# MCN Lab

## Molecular and Cellular Neuroscience Laboratory

by Kim Ryun Drasbek

The primary focus of the Molecular and Cellular Neuroscience Lab (MCN Lab) at CFIN is stroke and how inflammation impacts brain activity controlled cerebral blood flow changes. The lab uses a multitude of molecular techniques in combination with cellular assays and animal models.

Several of the projects in the lab is conducted in a large Novo Nordisk Synergy project, Conditioning based intervention strategies - ConBIS that investigates the molecular response to different interventions in relation to stroke and acute myocardial infarction (AMI). This consortium facilitates the collaboration with researchers at CFIN, Cardiology, iNANO, and Sport Science at Aarhus University and Medical Gastroenterology at Odense University Hospital. The project funding ended in 2020 and we have over the last 2 years finalized several publications surrounding the protective effects of remote ischemic conditioning (RIC), blood-flow restricted exercise, (BFRE) and traditional resistance training (TRT) in stroke and AMI cell and animal models. In addition to the effects of extracellular vesicles (EVs) reported by PhD graduate Tingting Gu, we have seen a protective effect of EVs in cell models of stroke (see Figure 1 in the description of Tintin Gu's PhD) released shortly after RIC and BFRE. EVs are of special interest as they are released by many cell types with molecular tags reflecting their origin and the state of the secreting cell. These small nanosize particles can function as long distance signaling carriers through their release in the blood and their ability to carry information between organs in the body as EVs are readily taken up by other cells. Interestingly, EVs carries miRNAs that are post-transcriptional regulators and plays a large role in fast cellular protein expression regulation. As each miRNA targets several proteins, they have the potential of re-programming the recipient cells. Due to their biological impact on the recipient cells, miRNAs present an interesting future treatment strategy. Using bioinformatic tools, we have found several miRNAs, which are differentially expressed following the conditioning procedures in human subjects.

The impact of selected miRNAs is tested in cellular stroke assays as part of Katrine Tang Stenz' PhD project. Inflammation has also been studied in relation to blood flow changes and neural activity using optical imaging techniques in awake mice as reported by PhD graduate Signe Kirk Fruekilde (see description of Signe Kirk Fruekilde's PhD in the following pages). Anesthesia is a confounding factor when working with animal models to study physiological responses. Especially the study of the neurovascular coupling is impacted by all anesthetics and we have therefore worked intensively on protocols for awake animal imaging. Here Signe Kirk Fruekilde has been central as she has implemented the techniques acquired in the lab of her Chinese co-supervisor Ninglong Xu in regards to animal handling, surgery, and training to obtain superb optical imaging data on awake animals subjected to different stimuli and interventions.

In 2020, we received funding from the Novo Nordisk Tandem call together with Grethe Andersen from the Neurological Department at Aarhus University Hospital. The 4-year project Ultra-early Stroke diagnostics Implementing novel Multiparametric tests for Acute Treatment decision – STIMULATE seeks to develop a point-of-care diagnostic test to be used in the ambulance. This is based on EV surface markers on blood from stroke patients drawn in the ambulance in the large clinical RESIST study. Since STIMULATE started in 2021, we have worked on securing and processing patient blood samples with the help of newly employed biomedical laboratory scientist Birgitte Hviid Mumm. We plan to include up to 500 patient blood samples from the clinic which will include both ischemic and hemorrhagic stroke as well as non-vascular patients (presenting stroke-like symptoms without a visible stroke on MRI), which will be used as control in our search for diagnostic blood biomarkers.

### NEW FACE at CFIN

**Birgitte Hviid Mumm**, Biomedical Laboratory Scientist, has been employed since January 2021 in the Molecular and Cellular Neuroscience Lab.

Birgitte has experience with a wide range of laboratory techniques mainly within molecular biology, protein chemistry and cell culture, an experience she has achieved through work in research since 2000, mainly at Aarhus University but also some years at Dresden University in Germany.

At CFIN Birgitte primarily work related to the project STIMULATE. Also, she is training students in the laboratory techniques, and assist them in their experiments.

