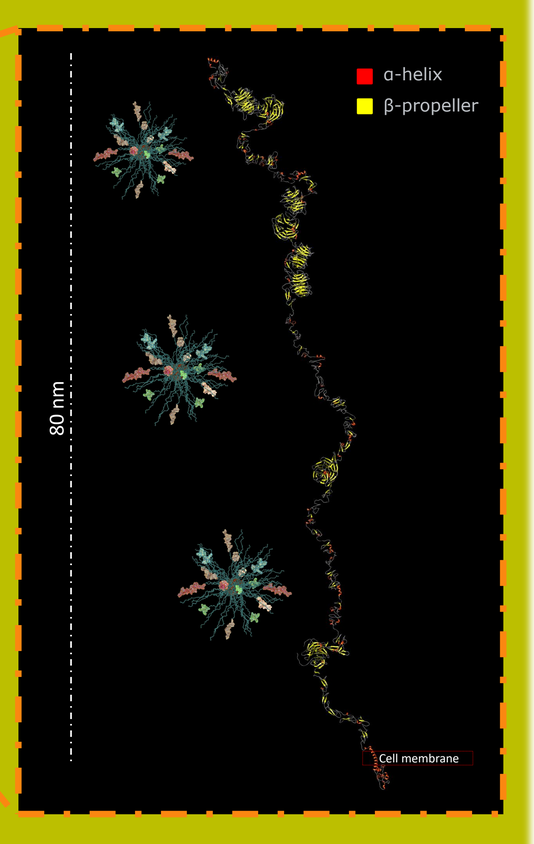
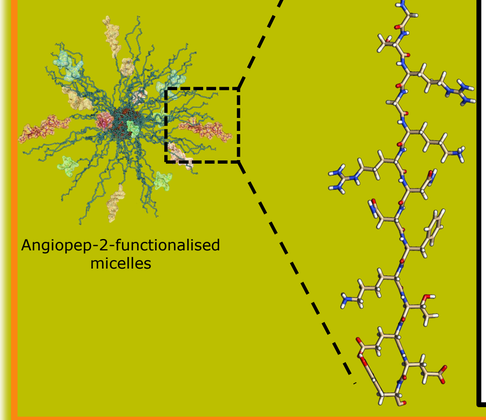
Background: The blood-brain barrier (BBB) plays a main role in regulating the transport of misfolded proteins from and into the CNS. Two of the different receptors involved have been singled out: the low-density lipoprotein receptor-related protein 1 (LRP1) and the low-density lipoprotein receptor-related protein 8 (LRP8), also known as apolipoprotein E receptor 2. Several data suggest a primary role of LRP1 in the shuttling of amyloid-β (Aβ) across the BBB. To stimulate such a physiological process, we formulate functionalised polymeric nanoparticles whose avidity, based on multiple ligand-receptor affinities, can reproduce what happens in vivo. To support our theory, we evaluate the expression of the main genes involved in the transcytosis and deeply characterise our BBB in vitro model. The investigations herein are an important step to further assess the enhancement of the clearance of Aβ from the CNS by using polymeric nanoparticles.

