

Massachusetts
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Technology

BBB Spheroids: A next-generation *in vitro* screening platform

NASEM:

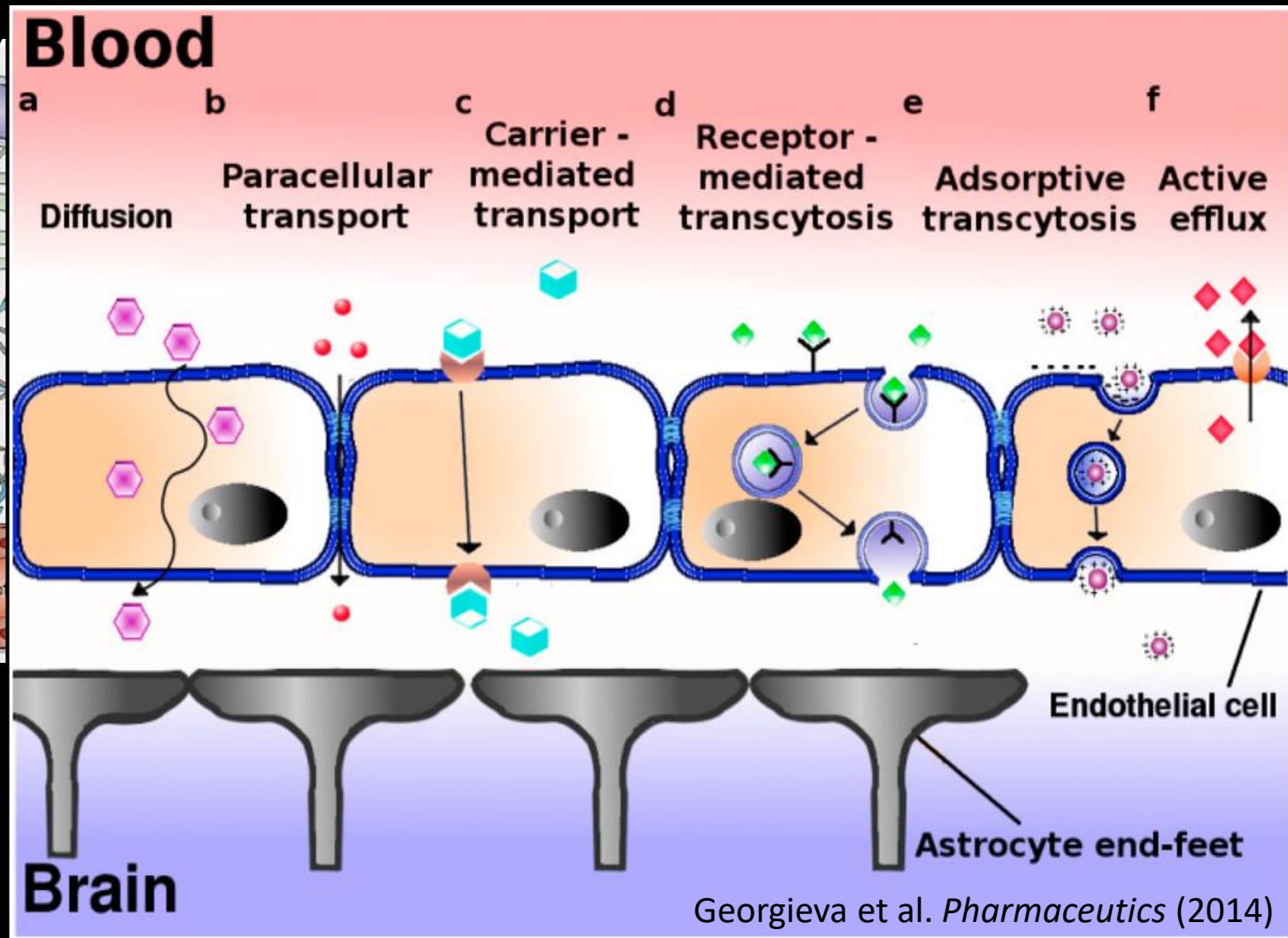
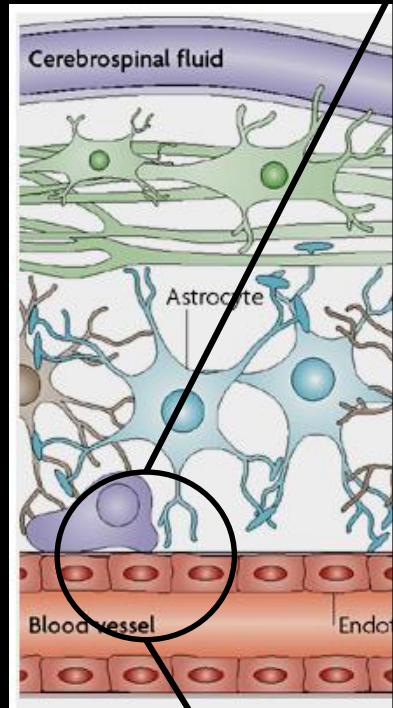
*Improving Methods for Traversing the
Blood-Brain Barrier (2017)*

