

Blood-brain barrier targeted nanoparticles for peptide delivery to glioblastoma: in vitro and in vivo study.

Pablo Hervella, PhD.

Neuroimage and Biotechnology Lab (NOBEL)

Clinical Neurosciences Laboratories (LINC)

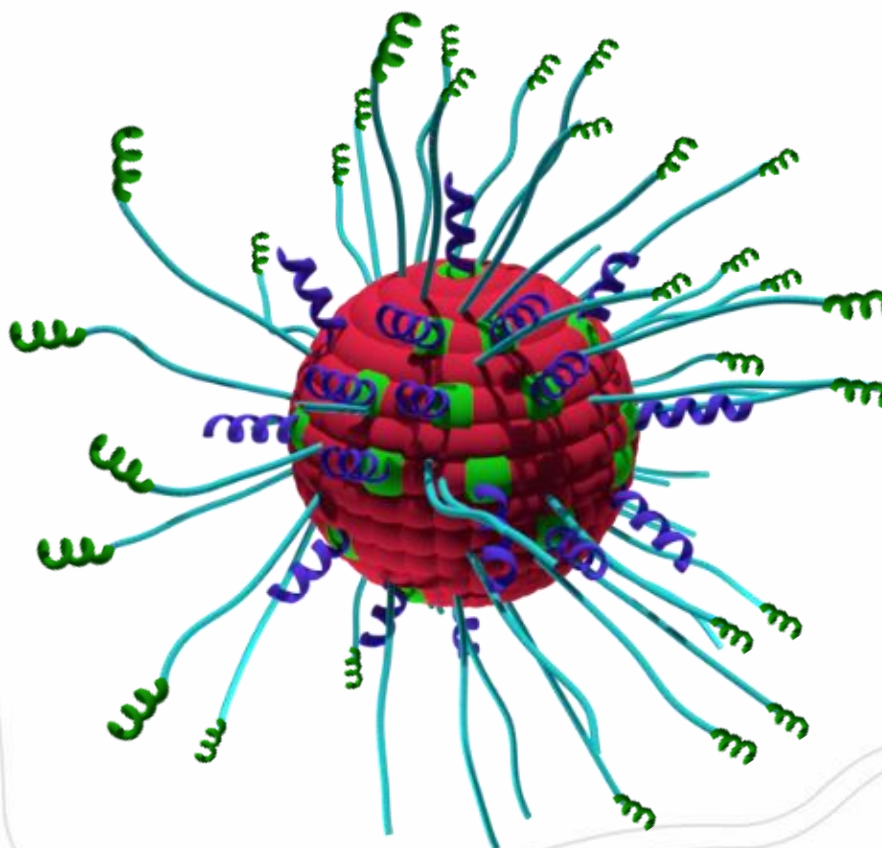
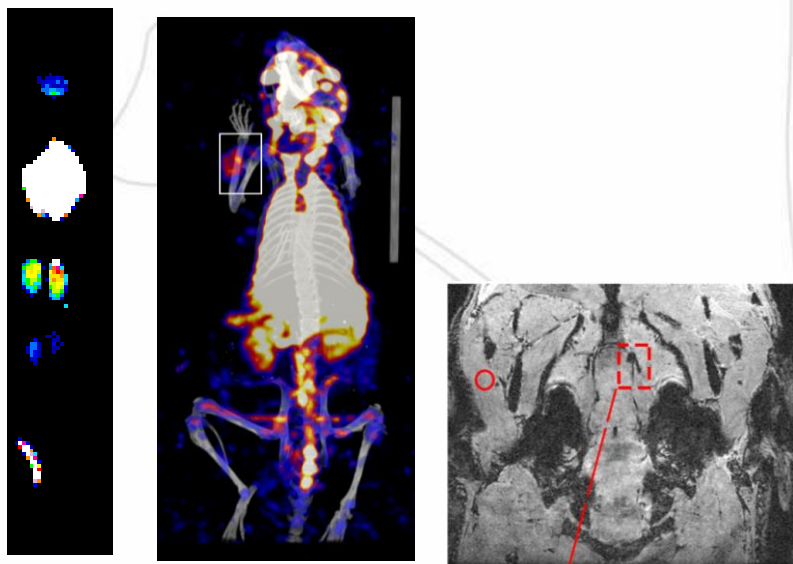
Health Research Institute of Santiago de Compostela (IDIS)

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LDL-like Lipid nanoparticles

Imaging:

- Fluorescence
- PET
- MRI (1H, 19F)



Therapy:

- Peptide delivery
- CAR-T therapy
- mRNA, siRNA
- Cytotoxic Drugs

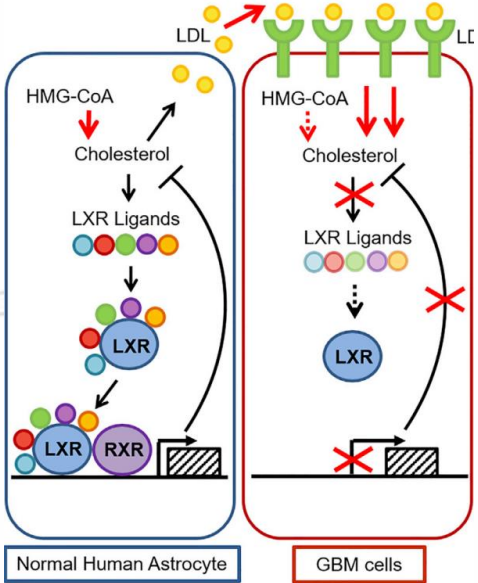
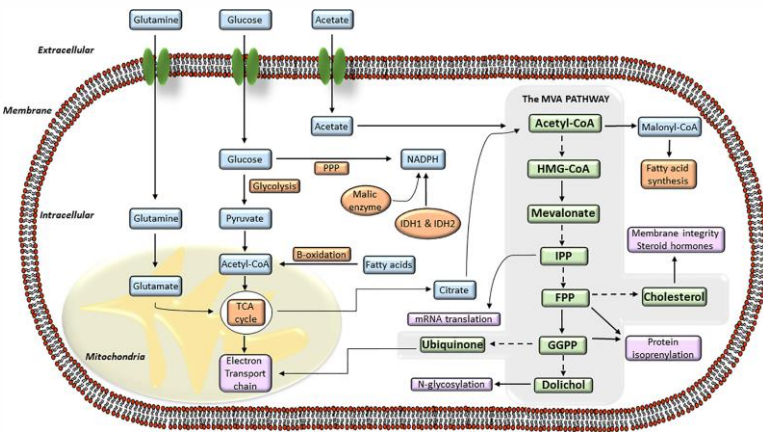
BBB blocks the transport of cholesterol into the brain.

Brain Endothelial Cells Do Not Transport but Degrade Low-Density Lipoproteins via Both LDLR and ALK1

Cholesterol in the brain is synthesized de novo through the mevalonate pathway.

Gliblastoma cells rely on cholesterol uptake for survival, mostly provided from astrocytes.

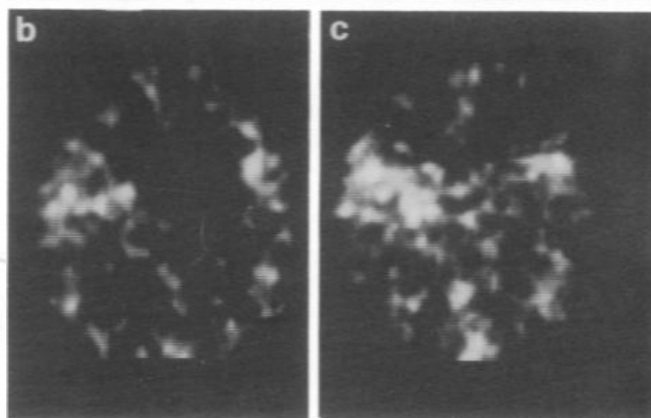
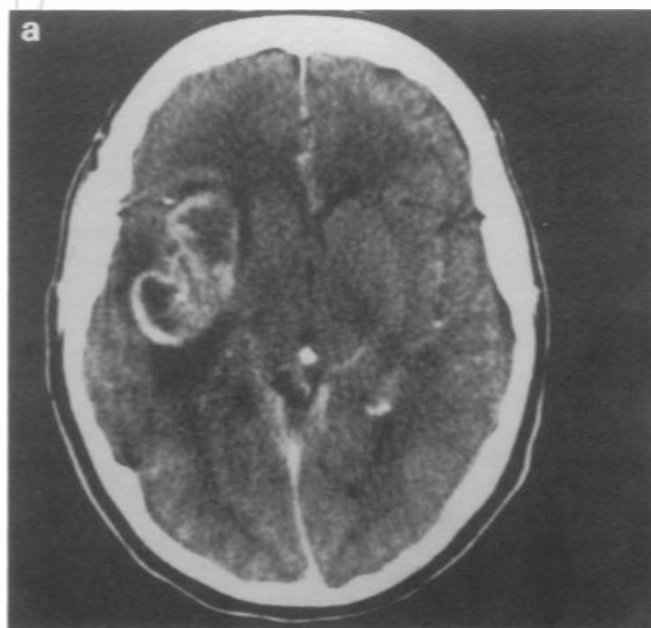
Cholesterol-depleted astrocytes can upregulate LDL receptor expression at the BBB, enhancing LDL transcytosis. This suggests that cholesterol levels in the brain can influence the efficiency of LDL transport



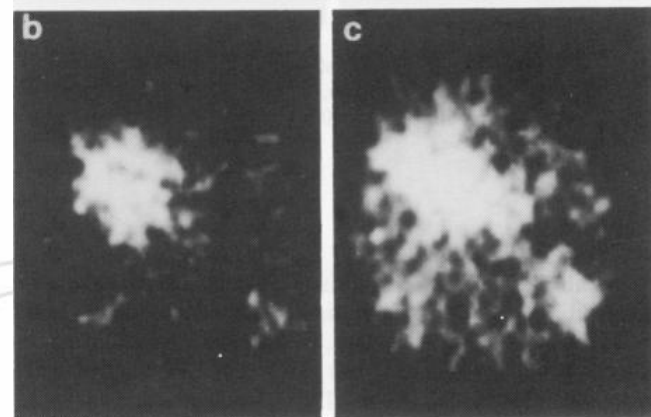
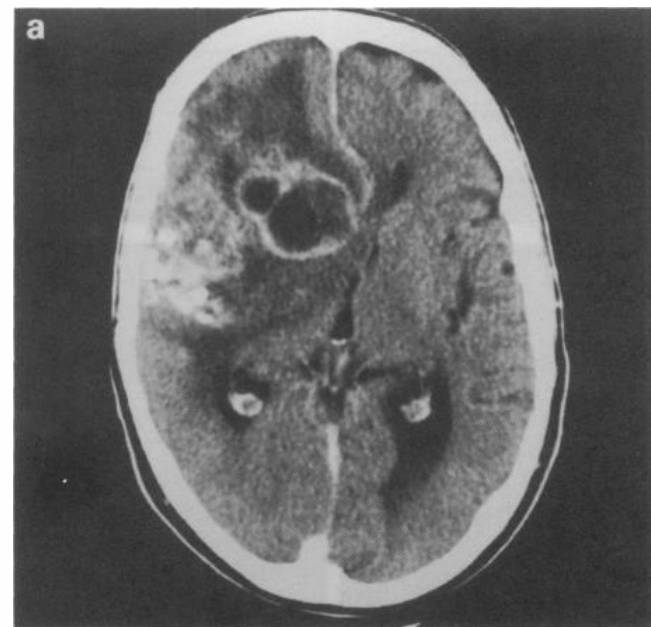
Leppälä, J., Kallio, M., Nikula, T. *et al.* Accumulation of ^{99m}Tc -low-density lipoprotein in human malignant glioma. *Br J Cancer* **71**, 383–387 (1995)

