Organoid platform for modeling the blood-brain-barrier

Transporter Conference 2019

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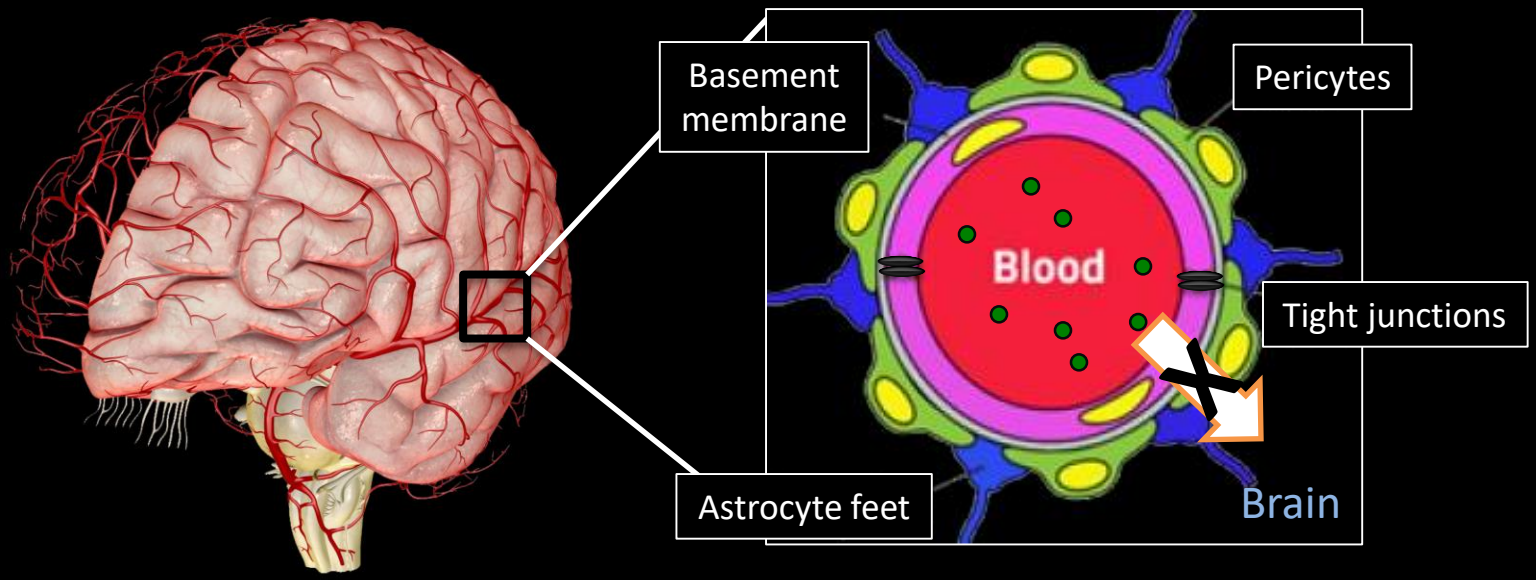
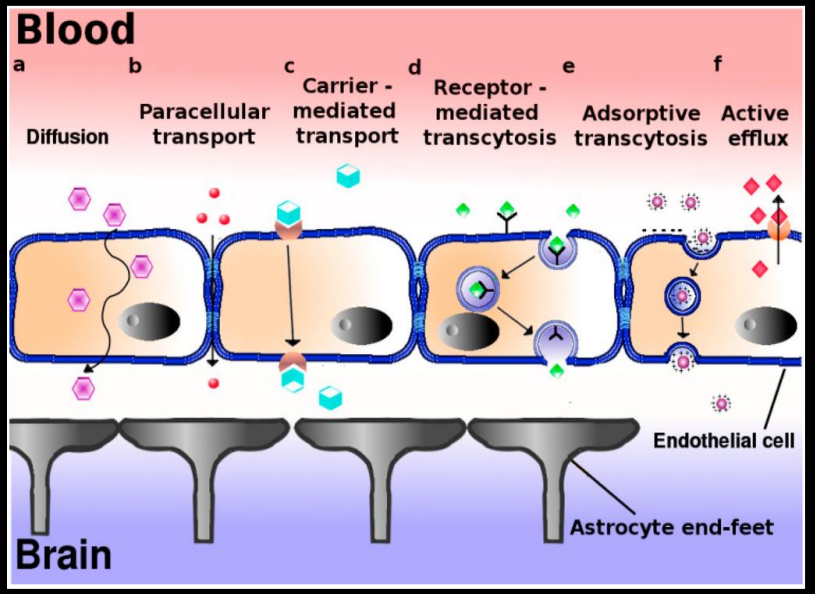
Blood Brain Barrier (BBB)



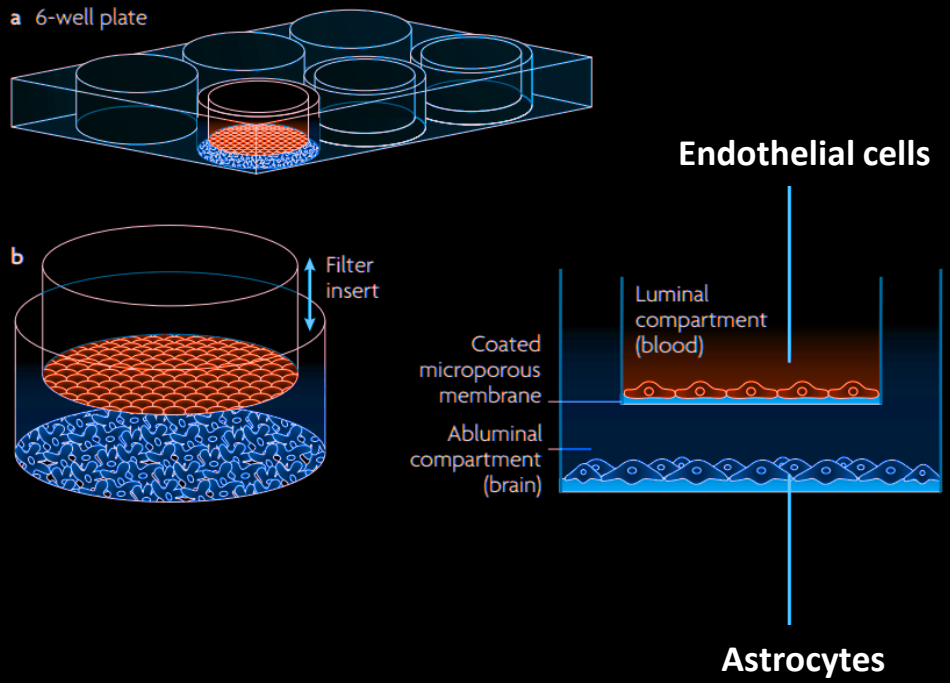
BBB:

- Protects brain from harmful substances.
- Estimated 98% of drugs cannot cross the BBB.
- Clinical need: Brain therapeutics that can overcome the BBB.



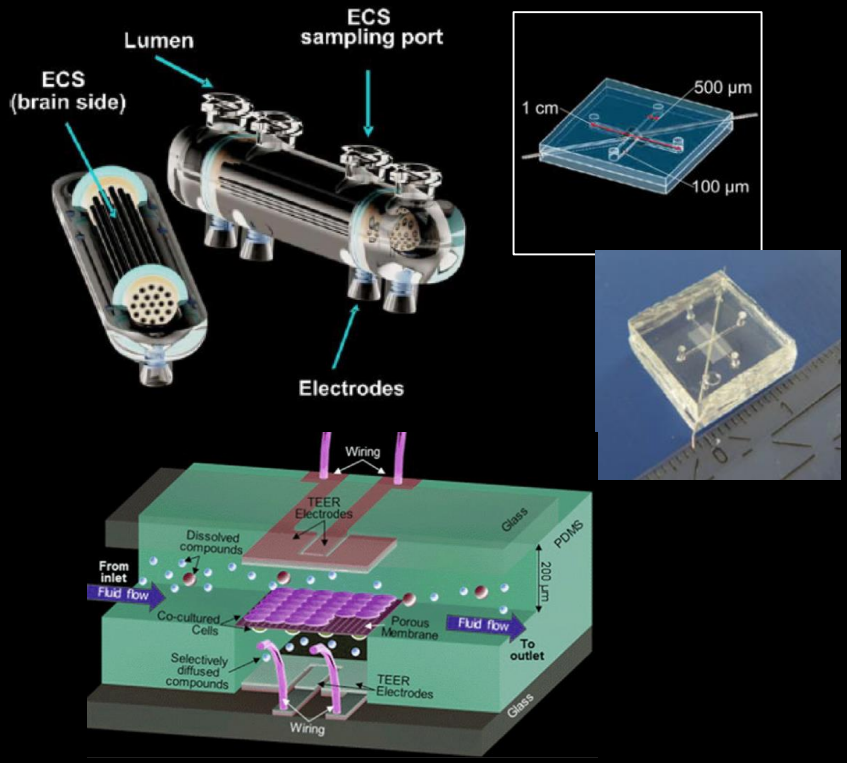
Models to study BBB

Trans-well (static) model



- 2D in vitro BBB model.
- Mid-throughput
- Monolayer of cells – dedifferentiation and loses BBB properties.
- Failure to account of cell-cell interactions.

Dynamic BBB model and microfluidic systems



- 3D - Account for cell-cell interaction, blood flow, shear stress.
- Low throughput.
- Technically demanding

Transwell model: Imperfect barrier formation

- Transwell was first developed in 1983.
- Imperfect EC monolayer across the filter.
- Areas consisting multiple layers of ECs.
- Holes in the monolayer.

Ann Neurol 14:396–402, 1983

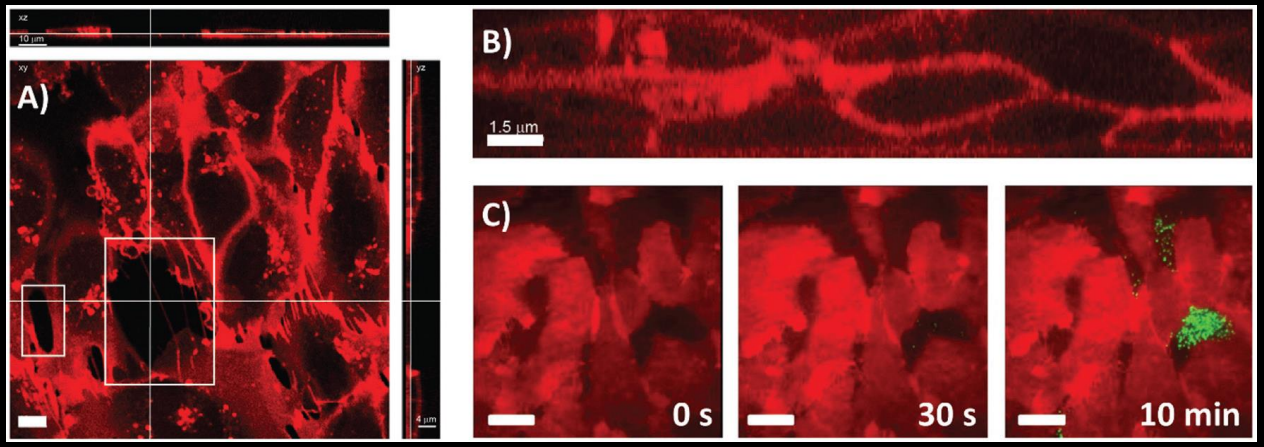
Brain Microvessel Endothelial Cells in Tissue Culture: A Model for Study of Blood-Brain Barrier Permeability

Phillip D. Bowman, PhD,* Steven R. Ennis, PhD,* Kyle E. Rarey, PhD,†
A. Lorris Betz, MD, PhD,* and Gary W. Goldstein, MD*

Mini Review

Diána Hudecz, Louise Rocks, Laurence W. Fitzpatrick, Luciana-Maria Herda and Kenneth A. Dawson*