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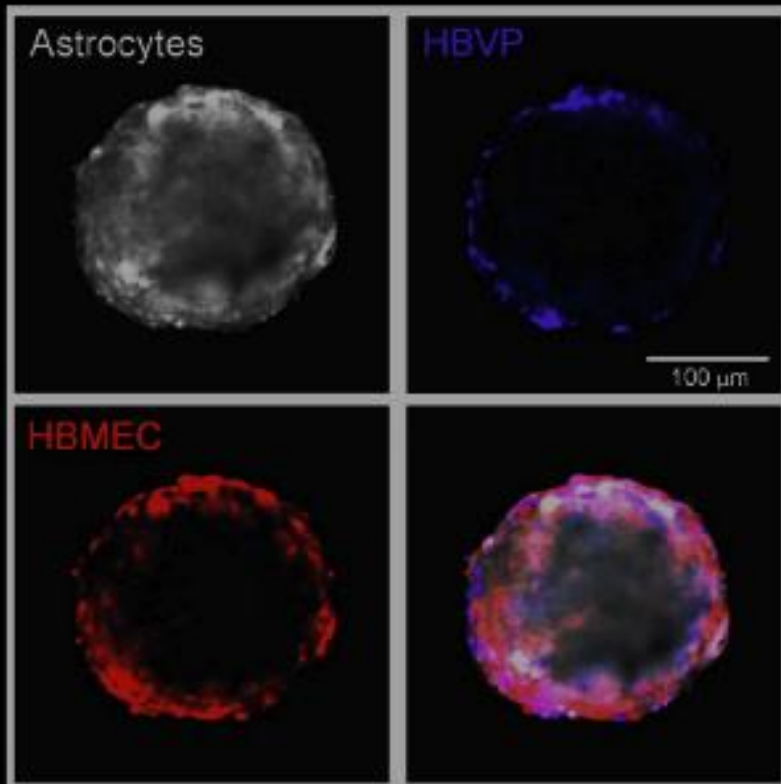
Therapeutic Delivery: How to Overcome Regulators of Blood-Brain-Barrier (BBB)?

Mechanism of entry regulating the BBB



“BBB spheroids”: Next-generation *in vitro* BBB model

Triple co-culture of
Astrocytes, Pericytes,
Endothelial cells

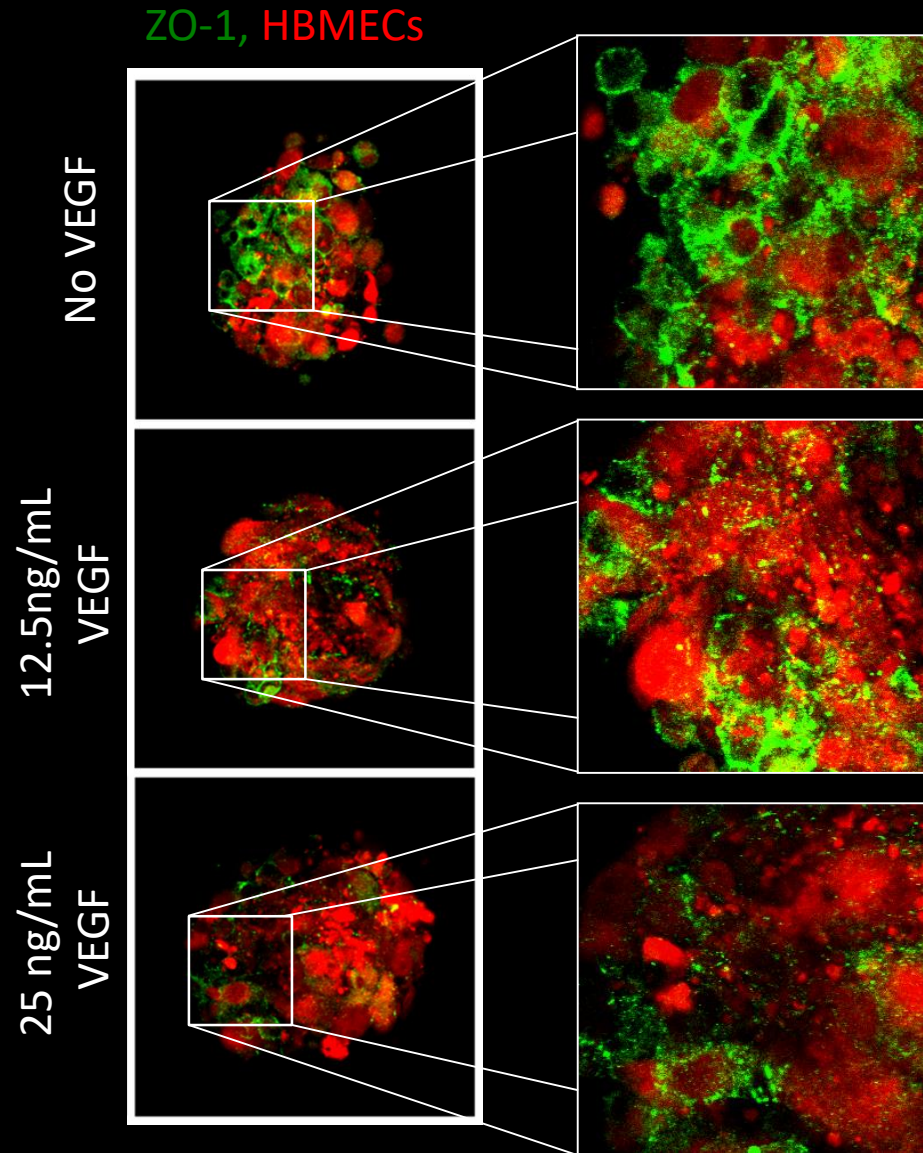


Key advantages:

