

# Visualizing sub-second dynamics of single nanoparticles crossing the blood–brain barrier in live zebrafish

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The blood–brain barrier (BBB) presents a grand challenge in treating brain diseases. To enhance drug delivery across the BBB, emerging nanomedicine have demonstrated early promising results, but to date the overall efficiency still remains relatively poor.<sup>1</sup> Here, aiming to gain new insights into this sophisticated problem, we have developed a real-time imaging technique to study the microscopic process of individual nanoparticles crossing the BBB *in vivo*. Lanthanide-based upconversion nanoparticles (UCNPs) of NaYbF<sub>4</sub>:Tm with optimized doping concentration and inert-shell passivation<sup>3,4</sup> were synthesized, and coated with PEGylated phospholipid to form stable photoluminescence agent of uniform size and brightness. They were intravenously injected into transgenic fluorescent zebrafish larvae, and imaged at video rate (20 fps) for up to 5 hours post inoculation under an epifluorescence microscope. The system was engineered in-house to enable unambiguous identification and tracking of single UCNPs as they crossed the BBB in real time. We obtained the dynamic trajectories of individual UCNPs with respect to the brain vasculature, revealing distinct characteristics between intercellular transport through compromised tight junctions versus transcytosis. In the latter, the UCNPs were found taken up by brain endothelial cells and mobilized intracellularly, followed by exocytosis to reach the brain parenchyma. Longitudinal evaluation revealed that the two pathways were separately regulated, both during embryonic development and ageing, and upon various perturbations and diseased conditions. We are currently studying the influence and underlying mechanisms of nanoparticle size, morphology, and surface moieties on BBB penetration, and their subsequent movement and interactions after BBB crossing in the brain. The results are expected to facilitate future development of nanomedicine to attend brain health for our ageing society.

## References

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