- a) CT scan
- b) ^{99m}Tc -HSA SPET
- c) ^{99m}Tc -LDL SPET

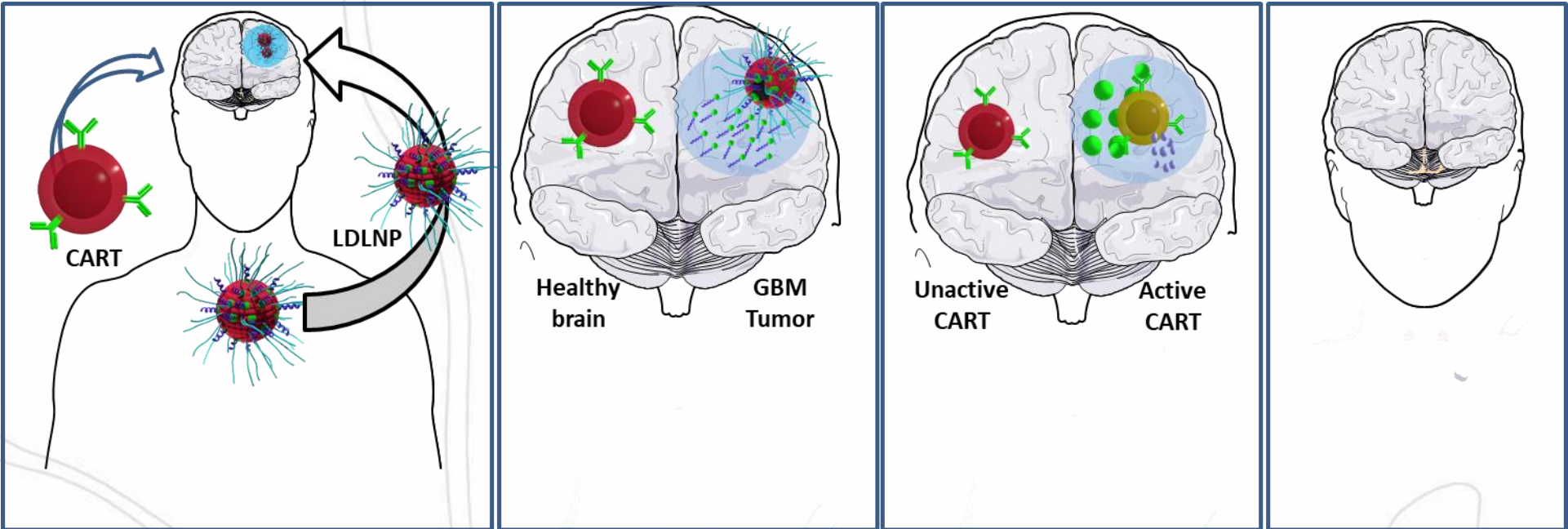
SPET performed 20 h after injection.



Untreated GBM



Recurrent anaplastic oligodendrioma



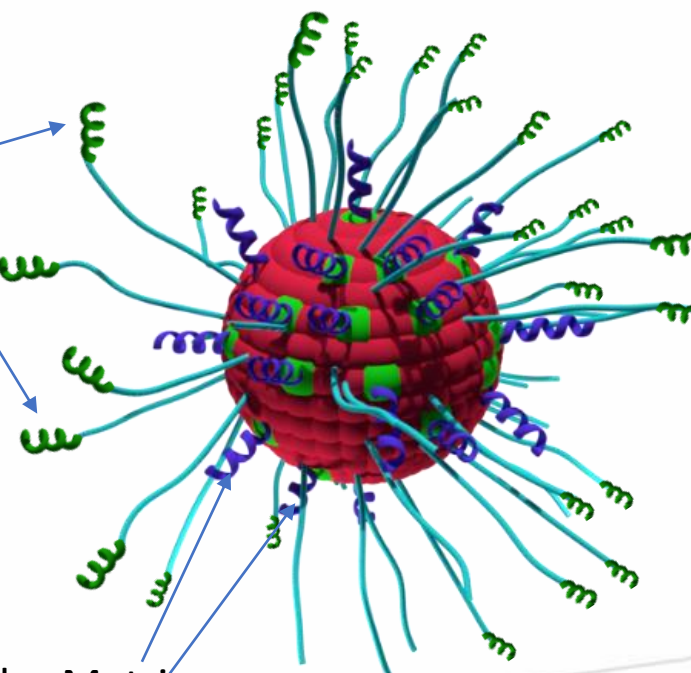
Structural Lipids : Phospholipids, triglycerides, cholesterol,

Synthetic Lipids: PEG, BBB-targeting peptides, Extracellular Matrix targeting peptides

New peptides targeting:

Blood brain barrier

Glioblastoma Extracellular Matrix



Tambet Teesalu

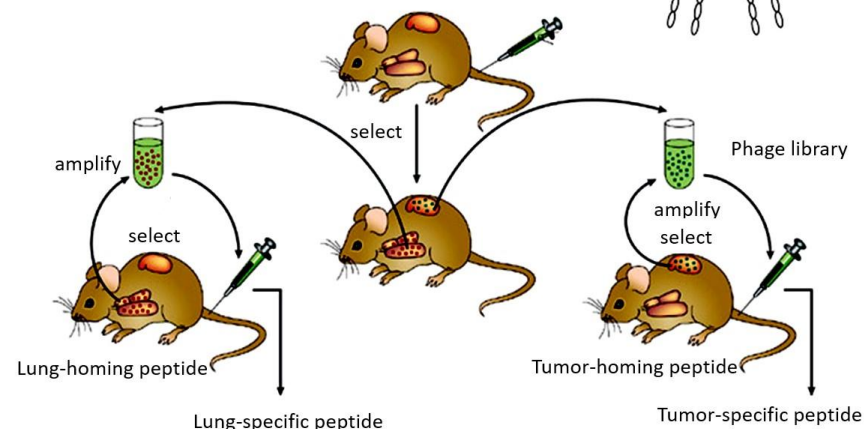
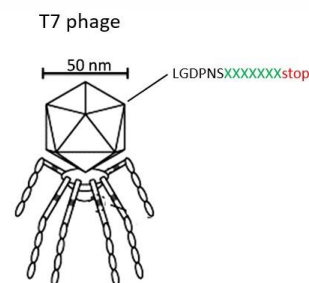
Laboratory of Precision and Nanomedicine. University of Tartu.



Phage display screening of GBM: identify new targets

T7 bacteriophage display of random peptides

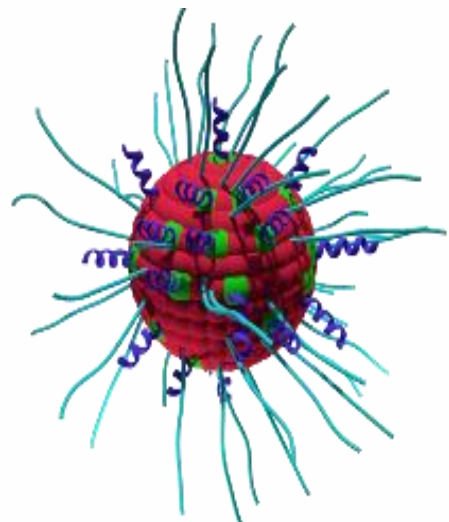
1. 10^9 different peptides /library
2. Random (NNK-encoded), linear and cyclic (CX7C, CX8C, CX10C, X7, X8, X10)
3. Peptides fused to the C-terminus of the major coat protein and displayed at ~200 copies/phage particle



a) Peptides targeting the LDLR in brain endothelial cells

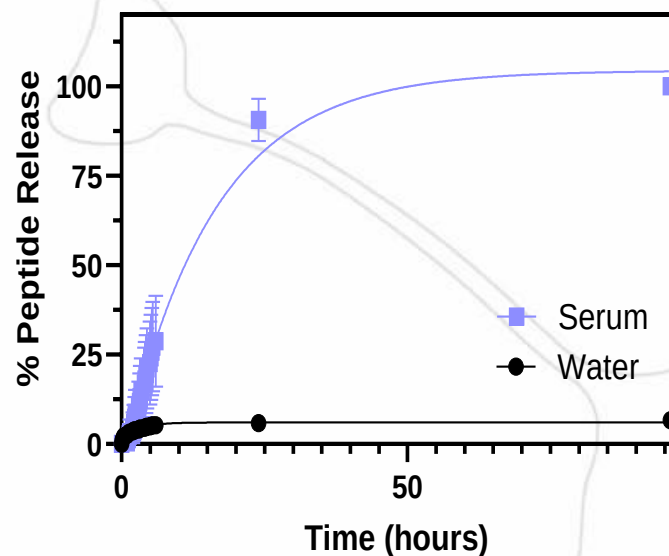
b) Peptides targeting extracellular components of brain tumors

Pasqualini R, Ruoslahti E. Nature. 1996
Teesalu T, Sugahara KN, Ruoslahti E. Methods Enzymol. 2012

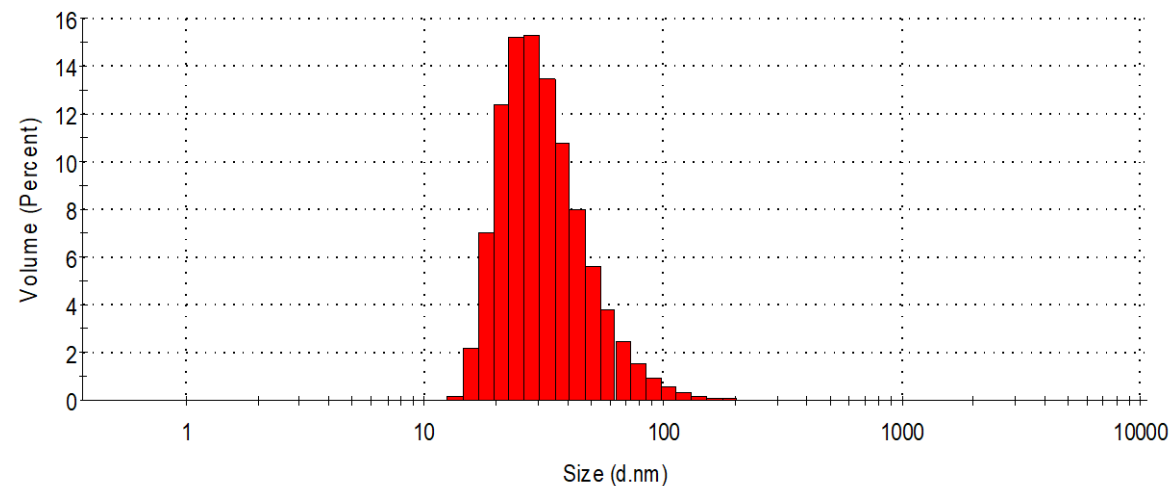


Peptides targeting the extracellular matrix

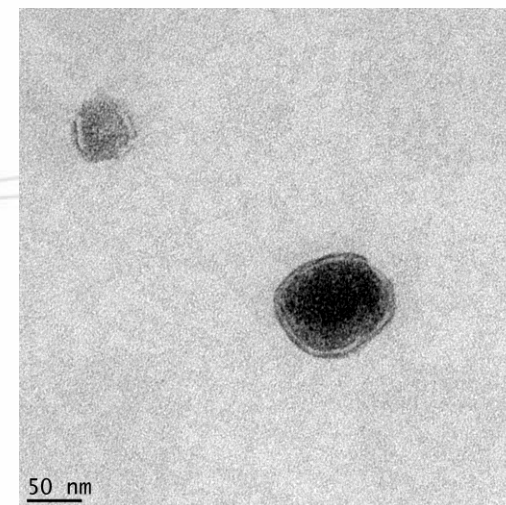
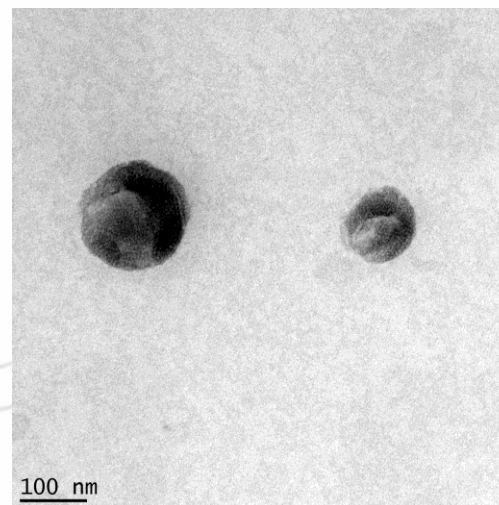
Release profile