### NEW FACE at CFIN

**Anna Bay Nielsen** joined CFIN in April 2020 as a Laboratory Technician.

She is experienced in both 2D and 3D cell culturing, hypoxia and different molecular biology methods, especially qPCR, ELISAs and different cell stainings.
Anna came from a position at the Orthopedic Research Laboratory, where she was responsible for both the cell lab and the molecular lab. The main research focus has been in cartilage and bone regeneration and stem cells. At CFIN, her main tasks are to order supplies for the pre-clinical groups, registration of chemicals and also helping where needed, mostly with immunohistochemistry, ELISAs, qPCR, animal behavior tests etc.

Since March 2021, Anna has been selected as an occupational health and safety representative at the Department of Clinical Medicine.

# FACTS

### Group members:

- Kim Ryun Drasbek, MCN Lab group leader
- Katrine Tang Stenz, PhD student
- Tingting Gu, PhD student
- Signe Kirk Fruekilde, PhD student
- Birgitte Hviid Mumm, Biomedical Laboratory Scientist
- Anna Bay Nielsen, Laroratory Technician

### Equipment and analyses:

**Extracellular vesicles**
- qNano Gold (Izon) TRPS vesicle size and concentration estimation
- Automated Fraction Collector (Izon) for size exclusion chromatography purifications

**Molecular analyses**
- Dedicated RNA workspace for RNA purifications and qRT-PCR estimations
- S1000 Thermal Cycler (BioRad) PCR machine
- Criterion Gel electrophoresis and Trans-Blot Turbo (BioRad) for protein separation and Western blotting
- PXI4 touch gel doc (Syngene) with fluorescence and chemiluminescence detection
- Synergy HTX Multi-Mode Reader (BioTek) for ELISA and fluorescent plate analysis

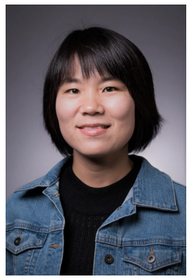
**Cell lab**
- Forma Steri-Cult CO2 incubator (Thermo Scientific)
- Modular Incubator Chamber (Billups-Rothenberg) hypoxia chambers
- xCELLigence platform (Agilent) for continuous cell monitoring

# MCN Lab

## PhD defense: Tingting Gu

By Tingting Gu

### Do conditioned extracellular vesicles protect brains from ischemic injury? – proof from a murine stroke model



Ischemic stroke is a severe disease caused by the occlusion of cerebral arteries. It can lead to long-term disability and even death[1]. Current treatments of ischemic stroke have a short treatment window of 4.5 to 6 hours and less than 30% patients are eligible for the treatments. Therefore, effective neuroprotective treatment is in urgent need[2]. Remote ischemic conditioning (RIC) is an intervention that induces brain ischemic tolerance by several brief cycles of controlled ischemia and reperfusion in the limb[3,4]. The mediator that transfers the effects of RIC from the limb to the brain remains unclear. Revealing the mediator of RIC can provide a new perspective for the clinical treatment of ischemic stroke.

This PhD project studied the role of extracellular vesicles (EVs) in RIC induced protection for brain ischemia and reperfusion injury. EVs are nano-sized vesicles that can carry various cargoes to distant tissues in the body and convey signals to target cells[5]. In this project, EVs were isolated from the plasma of healthy volunteers before (pre-RIC EVs) and after RIC treatment (post-RIC EVs) and then tested in a mouse stroke model induced by transient middle cerebral artery occlusion (tMCAO). First, a homing effect was found from post-RIC EVs that accumulated in the ischemic area at 4 hours after reperfusion (see Figure 1). This increases the likelihood of post-RIC EVs to have an effect in the ischemic area. The function of these post-RIC EVs was then tested in the tMCAO mice in an acute setting. The relative changes of cerebral blood flow and hemoglobin concentration were monitored by laser speckle imaging combined with multispectral reflectance imaging during occlusion and the first 4 hours of reperfusion.