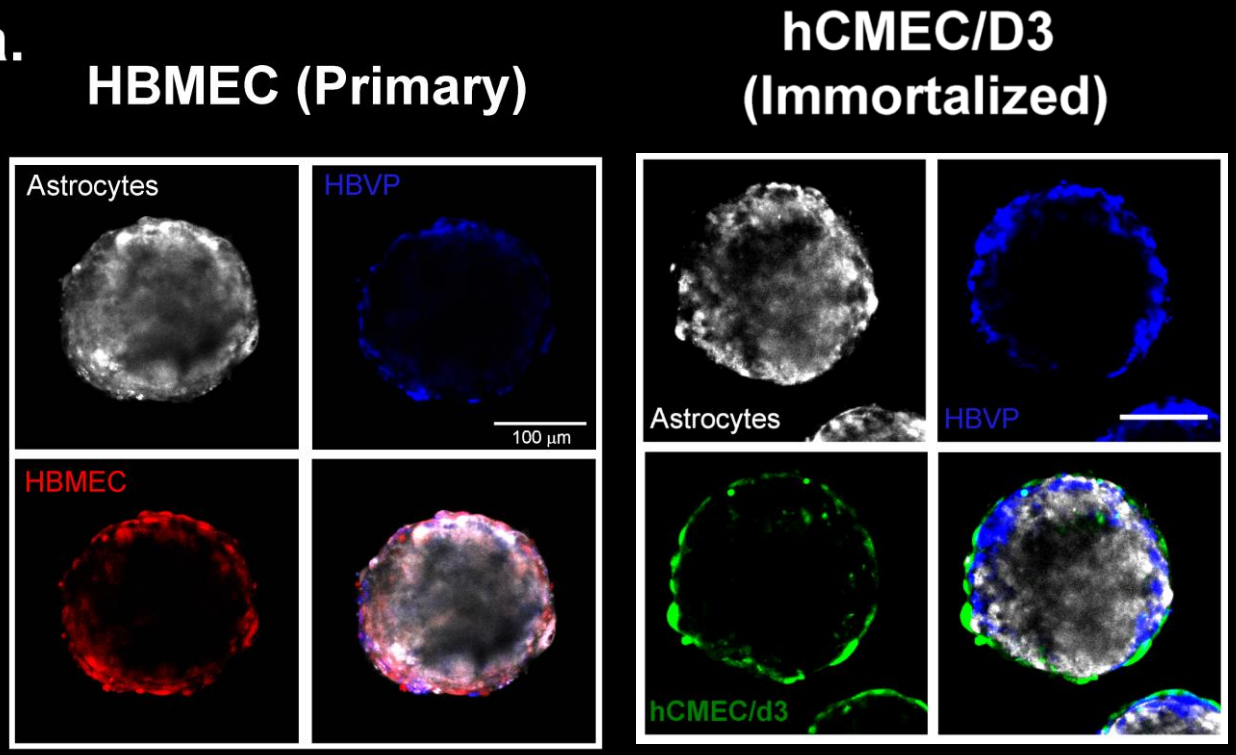
Reproducibility in biological models of the blood-brain barrier



BBB organoids

Triple co-culture of
Astrocytes, Pericytes, Endothelial cells

a.



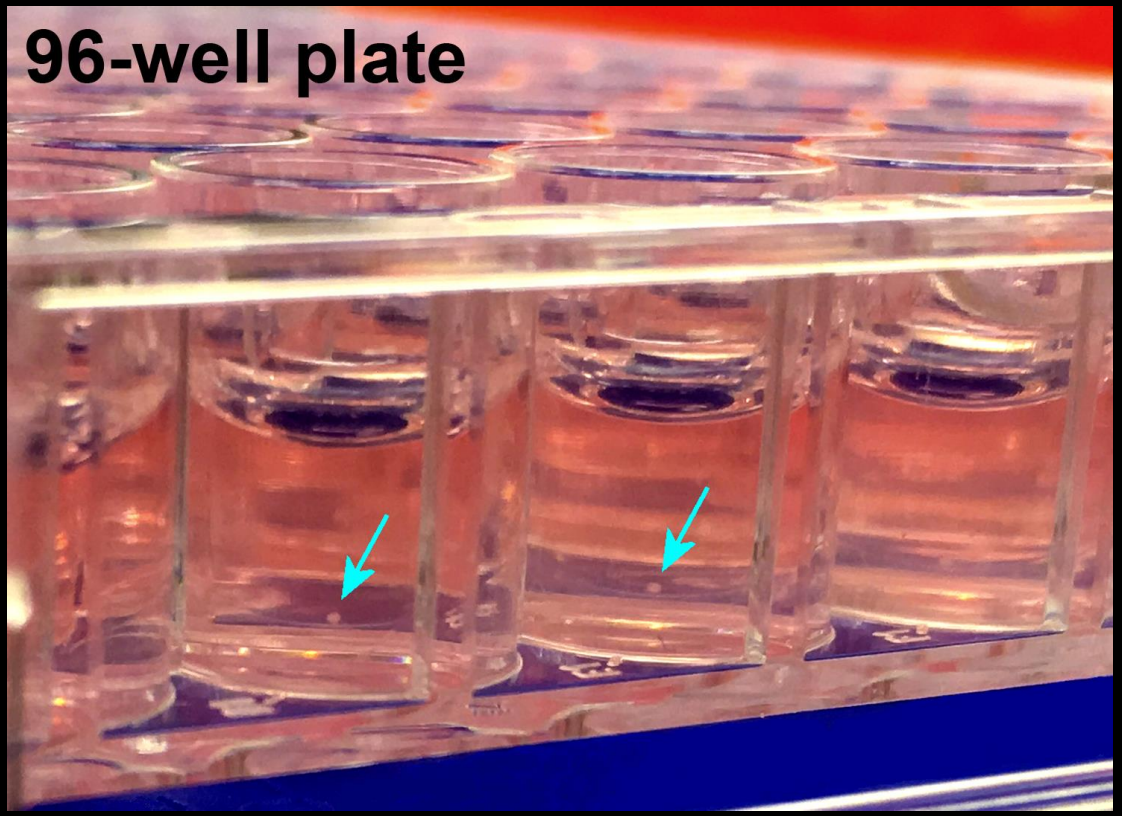
Cho et al. *Nat Comm* (2017)

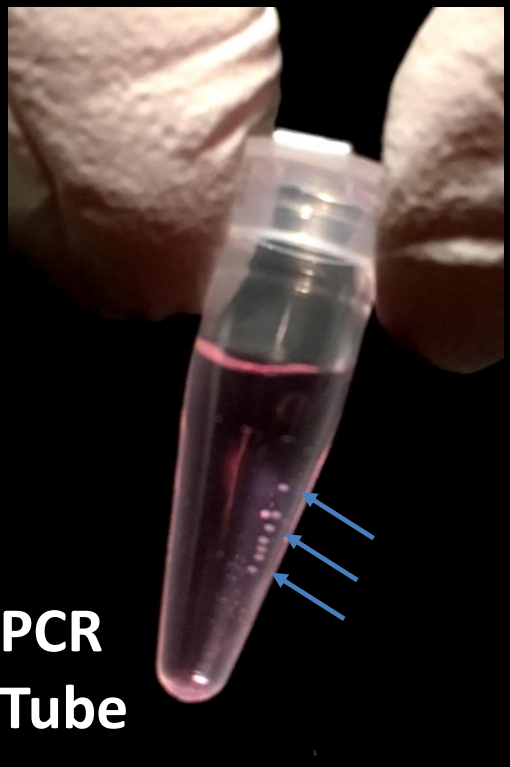
Advantages:

- 3D in vitro multicellular BBB spheroids.
- Accounts for cell-cell interactions.
- High-throughput.
- Cost-effective.
- Easily reproducible (spheroid formation: 90% success rate).

Culture of organoids

- 1 spheroid per well
- Low-adherence condition (agarose coated well)
- Can be pooled together for experiments

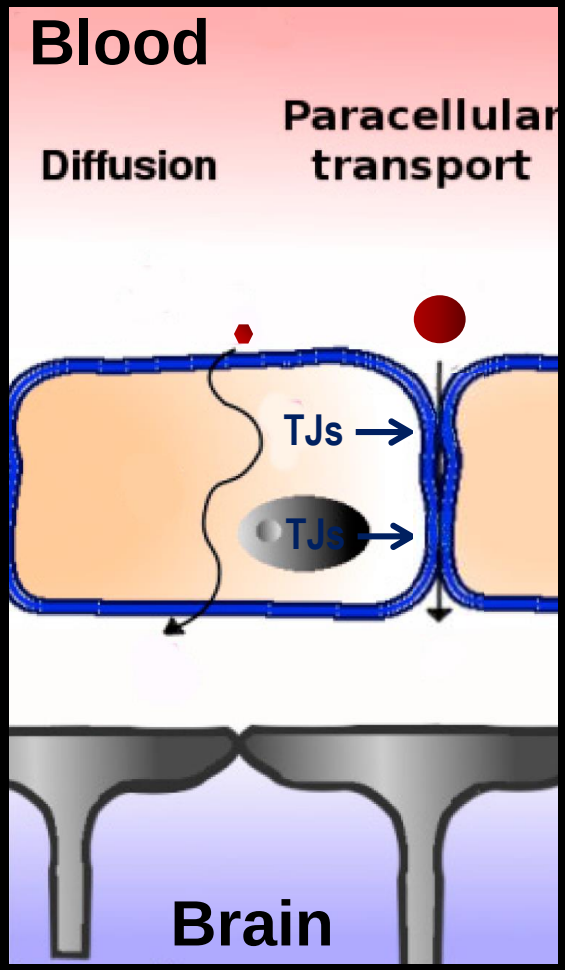




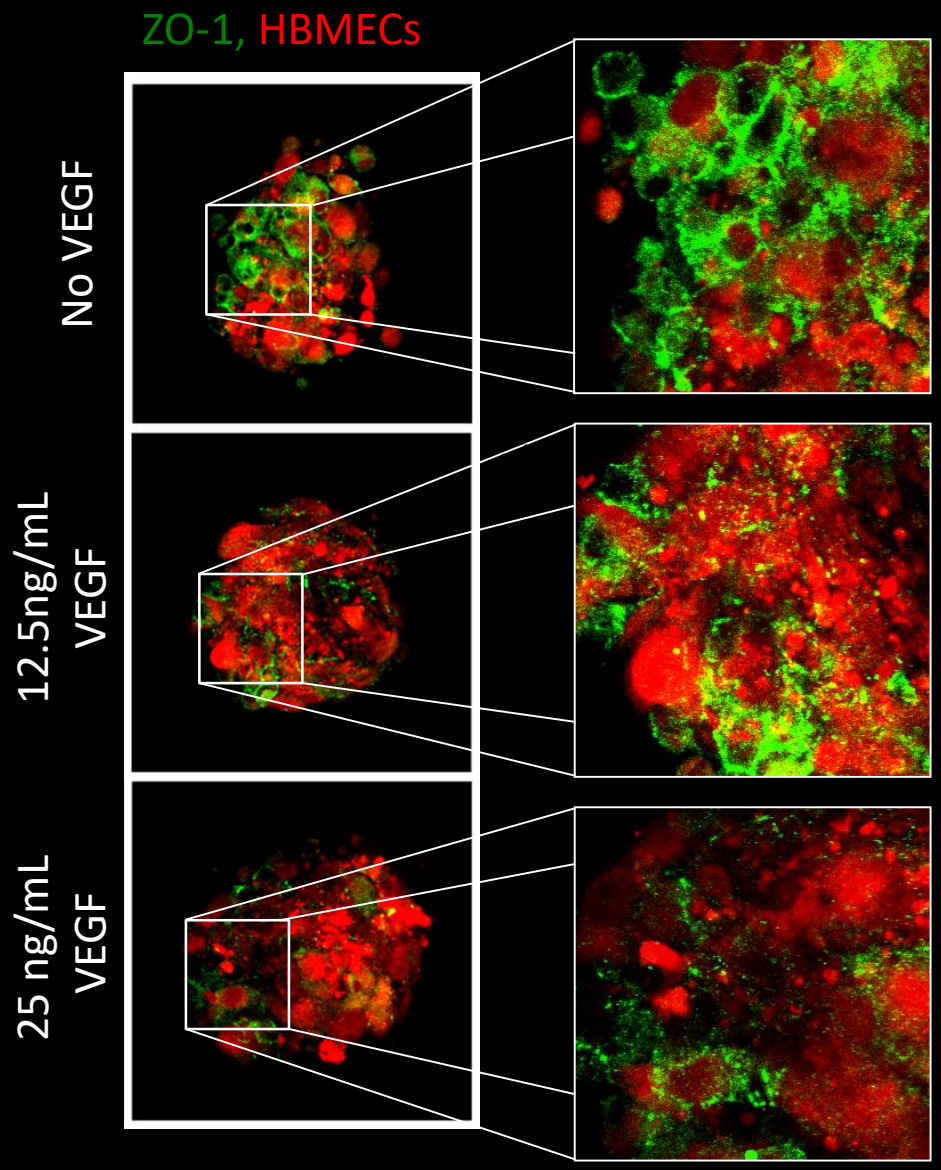
Bergmann et al. *Nat Prot* (2018)