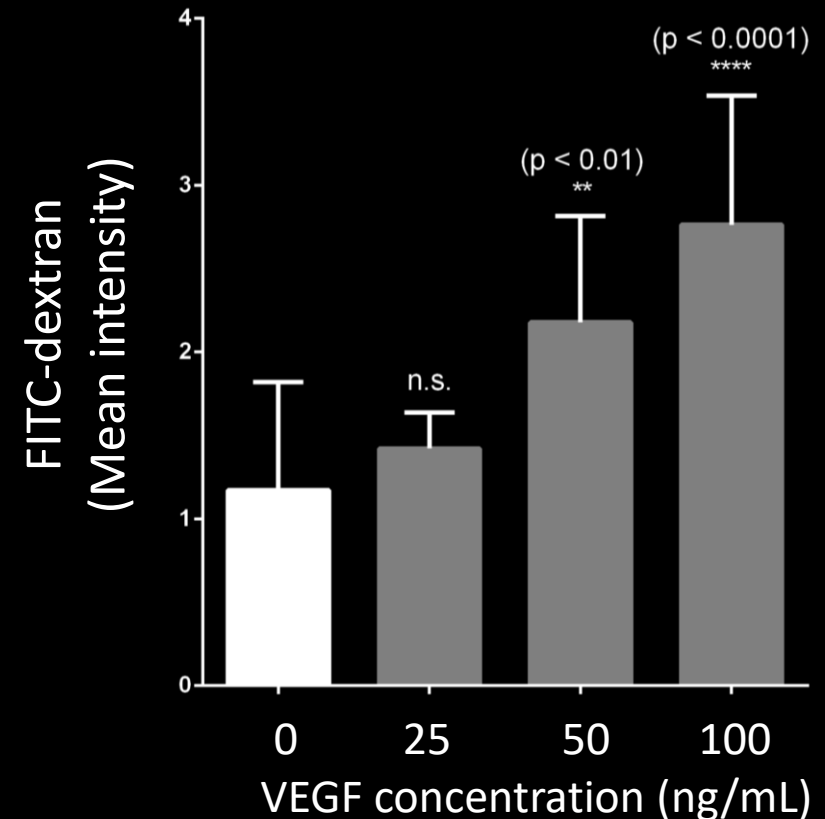
- 3D *in vitro* multicellular BBB spheroids.
- Cell-cell interactions critical for BBB properties.
- High-throughput capacity.
- Cost-effective, and require low amount of reagent
- Easily reproducible.

**BBB spheroids as a next-generation
drug-screening tool for brain-
penetrating agents**

Disruption of tight junctions increases spheroid permeability to dextran

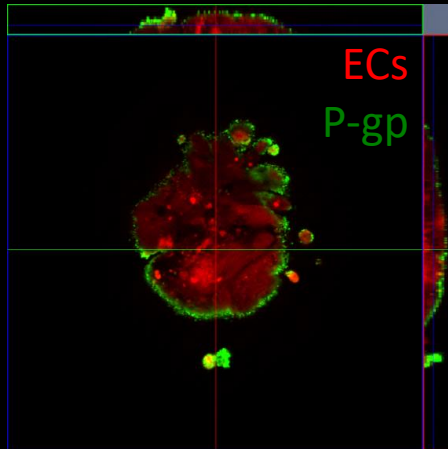


Disruption of tight junctions with VEGF increases dextran permeability

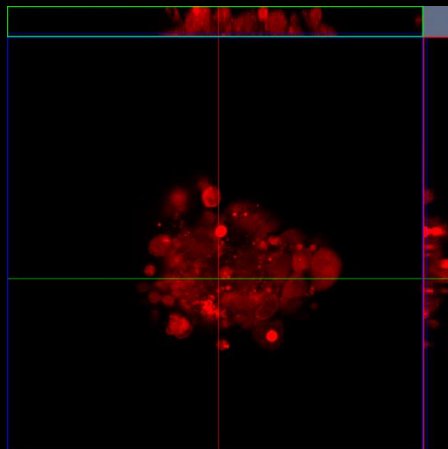


Efflux pump (P-glycoprotein) in BBB spheroid

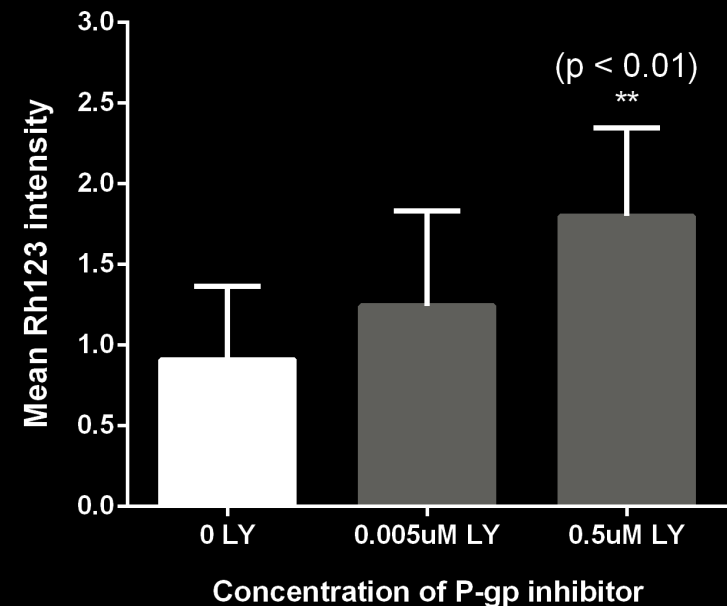
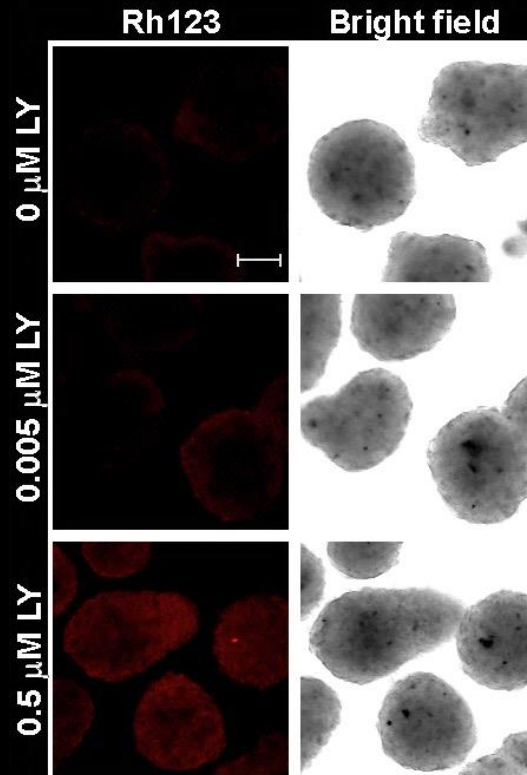
Anti-P-gp IgG