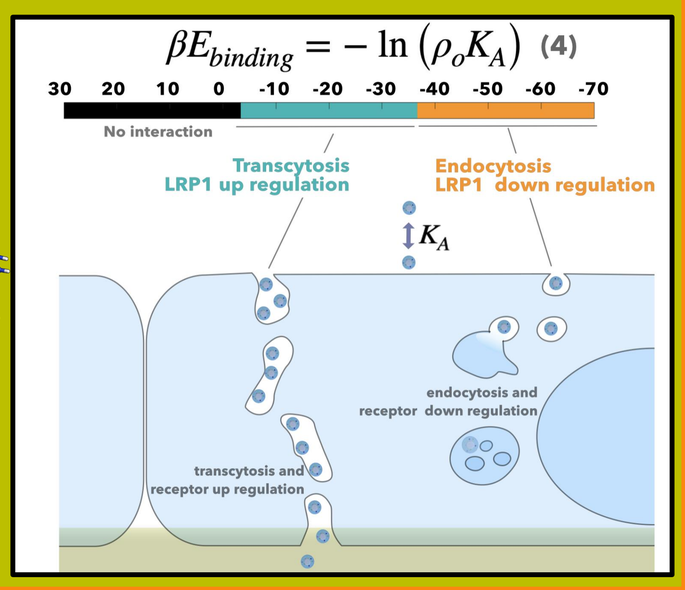




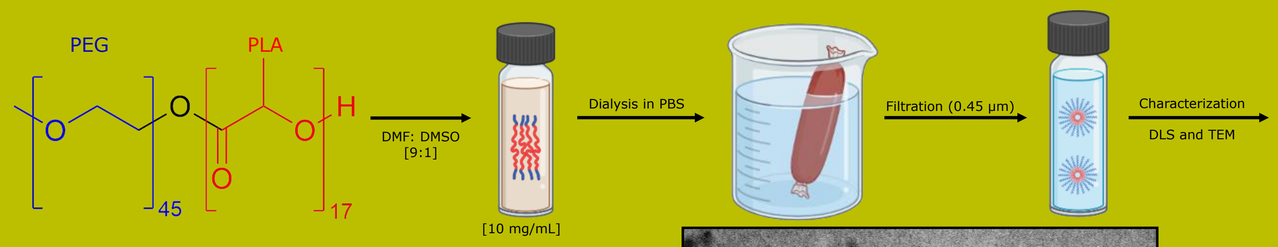
Aim: modulating transcytosis and LRP receptors expression by treating brain endothelial cells (BECs) with angiopep-2-functionalised nanoparticles.

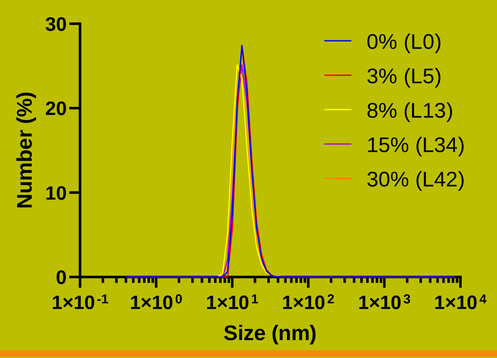
Angiopep-2-functionalised copolymer: the binding energy of angiopep-2-functionalised micelles is modulated to induce transcytosis rather than endocytosis through LRP1.

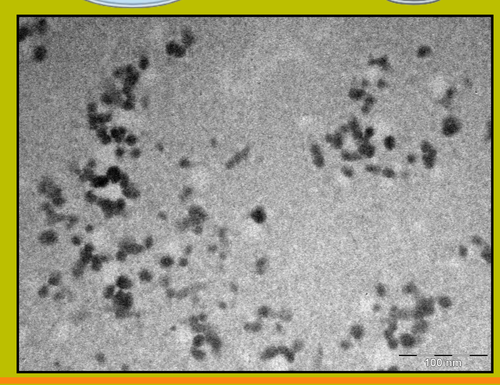




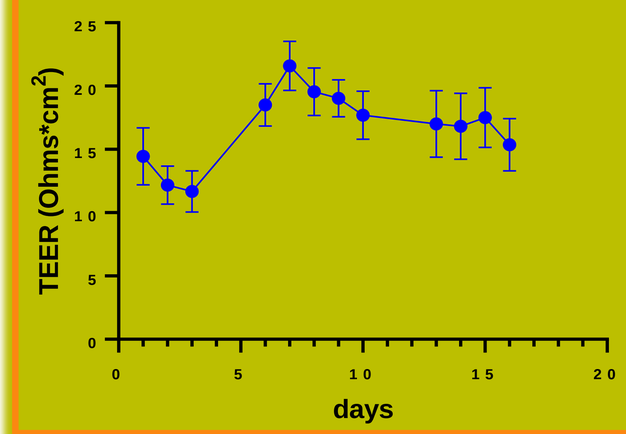
Micelles formulation: the copolymer is solubilised in organic solvent and then dialysed in PBS (o.n.). At the end, the micelles suspension is filtered and characterised by DLS and TEM.