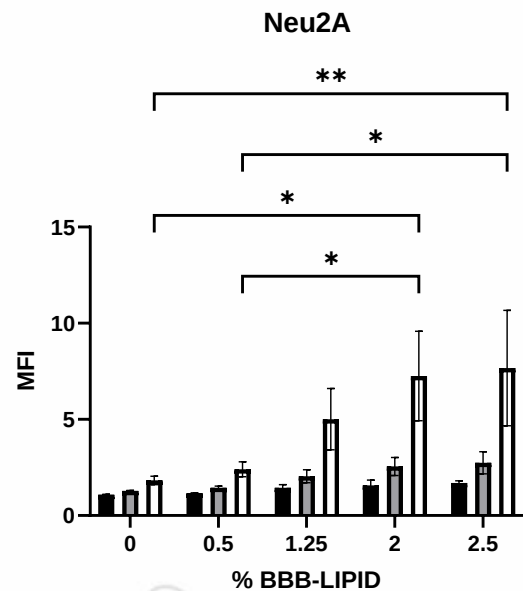
Size Distribution by Volume



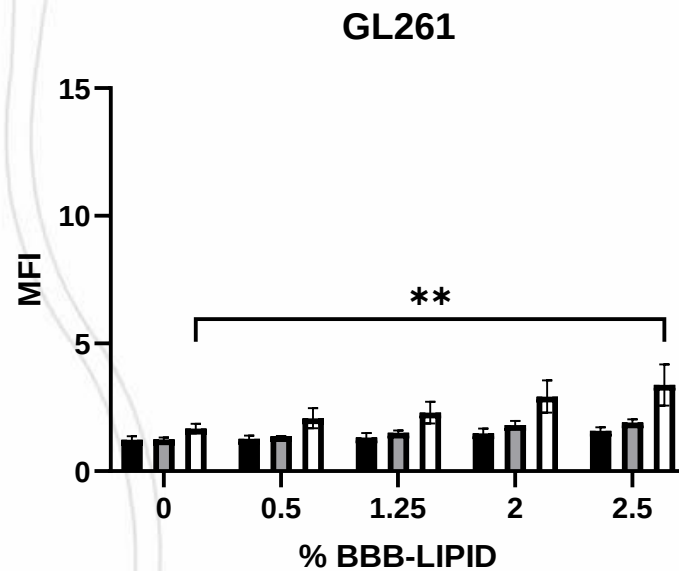
Particle Size: DLS and TEM



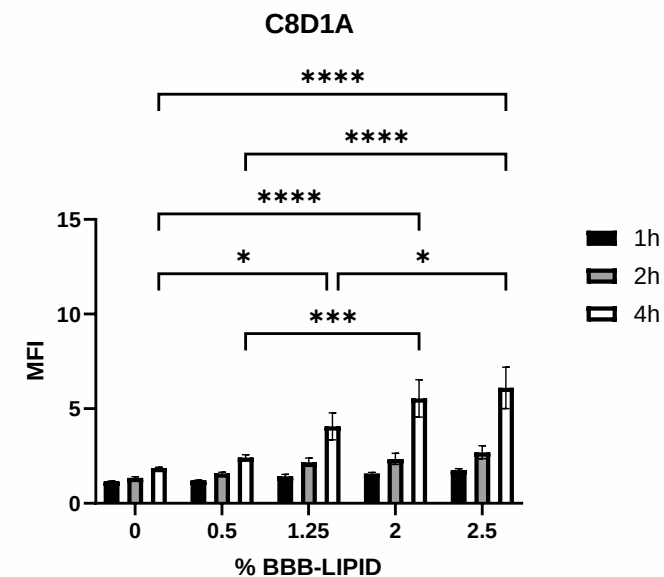
Brain tissue isolated Neuroblasts



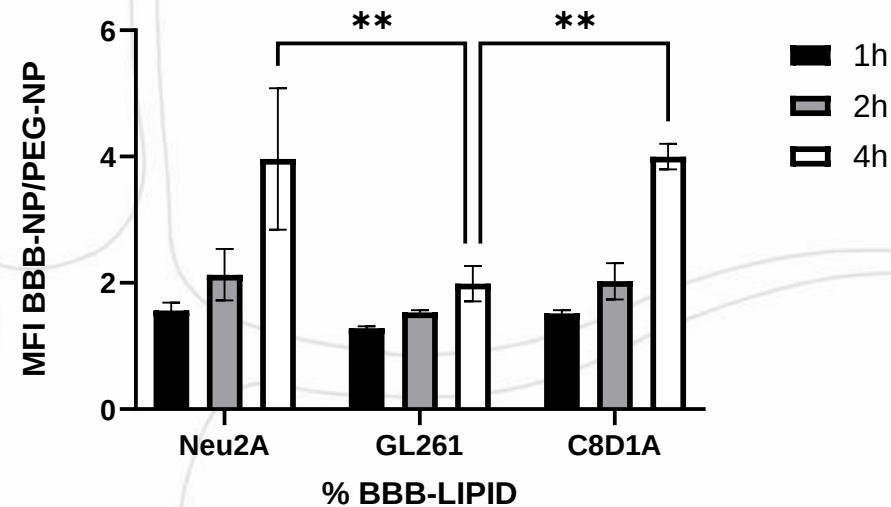
Glioblastoma



Astrocytes



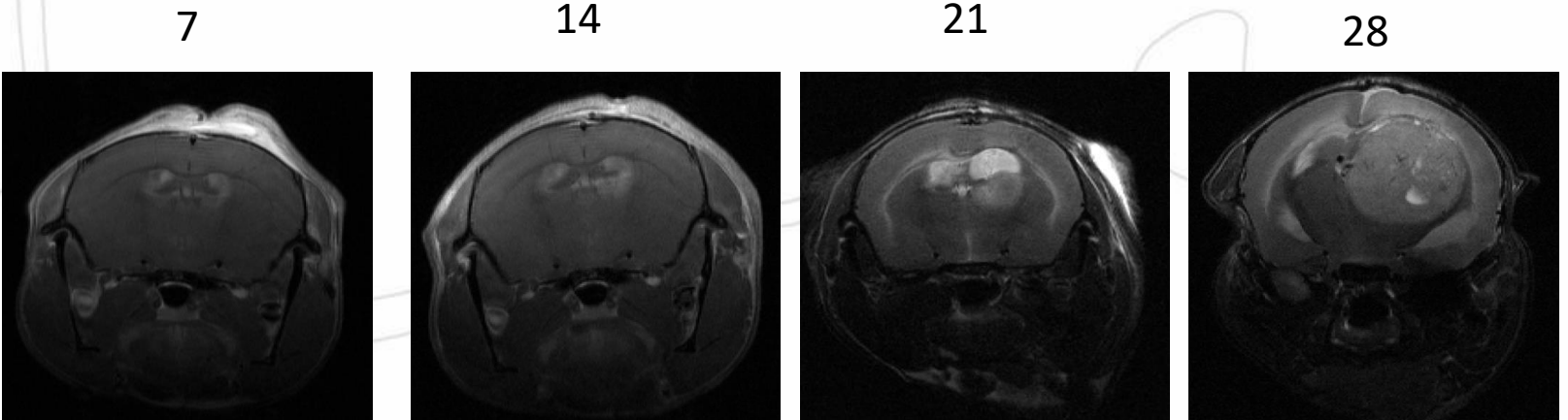
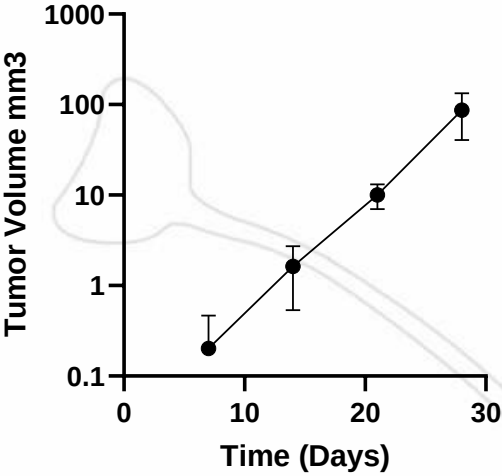
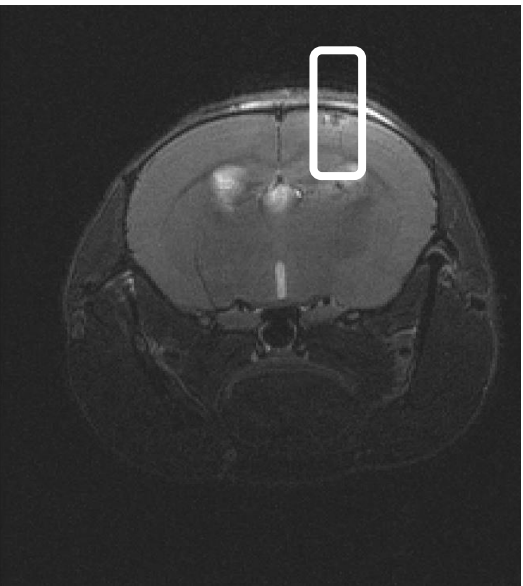
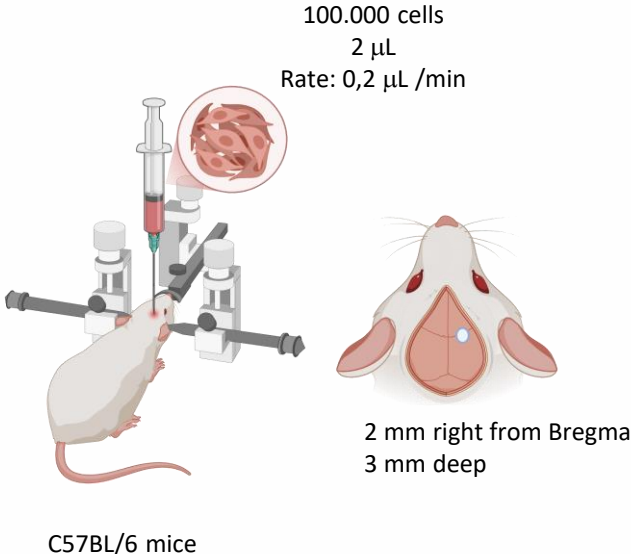
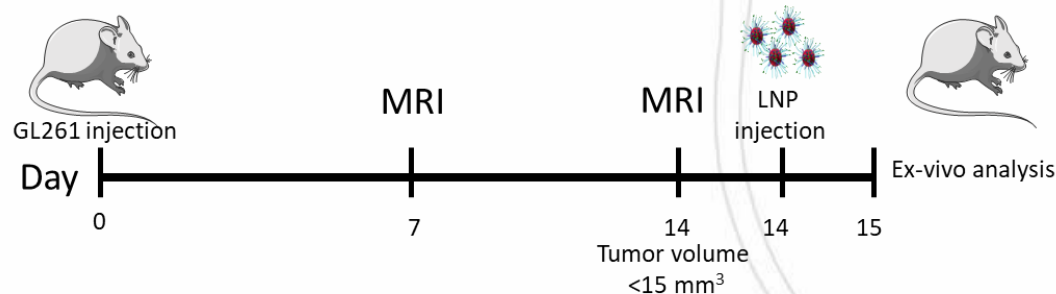
BBB-NP/PEG-NP