Disruption of tight junctions increases spheroid permeability to dextran

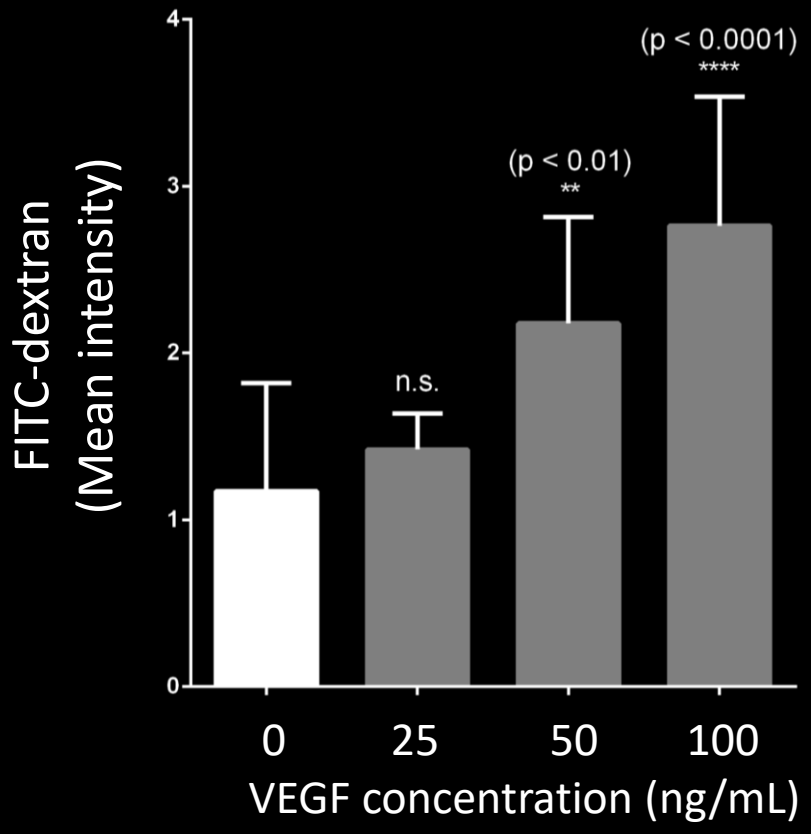
Paracellular pathway



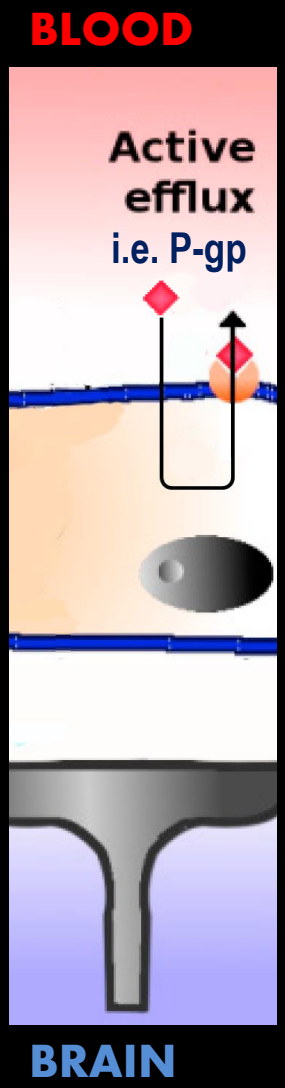
Disruption of tight junctions increases spheroid permeability to dextran



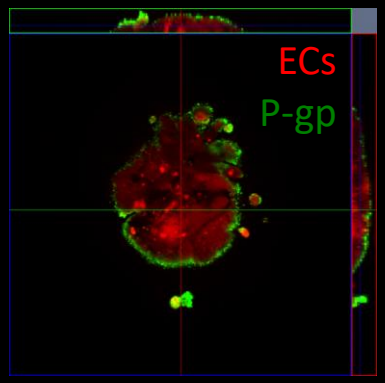
Disruption of tight junctions with VEGF **increases dextran permeability**



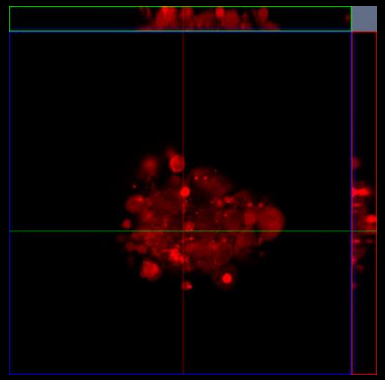
Efflux pump (P-glycoprotein) in BBB spheroid



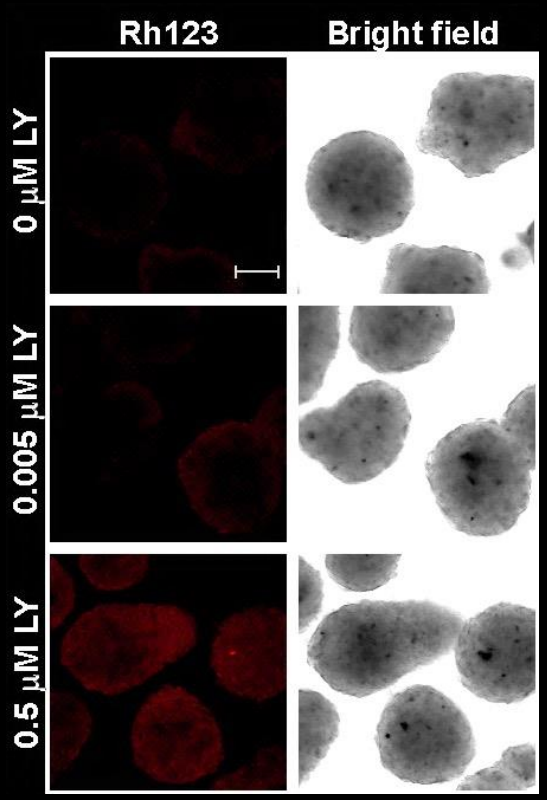
Anti-P-gp IgG

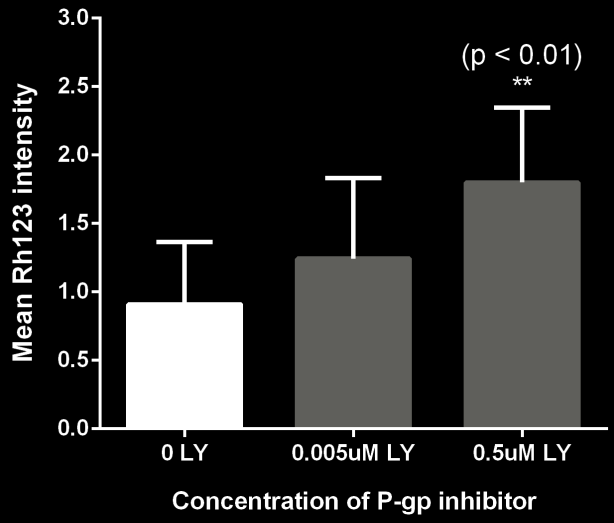


Secondary IgG only control



Rhodamine-123: Substrate of P-gp

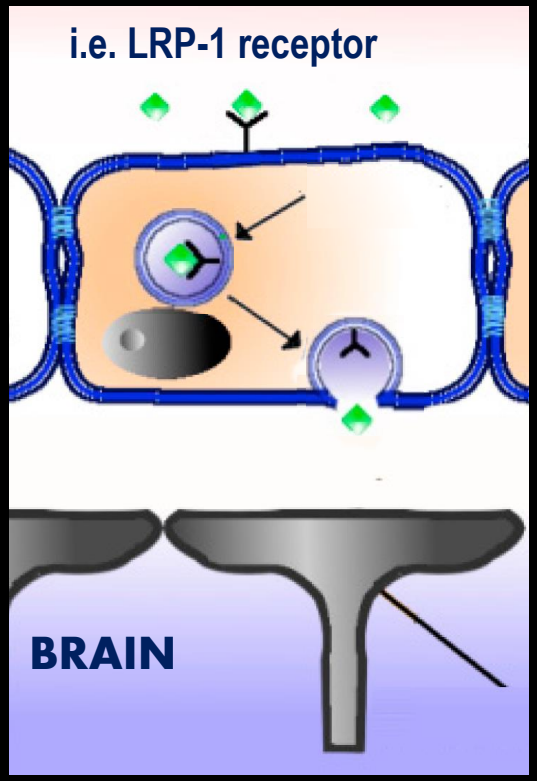




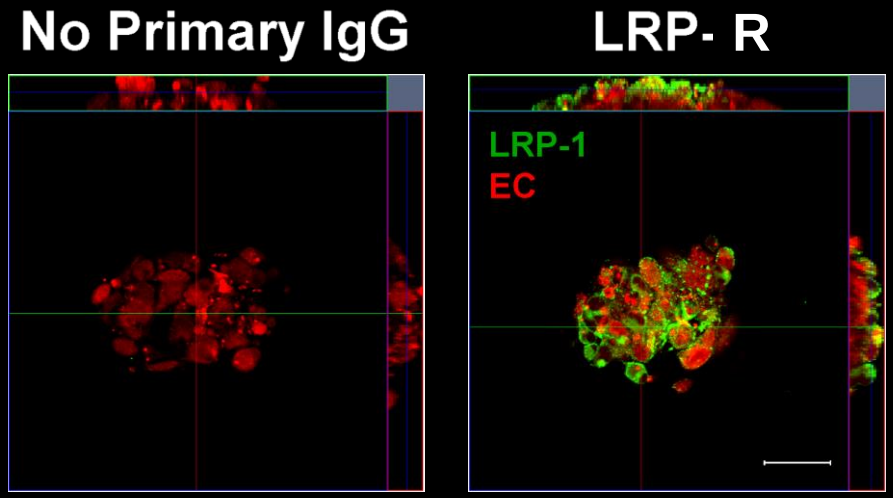
Inhibition of P-gp promotes entry of Rhodamine-123 into spheroid