Post-RIC EVs appear to have a tendency of slowing down the development of infarction and reducing the infarct size at 4 hours after reperfusion (see Figure 2). Further hemodynamic analysis showed a higher oxyHb level in the ischemic area of the post-RIC EVs group, while no group effects in cerebral blood flow were observed. However, neither functional assessment nor infarct size at 7 days after ischemia was improved when evaluating the effects of pre- and post-RIC EVs in tMCAO animals.

The current results suggest that RIC EVs might slow down the development of infarction in the acute phase after ischemic injury while no effects were found in the subacute phase after ischemic onset. Our study is the first to test RIC EVs in an animal model of ischemic stroke with reperfusion. Further validation of the results in different models of ischemic stroke with more end-points could provide more solid evidence for the effects of post-RIC EVs as a potential neuroprotective treatment option for ischemic stroke.

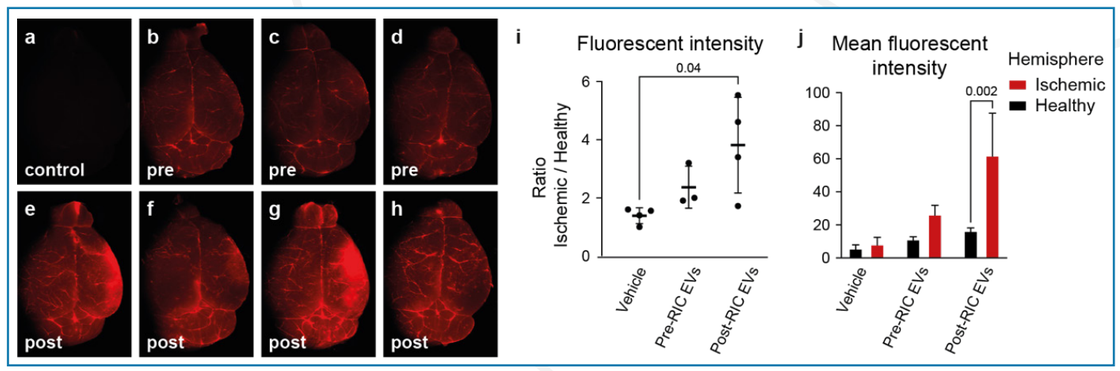



**Figure 1**
Accumulation of labelled EVs in murine brains following tMCAO. Fluorescent brain images of single tMCAO mice injected with vehicle (a, representative negative control labelling without EVs, n=4), pre-RIC EV mice (b-d, n=3), and post-RIC EV mice (e-h, n=4). The right side is the ischemia side of the brains in the figure. After normalization of the fluorescent intensities to the healthy hemisphere in each animal, a significant accumulation of EVs were seen in post-RIC EV treated animals compared to the vehicle control (i). Post-RIC EVs accumulated in the ischemic hemisphere when comparing the mean fluorescent intensity in the ROIs from the ischemic hemisphere and the healthy hemisphere (j). Data are presented as mean ±SD. P-values are shown for significant comparisons.