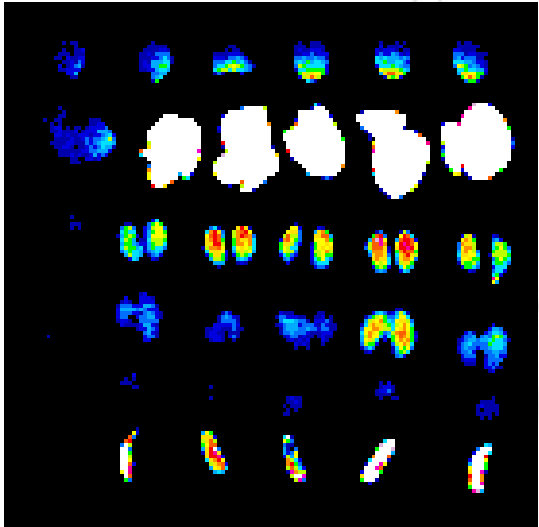
In vivo studies

In vivo GL261 glioblastoma model

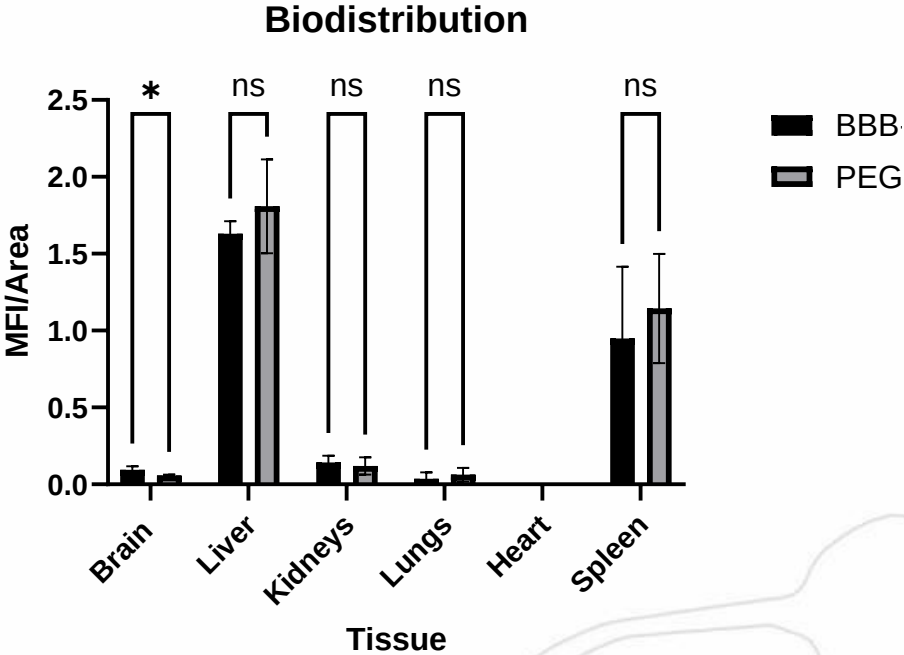
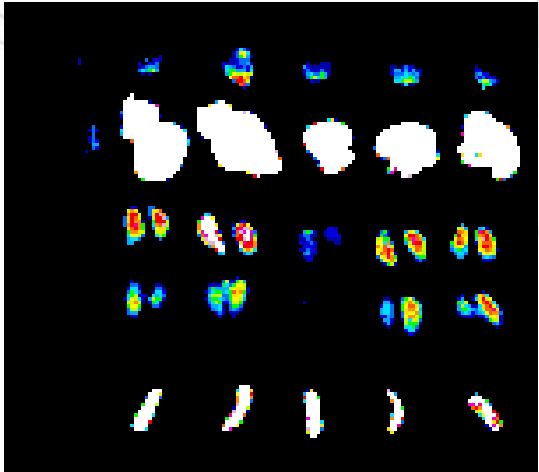
Tumor model

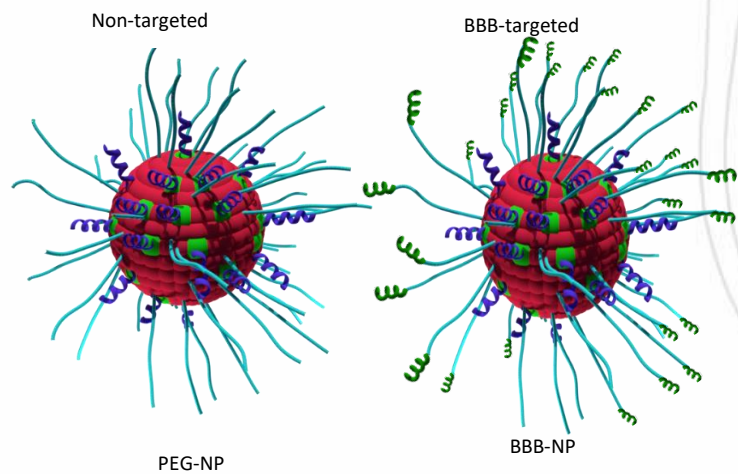


BBB-NP

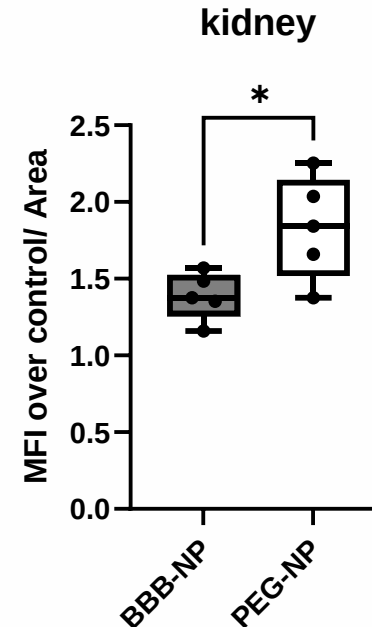
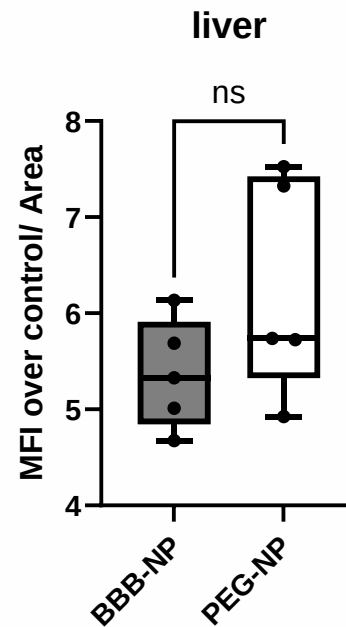
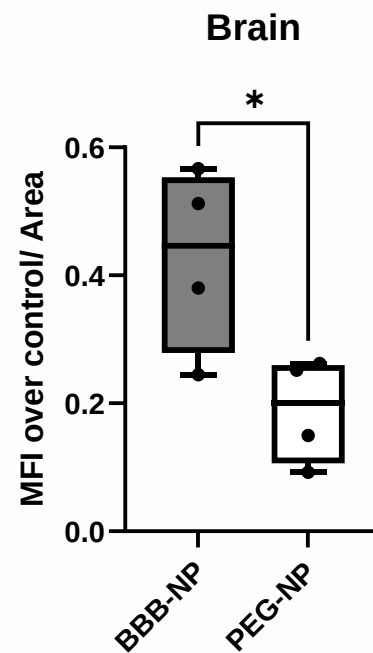
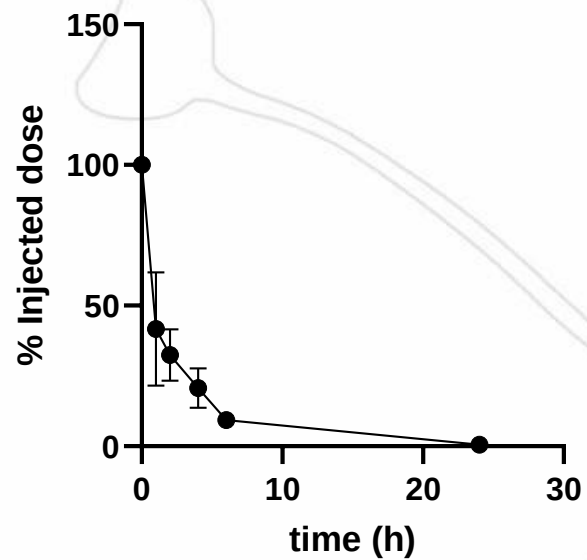


PEG-NP

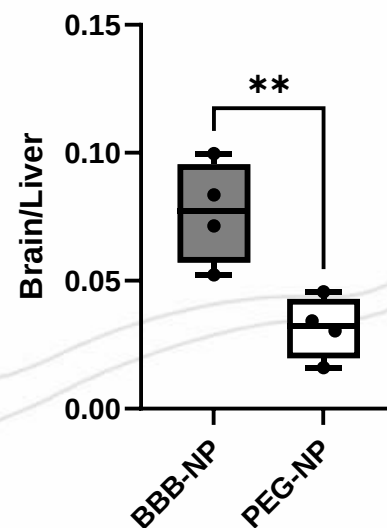


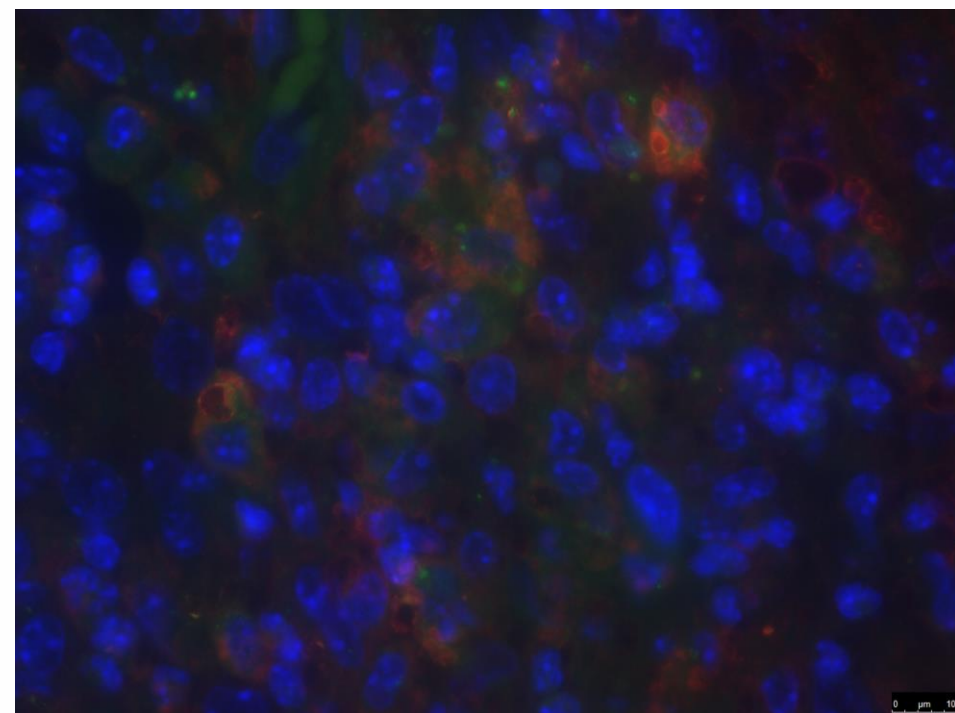
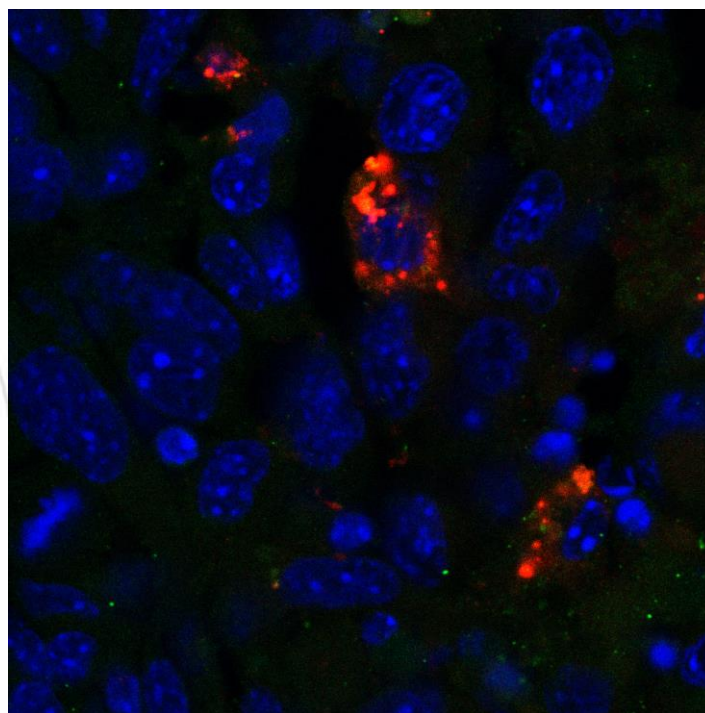
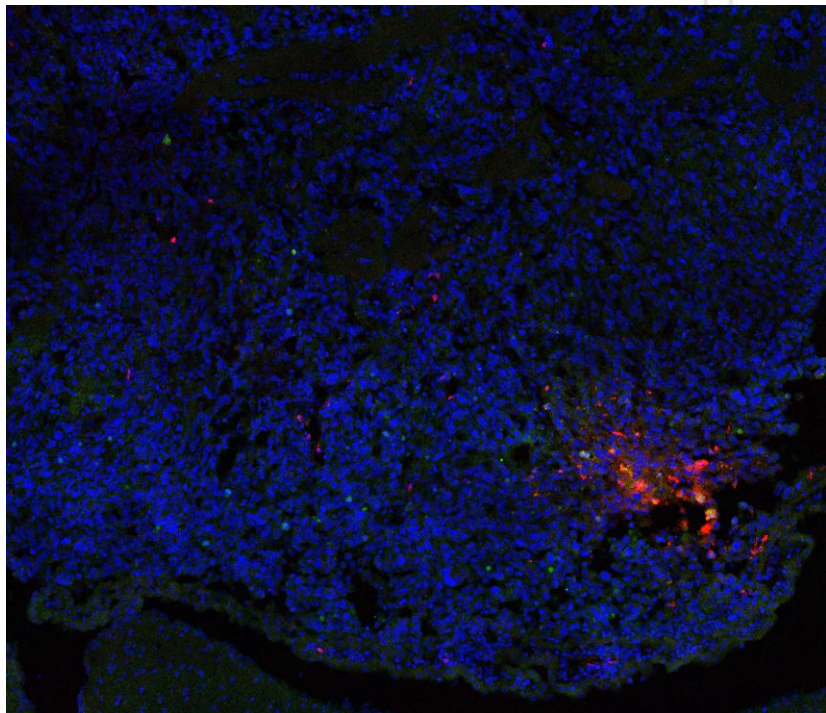