Receptor-mediated transcytosis: Angiopep-2

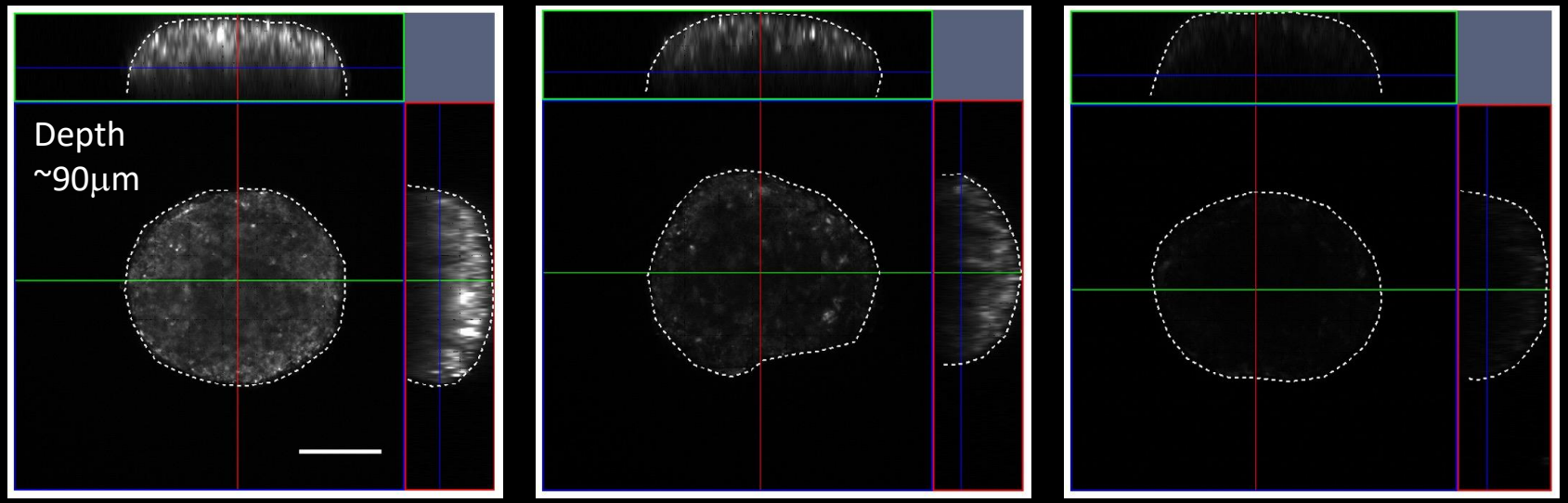
BLOOD



Receptor-mediated transcytosis: Angiopep-2



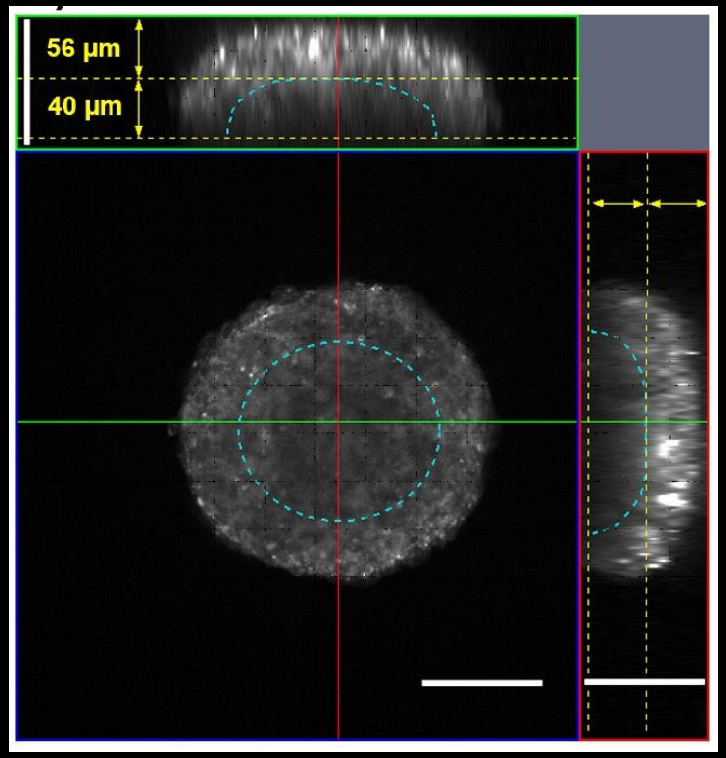
- High LRP-1 receptor expression.
- Angiopep-2, known BBB-penetrating ligand of the LRP-1 receptor.
- High angiopep-2 permeability observed.

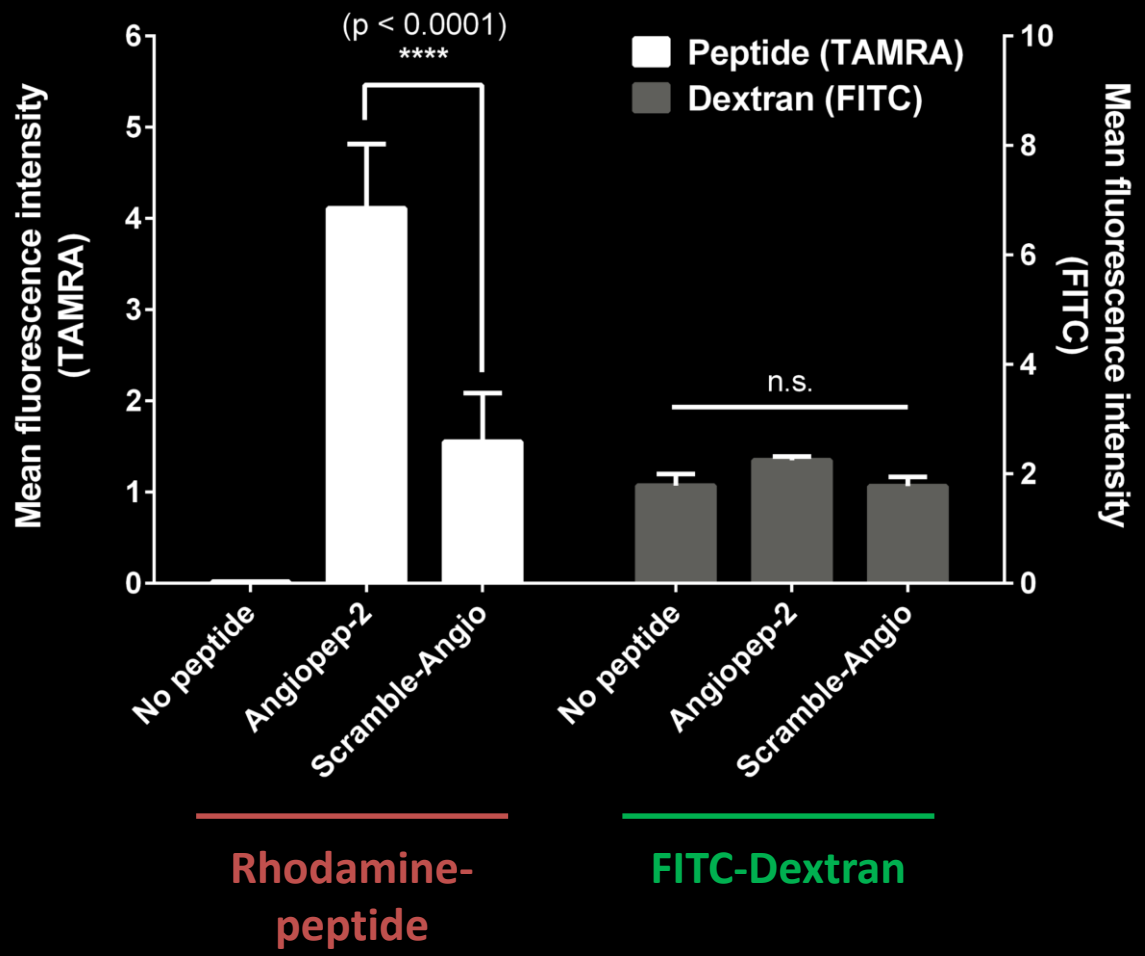


Bergmann et al. *Nat Prot* (2018)

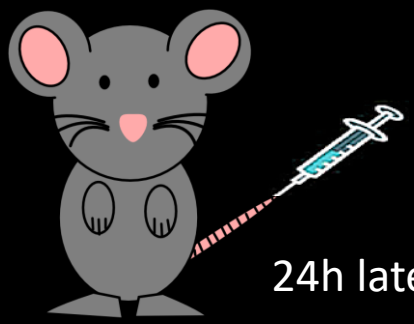
Quantification of angiopep-2 permeability