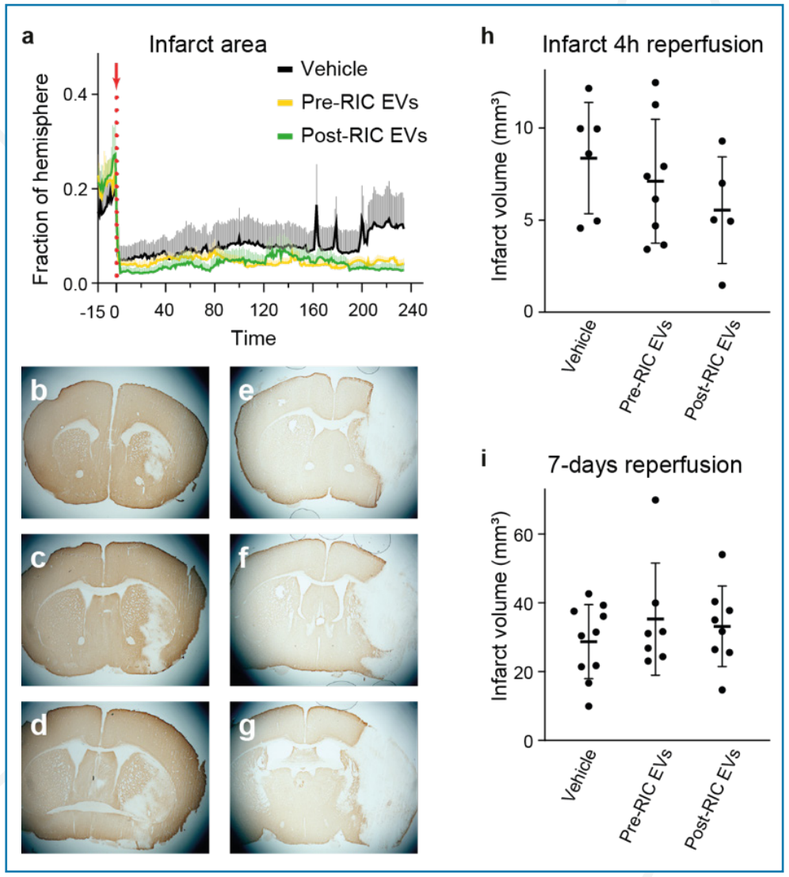



**Figure 2**
Ischemic core development during occlusion and reperfusion and final infarct volume in tMCAO mice. Laser speckle contrast imaging of the ischemic core during the first 4 hours of reperfusion (a, number of pixels with flow intensity less than 33% of baseline CBF). Infarct volumes at termination were assessed based on anti-Map-2 stainings showing early infarction in the striatum after 4 h reperfusion (b-d, adjacent sections from a post-RIC EV treated animal) which develops into a large cortical infarct after 7-day reperfusion (e-g, adjacent sections from a post-RIC EV treated animal). Mean and individual infarct volumes in MCAO animals after 4h reperfusion (h) and 7-days reperfusion (i). Data are presented as mean±SD.

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# MCN Lab

## PhD defense: Signe Kirk Fruekilde

by Signe Kirk Fruekilde

### Longitudinal imaging of cerebrovascular dynamics in awake mice using optical techniques

Neurons are extremely vulnerable to starvation. Therefore, a well-functioning vascular system that provides an adequate supply of nutrients and oxygen, as well as removes metabolic waste, is crucial for maintaining a healthy cerebral environment[1–3]. In the brain, the vasculature and tissue function in a vital symbiosis. The interaction is so essential that it is recognised as a joint "neurovascular unit"[4,5]. For this reason, systemic vascular diseases must be acknowledged in the discussion of neurological diseases.

Here, we describe an experimental setup and analysis protocol[6], which allowed for a longitudinal investigation of cerebrovascular dynamics in mice without the confounding effects of anaesthesia (see Figure 1). The presented empirical study was an investigation of how the vascular dynamics are affected by systemic inflammation induced by lipopolysaccharide (LPS).

Overall, we revealed how a severe systemic inflammation caused a disturbance in the hyperaemic response to functional activation (see Figure 2) and, in parallel, disrupted the normal flow of blood within the capillary network (see Figure 3). These findings are a potential danger to neuronal health, since demands of oxygen and nutrition may not be met by the inflammation affected vascular system. The methods and applications developed in this project can be applied to further investigations into the role of inflammation in cerebrovascular and neuronal health, both on their own and in combination with more imaging techniques. The potential of the developed protocols extends beyond inflammation to many questions related to the health and resilience of the cerebrovascular system.