PK



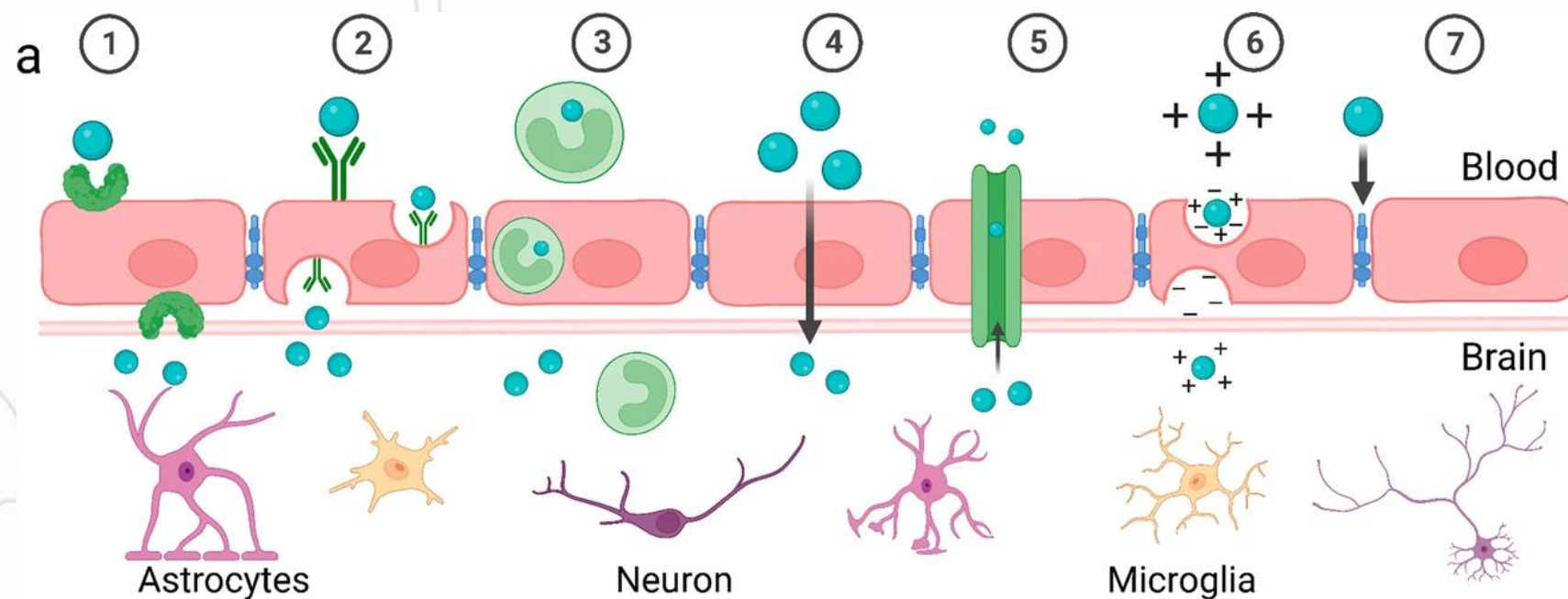
brain to liver





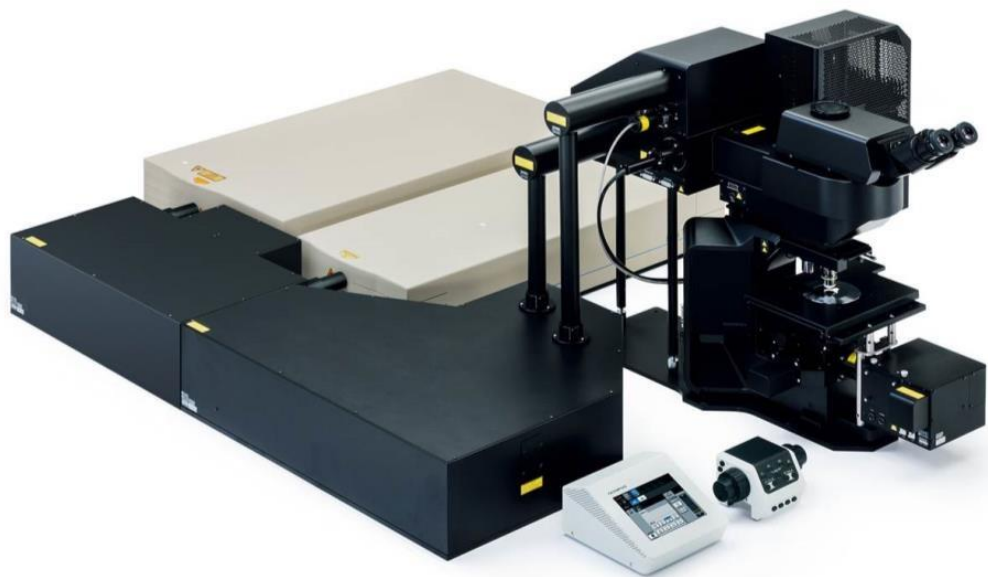
Blue: Hoesch (Tumor cell nuclei); Red: Peptides

Nanoparticle uptake mechanism



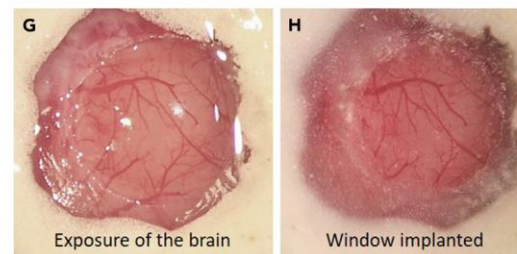
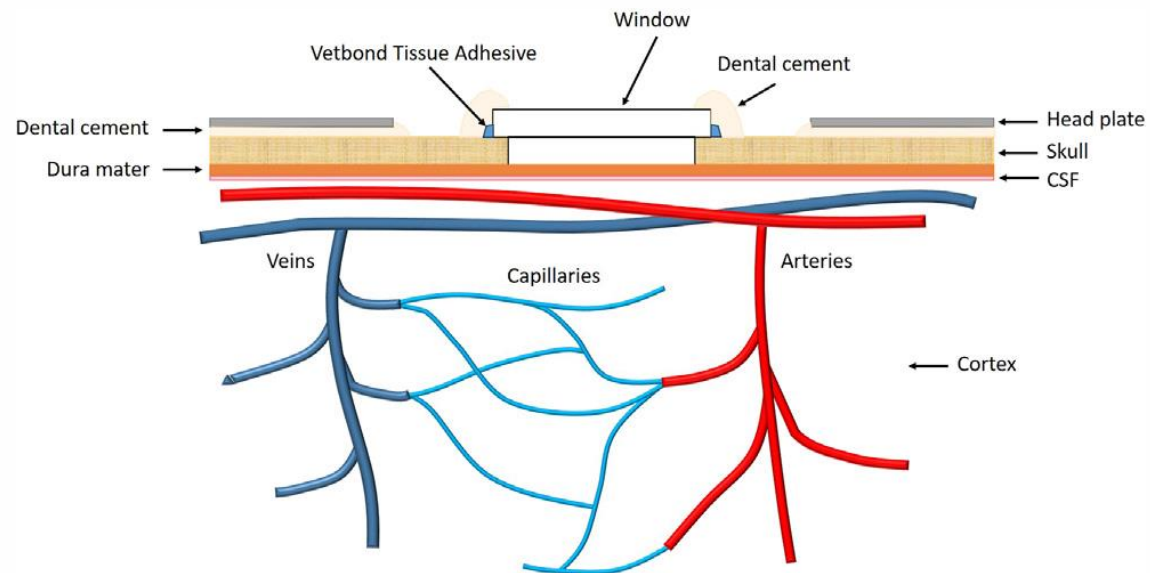
- | | | |
|--------------------------------------|-----------------------------------|-------------------------------|
| 1) Transporter-mediated transcytosis | 2) Receptor-mediated transcytosis | 3) Cell-mediated transcytosis |
| 4) Lipophilic pathway | 5) Efflux pumps | 6) Adsorptive transcytosis |
| 7) Paracellular aqueous pathway | | |

