european calcium society

PROGRAM & ABSTRACT BOOK

10th ECS Symposium

The Ca^{2+} signaling toolkit in cell function, health and disease

November 15 - 17, 2023

Organized on the occasion of the retirement of

Prof. Dr. Jan B. Parys

Location: Convent of Chièvres, Faculty Club, Leuven, Belgium

WELCOME

Dear colleagues & friends,

We would like to warmly welcome you to the symposium of the European Calcium Society on the "The Ca^{2+} signaling toolkit in cell function, health and disease" in the historic and UNESCO site of the Grand Beguinage in Leuven, Belgium. The meeting is also in honor of the scientific career of Prof. Jan B. Parys, who has become Professor Emeritus.

We are extremely thrilled to see the enthusiasm within the community for this event and to meet with 135 calcium aficionados during 3 days to present and discuss their recent findings. We welcome participants from about 18 countries from 4 different continents. We also very much value the participation of numerous early career researchers from KU Leuven, Flanders and abroad to the scientific program via oral and poster presentations. We also want to encourage everyone to actively participate to the discussions and questions. The program hosts 15 invited speakers from diverse institutions and career stage covering a broad range of scientific topics in the field of Ca^{2+} signaling and Ca^{2+}-binding proteins. The meeting is kicked off by the opening lecture by Jan B. Parys (KU Leuven, Belgium) and is concluded by the closing lecture by Gyorgy Hajnoczky (Thomas Jefferson University, Philadelphia, USA). In addition to this, the program has been complemented by 14 short talks selected by a delegation of the scientific committee, mostly awarded to early career researchers, as well as a short talk sponsored by Hamamatsu Photonics. Finally, we will have about 40 poster presentations with designated discussion time at the extended lunch breaks. We also secured two awards of 250 euro each from *BBA-Molecular Cell Research* and from *Cell Calcium* for the best short talk/poster award by an early career researcher.

We also provided ample time for informal interactions during the opening & closing receptions, coffee breaks, lunches and the open-to-all conference dinner at the Oud Gasthuys as well as during the sociocultural & sports activities planned. We hope these moments & events give a taste, flavor and impression of Leuven as a city and will contribute to the collegial and convivial atmosphere and help to promote interactions & discussions among participants.

We also aimed to organize the event maximally considering sustainability. The venue site and its caterer (Faculty Club) are awarded the Green Key label for sustainable hospitality using local produce and sustainable materials. We limited the use of natural resources (e.g. online-only program book.) The venue site was also chosen due to its accessibility via public transport. To actively promote sustainable travel to Leuven, we have offered 6 sustainable travel fellowships, sponsored by the Company of Biologists, awarded to attendees opting to travel by train.

We also wish to express our gratitude to all research funders and commercial sponsors who have generously supported our scientific event. Their support was absolutely essential to keep registration fees modest and to facilitate the participation of early career researchers.

Finally, I would like to thank the organizing committee and scientific committee for their support and input! A special mention is deserved to the LMCS team and specifically Tomas Luyten and Rita La Rovere, who have been managing all administrative, practical & organizational matters from the early preparations up to today! Thank you!

We hope you'll have an enriching, joyful and insightful symposium!

Cordial greetings,

Geert Bultynck

WORKSHOP VENUE

The **venue of the workshop** will be the Convent of Chievres/Faculty Club at the **Grand Beguinage of Leuven**, a unique, historical part of the city that is listed by the UNESCO as belonging to the World Heritage. It was completely renovated by the KU Leuven and now houses students and (visiting) faculty and allows for the organization of meetings.

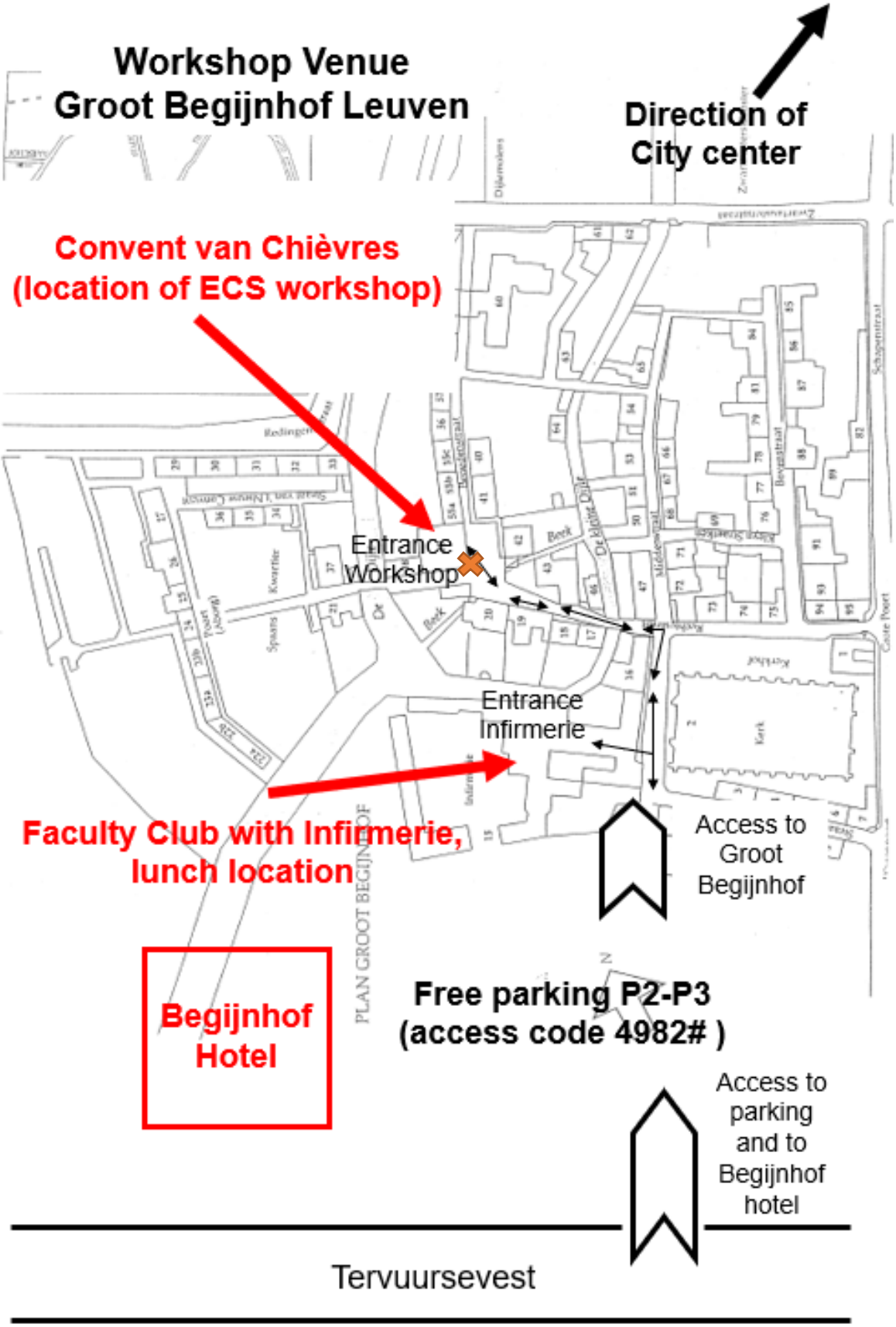
The workshop, including **lectures, poster sessions, coffee breaks, sandwich lunches, and exhibitions by sponsors**, will be take place in the **Convent of Chièvres** (see red cross on the plan below), Groot Begijnhof, Benedenstraat 39. Please note that there is no elevator in the building. There are also small rooms available for networking, discussions and/or co-working. The venue will open about 30 minutes before the start of the 1st session (thus at about 8 am).

Ground floor: registration desk, coffee breaks & lunches, sponsor exhibition
2nd floor: poster display & session
Top floor (Willem van Croÿ room): lectures

The **beer & cheese reception** (Wed November 15th) **and closing reception** (Fri November 17th) will take place in the nearby **Faculty Club (room Infirmerie)**.

The **conference dinner** (Thu November 16th) will be hosted at **'t Oud Gasthuys**, Brusselsestraat 63B – Leuven (Inner Square 30CC near to Roman Gate). The restaurant is about 15-20 min by foot from the main venue. However, participants attending the social activities will be chaperoned towards the restaurant by LMCS lab members.

Please note that if the door is closed you can open it with **code: 4982#, only during the hours of the workshop**. The venue operator also asks to close the door, avoiding access by tourists to the venue.


Workshop Venue
Groot Begijnhof Leuven
Direction of
City center
Convent van Chièvres
(location of ECS workshop)
Entrance
Workshop
Entrance
Infirmerie
Faculty Club with Infirmerie,
lunch location
Access to
Groot
Begijnhof
Begijnhof
Hotel
Free parking P2-P3
(access code 4982#)
Access to
parking
and to
Begijnhof
hotel
Tervuursevest

Arrival to Leuven

Our workshop strives to organize the meeting with a low impact on the environment. We therefore encourage attendees consider environmentally sustainable/eco-friendly traveling options.

By train:

Europe has an extensive railway system capable to transport you across the continent, sometimes even faster and with more comfort then using an airplane. International trains will arrive in Brussels. From there it is just a short 40 minute train ride to Leuven.

Leuven can easily reached by train from all major cities (e.g. Brussels-Leuven about 30-40 min, several trains every hour). More information available: https://www.belgiantrain.be/en

All hotels are within a 30 minute walking distance from the train station. However, there are frequent buses (operated by "De Lijn"; https://www.delijn.be/en/; see also getting around in Leuven).

By plane:

Brussels Airport is the nearest airport to Leuven (about 25 km). The airport has a train station located below the terminal (basement level-1) and is connected twice an hour to Leuven (travel time of about 15 min). Alternatively, there are also airport shuttles that can be booked in advance (e.g. https://airportstaxitransfers.com/).

Alternatively, participants can arrive at the airport Brussels-South located near Charleroi. This airport is often favoured by low-cost companies. Passengers flying in to Charleroi can either take a bus (about twice an hour, further info see http://www.brussels-city-shuttle.com) to Brussels (train station Bruxelles-Midi/Brussel-Zuid) where they can take the train to Leuven (about 1 hour per bus followed by 30 min by train) or take the bus to Charleroi train station and continue their journey by train.

By car:

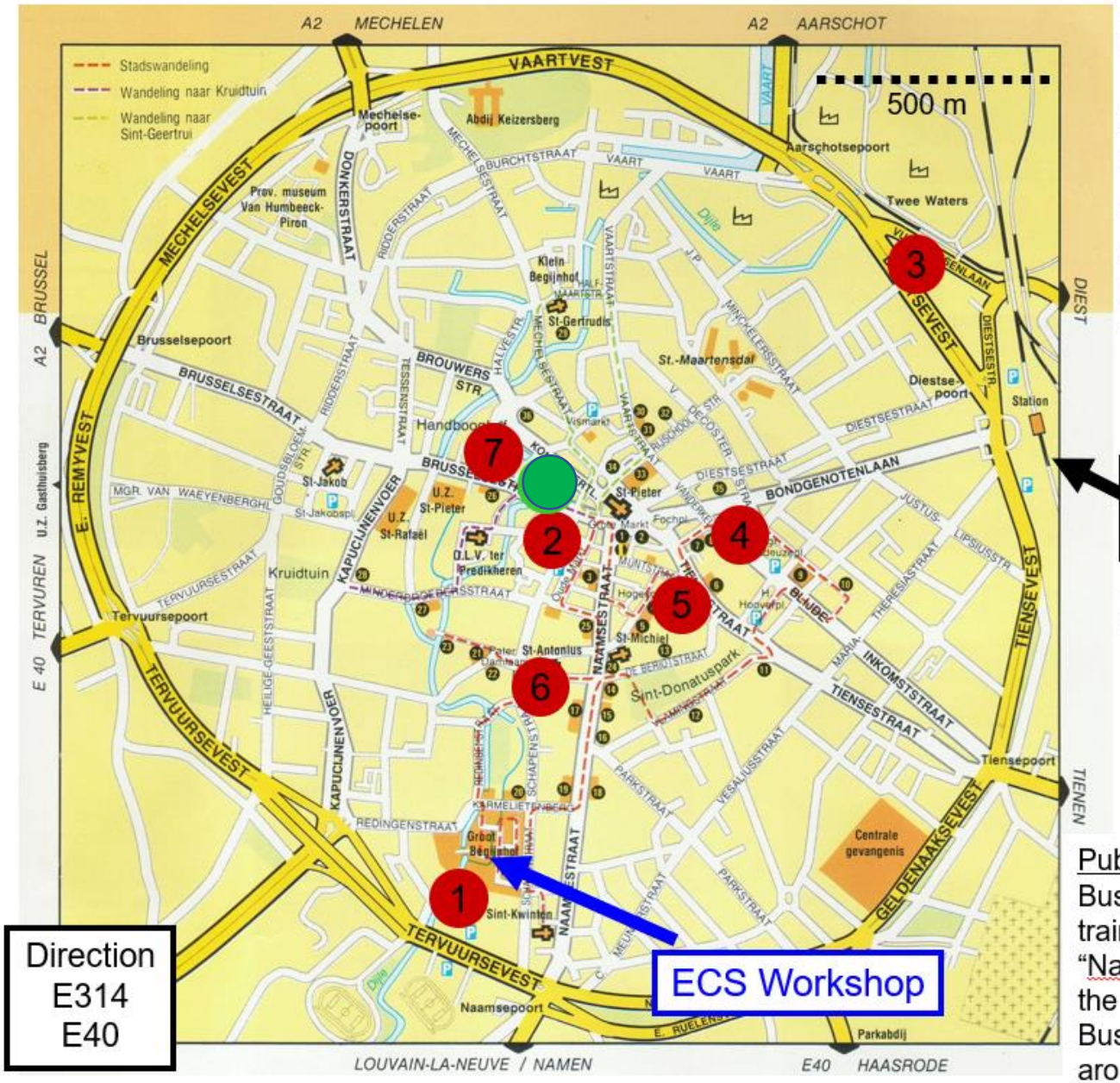
Leuven is easily accessible by car. It is located near the E40 highway Brussels-Liège. Coming from the E40 please follow the signs Hasselt when arriving at the junction with the E314, and take then the first exit (exit 15), which will bring you to the Leuven ring road, in the immediate vicinity of the workshop venue.

For those arriving by car, it will be interesting to know that the meeting venue has a free, private parking lot open to all meeting participants. The code that is needed to enter this parking lot P2 or P3 is **4982#**.

Getting around in Leuven

Leuven is an old city with many small streets. Walking (or biking) around is very easy, though driving is less recommended.

There are numerous possibilities for public transportation. Especially interesting are the bus lines 1 and 2 which allow easy communication between the train station, down-town Leuven and the "Naamse Poort" located quite near to the meeting venue. Other convenient bus lines, particularly for those staying at Begijnhof Hotel, are 600 and 601, traveling along the ring road (600 counter clockwise, 601 clockwise) every 10-15 minutes. They are however only available on weekdays between 6:00 and 20:00 and have a tendency to sometimes be cancelled on short notice.



Hotels:

1. Begijnhof hotel
2. Kloosterhotel
3. Novotel
4. Theaterhotel
5. Mercure
6. New Damshire
7. Ibis

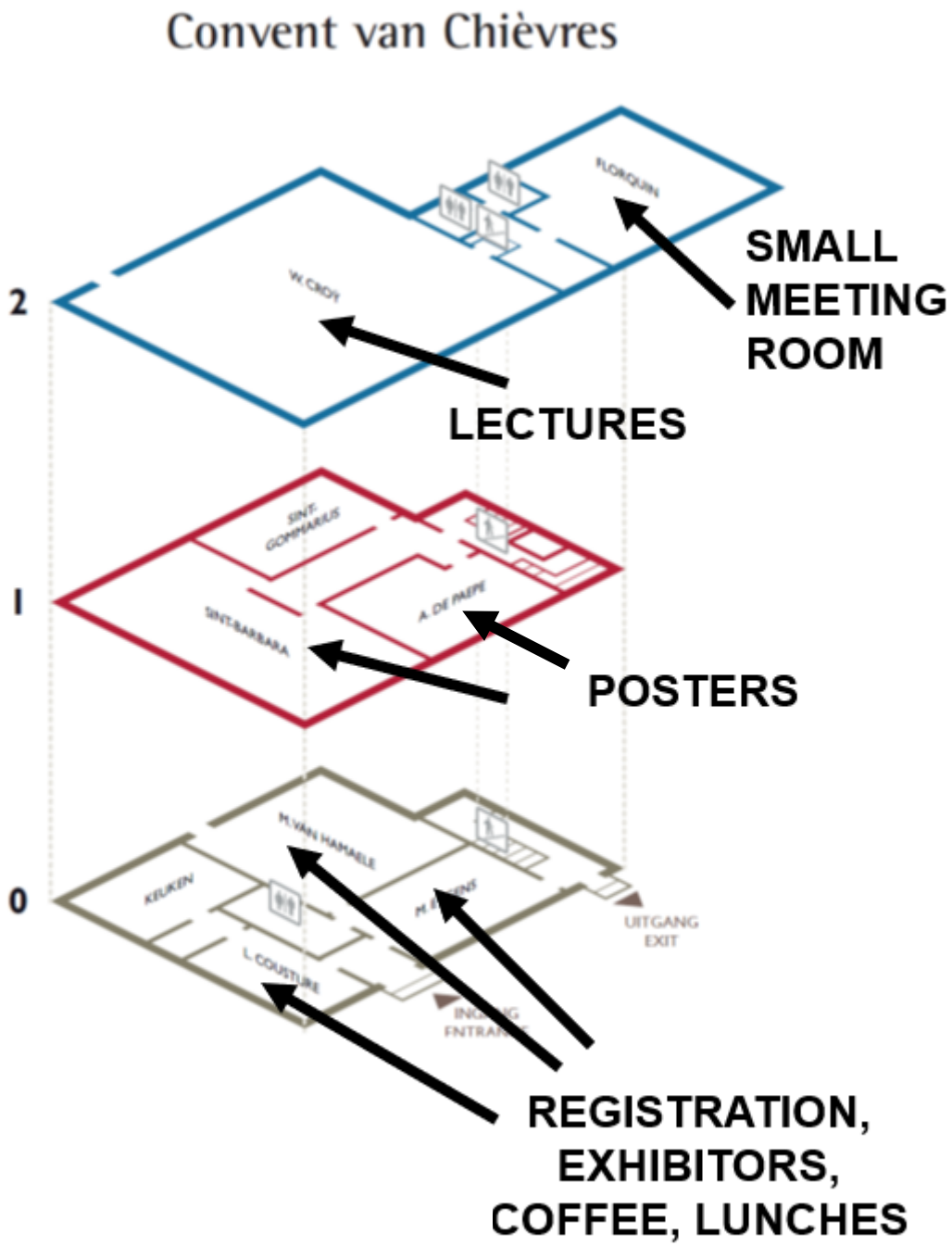
Restaurants:

10. 't Oud Gasthuys (Thursday ev.)

Public transportation:

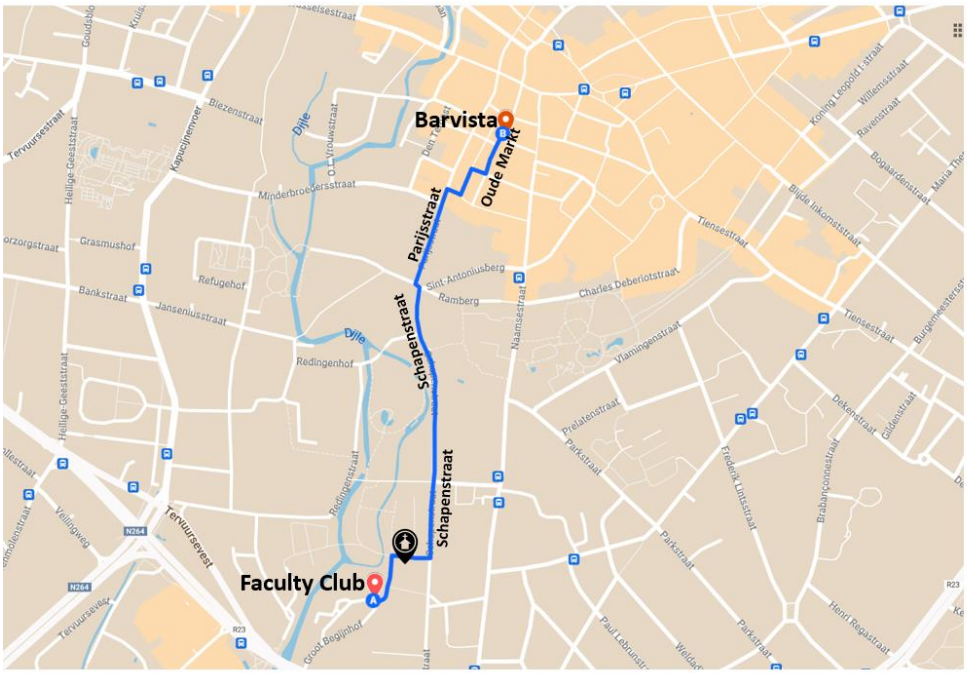
Buses 1 and 2 travel between train station, city center and "Naamsepoort" (which is near the meeting venue).
Bus 600 and 601 travels around the ring road.