High permeability of angiopep-2 in BBB organoids that exclude dextran

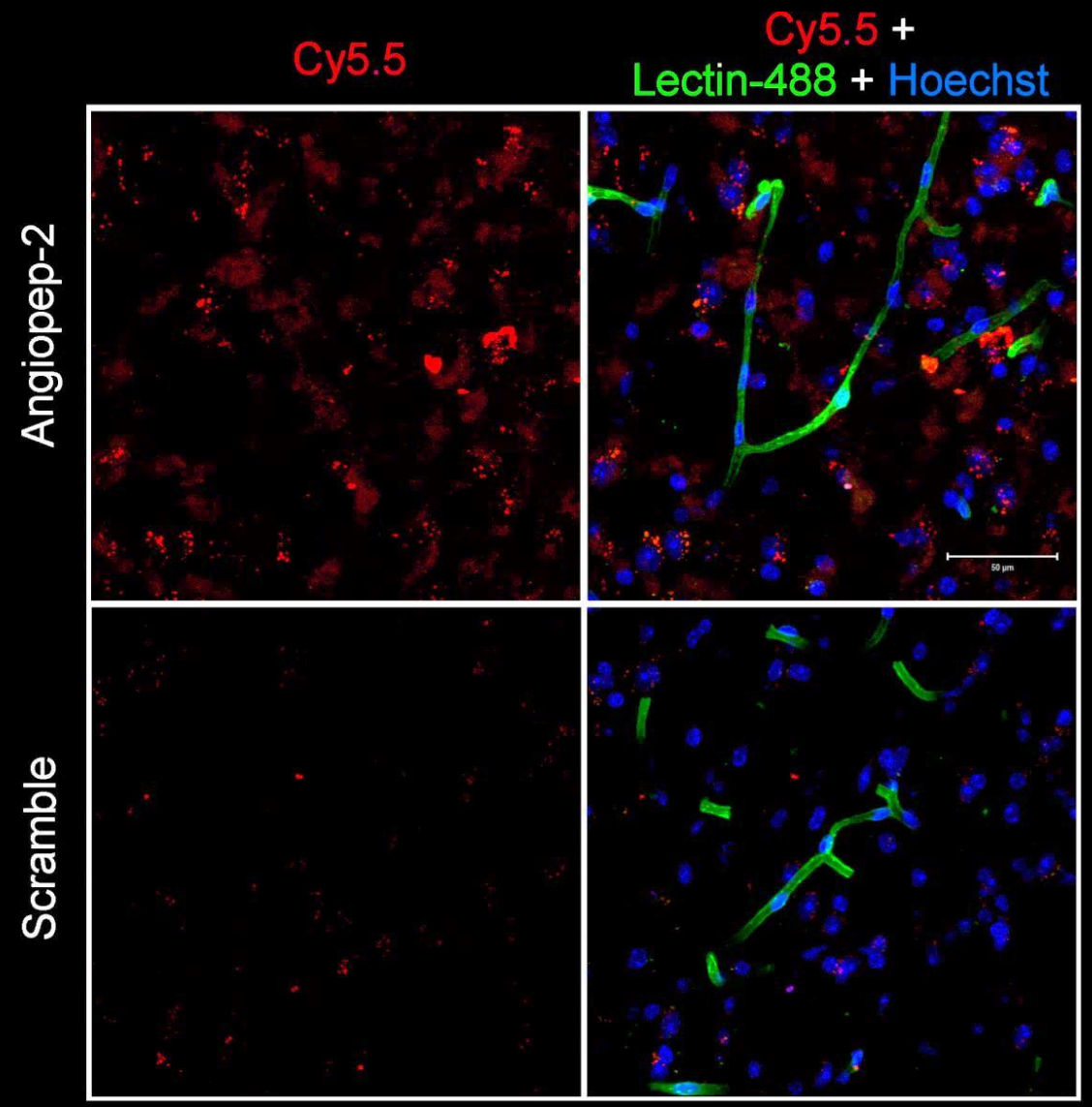




Receptor-mediated transcytosis in mouse brain: Angiopep-2 vs. scramble peptide



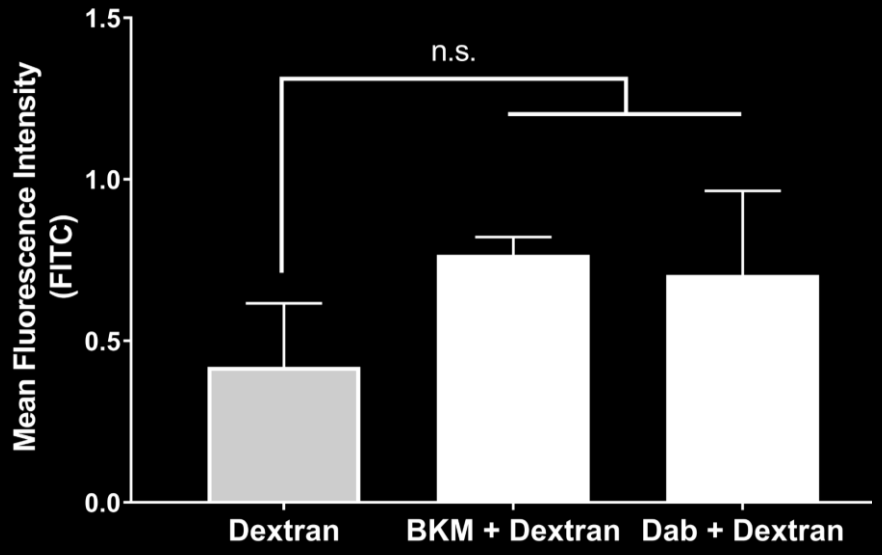
24h later…



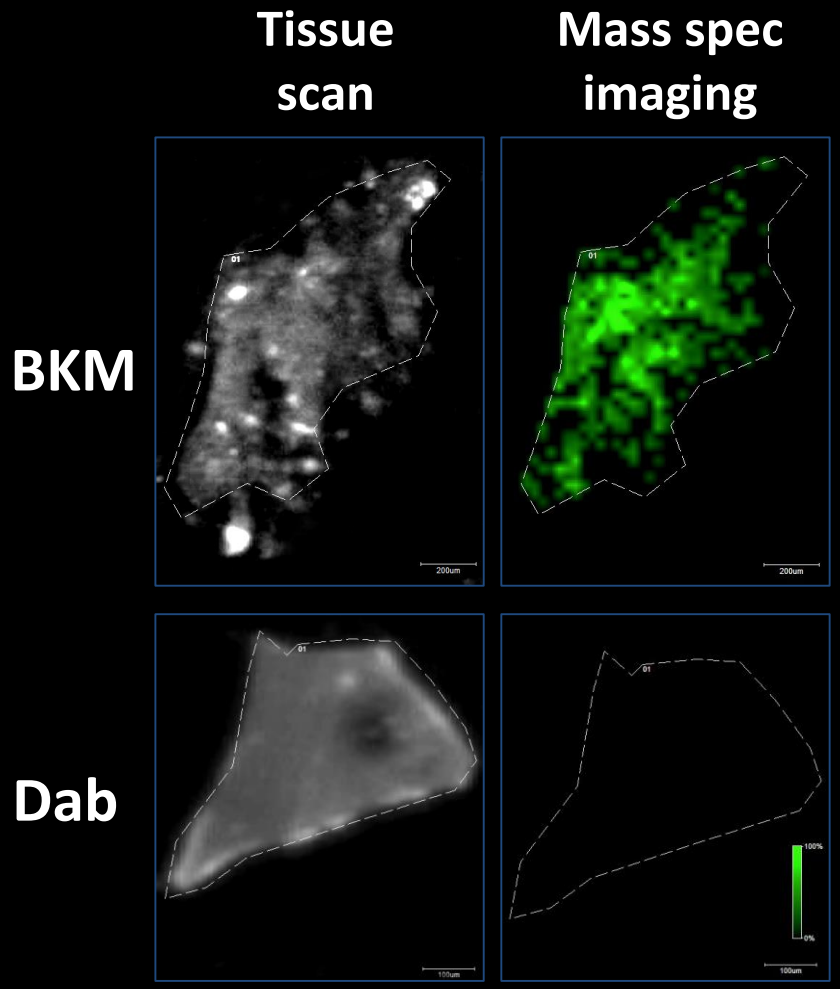
Mass Spectrometry Imaging: Small molecule detection

BKM-120 and Dabrafenib **do not disrupt BBB integrity**:

Dextran influx



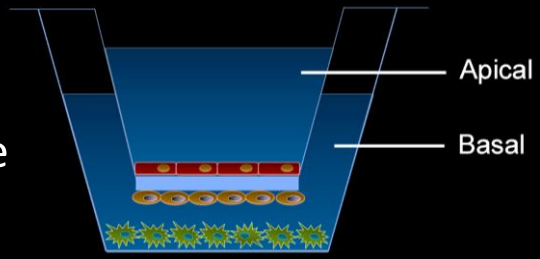
- **BKM-120**: BBB-penetrant drug
- **Dabrafenib**: Non-penetrant (control) drug

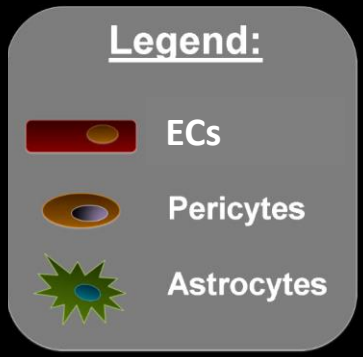


Detection of BKM-120 in BBB spheroids

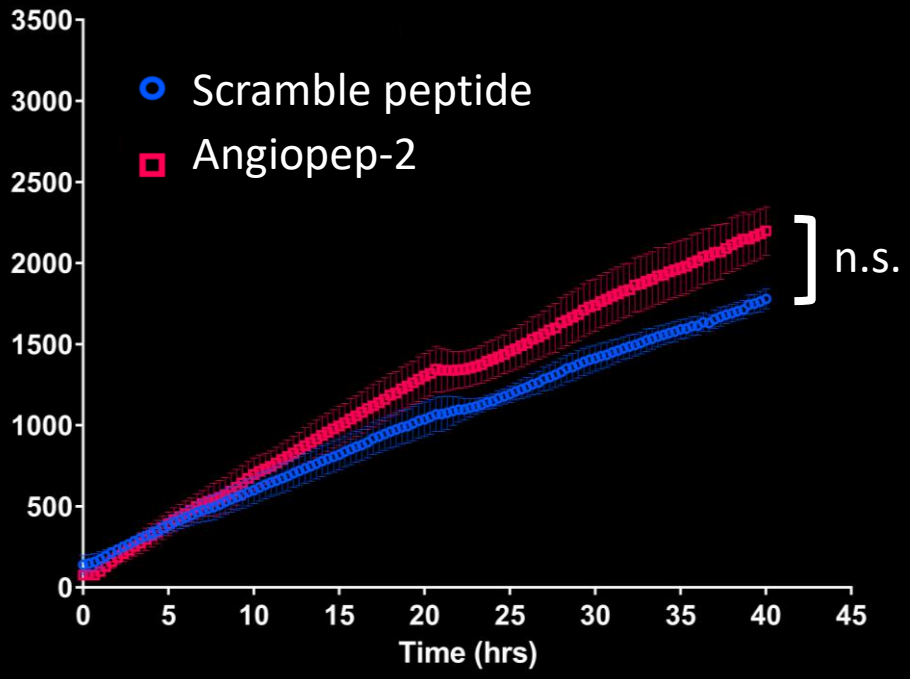
Comparison with well-known Transwell model