Secondary IgG
only control



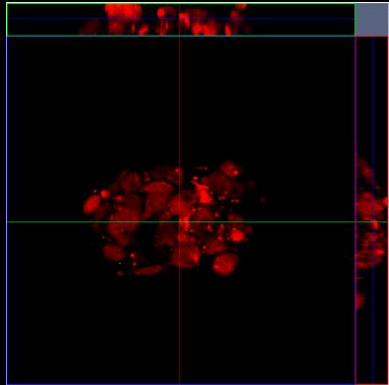
Rhodamine-123: Substrate of P-gp



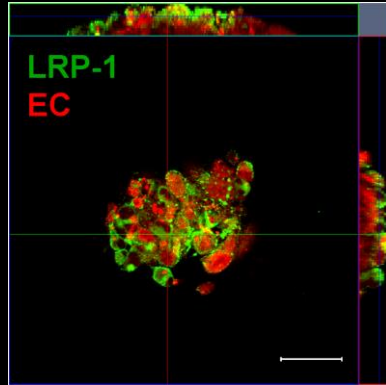
Inhibition of P-gp promotes entry of
Rhodamine-123 into spheroid

Receptor-mediated transcytosis: Transport of Angiopep-2

No Primary IgG



LRP-1 R



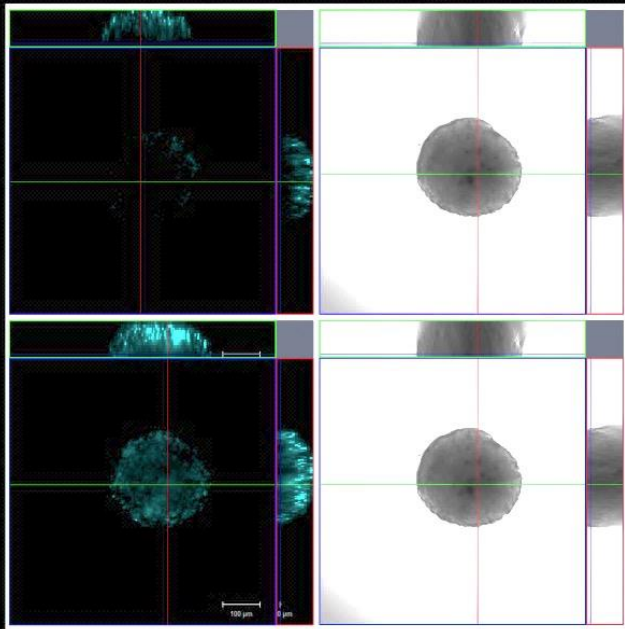
- High LRP-1 receptor expression
- Increased Angiopep-2 penetration in BBB spheroid