Layout of the Convent of Chièvres



How To get from the Venue to Barvista Friday Evening

Oude Markt 7/8, 3000 Leuven



On foot

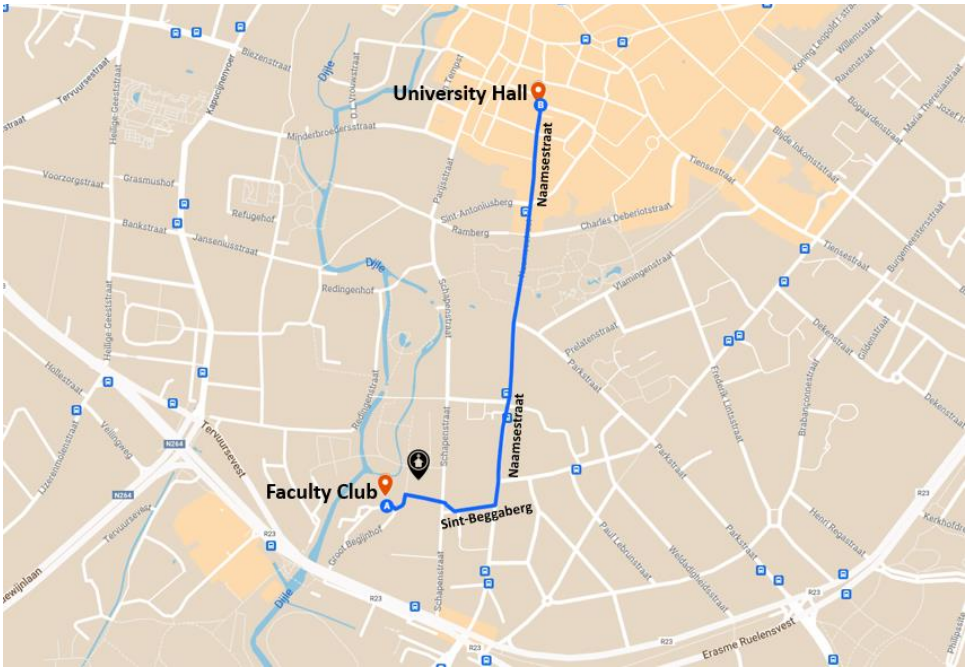
- Go in the direction of the church.
- Turn right and continue straight until the intersection with Schapenstraat.
- Turn left onto Schapenstraat.
- Keep going straight onto Parijsstraat.
- Turn right at the intersection with Lavorenberg.
- Immediately turn left onto Oude Markt.
- Almost at the end of the square, Barvista will be on your right side.

By Taxi

Taxi Jenny: +32 16 26 10 10

How To get to the University Halls Saturday Afternoon

Naamsestraat 22, 3000 Leuven
(Emeritus Celebration Jan Parys/ Academic session)



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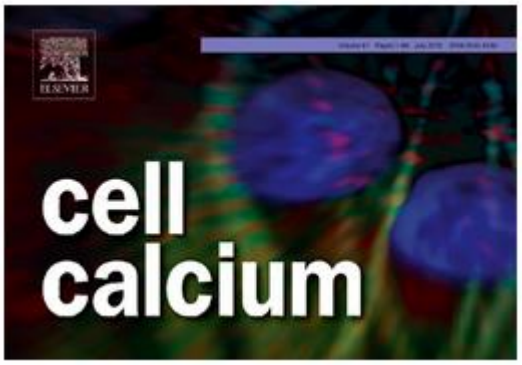
BIOMEDICAL SCIENCES



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Flanders
Opening new horizons

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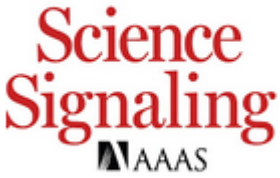

ORGANIZERS

Organizing Committee

Geert Bultynck (KU Leuven)

Jan Parys (KU Leuven)

Fernanda Lemos (KU Leuven)

Tim Vervliet (KU Leuven)

Flore Sneyers (KU Leuven)

Rita La Rovere (KU Leuven)

Tomas Luyten (KU Leuven)

Vivianne Aelbrecht (KU Leuven)

Martijn Kerkhofs (KU Leuven)

Thomas Voets (KU Leuven)

Patrizia Agostinis (KU Leuven)

Llew Roderick (KU Leuven)

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Philippe Gailly (UC Louvain)

Genevieve Dupont (ULB)

Martin Bootman (Open University, UK)

Andreas Guse (UKE Hamburg, Germany)

Scientific Committee

Geert Bultynck (KU Leuven, Belgium)

Jan B. Parys (KU Leuven, Belgium)

Llew Roderick (KU Leuven, Belgium)

Thomas Voets (KU Leuven, Belgium)

Patrizia Agostinis (KU Leuven, Belgium)

Djalila Mekahli (KU Leuven, Belgium)

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Barbara Niemeyer (Universität des Saarlandes, Germany)

Martin Bootman (Open University, UK)

Jim Putney (NIH, USA)

Carlos Villalobos (Universidad de Valladolid, Spain)

Natacha Prevarskaya (University de Lille, France)

Paola Pizzo (University of Padova, Italy)

Khaled Machaca (Weill Cornell Medicine, Qatar)

INSTRUCTIONS FOR SPEAKERS AND CHAIRS

It is the responsibility of the Chair of each session to ensure stringent timekeeping. Please allow 5 minutes for questions as part of the total time allocated to each speaker. Speakers should not go over time with their presentations.

Chairs should ensure that all presentations are loaded onto the computer prior to the session (the break before your session or even before). We have assigned also a local support person at the podium during each break just before a session. They will be available to assist the chair with this process, however the chair needs to be at the podium to answer questions from speakers.

It is the responsibility of each speaker to provide well in advance their presentations to the session chair, no later then the break before their session talk. If a speaker is not able to present that day, they need to inform the organizers as soon as possible so that a replacement can be found.

The convent of Chièvres opens half an hour before the first session (~8 am).

Presentations will be in English. The computer at the venue uses Windows 11 and has PowerPoint. Apple users should check for compatibility well in advance of their presentation and should bring their own equipment if needed.

If speakers want to test their presentation they can do this in the breaks in between sessions or during lunches. Please contact a person of the organizing staff if you want to do this. These tests should not be done when uploading of presentations is taking place.

INSTRUCTION FOR POSTER PRESENTERS

Please note. In cases where a delegate has been selected to give an oral presentation, the same presentation should not be given in poster form.

Both oral presentations (O) and poster presentations (P) are listed in this book of abstracts. Posters will be in portrait format (vertical, max 100 cm wide x 200 cm tall). Pins will be provided. Each poster board has been assigned a number corresponding to the number in this abstract book and posters should be affixed to the board of the same number, ensuring that the poster number is also visible.

Poster mounting and dismounting.

All posters will be on continuous display from the start of the workshop 12h30. When you arrive you can fix your poster to the assigned board on the second floor of the building in the poster room. Boards will be available from Wednesday noon until Friday after lunch just before session 6 starts. Please dismount posters immediately on Friday after lunch as the poster boards will have to be dismantled and removed immediately afterwards due to another booking of the room. Any posters left will be discarded and will not be recoverable.

Presenting authors are expected to attend their posters at the following times:

Odd Numbers: Thursday 12h10 -14h10

Even Numbers: Friday 12h15 -14h15

CODE OF CONDUCT

Respect for All Participants:

All participants, including attendees, speakers, and staff, are expected to treat each other with respect and professionalism. Harassment, discrimination, or any form of disrespectful behaviour will not be tolerated.

Equity:

We strive for an inclusive and diverse conference that represents the scientific community as a whole. All participants, regardless of race, ethnicity, gender identity, sexual orientation, disability, or any other personal characteristic, are welcome and valued.

Environmentally Conscious Behaviour:

We recognize the impact that conferences and large events can have on the environment. Participants are encouraged to reduce their carbon footprint by using public transportation, recycling, and reducing waste whenever possible.

Confidentiality:

All conference discussions and presentations should be considered confidential and not shared outside of the conference without the express permission of the presenter.

Responsible Use of Technology:

The use of technology, including smartphones and laptops, should not disrupt presentations or other conference activities. Participants should also be mindful of the privacy and security of their and others' information when using technology at the conference.

Compliance with Laws and Regulations:

All participants must comply with all applicable laws and regulations, including those related to data privacy and intellectual property.

Reporting Violations:

If a participant experiences or witnesses any behaviour that violates this Code of Conduct, they are encouraged to report it to a member of the conference staff or to the conference organizers. Confidentiality will be respected, and all reports will be promptly investigated and addressed.

Consequences for Violations:

Participants who violate this Code of Conduct may be subject to disciplinary action, up to and including removal from the conference without refund.

PRACTICAL INFORMATION

Internet access

The venue has Wi-Fi availability. The Password is available inside of the meeting room and will be communicated during the starting session.

Helpdesk

During the workshop you can always contact anyone of the local organizers for assistance. You can recognize them by the badges that have a green color. The badges will also have organization printed on them.

Certificates

A certificate of participation will be issued to you upon arrival to the workshop after registration.

Badges

At the start of the workshop you need to pass by the registration desk to receive your badge and attendance certificate. This badge needs to be on you during the entire workshop, including social events. It will help other attendees to recognize you, allow for easy interaction and will ensure your entry to the receptions, lunches, dinner and (if you booked this during registration) your social activity.

If you arrive late, your badge is still available at the registration desk or you can contact anyone from the organizer to obtain your badge.

Awards

We will give 3 awards for short talks (sponsored by Cell Calcium and ECS) and 3 awards for posters (sponsored by BBA-Mol Cell Res and ECS): 1st prize: 200 euro; 2nd prize: 100 euro; 3rd prize: 50 euro.

Sustainable Travel Fellowships.

Thanks to the support of the Company of Biologists (http://www.biologists.com), we have been able to offer 6 travel fellowships to early career researchers making their complete journey to the meeting by train to the venue site. Those who have been selected for a travel fellowship will receive information by email on how to get reimbursed for travel expenses by the organizers.

Hamamatsu FDSS µcell Site Visit

Information about the possibility to visit the FDSS/µcell device from Hamamatsu in the LMCS lab will be provided during the workshop. The timing of this depends on the program / availability of attendees and staff and your optional social activity. Sign-up sheets will be provided at the registration desk to book a slot if interested.

SCIENTIFIC PROGRAM

Wednesday, November 15th

- Registration + set-up of the posters (12:30 – 14:00)
- **Opening Session (start 14:00)**
  - 14:00 Introduction by Geert Bultynck
  - 14:15 Jan B. Parys (KU Leuven, Belgium): Endoplasmic reticulum biology and Ca^{2+}-release channels

- **Session 1 (start 15:15): Ca^{2+} in pathophysiology [chair: Thomas Voets]**
  - 15:15 Introduction by Chair
  - 15:20 Ilya Bezprozvanny (UT Southwestern Medical Center at Dallas, USA): Targeting ER signaling as an approach to treat neurodegeneration
  - 15:50 Barbara Ehrlich (Yale University, USA): Calcium signaling and neuropathy
  - 16:20 coffee break
  - 17:00 Natacha Prevarskaya (Universite de Lille, France): Calcium signaling in cancer cells: new challenges
  - 17:30 Carlos Villalobos (University of Valladolid, Spain): Mitoception or transfer of normal mitochondria to tumor cells reverses Ca^{2+} remodeling in colon cancer cells
  - 17:45 Jeremy Smyth (Uniformed Services University of the Health Sciences, USA): Astrocyte store-operated calcium entry is required for the development of chronic neuropathic pain
  - **Commercial partner talks [chair: Geert Bultynck]**
  - 18:00 CoolLED movie
  - 18:05 *Hamamatsu-sponsored short talk*, Jean Marc D'Angelo (Hamamatsu Photonics, France): Electric Field Stimulation Technique for High-Throughput Screening of Voltage-Gated Ion Channels
  - 18:10 *Hamamatsu-sponsored short talk,* Julius Rönkkö (University of Helsinki, Finland): Human IP3 receptor triple knockout stem cells remain pluripotent despite altered mitochondrial metabolism
  - 18:25 END
- **Opening Beer & Cheese reception (start 18:45), guided by Dimitri Staelens – Infirmerie Room @ Faculty Club**

Thursday, November 16th

- **Session 2 (start 8:30): Novel insights in Ca^{2+}-transport systems [chaired by Philippe Gailly and Luc Leybaert]**
  - 8:30 Introduction by Chair
  - 8:35 Shmuel Muallem (NIH, USA): ORP5/ORP8, phosphatidylserine and STIM1-Orai1 interaction and function
  - 9:05 David Yule (University of Rochester, USA): Lessons learned from in vivo measurements of Ca^{2+} signals in exocrine glands
  - 9:35 Julika Neumann (KU Leuven, Belgium): Disrupted Ca^{2+} homeostasis and immunodeficiency in patients with functional Inositol 1,4,5-trisphosphate receptor subtype 3 defects
  - 9:50 Mariella Weiß (University Medical Centre Hamburg, Germany): Adhesion to laminin-1 and collagen IV induces the formation of Ca^{2+} microdomains that sensitize mouse T cells for activation
  - 10:05 Coffee break
  - 10:40 Andreas Guse (University Medical Centre Hamburg, Germany): Novel insights in NAADP signaling
  - 11:15 Tim Vervliet: Ryanodine receptors regulate ER lysosomal contact and function
  - 11:30 Inga Pauels: Two pore channel 2 (TPC2) activity governs leukocyte adhesion in endothelial cells
  - 11:45 Franziska Möckl (University Medical Centre Hamburg, Germany): MASTER-NAADP – Membrane permeAble, Stabilized, biorEversibly pRotected derivative of the Ca^{2+} mobilizing second messenger NAADP
- **Sandwich lunch with poster session (12:00 – 14:00): odd numbers**
- **Session 3 (start 14:10): Cell death and survival [chaired by Patrizia Agostinis]**
  - 14:10 Introduction by Chair
  - 14:15 Rafael Fissore (University of Massachusetts, USA): The sophisticated Ca^{2+} toolkit required for the initiation of development in mammals
  - 14:45 Fernanda Lemos (KU Leuven, Belgium): Pyruvate kinase M2 (PKM2) as a modulator of Ca^{2+} signaling in cancer cell lines
  - 15:00 Christoph Brenker (University of Münster, Germany): Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation
  - 15:15 Yu Yuan (UC London, UK): Going global: How Ca^{2+} release from lysosomes and the endoplasmic reticulum is coordinated
  - 15:30 *Cell Calcium-sponsored keynote lecture* by Ana Garcia-Saez (University of Cologne, Germany): Calcium fluxes in cell death
- **Socio-cultural activities or free time (16:30-19:15)**
  - squash Lovanium (contact Tomas Luyten)
  - City Walking Tour option 1: Aperowalk; Leuven InsideOut; (contact Fernanda Lemos)
  - City Walking Tour option 2: Leuven in a Nutshell; Leuven Plus; (contact Maarten Van Munster).
  - Museum M, Leopold Vanderkelenstraat 28, 3000 Leuven; (contact Ian De Ridder).
  - FDSS site visit campus Gasthuisberg (contact Rita La Rovere)
- **Dinner at 7.30pm in Oud Gasthuys** (Brusselsestraat 63B – Leuven; Binnenplein 30CC nabij de Romaanse Poort)

Friday, November 17th

- **Session 4 (start 8:30): Mitochondrial Ca^{2+} [chaired by Amalia Dolga]**
  - 8:30 Introduction by Chair
  - 8:35 Peace Atakpa-Adaji (Cambridge University, UK) (Cambridge University, UK): Licensing of IP3Rs that deliver Ca^{2+} to mitochondria - a KRAP story
  - 9:05 Paola Pizzo (University of Padova, Italy): Calcium signalling and organelle contact sites: two interconnected elements in physiological and pathological conditions
  - 9:35 Sylvie Ducreux (Universite de Lyon, France): Are TRPV1 channels new players in the reticulum-mitochondrial Ca^{2+}-coupling?
  - 9:50 Adelina Ivanova (Cambridge University, UK): Novel regulation of IP3 receptors by phosphatidylinositol 4,5-bisphosphate contributes to controlling the transition from local to global Ca^{2+} signals
  - 10:05 Coffee break
- **Session 5 (start 10:40): Novel insights in Ca^{2+}-binding proteins & effectors [chaired by Genevieve Dupont)**
  - 10:40 Introduction by Chair
  - 10:45 Malene Brohus (Aalborg University, Denmark): Molecular segregation of calmodulin mutation phenotypes
  - 11:15 Martha Cyert (Stanford University, USA): Systematic discovery of calcineurin signaling pathways
  - 11:45 Tom Venneman (KU Leuven, Belgium): Interplay between axonal mitochondrial transport and neuronal activity.
  - 12:00 Oflaz Furkan (Medicial University of Graz, Austria): Annexin-A5 is fundamental for VDAC1-dependent mitochondrial Ca^{2+} homeostasis and determines the susceptibility to apoptosis
- **Sandwich lunch with poster session (12:15-14:15):** even numbers
- **Session 6 (start 14:30): Role of Ca^{2+} stores [chaired by Llew Roderick]**
  - 14:30 Introduction by Chair
  - 14:35 Marek Michalak (University of Alberta, Canada): Interplay between stress sensing, signaling and cardiac excitation-contraction (E-C) coupling
  - 15:05 Khaled Machaca (Weill Cornell Medical, Qatar): Ca^{2+} tunneling as an integral modality downstream of SOCE
- **Closing Lecture [chaired by Jan Parys]**
  - 15:40 Introduction by Chair
  - 15:45 Gyorgy Hajnoczky (Thomas Jefferson University, USA): IP_3 receptors and ER-mitochondrial contact sites
- **Awards and Concluding remarks [Geert Bultynck]**
- **Closing reception (17:00-18:30) – Infirmerie Room @ Faculty Club**
- **Party at Bar Vista Oude Markt 7/8, 3000 Leuven (for those registered), starting 20:00**

Are you ready to elevate your calcium-related research and assays to new heights?

Molecular Devices is thrilled to announce our presence at the Leuven Calcium Society Workshop. As a trusted leader in the field, we're excited to share with you how our cutting-edge technology and innovative solutions can transform your experiments.

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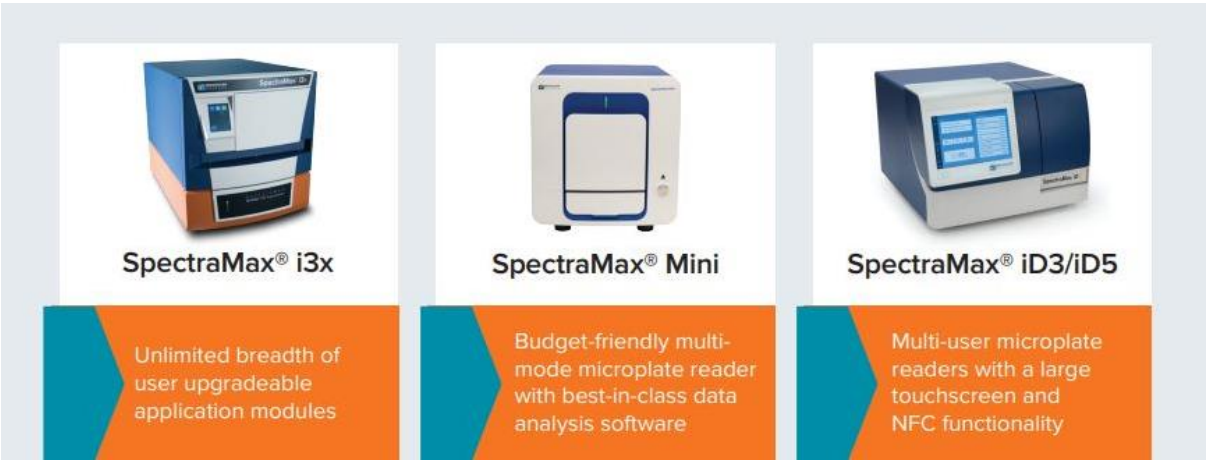

ABSTRACTS FOR ORAL PRESENTATIONS

O1

Disrupted Ca^{2+} homeostasis and immunodeficiency in patients with functional Inositol 1,4,5-trisphosphate receptor subtype 3 defects

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Calcium signaling is essential for lymphocyte activation, with genetic disruptions resulting in severe immunodeficiency. Inositol 1,4,5-trisphosphate receptors (IP_3Rs), formed by homo- or heterotetramers of the subtypes IP_3R1-3, are ER-based Ca^{2+} channels that open upon binding of the intracellular messenger IP_3 and induce an amplifying Ca^{2+} release pathway as a response to antigen stimulation. Although knockout of all IP_3R isoforms in mice results in immunodeficiency, human variants had not previously been associated with inborn errors of immunity (IEI). Here, we identify compound heterozygous variants in two unrelated Caucasian patients presenting with combined immunodeficiency, in one case requiring hematopoietic stem cell transplantation. We observed disrupted Calcium homeostasis and signalling in patient-derived fibroblasts and immune cells, with abnormal proliferation and activation responses in T cells. Reconstitution of IP_3R knockout cell lines identified the variants as functional hypomorphs with reduced discrimination between homeostatic and induced states, validating a genotype-phenotype link. These results demonstrate a functional linkage between defective ER Ca^{2+} channels and immunodeficiency, and identify IP_3Rs as diagnostic targets for patients with specific IEIs.