Transwell: Co-culture

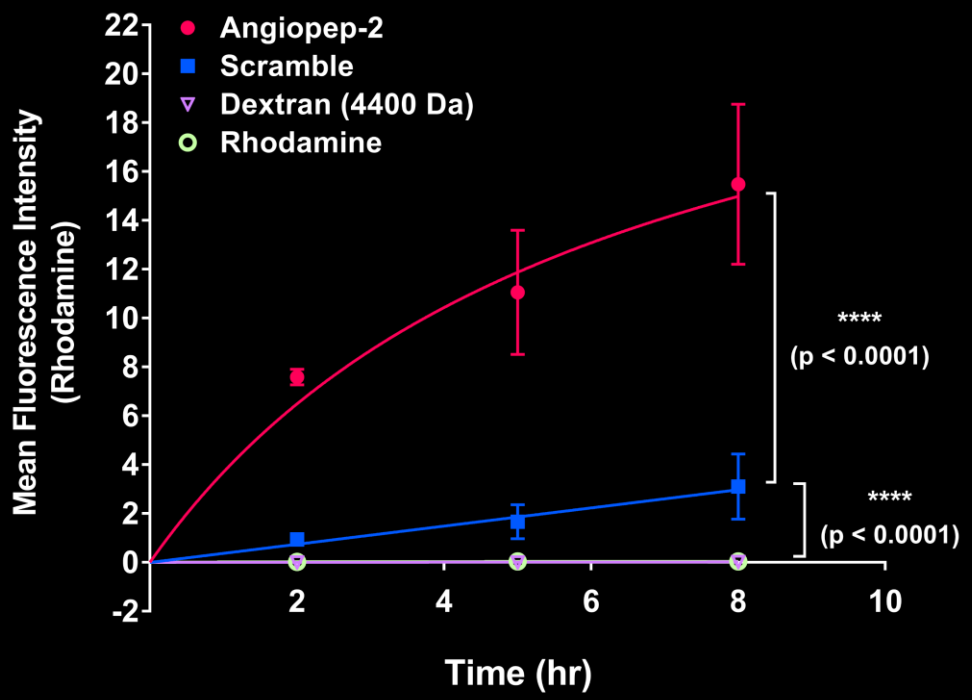




Transwell Model



BBB Organoid Model

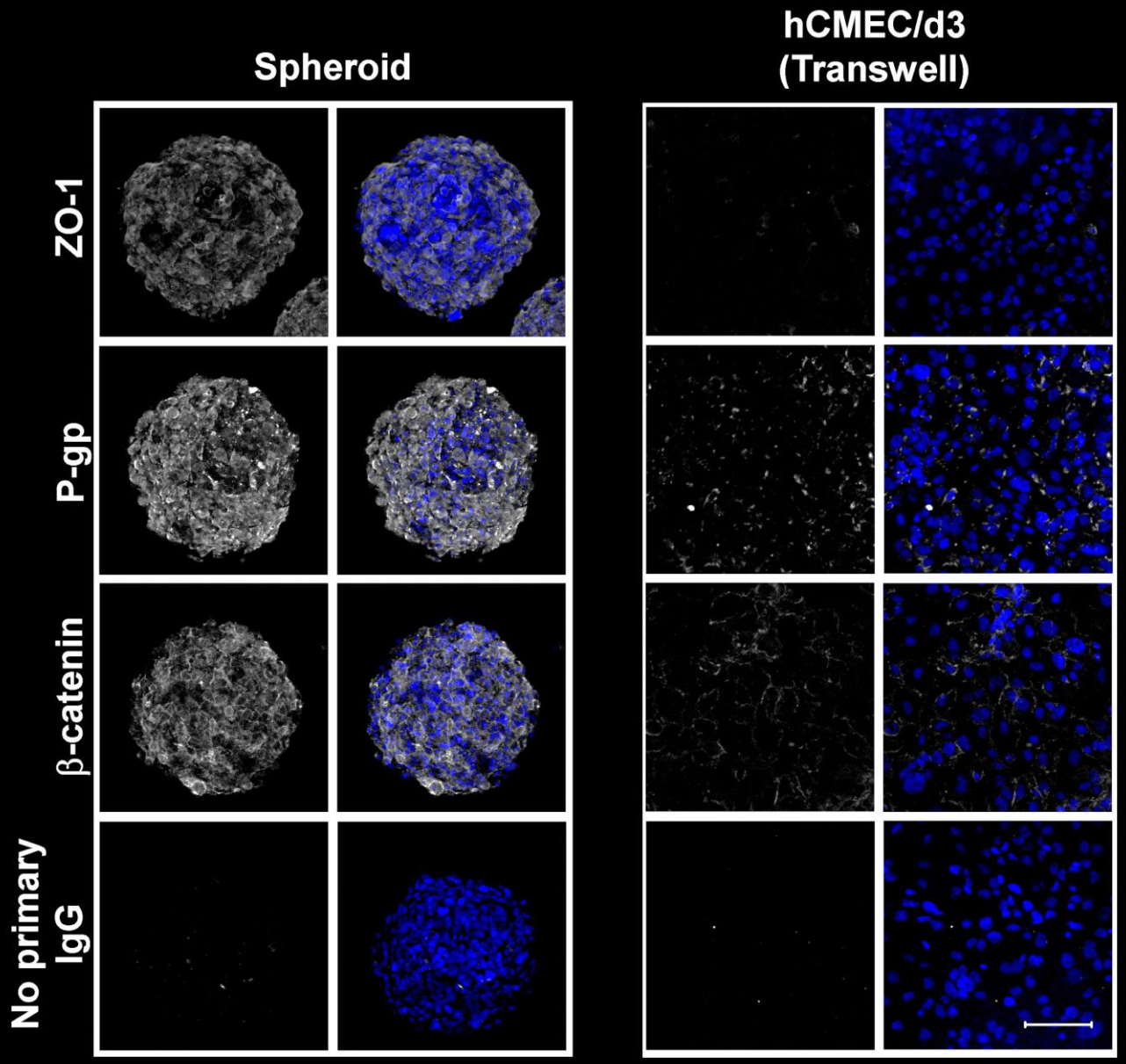


BBB characteristics: Spheroids vs. Transwell Model

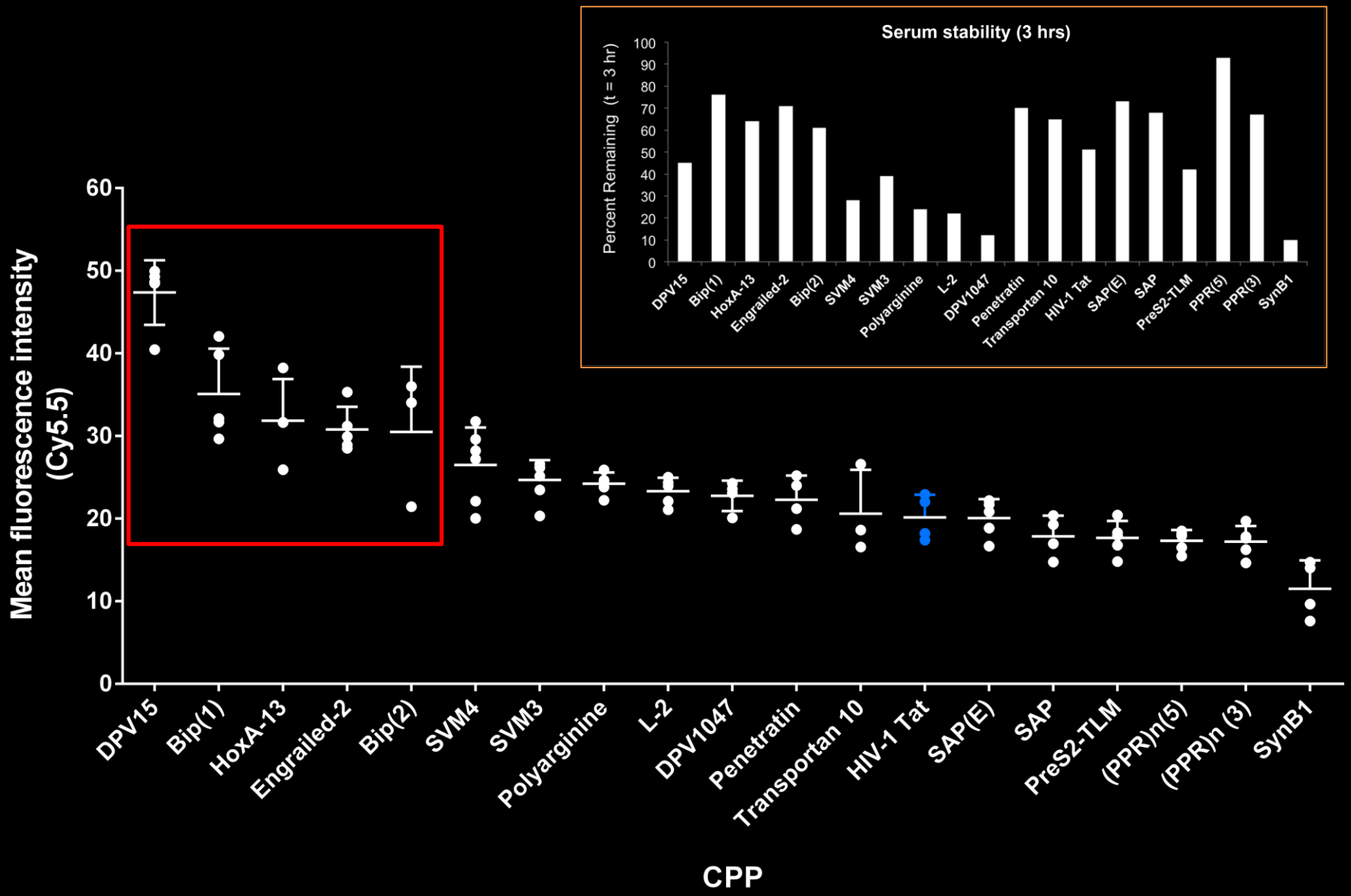
Tight junctions

Efflux pump

Adherens junctions

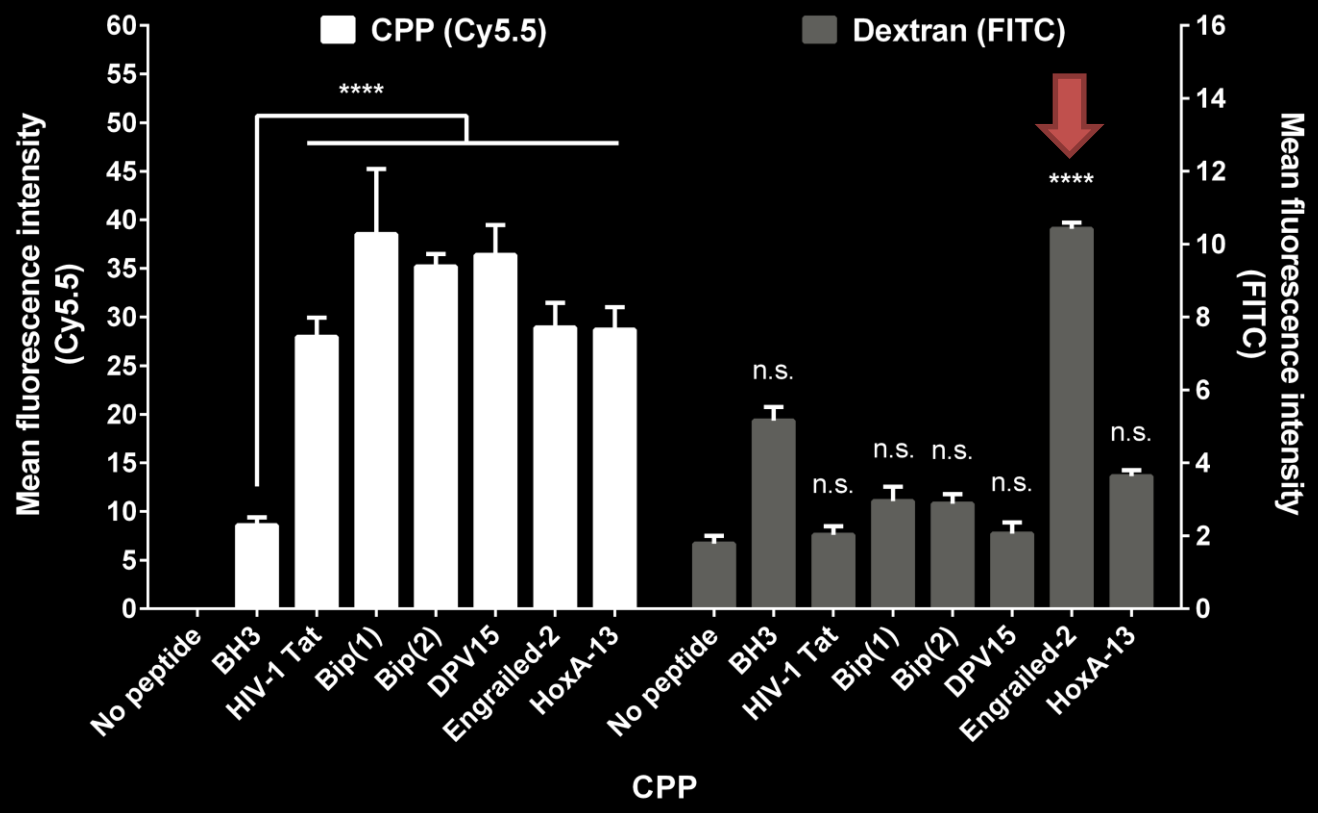


CPP screen using BBB spheroids



Analysis of top CPPs

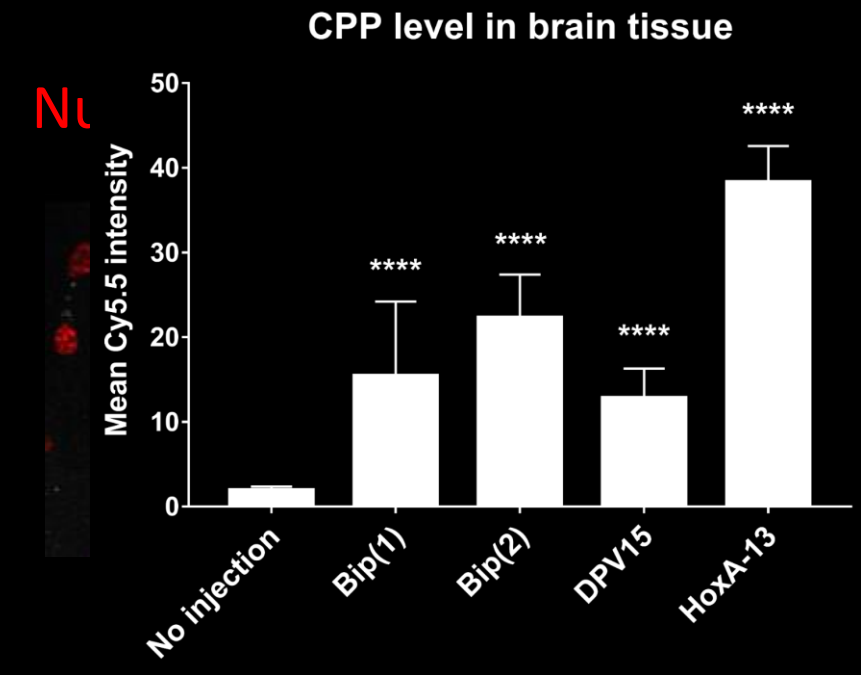
Co-incubation of CPP and dextran

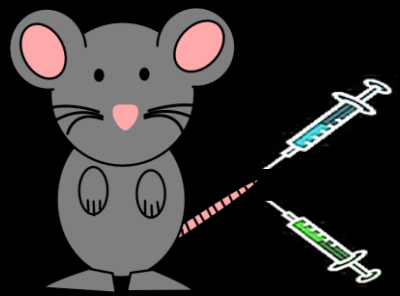


Engrailed-2 promoted influx of dextran

→ Indication of barrier disruption?

