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# Nanoparticle brain-targeting by exploiting the BBB impermeability to selectively label the brain endothelium


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## Introduction

- Current brain delivery strategies **target proteins expressed at the brain vasculature** (*e.g.* TfR1)
- Significant **'off-target' nanoparticle (NP) accumulation** occurs due to protein expression in peripheral organs
- A **new strategy** has been developed to increase brain specificity by **selectively labelling brain endothelium**
- The high **BBB impermeability due to lower endocytosis in brain endothelial cells (BEC)** is exploited to selectively retain biotin labels on the surface of BEC, resulting in specific brain endothelium labelling
- **Avidin-functionalized NP recognize the biotin labels** and are targeted specifically to the brain

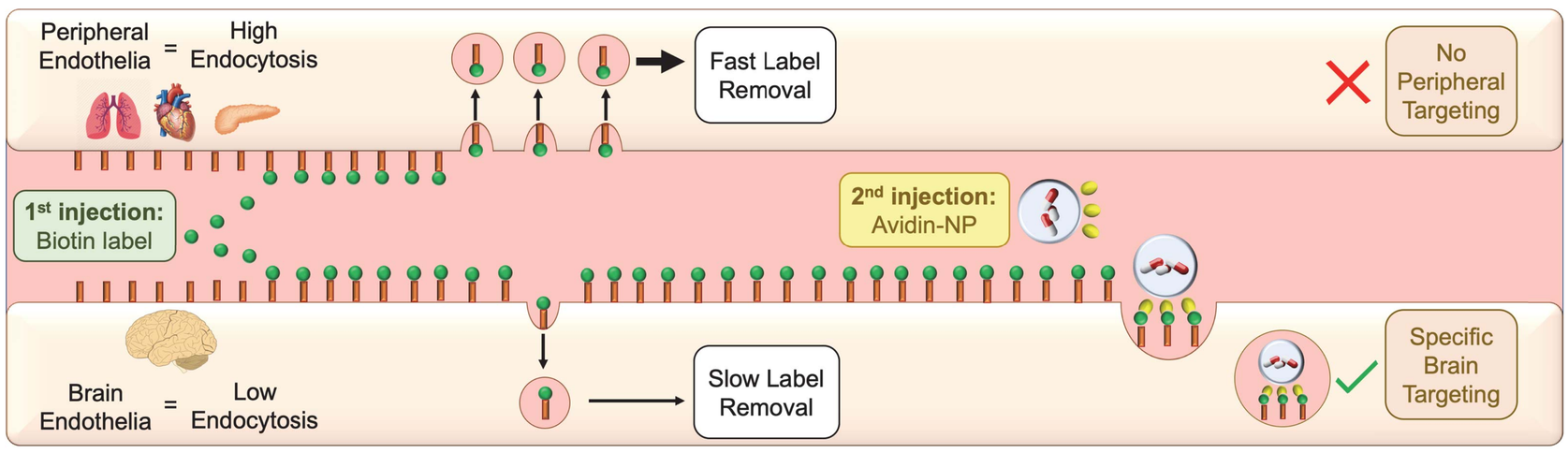



**Figure 1.** Synthesis of avidin-functionalized nanoparticles (avidin-NP)

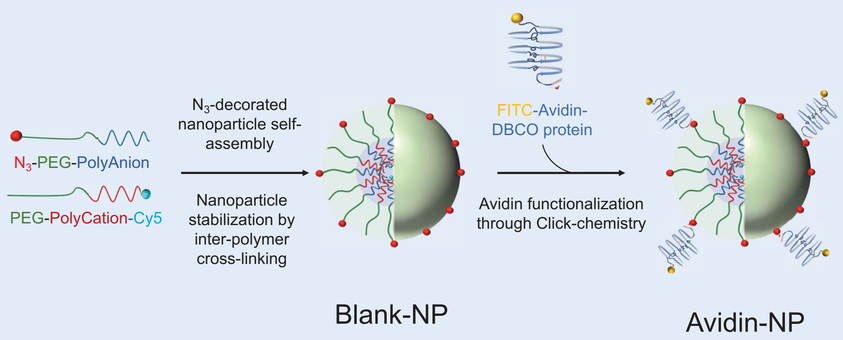



**Figure 2.** Biotin labelling of endothelial cell surfaces *in vitro*

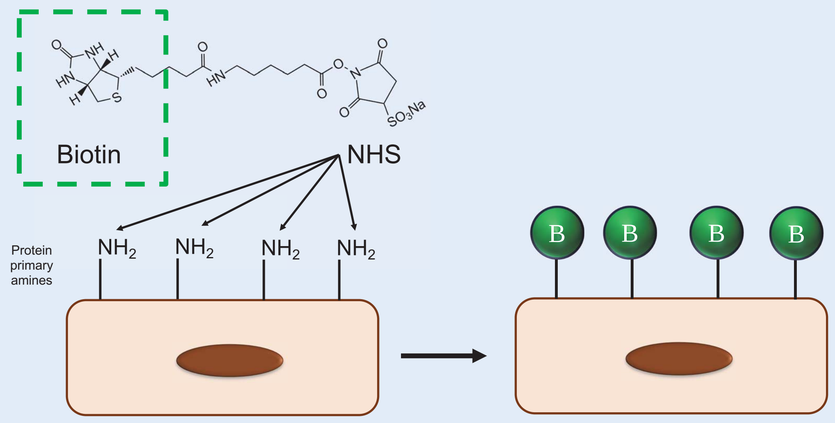



**Figure 3**. Avidin-NP uptake into biotin-labelled BEC

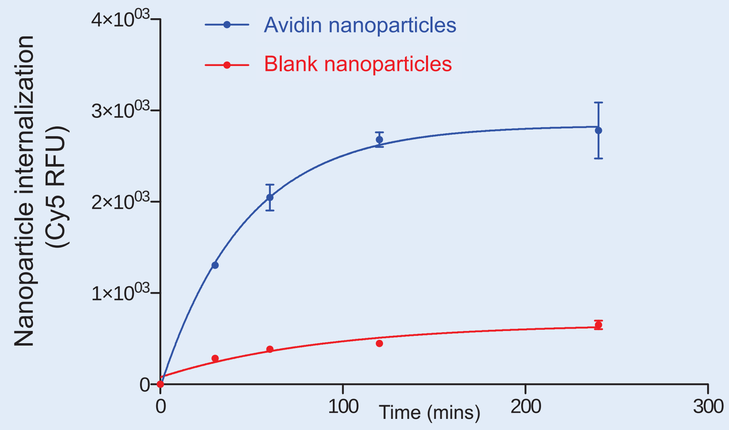
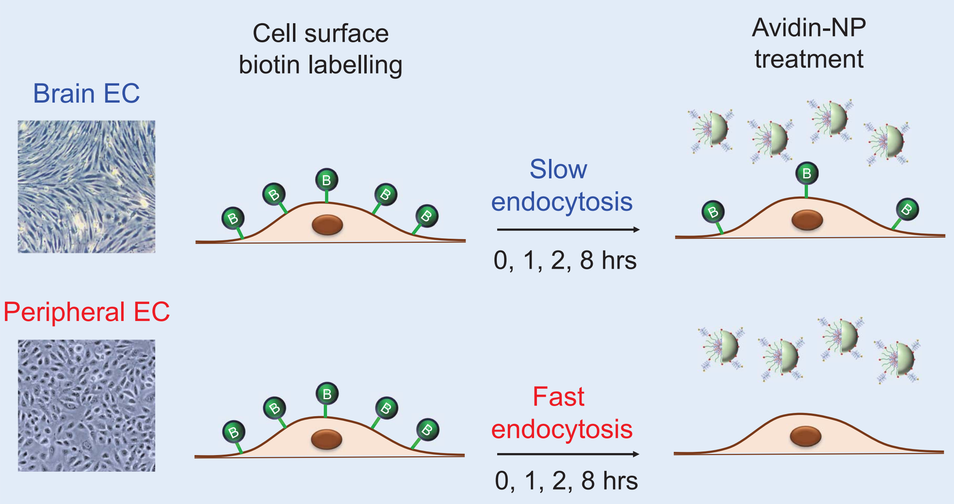
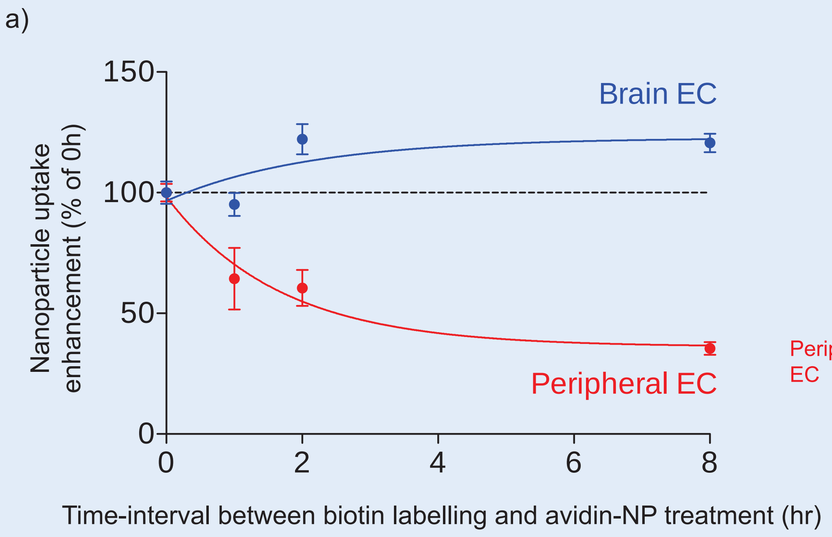