O2

Novel regulation of IP_3 receptors by phosphatidylinositol 4,5-bisphosphate contributes to controlling the transition from local to global Ca^{2+} signals

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Ca^{2+} signals through inositol 1,4,5-trisphosphate receptors (IP_3Rs) regulate a plethora of cellular processes due to variations in their spatial and temporal dynamics. Activation of plasma membrane (PM)-resident receptors generates IP_3 via phospholipase C-mediated hydrolysis of a PM lipid, phosphatidylinositol 4,5-bisphosphate (PIP_2). The stimulus intensity governs the amount of IP_3 produced and in consequence, the extent of IP_3R activation, starting from brief, localised Ca^{2+} puffs, and progressing all the way to cell-wide global Ca^{2+} waves. Tight control of the transition from local to global Ca^{2+} signals is central to the downstream consequences of receptor activation. However, it is not known whether additional components of the signalling pathway beside IP_3 contribute to the regulation of hierarchical transition of IP_3R-mediated Ca^{2+} signals.

To address this, we developed methods to uncouple stimulation of the G-protein-coupled receptors (GPCRs) that evoke IP_3 formation from delivery of IP_3 to IP_3Rs while retaining opportunities to stimulate IP_3Rs directly. Activation of the H1 receptor in HeLa cells reduced the frequency of Ca^{2+} puffs evoked exogenously by photolysis of caged i-IP_3 without affecting puff amplitude or kinetics. We show that the inhibition of i-IP_3-evoked Ca^{2+} puffs by histamine is entirely mediated by depletion of PIP_2, the precursor of IP_3, during signalling. Employing a rapamycin-inducible heterodimerisation tool for selective PIP_2 depletion at the PM, we confirmed that loss of PIP_2 regulates IP_3R-mediated Ca^{2+} puffs in HeLa and HEK293 cells, and this regulation extends to all three IP_3R subtypes. PIP_2 depletion significantly reduces the likelihood of Ca^{2+} puffs progressing to a global Ca^{2+} signal, but once the transition threshold is reached, the amplitude of the global signal is indistinguishable in the presence and absence of PIP_2. We conclude that PM-resident receptors regulate IP_3R activity by both providing IP_3 and by depleting PIP_2, and PIP_2 contributes to controlling the progression from local to global Ca^{2+} signals.

O3

Pyruvate kinase M2 (PKM2) as a modulator of Ca^{2+} signaling in cancer cell lines

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Cancer cells exhibit an upregulation of the pyruvate kinase M2 (PKM2), an alternatively spliced variant of the PKM gene. PKM2 drives aerobic glycolysis, conferring a metabolic advantage to malignant cells. Additionally, PKM2 promotes gene expression, cell cycle progression and cell survival. Recently, our group demonstrated that PKM2 interacts with and inhibits inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3R), a ubiquitously expressed endoplasmic reticulum (ER) Ca^{2+}-release channel, in various tumor cell lines (Lavik et al., 2022, Biochim. Biophys. Acta 1869:119206). Building upon this, we aimed to further characterize the functional interaction between PKM2 and IP_3R. To achieve this, we knocked out PKM2 from HeLa cells (HeLa PKM2 KO), and observed upregulation of PKM1, the other spliced variant of PKM. Comparing HeLa WT and PKM2 KO cells, we showed that PKM2 suppresses IP_3R-dependent Ca^{2+} release without affecting IP_3R expression levels, ER Ca^{2+} store content, or store-operated Ca^{2+} entry. Conversely, at identical IP_3-induced Ca^{2+} release, HeLa WT cells showed higher mitochondrial Ca^{2+} uptake compared to HeLa PKM2 KO cells, which can be linked to PKM2's role in promoting mitochondrial elongation and facilitating ER-mitochondria contact sites. However, it is worth noting that HeLa WT cells exhibited a lower mitochondrial membrane potential and reduced respiration. As an attempt to characterize the PKM2:IP_3R complex, we assessed the effect of a synthetic peptide based on the amino acid sequence 2078-2098 of IP_3R1, referred to as D5SD, which can displace PKM2 from the IP_3R. Notably, a cell-permeable version of D5SD (TAT-D5SD) induced comparable increases in cytosolic Ca^{2+} levels in both HeLa WT and HeLa PKM2 KO cells, while the mitochondrial Ca^{2+} uptake was slightly increased in HeLa WT cells. Correspondingly, TAT-D5SD treatment significantly caused apoptosis in both HeLa WT and HeLa PKM2 KO cell lines while HeLa IP_3R 3KO cells, which lack IP_3Rs entirely, are not affected. Importantly, the D5SD has the capability to disrupt the interaction between IP_3R and PKM1 as well, although PKM1 downregulation did not influence the cytosolic Ca^{2+} release triggered by TAT-D5SD. Collectively, these findings underscore the functional interplay between PKM2 and IP_3R in modulating cellular Ca^{2+} signaling within the cytosol and in the ER-mitochondria microdomain of cancer cells. It is noteworthy that while D5SD disrupts the PKM:IP_3R interaction and induces Ca^{2+} release, this effect does not solely rely on PKM. Hence, we are exploring novel strategies to target the functional interaction between PKM2 and IP_3R. These strategies include investigating PKM2 mutants as well as exploring the involvement of other proteins, such as GRP75 and Bcl-2, in the complex formed by PKM2 and IP_3R.

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O4

Astrocyte store-operated calcium entry is required for the development of chronic neuropathic pain

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Astrocytes act as sentinels in the central nervous system by modulating neural function in response to environmental cues such as sensory stimulation, environmental stressors, and nerve injury. This sentinel function depends on complex Ca^{2+} signaling mechanisms in astrocytes. Nerve injury often results in the development of chronic neuropathic pain, and astrocyte function is essential for the transition from acute nerve injury to chronic pain. However, the role of astrocyte Ca^{2+} signaling in chronic pain is poorly defined. Store-operated Ca^{2+} entry (SOCE) was recently shown to regulate neuromodulation by astrocytes, and we have now tested the hypothesis that astrocyte SOCE is activated by nerve injury and is required for the transition to chronic neuropathic pain. We have addressed this using a *Drosophila* model of chronic pain, whereby leg amputation injury results in development of neuropathic pain in the form of thermal allodynia by seven days following injury. We show using a transcriptional reporter of intracellular Ca^{2+} (TRIC) that leg amputation injury results in robust Ca^{2+} signaling activity in astrocytes on day four post-injury, and this astrocyte Ca^{2+} signaling was completely inhibited by astrocyte-specific suppression of the SOCE components STIM and Orai as well as inositol 1,4,5-trisphosphate receptor (IP_3R) Ca^{2+} release channels. Development of thermal allodynia seven days post-injury was also inhibited by astrocyte STIM and Orai suppression, consistent with a requirement for astrocyte SOCE-mediated Ca^{2+} signaling in chronic pain. Thermal allodynia on day seven was accompanied by significant loss of inhibitory GABAergic neurons, and GABAergic neuron loss has been shown to be both necessary and sufficient for disinhibition of excitatory sensory neural circuits that contributes to pain sensitization. Importantly, this loss of GABAergic neurons was also prevented in animals with astrocyte STIM and Orai suppression. Lastly, we found that astrocyte-specific expression of the G170M constitutively active *Drosophila* Orai mutant was sufficient to increase astrocyte Ca^{2+} and drive thermal allodynia in the absence of nerve injury. These results suggest that astrocyte SOCE is an essential signaling response that mediates the transition from acute nerve injury to the development of chronic neuropathic pain. Ongoing studies are focusing on identifying the astrocyte signaling targets of SOCE that modulate sensory and nociceptive function in the central nervous system.

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O5

Interplay between axonal mitochondrial transport and neuronal activity.

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Due to their large scale and uniquely branched architecture, neurons critically rely on active transport of mitochondria in order to match energy production and calcium buffering to local demand. Consequently, defective mitochondrial trafficking is implicated in various neurological and neurodegenerative diseases. A key signal regulating mitochondrial transport is intracellular calcium. Elevated Ca^{2+} levels have been demonstrated to inhibit mitochondrial transport in many cell types, including neurons. However, it is currently unclear to what extent calcium-signaling regulates axonal mitochondrial transport during realistic neuronal activity patterns. We created a robust pipeline to quantify with high spatial resolution, absolute Ca^{2+} concentrations. This allows us to monitor Ca^{2+} dynamics with pixel precision in the axon and other neuronal compartments. In this study, we confirm that neuronal activity can indeed regulate axonal mitochondrial transport, and reveal a spatial pattern to this regulation which went previously undetected. As expected, prolonged KCl-induced depolarization did inhibit axonal mitochondrial transport in primary hippocampal neurons. However, the inhibitory influence of physiologically relevant neuronal activity patterns on mitochondrial transport was only observed in axonal segments which made actual connections to a target neuron. In 'non-connecting' axonal segments, we were unable to trigger this inhibitory mechanism using realistic firing patterns. Ratiometric imaging revealed that axonal calcium levels scale with firing frequency in the range of 0.1-1µM, whereas KCl-induced depolarization generated levels almost a magnitude higher. Together, these findings indicate a potent, but localized role for activity-related calcium fluctuations in the regulation of axonal mitochondrial transport.

O6

Ryanodine receptors regulate ER lysosomal contact and function

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Intracellular Ca^{2+} signaling is critically involved in the regulation of several processes including, autophagy, a lysosomal degradation pathway. The endoplasmic reticulum (ER) serves as the largest, main cellular Ca^{2+} store where adequate Ca^{2+} levels are needed to ensure proper protein folding. In addition to this, Ca^{2+} is released from the ER thus affecting several cellular processes. At the ER two main families of tetrameric Ca^{2+} release channels are known to exist, inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs). The ubiquitously expressed IP_3Rs have been extensively studies for their roles in regulating different steps of autophagy. The RyR has been less well studied in this context. This is rather surprising considering that RyRs are predominantly expressed in long lived cells with specialized metabolic needs, such as neurons and muscle cells, in which autophagy plays important roles. In addition, excessive RyR activity in neurons has been implicated in the onset of several diseases including Alzheimer's disease, where impaired autophagy regulation contributes to the pathology. Our previous research has shown that RyR are indeed involved in regulating the distal steps of autophagic flux. Specifically, spontaneous RyR-mediated Ca^{2+} release impaired autophagic flux at the level of the lysosomes by inhibiting their fusion with autophagosomes. How exactly RyR-mediated Ca^{2+} release regulates lysosomal functions was at that time not elucidated and is the topic of this current research.

In this study we primarily utilized cortical neurons derived from human induced pluripotent stem cells to elucidate the effects of RyR-mediated Ca^{2+} release on lysosomal function. Lysosomal pH measurements using a combination of FITC (pH sensitive) and Rhodamine (pH insensitive) dyes conjugated to dextran showed in these wildtype neurons that blocking RyR activity did not affect lysosomal pH. However, the total lysosomal rhodamine signal was increased upon inhibition of RyR activity suggesting either increased uptake, breakdown or reduced extrusion of these dyes. Further analysis showed that blocking RyR activity increased lysosomal exocytosis. In addition, the RyR was shown to act as a tether for ER/lysosomal contact sites by interacting with the lysosomal V-ATPase. Overexpressing the v0a1 subunit of the V-ATPase in a RyR2 overexpression model could mimic the effects of RyR inhibition on rhodamine dye loading suggesting this interaction to also be involved in regulating lysosomal secretion.

O7

Mitoception or transfer of normal mitochondria to tumor cells reverses Ca^{2+} remodeling in colon cancer cells

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During tumor transformation, metabolic reprogramming occurs that provides survival advantages to tumor cells. The cornerstone of this reprogramming is the Warburg effect, which consists of the switch from aerobic metabolism to glycolysis due to defective mitochondrial ATP synthesis. Due to this effect, most tumor cells show an increased mitochondrial potential (ΔΨ) associated to enhanced driving force for mitochondrial Ca^{2+} uptake that prevents Ca^{2+}-dependent inactivation of store-operated channels leading to enhanced and sustained Ca^{2+} signaling involved in cancer hallmarks. Here we asked whether the transfer of mitochondria from normal cells may reverse the enhanced SOCE characteristic of cancer cells. To address this issue, we isolated mitochondria from normal human colonic NCM460 cells, labeled them with a fluorescent marker and transferred efficiently to human colon cancer HT29 cells. Mitocepted cells were used to investigate transfer efficiency, SOCE and mitochondrial potential using flow cytometry, confocal microscopy and calcium imaging. We found that isolated normal cell mitochondria can be efficiently introduced (Mitoception) in tumor cells by co-centrifugation and/or by co-incubation. The amount of mitocepted mitochondria depended on the amount of co-centrifugated or co-incubated mitochondria. Tumor cells mitocepted with normal cell mitochondria show decreased SOCE close to values observed in normal cells. This effect is partially observed at 3h post-mitoception, maximum at after 48 h and disappeared one week after mitoception. SOCE downregulation after mitoception is not due to increased mitochondrial mass since mitoception of these cells with tumor cell mitochondria (automitoception) resulted in increased SOCE. In contrast, normal cells mitocepted with tumor cell mitochondria show low SOCE similar to normal, not mitocepted cells. Tumor cells mitocepted with normal cell mitochondria show decreased mitochondrial potential whereas automitocepted tumor cells show increased mitochondrial potential. In contrast, normal cells mitocepted with tumor cell mitochondria show no change in mitochondrial potential. We conclude that transfer of mitochondria to tumor cells may modulate SOCE depending on the nature of mitocepted mitochondria. However, these effects are not observed in normal cells that set mitochondrial potential and SOCE to normal values.

O8

Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation

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Human sperm have to fulfil a series of demanding functions during fertilization: they navigate along various chemical and physical cues to localize the oocyte, and they break through its protective vestments by hyperactivation and acrosomal exocytosis. These processes are controlled by changes in intracellular pH (pHi), membrane potential (Vm), and intracellular Ca^{2+} concentration ($[Ca^{2+}]i$), mediated by a subset of unique ion channels. The sperm-specific ion channels CatSper serves as central signalling node and controls $[Ca^{2+}]i$. We assessed the function of the sperm-specific multisubunit CatSper-channel complex in sperm of almost 2,300 men undergoing a fertility workup, using a simple motility-based test. Thereby, we identified a group of men with normal semen parameters, but defective CatSper function. These men or couples failed to conceive naturally and upon medically assisted reproduction via intrauterine insemination and in vitro fertilization. Intracytoplasmic sperm injection (ICSI) was, ultimately, required to conceive a child. We show that the defective CatSper function was caused by variations in CATSPER genes. Moreover, we unveil that CatSper-deficient human sperm are unable to undergo hyperactive motility and, therefore, fail to penetrate the egg coat. Thus, we provide the first experimental evidence that sperm hyperactivation is required for human fertilization, explaining the infertility of CatSper-deficient men and need of ICSI for medically assisted reproduction. Finally, our study also reveals that defective CatSper function and ensuing failure to hyperactivate represents the most common cause of unexplained male infertility known thus far.

O9

Are TRPV1 channels new players in the reticulum-mitochondrial Ca^{2+}-coupling?

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Ca^{2+} release in microdomains formed by contact sites between compartments, such as mitochondria-associated endoplasmic reticulum membranes (MAMs), encodes a signal contributing to Ca^{2+} homeostasis and cell fate control. However, the composition and functionality of MAMs are not yet fully understood. In the present work, we are interested in Transient Receptor Potential Vanilloid 1 (TRPV1), a Ca^{2+}-permeable ion channel and a polymodal nociceptor. Recently, a study reported that pharmacological activation of plasma membrane TRPV1 channels indirectly disturbed the formation of MAMs in a Ca^{2+}-dependent manner, suggesting that TRPV1 activation could have prevented an excessive and deleterious mitochondrial Ca^{2+} uptake in renal disease. Paradoxically, TRPV1 activation restores the ER–mitochondrial tethering by increasing the expression of a MAM protein called PACS2 in pulmonary fibrosis or supports MAM formation through the AMPK-MFN2 pathway in response to myocardial hypertrophy. However, no study has investigated the possibility that TRPV1 channels may be components of MAMs.

Using several biochemical methods (Western blotting, immunostaining, cell fractionation, and proximity ligation assay), we found TRPV1 channels in the endoplasmic reticulum (ER) membrane, including at MAMs in a rat cardiomyoblast cell line (SV40-transformed H9c2). Thanks to chemical and genetic probes, we performed a comprehensive Ca^{2+} imaging approach in the four cellular compartments: ER (ErGap1), cytoplasm (Fura2-AM), mitochondrial matrix (CMV-mito-R-GECO1 or 4mtD3cpv), and mitochondrial surface (N33D3cpv). Our results show that Ca^{2+} released from ER via TRPV1 channels can be detected at the outer mitochondrial membrane and transferred to the mitochondria. Finally, we observe that a 30-minute prolonged TRPV1 modulation modifies the intracellular Ca^{2+} balance and can influence the MAMs structure or else hypoxia/reoxygenation-induced cell death.

In conclusion, our study provides the first evidence that TRPV1 channels contribute to Ca^{2+} exchange in MAMs.

O10

Two pore channel 2 (TPC2) activity governs leukocyte adhesion in endothelial cells

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Upon pro-inflammatory stimulation, endothelial cells present leukocyte-attracting receptors at the surface, consisting of the adhesion molecule P-selectin and its stabilizing cofactor CD63, that is primarily stored in late endosomes/lysosomes and is needed for proper leukocyte attachment and rolling. Both are delivered to the endothelial surface via transport onto lysosome-related organelles, called Weibel-Palade bodies (WPB), and fusion with the plasma membrane.
We identified the endolysosomal Ca^{2+} channel TPC2 as a regulator of the post-endolysosomal CD63 transport. Histamine-activated WPB release was associated with TPC2-mediated Ca^{2+} flux in primary human endothelial cells. Blocking TPC2 activation via RNAi-mediated gene silencing and pharmacological inhibition resulted in reduced CD63 transport from late endosomes/lysosomes to the plasma membrane, leading to diminished P-selectin retention on the surface and the concomitant defective P-selectin-dependent adhesion of human neutrophils. Accordingly, we found neutrophil recruitment to the activated vessel wall in TPC2 knockout mice to be strongly reduced, confirming the physiological relevance of TPC2 functionality for leukocyte-endothelium interaction. Interestingly, pharmacological TPC2 inhibition caused the accumulation of cholesterol in CD63-positive compartments, pointing to the regulatory role of endolysosomal lipid balance in post-endolysosomal trafficking.

O11

Adhesion to laminin-1 and collagen IV induces the formation of Ca^{2+} microdomains that sensitize mouse T cells for activation

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A hallmark of the adaptive immune response is the activation of T cells. Once activated, T cells migrate from blood vessel walls to inflamed tissue by crossing the basement membrane and subsequently interacting with elements of the extracellular matrix (ECM). In this process, binding to endothelial cells and ECM proteins is facilitated by integrins, adhesion molecules expressed on the surface of T cells. This adhesive interaction may be crucial to change T cell sensitivity and facilitates full T cell receptor (TCR)/CD3-mediated activation[1].

Local Ca^{2+} microdomains are the earliest Ca^{2+} signaling events observed upon full T cell activation. They occur within tens of milliseconds after T cell activation and can be detected by high-resolution live-cell imaging. Previously, our group observed rarely occurring Ca^{2+} microdomains before TCR/CD3 stimulation involving Orai1 channels and STIM proteins[2].

Here, we report that Ca^{2+} microdomains observed in the absence of TCR/CD3 stimulation are initial signaling elements to increase T cell sensitivity and emerge in response to adhesion to ECM proteins[3]. High-resolution live cell–imaging technique was used to detect local adhesion-dependent Ca^{2+} microdomains (ADCM). Blocking with anti-integrin monoclonal antibodies against integrin receptors such as α2/β1 (binds to collagen-IV/VI), α6/β1 (binds to laminin-1) and αL/β2 (binds to the intercellular adhesion molecule, ICAM-1), but not to α5/β1 resulted in significant decrease of ADCM. Next, only adhesion to collagen-IV and laminin-1 caused significant increases of Ca^{2+} microdomains and promoted translocation of nuclear factor of activated T cells subtype 1 (NFAT-1) to the nucleus. Moreover, inhibition of focal adhesion kinase (FAK), phospholipase C (PLC), or deletion of all three D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R) subtypes resulted in significantly decreased formation of ADCMs. Upon TCR stimulation, reduced global Ca^{2+} signals, as well as decreased translocation of NFAT1 on adhesive collagen-IV coating were observed when FAK was blocked, indicating a relevance of ADCM for the magnitude of T cell activation.

In summary, these data show that the sensitization of T cells might be achieved by adhesion to basement-membrane proteins like collagen-IV and laminin-1, involving signaling via FAK, PLC, IP_3Rs and Orai1 to produce Ca^{2+} microdomains.