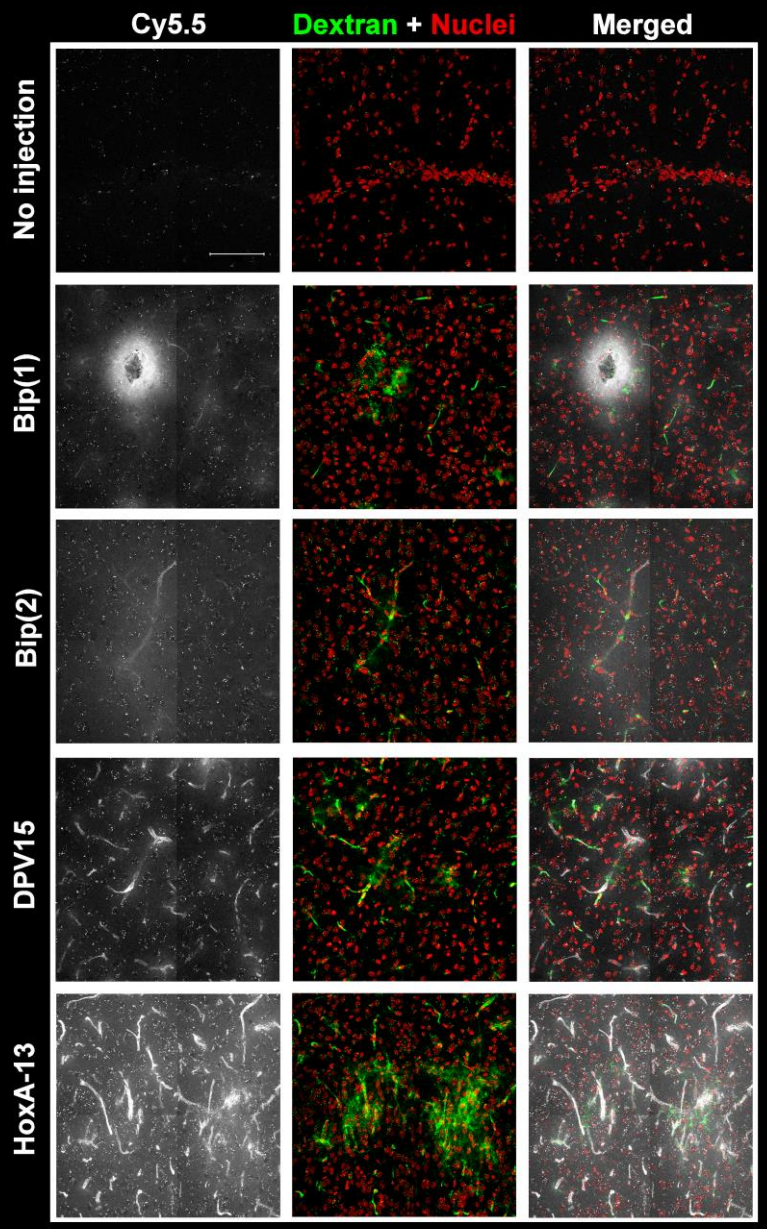
BBB-penetration *in vivo*





1) I.V. administration of **CPP**

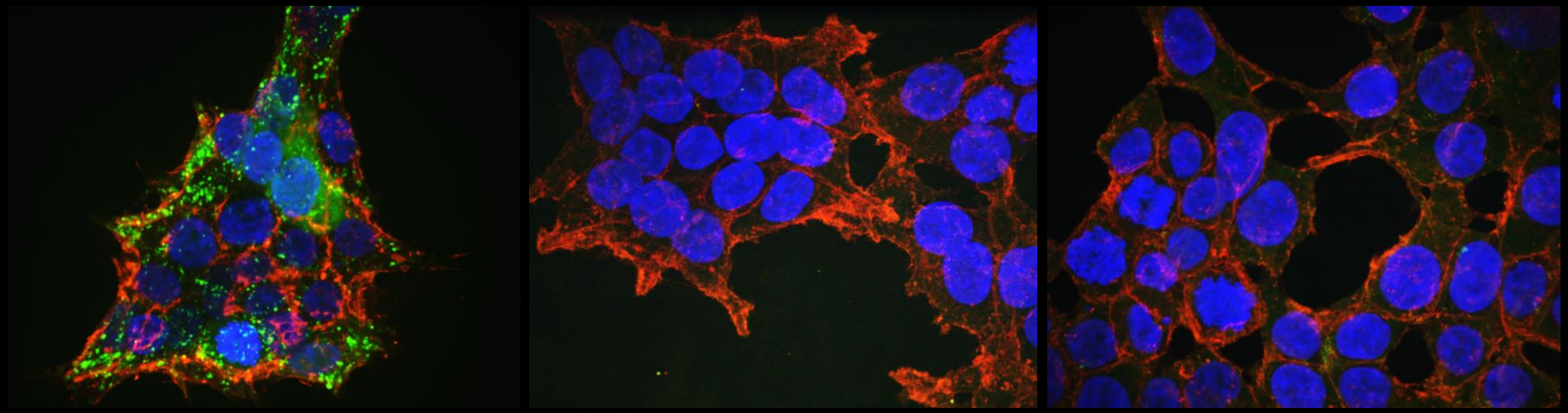
2) I.V. administration of **dextran**

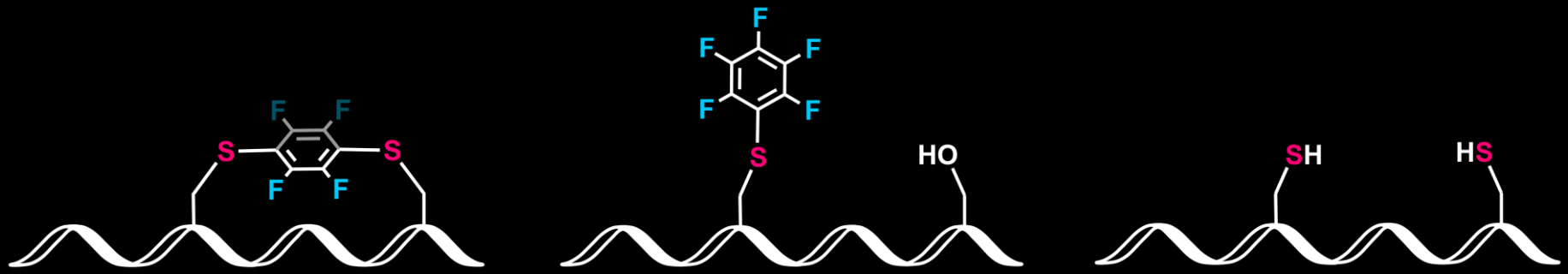


BBB spheroids as drug screening tool:

Chemically modify drugs to enhance BBB permeability?

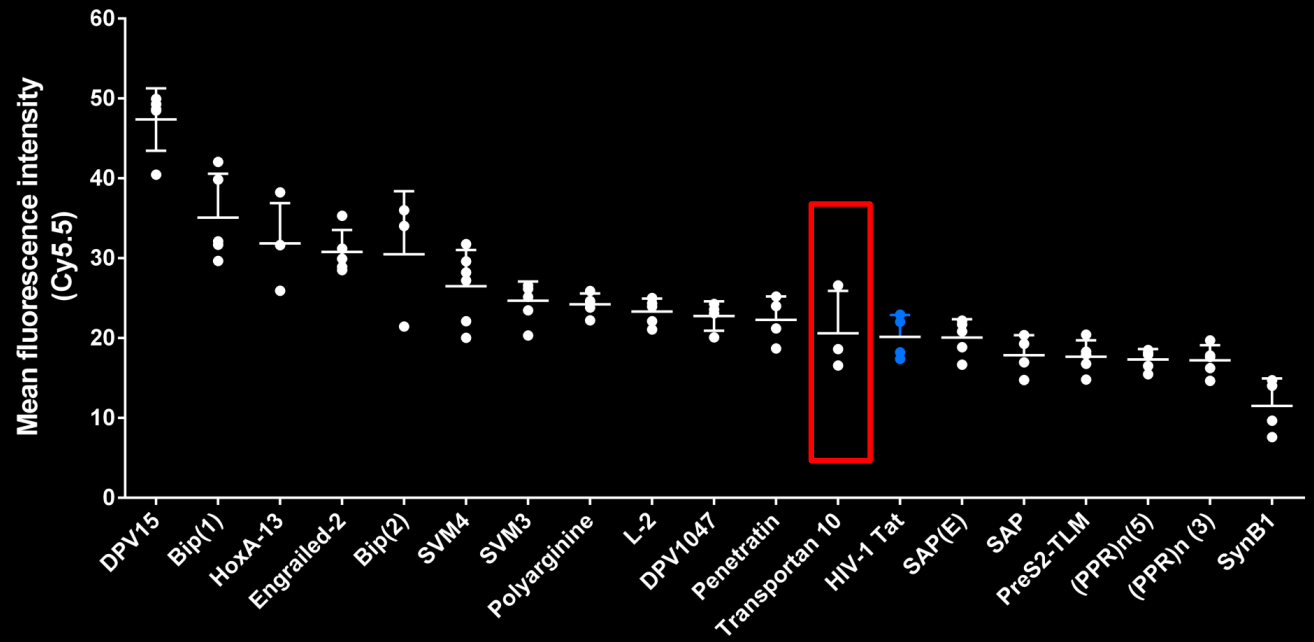
Pentelute Lab: JACS, 2013, 135 (16): 5946-9

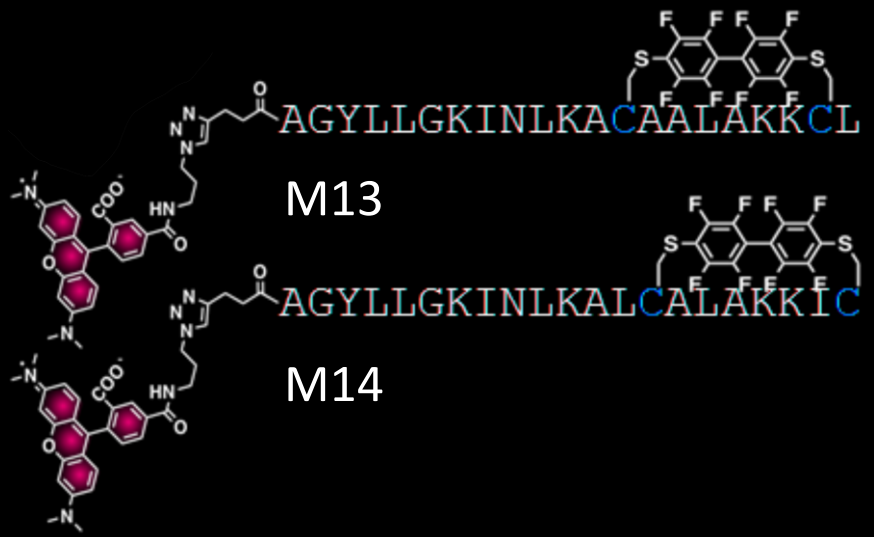




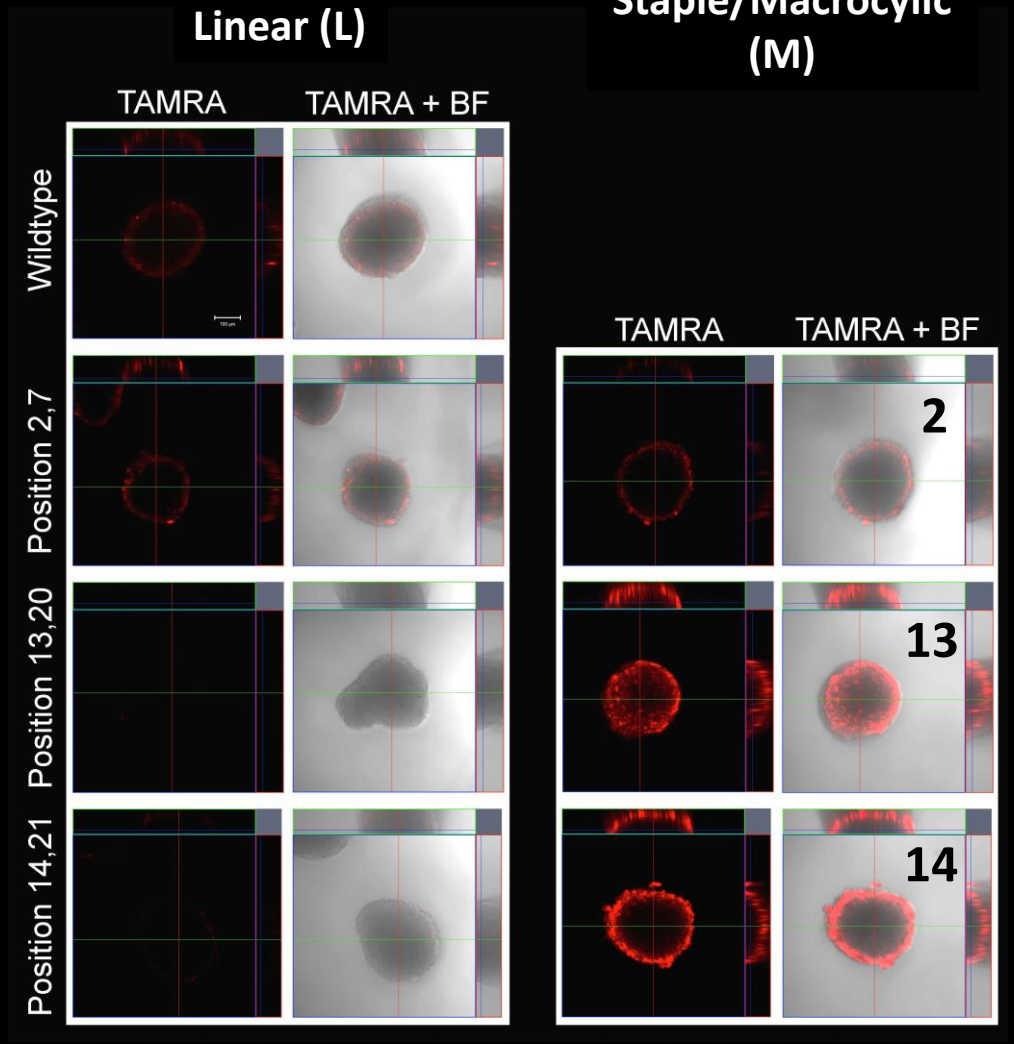
Peptide stapling enhances cell binding and permeability