Intravital Microscopy



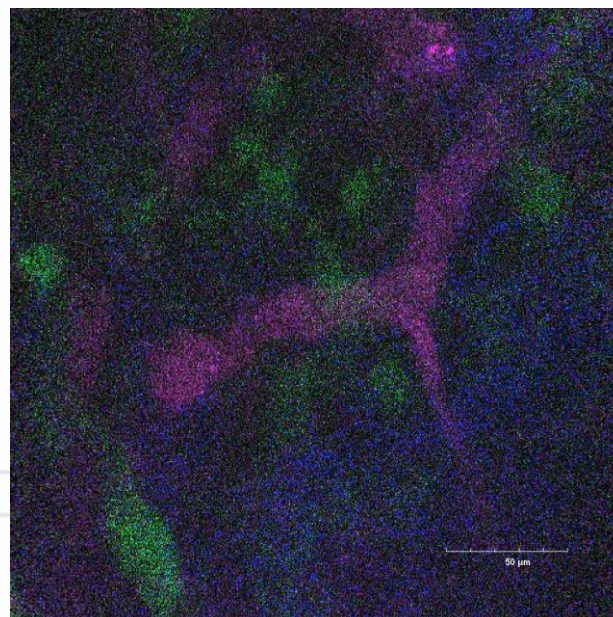
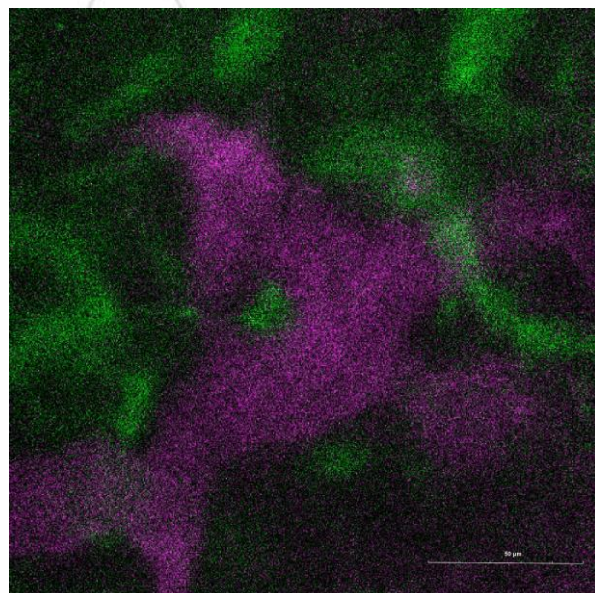
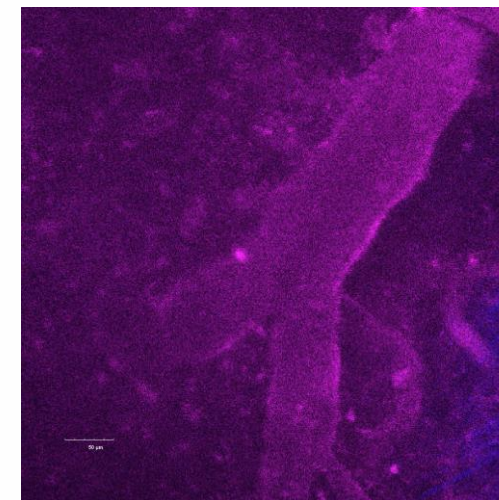
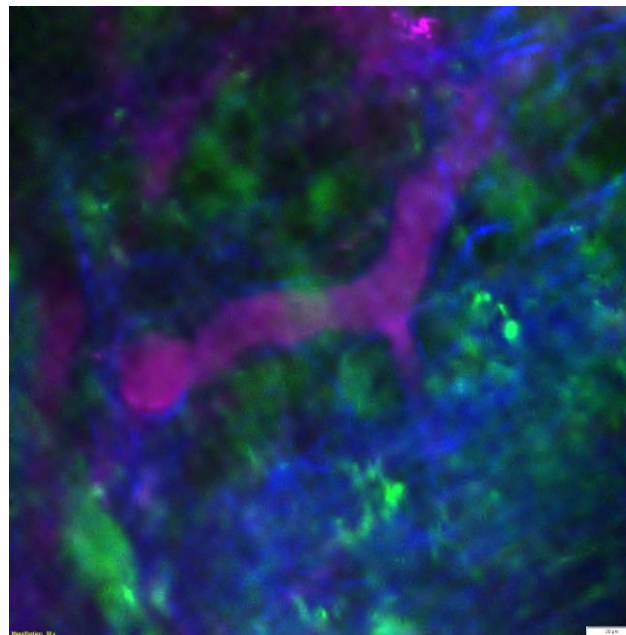
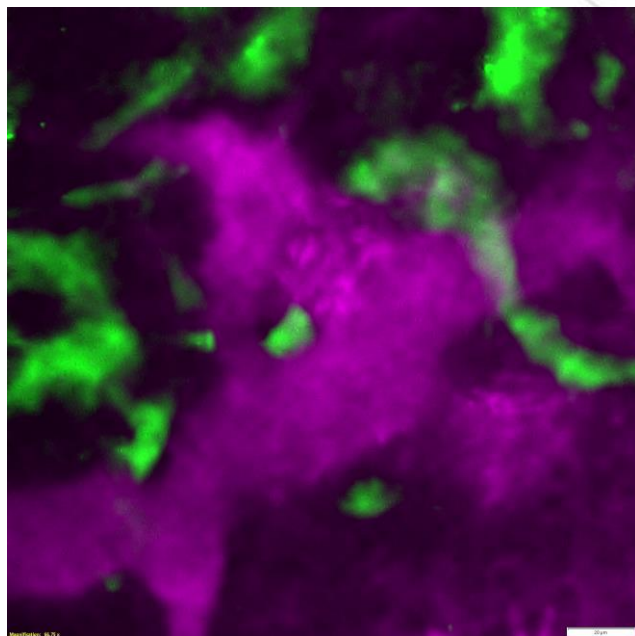
Multiphoton Microscope

Cranial window



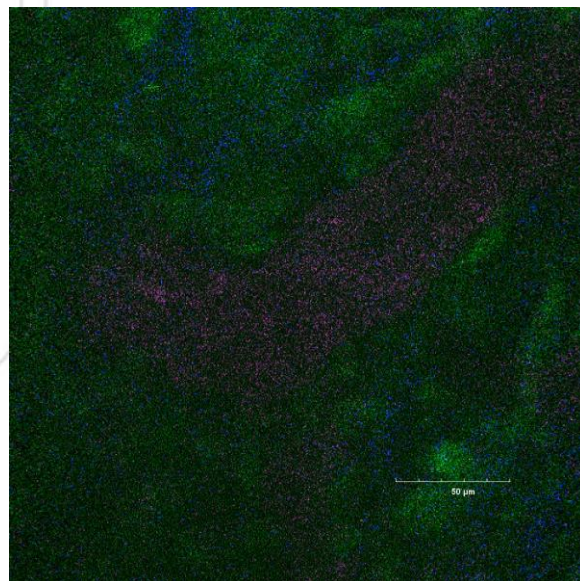
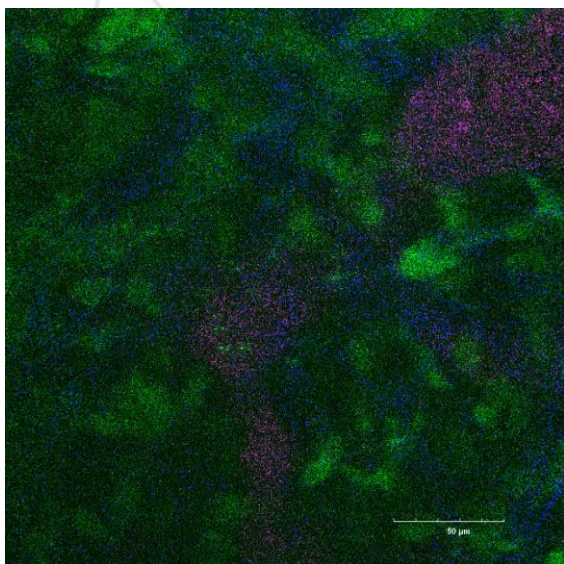
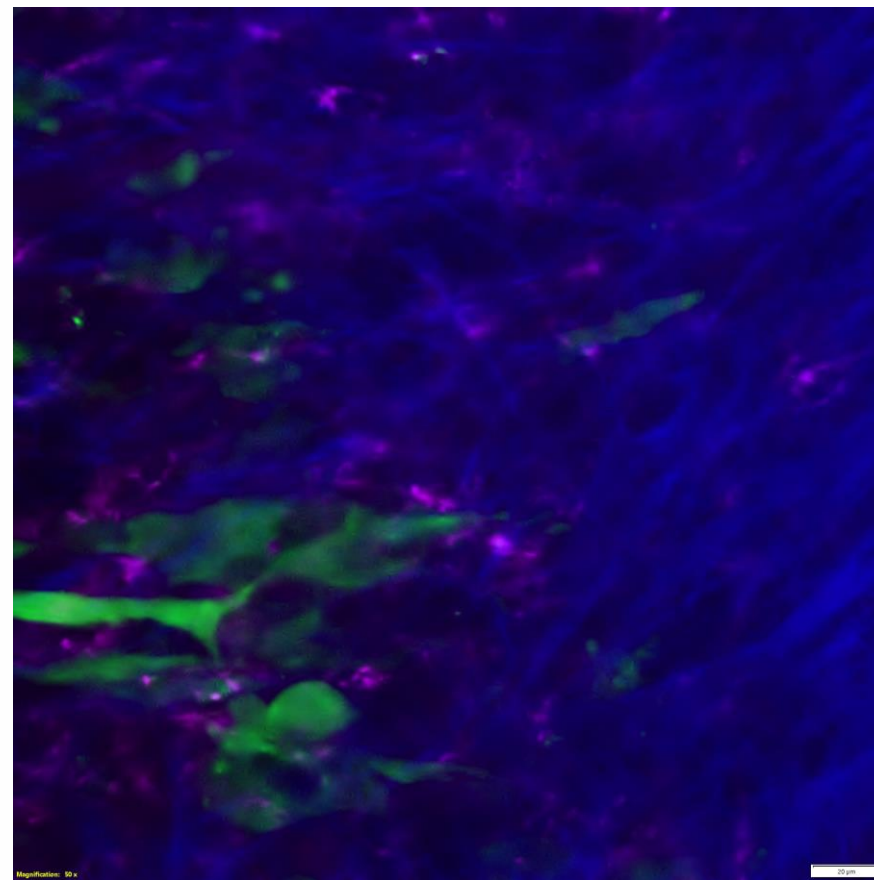
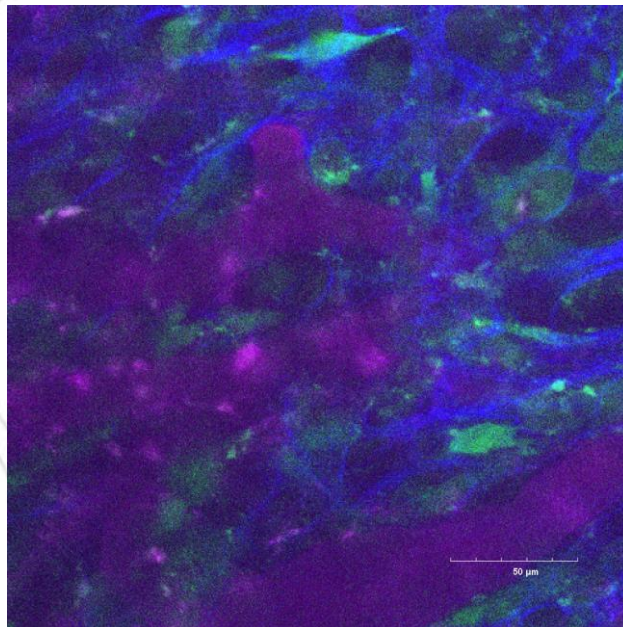
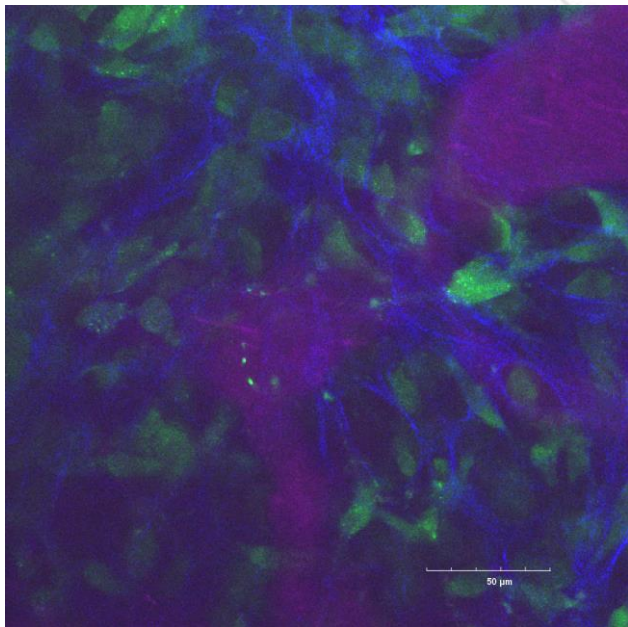
Gray AL, Schiessl I, Dyer DP. Chronic cranial window implantation for high-resolution intravital imaging of the endothelial glycocalyx in mouse cortex. STAR Protocols 2023;4(4):102712.