References

1. Randriamampita et al. (2003). Eur. J. Immunol. 33, 1215–1223.

2. Diercks et al. (2018). Sci. Signal. Dec 18; 11(561):eaat0358.

3. Weiß et al. (2023

O12

Going global: How Ca^{2+} release from lysosomes and the endoplasmic reticulum is coordinated

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Spatially localized changes in cytosolic Ca^{2+} are fundamental events that underpin Ca^{2+} signals across scales.

Lysosomes are thought to generate local Ca^{2+} signals that are globalized by neighboring Ca^{2+} stores of the endoplasmic reticulum (ER) through interplay between the second messengers, NAADP, IP3 and cyclic ADP-ribose. But little is known concerning this transition. Using Ca^{2+} chelators and cell-permeant activators of the lysosomal ion channel TPC2, we resolve endogenous cell-wide lysosomal-derived Ca^{2+} signals in to global and local changes. We show that these signals are largely reliant on ER Ca^{2+} stores. We resolve both IP3R-independent and IP3R-dependent elementary TPC2-evoked events and show that TPC2-IP3R coupling is Ca^{2+}-dependent. Local activation of TPC2 sensitized global Ca^{2+} signals to a physiological IP3-forming stimulus.

Our data identify inter-organelle Ca^{2+} signals as fundamental building blocks that couple local Ca^{2+} release events from lysosomes and the ER to global Ca^{2+} changes in the cytosol.

O13

MASTER-NAADP – Membrane permeAble, Stabilized, biorEversibly pRotected derivative of the Ca^{2+} mobilizing second messenger NAADP

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To date, nicotinic acid adenine dinucleotide phosphate (NAADP) is described as the most potent endogenous Ca^{2+} mobilizing second messenger. In T cells, NAADP concentrations in the low nanomolar range are sufficient to evoke Ca^{2+} signals [Gasser et al., 2006]. Rapid formation of NAADP by NADPH oxidases after stimulation of T cells via the TCR/CD3 complex [Gu et al., 2021] resulted in Ca^{2+} microdomain formation relying on the type 1 ryanodine receptor (RYR1) [Wolf et al., 2015] and merge into global Ca^{2+} signals amplified by IP_3, cyclic ADP-ribose [Guse et al., 1999] and store-operated Ca^{2+} entry (SOCE). However, the mode of activation and target channels of NAADP remains controversially discussed, as both, RYR1 on the endoplasmic reticulum (ER) membrane, as well as endo-lysosomal two-pore channels (TPCs), have been proposed as NAADP targets. Recent findings by our group describe NAADP binding to its binding protein HN1L/JPT2 and subsequent Ca^{2+} release through RYR1in T cells [Wolf et al., 2015; Diercks et al., 2018, Roggenkamp et al., 2021].

To study NAADP-induced signaling pathways, direct delivery of NAADP into the cytosol is crucial. Previously, laborious and difficult techniques, such as via patch-clamp or microinjection have been used.

Here, the newly developed compound MASTER-NAADP, a membrane-permeable and bio-reversible NAADP derivative is characterized by high-resolution live cell imaging in different cell types. The addition of MASTER-NAADP reliably evokes Ca^{2+} microdomains in Jurkat T cells, Neuro2A cells, KHYG-1 NK cells, and primary murine T cells, eventually further merging into global Ca^{2+} signals. Control experiments using MASTER-NADP, cleaved into an inactive NADP derivative, or HN1L/JPT2 knock-out cells confirm the specificity of MASTER-NAADP. Further, release of active NAADP mimic from protected MASTER-NAADP by esterase activity was confirmed by HPLC and mass spectrometry.

The results identify MASTER-NAADP as a novel NAADP derivative, which reliably evokes Ca^{2+} responses in various cell types. Hence, MASTER-NAADP is a useful new tool in the NAADP signaling toolbox.

References:

Diercks et al., Sci Signal. 2018 Dec 18;11(561):eaat0358.

Gasser et al., JBiol Chem. 2006 Jun 23;281(25):16906-16913.

Gu, F, et al. Dual NADPH oxidases DUOX1 and DUOX2 synthesize NAADP and are necessary for Ca^{2+} signaling during T-cell activation. Science Signal. 2021 Nov 16;14(709):eabe3800.

Guse et al. Nature. 1999 Mar 4;398(6722):70-3.

Roggenkamp, HG, et al. HN1L/JPT2: A signaling protein that connects NAADP generation to Ca^{2+} microdomain formation. Science Signal. 2021 Mar 23;14(675):eabd5647.

Wolf, I.M. et al. Frontrunners of T cell activation: Initial, localized Ca^{2+} signals mediated by NAADP and the type 1 ryanodine receptor. Science Signal. 2015; 8: ra102.

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O14

Annexin-A5 is fundamental for VDAC1-dependent mitochondrial Ca^{2+} homeostasis and determines the susceptibility to apoptosis

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Annexin-A5 (AnxA5) is a Ca^{2+}-dependent phospholipid-binding protein associated with the regulation of intracellular Ca^{2+} homeostasis. However, the precise role of AnxA5 in controlling mitochondrial Ca^{2+} signaling remained elusive. Here, we introduce a novel function of AnxA5 in regulating mitochondrial Ca^{2+} signaling. Our investigation revealed that AnxA5 orchestrates intermembrane space (IMS) Ca^{2+} access capacity upon high Ca^{2+} elevations induced by ER Ca^{2+} release, leading to AnxA5 translocation to the outer mitochondrial membrane. Through close association with the voltage-dependent anion channel (VDAC1), AnxA5 regulates the Ca^{2+}-permeable state of VDAC1. By modulating IMS Ca^{2+} signaling, AnxA5 actively shapes mitochondrial ultrastructure and influences the dynamicity of the mitochondrial Ca^{2+} uniporter. Furthermore, our findings regarding AnxA5's localization in the VDAC1 microenvironment reveal its protective role in regulating cell death by controlling VDAC1's oligomeric state triggered by cisplatin-induced apoptosis. Our study uncovers AnxA5 as a regulator of VDAC1 in physiological and pathological conditions.

Hamamatsu-sponsored short talk

Human IP3 receptor triple knockout stem cells remain pluripotent despite altered mitochondrial metabolism

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Inositol 1,4,5-trisphosphate receptors (IP3Rs) are ER Ca^{2+}-release channels that control a broad set of cellular processes. Animal models lacking IP3Rs in different combinations display severe developmental phenotypes. Given the importance of IP3Rs in human diseases, we investigated their role in human induced pluripotent stem cells (hiPSC) by developing single IP3R and triple IP3R knockouts (TKO). Genome edited TKO-hiPSC lacking all three IP3R isoforms, IP3R1, IP3R2, IP3R3, failed to generate Ca^{2+} signals in response to agonists activating GPCRs, but retained stemness and pluripotency. Steady state metabolite profiling and flux analysis of TKO-hiPSC indicated distinct alterations in tricarboxylic acid cycle metabolites consistent with a deficiency in their pyruvate utilization via pyruvate dehydrogenase, shifting towards pyruvate carboxylase pathway. These results demonstrate that IP3Rs are not essential for hiPSC identity and pluripotency but regulate mitochondrial metabolism. This set of knockout hiPSC is a valuable resource for investigating IP3Rs in human cell types of interest.

ABSTRACTS OF POSTER PRESENTATIONS

P1

Modified BH4 domain derived peptides of Bcl-2 as a potential tool against acute pancreatitis

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Abstract

Sudden onset of inflammation within the pancreas, Acute Pancreatitis (AP) is a life-threatening condition where the pancreas ultimately digests itself and surrounding tissue. Over 100,000 deaths worldwide, per year are attributed to AP related illness with morbidity approximately 5%. Up to 35% of patients with recurrent AP develop Chronic Pancreatitis, dramatically increasing the risk of Pancreatic Cancer. Despite years of research, currently no specific treatment is available in clinic. The main causes of AP include excessive alcohol intake and gallstone disease with blockage of the bile duct and subsequent toxic reflux of bile acids into the pancreatic duct. The initiation of pancreatic pathology leading to AP has two major hallmarks: sustained elevation of cytosolic calcium and significant ATP loss. These pathological events result in premature, intracellular activation of powerful digestive enzymes, mitochondrial dysfunction and ultimately cell death (Gerasimenko and Gerasimenko 2023).

The Bcl-2 family proteins, Bcl-2 and Bcl-X_L are crucially important in regulation of cell death. BH4 domain peptides derived from Bcl-2/Bcl-X_L have been shown to regulate ryanodine receptors (RyRs) in pancreatic acinar cells (PACs), reducing AP pathology at a concentration of 50µM (Vervliet et al. 2018). Since both inositol triphosphate receptors (IP_3Rs) and RyRs are the most important receptors responsible for the initial excessive Ca^{2+} release in PACs and initiation of AP, it is necessary to continue the search of more effective inhibitors.

We have recently developed a new line of modified Bcl-2 BH4 domain-derived peptides with increased inhibitory effects of Ca^{2+} signalling in PACs. These novel BH4 peptides at sub-micromolar concentrations effectively and reversibly block physiological responses in PACs induced by acetylcholine and cholecystokinin that rely on the opening of IP_3Rs and RyRs, respectively. Additionally, these novel peptides at submicromolar concentrations protect PACs against pathological Ca^{2+} overload as well as significantly reducing cell necrosis, induced by pathological agents such as bile acid. Therefore, these novel peptides provide a potential therapeutic avenue for development of new viable drugs against AP.

References:

Gerasimenko, J. V. and Gerasimenko, O. V. 2023. The role of Ca^{2+} signalling in the pathology of exocrine pancreas. Cell Calcium 112: 102740. (10.1016/j.ceca.2023.102740)

Vervliet, T., Gerasimenko, J. V., Ferdek, P. E., Jakubowska, M. A., Petersen, O. H., Gerasimenko, O. V. and Bultynck, G. 2018. BH4 domain peptides derived from Bcl-2/Bcl-XL as novel tools against acute pancreatitis. Cell Death Discovery 4: 58. (10.1038/s41420-018-0054-5)

P2

Role of adenine nucleotides for the activation of TRPM2 during chemotaxis

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As the first responders to pathogens, neutrophils are an essential part of the innate immune system. After leaving the blood vessel, they migrate towards the site of inflammation in a gradient of chemoattractants, including the bacterial degradation product N-formylmethionyl-leucyl-phenylalanine (fMLP), interleukin 8 and the anaphylatoxin C5a. The non-selective, Ca^{2+}-permeable cation channel TRPM2 is highly expressed in phagocytic cells of the immune system including neutrophils. TRPM2 can be activated by different intracellular adenine nucleotides like adenosine-5'-diphospho ribose (ADPR) (1), 2'-deoxy-ADPR (2) and 2'-phospho-ADPR (3) but is also subject to regulation by numerous other factors like pH, temperature and PIP_2 concentration. While the fact that TRPM2 is involved in the chemotaxis of neutrophils is well established, as this process is impaired in knock-out cells (4) or after blocking of the channel (5), the role of the different nucleotide agonists during this process is less clear. Complicating matters further, there are different biosynthetic pathways for the nucleotide agonists for TRPM2 including the membrane-bound glycohydrolase CD38, the nuclear poly-ADPR polymerase/poly-ADPR glycohydrolase (PARP/PARG) system and the cytosolic enzyme SARM1. The promyelocytic cell lines NB-4 and HL-60 can be differentiated *in vitro* into neutrophil-like cells that respond to chemoattractants and are used to investigate the role of different pathways by pharmacological inhibition of the pathways and enzymatic manipulation of the nucleotide levels in the cells. The effect on the chemotaxis in response to different stimuli will be followed by transwell assays and microscopy-based cell tracking.

References

1. Perraud AL et al. Nature. 31. Mai 2001;411(6837):595–9.
2. Fliegert R et al. Nat Chem Biol. September 2017;13(9):1036–44.
3. Tóth B et al. J Gen Physiol. Mai 2015;145(5):419–30.
4. Morad H et al. Sci Rep. 29. April 2021;11(1):9339.
5. Partida-Sanchez S et al. J Immunol 2007;179(11):7827–39.

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P3

Role of the Unfolded protein Response during *Shigella* infection of epithelial cells

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The Unfolded Protein Response (UPR) is a cell response to the alteration of ER protein folding and quality control (ER stress) that elicits an adaptive signaling pathway, restoring ER homeostasis and promoting cell survival. Due to its role in host inflammatory response and immune cells development and activation, the UPR is activated by various pathogens and regulates bacterial infection. Depending on the infection model, the UPR may orchestrate host responses to restrict infection, or conversely, may be diverted to promote intracellular pathogen survival and replication. *Shigella* is an invasive pathogen that is able to colonize the colonic mucosa, triggering severe inflammation and tissue destruction leading to dysentery. The role of UPR in *Shigella* pathogenesis is poorly understood. It has been shown that Shiga toxin, a virulence factor produced by the enteric pathogens *Shigella dysenteriae* serotype 1, triggers ER stress sensors activation and induces a pro-apoptotic signaling in monocytic and macrophage-like cells. In this study, we explore the role of UPR during epithelial cell infection with *Shigella flexneri* that does not express the Shiga toxin. In preliminary results, upon infection of HeLa cells with *S. flexneri*, we observe that this pathogen induces UPR activation suggesting a role during the colonization of epithelial cells by this pathogen. This effect seems to be partially mediated by depletion of ER Ca^{2+} content. Interestingly, initial UPR activation is followed by sensor degradation 2h post-infection, an effect that is promoted by specific bacterial effectors secreted by *Shigella* into the host cell. A better understanding of the role of UPR in this context could contribute to the development of new therapeutic strategy for counteracting *Shigella* infection

P4

Electrophysiological study of Cx43 hemichannel and Panx1 channel modulation by Gap19 and ^{10}Panx1 peptides

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Abstract:

Cx43 hemichannels (HCs) and Panx1 channels are two genetically distant protein families. They form Ca^{2+} permeable channels; Cx43 HCs are regulated by cytoplasmic Ca^{2+} in a bimodal manner. Panx1 and Cx43 have several common properties including (i) analogous tetra-spanning transmembrane topology with cytoplasmic N-and C-termini, two extracellular loops and one cytosolic loop, (ii) a large diameter channel pore in the order of 17-21 Å , (iii) poor ion selectivity, (iv) channel opening triggered by positive membrane voltages. Given the structural similarities and overlapping function of Cx43 and Panx1, the physiological roles of the two proteins are still subject of debate. Current methods to distinguish between the two channels rely on electrophysiological approaches (mainly single-channel conductance measurement) and peptide-based inhibitors targeting Cx43 or Panx1. We investigated the selectivity of the mimetic peptides Gap19 and 10Panx1 as a tool to discriminate between the two channels. Gap19 mimics a sequence on the intracellular loop of Cx43 (KQIEIKKFK) and inhibits Cx43 HC opening by preventing Cx43 tail-loop interactions. ^{10}Panx1 peptide consists of a sequence from the first extracellular loop of Panx1 (WRQAAFVDSY), which is known to affect channel opening. Single-channel current activity from stably expressing HeLa-Cx43 and C6-Panx1 cells was recorded using the patch-clamp technique in whole-cell voltage-clamp configuration, demonstrating 214 pS (Cx43 HC) and 68 pS (Panx1) average unitary conductance. Gap19 was applied intracellularly, while ^{10}Panx1 was applied extracellularly, and the average nominal open probability (NPo) was determined for each testing condition (100, 200, 500 µM). A concentration of 100 µM Gap19 reduced the NPo of Cx43 HCs below 50%, while 200 µM ^{10}Panx1 was necessary to obtain half-maximal NPo reduction of Panx1 channels. Gap19 started to significantly inhibit Panx1 channels at 500 µM, reducing the NPo by 26% while reducing the NPo of Cx43 HCs by 84%. In contrast, ^{10}Panx1 significantly reduced the NPo of Cx43 HCs by 37% at 100 µM and by 83% at 200 µM, a concentration that caused half-maximal inhibition of Panx1 channels. These results demonstrate that extracellular ^{10}Panx1 more strongly reduces Cx43 HC function than Panx1 channel function at all concentrations tested, limiting the use of this peptide as a pharmacological tool to identify involvement of Panx1 channels in cellular function. Careful inspection of the Panx1 channel traces further demonstrated fast flickering closures in the presence of ^{10}Panx1, which were not observed with Gap19. The flickering frequency increased with the concentration of ^{10}Panx1 (67, 84, 146 Hz for 100, 200, 500 µM ^{10}Panx1), indicating fast ON-OFF gating effects within the channel pore. Understanding single-channel gating of Panx1 will help drive further development of inhibitors with optimal target selectivity to extend the Ca^{2+} signaling toolkit.

P5

Functional neurotoxicity assays in human dopaminergic neurons

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Assessment of drug toxicity on the human nervous system predominantly relies on animal models. However, we've developed an alternative system using Lund Human Mesencephalic (LUHMES) neuronal cultures for high-throughput quantification of free intracellular Ca^{2+} concentrations as a representative of neuronal activity, with confirmation of key processes by patch clamp recordings.

We investigated the potential impact of six in non-EU states still widely used neonicotinoids, primarily developed to target the insect nervous system, on human nicotinic acetylcholine receptors (nAChRs). Utilizing the established LUHMES cells as human neuronal model, several neonicotinoids including acetamiprid, imidacloprid, clothianidin, and thiacloprid were found to trigger Ca^{2+} signaling at 10–100 µM concentrations.

Further research focused on the metabolites of these neonicotinoids, particularly imidacloprid (IMI) and its derivatives imidacloprid-olefin and desnitro-imidacloprid (DN-IMI). DN-IMI was found to significantly trigger human nAChRs at sub-micromolar concentrations, demonstrating a potency and, therefore, toxicity similar to nicotine.

Our current research activities investigate the phenomenon that under certain conditions LUHMES cells exhibit synchronous slow Ca^{2+} oscillations with frequencies close to 1 Hz that last up to 2 minutes in 2D cultures. In 3D cultures, the properties appear to be similar but with oscillation times of more than 15 minutes. Under both conditions, this activity can be modulated by substances with a variety of different mechanisms of action. Although the underlying mechanism yet remains unclear, we are aiming to use this phenomenon to develop assays to test modulatory action of compounds on neuronal network activity.

Overall, this alternative system enables the assessment of complex signaling in human neurons, both at single cell and the neuronal network level.
The neonicotinoid application example already demonstrates the potential of this assay for neurotox applications. It highlights the potentially neurotoxic effects of these substances and their metabolites on the human nervous system and underscores the need for toxicological evaluation of these compounds.

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P6

A genetically encoded fluorescent sensor for NAADP visualization

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Nicotinic acid adenine dinucleotide 2'-phosphate (NAADP) is the most potent Ca^{2+}-releasing second messenger, which was first observed in sea urchin egg homogenates[1]. Our group demonstrated that NAADPH oxidation forms NAADP, which interacts with ryanodine receptor type 1 (RYR1) whereupon the release of Ca^{2+} from endoplasmic reticulum occurs[2,3]. Recently, two NAADP binding proteins were discovered (i) hematological and neurological expressed 1-like protein/Jupiter microtubule associated homolog 2 (HN1L/JPT2) binding NAADP to interact with RYR1 or two-pore channel 1 (TPC1)[3,4], while (ii) Lsm12 mediates the interaction with TPC2[5].

By using these NAADP binding proteins, a fluorescent genetically encoded NAADP sensor, enabling intracellular NAADP visualization was generated. The strategy to build the sensor was based on existing cAMP sensors[6, 7]. The sensors were tested by either addition of a novel stable membrane permeable derivative of NAADP (MASTER-NAADP) or anti-CD3 OKT3, an antibody that stimulates NAADP production via TCR/CD3 complex activation[8].

The aim is to create a tool that allows specific imaging of low nanomolar concentrations of NAADP signaling in (T) cells.