Cy 5.5

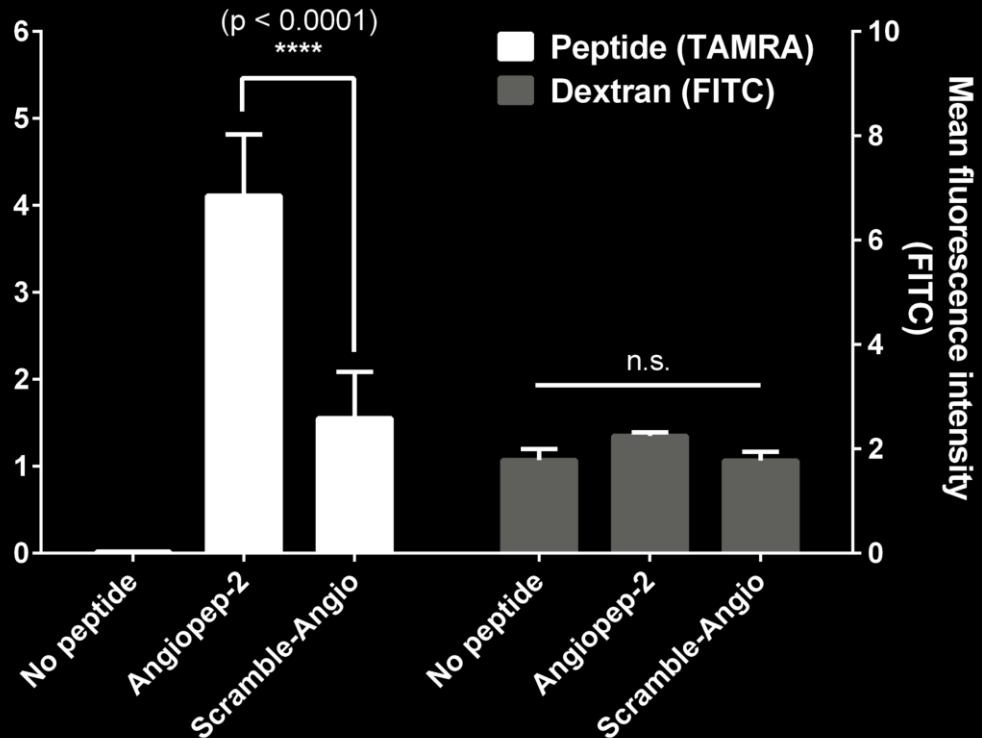
Bright field

Scrambled

Angiopep 2

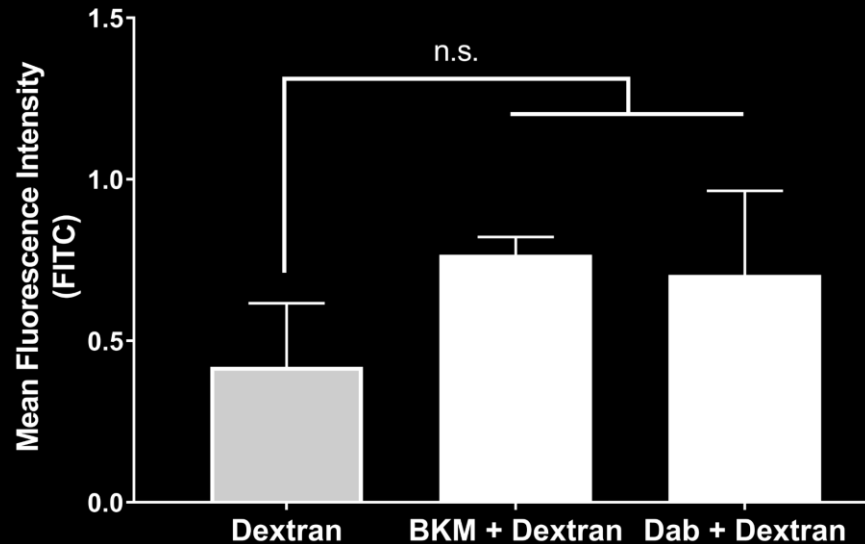


Mean fluorescence intensity
(TAMRA)



Mass Spectrometry Imaging: Transport of BKM-120

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



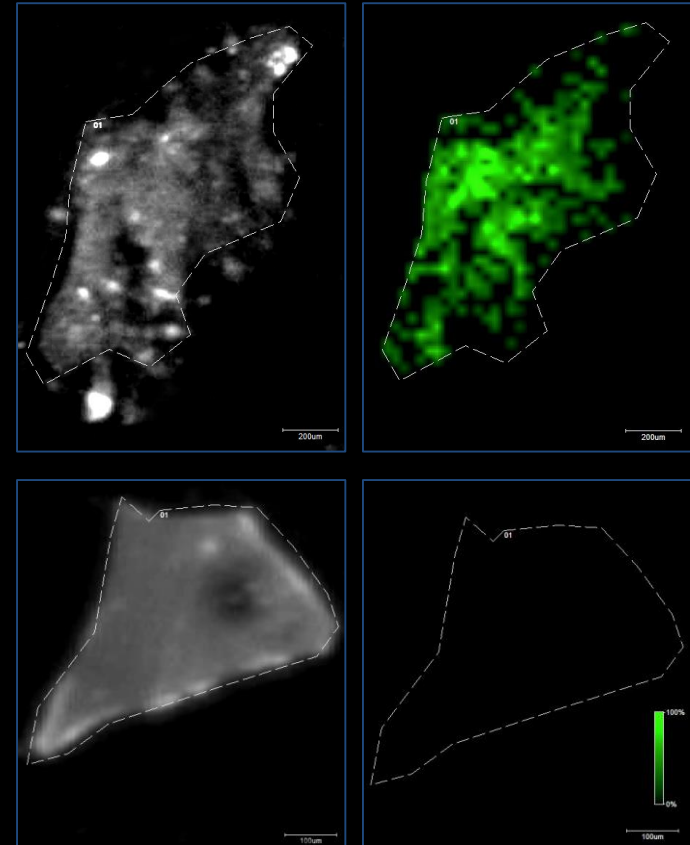
- BKM-120: BBB-penetrant drug
- Dabrafenib: Non-penetrant (control) drug