BBB-NP 1 h after injection

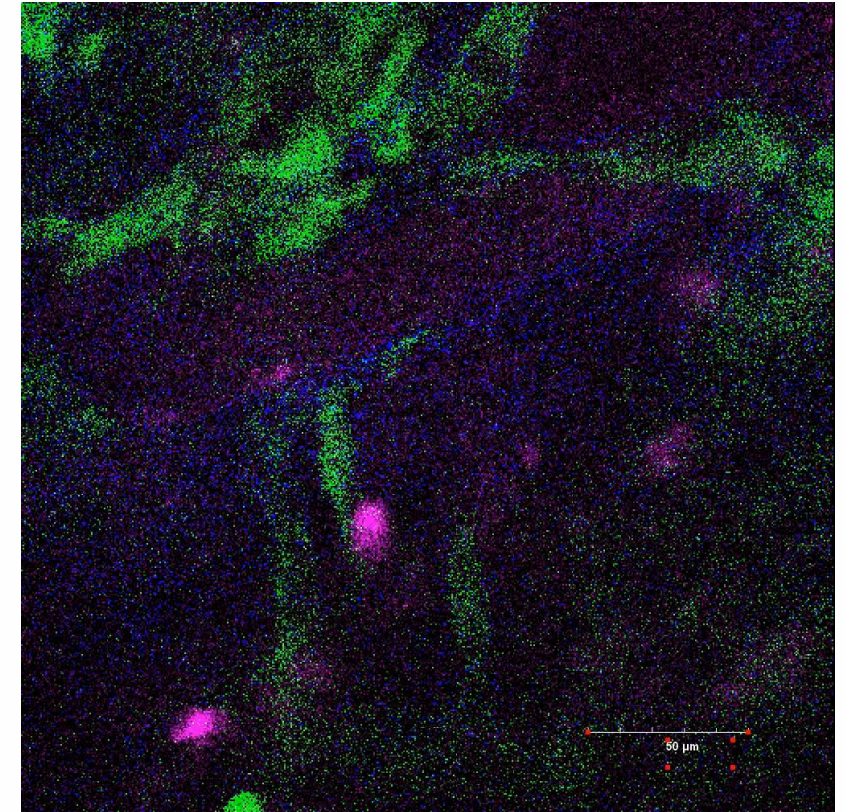
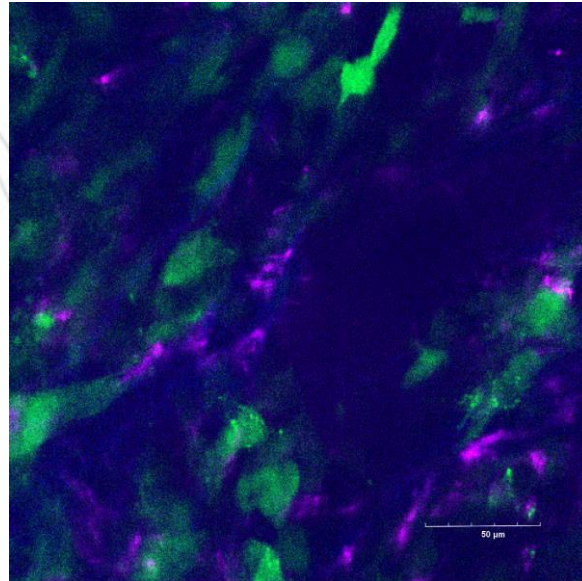
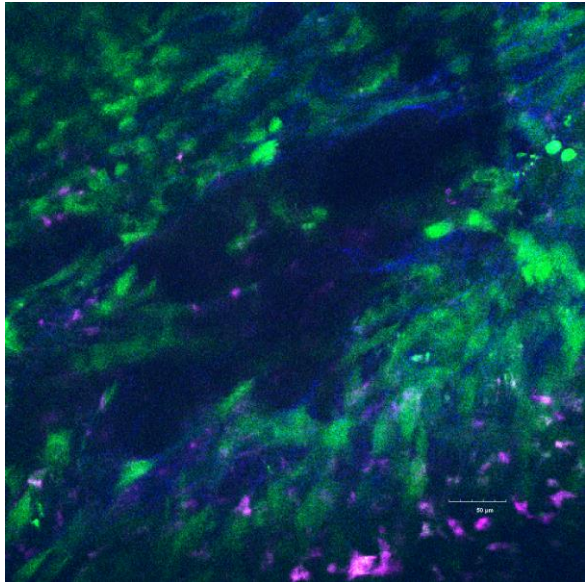


Magenta: LNP
Green: Glioblastoma
Blue: collagen

BBB-NP 24 h after injection

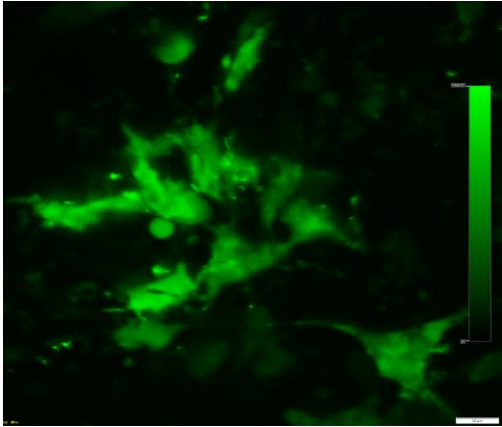
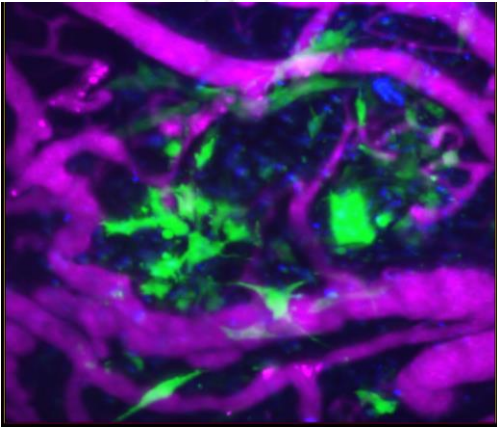
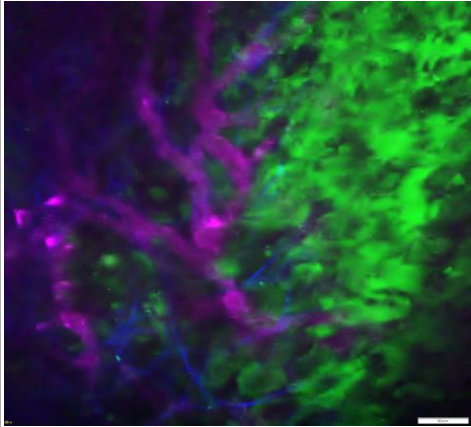
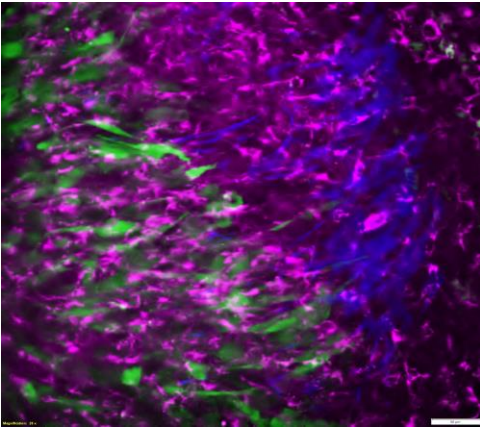
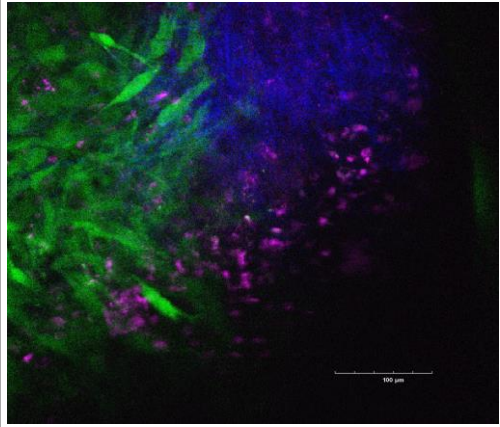
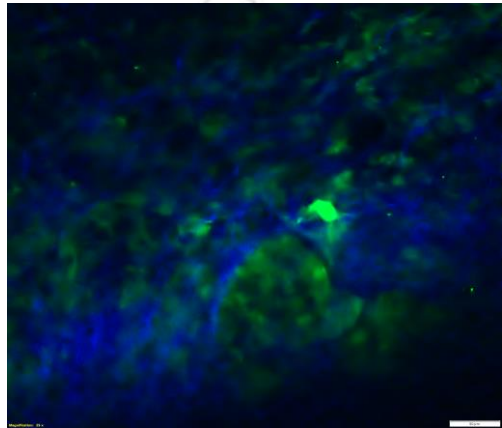
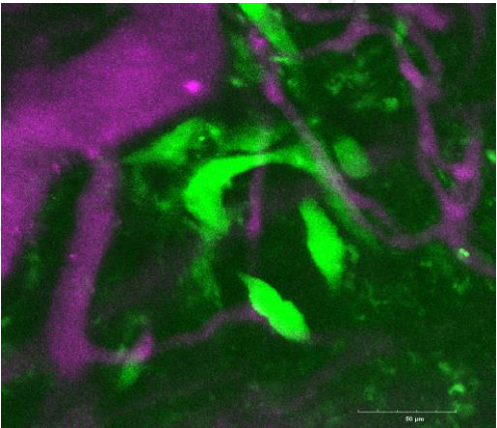
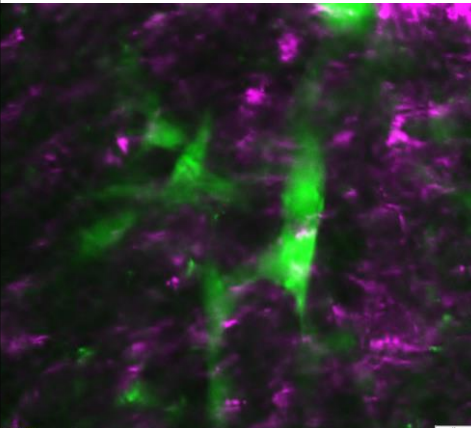
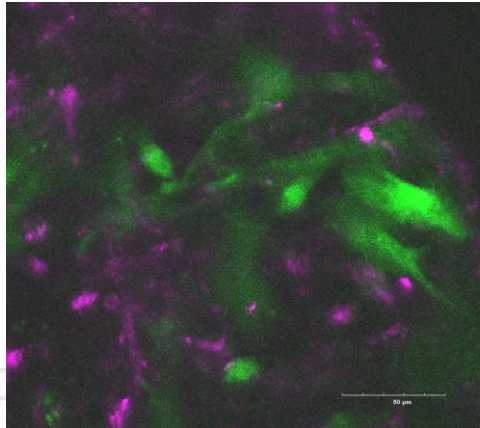
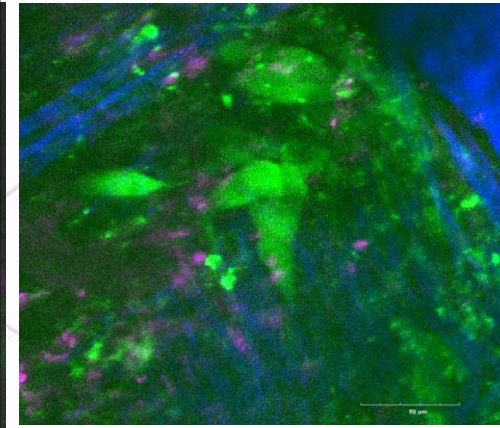


Magenta: LNP
Green: Glioblastoma
Blue: collagen



Magenta: LNP
Green: Glioblastoma
Blue: collagen

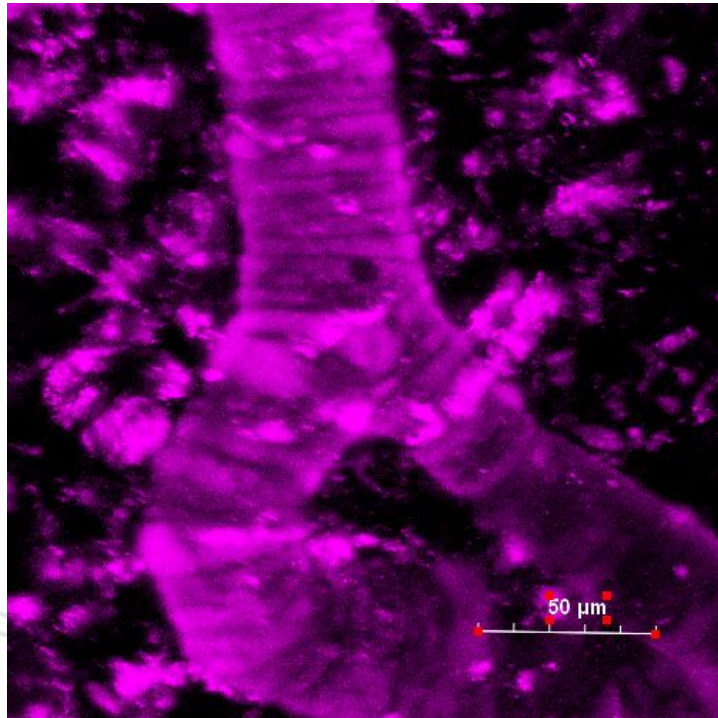
BBB-NP

Tumor	1 h after injection	24 h after injection	48 h after injection	96 h after injection
				