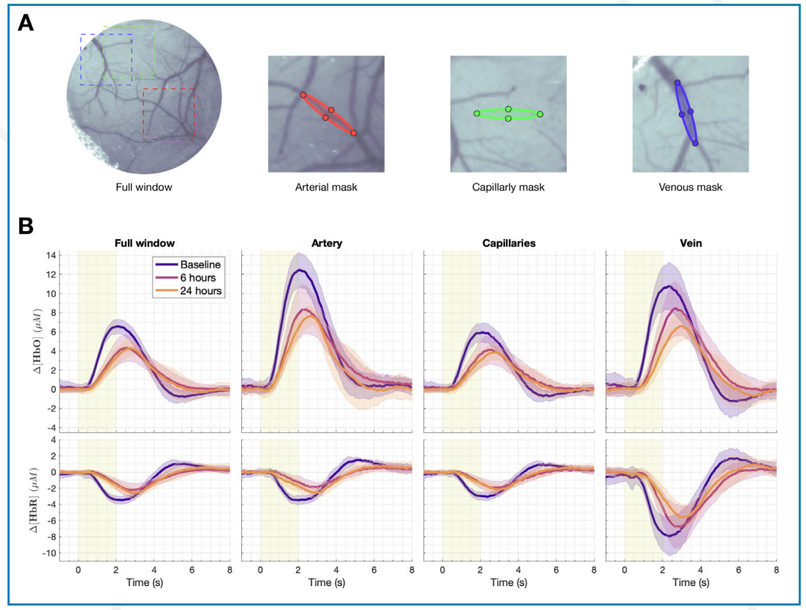


**Figure 2**
Response of oxy- (HbO) and deoxyhaemoglobin (HbR) to whisker stimulation. (A) The full cranial window and the vessel compartment masks used for data extraction from an example animal. (B) The averaged HbO and HbR responses from 10 animals at three timepoints in relation to LPS administration. Mean responses at baseline (purple), 6 h (pink), and 24 h (orange) are shown as solid lines and the standard deviation between animals is indicated as surrounding shades.

**Figure 1**
(A) Timeline of the experimental procedure. (B) Image of an example mouse with the cranial window implanted going through behavioural training. (C) Image of the somatosensory cortex through the cranial window acquired in combination with imaging sessions. The square marks one of two regions of interest (ROIs), from which a timeseries of capillary angiograms are collected. (D) Example of an angiogram series collected with the OCT system. (E) Example of haemodynamic response to whisker stimulation collected with the OISI system. Increases, relative to a pre-stimulus baseline, in oxyhaemoglobin (top, red colour scale) and deoxyhaemoglobin (bottom, blue colour scale) are visualised and quantified separately.

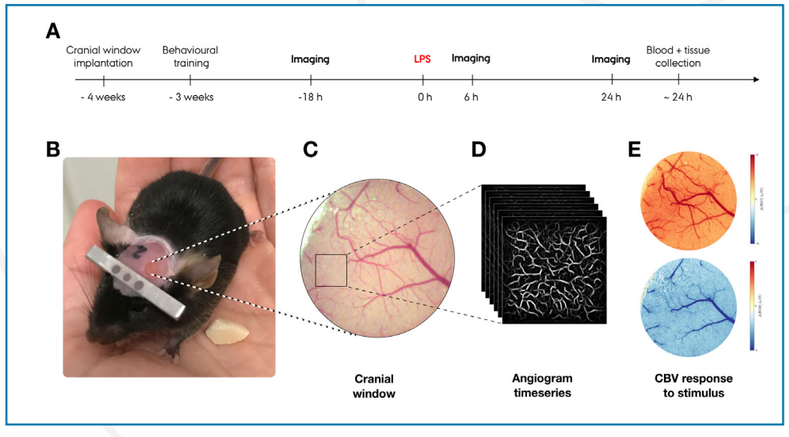


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**Figure 3**
Capillary stalled flow events at rest. (A) Three consecutive OCT angiograms showing examples of stalling capillary segments in a single region-of-interest (ROI). Each full arrow points to a segment with flow, and the corresponding hollow arrow points to the same capillary segment that is missing due to stalled flow. (B) Number of stall events observed during a 15 min imaging session at baseline, 6 h and 24 h after LPS injection. True stall events were considered throughout to have a minimum duration of 2 frames. (C) Proportion of capillaries in which at least one stall event was registered. (D) Duration of individual stall events measured in # frames (~15 sec / frame). The parameters are compiled across the 10 mice, with two independently acquired ROIs per animal. The median and inter-quartile ranges are marked with horizontal hatch marks in (B) to (D). Statistical comparisons are based on Tukey's post hoc correction for multiple corrections: *p<.05, **p<.01, *** p<.001, ****p<.0001, ns = not significant.