**Figure 4**. Selective biotin label retention on brain endothelial cells *in vitro*



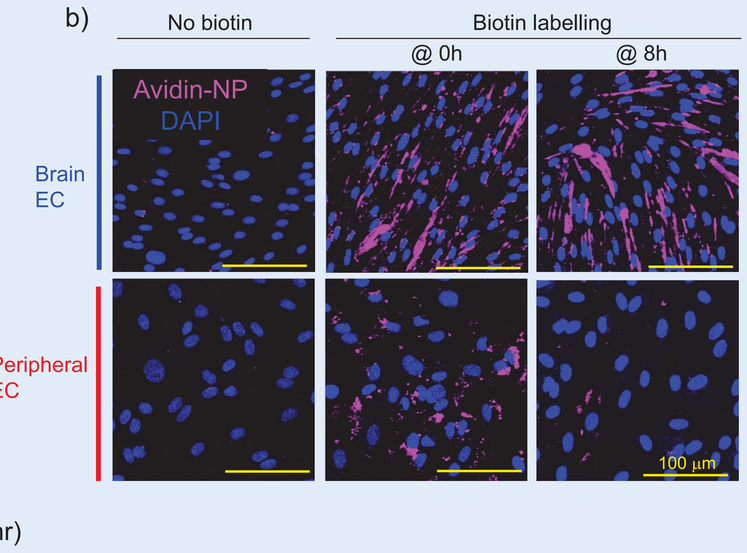
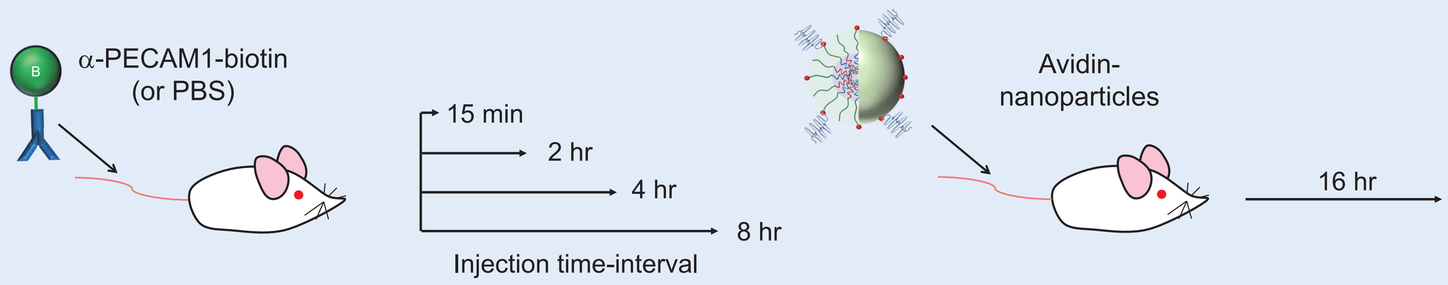
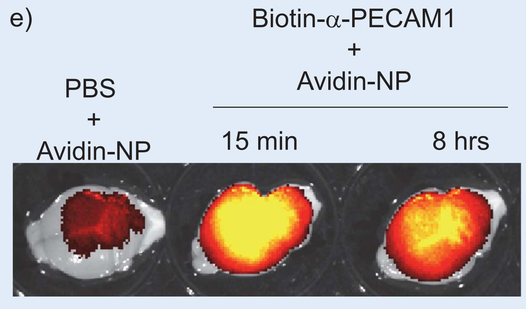
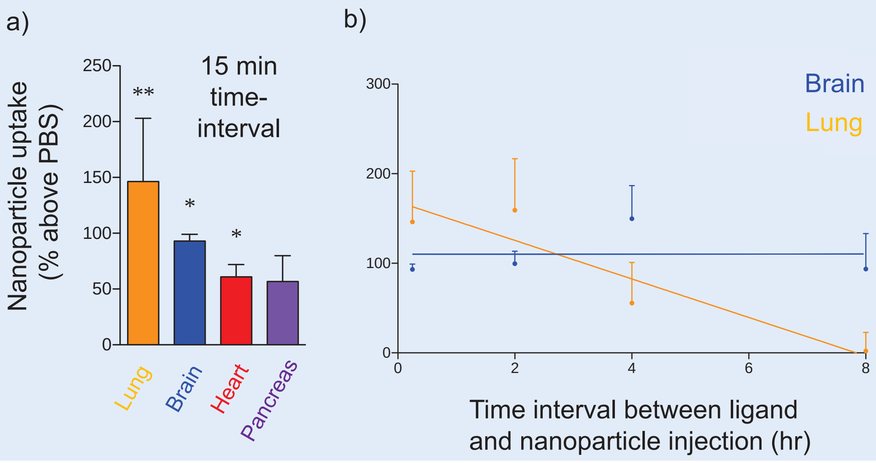