				

PEG-NP

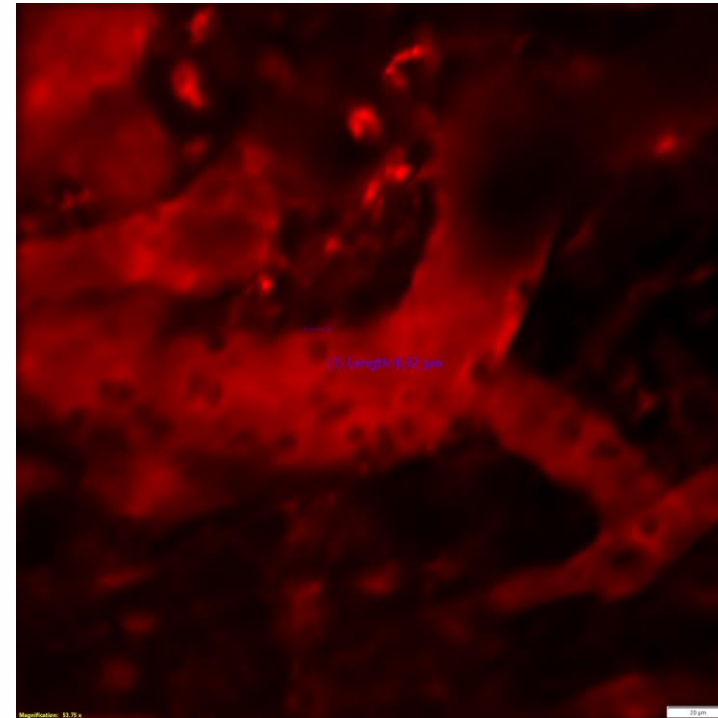
Magenta: LNP
Green: Glioblastoma
Blue: collagen

Nanoparticle stain



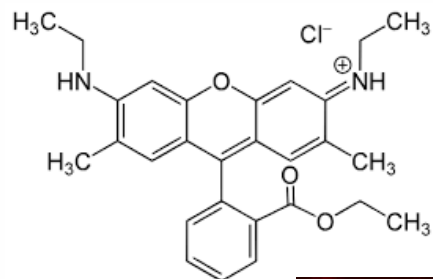
8 μm diameter

Dextran stain

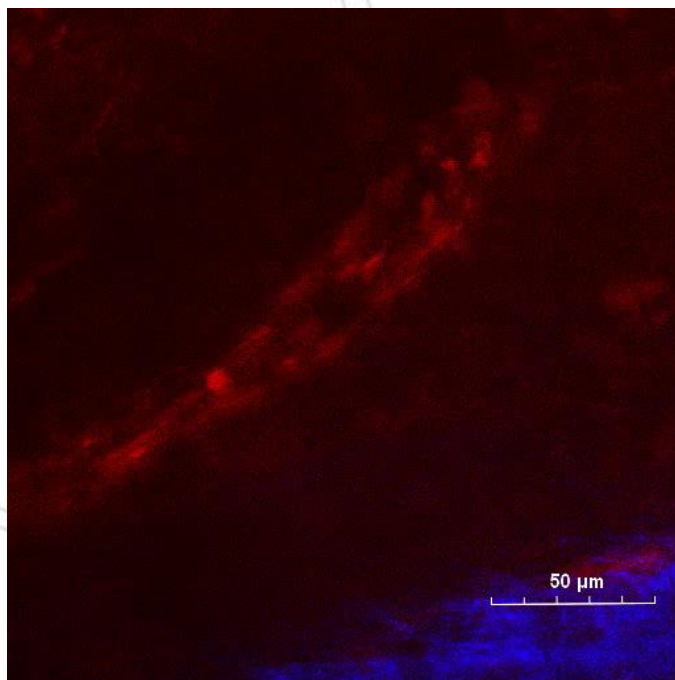


The presence of leukocytes at the endothelium was confirmed by dextran labelling

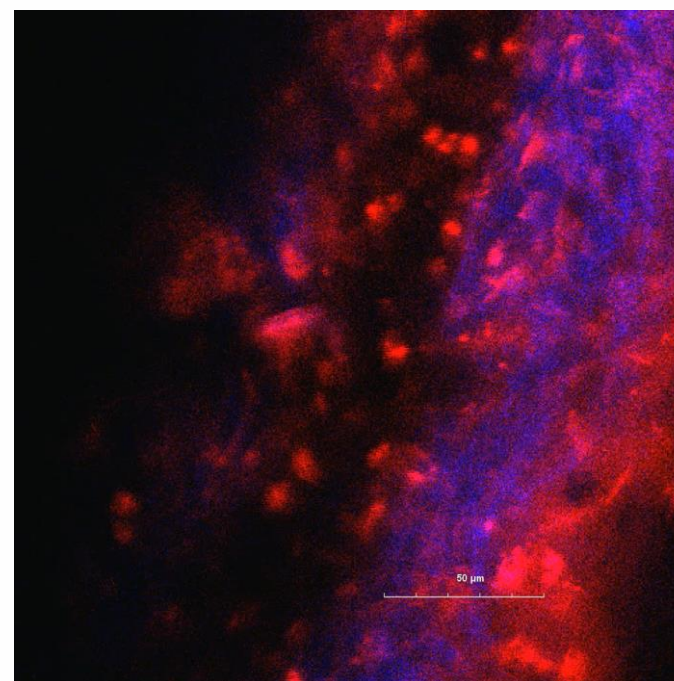
Leukocyte labelling

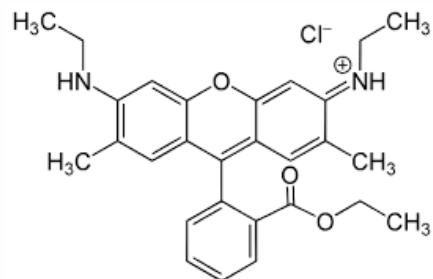


Rhodamine 6G



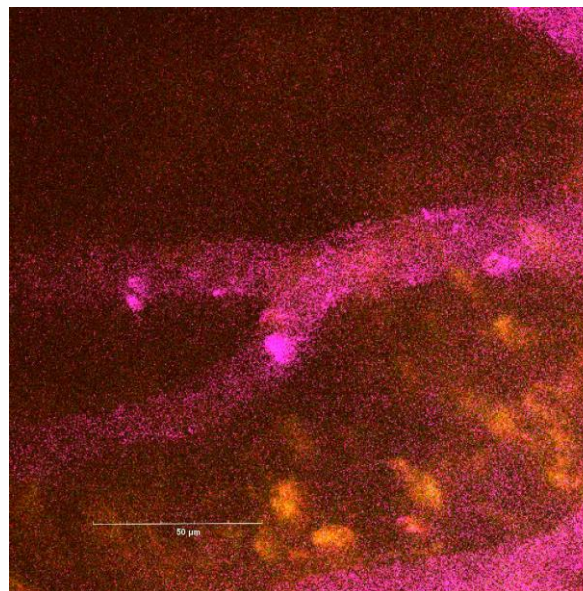
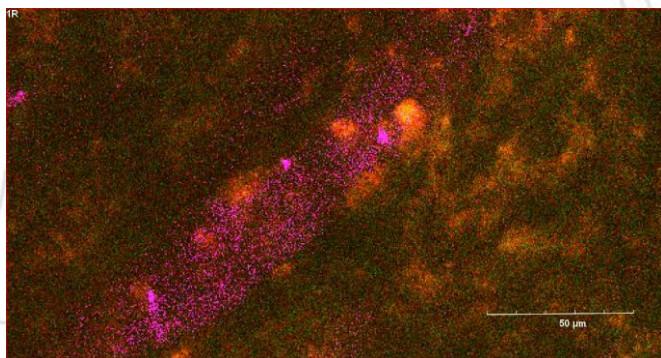
Leukocyte labelling

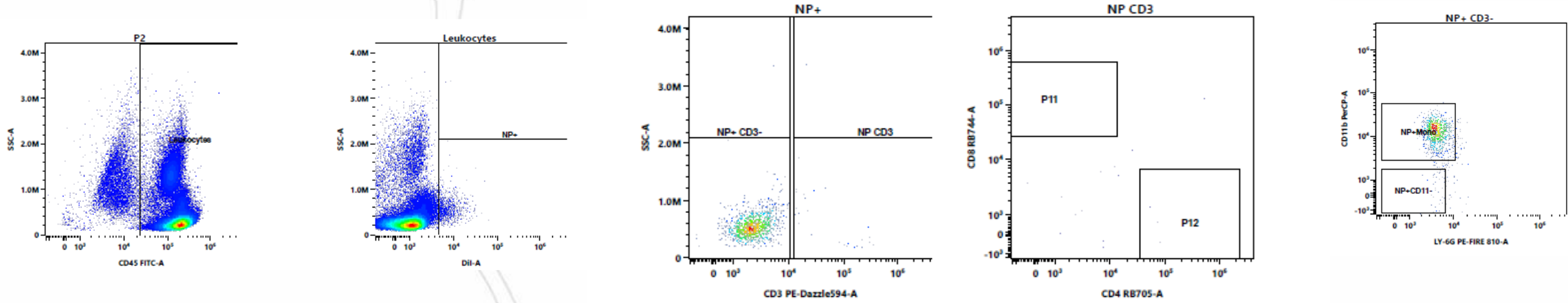




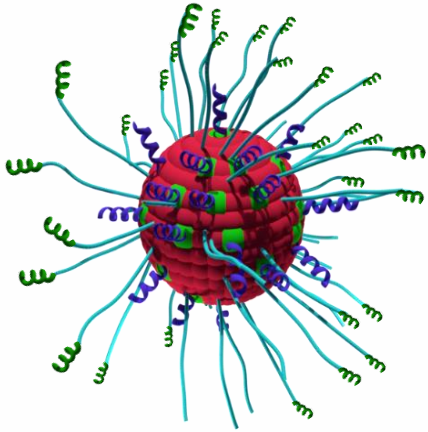
Rhodamine 6G

Leukocyte labelling





96% of the NP-stained cells were monocytes (CD45+,CD3-,CD11b+,Ly-6G-)

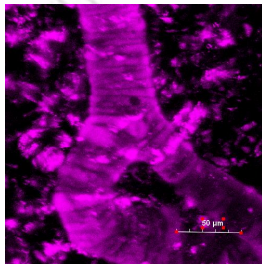
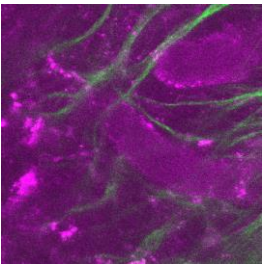


Synthetic Lipid nanoparticles could be an effective drug delivery system targeting glioblastoma

10.7 mm³ 9.70 mm³ 0.34 mm³ 2.24 mm³ 2.44 mm³



Biodistribution studies confirmed the presence of Nanoparticles in the brain



We observed both transcytosis and cell-mediated transcytosis.

NOBEL GROUP:

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Ana Sampedro Viana
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Teresa Abengoza Bello
Christian Oblitas
Sara Ortega Espina

Dinorah Friedmann-Morvinski (Tel-Aviv University).



Anat Globerson Levin (Tel-Aviv Souravsky Medical Center)

Tambet Teesalu (Tartu University)



Jacobo Cruzes (GalChimia S.L.)



Pelayo Garcia-Acevedo (International Iberian Nanotechnology Laboratory)



Andrés da Silva-Candal (bflow S.L.)



Thank you for your attention!

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