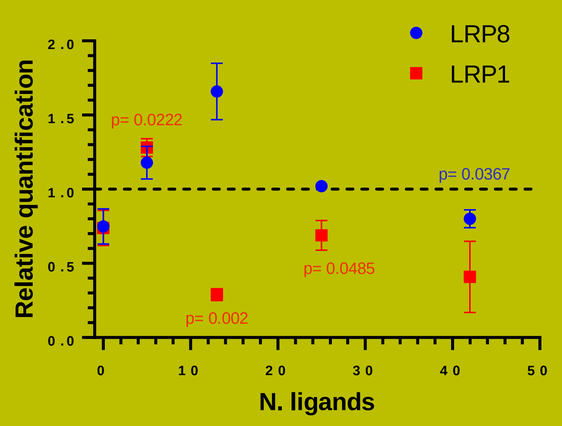




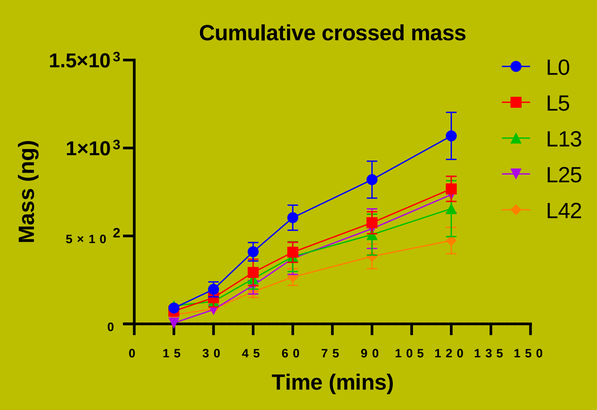
Trans Endothelial Electrical Resistance (TEER): To mimic the BBB endothelium, BECs are cultured in transwells. By assessing the TEER, the barrier integrity is evaluated.

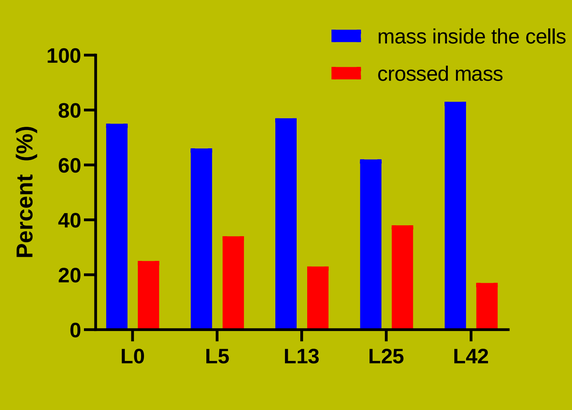


Real Time PCR: the BBB model was treated with micelles (0.1 mg/mL) for 2 hours. Then, the RNA was collected and analysed.



Permeability assay: the BBB model was treated with micelles (0.1 mg/mL) for 2 hours. To get the cumulative crossed mass, the micelles mass crossing the BBB was evaluated every 15 minutes. In the end, it was assessed the mass inside the cells as well.





Conclusions:

- Low avidity micelles (L5) showed to be able to increase LRP1 and LRP8 expression and cross the BBB more than high avidity micelles (L42).
- Interestingly, L13 micelles induce an opposite expression of LRP1 and LRP8.

Future work: we are going to replicate these experiments with angiopep-2-functionalised polymersomes to evaluate how the nanoparticles structure can influence both gene expression and vesicle destiny.

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