**Figure 5.** Brain targeting of avidin-NP by selective retention of a biotinylated α-PECAM1 antibody on the brain endothelium

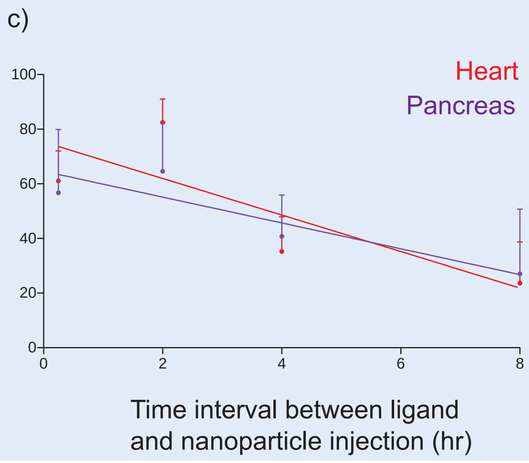


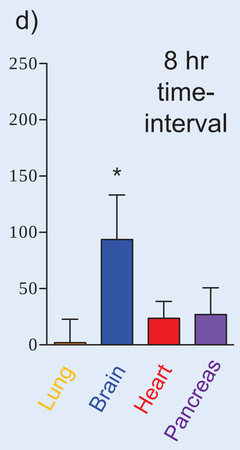



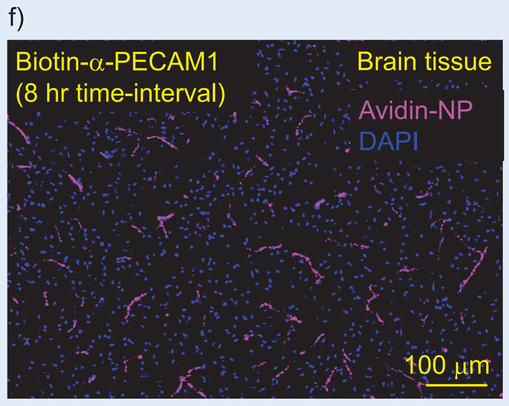










## Conclusions

- Successfully targeted nanoparticles (NP) specifically to the brain with minimal peripheral 'off-target' accumulation increase by exploiting the impermeability of the BBB to selectively retain free targeting ligands on brain endothelial cells
- This strategy may revolutionize brain targeting by moving away from directly functionalizing NP with targeting ligands, thereby increasing brain specificity of NP delivery and improving clinical translation of NP-based therapies
- New protein targets may be identified based not on differential protein expression but on differential endocytosis rate between the periphery and the brain

Methods

- $N_3$-decorated PEGylated polyion-complexed nanoparticles (nanomicelles) created by self-assembly of PEGylated poly-anionic/cationic block co-polymers followed by inter-polymer crosslinking
- DBCO-Avidin functionalization onto $N_3$-nanomicelles through Click chemistry
- Brain endothelial cells were isolated from rat brain microvasculature
- HUVECs were employed as peripheral endothelial cells
- Balb/C mice were employed for *in vivo* studies. Biotin-α-PECAM1 (25 μg) and avidin-NM (200 μg) injected intravenously