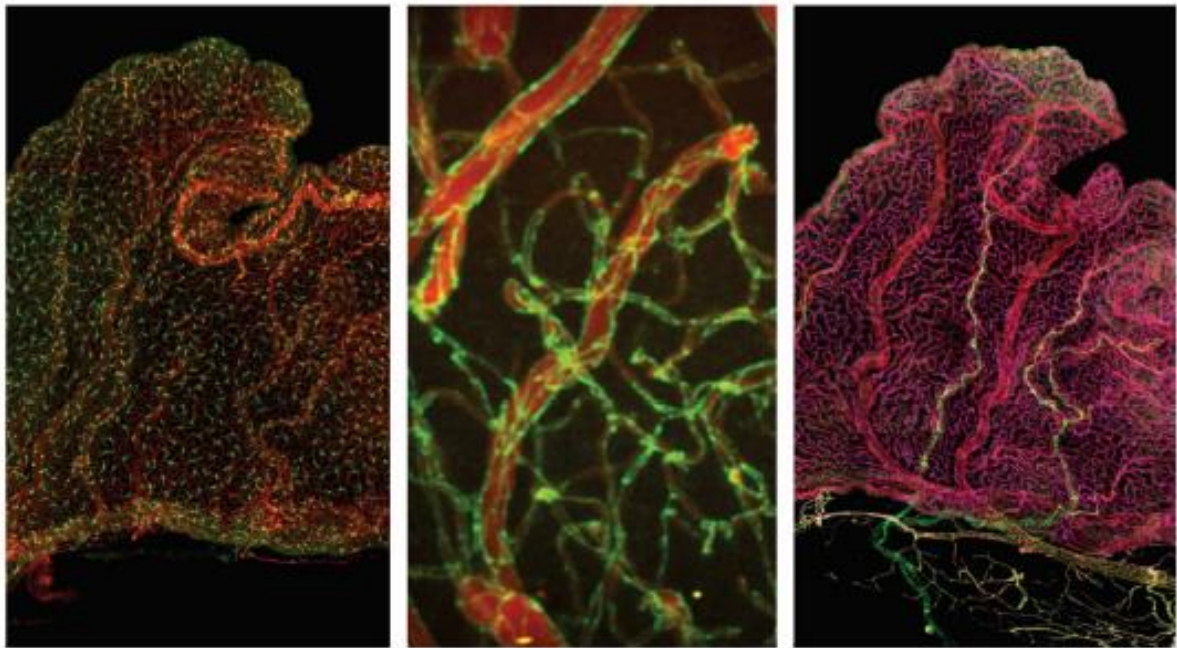


Abstracts of papers presented
at the 2021 *virtual meeting on*

BRAIN BARRIERS

April 7–April 9, 2021



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

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Arranged by

Dritan Agalliu, *Columbia University*

Maria Lehtinen, *Boston Children's Hospital*

Benoit Vanhollebeke, *Universite Libre de Bruxelles, Belgium*



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qMSI-uD a novel quantitative mass spectrometry imaging based method for mapping a regional and sub-regional unbound drug transport across the blood-brain barrier
Irena Loryan, Dominika Luptaková, Theodosia Vallianatou, Anna Nilsson, Reza Shariatgorji, Margareta Hammarlund-Udenaes, Per Andren.
Presenter affiliation: Uppsala University, Uppsala, Sweden.

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Modelling the inner blood-retinal barrier-on-a-chip with human primary retinal microvascular cells
Thomas L. Maurissen, Peter D. Westenskow, H  loise Ragelle.
Presenter affiliation: F. Hoffmann-La Roche Ltd., Basel, Switzerland.

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Immunohistochemical characterization of ACTA2 knockout mice reveals significant vascular, white matter and glial cell abnormalities
Siobhan McCarthy, Fanny Herisson, Patricia Musolino, Florian Eichler, Dianna Milewicz, Jamie Wright.
Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts.

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A modular microphysiological system featuring ultrathin silicon membranes to enable collaborative studies of the human blood-brain barrier (m-  SIM-BBB)
Molly C. McCloskey, Alec T. Salminen, Kevin F. Webb, Mehran Mansouri, Steven W. Day, Vinay Abhyankar, James L. McGrath.
Presenter affiliation: University of Rochester, Rochester, New York.

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Modelling ischemic stroke in a triculture neurovascular unit on-a-chip
Arya Lekshmi Nair, Nienke R. Wevers, Tania M. Fowke, Maria Pontier, Xander M. Spijkers, Gwenn  lle Rabussier, Remko van Vught, Paul Vulto, Helga E. de Vries, Henri  tte L. Lanz.
Presenter affiliation: Mimetis, Leiden, the Netherlands; Vrije University, Amsterdam, the Netherlands.