1. Lee HC, Aarhus R. A derivative of NADP mobilizes calcium stores insensitive to inositol trisphosphate and cyclic ADP-ribose. J Biol Chem. 1995 Feb 3; 270(5):2152-7. doi: 10.1074/jbc.270.5.2152. PMID: 7836444.
2. Gu, F., Krüger, A., Roggenkamp, H. G., Alpers, R., Lodygin, D., Jaquet, V., ... & Guse, A. H. (2021). Dual NADPH oxidases DUOX1 and DUOX2 synthesize NAADP and are necessary for Ca^{2+} signaling during T cell activation. *Science signaling*, *14*(709), eabe3800.
3. Roggenkamp, H. G., Khansahib, I., Hernandez C, L. C., Zhang, Y., Lodygin, D., Krüger, A., ... & Guse, A. H. (2021). HN1L/JPT2: A signaling protein that connects NAADP generation to Ca^{2+} microdomain formation. *Science signaling*, *14*(675), eabd5647.
4. Gunaratne, G. S., Brailoiu, E., He, S., Unterwald, E. M., Patel, S., Slama, J. T., ... & Marchant, J. S. (2021). Essential requirement for JPT2 in NAADP-evoked Ca^{2+} signaling. *Science signaling*, *14*(675), eabd5605.
5. Zhang, J., Guan, X., Shah, K., & Yan, J. (2021). Lsm12 is an NAADP receptor and a two-pore channel regulatory protein required for calcium mobilization from acidic organelles. Nature communications, 12(1), 4739.
6. Massengill, C.I., Bayless-Edwards, L., Ceballos, C.C. et al. Sensitive genetically encoded sensors for population and subcellular imaging of cAMP in vivo. Nat Methods 19, 1461–1471 (2022). https://doi.org/10.1038/s41592-022-01646-57.
7. Nikolaev, V. O., Bunemann, M., Hein, L., Hannawacker, A., & Lohse, M. J. (2004). Novel Single Chain cAMP Sensors for Receptor-induced Signal Propagation. Journal of Biological Chemistry, 279(36), 37215-37218.
8. Gasser A, Bruhn S, Guse AH. Second messenger function of nicotinic acid adenine dinucleotide phosphate revealed by an improved enzymatic cycling assay. J Biol Chem. 2006 Jun 23; 281(25):16906-16913.
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P7

Bcl-2-based strategies to target dysregulated Ca^{2+} signaling as an early event in Alzheimer's disease

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Objectives

Ca^{2+} homeostasis is crucial for maintaining different neuronal functions and aberrant Ca^{2+} signaling plays an important role in the early pathogenesis of Alzheimer's disease (AD). Familial AD is mainly caused by mutations in presenilin 1 (PSEN1). These PSEN1 mutations provoke Ca^{2+} dysregulation and were shown to sensitize the inositol 1,4,5-trisphosphate receptor (IP_3R) and ryanodine receptor (RyR), thereby eliciting exaggerated Ca^{2+}-signaling patterns. An important regulator of IP_3Rs and RyRs is the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). Bcl-2 can bind to and inhibit both receptors and thus could be exploited to counteract dysregulated Ca^{2+} release caused by PSEN1 mutations. Our ambition is to provide new perspectives on the early pathophysiology of AD and to exploit Bcl-2 to normalize aberrant Ca^{2+} release.

Results

Using Fura2-loaded HeLa PSEN dKO cells expressing either wild-type (WT) PSEN1 or PSEN1 mutants, we conducted single-cell Ca^{2+} measurements. Unexpectedly, our analysis revealed an inhibitory trend of PSEN1 mutants on IP_3R -mediated Ca^{2+} release. Additionally, we measured mitochondrial Ca^{2+} influx and endoplasmic reticulum (ER) Ca^{2+} release in HEK293 cells overexpressing RyR2, the major RyR isoform in neurons, after stably overexpressing PSEN1 WT or PSEN1 mutants. Our data indicates that PSEN1 mutants increase RyR2-mediated mitochondrial Ca^{2+} influx compared to PSEN1 WT, without severely affecting ER Ca^{2+} release. This suggest a role of PSEN1 mutations at the mitochondria-associated membranes (MAMs). Furthermore, we evaluated the amyloid protective and synaptoprotective effects of Bcl-2 and a Bcl-2 mutant (Bcl-2^{K17D}), which fails to inhibit IP_3Rs but not RyRs, by overexpressing Bcl-2 proteins in brains of 5xFAD mice. Both Bcl-2 proteins were shown to protect against synapse loss but mainly Bcl-2^{K17D} showed amyloid protection. These in vivo results support the pivotal role of RyRs in AD pathology and indicate the neuroprotective potential of Bcl-2 through the inhibition of RyRs.

Conclusions

Mutations in PSEN1 increase RyR2-mediated mitochondrial Ca^{2+} flux, and this may contribute to other defects observed in early-AD pathology. Moreover, Bcl-2-based strategies show promise in restoring normal Ca^{2+} signaling and counteracting the development of early AD features.

P8

Dynamic tubulin microtubules regulate endoplasmic reticulum calcium signaling and localization in the hippocampal neurons

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Store-operated calcium entry (SOCE) from the extracellular environment into the cytoplasm is activated by a decrease in the calcium concentration in the smooth endoplasmic reticulum (ER). To initiate calcium entry into the cytoplasm, STIM (stromal interacting molecule) proteins form clusters at the ER-membrane contact points. End-binding proteins (EB) attach to the growing microtubule tubulin positive end and migrate with it. The EB1 protein controls the oligomerization and translocation of STIM1 to the contact sites of the ER and the cell membrane in non-excitable cells. The STIM1-EB1 protein complex regulates the movement of the endoplasmic reticulum in them through the tip-attachment complex (TAC), when the ER tubule attaches and moves along with the growing end of the tubulin microtubule. In hippocampal neurons STIM2 homologue regulates SOCE in soma and dendritic spines. STIM2 interact with neuronal-specific EB3 in the Ca^{2+}-dependent manner and these proteins interaction is important for the normal morphology of dendritic spines (Pchitskaya et al., 2017; Pchitskaya et al., 2022). Using expansion microscopy (Rakovskaya et al., 2023), we demonstrated that during calcium depot empty, the density and size of STIM1-TR/NN protein clusters, with a mutation in the binding site to EB proteins, significantly increase in comparison to STIM1 clusters, which is consistent with data from other studies (Chang et al., 2018; Wang et al., 2018). In opposite to STIM1 at the same conditions the STIM2-IP/NN mutant variant tends to decrease in cluster density compared to STIM2. Disruption of the STIM2-EB interaction leads to the exclusion of STIM2 from the dendritic spines of hippocampal neurons in vitro, blocks its translocation into spines and oligomerization when the calcium depot is empty. Prevention of STIM2 interaction with EB proteins causes a significant decrease in the ER distribution in the neuronal soma and in the dendritic spines number in hippocampal neurons containing the spine apparatus, a specialized organelle formed by the ER.

So, it was demonstrated that STIM2 translocation and oligomerization, as well as the ER movement in the hippocampal neuron soma and dendrites, is regulated by the interaction of STIM2 with dynamic tubulin microtubules via EB proteins.

SOCE is impaired in various mouse models of Alzheimer's disease (AD), and its pharmacological restoration has a neuroprotective effect. EB3 protein overexpression potentiate SOCE and also has a strong effect on dendritic spines morphology – enhancing the size of dendritic spines head and therefore the number of mushroom dendritic spines, and protects them from degenerative changes in amyloid and presenilin based in vitro AD models. Observed neuroprotective effects way possible be linked with enhanced STIM2 clustering or ER distribution into spines.

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P9

Calcium signaling in necroptosis: one ion with many faces

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Necroptosis is an inflammatory form of regulated cell death implicated in organ injury, cancer, and immunity against infection. Execution of necroptosis depends on Mixed Lineage Kinase domain-Like (MLKL), a pseudokinase that has emerged as a potential target to treat necroptosis-related diseases. MLKL mediates plasma membrane permeabilization via a mechanism that remains controversial. MLKL activity can be linked to the increase of ion fluxes in the cell. In particular, calcium ions have a dual role in necroptosis, as they are required for both cell death and the activation of plasma membrane repair mechanisms. However, it is not clear yet how calcium signaling modulates cell fate. We combine cell biology, biophysics, and microscopy tools to dissect different aspects and the relevance of calcium signaling in necroptotic cells. We identified that pore formation is a core mechanism in this cell death modality, which is linked to the activation of calcium fluxes through the plasma membrane and the endoplasmic reticulum. Increase in cytosolic calcium is an early event that anticipates cell rounding and plasma membrane breakdown, two typical hallmarks of necroptosis that are related to osmotic forces. In parallel to this process, calcium signaling can be activated in a cell death and MLKL-independent mechanism via the engagement of the Tumor Necrosis Factor (TNF) survival pathway. This alternative mechanism requires the concomitant inhibition of different Inhibitor of Apoptosis Proteins (IAPs). This work provides new insight into the interconnection between different calcium signaling pathways and necroptosis, which balance ultimately determines not only cell fate but the way they communicate with the environment.

P10

Dual NADPH oxidase 2 (DUOX2) synthesizes NAADP in T cells

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent endogenous Ca^{2+} mobilizing second messenger known to date and effective in the low nanomolar range in many cells [1]. NAADP functions as a Ca^{2+} trigger in T cells by evoking an initial local Ca^{2+} signal, termed Ca^{2+} microdomain, together with a NAADP binding protein HN1L [hematological and neurological expressed 1-like protein, also known as Jupiter microtubule-associated homolog 2 (JPT2)] through type 1 ryanodine receptor mediated Ca^{2+} release [2,3]. This project aimed to answer the question about endogenous generation of NAADP upon T cell activation [4].

In vitro, CD38 can catalyze the formation of NAADP from NADP through base-exchange reaction. However, this reaction requires acidic pH and excess of nicotinic acid. Since both Ca^{2+} microdomains and global Ca^{2+} signals were identical in WT and *Cd38*$^{-/-}$ murine primary T cells, CD38 does not seem to be the NAADP forming enzyme involved in acute stimulation in T cells [4].

We discovered another class of enzymes [(dual) NADPH oxidases] able to generate NAADP *in vitro* under physiological conditions. We investigated the role of different T cell expressed NADPH oxidases in Ca^{2+} signaling. Only knock-out of two of these isozymes, namely DUOX1 and DUOX2, showed a strong phenotype in global Ca^{2+} signals upon TCR/CD3 activation. Furthermore, the phenotype of *Duox2*$^{-/-}$ T cells was identical with NAADP antagonism, whereas NAADP antagonist BZ194 further reduced global Ca^{2+} signals in *Duox1*$^{-/-}$ T cells [4]. This indicates that DUOX1 altered the global Ca^{2+} signals, at least partially, through other signaling pathway(s). Similar phenotype was observed in the absence of the NAADP binding protein HN1L in rat effector T cells [3]. Thus, we identified DUOX2 as NAADP forming enzyme in the early phase of T cell activation.

[1] Guse, A. H. & Lee, H. C. NAADP: a universal Ca^{2+} trigger. **Science Signal. 2008** Nov 4;1(44):re10
[2] Wolf, I.M. et al. Frontrunners of T cell activation: Initial, localized Ca^{2+} signals mediated by NAADP and the type 1 ryanodine receptor. **Science Signal. 2015**; 8: ra102.
[3] Roggenkamp, H. et al. HN1L/JPT2: A signaling protein that connects NAADP generation to Ca^{2+} microdomain formation. **Science Signal. 2021**; 14, eabd5647
[4] Gu, F. et al. Dual NADPH oxidases DUOX1 and DUOX2 synthesize NAADP and are necessary for Ca^{2+} signaling during T cell activation. **Science Signal. 2021;** 14, eabe38007
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P11

IP_3 receptor modulation by anti-apoptotic Bcl-2: impact of disease-related mutations and role of novel interaction partners

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The inositol 1,4,5-trisphosphate receptors (IP_3R), located in the endoplasmic reticulum (ER), play a central role in intracellular Ca^{2+} signaling. Through the control of Ca^{2+} flow from the ER lumen into the cytosol and mitochondria, these Ca^{2+}-release channels influence many intracellular processes among which cell proliferation, mitochondrial bioenergetics and apoptosis. Moreover, IP_3Rs function as a hub for plenty regulatory proteins, including at the level of the ER-mitochondria contact sites. The anti-apoptotic Bcl-2 protein is one of those proteins that binds and inhibits the IP_3R via its BH4 domain. We are elucidating this crossroad of cell signaling using various interaction techniques such as, microscale thermophoresis and biolayer interferometry, to further our understanding this key section in cellular control.

Several recurrent mutations have been identified in BCL-2 both in diffuse large B-cell lymphoma patients as well as in the context of acquired resistance towards the BH3 mimetic Bcl-2 antagonist drug venetoclax in chronic lymphocytic leukemia patients. We have investigated the functional implications for selected recurrent mutations located in the hydrophobic cleft of Bcl-2, namely G101V, D103Y, A113G, R129L and V156D. Hydrogen deuterium exchange- mass spectrometry analysis of the purified mutant proteins reveal clear structural rigidity shifts in comparison to Bcl-2wt in both the hydrophobic cleft and the structurally isolated BH4 domain. While all mutants retain the ability to inhibit staurosporine-induced cell death, which is largely Ca^{2+}-mediated, R129L- and V156D-mutated Bcl-2 display an increased anti-apoptotic effect. We also observe shifts in the ability of the mutant Bcl-2 proteins to inhibit BAX pore formation at the mitochondria. Our data display the clear functional implication of these recurrent Bcl-2 mutations on the level of Ca^{2+} signaling and cell death. However, further research is required to capture the full scope of implications that these mutations might have and pinpoint the underlying mechanisms.

We are also further elucidating the effects of another IP_3R-accessory protein, CISD2, that may impact the regulation of IP_3Rs by Bcl-2. Wolfram syndrome related protein, CISD2 directly binds both IP_3R1 via its central modulatory region and Bcl-2 via its BH4 domain. This interaction of CISD2 was found to regulate IP_3R-mediated ER-mitochondrial Ca^{2+} flux and the influence of Bcl-2 therein without impacting Ca^{2+} stores. We also show that CISD2 is involved in ER-mitochondria contact formation as well as autophagy.

Our data highlight IP_3R-mediated ER-mitochondrial Ca^{2+} flux and Bcl-2 as an axis in cell death control and disease.

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P12

IP3RPEP6, a novel peptide inhibitor of IP3 receptor channels based on an intramolecular self-binding sequence

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Abstract:

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are intracellular Ca^{2+}-release channels with crucial roles in physiological and pathophysiological signaling. Current IP_3R inhibitors suffer from off-target effects and poor selectivity towards the three distinct IP_3R subtypes. The IP_3-Ca^{2+} signaling axis also activates opening of connexin-43 hemichannels, which allow direct Ca^{2+} entry into the cell and facilitate ATP release that indirectly impacts cellular Ca^{2+} signaling through autocrine and paracrine pathways. Here, we developed and characterized a peptide called IP3RPEP6 that is based on a self-binding sequence of type-2 IP_3Rs and competitively inhibits IP_3Rs in a potency order of $IP_3R2 \approx IP_3R3 > IP_3R1$ at micromolar concentrations. IP_3-Ca^{2+} activation of hemichannel opening was found to enhance IP_3-triggered Ca^{2+} responses and to decrease the agonist concentration necessary to obtain half-maximal Ca^{2+} elevation, even at low connexin expression levels. IP3RPEP6 did not affect hemichannel opening nor ryanodine receptors and therefore genuinely reports effects at the level of IP_3Rs.

P13

TRPC3 regulates islet beta-cell insulin secretion

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Insulin release is tightly controlled by glucose-stimulated calcium (GSCa) through hitherto equivocal pathways. This study investigates TRPC3, a non-selective cation channel, as a critical regulator of insulin secretion and glucose control. TRPC3's involvement in glucose-stimulated insulin secretion (GSIS) is studied in human and animal islets. TRPC3-dependent in vivo insulin secretion is investigated using pharmacological tools and Trpc3−/− mice. TRPC3's involvement in islet glucose uptake and GSCa is explored using fluorescent glucose analogue 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose and calcium imaging. TRPC3 modulation by a small-molecule activator, GSK1702934A, is evaluated in type 2 diabetic mice. TRPC3 is functionally expressed in human and mouse islet beta cells. TRPC3-controlled insulin secretion is KATP-independent and primarily mediated by diacylglycerol channel regulation of the cytosolic calcium oscillations following glucose stimulation. Conversely, glucose uptake in islets is independent of TRPC3. TRPC3 pharmacologic inhibition and knockout in mice lead to defective insulin secretion and glucose intolerance. Subsequently, TRPC3 activation through targeted small-molecule enhances insulin secretion and alleviates diabetes hallmarks in animals. This study imputes a function for TRPC3 at the onset of GSIS. These insights strengthen one's knowledge of insulin secretion physiology and set forth the TRPC3 channel as an appealing candidate for drug development in the treatment of diabetes.

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P14

Stearoyl-CoA desaturase-dependent regulation of lipid droplet biogenesis and Golgi apparatus structure is controlled by TRPM8(85) isoform channel in ER

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Transient receptor potential melastatin 8 (TRPM8) is a cold thermosensor located in the dorsal root ganglia and the plasma and endoplasmic reticulum (ER) membranes of prostate tissue. This sensor can be regulated by cold mimicking substances or endogenous substances such as lipids. Our team has already characterized a TRPM8 isoform, 4TM-TRPM8, located in the ER-mitochondrial associated membrane (MAMs). Here we identify a new isoform of TRPM8 with 85 kDa size, named TRPM8(85), present in the ER of the epithelial cells of prostate at the interface with the ER-Golgi intermediate compartment (ERGIC). Targeting specifically this isoform by small interfering RNA (siRNA) in prostate cancer cell lines, C4-2 and LnCaP cells, induces Golgi apparatus (GA) fragmentation. In addition, electron microscopy has shown lipid droplets accumulation in the GA upon targeting TRPM8(85). Lipidomic analysis has shown increased unsaturated fatty acids fraction in LnCaP cells in which TRPM8(85) isoform was targeted. To further understand the changes seen in saturation fraction of lipids, the bottle neck desaturase enzyme expression: stearoyl-CoA desaturase 1, *SCD1,* was studied by western blot and it was shown that there is increase in SCD1 protein expression upon knocking down the 85 kDa isoform. Preliminary results indicate that the TRPM8(85) isoform does not exhibit calcium influx upon menthol stimulation, suggesting a potential regulatory function rather than ion channel activity. The exact function of this isoform is yet to be determined, including its differentiation from the canonical TRPM8 form and other isoforms.

P15

NAADP redox cycle during T cell activation

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NAADP is a potent Ca^{2+} releasing second messenger with an important role in T cell activation. After stimulation, NAADP is rapidly formed, contributing to the first Ca^{2+} signals in T cells and controlling downstream T cell functions [1-3]. The therapeutic potential of modulating NAADP signaling was demonstrated using the NAADP-antagonist BZ194 in a rat model of multiple sclerosis [4], further highlighting the importance of identifying the key components of this signaling pathway. So far, the mechanism of NAADP formation has remained largely elusive. The previously described candidate enzymes CD38 and SARM1 can form NAADP from NADP *in vitro* through a base-exchange reaction, but only under specific conditions (acidic pH and millimolar concentration of nicotinic acid), making it unlikely that they account for the fast rise in NAADP concentration observed during early T cell activation [5,6].

After confirming that CD38 is not involved in initial Ca^{2+} microdomains observed within tens of milliseconds after T cell stimulation using tissue from CD38 knockout mice, we set out to find other NAADP forming enzymes. Therefore, we established an *in vitro* assay for NAADP synthesis, in which we measure NAADP formation by membranes isolated from different cell types using high-performance liquid chromatography (HPLC). We discovered that members of the NADPH-oxidase enzyme family synthesize NAADP from its reduced precursor NAADPH under physiological conditions. Additionally, we identified glucose-6-phosphate-dehydrogenase as the only cellular dehydrogenase able to reduce NAADP to its inactive precursor. Using high-resolution calcium-imaging, we showed that knockout of dual NADPH-oxidases (DUOX1/2) reduces Ca^{2+} microdomains during stimulation of CD4+ T cells with an αCD3/αCD28 antibody-coated bead. Further, glucose 6-phosphate dehydrogenase (G6PDH) was identified as enzyme converting NAADP to NAADPH [3]. Initial Ca^{2+} microdomains were independent of H_2O_2 generation and not further decreased by pharmacological NAADP-antagonism, pointing towards a NAADP/NAADPH redox cycle between DUOX2 and G6PDH regulating the initial Ca^{2+} signals during T cell activation [3]. Currently, we are investigating the regulation of the NAADP redox cycle using a modified version of the HPLC assay in combination with Ca^{2+} imaging of different knockout models and pharmacological inhibitors and will share our first insights.

References

[1] Gasser, Bruhn et al. Journal of Biological Chemistry 2006. 281(25): 16906-16913.

[2] Wolf IM, …, Guse AH. Science Signal. 2015 Oct 13;8(398):ra102.

[3] Gu F, …, Guse AH. Science Signal. 2021 Nov 16;14(709):eabe3800.

[4] Cordiglieri et al. Brain 2010. 7(133): 1930-1943, 2010

[5] Aarhus, Graeff et al. Journal of Biological Chemistry 1995. 270(51): 30327-30333

[6] Zhao, Xie et al. iScience 2019. 15: 452-466

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P16

STIM1 and the Store-Operated Calcium Entry in the Pathology of Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA), a severe hereditary neuromuscular disorder, is caused by biallelic loss of the survival motor neuron 1 (*SMN1*) gene resulting in loss of α-motor neurons (MN), muscle weakness and paralysis. *SMN2*, an almost identical gene to *SMN1*, produces significantly less functional transcripts, and thus can only partially compensate for the loss of *SMN1*. Although, current treatment approaches – aiming to restore SMN – showed powerful results, they are not fully efficient. Thus, investigating and targeting of further pathways in combination with the previously approved therapies might stand as a better alternative in the future.

Previously, in asymptomatic individuals, carrying homozygous *SMN1* deletion, the upregulation of the SMN-independent SMA protective modifier, plastin 3 (PLS3) was found, which protective effect was confirmed across various SMA models. Although the overexpression (OE) of PLS3, an F-actin binding and bundling protein beneficially impacts disease manifestation, specific pathways through which PLS3 counteracts the SMA phenotype are still not fully understood.