BKM

Dab

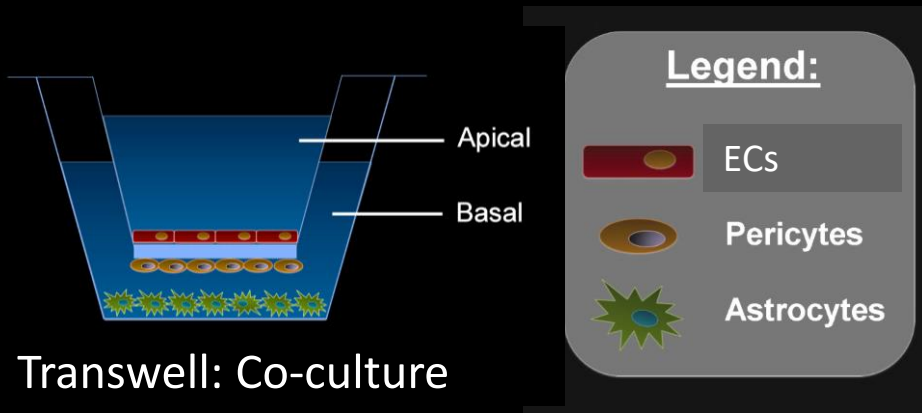
Tissue
scan

Mass spec
imaging



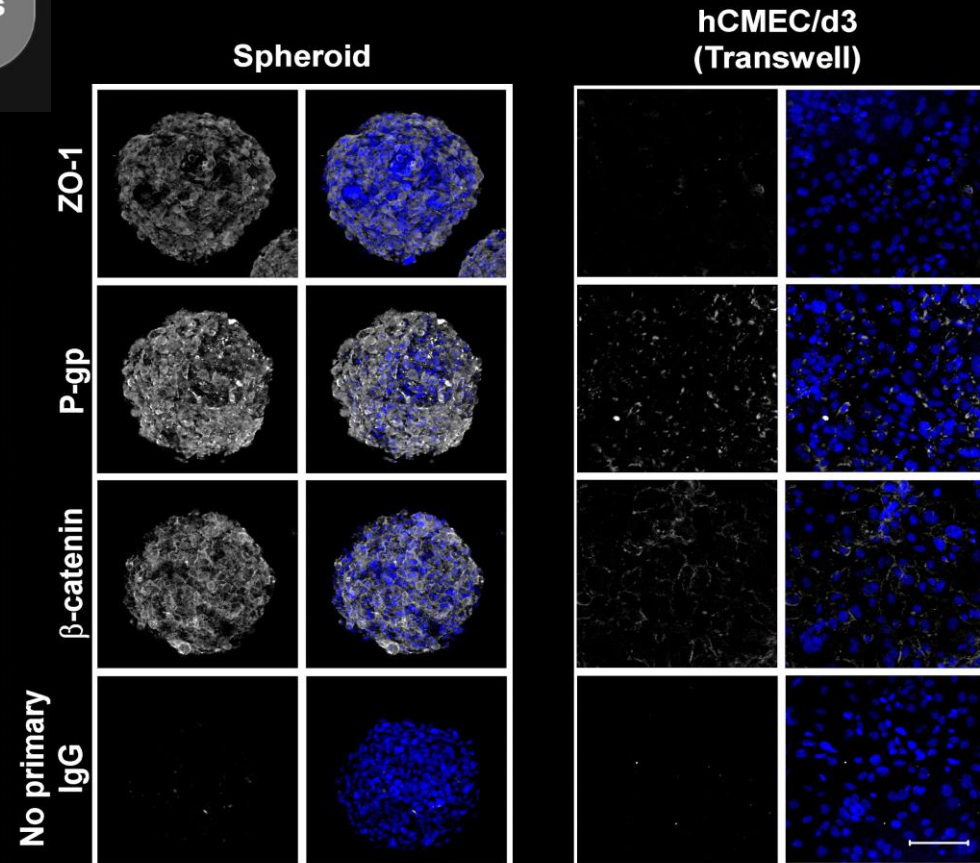
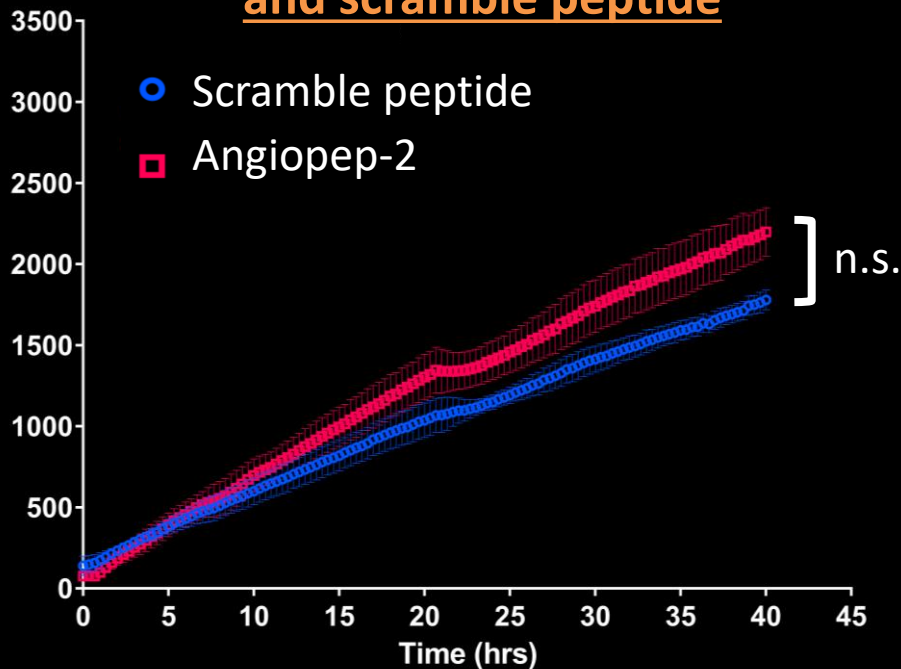
Detection of BKM-120 in
BBB spheroids

