BBB-targeting liposomes : Design, characterization and *in vivo* evaluation

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The blood-brain-barrier (BBB) remains a formidable obstacle for the treatment of brain diseases. Current technologies, characterized by high complexity, rarely achieve delivery of the drug in the brain beyond 1% of the total injected dose [1] and therefore prognoses for brain diseases remain extremely poor.

By using embryonic zebrafish (*Danio Rerio*) as a model to assess nanoparticle behavior *in vivo* [2], we report a simple liposome formulation composed of just two lipids, with a significant specificity for the brain endothelium. We demonstrate how the highly unusual morphology of these liposomes, characterized by phase-separation and a single protrusion per liposome, is essential for BBB targeting, as well as our current understanding of the mechanism behind this biological response. Finally, we show the incorporation of a pH sensitive lipid-dye and of a single gold nanoparticle per liposome, which enables the elucidation of the liposome fate using high-resolution imaging techniques.

Ultimately, the unprecedented specificity of this liposomal formulation for brain endothelial cells and our comprehension of its *in vivo* mechanistic behavior, may lead to the development of a novel powerful tool for drug delivery to the CNS.

References

[1] Lajoie J.M. & Shusta E.V., *Annual review of pharmacology and toxicology*, 55 (2015), 613-631
[2] Frederick Campbell et al., *ACS Nano*, 12 (2018), 2138-2150

Figures

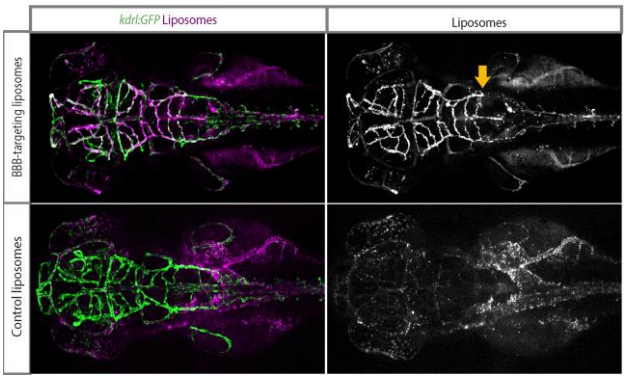

Figure 1. *10x fluorescent images of the embryonic zebrafish head (dorsal view), following intravenous administration of BBB-targeting liposomes and control liposomes (liposomes in magenta (left) or white (right)). Arrow indicates the specificity of the liposomes for the BBB, as targeting starts where the brain endothelium begins. Zebrafish embryo is at 3 days post fertilization (3dpf), expressing green fluorescent protein throughout its vasculature (kdrl:GFP).*