Here, through proteomic interactome analysis of PLS3 in spinal cords of mice, we identified stromal interaction molecule 1 (STIM1) as a novel interacting partner of PLS3. Since a multitude of previous studies using SMA models point towards disturbed calcium-dependent processes upon the disease, STIM1, an essential orchestrator of the store-operated calcium entry (SOCE) appeared as a highly interesting candidate. In depth investigation showed that SMN deficiency leads to dysregulation of STIM1 in various tissues of SMA mice and in neuronal and muscle cultures, whereas PLS3 OE restored STIM1 levels in the severely affected transversus abdominis muscle, heart and also cultured primary MNs. Surprisingly, despite decreased STIM1 levels, by using proximity ligation assay a significant degree of interaction was observed between STIM1 and its major calcium release-activated calcium channel, ORAI1 in SMA spinal cords, which interestingly was almost completely abolished by PLS3 OE. Finally, we aimed to investigate potential disturbances in Ca^{2+} handling and Ca^{2+} influx through SOCE upon SMA. Hereby, as a start, perforated patch-clamp recordings revealed that the previously suggested increased intrinsic excitability of SMA MNs arises in an SMN-dependent manner, only when residual SMN levels decrease to ~20-30% of controls (resembling a type I SMA phenotype), which we evoked by a lentiviral mediated sh*Smn* knockdown system. There appears to be a threshold of SMN above which electrophysiological deficits do not arise. As a next step, we will be performing live cell calcium imaging on SMA and control MNs with and without PLS3 OE.

By verification of disturbed Ca^{2+} influx, in future SOCE may not only represent a viable therapeutic target for combinatorial SMA therapies, but also provide evidence for its potential contribution to the multi-organ vulnerability in SMA.

P17

Title: Unraveling the impact of intracellular BAPTA on cell function and the development of novel intracellular Ca^{2+} chelators

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Abstract

Ca^{2+} signaling has been implicated in virtually all cellular processes, including autophagy, a lysosomal turn-over process in cellular homeostasis. Moreover, Ca^{2+} dysregulation contributes to pathophysiological conditions, such as oncogenesis and neurodegenerative disorders. Much of what is known about Ca^{2+} signaling has been based on the use of pharmacological tools that modulate cellular signals. One such tool that has been extensively used is BAPTA, a fast and high-affinity Ca^{2+} chelator. However, recent studies have shown that BAPTA directly impacts other cellular components and has Ca^{2+}-independent actions. Therefore, BAPTA can no longer be considered a reliable means of validating the role of Ca^{2+} in cellular processes and researchers should be cautious in interpreting findings based on the use of BAPTA. This highlights the need for (i) a re-evaluation of the role of Ca^{2+} in cellular processes such as autophagy, where Ca^{2+} has been implicated largely based on the inhibitory action of BAPTA (ii) the development of improved BAPTA variants (iii) a proteome-wide view of BAPTA's targets.

Our work aims to meet these existing needs. On the one hand, by comparing BAPTA with low Ca^{2+}-affinity BAPTA variants, we critically examine the exact role of Ca^{2+} signaling in autophagy. On the other hand, we investigate how the molecular structure of BAPTA should be adapted to avoid certain Ca^{2+}-independent actions. Based on these findings, we are developing novel Ca^{2+} chelators that will enable a more accurate identification of Ca^{2+}-dependent cellular processes.

Ultimately, this work will contribute to a better understanding of intracellular Ca^{2+} signaling in autophagy and potentially other cellular processes.

P18

The role of P2X4 and P2X7 on T cell activation - From initial Ca^{2+} microdomains to downstream signaling processes

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Initial T cell activation is triggered by the opening of Ca^{2+} channels like ryanodine receptor type 1 (RYR1) and ORAI1 leading to the formation of highly dynamic, spatiotemporally restricted Ca^{2+} signals milliseconds after T cell stimulation, termed Ca^{2+} microdomains [1,2]. ATP-gated cation channels P2X4 and P2X7, located at the plasma membrane, have been shown to enhance the free cytosolic Ca^{2+} concentration after T cell stimulation and the release of ATP from T cells has been demonstrated within seconds after activation. In the first step, we analyzed P2X4- and P2X7-dependent Ca^{2+} microdomains using high-resolution live-cell–imaging with different technical strategies, including knock-out mice, nanobodies and antagonists.

Initial Ca^{2+} microdomains were significantly decreased in $CD4^+$ T cells from *P2x4*$^{-/-}$ and *P2x7*$^{-/-}$ mice or by chemically inhibiting P2X4 (using 5-BDBD or PSB-15417) or blocking P2X7 with inhibitory nanobodies in WT T cells [3]. Moreover, the formation of Ca^{2+} microdomains was influenced by ATP released from the cytosol via pannexin1 and was blocked either by the addition of extracellular apyrase or using a mimetic inhibitor peptide (^{10}panx1) for pannexin1 [3]. Moreover, we show for the first time that a basal ATP release by pannexin-1 in unstimulated cells activates P2X4 and promotes the formation of TCR/CD3-independent Ca^{2+} microdomains in $CD4^+$ T cells. Next, we analyzed the role of P2X4 and P2X7 in $CD8^+$ T cell signaling from initial events to downstream responses, focusing on various aspects of T cell activation, including Ca^{2+} microdomains, global Ca^{2+} responses, NFAT-1 translocation, IFN-γ expression, and proliferation. Similar to $CD4^+$ T cells, in $CD8^+$ T cells from *P2x4*$^{-/-}$ and *P2x7*$^{-/-}$ mice the number of initial Ca^{2+} microdomains was significantly reduced. The lack of these initial Ca^{2+} microdomains is affecting NFAT-1 translocation as well as INF-γ expression. Interestingly, global Ca^{2+} responses were only slightly but significantly affected and, consequently, also cell proliferation.

In summary, our data suggest that purinergic signaling amplifies the initial Ca^{2+} events during $CD4^+$ and $CD8^+$ T cell activation and plays an essential role in fine-tuning the downstream activity on multiple timescales of T cell activation.

References

1. Wolf, I.M.A., Diercks, B.-P., Gattkowski, E., Czarniak, F., Kempski, J., Werner, R., Schetelig, D., Mittrücker, H.-W., Schumacher, V., Osten, M. von, et al. (2015). Frontrunners of T cell activation: Initial, localized Ca^{2+} signals mediated by NAADP and the type 1 ryanodine receptor. ***Sci. Signal.*** *8*, ra102. 10.1126/scisignal.aab0863.

2. Diercks, B.-P., Werner, R., Weidemüller, P., Czarniak, F., Hernandez, L., Lehmann, C., Rosche, A., Krüger, A., Kaufmann, U., Vaeth, M., et al. (2018). ORAI1, STIM1/2, and RYR1 shape subsecond Ca^{2+} microdomains upon T cell activation. ***Sci. Signal.*** *11*, eaat0358. 10.1126/scisignal.aat0358.

3. Brock, V.J., Wolf, I.M.A., Er-Lukowiak, M., Lory, N., Stähler, T., Woelk, L.-M., Mittrücker, H.-W., Müller, C.E., Koch-Nolte, F., Rissiek, B., et al. (2022). P2X4 and P2X7 are essential players in basal T cell activity and Ca^{2+} signaling milliseconds after T cell activation. ***Science Advances***. 10.1126/sciadv.abl9770.

P19

Mathematical modelling of the early phase of UPR activation triggered by progressive ER Ca^{2+} depletion

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Calcium (Ca^{2+}) is a key factor in the maintenance of Endoplasmic Reticulum (ER) homeostasis. Ca^{2+} depletion in the ER, often induced by the irreversible SERCA2B inhibitor thapsigargin in ER stress studies, has been shown to control the activation of the three ER stress sensors, IRE1, PERK and ATF6. While many studies have focussed on the activation of the 3 branches of Unfolded Protein Response (UPR) upon massive depletion of ER Ca^{2+}, we have investigated their induction following the application of moderate doses of the less potent SERCA2B inhibitor tBuBHQ. In this poster, we present the mathematical model built to back up the experimental investigation of the dynamics of the early phase of UPR activation in response to progressive ER Ca^{2+} depletion.

The model considers BiP titration-dependent sensors' activation and accurately reproduced the different activation levels and kinetics of IRE1, PERK and ATF6, induced by various concentrations of tBuBHQ. We selected the simplest models able to account for experimental observations, with values of parameters numerically optimized using a genetic algorithm. Surprisingly, our model predicted a deactivation of PERK and IRE1 upon ER Ca^{2+} replenishment. This prediction was validated experimentally. IRE1 and PERK indeed completely dephosphorylated in about 30 min after washout of tBuBHQ. To account for the rapidity of dephosphorylation, the model has to consider a Ca^{2+}-dependent active mechanism.

Besides, we introduced an innovative indirect approach to estimate the evolution of the concentration of Unfolded Proteins by quantifying the decrease in BiP mobility observed by FRAP experiments for different levels of ER stress.

In conclusion, our experimental and modelling results demonstrate the highly dynamic activation/deactivation cycle of PERK and IRE1, which in a physiological context could be used to tightly control cell fate upon acute and/or chronic stress.

P20

Mechanisms of local and global Ca^{2+} Signaling evoked by TPC2 agonist TPC-A1-N in T cells

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Perturbed endo-lysosomal ion homeostasis, in particular Ca^{2+} homeostasis, has been observed in several Lysosomal storage disorders (LSD) suggesting that dysregulation of lysosomal ion flux can impair endo-lysosomal pathway functions. To better understand defects in endo-lysosomal Ca^{2+} signaling involved in LSD, we analyzed Ca^{2+} signaling evoked by the two-pore channel 2 (TPC2) agonist TPC-A1-N (A1-N) in T cells (1). A1-N was shown to mimic effects of NAADP on TPC2 (1).

T cell receptor/CD3 evoked Ca^{2+} signaling starts in the first seconds by local Ca^{2+} signals evoked by NAADP mediated Ca^{2+} release through type 1 ryanodine receptors (RYR1) and store-operated Ca^{2+} entry (SOCE) via Orai1, termed Ca^{2+} microdomains (2,3). Global Ca^{2+} signaling is then observed over several minutes.

TPC2 agonist A1-N evoked Ca^{2+} microdomains in the human T-lymphoma cell line Jurkat. These microdomains were sensitive to (i) de-acidification of lysosomes, (ii) gene silencing of RYR1, and (iii) inhibition of Orai1, but not to deletion of the genes for NAADP forming enzyme DUOX*2* or NAADP receptor HN1L/JPT2. Ca^{2+} microdomains were also evoked in primary murine $CD4^+$ WT T cells by A1-N; currently, the numbers of Ca^{2+} microdomains in WT T cells vs *Tpcn2*$^{-/-}$ or *Tpcn1*$^{-/-}$ / *Tpcn2*$^{-/-}$ T cells are being determined.

Since NAADP was shown to activate TRPM2, though at high, non-physiological concentrations (4), the identity of a further lysosomal Ca^{2+} channel involved in T cell Ca^{2+} microdomains may be TRPM2. TRPM2 is mainly expressed in the plasma membrane. Thus, we hypothesized that A1-N may activate Ca^{2+} entry through TRPM2 during global Ca^{2+} signaling in T cells. Indeed, A1-N evoked Ca^{2+} entry was observed under conditions of (i) de-acidification of lysosomes or (ii) Ca^{2+} depletion of ER in the presence of SOCE blockade by Orai1 inhibition. Preincubation with specific TRPM2 antagonist 8-Br-ADPR (5) significantly inhibited the A1-N evoked Ca^{2+} entry component.

Taken together, A1-N activates local and global Ca^{2+} signaling in T cells. Local Ca^{2+} signaling requires intact lysosomes, RYR1, and SOCE via Orai1, whereas global Ca^{2+} signaling proceeds via activation of additional Ca^{2+} entry pathway(s), e.g. through plasmalemmal TRPM2.

References:

1. Gerndt, S.et al, Agonist-mediated switching of ion selectivity in TPC2 differentially promotes lysosomal function. e.Life9; 10.7554/eLife.54712 (2020).

2. Wolf, IM. et al, Frontrunners of T cell activation: Initial Ca^{2+} signals mediated by NAADP and the type 1 ryanodine receptor. Sci Signal;8(398):ra102 (2015).

3. Diercks BP. et al. ORAI1, STIM1/2, and RYR1 shape subsecond Ca^{2+} microdomains upon T cell activation. Sci Signal. (561):eaat0358 (2018).

4. Beck A. et al.. Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. FASEB J. 2006 May;20(7):962-4. doi: 10.1096/fj.05-5538fje. Epub 2006 Apr 3. PMID: 16585058.

5. Partida-Sanchez S. et al. Chemotaxis of mouse bone marrow neutrophils and dendritic cells is controlled by adp-ribose, the major product generated by the CD38 enzyme reaction. J Immunol. 2007 Dec 1;179(11):7827-39. doi: 10.4049/jimmunol.179.11.7827. PMID: 18025229.

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P21

Isoform-specific regulation of IP_3R Ca^{2+} release channels by Bcl-2

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Bcl-2 is an anti-apoptotic protein canonically impinging on mitochondrial Bak/Bax. Besides this canonical mode of action, Bcl-2 has also been identified as a modulator of ER-localized IP_3R Ca^{2+} release channels. In general, Bcl-2 has been reported to exert an inhibitory effect on the efficiency by which Ca^{2+} is released from the ER by IP_3Rs (1). Although the inhibitory action of Bcl-2 is already assessed, its influence on the three individual IP_3R isoforms is not completely understood.

Using HEK cell models with a knockout (KO) of all three IP_3R isoforms (HEK-3KO) and reconstituted with either one of them, the effect of Bcl-2 on cytosolic Ca^{2+} flux mediated by each of the IP_3R isoforms was investigated individually. In this approach, the used agonist concentration was calibrated against the sensitivity of each IP_3R isoform to IP_3, resulting in three different agonist concentrations: 3.3 µM, 5 µM, and 10 µM of carbachol to measure IP_3R2, IP_3R1, and IP_3R3 overexpressing cells respectively. After having established the concentrations of carbachol that result in approximately similar intermediate IP_3R activation, we measured the effect of Bcl-2 on cytosolic Ca^{2+} flux for all three IP_3R isoforms. Our results indicate that Bcl-2 possesses isoform-specific effects towards the three IP_3R isoforms, with IP_3R2 channel opening being least impacted by Bcl-2.

In conclusion, we have established that Bcl-2 might indeed possess an isoform-specific effect in its regulation of the three different IP_3R isoforms. Generating such insights is important and useful as each IP_3R isoform has a different propensity to transfer Ca^{2+} directly from the ER to the mitochondria, in the order of IP_3R2 > IP_3R3 > IP_3R1 (2). As such, they each differentially influence the mitochondrial Ca^{2+} concentration, which is a critical determinant in maintaining cell health, but also in prompting the cell towards programmed cell death. Hence, characterizing the effect of Bcl-2 on each IP_3R isoform might aid in further deciphering its anti-apoptotic actions. Follow-up analysis will consist of performing mitochondrial Ca^{2+} uptake experiments in each of the cell systems, in the presence of ectopic Bcl-2 overexpression. Preliminary data already demonstrate that Bcl-2 also exerts inhibitory effects on IP_3R2-mediated mitochondrial Ca^{2+} uptake.

1. Rong YP, Bultynck G, Aromolaran AS, Zhong F, Parys JB, De Smedt H, et al. The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP3 receptor. Proc Natl Acad Sci U S A. 2009;106(34):14397–402.

2. Bartok A, Weaver D, Golenár T, Nichtova Z, Katona M, Bánsághi S, et al. IP_3 receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer. Nat Commun. 2019;10(1):1–14.

P22

Inhibition of IP3R-mediated Ca^{2+} release impairs lysosome acidification and β1-integrin recycling, reducing breast tumor cell migration, invasion and metastasis.

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Breast cancer is the second leading cause of death in women worldwide. More than 90% of breast cancer-related deaths are due to metastasis, a process that depends on the ability of cancer cells to leave the primary tumor, migrate and colonize different tissues. Inositol 1,4,5-trisphosphate receptor (IP3R)-mediated Ca^{2+} signaling plays an essential role in maintaining the bioenergetic homeostasis of cancer cells and sustained proliferation. However, its role in migration, invasion and metastasis is less understood. Here, using the highly metastatic triple negative MDA-MB-231 breast cancer cells we demonstrate that a prolonged inhibition of IP3R-mediated Ca^{2+} signals significantly reduces migration and invasion in vitro and metastasis in vivo. We find that this phenomenon is independent of the bioenergetic control of IP3R over the mitochondria and AMPK activation. Moreover, using a tandem LC3-GFP-mcherry we determine that upon a prolonged IP3R inhibition the flux of autophagy is reduced, resulting from faulty lysosome acidification as measured by DQ-BSA and pHrodo. A significant level of regulation during cell migration involves the assembly and disassembly of focal adhesions and the internalization and recycling of integrins by autophagy. IP3R inhibition impairs autophagy, as observed by the accumulation of β1-integrins, the predominantly expressed integrins in mammary epithelial cells, in autophagosomes. Our findings reveal an unexpected effect of IP3R inhibition in cancer cells that can have a great impact in an uncurable manifestation of cancer such as metastasis.

P23

Calcium-induced tissue contractions drive *in vitro* human neural tube morphogenesis

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The generation of the neural tube during embryonic development involves large-scale and coordinated tissue movements through which the neural plate, an initially-flat neuroepithelium, bends and folds to create a tubular architecture. The morphogenetic process of neural tube closure has been shown to depend on Ca^{2+} signaling, and calcium transients have been observed during vertebrate neural plate folding. However, to date, it is unclear how Ca^{2+} flashes regulate the mechanics of neural tube morphogenesis.

Here, we used a recently-developed organoid model of neural tube closure to study the dynamics of neuroepithelial folding *in-vitro* and its relationship to tissue-scale Ca^{2+} activity. By geometrically-confining human iPSCs through photopatterning and by fast live imaging, we observed that Ca^{2+} transients take place during neural tube morphogenesis. We show that multicellular Ca^{2+} transients initiate only during neural plate bending, persist during folding, and stop after tube closure. We automated the quantification of cell displacements at high temporal resolution and established that Ca^{2+} transients precede local tissue contractions. Interestingly, tissue contractions result in cellular movements towards the organoid midline and contribute to neural folds approaching and closure. Through pharmacological treatments, we uncover the involvement of IP3R and gap junctions in the generation and propagation of the calcium waves, and their requirement for tissue displacement. By photo-activation of calcium cages in our bioengineered model, we controlled the Ca^{2+} transient location, size and displacement magnitude, and demonstrated that wave induction is sufficient for coordinated tissue mechanics. By taking advantage of a Connexin43 iPSCs cell line, we visualized spatially-restricted gap junction formations and we observed their dependence on the neural tube developmental time.

We currently focus on using this system to understand whether distinct cell types (neural and non-neural ectoderm) differentially express gap junctions and to uncover how Ca^{2+}-induced tissue deformations are affected during neural tube defects. Overall, this work highlights a new biomechanical role of calcium in human neural tube morphogenesis.

P24

Dysregulated mitochondrial-associated membrane & Ca^{2+} homeostasis underlying Wolfram syndrome type 2-associated CISD2 deficiency.

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Wolfram syndrome type 2 (WS2) is a rare, progressive disorder caused by loss-of-function mutations in CDGSH iron-sulfur domain 2 (CISD2). CISD2 is an iron-sulfur cluster binding protein that impacts an ample range of cellular processes, including ROS and iron homeostasis, autophagy, cellular health and Ca^{2+} homeostasis. Oppositely to the situation in WS2, increased expression levels of CISD2 correlate with poorer prognoses in several types of cancer. The CISD2 protein is specifically enriched in the mitochondria-associated ER membranes (MAMs) at ER-mitochondrial contact sites. There, CISD2 is thought to be crucial for Ca^{2+} homeostasis, and consequently, cellular health. Indeed, highly focalized ER-mitochondrial Ca^{2+} fluxes through the inositol 1,4,5-trisphosphate receptor (IP3R) occurring in the MAMs stimulate proliferative processes, but can also trigger cell death events.

We validated CISD2's presence in the MAMs, by performing fractionations of Hela cell lysates and isolating MAM fractions, in which we detected an enrichment of CISD2. In order to investigate the result of loss of CISD2, we generated HeLa CISD2 KO cells via a Crispr/Cas9 based method. Using a MAM-specific fluorescent SPLICS probe, we found that CISD2-deficient HeLa cells have decreased ER-mitochondrial contact sites. Using co-immunoprecipitation assays, as well as microscale thermophoresis experiments, we demonstrated that CISD2 directly interacts with IP3R. We simultaneously imaged cytosolic and mitochondrial Ca^{2+} signaling. Loss of CISD2 moderately affected cytosolically released Ca^{2+}, but drastically decreased ER-mitochondrial Ca^{2+} transfer. Importantly, we excluded the effects of a reduced ER Ca^{2+} content and decreased inner mitochondrial membrane potential. The loss of ER-mitochondrial contact and ER-mitochondrial Ca^{2+} transfer might lead to an autophagic defect, as we detected an increased autophagic flux in CISD2-deficient cells. To research the effect of CISD2 in WS2-relevant cells, we differentiated cortical neurons from CISD2 KO induced pluripotent stem cells. In cortical neurons, we can stimulate the IP3R by extracellularly adding glutamate. Responses of glutamate-induced Ca^{2+} release are dramatically decreased in CISD2-deficient cortical neurons, likely resulting in a defect in ER-mitochondrial Ca^{2+} as well, as we have observed in HeLa cells, albeit with different mechanisms. Future work will aim to further elucidate the impact of decreased IP3R-mediated Ca^{2+} release in a WS2 relevant cellular context.