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Brain capillary endothelial cell monolayers cultured under hypoxic conditions maintain barrier integrity and display increased expression of Glut1, Lat1 and transferrin receptor
Burak Ozgur, Hans Christian C. Helms, Erica Tornabene, Birger Brodin.
Presenter affiliation: University of Copenhagen, Copenhagen, Denmark.

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Deriving brain microvascular endothelial cells from human pluripotent stem cells using a combination of transcriptional induction and developmental patterning
Ronak Patel, Dritan Agalliu, Andrew Sproul.
Presenter affiliation: Columbia University Irving Medical Center, New York, New York.

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Development of an *in vitro* 3D neuro-vascular and meningeal lymphatics model
Georgios Pavlou, Jean C. Serrano, Cynthia Hajal, Yoojin Shin, Se Hoon Choi, Roger D. Kamm.
Presenter affiliation: Massachusetts Institute of Technology, Cambridge, Massachusetts.

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Pharmacological MRI with simultaneous measurement of brain parenchymal perfusion and BCSFB function using interleaved echo-time arterial spin labelling
Charith Perera, Ian F. Harrison, Mark F. Lythgoe, David L. Thomas, Jack A. Wells.
Presenter affiliation: UCL Centre for Advanced Biomedical Imaging, London, United Kingdom.

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Combination of alanine and glutathione as targeting ligands of nanoparticles enhances cargo delivery into the cells of the neurovascular unit
Ger  o P  rk  lab, Maria Meszaros, Aniko Szecsko, Szilvia Vesz  lka, Maria A. Deli.
Presenter affiliation: Biological Research Centre, Szeged, Hungary; University of Szeged, Szeged, Hungary.

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Anesthesia triggers drug delivery to experimental glioma in mice by hijacking caveolar transport
Lena Spieth, Stefan Berghoff, Sina Stumpf, Thomas Michaelis, Ali Shaib, Katrin Willig, Susana Minguet, Petra H  lper, Gesine Saher.
Presenter affiliation: Max Planck Institute of Experimental Medicine, G  ttingen, Germany.

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Flow induces blood-brain barrier and glycocalyx-related genes and negative surface charge in a lab-on-a-chip human culture model
Ana R. Santa-Maria, Fruzsina R. Walter, Ricardo Figueiredo, Andr  s Kincses, Judit Vigh, Marjolein Heymans, Maxime Culot, Peter Winter, Fabien Gosselet, Andr  s D  r, Maria A. Deli.
Presenter affiliation: Institute of Biophysics, Szeged, Hungary; Wyss Institute, Boston, Massachusetts.

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COMBINATION OF ALANINE AND GLUTATHIONE AS TARGETING LIGANDS OF NANOPARTICLES ENHANCES CARGO DELIVERY INTO THE CELLS OF THE NEUROVASCULAR UNIT

Gergo Porkolab^{1,2}, Maria Meszaros¹, Aniko Szecsko¹, Szilvia Veszeka¹, Maria A Deli¹

¹Biological Research Centre, Institute of Biophysics, Szeged, Hungary,

²University of Szeged, Doctoral School in Biology, Szeged, Hungary

The treatment of neurological diseases is hindered by inefficient drug delivery across the blood brain barrier (BBB). A promising strategy to increase the brain penetration of drugs is to encapsulate them into nanoparticles that target highly expressed transporters at the BBB. Our aim was to design nanoparticles that can both cross the BBB and interact with multiple brain cell types to reach their therapeutic targets. Vesicular nanoparticles were prepared with a protein cargo (EBA, 67 kDa or mCherry, 27 kDa) and functionalized with the combination of alanine and glutathione. Nanoparticles were characterized by their size, surface charge and shape by dynamic light scattering and atomic force microscopy. We used a real-time, impedance-based cell analysis to monitor the effects of nanoparticles on the viability of cultured brain endothelial cells, pericytes, astrocytes and neurons. We used fluorescent spectrophotometry and confocal microscopy to quantify and visualize cargo taken up by these cell types. To test the mechanism of uptake, inhibitors of cell metabolism and endocytosis were used. Alanine-glutathione dual-targeted nanoparticles enhanced cargo delivery into all brain cell types tested compared to non-targeted particles. The cellular uptake of nanoparticles was energy-dependent and was partially mediated by endocytosis. Dual-targeted nanovesicles also increased cargo delivery into astrocytes after crossing layers of brain endothelial cells and pericytes in a co-culture model of the BBB. Our data indicate that alanine-glutathione dual labeling of nanoparticles can potentially be exploited to deliver drugs, even biopharmaceuticals, across the BBB and into therapeutically relevant cell types in the brain.