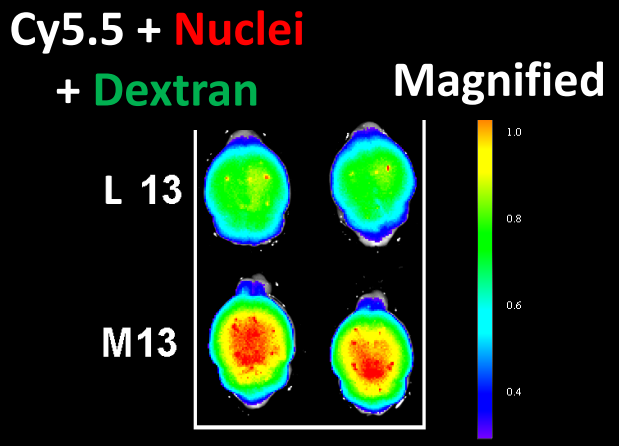
TP10 Analogues

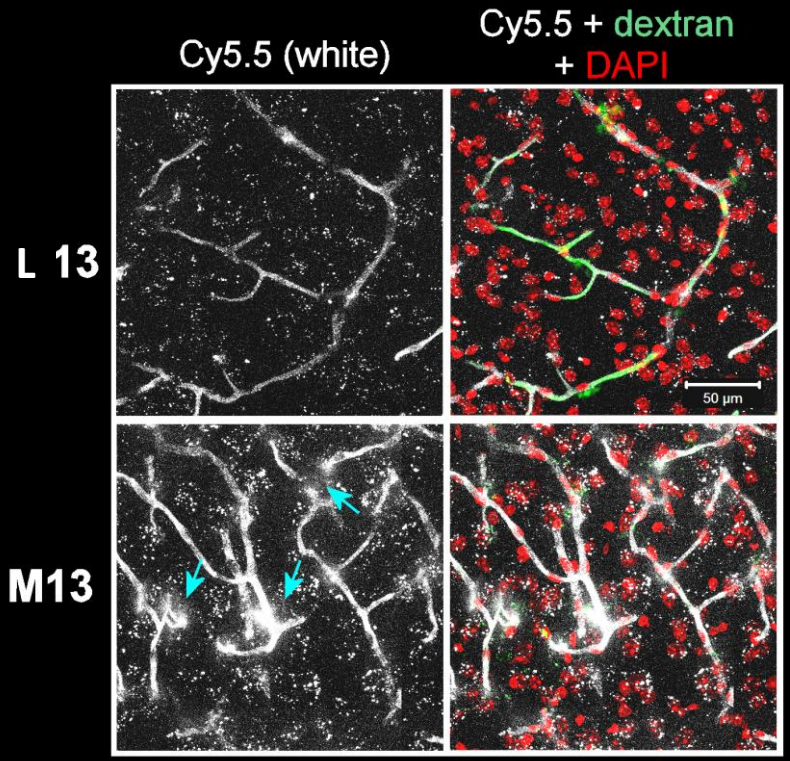




Stapling TP10 (M13) enhances brain delivery

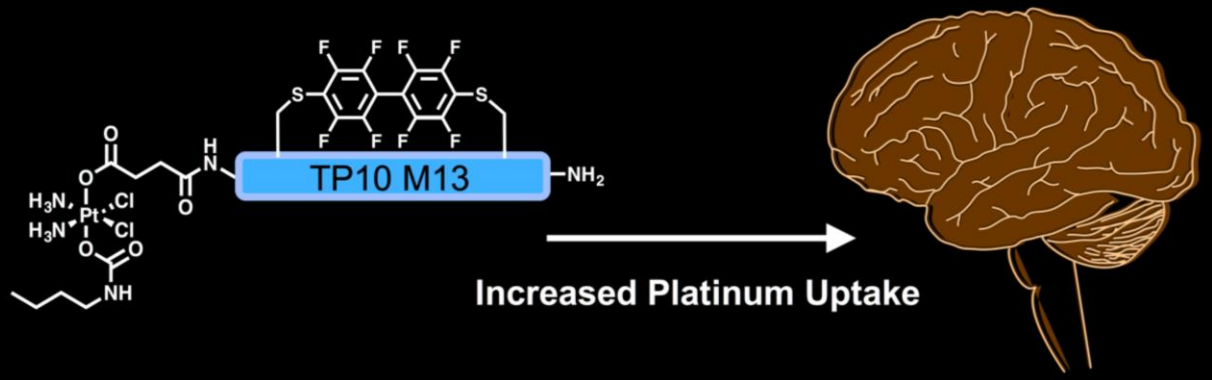




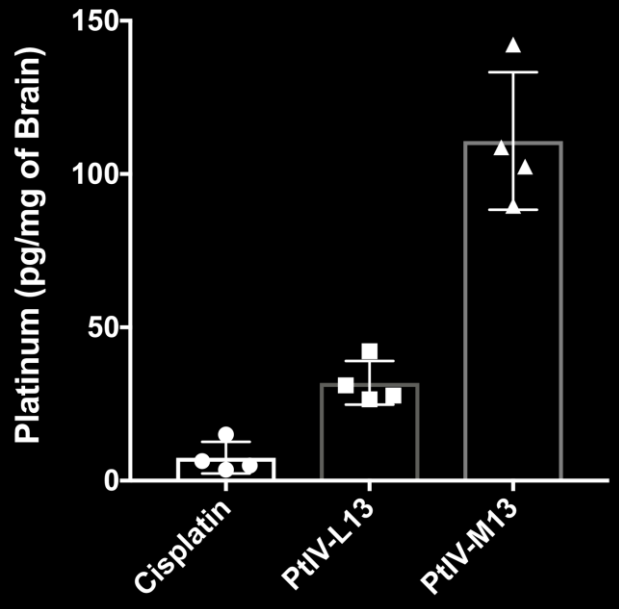


Fadzen, Wolfe, Cho et al. *JACS* (2017)

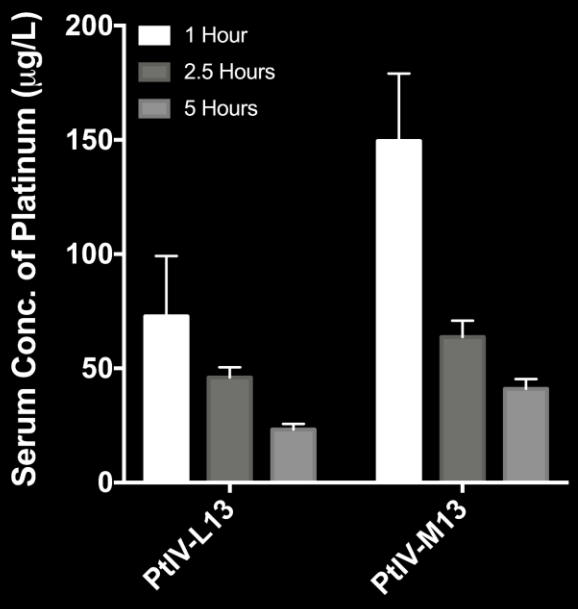
M13-cisplatin conjugate



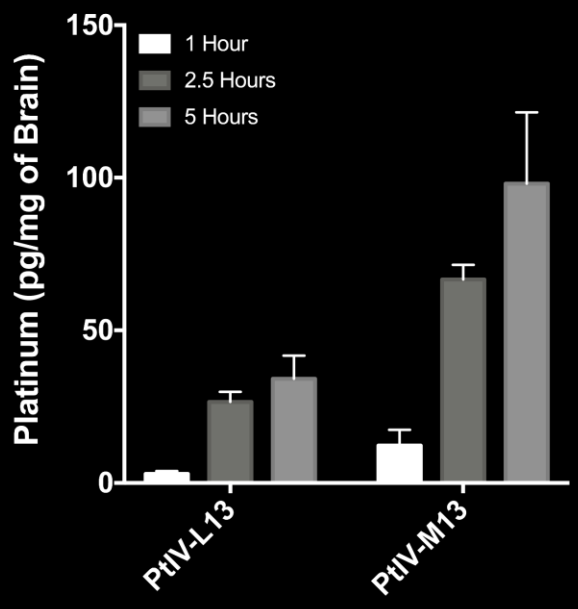
Brain uptake



Serum



Brain



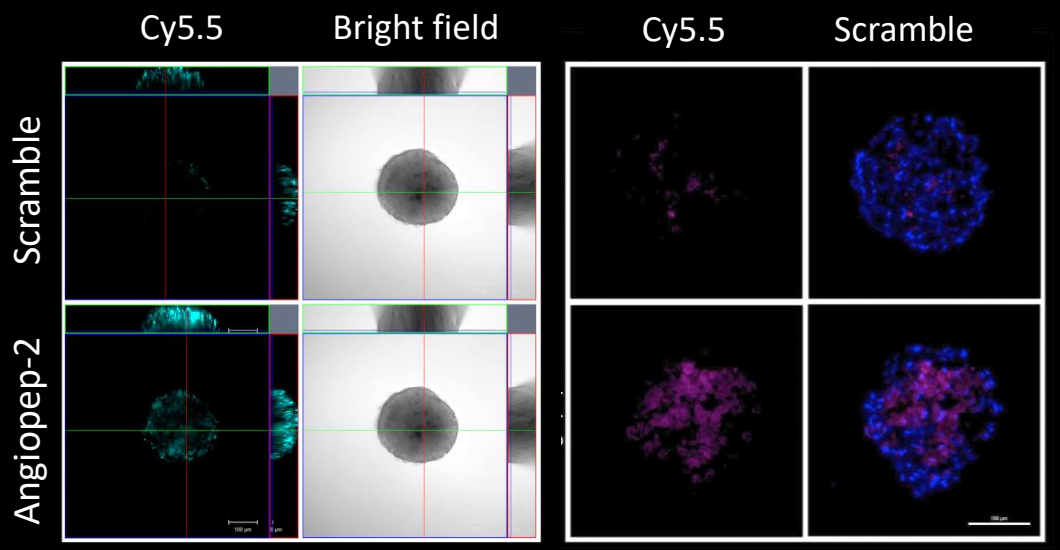
Fadzen et al. *J Med Chem* (2019); in revision

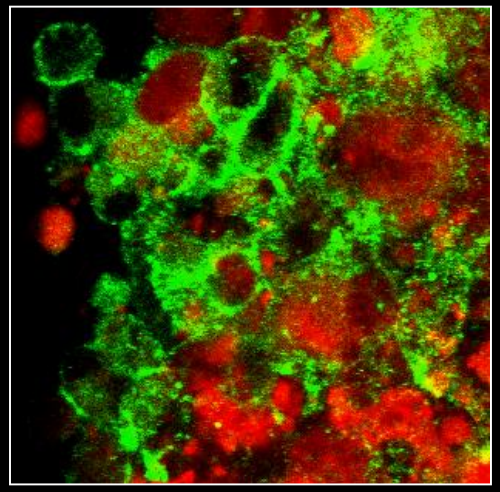
Summary

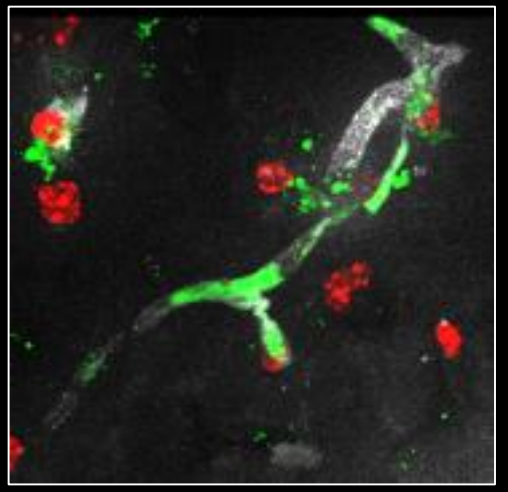
1. "BBB organoids" as next-generation analysis tool for brain-penetrating agents.

2. Organoids reproduce essential BBB properties and functions compared to widely-used transwell system.

3. Development of BBB-penetrating peptides and therapeutics.







Thank you…

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