For now, we conclude that CISD2 is a MAM-resident protein that is vital for MAM integrity and function. CISD2-deficient cells exhibit decreased ER-mitochondrial Ca2 transfer. Possibly, CISD2 can positively regulate IP3R in the MAMs, for example by taking part in a macrocomplex with other IP3R modulators. Alternatively, decreased ER-mitochondrial Ca^{2+} transfer can be independent of IP3R modulation and be solely attributable to decreased ER-mitochondrial contact.

P25

Cyclic ADPR does not activate the non-selective cation channel TRPM2

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Cyclic ADP-ribose (cADPR) is a metabolite of NAD that evokes Ca^{2+} release from the endoplasmic reticulum by activation of ryanodine receptors. In addition, cADPR has been shown to triggers Ca^{2+} entry across the plasma membrane in some cell types. It remains unclear whether this Ca^{2+} entry is due to the activation of store operated Ca^{2+} entry via the the STIM/Orai system or involves activation of a distinct cADPR-activated Ca^{2+} channel. TRPM2 is a non-selective, Ca^{2+} permeable cation channel. The presence of a C-terminal domain akin to the ADP-ribose pyrophosphatase NUDT9 led to the identification of ADP-ribose as the activating ligand for TRPM2. Initial reports suggested that cADPR might also activate TRPM2, either on its own or in conjunction with ADP-ribose, particularly at a temperature of 37°C [1,2]. Subsequently, these reports were questioned upon the discovery that commercially available cADPR contains ADP-ribose, and that the removal of ADP-ribose eliminates the effects of cADPR [3,4]. More recently, the identification of an additional nucleotide binding site in the N-terminal MHR1/2 domain of TRPM2 that binds ADP-ribose in a horseshoe-shaped configuration, as well as the cADPR antagonist 8-Br-cADPR [5], and a publication showing activation of TRPM2 by binding of cADPR to the NUDT9H domain [6], have rekindled our interest in the role of cADPR in TRPM2 activation.

Through HPLC analysis, we verified that commercial cADPR preparations can contain notable quantities of ADP-ribose. We conducted whole-cell patch clamp experiments using HEK293 cells with stable overexpression of human TRPM2, employing the purest available cADPR preparation (<2% ADP-ribose). Surprisingly, we observed no activation of the channel, regardless of temperature, calcium buffering, or extracellular cation composition [7]. Furthermore, we found no synergistic effects when co-infusing cADPR with subthreshold ADP-ribose under conditions of low calcium buffering. To explore the contributions of the MHR1/2 and NUDT9H domains, we examined channel variants with mutations in either binding site for their response to ADP-ribose and the super agonist 2'-deoxy-ADP-ribose. Modifications in either domain abolished TRPM2 activation by both agonists. To investigate the binding of cyclic nucleotides to the MHR1/2 domain, we expressed the isolated MHR1/2 domain from zebrafish TRPM2 in bacteria and quantified the binding using isothermal titration calorimetry (ITC). Remarkably, neither cADPR nor 8-Br-cADPR exhibited binding to the isolated MHR1/2 domain, while ADP-ribose and 8-Br-ADP-ribose displayed high-affinity binding under identical conditions [7].

In summary, our findings indicate that while both nucleotide binding sites are crucial for the activation of human TRPM2 by ADP-ribose and 2'-deoxy-ADP-ribose, we found no compelling evidence for the activation of the channel by cADPR or the binding of cyclic nucleotides to the MHR1/2 domain.

References

[1] Kolisek M et al. Mol. Cell 2005, 18, 61–9

[2] Togashi K et al. EMBO J. 2006, 25, 1804–15

[3] Heiner I et al. Biochem. J. 2006, 398, 225–32

[4] Tóth B et al. J. Biol. Chem. 2010, 285, 30091–102

[5] Huang Y et al. eLife. 2019 Sep 12;8:e50175

[6] Yu P et al. Cell Rep. 2019, 27, 3684–3695.e4

[7] Riekehr WM et al. Int. J. Mol. Sci. 2022, 23 :316.3

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P26

Human iPSC derived iCell Cardiomyocytes reproduce vagomimetic side effects

of S1PR agonists therapy for Multiple Sclerosis

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Sphingosine-1-Phosphate receptor (S1PR) agonists are used to treat remitting multiple sclerosis. The primary mechanism of action of S1PR modulators in multiple sclerosis is through binding S1PR1 on lymphocytes resulting in internalization of the receptor and loss of responsiveness to the S1P gradient that drives lymphocyte egress from lymph nodes. The reduction in circulating lymphocytes presumably limits inflammatory cell migration into the CNS. Fingolimod-1-Phosphate (FTY-P) that binds on all S1PR but S1PR2, was the first S1PR modulator to receive regulatory approval but knowledge of adverse effects associated with FTY-P (e.g., bradycardia) led for a search for S1PR modulators with greater selectivity for S1PR1. Other recent compounds include among others ponesimod, siponimod and ozanimod were all designed to target S1PR1. Our aim was to test whether these different agonists could induce bradycardia in cardiomyocytes.

iCell cardiomyocytes from FujiFilm Cellular Dynamics are highly purified human cardiomyocytes derived from induced pluripotent stem cells. There is a mixture of spontaneously electrically atrial-, nodal- and ventricular–like myocytes. Thawed cells were seeded at a density of # 150.000 cells per cm² and kept for 10 days in maintenance medium. Cardiomyocytes were loaded for 45 minutes with Cal-520 calcium dye at 37°C in maintenance medium. Cardiomyocytes were washed twice in maintenance medium and placed in Hamamatsu FDSSµCell plate reader at 37°C. Calcium fluorescence was recorded for 2 minutes every 5 minutes over a period of 30 minutes. Data were acquired at a 33Hz frequency.

Heart beat rates in control conditions were 34 BPM in five independent experiments. Our results showed that S1PR agonists (Sphingosine-1-Phosphate (S1P), FTY-P, ozanimond, siponimod and posenimod) all induced bradycardia (25 to 32 BPM) at 1nM. However, long pauses (BPM less than 20) were also observed in the presence of S1P, FTY-P and ozanimod. The occurrence of these long pauses increased at higher concentrations (6 and 30nM) for all agonists. 24h pretreatment with 200ng/ml pertussis toxin that induced by itself strong bradycardia totally blocked the effects of these agonists indicating a clear involvement of G proteins in this process. These long pauses observed at 30nM were blocked in the presence of 100nM tertiapin Q, a IKACh blocker activated by beta/gamma G protein subunits, resulting in normal heart beat rates with few episodes of bradycardia suggesting that these S1PR agonists mainly trigger their effects through this channel. W146 200nM, a S1PR1 antagonist inducing bradycardia by itself, almost totally blocked bradycardia and long pauses induced by 6nM ponesimod.

Our data suggest that siponimod and ponesimod at 1nM are the two S1PR agonists with the fewer adverse effects on heart beat rates in our conditions. It confirms that patients treated with these drugs must be monitored for potential bradycardia. This study also shows that these iCell cardiomyocytes can be used for drug testing and cardiotoxicity studies.

P27

Effects on rhythmic behavior and neuronal function of human calmodulin mutations in *C. elegans*

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Calmodulin is a key calcium signal integrator and a central regulator of numerous cellular pathways: It measures changes in cytosolic calcium concentration and relays this information to its more than 300 interaction partners, including receptors, ion channels, and kinases. Our dependence on calmodulin is emphasized by the fact that the exact same protein is expressed in all vertebrates, and in humans, it is encoded in three independent genes.

Point mutations in calmodulin cause severe cardiac arrhythmia in humans. Experimental studies have demonstrated that the disease arises from dysregulation of specific ion channels, primarily calcium channels. However, it remains puzzling that mutations in this ubiquitous protein gives so specific effects. Could there be effects on other targets or in other tissues?

To answer these questions, we designed a *Caenorhabditis elegans* model that expresses human calmodulin as well as a number of mutations found in heart patients. We hypothesized that the arrhythmic effects of calmodulin mutations were conserved in this nematode and that we could use it to measure other potential phenotypes.

We found that the mutations N54I and D96V disrupt rhythmic behavior in *C. elegans*, recapitulating their effects in humans. We did not see effects on skeletal muscle and motility, emphasizing that the effects were specific and that *C. elegans* is a relevant model. Interestingly, we found that mutations that cause arrhythmia in humans and *C. elegans* also affect neuronal function, including acetylcholine signaling and chemotaxis, but each mutation in different ways.

Together, we demonstrate that *C. elegans* is a good system to study effect of calmodulin mutations and that the effects of mutations are not limited to rhythmical behavior. Moreover, we propose that these mutations may be used as tools to study calcium signaling in e.g. neuronal diseases. We are intrigued by the idea that different calmodulin mutations may affect different targets and thus different pathways, enabling new approaches to address the complexities of calcium signaling.

P28

Title: Calcium handling in 3D human cardiac models of Duchenne cardiomyopathy

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Abstract:

Duchenne muscular dystrophy (DMD) is the most prominent and severe muscular dystrophy affecting 1 in 3500 male births. DMD is a progressive X-linked genetic disorder caused by mutations within the *DMD* gene. In recent years the use of respiratory systems and palliative care improved patient survival leading to an increase of late-stage lethal complications, such as cardiomyopathy. The absence of dystrophin in DMD cardiomyocytes leads to calcium overload, oxidative stress, and mitochondrial dysfunction. Notably, the dysregulation of NADPH oxidase 4 (NOX4) has been identified as one of the responsible factors for the excessive reactive oxygen species production leading to DMD cardiac derangement[1]. The research into DMD cardiomyopathies has been mainly hampered due to the scarcity of human samples and preclinical models that faithfully recreate the symptoms of human illness. In order to tackle this limitation, we generated long-term cultured 3D cardiac organoids (DMD-COs) based on patient-derived induced pluripotent stem cells and the relative CRISPR/Cas9 corrected control (DMD-Iso-COs) which were able to reproduce cardiomyopathic characteristics and progression. DMD-COs developed fibrotic and adipose tissues as well as a higher rate of cell death, sarcoglycan loss, and endoplasmic reticulum stress over time. Additionally, the enrichment in hypertrophic/dilated cardiomyopathy, arrhythmia, adipogenesis, and fibrosis pathways in the DMD-COs emerged from the bulk RNA sequencing analysis on day 56[2]. To prove the extent to which the DMD-COs can be applied for drug screening, we are testing newly developed NOX4 inhibitors developed from the crystal structure of the NOX4 enzyme[3]. Preliminary data from flow cytometry and calcium handling analyses shows that NOX4 inhibition appears to improve the viability and the contractility of the dystrophic cardiomyocytes.

References

Duelen et al. (2022) Stem Cell Reports 17(2):352

Marini V et al. (2022) Front Cell Dev Biol 10: 878311.

Reis, et al. (2020) Redox biology, 32, 101466.

P29

Ca^{2+} signals during Enteropathogenic *E. coli* (EPEC) infection of epithelial cells

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Enteropathogenic *Escherichia coli* (EPEC) is a bacterial enteropathogen, a major agent of infectious diarrhea among children in developing countries. EPEC infection is associated with inflammation and disruption of the intestinal epithelium integrity through the action of bacterial effectors injected via a type III secretion system. Ca^{2+} is involved in the regulation of virtually all known cell processes, including cytoskeletal reorganization, inflammation, or cell death. EPEC was previously shown to trigger Ca^{2+} responses in infected cells, altering Ca^{2+} homeostasis. In this study, we investigated in more detail EPEC-induced Ca^{2+} signaling and the role of ATP released by the type III pore-forming translocon. We found that EPEC induces isolated global Ca^{2+} responses of varying amplitude. While these Ca^{2+} responses depended on the T3SS and extracellular ATP, they were elicited in only a minority of infected cells forming bacterial-associated actin pedestals. We found no evidence for increased basal Ca^{2+} levels, even upon prolonged bacterial infection. Consistent with an inhibitory function on Ca^{2+} signals of EspC, an EPEC secreted protease degrading components of the T3SS translocon, an espC mutant elicited a sharp increase in the frequency of Ca^{2+}-responding cells. The results suggest that EPEC-induced global Ca^{2+} responses are mainly due to ATP release through pores in plasma membranes of infected cells formed by the T3SS translocon.

P30

Comparing the effects of ADPR derivatives on the non-selective Ca^{2+} permeable cation channel TRPM2 and the ADPR-binding macrodomain of SARS-CoV-2

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Worldwide vaccination efforts have been conducted against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2); nevertheless local outbreaks of new variants continue requiring additional pharmacological treatment options (1).

To keep up in this arms race, novel viral targets came into focus. One promising candidate is the non-structural protein 3 (nsp3) of SARS-CoV-2, which contains a macroD-like macrodomain, called Mac1 that removes ADP-ribose (ADPR) from post-translationally modified proteins (2).

Since post-translational modification by ADP-ribosylation is required for the antiviral interferon response, Mac1 activity may contribute to early immune evasion and pathogenicity of the virus (3). Thus, pharmacological inhibition of Mac1 may provide a novel antiviral approach by restoring the type-I interferon response.

The hydrolysis product of Mac1 (i.e. ADPR) is a second messenger and can gate the transient receptor potential cation channel, subtype melastatin 2 (TRPM2), a non-selective cation channel, leading to influx of Na^+ and Ca^{2+} into cells and, thereby causing membrane depolarization (4).

In the past, our group has already developed and tested a number of ADPR analogues as potential modulators of TRPM2 (5).

As both the viral macrodomain and human TRPM2 bind to ADPR (2-5) it is important to understand the difference in structure-activity relationship (SAR) of both binding sites for selectively targeting Mac1.

Therefore, we set up assays for the enzymatic activity of Mac1 and performed orthogonal binding studies to further explore and validate the inhibitory potential of our ADPR analogs. Initial results using established TRPM2 binding nucleotides will be presented.

(1) Flynn, J. M. et al. “Comprehensive fitness landscape of SARS-CoV-2 Mpro reveals insights into viral resistance mechanisms.” eLife vol. 11 e77433. 20 Jun. 2022

(2) Alhammad, Y. M. O. et al. “The SARS-CoV-2 Conserved Macrodomain Is a Mono-ADP-Ribosylhydrolase.” Journal of Virology vol. 95,3 e01969-20. 13 Jan. 2021

(3) Fu, W. et al. “The search for inhibitors of macrodomains for targeting the readers and erasers of mono-ADP-ribosylation.” Drug Discovery Today vol. 26,11 (2021): 2547-2558.

(4) Berlansky, S. et al. “Calcium Signals during SARS-CoV-2 Infection: Assessing the Potential of Emerging Therapies.” Cells vol. 11,2 253. 12 Jan. 2022

(5) Moreau, C. et al. “Structure-activity relationship of adenosine 5'-diphosphoribose at the transient receptor potential melastatin 2 (TRPM2) channel: rational design of antagonists.” Journal of Medicinal Chemistry vol. 56,24 (2013): 10079-102.

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P31

Optimization of the ER-mitochondrial Ca^{2+} transfer: challenges and translational perspectives

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Mitochondria-endoplasmic reticulum (ER) contact sites (MERCS) are highly organized morpho-functional structures where numerous important cellular processes occur from phospholipid biosynthesis and protein translation to autophagosome formation, apoptosis and ER stress/UPR transduction. ER-mitochondrial (ER-Mit) Ca^{2+} transfer occurs at MERCS through IP3R-VDAC1 complexes. Dysfunctions of ER-Mit interation and ER-Mit Ca^{2+} transfer have been proposed to play a key role in many pathological conditions including neurodegenerative diseases, tumors and metabolic disorders. It is hypothesized that the ER-Mit distance for Ca^{2+} transfer lays in a range of 10-25 nm, however, the optimal distance and its regulation remain unknown.

We employed available and newly-designed artificial ER-Mit linkers, overexpressed in HeLa and other cells, fixing the ER-Mit distances ad 5, 10, 15, 20 and 30 nm. Following ATP-induced Ca^{2+} dynamics in Mit matrix of HeLa and other cells, 20nm was identified as the optimal distance to promote ER-Mit Ca^{2+} transfer. Using Proximity Ligation Assay (PLA) we show that, in presence of 20nm ER-Mit linker (20nm-EML), there is a drastic increase of IP3R-VDAC interaction, justifying the increased Ca^{2+} transfer. Immunocytochemical analysis and fractionation suggests enrichment of IP3Rs in MERCS, while immunoprecipitation of endogenous IP3R1 suggests increased association of Grp75 with IP3R1 in cells overexpressing 20nm-EML.

Unexpectedly, 20nm-EML overexpression produced an increase of basal [Ca^{2+}] in the cristae and mitochondrial inter-membrane space (MIMS), but not in the matrix suggesting that a low-affinity mitochondrial Ca^{2+} transfer is not activated upon tonic Ca^{2+} increase in the inter-membrane space. A recently identified MCU activator amorolfine discharged the Ca^{2+} from MIMS and cristae into the matrix inducing Mit Ca^{2+} overload, and resulting in death of HeLa cells, sensitive to mPTP antagonist cyclosporin A.

To investigate physiological role and modulation of 20 nm contact sites, we generated a split-GFP contact sites sensor for 20nm distance (20nm-SPLICS), that allowed monitoring the dynamic increase of ER-Mit interactions at 20nm. In Airway Smooth Muscle Cell, upon stimulation of Absintin→hTAS2R46→EPAC signalling pathway, increased interaction at 20nm augmented Histamine-induced Mit Ca^{2+} uptake.

In conclusion: 1) 20nm is the optimal distance for ER-Mit Ca^{2+} transfer which allows spontaneous assembly of IP3R-VDAC1 complexes; 2) Dynamic modulation of 20nm ER-Mit contact sites physiologically regulates Mit Ca^{2+} signals; 3) Modulation of the ER-Mit distance is a valuable strategy to finely tune Mit calcium uptake, thereby regulating cell fate.

P32

Evolution and Biochemistry of Histidine-Rich Calcium Binding Proteins

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In the striated muscles of mammals, the histidine-rich calcium binding protein (HRC) resides in the lumen of the sarcoplasmic reticulum (SR), where it can modulate the function of two proteins critical for excitation contraction coupling. At low luminal Ca^{2+} concentrations, HRC predominantly associates with SR/ER Ca^{2+}-ATPase (SERCA) pumps, enhancing their transport activity. At high luminal Ca^{2}, HRC predominantly interacts with the SR integral membrane anchoring protein triadin, thereby promoting Ca^{2+}-release via associated ryanodine receptor (RyR2) channels. Enhanced interaction with triadin is promoted by phosphorylation of HRC at residue S96, by the secretory pathway kinase Fam20C. In humans, a S96A mutation of HRC is associated with arrhythmias in subjects with idiopathic dilated cardiomyopathy. Dysregulation of HRC is also linked with disorders in non-muscle systems, including lung cancer, liver fibrosis, hepatocellular carcinoma and gastric cancer. Aspolin is an aspartic acid-rich paralogue of HRC, initially identified in fish, that promotes demethylation of trimethylamine-N-oxide.

In the current study, homologues of HRC and aspolin were identified in the proteomes of diverse species, including archaea, bacteria, sponges, molluscs and vertebrates. By contrast, triadin appears to be a vertebrate innovation. In phylogenic reconstructions of the evolution of HRC, homologues tended to cluster poorly, even among closely related taxa. This suggests rapid rates of HRC evolution. This hypothesis was confirmed by analyses of apparent mutation rates of HRC, relative to other proteins involved in excitation-contraction coupling in striated muscles, such as triadin.

The high histidine content of HRC homologues indicates that these proteins potentially participate in pH-sensing. This might be of physiological significance, given the stoichiometry of Ca^{2+} and H^{+} exchange in SERCA pump reaction cycles. A conserved pattern of cysteine residues suggests that HRC homologues might also belong to a superfamily of ferredoxin-binding proteins, involved in iron-dependent redox reactions. Since many SR proteins involved in excitation-contraction coupling are exquisitely regulated by redox potential, this is also likely to be of physiological relevance.

P33

PAR2-mediated signaling in human ADPKD cells stimulates macrophage attraction

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder characterized by the gradual development of kidney cysts and leading to end-stage kidney disease. Most cases are caused by mutations in *PKD1* (*ca.* 75%) and *PKD2* (*ca.* 15%), encoding for polycystin-1 and -2, but their exact functions remain elusive. Aberrant Ca^{2+} homeostasis and signaling is one of the main and early hallmarks in ADPKD cells. Previously, we have also identified enhanced Monocyte Chemoattractant Protein-1 (MCP-1) release as an early-stage event in ADPKD. MCP-1 attracts macrophages towards the cystic environment, promoting cyst growth in ADPKD.