Combination of alanine and glutathione as targeting ligands of nanoparticles enhances cargo delivery into the cells of the neurovascular unit



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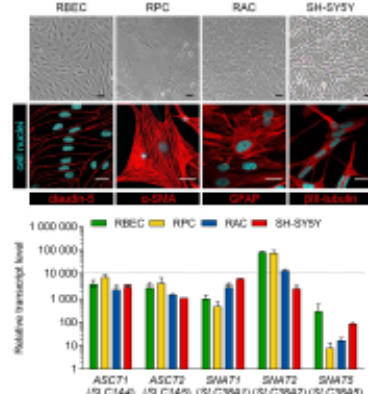


Introduction

A promising strategy to increase the brain penetration of drugs is to encapsulate them into nanoparticles (NPs) that target highly expressed transporters at the blood-brain barrier (BBB). However, penetration of NPs across the BBB might not be enough for therapeutic success by itself. The endothelial layer of cerebral microvessels is covered with pericytes, ensheathed by astroglial endfeet and all these cells are in communication with neurons and other glial cells. That means drugs must travel through multiple cell layers to reach their therapeutic targets, typically in neurons or glia. Our aim was to test whether our previously described alanine-glutathione dual-targeted NPs capable of crossing the BBB can potentially deliver their cargo to multiple cell types in the brain.

Results

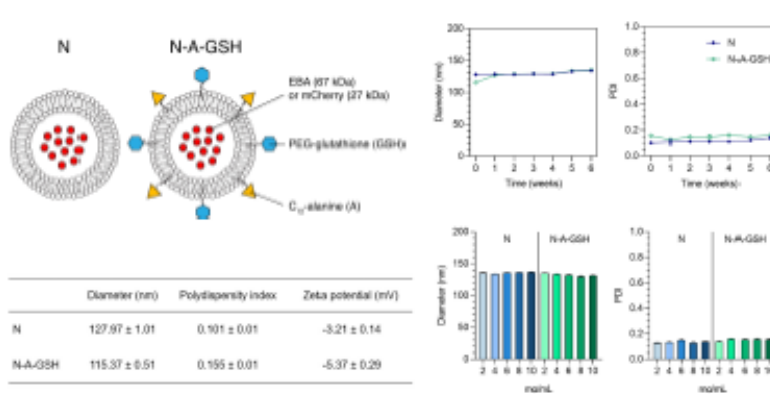
I. Expression of genes encoding alanine transporters in cultured RBECs, RPCs, RACs SH-SY5Y cells



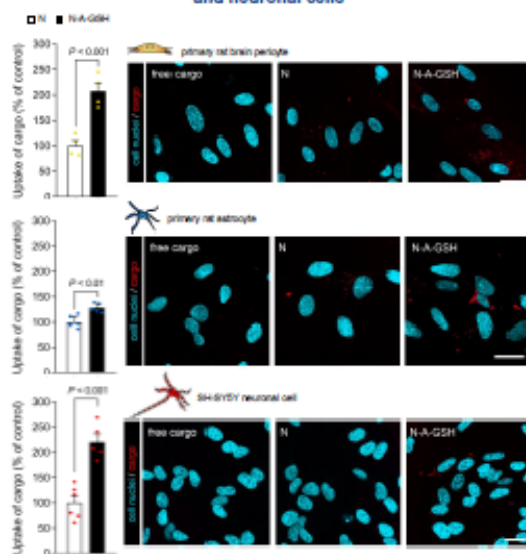
Methods

- Cell culture:** primary rat brain endothelial cells (RBEC), pericytes (RPC), astrocytes (RAC) and differentiated human SH-SY5Y neuronal cells, as well as BBB co-culture model (primary RBEC, RPC, RAC); characterization: phase-contrast microscopy, immunocytochemistry
- Expression of alanine transporters:** qRT-PCR
- Nanoparticles:** non-ionic surfactant and cholesterol-based vesicles (niosomes); characterization: measurement of size, zeta potential and stability
- Cellular uptake of nanoparticles:** fluorescent spectrophotometry from cell lysates after 4 h incubation (10 mg/mL), live-cell confocal imaging (mCherry cargo)
- Mechanism of cellular uptake:** inhibitory conditions (see above figure), fluorescent spectrophotometry
- Uptake after crossing the BBB:** permeability across the BBB model (24 h, fluorescent spectrophotometry from lower compartment); live-cell confocal imaging in astrocytes

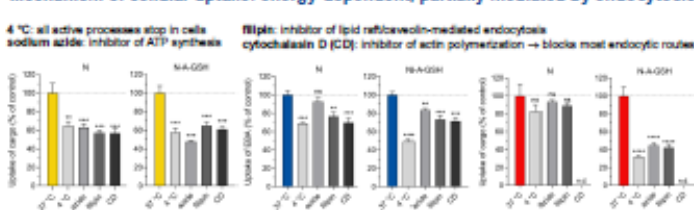
II. Composition and characterization of non-targeted (N) and alanine-glutathione dual-targeted (N-A-GSH) niosomes: size, surface charge, stability



III. Uptake of the cargo of NPs in pericytes, astrocytes and neuronal cells



IV. Mechanism of cellular uptake: energy-dependent, partially mediated by endocytosis



V. Uptake of cargo into astrocytes after crossing the BBB co-culture model