Comparison with well-known Transwell model

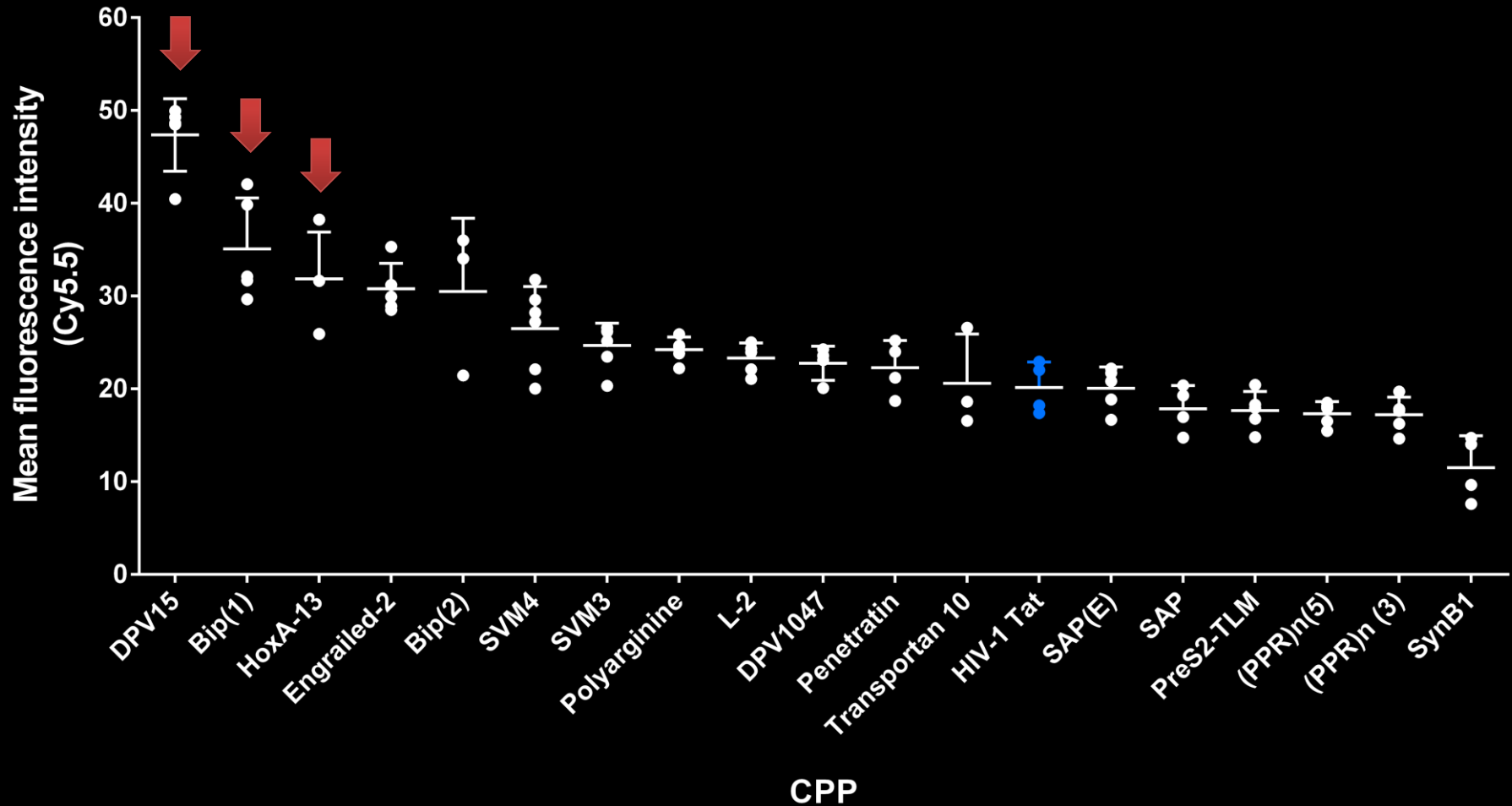


Spheroids maintain expression of key BBB regulators

No sig. diff. between Angiopep and scramble peptide

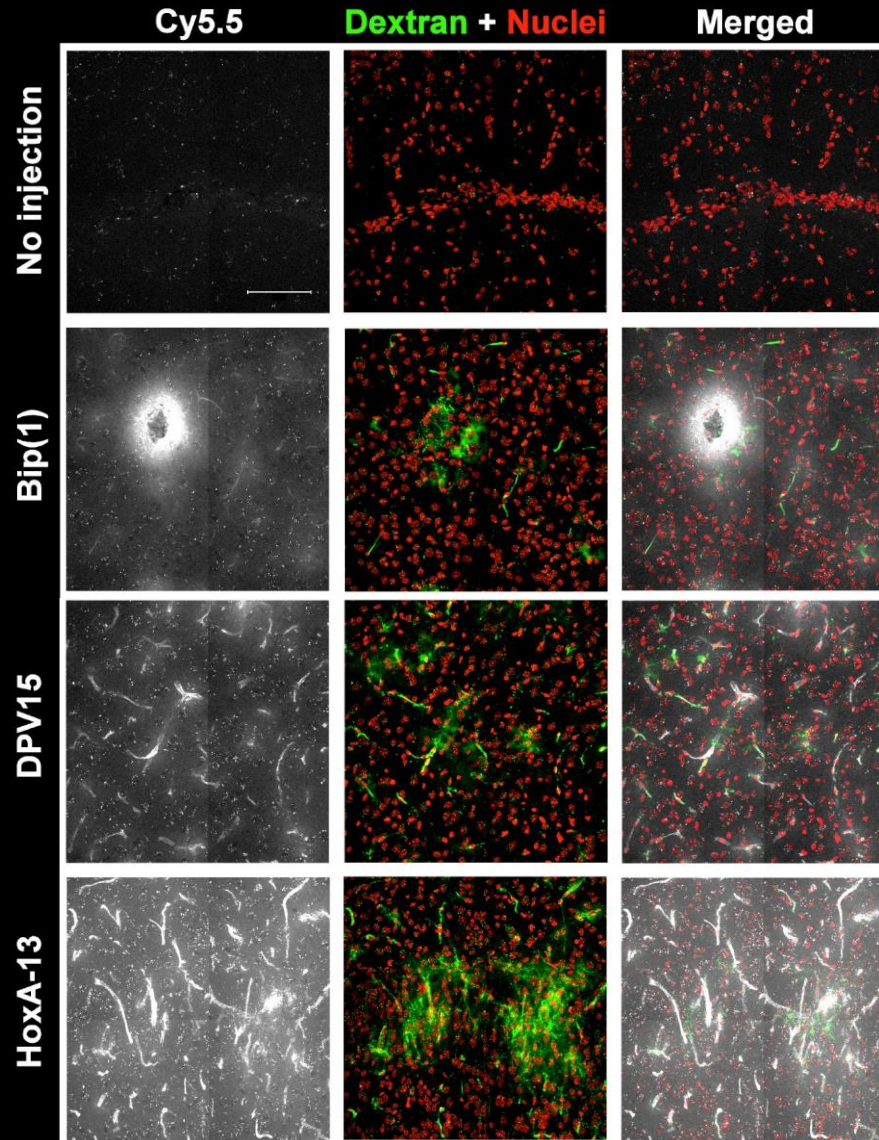


Screening for brain-penetrating CPP using BBB spheroids



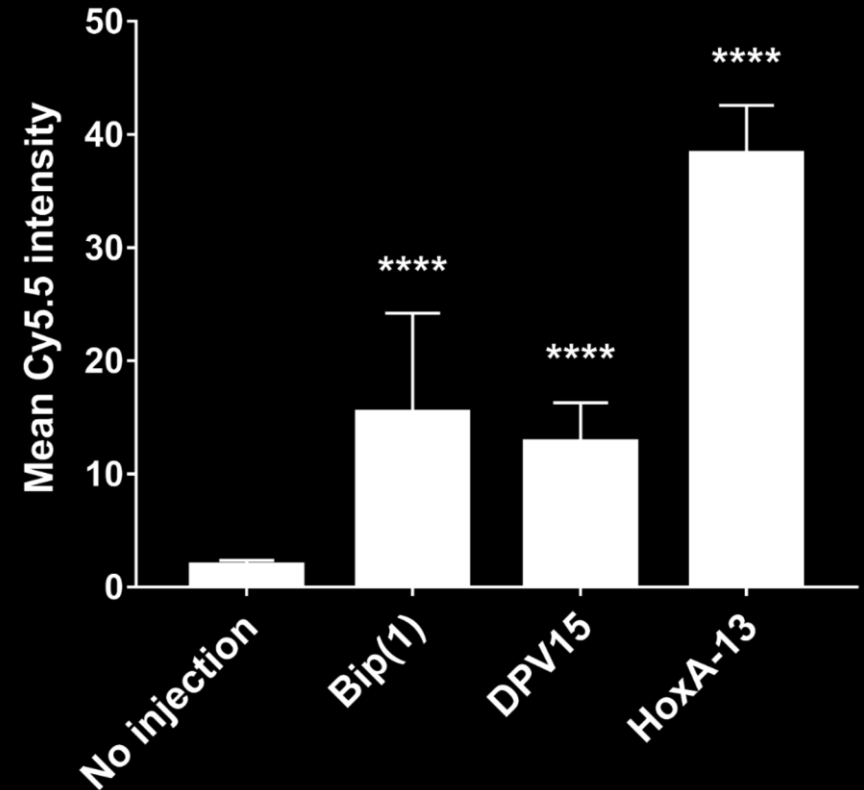
Top 3 CPPs were brought forward for in vivo analyses...

CPP: Accumulation in brain tissue



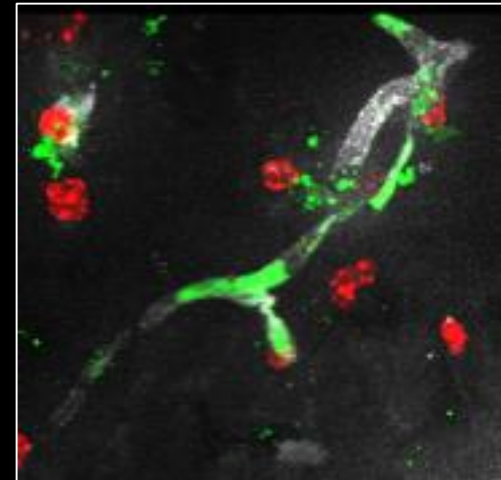
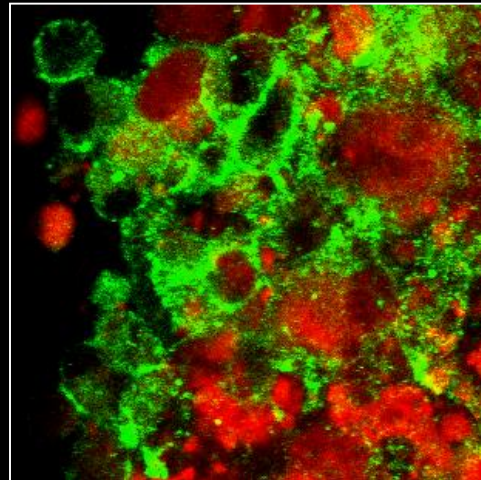
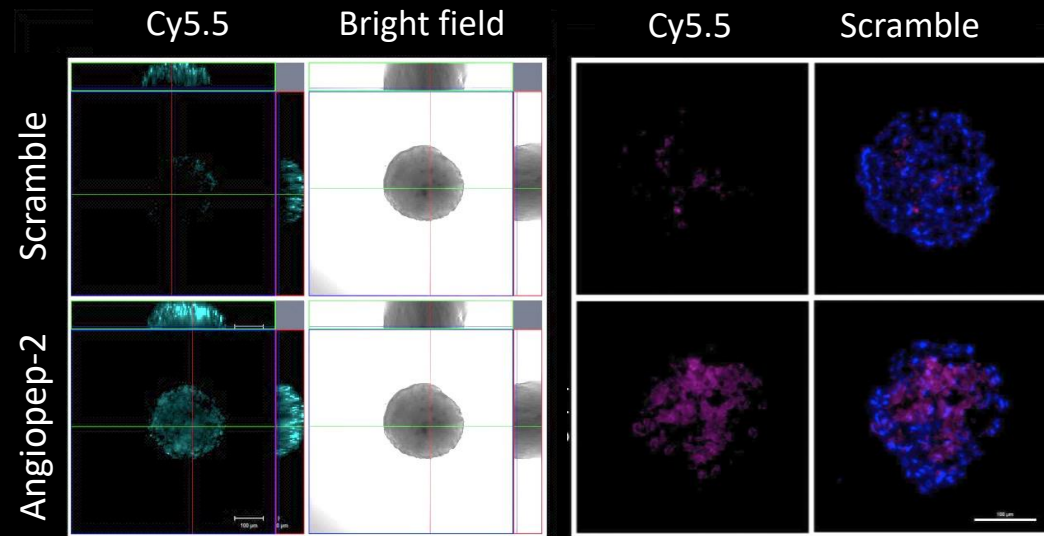
Level of CPP in brain tissue
(outside of blood vessels)

CPP level in brain tissue



Summary

1. “BBB spheroids” as next-generation screening tool for brain-penetrating agents.
2. Spheroids are superior in reproducing and maintaining essential BBB properties and functions compared to widely-used transwell system.
3. Identification of BBB-penetrating peptides.

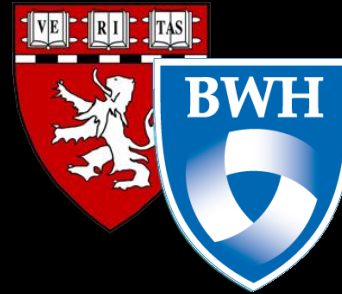


Team Effort...

BWH/Harvard Medical School

Dept. of Neurosurgery:

- Sean Lawler, PhD
- Nathalie Agar, PhD
- David Calligaris, PhD
- Kalvis Hornburg



MIT, Dept of Chemistry:

- Bradley Pentelute, PhD
- Justin Wolfe
- Colin Fadzen