Using a G-protein coupled receptor (GPCR) agonist library, we observed that Protease-Activated Receptor 2 (PAR2) stimulation triggered Ca^{2+} signals in urine-derived human kidney epithelial cell lines. ADPKD cells (carrying *PKD1* mutations) were more responsive to physiological PAR2 agonist trypsin (0.2-2 µg/ml) compared to healthy cells and this was associated with an enhanced oscillatory behavior. Thrombin, which stimulates other PAR family members, induced similar Ca^{2+} responses between healthy and ADPKD cells. As PAR2 is known to stimulate MCP-1 expression, we investigated the effect of PAR2 agonists (trypsin and 2-Furoyl-LIGRLO-amide) and antagonist (I-191) on MCP-1 production and secretion in human kidney epithelial cells. Indeed, incubation with PAR2 agonists induced MCP-1 release, which was higher in ADPKD cells compared to healthy controls, and this was potently blocked by inhibiting PAR2. Finally, using co-culture transwell experiments, incubation of ADPKD epithelial cells with I-191 reduced the migration of THP-1 macrophage-like cell lines.

Our *in vitro* data suggest that PAR2-dependent signaling is enhanced in human ADPKD epithelial cell lines. This leads to enhanced MCP-1 expression, thereby promoting macrophage attraction. As such, chemical PAR2 modulation could be a promising target to attenuate macrophage recruitment and reduce cyst formation in ADPKD.

P34

Title (max. 150 characters): Lipid particles induce Ca^{2+} signals in target cells

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Due to their involvement in a wide range of pathologies and the highly specific transported cargo, extracellular vesicles (EVs) gained tremendous attention in the field of intercellular signaling. As a low-cost, easy production, and customizable alternative for EVs, synthetic lipid vesicles (SLVs) are good candidates for the study of fundamental interactions between EVs and recipient cells. Most studies on lipid vesicles focus on the long-term effects on target cells, occurring after endocytosis. However, the acute events preceding the internalization of the vesicles, i.e. the initial interactions with the plasma membrane, are likely to be sensed by specialized receptors on the cell surface. In this study, we hypothesized that extracellular lipid particles activate mechano-sensory channels upon their interaction with the plasma membrane of recipient cells. To test this, we determined the effects of extracellular lipid vesicles on TRPA1, a Transient Receptor Potential cation channel known to be activated by mechanical perturbations in the plasma membrane. We used Fura-2-based fluorescence imaging to assess changes in intracellular Ca^{2+} concentrations triggered by possible TRPA1 activation by lipid vesicles. We found that acute (<20 min) extracellular applications of EVs derived from MCF-7 cells (120 nm) or SLVs (150 nm) trigger robust Ca^{2+} responses in recipient CHO cells expressing TRPA1, in a concentration-dependent manner. The responses to lipid vesicles are transient and occur randomly along the application time, suggesting for discrete stochastic interactions between the vesicles and the cells. TRPA1-transfected cells exposed to a control solution or the vesicles together with the specific TRPA1 blocker HC-030031 responded with significantly smaller and less frequent Ca^{2+} transients. Moreover, the transfection reagent Mirus, which forms polymer-based lipid particles, elicits the same kind of responses in TRPA1-expressing cells. Our results unveil a mechanism whereby extracellular lipid vesicles stimulate Ca^{2+}-dependent signaling in recipient cells. This suggests that TRP channels may be implicated in EV-mediated intercellular communication recruiting nociceptive neurons, which in turn may lead to pain and neurogenic inflammation.

P35

NON GENOMIC REGULATION OF TRPM8: FROM THERMOSENSATION TO CANCER Dimitra Gkika, CANTHER Lab, UMR9020 CNRS - UMR-1277 Inserm, University of Lille, France

TRPM8, a predominant detector of cold temperatures in vivo, is also expressed in sensory fibers innervating visceral organs and in epithelia such as prostate, bladder, testis and skin. In epithelia TRPM8 was involved in carcinogenesis and seems to be one of the most promising clinical targets for prostate cancer due to the variation in its expression. In an effort to characterize physiological factors other than cold playing a putative role in TRPM8 activation/modulation, several hormones were tested in our laboratory. In this context we have recently shown that testosterone regulates directly TRPM8 activity in both prostate carcinogenesis and cold thermosensation. In this presentation I will provide a mechanistic insight in the hormonal non genomic regulation of TRPM8 channel in two processes modulated during ageing, cold thermosensation and malignant transformation.

P36

Mitochondrial calcium homeostasis is altered during endothelial-to-mesenchymal-transition

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The transdifferentation of endothelial cells into mesenchymal-like cells, a process named endothelial-to-mesenchymal transition (EndMT) is an embryonic program necessary for organ development. Despite being dormant in adults, this mechanism can be reactivated during several diseases, including cancer, organ fibrosis and a number of cardiovascular disorders. Therefore, EndMT targeting holds therapeutic promise for treating some of the most prevalent diseases. However, a precise molecular definition of EndMT is lacking, and targeting the EndMT master-regulatory transcription factors remains challenging.
We performed single-cell RNA-sequencing on three in vitro models of induced EndMT differentiation and identified mitochondrial calcium signaling as a novel conserved EndMT regulator. We functionally validated that mitochondrial calcium uptake influences endothelial transdifferentiation, whereas blockade of the mitochondrial calcium uniporter (MCU) prevented EndMT. Conditional endothelial deletion of *mcu* confirmed loss of mesenchymal activation in a hind limb ischemia mouse model. Consistent with these findings, human tissues from patients with critical limb ischemia showing features of EndMT expressed significant higher levels of endothelial MCU.
Together, these data identify mitochondrial calcium signaling as a novel regulatory mechanism of endothelial transdifferentation, thus potentially allowing for the development of new therapeutic interventions for EndMT-based diseases.

P37

The role of Calcium Signaling in Cancer Resistance

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Resistance to cancer therapy is a major challenge for cancer treatment. Thus, the identification of new therapeutic targets is required to overcome resistance. There is limited information on the role of Calcium (Ca^{2+}) signaling in cancer resistance. In our study, we are targeting the role of Ca^{2+} signaling as a mediator of cancer resistance showing that the combination of anticancer therapy with Ca^{2+} signaling modulators can improve the effectiveness of current treatments, both chemo and hormonal therapies focusing on understanding the cellular processes behind resistance, including autophagy and mitochondrial integrity. Keeping in mind that every type of cancer or model of cancer cell resistance, has its own Ca^{2+} signaling signature depending on the type of drug used and and its mechanism of action, we described the ability of transient receptor potential calcium channels to alter Ca^{2+} influx and affecting the sensitivity of cancer cells to treatment in prostate cancer cells and drug resistant cell line. We have identified several calcium channels involved in chemo and hormonal resistance where TRPV6 represented the most promising candidate; as a novel autophagy modulator and target for improving the efficacy of treatment in prostate cancer patients. Therefore, the identification of the channel or network of channels involved in chemoresistance might allow for the establishment of new, promising therapeutic strategy mixing pharmacological modulators of Ca^{2+} channels with established drug therapies.

P38

Ion channel modulation in prostate cancer drug resistance : mechanisms and therapeutic implications

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Prostate cancer (PCa) is the most common non-cutaneous human malignancy and the second most lethal tumor among men, with its highest incidence in industrialized countries. Despite considerable progress in diagnosis and treatment, drug resistance remains a significant hurdle in cancer treatment. Henceforth, development of new therapeutic targets are necessary to improve both the survival and the everyday life of the patients. It is now well established that the ubiquitous second messenger Ca^{2+} is a critical regulator of cancer cell death and survival. As this field of research is emerging, our research delves into the relatively unexplored domain of Calcium (Ca^{2+}) signaling and its potential role in cancer therapy resistance. It's pivotal to understand that each cancer type or its resistant cell model carries a unique Ca^{2+} signaling fingerprint, influenced by the treatment received and its functional pathway. We have identified that in prostate cancer, several calcium channels namely TRPC1, TRPV6 and Orai channels to be involved in regulating cell fate decisions through altering mitochondrial integrity and autophagy process and thereby providing resistance to treatment. We therefore propose that integrating anticancer treatments with specific Ca^{2+} signaling modulators could enhance the efficacy of chemo and hormonal therapies.

P39

CMT1J – *ITPR3* mutations in peripheral neuropathy, immunodeficiency, and tooth abnormalities

Rönkkö, J., Hytönen, M.K., Rodriguez, Y., Kvist, J., Hundi, S., Suonto, E., Torregrosa-Muñumer, R., Pennonen, J., S., González, D., Teräväinen, E., Kuntsi, H., Kuuluvainen, E., Hietakangas, V., Van Den Bosch, L., Bultynck, G., Lohi, H., Tyynismaa, H. & Ylikallio, E.

Inositol 1,4,5-trisphosphate receptors (IP3Rs) are ER Ca2+-release channels that control a broad set of cellular processes. They are crucial signaling hubs in cells, releasing calcium from the endoplasmic reticulum upon IP3 binding. Recently, we have identified autosomal dominant mutations in the *ITPR3* gene, which encodes the IP3R3 protein, as a novel causative factor for CMT, leading to the designation of a new demyelinating CMT type, CMT1J (OMIM #620111). Charcot-Marie-Tooth disease (CMT) is a group of hereditary neuropathies, characterized by progressive distal sensory and motor impairment, which affects 1:2500 individuals.

Given the importance of IP3Rs in human diseases, we investigated their role in human induced pluripotent stem cells (hiPSC) by developing single IP3R and triple IP3R knockouts (TKO) and generated three CMT1J-patient specific knockin hiPSC lines. Genome edited TKO-hiPSC lacking all three IP3R isoforms, IP3R1, IP3R2, IP3R3, failed to generate Ca2+ signals in response to agonists activating GPCRs, but retained stemness and pluripotency. Steady state metabolite profiling and flux analysis of TKO-hiPSC indicated distinct alterations in tricarboxylic acid cycle metabolites consistent with a deficiency in their pyruvate utilization via pyruvate dehydrogenase, shifting towards pyruvate carboxylase pathway. These results demonstrate that IP3Rs are not essential for hiPSC identity and pluripotency but regulate mitochondrial metabolism. Next, we will investigate the CMT1J pathogenesis and IP3R function. Newly created CMT1J-specific iPSC lines will be differentiated into motor neurons and Schwann cells, and will be studied extensively, with unbiased methods such as metabolomics, proteomics, and electrophysiology.

P40

Inositol-trisphosphate signaling enhances Cx43 hemichannel opening in ventricular cardiomyocytes

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Connexins (Cxs) are channel forming proteins that are crucial in facilitating impulse conduction over the cardiac tissue. Cxs form hemichannels that assemble as gap junctions and directly connect cardiomyocytes as well as cells of specialized conduction tissues. In addition to their function as building stones for gap junctions, connexin hemichannels are also present as free membrane channels not being part of gap junctions; hemichannels are controlled by calcium ion (Ca^{2+}) signaling but also allow Ca^{2+} entry thereby affecting Ca^{2+} signaling. We and others recently found that hemichannel opening is involved in disturbances of the cardiac rhythm, especially by triggered action potential generation. Work of our research group is focused at understanding the conditions and signaling by which hemichannels are activated to open, especially in the context of cardiac disease. Recent work indicated that the triad of Ca^{2+} elevation, ryanodine receptor (RyR) activation and Cx43-RyR interaction strongly enhanced hemichannel opening and arrhythmogenic responses. New findings further indicate that crosstalk between inositol-trisphosphate (IP_3) induced Ca^{2+} release and RyRs facilitates arrhythmias in the failing human ventricle where IP_3-receptor expression is increased. Given the fact that IP_3/Ca^{2+} signaling is a strong stimulus for hemichannel opening in non-cardiac cells, we here investigated whether elevated IP_3 signaling could enhance hemichannel opening induced by caffeine stimulation of RyRs in mouse ventricular cardiomyocytes. Our electrophysiological recordings in these cardiomyocytes demonstrate that elevation of cytoplasmic IP_3 to 1 µM significantly enhanced hemichannel opening triggered by RyR stimulation with caffeine, which was prevented by a peptide designed to interfere with molecular interactions between Cx43 and IP_3 type 2 receptor (IP_3R2). STED imaging further suggested that IP_3R2 are closely associated with Cx43 at the cell ends/intercalated disks. Further work is in progress to investigate whether IP_3-induced hemichannel opening results in arrhythmogenic responses in ventricular cardiomyocytes isolated from hypertrophic/failing rodent hearts.

LIST OF DELEGATES

LAST NAME	FIRST NAME	AFFILIATION	COUNTRY
AGOSTINIS	PATRIZIA	KU Leuven	Belgium
ALLEWAERT	ROSALIE	Ghent University	Belgium
AMBUDKAR	INDU	NIDCR, NIH	France
AMOR BARRIS	SILVIA	VIB -Uantwerp	Belgium
ARCOT KANNABIRAN	SUKANYA	UKE, Hamburg Eppendorf	Germany
ATAKPA-ADAJI	PEACE	University of Cambridge	United Kingdom
BACHKÖNIG	OLAF	Medical University Graz	Austria
BADER	EMENI	Westburg	Belgium
BALCAEN	WOUT	KU Leuven	Belgium
BARBEAU	SOLÈNE	UC Louvain	Belgium
BAZELI	BAHAR	VIB-KU Leuven	Belgium
BEZPROZVANNY	ILYA	UT Southwestern Medical Center at Dallas	United States
BOOTMAN	MARTIN	The Open University	United Kingdom
BRENKER	CHRISTOPH	University of Münster	Germany
BROHUS	MALENE	Aalborg University	Denmark
BULTYNCK	GEERT	KU Leuven	Belgium
CALDWELL	ASHLEE	KU Leuven	Belgium
CALLENS	MANON	KU Leuven	Belgium
CANTELMO	ANNA RITA	Institut Pasteur de Lille	France
CAPIOD	THIERRY	Institut Necker Enfants Malades (INEM)	France
CARDENAS	CESAR	Universidad Mayor	Chile
COMBETTES	LAURENT	CNRS	France
COUGHLAN	ALED	Cardiff University	Wales
CYERT	MARTHA	Stanford University	United States
D'ANGELO	JEAN MARC	Hamamatsu	France
DE CLERQ	KATRIEN	KU Leuven	Belgium
DE MATOS	FIONA	KU Leuven	Belgium
DE RIDDER	IAN	KU Leuven	Belgium
DE SMEDT	HUMBERT	KU Leuven	Belgium
DECUYPERE	JEAN-PAUL	KU Leuven	Belgium
DIERCKS	BJÖRN-PHILIPP	University Medical Centre Hamburg-Eppendorf	Germany
DOLGA	AMALIA	University of Groningen	Netherlands
DUCREUX	SYLVIE	University of Lyon	France
EHRLICH	BARBARA	Yale University	United States
EKSHTEYN	ALEXANDRA	VIB -Uantwerp	Belgium
ERNEUX	CHRISTOPHE	Université Libre de Bruxelles	Belgium
FARES	NASSIM	Saint Joseph University of Beirut	Lebanon
FISSORE	RAFAEL	University of Massachusetts	United States
FLIEGERT	RALF	University Medical Centre Hamburg-Eppendorf	Germany
GAILLY	PHILIPPE	UC Louvain	Belgium
GARCIA SAEZ	ANA J.	Univ. Cologne	Germany

GKIKA	DIMITRA	Univerity of Lille	France
GRANATO	VIVIANA	University Medical Centre Hamburg-Eppendorf	Germany
GREBENIUK	SERGEI	KU Leuven	Belgium
GU	FENG	University Medical Centre Hamburg-Eppendorf	Germany
GUDAITIS	LUCAS	KU Leuven	Belgium
GUO	FANGRUI	Institute for Integrative Biology of the Cell – I2BC	France
GUSE	ANDREAS H	University Medical Centre Hamburg-Eppendorf	Germany
HAJNOCZKY	GYORGY	Thomas Jefferson University	United States
HALKJÆR JENSEN	HELENE	Aalborg University	Denmark
HEINZE	CORINNA	KU Leuven	Belgium
HINRICHS	MACARENA	University Medical Centre Hamburg-Eppendorf	Germany
IVANOVA	ADELINA	University of Cambridge	United Kingdom
KAJI	MARCUS	VIB -Uantwerp	Belgium
KANNANCHERI PUTHOORU	DHEERAJ	INSERM U1003	France
KRAUSHAAR	UDO	NMI	Germany
KREBS	JOACHIM	Max Planck Institute for Natural Sciences	Germany
KULOW	FREDERIKE	University Medical Centre Hamburg-Eppendorf	Germany
LA ROVERE	RITA	KU Leuven	Belgium
LEMOS	FERNANDA	KU Leuven	Belgium
LEYBAERT	LUC	Ghent University	Belgium
LIM	DMITRY	Università del Piemonte orientale	Italy
LONCKE	JENS	KU Leuven	Belgium
LUYTEN	TOMAS	KU Leuven	Belgium
MACHACA	KHALED	Weill Cornell Medicine Qatar	Qatar
MACKRILL	JOHN	University College Cork	Ireland
MARINI	VITTORIA	KU Leuven	Belgium
MATTOLINI	LORENZO	KU Leuven	Belgium
MCKEOWN	LYNN	University of Leeds	United Kingdom
MEKAHLI	DJALILA	UZ Leuven	Belgium
MICHALAK	MAREK	University of Alberta	Canada
MILICI	ALINA	VIB-KU Leuven	Belgium
MIZUNO	HIDEAKI	KU Leuven	Belgium
MOECKL	FRANZISKA	University Medical Centre Hamburg-Eppendorf	Germany
MU	QIANRU	University College London	United Kingdom
MUALLEM	SHMUEL	NIDCR/NIH	United States
NAERT	ROBBE	KU Leuven	Belgium
NEUMANN	JULIKA	VIB-KU Leuven	Belgium
NUÑEZ	LUCIA	University of Valladolid	Spain
OFLAZ	FURKAN ENES	Medical University Graz	Austria
ORNELAS GUEVARA	ROBERTO	Université Libre de Bruxelles	Belgium
OVERGAARD	MICHAEL TOFT	Aalborg University	Denmark
PARPAITE	THIBAUD	UC Louvain	Belgium

PARYS	JAN	KU Leuven	Belgium
PATEL	SANDIP	University College London	United Kingdom
PAUELS	INGA	University of Münster	Germany
PCHITSKAYA	EKATERINA	Peter the Great St.Petersburg Polytechnic University	Russia
PEREIRA	GUSTAVO	University College London	United Kingdom
PERSOONS	ELEONORA	KU Leuven	Belgium
PIZZO	PAOLA	University of Padua	Italy
PONTISSO	ILARIA	Institute for Integrative Biology of the Cell – I2BC	France
PREVARSKAYA	NATACHA	INSERM	France
RANGA	ADRIAN	KU Leuven	Belgium
RESCHER	URSULA	University of Münster	Germany
RIETDORF	KATJA	The Open University	United Kingdom
ROBBEN	STIJN	KU Leuven	Belgium
RODERICK	LLEWELYN	KU Leuven	Belgium
RÖNKKÖ	JULIUS	University of Helsinki	Finland
ROS QUINCOCES	URIS LIANNE	Univ. Cologne	Germany
ROSSI	ANA	University of Cambridge	United Kingdom
SALIBA	YOUAKIM	Saint Joseph University of Beirut	Lebanon
SANDMANN	MAXIMILIAN	University Medical Centre Hamburg-Eppendorf	Germany
SCHILLEBEECKX	LAURA	KU Leuven	Belgium
SCHMIDT	TAMÁS	Univ. Cologne	Germany
SERYSHEVA	IRINA	McGovern Medical School at UTHealth	United States
SMYTH	JEREMY T	Uniformed Services University	United States
SNEYERS	FLORE	KU Leuven	Belgium
SPEELMANS-ROOMS	FEMKE	KU Leuven	Belgium
STARTEK	JUSTYNA	VIB-KU Leuven	Belgium
TAJEDDINE	NICOLAS	UC Louvain	Belgium
TALAVERA	KAREL	KU Leuven	Belgium
TAO	SIYU	Ghent University	Belgium
VAN AERDEN	PHILIPPE	Molecular devices	Belgium
VAN GRORP	ROBBE	KU Leuven	Belgium
VANDEN BERGHE	PIETER	KU Leuven	Belgium
VANMUNSTER	MAARTEN	KU Leuven	Belgium
VENNEKENS	RUDI	KU Leuven	Belgium
VENNEMAN	TOM	KU Leuven	Belgium
VERVLIET	TIM	KU Leuven	Belgium
VILLALOBOS	CARLOS	Spanish National Research Council (CSIC)	Spain
VOETS	THOMAS	VIB-KU Leuven	Belgium
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WITSCHAS	KATJA	Ghent University	Belgium
YANG	XIAOJUAN	VIB-KU Leuven	Belgium
YASSINE	MAYA	University of Lille	France
YUAN	YU	University College London	United Kingdom
YULE	DAVID	University of Rochester	United States